Abstract Book

Posters

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dia-PASEF proteomic analysis of HNSCC tumor and stroma enriched sections from FFPE samples prepared with laser capture microdissection

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Head and neck squamous cell carcinoma (HNSCC), an epithelial cancer is the most common type of head and neck cancer. HNSCC cells first invade the basement membrane of native epithelium, and in >50% cases proceed to lymph node metastasis, which is associated with poor survival. Overall, the response to available treatments has been moderate. The genomic and transcriptomic landscape of HNSCC (The Cancer Genome Atlas) has been defined, but pinpointing the genetic aberrations linked to tumor phenotypes remains elusive. Deep proteome analysis of tumor and matched normal adjacent tissues (NATs) has been performed (Clinical Proteomic Tumor Analysis Consortium). Proteomic comparison of the cancer cells and its neighboring microenvironment may help identify novel targets for early detection, and intervention of HNSCC.

Methods

In this study, laser capture microdissection (LCM) was used to collect tumor and stroma enriched sections from formalin-fixed paraffin-embedded (FFPE) tissues. The samples were processed and digested with trypsin. Data independent LC-MS/MS analysis was performed using the timsTOF HT mass spectrometer connected to nanoElute 2 LC system. Each sample was analyzed in triplicate using a 32-minute gradient (500 ng peptide per injection, 40 min total run time), resulting in a throughput of 24 samples per day. The data analysis was performed using the directDIA+ workflow (Spectronaut 18 software) and the Uniprot-Human-reviewed database (20,383 protein entries).

Novel Aspect

Deep proteomic analysis of FFPE derived tumor cells and their microenvironment.

Preliminary Data or Plenary Speaker Abstract

We have completed analysis of 20 samples, and in total 8,300 protein groups were identified corresponding to ~96,000 precursors. Excellent technical reproducibility was observed between sample runs with median CV of <8% at protein quantitation level. In paired tumor and stroma samples 6,676 proteins were quantified across all samples. All stroma samples clustered together and clearly separated from tumor group by hierarchical analysis. Volcano plot analysis extracted the proteins that are significantly more abundant in stroma vs. tumor enriched samples. GO functional and pathway enrichment analysis of these proteins identified several functional groups relevant to stromal and tumor regions, e.g., relative high abundance of growth factor binding, collagen binding, heparin binding proteins, and ECM structural constituents in the stromal region. Overall, in this study we were able to achieve excellent proteome coverage in LCM FFPE samples. This is especially important for stromal samples where available amount of protein is very low. The methodology allows for comparative deep proteome analysis of tumor and its adjacent microenvironment in a scalable format.

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Confident transformation site localization of PROTAC drug metabolites facilitated by multi-stage fragmentation LC-HRAM-MS

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The study of a drug's metabolism is an integral part of drug discovery and development, to improve drug exposure and reduce potential toxicity from its metabolites. The identification of drug metabolites routinely relies on LC/MS experiments, with fragmentation data enabling their structure elucidation.
In recent years, hetero-bifunctional small molecule drugs, acting on a given protein of interest (POI) through recruitment of inherent protein degradation pathways rather than inhibition, have gained significant interest, with multiple such proteolysis targeting chimera (PROTAC®) drug candidates being investigated in clinical trials. However, due to PROTACs' unique properties, the identification of their metabolites can be challenging.
Here, we describe the utility of multi-stage fragmentation (MSn) for the elucidation of transformation sites on PROTAC drug metabolites.

Methods

Commercially available PROTAC drugs were incubated with liver S9 fractions to generate metabolite profiles, with aliquots quenched at different times. Samples were analyzed using a Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to a Thermo Scientific™ Orbitrap Tribrid™ mass spectrometer. Metabolites were separated on a C18 column with a 15 minute gradient using 0.1% formic acid in water and acetonitrile, respectively, as mobile phases. Mass spectral data were acquired using data-dependent MSn experiments with a flexible decision tree for HCD or CID-MS2 and MS3 triggering based on precursor m/z and on-the-fly analysis of acquired spectra. Thermo Scientific™ Compound Discoverer™ 3.3 SP3 software was used for metabolite detection and transformation site localization with the help of the fragment ion search algorithm.

Novel Aspect

Confident characterization of PROTAC drug metabolites based on LC/MSn fragmentation data for transformation site localization and soft-spot analysis.

Preliminary Data or Plenary Speaker Abstract

The commercially available PROTAC compound MZ1 and other structurally related compounds were investigated as model drug compounds in this study to investigate the impact of linker and drug chemical structure on metabolism as well as to examine the utility of different dissociation techniques and multi-stage fragmentation for the structure elucidation of their metabolites.
All test articles were separately incubated with liver S9 fractions in the presence of NADPH to induce metabolism, followed by the addition of cold acetonitrile to quench and precipitate proteins. Initial qualitative analysis of the supernatants suggested a slow but diverse metabolism of the parent compounds, forming a complex mixture of low abundant metabolites. In the case of MZ1, among the 25 metabolites found to exceed 1% relative intensity compared to the parent at t=0h, several different dealkylated metabolites resulting from amide- or ether-bond cleavage were detected. In addition, multiple isomeric oxidation products could be detected, however their MS2 fragmentation provided insufficient information to unambiguously localize their respective oxidation sites.
Based on these initial observations, the ddMSn method was adjusted to perform only HCD-MS2 fragmentation on precursors below 500 Da, while acquiring both CID-MS2 and HCD-MS2 fragmentation on precursors above 500 Da, with subsequent MS3 spectra acquired on any MS2 fragments above 400 Da.

Data were then acquired on multiple timepoints to enable comprehensive metabolite identification for all test articles. The resulting data showed that linker cleavage metabolism was more prevalent in ethylene-glycol-linked PROTACs compared to aliphatic linkages. Additionally, the MSn fragmentation data could be used to reduce ambiguity in the transformation site localization, especially for metabolites with higher molecular weights. For example, in case of one oxidized metabolite of MZ1 eluting at 5.62 min, the MS3 fragments from a transformation-shifted MS2 ion at 695 Da identified the POI-binding motif as the oxidation site.

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A Semi-Targeted Orbitrap Tribrid Method for Simultaneous Quantification and Discovery of Immunopeptides

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Immunopeptidomics is a rapidly evolving field that aims to identify and quantify naturally occurring peptides presented by the major histocompatibility complex molecules to the immune system. Many experiments focus on both the discovery of unknown peptides, or the quantification of a set of peptides of interest, requiring enough sample for at least two injections. Utilizing a modified Orbitrap AscendTM Tribrid mass spectrometer which combines both a high-resolution Orbitrap and a highly sensitive ion trap (IT), a data-dependent experiment for untargeted analysis can be combined with highly sensitive quantitative data in the same analysis using a single injection. Here, we present a new method utilizing the high-resolution Orbitrap detector discovery, while simultaneously collecting quantitative data in parallel with the IT.

Methods

HeLa protein digest was spiked with a dilution of Pierce™ Peptide Retention Time Calibration Mixture in concentrations from 40 to 0.4 fmol. Samples were analyzed using an IonOpticks Aurora Ultimate TS 25cm column connected to a Thermo Scientific™ Vanquish™ Neo LC and a modified Orbitrap Ascend MS. The semi-targeted method used the Orbitrap to collect data-dependent MS2 data while the IT was used for targeted analysis. Class 1 MHC peptides from HCT-116 cells were prepared at 1e6 cell equivalents, spiked with synthetic heavy labeled AQUA peptide standards of common HLA peptides at concentrations of 100 amol to 100 fmol, and analyzed using the same method. Data was processed using Thermo Scientific™ Proteome Discoverer™ 3.1, PEAKS Studio 11, and Skyline software.

Novel Aspect

New method for simultaneous Targeted and Untargeted analyses of immunopeptides in a single injection utilizing two mass analyzers.

Preliminary Data or Plenary Speaker Abstract

Data-dependent MS2 was obtained for a HeLa digest standard and the Class 1 MHC peptides sample, while targeted MS2 was obtained for all 15 PRTC peptide standards. Data for the HeLa standard showed minimal loss of identifications when adding the unscheduled targeted MS2 experiment. In the targeted ion trap experiment, MS2 scans yielded diagnostic fragments of all PRTC peptides sufficient for quantification down to 0.4 fmol.
For the HLA enriched sample, peptide identification was evaluated along with quantitative performance on 9 heavy labeled AQUA peptides and their endogenous counterparts. Over 3500 peptides were identified in a 72-minute run from 1e6 cell equivalents of sample. Additionally, simultaneous parallel reaction monitoring of the heavy-labeled peptide pairs enables accurate quantitation over 3 orders of magnitude for some peptides, and 2-orders of magnitude for others, with lower limits of quantitation as low as 100 amol. It is possible to further increase sensitivity by increasing injection time in the linear ion trap, but this may come at a cost to total peptide coverage. Therefore, the parameters of a SQUAD method may need to be tailored based on experimental needs.
These results demonstrate that high-resolution Orbitrap data-dependent MS2 can be combined with highly sensitive targeted IT MS2 scans can be combined into a single method using the dual detectors of the Orbitrap Ascend. The new method reduces sample requirements when discovery measurements are required in parallel with quantitation of known targets. The PRM quantitation in the linear ion trap is more sensitive and selective than MS1 quantitation, enabling analytically rigorous measurements while maintaining discovery depth at scale.

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Development of a better treatment for glioblastoma

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumour in adults. Despite a multidisciplinary approach including surgery, concomitant radiation therapy, and chemotherapy to treat GBM, and extensive efforts to identify new therapeutic approaches, most patients experience poor prognosis limiting the median survival to 14.6 months. The standard chemotherapy treatment, temozolomide (TMZ), increases patient median survival by only 2.5 months. Clearly, much better treatments are urgently needed for GBM.

Cyclic peptide cyclo-((2-Nal)-Leu-Ser-(2-Nal)-Arg) acetate (Fc2), an experimental prostate cancer drug, significantly outperformed TMZ in 2D and 3D cell culture experiments using three GBM cell lines. Excitingly, when mice are given a single dose of Fc2 the drug is found to cross the blood-brain barrier, entering the brain.

Methods
Cell culture experiments were conducted with the GBM cell lines U251 (astrocytoma), LN229 (GBM, right frontal parieto-occipital cortex), and T98G (brain) to assess cell viability using an MTS assay with variable concentration of both Fc2 and TMZ.

To better mimic the actual tumour environment, cell viability was assessed by drug treatment of 3D printed GBM microtumours. Cell migration experiments were also conducted with each of the cell lines to better understand their response to drug treatment.

Finally, mice treated with Fc2 were sacrificed and their organs harvested. A UPLC-MS/MS method was developed to enable measurement of drug concentration within liver, kidney and brain tissue.

Novel Aspect
Fc2 is more cytotoxic than TMZ, the standard GBM chemotherapy. And excitingly, Fc2 has been shown to enter the brain.

Preliminary Data or Plenary Speakers Abstract
For the U251 cell line the IC50 for Fc2 was calculated as 168.3 ± 3.2 µM and for TMZ as 417.3 ± 5.5 µM. For LN229, the IC50 for Fc2 was calculated as 199.6 ± 5.5 µM and for TMZ as 430.2 ± 6.1 µM. And for T98G, Fc2 gave an IC50 of 330.8 ± 33.3 µM, while TMZ showed poor potency even at while TMZ showed poor potency even at concentrations as high as 100µM, and the IC50 was greater than 1000 µM.

For the scratch assay experiments, in all three cell lines the wound size remained constant for Fc2, while TMZ and DMSO treated wells had similar rate of wound closure in LN229 and T98G.

For a 40mg/kg dose of Fc2 by IP injection, the serum drug concentration averaged 115 ± 66 ng/mL (n = 5). For the mouse tissues, Fc2 was measured as 995 ± 46 ng/g in liver (n=3), 322 ± 132 ng/g in kidney (n=5) and 50 ± 14 ng/g in brain (n=4), using the tissue wet weight.

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Quantitation of breast cancer biomarkers by selected reaction monitoring

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Diagnosis and treatment selection for breast cancer patients currently relies heavily on semiquantitative immunohistochemical (IHC) assessment of three breast cancer biomarkers: Receptor tyrosine-protein kinase erbB-2 (HER2), Oestrogen receptor (ESR1) and Progesterone receptor (PRGR). Clinical grading of breast tumours to assess tumour aggressiveness and metastatic potential includes histopathological review of morphology and, often, IHC quantitation of Proliferation marker protein Ki-67 (Ki-67). Although IHC is widely used, it has known limitations include lack of specificity, lack of quantitative standardisation and inter- and intra-laboratory variation. Here, we investigate a potential alternative - protein quantitation with selected reaction monitoring (SRM), a highly specific, reproducible technique utilising internal standards suitable for high-throughput rapid screens.

Methods

We developed a set of SRM assays for breast cancer markers, including HER2, ESR1, PRGR, Ki-67 and Proliferating cell nuclear antigen (PCNA). Tissue samples from primary and metastatic breast cancer patients (n=55), matched healthy breast tissue (n=8) and matched patient-derived xenografts (PDX) (n =59) were assayed on a Sciex 7500 triple quadrupole mass spectrometer. SRM quantitative data was acquired from 150 ng sample loads spiked with heavy isotope-labelled peptide standards (5-20 fmol) with microflow (5 µL/min) reverse-phase liquid chromatography. To assess the clinical utility of the SRM assays, quantitation of the breast markers was compared to IHC results, the more accurate in-situ hybridisation (ISH) classifications and to patient clinical data.

Novel Aspect

We demonstrate the utility of protein biomarker quantitation in cancer tissues using SRM and its potential clinical application.

Preliminary Data or Plenary Speaker Abstract

The SRM and IHC assays were concordant for 26 of the 28 ESR1 cases, a key metric in assessing a patient’s likelihood of responding to hormone therapies. For HER2 quantitation, to identify patients likely to respond from HER2-targeted therapies, more accurate ISH results are routine and available for comparison. Of the 36 HER2 cases with ISH classifications, IHC resulted in one false negative and six equivocal cases that could not be accurately classified. In contrast, SRM showed concordance with ISH results in all cases (Pearson’s correlation 0.77), with all HER2 amplified cases showing an average HER2 quantitation of 45.3 fmol compared to 7.1 fmol for healthy breast tissue. SRM was also able to detect differential quantitation for low to very low HER2 abundance in 20 cases that could be potential candidates for clinical trials of anti-HER2-chemotherapy conjugates.
A quantitative proliferative index was established utilising proliferative markers Ki-67 and PCNA. The SRM proliferative index had a Pearson’s correlation of 0.6 (p-value <0.01) with the clinical prognostic tumour grading of 36 breast cancer patients. The SRM proliferative index could potentially replace clinical grading of breast tumours, potentially reducing the costs and turn-around time for testing compared to current histopathological grading which requires assessment by a qualified pathologist and multiple molecular tests.

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Exploring the potential of soluble HLA through immunopeptidomics

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Using circulating molecular biomarkers to screen for cancer and other debilitating disorders in a high-throughput and low-cost fashion is becoming increasingly attractive in medicine. One major limitation of investigating protein biomarkers in plasma is that antigenic cancer protein are often invisible through routine plasma proteomics. In contrast, Human Leukocyte Antigen (HLA) presents peptides from the entire proteome on the cell surface. While peptide-HLA complexes are predominantly membrane-bound, a fraction of HLA molecules is released into body fluids which is referred to as soluble HLAs (sHLAs). Recent developments in mass spectrometry (MS)-based proteomics have enabled the acquisition of ever smaller input amounts and therefore enabling valuable information to be extracted out of the peptidome of these circulating sHLA.

Methods

Plasma from healthy donors were collected and subjected through various sample development methods, from the sample preparation side to the mass spectrometry acquisition methods, via incorporation of recent immunopeptidomics approaches. The final method would then be applied to 92 plasma samples of rare cancer patients undergoing immune check point inhibitor therapy to investigate whether there are shared immunopeptidomic profiles among them.

Novel Aspect

Plasma immunopeptidomics acts as a new reservoir of cancer biomarkers.

Preliminary Data or Plenary Speaker Abstract

To ensure reproducibility, we sought to optimize a semi-automated sample preparation based on modified SAPrIm method on these plasma samples which minimize manual handling time. Improvement in sample preparation and mass spectrometry workflow has allowed for deeper plasma immunopeptidomics coverage without sacrificing the purity of the immunopeptidome in healthy control.

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Use of mass spectrometry to determine the effects of storage temperature and time on FFPE tissue sections

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Formalin-fixed paraffin-embedded (FFPE) tissues are invaluable resources for cancer proteomic studies and biomarker discovery. Very large numbers of them are stored in pathology laboratories and biobanks worldwide and often have associated clinical data available. FFPE tissue blocks are stable for decades when stored at room temperature (RT) and can be sectioned to produce samples for liquid chromatography-mass spectrometry (LC-MS) analysis. Once an FFPE block has been sectioned using a microtome, it is not known if the proteome of resultant sectioned samples is affected by storage temperature or time. To address this, we stored FFPE tissue sections at RT and -80°C for up to 48 weeks and analysed them at different timepoints to determine the proteome stability.

Methods

A total of 297 FFPE 10 µm sections (triplicates of rat brain, kidney and liver) were cut from tissue blocks and stored at either RT or -80°C. Control samples were freshly cut sections from the same FFPE blocks. Samples were prepared for LC-MS analysis by tryptic digestion at 11 timepoints after storage (up to 48 weeks) and analysed by microflow HPLC on Triple TOF 6600 mass spectrometers (SCIEX) using data-dependent acquisition (DDA) mode. Kidney and liver digests were further analysed in data-independent acquisition (DIA) mode for quantitative analysis.

Novel Aspect

Gaining a better understanding of the impact of storing precut FFPE sections for prolonged time periods on proteomic studies.

Preliminary Data or Plenary Speaker Abstract

The number of proteins and peptides identified using DDA-MS were not affected by the storage temperature or time. Nine post-translational modifications (PTMs) that are specific for FFPE samples were identified from the DDA data analysis. They were monitored for quantitative changes in samples that were stored at different temperatures and for different time periods using the DIA data. Overall, the storage temperature and time did not have detectable impacts on the identified proteome and FFPE related PTMs. Stored cut sections gave similar results as control sections that were freshly cut on the experiment day. FFPE tissue sections can be safely stored at either RT or -80°C for up to 48 weeks and are suitable alternatives for fresh frozen tissues in proteomic studies.

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Enhancing Immunopeptide Profiling with Orbitrap Astral Mass Spectrometer for Unbiased Discovery of Neoantigens

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Peptides presented on the cell surface by the major histocompatibility complex (MHC) play a vital role in immune response, making identification of neoantigens critical for immunotherapy. Mass spectrometry (MS) serves as a valuable tool for immunopeptidomics, allowing the simultaneous identification and quantification of thousands of MHC peptides. However, these measurements are difficult due to low recovery in sample preparation and the lower starting amounts, particularly for tissue biopsies. Additionally, these peptides exhibit a wide dynamic range, are endogenous and are generated by non-canonical events like alternative splicing, which makes confident identification more challenging. The Orbitrap Astral™ mass spectrometer enables researchers to delve deeper, with the new levels of sensitivity and selectivity, providing comprehensive insights into the immunopeptidome and neoantigen discoveries.

Methods

Class I MHC peptides were obtained by immunocapture with W6/32-conjugated resin on 100 million HCT-116 cells. For proof-of-concept studies, performance on low-loads was performed by diluting the starting material down to lower amounts. The MHC-bound peptides were eluted and subjected Liquid Chromatography (LC) coupled to MS analysis on an the Orbitrap Astral mass spectrometer interfaced with a Thermo Scientific™ Vanquish™ Neo LC and FAIMS Pro™ Interface. The LC-MS data were acquired in data-dependent acquisition (DDA) mode across a 60-minute LC gradient (70-minute total run time). LC-MS data were analyzed using PEAKS Studio software with the DeepNovo Peptidome workflow (Bioinformatics Solutions Inc.) as well as with MSFragger.

Novel Aspect

Orbitrap Astral enables deep immunopeptidome profiling from minimal starting material

Preliminary Data or Plenary Speaker Abstract

Preliminary findings suggest that the Orbitrap Astral MS enables deep immunopeptidome coverage from low starting inputs without compromising data quality. DeepNovo searching with PEAKS identified over 3,500 peptides from 1e5 cell equivalents, >9,000 peptides from 1e6 cell equivalents. To further showcase instrument dynamic range, we will demonstrate its performance at higher loads t. It is important to note that the total number of MHC peptides detected within a sample is highly variable, influenced not only based on instrument sensitivity, but also by factors such as cell line and enrichment method. Preliminary comparisons with other top-of-the line instruments suggest that the Orbitrap Astral MS obtains unparalleled coverage that would otherwise be unattainable at this sample input.
Our initial data also suggests that the peptide identifications are of high quality. Results are validated on two different search engines and show similarly high coverage. Additionally, reducing analytical variability is the key to deciphering meaningful biological insights from MS measurements. We successfully achieved high reproducibility between runs with over 75% of peptides detected across all three technical injections. Additionally, a potential pitfall of a non-enzymatic database search is that internal fragments generated prior to the collision cell may falsely inflate identification numbers. These artifacts of front-end fragmentation can be identified when one peptide is a sub-sequence of another peptide and there is no retention time shift. We analyzed our data and found that less than 1% of all identifications fall into this category. Overall, our preliminary results suggest that the Orbitrap Astral produces deep coverage with exceptional data quality, enabling vital biological discovery in the field of immunopeptidomics.

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Resolving isomeric bis(monoacylglycero)phosphates and phosphatidylglycerols by hydrophilic-interaction liquid chromatography coupled with cyclic ion-mobility mass spectrometry

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Shotgun lipidomics analysis of cancer cell lines has revealed significant variations in the abundance of membrane phospholipids in metastatic melanoma cell lines (WM164) compared with primary non-cancerous melanocytes (Hema). Notably, putative phosphatidylglycerols (PG) were upregulated in metastatic relative to the non-cancerous cell lines. The shotgun analysis however, could not resolve the response of PGs from isomeric bis(monoacylglycero)phosphates (BMP) that are known to be abundant in endosomes and lysosomes present within the cells. Herein, we deployed hydrophilic-interaction liquid chromatography (HILIC) coupled with cyclic ion mobility mass spectrometry (cIM-MS) to resolve BMP and PG isomers and generate subclass lipid profiles that enabled quantification of changes in abundance in response to cancer.

Methods

Experiments were conducted on Waters Acquity Premier (UPLC; ACQUITY™ Premier BEH Amide Vanguard™ column), system coupled with a quadrupole time-of-flight mass spectrometer equipped with cyclic ion mobility (Waters Select Series cIM-MS; Wilmslow, UK) and operated in negative ion mode. The column temperature is maintained across the 11.25 minutes gradient elution at 30 ºC. Mobile phase A is 95:5 acetonitrile: water with 10 mM ammonium acetate and 0.04% ammonium hydroxide; mobile phase B is 50:50 acetonitrile: water with 10 mM ammonium acetate and 0.04% ammonium hydroxide. Standard stock solutions BMP 18:1/18:1(S,R), PG 18:1/18:1, lipid extracts of metastatic melanoma cell lines (WM164) and primary non-cancerous melanocytes (Hema) were injected by the autosampler with the injection volume set to 2 µL.

Novel Aspect

HILIC-cIMS resolves isomeric PG and BMP lipid isomers revealing distinct responses of each subclass in cancer.

Preliminary Data or Plenary Speaker Abstract

Under HILIC conditions, lipids in BMP and PG subclasses were found to be completely separated, with BMP eluting earlier than PG (0.8 + 0.3 min and 1.3 + 0.3 min, respectively). High-resolution mass spectra acquired across each chromatographic feature were used to generate profiles for BMP and PG at the sum composition level. Referencing these data to isotope-labelled internal standards enabled absolute quantification of each subclass with comparisons revealing PG was found to be twice as abundant as BMP in WM164 cells, while in the Hema cells BMP was four times the abundance of PG. At the sum composition level, the BMP profile in both WM164 and Hema cell lines was dominated by BMP 36:2 and BMP 40:7 while, in contrast, the PG profile exhibited predominantly PG 34:1. Through the utilization of cIM-MS in conjunction with post-mobility collision-induced dissociation (CID), the molecular lipid structures within each class were assigned. Specifically, BMP 36:2 consists of isomers of BMP 18:1_18:1 across both cell lines with its abundance found to be 5-fold lower in the metastatic cell line. In contrast, the polyunsaturated BMP 40:7 was found at similar levels in both cell lines and was assigned as BMP 18:1_22:6; likely indicative of docosahexaenoic acid.

CID identified the most abundant PG as PG 16:0_18:1 and its relative abundance significantly higher in WM164 compared to Hema.

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Predictive Modeling of Tumor Microenvironment Diversity Using Mass Spectrometry Imaging and Deep Learning: Advancing Immunotherapeutic Strategies in Oncology

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The complex interplay between cancer cells and the immune system within the tumor microenvironment (TME) presents a frontier for cancer immunotherapy. Traditional methods for analyzing the TME often lack the resolution to fully understand its complex molecular composition. This study uses a novel deep learning framework that utilizes mass spectrometry imaging (MSI) to predict immune cell infiltration and tumor heterogeneity, refining immunotherapeutic targets and improving patient-specific treatment strategies.

Methods

We developed a convolutional neural network (CNN) model trained on a dataset of high-resolution MSI scans from 200 histologically verified cancer tissue samples. The MSI data provided spatial distributions of proteins and metabolites, which were used to reconstruct the TME's molecular landscape. Feature engineering focused on identifying biomarkers predictive of immune response. The model was validated against a separate set of 50 samples, with performance metrics calculated using area under the ROC curve (AUC), precision-recall curves, and 95% confidence intervals (CI).

Novel Aspect

Novel deep learning framework utilizing mass spectrometry imaging to predict immune cell infiltration and tumor heterogeneity in the tumor microenvironment.

Preliminary Data or Plenary Speaker Abstract

The CNN model achieved an AUC of 0.94 (95% CI: 0.91-0.97) for predicting the presence of key immune cells, such as cytotoxic T lymphocytes and macrophages, within the TME. In classification tasks, the model accurately distinguished between responsive and non-responsive tumors to PD-1 inhibitors with a precision of 88% and a recall of 85%. The analysis revealed that specific lipid and protein clusters were strongly correlated with immune cell presence, influencing the TME's responsiveness to immunotherapy.

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The proteomic and immunopeptidomic landscape of non-small cell lung cancer

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Non-small cell lung carcinoma (NSCLC) is the most common type of lung cancer, with adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) being the most prevailing histological subtypes with distinct biological signatures. Correct classification and accurate molecular profiling are essential for effective therapeutic guidance. In this research, we performed an in-depth mass spectrometry (MS)-based proteomic study of a large patient cohort representing the two aforementioned subtypes of NSCLC. This MS-based profiling of LUAD and LUSC revealed proteomic alterations that might determine their clinical behavior. High-coverage proteome analysis opens the possibility to unravel the mechanisms of immune evasion in NSCLC. Moreover, additional immunopeptidomics study should elucidate distinct tumor-associated antigens, that are pivotal for the development of epitope-specific cancer immunotherapies.

Methods

In our work, the patient-derived samples were processed with an optimized, semi-automated single-pot, solid-phase enhanced sample preparation (SP3-beads) workflow utilizing a pipetting BRAVO robot. Thereby, extracted proteins were digested and the resulting peptides were analyzed via liquid-chromatography tandem-mass spectrometry (LC-MS/MS).
For immunopeptidome study, we established and optimized a workflow to enrich HLA-I and HLA-II-bound antigens. This workflow involves semi-automated affinity purification with crosslinked monoclonal antibodies, performed on a pipetting robot. This method enables processing of cell line samples and fresh frozen tissue from biopsies.

Novel Aspect

Extensive proteomic analysis enables molecular insights in LUAD and LUSC. Immunopeptidomics identifies subtype-specific tumor antigens for personalized immunotherapy.

Preliminary Data or Plenary Speaker Abstract

The MS-analysis of the patient cohort (consisting of LUAD (n = 83), LUSC (n = 70), and tumor-adjacent (n = 138)) resulted in high proteome coverage of a total of 10164 proteins. Initial statistical analysis shows significant upregulation of CSTA, ITGA6 in LUSC, suggesting roles in tumor progression. Conversely, LUAD reveals elevated levels of SNTB1, DPP4, and MSLN. Further analysis suggests a possible distinction based on the patient’s gender.
In our pilot study of the immunopeptidome, we successfully identified 4416 peptides after HLA class I enrichment and 7592 peptides after HLA class II enrichment. This indicates a robust coverage of the immunopeptidome, providing valuable insights into antigen presentation. The further immunopeptidomic investigation gives a promising outlook to reveal a subset of tumor-associated antigens unique to each NSCLC subtype, offering precise targets for epitope-specific cancer immunotherapies. This study should facilitate the development of personalized immunotherapies tailored to each patient's tumor profile.

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Paediatric Oncology Revolution: Unleashing Precision Medicine through Phosphoproteomics

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The battleground against childhood cancer is an ongoing struggle, where each child's unique genetic makeup and tumour profile present a complex puzzle. Emerging technologies and cutting-edge techniques, such as phosphoproteomics and other multi-omics approaches, have set the stage for a transformative paradigm shift in the field of paediatric oncology. More importantly, phosphoproteomics has enable the profiling of aberrant kinases, which are critical enzyme found to be often dysregulated in cancer. In this project, we delve into the captivating world of phosphoproteomics, unlocking their potential to guide the way towards brighter, more hopeful futures for childhood cancer.

Methods

In this study, we present a robust mass spectrometry-based phosphoproteomics methodology aimed at profiling a growing cohort of paediatric cancer patients. Notably, this undertaking represents one of the most comprehensive investigations into phosphoproteomics that is specifically tailored for paediatric cancer.

Novel Aspect

First study to profile large cohort of paediatric cancer patients

Preliminary Data or Plenary Speaker Abstract

Our analysis encompasses an extensive dataset, comprising of over 140 samples derived from more than 15 paediatric cancer subtypes, including medulloblastoma, osteosarcoma, atypical teratoid rhabdoid tumour (ATRT) and one of the most devastating brainstem tumours, diffuse midline glioma (DMG). Through our rigorous examination, we are able to monitor the protein and phosphorylation changes of more than 10,000 proteins and in excess of 21,000 distinct phosphosites across these samples.

Ultimately, we want to integrate this wealth of data with other extensive multi-omics datasets generated within the group. These include datasets stemming from RNA sequencing, CRISPR knockout (KO) screening, and drug screening initiatives. By harnessing the integrative potential of this multifaceted information, our aim is to predict actionable molecular targets, in the hope of facilitating the development of highly individualised and efficacious therapeutic strategies, such as drug repurposing and immunotherapy, for paediatric cancer patients. This scientific pursuit holds immense promise for enhancing the prospects of improved, personalised care for paediatric oncology.

Proteomic characterisation of clear cell renal cell carcinoma in patients with Von Hippel-Lindau Syndrome

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The Von Hippel-Lindau (VHL) syndrome describes a familial neoplastic condition, caused by germline mutations of the VHL tumor suppressor gene, resulting in a functional loss of the VHL protein (pVHL). pVHL, as part of a multiunit ubiquitin ligase complex, is involved in the proteasomal degradation of specific target proteins, notably hypoxia-inducible transcription factors (HIF-1α/2α). Impaired HIF degradation and the subsequent induction of a pseudo-hypoxic state predispose patients for the development of various tumors, including clear cell renal cell carcinoma (ccRCC). In addition to its role in tumorigenesis, dysregulated HIF signaling in VHL disease affects the extracellular matrix (ECM). We aim to elucidate the molecular alterations linked to hereditary VHL loss in ccRCC, with a focus on matrisomal proteins.

Methods

The present study entails a comprehensive analysis of formalin-fixed paraffin-embedded (FFPE) patient samples (n = 134) from the Freiburg VHL register through liquid chromatography – tandem mass spectrometry (LC MS/MS). The bead-based single-pot, solid-phase-enhanced sample preparation (SP3) protocol combined with a semi-automated liquid handling platform were utilized for FFPE sample processing. The samples were measured on a timsTOF Flex mass spectrometer coupled to the Evosep One chromatography system. Our investigation involved the proteomic characterization of 54 tumor specimens, 45 pseudo-capsule (PC) specimens, and 35 samples of non-malignant adjacent tissue (NAT).

Novel Aspect

Proteomic characterization of the ccRCC pseudo-capsule with a focus on ECM and ECM associated proteins.

Preliminary Data or Plenary Speaker Abstract

In our study, we employed MS-based proteomics to analyze ccRCC patient samples, achieving a proteome coverage of up to 8500 proteins per sample. To corroborate our findings, we demonstrated the alignment of tumor and NAT samples with those of a Clinical Proteomics Tumor Analysis Consortium (CPTAC) dataset using a principal component analysis (PCA) after batch correction. Differential abundance analysis comparing tumor and NAT and subsequent gene ontology (GO) enrichment analysis unveiled an upregulation of proteins involved in glycolysis within the tumor, alongside a downregulation of proteins associated with aerobic respiration. Moreover, we analyzed the PC proteome through comparison with both tumor and NAT and identified a predominance of upregulated proteins specifically associated with extracellular matrix (ECM) organization within the PC samples. These findings suggest the PC as a highly intriguing entity in the context of tumor microenvironment dynamics and ECM remodeling. Further, we employed a missingness statistics approach to identify proteins with a significantly higher missingness in either tumor, PC or NAT. In our analysis, we observed that chemokines (CCL14, CXCL12), semaphorins (SEMA3B, SEMA3C), Wnt signaling proteins (WNT9B, SFRP1), and collagens (COL4A3-A6) were

significantly depleted in tumor samples. Conversely, angiogenic factors such as VEGF, ANGPTL2, and ANGPTL4 exhibited a lower presence in NAT.

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Variation of 234U/238U isotope ratios in groundwater under contrasting redox environments measured by mass spectrometry

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

$^{234}U/^{238}U$ activity ratios (AR) have been applied as a useful tracer for groundwater hydrological research. Their relative differences in decay constants form a secular equilibrium condition (AR = 1) in a closed system for more than a few million years, and groundwater usually shows AR >1 related to differences in aquifer and residence time. Furthermore, AR in reducing groundwaters become greater due to the preferential removal of ^{238}U as insoluble $U^{4+}O_2$. In this study, the variation of AR in two groundwater samples under contrasting redox environments was investigated by mass spectrometry to understand the migration behavior of U in groundwater.

Methods

Shallow oxidative groundwater samples were collected in Kumamoto area, and deep reductive groundwater samples were collected in Horonobe area in Hokkaido. The aquifer in Kumamoto area has composed of Quaternary volcanic deposition, whereas the low permeable aquifers in Horonobe area have composed of Pliocene-Quaternary diatomaceous mudstone and Miocene-Pliocene siliceous mudstone. These groundwater samples were chemically pre-treated by Fe-coprecipitation and anion exchange separation method to remove major matrix elements prior to U isotope analysis. After the $^{234}U/^{238}U$ isotope ratios were analyzed with a multiple-collector ICPMS (NEPTUNE), AR was calculated from the decay constants. A reproducibility in $^{234}U/^{238}U$ isotope ratio measurements of about 0.18% was obtained from approximately 1.6 ng U within 10 minutes, which is greatly improved over conventional alpha-spectrometry.

Novel Aspect

In this study, wide $^{234}U/^{238}U$ activity ratios in groundwater were accurately determined by mass spectrometry with small U contents.

Preliminary Data or Plenary Speaker Abstract

As the result, in the shallow oxidative groundwater in Kumamoto area (Eh > +100 mV), AR was ~1.3 on the main flow path, while locally varied from ~1.0 to ~1.5. The local AR variation might reflect anthropogenic inputs from chemical fertilizer and animal manure. In Horonobe area (Eh −250 mV to −100 mV), AR varied from 2 to 11 and it correlated with the inverse of U concentration. Groundwater in this area is known to be a mixture of fossil water and meteoric water based on $\delta^{18}O$-δD. Therefore, this apparent trend might suggest a simple mixing of the two-component, one fluid with AR ~1 and high U concentration, and the other fluid with high AR and lower U concentration. In fact, under highly reducing conditions, U does not show conservative chemical behavior in groundwater, and the dissolved U with high AR in groundwater in this area is likely due to the recoil effect from the host rock secondarily. The correlation between AR and porosity or hydraulic conductivity suggests that AR in the groundwater may be controlled by the mass ratio of rock to water in porous rocks.
Based on the results of the two groundwater measurements, AR can be used as a tracer for anthropogenic inputs from surface in oxidizing environments due to the conservative chemical behavior of U, and may be used to assess the hydraulic characteristic of rock mass in low-flow and reducing environments in groundwater.

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Development of a simple and rapid simultaneous determination methods for Cr(III) and Cr(VI) by HPLC-ICP-MS

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The toxicity of chromium depends on its chemical forms, thus those species should be measured rather than total chromium concentration. Recently, environmental regulatory values in Japan have been lowered due to the toxicity of hexavalent chromium, therefore the analytical method for their species concentration is necessary. In this study, based on Shigeta et al. (2018), we investigated a rapid and sensitive method combining high performance liquid chromatography (HPLC) with an anion exchange column and inductively coupled plasma mass spectrometry (ICP-MS).

Methods

The hexavalent chromium (Cr(VI)) is dissolved as oxyanions in environmental water, while trivalent chromium (Cr(III)) as cations. Introducing high chloride samples into an ICP-MS can produce ClO ions, and tailing of a chloride peak may interfere with the most abundant chromium isotopes, 52Cr and 53Cr. To overcome this problem, 2,6-pyridinedicarboxylic acid (PDCA) was used to unify Cr(III) species in various chemical forms into a stable anion complex ([Cr(PDCA)2]-), which was separated from Cr(VI) oxyanions and chlorides in an anion exchange column. In this study, HPLC-ICP-MS condition was optimized to enable mutual separation and sensitive analysis of chromium chemical species with an ammonium acetate based eluent, which does not contain non-volatile salts.

Novel Aspect

A simple eluent and organic ligand enabled simultaneous and sensitive determination of chromium species in high chloride water samples.

Preliminary Data or Plenary Speaker Abstract

The pH of the eluent was varied from 6 to 7 to avoid internal conversion between Cr(VI) and Cr(III). At pH 6.22, 6.41, 6.71, and 7.03 of the eluent, the peak area of Cr(III)-PDCA was found to be larger than the peak area of Cr(VI) at pH 6.22 and 6.41, suggesting that Cr(VI) is converted to Cr(III). It is also reported that Cr(VI) may change between two forms, CrO42- and HCrO4-, at the boundary of pH 6.5. Based on this result, the pH of the eluent was set in the range of 6.7-7.0. The flow rate was tested from 0.3 to 0.7 mL/min and found that 0.6 mL/min was sufficient for the mutual separation among Cl-, Cr(III)-PDCA, and Cr(VI), and all analyses were eluted in about 6 minutes. Helium gas was introduced into the collision reaction cell (CRC) in ICP-MS at 4.3 mL/min to reduce the interference of polyatomic molecular ions formed in the ICP-MS interface. In addition, the Cr standard solution of 2.5 µg/L was used to obtain the highest S/N ratio at m/z=52. Calibration curves made from 0.5, 1.0, 2.5, 5.0, and 10 µg/L standard solutions under the optimized instrument conditions had the correlation coefficients greater than 0.999 for both Cr(VI) and Cr(III) at m/z=52. The detection limits obtained from repeated analysis of 0.5 µg/L Cr standard solutions were 0.02 µg/L for both species with 10 µL injection volume. In addition, we will show the results of environmental water measurements on brackish water with high salinity and high major element matrix.

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Mass Spectrometry as a tool for analysing marine microalgal toxins from Indian waters

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The outbreak of harmful algal blooms has become a common scenario all over the globe and improved detection techniques coupled with satellite data empower countries to establish a warning system. Such events harm aquatic life, prove to be fatal for human health, and shake up the nation's economy by affecting tourism and exports. Hence, the study of toxins produced by phytoplankton has garnered a huge interest in the scientific community. Although toxins are of diverse chemical structure, phycotoxins are best described as small molecules which do not denature upon heat treatment. Due to the low concentrations and yet immense impact on human health, phycotoxin characterization and quantification by Mass Spectrometry (MS) is gaining attention in recent times.

Methods

surface water is collected from estuarine regions of Goa (Lat: 15°30'12.14" N; Long:73°50'28.49" E) and phytoplankton cultures were then established in cell culture bottles under laboratory conditions. Upon establishment of growth kinetics of the unialgal batch cultures, the cells were centrifuged and pellets were lysed to obtain the intracellular secondary metabolites which were then treated with acetic acid and methanol. The supernatant is filter sterilised and analysed by liquid chromatography-mass spectrometry quadrupole time of flight (LCMS-QToF).

Novel Aspect

This study will help in characterizing the potential toxin-producer species from the west coast of India.

Preliminary Data or Plenary Speaker Abstract

Although no outbreak cases have been reported in India in the recent past, toxin-producing species have been identified in the Indian waters. Till date, isolation and quantification of phycotoxins from this area has not been carried and hence there is no data on the permissible levels. Hence this study has been conducted to isolate and identify toxins from this region. The spectral profiles are compared with spectra obtained from toxin reference standard material namely, Domoic acid (DA) and Okadaic acid (OA). The diatom Pseudo-nitzschia is known to produce DA whereas OA is produced by dinoflagellates like Dinophyis and Prorocentrum spp. Several other toxins like Saxitoxin, Gonyautoxin, Brevetoxin are also produced by the dinoflagellates.

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Optimization of Sr^+-O2 reaction process for direct 87Sr/86Sr analysis by ICP-MS/MS without chemical separation

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Strontium has four stable isotopes: ^{84}Sr, ^{86}Sr, ^{87}Sr, and ^{88}Sr. The isotopic abundance of ^{87}Sr varies widely among geological samples due to the beta decay of the long-lived radionuclide ^{87}Rb, and the $^{87}Sr/^{86}Sr$ ratio is widely used as a dating tool and geological tracers. Due to the isobaric interference of ^{87}Rb on ^{87}Sr, removal of Rb by chemical pre-treatment is necessary with conventional mass spectrometers. An online Rb-Sr separation method has recently been reported using a collision reaction cell (CRC) technique on a triple quadrupole mass spectrometer (ICP-MS/MS). This method is based on the mechanism that only Sr^+ reacts with the CRC-introduced gas molecules, but it generates mass-independent isotope fractionation that cannot be simply corrected.

Methods

The aim of this study was to optimize the instrument conditions of the ICP-MS/MS (Agilent 8800) to avoid mass-independent mass fractionation. We utilized oxygen as the reaction gas introduced into the CRC because the ion-molecule reaction process can be easily controlled owing to its endothermic reactivity with Sr^+ to SrO^+. The optimized analytical conditions of ICP-MS/MS were validated by comparing the results of $^{87}Sr/^{86}Sr$ isotope ratio analysis obtained by conventional methods (measured by TIMS after chemical pre-treatment) and optimized ICP-MS/MS methods using four actual rock or mineral samples with various Rb/Sr concentration ratios, formation ages, and major element compositions. We used an internal correction assuming that $^{88}Sr/^{86}Sr$ =8.3752 as well as a conventional mass-dependent fractionation correction technique.

Novel Aspect

This study applied detailed product ion pattern analysis with varying oxygen flow rates and kinetic energies for direct $^{87}Sr/^{86}Sr$ measurements.

Preliminary Data or Plenary Speaker Abstract

As a result of the experiments, it was found that the reaction process between Sr^+ and O_2 molecules depends on two main factors: the flow rate of oxygen gas introduced into the CRC and the kinetic energy imparted to the ions at the entrance of the CRC. Under default instrument conditions with a high oxygen gas flow rate and low kinetic energy, a significant number of by-product ions like $SrOH^+$ and SrO_2^+ are produced, as well as analyte SrO^+. In contrast, when the oxygen gas flow rate is kept low and the kinetic energy is set high, the number of by-product ions decreases, and the composition of the produced ions becomes simpler. Under these conditions, mass-independent isotope fractionation was minimized, and accurate $^{87}Sr/^{86}Sr$ ratio measurements were achieved using only the internal correction. These results suggest that mass-independent isotope fractionation is due to the isotope fractionation among the many different by-product ions in complex ion-molecule reactions. The results of the $^{87}Sr/^{86}Sr$ ratio analysis of the four actual rock or mineral samples, optimized ICP-MS/MS methods were in close agreement with the results of conventional data (measured by TIMS after chemical pre-treatment) over a wide range of Rb/Sr concentration ratios up to 480 and $^{87}Sr/^{86}Sr$ ratios from about 0.7 to 22, indicating that the method is applicable to a wide range of samples. This presentation will also discuss the details of the ion-molecule reaction process and the stabilization of the mass spectral peak shape by the helium gas collision process in CRC.

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Direct air analysis capability at Central Analytical Research Facility Queensland University of Technology

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The Central Analytical Research Facility (CARF) is part of the Research Infrastructure within the Research Portfolio of the Queensland University of Technology (QUT). CARF hosts a range of characterization, analysis, and 'omics' capabilities, underpinned by $30M of cutting-edge technologies in microscopy, mass spectrometry, DNA sequencing, etc.
CARF provides specialist equipment and expert scientists to offer services to QUT and external students, researchers, and commercial organizations.
Three of the many mass spectrometers at CARF offer capability in direct air analysis; Voice200 Ultra SIFT-MS from Syft Technologies, HR-TOF AMS, and Vocus CI-TOF from Aerodyne Research Inc. These instruments offer real-time measurements of volatile organic and inorganic compounds as well as aerosol particles. All three instruments are field portable.

Methods

Selected Ion Flow Tube Mass Spectrometer (SIFT-MS) is a form of direct mass spectrometry. It applies precisely controlled chemical ionization reactions to detect and quantify trace amounts of volatile organic compounds (VOCs) and inorganic gases.
Vocus CI-TOF is a Chemical Ionization (CI) Time-of-Flight (TOF) mass spectrometer. The Vocus instrument at QUT is equipped with a Proton Transfer Reaction (PTR) reactor and 2R version of the TOF with a resolving power of 10000 M/ΔM.
High Resolution (HR) Time-of-Flight (TOF) Aerosol Mass Spectrometer (AMS) measures in real-time, non-refractive, size-resolved particulate chemical composition and mass. The resolution range of the AMS is 2500-5000 M/ΔM.

Novel Aspect

Capability of offering multiple field-deployable instruments for real-time measurement for particles and gas-phase compounds in the air.

Preliminary Data or Plenary Speaker Abstract

Voice200 Ultra provides multiple reaction ions to allow for a variety of reaction mechanisms that yield different "fingerprints" for analytes. This helps to verify the identification of the isobaric compounds that is not distinguishable by a quadrupole mass filter.
The eight SIFT-MS reagent ions (H_3O^+, NO^+, O_2^+, O^-, O_2^-, OH^-, NO_2^-, and NO_3^-) are all formed by microwave discharge through moist or dry air. The reagent ion of choice is then selected using a quadrupole mass filter.
The selected reagent ion is injected into the flow tube and excess energy is removed through collisions with the carrier gas. The sample is then introduced at a known flow rate and the reactive compounds it contains are ionized by the reagent ion to form well-characterized product ions. Product ions and unreacted reagent ions are sampled into a second quadrupole mass spectrometer. Utilizing Syft's compound library, the software instantaneously calculates each analyte's absolute concentration.
The Vocus CI-MS does not switch between reagent ions as quickly as in the SIFT-MS, however, it has a higher reagent ion yield offering more sensitivity and a much higher mass resolution with a TOF detector.
The Vocus PTR reactor can be tuned for different sensitivity and transmission based on the target m/z range. Mass transmission curve under a specific condition can be determined by measurement of a gas calibration standard. The sensitivity of a compound of known reaction rate can be calculated for quantification of the compound concentration.

The HR-AMS can measure particles in the range of 70-700 nm and yield detailed information about the organic aerosol species, including primary and secondary organic aerosol. The quantitative and multidimensional data produced by HR-AMS has many applications in atmospheric science, including predictions of climate, atmospheric composition, and meteorology.

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Analysis of PFAS and other environmental contaminants in soil and oat plants using high resolution GC/MS

Dr Angus Hibberd[1], Dr Matthew Giardina[1], Dr Matthew Curtis[1], Mr Luann Wong, Ms Gabrielle Black, Dr Thomas Young, **Dr Courtney Milner**
[1]Agilent Technologies

Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Per- and polyfluoroalkyl substances (PFAS) are persistent synthetic organic pollutants with a potential to bioaccumulate. Soil is a significant reservoir of PFAS as well as of many other persistent environmental contaminants, and thus can contribute to contamination of ground water, atmosphere, and biota. Therefore, to better understand the source and transport of these contaminants, both soil and plant extracts have been evaluated. This study describes different approaches for extraction and analysis of PFAS and other environmental contaminants in soil and plants using the GC/Q-TOF.

Methods

Soil and oat plants were sampled from two fields in California that have historically received biosolids. The soil and plant samples were either extracted with methylene chloride (DCM) for liquid injections or subjected to headspace solid-phase microextraction (HS-SPME). GC/MS analysis was performed using an 8890 GC coupled to a 7250 high resolution Q-TOF. The chromatographic deconvolution and library search were performed using MassHunter Unknowns Analysis software and the NIST23 EI library. The suspect screening was performed using the GC/Q-TOF Screener tool of MassHunter Quantitative analysis software and accurate mass libraries for Pesticides and PFAS.

Novel Aspect

Evaluation of different methodologies for extraction and analysis of PFAS and other contaminants in soil and plants using high-resolution GC/MS

Preliminary Data or Plenary Speaker Abstract

A variety of SPME fibers have been evaluated for extracting PFAS and other environmental contaminants from soil. The SPME showed higher sensitivity at extracting some of the volatile PFAS, DCM extraction was more efficient at extracting heavier contaminants such as PCBs, PBDEs and flame retardants.
In addition to PFAS, pesticides and PAHs detected using the target screening methodology based on the accurate mass personal compound database and libraries (PCDL) of these pollutants, a range of other contaminants including PCBs and flame retardants were identified in the soil and plant samples in non-targeted screening and NIST23 library. Some of these contaminants were uniquely detected in soil. However, pesticides, PAHs and flame retardants were also found in plant samples.

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Automated analysis pipeline for identification of untargeted GC-EI-MS spectra

Dr. Deborah McGlynn, Professor Lindsay Yee, Lewis Geer, Yuri Mirokhin, Dmitrii Tchekhovskoi, Coty Jen, Allen Goldstein, **Anthony Kearsley**, Stephen Stein

Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The identification of organic compounds collected using electron ionization mass spectrometry in untargeted analyses is challenging despite the large number of compounds represented in mass spectral libraries. The problem becomes more acute for materials subjected to complex chemical reactions such as oxidation or pyrolysis as they are less likely to be represented in libraries. It is therefore common for only a small fraction of environmentally sampled compounds to be identified. Chromatographic retention index data improves the confidence in spectral identification process, but this information is typically manually incorporated in the analysis.

Methods

We present a library search pipeline that considers mass spectral matching, retention index, and molecular weight-based scoring methods. The new method is applied to identify components of the mass spectral dataset (FIREX) containing over 4000 electron ionization mass spectra collected by the University of California at Berkeley of particulate organic compounds emitted during wildfires. Retention indices of matches are considered alongside the RI of query spectra. Matches outside of a threshold are penalized, pushing the most likely matches to the top of the hitlist. Following estimation of spectral MW, the 'hybrid search' method is used to identify compounds similar to but not present in libraries. These new methods increase reproducibility, reduce analysis time, and increase identifications in such complex mixtures.

Novel Aspect

This method presents an effective means of identifying compounds in the GC/MS analysis of complex mixtures.

Preliminary Data or Plenary Speaker Abstract

Previously, 148 compounds were identified in the "FIREX" dataset. The wildfire emission dataset collected by UC Berkeley contains experimental retention indices, of direct use in the refinement of scoring in the latest NIST software. The dataset was run against the NIST 2023 EI-MS dataset using the identity search method with retention index correction. The RI threshold was +/- 15 with score reduction that is dependent on the mismatch between experimental and library values. This analysis led to 98 new identifications. 105 identities from previous work were confirmed in the NIST23 library while 32 previous ID's were changed. We then estimated molecular weights of ~130 mass spectra with a high signal to noise ratio in the dataset. These were then used for HSS matching. Confident matches were made using HSS based on a library spectrum with a high match factor, a similar retention index, and a DeltaMass (the MW difference between the query and library spectrum) corresponding to a reasonable and identifiable chemical structure. Then, the library structure was adjusted based on the DeltaMass to create a new structure for the unknown spectrum. This method is also being combined with automated retention index thresholding between unknown and library spectra. The number of remaining unidentified spectra is notable in this work (4456 spectra). Therefore, the work concludes with exploring methods for separating potentially identifiable from low-quality spectra.

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Deep scan screening of remediated water & biological samples for PFAS

Doctor Samiuela Lee[1], **Dr Atul Bhatnagar**[1], Dr David Alam[2], Doctor Owen Tang[3]
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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Per- and polyfluoroalkyl substances (PFAS) are a large, complex group of synthetic chemicals that have been widely used in consumer products because of their ability to repel water, grease, and oil. They have been around the world since about the 1950s and are used in non-stick cookware, waterproof clothing, food packaging, and firefighting foams.

Widespread use and occurrence of PFAS is quite concerning as they are classified as persistent organic pollutants. They bioaccumulate in living organisms and have potential adverse health effects, including links to certain cancers, immune system problems, and developmental issues.

In this study we aimed to identify various chemical classes of PFAS in various samples via the deep scan AcquireX data acquisition workflow.

Methods

The controlled water blank and biological samples was spiked with PFOA standard. The control water blank was then remediated by plasma treatment in which high voltage micropulse generator was used to produce plasma discharge at a peak voltage of 3.5kV, pulse frequency of 1000 Hz and duty cycle of 102 µs. Liquid samples were taken at 30 mm below the surface and stored in certified PFAS free HDPE sample bottles for further LC-MS analysis. Samples were collected after 5, 10 & 15 min of plasma treatment.
The samples were injected onto a C18 column conjugated with an isolator column. The eluent from the column was introduced into the mass spectrometer to acquire MS1 and MS2 level mass spectra.

Novel Aspect

1) use of deep scan for PFAS in biological samples
2) use of KMD to identify most of the PFAS.

Preliminary Data or Plenary Speaker Abstract

The preliminary data from the analysis on the Thermo IQ-X was processed using Compound Discoverer (CD) software. PFAS standard QCs were used as a reference for comparison to the remediated samples, and the deep dive scan samples and blank water samples were used to correct for background peaks. A customized PFAS workflow template was chosen in CD. The standards, samples, and blanks were categorized according to scan and sample type and processed on CD. A customised PFAS filter was loaded and applied to the raw results to eliminate the non-related PFAS compounds, and a few thousand hits were narrowed down to less than one hundred. The resulting chromatograms for each analyte hit were classified by their sample type names, such as quality control, blank, identification only, and samples, and they were colour-coded for easy identification. A Kendrick mass plot was constructed against the m/z ratio. The CF2 subunit, the distinguishing repeating subunit that occurs in all PFAS chemical families, was used to calculate the Kendrick Mass Defect (KMD) for each PFAS compound. When rounding to the plot floor, the plot showed a definitive separation of the various PFAS functional groups. From the plot, the sulfonic acids gave a KMD of 0.971, carboxylic acids KMD of 0.720, straight and branched alkane KMD of 0.608 and fluorinated telomere sulfonates KMD of 0.443. Future work will endeavor to discover new PFAS chemical families in remediated and biological samples via the deep dive scan method.

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ANALYSIS OF PHOTODEGRADATION PRODUCTS OF POLYETHYLENE TEREPHTHALATE IN SEAWATER USING HIGH RESOLUTION MASS SPECTROMETRY

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Polyethylene Terephthalate (PET) is one of the most widely used plastics globally, employed in a variety of applications such as beverage bottles, food packaging, and fibers. However, the increased marine plastic waste due to the mass production and consumption of PET negatively affects marine ecosystems. PET discarded in the sea undergoes photodegradation due to the action of ultraviolet light and seawater, breaking down into various chemical substances. These photodegradation products can persist in the marine environment for an extended period, potentially causing a range of biological impacts, which ultimately may affect humans as well. These research findings will provide crucial foundational data for future marine environmental protection and plastic waste management efforts.

Methods

To prepare the samples, PET was immersed in artificial seawater and exposed to UV lamp. Solid-phase extraction was utilized to extract the photodegradation compounds from the artificial seawater. Qualitative analysis was conducted using a high-resolution mass spectrometer. The compounds were identified through a database search software, and reliable data was obtained by confirming MS/MS patterns. Quantitative analysis was carried out using a triple quadrupole mass spectrometer. Four compounds, representative of the characteristics of PET photodegradation, were selected for quantitative analysis. The extent of environmental accumulation was determined by conducting targeted analysis of these four compounds in seawater samples.

Novel Aspect

The types and concentrations of PET photodegradation compounds were identified through non-targeted analysis using a mass spectrometer.

Preliminary Data or Plenary Speaker Abstract

A total of 309 compounds were detected as a result of PET photodegradation. Using database search software, a total of 22 compounds were identified based on MS/MS patterns and structural similarity to PET. 20 of these were photodegradation compounds derived from PET, and 2 were identified as plasticizers, which are additives. Among the identified compounds, four with high concentrations were selected for quantitative analysis. The compound with m/z 179.0349 was found at 11.36ppm, the compound with m/z 209.0454 at 11.32ppm, the compound with m/z 357.0611 at 16.69ppm, and the compound with m/z 401.0880 at 0.4ppm in the samples. Annually, 8 million tons of plastic waste are dumped into the oceans, 12% of which is PET. Based on this information, the annual production of PET photodegradation products in the oceans was predicted. The compound with m/z 179.0349 is estimated to be produced at 5452.8kg, the compound with m/z 209.0454 at 5433.6kg, the compound with m/z 357.0611 at 8011.2kg, and the compound with m/z 401.0880 at 192kg annually in the world's oceans. An MRM analysis targeting these four compounds was conducted in seawater samples. The detection of all four compounds in seawater samples indicates a significant accumulation of PET photodegradation compounds in the environment. Therefore, it demonstrates the need for monitoring and further research on the discharge of PET into the oceans.

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Highly sensitive tandem mass spectrometry detection for high resolution HILIC separation of biomass burning markers

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Tracing current and past biomass burning (BB) events is crucial for understanding the links between human activity, fire frequency, and climate. One method to trace biomass burning involves measuring concentrations of levoglucosan and its isomers, products of cellulose and hemicellulose pyrolysis. The ratios between these isomers can indicate the type of biomass burnt, aiding fire activity reconstructions. Selective and sensitive analytical methods are needed to separate and quantify these compounds. We present, for the first time, high-resolution separation of levoglucosan and three of its isomers using hydrophilic interaction liquid chromatography (HILIC). Triple quadrupole-mass spectrometry was hyphenated with the new HILIC separation to achieve the low-ppb detection limits necessary for detecting these markers in atmospheric aerosols, lake sediments, and ice cores.

Methods

The study utilized a DionexTM Ultimate 3000 liquid chromatography system coupled with a Thermo Scientific TSQ QuantivaTM triple-stage quadrupole mass spectrometer, equipped with an Ion Max NG source operating in heated electrospray ionization (H-ESI) mode. Samples were injected via an AS-AP autosampler onto two 150 mm x 1.5 mm ID -2.7 µm particle packed Penta-HILIC columns connected in series (300 mm total length). The advantages of the method are demonstrated in this case study focusing on the previously unresolved separation of four biomass burning markers, namely levoglucosan (LEV) and its main isomers mannosan (MAN), galactosan (GAL) and 1,6-anhydroglucofuranose (AGF). Separation and detection parameters were optimised to obtain baseline separation and required sensitivity to analyse these BB markers in environmental samples.

Novel Aspect

This study achieved baseline separation and sensitive detection of BB-markers by using HILIC-MS/MS, enabling the accurate determination of isomer ratios.

Preliminary Data or Plenary Speaker Abstract

While previous studies have utilized various chromatographic techniques, such as liquid, gas, and ion chromatography, to separate levoglucosan and some of its isomers, none have reported the simultaneous separation of all four isomers mentioned above in a single run. This study optimized injection volume, mobile phase composition, flow rate, and column temperature to achieve baseline separation of LEV, MAN, GAL, and AGF in less than 10 minutes. The optimized conditions involved isocratic separation using a mobile phase consisting of 90:10 acetonitrile-water, a column temperature of 15°C, and injection volume of 5 µL of sample. An optimal flow rate of 100 µL/min was determined to achieve the highest number of plates from the 1.5 mm ID HILIC columns, resulting in approximately 50% reduced solvent consumption compared to traditional 2.1 mm ID columns, a significant advantage considering the typically large number of samples analysed in such environmental studies. Electrospray ionization (ESI) was optimized by evaluating ionization modes, capillary voltage, gas flow rates, and temperature. Detection and ion transmission were optimized through the evaluation of the optimal SRM transitions, addition of modifiers, dwell time, quadrupoles resolution, and RF lens voltage. It was found than the addition of 0.8 mM formic acid and 0.5% MeOH in the mobile phase provide the best sensitivity to monitor levoglucosan and its isomers in ESI negative mode using the SRM transition 207 □ 45 m/z ([M+COOH]- □ [COOH]-). Under

the optimal conditions, instrumental limits of detection (LODs) for all targeted analytes were below 1 µg/L, and both intra- and inter-repeatability were consistently below 5% RSD. The method proved effective in quantifying LEV, MAN, GAL, and AGF and determining their ratios in over 120 samples of marine and terrestrial atmospheric aerosols, lake sediments, and ice cores, covering a wide range of BB particle concentrations.

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Proteomic changes based on health implications of abandoned mine local residents

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Residents in areas with abandoned mines are at significant risk due to exposure to high levels of heavy metals present in the environment. However, the clinical indicators currently used in health surveys have limitations in monitoring health changes associated with abandoned mine exposure. Therefore, this study aimed to identify and analyze biological changes in the residents of abandoned mine areas by conducting proteomic analysis of their blood samples.

Methods

Blood samples were collected from abandoned mine and control areas, and mass spectrometry was used for protein profiling. A comparative analysis was performed between the exposed and unexposed groups, resulting in the selection of candidate biomarkers. The STRING software was employed for protein network mapping.

Novel Aspect

Apolipoprotein E and cholesteryl ester transfer protein can be used as indicators reflecting the degree of mine exposure.

Preliminary Data or Plenary Speaker Abstract

Apolipoprotein E and cholesteryl ester transfer proteins, biomarkers related to lipoprotein metabolism, showed significant changes in the low-exposure group compared to the control group. Protein networking analysis revealed functional connections between these biomarkers and proteins specifically associated with lipoprotein metabolism in the high-exposure group. Moreover, alterations were observed in proteins involved in the complement cascade, fibrin clot formation, and heme scavenging from the plasma in the high-exposure group. Apolipoprotein E and cholesteryl ester transfer protein can be used not only to differentiate the low-exposure group from the control group, but also as indicators reflecting the degree of mine exposure, thereby allowing for distinguishing the areas affected by abandoned mines. Furthermore, the observed changes in lipoprotein metabolism, present in both the low- and high-exposure groups, can be utilized to predict the risk of cardiovascular disease and assess the potential occurrence of renal dysfunction. These findings are crucial for monitoring the health status of residents exposed to harmful factors, evaluating disease risks, and assisting in disease prevention and treatment.

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Investigating matrix interference from analysing nanoplastics and microplastics in spleen samples by Pyrolysis-Gas Chromatography-Mass Spectrometry

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Micro- and nanoplastics (MNPs) are contaminants of emerging concern due to their presence in the environment. Humans can be exposed to MNPs via inhalation, ingestion, and dermal contact. Recently, a study reported the occurrence of MNPs in human blood. However, the spleen, which acts as a filter for the blood, has not been investigated. MNPs present in the blood may be captured and accumulated in the spleen. There is a lack of investigation into background interference impacts on the accurate quantification of MNPs due to the complexity of biological sample matrices. This study aims to reduce interferences from spleen tissues to measure MNPs accurately using pyrolysis coupled to gas chromatography and mass spectrometry (Py-GC-MS).

Methods

Cow spleen was used for method development. Each spleen sample was weighed (0.25 g) and homogenised using a dichloromethane (DCM) pre-cleaned stainless-steel homogeniser. Enzymatic digestion was used to digest proteins and break down long-chain fatty acids to short-chain fatty acids in spleen tissues. Following enzymatic digestion, potassium hydroxide (10%; KOH) was added to the sample to digest the organic material, and hydrogen peroxide (30%; H2O2) was added to remove the remaining organic material in the sample. The digested sample was filtered through glass fibre filters (GFFs) with pore sizes of 1 µm, 0.7 µm, and 0.3 µm in sequence. The collected GFFs were then dried in an incubator at 40 °C and analysed for MNPs using Py-GC-MS.

Novel Aspect

The study proposes an approach to remove matrix interferences from spleen tissues and increase detection accuracy of MNPs using Py-GC-MS.

Preliminary Data or Plenary Speaker Abstract

None to date

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National Reconnaissance of Antimicrobial Occurrence in Australian Wastewater and their Socioeconomic Correlates

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Although it is well established that inappropriate and overuse of antimicrobials accelerates the spread of antimicrobial resistance (AMR), Australia's per-capita consumption of these drugs remains among the highest globally. However, community antimicrobial use in Australia is largely based on passive data from subsidized prescriptions, which do not capture all sources of community antimicrobial use. Wastewater-based epidemiology (WBE) has been used for over a decade to reveal regional patterns of community-level drug consumption in almost near-real-time. WBE has been successfully applied to estimate drug and pharmaceutical use in Australia and has gained popularity in estimating antimicrobial use in other countries. Given WBE's features of timeliness and finer resolution, its application in monitoring antimicrobial use could enhance antimicrobial stewardship efforts in Australia.

Methods

This study aims to utilize wastewater analysis to explore both the occurrence and spatial trends of antimicrobial use throughout Australia and to identify potential population-level socioeconomic factors that may be driving antimicrobial usage. Wastewater samples collected on the 2021 Australian Census Day from 50 WWTPs covering approximately 50% (11.3 million) of the national population were analyzed for 102 commonly used antimicrobials as well as their TPs, via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Subsequently, the wastewater data were cross-referenced with Census variables to discern potential social, demographic, and economic influences on antimicrobial usage patterns in Australia.

Novel Aspect

First nationwide study on the occurrence of 102 antimicrobials and their transformation products in Australian wastewater.

Preliminary Data or Plenary Speaker Abstract

The improper and excessive use of antimicrobial agents exacerbates the development and dissemination of antimicrobial resistance (AMR), which has become a global environmental challenge. A critical approach to combating AMR involves monitoring antimicrobial usage. However, despite Australia's already elevated and rising per capita antimicrobial use, research focusing on its surveillance remains scarce. The present study investigated the occurrence and use of 102 common antimicrobials and their transformation products (TPs) in wastewater influent samples collected from 50 wastewater treatment plants (WWTPs) across Australia on Census Day 2021, encompassing ~50% of the national population. This wastewater data was subsequently cross-referenced with catchment-matched Census data to identify population-level socioeconomic factors correlated with antimicrobial use. The results indicated that 41 antimicrobials and 15 TPs were detected in Australian wastewater influent, spanning various antimicrobial groups such as β-lactams, quinolones, sulfonamides, macrolides, tetracyclines, azoles, lincosamides, and aminoglycosides. Notably, 30 analytes were detected at a frequency >50%. Amoxicilloic acid and cephalexin occurred at the highest concentrations and population-normalized mass loads. Correlation analysis revealed a significant association between the consumption of certain antimicrobials, especially quinolone antibiotics, and various aspects of the population's socioeconomic status, including income, education, occupation, housing, race, health condition, and transport. For example, antimicrobial use correlated positively with higher income, education level, and occupational status, as well as

crowded living conditions and frequent use of public transportation. Conversely, it negatively correlated with people having long-term health conditions. Our study offers a comprehensive insight into the patterns of antimicrobial use at the population level in Australia and highlights potential socioeconomic drivers of their use.

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Field-deployable compact LC-MS for determination of per, and polyfluoroalkyl substances (PFAS)

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

PFAS are pervasive in the environmental media where their egress into global supply chains has led to measurable levels in nearly the entire human population accompanied by a potential impact on human health and the environment. LC-MS is currently the industry standard for measurement of PFAS in environmental samples. However, global increase in awareness of potential PFAS impacts has led to significant increase in sample volume, with end-users waiting up to eight weeks for analytical results. Ideally, PFAS contaminated site investigations and remediation works require same-day analytical data to facilitate decision making. To meet this market need, a field-deployable LC-MS platform (p-LCMS) is implemented in a mobile lab setting to target in-situ environmental monitoring workflows and facilitate efficient decision making.

Methods

A compact briefcase-sized capillary LC system (Trajan Scientific and Medical) coupled with a miniaturised single quadrupole MS (Microsaic 4500 MiD) was configured in a mobile lab for in-field screening, powered by petrol generator and uninterruptible power supply. 2 g of soil sample is weighed into polypropylene tube and extracted with surrogate in 10 mL of methanol-ammonia solution over a tumbling device. Following centrifugation, the supernatants are syringe filtered. Filtrates are concentrated under nitrogen, then reconstituted with spiking non-extracted internal standard for analysis. Ionization and data acquisition were performed in negative and single ion monitoring mode, respectively. Results are available same-day when running a screening method and next day for trace analysis.

Novel Aspect

A rapidly deployable PFAS monitoring solution based on a compact and portable LC-MS platform for targeting on-site environmental monitoring workflow.

Preliminary Data or Plenary Speaker Abstract

The field-deployable LC-MS platform was applied for suitability test in the laboratory setting using soil samples from contaminated sites including a certified reference material (National Measurement Institute, NSW, Australia). Calibration curves based on eight PFAS compounds, including perfluorobutanoic acid (PFBA), perfluoropetanoic acid (PFPeA), perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), perfluorohexanesulphonic acid (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorooctanesulfonic acid (PFOS), were established for quantitative evaluation of the soil samples. Perfluoro-1-[2,3,4-13C3]butanesulfonate (M3PFBS) and 13C4-perfluorooctanoic acid (MPFOA) were used as surrogate and internal standard, respectively.
Recoveries for the eight PFAS compounds in spiked samples ranged from 65-165%, with the relative standard deviation ranging from 7.4-10.3%. Method sensitivity for eight PFAS compounds ranged from 1.9-12.4 ng/g, with wide dynamic range (50-12,500 ng/g) and excellent linearity (R2 > 0.99). Following the laboratory trial, the LC-MS platform was deployed for testing of soil samples collected in-situ at the contaminated site. Site setup was remote from any mains power and took 45 minutes following which the first standard was injected. A total of 40 soil samples were processed within ~8-hours' timeframe and ready for injection to the LC-MS for analysis.

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Distribution and metabolism of fungicides in plant tissue

Mr Jordan Campbell[1,2,3]
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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Most fungicides used in broadacre agriculture penetrate the leaf and are systemic to some extent. They may be truly systemic and migrate throughout the entire plant, they may be translaminar and stay close to the treated site, or they may be contact fungicides that remain where they are applied. Although we know fungicides work, we often don't understand their behaviours. For example, many fungicides are marketed as 'systemic', but have been shown to have very different mobilities and poorly understood metabolism. Without understanding fungicide mobility and metabolism within plants, we can't predict their longevity in protecting crops against fungal disease. To improve the efficiency with which we use fungicides in agriculture, we need to better understand their mobility and metabolism.

Methods

To better understand the mobility and metabolism of fungicides in plants, I am using QuEChERS based solvent extractions to extract fungicides and their metabolites from plant tissues. I am using liquid chromatography and tandem mass spectrometry to quantify 12 commercial systemic fungicides and their metabolites from samples of Arabidopsis, wheat, barley and canola. Single ion monitoring and full-scan acquisition modes are used to gather mass spectrometry data on an Orbitrap Exploris 120, and mass spectrometry software such as Tracefinder, Freestyle and Compound Discoverer are used to identify and quantify analytes. I will be using chemometric analyses, such as PCA and PLS to find correlations between physiochemical properties of fungicides and their mobility, and to assess their metabolic profiles.

Novel Aspect

1) Quantifying fungicide mobility within plant tissue, associated with fungicide physiochemical properties
2) Metabolic profiling of commercial fungicides in crops

Preliminary Data or Plenary Speaker Abstract

Most fungicides used in broadacre agriculture penetrate the leaf and are systemic to some extent. They may be truly systemic and migrate throughout the entire plant, they may be translaminar and stay close to the treated site, or they may be contact fungicides that remain where they are applied. Although we know fungicides work, we often don't understand their behaviours. For example, many fungicides are marketed as being 'systemic', but have been shown to have very different mobilities and poorly understood metabolism. Without understanding fungicide mobility and metabolism within plants, we can't predict their effective longevity in protecting crops against fungal disease. This project aims to improve our understanding of fungicide mobility and metabolism, to increase the efficiency with which we use fungicides in agriculture. This project uses QuEChERS based solvent extractions to extract fungicides and their metabolites from plant tissues of Arabidopsis, wheat, barley and canola. Mass spectrometry software such as Tracefinder, Freestyle and Compound Discoverer are used to identify and quantify analytes. Chemometric analyses such as PCA and PLS are used to find correlations between fungicide physiochemical properties and mobility, and to determine fungicide metabolic profiles. I have shown that each of the 12 'systemic' fungicides have different mobilities in plant systems, despite having the same mobility classification. Generally, they tend to concentrate (mg/kg) more towards the tip of leaves, but some fungicides migrate down leaves, and between leaves, much more favourably than other fungicides. I am expecting to find correlations between fungicide mobility and their physiochemical properties before the conference in August, as plant transport systems are known to be heavily dependent on metabolite physiochemical properties. I will also be exploring the metabolism of commercial fungicides in their

relevant crops, to try identify novel metabolites and/or metabolic pathways that contribute to fungicide degradation.

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Simultaneous analysis of 12 NBFRs in foods by GC-EI-MS/MS

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Novel Brominated Flame Retardants (NBFRs) were widely used in plastics, electronics, etc. However, they are considered strong candidates listing on Persistent Organic Pollutants (POPs) due to harmful effects such as bioaccumulation, long-range transport, and toxicity. Therefore, many studies about human exposure assessment of NBFRs are ongoing. This study aimed to estimate human exposure to NBFRs by quantifying NBFRs in most consumed foods in South Korea. The samples were extracted by accelerated solvent extractor (ASE) and purified through multi-layer silica column, gel permeation chromatography (GPC), and dispersive solid phase extraction (d-SPE). Ultimately, the samples were analyzed by GC-EI-MS/MS. The analytical method was verified during method validation. 12 NBFRs in 469 food samples were quantified by the method.

Methods

Samples were extracted using Dionex ASE 350 with hexane and dichlromethane (1:1) The extracts were evaporated by a stream of nitrogen gas. After evaporation, 4 g of sodium sulfate, 5 g of florisil, and 5 g of sodium sulfate were added to an open column and the extracts were purified through the multi-layer silica column. In addition, residual lipid was removed through GPC with Bio-Beads S-X3 and d-SPE with 85 mg of Z-sep. After the reparation method, the samples were analyzed by GC-EI-MS/MS. The samples were injected by pulsed splitless mode and passed through DB-5HT column (15 m x 0.25 mm, 0.1 μm),and finally analyzed by multiple reaction monitoring (MRM) mode.

Novel Aspect

12 NBFRs were analyzed simultaneously and NBFRs in various food samples were quantified.

Preliminary Data or Plenary Speaker Abstract

Brominated flame retardants (BFRs) have been listed on POPs. Therefore, they are banned and restricted. NBFRs have been currently used as a replacement for BFRs. However, NBFRs are highly similar to BFRs and harmful to human. Thus, we detected and quantified NBFRs in foods. NBFRs are lipophilic. Therefore, they are mostly accumulated in lipid. ASE is an efficient equipment to extract lipid of food samples. Hence, ASE was employed to extract NBFRs in food samples. ASE parameters such as oven temperature, static period, and purge time were optimized to achieve good recoveries of 12 NBFRs. In some previous studies about NBFRs, acidified silica gel was used to remove lipid, but some NBFRs which have ester groups were hydrolyzed under acidic condition. Thus, in this study, the samples were purified using multi-layer silica column without acidified silica gel. In addition, GPC and d-SPE were used for clean-up method. Most of 12 NBFRs are stable at high temperature. However, some NBFRs were subjected to thermal decomposition. GC column and oven temperature program were optimized to address the problem. As a result, shorter residence time in a column inhibited thermal degradation. Selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, and precision of the analytical method were verified through method validation. 12 NBFRs in 469 food samples were detected and quantified by the method. Consequently, detection rates of NBFRs in a livestock food group was highest and 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE) was detected most frequently.

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Proteomic Profiling of Mango: Unveiling Bioactive Peptides in Pulp and Peel

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Mango (Mangifera indica L.), known as "the king of fruits" for its delectable taste and nutritional richness, belongs to the Anacardiaceae family and is a widely cherished tropical fruit. During mango processing, a significant portion of the fruit, ranging from 35% to 60%, is disposed, often without any treatment, resulting in environmental challenges and financial setbacks. Peels and seeds, accounting for 40-50% of the fruit's weight, are typically discarded as by-products, with the peel alone contributing about 20% to the overall waste. This waste holds potential for conversion into valuable resources. Moreover, recent studies have revealed additional health benefits associated with mango consumption, owing to the presence of bioactive compounds in various parts of the fruit.

Methods

Although mango is known to have bioactive compounds, our understanding of the proteome and bioactive peptides within different mango tissues remains limited. This research aims to explore the mango proteome and identify potential bioactive peptide-encoding genes present in both the pulp and peel, utilizing publicly available transcriptome data resources. By employing bioinformatics tools, we conducted an in-silico analysis of previously published mango genomics data. . Through this analysis, we assessed gene expression and mapped the bioactive peptides to the mango reference proteome identifying bioactive peptides and their corresponding coding genes.

Novel Aspect

This research explores bioactive peptides in mango tissues, revealing health benefits and advancing mango tissue proteomics with mass spectrometry.

Preliminary Data or Plenary Speaker Abstract

Our findings reveal the presence of over 250 bioactive peptide coding genes in the pulp and peel of the analysed cultivars. Remarkably, our data suggests differential expression of potential bioactive peptide coding genes across various mango tissues, with a higher abundance observed in the peel tissue compared to pulp. These bioactive peptides exhibit diverse functionalities, including antioxidant, ACE inhibitory, anticancer, antibacterial, antifungal, and antiviral properties. In the subsequent phase, we plan to conduct mass spectrometry-based proteomics to gain further insights into the differential proteomic profiles among different mango tissues, thus providing a deeper understanding of the proteins from which bioactive peptides originate.

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Rapid detection by SIFT-MS of toxic inorganic and organic compounds relevant to worker safety in the shipping industry

Dr Caleb Allpress[1], Dr Paul Wilson[1], Dr Sam Edwards[1], Daniel Comesky[1], **Matt Lynn**
[1]Syft Technologies Ltd.

Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Workers in the shipping and border security industries are at risk of exposure to toxic compounds including those used for fumigating shipping containers. Rapid, accurate and reliable quantitation of fumigant and other toxic compounds is crucial to protecting workers who handle or inspect these shipping containers. Compounds of interest span a range of polarities, volatilities, and reactivities which make them challenging to comprehensively analyse using a single technique.

Selected ion flow tube mass spectrometry (SIFT-MS) is a direct mass spectrometry technique capable of analysing most volatile organic and inorganic compounds in real time. This makes it an ideal candidate for quantifying fumigant compounds commonly used in the shipping industry.

Methods

This work utilizes a Syft Technologies ContainerSure selected ion flow tube-mass spectrometer (SIFT-MS), equipped with an 8 reagent ion source, and operating with helium carrier gas. Rate coefficients, product ions, and branching ratios are measured for the ion molecule reactions of a subset of H3O+, NO+, O2+, OH-, and O2- with toxic organic and inorganic compounds. Stock gas mixtures of analytes of interest were prepared using permeation tubes in a Dynacal permeation oven using nitrogen gas as a diluent. These stock solutions were diluted into Tedlar bags with known volumes of whole air for analysis.

Novel Aspect

Rapid characterization of fumigants using a SIFT-MS equipped with an ion source that operates in both positive and negative modes.

Preliminary Data or Plenary Speaker Abstract

This work explores the application of a SIFT-MS instrument equipped with an 8 reagent ion source to the analysis of common toxic and fumigant compounds used in the shipping industry, including benzene, chloropicrin, ethylene dibromide, ethylene oxide, formaldehyde, hydrogen cyanide, methyl bromide, phosphine, sulfuryl fluoride, and toluene. All compounds are found to be measurable by SIFT-MS, although sulfuryl fluoride is found to be unreactive with positive reagent ions and requires the use of the negative reagent ions OH- and O2-. Bimolecular (or pseudo bimolecular) rate constants are reported for the ion molecule reactions of these analytes with a selection of the reagent ions H3O+, NO+, O2+, OH-, and O2-.

The suitability of the method for routine analysis of whole air samples has been assessed, and limits of quantitation have been determined for all analytes. With an analysis time of 3 minutes, detection limits in the single digit ppbV range or lower are achievable for all analytes, indicating the sensitivity of the method is sufficient for exposure surveillance. Further experiments have assessed the selectivity of the analysis, and expand the principles developed here to the analysis of additional inorganic compounds.

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Protein variations in a marine-derived Aspergillus terreus in response to salinity and chromium concentrations as deduced by peptide mass fingerprinting

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Adaptations to saline environments is the key factor that differentiates marine-derived microbes from terrestrial habitats. The presence of soluble osmolytes, affinity for sodium ions and streamlined genomes aid marine biota to survive hypertonic environments. With the current rise in anthropogenic activities, marine biota now face multiple stresses that demand immediate acclimation and evolution to adapt. This study evaluated the cellular response of marine-derived fungal isolate to variable salinity and Cr (VI) stress using peptide mass fingerprinting on an LCMS-MS QToF.

Methods

The whole-cell proteins were extracted using bead-bashing technique and subjected to trypsin digestion. A total of 659 proteins across 9 variable conditions with salinity (0, 35, 100 PSU) and Cr (VI) (0, 100, 500 ppm) combinations were detected. Fifty-three housekeeping proteins were expressed across all conditions, categorised into carbohydrate metabolism, nucleotide metabolism, genetic information processing, and cellular processes using KEGG pathways.

Novel Aspect

Expression of multiple tolerance mechanisms to combat the synergistic effects of salt and chromium stress was observed in this study.

Preliminary Data or Plenary Speaker Abstract

Variation in salinity leads to the expression of proteins associated with DNA damage control and Reactive Oxygen Species scavenging mechanisms, but the addition of Cr (VI) stress triggered the expression of proteins which were involved in the enzymatic reduction and transport/metal efflux. Thioredoxin (NCBI id 114190288) expression was upregulated with fold change values of 5.011 and 5.032 at 100 and 500 ppm Cr (VI) at 35 PSU and 2.358 and 1.839 at the same Cr (VI) concentrations at 100 PSU. This indicated towards up-regulation of ROS scavenging proteins being the pre-dominant mechanism amongst the others employed by marine fungi in the presence of elevated stresses.

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Advancing Environmental Protection Through Rapid Volatile PFAS Detection and Real-Time Mobile, Fenceline, and Ambient Air Monitoring

Mr. Paul Johnson[1]
[1]Syft Technologies

Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The effectiveness of environmental emissions monitoring has been limited by the use of slow off-line techniques which lack the speed and resolution needed for rapid detection and source identification. Detection of PFAS compounds in the environment and consumer products using direct measurement frequently encounters the challenge of highly concentrated volatiles that can result in reagent ion depletion and saturation effects.

Selected ion flow tube mass spectrometry (SIFT-MS) is a real-time technique that can speciate and quantify volatile organic compounds (VOCs) and inorganic gases simultaneously and continuously. Its use by both governmental agencies and industry to test for toxic product impurities and for emissions monitoring in mobile, fenceline, and ambient air settings is discussed.

Methods

The combined studies utilize SIFT-MS operating on nitrogen carrier gas. Lee et al. targeted 84 pollution VOCs arising from small-scale ship repair yards in Hyeon, southeast Korea using mobile SIFT-MS. Yu et al. of NIER utilized mobile SIFT-MS for the fenceline characterization of 17 air pollutants emitted from textile dying facilities in Daegu.

Standards were prepared for PFPA and HFBA into Nalophan bags filled with zero air. Scans from m/z 10 – 400 were run on a Syft Tracer instrument to identify product ions and determine branching ratios for the reagent ions. Consumer products were decanted into 250 mL glass bottles. Samples were heated to 40°C for 10 mins, and the sample bottle septum was connected directly to the inlet.

Novel Aspect

Real-time characterization of environmental pollutants in four distinct applications and PFAS impurity analysis using a single SIFT-MS instrument configuration.

Preliminary Data or Plenary Speaker Abstract

Temporal variations in VOC concentrations were considerable due to activities being undertaken at the repair yards and hence demonstrate the high value of real-time monitoring compared to the time-averaged TD-GC approach. Seasonal (winter-summer) behaviors and the annual average distribution of 105 HAPs in Ulsan (southeast Korea) were investigated at three locations (industrial, urban, and ‘non-urban’) using a SIFT-MS instrument in a mobile laboratory. Concentrations of the dominant VOCs were highest in the industrial area. The measured formaldehyde concentrations were consistent with other Korean cities, while average benzene levels just met the Korean standard of 1.5 ppbV. Little summer-winter variation in VOC emissions was observed at the coastal industrial location, whereas in the urban and inland ‘non-urban’ areas the emissions were higher in the summer. There was little diurnal change in VOC levels in the non-industrial areas for the dominant VOCs, whereas they nearly doubled at night in the industrial area.

In agreement with the Korean chemical inventory, Yu et al. observed significant concentrations of toluene (up to nearly 1 ppmV), as well as methyl ethyl ketone (MEK) and dichloromethane (up to 130 ppbV). The most frequently detected pollutants at higher concentrations were toluene, methanol, acrolein, dichloromethane, and acetone.

Two of the five negative reagent ions used for PFAS analysis showed saturation effects due to the many other volatiles present in the matrix, so quantitation of PFPA and HFPA was performed using the three ions mentioned previously. Concentrations of 5 – 60 ppbV and 18 – 150 ppbV for PFPA and HFBA were measured in the four products. The eight reagent ions in Syft Tracer provide enhanced selectivity, because three negative reagent ions can measure low concentrations of PFAS without any ion suppression effects. This prevents the need for sample dilution or pre-concentration of these toxic VOCs, facilitating rapid sample screening.

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Discovery of emerging endocrine disrupting chemicals in surface waters using effect-directed analysis

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Various environmental contaminants are considered as “pseudo-persistent” because of their continuous use and release to environment. It is essential to detect and identify novel emerging contaminants to provide early warning of environmental pollution. Generally, environmental samples are complex mixtures containing naturally occurring substances and synthetic chemicals, whereas only few of them are responsible for the detectable toxicity. To accurately specify major contributing pollutants of the observed toxic effects, efficient tools to reduce sample complexity, to measure target toxicity, and to identify active chemicals are necessary.

Methods

Effect-directed analysis, of which toxicity tests are combined with chemical fractionation and identification procedure is suggested to be a promising technique to identify novel environmental contaminants. In this work, endocrine disrupting activities of river water samples were analyzed using recombinant yeasts co-transfecting with human receptors and a lacZ reporter plasmid carrying the response element for the corresponding complex. Target contaminants quantification and non-target screening were carried out using a TSQ Quantum Ultra Triple Stage Quadrupole MS (Thermo Fisher Scientific) and a JMS-TQ4000GC Triple Quadrupole MS (JEOL), respectively. Non-target screening was performed manually, and available open-source softwares were used to allow information extraction from raw data.

Novel Aspect

Emerging endocrine disrupting contaminants, such as novel antioxidants and their transformation products were identified in surface waters of Taiwanese rivers.

Preliminary Data or Plenary Speaker Abstract

Our results showed that aryl hydrocarbon receptor agonist activities were frequently detected in aqueous (W) (18-437 ng β-naphthoflavone equivalent/L) and suspended solids (SS) (58-720 ng β-naphthoflavone equivalent/L) samples from Taiwanese rivers. Anti-androgenic and anti-thyroid activities were mainly found in W samples (<69.1-319.4 μg flutamide equivalent/L and <12.5-102 μg 3,5-di-tert-butyl-4-hydroxybenzoic acid equivalent/L), whereas thyroid hormone-like activities were only detected in the SS samples (<32.5-281 ng triiodothyronine equivalent/L). After fractionation, methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate (BHT-MP), 3,5-di-tert-butyl-4-hydroxybenzyl alcohol (BHT-OH) and 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionic acid (Fenozan) were identified in samples showing anti-thyroid activities, and bisphenol A was found in samples showing anti-androgenic activities. Furthermore, diverse synthetic compounds were found in the downriver samples using non-target analysis, including phenolic compounds, antioxidants, bisphenols, surfactants, etc., suggesting possible contamination of aquatic environment.

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Non-targeted Analysis and Toxicity Evaluation of UV Irradiated Nylon-6 in Seawater

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

South Korea collected 78,000 tons of marine debris in 2016, 44% of which was plastic, including nylon 6 fishing nets. These nets, globally produced and highly UV-sensitive, contain additives to enhance properties but degrade into toxic and persistent compounds under UV exposure. This study aimed to identify these degradants in fishing nets using ultra-high performance liquid chromatography (UPLC) coupled to high resolution mass spectrometry (HRMS). A non-targeted approach was used to develop an in-house database, addressing the environmental impact of these pollutants.

Methods

Discarded fishing nets from coast were analyzed alongside nylon-6 under conditions mimicking environmental exposure to UV light and seawater, as per Korea Meteorological Administration's data (263.60 MJ/m^2 over what equates to 21 days). A comparative dark control was also employed. Both UV-exposed and control samples, including real fishing nets, underwent methanolic SPE for isolating photodegraded products, which were then analyzed via UPLC-LCMS. Data processing and non-targeted compound identification were carried out using Compound Discoverer 3.3. Additionally, the T.E.S.T software facilitated toxicity predictions, while 2DLC-based EDA pinpointed toxicants, offering an extensive environmental impact assessment.

Novel Aspect

Exploration of non-targeted toxic compounds produced from the effects of mechanical stress and UV exposure in disposed fishing nets.

Preliminary Data or Plenary Speaker Abstract

Data processing via CD 3.3 revealed 500, 729, and 519 peaks in dark control, UV-irradiated, and actual fishing net samples, respectively. Analysis against environmental compound databases identified nylon 6 oligomers and plasticizers in both control and UV-irradiated samples, highlighting the formation of numerous new compounds under UV exposure. These findings, consistent across lab and environmental samples, were validated against standard references and incorporated into an in-house database.
Toxicity predictions, initially focusing on rat models before extrapolating to humans, indicated several compounds with low LD50 values, suggesting high toxicity. Consequently, an online two-dimensional liquid chromatography (2DLC) based effect-directed analysis (EDA) was employed to identify toxic compounds. Fractions from the initial separation underwent cytotoxicity assessments using the MTT assay, renowned for its cell viability measurement capabilities. Subsequent analysis of toxic fractions via online 2DLC coupled to high-resolution mass spectrometry (HRMS) provided further insights.
This comprehensive methodology, integrating the precision of 2DLC and the reliability of the MTT assay, underscores the environmental and health risks associated with UV-exposed nylon 6. The identification and confirmation of toxic compounds, especially those verified against available reference standards, highlight the necessity of thorough evaluations to understand the impacts of these hazardous substances comprehensively.

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Identification of short chain per and polyfluorinated alkyl substances (PFAS) using ion ratios with low mass product ions

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

As the environmental impact and health hazards of legacy long chain (≥C8) PFAS emerged, they started to be substituted for short (C4-C7) and ultrashort chain (≤3) PFAS in manufacturing. The belief was these would have less of an impact but initial evidence suggests that there are still concerns regarding their effects. The analysis of the carboxylate PFAS with chain lengths C3-C4 (PFPrA & PFBA) is problematic in terms of positively identifying the analyte due to the lack of a second product ion with suitable intensity to use for ion ratio calculations to meet common identification criteria. The use of m/z 19 is possible and this response can be significantly improved using a modified high performance tandem quadrupole mass spectrometer.

Methods
A direct inject approach was taken to analyze a wide range of PFAS including short and ultrashort chain PFAS in various types of water samples, including drinking water, ground water, surface water, and wastewater. Each water sample was subsampled with 0.25 mL of sample added to a polypropylene vial, then diluted with an equal volume of methanol. This sample was injected onto a modified high performance tandem quadrupole mass spectrometer with a compact design, enhanced negative ion detection and removable source shield to reduce source contamination from sample matrix and mobile phase additives.

Novel Aspect
Demonstration of enhanced detection of low mass product ions from short chain PFAS analytes for analyte identity by ion ratio.

Preliminary Data or Plenary Speakers Abstract
For a number of short and ultrashort chain PFAS, predominately carboxylates with a carbon chain length of 5 or less (PFPrA, PFBA, PFPeA), there has been no mass product ion (m/z ≥50) identified to use to create ion ratios. Initial work has been carried out on a high performance negative ion tandem quadrupole mass spectrometer to show that the detection of a fluoride ion (m/z 19) was possible using the standard configuration, but the ion ratios measured were between 0.001 to 0.002. By modifying the high performance tandem quadrupole mass spectrometer improved sensitivity for the fluoride ion was achieved, and initial results show that the increase in response was at least 2 orders of magnitude. Using this modified system, the ion ratios measured were between 0.054 and 0.074 making confirmation of analyte identity by ion ratio a realistic possibility.

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Proteome profile of mucous proteins in oyster mantle and their response following DDT exposure

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Mucus is secreted from mucous cells. In bivalves, mucus plays important roles in filtering food particles and interacts with waterborne microorganisms. It may also involve in protective role against microorganism and chemical pollutants in seawater. Studies of mucous cell structure and function are scarce in bivalves. This work aimed to investigate histological structure of mucous cells in mantle of the Hooded oyster (Saccostrea cucullata), and to explore proteome profile in mucous as well as their response following DDT exposure.

Methods

Oysters were collected and acclimated for 1 week and exposed to 0 and 10 ug/L DDT for 96 hours. Exposure condition and protocols were based on our previous report (Chueycham et al., 2021). After the exposure, 3 oysters from control (0 ug/L) and from DDT-exposed group (10 ug/L) were randomly collected and dissected for histological examination (n = 3). Later, oysters from control and from DDT-exposed group (n = 3) were also randomly collected for mucous protein extraction and separating on an SDS-PAGE for 10 minutes. Gel bands were cut, and in-gel digestion was performed by using trypsin. Peptides were extracted and analyzed by using Q Exactive Orbitrap LC-MS/MS according to Gutiérrez et al. (2019).

Novel Aspect

This is the first report on mucous proteome of the Hooded oyster Saccostrea cucullata and their response to DDT exposure.

Preliminary Data or Plenary Speaker Abstract

Histological results showed that the mucous cells were distributed in mantle epithelia. Within mucous cell, nucleus was found at the basement of cell and numerous mucin granules were found in cytoplasm. DDT affected oyster tissue and increased number of mucous cells in mantle. Proteomic results revealed that 1,285 proteins were identified in mucous sample. The proteins are involved in various biological processes. After exposed to DDT, 83 proteins were significantly dysregulated which include 45 up- and 38 down-regulated proteins. Possible role of mucous proteins will be discussed.

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Leveraging MS1 Dimension & Formula Prediction in NTA of PFAS using novel FluoroMatch Algorithms: Assessing Confidence and Coverage

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Liquid chromatography high-resolution accurate-mass mass spectrometry (LC-HRAMMS) provides measurements related to universal properties of chemicals which allows for de novo structure elucidation. Fragmentation data is key for de novo structure elucidation, but often only the topmost abundance ions are selected for fragmentation, which may not cover all molecules of interest. Furthermore, for low abundance ions, there may not be enough ion signal to obtain quality fragmentation data. Therefore, in non-targeted analysis often only accurate mass information from the full-scan (MS1) data is available for many features. We integrated a range of techniques leveraging retention time and MS1 data applied to PFAS analysis to test whether these two measurements alone could be used for de novo chemical elucidation.

Methods

Kauffman plots, homologous series, and theoretical versus experimental isotopic patterns were integrated into FluoroMatch alongside the MS2 visualizations. Filters including occurrence of isotope peaks, isotopic ratios and spacing, formula matching metrics, mass defect, number of features in a homologous series, Kauffman equivalent carbon ratios, and other MS1 based measurements/metrics were added for refining features for compound discovery and validation. Furthermore, a formula prediction workflow was developed and optimized for PFAS using the following R packages (Rdisop, MassTools, enviPat, MetaboCoreUtils) plus modified Fiehn lab golden rules. The entire visualization and formula prediction workflow was validated against dried blood spots, standards spiked into neat solution, and municipal sewage samples, acquired on Revident and 6546 Agilent LC/Q-TOF mass spectrometers.

Novel Aspect

A MS1 based workflow was optimized for PFAS analysis showing highly accurate formula predictions.

Preliminary Data or Plenary Speaker Abstract

Two scoring methods were implemented to assess their usefulness in decision making for creating the best formula candidates. For PFAS formula prediction the following parameters were most important for both reducing the number of potential formulae and reducing false positive rates: elemental heuristics applicable to PFAS, mass defect filtering of MS1 spectrum, and strict scoring method. After applying to dried blood spots, standards spiked into a neat solution, and municipal sewage samples, the formula prediction algorithm was assessed for false positive rate. The false positive rate amongst the top 10 formulas was below 10% in a preliminary subset of the data, although results were highly sensitive to isotopic fidelity. A 52% false positive rate for the topmost formula was obtained benchmarking against 29 high confidence hits in a complex AFFF sample. It is important to note, this was for the most complex sample, and hence may be conservative. Using homologous series and retention time order to manually review formula patterns using the visualizer further reduced the false positive rate, allowing for a top formula assignment with a false positive rate of less than 20%. Features with low abundance and/or with high m/z ratios, or in regions with dense MS1 spectra with overlapping isotopic peaks, were most difficult for formula prediction. A further challenge was computational time; feature tables with thousands to tens of thousands of features across tens to hundreds of samples led to unsustainable computational times. Therefore, representative spectra from each respective most abundant M across all samples were chosen for formula prediction and algorithms were developed to further filter the candidates for formula

prediction to only those likely to be PFAS. Future work automating the integration of MS2 fragments, accurate mass database matching, homologous series, and predicted structures from MS2, into the formula prediction algorithm will increase accuracy.

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Critical Assessment of the Chemical Space Covered by LC–HRMS Non-Targeted Analysis

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Non-target analysis (NTA) using liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) is one of the most widely used methods for screening environmental samples for unknown chemicals. We were interested in finding out how well this method covers the chemical space of chemicals of emerging concern. For comparison we used close to 60000 chemicals from NORMAN SusDat which had a PubChem chemical identifier. We then projected these along with compounds found in LC-HRMS NTA studies with principal component analysis. We also investigated the most common experimental parameters used to see if these could be potentially limiting the space covered. We found that only around 2% of the NORMAN SusDat chemical space was covered by recent LC-HRMS NTA studies.

Methods

61 non-target LC-HRMS studies were selected from Scopus between 2017 and 2023. Several experimental parameters were collected within 4 categories: Sample collection and extraction, liquid chromatography, high resolution mass spectrometry and data processing. In addition, all of the detected compounds at a level of 1 or 2 on the Schimanski scale were noted for each study. Using chemical identifiers provided by authors in each paper (names, SMILES, InChIKey), the XlogP3 and MW for each of the compounds were retrieved from PubChem. We also calculated the elemental mass defects for each compound as a way to cluster chemical classes. We were then able to perform principal component analysis using all the collected variables. This allowed for clustering according to chemical classes.

Novel Aspect

To our knowledge, this is the first time the covered chemical space by LC-HRMS NTA has been estimated

Preliminary Data or Plenary Speaker Abstract

Non-targeted analysis (NTA) has emerged as a valuable approach for the comprehensive monitoring of chemicals of emerging concern (CECs) in the exposome. The NTA approach can theoretically identify compounds with diverse physicochemical properties and sources. Even though they are generic and have a wide scope, non-targeted analysis methods have been shown to have limitations in terms of their coverage of the chemical space, as the number of identified chemicals in each sample is very low (e.g., ≤5%). Investigating the chemical space that is covered by each NTA assay is crucial for understanding the limitations and challenges associated with the workflow, from the experimental methods to the data acquisition and data processing techniques. We examined recent NTA studies published between 2017 and 2023 that employed liquid chromatography–high-resolution mass spectrometry. The parameters used in each study were documented, and the reported chemicals at confidence levels 1 and 2 were retrieved. The chosen experimental setups and the quality of the reporting were critically evaluated and discussed. Our findings reveal that only around 2% of the estimated chemical space was covered by the NTA studies investigated. Little to no trend was found between the experimental setup and the observed coverage due to the generic and wide scope of the NTA studies. The limited coverage of the chemical space by the reviewed NTA studies highlights the necessity for a more comprehensive approach in the experimental and data

processing setups in order to enable the exploration of a broader range of chemical space, with the ultimate goal of protecting human and environmental health.

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Ultra-fast multiresidues accurate mass screening strategy

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

In the area of mass food production and global goods exchange, it is crucial to develop ultra-fast screening to assure raw material food safety. Indeed, annually in Europe for instance, more than 88,000 samples are analyzed for the presence of pesticide residues. Due to that fact, it is crucial to develop rapid methods to facilitate swift safety assessment of raw goods being introduced to the market. Utilizing high resolution accurate mass spectrometry offers a unique opportunity to employ an ultra-fast chromatographic gradient to improve on reliable residue detection in complex matrices.

Methods

A ballistic short reverse phase chromatographic gradient (4 minutes) combined with a MS method was developed with a Revident LC/Q-TOF system. Various vegetables and fruit matrices such as Broccoli, Celery, and Strawberry were extracted with QuEChERS sample preparation for pigmented fruits and vegetables and spiked with a pesticide mixture of over 150 compounds and 4 heavy labeled internal standards. A calibration curve was generated from 8 different concentrations ranging from 1 ng/ml to 100 ng/ml and all injections were completed in triplicate. Initial data were analyzed using embedded screening software, after which they were reinjected automatically in the case of a non-compliant result using a longer LC-MS method including identifying fragment information, without interjection by the analyst.

Novel Aspect

Ballistic gradient coupled to accurate mass MS1 for pesticide screening.

Preliminary Data or Plenary Speaker Abstract

With the first pass, over 150 compounds were identified in complex matrices in the calibration range based only on mass accuracy and matching retention time. The review of results was assisted by a commercially available software with 90% of the detected compounds with a match score higher than 90.0 and 96% with a mass accuracy less than 1 ppm independent of concentration (1 ng/mL to 100 ng/mL). Utilizing effective heavy labeled internal standards, the majority of identified compounds showed good linearity and R2 values above 0.99.

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Mass spectrometry-based digesta-peptidome pipeline for decoding dietary protein quality, bioavailability, and functionality.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Current mass spectrometry (MS)-based proteomics enables identification and quantification of peptides derived from several thousand proteins or their fragments in a single experiment. Such peptide information is generally used to extract protein dynamics in complex biological and clinical samples. We leveraged dissecting such massive peptide data sets to decipher functional proteolytic events during food processing and gastrointestinal digestion.

Methods

A peptide-centric analytical strategy was devised to interpret proteolytic events at the molecular level, focusing on mapping the cleavage site and the resultant peptides fragments during endogenous protein breakdown. Following that, a systematic in vitro gastrointestinal digestion (Infogest)-based peptidomics pipeline to evaluate the fate of dietary proteins in the gastrointestinal system. We applied these approaches to tempeh, a fermented soy-based product known for its enhanced nutritional profile compared to its unfermented counterpart (soybean). Our aim was to elucidate the mechanistic insights by which fermentation process enhances bioavailability of proteinaceous nutrients.

Novel Aspect

Our strategic digesta-peptidome MS pipeline, complemented by advanced analytics, provides a detailed interpretation of nutritional bioaccessibility of dietary proteins.

Preliminary Data or Plenary Speaker Abstract

Our results demonstrate that tempeh fermentation leads to significant proteolysis of soy proteins, resulting in a diverse array of protein hydrolysates that increased nutrients bioavailability. Deep proteomics analysis revealed a global proteolytic alteration in tempeh's proteome, with no specific protein groups predominantly affected. Furthermore, comparative analysis of commercial soybean and tempeh samples showed distinct proteome profiles, suggesting consistent proteolytic events during fermentation across different products, underscores the reliability of fermentation processes in enhancing protein digestibility.
Analysis of soy and tempeh through digesta-peptidomics pipeline further confirmed enhanced digestibility, characterized by a higher prevalence of shorter peptides chains liberated during digestion, which are more readily absorbed in the gastrointestinal tract. In addition, digestion-resistant proteins at the end of in vitro digestion reduced, thereby improving overall protein utilization. These findings underscore the potential of fermentation process to result in a superior plant-based protein source, providing valuable insights into its nutritional advantages and supporting its inclusion in a healthy diet.

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Rapid and high-throughput measurement of cyanide in liquor by direct sampling photoionization time-of-flight mass spectrometry

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Cyanide is invariably present in fermented liquors derived from cyanogenic plants, necessitating stringent control measures for safe consumption. Rapid assessment of cyanide levels during liquor production is paramount for ensuring product safety and quality control. While conventional methods such as titration, spectrophotometry, gas chromatography with electron capture detector, and ion chromatography can fulfill sensitivity requirements for trace cyanide detection, they often entail complex pretreatment processes, time-consuming analyses, extensive utilization of organic solvents, or susceptibility to matrix interference. Hence, there is an urgent need to devise a simple, rapid, and high-throughput measurement method for cyanide to streamline quality control procedures in liquor production.

Methods

A rapid and high-throughput method based on negative photoionization time-of-flight mass spectrometry (NPI-TOF) was developed for the measurement of cyanide in liquor. A simple alkalization-dilution-acidification pretreatment process converts cyanide to gas phase HCN. Then the characteristic ion CN- (m/z 26) was obtained by the NPI-TOF and utilized for the qualitative and quantitative analysis. The liquor samples were diluted 50 times to reduce the matrix effect of cyanide detection in liquor. A set of heating and stirring modules was designed to promote HCN volatilization from the solution, with a heating temperature of 50 ℃, and a stirring speed of 100 rpm. This procedure facilitates high-through pretreatment and detection of batch samples, which only takes about 30 min for 20 samples.

Novel Aspect

Rapid derivatization combined with negative photoionization mass spectrometry enables the fast detection of cyanide in liquor within one minute.

Preliminary Data or Plenary Speaker Abstract

The method performance, including precision, method detection limit (MDL), linearity, and recovery, was systematically evaluated. Precision, expressed as the relative standard deviation (RSD) of 7 parallel determinations of experimental liquor, achieved 4.74%. To determine the MDL, the standard deviations of 7 replicates of 0.10 μgL−1 cyanide standard solutions were utilized, resulting in an MDL value of 0.05 μgL−1, with the practical quantitation limit set at 2.5 μgL−1 for liquor samples (50-fold-diluted). A calibration curve with a concentration range of 0.1 - 100 μgL−1 was established corresponding to the quantitative concentration range of 5.0 - 5000 μgL−1. Recoveries of mixed liquor samples were assessed by the standard addition method, which was 115.85%, 106.92% and 94.28% with the addition amount of 5.00, 10.00 and 50.00 mgL−1, respectively.
The developed method was employed to determine cyanide concentrations in 200 liquor samples collected from different distillation rounds (2nd to 7th) in the same production cycle. The average cyanide concentrations in the liquors collected from the 2nd to 7th distillation rounds were 1485, 538, 309, 306, 538 and 594 μgL−1, respectively. The liquors collected from the 4th and 5th distillation rounds had the lowest cyanide content, which was also consistent with the final quality assessment (color, aroma, alcohol, taste) results of the liquor.
The quantitative results of the newly developed method were compared with the ion chromatography (IC) method, which has been widely used in the determination of cyanide in liquor. As a result, the cyanide concentration profile of the 200 liquor samples by the NPI-TOF method showed a strong positive correlation with the IC method with a correlation coefficient of 0.9738. It is

remarkable that the detection time was significantly reduced from 9 min to 1 min per sample, which is necessary and crucial to achieving high-throughput measurements of large numbers of samples.

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Quantitative Method for Assessing Pentachlorothiophenol(PCTP) Risk in Food Samples Using GC-MS/MS Analysis

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Pentachlorothiophenol (PCTP) is an organic compound featuring five chlorine atoms and a thiol group on a benzene ring. It is used industrially as a sulfur cross-linking agent to improve rubber pliability and can also be unintentionally generated through incineration. Due to the teratogenic nature of its degradation products, its industrial use has ceased. PCTP meets the persistent, bioaccumulative, and toxic (PBT)/vPvB criteria in Europe. Increased chemical usage in industries raises concerns for food safety, as PBT chemicals can transition into food and human exposure. Although research on PCTP is still in its early stages, there is an urgent need for further study and stringent safety management.

Methods

Weigh 2g sample, add 200µl ISTD, extract with 50ml hexane for 20min by ultrasonic wave. Repeat extraction twice, remove water with anhydrous sodium sulfate, concentrate with rotary vacuum evaporator, dissolve in 2ml benzene. Activate 5ml DCM, 15ml hexane in silica cartridge, add sample. Elute with 5ml hexane, 15ml hexane/DCM (3:1), concentrate to 1ml. Add 100µl methanol, 300µl TMS diazomethane, react at 35°C for 30min. Add 150µl distilled water to 7ml. Centrifuge at 3500RPM for 5min, collect supernatant, filter through 0.45µm filter. Concentrate, add 100µl benzene for GC/MS/MS analysis.

Novel Aspect

New analytical method for determining PCTP in food was developed

Preliminary Data or Plenary Speaker Abstract

The primary aim of this study was to develop a analytical method utilizing Gas Chromatography-Mass Spectrometry (GC/MS) for the detection of Pentachlorothiophenol (PCTP) in various food items. A wide spectrum of food products, encompassing apples, rapeseed oil, eggs, pork, clams, and mackerel, was selected to serve as representative samples. The determination of the limit of detection (LOD) for these samples yielded values ranging between 1.7 and 1.9 ng/ml, while the limit of quantification (LOQ) fell within the range of 5.1 to 5.9 ng/ml. Upon scrutinizing the Day 1 data, it was discerned that the accuracy levels of the method ranged from 72.8% to 107.6%, exhibiting a precision of below 19.2%.

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Detecting Adulteration of Essential Oils Using Gas Chromatography Mass Spectrometry

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Adulteration is a prominent food safety problem affecting various foods and beverages. Adulterated foods have caused health problems in 57% of the global population, with this trend increasing as the demand for food products continues to rise. Chemical fingerprinting presents a valuable tool for identifying the presence of adulteration, with gas chromatography–mass spectrometry offering a promising approach.

Methods

Distilled hop oil samples were obtained from an industry partner, as per the recommendations of the International Organization for the Flavor Industry (IOFI). Authentic Galaxy hop oil was artificially adulterated by spiking it with increasing amounts of secondary hop oil. Adulteration was assessed at 10% – 50% ranges.
Qualitative data analysis was performed using Adams' method for essential oils using an Agilent 7890B GC coupled with a 5977B Quadrupole MS. Peak identification was performed by spectra matching using NIST MS Search and Kováts retention indices (RIs) with Adams' essential oil library.
Quantitative data analysis was carried out by adapting the Adams' method for use on an Agilent 7890B GC-FID. Quantification followed the IOFI method of using relative response factors (RRFs).

Novel Aspect

This research offers an untargeted GC–MS and GC–FID workflow for assessing adulteration instead of using targeted analyte monitoring.

Preliminary Data or Plenary Speaker Abstract

Twenty-six compounds were putatively identified and quantified in 28 genetically characterised Galaxy hop oils to form a Galaxy hop fingerprint.
Artificially adulterated samples were compared to the Galaxy hop fingerprint using univariate and multivariate statistics. Initially, the abundance of each VOC in the adulterated samples was compared to the Galaxy hop fingerprint using a two-tailed t-test ($\alpha=0.05$), which showed ten compounds significantly differed ($p\text{-value} \leq 0.034$) at the lowest adulteration level.
PCA showed that this workflow could accurately differentiate between artificially adulterated samples with greater than 20% adulteration. However, there was an overlap in the Galaxy fingerprint and 10% adulterated samples, indicating that further work is required to improve the capability of this workflow to detect low levels of adulteration.

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Development and Validation of an LC-QQQ-MS Method for Polyphenol Quantification

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Polyphenols, abundant in plant-based foods, are renowned for their diverse health benefits, including antioxidant and anti-inflammatory properties. Analysing polyphenols is crucial for understanding their presence and potential impacts in various products, necessitating the development of accurate quantification methods. Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-QQQ-MS) has emerged as a reliable technique for this purpose, offering high sensitivity and selectivity. This study focuses on the development and validation of an LC-QQQ-MS method for identifying and quantifying specific polyphenols, such as flavonoids and phenolic acids. By optimising mass spectrometry and liquid chromatography parameters and conducting thorough validation, including determining limits of detection and quantification, assessing linearity, and evaluating precision, this method provides a robust means of quantifying polyphenols in complex matrices.

Methods

In this study, we utilised a commercial polyphenols kit comprising pooled flavonoids and phenolic acids standards. We optimized the collision energy for Multiple Reaction Monitoring (MRM) transitions by scanning the compounds in product ion mode at different collision energies. Selection of MRM transitions was based on product ion abundance at each collision energy. Subsequently, we optimized the liquid chromatography (LC) parameters, beginning with isocratic conditions and then developing a gradient method. Method validation included establishing limits of detection and quantification, assessing linearity, and evaluating precision. While our analysis covered 90 polyphenols, this report focuses on selected 11 flavonoids and 11 phenolic acids. This approach ensured accurate quantification of the selected polyphenols in complex matrices.

Novel Aspect

These results comprise of a part of a larger method where 90 polyphenol MRM transitions and validations are presented to facilitate analyses of any polyphenol enriched extracts

Preliminary Data or Plenary Speaker Abstract

In our preliminary analysis, we utilised Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry (LC-QTOF-MS) to identify polyphenols in sugarcane-derived polyphenol extracts formulated into two bioactive products. Initially, our analysis identified 12 polyphenols, including flavonoids and phenolic acids, based on an in-house prepared polyphenol standards library. However, numerous unidentified peaks were observed in the chromatogram, prompting us to expand our polyphenol library to include 90 polyphenols. This expanded library enables us to identify and quantify a larger number of peaks in our polyphenol extracts. The data not only confirms the presence of previously identified polyphenols but also reveals additional compounds that were previously unidentified. Our method validation results demonstrated excellent sensitivity, indicating that our LC-QQQ-MS method is robust, and is reliable for quantifying polyphenols in complex extracts. This suggests that our method can be effectively used for the accurate quantification of polyphenols in various polyphenol-based samples. Further analysis in biological systems will provide a more comprehensive understanding of the polyphenol profile in these extracts and their potential bioactivity.

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Effect of perilla protein hydrolysate with oxidized coconut oil and amino acid for beef flavor production

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Perilla meal is an agricultural by-product that originated from the extraction of perilla seed oil. It contains a high protein (36.3%), phenolic compounds, phytic acid, and polysaccharides. Perilla meal has been hydrolyzed to produce bioactive hydrolysates, called as perilla meal hydrolysates (PMHs). Maillard reaction (MR) is the main process responsible for the generation of meaty flavor and is affected by several factors, including pH, temperature, reactant type, and concentration. Among these, the type of amino acid is a major factor in determining the pathways and reaction rates in MR. The objective of this study was to evaluate volatile profiles generated by the thermal reaction of PMH with oxidized coconut oil (CO) and amino acids to mimic a beef flavor.

Methods

Ten grams of CO was put in a 30 mL vial and sealed air-tight with rubber septa and aluminum cap. It was heated in a convection oven (CO-81, Han Yang, Seoul, Korea) at 180°C for 16 h. CO oxidized for 2, 8, and 16 h were designated as CO2, CO8, and CO16, respectively. Beef flavor was prepared by heating top round meat added with 20% (w/w) beef tallow. Amino acid were aspartic acid, proline, glutamic acid, alanine, and methionine. Solid phase microextraction (SPME)-GC/MS and electronic nose (EN) techniques were applied to select proper reaction conditions. PMH was prepared from hydrolysis using a double enzyme (Alcalase and Flavourzyme).

Novel Aspect

Heated PMH with oxidized CO and glutamic acid in the MR system could generate desirable beef flavor.

Preliminary Data or Plenary Speaker Abstract

Beef flavor was prepared by adding 20% (w/w) beef tallow to top round meat. The volatiles produced after heat treatment could be divided into three groups such as sugar dehydration or fragmentation products (e.g. furans, pyrones, cyclopentenes), amino acid degradation products (e.g. aldehydes), and volatiles produced by further interactions (e.g. pyridines, imidazoles, pyrazines, and thiazoles). PMH generated the highest sulfur-containing heterocyclic volatiles when heated with CO oxidized for 8 h (XCP-CO8) compared to other samples. Furthermore, XCP-CO8 with glutamic acid produced the highest sulfur-containing heterocyclic volatiles and mimicked the aroma pattern closest to beef flavor compared to other amino acids. N-containing volatiles emerged as the predominant compounds responsible for imparting beef flavor.

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A Mass spectrometry approach to protect fungicides by understanding the role of barley genetics in fungicide distribution and dissipation.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Fungicides are pivotal for managing diseases causing millionaire losses to the barley industry; however, their chemistries are at risk given the rise in fungicide resistance strains. Our group is using mass spectrometry (MS) to inform deployment strategies to protect their active compounds. This project which has just started, focuses on how fungicide efficacy varies across diverse barley cultivars, environmental conditions, and agronomic practices. The study will assess the distribution and dissipation of fungicides about cultivar-specific, agronomic, and environmental traits. We aim to delve into the dynamics of fungicides and understand how this influences the efficacy of fungicides in barley cultivation. Leveraging MS techniques, these insights promise to revolutionize fungicide management strategies, empowering stakeholders with tailored solutions for sustainable agricultural practices.

Methods

To measure the in planta concentration of the fungicides, samples will be collected at different time points and extracted using the original QuEChERs method, and then will be analysed by LC-MS. After extraction, samples will undergo separation using a Vanquish LC system. Single ion monitoring (SIM) analysis will be performed using an Orbitrap Exploris 120 (Thermo Fisher Scientific) coupled to Hypersil GOLDTM aQ C18 HPLC column (at 30°C) with particle size of 1.9 µm and analysed using Freestyle and TraceFinder softwares. Plants will be infected with net blotch and the disease would be monitored and scored. Correlations between barley genetics (traits), in planta dissipation curves, and disease scores would be drawn to inform sustainable application strategies.

Novel Aspect

No current data is available

Preliminary Data or Plenary Speaker Abstract

No current data is available

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Comprehensive proteome profiling of plant-based meats for food allergenicity risk assessment of novel foods

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The global food supply landscape is facing a crisis with production struggling to meet demand. Increasing production of meat and dairy products places considerable strains on environmental resources, raising ecological and sustainability concerns. There is thus increasing interest in developing novel food products, of which plant-based meats have rapidly gained traction as a viable alternative protein source. However, such novel foods may present unknown risks to food sensitive individuals. Comprehensive proteome profile characterisation of plant-based meats and their ingredients will thus be valuable for food safety assessments. To achieve this, we explored several different protein extraction conditions. We showed that careful consideration of the sample nature and combinatorial extraction approaches is critical to maximise total protein extraction.

Methods

Comprehensiveness of protein extraction is critical to ensure that food allergencity test on the extract are as representative as the native sample. In this project, we have investigated various protein extraction methods to optimize and develop a highly efficient protein extraction protocol that works on a wide variety of complex food matrices ranging from solid, powder, and liquid food samples.
We further investigated the protein extract to using proteomics approach to investigate the amount of allergens extracted from plant based meat. As these alternative proteins are highly processed, these food processing effects will cause proteins within the product to interact and may affect its ability to be solubilized by convention method.

Novel Aspect

We have developed a highly efficient protein extraction protocol that is effective for a wide variety of complex food matrices.

Preliminary Data or Plenary Speaker Abstract

Our results showed that the newly developed protocol is able to achieve at least 80% protein extraction efficiencies in a wide variety of foo matrices. High extraction efficienices also resulted in greater number of proteins that are identified using proteomics approach.
More importantly, in the aspect for food allergenicity, our results showed that there are significant differences in the amount of allergens idenitifed using different protein extraction protocols and this informs the need for comprehensive protein extraction efficiencies especially in ensuring the food safety of novel food alternative proteins.

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Comparision of applicability between external and Internal standard method for the determination of multi-mycotoxins in various foods using HPLC-MSMS

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Mycotoxin is toxic secondary metabolites of fungi that live on plants. Including aflatoxins (B1, B2, G1, G2), 11 mycotoxins are regulated for various foods in Korea. In this study, we simultaneously determined aflatoxins, ochratoxin A, fumonisins, and zearalenone using high-performence liquid Chromatography (HPLC) with tandam mass spectrometry (MS/MS). But, there are limitations of its application to complex matrices in various foods items. Specially, the recoveries of aflatoxins were low in some foods.

Methods

To improve the applicability of analytical method, we selected 36 food items according to moisture, protein, carbohydrate, and fat components. The recoveries of the external standard and internal(13C labeled) standard calibration method were compared for the 36 selected items.

Novel Aspect

Internal standard calibration has higher accuracy and precision than external standard for the determintion of multi-mycotoxins in various foods.

Preliminary Data or Plenary Speaker Abstract

In 36 food items, the accuracy and precision of Internal standard calibration method were satisfied within range of codex guideline for method validation. So,it is need to use Internal standards to apply the analytical method of multi mycotoxins for raw plant and the processed food.

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Stable Isotope Ratios and Multi-Elemental Content of High-Grade Asian Rice Varieties for the Identification of Geographic Fingerprints

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The majority of the world's rice production takes place in the Asia-Pacific region, including premium grade varieties such as Jasmine rice (Thailand), Paw San Hmue (Myanmar) and Ciherang (Indonesia). Considering their high quality and high production costs, these varieties are sold at premium prices in the world market and are susceptible to economic food frauds. Therefore, methods that contribute to the traceability of such products are important in identifying fraudulent cases and enhancing consumer trust in the agrifood system. The direct effect of the growing conditions and surrounding environment on the isotopic and elemental composition of plants, render stable isotope ratio analysis and multi-elemental analysis as powerful tools in geographic authentication cases.

Methods

In this work, we collected 350 authentic samples of premium-grade Asian rice varieties produced in regions of Thailand, Myanmar, Indonesia and China. These were analysed by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS) and Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) to obtain their light element (C, N, O, H, S) isotopic composition and concentration of 25 elements (Na, Mg, Al, P, S, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Cd, Sn, Ba, Hg, Pb), respectively. Thereafter, Low-Level Data Fusion (LLDF) was applied by combining the two datasets and a random forest (RF) model was performed to determine the best combination of variables that could classify the dataset by sample provenance.

Novel Aspect

This study contributes significantly to the food traceability database of emerging countries, also showcasing the complementarity among different analytical techniques.

Preliminary Data or Plenary Speaker Abstract

Preliminary results suggest noticeable differences stemming from both intra- and inter-country comparisons. Specifically, the stable isotope values of O and H could distinguish between the North and Northeast regions of Thailand, as well as the Ayeyarwady from Shwebo divisions of Myanmar. Moreover, significant differences were found between Java and the Bangka-Belitung Islands in Indonesia based on the elements Sn, Ni, Co, Se and As, while higher concentrations of Sn, Ni, Ba and Cd were found in Thailand compared to Indonesia, China and Myanmar. The highest classification accuracy was achieved from the LLDF-RF method compared to the standalone isotopic and multi-elemental data,

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Internal energy distributions of negative ions generated by MALDI with different matrices. Implications to PIP3 analysis.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is a powerful technique for mapping the spatial distribution of molecules in biological tissues. Although this technique is widely used for analyzing various lipid classes, it has not yet been fully established for certain clinically relevant classes, such as phosphoinositides (PIPs).

Methods

We are using a set of thermometer ions (phenyl sulphate derivatives) to evaluate and compare the internal energy distributions of negative ions generated by MALDI with different matrices such as DAN, NEDC, CMBT, and DHA and correlating these energies with the extent of in-source decay (ISD) and the limit of detection (LOD) of PIPs standards.

Novel Aspect

This research aids in choosing suitable MALDI matrices for imaging PIPs in tissues. However, enhanced methodologies are still needed.

Preliminary Data or Plenary Speaker Abstract

Overall, the matrices investigated increased the LOD of PIPs compared to DHB previously investigated. However, the LODs are still high, not allowing the visualization of PIP3 directly from tissues.
Initial results indicated that the LOD and the extent ISD of PIPs do not correlate well with the internal energy distributions observed with different matrices.

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Expanding TMTpro reagents to 32-plex for high-throughput quantitative proteomics on Orbitrap platforms

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

TMTpro reagents are popular reagents for highly multiplexed mass spectrometry (MS)-based proteomics, enabling relative quantification of many samples in a single LC-MS/MS acquisition. Each tag in the multiplex set incorporates nine 13C & 15N stable isotopes at unique positions within the structure to enable generation of 18 reporter ions with distinct masses that differ by a minimum of 6 mDa during MS/MS fragmentation. Through incorporation of a single 2H isotope into the reporter group, an additional set of isobaric TMTpro reagents that differs in mass by 3 mDa was developed that can be resolved in Orbitrap mass analyzers at RP ≥90K. Here, we present data on the novel TMTpro deuterium isotopologues and assess their performance for 32-plex relative quantitation.

Methods

TMTpro reagents incorporating one deuterium stable isotope were synthesized using existing manufacturing methods. Each reagent was analyzed by direct infusion MS to verify chemical purity, measure isotopic incorporation, and determine isotopic impurities and interferences. BSA digest and HeLa digest samples labeled with deuterated and non-deuterated TMTpro reagents were subjected to LC-MS/MS analysis using a Vanquish Neo UPLC with a 50cm EASY-Spray HPLC column interfaced with an Orbitrap Eclipse MS system. MS2 and RTS SPS-MS3 acquisitions were performed at various eFT and TurboTMT resolving powers. Data was processed with Proteome Discoverer to determine labeling efficiency and numbers of identified & quantified peptides & proteins.

Novel Aspect

New TMTpro reagent variants with 3 mDa mass differences enable 32-plex quantification on Orbitrap instruments.

Preliminary Data or Plenary Speaker Abstract

18-plex TMTpro reagents have been previously demonstrated to provide accurate and precise quantification of proteins and peptides by high-resolution MS/MS analysis on Orbitrap platforms. The structure of the TMTpro tag results in generation of reporter ions at high intensity and in balanced proportion to peptide backbone ions, enabling both confident quantification and peptide sequence identification. To further increase multiplexing capacity, we designed 17 structurally identical isotopologues that substitute one 2H stable isotope to impart a 3 mDa mass difference between the new variants and the existing 18-plex tags. We strategically located this deuterium isotope on the isobutyl group proximal to the nitrogen atom on the reporter to minimize chromatographic shifts and for synthetic tractability using the existing synthesis route and isotopic starting materials. Following synthesis of 14 isobaric deuterated isotopologues, we verified that their chemical purity matched the existing reagents (>98%) and characterized isotopic purities & interferences by direct infusion MS. BSA digest labeled with a subset of the TMTpro reagents was analyzed using a targeted MS/MS acquisition method to evaluate chromatographic shifts between peptides labeled with the deuterated and non-deuterated tags and to determine the impact on quantitative metrics. HeLa digest samples were also labeled with higher multiplex TMTpro reagents to determine appropriate instrument parameters, evaluate peptide identification rates, and assess quantitative performance. An Orbitrap resolving power (RP) of ≥90K was sufficient to baseline resolve the reporter ions differing in mass by 3 mDa in MS/MS spectra, while TurboTMT acquisition at RP 45K permitted distinguishing reporter ions with greater instrument duty cycle efficiency to realize greater proteomic depth. Our preliminary LC-MS/MS data using 18-plex and 32-plex TMTpro reagents demonstrate comparable

labeling efficiency, peptide identification rates, and quantitative performance with fewer missing values for the expanded reagent set.

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Targeted DESI mass spectrometry imaging to visualize isobaric low abundance compounds and isobaric/isomeric lipids.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Desorption electrospray ionisation (DESI) technique ionizes small molecules such as drugs and lipids efficiently directly from surfaces such as tissue sections, without any significant sample preparation step.
Tandem quadrupole (TQ) MS are renowned for their sensitivity and specificity in targeted applications using multiple reaction monitoring (MRM) mode of acquisition. When implemented with a DESI mass spectrometry imaging workflow, spatial localisation of drug molecules and their metabolites at low concentration, such as therapeutic dosage, can be easily visualized. Furthermore, by monitoring diagnostic product ions, it is possible to image isobars/isomers molecules, in particular endogenous lipids and low abundant eicosanoids.

Methods

Using this approach, we have implemented targeted MRM DESI MSI methods using the DESI XS source mounted on the Xevo TQ Absolute mass spectrometer allowing the determination of the distribution of low abundant and/or isobaric and isomeric compounds from brain tissue sections. Organs were immediately frozen in liquid nitrogen and stored at -80°C until sectioning at 18 µm using a cryostat. The novel DESI High-Performance sprayer (HPS) was used for improved sensitivity, DESI spray focus, robustness and ease-of-use. DESI spray conditions were set at 2 µl/min, 95:5 MeOH: water v/v and the N2 nebulising gas pressure was set at 10 psi. The samples were imaged at 25 µm pixel size at different acquisition speeds.

Novel Aspect

Differential localisation of isomeric lipids using a targeted DESI TQ imaging workflow for sensitive and fast MSI.

Preliminary Data or Plenary Speaker Abstract

Phosphatidylcholines (PCs) are a major component of biological membranes and are highly abundant. They also ionize very efficiently by DESI with proton, sodium and potassium cations when analysed directly from tissue section in positive ion mode. This convolutes the full MS spectrum and identification as multiple peaks within a 1 Da window can correspond to various PCs containing different cations. When subjected to MS/MS, the headgroup of the PCs fragments easily and fragment ions are diagnostic to the type of cation with fragment (m/z 184 belonging to protonated PC, m/z 147 is diagnostic to sodiated PC and m/z 163 to potassiated PC).
By setting the MRM transition using the headgroup cation specific ion, it was possible to display the different distribution of two isobaric lipids at m/z 782.55: sodiated PC [34:1] and protonated PC [36:4] as well as isobaric lipids m/z 806.55: sodiated PC [36:3] and protonated PC [38:6].
In negative mode, phospholipids produce intense fragments corresponding to the fatty acid (FA) chains R1COO- and R2COO- or the loss of the FA. Isobaric lipids at m/z 788.5 PE (18:1_22:6) and PS (18:0_18:1) were imaged with respectively MRM transitions 788.5 > 327.3 and 788.5 > 419.25.
Moreover, a couple of examples of isomeric lipids at m/z 750.5 PE(P-18:0_20:4) and PE (P-16:0_22:4) and at m/z 766.6 PE (18:0_20:4) and PE (16:0_22:4) were successfully imaged and showed different localisation in the rat brain tissue section.
Finally, in this study five isomer HETE compounds were monitored and imaged simultaneously using DESI XS Xevo TQ Absolute in MRM mode with specific diagnostic product ions for each HETE: m/z

319.2 > 115 (5-HETE), m/z 319.2 > 155 (8-HETE), m/z 319.2 > 167 (11-HETE), 319.2 > 179 (12-HETE) and 319.2 > 175 (15-HETE). All five HETEs were easily detected with signal to noise (S/N) above 1:5.

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Optimisation of Multi-omic Spatial Analyses of Endometriosis FFPE Tissues using MALDI MSI

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Endometriosis is a complex, chronic condition characterised by growth of benign endometrial-like glands or stromal lesions outside the uterine cavity. Approximately, 11.4% of reproductive-age Australian women are diagnosed by age 40-44. Despite research, the origins, pathogenesis, and mechanisms of endometriosis remain poorly understood, leading to a lack of non-invasive diagnostic biomarkers. Mass Spectrometry Imaging (MSI) is a technique allowing spatial biomolecule identification within tissue sections on histology slides. Utilising MSI, we investigated endometriotic lesions, uncovering possible molecular pathways and disease mechanisms.

Methods

We optimised published MSI methods for analysing 'omic profiles (metabolomic, lipidomic, proteomic, and glycomic) of reproductive tissues and lesions using biobanked formalin-fixed paraffin-embedded (FFPE) samples. Digestive enzymes (trypsin/PNGaseF) and MALDI matrices (cyano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid, 9-aminoacridine, etc) were deposited using a HTX TM-Sprayer. We then assessed 'omic MSI data obtained from human endometriosis -tissues paired with corresponding histological images against a healthy endometrial tissue microarray (with cores representing each endometrial cycle stage) to gain insight into and characterise molecular workings of the disease. Tissue samples were analysed using a Bruker SolariX 2XR MALDI-FT-ICR MS system then were processed using SCiLS Lab 2024 (Bruker) using statistical methods such as, receiver operating characteristic (ROC) curves and linear discriminant analysis (LDA) classification models.

Novel Aspect

Research highlights molecular differences and potential endometriosis biomarkers, marking the first published investigation using MALDI MSI and a multi-omic approach.

Preliminary Data or Plenary Speaker Abstract

Preliminary data suggests the discovery of protein peptides and N-glycans capable of discriminating between lesion and non-lesion ti¬ssue regions. Notably, 15 peptides distinguished endometrial cycle phases on the microarray, with elevated levels during the mid- and late-secretory phase (ROC AUC values ≤0.25, ≥0.75). Eight co-localised peptides within endometriotic lesions, present in up to 66.67% of lesions, hold promise as potential biomarkers upon further validation. No single peptide could alone pinpoint lesion sites highlighting disease heterogeneity.
Similarly, our N-glycome research identified 15 differential N-glycans across healthy endometrial cycle phases on the microarray, with increased levels during the mid-secretory phase, correlating with the window of implantation. Increased glycosylation during the secretory compared to proliferative phase was mimicked in endometriotic lesions. We detected 16 N-glycan suggestive peaks predominantly in lesion sites, with some exclusive to lesion sites. Classification models using significant peptides and N-glycans showed success in a small sample cohort, suggesting future validation with a larger cohort.
These findings hint at a potential similarity between endometriotic lesions and endometrial profiles measured during the secretory phase warranting further analysis. While further investigation of identified peptides and N-glycans are needed, combined with annotation and validation using

orthogonal tandem mass spectrometry (currently underway), they hold some promise of becoming tools in understanding endometriosis.

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Development of a Robust Protocol for Profiling Peptides by MALDI-MSI

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

MALDI-MSI has emerged as a powerful tool for the spatial mapping of a wide range of molecules in a variety of tissue types, including fresh frozen tissues, FFPE tissue, and tissue microarrays to aid in the identification of potential biomarkers. Here we describe the development of a robust protocol for profiling the spatial distribution of peptides within fresh frozen and FFPE tissues. Specifically, varying trypsin digestion time and relative humidity during digestion were optimized. Relative humidity was modified during trypsin digestion through the addition of different salts. The selection of optimized conditions involved assessing the number of identified peptide IDs in METASPACE and evaluating analyte delocalization by examining off-tissue signals within SCiLS.

Methods

FFPE and fresh frozen tissue were sectioned at 10 µm thickness. FFPE slides were heated, dewaxed, and rehydrated for heat-induced antigen retrieval. Fresh frozen tissue only underwent ethanol washes. Trypsin was applied to the slides using a TM-Sprayer. Trypsin incubation times tested were 2, 6, and 16 hours. In addition, NaCl, KCl, and K2SO4 were introduced to humidity chambers to saturation to change the relative humidity at 37 °C. CHCA matrix was sprayed onto the slides. Bruker timsTOF Flex MALDI2 was operated in positive ion mode. Imaging data was imported into the SCiLS lab software, exported to imzML, and the resulting files were then submitted to METASPACE for data processing using a custom database generated by LC-MS/MS of bulk tissues.

Novel Aspect

The optimization of MALDI-MSI sample preparation methods for the detection of peptides in fresh frozen and FFPE tissue.

Preliminary Data or Plenary Speaker Abstract

MALDI-MSI is becoming an increasingly popular method in clinical research with many interested in mapping where particular proteins are present within their sample of interest. Here we examined both FFPE and fresh frozen tissue to optimize the sample preparation protocol to maximize the number of peptide annotations found as well as reduce the analyte delocalization. This optimization was achieved through testing several different trypsin digestion times and through varying the humidity levels during trypsin digestion. Trypsin incubation times tested were 2, 6, and 16 hours. In addition, NaCl, KCl, and K2SO4 were added into the humidity chambers to maintain a relative humidity of 75%, 82%, and 96% respectively at 37 °C. Chambers with each solution were set up in an incubator the night before the incubation experiment to ensure the relative humidity remained constant during digestion.
Several different tissue types were also tested throughout this optimization process to characterize those matrix effects as well. Fresh frozen mouse brain and kidney were tested and FFPE mouse kidney and human prostate and eye were examined. The number of confident annotations were found to be increased by 20% as the trypsin digestion time increased. Increasing the humidity levels in the incubation chamber also greatly helped with reducing analyte delocalization. Correlations of an on and off tissue signal were examined in SCiLS for each tissue and analyte delocalization was found to be minimized the most with the addition of K2SO4 at 96% humidity.
Peptide MALDI-MSI annotation poses inherent challenges, and in this study, we employed utilization of custom databases through METASPACE for efficient data processing. Utilizing custom databases derived from the peptide lists obtained through a standard LC-MS/MS proteomics with extracted peptide chemical formula through Skyline, we successfully identified peptides with confidence within the dataset.

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Multiplexed targeted imaging of intact proteins in tissue by multi reflecting time of flight (MRT) MALDI-IHC

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

A promising mass spectrometry imaging technique has recently become available known as multiplexed MALDI-immunohistochemistry (IHC), whereby photo-cleavable peptide tags attached to antibodies are bound to tissue prior to MALDI imaging. These tags are then released prior to MALDI imaging and provide localisation information for the original protein. Typically, these proteins have a molecular weight outside of the instrument mass range and would traditionally have required on-tissue digestion prior to analysis which can reduce localisation and specificity.
MALDI-IHC is shown to yield results similar to traditional fluorescence immunohistochemistry, but with the advantage of allowing superior multiplexing on a single sample image. Here we demonstrate the compatibility and advantages of this technique on a SELECT SERIES™ MRT mass spectrometer.

Methods

Sections of Human Tonsil (Ambergen) and Human Kidney (ccRCC ISUP grade 3) FFPE tissues were prepared in accordance with the AmberGen MALDI-HiPLEX-IHC Miralys™ Imaging laboratory Workflow user guide (Control number v173(J)) protocol. The tonsil control sections were purchased pre-stained, the kidney sections were stained in-house. The samples were analysed using a SELECT SERIES MRT mass spectrometer, in positive mode, with a mass range of 50-2400. Due to the tags small mass distribution, a fixed quad setting of 1000 Da was set. The laser repetition rate was 2 kHz with a scan speed of 10 s/s. Images were acquired at 50 µm pixel size and 20 µm pixel size with a laser focus setting of 4.0 mm and 5.8 mm respectively.

Novel Aspect

Multiplexed MALDI IHC performed on a multi reflecting time of flight mass spectrometer.

Preliminary Data or Plenary Speaker Abstract

Here we demonstrate full compatibility of MALDI-IHC analysis on a SELECT SERIES MRT and highlight potential advantages over a standard OA-MALDI-Tof. The high mass accuracy (>500 ppb) allows for unambiguous identification of released tags, easily identified from endogenous signals. Coupled with the high mass resolution (>200,000 FWHM) which significantly reduces potential signal overlap with endogenous signals of similar mass. This also provides higher confidence in protein localization information compared to traditional immunofluorescence, where tissue auto-fluorescence (background fluorescence of molecules in the tissue) can cause high interfering signals and result in non-specific detection.
Traditional immunofluorescence histochemistry is typically limited to 2-5 markers per tissue section, this is due to spectral overlap in excitation and emission wavelengths, in contrast MALDI-IHC is able to image >40 markers per tissue section and is only limited by the number of available masses and availability of tags for all proteins of interest. Conversely, MALDI-IHC detection does take longer than traditional fluorescence methods, and once the tag is consumed the data cannot be re-acquired.
From the tissue sections analyzed the bio-localization of the tags was consistent with the expected biology of the tissue. Excellent signal strength was achieved with both the 50 µm and 20 µm pixel sizes suggesting that good data could be achieved with smaller pixel sizes or faster analysis times if desired.

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Spatially Mapping Isomeric Lipids by Ozone-Induced Dissociation in the HCD Region of on an Orbitrap Fusion

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

In biology, it has been estimated that there are more than 180,000 possible lipids, including isomers which are composed of varying fatty acyl chains, unique sites of unsaturation or double-bonds (db-isomers), and differing esterification positions of fatty acyl chains (sn-isomers). Most recently, these structural isomers have been successfully elucidated by the implementation of ozone-induced dissociation (OzID) for db-isomers, and consecutive collision-induced dissociation (CID) and OzID (CID/OzID) for sn-isomers. Previous implementations of OzID on hybrid Orbitrap systems were limited by the amount of ozone that could be introduced into the linear ion trap. Here, we have overcome this by introducing OzID in the HCD region of an Orbitrap Fusion and realised a 10-100 fold reduction in the ozone exposure time required.

Methods

Mouse brain tissue was sectioned (12µm) onto indium-tin oxide (ITO) glass slides and stored in a -80°C freezer until use. Slides were desiccated for 30 min and then sprayed with sodium acetate dissolved in methanol/chloroform (2:1) using a TM-Sprayer (HTX Technologies). Subsequently, 2,5-dihydroxyacetophenone (2,5-DHA) matrix was deposited using an in-house sublimator where slides were cooled to 10°C and matrix was heated to 140°C for 5 min. Following sublimation, the 2,5-DHA matrix coated slide was recrystallised at 50°C for 90 sec. Then, sn-isomers were spatially mapped and resolved using CID/OzID on an Orbitrap Fusion (Thermo Scientific) with an atmospheric pressure (AP)-MALDI source (MassTech) that was modified to enable the introduction of ozone into both the linear ion trap and HCD regions.

Novel Aspect

Introduction of OzID in the HCD region of an Orbitrap Fusion increases the throughput of lipid sn-isomer MALDI-MSI.

Preliminary Data or Plenary Speaker Abstract

MALDI mass spectrometry imaging (MSI) of lipid sn-isomers using CID/OzID requires the formation of alkali adducted lipid ions to produce the required MS2 headgroup loss fragment. Prior to MALDI-MSI, the amount of sodium acetate deposited onto the tissue before matrix application was optimised to yield the highest intensity for [M+Na]+ ions.

Ozone introduction into the HCD region allows for significantly higher density of ozone compared to the linear ion trap and this was utilised to enable more efficient OzID reactions. For mouse brain tissue, the [PC+Na-183]+ fragment formed by CID was mass-selected in an MS3 experiment and allowed to react with ozone either in (i) the HCD ion routing multipole where the reaction time was limited by the transit time though the ion guide (several milliseconds); or (ii) the linear ion trap (up to 500 milliseconds). Notably, CID/OzID product ion yields for the HCD reaction of several milliseconds were equivalent to the several hundred millisecond reaction time in the linear ion trap. As such HCD-enabled OzID significantly increases the throughput of CID/OzID for lipid sn-isomer MALDI-MSI.

Lipid sn-isomer MALD-MSI using CID/OzID in the HCD region was performed on a variety of phosphatidylcholine (PC) lipids within the mouse brain and revealed distinct spatial localisation of sn-isomers, consistent with earlier reports.

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MS Imaging-Based Prediction of Immune Cell: Bringing Real-Time Intraoperative Prognosis to Life

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Glioblastoma is a highly heterogeneous and infiltrative form of brain cancer associated to a poor outcome with limited efficiency of therapies. The extend of the surgery is known to be related to the patient survival. Reaching an accurate diagnostic and prognostic, a better understanding of the TME by the time of the initial surgery is therefore paramount in management of glioblastoma. Indeed, patient prognosis is predictable through the measurement of immune cells infiltration in TME. It's achieved post-operatively by immunohistochemistry (IHC) which is time-consuming and limited. We thus investigated the development of an immunoscoring based on SpiderMass-MSI which could be performed in real-time during the surgery, to predict the distribution of each cell type within pixels to develop personalized surgery.

Methods

The SpiderMass enables real-time in vivo analysis with minimum invasiveness. The technology uses mid-IR to obtain the resonant excitation of water which is in large excess in the tissues. Different immune cell populations (Macrophages vs. Lymphocytes) were analyzed from blood samples using SpiderMass after cell sorting. Classification models were built from the cell population MS spectra and the discriminative lipid markers identified. In addition, a combination of supervised and unsupervised ML and AI-explained algorithms was used to find robust biomarkers. Interestingly, a LightGBM model was built to predict the ratio of the different cell populations for each pixel of different image from the glioblastoma patient tissues (both favorable and unfavorable prognosis).

Novel Aspect

Prognosis by ambient Mass Spectrometry Imaging.

Preliminary Data or Plenary Speaker Abstract

The analysis of various immune cell populations and sub-populations directly from cell plates compared with a cancer GBM cell line, reveals distinct molecular profiles. This facilitates the development of a classification model through supervised machine learning approache. Discriminative markers of the different cells were then identified thanks to MS/MS. For example, M1-like macrophages are discriminated by m/z 818.65 and m/z 846.65 which correspond to GlcCer d40:1 (d18:1/22:0) and GlcCer d42:1 (d18:1/24:0) and M2-like by a significative increase in relative abundances of m/z 819.55, m/z 841.55, m/z 843.55 and m/z 867.55 identified respectively to be different PG and a PI. Subsequently, SpiderMass imaging is employed to analyse different tissues from glioblastoma patients with varying overall survival rates. SpiderMass imaging was performed at 2.6 pixel/s in microprobe mode thanks to a robotic arm which moves the fibered laser probe above the tissue to be imaged. Immunoscoring is then conducted by leveraging LightGBM from the MS image data, enabling the prediction of cell population ratios (including cancer and diverse immune cells) in each pixel. When applied to glioblastoma, the predicted cell ratio align with established immune cells ratios in these tissues and are corroborated through cross-validation by MALDI-IHC. Furthermore, these ratios correlate with patient's overall survival, allowing the description of a prognosis score by evaluating the M1 versus M2 or macrophage versus lymphocyte immune cell ratios. This breakthrough enables real-time prognosis assessment for patients through the direct integration of SpiderMass technology during surgery. Same type of results are also obtained by using the SpiderMass for the analysis of metabolites.

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Integrated mass spectrometric investigation of eucalypt chemistry.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

There are over 800 species of eucalypts, encompassing a variety of bioactive chemistry that is unique to subgenera within the broader group. Several natural products isolated from the flowers are known to exhibit antimicrobial and insecticidal bioactivity and likely play ecological roles to aid in the host plants survival.
There is lacking research on the localisation of these natural products. Current methods limit spatial information to which part of the plant is extracted. To ascertain specific localisation for compounds of interest, MS imaging can be a valuable tool.
Additionally, the diversity of these compounds across the eucalypts may also hold value as taxonomic differentiation, and chemometric analysis of plant phylogeny is coming more available through LC-MS and molecular network mapping.

Methods

Here, both MALDI and DESI MSI were used to investigate the localisation of bioactive natural products within eucalypt flowers. Due to the delicate nature of the samples, emphasis was placed on sample preparation and method development. Histological sections were sliced at 20µm. Various embedding media, matrices, and ionisation modes were compared.
Through a wider lens, LC-MS and MS/MS were also applied to a comprehensive taxonomic representation of the eucalypt group to show patterns in chemistry across various eucalypt genera and sub-genera. Molecular network mapping can be applied and used as an overlay to compare with current molecular phylogeny research.

Novel Aspect

Using multiple MS techniques to characterise chemical diversity within the eucalypts, whilst demonstrating and advancing MSI applications in plant biochemistry.

Preliminary Data or Plenary Speaker Abstract

Images acquired by MSI revealed newly isolated natural products were compartmentalised in specific regions of eucalypt flowers. Notably, new phloroglucinols isolated in this study, were found to be in oil glands and areas associated with pollination. Additionally, unknown ions displayed in the data showed to be highly concentrated in pollen regions. The m/z values of the unknown ions were targeted using LC-MS in crude extracts and image-guided elucidation successfully led to the isolation of both new and known compounds using HPLC and NMR.
The successful histological preparation, achieved through the exploration and refinement of methods specific to delicate, stamen-abundant flowers, contributes to the developing research on MSI particularly in the field of plant biochemistry.
The use of LC-MS with MS/MS is proving to be a comprehensive fingerprinting technique for demonstrating chemical profiles across plant groups. The taxonomic placement of species within the eucalypts have been hotly debated, however chemical diversity across the group supported by this study may be used to support current taxonomic division by providing detailed fragmentation data and comparing across molecular network mapping and current phylogeny research.

Mass Spectrometry Imaging of the Lipid Distribution in Osteoarthritic Human Knee Cartilage

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Osteoarthritis (OA) is a degenerative disease of the joints that is estimated to affect 7% of the global population. The characteristic degradation of joint cartilage leads to pain, stiffness, swelling, and a loss of joint function, resulting in disability, lost productivity, and substantial healthcare costs.

Lipids help maintain cartilage integrity by contributing to joint lubrication and cushioning, chondrogenesis, chondrocyte viability, matrix synthesis, and cell signal transduction; however, much is still unknown about changes in the spatial distribution of lipids within the osteochondral unit during OA progression. Herein, we describe an MSI approach to mapping lipid, and other biomolecule, distributions in sections of human knee cartilage tissue with varying levels of OA severity.

Methods

Femoral condyle samples were collected from tissue recovered during total knee arthroplasty procedures. The degree of OA severity in these samples was classified using the modified Mankin score. Ion images were acquired using both matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) and time-of-flight secondary-ion mass spectrometry (ToF-SIMS) from tissue samples sectioned onto cryofilm. Lipid classes and subtypes within non-sectioned cartilage were also identified using shotgun lipidomics.

Novel Aspect

The combination of ToF-SIMS and MALDI-MS to co-register images of both low- and high-mass molecules in the osteochondral (bone/cartilage) unit.

Preliminary Data or Plenary Speaker Abstract

The utility of ToF-SIMS in mass spectrometry imaging of tissue sections is its ability to achieve spatial resolutions beyond those currently available with other ion-imaging techniques. It is also generally better suited than these techniques at mapping the distributions of low-mass molecules. Both qualities were exemplified by the observation of bone fragments (from signals characteristic of hydroxyapatite ions), ca.10 µm–20 µm in size, in the deep/radial zone of OA cartilage tissue.

On the other hand, the high energy of the incident ion beam in ToF-SIMS produces fragmentation within the sample, especially of large organic molecules, and a concomitant reduction in signal intensity. Consequently, the only signals observed that could be unambiguously assigned to lipid species are those characteristic of phosphocholine, which is the headgroup in both phosphatidylcholine (PC) and sphingomyelin (SM) lipids. In non-OA samples these mostly encircled cartilage lacunae, whereas in OA samples, the relative areas of PC coverage increased, leading to the appearance of continuous regions measuring up to 200 µm. An increase in the levels of PC lipids in OA tissue was also observed in the shotgun lipidomics results.

MALDI-MS produces much less sample fragmentation than ToF-SIMS, thus yielding intact lipid species, but with a diminution in the achievable spatial resolution. Maps of several species from the plasmenylethanolamine (PE-O), phosphatidylinositol (PI), and phosphatidylserine (PS) lipid classes showed higher signal intensity within OA cartilage than non-OA cartilage. Shotgun lipidomics data for these also showed a greater abundance in OA tissue.

The general accumulation of lipids observed in OA tissue is attributed to an increase in lipogenesis, coupled with impaired lipid transport and catabolism.

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Utilising matrix adducts and glufib peptide for lock mass calibration of MALDI-MSI

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Spatial proteomics via matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) offers a promising avenue for enhancing our comprehensive understanding of biological systems in health and disease. MALDI-MSI enables direct detection and spatial mapping of analyte within tissues, preserving spatial context for unbiased analysis of heterogeneous samples. However, accurate mass identification poses a significant challenge in spatial MSI due to mass shifts during data acquisition. Inclusion of lock masses at each pixel enables accurate calibration throughout and between the imaging experiments. Such thorough identification of masses would enable accurate protein annotation and biological interpretation. To address this, our group has focused on optimizing the inclusion of lock mass-based calibration with each pixel during data acquisition for peptide-based spatial MSI.

Methods

We utilised peptide-mix standards (Bruker) as analytes. A 2 µL spot of the peptide-mix was spotted on ITO coated slide and sprayed with α-CHCA using HTX M3+ sprayer (HTX imaging). The α-CHCA 10 mg/mL in Acetonitrile: Water (7:3) was sprayed with 7, 10, 15, and 20 passes, to achieve different matrix densities. Next, we tested α-CHCA spiked with heavy glufib peptide achieving a final concentration of 250 fM - 1000 µM on peptide-mix spots. Further, the optimized settings were applied to fresh-frozen brain and formalin-fixed lung tissue sections. Data acquisition was performed using a Solarix 2xR-7T-FTICR mass spectrometer (Bruker) in a positive mode for m/z 365-5000, spatial resolution 50µm. The statistics were run graphpad, R, compass-data analysis and Scils-lab (Bruker).

Novel Aspect

We introduce the utilization of lock-masses from the sample for each spot-pixel calibration addressing a critical need in spatial MSI.

Preliminary Data or Plenary Speaker Abstract

Our results demonstrate that spraying 10 mg/mL α-CHCA, with 10 passes, achieves a matrix density of 2.1µg/mm³, allowing reliable and confident identification of adducts 379.0925 m/z [M+H]+ and 401.0744 m/z [M+Na]+, and monoisotopic peaks for 6 peptide standards from the peptide-mix, including Bradykinin (757.3992 m/z), Angiotensin-I (1296.6848 m/z), Angiotensin-II (1046.5418 m/z), Substance P (1347.7354 m/z), and Bombesin (1619.8223 m/z). Most no. of peptide-mix standards were identified with matrix deposition with 10 passes - 2. 1µg/mm³ compared to 7 passes - 1.5 µg/mm3, 15 passes -3.230 µg/mm3 and 20 passes - 4.30 µg/mm3. This was established by measuring peak area/intensity, full width half-maximum (FWHM), signal-to-noise ratio (S/N) and error ppm for detected m/z from peptide-mix standards and α-CHCA adducts. Our results shows that the peak intensities for three commonly identified pep-mix standards across all passes - Bradykinin ($p < 0.0011$), Angiotensin-I ($p<0.0001$), and Angiotensin-II ($p<0.0151$), was significantly higher in 10 passes compared to 7 passes. We observed significantly higher S/N ratio for 10 passes of matrix density compared to the 7 passes; Bradykinin ($p<0.0007$), Angiotensin-I ($p<0.0001$), and Angiotensin-II ($p<0.00221$). The peak intensities for same three peptide-mix was higher for 10 passes compared to 15/20 passes of matrix depsoition. Further, we didn't observe any change in FWHM values across 7, 10, 15, and 20 passes of matrix deposition. Additionally, in a separate experiment included heavy glufib peptide (1570.6768 m/z) in the reference mass list, combined with α-CHCA adducts for pixel data calibration during acquisition showed optimum signal intensity and reliable calibration at 500nM for glufib peptide. The matrix-optimized settings applied to optimise biological samples show that 98% of spots in 10 passes were calibrated using lock mass -401. 0774 m/z and 1570. 6768 m/z,

whilst only ~60% spots were calibrated with 7 passes of matrix deposition evaluated using our in-house R-based package.

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Improving workflows of quantitative elemental bioimaging using optical profilometry

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Modern laser ablation – inductively coupled plasma - mass spectrometry (LA-ICP-MS) instruments have vastly improved acquisition times for quantitative two-dimensional elemental bioimaging of thin biological specimens. It is a mature technology with an ever-increasing application potential in medical, biological, clinical, forensic, and environmental sciences. However, precise and accurate quantification is a persistent challenge due to instrumental drift, matrix effects, and sample thickness variations of disparate cell populations and densities, or irregular and inconsistent cutting procedures.
Typical analytical workflows remove all tissue from the sample substrate, usually glass microscope slides. Effective methods to overcome instrumental drift and matrix effects are well developed, however, specimen thickness variations and cutting artefacts are usually ignored or presumed non-significant contributors to method statistical uncertainties.

Methods

We propose a novel approach to minimise contributions of statistical method uncertainties associated with calibration standards and specimen thickness variations and anomalies by introducing volume normalisation to the analytical workflow using optical profilometry.
Ablated volumes per voxel were measured to normalise differences of ablated mass by calculation of the volume of standard removed during calibration post-ablation, and pre-ablation acquisition of detailed maps of sample thickness and surface topography. This approach was cross-validated by non-contact mode atomic force microscopy (NC-AFM) measurements.

Novel Aspect

Ablation volume normalization enhances precision, accuracy, and reliability of quantification, addressing final challenges in absolute quantification workflow for tissues.

Preliminary Data or Plenary Speaker Abstract

Our preliminary data demonstrates that integration of ablation volume normalisation improved the precision, accuracy and reliability of quantification, particularly for heterogeneous tissue structures. This integrated approach is the first report that overcomes the final challenge of absolute quantification workflows to advance our understanding of elemental distributions in biological samples, ultimately facilitating discoveries of hidden biochemical pathways where elements are intimately involved, such as cancer pathogeneses and regenerative medicine.

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On-tissue chemical derivatization of carboxylic acids for MS imaging

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Various types of carboxylic acids are present in the tissues of living organisms. In particular, the intestinal tract is rich in food-derived long-chain fatty acids and volatile short-chain fatty acids produced by microorganisms. Conventional MALDI-MSI using organic matrices has low detection sensitivity for carboxylic acids, making it difficult to determine the distribution of endogenous substances. A method for condensing N,N,N trimethyl-2-(piperazin-1-yl)ethane-1-iodoamine (TMPA) and carboxylic acid on tissue sections has been reported, but it used reagents with a risk of explosion. Therefore, we investigated a method to improve reactivity by adding an organic amine to the reaction system and to detect carboxylic acids on tissue sections with good reproducibility using an automated system.

Methods

10-µm-thick fresh frozen sections of murine small intestine and cecum were prepared and dried in a cryostat chamber at -20°C. Derivatization reagents, containing TMPA (2 mM), HATU (2 mM), and 4-methylmorpholine (2mM) in acetonitrile were applied to tissue section by iMLayerTM AERO.
The derivatization reagent coated tissue sections were incubated in saturated acetonitrile gases at room temperature for 4 h. These were coated with the 1,5-Diaminonaphthalene (DAN), 9-aminoacridine (9-AA), 2,5 dihydroxybenzoic acid(DHB), and α-Cyano-4-hydroxycinnamic acid (CHCA) by sublimation. MSI analysis was performed using the iMScope QT atmospheric MALDI equipped with an optical microscope and the LCMS-9030 (Shimadzu). Tissue sections were stained with hematoxylin and eosin after MSI acquisition. Data analysis was performed using the IMAGEREVEALTM MS (Shimadzu).

Novel Aspect

We have developed an automated method for MS imaging to visualize intrinsic carboxylic acid present in frozen tissue section.

Preliminary Data or Plenary Speaker Abstract

Short- and long-chain fatty acids in murine intestine and intestinal contents were successfully derivatized by the automated on-tissue derivatization method and visualized by MS imaging. The basic derivatization was performed according to the methods of previous publication (Sun et al., Analytical chemistry 2020.92, 12126). We modified the composition of the reaction solution by adding 4-methylmorpholine, an amine that does not react with carboxylic acids to N,N,N trimethyl-2-(piperazin-1-yl)ethan-1-aminium iodide (TMPA), which has quaternary ammonium group in the presence of a condensation reagent 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b] pyridinium 3-Oxide Hexafluorophosphate (HATU). Since the short-chain carboxylic acids are highly volatile, tissue sections were thaw mounted to ITO glass slides, then kept in a cryochamber at -20°C untill on-tissue derivatization. We applied matrices by sublimation, to avoid diffusion of target compounds. Using this technique, short-chain fatty acids, which would otherwise volatilize under vacuum, were converted to nonvolatile quaternary amines, which were successfully detected by MALDI-MSI at 10 micrometer resolution.

We compared the detection sensitivity of four different matrices deposited on slides after on tissue derivatization. The signal for TMPA-bound carboxylic acids were detected in all matrices used, among them, especially good results were obtained when CHCA and DAN were used; when CHCA was used,

not only TMPA-bound fatty acids, but also endogenous amines and cholesterol were detected simultaneously.
In colon tissue sections, butyrate-TMPA and propionate-TMPA were detected in the intestinal contents and lactate-TMPA in the smooth muscle region; long-chain fatty acids bound to TMPA were detected in the colonic contents. In the small intestine, lactic acid and long-chain fatty acids bound to TMPA were detected in the villi. Cholesterol (m/z 369.351) was strongly detected in the intestinal epithelial region of the colon and small intestine when CHCA and DHB were used as matrices.

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Evaluation of plasma polymer coating of Indium tin oxide (ITO) slides in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI)

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

MALDI-MSI is hampered by poor ionisation of analytes. Multiple advancement have been made to improve this by modifying each step of the sample preparation and data acquisition. As the ionisation of analytes correlates with their ability to co-crystallize with the matrix and this process is dependent on wettability, we decided to modify the wettability of the the commonly used ITO slide to modify which ions can be detected in MALDI-MSI experiment.

Methods

Plasma polymer deposition on ITO slides followed by MALDI-MSI of mouse brains

Novel Aspect

Surface modification of ITO slide for MALDI-MSI

Preliminary Data or Plenary Speaker Abstract

Here, we report the development of a sampling probe by modifying the wettability of ITO coated glass slides, which are widely used for tissue analysis, using plasma polymer coatings. These plasma polymers are stable under vacuum and during the ionisation process. However, modification of the surface had minimal impact on the signal/noise ratio observed of peptides, proteins, and lipids in profiling experiments, in which a small volume containing the analytes was directly spotted on the surface. In contrast, when analysing fresh frozen murine brain sections, the different surfaces influenced the number of ions observed in the mass spectrometer. For example, using a very hydrophobic surface modification increased the signal/noise ratio for more than 60 putative phospholipids up to 25-fold, when compared to uncoated ITO slides. In fact, previously not-detected analytes such as name them PE(40:4) and PS(40:5) were easily detected in negative mode . Plasma polymers are used for the first time to modify ITO slides for MALDI-MSI and boost lipid ion intensity. In summary, the modification of wettability of the ITO slide can improve data collection and aid classification data analysis .

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Sample preparation protocols for detailed MS imaging of mouse intestinal tissue by MicroGRID/MALDI-2 technology

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Intestinal tissue is interesting when studying absorption and conjugation of molecules in relation to disease but also challenging due to its fine structure and inhomogeneous morphology. We hypothesize that tissue morphology can be preserved by fixation to maintain structural and molecular integrity during MS imaging using MicroGRID and MALDI-2. We explored sample preparation protocols for handling mouse intestinal tissue, including fixation, embedding, and cryosectioning, optimization of matrices for mass profiling of different compound classes, i.e. small molecules and lipids in different ionization modes, and investigation of artifactual mass shifts caused by fixation chemistry. The challenge lies in preserving the fine details of mouse intestinal tissue morphology while maintaining the endogenous molecule profiles.

Methods

Dissected intestinal tissue from C57BL/6JRj male mice was frozen on crushed dry ice before storage at -80°C until pre-processing. Fixation was done in 4% paraformaldehyde or Methacarn solution for varying time periods at 5°C. The fixed and rinsed tissues were embedded in 3% agarose solution and kept at -80°C until sectioning. Intestines were cryosectioned at 10 µm on a Leica CM1950 cryostat and thaw-mounted on plain microscope slides. Matrices (1,5-diaminonapthalene, 2,5-dihydroxybenzoic acid, and 2,5-dihydroxyacetophenone) were sprayed on samples using a TM-sprayer. Mass spectrometry imaging analysis was performed with a Bruker timsTOF fleX MALDI-2 MicroGRID instrument at 10 µm resolution and data processing performed with Bruker SCiLS Lab and MetaboScape®. Consecutive slides were H&E stained and analysed by Olympus APX100 microscope.

Novel Aspect

Optimized protocol for fixation and embedding of mouse intestinal tissue preserves morphology as studied by MS imaging and H&E staining.

Preliminary Data or Plenary Speaker Abstract

Initial MSI analysis of unfixed fresh frozen tissue using various matrices showed a diffuse spread of molecules in the intestinal tissue indicating a damaged fine structure of the tissue. We concluded that tissue fixation, embedding, and cryosectioning was required to preserve intestinal morphology. Data obtained from Methacarn fixed mouse intestinal tissue suggested that ionization and detection of molecules in the m/z range 100-1000 and of lipids in positive and negative mode were negatively affected by fixation for 30 min, 60 min, or 3 h. Fixation of tissue with 4% paraformaldehyde improved preservation of morphology already after 10 min fixation as seen from H&E-stained tissue. This observation was supported by MSI where ion density maps showed spatial localization of molecules to the tubular structures of concentric layers in mouse intestinal tissue. Compounds supporting the evaluation of this methodology included small molecules of m/z < 1000 and lipids in positive and negative mode. Generally, fixation of tissue resulted in reduced intensity of molecules, and only for some compounds, fixation chemistry caused molecular mass shifts.
We will demonstrate that for small molecules of m/z < 1000 analysed in negative mode with 1,5-diaminonapthalene as matrix, the integrity of molecules was unchanged, but fixation came with a cost of reduced intensity. For lipids in the mass range m/z 300-2500 analysed in positive mode using 2,5-dihydroxybenzoic acid as matrix, no pronounced molecular modification was observed, however sensitivity of detected molecules was reduced as compared to unfixed tissue. We will also report results on lipid detection in the mass range m/z 400-2300 in negative mode using 2,5-dihydroxyacetophenone as matrix.

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Abstract Novel Photoionisable Derivatization Reagents for Small Metabolite Detection by Laser Post-Ionization Combined Mass Spectrometry

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Mass spectrometry imaging provides a powerful tool for the analysis of biological tissues, allowing for the simultaneous identification and mapping of hundreds of molecules within a sample. However, many analytes possess physical and chemical properties which greatly limits their ionisation efficiency. Currently this challenge is addressed though either: (i) on-tissue chemical derivatisation with fixed-charge reagents or (ii) post-ionsiation techniques.(1,2) Here we have developed a new method that combines both laser post-ionisation and on-tissue chemical derivatisation with the goal of enhancing the sensitivities of hard to ionise molecules. This approach relies on the access to REMPI-like ionsiation processes of suitable chromophores enabled by MALDI-2 and utilises derivatisation reagents that instead of a fixed-charge contain photoionisable chromophores.

Methods

Novel reagents were designed using the chromophore core of vitamin E, with the alkyl chain replaced with a reactive moiety for the selective targeting of metabolite functionalities. Initial synthetic work involved developing optimised pathways to reagents for the selective targeting of primary amine-containing metabolites through multi-step synthesis. Once synthesised and purified to a high degree, compounds were tested for reactivity with commercial standards of small metabolites, prior to on-tissue testing and optimisation on fresh-frozen murine brain sagittal sections. All samples were analysed using MALDI-2 mass spectrometry on an Orbitrap Elite mass spectrometer with a desorption laser energy below the MALDI-threshold which has been shown to enhance the yield of REMPI-produced ions.(3) Post-ionisation was achieved using a 266 nm laser.

Novel Aspect

Derivatisation reagents currently available undergo ionisation by traditional MALDI-like processes, this work enters a novel class of selectively photoionisable reagents.

Preliminary Data or Plenary Speaker Abstract

An efficient and scalable synthesis has been designed, and successfully optimised producing a O-succinimidyl carbamate-containing reagent in high purity (>98 %). Analysis by MALDI-2 found the reagent experienced photoionisation (via (1+1) REMPI) as the dominating pathway. Furthermore, on-slide derivatisation of key biogenic amines dopamine ([M]•+ m/z 471.2371), serotonin ([M]•+ m/z 494.2536), and γ-aminobutyric acid ([M]•+ m/z 421.2208) standards was successful, with target analytes experiencing a 6-, 391-, and 594-fold increase in signal intensities after derivatisation and photoionisation. Work to translate procedures for on-tissue chemical derivatisation to be utilised for tissue imaging is on-going and results will be presented. Additionally, further work to develop a wider class of reagents to deploy this approach for additional analyte classes is on-going and will be presented.

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Markedly enhanced analysis of mass spectrometry images using weakly supervised learning

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Supervised and unsupervised machine learning (ML) algorithms are routinely applied to mass spectrometry imaging (MSI) data. These algorithms have enabled large scale single-pixel characterisation, classification and regression. In many circumstances, however, analysts are faced with so-called weakly supervised problems, where ground-truth class labels exist at the image level (e.g. diseased versus healthy tissue), but not at the individual pixel level. Most of the ML algorithms typically used for MSI data analysis are not well-suited for this scenario. Here, a novel method specifically designed for weakly supervised MSI data is presented. A dual-stream multiple instance learning (MIL) approach is adapted from computational pathology that reveals the spatial-spectral characteristics distinguishing different classes of MSI images. Results from various sample systems are presented.

Methods

Several ToF-SIMS imaging data sets were acquired from multiple systems, including printed inks, mixed powders and mouse tumour tissue sections (ongoing study). In each case, samples were each labelled as one of two binary classes (e.g. black ink or no black ink, or classes of tumour tissue). The MIL approach was then applied to identified characteristic pixels associated with each of the two binary classes, using only image-level labelling (i.e. without explicit single-pixel labelling). This allowed for exploration of the spatial-spectral characteristics of each class. The tumour tissue data set (study currently in progress) focuses on breast cancer tumour tissue sections excised from mice, with or without BMP4 (a breast cancer metastasis inhibitor) protein expression, representing the binary class labels.

Novel Aspect

This is a novel approach for classifying weakly-labelled MSI data, to identify characteristic pixels without needing single-pixel labelling for training.

Preliminary Data or Plenary Speaker Abstract

A proof-of-concept of the proposed methodology was recently published.[1\ This work focused on two sample systems: ink-jet prints on paper (with/without black ink), and a mixed-powder sample (lactose and vitamin C). Results showed that the MIL method successfully (a) classified whole MSI images as one of two binary classes, (b) identified characteristic pixels associated with each class, and (c) extracted characteristic spectra and mass peaks associated with each class.
The method was able to clearly identify black ink, as well as formulation components specific to the black ink that were not visible using light microscopy. For the mixed powder sample, the method accurately identified vitamin C powder amongst the chemically similar lactose powder. Furthermore, we showed explicitly that our novel use of information-entropy regularised attention maps proved critical (for both data sets) for accurate identification of characteristic pixels, represent a significant step forward in terms of ML algorithm development.
An ongoing study focuses on breast cancer tissue excised from mice, with and without BMP4 protein expression. Early results have validated proposed workflows for collecting correlated whole slide scan H&E, scanning electron microscopy (SEM), and ToF-SIMS images, covering the entire tumour tissue section area. Acquired data will be used to further validate and apply our MIL method, while also considering an extension to multimodal scenarios. This will enable exploration and comparison of the regions in the tissue classified as important/characteristic by each imaging mode. Given our approach was adapted from computational pathology[2], it is well-suited for such a multimodal study.

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A sensitive and high coverage spatial resolved metabolomics workflow for highly heterogeneous tissue.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Mass spectrometry imaging (MSI) is a developing technique that spatially resolves the molecular composition of samples in a label-free manner. Matrix-assisted laser desorption/ionization technique is one of the most investigated ionization techniques in MSI. However, due to challenging sample preparation, rigid samples, such as teeth, bones, and plants, usually cannot be easily analyzed.

Methods

We proposed an optimized workflow for mass spectrometry imaging of rigid biological samples, such as skulls and bones, with high sensitivity and spatial resolution. We prepared an unprocessed frozen section of hard samples using a cryofilm-assisted method. Cryofilm- cryofilm-section complex was mounted on conductive indium tin oxide-coated glass with a specially designed centrifugation device for uniform adhesion of cryofilm to slides . Using N-(1-Naphthyl) ethylenediamine dihydrochloride as the matrix, spatial information of organic metabolites and inorganic content were investigated in rigid biological samples.

Novel Aspect

Our method has a higher coverage of endogenous molecules, from metal to metabolites, and is suitable for rigid biological samples.

Preliminary Data or Plenary Speaker Abstract

An abundant calcium chloride cluster signal was detected in the mineralized region of bone and absent in other compartments. Various types of glycerophospholipids were detected, including phosphatidylinositol, phosphatidylserine, phosphatidylethanolamine, phosphatic acid, phosphatidylglycerol, and corresponding lysoglycerophospholipids. small polar metabolites, such as fatty acids, glycolysis products, nucleotide phosphates, and amino acids, were also detected and imaged.

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Structural determination of small unsaturated acids isolated from Australian trees: Ozone-Induced Dissociation as a complement to traditional analyses

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Natural products analysis workflows can lead to the discovery and structure elucidation of therapeutic compounds. These routines are centered around well-established analytical tools including liquid chromatrography, high resolution mass spectrometry (HRMS), and nuclear magnetic resonance spectroscopy (NMR). NMR is the gold-standard for small molecule structure determination, with HRMS used as confirmation of the molecular formula. There are cases, however, where NMR analysis is confounded by (i) the presence of isomeric species due to incomplete chromatographic separation and/or (ii) signal shielding effects from functional groups. In these situations, novel analytical tools rooted in MS such as Ozone-Induced Dissociation (OzID) can provide complementary information towards total structure elucidation.

Methods

Ozone-Induced Dissociation (OzID) was deployed in a modified ThermoFisher Orbitrap Fusion mass spectrometer. Five fractions from HPLC-purified ethanolic extracts from the exocarp of the Australian rainforest tree Endiandra insignis were each diluted in methanol containing sodium acetate. For each fraction, sodium adducts from □□□-unsaturated 24 carbon fatty acids were liberated from solution via electrospray ionization, mass selected in the quadrupole, and sent into the HCD cell containing a mixture of 8% ozone in oxygen. Control over trapping (i.e. reaction) time out to 4000 ms was afforded through the Xcalibur Method Editor via a software patch provided by ThermoFisher. The ions resulting from this MS/MS workflow were sent to the Ion Trap for mass analysis.

Novel Aspect

OzID can complement traditional analytical tools towards complete structure elucidation in synthetic & natural products chemistry workflows.

Preliminary Data or Plenary Speaker Abstract

Three α,β-unsaturated acids and 2 ethyl esters were identified via NMR from the ethanolic extract of an Australian rainforest tree fruit (Endiandra insignis). There was some ambiguity in the NMR spectra relating to the double bond along the aliphatic chain in each: the data allowed assignment of the stereochemistry as cis but not the exact location along the chain. OzID spectra acquired for the sodiated ions from the 5 compounds revealed a mixture of carbon-carbon double bond positional isomers for each. Ozonolysis reaction times of 1 second provided adequate signal-to-noise OzID spectra for confident assignment of double bond position within the aliphatic chain, revealing the presence of up to 6 positional isomers in some of the extract fractions. Each fraction was screened for anti-inflammatory properties with at least one fraction performing well relative to the Curcumin control. It was also realized that some of the fractions promoted cell cytotoxicity, leading to testing against 2 cancerous cell lines, where modest performance was observed relative to the doxorubicin control.

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Dissociation of biomolecules by ribbon-shaped low-energy electron beams in conventional linear radiofrequency ion traps

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Electron capture dissociation techniques can also be achieved without involving magnetic fields. Ding Li and others used digital square waves to drive 3-dimensional ion traps and introduced electrons during the positive period of the square wave. When electrons are transferred to the center of the ion trap, they are first focused and then decelerated, thus realizing ECD technology.
Again, without the involvement of a magnetic field, I used a conventional RF ion trap driven by a sine wave as the ion trapping device. The electron introduction efficiency is improved by expanding the proportion of electrons successfully introduced in the high-frequency drive waveform used for electron capture and dissociation.

Methods

Positive-phase high-frequency voltage and reverse-phase high-frequency voltage are applied alternately to the strip electrode of the linear ion trap in sequence, so that there is a DC saddle point potential at the central axis of the ion trap space. The electrons emitted by the electron emission source are accelerated by the control electrode. Then it is focused by the focusing lens and enters the ion trapping space through the electron introduction slot, and decelerates to the energy range where electron capture and dissociation can occur before reaching the central axis of the ion trapping space.

Novel Aspect

Electron capture dissociation is achieved in a conventional radio frequency linear ion trap without the involvement of a magnetic field.

Preliminary Data or Plenary Speaker Abstract

The electrons emitted by the electron emission source are first accelerated by the control electrode, then focused into an electron beam by the focusing lens, and finally pass through the Y-direction strip electrode and enter the central axis position of the linear ion trap. At this time, the potential in the Y direction of the strip electrode should be the positive half axis of the sine wave, then the electrons will quickly pass through the slit on the Y strip electrode and enter the central axis of the ion trap. At the same time, in the X direction, the electrons will be repelled by the negative electrodes on both sides and gather in the middle.If the low-energy electrons at this time are not captured by multivalent ions, they may enter the linear ion trap multiple times until they diverge and cannot be used again.
The following data were calculated by the commercially available SIMION ion simulation program (Scientific Instrument Services, Inc., Ringoes, New Jersey):
1. In the X-Y coordinate system, the electron source generates 100 electrons at each point, and counts the number injected into the central axis of the RF ion trap. The results show that when the high-frequency voltage of the ion trap is between +30V and +500V, the number of electrons entering the central axis of the ion trap can reach more than 90%.
2. In the X-Y coordinate system, electrons generate an electron every 7.5° in the range of 15° to 165°, for a total of 21 electrons. The lowest electron energy injected into the ion trap at the central axis is calculated. The results show that when the high-frequency voltage of the ion trap is between +30V and +500V, the number of electrons with electron energy less than 3ev accounts for more than 95%.

Structural Analysis of Ester Lipids Using Ultraviolet Photodissociation of Their Lithium Adducts

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Lipids are a diverse group of hydrophobic and amphiphilic molecules that serve essential functions in cellular structure, energy storage, and signaling pathways. Understanding lipids is key to unraveling their complex roles in developing and progressing various diseases. Mass spectrometry with low-energy CID is a powerful technique for elucidating lipid structures, but it faces limitations in describing subtle yet crucial structural variations. CID usually fails to identify positions of double bonds in aliphatic chains of lipids. Recent advances in mass spectrometry have introduced novel fragmentation techniques, such as ultraviolet photodissociation (UVPD). Interactions of ions with UV photons lead to interesting photochemical reactions and photoproducts that can be used for the structural analysis of lipids.

Methods

Mass spectra of lipids were collected with the Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific). For UVPD experiments, mass-selected precursors stored in the linear ion trap were irradiated by pulses of 213 nm UV light. Fragments were detected in the Orbitrap operated at a resolution of 120,000. Standards of wax esters from Nu-Chek Prep (Elysian) and triacylglycerol estolides synthesized in-house were dissolved in a mixture of solvents containing lithium formate. The sample solutions were delivered using a Triversa Nanomate ion source (Advion). UHPLC experiments were performed with Dionex Ultimate 3000 LC system (Thermo Scientific), Acquity BEH C18 column (Waters), and a heated ESI source; lithium formate solution was infused post-column using a tee-union.

Novel Aspect

Description of ester group fragmentation in UVPD and use of the fragments for structural analysis of lipids.

Preliminary Data or Plenary Speaker Abstract

Activation of lithium adducts of wax esters (WE) and triacylglycerol estolides (TG-EST) in UVPD with 213-nm laser provided structurally informative mass spectra. We suggest that Norrish and Norrish-Yang photochemical reactions occur in the ester groups. Absorption of UV photons led to the excitation of carbonyl, which abstracted a γ-hydrogen from an aliphatic chain. The biradical formed in this way either fragmented (Norrish type II reaction) or recombined internally to substituted oxetan-2-ol or cyclobutane (Norrish-Yang reaction) and produced secondary fragments. In the spectra of WE, these photoreactions formed acid and alcohol chain-related aldehyde fragments and acid fragments. TG EST contain several ester groups where the photochemistry can take place. Consequently, their UVPD spectra were more complex. We investigated TG EST with four ester groups, two linking fatty acid to glycerol backbone, the third connecting glycerol to a fatty acid ester of hydroxy fatty acid (FAHFA), and the fourth in the FAHFA moiety. UVPD fragments made it possible to distinguish isomeric estolides differing in the position of the ester bond in the FAHFA unit. The differences were even more pronounced in MS3 CID/UVPD spectra with lithiated FAHFA as precursors for UVPD. MS3 CID/UVPD spectra made it also possible to distinguish sn 1/3 from sn-2 substituents on the glycerol backbone. Absorption of UV photons to double bonds provided fragments useful for deducing double bond positions. Monounsaturated lipids provided a pair of fragments differing by 24.0000 Da, which was easy to recognize in MS2 UVPD spectra of WE. As for TG-EST, these fragments were difficult to detect in MS2 spectra. However, double bond-related fragments were accessible by MS3 HCD/UVPD or MS4 CID/CID/UVPD. The practical applicability of UVPD in the structural analysis of lipids was

demonstrated by UHPLC analysis of WE from jojoba oil and direct infusion analysis of WE from vernix caseosa.

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Cross-validation of lipid structure assignment by exploiting orthogonal ion activation modalities on the same instrument.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The onset and progression of numerous pathologies, including cancer, are associated with changes in the cellular lipidome. However, detailed structural characterisation of complex lipids in biological extracts is confounded by the presence of lipid regioisomers that differ only in the relative position(s) of functional groups, such as carbon-carbon double bonds, within the molecular structure. Such isomers are often difficult to separate by chromatography, and conventional (low energy) collision-induced dissociation is insufficient to yield isomer-specific product ions. Ozone-induced dissociation (OzID) and UV-photodissociation (UVPD) are alternative ion activation modalities for distinguishing regioisomers in unsaturated lipids. Here, they are deployed on a common platform to validate the discovery of non-canonical lipids in DLD1 colorectal cancer cell lines.

Methods

Experiments were undertaken using a high-resolution Q Exactive mass spectrometer (Thermo Fisher Scientific) with direct infusion sample introduction using a robotic nano-electrospray ionisation source (Advion TriVersa NanoMate). The mass spectrometer was modified to provide both optical access and delivery of ozone to the higher-energy collision dissociation (HCD) region. UVPD was conducted using a 193 nm Excistar XS ArF excimer laser system (Coherent) while externally generated ozone (10% w/w in O_2) was introduced into the HCD cell via a PEEK restriction (127 µm x 2 m). For direct comparison, monoisotopically mass-selected lipid ions were injected into the HCD cell (NCE = 2) and subsequently subjected to OzID or UVPD prior to mass analysis in the Orbitrap.

Novel Aspect

Ozone-induced dissociation and UV-photodissociation implemented on the same high resolution mass spectrometer enables cross-validation of non-canonical monounsaturated lipids.

Preliminary Data or Plenary Speaker Abstract

Benchmarking using lipid standards was undertaken by analysis of the isomer-specific product ion pairs associated with chemical (OzID) or photochemical (UVPD) cleavage at, or adjacent to, the carbon-carbon double bond identified by accurate mass analysis. The yield of these product ions by each ion activation technique varied for dilithiated fatty acids but was comparable for monounsaturated phosphatidylcholines (PC). In addition, low abundant secondary fragmentation pathways were identified that have the potential to be misassigned as arising from double bonds in positions n-(x+1) for OzID and n-(x±1) for UVPD experiments (where n is the number of carbons in a lipid acyl chain, and x is the position relative to the methyl terminus). Mechanistic considerations provide a consistent framework for minimising false positive assignments for each modality and emphasise the benefits of cross-validation between these orthogonal approaches.

Shotgun analysis of lipid extracts from KRAS mutant isogenic DLD1 colorectal cells lines was undertaken, with targeted MS/MS spectra acquired on the [M+Li]+ cations of PC 32:1 and PC 34:1 using both ion activation modalities. De novo analysis by both OzID and UVPD revealed the presence of multiple regioisomers for abundant monounsaturated lipids PC 16:0_16:1 and PC 16:0_18:1 that

were identified to have both n-7 and n-9 double bond positions. The change in the ratio of isomer-specific product ions was found to be consistent between the two ion activation modalities providing high confidence that the changes observed between cell lines reflected significant modulation of the underlying lipid metabolism. Notably, both ion activation methods identified the presence of a n-5 isomer in the KRAS mutant cell line following false-positive filtering. Thus, cross-validation between OzID and UVPD provides high confidence in the assignments of these non-canonical lipids and point to hitherto undescribed modulation of lipid desaturation and elongation pathways in these cells.

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High-efficiency Electron Capture Dissociation of Peptides and Proteins after Collision-Induced Unfolding and Ion Mobility

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Collision induced unfolding (CIU) combined with ion mobility-mass spectrometry (IM-MS) provides valuable information about biomolecule structure. Combining CIU-IM-MS with a top-down fragmentation technique like electron capture dissociation (ECD) can provide additional information about sequence and modifications for unfolded protein structures. CIU can also improve the sequence coverage of structures otherwise recalcitrant to ECD. However, it has historically been difficult to perform electron-based fragmentation in an ion trapping device after IM separation because reaction times of tens of milliseconds are required to achieve practical efficiencies. The “fly-through” design of the ExD cell with microsecond-scale transit times makes it uniquely suited to combining with millisecond-scale IM separation. Here we leverage a new ExD cell design with increased efficiency and simplified operation.

Methods

A 6560C IM-QTOF instrument (Agilent Technologies, Santa Clara, CA) was modified with prototype ECD hardware (“ExD cell”, Agilent Technologies, Santa Clara, CA) installed after the collision cell. This modified instrument provides in-source CIU, drift tube ion mobility separation, CID and ECD capabilities. ECD efficiency and IM resolving power were optimized using a series of standard compounds including tunemix, Substance P, ubiquitin, carbonic anhydrase, and NISTmAb by adjusting gas pressure & composition and ion optics voltages. Intact protein samples under native-like and denaturing conditions were infused using an AJS source with a micro-flow nebulizer; a syringe pump was used for sample delivery. Top-down ECD data were analyzed using ExDViewer (Agilent Technologies, Santa Clara, CA).

Novel Aspect

CIU-IM-ECD using a new ExD cell design with enhanced ECD efficiency

Preliminary Data or Plenary Speaker Abstract

Nitrogen collision gas was plumbed directly into the ExD cell, resulting in an order of magnitude increase in ECD product ion S/N for 2-4+ peptides, compared to the previous cell design. The increase in efficiency is attributed primarily to improved electron thermalization and spatial distribution of reactants. Tuning the gas pressure and ExD cell voltages was necessary to optimize drift resolution, transmission, and ECD efficiency. To demonstrate the utility of combining IM separation and highly-efficient ECD for peptides, IM+ECD was performed on a mixture of 2+ and 3+ peptide isomers with aspartyl- or isoaspartyl-residues to directly differentiate the isomers and perform relative quantification. The effect of increasing gas pressure in the ExD cell was less dramatic for highly-charged (5-50+) intact proteins, because electron capture cross-section is not a major limiting factor in the detection and interpretation of structure-informative fragments for such highly-charged proteins. Primary sequence coverage and product ion S/N were similar to results obtained with the previous cell design. However, supplemental collisional activation of proteins, performed at either the exit of the inlet capillary or in the collision cell, yielded greater primary sequence coverage values via ECD, an increase in the average m/z value for detected product ions, and fewer products of electron capture charge reduction (“EC-no-D”). The relationship between protein collision cross section and ECD sequence coverage will be explored further in this work.

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Structural Analysis of Synthetic Opioids Belonging to the Fentanyl Class using Travelling Wave Ion Mobility-Mass SPECTROMETRY (TWIM-MS)

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Illicit fentanyl analogues are a growing global concern as opioid related deaths rise. Given that fentanyl analogues are readily obtained by modifying the structure of fentanyl, illicit fentanyl analogues appearing on the black market often contain similar structures, making analogue differentiation and identification difficult. While obtaining both precursor and product ion data during analysis is becoming increasingly valuable in fentanyl analogue characterization, due to the increasing complexity of seized drugs, so is the need for additional dimension of separation during mass spectrometric analysis. Here we provide collision cross section (CCS) measurements obtained via IM-MS to provide scientists with extra molecular descriptors that can be used to decipher complex mixtures that are otherwise difficult to deconvolute using solely HPLC-MS or GC-MS.

Methods

The Fentanyl Analogue Screening Kit and Emergent Panels 3, and 4 were obtained from Cayman Chemicals.TWCCSN2, TWCCSHe ,TWCCSCO2 values for fentanyl and 150 fentanyl analogs were determined employing TWIM-MS instrumentation: Synapt G2-Si (resolution ∼40 Ω/ΔΩ fwhm) in positive electrospray mode (ESI+). The Synapt system consists of a quadrupole orthogonal acceleration time-of-flight mass spectrometer with a mobility cell positioned after the quadrupole and between trap and transfer regions. During analysis, precursor ions underwent collision induced dissociation (CID) dissociation by incrementing the cone voltage (10, 20, 30, 40, 50 V) throughout a single run. These were used for fragmentation trend analysis of fentanyl related opioids. Theoretical CCS values were calculated using the MobCross model and were then compared to experimental values obtained.

Novel Aspect

Three different ion mobility gasses (N2, He, CO2) were implemented to obtain CCS values for 150 fentanyl analogues.

Preliminary Data or Plenary Speaker Abstract

Experimental vs Computational CCS Values
Computational work is still underway and thus no preliminary data can be provided for the comparison of theoretical vs experimental CCS values at this time, but this section will surely be completed well before the conference. Yet so far, experimental CCS values obtained using TWIM-MS correlate with published values obtained using DTIM-MS.
Fragmentation Trend Analysis
At low-collision energies 62 % of the analogues analyzed cleaved at the N-C4 bond. 33% cleaved at the N-alkyl chain (N-αC or αC-βC bond) and 5% cleaved at the piperidine ring, Fentanyl analogues with any substitution at the tertiary amine or with only one functional group at the N-alkyl chain phenyl ring resulted in N-C4 bond cleavage, while analogues with more than one functional group attached to the phenyl ring of the alkyl chain typically fragment at the N-αC bond during low-energy CID scans. The wide range of cone voltage (10-50V) implemented in this experiment was sufficient in producing stable precursor ions as well as primary and secondary product ions of structure A, B, C, D and E. Thus, we suggest implementation, if not the same, a similar collision energy profile. At 10V expect only the molecular ions to be present. At 20 V, structure A or B are commonly produced as etc…
Furthermore, positive ESI mass spectrums for fentanyl structures were compared to electron ionization (EI) mass spectrums traditionally implemented GC-MS analysis. A crucial distinction between the two ionization methods is their ability to readily produce precursor ions. ESI readily

produces precursor ions while such molecular structure is typically not observed in EI. Compared to ESI, EI makes identification of the precursor molecule complex without a spectral library and doesn't behave in a predictable pattern as ESI does where it prefers to fragment at the N-C4 and N-αC bonds.

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Real-time monitoring at high concentrations around the short-term exposure limit of toluene using ion-mobility spectrometry

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

We have developed an ion-mobility spectrometry (IMS) system that can monitor high-concentration chemicals in real time in the workplace. This IMS device performs qualitative and quantitative analyses at atmospheric pressure and so has no vacuum requirement. The device can examine worker's short-term exposure to chemicals.
This study examined the applicability of the IMS device to real-time monitoring of fluctuating high concentrations in the vicinity of the short-term exposure limit (STEL) of toluene during a simulated manual operation.

Methods

Our IMS device obtains the peak of a chemical substance overlapped with the water-cluster peak, with the observed peak being a "nominal" peak. We used a quantitative analysis technique in which a calibration curve was derived from the shift of the arrival-time spectrum of chemical substances overlapped with that of water clusters. It takes the IMS device 1 min to complete single measurement which is much shorter than the single measurement time of GC-MS (about 30 to 60 min).
In this study, toluene concentrations were measured in real time, and quantitative analysis was performed using the calibration curve technique.

Novel Aspect

We can estimate chemical substance accurately by using the nominal arrival-time shift at both low and high concentrations.

Preliminary Data or Plenary Speaker Abstract

The capability of the IMS device in real-time monitoring of the workplace atmosphere was demonstrated for a simulated cleaning operation using toluene.
As the toluene concentration approaches the limit of the ionizability, the arrival-time shift of nominal toluene spectrum asymptotically converged to a certain value corresponding to the arrival time shift of genuine toluene spectrum. No arrival-time shift was observed at concentrations less than 13.3 ppm, suggesting that the concentration of 13.3 ppm is the lower limit of quantification under the condition applied.
Toluene concentrations measured for 10 minutes at an interval of 1-min fluctuated from 20.3 to 83.6 ppm during the cleaning operation. The toluene concentration measured at the opening of the toluene container (60–80 ppm) was the highest. The toluene concentration dropped when the container was closed. The toluene concentration during the cleaning operation greatly fluctuated.
The 10-min average of toluene concentration measured by using GC–MS was 44.3 ppm, whereas that measured using the IMS was 45.8 ppm. These two average concentrations were consistent within the tolerance of 3%. The results obtained in this study suggests that the IMS device can measure chemicals in real-time with an accuracy similar to GC–MS does.

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Non-small cell Lung cancer single spheroid analysis using the cellenONE with proteoCHIP EVO 96 workflow on the timsTOF Ultra

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

For cancer treatment, common strategies are the establishment of cell cultures from cancer tissue biopsies to assess probabilities in treatment success or drug responsiveness. Typically, 2D tissue culture is performed, however, this artificial lab environment is altering inter cell connectivity, communication, and cell to cell microenvironments and hence responses to treatment as well. 3D spheroid-based cell culture simulates structural properties of solid tumors better. Single cell proteomics aims to decipher heterogeneity on cell microenvironment. Therefore, we want to assess treatment responses in small spheroids versus 2D culture derived single cells using the cellenONE proteoCHIP EVO 96 platform for small spheroid and single cell isolation, direct transfer to Evotips and proteome analysis using the timsTOF platform.

Methods

A549 lung cancer cells from 2D culture as well as spheroids, generated from 50 seeded A549 cells in a microplate and grown for one day, were treated with lipopolysaccharides (LPS). 2D cultured cells as well as small spheroids (3 – 10 cells?) were isolated into the proteoCHIP® EVO 96, directly lysed and digested using the cellenONE platform. Samples were transferred by centrifugation onto Evotips (96 tip box). Prepared boxes were placed on top of an Evosep One and analyzed using a Whisper 40 SPD method. Peptides were separated on a 15 cm Aurora Elite C18 column (IonOpticks) and were eluted into a timsTOF Ultra with data acquisition in dia-PASEF® mode. Collected data were processed with Spectronaut 18 (Biognosys) using directDIA+.

Novel Aspect

Single small spheroid analysis on the timsTOF Ultra using the cellenONE with proteoCHIP EVO 96 for direct transfer onto Evotips.

Preliminary Data or Plenary Speaker Abstract

Low sample amount transfer into Evotips by centrifugation using the proteoCHIP EVO 96 has been proven to have less technical variation compared to manual transfer. Here we show with HeLa cell digest dilutions (Pierce) as well as isolated HeLa cells at various concentrations good technical repeatability and stability, and quantitative accuracy, identifying reproducibly more than 4,000 proteins out of single HeLa cells and over 7,000 proteins from 20 cells.

This setup was then used to investigate cellular responses to LPS treatment on single cell level from 2D cultures (40 single A549 cells treated, 40 single A549 cells as controls) and small spheroids (3 – 10 cells per spheroid; 48x treated and 40x controls). Results were compared to responses seen in 50 cell isolation bulk samples (4x treated, 4x controls). As expected, sample to sample variability decreased with an increasing cell number. However, comparing protein groups identified in the undisturbed non-treated samples, 65% of all proteins identified in 50 cell bulk were identified at single cell level and increased to 85% on small spheroid level. The protein group identification overlap between Spheroids and single cells was 75% demonstrating good protein coverage depth.

LPS treatment responses on 50 cell bulk samples, spheroids and 2D cultured single cell level were comparable showing expected activation of inflammatory pathways. However, severity varied more in the single cells and spheroids than in the 50 cell bulk samples presumably due to drug distribution and accessibility, or cell cycle stage, which is averaged out in the 50 cell bulk.

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Unveiling Hidden Depths: A High-Throughput Plasma Proteomics Workflow on Orbitrap Astral Mass Spectrometer for Enhanced Biomarker Discovery

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Cancer is the second leading cause of death, accounting for millions of deaths annually. Late diagnosis hinders prognoses and highlights the urgency for improved early detection tools. Existing diagnostic tests lack sensitivity or require invasive biopsies. While analyzing blood plasma for cancer biomarkers holds promise, complex workflows involved limit progress. We address these challenges by developing a high-throughput plasma proteomics workflow on the cutting-edge Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer, which yields the deepest plasma proteome coverage yet. We have evaluated the performance of this workflow in a mini cohort of diverse cancer and non-cancer samples. This paves the way for rapid cancer cohort and translational research studies, offering the depth needed for early disease detection through comprehensive plasma analysis.

Methods

Plasma samples were processed with a standard plasma proteomics workflow using the Proteograph XT Assay from Seer or top 14 High Select™ Depletion Spin Columns from Thermo Fisher Scientific. Resulting peptides were then analyzed using a robust liquid chromatography (LC) method with EASY-Spray™ PepMap™ Neo columns on a Vanquish™ Neo UHPLC system coupled to the new Orbitrap Astral mass spectrometer in Data Independent Acquisition (DIA) mode with few variations of LC-MS methods which provided a variety of high- throughput options to enable translational researchers to choose a workflow optimized for their cohort size and preferred turnaround time. The LC-MS data analysis was done using Thermo Scientific™ Proteome Discoverer software 3.1 and Seer's Proteograph Analysis Suite.

Novel Aspect

Unlocking the plasma proteome with a novel high throughput LC-MS workflow for Translational Research

Preliminary Data or Plenary Speaker Abstract

Limited protein coverage often plagues standard plasma proteomics and hinders the ability to uncover subtle disease signatures. Targeted assays excel in high-throughput validation but rely on discoveries made with potentially insufficient statistical power and overlook subtle changes. Addressing this conundrum, we have developed a fully automated plasma proteomics workflow enabling proteomics researchers to do larger cohort studies. This workflow strikes a crucial balance of enhanced depth of coverage without compromising high-throughput analysis.
Our preliminary results show over 700 protein groups identified in neat plasma with the standard high-throughput proteomics workflows through LFQ-DIA analysis on an Orbitrap Astral mass spectrometer. The top 14 depletion column enables identification of 1500 protein groups, which doubled the plasma proteome coverage without sacrificing the throughput, showing an economical method to capture subtle disease biomarkers. When coupled with the Proteograph XT Assay sample processing, we were able to identify 7,000 protein groups, which represents a 10-fold increase in proteome coverage. Furthermore, the Orbitrap Astral mass spectrometer delivers impressive performance regardless of sample preparation, excelling in coverage, precision, throughput and statistical power to detect subtle changes in plasma proteome compared to other MS methods. While proteome depth benefits biomarker discovery, quantitation accuracy is also an essential

criterion for success. Here, we further evaluated the quantitation accuracy by mixing different quantities of human and chicken plasma. The results showed that we were able to obtain a ratio that is close to the expected ratio, highlighting the quantitation accuracy from our plasma proteomics workflow.

Together, the novel DIA LC-MS workflow represents a powerful combination of scale and depth of coverage, empowering the development of improved classification models and enhancing biomarker discovery through robust and larger statistically powered early-stage biomarker discovery studies.

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Label-Free Quantitation with High Accuracy and Precision at the 100pg to 500ng Scale using Orbitrap Astral MS: An Inter-laboratory Study

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Analysis of protein expression at the single-cell level which reveals cellular heterogeneity, has gained increased importance in recent years. Nanoflow UHPLC coupled with high-resolution mass spectrometry is the method of choice for proteomics studies at the single-cell level, due to its sensitivity, dynamic range, and throughput. In 2023, Orbitrap Astral MS was launched, and its performance for label-free quantitation application was demonstrated for a mixture of 3-proteome digests, for high loads of 50 to 800 ng per injection. The work presented here demonstrates the accuracy and precision of quantitation at levels from 100 pg to 500 ng. The interlaboratory study demonstrates that such performance can be achieved routinely on sample loads representative of single-cell protein amounts.

Methods

Digests of Human, Yeast and E. coli proteomes were mixed at different ratios and analyzed at total protein loads from 100 pg to 500 ng/injection. Peptides were separated on an Aurora™ Ultimate TS 25 cm column (IonOpticks) in a direct injection workflow, using a Thermo ScientificTM Vanquish™ Neo UHPLC system under nanoflow conditions, at a gradient length ensuring 50 samples per day throughput. MS analysis was performed on the Thermo ScientificTM OrbitrapTM AstralTM mass spectrometer with FAIMS Pro Duo, using DIA methods with different isolation windows and ion injection times, ensuring the acquisition of up to 9 (median value) data points per LC peak. Data were analyzed using SpectronautTM 18 software (Biognosys) with directDIA, library-free processing.

Novel Aspect

Orbitrap Astral MS allows accurate and precise LFQ of proteomics samples for a sample load range from 100pg to 500ng.

Preliminary Data or Plenary Speaker Abstract

In this study, we assessed the accuracy and precision of quantitation for low- to high input samples, from single-cell level (hundreds of picogram) to 500 ng per injection. Wide (20-30 Th) isolation windows for DIA with longer (up to 60 ms) Astral ion injection times were employed to balance proteome coverage with quantitative accuracy and precision for low-input samples. With these settings, more data points per LC peak are acquired at MS1 level with the Orbitrap analyzer, ensuring high quantitation accuracy and precision, and MS2 information is used only for identification purposes. A maximum injection time of 100ms was used for the Orbitrap MS1 scans to ensure reliable quantitation at single-cell level loads. Linear correlation (up to R2=0.99) was observed for analyte peaks measured with the Orbitrap analyzer with FAIMS, at 100pg-10ng sample loads demonstrating a linear dynamic range of quantitation at low sample loads. A systematic study of parameters critical for high quantitation accuracy was performed, and the best MS methods were selected. As a result, more than 7,000 protein groups and more than 40,000 unique peptides were quantified with FDR<1% for a 100 pg sample load per run, with a median CV of less than 7%, and over 80% protein groups identified with CV<20%. In the high load range, over 15000 protein groups and over 200,000 unique peptides were quantified at FDR<1%. Quantitation accuracy at the single-cell level loads (100-250pg) were in agreement with expected values, with less than 10% relative error, and less than 3% relative error across the triplicate injections. Quantitation accuracy remained high

throughout the entire range of sample loads, regardless of the number of data points per peak. The inter-lab study of single-cell level LFQ showed similar results and demonstrated that high accuracy and precision of quantitation can be achieved on a routine basis.

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Streamlined Automated Proteomics Sample Preparation with Novel Magnetic Beads

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Standardizing reagents and methods for proteomic sample preparation remains challenging. Current methods are time-consuming, laborious, prone to variability, inefficient at removing contaminants, and impractical for processing large sample numbers. Magnetic beads-based methods are becoming more widely adopted for proteomics sample preparation due to the ability to automate protein and peptide clean-up. However, these methods face challenges related to sample and reagent compatibility, different magnetic bead options, and lack of standardized procedures and reagents. To address these challenges, we introduce a novel Proteomics Magnetic Clean-up Beads with an EasyPep™ Magnetic Sample Preparation Kit workflow (EasyPep protein digestion followed by magnetic beads-based peptide cleanup) or an SP3 workflow (magnetic beads-based protein cleanup and digestion) for streamlined and automated proteomics sample preparation.

Methods

Various samples including mammalian cells, plasma, tissue, and bacteria were prepared following the EasyPep Magnetic MS Sample Preparation Kit protocol and optimized SP3 protocol utilizing the new Proteomics Magnetic Clean-up Beads to facilitate efficient protein or peptide clean-up. A Thermo Scientific™ Vanquish™ Neo UPLC system coupled to a Thermo Scientifc™ Orbitrap Exploris™ 480 mass spectrometer or Orbitrap Astral mass spectrometer, and a Thermo Scientific™ Dionex™ Ultimate™ 3000 Nano LC system coupled to a Thermo Scientifc™ Q Exactivex™ Plus Hybrid Quadrupole-Orbitrap mass spectrometer were used for analysis of different protein digest samples. Raw files were analyzed using Spectronaut 18 and Proteome Discoverer 3.0. Raw files were analyzed using Proteome Discoverer™ (PD) 3.0, Spectronaut 18, and CHIMERYS on PD 3.1.

Novel Aspect

Our Proteomics Magnetic Clean-up Beads-based sample preparation approaches enable efficiently automated processing of protein samples for proteomics analysis.

Preliminary Data or Plenary Speaker Abstract

Compared to other magnetic resins, our novel Proteomics Magnetic Clean-up Beads exhibit superior compatibility with a wider range of solvents (water, acetone, acetonitrile, methanol), pH (pH 3-12), and are free from leachables. We optimized the peptide binding and elution for different sample input amounts (1µg-1.5mg) and preparation of up to 96 samples using KingFisher™ and Hamilton automated systems in under 3 hours. Validation studies using mammalian cells, plasma, and tissue samples (10-100µg) demonstrated exceptional digestion efficiency with a missed cleavage rate of ~10% and complete cysteine reduction and alkylation. Notably, the eluted peptides can be directly injected into liquid chromatography-mass spectrometry (LC-MS), eliminating the conventional and time-consuming SpeedVac drying and reconstitution steps. Our sample preparation process exhibits high robustness and reproducibility with peptide and protein identifications having less than 5% coefficient of variation (CV) and quantification of protein abundances showing less than 10% CV. The magnetic beads-based EasyPep Magnetic workflow and SP3 workflow were applied to the quantitative analysis of lung tumor/normal plasma and tissue samples, ensuring exceptional quantification reproducibility and validation against known protein markers. To enhance the sample throughput of our automation solution, we integrated tandem mass tag (TMT) for multiplexed proteome analysis. Additionally, we verified the compatibility of the Proteomics Magnetic Clean-up Beads with various detergents (e.g., SDS) and lysis solutions (e.g., Thermo Scientific™ IP lysis buffer, T-PER™ Tissue Protein Extraction Reagent, Subcellular Protein Fractionation Kit, NE-PER™ Nuclear

and Cytoplasmic Extraction Reagents, and Mem-PER™ Plus Membrane Protein Extraction Kit). The MS results showed no carry-over of any detergents and comparable protein and peptide identifications across different samples, buffers, and fractions. Overall, both our magnetic beads-based comprehensive EasyPep Magnetic solution and optimized SP3 solution maximize laboratory productivity while significantly improving the speed and reproducibility of high-quality proteomics sample preparation.

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Evaluation of Parallel Reaction Monitoring Assays at Discovery Scale on a New Hybrid Nominal Mass Instrument for Phosphoproteomics Studies

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The measurement of phosphosignatures is crucial to better understand cellular mechanisms, as protein phosphorylation is well known to be a strong mediator of cellular signaling. MS-based proteomics approaches have led to the discovery of thousands of known human phosphosites, however discovery approaches fail in detecting uniformly any given phosphosite across an entire sample cohort. Recently, a 300-plex phosphopeptide targeted MS assay suitable for both discovery and preclinical studies was developed using triple quadrupole technology (Keshishian et al. 2022). Herein we evaluate Parallel Reaction Monitoring-based approaches using a new hybrid high-speed nominal mass instrument. Both targeted-MS2 and MS3 approaches were assessed for the absolute quantitation of more than 300 phosphopeptides in cancer cell lines using synthetic stable isotope labeled (SIL) peptides.

Methods

Spectral libraries were previously generated on a HRAM mass spectrometer using synthetic SIL peptides (Keshishian et al. 2022). The list of precursors was imported to create targeted parallel reaction monitoring assays on the new hybrid high-speed nominal mass instrument. The SIL peptides mixture on neat solution was then measured to determine retention times in a 30-minute gradient method. Scheduled targeted MS2 and MS3 assays were created using PRM conductor, a new Skyline-based plugin tool. This new tool also added the corresponding endogenous peptides which led to a final assay of 670 phosphopeptides in both tMS2 and tMS3 methods. The performance of both assays was evaluated by measuring the phosphopeptides in a mixture of five cancer cell lines.

Novel Aspect

Discovery-scale quantitative PRM-based approaches for phosphoproteomics studies on a novel hybrid high-speed nominal mass instrument

Preliminary Data or Plenary Speaker Abstract

Targeted MS approaches have been widely used for biomarker verification, however with the development of faster and more sensitive mass spectrometers, highly-multiplexed targeted assays are more frequently used to validate findings in biological and preclinical studies.
The quantitative targeted MS2 and MS3 assays developed were based on the SigPath assay developed by Keshishian et al. The assay is a highly multiplexed SRM method designed to measure phosphosites in nodes of biological pathways known to be modulated via phosphorylation. Additionally, P100 phosphopeptides (Abelin et al, 2016) were also included, totaling 670 phosphopeptides monitored in a 30-min gradient method.
Assessment of the limit of quantitation and linearity of both MS2 and MS3 methods was performed. For this, a serial dilution of cell lysate with SIL peptides into cell lysate was carried out. Preliminary results demonstrated excellent sensitivity in the atto-mole range of peptide amount on column. Additionally, results also indicated that enhanced selectivity can be achieved with MS3 acquisition.
Sensitivity and reproducibility were also evaluated in a mixture of five cancer cell lines as reported by Keshishian et al. More than 200 endogenous light peptides were detected in this mixture after IMAC enrichment and the method was able to quantify more than 100 light peptides in the lowest input, 25 µg sample. The current results suggest 4x improvement of at least a 4x in sensitivity in ~5x in sample analysis throughput than the previous triple-quad-based method.
We are planning to extend the investigations to the use of FAIMS MS2 and MS3, to contrast with the current without-FAIMS methodologies. Further improvements to the assay will be studied, such as

the effect of gradient length, and the incorporation of the Adaptive RT algorithm to reduce the scheduled acquisition windows from 5 minutes to around 1 minute.

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Leveraging the sensitivity, resolution, speed and accuracy of the Orbitrap Astral Mass Spectrometer to analyse low concentration, high value samples.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

During cancer diagnosis, tumour samples are subjected to pathology stains to assist disease characterisation. These techniques may leave insufficient tissue for proteomic analysis using conventional mass spectrometers. The sensitivity and efficiency of mass analysers play pivotal roles in proteomics research, particularly in accurately identifying and quantifying peptides and proteins. The sensitivity of the Orbitrap Astral Mass Spectrometer offers the promise of analysing minimal quantities of samples. We tested its sensitivity using digests of a HEK-293 human cell line ranging from 500 ng to 2 ng and evaluated its quantitative accuracy with a three-species mix at different loads. We demonstrate the Orbitrap Astral's effectiveness across varying on-column loads.

Methods

The 100 SPD (samples per day) 15 min workflow was employed to analyse HEK-293 cell digests, with varying loads of 2 ng, 10 ng, 50 ng, 100 ng, and 200 ng on-column. Utilising a trap-elute setup, a 150 µm x 15 cm, 2 µm particle size column and a 300 µm x 5 mm trap at a flow rate of 1.5 µL/min were used to analyse the samples. For quantitative accuracy assessment, three pools of a three-species mix comprising Human HEK-293, E. coli, and Yeast cells were prepared. The relative amount of E. coli and yeast was altered across the pools, while Human was kept constant. This design enabled evaluation of the workflow's performance across a range of sample loads.

Novel Aspect

Low ng tissue digest analysis by leveraging the robustness of microflow and the sensitivity of the Orbitrap Astral mass spectrometer.

Preliminary Data or Plenary Speaker Abstract

We employed HEK-293 cell lines to assess the sensitivity of the Orbitrap Astral mass analyser. To mitigate challenges associated with nanoflow delivery, we utilised a low microflow rate of 1.5 µL/min. Employing a 150 µm ID column at this flow rate achieved an optimal balance between sensitivity and minimising idle time at the start of the run.
To test the robustness and sensitivity of the orbitrap Astral mass analyser, we used a wide range of peptide loads. Upon loading 500 ng of HEK-293 digest, we identified nearly 9,000 proteins and over 150,000 peptides. This decreased to 8,500 proteins and 120,000 peptides when reducing the load to 200 ng. Even with minimal loads of 10 ng and 2 ng, we identified more than 4,500 and 2,500 proteins, respectively.
To enhance sensitivity further, especially at lower on-column loads, we tested various accumulation times, DIA window sizes and precursor mass ranges. By optimising these parameters, an improvement of up to 30% in the number of identified proteins was observed. This indicates the importance of fine tuning these parameters, especially for low load samples.
We evaluated the Orbitrap Astral mass analyser quantitative accuracy using a three-species mix across different on-column digest loads. The instrument consistently maintained good quantitative accuracy across varying loads. Notably, higher on-column loads exhibited slightly better performance.
This study demonstrates the effectiveness of the Orbitrap Astral mass analyser in achieving high sensitivity and accuracy in proteomics research. Through parameter optimisation and load adjustment, researchers can further enhance the capabilities of mass analysers for comprehensive proteome analysis.

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The Proteomic Analyst Suites: A collection of easy-to-use interactive web applications for proteomic data analysis and visualisation

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Mass spectrometry (MS) technique has been widely used to characterise and quantify proteins in biological and clinical samples. Furthermore, with the rapid advancements in mass spectrometry, the complexity of proteomic datasets has increased. Although software (such as Perseus) and/or R-based packages have been developed to assist with post-processing and downstream analysis, their general availability is limited to highly skilled individuals proficient in proteomics, mass spectrometry, and/or bioinformatics. As mass spectrometry-based proteomics becomes more widespread, there is a growing need for easy-to-use tools to pre-process, analyse and visualise data. To cater for this unmet need, we have therefore created the “Proteomic Analyst Suites” that promise to bridge expertise gaps and provide broader proteomic insights to meet diverse research needs.

Methods

The Proteomic Analyst Suits offer a comprehensive set of methods for different types of proteomic data analysis and visualisation. Fully automated pipelines of contemporary bioinformatic approaches are performed using R statistical analysis packages. The process begins with data pre-processing, imputation, and normalisation to ensure data quality. Subsequently, statistical algorithms are applied to identify differentially expressed proteins and assess significance. Interactive visualisation tools, such as heatmaps, volcano plots, and principal component analysis (PCA) facilitate quantitative and qualitative data interpretation and exploration. Additionally, features for enrichment analysis are included. It is worth noting that while these methods have predefined parameters for users, experienced researchers retain the flexibility to select additional methods and/or parameters as needed.

Novel Aspect

Cover the analysis of diverse proteomic datasets, compatible with numerous search engines, and integrate with a novel pathway analysis module.

Preliminary Data or Plenary Speaker Abstract

During the last decades, several label-free and label-based proteomic workflows and acquisition strategies have been developed to comprehensively interrogate the proteome of virtually any biomedical sample. Due to the complexity of mass spectrometry data, software algorithms (often called search engines) are used to convert mass spectrometry raw files into data matrices with quantitative and quantitative information on proteins and peptides. However, it is still a challenge for many researchers to interpret the results without basic training or expert help. This is mostly due to a lack of easy-to-use tools for data pre-processing and analysis, and especially around the visualization of such information-rich proteomic datasets. Moreover, diverse types of proteomic datasets can be generated to cater for different research needs, and a major proportion of raw mass spectral and peptide-level data has been overlooked in the past, partly due to a lack of computing resources to make them easy to process and summarize.
Recognizing this need, we have developed the Proteomic Analyst Suites, which is a collection of easy-to-use, interactive web applications to analyse and visualise proteomic datasets with ‘one click’ (encompassing DDA, DIA and TMT data) based on output files from the most popular search engines including MaxQuant, FragPipe, Spectronaut (Biognosys), DIA-NN and Proteome Discoverer (Thermo

Scientific). Each individual web application (LFQ-Analyst, DIA-Analyst, TMT-Analyst, FragPipe-Analyst, Phospho-Analyst) provides a wealth of user-analytical features and publication-quality output graphics to facilitate exploratory and statistical downstream analysis and interpretation. Additionally, each web application will seamlessly tie into a novel module entitled Pathway-Analyst to easily perform pathway and network analyses using various databases (e.g. Gene Ontology, KEGG, String) and tools (e.g. g:Profiler, GSEA). Despite their simplicity, the Proteomic Analyst Suites use state-of-the-art bioinformatic pipelines, and they are freely accessible at https://analyst-suites.org/.

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A Rapid and Economical Workflow to Discover Protein Biomarkers Using High-performance Triple Quadrupole LC/MS System

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

The development of triple quadrupole LC/MS system and the availability of peptide MS/MS spectral library have enabled the use of comprehensive MRM-based assay for protein biomarker discovery. This approach allows the measurement of hundreds of peptides simultaneously and accurately in complex biological samples.

In this study, we evaluated a discovery workflow using publicly available databases, Skyline software and a high-performance standard flow liquid chromatography triple quadrupole (LC/TQ) system. A comprehensive peptide quantitative assay was developed directly from publicly available proteome database and peptide spectral library without using stable isotope-labeled standard peptides. This assay was applied to analyze plasma samples from human individuals. The results demonstrated that this rapid and economical approach can discover protein biomarkers in complex biological matrices.

Methods

Normal human Plasmas from 20 male and 20 female individuals were purchased from BioIVT. All the samples were trypsin-digested and desalted. Aliquots from all digested samples were pooled together to produce a quality control (QC). Skyline software was used to create a targeted peptide transition list based on human proteome from Uniprot and human plasma spectral library from PeptideAltas. A C18 column (2.1 × 150 mm) was used for LC separation. The integrated Agilent automation tool in Skyline was used to develop dynamic MRM (dMRM) method. An in-house retention time (RT) predictor was created to guide the peptide peak selection in Skyline. The final standard flow LC-dMRM method was applied to analyze the pooled QC sample and the individuals' plasmas.

Novel Aspect

A rapid and economical workflow to discover protein biomarkers in complex biological matrices.

Preliminary Data or Plenary Speaker Abstract

To create a list of targeted peptide transitions, we first imported a list of 581 human protein names into Skyline software. To ensure the quality and accuracy, we used rigorous criteria on peptide and transition settings to narrow down candidates and select only the top peptides and transitions. In the QC sample, we detected 743 endogenous peptide peaks that potentially corresponded to 417 proteins. We used these peaks to create the acquisition method, which included more than 3000 dMRMs.

This method was applied to examine the 40 plasmas (20 male vs. 20 female). Repeated injections (n=10) of the pooled QC were performed during the entire study.

Quantitative analysis of the pooled QC samples shows:

- A median CV of 11.3% on peak area was observed for all the targets

- 72.3% of the targeted endogenous peptides have a CV < 20% on peak area

- The median CV of RTs is 0.1%, demonstrating the excellent RT reproducibility of the standard flow LC system

Group comparison between male and female groups found that the female group exhibited higher levels of Pregnancy Zone Protein (PZP_HUMAN) than the male group, as indicated by two peptides from PZP that had high statistical significance for this difference (adjust p-value < 0.001). This finding agrees with the existing literature. Identities of the interesting peptides were further confirmed by using MRM methods with more product ion transitions of the target peptides and by performing full product ion scan of the targeted precursors on the pooled QC sample.

This study demonstrated protein biomarkers can be discovered by quantitative measuring cohort samples directly using MRM-based LC/MS/MS approach based on publicly available databases.

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Quantitative proteomics and histopathology reveal hepatic adaptations to type 2 diabetes

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease, and T2D represent two interrelated metabolic diseases with paralleled escalations in global prevalence. MASLD affects 1 in 3 adults, with projections indicating an increase to 1 in 2 adults by 2040. MASLD typically initiates with simple steatosis and can progress to metabolic dysfunction-associated steatohepatitis, cirrhosis, or hepatocellular carcinoma if left untreated. Notably, MASLD is an independent risk factor for type 2 diabetes (T2D), which involves insulin resistance and chronic hyperglycaemia. Conversely, T2D accelerates MASLD progression. Despite their clinical importance and advances in our understanding of the individual pathophysiology of MASLD and T2D, the hepatic adaptations underlying the coexistence of MASLD and T2D remain insufficiently characterised.

Methods

To address this gap, we induced T2D in 8-week-old male Sprague-Dawley rats using a high-fat diet (HFD) to induce obesity over 8 weeks and low-dose streptozotocin (STZ) at week 4 of feeding to induce pancreatic insufficiency. Chow diet and citrate were used as controls for each treatment, generating four biological cohorts. Alongside fasting blood glucose quantitation, plasma insulin, triglyceride, alanine transaminase, and aspartate aminotransferase levels were determined via colourimetric assays (n≥6 per group). Histological assessments of microvesicular and macrovesicular steatosis were performed on digitised haematoxylin and eosin-stained rat liver sections (n≥7 per group) using QuPath. This was complemented by quantitative proteomic analysis of liver specimens from matched animals (n=4 per group) utilising two-dimensional liquid chromatography-tandem mass spectrometry.

Novel Aspect

Overall, we defined a hepatic signature that reflects both molecular and cellular adaptations, providing crucial insights into the MASLD-T2D nexus.

Preliminary Data or Plenary Speaker Abstract

Our findings revealed that HFD and STZ synergistically elevated fasting blood glucose and plasma insulin, triglyceride, and alanine transaminase levels. Also, STZ alone raised plasma alanine transaminase and aspartate aminotransferase levels, but HFD alone did not impact the levels of these biomarkers of hepatic injury. Correspondingly, histological assessment unveiled distinct histological patterns: STZ-induced hyperglycemia promoted hepatic microvesicular steatosis, while HFD-induced obesity and dyslipidaemia led to hepatic macrovesicular steatosis. In the combined HFD-STZ group, liver histology demonstrated a synergistic response, with fewer physiological hepatocytes and evidence of mixed steatosis. Proteomic analysis identified 1323 significantly altered hepatic proteins across the four biological cohorts (ANOVA, $p<0.05$ and; z-Score log-2 fold change ±1.00). Hierarchical clustering revealed that the synergistic impact of HFD and STZ dampened the impact of standalone STZ or HFD. Pathway analysis highlighted specific alterations in proteins governing ketone body and fatty acid metabolism within the T2D environment. Furthermore, enzymes regulating mitochondrial beta-oxidation of fatty acids correlated with hepatic macrovesicular steatosis, while those involved in peroxisomal beta-oxidation were specific to hepatic microvesicular steatosis.

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Monash Proteomics & Metabolomics Platform: Providing answers to biological questions with analytical tools

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

As the field of biological mass spectrometry advances, we see an impressive expansion in the techniques available to characterise, catalogue, and quantify biomolecules such as proteins, lipids, and small molecules. At the Monash Proteomics & Metabolomics Platform, we're dedicated to harnessing these advances, offering the scientific community access to cutting-edge equipment and expert support. Our comprehensive approach includes proteomic, metabolomic, and lipidomic support for researchers, clinicians, and industry. We work through a mix of collaborative projects and fee-for-service engagements, providing robust pipelines for a variety of sample types. From identifying drug targets to single-cell proteomics, our methods span the entire spectrum of bio-mass spectrometry, ensuring that the projects get the tools they need to answer the questions asked.

Methods

Workflows include basic identification, label-free quantification (DDA & DIA), automated bead-based enrichment, label-based quantification (TMT SILAC), structural (Crosslinking, Intact, HDX) meta-proteomics, limited proteolysis (LIP-MS), drug target discovery, single-cell proteomics post-translational modification analysis (including phosphorylation) and bespoke targeted methods for validation including Olink. We also develop data analysis tools for exploration by collaborators and the wider community with our Proteomic Analyst Suites and IDEOM. Our Analyst Suites provide s with the ability to qualitatively analyse experimental data from various sources from whole protoeme analyses LFQ / TMT / DIA-Analyst, PTM analyses (Phospho-Analyst), Pathway-Analyst and future tools of NPX-Analyst (Olink) and Single Cell Proteomics (SCP-Analyst). With a fleet of mass spectrometers, including the Orbitrap-Astral, we have the tools to answer complex questions

Novel Aspect

Providing new bioinformatics tools and benchmarked data on orthogonal methods

Preliminary Data or Plenary Speaker Abstract

Here, we share information on our pipelines and the depth of analysis achievable, exemplified by representative projects analysed within our platform. Including examples of the same samples analysed by multiple strategies including labelling-based methods with the use of real-time searching and DIA analysis on the Orbitrap-Astral.

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High-throughput high-resolution data-independent acquisition workflow on an Orbitrap Exploris 480 Hybrid mass spectrometer for accurate label-free quantitation

Mr Julian Saba[1], Kevin Yang[2], Julia Kraegenbring[3], Maciej Bromirski[3], Amirmansoor Hakimi[2]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific, [3]Thermo Fisher Scientific

Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Traditional data-dependent analysis (DDA) approaches have been widely employed for LFQ experiments, but they suffer from run-to-run inconsistencies due to intensity-based stochastic triggering of precursors, often leading to under sampling especially of low-abundant proteins. Missing values become more likely as sample size increases, and so data independent acquisition (DIA) has emerged as a popular technique for large scale quantitative analyses. The need for analyzing large numbers of samples, especially in clinical and biomarker discovery studies, makes DIA-based workflows an obvious choice for ensuring reproducible quantitative analyses. A suitable analytical workflow addresses the need for reproducible sample preparation, robust separations, high quality quantitative measurements, and reliable data analysis.

Methods

HeLa digest and three-proteome mixtures were loaded onto a 50 cm µPAC Neo HPLC column and separated at a 350 nL/min flow rate in direct injection mode using a Vanquish Neo UHPLC system over 9 min, 30 min, and 60 mins active LC gradients, respectively, before being transferred into the Orbitrap Exploris 480 Hybrid mass spectrometer.

Source parameters, including spray voltage and ion transfer tube temperature, are tunable parameters and must be optimized for the individual setup. Acquired data has been processed by Spectronaut (Biognosys, v18) using a directDIA approach, DIA-NN (v1.8.1) or Thermo Scientific™ Proteome Discoverer™ software (v3.1.0.638) using CHIMERYS™ intelligent search algorithm by MSAID.

Novel Aspect

The high-resolution DIA workflow for label-free quantitation setup on an Orbitrap Exploris 480 Hybrid mass spectrometer.

Preliminary Data or Plenary Speaker Abstract

The initial set of experiments consisted of maximizing identification and quantitative performance for various active gradients of 9, 30, and 60 minutes for tryptically digested Hela standards. With 30 min active gradient, 6,300+ proteins and 40,000+ peptides were identified, along with a protein group CV of approximately 5%, suggesting that the 30 min active gradient method provides the perfect amount of time to achieve throughput while maximizing identification and quantitative. We extended this workflow to 60 min active gradient and successfully identified close to 7,200 proteins and >60,000 peptides, highlighting that deeper proteome coverage can be achieved in the Velocity DIA workflow by using a longer gradient.

To test the reliability of quantitation accuracy, in this data set, we used two samples with different amounts of spiked microbial proteins to mimic biological samples where proteins might be up- or downregulated under different conditions. We tested the quantitative performance for different active gradients (9, 30, and 60 mins). The Velocity DIA workflow used for relative quantitation of E.coli and Yeast proteins in a high amount of human peptides as background yields excellent quantitation accuracy across all ratios with median values extremely close to the theoretical ratios, as well as a narrow distribution of all data points around the median values, indicating high quantitative accuracy and precision of the workflow.

Additionally, the three-proteome mix experiment further highlights the proteome depth that can be achieved with an Orbitrap mass spectrometer. In the 60 min active gradient, > 10,000 protein groups were identified. The numbers of quantified proteins differ by species, with nearly 7,200 human protein groups, approximately 2,800 yeast protein groups, and close to 870 E. coli protein groups.

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Automated high-throughput proteomic analysis of stored blood cells from a large cohort of non-domestic felids

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Blood transfusions occur infrequently in non-domestic cats housed at zoos, but remain a necessary, life-saving treatment. For this reason, long-term blood storage for non-domestic felid species is required. Little is known about how long blood can be stored from these species before degradation limits its clinical efficacy, however. Non-targeted proteomics provides a sensitive, unbiased method to look at molecular changes in blood cells that result from prolonged storage. Here, over 500 blood cell samples were analyzed (resulting in over 1600 injections) using a highly automated, high-throughput LC-MS/MS and data analysis workflow.

Methods

Blood samples were obtained from 135 non-domestic cats, consisting of 18 different species, housed at US zoos. Fresh, anticoagulated, whole blood samples were aliquoted and stored at 4^{o}C in a clinical blood bank refrigerator for 0, 7, 14, or 28 days, after which the red blood cells were pelleted. Pelleted blood cells were then prepared for bottom-up proteomic analysis using the Thermo Scientific™ AccelerOme™ automated sample preparation platform and analyzed using a high-throughput (85 samples per day) LC-MS/MS DIA method on a Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer. Data analysis was automated using the Thermo Scientific™ Ardia™ Platform via the connection of acquisition using the Thermo Scientific™ Xcalibur™ software to processing by the Thermo Scientific™ Proteome Discoverer™ software.

Novel Aspect

Large cohort study of stored, non-domestic felid blood cells using a highly automated workflow from sample preparation through data analysis.

Preliminary Data or Plenary Speaker Abstract

The AccelerOme sample preparation platform allowed for hands-off, automated sample preparation (including protein lysis, DNA removal, protein reduction, alkylation, protease digestion, and sample cleanup) of over 500 individual blood cell samples. These samples were then analyzed in triplicate (resulting in greater than 1600 injections) on a Thermo Scientific™ Vanquish™ Neo LC flowing at capillary flow rates, coupled to an Orbitrap Astral mass spectrometer operating in data independent (DIA) acquisition mode. Upon the completion of each injection, result files were automatically uploaded to the Ardia platform by the Xcalibur acquisition software, and analysis by the Proteome Discoverer software was automatically triggered. Proteome Discoverer searches leveraged the CHIMERYS™ algorithm running locally on the Ardia server for both spectral identification and quantification.
Each injection resulted in the identification of approximately 1200 to 1500 proteins, and 8000 to 12,000 peptide groups. The protein and peptide identification rates were approximately 2.5-fold and 5-fold higher, respectively, than a pilot study that was performed on a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer, despite using a method that was nearly twice as long. The variation in the number of identifications observed here can be attributed to differences in the completeness of the proteomes which were available for each species. However, intensities and identification rates across samples derived from the same species were very consistent, highlighting the robustness of the sample preparation and LC-MS setup used. Label-free relative quantification revealed few changes in protein abundance across storage times, which was consistent with limited metabolic activity due to storage at 4^{o}C. Interestingly, numerous peptides showed changes in abundance as storage lengths increased, which could indicate protein degradation or chemical changes that occur during storage.

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Enhancing Translational Research through High-Throughput Plasma Proteomics

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Plasma proteomics remains vital for biomarker and drug target discovery. Large-scale plasma proteomics studies, however, demand robust LC-MS methods without sacrificing coverage or accuracy. Plasma proteins are expressed at a wide dynamic range of concentrations and most analytical methods are not yet capable of providing deep proteome coverage in a high throughput way. Here we present a scalable high-throughput plasma proteomics workflow using the Orbitrap Exploris 480 MS and a multi-nanoparticle approach. We have evaluated the performance of this workflow using a pooled control plasma and a mini cohort of diverse cancer and non-cancer samples. Our workflow has generated promising results for in-depth plasma analysis, providing a solution for efficient biomarker discovery and drug target identification.

Methods

Plasma samples from a mini cancer cohort with aged-matched healthy controls were processed separately and pooled, with standard plasma proteomics and the Proteograph XT workflow from Seer. Tryptic peptides were then analyzed using a robust liquid chromatography method with the EasySpray PepMap Neo columns on a Vanquish Neo UHPLC system coupled to the benchtop Orbitrap Exploris 480 MS, all from Thermo Fisher Scientific. Data Independent Acquisition (DIA) with few variations of LC-MS methods were evaluated, providing variety of analysis throughput, enabling translational researchers to choose a workflow optimized for their cohort size. The LC-MS data analysis was done using Proteome Discoverer software 3.1 from Thermo Fisher Scientific and Seer's Proteograph Analysis Suite.

Novel Aspect

Next generation translational research enabling precision medicine through High-Throughput Plasma Proteomics.

Preliminary Data or Plenary Speaker Abstract

Our preliminary results show over 3000 protein groups identification with a high-throughput proteomics workflows of 50 min per sample (high throughput method, 2 injections per sample) with a robust Vanquish Neo UHPLC system in capillary flow liquid chromatography mode coupled to the Orbitrap Exploris 480 MS with the Proteograph XT Assay sample processing, the protein groups were further boosted to approximately 3900 identification with a 102 min nano flow gradient (Max-ID method), supporting the need for deeper proteome coverage. Furthermore, the Orbitrap Exploris 480 MS delivers statistical power regardless of sample preparation, excelling in precision, accuracy and throughput to detect subtle changes in plasma proteome needed for biomarker discovery. Our results show that high-throughput and Max-ID method affords over 87% and 89% of protein groups, respectively, with the coefficient of variance (CV) being less than 20%. This novel DIA LC-MS workflow represents a powerful combination of scale and depth of coverage, while empowering the development of improved classification models and enhancing biomarker discovery through robust and larger statistically powered, early-stage biomarker discovery studies. The pilot cancer mini cohort data showcases how advancements in sample preparation and mass spectrometry enable unprecedented depth of coverage for plasma proteome with the reproducibility, robustness, and throughput needed for large cancer cohort studies. While methods like high abundant protein depletion and peptide fractionation improve coverage, they sacrifice analysis throughput, restricting early-stage biomarker discovery to smaller cohorts. This workflow strikes a crucial balance of

enhanced depth of coverage without compromising high-throughput analysis, offering the depth needed for early disease detection through comprehensive plasma analysis.

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Proteomic biomarkers of distant recurrence in early-stage rectal cancer extracted from FFPE blocks

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

In Stage I/II rectal cancer (RC), patients are treated with curative intent surgery, which does not require adjuvant chemotherapy. This is due to the low risk of recurrence which is estimated to be less than 9%. However, for those patients who recur with distant metastasis, their outcomes are dire. Thus, knowledge of biomarkers that can predict patients who are at greater risk for recurrence may be clinically useful. There are no currently used prognostic biomarkers for distant recurrence of early-stage rectal cancer. Hence, identification of biomarkers that are involved in metastatic pathways will allow us to identify patients who may benefit from adjuvant chemotherapy, as this is not a standard treatment protocol used with early-stage cancers.

Methods

FFPE specimens of Stage I/II resected rectal tumours were used. The cohort included 10 patients with recurrence to distant sites and 13 patients with no recurrence after 3 years from surgery. Tumour and normal tissue areas were collected by laser microdissection from 8 µm H&E stained sections on PET slides. Samples were heated at 95□C for 30 minutes with 1% SDC, 100mM Tris-HCl, digested overnight with trypsin, then desalted using StageTips. Peptides were analysed by LC-MS/MS using DIA on a Orbitrap HF-X mass spectrometer over a 140-minute time period. DIA-NN was used to identify and quantify peptides to 1% false-discovery rate, and differential expression analysis was conducted using Student T-test. PRM was conducted to confirm expression of selected proteins.

Novel Aspect

Identification of putative proteomic biomarkers of distant recurrence in early-stage rectal cancer from FFPE blocks.

Preliminary Data or Plenary Speaker Abstract

A total surface area of 100 000 000 µm2 for each FFPE sample was determined as sufficient to produce a final protein extract of ∼30 µg. This ranged from 3-7 slides for each sample, depending on the size of each tumour. A total of 7977 proteins were identified in the rectal tumour specimens, of which 5508 proteins were quantified in all 23 samples.

Eight proteins with more than 1.5-fold expression differences were identified between the groups, while five proteins showed more than 2-fold differential abundance.

Gene set enrichment analysis was conducted to identify enriched pathways in the recurrent group, based on MSigDB signatures of hallmark gene sets. The following pathways - epithelial-mesenchymal transition (EMT), coagulation, TGF-□ signalling, and angiogenesis were found as enriched in proteins that are significantly upregulated in the group with distant recurrence.

Examples of upregulated proteins in the recurrence group included heat shock proteins (HSPA6), carboxypeptidase B2 (CPB2), extracellular matrix proteins (RCN3, FN1) matrix metalloproteases (MMP9, MMP11, MMP14) growth factor receptors (PDGFRB).

Between the tumour and the adjacent normal mucosa, we also identified 254 proteins with more than 1.5-fold expression differences, including 113 proteins with more than 2-fold differential expression between the groups.

To verify the DIA findings, we designed a PRM assay targeting 45 peptides from 19 proteins, and will evaluate the consistency of the quantitative methods.

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Exploring the molecular mechanism of lycorine against hepatocellular carcinoma based on quantitative phospho-proteomics

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death in China. Lycorine has shown potent anti-HCC activity in vitro and in vivo, but the relating targets and molecular mechanism have not been fully defined. To this end, we investigated the molecular mechanism of lycorine anti-HCC effect using quantitative proteomics and phospho-proteomics strategies.

Methods

A number of biological or biochemical tests with Hep G2 cells were conducted to evaluate the effects of lycorines on cell growth inhibition, cell cycle progression and other cellular processes. To further reveal the molecular mechanism of the anti-HCC effect of lycorine, we performed TMT labeled high-throughput quantitative phosphorylated proteomics to identify proteins with significant changes in phosphorylation after being treated with lycorine in HepG2 cells.

Novel Aspect

Molecular mechanisms of anti-HCC activity of lycorine firstly explored through MAPK, mTOR and CDK signaling pathways with quantitative phospho-proteomics.

Preliminary Data or Plenary Speaker Abstract

Our previous results showed that lycorine inhibited cell proliferation in a time- and dose-dependent manner, G2/M cell cycle progression, and stimulated cellular ROS generation in HCC HepG2 cells. In the phopspho-proteomics study, a total of 3366 phosphorylated proteins were identified, including 249 up-regulated (fold change >1.5) and 93 down-regulated (fold change <0.5) phosphorylated proteins. Bioinformatics analysis indicated that the differentially expressed phosphorylated proteins are mainly involved in MAPKs signaling, mTOR signaling and CDKs proteins relating to G2/M regulation.

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Identification of Common Host Cell Proteins Detected in CHO Produced Monoclonal Antibodies

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Host cell proteins (HCP) play an important part in drug stability, safety and potency and are a critical quality attribute that requires monitoring during both the establishment of a purification process and in the final drug substance. In this study we utilised an MS/MS DDA platform to identify and semi-quantify the levels of HCPs in drug substance and downstream purification stages. In total, data was generated for n = 20 studies from three different IgG antibodies encompassing IgG1 and IgG4 subtypes and both kappa and lambda antibody light chains. Identified HCPs were classified based upon their impact to the drug quality and patient safety and compared between mAbs to identify common high-risk HCPs copurified across molecules.

Methods

Identification of HCPs was performed using a bottom-up peptide mapping approach. Samples underwent tryptic digestion, followed by reduction and alkylation. The resulting peptide mixtures were separated via reverse phase analytical flow HPLC and peptides analysed by MS/MS data dependent acquisition on either a Bruker II QTOF or QE-HF Orbitrap Mass spectrometer. Identification of HCPs was performed in the software Proteome Discoverer using a publicly available CHO proteome, with identification considered true if identified by at least two unique peptides and in independent sample replicates. Semi-quantification was performed using the XIC area of the top three most intense peptides normalised to the intensity of the top three most abundant peptides of the product mAb.

Novel Aspect

Retrospective analysis of the distribution of host cell proteins in 20 independent studies of CHO produced mAbs.

Preliminary Data or Plenary Speaker Abstract

This cross-product study revealed the presence of over 100 HCPs. Identified HCP were compared to the publicly available BioPhorum database allowing for identification of several commonly copurified HCPs found in the studied protein A purified mAbs.

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Lipid Profiling in Niemann-Pick Disease Type C cell cultures and mouse brains using MALDI Mass Spectrometry Imaging.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Niemann-Pick Disease (NPD) involves rare genetic disorders characterized by abnormal lipid accumulation, notably cholesterol and sphingolipids, particularly in brain tissue. Spatial analysis of cholesterol levels is crucial in NPD, providing insight into disease progression and potential interventions. Employing MALDI-2 microGRID mass spectrometry imaging (MSI) with a 5µm spatial resolution, we conducted a comprehensive analysis of lipid expression profiles in APRE19 cells (WT, NPC1-/-, NPC2-/-). Gangliosides, found in cell membranes, serve multiple functions relevant to NPD pathology, including cell-cell interactions and neuronal signaling. Our study investigates the effects of gene therapy on ganglioside levels and neuroinflammation in NPC2-/- mice using MALDI-MSI and trapped ion mobility separation (TIMS).

Methods

APRE19 cells (WT, NPC1-/-, NPC2-/-) were cultured until subconfluency in 8-well chambers on glass slides. Cells underwent PBS washing, formalin fixation, and fluorescence staining. DHB matrix was then applied using a home-built sublimator. MALDI-MSI was performed using a timsTOF fleX MALDI-2 microGRID (Bruker Daltonics) in positive ion mode. Lipidomic alterations were investigated in 16 mouse brains, including WT, NPC2-/- PBS treated, and NPC2-/- treated with AAV-BR1-NPC2—a virus targeting brain endothelial cells (BECs) and expressing NPC2 protein. Tissue sections were measured in negative ion mode with a 300ms ramp time for ganglioside isomer separation. DHAP matrix was applied using an M3+ sprayer (HTX technologies). Data processing utilized SCiLS lab software (Bruker Daltonics) for visualization and RMS normalization, followed by statistical testing.

Novel Aspect

Investigating lipid alterations and gene therapy effects in Niemann-Pick Disease, particularly in ganglioside metabolism, offers insights into potential therapeutic strategies.

Preliminary Data or Plenary Speaker Abstract

Preliminary results demonstrated elevated cholesterol ion intensities in NPC1-/- and NPC2-/- cells compared to WT, indicative of the disrupted cholesterol metabolism seen in NPD. This aligns with the known pathophysiology of NPD, where mutations in NPC1 and NPC2 genes impair cholesterol trafficking and lead to its abnormal accumulation within cells. The utilization of MALDI-2 MSI and microGRID technology offers enhanced sensitivity and spatial resolution down to 5µm, facilitating precise characterization of lipid distributions. Multiple other lipid species were detected, including Phosphatidylethanolamine (PE), Phosphatidylcholine (PC), Sphingomyelin (SM), and Lysophosphatidylcholine (LPC), with some SM species showing higher intensity in the NPC2-/- cell line compared to WT. These observations suggest alterations in sphingomyelin metabolism, further elucidating NPD pathophysiology.

The employment of trapped ion mobility separation (TIMS) allowed for the separation of different forms of gangliosides, providing detailed insights into their composition and distribution. Preliminary data explores AAV-BR1 vector-mediated gene therapy, demonstrating efficacy in transducing BECs to express NPC2, thereby mitigating NPC2-/- mouse brain pathology. MALDI MSI reveals variations in

ganglioside ion intensities (GM1, GM2, and GM3) absent in WT brains but elevated in NPC2-/- PBS treated, suggesting disrupted lipid metabolism. Some AAV-BR1-NPC2 treated NPC2-/- mice show decreased ganglioside ion intensities, aligning with NPD type C-associated neuroinflammatory processes.

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A High-Throughput, In-Depth Investigation into the Immunological Effects of Lipopolysaccharide Challenged Mice in Neat Plasma on Orbitrap Astral Mass Spectrometer

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Lipopolysaccharide (LPS) inhalation stimulates an immune response in mice and serves as a preclinical model for investigating potential therapeutics for inflammatory related disorders such as acute bronchitis. Here mice were treated and challenged with LPS and compared to healthy, unchallenged mice to gain insights into immunological response and identify key down regulated and upregulated proteins of importance. Traditionally, neat plasma requires long runtimes of over 1hr in order to achieve the depth of coverage needed for meaningful insights by mass spectrometry. Here the Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer was optimized and assessed for high-throughput analysis while still maintaining depth of coverage.

Methods

Neat plasma from five biological replicates of LPS challenged and unchallenged mice were prepared by automated sample preparation on the Thermo Scientific™ AccelerOme™ with LysC/trypsin digestion for separation by Thermo Scientific™ Vanquish™ Neo™ and detection by Orbitrap Astral™ mass spectrometer. Separation was performed by reverse phase gradient with Ionopticks Aurora and EASY-Spray™ PepMap™ columns for various sample throughputs of 20SPD, 60SPD, and 180SPD to compare depth of coverage and quantitative accuracy. Three technical replicates were acquired to assess instrument reproducibility. A gas phase fractionation library was created for the 60SPD method and used to search the data for maximum protein identification. Data collected was analyzed by Thermo Scientific™ Proteome Discoverer™ 3.1, DIANN, and Spectronaut® 18 software for comparison.

Novel Aspect

Orbitrap Astral mass spectrometer optimized for high-throughput and depth of coverage yielded >3600 protein groups in LPS challenged neat plasma.

Preliminary Data or Plenary Speaker Abstract

Over 3600 protein groups and 25400 peptides were detected with the 20SPD method searched with DIANN with no library applied. This was over 40% and 86% improvement over the 60SPD and 180SPD method respectively. Results showed over 93% quantifiable IDs and median coefficient of variance (CV) below 10 % for technical replicates for the different run times. The gas phase fractionation library for 60SPD was used to process results and yielded a 44 % improvement from 1680 protein IDs to 2427 protein IDs. The five biological replicates for the LPS challenged mice versus the unchallenged mice yielded 2169 and 1914 protein IDs respectively and were reproducible and robust across all five biological replicates. The LPS challenged results for the 60SPD method were 76 % quantifiable in all five replicates and 91 % quantifiable in three out of five replicates. Proteins were ranked on a ranked protein list and spanned nearly six order of magnitude and were clustered on a PCA plot to show the differences observed between the two treated and untreated groups. Next, differential expression was performed with a p-value set to < 0.001 and +/- 2 Log2FC as cut-off, revealing 35 proteins upregulated and two downregulated proteins in LPS challenged plasma versus unchallenged plasma.

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Kinetic analysis and ultrasensitive detection of SARS-CoV-2 3CLpro-mediated proteolysis

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

SARS-CoV-2 3CLpro (Mpro/ NSP5 / Main protease) catalyses proteolysis of the SARS-CoV-2 polyprotein at 11 sites, releasing 12 non-structural proteins (NSPs) that promote viral replication. In addition, 3CLpro cleaves human proteins to subvert host anti-viral responses. To date, ~800 3CLpro cleavage sites have been identified in human proteins using in vitro assays; however, <1% of these proteolytic events have been unambiguously validated by mass spectrometry in SARS-CoV-2 infected cells. Thus, the full extent to which 3CLpro alters host cellular pathways during infection remains poorly defined. We hypothesised that time-course analysis of 3CLpro substrate cleavage would reveal kinetically favoured substrates and, in so doing, facilitate targeted MS methods for ultrasensitive detection of 3CLpro-mediated proteolysis in infected cells.

Methods

Peptide-level assay: Known SARS-CoV-2 polyprotein (Orf1ab) 3CLpro cleavage site sequences were used to train models to predict candidate peptide substrates of 3CLpro . 203 of these 14-mer peptide sequences were synthesised, and pooled peptides were incubated (0-4h, 6 timepoints) with purified active or proteolytically inactive (p.C145A) 3CLpro(N = 2). 3CLpro activity was quenched using NEM samples were labelled with TMTpro (16-plex). Protein-level assay: HEK293 native cell lysate was incubated (0-16h, 6 timepoints) with active or inactive 3CLpro(N = 2). Samples were quenched and TMTpro-labelled, then protein N–termini were enriched using Terminal Amine Isotopic Labeling of Substrates (TAILS). Peptides were separated by the Vanquish Neo UHPLC system. Data was acquired on an Orbitrap Ascend in RTS SPS MS3 mode.

Novel Aspect

Novel workflow for kinetic analysis of viral proteases and ultrasensitive detection of substrate cleavage in biologically relevant model systems.

Preliminary Data or Plenary Speaker Abstract

Outcomes from peptide cleavage assays evidenced the high precision of our substrate prediction model – 83% of the highest ranked peptides were efficiently cleaved by 3CLpro, compared with only 41% of lower-ranked sequences and 0% of negative controls. A Comparison of the kinetic parameters (apparent kcat/Km) of peptide cleavage revealed new insights into the sequence specificity of 3CLpro. Protein-level kinetic analysis of 3CLpro substrate cleavage distinguished a subset of proteins that were efficiently cleaved from other proteins that were cleaved only upon extended incubation with highly concentrated active protease. Thereby, we identify the protein substrates of 3CLpro that are most likely to be cleaved during infection at physiologically relevant protease concentrations and timeframes. Ongoing proteomic analyses will allow the ultrasensitive detection of 3CLpro-mediated proteolysis in cell models and during SARS-CoV-2 infection. These approaches can readily be applied to the study of other viral proteases to increase mechanistic understanding of their role in infection.

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Proteomic Insights Unravels 5-Fluorouracil Resistance Mechanisms in Colorectal Cancer: Targeting DNA break for Therapeutic Advancement

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Colorectal cancer remains a leading cause of cancer-related mortality worldwide. 5-Fluorouracil (5-FU) stands as a first-line chemotherapy agent for colorectal cancer due to its inhibition of thymidylate synthase, leading to the disruption of DNA and RNA synthesis and repair. However, the emergence of 5-FU resistance has become the major barrier to therapeutic outcomes. This study aimed to elucidate the molecular mechanisms underlying 5-FU resistance and explore potential clinical implications.

Methods

By establishing 5-FU-resistant cell lines and employing both total proteomics and phosphoproteomics, we gained deep insights into the proteomic landscape and signaling pathways of resistant cancer cells. To confirm the contribution of differentially expressed proteins in drug resistance, we established stable knockdown cell lines using shRNA. The drug resistance signatures were assessed by qPCR and western blot.

Novel Aspect

Our findings provide valuable insights into the molecular patterns of 5-FU resistance and offer potential avenues for therapeutic intervention.

Preliminary Data or Plenary Speaker Abstract

Our data show that TYMS expression is significantly elevated in resistant cells, confirming and supporting its role in mediating 5-FU resistance. Moreover, functional and kinase enrichment analyses indicated pronounced alterations in cell cycle regulation, highlighting CDK1 and CDK2 as potential modulatory kinases. Intriguingly, we observed the phosphorylation of RB1 at S807, which might influence its interaction with E2F, subsequently affecting TYMS expression. This altered RB1-E2F dynamic could play an important role in 5-FU resistance.

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Multiplexing TMT for longitudinal measurement of biomarkers in Duchenne muscular dystrophy.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Blood circulating protein biomarkers are becoming attractive surrogate tools for longitudinal monitoring of disease progression and response to therapies especially in pediatric diseases, such as Duchenne muscular dystrophy (DMD), where other outcome measures are burdensome to patient and subjective. Non mass spectrometry method such as multiplexing aptamer-based technology (e.g., SomaScan) and multiplexing antibody-based assay while robust they are costly and lack specificity for some targets. In this study we tested two mass spectrometry-based proteomics methods (e.g., label free and TMT-MS) for their specificity and accuracy measuring changes in the level circulation biomarkers in longitudinal serum samples of DMD patients and age matched healthy controls. The data was compared to SomaScan data for overlapping biomarkers.

Methods

10 µL aliquots from baseline and years 1 serum samples from same patients were spiked with 2 µg and 4 µg of each of the standard protein (Staph Prot A and bovine beta casein) respectively then processed for depletion of the 14 most abundant proteins. Aliquots containing 50 ug of each depleted sample were reduced and alkylated and further digested with trypsin overnight. The obtained peptides samples were labeled with a six plex TMT Tags. Mixed samples were then fractionated using high pH reversed-phase fractionation kit followed by LC-MS/MS analysis. Same processing was performed on same samples for label free proteome profiling but without TMT tagging. MS data was analyzed using Proteome Discoverer 2.2 against Swiss-Prot Homo sapiens proteins database.

Novel Aspect

TMT method has a poor upper limit of quantification but performed very well when dealing with lower limit of quantification.

Preliminary Data or Plenary Speaker Abstract

Using these two mass spectrometry methods above we identified and quantified 1270 proteins across the different serum samples. Comparing glucocorticoid naïve DMD patients to age matched healthy controls, we identified 70 significantly elevated and 67 significantly decreased in the DMD group relative to the control group. Many of the previously reported biomarkers were confirmed using these two methods. Again, as expected most elevated proteins corresponded to muscle injury proteins (28%) followed by some developmental and inflammation markers while decreased proteins consisted mostly of cell adhesion and extracellular matrix proteins suggesting alteration in the extracellular matrix of the degenerating muscle fibers. TMT and not label free method had a limit in the dynamic range of quantification when dealing with larger fold changes. For example, creatine kinase was found to be 60 to 80-fold elevated in serum of DMD patients compared to healthy controls using affinity-based assays while it was found to be 200-fold and only 8 fold elevated in serum samples of the same DMD patients versus controls when measured by label free and TMT method respectively. The TMT signal suppression of reporter ions has been previously reported and is observed in this study as well. Conversely, TMT performed better than label free method in terms of accurately measuring moderate longitudinal changes in levels of circulating biomarkers. Indeed, longitudinal data generated by TMT method on 80 biomarker targets correlated very well with longitudinal data generated by SomScan on same set of biomarkers using same longitudinal serum samples.

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High-throughput high-resolution data-independent acquisition workflow on an Orbitrap Ascend Tribrid mass spectrometer for accurate label-free quantitation.

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Poster Session: Monday Posters, Exhibition Hall, August 19, 2024, 13:00 - 15:00

Quantitative proteomics is an essential tool for understanding global protein expression and the mechanisms of biological processes and disease states. Accurately quantifying the abundances of proteins of interest in complex samples is a prerequisite for developing suitable statistical models to gain biological insights from experimental data sets. Statistical significance is improved by decreasing variability in measurements and/ or increasing the sample set. The need for analyzing large numbers of samples, especially in clinical and biomarker discovery studies, makes DIA-based workflows an obvious choice for ensuring reproducible quantitative analyses. A suitable analytical workflow addresses the need for reproducible sample preparation, robust separations, high quality quantitative measurements, and reliable data analysis.

Methods

HeLa digest and three-proteome mixtures were loaded onto a 50 cm µPAC Neo HPLC column and separated at a 350 nL/min flow rate in direct injection mode using a Vanquish Neo UHPLC system over 9 min, 30 min, and 60 mins active LC gradients, respectively, before being transferred into the Orbitrap Ascend Tribrid mass spectrometer.
Source parameters, including spray voltage and ion transfer tube temperature, are tunable parameters and must be optimized for the individual setup. Acquired data has been processed by Spectronaut (Biognosys, v18) using a directDIA approach, DIA-NN (v1.8.1) or Thermo Scientific™ Proteome Discoverer™ software (v3.1.0.638) using CHIMERYS™ intelligent search algorithm by MSAID.

Novel Aspect

Deep proteome coverage and excellent quantitation accuracy afforded by the Orbitrap Ascend Tribrid mass spectrometer operated in DIA mode.

Preliminary Data or Plenary Speaker Abstract

Various run times of 9, 30, and 60 minutes were evaluated for a Hela digest standard to meet different throughput needs. With 30 min active gradient, 7,000+ proteins and 47,000+ peptides were identified, along with a protein group CV of approximately 5%, suggesting that the 30 min active gradient method enables relatively high throughput while maximizing identification and quantitative performance. We extended this workflow to 60 min active gradient and successfully identified close to 7,800 proteins and >76,000 peptides, highlighting that deeper proteome coverage can be achieved in the Velocity DIA workflow by using a longer gradient.
To test the quantitative accuracy of the Velocity DIA workflow, we created a 3-proteome mix to mimic biological samples where proteins might be up- or downregulated under different conditions. The Velocity DIA workflow yielded excellent quantitative accuracy across a wide dynamic range with median values extremely close to the theoretical ratios, as well as a narrow distribution of all data points around the median values, indicating high quantitative accuracy and precision of the workflow.
Additionally, the three-proteome mix experiment further highlights the proteome depth that can be achieved with an Orbitrap mass spectrometer. In the 60 min active gradient experiment, > 11,000 protein groups were identified. The numbers of quantified proteins differ by species, with close to

7,500 human protein groups, approximately 3,500 yeast protein groups, and nearly 890 E. coli protein groups (. The data demonstrated deep proteome coverage and excellent quantitation accuracy afforded by the Orbitrap Ascend Tribrid mass spectrometer.
Library-based search in DIA proteomics may enhance proteome coverage and improve peptide identification in certain scenarios. Thus, we re-processed the raw files with library-based search on DIA-NN (v1.8.1). In line with the benefit of library-based search, we observed a 3-10% increase in protein identification with more benefits for shorter gradients.

Clinical ‘omics

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A COMPREHENSIVE LIPIDOMICS APPROACH REVEALS SIMILARITIES BETWEEN THE EFFECTS OF METFORMIN AND DIETARY RESTRICTION IN C. elegans

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Organismal homeostasis relies on a complex metabolic network across different organs, regulated by nutrient availability and extracellular signals, notably from tissues specialized in energy storage. Dietary restriction (DR) is linked to longevity and metabolic adaptations, altering biomarkers of age-associated diseases. Metabolites, lipids, and proteins facilitate intercellular communication, disrupted in metabolic diseases. Understanding DR-induced metabolic changes may reveal mechanisms behind lifespan extension. Metformin, a complex I inhibitor, mimics DR effects on conserved aging pathways. We used lipidomics to examine the effects of metformin on C. elegans lipid fluxes and profiles aiming to identify common mechanisms underlying this intervention and longevity

Methods

The study employed LC/MS-MS and isotopic labeling to analyze the lipidomic profile of C. elegans under dietary restriction (DR) (eat-2 mutant), or metformin (Met) intervention. Wild-type worms (N2) were treated with metformin and labeled for 24 hours with an isotope of palmitic acid 13C16 after the L4 stage. Lipids were extracted using an adapted Matyash Method, which involves a liquid-liquid extraction using a specific mixture of MTBE/MeOH/H2O added in specific proportions and at specific times. We used metabolic flux analysis (MFA) to monitor lipid class metabolic rates in control worms and those under metformin treatment, quantifying labeling and distribution across different lipid species. Data were analyzed using LipidSearch, FreeStyle, and Metaboanalyst software.

Novel Aspect

The study uses comprehensive lipid metabolism analysis to unravel specific lipid pathways associated with C. elegans longevity.

Preliminary Data or Plenary Speaker Abstract

We observed similarities in the lipid profile of worms treated with Met and the eat-2. Met and eat-2 showed lower concentrations of free fatty acids (FFAs) compared to control (N2), except for fatty acids (FA) (16:0) and (18:0) in eat-2 worms. Most phospholipids, ceramides, hexosylceramides, and triglycerides exhibited decreased concentrations in eat-2 and upon Met. However, sphingomyelin (SM) showed increased concentration in Met-treated and eat-2 worms, especially SM containing a chain with 22 carbons. Certain triglycerides and plasmalogens, particularly those containing the polyunsaturated fatty acids (PUFAs) 20:2 and 20:3, were more abundant in Met-treated worms. The lipids labeled with 13C16 palmitate isotopes that increased in worms treated with Met, when compared to N2, included FFAs (16:0; 18:0; 18:1; 18:3; 18:4; 20:1; 20:2; 20:4; 20:5; 22:0; 22:1), phosphatidylcholine containing side chains with the following number of carbons: unsaturations

(16:0; 18:2; 18:3; 20:2; 20:4; 20:5; 22:0), phosphatidylethanolamines (PE) (16:0; 18:0; 18:1; 18:2; 18:3; 22:0), and plasmalogens of PE (pPE) (18:1; 20:4; 20:5; 22:0). In summary, metabolic pathways employed by the worms under DR conditions can also be activated by metformin, affecting metabolism without altering caloric intake. Our research reveals alterations in sphingolipid metabolism, PUFAs bound to TAGs, and pPE in long-lived C. elegans. These findings shed light on potential mechanisms influencing lifespan in C. elegans and suggest new avenues for longevity research.

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Mapping the landscape of pediatric diseases: cross-sectional urinary proteomics reveals case-control differences and developmental dynamics

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The urinary proteome serves as a dynamic reflection of the body's physiological state. In children, it is strongly regulated by rapid age-related changes, organ development, and metabolic maturation, leading to the differential expression of proteins involved in these processes. Identifying these distinctions is crucial for understanding normal physiological mechanisms, determining age-specific biomarkers, and recognizing molecular signatures of pediatric diseases. Furthermore, urinary proteomics is considered an attractive approach in pediatric studies because urine can be collected easily and non-invasively. This study aims to analyze the urinary proteome profile of children in depth through a cross-sectional proteomics approach using a dimethyl-based multiplexed-DIA workflow, with the goal of uncovering age-specific molecular profiles and their implications for pediatric health and disease.

Methods

Our cohort consists of 1000 children aged 3 to 17 years, with a balanced male and female demographic, comprising 112 healthy controls and 888 patients with various pediatric diseases. We prepared the urine samples using a semi-automated on-bead SP3 protocol with MagReSyn® HILIC beads. We then employed a dimethyl-based multiplexed-DIA (mDIA) workflow that incorporates a reference channel labeled with dimethyl Δ0 peptides. For data acquisition, we used the 30SPD gradient of the EvoSep One system on a PepSep column (15 cm length, 75 µm id, 1.5 µm C18 beads) coupled to a Bruker timsTOF HT mass spectrometer. For raw data analysis, we used DIA-NN 1.8.2 beta 34 for protein search, RefQuant for channel normalization, and directLFQ for protein intensity measurement.

Novel Aspect

This study characterizes the urinary proteome in the pediatric population, revealing age-specific dynamics and disease associations using the mDIA workflow.

Preliminary Data or Plenary Speaker Abstract

Using the mDIA workflow, our study identified up to 5,000 distinct protein groups in the entire cohort. Conversely, we identified a median of about 1,200 protein groups per sample, indicating the sparsity of the urine proteome matrix, especially in the pediatric population, which underlies heavy changes in age-related growth, organ development and metabolic maturation. Stratification by age and sex revealed a median coefficient of variation (CV) of protein intensities at 60%, underscoring the considerable biological variability inherent in the urine proteome compared to a median technical CV of 20%.

Regression analysis further elucidated age-dependent variations in proteome composition, reflecting physiological maturation. Notably, proteins crucial for reproductive system development, such as kallikreins in males and mucins in females, exhibited differential enrichment in the urine proteome of children as they undergo puberty. This highlights the intricate hormonal and physiological changes occurring during this critical period of growth and development.

Moreover, our case-control comparisons revealed distinct proteomic signatures linked to prevalent pediatric diseases including cystic fibrosis, celiac disease, and type 1 diabetes mellitus. These findings

not only suggest potential biomarkers and pathways implicated in disease pathogenesis, but also underscore the promising role of urine proteomics in disease diagnosis and monitoring.

This study advances precision medicine through MS-based proteomics in pediatric research. Mapping the pediatric disease landscape provides valuable insights into the molecular mechanisms underlying disease onset and progression, thereby paving the way for early diagnosis and tailored treatment strategies in pediatric patients.

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Evaluation of EvoSep One and Orbitrap Exploris 480 for quantitative DIA phosphoproteomics of low input formalin-fixed paraffin-embedded tissues.

Miss Erin Humphries[1,2], Marius Schliemann[3], Dr Bernhard Kuster[3], Dr Peter Hains[1], Professor Phillip Robinson[1,2]

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Biobanks with formalin-fixed paraffin-embedded (FFPE) tissues have clinical data on patient diagnosis and treatment outcomes making the samples suitable for large-scale cancer biomarker studies. The phosphoproteome is known to play a key role in cell signalling and potentially in tumour development, however, only a handful of studies have investigated the use of FFPE tissues for phosphoproteomics. Here we investigate the suitability of the EvoSep One for sensitive and reproducible phosphoproteomics with FFPE tissues.

Methods

FFPE rat tissue sections were deparaffinised using heptane and methanol, then lysed with a combination of bead beating, Adaptive Focused Acoustic sonication and heating for 10 minutes at 95℃. The protein lysate was SP3 digested and desalted. The phosphoproteome was extracted from 5-50 µg of tryptic peptides using Ti and Zr-IMAC MagReSyn HP magnetic beads and desalted by EvoTip. Phosphopeptides were separated on an EvoSep 15 cm Endurance or Performance column using 15 or 30 samples per day (SPD) and analysed in data-independent acquisition (DIA) mode on an Orbitrap Exploris 480. The data was searched using Spectronaut using the direct DIA workflow with 0.01 identification cut offs.

Novel Aspect

The sensitivity and reproducibility of EvoSep One and Orbitrap Exploris 480 for DIA single-shot phosphoproteomics of FFPE tissues was evaluated.

Preliminary Data or Plenary Speaker Abstract

Using 15 SPD and the Endurance column, >5,000 protein groups were quantified from a single shot injection of 1 µg peptides from FFPE tissue across rat brain, kidney, liver, lung, spleen, and testis. Nearly 3,000 protein groups were quantified in FFPE heart and leg muscle. A single shot phosphopeptide enrichment of 50 µg FFPE tryptic peptides quantified 3,000-8,000 phosphoprecursors with an average Pearson correlation coefficient of 0.99 across replicates. Across eight rat organs, testis had the highest summed intensity of phosphoprecursors. The phosphoproteomes of organs from different rats clustered by organ with correlations ranging from 0.86-0.97, providing evidence that tissue-specific phosphoproteomes of FFPE tissues can be retained through fixation and storage.

To compare the sensitivity of the Performance and Endurance columns, phosphopeptide enrichments of 5, 10, 20, and 40 µg FFPE rat spleen were analysed in duplicate using 15 and 30 SPD. Phosphoprecursor identifications were highest using the Performance column and 15 SPD with no difference in identifications when enriching 20 or 40 µg tryptic peptide. Whilst both columns identified similar numbers of phosphoprecursors across enrichment quantities, the Performance column was quantitatively superior with increased summed quantitation and median log2 intensity of phosphoprecursors. This superiority in quantitative power of the Performance column was evident using 15 SPD however, it was minor using the faster 30 SPD. Reproducibility was highest in higher enrichment loads with a median coefficient of variation below 10% for enrichments of 20 µg or greater.

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Investigation of Altered Metabolites in Healthy Koreans with and without Obesity Following Dapagliflozin Administration

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Dapagliflozin, a selective sodium glucose cotransporter-2 (SGLT2) inhibitor, lowers the blood glucose and body weight by increasing urinary glucose excretion in diabetic people. Several studies have reported SGLT2 inhibitor-induced weight loss in obese people without diabetes, and there is increasing interest in using dapagliflozin as a potential medication for body weight control. Therefore, we aimed to investigate the altered metabolites in both obese and non-obese healthy Koreans before and after dapagliflozin was administrated. After administration of dapagliflozin, 11 and 14 putative metabolites were identified in obese and non-obese groups, respectively. Putative metabolites identified in both groups were related to energy metabolism, including carbohydrates, indoles, carboxylic, and fatty acids.

Methods

Nine obese and thirteen non-obese subjects received oral dapagliflozin (10 mg/day) for five days. Urine samples were collected before and after administration of dapagliflozin. Untargeted metabolic profiling was performed on the two groups: obese (body mass index (BMI)>30 kg/m2) and non-obese (BMI 18.0–25 kg/m2) using ultra high performance liquid chromatography and quadrupole time-of-flight/mass spectrometry. Multivariate statistical analysis and putative metabolites identification (variable importance in the projection value≥1.0, p-value<0.05) were performed using SIMCA and MetaboScape®, respectively.

Novel Aspect

we aimed to investigate the altered metabolites in both obese and non-obese healthy Koreans before and after dapagliflozin was administrated.

Preliminary Data or Plenary Speaker Abstract

Dapagliflozin, a selective sodium glucose cotransporter-2 (SGLT2) inhibitor, lowers the blood glucose and body weight by increasing urinary glucose excretion in diabetic people. Several studies have reported SGLT2 inhibitor-induced weight loss in obese people without diabetes, and there is increasing interest in using dapagliflozin as a potential medication for body weight control. However, studies on the impact of dapagliflozin on weight loss in healthy individuals who are obese are lacking. Therefore, we aimed to investigate the altered metabolites in both obese and non-obese healthy Koreans before and after dapagliflozin was administrated.
Nine obese and thirteen non-obese subjects received oral dapagliflozin (10 mg/day) for five days. Urine samples were collected before and after administration of dapagliflozin. Untargeted metabolic profiling was performed on the two groups: obese (body mass index (BMI)>30 kg/m2) and non-obese (BMI 18.0–25 kg/m2) using ultra high performance liquid chromatography and quadrupole time-of-flight/mass spectrometry. Multivariate statistical analysis and putative metabolites identification (variable importance in the projection value≥1.0, p-value<0.05) were performed using SIMCA and MetaboScape®, respectively.
The orthogonal partial least squares-discriminant analysis score plot showed a clear separation between before and after administration in both groups. After administration of dapagliflozin, 11 and 14 putative metabolites were identified in obese and non-obese groups, respectively. Among putative metabolites, dimethyl hetanoyl carnitine, L-theronine, theobromine and glucose-1-

phosphate showed significant fold change in the comparison before and after administration of dapagliflozin, between obese and non-obese groups. Putative metabolites identified in both groups were related to energy metabolism, including carbohydrates, indoles, carboxylic, and fatty acids. Further study is required to investigate and compare the network related to altered metabolites in obese and non-obese groups after multiple administrations of dapagliflozin.

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Various drug regimens for depression reveals association with complement and immune system networks

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Major depressive disorder (MDD) can present a variety of clinical presentations and has high inter-individual heterogeneity. Multiple studies have suggested various subtype models related to symptoms, etiology, sex, and treatment response. Employing different regimens is common when treating MDD, and identifying effective therapeutics requires time. Frequent treatment attempts and failures can lead to a diagnosis of treatment resistance, and the heterogeneity of treatment responses among individuals makes it difficult to understand and interpret the biological mechanisms underlying MDD. Thus, knowledge of the common mechanisms and changes in biomolecules for different types of medications will help clinicians better understand the medication-induced neurobiological changes in patients with MDD. This will improve our understanding of the pathophysiological mechanisms of drug regimens for MDD.

Methods

This study explored the differentially expressed proteins and commonly altered protein networks across drug treatments by comparing the serum proteomes of patients with MDD treated with drug regimens and untreated patients . Differentially expressed proteins were profiled in non-drug-treated and drug-treated patients with depression using liquid chromatography-mass spectrometry.

Novel Aspect

This study provides information on common molecular and neurobiological mechanisms of action for different types of drug treatments.

Preliminary Data or Plenary Speaker Abstract

Common protein networks affected by different types of medications were studied. Twelve proteins were significantly differentially expressed between drug-treated and non-drug-treated patients with depression. Network analysis of these proteins revealed that networks common to various types of drug treatments for depression are related to the complement system and immunity.

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Discovering Hidden Depths: High-Throughput Proteomics Study for Enhanced Biomarker Discovery

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Cancer claims millions of lives globally every year. An earlier and more timely diagnosis greatly improves the prognostic outcome, emphasizing the need to detect cancer at an earlier stage. Current tests, such as invasive biopsies or costly imaging scans can lack sensitivity or selectivity and are not readily available. For this reason, analyzing blood plasma is widely accepted as a promising technique for biomarker detection, but the complex workflows involved in handling non-invasive blood-based samples have hindered the progress. Here, we assessed the performance of a high-throughput, in-depth workflow for proteomic analysis on an Orbitrap Astral mass spectrometer in a small group of diverse cancer and non-cancer samples, which sets the stage for larger-scale cancer cohort studies and translational research.

Methods

Plasma samples from a mini cancer cohort with aged-matched healthy controls were processed with Proteograph XT Assay from Seer for proteomic analysis. The resulting peptides were separated by EASY-Spray™ PepMap™ Neo columns on a Vanquish™ Neo UHPLC system coupled to an Orbitrap™ Astral™ mass spectrometer operated in DIA mode at 60 or 100 sample per day (SPD) to provide solutions for different throughput need., Each workflow enables translational researchers to choose ann optimized solution for their cohort size and overall throughput need. Quantitation accuracy was accessed through comparing mixtures of chicken and human plasma at different ratios. Proteomics data was analyzed by Chimerys in Proteome Discoverer and DIANN software. Protein group intensity was used for unsupervised classification of patients.

Novel Aspect

Unlocking biomarker discovery with novel high-throughput LC-MS workflows for translational research on an Orbitrap Astral mass spectrometer.

Preliminary Data or Plenary Speaker Abstract

In-depth proteomic analysis has resulted in an increased number of features for precise and accurate sample classification, suggesting a proof-of-concept data on a workflow that provides statistical power for early biomarker discovery in cancer research from blood samples. In the mini cohort study, the plasma samples of various cancers, including melanoma, non-small cell lung cancer (NSCLC), B-cell lymphoma, ovarian and pancreatic cancer together with age- and ethnicity-matched normal plasma samples were prepared and analyzed in an automated, high-throughput approach using the Seer Proteograph XT Assay coupled with an Orbitrap Astral mass spectrometer for proteomic analysis. In total, we were able to identify approximately 7000 protein groups. Our results suggest that the workflow may allow for the identification of subtle disease biomarkers from blood samples as compared to healthy individuals.

To evaluate the quantitation accuracy, which is crucial to translational proteomics for high-confidence biomarker identification, we further compared the data of human and chicken plasma mixed at different ratios. The quantitative results of the observed ratio accurately reflected the expected ratio and demonstrated excellent quantitation accuracy. Furthermore, the coefficient of variation (CV) was less than 10 percent and highlighted the quantitation precision. Together, we demonstrate the excellent quantitation precision and accuracy of our plasma proteomics workflow to support the high-throughput needs of translational research studies.

With the in-depth proteome coverage and excellent quantitation, we further conducted principal component analysis, with unsupervised classification. Our results indicate that we were able to classify the samples based on cancer types, highlighting the performance of the workflow in biomarker discovery. Altogether, our LC-MS workflows on Orbitrap Astral offer extensive coverage along with excellent throughput and quantitation accuracy for proteomics empowering enhanced classification and biomarker discovery. The mini cancer cohort results demonstrate the advancements unprecedented depth of coverage and biomarker detection from Orbitrap Astral mass spectrometer.

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MALDI-IHC/ISH: The Next Generation of High-plex Multiomic Imaging for Spatial Biology

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

MALDI-IHC is a recently introduced top-down MALDI mass spectrometry imaging (MALDI-MSI) approach based on novel photocleavable mass-tags (PCMTs) conjugated to antibody probes. It provides a basis for achieving highly multiplex and multiomic workflows to image both untargeted small molecules and intact targeted proteins on the same tissue sample and even on individual cells. Moreover, multimodal dual-labeled antibody probes enable same-slide mass spectrometry and fluorescent imaging of the targeted intact proteins. Here, we demonstrate new workflows which combine the ability to perform MALDI-MSI of small molecules including lipids, metabolites, drugs, and RNA transcripts using MALDI-based in situ hybridization (MALDI-ISH), specific glycans using PCMT lectin probes, and potentially hundreds of proteins using MALDI-IHC, all on the same sample.

Methods

MALDI-IHC antibody and lectin probes (AmberGen Inc, Billerica MA) have been previously reported for highly multiplex tissue MALDI-MSI. To create the MALDI-ISH probes, PCMTs with an azide reactive group were linked to 5'-thiol modified oligonucleotides available from Advanced Cell Diagnostics (ACD). Fresh frozen (10 µm) tissue slices from hAbetaSAA mouse brain blocks (The Jackson Laboratory) were deposited on microscope slides for MALDI-ISH. Amplification and imaging of selected mRNA transcripts was achieved with a modified RNAscope HiPlex Flex assay (ACD). Multiplex and multiomic imaging of targeted transcripts and protein biomarkers was achieved by combining workflows for MALDI-IHC and MALDI-ISH. In additional workflows, selective glycan detection was achieved using PCMTs conjugated to highly specific recombinant prokaryotic lectins.

Novel Aspect

MALDI-IHC and MALDI-ISH together promise a new highly multiplex and multiomic approach to rapid spatial imaging of tissues and cells.

Preliminary Data or Plenary Speaker Abstract

Fresh frozen whole hAbetaSAA mouse brain tissue sections were utilized to evaluate various workflows. First, conventional MALDI-MSI was performed to image small molecule metabolites and exogenously added drugs followed by matrix removal. For workflows involving MALDI-ISH, tissues were processed with up to 12 PCMT-oligo probes and imaged by MALDI-MSI in ~3 hrs at 20 µm spatial resolution. The spatial distribution of targeted transcripts agreed well with the adjacent slices imaged using similar fluorescent probes purchased from ACD. The MALDI-ISH images of each selected transcript also agreed with the expected distribution based on both the Mouse Whole Brain Atlas and MALDI-IHC imaging from adjacent slices using corresponding PCMT-conjugated antibody probes. For example, both transcripts and the cognate expressed parvalbumin protein were found concentrated in the cerebellum, whereas GFAP transcript and protein were found concentrated in the periventricular regions. Individual amyloid plaques imaged using the PCMT-antibody probes for amyloid β42 appeared colocalized with the GFAP transcripts characteristic of astrocytic immune inflammatory response. Different workflows were evaluated for the same-slide detection of mRNA transcripts, proteins, and glycans using a combination of MALDI-IHC and MALDI-ISH protocols. Autofluorescence as well as the need for reiterative cycling is eliminated compared to conventional fluorescence-based approaches. Importantly, MALDI-IHC/ISH can be performed using conventional MALDI-MSI instruments instead of specialized instruments needed for cyclic fluorescence imaging or Imaging Mass Cytometry (IMC). Post-MALDI H&E staining of samples and optical bright-field imaging also enabled single cell multimodal and multiomic image analysis. In general, these workflows with

MALDI-IHC and MALDI-ISH provide a promising new highly multiplex and multiomic whole-slide spatial imaging approach which can rapidly profile a variety of different biomarkers from tissues and cells.

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Integrated proteomics and single cell RNAseq reveal that steroid resistance in severe asthma is driven by immune cell “Stasis”

Dr Matthew O'Rourke[1], Prof Phil Hansbro
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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Severe asthma accounts for only 10% of all asthma cases but is responsible for 80% of all asthma associated costs. Typified by a resistance to inhaled glucocorticoid (GC) drugs, the pathology of severe asthma includes airway remodeling, neutrophilic infiltration, and chronic inflammation. Currently, little is being done to identify the mechanisms that drive treatment resistance, with previous efforts predominantly focused on developing new GC drugs/drug combinations or attempting to sensitize patients who are resistant. With this in mind, it is vitally important to identify the mechanisms of resistance in severe asthma in order to develop new therapeutic approaches and move beyond GC treatments.

Methods

In this study we utilized an ovalbumin/chlamydia murine model to induce severe asthma in BALB/C6 mice followed by either control or dexamethasone (DMSO) treatment to mimic GC treatment. Mice were euthanized, lungs removed, and parallel shotgun proteomics and single cell RNA sequencing was performed on whole lung lobes. Single cell data was integrated with proteomics using a “deep exploration” approach focusing on validating changes to actual biology rather than high level KEGG pathway or GO term analysis.

Novel Aspect

This work has described the first possible mechanism for steroid resistance in severe asthma, Cell "Stasis".

Preliminary Data or Plenary Speaker Abstract

Deep exploration revealed that when treated with DMSO, T-Cells and neutrophils both exhibited significant downregulations of almost all cell cycle checkpoint genes and cell development pathways. This was in conjunction with an upregulation of genes responsible for neutrophilic recruitment and infiltration, and a downregulation of genes responsible for neutrophilic inflammation. P53 mediated cell senescence was also upregulated in both proteomics and single cell datasets as well as a downregulation of cytokine based immune suppressors. It was also found that immune cell senescence protein and gene markers were upregulated in all other immune cell types as well. Based on these findings, we determined that in severe asthma, treatment with steroids may cause immune cells to lose function and become "Static". These immature cells then initiate feedback loops causing increased recruitment of neutrophils and T-cells which exhibit a chronic low level inflammatory response with a loss of inflammation regulatory control. High populations of neutrophils are histologically diagnostic for severe asthma further confirming these observations. This has led us to hypothesize that the current therapeutic protocol of high dose GC drugs for severe asthma patients may cause worsening of symptoms over time necessitating further research into GC alternative therapies.

Comparison of Plasma and Serum Samples for Lipidomic Profiling: Early Screening for High-risk Groups of Pancreatic Cancer

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Lipids are biologically active compounds that are involved in many metabolic processes, and the dysregulation of lipid concentrations in body fluids may reflect various pathological states, such as cancer development. Special attention should be paid to the preanalytical part to avoid the degradation or contamination of samples, which can negatively affect analytical results. Plasma and serum are the most used blood derivatives for clinical analysis, but different blood collection tubes and protocols are used for their isolation. UHPSFC/MS represents a high-throughput techniques for lipidomic analysis of biological samples, which uses the lipid class separation approach and leads to the coelution of the internal standards and analytes, guaranteeing the same matrix effect and providing the best conditions for accurate quantitation.

Methods

The Acquity UPC2 was connected to the high-resolution mass spectrometer Xevo G2-XS QTOF. Lipid class separation approach was performed under the following conditions: Viridis BEH column (100×3 mm, 1.7 µm), flow rate 1.9 mL/min, injection volume 1 µL, and column temperature 60°C. Gradient elution with total run time 8.0 min was set, where the mobile phase A was carbon dioxide, and the mobile phase B was methanol containing 30 mM ammonium acetate and 1% water.
Modified Folch extraction procedure was used for the lipidomic extraction of 25 µL of human plasma or serum spiked by 20 µL of internal standard mixture. The final extract was dissolved in 500 µL of mixture CHCl3/MeOH (1:1,v/v) and diluted 10 times before UHPSFC/MS analysis.

Novel Aspect

Adjustment of preanalytical part and confirmation of correctness of the method for early screening of high-risk groups with pancreatic cancer.

Preliminary Data or Plenary Speaker Abstract

The aim of this study is to investigate the effect of blood sample types (EDTA plasma vs. serum) on lipidomic profiling based on analysis of 218 healthy controls and 177 pancreatic cancer patients collected at the same time. We quantified 143 lipid species from 8 lipid classes using ultrahigh-performance supercritical fluid chromatography coupled with mass spectrometry (UHPSFC/MS). We observed comparable concentrations of lipids in EDTA plasma and serum samples for investigated lipids. However, the evaluation of the effect of gender on the lipid profile showed statistically significant differences (downregulation of TG and DG and up-regulation of SM for females) leading to the evaluation of lipidomic profiles separately for males and females. Finally, classification models were prepared for the results representing individual matrices. Although both matrices show accuracy higher than 90% in both matrices, we decided to use EDTA plasma for early screening of high-risk groups of pancreatic cancer. Moreover, the prediction of plasma samples in models built from serum samples and vice versa was investigated and an accuracy is still over 90%, showing the possibility of combining both matrices, for example, in studies with rare retrospective samples.
The lifetime risk of developing pancreatic cancer among high-risk groups is >5% compared to the general population, where the risk is around 1.5%. High-risk groups are defined according to the

recommendations of the American Society for Gastrointestinal Endoscopy, and they include individuals with genetic mutations (BRCA1, BRCA2, PALB2, Peutz-Jeghers syndrome, Lynch syndrome and hereditary pancreatitis) and familial pancreatic cancer. Preliminary data show successful results for the diagnosis of high-risk groups of PaC, where 93 samples were evaluated with 94% specificity.

This work was supported by the Project No. NU21-03-00499 (Czech Health Research Council) and Project No. 101095860 (ERC Advanced grant).

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Proteomic analysis of high-density lipoprotein (HDL) particles to investigate the therapeutic mechanism of action in a Phase 2B clinical trial

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The function of HDL particles is thought to play an important protective role against atherogenesis and clinical atherosclerotic cardiovascular disease (CVD). The molecular process known as reverse cholesterol transport (RCT), whereby excess cholesterol is removed from atherosclerotic plaques and transported back to the liver for excretion, is thought to be pivotal in modulating disease. RCT encompasses three key stages including cholesterol efflux from plaque, cholesterol esterification within HDL particles, and hepatocyte cholesterol uptake and clearance. Thus, enhancing RTC has been the focus of therapeutic efforts to combat CVD.

Methods

A subset of samples from 50 patients, which were administered either drug or placebo and plasma collected at various longitudinal time points, was selected. A method was developed to immuno-isolate HDL from 450 plasma samples. HDL particles were then eluted, denatured, and digested with trypsin. Peptides were separated using an Evosep One (30 SPD method), and mass spectrometry was performed on a Q-Exactive instrument. Label-free quantitation was conducted using MaxQuant, and biostatistics performed using in-house developed pipelines.

Novel Aspect

This study provides critical experimental evidence to support the molecular understanding that this therapeutic treatment promotes RCT.

Preliminary Data or Plenary Speaker Abstract

A total of 97 proteins were identified and quantified across patients and longitudinal time points, including many primary well-characterised HDL constituents. Drug treatment induced comprehensive HDL protein remodelling, with 18 out of the total 97 proteins displaying a statistically significant change in abundance. Most importantly, drug treatment displaced several RCT inhibitors in the remodelled HDL, while simultaneously enriching several RCT activators. Therefore, drug treatment promotes RCT, and this finding was further supported by ex vivo and in vitro assays.

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MALDI Glycotypying of O-antigens of E.coli and Y. pseudotuberculosis

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS) enables high-resolution and high-sensitivity measurement of biomolecules. Recently, MALDI-MS has become widely adopted as a tool for microbial species identification in clinical settings due to its rapidity and simplicity. However, its application is limited to species identification, and distinguishing antigen types remains challenging. The identification of O-antigens commonly involves the use of PCR or immunologic test. However, these methods require specific antibodies or PCR probes corresponding to each antigen. O-antigens exhibit diverse structures, making it complex and time-consuming to prepare and utilize probes for each of them. In this study, we developed MALDI glycotyping, a rapid and simple method for identifying O-antigens in Escherichia coli and Yersinia pseudotuberculosis without the need for probes.

Methods

The colonies of Escherichia coli and Yersinia pseudotuberculosis strains were inoculated and suspended in water. After centrifugation and removal of the supernatant twice, the solution was adjusted to an O.D. value of 1.8-2.0. 1.5 µL of the bacterial strain solution was transferred to a PCR tube and mixed with 0.5 µL of hydrochloric acid (final concentration 100 mM), then incubated at 90 ℃ for 10 minutes. After centrifugation, 0.35 µL of the supernatant was added to a MALDI plate and dried. Immediately, DAN/DHB/Na matrix was added and dried. MALDI-TOF MS (Ultraflex III, Bruker) was used for the measurements. The DAN/DHB/Na matrix was adjusted to a final concentration of 2 mM 1,5-diaminonaphthalene, 10 mM 2,5-dihydroxybenzoic acid, and 1 mM sodium bicarbonate.

Novel Aspect

We developed a rapid and simple method for identifying O-antigens without the need for probes using MALDI-MS.

Preliminary Data or Plenary Speaker Abstract

In this study, we developed a method called MALDI glycotypying to identify O-antigens from a single colony of E. coli using MALDI-MS, without the need for column or HILIC operations, within one hour. This method enabled the rapid determination of the molecular masses of the repeating unit and components of O-antigens, facilitating their identification. Measurements of O1-type, O6-type, and O157-type E. coli strains revealed distinct signal patterns attributed to variations in constituent elements. Notably, the measurements of two different O157-type E. coli strains showed closely similar signal patterns, indicating identical constituent elements despite their differences. These signal patterns facilitated the identification of variants present in strains with several reported variants of O-antigens, such as O1 and O6. Additionally, this method allowed for the parallel identification of O-antigens present in ten colony from agar medium inoculated with mixed E. coli strains. Additionally, we demonstrated the ability to simultaneously measure ribosomal proteins and O-antigens from a single colony. Therefore, by integrating the detection of ribosomal proteins with this O-antigen identification method, MALDI glycotypying, we anticipated achieving microbial species identification and serotype identification solely using MALDI-TOF MS. Furthermore, MALDI glycotyping enabled the measurement of O antigens in Y. pseudotuberculosis, a Gram-negative bacterium other than E. coli. Interestingly, despite the identical molecular masses of constituent elements in the repeating structures of O-antigens in Y. tuberculosis, we demonstrated the ability to distinguish each of two different O-antigen based on differences in the detected signal patterns.

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Comparing the active and passive sampling of volatiles emitted from disaster victims

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The search for victims in mass disasters is a long-established challenge that is aided greatly by the use of canines. Search-and-rescue (SaR) dogs and cadaver detection dogs (CDDs) use their keen sense of smell to locate living and deceased victims, relying on the volatile organic compounds (VOCs) produced through ante-mortem metabolic processes and post-mortem decomposition, respectively. Despite their successes, these canines are expensive to train and deploy. There is also a lack of knowledge surrounding what these canines are scenting, with little work being done on characterising the VOCs emitted from victims in these disaster events. This research aimed to determine the most suitable method for the in situ collection of VOCs in a disaster.

Methods

The work was conducted at the Australian Facility for Taphonomic Experimental Research (AFTER), where two trials were performed to simulate mass causality events. In Trial 1, four human donors were placed in a simulated building collapse and allowed to decompose for two weeks. Trial 2 utilised six donors and aimed to simulate a helicopter crash, where remains were placed under rubble and allowed to decompose for one week. Passive samples were collected during both trials, where sorbent tubes were placed over the rubble and passively collected volatiles for 24 hrs. Comparatively, VOCs were actively collected at 100 mL/min for 1 min (Trial 1) and 10 min (Trial 2), and all samples were analysed using two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOFMS).

Novel Aspect

Profiling ante- and post-mortem volatiles will aid in developing future portable technologies to locate living and deceased mass disaster victims.

Preliminary Data or Plenary Speaker Abstract

Both one minute and ten minutes of active collection yielded similar results to 24 hrs of passive collection, which highlights the benefit of active sampling (low-sampling time and a customisable sample volume and rate). Active collection should thus be utilised to determine the volatile profiles of both living and deceased individuals.

Forensics, Sports Doping, Homeland Security

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Verification of Novichok precursors via alkyl chloroformate derivatisation and GC MS/GC NPD analysis

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The Organisation for the Prohibition of Chemical Weapons (OPCW) is an international organisation responsible for enforcing the Chemical Weapons Convention (CWC). The OPCW consists of 193 member States, all committed to eliminating the development, production, and use of chemical weapons (CWs). As a member state, Australia strongly supports the OPCW verification regime, and maintains an OPCW designated laboratory within Defence Science and Technology Group (DSTG). Novichoks are fourth-generation organophosphorus compounds that are a challenge to identify and verify, with little published literature available. The precursor amidine compounds have poor chromatography. The application of alkyl chloroformate chemistry in the development of novel derivatisation methodologies for identification of Novichok precursors is an important contribution to verifying alleged usage of these chemical agents.

Methods

Alkyl chloroformate (25 µL) was added to a mixture of amidine (CH2Cl2, 100 µg/mL, 500 µL) and aqueous NaOH (1 M, 500 µL). The mixture was shaken (2000 rpm, 10 min, RT). The organic layer was then washed (H_2O, 1 mL), dried ($MgSO_4$), and filtered (0.45 µm, PTFE). Sample was diluted to 10 µg/mL and subsequently analysed by GC-MS/GC-NPD.
Dual analytical columns (DB-5ms Ultra Inert 30 m x 0.25 mm x 0.25 µm) were connected to MS and nitrogen-phosphorous detectors. GC oven was programmed at 40°C (3 min hold), ramped to 300°C (10°C/min, 3 min hold). MSD operated in EI mode (70 eV), full scan from m/z 40-650. NPD operated at 300°C, detector gas flow rates at 3:60:10 mL/min (H_2:Air:N_2).

Novel Aspect

Application of alkyl chloroformate chemistry in the development of novel derivatisation methods for the verification of Novichok precursors by GC-MS/GC-NPD.

Preliminary Data or Plenary Speaker Abstract

Eleven different amidines were prepared and characterised in this study. Amidines of interest ranged from N,N-dimethylethyl to N,N-dihexylethyl substituted variants; N,N-diethylethyl to N,N-diethylhexyl substituted variants; or those with a combination of substitutions at both positions. The corresponding methyl chloroformate derivatives were also prepared and characterised, as well as the ethyl chloroformate derivative of N,N-diethylethanimidamide.

Mass spectral data was collected for each precursor and derivative, along with retention time (MSD, NPD), retention index (MSD), and limits of detection (MSD, NPD). Poor chromatography was observed during the GC analysis of all amidines. The limit of detection for each amidine ranged from 20-5 µg/mL on the MSD, with the mode being 7.5 µg/mL. The limit of detection on the NPD ranged from 20-1 µg/mL, with the mode being 5 µg/mL.

In contrast, the respective carbamate derivatives showed improved chromatography with sharper, more symmetrical peaks. The limit of detection for each derivative on the MSD ranged from 0.25-0.10 µg/mL, with the mode being 0.25 µg/mL, representing a 30-fold improvement in sensitivity. Similarly, the detection limit on the NPD ranged from 0.25-0.10 µg/mL, with the mode being 0.10 µg/mL, representing a 50-fold improvement in sensitivity.
This improved sensitivity exceeds the requirements outlined by the OPCW with regards to their proficiency testing scheme, where test sample matrices are to be spiked with the analyte of interest at a minimum concentration of 1 µg/mL.

To demonstrate method repeatability, each amidine was successfully derivatised in triplicate using methyl chloroformate. N,N-diethylethanimidamide was also successfully derivatised in triplicate using ethyl chloroformate. All derivatised samples were easily detected at 10 µg/mL (MSD, NPD). Further work will include spiking the amidine into previous OPCW proficiency test samples to determine method suitability for more complex matrices, including aqueous and organic liquids, and solids. Stability studies of the derivatised products are also in progress.

Cannabinoid and Terpene Content of Homemade DIY BHO and Rosin Extracts

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Cannabis extracts (or concentrates) are often produced by consumers at home via different Do-It-Yourself (DIY) methodology, for the purpose of vaporisation. Two key methods for making extracts are solventless rosin press extraction and gas-solvent extraction, often referred to as Butane Honey/Hash Oil (BHO). A range of sources online detail methodology on how to create these extracts at home using readily available materials. Between methods presented online, variables (such as temperatures, extraction times, solvent evaporation methods etc.) exist that may affect the final composition (terpene and cannabinoid levels) of the produced extract.

Methods

This project involved investigation and compilation of online sources (YouTube, Reddit, online forums) showcasing individuals' methods for creating cannabis extracts for vaporisation to identify different variables for extraction procedures. Common DIY methods were then replicated in the laboratory using commercially available medicinal cannabis flower and materials that can be obtained by the Australian general public from sources such as supermarkets, hardware stores and online vendors. Differences in cannabinoid content in cannabis extracts prepared using different methodologies were investigated using LC-MS/MS MRM. Variances in terpene levels within cannabis extracts prepared using different methodologies were also investigated using GC-MS.

Novel Aspect

This project is the first to analytically determine the influence of manufacture procedures on final content of DIY cannabis extracts.

Preliminary Data or Plenary Speaker Abstract

This project is the first of its kind examining and comparing the range of methods currently employed by the general public to generate cannabis homemade extracts for vaporisation. When comparing the gas based BHO extracts to rosin extracts it was identified that there is a difference in final composition, with rosin samples containing more decarboxylated (activated) cannabinoids than BHO samples. Rosin extracts, which are produced with heat, have a cannabinoid profile considerably different to the initial plant material used, with higher decarboxylated cannabinoid content and lower terpene levels. BHO samples have terpene and cannabinoid content close to the original cannabis material. In addition variables within method types effect the final content of extracts. An example for BHO methods is that winterisation (freezing before extraction) produces extracts with higher cannabinoid content than non-winterised BHO extraction. One example for rosin production is that higher temperatures used in rosin pressing gives higher THC to THCa ratios. Overall, the results of these analyses indicate that differences in cannabis extract preparation (such as temperature, extraction time and solvent evaporation methods) do have an effect on the cannabinoid and terpene levels and profiles in extracted products that are subsequently vaporised by consumers.

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Rapid analysis of Δ9-THC-COOH and Δ8-THC-COOH in urine sample by the automated SPE and GC-MS

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Cannabis is the most widely used drug worldwide, with an estimated 219 million users in 2021 (4.3 % of the world's adult population). With the increase in drug abuse cases, there is a critical need for advancements in drug analysis technology and efficiency. In this study, automated solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS) were employed to analyze Δ9-THC-COOH, a criterion for identifying cannabis abuse in urine samples, as well as its isomer, Δ8-THC-COOH. The introduction of automated SPE is anticipated to improve analysis efficiency by over 60 times compared to current manual methods. Consequently, this research is expected to significantly contribute to the acceleration of drug investigations and the reduction of global drug abuse.

Methods

After alkaline hydrolysis of 3 mL of urine using 5 M KOH, automated SPE was conducted as follows: conditioning with methanol and water, washing with 0.1 M NaOH, water, and hexane after loading, and elution using ethyl acetate. Then, trimethylsilyl (TMS) derivatization was performed using BSTFA with 1 % TMS (N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane) and was analyzed by GC-MS. A DB-5MS UI (30 m × 0.25 mm, 0.25 µm) was utilized as the GC column. The injection volume was 1 µL, and the injection mode and acquisition mode were set to splitless and SIM/SCAN mode, respectively. The entire process was completed within 2 hours.

Novel Aspect

The introduction of automated methods is expected to increase efficiency by over 60 times compared to current manual methods.

Preliminary Data or Plenary Speaker Abstract

When cannabis is inhaled, approximately 99 % of the inhaled amount is metabolized into THC-COOH-glucuronide and excreted in urine. Consequently, alkaline hydrolysis was performed to convert THC-COOH-glucuronide into THC-COOH. Subsequently, SPE was carried out to effectively remove interfering substances in urine, followed by analysis using GC-MS. Various types of SPE cartridges were compared and optimized, and parameters of the automated SPE device such as positive pressure and drying time were fine-tuned to ensure optimal recovery and reproducibility. Additionally, the pipette tip height was adjusted to prevent cross-contamination during the automated SPE process. Moreover, optimization of the derivatization reaction solvent, temperature and time was conducted for detection at low concentrations. The optimized method was verified for selectivity, linearity, recovery, accuracy, precision, dilution integrity, and stability according to the FDA Guidance for Industry (2018). The limit of detection for both Δ9-THC-COOH and Δ8-THC-COOH was established as 1 ng/mL, with the R2 (correlation coefficient) more than 0.999. Comparison before and after pretreatment revealed a recovery rate approaching 100 %, and intra-day and inter-day repeated experiments demonstrated both accuracy and coefficients of variation (CV) below 15 %. Furthermore, when 1 µg/mL of urine outside the calibration range was diluted 20 times, and before and after storage for 24 hours in the autosampler, accuracy and CV below 15 %. The applicability of the verified method was confirmed by analyzing the urine of 12 actual cannabis smokers who were confirmed positive on an immunoassay analyzer (cobas). The implementation of automated preprocessing methods enables efficient drug evaluation and monitoring, with the potential to contribute to the prevention of drug abuse.

IsoRep – A Low-Code Approach to Exploring and Visualizing Stable Isotope Analysis Data

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The Defense POW/MIA Accounting Agency (DPAA) Laboratory is the largest skeletal identification laboratory in the world whose mission is to provide the fullest possible accounting of missing and unidentified United States' military personnel. The DPAA Laboratory's Isotope Program was accredited in 2019, demonstrating that stable isotope analysis data can help answer specific questions regarding population origin and commingling of human remains.

As the Isotope Program expands, it has identified the need for a custom, scalable data repository that meets forensic standards. The research surrounding development of that repository is presented here. This research provides a reference for validating and managing stable isotope analysis data using a low-code platform, enabling researchers to design solutions without coding expertise or costly software.

Methods

The Isotope Repository (IsoRep) is a relational database that stores sample information and test results from sample preparation and analysis. IsoRep was designed on the Microsoft Power Platform, a collection of cloud services typically included with government, business, and educational Microsoft 365 licenses. Isotope data are uploaded directly from controlled Excel templates into SharePoint, which acts as
the data storage and primary access control. Authorized personnel initiate automated data upload protocols via a Power App user interface that triggers Power Automate flows. IsoRep data are automatically extracted daily into Power BI, where they are transformed and loaded into the relational model for exploration and visualization.

Novel Aspect

IsoRep is innovative because it utilizes a low-code platform for development and is being validated under forensic testing laboratory standards.

Preliminary Data or Plenary Speaker Abstract

IsoRep currently houses approximately 2,500 isotope sample preparation results, with upload of analysis test results forthcoming. Once complete, IsoRep will facilitate isotope data retrieval and drive research initiatives, novel method development, laboratory task coordination, and quality control monitoring in the DPAA Laboratory's Isotope Program.

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Detection and accurate mass measurement of difficult to handle negatively charged compounds using Chemical Ionization (CIMS)

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Here we present an interface system that will allow for the nominal and accurate mass measurement of negatively charged compounds that, due to physical properties of volatility and or air sensitivity, are difficult to detect and measure.
These physical properties has resulted in the need to develop an interface system to deal with this problem.
The technique eliminates the need to have the expense of a fume hood, stainless-steel tubing and connections, an Ammonia cylinder and head.

Methods

A Thermo MAT 900XP sector mass spectrometer was coupled to a cooled box sample inlet system maintained at 30^{o}c.
A small glass reservoir containing ammonium hydroxide was coupled into the fused deactivated silica inlet line and allowed to bleed directly into the CI source. A low flow of nitrogen gas was passed through the line producing a source of ammonia into the mass spectrometer.
Samples were presented into the source using a direct insertion probe and measured.

Novel Aspect

Development of the cooled inlet system to measure negatively charged compounds.

Preliminary Data or Plenary Speaker Abstract

Samples that are difficult to detect, were chosen. These samples were a mixture of perfluoronated and lithium salt compounds. These samples were presented into the instrument using a direct insertion probe.
The difficulty of negative CI MS is the requirement to have an ammonia gas supply.
Normally, this requires the use of expensive stainless-steel tubing and fittings, an ammonia cylinder and head and the need to have a locally sited fume hood to house the cylinder.
We no longer need to have this. By inserting a small glass reservoir of ammonia hydroxide into a deactivated silica glass line within the cooled inlet system, has eliminated the need for the ammonia cylinder.
Passing a low flow of nitrogen gas through the line, gives us a source of ammonia.
Presenting the samples into the mass spectrometer using a direct insertion probe allowed us to observe the peaks of interest and measure their nominal and accurate masses.

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Development of an LC-MS Extensive Fecal Library for Advancing Microbiome Metabolomics

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Recent studies underline the crucial role of the human gut microbiome in maintaining a healthy gastrointestinal tract and influencing pathological processes. The fecal metabolome is also increasingly studied for clinical diagnosis potential, revealing insights into the microbiome's impact on health.
Targeted MS-metabolomics identifies and quantifies fecal metabolites such as amino acids, fatty acids, and bile acids. However, limitations including instrument speed and selectivity can lead to lowering the number of targets. A comprehensive fecal metabolites library was built on a novel mass spectrometer combining fast scan speed, high selectivity, and detection sensitivity. The developed library was then used to evaluate metabolic variations in mice fecal samples collected from hypoxia- and dietary-based interventions.

Methods

A Fecal Metabolites Library from MetaSci was utilized for the library development. Fecal samples were collected from 8-week-old mice in either room air (21% FiO2) or normobaric hypoxia chambers (8% FiO2). Within each oxygen treatment, mice were fed one of three diets, each with 15% fat kcal%. Diets differed by fat source: standard (soybean oil), SFA-rich (90% cocoa butter, 10% soybean oil), and MUFA-rich (90% olive oil, 10% soybean oil). After 29 days in each condition, fresh fecal samples were collected.
Metabolites were extracted using 80% methanol and separated via Thermo Scientific™ reversed-phase and HILIC columns. A novel mass spectrometer provided fast scanning MS/MS and MSn levels for precise annotation and extended quantitation used for data acquisition.

Novel Aspect

A comprehensive fecal library using a fast, sensitive, and selective novel mass spectrometer enabling high throughput and accurate microbiome metabolomics.

Preliminary Data or Plenary Speaker Abstract

The new mass analyzer's high scan speed reduced LC acquisition time, crucial for high-throughput analyses with sample stability challenges. In addition, the MSn-based quantitation provided the selectivity necessary for detecting and accurately quantifying co-eluting isomers and isobars, improving discrimination between analyte signals and those from matrix interferences.
The established library allowed reliable quantitation over a broad dynamic range (5 orders of magnitude) in fecal components, with an improved sensitivity reflected in a low limit of quantification of 10 femtomoles and a low limit of detection of 0.25 femtomoles for most targets. Assessment of isotopically labeled internal standards demonstrated high data quality, reliability, and robust measurement, evidenced by minimal chromatographic shift and consistent signal responses, as indicated by low % CV for quality control and sample replicates.
This study enables the assessment of metabolic profile differences in the mice fecal samples across various groups. For instance, variations in bile acids were noted among the different dietary samples, indicating potential metabolic impacts of diet on the studied parameters.

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Enhancing Biotransformation Product Identification Using Multi Reflecting Time-of-Flight MS PPB Mass Accuracy and Fine Isotope Structure

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[1]Waters Corporation, [2]Waters Corporation

Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Unbiased data independent acquisition (DIA) to identify small molecule drug metabolites, has been used to assess the LC-MS accurate mass measurement specificity attained using a high mass resolving power (>200,000 FWHM) hybrid quadrupole multi-reflecting time-of-flight (Q-MRT) mass spectrometer. High mass resolving power enhances ion selectivity and subsequently the detection of analytes in complex matrices. Precursor/fragment ion part per billion (ppb) mass accuracy enhances analyte identification confidence and facilitates use of stringent data processing tolerances, in metabolite identification research. Resolution Enhancement Mode (REM) has a system resolving power >300,000 FWHM and delivers fine isotope structure (FIS) with ppb mass accuracy providing highly specific identification criterion and is a powerful tool to determine elemental composition and facilitate confident assignment of metabolite identifications.

Methods

LC-MSE ES+ and ES- data independent analysis (DIA) precursor/fragment ion data acquisition was performed using a multi-reflecting quadrupole time-of-flight mass spectrometer (system resolving power >200,000 FWHM and 300,000 FWHM (REM)). Human urine samples (time course points 0 hours, 2 hours, 4 hours, and 6 hours post dose) were analysed, using reversed phase separation liquid chromatography (0.1% v/v formic acid in H2O) and (0.1% v/v formic acid in acetonitrile)), comprising a 12-minute gradient at a flow rate of 0.5 mL/min, using a C18 (100 mm x 2.1 mm, 1.8 µm) column at 40ºC. 5 µL injection volumes of urine samples 1:10 diluted (H2O) were utilised.

Novel Aspect

DIA of xenobiotics and metabolites in complex biological matrices using a high mass resolving power quadrupole multi-reflecting time-of-flight mass spectrometry.

Preliminary Data or Plenary Speaker Abstract

Initially, using a single time course point sample post-acquisition processing involved comparison with a comprehensive library of 1343 pharmaceutical compounds. The library comprised retention time (tr), precursor ion and fragment ions values. Acetaminophen, naproxen and carbamazepine were identified, using tolerances of tr (0.1 min) and precursor mass accuracy (+/-2ppm), product ion count ≥1 and expected fragment ion tolerance (0.2 mDa).
Subsequently these stringent screening tolerances were applied in a non-targeted post-acquisition metabolite identification workflow to identify administered pharmaceutical compounds and corresponding biotransformation products. Repeat analysis of human urine time course point samples has been performed. System mass accuracy performance has been monitored over a 24-hour period for the urinary screening analysis. An RMS error of 549 ppb has been determined for 2651 detections of parent drugs and identified metabolites as well as ppb fragment ion mass accuracy has been observed.
Metabolites of xenobiotic therapeutics acetaminophen, naproxen and carbamazepine have been identified. RMS mass measurement error for acetaminophen (444 ppb), acetaminophen sulphate (527 ppb) and acetaminophen glucuronide (538 ppb) have been attained.
Using high mass resolving power MSE DIA fragment ions are also generated and mass resolved from matrix interferences, for [acetaminophen glucuronide + H]+ acquired at 10 Hz, fragment ion mass measurement errors of -88 ppb (m/z 110 (>126,000 FWHM)) and 118 ppb (m/z 152 (>162,000)) have been observed.

In addition to precursor and fragment ion mass accuracy, fine isotope structure has provided an additional characteristic identification criterion, where further investigations utilising a method to increase the mass scale resolution to >300,000 FWHM have been performed. In this study we demonstrated the benefit of REM and present identified biotransformation product, including their resolved fine isotope distributions which incorporate 2H, 13C, 15N, 18O, 33S and 34S.
“For Research Use Only. Not for use in diagnostic procedures.”

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Simultaneous quantitation and discovery (SQUAD) metabolomics workflow for the analysis of fecal bile acids

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Bile acids (BA) are synthesized from cholesterol in the liver and play a crucial role in lipid digestion and absorption. Fecal bile acids are known to serve as biomarkers and signaling molecules due to their intricate interplay with gut microbiota. Disordered microbiomes can alter the composition and size of the bile acid pool, producing a variety of conjugated bile acids and structurally similar metabolites. These changes may indirectly confer disease states.
Here an LC-MS metabolomics workflow was developed for the simultaneous quantitation and discovery (SQUAD) of fecal BA and BA conjugates. The workflow utilizes Real-Time Library Search (RTLS) for spectral similarity measures and heightened identification confidence for molecular species during method execution.

Methods

Human stool material and BA standards (unlabeled and isotopically labeled) were obtained from NIST and CIL, respectively. Feces were spiked with the labeled standards and extracted with 80% methanol. Metabolites were separated on a reversed-phase column contained within the UHPLC system. Data were acquired on an Orbitrap IQ-X™ Tribrid™ mass spectrometer, which facilitates sensitive and highly dynamic PRM-quantitation utilizing the linear ion trap. It also allows orbitrap MS1 scanning for higher annotation rates. AcquireX™ and RTLS workflows were used to maximize the number of relevant compounds interrogated by MS2 and MS3, for confident annotation.

Novel Aspect

SQUAD metabolomics workflow for parallel targeted quantitation, deep coverage, and confident annotation of fecal bile acids.

Preliminary Data or Plenary Speaker Abstract

The standard NIST fecal sample spiked with isotopically labeled BA was used as a proof of concept to evaluate a simultaneous quantitation and discovery (SQUAD) metabolomics workflow for BA analysis on an IQ-X. Unlabeled and labeled BA standards were used to generate calibration curves for absolute quantitation. This workflow facilitates a reliable ion trap quantitation over a wide dynamic range (i.e., 5 – 6 orders of magnitudes) of the targeted BA in feces. A lower limit of quantification (LLOQ) of 10 femtomoles and a lower limit of detection (LLOD) of 0.25 femtomoles was observed for most of the targets. Moreover, MSn-based-quantitation of the mass analyzer enabled the selectivity required to detect and accurately quantify co-eluting isomers and isobars. It also leads to improved discrimination between signals derived from analytes and those resulting from matrix interferences. Simultaneously, the HRAM Orbitrap data and the increased percentage of fragmented compounds using the advanced deep scan AcquireX workflow resulted in improved annotation capability compared to traditional DDA on a wider dynamic range of fecal compounds. In addition, Real-Time Library Search provided spectral similarity measures and identification confidence scores for BA species upon which acquisition decision-making can be based during method execution.
This study enables the assessment of bile acids and other metabolic profile differences in mice fecal samples collected from a dietary-based intervention across various groups (i.e., soy oil, soy and olive oils, and coconut butter and soy oil) due to improved annotation capabilities of the HRAM data. Finally, confidence in the targeted and untargeted MS-based workflow's quality was obtained by regularly monitoring the spiked IS.

High data quality, reliability, and robustness of measurement were observed by evaluating the performance of isotopically labeled compounds over time through metric tracking (e.g., retention time, mass accuracy, signal response).

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Simultaneous quantitation and discovery (SQUAD): an intelligent combination of targeted and untargeted metabolomics workflow for the analysis of fecal metabolites.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

In the absence of internal standards and libraries, untargeted metabolomics faces challenges in accurately quantifying and identifying metabolites crucial for studying biological systems. This deficiency can complicate study designs and data processing, prompting many researchers to opt for targeting a limited number of analytes, despite the risk of overlooking important compounds. Hence, a single-injection simultaneous quantitation and discovery (SQUAD) metabolomics workflow is favored, ensuring confident identification and precise quantitation of predetermined metabolites using authentic standards, while preserving the integrity of untargeted analysis that facilitates the discovery of metabolites with potential biological importance.
The faster MS2 scanning of the high-resolution MS Orbitrap Astral™ enables sensitive quantitation of numerous metabolites through Astral PRM, simultaneously, ensuring robust unknown annotation with comprehensive sample coverage.

Methods

We utilized a Fecal Metabolites Library from MetaSci to develop a library. Fecal samples were obtained from 8-week-old mice exposed to either room air (21% FiO2) or normobaric hypoxia chambers (8% FiO2). Under each oxygen treatment, mice were assigned one of three diets, each containing 15% fat kcal%. These diets varied in fat source: standard (soybean oil), SFA-rich (cocoa butter and soybean oil), and MUFA-rich (olive and soybean oils). Fresh fecal samples were then collected after 29 days in each condition.
Metabolites were extracted with 80% methanol and separated using Thermo reversed-phase and HILIC columns. Data was acquired on a Thermo Scientific™ Orbitrap Astral™, which allows faster scanning on the MS2 level for a higher annotation rate and accurate quantitation.

Novel Aspect

SQUAD metabolomics workflow for targeted quantitation, and deeper coverage with confident annotation of fecal metabolites using the Orbitrap Astral MS.

Preliminary Data or Plenary Speaker Abstract

In this study, SQUAD analysis on Orbitrap Astral enables reliable quantitation of a broad range of targeted components in mice feces with heightened sensitivity. Furthermore, the SQUAD workflow revealed a substantial compound fragmentation rate (>80%), ensuring fragmentation of lower-abundance compounds within complex matrices like feces. The enhanced MS2 fragmentation ratio is attributed to the novel mass spectrometer's faster scanning rates, enabling more confident annotation of unknown compounds.
The instrument's performance was assessed by evaluating data quality, reliability, and measurement robustness for selected metabolites where metrics such as retention time, mass accuracy, and signal response were utilized. Minimal chromatographic shift and consistent signal responses were observed, as indicated by low % CV for sample replicates. Sub-ppm mass accuracy was consistently achieved for all targets throughout the acquisition period.
This study allows for the evaluation of variations in metabolic profiles among fecal samples from different groups of mice, which is used to facilitate the understanding of diet and oxygen levels in the mice model.

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Metabolic Tracing in P. falciparum Using a Stable Isotope Labelling Strategy

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Metabolic profiling has emerged as a pivotal tool in understanding the biochemical pathways in Plasmodium, the causative agent of malaria, which remains a major public health challenge. This study employs stable isotope labeling (SIL) with 13C_6-glucose to dissect metabolic changes in infected red blood cells (iRBCs) versus uninfected red blood cells (uRBCs). By integrating high-resolution mass spectrometry (HRMS) via Orbitrap analysis, we aim to unravel the metabolic alterations induced by Plasmodium infection and glucose metabolism, contributing to the development of novel therapeutic strategies.

Methods

Plasmodium cultures were incubated with ^13C_6-glucose for varying periods during different hours post-infection of the intraerythrocytic cycle to obtain temporal information. Control groups were treated with ^12C_6-glucose in both infected red blood cells (iRBC) and uninfected red blood cells (uRBC). After incubation, metabolites were extracted from the Plasmodium culture and analyzed using Orbitrap high-resolution mass spectrometry (HRMS) for untargeted metabolomics profiling. The IDEOM tool was employed for feature identification, and the average intensity ratios of ^13C to ^12C labeled metabolites in iRBC samples were calculated. This approach was used to identify metabolites derived from ^13C-glucose, enabling the investigation of Plasmodium's metabolic adaptations.

Novel Aspect

Unveiling Plasmodium metabolic pathways and host-parasite interactions using ^13C-glucose labeling and Orbitrap HRMS.

Preliminary Data or Plenary Speaker Abstract

The application of 13C_6-glucose in Plasmodium culture followed by Orbitrap HRMS analysis facilitated the identification of metabolites in iRBC and uRBC. Preliminary results demonstrate distinct metabolic profiles between infected and uninfected cells. In the 13C-glucose iRBC group, multiple metabolites exhibited significant labeling, indicating active glucose-related metabolism and utilization by Plasmodium. Comparative analysis with control groups revealed specific metabolic pathways altered by the parasite, including glycolysis, pentose phosphate pathway, and amino acid metabolism. These findings highlight the parasite's metabolic flexibility and potential vulnerabilities. Moreover, the study underscores the utility of SIL coupled with HRMS in elucidating host-parasite interactions and metabolic networks, providing a comprehensive overview of Plasmodium metabolism and identifying potential targets for therapeutic intervention.

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Semi-targeted, high-resolution mass spectrometry analysis of thiols in wine

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Thiols can be potent aroma compounds contributing to desirable flavours sought in some wine varieties. Sensitivity and isolation of these compounds are improved by using selective derivatising agents which bind to the thiol group, increasing ionisation efficiency and affording characteristic fragmentation ions. While various derivatising agents have been applied previously, ebselen, a selenium-containing thiol derivatising agent, coupled with high resolution mass spectrometry (HRMS), enables thiol-specific semi-targeted analysis. Data obtained through such experiments can then be easily interpreted due to the distinct fragmentation pattern, capitalising on both the distinctive Se isotope pattern and unique MS^2 loss. This study focused specifically on the measurement, presence, and contribution of thiols, particularly those bound to ebselen, to tentatively identify compounds of sensory significance.

Methods

Twenty-four wines were selected to study flint aroma in Chardonnay from a larger survey set using notes from a preliminary informal tasting by AWRI staff from the sensory and research teams. The wines underwent quantitative descriptive analysis using a trained sensory panel.
Semi-targeted analysis was undertaken using the method of Vichi et al. (2015; https://doi.org/10.1016/j.foodchem.2014.11.095) with minor modifications. Extracts were separated and analysed using reverse-phase liquid chromatography coupled to an Orbitrap ID X. Data-dependent MS^2 was triggered using a targeted isotope ratio filter set to monitor the selenium-78:80:82 isotope ratio. Data was analysed using Compound Discoverer with a modified vendor-supplied workflow.

Novel Aspect

Using semi-targeted analysis to tentatively identify compounds of sensory significance.

Preliminary Data or Plenary Speaker Abstract

To process acquired HRMS data, an isotope-specific workflow supplied by the vendor was modified to ensure that Se-containing compounds were identified. From this workflow, 4,958 features were detected in the samples. Using appropriate filtering, the number of features was reduced to 136 corresponding to ebselen-bound thiols. Filtering to include derivatised components was greatly enhanced through analysis of a pooled control, both with and without the addition of the derivatising agent.
While the derivatising agent increases sensitivity, it also complicates structural elucidation as there is no automated way to account for the derivatising agent. Thus, one must manually remove the derivatising agent mass from the MS^1 mass prior to library searching. To reduce the amount of time required for this manual adjustment, the 136 features were further reduced by statistical comparison to sensory data.
As the end goal from this approach was to identify thiols contributing to flint aroma, the features were statistically compared to sensorial qualities, with a focus on increase flint character. This resulted in 19 features being identified as having a positive correlation with flint aroma. Extensive user input was then required to propose tentative molecular identifications for these 19 features; 14 features were tentatively assigned compounds while 5 were tentatively assigned by chemical formulas.
Two features were most strongly related to flint character and could be tentatively assigned as 4-oxo-2-sulfanylpentanoic acid and 4-(1-hydroxy-2-sulfanylethyl)-2-methoxyphenol.

Future work could entail isolating the tentatively identified compounds to confirm their identification, determine their sensory characteristics in wine, and develop a quantification method if warranted.

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DLLME factors optimization using RSM and DF for UHPLC–QTOF–MS organic pollutants detection in water samples: environmental assessment.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Pharmaceuticals and Personal Care Products (PPCPs) are organic micropollutants that are becoming a global health risk humans to aquatic ecosystems and humans. Numerous studies utilizing a range of analytical techniques have been reported in the literature, describing the existence of PPCPs in various environmental matrices and compartments. So far, nevertheless, not much research has been done in South Africa to quantify the degree of environmental contamination caused by these pollutants. The primary focus of this research is to develop a simple and efficient analytical method for the determination of residual PPCPs contamination using dispersive liquid–liquid microextraction (DLLME) and liquid chromatography mass spectrometry (LC–MS).

Methods

: Prior to analytical determination, the DLLME sample enrichment procedure was also developed and optimized for the determination of mifepristone, benzophenone, eicosapentaenoic acid, and docosahexaenoic acid in wastewater treatment plant influent and effluent, as well as river water receiving WWTP effluent discharge, using central composite design (CCD) with desirability function (DF). The optimal conditions were achieved with 195 µL of tetrachloroethylene as an extraction solvent, 1439 µL of acetonitrile as a disperser solvent, and sample pH of 5.8.

Novel Aspect

The compounds under investigation, specifically polyunsaturated fatty acids (PUFAs), docosahexaenoic acid, and eicosapentaenoic acid, may represent novel sources of pollution.

Preliminary Data or Plenary Speaker Abstract

The developed method's analytical method detection limits (MDLs) ranged from 0.11–0.48 µg/L, as indicated by method performance data analysis. The developed method was applied for the determination of the selected analytes in environmental aquatic samples. Only benzophenone was detected in the concentration range of 0.79–0.88 µg/L across all the water samples. The aquatic ecology and human health were shown to be at minimal potential risk, according to the computed risk quotient arising from benzophenone exposure in water samples. Analytical Eco–Scale (AES), Green Analytical Procedure Index (GAPI), Analytical GREEnness (AGREE), and Analytical Greenness for Sample Preparation (AGREEprep) were among the metrics tools used to further assess the method's environmental impact. Of these, only AES qualified it as green. The current method, though not entirely green, is simple and effective for determining the selected compounds and has the potential to be expanded to detect other compounds with similar physicochemical properties as the targeted compounds in various water samples.

GCxGC-High Resolution-TOFMS with Multi-Modal Ionization: Comprehensive Nontargeted Screening of Micro- and Nanoplastics and POPs in Environmental Samples

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Plastic production started early in the 20th century and has dramatically changed the materials we use every day. These materials can be tailor-made for specific applications by adjusting their composition to increase strength, durability, etc. Enormous quantities of plastic waste have been generated each year and are expected to grow to up to 26 billion tons by 2050. Breakdown products of plastic waste, micro- (100nm – 5mm) and nanoplastic (<100nm) are of particular concern because of the ease of their distribution in the environment. Micro/nanoplastics are ubiquitous, and chronic exposure via inhalation or ingestion can be detrimental to human health. This is compounded by the fact that persistent pollutants can be adsorbed by these materials and cause further harm.

Methods

The combination of comprehensive two-dimensional chromatography with high-resolution time-of-flight mass spectrometry is a powerful analytical technique for nontargeted screening of complex samples due to the combination of enhanced chromatographic separation with high-speed acquisition of full mass range, and accurate mass spectra. Analyte identification using Electron Ionization followed by commercial or custom library search is a common but not always highly reliable approach. The absence or low abundance of molecular ions and similarities of fragmentation patterns often lead to erroneous analyte assignments. The novel multi-mode source capable of providing Electron Ionization and Chemical Ionization was coupled with multi-reflecting HR-TOFMS for GC-MS and GCxGC-MS. The use of complimentary soft ionization methods is greatly improving confidence in the nontargeted analysis results.

Novel Aspect

Combination of GCxGC, high mass accuracy of HR-TOFMS, and multimode ionization source for nontargeted analysis of complex samples.

Preliminary Data or Plenary Speaker Abstract

The goal of this study was to identify micro- and nanoplastics in environmental air, collected via particulate matter sampling at several urban and rural sites, as well as characterize adsorbed persistent organic pollutants (POPs) associated with these materials. The characterization of these pollutants is challenging due to their chemical diversity and trace concentrations in complex plastic samples. Many POPs are present in different forms (i.e., congeners, structural isomers, and stereoisomers). Furthermore, analysis by conventional GC-MS or LC-MS methods is hampered by overlapping chromatographic peaks and isobaric interferences. In this study, we implemented the use of thermal desorption and pyrolysis to identify adsorbed compounds, as well as characterize the plastic materials. Sample analyses were carried out in two steps: 1) Thermal desorption and 2) pyrolysis, with each stage followed by chromatographic separations and detection using comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometry (GCxGC-TOFMS). Electron Ionization (EI) data were utilized to annotate compounds through untargeted processing with spectral similarity searches of large databases. Additional confirmation steps such as matching retention indices, literature confirmation, elemental composition characterization via the accurate mass of the molecular ions and fragments, and others increase analyte assignment reliability and confidence. Positive Chemical Ionization and Electron Capture Negative Ionization spectra provided complementary molecular formula information to increase confidence in compound annotation. The technology resulted in comprehensive data with exceptional peak

alignment between ionization modes, better S/N, and an increase in the total number of compounds identified. The analyses resulted in the identification of polymers via detecting polymer additives (plasticizers, light and heat stability agents, flame retardants, slip additives, etc.) and polymer pyrolytic markers. The adsorbed analytes such as aromatics, polyaromatics, including hetero aromatics, and bisphenols were also detected and assigned with high confidence.

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Developing and Implementing a Signal Handling Approach for More Accurate Metabolite Elemental Composition Determination using Orbitraps

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

High-resolution mass spectrometry (HRMS) plays a pivotal role in metabolome profiling, offering precise mass measurements and facilitating determining metabolite elemental compositions. However, Ultrahigh-Resolution Mass Spectrometry (UHRMS), particularly Orbitrap MS (OT-MS), faces challenges in accurate metabolite elemental composition determination due to ion decay during UHRMS OT scans. This study presents a novel signal-handling approach to minimize mass and Relative Isotopic Abundance (RIA) errors in UHR-MS mode OT-MS, focusing on lipidome analysis.

Methods

To enhance metabolite elemental composition determination with UHR-MS, we developed a software tool named isotopic ratio and mass correction (imCorrect). This tool preprocesses LC-OT-MS raw data, reducing noise and signal instability, and utilizes a set of reference signals with known mass and RIA values to learn mass and RIA shifts in different ion intensities and backgrounds. Subsequently, imCorrect corrects detected metabolite signals, enabling accurate elemental formula determination. Performance evaluation was conducted on spiked and endogenous lipids from plant extracts.

Novel Aspect

A new approach to minimize isotopic pattern errors in OT-MS analysis, improving the accuracy of metabolite elemental composition determination

Preliminary Data or Plenary Speaker Abstract

The imCorrect software was initially employed for analyzing tomato leaf lysates spiked with a blend of 15 lipid standards, utilizing LC systemic coupling to an LTQ Orbitrap Elite operated at a resolution of 240K. Reference signals generated from cluster ions injected with sodium acetate solution pre and post lipid elution were utilized for mass and RIA correction. Upon processing and correcting the LC-MS raw data, the root mean square (RMS) of RIA errors decreased from 8.09 to 2.25, demonstrating the efficacy of imCorrect. Moreover, formula assignments improved significantly, with only 7 lipids correctly assigned without imCorrect compared to 14 lipids with its application. For 96 endogenous lipids identified through data-dependent acquisition (DDA) LC-MS/MS analysis, imCorrect reduced the RMS of RIA errors from 3.96 to 2.70, leading to an increase in correct formula assignments from 66 to 76. Additionally, when analyzing Arabidopsis leaf extracts using LC-MS based on Orbitrap IQ-X, imCorrect facilitated the correct identification of 238 and 203 lipids at resolutions of 240K and 480K, respectively.

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Direct analysis in real-time mass spectrometry (DART-MS) applications in the pasture-to-plate continuum

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

DART-MS enables real-time ionisation of samples with nil/minimal sample preparation, under ambient atmospheric conditions. Solid, liquid or gas samples are exposed to heated and metastable helium (He) atoms. Penning ionisation is the dominant reaction, where He atoms generate cluster (hydronium) ions with atmospheric water, and the desorbed surface analytes react with these clusters to form protonated ([M+H]+) molecules. A pioneer in the ambient MS group of techniques, DART-MS has found numerous applications across forensics, pesticide detection, drug detection, pharmacology, food quality and safety, and volatile compound analysis. Here, a compendium of studies using DART-MS in the pasture-to-plate continuum is presented.

Happy to present as poster if required.

Methods

Targeted/Nontargeted metabolomics approaches/workflows have been used in conjunction with a DART SVP source attached to a low resolution QDa (Waters) or high resolution LTQ mass spectrometer (Thermo Fisher). Samples with nil/minimal sample preparation were analysed in positive and negative ionisation modes with the DART source maintained at 250^{o}C.

Novel Aspect

High throughput detection of targeted chemicals and sample discrimination using DART-MS in agricultural metabolomics, expediates no/go decisions, fostering sustainable solutions.

Preliminary Data or Plenary Speaker Abstract

DCD (Dicyandiamide; C2H4N4), a synthetic nitrification inhibitor, with detrimental effects on human health was identified in soil samples. Residues of DiuronTM (N-(3,4-Dichlorophenyl)-N,N-dimethylurea; C9H10Cl2N2O), a broad spectrum herbicide, were detected in ryegrass seeds. Ryegrass cultivars with preferential intake by livestock were screened using raw leaves, and animal samples were used to shortlist biomarkers that distinguish low methane and/or nitrogen production. Finally, beef samples that were cooked by three different methods (pan fry, oven cooked and sous vide) were classified based on their volatile organic compound (VOC) fingerprints.

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Use of Unique GC-TOFMS Technology Enabling Fast GC & Hydrogen Carrier Gas – A Method Transfer Overview & Application Examples.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Helium is a limited natural resource with variations in both supply and expense. At the same time, the advantages of hydrogen to achieve increased chromatographic throughput in Gas Chromatography applications is attractive. The transferal of methods to hydrogen carrier gas can be problematic due to limitations and challenges associated with some types of mass spectrometry technology used. In particular, the mass spectral quality & fragmentation, in comparison with that obtained using helium, as well system sensitivity, robustness and acquisition rate are important factors which can differ and negatively affect results. In this study we have investigated the use of a particular type of GC-TOF-MS technology to overcome these issues. The method transfer approach, technology benefits, and application examples are presented.

Methods

A LECO Pegasus BT Gas Chromatography-Time-of-Flight Mass Spectrometry (GC-TOFMS) instrument was used to investigate the method transfer of the analysis of semivolatile compounds in waste water samples from Helium to Hydrogen carrier gas. The method was first optimised using Helium carrier gas and a Restek EZGC method translator was used to investigate which chromatography conditions would be optimal when using Hydrogen as a carrier gas. The fast data acquisition speed, full mass range and unskewed data collected along with the Stay-Clean open ion source design incorporated withing the instrument, eliminating the need for cleaning and ensuring very similar spectral fragmentation when using either Helium or Hydrogen as a carrier gas, were investigated.

Novel Aspect

GC methods transfer to hydrogen as a GC carrier gas and performance evaluation of the GC-TOFMS in wastewater analysis applications.

Preliminary Data or Plenary Speaker Abstract

A step wise method transfer process, transferring to smaller internal diameter capillary columns allowed chromatographic resolution to be maintained whilst improving analysis speeds by greater than a factor of four.
Further, mass spectrometry performance using both helium and hydrogen was evaluated by comparing mass spectral fragmentation similarities, response linearity, precision and sensitivity.
In all cases, performance data was found to be highly similar, resulting in the conclusion that the instrumental technology used is highly suitable for the use of hydrogen as a carrier gas.

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Rapid high-resolution screening for drugs of abuse with library searching on a novel acoustic ejection HRMS with triple quadrupole confirmation

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Laboratories typically choose immunoassays for drug screening in the urine despite drawbacks such as low specificity and the time needed for antibody incubation. Furthermore, high immunoassay cutoff values lead to false negatives.
Here, we utilized a novel acoustic ejection HRMS to rapidly screen urine samples at a rate of five seconds per sample for the presence of drugs of abuse and metabolites. Mass spectral library searching of the MS/MS fragmentation pattern was performed to identify the substances in the sample.
For confirmation, a microflow liquid chromatography device was coupled to a triple quadrupole mass spectrometer. Samples were injected onto a C18 column after dilution with water in the 384-well plate. A multiple reaction monitoring (MRM) method confirmed the screening results.

Methods

Directly in a 384-well plate, human urine samples were diluted 10-fold in 5% methanol in water. A total of 5 µL of hydrolysis enzyme was added to the plate. The plate was then shaken at 600 RPM for 30 minutes at 55°C.
A total of 70 nL of diluted urine was acoustically ejected at a rate of five seconds per sample. Samples were analyzed using a data-dependent acquisition (DDA) method with and without an inclusion list. The data were processed where a high-resolution TOF-MS/MS library search identified the drugs and metabolites in each sample. The data were filtered by drug and metabolite groups.

Novel Aspect

The novel acoustic ejection HRMS analyzed 208 urine samples in under 20 minutes and provided high-resolution spectral library confirmation

Preliminary Data or Plenary Speaker Abstract

The total time to scan all 208 samples and barcodes was 18.31 minutes. All compounds and their metabolites present in each sample are presented in one wide peak. The library searches were used as a means of separation. Isobaric compounds, such as morphine and hydromorphone, and codeine and hydrocodone were differentiated via their library spectrum. Many samples showed library hits for multiple drugs and metabolites.
We chose to hydrolyze our sample set to cleave the glucuronide and measure the parent drug or metabolite. Good data quality could be achieved with 10% enzyme in the sample well. However, if hydrolysis is not required, the analysis could be completed on diluted urine samples without the addition of a hydrolysis enzyme. The spectral library detected nonconjugated and conjugated drug forms from a nonhydrolyzed sample.

Calibrators for the acoustic ejection HRMS ranged in concentration from 4.8 ng/mL to 5000 ng/mL. MDMA was detected as low as 4.8 ng/mL, while most other drugs were detected at 39 ng/mL. For definitive LC-MS/MS testing, the 384-well plate was directly diluted and injected from the 384-well plate without any additional sample preparation. The definitive microflow method separated isobaric compounds in less than 0.9 minutes and could detect drugs and metabolites at 390.6 pg/mL.

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Exploring sex differences in zebrafish livers using a novel targeted discovery metabolomics approach

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Targeted workflows using triple quadrupole (QQQ) instruments have recently gained more attention in the field of metabolomics. The targeted aspect, namely having to decide which metabolites to measure before data acquisition, is a barrier for untargeted metabolomics. However, we have shown previously that even a QQQ instrument can be used to acquire data with the same level of metabolite coverage as conventional untargeted metabolomics experiments. As new nominal mass instruments with high acquisition rates, sensitivity, and advanced features like MSn emerge, our goal is to expand the boundaries of what can be achieved through a targeted approach and propose an alternative to untargeted analysis. Using a targeted discovery metabolomics experiment, we measured liver extracts from male and female zebrafish.

Methods

Livers from adult male and female zebrafish (Danio rerio) were harvested. Polar metabolites were extracted with 2:2:1 acetonitrile/methanol/water (40 µL per mg wet weight). A pooled sample was prepared using an aliquot of all samples. Untargeted metabolomics data were acquired using hydrophilic interaction liquid chromatography coupled to a high-resolution mass spectrometer. After feature detection and grouping using Compound Discoverer 3.4, a list of identified and unidentified compounds was generated and used as a precursor list for the new hybrid nominal mass instrument. Data were acquired in parallel reaction monitoring (PRM) mode using fast polarity switching and stepped collision energies.

Novel Aspect

A streamlined approach from untargeted metabolomics to a targeted discovery methodology to determine biological sex differences.

Preliminary Data or Plenary Speaker Abstract

Currently, most mass spectrometry-based untargeted metabolomics studies are performed on high-resolution accurate mass instruments. There are certain limitations to this approach such as complicated data processing. Using a triple quadrupole mass spectrometer requires knowing each compound’s precursor and product ions prior to data acquisition. While we have previously shown that this is doable, a PRM approach streamlines this process and only the compound precursor ions need to be known while all product ions are simultaneously detected.
Using a new hybrid nominal mass instrument, we used a targeted PRM method to profile 763 metabolites in liver extracts from male and female adult zebrafish. The precursor list with 0.5 to 3 min wide isolation windows was generated based on discovery untargeted high-resolution data. A brief collision energy (CE) optimization using HCD and CID with different CEs was performed. Multiple HCD collision energies can be used to compensate for different optimal CEs for each individual metabolite and its fragments. We found that HCD with NCE 10, 20, and 40 yielded the highest product ion intensities. We obtained excellent coefficients of variation of less than 10% for most metabolites for our pooled quality control samples. The data showed major differences in metabolite levels between male and female livers with more than 30% of the compounds having a p-value < 0.05 (after Benjamini-Hochberg correction) and a $|FC| \geq 1.5$. Amino acids, such as threonine, were higher in female livers. Uridine 5'-diphosphoglucuronic acid was 7 times higher in female livers whereas methylhistidine was 2 times higher in males.

Additionally, the new hybrid nominal mass instrument can perform MSn fragmentation. Using an MS3 method to characterize the fatty acid composition of triglycerides, we found that while most lipids are increased in female livers, lipids containing FA 18:2 are lower in females compared to males.

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The Importance of High-Throughput Mass Spectrometry in Synthetic Biology

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Synthetic biology is becoming an essential field of research especially in a small island country like Singapore. The wide applications of synthetic biology provide potential solutions to a number of Singapore's sustainable development goals including responsible consumption and production, zero hunger, and industry innovation. Metabolic and enzyme engineering are two key research themes at NUS SynCTI, which generates thousands of samples especially when mutagenesis is involved. As such, high-throughput screening methods are imperative to identify improved mutant candidates relative to wild-type. The Agilent RapidFire (RF) is one of SynCTI's workhorse for high-throughput mass spectrometry analysis, and a diverse range of analytes is possible on this platform.

Methods

The enzymes and substrate were placed in 2-mL tubes and allowed to react at 50C for either 30 or 60 mins in a thermomixer. Isopropanol was then added to extract the analytes and then further diluted with acetonitrile. The diluted samples were transferred to a 96-well plate for injection into the Agilent RapidFire (RF) system coupled to either an Agilent QTOF 6550 or an Agilent Triple Quadrupole 6495. The RF-MS method was optimised using dilute solutions of analytical standards. The samples were injected twice to analyse compounds both on positive and negative modes.

Novel Aspect

Improved throughput for fast screening of enzymes in synthetic biology

Preliminary Data or Plenary Speaker Abstract

RF-MS can be used for detection of different analytes with a wide variety of polarity, i.e., from oligonucleotides to nonpolar lipids. In addition, small polar metabolites can be derivatised to improve their adsorption onto the RF cartridge. In this study, RF cartridges C, E, and F were tested for their ability to retain the lipid analytes. Results showed that cartridge C, which has C18 chemistry can retain most of the analytes; thus, yielding to higher signal intensities. In addition, elution solvents were also varied to improve the signal of the analytes. The most common elution solvent for the RF system is acetonitrile. However, because of the nonpolar nature of the analytes, isopropanol was the best solvent for elution as evidenced by the higher signal intensities. By using the optimised RF-MS methods, analysis of a 96-well plate can be accomplished within 30 mins with 2 blank runs after each sample injection.
Results show the difference in the activity of the enzymes. Enzyme B yielded more products in the form of free fatty acids compared to Enzyme A. Aside from this, results show that the presence of water improves the production of the hydrolysis products. Future studies will involve mixing other enzymes and testing other reaction parameters including reaction temperature and time in order to find the best combination set to obtain the highest yield of products.

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Improved proteome coverage and reproducibility in large scale analyses using packed emitter columns

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Proteomic analyses of large cohorts of human patient samples plays a critical role in discovering novel biomarkers for diagnosis and response to treatment. Deep proteome coverage and reproducibility of results is essential for the success of these studies. Here, we introduce a novel packed emitter chromatography column, the IonOpticks Aurora Rapid 8x150 (8 cm x 150 µm inner diameter, 1.7 µm C18), designed to improve the performance of large-scale plasma proteomic analyses. By analysing weekly quality control injections between plasma samples, this study evaluated the efficiency and reproducibility of the column for high-throughput proteomic analysis of large-scale plasma cohorts coupled with an Evosep One and a Thermo Fisher Orbitrap Astral mass spectrometer.

Methods

LC-MS Analysis: A HeLa tryptic digest (200 ng) was separated on Aurora Rapid 8 cm x 150 µm column on a 60SPD method using Evosep One and Thermo Fisher Orbitrap Astral Mass Spectrometer.
MS parameters: mass range of MS(Orbi)/MS2(Astral) from 380 to 980, isolation windows of 2Th, a maximum injection time (IT) of 3 ms, FAIMS compensation voltage (CV) set at -40, and a resolution for MS1 set at 120,000.
Data Processing: The data were processed using DIA-NN software (version 1.8.1) with match between runs enabled. The pg.matrix.tsv, stats.tsv and pr.matrix.tsv tables were used to calculate protein and peptide identifications and metrics.

Novel Aspect

Packed emitter columns enable consistent performance and reproducibility across large patient sample cohorts

Preliminary Data or Plenary Speaker Abstract

To assess system performance and data reproducibility, patient plasma samples were analysed using 100 samples per day method with approximately 700 samples run between quality control (QC) injections. Weekly injections of HeLa tryptic peptides were performed using a 60 samples per day method to monitor system performance. The system achieved robust protein identifications, with over 9,300 proteins and 100,000 unique peptides identified per QC run across multiple columns. To assess the reproducibility of protein quantification across multiple QC runs and columns, protein intensities were used to calculate a coefficient of variation (CV) and Pearson correlation, revealing uniform performance with low CV values and high correlation between runs and between columns. This reproducible performance, along with consistently narrow peak widths averaging at 2.9 seconds, indicates good column quality and reproducibility, important for large patient cohort analyses. Furthermore, to ensure reliable data analysis, we evaluated retention time stability. This analysis demonstrated a high level of consistency, with a 2.52% median CV of retention times. Additionally, a four-month experiment on 16 selected peptides demonstrated stable retention times. Our data demonstrates that the novel packed emitter column combined with the Evosep One and Thermo Fisher Orbitrap Astral Mass Spectrometer enabled maximum protein and peptide identifications whilst also ensuring robust and reproducible protein quantification across a large patient plasma cohort analysis.

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High-speed compound quality assessment using Acoustic Ejection Mass Spectrometry

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The drug development cycle is a lengthy and expensive process, taking 12-13 years and costing $1-2 billion. It starts with high-throughput screening of thousands to millions of candidates. Rapid and accurate identification of drug candidates is crucial. NMR is impractical due to low throughput and large sample volume requirements. LC-MS/UV, while accurate, is inefficient for large libraries due to long analysis times. Advances in automation and miniaturization have led to chemical libraries in 384- or 1536-well formats, allowing compounds to be stored in assay-ready plates, reducing costs. Acoustic Ejection Mass Spectrometry (AEMS) offers rapid results with high mass accuracy. Using the Echo® MS+ with the ZenoTOF 7600 system, we achieved the rapid identification of 45 compounds within 4 minutes.

Methods

To simulate a compound library setting, 45 compounds for positive or negative ionization modes were selected, along with deuterated internal standards for 2 analytes and a negative control. The analytes were dissolved in DMSO to a final concentration of 10 µg/mL. A 50 µL aliquot was transferred into an Echo® MS qualified 384-well plate, centrifuged at 4,000 rpm for 10 minutes, and shaken for 5 minutes. Acoustic ejection used 0.1% formic acid in methanol at 350 µL/min with an ejection volume of 2.5 nL and a 1-second sample rate. The DMSO fluid class was selected for compatibility. TOF MS experiments were conducted with positive and negative ionization modes, and data were processed using SCIEX OS software.

Novel Aspect

Echo® MS+ system with the ZenoTOF 7600 system rapidly identified 45 compounds in DMSO from 384-well plate, showcasing HTS capabilities.

Preliminary Data or Plenary Speaker Abstract

In this study, A TOF MS experiment was performed to identify analytes, and the data were analyzed using SCIEX OS software. The total acquisition was completed within 2 minutes for each polarity. Integration parameters and compound details (precursor mass, formula, adduct, charge) were used to set processing methods. Identification criteria included peak detection (S/N ratio), <5 ppm mass error, and <20% isotope ratio difference. Analytics in SCIEX OS handled peak integration and TOF MS spectrum evaluation. Reprocessing in the Explorer module generated a results file with mass error, isotope ratio, and confidence status for each analyte. Automated processing in SCIEX OS was subsequently applied to process batches. The Analytics workspace displayed results including polarity, formula, peak area, adduct/charge, precursor, and confirmed mass identities.

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Comparative Analysis of Recalled Metformin Using SIFT-MS and Liquid Chromatography

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Mutagenic N-nitrosamine impurities can occur at trace concentrations in pharmaceutical products as byproducts of synthesis or, less commonly, through migration from packaging materials. N-nitrosodimethylamine (NDMA) is the most volatile of these, the most common culprit in product recalls, and is challenging to quantify using conventional procedures. In contrast, selected ion flow tube mass spectrometry (SIFT-MS), a direct mass spectrometry technique applying soft chemical ionization, quantifies NDMA from direct headspace analysis in tens of seconds with a limit of quantitation of 2 ng/g. In this study, SIFT-MS is applied to quantitative analysis of six recalled metformin products and results are compared with those obtained using conventional liquid chromatography (LC) analysis.

Methods

SIFT-MS analyzes air and headspace continuously using soft chemical ionization with up to eight reagent ions (H_3O^+, NO^+, $O_2^{+\bullet}$, $O^{-\bullet}$, OH^-, $O_2^{-\bullet}$, NO_2^- and NO_3^-). In this study, the positively charged reagent ions on a commercial SIFT-MS instrument (Voice200ultra model; Syft Technologies Limited, Christchurch, New Zealand) are utilized for analysis of NDMA in six metformin batches (recalled in 2020). Headspace analysis is automated using a multipurpose (MPS) autosampler (Robotic Pro; GERSTEL, Mülheim, Germany) with sampled headspace injected into the SIFT-MS instrument through a septumless sampling head (GERSTEL).
Quantitative analysis of NDMA content is achieved by using multiple headspace extraction (MHE). SIFT-MS data are compared with results from a validated analysis using direct solution injection onto the LC.

Novel Aspect

Rapid quantitative screening of NDMA using SIFT-MS is compared with LC analysis for the first time.

Preliminary Data or Plenary Speaker Abstract

Current United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) regulations require measurement of NDMA with limits of quantitation in the very low nanogram range since the acceptable daily intake is 96 ng/g. Since the SIFT-MS technique can only analyze gas-phase samples (it cannot accommodate direct liquid injection) a headspace approach must be utilized. However, the high polarity of NDMA and affinity for water means that it is challenging to analyze with adequate sensitivity from aqueous headspace. (Water is the preferred solvent for direct headspace-SIFT-MS analysis because the SIFT-MS reagent ions are very insensitive to water, in contrast to most organic solvents.) This study therefore utilizes an enhanced form of multiple headspace extraction (MHE) in which the multi-step MHE process can be applied routinely as a single headspace analysis for a given product following calibration with the full MHE procedure. Previous work has found that this approach has a limit of quantitation for NDMA of 2 ng/g when analyzing the headspace of powdered drug product without dissolution.
Results obtained for the four batches analyzed to date show good agreement between the LC and SIFT-MS results given the very different sample preparation and analytical approaches. These early results suggest that rapid screening (12 samples per hour) using headspace-SIFT-MS may be feasible.

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High-throughput proteomics using narrow DIA window on the Orbitrap Astral mass spectrometer

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Single-shot LC-MS based proteomics has been an essential tool to help researchers unravel the proteome composition of complex biological samples. As it allows unbiased identification and quantification of thousands of proteins in a single run, it remains the workhorse of modern proteome research. Even though innovation in LC-MS instrumentation and bioinformatics processing have brought major increases in sensitivity and workflow robustness to the field, there is a growing necessity to identify more proteins in less analysis time. In this contribution, we report on the use of the Orbitrap Astral mass spectrometer to achieve both comprehensive as well as high throughput proteome analysis with a single LC-MS set-up.

Methods

Lyophilized mammalian cell digests were resuspended in 200 µl 0.1 % FA to obtain a stock solution of 100 ng/µl; 2 µl of this was injected on column. The separation was performed on a Thermo Scientific™ Vanquish™ Neo UHPLC system with cycle times of 8, 14.4, and 24 min using a 5.5 cm long µPAC Neo High-Throughput column in a trap-and-elute workflow. The LC was coupled to Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer operated in DIA mode with a narrow isolation width of 2 Th. The raw files were processed with Proteome Discoverer 3.1.

Novel Aspect

Increasing throughput for comprehensive proteomics using narrow DIA window on the Orbitrap Astral MS and a short microfabricated LC column.

Preliminary Data or Plenary Speaker Abstract

Aiming at optimal use for large cohort studies, a set of robust, high-to-medium throughput LC-MS methods with variable flow rates was developed. This was achieved by combining the extended flow capabilities and minimal delay volumes of the Vanquish Neo with the flow rate versatility of the 5.5 cm µPAC Neo High Throughput column. A significant increase in instrument productivity was achieved by operating in trap-and-elute configuration and performing column re-equilibration in parallel to sample loading. In addition, utilizing variable flow rate during the gradient formation pushes peptide elution forward and allows for more effective peptide elution, at flow rates ranging from 2.5 to 0.3 µL/min. LC-MS instrument productivity of 68, 79, and 87% could be achieved for respective cycle times of 8, 14.4, and 24 min.
The performance of these different methods on the Orbitrap Aatral mass spectrometer was evaluated by injecting 200 ng of HeLa digest sample. Due to the high transmission efficiency and high scan rate of the Orbitrap Astral MS, all DIA experiments were performed with the same isolation width as DDA experiments, 2 Th. At a sample throughput of 180 samples per day (8 min method) we were able to identify 7900 protein groups on average from 5 technical replicates. Considerable increases in proteome depth could be achieved by extending the gradient length and reducing the active elution flow rate gradually. Within 14.4 and 24 min of total LC-MS time, we identified an average of 8579 and 9167 protein groups, respectively. These preliminary results show that the 5.5

cm µPAC Neo High Throughput column has an extremely high gradient flexibility, and the novel HRAM-MS is suitable for all high-throughput proteomics methods.

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Accelerating biomarker discovery in clinical cancer cohorts using volumetric absorptive microsampling (VAMS) devices and high-throughput mass spectrometry.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Volumetric absorptive microsampling devices (VAMS) allow patients to collect blood via fingerprick sampling at home. This approach enables collection of frequent longitudinal samples that can be used for health surveillance of disease, including cancer, for early diagnostics, ongoing monitoring, and recurrence. We have demonstrated that VAMS devices aid sample preparation for proteomics, facilitating the depletion of high abundance proteins via washing, prior to digestion. Reducing the dynamic range allows detection of more than 3000 proteins using a mid-throughput mass spectrometry (MS) method running 18 samples per day (SPD). Using a clinical cohort of non-small cell lung cancer (NSCLC), we compared the use of both mid- and high-throughput methods to identify relevant biomarkers and differentiate healthy from disease patients.

Methods

Blood from 16 patients with NSCLC and 18 age and sex matched controls were loaded onto VAMS devices and dried at room temperature for 24 hours. VAMS devices were washed overnight in LiCl wash buffer, followed by two subsequent shorter washes. The proteins remaining in the tip were extracted using lysis buffer, reduced, alkylated and digested with 1 µg overnight at 37 °C. Samples were desalted using solid phase extraction, quantified and 500ng used for MS injection. Two MS methods were employed, first a mid-throughput method using 18 SPD method on a QEHFX (Thermo) and the second a 60 SPD method on a 7600 Zeno-TOF (SCIEX) equipped with an Evosep LC.

Novel Aspect

Combination of dried blood spots and high-throughput mass spectrometry to identify cancer biomarkers.

Preliminary Data or Plenary Speaker Abstract

Due to the use of a longer gradient and LC column for the mid-throughput method, significantly more proteins were identified in comparison to the high-throughput method (mean and SD of 3370 ± 117 and 1483 ± 137, respectively). Per minute of run time however, the high-throughput produced 1.5-fold more IDs. The high-throughput method had greater sparsity with less than 50 % of the data quantitated in all samples compared to more than two thirds for the mid-throughput method. Overall, 36 (high-throughput) and 455 (mid-throughput) differentially expressed proteins were discovered for each method. There were 31/36 of these identified proteins thar overlapped with the mid-throughput results, with several of the overlapping proteins previously identified as biomarkers of NSCLC. Using a machine-learning package, ProMor, a fourteen-marker prediction model was identified for the high-throughput method which gave an area under the ROC Curve (AUC) of 72.2% using k-nearest neighbours (knn) compared with 94.4% using a nine-maker panel for mid-throughput. There was only one protein common between the mid- and high-throughput models, a serine protease, Myeloblastin (PRTN3), which has been linked to KRAS mutations in lung cancer patients. There were 5 pathways identified that were statistically significant and were common to each method. All these pathways are closely related, with neutrophil degranulation, antimicrobial peptides, and ROS and RNS production in phagocytes being pathways under the innate immune system, indicating a strong immune system response in the disease cohort that is detected by both methods.

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Instrumentation That Scales Analytical Throughout Reliably And Practically

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Targeted mass spectrometry (tMSn) traditionally is employed at the end of the biomarker discovery pipeline for small numbers of diagnostic compounds. Although tMSn produces the best quantitative data, it has had limited applicability to upstream experiments due to its low throughput. We describe a novel high-speed hybrid nominal mass platform designed for tMSn that has ~10x higher throughput for targeted peptide quantitation than triple quadrupole platforms. The increased performance is due to a combination of hardware/instrument control developments coupled with a real-time Adaptive RT algorithm that adjusts for retention time shifts, allowing narrower acquisition windows. We also present a Skyline-based software plugin that streamlines instrument method creation. These advances enable large-scale tMSn with applications to translational and even discovery proteomics.

Methods

Parallel ion management is used to achieve high duty cycles for ion injection and analysis, maximizing sensitivity and acquisition rates. A new long-life detector maintains stable gain while achieving single-ion detection limits. The instrument acquires nominal resolution mass spectrometry data at a rate of 65-100 Hz for peptide analysis, depending on the choice of acquisition parameters. The Skyline plugin filters transition data according to metrics like area and signal-to-background, and filters precursors by a minimum number of qualifying transitions. Precursors are scheduled for analysis with a load-balancing technique that chooses the best peptides per protein and ensures a minimum points-per-peak. The Adaptive RT algorithm estimates retention time shifts and updates acquisitions using cross correlation-based comparisons of real-time with reference data.

Novel Aspect

A novel high-speed hybrid nominal mass platform enables routine targeted MSn quantitation with throughput greater than 5000 peptides per hour.

Preliminary Data or Plenary Speaker Abstract

Large scale discovery experiments are now capable of probing the depths of the proteome in unprecedentedly short times. However, the data independent and data dependent acquisition strategies used to perform those experiments carry tradeoffs in sensitivity and selectivity, as well as instrument complexity and cost. On the other hand, targeted experiments on triple quadrupoles are more cost effective and have good sensitivity but have not traditionally been easily scalable to more than a few hundreds of peptides. The new MS platform fills the gap between the capabilities of triple quadrupoles and high-resolution accurate mass (HRAM) instruments, with a capacity to target more than 5000 peptides per hour.

We present a unified workflow for creating large scale tMSn assays on the new instrument from discovery data. With a new Skyline-based software plugin, precursors are selected that have the best chance of being good quantitative targets. The plugin can utilize HRAM data or discovery data generated by the new instrument, especially when the gas-phase fractionation strategy is employed. The plugin is aware of the instrument acquisition speed, so that the generated instrument method files respect the user-requested points per LC-peak. Additionally, the plugin compresses and embeds the discovery data into the instrument method files, enabling an Adaptive RT algorithm to adjust the acquisition times for each target in real-time and increase throughput by 2-3x.

We present experimental use cases that include relative and absolute quantitation, as well as MS2 and MS3 acquisition modes. We demonstrate the ability to apply the same tMSn workflow to

accurately quantify tandem mass tag labeled samples, increasing sample throughput by an additional 10x. While the platform's additional throughput can be used to extend the number of targeted compounds, we show that the gains can be used to shorten the gradients used with smaller numbers of targets.

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A non-derivatised high-throughput LC-MS/MS method for simultaneous quantitation of short chain fatty acids and tryptophan across various sample types

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Recent studies have identified short chain fatty acids (SCFAs), tryptophan and related metabolites as important analytes of gut microbial health. SCFAs are known to be biomarkers and regulators of metabolic health. Gut-brain axis research identified the gut microbiome modulation of tryptophan metabolism as a key pathway of interest. We recently demonstrated that circulating and faecal SCFAs are important biomarkers of metabolic health through a systematic review and meta-analyses involving 1186 participants from 23 studies. Current protocols are sample-, cost- and resource-prohibitive; robust methods for high-throughput, sensitive detection of these analytes across different bio-samples are highly desired. Here we present a simple, versatile, non-derivatisation and high-throughput UPLC-MS/MS method to simultaneously quantify SCFAs, tryptophan and kynurenine across various sample types.

Methods

The UPLC-MS/MS analysis was carried out using a SCIEX triple quadrupole 7500 QTRAP system coupled to a Waters ACQUITY UPLC I-Class system equipped with an ACQUITY Premier HSS T3 1.8 µm (2.1 x 100 mm) column. Chromatographic separation was achieved with a gradient of mobile phase A) water 0.1% formic acid and B) acetonitrile 0.1% formic acid. Samples were extracted and purified using an acidified solution containing 70% (v/v) ethanol or methanol, and 0.1% (v/v) trifluoroacetic acid (TFA) containing internal standard (IS) 2-ethylbutyric acid (2-EBA). Samples were diluted in 0.5% ortho-phosphoric acid buffer and spiked with deuterated IS. Analytical method validation was conducted; precision, accuracy and reliability were assessed. Recovery rates and matrix effects in various sample types were calculated

Novel Aspect

We present a robust/versatile, non-derivatised, cost-effective, high-throughput method to simultaneously assess SCFAs, tryptophan and related metabolites across various sample types.

Preliminary Data or Plenary Speaker Abstract

SCFAs (acetic-, propionic-, butyric-, valeric, and isovaleric acid), tryptophan and kynurenine were measured using this high-throughput method (over 100 samples/day) from various mouse sample types (faeces, gut contents, and serum). All analytes, including the constitutional isomers valeric- and isovaleric- acid, were resolved in under 12 minutes. We optimised the extraction protocol utilising different extraction solvents. Compared to methanol, ethanol yielded higher recovery rates of analytes and produced superior peak shape and chromatography for all analytes, especially for acetic acid. Subsequently, ethanol was chosen as the extraction solvent. Due to the high sensitivity of the assay, samples required dilution of up to 200 times. We were able to minimise costly deuterated IS used by adding them after dilution. 2-EBA was shown to accurately account for recovery. The dual IS approach enabled the cost benefits of non-deuterated IS as well as robustness, accuracy, and reliability of deuterated IS. The method was shown to be robust, reproducible, reliable, and accurate. Preliminary results indicate that SCFA concentrations between sample type are correlated. A negative association between serum and faecal acetate and a positive correlation between serum and colon propionate were observed. Notably, this method can be quickly adapted and customised for additional metabolites of interest, especially neurotransmitters. We demonstrated that additional

tryptophan related metabolites (i.e. serotonin, melatonin, indole-3 proprianate and indole-pyruvate) could easily be added to this proposed method. This is due to the absence of derivatisation and use of commonly utilised chromatographic conditions.

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Development of the OMIX3 platform for parallel, high-throughput, multi-omics analysis of clinical samples.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Multi-omics studies are increasingly being used to understand the complex molecular and physiological changes that are associated with a wide range of acute and chronic diseases. These analyses are typically performed on distinct aliquots of different biofluids/tissue samples across different facilities, significantly increasing variability. Here, we describe the establishment of a new Clinical MultiOmics platform, termed OMIX3, which allows the semi-automated extraction of proteins, polar metabolites and lipids from a sample of interest and parallel analysis of each subfraction on three mass spectrometry platforms.

Methods

The OMIX3 platform is capable of undertaking multi-omics analysis of a variety of human samples, including plasma, PBMC, urine and saliva. Each sample type is extracted in MTBE:methanol:water, and polar metabolites and lipids are recovered by phase partitioning of the supernatant extract, while proteins are recovered in the pellet. All steps involved in the extraction and phase separation recovery of lipids and metabolites and processing the protein pellet using paramagnetic bead technology are undertaken using Janus/Revvity liquid handling instruments. Polar metabolites and lipids are subsequently analyzed on Orbitrap IQ-X and Shimadzu 9050 OAD-TOF platforms, while proteomics analysis of tryptic digests is analyzed on the Orbitrap Astral MS

Novel Aspect

The platform's automation streamlines high-throughput clinical multi-omics sample preparation for MS analysis.

Preliminary Data or Plenary Speaker Abstract

The OMIX3 platform has validated a workflow for clinical multi-omics analysis from a single sample to streamline sample preparation, improve data quality, and reduce time, enhancing the efficiency and accessibility of multi-omics analysis. This integrated approach accelerates the sample preparation process and expands the scope of detectable biomolecules, enhancing the platform's utility in clinical and translational research.
The OMIX3 multi-omics platform exploits complementary high-resolution mass spectrometry instruments, including the latest state-of-the-art Thermo Orbitrap Astral for Proteomics analysis. Other available MS instruments include the Thermo Orbitrap IQ-X Tribrid Mass Spectrometer, the Agilent 6490 Triple Quadrupole Mass Spectrometer, and the Shimadzu 9050 OAD-TOF. The platforms consist of 2 Revvity Janus G3 workstations for multi-omics sample preparations, one each for i) metabolomics and lipidomics analysis and ii) proteomics analysis. In addition, the platform has an automation platform for blood fractionation into plasma, buffy coat and RBCS components with the recent acquisition of the Janus G3 Blood iQ Workstation, enabling efficient on-site sample handling and preparing the samples for downstream analysis and storage requirements. These liquid handling platforms and MS instruments ensure the platform can deliver throughput and reproducibility for large-scale clinical projects. Backed by a team of experienced professionals, the platform aims to

serve academic and industry clients by offering multi-omics solutions, complementing the services offered by existing high capacity genomic facilities. These solutions incorporate a variety of clinical samples, catering to specific clinical and translational research requirements under a fee-for-service model.

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SILAC quantification using narrow width data-independent acquisition on the Orbitrap Astral Mass Spectrometer.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Identification and quantification of proteins provide a broad understanding of protein interactions, cellular signaling, and many other biological pathways Due to their multiplex capabilities, TMT and SILAC, especially when proteins in cells are expressed under defined conditions, are commonly being used for protein quantification. In SILAC experiments, cells to be compared or multiplexed are grown in different cultured mediums, using either naturally occurring or stable isotope labeled amino acids. The differentially labeled cells are mixed, prepared, and analyzed together by LC-MS/MS, commonly, using data dependent analysis (DDA). Here we evaluated the use of a narrow window DIA on the Orbitrap Astral MS to identify and quantify changes in proteins labeled HeLa cells without sacrificing throughput and depth of coverage.

Methods

HeLa cells were cultured in heavy- and light-SILAC culture media. Labeled and unlabeled cells were combined in different ratios and processed using the Thermo Scientific™ EasyPep™ Mini MS sample Prep kit. Peptides were separated using Thermo Scientific™ Vanquish™ Neo UHPLC system on an IonOpticks Aurora 25 cm TS C18 column coupled to a Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer. Data was acquired in data independent analysis mode with an isolation width of 2 Th. Raw files were processed with Spectronaut 18\DIA-NN using a human database with 20k sequences (no isoforms).

Novel Aspect

Data independent acquisition with narrow isolation windows enables high-throughput SILAC quantitation without sacrificing the depth of coverage.

Preliminary Data or Plenary Speaker Abstract

Increased samples complexity, which is induced by mixing proteomes labeled either with chemical or metabolic incorporation of labeled amino acids, to increase throughput or for quantitative reasons, has an adverse effect on the depth of coverage, when compared with single proteome analysis. Therefore, we started off by evaluating the losses associated with multiplexing labeled samples at a defined gradient. We analyzed the individual proteome in DIA and DDA mode of acquisition using 60 and 30 SPD LC\MS method. For the DIA acquisition mode, multiple isolation widths were used and only one isolation width for DDA acquisition mode. We identified more than 8,500 and more than 10,000 protein groups from the individual unlabeled samples in DIA for 60 and 30SPD, respectively. For the DDA experiments of individual unlabeled samples we identified 10-15% less protein groups for the same gradients. Furthermore, SILAC labeled samples were mixed in different ratios and analyzed using DIA. More than 8,400 and 9,500 proteins groups were matched to more than 137,000 and 216,000 precursors from 60 and 30SPD methods, respectively. Interestingly, we did not only reach the same depth of coverage compared to the individual samples (less than 10 % difference) but achieved this with 2x higher throughput. Because of the increased complexity, high resolving power was used in MS 1 (240k) to improve the selectivity and specificity, thereby improving quantitation. Of the identified proteins, more than 75 % were quantified.

Next-generation Acoustic Ejection Mass Spectrometry: A fully automated platform for high-throughput sample analysis

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Proteins play a key role in almost every biochemical process. The analysis of intact protein molecules allows for the characterization of these proteins and understanding of their role in the biophysical landscape. The need for high-throughput intact protein analysis has increased in the pharmaceutical and biopharmaceutical industries. Throughput currently limits peptide mapping LC-MS analysis, as minutes or hours are needed to achieve the necessary characterization. An alternative approach is to analyze the intact mass of the protein and compare the data against a known sequence while preserving high sensitivity and mass accuracy. However, high-throughput screening analyses are not typically performed in buffers compatible with LC-MS. This study demonstrates a high-throughput sample preparation on an acoustic ejection time-of-flight mass spectrometer.

Methods

Samples were prepared using a Biomek i7 Automated Workstation (Beckman Coulter® Life Sciences), configured with a 96-multichannel pipetting head and Span-8 Pod. Prepared plates were loaded onto the Echo® MS+ system coupled with a ZenoTOF 7600 mass spectrometer. The system was controlled by SAMI EX scheduling software, which used the SCIEX OS software control (Application Programming Interface) API. Commercially available proteins (ubiquitin, lysozyme, BSA and NISTmAb IgG1k) were selected to benchmark the system at various concentrations and in biologically relevant buffers. Datasets were collected and compared with and without sample cleaning steps, such as SPE, buffer exchange or dilute-and-shoot, before Acoustic Ejection Mass Spectrometry (AEMS) analysis. Data collection and processing were performed using SCIEX OS software and Genedata.

Novel Aspect

Fully automated system with demonstrated key attributes of system performance for intact protein analysis, including quality, reproducibility and system versatility.

Preliminary Data or Plenary Speaker Abstract

This study demonstrates the use of an Echo® MS+ system coupled with a ZenoTOF 7600 mass spectrometer for the analysis of intact proteins. The prototype system uses an alternative approach to rapidly analyze proteins and solve some challenges faced by traditional techniques. Samples are placed into well plates held by the system and acoustic energy is applied to the bottom of the well plate, causing a droplet of the sample to be ejected. The Open Port Interface (OPI) is located above the well plate and captures the ejected droplet for dilution. The diluted sample is transported by a carrier solvent (~400
µL/min) to an electrospray ionization source for ionization and delivery of sample ions to a high-resolution mass spectrometer for analysis.
This study explores important parameters (carrier solvent composition, chemical additives and varying matrices) that affect the data quality of high-resolution AEMS on a high-throughput scale for intact protein analysis. Preliminary AEMS data demonstrate the rapid analysis of analytes, as a 384-well plate can be analyzed in 10-30 minutes (1-5 seconds/sample). This study further demonstrates a method to increase throughput by automating the AEMS system. Preliminary AEMS data collected without system automation highlight the rapid analysis of compounds, based on the analysis of a 384-well plate in <10 min. Subsequent ejections result in baseline resolution between ejection peaks and no carry-over between measurements. Very high reproducibility is observed across a single plate and when measuring many plates in series, with an average of <10% CV across 146 384-well microtiter plates (~56,000 samples) analyzed over 4 working days. The use of a liquid handler to automate the workflow increases sample throughput by enabling the preparation and scheduling of

subsequent plates around the clock. As a result, significantly more samples were analyzed with comparable data quality, reproducibility and system robustness.

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Application of small footprint LC-MS for determination of residual chemicals in green solvent

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The hyphenation of chromatography and mass spectrometry leverages the strengths of both techniques to achieve superior analytical performance. Portable liquid chromatography-mass spectrometry (LC-MS) can significantly contribute to solving practical issues arising from sample degradation and contamination during transportation and long-term storage. This is particularly valuable when the sample quantity is limited. This enables rapid on-site decision-making and substantial financial savings.
Cyrene™ is an innovative bio-based solvent, increasingly recognized as an eco-friendly alternative to conventional petrochemical solvents due to its minimal toxicity and reduced environmental impact. This project seeks to apply the portable LC-MS technology to the analysis of Cyrene™ and residual chemicals present from its production.

Methods

Building on previous research known internally as the 'Hummingbird Project' within the ASTech ARC Training Centre, this project aims to explore additional modes of selective detection, such as multiwavelength UV/Visible absorbance and portable mass spectrometry. As its name implies, Hummingbird is a compact, lightweight device that can deliver gradient capillary separations based on a capillary-scale LC column and flow rates. The portable LC system is linked to a small-footprint mass spectrometer featuring an electrospray ionization (ESI) source, with a built-in PC and vacuum pump, ensuring maximum portability of the MS instrument. In this study, a liquid chromatographic method has been developed for the analysis of Cyrene™ and its most abundant residual chemicals.

Novel Aspect

The portable LC-MS approach offers an innovative and real-time analytical tool for the analysis of Cyrene™.

Preliminary Data or Plenary Speaker Abstract

The significance of using LC-MS for the analysis of the Cyrene™ and related impurities lies in its higher sensitivity for non-volatile and thermolabile compounds without requiring derivatization. LC-MS operates under milder temperature conditions compared to gas chromatography (GC), which helps minimize the thermal degradation of sensitive compounds present in Cyrene™. In addition to the ESI-MS detection, the Hummingbird device's capability to monitor analytes at multiple wavelengths allows for the sensitive and selective detection of each analyte at its maximum absorbance.
LC separation was carried out on a C18 packed column (100 mm × 0.53 mm, 3 µm), with a column temperature of 40°C using gradient elution. The mobile phase consisted of mixtures of 2 mM ammonium acetate in acetonitrile and 2 mM ammonium acetate in water with a flow rate of 0.03 mL/min. This developed chromatographic method, the first LC-ESI-MS analysis for Cyrene™, has made significant strides by separating approximately 20 novel peaks in under 10 minutes and confirming the presence of three major residual chemicals from the production process.

The new portable LC-MS technique represents a substantial advancement in analysing chemical entities in environmental samples, biochemical toxins, and contaminants from production processes. Moreover, it facilitates the identification of trace chemicals at various production stages on-site, ensuring compliance with quality, safety, and regulatory standards while supporting cost-effective production.

The natural extension of this work involves applying Cyrene™ and residual chemical analysis within manufacturing sites to enhance process optimization and overall performance.

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SEC x IP Two-Dimensional LCMS for the Analysis of Non-Denatured and Denatured Cyclic-Peptide siRNAs in a Single Step

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Several separate non-denaturing and denaturing methods with varying MS compatibility have been reported to determine the excess of the individual strands in the formation of a small interfering RNA (siRNA) duplex, the impurities of the individual strands and of the duplex. Additional conjugation of a ligand to the duplex, however, makes finding suitable methods even more challenging. For an siRNA duplex containing a cyclic-peptide (CP) ligand, standard Ion-Pair and Ion-Exchange non-denaturing chromatography methods could not separate the strands from each other or the duplex from one of the strands. Here, we report a two-dimensional LC MS-compatible system combining Size Exclusion and Ion-Pair able to perform non-denaturing and denaturing analysis of the CP-siRNA in a single method.

Methods

An Agilent 1290 Infinity two-dimensional LC system was used for the experiments interconnected via a trap column (Agilent Poroshell 120 EC-C18, 4.6x50mm, 2.7□m) which allows the capture of broad 1D peaks. Two SEC columns were used in series for the 1D analysis (Tosoh TSKgel UP-SW2000, 4.6x300 mm, 2□m). 2D analysis was conducted using a Waters OST C18, 2.1x100mm, 1.7□m column. The temperature of the 1D column was 25°C (non-denaturing) and that of the 2D, 50°C (denaturing). The 2DLC system was interfaced to an Agilent time-of-flight (TOF) mass spectrometer. IP-RPLC HRMS with tributyl (tBuAA) or triethyl (TEAA) ammonium acetate was used for the analysis. The mass spectrometer was operated in the negative ionization mode.

Novel Aspect

Non-denatured and denatured CP siRNA analysis achieved in a single step.

Preliminary Data or Plenary Speaker Abstract

Initial work with tBuAA as IP reagent at 15°C (non-denaturing conditions) separated the CP-siRNA duplex from the two excess strands, but not the two strands from each other. Subsequent work with TEAA as IP reagent separated the two strands but led to the decomposition of the duplex even at 12°C using acetonitrile in the mobile phase (MP). Evaluation of strong anion exchange (SAX) separated the two strands but the duplex co-eluted with one of the strands. Work with a SEC column revealed good separation of all three CP-siRNA species based on MW and hydrophobicity differences. Different buffer systems (sodium bromide, sodium phosphate, ammonium acetate, sodium perchlorate) were evaluated for the maximum attainable chromatographic resolution of the three species by SEC. Ammonium acetate at 0.1M provided the highest resolution and was selected as an appropriate mobile phase for the SEC analysis. Separation could also be achieved at lower concentrations than 0.1M using ammonium acetate allowing for the optional direct 1D introduction to the mass spectrometer.

tBuAA as IP reagent at 50°C (denaturing conditions) degraded the duplex but led to closely eluting peaks for the two CP-siRNA strands. Excellent separation was obtained at 50°C denaturing conditions using TEAA as IP reagent. Combination of these results naturally led to a 2D LCMS method involving 1D non-denaturing analysis with SEC to separate species of interest (duplex, individual strands, and impurities) and 2D analysis with TEAA IP coupled to MS for the selective analysis of the duplex, individual strands, or their impurities under denaturing conditions. The SEC x IP 2D LCMS method developed was successfully applied to guide process development of CP-siRNA duplex formation, including various studies involving the effects of salt and temperature on duplex annealing.

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Determination of N-nitroso Fluoxetine in Fluoxetine Products by LC-MS/MS

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

N-nitroso fluoxetine is a nitrosamine impurity found in fluoxetine products. It is a potent carcinogen compound. However, there was no sensitive method available to determine N-nitroso fluoxetine in fluoxetine products. Hence there is a need to develop a sensitive LC-MS/MS method to determine N-nitroso fluoxetine in fluoxetine products to ensure product safety and protect public health.

Methods

Determination of N-nitroso fluoxetine in fluoxetine products was performed using SCIEX QTRAP 7500 MS/MS coupled with ExionLC. The column used was Kinetex Biphenyl column (150 × 2.1 mm, 2.6 µm). Mobile phases A and B were 0.1% formic acid in deionized water and 0.1% formic acid in acetonitrile, respectively, with gradient elution. Flow rate was 0.3 mL/min, column temperature was set at 40 ºC and injection volume was 5 µL. The mass spectrometer was operated in multiple reaction monitoring (MRM) positive mode using electrospray ionization (ESI) source. N-nitroso fluoxetine-D5 was used as the internal standard.

Novel Aspect

A straightforward, rapid, highly sensitive and selective LC-MS/MS method for the determination of N-nitroso fluoxetine in fluoxetine products was developed.

Preliminary Data or Plenary Speaker Abstract

In this study, a highly sensitive and selective LC-MS/MS method was developed to accurately quantify N-nitroso fluoxetine in products containing fluoxetine. The sample was extracted by MeOH: deionized water (8:2, v/v) containing 10 ng/mL of N-nitroso fluoxetine-d5 (Isotope internal standard). The quantification was performed by MRM in positive ESI mode. The method was validated and found to have sufficient linearity (R2 > 0.995), accuracy (recovery 80–115%), precision (RSD 5%) and low LOD and LOQ (0.02 and 0.1 µg/g respectively, with respect to fluoxetine). The developed method allowed the lab to conduct the analysis of N-nitroso fluoxetine at trace level in fluoxetine products with different strengths and dosage forms. The testing capability developed will enable prompt regulatory actions to be taken on unsafe products to safeguard public health.

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Evaluation of the Effect of Using Nitrogen or Hydrogen as Carrier Gases in GC-MS on Qualitative Analysis

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The supply of helium, the most common carrier gas used for GC-MS analysis, has become unstable in recent years. Therefore, there is a growing need for alternative gases to helium for stable operation of GC-MS. On the other hand, there are concerns that the use of alternative gases may lead to decreases in sensitivity and/or accuracy due to changes in the mass spectrum. In this study, aromatic oils were measured using nitrogen and hydrogen as carrier gases. Results were compared with those obtained when helium was used as the carrier gas, and the effects of using nitrogen and hydrogen as carrier gases on the analysis results were evaluated.

Methods

Commercially available aromatic oils were measured by GC-MS using three different carrier gases: helium, nitrogen, and hydrogen. Both electron ionization (EI) and photo ionization (PI) were used for measurements. Results of the hydrogen and nitrogen measurements were compared with the helium results for 30 components based on relative retention times and EI mass spectral library matches. In addition, qualitative analysis was performed for each peak using a method that combines mass spectral library search results based on the EI mass spectrum, and the molecular weight determined using PI data.

Novel Aspect

The effect of using hydrogen and nitrogen as carrier gases for GC-MS on qualitative analysis was evaluated.

Preliminary Data or Plenary Speaker Abstract

From the results of the hydrogen and nitrogen measurements, 30 component peaks were compared with results using helium. For hydrogen, the match factor from the library search was found to be much lower than that for helium for several peaks, and the estimated compound idenities were also changed. This may be due to changes in the mass spectrum due to the reducing effect of hydrogen. In addition, for some peaks, the retention time on the chromatogram changed in addition to the mass spectrum. If only the mass spectrum changes, the change is considered to be only on the MS side. However, because both mass spectra and retention times have changed, it can be concluded that hydrogen affects both GC and MS results. On the other hand, although a decrease in sensitivity was observed when using nitrogen as a carrier gas, no change was observed in the mass spectra, and the results were almost equivalent to those for helium. These results suggest that nitrogen is the best substitute for helium in qualitative analysis using GC-MS. Compound indentificaiton of each peak in the hydrogen measurement results was also performed using an analytical method that combines the molecular weight obtained by PI and a mass spectral library search. It was confirmed that in some cases, correct estimation of the compound identity could be derived from the molecular weight information for compounds whose mass spectra were altered by the reduction effect.

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Improved chromatographic resolution of underivatized phosphatidylinositol mono-, di-, and triphosphates by minimising surface interaction.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Phosphatidylinositol phosphates (PIPs) are signalling messengers that orchestrate cellular growth and metabolism. Reliably measuring phosphatidylinositol monophosphate (PIP), diphosphate (PIP2), and triphosphate (PIP3) pose substantial challenges in lipidomics due to their low abundance and poor chromatographic peak resolution with liquid chromatography (LC). One approach to enhance PIP identification involves derivatization (i.e., trimethylsilyldiazomethane), which prevents unwanted interactions between phosphate groups with the LC system, leading to off targeted retention along the flow path and thus, lower/variable chromatographic signal. Conversely, derivatization presents logistical challenges involving technical consistency, reactivity, and toxicity of reagents. To address these limitations, we explored extraction approaches, solvent pH conditions, and column chemistry to improve PIP, PIP2 and PIP3 peak resolution, minimise technical variation and increase sensitivity without derivatization.

Methods

A mixture containing PIP(5')-16:0/16:0[d5], PIP2(3',5')16:0/16:0[d5] and PIP3(3',4',5')18:1/18:1 (Avanti Polar Lipids) was spiked into RAW 264.7 macrophages tested with a modified butanol:methanol extraction with increasing water content. Samples were examined using an Agilent Infinity II HPLC with an Agilent 6495C mass spectrometer. Chromatography was performed with H2O/ACN/IPA in solvent ratios of A (50/30/20) and B (1/9/90) with 10mM ammonium formate. Solvent A was tested with combinations of 5uM medronic acid or 0.1% ammonium hydroxide. Analysis was conducted using single ion monitoring in both positive and negative mode. Columns tested included an Eclipse Plus C18 (2.1mm x 100mm, 1.8µm, RRHD, Agilent), Poroshell 120 HPH C18 (2.1 x 150 mm, 1.9µm, Agilent) and ACQUITY Premier BEH C18 (2.1 x 100 mm 1.7µm, Waters).

Novel Aspect

In this study, we developed an approach to better examine the PIP subclasses, without derivatization, using LC-MS.

Preliminary Data or Plenary Speaker Abstract

Initial development with the inclusion of medronic acid in the solvents improved PIP resolution, with minimal impact to PIP2 and PIP3. To improve the recovery of PIP, PIP2 and PIP3, the extraction conditions were adjusted from standard single phase BUME protocols by increasing the buffered water content. An increase in water composition with ammonium formate/medronic acid from 10% to 30% of total sample volume improved signal height of the most problematic PIP class (PIP3) by up to 90% from original extraction conditions.
We subsequently examined conditions that could improve peak resolution. A minor improvement in peak resolution was observed, mostly with PIP, with the addition of 0.1% ammonium hydroxide coupled with the InfinityLab Poroshell 120 HPH C18 column. While peak resolution remained largely unchanged, the sensitivity in negative ion mode was substantially higher with both [M-H]- and [M-2H]2- adducts of PIP, PIP2 and PIP3.

Interestingly, utilizing the ACQUITY Premier BEH C18 Column, coupled with the medronic acid in solvent A, resulted in a near-symmetrical peak across all measured PIP classes. Peak symmetry improved to 1.14 from 4.43 (ratio of leading/trailing half widths of the peak) and baseline peak width to 0.47 minutes from 1.09 minutes (full width half maximum to 0.11 minutes from 0.6 minutes) in this specific column compared to the other columns with high pH.

Together, the ACQUITY Premier BEH C18 column coupled to mobile phases that included medronic acid, with increased water content in extraction solvent allowed for improved measurements across all PIP, PIP2 and PIP3 standards with minimal smearing, improved peak symmetry and enhanced peak intensity without the need for derivatization.

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A comprehensive lipidomics workflow using the Orbitrap Astral mass spectrometer for deep coverage and high confidence annotations

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Discovery lipidomics using mass spectrometry aims to gather comprehensive insights into the lipid diversity of a sample. The presence of a large number of isobaric and isomeric species in the lipidome necessitates the use of fragmentation for confident annotation. Challenges such as mass spectrometer speed, sensitivity, and accuracy may result in suboptimal production of high-quality MS2 spectra, leading to reduced annotation percentage and confidence. To address this, an untargeted lipidomics approach was established using the Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer, which provides very fast and sensitive MS2 scanning in parallel with high resolution accurate mass full scan Orbitrap detection.

Methods

Lipid standards and bovine liver lipid extracts were purchased from Avanti Lipids. Animal and plant-based milk samples were obtained from local markets (San Jose, California). Lipids were extracted from the biological matrices using a Folch method. The extracted milk lipids were spiked with equal amounts of liver lipid extract to create a complex matrix sample set. Lipids were separated on a Thermo Scientific™ Accucore™ C30 column connected to a Thermo Scientific™ Vanquish™ Horizon system. Data were acquired (both positive and negative polarities) on the Orbitrap Astral mass spectrometer using data-dependent acquisition. Thermo Scientific™ Compound Discoverer™ 3.3 software, Thermo Scientific™ LipidSearch™ 5.1 software, and MS-Dial software were used for data processing, statistical analysis, and unknown annotation.

Novel Aspect

Utilization of the Orbitrap Astral MS for double the depth of lipidomic profiling for complex samples

Preliminary Data or Plenary Speaker Abstract

In this study, the complex lipid samples were analyzed on the Orbitrap Astral MS, with a full-scan Orbitrap HRAM MS1 analysis at 120K resolution and either a data-dependent Astral HRAM MS2 or Orbitrap MS2 scan for comparison. The 30-minute LC-MS method using Astral analyzer MS2 collected over 250,000 MS2 events, leading to a very high percentage of detected compounds being fragmented (i.e., >90%). MS2 spectral quality from the Astral analyzer was assessed by comparing to data from the Orbitrap analyzer MS2s. High quality MS2 fragmentation was observed for both abundant and very low abundant lipids. The improved ratio of MS2 fragmentation is a result of the faster scanning rates of the Astral mass spectrometer, which is also accompanied by a higher sensitivity that also enables the confident annotation of more unknown lipids. Despite the higher 200 Hz repetition rate of the Astral analyzer, it collects data with a higher resolution of 80,000 and higher sensitivity compared to the Orbitrap analyzer. Nearly 5,000 lipids were annotated when using the Astral analyzer MS2 method compared to just over 2,000 lipids using the Orbitrap analyzer MS2 method. Despite the higher number of annotated lipids from the Astral analyzer, the number of identified lipids with high quality identifications, as defined by grade A or B meaning that the head group and all or some of the substituent ions are identified, is similar for the two analyzers with 57% for the Astral analyzer and 60% for the Orbitrap analyzer. These results demonstrate the utility of the Orbitrap Astral mass spectrometer for lipidomics.

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Comprehensive Characterisation of sn-isomer populations by DIA-based Shotgun Lipidomics with Ozone-Induced Dissociation

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Conventional lipidomics strategies characterise lipids to only a limited level of structural detail, masking a great deal of structural information crucial to understanding biology. For example, enzymatic processes involved in lipid metabolism, such as phospholipase-mediated acyl chain remodelling produce specific isomer variants. In recent years there has been significant interest in the development of innovative techniques capable of resolving lipid isomers (including double bond- and sn-isomers). However, these techniques have largely been used to probe a limited number of lipids, especially in the case of sn-isomers. Here we describe a novel shotgun lipidomics workflow employing data-independent analysis (DIA) and combinational collision-induced dissociation and ozone-induced dissociation (CID/OzID) to resolve hundreds of sn-isomer pairs in biological extracts in <20 minutes.

Methods

Shotgun lipidomics in positive-ion mode was performed by nanoelectrospray of methyl-tert-butyl ether lipid extracts in isopropanol:methanol with sodium formate. Ozone was supplied to either the linear ion trap or higher-energy dissociation regions of an Orbitrap Fusion, allowing ozonolysis of mass-selected ions under varied conditions.

A positive-ion DIA method was designed to subject sequential 1 Da windows to CID/OzID, targeting the characteristic headgroup-loss product ions of sodium adducted glycerophospholipids. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) lipid classes were analysed using 466 individual scans in less than 20 minutes of runtime.

An automated data analysis pipeline for identification of lipid species was developed using ALEX123 with a curated fragmentation library and a set of strict identification criteria.

Novel Aspect

An accessible workflow revealing unprecedented detail of phospholipid isomer diversity, enabling discovery of novel species and study of remodelling dynamics.

Preliminary Data or Plenary Speaker Abstract

Using DIA-CID/OzID to study the lipidomes of 4 diverse cell lines (SHSY5Y, RAW264.7, HEK293T, and a human dermal fibroblast line), 966 unique sn-resolved species, and 136 ether-linked species were observed across PC and PE lipid classes, reflecting an over an order of magnitude increase in the number of sn-resolved species compared to previous studies. The MS3 isolation strategy allows straightforward interpretation by the ALEX123 pipeline due to the exclusion of isobaric features, and the abundant product ions provide a boon to both sensitivity and structural characterisation over conventional CID-based shotgun lipidomics.

Critically, the data reveal the ratio of many sn-isomers pairs were found to differ significantly across cell lines, and for PC and PE. Key observations included fibroblasts having the highest fractions of the unconventional isomeric species (apocromer) with the more unsaturated chain at the sn-1 position (e.g., PC 20:4/16:0), whilst SHSY5Y cells routinely had the highest fractions of the canonomeric species with the more saturated chain at the sn-1 position. Moreover, across all cell lines PE lipids were generally found to have a higher fraction of the canonomeric species relative to PCs for the same acyl chain pairings.

These data show the sn-positioning of PUFA lipids is influenced by the specific saturated and monounsaturated acyl chains present, suggesting that cells have fine control over sn-isomer metabolism and a cellular demand for highly specific sn-isomer populations.

The untargeted DIA strategy and data-driven analytical approach enabled discovery of a range of previously undescribed PCs containing ultra-long fatty acyl chains, such as 32:1 and 34:1 with a strong bias towards the sn-1 position (e.g., PC 32:1/18:1 and PC 34:1/16:1).

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Comparison of high-throughput extraction methods for bacterial multi-omic lipidome and proteome analysis

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The composition of the bacterial lipid membrane plays a significant role in the development of antibiotic resistance. Evaluating the lipidomes of bacteria, and simultaneous analysis of the proteome to reveal the key metabolic enzymes underlying their regulation, is therefore critical to understanding resistance more broadly. However, due to the large number of pathogenic bacteria, and various clinically relevant resistant strains, there remains a need for high-throughput analytical methods for their analysis. Monophasic lipid extraction methods are ideal for high-throughput applications, however few have been evaluated to determine their applicability in bacterial multi-omic studies. In this work we compare three published single-phase extraction methods and evaluate their applicability for high-throughput bacterial lipidomics and proteomics.

Methods

E coli (BL21) was grown to exponential phase and harvested. Cell suspensions (100 uL, OD = 1) were homogenized then subjected to lipid extraction with three methods. The Lydic et al. method using 2:1:0.74 (v:v:v) methanol/chloroform/water, the Alshehry et al. method using 1:1 (v:v) 1-butanol/methanol and the Bimpeh and Hines method using 1:4 (v:v) ACN/MeOH + 0.5% acetic acid. The suspensions were then centrifuged to generate a protein pellet. Lipid extracts were analysed by ultra-high resolution accurate mass direct infusion lipidomics. Protein pellets were subjected to a BCA assay to determine total protein recovery. Lipid internal standards were added prior or post extraction to determine the percentage recovery for each lipid class. Proteomes were analysed using DIA.

Novel Aspect

Development of multi-omic extraction technique for bacterial samples

Preliminary Data or Plenary Speaker Abstract

The three E coli major lipid classes phosphatidylglyceroethanolamine (PE), phosphatidyl glycerol (PG) and cardiolipin (CL) were analysed and quantified. The three extraction methods all showed similar recovery of PE and PG, however the Bimpeh and Hines method showed significantly poorer recovery of CL. The Alshehry et al. and Lydic et al. methods showed similar recovery of protein, but Bimpeh and Hines method resulted in significantly less recovery of protein compared to the other methods. Bimpeh and Hines method was determined to be less suitable for multi-omic experiments due to the poor protein recovery and incomplete recovery of CL compared to the other methods.

Determination of Total and Free Cholesterol in one single run using Supercritical Fluid Chromatography coupled to Mass Spectrometry

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Cholesterol is present in two forms in human plasma: free (FC) and esterified (cholesteryl esters). Determination of total cholesterol (TC; esterified + free) is usually performed by spectrophotometric assays or gas chromatography mass spectrometry (GC-MS) based on hydrolysis of cholesteryl esters into cholesterol followed by derivatization. The FC can also be analyzed using GC-MS, but it requires separate detection runs and a complex sample preparation including separation of cholesteryl esters from FC using solid-phase extraction. These facts make the analysis of FC and TC more complicated and time-consuming. Supercritical Fluid Chromatography coupled to Mass Spectrometry brings a solution to analyze TC and FC in one single run without hydrolysis and derivatization but using the in-source fragmentation of cholesteryl esters.

Methods

Supercritical Fluid Chromatography coupled to Mass Spectrometry (SFC-MS), using carbon dioxide as a mobile phase, offers very fast separation analysis including separation of non-polar and polar lipid classes. Generally, there are two important elements for analysis of FC and TC in one single run, without any hydrolysis and derivatization step: 1) separation of cholesteryl esters and free cholesterol and 2) induction of in-source fragmentation of cholesteryl esters into cholesterol with high reproducibility. The SFC-MS platform is compatible with these requirements as cholesterol esters (esterified cholesterol) and free cholesterol are very well separated from each other and cholesteryl esters are also prone to in-source fragmentation generating high amounts of signal corresponding to free cholesterol.

Novel Aspect

SFC-MS is a useful tool for the FC and TC analysis in one single run without hydrolysis and derivatization steps.

Preliminary Data or Plenary Speaker Abstract

Analysis of FC and TC using SFC-MS was applied to four different reference materials: SRM 1950 Metabolites in Frozen Human Plasma and candidate RM 8231 Frozen Human Plasma Suite for Metabolomics (hypertriglyceridemic, diabetic Type I, and young African American plasma samples). Retention time of cholesteryl esters and FC is 1min and 1.5min, respectively, fulfilling the first important element required for the analysis. Ratio of cholesteryl ester and in-source fragmented cholesterol is approximately 2:8 and highly reproducible with a coefficient of variation (CV) below 10% fulfilling the second element. Concentration of the two cholesterol forms was based on calculation using calibration curve and one-point calibration. Concentration of TC in SRM 1950 human plasma was 3616.2 ±130.8µM (one point calibration) and 3671.9 ± 8.6µM (calibration curve) giving us very close results regardless of calculation approach. SRM 1950 human plasma certificate offers a certified value for TC, which is 3917 ± 85µM, and accuracy investigated as a closeness of our values with the certified value has shown great results with accuracy above 90%. Concentration of FC in SRM 1950 human plasma was 638.8 ± 14µM (one point calibration) and 657.7 ± 5.11µM (calibration curve). These results were compared with literature showing also high accuracy of our measurements. Other reference materials show similar outcomes. In general, higher level of TC was determined in hypertriglyceridemic (4538.5±147.7µM, one-point calibration) and young African American (4538.5±147.7µM, one point calibration) plasma samples. However, concentration of FC differs in the two plasma samples: 718.5±31.9µM (hypertriglyceridemic) and 2055.5±66.5µM (young

African American), showing the significant higher level of FC in Young African American plasma samples. This fact also corresponds to already published results showing similar findings.

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Quantifying sn-positional isomers in glycerophospholipids by reversed-phase chromatography combined with collision-/ozone-induced dissociation mass spectrometry

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Glycerophospholipids are the major component of lipid membranes with the structure and composition carefully regulated to maintain the critical biophysical properties of the membranes and promote specific interactions between lipids and membrane proteins. Assigning the specific structure of individual glycerophospholipids can be challenging due to the presence of regioisomers such as those arising from alternate positions of esterification of fatty acyl chains at the stereospecific numbering (sn)-1 and -2 positions on the glycerol backbone. Herein we combine reversed-phase chromatography and sequential ion activation by collision- and ozone-induced dissociation (CID/OzID) modalities to resolve sn-positional isomers and quantify their relative contributions to the isomer pool.

Methods

Phosphatidylcholine (PC) IsoPureTM standards were acquired from Avanti Polar Lipids with stated regiopurities ranging from 95-98%. Human plasma was obtained from Sigma-Aldrich and extracted using methyl-tert-butyl ether. Samples were subjected to chromatography using ACQUITY UPLC® CSHTM C18 (1.7 µM, 2.1×100 mm) and a gradient comprised of solvent A: 50% water / 30% acetonitrile / 20% isopropanol (v/v/v) containing 10 mM ammonium formate and solvent B: 1% water / 9% acetonitrile / 90% isopropanol (v/v/v) containing 10 mM ammonium formate. Eluant from the chromatograph (0.3 mL/min) was combined with a solution of methanolic sodium acetate (0.1 M, 5µL/min) prior to electrospray ionisation into a linear ion-trap mass spectrometer modified to seed ozone (15% O3 in O2) in the helium buffer gas.

Novel Aspect

Resolution of sn-positional isomers of glycerophospholipids by LC-CID/OzID enables the relative quantification in complex extracts.

Preliminary Data or Plenary Speaker Abstract

Reversed-phase chromatography methods were optimised to enhance the resolution of sn-positional isomers PC 16:0/20:4 and PC 20:4/16:0. Through a gradual increase of the non-polar solvent system (B) over the first 7 minutes of the gradient a peak-to-peak resolution of 0.8 could be achieved in 16 minutes and presenting a significant efficiency over previously published methods requiring upwards of 45 minutes to attain an equivalent resolution. While prior investigations had focussed on conventional negative ion MS3 fragmentation of [PC+HCOO]− or [PC+CH3COO]− adduct ions, in our hands, these ions were inefficiently formed leading to an overall low abundance of the desired isomer-specific product ions. Instead, the column eluant was combined with a flow of methanolic sodium acetate that enhanced formation of abundant [PC+Na]+ ions that could then be subjected to sequential CID/OzID activation in a specifically modified linear ion trap mass spectrometer. Comparison of CID/OzID mass spectra obtained for the chromatographically separated isomers of PC 16:0_20:4 revealed abundant and isomer-specific product ions that could be used to uniquely identify the molecular structure in each instance. Moreover, through the analysis of extracted ion chromatograms generated from these isomer-specific product ions, the chromatographic peak positions could be accurately determined to inform peak-fitting algorithms that enabled

quantification of the relative abundance of each isomer with a dynamic range extending down to a 20:1 ratio of the sn-positional isomers. The method has been deployed on a range of complex biological extracts including a lipid extract from a reference human plasma sample that has previously been widely investigated. Preliminary analysis of these data revealed significant variation in the relative abundance of sn-positional isomers with, for example, PC 16:0_20:4 in plasma found to dominated by the expected PC 16:0/20:4 isomer while in contrast, PC 16:0_18:1 was found to be comprised of up to 5% PC 18:1/16:0.

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Lipidomic Patterns and Skin Aging: Combining MALDI MSI FTICR and HPLC MS/MS

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Aging is a complex, multifactorial process that profoundly impacts the skin, our largest organ, by altering its structure, function, and appearance. Lipid components are essential for maintaining skin health, barrier function, and cellular signaling, playing a critical role in age-related changes. Nonetheless, the intricate lipidomic variations associated with the aging process are yet to be fully explored.
Here, we present a systematic study utilizing the high resolving power and mass accuracy of MALDI MSI FTICR along with the identification capabilities of HPLC MS/MS to explore lipidomic variations in young versus aged human skin, and fibroblast cultures subjected to normal and stress-induced premature senescence (SIPS) conditions. Thus, offering novel insights into the role of lipids in the skin aging process.

Methods

HPLC MS/MS analysis employed an Orbitrap Exploris 240 (ThermoFisher) coupled with a UHPLC column in reversed-phase (Kinetex C18, Vanquish) to investigate lipid profiles in control and SIPS fibroblast cultures. This provided a detailed view of lipid alterations under stress-induced senescence. MS-DIAL LipidBlast library was employed for lipid identification. Separately, MALDI MSI FTICR analysis was conducted on skin samples from four donors aged 28, 31, 58, and 62. 5 µm thickness sections were mounted on ITO-coated microscope slides and coated with 1,5-Diaminonaphthalene (0.16 mg/cm²). Analyses were performed using a 7T scimaX FTICR MRMS (Bruker) in both positive and negative ion modes at a lateral resolution of 10 µm.

Novel Aspect

Mapping lipidomic signatures of aging skin by combining MALDI MSI FTICR and HPLC MS/MS analyses.

Preliminary Data or Plenary Speaker Abstract

Following a standard lipidomic approach, we initially conducted HPLC MS/MS analysis on fibroblast cultures. This revealed lipid alterations between control and stress-induced premature senescence (SIPS) conditions, showing significant fold changes. Subsequently, we employed MALDI MSI FTICR at a lateral resolution of 10 µm and mass accuracy below 1 ppm to analyze skin samples. This included samples from four female donors, aged 28, 31, 58, and 62, uncovering distinct lipidomic profiles between young and old samples.
By combining both methodologies, we identified lipids from the HPLC MS/MS dataset that were also detected in the MALDI MSI FTICR data. This produced a distinct subset of lipids found in both datasets.
Within this subset, we observed distinct variations in the distribution and presence of key lipid categories, such as phosphatidylcholines (PC), phosphatidylethanolamines (PE), fatty acids (FA), and ceramides. These specific lipids showed significant quantitative changes between control and SIPS conditions, along with altered distribution patterns, both across different age groups and within the morphological compartments of the skin. These observations highlight the shifts in lipid metabolism associated with the aging process, and provide translational insights for SIPS-induced changes in cell culture versus in vivo aging.

Although comprehensive identification and characterization of all differentially expressed lipids are ongoing, our preliminary findings emphasize the complexity and critical role in the aging process. These observations contribute to the skin lipidomics field within the context of aging and stress the importance of further research to fully investigate the intricacies of lipid changes and their role in aging.

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Combining charge-switch derivatisation with epoxidation for monounsaturated fatty acid isomer discrimination by liquid chromatography/mass spectrometry

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Lipids are ubiquitous molecules essential for life that exist with an extreme structural variety that, until recently, we have lacked the technology to fully appreciate. Various advanced mass spectrometry methods have been developed to better elucidate lipid isomers, including ozone-induced dissociation, electron-activated dissociation and ultraviolet photodissociation. Others have employed various chemical reactions to achieve lipid isomer discrimination, including photochemical reactions (e.g. Paternò-Bùchi), charge-switch derivatisation, and epoxidation. However, many of these methods require specialist instrumentation, instrument modification, or complex chemical reactions that limit the widespread adoption of the technique. In this work, we sought to develop a method for fatty acid double-bond isomer discrimination that can be easily coupled with standard liquid chromatography/mass spectrometry workflows.

Methods

Isomeric monounsaturated fatty acid (MUFA) standards were derivatised with AMP+ as previously described. In methanol, epoxides of AMP-derivatised fatty acids were generated using 10 ug/ul meta-Chloroperoxybenzoic acid (mCPBA). Derivatised epoxides were then separated using a C30 solid-core HPLC column (2.6 µm 2.1 x 150 mm) coupled to an Agilent 6560 IM-QTOF using data-dependent acquisition in QTOF-only mode.

For cell extracts, Panc1 cells were grown to confluency in 6 well dishes and lipids were extracted using a modified MTBE method. Lipid extracts then underwent saponification before being derivatised and epoxidated as described above.

Novel Aspect

The combination of two lipid derivatisation techniques results in baseline separation of isomeric lipid species.

Preliminary Data or Plenary Speaker Abstract

Initial optimisation experiments demonstrated a yield of ~23% epoxide product following 10 min incubation in methanol. 16:1-AMP n-7, n-9, and n-10 isomers could be separated by C30 chromatography at full-width half-maximum, however 18:1-AMP n-7, n-9 and n-10 isomers co-eluted under near-isocratic chromatography conditions. In contrast, 16:1+O-AMP (i.e. epoxy 16:1-AMP) and 18:1+O-AMP n-7, n-9, n-10 and n-12 isomers could be baseline separated. Collisional activation of epoxy-AMP isomers produced more complete charge-remote fragmentation across the acyl chain when compared with AMP alone, and additionally produced diagnostic 16 Da spaced ions indicative of carbon-carbon double bond position.

We then used the method to detect 16:1n-10 and 18:1n-10 isomers in Panc1 pancreatic ductal adenocarcinoma cells. Treatment of cells with a novel compound suppressed both FADS2 expression and n-10 isomers, providing evidence for FADS2 synthesis of n-10 isomers in pancreatic cancer.

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Reliable and Convenient GC-MS Analysis of Human Faecal Short Chain Fatty Acids and Other Organic Acids

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Short chain fatty acids (SCFA) are generated via fermentation of indigestible carbohydrates by endogenous microbiota within the gastrointestinal tract. The large intestine harbours the largest microbial community in the human body and is its main source of SCFA. As well as important metabolic precursors, research has highlighted the influence of SCFA on multiple physiological pathways. The concentration of SCFA in the colon is influenced by various factors, in particular microbiota composition. Consequently, SCFA have become important targets in research that examine the association of gut microbiota to health and disease. Analysis of SCFA in faecal samples represent a convenient measure of microbiome metabolism and colon SCFA generation.

Methods

To satisfy requests from multiple researchers, a robust and accurate method was developed at BMSF, that enabled faecal samples to be conveniently prepared in different, local, offsite, non-analytical laboratories for analysis at a centralised analytical facility. BMSF trained each researcher and supplied heavy isotopic internal standards and calibrations to maintain quality control. Initially, a small aliquot of supernatant from a 10% faecal homogenate was derivatised (aqueous) with pentafluorobenzylbromide (PFB-Br) to generate more stable SCFA – PFB esters. After hexane extraction, SCFA and other organic acid metabolite PFB derivatives were monitored by single quadrupole GC-MS using selective ion monitoring and negative ion chemical ionization (NICI) with methane as reagent gas.

Novel Aspect

Convenient and validated GC-MS method has provided considerable human data for faecal SCFA plus rarely reported organic acid metabolites.

Preliminary Data or Plenary Speaker Abstract

Ionization of SCFA and organic acid PFB derivatives using NICI typically generated a major acid ion, with a loss of m/z 181 that represents the loss of PFB. These specific acid ions were used as the SIM target ions and provided high sensitivity. All analytes were resolved using a mid polarity 50% phenyl, 50% methyl stationary phase capillary GC column. Full analytical validation demonstrated strong accuracy, precision and reproducibility. Over 400 human faecal samples collected at MRC, as a part of the Australia IBD Microbiome Study have been analysed to date. Preliminary data analysis of 90 healthy individuals (18-80 years) confirms that acetate, propionate and n-butyrate are the most abundant SCFA in the colon and that there is considerable inter-individual variation in all SCFA. Faecal samples collected from each volunteer at baseline, six and twelve months demonstrated that SCFA composition was stable over time amongst healthy individuals, with non-significant time shifts. Mean levels of the minor SCFA n-valerate, isobutyrate, isovalerate, and 2-methylbutyrate were at least 10 fold lower than the major 3 SCFA. Other organic acids including succinate, hexanoate, heptanoate, octanoate, phenylacetate, phenylpropionate and benzoate were also much lower.

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Spatial proteomics and metabolomics to characterise glucose uptake and utilisation in multiple species of the ocular lens

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Our sense of sight depends on the ability of the ocular lens to actively maintain its transparent and refractive properties so that light focuses onto the retina. The energy required to maintain the optical properties of the lens is predominantly generated by glucose. Diabetics suffer from poor blood glucose regulation, with cortical cataract formation a common ocular pathology. With the increasing incidence of diabetes, there is an urgent need to increase our understanding of lens glucose uptake and metabolism. Our research uses spatially resolved proteomics and MALDI imaging-based metabolomics to characterise the complement of glucose transporters (GLUTs) and sodium-dependent glucose transporters (SGLTs) present in lenses from different species, and spatially correlate these transporters to sites of preferential glucose uptake.

Methods

For spatial metabolomic studies, bovine lenses were incubated in 5mM stable isotopically-labelled (SIL) glucose (5min – 20h). Lenses were then frozen, and 20µm-thick sections sprayed with matrix (7mg/mL NEDC in 90% MeOH). Tissue sections were analysed by MALDI FT-ICR mass spectrometry (Bruker 7T Solarix-XR). Data were analysed using a combination of SCiLS software (Bruker) and custom scripts generated in R. For spatial proteomics, lenses from multiple species (mouse, rat, rabbit, bovine, human) were microdissected into cortical and nuclear regions. Homogenised lens fractions underwent multiple centrifugation and washing steps (4M and 7M urea), and membrane protein fractions separated via SDS-PAGE. Gel regions corresponding to predicted molecular weights for GLUTs/SGLTs underwent Gel-LC analysis. Tryptic peptides were detected using SWATH-MS (ZenoTOF 7600, Sciex).

Novel Aspect

Confirmation and relative quantitation of GLUTs and glucose metabolism in multiple species of ocular lens.

Preliminary Data or Plenary Speaker Abstract

A signal for SIL glucose [M+Cl]- was detected in all incubated lenses. Initial uptake of SIL glucose was localised to the germinative region of the lens, a region of active cell division and elongation. Over time, SIL glucose signal was detected throughout the whole lens, with most abundance in the lens cortex. In addition, SIL metabolites representing the major metabolic pathways present in the lens were detected. For example, SIL glucose-6-phosphate was detected in the germinative region after 15min of incubation, while SIL sorbitol was detected predominantly in the lens epithelium after 4h, and then spread throughout the whole lens. Spatial proteomics analysis confirmed that GLUTs 1, 3 and 12 were present in the human lens, and suggested a relative abundance of GLUT1 > GLUT12 > GLUT 3. Interestingly, no SGLTs were detected in human lenses, in contrast to rodent lenses which contained isoforms 1 and 2. These results enabled spatial correlation between glucose uptake and GLUT/SGLT localisation to be performed, with future functional studies planned to determine the relative roles that each transporter makes to glucose uptake in normal and cataractous lenses.

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Metabolomics used to investigate reactivity of the bioactive agent sulforaphane with the plasma metabolome

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Sulforaphane (SFN) from broccoli sprouts is a primary plant-derived isothiocyanate (ITC) renowned for its cancer-preventive properties. Diverse cytoprotective effects are attributed to gene activation through interactions with protein sulfhydryl groups in a rapid and reversible manner. SFN also binds to serum albumin lysine residues. SFN's reactivity with endogenous small molecules is far less explored, except its conjugation with glutathione. SFN’s electrophilic nature renders it highly susceptible to nucleophile attack. Primary amines can form irreversible urea adducts and hydrolysis leads to amine at physiological conditions, resulting in loss of SFN pharmacological activity and potentially depleting certain plasma metabolites.
This study employs untargeted metabolomics to investigate how SFN interacts with the plasma metabolome exclusive enzyme activity, uncovering novel biomarkers of pharmacological action.

Methods

Metabolomics

Novel Aspect

We use metabolomics to investigate how SFN interacts with plasma metabolome exclusive enzyme activity, uncovering novel biomarkers of pharmacological action.

Preliminary Data or Plenary Speaker Abstract

We observed loss of SFN upon addition to plasma other than to thiol binding.

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Alterations in Plasma Lipid Profile before and after Surgical Removal of Soft Tissue Sarcoma

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Soft tissue sarcoma (STS) is a relatively rare malignancy, accounting for about 1% of all adult cancers. It is known to have more than 70 subtypes. Its rarity, coupled with its various subtypes, makes early diagnosis challenging. The current standard treatment for STS is surgical removal. To aid in identifying prognosis and pathogenesis, we conducted untargeted metabolic profiling on pre-operative and post-operative plasma samples of STS patients.

Methods

We collected pre-operative and post-operative plasma samples from 24 STS patients who underwent surgical removal of masses. Plasma metabolic profiling was conducted using ultra-high performance liquid chromatography-quadrupole time-of-flight/mass spectrometry. Eleven patients experienced recurrences after the operations.

Novel Aspect

Our investigation revealed distinct lipid profile alterations in STS patients after surgical removal of masses.

Preliminary Data or Plenary Speaker Abstract

Thirty-nine putative metabolites, including phospholipids and acyl-carnitines were identified, indicating changes in lipid metabolism. Phospholipids exhibited an increase in the post-operative samples, while acyl-carnitines showed a decrease. Notably, lysophosphatidylcholine (LPC) O-18:0 and LPC O-16:2 exhibited predictive capabilities for STS recurrence, with area under the curve values of 0.748 and 0.797, respectively.

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Discovering volatile signatures of gut microbiota products in honeybees by secondary electrospray ionization high resolution mass spectrometry

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Understanding the complex relationship between gut microbiota and their host frequently poses a challenge because of the need for invasive sampling techniques. Honeybees often serve as a model organism for studying the role of gut microbiota due to their relatively simple genome, advanced social structure allowing for bacteria transmission, and well-defined roles within the colony. This makes them ideal candidates for investigating how gut microbiota influences various aspects of metabolism, such as immune function, stress response, and colony productivity.

Methods

We present a non-invasive approach to analyzing the volatile metabolites arising in honeybee headspace. By introducing a steady, heated and humidified flow of synthetic air into a measuring tube containing bees, we collect the volatile compounds and direct them to the mass spectrometer. For the headspace analysis, we combined high-resolution mass spectrometry (HRMS) with a soft ambient ionization technique, secondary electrospray ionization (SESI). SESI allows for the ionization of a gaseous analyte by colliding it with a charged nano-electrospray without the need for any sample preparation or preconcentration.

Novel Aspect

Development of a non-invasive method for analyzing honeybee gut microbiota and a new application of volatilome analysis by SESI-HRMS.

Preliminary Data or Plenary Speaker Abstract

We found 2836 significant mass-to-charge features across 32 individual bees, split into two groups: bees depleted of gut microbiota (MD) and colonized with a synthetic gut microbial community (SC). We saw significant changes in the average intensity of various m/z features, especially those corresponding to short-chain fatty acids, the main metabolic products of gut microbiota activity. By comparing matrices of feature intensity across MD and SC bees, we present volatile profiles of the two groups, providing a global overview into the metabolome of honey bees.

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Unveiling chemical impacts of the octocoral Carijoa riisei on artificial reefs in Southeast Queensland, Australia.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Invasive species present challenges to marine ecosystems, particularly in artificial reef environments where they can proliferate rapidly and outcompete native species, disrupting ecological balance. The octocoral Carijoa riisei is widely distributed across the world, including Australia, and is considered an invasive species in some regions and known to use chemical defences of great chemical diversity. Understanding the chemical ecology of invasive species is therefore paramount for assessing their impact on communities. Mass spectrometry is a technique that allows for the identification and quantification of molecules, providing information on the chemical makeup of complex samples. This study applies mass spectrometry-based metabolomics to elucidate what role chemicals produced by C. riisei have in shaping reef dynamics within artificial reefs in Southeast Queensland.

Methods

- LC-MSe Untargeted metabolomics workflow and feature based molecular networking of metabolomics data.
- Structure elucidation using Nuclear Magnetic Resonance (NMR) Spectroscopy.
- Spatial metabolomics using DESI and MALDI Mass Spectrometry Imaging (MSI).

Novel Aspect

Application of MSe untargeted metabolomics data and spatial metabolomics to enrich and shed light on chemical interactions in reef systems.

Preliminary Data or Plenary Speaker Abstract

Utilising SCUBA, in-situ investigations revealed that C. riisei employs chemical defences to deter predators and inhibit settlement and growth of neighbouring organisms. Therefore, we decided to implement an untargeted metabolomics workflow combined with feature based molecular networking and NMR spectroscopy to comprehensively profile metabolites and elucidate the structures of secondary metabolite classes produced by the coral. Furthermore, employing Mass Spectrometry Imaging (MSI), we detect and spatially map the distribution of specialised metabolites, providing valuable insights into their ecological roles within reef ecosystems.

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Carbon and Nitrogen Positional Isotopomer Determination in Metabolites using Orbitrap IQ-X and a novel hybrid nominal mass instrument.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Fluxomics or flux analysis aims to quantify metabolism by computing the flow of carbon or nitrogen atoms through various metabolic pathways. Use of stable isotopes for flux analysis in biology has contributed greatly to the understanding of cellular metabolism and regulation. However there remains a challenge to determine accurate flux rates for metabolites which are formed from multiple precursors utilizing different enzymatic pathways. Positional isotopomer distribution of metabolites obtained by utilizing multiple tracers are invaluable pieces of information for the computation of these fluxes. Classically done by GC-MS and NMR, here we show the application of liquid chromatography-based separation of metabolites with the use of hybrid dissociation strategies using HCD and CID MS^3 fragmentation for positional isotopomer determination.

Methods

HEK cells were grown under different oxygen tensions (50%, 21% and 1.5%). Cells were unlabeled or labeled with $^{13}C_6$ Glucose, $^{15}N_2$ Glutamine or both $^{13}C_6$ Glucose and $^{15}N_2$ Glutamine. Initial data were acquired on a Thermo Scientific™ Orbitrap IQ-X™ Tribrid Mass Spectrometer. The use of a tribrid allows the use of hybrid strategy utilizing higher-energy collisional dissociation (HCD) and collisionally induced dissociation (CID) for fragmentation of metabolites up to MSn level. Thermo Scientific™ Mass Frontier™ 8.0 and Compound Discoverer™ 3.3 software were used for the metabolite annotation and data processing. A targeted MSn based assay utilizing the above data to get the structural information of the metabolites was developed on a novel hybrid nominal mass instrument.

Novel Aspect

Workflow and application of positional isotopomer determination of metabolites using LC-MS with a hybrid dissociation strategy.

Preliminary Data or Plenary Speaker Abstract

GC-MS and NMR have been used extensively for positional isotopomer determination in fluxomics. However, NMR lacks the sensitivity to detect low abundant metabolites and GC-MS cannot give all the positional isotopomers of a metabolite. Moreover, GC-MS lacks the resolution for dual tracer experiments (for e.g., using ^{13}C and ^{15}N). Use of high-resolution accurate mass instruments with MSn capability can help overcome the challenges of using GC-MS.
Initial experiments were done using ^{12}C and U-^{13}C labeled E.Coli cell extracts. Metabolites were separated on HILIC-AEX column. Based on biological importance and complexity ATP, CTP and glutamate were chosen for the analysis. HCD and CID MS/MS spectra of these metabolites was annotated and curated using Mass Frontier 8.0 which is a spectral interpretation software. Based on the structural information of the fragments obtained from Mass Frontier 8.0, MS^3 experiments were designed to target fragments which could potentially give us all the positional isotopomers. HCD and CID MS^3 spectra from these fragments were again annotated and curated using Mass Frontier 8.0 for determining the optimum secondary ions to fragment. For ATP and CTP molecules, six MS^2 fragments were selected for further fragmentation to obtain the positional information of carbon and nitrogen atoms. For glutamate, three MS^2 fragments were selected.
An initial HCD/CID MS^2 profiling experiment was done on the HEK cell extracts. A targeted MS^3 experiment was designed, from the data obtained, for all the detected isotopologues and the optimized labeled MS^2 fragments of the three metabolites. The targeted assay was run on Orbitrap IQ-X Tribrid Mass Spectrometer and a novel hybrid nominal mass spectrometer. Differences in the

nitrogen and carbon labeling of the metabolites in three different oxygen tension were quantified and correlated to metabolic pathways.

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Mass spectrometer interface gas flow visualisation techniques in atmospheric and vacuum parts.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Understanding of gas flow profiles is an important aspect of MS device improvements. Visualization is the key to understanding these processes in general. Charged particle optics can be understood through simulations, the remaining element that is challenging to model is gas dynamics. Computational fluid dynamics (CFD) simulations can provide some insights of the internal gas dynamics within the instrument, but this still requires experimental comparison. To master MS instrument development, a number of visualisation techniques were combined to reveal gas flow in an ESI-MS system on both the atmospheric and vacuum sides of the instrument. The techniques we used were fast photography, Schlieren photography and electric discharge . ESI gas stream as well supersonic gas jet plume visualisations are presented.

Methods

The atmospheric pressure side of MS with an ESI source was investigated by means of fast photography and Schlieren photography. We used a high-resolution monochromatic camera with short 20 µs exposure times and strong LED light sources with different wavelengths. This setup was used for ESI droplet visualisation. The ESI gas exhaust and MS inlet capillary gas intake was visualised with the use of a Schlieren photography setup built with two parabolic and two planar mirrors. Imaging was carried out through enlarged ESI chamber windows as well for an exposed ESI head. The MS vacuum side was reproduced with the use of a dummy chamber containing transparent windows. The electric discharge was generated by Tesla coil 10 cm sparks.

Novel Aspect

Light wavelength and exposure time in ESI Schlieren photography.
Electric discharge visualisation technique to reveal MS capillary supersonic jet plume.

Preliminary Data or Plenary Speaker Abstract

An ESI gas exhaust was visualised, in different conditions, inside the chamber as well in an open environment. Light sources with different wavelengths were tested as well different camera exposure times. Visualization of this process allows better ESI gas flow adjustment and understanding of the working principles.
The MS capillary takes ions from ESI atmospheric pressure to the MS vacuum side. On the vacuum side a supersonic gas jet plume is created that determines the initial distribution of ions. The plume expands into an ion optic device with the main role of capturing as many ions as possible. Therefore, the shape of the gas jet plume plays an important role in system design and optimisation. With the use of the simple technique of electric discharge we were able to extract the supersonic jet plume shape. The process was visualised inside the pumped dummy chamber where electric field pulses were provided by the Tesla coil discharges.

Application of Gas Chromatography–Mass Spectrometry (GC–MS) to Detect Foodborne Pathogens

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Salmonella is a significant food safety concern associated with foodborne illnesses. The gold standard for Salmonella testing approaches, culture-based methods, require up to one week for identification, a major challenge during mass production. Increasing literature has focused on identifying microbial metabolic footprints using gas chromatography–mass spectrometry (GC–MS) to enable rapid microbial detection. However, most research has mainly focused on identifying polar metabolites (e.g., sugars and amino acids), which have challenging sample preparation workflows and lengthy analysis times. Therefore, the current research aims to explore the identification of non-polar secondary metabolites (e.g., fatty acids and terpenoids) that are often more unique to the organism and require less intensive sample preparation workflows to enable rapid Salmonella detection using GC–MS.

Methods

A known foodborne pathogen, Salmonella enterica serovar Typhimurium was chosen as the model bacteria. A known pathogen concentration (1 cfu/mL) was spiked in routine broth and incubated at 37°C for 24 hours. Sterile broth served as the non-spiked control sample. In the preliminary study, a solvent-based extraction method was optimised, and putative non-polar biomarkers of Salmonella were identified using GC–MS. Based on this, headspace-solid-phase microextraction (HS-SPME) was adopted and optimised to enable a more rapid and less labour-intensive workflow that can offer optimal detection sensitivity without reliance on toxic solvents. Compound identification was based on the unknown compound's retention time and retention index relative to the references using the National Institute of Standards and Technology (NIST) 11 MS Library.

Novel Aspect

Our study shows that non-polar biomarkers can be targeted using HS-SPME-GC–MS to provide a novel high-throughput pathogen detection tool.

Preliminary Data or Plenary Speaker Abstract

Three statistically significant non-polar metabolites, namely, 3-ethylbenzaldehyde, 4-terpinenyl acetate, and beta-ionone (false discovery rate-adjusted p-value of less than 0.05), were identified as putative biomarkers based on NIST 11 MS Library matching. In summary, targeting non-polar secondary metabolites of pathogenic bacteria using HS-SPME-GC–MS offers a promising strategy for the early detection and screening of Salmonella. With automation and further validation, the proposed workflow has the potential to improve high-throughput testing, especially around sample handling and preparation. However, this proof-of-concept study still requires further research to explore the limits of detection, validation of putative biomarkers, and specificity of the assay.

Analysis of volatile aldehydes using secondary electrospray ionization mass spectrometry.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Aliphatic straight-chain aldehydes have been consistently identified in the breath of individuals with lung diseases through multiple detection methods, including mass spectrometry, ion mobility spectrometry, and electrochemical sensors. Research has revealed elevated levels of these exhaled aldehydes in patients with various lung conditions, including lung cancer, inflammatory and infectious lung diseases, as well as mechanical lung injuries. Quantifying straight-chain aliphatic aldehydes in the clinical setting is thus highly important. This work focuses on the qualitative detection and quantification of volatile straight-chain aliphatic aldehydes using an online secondary electrospray ionization source coupled to a high-resolution mass spectrometry system.

Methods

A secondary electrospray ionization (SESI) source coupled to a high-resolution mass spectrometry (HRMS) system was employed for the first time to detect and monitor, both qualitatively and quantitatively, selected volatile straight-chain aliphatic aldehydes in the gas phase. The generation of gas standards was done using a built-in-house vapor generator based on the controlled evaporation of volatile or semi-volatile chemical analytes and their diffusion into a carrier gas stream. SESI-HRMS is a powerful, well-established, and robust analytical technique ideal for in-depth breath metabolomics characterization, offering high sensitivity (low limits of detection), selectivity, fast (within seconds), and accurate analysis.

Novel Aspect

Online SESI-HRMS for volatile aldehyde detection in breath.

Preliminary Data or Plenary Speaker Abstract

Experiments were undertaken for propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, and decanal at different concentrations and flow rates. Both individual compounds and mixtures were tested. Gas-phase experiments were performed at concentration levels from low ppt to low ppm and in both dry and humid conditions. The experimental results obtained showed a precise and repeatable production of gas standards with excellent linearity within the examined concentration range, low ppt detection limits, and fast response times.

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Simultaneous quantitation and untargeted discovery (SQUAD) workflow for diagnostic fragments of coeluting sugar-phosphates using orthogonal fragmentation techniques

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Sugar-phosphates are key intermediate metabolites in carbohydrate metabolism. Identifying these compounds is challenging, because they are similar in structure and often not resolved chromatographically. Also, they don't produce unique fragments with a single fragmentation type. Employing multiple fragmentation techniques to generate unique fragment ions followed by targeted quantitative analysis of the diagnostic ions yield better identification.
Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ mass spectrometer with a high-resolution Orbitrap detector, a sensitive Ion trap detector along with multiple fragmentation options; Higher-energy collision dissociation (HCD), Collision induced dissociation (CID), and Ultraviolet photodissociation (UVPD) is a well-suited instrument for these analyses. Here, a simultaneous quantitation and untargeted discovery (SQUAD) analysis was developed to characterize and quantify sugar phosphates based on their unique fragmentation ions.

Methods

10mM ammonium acetate with 0.1% Acetic acid in Acetonitrile and Water (from Fisher Scientific). Six sugar-phosphate standards (from Sigma-Aldrich)

A SeQuant® ZIC®-pHILIC column connected to Thermo Scientific™ Vanquish™ Horizon LC, Orbitrap IQ-X with HCD, CID, and UVPD fragmentation options was used to generate, quantify diagnostic fragments.

Individual standards and their adducts were evaluated using multiple fragmentation techniques. SQUAD experiments using UVPD, HCD, and CID were performed on dilution series (30uM to 1mM). Samples of three coeluting sugar-phosphate standards, varying the concentration of each isomer were subjected to SQAUD experiments.

Spectra were evaluated for unique fragments, quantified using Thermo Scientific™ FreeStyle™ 1.8 software. Further annotated using Thermo Scientific™ Mass Frontier™ 8.0 software. Calibration curves were also generated using TraceFinder software.

Novel Aspect

HRAM, HCD, CID, MSn, and UVPD to differentiate and quantify sugar phosphate isomers during a SQUAD analysis.

Preliminary Data or Plenary Speaker Abstract

Fragmentation spectra collected from targeted analysis of sugar phosphates using multiple activation types are evaluated for unique ions. The fragment ions are treated as uniquely diagnostic if they are observed to be found alone for that sugar phosphate. Such unique diagnostic ions are observed for UVPD, CID, and HCD fragments, providing enough evidence for the differentiation of coeluting sugar phosphates. Peak areas for the diagnostic ions for all the dilution series (30uM to 1mM) were calculated and calibration charts were generated. The linear dynamic range for sugar phosphates is five orders of magnitude. From the three coeluting sugar phosphates data, the linear range for the

Precursor ion, most intense ion and the unique ion as well is measured for each sugar phosphate. The linear It was observed that Fructose-6-Phosphate unlike other two sugar phosphates, showed significantly abundant peak for its water loss precursor instead of its monoisotopic precursor. Moreover, the base peak in its MS2 spectra is relatively intense and its abundance increases as the Fructose -6-Phosphate concentration increases. Similar behavior is observed for its unique diagnostic ion as well. Thus, making the unique ion quantifiable in the presence of other two sugar phosphates. A linear response down to mMolar was observed for both the intense ion and the diagnostic ion. Future work is planned to perform SQAUD analysis to identify sugar phosphates in biological samples.
Orbitrap IQ-X Tribrid mass spectrometer with a high-resolution Orbitrap and a sensitive ion trap along with multiple fragmentation options makes the instrument a powerful tool in differentiating and quantification of isomeric structures with ion-trap detection while simultaneously performing untargeted discovery analysis in the Orbitrap.

Metabolomics studies on Brazilian native plants as a source of anti-COVID-19 multitarget agents

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Plant secondary metabolites have promising pharmacological activities due to their specific mechanisms of action in biological systems. In a context which viral infections are part of modern society, aggravated by globalization, the discovery of new antiviral substances is essential. Currently, the Phytochemistry and Pharmacognosy (UFRJ/BR) and the Phytochemistry and Countercurrent Chromatography (UFRJ/BR) labs hold a bank with about 200 samples, among extracts, fractions, and isolated substances of Brazilian plant species, which have been used in several studies following the random and ethnopharmacological approaches and resulting in promising findings. Thus, the aim of this work is to conduct a metabolomics investigation guided by in vitro and chemometric analyses, to identify potential agents in the tackling of COVID-19 and SARS-CoV-2.

Methods

In vitro assays include the inhibition of the interaction between the Spike protein and ACE2, and proteases activity (3CLpro and PLpro). Chemical data were obtained in a UHPLC-IT-MS/MS system, in the positive and negative modes, using ESI and/or APCI sources, then processed using MZmine and GNPS for data mining and molecular network. PLS regression employed m/z ions and retention time of the samples as independent variables (X) and inhibition rates for the targets as the dependent variable (Y), combining principal component analysis (PCA) and linear regression to correlate predictors and biological activity.

Novel Aspect

The research supports metabolomics and computational tools usage for drug discovery from Brazilian flora, highlighting its potential against COVID-19.

Preliminary Data or Plenary Speaker Abstract

The bank covers species from 11 botanical families, of which 147 extracts were screened, representatives of 8 families and 28 species, against three of SARS-CoV-2 most important targets: the proteases 3CLpro and PLpro, related to virus replication, and the interaction between the Spike protein and its receptor ACE2, related to viral cell attachment and entry. Among the tested samples, 39 were able to inhibit the interaction between Spike protein and ACE2 over 50%, while 58 and 65 extracts were able to inhibit 3CLpro and PLpro activities, respectively, in the same rate. Among the most active extracts, 13 were classified as multitarget once they were capable of inhibiting the three targets and these samples belong to representative families on the extracts bank: Siparunaceae (A.DC.) Schodde and Myrtaceae Juss. The ions predicted by the PLS model were tentatively annotated using the fragmentation patterns, references check and molecular networks. For Siparunaceae family, the model predicted the isoquinoline alkaloids ([M+H]+ m/z 312, 328, 314, 268, 326, 286, 330) to be the most related to the multitarget activity, being annotated as actinodaphine, boldine, laurolitsine, assimilobine, bulbocapnine, coclaurine and reticuline, respectively. These substances can be found in several Siparuna spp. extracts, some of which present inhibitory activity against respiratory viruses such as influenza and SARS-CoV-2. Of these substances, bulbocapnine (m/z 326) presented the highest activity potential, as it exhibited significant contribution values in explaining activity across all constructed chemometric models. Based on fragmentation patterns, for the Myrtaceae family, PLS regression pointed out 5 myrtocummulones as the ones responsible for the observed activity that were annotated as semimyrtucommulone and nor-semimyrtucommulone,

and three more that were not annotated due to the lack of references, suggesting they might be novel compounds, not yet recorded in the family.

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Metabolomic Profiling of Osteocyte Extracellular Vesicles and Matrix Bound Vesicles using a Prototype Benchtop Multi Reflecting Time-of-Flight (MRT) Mass Spectrometer

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Extracellular vesicles (EVs) are lipid delimited nanoparticles that function in development and intercellular signaling events throughout the body. Within bone there exists a subset of EVs, known as matrix binding vesicles (MBVs), which have been long proposed to associate with the underlying collagenous extracellular matrix to drive early mineralization events during bone development. However, the precise relationship between EVs and MBVs and their differential roles in bone development and metabolism remains a point of contention[1].

This study uses a prototype benchtop MRT to construct a comparative metabolite profile for EVs and MBVs obtained from MC3T3 pre-osteoblasts under osteogenic culture conditions.

Methods

MC3T3 osteoblast cells were cultured to confluence and differentiated towards a pro-mineralising phenotype in the presence of 10mM beta-glycerophosphate and 50µg/mL ascorbic acid. EVs were isolated from the media using a differential ultracentrifugation (UC) process and lysed using sonication which incorporated no lysis buffer. MBV's were liberated from the ECM using collagenase digestion and subjected to UC isolation.
Lysed extracts underwent a water:MeOH:MTBE extraction and the aqueous layer analysed in triplicate by LC-MS using a prototype benchtop MRT. Polar metabolites were chromatographically separated using HILIC and Reversed-Phase chromatographic methods, in positive and negative electrospray ionization (ESI) modes. The data were processed using a variety of informatic tools prior to statistical analysis for multi variate analysis (MVA) and pathway analysis.

Novel Aspect

A prototype benchtop MRT to perform metabolic profiling of extracellular vesicles and matrix bound vesicles derived from MC3T3 osteocyte cells.

Preliminary Data or Plenary Speaker Abstract

EVs and MBV's were extracted from MC3T3 osteocyte cells and analysed by both HILIC and Reversed Phase chromatographic methods - to ensure maximal compound coverage – on a prototype benchtop MRT mass spectrometer. Chromatographic separation was investigated using 'conventional' scale (10 minute gradient on a 2.1x100mm column) and also a higher throughput methodology which utilized a 3.5 minute gradient, using a 1x50mm column. Data were acquired using a data independent analysis (DIA) strategy, across the mass range 50-1200 Da, and the instrument consistently produced a mass spectral resolution in the region of 100,000 FWHM.

This feasibility dataset has putatively identified several significant biologically relevant polar metabolites, including amino acids, carboxylic acids, fatty acids, phenols, pyridines and indoles being present within both the EV and MBV extracts. LC-MS data were processed using MARS (Mass Analytica). The data were peak picked, normalized and putative compound identifications were gained with database searches conducted using a library consisting of the human metabolome (HMDB) database. A mass tolerance of +/- 1ppm was used as a tolerance for compound matching,

which resulted in mass accuracies being returned at the sub-ppm level. Compound expression levels were then investigated using MVA. Unsupervised principal component analysis (PCA) revealed potential differences between the EV and MBV samples. A PLS-DA analysis was then performed which enabled the generation of an S-plot to highlight discriminating features, tentatively identified as fatty acids, amino acids and glycerophosphocholines.

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IP-MALDI-Based Analysis of Endogenous Tau Fragments in Cerebrospinal Fluid for Discovery of Biomarkers for Neurodegenerative Diseases

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Tau protein, a critical microtubule-associated protein for neuronal structure and function, undergoes hyperphosphorylation and fragmentation in Alzheimer's disease (AD). These changes are believed to play a key role in disease progression. Mid-domain tau phosphorylation (e.g., pTau-217) is a recognized marker of amyloid pathology, while microtubule binding region tau (MTBR-tau) shows promise for specific tau tangle detection. This study aims to develop a sensitive and robust MALDI-MS method for analyzing mid-domain and MTBR endogenous tau fragments in Cerebrospinal Fluid, offering insights into tau-related mechanisms in AD.

Methods

Immunoprecipitation (IP) with monoclonal antibody HT7/77G7 enriches mid-domain/MTBR tau, respectively. Conditions were optimized to minimize adsorption losses during IP. Isolated tau fragments were then analyzed by Nano-LC/MALDI-MS with a bio-inert autosampler. Nano-LC eluent was spotted directly onto a 150 um-diameter DHB matrix using a column-integrated spotting probe.

Novel Aspect

high-yield IP and bioinert Nano-LC/MALDI-MS techniques to identify novel CSF tau fragments.

Preliminary Data or Plenary Speaker Abstract

Our high-yield IP and bioinert Nano-LC/MALDI-MS approach identified numerous potential mid-domain/MTBR tau fragment peaks from 0.5 ml CSF. The results revealed the presence of novel and diverse fragment species, some of which were mono-/multi-phosphorylated. Additionally, we developed a tool to generate theoretical spectral peaks of all possible endogenous fragments containing the targeted mid-domain/MTBR tau epitope sequence. This tool, along with filtering for series peaks with identical sequences but varying modifications, will significantly refine candidate identification. We aim to discuss the potential of these phosphorylation patterns and fragment species as novel markers and the
advantages of this unique analytical method.

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The ion mobility/mass spectrometry method for analysis of phosphorylated tau peptides in cerebrospinal fluid.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Aberrant tau phosphorylation is a hallmark in tauopathies. Hyperphosphorylation promotes the formation of paired helical filaments which are the main constituents of neurofibrillary tangles. While cerebrospinal fluid levels of total tau and tau phosphorylated at threonine 181 are established core biomarkers for AD, the values of alternative phosphorylation sites, which may have more direct relevance to pathology, for early diagnosis is not yet known. To characterize specific phosphorylations of tau peptides in CSF, we have applied an innovative mass spectrometry workflow. Using ion mobility separation, we were enabled to provide valuable structural information and separate different isobaric tau peptides. This is the most critical step for the development of ultra-sensitive immunoassays allowing the quantification of novel biomarkers at sub-picogram levels.

Methods

For extraction of tau peptide sample we used 500µl of human CSF. 25 µl of 70% perchloric acid were added for protein precipitation and samples were kept on ice for 15 min before centrifugation. Supernatants were collected and mixed with 50µL of 1% trifluoroacetic acid (TFA). Peptide extraction was performed using 96-well OasisHLBElution plate and extracts were digested for 24 hrs. with trypsin
solution. Finally, the digested result was acidified with 5µLof 10% acid formic. The high-resolution mass spectrometry analysis was performed using a Synapt-G2Si instrument coupled to the Acquity M-class nano
LC system and equipped with ionKey source (Waters, Prague, Czech Republic). iKey HSS T3 Separation Device was used for peptide separation.

Novel Aspect

Using ion mobility separation, we were enabled to provide valuable structural information and separate different
isobaric tau peptides.

Preliminary Data or Plenary Speaker Abstract

We developed a method based on a combination of collision-induced dissociation, isomeric triply protonated ions (m/z 500.9) mobility separation and post-mobility dissociation to aid in analyzing the isomeric phosphopeptides of tau in human CSF.

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Analysis of amino acids in Mucuna pruriens supplements for the treatment of Parkinson's disease

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Parkinson's disease (PD) is a neurodegenerative disease characterised by the progressive loss of dopaminergic neurons of the substantia nigra, resulting in severe motor complications including resting tremors, rigidity, and bradykinesia. Due to resulting dopamine deficiency, the gold standard for PD treatment is supplementation of the precursor amino acid L-DOPA. The climbing legume Mucuna pruriens naturally produces high levels of L-DOPA, and has been used as an alternative medicine for PD as it may have more favourable long-term outcomes compared to traditional L-DOPA drugs which have reduced efficacy in chronic treatment. This may be due to cognate protein amino acids preventing L-DOPA misincorporation in proteins. Here we analysed Mucuna pruriens supplements by HILIC-MS/MS to determine their L-DOPA, phenylalanine and tyrosine content.

Methods

Five commercially available preparations of Mucuna pruriens supplements, consisting predominantly of Mucuna pruriens seed extract, were analysed for their respective phenylalanine, tyrosine, and L-DOPA amino acid levels. Each preparation was analysed by pooling dose units of each supplement and taking a representative dry weight per sample in triplicate. Samples were prepared for the determination of the free and protein bound amino acids, with the latter fraction retrieved using acid hydrolysis. These samples were analysed on a newly developed and validated HILIC-MS/MS method which has transferable applications for the detection and quantification of the aforementioned compounds as well as tyrosine structural isomers (implicated in aging) in a range of biological matrices.

Novel Aspect

The developed HILIC-MS/MS method has a broad range of applications in the analysis of biological samples for PD and aging.

Preliminary Data or Plenary Speaker Abstract

The developed and validated analytical method demonstrates comparable quantification limits to established reverse phase chromatography methods in the literature, while expanding on detectable compounds in what is traditionally considered a separation method with reduced analytical sensitivity. HILIC-MS/MS analysis of the five different supplements found L-DOPA levels to range between 66.2-82.7% of the amount reported by the respective manufacturers (when it was provided). All the L-DOPA detected was found in the free amino acid fraction. The L-DOPA protein amino acid precursors phenylalanine and tyrosine (para- or L-) were found in both the free and protein bound fraction of each supplement while none of the structural isomers of tyrosine were detected. Phenylalanine was found in levels ranging from 0.069-2.602% of the respective supplement's amount of L-DOPA, with tyrosine found at levels ranging from 0.068-2.923%. Commercially available Mucuna pruriens supplements are unregulated and not currently recommended for clinical use, however, are popular alternatives around the world due to availability and affordability compared to traditional L-DOPA formulations. This work demonstrates both the need for the ability to analyse these supplements due to the lack of regulation, as well as providing evidence for the suggested improved long-term outcomes of these supplements in PD patients through co-supplementation of the cognate protein amino acids phenylalanine and tyrosine. These protein amino acids may prevent misincorporation of L-DOPA in peptides, a hypothesised mechanism for the worsening of PD pathogenesis observed in chronic L-DOPA treatment.

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Capturing the oligomerization dynamics of ALS-related Cu/Zn superoxide dismutase with temperature-controlled nESI and cyclic IM-MS

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Neurodegenerative diseases share a complex molecular mechanism of protein misfolding, oligomerization, and aggregation. For example, the fatal neuromuscular disorder Amyotrophic Lateral Sclerosis (ALS) is characterised by the accumulation of soluble (oligomers) and insoluble (aggregates) multimeric assemblies harbouring non-native conformations of Cu/Zn superoxide dismutase (SOD1), arising from more than 120 gene mutations. The underlying mechanisms behind early aggregation, a key neurotoxic feature of ALS, remain poorly understood. This is due to the lack of techniques that can capture the misfolding and oligomerization dynamics of SOD1. In this study, bovine SOD1 was used to develop a continuous assay that utilises temperature-controlled nESI (TC-nESI) and ion mobility spectrometry (IMS) to elucidate the conformational dynamics of early aggregation, in different metalation states.

Methods

Cu/Zn superoxide dismutase from bovine erythrocytes was purified by size exclusion chromatography (SEC) and diluted to 40 µM of native SOD1 dimer in 200 mM ammonium acetate (pH 7). To remove the metal co-factors, roughly 1 mg of bovine SOD1 was incubated in 10mM EDTA 50mM ammonium acetate chelating solution for one or two days to yield Zn-SOD1 and apo-SOD1, respectively. The chelated samples were buffer exchanged to 200 mM ammonium acetate by SEC prior to MS analysis. Thermal unfolding experiments were conducted utilizing a custom-built temperature-controlled nESI source coupled to cyclic IMS, which allowed for structural and conformational characterization of misfolded and oligomeric species, while different solution heating rates (1, 0.5 and 0.3 °C/min) and metal-induced stability were explored.

Novel Aspect

Our proposed assay allows for rapid and simultaneous structural, conformational, and kinetic characterization of oligomerization pathways applicable to disordered proteins.

Preliminary Data or Plenary Speaker Abstract

Native bovine SOD1 is a homodimer containing a Cu2+ and a Zn2+ ion in each subunit. Since the loss of metal co-factors promotes oligomerization in the disease mutants of wild-type SOD1, three different metalation states were assessed. Specifically, native holo-SOD1, Zn-SOD1 (one Zn2+ ion per subunit), and apo-SOD1 (stripped of metal co-factors) were investigated. Initial optimization showed that the oligomeric assemblies could only be observed at a heating rate of 0.3 °C/min, revealing a kinetic effect. For holo-SOD1, a melting temperature (Tm) of 76.4 ± 2.5 °C suggests high stability for the dimer. Conversely, Zn-SOD1 (Tm of 51.6 ± 1.4 °C) and apo-SOD1 (Tm of 51.5 ± 0.7 °C) showed a significant decrease in thermal and structural stability. In all metalation states, the dimer presented three conformations before dissociating into monomers of two conformational populations, hinting at asymmetric unfolding. Concerning the oligomerization profiles, holo-SOD1 generated mostly trimers, hexamers, and octamers, while initially observed tetramers dissociated into trimers and monomers. As for Zn-SOD1, progressive loss of Zn2+ ions accelerated oligomerization while pentameric and heptameric assemblies were additionally observed. Finally, for apo-SOD1, misfolding and oligomerization were actively promoted. For the apo-dimer, there are indications of a two-step transition to monomers, confirming variable unfolding. In its oligomers' profile, all the assemblies up to octamers are observed, while trimers are seemingly consumed throughout the pathway, hinting at their integration in larger assemblies. In terms of geometry, linear growth was predicted by performing CCS calculations of the holo-SOD1 oligomers. Finally, monitoring these pathways in a time-resolved manner allowed us to characterize them kinetically. Initial dimer unfolding and loss of

metals (slow) is followed by dimer dissociation (fast) and misfolding of monomers (slow), which, in turn, drives oligomer assembly (fast).

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Comprehensive Proteomic Profiling in Accelerated Aging of Kidney Cortex and Medulla in Non-Human Primates

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Whole-body ionizing radiation (IR) causes damage to DNA, lipids, and proteins. IR exposure may lead to oxidative stress, inflammation, and cellular senescence, processes that accelerate biological aging. Slowly reproducing tissues, including kidney, are more likely to react to DNA damage, which may exacerbate aging processes. Here, quantitative data-independent acquisitions were employed to gain molecular insights into how IR exposure may drive renal aging processes in two kidney regions, the cortex and medulla. The elevation of senescence-associated secretory phenotype (SASP) factors and loss of proteostasis was investigated in the context of non-human primates with IR only, kidney disease (KD) only, and the combination of IR with KD to uncover new targets for interventions to enhance resilience and promote healthy kidney aging.

Methods

Kidney cortex and medulla tissues (N=38 each) were obtained from the Wake Forest Non-Human Primate Radiation Late Effects Cohort (NHP RLEC) and experiments were conducted in compliance with state and federal animal welfare laws. Tissues were homogenized for protein extraction. Proteins were digested using S-Trap and desalted by HLB C18-cartridges, then analyzed on a timsTOF HT mass spectrometer (Bruker) in data-independent acquisition (DIA) mode using parallel accumulation serial fragmentation (PASEF) to quantify dynamic protein changes across the following conditions: control with no kidney disease (CTL), irradiated with no kidney disease (IR), irradiated with kidney disease (IR KD), and control with kidney disease (KD). Raw data were analyzed using Spectronaut v18 (Biognosys) and significantly-altered proteins were subjected to bioinformatic analysis.

Novel Aspect

This is the first proteomic analysis to study SASP factors in kidney cortex and medulla from aging survivors of radiation.

Preliminary Data or Plenary Speaker Abstract

We leveraged this NHP cohort, a unique national resource, to study accelerated aging and heterogeneity in resilience and aging in the kidney after ionizing radiation exposure. In this analysis, 5,472 and 5,411 M. mulatta protein groups with at least two unique peptides were identified in the kidney cortex and medulla, respectively. As the M. mulatta proteome is not well annotated, proteins from this dataset were mapped to homologous human proteins, thus providing a better annotation of the biological processes and associated gene ontology. There was an overlap of 4,374 H. sapiens protein groups identified in both the kidney cortex and medulla, with 547 and 314 protein groups uniquely identified in the cortex and medulla, respectively. Groupwise comparisons were performed to study the influence of 1) kidney disease in irradiated individuals (IR KD vs. IR), 2) only kidney disease (KD vs. CTL), and 3) only irradiation (IR vs. CTL). Interestingly, in almost all comparisons in both the cortex and medulla, over 75% of significantly-altered proteins (i.e., Q value < 0.01 and absolute average log2fold change > 0.58) are significantly upregulated. This includes the extracellular matrix and SASP proteins tenascin, upregulated in all conditions in the cortex and medulla, and periostin, upregulated in all conditions in the cortex. In both tissues, proteins that were significantly-altered and associated with kidney disease in irradiated and non-irradiated animals included SASP proteins and were linked to biological processes involving proteolysis, EGF pathways, integrin

signaling, fibrosis, and inflammation. In both tissues, irradiation exposure alone, regardless of kidney disease status, was linked to significantly-altered SASP proteins involved in fibrosis and proteolytic pathways. Similarities in the fibrotic and proteolytic processes from significantly-altered proteins in either kidney disease or irradiation highlight a potential synergistic effect of irradiation on kidney disease. Overall results from this study will be presented.

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How to improve the resolution of MS/MS based molecular networks? Add the Ion mobility dimension. A cocoa polyphenol isomers study.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

During processing (i.e. fermentation, sun drying...), the polyphenols from cocoa beans undergo biochemical and chemical transformations, leading to complex isomeric compounds that may co-elute in liquid chromatography. MS/MS spectra obtained from those co-eluting isomers are poorly informative and correspond to mean fragment ion spectra.
A feature-based molecular networking (FBMN) analysis reveals large clusters containing tenth of compounds.
The objective of this study was to improve the resolution of the FBMN by incorporating trapped ion mobility spectrometry (TIMS) data into a UHPLC-HRMS/MS workflow.

Methods

Two sets of fine cocoa beans, having the particularity to generate chocolates of black or brown colours [Dias et al., Metabolites 2023, 13(5), 667; https://doi.org/10.3390/metabo13050667], were analysed on a UHPLC-TIMS-QTOF mass spectrometer, with and without activation of the ion mobility module. The processed HRMS and HRMS/MS data underwent univariate and multivariate statistical analyses as well as FBMN analysis.

Novel Aspect

An IMS based method to enhance the resolution of molecular networks of isomeric plant specialized metabolites.

Preliminary Data or Plenary Speaker Abstract

The additional mobility dimension of TIMS improved the resolution of the molecular networks compared to the approach without TIMS, by the presence of additional nodes and clusters in the networks corresponding to isomeric compounds. Several types of isomers separated by TIMS were annotated based on their distinct MS/MS fragmentation patterns. They included polyphenol monomers ((epi)catechin-O-hexoside and (epi)catechin-C-hexoside), dimers (B-type procyanidin dimers and dehydrodicatechins B), trimers (B-type procyanidin trimers and dehydrotricatechins B), and tetramers (B-type procyanidin tetramers). A metabolomics statistical analysis workflow revealed that the majority of those isomers were discriminating compounds on cocoa beans, for black or brown chocolate.
Moreover, the accurate identification of TIMS separated isomers improved the understanding of cocoa bean metabolite evolution. Dehydrodi(or tri)catechins B are oxidation products formed during cocoa bean fermentation, while their corresponding isomers, B-type procyanidin di(or tri)mers, are native metabolites.

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Advances in top-down HRMS with AP/MALDI orbitraps for spatial analysis of peptides/metabolites/neurotransmitters in human pathology FFPE tissue archives

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

For (light) microscopy, formaldehyde-fixation and paraffin-embedding (FFPE) is the sample preparation method of choice by histologists world-wide. A wealth of FFPE histological data is available in excellent Atlases with a focus on different tissues, species, pathologies, etc...
As a direct consequence, many millions of pathologically, medically, or biologically well-documented samples are archived (or piled up) in FFPE tissue banks across the planet.
On a mission to make this wealth of FFPE information accessible for spatial analysis of endogenous peptides and metabolites, we have been working on the optimization of top-down mass spectrometry imaging (MSI) protocols in a wide variety of healthy and diseased tissues of Homo sapiens (and other species). We call this approach Mass Spectrometry HistoChemistry (MSHC).

Methods

Employing recent generation high-resolution mass spectrometers in combination with careful sample selection and processing, revisiting FFPE samples, such as those ubiquitously archived in biobanks all over the world, does allow successful top-down MSHC of biologically relevant signaling molecules in histological sections.
We will elaborate on the very latest developments in FFPE MSHC, revealing data generated on multiple platforms consisting of atmospheric pressure MALDI (AP/MALDI) sources fitted to various orbitrap HRMS systems (among which Velos, Elite, Exactive Plus, QExactive, Fusion Lumos, Exploris480).
MALDI matrix (typically DHB) was applied via pneumatic spraying on HTX and Sunchrom automated spraying devices. AP/MALDI HRMS was performed in the m/z ranges of peptides (150-2500) and of metabolites (40-250). Analyses were primarily in positive mode.

Novel Aspect

Never before neurotransmitters were analyzed by MALDI HRMS in human tissue. MSHC unlocks clinical FFPE biobanked tissues for biomarker discovery.

Preliminary Data or Plenary Speaker Abstract

Already several years ago we reported that (neuro)peptides can be detected by direct FFPE tissue spatial top-down AP/MALDI high resolution (HR) MS. Yet, FFPE tissues are still hardly ever used for spatial MS, considering the ample chemical cross-linking of biomolecules by formaldehyde fixation and the abundant extraction of (entire classes) of biomolecular and other analytes of interest from the sample by the paraffin embedding process.
Early MSI work indeed failed to detect, in particular, low-abundant and/or poorly ionizable biomolecules in FFPE material.
Cross-platform comparative MSHC analyses illustrate that with new instrument generations extra performance is achieved in terms of sensitivity, MS accuracy, as well as data acquisition speed.

Our most recent MSHC experiments, which observe short deparaffinization times and precise automated MALDI matrix application conditions, indicate that not only (secretory) endogenous peptides and certain metabolites, but even small biomolecules such as neurotransmitters can be directly imaged in FFPE sections, i.e., without the use of enzymes or derivatization reagents to boost their ionization efficiency.

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Towards Commercialization of a Traditional Aboriginal Pain Medicine: LC-QTOF Profiling of Saponin Metabolites in Barringtonia acutangula

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The bark of Barringtonia acutangula (Mudjala) has been used by Traditional Knowledge holders in the Kimberley region of Western Australia as a potent analgesic medicine for generations. The Traditional Knowledge holders in conjunction with researchers at Griffith University hope to develop this traditional medicine into an over-the-counter topical analgesic; this product would be the first Traditional Aboriginal medicine with regulatory approval for sale in Australia. To market a traditional herbal medicine, a chromatographic “fingerprint” of the bioactive compounds is required. The fingerprinting method must be targeted for detection of bioactive compounds, but also enable detection of possible adulterants and distinguish between extracts of related plants. HPLC coupled HR-MS provides a platform for sensitive detection of plant metabolites meeting these requirements.

Methods

B. acutangula has high saponin content with several of these saponins contributing to the analgesic activity of the plant. Many of the saponins in this plant are too large for detection on common bench top MS instruments which have upper ranges of m/z 1250. As such, a HPLC coupled Bruker MaXis II ESI-QTOF with a detection range of m/z 50-3000 was utilised for this analysis. Minimal optimisation of detection parameters enabled the detection of saponins with masses exceeding 1400 Da in the crude plant extract with high mass accuracy (~7 ppm error) and short acquisition times.

Novel Aspect

High resolution mass spectrometry for profiling plant metabolites of a traditional Aboriginal medicine

Preliminary Data or Plenary Speaker Abstract

These methods enable the detection of approximately 100 natural products in the crude extract and saponin enriched fractions acquired by size exclusion chromatography. This includes 20 saponins with molecular masses ranging from 900-1500 Da. Analysis of freshly extracted herbal material compared to stored materials indicates the formation of transesterification products of at least one saponin (m/z 1105.5144), as determined by the presence of multiple extracted ion chromatogram peaks with this molecular ion in the older extract but only one peak in the fresh extracts. Simultaneous detection of bioactive saponins and smaller molecular weight compounds in the extract provides a sensitive method for authentication of the plant extract and detection of potential adulterants.

Plant ‘omics

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Characterization of Glucosinolates in Arabis sagittata Extracts using a Multi-Reflecting Q-Tof Mass Spectrometer

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Glucosinolates (GSLs) are a diverse group of sulfur-rich plant secondary metabolites associated with defense against herbivore or pathogenic attack. Over 100 GSLs have been identified, with many more uncharacterized. The diversity of GSLs is attributed to different amino acid precursors and further multiple modifications of the side chain during their biosynthesis.
The high variability of GSLs poses a complex analytical challenge, which is compounded by the presence of a wide chemical diversity of primary and secondary metabolites, present in differing concentrations in plant extracts.
Here we demonstrate the benefit of using a high resolving power (>300,000 FWHM) multi-reflecting Q-Tof to detect GSLs and confidently identify them based on accurate mass and their naturally occurring fine isotope structure.

Methods

Glucosinolate standards and Arabis sagittata extracts were analysed by UHPLC/MS using an charged surface hybrid UPLC column, coupled to a multi-reflecting Q-Tof. Arabis sagittata extracts were prepared in a two-step protocol by grinding leaves in liquid nitrogen, subsequent extractions with 80% and 50% methanol (including 5µM sinigrin as IS), with immediate inactivation of the enzyme myrosinase at 80°C. Mobile phases were water + 0.1% formic acid (MPA) and methanol + 0.1% formic acid (MPB) with an LC gradient from 2% MPB to 100% MPB coupled to an electrospray source operating in negative ion mode. Data were acquired as data independent acquisition and were lockmass corrected with leucine enkephalin to provide ppb mass accuracy.
Data were processed using waters_connect™.

Novel Aspect

High resolving power (>300,000 FWHM) allows improved assignment of glucosinolates in complex plant extracts at fast acquisition rates.

Preliminary Data or Plenary Speaker Abstract

The core structure of GSLs is made up of a thio-glucose group attached to a hydroximinosulfate ester and a side chain based on the parent amino acid, which then undergoes further modification, resulting in high variability of the structures of GSLs with multiple nitrogen, oxygen and sulfurs generating complex isotope patterns that can be used as fingerprints to characterize candidate elemental formulae.
A mix of 26 GSL standards was analyzed by data independent acquisition (DIA) with a collision energy ramp (20 to 50 V) applied to generate product ions. The overall mass accuracy for the observed standards was 30 ppb RMS, with -226, 621 and -317 ppb observed for methoxybenzyl glucosinolate, glucobrassicin and 4-hydroxyglucobrassicin respectively. GSLs exhibit common fragment ions (m/z 195.03, 241.00, 259.01 and 274.99), that originate from the common core structure. Excellent mass accuracy was also observed for these fragment ions, for example the mass accuracy for sinigrin of these fragment ions was 780, 998, 480 and 692 ppb respectively. Interrogation of the fine isotope structure of 11-(methylsulfinyl)undecylglucosinolate (C19H36NO10S3, -164 ppb) when chromatographic data were acquired at >300,000 FWHM resulted in the identification of 19 isotopes,

which further confirms the empirical formula especially the presence of three sulfurs within this analyte.
Further DIA experiments from an extract of Arabis sagittata were performed, fourteen GSLs were detected with an overall mass accuracy of 94 ppb, with 344, 328 and -35 ppb observed for glucoarabin, neoglucobrassicin and 4-hydroxyglucobrassicin respectively. As with the standards the common fragment ions were all observed for these analytes with excellent ppb mass accuracy. Interrogation of the fine isotope structure of neoglucobrassicin ($C_{17}H_{22}N_2O_{10}S_2$, 328 ppb) when chromatographic data were acquired at >300,000 FWHM resulted in the identification of 20 isotopes, which further confirms the empirical formula especially the presence of two sulfurs within this analyte

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Liquid chromatography-tandem mass spectrometry analysis of dicaffeoylquinic acids in Adenocaulon himalaicum for the development of joint health improvement

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Osteoarthritis is a common chronic joint inflammatory disease characterized by progressive destruction of the articular cartilage, bone remodeling, and excessive chronic pain. Adenocolon himalaikum extract(AHE) has shown strong effects on anti-inflammatory and cartilage protection, and ultra performance LC-MS/MS method has been developed for the analysis and quantification of five kinds of quinic acids which are its major compounds.

Methods

This study was evaluated on anti-inflammatory activity of Adenocaulon himalaicum in macrophage (RAW264.7) and human chondrosarcoma (SW1353) cell lines. The rapid separaion was achieved based on C18-silica column (100 mm × 2.1 mm id, 1.7 µm). The target compounds were detected and quantified by a triple-quadrupole mass spectrometer.

Novel Aspect

This study provides an efficient and accurate method for quality control for development of joint health using Adenocaulon himalaicum.

Preliminary Data or Plenary Speaker Abstract

AHE dose-dependently inhibited NO release in lipopolysaccharide(LPS)-stimulated RAW 264.7 macrophages and showed three kinds of dicaffeoylquinic acids(3,5-dicaffeoylquinic acid, 3,4-dicaffeaoylquinic acid, 4,5-dicaffeoylquinic aicd) and two chlorogenic acids(chlrogenic acid, cryptochlorogenic acid). The quantity was 20.9 ± 1.04(chlorogenic acid), 2.4 ± 0.02(cryptochlorogenic acid), 11.5 ± 0.02(3,5-dicaffeoylquinic acid), 43.8 ± 2.1 (3,4-dicaffeoylquinic acid), 16.4 ± 1.97(4,5-dicaffeoylquinic acid) mg/g, respectively.

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Phytocannabinoids: Unlocking the Role of Oxylipins on Biosynthesis

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Phytocannabinoids (PCs) are a class of secondary metabolites produced by plants of Cannabis sativa L. (Cannabis). They are stored and synthesised in glandular trichomes that occur on surfaces of vegetative leaves and floral tissues of female flowers. While most of the enzymes in PC biosynthesis have been characterised, the control mechanisms which underly PC production are poorly understood. Oxylipins are lipid-based oxygenated biologically signalling molecules that participate in plant defence. This study aimed to clarify the role of oxylipin metabolism in PC biosynthesis through chemical inhibition of lipoxygenases (LOXs) that catalyse the oxygenation of polyunsaturated fatty acids and provide substrate for oxylipin metabolites.

Methods

Two inhibitors of LOX, phenidone and diethyl dithiocarbamate sodium salt (DIECA), were applied at different concentrations (0.1 1, 7.5 and 15 mM) by foliar spray to the leaves of juvenile plants and compared against the leaves of control plants (0 mM). Plant leaves were harvested, snap-frozen and extracted in MeOH. Targeted and untargeted metabolic analysis was performed using high-resolution mass-spectrometry (HRMS) and samples were run on a Thermo Fisher Vanquish Flex UHPLC system coupled to an Orbitrap ID-X Tribrid mass spectrometer (Thermo Fisher Scientific Inc., MA, USA). The MS was operated in positive and negative ion modes and data was analysed using Compound Discoverer (ver. 3.3) and Xcaliber (ver.4.4) (Thermo Fisher Scientific Inc., MA, USA).

Novel Aspect

This is the first reported study which examines the effect of LOX inhibitors on PC production.

Preliminary Data or Plenary Speaker Abstract

Approximately 2,500 compounds were putatively annotated from the leaf metabolomes of LOX inhibitor and control treated cannabis plants. Principal component analysis showed a clear separation of the control leaf samples with the LOX treated samples, suggesting that the inhibitors impacted leaf metabolism. Targeted PCs were identified and confirmed based on comparison with certified reference standards. This preliminary result confirms that the production of the PC precursor cannabigerolic acid was significantly reduced ($p < 0.05$), with a ~50% reduction observed in plants treated with the highest LOX inhibitor dose (15 mM). These results suggest that PC production is impacted by oxylipin metabolism. Future work will comprehensively examine the effect of LOX inhibitors and other oxylipin metabolites on the metabolomes of different PC producing tissues, such as the female flowers and isolated glandular trichomes.

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Proteomic and Physiochemical Responses of the Red Alga Pyropia yezoensis to Nitrogen Deficiency

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Nitrogen deficiency is the primary factor responsible for the discoloration of the red alga Pyropia yezoensis in coastal environments. However, the precise molecular mechanisms governing alterations in physiochemical properties and the development of chlorosis in this alga remain incompletely understood. In this current investigation, I conducted quantitative proteomic analyses utilizing the iTRAQ method to elucidate the underlying molecular mechanisms behind chlorosis in its natural habitat.

Methods

The red algae were collected from the western coast of Korea and proteins of chlorotic and non-chloric algae were precipitated by adding pre-chilled acetone. The purified proteins were digested with trypsin and labeled with iTRAQ reagent. The iTRAQ-labeled peptides were analyzed using LC-ESI-MS/MS consisting of an EASY-nLC system online coupled to a Q-Exactive mass spectrometer. Peptides were identified and quantified by comparing MS/MS spectra against the UniProt database of Pyropia genus. Gene Ontology enrichment and KEGG pathway analysis were performed for differentially expressed proteins.

Novel Aspect

Quantitative proteomics revealed the direct impact of nitrogen deprivation on the degradation of the biosynthetic machinery in this red alga.

Preliminary Data or Plenary Speaker Abstract

Notably, when chlorosis manifested in Pyropia, there was a pronounced decrease in seawater nitrogen concentration. The proteomic analysis uncovered notable shifts in the expression of structure-associated proteins within P. yezoensis in response to the reduced availability of soluble nitrogen. This involved the upregulation of polyubiquitin (associated with protein degradation) and the downregulation of ribosomal proteins (associated with protein synthesis), ultimately resulting in a reduced growth rate. Furthermore, proteins related to chlorophyll-associated photosynthesis, such as MgPME cyclase and chlorophyll a binding protein complexes, exhibited downregulation. This led to a decrease in chlorophyll a synthesis within discolored P. yezoensis, indicating significant damage to the entire photosystem. The changes in the expression levels of chlorophyll-associated proteins corresponded with reductions in photosynthetic pigment content.

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Rapid in situ near-infrared assessment of tetrahydrocannabinolic acid in cannabis inflorescences before harvest using machine learning

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Cannabis is cultivated for therapeutic and recreational markets. Delta-9 tetrahydrocannabidiol (THC) is a main target for cultivators. As the global cannabis industry expands, more efficient and cost-effective analysis methods for determining cannabinoid concentrations are needed to increase efficiencies and maximize productivity. Development of machine learning tools for near-infrared (NIR) spectroscopy-based prediction models that have been validated from accurate and sensitive chemical analysis, such as gas chromatography (GC) or liquid chromatography mass spectroscopy (LCMS), is essential. The current study focuses on building prediction models for THCA concentrations in whole cannabis inflorescences prior to harvest, by employing non-destructive screening techniques allowing cultivators to rapidly characterize high-performing cultivars in real time, thus facilitating targeted optimization of harvest and crossbreeding efforts.

Methods

Cannabis plants (264 individuals) representing 88 unique genotypes, cloned in triplicate, and grown in indoor cultivation facilities. The chemovars present within population were high-producing THCA (n=222) cultivars and even-ratio (n=42) cultivars. Data collection took place over two days just prior to harvest. The data was collected at 10 am to 11 am each day at a temperature 25°C with 60% relative humidity. A VIAVI MicroNIR Onsite-W was utilised for scanning and data were collected within the 950 – 1650 nm range. The top apical inflorescence of each cannabis plant was taken, dried for 72 hours, and weighed to assess moisture loss, and then analysed via LCMS (Thermo Q-Exactive) for cannabinoid concentration. PLS_toolbox was used to create prediction models.

Novel Aspect

Using portable handheld NIR devices and LCMS quantitation data to predict THCA concentration before harvest and increase high-throughput capability.

Preliminary Data or Plenary Speaker Abstract

Combining NIR spectroscopy and LCMS to create prediction models we can differentiate between high-THCA and even ratio classes with a 100% prediction. High THCA was defined as a class that had a concentration ratio of CBDA to THCA lower than 1:1; and even ratio had a concentration ratio of more than 1:1 (CBDA:THCA ratio). The sample with highest CBDA:THCA ratio present was 1.723:1 (even ratio) and the lowest was 0.002:1 (high THCA). For prediction models of THCA concentration, three modelling techniques were assessed; Partial-least squares regression (PLS-R), support vector machine regression (SVM-R) and eXtreme Gradient Boosting Regression (XGB-R). PLS-R delivered the best result of R2 = 0.78 with a prediction error average of 13%.

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Characterization of biochemical and physiological changes in pea (Pisum sativum L.) seeds after drought stress application during seed filling

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

In recent years, rising temperatures and changing precipitation patterns triggered significant climate changes and the expansion of arid areas. This results in widespread of droughts that represent substantial threats to global food security, notably impacting crop yields worldwide. Grain legumes, impacting on food protein and sustainable agriculture, are particularly susceptible to drought. It is known, that drought, occurring during seed filling, negatively affects seed productivity and chemical composition. Hence, new drought-tolerant genotypes are desired to secure sustained grain productivity under drought conditions. Integrated analyses of proteome and metabolome dynamics are powerful tools for understanding plant responses to drought. This strategy enhances breeding strategies aimed at mitigating the adverse effects of water deficit on the development and nutritional properties of seeds.

Methods

Pea (Pisum sativum L., line SGE) plants were subjected to irrigation withdrawal during the seed-filling stage. Following 14 days of drought stress, irrigation was resumed, and plants were grown till the completion of seed maturation. The seeds were harvested directly after stress application, after five-day recovery and upon maturation. The quality of mature seeds was assessed using the germination test, accomplished with and without the procedure of accelerated aging (AA), while nutritional properties were evaluated via standard methods. Metabolomics analysis of seeds relied on several analytical strategies including targeted LC-MS and untargeted GC-MS analyses of primary polar metabolites along with untargeted LC-MS analysis of semi-polar secondary metabolites. Proteomics analysis was based on a bottom-up approach and relied on nanoLC-ESI-MS/MS.

Novel Aspect

Integrating multi-omics approaches to comprehensively elucidate drought effects on pea seeds.

Preliminary Data or Plenary Speaker Abstract

The assessment of biochemical stress markers revealed that applied experimental drought did not induce oxidative stress in developing seeds, but enhanced the rates of seed maturation. However, the germination test showed that AA had a negative impact on germination rates and seedling quantity from seeds collected from drought-affected plants, while it did not significantly affect the seeds from control plants.
Characterization of the nutritive properties of seeds demonstrated that seeds obtained from drought-exposed plants had lower relative contents of crude protein, but higher contents of crude fat compared to seeds from the control plants. Moreover, the seeds from the stressed plants showed higher relative contents of oligosaccharides, while there were no significant differences in the contents of starch and monosaccharides.
Proteomics analysis demonstrated differences in relative contents of individual seed proteins after stress application and during recovery but not in mature seeds. Interestingly, both developmental stages demonstrated prominent down-regulation of most proteins, with only a small number exhibiting up-regulation stimulated by drought application.
Metabolomics analysis revealed differences between the stressed and control seeds harvested after maturation, but not after the stress application and during the recovery period. Among the top 100 differentially abundant metabolites selected based on the results of principal component analysis, the larger cluster of metabolites demonstrated increasing contents, while the smaller cluster showed

decreasing contents. The pathway analysis performed with these metabolites unveiled the impact of drought on the metabolism of amino acids, carbohydrates, terpenoids and polyketides, as well as pyrimidine metabolism. Furthermore, untargeted analysis of semi-polar secondary metabolites showed that drought led to increased contents of 51 and 16 metabolites analyzed in negative and positive mode, respectively, with only four features decreasing after drought application detected in negative ion mode and one feature in positive ion mode (Welch's t-test, $p\ adj < 0.05$, $FC > 2$).

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Ultra-high throughput workflow for robust and sensitive quantitative single cell analysis.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Thousands of proteins can be measured from different cell lines using MS based proteomics. However, to understand and characterize cell heterogeneity, these cells need to be studied individually. In recent years single cell analysis has profited from advances in LC-MS based proteomics approaches. Nevertheless, there are still challenges in this field of application. Besides sample preparation, the key challenges in looking at individual single cell proteomes are sensitivity, coverage, dynamic range, and throughput. To address some of these challenges, new technological developments, as well as improvements on existing LC-MS-based proteomics workflows are a necessity. Here we described a high-throughput workflow of TMTpro multiplexed single cells using an Orbitrap Astral mass spectrometer.

Methods

Individual HeLa cells were sorted, followed by reduction, alkylation, trypsin digestion and TMT labeling using CellenONE as per the manufacturer's protocols. To enhance the MS1 signal of the peptides a booster channel was used.
The multiplexed single cell digests were analyzed using an Orbitrap Astral MS with FAIMS Pro Duo interface coupled to a Vanquish Neo UHPLC system. Data was acquired in DDA mode and searched with Proteome Discoverer Software 3.1.

Novel Aspect

A complete ultra-high throughput workflow for sensitive and reliable quantification of single cells.

Preliminary Data or Plenary Speaker Abstract

After method optimization 250 single cell could be analyzed per day. On average more than 3500 proteins and 18.000 peptides were quantified. 80% of those proteins had less than 20% CV.

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Robust workflow for high-throughput quantitative analysis of low sample amounts and single-cells

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

In MS based proteomics, millions of cells are analyzed at the same time and protein changes are presented as a sum of the total number of cells analyzed. However, it is known that each cell carries out its own biological processes. Therefore, a better understanding of this diversity can be achieved by studying as many individual cells as possible. Improvements in MS instrumentation have enhanced sensitivity, dynamic range, and large-scale profiling with improved throughput. However, maintaining the proteome depth at throughput is still challenging. Thus, we optimized and benchmarked the performance of a low nanoflow LC-MS methods in data-independent acquisition (DIA) on the Orbitrap Astral MS to achieve deep proteome profiling at highest throughput for single cell.

Methods

For the dilution, Pierce™ HeLa digest (20 ug) was reconstituted by adding 200 µL of 0.015% DDM (Dodecyl β D maltosid) solution, sonicated for 5 min, then diluted to 5 ng/µL in 0.015% DDM solution. Individual HeLa cells were isolated using CellenONE® system from Cellenion, followed by reduction, alkylation, and trypsin digestion as per manufacturer's instructions. The data was acquired on a Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer with a Thermo Scientific™ FAIMS Pro™ interface. Samples were separated using Thermo Scientific™ Vanquish™ Neo UHPLC system on an IonOpticks Aurora 25 cm TS C18 column. Data was acquired in DIA mode. Raw files were processed with Spectronaut 18, with and without library, using a human database with 20k sequences (no isoforms).

Novel Aspect

Sensitive, reliable, and robust high-throughput workflow for in-depth analysis of single cell/single cell-like samples using Orbitrap Astral MS.

Preliminary Data or Plenary Speaker Abstract

To increase the chances of extracting relevant biological information in single-cell analysis, it is essential to analyze as many cells as possible. Therefore, it is necessary to develop high-throughput methods that are not only fast, but also reproducible, reliable, and provide the highest protein coverage and quantitative accuracy. Though throughput is determined by sample preparation, LC separation and MS acquisition, we focused more on the LC optimization since the MS part has been previously optimized. The Vanquish Neo UHPLC was selected because of its capacity to generate precise ultra-low flow LC gradient separations at flexible flow rate in combination with the Aurora 25 cm TS column. During the separation, the flow rate is ramped multiple times, starting from 450nl/min at the beginning of the gradient. During peptide elution, the flow rate dropped to 200nl/min and later ramped up to 300nl/min during the washing step. With library-free Spectronaut data proessing, from 250pg HeLa digest on column, separated using a 19.5min active gradient, we could identify more than 5,600 protein groups from individual files. When files from 3 technical replicates are combined and processed together, the number of protein groups at 1 % FDR goes up to over 6100, with median CVs below 10%. With a library generated from 10ng runs, the number of protein groups increased to over 7,400. To evaluate the reproducibility and reliability of the optimized method, we performed the same measurements on different instruments operated by different users at multiple Thermo Fisher Scientific sites across the globe. All results from the different sites were within ± 10%, demonstrating the reproducibility and reliability of this method. Analyzing real HeLa single cells, we

identified on average about 5,000 protein groups without using a library and 6,000 using a library generated from 3 runs of 20 cells each.

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Label-free DIA-based workflow for single-cell proteomic analysis on an Orbitrap Ascend Tribrid mass spectrometer.

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Recent advances in LC-MS have enabled label-free single-cell proteome analysis, revealing unexpected functional diversity in cells. However, there are still key challenges in this field, such as sensitivity, coverage, dynamic range, and throughput. To address some of these challenges, new method developments as well as optimization of existing LC-MS-based proteomics workflows are necessary. Here, we demonstrate the use of the Orbitrap Ascend Tribrid mass spectrometer and the Vanquish Neo UHPLC system for high-throughput single cell applications.

Methods

Individual HeLa cells were sorted, followed by reduction, alkylation, and trypsin digestion using CellenONE as per the manufacturer's protocols. Pierce HeLa digest was used for dilution series from 50 pg to 10 ng loaded on column. Single cell digests and the diluted standard HeLa digest samples were analyzed using the Orbitrap Ascend Tribrid MS with the FAIMS Pro interface coupled to the Vanquish Neo UHPLC system. Separation was performed on the Aurora Ultimate TS 25cm column. Data was acquired in a DIA mode and searched with a beta version of Proteome Discoverer Software 3.1 and Spectronaut 18.

Novel Aspect

End-to-end workflow for single-cell proteomics on an Orbitrap Ascend Tribrid MS with sensitive protein detections from low-input samples.

Preliminary Data or Plenary Speaker Abstract

The performance of this ultra-sensitive LC-MS workflow was first optimized using a dilution series of HeLa digests. From 250 pg of HeLa digest load, we could identify on average 3,200 protein groups by a library-free approach, whereas using DIA-library generated from 5 and 10 ng of HeLa digest, we were able to identify around 5,000 protein groups using a method with a throughput of 50 samples per day. HeLa single cells resulted in an average of 3,700 protein groups and 21,000 peptides using library-free searches and 4,400 protein groups and >26,000 peptides in library-based searches.

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Enabling scalable single-cell proteomics by utilizing the unique analytical properties of the Evotip Pure

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Single-cell proteomics is a rapidly developing field with recent advancements improving robustness and sensitivity in all parts of the workflow. The Evotip Pure technology has become a cornerstone in integrating upstream sample preparation with LC-MS analysis by eliminating sample handling steps and ultimately sample losses to pipette tips and plastics.
Here we assess the capture and recovery of low-input samples on the C18 based stationary phase of the Evotip Pure and benefits of using it as the starting point of the LC-MS analysis as opposed to using a traditional liquid sample. This not only circumvents the difficulty of aspirating low-volume samples but also reduces sample handling and storage difficulties which are essential to scale the number of cells analyzed reproducibly.

Methods

250 pg of HeLa peptides containing 0.015% n-Dodecyl β-D-maltoside (DDM) in 2 µL 0.1% formic acid was deposited in Protein LoBind plates (Eppendorf) or loaded directly on Evotip Pure. Samples were stored for 0-3 days, and then loaded on Evotips for analysis. Samples with volumes less than 20 µL were loaded on Evotips with pre-deposited solvent A so all samples had a final loading volume of 20 µL. Finally, single HeLa cells were isolated, digested, and loaded on Evotips using the ProteoCHIP Evo96 protocol (Cellenion).
All samples were analyzed using an Aurora Elite column, the Whisper 40 SPD method, and dia-PASEF on a timsTOF Pro2 mass spectrometer.

Novel Aspect

The Evotip Pure increases sample preparation sensitivity for high-sensitivity LCMS workflows by facilitating lossless sample delivery to instruments.

Preliminary Data or Plenary Speaker Abstract

Unlike most autosamplers that utilize a sample loop for injecting limited sample volumes, the Evotip can be loaded with large volumes (20 µL) and used for concentrating dilute samples. We loaded 250 pg HeLa digest on Evotips in various sample volumes showing a stable precursor signal intensity independent of loading volume. This highlights the Evotip ability to efficiently capture and concentrate minute peptide amounts.

Next, we investigated the advantages of storing low amounts of peptides on the Evotip Pure compared to storage in solution. Single-cell samples are typically prepared for other LC systems with 0.015% DDM to reduce sample loss to plastics. We compared this storage methodology to storing samples on Evotips with and without DDM.
The precursor identification rate of samples stored in a sample plate was significantly reduced compared to samples stored on an Evotip after 24 hours of storage.

When working with single cells, robustness and scalability of workflows are crucial points to be addressed. Earlier this year, proteoCHIP EVO 96 was launched, specifically designed for streamlined sample clean-up on Evotips. It is an easily implementable combination of two technologies enabling user-friendly, fast, and reliable upstream sample preparation as well as reproducible and high-throughput downstream LC sample transfer for single-cell proteomics workflows.

We evaluated the proteoCHIP EVO 96 single-cell sample preparation workflow for 384 single HeLa cells isolated in four different chips. During the sample preparation workflow in the cellenONE, cells are isolated, lysed and proteins are digested. Once these steps are complete, the proteoCHIP EVO 96 is taken out of the instrument and the content of each well is transferred to the Evotips via centrifugation. We observed a stable performance in peptide identification rates and precursor ion intensity signal during the 10 days of analysis.

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Metalloprotein Profiling of Pathogenic Gram-Negative Bacteria

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Our laboratory investigates the role of transition metal ions in bacterial physiology, in particular, their capacity to modulate responses to antimicrobial agents. As such, we have developed an analytical pipeline to quantify trace elements and their metalloprotein binding partners in bacterial cell extracts, using liquid-chromatography systems connected to inductively coupled plasma mass spectrometers (LC-ICP-MS). This technique allows us to investigate changes in element distribution occurring from mutations in metal transporter systems, as well as the capacity for small molecule ionophores to alter the metal ion homeostasis of bacterial cells. Further, proteins with predicted metal-binding motifs are tested after recombinant expression to directly measure the metalloprotein complex to assess metal affinity, stoichiometry and stability.

Methods

To profile the metalloprotein environment of bacterial cells we perform sequential non-denaturing chromatographic fractionation of cell extracts via anion-exchange chromatography, followed by size-exclusion chromatography directly linked to a triple-quadrupole ICP-MS. This setup allows our laboratory to build two-dimensional maps for each of the elements of interest, including Mn, Al, Fe, Cu, Zn, Ni, Co and Cd. This analysis is applied to our experimental systems, whereby the metal ion homeostasis in bacterial cell cultures is perturbed via genetic knockout (e.g. of putative or known metal ion transporters), or via supplementation with small molecule metal ionophores. By overlaying and contrasting the elemental maps from different conditions, we can isolate fractions of interest to narrow our downstream proteomics investigations for metalloprotein identification.

Novel Aspect

Two-dimensional metalloprotein profiling for the elucidation of metal-ion homeostatic pathways in bacterial pathogens.

Preliminary Data or Plenary Speaker Abstract

Here we present the metalloprotein profiles of Klebsiella pneumoniae, Pseudomonas aeruginosa and Streptococcus pneumoniae to demonstrate the utility of this technique for comparing the trace element physiology of pathogenic bacteria.

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Rapid Data Rationalization for Biotransformation using a Novel Benchtop Multi-Reflecting Time-of-Flight Mass Spectrometer with Dedicated Data-Mining Software

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Whilst the support of biotransformation studies using high-resolution mass spectrometry (HRMS) for structural characterization of drug metabolites is well established, key limitations exist around these approaches. Provision of fit-for-purpose HRMS data is often subject to compromise, where the interplay between mass resolving power and scan-speed brings an inherent reduction in performance, particularly when using ultra-performance liquid chromatography (UPLCTM). In addition, providing actionable data remains challenging due to protracted data rationalization via manual data interrogation, often introducing bias. Here, we present a novel data-independent workflow for metabolite characterization, utilising routine part-per billion (ppb) mass accuracy, rapid scan speeds and dedicated software to leverage these unique data attributes; in turn, reducing analytical compromise, increasing confidence and reducing time to first-answer.

Methods

Clozapine was incubated (37 °C, t=1 h) in rat and human microsomes (20 µM with GSH (5 mM)) before termination and subsequent centrifugation. Samples were analyzed using reversed-phase UPLC separation (0.4 mL/min, C18 (2.1 x 100 mm, 1.7 µm, 40 oC). Metabolite detection was performed in positive or negative electrospray ionization using a prototype benchtop quadrupole multi-reflecting time-of-flight (Tof) mass spectrometer (mass resolving power of 100,000 FWHM, scan speeds of 200 Hz and mass accuracy of 200 ppb). Data were acquired using the MSE acquisition mode (a data independent acquisition method) and processed using dedicated Mass-MetaSite software. Resultant rationalized data were compared with outcomes from manual data interrogation, applying pragmatic (but limited) requisite manual oversight prior to reporting.

Novel Aspect

A step-change in HRMS data quality for biotransformation and automatic leveraging of data attributes to drive decisions using dedicated software.

Preliminary Data or Plenary Speaker Abstract

UPLC-MSE acquisition provided highly-selective data-independent information for putative metabolites, which were rationalized automatically using Mass-MetaSite software. Data independent acquisition using MSE allowed high-quality structural information to be gleaned within a single acquisition. Multiple phase one metabolites and glutathione adducts of clozapine were detected and identified with sub ppm mass accuracy measurement (RMS) and resolution achieving 100,000 (FWHM) across a wide m/z range in both positive and negative ionization mode. Candidate metabolites were identified based upon accurate mass measurement and leveraging isotopic fidelity to confirm or rule-out elements in the proposed molecular formulae for both parent and fragment ions. Here, the impact of ppb mass accuracy combined with high mass-resolution increased overall confidence in metabolite identification, where elegant isotopic fidelity allowed resolution of isotopes such as 34S and 15N to facilitate the automatic proposition of the most relevant formulae (improved elemental fit). When applied automatically to parent and associated fragment ions, these improvements gave quicker data rationalization and time to first-answer in the structural elucidation of both expected and unexpected metabolites of clozapine. Using dedicated Mass-MetaSite software to automatically leverage these unique data attributes, the need for extensive manual data verification is reduced markedly. Instead, this increased data confidence introduces an unsupervised

aspect to this novel workflow, prior to first-answer; therefore, increasing the efficiency of these biotransformation studies and their potential impact to drug discovery.

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Identification of Three New Psychoactive Substances and Obtaining Their Metabolites through Zebrafish

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

To confirm the structures of new psychoactive substances (NPSs) and their corresponding biological metabolites based on analytic data, and subsequently incorporating these substances into regulatory frameworks are the methods for counteracting the criminal trend. This study involves three powder samples that don't match with well-known drugs on their mass spectrum. The exact molecular structures of the three specimens were determined as NPSs. Subsequently, zebrafish metabolism model was used to simulate the human metabolic behavior in response to the three NPSs, and thus their suggested metabolites were obtained for analysis of urine samples.

Methods

Gas chromatography-mass spectrometry (GC-MS) and liquid-chromatography (LC)-orbitrap MS analyses were used to obtain the exact molecular weights and molecular formula of three NPSs: 1-[(4-fluorophenyl)methyl]-4-methylpiperazine, N-(1,2-diphenylethyl)propan-1-amine and 1-(4-fluorophenyl)-4-methyl-2-(pyrrolidin-1-yl)pentan-1-one. NMR analysis confirmed the molecular structures and conformation of these three drugs. Subsequently, metabolites of these three NPSs were obtained through zebrafish, and their structures were analyzed using LC-orbitrap MS measurement.

Novel Aspect

The three new designer drugs and their metabolites were discovered for abused-drug detection

Preliminary Data or Plenary Speaker Abstract

The date obtained from GC-MS measurement for the three NPSs included retention time, mass spectra, and their operational parameters. In addition, LC-Orbitrap MS results provided exact molecular weight, retention time, and their operational parameters. NMR analysis provided 1H chemical shifts and corresponding integration values, as well as their 13C NMR data including DEPT 45, 90, 135 spectra. In addition, two-dimensional NMR spectra including COSY, NOESY, HSQC, HMBC were presented. Zebrafish metabolism model was used to obtain metabolites of the three NPSs, involving experimental procedures, feeding environment temperature, water quantity, drug feeding concentration, sampling time, and pre-treatment methods. The results show types of metabolites, the relationship between metabolite concentration and sampling time, mass spectra of metabolites, structural analysis using LC-Orbitrap MS.

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Reducing N-nitrosodimethylamine in metformin through manufacturing adjustments

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Nitrosamine impurities have emerged in pharmaceuticals after NDMA was detected in valsartan and others between 2019 and 2020. Although the active pharmaceutical ingredient (API) for metformin seldom surpasses established NDMA safety thresholds, NDMA has been identified in drug products, leading to recalls. NDMA are often generated in the presence of nitrite in excipients and dimethylamine (DMA) in APIs during drug formulation stage. We delineate risk factors for impurity formation in the manufacturing process, with a focus on the wet granulation process, which entails heat and moisture. Consequently, this research suggests that managing formulation variables are efficacious strategies for mitigating NDMA levels, thereby aiming to enhance pharmaceutical safety and public health.

Methods

For the quantification of N-Nitrosodimethylamine (NDMA) in pharmaceutical formulations, 500 mg powder was mixed with 5 mL of DCM and NDMA-d6, shaken, and added with 5 mL of 1 M HCl. After separation, the DCM phase was taken for analysis. The analytical process was conducted using GC-EI-MS/MS. The analysis was conducted using an GC8890 gas chromatograph coupled with a 7000D tandem mass spectrometer (Agilent Technologies in Santa Clara, USA). Column was a TG-WAXMS (30 m, 0.25 ID, 0.25 µm film), injection 2 µL pulsed splitless. The quantification of NDMA utilized transitions from m/z 74 to m/z 44 (quantifier) and m/z 42 (qualifier).

Novel Aspect

This study establishes a quantitative correlation between NDMA formation in metformin and the levels of excipients used in its formulation.

Preliminary Data or Plenary Speaker Abstract

This study aimed to optimize the formulation conditions for metformin products, with a specific focus on minimizing NDMA concentrations, a known carcinogenic impurity. Notably, NDMA was not detected in metformin API, but it was detected in the final drug products. We conducted a comparative analysis of NDMA levels across immediate-release (IR) and extended-release (ER) metformin formulations, utilizing a consistent excipient type. The manufacturing process variables including water purity, granule size, drying temperature, and tablet hardness were evaluated for their impact on NDMA formation. By optimizing process parameters, we manufactured final drug products under conditions that minimized NDMA production. Furthermore, quantitative correlations between NDMA concentrations and specific excipient ratios were established through batch analysis. The level of NDMA was maintained below the stringent control threshold set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline M7. The findings demonstrate that through careful control of formulation and manufacturing variables, it is feasible to significantly reduce NDMA formation in metformin products. This study not only contributes to the understanding of NDMA formation dynamics but also underscores the importance of manufacturing controls in ensuring drug safety. This research was supported by a grant (23194MFDS086) from the Ministry of Food and Drug in 2024.

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Analytical method development and dermal absorption of 4-amino-3-nitrophenol (4A3NP), a hair dye ingredient under the oxidative or non-oxidative condition

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

4-amino-3-nitrophenol (4A3NP) is classed as amino nitrophenol and is mainly used as an ingredient of hair dye colorant. In Korea and Europe, it is only used in non-oxidative or oxidative hair dye formulation at a maximum concentration of 1% or 1.5%, respectively. Until now, the risk assessment of 4A3NP was not terminated because proper dermal absorption was not provided. So, in this study, the analytical method validation and in vitro dermal absorption study of 4A3NP were conducted according to Korea MFDS guidelines.

Methods

Analytical methods were developed for quantitation of 4A3NP in various matrices. For non-oxidative and oxidative (6% hydrogen peroxide (H_2O_2): water, 1:1) hair dye conditions, 1% and 1.5% of 4A3NP were applied to the skin at 10 µl/cm², respectively.
After the application for 30 min, an interim wash step was progressed to mimic general hair dye conditions (WASH_30 min) and 24 hours (h) later, the skin was wiped off with a swab (WASH_24 h) and stratum corneum (SC) was collected using tape stripping. The mini pig skin was then cut into 8 pieces (SKIN) and receptor fluid (RF) was sampled at 0, 1, 2, 4, 8, 12, and 24 h.

Novel Aspect

Analytical method for 4A3NP in various matrices was developed and absorption rates of 4A3NP were determined.

Preliminary Data or Plenary Speaker Abstract

The developed methods showed well-fitted linearity (r^2=0.9962-0.9993), accuracy (93.5-111.73%) and precision (1.7-14.46%) by the validation guideline. The current analytic method was used for the quantitation of 4A3NP.
In In vitro dermal absorption study, WASH_30 min was mainly quantitated ranging from 88.51 ± 3.20% to 93.39 ± 6.31% in oxidative and non-oxidative hair dye conditions. Whereas, the smallest amount of 4A3NP was detected in stratum corneum (SC) as follows; 0.14% ± 0.23% (oxidative) and 0.05% ± 0.08% (non-oxidative). In addition, total recoveries in oxidative and non-oxidative samples were 92.989% ± 3.42% and 100.30% ± 4.08%, respectively. Under the different conditions of concentration in oxidative (1.5%) and non-oxidative (1.0%), the total absorbed 4A3NP in accordance with application dose in SKIN and RF was 2.83% ± 1.48% and 5.62% ± 2.19%, respectively. The total dermal absorption rates of 4A3NP in non-oxidative (1%) and oxidative (1.5%) conditions were determined to be 5.62 ± 2.19% (5.62 ± 2.19 µg/cm²) and 2.83 ± 1.48% (4.24 ± 2.21 µg/cm²), respectively.

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Metabolomic and Molecular Networking Study for the Identification of Reactive Metabolites of Etodolac

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

The occurrence of drug-induced liver injury (DILI) is associated with the covalent binding of drugs or their metabolites to proteins. When drugs are metabolized to hydrophilic, reactive metabolites, they bind to nucleophilic proteins and cause problems. Cases of liver damage caused by etodolac that selectively inhibit cyclooxygenase-2, have been reported. However, the underlying mechanisms of etodolac-induced liver injury remain unknown.

Methods

In this study, the in vitro metabolism of etodolac was investigated in rat and human liver microsomes using liquid chromatography-tandem mass spectrometry based on a non-targeted metabolomics approach. To identify metabolites, the data were subjected to multivariate data analysis and molecular networking.

Novel Aspect

We newly identified reactive metabolites of etodolac including glutathion conjugate of hydroxyetodolac in human liver microsomes.

Preliminary Data or Plenary Speaker Abstract

A total of 20 etodolac metabolites were detected including 11 newly identified metabolites. Metabolites were formed through four major metabolic pathways (hydroxylation, demethylation, dehydrogenation, and conjugation). Potential reactive metabolites including etodolac acylgluronide and etodolac gluththione conjugates were also found. Based on these results, we are performing reactive metabolite-target protein adduct formation. The characterization of etodolac-protein adducts may help in understanding drug-induced liver injury and serve as a potential tool to assess the risk of covalent binding of drugs to proteins.

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Headspace-SIFT-MS: Rapid Screening of Hazardous Volatile Impurities in Haircare and Skincare Products

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Personal care products (PCPs) are formulated for regular use on the body, and it is hence very important that the safety of such products is assured. Several volatile organic compounds (VOCs) feature among impurities or ingredients of particular concern, including the known or suspected carcinogens benzene, 1,4-dioxane, and formaldehyde. Tackling this suite of analytes with conventional chromatographic techniques typically requires sample analysis using gas and liquid chromatography. In contrast, selected ion flow tube mass spectrometry (SIFT-MS), a direct mass spectrometry technique applying soft chemical ionization, quantitatively analyzes all species in a single headspace analysis in tens of seconds. In this study, SIFT-MS is applied to quantitative analysis of nine haircare products and easily achieves the limits set in current regulations.

Methods

SIFT-MS analyzes air and headspace continuously using soft chemical ionization with up to eight reagent ions (H3O+, NO+, O2+•, O−•, OH−, O2−•, NO2− and NO3−). In this study, the positively charged reagent ions on a commercial SIFT-MS instrument (Voice200ultra model; Syft Technologies Limited, Christchurch, New Zealand) are utilized for analysis of target compounds in nine retail PCPs. Headspace analysis is automated using a multipurpose (MPS) autosampler (Robotic Pro; GERSTEL, Mülheim, Germany) with sampled headspace injected into the SIFT-MS instrument through a septumless sampling head (GERSTEL).
Quantitative analysis of volatile impurity content in the emulsions is achieved by using the method of standard additions. Standards were prepared in-house from high-purity chemicals (>99%) sourced from Sigma-Aldrich (Gillingham, UK).

Novel Aspect

Automated SIFT-MS analysis is a new option for rapid screening of benzene, 1,4-dioxane, and formaldehyde in personal care products.

Preliminary Data or Plenary Speaker Abstract

Benzene, 1,4-dioxane, and formaldehyde are targeted using four, five, and one product ions, respectively. In the results presented below, quantitation ions are abbreviated as reagent ion (H= H3O+, N=NO+, O=O2+•) and product ion m/z (e.g., N78 represents NO+ reacting with benzene to yield the electron transfer product at m/z 78). Real-time reagent ion switching in SIFT-MS enables maximum specificity to be achieved for each sample within a single 50-s headspace injection. Compatibility of automated headspace-SIFT-MS with the method of standard additions is demonstrated in this study. LOQs for benzene, 1,4-dioxane, and formaldehyde in haircare and skincare products are 1.0−15 ng g−1, 0.19−4.5 µg g−1, and 2.8−3.7 µg g−1 range, respectively (product and quantifying ion dependent). Higher LOQ ranges for 1,4-dioxane and formaldehyde arise from poorer headspace partitioning compared to benzene. LOQs easily achieve the limits required by current regulations.

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Determination of maximum residual limit about toltrazuril and diclazuril in fish with liquid chromatography-tandem mass spectrometry

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

Positive list system (PLS) had been in effect in Korea since January 2024. The PLS sets maximum residue limit (MRL) of approved veterinary drugs in food and manages the levels of drug present in livestock and fishery product. Compared to livestock antibiotics, there is a shortage of approved aquaculture antibiotics. This necessitates the establishment of safety standards and residue tolerance limits for aquaculture veterinary drugs. Toltrazuril and diclazuril are used as antiparasitic drugs in fish. This study analyzed antibiotic residue levels in fish administered toltrazuril and diclazuril orally. MRL was derived using the Joint FAO/WHO Expert Committee on Food Additives (JECFA) calculator. This study can contribute to the establishment of MRLs for the approval of toltrazuril and diclazuril in aquaculture.

Methods

Antibiotics of 15 mg/kg (1X) and 30 mg/kg (2X) at 22 °C appropriate water temperature and 1X at 13 °C comparative water temperature were injected Rockfish and rainbow trout. Sample was collected through 1, 3, 7, 14, and 28 days, depending on the time. Liquid-liquid extraction (LLE) and dispersive-solid phase extraction (d-SPE) were used for pretreatment. The residue was dissolved in a mixture of water/methanol (1:4, v/v) and filtered through a 0.2 μm PTFE filter for analysis. The column used in LC-MS/MS is Waters Atlantis C18 (150 x 2.1 mm, 3 μm). (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile were used for mobile phase. ion source used ESI and analyzed in MRM mode.

Novel Aspect

Establishment of residual acceptance criteria through analysis of antibiotics for aquatic life in LC-MS/MS fish

Preliminary Data or Plenary Speaker Abstract

This study analyzed the degree of residual in fish by time and water temperature for toltrazuril and diclazuril. For analysis, sample preparation was conducted LLE and d-SPE and analyzed LC-ESI-MS/MS. Method validation was performed according to the Codex Alimentarius Commission (CODEX) guidelines (CAC/GL-71). The acceptance criteria for the target analytes were met. The recoveries of the analytes ranged from 70 to 110%. The relative standard deviations were less than 15%. The R2 for linearity was above 0.99. The limits of quantification were 0.002 to 0.022 mg/kg. In rockfish, the residues were similar in the optimal temperature 1X and 2X groups on days 1 and 3. The residues decreased rapidly on day 7 and were almost undetectable. In the comparison temperature 1x group, the residues decreased rapidly from day 3 and were almost undetectable on day 14. In rainbow trout, the residues decreased rapidly from day 3 and were very low on days 14 and 28, compared to the administered dose. MRLs were set based on the residue analysis results using the JECFA calculator. Considering the MRLs of 0.5 mg/kg for bovine and ovine muscle, and the tolerance limit of approximately 1.0 mg/kg in the optimal temperature 1x group on day 7 in the JECFA calculator, an MRL of 1.0 mg/kg is proposed for diclazuril in fish muscle. Based on this proposed MRL, the estimated daily intake (EDI) and theoretical maximum daily intake (TMDI) are 16-20% of the acceptable daily intake (ADI). Therefore, it is considered that there will be no adverse effects on human health from diclazuril, even if it is consumed daily.

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2-Methyl-4'-(Methylthio)-2-Morpholinopropiophenone: A Commercial Photoinitiator Being Used as a New Psychoactive Substance

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Poster Session: Tuesday Posters, Exhibition Hall, August 20, 2024, 13:00 - 15:00

2-Methyl-4’-(methylthio)-2-morpholinopropiophenone (MMMP) is a commercial industrial photoinitiator with the trade name of Varnifm Photoinitiator 907. MMMP has a basic cathinone backbone and likely poses a severe threat to human health. However, few studies have investigated MMMP. In the current study, likely metabolites of MMMP —hydroxy-MMMP (HO-MMMP), HO-MMMP-sulfoxide (HO-MMMP-SO), and HO-MMMP-sulfone (HO-MMMP-SO2)—were synthesized as standard chemicals to enable investigation of the metabolites of MMMP. Subsequently, an analytic method for quantitatively analyzing MMMP, HO-MMMP, HO-MMMP-SO, and HO-MMMP-SO2 in urine samples that was based on liquid chromatography (LC)-tandem mass spectrometer (MS/MS) was established. Forty urine samples identified as containing MMMP metabolites from 1,691 individuals who abuse drugs in Taiwan were used to study the metabolism of MMMP in humans.

Methods

Three metabolites of MMMP including HO-MMMP, HO-MMMP-SO, and HO-MMMP-SO2 were synthesized as standard chemicals. Then, an analytical method for urine samples was established and validated using a LC-MS/MS system comprised a mass spectrometer (SCIEX Triple Quad 5500, Framingham, MA, USA) and an LC system (Agilent 1260 Infinity, Santa Clara, California, USA) equipped with a separation column (3.0 × 100 mm, 2.7 µm, Poroshell 120SB-AQ Agilent). Qualitative and quantitative analyses of urine samples were conducted for five species, namely MMMP, HO-MMMP, HO-MMMP-SO, and HO-MMMP-SO2 and MDPV-d8 (served as the internal standard) in the MRM mode and the electrospray ionization mode.

Novel Aspect

An analytical method for urine samples from drug abusers who consume MMMP was established.

Preliminary Data or Plenary Speaker Abstract

Parameters, including the precursor ions, fragments, declustering potential (DP), collision energy (CE), entrance potential (EP), retention time, and ion ionization mode, for MMMP, HO-MMMP, HO-MMMP-SO, HO-MMMP-SO2, and MDPV-d8 were optimized. LC-MS/MS in the extracted ion mode revealed excellent separation for each analyte under the optimized conditions. Validation data, including the IS, correlation coefficients (R2), calibrated linear ranges, LODs, carryover effects, accuracy, and precision for MMMP, HO-MMMP, HO-MMMP-SO, and HO-MMMP-SO2 were shown. The calibration curves for the four analytes were linear (R2 > 0.999), as revealed through quantitation in linear ranges from 2 to 600 ng mL−1. The LOD was calculated to be 0.79 ng mL−1 for MMMP, 0.81 ng mL−1 for HO-MMMP, 1.01 ng mL−1 for HO-MMMP-SO, and 0.94 ng mL−1 for HO-MMMP-SO2, indicating that this LC-MS/MS method for quantifying the four analytes in urine samples was sensitive.
Urine samples obtained from 2,376 individuals who suspected of abuse drugs in Taiwan in 2023 were screened and then confirmed for drug abuse by using an LC system connected to an orbitrap mass spectrometer and an LC-MS/MS, with the capability to identify more than 700 forensic chemicals. Of these samples, 1,691 had results indicating drug abuse, and 40 were identified as containing MMMP, HO-MMMP, HO-MMMP-SO, or HO-MMMP-SO2. To study the metabolism of MMMP in humans, quantitative analysis of these 40 samples was conducted. This result indicates that HO-MMMP-SO is the major metabolite of MMMP in human urine. MMMP was not detected in any of the 40 samples, mainly because it is easily metabolized to HO-MMMP, HO-MMMP-SO, and HO-MMMP-SO2. The present study also recommends a concentration of 2 ng mL−1 for the major metabolite HO-MMMP-SO as a target and cutoff value for identifying individuals who abuse MMMP.

Prebiotic Formation of Peptides through Bubbling and Arc Plasma

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The possible prebiotic pathway from amino acids to peptides, which is a prerequisite for accepted origin of life chemistry, remains unclear. Several potential prebiotic scenarios for peptide synthesis have been proposed to overcome the thermodynamic constraints, such as submarine hydrothermal vents, mineral surfaces, aerosols or sea spray, and meteorites. However, these scenarios are limited by the low probability of occurrence due to harsh conditions, or the need for high concentrations of reactants and additional additives. To simulate a more common natural scenario, we wondered if the combination of arc plasma and bubbling which akin to lightning phenomenon over the sea could enable prebiotic peptide bond formation, and monitor the process using online mass spectrometry.

Methods

The device consists of a bubble generation unit and an arc plasma unit, which was placed at the atmospheric pressure inlet of MS. A 15 mL Buchner funnel with G3 Sand Board was used to generate microbubbles. Through the G3 pore (15-40 μm), CO2 (≥99.9%, 0.8 L/min) was injected into the amino acid solution (water). The surface of the sand core produces microbubbles, which burst at the water-air interface to produce microdroplet aerosols. Placing arc plasma at the bubble bursting interface. 4-10 kV AC voltage with a frequency of 20 Hz was applied between the two electrodes to generate a plasma. MS data were acquired using an Orbitrap Exploris™ 120 mass spectrometer (Thermo Fisher Scientific, San Jose, CA).

Novel Aspect

The innovative integration of online MS monitoring with arc discharge at the bubble interface enables peptide bond abiotic synthesis.

Preliminary Data or Plenary Speaker Abstract

Glycine (Gly) and alanine (Ala) were used as model amino acids for our initial investigation. Peaks of the dipeptides glycylglycine (m/z 133.06) and alanylalanine (m/z 161.09), formed by the reaction of the amino acids during the process of bubble bursting and droplet traveling through the arc plasma to the MS inlet, were unambiguously observed in the full scan mode. To further explore the possibility of peptide formation, 4 other amino acids (Leucine, Valine, Isoleucine, Proline) that were probably first present on early Earth were chosen to extend the condensation reaction. The conversion ratio (CR), which is calculated as the intensity of peptides relative to the sum of intensities of peptides and amino acids, roughly reflects the yield of the reaction. The CRs of Gly, Ala, Leu, Val, Ile and Pro ranged approximately from 2.6% to 25.5%. All product structures were verified by MS/MS.
We optimized the reaction conditions and explored the role of arc plasma in this reaction process. We performed the reaction with a mixed solution containing all 20 amino acids to study the reactivity of the entire system. A total of 102 possible dipeptides (regardless of sequence differences) were detected. A comparison of the distribution of the resulting dipeptide products revealed significant differences in amino acid reactivity. In the total mixture, Ala (A), Gly (G), and Ser (S) have particularly good overall reactivity with other amino acids. We used 2H4-L-alanine and unlabeled D-alanine to investigate the chiroselectivity of the condensation reaction occurring under the combined effect of bubble and arc plasma. Interestingly, when calculated based on their respective intensities, the

actual observed intensity ratio appears to be 0.86±0.19 : 2.24±0.16 : 0.90±0.17. This result indicates that the dipeptide formation reaction in the current method does not exhibit significant homochirality.

Profiling soluble protein fractions from commercial oat milks using mass spectrometry-based proteomics

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Oat milk demand continues to rise as consumers seek out dairy-free alternatives due to lactose intolerance or lifestyle preferences. Among the plant-based milks, oats stand out due to its nutritional value and health benefits. Oats are rich in protein, fats, and soluble fibre like β-glucan, which can act as a fat replacer and stabiliser. The protein content of oat grain typically ranges from 12-20% and globulins are the major group of oat proteins. The protein content and composition of oat milk is influenced by various factors including raw material characteristics (oat variety, growing conditions, and physical format), as well as processing conditions including heat treatments applied.

Methods

As a foundation for oat milk enhancement, it is important to explore the soluble protein profile of commercially available oat milk to define product quality and nutritional value. This study employs mass spectrometry-based proteomics to identify the soluble protein profile in nine different commercial oat milks, sourced from both international and local manufacturers. In this foundational study, oat milk samples will undergo protein extraction using water-based extraction buffers, protein clean-up, and enzymatic digestion using an on-filter digestion method, and analysis will be performed via liquid chromatography coupled with mass spectrometry (LC-MS/MS).

Novel Aspect

By identifying the soluble proteins and peptides present in commercial oat milks sets the foundation for enhancing the nutritional value.

Preliminary Data or Plenary Speaker Abstract

The aim is to observe variations in specific protein abundance among commercial oat milks and define differences resulting from the manufacturing processes.

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IsoFoodTrack: Innovating Saffron Traceability by Stable Isotopes, Multi-Elemental Analysis, and Explainable AI

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Food authenticity testing is crucial for uncovering fraudulence in the food industry, utilizing advanced techniques like Isotope Ratio Mass Spectrometry (IRMS) to determine isotopic composition of light elements such as H, C, N, O and S (expressed as δ2H, δ13C, δ15N, δ18O, δ34S). The use of isotopes can be even more effective when combined with elemental composition. However, a common requirement in food authenticity and traceability studies is the need for a product reference database.

Methods

Addressing a critical need in this domain, the IsoFoodTrack database provides a standardized, up-to-date repository of authentic samples, encompassing isotopic and multi-elemental data for susceptible foods such as dairy, meat, spices, oils, and seafood. Additionally, the database integrates metadata, including annual/seasonal variations and production regions, for statistical evaluation to ensure authenticity verification, production type determination, and geographical origin identification. In this study, the stable isotope and elemental composition data of 750 saffron samples sourced from various countries were included.

Novel Aspect

The novel aspect is IsoFoodTrack as a comprehensive database, combining isotopic and multi-elemental data for food authenticity and traceability enhancement.

Preliminary Data or Plenary Speaker Abstract

The latest data (75 samples) were subjected to the Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) model, which effectively distinguished saffron samples based on geographical origin. Notably, Moroccan, Iranian, Spanish, and Greek samples were accurately differentiated with a 97% success rate, demonstrating the efficacy of our approach in sample categorization. Using Explainable AI, our model consistently surpassed the majority class baseline accuracy, indicating its ability to discern meaningful patterns from the data. With an average accuracy of approximately 90%, the model demonstrated strong overall performance. These findings underscore the potential of IsoFoodTrack in enhancing saffron traceability and verification practices. Although this approach has been used for saffron as an example, it is readily adaptable for other animal and plant commodities.

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Proteomic exploration reveals a metabolic shift due to low oxygen during controlled germination of malting barley (Hordeum vulgare L.)

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Barley (Hordeum vulgare L.) is an economically important cereal crop in world malt production. Mature barley grain is converted to malt through controlled germination called malting. During the early stages of malting, the grain is submerged in water to raise the moisture content. This triggers the activation of enzymes to solubilise starch and protein, leading to grain modification. Malting is a water and energy-intensive process, achieving grain modification at reduced moisture could contribute to enhanced malting practices. Different barley varieties, defined by a distinct genotype, exhibit different metabolic adaptations during malting. Enzymes control a broad range of metabolic adaptations to germination. Understanding the differences in genotype-specific enzyme production is important to barley breeders in developing varieties with desirable malting phenotypes.

Methods

This study combined quantitative Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH-MS) proteomics, biochemical phenotypic data, and statistical bioinformatics analyses. We aimed to identify significant changes in biological processes occurring within two malting barley genotypes characterised by distinct water uptake and modification rates. The study was designed to reveal the intricate molecular mechanisms underlying controlled germination and explore protein groups roles in molecular adaptation. Specifically, we conducted analyses at 24-hour intervals over the initial 72 hours of controlled germination, where the majority of malthouse water is used.

Novel Aspect

The study provides a comprehensive proteome reference and new insights into the molecular mechanisms underlying malting to improve malting practices.

Preliminary Data or Plenary Speaker Abstract

Pairwise comparisons identified 793 significantly differentially abundant protein groups, revealing dynamic variations within and between genotypes. These proteins are involved in key biological processes, including 'protein synthesis,' 'carbohydrate metabolism,' and 'hydrolysis,' and were integrated into metabolic pathways such as glycolysis, fermentation, starch, and sucrose metabolism. Our data highlighted pilot malting-induced metabolic shifts, particularly in response to low oxygen during early controlled germination stages. The stress induced by this oxygen deficiency led to various metabolic adaptations and rerouting of the carbohydrate metabolism for sustaining protein synthesis. Furthermore, we found a switch to fermentation and pyrophosphate (PPi)-linked energy pathways to support the boosted protein synthesis for efficient modification. These findings suggest that genotype-specific adaptability or ‘reduced sensitivity’ to low oxygen conditions experienced during submergence in controlled germination may contribute to more efficient endosperm protein hydrolysis at lower grain moisture levels. However, the enhanced low oxygen tolerance is likely a result of several interactions at the molecular, biochemical, and anatomical levels influencing the malting phenotype. Further studies are required to understand the signalling pathway and the potential impacts of reduced antioxidant activity on malt quality. These findings deepen our understanding of barley germination physiology and provide novel insights into the effects of submergence during controlled germination, shedding light on the mechanisms for low-oxygen sensing, the underestimated role of fermentative metabolism for efficient grain modification, and

highlighting several potential adaptive metabolic traits as breeding targets for optimising malting practices.

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Identification of cell wall-bound phenolics by LC-MS and IM-MS reveals selective cell wall hydrolysis by green and conventional solvents

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Plant cell wall structural polymers are crosslinked by bound phenolic compounds, contributing to cell wall structural resistance and plant protection. However, this resistance poses a major challenge to valorising lignocellulosic biomasses into high-value products. Major bound phenolics are a class of hydroxycinnamic acids dominated by ferulic and coumaric acids and connect polysaccharides and lignin within the cell wall. To valorise this ubiquitous, yet underutilised biomass, there is an increasing research interest in the effectiveness of solvents to enhance the deconstruction of the lignocellulosic cell wall. In this context, the identification and characterisation of bound phenolics can help our understanding of the impact of solvents on the fractionation of lignocellulosic cell walls into useful individual constituents.

Methods

Brewers spent grains (BSG), a solid byproduct of the brewing industry, was fractionated using a conventional sequential process with 60% acetone (v/v) and alkaline hydrolysis (0.75 N NaOH) to release bound phenolics, or directly treated by novel deep eutectic solvents based on choline chloride maleic acid (CCM), choline chloride glycerol (CCG) and choline chloride urea (CCU). The extraction process was performed for 3 hrs at 60 ºC and extracts were purified using C18-E SPE cartridges. Chromatographic separation was performed on a C18 column followed by mass analysis in negative ionisation mode, on an Agilent 6545A LC-DAD-QTOF-MS and Masshunter Qualitative software used to process data.

Novel Aspect

Hydrolysis of cell wall ether and ester bonds produces glycosylated and isomeric compounds, these can be readily identified with IM-MS.

Preliminary Data or Plenary Speaker Abstract

The hydrolysis of BSG cell walls using conventional alkaline solvents results in the release of hydroxycinnamic acids, primarily coumaric and ferulic acids, and various isomers of dehydrodiferulic acid in aglycon form. In contrast, the deep eutectic solvent (CCM) hydrolysis yields a relatively low concentration of ferulic acid aglycon, while a pentose-conjugated ferulic acid is the major compound identified. These findings suggest that CCM has the potential to selectively hydrolyze the cell wall by cleaving ether linkages between arabinose sugars and the xylan linear backbone of BSG arabinoxylans, resulting in the release of arabinose glycosylated ferulic acid. Further analysis using ion mobility and collision cross-section data may aid in identifying different isomers of bound phenolics with increased accuracy.

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Analysis of o-Phenylphenol, Thiabendazole, Biphenyl and Imazalil in Wood Camping Cooking Utensils

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Recently the camping population has increased due to changes in leisure life by COVID-19. Accordingly, the supply and demand of camping cooking utensils are also increasing but safety verification of camping cooking utensils is insufficinet. When camping, campers often drink, talk and eat for a long time, so cooking utensils come into contact with food for a long period of time. Therefore hazardous substances may migrate from food contact materials into food.

Methods

In this study, migration time varied from 30 minutes to 2 hours and 20% ethanol were used as migration simulants by reflecting the way consumers cook and use while camping. We prepared the test solution using 20% ethanol as food simulant. 20 mL of water was added to 20 mL of the test soultion. The mixture was mixed well and purified with C18 cartridge. The amounts of migrated fungicides(o-phenylphenol, thiabendazole, biphenyl and imazalil) from wood cooking utensils(cutting boards, plates, etc) were analyzed using HPLC and GC/MS.

Novel Aspect

Migration time varied from 30 minutes to 2 hours and reflected the way consumers cook and use while camping.

Preliminary Data or Plenary Speaker Abstract

The concentration range of calibration curve for each fungicide was 0.1∼5 μg/mL and R2 value was over 0.999. The LODs and LOQs were 0.003∼0.008 μg/mL and 0.009∼0.025 μg/m, respectively. The detection levels did not exceed the standards and specifications for wood utensils, so domestic camping cooking utensils were evaluated as safe to use for cooking.

Scent Profiles of Annonaceae: Investigating the Diversity of Floral Volatile Organic Compounds in Annonaceae Plants Through GC-MS Analysis

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The effectiveness of plant reproductive success, which is crucial for food production, depends on the interactions between plants and pollinators. Plant volatile organic compounds (VOCs) primarily serve as attractants for pollinators, but they may also serve to defend the plants against biotic and abiotic stress. The Annonaceae family of diverse flowering plants in tropical rainforests exhibits dynamic scents. Gas chromatography-mass spectrometry (GC-MS) is a powerful analytical technique used to identify and quantify volatile organic compounds (VOCs) in various samples. The primary objective of this research is to employ Gc-Ms to isolate and quantify the floral VOCs and to identify the specific VOCs responsible for the unique scent, elucidating their established functions in pollination and protection mechanisms.

Methods

Floral scents from ten Annonaceae species in Bangladesh were collected via dynamic headspace sampling and analyzed at Ghent University using GC-MS. Analysis was performed on an Agilent GC-MS system with an HP-5 MS UI column. Hexane was used for volatile compound extraction, with toluene solution as an internal standard. Compound identification utilized Chemstation software and the NIST02 MS library. The species include Annona glabra L., Annona muricata L., Annona reticulata L., Annona squamosa L., Artabotrys hexapetalus (L.f.) Bhandari, Cananga odorata (Lam.) Hook.f. & Thomson, Miliusa velutina (A.DC.) Hook.f. & Thomson, Monoon longifolium (Sonn.) B.Xue & R.M.K.Saunders, Polyalthia suberosa (Roxb.) Thwaites, and Uvaria hamiltonii Hook.f. & Thomson.

Novel Aspect

GC-MS plays a crucial role in this research in both identifying and quantifying volatile compounds extracted from floral scents.

Preliminary Data or Plenary Speaker Abstract

A total of 65 compounds were identified from the floral scents of all species, categorized into three groups based on their biosynthetic pathways: compounds derived from fatty acids, terpenoids, and those from the phenylpropanoid or benzenoid pathway. In terms of human perception of scent, Cananga odorata is notably the strongest scent-producing species, emitting a pungent perfume-like aroma. It is followed by Artabotrys hexapetalus, which emits a strong fruity scent, and Monoon longifolium, which emits a perfume-like aroma. Annona muricata emits a sour, fruity scent, while all other species emit a sweet, fruity scent. Regarding VOC production, Cananga odorata, Artabotrys hexapetalus, and Monoon longifolium, respectively, yield the most abundant compounds. Specifically, Cananga odorata produces the most diverse compounds, predominantly terpenoid-derived. Annona muricata also demonstrates dominance in terpenoid-derived compounds. Conversely, the remaining species predominantly display higher proportions of fatty acid-derived compounds. In terms of compound occurrences, fatty acid-derived compounds are prevalent, followed by terpenoids and phenylpropanoids. Prominent among the fatty acid compounds were hexanoic acid methyl ester, acetic acid butyl ester, and butanoic acid butyl ester. An array of common terpenoid compounds, such as caryophyllene, beta-myrcene, germacrene D, alpha-curcumene, alpha-farnesene, and cyperene, were identified. Regarding the established roles of different compounds, fatty acids and phenylpropanoids-derived compounds emit diverse sweet fruity fragrances, attracting various beetles, flies, and other species for pollination. Terpenoids not only contribute to pollination but also shield the plant against biotic and abiotic stresses. Thus,

Annonaceae plant species emit a significant array of volatiles crucial for pollination and protection against diverse stressful conditions.

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Time-Course Peptidomics Analysis of Gastrointestinal Digesta: Decoding Dietary Protein Breakdown with Mass Spectrometry

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Recent advancements in peptide search tools and engines, featuring increased search speed, have revolutionized peptidomics analysis. These advancements enable comprehensive non-specific enzyme searches of peptides within multiple proteome databases in a single experiment, facilitating the examination of native and endogenous peptides in complex food matrices made from multiple ingredients. Capitalizing on these technological improvements, we explore human gastrointestinal digestion of dietary proteins—a complex proteolytic cleavage process driven by the combined action of pepsin, trypsin, chymotrypsin, and exopeptidases across different digestive phases (oral, gastric, and small intestinal). Our aim is to gain a deeper understanding of how dietary proteins are broken down and how this process affects nutrient absorption and overall health.

Methods

We have established a digesta-peptidomics pipeline designed for the systematic evaluation of dietary peptides released throughout the digestive process. This peptidome dataset, combined with bioinformatics analysis, enables interpretation of dietary protein quality, defined by the amino acid profile of digestion-liberated nutrients and influenced by both protein digestibility and the overall amino acid profile. Our analysis demonstrates the feasibility of employing peptide length analysis of digestion-liberated peptides as a proxy for protein digestibility. Beyond digestibility, digesta-peptidome analysis provides insights into protein fragments that resist digestion, including the identification of their origins, the specific protein domains or motifs that resist digestion, and peptidyl terminals containing hydrolysis-resistant peptide bonds.

Novel Aspect

Our pipeline elucidates mechanism underlying complex digestive environment, guiding enhancements in dietary protein formulations and processing techniques for optimized nutrition.

Preliminary Data or Plenary Speaker Abstract

To demonstrate the application of this pipeline, we compared various protein sources, including animal, plant, fungal and microbial proteins, as well as different food processing techniques and cooking methods. Our data suggests that animal protein have the best digestibility and protein quality, followed by, plant protein, algae, and mushroom’s protein. In all protein sources, C-terminus peptidyl bond between proline and any other amino acids appear to be the bottleneck of digestion as it consistently being over-represented at the C-terminal of peptides. This finding highlights a universal mechanism in protein digestion and suggests that targeting this specific peptidyl bond could enhance the overall digestibility of various protein sources. Comparing same protein sources of different food processing methods, we observed that sources of the protein played a more important role in deciding the digestibility of a protein matrices. Animal proteins consistently exhibited higher digestibility regardless of whether they were raw protein or processed protein (extrudate), whereas plant proteins demonstrate variation in digestibility depending on the plant sources and its inherent protein composition, but less variation caused by processing method. Overall, our study suggests that intrinsic factors such as protein structure, amino acid composition, and thus the presence of specific peptidyl bonds are more influential in determining the overall digestibility and nutrients availability. These insights offer mechanistic understanding of digestive enzymatic mechanism and efficiencies within gastrointestinal system, thereby guides improvements in dietary protein quality through

targeted strategies to improve, such as the addition of enzymatic hydrolysis step, and the production of fermented hybrid protein formulation. This research not only enhances our understanding of protein fate during gastrointestinal digestion, but also guides improvements in dietary protein utilization, supporting the development of more sustainable, effective, and nutritionally optimized food products.

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Project overview: LC-MS as a key tool for improving stewardship of fungicide chemistries and crop disease management.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The pressure of fungal diseases on agricultural production is rising; developing new disease-resistant varieties and effective fungicide strategies are at the core of crop protection. However, fungi are evolving to counteract these measures through the development and selection of mutations associated with new virulence and fungicide resistance. Mass spectrometry can provide appropriate and accurate measurement of fungicide distribution and concentration within plant tissue and the dynamics of its dissipation and degradation, to inform the best strategies to protect, not only the crops from disease. This information will inform new models enabling the next generation of fungicide strategies to optimise the efficacy of compounds with flow-on benefits for also optimising the life space of new forms of crop genetic resistance.

Methods

In our lab we are focusing on barley to understand the three big D's of fungicide dynamics (distribution, dissipation and degradation) and how these are influenced by plant genetics, agronomy, and environmental factors. Plants are grown in the laboratory, in the glasshouse, or in the field, and treated with commercial fungicides. Fungi are grown in the lab on solid media from which spores are collected for the inoculation of plants in both, attached and detached leaf assays. Plant samples are collected and frozen at -70 °C prior original QuEChERS extraction and C18 dSPE cleanup. We are using a Vanquish UHPLC coupled to an Orbitrap Exploris 120 (Thermo Scintific) to determine fungicide concentration within plant tissues and its changes through time.

Novel Aspect

Our project uses simple mass spectrometric analysis to understand fungicide dynamics, aiding in customising treatments for barley varieties and environments.

Preliminary Data or Plenary Speaker Abstract

Initial results have shown mostly xylem mobility with very little to neglectable phloem mobility of common commercial fungicides. Uneven distribution of fungicides along the plant canopy and within leaves was observed for fluxapyroxad seed treatments. The effect of this heterogeneous distribution through time on crop protection is being investigated. No evidence has been found that suggest relevance of metabolic/degradation products on crop protection. The next aim is to look for correlations of dissipation and disease protection curves with plant genetics and environmental factors.

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Combined LC/MS and GC/MS approach for analysis of extractables and leachables in complex matrices using high resolution mass spectrometry.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

One of the challenges in extractables and leachables applications is the variety of matrices that could be quite complex, containing a multitude of structurally diverse compounds. This requires both LC/MS and GC/MS techniques for the adequate compound coverage. The goal of this study was to develop analytical methods and workflows for acquiring and efficient processing of the complex E&L data with a focus on a non-targeted approach.

Methods

Rubber syringe gaskets were extracted using tetrahydrofuran (THF) solvent at room temperature for six months. Four different types of catheters were extracted with water:ethanol 1:1 at 40 °C for 48 hours. An aliquot of each extract as well as solvent blanks were analyzed using both GC/Q-TOF and LC/Q-TOF systems. The LC/MS analysis was preformed using the Revident Q-TOF. GC/MS analysis was performed using the 7250 Q-TOF. The GC/MS data were acquired in standard EI mode, low energy EI as well as positive chemical ionization (PCI) modes. MS/MS data have also been acquired for further structure elucidation of the unknowns. All the data have been collected under a unified compliance environment.

Novel Aspect

Combined approach for analysis of extractables and leachables in complex matrices using high resolution mass spectrometry.

Preliminary Data or Plenary Speaker Abstract

For GC/MS analysis both 30 m 0.25 mm x 0.25 m and 20 m 0.18 mm x 0.18 m DB-5ms UI columns have been evaluated with respect to the chromatographic separation capability of the complex E&L samples as well as sensitivity, in addition to other benefits, such as run time. The GC methods have been optimized for each column. Preliminary results indicated that, while the 20 m column provided sharper peaks and greater sensitivity for trace-levels compounds, 30 m column offered better separation with higher number of components been reliably identified. Over 200 hundred compounds have been identified after blank subtraction using GC/MS including many antioxidants used in rubber production, PAHs, plastic additives, nonylphenols, rubber accelerators and strength agents, crosslinking agents and rubber oligomer impurities. Compound identification was confirmed using accurate mass information and retention indices (RIs). Some of the compound annotations overlapped between GC/MS and LC/MS, but there were also a number of compounds uniquely detected by either LC/MS or GC/MS technique (for example, hydrocarbons have only been detected by the GC/MS). The higher chromatographic resolution of GC also enabled the separation of structural isomer of phthalates and breakdown products that were not readily resolved using reverse phase chromatography. In contrast, intact higher molecular weight oligomeric species were better detected using LCMS reinforcing the importance of using both techniques for E&L compound ID.

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Profiling and optimized extraction of bioactive polyphenolic compounds from agriculture waste using eco-friendly deep eutectic solvents

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The apple processing industry annually generates tons of waste. The valorisation of this waste is crucial for promoting sustainability within the industry. Apples are rich in flavonoids, which possess significant potential in both pharmaceutical and food industries due to their versatile health benefits. However, many flavonoids exhibit low solubility in aqueous solutions, leading to the utilization of organic solvents in conventional extraction methods, which generates substantial amounts of pollutants. Deep eutectic solvents (DES) are increasingly garnering attention as a green alternative to hazardous organic solvents for extracting bioactive compounds from biomass. This study endeavours to employ LC-MS to profile the polyphenolics present in apple waste using both conventional organic and DES extraction methods, and compare their respective bioactivities.

Methods

DES, comprised of natural compounds, consist of a hydrogen bond donor (e.g., a quaternary ammonium salt) and a hydrogen bond acceptor (e.g., a carboxylic acid). Three choline chloride-based DES solvents were synthesized and employed to extract polyphenolic compounds from both apple pomace and unripe apples. Extraction conditions were optimized to attain superior yields compared to conventional organic solvent extraction methods. Phenolic compounds within the apple extracts were identified and quantified using ultra-high performance liquid chromatography (UHPLC) coupled with a diode-array detector (DAD) and Quadrupole Time-of-Flight mass spectrometry (QTof). Biological activities, including antioxidant, antimicrobial, and neuroprotective properties, were compared between apple extracts obtained via organic solvent extraction and DES extraction methods.

Novel Aspect

DES offer a sustainable alternative to organic solvents for both extracting and structurally modifying bioactive polyphenolics derived from agricultural waste.

Preliminary Data or Plenary Speaker Abstract

Unripe apples are a significant byproduct of the fruit thinning process, exhibiting four times higher phenolic content (2078.4 ± 4.0 mg gallic acid equivalent /100g) compared to ripe apples (545.0 ± 32.0 mg gallic acid equivalent /100g). The use of choline chloride and glycerol DES extraction solvents supplemented with 40% w/w H2O yielded similar amounts of total phenolics from unripe apples at 40°C compared to methanol. Additionally, the polyphenolic profile and bioactivities of the DES extracts from unripe apples were similar to those of methanol extracts.
Apple pomace, another byproduct of the juicing process in the beverage industry, was subjected to extraction using acidified methanol and acidic DES solvents. Eight glycosylated quercetins and quercetin aglycon were identified as major polyphenolic compounds in both extracts through UHPLC-MS and NMR analysis. Compared to methanol extracts, the content of glycosylated quercetins and quercetin aglycon was similar in DES, but the relative amounts of these compounds varied distinctly due to de-glycosylation during DES extraction. The proportion of quercetin aglycon in DES extracts could be controlled by adjusting the DES composition, extraction temperature, and duration of extraction. Notably, quercetin-3-O-α-arabinofuranoside was found to be more susceptible to de-glycosylation during DES extraction than other quercetin derivatives. Furthermore, the antioxidant activity of the DES extracts surpassed that of methanol extracts, likely due to the increased quercetin aglycon content generated during extraction.
Our findings suggest that DES extracts from apple waste, including unripe apples and apple pomace, hold promise as sources of bioactive polyphenolics with applications in various sectors such as

functional food production, smart material engineering, and natural therapies. Moreover, the results indicate the potential for extracting and de-glycosylating quercetin derivatives in a single eco-friendly step using DES, leading to the production of bioactive compounds with enhanced activity.

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Mass spectrometry as an informing technology for ultra-high resolution GC×GC analysis for Hop volatiles - an example of metabolomics profiling

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Food analysis is heavily dependent on mass spectrometry, from e.g. direct analysis of volatiles, to proteins by MALDI. But perhaps the most widely employed and popular use of MS will be for hyphenation of separations (separation-domain) with MS (mass-domain). This includes for e.g. liquid, gas and supercritical chromatography. Today, technical and instrumental procedures are generally well developed for MS, MS/MS and related approaches, with separations. The most recent development and highest resolution method for separations is 'super-resolved' comprehensive 2D GC (GC×GC). This puts extra demands of the MS domain, particularly related to acquisition speed, and the lack of access to certain approaches - for instance MS/MS might not be so readily available. Here we present our work on Hop analysis.

Methods

Food analysis relies heavily on MS. Direct analysis of volatiles by e.g. PTRMS has limited capability for overall sample profiling. Recent developments in comprehensive 2D GC (GC×GC) provide ultra-high resolution for volatile separations. Here Hop volatiles sampling employed a PDMS/DVB fibre coating for solid-phase microextraction, conducted at 50 □C, with 10 min equilibration and 30 min sorption times. The column set comprised a long low-polarity DB-5ms 1D column and a short SUPELCOWAX 10 2D column. Modulation used a SSM1800 solid-state modulator (J&X Technologies, Nanjing). GC×GC peak widths are typically 0.1-0.2 s, suggesting MS acquisition of at least 50 Hz; TOFMS and fast QMS are necessary. Target methods (e.g. MS/MS) are rarely used for GC×GC which excels for untargeted analysis.

Novel Aspect

SPME sampling with GC×GC analysis for Hop; PCA data interpretation; Marker identification

Preliminary Data or Plenary Speaker Abstract

Volatilomics analysis using GC×GC should be logical; ultra-high resolution allows a considerable proportion of the volatile composition of samples to be theoretically measurable. Separation is one aspect; valid compound identity is another. This demands authentic standards and/or retention index correlation, reproducibility, and MS library matching. However confirmation may still be lacking. The 2D structure/retention properties of GC×GC are still seldom explored, though we have investigated possibilities for this. The AZAC, CASC, ENIG, LORA and ZAPP Hop varieties generated about 150 integrated peaks with GC–MS and 300-400 with GC×GC–MS. About 50% more were subsequently 'identified' using GC×GC vs GC. Suggested marker compounds were proposed, and the analytical coverage, composition heterogeneity, profiling, and preliminary interpretation of PCA and HCA data discussed.

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Tagging fatty acids to complex lipids carriers in edible nuts

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Edible nuts are energy-dense foods with high concentrations of lipids, which comprise ca. 40-70% dry weight. Of this fraction up to 98% is comprised of triacylglycerols (TGs) and thus determination of the structure and composition of this lipid class is essential to understanding the nutritional value of these critical foodstuffs. Herein, we couple reversed-phase chromatography with collision-induced dissociation (CID) and ozone-induced dissociation (OzID) modalities to assign the structure and composition of TGs across 9 classes of nuts.

Methods

Almonds, Brazil nuts, cashews, macadamias, peanuts, pecans, pine nuts, pistachios, and walnuts were obtained from local supermarkets, cyrogenically milled and extracted using a biphasic lipid extraction. Lipid extracts were subjected to reversed-phase liquid chromatography (LC) using a C18-column over a 15-minute gradient with a low percentage of sodium acetate present in the mobile phase. Electrospray ionisation generated abundant [TG+Na]+ cations that were subjected to either collision- (CID) or ozone-induced dissociation (OzID) on a modified ion mobility enabled quadrupole time of flight mass spectrometer (MS, Waters SYNAPT G2-Si). In parallel, lipid extracts were hydrolysed and subjected to fatty acid analysis using the ozone-enabled fatty acid discovery method as previously described.

Novel Aspect

The combination of CID and OzID describes non-methylene interrupted fatty acids esterified to TG carriers for the first time.

Preliminary Data or Plenary Speaker Abstract

LC-MS analysis of saponified lipid extracts across all nine nut varieties revealed over 60 structurally distinct fatty acids (FAs). Application of OzID in this workflow resolved multiple isomeric FAs that differed only in the location of carbon-carbon double bonds. Numerous FAs characterised by unusual site(s) of unsaturation were detected and pine nuts, in particular, were found to be rich in non-methylene interrupted polyunsaturated fatty acids (NMI-PUFA) with FA 18:2n-9,13 (taxoleic acid), FA 18:3n-6,9,13 (pinolenic acid) and FA 20:3n-6,9,15 (sciadonic acid) found to be prevalent. High resolution LC-MS analysis of the unhydrolyzed lipid extracts enabled identification of >60 TG molecular lipids with principally even-carbon numbers ranging 50 to 60 and characterised by degrees of unsaturation between 0 and 9. Pine nuts were characterised by abundant polyunsaturated TGs with carbon numbers of 52, 54 and 56 and thus likely carriers of PUFAs with 18-and 20-carbon FA chains. Data-dependent LC-CID analysis of these extracts enabled molecular lipid assignments of these sum compositions and confirmed the presence FA 18:2, 18:3 and 20:3 carried predominantly by TG 52:5, TG 52:6 and TG 54:8. Characteristic neutral losses confirmed FA 18:3 was carried by TG 52:5 with an overall molecular lipid composition confirmed as TG 16:0_18:2_18:3 (among other isomers). Targeted LC-OzID analysis enabled explicit assignment of carbon-carbon double bond locations in with retention-time aligned aldehyde and Criegee ions for TG 52:5n-6,9,13 consistent with a proposed molecular lipid of the form TG 16:0_18:2n-6,9_18:3n-6,9,13. These findings provide the first evidence of NMI-PUFAs bound to complex lipids and is a critical step toward the complete structural lipid description of these unusual molecular species. Preliminary CID/OzID data will be presented that provide the first indication of the sn-positional assignments for these complex lipids.

Characterization of occupational exposure to glyphosate and AMPA among forestry workers in Australia

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Glyphosate, a widely used herbicide in agricultural and bushland for weed control, raises significant concerns regarding potential exposures. Despite its widespread use, our understanding of potential exposures during common occupational uses remains limited. This study aims to characterize occupational exposures to glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA), among forestry workers in Australia.

Methods

The study will span various locations, starting with a pilot trial in SA, then expanding nationwide to include SA, QLD and TAS. A biomonitoring approach will be adopted to quantify the exposure to glyphosate and AMPA under field conditions. Additionally, environmental monitoring, including assessment of skin and working surface wipes will be undertaken as a complementary approach to inform exposure pathways. LC-MS/MS methods will be developed and validated for glyphosate and AMPA in urine and wipe samples to assess potential exposure. By combining data from these locations, our goal is to understand the magnitude of glyphosate exposure in forestry settings, how usage patterns and personal protective equipment (PPE) influence exposure, and propose effective risk reduction strategies.

Novel Aspect

Efficient urine extraction with AFFINIMIP cartridges and LC-MS/MS analysis with in-sample MA addition for glyphosate biomonitoring in Australian forestry studies.

Preliminary Data or Plenary Speaker Abstract

An LC-MS/MS method was developed using the SCIEX 4500 Triple Quad for the determination of glyphosate and AMPA in human urine. Chromatographic separation was achieved within 6 minutes on an Obelisc N column. The effect of adding medronic acid (MA) was assessed to improve peak shape and intensity of both analytes. We found that even adding 0.1 mM MA consistently enhanced results. MS determination was performed using multiple reaction monitoring (MRM) in negative electrospray ionization (ESI) mode, with optimized source and MRM parameters. For urine extraction, various SPE cartridges were tested, including Oasis MAX (mixed-mode anion exchange sorbent), Oasis MCX (mixed-mode cation exchange sorbent) and Oasis HLB (hydrophilic-lipophilic balanced reversed-phase sorbent). A two-step process with MCX for pre-cleanup followed by MAX extraction proved to be most efficient. AFFINIMIP SPE Glyphosate-AMPA cartridges, based on molecularly imprinted polymers, showed promise in selectively cleaning and concentrating glyphosate and AMPA in different water matrices and bee bread samples, yet its efficacy in complex biological matrices like urine remains unreported. We optimized extraction using AFFINIMIP cartridges with different parameters, including pH of the loading solution, acidic strength in elution solutions, different washing steps and volumes of reconstitution solution. Improving recovery approximately fivefold was achieved compared to the optimized two-step method. Employing heavy-labelled internal standards successfully corrected both recovery and matrix effect for both analytes. The method was validated in terms of specificity, linearity, limit of detection (LOD), lower limit of quantification (LLOQ), intra-batch and inter-batch accuracy and precision, matrix effect, recovery, carryover and stability following US FDA guidelines for bioanalytical method validation. The LOD and LLOQ was validated at 0.1 and 0.4 ng/mL for glyphosate and 0.2 and 0.8 ng/mL for AMPA, respectively. These levels are considered suitable for application in biomonitoring studies within occupational exposure settings.

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Analysis of NX-toxins using high resolution mass spectrometry from Fusarium graminearum isolates in Western Canada

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Fusarium graminearum is considered an important plant pathogen, causing fusarium head blight (FHB), producing mycotoxins, in cereal crops in Canada, USA, and other parts of the world. Every year significant quantities of grain becomes unfit for human and animal consumption due to mycotoxin contamination leading to huge economic losses world-wide. Economic losses due to FHB estimate over $1 billion CAD in Canada, each year. Fusarium graminearum is mostly known to produce trichothecenes including deoxynivalenol, 15-acetyl deoxynivalenol, 3-acetyl deoxynivalenol, and nivalenol. Recently, a small percentage of Fusarium graminearum strains from Canada and U.S. were found to produce a novel 3ANX toxin in cereal crops. These F. graminearum strains could pose a new threat to food safety in Canada and world-wide.

Methods

Lack of analytical methods to detect NX toxins limited the early detection and study of the novel NX-producing isolates in Canada. High resolution mass spectrometry with ultra-high performance liquid chromatography (UHPLC-HRMS) was employed to detect and quantify NX toxins. Extraction and analytical methods were developed and validated to help detect NX-producing isolates. Seven days old Fusarium graminearum isolates were sub-cultured in sterile rice. Ground samples were extracted for mycotoxins (3-ANX, NX, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and nivalenol) with a solvent mixture containing acetonitrile, methanol, and water followed by sonication and centrifugation. The separated supernatant was filtered and evaporated to dryness under vacuum. Dried extracts were re-suspended in solvent and a labelled internal standard was added followed by analysis using an UHPLC-HRMS.

Novel Aspect

The UHPLC-HRMS method can detect and quantify novel NX-toxins including 3-ANX and NX to identify fusarium isolates producing NX toxins.

Preliminary Data or Plenary Speaker Abstract

The developed method is capable of extracting, detecting, and quantifying NX toxins along with 4 other trichothecene mycotoxins. Preliminary results indicate a strong correlation between 3-ANX toxin and other trichothecene mycotoxin. The details of this correlations and significance of these finding will be presented. The developed methods would help in detecting NX producing isolates and characterizing their trends in the spatial and temporal dynamics, as well as promote early detection of emerging threats to plant, human, and animal health. In addition, these findings will help develop proactive mitigation strategies to increase resiliency in agriculture and achieve food security.

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Characteristics of new ion source match factor of GC/MS library search with hydrogen carrier gas for chemical active compounds

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The supply of helium gas used in GC/MS is still unstable globally. Hydrogen is one of the choices of alternative for carrier gas, which is chemically more active than helium, resulting in the mass spectrum change. GC/MS is widely used for compound identification by EI mass spectrum library because of its stable ionization with helium carrier gas. Changing the spectrum by hydrogen influences on the compound identification. 193 compounds from Japanese flavor GC/MS library are the selected according to knowledge of application experts, observed the changes in the mass spectrum with hydrogen carrier gas. The effect of mass spectrum of hydrogen carrier gas with conventional inert ion source (XT) and newly developed HydroInert ion source (HI) of GC/MS is discussed.

Methods

193 compounds were analyzed with hydrogen carrier gas by single quadrupole GC/MS with two types of ion source: a conventional inert ion source and a new ion source (HI: HydroInert source) developed for better performance with hydrogen carrier gas. Mass spectra were obtained using MassHunter Unknowns Analysis software and library searches were performed using NIST 20. The Match Factor (MF), which is an index of spectral similarity, was used as the performance metric. Based on the profile of MF of both ion sources, compounds were classified by hierarchical cluster analysis. 155 molecular descriptors of 193 compounds, representing the molecular chemical characteristics were obtained. Molecular descriptors correlated with MF of HI were selected by RandomForest for interpretation of MF profile.

Novel Aspect

Classify the compounds and interpret the GC/MS mass spectrum changes on hydrogen carrier gas by cheminformatic method.

Preliminary Data or Plenary Speaker Abstract

193 compounds of in the Japanese Flavor Library were selected according to the availability of chemical standards at our laboratory in Japan. Hydrogen carrier gas spectrum of 193 compounds at 50 ppm acquired by the conventional ion source (eXTractor source: XT) and HydroInert (HI) ion source were library-searched by MassHunter Unknows analysis with chromatographic deconvolution, and they were classified into nine clusters according to the Match Factor (MF) of library search. MF of library search was used as the metrics of fitting the spectrum with the library. HI MF showed excellent performance over XT MF for most compounds. Two metrics were used for characteristics of mass spectra on hydrogen carrier gas, HI MF and the MF ratio. MF ratio is the ratio of HI MF over XT MF. The profile of HI MF and MF ratio were divided in nine clusters by hierarchical cluster analysis. 129 compounds belonged to Cluster 1 , HI MF was over 95% and the MF improvement (HI MF / XT MF rate) was 1.18 times on average. For understanding on the EI ionization efficiency of hydrogen carrier gas generally (common both HI and XT), compounds which were MF ratio less than 1.05 (165 compounds) were selected with 149 molecular descriptors. Eight molecular descriptors were selected by random forest for interpretation. Among the selected eight molecular descriptors, five molecular descriptors with high Pearson correlation coefficients were found to be associated with the water-octanol coefficient, i.e. Log P, and the proportion of hydrogen in the molecule. The MF on hydrogen carrier can be associated with these parameters which can be used for interpretation.

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Effect of postharvest storage on cellular pathways in Choysum (Brassica rapa)

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

After being harvested, green leafy vegetables begin to lose their freshness and nutritional properties. Postharvest losses present a significant challenge in agriculture, with estimates indicating rates as high as 25% in developed countries and 50% in developing nations. This dilemma imposes substantial economic, social, and ecological burdens. Employing suitable postharvest storage methods, such as cold storage, is a common approach to slow down deterioration. However, the intricate cellular pathways involved in vegetable deterioration are not fully understood. Thus, we aim to elucidate the impact of postharvest storage on cellular pathways involved in degradation and senescence in Choysum, a popular green leafy vegetable, using proteomics and metabolomics.

Methods

Protein and metabolite extractions were done on Choysum samples stored for various time points at 4oC. SWATH quantitative proteomics was done to elucidate changes in cellular pathways upon postharvest storage of Choysum. Key metabolites were quantified and compared.

Novel Aspect

Elucidating cellular pathways involved in postharvest quality loss of vegetables improves the understanding of the plant's response to storage.

Preliminary Data or Plenary Speaker Abstract

Preliminary data on duplicate protein extracts showed that 2372 proteins were identified and quantified using SWATH MS and 20 proteins were found to be differentially abundant (based on 1.5-fold change and a Q-value of less than 0.05). Gene annotation of differentially abundant proteins revealed several cellular pathways that were involved in the degradation of Choysum upon post-harvest storage. Proteins involved in organic metabolic processes, amino acid biosynthesis and translation were found to be decreasing in abundance. On the other hand, proteins involved in carbohydrate and ROS metabolic process were found to be increasing in abundance.

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Evaluating the impact of oat protein isolate composition using SE-HPLC and LC-MS approaches

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The rising global population emphasizes the need for sustainable agriculture, and in this context plant-based proteins have gained widespread attention. Currently, the global plant-based protein market is dominated by soy, however, alternative proteins, such as oat protein are gaining traction. Oat is hypoallergenic and has a good nutritional profile, making it a promising alternative to allergenic proteins like soy. The utilization of oat protein in food product development is currently limited, and research focused on this topic is also sparse. Therefore, there is a need to study oat, in the context of plant-based proteins. To this end, the objective of this study was to determine how the chemical composition of oat protein isolate is impacted by genotype and growing environment.

Methods

The study included grains from three most established oat cultivars in Canada (Summit, AC Morgan and CS Camden). The samples were collected from the Canadian prairie provinces, namely Brandon-Manitoba, Saskatoon-Saskatchewan and Lacombe-Alberta in the growing year 2020. The effect of genotype and environment on oat protein isolate (OPI) composition was analyzed through Size Exclusion- High-Performance Liquid Chromatography (SE-HPLC) which was used to fractionate OPI into its constituents, and Liquid Chromatography – Mass Spectrometry (LC-MS) was used to gain additional information of the globulin protein fraction in OPI. Statistical analyses were used to determine the effect of genotype x environment on OPI composition and globulin protein chemistry.

Novel Aspect

The results demonstrate the significance of understanding OPI composition to develop functional protein ingredients from oat.

Preliminary Data or Plenary Speaker Abstract

Upon extracting OPI from oat flour using an optimized alkaline extraction-isoelectric precipitation method, the OPI samples were analyzed through SE-HPLC to identify four fractions, namely polymeric globulins, avenins, glutelins and albumins, and smaller proteins. Oat protein is mainly (approximately 50-80%) composed of globulin proteins and in this study, the percentage of globulin in OPI ranged between 40-62%, with Alberta showing the highest percentage. As for the other protein fractions, samples from Manitoba showed the highest percentage. Overall, OPI composition was significantly impacted by growing environment, and genotype did not appear to play a prominent role. The globulin to avenin ratio (G/A) was also determined, and that too was mainly dependent on the growing environment.. The proteins identified through LC-MS were grouped into eight categories including globulins, prolamins/avenins, glutelins, enzymes/ albumins, enzyme inhibitors, heat shock proteins, grain softness proteins and allergenic proteins. In this study, three main globulin protein types were identified including the P14812|SSG2-12S seed storage globulin, the Q6UJY8_TRITU-globulin and the M7ZQM3_TRIUA-Globulin-1 S. The LFQ intensity was used to determine the impact of genotype and environment on globulin proteins, and the most abundant globulin, P14812|SSG2-12S seed storage globulin, was mainly impacted by genotype, rather than environment. Principal Component analysis (PCA) showed specific genotype-environment combinations have positive associations with P14812|SSG2-12S seed storage globulin, which warrants further investigation.

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N-glycans of two isoforms from sheep testicular hyaluronidase identified and quantified by liquid chromatography-electrospray ionization-high energy collision dissociation-tandem mass spectrometry

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Hyaluronidases have found wide application, either as standalone agents or as adjuncts with other medications. Animal-derived hyaluronidase has been used in medical applications despite its limited purity. N-Glycans affect the functionality of hyaluronidases. For instance, the bioactivity of human hyaluronidase, synthesized in Chinese hamster ovary cells, diminishes by nearly 80 % post-deglycosylation. Similarly, deglycosylation of PH-20 on the macaque sperm surface leads to a total loss of its activity. Yet, there are no reports on the influence of N-glycans on STH activity. In this study, STH was purified from the commercially available STH preparation using heparin-affinity chromatography. The structure and quantity of N-glycans of STH were investigated using liquid chromatography-electrospray ionization-high energy collision dissociation-tandem mass spectrometry.

Methods

We performed sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) with a crude form of STH. STH was purified from the commercially available STH preparation (containing at least 14 impurity proteins) using heparin-affinity chromatography followed by size exclusion chromatography. Subsequently, in-gel digestion was conducted for the preparation of peptide sample. Hyaluronidase activity was determined using zymography and turbidimetric method. The N-glycan chromatogram was generated using ultra performance liquid chromatography, and the structure and quantity of N-glycans of STH were investigated using liquid chromatography-electrospray ionization-high energy collision dissociation-tandem mass spectrometry. Following treatment with trypsin/chymotrypsin, the peptides and glycopeptides derived from H3S1 and H3S2 were analyzed using nano liquid chromatography high energy collision dissociation-tandem mass spectrometry.

Novel Aspect

This is the first report of N-glycan characterization of two highly purified isoforms of STH.

Preliminary Data or Plenary Speaker Abstract

Two isoforms exhibiting STH activity, namely H3S1 and H3S2, were successfully purified from commercially available STH using HAC and SEC. The resulting products exhibited a purity exceeding 98 %, with specific activities of 239,531 Units/mg and 339,659 Units/mg, and yields of 3.4 % and 5.1 %, respectively. N-glycans were derived from both H3S1 and H3S2 samples, which were treated with PNGase F. These glycans were subsequently labeled with ProA, a highly efficient fluorescent reagent. We identified 14 distinct N-glycans, including one core structure, six high-mannose type, two hybrid type, and five complex type, in both H3S1 and H3S2, with similar quantities of each N-glycan present. Approximately half of the total N-glycans in both H3S1 and H3S2 were core-fucosylated. Deglycosylated versions of H3S1 and H3S2 were produced using PNGase F, and their hyaluronidase activity was evaluated both qualitatively (via zymography) and quantitatively (via the turbidimetric method). The peptides and glycopeptides derived from H3S1 and H3S2 were analyzed using nanoLC-HCD-MS/MS. Both samples contained nine identical peptides. This finding suggests that H3S1 and

H3S2 are isoforms of STH sharing the same peptide sequences, except for 2 (glyco)peptides. This represents the first study to elucidate the structure, relative quantity, and structural role of these 14 N-glycans, including core-fucosylation, in two purified isoforms of STH, H3S1 and H3S2. Despite their lower yield, these high-purity isoforms hold promise for future research into the biological characterization and medical application of STH, potentially avoiding the unwanted effects associated with partially purified STH. Given the spreading property of STH when applied in drug injections and the distinct characteristics of N-glycans derived from STH, the development of a more accessible drug formulation appears viable.

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Distinguishment and quantification of sialylated N-glycan linkage isomers in glycoengineered therapeutic glycoproteins using LC-MS/MS

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Glycosylation is an important post-translational modification of therapeutic glycoproteins, and N-glycans are crucial for the physicochemical properties, such as stability, solubility, bioavailability, pharmacokinetics, and immunogenicity, of glycoproteins. Sialic acids are mainly linked to galactose by α2-3 and α2-6 linkages at the terminal position on N-glycans in glycoproteins. The sialylated N-glycan isomers with α2-3 and α2-6 linkages possess distinctive roles in glycoproteins with regard to biopharmaceutical efficacy.
The liquid chromatography (LC)-electrospray ionization-tandem mass spectrometry (MS/MS), given their high accuracy and reliability, have recently been used for quantitative and qualitative N-glycan analysis. However, there are many difficulties in identifying sialylated N-glycans, and analytical difficulties were increased by the presence of sialyl-linkage isomers with α2-3 and α2-6 linkages during mass spectrometry analysis.

Methods

Using LC-MS/MS coupled with the hydrophilic interaction chromatography (HILIC) column, this study investigated the structure and quantity of N-glycans, especially sialylated N-glycan isomers with α2-3 and α2-6 linkages, in glycoengineered therapeutic glycoprotein, cytotoxic T lymphocyte-associated antigen-4-Ig (CTLA4-Ig), and compared these with the isomer structures in wild-type (WT). The N-glycans were released, labeled with ProA for enhancing ionization efficiency, and compared in two different parameters generated from sialyl-linkage isomers: the ratio of intensities of fragment ions and retention time (RT). The scatter plot of Ln/Nn value versus RT shift of N-glycan mass peaks based on the present results was suggested for distinguishing sialyl-linkage isomers. Additionally, these sialyl-linkage isomers were compared with those by α2-3 neuraminidase-treated N-glycans.

Novel Aspect

This study generated a novel plot of Ln/Nn versus retention time to distinguish sialylated N-glycan linkage isomers in glycoprotein.

Preliminary Data or Plenary Speaker Abstract

Sialylated N-glycan isomers with α2-3 or α2-6 linkage(s) have distinctive roles in glycoproteins, but are difficult to distinguish.
Wild-type (WT) and glycoengineered (mutant) therapeutic glycoprotein, CTLA4-Ig, were produced in Chinese hamster ovary cell lines; however, their linkage isomers have not been reported.
In this study, sialylated N-glycan isomers with α2-3 and α2-6 linkages in CTLA4-Igs were distinctively and successfully identified and quantified by LC-ESI-MS/MS with HILIC columns by enhancing ionization efficiency with ProA, relative intensities of oxonium ions (Ln/Nn value) in MS/MS spectra, and separation of mass peaks due to RT shift in extracted ion chromatogram. The present study obtained the following results: 1) the relative intensities of two sialic acid ions in α2-6 linkage were decreased compared to those in α2-3 linkage, however that of [N-acetylhexosamineHexose]+ ion in α2-6 linkage was increased compared to that in α2-3 linkage, 2) the intensity of oxonium ions was used to calculate the Ln/Nn value, and the values showed the ranges for sialylated N-glycans containing only α2-3 linkage(s) group (0.5–1.3), only α2-6 linkage(s) group (1.6–4.7), and both α2-3 and α2-6 linkages group (1.0–2.2), and 3) mono-, di-, and tri-sialylated N-glycans showed a different

RT between α2-3 and α2-6 linkage(s), and RT shift (1.3–1.5 min) in mono- and bi-antennary structures was slightly different those (1.1–1.2 min) in tri- and tetra-antennary structures. Consequently, the N-glycans from WT were obtained as 20 sialylated N-glycans (50.4%) with only α2-3 linkages, but 39 sialylated N-glycan isomers (58.8%) with only α2-3 (10 N-glycans; 4.8%), both α2-3 and α2-6 (14; 18.4%), and only α2-6 (15; 35.6%) linkages were obtained in mutant. These results are consistent with those by α2-3 neuraminidase-treated N-glycans, and all of the present results were summarized as a scatter plot of Ln/Nn (y-axis) versus RT (x-axis).

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Identification and quantification of N-glycans in two glycoproteins by peptide-N-glycosidase treatment and procainamide-labeling using LC-MS/MS

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Glycosylation of proteins is one of the most important post-translational modifications that occurs in the majority of proteins. This process affects the biological properties of proteins, such as folding, localization, and degradation.
However, reports of N-glycan structures are often inconsistent depending on the type of glycoprotein, particularly bovine fetuin, and the preparation methods including release and derivatization of N-glycans, and almost no studies on the structural compassion and quantification of each N-glycan obtained by peptide-N-glycosidase (PNGase) and fluorescent tags using the recently developed ultra-performance liquid chromatography (UPLC) and LC-tandem mass spectrometry (MS/MS) have been reported.
To obtain consistent results for qualitative and quantitative analyses of N-glycans, N-glycans obtained by different preparation methods were compared for two types of mammalian glycoproteins.

Methods

N-glycans are released by peptide-N-glycosidase F (PF) or A (PA) from two model mammalian glycoproteins, bovine fetuin (with three glycosylation sites) and human IgG (with a single glycosylation site) and labeled with a fluorescent tag [2-aminobenzamide (AB) or procainamide (ProA)]. The structure and quantity of each N-glycan were determined using UPLC and LC-ESI-HCD-MS/MS coupled with hydrophilic interaction chromatography (HILIC) column.
The relative quantity (%) of each N-glycan was calculated from the sum of the individual LC-MS peak areas of the extracted ion chromatogram (EIC). Each EIC area was generated using LC-electrospray ionization (ESI)- high-energy collisional dissociation (HCD)-MS/MS, and the quantity (%) of each N-glycan (>0.1 %) was determined relative to the total amount of N-glycans (100 %).

Novel Aspect

This study is the comparative analysis for identification and quantification of N-glycans by PF and PA-ProA and AB using LC-MS/MS.

Preliminary Data or Plenary Speaker Abstract

The present study investigated methods of characterization of N-glycans in model glycoproteins, bovine fetuin and human IgG, released by PF (widely used for N-glycan analysis) or PA (only used for plant N-glycan analysis), labeled with AB (used to characterize N-glycans for approval of therapeutic glycoproteins in FDA) or ProA, and analyzed using UPLC and LC-MS/MS. The present results indicate that 21 (4 non and 17 sialylated; 16 non- and 5 core-fucosylated) N-glycans (>0.1 %) of fetuin and 21 (11 non- and 10 sialylated; 7 non- and 14 core-fucosylated) N-glycans of IgG (>0.1 %) were obtained using both PF and PA, but all of them were identified and quantified when using ProA-labeling, not AB-labeling, particularly in the case of bovine fetuin. There were no non- to tetra-sialylated or non- to mono-core-fucosylated N-glycans in fetuin and IgG that could not be identified by PF or PA when using ProA-labeling. The relative quantity (%) of each N-glycan released by PF (or PA) and labeled using AB (or ProA) was obtained with a similar tendency. However, the absolute quantities were more effectively determined using ProA-labeling than those obtained using PF-AB. The present

results demonstrate that either PF-ProA or PA-ProA treatment allows for efficient identification and quantification of N-glycans in glycoproteins, particularly fetuin. The combination of UPLC and LC-MS/MS used in this study can provide useful quantitative information about N-glycans. Additionally, MS analysis was performed using the positive ion mode of ESI, and a negatively charged sialic acid moiety can decrease its ionization efficiency and detection. However, ProA-labeling showed enhanced ionization in the positive ion mode, and the results of this study suggest that PF-ProA (or PA-ProA) can be used for MS quantification and qualification of N-glycans in fetuin and IgG, together with LC-ESI-HCD-MS/MS.

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Analysis of sulfated N-glycans as a potential biomarker for the early detection of Breast Cancer

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Breast cancer (BC) is a significant global health concern among women, and early detection plays a pivotal role in enhancing patient survival rates. Alterations in the structure and abundance of sulfated glycans, similar to native glycans, have been linked to various diseases, including cancer. However, the analysis of sulfated glycans poses challenges due to their low abundance. Therefore, the investigation of sulfated glycan profiles has become an area of great interest in the search for new biomarkers for the early detection of BC.

Methods

In this study, we utilized a glycoblotting-based sulphoglycomics workflow to examine the sulfated N-glycans in the serum of Ethiopian patients with BC. This approach integrates a high-throughput glycoblotting enrichment technology, WAX separation, and MALDI-TOF MS. The sulfated N-glycan profiles in the whole serum of 76 BC patients and 20 age-matched healthy controls were analyzed. The mass spectra data was then carefully analyzed using various statistical tools.

Novel Aspect

analyzing sulfated N-glycans in BC patients quantitatively, identifying novel glyco-biomarkers with discriminatory potential in the early stages of BC.

Preliminary Data or Plenary Speaker Abstract

By separating the trace amounts of sulfated N-glycans from the more abundant non-sulfated ones, we successfully identified seven mono-sulfated glycans that exhibited a significant increase in serum levels among BC patients compared to the control group. These glycans displayed high abundance (AUC ≥ 0.8) and demonstrated strong diagnostic accuracy in predicting early-stage BC. Most sulfated glycans primarily displayed terminal lewis-type glycan epitopes, unlike their negligible presence in non-sulfated N-glycans in serum, whose abundance has been strongly associated with BC progression, metastasis, and immune invasion. In addition to the seven mono-sulfated N-glycans, the total amount of fucosylation and sialylation on sulfated glycans is also a statistically reliable marker candidate for diagnosis in the early stages of BC. Importantly, we analyzed sulfated glycans without removing the sialic group, enabling a comprehensive evaluation of the sialylation status of the identified sulfated glycans.

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Structure identification of N-glycans in bovine testicular hyaluronidase by liquid chromatography-tandem mass spectrometry and de-sialylation effect on hyaluronan degradation activity

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Bovine testicular hyaluronidase (BTH), which accelerates the absorption and dispersion of drugs by decomposing hyaluronan in subcutaneous tissues, has been used in medical applications. The requirement of N-glycans for the activity of human hyaluronidase has been reported, and BTH has greater activity than human hyaluronidase. However, the N-glycan characteristics of BTH are unclear. N-glycans, which are covalently attached to proteins at Asn residues, influence numerous properties of glycoproteins, including their structure, solubility, antigenicity, activity, and recognition by glycan-binding proteins Specifically, the presence of sialic acid prevents glycoprotein degradation, thereby preserving its half-life. It also influences protein attributes such as thermal stability, resistance to proteolysis, and solubility. This study focused on the structural characteristics and roles of N-glycans of BTH.

Methods

BTH source containing additional proteins was procured, and purified BTH (pBTH) was obtained using size exclusion chromatography. For N-glycan analysis, liquid chromatography (LC)-electrospray ionization (ESI)-higher energy collisional dissociation (HCD)-tandem mass spectrometry (MS/MS) were employed. The structures of all N-glycans labeled using procainamide were confirmed by MS/MS spectra showing fragment ions related to the corresponding N-glycans. The absolute N-glycan quantities were determined using fluorescence intensity and relative quantities. The proteolytic (glyco)peptides of pBTH digested with trypsin and chymotrypsin or pronase E were analyzed by nano-LC-HCD-MS/MS. The structural role of N-glycans in the enzymatic activity was investigated using turbidimetric assays. Zymography was also employed to further validate the molecular mass of proteins possessing hyaluronidase activity.

Novel Aspect

This is the first study to identify and quantify 32 N-glycans of pBTH and investigate structural roles in its activity.

Preliminary Data or Plenary Speaker Abstract

This study has identified and quantified the microheterogeneous N-glycan structures of pBTH, thereby establishing their importance for hyaluronidase activity. Using LC-ESI-HCD-MS/MS, we identified and categorized 32 distinct N-glycan structures, including five mono-sialylated (6.4%), three di-sialylated (20.6%), and four tri-sialylated (12.0%) structures, all of which are involved in improving the half-life, stability, and solubility of glycoproteins. Nine core-fucosylated structures (31.5%), believed to enhance glycoprotein flexibility, were also identified. Additionally, six terminal-galactosylated (14.6%) structures, reportedly promoting sialylation enhancement, five high-mannosylated structures (13.7%) associated with protecting the glycoprotein from degradation, and four bisecting N-acetylglucosamine (GlcNAc) structures (7.8%) implicated in various biological processes were identified. The sialylation level of N-glycans in pBTH was 24.4 times greater than that recombinant human PH-20 (rHuPH20). The presence of proteolytic peptides containing sialic acids in pBTH was also detected by nano-LC-HCD-MS/MS. The total absolute quantity of all N-glycans was calculated as 1.4 pmol per 1.0 pmol of pBTH; including sialylations, core-fucosylations, terminal galactosylations, high-mannosylations, and bisecting GlcNAc structures as 0.6, 0.4, 0.2, 0.2, and 0.1

pmol, respectively, per 1.0 pmol of pBTH. Additionally, the hyaluronidase activity of de-sialylated pBTH was reduced (to 41.2 ± 4.2%), a change comparable to that observed in fully de-glycosylated pBTH (reduction to 35.3 ± 3.3%). The negative charge of sialic acids in pBTH is considered beneficial for the optimal activity of the enzyme by involving electrostatic forces between pBTH and hyaluronan. The presence of larger amounts of sialylated N-glycans in pBTH than in human hyaluronidase suggests a greater utilization of pBTH. This is the first study to identify and quantify 32 N-glycans of pBTH, including sialylation, and investigate their structural roles in enzymatic activity. The present results improve our understanding of the structural characteristics of BTH and provide insights into the medical applications of animal-derived hyaluronidase.

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Glycoblotting-based Sulphoglycomics Reveals Waterfowl Influenza Prevalence

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Sulfated/phosphorylated glycans are important biomarkers for tissues, blood, etc., and their low abundance ratio has limited omics analysis using mass spectrometry. “Glycoblotting” is a reducing end-selective glycan capture/modification technology widely used to detect neutral/sialylated glycan biomarkers. In this study, we report the results of a correlation analysis between the sulphoglycomics method, which combines glycoblotting and weak ion exchange (WAX), and the prevalence of influenza virus using waterfowl egg white analysis.

Methods

Selective and continuous glycomics of neutral and acidic glycans were performed using a combination of Glycoblotting and WAX analysis.
We demonstrated that sulfate and phosphate groups can be distinguished by phosphatase treatment.
Multivariate analysis of large-scale sulphoglycomics in waterfowl egg whites and information on influenza incidence in waterfowl revealed a group of sulfated/phosphorylated glycans that are highly associated with influenza infection.

Novel Aspect

Development of sulphoglycomics workflow combination with glycoblotting.

Preliminary Data or Plenary Speaker Abstract

Sulfated/phosphorylated glycans are important biomarkers for tissues, blood, etc., and their low abundance ratio has limited omics analysis using mass spectrometry. “Glycoblotting” is a reducing end-selective glycan capture/modification technology widely used to detect neutral/sialylated glycan biomarkers. In this study, we report the results of a correlation analysis between the sulphoglycomics method, which combines glycoblotting and weak ion exchange (WAX), and the prevalence of influenza virus using waterfowl egg white analysis.

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Structural characterisation of biofilms from pure and co-cultures of clinical isolates of Klebseilla pneumoniae and Acinetobacter baumannii

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The formation of a biofilm is an essential facet of the bacterial life cycle. They provide protection from various environmental stresses or from combative measures released locally by other microorganisms, and from a clinical perspective are a well-documented driver of the spread of antimicrobial resistance. Biofilms are composed of a mixture of proteins, DNA and polysaccharides. Polysaccharides form the primary macromolecular constituent by mass and consist of either polysaccharides synthesised explicitly for biofilms (e.g. alginate, poly-N-acetylglucosamine) or of shed cell surface components (i.e. O- and/or capsular antigens). Many of these biomolecules feature incredible structural diversity, with variability even between related strains, and our structural knowledge of the biofilms produced by clinical isolates, or by complex bacterial communities, is exceedingly limited.

Methods

Crude biofilms were extracted following culture of K. pneumoniae, A. baumanii or co-cultures seeded in various proportions on nutrient supplemented agar. Qualitative comparisons of biofilm extracts were facilitated by gel electrophoresis and visualisation using the carbocyanine dye, Stains-All.
For structural characterisation of biofilm polysaccharides, extracts were first hydrolysed by boiling in trifluoroacetic acid. Following solvent evaporation, sugar monomers and oligosaccharides were characterised by monosaccharide analysis and liquid chromatography-tandem MS respectively. For monosaccharide analysis, sugar monomers were initially derivatised by sequential oximation and silylation. Derivatised monomers were then identified by gas chromatography-MS using a GCMS-QP2020 NX. Oligosaccharides were characterised by tandem MS using a Velos™ Pro following separation over porous graphitic carbon.

Novel Aspect

Structural characterisation of biofilms produced by clinical isolates of two prominent human pathogens using a combination of GC-MS and LC-MS.

Preliminary Data or Plenary Speaker Abstract

Here we sought to characterise the polysaccharide component of biofilms derived from clinical isolates of two ESKAPE pathogens, K. pneumoniae and A. baumannii. Initial qualitative assessment of biofilms from pure cultures using gel electrophoresis and visualisation using the carbocyanine dye, Stains-All, revealed banding patterns in both extracts were consistent with negatively charged polysaccharides. Biofilms from individual organisms were readily distinguished based on ladder size, staining hue and intensity.
To better understand the composition and structural arrangement of sugars in these biofilms, extracts were analysed by both GC-MS and LC-MS following acid hydrolysis. Biofilms obtained from K. pneumoniae were observed to have intense peaks for mannose, galactose, glucose and galacturonic acid, consistent with the predicted components of the capsular polysaccharide, when analysed by monosaccharide analysis. When analysed by LC-MS we observed multiple oligosaccharide signals with arrangements of hexoses and hexuronic acids in repeat units also consistent with previous structural characterisations of the predicted capsular type for this isolate; collectively these data suggest this K. pneumoniae isolate sheds its capsular polysaccharides to form the major polysaccharide component of its biofilms

In contrast to our findings for K. pneumoniae, A. baumannii extracts did not produce strong signals when analysed by monosaccharide analysis. When analysed by LC-MS we observed oligosaccharides with linear arrangements of heavily N-acetylated deoxyhexose and hexuronic acids in either an alternating configuration, or in repeats consistent with the previously determined capsular type for this strain. Collectively these data suggest this A. baumannii isolate produces multiple, hardy polysaccharides likely derived from its capsular biosynthetic pathway.
Analysis of biofilm extracts from co-cultures of both isolates seeded in various proportions revealed that, although these biofilms most closely resembled A. baumannii extracts, the presence of unique markers in both GC-MS and LC-MS suggest additional polysaccharides are produced during co-culture.

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Developing novel in-house packed diamond column for separation of polysaccharides based on theoretical predication

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Protein glycosylation is most abundant post-translational modification. Altered glycans modulate biological processes from the cell adhesion to host–pathogen interactions and protein trafficking. It is important for carbohydrate analysis. In this study, we applied molecular dynamics (MD) to predict adsorption capacity of polysaccharides on surface of porous graphic carbon (PGC) and diamond particles.and we also demonstrate that packed in-house diamond column coupled with liquid chromatography mass spectrometry is able to separate large polysaccharides at different temperatures and mobile phase gradients.

Methods

Diamond column (1.0mm x 100mm) is packed with 2.5µm powder (Microdiamant AG,Switzerland),and the PGC column (Thermo Hypercarb ,100*1mm, ID 3µm). The LC-MS system were controlled by Ultimate 3000 (Thermo Fisher Scientific,USA) and LTQ Velos-Pro(Thermo Fisher Scientific,USA).The mobile phase solutions consists of (A) 100%H2O/0.03%NH3OH,(B)100%MeOH,(C)100%H2O/0.1%FA and (D)100%ACN. A and B solvents are used in Diamond column,while C and D solvents are used in PGC column.Gradient runs from (i) 2% B to 80% B (ii) 2% D to 40% D, both over 30 min at a flow rate of 100µL/min and 50µL/min.MD simulations were conducted by Materials Studio (Dassault systèms, France).The COMPASS II forcefield was used in this study. Furthermore,the Andersen thermostat and Berendsen barostat were used to control temperature and pressure.

Novel Aspect

The in-house packed diamond column can be be used for the separation of polysaccharides.

Preliminary Data or Plenary Speaker Abstract

The theoretical calculation results shown binding energy is more negative on surface of PGC particle when temperature was increased. This represent polysaccharides can be retained on the stationary phase. The experimental data also demonstrated that low molecular weight of polysaccharides can well-separated in commercially available PGC column. Furthermore, diamond particle is target of theoretical prediction.According to calculation results, there is not strength binding on surface of diamond for polysaccharides after increasing temperature.Also, the experimental data showed that high molecular weight of polysaccharids can well-detected by LC and Mass spectrometer.Upon comparison with experimental results, the low binding energy may favor the retention of large polysaccharides on the surface of diamond particles. Accroding to experimental data, We also observe the diamond column exhibits optimal signal for high molecular weight of polysaccharides at 90°C because the chromatograms clearly show the singal for (Glc)11 to (Glc)17.

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De-N-glycosylation of protein samples reveals differential expression of CD36 glycoprotein in human adipogenesis

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Adipogenesis is the process of adipose-derived stem cell (ADSC) proliferation followed by differentiation into mature adipocytes. When carried out in vitro, this process encapsulates many of the changes seen in vivo, making in vitro models ideal to study the mechanisms underpinning stem cell differentiation and adipocyte biology. An integrated glycomics and proteomics approach was undertaken here to investigate specific differences between the stem cell progenitors and their in vitro and in vivo progeny.

Methods

Membrane proteins from native adipocytes, ADSCs and in vitro-differentiated ADSCs (dADSCs) from the same individual were separated using SDS-PAGE and the separated protein bands analysed by glycomic profiling and shotgun proteomics techniques. This provided the rare ability to determine a correlation between expressed proteins and their glycosylation profile. Released N-glycans were analysed using porous graphitised liquid chromatography (PGC-LC) coupled with negative ion electrospray tandem mass spectrometry (ESI-MS/MS), while peptides were analysed using reverse phase-liquid chromatography (RP-LC) coupled to ESI-MS/MS. Proteomic comparisons were also performed with and without prior in-gel de-N-glycosylation using PNGase F, and a membrane protein N-glycomic analysis done to obtain the global glycan profile across all molecular weights.

Novel Aspect

An integrated glycomics and proteomics approach via de-N-glycosylation of protein samples prior to trypsin digestion unearths key hidden glycoproteins

Preliminary Data or Plenary Speaker Abstract

The analysis of N-glycans released from adipocyte, ADSC and dADSC membrane proteins across the SDS-PAGE gel molecular weight range showed similarity to the global N-glycomes for each cell type. In the native adipocytes, three major ion signals corresponding to bisecting GlcNAc structures in the global profile were similarly prominent across all of the molecular weight sub-divisions of the separated membrane proteins and not seen in the profiles of ADSCs and dADSCs. This indicates that bisecting GlcNAc structures are present on the majority of glycoproteins expressed on adipocyte membranes. Separated ADSC membrane protein glycomic profiles contained ion signals corresponding to glycan structures also reflective of the global N-glycomic profile, while more variation was seen in the glycosylation of the dADSCs between the gel fractions and global profile. In the regions of the gel that displayed obvious differences in protein abundances, glycoproteins were specifically targeted for identification so that glycan structures could be attributed to differentially expressed glycoproteins within a defined molecular weight range. Of all the membrane glycoproteins identified, CD36 displayed the most striking differential expression across the three cell types. Only 68 total peptides were identified in the glycosylated sample whilst 580 were identified when the CD36 glycoprotein was de-N-glycosylated prior to trypsin digestion, resulting in a 41.74 % increase in sequence coverage and allowing the elucidation of CD36 as the major protein and glycoprotein in adipocytes. Western blotting and qPCR confirmed the high CD36 abundance levels in adipocytes compared to the other cell types. This work demonstrates the crucial role protein de-N-glycosylation plays in uncovering the true abundance levels of key components of the (glyco)proteome, and that in in vivo differentiation products (adipocytes) are not reflected in their in vitro counterparts

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Glyco-PASEF®: Advancing Glycoproteomic Profiling with Oxonium-Ion-based Glycan-Specific Polygon (GP) Gating and Stepped-Energy Collision-Induced Dissociation (SEC) on the timsTOF Ultra

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Characterizing and quantifying intact glycopeptides from complex samples poses challenges due to diverse glycan structures, ionization and separation behaviors, and relatively low abundance. Ion mobility (IM) devices allow rapid segregation of ions based on their collisional cross-section (CCS). Integration of IM with liquid chromatograph-mass spectrometry (LC-MS) achieves another layer of separation and has emerged as a promising approach for analyzing glycoconjugates. Glycopeptides, distinguished by their inherent ion mobility, can be isolated more effectively from non-glycosylated peptides, reducing background noise and non-glycopeptide interference. Also, different structures of glycan and peptide backbone demand different optimal fragmentation energy, facilitating informative spectra for structural characterization. These advancements permit direct, detailed elucidation of glycopeptides, eliminating the need for preliminary enrichment and thereby enhancing analytical efficiency.

Methods

Unenriched tryptic peptides from purified glycoproteins and plasma were analyzed using nanoUHPLC coupled to a timsTOF Ultra to a Parallel Accumulation Serial Fragmentation (PASEF) method previously optimized for glycopeptides in a timsTOF HT. This optimization included a glycan-specific polygon (GP) for precursor selection as well as stepped collision energy (SCE) for enhanced fragmentation. Four strategies focusing on optimization of ion transfer settings, collision energies and precursor selection were evaluated: standard PASEF, SCE-PASEF, GP-PASEF, and GP-SCE-PASEF. Analysis involved generating MGF-meta files containing precursor-intensity, CCS, and monoisotopic mass, with a focus on identifying N-glycopeptide precursors through glycan-oxonium ions in the MS/MS-spectra. Raw data were processed with MSFragger by identifying N-glycopeptide precursors through glycan-oxonium ions in the MS/MS-spectra.

Novel Aspect

Glyco-PASEF® innovatively merges IM, SCE, and oxonium-ion-gating on the timsTOF-Ultra, setting new standards in glycoproteomics for throughput, resolution, and sensitivity.

Preliminary Data or Plenary Speaker Abstract

Method optimization on the timsTOF-Pro indicate that Trapped-Ion-Mobility Spectrometry (TIMS) facilitates effective physical separation of N-glycopeptides from nonmodified peptides, thus significantly enhancing analytical depth for purified single glycoproteins and more complex samples such as plasma.
A dedicated GP in the PASEF mode, together with SCE, substantially augmented N-glycopeptide identification by effectively improving spectrum quality and maximizing the focus on analytes of interest, leading to a more efficient and effective analysis. The GP-SCE-PASEF (glyco-PASEF®) method yielded an almost 10-fold increase in unique annotated glycopeptides identified compared to the original PASEF-only approach, underscoring the performance of our integrated enhancements.
Over 500 unique N-glycopeptides were identified from plasma using the SCE-GP-PASEF method, marking a 7-fold increase over the results of PASEF without SCE. Of note, combination of glyco-polygon-filtering with SCE resulted in a 20 % and 51% increase in identified N-glycopeptides over SCE-PASEF without glyco-polygon-filtering, at longer (90min) and shorter (15min) gradients, respectively, demonstrating the advantages of the combined GP-SCE-PASEF workflow.

Application of the glyco-polygon to SCE-PASEF consistently outperformed SCE-PASEF alone, enabling either faster analysis with shorter gradients or increased analytical depth with longer gradients. The former, in particular, makes our workflow very attractive for glycoprotein biomarker discovery where large cohorts need to be analyzed.

The timsTOF-Ultra system is a highly sensitive instrument developed for in-depth analysis of low sample inputs. Based on method optimization on the timsTOF-Pro a dedicated glyco-PASEF method has been developed for the timsTOF-Ultra. Due to its speed and sensitivity, it is possible to identify glycopeptides from approx. 1/10th of the sample in 1/3rd of the gradient time.

While it is still challenging for any MS method to ascertain the unbiased “true” glycosylation profile of any complex sample, our study introduces a robust and efficient new means for rapid glycoproteomic analyses with high resolution and sensitivity.

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Glycan Analysis using a 3D-Printed Offline Nano-ESI Source for Bruker MS Instruments

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Nanoelectrospray ionization (nano-ESI) is crucial for the mass spectrometric analysis of minute analyte quantities by facilitating the direct infusion of complex mixtures. Its application extends significantly in the domain of native mass spectrometric analysis of proteins, despite the absence of a commercially available offline nano-ESI source for Bruker instruments. Addressing this gap, we introduce a novel, 3D-printable nano-ESI source compatible with Bruker's ionBooster ESI source, fostering broader accessibility and utility in mass spectrometric analysis.

Methods

We designed a cost-effective 3D-printable nano-ESI source that integrates seamlessly with Bruker mass spectrometers. We provide detailed instructions for assembly and all necessary files are available through the open platform printables.com (https://www.printables.com/model/410886-nanoesi-source-for-bruker). We demonstrate the utility of the nano-ESI source across a spectrum of samples, including small molecules and complex biological samples, by achieving high-resolution mass spectra in both positive and negative ion modes. Utilizing the Bruker timsTOF instrument, we address challenges in glycosaminoglycans (GAGs) and O-glycan analysis through trapped ion mobility-mass spectrometry (TIM-MS), significantly enhancing analytical speed and reducing complexity.

Novel Aspect

3D-printed nano-ESI source for rapid, efficient analysis of complex biological molecules on Bruker systems.

Preliminary Data or Plenary Speaker Abstract

In this study, we introduce a 3D-printable nano-ESI source for Bruker mass spectrometers, enabling precise and efficient analysis of complex biological samples. Here we focus on the analysis of two types of glycans: 1) glycosaminoglycans (GAGs) and 2) mucin-type O-glycans. These molecules play pivotal roles in a myriad of biological processes and are linked to various pathological states such as chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) or cancer. Traditional analysis of these biopolymers relies heavily on HPLC methods, either through coupling with LC-MS or via detection of fluorescently labelled derivatives, which are time-consuming and can lack reproducibility. Leveraging trapped ion mobility-mass spectrometry (TIM-MS), our approach markedly accelerates the analysis, reducing it from hours to minutes when compared to the conventional HPLC-based techniques. This rapid methodology not only streamlines the analytical process but also enhances our ability to dissect the complex structural diversity of GAGs and the dense O-glycosylation patterns of mucins. Our findings offer profound insights into the structural and functional heterogeneity of these critical biomolecules, enabling advanced research into their roles in diseases such as COPD, cancer, and cystic fibrosis. Our approach highlights the superiority of ion-mobility based techniques over traditional HPLC analysis in terms of speed and efficiency.

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Interrogating the mechanism of nano electrospray ionisation for the formation of supercharged N-glycan ions

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Glycosylation plays a crucial role in cellular processes, yet analysis of glycans often gives an incomplete picture due to the complexity of sequence, branching and linkages. Electrospray ionisation mass spectrometry (ESI-MS) is widely used for glycan analysis owing to the formation of multiply charged gas phase ions that fall within the mass range of MS. Accurate quantitation of glycans remains challenging.
One limitation is native glycans ionise inefficiently, limiting charge and subsequent MS/MS information. Mechanisms of ion formation for N-glycans are understudied, particularly in negative mode ESI. We investigated the use of supercharging additives to released N-glycans to interrogate glycan ion formation in negative mode, and assist with development of in depth analysis and quantitation of glycans.

Methods

N-glycans from bovine Fetuin and human serum IgM were prepared for MS by releasing with PNGase F, then reduced and cleaned before resuspension in 10 mM ammonium bicarbonate with 50% ACN and 5% (v/v) or 10% (v/v) supercharger compound. 15 µL of each glycan solution was loaded onto in-house-pulled nanoESI borosilicate coated emitters (1.2 µm I.D) with an exit orifice of ~200nm. NanoESI was performed by applying a voltage of ~0.9-1.2 kV on the capillary using a Select Series Cyclic IMS, and 200 scans were acquired per spectrum.
MassLynx and SkylineMS were utilized for analysis. Average charge state of glycans was calculated and differences analysed using two-way ANOVA against control solutions. This enabled comprehensive characterization of N-glycans from various glycoproteins.

Novel Aspect

Known supercharging agents were tested on MS of released N-glycans, furthering understanding of ion formation, which would assist future analysis.

Preliminary Data or Plenary Speaker Abstract

The effect of conventional supercharging agents on released and purified N-glycans was analysed via negative mode ESI. N-glycans of different structure types were directly compared, with oligomannose, neutral complex and hybrid N-glycans showing no significant changes to observed charge states for all superchargers tested (DMSO, propylene carbonate, 4-vinyl-1,3-dioxolan-2-one and 4-nitrophenol). In contrast, N-glycans which carried a core fucose and at least one terminal sialic acid were consistently observed with higher average charge states (+0.4 on average) when ionized in the presence of supercharging agents, propylene carbonate and 4-vinyl-1,3-dioxolan-2-one.
The data suggests that glycan ion formation occurs via an ion-pairing mediated H-abstraction event. During the electrospray process, ion pairs between the glycan and other charged species in the droplet are formed. This occurs preferentially on carboxylic acids found on sialic acids of complex N-glycans. At the moment of ion formation, these higher charged ion-paired glycans would be situated close to the surface of the droplet due to Coulombic repulsion, resulting in the formation of glycan ions ejected from the surface of the droplet. Further, glycans that do not contain carboxylic acids will be buried deeper within the droplet at the moment of ion formation, resulting in observed limited charge abstraction on these glycans. The data suggests that these ion-pairs mediate H-abstraction and ejection from the droplet, which is consistent with the ion evaporation model for ion formation.

Further research is needed to understand the interactions between superchargers and glycans, particularly regarding the specific acidic glycan motifs influence on supercharging efficiency. This study suggests that optimizing supercharging methods could improve observed charge states and increase information acquired in glycan MS analysis, benefiting glycan structural characterization. Future research directions include investigating other supercharging additives and physical parameters, as well as integrating supercharging into analytical workflows to enhance glycan structural analysis.

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Unveiling host-cell glycosylation changes upon parainfluenza virus infection using Mass spectrometry

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Human parainfluenza virus (HPIV) remains to be one of the major causes of respiratory illness all over the world. There were 725, 000 hospitalizations and 34,400 deaths reported in 2018 alone globally. Despite the significant efforts in designing therapeutics, there is neither an effective antiviral nor a vaccine available against HPIV. Cellular glycosylations are known to play a pivotal role in HPIV biology. Glycans like sialic acid (Neu5Ac) have been identified as cellular receptors to HPIV and utilised as molecular template for structure-based drug design. However, dynamics of the host-cell glycome upon HPIV infection has never been studied.

Methods

Herein, we profile surface glycans (N-, O-, glycosphyngolipids) decorating the cell upon HPIV infection in both immortalized and primary epithelial cells using state-of-the-art mass spectrometry techniques and instruments. Mass spectrometry has emerged as a key tool in the structural analysis of glycans due to its spectacular sensitivity, resolution, and robustness. Glycans were separated by porous graphitized carbon chromatography and analysed by ion-trap mass spectrometer in negative ion mode. The structures of oligosaccharides were identified manually based on the tandem mass spectrum with the help of Glycomod and GlycoWorkbench tools. Quantitation was the done in Skyline.

Novel Aspect

This is the first of its kind comprehensive study deciphering the glycan changes on host cells followed by infection.

Preliminary Data or Plenary Speaker Abstract

We detected approximately 200 different glycan species across all immortalized and primary nasal epithelial cells. A significantly higher expression of oligomannose type N-glycans at the surface of HPIV-infected cells when compared to mock-infected control, with correspondingly lower expression of complex type N-glycans. Furthermore, expression of doubly sialylated core-2 type O-glycans were reduced on infected cells which was compensated by increased expression of core-2 singly sialylated glycans. Unique glycosphingolipids glycosylation features were also found on HPIV-infected cells when compared to their mock-infected counterpart. Based on the findings so far, we hypothesize that distinct glycan motif on infected cells may guide immune responses leading to disease severity followed by parainfluenza infection. Thus, our study could pave the way to potentially identify novel target(s) for designing therapies to mitigate the impact of HPIV infection.

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O-GlcNAc proteomics of Parkinson's disease model cells using data-independent acquisition mass spectrometry

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Glycosylation plays a crucial role in various biological processes and diseases. Recent studies have shown that N-acetylglucosamine modification on serine and threonine residues (O-GlcNAc) may be associated with Parkinson's disease (PD). O-GlcNAc appears to inhibit the aggregation of α-synuclein implicated in PD pathogenesis. However, the precise role of O-GlcNAc in PD remains unclear. This is partly due to the challenge of analyzing O-GlcNAcylated proteins, given their low abundance, and the limitations of animal models in perfectly replicating the complexities of human diseases. Recently, data-independent acquisition mass spectrometry (DIA-MS) is emerging as a valuable technique in quantitative post-translational proteomics. This study utilizes DIA-MS for O-GlcNAc proteomics to identify O-GlcNAcylation changes specific to PD model cells generated through genome editing of iPSCs.

Methods

For spectral library generation, iPSCs (RIKEN) were used. The PD model consisted of neural progenitor cells (AXOL) harboring a familial mutation. O-GlcNAc enrichment was achieved with wheat germ agglutinin (WGA)-agarose (5 mL) and biotinylated WGA (5 µg). LC/MS/MS analysis (EASY-nLC and Q-Exactive) employed mobile phases A (0.1% FA) and B (0.1% FA/ACN) with a gradient for B of 0% to 35% in 150 min at 300 nL/min. Data-dependent acquisition mass spectrometry parameters included a scan range of m/z 350–2000, NCE of 27, and dynamic exclusion of 30.0 s for MS/MS. DIA-MS was performed with a scan range of m/z 495-–905 and NCE of 27. Centroid mode was used for data acquisition in both DDA and DIA modes.

Novel Aspect

DIA-MS facilitated O-GlcNAc proteomics of PD model cells directly generated from human iPSCs, eliminating the need for cumbersome enrichment methods.

Preliminary Data or Plenary Speaker Abstract

O-GlcNAcylated proteins from iPSCs (201B7 strain) were first enriched via affinity chromatography and batch methods employing WGA. Following desalting, the trypsin-digested samples were subjected to data-dependent acquisition mass spectrometry. Utilizing the Byonic analysis algorithm, 73 O-GlcNAcylated peptides originating from 47 distinct proteins were identified and compiled into a spectral library using Skyline analysis software. Next, we investigated a commercially available PD model: neural progenitor cells (PD-NPCs) harboring a G2019S mutation in leucine-rich repeat kinase 2 (LRRK2), a gene associated with familial PD. These PD-NPCs were further differentiated into mature neurons (PD-NCs). Employing DIA-MS alongside the constructed spectral library, we performed a comprehensive O-GlcNAc proteomic analysis of PD-NPCs, PD-NCs, wild-type NPCs (WT-NPCs), and wild-type neurons (WT-NCs). Interestingly, the analysis revealed several O-GlcNAcylated proteins, including Host cell factor 1 and Nucleosome-remodeling factor 1 BPTF. The O-GlcNAcylation levels of these proteins were higher in NC compared to NPC, suggesting a potential role in disease development. However, some of these O-GlycNAcylation decreased upon differentiation into PD-NCs, suggesting a possible regulatory mechanism. This study demonstrates the effectiveness of DIA-MS for O-GlcNAc proteomics. This method facilitates the analysis of trace amounts of O-GlcNAcylated proteins, eliminating the requirement for WGA enrichment. Interestingly, some of the identified O-GlcNAcylated proteins have established roles in transcriptional regulation[1]. To further clarify the

functional significance of O-GlcNAcylation in PD, we plan to enrich the spectral library with additional LC/MS/MS data acquired from PD neuron models.

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Decipher the intricate glycoproteins from the SARS-CoV-2 family using data-independent acquisition-proton transfer charge reduction and native top-down mass spectrometry

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Glycoproteins play a significant role in the family of coronaviruses, including those associated with SARS-CoV-2. However, glycoprotein characterization using native mass spectrometry (nMS) and native top-down MS remains challenging as its heterogeneity leading to complex spectra. This study leverages the high quadrupole mass filter coupled to proton transfer charge reduction (PTCR) on Orbitrap platforms to unravel the complexity of glycoproteins from COVID family using nMS. Top-down analysis using electron transfer dissociation (ETD) reveals the structural information as well as sequence information. In summary, combination of DIA-PTCR and native top-down enabled comprehensive characterization of the virus glycoproteins.

Methods

Human Fetuin was purchased from Sigma-Aldrich. Nucleocapsid protein (Nc), spike protein receptor-binding domain (S-protein RBD), and full-length spike protein (S-protein) were purchased from Acro Biosystems. Thermo Scientific Orbitrap Ascend mass spectrometer has been modified with a high quadrupole mass filter up to m/z 8000. The complex glycoproteins were quadrupole-isolated in narrow windows to minimize interference from various glycoform signals. Subsequently, the isolated ion packets were charge reduced in the ion trap and scanned out in the Orbitrap. Data independent acquisition-PTCR (DIA-PTCR) streamlined the stepped isolation window across a wide m/z range followed by PTCR, facilitating the capture of comprehensive MW profiles. Top-down analysis employed ETD and HCD on Ascend for de-novo sequencing and structural elucidation.

Novel Aspect

The integration of the high mass range, high quadrupole mass filter, and PTCR empowers the elucidation of glycoprotein complexity.

Preliminary Data or Plenary Speaker Abstract

Initial benchmarking of heavily glycosylated Fetuin demonstrates that a quadrupole 5-Th isolation followed by PTCR could reduce the charge from +12 to +4. The resulted signal-to-noise ratio is superior compared to ion trap isolation. We successfully separated two distinct charge envelopes originating from precursors, each representing unique PTMs. Stepping through m/z 3300-4500 with a 10-Th isolation prior to PTCR unveiled numerous glycoforms from 38 kDa to 46 kDa. Ultra-high resolution at 480K facilitated isotope resolution for each glycoform, allowing unambiguous determination of monoisotopic molecular weights. Comparing full scan and DIA-PTCR scan results indicated a tenfold increase in the number of deconvoluted peaks.
We applied the same methodology to the proteins from the COVID family including Nc, S-protein RBD and S-protein. Nc protein is arguably glycosylated depending on its leader sequence and expression system. A full MS scan of 49 kDa Nc revealed partially resolved peaks atop the elevated baseline. DIA-PTCR across the entire m/z range not only resolved the charge states and identified PTMs on the 49 kDa species but also revealed dimers near 100 kDa. Some unexpected peaks were observed ~70 kDa and ~90 kDa. Top-down unveiled the unknown N-terminal tag information and indicated the dimerization domain at C-terminus but didn't indicate any glycosylation. RBD, with mixed N- and O-glycans, displayed a broad MW distribution centered at 31.8 kDa in DIA-PTCR analysis. Cleavage of N-glycans using PNGaseF shifted the MW center to 27.6 kDa, indicating N-glycan removal. DIA-PTCR in conjunction with accurate MW measurement at resolution 480K on O-linked RBD leads to O-glycan composition assignment. Furthermore, DIA-PTCR facilitated easy determination of spike protein

monomer MW (160-200 kDa) and revealed impurities at 70 kDa and 144 kDa. Future work will delve into data analysis to explore the capabilities and limitations of glycoform assignment at the intact level.

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Full Identification of N-Glycopeptide with a New Mass Spectroscopic Method

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The biological functions of glycoproteins have been of scientific interest for many years. Although the biosynthetic pathways of N-glycosylation have been established, N-glycans that do not conform to the rules have been discovered and reported. The current methods of analysis, as well as the algorithms, are still not sufficient to deal with the great amount of variation in glycopeptides. Recently, we have developed a new mass spectrometry method, namely Logically Derived Sequence (LODES) Multistage Tandem Mass Spectrometry (MSn), which is applied to glycopeptide analysis, providing information on glycosylation sites while solving glycan structures. The N-glycosylation of bovine lactoferrin has been characterized with our new method.

Methods

Lactoferrin was digested with Proteinase K. Briefly, a reducing agent (SDS/DTT) was added to the samples followed by the alkylation reagent (iodoacetamide) before reacting with Proteinase K. The glycopeptide mixture was purified by adding a 5-fold volume of ice-cold acetone and incubating in the fridge for over 16 hours. The precipitate was collected and treated with EDC and HOBt in ethanol for esterification. The product was separated and fractionated via HPLC (C-18 column, Amide-80 column, and PGC column) followed by injection into mass spectrometry.

Novel Aspect

We determined different structures of N-glycopeptides with LODES, and we could extend this method for examining O-glycosylation as well.

Preliminary Data or Plenary Speaker Abstract

Glycopeptides after esterification treatment were analyzed using a mass spectrometer. Unlike traditional protonated precursor cleavage with HCD or EThcD for small fragments, we used CID for analysis. The doubly-charged precursor of each glycopeptide was selected with one proton and one sodium attached. After CID was applied, the selected glycopeptide was dissociated into two parts: sodiated glycan fragments and protonated peptide fragments. The glycosylation site could be found while solving the peptide sequence using the old methods, while the glycan structures could be characterized with our newly developed scheme: Logically Derived Sequence (LODES). We have used the scheme and successfully determined free oligosaccharides as well as N-glycans with no difficulty. Here, we provide an example of the characterization of a glycopeptide.

The precursor ion with m/z=1040.8 was selected with CID applied and fragments were generated. The glycan portion could be observed with fragments m/z=1742 (c-ion of Man8 N-glycan), m/z=1642 (the cross-ring fragments of Man8 N-glycan), and m/z=1540 (Man8 N-glycan with the loss of reducing GlcNAc). The fragment m/z=1540 was selected for further CID, seeking the c-ion of mannose oligosaccharides, which is m/z=1337. This m/z=1337 mannose oligosaccharide fragment is important to distinguish between isomers of different Man8 while applying CID again. The diagnostic fragments of m/z=923 and m/z=761 were used to differentiate the isomers. With the mass spectra provided by the LTQ mass spectrometer, isomers of Man8 N-glycopeptide were resolved. With the method we proposed, all the glycopeptides generated from Proteinase K were solved in the study.

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Who took my proton? Protonation isomers, and their presence in electrospray ionisation mass spectrometry.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Protonation affects the gas-phase stability of molecular cations and proton site preference often changes when an analyte transitions from solution to the gas-phase. For molecules with increasing chemical complexity and basic sites, multiple protonation sites give rise to protonation isomers with often distinct fragmentation pathways, structures, electronic transition energies, and reactivity. Protonation isomers (protomers) can confound analyte detection and at least reduce the efficiency of detection. This work aims to address the current strategies to control protonation isomer ensembles using the archetype protomer molecule—para-aminobenzoic acid—that can protonate at the amino and carboxylic acid groups. This is explored in atmospheric and ion trap pressure regimes, providing mechanistic insight behind the process of formation and solvent-mediated protonation isomer interconversion.

Methods

Para-aminobenzoic acid protomers were formed by electrospray ionization and guided to the ion trap of a ThermoFisher Scientific LTQ-XL mass spectrometer, which was modified to enable ion-molecule reactions. Protomers were mass isolated and stored (1−10000 ms) in the presence of gaseous solvents commonly used in direct infusion mass spectrometry (water, formic acid, methanol, ethanol, propanol, ammonia, acetonitrile). Following the reactions with solvent gas, protomers were re-isolated and subjected to collision-induced dissociation to produce mass spectra, which inform on the site of protonation. Plotting the fragment ion abundances over time yields kinetic rate constants, and repeating these experiments over a range of solvent gas concentrations produces second order rate constants. Quantum chemical calculations (DSD-PBE-P86/D3(BJ)) were used to support mechanistic assignments.

Novel Aspect

This work develops a holistic picture of controlling protonation isomers in mass spectrometry.

Preliminary Data or Plenary Speaker Abstract

Ion populations of electrosprayed para-aminobenzoic acid protomers consist of amino (N-protomer) and carboxylic acid (O-protomer) protonation site isomers and these are subjected to gas-phase reactions with methanol in an ion trap. During early reaction times, the para-aminobenzoic acid fragment patterns are indicative of a dominant N-protomer population and this transforms to the O-protomer population over reaction time. The kinetic rate constants for this interconversion were plotted against a range of methanol concentrations (number densities) and this exhibited a linear response. This highlights that pseudo-first-order conditions are met and a single methanol molecule is required to induce interconversion—through a vehicle mechanism, rather than a multi-molecule Grotthuss mechanism. The vehicle mechanism is supported by quantum chemical calculations and isotopic labelling experiments, which shows the vehicle mechanism barrier is accessible in room temperature experiments.
Extrapolating from this study, seven solvent systems (water, formic acid, methanol, ethanol, propanol, ammonia, acetonitrile) were investigated to gain further mechanistic insight. Reaction efficiencies (0.39−80%) for each of the solvent catalysis reactions were plotted against the calculated transition state barrier heights, which showed an inversely proportional relationship. This means the transition state barrier height predicts whether internal proton transfer occurs. The solvent proton

affinity showed a direct correlation to this barrier height and thus, solvents with larger proton affinities lower the vehicle mechanism barrier height and thus increase the reaction efficiency. The last arc explores how to control methanol-mediated proton transfer catalysis with substrate substitution across ten different protomer molecules. A range of reaction efficiencies are reported (0.01−2.12%) and the direction of proton transfer across the aromatic ring are substrate dependent. Quantum chemical calculations indicate that the interconversion barrier was inversely proportional to the reaction efficiency, and the direction of proton transfer is predicted by thermodynamics, favouring lower energy protomers.

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Investigating Fragmentation of Perfluoroalkyl Carboxylic Acid Compounds via Radical Oxygen Additive Dissociation.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The environmental impacts and human toxicity of per- and polyfluoroalkyl substances (PFAS) has caught both scientific and public attention. As such, further research into the structural properties of these compounds is particularly desirable. While mass spectrometry studies of PFAS typically leverage collision-induced dissociation (CID) to reveal structural information, recent works on neutral radical-based fragmentation of lipids have suggested that oxygen radical reactions may result in unique molecular fragmentation to CID.
Our studies of perfluoroalkyl carboxylic acids (PCA) via negative mode ESI-MS have demonstrated unique fragmentation of these compounds via oxygen addition dissociation (OAD) pathways as compared to CID fragmentation. In tandem with theoretical calculations, OAD mass spectrometry experiments are exploited to investigate the fragmentation of PCAs.

Methods

Mass spectrometry experiments were performed on a quadrupole time-of-flight Shimadzu LCMS-9050 equipped with an OAD unit. The OAD unit is composed of a spiral antenna type microwave driven electron cyclotron resonance (ECR) radical source at 2.45 GHz. Upon feeding water to the plasma source, atomic oxygen and hydroxide radicals are formed, and were introduced to ionised analytes. PCA anions were formed via ESI of a methanol and water diluted mixed PCA standard, and fragmentated via either CID or OAD. Separation of individual PCAs was achieved via LC with an elution gradient of 50-100% methanol and water at 0.4 mL/min through a C18 column.
Density functional theory (DFT) calculations were performed with Gaussian 16 at M06/6-31+g(d) level of theory.

Novel Aspect

First example of unique fragmentation of perfluoroalkyl carboxylic acids in mass spectrometry via an oxygen atom addition dissociative pathway.

Preliminary Data or Plenary Speaker Abstract

Perfluoroalkyl carboxylate anions [CnF2n-1O2]– (n = 4-8) were formed upon negative-mode electrospray ionisation of a standard of mixed PCAs. Decarboxylation to the corresponding perfluoroalkyl anions (PA) was observed readily for all PCAs. As such, in-source fragmentation allowed for pseudo-MS3 experiments of the decarboxylated anions to be performed. Both CID and OAD experiments were conducted, and unique fragmentation was observed under OAD.
MS2 experiments demonstrated the expected decarboxylation of PCA to PA, with the OAD experiments showing additional unique [CnF2n-1O2 – CO¬2 - F]– fragmentation across all examined PCAs.
Longer chain PCAs (n = 6-8) demonstrated the presence of ‘doublet’ peaks; a peak corresponding to [CnF2n-1O2 – CO¬2 - F]– as well as one -3 Da smaller. Examining PFOA (n = 8) as an example, MS2 experiments demonstrated precursor at m/z 413, as well as the decarboxylated perfluoroalkyl anion at m/z 369, [CnF2n-1O2 – CO¬2 - F]– at m/z 350, a corresponding ‘doublet’ at m/z 347, as well as a smaller mass fragment at m/z 331. Notably, the latter two peaks are of 16 Da difference, and thus we hypothesise that the lower mass ‘doublet’ peak is the result of an oxygen adduct to a smaller perfluoroalkyl fragment. As the perfluoroalkyl chain length increases, the abundance of the oxygen adduct peak increases relative to the [CnF2n-1O2 – CO¬2 - F]– peak, suggesting a preference of

oxygen addition to proceed with longer chain PAs; furthermore, oxygen adduct peaks were not observed in shorter chain PCAs (n = 4-6). PFHxA and PFHpA (n = 6, 7 respectively) exhibited the oxygen adduct peak without the smaller mass fragment.
Pseudo-MS3 experiments of the decarboxylated anions demonstrated, depending on chain length, the defluorinated product and/or oxygen adducts, suggesting that the oxygen atom addition pathways proceeded upon reaction with the decarboxylated anions.

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Development of a Mass Spectrometer Combined with low-intensity Femtosecond Laser to Observe Non-equilibrium Desorption/Ionization Process

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Femtosecond laser desorption/ionization has gained attention in the last decade as a relatively new ionization technique in mass spectrometry imaging. Most of the femtosecond laser applications in mass spectrometry are used as ionization methods by multiphoton absorption in a high electric field above the sample ablation threshold. However, to minimize damage in samples, a decrease in the laser intensity is required. Since the research on the low-intensity femtosecond laser ionization process has not yet been advanced, we have developed a time-of-flight (TOF) mass spectrometer using the femtosecond laser pulse as the ionization source to observe the non-equilibrium desorption/ionization process with extremely low-intensity laser irradiation, which may pave the way for low/non-destructive mass spectrometry imaging.

Methods

The strong ultrafast laser electric field interacts with matter: an atom or molecule will liberate electrons via tunnel ionization and become ions. The electrons are accelerated in the laser field and driven back to the ions. To explore the relationship between the low-intensity non-equilibrium laser desorption process and the laser field oscillation direction, using CsI and $CH_3NH_3PbI_3$ deposit as samples, we employed a waveplate to control the laser polarization state and designed an optical path to split the original pulse train into two for double laser pulse ionization experiments. By changing the directions of laser field propagation, we expect to prevent laser field-driven electrons from returning to the ions and neutralizing, which may improve ion yield efficiency in mass spectrometry.

Novel Aspect

We confirmed femtosecond desorption in the electric field range of low-intensity region(10^{10}~10^{11} W/cm²) and revealed its polarization-dependent desorption rate.

Preliminary Data or Plenary Speaker Abstract

For femtosecond lasers to achieve sample material transformation, the power density is typically between 10^{10} and 10^{16} W/cm², and in 10^{13}~10^{14} W/cm² region will exceed the ablation threshold and damage the sample. We have detected the ion signals by irradiating the CsI deposit with the laser (pulse duration 180fs, wavelength 800 nm, 200 kHz repetition rate) at power densities ranging from 10^{10} to 10^{11} W/cm², which almost achieves the least laser intensity required for material transformation. We observed the differences in the velocity distribution of the emission Cs+ ions with different power densities and found that the faster velocity ions could be related to the non-equilibrium electrostatic ablation process.
The ions generated by the laser pulse depart from the sample plate and accelerate by the applied voltage electrode; the oscillating electric field of the vertically polarized laser pulse has the same direction as the electrode-accelerating electric field, which will drive the electrons back to the ions and neutralize them. The horizontally polarized state is perpendicular to the electrode-accelerating electric field, causing the electrons to scatter and prevent returning to the ions. Based on low-intensity laser, we performed polarization-dependent experiments with $CH_3NH_3PbI_3$ deposit using laser pulses with intensity ranging from 15 nJ (2.4×10^{10} W/cm²) to 70 nJ (1.12×10^{11} W/cm²) under vertical, horizontal, and circular laser polarization states. For each laser intensity and polarization

condition, we obtained TOF spectra and compared ion count; we observed that all three species of positive ions CH_3+, CH_3NH_3+, and Pb+ have a better ion yield in the horizontal state, which is 1.2 times the vertical state and 2 times the circular state. These results reflect that the horizontal case prevents ion neutralizing and improves ion yield efficiency compared with the vertical case, and we are analyzing the reason for the inefficiency of the circular state.

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Real-time identification and quantitation of ethylene oxide through selective gas-phase ion-molecule chemistry

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Ethylene Oxide is a highly reactive gas with no color or odor that is used or produced in a wide range of industrial activities from drug manufacturing to food importation. The U.S. EPA has identified that ethylene oxide poses a carcinogenic risk even at trace levels (pptv). Therefore, a rapid, selective, sensitive, and versatile analysis method is needed to ensure human safety across a broad range of applications. Selected ion flow tube-mass spectrometry (SIFT-MS) is a suitable method for rapid identification and quantitation of trace gases. This work demonstrates how fundamental gas-phase ion chemistry research continues to advance analytical sensing technologies, such as SIFT-MS. The impact to different real-world applications will also be discussed.

Methods

This work utilizes a Syft Technologies Syft Tracer™ selected ion flow tube-mass spectrometer (SIFT-MS) operating with nitrogen carrier gas. Rate coefficients, product ions, and branching ratios are measured for the reaction of ethylene oxide (C_2H_4O) and the isobar acetaldehyde with eight reagent ions (H_3O+, NO+, O_2+, O–, OH–, O_2–, NO_2–, NO_2–). The experiments are supported by density functional theory (DFT) calculations of reaction enthalpies using the B3LYP method with the 6-311++G(d,p) basis set. From these results, limits of detection and quantitation are determined for the reaction of ethylene oxide and acetaldehyde with OH–. Subsequently, identification and quantitation of ethylene oxide and acetaldehyde in an air matrix are explored.

Novel Aspect

Real-time isobaric separation and quantitation of ethylene oxide and acetaldehyde is enabled through newly explored gas-phase ion chemistry and SIFT-MS.

Preliminary Data or Plenary Speaker Abstract

Rate coefficients for the reactions of ethylene oxide and acetaldehyde with H_3O+ and NO+ are reconfirmed, and the rate coefficients for the reaction with O–, OH–, O_2–, NO_2–, NO_3– are established. The reagent ions O_2–, NO_2–, NO_3– show no measurable reactivity; however, the reactions with O–, OH– are fast and produce unique and different product ions. In particular, the reaction of acetaldehyde with OH- proceeds exclusively through the proton transfer reaction pathway to produce a single product ion at m/z 43 (C_2H_3O −), whereas the major reaction with ethylene oxide proceeds through proton transfer with oxygen addition to produce a product ion at m/z 59 ($C_2H_3O_2$−). This unique product was confirmed in a separate laboratory and is supported by DFT calculations.

Further experiments demonstrate that low concentrations of ethylene oxide (<50 ppb) can be determined in the presence of acetaldehyde up to 1500 ppb, while low acetaldehyde concentrations can be measured in an ethylene oxide matrix up to 2000 ppb. Therefore, ethylene oxide and acetaldehyde are distinguishable and quantifiable in real time utilising only one reagent ion.

Additionally, the reaction with O– also produces two distinct major product ions; however, the resulting reactions are more diverse, and the applicability of the different ion chemistries will be further discussed.

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A novel matrix alkylated hydroxy chalcone for peptides analysis by MALDI-MS

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

In MALDI-MS, matrices more suitable for analytes are selected. Matrices such as alkylated trihydroxy acetophenone (ATHAP) are suitable for hydrophobic peptide analysis (1). In this study, alkylated hydroxy chalcone (AHC), a compound with alkyl chains like ATHAP, was used as a matrix, and Humanin, a hydrophobic peptide was detected as [M+H] + from outer edges of a matrix/analyte crystal spot. However, the poor crystallinity of AHC may reduce reproducibility. In order to overcome this weakness, AHC was mixed with 2, 5-dihydroxy benzoic acid (DHB). In addition, AHC with a high absorbance at 355 nm was expected to improve the sensitivity.

Methods

A MALDI-8020 (Shimadzu/Kratos, UK) was used as the MALDI-MS instrument. Mixed samples (1:1 (v/v) mixture of 1 nmol/mL aqueous solution of each) of the hydrophobic peptide humanin (MW 2686) and the hydrophilic peptide β-amyloid (1-16) (MW 1955) were analyzed. Matrix DHB and AHC solutions were mixed 10:0-0:10 (v/v) to make DHB/AHC solution, which was then mixed 1:1 (v/v) with the sample solution, and 1 μL was dropped onto the plate, dried, and measured on a MALDI-8020.

Novel Aspect

A novel matrix AHC for peptides, mixed with DHB, can improve the sensitivity of hydrophobic peptides.

Preliminary Data or Plenary Speaker Abstract

First, the mixing ratio of matrix AHC and DHB for hydrophobic and hydrophilic peptides was optimized. When DHB:AHC (9:1, v/v) was used as a matrix, hydrophobic peptide ions were detected with higher sensitivity. This result indicates that the addition of AHC to DHB improves the detection sensitivity of hydrophobic peptides.

Next, we evaluated DHB:AHC (9:1, v/v) for multiple hydrophobic peptides. Peptides with higher hydrophobicity were detected with higher sensitivity. We hypothesized that the hydrophobic interaction between the alkyl chains of the AHC and the peptide was related to the correlation between the high hydrophobicity and the high intensity of detection.

These results suggest that the mixture of DHB and AHC changed the crystal form and matrix features. It has been reported that when DHB is used as a matrix, there is a so-called "sweet spot" of crystals where the signal of the analyte is easily found, and the sweet spot can be determined by Raman spectra (2). When DHB:AHC (9:1, v/v) was used as the matrix, Raman spectra derived from the sweet spot were obtained. This indicates that DHB:AHC (9:1, v/v) has more sweet spots in crystals than only DHB. Therefore DHB:AHC (9:1, v/v) can detect peptides more sensitively than only DHB.

In addition, X-ray diffraction analysis of the crystal structure in more detail revealed that the space group changes due to the mixing of AHC, and that the hydrogen bond distance between the hydroxy groups of the DHB molecule is longer than that of only DHB. These results suggest that the mixture of AHC increases the hydrogen bond distance between DHB molecules, and the hydroxy groups available for ionization are more easily dissociated after laser irradiation.

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Curtius and Wolff Rearrangement Reactions investigated by Tandem-MS, IR Ion Spectroscopy (IRIS) and Theory

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Acyl azides as well as α-diazo carbonyl compounds are important in organic chemistry and widely used as effective precursors for isocyanates and ketenes formed upon loss of nitrogen N_2. The respective Curtius and Wolff rearrangement reactions, have been broadly investigated, both experimentally and theoretically. The nitrene and carbene intermediates as well as the labile nature of the rearrangement products, especially against hydrolysis, makes them perfect candidate reactions to be studied in the absence of solvent and on molecular level via tandem-MS in the gas phase of a quadrupole ion trap, QIT. We use a stepwise MS^2 approach to initiate N_2-loss and examine the product ions with IR ion spectroscopy, IRIS enabling selective analysis of reaction intermediates and isomeric reaction products.

Methods

Three charge-tagged acyl azides and one α-diazo carbonyl compound were synthesized for this gas-phase study via (+)ESI-MS/MS and IRIS. The precursor ions expel molecular nitrogen upon collision induced dissociation, CID in a QIT. The N_2-loss product ions were subsequently probed with IRIS. The IRIS data sets are interpreted on the basis of computed linear IR spectra of ion structures identified by theory (Density Functional Theory, DFT). The multidimensional analytical strategy is outlaid to identify nitrene besides isocyanate, or carbene besides ketene isomers produced via CID in the gas phase.

Novel Aspect

Multistep gas-phase Curtius and Wolff rearrangements with structure elucidation of reaction products by IR ion spectroscopy and theory

Preliminary Data or Plenary Speaker Abstract

A set of three acyl azides and one α-diazo carbonyl compound are synthesised having either a quinuclidine or an aromatic backbone and a remotely attached charge tag to prevent any influence of the charge on the moiety that undergoes the N_2-loss. After the phase transfer with (+)ESI the positively charged molecular ions were selected and isolated in the QIT. Subsequent collision activation triggered N_2-loss and the product ions were analyzed with IRIS. In case of the acyl azides, the IR ion spectra of the N_2-loss products document the exclusive presence of the respective isocyanates, i.e. the ultimate products of the Curtius rearrangement reaction. Similarly, the IR ion spectrum of the N2-loss product ion of the α-diazo carbonyl precursor matches the computed IR spectrum of the respective ketene derivative, the rearrangement product of the Wolff reaction. In all 4 case studies presented here, computed linear IR spectra of ion structures identified by high level DFT calculations are consistent with the recorded IRIS data, allowing confident structure identification and assignment. Depletion experiments indicate that only one tautomer is formed and present in the gas phase. The detection of the isocyanate as well as the ketene based on the IRIS study suggests that the internal energy of the precursor ions necessary for efficient release of N_2 via CID is sufficiently high for the subsequent rearrangement to the ultimate reaction products. Further investigations are underway to probe different and more gentle ways of precursor activation to get access to the labile nitrene and carbene intermediates.

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Investigation of ligand transfer mechanism during collisional activation of protein complexes in native mass spectrometry

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Native mass spectrometry (native MS) is a powerful tool to study noncovalent interactions and can monitor enzymatic reactions to inform drug design against important protein targets. Collisional activation can release subunits for multimeric complexes to dissect the contribution of each component. However, gas-phase specific protein unfolding can occur, especially under slow heating conditions. The connection between gas-phase behavior and solution structures have been investigated via collision induced unfolding (CIU) studies. But only a few reports have examined ligand bound multimeric complexes, preventing the application of native MS for more heterogeneous systems. Herein, we aim to bridge the gap by interrogating the mechanism of ligand transfer and structural rearrangement of an allosteric heterodimer nsp10/16 from SARS-CoV-2 during gas phase collisional activation.

Methods

Purified nsp10/16 proteins were buffer exchanged into 200 mM ammonium acetate using BioRad Biospin P-6 columns. Charge reduction was achieved by adding triethyl ammonium acetate to the protein solution. Static nanospray was used to ionize the proteins, using glass capillaries pulled using Sutter Instrument P-1000, and an inserted platinum wire at ~1 kV. The mass spectrometer is a Waters Synapt quadrupole ion mobility time-of-flight mass spectrometer, modified with surface induced dissociation (SID) after the mass-selective quadrupole. Gas collision was achieved using the collision induced dissociation (CID) function available in the commercial instrument. Data were analyzed in MassLynx and UniDec.

Novel Aspect

Mechanistic study bridging the knowledge gap to better connect solution phase and gas phase structures for unknown protein complexes.

Preliminary Data or Plenary Speaker Abstract

Nsp10/16 (nonstructural protein 10/16) is a methyltransferase that help SARS-Cov-2 evade the host's immune system, and is a good drug target candidate due to high conservation in coronaviruses. Nsp10 is a known allosteric regulator for nsp16. When the two forms an active heterocomplex, the nsp16 provides the binding pocket for cofactors for carrying out the methyltransferase reaction on RNA substrates. Native MS has been recently shown to capture all the monomeric and dimeric states of nsp10/16 in equilibrium. Only the active dimeric form can bind to cofactors (ChemRxiv DOI: 10.26434/chemrxiv-2023-hjnxh). Interestingly, we also noted that the two bound Zn in nsp10 monomer can transfer to nsp16 during collisional activation, which complicates the structural elucidation. The charge state and the activation method significantly affect the degree of Zn transfer. Surface collision at reduced precursor charge largely eliminated the Zn transfer.
While the metal transfer appears to be gas-phase related, the connection to the dynamics of solution phase structure cannot be excluded completely. Herein we are systematically examining the effect of collision energy, charge states, presence of cofactors on the dissociation behavior in gas collision and surface collision. We will also utilize ion mobility and electron capture dissociation to gain more structural insights on the activated proteins to understand this metal transfer mechanism, with the end goal to inform native MS of unknown and dynamic protein structures.

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Real time monitoring of Cu catalysis reaction using time resolved mass spectrometry and infrared spectroscopy.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The work presented is centered on monitoring the oxidative addition and reductive elimination processes of intricately designed 'NHC-Cu-X' complexes. We aim to identify and characterize transient Cu(III) species, shedding light on crucial mechanistic steps. This study aims to advance our comprehension of copper catalysis reaction mechanisms with time resolved mass spectrometry and infrared spectroscopy techniques. Organometallic reagents serve as indispensable catalysts in various domains including pharmaceuticals, agrochemicals, and advanced materials synthesis. The quest for more sustainable organometallic catalysts arises from the scarcity and toxicity associated with commonly used 4d and 5d metals. Given its abundance as an earth metal, copper is currently under intense scrutiny as a potential sustainable replacement catalyst for such reactions.

Methods

NHC-Cu-X complexes are being studied, where X is either a methyl group or a 2-pyridyl group. The NHC-Cu-CH3 complex was synthesised electrochemically, and the NHC-Cu-C5H5N complex was synthesised by Nucleophilic Aromatic Substitution (SNAr).
Time-resolved mass spectrometry will enable the observation of intermediates in the oxidative addition and reductive elimination processes. The experiments are conducted using a positive pressure cannula transfer method into a Bruker AmaZon Speed quadrupole ion trap mass spectrometer. Future work will couple the time resolved mass spectrometry with IR spectroscopy at the FELIX laser facility.

Novel Aspect

Using time resolved mass spectrometry in the identification of reaction intermediates for Cu catalysis reactions.

Preliminary Data or Plenary Speaker Abstract

This study aims to deepen our understanding of organometallic catalysis reaction mechanisms by integrating Infrared (IR) spectroscopy with time resolved mass spectrometry techniques and Infrared spectroscopy using FELIX-2 free electron laser. The primary objective is to elucidate crucial reaction intermediates in pharmaceutically relevant organic transformations, focusing particularly on N-Heterocyclic Carbene-copper (NHC-Cu) complexes due to their stability and catalytic reactivity.
The research aims to characterize two pivotal steps in Cu-based catalysis: oxidative addition and reductive elimination. By harnessing the reactivity of the NHC ligand, transitioning it from a mere spectator to an active participant in carbon-carbon bond formation, this study will facilitate advancements in catalyst development and mechanistic understanding. Time resolved mass spectrometry and infrared spectroscopy are being used to monitor these catalysis reactions in real time, allowing observation of reaction intermediates, with the goal of identifying and characterizing transient Cu(III) species.
This combined approach promises to enhance our comprehension of reaction mechanisms and pave the way for further advancements in organometallic catalysis.

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What's with the ozone BrO? The gas-phase reactivity and photochemistry of halo-oxide anions

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Ozone in the upper atmosphere serves to protect life on Earth from harmful short-wavelength radiation, while conversely, at lower altitudes, it can be damaging to plants and animals with negative impacts on human health. Recently measured levels of stratospheric halides (Cl, Br, I) suggest these species could be responsible for a major fraction of halide-induced ozone loss, but a paucity of knowledge concerning the relevant gas-phase chemical and photochemical reactions means that these species are not currently included in atmospheric models, limiting their predictive power. Field-based observations of gaseous halide anions (along with their oxides) further emphasize the absence of precise measurements of reaction rates and products that are required to model their impacts on global ozone budgets.

Methods

Ion-molecule reactions were conducted across several mass spectrometers, that were modified to allow the introduction of reagent gases into the ion-trapping region, to access a range of ozone concentrations across varying pressure regimes. Reagent ions were generated by electrospray ionization of halide salts or corresponding oxides ($NaClO_4$, $KBrO_3$, $NaIO_4$) with the in-source collision energy set to 75-95 V for the latter. Gas phase action spectra of halo-oxide anions were obtained at both room and cryogenic temperatures using customized equipment.

Novel Aspect

New insights into the mechanisms of reactions of halide and halo-oxide anions with ozone in the gas phase.

Preliminary Data or Plenary Speaker Abstract

Experimental observations of ion-molecule reactions reveal that the initial oxidation of bromide and iodide by ozone is intrinsically slow with second order rate constants of $(1.1 \pm 0.5) \times 10^{-12}$ and $(6.2 \pm 0.4) \times 10^{-15}$ cm³ molecule^{-1} s^{-1} at ca. 300 K, whilst oxidation of chloride was unobservable under the current conditions. For the heavier halides, the major product was BrO_3^- or IO_3^- by step-wise oxidation. All four halo-oxides were shown to react several orders of magnitude faster with ozone (ca 10^{-10} cm³ molecule^{-1} s^{-1}) than the starting halides in the order $BrO_2^- < BrO^- < IO_2^- < IO^-$ with the latter approaching the theoretical collision rate. These results are consistent with recent ab initio calculations on the iodo-oxides indicating that the first step is rate-determining, whilst the subsequent oxidation steps have lower barriers, extended here to incorporate the corresponding bromo-oxides. There is also a minor (< 1 %) reduction pathway present in all reactions of the halo-oxides with ozone, though it is up to 6% of the BrO^- reaction partitioning. The chloro-oxides show very different reactivity with ozone with oxidation, reduction, and electron transfer [e.g., $EA(O_3)$ = 2.103 eV and $EA(ClO_2^-)$ = 2.140 eV] pathways competing. The second order rate constants of ClO^- and ClO_2^- with ozone are ca 1×10^{-10} and 6×10^{-10} cm³ molecule^{-1} s^{-1}, respectively, in the opposite ordering to the heavier halo-oxides. The gas phase photoaction spectra reveal a competition between electron detachment and photodissociation of the halo-oxide anions at wavelengths within the actinic spectrum. Taken together, these data suggest a complex interplay between the chemistry and photochemistry of gas phase halide and halo-oxide anions under atmospheric conditions.

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Formation and Hydrolysis Reactions of Mononuclear Arylalkali Species: Unexpected Subsequent SN2 Reaction Involving Alkali Hydroxides.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Organoalkalis have a rich history, with Schlenk describing the first synthesis of methyllithium, ethyllithium and phenyllithium from the reaction of lithium metal with the appropriate organomercury compound in 1917. Since then other methods for their preparation have been developed, resulting in organolithiums becoming a mainstay in organic synthesis. Although there are fascinating reports of divergent modes of reactivity for organolithiums versus organosodiums, a key challenge in directly comparing their reactivity is that they can exist in various aggregation states that are solvent dependent. One approach to study the fundamental reactivity of 'naked' monomers is to study them using mass spectrometry to examine the CID reactions of the fixed charge metalated betaines [4-(CH3)3N(C6H4)CO2M]+ and their decarboxylated fixed charge arylalkalis 4-(CH3)3N(C6H4)M]+.

Methods

The mass spectrometry samples were prepared according to the following procedure. One to one equivalent of the metal salts with the ligand 4-(carboxyphenyl) trimethylammonium iodide in methanol. An Orbitrap Elite ETD linear ion trap mass spectrometer (Thermo Scientific) was employed to conduct ESI-MS experiments. Via a heated electrospray ionisation (HESI) source, samples were introduced into the mass spectrometer. Multistage mass spectrometry (MSn) with the linear ion trap was used to mass select the precursor ions with an isolation width of 1.0 m/z which then were subjected to collision-induced dissociation (CID). Additionally, Gaussian 16, was used for the full optimisation of all structures with the MN1510 Density-Functional Theory (DFT) functional and the def2-TZVP basis set.

Novel Aspect

Chemistry of metal hydroxides has been rarely explored due to the challenges of vaporising metal hydroxides and their corrosive nature.

Preliminary Data or Plenary Speaker Abstract

Using ESI-MS, the cation [4-(CH3)3N(C6H4)CO2Li]+, m/z 186, was mass selected and subjected to CID. Loss of a methyl radical to form [4-(CH3)2N(C6H4)CO2Li]+ at m/z 171 was a minor channel (eq. 1). The main fragmentation channel corresponds to formation of the aryllithium, [4-(CH3)3N(C6H4)Li]+ at m/z 142 via decarboxylation (eq. 2). The second most intense peak at m/z 136 was assigned to [4-(CH3)3N(C6H5)]+. This cation results from an ion-molecule reaction between the organometallic cation [4-(CH3)3N(C6H4)Li]+ and background water. Given the success in forming the aryllithium via the desired decarboxylation pathway, we next turned our attention to examining whether decarboxylation could occur for the cations [4-(CH3)3N(C6H4)CO2M]+, of the heavier alkali metals (M = Na, K, Rb and Cs). In all cases decarboxylation was the major fragmentation channel giving rise to the organoalkali cations. To shed further insights into the decarboxylation reactions, DFT calculations were carried out to study the formation of the organoalkali ions [4-(CH3)3N(C6H4)M]+ and the results show that transition states TS are four-centred and have a similar energy barrier range from ΔH +48.1 kcal/mol for caesium to +50.2 kcal/mol for sodium. Overall, the decarboxylation reaction is found to be endothermic and the DFT results are consistent with the mass spectrometry results. Additionally, to confirm that origin of the ion [4-(CH3)3N(C6H5)]+, IMR experiments between the arylalkali cations 4-(CH3)3N(C6H4)M]+, and background water were carried out. An examination of other minor peaks in the spectra provides a clue as to additional pathways that are part of the initial hydrolysis reaction: SN2 reaction pathways and roaming mechanisms of the metal hydroxide side product of the hydrolysis reaction.

Developing New Methods to Destroy Polyfluoroalkyl Substances

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Per- and polyfluoroalkyl substances (PFAS) are widely used for their heat, oil, stain, grease and water resistance properties. PFAS consist of carbon chains with multiple fluorine atoms, and since the carbon-fluorine bond is one of the strongest bonds, they are not easily degraded in the environment, resulting in environmental persistence and bioaccumulation. With growing concerns about PFAS contamination in water resources and agricultural products, new methods to eliminate contamination by disrupting PFAS are being actively explored. Previous work demonstrated that metal complexes can activate C-F bonds and here we use MS experiments and theoretical calculations to examine whether metal-mediated activation of head groups and C-F can be used as a means of triggering the decomposition of PFAS.

Methods

Samples were prepared by dissolving PFAS chemicals in methanol and Electrospray Ionisation Mass Spectrometry (ESI-MS) experiments were performed by Orbitrap Elite ETD linear ion trap mass spectrometer (Thermo Scientific). Samples were introduced into the mass spectrometer via a heated electrospray ionization (HESI) source at an injection rate of 5 µL/min. Mass selection of precursor ions with an isolation width of 1.0 m/z was performed using multistage mass spectrometry (MSn) with a linear ion trap, followed by collision-induced dissociation (CID). Gaussian 16 was used to perform all Density Functional Theory (DFT) calculations and all molecular geometries were optimized with the ωB97XD functional and carried out with the basis set aug-cc-pVDZ.

Novel Aspect

Metal mediated head group and C-F activation reactions are used as means to trigger PFAS decomposition.

Preliminary Data or Plenary Speaker Abstract

ESI-MS of a methanolic solution of silver trifluoromethanesulfonate gave rise to the trifluoromethanesulfonate anion, $CF_3SO_3–$, at m/z 149 and the metal complex $[CF_3SO_3AgO_3SCF_3]–$ at m/z 405. Then the anion $CF_3SO_3–$ was mass selected and subjected to CID. Four fragment ions were observed. The $SO_3–$. product ion (m/z 80) requires 110 kcal/mol to be formed via bond homolysis. Other fragment ions observed are $FSO_3–$ (m/z 99), $FSO_2–$ (m/z 83) and $CF_3O–$ (m/z 85) ions and their mechanisms of formation involve rearrangement reactions. CID of $[CF_3SO_3AgO_3SCF_3]–$ results in the major loss of $CF_3SO_3–$ and desulfonation to yield the organometallate anion $[CF_3AgO_3SCF_3]–$ (m/z 325). DFT calculations reveal that desulfonation occurs via a four-centred transition state, with an activation energy of 48.7 kcal/mol, which is slightly higher in energy than the Ag-O bond heterolysis reaction (48.4 kcal/mol) to form $CF_3SO_3–$, consistent with the experimental results. In a MS^3 experiment, $[CF_3AgO_3SCF_3]–$ was mass selected and subjected to CID. Once again the major loss is $CF_3SO_3–$. Desulfonation to produce the organometallate anion $[CF_3AgCF_3]–$ is not observed. Instead, extrusion of difluorocarbene via an alpha-elimination reaction is observed, yielding the fluoride complex $[FAgO_3SCF_3]–$ (m/z 275). Current work is examining the mechanisms and energetics of these reactions using DFT calculations and extending the work to other metal complexes, $[RSO_3MSO_3R]–$ and $[RCO_2MO_2CR]–$, where M = Cu, Ag and Au, and R = longer chain perfluoro alkyl groups.

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Discovering lactose metabolism associated breath markers using secondary electrospray ionization mass spectrometry

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Lactose malabsorption (LM), a reduced ability to digest lactose, is present in ca. 68% of the adult world population. Currently available tests suffer from limited sensitivity and specificity for assessing symptoms of LM. Moreover, not every person who is a malabsorber develops symptoms and, therefore, lactose intolerance (LI). Secondary electrospray ionization (SESI) coupled to high-resolution mass spectrometry (HRMS) is a powerful tool for the detection of volatile biomarkers in exhaled breath. Performing postprandial on-line breath analysis after consumption of lactose can generate characteristic breath metabolomic profiles, providing a holistic approach to understanding and diagnosing LM and LI. Since the metabolic response can be highly individual, inter- and intra-individual variations need to be assessed.

Methods

After a standardized dinner and overnight fasting, 3 adults underwent a lactose challenge (consumption of 25 g or 12.5 g of lactose dissolved in 150 ml water) on 3 different days. On-line postprandial breath analysis was carried out for 6h with a SESI source (Fossil Ion Tech, Spain) coupled to a Q-Exactive Plus Orbitrap mass spectrometer (Thermo Fischer Scientific, Germany). Additionally, breath was condensed at -80°C and analyzed using an Acquity UPLC system (Waters Corporation, USA) coupled to the same mass spectrometer under reverse phase and hydrophilic interaction conditions. Furthermore, the hydrogen concentration in breath was determined using a QuinTron BreathTracker SC (Quintron Instrument Company, USA) and symptoms were assessed using the adult carbohydrate perception questionnaire.

Novel Aspect

Using SESI-HRMS for exhaled breath analysis to discover biomarkers in breath related to lactose metabolism.

Preliminary Data or Plenary Speaker Abstract

Using SESI-HRMS on-line breath analysis, features that increased in intensity after the consumption of lactose were detected in both positive and negative ion mode. Differences in the metabolic response between individuals, but also within the same individual on separate intervention days were found. Tentative chemical formula annotation was performed using the m/z values obtained and heuristic algorithms. Compound identification was facilitated by UPLC-MS analysis of collected breath condensate and employing spectral libraries. The rise of hydrogen concentration in breath was found to be dependent on the dosage of lactose. We found that a minimum amount of lactose needs to be consumed before detecting an increase in hydrogen concentration and also the onset of symptoms. At a fixed dose, the response of the same individual still shows great variation. These preliminary results will aid a larger clinical intervention study with 120 participants with a targeted feature analysis within the detected breath metabolic profiles.

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Detection of estrogens and estrogen-like endocrine disruptors in human prostatic tissue

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Endocrine disruptors (EDs) are widespread substances found in both the environment and everyday products, that interfere with the hormonal system. Increasing evidence indicates their harmful impact on various bodily functions, particularly affecting the reproductive system and prostate due to their (anti)estrogenic or antiandrogenic properties. Given that EDs can directly affect steroid hormone functions within tissues, assessing the levels of EDs within the prostate together with steroid hormones could be of significant importance.

Methods

The aim of this study was to develop and validate a method for determining estrogens, various groups of EDs (bisphenols, parabens, oxybenzone and nonylphenol) and phytoestrogens in their unconjugated and conjugated forms in prostate tissue by liquid chromatography-tandem mass spectrometry, and subsequently analyze 20 human prostate tissue samples. The method enabled 20 compounds to be analyzed: estrogens (estrone, estradiol, estriol), bisphenols (bisphenol A- BPA, BPS, BPF, BPAF, BPAP, BPZ, BPP), parabens (methyl-, ethyl-, propyl-, butyl-, benzyl- paraben), oxybenzone, nonylphenol and phytoestrogens (daidzein, genistein, equol) with LLOQs between 0.017-2.86 pg/mg of tissue. Detection of the analytes was performed on an QTRAP6500+ mass spectrometer (Sciex, Concord, Canada), with an electrospray ionization (ESI) probe operating in negative ionization mode.

Novel Aspect

To the best of our knowledge, this is the first study detecting EDs, phytoestrogens and estriol conjugate in the prostate.

Preliminary Data or Plenary Speaker Abstract

The most frequently detected EDs in prostate tissues were propylparaben (conjugated and unconjugated forms in 100% of tissues), methylparaben (unconjugated in 45% and conjugated in 100%), ethylparaben (unconjugated in 25% and conjugated in 100%), BPA (unconjugated in 35% and conjugated in 60%) and oxybenzone (both forms in 45%).

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Comprehensive Steroid Profiling: Simultaneous Quantification of 32 Steroids in Human Plasma Using LC-MS/MS

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Steroid hormones play pivotal roles in various physiological functions, including reproductive health, immune responses, and endocrine regulation. Analyzing them via mass spectrometry methods often requires derivatization steps to enhance separation and ionization. Moreover, different steroid groups necessitate distinct derivatization approaches, complicating comprehensive analysis. If analyzed without derivatization, it often limits the analysis to certain groups of steroids. Hence, there's a need for a robust method capable of quantifying the entire steroid metabolome. However, the development of such a method is complicated due to the necessity to choose a suitable compromise between the number of analytes, the sensitivity of the method and the complexity of the preanalytical phase.

Methods

In this study, a highly sensitive LC-MS/MS method for the determination of 32 steroid hormones of the C18-, C19-, C21- family in human plasma was developed and validated. Twenty-two of them were analyzed directly without the need for derivatization, while ten were derivatized with 2-fluoro-1-methylpyridinium p-toluenesulfonate. The steroids were separated on a C18 column with a gradient elution consisting of methanol and water with the addition of
0.1% formic acid. The mass spectrometer was operated in positive electrospray ionization mode.

Novel Aspect

This sensitive method is now used for exploring the mechanisms of a broad spectra of steroid-related diseases.

Preliminary Data or Plenary Speaker Abstract

Validation demonstrated that the method was applicable for the quantitative analysis of two C18-steroids (estrone, estradiol), nineteen C19- steroids (testosterone, epitestosterone, dihydrotestosterone, 11-ketodihydrotestosterone, 11β-hydroxyandrostenedione, 11βhydroxytestosterone, 11-ketotestosterone, dehydroepiandrosterone, 7α-hydroxydehydroepiandrosterone, 7βhydroxydehydroepiandrosterone, 7-ketodehydroepiandrosterone, androsterone, epiandrosterone, androstenedione, androstenediol, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, 5β-androstane-3α,17β-diol, 5β-androstane3β,17β-diol), and eleven C21- steroids (cortisol, 21-deoxycortisol, 11-deoxycortisol, cortisone, corticosterone, 11- deoxycorticosterone, pregnenolone, 17-hydroxypregnenolone, progesterone, 17-hydroxyprogesterone, 5α-dihydroprogesterone). The lower limits of quantification are appropriate for analyses in both physiological and various pathophysiological conditions.

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Metabolic Analysis of the Effects of Heavy Metals on the Antioxidant Activity of Vitamin D in Human Plasma

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Vitamin D (VD) has various positive effects on the body, including the activation of the immune system and the antioxidant pathway. Heavy metals (HMs) that are known to disrupt VD metabolism can limit VD function. VD has also been reported to stimulate HMs absorption. No studies have investigated the impact of HMs on VD activity from a metabolomics perspective, despite the fact that there are a variety of opinions on how exposure to HMs affects VD activity. Therefore, in this study, we identified the endogenous metabolites altered by the effect of HMs exposure on VD activity and investigated their networks.

Methods

Using ultra-high performance liquid chromatography and quadrupole time-of-flight/mass spectrometry, 46 plasma samples were analyzed. Untargeted metabolic profiling was performed using two groups: one with relative severe VD deficiency and low level of HMs (lead, cadmium, mercury, and arsenic) (SVDD-low HM), and the other with VD deficiency and high level of HMs (VDD-high HM).

Novel Aspect

The identified metabolites appear to support the activation of the antioxidant pathway of VD when HMs exposure is high.

Preliminary Data or Plenary Speaker Abstract

Six metabolites were putatively identified: docosahexaenoic acid (DHA), phosphocholine (PC) 38:8, PC 36:5, bilirubin, eicosapentaenoic acid (EPA) and diacylglycerol (DG) 33:3. Five metabolites were increased in VDD-high HM compared to SVDD-low HM, and only DG 33:3 was decreased. The bilirubin, EPA, and DHA, known as antioxidants, are increased by the antioxidant pathway of VD, which is further activated as a defense mechanism against increased oxidative stress caused by HMs. Also, increased EPA and DHA promote the synthesis of DG and PC. In receiver operating characteristic curve analysis, DHA was exhibited an area under the curve of 0.826 with an accuracy of 76.8%. This suggested that DHA is the most suitable for predicting VD activity by HMs exposure.

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Multi-Omics for Plasma: A Three-in-One End-to-End Automated Sample Preparation and LC/MS Metabolomics, Lipidomics, and Proteomics Workflow

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The breadth of biological insights obtained in an LC/MS study is increased by multi-omic analyses, which can inform therapeutic modes of action, off-target effects, and correlations between treatment dosing and efficacy or toxicity. Comprehensive omics analyses including metabolomics, lipidomics and proteomics require extraction, separation, and detection of all three compound classes from individual samples. We deployed a novel automated sample preparation workflow that extracts metabolites, lipids, and proteins from single 20 µL plasma samples. Each biomolecule fraction was analyzed on the same LC/TQ instrument using tested, robust transitions to switch between analysis of each compound class.

Methods

The automated sample extraction was accomplished using a liquid handler, novel automation protocols, and commercial accessories, including a lipid-binding solid phase extraction plate. For analysis, a standardized Bio LC was coupled with an ion funnel LC/TQ and easily transferrable metabolite, lipid and protein methods. Metabolomics analysis entails a HILIC separation with 500+ metabolites in positive and negative mode. Lipids and proteins use C18 separation with databases of 763 lipids and 375 peptides, respectively. These methods can be customized to add as many overlapping transitions as desired up to a limit of 0.5 ms dwell time. In this study, techniques for switching between the metabolite, lipid and protein LC/MS methods were optimized, enabling multi-omics analyses on a single LC/TQ.

Novel Aspect

Multi-omics workflow for biological insights: metabolite, lipid, and peptide fractionation from 20µL plasma samples and analysis on a single LC/TQ.

Preliminary Data or Plenary Speaker Abstract

Combining the three omics analyses onto one LC/MS system was successful. We transitioned the LC/MS from HILIC for polar metabolites to peptides then lipids and back to HILIC without any performance issues in terms of retention time shifts or sensitivity losses. The databases were enabled to measure as many analytes as possible. dMRM methods pushed the dwell times low for each injection but this LC/MS maintained stable measurement; as an example, cis-aconitic acid had an RSD of 1.7% at 0.5 ms dwell time.
The HILIC database was used to create a custom method of 437 metabolites. Lipid analysis was completed with C18 separation with 763 plasma relevant lipids, and MRM Proteomics kits cover 375 peptide biomarkers in mouse plasma. The fully developed workflows allow researchers to collect relevant molecular scale data fast, enabling deeper biological insights without rigorous setup. In a reproducibility analysis of a subset of metabolites, 78% of compounds exhibited %RSDs <5% and 95% of compounds exhibited %RSDs <11%, indicating excellent reproducibility across the end-to-end workflow. Targeted lipidomics detected 615 lipids across a broad range of lipid classes. A lipid reproducibility study with a spike-in of 2H-labeled lipids found 100% of 2H-lipids exhibited a %RSD ≤14%. 99% of the peptides were detected <20% RSD in QC samples. In summary, the 3-in-1 LC/MS omics workflow with automated sample preparation offers reliable results from small plasma volumes with great coverage and reproducibility for metabolites, lipids and proteins. This flexible

workflow enables collection and analysis of any combination of metabolite, lipid and protein sample fractions, depending on which biomolecules are of interest for a given study.

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Routine, rapid and high coverage quantification of tryptophan and redox pathway analytes

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The human gut microbial metabolism plays a critical role in various biological functions including nutrient absorption, homeostatic maintenance and redox balance, intracellular signaling, and the regulation of immune functions. Metabolites produced by gut microbiota mediated transformation of tryptophan (via indole, serotonin, and kynurenine metabolic pathways) are important to regulate various physiological processes like protein interactions and neurotransmission. On the other hand, redox balance in the gut relies on normal metabolism of tryptophan catabolites. The dysregulation of these gut metabolites may result in various diseases such as cardiovascular diseases, neurodegenerative diseases, and cancer. Thus, it demands development of a fast and reproducible method for quantification of tryptophan and redox metabolites with comprehensive coverage.

Methods

A fast and sensitive method for the targeted analysis of tryptophan catabolites and redox pathway metabolites has been developed on a pentabromobenzyl (PBr) column with reversed-phase conditions. Assays operate in gradient elution mode using water and methanol (both containing 0.2% formic acid) as mobile phase composition on an ultra-high-performance liquid chromatography-triple quadrupole mass spectrometry (UHPLC-MS/MS) with dynamic multiple reaction monitoring (dMRM). The method uses Skyline for small molecules open-source software for the post-acquisition data processing and quantification based upon stable isotopic dilution.

Novel Aspect

Greater analyte coverage and shorter runtime for routine quantification of tryptophan and redox analytes in human serum and plasma.

Preliminary Data or Plenary Speaker Abstract

Existing methods are typically based on hydrophilic interaction liquid chromatography (HILIC) separations due to the polar nature of tryptophan and redox metabolites. However, the mobile phase compositions common for HILIC, e.g., high initial organic concentrations lead to poor solubility of highly polar analytes, challenging sample loading and tending towards variable retention and peak shapes. The high specificities of HILIC stationary phases also means that achieving reproducible interactions over time is challenging and batch-to-batch variability greater than reversed phase separations whilst overall analyte coverage is reduced. The developed method uses PBr column with dispersion force and hydrophobic interactions, which enables faster elution of ~45 metabolites in 5 minutes run time with gradient mode.

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A quantitative targeted GC-MS approach to characterize lactose malabsorption

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Approximately 68% of the world population has a reduced ability to digest lactose in adulthood. The genetic and hydrogen breath tests currently in use for assessing symptoms of lactose malabsorption (LM) exhibit limited sensitivity and specificity. In a randomized controlled intervention study (Lactobreath) involving 120 participants undergoing a lactose challenge followed by on-line breath analysis, intestinal gas monitoring via the Atmo® Gas Capsule, and the assessment of gastrointestinal (GI) symptoms, we aim to identify distinct metabolic breath profiles indicative of LM symptoms. To validate these breath profiles, we will also quantify lactose and lactose-derived metabolites in urine. Excreted levels of lactose, galactose, galactitol, and galactonate provide insights into the digestive pathways stimulated by lactose consumption.

Methods

A quantitative targeted approach was developed. Calibration curves were obtained from fasting urine spiked with pure standards of lactose, galactose, galactitol and galactonate. Samples underwent a two-step derivatization procedure before injection in triplicate into a GC-MS 8890/5975 (Agilent Technologies). Helium carrier gas was used at 0.9ml/min, and electron ionization at 70 eV. The temperature program began at 60˚C for 2 minutes, increased to 160˚C at 5˚C/min and finally to 300˚C at 10˚C/min for 36 minutes. The mass range covered was from 28.6 to 600 m/z. Quadrupole analyzer and source temperatures were set to 230˚C and 150˚C, respectively.
Absolute quantities were then determined in urine samples collected from two healthy adults before and up to six hours after a 25g-lactose challenge.

Novel Aspect

Quantitative assessment of lactose and its metabolites in urine after lactose consumption using gas chromatography coupled to mass spectrometry.

Preliminary Data or Plenary Speaker Abstract

Calibration curves were built within the ranges of 0 to 4 µg/100µL for lactose and galactonate, within a range of 0 to 3 µg/100µL for galactose and a range of 0 to 0.4 µg/100µL for galactitol. The signals for these four compounds increased linearly within these ranges (lactose: R2 =0.976 [±0.223]; galactose: R2 =0.999 [±0.0278], galactitol: R2 =0.969 [±0.0245]; and galactonate: R2 =0.998 [±0.0635]). The relative coefficient of variation of triplicate measurements ranged from 0.02% to 9% and was below 5% for the majority (75%) of triplicate measurements. Using this method, we were able to successfully quantify lactose and lactose-derived metabolites in baseline and postprandial samples after a lactose challenge. These results confirmed the efficacy and repeatability of the approach, which could potentially enhance our comprehension of the variability in digestive pathways in individuals with LM.

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A comprehensive method to quantify underivatized acylcarnitines and carnitine intermediates using liquid chromatography and ion funnel triple quadrupole

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Acylcarnitine profile analysis is a commonly requested analysis in genetic disease research laboratories due to their wide metabolic involvement. Acylcarnitines detection can be important markers for numerous disorders as they are intermediates of fatty acid oxidation and amino acids metabolism in tissues and body fluids. Currently, acylcarnitines are often tested using flow injection analysis, which compromises their quantification because various species occur as isomers and/or have very low concentrations. In order to address many issues inherent to such analysis, a new comprehensive LC-MS/MS method was developed to quantify 32 acylcarnitine species, with acyl-chain lengths from C0 to C20 in plasma samples without derivatization step.

Methods

Acylcarnitines were extracted using acetonitrile acidified with formic acid to precipitate proteins. After mixing and centrifuging, samples were diluted and analyzed on a triple quadrupole mass spectrometer coupled to a biocompatible LC system, which is coated with an iron free alloy. Optimal source conditions and MRM transitions for all compounds were determined individually using enhanced optimization software. Robust chromatography separation for the main isomers compounds was achieved without ion pairing reagents. A gradient elution was obtained using a solution of methanol:isopropanol:water (B) and water (A) both phases containing 5mM of Ammonium acetate and 0.1% formic acid. Absolute quantification was obtained using commercial labeled compounds spiked into samples. Relative response calibration curves were made for quantitative analysis of each analyte.

Novel Aspect

New sensitive method developed to quantify acylcarnitines using a wide acyl-chain length without derivatization in a standardized metabolomics system.

Preliminary Data or Plenary Speaker Abstract

The method achieved the desired separations of the main isomers, such as: (C5) isovaleryl, valeryl and 2-methylbutyryl-L-carnitine; (C4) butytryl and isobutytryl-L-carnitine and (C6DC) 3-methylglutaryl and Adipoyl-L-carnitine without ion pairing additives, which can cause ion suppression. This separation used a standardized configuration intended for discovery analysis and workflow to allow flexibility for different studies. The chromatography was reproducible, and the figures of merit evaluated for each analyte were linearity, detection and quantification limits, recovery, and precision. Simple sample preparation was obtained avoiding any derivatization. Protein precipitation is well known for its efficiency in extracting metabolites from plasma and reduces processing time. Method reproducibility showed RSD < 10%. Limits of quantification ranged from 0.01 to 0.5 ng/L for all metabolites, except lineoyl-L-carnitine which presented 5 ng/L. Limits of detection ranged from 0.005 to 1 ppb for most compounds. All the compounds showed a great linearity ($r^2 > 0.99$) and RSD below 15%. This data shows the method is capable of quantifying acylcarnitines and carnitines intermediates at levels relevant to plasma for monitoring metabolic alterations. Compared with literature and previous data, the ion funnel LC/TQ contributes to the analyte sensitivity and reproducibility. The method developed on the standardized system can be easily transferred and applied to perform different experiments from pathway discovery metabolomics to absolute quantitation.

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Nitrilotriacetic Acid Affinity Probe-based mass spectrometry for Rapid Enrichment and Profiling of Cellular Porphyrin

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Porphyrins are vital for heme synthesis, crucial for functions like oxygen transport and electron transfer. Disruptions in heme biosynthesis can lead to intermediate porphyrin accumulation, serving as biomarkers for diseases like porphyria and cancer. Conventional methods like HPLC face challenges due to overlapping absorbance maxima. We propose a novel approach integrating IMAC with NTA@MNP for efficient porphyrin purification and enrichment. Fe3+-activated NTA@MNP aids porphyrin identification via MALDI-TOF MS. Further refinement involves LC-ESI-MS for separating porphyrin species from cancer cell lines. Our method identifies 12 putative porphyrin metabolites alongside PPIX in the PC9 cell line. This innovative technique offers a potent tool for comprehensive porphyrin metabolomics analysis in biological samples, aiding in the study of porphyrin-related diseases.

Methods

Methods
The NTA@MNPs were first activated with Fe3+ ion by adding FeCl3 as an iron source in the presence of ammonium acetate to provide a high affinity for the enrichment of porphyrins. The activated Fe3+-NTA@MNP was magnetically separated and incubated with an endogenous porphyrins solution which was extracted from PC9 cell lines after incubation with the 5-ALA prodrugs to form a porphyrin and NTA@MNP complex. Then, the porphyrins were eluted by adding 50 mM Ethylenediaminetetraacetic acid (EDTA) (pH = 10) and vortex for 30 min. The eluted solution was separated from free particles using a magnet and followed by drying step by speed vac. Finally, the samples were reconstituted and direct injection into the LC-MS/MS instrument for further structural analysis.

Novel Aspect

NTA-conjugated MNP affinity-based mass spectrometry noble method for the rapid enrichment and identification of endogenous porphyrins to achieve efficient profiling.

Preliminary Data or Plenary Speaker Abstract

Preliminary findings indicate that in IMAC, the coordination and binding strength between NTA, metal ions, and negatively charged analytes, crucially influenced by metal ions and environmental pH, impact detection sensitivity and specificity. Metal selection for NTA@MNP activation was assessed, with Ti4+, Ni2+, Cu2+, and Fe3+ evaluated using a protoporphyrin IX (PPIX) standard solution. Based on PPIX ion intensity, ferric ion was chosen to activate NTA@MNP. To optimize nanoprobe performance, the influence of salts on simultaneous porphyrin extraction and detection was investigated, with ionic salts added to stabilize the Fe-NTA complex. Media containing ammonium acetate showed superior enrichment and ionization of protoporphyrin. Following optimization, the enrichment protocol's performance was evaluated, including matrix effect, recovery, and processing efficiency. Extraction yields, determined by comparing analyte intensity among direct spot, post-spiking, and pre-spiking of standards onto the enrichment protocol, were nearly 100% and 80% for protoporphyrin and coproporphyrin, respectively, indicating NTA@MNP

enrichment as a viable single sample purification strategy for hydrophobic and hydrophilic porphyrin types. Further analysis involved spiking PPIX into PC9 cancer cell lysate, with one sample enriched using Fe3+-NTA@MNP and the other processed without enrichment. Mass spectrometry revealed PPIX detection only post-enrichment, highlighting the necessity of enrichment due to low porphyrin concentration and the presence of other biomolecules and salts causing ion suppression in the mass spectra.

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Molecular Targets Program: Drug Discovery Using Native Mass Spectrometry

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Target identification is crucial for rational drug design and is a current bottleneck for advancing bioactive compounds through the discovery pipeline. This research uses the power of native mass spectrometry (native MS) to establish and validate a disruptive new platform to elucidate targets of bioactive compounds by direct detection of protein-small molecule binding. The approach accelerates current target identification as there is no need to modify bioactive compounds or proteins to achieve this outcome.

Methods

Native mass spectrometry (native MS) relies on non-denaturing electrospray- ionization (ESI) to recognize multi-charged proteins in their near-native states. It is a label-free, fast, and accurate method that permits the direct observation of non-covalent and covalent protein-ligand complexes. Collision induced affinity selection mass spectrometry (CIAS-MS) is a method developed by the group to identify a ligand binding to proteins. Whereas native MS detects protein-ligand complexes, CIAS-MS only detects bound ligand(s). The advantage of this method is that it does not require the protein to be visible under MS conditions. This is particularly useful for studying challenging proteins which have poor visibility under native MS conditions, such as membrane proteins, as well as complex protein mixtures such as cell lysates.

Novel Aspect

A novel workflow by combining CIAS-MS and native MS enables unbiased target identification, applicable to any small molecule.

Preliminary Data or Plenary Speaker Abstract

Over the last 5 years, our group has advanced the application of native MS and CIAS-MS from measuring binding interactions of individual small molecules (ligands) with proteins in their native state, to screening libraries of compounds and compound mixtures, to protein mixtures, and ternary protein-ligand-protein molecular glue type complexes. Our outcomes which involved collaborations with disease experts in academia and industry include identification of new cancer, malaria, neurodegenerative diseases, tuberculosis and COVID-19 therapeutic candidates. The current focus lies on further refining our platform to probe increasingly complex mixtures of proteins using known small molecule drugs and bioactive compounds identified from phenotypic assays where the target protein is unknown. The ultimate aim is to precisely and directly identify the target protein for every small molecule drug candidate within disease-relevant cell lysates. This ongoing development holds immense promise for advancing drug discovery and understanding disease mechanisms.

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Deciphering the microbiome: a targeted LC/MS/MS method for the comprehensive analysis of bile acids in biological samples

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Bile acids (BAs) play pivotal roles in the digestion and absorption of lipids and lipid-soluble vitamins. Primary BAs are synthesized in the liver and stored in the gallbladder and secreted into the duodenum. 95% of BAs are reabsorbed in the terminal ileum, and the remaining 5% is subjected to a series of structural modifications by the gut microbes leading to a series of related metabolites termed secondary bile acids. Although the functions of secondary BAs remain elusive, emerging research shows they make important contribution to immune regulation, carcinogenesis, and tumor progression. LC-MS and sample preparation methodologies that are highly curated and robust offer opportunities to expand our comprehension of bile acid dynamics and their implications in both health and disease.

Methods

We have developed a novel LC/MS/MS method for the analysis of 70 unique BAs in plasma, serum, and fecal samples. The LC/MS system consisted of an Agilent 1290 Infinity II Bio UHPLC with the Agilent Standardized Omics LC configuration and an Agilent 6495D triple quadrupole mass spectrometer with the latest iFunnel technology. Optimal MS ESI source and compound MRM acquisition parameters were achieved using authentic standards with Agilent MassHunter 12.1 acquisition software. BAs were extracted with the Bravo automated liquid handler platform and separated by reverse-phase liquid chromatography. Raw MS data was acquired with fast polarity switching and processed with MassHunter Quantitative Analysis 12.1 software to quantify levels of bile acids in the samples.

Novel Aspect

Novel LC/MS/MS method for the targeted analysis of 70 unique bile acids in relevant matrices with a single chromatographic run.

Preliminary Data or Plenary Speaker Abstract

Our LC/MS/MS method allowed the measurement of biologically relevant BAs in plasma, serum, and fecal samples, highlighting its applicability in microbiome studies. Nearly all isobaric and isomeric BAs in the panel were separated at base-peak level in a single chromatographic run. The method exhibited excellent reproducibility and robustness when applied to sample matrices. The implementation of acidic extraction conditions and Captiva EMR-lipid plates in the LC/MS sample preparation protocol allowed simultaneous removal of proteins and interfering lipids from the samples. Efficient BAs extraction from biological samples was achieved, with an extraction recovery of approximately 85% and consistently low relative standard deviations (RSDs) below 5% across samples. This optimized extraction protocol significantly enhanced instrument sensitivity for the detection of low-level BAs. Accurate quantification of BAs was achieved over a wide concentration range, spanning from low nanomolar to micromolar levels, with a remarkable limit of detection in the picomolar range. Importantly, the sample preparation protocol is amenable to automation, ensuring not only higher accuracy but also increased throughput. This comprehensive approach addresses the challenges associated with isomeric and isobaric bile acid analysis in complex biological matrices, providing a robust and sensitive method applicable to diverse research and clinical settings.

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Employing Machine Learning for the Peak Quality Assessment in Untargeted Metabolomics Data

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Metabolomics is a study that investigates the complete set of small molecules within a biological system. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a powerful technique used in metabolomics to identify and quantify small molecules with high sensitivity and precision. Several software tools have been proposed for metabolite quantitation from LC-MS/MS-based metabolomics data, but only few tools focus on peak quality evaluation. Thus, we conducted an analysis of peak quality assessment, employing nine metrics and five machine learning algorithms, aiming to identify an appropriate algorithm and key metrics for evaluating peak quality.

Methods

In our peak quality assessment analysis, we integrated nine metrics, including aspects such as Shapiro-Wilk (SW) statistic test, intensity slope, signal-to-noise ratio (SNR), sharpness, peak significance level, symmetry, triangle peak area similarity ratio (TPASR), zig-zag index, and apex boundary ratio, along with five machine learning algorithms, including logistic regression, support vector machine, AdaBoost, random forest, and multilayer perceptron. We utilize accuracy and the area under curve (AUC) as the measurements for performance evaluation. Accuracy measures the ratio of correctly classified instances to the total instances. AUC, on the other hand, evaluates the model's ability to distinguish between two classes by analyzing the area under the receiver operating characteristic curve (ROC).

Novel Aspect

An innovative automated peak quality evaluation integrating nine quality assessment metrics and five machine learning algorithms.

Preliminary Data or Plenary Speaker Abstract

We conducted the analysis using an in-house metabolomics dataset, comprising 360 LC-MS/MS-based samples, each pooled with human serum and ten internal standards. The 360 LC-MS/MS samples were first converted into mzML file format using ProteomeWizard, and quantified using MS-Picker and MS-Aligner, generating a feature table with detected metabolite features in rows and samples in columns. We then categorized these features into "good" or "bad" based on manual assessment. A feature was considered "good" if it garnered at least six positive evaluations across the nine metrics; conversely, receiving fewer than three positive evaluations relegated a feature to the "bad" category. The criteria for these evaluations included considerations like SW values below 0.05, intensity slope exceeding 466.45, SNR greater than 1.97, sharpness over 9.03, peak significance level above 8.45, symmetry higher than 0.87, TPASR lower than 0.27, zig-zag index under 0.03, and apex boundary ratio below 0.08. Unlabeled peaks were excluded from consideration. Next, we applied the five machine learning models, using a five-fold cross-validation approach. The outcomes indicated that logistic regression achieved an accuracy of 0.96 and an AUC of 0.98. Random forest models excelled with an accuracy and AUC of 0.99. Support vector machine posted results of 0.97 in accuracy and 0.98 in AUC, while both AdaBoost and multilayer perceptron reached an accuracy and AUC of 0.98. The computational times for these algorithms were recorded as 0.62 seconds for logistic regression, 18.85 seconds for random forest, 495.47 seconds for support vector machine, 98.69 seconds for AdaBoost, and 176.13 seconds for multilayer perceptron, highlighting random forest as the top performer among the evaluated algorithms. We also conducted an analysis of nine metrics utilizing logistic regression, AdaBoost and random forest, and observed that the apex boundary ratio, zig-zag index, and peak significance level as crucial metrics for assessing peak quality.

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A system suitability scheme for assessment of longitudinal LC-TIMS-MS performance to promote reproducibility in omics sciences

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Reproducibility is a cornerstone of all scientific areas, yet it is a challenging one to implement in discovery “omics” sciences owing to the large number of variables being measured and the focus on not restricting measurements to known chemistry. LC-MS instruments are powerful bioanalytical tools for the selective measurement of metabolite and lipid profiles in complex biological samples. To ensure accuracy and comparability of the data produced, the consistency of their performance over time must be routinely established. In this study, we present the development of a complete solution for LC-TIMS-MS suitability testing and longitudinal monitoring based on a mixture of synthetic reference standards, standard methods and automatic processing and reporting of data.

Methods

We developed a software-guided solution for data acquisition, processing and reporting that uses a mixture of eight synthetic reference standards to monitor data quality metrics. For chromatographic separation, fast gradients were used for lipidomics and metabolomics applications (C8-/C18-based) with a goal to quickly qualify the system. A targeted prm-PASEF MS/MS acquisition mode in positive and negative ionization was used to acquire. Data from different labs from China, Japan, the USA and Germany was collected, and the overall data reproducibility was investigated manually as well as via an online repository for long-term surveillance of system wellness (Bruker TwinScape). Investigated parameters such as LC peak shapes and areas or retention time accuracy, exact masses and Collisional Cross Section (CCS) values.

Novel Aspect

The poster shows a streamlined workflow to survey the actual state as well as the long-term performance of LC-TIMS-MS devices.

Preliminary Data or Plenary Speaker Abstract

System suitability assessment was performed in a cross-lab study using an Agilent 1290 coupled to a timsTOF HT fitted with a VIP-HESI source. Implementing system qualification as a standard procedure prior to data acquisition of large sample cohorts will ensure high quality and reproducible data to generate meaningful results. Our preliminary results demonstrate that the reference standard mixture of eight synthetic compounds is efficient in generating reproducible data across different labs and countries. All labs followed the same chromatographic methods to ensure data could be assessed and compared equally in the global ring trial.
Instant feedback on the current state of instrument performance was assessed by the software to determine if it passed specified criteria. Specifically, the accuracy of retention times, peak shapes, areas and intensities were used to rate LC performance, while the MS performance was assessed based on the accuracy of exact masses and CCS values, as well as the quality of MS/MS spectra of the reference compounds. Retention times varied well below 0.1 min, mass accuracies below 1.5 ppm and the mobility below 2% Å².
By using synthetic analytes with superior chemical stability provides a consistent standardized sample that is not subject to degradation. It also has broad applicability with different solvent systems so the user can qualify systems for both metabolomics and lipidomics applications.
In the end, the software-guided workflow provided a reliable and efficient way to monitor the instrument performance over time which can be used at regular intervals (e.g. a weekly basis) to detect dwindling performance over time with easy to interpret visualizations. This gives analysts a

chance to undertake the required interventions, such as source cleaning or changing columns, to restore the performance.

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Derivatized versus non-derivatized LC-MS/MS techniques for the analysis of estrogens and estrogen-like endocrine disruptors in human plasma

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Bisphenols, parabens, alkylphenols, and triclosan are synthetic compounds containing a phenolic group, which have emerged in the environment over the past few decades. Due to their hormone-mimicking properties, they are classified as endocrine disruptors (EDs), capable of disrupting steroid pathways in organisms. To evaluate the potential impact of EDs on steroid biosynthesis and metabolism, sensitive and robust methods enabling the concurrent measurement of EDs and steroids in plasma are needed. Of crucial importance is the analysis of unconjugated EDs, which possess biological activity.

Methods

The aim of the study was to develop and validate LC-MS/MS methods with and without a derivatization step for the analysis of unconjugated steroids (estrone-E1, estradiol-E2, estriol-E3, aldosterone-ALDO) and different groups of EDs (bisphenols, parabens, nonylphenol-NP and triclosan-TCS), and compare these methods on a set of 24 human plasma samples using Passing-Bablok regression analysis. Different liquid-liquid extraction reagents were tested (MTBE, toluene, hexane, ethyl acetate). Furthermore, different additives and mobile phases, gradients, and chromatographic columns were tested. Detection of the analytes was performed on an QTRAP6500+ mass spectrometer (Sciex, Concord, Canada), with an electrospray ionization (ESI) probe operating in negative ionization mode in the method without derivatization and in positive ionization mode in the method with derivatization.

Novel Aspect

Both methods provide a useful tool for evaluating the relationships between EDs and steroid metabolism.

Preliminary Data or Plenary Speaker Abstract

Both methods were validated according to FDA and EMA guidelines. The method with dansyl chloride derivatization allowed 17 compounds to be measured: estrogens (E1, E2, E3), bisphenols (bisphenol A-BPA, BPS, BPF, BPAF, BPAP, BPZ, BPP), parabens (methylparaben-MP, ethylparaben-EP, propylparaben-PP, butylparaben-BP, BenzylparabenP-BenzylP), TCS and NP, with lower limits of quantification (LLOQs) between 4-125 pg/mL. The method without derivatization enabled 15 compounds to be analyzed: estrogens (E1, E2, E3), ALDO, bisphenols (BPA, BPS, BPF, BPAF, BPAP, BPZ), parabens (MP, EP, PP, BP, BenzylP) with LLOQs between 2-63 pg/mL, and NP and BPP in semiquantitative mode. Adding 6 mM ammonium fluoride post column into mobile phases in the method without derivatization achieved similar or even better LLOQs than the method with the derivatization step.

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Complexome profiling with Split-TurboID identifies interacting partners of Plasmodium falciparum Alba4 protein

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The steady increase in antimalarial artemisinin resistance is a contemporary problem underpinning the need for continuous development of novel drugs and drug targets by pharmaceutical researchers. Next generation methodology in proteomics workflows such as Split-TurboID and complexome profiling, allows the identification of protein-protein interactions (PPI) with good sensitivity. Split-TurboID is a novel technique which uses a catalytic chemical reaction linked to protein of interest to introduce a biotin moiety onto neighbouring protein or peptides. Complexome profiling combines separation of native proteins by size exclusion chromatography with mass spectrometry (SEC-MS) for identification and quantification. Here, the study of parasite Alba4 protein was investigated, as the Alba family are parasite specific small nucleic acid-binding proteins, that could make attractive drug targets.

Methods

Previously we generated crosslinked whole cell lysate proteome and analysed potential nuclear protein binding partners using SEC-MS. Here, both peptide-level and protein-level pulldown assays were performed in parallel using magnetic streptavidin coated beads on Split-TurboID whole cell lysates of infected P. falciparum red blood cell cultures. Samples were then analysed on the next generation Orbitrap Astral Mass Spectrometer with unmatched range, sensitivity and sample processing time.

Novel Aspect

The Orbitrap Astral MS data generation is advancing the capability of structural biology deconvolution, much needed for antimalarial drug development

Preliminary Data or Plenary Speaker Abstract

We highlight peptide pulldown data corroborated with previous SEC-MS data. Furthermore, peptide pulldown data achieved the greatest sensitivity in identifying PPI pairs. The results suggest interacting pairs observed for Alba4 play a role in the life of mRNA, from transcription (AP2 transcription factors, PABP2) and nuclear export (karyopherin beta, PABP1) to translation (eIF2A, RACK1), but also associated with many Plasmodium-specific proteins of unknown function, potentially implicating these proteins in mRNA processing.

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In-Depth Characterisation of Naja Nivea Venom Using a Multifaceted Mass Spectrometry Approach

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Snake venom contains a complex mixture of bioactive proteins and peptides, evolved to allow for efficient immobilisation, death and digestion of prey. In 2017, the World Health Organisation classified snake bite envenomation as a neglected tropical disease. Due to issues with current antivenom treatments, snakebite envenomation is responsible for more deaths per annum than any other neglected tropical disease. Naja nivea, a snake from South Africa, is classified as medically important due to the lethality of its venom and its tendency to enter human settlements. Despite this, there has been little research into the venom composition of N. nivea. Our work utilised a multifaceted mass spectrometry approach to unravel the composition of N. nivea's venom proteome.

Methods

N. nivea venom was first separated using size exclusion chromatography before being profiled via a bottom-up proteomic workflow. In addition to this, SDS-PAGE, native and denatured mass spectrometry experiments were conducted to characterise toxins within the venom.

Novel Aspect

We report the first investigation into protein structures in N.nivea venom beyond their primary sequence.

Preliminary Data or Plenary Speaker Abstract

As expected by its clinical presentation, N. nivea venom was found to consist mainly of neurotoxins, with three-finger toxins making up 76.01% of the total venom proteome. Additionally, cysteine-rich secretory proteins, vespryns, cobra venom factors, 5'-nucleotidases, nerve growth factors, phospholipase A2s, acetylcholinesterases, Kunitz-type serine protease inhibitor, phosphodiesterases, L-amino acid oxidases, hydrolases, snake venom metalloproteinases, and snake venom serine protease toxins were also identified in decreasing order of abundance. Interestingly, contrary to previous reports, we found phospholipase A2 toxins in N. nivea venom. This highlights the importance of repeatedly profiling the venom of the same species to account for intra-species variation.

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Spatial proteomics to study immune cells driving inclusion body myositis

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Inclusion Body Myositis (IBM) is a chronic, progressive inflammatory muscle disease characterised by skeletal muscle inflammation, leading to weakness. Notably, the presence of immune cells within IBM muscle does not confer responsiveness to conventional immunotherapy, a phenomenon that remains poorly understood. Here we undertake a detailed proteomic analysis of affected muscle tissue and infiltrating immune cells, to elucidate the immune pathways involved in IBM. Our goal is to provide novel insights into disease pathogenesis that could pave the way for more effective treatments.

Methods

Formalin-fixed paraffin-embedded samples from five IBM patients and five sex- and age-matched controls were analysed. Sections of 2 um thickness were prepared on polyethylene terephthalate membranes and stained with hematoxylin and eosin (H&E). Single muscle fibres were isolated via laser microdissection and analysed using the Astral in data independent acquisition mode. Immune cells, which were only present in IBM patients, were excised and analysed separately.

Novel Aspect

This study demonstrates the power of LMD and high-sensitivity proteomics to investigate immune cell roles in IBM with spatial resolution.

Preliminary Data or Plenary Speaker Abstract

Proteomics analyses revealed > 2000 detected proteins in muscle and immune cell samples with cell type specific signatures. Data analysis of immune cell samples with CIBERSORTx enabled the identification of infiltrating immune cell types. This information will be used to perform immune cell type specific immunofluorescence staining, excision and proteome analysis, to uncover immune signalling.

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Identifying TDP-43's mitochondrial import pathway in ALS patient iPSCs-derived motor neurons

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Transactive response (TAR) DNA binding protein 43 (TDP-43) is a ubiquitously expressed DNA/RNA binding protein that regulates vital cellular processes such as mRNA stabilisation and alternative splicing. Recent studies have demonstrated TDP-43 to be a key trigger in neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD). Although their aetiology is unknown, pathogenic hyperphosphorylated and ubiquitinated cytoplasmic TDP-43 aggregates is a critical hallmark of neurodegenerative diseases; observed in 97% of ALS and 50% of FTLD patients. TDP-43 is a predominantly nuclear protein that can also translocate to the cytoplasm. Over-accumulation in the cytoplasm has been shown to cause aberrant TDP-43 aggregation. Importantly, aberrant mitochondrial mislocalisation also leads to mitochondrial dysfunction and exacerbates TDP-43's neurotoxic effects.

Methods

We isolate mitochondrial, nuclear, and S100 cytoplasmic fractions at 10 timepoints post-differentiation of ALS-derived iPSC-motor neurons and controls. Post-isolation, proteins undergo reduction, alkylation, and trypsin digestion before isobaric TMT labelling for multiplexing into a single analysis run on a Nano Dionex UltiMate LC system coupled with an OrbiTrap Eclipse instrument. Using HCD fragmentation and dynamic exclusion for MS/MS scanning analysis, we identify and quantify the proteome within cellular fractions. Initial analysis employs PEAKS Xpro software, ensuring accuracy through false discovery rates and variable modes for labelling efficacy. Subsequent steps involve data normalization and batch correction, culminating in the mapping of protein interactions for each iPSC-motor neuron cell lineage using our established pipeline with Mass Dynamics.

Novel Aspect

We aim to identify the comprehensive TDP-43 interactome and define its mitochondrial import pathway in ALS patient derived iPSC-motor neurons

Preliminary Data or Plenary Speaker Abstract

We have begun to generate proteomics data that precisely tracks the spatial and temporal development of TDP-43 pathogenesis. To achieve this, we have differentiated 3 ALS and 3 control iPSCs into motor neurons and closely monitored TDP-43 pathology from 4 weeks post-differentiation. We are conducting direct analysis on isolated nuclei, cytoplasm, and mitochondria, significantly expanding the pool of proteins we can study using mass spectrometry-based proteomics. This comprehensive approach promises to greatly enrich the depth of insights gathered from our proteomic study, providing insight into the changes in mitochondrial import observed in ALS patients' motor neurons. Our initial findings will not only illuminate the composition of mitochondrial proteins but also reveal how these proteins interact within protein networks. This understanding is crucial for translating proteomic data into practical insights into ALS pathogenesis. Ultimately, our proteomic research aims to contribute essential knowledge about interaction networks and offer valuable insights into the mechanisms underlying ALS progression.

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Disulfide reduction using reducing agent combinations significantly increases protein coverage and detection of cysteine containing peptides in bottom-up proteomics

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Sample preparation in routine bottom-up proteomic workflows have three key components: (1) protein extraction using chaotropes, and/or surfactants, (2) reduction of disulfide bonds followed by alkylation of cysteine residues, and (3) enzymatic digestion to peptides. If not completely reduced (and alkylated), peptides linked together by disulfide bridges become difficult to identify in database searching. Thus, an appropriate concentration and combination of effective reducing agent(s) is essential. Previously, various reducing agents' concentrations and combinations were optimized to improve proteoform detection during fractionation via two-dimensional gel electrophoresis. In this work, we investigate the concentration and combination of reducing agents to improve the detection of cysteine-containing peptides in bottom-up proteomics.

Methods

To determine the treatment yielding optimal detection of cysteine-containing peptides, protein extracts were treated with various combinations and concentrations of excess free thiols (dithiothreitol, DTT), and trivalent phosphorus reagents (tributylphosphine, TBP). Following reduction and alkylation, samples were digested with trypsin. Tryptic peptides were analyzed via LC-MS/MS and subsequently searched and identified using Peaks Studio 11.

Novel Aspect

This work indicates that increasing the concentration and using a combination of reducing reagents significantly improves bottom-up proteomic analyses.

Preliminary Data or Plenary Speaker Abstract

Preliminary data using bovine serum albumin (BSA) and mouse brain protein extracts indicate that reducing proteome extracts with 100 mM DTT + 5 mM TBP prior to tryptic digestion and analysis using LC-MS/MS yielded improved peptide detection in bottom-up proteomics. While no significant differences were observed in the number of proteins identified using different concentrations and combinations of reducing agents, protein percent coverage and the number and intensity of cysteine-containing peptides identified were increased with 100 mM DTT + 5 mM TBP. Next steps will include comparisons of different reducing agent combinations in other sample types to demonstrate the efficacy of this reducing agent cocktail.

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Standardized, fully automated undepleted plasma and MagNet enrichment workflows enabled by the Evotip Pure.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Plasma is a rich source of potential biomarkers for monitoring disease onset and progression. The implementation of non-targeted plasma proteomics in disease diagnostics promises to improve both speed and accuracy, thereby contributing to improved patient prognosis. However, the lack of reproducible, robust, and high throughput proteomics workflows is a major limiting factor in the transition from biomarker discovery to verification and ultimately clinical application. Additionally, the large dynamic range of plasma proteins prevents the identification of many proteins with bottom-up proteomics in undepleted plasma. We have implemented automated end-to-end sample preparation methods, integrated with the Evotip Pure, for profiling of undepleted plasma and deep plasma proteomics. These advancements will significantly enhance scalability for large scale cohort analysis.

Methods

For the undepleted plasma workflow, we combined on-robot reduction and alkylation with protein aggregation capture (PAC) based digestion. An additional MagNet enrichment step was added for deep plasma profiling using quarternary ammonium functionalized magnetic beads (Resyn Biosciences) to capture membrane-bound particles. Both methods were fully automated on a liquid handling robot, covering all steps from neat plasma to the mass spectrometer. This includes a step for enrichment of membrane-bound particles and simultaneous abundant plasma protein depletion as well as on-bead protein capture, clean-up, protein digestion, and Evotip loading based on our unique layered sandwich approach. The two workflows were evaluated with the standard Evosep One methods (30 SPD – 500 SPD).

Novel Aspect

These workflows enable large-scale clinical cohort analysis via deep, robust, high-throughput, and cost-efficient profiling of plasma using minimal starting material.

Preliminary Data or Plenary Speaker Abstract

Here, we present automated end-to-end workflows for plasma proteomics sample preparation, designed for the preparation and digestion of up to 384 samples in parallel, starting from undepleted plasma to desalted tryptic peptides on Evotips ready for LC-MS analysis.
The protocols are part of a modular sample preparation strategy based on the technology of the Evotip Pure and are optimized for speed, simplicity, and low sample input. The two automated workflows can process several 96 well plates in parallel by using just 1 µl (undepleted) and 4 µl (deep plasma proteomics) plasma as starting material, respectively. Sample preparation time was optimized with PAC digestion at 37 ℃ resulting in 1 hour digestion time with reproducibility and digestion efficiency comparable to protocols using overnight digestion. With careful consideration of efficient pipette tip usage and mixing steps, we present complete ‘one-touch’ protocols that produce ready-to-analyze peptides within as little as 3 hours (undepleted) and 5 hours (deep plasma proteomics), respectively.
As digested samples are loaded directly onto Evotips, the sample can be stored for up to several weeks before LC/MS analysis with no further preparation required. This eliminates the need for sample evaporation and resuspension, reducing pipette tip usage and sample loss while forming a resource- and cost-efficient automated end-to-end workflow for high productivity.

Preliminary results indicated that in combination with library-free data independent acquisition, up to 600 protein groups could be reliably quantified from undepleted plasma, while the coverage is the extended to more than 4,000 protein groups for deep plasma profiling.

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The Small Protein Enrichment Assay Rapid (SPEAR) allows for high-throughput analysis of low-abundance plasma proteins and hormones.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Unbiased and sensitive quantification of low abundance small proteins including hormones and immune factors in blood plasma is an exciting and difficult proposition. Blood plasma is a notoriously difficult tissue to study as many interesting factors are up to 13 orders of magnitude less abundant than high abundance proteins including albumin and immunoglobulins. Therefore, quantification of these proteins is done with expensive antibodies that lack specificity and cannot discover new proteins or modifications. Previously, we developed a method to address this problem which identified proteins not previously quantified in untargeted runs. The Small Protein Enrichment Assay Rapid (SPEAR), greatly increased the throughput of our assay and was applied to a mouse innate-immune activated cohort and human mixed meal tests.

Methods

The Small Protein Enrichment Assay (SPEA) removes most of the high abundant plasma proteins using an alcohol-acid precipitation, removes metabolites by chloroform precipitation and size-exclusion chromatography (SEC) to enrich for low-molecular weight proteins. SPEA-rapid (SPEAR) follows the same principles of SPEA but replaces the original method with a 96-well plate-based setup. Instead of chlorform-methanol precipitation, on-plate separation with solid phase extraction. This allows for efficient separation of up to 96 samples at once. SEC throughput was improved by using a shorter column whose decreased resolution allows for the capture of a greater proportion of the secreted protein phase. This was then coupled to high-throughput LC/MS-MS analysis using a Q-Trap mass spectrometer to analyse the plasma from humans and mice.

Novel Aspect

SPEAR is a high-throughput methodology for the discovery of new secreted protein factors and modifications in humans and mice.

Preliminary Data or Plenary Speaker Abstract

SPEAR provides the ability to study low-abundance proteins and hormones from hundreds of samples in a single assay, which improves upon an already invaluable tool for plasma protein and protein modification discovery. Utilising this tool we have previously identified numerous small proteins including insulin which had not previously been captured in an untargeted mass spectrometry method. This analysis has shown innate differences between human and mouse plasma which is vital to both the discovery of new secreted protein factors and translating these discoveries to humans. We have applied SPEAR to a mouse cohort exposed to an immune activator to capture the full range of secreted immune protein factors as well as a human cohort exposed to mixed meal tests to capture a wide range of secreted metabolic protein factors.

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Bringing Simplicity into Plasma Proteomics by Applying a Fast, High-Throughput and Standardized Automation Platform

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The blood plasma proteome is a promising source of biomarkers, offering the advantage of minimal invasive collection and widespread clinical availability. However, sample-heterogeneity and wide dynamic range of protein abundances hinders comprehensive profiling within the necessary throughput for large cohort studies. To address this challenge, we present a fully automated sample preparation pipeline on the Fluent® liquid handling system applying the standardized ENRICH-iST technology that facilitates the identification and quantification of low abundant proteins from plasma. Combined with the next-generation dia-PASEF® MS acquisition, it is designed for high-throughput yet profound characterization of the plasma proteome of large cohorts.

Methods

The automation platform consists of the Fluent® liquid-handling system (Tecan) with an integrated positive pressure and evaporator module, enabling automation from sample to dried peptides. The automated sample processing covers lysis, enrichment of low abundant proteins, tryptic digestion and clean-up including peptide drying by evaporation for LC-MS ready peptides employing ENRICH-iST technology (PreOmics). A total of 96 samples/run can be prepared for LC-MS analysis within one working day processing 20 µL of blood plasma/ sample. For mass spectrometry-based analysis 300 ng of peptides were separated with a 20 min or 65 min gradient on the nanoElute® 2 system and analyzed using the dia-PASEF® acquisition program on the timsTOF HT (Bruker). The data were searched with Spectronaut using directDIA+ (Biognosys AG).

Novel Aspect

This automated plasma proteome pipeline combines scalable throughput with profound proteomic characterization, bringing simplicity into the field of plasma proteomics.

Preliminary Data or Plenary Speaker Abstract

The high-throughput proteomic plasma pipeline underwent a comprehensive evaluation, comprised of a comparative analysis between the automated and manual ENRICH-iST workflow, processing plasma samples of three healthy donors. Remarkably, identification rates and quantitative performance of the automated workflow were found to be equivalent to those obtained through the manual process. When comparing against neat plasma preparation, both manual and automated workflow demonstrated efficient dynamic range compression, yielding an approximate two-fold increase in identifications with application of ENRICH-iST over neat plasma preparation. To further assess the robustness of the automated pipeline, an inter-plate comparison spanning three days was conducted. The Inter-Day study revealed low variability in protein group identifications and their abundance across independent sample preparation runs, affirming the reliability of the automated plasma pipeline. The workflow flexibility, accommodating samples sizes ranging from 1 to 96, enables seamless processing of both small- and large-scale cohorts. This adaptability was exemplified by processing two distinct biological cohorts, yielding high-quality data to provide valuable biological insights across diverse experimental settings.

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Recent development of native mass spectrometry analysis for membrane proteins in complex membrane mimetics

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Membrane proteins (MPs) are prominent in drug targeting due to their role in cellular processes. However, their analysis via native mass spectrometry (nMS) in mimetic environments presents spectral complexity. Leveraging high quadrupole selection, proton transfer charge reduction, and extended mass range scanning aids in resolving this complexity. Data independent acquisition-proton transfer charge reduction (DIA-PTCR) offers rapid molecular weight assessment of MPs in detergents or nanodiscs. Furthermore, high quad selection of precursor ions from MPs in native charge states followed by hybrid fragmentation enables the sequence identification and structural analysis.

Methods

Aquaporin Z (AqpZ) in detergent C8E4 and LDAO were internally generated. MPs in nanodiscs were provided by Prof. Michael Marty. Thermo Scientific Orbitrap Ascend mass spectrometer has been modified with a high quadrupole mass filter up to m/z 8000. The complex MPs in detergent or nanodiscs were quadrupole-isolated in narrow windows to mitigate signal interference. Subsequently, the isolated ion packets were charge reduced in the ion trap and scanned out in the Orbitrap. Data independent acquisition-PTCR (DIA-PTCR) streamlined the stepped isolation window across a wide m/z range followed by PTCR, facilitating the capture of comprehensive MW profiles. Top-down analysis employed ETD and HCD on Ascend for structural elucidation. Data were analyzed using Thermo Scientific™ BioPharma Finder™ 5.0.

Novel Aspect

Native mass spectrometry analysis of membrane proteins in complex mimetics incorporates DIA-PTCR and top-down approaches for lipid and structural elucidation.

Preliminary Data or Plenary Speaker Abstract

In the initial experiment with AqpZ conducted in C8E4, employing a quadrupole 20-Th isolation of precursors at m/z 7066 followed by a 5 ms PTCR resulted in a reduction of charge from +14 to +7. This led to a superior signal-to-noise ratio compared to ion trap isolation. When extending this method to empty nanodiscs comprised of DMPC and DPPC lipids, scanning through m/z 7000-8000 with a 10-Th isolation prior to PTCR revealed numerous previously obscured peaks in the full scan. Deconvolution of the mass-to-charge distribution exposed molecular weights ranging from 140 to 170 kDa. The spacing between adjacent deconvoluted peaks, falling within the range of 700-750 Da, corresponded to lipid molecular weights. The application of higher desolvation energy aided in cleaning up the spectrum without altering the molecular weight distribution. Moreover, an LC-nMS method was developed for analyzing MPs in nanodiscs, streamlining the analysis process. It was observed that nanodiscs dissociated into monomeric membrane scaffold proteins and phospholipids upon inclusion of detergent in the mobile phase. Intriguingly, removing the detergent from the mobile phase and switching from high flow to low flow source preserved the integrity of the empty nanodiscs.
Additionally, AqpZ prepared in C8E4 and LDAO exhibited distinct charge state distributions. LDAO is known to shown charge reduction effect on membrane proteins. But its effect on protein structure remains unclear. Our objective is to conduct top-down analysis to compare fragment patterns in these two detergents. It would shed light on any structural differences introduced by detergents.

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Using solvent-induced proteome profiling to identify antimalarial protein targets in live cells and lysate

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Plasmodium falciparum parasites cause the majority of malaria mortality worldwide. Antimalarial drugs have historically played a key role in controlling malaria in endemic regions, and for nearly all available antimalarials, cellular targets are unknown.

Methods

We applied solvent-induced proteome profiling in lysates and live cells with data independent acquisition mass spectrometry (DIA-MS) to identify antimalarial protein targets.

Novel Aspect

We identified the known targets of current antimalarials and extended the proteome information of solvent-induced protein precipitation for target identification.

Preliminary Data or Plenary Speaker Abstract

We generated the solvent denaturation curve of every single protein identified in the asexual stage of the parasite proteome using increasing percentage of solvent, following which, we choose 4-5 percentages to identify the known targets of current and novel antimalarials in lysate and live infected red blood cells. The experimental workflow involves treatment of P. falciparum infected red blood cells with compounds of interest, solvent exposure, soluble protein isolation, digestion, and DIA-MS using the Orbitrap Astral MS allowing faster throughput with deep coverage and accurate quantification. Further, methodological optimisation in the live cell workflow for the analysis of this intracellular parasite was also necessary, including an enrichment step. We have also developed new approaches for the analysis of the resulting datasets, affording better discrimination of specific compound-induced stabilisation from various experimental artefacts.

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D-Amino Acid Site Characterization in Peptide Epimers by using Higher-Energy Collisional Dissociation Tandem Mass Spectrometry

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

There are growing interest in D-amino acid-containing peptides (DAACPs) since more and more specific biological or pharmaceutical functions of DAACPs have been reported. Accompanied by the increasing number of DAACP-related studies, there is an urgent need for an effective strategy for characterizing DAACPs, especially the modified site. Previous developed analytical strategies focus on instruments with electron capture dissociation (ECD), radical-directed dissociation (RDD), or in mobility. Higher-energy collisional dissociation (HCD) is gradually became widely used and provides fragment-rich spectra. However, its capability on DAACPs characterization, particularly D-form residue localization, has not been evaluated. In this work, we systematically evaluated the capability of HCD in the differentiation of peptide epimers.

Methods

Standards of a therapeutic peptide, liraglutide, and its 14 DAACPs with different D-amino acid sites were analyzed by high-resolution tandem mass spectrometry using HCD fragmentation. The triply and quadruply charged ions of liraglutide/DAACPs were observed in full scan spectra and selected as precursor ions for the following MS/MS analysis. A series of normalized collision energies (NCE), 10~100%, were applied to understand the chirality-related characteristic of DAACPs in HCD. The raw MS/MS spectra were processed by an in-house software, which generated comparison results between fragments from liraglutide (all-L) and each DAACP. Differentiations in fragment ions, including b or y ions and charge states, were evaluated, and the relationship between fragment ion intensity and sites of D-amino acid were visualized by heatmap.

Novel Aspect

Our data shed lights on the potential of HCD to be applied to the site characterization of DAACP.

Preliminary Data or Plenary Speaker Abstract

The systematic evaluation result of the HCD data of liraglutide and 14 corresponding DAACPs indicated that the HCD MS/MS spectra of 9 of 14 DAACPs have significant differences in the intensity of fragment ions compared to that of all-L liraglutide. Specifically, the normalized intensity of D-amino acid site-corresponded doubly charged y-ion (y2+) fragments from quadruply charged DAACP precursor ion, generated by peptide bond cleavage between D-amino acid at N-terminal residue and L-amino acid at C-terminal residue, showed significantly higher intensities than the corresponding fragment ions from the all-L liraglutide. The comprehensive investigation of the capability of HCD in DAACP characterization also indicated that this characteristic can only be observed in the specific experimental conditions, including (a) the D-amino acids located in the middle of the liraglutide sequence, in the case of liraglutide is 11 to 21; (b) intensity difference of fragment ions are more obvious at lower normalized collision energies; and (c) only the doubly charged y-ion fragment generated from quadruply charged DAACP precursor ions show the D-amino acid site-related difference in fragment ion intensity. These specific experimental conditions might suggest the unknown chirality-related mechanism underlying HCD-based DAACP fragmentation. The evaluation of HCD-based DAACP fragmentation showed the potential of the application of HCD in the differentiation of peptide epimer and D-amino acid position determination.

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AutoMod: Exploring New Frontiers in Protein Post-Translational Modifications

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Protein database search is commonly used in shotgun proteomics with liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), where peptide identification mainly relies on matching tandem mass spectra to peptide candidates using database search tools. Numerous search tools have been proposed, but prior knowledge of protein post-translational modifications (PTMs) potentially exist in a sample is often required when users perform protein database searches. In most cases, users specify possible PTMs based on personal experiences or use default parameters suggested by the search tools. Hence, the search results might not be optimal. To addressee this limitation, we introduce a software tool, called AutoMod, designed to automate the detection and recommendation of PTMs possibly exist in proteomics data.

Methods

To detect PTMs from LC-MS/MS-based proteomics data, an open search is firstly performed. Using open search pepXML files as input, AutoMod generates PTM combinations according to user-specified PTMs. Subsequently, AutoMod correlates the mass alterations of peptide-spectrum matches (PSMs) in the pepXML files with the masses of PTM combinations. A PTM combination is deemed a match with a PSM if the mass discrepancy falls within a user-defined mass tolerance (typically 20 ppm) and the PTM sites are present in the peptide sequences. AutoMod organizes the matched PTM combinations and presents the top N PTM candidates (N is specified by the user) for downstream closed searches.

Novel Aspect

AutoMod is crucial for shotgun proteomics, enabling the discovery of novel post-translational modifications, enhancing understanding of biological processes.

Preliminary Data or Plenary Speaker Abstract

The performance of AutoMod was evaluated using a label-free HEK293 proteome dataset downloaded from ProteomeXchange (PXD001468). We first conducted an open search of the HEK293 proteome dataset using MSFragger, specifying a precursor mass tolerance of -150 to 500 Da and no modifications. AutoMod then processed the results of the open search, exporting top five recommended PTM combinations, which included n-terminal carbamidomethylation, methionine oxidation, a combination of n-terminal carbamidomethylation with cysteine carbamidomethylation, and pyroglutamate formation (pyro-Glu). Next, we performed three closed searches using MSFragger, specifying three sets of variable modification settings: no modification, default modifications, and AutoMod-recommended modifications. As a result, 489,605 PSMs, 126,225 peptides, and 9,618 proteins were identified without modification, while 579,702 PSMs, 133,050 peptides, and 9,677 proteins were identified with default modifications, and 615,416 PSMs, 136,570 peptides, and 9,841 proteins were identified with AutoMod-recommended modifications. Thus, more PSMs and peptides were identified using the AutoMod-recommended modifications. Further comparison between default and AutoMod revealed 90% PSMs shared, with 8% additionally identified by AutoMod and 2% by default. Among the 90% commonly identified PSMs, 89% exhibited identical peptide sequences and modifications, while 1% showed discrepancies. Investigation into the 1% of PSMs with varied peptides and modifications revealed 56% reassigned with pyroglutamate formation, which is a frequently observed post-translational modification in peptides and proteins,

exerts diverse impacts on their stability, activity, and function. For example, when occurring at the N-terminus of peptides, it enhances resistance to enzymatic degradation, prolonging their lifespan in biological environments. The detection of pyroglutamate formation underscores AutoMod's ability to detect potential PTMs, thereby aiding in the discovery of novel modifications.

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Rapid and robust profiling of small extracellular vesicles from ultralow samples

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

In recent decades the study of extracellular vesicles (EV) has gained attention due to their function and role in intercellular communication and cargo transfer. Advancements in liquid chromatography and mass spectrometry (LC-MS/MS) has demonstrated the ability to comprehensively define EV proteomes. However, these studies are often limited by sample availability, requiring upscaled EV production from cell culture or biofluids, limiting its applicability to lower yield EV sources.

Methods

Using our Q-Exactive HFX, we establish a method which enables precise and comprehensive proteomic characterisation of small EVs (sEVs) from ultralow starting quantities. This pipeline is defined by its optimised sample preparation methods, short chromatography lengths, and data-independent acquisition (DIA). This refined DIA-based MS approach combined robust single-pot, solid-phase-enhanced sample preparation with temporally optimised enzymatic digestion and short chromatography gradients (15 to 44 min) using low input loads of 0.5 to 50 ng of EV peptide across each LC gradient.

Novel Aspect

Streamlined EV proteome capture for low samples; maintains depth/accuracy. Adaptive pipeline suits various lab conditions, where sample availability is constrained

Preliminary Data or Plenary Speaker Abstract

For 50 ng loading, more than 3730 proteins for all gradient lengths were observed, with 4599 identifications in our 44 min workflow. The short gradient lengths favoured low peptide loads, with a 15 min gradient consistently quantifying >1100 protein groups from 500 pg of EV peptide, and >3800 protein groups from 50 ng, including the robust quantification of 22 core EV marker proteins. Furthermore, we optimised bead-based sample preparation for ultralow quantities of EV (0.5 to 1 µg) to obtain sufficient peptides for MS quantification. Our approach enables the generation of meaningful proteome insights from <1 µg starting EV protein, encompassing the identification of >1900 protein groups and capturing sufficient proteomic diversity of EV from different cell sources to determine known EV biology.

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Assessment of High-Resolution DIA Methods and Short Gradients on High-Throughput µPAC Columns for Maximum Proteome Coverage and Quantitative Performance

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Understanding biological processes on a global proteome-wide scale is crucial in scientific research. Achieving statistically significant quantitative data often involves studying large cohorts comprising hundreds of biological samples and replicates. An effective workflow must be capable of processing, measuring, and analyzing a high volume of samples while ensuring data reproducibility and consistent performance throughout the study. The primary aim of this study was to introduce a robust workflow for high-throughput label-free quantification using a Vanquish Neo LC system equipped with a 5.5 cm uPAC Neo High Throughput column and an Orbitrap Exploris 480 mass spectrometer.

Methods

We evaluated three gradient lengths, namely 3.5, 5.5, and 10 minutes. The mass spectrometric method, predominantly the isolation window widths, have been optimized for two different objectives: one method for giving maximum proteome coverage of the sample, another for gaining high-quality quantitative information on the protein abundances. To further showcase the quantitative capabilities of these methods, we employed a three-species proteome mix, achieving accurate quantification results across all three gradient lengths using the MS method for quantitative analysis. Furthermore, we demonstrated the workflow's robustness through an analysis of several 100 consecutive injections, proving this workflow to be suitable for the reliable analysis of large cohort studies. Data processing was carried out using different software tools with library-free analysis approaches.

Novel Aspect

Using pillar array-based columns with well-designed LC and MS methods optimized for quantitative performance results in very high sample throughput.

Preliminary Data or Plenary Speaker Abstract

The main objective of this study was to evaluate the suitability of µPAC columns with 5.5cm flow path for maximizing the throughput of MS analysis gaining reliable and robust quantitative information. Each gradient length was analyzed for the number of identified proteins and peptides from different amounts of HeLa digests. Despite the short gradients, the data clearly illustrate that a median of 5-6 points per peak could be measured for isolated HeLa peptide XICs, achieved by meticulous duty cycle adjustments in each of the three DIA methods tested.
In experiments focused on label-free quantification (LFQ), precision and accuracy in measuring relative protein and peptide abundances are crucial. To assess the quantitative performance of the described methods, the study analyzed a mix of three species proteomes, i.e. tryptic digests of human, yeast, and E.coli proteins mixed in specific ratios. The quantification precision, evaluated through coefficients of variation (CV) across different technical replicates at both the protein and peptide levels, consistently demonstrated CVs well below 10% and minimal missing values, indicating high technical reproducibility.
For quantification accuracy, the study compared experimental ratios of MS2-based protein quantification results for human, yeast, and E.coli proteins to expected ratios of 1:1, 1:0.5, and 1:4,

respectively. Across the three different gradients and MS methods, median values of experimental ratios closely aligned with theoretical ratios, emphasizing the accuracy of the quantification process. While demonstrating high quantitative value of the acquired data, a high proteome coverage (> 5000 proteins for 10 min, >2500 proteins for 3.5 min for single proteome samples) could still be achieved.

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LC separation optimization for XL-MS Analysis

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Cross-linking mass spectrometry (XL-MS) has grown dramatically as a key workflow for elucidating protein higher-order structure and mapping protein-protein or protein-nucleic acid interaction networks on a proteome-wide scale. However, significant LC separation challenges remain due to sample complexity and low abundance of XL peptides. In this work, we evaluated XL-MS workflows with various newly released columns including the Thermo Scientific™ µPAC™ Neo HPLC Columns and IonOpticks Aurora Ultimate™ columns.

Methods

Four amine-reactive crosslinkers: DizSEC (2,5-Dioxopyrrolidin-1-yl (2-(3-methyl-3H-diazirin-3-yl)ethyl)carbamate), DSPP (Disuccinimidyl Phenyl Phosphonic Acid, PhoX), DSSO (disuccinimidyl sulfoxide) and DSBU (disuccinimidyl dibutyric urea) were used to crosslink standard proteins and E.coli cells. XL samples were digested and spiked into Hela digest in various ratios. Separation was achieved using Thermo Scientific™ Vanquish™ Neo LC system with various gradients using EASY-Spray™ PepMap™ Neo columns, µPAC™ Neo HPLC Columns or IonOpticks Aurora Ultimate™ columns. Following separation, the peptides were detected on Thermo Scientific™ Orbitrap Ascend or Astral™ mass spectrometers and data were analyzed using multiple search engines: XlinkX node in Proteome Discoverer v3.1 (Thermo Scientific), MeroX, xiSEARCH/xiFDR, and Scout v1.4.10.

Novel Aspect

Comparison of newly released C18 columns for XL-MS workflows.

Preliminary Data or Plenary Speaker Abstract

XL-MS has become a universal tool for studying protein structure and protein-protein interaction networks. However, two challenges in this workflow are the low abundance of crosslinked peptides and high sample complexity, especially for intra-cellular XL. To detect low abundance XL peptides, the choice of C18 column and LC gradients becomes critical for XL analysis. We tested three types of C18 columns (25 cm Easy PepMap and Aurora columns as well as 50 cm µPAC Neo HPLC Column) using crosslinked samples spiked into HeLa digest at different ratios. Each column performance was evaluated for peptide peak width, carryover and XL identifications using 1 hr gradient. µPAC and 25 cm Aurora Ultimate columns demonstrated a 50% narrower peak width compared to the PepMap column. However, we also observed significant carryover from the Aurora column for standard cross-linked protein samples. Importantly, we identified increased numbers of HeLa proteins as expected using Aurora or µPAC columns compared to the PepMap column but fewer of crosslinked peptides. The Aurora column resulted in the separation and identification of the most looplinks (50%) and monolinks (33%), but with higher false positive rates compared to the other two columns. These results can be explained by differences in columns' stationary phases, bead types, and packing. The identified crosslinks from these three columns had over 75% XL identifications that overlapped. Moreover, we also tested PepMap columns 75 µm × 25 cm vs 50 µm × 15 cm for low-load samples down to 3 ng alone or spiked in. After optimizing the gradient, PepMap column 50 µm × 15 cm gave the best results at a flow rate of 100 nL/min in a 40 min gradient. Overall, our results show that optimizing LC setups for various crosslinked samples using different columns has a high impact on crosslink identification rates.

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Implementation of mass spectrometry-based proteomics of low input samples as a standard service in a core facility

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Proteomics requires millions of cells as starting material to generate comprehensive quantitative proteome data. This leads to mixed populations of cell types being analysed together and such bulk measurements average out stochastic variations of individual cell types. Further, only a few hundred cells are available in several clinical samples. Analysis of small sub-populations of cell types (nanoproteomics) was an unmet need at our facility. Although specialized devices and equipment are available for such applications, they significantly increase the overall running costs of the facility. In addition, procuring capital-intensive custom liquid handling systems needs a high number of projects to substantiate the cost. Therefore, we set out to identify, optimize, and implement a robust and inexpensive nanoproteomics method at our facility.

Methods

We carried out a comprehensive literature review and finally implemented the sample prep method published by Martin et al (2021). Briefly, low tens to few hundreds of cells were deposited into a 96-well plate pretreated with 0.1% DDM. Cells were treated with DDM overnight and lysed by heating and water bath sonication. Digestion was carried out by Trypsin/Lys-C and quenched with formic acid. Sample volume was reduced, and LC-MS/MS was carried out employing DIA on an Orbitrap mass spectrometer coupled to a nanoLC. Data processing was carried out using DIA-NN utilizing sample size-comparable spectral libraries. Data analysis was carried out using R.

Novel Aspect

This abstract describes the implementation of a novel technique useful for translational research in an academic core facility.

Preliminary Data or Plenary Speaker Abstract

Our comprehensive review of literature showed that most low-input proteomic methods leverage low-volume, less to no sample transfer, and minimal manual manipulation to reduce losses during sample preparation. This is achieved in several ways including custom liquid handling systems, microfabrication, or microfluidics. In addition, some methods rely on chemical tagging-based sample multiplexing where one of the channels is used to label a higher amount of peptides thus “boosting” the signal. However, this brings in an additional experimental cost.

We implemented a surfactant-assisted one-pot sample processing at standard volume sample preparation method for convenient robust proteome profiling. In this method, the plastic surface is precoated with a mass spec-compatible surfactant thus reducing surface adsorption losses. A basic cell sorter is employed to count and deposit designated number of cells into wells of a 96-well plate. The cell sorter provides an added advantage of cell type-specific marker-based cell isolation that could be valuable to study rare cell populations. Sample preparation was carried out in the same plate thus preventing sample transfer-induced losses.

In combination with ultrasensitive LC-MS, we could confidently identify and quantify thousands of proteins from tens of cultured human cells. We evaluated the performance of the method over a range of starting material. As expected, the number of input cells affected the depth of proteomic coverage and variability in measurements. Quantitative variability was determined by calculating a correlation coefficient of protein intensities between any two replicates and was found to be low.

In summary, we could implement an accessible proteomic pipeline with widespread applicability to various sample-limited systems that could be utilized by researchers to uncover biological insights of rare cell populations.

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Entire 2D-PAGE gel analysis for whole proteome/PTM analysis

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

With the field shifting towards proteoform analysis, there is a strong desire for a method that separates proteoforms out by their physical and chemical properties. Using their properties as a filter to separate intact proteins this allows a range of Post Translational Modifications (PTMs) to be analysed such as proteolytic cleavage changing the mass, and covalent modifications changing the isoelectric point (charge).

2D-PAGE facilitates this through isoelectric focusing and electrophoresis, this is critical to the process as covalent PTMs will in most cases change the charge of the protein. This significantly reduces the complexity of the samples allowing for products of proteolytic cleavage to be analysed and potential low abundance proteoforms to be discovered.

Methods

The initial loading of protein into the IEF strips and onto the acrylamide gel along with Coomassie staining is unchanged from general protocols, this is where our method diverges. Once staining has verified that protein has loaded correctly onto the gels, they undergo a whole gel destain, followed by press slicing into a squared 96 well plate, gel slices then undergo the process for in gel trypsin digestion followed by stage tip cleanup.

Novel Aspect

Rapid untargeted PTM discovery analysing an entire gel

Preliminary Data or Plenary Speaker Abstract

Pilot experimentation has shown that this allows for proteoforms to be analysed separately (with 88 separate samples being made from 1 gel) and effectively provides a form of untargeted PTM discovery.

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Rapid and robust plasma proteomics methods by leveraging microflow and ion mobility.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

As the need for faster and more robust label-free bottom-up shotgun proteomics increases, many researchers are turning away from nanoflow based approaches and embracing microflow. This approach is particularly useful and popular with plasma samples as their proteome is generally less complex than other tissue samples and there is usually ample sample to compensate for the reduced sensitivity. Furthermore, pairing the faster chromatography with ion mobility separation (IMS) compensated for the reduced peak capacity. We developed and optimized an LCMS method utilising IMS for discovery proteomics in human plasma. Two experiments were run and the resulting pathways analysed, providing insights into proteome changes between human plasma samples derived from control, multiple sclerosis (MS) and participants experiencing pain hypersensitivity.

Methods

LCMS analysis was performed using a nanoACQUITY UPLC coupled to a Waters Synapt G2-Si HDMS instrument. 1 µL of sample was injected onto an ACQUITY UPLC HSS T3 1.8 µm 100 mm × 300 µm Column at 40 °C and eluted at a flow rate of 5 µL/min with a 39.5 min gradient (including wash and equilibration). Mobile phase A consisted of Water + 0.1% Formic Acid and mobile phase B consisted of Acetonitrile + 0.1% Formic Acid. HDMSe data from 50-2000 m/z were collected in positive ESI resolution mode. The scan time was 0.5 s and the elevated energy transfer collision voltage was 17-45 eV (synced to IMS). Glu-1-Fibrinopeptide B solution was used for lockspray

Novel Aspect

We demonstrate the superior plasma proteomics achieved by combining microflow and ion mobility over traditional nanoflow based protocol.

Preliminary Data or Plenary Speaker Abstract

Glu-1-Fibrinopeptide B solution was used to optimize source settings (using a low flow capillary in the probe) and maximum sensitivity was achieved with capillary: 2.8 kV, source temperature: 100 °C, sampling cone: 30 V, source offset: 30 V, desolvation temperature: 500 °C, desolvation gas flow: 600 L/h, cone gas flow: 50 L/h. A commercial E-coli digest was used for initial optimization of microflow parameters. After several gradients were compared, the one which resulted in the highest protein identifications was chosen: mobile phase A initially at 99%, decreased to 95% over 1.5 min, decreased to 92% over 1.8 min, decreased to 75% over 21.9 min, decreased to 55% over 8.7 min, decreased to 15% over 0.1 min and held for 3 min before being instantly increased to 99% and held for 2.9 min.

Plasma samples from two experiments were analysed using the optimized settings and raw data was processed in Progenesis QI for proteomics using a uniport human reference proteome as a search database. In the first experiment, we compared the proteome signature of plasma samples from individuals with and without multiple sclerosis. In the second, we compared the proteome signature of plasma samples from individuals with and without pain hypersensitivity. Differentially expressed proteins identified significant changes in functional pathways between control and MS/HS samples (Metascape, Panther, KEGG, STRING).

Results were compared to a similar previous experiment run using our nanoflow protocol (1.5 hours per sample). The much faster microflow protocol provided significant system improvements over the nanoflow protocol resulting in less instrument issues and improved robustness. The microflow

protocol results also showed superior reproducibility and better retention time alignment with a similar number of protein identifications.

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Do-it-yourself de novo antibody sequencing workflow with 100% accuracy and coverage

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Antibodies are widely used as research tools or therapeutic agents. Knowing the sequences of the variable regions of an antibody, for both the heavy chain and the light chain, is a prerequisite for production of recombinant antibodies. Mass spectrometry (MS) based de novo antibody sequencing is a frequently used, sometimes the only approach to gaining this information. Here, we describe a workflow that enables accurate sequence determination of monoclonal antibodies based on MS data and freely available software tools. This workflow, which we developed using a homemade anti-FLAG monoclonal antibody and achieved 100% sequence coverage and accuracy (distinguishing I/L), consists of the following steps.

Methods

I. SDS-PAGE separation of the heavy chain and the light chain of a monoclonal antibody after a contingent deglycosylation treatment using PNGase F.
II. In gel digestion of the excised heavy chain and light chain using different proteases or protease combinations, including trypsin, elastase, pepsin, chymotrypsin, asp-N, gluC, trypsin+aspN, trypsin+gluC.
III. LC-MS/MS analysis of the digested peptides on EASY-nLC 1200 system interfaced with a Fusion Lumos mass spectrometer.
IV. Analysis of the resulting MS dataset using four de novo sequencing software tools. Of the ones we tested pNovo, Casanovo, and Novor.Cloud performed well, and we recommend parallel use all of them to ensure accuracy.

Novel Aspect

Do-it-yourself de novo antibody sequencing workflow with 100% sequence coverage and accuracy and no ambiguity of I/L

Preliminary Data or Plenary Speaker Abstract

V. Antibody sequence assembly using the Stitch software (v3.1.5), with peptide sequences obtained from step IV as input data. The different Average Local Confidence (ALC) cutoff values for peptide sequence selection were compared, and the results will be reported for each of the de novo sequencing software tools tested.
VI. Antibody sequence validation and correction. They includes (1) clarification of common ambiguities such as GA/Q, GG/N, N/D and Q/E. (2) Comparison of the Ab sequences obtained with different de novo sequence software to identify positions of inconsistent amino acid assignment. (3) Open-pFind search using the assembled Ab sequences as reference to resolve conflicting amino acid assignment. (4) Manual inspection of the original mass spectra to resolve the last bit of antibody if there is.
We tested this workflow on a monoclonal anti-HA Ab (TANA2), whose sequence was unknown to us. A recombinant anti-HA antibody was made based on the decoded Ab sequence, and it performed indistinguishably from the commercial Ab.

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A cumulative sample preparation approach for higher coverage of protein identification in giant virus global mass spectrometry proteomics

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Giant viruses are nucleocytoplasmic large DNA viruses (NCLDVs) isolated from eukaryotic hosts. Discovery of Acanthamoeba polyphaga Mimivirus has challenged the definition of a virus and unveiled the presence of more than 180 giant viruses to date. Giant viruses have a capsid size comparable to some microbes which encapsidates a large reservoir of proteins including ORFans and cellular homologues. Although, proteome study of giant viruses are being reported, however the type of proteins and their abundance inside giant virus capsids differs, which suggests that a combined approach is required to detect and identify all the packaged proteins.

Methods

We performed a mass spectrometry global proteomic analysis on five giant viruses- Acanthamoeba polyphaga Mimivirus (APMV) (La Scola et al., 2003), Mimivirus Bombay (MVB) (Chatterjee et al. 2016b), Powailake Megavirus (PLMV) (Chatterjee et al. 2016a), Marseillevirus (MV) (Boyer, et al. 2009), and Kurlavirus (KV) (Chatterjee and Kondabagil 2017)- using two different sample preparation methods, in-gel and in-solution. We combined two different sample preparation methods to compare and to increase the coverage of protein identification.

Novel Aspect

For purified virus particle samples, a cumulative approach for Mass Spectrometry sample preparation leads to higher coverage of protein identification.

Preliminary Data or Plenary Speaker Abstract

Our study reports the identification of higher number of capsid packaged proteins than previously reported in APMV and MV. Our results also shed light on evolutionary selection of protein packaging between Mimiviridae and Marseilleviridae families.

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Comparison of sample preparation methods for proteomics analysis using PreOmics ENRICH-iST beads and volumetric absorptive microsampling (VAMS) devices.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Protein corona-based proteomic workflows that utilise beads are being more popular as sample preparation tools to help mitigate the dynamic ranges issues of plasma. There are a number of options available in the market that all work, using similar mechanisms, by enriching for the 'sample' on the bead corona and thus depleting the problematic high abundance proteins. Although effective, these have a high cost per sample. We have developed novel methods that utilise volumetric absorptive microsampling devices (VAMS) to collect samples and to deplete high abundance proteins from blood samples at a much lower per sample cost. In this study we sought to compare a representative bead technology with our VAMS methods to assess differences and benefits of each technology.

Methods

Whole blood and plasma (single-spun and double-spun) from healthy volunteers was collected and processed using 2 workflows, (1) our standard workflow using VAMS and (2) the PreOmics ENRICH-iST workflow.

1. Samples were applied to 30µL Mitra VAMS tips and dried. Samples were incubated in an extraction solution (whole blood: 24 hours, plasma: no incubation), tips were then washed and proteins were digested in tip with trypsin (1 µg/µL in lysis buffer).
2. Samples were combined with prepared EN-BEADS and incubated for 30 minutes. The sample-bound beads were then lysed, alkylated, and digested with the proprietary provided reagents and methods.

For both workflows, peptide concentration of all samples were quantified and normalised to 0.2 µg/µL and were analysed by LCMS.

Novel Aspect

Novel blood sample enrichment methods providing better affordability and reproducibility and producing more protein identifications than corona-based workflows.

Preliminary Data or Plenary Speaker Abstract

Each workflow is compatible with a 96-well plate system and for plasma samples, both protocols can be completed within 24 hours. A cost comparison of the methods determined that the VAMS methods are 10-fold more affordable than the ENRICH-iST kit per sample.

ENRICH-iST is only validated for use with plasma samples, as such this was the primary comparison we used to determine reproducibility and validity of each method. Plasma was single-spun or double-spun before sample enrichment, processing, and analysis for both workflows. There were fewer missed cleavages in the ENRICH-iST processed samples compared to the VAMS samples (23% vs 33%), indicating greater trypsin digestion for this protocol.

The lowest mean %CV for plasma was observed for double-spun plasma on the ENRICH-iST beads with the double-spun plasma on the VAMS as a close second. VAMS produced a higher protein and peptide count (1,218 vs 1,030 for proteins and 11,306 vs 8,246 for peptides) and a lower overall %CV. Single-spun plasma produced more protein identifications than the double-spun for all samples (2634 vs 1664 for identifications with VAMS and ENRICH-iST respectively) but was significantly more variable for the ENRICH-iST beads.

Although the ENRICH-iST beads are not recommended for whole blood, they did produce a similar number of protein identifications to VAMS devices, with 3019 proteins detected. However, there was

significant variability in the data in that 46.5% of the proteins had a %CV greater than 20%. The VAMS whole blood samples were highly reproducible (mean %CV of <10%) and produced the highest number of identifications of all samples with 3614 proteins detected.

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A novel computational pipeline for immunopeptidomics with DIA-LC-MS/MS

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

In recent years, data-independent acquisition (DIA) has gained significant popularity in LC-MS/MS due to its superior proteomic coverage and enhanced reproducibility across experiments. However, analyzing immunopeptidomics DIA data remains a challenging problem due to its large search space. Moreover, in clinical applications we are often interested in mutated antigens which typically do not exist in databases. Here we introduce a novel solution that supports non-enzyme peptidome searches along with de novo peptide peptide sequencing for DIA data.

Methods

1. Given an DIA-LC-MS/MS data set, direct database search was performed against a canonical protein sequence database and cryptic database from RNA_seq. Deep learning-based algorithms were used for the prediction of peptide occurrence, retention time, spectrum, and ion mobility.
2. For each precursor remaining unidentified in step 1, de novo sequencing was performed with DeepNovo DIA algorithm.
3. Peptides with high confidence from steps 1 and 2 were collected and re-scored based on the prediction of peptide physic-chemical properties and finally were reported with a false discovery rate.
4. A personalized machine learning approach was used to predict the collective response of a patient's CD8+ T cells by modeling the positive and negative selection.

Novel Aspect

immunopeptidomics for DIA. Sensitive peptide identification and quantification software for DIA data

Preliminary Data or Plenary Speaker Abstract

We implemented this pipeline into PEAKS software and tested with a few data sets. The results showed that it can accurately identify and quantify HLA peptides that binds to patients' MHC haplotype, as well as detect mutated peptides of potential clinical interests from the DIA datasets. For example, with the data from human tumor tissue sample published by Kraemer et al, our pipeline identified 22k HLA-I peptides from the DIA spectra, which is ~15% increase from the number of peptides identified from the DDA data generated using the same samples. We further validated the correctness of these HLA-I peptides by predicting binding affinities using the widely adopted MHCPred tools and find that 96.6% of the identified 9-mer peptides binds to at least one allele of patient. In addition, our pipeline features an intuitive graphical user interface (GUI) for user-friendly validation of identification

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FAIMS-DIA proteomics approach from formalin-fixed paraffin embedded lung tissue of mucopolysaccharidosis (MPS) disease murine models

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Mucopolysaccharidosis (MPS) diseases are recessive lysosomal storage disorders caused by dysfunctional lysosomes. Specifically, enzyme dysfunction in MPS diseases cause accumulation of glycosaminoglycans in lysosomes and effects the downstream metabolism pathways. in MPS I disease, distinct clinical signs include altered facial features and impaired cognitive development. Despite lung dysfunction is not a primary clinical sign, patients suffer from recurrent respiratory infections and dysfunctions contributing to mortality. The exact cause of the respiratory dysfunction in MPS diseases has not been investigated. We aim a better understanding of the disease by performing proteomics of the lung tissues investigating the proteome in the distal lung.

Methods

20 weeks old C57BL/6 MPS I mice were used and lungs were inflated in situ with 4 % paraformaldehyde neutral buffer and processed as formalin-fixed paraffin embedded tissue. The sections were dewaxed, rehydrated and antigen retrieval was performed with citric acid. The tissues went through denaturation, reduction, alkylation, and tryptic digestion. The samples were cleaned up with zip tip and untargeted proteomics approach by high-field asymmetric waveform ion mobility spectrometer (FAIMS) with data-independent acquisition (DIA) mass spectrometry was performed to investigate the changes of proteome of formalin fixed paraffin embedded lung tissue of MPS I disease models. FAIMS-DIA was performed with Orbitrap Exploris™ 480 mass spectrometry (Thermo Scientific). Spectronaut™ were used to process the data.

Novel Aspect

Proteomics approach using FFPE lung tissue in MPS I model to investigate the disease’s impact in lungs.

Preliminary Data or Plenary Speaker Abstract

In this study we successfully extracted protein for digestion from FFPE tissue for proteomics. There were 46000 – 48000 peptides and ~7350 proteins were identified in each sample with 500 ng injection performed in technical triplicate. Results demonstrated the known disease related proteins and pathways are being identified. Furthermore, other protein candidates suggest potential mechanism on how MPS I disease contributing to respiratory symptoms in patients.

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Tandem mass spectrometry and Peptide Ion Intensity as a Tool to Measure Protein Abundance from The Venom of Malaysian Snakes

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Snake envenomation has been identified as a neglected tropical disease responsible for a high morbidity and mortality rate, especially in Southeast Asia, including Malaysia. The only treatment against snake envenomation is antivenom produced from horses; however, it is associated with many drawbacks, including anaphylactic reactions and serum sickness. Snake venom is a complex mixture of proteins and polypeptides with various biological activities. Information on the venom proteome is essential for understanding and predicting the clinical consequences of envenomation and formulating effective treatment/and antivenom that will neutralize toxic venom components. Advancements in proteomic techniques, i.e., shotgun proteomics and tandem mass spectrometry (LC-MS/MS), and advanced proteomic software have allowed better identification and characterization of venom proteins.

Methods

In this present study, we aimed to determine the abundance of different proteins from the venom of five venomous snake species of medical importance in Malaysia (Naja kaouthia, Naja sumatrana, Ophiophagus hannah, Calloselasma rhodostoma, and Cryptelytrops purpureomaculatus) using LC-MS/MS and peptide ion intensity approach. Although the enzymatic activities and the proteomic characterization of the venom from all these species are available, none has determined the protein abundance using the present approach. With PEAKS (PEAKS Studio, Bioinformatics Solution Inc., Canada), the relative protein abundance was measured using the peptide ion intensity feature available in the software.

Novel Aspect

The present study provides new data and strategies that can be formulated to develop effective treatment against snake envenomation.

Preliminary Data or Plenary Speaker Abstract

Phospholipase A2 was identified as the common venom protein family in all five species with the highest percentage identified in C. purpureomaculatus venom (19.78%). L-amino acid oxidase, snake venom metalloproteinase, snake venom serine protease and peptidase were identified as common venom protein families in all species except N. sumatrana, with varied relative abundance.

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Evaluating a new Workflow for Top-Down Protein Sequence Confirmation and de novo Sequencing

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Recombinant protein primary sequence determination typically was the domain of Edman Sequencing. The reduced availability of Edman sequencers raised interest in alternative mass spectrometric approaches that additionally offer capabilities to sequence N-terminally modified proteins and obtain C-terminal sequence information. The spectral complexity encountered in high resolution/accurate mass spectrometry data previously limited ESI-Top-Down analyses to sequence confirmation. Thus, the de novo determination of protein sequences without pre-existing sequence information demands new developments in the data analysis software to reliably extract and timely process such high-density information. Here, we report on the development of new workflows for protein sequence confirmation and de novo sequencing by processing complex Top-Down MS/MS data.

Methods

Analyses were performed on a high resolution QTOF instrument and a modified timsTOF enabled for ExD fragmentation. Proteins such as ubiquitin and NISTmAb subunits were electrosprayed under denaturing conditions. In CID collision energies were optimized for each analyte.
The new analysis software platform was developed for the confirmation and spectral annotation of known protein sequences, and a newly developed de novo sequencing workflow for processing complex protein spectra. In the first step it extracts all possible fragment ion masses including all charge states and isotopes and in the second step determines all possible monoisotopic masses of fragment ions to match plausible sequences.

Novel Aspect

First de novo protein sequencing workflow for the analysis of high-resolution Top-Down mass spectra.

Preliminary Data or Plenary Speaker Abstract

The confirmation workflow involved calculating and assigning theoretical isotopic distributions to the experimental data based on known peptide/protein sequences. It provided a list of peptide sequence tags that assemble to a sequence coverage map. This map was used as reference to optimize the de novo sequencing algorithm for each model protein.
For sequence confirmation of the NISTmAb LC and Fd subunits after IdeS digestion and reduction, an LC-ETD-MS/MS analysis on the QTOF was used with a mass tolerance of 3 ppm. In depth automated peak picking and manual review resulted in sequence coverages of the LC and the Fd chains of 71 and 76%, respectively. Fragment ions were 45% z+1, 39% c and 17% y ions.
Corresponding ubiquitin analysis yielded a sequence coverage of 93% in the confirmation workflow.
The de novo algorithm involved peak picking, assigning charge states to individual peaks, and determining the monoisotopic masses using the Averagine chemical formula model. A list of sequence tags was then generated for the ubiquitin dataset based on distances between neutral monoisotopic peaks. In this context a mass accuracy of <6 ppm turned out to be crucial to avoid excessive rates of false positive sequence tags. Typically, the sequence tags could be derived from related ion types, resulting in 4 reversed sequence tags (C->N-terminal readout, z+1 ions), 3 forward tags (a, c-ions) and 1 of unspecified direction. Ile and Leu ambiguity wasn´t resolved in the calculated sequence tags and some Lys/Gln ambiguities for higher charged fragments as well. In the software,

the sequence tags were automatically assembled to an MS-BLAST search string and the subsequent sequence homology search in UniProt yielded the correct ubiquitin sequence. Of ubiquitin´s 76 residues, sequence tags covering 25 residues were correctly determined automatically (SC =33%) from the dataset.

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Streamlining the Analysis of Proteins from Snake Venom

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Snake envenomation has been designated as a neglected tropical disease by the World Health Organization, which disproportionately affects rural and impoverished communities worldwide. Snake venom is also a potential source of new therapeutics. Because of the danger it may cause to people and its untapped therapeutic potential, it is important to characterize and understand snake venoms on a detailed molecular level. The demand for novel and efficient analytical methodologies capable of rapidly unraveling venom protein composition has led researchers to employ mass spectrometry (MS), focusing on native top-down and bottom-up proteomics. Despite considerable efforts in previous research, several technical challenges remain in characterizing the individual components within such complex mixtures, requiring new analytical workflows to be developed.

Methods

King Cobra (Ophiophagus hannah) whole venom was fractioned and exchanged into 200 mM ammonium acetate using size exclusion chromatography (SEC) on a Superdex Increase 200 10/300 GL column (Cytiva, Massachusetts, United States). The MS experiments were performed on a SELECT SERIES Cyclic IMS mass spectrometer (Waters, Manchester, UK) optimized for native MS. Charge reduction and top-down experiments were performed by selecting the target charge state of a protein with a quadrupole, separating the ions with one cycle in the ion mobility cell, and subjecting them to either CID or ECD in the instrument's transfer region. A temperature-controlled nESI-MS source was used to adjust the spray solution temperature from 25 °C to 75 °C to monitor disulfide bond reduction.

Novel Aspect

This work demonstrates how IMS, ECD charge reduction, and TC-nESI-MS can be used to characterize complex mixtures.

Preliminary Data or Plenary Speaker Abstract

Here, we demonstrate how temperature-controlled nanoelectrospray ionization mass spectrometry (TC-nESI-MS), cyclic ion mobility spectrometry (cIMS), and electron capture dissociation (ECD) can be used to elucidate the composition of King Cobra (Ophiophagus hannah) venom.

First, cIMS was used to isolate the individual charge state series in the highly convoluted mass spectra of the venom proteins eluting in the earliest SEC peak. Five distinct charge state series were extracted from the cIMS mobilogram, with calculated masses of 126.6 kDa, 110.1 kDa, 105.2 kDa, 60.0 kDa, and 54.7 kDa, which corresponded to different L-amino acid oxidases and snake venom metalloproteases. However, there was still significant charge state overlap in the cIMS mobilogram, lowering the accuracy of the mass calculations. Therefore, ECD was also applied to generate a charge state series for each proteoform following cIMS separation. This charge reduction experiment yielded discrete charge state distributions, allowing for more accurate mass calculations.

Next, we performed a top-down analysis on the glycosylated toxin that eluted next in the SEC. The toxin charge states had broad profiles, common for glycosylated proteins, with an average mass of 25.5 kDa. The only fragmentation observed on this toxin corresponded to the loss of glycans, which provided a unique opportunity to perform a top-down glycomics experiment. The fragments found during the top-down glycomics experiments indicated that this toxin contains complex fucosylated glycans.

Finally, TC-nESI-MS was used to monitor the reduction of disulfide bonds in three-finger toxins by DTT for top-down sequencing. This is important, as the disulfide bond patterns of these toxins make them challenging to fragment by collisional energy. Also, due to their high number of disulfide bonds, these toxins can be tricky to denature fully. These experiments allowed us to continuously monitor the reduction of the disulfide bonds and perform top-down sequence analysis of a β-cardiotoxin.

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An efficient method for sequencing the middle portion of mAbs by performing middle-down analysis of partially reduced mAb subunits

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Electron-based top-down and middle-down mass spectrometry have gained more interest in recent years with the advent of new hardware.
One challenge of such analysis is getting sequence of the middle portion of the protein, where the diagnostic fragments are particularly vulnerable for secondary and tertiary fragmentation yielding shorter terminal fragments together with uninformative internal fragments.
In this work, we performed partial disulfide bond reduction of mAb subunits under non-denaturing condition to retain the intra-chain disulfide bonds. Unreduced disulfide bonds protect larger fragments from secondary fragmentation in EAD MSMS and vastly improve sequence coverage in the region between the two disulfide bridges. When combined with data from conventional experiments (complete reduction of disulfide bonds) ~20% increase in sequence coverage was observed.

Methods

For preparation of partially reduced IdeS subunit samples, NIST mAb was digested with IdeS then reduced using TCEP and alkylated using Iodoacetamide following a standard protocol. For NIST mAb HC subunit, the reduction was performed using DTT. For completely reduced samples an extra denaturing step in guanidine was applied prior to the reduction step.
Top-down data was acquired on Sciex ZenoTOF 7600 instrument using EAD fragmentation. EAD was performed at KE=0V, electron beam current =1500nA, reaction time=5ms and ETC =100% for IdeS subunits and ETC=50% for HC.
Data was processed in research version of PeakView (Sciex). To minimize false positives, only c' and z* fragment ions were considered for matching. Fragment mass tolerance was set at 10 ppm.

Novel Aspect

A simple method to efficiently sequence middle portions of the mAb in middle-down analysis of subunits.

Preliminary Data or Plenary Speaker Abstract

Electron capture efficiency is proportional to Z2 in ECD process. Following the fragmentation event, the product charge is proportional to its size and hence larger products have higher secondary reaction rate. Given linear structure of the protein, such secondary reaction converts larger terminal fragment to shorter terminal fragments and uninformative internal fragments. This effectively depletes the signal for longer fragments necessary for sequencing the middle portion of a large protein.
Intra-chain disulfide bond severely inhibits the fragmentation process possibly acting as a secondary link, which upon secondary fragmentation of the backbone holds the longer fragment together, making it detectable. Such property can be exploited for sequencing of an unprotected by disulfide bond middle portion of IgG mAb protein. For this standard partial disulfide bond reduction can be performed, which reduces the inter-chain disulfide bonds but retains the intra-chain disulfide bonds.
To demonstrate the method NIST mAb protein was analyzed. Its Fd' subunit contains two intra-chain disulfide bonds: C[22]-C[97] and C[147]-C[203]. When fully reduced, limited fragments were identified between C[97] and C[147].
Significant improvement in fragment identification was obtained between C[97] and C[147] when these two disulfide bonds were still linked.

The overall sequence coverage was improved by combining the sequence coverage from intra-chain disulfide bond reduced and non-reduced data. The sequence coverage was 63.4% for reduced and 44.1% for non-reduced Fd' subunit with a combined sequence coverage of 81.1%.
The heavy chain of NIST mAb contains four intra-chain disulfide bonds: C[22]-C[97], C[147]-C[203], C[264]-C[324] and C[370]-C[428] and has a MW of ~50KDa. All three region between these four disulfide bonds showed improvement in fragment identification, especially between C[97] to C[147] and C[324] to C[370]. The sequence coverage obtained was 30.4%, 29.1% and 45.2% for reduced, nonreduced and combined HC respectively.

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Optimising mass spectrometry to study the proteoform changes in VPS35-D620N protein mutation of Parkinson's disease

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The D620N mutation in the VPS35 protein is a crucial factor in Parkinson's Disease (PD) pathology, yet the precise signaling pathways involved remain inadequately understood. While top-down mass spectrometry (MS) presents a promising avenue for analyzing proteoforms within proteomic samples, it encounters obstacles such as sensitivity, resolution, and ion suppression. Consequently, meticulous attention to sample preparation and separation protocols becomes imperative to ensure robust top-down proteomic analyses. In this regard, we have meticulously refined various sample preparation techniques including immunoprecipitation, gel separation, acetonitrile precipitation, and solid-phase cartridge separation. These methodologies have been tailored to selectively target proteins with a molecular weight below 30 kDa, thus facilitating comprehensive investigations into proteoforms.

Methods

Littermate-matched Mouse Embryonic Fibroblasts, harboring either wild-type VPS35 or a homozygous knock-in of the Parkinson-associated VPS35[D620N] mutant, were generated. For immunoprecipitation experiments, samples were incubated and eluted with 0.5% TFA solution, 1% SDS, or 8M urea. Detergents were removed using a HiPPR detergent removal spin column kit. High molecular weight proteins were removed using MWCO filters or acetonitrile precipitation methods. Offline fractionation with C8 cartridges was applied to reduce the complexity of the samples. Subsequently, samples were loaded onto an MAbPac RP column into an Orbitrap Exploris 240 MS. Data were analyzed using the MASH Suite software.

Novel Aspect

New, simple sample preparations have been optimized for top-down proteomics, enhancing the detection of low molecular weight (<30 kDa) proteins.

Preliminary Data or Plenary Speaker Abstract

Acetone or chloroform precipitation and MWCO filter washes with a high molarity of urea solution are the most common methods for removing detergents from top-down proteomics samples. These methods effectively eliminate all types of detergents but also lead to a significant protein loss, particularly of low molecular weight proteins, which are soluble in high organic solvents and easily trapped in the membrane of MWCO filters. We found that using a HiPPR detergent removal spin column kit can reduce the loss of low molecular weight proteins by at least 20%.

SDS-PAGE gel is the most common tool used by biologists to separate proteins in complex samples. However, the extraction of intact proteins from the gel presents challenges. Previous publications have demonstrated the combined use of high pH extraction solutions, followed by chloroform precipitation and finally concentration using MWCO filters. While this method significantly enhances intact protein recovery, it results in a dramatic loss of low molecular weight proteins. In our experiments, we found that acetonitrile mixed with 100 mM ammonium bicarbonate solution was the most effective extraction buffer for intact proteins. Extracted samples can be directly concentrated using MWCO filters without chloroform precipitation, thereby reducing the loss of low mass proteins.

With the optimized sample preparation methods, we can easily observe over 150 proteins with more than 400 different proteoforms in a single-shot experiment. The most common modifications

observed are oxidation, acetylation, and phosphorylation. Surprisingly, a modification with a mass shift of +52.9 Da was also commonly observed in the mutant samples, which we believe is a modification caused by iron metal binding. Further detailed analysis is required to validate this hypothesis.

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Ion mobility-based enrichment-free terminomics analysis of protease substrates.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Proteases contribute to numerous biochemical processes by catalysing the irreversible post-translational modification of proteolysis, broadly regulating protein homeostasis and various signalling cascades. As such, aberrant proteolysis is often associated with various disease states including inflammation, neurodegeneration, and cancer. It therefore remains critical to assess protease function by profiling proteolytic substrates in physiological settings. Conventional methods for protease substrate identification employ peptide-based N/C-terminomics methods which utilise enrichment methods for protease-generated (neo) N/C-termini detection. Whilst these methods have proven useful, they often require specialised material and user expertise, and often sacrifice protein abundance information. Considering this, we aimed to employ an online fractionation method for deeper proteome coverage and terminal peptide detection without prior enrichment for a more streamlined N-terminomics workflow.

Methods

Using high-field asymmetric waveform ion mobility spectrometry (FAIMS), we demonstrate a streamlined approach for proteome and terminome analyses without the need for termini enrichment methodologies. By combining protein dimethyl labelling with paramagnetic SP3 (single-pot, solid-phase sample-preparation) bead clean-up followed by peptide-based fractionation, deep proteomic and terminome analysis can be achieved with as little as 20 µg using a Orbitrap 480™ mass spectrometer equipped with a FAIMS Pro interface with six static compensational voltages (-35, -45, -55, -65, -75 and -85). Analysis of proteomic datasets using MSFragger (FragPipe v.18.0) with label-free quantification (IonQuant v.1.8.0) followed by statistical analysis using Perseus reveals both proteome and terminome changes can be quantified within a single analytical workflow.

Novel Aspect

Combined, these data demonstrate the utility of FAIMS to improve proteome coverage and terminome depth for protease substrate identification.

Preliminary Data or Plenary Speaker Abstract

We compared unfractionated and FAIMS-fractionated RAW264.7 cell lysates treated with or without the legumain-specific inhibitor (SD-134) (n = 4/group) and found an approximate two-fold increase in both peptide and N-termini detection. Whilst 32,849 unique peptides corresponding to 3,762 proteins were identified in the unfractionated sample, FAIMS identified 66,819 peptides from 6,164 proteins. Concurrently, the enhanced proteome coverage enabled more extensive N-termini identification, increasing from 1,145 to 2,882 following FAIMS fractionation. Of these N-termini, 2,038 were solely identified following FAIMS fractionation, enhancing the detection of protease-generated (neo) N-termini. This is most likely a result of each fraction identifying a pool of unique peptides and N-termini, thus expanding the proteome coverage. By comparing our FAIMS-fractionated data to previously reported TAILS (Terminal Amine Isotope Labelling of Substrates) N-terminomics data, we observed similar N-termini identifications despite using approximately ten times less starting material. These results support FAIMS-facilitated N-terminomics analysis for

streamlined and robust detection of N-termini, bypassing the need for N-terminal peptide enrichment steps by instead improving proteome coverage for enhanced N-termini identifications.

We applied this method to profile the degradome of the cysteine protease legumain in naïve spleens from wild-type (WT) and legumain-deficient (Lgmn-/-) mice. Our approach was successful in identifying 64,649 peptides and 6,366 proteins, of which, 2,528 represented N-termini. We identified 235 N-termini to be enriched in WT spleen and of those, 119 (50.6%) resulted from asparaginyl cleavage events. Considering legumain is the only know mammalian protease to exhibit asparaginyl endopeptidase activity, these 119 N-termini represent putative legumain substrates and hint at novel functions for the cysteine protease. As such, this study is the first to characterise physiological legumain substrates in an unbiased and systematic manner, utilising a novel enrichment-free terminomics workflow for detection of both protein abundance changes and native cleavage events in the one experiment.

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Identification of the Doublecortin like kinase 1 (DCLK1) interactome reveals novel kinase dependent processes involved in cell cycle processes.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Gastric cancer (GC) is the third leading cause in cancer related deaths worldwide. Since TCGA molecularly profiled GCs many new promising targets have emerged for different GC subtypes. Unfortunately, no new therapeutic targets have emerged for the genomic stable (GS) subtype, which has the lowest overall survival. However, Ser/Thr-protein kinase Doublecortin like kinase 1 (DCLK1) is significantly upregulated in GS GCs. DCLK1 is a microtubule associated protein family member, hence important for cellular shape, polarity, migration, mitosis, and vesicular transport. DCLK1 has been shown to promote epithelial-to-mesenchymal transition and inducing migration and invasion in many different solid cancers. Therefore, we aimed determine how molecularly DCLK1 contributes to gastric cancer progression and identifying kinase substrates to assess future therapeutic options.

Methods

: Total- and phospho-proteomics was performed on lysates of parental GC cell line MKN1, DCLK1 overexpressing MKN1 with and without the highly specific DCLK1-IN-1 kinase inhibitor (n=6/condition) with 10ug and 200ug protein lysate respectively, according to the “easy-phos” method. LC-MS/MS was performed on the Thermo Orbitrap Eclipse. MS Amanda 2.0 and MSFragger were used for label free (phospho)peptide identification & quantification.
For the DCLK1 interactome, FLAG-tagged DCLK1, DCLK1-IN-1 treated, and kinase dead mutant were transiently expressed in HEK293T and MKN1 cells (n=4), prior to anti-Flag co-immunoprecipitation. Polled elutions were subsequently analysed on the Q-Executive HF. DCLK1 interactors were confidently identified with the Significance Analysis of INTeractome (SAINT) software.

Novel Aspect

DCLK1’s reversible involvement in cell cycle regulation makes it a promising targetable regulator for therapy resistant GCs.

Preliminary Data or Plenary Speaker Abstract

Stable DCLK1 overexpression resulted in increased cellular protrusions and cell migration in vitro, which was reversed upon kinase inhibition with the highly specific DCLK1-IN-1 inhibitor. Subsequent label-free quantitative (phospho)proteomics revealed significant changes in RNA-processing, cell-cell adhesion, cell cycle processes, cellular matrix organization, chromatin organization, and vesicular transport upon either DCLK1 overexpression or inhibition. 91 DCLK1 interactors were confidently identified for the on FLAG-tagged DCLK1, DCLK1-IN-1 treated, and kinase dead mutant. Next we compared the interactome and phospho-proteome data and identified 9 overlapping potential DCLK1 kinase substrates. These 9 proteins are again involved in membrane trafficking, RNA processing, cytoskeletal-, mitotic spindle-, and chromosome organization.
Many of these processes contribute to the cell cycle phase, flow cytometry analysis confirmed a reduction of cells in G1-phase, and increase of cells residing in the G2/M-phase. With the decrease in kinase activity of DCLK1, DCLK1-IN-1 treated, and kinase dead mutants, DCLK1 localizes more prominently within the nucleus. Suggesting a kinase dependent role for DCLK1 in G2/M-phase check-point transitions.

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Cell surface proteomics of macrophages upon dead cell clearance

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Dying cells release inflammatory factors and must be cleared to resolve inflammation. Defects in dead cell clearance can lead to chronic inflammation and autoimmune disease. Dead cell engulfment and its environmental effects must be mechanistically understood to control inflammation. Macrophages, the professional phagocytes, sense phosphatidylserine (PS) exposed on the surface of dying cells through PS receptors such as Mertk and Axl. PS receptor activation and dead cell engulfment results in inflammatory reprogramming, which is marked by the upregulation and release of anti-inflammatory factors, increased PS receptor production and change of surface protein levels. Here we aim to identify receptor complex- and surface proteome changes that influence signalling and the inflammatory potential of engulfing macrophages.

Methods

We induced dead cell clearance by incubating SILAC labelled dead Jurkat cells with bone marrow derived macrophages. For receptor complex enrichment, Mertk and Axl were immunoprecipitated after 1 hour of co-incubation with dead cells and binding proteins were analysed in data dependent acquisition. To analyse overall changes of the surface proteome, macrophages' surface proteins were linked to aminooxy-biotin and enriched via streptavidin Sepharose beads 3 and 20 hours after co-incubation with dead cells. The surface proteome was subsequently acquired in data independent acquisition mode on the Astral.

Novel Aspect

The unravelling of receptor complex regulations upon dead cell clearance will enable us to influence inflammatory programming of macrophages.

Preliminary Data or Plenary Speaker Abstract

Enrichment of surface proteins resulted in the increased detection of > 700 surface proteins, which included a macrophage specific receptor signature among the most enriched proteins. A 1D annotation analysis revealed a significant reduction of proteins annotated as exocyst proteins in samples of 20 hours post incubation with dead cells. This might indicate reduced vesicle formation of macrophages several hours after dead cell clearance.
Receptor complex immunoprecipitations revealed dynamic interaction partners with the key PS receptors and will be further analysed in functional cell-based assays.

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Exploring the Cellular ADP-Ribosylome in Deinococcus radiodurans

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Protein ADP-ribosylation, a conserved post-translational modification, plays a vital role in regulating various cellular processes, including gene expression, cellular signaling, and DNA repair. While extensively studied in higher organisms, recent evidence suggests its conservation in primitive prokaryotes, impacting critical cellular functions. However, research on poly-ADP-ribose (PAR) metabolism in prokaryotes is limited. Our study focuses on the extreme radiation-resistant bacterium D. radiodurans, revealing the presence of PAR and upregulated expression of PAR-processing enzymes post-radiation. Crystal structure analysis of ADP-ribose-bound DrPARG indicates potential endo-glycohydrolase activity, further confirming PAR existence. Mass spectrometry-based proteomics could offer valuable insights into ADP-ribosylation, shedding light on its physiological relevance and substrates regulation. Efforts to develop robust methodologies for mapping ADP-ribosylome are crucial for future research.

Methods

Experimental procedures involved protein expression and purification of DrPARG gene, SDS-PAGE, in-gel digestion, and mass spectrometric analysis. Bioinformatics methods were employed to analyze the obtained data. Additionally, bacterial growth conditions were optimized for irradiation experiments. The DrPARG gene was PCR-amplified from D. radiodurans genomic DNA and cloned into the pET28a vector system. Recombinant DrPARG_E112A protein with a His-tag was expressed in Escherichia coli BL21(DE3) cells and purified using affinity chromatography. Mass spectrometry analysis provided insights into protein interactions. Bioinformatics tools such as DAVID and Panther Gene Ontology were used for functional annotation. Overall, these techniques facilitated comprehensive understanding of DrPARG protein function and its interaction network.

Novel Aspect

Our research highlights the role of poly-ADP-ribosylation in bacteria and its correlation with DNA damage response.

Preliminary Data or Plenary Speaker Abstract

The results shed light on the development of methodologies to investigate protein ADP-ribosylation in Deinococcus radiodurans. Initially, we noted varying levels of poly-ADP-ribosylation (PAR) in response to genotoxic stress, notably under UV irradiation. Western blot analysis confirmed distinct PAR levels in the DrPARG deletion strain (Δparg) compared to the wild-type strain, suggesting the Δparg mutant's suitability for further analysis. Particularly, UV irradiation induced the highest PAR levels in the Δparg strain, implying a role for poly-ADP-ribosylation in responding to UV-induced DNA damage.
Subsequently, we employed affinity-purification mass spectrometry using the DrPARG_E112A mutant and an anti-PAR antibody for unbiased profiling of the ADP-ribosylome. This approach identified significant candidates involved in diverse biological processes like translation, proteolysis, DNA replication, and repair. Functional annotation revealed enrichment of intracellular signaling and nucleotide-binding proteins within the ADP-ribosylome, underscoring potential regulatory roles in DNA and RNA biology.
Furthermore, comparative analysis between the DrPARG-associated proteome and the anti-PAR-associated poly-ADP-ribosylome uncovered convergent pathways, particularly in DNA repair and metabolism. The overlap suggests common regulatory mechanisms mediated by ADP-ribosylation in response to genotoxic stress.
In summary, our findings demonstrate the feasibility of sensitive and unbiased methodologies for studying protein ADP-ribosylation in D. radiodurans. These insights lay the groundwork for further

exploration of ADP-ribosylation's functional significance in bacterial physiology and stress response, with potential implications for understanding DNA damage repair mechanisms and microbial resilience to environmental challenges.

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A pharmacological dimension to personalised phosphoproteomics of human skeletal muscle.

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Recent studies by our group suggest that the kinase mTORC1 (the mammalian target of rapamycin) may regulate changes to insulin sensitivity in skeletal muscle following exercise. To investigate this directly in humans we employed an approach recently developed by our group ('personalised phosphoproteomics') to study the effect of Rapamycin on muscle signalling networks and their interactions with both insulin and exercise.

Methods

We recruited 13 healthy human subjects and administered a single dose of rapamycin using a double blinded placebo crossover study design. Subjects performed one-legged exercise for 2 hours, and 4 hours post-exercise a 2 hour hyperinsulinemic-euglycemic clamp was administered. Muscle biopsies from m. vastus lateralis were obtained immediately prior to the clamp in both the rested and exercised leg, and at the conclusion of the clamp. Frequent blood samples from catheterised femoral and arterial veins were additionally taken, concomitantly with blood flow measurements, enabling the direct measure of glucose utilization by the muscle bed. Phosphopeptides were prepared using our EasyPhos method, and measured phosphoproteomes using a Vanquish Neo HPLC coupled to an Orbitrap Astral in narrow window data-independent acquisition mode.

Novel Aspect

We employed personalised phosphoproteomics to explore the modulation of human muscle insulin sensitivity by both exercise and Rapamycin.

Preliminary Data or Plenary Speaker Abstract

Earlier personalised phosphoproteomics studies by our group under similar experimental conditions revealed that the phosphosite AMPKa2 Serine 377 is an mTORC1 substrate and may regulate skeletal muscle insulin sensitivity in response to exercise. Here we extended the personalised phosphoproteomics paradigm to include a pharmaco-dimension through the addition of treatment with the mTORC1 inhibitor Rapamycin, to directly assess the role of mTORC1 on insulin sensitivity following exercise in humans. Our global phosphoproteomics analysis quantified over 60,000 Class I phosphopeptides in human skeletal muscle tissue, and through comprehensive mass spectrometry-based analyses we identified and quantified phosphosites within the mTOR pathway across eight different conditions in 13 subjects (104 biopsies in total). As we observed previously, skeletal muscle phosphoproteomes exhibited high inter-subject variation, however our paired experimental design enabled us to uncover numerous novel phosphorylation events modulated by mTORC1 and its downstream effectors. Ongoing investigation into these phosphosites and their potential kinases are shedding light on the complex interplay between mTOR, insulin and exercise at an individual and group level.

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Integrating Mass Spectrometry methods for characterisation of PROTAC-induced ubiquitination architecture

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Proteolysis-Targeting Chimera (PROTAC) technology holds the promise of rapid and reversible depletion of therapeutically-relevant protein targets through induced ubiquitination. However, PROTAC development is hampered by a limited understanding of detailed PROTAC mechanism-of-action, thus relies heavily on costly trial and error.
In 2021, Kaiho-Soma et al. identified that upon PROTAC treatment, an additional E3 ligase, TRIP12, works alongside CRL2VHL to assemble K29/K48 branched ubiquitin chains on in vitro substrate, enhancing the PROTAC efficiency. Such observation highlights the cooperativity in the underlying mechanism of PROTAC function.
My project employed a range of Mass Spectrometry methods to provide the first insight into the ubiquitination events induced on a single in vivo target and paves the way for a deeper understanding of PROTAC activity.

Methods

LC-MSMS, AQUA MS, Intact MS

Novel Aspect

This is the first insight into PROTAC-induced ubiquitination on a single in vivo target, providing deeper understanding of PROTAC functionality.

Preliminary Data or Plenary Speaker Abstract

Using a range of mass spectrometry methods and ubiquitin clipping I have defined the distinct architecture and composition of polyubiquitin chains formed on a model substrate as well as specific ubiquitination sites on the model substrate upon PROTAC treatment.

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Compartmentalized Acetyl-CoA Metabolism in Beige Adipocyte Thermogenesis: Potential Obesity Treatment Targets

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The global obesity epidemic necessitates novel therapeutic interventions. One promising avenue is harnessing the thermogenic potential of beige adipocytes, characterized by high energy expenditure and mitochondrial density. Integrating proteome, phosphoproteome, and acetylome analyses, this study investigate the crosstalk between post-translational modifications and revealed that acetyl-CoA (AcCoA) metabolism in promoting beige adipocyte thermogenesis: a key cellular process implicated in weight regulation.

Methods

Our investigation centers on the subcellular distribution of AcCoA and its regulatory roles in adipocyte beiging. Through pharmacological manipulation of AcCoA levels in different cellular compartments, we explored the subsequent effects on beige adipocyte function. We induced differentiation of immortalized preadipocytes into mature beige adipocytes, using oil red O and BODIPY staining to track lipid droplet accumulation, coupled with quantitative image analysis by Cell Profiler. Quantitative proteome analysis, enabled by isobaric labeling and basic reverse-phase fractionation, identified differentially expressed proteins. Additionally, we employed Seahorse XF Analyzers to measure key metabolic indicators such as the oxygen consumption rate and extracellular acidification rate. Mitophagy activity, a critical process in mitochondrial quality control, was assessed through the colocalization of LC3 and mitochondria.

Novel Aspect

This study establishes mitochondrial AcCoA as a pivotal factor in beige adipocyte thermogenesis and a potential therapeutic target for obesity.

Preliminary Data or Plenary Speaker Abstract

Our findings demonstrate that augmenting mitochondrial AcCoA reduces lipid accumulation and promotes a thermogenic gene expression profile in beige adipocytes. Moreover, increasing mitochondrial AcCoA mirrors the effects of β3-adrenergic agonists, boosting the thermogenic capacity of beige cells. We also elucidate that mitochondrial AcCoA modulates mitophagy, with potential implications for mitochondrial homeostasis and function. By modulating mitochondrial AcCoA, we can potentially amplify energy expenditure in adipose tissue, offering a strategic approach to combat obesity and related metabolic disorders.

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Surfaceome mapping of the vascular endothelium to identify therapeutic targets for treating ischaemia / reperfusion injury.

Ms Iasmin Inocencio[1], Dr Alin Rai[1], Dr David Greening[1]
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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Myocardial infarction (MI) is a leading cause of death worldwide. About 30% of MI survivors will develop heart failure due to ischaemia-reperfusion (I/R) injury. Unfortunately, delivering selective and targeted therapeutics to treat I/R injury remains a clinical challenge. To overcome this challenge the vascular endothelial cell surface poses as a central target for intervention, given its direct accessibility to circulation. Targeting the transformation of cell surface protein landscape offers an opportunity for precise therapeutic delivery and curbing off-target effects. Here, we report the surfaceome map of mouse coronary vasculature. We also report the shifts in vascular surface proteins in the model of I/R injury, providing insights to identifying selective molecular targets to enable safe, non-invasive, and effective targeting strategies.

Methods

Here, we describe the combination of a chemical labelling technique using membrane-impermeable biotin and quantitative mass spectrometry (MS)-based proteomics. To profile the surface proteome (surfaceome) of the mouse coronary endothelium, we labelled surface proteins with biotin-saline solution and enriched via a neutravidin-based enrichment. To investigate human venous (HUVEC) and arterial (HAEC) endothelial cells in I/R injury, cell lines were exposed to 4 – 24 hours hypoxia in a nitrogen purged chamber followed by 6 – 24 hours reoxygenation to simulate injury. Cell surface proteins were biotin-labelled and enriched as the previous and tryptic peptides were analysed by data independent acquisition MS.

Novel Aspect

Surfaceome mapping facilitates the identification of vasculature specific targets for clinical treatment of I/R related injuries.

Preliminary Data or Plenary Speaker Abstract

We report experimental evidence of surface localisation and extracellular domains for 353 proteins from mouse coronary vascular surfaceome, with known localised factors associated with vascular surface identified to support cell migration, angiogenesis, and vessel stability (i.e., TEK, VASP and ANXA2). Overall, 2091 and 1600 surface proteins were identified in HUVEC and HAEC, respectively. From those surfaceome populations, a 537 and 1140 surface proteins were significantly dysregulated (student's t-test, FDR<0.05) in HUVEC and HAEC under I/R injury. Further 206 surface proteins were commonly dysregulated in these two cell lines. Functional enrichment analysis reveals significantly enriched terms including "cell adhesion" (GO:0050839, p-adjusted value: 3.19E-27), "angiogenesis" (GO:0001525, p-adjusted value: 1.32E-07) and "integrin cell surface interactions" (REAC: R-HSA-216083, p-adjusted value: 4.56135E-05). More importantly, we have identified key proteins relating to inflammatory processes (i.e., MARCKS, IL6ST, TEK, WDFY1) which is a hallmark of I/R injury.

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Uncovering the Anti-Stroke Potential of Electrophilic Dietary Natural Products via Chemical Proteomics Approaches

Miss Ivy Guan[1,2], Miss Joanna Liu[1,2], Miss Renata Sawyer[1,2], Dr Xuyu Liu[1,2]
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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Acute ischaemic stroke is a global leading cause of death and disability. Approximately 85% of these cases result from a blood clot obstructing an artery, depriving the brain of essential nutrients. Although antiplatelet drugs offer potential in enhancing vessel clearance, the associated risk of brain bleeding limits their approval for clot-lysis therapy in stroke clinics. In this study, we focus on investigating the natural antiplatelet agents found in healthy diets with a view to identify safer protein targets for future antithrombotic discovery campaigns.

Methods

Medium-throughput phenotypic screening using light-transmitted platelet aggregometry was conducted to examine the antiplatelet activity of cardioprotective natural products. Sulforaphane (SFN), a predominant electrophilic lipid isolated from broccoli, was found selectively suppresses platelet activity in response to ADP biochemical stimuli and under flow disturbances - conditions that resemble stroke onset. An alkyne-based probe of SFN was developed to map the bone fide target responsible for the antiplatelet phenotype using proteomics. To streamline the process of identifying protein targets for multiple natural products, a novel multiplexed competitive proteomic approach was developed. This involved competitively labeling natural products with 6plex iodoTMT, followed by enrichment of labeled peptides and LC-MS/MS analysis. For validation, corresponding probes based on the alkyne strategy were synthesized.

Novel Aspect

With the traditional alkyne probe strategy having distinct advantages, multiplex proteomics allows high-throughput protein target identification for drug discovery.

Preliminary Data or Plenary Speaker Abstract

Platelet proteomic analysis using the SFN alkyne probe led to the identification of the protein disulfide isomerase (PDI) A6 isoform as the principal target of SFN in platelets through covalent modulation of two isoform-specific cysteines, Cys291 and Cys297. Isoform selectivity remains a challenge in PDI-focused drug discovery, which our approach introduces a novel perspective for developing precision medicine of PDI proteins. The PDIA6 catalytic acivity was partially inhibited by SFN, demonstrated by insulin aggregation assay in comparison with a commercially available PDI covalent inhibitor, PACMA31. Interactome mapping analysis further revealed the important role of PDIA6 in regulating platelet activation under shear-induced thrombotic conditions. Subsequent in vivo studies with our electrolytic injury murine models have shown that SFN improves recanalization outcomes when administered as a prophylactic agent. Importantly, SFN incurs minimal bleeding risk in the tail bleeding experiment, suggesting a novel, safer strategy for stroke intervention.
With sustained interest in natural products, several natural compounds showing potential safe antiplatelet effects were identified - 6-shogaol (from ginger), 9-nitrooleate (from brassica sprout seeds), xanthohumol (from hops flower) and oleocanthal (from olives). Preliminary competitive proteomic analyses were conducted with these five antiplatelet natural products, including sulforaphane, while further target validation experiments will be needed, such as protein targeting site identification and in vitro protein activity assay.

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Secretome analysis sheds light on macrophage reprogramming upon dead cell clearance

Jenny Riedel[1,2], Mr Kael Schoffer[1], Dr. Lianju Shen[1], Charlotte Williams[1,3], Dr. Maria Tanzer[1,3]
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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Macrophages play an important role in the clearance of dead cells in regular cell turnover. However, they also serve a pivotal role in resolving inflammation, which is mostly accompanied by an increase of dying cells. These dying are removed by macrophages to resolve inflammation. Dysregulation in the clearance of dead cells drives inflammation and can result in autoimmune diseases and atherosclerosis. Macrophages not only phagocytose dead cells, but also undergo reprogramming, which results in the release of proteins, amongst them wound-healing factors. Here, we conducted a comprehensive analysis of proteins released by macrophages following phagocytosis of dead cells and analysed the effect of these proteins on neighbouring cells.

Methods

We irradiated SILAC labelled T-cells to induce cell death and incubated them with macrophages for 2 hours. The dead cells were washed off and the macrophages were left overnight. Supernatants were harvested the next day, precipitated, and analysed with the Astral in data independent acquisition mode. To examine the effect of proteins released by phagocytosing macrophages on neighbouring cells, we incubated fibroblasts and macrophages with the supernatant and subsequently analysed their proteome.

Novel Aspect

We provide a comprehensive analysis of the supernatant of macrophages after dead cell clearance and their effect on neighbouring cells.

Preliminary Data or Plenary Speaker Abstract

In first experiments we were able to detect up toOverall, we detected up to 3211 proteins in the secretome of efferocytosing macrophages. We see over 300 proteins significant changes in protein releasewere significantly upregulated between efferocytosing engulfing and non-engulfingefferocytosing macrophages. Known markers of macrophage reprogramming such as TGFbeta, Igf1 and Gdf15 were released upon dead cell engulfment. . Pathway enrichment analysis shows upregulation in of a range of proteins involved in extracellular matrix organisation, fibrinolysis, blood coagulation and wound healing upon efferocytosis clearance of apoptotic cells. We also detected enrichment of receptor proteins in the supernatant of engulfing cells. Protease inhibitor experiments revealed metalloproteinase mediated cleavage of selected receptors. Macrophages and fibroblasts exposed to proteins released upon dead cell phagocytosis revealed very specific protein changes, such as the upregulation of Cxcl4, a potential wound healing factor.We also see an increase in the release of markers of efferocytosis (GDF-15), anti-inflammatory factors and receptors. In macrophages that engulfed necroptotic cells for two hours we saw an increase in the release of inflammatory factors, such as IFNɣ. Proteins contributing to fibrinolysis and extracellular cellular matrix reorganisation are also released from macrophages, exposed to necroptotic cells. We show that there is a difference in the way macrophages respond to clearing different dead cells. We provide a protein view of this reprogramming.

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Investigating the macrophage phosphoproteome upon dead cell clearance

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Efferocytosis is a process by which macrophages – key cells of the immune system – engulf and remove dead cells. This process prevents the release of inflammatory molecules by dead cells and induces reprogramming of macrophages towards a wound healing/anti-inflammatory state. Signalling events induced upon dead cell sensing via phosphatidylserine receptors that drive engulfment and macrophage reprogramming are poorly studied. Here, we take a comprehensive and unbiased approach to study signalling by analysing the phosphoproteome changes of macrophages upon exposure to dead cells.

Methods

Mouse bone marrow derived macrophages (BMDMs) were incubated with SILAC-labelled dead T-cells, for either 5, 15 or 60 minutes, or left untreated. The macrophages were then harvested and phosphopeptides were enriched using TiO2 beads. The phosphopeptides were injected into a Thermo Exploris and acquired in data-independent acquisition (DIA) mode.

Novel Aspect

This data provides insights into important signalling events that drive dead cell engulfment and inflammatory reprogramming upon efferocytosis.

Preliminary Data or Plenary Speaker Abstract

We detected about 27,500 phosphosites per sample and 48,700 phosphosites across the whole experiment, while 9,780 were changing significantly. Of note, Erk1/2, Itpk, and Pi4kb phosphorylation was strongly induced after 15-minute co-incubation with dead T cells. Enrichment analyses revealed increased phosphorylation of GTPase proteins and proteins involved in cell migration after 15-minute of dead cell co-incubation. Furthermore, proteins involved in mTOR signalling were increasingly phosphorylated in macrophages co-incubated with dead cells for 60 minutes. These results show that phosphorylation is critically involved in most important processes that drive efferocytosis.

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Unraveling the Mysteries of PHACTR1: A Multi-Omics Perspective on Vascular Disease

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Genetic studies have ranked the PHACTR1 gene in the top 2 most important genes for vascular disease. Uniquely to PHACTR1, elevation and decrease of gene expression are linked to two different subsets of disease. Increased PHACTR1 is associated with migraines, fibromuscular dysplasia and spontaneous coronary arterial dissection, whereas decreased gene expression is causal for coronary artery disease and hypertension. These differing disease profiles highlight PHACTR1's central role in vascular homeostasis. Despite a decade of research, the underlying molecular mechanisms by which PHACTR1 signals are poorly understood. To address this knowledge gap, we have developed a novel multi-omics integration pipeline to elucidate how PHACTR1-mediated signalling governs the flow of information within the cell from translation to end metabolic effectors.

Methods

Parallel sets of stably transduced HT1080 cells (PHACTR1 overexpression [OE] or knockdown [KD] and siRNA control) underwent multi-omics analysis consisting of bulk RNAseq (Illumina NovaSeq 6000), untargeted nano-flow proteomics (fritless nano column containing C18AQ with Thermo Orbitrap Fusion Lumos), untargeted metabolomics (ZORBAX Eclipse XDB-C18 or InfinityLab Poroshell 120 HILIC-Z column with Agilent 6546 QTOF) and targeted lipidomics (ZORBAX Eclipse plus C18 column with Agilent 6490 QQQ). 'Omics datasets were analyzed individually and then integrated using a series of R-based tools, including Ingenuity Pathway Analysis (IPA) and OmicsNet 2.0. Significantly changed pathways of interest were validated using targeted experimental approaches, such as qPCR, immunoblotting, cell microscopy, and Agilent Seahorse XF analysis.

Novel Aspect

Our novel multi-omics pipeline unlocks the biological significance of omics data, offering deeper understanding of critical gene functions.

Preliminary Data or Plenary Speaker Abstract

Multi-omics analysis of HT1080 cells resulted in a comprehensive dataset encompassing 14,737 transcripts, 5,666 proteins, 1,752 metabolites, and 617 lipid species. Overexpression and knockdown of PHACTR1 was validated in both the transcriptomic and proteomic datasets.
Significantly altered entities were detected in all individual 'omics datasets upon comparing PHACTR1 OE or KD cells to controls, at both feature and pathway levels. Many of these changes are linked to vascular disease. However, the unique strength of our approach lies in integrating these 'omics datasets and our initial findings revealing the diverse cellular processes governed by PHACTR1 signalling validate our need for integrative analysis.
While database mapping of genes and proteins is routine, current methods struggle with pathway analysis of metabolites and lipids. To ensure successful multi-omic pathway analysis, significant effort has been undertaken to map detected entities against known biological system databases. Ultimately, we employed Kyoto Encyclopedia of Genes and Genomes (KEGG) IDs in combination with Human Metabolome Data Base, ChEBI and Lipidmap IDs to maximise pathway coverage. Using this combined technique, we successfully mapped a high proportion of metabolites (108 of 187 significantly changed). Pathway analysis of lipids remains challenging, with less than 25% mapped. Despite these limitations, integrative analysis using Ingenuity Pathway Analysis (IPA) identified 12 pathways significantly altered in at least three omics datasets in PHACTR1 OE cells. These included

nitric oxide signalling, ferroptosis signalling, and extra-nuclear estrogen signalling, all essential for vascular homeostasis and, to our knowledge, have not previously been linked to PHACTR1.
This data unveils the multifaceted role of PHACTR1 within cells, providing valuable insights into its critical function in the vasculature and, ultimately, cardiovascular disease.

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Analysis of early epileptogenesis in mice by proteomics and phosphoproteomics identified RNA processing and synaptic plasticity as major signalling pathways

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

We investigated the phosphorylation-based signalling and protein level changes occurring early in a mouse model of epileptogenesis to gain new insight on how seizures transform affected brain tissue. Information on early phospho-signalling has been lacking.

Methods

The hippocampi of mice treated with pilocarpine were examined by quantitative mass spectrometry at 4 and 24 h post-status epilepticus to provide proteome and phosphoproteome data at great depth. Gene ontology enrichment analysis was performed.

Novel Aspect

The observations suggest that early epileptogenesis is characterized by signalling that stimulates translation and weakens neuronal excitability.

Preliminary Data or Plenary Speaker Abstract

Hundreds of proteins involved in RNA processing were the major early targets of increased phosphorylation. At 24 h, many protein level changes were detected and the phosphoproteome continued to be perturbed. The major targets of decreased phosphorylation at 4 h and 24 h were a subset of postsynaptic density scaffold proteins, ion channels and neurotransmitter receptors. Many proteins targeted by dephosphorylation at 4 h also had decreased protein abundance at 24 h, indicating a phosphatase-mediated weakening of synapses. Increased translation was indicated by protein changes at 24 h.

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Phosphomatics: Integrating Enhanced Features for Comprehensive Phosphoproteomics Data Analysis

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

The intricate dynamics of phosphorylation play a crucial role in cellular signaling and regulation, making phosphoproteomics an essential field for uncovering mechanisms underlying health and disease. Despite advances in mass spectrometry, the interpretation of phosphoproteomics data remains a formidable challenge due to its complexity and the vast quantity of data generated. Addressing this challenge requires sophisticated analytical tools that can decipher the functional implications of phosphorylation events and their regulatory networks. Herein, we highlight advances and new features in Phosphomatics, a free, web-based platform designed to bridge the gap between raw phosphoproteomics data and actionable biological insights.

Methods

Phosphomatics offers a comprehensive solution for the analysis of phosphoproteomics data, combining a user-friendly interface with powerful computational tools. As a freely available resource, it streamlines the data analysis process by incorporating various elements of statistical analysis, data visualization, and biological interpretation into a single platform. This update introduces advanced algorithms for missing data imputation, alongside innovative methods for upstream kinase prediction directly from protein sequences. Additionally, we have expanded the platform's capabilities to include enhanced plot customization and a broader array of visualizations, allowing for more nuanced data exploration and interpretation.

Novel Aspect

Enhanced analysis and visualization features for more flexible investigation of phosphoproteomics data with Phosphomatics.

Preliminary Data or Plenary Speaker Abstract

The latest version of Phosphomatics introduces key features for phosphoproteomics research. Phosphomatics now integrates the recently published Kinase Library offering the ability to perform primary sequence-based upstream kinase prediction and hyperactivation analysis. These analyses can reveal candidate upstream kinases for individual phosphorylation sites and also consider many phosphorylation sites simultaneously to identify kinases that may be hyperactive between experimental conditions. This capability is significant since it allows researchers to leverage more of their experimental data in generating biological hypotheses.
Secondly, we have implemented substantial new methods that allow users to handle missing quantitative values in uploaded datasets. This includes a range of visualisations to assess patterns of missingness in experimental data, as well as new algorithms and approaches to impute missing values where necessary. By improving how missing values are handled, Phosphomatics helps researchers obtain more accurate insights from their data.
Lastly, Phosphomatics now offers a wide range plot customisation options. These tools make it easier for users to interpret and present their data, facilitating clear communication of complex datasets.
To date, Phosphomatics has achieved 42,240 page views and 2,173 users from 42 countries. The number of active monthly users has increased from an average of 47 in 2021 to 122 in 2023 and now stands at 285 for the 2024 year to date.
Phosphomatics is freely available at www.phosphomatics.com.

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Identification of novel phosphopeptides in the immunopeptidome using hybrid EAD-CID fragmentation

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Immunopeptidomics is the mass spectrometry-based study of Human Leukocyte Antigen (HLA)-bound peptide antigens. These peptides, presented on the cell surface, allow T cells to monitor the cellular health status. Mutation-containing peptides or peptides bearing aberrant post-translational modifications (PTM) arising from cancer-associated signaling events, can elicit anti-tumour immune responses. However, analyzing PTM peptides, particularly immunopeptides that have diverse termini (non-tryptic), can be challenging. The traditional workflows in tandem mass spectrometry struggle with the loss of the PTM during fragmentation and, consequently, loss of accurate identification and localization of the modification. To circumvent some of these issues, we leveraged an alternative fragmentation method called Electron-Activated Dissociation (EAD) on the SCIEX ZenoTOF 7600 system to characterize PTM and non-PTM immunopeptides.

Methods

We used HLA-peptides isolated from mono-allelic B-cell lymphoma lines to optimize the electron kinetic energy (KE) and EAD reaction time to achieve a hybrid EAD-Collision-Induced Dissociation (CID) method (EAD-CID). First, the combination of 3 KE values (3eV, 6eV, and 9eV) and two EAD reaction times (10ms and 20ms) was examined. Next, we applied EAD-CID with the optimum values to Zr-IMAC HP enriched phosphopeptides isolated from HLA class I and class II molecules. In all the experiments (EAD-CID and CID), the collision reaction occurred for 30ms and the collision energy (CE) was automatically calculated with the dynamic CE settings.

Novel Aspect

The versatile and tunable hybrid EAD-CID fragmentation improves MS/MS fragment coverage and PTM site localization in immunopeptidomics studies.

Preliminary Data or Plenary Speaker Abstract

For peptides isolated from HLA Class I, we observed that a KE of 6eV and reaction time of 20ms boosted the number of identified phosphorylated and cysteinylated species. The same values worked equally well for the peptides isolated from HLA class II. Furthermore, we observed that both the optimum EAD-CID and the control CID methods identified peptides with similar characteristics in the IMAC-enriched HLA-peptides. However, the hybrid EAD-CID method outperformed the control CID method for the identification and characterization of phosphorylated HLA peptides. Overall, more than 200 phospho-HLA peptides with more than 20 novel phospho-sites were identified. Additionally, EAD-CID improved the spectral evidence for phospho-site localization to differentiate closely-eluting phosphopeptide isomers.

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Methyl Masters: Unveiling the Role of Yeast Methyl Transferases in Bioproduction

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Poster Session: Wednesday Posters, Exhibition Hall, August 21, 2024, 13:00 - 15:00

Astaxanthin is one of the most potent antioxidants found in nature. Our previous research at QUT successfully developed a yeast mutant of Phaffia rhodozyma that showed a remarkable 50-fold increase in astaxanthin synthesis. This mutant strain was previously extensively characterized by us using advanced genomics, transcriptomics, and metabolomics techniques, and recently, through proteomics. The analysis has highlighted epigenetic regulation as a pivotal factor influencing yield in this industrially crucial resource.

Methods

Quantitative protein profiling involved the analysis of wild type and mutant Phaffia rhodozyma specimens across different growth phases (n=3 per condition) using a SCIEX 5600+ QTOF mass spectrometer configured for capillary flow applications. Data analysis included peak area extraction using DIA-NN software and differential analysis using MSstats R package.

Novel Aspect

We propose that spatio-temporal organization of enzymes creates unique microenvironments that can enhance specific metabolic pathways and promote epigenetic modifications.

Preliminary Data or Plenary Speaker Abstract

We were able to quantify nearly 4,500 proteins, which largely validated previous findings and revealed significant regulatory patterns in genes crucial for maintaining methyl group homeostasis. Notably, we observed downregulation of ER-localized MTases involved in lipid metabolism, alongside the upregulation of nuclear and mitochondrial MTases potentially influencing RNA and histone modification processes.

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Characterisation of monoclonal antibody subunits using electron activated dissociation

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Therapeutic monoclonal antibodies (mAb) have become important in the treatment and prevention of a wide range of diseases. During manufacturing process and stability, they are prone to chemical or physical alterations that can impact their efficacy and safety. Middle-down (MD) protein analysis is rapidly emerging as a complimentary to bottom-up analysis for the mAb products characterisation such as protein sequencing, post-translational modifications (PTMs) and quantification, because of minimal sample preparation required and preservation of PTMs. Previous MD methods often employed multiple fragmentation methods (examples, CID, ETD etc) before achieving high sequence coverage for PTMs identification. Here, we optimised an MS/MS method for subunits sequence coverage determination of biotherapeutic mAbs on a ZenoTOF 7600 (Sciex).

Methods

mAb samples (50 µg, PBS pH 7.4) were digested with 1µL of IdeS protease (50 units/µL) at 37oC for 30 minutes. Digested samples were reduced in guanidine hydrochloride/DTT and desalted with 7K MWCO Zeba Microspin columns. Sample (1 µg) was injected on to the column and subunits chromatographically separated with a gradient elution of 0.1% FA in H2O and 0.1% FA in ACN on Waters Acquity BEH C4 column (300Å, 1.7µm 300 µm x 100 mm) at a flow rate of 7 µL/min. MS optimisation and analysis was carried on ZenoTOF7600 using MRMHR EAD experiment on Sciex OS software. Data obtained were processed for subunit masses and sequencing using the Biologics Explorer software.

Novel Aspect

Sequencing antibody subunits that can potentially be rapidly applied to characterise site-specific PTM in a single run.

Preliminary Data or Plenary Speaker Abstract

Subunit masses were all measured with less than 20 ppm accuracy. EAD fragmentation parameters were all optimized to produce greatest sequence coverage, with electron kinetic energy and reaction times influencing the coverage most. The final optimized parameters gave 73.6%, 71.7% and 79.6% coverage for NISTmAb subunits LC, Fd and Fc/2 (G1F) respectively, with c- and z- ions being the most predominant fragment ions observed.

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Applying UHPLC-HRAM MS/MS/MS method to assess host cell protein clearance during the purification process development of therapeutic mAbs

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Host cell protein (HCP) is one of the process-related impurities that needs to be well characterized and controlled throughout biomanufacturing processes to assure the quality, safety and efficacy of the monoclonal antibodies (mAbs). Although ELISA remains the gold standard method for quantification of total HCPs, it lacks the specificity and coverage to identify and quantify individual HCPs. As a complementary method to the ELISA, LC-MS/MS/MS method has emerged as a powerful tool to identify and profile individual HCP during the downstream purification process. In this study, we applied the UHPLC-MS/MS/MS method to in-house trastuzumab samples to assess HCPs clearance using the newly introduced POROS™ Caprylate Mixed-Mode Cation Exchange Chromatography Resin for polishing purification.

Methods

Two in-house trastuzumab samples (one was purified by Protein A only; another one was purified by Protein A, followed by the POROS Caprylate Mixed-Mode Cation Exchange Chromatography) were digested with trypsin under native conditions. An Acclaim™ VANQUISH™ C18 column (2.1 × 250 mm) was used for peptide separation at 300 µL/min flow rate over a 90 min linear gradient. An Orbitrap™ Ascend Tribrid™ mass spectrometer coupled to Vanquish™ UHPLC was used for data collection. Data dependent MS/MS approach was used, in which a HRAM full MS scan was followed by top 12 data dependent HRAM MS/MS scans. Trastuzumab samples were analyzed in triplicate, respectively. Thermo Scientific™ Proteome Discoverer™ 3.1 software was used for HCP identification and relative quantification.

Novel Aspect

Successfully applied 1D UHPLC-MS/MS/MS method for HCP characterization and profiling to monitor the HCP clearance during the mAb purification process.

Preliminary Data or Plenary Speaker Abstract

The major challenge with UHPLC-MS/MS/MS analysis of HCPs is overcoming the huge intrasample dynamic range to detect low abundant (<10 ppm) residual HCPs among the dominant therapeutic proteins. To address this challenge, we adapted native tryptic digestion protocol for reducing the dynamic range of the proteins in the mAb samples and used Acclaim™ VANQUISH™ C18 column for efficient and reproducible chromatographic separation. The Orbitrap Ascend Tribrid mass spectrometer offers faster HRAM MS/MS scan rate and improved sensitivity, allowing more low abundant HCPs detection from the digest sample. To ensure the quality of MS and MS/MS data, high resolving power was used for both full MS scan (120K resolution at m/z 200) and MS/MS scan (30K resolution at m/z 200) data acquisition. The data was processed using Proteome Discoverer 3.1 software. For HCP identification, the HRAM MS and MS/MS data were searched against a UniProtKB/SwissProt database containing all Cricetulus griseus entries (89,146 entries, Tax ID = 10029, 2024/01/10). Only proteins which were assigned as master proteins with high protein FDR confidence and had ≥ two unique peptides were accepted as positive HCP identifications. 186 HCPs, including 12 problematic HCP proteins such as Phospholipase B-like 2 (PLBL2), were identified from the trastuzumab sample after protein A purification. With the polishing purification using POROS Caprylate, 126 HCPs of the identified 186 HCPs, including PLBL2 were removed from the trastuzumab sample. Of the HCPs that remained in the POROS Caprylate purified sample, most showed significant decrease in abundance, demonstrating that the polishing purification using the POROS Caprylate resin can efficiently remove the HCPs from the Protein A purified mAb sample.

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Broadly neutralizing humanized SARS-CoV-2 antibody binds to a conserved epitope on spike protein and provides antiviral protection through inhalation-based delivery

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The COVID-19 pandemic caused by the SARS-CoV2 virus was one of the greatest health crises of the last century. One of the unique aspects of SARS-CoV2 is its ability to rapidly mutate, quickly evading many of the antibody therapeutics that were first developed. In this study, two monoclonal antibodies against spike protein were studied using hydrogen-deuterium exchange mass spectrometry (HDX-MS) to elucidate the epitope sites and associated allosteric “hotspots” upon binding to the RBD and full-length spike protein. We also demonstrate that when a broadly neutralizing antibody binds the full-length spike of multiple variants of concern there is a unique neutralization mechanism through destabilization of the coiled-coil region. This antibody was further developed by Icosagen into a commercialized inhalation-based vaccine.

Methods

HDX-MS was carried out using the commercial Waters system. Samples (RBD or full-length spike protein alone and at a 1:1 antibody complex) were provided by Icosagen. The samples were mixed with deuterium and injected by a PAL3 autosampler followed by UPLC separation and mass spectrometry analysis using a Waters M-Class ACQUITY UPLC and a Waters Select Series Cyclic IMS. Labeling times of 1, 10, and 60 minutes at room temperature were used, followed by quenching (7.5 M guanidine hydrochloride and 0.5M TCEP) at 0˚C and digestion using an Enzymate BEH Pepsin column. The peptides were reverse-phase separated using an ACQUITY UPLC BEH C18 column. Peptide identification was carried out using ProteinLynx Global Server (PLGS), followed by HDX analysis using DynamX.

Novel Aspect

Use of HDX-MS to identify epitope sites for a broadly neutralizing antibody to aid in development of an inhalation-based vaccine.

Preliminary Data or Plenary Speaker Abstract

The first antibody ICO-hu23 was Wuhan-selective and the epitope was identified in the disordered loop region, 472-502, on the surface of the RBD which is a known ‘orthosteric’ site for spike protein neutralizing antibody binding. Weaker allosteric decreases were also observed at beta sheet region 433-452. Interestingly, ICO-hu23 was found to have lost its virus neutralizing activity when tested against the Omicron RBD which showed no significant binding. This might come as no surprise since Omicron has six mutations across the original Wuhan epitope site. Conversely, the second antibody ICO-hu104 which was screened against emerging variants was found to be broadly neutralizing and produced large magnitude HDX decreases in the same region for both Wuhan and Omicron, this time at the exposed beta sheet further removed from the common ‘orthosteric’ site which does not contain any mutations. Building upon previously observed studies, this highlights the use of HDX-MS to detect potential interface adjacent “hotspots” for neutralizing antibodies. The epitope sites elucidated for ICO-hu104 across variants of concern helped in the development of an inhalation-based nasal spray which has several benefits compared to traditional vaccines due to its non-invasiveness, potential for rapid distribution, and increased antibody delivery to areas most affected by the virus. Furthermore, these experiments were repeated for each antibody with the full-length spike protein including both S1 and S2 subunits. ICO-hu23 produced stabilization of various regions including the coiled-coil domain in S2 with no significant destabilizations present. In contrast, antibody ICO-hu104 destabilized regions of the coiled-coil domain for both the Wuhan, Omicron and

Delta variants suggesting a unique neutralization mechanism. Interestingly, this phenomenon has also been observed in previous studies upon binding of spike protein to human ACE2 receptor and is hypothesized to be involved in the transition to its post-endocytosis conformation.

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Sub ng/mL quantification and characterization of oligonucleotides in plasma using microflow LC coupled to a QTOF mass spectrometer

Phd Fiona Teh[1], Remco van Soest[2], Eshani Nandita Galermo[2], Kerstin Pohl[2], Elliot Jones[2]
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Oligonucleotide therapeutics such as antisense oligonucleotides (ASOs) and siRNA are becoming increasingly important for therapeutic applications involving untreatable diseases. Oligonucleotides require high performance and robust quantitative methods for pharmacokinetic analysis. Triple quadrupole mass spectrometers using multiple reaction monitoring (MRM) mode have been routinely employed for bioanalytical studies of ASOs, as they offer excellent sensitivity and overall quantitative capability. Quadrupole time-of-flight (QTOF) mass spectrometers are very well suited for characterization, but historically have not been used for quantification due to limited sensitivity. In this study, a highly sensitive workflow was developed for the quantification and characterization of several ASOs in extracted rat plasma using microflow LC coupled to a QTOF mass spectrometer.

Methods

Rat plasma was extracted using solid phase extraction. Extracted plasma samples were spiked with a mixture of fomivirsen, nusinersen, eluforsen, a fully phosphorothioated 2'O-methylated 20-mer model oligonucleotide, and an internal DNA standard. Calibration curves were measured in triplicate for all analytes by spiking the analytes into a plasma extract in the concentration range between 0.01 ng/mL and 300 ng/mL. Samples were analyzed using a M5 MicroLC system (SCIEX) used in the trap-and-elute mode, which allowed for the injection of 30 µL extracted plasma on the trap column. A ZenoTOF 7600 system (SCIEX) with an OptiFlow ion source was used in Zeno MRMHR mode, and SCIEX OS software and Molecule Profiler software were used for data analysis.

Novel Aspect

There is an increasing interest in oligonucleotide therapeutics but not many detection methods using microflow workflow with QTOF detection.

Preliminary Data or Plenary Speaker Abstract

All oligonucleotides were baseline separated within a 3 min gradient. Overall, the linear dynamic range
was greater than 3.5 orders of magnitude. Baseline separation is important because overlap among the precursors can often occur due to the large number of charge states of each oligonucleotide. Calibration curves were measured in triplicate for all of the analytes by spiking the analytes into plasma extract in the concentration range between 0.01 ng/mL and 300 ng/mL. Exceptional linearity and accuracy and precision were achieved for all analytes. The LLOQ for nusinersen was 10 pg/mL, while the LLOQs for the other ASOs was 30 pg/mL. No significant interferences were seen, illustrating efficient cleanup of the plasma matrix using the Clarity OTX SPE cartridges. The TOF MS spectra can provide valuable information about non-targeted impurities. After reconstruction, using the Bio Tool Kit option in SCIEX OS software, two additional species were found in the nusinersen peak. Using the SCIEX Molecule Profiler software, they were identified as the desulfurized products of the main sequence.

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Complete Characterization of Trastuzumab Deruxtecan, a Cysteine-linked antibody drug conjugate, using high resolution accurate mass (HRAM) Mass Spectrometry

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Over the last decade, antibody-drug conjugates (ADC) have evolved into promising and efficient therapeutic agents for targeted chemotherapy in cancers, with 11 and 9 ADCs currently approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), respectively, and more than 80 ADCs in clinical studies. ADCs are generated through the conjugation of monoclonal antibodies (mAbs) targeting specifically the tumor-associated antigens (TAAs) of the tumor cell with highly potent cytotoxic drug payloads via a cleavable or non-cleavable chemical linker. Here we demonstrated the comprehensive characterization of trastuzumab deruxtecan(T-DXd), a latest-generation homogenous Cysteine conjugated-ADC with a high DAR, using a Vanquish UHPLC coupled to a Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer equipped with BioPharma Option.

Methods

Sample preparation:
Intact native MS: Commercially available T-DXd was diluted to 1mg/mL using ddH2O.
Reduced chains analysis: T-DXd was diluted to 1mg/mL using denaturing buffer (7M guanidine hydrochloride, 50mM Tris-HCl, pH=8.3) followed by DTT reduction.
Subunit analysis: T-DXd was diluted to 0.5mg/mL using 50mM Tris-HCl (pH=7.9), followed by IdeS digestion then DTT reduction.
Peptide mapping: T-DXd was diluted to 1mg/mL using denaturing buffer, then reduced, alkylated and digested with trypsin.
UHPLC Separation:
MAbPac™ SEC-1 (P/N 088790), MAbPac™ RP (P/N 088648) and Acclaim Vanquish C18 (P/N 071399-V) columns were used for separation.
Mass Spectrometry:
An Orbitrap Exploris™ 240 mass spectrometer with Biopharma option was used for all analysis. Data analysis was performed using Thermo Scientific™ BiopharmaFinder™ software.

Novel Aspect

Comprehensive characterization of Trastuzumab deruxtecan, a latest-generation cysteine-linked ADC using HRAM mass spectrometry.

Preliminary Data or Plenary Speaker Abstract

In this study, comprehensive characterization of a cystine conjugated ADC at multiple levels were achieved using High-resolution accurate-mass (HRAM) mass spectrometry.
At native intact level, the main peak corresponds to mAb bearing 8 conjugated drugs (expected mass of 156,339 Da for 2*A2G0F). Low level of intact ADC contains 6 payloads (~5%) was also detected. The average DAR is 7.94, which is calculated by BioPharma Finder software automatically, in agreement to previous publication.
In reduced intact mass analysis, LC D1 and HC D3 were the main payloads, which is in agreement with native intact MS results. Minor LC D0 and HC D1/D2 were also detected. Other modifications, like oxidation and glycation on LC were also observed.

In subunit analysis, after IdeS digestion and DTT reduction, in addition to N-glycoforms distribution in Fc region and payload distribution in light chain and Fab region, more PTMs, such as oxidation and glycation in Fab region, were detected.

In peptide mapping analysis, we identified all theoretical conjugation sites and calculated the site occupancy. or this homogenous Cys-ADC with a high DAR, light chain C214, heavy chain C223, C229 and C232 were highly occupied (>95%).

Other common modifications in biotherapeutic products, such as deamidation, succinimidation, aspartic acid isomerization, glycation and oxidation were identified and quantified. It is worth noting that deamidation% of light chain N30 is 10.63% and 1.09% of heavy chain N55. The most abundant N-glycoform is A2G0F (77.86%).

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In-depth characterization of monoclonal antibodies using intact mass analysis and middle-down approaches on a modified Orbitrap Tribrid mass spectrometer

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

In-depth characterization of monoclonal antibodies (mAbs) and their post-translational modifications (PTMs) is critical to ensure the safety and efficacy of biotherapeutics. Intact mAbs analysis can be used to confirm intact mass and possible mass shifts. However, the utilization of the middle-down mass spectrometry (MS) technique gained traction as an encouraging method for the characterization of biotherapeutics. The method involves the digestion and/or reduction of mAb subunits, followed by LC-MS/MS offering high sequence coverage with straightforward sample preparation. In this work, we performed intact mass analysis and middle-down MS to characterize Trastuzumab and its subunits on a modified Orbitrap Tribrid mass spectrometer using different MS/MS fragmentation approaches.

Methods

Commercially available Trastuzumab was used for all the experiments. For native MS experiments, intact Trastuzumab was separated using size exclusion chromatography. Reverse phase LC-MS was employed for intact/subunit analyses under denaturing conditions. Trastuzumab subunits were prepared using DTT for reduction combined with or without previous IdeS digestion. LC-MS data were acquired using a modified Orbitrap Tribrid mass spectrometer. All the analyses were performed in the intact protein mode, with high mass range enabled specifically for intact Trastuzumab. Middle-down MS experiments were conducted using electron transfer dissociation (ETD), electron transfer higher energy collision dissociation (EThcD), ultraviolet photodissociation (UVPD) and proton-transfer charge reduction (PTCR) technologies. Data analysis was performed using commercially available software.

Novel Aspect

Improved sequence coverage of mAb subunits was achieved using advanced MS/MS techniques on a modified Orbitrap Tribrid MS.

Preliminary Data or Plenary Speaker Abstract

For intact mAb analysis under the native and denaturing conditions, high mass accuracies were obtained for major glycoforms of Trastuzumab using an Orbitrap resolving power setting of 30,000 at 200 m/z. A higher Orbitrap resolving power setting of 240,000 at m/z was used to acquire isotopically resolved full MS spectra of the Fc/2, light chain (LC) and Fd' subunits for deconvolution with Xtract and Sliding Window algorithm. A resolving power setting of 7,500 at 200 m/z was used to acquire full MS spectra of heavy chain (HC) subunit for deconvolution with ReSpect and Sliding Window algorithm. Mass accuracies below 3ppm were obtained for all subunits.
For middle-down analysis of all subunits, a quadrupole isolation window of 100 m/z centered around m/z 900 was used. Replicate ETD/EThcD and UVPD data were acquired using five different reaction/activation times.
The combined results from five ETD experiments led to high sequence coverage of 80% for Fc/2, 75% for LC, and 75% for Fd'. With EThcD, the sequence coverage was improved to 90% for Fc/2, 84% for LC and 84% for Fd. UVPD and its diverse fragment ions provided even higher sequence coverage with 93% for Fc/2, 91% for LC and 87% for Fd'. For LC and HC subunits a similar trend of improvement in sequence coverage was observed, with ETD experiments leading to 78% for LC and 48% for HC, EThcD experiments with 89% for LC and 58% for HC, and UVPD experiments with 91% for LC and 71%

for HC subunits. Combination with further experiments using EThcD and UVPD coupled with PTCR provided a nearly complete sequence coverage of mAb subunits, with 98% for Fc/2, 95% for LC, 92% for Fd'.

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Lysoalkylphosphatidylethanolamines: novel precursors to enrich endogenous plasmalogens

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Plasmalogens are structurally unique glycerophospholipids with multiple biological functions, with levels found to be reduced in different disease conditions. Although dietary supplementation of plasmalogens or naturally occurring precursors, alkylglycerols (AKGs), can increase plasmalogens and reduce disease pathology, both have limitations, highlighting the need to consider alternatives. These alternatives include lysoalkylphosphatidylcholine (LPC(O)) and lysoalkylphosphatidylethanolamine (LPE(O)), which are both metabolic precursors to plasmalogens. Even though krill oil, a natural source of LPC(O), has been used in several previous studies, the effect of supplementing only LPC(O) and LPE(O) has yet to be explored. Therefore, this study aimed to compare the bioavailability of deuterium-labelled AKG, LPC(O) and LPE(O) and their incorporation into circulatory plasmalogens of mice.

Methods

Eight-week-old C57BL/6 mice were orally gavaged with a single dose of lecithin (vehicle control; 1.0 and 3.3 mg/mouse) or deuterium-labelled mixes of AKG (1.0 and 3.3 mg/mouse), LPC(O) (1.5 and 5.0 mg/mouse), or LPE(O) (1.4 and 4.5 mg/mouse) at low and high doses respectively (n=8/group; 4 females and 4 males). Blood was collected through tail-tip bleeding before gavage and at 1, 4, 24, and 48 hours after gavage for lipidomic analysis. Lipids from plasma samples were extracted and analysed using ultra-high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHLPC/MS/MS). Statistical analysis involved a one-way ANOVA with Tukey's post-hoc test.

Novel Aspect

These findings suggest LPE(O) supplementation as an AKG alternative for increasing plasmalogens due to improved bioavailability and incorporation into PE(P).

Preliminary Data or Plenary Speaker Abstract

The results showed that LPE(O) supplementation in both males and females led to the highest deuterium label incorporation and bioconversion into phosphatidylethanolamine plasmalogens (PE(P)s) compared with the AKG and LPC(O) precursors. Specifically, the maximum concentrations of labelled PE(P-16:0) (Cmax) following a low dose of LPE(O) was shown to be ~1.9-fold higher than a low dose of LPC(O), which was ~3.7 fold greater than a low dose of AKG. Additionally, bioavailability was shown to be highest for LPE(O) compared to the LPC(O) and AKG precursors. Bioavailability, in this study, specifically referred to the bioconversion of plasmalogen precursors into plasmalogen, which was determined using the area under the curve (AUC). In particular, a low dose of LPE(O) resulted in a ~2.1-fold greater AUC than a low dose of LPC(O), which was ~3.3-fold higher than a low dose of AKG. Similar trends in Cmax and AUC were evident in the high-dose treated mice.

In summary, a low dose of LPE(O) resulted in an approximately 7-fold higher Cmax and AUC than a low dose of AKG, suggesting that LPE(O) is more readily converted into PE(P) and can be considered more bioavailable than the other precursors tested. This could be the result of LPE(O) supplementation leading to higher label incorporation into PE(O), which is the immediate

endogenous precursor to PE(P) in the biosynthesis pathway, and less incorporation into DG(O) and monoalkyl-diacylglycerol (TG(O)) than other precursors.

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Redefine bioanalysis with enhanced robustness on the SCIEX 7500+ system

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Dr Justin Lim[1], Dr Brad Patterson[2]
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Quantitation of pharmaceutical drugs is often performed in complex matrices. Because of challenging matrix contaminants, highly robust analytical techniques are needed to ensure accurate and precise measurements. Systems such as triple quadrupole mass spectrometers are commonly used for this quantitative bioanalysis. Therefore, achieving longer stable sensitivity and reduced downtime based on cleaning are thus significant benefits in extending the uptime for prolong bioanalysis and crucial in such laboratories.

Here, by introducing the Mass Guard technology and newly designed removable DJet+ assembly, the long-term robustness of the SCIEX 7500+ system and SCIEX 7500 system was evaluated under contamination-accelerated matrix conditions using 2:1 (v/v) methanol/rat plasma protein precipitation.

Methods

Rat plasma matrix was prepared by extracting 500 µL of rat plasma with 1000 µL of methanol. The mixture was vortexed for a minute and centrifuged at 12000 rcf for 10 minutes. 1200 µL of supernatant was collected and diluted with 1200 µL of water. A mixture of alprazolam, diazepam and sulfamethoxazole and their respective deuterated internal standards (pharma mix, SST) were spiked in rat plasma extract to achieve a final concentration of 0.5 ng/mL for analysis.

Analytes were separated using a Gemini C18 column (3 µm, 110 Å, 3 x 50 mm). The LC system was operated at 1.0 mL/min. MS analysis was performed in positive mode. Collision energy, source and MS parameters were optimized for MRM-based quantitation.

Novel Aspect

Accomplish long-term bioanalytical sample analysis with high accuracy and reproducibility highlighting enhanced robustness of SCIEX 7500+ system.

Preliminary Data or Plenary Speaker Abstract

Instrument robustness was evaluated by intermittently monitoring SST samples spiked with native and internal standards between large blocks of consecutive rat plasma matrix injections. The peak area ratios obtained for all analyte and internal standards were analyzed to evaluate the performance of the instrument.

SCIEX 7500+ system features an improvement in the front end (incorporating Mass Guard technology; removable DJet+) to reduce contamination and maximize instrument robustness. The configuration was evaluated on its impact on the analysis of samples with a higher percentage of matrix components over a long period of time.

For rigorous testing, extraction was carried out using a 1:2 (v/v) ratio of rat plasma and methanol to evaluate the robustness of the instrument under extreme conditions and analysis was performed without using divertor valves. The aim was to assess the ability of the instrument to withstand

consecutive days of analysis without showing any signs of performance degradation or loss of sensitivity. The %CV based on the peak area ratio was less than 10% for all the tested compounds throughout the analysis of more than >10,000 rat plasma matrix injections, indicating that the SCIEX 7500+ system was highly robust and reliable. Furthermore, multiple days of analysis showed no signs of performance degradation or loss of sensitivity, as indicated by the consistent reproducibility of the peak area ratios.

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Removal of Polysorbate 80 for mass spectrometry (MS)-based proteomic analysis

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[1]Csl

Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The Non-ionic surfactant Polysorbate 80 (PS80) is often added to the pharmaceutical recombinant proteins to stabilize the protein and mitigate protein adsorption and aggregation. Elevated levels of PS80 in the sample results in subsequent contamination of the mass spectrometers (MS) and a significant reduction in signal intensity. As mass spectrometers are routinely utilised to characterise biopharmaceuticals and associated host cell proteins, it is necessary to reduce or remove PS80 prior to introduction into the MS. In this study, several approaches including solvent extraction, commercially available desalting devices and sample preparation kits have been evaluated for their ability to remove the PS80.

Methods

Four different organic solvents, Toluene, Dichloromethane, Ethyl Acetate and Chloroform, two commercial kits, and two spin columns were evaluated for their ability to remove PS80 from samples. The PS80 levels were quantified using charged aerosol detection (CAD)-HPLC. Cleaned samples were subjected to proteolysis using Trypsin and analysed using Thermo Q-Exactive mass spectrometer interfaced with Vanquish HPLC running a gradient of 3-30% over 166 minutes.

Novel Aspect

Evaluation of several procedures to effectively remove PS80 from biopharmaceutical samples.

Preliminary Data or Plenary Speaker Abstract

The results presented will demonstrate relative removal of PS80 from the samples enabling cleaner input into the MS. CAD-HPLC results demonstrated a minimum of 3-fold reduction of PS80 levels which correlated with cleaner signal observed in mass spectrometer.

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A cell culture metabolite profiling assay developed using a new nominal mass hybrid mass spectrometer

Elsa Gorre[2], Sven Hackbusch[1], Bo Zhai[2], Andrew Mahan[2], Dr. Cristina Jacob[1], Snr Manager Pharma and BioPharma Applications Min Du[3], **Ms Nicole Zhang**
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Media in cell culture provides essential nutrients to maintain cell viability and maximize biotherapeutic production. Profiling amino acids and other essential metabolites helps to ensure cells have the nutrients they need and can detect accumulation of other metabolites with detrimental effects on viability and product quality.
However, spent media is a complex mixture of metabolites, making their simultaneous detection difficult. Analyzing different chemical classes using one method creates challenges for sample preparation and requires a mass spectrometer with wide dynamic range and rapid polarity switching. Here, we developed a fit-for-purpose targeted nominal mass method to monitor changes in amino acids and other metabolites from key metabolic pathways and applied it to study a 12-day bioreactor run producing the NIST antibody.

Methods

Spent media was harvested daily from 12-day NIST ambr15 bioreactor runs along with collection of cell pellets. The samples were protein precipitated using acetonitrile, centrifuged and then diluted using 0.1% FA prior to analysis. LC/MS analysis was carried out to quantify a list of metabolites in targeted fashion, using a Thermo Scientific Vanquish Horizon UHPLC system coupled to a new nominal mass hybrid mass spectrometer with data acquired using targeted MS2 experiments and rapid polarity switching. Precursors were fragmented using stepped or fixed normalized collision energies. Both reversed phase and HILIC separation of the metabolite targets were explored. Collected data were processed using the Skyline 23.1 software to detect compounds based on one or more transitions for each metabolite.

Novel Aspect

Semi-quantitative metabolite assay to monitor cell culture and optimize biologics production using a new nominal mass hybrid mass spectrometer

Preliminary Data or Plenary Speaker Abstract

Initial LC/MS method development was carried out using the KAIROS amino acid standard mixture and other relevant metabolite standards -totaling over 110 precursor ions to be monitored with the assay - to establish suitable chromatography conditions and optimize the MS settings. For the separation of the metabolites, different columns were evaluated to provide a fast run time for the assay, while still resolving all isomeric species. In developing the MS experiment, acquisition time windows, polarity, collision type and energy, among others, were optimized to provide sensitive and selective monitoring of each metabolite.
Data were acquired for all fragment ions using a targeted MS2 approach, which allowed to choose preferred transitions for each target during the data analysis step. This also enabled longer injection times for each metabolite precursor, as only one scan event was required to detect all product ions, which boosted sensitivity in case of reversed phase (RP) separations with many closely eluting precursors.
The sensitivity of the RP-LC/MS method was evaluated using a dilution series of 1 µL injections of the KAIROS standard mixture. For most of its compounds, the limit of detection was determined in the range of 5-200 nM.
The developed method was then used to analyze daily spent media samples from ambr15 bioreactor producing the NIST antibody. Four bioreactors with different feeding strategies were compared. Amino acid and metabolites measurements were collected and processed with Skyline. Profiles for

each analyte were generated and compared, including for metabolites such as glucose, glucuronic acid, and citric acid cycle intermediates (e.g. lactic acid and succinic acid), that could also be measured in the lysed cell pellets. Data from the latter provided insight into the effectiveness of the cell's energy conversion. The cross-correlation of the intra-cellular metabolite profiles with the spent media results will be discussed.

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Characterization of Adeno-Associated Virus Capsid Proteoforms Using Multiple Ion Activations on an Orbitrap Tribrid Instrument

Mr Rafael Melani[1], Ms Jingjing Huang[1], Ms Kristina Srzentić[2], Jake Kline[3], Christopher Mullen[1], Luca Fornelli[3]

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Adeno-associated viruses (AAVs) are small, non-enveloped, and single-strained DNA viruses that have gained significant attention in gene therapy research and applications. There are 11 serotypes of AAVs that have been isolated from human and primate samples with different specificity to transduce into different tissues with a lack of pathogenicity. AAVs capsid is structurally composed of three proteins: VP1, VP2, and VP3, with masses ranging from 59-87 kDa. The overall mass of the capsid is approximately 3.7-3.9 MDa, which is the result of approximately 60 VP proteins coming together. Here, we describe a mass spectrometry-based method to characterize intact VP proteoforms isolated from AAV2, a serotype particularly suited for gene therapy directed to muscular dystrophy, liver diseases, and neurological disorders.

Methods

AAV2 was acquired from VIROVEK. Particles were disassembled using denaturing buffer (50% acetonitrile v/v, 0.1% formic acid). About 1 µg of capsid proteins was loaded onto a C4 reversed-phase column for liquid chromatography (LC) separation. The outlet of the column was coupled to a modified Orbitrap Ascend Tribrid mass spectrometer (Thermo Fisher Scientific). VP intact mass (MS1) proteoforms were measured at low resolving power (7,500 at m/z 200). Next, the primary proteoforms were selectively fragmented, employing a single ion activation method for each LC run. HCD, ETD, EThcD, and UVPD were individually employed as standalone techniques for targeted MS2. Subsequently, these methods were combined with PTCR for MS3 analysis.

Novel Aspect

A method for characterization of AAV2 proteoforms utilizing multiple ion dissociation techniques and PTCR MS3 on chromatographic time scale

Preliminary Data or Plenary Speaker Abstract

AAV2 was acquired from VIROVEK. Particles were disassembled using denaturing buffer (50% acetonitrile v/v, 0.1% formic acid). About 1 µg of capsid proteins was loaded onto a C4 reversed-phase column for liquid chromatography (LC) separation. The outlet of the column was coupled to a modified Orbitrap Ascend Tribrid mass spectrometer (Thermo Fisher Scientific). VP intact mass (MS1) proteoforms were measured at low resolving power (7,500 at m/z 200). Next, the primary proteoforms were selectively fragmented, employing a single ion activation method for each LC run. HCD, ETD, EThcD, and UVPD were individually employed as standalone techniques for targeted MS2. Subsequently, these methods were combined with PTCR for MS3 analysis.

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Development of DAR assay and refinement of reaction kinetics to support production of a Phase I theranostic antibody

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The 10D7 antibody, that binds CUB-domain containing protein 1 (CDCP1), has shown promise for targeting ovarian cancer cells. Hence, it is being developed as a theranostic antibody for ovarian cancer, by covalently attaching a deferoxamine (DFO) chelator to enable labelling with radioactive isotopes, either for PET imaging or therapy. The Regulated Biomanufacturing Group at CSIRO was tasked with producing 10D7-DFO under a regulated Quality Management System, in CSIRO's National Vaccine and Therapeutics Laboratory, for phase I trials with a specific, and defined, drug-antibody ratio (DAR).
Here we present the development of a mass spectrometry-based DAR assay for 10D7-DFO and its application in optimising various reaction parameters to achieve a multigram scale production run of 10D7-DFO with the targeted DAR.

Methods

Conjugated antibody samples were reduced and analysed by rpLC-MS. Acquired average peak spectra were deconvoluted with maximum entropy algorithm. Antibody DAR values were computed from peak areas of the light and heavy chains using a custom script.

Novel Aspect

Scale up, conjugation reaction kinetics,

Preliminary Data or Plenary Speaker Abstract

N/A

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Increased sensitivity and throughput for native intact mass analysis of mAb and ADCs using an online buffer exchange column

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[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific, [3]Thermo Fisher Scientific

Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Antibody-drug conjugates (ADCs) are heterogeneous mixtures of chemically distinct molecules that vary in both drugs/antibodies and conjugation sites. Most of ADCs on market or in clinical trials are lysine-linked, cysteine-linked or site-specific ADCs. For cysteine-linked ADC, the interchain disulfide bonds are reduced followed by linker-payload conjugation. Therefore, native intact mass analysis has been routinely used in cysteine-linked ADC characterization due to its ability to retain non-covalent interactions. Here we used the NativePac OBE-1 SEC column and the Orbitrap Exploris 240 mass spectrometer for native intact analysis of two ADCs, polatuzumab vedotin (cysteine-linked) and trastuzumab deruxtecan (site-specific). Trastuzumab was also analyzed as reference.

Methods

Sample preparation:
Commercially available polatuzumab vedotin, trastuzumab deruxtecan and trastuzumab were diluted at different concentration ranges (0.01,0.1 and 1mg/mL) using ddH2O.
UHPLC Separation:
Thermo Scientific™ Vanquish™ Flex Binary UHPLC system
Thermo Scientific™ NativePac OBE-1 SEC (P/N 43803-052130) column
Flow rate: 0.1mL/min
Column temperature: 25 °C

Mass Spectrometry:
An Orbitrap Exploris™ 240 mass spectrometer with Biopharma option was used for data acquisition. Data analysis was performed using Thermo Scientific™ BiopharmaFinder™ software.

Novel Aspect

The method allowed accurate intact mass and DAR measurement of polatuzumab vedotin and trastuzumab deruxtecan with high sensitivity and throughput.

Preliminary Data or Plenary Speaker Abstract

In this study we evaluated the sensitivity of OBE column for the native intact mass analysis of ADCs and mAb. The linker-payload conjugation induces not only structure heterogeneity but also lower MS signal level compares to naked mAb. Therefore, sensitivity plays even more important roles in native intact mass analysis of ADCs than mAbs. The low flow rate (50-100µL/min) for OBE column separation provides better sensitivity compares to regular SEC columns. As we observed, 1µg polatuzumab vedotin on OBE column gives same level MS intensity as 10 µg on a 2.1mm ID SEC column.
Down to 2ng trastuzumab was detected with MS S/N>3 and 5ng trastuzumab on column was measured with MS S/N>10. For ADCs, the lower detection limit of polatuzumab vedotin is 5 ng and 10ng of trastuzumab deruxtecan. Both payload and N-glycosylation distributions can be detected even with 5~10ng ADC sample loading.
The OBE column can also improve throughout by decreasing analytical time. In this study, the analytical time was 3 minutes for each run, much shorter than regular SEC columns, which usually takes 15~20 minutes per run. The sensitivity and throughout were improved significantly compares to previous SEC-native mass analysis workflow, providing benefits in fast sample screening during drug development and process optimization.

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High-throughput PROTAC compound screening workflow for targeted protein degradation on an Orbitrap Astral mass spectrometer with accurate label-free quantitation

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Targeted protein degradation (TPD) is an emerging and transformative strategy in drug discovery that utilizes cellular protein degradation processes to selectively eliminate deleterious proteins. Through enabling induced proximity to the ubiquitination-proteasome system, the small molecules facilitate the degradation of the disease-causing proteins, opening the possibilities to target the previously undruggable proteins for a wide range of diseases with unprecedented precision and efficacy. Directly designing compounds that can promote the induced proximity with selective is challenging in practice thereby compound screening with reliable quantitation accuracy is a crucial phase in drug discovery in the TPD space. The need for screening large numbers of compounds with accurate quantitation makes high throughput mass spectrometry-based workflows an obvious choice for ensuring accurate lead identification.

Methods

VCaP prostate cancer epithelial cells were treated with different concentrations of ARCC-4, a PROTAC protein degrader for androgen receptor. Cells were lysed and digested with trypsin using an AccelerOme automated sample preparation platform. The ensuing peptides were loaded onto a 15cm EASY-Spray™ or a 5 cm Ionopticks Aurora UHPLC column and separated with nano or capillary flowrate in a trap and elute mode using a Vanquish Neo UHPLC system for a throughput of 60, 100, 180 and 300 samples per day. Eluted peptides were ionized with an Easy-Spray source and transferred into the Orbitrap Astral mass spectrometer for data-independent acquisition analysis. Acquired data was processed by Spectronaut, DIA-NN or Proteome Discoverer™ software using CHIMERYS™ intelligent search algorithm by MSAID.

Novel Aspect

LFQ-DIA workflow on Orbitrap Astral mass spectrometer for targeted protein degradation discovery and validation with high sensitivity and quantitation accuracy.

Preliminary Data or Plenary Speaker Abstract

The tryptic peptides from VCaP cells treated with different concentrations of ARCC-4, a known androgen receptor (AR) degrader with high specificity, were analyzed on an Orbitrap Astral mass spectrometer for label free quantitation. Here, we developed an LFQ-DIA workflow with various throughputs to meet the needs of different TPD compound screening speeds. With the 100 SPD method, over 9000 proteins were reproducibly identified and quantified with a protein group CV of approximately 10%. More importantly, we observed decreased androgen receptor expression upon increasing ARCC-4 concentration while the vast majority of proteome remained unaffected, highlighting the suitability of Orbitrap Astral mass spectrometer for ultra-high throughput PROTAC compound screening with excellent proteome coverage and quantitation.
To explore the possibility of ultra-fast compound screening, we further developed an even higher throughput of 180 SPD and 300 SPD. We were able to identify over 8500 proteins and close to 8000 proteins with the 180 SPD and 300 SPD methods, respectively. Moreover, AR was the major protein being degraded at the higher concentration of ARCC-4 in both the ultra-high-throughput methods. The results suggest that the advancement of mass spectrometer instrumentation allows for the unprecedented speed of TPD compound screening while achieving good proteome coverage and reliable quantitation for accurate lead identification.

To maximize the proteome coverage, which is important for the validation phase, we further extended this workflow to 60 SPD. We successfully identified approximately 10,000 proteins with the 60 SPD method, which represents close to complete proteome identification in the cell. In addition, AR protein was still found degraded with increasing ARCC-4 concentration, suggesting a high-throughput and comprehensive on-target/off-target validation method from our LFQ-DIA workflow on an Orbitrap Astral mass spectrometer.

Together, our data suggests that Orbitrap Astral mass spectrometer enables ultra-high-throughput TPD compound screening with excellent sensitivity, reproducibility, and quantitation accuracy.

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Chemoproteomic Target Deconvolution Approaches in Giardia duodenalis

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Giardia duodenalis is a gastrointestinal parasite causing ~200 million symptomatic infections annually, disproportionately in lower socioeconomic tiers and children, leading to malnutrition, stunting in physical and cognitive growth. Chemotherapeutic interventions are limited to nitroheterocyclic antibiotics such as metronidazole. However, high doses are toxic and treatment failure related to drug-resistance occurs in up to 20% of cases, highlighting the urgency of novel and safer chemotherapeutics.

Methods

We previously identified a potent, drug-like series of kinase inhibitor compounds (IC50=114nM). To identify the kinase target(s) in Giardia, we selected a representative compound and immobilise through “Click” chemistry, to azide-agarose (ClickChemistryTools) and azide-magnetic beads (ClickChemistryTools) to “pulldown” the high affinity target(s). Pilot MS studies of this approach showed different enrichment and non-specific binding profiles for either sets of beads, and manufacturers reported different compound support affinities. To optimise this pulldown, we have investigated washing buffers and different protein elution/digest strategies to reduce non-specific binding. We use “blank” beads as controls and “baited” beads for the samples. For azide-agarose beads, we implemented a secondary control by binding baited beads in the presence of excess inhibitor.

Novel Aspect

The choice of beads selected in a pulldown may alter the protein targets identified, and may hinder downstream drug-discovery experiments.

Preliminary Data or Plenary Speaker Abstract

Data-independent acquisition (DIA), library-free searches of these samples identified 2065 and 931 proteins from these respective pulldowns, out of the 4900 proteins in the annotated G. duodenalis proteome. Relative to “blank” agarose and magnetic beads, we pulled down 819 and 132 significant proteins respectively. When further cross-referenced to secondary controls, we identify eight significantly enriched proteins from the baited agarose, and ten significantly enriched proteins from the baited magnetic beads. Two of the eight proteins from the agarose were annotated as kinases in the G. duodenalis proteome; no kinases were identified from baited magnetic beads. Overall, despite both beads being appropriate for Click chemistry approaches, we had to use different workflows for both reagents. This was largely due to non-specific binding between reagents, and sample-processing had to be adapted for magnetic or agarose beads

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Advancing blood proteome analysis past the plasma age: Mass spectrometry of whole blood collected via volumetric absorptive microsampling devices (VAMS).

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Approximately 40% of clinical tests rely on plasma analysis, however, further biomarker discovery using plasma is stymied due to being comprised of 22 highly abundant proteins that limit the detection of much else. Further, the very processing steps required to isolate plasma, such as chosen anticoagulant, can introduce variability into the results. Whole blood is an attractive alternative to plasma as it can be collected without additional processing required, although it has the same high abundance protein issue with a very wide dynamic range. The increased focus on remotely collected blood samples using dried blood spots and volumetric absorptive microsampling (VAMS) devices has further driven the need to shift focus to developing methods for whole blood analysis.

Methods

Blood was collected from healthy volunteers and processed into either whole blood or plasma using a variety of anticoagulant vacutainers. All samples were applied to 30µL Mitra VAMS tips and left to dry for 24 hours. In a test of sample stability, a number of samples were stored for 3 months at room temperature, 4 °C, or -80 °C. All other samples were processed for analysis immediately after drying. To process for analysis, sample-bound tips were incubated then washed in an extraction solution (lithium chloride + Tris) before being digested in tip with trypsin (1 µg/µL in lysis buffer). Peptides were quantified and normalised to 0.2 µg/µg and were analysed by LCMS on HF-X Orbitrap mass spectrometer.

Novel Aspect

Novel methods for whole blood analysis enable the production of more datapoints than plasma and analysis of remotely collected samples.

Preliminary Data or Plenary Speaker Abstract

By utilising VAMS to immobilise the sample for washing and digestion, the high abundance protein problem of plasma and whole blood was mitigated which was demonstrated by a significant improvement in the dynamic range of both whole blood and plasma. Immobilisation of plasma into VAMS produced up to 2519 protein identifications for plasma. Whole blood performed even better with up to 3318 protein identifications. In fact, for every metric measured whole blood outperformed plasma.
In a reproducibility test of whole blood and plasma from VAMS, whole blood was found to be superior with a median %CV of 6.1% compared to 10.7% for plasma. In a comparison of the effect of various anticoagulants, the number of protein identifications for plasma changed by 1.9-fold between the highest and lowest results compared to 1.1-fold for whole blood, with mean % CVs of 35% and 11% respectively. It was also found that small deviations in plasma production protocols such as double spinning plasma (compared to single spinning plasma) can result in a loss of 1307 identifications in double spun plasma and 1400 differentially expressed proteins between the processing methods.
Further, in a test of storage stability, plasma was found to be highly affected by storage temperature with a 76% reduction in number of proteins detected after 3 months of storage at room temperature and 42% reduction at 4 °C. Whole blood conversely lost 29% of the IDs after room temperature storage and no detectable loss at 4 °C. Overall, these results demonstrate that when immobilised in VAMS and dried, whole blood is more reproducible, produces more datapoints, and is more resilient to temperature associated changes than plasma.

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Development of certified reference material for amyloid β (1-42) NMIJ CRM 6210-a

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Amyloid β is known as the peptide biomarker of Alzheimer's disease (AD). One of the histopathological features of AD brains is the formation of dense senile plaques composed of amyloid β. In particular, amyloid β (1-42) is known as a major cause of senile plaque formation. Therefore, amyloid β species including amyloid β (1-42) in matrix were quantitated using various methods such as immunological assays, positron emission tomography, and mass spectrometry. However, variations in measured amyloid β (1-42) concentrations in different procedures could be overcome by standardizing measurement procedures and establishing traceability to a reference material. Hence, we developed a certified reference material (CRM) for amyloid β (1-42), namely NMIJ CRM 6210-a.

Methods

The human amyloid β (1-42) solution dissolved in 0.1 % ammonia aqueous solution was divided into 120 vials on a balance and lyophilized. The lyophilized materials were used for various analysis after reconstitution by adding 1.00 g of 0.1 % ammonium aqueous solution. The certified values of this material were determined via amino acid analysis employing an isotope-dilution mass spectrometry. The results of amino acid analyses were converted into the mass concentration by using the density and molecular weight of the reconstituted solution to give the certified values. The content of amyloid β (1-42) and its impurities in this material was determined by the relative area percentage using high performance liquid chromatography (HPLC) and LC-MS.

Novel Aspect

Development of the certified reference material of amyloid β which is aimed to be used as calibration standards for LC-MS

Preliminary Data or Plenary Speaker Abstract

We examined the primary structure of the candidate CRM by LC-MS. The observed masses of the multi charged ions (2+ to 6+) were identical to their calculated masses of human amyloid β (1-42). This result indicated that the structure of the candidate CRM has human amyloid β (1-42) sequence (thereafter, human amyloid β (1-42) is abbreviated as amyloid β). To investigate the peptidic impurities in the material that affect the amino acid analyses, we analyzed the candidate CRM by HPLC and LC-MS. As a result, it was found that this candidate CRM contains oxidated, deamidated and isomerized forms of amyloid β in addition to amyloid β. Thus, we assigned two certified values that represent the mass concentration of total amyloid β (mixture of amyloid β, oxidated amyloid β, deamidated amyloid β, and isomerized amyloid β) and amyloid β.
Amino acid analyses of the candidate CRM were performed by the two hydrolysis methods, liquid-phase and gas-phase methods. The quantitative results were obtained by calculating the weighted mean of the results by two amino acid analyses. This value is the concentration of total amyloid β. The concentration of amyloid β was calculated by the ratio between amyloid β to total amyloid β determined by HPLC. In addition, homogeneity and stability studies (long-term and short-term) were performed with HPLC and assessed by measuring the area ratios of the total amyloid β or amyloid β to external standard, hydrocortisone. The uncertainties obtained by these studies were combined into the uncertainties of the certified values. Overall, the certified value of total amyloid β and amyloid β were assigned to be (46.1 ± 10.24) mg/L and (42.6 ± 6.9) mg/L, respectively, the number following “±” represents the expanded uncertainty (k = 2.45).

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β-Lactamase Sensitive Prober for Rapid Detection of Antibiotic-Resistant Bacteria with Gas Chromatography-Tandem Mass Spectrometry

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Typical bacterial identification methods (culture-based, serological and genetic methods using DNA replication) are highly time-consuming (several hours, sometimes even days) and expensive. Therefore, developing efficient diagnostic protocols for drug-resistant bacteria is of great significance. Herein, based on gas molecules in bacteria, our research group has carried out a novel research strategy to develop a gas molecule-based probe by grafting 2-methyl-3-mercaptofuran (MF) onto cephalosporin intermediates via nucleophilic substitution reaction. The probe can release the corresponding MF by reacting with Bla. The released MF, as a marker of drug-resistant bacteria, was analyzed by headspace solid phase microextraction coupled with gas chromatography-mass spectrometry. The probes with different substrates to further identify different types of bacteria, thereby broadening for monitoring physiological processes.

Methods

Herein, based on gas molecules in bacteria, our research group has carried out a novel research strategy to develop a gas molecule-based probe (CS) by grafting 2-methyl-3-mercaptofuran (MF) onto cephalosporin intermediates via nucleophilic substitution reaction.

Novel Aspect

The probes with different different substrates to further identify different types of bacteria, thereby can monitor physiological processes.

Preliminary Data or Plenary Speaker Abstract

The probe can release the corresponding MF by reacting with Bla. The released MF, as a marker of drug-resistant bacteria, was analyzed by headspace solid phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS/MS). The Bla concentration as low as 0.5 nM can be easily observed, providing an efficient method for detecting enzyme activity and screening drug-resistant strains in vivo.

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Unusual Epigenetic DNA Modifications:

Elucidation, Synthesis and Annotation using UHPLC-HRMS2

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

DNA modifications have multiple functions in organisms across all domains of life, including bacteria. Especially methylation of the DNA bases cytosine at the positions 4 or 5 as well as adenine at position 6 are common modifications and therefore frequently investigated. They are associated with many cellular processes like changes in binding behaviour of the DNA to proteins, the replication and repair mechanisms or the regulation of transcription. However, other modifications also exist in bacteria,as we could detect in two distinct species.

Methods

The genomic DNA of Escherichia coli and Klebsiella grimontii strains were extracted and their DNA were hydrolysed to yield the nucleobases. The composition of nucleobases and their modifications were analysed via Ultra High Performance Liquid Chromatography coupled to High Resolution Mass Spectrometry (UHPLC-HRMS) using a buffered gradient and a polar endcaped column.
Possible matching DNA modifications were examined and evaluated using HRMS2 techniques, then synthesised and also hydrolysed. Finally, utilizing a comparison of retention time, HRMS1 and HRMS2 data, the modifications were identified.

Novel Aspect

Two DNA modifications not decribed so far were discovered in two bacterial genera and one new DNA modification was elucidated.

Preliminary Data or Plenary Speaker Abstract

Investigation of the DNA modification of bacteria (E.coli, K. grimontii) showed two so far not reported DNA modifications. Additionally, 2-methylguanosine was synthesised as described by Lu et al. and then hydrolysed to obtain the methylated nucleobase. Retention time, MS1 and MS2 data were in agreement with the K. grimontii sample.

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Advancing heart failure pharmacotherapy: a comprehensive LC-MS/MS platform for simultaneous detection of 28 drugs and their metabolites in plasma

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Heart failure (HF), a multifaceted clinical syndrome, poses a considerable challenge in managing frail patients with increasing multimorbidity. It often requires a complex regimen of polypharmacy. While current HF pharmacotherapy, encompassing beta-blockers (BBs), angiotensin receptor-neprilysin inhibitors (ARNIs), mineralocorticoid receptor antagonists (MRAs), and sodium-glucose co-transporter-2 inhibitors (SGLT2) as four pillars of HF management demonstrates prognostic benefits, the overlooked concerns of adverse drug reactions (ADR) and drug-drug interactions (DDR) require a more scrupulous approach.
To bridge this gap, we introduce an innovative LC-MS/MS based method capable of simultaneously detecting 28 commonly used HF drugs and their metabolites. This pioneering approach seeks to enhance patient care, ensure safety and compliance, and address the unique challenges associated with polypharmacy in this vulnerable population.

Methods

The LCMS analysis was performed on Sciex 7500 QQQ via electrospray ionisation in multiple reaction monitoring (MRM) mode, optimised using individual authentic drug standards and the stable isotope labelled standards. The LC separation was achieved on a UHPLC (LC-40Dx3, Shimadzu) equipped with an Agilent HPH C18 column connected to a compatible guard column at a gradient elution program. Isotopically labelled drug standards were served as internal standards for precise quantification in human plasma. Rigorous validation, encompassing specificity, optimal recovery, limit of detection, and inter- and intra-day variability, attests to the reliability, specificity, and accuracy of this methodology.

Novel Aspect

Introducing advancements in heart failure pharmacotherapy through the application of LC-MS/MS detection, with a focus on mitigating ADR.

Preliminary Data or Plenary Speaker Abstract

Our exploration of plasma sample preparation methods, leading in a streamlined one-step solvent protein precipitation after optimisation, underscores the efficiency and practicality of the proposed approach. The linear range of standard curves for all the drugs in human plasma was from 0 – 250 nM. The inter, intra assays performed with three different concentrations on five different days were within 1.1%- 8.6%, and 4.6% - 11.8%, respectively. In conclusion, our results affirm that these highly sensitive methods are not only efficient and accurate but also eminently applicable for quantifying HF drugs and their metabolites in human plasma, thus contributing to the advancement of tailored and safer heart failure pharmacotherapy.

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Combining Pyrolysis-GC/MS and Machine Learning to Construct an in silico EI Mass Spectral Library for Polymeric Materials Analysis

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Pyrolysis-GC/MS (Py-GC/MS) is an analytical method in which nonvolatile samples can be measured to produce valuable mass spectral information about the internal structure and compounds contained within. This method involves flash heating the sample in an inert environment to produce pyrolyzates that are then directly injected into the GC-MS. A common application for Py-GC/MS is the characterization of polymer materials.
One problem that frequently occurs when analyzing polymers is that the observed pyrolyzates are often not registered in commercial EI mass-spectral libraries, making them difficult to identify by library search. Additionally, these pyrolyzates may not be registered in the PubChem database. Consequently, Py-GC/MS polymer structure analysis using EI mass spectra can be difficult, resulting in many unknown compounds.

Methods

In this work, we attempted to solve this problem by constructing a virtual mass-spectral library that combines calculating the pyrolysis reaction of the polymers with predicting EI mass spectra using machine learning. The computational aspects of the pyrolysis reactions for the polymers were carried out as follows: first, an oligomer with six monomers was created, and then the oligomer was computationally fragmented. Since many simple cleavages have been reported in the literature, we specifically focused on this cleavage type. A machine-learning model was then used to predict the EI mass spectra from the structural formulas of these in silico pyrolyzates. Afterwards, each pyrolyzate structural formula and predicted EI mass spectra were registered in a virtual mass-spectral library.

Novel Aspect

Efficient and accurate qualitative analysis of pyrolyzates derived from polymers using an in silico MS library developed using machine learning.

Preliminary Data or Plenary Speaker Abstract

An acrylic resin was prepared as a model sample and measured using a GC-HRTOFMS equipped with a pyrolyzer. Measurements were performed using EI, followed by Field Ionization (FI) to confirm molecular ions. The chromatographic data was processed using peak deconvolution and peak alignment, and then the FI+ accurate mass data for each compound was used for molecular ion confirmation and then subsequently, molecular formula determination.
Afterwards, the EI+ fragmentation data for each compound was compared to the NIST23 MS database as a starting point. From the library search results, we found that some of the monomers and dimers had structural formulas consistent with formulas reported in the literature. On the other hand, the structural formulas reported in the literature for most dimers and trimers could not be confirmed.
Next, a virtual library was constructed by performing computational pyrolysis on 60 types of representative homopolymers and copolymers. These computations produced 10 million in silico pyrolyzates that were not registered in the compound library. The mass spectra for these pyrolysis products were predicted from the structural formulas and then registered in the virtual library. After a database search using this new virtual library, the structural formulas reported in the literature for dimers and trimers were found at the top of the search results.
Using this method, we were able to correctly identify the thermal decomposition products derived from the resin that are not registered in the NIST mass spectral database or PubChem compound library.

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Shin-MassBank project: Generation of high-quality spectra from human metabolome datasets.

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

MassBank is a comprehensive mass spectrometry database that houses over 90,000 mass spectral data records obtained from standard compounds. The Shin (Neo)-MassBank project aims to enrich the MassBank records by developing a re-analysis pipeline capable of generating high-quality and well-annotated product ion spectra from publicly available metabolomics datasets. One of the key activities is enhancing data quality by generating averaged spectra from redundant MS2 spectra present in data-dependent acquisition (DDA) datasets. This approach aims to improve the accuracy and reliability of the mass spectral data by combining multiple measurements of the same compound, thereby reducing the impact of potential noise or experimental variations.

Methods

Seven human metabolome DDA datasets were downloaded from the Metabolomics Workbench (https://www.metabolomicsworkbench.org/) and MetaboLights (https://www.ebi.ac.uk/metabolights/) repositories. A dataset comprises 45 lipidome files acquired from similar yeast samples using the same DDA method of LC-Q-TOF/MS. The spectra averaging process was performed using in-house Python3 scripts. Two raw spectra data were considered similar if they met the following criteria: Here, two raw spectra data are considered similar if the retention time gap ($\Delta RT \leq 1.0$ min), the m/z gap of precursor ions ($\Delta m/z < 5$ ppm), and the cosine product score of two product ion spectra ($S \geq 0.9$) are within thresholds levels.

Novel Aspect

The Shin-MassBank provides high-quality of product ion spectra from human DDA datasets by spectra averaging.

Preliminary Data or Plenary Speaker Abstract

In mass spectrometry-based metabolomics studies, the analysis of product ion spectra was hampered by the presence of artifact signals and inaccuracies in measured mass-to-charge (m/z) values. To address this issue, we developed a method to average similar product ion spectra recorded in data-dependent acquisition (DDA) metabolome datasets. The averaging of product ion spectra was performed using the following approach: For example, 504,072 product ion spectra were gathered from 45 yeast lipidome data files acquired by the DDA mode of LC-Q-TOF/MS. Subsequently, a graph-based clustering approach was employed to group closely resembling spectra, resulting in the formation of 2,400 complete graphs (cliques). For each clique, fragment ions present in over 70% of the spectra were identified, and the median m/z values were calculated.
A comparable averaging procedure was implemented on eight DDA datasets obtained from human metabolomics studies. Comparative analysis revealed a more pronounced intra-file redundancy in quadrupole (Q)-Orbitrap-MS datasets compared to Q-TOF-MS datasets. Examination of the averaged dataset unveiled systematic errors in the m/z values of the DDA dataset, likely originating from

calibration tasks. This finding highlights the necessity for recalibration via subformula assignment to enhance the precision of m/z measurements.
The averaged data will be made accessible through the MassBank-Human (a library of mass spectra derived from human samples) webpage (https://human.massbank.jp/). The Shin-MassBank project (https://shin.massbank.jp/) plans to establish other three websites: MB-POST (a raw data file repository), MassBank in silico (a library of predicted mass spectra), and MassBank links (a portal site).

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A standalone software tool for annotating tandem MS spectra of modified nucleic acids.

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

This study aims to develop a software tool that will enable users at all levels to explore MS/MS spectra of RNA oligos, especially modified RNA oligos, and to obtain annotated spectra at publication quality.

Methods

We utilized two datasets for testing the annotation software. In the first, chemically synthesized RNA and DNA oligos (~20 nt) were dissolved to a concentration of 5 µM. The preparation involved using 0.1% formic acid as the buffer for positive ion mode and 3.75 mM N-hexylamine with 35-45% ACN for negative ion mode. These samples were then infused into the Fusion Lumos and fragmented by HCD and CID. Fragment NCE for CID and HCD was set from 5-45 (step 5) and 10-80 (step 10), respectively. The second dataset, from M Zuo et al., contains HCD and CID spectra of RNA oligos (4-9 nt). The annotation software, developed in Python with a PyQt5 GUI, was packaged for both macOS and Windows.

Novel Aspect

We developed nucleic acid MS/MS spectrum annotation software that enables high peak interpretation rates for both MS beginners and experts.

Preliminary Data or Plenary Speaker Abstract

This is the workflow of the annotation software: 1) The software reads spectra files, extracting peak lists for each spectrum. 2) It calculates theoretical ion m/z values, using user-input information of sequence, 5’ and 3’ termini, modifications, ion mode (positive or negative) and ion types. 3) The software matches theoretical and experimental m/z values within a user-defined tolerance. 4) The matched ion identities will be labelled around the peaks, with different identities represented by distinct colors. The match errors and fragmentation map will also be visualized. These plots can all be downloaded in vector format. In our testing data (RNA/DNA) under negative MS mode, all peaks with intensities above 10% can be annotated.
Unique features of this annotation software: 1) Due to the similarity in fragmentation patterns between DNA and RNA, the software now also supports annotating DNA tandem MS data. 2) Because base losses are common in nucleic acid tandem mass spectra, users can add them in configuration files to explain peaks not matched by typical sequencing ions. 3) To enhance support for nucleic acid modifications, this software accommodates all modifications published on the MODOMICS website. It also allows users to define new modifications via configuration files. 4) Other useful tips: Users can customize font size and ion type colors, zoom in/out the spectra, and download theoretical ion masses in Excel format. They can also navigate to any spectrum through a table window listing all spectra.
Furthermore, we will add metrics such as sequence coverage and peak interpretation rate of target ions to evaluate spectrum matching.

INVESTIGATING THE COMPOSITION PROFILES OF CRUDE OIL MIXTURES FROM LACUSTRINE AND MARINE ORIGINS USING MULTIVARIATE CURVE RESOLUTION AND FT-ICR MS

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The blending of crude oils complicates their composition and physicochemical properties, making the understanding of the underlying geochemical phenomena crucial for elucidating petroleum formation and geological history, assisting in the mitigation of exploratory risks. Our study proposes a novel method using multivariate curve resolution – alternating least squares (MCR-ALS) and Fourier Transform Ion Cyclotron Mass Spectrometry (FT-ICR MS) techniques. This approach aims to evaluate the distinct contributions of lacustrine and marine crude oil sources in mixtures from various origins. Employing MCR-ALS in combination with FT-ICR MS allows exploration of the pure contributions of marine and lacustrine oil mixtures. This integrated approach enables more precise differentiation and characterization of the complex compositional variations present in crude oils of diverse origins.

Methods

To introduce variability into the dataset, ten lacustrine crude oils were mixed, resulting in a mixture of lacustrine origin (LAC), while ten marine origin crude oils were mixed, resulting in a mixture of marine origin (MAR). Thirty-seven MAR/LAC binary mixtures were prepared by varying the mass ratio of LAC from 0 to 100%. These samples were analyzed using FT-ICR MS with atmospheric pressure photoionization (APPI) in positive mode at a concentration of 0.50 mg.mL-1. Molecular formulas were assigned using Composer® software. Subsequently, the data were organized using Matlab®, and MCR-ALS analysis was carried out using MCR-ALS GUI 2.0 toolbox. The number of components was chosen by singular value decomposition (SVD), and the initial estimate spectra were obtained using SIMPLISMA algorithm.

Novel Aspect

This work presents an innovative approach to evaluate lacustrine and marine contents in mixtures using APPI (+) FT-ICR MS.

Preliminary Data or Plenary Speaker Abstract

Based on the SVD algorithm, the binary mixtures consisted of two pure components. In practice, these two pure components are related to both MAR and LAC crude oil origins employed in binary mixtures. Consequently, the MCR-ALS method decomposes the data into pure signals for each component and pure concentration profiles. The pure signals are related to the mass spectra of LAC and MAR origins, while the pure concentration profiles are related with the proportion of LAC and MAR crude oil origin in binary mixtures.
MCR-ALS analysis displayed a lack-of-fit of 14.77%, with an explained variance of 97.82%. Regarding the pure spectral profiles recovered by MCR-ALS, both exhibited strong Pearson's correlations exceeding 0.98, with their respective components. The first pure component signal demonstrated a correlation of 0.99 with the spectrum of the LAC mixture. In turn, the second pure component signal demonstrated a correlation of 0.99 with the spectrum of the MAR mixture.
Furthermore, the concentration profile exhibited a correlation coefficient of 0.97, between the proportion of the LAC mixture in the LAC/MAR binary mixture and the concentration profile of the first component. Consequently, the MCR-ALS method is a good chemometric tool for differentiating

MAR and LAC crude oil mixtures. However, this approach has not been explored in studies involving petroleum mixtures of different origins.

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Answers to your questions about running big batch LC-MS, that you've been too afraid to ask.

Dr Katy Jeppe[1], Dr Christopher Barlow[1], Miss Eleanor Sabey[2], Dr Xin Lin[2], Dr Yuan Zhou[2], Dr Bruce Taylor[2], Director of Platform Ralf B. Schittenhelm[1]

[1]Monash Proteomics and Metabolomics Platform, [2]Menzies Institute for Medical Research

Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Batch effects both within- and between- LC-MS runs are a common problem when analysing biological samples, and the larger the experiment the bigger the risk and the effects. Here, we describe batch effect mitigation and management in a large column switching analysis (770 samples, 14 days run time) for semi-targeted/untargeted metabolomics (Vanquish LC and Q-Executive Plus2 Orbitrap, Thermo).

Methods

By including two sets of biological quality controls, a suite of stable isotope labelled (SIL) internal standards, and "just in time" sample preparation we were able to monitor and mitigate many common batch effects. However, some remained for both between- (column retention time differences) and within-batch effects (drift in m/z, retention time and intensity over time).

Novel Aspect

Batch effects are common and can be disastrous for experiments. We demonstrate precautions and solutions that can help avert disaster.

Preliminary Data or Plenary Speaker Abstract

Samples were prepared 'just in time' to limit the damage a stoppage would cause. We worked (and managed) to maintain a single batch (avoiding instrument shutdowns) to limit between-batch shifts in MS. We assessed m/z, retention time, and intensity changes in 'real-time' by regularly processing SILs and their endogenous compound during the run. We then used these assessments to make decisions on rerunning samples and after the acquisition to make informed changes to mzxml files that improved peak alignment and annotation. Finally, we adjusted intensity to improve statistical results masked due to batch effects.

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A Self-Adjusted Feature Detection, Noise Identification and Noise Reduction Algorithm for Gas Chromatography High Resolution Mass Spectrometry Data.

Mr Liam O'Brien[1], Dr Jake O'Brien[1], Dr. Saer Samanipour[2], Ms Denice van Herwerden[2], Professor Kevin Thomas[1]
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Feature detection is a critical pre-processing step for HRMS data analysis tasked with distinguishing analytical signal from noise.Feature detection algorithms have proven to be challenging to successfully run on raw GC HRMS data due to having a sparse distribution, noisy baselines that change substantially in height over a run and data that is complex and large. The Self Adjusting Feature Detection (SAFD) algorithm is a non-target feature detection algorithm, originally developed to identify relevant signals within LC chromatograms. This presentation focuses on presenting and discussing the challenges observed and the solutions both attempted and finally utilized to address difficulties and limitations found with applying SAFD to GC HRMS Orbitrap data. The new Algorithm is called SAFD GC.

Methods

Data previously collected on a GC Orbitrap was analysed using two tools for non-target feature detection. An existing algorithm, the Self Adjusting Feature Detection algorithm was chosen to be updated. The reason for using this algorithm was this algorithm does not require a pre-processing step, and interprets the profile data.

Novel Aspect

A non-target feature detection algorithm for GC HRMS profile data is novel and unique.

Preliminary Data or Plenary Speaker Abstract

Compares well to MS-Dial, one of the leading algorithms for feature detection in GC non-target. SAFD GC found less false discovery rate compared to MS-Dial. 13 GC Orbitrap files were analyzed through the two algorithms then their output compared for similar and different peaks. To compare the accuracy of the respective algorithms, 325 random peaks were plotted and visually analysed from the unique and common found peaks. The false discovery rate for MS Dial unique peaks was around 85% compared to around 50% in SAFD GC.

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Phytochemical and bioactivities of promising flavonoid glycosides and their content from unmatured fruits of Vicia bungei with their bioactivity

Mina Lee[1]
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Flavonoids have extensive biological qualities that support human health. Using UHPLC-MS guided identification of flavonoid glycosides from Vicia bungei, a molecular networking strategy produced representative networks despite mass fragmentation of spectra of untargeted data-dependent acquisition approach to target flavonoid derivatives. Using contemporary methods, seven chemicals were extracted and identified. Antioxidative and anti-inflammatory effects of these isolates on inflammatory mediators , cytokines, enzymatic proteins, and free radicals were assessed in vitro. Two active compounds, apigenin 6-C-β-D-galactopyranosyl-8-C-β-D-xylopyranoside, and sphaerobioside, were further assessed for their binding affinity to target protein in in silico study. Furthermore, an analytical method was successfully established and employed to quantify the total extract using these seven chemicals present in this plant as markers.

Methods

Using contemporary methods, seven chemicals were extracted and identified. Antioxidative and anti-inflammatory effects of these isolates on inflammatory mediators , cytokines, enzymatic proteins, and free radicals were assessed in vitro. Two active compounds, apigenin 6-C-β-D-galactopyranosyl-8-C-β-D-xylopyranoside, and sphaerobioside, were further assessed for their binding affinity to target protein in in silico study.

Novel Aspect

Discovery of bioactive phytochemicals via molecular networking and molecular docking

Preliminary Data or Plenary Speaker Abstract

The molecular mechanism of sphaerobioside was found to involve suppression of LPS-stimulated inflammation by NF-κB inactivation by inhibiting nuclear translocation of p65 and prevention of phosphorylation of κB inhibitor α (IκBα) and IκB kinase (IKKα/β).

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Development of quantitative analysis of 15 types of phthalates in hygiene products by using gas chromatography–mass spectrometry

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Phthalates, or phthalate esters, are a group of compounds with a wide range of industrial applications, including their use as solvents and plasticizers, particularly as additives in polyvinyl chloride(PVC) to give it flexibility.
However, they also have many negative effects on the environment and human health, which has become a global problem.

The Ministry of Food and Drug Safety's 'Cleansing & Hygiene Products Control Act' regulates permissible detection limits, which should not exceed 0.1% of seven types of phthalates* in hygiene products children's such as diapers and swabs.
* Diethylhexyl phthalate(DEHP), Dibutyl phthalate(DBP), Benzyl butyl phthalate(BBP), Diisononyl phthalate(DINP), Diisodecyl phthalate(DIDP), Di-n-octyl phthalate(DnOP), Diisobutyl phthalate(DIBP)

Methods

In this study, we developed a simultaneous GC-MS analysis method for fifteen types of phthalates, including seven types that are regulated under the 'Cleansing & Hygiene Products Control Act' and the additional eight types for which toxicity research is being actively pursued by chemicals management organizations such as the European Chemicals Agency(ECHA).
** DEHP, DBP, BBP, DINP, DIDP, DnOP, DIBP, Diethyl phthalate(DEP), Dimethyl phthalate(DMP), Di(methoxyethyl) phthalate(DMEP), Diisopentyl phthalate(DIPP), N-pentyl-isopentyl phthalate(PIPP(IPPP)), Di-n-pentyl phthalate(DNPP(DPENP)), Di-n-hexyl phthalate(DNHP(DHEXP)), Dicyclohexyl phthalate(DCHP)

Novel Aspect

It is considered that this method would contribute to the quality control and safety evaluation of Cleansing & Hygiene Products.

Preliminary Data or Plenary Speaker Abstract

Samples were taken from the synthetic resin of hygiene products, such as diapers, swabs, and toilet paper, and pulverized using a freezer mill, followed by n-hexane soxhlet extraction. The GC-MS analysis was performed on a DB-5MS column. The oven temperature of the GC-MS was heated from 110 ℃ to 320 ℃. The acquisition was performed in both full scan mode(m/z=50-500) and SIM mode.

Simultaneous analysis for fifteen types of phthalates was developed and validated using GC-MS, according to the 'Guidelines for the validation and verification of chemical test methods (KOLAS-G-014:2020)' and AOAC guidelines 'Appendix K'. The validation results showed excellent linearity, accuracy, and precision, confirming that these methods are applicable to the detection of fifteen types of phthalates.

As a result of the validation, the linearity showed good linearity($R^2 > 0.993$) over a concentration range from 4 to 160 μg/g. The limits of detection(LOD) and quantification(LOQ) were approximately 0.02-0.15 mg/L and 0.07-0.46 mg/L, respectively, with a linearity(R^2) greater than 0.99 in the range of 0.5~20.0 mg/L. The intra- and inter-day accuracies ranged from 93% to 114%.

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Hive: A Novel File Format and Reader API for Mass Spectrometry Data

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

In recent years, the sizes of raw data files containing the results of mass spectrometry measurements have increased significantly owing to the increased functionality and performance of instruments. While this enhancement is a welcome development, the larger data size not only makes analysis and processing more time-consuming but also makes even realistic processing using conventional personal computers and software difficult. Mass spectrometry data analysis software often replaces raw data with generic formats, such as mzML, to enable interpretation and analysis of raw data in formats that vary by manufacturer and instrument. This replacement further increases the file size and reduces processing speed, thus necessitating a lightweight and high-speed raw data processing solution that is manufacturer- and instrument-agnostic.

Methods

In this study, a completely new file format, Hive, which is independent of the manufacturer or instrument, has been developed for the lightweight and fast analysis of mass spectrometry measurement data. This format is created by converting raw data in the same way as conventional general-purpose XML files such as mzML; however, the converted data is in binary format, thereby reducing the file size while maintaining the accuracy of the information in the raw data. However, the binary format does not allow the content to be read directly by a text editor. Therefore, an application programming interface (API) was also developed using C#, Java, and Python, to read the binary data.

Novel Aspect

New generic format Hive and API developed for smaller file size and faster processing of mass spectrometry measurement data.

Preliminary Data or Plenary Speaker Abstract

To evaluate the efficiency of the proposed Hive, file sizes and read speeds of raw data and Hive format were compared for different measurement modes, manufacturers, and instruments. On average, file sizes were reduced to less than one-third of their original raw data sizes, and in some cases, to less than one two-hundredth. At no point did the converted file size exceed that of the original raw data. In all cases, the API reading data speed in Hive format was faster than extracting information from the raw data. In some cases, the reading speed using API was more than 50 times faster than the rate of extracting information from the raw data. We attribute this to the file size, data structure, and processing methods that were considered to enable fast processing in sequence and random access. Although there is an overhead in converting the raw data into Hive format, the conversion allows for fast and lightweight data analysis and helps with the problems caused by the increased size of the measured raw data. With the effectiveness of the Hive format confirmed, we are finalizing the release of Hive-formatted data in MetaboBank, DNA Data Bank of Japan (DDBJ)'s public repository, to facilitate broader research applications. The integration of Hive format data with raw data in public databases is anticipated to significantly improve the efficiency of data analysis for researchers worldwide.

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Exploring the performance of linear and non-linear models of ToF-SIMS spectra

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

ToF-SIMS data is inherently hyperspectral, and many machine learning (ML) techniques – both linear and non-linear, have been previously employed. Given the feature complexity and dataset size, there is generally a disparity between the applicability and operability of the methods, leading to overfitting or underfitting of the data in what is known as the bias-variance tradeoff. Especially when the training set size is diminished, the performance of the models can degrade significantly. Therefore, it is critical that we evaluate the performance of these models subject to various conditions that may affect their generalization ability. This work explores the performance of linear partial least squares (PLS) and non-linear random forest (RF) models in a regression setting.

Methods

The sample system was prepared in a microarray format by spotting 21 mixtures of blue/red dyes on an ITO-coated glass slide using a liquid handling system (BioDot), resulting in 12 replicate arrays containing 4 replicates for each dye mixture (total 1008 spots with diameter approx. 400 µm). ToF-SIMS stage rastering was used to capture large area scans across a grid of 9.2 × 9.2 mm for each array. The data collected was analyzed using partial least squares (linear) and random forest (non-linear) regression methods. Baseline models were initially constructed to make direct comparisons between the 2 methods, followed by a study of their feature importance. Lastly an investigation of the relationship between training set size and model performance was conducted.

Novel Aspect

We performed a comprehensive regression study of ToF-SIMS spectra subject to changes in the training set using common ML methods.

Preliminary Data or Plenary Speaker Abstract

Initially, the dataset was randomly split into 75% training and 25% test data, which was used to generate baseline models for random forest and partial least squares methods. In this case, the non-linear RF model achieved higher performance than the linear PLS model, indicating its robustness in its ability to generalise hyperspectral ToF-SIMS data.
Additionally, we generated a concatenated dataset comprising both positive and negative secondary ion peaks from the ToF-SIMS spectra. Interestingly, this dataset produced the best performing models for both RF and PLS, which indicates that there exist important peaks within either dataset that supported the construction of a better performing model, despite increasing the number of features/peaks.
As for peak importance measures for either model, the concatenated dataset also placed higher importance to peaks which are strongly associated with the structure of the dyes, such as the fragmented sulphonate anions (R-SO3-) found in the blue dye.
Lastly, the model performance between PLS and RF was explored by adjusting the size of the training set. In the low data limit (~20% training set size or the equivalent 10 sample replicates), the predictive power of the RF model degraded much quicker than PLS, and eventually loses to the PLS model. This phenomenon is tied to the bias-variance tradeoff, where the extrapolation of the test data from the non-linear RF model becomes weaker and more variable, due to a less informative training set. This result reflects the need to keep enough sample replicates in our experimental work to ensure the performance of ML models in subsequent statistical analyses.

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A Comprehensive Quantitation Platform for Untargeted Metabolomics Data

Miss Rui-yi Chen[1], Miss Pin-yu Wang[1], Sin-Chen Chiang[2], Jie-Wei Chiu[2], Dr. Hui-Yin Chang[1,2]
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Metabolomics refers to the qualitative and quantitative analysis of small molecule metabolites within a biological system at a specific point in time. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has become a popular analytical platform in metabolomics, and quantification stands as a crucial endeavor encompassing various stages such as noise elimination, peak identification, and peak alignment. Visualization is also important for evaluating quantitation results. We thus proposed a comprehensive quantitation platform with three implemented software tools, including MS-Picker, MS-Aligner, and DeNox, as important components for untargeted metabolomics quantitation.

Methods

Upon importing raw files, MS-Picker initiates feature detection by extracting their m/z, retention time, charge states, and abundances, and evaluates the quality of detected features using logistic regression along with nine metrices, exporting quality scores for detected features. Next, MS-Aligner aligns the detected features across files, generating an integrated feature table with features in rows and files in columns. It also summarizes the quality scores across files, providing a general quality indicator for downstream statistical analysis. DeNox empowers users to interact with the raw files and visualize quantitation outcomes. All three software tools are cross-platform applications developed in Java, where MS-Picker and MS-Aligner operate as command-line tools and DeNox provides a graphical user interface for ease of use.

Novel Aspect

Our platform offers accurate quantitation, quality scores for detected features, and user-friendly visualization, empowering quantification and quality assessment.

Preliminary Data or Plenary Speaker Abstract

We evaluated the performance of our platform using a public blackcurrant dataset with eleven samples downloaded from MetaboLights (MTBLS773). The eleven blackcurrant samples were analyzed using positive and negative ion modes. Four technical replicates were generated for each sample by analyzing four times in each mode. The abundance ratios served as the metric for assessing performance, defined as the ratio of abundance between any two technical replicates. The closer the ratio gets to one, the more accurate the quantification is. We processed the blackcurrant dataset using our platform and three public software tools, including iMet-Q, XCMS Online, and MZmine3. As a result, in the positive-mode dataset, the average sample ratios of MS-Picker/MS-Aligner, iMet-Q, XCMS Online, MZmine3 are 0.92, 0.92, 0.99, and 0.9, respectively. In the negative-mode dataset, the average sample ratios of the four platforms are 0.97, 0.97, 0.98, and 0.99, respectively. The findings indicate that all four platforms yield comparable quantification outcomes, and the performance is satisfactory. A uniqueness of our platform is that it provides the quality scores of detected features. DeNox allows users to view both quantitation and quality assessment results. To simplify its use, we have updated the software, which includes reducing the number of operations, placing parameter settings in a collapsible page, and making the size of each section on the main page adjustable.

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Exploring the Relationship between Polymer Surface Chemistry and Bacterial Attachment Using ToF-SIMS and Self-Organizing maps

See Yoong Wong[1], Dr Wil Gardner[1], Andrew Hook[2], Martyn Davies[2], Prof David Winkler[1,2], Morgan Alexander[2], Chien-Yi Chang[3], Ying Mei[4], Paul Williams[2], Davide Ballabio[5], Dr Benjamin Muir[6], Prof Paul Pigram[1]

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Biofilm formation is a major cause of hospital-acquired infections. Research into biofilm-resistant materials is therefore critical to reduce the frequency of these events. Materials that are inherently resistant to bacterial attachment and biofilm formation provide an alternative approach to preventing device-associated infections that avoids the development of antimicrobial resistance. However, advanced level of understanding of biofilm formation on polymeric materials is required to enable ab initio design of biofilm-resistant materials. Polymer microarrays offer a high-throughput approach to enable the efficient discovery of novel biofilm-resistant polymers.
ToF-SIMS provides a comprehensive assessment of the surface chemistry of polymers, enabling the construction of structure–function relationships. We use Self Organizing Maps (SOMs) to identify relationships between polymer surface chemistry and biofilm attachment property.

Methods

Microarrays consisted of over 400 different combinations of 22 different monomers were created. One of the arrays was used for bacteria (P. aeruginosa) attachment assay and the other was used for ToF-SIMS measurements, which were conducted on a ToF-SIMS IV (IONTOF GmbH, Germany) instrument.
ToF-SIMS spectra were transformed into data array and each sample was associated with its measured bacterial counts. We compared the performance of different SOMs algorithms and trained another set of SOMs with random assigned classes to test the quality of classification.
The output weights from neuron clusters representing polymers with lowest and highest bacterial attachment were selected to compare the chemical groups associated with high bacterial resistance and attachment.

Novel Aspect

Application of SOMs to study the indirect relationship between input data (secondary ions fragments) and assigned classes (bacteria count).

Preliminary Data or Plenary Speaker Abstract

The relationships between ToF-SIMS data and biofilm formation are analyzed using conventional linear multivariate analysis (PLS regression) and a nonlinear self-organizing map (SOM). We explored different SOM variants and their ability to reveal information about structure–property relationships in a polymer microarray.
Comparing the weights of clusters representing the highest and lowest bacterial attachment for the counter propagation Kohonen network (CPN) and supervised Kohonen network (SKN) suggested that SKN had a stronger ability to cluster and identify major chemical functional groups associated with bacterial resistance and bacterial attachment. The weights of the SKN suggested that fluorine- and nitrogen-containing groups hinder bacterial attachment while hydroxyl and long glycol groups promote bacterial attachment. The weights also showed that the contaminated samples or other outliers could be identified in the SKN topographic map as isolated neurons. This powerful ability of the SKN to identify contaminated samples allows them and other outliers, which could complicate conventional regression analyses, to be eliminated with justification.
The SOM models revealed several combinations of fragment ions that are positively or negatively associated with bacterial biofilm formation, which are not identified by PLS. With these insights, a

second PLS model is calculated, in which interactions between key fragments (identified by the SOM) are explicitly considered. Inclusion of these terms improved the PLS model performance and shows that, without such terms, certain key fragment ions correlated with bacterial attachment may not be identified. The chemical insights provided by the combination of PLS regression and SOM will be useful for the design of materials that support negligible pathogen attachment.

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Open-source data processing software for single particle ICP-ToF

Dr Thomas Lockwood[1], Dr David Clases[2]
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Thomas Lockwood 415 - Open-source data processing software for single particle ICP-ToF, Meeting Room 110, August 22, 2024, 16:03 - 16:22

The comprehensive analysis of nano-materials is increasingly desired as we discover new applications, and more about their health and environmental impacts. Single-particle (sp)ICP-MS has become the technique of choice for investigating nano-materials in environmental matrices and is able to provide information about their size, mass and number. However, traditional quadrupole instruments can only monitor a single isotope, resulting in time consuming analyses and incomplete information on particle compositions. The introduction of time-of-flight ICP instruments has revolutionised single particle analyses, by capturing the entire elemental mass range. As there is currently no vendor agnostic solution for data processing we have built upon existing open-source software to introduce an spICP-ToF processing solution and demonstrate its capabilities through several applications.

Methods

Data was collected on a Nu Instruments Vitesse ICP-ToF. The software, SPCal, is written in Python using NumPy and C extensions for performant code. The GUI is implemented in Qt6 using the PySide 6 library. Import of raw data, with an elemental selector, is implemented for both Nu Instruments and TOFWERKs ICPs. To calculate the detection thresholds produced by a ToF instrument a novel log-normal approximation was created to simulate Poisson sampling from a log-normal single-ion area. Commercial spirits were obtained from a local distributor and non-target analyses performed by scanning for elements with detection numbers greater than a stipulated threshold. Particle compositions were determined using hierarchical-agglomerative clustering. Cobalt particles were extracted from soil collected at six locations across Australia.

Novel Aspect

The first open-source spICP-ToF processing platform.

Preliminary Data or Plenary Speaker Abstract

Data importers, calibration pathways and tools implemented in the previous version of SPCal were updated to work with spICP-ToF data.
The threshold produced by the log-normal approximation was compared to that of a Monte-Carlo simulation (1 billion samples) of the compound Poisson process. There was excellent agreement between the two for error rates of 0.05 to 1e-6 and mean background ranging from 0.1 to 5 counts. Above this, Gaussian statistics better describe the detection threshold.
Existing SP analysis algorithms are unable to easily pinpoint NP events for every element in gigabyte sized data sets. SPCal incorporates a “non-target screening” approach that rapidly defines a decision limit for all recorded m/z. Using a threshold of 25 ppm whilst acquiring a mass range from 45-210 amu pinpointed Ti, Fe, Ag, Sn and Au as particulate elements in commercial spirits within seconds and without previous knowledge.
One of the fundamental benefits of spICP-ToF is the ability to determine particle compositions. Clustering of particle compositions revealed the presence of three major Co containing compositions in soil samples from an Australian weathering pyrite environment. Mn was found with Co in most fractions, showing that the Co particles have a key association with Mn, and most likely exist in the form of various Co-Mn oxides.
A major drawback of ICP-ToF is the relative lack of sensitivity compared to quadrupole based instruments. A calculator tool in SPCal allows the user to perform arithmetic operations on acquired signals. When analysing Gd and Yb containing UCNPs, the sensitivity of the analysis could be

increased 32 times by considering the sum of all Gd and Yb isotopes, compared to 158Gd alone. While backgrounds did increase, the size detection limit was still improved from 47.7 to 25 nm.

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Transforming Raw Data to Actionable Insights: A Total Ion Current-Based Assessment to Evaluate Sample Quality and Instrument Performance

Mr Vimalnath Nambiar[1,2], Dr Shabarinath Nambiar[1,2], Dr Luke Whiley[1,2], Professor Kok Wai Wong[3], Dr Guanjin Wang[3], Prof Elaine Holmes[1,2,4], Professor Julien Wist[1,2,5]
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The integration of liquid chromatography (LC) with mass spectrometry (MS) has revolutionised the field of metabolic phenotyping studies, yet spectral data acquired from these instruments pose significant challenges due to their susceptibility to sample and instrument variations. Consequently, ensuring data quality and reliability has become a time-consuming bottleneck in high-throughput LC-MS data processing pipelines, requiring the manual in-person assessment of each acquired sample. To address this, we present an analytical methodology employing total ion current (TIC) for rapid sample quality and instrument performance evaluation.

Methods

Our study utilised an exploratory dataset comprising of 3439 control samples obtained using LC-Quadrupole Time-of-Flight (QToF)-MS instruments. These samples include quality control (matrix-free) and calibration samples of different concentration levels along with long-term biological references (LTR) of different matrices. Using an in-house data preparation pipeline, we filtered the mass-to-charge (m/z) values stored within the spectral data against 188 known compounds of interest that encompass mass calibration references, internal standards, endogenous analytes, and product ions.

Our analytical workflow involved a preliminary check on the total amount of spectral data available for each sample followed by univariate and multivariate statistical analyses, including violin plots, time series, and principal component analyses.

Novel Aspect

Introducing total ion current (TIC) for rapid LC-MS data quality assessment, enhancing reliability and efficiency through automated analysis.

Preliminary Data or Plenary Speaker Abstract

The proposed analyses efficiently assessed the TIC values across chromatographic regions, revealing influences of sample quality and instrument variations through the identification of significant outliers using deviation-based threshold limits. Further examination of the TIC of extracted ions also enabled the observation of compound stability and detection of mass accuracy drift.

Our study addresses a significant issue in metabolic phenotyping research by introducing a novel LC-MS data quality assessment methodology. By combining the use of TIC values for spectral data analysis with statistical modelling approaches, we offer a swift systematic approach to evaluate sample quality and instrument performance without the need for manual assessment. Through automation and reporting features, the proposed methodology significantly lowers the time and resources required while enabling researchers to consistently ensure the quality and reliability of their data. By mitigating the impact of sample and instrument variances, we can facilitate more accurate and reproducible metabolic phenotyping studies, paving the way for deeper insights into biological systems.

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Identifying Drug Targets with Extracellular Vesicles: Developing a Native Mass Spectrometry Platform to Investigate Ligand Interactions with Tuberculosis-Related Membrane Proteins

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

This project proposes the utilization of extracellular vesicles as an innovative platform for identifying drug targets, particularly focusing on tuberculosis (TB)-related membrane proteins. Through native mass spectrometry (MS) techniques, it aims to elucidate the complex biological interactions at the membrane protein level, facilitating the identification of effective ligands for previously unidentified targets and discovering new ligands for membrane proteins with unknown binding partners.

Methods

By using extracellular vesicles as a model system, the project proposes a direct path to understanding the influence of the native lipid environment on membrane protein structure and function. This platform will significantly reduce the complexity and time associated with drug target identification, enabling rapid screening and discovery of new therapeutic targets and ligands.

Novel Aspect

This method identifies ligands binding to native membrane proteins and membrane protein targets for ligands with unknown targets.

Preliminary Data or Plenary Speaker Abstract

Our lab has successfully screened ligands for soluble proteins, and the process runs quite well.

Data Storage and Retrieval with Proteins and LC-MS/MS

Yin Zhou[1], Prof. Zhongping Yao[1], Prof. Francis Lau[1], Dr. Cheuk Chi Ng[1], Chengxi Liu[1], Dr. Wai Man Tam[1]
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

In this era of information explosion, data are generated at an exponential rate. Current data storage devices are expected to reach a plateau soon. New data storage methods that can store data with high density and long duration are desirable. Data storage in molecules is a potential alternative. DNA is the most popular molecule applied for data storage in recent years. Compared to DNA, proteins can achieve higher storage capacity and stability with the availability of more monomers (amino acids) for the data storage. However, data storage using proteins, particularly the expression and sequencing of de novo designed data-bearing proteins, is very challenging.

Methods

In this method, by assigning amino acids as specific sequences of digital bits in binary digital data, the digit data are translated into amino acid sequences, which are expressed as proteins by E. Coli, and stored in the format of powder. To retrieve the data, these proteins are digested by trypsin, and the digestion mixtures are analyzed by LC-MS/MS, with the acquired MS/MS spectra processed by our developed software for assignment of amino acid sequences. The protein sequences obtained are converted back to binary digits and decoded to the raw data.

Novel Aspect

New data storage method using proteins, de novo protein design and de novo sequencing.

Preliminary Data or Plenary Speaker Abstract

We designed several templates for data-bearing proteins. In template I, data-bearing peptides composed of 8 amino acids were directly jointed together to form a protein encoding a text file of 1088 bits. However, no intact protein was observed after expression, and no data-bearing peptide was detected by LC-MS/MS. After changing the hydrophobic amino acids to more hydrophilic ones, although the intact data-bearing protein still could not be detected, most of the amino acid sequences was detected by LC-MS/MS after trypsin digestion, suggesting that the use of more hydrophilic amino acids improved the expression of proteins but not sufficient for robust protein expression.

We improved the yield of data-bearing proteins by imitating the sequence pattern of collagen. Template II followed the pattern of typical collagen-like (GXX)n sequences, and included V-domain before the data-bearing region. Data-bearing proteins that were constructed using template II were successfully expressed with high yields, and reached over 95% sequencing correct rates by LC-MS/MS analysis. Native MS analysis showed that template II data-bearing proteins were predominantly unfolded. To demonstrate the possibility of data storage with highly ordered proteins, we further designed template III that introduced leucine-zipper motifs to form coiled-coil structures. The template III data-bearing protein was successfully expressed, with the stored information fully retrieved. The CD and native MS analysis indicated that the template III data-bearing protein could form α-helix and was folded to a compact structure.

By designing the protein templates and optimizing the protein expression and sequencing, we successfully stored text files into and retrieved data from data-bearing proteins, demonstrating the feasibility of data storage using proteins. This new data storage method offers new possibilities for multi-purpose high-density data storage.

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Quality control of non-target analysis using the same model of mass spectrometer

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The improvement in the quality of data obtained by mass spectrometry has made possible new scientific methodologies that do not require any targets to be set at in advance, such as structural estimation of unknown compounds and extraction of relationships among samples. A considerable element of this methodology is an extensive and high-quality mass spectrometry database. However, the records in the currently available databases are not acquired on a single instrument. Although there is a possibility of re-calibration, data quality is not always evaluated from an authorized perspective. In this study, we analyzed common samples using the same model of mass spectrometer and compared the data qualitatively.

Methods

Liquid chromatography-mass spectrometry (LC/MS) with electrospray ionization (ESI) were examined. Eight institutions with the same system consisted of LC and quadrupole time-of-flight type tandem mass spectrometer (SCEIX X500R) participated. Mixed standard solutions of pesticides were analyzed. Data were acquired for positive and negative ion modes using common reversed-phase mobile phases and columns (GL Science, InertSustain Swift C18 metal free) in data-dependent and data-independent data acquisition modes (DDA and DIA). Different calibrations were applied for DDA and DIA. In LC, an additional column (GL Science, Delay Column for PFAS) was installed upstream of the autosampler. This allowed separation of the signals into those derived from LC system and those from the sample injected by the autosampler.

Novel Aspect

Quality control in non-target analysis using high resolution mass spectrometry was conducted using the identical model of mass spectrometers.

Preliminary Data or Plenary Speaker Abstract

ESI is used extensively in mass spectrometry ionization, but the actual ionization steps involved are indeed complex. Adduct ion formation, in-source fragmentation, and dimer formation, which are not of concern in usual quantitative analysis where target analytes are well-defined, happen in varying degrees depending on the state of the instrument. If this is not addressed and the analysis is performed without any targets to be set at in advance, there is a high likelihood that the in-source fragment ions and adduct ions will be misidentified as protonated or deprotonated molecules. In addition, different calibrations were separately applied to MS1 and MS2 in the instruments used in this study. Their degrees of trueness did not always agree and varied within the range of the instrumental specification. Furthermore, compounds like surfactants are often a source of LC system persistence signals. Even with the same setup of the same model, the same results were not always obtained in multiple labs. The two main issues reported in this presentation are: 1) changes in the accurate mass trueness under different calibration conditions, and 2) variation of ion species produced at ESI using the identical instruments.

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Haloacetic acids analysis in water samples via portable and field-deployable liquid chromatography – mass spectrometry

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The portable analytical technologies allow for the rapid on-site screening of the target analytes and reduce the analysis cost by eliminating the shipping of bulky samples to the centralized labs. These portable systems are characterized by the minimal exposure of the samples to degradation or contamination. Currently, various new methods based on portable liquid chromatography – mass spectrometry (LC-MS) are being developed for monitoring emerging environmental contaminants. Haloacetic acids (HAAs) represent a major group of disinfectant byproducts (DBPs) in drinking water. United States Environmental Protection Agency (USEPA) has classified dichloroacetic acid as a probable human carcinogen and trichloroacetic acid as a possible human carcinogen. Furthermore, decarboxylation of HAAs results in the formation of trihalomethanes, which are known carcinogens.

Methods

In this study, a portal capillary LC-MS system equipped with a polar-modified C18 column was utilised for the analysis of the major HAAs reported by USEPA using a gradient elution program. This presentation will illustrate results from the validation of our portable system, and discuss different sample enrichment strategies investigated, particularly, solid phase extraction (SPE). The SPE-LC-MS method allows for the sensitive determination of HAAs at their regulated limits in disinfected water matrices, namely swimming pool samples and spiked tap water samples. The % recovery of HAAs using the SPE protocol was evaluated using pre-spiked and post-spiked standard solutions and compared with other reported methods.

Novel Aspect

The portable LC-MS set-up will enable a cost-effective site evaluation where quick data feedback is required.

Preliminary Data or Plenary Speaker Abstract

Different chromatographic conditions were optimized for HAAs analysis. The best resolution and sensitivity were achieved using a gradient elution program based on 0.05% formic acid in water as solvent A and methanol as solvent B. Injection loop of 0.6 µL was installed in the portable chromatographic system. The % recovery of HAAs using our SPE protocol and the other reported protocols were comparable. HAAs were detected in spiked tap water samples at par per billion (ppb) levels. The level of HAAs in drinking water is regulated in many countries and consequently must be monitored strictly. USEPA has set the total maximum contaminant level (MCL) of five HAAs (HAA5), namely, monochloroacetic acid (MCAA), monobromoacetic acid (MBAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), and dibromoacetic acid (DBAA) in drinking water to be 60 µg/L. Moreover, 76.0 ppb of DCAA and 228.1 ppb of TCAA were determined in local swimming water using the portable LC-MS system. Having a portable LC-MS set-up for the on-site monitoring of the HAAs within the treatment plants and along the distribution systems has considerable potential value, particularly in comparison with traditional ‘grab and lab’ based approaches. The new approach eliminates the need for shipping bulky samples and minimizes the risk of samples’ degradation and/or contamination.

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Modular open-access and open-source Julia HRMS toolbox

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Non-target analysis (NTA) of biological or environmental samples analyzed with chromatography coupled with high-resolution mass spectrometry (HRMS), generally yields complex data. While there are a variety of tools and software available for the processing of these complex datasets, not all programs are open-source or open-access, provide sufficient freedom to the user to tailor their data processing to the needs of the research question, or are fully suited for processing NTA data as they may be more tailored to targeted or suspect screening approaches. Therefore, we developed a modular open-source and open-access framework for the processing of HRMS data. The jHRMS toolbox allows the user to perform a variety of different workflows, containing steps from feature detection to trend analysis.

Methods

The modules related to processing data independent or data dependant raw data are feature detection using the self-adjusting feature detection algorithm (SAFD), componentization using CompCreate, and suspect screening. Identification can be performed with the Universal Library Search Algorithm (ULSA.). Alignment of features or components, and statistical analysis (i.e., PLS-R, PLS-DA, HCA, SVD, and k-means) can also be performed. The library database contains spectra from MassBank EU, MassBank of North America, and the Global Natural Products Social Molecular Networking (GNPS) or user can provide their own. Moreover, the jHRMS toolbox allows significant modularity, which enables users to perform steps within a workflow using different algorithms or programs and use this output within the jHRMS toolbox (e.g. from/to Patroon).

Novel Aspect

jHRMS Toolbox provides an open source open access modular platform to both process and evaluate HRMS data.

Preliminary Data or Plenary Speaker Abstract

Additionally, the toolbox comes with a set of built-in visualization tools that enable the user to evaluate their raw data and the results from the different algorithms. The toolbox also allows users to perform a variety of statistical analysis approaches for initial screening to evaluate if these could provide useful insight or trends in a dataset. Overall, the jHRMS toolbox is an open-access and open-source program developed for the analysis of HRMS data, which can be used in a highly modular way as well as being tailored to user specific needs. To showcase the toolbox, a set of wastewater and biological samples has been processed.

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Identification of anthropogenic compounds, introduced into pristine stream waters, using non-target HRMS strategies.

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Surface waters are environmentally impacted by wastewater sources. Pristine, upstream water sources can be compared to downstream locations to find anthropogenic compounds potentially contaminating a river. A common approach is to analyze these samples using high resolution mass spectrometry techniques to measure the maximum number of potential contaminants. However, thousands of compounds can be measured with modern technology, requiring tools that provide focus on significant compounds. Further, one of the most demanding tasks is the identification of potential contaminants in water samples. We applied statistical tools to find compounds introduced into a river by urban sources, then we compare compound identification strategies from a variety of tools aiming to identify all the anthropogenic compounds.

Methods

Water samples, collected between 2017-2022 along the Big Thompson River in Colorado, were prepared with solid phase enrichment. Two sample sites are from pristine locations, used as controls. Four sample sites were downstream, passing through an urban area and past wastewater treatment plants. The extracts were analyzed by high-resolution mass spectrometry using an LC/Q-TOF instrument operating in positive ion mode. Two modes of acquisition were used: data independent (All Ions) and data dependent (MS/MS) modes. MassHunter Explorer was used to find compounds unique to downstream locations. A combination of compound identification techniques including the use of retention time and accurate mass databases, NIST Tandem MS/MS libraries, SIRIUS and CSI:FingerID were used to corroborate putative identifications of the anthropogenic compounds.

Novel Aspect

A new workflow for the high confidence, putative identification of statistically significant anthropogenic compounds.

Preliminary Data or Plenary Speaker Abstract

More than 5,000 compounds across 78 samples were measured. The data was analyzed using a combination of statistical techniques to find the anthropogenic compounds introduced by urban and wastewater sources. LOESS normalization removed batch-to-batch variation observed in the acquired data, collected over the years. 1-way analysis of variance (ANOVA) of the normalized data, focused the analysis on compounds that were statistically different ($p<0.05$) between the pristine upstream sites and all downstream sites after the wastewater treatment plant. A comparison of the abundance ratios allowed for prioritization in identifying >300 compounds 10x more abundant in downstream locations. This group of compounds reflect what is introduced by wastewater treatment plants and other urban activities and contribute to anthropogenic contamination of pristine water sources.

Identification of these compounds was done using several techniques: (a) Databases (both home-made and commercial) were applied first and identified about 10% of the anthropogenic compounds; (b) Iterative MS/MS of pooled samples were investigated for the presence of these compounds and the resulting MS/MS spectra were then compared to the NIST Tandem Mass Spectral Library; (c) MS/MS spectra were also processed by SIRIUS and CSI:FingerID to obtain putative identifications via fragmentation patterns.

The results from the multiple identification techniques were compared to corroborate putative identifications of the anthropogenic compounds. Many pharmaceuticals, pesticides and their degradation products form a portion of the putative identifications, some confirmed with retention times from reference standards, some with significant evidence from correlating fragment ions with chemical structures or curated library spectra. However, putative identifications for some compounds have a lower confidence from a single compound identification technique, so we compare results from multiple identification techniques to improve confidence in the putative identification of these anthropogenic compounds.

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Non-targeted analysis of decomposition products of polypropylene and investigation of compound changes according to UV exposure period

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Large plastics entering the ocean are exposed to sunlight and produce microplastics (<5 mm) through photo-oxidation. Polypropylene (PP) has a lower density than water, so it mainly exists on the ocean surface and is directly exposed to sunlight. When the propylene polymer structure of polypropylene is exposed to sunlight, the bonds are broken by UV energy. These structural features result in many secondary microplastics of polypropylene in the marine environment. To investigate the environmental impact of photodegradation products generated at this time, compounds were identified through non-target analysis using high-resolution mass spectrometry (HRMS). In addition, samples photodegraded for three periods were analyzed to observe changes in the amount of compounds according to the photodegradation period.

Methods

2.0 g of polypropylene plastic was cut into small pieces and placed in a quartz crucible along with artificial seawater. Plastic pieces in artificial seawater were photodegraded by UV irradiation and magnetic stirring in a dark environment. After photolysis during the accelerated photolysis period calculated based on the annual UV irradiation in Korea, the analytes were extracted and solvent replaced using solid phase extraction (SPE). The eluted sample was concentrated by blowing out the solvent with nitrogen gas, then diluted with 1 mL of MeOH and used for LC-MS/MS analysis. Orbitrap Exploris 120 (Thermo) coupled with UPLC (Waters) was used for LC-MS/MS analysis. Raw data was processed through the database searching software Compound discoverer (Thermo).

Novel Aspect

Decomposition products from light exposure were analyzed with a high-resolution mass spectrometer, yielding quantitative data on common compounds.

Preliminary Data or Plenary Speaker Abstract

LC-MS/MS acquisition was performed quadruplicate for each sample during non-targeted analysis. Through database searching, decomposition products were identified for each photodegradation period, and structures similar to those of polypropylene were specifically identified and investigated. In the analysis of decomposition products, it was confirmed that the number of identified compounds increased as the photodecomposition period increased. A database was established to use the identified compounds to identify polypropylene degradation products in samples collected from real environments. Among the identified compounds, compounds that commonly appeared in 1-, 2-, and 3-year photodegraded samples were identified. For common compounds, the amount of change in the compound as the photodecomposition period increased was investigated through quantitative data, and change trend information was observed.

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Total PFAS coverage using novel targeted and non-targeted workflows on an LC/TQ and timsTOF

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

PFAS are highly present in the public and pose a threat to mankind and nature. Manufactured since the 1940s as water and grease repellents in consumer products, they are considered “forever chemicals”. With their persistent, bio-accumulative, toxic (PBT) properties and ubiquitous presence in the environment and organisms, there is mounting evidence that exposure to PFAS may generate adverse health effects. EPA and EU regulations exist for the routine monitoring of certain PFAS in water and food. But the EPA estimates almost 15,000 species exist in this group as manufactured compounds, precursors, and degradation products. This makes their systematic and comprehensive analysis an extremely challenging task.

Methods

Presented are two alternative workflows for a total coverage of PFAS. Routine targeted analysis by a novel, fast, robust and highly sensitive triple quadrupole is demonstrated. This covers and exceeds the current EPA and EU regulations for reliable and confident testing of water and food samples. For a comprehensive PFAS overview, a novel non-targeted approach was developed that adds the orthogonal selectivity of trapped ion mobility spectrometry (TIMS) to the information from UHPLC-HRMS. The data is screened against the NIST PFAS suspect list which currently contain ca. 5,000 registered compounds, allowing their identification without using reference libraries.

Novel Aspect

Highly sensitive LC/TQ and tims-QTOF as complementary tools for the comprehensive target and suspect screening of PFAS in any matrix

Preliminary Data or Plenary Speaker Abstract

Both DART-TQ and conventional LC/TQ methods were applied to cover the required quantification limits of 40-50 PFAS compounds in different matrices like water, food and soil according to regulations like US EPA 1633 and EU 2020/2184. While DART provides fast screening of a large number of samples, LC/TQ fulfills the sensitivity demands of the regulations for a detailed quantification.
For extending the number of potential PFAS compounds to be detected, a high resolution QTOF coupled to trapped ion mobility (TIMS) was used. The ion mobility feature of the system was utilized for several purposes. First, it can separate coeluting isobars and isomers. Second, the TIMS filter results in higher sensitivity and lower detection limits of the targeted PFAS as well as significantly higher quality of full-scan MS and MS/MS spectra. Finally, collisional cross sections (CCS) as additional identification criteria enhanced the identification confidence with is based on the exact mass, diagnostic fragment ions and the isotope pattern fit. The NIST suspect list contains information about the elemental composition and the InChI structures for PFAS which were compared with the exact mass, MS/MS fragmentation and CCS values of the experimental data for an automated and untargeted identification of all PFAS present in the sample.
The wide-scope suspect screening of real-life samples against the suspect lists proved to be a comprehensive approach for a fast and efficient identification and quantification of PFAS against the total set of 5,000 compounds in complex environmental matrices. Therefore, it will assist in understanding the chemical universe of PFAS in the environment and protecting environment, wildlife, and human health.

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Automated sample preparation of water samples using ePrep ONE robotic system for the US EPA 3511 method

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[1]Eprep

Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

An Automated Liquid Liquid Extraction (ALLEx) technique using the robotic sample preparation workstation - ePrep ONE, has been developed for the analysis of a suite of semi-volatile organic compounds in drinking water based on the micro extraction US EPA 3511 method. This manual method is commonly used in environmental laboratories to extract the organic compounds from water into an organic solvent (dichloromethane) followed by analysis by GC-MS (gas chromatography coupled to a single quadrupole mass spectrometer). The method though is labour intensive and has previously been difficult to automate.

Methods

The ePrep ONE instrument has some unique advantages over conventional or existing semi-automated sample preparation methodology. At the heart of the unit is a glass analytical syringe which unlike auto pipette systems, can easily handle volatile organic solvents like dichloromethane (DCM). The ePrep ONE incorporates automated syringe exchange technology which allows different volume syringes to be incorporated into the same workflow.
The developed workflow was also applied to real world water samples to investigate the efficiency of the automated methodology.

Novel Aspect

Fully automated ePrep ONE sample preparation methodology suitable for GC-MS analysis of semi volatile organic components.

Preliminary Data or Plenary Speaker Abstract

This poster will show the steps involved for the fully automated method using the ePrep ONE. Laboratory control samples (LCS) were prepared for a range of US EPA 8270 semi-volatile components and excellent recoveries of analytes and RSDs were observed.

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Direct determination of organophosphorous herbicides and their metabolites in surface water by HPLC-ICPMSMS

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Organophosphorus herbicides such as glyphosate and Glufosinate are widely used worldwide due to their versatility and availability. Their toxicity has been discussed since the International Agency for Research on Cancer (IARC) classified as a suspected carcinogen. Difficulty in the analysis of organophosphorus pesticides and their metabolites is their high water solubility, poor volatility, and lack of UV absorption and fluorescence. The conventionally used HPLC, GC, and GC/MS, therefore, require complicated pre-treatment, such as derivatization. To solve these problems, the use of an inorganic mass spectrometer equipped with a powerful ionization source (inductively coupled plasma: ICP) has been investigated, combined with mutual separation of the analytes by HPLC.

Methods

We used an anion-exchange column (TSK gel Super IC-AZ) installed in Agilent 1260 II infinity Bio-inert LC system. The HPLC-ICP-MS/MS was then configured by directly connecting the column output tube to the nebulizer of Agilent 8800 ICP-MS/MS (Agilent Technologies). The four compounds (glyphosate, glufosinate, AMPA, and MPPA as metabolites) were separated with ammonium acetate eluent. Since ICP-MS decomposes analytes into elemental units and ionizes to monovalent positive ions unlike soft ion sources such as ESI, organophosphorus analytes are detected as 31P+ ion (m/z 31) signals. In the instrument, the analyte P+ is converted to PO+ through a reaction with O2 gas to avoid background polyatomic interferences on m/z 31.

Novel Aspect

Component-independent quantitative analysis of organophosphorous herbicide was achieved without derivatization by ICP-MS/MS.

Preliminary Data or Plenary Speaker Abstract

With the optimum eluent composition of 70 mM at pH 3.7, the four organophosphorus compounds and phosphoric acid were separated within 15 minutes in the order of AMPA, glufosinate, glyphosate, phosphoric acid, and MPPA with retention times of 2.83, 5.52, 6.84, 8.12, and 13.66 minutes, respectively. All analyte's calibration curves up to 10 ng/g showed good linearity in the tested range with a correction coefficient of more than 0.998, and detection limits were also in the range of 0.06-0.12 ng/g.
The component-independent sensitivity of this method suggests a possibility of semi-quantitative analysis for unknown phosphorus-containing organic components owing to the high ionization efficiency of ICP. This advantage allows the method to be applied for rapid screening analysis; for example, semi-quantitative analysis of an analyte compound can be carried out based on phosphoric acid's calibration curve without individual calibrations.

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Targeted and untargeted analysis of paleoclimatic proxies in ice cores using dual capillary ion chromatography-mass spectrometry

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The trapped dissolved and particulate matter in ice cores renders them invaluable for understanding past atmospheric composition and paleoclimatic conditions. Research often focuses on identifying specific proxies, such as inorganic ions and organic acids like methanesulfonic acid-MSA, to track past changes in sea ice extent, terrestrial dust variability, and atmospheric aerosol transport. The high costs of collecting ice cores from Antarctica require scientists to maximize data extraction, making recent advances in deriving high-value data from sub-1 mL ice core sample volumes significant. This study demonstrated the utility of dual capillary ion chromatography with suppressed conductivity detection and mass spectrometry (CapIC-MS) for the analysis of trace inorganic and organic anions and cations in microliter sample volumes of Antarctic snow and ice.

Methods

A DionexTM ICS-5000 ion chromatography system coupled with an ISQTM EC single quadrupole mass spectrometer (Thermo Fisher Scientific, Sunnyvale, USA) was used throughout. The IC instrument was equipped with two capillary pumps, two eluent generator two IC Cube modules and two capillary conductivity detectors (CDs). Each IC Cube included an injection valve, a degas cartridge, a column heater, a guard column, a separator column and a suppressor. The eluate from the anion conductivity detector was combined with the make-up solvent (acetonitrile) via a micro-mixing tee and passed through a grounding union before entering the MS detector via the ESI interface. The MS was operated in the negative electrospray ionisation mode. The samples were directly injected via an AS-AP autosampler.

Novel Aspect

Advancing Antarctic paleoclimate knowledge through comprehensive analysis of inorganic and organic compounds in ice core samples.

Preliminary Data or Plenary Speaker Abstract

Herein, we present an improved dual Cap-IC-MS method for the simultaneous quantification of fluoride, acetate, formate, MSA, chloride, nitrite, nitrate, bromide, sulphate, oxalate, phosphate, sodium, ammonium, potassium, magnesium and calcium in ice core and snow samples. This method requires simple direct injection of only 190 µL of sample, 35 times less volume than standard ion chromatography methods. A range of chromatographic parameters were optimised, including separation column, flow rate, eluent concentration, gradient conditions, and column temperature, for both anion and cation systems, to achieve baseline separation of all target analytes in a suitable run time. Baseline separation of impurities, matrix, and trace ions was achieved in under 35 minutes, ensuring the high chromatographic selectivity necessary for precise and reliable quantification of the target analytes. Impurities/matrix ions, such as acetate-formate/chloride-sulfate anions, did not interfere with the quantification of targeted analytes present at low-ppb levels, such as MSA or bromide. Limits of detection (LODs) for all analytes determined using conductivity detection were similar to those achieved by previously published standard bore IC-based methods. The dual Cap-IC system was hyphenated with mass spectrometry detection for targeted ion identity confirmation, lowering LODs, and untargeted screening analysis to broaden knowledge of the organic aerosol

chemical composition in ice cores. Time-single ion monitoring (t-SIM) and Full Scan acquisition modes were used for targeted and untargeted analysis, respectively. The new developed method was applied to analyse 100 ice core samples from Antarctica, demonstrating its applicability and laying the groundwork for future research within the significant 'Million Year Ice Core' (MYIC) project.

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A UHPLC-HRMS method to record cyanotoxins and related secondary metabolites: A case study in Lake Stechlin (Ger)

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Water is essential for life on earth. Besides the use as drinking water, clean rivers and lakes are of great importance for humans e.g., as a source for nutrition and recreational activities. Climate change and increased nutrient inputs may promote increased development such as algae. Among algae blooms in freshwater, particular attention is paid to the cyanobacteria, photosynthetically active microorganisms that are known to produce a large number of secondary metabolites. Some cyanobacteria genera can produce so-called cyanotoxins that can be harmful for humans and animals. Most frequently observed cyanotoxins are microcystins, cyclic heptapeptides that can lead to acute poisoning.

Methods

For a more precise characterization of these toxins liquid chromatography coupled with mass spectrometry was used as it allows both qualitative and quantitative determinations. For analysis of cyanotoxins from Lake Stechlin in northern Brandenburg (Germany), the particulate material of the water was collected by filtration. The filter cake was extracted, and analysed using UHPLC-HRMS We used an elevated water acetonitrile gradient with a C18 column for chromatography and state of the art orbitrap HRMS analysis in combination with different tandem-MS experiments for an in deep data analysis.

Novel Aspect

We developed an UHPLC-HRMS method to analyze cyanobacterial toxins like microcystins and 5 additional compound classes, enabling further risk assessment.

Preliminary Data or Plenary Speaker Abstract

By investigating additional cyanotoxins besides the standards available, two more microcystins, three cyanopeptolins, four anabaenopeptins, one aeruginosin as well as three additional secondary metabolites could be identified. Moreover, one unknown microviridin was detected and parts of its structure could be elucidated with HRMS2 experiments. In addition to these molecules six unknown metabolites were detected and could be linked to Planktothrix rubescens as native in Lake Stechlin. The application of this method yielded insights on cyanotoxin prevalence in the region of Northern German lowland Lake Stechlin and enabled us to elucidate additional ecological coherences by correlating the recovered data with additional bio-geo-ecological data. Furthermore, the method delivered important information about the quality and toxicity of water samples. J. F. M. Otto, C. Kiel, J. C. Nejstgaard, G. Pohnert, S. A. Berger, N. Ueberschaar, J. Hazard. Mat. Adv. 2023, 12, 100370.

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Tracing Contaminants of Emerging Concern and Their Transformations in a Wastewater Treatment Plant Using Nontarget Screening and Molecular Networking Strategies

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

This study utilized nontarget screening with high-resolution mass spectrometry to characterize contaminants of emerging concern (CECs) in a WWTP in Melbourne. The treatment process's effectiveness was assessed by identifying and quantifying various compounds in different categories. Transformation products of CECs were tentatively identified, and a molecular network was used to recognize common fragments between CEC pairs. These findings highlight the actual treatment efficiency of wastewater treatment plants for CECs and potential transformation products, providing valuable insights for future wastewater management improvements.

Methods

Sample Collection and Preparation: Influent and effluent wastewater samples from a WWTP in Melbourne were collected.
Instrumental Analysis: Samples were analyzed using an Agilent HPLC platform with a 6546 quadrupole TOF HRMS. A C18 column was used for separation at 40°C. Mobile phases included ammonium acetate, formic acid in water, and methanol, with a gradient program. Data were acquired using Data-dependent (DDA) and Data-independent acquisition (DIA) methods.
Data Processing: Raw data were converted to mzML format and processed with PyHRMS for feature extraction, prioritization, and alignment. Identifications were made using an in-house database, MoNA, and NORMAN-SLE.
Molecular Networks: PyHRMS was used to construct molecular networks, linking features with shared fragments, to identify transformation products.

Novel Aspect

Using combined DDA and DIA methods and molecular networks enhances our understanding of CECs related to WWTPs.

Preliminary Data or Plenary Speaker Abstract

This research significantly advances our understanding of the prevalence of CECs in WWTPs, crucial pathways for these contaminants into aquatic environments. Utilizing non-target screening (NTS) provides an immediate comprehensive overview and establishes a foundation for future retrospective analyses. As our reference databases grow, we expect to uncover more CECs and gain deeper insights into the effectiveness of specific WWTP units in removing them. By integrating our extensive dataset with degradation data, we open avenues for innovative applications, particularly in machine learning. This data, combined with molecular descriptors, can be used to create models that predict the removability of new contaminants. As degradation data quantity and quality improve, and as confidence in compound identification increases, the accuracy of these predictions is expected to enhance. Additionally, our approach of using both data-dependent and data-independent acquisition modes for MS2 data enables the use of molecular networking for identifying transformation products (TPs), offering a dynamic view of their concentration changes. This comprehensive strategy not only deepens our understanding of CEC transformations but also paves the way for groundbreaking data exploration, all aimed at improving the health and sustainability of aquatic ecosystems.

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Identification and quantification of halogenated polymers by pyrolysis gas chromatography–mass spectrometry

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Halogenated polymers, such as PVC, Teflon, polychlorotrifluoroethylene, and brominated polystyrene, are extensively used in consumer products as food packaging and coatings due to their high performance, such as thermal and corrosion resilience. When released into the environment, these polymers are subjected to biological, physical, chemical, and photochemical degradation processes that have the potential to result in fragmentation into halogenated MNPs. At present, there is limited information on the characterisation and quantification of these polymers in the environment using mass spectrometry-based techniques such as Pyrolysis Gas Chromatography-Mass spectrometry (Py-GC-MS). Therefore, this study aims to develop Py-GC-MS methods to characterise halogenated plastics in the environment.

Methods

Samples were micro-sectioned and placed in pyrolysis cups. The cups were spiked with deuterated-PS and fluorinated polystyrene internal standards and analysed using methods developed for the identification and quantification of high production polymers using the double-shot mode of a multi-shot micro-furnace pyrolyser- PY-3030D (Frontier Lab Ltd., Fukushima, Japan), coupled with a GC-MS - GC2030 (Shimadzu Corporation, Japan). Standards for a range of halogenated polymers were assessed, including brominated polystyrene, fluorinated polystyrene, fluorinated polyethylene, and polychlorotrifluoroethylene, to identify the individual polymers using specific pyrolysis products. The Py-GC-MS method developed will be able to quantify halogenated polymers in a range of complex environmental matrices for the first time.

Novel Aspect

This study will develop Py-GC-MS methodologies for the simultaneous quantification and identification of halogenated polymers.

Preliminary Data or Plenary Speaker Abstract

N/A

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Study on regional and seasonal differences in organic constituents of PM2.5 in East Asia using UPLC FT-ICR MS and ANN

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Aerosol particulate matter (PM) is a major environmental pollutant in East Asia and its concentration is increasing every year. $PM_{2.5}$ (aerodiameter ≤ 2.5 ㎛) is a PM that is small and easily suspended in the atmospheric environment. It affects various health problems through the respiratory tract, so it is important to understand $PM_{2.5}$ and its effects. Although research on inorganic salts contained in PM is actively underway, research on organic constituents is relatively lacking. Therefore, organic constituents analysis with ultrahigh resolution mass spectrometry (UHR-MS) and artificial neural network (ANN) modeling technique in this study.

Methods

173 $PM_{2.5}$ samples collected daily in summer and winter were used. The high-throughput ultra-performance liquid chromatography (UPLC) coupled with negative mode-atmospheric pressure chemical ionization ((-)APCI) fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was used to analyze organic compounds constituting $PM_{2.5}$. The peaks lists were established using HTP UPLC for fast, high-sensitivity and -reproducibility analysis of trace $PM_{2.5}$. Classification analysis of regional/seasonal differences through relative abundance of $PM_{2.5}$ UHR-MS spectra was performed using ANN. The deep learning Toolbox in the MATLAB program (version R2022b; MA, USA) was used. An ANN model with three layers consisting of an input, a hidden layer, and on output layer was used. The hidden layer had 20 nodes.

Novel Aspect

The first large-scale analysis study on regional/seasonal differences of $PM_{2.5}$ organic constituents using HTP UPLC UHR-MS and ANN.

Preliminary Data or Plenary Speaker Abstract

Analysis of organic constituents of $PM_{2.5}$ according to regional/seasonal differences was performed using the HTP UPLC (-)APCI FT-ICR MS dataset and ANN classification analysis. Molecular level analysis was performed using Van Krevelen diagrams and elemental composition class distributions. As a result of classification analysis of the organic compounds of $PM_{2.5}$ according to regional differences, aromatic organic oxides and aliphatic sulfur oxide structural compounds were confirmed to be the main specific organic compounds in Ulaanbaatar (Mongolia), which are derived from solid fuels combustion such as coal and biomass. On the other hand, in Beijing (China) and Seoul (Republic of Korea), aliphatic organic oxides were identified as region-specific organic compounds, consistent with typical compounds from petroleum combustion. In addition, in the case of Beijing, aromatic nitrogen oxides were additionally detected as region-specific organic compounds, which matched representative chemical species produced by diesel combustion. As a result of classification analysis of the organic constituents of $PM_{2.5}$ according to seasonal differences, aromatic oxides generated by fuel combustion were detected as specific organic compounds in winter, whereas lipids, proteins, lignin produced in nature were detected in summer. However, because only seasonal difference analysis was performed for winter and summer, there are limitations in analyzing the organic constituents of $PM_{2.5}$ according to seasonal changes. Additionally, by expanding the spring and fall $PM_{2.5}$ datasets, it will be possible to analyze the detailed classification of organic constituents of $PM_{2.5}$ by season. Additionally, it is expected that regional/seasonal contributions to secondary organic aerosol generation will be revealed.

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Top-down Proteomic Approach for the Identification of Saxitoxins-producing Dinoflagellates

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Harmful algal blooms (HABs) caused by toxic dinoflagellates result in severe adverse effects on the environment, economy, and human health. Constant monitoring of toxic dinoflagellate can help mitigate the adverse impacts of HABs. Top-down proteomics can be a streamlined detection method for toxic dinoflagellates. However, toxic dinoflagellates are rich in pigments and secondary metabolites, so additional clean-up steps may be required to obtain proteins with high purities for top-down proteomic analysis. To develop a rapid, reliable and reproducible detection method for toxic dinoflagellates, this study aims to evaluate the compatibility of different protein extraction methods with top-down proteomics for screening potential biomarkers of toxic dinoflagellates.

Methods

Four saxitoxins-producing dinoflagellate species, including Alexandrium lusitanicum, A. tamarense, A. minutum, and Gymnodinium catenatum, were harvested at the lag, log, stationary, and death phases. Three protein extraction methods, namely acetone method, chloroform-methanol method, and phenol method, were performed. The extracted proteins with a molecular weight lower than 30kDa were collected using Amicon® Ultra Centrifugal Filter and subjected to UPLC-MS/MS analyses by Thermo Scientific Orbitrap Fusion Lumos MS. Peak identification of MS/MS data was processed by Proteome Discoverer 2.5. PCA analysis was conducted using SIMCA 17 to evaluate the effectiveness of different protein extraction methods for identifying selected toxic dinoflagellates.

Novel Aspect

The findings of this study demonstrated the potential of top-down proteomics to identify toxic dinoflagellate efficiently for HAB mitigation.

Preliminary Data or Plenary Speaker Abstract

While the MS/MS spectra obtained using acetone and chloroform-methanol methods were consistent and can be used for downstream peak identification, the number of MS/MS spectra generated using the phenol method was significantly lower than the other two methods by around 90%. The protein extracts from the acetone and chloroform-methanol methods had more proteins which could be detected and fragmented in the Orbitrap compared to the phenol method. More detectable proteins in the extracts would increase potential candidates for the identification of toxic microalgae. Thus, the acetone and chloroform-methanol methods would be a better choice for top-down proteomic analysis. Fractionation of extracted proteins before UPLC-MS/MS analyses also helped detect potential protein biomarkers of toxic microalgae. Large proteins seem to have low ionization and fragmentation efficiency for signal generation. The number of MS/MS spectra increased by nearly 20% for both acetone and chloroform-methanol methods when analyzing smaller extracted proteins with a molecular weight lower than 30kDa. The results of PCA showed a successful separation of four selected toxic dinoflagellates based on the identified MS features using acetone and chloroform-methanol methods.

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Assessing the potential for micro/nano plastics (MNPs) to cross human biological barriers

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Micro and nanoplastics (MNPs) are a growing concern to human health due to our constant contact with plastic products including textile, food packaging and car tyres. Literature has reported MNPs in different human organs, however, there is limited reliable evidence to date to confirm the translocation of MNPs in the human system. Meanwhile, studies have shown MNPs penetrating cells that make up crucial biological barriers. The process whereby MNPs could cross these barriers is complex and depends on numerous factors, e.g. particle size, charge, surface chemistry and cell types. This study uses human cell cultures to investigate the behaviour and fate of MNPs in contact and across key biological barriers, thereby expanding the understanding about the health risks of MNPs.

Methods

The first part of the study focuses on the in vitro blood-brain barrier (BBB) model. The human brain endothelial hCMEC/D3 cells was seeded into the collagen-coated apical (top) chambers of the Transwell plate (12 wells). Nano-sized (20-70 nm) polystyrene and polymethyl methacrylate were mixed with cell culture media at 10 µg/mL and applied to the apical chambers. Trans-endothelial electrical resistance was documented regularly. The solution was removed from both the apical and basolateral (bottom) chambers after 24 hours, fresh media was added to assess the recovery of barrier integrity. The media, collected from both chambers were analysed using pyrolysis–gas chromatography–mass spectrometry to assess if any plastics have penetrated the cell barrier.

Novel Aspect

This study aims to use environmentally relevant MNPs to investigate the potential for MNPs to cross human biological barriers.

Preliminary Data or Plenary Speaker Abstract

N/A

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Validation of DART-MS Average Signal Intensity Data for Semi-Quantitative Monitoring of Illicit Drug Use

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Illicit and pharmaceutical drug misuse are increasingly prevalent, posing inherent risks of harm to users. Consequently, there is a need for harm reduction strategies capable of high throughput and highly accurate qualitative and quantitative drug monitoring and analysis. Current gold-standard methods (GC- and LC-MS) are not amenable for high-throughput monitoring. Advances in ambient-ionisation techniques, including DART-MS, may realise the potential of MS based methods for this purpose. However, these potentially suffer from variability in quantitative signal response. Here we report the potential of DART-MS for the quantitation of illicit drugs, to validate a previously reported approach for semi-quantitative monitoring of population-level drug market trends utilising average signal intensity (ASI) values from individual drug samples obtained from discarded drug paraphernalia.

Methods

1 mL syringes, a typical item of paraphernalia associated with intravenous drug use, were loaded with known volumes and concentrations of various illicit drug reference materials and other pharmaceutical drugs. Syringe barrels were then swabbed with cotton tips, and the tips were affixed to a motor mounted on the DART rail. Optimization of motor speed and lateral translation (rail) rate was performed by individual incremental increases to determine the combination of the two which provided the most comprehensive analysis of the samples, while also providing the least variability in ASI measurements. Following optimisation of the delivery mechanism, concentration linearity testing was completed.

Novel Aspect

We demonstrate the effectiveness of DART-MS for rapid and cost-effective semi-quantitative illicit drug monitoring with motorised sample delivery.

Preliminary Data or Plenary Speaker Abstract

Linearity between concentration and ASI over a single order of magnitude was observed when using a stationary, manually rotated cotton tip. Incorporating motorised rotation and lateral movement of the tips enabled more comprehensive analysis of the entire tip surface, maximising the exposure of the tip surface to the source gas, and minimising ASI variability. Approximating the diameter of the gas flow incident on the cotton tips at a fixed DART source-tip distance enabled an estimation of the proportion of tip surface exposed to the gas at different motor and rail speeds to be determined. Fixing the number of scans acquired per individual rail speed facilitated direct comparison of ASI and peak area data between analyses at the same rail rate but different motor speeds. Incrementally increasing rail speeds resulted in reduced acquired scans, and a less thorough sample analysis as reflected in decreased peak area of the ion signal of interest. Incrementally increasing the motor speed led to a small decrease in ASI, but with reduced variability compared to slower motor speeds. Minimising variability via a fast motor speed (~87 rpm) and maximising the exposure of the tip to source-gas via slower rail speed (0.5 mm/s) enabled a sub-minute analysis per sample.

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Evaluation of Emitter Tip Geometry to Enhance the Performance and Robustness of nano-ESI-MS

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Nano-Electrospray Ionization-Mass Spectrometry (nano-ESI-MS) has played a pivotal role in proteomics due to its enhanced sensitivity over conventional ESI.[1] While the technique relies on the use of emitter tips with small dimensions,[2] the sensitivity and reproducibility of nano-ESI-MS proteomic data are influenced by the emitter tip's geometry and material. This study was established to develop machine learning models to evaluate the impact of silica glass emitter tip geometry variables on sensitivity, including investigating the operating voltages and comparing different coating metals. Finally, the glass emitters developed here were compared with commercially available pulled glass emitters and stainless-steel emitters to evaluate their relative performance.

Methods

36 constant bore metal-coated glass emitter tips with different geometries were fabricated and assessed using nano-ESI-MS proteomic analysis of 100-200 ng HeLa cell trypsin digest. Peptide counts from each emitter (n=2 replicates) were averaged. A multiple linear regression model was fitted to assess the impact of emitter tip design on mean peptide count. In a subsequent voltage study, four emitters with distinct configuration were tested across varying voltages to determine spray onset and failure points. To improve performance, six emitters were coated with a different metal, and peptide counts were compared. Finally, several emitters with different coating positions and internal diameters were compared with commercial pulled glass emitters and commercial stainless-steel emitters.

Novel Aspect

A comprehensive study on the impact of varying emitter tip geometries on nano-ESI-MS performance.

Preliminary Data or Plenary Speaker Abstract

Results from the multiple linear regression models revealed that the primary and significant contributor to variation in peptide counts was the variance in MS performance. None of the models demonstrated a significant association between tip angle and mean peptide count. However, the p-value ($p = 0.072$) of glass emitter tip wall thickness (i.e., = tip diameter/internal diameter) approached significance, indicating that this predictor might be useful in explaining differences in the mean peptide count. Among the tested emitters in the voltage study, a gold coated glass emitter with a wall thickness ratio of 2.24 showed comparable operating voltage ranges (1.6-2.6 kV) and on-par sensitivity with commercial stainless-steel emitter tips. Subsequent investigation of different glass emitter coating metals demonstrated enhanced sensitivity when the coating material was gold compared to platinum. Furthermore, comparative assessment between tip/distal gold coated glass emitters with varying internal diameters, and commercial stainless-steel emitters, resulted in consistently higher performance of gold coated glass emitters compared to commercial pulled glass emitters. This was attributed to the requirement of hand cleaving pulled glass emitters in the laboratory.

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Monitoring indoor farming processes using a Direct Analysis in Real Time mass spectrometry (DART-MS) platform

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The stability of food production systems is increasingly threatened in land-scare countries such as Singapore, where arable spaces for food production are limited. The indoor farming system is therefore a space-efficient solution for edible crop production in controlled environments. To maximise productivity in indoor farms, there is increasing emphasis on innovating efficient technologies to monitor production and growth processes. Direct analysis in real time mass spectrometry (DART-MS) presents an attractive approach for at-line monitoring of indoor farming processes, with its capability for rapid and high throughput analysis under ambient conditions.

Methods

Here, we explore the application of DART-MS for profiling indoor farming agriculture samples and growth inputs. We examine a variety of sampling approaches, such as direct analysis of solid surfaces, and analysis of powderised solids and solutions on wire mesh sample cards and glass capillaries. We also investigate the effects of DART ionisation parameters on the overall data quality.

Novel Aspect

DART-MS is an efficient and informative approach for at-line monitoring of indoor farming processes.

Preliminary Data or Plenary Speaker Abstract

To develop a mechanism for rapid determination of optimal DART-MS sampling and acquisition workflows for diverse indoor agriculture samples, we compare the data acquired from several sample types, such as surfaces and tissues of vegetables (i.e. leaf, stem and roots), growth substrates and soil, and nutrient solutions. We observe unique molecular profiles generated with different sampling approaches selected depending on the sample nature. Further analysis is in progress to determine if these profiles could be used as molecular signatures of plant growth, nutrition and health status.

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Building a Multidimensional Oxylipin Library Containing Liquid Chromatography, Ion Mobility Spectrometry, and Mass Spectrometry Information

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Oxylipins are oxygenated products of fatty acid metabolism that may play a significant role in inflammation (anti- and pro-inflammatory) and immune response (pro-resolving). Oxylipins possess a wide range of molecular diversity and feature several classes including octadecanoids, eicosanoids, and docosanoids. While eicosanoids (derived from 20-carbon PUFAs) are well studied, the full range of oxygenated products of 18 to 22 carbon PUFAs (e.g., linoleic acid, α-linolenic acid, and docosahexaenoic acid) and their metabolic products are relatively uncharacterized. Furthermore, the high number of possible isomers renders it challenging to separate oxylipins in traditional LC-MS workflows. In this work, we utilize a multidimensional separation approach featuring ion mobility spectrometry (IMS) in conjunction with LC-MS to better distinguish and characterize oxylipins.

Methods

All oxylipin standards were assessed in triplicate with LC-IMS-MS platform (Agilent 1290 UPLC and Agilent 6560 IM-QTOF) utilizing nitrogen as the buffer gas. Agilent ESI-L Low Concentration tuning mix solution was then used for single-field calibration of all CCS values and data was analyzed with both Agilent MassHunter Workstation Software IM-MS Browser Version B.10.00 and Skyline-MS (MacCoss Lab Software, v. 23.1). Oxylipin standards with triplicate CCS values having less than 1% error were populated into a multidimensional Skyline library containing LC retention times, IMS CCS values and MS information. Isomeric oxylipins with close CCS values were further evaluated with a MOBILion structures for lossless ion manipulation (SLIM) traveling wave ion mobility platform.

Novel Aspect

A multidimensional library was created to improve the separation and identification of oxylipins in complex samples.

Preliminary Data or Plenary Speaker Abstract

Oxylipins are of high interest due to their potential biological roles in inflammation and immune response; however, they can be challenging to measure due to their low concentrations in complex matrices as well as the presence of multiple isomers. To explore the diversity of oxylipins present in biological samples, we created a multidimensional library with LC, IMS and MS information. To create the library, several classes of oxylipins including eicosanoids (e.g., prostaglandins, leukotrienes, hydroxyeicosatetraenoic acids, epoxyeicosatrienoic acids), docosanoids (e.g., resolvins, maresins) and several octadecanoids were characterized in this study as well as isomers within each subgroup. The oxylipins analyzed had a range of varying isomerism including stereoisomers (R versus S), regioisomers (double bond position), and geometric isomers (cis vs trans double bond orientations). Many of the oxylipins were separated in the 15-min LC-IMS-MS analysis ; however, a number of isomers evidenced overlap especially in the LC and MS dimensions.

For the IMS measurements, the drift tube IMS (DTIMS) platform separated most of the isomers studied in this work. However, the resolving power of the current Agilent 6560 platform is 60 CCS/ΔCCS, which is modest in comparison to newer IMS platforms such as the MOBILion SLIM

traveling wave IMS platform (~300 CCS/ΔCCS). Therefore, the same oxylipins were injected onto the MOBILion SLIM platform to evaluate if better separations were possible. Initial replicate reproducibility results indicate that CCS values obtained on the SLIM platform possess more variance in comparison with the low resolution DTIMS system; however, a substantial increase in IMS resolving power was observed. This enabled the discrimination of more isomer pairs, which were unresolvable using DTIMS. Hence, each IMS platform possessed unique advantages and provided complementary information for database development and applications of isomer separation and characterization.

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An Assessment of Serum Lipidomic Perturbations due to Drinking Water and Occupational PFAS Exposures

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals with carbon-fluorine bonds, which cause some species to be resistant to breakdown. Some PFAS are also known to accumulate in the human body, and are linked to health concerns, including associations with lipid dysregulation. However, PFAS are widely used in a variety industrial and consumer products, including firefighting foams and waterproof apparel, and thus pose a threat to communities near their production, use, and release. The full scope of their effect on lipid pathways is unknown. To evaluate associations between PFAS and lipidomic changes in people having occupational and drinking water exposure, we used a high-resolution mass spectrometry (HRMS) platform for analysis of both molecule types in human serum.

Methods

Blood samples were collected from firefighters working in Durham, NC and civilians living in Pittsboro, NC. These samples were centrifuged to isolate serum and stored at -80°C. 50µL of each sample was aliquoted in 2 vials for extraction of PFAS and lipids. For PFAS analysis, each sample was spiked with 5µL of a heavy labeled internal standard mix containing 24 PFAS. A liquid-liquid extraction and acetonitrile protein crash was performed to isolate PFAS prior to reconstitution. A modified-Folch extraction was performed to isolate lipids for the complimentary analysis. Analysis was performed using a liquid chromatography, ion mobility spectrometry, collision induced dissociation and mass spectrometry platform. Data was annotated using Skyline software and our in-house libraries containing PFAS and lipids.

Novel Aspect

An exploration of lipidomic changes in serum from PFAS exposed firefighters and civilians to understand molecular relationships.

Preliminary Data or Plenary Speaker Abstract

The goals of this project were to identify PFAS present in human serum samples from different exposed populations and analyze the relationship between the detected PFAS and dysregulation to human lipidomes. For the study, 28 firefighter (Durham, North Carolina) and 49 civilian serum samples (Pittsboro, North Carolina) were analyzed for over 100 PFAS using Skyline libraries. Semi-quantification calculations showed that 84% of these samples (47/49 civilians, 18/28 firefighters) have higher total PFAS levels than the average U.S. population from the 2017-2018 NHANES study for 5 common PFAS. Surprisingly, the total PFAS concentrations were significantly higher in the civilians compared to the firefighters. In fact, only 7% of the firefighter samples had total PFAS concentrations above the median of the civilian samples. These results suggest that the civilians in this study are being exposed to more PFAS than the occupational exposure of firefighters. This is also consistent with the Town of Pittsboro's lawsuit against PFAS manufacturers for the pollution of their drinking water supply. Lipid data was also analyzed for the serum samples using the same LC-IMS-MS platform as used to collect the PFAS, with the addition of collision-induced dissociation (CID) fragmentation to aid in the confident lipid annotations. To begin lipidomic statistical analyses, absolute quantification concentration values for 13 PFAS previously determined by collaborators were used to evaluate trends between PFAS totals and lipid dysregulation. Data work up and analysis is ongoing, however, currently 55 unique lipids from 11 classes have been deemed significant in relationship to PFAS sums. We are also evaluating samples with distinct PFAS in their serum to see if we observe different lipidomic trends. This lipidomic study will aid in our understanding of lipid dysregulation due to various PFAS since currently only cholesterol has been assessed in this cohort.

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Untargeted Metabolomic Analysis Reveals the Lipidome Dynamics of Tomato Infected with Late Blight Disease

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Late blight is a destructive disease affecting tomato and potato crops, caused by Phytophthora infestans, a fungal-like oomycete pathogen. The life cycle of P. infestans is hemibiotrophic, characterized by an early asymptomatic biotrophic phase and a late necrotrophic stage, resulting in tissue degradation and disease symptoms in plants. Despite the recognized role of lipids in plant-pathogen interactions, their involvement in the distinct pathogenesis phases of late blight disease remains unclear.

Methods

To address this, we employed DDA LC-MS/MS to investgate the lipidome dynamics of detached tomato leaflets inoculated with P. infestans. Our analysis confidently identified 456 non-redundant lipid species using MS/MS and database search matching of predicted retention time.

Novel Aspect

This work may lead to the development of more efficient strategies to manage late blight diseases.

Preliminary Data or Plenary Speaker Abstract

We found that Cer-PE and TAG species containing the FA 20:5 moiety were elevated at the early infection phases (biotrophic and transition phases), and those were originated from the pathogen. As the TAG and lipid droplets are known to regulate the cellular FA homeostasis under stress, furthermore, the enrichment of pathogen-derived FA20:5 in TAG implied that the lipid droplets play crucial roles in establishing compatible plant-pathogen interactions. During necrotrophic phase, a significant portion of differentially regulated lipids associated to the regulation of plant defense response, including programmed cell death, ROS activation, and phytohormone signaling pathways. Our study revealed the dynamics of lipidome regulation in the pathogensis of late blight disease, providing novel insights into the molecular mechanisms under in this disease.

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Effects of Empagliflozin on Lipid Metabolism in Heart and Kidney via Targeted Lipidomic Analysis across Arteriovenous Gradients

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Elevated blood pressure and metabolic disturbances including hyperglycemia, dyslipidemia, and obesity pose significant risk for cardiorenal disease development. Sodium-glucose co-transporter 2 inhibitors (SGLT-2i) offer therapeutic promise in this context; however, the precise mechanisms underlying their protective effects remain elusive. Recent studies provide compelling evidence for SGLT-2i induced effects on lipid regulation and metabolism, particularly in cardiac and renal-specific contexts. Examining organ-specific arteriovenous gradients could offer valuable insights into metabolic dynamics and lipid metabolism by assessing lipid composition disparities between arterial and venous blood. This study presents a comprehensive lipidomic analysis leveraging arteriovenous gradients obtained from coronary sinus (CS) and renal sampling.

Methods

Arteriovenous samples from an ongoing double-blind crossover trial, investigating Empagliflozin's impact on cardiac and renal sympathetic nerve activity, were utilised to assess potential lipid metabolism disparities between the heart and kidneys. Participants (n=18) with BMI ≥30kg/m² and diabetes or metabolic syndrome received either 10mg Empagliflozin or placebo daily for 4 weeks, followed by the alternate treatment for another 4 weeks, separated by a 4-week 'washout' period. At the end of each treatment phase, CS, renal vein, and matching radial artery samples were obtained simultaneously via catheterisation.
Targeted lipidomic analysis was performed, with quantitative measurements calculated based on the peak area relative to the appropriate internal standard. Arteriovenous gradients from serial paired CS, renal and arterial blood samples were then determined.

Novel Aspect

Elucidating SGLT-2i effects in high-risk populations through targeted lipidomic analysis of arteriovenous gradients within the coronary and renal circulations.

Preliminary Data or Plenary Speaker Abstract

This unique sample collection is, to our knowledge, the first reported utilisation of arteriovenous sampling to conduct lipidomic analysis across the heart and kidney to further elucidate the mechanism responsible for SGLT-2i associated cardiorenal protection. A total of 978 lipid features from 20 sub-classes were identified. Strong multivariate modelling demonstrates promising results with significant separation of discriminating lipid features observed between participants for individual arteriovenous gradients at each time point. Lipid sub-classes of particular interest, reflecting either an uptake or release of lipids from the heart or kidney, include phosphatidylglycerol (PG), phosphatidylinositol (PI), and triacylglycerides (TAG). These preliminary results were obtained from data collected from 18 participants, with recruitment ongoing.

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Investigating the effects of colchicine treatment on central carbon metabolism using stable isotope tracing and flux analysis

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The herbal drug colchicine, traditionally used to treat Mediterranean fever and gout, has more recently been repurposed in the treatment of cardiovascular diseases, including vulnerable atherosclerotic plaque, vascular stenosis and recurrent pericarditis. Colchicine disrupts microtubule assembly and NLRP3 inflammasome activation, reducing inflammatory factor expression and synergistically acting as an anti-inflammatory, as well as stabilising atherosclerotic plaque. Despite the promising capacity of colchicine and its current use in medicine, significant adverse effects can occur, including gastrointestinal events, diarrhoea, infections and death. Due to this, further research is needed to describe the effects of colchicine treatment on a metabolic and cellular level. Further characterisation of this drug can aid in increasing its efficacy, whilst reducing the risk of adverse events.

Methods

To describe metabolic changes in central carbon metabolism upon colchicine treatment, we used stable isotope tracing (^{2}H and ^{13}C) and flux analysis to probe for cellular metabolism. Changes in labelling patterns of the TCA cycle, glycolysis, energy, fatty acid and lipid synthesis were analysed using liquid chromatography-mass spectrometry (LC-MS) with varying chromatographic separation techniques, including HILIC (polar metabolites) and C18 reversed phase chromatography. In vitro analysis was conducted on various cell types, including mouse-derived 3T3 fibroblasts and endothelial cells, and human-derived HEK293F and smooth muscle cells upon colchicine treatment. Data were processed and analysed using Agilent MassHunter and MAVEN software, followed by kinetic flux profiling (KFP) analysis.

Novel Aspect

This study enables further characterisation of the metabolic consequences of colchicine, providing insight into the mechanistic actions of this drug.

Preliminary Data or Plenary Speaker Abstract

Preliminary data exhibited the effective incorporation of stable isotopic tracers into central carbon metabolism. Early analysis indicated incorporation of U-^{13}C-oleic acid into complex lipids with triglycerides being the major pool labelled. Interestingly, lipid analyses from D_2O and U-^{13}C-glucose tracing experiments revealed that newly synthesised fatty acids predominantly contribute to the synthesis of the triglyceride pool, whereas the phospholipid pool (phosphatidylcholine and phosphatidylethanolamines) remains largely unlabelled upon colchicine treatment. However, further analyses are yet to be presented to confirm these observations.

Though labelling was observed across several metabolites, the significance of their occurrence is yet to be extrapolated. As data is collected across the various cell types, comparative flux analyses of major metabolic pathways will be completed using the kinetic flux profiling model to estimate changes in fluxes of metabolites across various metabolic and lipidomic pathways. This will allow the metabolic fluctuations of intermediates upon colchicine treatment to be assessed and described.

Advancements in High-Throughput Dried Blood Spot-Based 4D Lipidomics

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Dried blood spot (DBS) microsamples offer a minimally-invasive and cost-effective method for collecting and storing blood volumes, typically <50 µL, in metabolic phenotyping research. Mass spectrometry (MS)-based analysis of DBSs provides a valuable tool for enhancing our understanding of metabolism. DBS collections allow for frequent and longitudinal sampling, are easily transportable (suitable for remote or resource-limited settings), and thereby extend opportunities for enriched tracking of metabolites by MS. These capabilities may contribute to the development of personalised interventions aimed at improving patient outcomes.

Methods

We developed a high-throughput untargeted 4D lipidomic method for DBS microsamples using ultra-high-performance liquid chromatography (UHPLC) and Trapped Ion Mobility Spectrometry coupled with Time-of-Flight mass spectrometry (Bruker timsTOF Pro). We assessed annotation quality with and without ion mobility; optimised the extraction method to obtain maximum annotation of reproducible lipids (<30% cv); intermittently assessed the stability of lipids up to three months of storage at multiple temperatures (-80˚C, -20˚C, 4˚C, RT, 45˚C); and compared differences between DBS collection matrices commonly employed in commercially available advanced microsampling devices (Whatman 903®, Perkin Elmer 226®, Ahlström 222®). Following method optimisation, DBS collections were applied in a preliminary cohort representing a diverse range of metabolic disorders and healthy controls.

Novel Aspect

Our research presents a high-throughput 4D lipidomic method optimised for DBS samples, facilitating comprehensive lipid analysis in metabolic research.

Preliminary Data or Plenary Speaker Abstract

A 15-minute high-throughput 4D-lipidomics method was developed to enable acquisition of 96 samples per day. Subclasses of annotated lipid species included free fatty acids (FFA), monoacylglycerides (MG), diacylglycerides (DG), triacylglycerides (TG), cholesteryl esters (CE), lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), lysophosphatidylglycerides (LPG), lysophosphatidylinositols (LPI), lysophosphatidylserines (LPS), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerides (PG), phosphatidylinositols (PI), phosphatidylserines (PS), ceramides (CER), dihydroceramides (DCER), hexosylceramides (HCER), lactosylceramides (LCER), and sphingomyelins (SM).

The application of ion mobility enhanced the annotation accuracy of triglyceride (TG) species. Among the tested extraction solvents, it was found that 80% isopropanol led to the highest number of reproducible lipid annotations with coefficients of variation (CV) below 30%. Short-term storage of dried blood spot (DBS) microsamples is feasible, ensuring minimal exposure to temperatures above -20˚C. Moreover, a significant overlap was observed in the identified lipids across various collection matrices for DBS microsamples. Lipidomic differences observed across the various disease states in

our preliminary cohort indicate the potential biological application of our DBS method for discerning metabolic alterations.

In summary, we have developed a high-throughput 4D LC-MS workflow specifically tailored for analysis of 20 subclasses of lipids in DBS microsamples. This research has the opportunity to facilitate frequent, self-administered microscale blood collections for lipid biomarker discovery in metabolic disorders across a variety of clinical and research settings. This may extend future opportunities to remote sampling and personalised health monitoring.

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SARS-CoV-2 induced lipid perturbation in lung, liver, serum, brain, and spleen of mice

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The emergence of SARS-CoV-2, the virus responsible for COVID-19, has posed a significant threat to public health worldwide. COVID-19 is characterized by symptoms such as fatigue, coughing, chest tightness, shortness of breath, palpitations, muscle pain, and difficulty concentrating. These symptoms may be associated with various conditions, including organ damage, post-viral infection syndrome, post-critical care syndrome, and related conditions. Lipids serve as both a source of energy and essential components of cells, playing a crucial role in cellular signaling pathways and regulating cell membrane permeability. Therefore, investigating lipidomic alterations related to organ damage and recovery caused by metabolic changes after SARS-CoV-2 infection is of interest.

Methods

In this study, lung, liver, serum, brain, and spleen samples from K18-hACE2 mice were examined. To compare lipid change patterns between mild and severe infection, two concentrations were used: 1x10^2 PFU/mL and 1x10^5 PFU/mL injected into the nasal cavity. Lipid changes were observed at 1, 2, 5, 7, 10, and 14 days post-infection (dpi). Samples injected with 1x10^5 PFU/mL were observed up to 7 dpi due to mortality occurring after 7 days. Lipids were extracted using a modified Folch method with MTBE (Methyl-tert-butyl ether) and analyzed qualitatively and quantitatively using nanoflow ultrahigh-performance liquid chromatography-electrospray ionization tandem mass spectrometry (nUHPLC-ESI-MS/MS).

Novel Aspect

This study will contribute to understanding organ damage and recovery resulting from COVID-19 infection.

Preliminary Data or Plenary Speaker Abstract

The recovery process from COVID-19 infection was observed in the lung, liver, serum, brain, and spleen of K18-hACE2 mice by monitoring changes in lipid composition using nUHPLC-ESI-MS/MS. Qualitative analysis identified a total of 698, 869, 466, 593, and 926 lipids in the lung, liver, serum, brain, and spleen samples, respectively. With low-dose infection (1x10^2 PFU/mL), most lipid classes exhibited significant decreases in lung tissue at 2 dpi, followed by subsequent recovery. Similarly, with high-dose infection (1x10^5 PFU/mL), most lipid classes showed significant reductions in lung tissue at 1 dpi, followed by subsequent recovery. However, triacylglycerol (TG) exhibited a steady decreasing trend in lung tissue. Even in the liver and brain, most lipid classes decreased and then recovered in late dpi. Quantitative analysis was accomplished to assess the degree of recovery in mice infected with COVID-19.

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Impacts of acute and chronic collagen and thrombin treatments on platelet and releasate lipidomics

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Platelets are small and anucleate blood cells that are important to physiological functions such as haemostasis. Platelet activation and thrombus formation may lead to severe cardiovascular events such as stroke and myocardial infarction.

Lipid was known to regulate platelet activation. However, detailed knowledge of the lipid species involved is still lacking. Besides intracellular lipid, lipid released by platelets upon stimulation, or releasate, appears to also modulate platelet activation. Few studies emphasized on releasate lipid, its relationship to platelet lipid and their collective roles in platelet activation.

With LC/MS-based lipidomics, we simultaneously studied alterations to human platelet and releasate lipidomes during acute/chronic collagen and thrombin stimulations, as well as how these changes contributed to platelet activation, utilizing P-selectin and PAC1 staining.

Methods

Washed human platelets (n=14) from individuals without known cardiovascular and platelet related diseases were stimulated in vitro with thrombin (0.2 or 1.0 U) or collagen (10 µg/mL) for 10 or 60 minutes. Platelet lysates and releasates were subsequently collected and then analysed with a purposely designed targeted platelet lipidomics platform encompassing >550 lipid species across >30 lipid classes/subclasses found in human platelet and releasate. Platelet activity in reaction to the stimulations were monitored by flow cytometry using antibodies against p-selectin and PAC1, the activated conformation of integrin αIIbβ3.

Novel Aspect

1. Comprehensive human platelet and releasate lipid profiles
2. Potential lipid biomarkers reflecting platelet reactivity to different stimulations

Preliminary Data or Plenary Speaker Abstract

Our results corroborated several well-documented changes during platelet activation, such as the consumption of phosphatidylinositol 38:4 and lipid profiles that reflected elevated phospholipase A2 activity and the production of lipid mediators. Alterations to platelet and releasate lipid levels were simultaneously presented, which revealed an overall suppression of lipid secretion by acute collagen treatment. Moreover, thrombin treatment appears to show a 2-stage effect, with prolonged treatment showing a releasate lipid profile akin to cell death/apoptosis, i.e., increase in diacylglycerol, ceramide, sphingosine-1-phosphate and phosphatidic acid. Correlation tests between the lipid profiles and different markers for platelet activity identified ether lysophosphatidylcholine (lyso-platelet activating factor) and phosphatidylethanolamine 38:7 as possible predictors for platelet reactivity in response to collagen and thrombin stimulations.

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Characterising the metabolic effects of a novel ceramide synthase inhibitor

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Ceramide, a sphingolipid, has a causal role in cardiometabolic disease. Ceramides containing saturated acyl tails of 16 and 18 carbons are considered particularly deleterious, due to frequent association with metabolic diseases and negative cardiovascular disease outcomes. Ceramide synthase (CerS), the enzyme which catalyses ceramide production, has six isoforms, each demonstrating specific fatty-acid substrate preference and tissue localisation. Selective pharmacological inhibition of CerS isoforms which produce deleterious ceramides, could potentially prevent cardiometabolic disease. Our study aims to characterise ET2.39, a novel CerS inhibitor. Through the use of liquid chromatography-mass spectrometry (LC-MS), we have developed a series of methods to enable rapid examination of this compound's pharmacokinetics, alongside its cellular and metabolic effects on sphingolipid metabolism.

Methods

HEK293 cells were treated with ET2.39 and the effects on ceramide levels and cell viability were evaluated. Oral pharmacokinetics of ET2.39 was determined in mouse plasma via targeted LC-MS. A 4 week high-fat diet (HFD) study was undertaken with male C57BL/6 mice provided Chow, HFD, or HFD + ET2.39 (~10 mg/kg/day) and physiological measurements (weight gain, fat and lean mass, glucose tolerance) taken throughout. Lipids extracted from cells in addition to snap-frozen mouse tissues, were analysed via a high throughput sphingolipid screening method utilising the Agilent 6495 QQQ LC/MS system.

Novel Aspect

Utilisation of targeted lipidomics to characterise the pharmacokinetics and inhibitory effects of a novel ceramide synthase inhibitor.

Preliminary Data or Plenary Speaker Abstract

At 1mM ET2.39 significantly inhibited the production of C16:0 and C18:0 ceramides in HEK293 cells, without impacting on cell viability. In mice, oral delivery of 5 mg/kg ET2.39 resulted in a peak plasma concentration of 58 nM at 8 hours, with the half-life determined to be 20 hrs. Administration of ET2.39 in the diet prevented HFD-induced weight gain but not glucose intolerance. ET2.39 also slowed the accretion of fat mass and resulted in significantly decreased mass of epididymal fat pads in mice. Marked decreases in C18:0 ceramide levels in heart (20% lower) and quadriceps tissue (50% lower) were observed, with no major impact of ET2.39 on C16:0 ceramide levels. Overall, the novel CerS inhibitor, ET2.39 demonstrates significant anti-obesogenic effects in mice fed a HFD, in conjunction with limiting the accumulation of deleterious C18:0 ceramides in key metabolic tissues.

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CSF sphingolipid levels are correlated with neuroinflammatory cytokines and differentiate neuromyelitis optica spectrum disorder from multiple sclerosis

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system CNS characterised by immune-mediated demyelination, neurodegeneration, and progressive loss of neurological function. There is a need for molecular biomarkers that can accurately distinguish MS from other demyelinating disorders and predict clinical progression. As myelin is a particularly lipid-rich membrane, cerebrospinal fluid (CSF) lipids may prove useful as biomarkers for MS and related conditions such as neuromyelitis optica spectrum disorder (NMOSD). The aims of this study were to identify CSF lipid signatures that (i) differentiate MS from NMOSD, other inflammatory neurological disorders (OIND), and non-inflammatory neurological disorders (NIND); and (ii) correlate with expanded disability status scale (EDSS) scores, MRI lesion load, or inflammatory mediators.

Methods

Lipids (161) and inflammatory cytokines/chemokines (15) were quantified with liquid chromatography-tandem mass spectrometry and multiplex ELISA, respectively, in CSF from people with untreated MS (33), NMOSD (11), OIND (27), and NIND (28). Analyte levels were compared between groups using ANOVA adjusted for age and sex, and linear regression was used to assess Pearson's correlations with age, EDSS scores, MRI lesions, cytokines and CSF protein. Correlations of lipids with EDSS, lesions, CSF protein and age were validated with an independent cohort of 29 untreated MS cases.

Novel Aspect

A novel lipidomic comparison between MS and NMOSD in CSF. Significant lipids correlated with MIF. CE(16:0) associated inversely with EDSS.

Preliminary Data or Plenary Speaker Abstract

Twenty-five sphingolipids and four lysophosphatidylcholines were significantly higher in NMOSD compared to MS and NIND cases, whereas no lipids differed significantly between MS and NIND. A combination of three sphingolipids differentiated NMOSD from MS with Area Under the Curve 0.92 in random forest models, corresponding to 91% sensitivity and 91% specificity. Ninety-four lipids, including those that differentiated NMOSD from MS, were positively correlated with macrophage migration inhibitory factor (MIF) (r = 0.3-0.7), and 37 lipids were positively correlated with total CSF protein (r = 0.4-0.8) in two independent MS cohorts. EDSS was inversely correlated with cholesterol ester CE(16:0) in both MS cohorts, whereas no lipids were reproducibly associated with the presence gadolinium-enhancing lesions or number of T2 lesions. In contrast, MIF and soluble triggering receptor expressed on myeloid cells 2 were positively associated with EDSS. Five sphingomyelin species were significantly increased with age in both cohorts.

Conclusively, CSF sphingolipids were positively correlated with markers of neuroinflammation and differentiate NMOSD from MS. The inverse correlation between EDSS and CE(16:0) levels may reflect

poor clearance of cholesterol released during myelin break-down and warrants further investigation as a biomarker of therapeutic response.

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Towards targeted and quantitative 4D Lipidomics - From PASEF to prm-PASEF

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Parallel accumulation–serial fragmentation (PASEF) in combination with trapped ion mobility spectrometry (TIMS) enables mobility-resolved fragmentation and a higher number of targeted precursors per time unit compared to conventional MS/MS experiments. PASEF is proven for 4D-Lipidomics profiling experiments and screening of unknown samples. High confidence lipid annotations derived from 4D-Lipidomics data in MetaboScape can be utilized as basis for targeted screening and quantitation methods. For targeted lipidomics, the highest data quality could be achieved using a parallel reaction monitoring (prm-PASEF) data acquisition offering potential for clinical lipidomics applications. Yet, the creation of prm-PASEF methods requires concerted acquisition and data processing methods. We present the integration of MetaboScape and TASQ softwares to offer a seamless workflow for high confidence lipid quantitation.

Methods

For benchmarking, phospholipids of the well-characterized standard reference plasma from the National Institute of Standards & Technology (NIST SRM 1950) was quantified by HILIC-TIMS-MS/MS using prm-PASEF acquisition with timsControl 5.0 on a timsTOF fleX instrument. For lipid quantification, coelution of the isotope-labeled internal standards with the corresponding lipid class was achieved by hydrophilic interaction liquid chromatography (HILIC). MetaboScape 2024b conducts untargeted analysis on the PASEF lipidomics dataset, including data processing and rule-based lipid annotation. Lipids of interest including species-dependent characteristic fragment ions are selected and transferred to TASQ's targeted analysis. TASQ 2024b curates a transition list for prm-PASEF acquisition and subsequent quantitative data analysis.

Novel Aspect

Demonstration of a HILIC-TIMS-MS/MS workflow with high confidence in lipid quantitation by prm-PASEF and combination of MetaboScape and TASQ software.

Preliminary Data or Plenary Speaker Abstract

By phospholipid profiling of NIST SRM 1950 plasma lipids, a deep lipidome coverage with high confidence annotation based on retention time, isotope pattern, collisional cross section and fragmentation behavior, i.e. the 4D lipidomics approach, was obtained in MetaboScape. The transfer of the identified lipids including lipid species-dependent fragment ions to the targeted/quantitative software solution TASQ facilitates the seamless generation of a prmPASEF MS/MS transition list and the generation of a TASQ method. MS/MS transitions were used to generate indicative qualifier ions as an additional tool for identification to support reliability in lipid quantitation on species or molecular species level.
Using HILIC-MS/MS, a lipid class separation was achieved which favors quantitation by using one isotope-labeled internal standard per lipid class. However, this coelution also favors isobaric type-II overlaps that result from the natural isotopic pattern of lipid species with an additional double bond ($\Delta m/z$ = 2 Da). Through the additional TIMS dimension, a separation of the isobaric overlap could be achieved, highlighting the benefits of the multidimensional HILIC-TIMS-MS/MS approach for lipid quantitation. For accurate quantification of non-baseline separated overlaps in TIMS, we recommend the use of the M+1 signal for quantitation, which is also implemented in the TASQ software. All in all, the combination of MetaboScape and TASQ software solutions for targeted Lipidomics by HILIC TIMS MS/MS and prmPASEF served for a precise and user-friendly lipid quantitation with high confidence, offering potential for applications in clinical lipidomics.

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Capillary electrophoresis-mass spectrometry profiling of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine products in human lipoproteins

Mine-yine Liu, Capillary electrophoresis-mass spectrometry profiling of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine products in human lipoproteins Chao-Jung Chen, Capillary electrophoresis-mass spectrometry profiling of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine products in human lipoproteins Chiz-Tzung Chang, Capillary electrophoresis-mass spectrometry profiling of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine products in human lipoproteins Wen-Chien Chiu, Capillary electrophoresis-mass spectrometry profiling of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine products in human lipoproteins Zhi-Ru Lin, Capillary electrophoresis-mass spectrometry profiling of oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine products in human lipoproteins Chuan-Jun Wang
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Phospholipids are crucial components on human lipoproteins. Oxidized phospholipids on lipoproteins were suggested to be closely associated with atherosclerosis. Many oxidized phospholipids were reported biologically active and pro-inflammatory. The phospholipid, 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (PAPC) contains a polyunsaturated fatty acyl chain which is easily oxidized. Oxidation of PAPC produces large amounts of molecules and many of them are bioactive. Many ox-PAPC products have not been characterized yet. Their biological effects and associated pathological conditions have not been understood. However, separation and identification of ox-PAPC molecules are difficult due to their similar structures. Determination of oxidized phospholipids in biological samples has opened an interesting and important new research window.

Methods

A simple and fast low-flow capillary electrophoresis-mass spectrometry (low-flow CE-MS) method has been developed to analyze oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (ox-PAPC) products in human very low-density lipoproteins (VLDLs). The optimal CE conditions included separation buffer (60% (v/v) acetonitrile, 40% (v/v) methanol, 0.1% (v/v) water, 0.5% (v/v) formic acid, 20 mM ammonium acetate), sheath liquid (60% (v/v) acetonitrile, 40% (v/v) methanol, 0.1% (v/v) water, 20 mM ammonium acetate), separation voltage (20 kV), separation capillary internal diameter (i.d.) (75 μm), separation capillary temperature (23˚C) and sample injection time (6 s). The selected MS conditions included heated capillary temperature (250 °C), capillary voltage (10 V), and injection time (1 s). Sheath gas was not used in this study.

Novel Aspect

This is the first study to determine ox-PAPC products in human lipoproteins by CE-MS.

Preliminary Data or Plenary Speaker Abstract

The total ion chromatograms (TICs), extracted ion chromatograms (EICs) and MS spectra of native PAPC standard and its in vitro oxidation products showed good repeatability and sensitivity. To determine the ox-PAPC products in human VLDLs, the EICs and MS spectra of VLDLs were compared with the in vitro oxidation products of native PAPC standard. For native PAPC standard, the measured linear range was 2.5 - 100.0 μg/mL, and the coefficients of determination (R2) was 0.9994. The concentration limit of detection (LOD) was 0.44 μg/mL, and the concentration limit of quantitation (LOQ) was 1.34 μg/mL. A total of 21 ox-PAPC products were analyzed for the VLDLs of healthy and uremic subjects. The levels of 7 short-chain and 5 long-chain ox-PAPC products on uremic VLDLs were significantly higher than healthy VLDLs.

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Lipidomics to identify early pregnancy plasma biomarkers in horses

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Early pregnancy diagnosis in mares is an important component of equine breeding practice, as early embryo loss is relatively common and incurs substantial economic loss. This loss is compounded by a short breeding season that requires pregnancies to be achieved early. To date, a precise signal for maternal recognition of pregnancy has not been elucidated in horses and detection is not possible until day 14.
Lipidomics analyses were performed to compare the blood plasma profiles of pregnant (P) and non-pregnant (NP) mares at day 7 and day 14 post-ovulation to identify pregnancy-induced biomarkers. Using a batch mode approach, established bioinformatical pipelines processed data to generate plasma lipid profiles containing many molecular lipid species.

Methods

Horse plasma samples (20 µL) were extracted with 180 µL of 1:1 (v/v), butanol/methanol containing 10mM ammonium acetate and internal standards. The supernatant (100 µL) was analyzed. Separation was performed on a Phenomenex Kinetex C18 column (2.6 µm, 100 Å, 100 x 2.1 mm) using a 5 µL injection. Mobile phase A was 1mM sodium acetate in 50% water, 30% acetonitrile and 20% isopropanol. Mobile phase B was 10mM ammonium acetate in 90% isopropanol, 9% acetonitrile and 1% water. The 15-minute gradient transitioned from 10% to 100% B. Samples were analyzed in data-dependent mode using a QTOF instrument with a Zeno trap and electron-activated dissociation (EAD). Data were analyzed using MS-DIAL software, version 5.1.

Novel Aspect

Using high-resolution mass spectrometry, lipidomics analysis on horse plasma shows significant lipid changes in pregnant horses by 7 days post-fertilization.

Preliminary Data or Plenary Speaker Abstract

These novel findings support the utility of mass spectrometry-driven omics platforms for pregnancy biomarker discovery and indicate that systemic physiological changes occur as early as day 7 post-fertilization in the pregnant mare. Overall, this study represents significant progress toward establishing a panel of biomarkers for the accurate detection of early pregnancy in the mare.

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Using an In Vitro Model of Ferroptosis to observe Lipidomic Changes in HT22 Cells in the Context of Alzheimer's Disease

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Alzheimer's disease (AD) is a neurodegenerative disorder that has been associated with changes in the cellular lipidome including increased levels of oxidative stress. Ferroptosis is a type of cell death characterized by uncontrolled lipid peroxidation and has several commonalities with AD pathology. Dysregulation of lipids such as increased ceramides, accumulation of lipid droplets containing triglycerides and changes in ether lipids have been associated with the pathogenesis of AD. The alkyl and alkenyl glycerophospholipids are structural isomers which can be difficult to distinguish, both play important roles in AD and ferroptosis but have different biological functions. We refined our targeted lipidomics methods to differentiate several lipid isomers and mapped out the comprehensive changes to the cellular lipidome seen in ferroptosis.

Methods

We optimized an in vitro model of ferroptosis using HT22 neuronal cells to explore changes in the cellular lipidome in the context of AD. Cells were treated both independently and together with 5µM of ML210, a GPX4 inhibitor that limits cell antioxidant defense, 1-5 µM of Aβ1-42, and 200µM of ferric ammonium citrate with and without 10µM Ferrostatin-1 (Fer-1), an inhibitor of ferroptosis. Butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid (EDTA) were added to samples when harvested to prevent further lipid peroxidation. Selective acid hydrolysis with HCL was utilized to distinguish alkyl and alkenyl glycerophospholipids within the study. Over 800 lipids were examined using our previously reported lipidomic platform (targeted liquid chromatography with tandem mass spectrometry).

Novel Aspect

Using comprehensive targeted lipidomics with the HT22 cell line we mapped out the impact of ferroptosis on lipid metabolism.

Preliminary Data or Plenary Speaker Abstract

Unique changes to the HT22 cellular lipidome were observed across several treatment groups. Ferroptosis is defined by its uncontrolled lipid peroxidation, and we were able to measure specific oxidised lipids to further understand this process. We noted an increase in oxidized phosphatidylcholine species such as PC (36:4;O2) (10.6-fold increase, p value= 1.16 x10-13) and an increase in oxidatively truncated species such as PC (25:4O2 [a]) (3.2-fold increase, p value= 1.09 x 10-7). We observed highly specific changes predominantly in the ether lipids, distinguishing between alkenyl (also known as plasmalogens) and alkyl glycerophospholipids. ML210 generated an extensive decrease to total ethanolamine plasmalogen levels (PE P) (0.57-fold difference, p value=1.2 x 10-8) whilst alkylphosphatidylethanolmine species (PE O) were increased (2.6-fold increase, p value=1.53 x 10-11) compared to corresponding controls. These changes were not observed in cells treated with Fer-1 indicating the change was unique to ferroptosis/GPX-4 inhibition and suggests a functional difference in metabolism between the two sub classes. A significant increase in ceramide species were observed with ML210 and Aβ peptide treatment including Cer(18:1/16:0;O2) (2.8 fold increase, p value = 6.15x10-13), Cer(18:1/18:0;O2) (4.0-fold increase, p value = 4.33x10-15) and Cer(18:1/24:0;O2) (3.3-fold increase, p value = 4.33x10-15) which were prevented with Fer-1 intervention. In addition, total triglycerides also increased in response to ML210 treatment (2.2-fold increase, p value=1 x 10-4) compared to Fer-1 treated samples. The changes with plasmalogens and

increases in ceramides and triglycerides support previously reported brain lipid phenotype with AD highlighting the connection between AD and ferroptosis.

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Lipidomics of Pro-inflammatory and Anti-inflammatory Macrophages

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

An increasing incidence of cardiovascular diseases in the developed countries population is obvious. Factors contributing to their development are obesity and pro-inflammatory processes occurring in the adipose tissue. Macrophages, cells of the non-specific immune system, are also an important component of adipose tissue. Macrophages can be classified as pro-inflammatory and anti-inflammatory. They play a significant role in biological processes. For a better understanding of these phenomena, it is necessary to analyze the lipidome of macrophages of both mentioned groups. We optimized the LC-MS method for the lipidomic analysis of macrophages and found the lipidome differences.

Methods

The adipose tissues were collected from the kidneys during their transplantation. Macrophages from each individual were isolated and divided into pro-inflammatory and anti-inflammatory by flow cytometry. The samples were extracted by MTBE method. LC-MS system consisted of autosampler TriPlus RSH Smart 850, quaternary HPLC pump Vanquish, and mass spectrometer Orbitrap IQ-X (Thermo Scientific, USA). Column Acquity UPLC BEH C18 (1.7 µm, 2.1 mm × 100 mm) operating at the flow rate of 300 µl/min and temperature of 55°C was finally used. The gradient of the mobile phase was set from A) acetonitrile/water (60:40 (v/v)) and B) acetonitrile/ 2-propanol (90:10 (v/v)), and additives (formic acid (0.1%) and ammonium formate (10 mM)). The optimal desolvation temperature was set to 350°C.

Novel Aspect

Characterization of the lipidome of a small number of cells. Expansion of knowledge about inflammatory processes in adipose tissue.

Preliminary Data or Plenary Speaker Abstract

The lipidomic method was optimized and partially validated for analysis of the limited number of cells. The analysis of the 71 standards allowed the construction of retention dependency curves for individual lipid classes. They were successfully fitted with a linear model ($R^2 > 0,99$) and subsequently used to confirm the accuracy of lipid identification. The optimized LC-MS method was used to analyze the lipidomes of macrophages isolated from the adipose tissue of kidney donors. The principal component analysis provided insight into the division of macrophages according to their type. The volcano plot showed a significant increase in the concentration of some lipids (phosphatidylcholines, phosphatidylethanolamines, and sphingomyelins) in the case of pro-inflammatory macrophages. These lipids contain predominantly saturated and monounsaturated fatty acids.
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Cardiac Specific Knockout of GM3 Gangliosides Does Not Protect Against Myocardial Infarction Potentially From Unexpected Alteration to the Cardiac Lipidome

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

GM3 gangliosides are a class of lipids found predominantly in the brain and liver, and previously have been positively associated with metabolic diseases such as diabetes and obesity. GM3s are the main ganglioside in the heart. More recently, they have also been implicated in the pathophysiology of heart failure and atrial fibrillation, which remains the greatest cause of mortality and most common heart rhythm disorder globally. Whether GM3 lipids directly contribute to cardiac pathology remains unknown. Greater understanding of the role they play in cardiac pathophysiology may uncover new therapeutic targets for heart failure.

Methods

Cardiac-specific GM3 synthase Knockout (GM3s cKO)/ floxed controls (FC) were generated and mice underwent basal phenotyping (n=8-13/group). Additionally, a separate cohort also underwent surgical induction of 1-hour myocardial ischemia with subsequent reperfusion (IR) in ~13 weeks old males to mimic a heart attack with subsequent clinical treatment (n=5-7/group). Mice underwent echocardiography to assess heart left ventricular (LV) function at baseline and study endpoint (28 days post-IR). Tissues were collected and molecular phenotyping was conducted on cardiac tissue and plasma. Additionally, lipids were extracted via the chloroform:methanol method and run using liquid chromatography electrospray ionisation tandem mass spectrometry. Data was analysed via unpaired t-tests with Benjamini-Hochberg correction applied where necessary ($p<0.05$ was considered significant).

Novel Aspect

This is the first study to specifically assess the role of GM3 lipids in the heart.

Preliminary Data or Plenary Speaker Abstract

GM3 lipids were reduced by approximately 84% in cKO hearts. GM3s cKO heart weights normalised to tibia length ratios were significantly reduced vs FC mice under basal conditions, as well as after IR injury (15.9% and 10.2% respectively; $p<0.05$). There were no significant differences in heart function or gene expression of ANP or fibrotic markers such as collagen 1 following IR injury. The reduction of GM3 lipids in GM3 cKO hearts was associated with a 16-fold increase in its metabolic precursor, dihexosylceramide, while other lipids within the sphingolipid pathway remained unchanged. Lipidomic profiling revealed a decrease in odd and branched chained phospholipids in GM3s cKO vs FC hearts following IR, suggesting dysregulation of branched chain amino acid catabolism in the mitochondria. Further analysis of circulating lipids from GM3s cKO vs FC mice following IR demonstrated a differential lipidome, including a significant reduction in 33 lysophosphatidylcholines species, and an increase in ether lipid species (Alkylphosphatidylcholines, Alkenylphosphatidylcholines, Alkylphosphatidylethanolamines and Alkenylphosphatidylethanolamines). Collectively, these studies suggest that Cardiomyocyte depletion of GM3 lipids had no effect in a setting IR injury.

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A New Electron Ionization (EI) Source Equipped with a Novel RF Lens Promoting Prolonged Robustness

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Mass spectrometers (MS) equipped with an Electron Ionization (EI) source are usually connected with a gas chromatograph (GC). The carrier gases used in GC, commonly helium or hydrogen, are present at a higher concentration than the analytes of interest. When ionized by the EI source, these overwhelming carrier gas ions pose risks of sensitivity loss of analytes as well as contamination on source/analyzer surfaces. In this study, we present a new EI source equipped with a novel dipolar RF (Radiofrequency) lens that redirects the carrier gas and low mass ions and, as a result, provides reduced noise and extended instrument robustness while maintaining sensitivity.

Methods

The dipolar RF lens was simulated via SIMION using various geometries, frequencies, and voltage amplitudes. The selected design was implemented in Agilent's High Efficiency Source (HES) (becoming the High Efficiency Source 2.0 or HES 2.0) and tested in an Agilent 7010 Triple Quadrupole GC/MS system for effectiveness in carrier gas ions deflection and autotune performance. The 7010 Triple Quadrupole GC/MS system was then injected with black pepper extract as samples with heavy matrices to evaluate its analytical performance and system stability. This new ion source (HES 2.0) was installed in collaborating laboratories to be used in routine analytical testing.

Novel Aspect

This is the first study using a dipolar RF lens inside an EI source to deflect carrier gas ions.

Preliminary Data or Plenary Speaker Abstract

Both SIMION and experimental data prove that the lens is effective in deflecting light carrier gas ions by >95% at the optimized 4.5 MHz frequency and 300V peak-to-peak amplitude. The deflected carrier gas ions land on an adjacent lens and are pumped out prior to entering the mass analyzer. While effective in deflecting carrier gas ions, low mass ions are also subject to some deflection potentially causing reduced abundance. Therefore, a ramped RF amplitude vs mass is implemented to avoid analytes spectra tilt. Up to 3x neutral noise reduction was also achieved.
The 7010 system equipped with this new ion source showed robust analytical performance when it was tested with black pepper extract injections. No degradation was observed in analytical performance, sensitivity, or system stability after thousands of injections of black pepper extract spiked with PAHs and pesticides. This is demonstrated in RSDs of less than 10% in PAHs and most pesticides qualifier ratios. Additionally, mass spectrometer peak widths and transmission remained constant with RSDs < 3% and < 10%, respectively. Aside from regular source cleaning and GC maintenance, no additional mass spectrometry service or cleaning was necessary.
Collaborating analytical laboratories operating the new HES 2.0 ion source achieved comparable analytical performance and superior system stability compared with a reference HES system.

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Rapid and Reliable Nitrite Content Analysis in Pharmaceutical Excipients with the ACQUITYTM QDaTM II Mass Detector

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Nitrite is the most critical risk factor in N-Nitrosamine formation, present as an impurity in pharmaceutical excipients typically in parts per million (ppm). Screening for total nitrite content as a single underlying precursor to N-Nitrosamine formation can be a simple and pragmatic approach to support risk assessment in the pharmaceutical manufacturing process.
A reliable solution is proposed to manage high-throughput routine workflows in excipient testing, while maintaining a low cost of analysis per sample. These results describe a UHPLC coupled to QDa II Mass Detector employed to assess the suitability of this analytical configuration for long term analysis of pharmaceutical excipients over the course of 29 days of analysis, demonstrating the performance, robustness, and reliability required for effective analysis.

Methods

Selected representative pharmaceutical excipients were extracted following a modified protocol from Jireš & Douša, where powdered excipients were taken through a 2,3-diaminonaphthalene (DAN) derivatisation method. The principle of this method is reaction of nitrite ions with DAN to form 1H-Naphtho(2,3-d)triazole (NAT) which can then be separated and detected as a proxy for total nitrite content, using mass detection.
Unspiked excipient samples were quantified against a calibration curve of known nitrite concentration; the recoveries of spiked excipient samples were also assessed. Instrument QC standards of acetaminophen and caffeine were analysed throughout the analysis, to monitor system stability for the purposes of robustness testing. Samples were prepared in four sequential batches and assessed over 29 days of analysis.

Novel Aspect

ppm level analysis of nitrite in pharmaceutical excipients achieved with robustness and long-term stability utilizing the QDa II Mass Detector.

Preliminary Data or Plenary Speaker Abstract

Determination of nitrite content in selected excipients was achieved by quantifying NAT (m/z 170) with the QDa II Mass Detector operated in positive electrospray ionization (ESI+). The derivatized compound had a retention time (RT) of 2.73 mins in a 6-minute method. Nitrite was easily detected in maize starch excipients at 0.14 µg/g, in line with mean levels reported in excipients databases. The %RSD for n=27 injection of maize starch samples containing this amount of nitrite was 8.7%.
Excipients samples of sorbitol, lactose monohydrate, maltodextrin, and calcium carbonate did not exhibit detectable levels of nitrite during the analysis.
All excipient samples were spiked at 0.2, 2.0 and 5.0 µg/g, and recoveries measured across a 190-hour sample batch ranged from 70 - 129%. %RSDs across the spike levels of the excipients analysed did not exceed 18.5%, 11.1%, and 6.0% respectively. The total average recovery across all samples and spikes was 94%.
Over the course of 29 days of analysis, ~6000 injections were performed on the QDa II with only 2 hours of instrument downtime required for source component cleaning. Throughout 520 hours of continuous analysis following the 2 hours of downtime, the %RSD of absolute peak area for any given QC level remained below 12.5%, demonstrating stability of the system throughout the analysis. The %RSDs of absolute peak area for all nitrite standard injections was consistently less than 14.7% for any given sample batch analysed. The %RSD of instrument QC injections in each sample batch did not exceed 9.8%, demonstrating consistent performance. The results from the experiments performed

indicate that the ACQUITY QDa II Mass Detector is a robust and reliable instrument suitable for routine high throughput applications.

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Investigating Factors Influencing Intensity-to-Charge Ratio in Orbitrap-based Charge Detection Mass Spectrometry

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Charge detection mass spectrometry (CDMS) is a single-particle detection technique that determines the mass of each ion by simultaneously measuring the mass-to-charge ratio (m/z) and the charge, eliminating the need for charge-state resolved mass spectra. Within the Orbitrap, the signal intensity of individual ions exhibits a validated linear relationship with their corresponding charges. Consequently, the measured signal intensity from individual ions can be converted into charge states using appropriate calibration factors. However, numerous parameters within the controlled panel may influence the signal amplitude of individual ions, potentially leading to fluctuations in the intensity-to-charge ratio. Hence, it is imperative to thoroughly investigate the key factors that exert influence.

Methods

CDMS measurements were conducted using an Orbitrap Q Exactive UHMRTM mass spectrometer (Thermo Fisher Scientific). Standard samples with charge-state resolvable native mass spectra were used for intensity-to-charge conversion. These samples were buffer exchanged into ammonium acetate solution before being introduced into a platinum-coated borosilicate capillary for nanoelectrospray ionization. A resolution of 200,000 at 400 m/z was set for recording 1s transients. After multiscan acquisition, an appropriate calibration factor was determined to correlate the measured intensities and charges of individual ions. Subsequently, based on the determined charge state, the formula mass = m/z × z − z was employed to calculate the mass of each ion individually. All data and plotted results were analyzed and processed using our in-house Python scripts.

Novel Aspect

We investigated and quantified the majority of factors influencing the intensity-to-charge ratio in Orbitrap-based charge detection mass spectrometry.

Preliminary Data or Plenary Speaker Abstract

Charge-state resolvable samples such as Trastuzumab and β-galactosidase were utilized in our preliminary tests. We investigated various experimental conditions, including the activation of in-source trapping (IST), adjustments to ion transfer voltages, selection of detection mode (for high or low m/z), variations in gas types (N2 and SF6), and manipulation of tapping pressure. Our findings indicate that IST, ion transfer voltages, different gas compositions, and pressures significantly impact the survival rate of individual transient ions. Additionally, we observed that detector m/z optimization exerts a considerable influence, resulting in significant changes in the intensity-to-charge ratio.

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Algorithm-assisted optimization of high-resolution planar electrostatic ion trap mass analyzer

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The study and design of high-resolution mass analyzers is a very important task in mass spectrometry. A planar electrostatic ion trap (PEIT) mass analyzer with image charge detection and FT-based data processing has been developed, theoretically simulated, and experimentally validated. However, the previous 10 ring electrode configuration (PEIT-10) is difficult for mechanical construction and voltage tuning; moreover, few methods have been reported for optimizing the performance of multi-electrode mass analyzers. Here, a simplified 6 electrodes structure (PEIT-6) mass analyzer was designed, and a genetic algorithm parallel optimization (GAPO) method was developed for optimizing multiple voltage settings of the new PEIT-6 mass analyzer to achieve spatial and energy isochronicity as well as iso-coordinate property.

Methods

Genetic algorithm parallel optimization(GAPO) method was developed for optimizing multiple voltage settings of the new PEIT-6 mass analyzer to achieve spatial and energy isochronicity as well as iso-coordinate property.

Novel Aspect

high-resolution planar electrostatic ion trap mass analyzer; Genetic algorithm parallel optimization; SIMION

Preliminary Data or Plenary Speaker Abstract

The automatic voltage optimization processes for the reduction of time aberration and spatial aberration showed that the developed GAPO method can significantly improve the optimization efficiency (the optimal voltage set being found within 5 hours with a maximum time aberration of 5 ps and a maximum z aberration of 0.10 μm). Based on the results obtained from the GAPO method, the resolving power of the PEIT-6 mass analyzer for six groups of ions with closely packed masses (m/z = 117.000 Th to 117.010 Th) was demonstrated, and a mass resolution of 171k was achieved at an acquisition time of 200 ms. The established GAPO method facilitates the design and optimization of high-resolution mass analyzers and may be useful for the design of other multi-electrode ion optical devices.

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Unleashing Mobile Trace Gas Analysis in Real-Time

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The Syft Explorer mobilises the direct real-time mass spectrometry technique of SIFT-MS. Using state-of-the-art power management and independent gas systems, mass spectrometry can now be brought to any location with no shutdowns or configuration changes.

Methods

SIFT-MS real-time direct mass spectrometry.

Novel Aspect

The Syft Explorer is the world's only mobile real-time direct mass spectrometer capable of measuring both VOC and Inorganic componds.

Preliminary Data or Plenary Speaker Abstract

Syft Explorer is an all-in-one solution designed for ambient air applications. It delivers high sensitivity, exceptional performance stability, and highly reproducible data through the SIFT-MS technology. With its mobile functionality, it enables rapid, selective, and reliable analysis of contaminants at ambient air sites.

Syft Explorer offers a unified configuration for analyzing volatile organic compounds and inorganics with a single method or between multiple methods, eliminating the need for column changeover or other chromatographic delays. It enables continuous, on-line analysis and provides a dedicated user-friendly interface for instrument control, status information and data visualization. The Syft Explorer unit provides trace-level analysis of chemically diverse compounds. The dedicated in-built software allows method generation and adjustments on site to suit the environment.

Equipped with onboard clean dry air, nitrogen or helium cylinders and intelligent, automatic power management, it facilitates leak detection, and targeted pollution and ambient air measurements. Its comprehensive braking system, bumpers, sample line storage and power cord storage ensure easy movement around sampling sites.

Come see how we measured trace voltailes such as individual flourianted compounds/PFAS, VOCs, inorganics, and our office lunch room all in a day with the Syft Explorer.

Enzymatic Phospho-Probing for Detecting Protein Conformational Changes on a Proteome Scale

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Mass spectrometry (MS) is increasingly being utilized to monitor protein structural changes from a limited amount of samples. These methods include the steps of labeling the protein surface with probes and analyzing the probe-labeled peptides using MS. However, the low specificity of probing and the high complexity of samples make it challenging to detect protein conformational changes on a proteome scale with high sensitivity. In this study, we developed an MS-based structural analysis method that utilizes site-specific probing with phosphate groups, taking advantage of the substrate recognition provided by protein kinases. The phosphate groups serve as enrichment handles, enabling selective extraction of labeled sites and highly sensitive analysis.

Methods

Proteins were extracted from HEK293T cells under non-denaturing conditions. For examining protein conformational changes due to the loss of RNA-protein interaction, the extracted protein solution was treated under RNA digestion conditions (RNase cocktail treatment, 4 °C, 30 min). Phospho-probing was performed by in vitro kinase reaction with recombinant tyrosine kinase SRC (Carna Biosciences) in the presence of 1 mM ATP and 20 mM MgCl2 at 37 °C for 15 min. After trypsin digestion, phosphopeptide enrichment was performed by hydroxy acid-modified metal oxide chromatography using titanium dioxide. To evaluate the labeling efficiency on the unstructured substrates, peptide substrates were prepared by trypsin digestion of the extracted protein solution, followed by phospho-probing and the phosphopeptide enrichment. Samples were analyzed by nanoLC/MS/MS.

Novel Aspect

In vitro phospho-probing can detect protein conformational changes with high sensitivity on a proteome-wide scale.

Preliminary Data or Plenary Speaker Abstract

We first examined how differences in substrate higher-order structure affect phospho-probing efficiency. We identified 440 tyrosine phosphorylation sites in the non-denatured sample and 678 sites in the trypsin-digested sample. The overlap between these two data sets was 247, with 193 and 431 sites uniquely identified in the non-denatured sample and trypsin-digested samples, respectively. We then compared the structural information of tyrosine residues in the predicted protein structures obtained from AlphaFold2 for the 440 sites identified in the non-denatured sample and the 431 sites identified uniquely in the trypsin-digested sample. The result showed that 50 % of the tyrosine phosphorylation sites identified in the non-denatured sample were in the disordered region, while more than 95 % of the tyrosine phosphorylation sites uniquely identified in the trypsin-digested sample were in the structured region. These results indicate that disruption of the higher-order structure of the protein increases the phosphorylation efficiency of tyrosine residues in the structured region, and that differences in substrate structure are reflected in the phospho-probing profiles.

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Kinetics and Conformational Dynamics Study of SARS-CoV-2 Main Protease and Its Interactions with Inhibitors

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The main protease (Mpro), an essential enzyme for the replication of SARS-CoV-2 and highly conserved among coronaviruses, is an appealing target for the development of broad-spectrum antiviral drugs. Although various inhibitors have been developed for SARS-CoV-2 Mpro, no experimentally systematic study on conformational dynamics of interactions between SARS-CoV-2 Mpro and its inhibitors in solution has been reported yet. SARS-CoV-2 Mpro is in an equilibrium between the active dimer and inactive monomer in solution. Knowledge about the kinetic aspects of the dimerization process, especially some important physicochemical parameters for understanding the dimerization mechanism, has not been available.

Methods

Subunit exchange between SARS-CoV-2 Mpro and 13C-labeled Mpro monitored by native mass spectrometry (Native MS) was performed to measure the dissociation rate constant and other kinetics parameters (i.e., association rate constant, activation energy, and relevant half-life time) of the SARS-CoV-2 Mpro dimer. Continuous labeling hydrogen/deuterium exchange mass spectrometry (HDX-MS) was employed to investigate the conformational dynamics of Mpro and its interactions with four inhibitors (i.e., PF-07321332, boceprevir, carmofur, and ebselen) that represent different binding modes and different inhibitory effects.

Novel Aspect

Kinetics and conformational dynamics study of SARS-CoV-2 main protease

Preliminary Data or Plenary Speaker Abstract

Native MS spectra of Mpro (2 μM) showed the predominant existence of dimeric Mpro in the solution and the complete binding of Mpro with the inhibitor PF-07321332 without detecting any Mpro monomer form, indicating PF-07321332 could make the dimeric Mpro less dynamic. Dissociation kinetics of dimerization was determined by subunit exchange combined with native MS. The dissociation was fitted to a first-order exponential decay kinetic model and the dissociation rate constant of Mpro dimer, a fundamental kinetic parameter, was determined to be 0.0025 min–1, with a half-life (t1/2) of 277 minutes, indicating that the rate-limiting step was the dimer dissociation and Mpro had a higher tendency to form the dimer. Moreover, the subunit exchange of Mpro was completely inhibited upon binding with PF-07321332. HDX-MS study revealed that the overall conformation of Mpro was more rigid after bound with PF-07321332, boceprevir and carmofur, but more flexible with ebselen. Active site regions showed a significant decrease in deuterium uptake of Mpro upon binding with PF-07321332, boceprevir, and carmofur. Long loop region (residues 183-197) showed the most significant decrease in deuterium uptake upon binding with PF-07321332, boceprevir, and carmofur, and a significant increase in deuterium uptake upon binding with ebselen. N-terminal and C-terminal regions were significantly affected upon the binding with PF-07321332 and ebselen, compared to boceprevir and carmofur, and changes in the conformational dynamics of these regions could potentially affect the dimerization process. These studies provided insights into the kinetic and dynamic properties of Mpro and revealed the allosteric sites (N-terminal region, C-terminal region and long-loop region) as new potential targets for drug design.

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The direct molecular isomer distinction of bipyridine dicarboxylic acid and biphenyldicarboxylic acid by ion mobility spectrometry in negative ion mode

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

The differentiation of positional isomers represents a central focus within contemporary analytical science. Nevertheless, most conventional analytical methods for accurate isomer identification either cumbersome or complex. We report a new method for the distinction of positional isomers of Bipyridine dicarboxylic acids (BDA) and Biphenyldicarboxylic acids (BA) without any pre-separation, chemical derivatization and host-guest complexation by simply measuring the ion mobility spectrometry of their negative ions in gas phase. The experimental results showed that the different isomer negative ions have different cross sections which can be used to distinguish their molecular structures. The theoretical calculation results showed that the formation of intramolecular hydrogen bond could affect the molecular conformation and result in different cross section of isomers.

Methods

The 3,3’-BDA/ 4,4’-BDA/ 5,5’-BDA, 2,2’-BA/ 3,3’-BA/ 4,4’-BA isomers were separately solubilized with DMSO to 10-4 M concentration under sonication conditions and stored in a refrigerator at 4 ℃. Before analysis, all solutions were diluted with acetonitrile/water solution (40:60, v/v) to a concentration of 10-5 M. All samples were analyzed with an ESI-TIMS-TOF MS instrument (Bremen, Germany) in the negative ESI mode. Both of the mass spectra and ion mobility spectra of BDA and BA were recorded and analysis in the experiments. For quantitative analysis, the isomer mixtures with different relative concentrations were also investigated, the signal intensity were measured by calculating the IMS peak volume. Meanwhile, the theoretical calculations were performed to explain the results of the experiments.

Novel Aspect

The molecular isomers can be easily identified by simply measuring its anion collision cross section using IMS technology.

Preliminary Data or Plenary Speaker Abstract

The mass spectra of the two sets of positional isomers (3,3’-BDA/4,4’-BDA/5,5’-BDA, 2,2’-BA/3,3’-BA/4,4’-BA) negative ion peaks were clearly observed in the experiments, and the ion mobilities of the negative ions of 3,3’-BDA/ 4,4’-BDA/ 5,5’-BDA, 2,2’-BA/ 3,3’-BA/ 4,4’-BA were measured. The results showed that the ion mobilities of different isomer anions are different, so it can be used to distinguish their molecular structures and conformations. The theoretical calculations were performed to explain the reason of molecular conformation difference, and the results showed that there are intramolecular hydrogen bonds in the negative ions of 2,2'-Bipyridine-3,3'-dicarboxylic acid (3,3’-BDA) and 2,2'-Biphenyldicarboxylic acid (2,2’-BA), and meanwhile, there are not intramolecular hydrogen bonds in the negative ions of 2,2'-Bipyridine-4,4'-dicarboxylic acid (4,4’-BDA) and 2,2'-Bipyridine-5,5'-dicarboxylic acid (5,5’-BDA); 3,3'-Biphenyldicarboxylic acid (3,3’-BA) and 4,4'-Biphenyldicarboxylic acid (4,4’-BA). The intra-molecular hydrogen bond will certainly tighten the molecular structure and lessen its volume. Finally, relative quantitative analysis experiments were performed on the isomer mixtures, and a satisfactory linear relationship ($R2 > 0.99$) was obtained, and demonstrating the possibility of quantitative analysis of isomer contents in mixture.

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Understanding the conformational variability of Histone's Globular Domain as a function of net charge and charge location using native IM-MS

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Linker histone (H1) plays a crucial role as a chromatin-binding protein, serving as an epigenetic regulator by engaging in electrostatic interactions with nucleosomes. Previous work by Martinsen et.al, shows that the structure and stability of Histone's globular domain (GD) is not substantially perturbed following the truncation of the disordered tails. The role of multiple positive charges on the globular domain (GD) to its structure and conformational stability has been assessed through the analysis of 11 charge variants. CD and NMR results suggest that increase of the Net Charge (NC) negatively affects their stability. Native ion mobility mass spectrometry has a unique capability to measure conformational variations induced by charge/sequence modifications and is here applied to linker histones.

Methods

Seven charge variants of the Histone GD ranging from +5 to +13 were buffer exchanged in a MS-compatible buffer of identical ionic strength and pH as their original storage solution. Using ion mobility mass spectrometry (IM-MS) we determined mass, stoichiometry, and structural preferences of the GD through their charge state distribution (CSD), which can indirectly inform on the range of conformations that are adopted as well as the influence of charged residue on desolvated NC. Ion mobility measurements directly report on the conformations of each m/z selected species, and on the conformational spread across the CSD. We were also able to select and activate each charge variant and determine their intrinsic stability as a function of sequence.

Novel Aspect

The conformational heterogeneity of these seven histone variants has not been explored before, through native IM-MS techniques.

Preliminary Data or Plenary Speaker Abstract

From native MS, each charge variant presented a different CSD but in every case the dominant charge state was z=5, suggestive of a globular form. Ion mobility measurements on this charge state for each GD variant show an exceptionally broad conformational spread spanning from 730 - 2050 Å2. For each charge variant IM-MS measurements allow the effect of net charge and charged residue location to be distinguished. The charge variants with the lowest NC in solution present a narrow CSD envelope where $\Delta z = 3$, and those with higher NC present with $\Delta z \leq 9$. This shows how net sequence charge can be read out in the desolvated species. The widest CSD was found in GD12K2 and GD2E2K with the highest NC in solution of 12+ and 13+, indicative of conformational heterogeneity in solution. Charge permutants, GD6K1/GD6K2 and GD8K2/GD8K3, possessing the same NC in solution, but with Lys and Gln at different locations in the primary sequence presented with highly similar CSD, indicating how stabilising interactions between acidic and basic residues and repulsive interactions between proximal charges conspire to provide each NC state for the self-solvated forms of these IDPs. These findings are also evident in the CCS distributions where the higher NC variants exhibit a broader CCS distribution.
The GDWT sequence with NC of +9 can be best replicated by the GD8K2 and GD8K3 mutants (NC=+7) with most of the conformers in the CCS range from 730-1700 Å2 and a small population as extended conformers at 1800 Å2. The lower NC GD6K1 and GD6K2 (NC=+5) have narrower CCSDs compared to the WT with no observable conformers above 1500 Å2. By contrast the higher NC GD12K2 and GD2E2K present broader CCSDs, with a far more populating extended conformers with CCS of 1700 Å2.

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Expanding native mass spectrometry capabilities for soluble and membrane proteins using a quadrupole-ion mobility-time-of-flight mass spectrometry system

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

After many years of increased adoption native mass spectrometry (MS) is an established technique in both academia and industry. Fundamental biomedical research is exploiting native MS to learn about ever more complex biological systems in environments approaching the endogenous with instrumental innovations being central to this. Increases in mass resolving power, high mass sensitivity, ion mobility performance and expansion of the available fragmentation methods are key developments that have occurred over almost two decades of rapid adoption of native MS. These advancements have also facilitated in many cases the convergence of native MS and top-down proteomics. In this work we describe the performance characteristics of a quadrupole-ion mobility time-of-flight system as applied to key problems in native MS research.

Methods

All experiments were performed on a SELECT SERIES™ Cyclic™ IMS mass spectrometer equipped with an ECD cell positioned after the ion mobility device. The instrument was also fitted with a surface-induced dissociation device prior to the post-quadrupole differential pumping aperture. ECD efficiency was optimized using substance P. All samples were introduced into the mass spectrometer using uncoated glass capillaries with spray voltage applied though direct contact with platinum wire inserted into the rear opening of each capillary. Maximum desolvation and micelle removal were optimized with a combination of capillary voltage and cone and trap collision voltages. For high m/z transmission, the trap gas flow rate and ion guide RF amplitudes were maximized.

Novel Aspect

First presentation of a full suite of capabilities and fragmentation modes for study of native proteins

Preliminary Data or Plenary Speaker Abstract

The high mass capability of the instrument setup was investigated with a number of protein complexes. The tetradecamer GroEL and its complexed dimer demonstrated transmission up to 1.6 megadaltons. The key tuning parameters to maximize transmission include increase of the array offset during the inject and separate steps of the cyclic ion mobility experiment.
Protein sequencing of native protein subunits is made possible on the instrument in different ways. Firstly, native protein ions are introduced into the gas phase and selected with the quadrupole. Next, the complex is dissociated with CID in the trap collision cell before being subjected to cyclic ion mobility separation. Then, post mobility, the ions can be further fragmented either with CID in the transfer or by ECD in the pre-transfer guide. We demonstrate this for released GroEL monomers. The differences between the data for CID and ECD will be discussed.
Membrane proteins represent greater than 60 % of all drug targets and their study by native MS is growing. Membrane proteins must be introduced into the mass spectrometer in either a detergent or other solubilizing agent. This must be removed though collisions by the application of accelerating voltage in a gas cell to obtain meaningful mass information. We demonstrate the high data quality obtained through collisional activation with the cone and trap collision voltages with the membrane proteins OmpF and Pf-MATE.
Next, we investigated the feasibility of a novel automated surface-induced dissociation acquisition strategy for profiling dissociation pathways. Using streptavidin as a model system we show that

thanks to the integration of the SID device within the instrument control software and method editor, a dissociation profile can be acquired with minimal user intervention and tuning.

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Native Mass Spectrometry as a Tool for the Development of Novel Triplex-Forming Oligonucleotides

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Triplex-forming oligonucleotides (TFOs) are short nucleic acid strands that bind to double-stranded DNA sequences, forming triple-stranded structures known as triplexes. Although highly promising as antigene agents, their use in molecular biology and medicine is limited, as triplex formation requires consecutive purine nucleotides in the target strand to form the hydrogen bonds necessary for this structure. Our group has previously demonstrated the use of ion mobility and native mass spectrometry (native MS) as methods to study triplex formation under various experimental conditions. In this study, we further explore the advantages and limitations of native MS as a tool to study triplex structures and its potential to inform the development of synthetically modified TFOs that can improve triplex formation and stability.

Methods

Bioinformatics: A script was designed to identify target triplex-forming sequences in a given genome downloaded from NCBI, followed by gene function analysis using BLAST.

Sample preparation: Commercial oligonucleotides were dissolved in ultrapure water and their concentrations were quantified using a NanoDrop One UV-Vis Spectrophotometer (Thermo Scientific). Equimolar amounts were then mixed and diluted in the desired buffer and incubated at 4 °C to prepare both duplex and triplex DNA samples.

Mass spectrometry: Mass spectra of the samples were obtained using a Bruker Impact HD Q-ToF mass spectrometer equipped with a nanospray ion source.

UV–Vis spectroscopy: Thermal melting experiments to assess triplex stability were conducted using a Shimadzu UV-3600i-Plus spectrophotometer paired with a temperature controller.

Novel Aspect

Use of native MS to assess triplex formation using pyrimidine-containing DNA targets and chemically modified TFOs.

Preliminary Data or Plenary Speaker Abstract

In this study, we have demonstrated the use of native MS in the assessment of triplex formation for a given combination of TFO, duplex, and pH, to study the effect of pyrimidine interruptions in poly-purine target sequences.

A 34-bp DNA sequence was selected from the reference genome of an antibiotic-resistant bacterium strain (P. aeruginosa PAO1, ID 208964). To study the effect of pyrimidine interruptions, two types of duplexes were prepared based on this sequence: 5’-CGGTGAAGGGGAAGAAGGAXGGAXGGAAGCGCAC-3’, where X is either cytosine (DNA1) or adenine (DNA2). A TFO was designed to target the central 20-bp region in each duplex, with the following sequence: 5’-CCTTCTTCCTTCCTTCCTTC-3’. Triplex formation with the TFO was carried out for each duplex in a 250 mM ammonium acetate buffer at a pH of either 6.7 (neutral) or 5.5 (acidic). Native MS allowed us to identify stable triplex formation for both duplexes under acidic conditions. Under neutral pH conditions, it was possible to study the effect of pyrimidine interruptions: triplex formation was observed with DNA2 but not with DNA1. These results were consistent with the results of thermal melting studies using UV-Vis spectroscopy, which helped quantify the stability of

each triplex. Native MS also allowed us to observe the presence or lack of unbound TFO in each sample.

We have shown that native MS can be used to reliably determine the presence of even relatively unstable triplexes at neutral pH. The ability to perform a rapid assessment before further stability studies makes this a promising method to compare the triplex-forming ability of a variety of chemically modified TFOs, aimed at targeting pyrimidine interruptions, which is the next step in this study. Further experiments are also necessary to assess the potential of using different parameters and techniques in native MS (relative peak intensity, CID, etc.) to assess triplex stability.

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Fundamental understanding of DNA triplex formation and its antigene properties using mass spectrometry.

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Deoxyribonucleic acid (DNA) triplexes have potential roles in a range of biological processes involving gene regulation. A major challenge in exploiting the formation of these higher order structures to target genes in vivo however is their low stability, which is dependent on many factors including the length and composition of bases in the sequence. Here, different synthetic and commercially available DNA modifications have been explored, primarily using native mass spectrometry, in efforts to enable stronger binding between the triplex forming oligonucleotide (TFO) and duplex target sites.

The aim is to monitor triplex formation within the bacterial environment, facilitating the development of a potential antigene therapeutic strategy. Additionally, the study evaluates conjugation of cell-penetrating peptides with modified oligonucleotides for efficient delivery.

Methods

The formation and stability of various modified TFOs in a range of model and biologically relevant triplex sequences containing pyrimidine base interruptions were investigated using native electrospray ionization mass spectrometry (ESI-MS) in combination with UV-vis spectroscopy.

Oxidative amination and thiol alkylation was used to modify the oligonucleotide and to add the alkyne and azide handles. Then, click chemistry was used to further expand the library of molecules that could be obtain to form stable triplexes.

To monitor triplex formation within the bacterial environment, cell penetrating peptides conjugated to oligonucleotides were utilised to enable cellular delivery, then antimicrobial assays were used to determine the minimum inhibitory concentration.

Novel Aspect

The project gives better fundamental understanding of the triplex formation along with the interaction of the triplex in bacterial cells.

Preliminary Data or Plenary Speaker Abstract

Using model sequences with a single pyrimidine interruption, TFOs containing locked nucleic acid (LNA) base modifications were shown to have a higher triplex forming propensity than DNA-only and dSpacer-containing TFOs. However, the triplex forming ability of these systems was limited by the formation of multiple higher order assemblies. Triplex forming sequences that correspond to specific gene targets from the Pseudomonas aeruginosa genome were also investigated, with LNA-containing TFOs the only variant able to form triplex using these sequences. This indicates the advantages of utilizing synthetically modified TFOs to form triplex assemblies in vivo for potential therapeutic applications and highlights the advantages of native mass spectrometry for the study of their formation.

Moreover, various base pair modifications were used to address multiple pyrimidine interruptions within the sequence, utilizing modified base pairs and diverse handles. This comprehensive approach seeks to optimize the design of synthetic oligonucleotides, considering the impact of different modifications on the stability of triplex formation in bacterial genomes. To ensure an efficient delivery into bacterial cells, the study successfully achieves the conjugation of a cell-penetrating peptide with the oligonucleotide.

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Utilizing U-Shaped Mobility Analyzer (UMA) for High Performance Dust-Mite Allergen Der p2 Quality Control

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Recombinant expression has been routinely used for biotherapeutic development. Therefore, analytical methods that can efficiently evaluate the quality of the products, especially the higher-order structure (HOS) to ensure its correct folding, are critical for its downstream safety and efficacy. Dust-mite allergen Der p 2 is a clinically important immunotherapy hypoallergen vaccine candidate. It has three disulfide bonds and is expressed as inclusion bodies in E. coli, thus protein refolding is required to obtain correctly folded protein for its production. Using U-shaped mobility analyzer (UMA) on a Q-TOF instrument, we developed a rapid ion mobility (IMS) based approach that can evaluate the quality of Der p2 expressed under different conditions at a precise and accurate manner.

Methods

Recombinant Der p2 was expressed in E.coli with (rDer p2-E1) and without its native ligand cholesterol (rDer p2-E2), and in Yeast (rDer p2-Y), respectively. Native MS, IMS-MS, collision-induced unfolding (CIU) data were acquired on both Waters synapt G2 si Q-TOF and Shimadzu Q-TOF equipped with UMA. IgE was titrated to rDer p2 samples to identify the correct rDer p2 conformer. LC-MS/MS was acquired for rDer p2 digested under native condition to evaluate the existence of mispaired disulfide bonds.

Novel Aspect

High resolution U-shaped mobility analyzer enables the rapid HOS quality control of the recombinant dust-mite allergen Der p2

Preliminary Data or Plenary Speaker Abstract

rDer p2 samples were firstly examined on Synapt G2 si Q-TOF instrument, the CIU results revealed that, for the 8+ charge state, Yeast Der p2 ions didn’t experience folding transition until a higher collision energy above 60 eV; while apart from the stable conformer as appeared in Yeast Der p2 spectrum, a folding transition appeared at a collision energy of 30 eV for both of the rDer p2 expressed in E.coli, suggesting the existence of misfolded rDer p2. But the percentages of misfolded rDer p2 present in the two E.coli expressed samples are different. As the conformers were not well separated and interrupt the downstream quality control, we then shifted to the U-shaped mobility analyzer (UMA) of higher separation resolution. UMA separation shows that there are 3 conformers for the 8+ E.coli Der p2, the abundance of conformer 2 drops gradually with the titration of Der p2 specific antibody, IgE. Additionally, as the UMA device has multiple channels for mobility separation or collisional activation, collision induced dissociation (CID) following conformer selection may further facility the characterization of correct and misfolded rDer p2 conformers. Nevertheless, LC-MS/MS analyses of rDer p2 digested under native condition disclosed that there are mispaired disulfide bonds in both of the E.coli expressed rDer p2 but at a different extend. Additionally, although the binding of cholesterol to rDer p2 was not observed under native MS condition, it appears to facility the correct folding of rDer p2.

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Analysis of High-order Protein Complexes Using Native Mass Spectrometry and Hydrogen/Deuterium Exchange Mass Spectrometry – Fresh vs Commercial Haemoglobin Samples

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Native MS analysis of protein complexes is highly susceptible to matrix effect, and addressing this predicament using buffer exchange is a common approach. Using haemoglobin (Hb) as the model entity, it was discovered that native mass spectrum of protein assembly is highly dependent on the buffer exchange protocol. This work attempts to use HDX-MS for comparative studies of haemoglobin complexes in untreated fresh and commercial samples. The information obtained from HDX study was found to correlate well with the native mass spectrometry analysis of the properly buffer-exchanged Hb samples. Both native MS and HDX-MS showed that the fresh Hb sample has retained the expected tetrameric structure, whereas the commercial Hb has largely been denatured to the dimeric form.

Methods

Commercial human Hb was procured from Sigma-Aldrich, whereas fresh Hb was extracted directly from donated blood samples. The fresh Hb extraction protocol includes the separation of red blood cells from plasma, the lysis of red blood cells to release Hb, and separation of Hb from the red blood cells lysate. Prior to native MS study, the two Hb sources were repetitively buffer-exchanged with 100 mM ammonium acetate using centrifugal ultrafiltration. HDX-MS analysis was conducted using untreated Hb samples. Labelling was conducted using D2O. The labelled protein was digested using the water-in-oil droplet approach to alleviate undesirable D/H back exchange. The labelled peptides in the water-in-oil droplet were analysed using liquid microjunction-surface sampling probe (LMJ-SSP) mass spectrometry.

Novel Aspect

Native-MS and HDX-MS are complimentary techniques for the study of the integrity of the high-order protein complexes.

Preliminary Data or Plenary Speaker Abstract

Direct ESI-MS analysis of Hb samples prepared using physiological buffer solution did not generate useful native mass spectra. To alleviate the matrix effect, multiple cycles of buffer exchange with 100 mM ammonium acetate were performed using centrifugal untrafiltration. The ESI-MS spectrum of Hb was found to vary significantly with the number of ultrafiltration cycles. ESI mass spectrum of fresh Hb after five rounds of ultrafiltration showed a base peak corresponding to the tetrameric Hb; whereas that of commercial Hb after four rounds of ultrafiltration showed a small signal of tetrameric Hb with a base peak of dimeric Hb. Extra rounds of ultrafiltration did not increase the abundance of the tetrameric Hb in both cases. The ESI-MS results indicated that the native tetrameric Hb structure is well-preserved in the fresh Hb, and is largely dissociated to dimer in commercial Hb samples.

In a comparative HDX-MS of untreated fresh and commercial Hb samples, several digested fragments exhibited significant differences in the deuterium uptake levels. In all cases, the digested fragments of commercial Hb sample show higher deuterium uptakes than that of the fresh Hb sample. Correlating the positions of these fragments with the crystal structure, the commercial Hb exhibited a significantly higher deuteration at the α1β2 interface than that of fresh Hb. This implies that a sizeable portion of tetrameric Hb complexes has been dissociated along the α1β2 interface to form

the α1β2 dimers in the commercial Hb sample. Results obtained in HDX-MS are found to correlate well with that of native mass spectrometry of Hb samples after proper sample purification.

Both the native-MS and HDX-MS data supported the denaturation of commercial Hb, thereby stressing the necessity to validate the integrity of the high-order structures of the proteins prior to the use of the protein samples for other biomedical studies.

Investigating the polymerization process during ethyl-cyanoacrylate fuming by ESI-MS/MS: the role of solvents employed for sample preparation

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Cyanoacrylate fuming is a widely used forensic process for the development of latent fingerprints deposited on non-porous surfaces. In this process, questioned samples are exposed to gaseous cyanoacrylate in an enclosed chamber of controlled humidity and a white polymer rapidly forms on fingerprint ridges, making them visible to the eye. The mechanistic details of ethyl-cyanoacrylate (ECA) polymerization during this process is still debated. In particular, it is still unclear whether the nucleophile initiating the polymerization reaction acts as a catalyst or is integrated as one termination of the final chains. This question could typically be addressed using tandem mass spectrometry (MS/MS) experiments for end-group analysis but first requires dissociation rules to be established for PECA polymer.

Methods

Drops of pure water were deposited on an aluminum plate and subjected to cyanoacrylate fuming using the MVC® Lite. PECA thin films were then left to dry for various controlled times before being scraped from the surface as powders. These samples were then dissolved in different solvents (either pure or in binary mixtures) supplemented with various salts for ESI-MS(/MS) analysis using a high resolution Q-TOF instrument (Waters Synapt G2). Detailed inventory of polymeric distributions observed in the MS mode was performed by Kendrick mass defect (KMD) analysis using the Kendo software. Samples were also analyzed by 1H and 13C NMR.

Novel Aspect

Dissociation rules of oligomers formed upon ethyl-cyanoacrylate fuming for MS/MS analysis of chain terminations

Preliminary Data or Plenary Speaker Abstract

ESI mass spectra of PECA samples exhibit different distributions of peaks spaced by 125.0477 Da, as typically expected for chains composed of the C6H7NO2 ECA repeating units. Detected species are either protonated molecules or cationic adducts in the positive ion mode and some species could also be deprotonated in the negative ion mode. Oligomer signals are measured in the 100-2500 m/z range but their chain length is observed to increase with the volume of the fumed water droplet. This shows that the polymerization chemistry does not stop when samples are removed from the fuming chamber but is still going on during the drying step, which implies that free ECA monomers are still available within the solid thin film. Moreover, five different PECA polymers (named A-E) were observed as a function of experimental conditions implemented for sample preparation. These results suggest that the solvent and/or cationizing agents employed to solubilize solid samples and promote oligomer ionization would also act as nucleophiles towards residual ECA monomers, hence producing new oligomers with different chain ends. In other words, sample composition is affected by sample preparation due to the high reactivity of ECA. The first step towards defining most inert conditions for sample preparation so as to detect original chains formed during ECA fuming was then to rationalize relationships between ESI conditions and MS detected polymers. To do so, collision-induced dissociation (CID) experiments were performed to establish fragmentation rules of PECA, using accurate mass measurements for reliable fragment assignment. In the negative mode, H-ended oligomers ionized as deprotonated molecules are observed to undergo an unzipping process, the total number of released units enabling determination of the polymerization degree as an additional evidence to support end-group findings. On-going works investigate how adducted cations influence CID of PECA oligomers in the positive mode.

Caerin 1.9-polycaprolactone-coated fully annealed AZ31 implants enhancing antibacterial performance and reducing foreign body responses in Sprague-Dawley rats

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Magnesium (Mg) alloys exhibit significant potential for degradable implants in hard tissue engineering, yet their clinical use is hindered by rapid corrosion and associated mechanical property reduction. Addressing these challenges and aiming to enhance antibacterial properties, we developed a three-dimensional coating using polycaprolactone and caerin 1.9 on three Mg specimens—pure Mg, cold-extruded AZ31, and fully annealed A AZ31. Our comprehensive investigation involves in vitro and in vivo assessments, including the introduction of methicillin-resistant Staphylococcus aureus at implantation sites in a rat model, employing multi-omic techniques for detailed analysis.

Methods

The Mg alloy specimens underwent thorough characterization using various techniques, including EBSD mapping, microhardness testing, uniaxial tensile testing, FTIR tests, and water contact angle tests. To assess their in vitro and in vivo behaviors, a range of analyses such as mitochondrial membrane potential (ΔΨm) assay, SEM-EDS analysis, computerized tomography imaging, and cytokine ELISA were conducted. Furthermore, quantitative proteomic and metabolomic approaches were employed to compare the acute and long-term responses of rats to the implants.

Novel Aspect

The higher percentage of first-order pyramidal slip in 3A may potentially contributed to its outstanding performance.

Preliminary Data or Plenary Speaker Abstract

The outcomes demonstrate that all coated Mg specimens exhibited enhanced corrosion resistance, notable antibacterial efficacy both in vitro and in vivo, and increased biocompatibility with minimal impact on crucial organs and foreign body reactions. Particularly noteworthy was the exceptional in vivo performance of the polycaprolactone and caerin 1.9-coated fully annealed AZ31 (referred to as 3A-PCL-F3), which showcased heightened immune response activation during the acute phase (within three days after implantation) of bacterial infection and optimal biocompatibility throughout the chronic phase (within three months after implantation) in a rat model. Proteomic analysis unveiled that the annealed AZ31 alloy stimulated the proliferation of mice osteoblast precursor cells, upregulated the expression of Brpf1 protein, and triggered other signaling pathways related to bone mineralization and hemostasis, promoting bone tissue formation. Proteomics and metabolomics analyses of tissues in direct contact with implants revealed that F3-PCL-3A did not activate inflammation or immune-associated signaling pathways in SD rats three months post-implantation. Meanwhile, it activated inflammatory responses, especially phagocytosis pathways up to 72 hours

post-implantation, indicating enhanced antibacterial capability during the acute stage after implantation. These findings underscore the significant potential of 3A-PCL-F3 as a degradable biomaterial for medical implants.

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Investigation of discrimination between recycled and virgin PET fiber using MALDI-TOF MS

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Due to recent strong social demands for sustainable growth, recycling of polymer adopted in consumable products is one of the significant issues. Polyethylene terephthalate (PET) is one such consumable polymer, which is already regularly recycled, and reproduced into many products, such as bottles, and clothing fibers. As this circular economy of PET recycling increases, there is a growing need for proper analytical methods to confirm the correct labelling of recycled fibers. Whereas many analytical studies of PET oligomers have been reported, a practical capability to discriminate recycled PET from virgin one is still unclear. Here we report our investigation using MALDI-TOF MS and statistical analysis to discriminate recycled PET fibers.

Methods

All fabric samples were provided from Boken (Osaka, Japan). More than 20 samples, including four virgin PET fabrics, were subjected to the analysis. The dissolution/reprecipitation method was applied to an extraction of oligomers from the PET in the fabrics. Briefly, the fabric was dissolved in HFIP/CHCl3, then the solution was carefully added to poor solvent, and finally a white precipitation was removed using syringe filter. An extracted oligomer solution was immediately mixed with Dithranol and NaI. All MS analysis were performed on a MALDI-8020 (Shimadzu Corporation) in positive ion mode. MS intensities of the oligomers were extracted from exported peak-list files using an in-house Excel macro, then subjected to a statistical analysis (eMSTAT Solution™, Shimadzu Corporation).

Novel Aspect

Analysis using MALDI-TOF MS and statistical analysis is a practical method to discriminate recycled PET fibers from the virgin one.

Preliminary Data or Plenary Speaker Abstract

PET oligomer has been widely studied in terms of chemical reaction, production, and food contamination. The relative content of oligomers is known to vary with reaction conditions. For the discrimination of recycled PET, an attempt of fingerprinting bottle-grade PET using MALDI-TOF MS has been previously reported[1]. Generally, the total amount of these oligomers is several % in the whole polymer. Cyclic oligomer is a major component in the overall oligomers, and linear oligomer and oligomers attached with diethylene-glycol (DEG) are minor ones. Although MALDI-TOF MS is highly sensitive for oligomer detection, a high reproducibility of MS signal intensity is required to determine the oligomer contents. After pre-treatment using the described method, the oligomers were observed with high enough reproducibility to allow the discrimination using MALDI-TOF MS. We found subtle changes in the intensity of DEGs and linear oligomers in some recycled fabrics compared with virgin fabrics, suggesting that the signal intensity of minor oligomers is a potential marker for discriminating virgin from recycled PET polymers. Data on more than 20 samples were accumulated for multivariate analysis. All data were successfully categorized into two groups, namely virgin and recycled. Whereas a score plot of virgin fabrics indicated a narrow dispersion, that of the recycled samples had a wide dispersion, which is possibly caused by various recycling processes. Notably, our method was also applicable to some blended fabric, consisting of PET and other polyesters.

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Assessing the effects of a snake venom in the lungs, in a murine model: label-free-quantitative proteomics employing DDA and DIA.

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Snakebite envenoming, a potentially life-threatening condition caused by venom injection, is classified as a neglected tropical disease by the WHO and predominantly occurs in impoverished countries. In Brazil, Bothrops snakes account for approximately 90% of human accidents. This study investigates the venom impact on the mouse lungs using label-free proteomics. Filter Aided Sample Preparation (FASP) or Single-pot, solid-phase-enhanced sample preparation (SP3), was employed for efficient protein digestion and recovery. Mass spectrometry analyses, employing Data Dependent Acquisition (DDA) or Data Independent Acquisition (DIA), captured individual peptides and provided comprehensive proteome sampling. Following these methodologies, an attempt was made to identify a combination of methods that yielded optimal results for their potential synergy in enhancing protein recovery and proteome coverage.

Methods

Bothrops jararaca venom was injected in the thigh muscle of mice (1.6 mg/kg), mimicking a snakebite, and anti-bothropic antivenom was injected 1 h later (i.v. tail). After 3 h and 24 h the lungs were recovered for evaluation. Proteomic analyses were carried out using FASP or SP3 trypsinization protocols followed by LC-MS/MS using DDA or DIA. FASP involved protein aliquoting onto filters, while SP3 employed beads for sample preparation. LC-MS/MS employed a Vanquish Neo/Orbitrap Exploris 480 system. DDA and DIA modes were employed, with subsequent bioinformatic analysis conducted using FragPipe for DDA and DIA-NN 1.8.2 for DIA. R Studio facilitated data processing, normalization, and visualization. This comprehensive approach enabled an in-depth exploration of venom-induced alterations in the lungs.

Novel Aspect

The findings underscore mouse response to a protease-rich venom at the molecular level helping to better understand venom-induced pulmonary toxicity.

Preliminary Data or Plenary Speaker Abstract

Comparisons between two techniques for sample preparation (FASP and SP3) for LC-MS/MS and different approaches for data acquisition revealed that DIA consistently outperformed DDA regarding protein identification and quantification.
Notably, the use of SP3 further enhanced the sensitivity and coverage of proteomic analyses, as particularly evidenced in DIA mode, by which over 8,000 proteins were identified in all samples whereas 5,250 proteins were quantified, showcasing the superior capability of SP3 and DIA-MS in capturing a comprehensive snapshot of the proteomic landscape, comprised of 98% of tissue proteins and 2% of secreted proteins. Despite the increased sensitivity and coverage provided by DIA and SP3 in surveying tissue composition no significant differences in protein abundance were observed between treated and control groups after 3 h and 24 h of venom (1,6 mg.kg - 1) or saline injection, indicating the stability of the lung proteome under the experimental conditions of this study. Nevertheless, the results underscore the robustness and reliability of the proteomic approach, as evidenced by the consistent identification of proteins across different methods of analysis.

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Labelling and chemical composition of contraband electronic cigarettes: Analysis of products from Australia

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Disposable electronic cigarettes (e-cigarettes or vapes) popularity has dramatically increased in Australia since they appear on the market in Australia around 2019 particularly amongst the younger population with vaping rate in Australia tripling in the last 5 years. The sale of nicotine-containing e-cigarettes is prescription only in Australia, regulated under the TGO110. Australian e-cigarette users, however, are purchasing e-cigarette products outside of the intended pathways. Despite regulations restricting the sale of nicotine containing e-cigarettes, these devices are still available illegally from a wide variety of over-the-counter retailers.

Methods

The labelling of e-cigarette packaging (N=388 boxes) and the chemical composition of disposable e-cigarettes and pods (N=428) were analysed for adherence to the current Australian regulations. These samples were obtained from over-the-counter retailers in NSW. Identification and quantification of the chemical content was carried out in a single gas chromatography-mass spectrometry method for the targeted quantification of 34 common flavouring chemicals, nicotine, 2 synthetic coolants, adducts formed in situ (compared against laboratory synthesised standards) and 8 chemicals currently prohibited by the current Therapeutic Goods Administration Regulation for Therapeutic Vaping Goods (TGO110). Other non-targeted compounds were tentatively identified by comparison against the National Institute of Standards and Technology (NIST17).

Novel Aspect

This study employed a single method for the simultaneous quantification of 48 compounds in contraband Australian vaping samples.

Preliminary Data or Plenary Speaker Abstract

Following the announcement of the prescription only model for nicotine-containing e-cigarettes in Australia in mid-2021 there was a clear shift in the labelling of products. Any mention of the word ‘nicotine’ was removed from e-cigarette packaging by early 2022 and nicotine warnings were replaced with generic underage sale warnings. Despite this labelling the vast majority (98.8%) of devices analysed contained nicotine, most (89%) at high concentration (>30 mg/mL) and 4.2% contained at least one chemical prohibited by the TGO110. Manufacturers have purposely removed any mention of nicotine from the original e-cigarette packaging to circumvent Australian regulations and continue their sale. The packaging of disposable e-cigarette products in Australia is generally not indicative of their contents, particularly nicotine, and most did not display required warnings. Ingredients with associated health risks, prohibited in legal vapes by the TGO110, were found in samples. Consequently, the risks of e-cigarette use cannot be appropriately identified from the information supplied on the packaging or device.

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LDI-TOF MS signal amplification for multiplexed exosome detection using antibody-presenting gold nanoparticles

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Exosomes, a subset of extracellular vesicles, hold immense potential as biomarkers for early disease detections, particularly in cancer diagnosis. In this study, we present a novel approach utilizing gold nanoparticles (AuNPs) functionalized with mass tags for signal amplification (Am-tags) and LDI-TOF MS, which afforded precise and sensitive exosome protein detection. The target exosomes were captured by specific antibodies both on AuNPs and a biochip, where the AuNPs contained highly excess Am-tags. LDI-TOF MS analysis then allowed the mass signal of Am-tags indicating the presence of target exosomes. In this way, a target signal was amplified by observing a large number of Am-tags resulting in enhanced sensitivity. In addition, Am-tags with isotope label were utilized for multiplexed analysis of exosome proteins.

Methods

We prepared stable antibody-presenting AuNPs with highly excess Am-tag molecules, called Ab/Am-tag@AuNPs. The synthetic process involved creating Am-tag molecules with various molecular weights via isotope labelling. Antibodies were immobilized to the AuNPs and biochips by using EDC/NHS coupling reaction between carboxyl groups on the surface and amine groups of antibodies. Exosomes were isolated from three mammalian cell lines, NIH3T3, HeLa, and MCF7, which then were characterized with nanoparticle tracking analysis (NTA). The selective capture of exosomes was achieved by both antibodies on the biochip and Ab/Am-tag@AuNPs by way of sandwich assay. The presence of exosome biomarkers was confirmed by the amplified mass signal of Am-tag on the AuNPs using organic matrix-free LDI-TOF MS, allowing accurate quantification of exosome protein abundance.

Novel Aspect

Simultaneous multi-quantitative detection of exosome biomarkers was enabled through mass signal amplification strategy.

Preliminary Data or Plenary Speaker Abstract

We focused on two primary criteria to enhance the accuracy of exosome detection with Ab/Am-tag@AuNPs: maintaining a stability and high Am-tag signals of the AuNPs. We first optimized the protocol to prepare stable Ab/Am-tag@AuNPs including concentration and ratio of thiol molecules to functionalize AuNPs, solvents for the EDC/NHS reaction, and the amount of antibodies of conjugating to AuNPs using LDI-TOF MS analysis. NTA was utilized to characterize isolated exosomes in terms of size and concentration. The mean size and total concentration of exosomes were found to be 99.0 nm and 3.10 E+11 particles/mL for NIH3T3, 105.6 nm and 1.13E+11 particles/mL for HeLa, and 134.1 nm and 4.47E+10 particles/mL for MCF7. We next evaluated the capability of our method to detect exosomes isolated from three cell lines using anti-RAB5 immobilized gold chip and anti-CD63/Am-tag@AuNPs, affording Am-tag mass signals with high intensity compared to the control of lacking antibody on the AuNPs. The calibration curves for three cell lines were constructed through linear regression on sum of signal intensities of Am-tag against concentration of exosomes, showing linear relationship with excellent limit of detections. Additionally, we quantified exosome proteins and confirmed their specificity with the use of different Am-tag and antibody combinations. Finally, we emphasized the versatility of our method in multiple quantitative detections of exosome proteins, CD63 and MUC1, using two kinds of Am-tags. LDI-TOF MS revealed the presence of CD63 in all three cell lines and MUC1 was identified in the cancer cells HeLa and MCF7. Furthermore, HeLa exhibited higher CD63 expression, while MCF7 cells showed higher MUC1 expression which were well consistent with the results of other reports. Our findings clearly indicate the potential of Ab/Am-

tag@AuNPs as a sensitive and reliable approach for identifying cancer biomarkers in exosomes and offer insights into their utility in biomedical research and clinical applications.

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GC-MS: a key technique for studying microbial communities in soil

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Understanding soil microbial communities is crucial for comprehending ecosystem dynamics, nutrient cycling, and soil health. Traditional methods like MIDI-Sherlock, Plate Count, and previous GC-MS have limitations in terms of sensitivity, specificity, time consumption, and details about microbial diversity. The current proposed GC-MS methodology provides a powerful and innovative approach to investigating soil microbial communities. It allows for the determination of 67 phospholipid fatty acids (PLFAs) in a single analysis. The method suggests molecular masses of fatty acid fragments that can be utilised for quantifying commercial and non-commercial PLFAs. The proposed method offers high sensitivity, enabling accurate quantification of a wide range of PLFAs, and providing valuable insights into the structure and function of microbial communities.

Methods

Soil samples were collected from The University of Queensland, Gatton Campus, at depths of 0-10 cm (LP-Wood-1), 10-30 cm (LP-Wood-2), and 30-60 cm (LP-Wood-1). The samples were freeze-dried and ground into fine powder. Lipids were extracted using a citrate buffer, methanol, and chloroform mixture at pH 4.0. Phospholipids were isolated from neutral lipids and glycolipids using solid-phase extraction cartridges with eluents of chloroform, acetone, and methanol, respectively. The phospholipids were converted into fatty acid methyl esters and analyzed using a Shimadzu GCMS-QP2010. PLFAs were identified based on retention indices, molecular masses, key fragments, and mass spectrograms compared to previously available standards and archived in online databases, such as the National Institute of Standards and Technology (NIST) library.

Novel Aspect

The proposed method offers high sensitivity, enabling accurate quantification of a wide range of PLFAs.

Preliminary Data or Plenary Speaker Abstract

The GC-MS method was developed to identify 67 fatty acids, which was then applied to identify and quantify biomarkers in soil samples. The results revealed the identification of most biomarkers, suggesting the presence of diverse microbial groups in the soil samples. Furthermore, the concentrations of these biomarkers exhibited a significant decrease with increased soil depth, indicating a higher microbial population on the surface. The analysis also showed notable differences in the concentrations of PLFA signatures within the sample, reflecting the significant variation in differential growth of microbial populations at the same soil depth.

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Development of analytical method for 8 polycyclic aromatic hydrocarbons in herbal medicine using gas chromatography-tandem mass spectrometry

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Herbal medicines from both natural and cultivated sources play a very important role in health protection and disease control. Polycyclic aromatic hydrocarbons (PAHs) are formed during the imcomplete combustion of orgainc material such as organic material, and are known to be carcinogenic, mutagenic, and teratogenic. In the case of herbal medicines, PAHs may be generated during the frying, roasting, steaming and smoking processes because some processed herbal medicinal products require long-term cooking at high temperature. A gas chromatography coupled tandem mass spectrometry (GC-MS/MS) method is developed to determine 8 representative PAHs such as Benzo(a)pyrene (BaP), Benzo(a)anthracene (BaA), Chrysene (Chry), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Indeno(1,2,3-c,d)pyrene (IcdP), Dibenzo(a,h)anthracene (DiahA), and Benzo(g,h,i)perylene (BghiP) in herbal medicines.

Methods

The method offers high sensitivity and selectivity under selected reaction monitoring (SRM) mode to satisfy the requirements of both quantitation and qualititation.
The sample was extracted with hexane, and purified with the florisil solid phase extraction (SPE) cartridge. Chromatographic separation was performed on a DB-5MS column (30 m x 0.25 mm x 0.25 um), and quantification was carried out by a Shimadzu TQ8040NX tandem mass spectrometer.

Novel Aspect

This method can be applied to various herbal medicine for determination of trace of 8 PAHs.

Preliminary Data or Plenary Speaker Abstract

The method validation was performed based on the Association of Offical Agricultural Chemists (AOAC) international guideline and evaluated the matrix effect prior to conducting validation.
The results showed that the method has good leanearity (r2>0.995) in the range of 1-100 ng/mL, and the detection and quantitation limits for the studied PAHs were calculated 0.22-0.31 ng/g and 0.66-0.95 ng/g. The recovery rate of 8 PAHs was from 72.5-113.9%, with the relative standard deviation (RSD) of 0.1-11.2%.

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LC-MS/MS characterization of forced degradation products of Pretomanid: stability-indicating RP-HPLC method using quality by design (QBD) approach.

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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

Tuberculosis (TB) continues to pose a significant health risk, despite the existence of over 20 drugs specifically designed to treat the disease. The discovery of pretomanid (PMD) aims to address the issue of resistant isolates of M.tb and reduce the duration of treatment. PMD also known as PA-824, belongs to bicyclic nitroimidazoles. It is composed of nitroimidazole pyran A/B rings, an ether link, and a hydrophobic side chain. It is one of the components of the BPaL regimen approved by the U.S. FDA for the treatment of extensively drug-resistant (XDR) and treatment-intolerant or nonresponsive multidrug-resistant (MDR) TB. According to the ICH, it is recommended to subject the drug to various stress conditions such as hydrolysis, oxidation, thermal, and photolysis.

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Molecular diagnosis based on non-enzymatic isothermal amplification using photocleavable mass tags and LDI-TOF MS

SEOKHWAN JI[1]
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Poster Session: Thursday Posters, Exhibition Hall, August 22, 2024, 13:00 - 15:00

We describe a new molecular diagnostic strategy for detecting specific nucleic acid targets without the use of a conventional method based on polymerase chain reaction and fluorescence, which necessitates enzymes and thermal cycling with limited multiplexity. For signal amplification and specific target detection, we harnessed one of enzyme-free isothermal amplification methods, hybridization chain reaction (HCR) that is based on a chain reaction of hybridization between two sets of DNA hairpin species. For signal transduction, hairpin DNA-photocleavable mass tag (PMT) conjugates were utilized instead of fluorescence. The presence of target DNA triggered HCR with two hairpin DNA-PMT conjugates, and PMT on hairpin DNA underwent photocleavage upon laser irradiation in LDI MS analysis, transducing biological target binding events to amplified mass signals.

Methods

In our strategy, trityl-based PMTs with mass-varying derivatives were prepared by simple synthetic protocols. The trityl group can facilitate cleavage resulting in the formation of a cationic species that is readily detectable in the MS analysis. The PMT molecule was conjugated to hairpin DNA, and amine functionalized loop DNA was tethered to acid-functionalized magnetic particles (MPs) via carbodiimide-mediated coupling reaction. To the resulting loop@MPs, a target DNA was treated followed by two hairpin DNA-PMT conjugates. After incubation and washing, the HCR product concatemers on MPs were thermally released. LDI MS analysis of the supernatant afforded mass signals of PMTs with high intensity indicating not only the presence of targets in the solution but also specific recognition and signal amplification.

Novel Aspect

Enzyme-free, isothermal, and non-fluorescent molecular diagnostic method was realized by mass signal amplification using photocleavable mass tags.

Preliminary Data or Plenary Speaker Abstract

As a model system, complementary DNA sequence of human immunodeficiency virus (HIV) was examined. We designed loop DNA and two sets of DNA hairpin species accordingly, and the progress of target specific HCR was validated with gel electrophoresis using these oligonucleotides. We first performed HIV target DNA detection using our strategy where the presence of the target revealed major peaks corresponding to PMT molecules on hairpin DNA with high intensities and S/N ratios compared to the control of lacking the target DNA. We next optimized the experimental processes to enhance the accuracy of specific target detection and to achieve highly amplified PMT signals at low target abundance in terms of concentration loop DNA for conjugation to MPs, ratio of hairpin DNA to loop DNA, incubation time for HCR, and a final reaction volume. Additionally, we assessed quantitative aspect of our strategy which showed that mass signal intensities increased linearly as the target concentration increased with excellent limit of detections. Finally, we emphasized the versatility of our method in multiple detections of target DNAs by utilizing various PMTs with different molecular weights which can be clearly distinguished in MS measurement. Additional two sets of loop DNA and hairpin DNA were designed for hepatitis B virus (HBV) and hepatitis C virus (HCV), and capability of simultaneous monitoring multiple analytes was validated using three kinds of PMTs. LDI-TOF MS clearly showed two and three MS signals for the simultaneous detection of two and three targets, respectively, corresponding to the PMT molecules that were conjugated to hairpin DNA complementary to designated targets. We strongly believe that our findings here demonstrate the potential of our strategy for nucleic acid diagnostics offering a more sensitive and reliable approach that is isothermal, enzyme-free, and non-fluorescent with multiplexing ability.

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Molecular diagnosis based on non-enzymatic isothermal amplification using photocleavable mass tags and LDI-TOF MS

SEOKHWAN JI[1]
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