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ORAL

001

FORENSIC PROVENANCING WITH ISOTOPE RATIO MS

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The natural environment as defined by geology, climate and ecology provides a wealth of chemical markers that are unevenly distributed on the earth's surface and thus provide a potential for spatial classification of natural materials and derived products. Forensic scientists have used individual markers for ad-hoc comparisons of evidence for specific cases but no forensically essential spatial reference sample-bases have been established, mainly due to financial constraints.

Recently Geographical Information Systems containing geological, geochemical and climatic data have become readily available and they provide new opportunities to predict the spatial distribution of natural chemical markers in a cost effective manner. Our research focusses on evaluating and validating the forensic potential of these and other spatial data in collaboration with colleagues around the world.

In theory, plants and animals are all related to regional soil and climatic conditions through the food (supply) chain and indeed regional foodwebs often show systematic relations between the isotopic profiles at different trophic levels and even humans. At the basis of every foodweb there is soil and water. On the basis of the isotopic composition of precipitation, combined with high resolution climate models, we have developed new hydrogen and oxygen isotope prediction models, that allow us to discriminate the geographical origin of natural products on a ca 300km scale. To improve the discrimination power in Europe we have used representative soil samples collected by the GEMAS consortium to create the first low resolution measured Sr isotope geochemical map of Europe. The measured data is being used to create a more comprehensive prediction map for the whole of Europe. The water and soil data, combined with systemic knowledge of the foodweb relations and isotopic fractionations, are now being used to make probabilistic predictions of the isotopic composition of regional flora and fauna. In the presentation we will give examples of how we validate and apply our models to support provenancing of food and unidentified human remains and discuss new analytical developments

002

GAS PHASE CHEMISTRY OF CYSTEINE RADICAL CATIONS.

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Electrospray ionization (ESI) of tryptophan gives rise to multiply charged, non-covalent tryptophan cluster anions, $[Trp_n\text{-}xH]^{x-}$ in a linear ion trap mass spectrometer, as confirmed by high resolution experiments performed on a Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer. The smallest

multiply charged clusters that can be formed in the linear ion trap as a function of charge state are: x = 2, n = 7; x = 3, n = 16; x = 4, n = 31. The fragmentation of the dianionic cluster $[Trp_9\text{-}2H]^{2-}$ was examined via low energy collision-induced dissociation (CID), UV photodissociation (UVPD) at 266 nm and electron-induced dissociation (EID) at electron energies ranging from > 0 to 30 eV. CID proceeds mostly via charge separation and evaporation of neutral tryptophan. The smallest doubly charged cluster that can be formed via evaporation of neutral tryptophans is $[Trp_7\text{-}2H]^{2-}$, consistent with the observation of this cluster in the ESI mass spectrum. UVPD gives singly charged tryptophan clusters ranging from n = 2 to n = 9. The latter ion arises from ejection of an electron to give the radical anion cluster, $[Trp_9\text{-}2H]^{-\cdot}$. The types of gas phase EID reactions observed are dependent on the energy of the electrons. Loss of neutral tryptophan is an important channel at lower energies, with the smallest doubly charged ion, $[Trp_7\text{-}2H]^{2-}$, being observed at 19.8 eV. Coulomb explosion starts to occur at 19.8 eV to form the singly charged cluster ions $[Trp_x\text{-}H]^-$ (x = 1-8) via highly asymmetric fission. At 21.8 eV a small amount of $[Trp_2\text{-}H\text{-}NH_3]^-$ is observed. Thus CID, UVPD and EID are complementary techniques to study the fragmentation reactions of cluster ions.

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003

SYNCHROTRON PIMS STUDY OF PHENYL AND METHYLPHENYL OXIDATION WITH ISOMER-SPECIFIC DETAIL

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Aromatic molecules make up a large portion of gasoline. In Australia, for example, aromatic species can comprise up to 45% v/v of domestic petrol (with benzene specifically limited to 1% v/v). Despite their significant abundance, a molecular-level understanding of the chemistry that renders these molecules useful as fuel-components is incomplete. Product branching fractions of aromatic radical oxidation are vital data in revealing the key mechanistic steps that underpin the ignition properties of these molecules. In this study we reveal the major room-temperature product channels of O2 reactions with phenyl (C6H5) and o-methylphenyl (C6H5CH3) radicals and attempt to reconcile the observed product distributions with reported potential energy surfaces. This undertaking employs synchrotron photoionisation mass spectrometry (PIMS) performed at the Chemical Dynamics Beamline of the Advanced Light Source (Berkeley, USA). Exploiting the tunable VUV radiation source, photoionisation efficiency curves (PIE) are constructed, thus enabling isomer-specific determination of detected products. The experiment also provides kinetic information of the formation of these product species. Phenyl and methylphenyl radicals are formed from laser photolysis of suitable precursors. In both cases, oxidation products are expected on more than one mass channel. For phenyl oxidation, some of the possible pathways are detected that correspond to H loss, HCO loss and CO2 loss from the peroxyl radical intermediate. For o-methylphenyl + O2, the presence of the methyl group provides an additional pathway for the peroxy radical intermediate to rearrange. This unique H-shift pathway, absent in the phenyl + O2 case, can lead to a QOOH-type intermediate that forms o-quinone methide via OH elimination. We detect this species in our experiments. For both reactions we discuss the likely chemical pathways to these products with reference to computationally derived potential energy surfaces.

004

PROTEIN DERIVATIZATION AS A METHOD FOR INCREASED COVERAGE IN ELECTRON TRANSFER DISSOCIATION SEQUENCING

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Introduction

Previous work on peptide derivatization has demonstrated the utility of this technique for increasing the sequence coverage obtained through electron transfer dissociation (ETD)[1,2]. The addition of histamine to acidic functional groups increases the overall positive charge of the peptides examined. Of note, peptides with disparate charge distribution benefited immensely from this technique.

The disparity of charge within peptides is amplified when analyzing whole proteins, leaving regions of protein with little charge. This hampers sequencing of proteins, particularly when we are further limited by the analyzer of choice. The 2,000 m/z upper limit of these analyzers rely on peptide fragments progressively reaching higher charge states to remain in the mass window. Thus, as we sequence further into the protein, the fragments require progressively more positively charged amino acids to increase the overall charge of the peptide. Where this is not the case, gaps are observed in protein sequence, until sections with positively-charged amino acids are reached. Accordingly, the application of protein derivatization, targeted at converting negatively-charged or neutral amino acids to positive charge-bearing amino acids, could enhance the sequence coverage obtained for proteins *via* ETD.

Results

In this study, we examine the combination of protein derivatization with ETD sequencing. To achieve this, we utilized a protein standard, ubiquitin; to develop our method. Once the parameters were optimized with ubiquitin, we extended our analysis to larger proteins.

Initial studies have shown only partial coverage of eIF-1 and the light chain of IgG. For eIF-1, we observe 117 distinct ions. Furthermore, we observe overlap of sequence ions from the N- and C- terminus, however, the coverage from both termini has many gaps. By comparison, the sequence ions observed for the light chain of IgG are all clustered between amino acids 94-133.

Preliminary results have demonstrated the successful derivatization of the sample proteins with histamine. In addition, we observe a shift in the average charge state proportionate to the addition of histamine to the target functional groups. We are currently analyzing and annotating the ETD spectra of these proteins, early results show increased sequence ions observed.

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005

A UNIQUE STABLE C AND H ISOTOPIC PROFILE OF NATIVE AUSTRALIAN PLANT LEAF *N*-ALKANES

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The distribution and stable carbon and hydrogen compositions of *n*-alkanes in higher plant leaf waxes are a valuable source of information for past growth environment (hence climate) reconstruction (Nabbefeld *et al*., 2010), photosynthetic mode discrimination (Chikaraishi *et al*., 2004) and ascertaining metabolic pathways (Zhou *et al*., 2010; Kroumova *et al*., 1994; Kroumova and Wagner, 1999).

Odd-carbon-numbered (OCN) *n*-alkanes are typically much more abundant than even-carbon-numbered (ECN) *n*-alkanes in higher plant leaves. Up to now, all plant species investigated showed that OCN-*n*-alkanes are relatively enriched in ^{13}C but depleted in ^{2}H than their neighbouring ECN-*n*-alkanes regardless of environmental conditions (Zhou *et al*., 2010; Kuhn *et al*., 2010). This led to Zhou *et al*. (2010) hypothesising that a common pyruvate precursor is involved in the biosynthesis of both OCN- and ECN-*n*-alkanes in higher plant leaves. Furthermore, a kinetic isotope effect (KIE) associated with competition for the common pyrvuate precursor pool was suggested to account for the observed odd-over-even enrichment of ^{13}C and depletion of ^{2}H.

However, in a recent attempt to characterise isotopic profile of biomarkers of native Australian plant species for potential (paleo)climatic reconstruction (Mackenna, 2009), only one species (*Melaleuca sp*, Figure 1) exhibited an isotopic profile consistent with the aforementioned profile. Several species showed the opposite pattern (odd-over-even depletion of ^{13}C) whilst the others showed no obvious isotopic pattern. The unique *n*-alkane C isotopic profile of Australia native plant species raises an interesting issue regarding the roles of the enzymes involved in leaf *n*-alkane synthesis and calls for a re-evaluation of the hypothesis of Zhou *et al*. (2010). Work is currently underway to expand our investigation of the C (and H) isotopic characteristics of native species.

006

TARGETED PROFILING OF PLANT HORMONES AND UNTARGETED METABOLIC PROFILING IN STRESSED TOMATO LEAVES USING A DUAL-PRESSURE LINEAR ION TRAP LC-MS SYSTEM

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The advance of LC-MS instrumentation has made it an increasingly popular tool for metabolic profiling, both targeted and non-targeted. Plant hormones such as Jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA) and ethylene play vital roles in the regulation of plant responses to environmental stimuli and stresses [1].

In this study, a Thermo Scientific LTQ Velos dual-pressure linear ion trap Mass Spectrometer and a Thermo Scientific Accela UHPLC system were used for simultaneously targeted profiling of the four plant hormones with Selection Reaction Monitoring (SRM) and non-targeted profiling with full scan of the potential components that respond to the stress. In SRM mode, the temporal abundance changes of JA, SA, ABA and 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor to ethylene, were determined under two stress situations during a 16 hr time course study: wounded leaves ("Wound") and methyl jasmonate (MeJA) pre-treated wound (MeJA+Wound). In non-targeted profiling, the full scan chromatographic TIC are subjected to alignment and peak identification, followed by the Principle Component Analysis (PCA) for differentiation analysis.

Results: The targeted profiling analysis found changes of ABA and JA in stress-induced leaves. The MeJA pre-treated stress leaves shows 20x fold increase of JA at 2hr compared to the control and untreated stress samples. The JA levels decreased over the time, but still remained 7-fold higher at 16 hr. The PCA analysis of full scan TIC traces from the untargeted profiling analysis illustrated clear patterns to allow the differentiation of various sample groups, including control vs. stress, wound vs. MeJA pre-treated wound, and at different time points within the each group. The combination of Thermo Scientific SIEVE^{TM} and PCA provides a powerful tool in analyzing the complex full scan MS traces to differentiate samples of different stress groups and at different time points.

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007

UTILIZATION OF AMBIENT OZONE AND DESORPTION ELECTROSPRAY IONIZATION MASS SPECTROMETRY FOR DETERMINING DOUBLE BOND POSITIONS IN UNSATURATED LIPIDS

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Previously our group has reported the reaction of ozone with ionized lipids within the confines of an ion-trap mass spectrometer for the elucidation of double bond positions [1]. While this has proven to be a powerful analytical approach it requires an expensive high-concentration ozone generator and a specifically modified mass spectrometer. Interestingly, ambient ozone - present in the troposphere within urban environments - has previously been identified as a source of oxidation when samples are left in the ambient laboratory environment [2] . In this study the surface analysis technique Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) is used to investigate the reaction of ambient ozone with unsaturated phospholipids deposited onto Teflon and silica thin-layer chromatography TLC plates. Products originating from the ozonolysis of the deposited lipids can be observed after only 5 minutes following deposition and increase in abundance the longer the sample is exposed to the ambient environment prior to analysis. The masses of the ozonolysis products allow the position of double bond(s) with the parent lipid to be assigned. Differences in the products formed upon ozonolysis on PTFE and silica substrates are observed, most significant is the observation of an abundant methanol adduct of the aldehyde(s) formed upon ozone induced cleavage of a double bond that is observed from the silica TLC plates but not the PTFE substrate. This technique is applied to the analysis of a human lens lipid extract where the individual lipids are first separated by TLC and distribution of lipids is analysed by directly imaging the TLC plate by DESI-MS. Along with a number of individual lipid classes being detected, ions corresponding to n-5, n-7 and n-9 double bonds in unsaturated lipids are observed and are consistent with previous studies [1, 3]. This work describes the powerful combination of DESI-MS and TLC in allowing both the lipid composition and double bond positions within lipids to be rapidly investigated simply by exposing the sample to the ambient laboratory environment prior to analysis, thus negating the need for expensive ozone generators.

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008

USING ION MOBILITY SPECTROMETRY - MASS SPECTROMETRY TO UNRAVEL BIOMOLECULAR ASSEMBLY PATHWAYS

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In vivo the majority of proteins function in non-covalently bound macromolecular complexes rather than alone. Unravelling the assembly pathways of these complexes is important for understanding how proteins interact with other proteins and ligands, and also, in cases where complexes are associated with disease, for devising means of assembly inhibition. Here, the assembly pathways of two macromolecular complexes have been probed using ESI-IMS-MS. ESI-IMS-MS offers the unique capability of separating ions derived from co-populated protein conformers or oligomeric species which give rise to the same m/z ratios, as well as allowing the cross-sectional area (Ω) of ions to be measured.

Amyloidosis is caused by the self-aggregation of certain proteins into amyloid fibrils *in vivo*. Key to elucidating this mechanism is the definition of assembly intermediates, some of which are known to be amyloid culprits. Monitoring amyloid fibril formation from beta-2-microglobulin, ESI-IMS-MS has been used to study individual species within transient, heterogeneous protein ensembles. Changes in the oligomers present in the early stages of amyloid fibril formation have been monitored as fibril assembly proceeds.

Mapping viral capsid pathways is key to the development of anti-viral therapeutics. Non-covalently bound protein-nucleotide intermediates have been characterised in real-time during the assembly of the MS2 bacteriophage capsid from its coat protein. The stoichiometry of these species was determined by mass measurements and by using different nucleotides for the assembly process. Modelling, in conjunction with cross-section measurements, has provided new structural insights into virus assembly intermediates and competing pathways.

009

THE USE OF FIELD ASYMMETRIC ION MOBILITY SPECTROMETRY TO IMPROVE THE UNDERSTANDING OF FRUCTAN BIOSYNTHESIS

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In the recent past there has been an increasing interest in measuring the levels of oligosaccharides in grasses. This is due to their potential use as fed stocks for the production of bio-fuels, as well as the resulting reduction of pollutants such as greenhouse gases that has been observed when animals are feed with high sugar grasses.

The monitoring of these oligosaccharides has been hampered by the fact that they can occur in groups of isomers and to gain a fuller understanding of the biosynthesis it is important to know which particular isomers are changing and how. There have been several reports in the literature that show separations of smaller oligomer isomers. In this paper we present our work with ryegrass fructans and show how Field Asymmetric Ion Mobility Spectrometry (FAIMS) may be useful in helping to dissect the complex patterns of isomers.

010

THE SIGNIFICANCE OF PERYLENEQUINONES AND THEIR DIAGENETIC ALTERATION PRODUCTS IN DEVONIAN REEFS (CANNING BASIN, WA)

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Distributions of unusual compounds including low-molecular-weight A-*nor*-steranes - potential novel sponge biomarkers originating from stromatoporoids - and relatively high abundances of the polycyclic aromatic hydrocarbon (PAH) perylene and other structurally related aromatic compounds have been detected in Devonian samples from ancient reefs of the Canning Basin, Western Australia. Perylene has been frequently reported in a variety of Mesozoic and Cenozoic sediments, but it has only occasionally been found in Paleozoic samples [1] . Despite a wide occurrence, its origin in sediments remains unclear. A combustion source, which is the origin of most unsubstituted PAHs, can be excluded since abundance and occurrence of perylene in sediments usually differ from those of pyrogenic PAHs [2, 3] . Perylenequinone pigments present in a variety of extant organisms including wood-degrading fungi, crinoids, plants and insects have been suggested as potential precursors on the basis of structural similarities [1, 3] . The aromatic quinone-type compounds identified in the Devonian Canning Basin samples could represent intermediate products in a formation pathway to perylene. A similar suite of compounds along with perylene was also detected in Cretaceous samples from the Lower Indus Basin, Pakistan. The distributions of these compounds and other biomarkers (including the series of A-*nor*-steranes) are being analysed for evidence of environmental changes preceding the Late Devonian mass extinction, which significantly affected reef-building organism such as stromatoporoids. Furthermore, catalytic hydropyrolysis (HyPy) will be performed on extant sclerosponges to investigate their sterol composition and to search for novel highly specific biomarkers potentially found in the stromatoporoid-rich Canning Basin samples. Whereas various studies have revealed a great diversity of sterol structures within the class Demospongea [4, 5] , the sterol compositions of sclerosponges have not been robustly investigated. In future studies we also hope to

examine the biomarker composition of additional samples from the Canning Basin to span the Givetian-Frasnian boundary.

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011

THE APPLICATION OF ICP-MS FOR THE DETERMINATION OF COUNTRY OF ORIGIN OF SELECTED FOODSTUFFS

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Fraudulent activities such as the intentional counterfeiting, substitution, adulteration or mislabelling of food to gain an economic advantage are major issues facing the food industry internationally. This is especially true in Eastern Europe, Asia and South America where cheap alternatives and labour mean that counterfeiting or substitution of high quality or brand name commodities can be highly profitable. However, this behaviour is not confined to these regions, as substitution of cheap foreign produce has recently been reported on sale as premium Australian produce in supermarkets in Britain and Singapore. In addition the recent introduction and possible mislabeling of Chinese apples as Australian produce has caused significant concern in Australia. In 2008 the Organisation of Economic Cooperation and Development report on piracy and counterfeiting estimated that counterfeit foods and beverages represent anywhere from €2.6 – 13.9 billion in losses per year. The provenance identification of selected types of food and drink products using data from both solution and laser ablation based ICP-MS analysis of pork, wine, tea, coffee and olive oil will be discussed. Quantitative determination of up to 55 elements has been undertaken using solution based ICP-MS while counts per second data for 49 elements were used for the direct analysis of olive oil and coffee beans samples using LA-ICP-MS.

Additional information, to establish higher resolution in provenance determination especially for tea and coffee (plantation of origin), has been developed using data for light stable isotope distribution patterns (2H (D), 13C and 15N). Results from this research indicated that it was possible not only to establish country of origin of the study materials but in some cases to improve resolution of provenance to state of origin (wine, pork and olive oil), and even plantation of origin (tea and coffee). In addition, case studies involving the mislabeling of Australian wines will also be detailed.

012

INVESTIGATING ACTIVE SITE MIMICS OF C–H BOND ACTIVATING ENZYMES IN THE GAS PHASE

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The selective and efficient activation of C–H bonds, including the direct oxidation of gaseous methane to liquid methanol, has been considered one of the “holy grails” of chemistry largely because of the intense interest in developing alternative energy sources and greener industrial routes for the production of organic molecules from abundant natural gas resources. One of the key challenges of C–H bond activation is to selectively functionalize alkyl groups without over-oxidizing the reactant or forming other undesired products as a result side reactions with more reactive functional groups. Despite such obstacles, biological enzymes, such as methane monooxygenase and ribonuclease reductase, which both contain a diiron, dioxo bridged active site, and

cytochrome P450 enzymes, containing a heme-bound high valent Fe=O active site, can readily activate and functionalize C–H bonds. However, because of the large size of such enzymes, obtaining detailed and definitive information about the mechanism of substrate oxidation at the iron-oxide active site of such enzymes proves difficult. Here, we report on the reactivity between gaseous enzyme iron-oxide based active site mimics towards various alkanes and alkenes using mass spectrometry and theoretical methods in order to gain a better understanding of the mechanism of C–H bond activation by various biologically relevant iron oxide functional motifs.

013

INVESTIGATING A NEW RAT MODEL OF AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE USING NON-TARGETED METABOLOMICS

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Introduction : Of patients requiring kidney dialysis and/or transplantation, 5-8% suffer from autosomal recessive polycystic kidney disease (ARPKD). An estimated 50% of ARPKD sufferers progress to end stage renal disease (ESRD) by the age of 10 years. Metabolomics has emerged in light of recent technological advancements in the separation and identification of small low molecular weight molecules. There is no published evidence to suggest that metabolomics has been used to study ARPKD to date. Therefore, the purpose of this research was to utilise non-targeted metabolomics to study a rat model of the ARPKD phenotype. Methods: Blood and urine was collected from Lewis Polycystic Kidney (LPK) rats and age matched Lewis controls weekly from the age of 3 weeks. Metabolites were extracted, derivatised, and analysed using gas chromatography-mass spectrometry (GC-MS). Data files were processed using AnalyzerPro and statistical analysis used The Unscrambler®. Results and discussion: Recent metabolite profiles generated from the urine of 5-week old male LPK rats have revealed the presence of three metabolites which may be important in understanding disease progression. These metabolites, α-ketoglutarate, allantoin and uric acid, have been reported to be involved in hypertension [1] and renal/liver dysgenesis [2] . It is important to investigate the involvement of these and other metabolites at various stages of the disease to determine 1) their role and 2) possible intervention strategies. A 14-week temporal study, collecting urine and plasma samples, has been completed to investigate the generality of these findings and the samples are being analysed using additional mass spectrometry based techniques to increase the extent of the urinary metabolome measured.

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014

HIGH SENSITIVITY GC-MS MEASUREMENT OF SUB-NANOMOLAR CONCENTRATIONS OF 2,6-DICHLOROPHENOL (2,6-DCP) IN WINE – TRACING THE SOURCE OF A FLAVOR AND AROMA TAINT.

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Bottles of Australian wines had been returned by consumers complaining of a ‘plastic' or ‘disinfectant like' taste and aroma. Following initial investigations by The Australian Wine Research Institute (AWRI), UNSW was contracted to independently confirm the presence of 2,6-DCP, determine its concentration and investigate its potential source. An aroma threshold for 2,6-DCP in wine of 37 ng/L had been established by AWRI [1,2].

Wine samples were spiked with 2,4-dichlorophenol-$^{13}C_6$, the internal standard. A novel solid phase extraction (SPE) technique, employing phenol-specific reversed phase cartridges with pentane elution, was used to selectively extract chlorophenols. Analytes were analysed as their pentaflourobenzyl (PFB) esters using a Thermo Trace DSQ II GC-MS, equipped with a 60 m x 0.25 mm J&W DB5-MS+DG column, operated in electron capture negative ionization (ECNI) mode. Chromatograms for m/z 161, 163, 167, 169, corresponding to the two most intense $[M\text{-}PFB]^-$ ions for dichlorophenol and dichlorophenol-$^{13}C_6$, were recorded in single ion monitoring (SIM) mode.

Linear calibration curves (R^2 = 0.9995) were obtained for 2,6-DCP over the range 1-500 ng/L. The detection limit ($y_B+3s_B-a)/b$ was 0.7 ng/L and LOQ ($y_B+10s_B-a)/b$ was 2.3 ng/L. Suspect wines showed 2,6-DCP levels between 2.3 and 367 ng/L (mean 177 ng/L). Control wines bought at random from local stores gave 2,6-DCP concentrations of 6-10 ng/L.

Analysis of solutions of tartaric acid (TA) used in production of the tainted wines showed negligible 2,6-DCP content. To investigate whether 2,6-DCP was formed during fermentation, suspect TA samples and control TA, were added to separate volumes of yeast-inoculated grape juice and fermented in the laboratory. The resultant fermentation products revealed 2,6-DCP concentrations at or above the aroma threshold for the suspect TA samples, and much lower levels for the control TA.

The experiments indicate that the suspect TA contains a ‘taint precursor' compound which is converted to 2,6-DCP during fermentation.

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015

A BRIEF MOMENT FOR A LONG TIME: MULTISTAGE MASS SPECTROMETRIC STUDIES OF GEOLOGICAL BIOMARKERS

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Structural elucidation of natural product-derived organic molecules isolated from sediments has been of crucial importance in enabling the interpretation of their structures and stereochemistry to facilitate our understanding of the nature of ancient environments and geological processes including oil generation and environmental change. Mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy have both played pivotal roles, commonly being applied in conjunction to solve the structures of novel molecules. With the advent of atmospheric pressure ionisation techniques and the consequent development of robust liquid chromatography-mass spectrometry (LC-MS) methods, it is now possible to monitor highly functionalised and labile biological markers at trace levels.

In order to facilitate the development of high-throughput approaches it is desirable to perform structure assignment without recourse to the time-consuming procedures of isolation and purification that are required for NMR. Tandem mass spectrometry (MS/MS) in conjunction with LC-MS has largely unrealised potential for structure assignment of geological age biological markers. Two cases demonstrate this potential:

the analysis of chlorophyll derivatives and the analysis of glyceroldialklyglycerol tetraether (GDGT) lipid cores of Archaea.

Ion trap mass spectrometers achieve tandem mass spectrometry by multistage mass spectrometry, whereby ions are fragmented sequentially. This approach gives a high degree of certainty in assignment of fragmentation pathways, thereby enabling structurally informative losses and product ions to be identified readily. The power of the LC-MS/MS approach will be illustrated through its application to reveal long-term changes in natural microbial communities in response to changing environmental pressures and to explore the structures and chemotaxonomic significance of GDGTs, including entirely novel structures, in microbes and in ancient sediments.

016

NANDROLONE INTERFERENCE IN QUANTIFYING PLASMA ESTRADIOL BY LC-MS/MS

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Background

Liquid chromatography tandem mass spectrometry (LC-MS/MS) has become the method of choice for sex steroid quantitation, largely due to its specificity with increased sensitivity now able to match immunoassays. We recently reported a highly sensitive method for the simultaneous quantitation of androgens (testosterone, T; dihydrotestosterone, DHT) and estrogens (estradiol, E2; estrone, E1) using atmospheric pressure photoionization(APPI). Nandrolone is a popular drug in sports doping and among androgen abusers. We observed interference with the measurement of E2 when present at supraphysiological levels. We now report a novel interference of nandrolone in quantification of E2.

Method and Results

Increasing amounts of nandrolone (0-100 nmol/L) were spiked into a 4% BSA solution (w/v) containing known concentrations of T, DHT, E2 and E1. When nandrolone was present at levels >10 nmol/L, artefactual increases in E2 levels was observed (see table). Levels of T, DHT or E1 were unaffected by the presence of nandrolone.

Added [Nandrolone] nmol/L	Observed [E2] nmol/L (% Accuracy)	Observed [E1] nmol/L (% Accuracy)	Observed [T] nmol/L (% Accuracy)	Observed [DHT] nmol/L (% Accuracy)
0	1.29 (100)	17.44 (101)	23.52 (100)	1.30 (100)
1	1.30 (101)	17.38 (100)	23.49 (100)	1.24 (95)
10	1.50 (116)	17.15 (99)	23.34 (100)	1.22 (94)

50	2.30 (180)	17.33 (100)	23.62 (101)	1.28 (98)
100	3.59 (277)	17.29 (100)	23.18 (99)	1.37 (105)

Conclusions

The presence of nandrolone at relatively high concentrations affects E2 quantitation in current setup. A possible explanation for interference is that a small fraction of nandrolone is converted into E2 within the ionization source; both steroids co-elute and are structurally similar. To avoid this interference a baseline separation of these two steroids is required. This aspect of assay validation may significantly influence studies related to reproductive biology, hormone replacement therapy and androgen misuse and abuse.

(1) Development and validation of a sensitive liquid chromatography-tandem mass spectrometry assay to simultaneously measure androgens and estrogens in serum without derivatization. Harwood DT, Handelsman

017

MONITORING THE SYNTHESIS AND PROBING THE REACTIVITY OF GOLD NANOCLUSTERS WITH ESI MS, CID, AND ION MOLECULE REACTION

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Nanoclusters and nanoparticles are interesting molecular systems that offer novel physical, surface, chemical, optical, and electrochemical properties and are important for a range of potential applications, such as biolabeling and catalysis [1, 2]. Their rich and novel properties are attributed to their small size that lies between an atom and bulk metal.

Until recently, synthesis of gold nanocluster has been difficult to achieve due to spontaneous rapid growth to form gold nano- and larger particles. Here we report the synthesis of a range of stable gold nanoclusters based on a one-pot method and the use of a bidentate ligand as their stabiliser [3]. Electrospray ionisation mass spectrometry (ESI MS) is employed to monitor the formation of the gold nanoclusters and their unimolecular dissocation investigated by collision induced dissociation (CID). The solution phase reactivity of gold nanoclusters with a range of different substrate was monitored and studied using ESI MS. Due to their interesting novel chemical and catalytic properties [4] we studied the gas-phase reactivity of gold nanoclusters using ion-molecule reaction [5] with a range of neutral reagents with a focus on investigating their catalytic properties.

keywords:

gold, nanocluster, electrospray, ion-molecule reaction, reactivity, catalyst

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018

GLYCAN PROFILING OF MONOCLONAL ANTIBODIES USING ZWITTERIONIC-TYPE HYDROPHILIC INTERACTION CHROMATOGRAPHY COUPLED WITH ELECTROSPRAY IONIZATION MASS SPECTROMETRY DETECTION

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Monoclonal antibodies (MAbs) represent a rising group of pharmaceuticals and their potency, stability and immunogenicity largely depend on their posttranslational modifications, especially N-glycosylation. The N-glycans are generally complex type, mostly core-fucosylated. Standard procedures for glycan profiling include labelling of enzymatically released oligosaccharides with fluorescent tag 2-aminobenzamide (2-AB) and separation on hydrophilic interaction chromatography (HILIC) columns followed by fluorescence or MS detection. In this work we present a new method for the analysis of glycans enzymatically released from monoclonal antibodies (MAbs) employing a zwitterionic-type hydrophilic interaction chromatography (ZIC–HILIC) column coupled with electrospray ionization mass spectrometry (ESI–MS). Both native and reduced glycans were analyzed, and the developed procedure was compared with a standard HILIC procedure used in the pharmaceutical industry. The separation of isobaric alditol oligosaccharides present in monoclonal antibodies and ribonuclease B is demonstrated, and ZIC–HILIC is shown to have good capability for structural recognition. Glycan profiles obtained with the ZIC–HILIC column and ESI–MS provided detailed information on MAb glycosylation, including identification of some less abundant glycan species, and are consistent with the profiles generated with the standard procedure. This new ZIC–HILIC method offers a simpler and faster approach for glycosylation analysis of therapeutic antibodies. Comparison with a novel method based on capillary electrophoresis (CE) with ESI-MS detection will also be presented.

019

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY GCXGC-TOF IN A MOLECULAR AND ISOTOPE STUDY OF CRUDE OILS AND SOURCE ROCKS FROM TERTIARY DELTAIC BASINS

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Establishing the age of a crude oil is an important tool for oil-source rock correlation. The present study aims to develop a molecular and isotope approach for high resolution age estimation of Tertiary deltaic oils and source rocks.

In order to identify and quantify higher plant biomarkers like oleanane, lupane and their diagenetically related compounds, several gas chromatographic systems are applied, namely gas chromatography-mass spectrometry (GC-MS) and GC-MS-MS (MRM, metastable reaction monitoring). For compound specific isotope analysis ($\delta^{13}C$ and δD) a GC-irMS instrument is used.

Explicit identification of land plant biomarkers or exact differentiation between two isomers of a compound using GC-MS is sometimes a challenge due to coelution and/or low abundances. For this reason, GC-MRM-MS (metastable reaction monitoring) and comprehensive two-dimensional GCxGC analyses are being carried out on the samples in this study.

Two dimensions of selectivity can be achieved by two-dimensional gas chromatography (GCxGC). GC×GC chromatographic separations typically resolve approximately 10 times more components in a complex mixture such as crude oil when compared with traditional one dimensional gas chromatography. Coupled with time of

flight mass spectrometry (GCxGC-TOF) we gain three dimensions in separation resulting in a very powerful tool for a wide range of applications from easily accessible but detailed fingerprinting of crude oils to separations of specific coelution problems. Further, a statistical approach to assess the amount of information in a large set of GCxGC data was carried out and shows additional potential for the use of GCxGC-TOF.

Results of stable carbon and hydrogen isotopes of biomarkers and molecular characterisation of deltaic crude oils and source rocks by MRM and GCxGC-TOF will be presented.

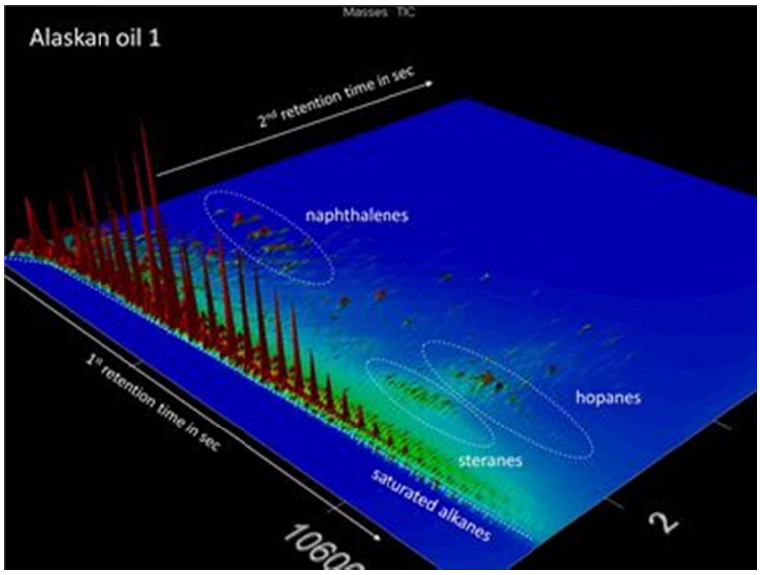

Figure 1 : GCxGC-ToF chromatogram of an Alaskan oil.

020

IMPROVING THE ELECTROSPRAY IONIZATION OF STEROID HORMONES BY SIMPLE OXIME DERIVATIZATION

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LC-MS is quickly becoming the method of choice for clinical steroid analysis, as many commonly used immunoassays are now known to be suffering from issues compromising their selectivity and precision. While GC-MS has a long history in steroid analysis, the current trend is towards LC-MS because of its potential for higher throughput and the lack of need for analyte derivatization. However, most steroids are nonpolar and have no ionizable groups in their structure, which makes them poorly suited for the commonly used electrospray ionization (ESI). An obvious solution has been the use of APCI or APPI, but for many steroids, the highest responses have been achieved with ESI after chemical derivatization of the analytes. Derivatization for LC-MS, however, is usually avoided as it complicates the sample preparation, diverting from the original motive of using LC-MS over GC-MS.

This work presents a simple and straightforward derivatization technique for LC-MS that can be incorporated into a typical serum sample preparation. When compared to a common liquid-liquid extraction (LLE) process, the only additional step is a short heating of the autosampler vials before their injection. The extraction is performed in common 1.5 mL autosampler vials using a test tube shaker. After the extraction, the organic solvent is transferred into conical-bottomed autosampler vials, evaporated, and reconstituted in methanolic hydroxylamine solution. Vials are then capped, shortly heated at 60 °C, and injected for analysis. By using the described method, the ionization efficiency of the serum ketosteroids can be improved due to the higher gas-phase proton affinities of the formed oxime derivatives. For the eight steroid compounds studied, increase in the signal intensity was from 1.5 to 15 times of that of the underivatized steroids.

021

INCREASING LC-MS/MS SAMPLE THROUGHPUT IN RESEARCH AND DEVELOPMENT USING U-HPLC AND MULTIPLEXING

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Traditionally LC-MS/MS sample analysis has been done using conventional size liquid chromatography (LC) columns (4.6mm internal diameter). However, with more emphasis on increasing sample throughput and reducing analysis time people are moving towards smaller diameter, and in some cases, smaller particle LC columns.

A generic 14 minute LC gradient was used as the starting point for the analysis and altered using a gradient method transfer calculator for use on a smaller diameter, smaller particle size column. By utilising a Thermo Scientific Transcend TLX-2 and Thermo Scientific TSQ Vantage mass spectrometer we will show that smaller particle columns can significantly increase sample throughput. We will further show that by utilising multiplexing we can increase sample throughput by upto 393% from initial conditions. We will also show savings in solvent and data storage can be achieved by utilising the methodologies above.

022

MASS SPECTROMETRY COLLIDES WITH LASER SPECTROSCOPY – STRUCTURAL STUDIES OF CHARGED MOLECULES AND CLUSTERS

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Attempts to probe molecular ions in mass spectrometers using tuneable laser radiation commenced in the 1970s, with pioneering contributions from Professor Jim Morrison and colleagues at Latrobe University. The talk will trace the history of the laser spectroscopy of molecular ions through to modern day investigations which couple sophisticated mass spectrometers with advanced light sources (optical parametric oscillators and free electron lasers). The latter part of the talk will focus on our recent laser spectroscopic studies of small molecular complexes in which one or more hydrogen molecules are attached to a metal cation such as Li^+-H_2, Na^+-H_2, Cr^+-H_2, B^+-H_2 and Ag^+-H_2.[2] These simple complexes serve as model systems for understanding the bonding of dihydrogen to charged sites in solid materials including zeolites and metal organic frameworks which are currently being investigated for hydrogen storage.[3] The infrared spectra, which feature full resolution of rotational structure, are recorded by monitoring charged photofragments, and deliver detailed information on the manner in which H_2 molecules interact with metal cations. In some cases, the onset of dissociation at a particular rovibrational level allows us to place extremely narrow bounds on the dissociation energy of the complex, providing a benchmark against which current computational techniques can be measured.

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023

EXPLORING THE INTERACTION BETWEEN AU AND RH CLUSTERS SUPPORTED ON MODEL LANTHANIDE-OXIDE "SURFACES" IN THE GAS PHASE

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Since the development of the laser ablation method for vaporising refractory materials in the early 1980s, it has been possible to perform spectroscopic experiments on metal clusters in the gas phase. It was quickly learnt that metal clusters possess chemical and physical properties that are strongly size-dependent and quite different from the bulk metal. However, it was also quickly realised that the high density of electronic states made traditional laser-based spectroscopic techniques inapplicable, except for the smallest clusters.

For the last few years, our research program at Adelaide has focused on the application of photo-ionisation efficiency (PIE) spectroscopy to determine accurate ionisation energies (IEs) of size-specific clusters in the gas phase. We have successfully measured IEs for pure metal clusters (Nb_x, x = 3, 4)[1], metal-carbide clusters (Nb_xC_y & Ta_xC_y, x = 3 - 5, y = 1 - 6).[2,3] and even bi-metallic clusters ($ZrTa_3C_y$, y = 0 - 4)[4]. We have also developed density functional methods for accurately calculating the IEs of clusters.[5] Excellent comparison between experiment and theory has enabled us to determine geometric and electronic information for these species.

We have recently developed our laser ablation source to prepare oxide clusters of the lanthanide metals; Ce, Pr, and Ho. These can be considered as models of lanthanide-oxide surfaces which are important in many catalytic applications. Further, we have been able to dope these "surface clusters" with clusters (and atoms) of the metals Rh and Au. We have successfully generated species such as $Rh\text{-}Ho_3O_2$ and $Au_3\text{-}Pr_2O_2$, isolated them in a supersonic expansion and measured their IEs.[6] Comparison with DFT-calculated IEs has allowed us to infer the geometric and electronic structure of these species, thus providing insight into the bonding and charge transfer between cluster and metal-oxide surface. This presentation will highlight our recent experimental and computational results on Rh_x (x = 1 - 3) bound to HoO_y, Ho_2O_y & Ho_3O_y (y = 0 - 4) clusters, and Au_x (x = 1 - 3) bound to Pr_2O_y & Pr_3O_y (y = 0 - 4) clusters. Also, our very recent work involving cerium-oxide clusters will be presented.

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024

MASS SPECTROMETRY AND ANION PHOTOELECTRON SPECTROSCOPY OF ANION-MOLECULE COMPLEXES

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At the University of Western Australia we have recently finished construction and testing of a time of flight mass spectrometer (TOF-MS) coupled to a photoelectron spectrometer. We have also constructed a specialised ion source which can create stable clusters consisting of simple anions (Cl^-, Br^-, I^-) solvated by neutral molecules (CO, N_2, C_2H_2).Using mass spectrometry the ions of interest are selected and subsequently intersected at the TOF-MS space focus with a pulse of 266nm UV radiation. Electrons are ejected from the anions and are channeled and analysed using the photoelectron spectrometer. Preliminary results will be presented which highlight the capabilities of the ion source, mass spectrometer, and photoelectron spectrometer. The photoelectron spectroscopy will be employed to provide insights into reaction chemistry of the 1:1 complexes, while modes of solvation will be elucidated for larger clusters of the form $X^-...M_n$.

025

DEVELOPMENT OF AN ALTERNATIVE ION ACTIVATION STRATEGY TO IMPROVE THE CAPABILITIES OF BIOANALYTICAL MASS SPECTROMETRY FOR PHOSPHOPROTEOME ANALYSIS

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The development of strategies directed toward comprehensive phosphoproteome analysis have undoubtedly been facilitated by the application of tandem mass spectrometry (MS/MS) for phosphopeptide identification, characterization and quantification. However, when multiple potential sites of phosphorylation exist within a peptide sequence, unambiguous phosphorylation site characterization remains a considerable challenge. Commonly, phosphorylation sites are identified by MS/MS using either collision induced dissociation (CID) or electron transfer dissociation (ETD) as the ion activation technique. Unfortunately, unambiguous phosphorylation site localization by using CID-MS/MS can be hindered due to facile losses of the phosphate group or intramolecular phosphate group 'scrambling', particularly under low proton mobility conditions in ion trap mass spectrometers [Anal. Chem. 2008, 80, 9735-9747]. ETD has an improved ability to localize phosphate groups, but can be limited in that it typically requires the precursor ions to be highly multiply-charged (i.e., $\geq$ 3+), where the ion/ion reaction cross-section is sufficiently large for efficient dissociation to occur.

Here, to address these limitations, we describe the development of an alternative ion trap based ion activation strategy to CID and ETD, termed femtosecond laser-induced ionization/dissociation (fs-LID) [*J. Am. Chem. Soc.* 2009, *131*: 940-942 .], for the enhanced characterization of singly protonated or multiply deprotonated phosphopeptide ions. Using this technique, exposure of a mass selected phosphopeptide precursor ion to a coherent packet of high electric field strength near-infrared photons (~800 nm; 1.55 eV/photon) on an ultrashort timescale (~35 fs, FWHM pulse duration), is capable of warping the ion's pseudo-potential energy surface to a sufficient extent so as to allow electron ejection (i.e., photoionization). The resultant oxidized radical intermediate species (e.g., $[M+H]^{+} \rightarrow [M+H]^{2+\bullet}$ or $[M-nH]^{n-} \rightarrow [M-nH]^{(n-1)-\bullet}$) then undergo relatively non-selective dissociation on a timescale similar to or faster than that of bond vibrations (10's-100's of fs) to produce a wide variety of peptide sequence ions (*a*, *b*, *c*, *x*, *y*, and *z)*, as well as ions which are potentially diagnostic of the positions of phosphorylation (e.g., 'a_n*+1-98*') [*J. Am. Soc. Mass Spectrom.* 2010, *In Press*.]. Importantly, the lack of non-selective phosphate moiety losses or phosphate group 'scrambling' provides unambiguous information for sequence identification and phosphorylation site characterization.

026

STRUCTURAL CHARACTERISATION OF PROTEIN COMPLEXES BY ION MOBILITY-MASS SPECTROMETRY

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Cellular processes are often regulated at the molecular level by protein-protein interactions. However, structural characterisation of the resultant complexes often proves difficult due to their heterogeneity and transient nature. Ion-mobility mass-spectrometry (IM-MS) has greatly enhanced our ability to study these systems and extract structural information regarding complex stoichiometry, size and arrangement. In addition, chemical cross-linking can be used to discern binding interfaces and provide restraints for molecular modelling applications in which model structures can be generated.

In this work we describe the design and synthesis of novel chemical cross-linking reagents for use with negative-ion mass spectrometry. These are built around a disulfide bond that undergoes facile cleavage in the negative mode. Validation of the disulfide containing cross-linkers with protein complexes of known structure has shown it is possible to readily identify site specific inter-subunit interactions, and examine protein-protein interactions for the first time by negative ion MS. The use of these chemical cross-linking reagents has also been applied in combination with IM-MS to study intact protein-peptide complexes of Calmodulin, a ubiquitous Ca^{2+}

binding protein. From these data, interaction maps and collision cross-section measurements are utilised to provide low resolution structural models of previously unknown protein assemblies.

027

CONSTRAINING STABLE CARBON AND HYDROGEN ISOTOPE EXCURSIONS OF FIRE EVENTS FROM CONTROLLED BURNING EXPERIMENTS AND APPLICATIONS TO THE TRIASSIC-JURASSIC EXTINCTION EVENT

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Sediments obtained from across the Triassic/Jurassic (Tr/J) extinction boundary from St. Audries bay, Lyme Regis and Westburyon-Severn in England and Larne, Ireland, are currently being processed and analysed focusing on terrestrial plant (i.e. waxes), marine-derived biomarkers and high-molecular-weight polyaromatic hydrocarbons. The molecular and compound-specific carbon and hydrogen isotopic compositions for these markers are tracked before, during and after the Tr/J extinction event (also see Grice et al., 2010 in this volume; Williford et al., 2010). The $\delta^{13}C$ are compared with marine carbonates to investigate environmental changes and δD excursions are hypothesized to be influenced by massive fire events associated with the Tr/J extinction.

To constrain isotopic shifts for C3 and C4 plants after burn events, a controlled burning experiment was conducted separately on native Australian C3 eucalyptus trees (Marri, Karri, Jarrah), conifer, and wild oats and C4 *Themeda triandra*. The resulting ashes and volatile organic compounds (VOCs) emitted, along with the unburnt leaves were analysed for changes in biomarker distributions and their stable carbon and hydrogen isotopic compositions. As expected, the leaves primarily contain odd/over/even predominance of long chain *n*-alkanes and their $\delta^{13}C_{n\text{-alkanes}}$ are consistent to those reported for C3 (-22 to -42 ‰) and C4 (-9 to -29 ‰) plants (e.g. Rieley et al., 1991; Krull et al., 2006). The relative abundances of the aromatic compounds were minimal. In the ashes, *n*-alkanes are still predominant, but are shorter with $\delta^{13}C_{n\text{-alkanes}}$ more enriched in ^{13}C compared to the unburnt leaves. VOCs were trapped on a Tenax resin and analysed by Thermal Desorption- compound-specific isotope analyses to yield $\delta^{13}C$ (Vitzthum von Eckstaedt et al., 2010 submitted).

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028

THE COMPLETE STRUCTURAL IDENTIFICATION OF (*O*-ACYL)-W-HYDROXY FATTY ACIDS IN HUMAN TEARS

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The tear film, located on the anterior surface of the eye, is vital in ocular health and vision. This film includes a thin outer layer of lipids that are secreted by the meibomian glands and prevent the excess evaporation of the aqueous phase and tear film instability. The identification of tear film lipids is limited and controversial, with the small volume of samples available and low lipid concentration hampering studies. Negative ion electrospray ionization (ESI) mass spectrometry of human tear lipid extracts reveals a series of lipid-like molecules that were previously thought to be phospholipids[1] but have more recently been suggested to be a sub-class of wax-esters, namely (*O*-acyl)- w -hydroxy fatty acids.[2] Despite this, limited structural information on these molecules has been reported. In this study, we used a suite of mass spectrometric techniques to comprehensively study the structure of these molecules. Lipids were extracted from tear samples by standard biphasic methods and analysed using nano-ESI. Hydrogen/deuterium exchange was used to determine if the molecules contained any exchangeable hydrogen sites. Precursor ion scans confirmed the ions were part of a series containing an 18:1 fatty acid (*m/z* 281) while MS^n fragmentation suggested the ions were not phospholipids. This was confirmed by accurate mass where the mass of the parent ions and their fragments pointed to a molecule with the formula $C_nH_{2n-7}O_4$. Analysis of authentic hydroxy fatty acids and authentic (*O*-acyl)- w -hydroxy fatty acids obtained from organic synthesis showed similar MS^n fragmentation patterns. The use of Ozone Induced Dissociation confirmed the presence of double bonds within these structures and for the first time assigned the position(s) of unsaturation. Through the application of various mass spectrometry techniques we have identified the complete structure of these poorly characterised molecules, facilitating a greater understanding of molecular interactions within the tear film and hence its stability.

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029

PRECURSOR ACQUISITION INDEPENDENT FROM ION COUNT: EXTENDING PROTEOMIC DYNAMIC RANGE WITHOUT PRIOR FRACTIONATION.

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Data-dependent precursor ion selection is widely used in shotgun proteomics to profile the protein components of complex samples. Although very popular, this bottom-up method presents major drawbacks in terms of detectable dynamic range. In Panchaud et al (Anal Chem. 2009 Aug 1;81(15):6481-8.) we demonstrated the superior performance of a data-independent method we term precursor acquisition independent from ion count (PAcIFIC). Our results showed that almost the entire, predicted, soluble bacterial proteome could be thoroughly analyzed by PAcIFIC without the need for any sample fractionation other than the C18-based liquid chromatograph used to introduce the peptide mixture into the mass spectrometer. Importantly, we also showed that PAcIFIC provides unique performance for analysis of human plasma in terms of the number of proteins identified (746 at FDR < or = 0.5%) and achieved dynamic range (8 orders of magnitude at FDR < or = 0.5%), without any fractionation other than immuno-depletion of the seven most abundant proteins. Since this publication we have gone on to show that PAcIFIC can 1) be used with spectral counting for quantitation, 2) may be combined with tandem mass tags to provide multiplexed quantitation and 3) detect the full dynamic range of yeast without prior fractionation.

030

ON THE QUEST TO UNDERSTAND THE REPAIR MECHANISM OF DNA DAMAGED BY UV RADIATION

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The major lesion in DNA caused by exposure to ultraviolet radiation is formation of cyclobutane pyrimidine dimers (CPD), namely *cis*,*syn*-thymine-thymine cyclobutane dimers (*c*,*s*-T<>T), resulting from [2π+2π] cycloaddition of two adjacent bases in the same oligonucleotide strand [1]. This lesion has serious biological consequences, such as incorrect DNA replication, which could lead to mutation, cancer or cell death [2]. Whereas placental mammals, including humans, remove CPD lesions through nucleotide excision repair, in prokaryotes, plants, and a variety of animals dimerized pyrimidines are efficiently converted to their monomeric form by the enzyme *DNA photolyase* in a light-driven catalytic reductive electron transfer cycle [3] .

Our recent study [4] revealed that the attachment of a free electron to the cyclobutane pyrimidine dimers, *c*,*s*-DMT<>DMT and *c*,*a*-DMT<>DMT, leads to the formation of dimer radical anions, very likely through dipole bound states [5]. These radical anions exhibit a lifetime of at least 80 μs, showing that they are much more stable than previously believed and that the splitting of the CPD radical anion into the respective pyrimidine monomers is associated with an activation barrier. In the present study we investigate the attachment of free electrons to uracil cyclobutane pyrimidine dimers (DMU<>DMU) with different geometry at the cyclobutane ring. How the stereochemistry at the cyclobutane ring affects the stability of the CPD radical anion, and its dissociation, will be presented.

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031

EXTENDED FUNGAL METABOLOME COVERAGE AND IDENTIFICATION OF DERIVATIZATION ARTEFACTS USING GCXGC-TOF-MS.

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Through optimisation of tissue sampling, metabolite extraction and preparation of metabolites, increasingly complex samples are now being produced for analysis. Alongside the development of more sensitive mass analysers and the need for high throughput gas chromatography methods, peak resolution and deconvolution play major roles in data analysis and mining. Comprehensive two-dimensional gas chromatography (GCxGC) eases the reliance on deconvolution software by significantly increasing peak capacity.

GCxGC allows for increased peak detectability by adding a second dimension of separation. This second dimension allows for better peak resolution, possibly giving the ability to separate co-eluting analytes. Signal enhancement is achieved through the cryo-focusing effect of the modulator, which permits better detection of trace-level compounds. Cleaner spectra are also obtained, as the analytes of interest are separated from residual solvent, column bleed and derivatisation artefacts.

Exploring comparative data from GC-QMS, GC-TOF/MS and GCxGC-TOF/MS for pig plasma and urine from a rat model of Polycystic Kidney Disease has highlighted the varying degrees of information that are collected

using each technique. Comprehensive metabolite profiles for several mutant strains of the wheat fungal pathogen *Stagonospora nodorum* have been generated for comparison. GCxGC has allowed for the separation of more than twice as many peaks as found by conventional one-dimensional gas chromatography with a higher match factor from commercial mass spectral libraries. Cleaner, sharper peaks obtained using GCxGC has led to considerable increases in S/N.

032

ARE TWO METALS BETTER THAN ONE? SYNTHESIS AND REACTIVITY OF THE HETERO-METAL CLUSTERS $RCC_XAG_NCU_M^+$.

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In order to develop robust organometallic based synthetic organic methods a better understanding of the link between organometallic structures (e.g., clustering) and their reactivity is essential [1]. Mixed metallic species are gaining grounds in their use as catalysts in preference to the homometallic ones. For example, a recent review by Mulvey[2] highlighted the existence of a "synergic" effect of the metals in "mixed-metal" organometallic catalysts for synthetic organic chemistry. Using ESI in conjunction with tandem mass spectrometry we can examine the fundamental unimolecular and bimolecular reactivity of size- and composition-selected cluster ions. In this work, we (i) synthesized mixed-metallic clusters of the form ($[RC{\equiv}C_xAg_nCu_mCl]^+$, m+n=14), (ii) studied their fragmentation patterns, and (iii) studied the chemistry of the smaller clusters ($m+n \leq 6$).

We used electrospray ionization (ESI) and multistage mass spectrometry in: (i) a Finnigan LTQ-FTICR mass spectrometer and (ii) an Agilent Q-TOF. DFT calculations using Gaussian03 with the B3LYP functional shed light on the structures and pathways observed.

ESI of a solution of RCCAg + $Cu(MeCN)_4PF_6$ (e.g., R=C_3H_7) generates clusters of the form $[RC{\equiv}C_xAg_nCu_mCl]^+$, m+n=14 and $m{\leq}6$. These fragment into $[RC{\equiv}C_xAg_nCu_m]^+$ ($m+n \leq 8$) upon Collision-Induced Dissociation. Unimolecular chemistry studies on these clusters revealed interesting size/composition dependant chemistry. For example, whereas CID of $[(C_3H_7C{\equiv}C)_4Ag_3Cu_2]^+$ generates ions corresponding to C-H (eq. 1) and C-C (eq. 2) bond activation as well as bond formation, a larger cluster (i.e.; ($[(C_3H_7C{\equiv}C)_5Ag_4Cu_2]^+$) only undergoes cluster fragmentation (eq. 3). Further, we will present a comparison of the unimolecular as well as bimolecular chemistry of $[(RC{\equiv}C)AgCu]^+$, $[(RC{\equiv}C)Ag_2]^+$, and $[(RC{\equiv}C)Cu_2]^+$ both experimentally and theoretically.

$[(C_3H_7C{\equiv}C)_4Ag_3Cu_2]^+$

$$\text{--> } [C_3H_6(C{\equiv}C)_2(C_3H_7C{\equiv}C)_2Ag_3Cu_2]^+ + C_3H_8 \quad (1)$$

$$\text{--> } [(C_3H_7C{\equiv}C)_2Ag_3Cu_2]^+ + (C_3H_7C{\equiv}C)_2 \quad (2)$$

$[(C_3H_7C{\equiv}C)_5Ag_4Cu_2]^+$

$$\text{--> } [(C_3H_7C{\equiv}C)_4Ag_3Cu_2]^+ + C_3H_7C{\equiv}CAg \quad (3)$$

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033

CHEMICAL DEGRADATION OF NERVE AGENTS: WHY IS VX PERHYDROLYSIS SO EFFICIENT AND SELECTIVE?

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Mass spectrometry has a pivotal role in chemical defence as evident in the predominance of MS methods for the detection and verification of chemical warfare agents (CWAs). MS methods also provide a powerful means of interrogating reactive chemistries of CWAs at the fundamental level that otherwise challenge experimentation. For example, the chemical degradation of the nerve agent VX in alkaline solution has been well studied but the disparity in product distribution between hydrolysis and perhydrolysis processes is not fully understood. Specifically, why does VX perhydrolysis occur more rapidly and exclusively yield the relatively non-toxic ethyl methylphosphonic acid (EMPA)? In the absence of solvent effects, gas phase reactions of HOO^- may provide insight into the efficacy and selectivity of VX degradation by alkaline perhydrolysis.

The ion-molecule reactions of HOO^- with *O*,*S*-dimethyl methylphosphonothioate, a VX-model system, were conducted using a modified linear ion-trap mass spectrometer. A combination of collision induced dissociation of product ions and isotope-labeling experiments provide evidence for two specific nucleophilic reaction pathways, namely: (i) S_N2 at carbon to yield the *S*-methyl methylphosphonothioate anion and (ii) nucleophilic addition at phosphorus affording a reactive pentavalent intermediate that readily undergoes internal sulfur oxidation and concomitant elimination of CH_3SOH to yield the methyl methylphosphonate anion. Hybrid density functional theory calculations complement the experimental results and provide further insight into the pertinent reaction pathways. Significantly, this is the first direct evidence for the intramolecular sulfur oxidation process that can explain the selective perhydrolysis of VX to relatively non-toxic products.

034

BIOCHEMICAL AND MORPHOLOGICAL EVIDENCE OF A FATAL BLUE-LINED OCTOPUS (HAPALOCHLAENA FASCIATA) ENVENOMATION OF AN ADULT GREEN TURTLE (CHELONIA MYDAS)

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This is the first recorded evidence of an envenomation of an adult green turtle (*Chelonia mydas*) by a blue-lined octopus (*Hapalochlaena fasciata*). The salivary glands and soft tissues of *H. fasciata* contain a powerful neuromuscular blocker tetrodotoxin (TTX), which it uses to both hunt prey and to protect itself from predators.[1] The bacterium produced TTX causes muscle weakness and respiratory failure, and with an LD_{50} of 9 μg/kg, is one of the most potent toxins known for mammals. Many case studies have been presented on *Hapalochlaena* sp. envenomation on human beings, but to date there have been no recorded incidents of an envenomation of a wild marine animal.[2,3]

Here we present evidence for a mortality of a green turtle (*C. mydas*) caused by a blue-lined octopus *(H. fasciata*). The large, sexually mature female turtle was outwardly healthy, with no obvious external injuries and internal pathologies indicating that the death had been sudden. On closer inspection of the oesophagus, a blue-lined octopus (*H. fasciata*) was found within a bolus consisting of the seagrass *Halophila ovalis*. Water was found within the lungs, indicating that the turtle was in the water when the bite occurred. The liver, kidney, muscle tissues and food bolus from the *C. mydas* specimen and the salivary gland from the *H. fasciata* were prepared for HPLC analysis. TTX was identified in the octopus and turtle samples based on comparison of the mass-to-charge ratio (m/z), fragmentation pattern and HPLC retention time of the unknowns with authentic TTX standard. It is proposed that this is a case of accidental ingestion with subsequent envenomation followed by death of the turtle due to the toxic effect of TTX.

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035

EXPLORING MASS EXTINCTION EVENTS USING BIOMARKERS & STABLE ISOTOPES (CARBON AND HYDROGEN)

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The Late Permian mass extinction event was the most profound extinctions of the entire Phanerozoic. Biomarker evidence for photic zone euxinic (PZE) conditions within Permian/Triassic (P/Tr) sections, where concentrations of sulfide, are sufficient to support anoxygenic photosynthesis, come from components derived from pigments of green sulfur bacteria (Grice et al., 2005; Hays et al., 2007; Fenton et al., 2007; Nabbefeld et al., 2010a,b,c) at 6 localities.

We provide sedimentological data, biomarker abundances and compound specific isotopic data ($\delta^{13}C$ and δD) along with bulk isotopes ($\delta^{34}S_{pyrite}$, $\delta^{13}{}_{carbonate}$, $\delta\ ^{13}C_{org}$) for several Late Permian sections. $\delta^{13}C$ data of algal and land-plant derived biomarkers, $\delta^{13}C$ carbonate & organic matter support synchronous changes in $\delta^{13}C$ of marine and atmospheric CO_2, attributed to a ^{13}C-depleted source (^{13}C depleted methane and/or CO_2 derived from the degradation of organic matter due to the marine collapse (Nabbefeld et al., 2010a).

A number of mechanisms have been proposed to account for the Triassic/Jurassic (T/J) extinction extinction, including the release of CO_2 associated with emplacement of the Central Atlantic Magmatic Province. A negative $\delta^{13}C$ excursion has been detected in many sections supporting a perturbation in the global carbon cycle. It is clear that major and abrupt ecological change including 80% extinction among terrestrial plant species coincides with increased atmospheric CO_2 concentration and a negative excursion in $\delta^{13}C$ of fossil wood from a Tr/J section at Astartekløft, East Greenland. $\delta^{13}C$ of the C_{29} land plant *n*-alkane shows a 5‰ negative shift that corresponds with similar shifts in wood, increasing CO_{2atm} and peak plant extinction. During the interval containing the rise in CO_{2atm} leading up to peak plant extinctions, δD of the *n*-C_{29} shifts positively, which we attribute in part to an increasing degree of evaporative fractionation in leaf water due to increasingly elevated leaf temperatures. Resin-derived biomarkers are abundant in the bed containing peak plant extinctions attributed to resins of gymnosperms produced under extreme environmental stress (Williford et al., 2010). The effect of combustion on δD and $\delta^{13}C$ of land plant compounds is being determined and a global T/J extinction study is underway (Jaraula et al., 2010).

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036

CLARIFYING THE MECHANISMS OF HALS OXIDATION BY ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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Hindered Amine Light Stabilizers (HALS) are piperidine-based radical scavenging anti-oxidants, used to increase the longevity of polymeric surface coatings. While their mechanisms of action have been widely discussed,[1,2] less work has focussed on the inevitable deactivation of HALS, leading to the coating fading or discolouring.

Traditional characterization methods such as electron spin resonance fail to identify the oxidized by-products of HALS formed at long service lifetimes, decreasing their efficiency. Previously, positive ion Electrospray Ionization Mass Spectrometry (ESI-MS) and tandem mass spectrometry (MS/MS) successfully characterized unaltered HALS in solution and from polymer extracts.[3] In the present study we have applied these methods to characterise some of the products of solution-phase HALS oxidation.

A systematic survey of the ESI-MS/MS spectra of commonly employed HALS revealed an imine fragment ion of *m/z* ($57+R_N$, R_N = piperidine nitrogen functionality), and therefore represents a useful target for parent ion scanning in complex extracts. When subject to oxidizing conditions, *N*-alkyl or *N*-ether HALS show an increased abundance of species containing *N*-H fragment ions (*m/z* 58), at the expense of the initial *N*-substituent species. This modification of the active site of HALS is not accounted for in currently accepted theories of HALS' action.

Tandem mass spectrometry also reveals oxidative modification occurring on the backbone linked to the 4-position of the piperidine for thermal stability, away from the active nitrogen of HALS. In one example, the entire backbone is eliminated by hydroxyl radical attack, with the observed formation of a ketone on the piperidine moiety. This represents a significant new pathway for HALS de-activation via volatilisation, and must be avoided when considering potential structures for HALS.

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037

ENVIRONMENTAL AND ORGANIC GEOCHEMICAL APPLICATIONS OF ADVANCED ANALYTICAL PYROLYSIS AND MASS SPECTROMETRY

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Analytical pyrolysis is a useful procedure for breaking down organic macromolecules into smaller fragments amenable to gas chromatography (GC) detection and is frequently used to help characterise geo- and biomacromolecules. However, traditional fast pyrolysis GCMS techniques are limited to the detection of just thermally labile, non-polar products; are prone to secondary fragmentations which reduce structural detail; and do not directly address the morphological heterogeneity of organic sediments. Many pyrolysates are also unresolvable by one dimensional GC, but can often be selectivity detected by selected ion or tandem mass spectrometry (MS). However, the lack of GC baseline resolution can limit the accuracy of complimentary $\delta^{13}C$ measurements by GC isotope ratio MS.

We have recently developed several pyrolysis technologies to address these issues. The mild thermal regimes of microscale sealed vessel pyrolysis (MSSVpy) and catalysed hydropyrolysis (Hypy) minimise secondary cracking and structural and isomeric rearrangements, and generate high yields of primary aliphatic and aromatic pyrolysates from many organic materials. They have proved very beneficial to the biochemical characterisation of immature organic sediments and extant biomass, particularly improving the detection of heteroatom compounds. Applied applications include the detection of pharmaceutical, sewage and other anthropogenic chemicals in potential potable water systems; and the regeneration of hydrocarbon biomarkers from the

asphaltene fractions of biodegraded oils for exploration purposes. Isolated HyPy products can also be subject to additional preparation procedures appropriate for d ^{13}C measurements.

Laser micropyrolysis was developed to support the analysis of small or small parts of samples (< 100 m m). It affords ultra-high sensitivity and an ability to separately analyse microscopically discrete morphological entities (e.g., coal macerals), improving trace detections and the organic appraisal of heterogeneous sediments. We recently used it to separately analyse the molecular composition of oil bearing fluid inclusions of different colour for the first time.

038

LILBID, A NEW NATIVE MASS SPECTROMETRY TO STUDY BIOMOLECULAR COMPLEXES AND LARGE PROTEINS FROM SOLUTION.

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In recent years we developed a new laser mass spectrometry, in which ions a laser desorbed/ablated from tiny, liquid droplets (d= 50 μm) containing the analyte. The method called LILBID (laser induced liquid bead ion desorption) turned out to be very powerful for native mass spectrometry of large protein complexes both from the membrane and from solution. Depending on the intensity of the desorption laser (3m) the method has two modes of operation: 1. an ultrasoft mode, for making large complexes "fly" unfragmented into a TOF mass spectrometer, such as mitochondrial complexes, ribosomes, DNA/RNA- protein complexes, polymerase etc. and 2. a hard mode, which allows to disintegrate these large noncovalent complexes into their covalent subunits. At medium laser intensity, groups of subunits may appear giving insight into proximity relations. The method is working very well with membrane molecules solubilized in detergent, with highly charged species like plasmids, and with buffer conditions of a native environment (divalent salts, pH, phosphate buffer etc.) which are in general absolutely crucial for specific binding and molecular recognition. The consumption of analyte is typically 3 μl at μM concentration. LILBID can be coupled with BNG-gel separation techniques. Examples are given for the high sensitivity, softness and specificity , allowing the determination of native stoichiometries of membrane complexes, their subunits and for receiving indications on their charge state in solution.

039

ELUCIDATION OF A DIFFERENTIAL RESPONSE TO XANTHANMONUS INDUCED DISEASE IN BRASSICA SPP BY LCMS METABOLOMICS APPROACHES.

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Xanthomonas campestris is the casual agent of black rot and leaf spot of crucifers. These bacterial diseases are characterised by marginal leaf chlorosis, vascular blackening, leaf lesions, wilting and necrosis, result in substantial yield loss. Identification of pathovars of X. campestris is an important diagnostic challenge that cannot be resolved at the 16sRNA level. Metabolomics has been investigated as a potential tool for bacterial diagnosis and to better elucidate the mechanism of plant immune response to bacterial infection.

Metaboloimcs analysis employed a rapid (15 minute) HPLC gradient with LTQ Velos Orbitrap MS data acquired in positive-negative switching mode. Statistical analysis of the data was carried out separately on the extracted negative data and positive data. This approach proved productive for biomarker discovery as clear indication of differential plant response at the metabolite level was observed with PCA and ANOVA analysis

for both datasets. There were metabolites contributing to this separation that were observed only in positive or only in negative mode reinforcing the need for both ionisation modes in metabolomic discovery approaches. The significance of several key metabolites, including glucosinolates, and their identification by high resolution MS^n approaches will be discussed.

The use of LCMS metabolomics coupled with statistical modeling has allowed differentiation of several important X. campestris pathovars. In addition key metabolic shifts were observed in resistant plants upon infection. The effect is pre-symptomatic and can be correlated with plant survival and health. This data indicates that metabolomic approaches may play an important part in the development of novel diagnostic tools and further enhance our understanding of plant-pathogen interactions.

040

PERFLUORINATED GROUP 11 ORGANOMETALLICS: GAS PHASE STUDIES AND DFT STUDIES TO UNDERSTAND A COMPLEX MECHANISTIC INTERPLAY

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Incorporating fluorine into organic molecules with efficiency is a continuing challenge for synthetic chemists today, with a growing number of pharmaceuticals, pesticides and materials compounds requiring this functionality. Perfluoroorganometallic reagents represent a promising means to introduce fluorine and trifluoromethyl groups using relatively mild conditions. With the introduction of fluorinated substances into our biosphere, there is also interest in how they degrade. There is currently a relative lack of mechanistic insight into these processes.

Trifluoroacetic acid (TFA) is a cheap source of trifluoromethyl group for catalytic or stoichiometric synthetic applications [1] and a potential environmental contaminant. This study represents the first ESI-MS and density functional theory (DFT) study of the gas phase decomposition of anionic group 11 perfluorinated dicarboxylates, $[CF_3CO_2MO_2CCF_3]^-$ (M= Cu, Ag and Au). This was conducted with the aim of decarboxylatively forming organometallic products via collision induced dissociation (CID) (eq 1 & 2). We have successfully used this double decarboxylation method for a range of group 11 complexes with hydrocarbon ligands. [2-4]

$$[CF_3CO_2MO_2CCF_3]^- \rightarrow [CF_3MO_2CCF_3]^- + CO_2 \quad (1)$$

$$[CF_3MO_2CCF_3]^- \rightarrow [CF_3MCF_3]^- + CO_2 \quad (2)$$

Utilizing a Finnigan LCQ mass spectrometry fitted with a standard ESI source, methanolic solutions of precursor metal salts and TFA were introduced to the mass spectrometer. Subsequently, their unimolecular reactivity was studied using multistage mass spectrometry (MS^n) and sets the stage for bimolecular ion-molecule studies. In this report, the formation of Cu, Ag and Au species are compared to each other, as well as to their fellow hydrocarbon analogues.

The possible competing reaction mechanisms were explored, including but not limited to:

- The nature of the metal carboxylate complexation (F vs O)
- α-difluorolactone vs fluoride transfer mechanisms
- Bond dissociation processes

The level of insight was greatly aided by indications from complementary DFT studies and these will be discussed in light of experimental results.

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041

MASS SPECTROMETRY AT THE CUTTING EDGE OF FORENSIC, ENVIRONMENTAL AND ANALYTICAL INVESTIGATIONS

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Currently, conventional techniques used for forensic, environmental and analytical investigations are based on an hierarchy with the least destructive, least expensive and most discriminatory techniques utilized first followed, if necessary, by more sophisticated, more expensive and more destructive techniques. Based on these criteria Laser-Ablation Multi-Collector High-Resolution Inductively Coupled Plasma Mass Spectrometry (LA-HR-MC-ICPMS) may seem to be the last choice to be used when all else has failed. However, this paper will argue, with examples, that if the results that can be obtained from the use of one or two high-end techniques such as LA-HR-MC-ICPMS and Isotope Ratio Mass Spectrometry (IRMS) analysis can provide unquestionable discrimination then these sophisticate techniques may eliminate the need for a range of other techniques to be carried out on all material and, therefore, may actually turn out to provide sufficient discrimination quicker and cheaper. Examples of where LA-HR-MC-ICPMS may be the first choice include those where the size of the sample is small and a range of techniques is inapplicable or where the single discriminative power of an isotope ratio would be sufficient to screen samples into those that require further investigations and those who have been sufficiently differentiated. Examples given will range between those for whom a quick yes/or no answer is needed to those for which a very specific elemental isotope ratio will be absolutely discriminatory.

042

STRUCTURAL CHARACTERIZATION OF COMPLEX LIPIDS BY RADICAL DIRECTED DISSOCIATION

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Traditional GC-MS approaches to the analysis of simple lipids (*e.g.*, fatty acids) rely on electron ionization and thus the fragmentation of odd-electron ions (typically radical cations) to provide structurally diagnostic spectra. These radical ions undergo significant fragmentation of the hydrocarbon portion of the molecule and can thus, under the right conditions, be used to identify the position and stereochemistry of double bonds within these chains.[1] Radical ion dissociation can even be used to identify cyclic and branched motifs.[2] In contrast, structural analysis of complex lipids (*e.g.*, triacylglycerols and phospholipids) is accomplished using the collision induced dissociation of even-electron ions generated by electrospray ionization. A key limitation is that the even-electron ions to not typically give rise to fragmentation of the fatty acyl chains themselves and as such reveals little information about the bonding within these substituents (*e.g.*, double bond position and stereochemistry). To obtain further structural information, we have applied the technique of radical directed dissociation (RDD) [3] to the study of lipids for the first time. In this approach a free radical initiator is appended to an adducting agent *e.g.*, 4-iodoaniline or 4-iodobenzoic acid. The adduct ions with complex lipids are then isolated and subject to photodissociation using 266 nm laser to yield odd electron ions via cleavage of the carbon-iodine bond. Upon isolation and further activation the nascent radical ions exhibit free radical-driven dissociation. Preliminary data suggest that RDD provides unique fragments linked to double-bond position in unsaturated triacylglcerols and

phospholipids and can thus complement traditional, even-electron fragmentation of the same adducts (*i.e.*, without the photolysis step). This technique is demonstrated for lipid standards as well as lipid extracts from a range of sources including olive oil and human very-low density lipoprotein.

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043

RAPID NON-TARGETED METHODOLOGY FOR THE ANALYSIS OF TEA EXTRACTS USING UHPLC AND HIGH RESOLUTION MASS SPECTROMETRY

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Non targeted analytical techniques are ideally positioned to be used in many areas of food science and have recently begun to be utilised as a tool for food quality, processing and safety of raw materials and final products. However this requires the development of fast and reliable unbiased analytical methods that are able to yield qualitative and quantitative data on as many metabolites as possible. High throughput mass spectrometry is one option, and the emergence of ultra-high pressure liquid chromatography (UHPLC) enhances both throughput and chromatographic resolution over conventional chromatography methods. When this chromatography technique is coupled to a fast scanning high resolution mass spectrometer the method then has considerable resolving power in two different domains, time and mass.

A fast non targeted analytical method was developed, based on the latest UHPLC system and utilizing high resolution mass spectrometry, to examine the profiles of commonly available tea varieties and identify the major components present, along with their relative levels. We applied this methodolgy to multiple samples and processed the data using the software package AnalyserPro ™ to detect and resolve overlapping peaks, and create a library of tea related metabolites.

This method serves as a useful tool for rapidly analysing large sample-sets to search for discriminatory ions which may be potential indicators of biological significance.

044

BIOMARKER DISTRIBUTIONS AND STABLE ISOTOPES (C, H) ESTABLISH THE AGE AND PALAEOENVIRONMENTAL CONDITIONS SPANNING THE PERMIAN/TRIASSIC IN THE NORTHERN ONSHORE PERTH BASIN.

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The Perth Basin is located in southwest Western Australia (WA). The Perth Basin petroleum system has been intermittently explored for the last few decades, resulting in the production of gas and oil from several onshore fields (e.g. Summons et al . , 1995). The Perth Basin sediments used in this study are from the Senecio-1 drill core , which is located approximately 15.5 km to the north of Dongara.

In the present study bulk geochemical, biomarker and compound specific isotopic analysis (CSIA) of biomarkers will be used to further constrain the age and palaeoenvironmental conditions spanning the Permian/Triassic in the northern onshore Perth Basin and to compare these results with the Hovea-3 drill core ‘type-section' (Grice et al., 2005a). In particular, stable carbon and hydrogen isotopic composition of

biomarkers measured by CSIA has been shown to be an effective tool for establishing biogeochemical changes across the Permian /Triassic boundary (Nabbefeld et al., 20 10). For this purpose 31 samples from the Senecio-1 cored interval were selected at 1 m spacing. The ages of the samples have been assessed by conodont biostratigraphy. Rock-Eval & TOC analysis have been carried out to identify the type and maturity of organic matter and to assess petroleum potential. The samples were then analysed following the methodology of Grice et al. (2005b) . Briefly, each sample was ground to a fine powder and extracted using an Accelerated solvent extractor. The extracts were separated into 6 fractions by liquid chromatography . Saturate and aromatic hydrocarbon fractions were characterised by GC-MS . The saturated hydrocarbon fractions were separated from branched and cyclic hydrocarbons by treating with 5A molecular sieves and CSIA o f biomarkers was performed for these fractions . Bulk stable isotopic compositions were measured on the kerogens isolated from the extracted powders. The results will be presented at the conference.

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045

MASS SPECTROMETERS IN PLANETARY EXPLORATION

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Mass spectrometer based scientific investigations are an important element in space missions to explore the chemistry of atmospheres and surfaces of planets and their moons. Such investigations have enabled us to explore Venus from orbit, to probe Saturn's moons Titan and Enceladus, and to measure the chemical composition of Jupiter's atmosphere.

We are presently developing instruments to orbit the moon and Mars, and a suite of instruments for the 2011 Mars Science Laboratory rover to explore the habitability of a site on the surface of this planet. These investigations are designed to reveal the chemical and isotopic composition of samples from planetary targets and the distribution and processing of organic molecules in the solar system. They are also directed toward understanding habitability of diverse environments in our solar system and understanding the origin and evolution of planets and more primitive solar system objects.

Supporting these studies is a program of planetary analog research that includes laboratory simulations of planetary environments and chemistry, geochemical and isotopic analysis, and field studies of planetary analog sites.

This presentation will focus most specifically on the science goals of the 2011 Mars Science Laboratory (MSL) with a rover named “Curiosity” that ties directly to the ongoing search for life on Mars. We wish to characterize the landing site selected as a potential habitat for past or present life.

We will discuss the goals of the Sample Analysis at Mars (SAM) suite of instruments that consists of a quadrupole mass spectrometer (QMS) coupled to a six-column gas chromatograph (GC) and a tunable laser spectrometer (TLS) that utilizes a common gas processing system with the other instruments.

The top-level scientific mission goals and specific investigations required are (1) to assess biological potential of at least one target environment (past or present), (2) to investigate the chemical, isotopic, and mineralogical composition of Martian surface and near-surface geological materials, and (3) to study planetary processes that influence habitability.

046

NEGATIVE ION CHEMISTRY OF (M-H)$^-$ IONS OF PEPTIDES CONTAINING PHOSPHOSER, THR AND TYR. CHARACTERISTIC FRAGMENTATIONS AND UNUSUAL REARRANGEMENT REACTIONS.

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(M-H)$^-$ ions of peptides containing phosphoSer and phosphoThr fragment characteristically by the processes [(M-H)$^-$- H_3PO_4) and (M-H)$^-$ → $H_2PO_4^-$ whereas the diagnostic fragmentations of the (M-H)$^-$ ion of a peptide containing phosphoTyr are [(M-H)$^-$- HPO_3]$^-$ and (M-H)$^-$ → PO_3^-. PhosphoTyr containing peptides undergo S_Ni cyclisation of the C-terminal carboxylate anion at the P of the phosphoTyr to effect transfer of PO_3H_2 to the C-terminal position. Following proton transfer, several rearrangements initiated by this phosphate anion can occur, including a specific cyclisation to, and cleavage of, the peptide backbone at the central C of the penultimate amino acid residue. Care must be taken when using negative ions to identify phosphoTyr. When a peptide has two phosphate side chains (at Ser, Thr or Tyr), the (M-H)$^-$ ion undergoes phosphate/phosphate cyclisation as evidenced by a pronounced peak at *m/z* 177: this corresponds to the anion $H_3P_2O_7^-$. The mechanisms of all fragmentation processes have been probed using *ab initio* theoretical methods.

048

ANALYSIS OF BROMINE AND OTHER HALOGENS IN ANTARCTIC ICE-CORES USING SECTOR FIELD ICPMS.

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Halogen species are important to a number of atmospheric processes, particularly in the polar regions where the advent of the polar sunrise results in a cascade of photochemical / halogen catalyzed reactions. Ice core records hold the potential to investigate sources of halogen species and changes in halogen chemistry over the past million years. Previous measurements of halogens in ice cores have focused on their determination in discrete samples using ion and gas chromatographic methods. Here we present the determination of soluble bromine, chlorine and iodine in ice cores from the West Antarctic Ice Sheet and the interior of East Antarctica. The three halogens were determined by coupling a continuous ice-core melter to two sector-field inductively coupled plasma mass spectrometers (SF-ICPMS). Using this system Antarctic ice core samples were continuously melted in sequence and the meltwater fed in parallel to the SF-ICPMS's. The first SF-CPMS was operated with a fixed mass resolution of 300 amu (10% valley) and was used to determine iodine (^{127}I), while the mass resolution of the second SF-ICPMS was set at 4200 amu for the determination of bromine (^{79}Br) and chlorine (^{35}Cl). The results suggest that the preservation of halogens in the ice core record is dependent on the amount of snowfall at the site. This is especially true of bromine. At locations with low snow accumulation, such as the interior of Antarctica, halogens are re-emitted to the atmosphere during the polar sunrise and as a result the interior of Antarctica becomes an emission source.

BIOMARKERS AND STABLE ISOTOPES OF EUXINIA AND THEIR ROLE IN FOSSIL PRESERVATION

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The Gogo Formation of Western Australia preserves a characteristic Late Devonian (380 MYA) reef fauna. The extraordinary preservation has been as a consequence of the taphonomy of the site as well as the lack of high tectonic forces in the region (Long, et al. 2010). Moreover, exceptional preservation of soft-tissue, including muscle bundles, nerve cells, and umbilical structures, has been identified in this macrofossils during paleontological studies (Trinajstic et al., 2007; Long et al., 2010). Through improved sampling and preparation techniques extensive areas of soft tissue have now been recovered from placoderms, acanthodians (spiny-finned fish) and actinopterygians (ray finned fish) from the Gogo Formation (Devonian Canning Basin, WA).

More recently Maslen et al (2009) have demonstrated that the Gogo Formation of the Canning Basin and the equivalent aged- Duvernay Formation of the Western Canada Sedimentary basin (and their associated oils) were deposited under highly euxinic based on the presence of biomarkers associated with green sulphur bacteria.

To investigate the presence of biomarkers in fossilised soft tissue half of a whole crustacean within a Devonian nodule was extracted and separated into different fractions. GC-MS and GC-IRMS have been performed in the saturated and aromatic fractions. *N*-alkanes (C_{15} to C_{32}) were identified in the saturated fraction as the main components, which have a significant depletion in ^{13}C associated to the contribution of sulphate reducing bacteria. Cholestane, was found to be the most dominant compound in the saturated fraction attributed to a source from algal derived sterols probably retained upon grazing (Grice et al., 1998). Markers of green sulfur bacteria globally identified across Permian/Triassic mass extinction boundary have also been identified here supporting photic zone euxinic conditions (H_2S and light) (Grice et al., 2005; Nabbefeld et al., 2010). Various biomarker proxies within the sample support preservation under highly euxinic conditions similar to those that occurred during the Permian/Triassic mass extinction event.

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050

QUANTITATIVE ANALYSIS OF HEPCIDIN TO EVALUATE AND MONITOR IRON METABOLISM IN A RANGE OF CLINICAL CONDITIONS

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Hepcidin is the principle regulatory hormone essential for iron homeostasis and the quantitative analysis of hepcidin in bodily fluids provides an insight into the pathogenesis of disorders of iron metabolism such as hereditary hemochromatosis and anemia of chronic disease. Hepcidin exists in a 20, 22 and 25aa form in urine and the 25aa form is the active form.

This study describes the use of solid phase extraction (SPE) as a preparative step followed by matrix assisted laser desorption/ionization-orthogonal-time-of-flight mass spectrometry (MALDI-TOF MS) with internal standard for the quantitative analysis of unlabelled urinary hepcidin. Recoveries greater than 70% of hepcidin (hepcidin-25) was achieved. Urinary creatinine was analyzed using HPLC-UV/Vis with hepcidin-25 levels of 2.2 to 2.7 nmol/ mmol of creatinine observed in healthy controls.

Spot-to-spot variation of hepcidin standard additions was less than 3.5%. Intra- and inter-day precision assay of less than 9.5% relative standard deviation was achieved with less than 0.5% variation between the intra-day assay data. The impact of sample handling of the urine will be discussed.

The development of a LC/MS based method will be addressed together with the implications of urine vs plasma samples.

The use of this method as a tool for monitoring chronic anaemia patients and dialysis patients will be introduced.

A validated non-invasive method has been developed for the quantification of unlabelled urinary hepcidin-25 which can be used to evaluate and monitor iron metabolism in a range of clinical conditions.

051

COMBINING NITROGEN AND CARBON ISOTOPE RATIOS FOR DISCRIMINATION OF AMMONIUM NITRATE AND AMMONIUM NITRATE BASED EXPLOSIVES

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Ammonium nitrate (AN) is a strong oxidiser commonly used in fertilisers that can be readily converted to an explosive with the addition of a suitable fuel. The explosive mixture under investigation in this research is AN mixed with aluminium (AN/Aluminium). This explosive was analysed and characterised pre- and post-blast using various analytical techniques including Isotope Ratio Mass Spectrometry (IRMS).

IRMS has been shown to have the potential to discriminate between samples of ammonium nitrate based on variations in their isotopic composition. This research investigated the discrimination of AN using nitrogen and carbon isotope ratios. Empirically, AN contains no carbon, however carbon is introduced, as AN prills are often coated with additional chemicals to improve storage (e.g. lilamine in paraffin oil).

IRMS results indicate that combined nitrogen and carbon isotope values can be used to discriminate between batches, manufacturers and sources of AN. It was also shown that IRMS could link four prepared AN/Aluminium explosives to the source AN material. Investigation of twenty one realistic samples demonstrated that the technique is sufficiently robust to be used in “real world” situations. Thus, the IRMS technique could be used as a valuable tool in determining the provenance of and linking similar explosive samples.

POSTER

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LIQUID EXTRACTION SURFACE ANALYSIS (LESA) COMBINED WITH AUTOMATED NESI-MS/MS AS A NOVEL TOOL IN THE BIOANALYTICAL LABORATORY

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The TriVersa NanoMate combines a robotic platform for sample delivery and a chip based nanoelectrospray emitter in one integrated system. It enables the online-coupling to HPLC systems as well as parallel fraction collection and direct infusion from sample plates. In comparison to conventional LC-MS setups, a much better reproducibility and stability as well as higher value information is achieved. The system has recently been extended to include the ability to perform Liquid Extraction Surface Analysis (LESA), first reported by Kertesz and Van Berkel 1, to enable the direct measurement of samples from surfaces such as Dried Blood Spots (DBS), tissue and TLC plates

Here we present LESA applied to two bioanalytical challenges:

DBS Analysis

Method

12 µL fortified lithium heparin blood was spotted onto filter paper and dried for 2 hours at room temperature (standard curve range: 10-5,000 ng/mL). DBS samples were mounted on a 96-well plate and spray-coated/soaked with silicone followed by drying for 1 hour at room temperature. Analyte was then extracted and electrosprayed.

Results

Hydrochlorothiazide (HCTZ) can be directly quantified from silicone-treated DBS media with an LOQ of 10 ng/mL. Method development is rapid due to elimination of off-line extraction method and LC separation

Small Molecule Profiling from Thin tissue Sections

Method

Whole guinea pig lung is extracted from the animal and dosed intra-tracheally with 3 mg fluticasone propionate in 5 mL air using a micro-sprayer needle. Tissue was then frozen, sliced in 16 µm thicknesses and placed on glass slides for vacuum drying and further LESA analysis.

Results

Spatial distribution of fluticasone is shown in the bronchial part of the lung tissue by monitoring SRM transition 499.1/413

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MS^4 FRAGMENTATION ON AN LTQ ION TRAP QUANTIFICATION AND UNEQUIVOCAL IDENTIFICATION OF TETRODOTOXIN (TTX) IN COMPLEX SAMPLES

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Tetrodotoxin (TTX) and its analogs (TTXs), widely distributed among marine as well as terrestrial animals, induce potentially lethal intoxications. In Japan these toxins are also the causative agent of puffer fish (Fugu) poisoning. TTX is a potent neurotoxin with no known antidote and is a powerful and specific sodium channel blocker. The reversible inhibition of the voltage-activated sodium channels induces paralytic symptoms, such as respiratory insufficiency.[1] Although tetrodotoxin was discovered in these fish and found in several other animals (e.g., blue-ringed octopus, rough-skinned newt and Naticidae[2]) it is actually produced by certain symbiotic bacteria, such as Pseudoalteromonas tetraodonis and certain species of Pseudomonas and Vibrio.

Presently, the mouse bio-assay is the standard method for determination of tetrodotoxin although recent methodology in the identification of TTX standard has been made using single ion monitoring (SIM) or multiple reaction monitoring (MRM). The Quantification of TTX in biological samples however, is essential and necessary for the establishment of a more ethically sound, simple and analytically accurate method compared to the standard mouse assay.

Therefore to address these issues and obtain more quantifiable information, in conjunction with fast turnaround times, a new method for analysis of TTX and its analogues has been made utilising LC-MS and LC-MS/MS.[3,4] We report in this study an approach for a simple yet powerful method which facilitates the unequivocal identification and quantification of TTX. This technique is based on hydrophilic interaction and strong cation exchange chromatography coupled with tandem mass spectrometry and MS^4 stage fragmentation. With minor modification, the quantification LC-MS^n method can now be applied generally to detect TTXs in different species.

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MASS SPECTROMETRY DISCOVERY AND VALIDATION OF PLASMA BIOMARKERS FOR DIABESITY

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Diabesity, a termed coined to encapsulate the twin epidemics of diabetes and obesity, is a metabolic time bomb. In the last decade diabetic sufferers have doubled with the expected growth by 2015 to reach 380 million (currently 250 million). Plasma biomarkers in diabesity were sought for use in the prediction of early-onset of the disease and its complications, and ultimately discover new therapeutic targets. Ten cohorts from diverse Western Australian populations were recruited. These cohorts include both children and adults who have either type1or type 2 diabetes or who are obese.. Plasma from subgroups of each cohort were analysed for differential protein expression following immunodepletion of high-abundance proteins. Candidate biomarkers were

identified from iTRAQ labelled samples by 2D-LC-MALDI MS/MS with an ABSCIEX 4800 MALDI-TOF/TOF, following the analysis of over 125,000 MSMS spectra. Verification and validation of potential biomarkers used an orthogonal MRM approach on an ABSCIEX 4000 Q-TRAP. 200-300 proteins per cohort were identified, and across all studies over 60 proteins showed significant differences in expression when compared to controls. Approximately one third of these proteins have been previously described as involved with diabetes, obesity and their complications.

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PROTEOMIC MAPPING AND LARGE SCALE QUANTITATIVE PROTEOMICS

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Understanding of proteome wide systems through large scale proteomic mapping efforts has become possible with new generation sensitive and accurate mass spectrometers. Coupled with this are technologies to quantitatively measure the relative levels of proteins on a proteome wide basis (eg iTRAQ). The accuracy and coverage modern mass spectrometric methods can achieve is far more extensive than has previously been possible with older systems such as 2D gel electrophoresis. A complex system such as plasma may yield 300 protein identifications from as little as 20 microlitres after immunodepletion of highly abundant constituents such as albumin. For proteomes of other cell types and organisms the coverage rises to over 1000 proteins of confident identification. The selective chemical tagging of proteomes from, for example, a control and diseased state, allows relative quantitative information about which proteins may be up or down regulated in the system. These techniques have been applied to a variety of biological systems in projects at the Lotterywest WA Proteomics Facility and examples are presented.

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A COMPARATIVE STUDY OF THE ACCURACY OF SEVERAL DE NOVO SEQUENCING SOFTWARE PACKAGES FOR MALDI AND ELECTROSPRAY-DERIVED DATASETS.

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Proteomic characterisation of complex biological samples commonly utilises enzymatic protein digestion and analysis of the resultant peptides via MS/MS. Experimental MS/MS data is then compared to theoretical fragmentation patterns derived from protein sequences within databases, using programs such as Mascot and Sequest. The key drawback to this approach is its limited utility when protein sequences are unknown, such as in a biodiscovery environment where the organisms are often without adequate proteomic or genomic coverage. The alternative approach when investigating uncharacterised proteomes is to utilise de novo sequencing programs to estimate a probable peptide sequence. The derived sequence can then be homology matched to existing databases using BLAST-related algorithms to perform putative functional classification. However, the successful utilisation of this approach is critically dependent upon the accuracy with which de novo sequencing is able to derive peptide sequences from MSMS spectra. A number of de novo software packages are available, however, there are few independent reports detailing their accuracy. We evaluated the accuracy of three de novo sequencing packages - DeNovo ExplorerTM, PEAKS, and PepNovo - utilising data derived with both MALDI TOF/TOF and electrospray LCMS platforms. Using a selected set of 68 peptide MSMS spectra from known proteins, we calculated the number of correct residues scored by each program. For MALDI TOF/TOF data, PEAKS and PepNovo performed almost identically (~65%), while the performance of DeNovo ExplorerTM was reduced (52%). This trend was also observed for ion trap data, with PEAKS and PepNovo correct for 64% of residues, and DeNovo ExplorerTM considerably worse with only 27% correct. Thus we found that the de novo sequencing algorithms tested can be expected to report up to two-thirds of residues correctly from a typical experiment. For increased confidence, a combination of algorithms may be useful.

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SIGNIFICANT METABOLITE DIFFERENCES IN INFLAMMATORY BOWEL DISEASE PLASMA RELATED TO DISEASE ACTIVITY

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Metabolism of the essential amino acid Tryptophan via the Kynurenine pathway has long been of bioscientific interest due to the reputed roles of its catabolites in neurotransmission and inflammatory processes. Previous metabolomic studies into Inflammatory Bowel Disease have demonstrated significant overexpression in selected Kynurenine metabolites suggesting an integral involvement in this pathway with autoimmune gut motility [1-4]. Quinolinic acid (QA) and Picolinic acid (PA) are end products of this pathway. In this study we quantified and compared QA and PA expression levels and performed a plasma metabolomics study between populations of CD, UC, and healthy controls using GC-MS and LC-MS on an Orbitrap instrument, respectively. Multivariate statistical techniques demonstrated significant overexpression of QA in CD, signaling a possible irregularity in interleukin 2 (IL-2) induced T regulatory cells. While variable PA and QA expression levels in UC provide more supportive evidence for the theory of an atypical T helper Cell 2 immune response involving IL-4 malfunction. Furthermore, the discovery of upregulated and contrasting expression levels of a specific metabolite between CD and UC reinforces suggestions of an autophagic abnormality in CD, that we suggest possibly does not exist in UC.

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MULTI-RESIDUE SCREENING OF PESTICIDES IN AGRICULTURAL COMMODITIES BY A BENCH-TOP HIGH RESOLUTION ORBITRAP LC-MS

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This study applies and validates simple sample preparation procedures and detection with ultrahigh performance liquid chromatography-high resolution bench-top Exactive ™ Orbitrap mass spectrometry (UHPLC-HRMS) for comprehensive pesticide residue screening. Pesticides are extracted using current extraction procedures such as *Qu*ick, *E*asy, *Ch*eap, *E*ffective, *R*ugged and *S*afe (QuEChERS) [1] and dilute-and-shoot [2] and the performance is evaluated based on recovery, repeatability, sensitivity, mass accuracy and resolution of the pesticides in the presence of the agricultural matrices such as fresh produce and dried botanicals. Most of the pesticides studied

could be detected in the 1 – 10 µg/kg (part-per-billion) range. Accurate mass measurements using Orbitrap ® HRMS of known pesticides were carried out in three different matrices (orange, carrot, spinach) at various concentrations with MS accuracy of <1 ppm or better, well within the acceptable limits for confirmation. At low concentration levels in complex plant-based matrices, a minimum nominal MS resolution of 50,000 was required to resolve pesticide analytes from interfering co-extractives. Collision-induced dissociation experiments were performed to separate two isomers, desmedipham and phenmedipham based on the uniqueness of their product ions. The results from this work demonstrate the applicability of this method and instrumentation for the routine analysis of targeted and non-targeted (data acquisition) screening of pesticides in complex agricultural commodities.

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A COMPARISON OF THE REACTIVITY OF ALKYL SUBSTITUTED AROMATIC RADICALS IN THE GAS PHASE USING A DISTONIC ION APPROACH

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Aromatic organic compounds comprise approximately 30% of petroleum-based fuel formulations sold in Australia. The toxicity of benzene has held cause for it to be replaced by less harmful alkyl substituted aromatics like toluene and xylenes in both diesel and spark-ignition fuels. In these radical driven combustion processes, the ignition properties of the fuel depend upon the stability of the radical intermediates . Despite their prominence in these fuels, their oxidation characteristics and reactive pathways remain relatively poorly described . In this study we have used a distonic ion approach to investigate the reactivity of a variety substituted phenyl radicals as well as some of their isomers. In this approach, the desired radical is tagged with a fixed trimethylammonium charge to allow isolation and detection of reaction products within an ion-trap mass spectrometer.

Figure 1 : Synthesis of the 2-methylphenyl radical by laser photolysis of the C – I bond

A series of aromatic radical cations were prepared in an ion-trap mass spectrometer by 266 nm laser-photolysis of halogenated precursor ions (e.g., Figure 1). Synthesis of the radical via this method is compared to that using collisional induced dissociation where other unique product channels have been identified. The radical cations prepared demonstrated vastly differing reactivity towards O_2. For example the 2-methylphenyl radical reacts rapidly with O_2, leading to the OH-loss product. While the isomeric benzyl radical appears to decompose back to the radical + O_2. Identification of the products and postulation of the reactive pathways has been assisted by computed potential energy surfaces. Computational approaches have also been employed to probe the effect(s) of the charge on the gas phase chemistry of the radical and thus describes the suitability of distonic ions as models for the archetypal neutral systems.

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MARINE BIOTOXINS BY LC QQQ

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Recently, legislation in the EU introduced liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS) as the soon to be reference method for the detection of lipophilic marine biotoxins in live bivalve molluscs, tunicates and echinoderms. This change in legislation allows for significant developments in the area of mass spectrometric detection for phycotoxins. This presentation will show two LC-MS/MS methods

proposed for the analysis of lipophilic marine biotoxins and both methods are currently evaluated in trials. Challenges including those overcome when quantifying compounds which are unavailable as reference standards are presented. Confirmation is achieved using new library search functionality of a modern QQQ instrument. Both methods have been validated and shown to be robust and sensitive.

(1) Highly Sensitive and Robust Analysis for Lipophilic Marine Toxins in Shellfish, Agilent Technologies Application Note, Publication number, 5990-6377EN

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ACTION SPECTROSCOPY OF GAS-PHASE DISTONIC PEROXYL RADICAL-CATIONS: EFFORTS TO UNCOVER EXCITED STATE REACTIVITY OF PEROXYL RADICALS

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Abstract: Current research into peroxyl radicals implicates them as key intermediates in the atmospheric oxidation of organic compounds. It has been suggested that dissociation of alkyl peroxyl radicals in a low lying excited electronic state may give rise to hydroxyl and other small radical species [1]. In the atmosphere, this process can be driven by solar radiation. Direct investigation of these potentially important processes however, have been hampered by experimental challenges in both forming and observing both aryl and alkyl peroxyl radicals. Previous research in our group has shown that the reaction of distonic radical-cations with dioxygen provides a pathway to the formation and isolation of charge-tagged alkylperoxyl radicals in good yield. These distonic peroxyl radical ions have proven to be effective models for the analogous neutral species [2]. We have undertaken a study to probe the excited state reactivity of distonic peroxyl radical-cations by irradiation of mass-selected species at 266 or 355 nm (Nd-YAG laser) within a linear ion trap mass spectrometer. For example, the mass-to-charge ratio of photo-dissociation product ions arising from 4-trimethylammonium phenylperoxyl radical cation [$(^{+}NMe_3)C_6H_5O_2^{\bullet}$] is found to be dominated by CO-O homolysis (Figure 1.).

Figure 1. The photo-dissociation of the 4-trimethylammonium phenylperoxyl radical cation in to 4-trimethylammonium phenoxy radical cation and triplet oxygen.

This is in contrast to the C-OO bond cleavage that is prevalent when the same ion is subject to collision-induced dissociation (a vibrational heating process). Further experiments using other photon energies and different distonic peroxyl radical ions will be presented. Future experiments will look to incorporate a tunable photon source to further explore peroxyl excited state chemistry.

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METABOLITE PROFILING OF G-PROTEIN-SIGNALING MUTANT STRAINS OF *STAGONOSPORA NODORUM* BY HYBRID QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

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The necrotrophic fungal pathogen *Stagonospora nodorum* costs Australian agriculture in excess of 100 million dollars (AUD) in losses each year due to its pathogenicity to wheat. Functional genomics tools are currently being used to dissect critical aspects of the fungal lifecycle.

G-protein signaling has long been implicated in the pathogenicity of phytopathogenic fungi such as *S. nodorum*. Strains of this fungus have been created lacking the Gα, Gβ and Gγ proteins, with all strains exhibiting abnormal development and defects in pathogenicity.

The ABSCIEX TripleTOF 5600 system has been used to analyse metabolite extracts from each of the strains of *S. nodorum* and complex metabolite profiles have been generated, using UPLC with a total run time of 12 minutes, for comparative analysis. The metabolomic data was collected in positive ion mode and negative ion mode using electrospray ionisation and APCI. Accurate mass with fast MS and MS/MS acquisition speeds of the TripleTOF 5600 has resolved in excess of 1000 peaks and generated structural information for metabolite identification. Preliminary statistical analysis differentiates the fungal strains and the metabolites contributing to differences between the strains are now being linked to the observed phenotypic effects of the mutations.

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COMPARISON OF TRIPLE QUADRUPOLE GC/MSMS, SINGLE QUADROPOLE GC/MS, GC/ECD IN THE DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCBS) IN MARINE BIOTA AND SHARK OIL (SQUALENE) AND THE MEASUREMENT OF SYNTHETIC PYRETHROIDS (SPS) IN EFFLUENT STREAMS.

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Measuring Polychlorinated Biphenyls (PCBs) and Synthetic Pyrethroids (SPs) represents a challenge in many laboratories. This poster summarizes work comparing measurement of PCBs and SPs in marine samples using triple quadrupole GC/MSMS, single quad GCMS and GC/ECD. The power of MRM (multiple reaction monitoring) using GC/MSMS offers significant advantages over the older techniques for PCB's in marine biota and shark oil (squalene) and SP's in effluent streams.

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IDENTIFICATION AND CHARACTERISATION OF GLYCOPEPTIDES VIA HIGHER-ENERGY C-TRAP DISSOCIATION AND ORBITRAP MASS ANALYSIS

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Post-translation modification (PTM) by carbohydrates has been implicated in numerous intracellular, cell-cell and cell-matrix recognition events, which has underscored the recognition of protein glycosylation as a critical area of research.[1] The thorough characterisation of intact glycopeptides following glycoprotein proteolysis, allowing information regarding site specific glycan composition to be retained,[2-3] shows considerable promise as a means by which comprehensive broad scale characterisations of glycoproteins may be achieved. Present implementations of glycopeptide analysis, however, struggle to achieve significant analytical breadth. This can largely be attributed to constraints associated with glycopeptide identification in complex peptide mixtures, and the need for high resolution MS data for effective high throughput analysis. To address the constraints associated with the analysis of glycopeptides in high throughput workflows, we utilise Orbitrap mass analysis to achieve selective and sensitive identification of glycopeptides through high mass accuracy oxonium ion observation in higher-energy C-trap dissociation (HCD) derived tandem MS spectra; high mass accuracy single stage MS data is obtained concomitantly. The utility of this data in the context of high throughput glycopeptide analysis is illustrated through the characterisation of tryptic digests of a model glycoprotein, ribonuclease B, and proteolytic digests of a mixture containing previously uncharacterised hen egg glycoproteins. The applications of Orbitrap mass analysis described herein strongly compliment recent developments in automated glycopeptide analysis workflows,[4-7] and can be expected to play a key role in progress towards comprehensive broad scale glycoproteomic studies.

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LINEAR ION TRAP PHOTODETACHMENT STUDY OF DICARBOXYLATE DIANIONS

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Linear dicarboxylate dianions have been shown to be stable in the gas phase.[1] Studies investigating these dianions have largely employed photoelectron spectroscopy.[2, 3] Inspired by these studies, we explore these dianions using pulsed laser photodetachment and track the resulting monoanions using ion trap mass spectrometry. The range of dianions targeted include $^{-}OOC\text{-}(CH_2)_n\text{-}COO^{-}$ where n=4-8, 10, acetylene dicarboxylate ($^{-}OOC\text{-}CC\text{-}COO^{-}$) and cyclic dicarboxylates. In the majority of cases, decarboxylation follows promptly after photodetachment and the intact radical anion is not observed. The decarboxylated radical anions

are subsequently characterised by ion-molecule reactions with O_2. All of the samples investigated except $^-OOC-(CH_2)_{10}-COO^-$ and 1,4-cyclohexane dicarboxylate displayed evidence of radical migration. Interestingly, in the case of acetylene dicarboxylate, the radical monoanion ($M^{\bullet -}$) was detected in the mass spectrum (m/z 112) following photodetahment and thus far is the only dicarboxylate monoanion that we have intercepted. The radical anion ($M^{\bullet -}$=m/z 112) gains stability through resonance of the radical through the conjugated backbone. These results are compared with MP2 and B3LYP calculations and photoelectron studies from the literature.

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METABOLITE DETECTION AND AUTOMATIC LOCALIZATION OF MODIFICATION USING FRAGMENT ION SEARCH (FISH)

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Introduction

Efficient metabolite identification requires combining modern mass spectrometric techniques with advanced software tools. Here a novel software approach is presented for single-run metabolite detection and identification from LC/MS data using Fragment Ion Search (FISh) in conjunction with automated prediction of fragments from parent structure.

In contrast to traditional post-acquisition processing techniques, this approach does not require the prediction of biotransformations from parent compound. Similarly to Mass Defect Filter (MDF), it utilizes the compound structure to filter out matrix-related background ions. However, the method presented here is fundamentally different from MDF and no subjective judgment of expected m/z range and mass defect range is required. The site of biotransformation can also be automatically localized using such approach.

Methods

It has been shown[1] that parent drug and its metabolites exhibit common fragments ions even if the m/z of their precursor ions are different. First, fragment ions are automatically predicted from parent structure using a beta version of Mass Frontier 7.0 software. Then a table consisting of accurate m/z values of predicted fragments is generated and subsequently applied as an extraction filter to the high resolution accurate mass LC/MS data. FISh enables selective removal of vast majority of matrix-related background ions. Furthermore, the matching of the predicted fragment ions to the observed fragment ions in the putative metabolites enables the automatic localization of biotransformations.

Preliminary data

The performance of such approach was evaluated using in vivo (1mg/kg by IV) rat bile samples. The results from FISh is compared and contrasted to the results from MDF. The inclusion of predicted fragment ions enhanced the number of “signature” fragment m/z values and thus significantly increased detection efficiency for potential metabolites. The base-peak chromatogram resulting from FISh, as well as the number of metabolites detected are comparable to those obtained by MDF. While MDF only works with the molecular ion of the metabolites thus not useful in suggesting the site of biotransformation, FISh was able to propose the likely structures of the metabolites by drawing the relationship between ions with the matched m/z values and the corresponding fragment structures.

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DRIVING EFFICIENCY IN A HIGH-THROUGHPUT METABOLIC STABILITY ASSAY THROUGH A GENERIC HIGH RESOLUTION/ACCURATE MASS METHOD AND AUTOMATED DATA PROCESSING

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Introduction

Improving analytical throughput is strongly desired in many quantitative workflows being developed for early drug discovery and a common practice is to connect ultrahigh performance liquid chromatography (UHPLC) to triple quadrupole mass spectrometry for drug candidate screening. This approach certainly results in short analytical run times, however, in assessing the true throughput one needs to consider all aspects of the workflow, including instrument set up and the necessity to rerun samples when information is missed. Here we demonstrate a high throughput metabolic stability assay with a simplified instrument set up which significantly improves overall assay efficiency. In addition, as the data collection is non-biased, information on the parent compound and metabolites is gathered at the same time.

Method

A series of standard drug compounds were incubated individually with human liver microsomes (HLM) using a standard protocol. Incubation of each drug was quenched at 0, 5, 10, 20 and 30 min. The Accela UHPLC was coupled to an Exactive Orbitrap mass spectrometer scanning a mass range of 100-1000 m/z at 25,000 or 50,000 resolution. Novel algorithms were used for automated data analysis, including metabolic stability plots of parent and potential metabolites. The high resolution/accurate mass (HRAM) approach was validated by comparison with a traditional triple quadrupole SRM method.

Results

The HRAM approach uses resolutions >20,000 and sub ppm mass accuracies to provide the selectivity and sensitivity required for these analyses. There were sufficient scans (12-20) across a typical UHPLC peak (width 2-4 sec) to provide high quality quantitative data. In contrast to triple quadrupole methods where selectivity is provided by a targeted scan specifically tuned to the analyte of interest, the HRAM approach uses an unbiased scan mode. Using the same method for all compounds simplifies the assay set up and reduces data entry errors; no need for developing compound specific MS/MS methods results in significant time savings. In addition, because of the unbiased nature of the analysis little information is needed about potential metabolites at the time of the assay. Therefore as knowledge is gained about the compound under study the metabolic stability data can be re-interrogated for the presence or absence of metabolites.

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NEW AUTOMATED SOFTWARE FOR BIOMARKER DISCOVERY FROM HIGH RESOLUTION LC-MS DATA

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Many stages of drug discovery require robust biomarkers and analytical capabilities to quantify them in various biological samples. High resolution LCMS provides the sensitivity, accuracy, and wide dynamic range required for quantitation and is suitable for high-throughput automation. A typical 10-minute high resolution LCMS profile of a biological sample may contain over a million data points. Reduction of the data to accurately represent the endogenous metabolites under study, their proper identification and statistical comparison across samples has been a major challenge for LCMS metabolomic analyses.

Blood samples were taken from 4 different groups of rats (male fully satiated, female fully satiated, male food deprived and female food deprived) and analyzed using LCMS. All samples were analyzed in both positive and negative modes and the data was acquired during 10 minutes reverse phase gradient on a 1.7µ 150x2.1mm BEH C18 column using high resolution (>15,000) and sub-5ppm mass accuracy. The data was then analyzed using Component Elucidator software from Bristol Meyers Squib to determine the metabolic effects of food deprivation on the rats.

A typical metabolomics data file from urine, plasma or tissue extract yields over one million signals. Such complex datasets contain redundant and irrelevant information due to isotopes, adducts, multiple charge states that represent a single component, and chemical noises unrelated to the sample. Proper reduction of the number of detected signals through identification of sample-related peaks, and filtering out signals related to system background is essential. A variety of noise-filtering approaches including blank subtraction were used to reduce the complexity of the sample with an intuitive GUI. In addition, grouping related signals, including isotope peaks, adduct, dimmers, fragments further reduced the number of components by a factor of 10. This approach presents a comprehensive, integrated solution to processing LCMS metabolomics data. The data analysis time for the 24 samples in this set is less than 30mins on a standard desktop computer. Visualization tools showed substantial differences in endogenous metabolites levels between groups of animals. Automated annotation of components was accomplished using a search in the ChemSpider database. Obtained tentative metabolite assignments were subjects for verification with synthetic standards.

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PROTEIN DERIVATIZATION AS A METHOD FOR INCREASED COVERAGE IN ELECTRON TRANSFER DISSOCIATION SEQUENCING

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Introduction

Previous work on peptide derivatization has demonstrated the utility of this technique for increasing the sequence coverage obtained through electron transfer dissociation (ETD)[1,2]. The addition of histamine to acidic functional groups increases the overall positive charge of the peptides examined. Of note, peptides with disparate charge distribution benefited immensely from this technique.

The disparity of charge within peptides is amplified when analyzing whole proteins, leaving regions of protein with little charge. This hampers sequencing of proteins, particularly when we are further limited by the analyzer of choice. The 2,000 m/z upper limit of these analyzers rely on peptide fragments progressively reaching higher charge states to remain in the mass window. Thus, as we sequence further into the protein, the fragments require progressively more positively charged amino acids to increase the overall charge of the peptide. Where this is not the case, gaps are observed in protein sequence, until sections with positively-charged amino acids are reached. Accordingly, the application of protein derivatization, targeted at converting negatively-charged or

neutral amino acids to positive charge-bearing amino acids, could enhance the sequence coverage obtained for proteins *via* ETD.

Results

In this study, we examine the combination of protein derivatization with ETD sequencing. To achieve this, we utilized a protein standard, ubiquitin; to develop our method. Once the parameters were optimized with ubiquitin, we extended our analysis to larger proteins.

Initial studies have shown only partial coverage of eIF-1 and the light chain of IgG. For eIF-1, we observe 117 distinct ions. Furthermore, we observe overlap of sequence ions from the N- and C- terminus, however, the coverage from both termini has many gaps. By comparison, the sequence ions observed for the light chain of IgG are all clustered between amino acids 94-133.

Preliminary results have demonstrated the successful derivatization of the sample proteins with histamine. In addition, we observe a shift in the average charge state proportionate to the addition of histamine to the target functional groups. We are currently analyzing and annotating the ETD spectra of these proteins, early results show increased sequence ions observed.

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GAS-PHASE SYNTHESIS AND CHARACTERISATION OF ALKYL AND ARYLPEROXYL RADICALS USING DISTONIC RADICAL IONS.

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Small organic radicals are key reactive intermediates in many important gas phase processes including combustion and the formation of photochemical smog. The inherent reactivity and thus transient lifetimes of many of these radicals has forced the experimentalist to develop increasing complex (although undoubtedly ingenious) methods with which to isolate and interrogate these reactive intermediates. Although key reactive intermediates in the reactions mentioned above, the study of the uni- and bi-molecular reactivity alkyl and arylperoxyl radicals remain theoretical, as current methods used to selectively synthesis and isolation these species do not allow for the systematic investigation of their subsequent reaction pathways. In an effort to characterise the fundamental gas phase chemistry of these elusive radicals, we have undertaken a systematic study of distonic radical ions that incorporate, for example, adamantyl, cyclohexyl and phenyl radical motifs and an inert remote charge \"tag\" which allows for the manipulation of the radical within an ion-tap mass spectrometer.

Using this approach we have studied the reaction products, kinetics and efficiencies of these radicals with neutral species such as dioxygen, nitric oxide and nitrogen dioxide. Products synthesised in these studies include cyclohexyl and phenylperoxyl radicals which we have isolated and allowed to react in the presence of nitric oxide and nitrogen dioxide, reactions purportedly involved in the formation of photochemical smog. In this presentation, the formation and reactions of model alkyl and aryl radicals bearing both positively charged tags (i.e., trimethylammonium) and negatively charged tags (i.e., carboxylato) with dioxygen and ^{18}O-labelled dioxygen will be discussed, including the uni- and bi-molecular reactions of adamantyl, cyclohexyl and phenylperoxyl radicals. Importantly, we have directly observed reactions of peroxyl radicals with nitric oxide and nitrogen dioxide, reactions which until now were based primarily on end-product analysis. This investigation aims to demonstrate the utility of distonic radical ions as models of the reactivity of their neutral counterparts.

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DETERMINATION OF A PHOSPHOLIPID SIGNATURE FOR HUMAN METABOLIC SYNDROME USING MASS SPECTROMETRY-BASED METABOLOMIC APPROACHES

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Metabolic syndrome (MetS) is an obesity-related disorder that predisposes an individual to life-threatening diseases such as cardiovascular disease, hypertension, and type 2 diabetes. The contribution and biochemical mechanism of phospholipid involvement in MetS is not understood. In this study, we employed high field FTICR mass spectrometry for comprehensive profiling and QTOF mass spectrometry for relative quantitation of human plasma phospholipids in a panel of clinically-diagnosed MetS patients and healthy lean controls, in order to reveal a potential phospholipid signature of MetS.

Phospholipids were isolated from 10 MetS and 8 control human plasma samples by liquid-liquid extraction with chloroform/water/methanol. For profiling, the organic phase was analyzed using DI-FTMS and LC-FTMS in both (+) and (-)ESI modes. Data extraction was conducted using custom-designed software in conjunction with Bruker DataAnalysis software suite. The monoisotopic, accurate masses obtained were searched against the LIPID MAPS database. A list of database-matched phospholipid masses was compiled.

For quantitation, the organic phase was analyzed by UPLC-QTOF MS in both (+) and (-) ESI modes. The database-matched phospholipid masses were used for mass-directed analysis of the two UPLC-MS datasets using Waters QuanLynx software to extract m/z-retention time pairs and corresponding peak areas. These datasets were exported into Simca P+ software for multivariate analysis to detect a statistically significant difference between the two sample groups. Univariate Statistics were then conducted on the top 10 most significant phospholipids in each ionization mode. UPLC fractions were collected offline for DI-FTMS/MS for structural confirmation of these top 20 phospholipids.

Of approximately 5000 metabolite features detected, 488 matched unique phospholipid masses in the LIPID MAPS database within 6 ppm. Multivariate analyses showed clear separations between the sample groups in both ESI modes. Univariate statistical analysis revealed at least 20 potential phospholipid species exhibiting statistically-significant differences in abundance between MetS patients and healthy controls. These results suggest that a plasma phospholipid signature of MetS exists.

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INSIGHTS INTO QUATERNARY STRUCTURE OF A POLYDISPERSE PROTEIN ASSEMBLY USING MASS SPECTROMETRY AND ION-MOBILITY SPECTROMETRY

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Mass spectrometry (MS) and ion mobility spectrometry (IMS) has revolutionalised structural investigation of biomolecules of varying size and complexity, including DNA, protein mixtures, and supramolecular assemblies. This hybrid technique has enabled characterisation of complex molecular systems that have been resistant to studies by existing methods.

Here we report characterisation of the quaternary structure of a molecular chaperone, MsHSP16.1, using a combination of nanoelectrospray mass spectrometry (nES MS) and IMS. MsHSP16.1 is a mycobacterium small heat shock protein (sHSP) that functions mainly to bind partially unfolded protein and thus it prevents formation of potentially pathogenic aggregates [1]. Its monomer size is ~16.1 kDa, but the monomers readily self-assemble to form large assembly of approximately 300 kDa.

Techniques, such as multi-angle light scattering and electron microscopy, establish that MsHSP16.1 forms three distinct oligomer sizes. nES MS experiments conducted on a modified QTOF-MS [2] reveals that the polydisperse assembly is composed of exactly 12-, 18-, and 24-subunits. The absolute collisional cross section

(Ω) of each of these oligomers were measured using a modified Waters Synapt HDMS with a linear drift cell. By using well-conserved structural properties of other sHSP, a series of model oligomers structures based on well-defined polyhedra were constructed and their theoretical Ω were calculated. This approach allows us to establish the overall shape of the 12-mer, 18-mer, and 24-mer of MsHSP16.1 assemblies to be tetrahedron, trigonal bipyramid, and octahedron, respectively. This knowledge will help high resolution structural refinement of MsHSP16.1 by EM reconstruction.

keywords: small heat shock protein, nanoelectrospray, ion-mobility, quaternary, assembly, protein complex, polydisperse

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AUTOMATED LIBRARY GENERATION FOR UNTARGETED METABOLOMICS STUDIES

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A major limitation for untargeted metabolomics studies is the generation of a library or target component list from the deconvoluted mass spectral data files. In order to achieve this, a script was written to compile a library from the results output of SpectralWorks AnalyzerPro. A set of 24 plasma samples from pigs were extracted and analysed using an Agilent 5973N GC-MS single quadrupole instrument. Generation of a library from this data set initially yielded a library with 2146 components, which reduced to 660 components after manual quality control. Analysis of the same samples using an in-house target component list of 390 components generates an average of only 102 matches that can be used for subsequent analysis (e.g. PCA). The same samples were analysed by GCxGC-TOF-MS using a LECO Pegasus 4D. Automated library generation was performed using the LECO ChromaTOF software. Generation of a library from these samples yielded 3689 components, which reduced to 679 components after manual quality control. The key limitation of this technique is the requirement to manually quality control the data set. To overcome this, we are currently exploring the integration of the process into AnalyzerPro. Automated library generation is a relatively simple option for strengthening metabolomics data sets and taking better advantage of the data generated by mass spectral analysis.

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NANOSCALE ZWITTERIONIC-TYPE HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY OF COMPLEX AND HIGH MANNOSE TYPE GLYCANS COUPLED WITH HIGH RESOLUTION TIME OF FLIGHT MASS SPECTROMETRY

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Therapeutic glycoproteins are a fast growing group of pharmaceuticals and the glycosylation plays a key role in biological activities of the drugs. The expression of glycoproteins in various mammalian systems can produce glycosylation patterns different to humans which can reduce effectiveness and stability of the drugs and can

cause immune reactions in patients. The standard approaches for glycan profiling include fluorescent labelling of the enzymatically released glycans and separation by hydrophilic interaction liquid chromatography (HILIC) coupled with fluorescence detection to achieve appropriate sensitivity and relative quantification. The standard procedure is time consuming and it does not provide direct structural information, therefore coupling with mass spectrometry (MS) is required for the structural elucidation. In order to simplify the sample preparation and improve sensitivity in electrospray ionisation (ESI) MS, we used nanoscale zwitterionic type hydrophilic interaction liquid chromatography (ZIC-HILIC, 75 μm ID) for separation of reduced high mannose and complex-type glycans from ribonuclease B and monoclonal antibodies. Compared to the standard bore column, by using nano ZIC-HILIC approximately 70 fold increase in sensitivity was achieved. Due to improved sensitivity, a range of minor glycan species which could not be identified by conventional ZIC-HILIC was observed. The use of high resolution time of flight mass spectrometer enabled the prediction of structures of less common glycans with high confidence.

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RAPID MONITORING OF SULFUR MUSTARD DEGRADATION IN SOLUTION BY HS-SPME GC-MS

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A method using headspace solid-phase microextraction (HS-SPME) followed by gas chromatography mass spectrometry (GC-MS) analysis has been developed to monitor the degradation of the chemical warfare agent sulfur mustard in solution. This approach involved the periodic sampling of the reaction headspace and allowed for the near real-time analysis of up to 12 samples per hour. The alternative liquid-liquid extraction (LLE) method involved quenching the degradation reaction by extraction into dichloromethane and subsequent sample preparation for GC-MS analysis. While both HS-SPME and LLE approaches provided similar results, the HS-SPME method significantly simplifies the analysis for the more rapid monitoring of sulfur mustard hydrolysis.

The utility of HS-SPME monitoring was further demonstrated by the investigation of the degradation process in a complex matrix with surfactant added to assist solvation of sulfur mustard. Again, both HS-SPME and LLE approaches provided similar results indicating a more rapid reduction in sulfur mustard abundance. This demonstrates the potential of HS-SPME as a rapid screening tool for aqueous decontaminant formulations.

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USING LC-MS-MS AND MALDI-TOF-MS TO STUDY THE LINK BETWEEN THE INCREASED RISK OF CARDIOVASCULAR DISEASE AND HIV PATIENTS PRESCRIBED THE ANTIRETROVIRAL ABACAVIR

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Abacavir is a reverse transcriptase inhibitor of HIV and is administered to HIV-patients in conjunction with other inhibitors as part of their Highly Active Antiretroviral Therapy (HAART). A recent Data Collection on Adverse Events of Anti-HIV Drugs (DAD) study showed that patients exposed particularly to abacavir had a significantly higher risk of myocardial infarction. This study describes the use of LC-MS-MS to identify the aldehyde intermediate arising from the alternate ADH-dependent metabolism of abacavir. This intermediate

drew many similarities in both structure and electrophilic properties to acrolein, which has been implicated in the increased risk of myocardial infarctions in smokers due to its modification of cholesterol efflux proteins. MALDI-TOF-MS together with timed-course cellular assays showed that the intermediate modified the protein Apo-A1, hampering its efficiency to efflux cholesterol from macrophages. In summary, the results provide a novel insight to the peculiar metabolism of abacavir which can only be obtained from long-term post-market studies. In addition, this study provides a framework in which other pharmaceuticals with long-term adverse effects can be studied.

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THE USE OF LC-MS-MS AND ELISA FOR THE ENVIRONMENTAL MONITORING OF DINOPHYSISTOXINS IN SINGAPORE'S COASTAL WATERS

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Okadaic acid (OA) and its derivatives are potent lipid soluble toxins released by the marine *Dinophysis* dinoflagellate species. These toxins are involved in occurrence of Harmful Algal Blooms (HABs) in various coastal regions across the world including Singapore. Human consumption of OA accumulated shellfishes has been reported as a key contributor to Diarrhetic Shellfish Poisoning (DSP). This study describes the use of a simple liquid-liquid extraction sample preparation method followed by liquid chromatography-tandem mass spectrometry (LC-MS-MS) for the quantitative analysis of okadaic acid in seawater. Biochemical assays using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) were also carried out to validate the LC-MS-MS results. The lowest limit of detection (LLOD) was 0.5 ppb and the limit of quantification (LOQ) was 50 ppb. Inter-day precision was 15% RSD with linearity greater than 0.999 for OA. Application of the method to samples collected around Singapore's coastal waters commonly known for public recreation or as ship docking sites showed undetectable levels of free OA.

In summary, a simple and high throughput method has been developed for the quantification of OA in seawater samples. This method can be employed for other large scaled environmental screening programs of chemically related toxins.

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N-OR HOMOLYTIC BOND CLEAVAGE OF HINDERED AMINE LIGHT STABILISERS PRESENT IN POLYESTER-BASED COIL COATINGS

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Detection and characterisation of structural modifications to ten hindered amine light stabilisers (HALS) directly from a polyester-based coil coating has been achieved by desorption electrospray ionisation mass spectrometry (DESI-MS). In situ detection is made possible by exposing the polymer-based coating to an acetone vapour atmosphere prior to analysis. This is a non-destructive treatment that allows diffusion of analyte to the surface without promoting lateral migration. A major structural modification to the N-ether HALS (Tinuvin® 144 (bis(1,2,2,6,6-pentamethyl-4-piperidinyl)-((3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl)methyl)butylmalonate), Tinuvin® 123 (bis (1-octyloxy-2,2,6,6-tetramethyl-4-piperidinyl) sebacate) Tinuvin® 152 (2,4-bis(N-butyl-N-(1-cyclohexyloxy-2,2,6,6-tetramethyl-4-piperidinyl)amino)-6-(2-hydroxyethylamine)-1,3,5-triazine) and Tinuvin® NOR371) was discovered where at least one N-ether piperidine moiety (N-OR) in each case is converted to a secondary piperidine (N-H). The modification was observed with the use of 2-dimensional DESI-MS imaging, occurring due to high curing temperatures (ca. 260 °C) and under simulated physiological conditions (80 °C, full solar spectrum). It is proposed that the secondary piperidine derivative is a result of a highly reactive aminyl radical intermediate produced by N-O homolytic bond cleavage. The nature of the bond

cleavage is also suggested by ESR spin-trapping experiments employing α-phenyl-N-tert-butyl nitrone (PBN) in toluene at 80 °C. The homolysis of N-O and O-R bonds of the alkoxyamines formed resulting from termination of polyester radical fragments (R1, R2 and R3) by HALS in the nitroxyl radical form were also studied by computational methods. Calculated Gibbs free energies for bond homolysis indicate that O-R cleavage is predominant for R1 and R2, however for R3 both types of homolysis are characterised with very similar values of reaction energy with a slight preference for N-O cleavage. The presence of a secondary piperidine derivative in situ and the implication of N-OR competing with NO-R bond cleavage suggests alternative pathways for activation and regeneration to the nitroxyl radical – an essential requirement in the HALS anti-oxidant catalytic cycle.

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QUADRUPOLE ION TRAP MASS SPECTROMETRY STUDY OF LA^{3+} AND ER^{3+} ATTACHMENT TO 3-HYDROXYFLAVONE, 5-HYDROXYFLAVONE AND 5-METHOXYFLAVONE.

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Positive-ion electrospray ionisation ion trap mass spectrometry has been used to investigate the reactions of the model flavonoids 3-hydroxyflavone, 5-hydroxyflavone and 5-methoxyflavone with La^{3+} and Eu^{3+} metal cations. La^{3+} and Er^{3+} are found to show similar complexation behaviour. The ESI mass spectra of 3-hydroxyflavone and 5-hydroxyflavone show singly and doubly charged species e.g., $[L+H]^+$, $[5L-H-M]^{2+}$, $[6L-H-M]^{2+}$, $[3L-2H-M]^+$, $[4L-2H-M]^+$, $[5L-2H-M]^+$ and $[6L-2H-M]^+$, whereas 5-methoxyflavone displays singly, doubly and triply charge species $[L+H]^+$, $[3L+M]^{3+}$, $[4L+M]^{3+}$, $[5L+M]^{3+}$, $[6L+M]^{3+}$, $[3L+M+NO_3]^{2+}$ and $[4L+M+NO_3]^{2+}$. CID experiments have been used to reveal the fragmentation behaviour of the metal-flavonoid complexes. The complex ion $[3L-2H+M]^+$ both for La^{3+} and Er^{3+} with 3-hydroxyflavone and 5-hydroxyflavone show loss of ligand or loss of ligand with subsequent addition of water. The 5-methoxyflavone complexes of the type $[4L+M]^{3+}$ also show ligand loss as the dominant process. The multistage tandem mass spectrometry (MS^4) of $[6L-H+La]^{2+}$ for 3-hydroxyflavone and 5-hydroxyflavone show common ions $[5L-H+La]^{2+}$, $[3L-2H+La]^+$ and $[4L-2H+La]^+$. When 5-methoxyflavone complex $[6L+La]^{3+}$ is subjected to MS^4 the predominant losses are one or two ligands to generate $[3L+La]^{3+}$, $[4L+La]^{3+}$ and $[5L+La]^{3+}$. When $[6L+Er]^{3+}$ is selected for MS^4, the only loss is the ligand L in each step of the CID process. This work has been extended to more complex, naturally occurring flavonoids having multiple binding sites for metal complexation.

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A SIMPLE STRATEGY FOR ACQUIRING *PRODUCTS OF ALL* USING TOF; IN APPLICATION TO LIPID CHARACTERIZATION.

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It remains highly desirable to develop a sensitive and reproducible approach to collecting CID fragmentation spectra, of every precursor, in an untargeted fashion. Using hybrid time-of-flight technology, it is now possible to collect survey scan independent driven MS/MS while combining high resolution, mass accuracy, and

quantitation over several orders of linear dynamic range. When applied to *shotgun* lipidomics, TOF MS and MS/MS approaches are now capable of characterizing complex lipid extracts by direct infusion without a preliminary need for HPLC or UPLC fractionation of lipid class and sub-classes[1]. This is carried out by a simplified acquisition technique which combines high sensitivity precursor ion scanning with information independent product ion spectra of every precursor, selected at 0.7 Da, throughout a given mass range. By storing of all TOF product ions, fast quadrupole scanning enables lipid-class specific profiling through the extraction of any lipid fragment ion to back-profile those precursors in a 300-1250 m/z range. Additionally, a high resolution TOF MS survey scan is looped within the acquisition to supplement the precursor profile with high mass accuracy measurements for optimal lipid identification. This approach can yield lipid quantitation through a broad dynamic range if precursor ion selection can be carried out better than UNIT resolution with high sensitivity in high speed. Also, if proper lipid internal strategies are employed and signals are corrected for isotopic distributions, this workflow can be both quantitative and qualitative for polarity specific lipid profiling in a single acquisition without the requirement for chromatographic separation. This technique represents a fast and easy acquisition strategy to profile lipids from complex biological extracts. Through the nanoelectrospray infusion analysis of very small lipid extract volumes, global lipid profiling experiments shows the identification of lipid species from 6 different lipid classes and 15 sub-classes encompassing a total of over 835 lipid species identified in a single polarity specific acquisition. This single acquisition approach offers reproducible quantitation, with% CV under 7%, when measuring the response of lipid species against their lipid class – specific internal standards.

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LOVE, MARRIAGE AND BETRAYAL IN THE CORAL SEA: UNDERSTANDING THE POSSIBLE ROLES OF LIPIDS IN THE CORAL-DINOFLAGELLATES SYMBIOSIS AND CORAL BLEACHING

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Coral reefs are built upon the relationship between hard coral hosts and their photosynthetic dinoflagellate symbionts. Intracellular dinoflagellates utilise host waste products and provide photosynthate derived metabolites in return. The coral-dinoflagellate symbiosis, a marriage across species as it were, requires that the dinoflagellate negotiates the corals immune defenses. A breakdown in the communication is tantamount to a betrayal of the relationship! This manifests as coral bleaching events where dinoflagellates are expelled from the coral tissues in responses to environmental stress.

Currently, the molecular mechanisms by which symbiosis is both controlled and disrupted are not fully understood. It has been shown that lipid quantity and composition change under different environmental stimuli and stress. However, the role of lipids in symbiosis remains unknown. Therefore, a HPLC-MS method for the identification of unknown polyunsaturated fatty acids was developed as part of a wider study.

Fragmentation patterns for a range of fatty acid standards were studied using tandem ion trap mass spectrometry. It was found that for fatty acids with five or more double bonds, four neutral losses were consistently observed. These were the neutral losses of 44, 78, 98 and 136 Da. Knowledge of these fragmentation processes was then applied to a model system by identifying fatty acids from an extract of the symbiotic sea anemone, *Aiptasia pulchella*. Using HPLC-MS, fatty acids were first separated and then analysed by mass spectrometry in a data dependant fashion. Precursor ions displaying all four neutral losses were identified as fatty acids with five or more double bonds. Chain length and degree of unsaturation for these fatty acids was easily determined from the *m/z* of the precursor ions. Using this technique the unusual fatty acids, 24:6, 26:6, 26:7, 28:7 and 28:8 were unambiguously identified in an *Aiptasia* lipid extract in a single 40 minute HPLC-MS analysis.

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HIGH THROUGHPUT PESTICIDE RESIDUE SCREENING USING GC-QQQ-MS AND LC-QQQ-MS IN DIFFERENT GRAIN MATRICES AND THE IMPACT FOR THE ANALYSIS OF GRAIN DUST SAMPLES

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Pesticide, herbicide and fungicide residue analysis of grains are subject to false positives and false negatives due to matrix effects causing enhanced response with GC-MS and ionisation suppression with LC-MS.

In GC-MS analysis of residues, the sample matrix can play a dual role to provide enhanced sensitivity and therefore limits of detection and quantification. The matrix protects pesticides in the inlet system and on column from thermal degradation and also deactivates active sites. As a consequence, matrix matched standards are necessary to avoid false positives. Some groups use a mixture of sugars and acids as a substitute for matrix matched standards although the effect is substantially reduced with highly deactivated inlets.

In contrast, for LC-MS the matrix suppresses electrospray ionisation and this has the effect of reducing sensitivity and therefore limits of detection and quantification. Dilution of samples can reduce matrix suppression, and cleanup with gel permeation chromatography (GPC) is used to remove matrix from the sample. However, removal of matrix adds considerably to sample preparation throughput and cost.

For LC-MS analysis of a given sample type, the matrix suppression as a function of retention time is reproducible and can be modelled to correct for recoveries in place of matrix matched standards.

The availability of blank matrix for food samples is a challenge and the substitution of matrix types can also lead to false positives and false negatives as well. The mixing of matrices has also been used to compensate for matrix effect; however, to date this has proved unsuccessful for grains.

In this work, matrix matched standards have been used to minimise false positives with GC-MS and false negatives with LC-MS and achieve limits of detection as low as 0.5 ppb for routine quantitative screening for in excess of 100 residues in grain. Results will be presented for a range of grain types to highlight the effectiveness of this approach.

The benefit of scheduled MRMs in lowering limits of detection and quantification will be presented.

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LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS-MS) FOR THE SIMULTANEOUS QUANTITATIVE ANALYSIS OF KETAMINE AND MEDETOMIDINE IN OVINE PLASMA

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Ketamine and medetomidine are commonly combined to sedate or anaesthetise a wide range of animal species. Despite this, there are few methods for the simultaneous quantitative analysis of the two drugs. This study describes the use of solid phase extraction (SPE) sample preparation followed by liquid chromatography-tandem mass spectrometry (LC-MS-MS) for the quantitative analysis of both drugs in ovine plasma. Extraction recovery was 93% for ketamine and 95% for medetomidine. Lowest limit of detection (LOD) for ketamine was 1 ng/mL and 2 ng/mL for medetomidine, with linearity greater than 0.99 for both. Intra-day and inter-day precision for both drugs was less than 10% and 7% respectively. Application of the method to samples obtained from pregnant ewes and their fetuses showed placental transfer of the drugs over time such that there was no significant difference in plasma concentration at delivery.

In summary, a validated method has been developed for the simultaneous quantification of ketamine and medetomidine in ovine plasma samples which can be used to study the pharmacokinetics of these drugs.

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WILDLIFE PROTECTION: PESTICIDE POISONING IDENTIFIED USING A UPLC-TOF SCREENING APPROACH

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Introduction In the environmental monitoring arena the use of ToF screening approaches has steadily increased. Screening of environmental matrices for pesticide contamination is especially important because the use of pesticides has steadily increased, in an effort to meet global food demands. Pesticides, which were applied to crops, can potentially cause harm to plants and wildlife. Birds of prey are particularly vulnerable to poisoning by pesticides because they frequently feed on the insects, grubs, rodents and small mammals that are killed by pesticide compounds. These birds also fall prey to intentional poisoning, when people spike pesticides into carrion, either to kill animals such as foxes, or to target the birds themselves. Part of the work undertaken by SASA involves analysing samples taken from suspected poisoning cases. In this work the carcass of a wild red kite was analysed after suspicion that it had been poisoned by pesticides. Objective To use UPLC-Xevo QTof pesticide screening to analyse extracted samples from the gullet contents of a wild red kite suspected of poisoning by agricultural pesticides. Data were processed using POSI±IVE software and screened using a targeted list of approximately 500 pesticides. Further confirmation of identity of the poisons was achieved using the Elemental Composition tool in MassLynx and MassFragment software. Results and Conclusion Broad-scope pesticide screening of the extracted red kite samples enabled the detection and identification of the pesticides used to poison the bird of prey. Carbosulfan and carbofuran were identified as the pesticides used in this poisoning case. The accurate exact mass ToF data for both the parent ions and related fragment ions, along with the retention times of the compounds, proved a match to the database entries for carbosulfan and carbofuran. Additional confidence in the results was afforded by the use of the Elemental Composition tool to provide the formulae.

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PHARMACEUTICAL DRUG UPTAKE AND METABOLISM IN THE FRESHWATER CRUSTACEAN GAMMARUS PULEX

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Human pharmaceutical products are frequently released into surface waters, via the processing and discharge of human waste, or the intentional disposal of medicines into domestic wastewater. Their constant emission into the environment in these ways results in pharmaceutical products being considered “pseudo-persistent”, which causes significant, on-going analytical challenges. Although ever-present, these compounds are found at very low levels, which mean that suitably sensitive and selective analysis techniques are required; and since pharmaceutical drug molecules are designed to cross cell membranes, and be biologically active in humans, they could potentially be harmful to environmental organisms. While significant consideration has been given to the fate of parent drug molecules in environmental waters, there has been less focus on the behaviour of potential metabolites. In this work we describe the application of Waters ACQUITY UPLC-Xevo QTof, with the Elemental Composition tool within MassLynx and MassFragment software, to the analysis of both freshwater crustacean (gammarus pulex) extracts and their corresponding water habitat. Gammarus pulex were collected from their native freshwater habitat and transferred to a controlled laboratory water habitat. The laboratory water was spiked with 0.5 mg/L diazepam. Samples of the crustacean were removed at regular time intervals and a portion of this sample was extracted and analysed immediately, while the other portion of the sample was transferred to a clean water laboratory habitat for a depuration study. Using this methodology we were able to both confirm the presence of the parent drug, diazepam, and detect and unequivocally identify the metabolite,

nordiazepam, in the gammarus pulex and the water samples. Using the Xevo QTof mass spectrometer in MSE mode enabled the simultaneous acquisition of low energy precursor ion data and high energy fragment ion data, providing extensive sample information in one single run. By acquiring data from samples over regular time periods it was possible to illustrate the gammarus pulex's uptake of diazepam over time, and its production of the nordiazepam metabolite.

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THE USE OF LC-MS AND RELATED SOFTWARE TO DETECT AND IDENTIFY PROTEIN-BASED MARKER COMPOUNDS IN FRAUDULENT FOODSTUFFS

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The authenticity of the food we eat, and the accuracy of food labelling, is of major importance to both consumers and food producers alike. As food testing techniques become increasingly advanced new areas of concern can be brought to light. One such area is that of meat speciation – the ability to show, unequivocally, that a certain meat product contains components solely from the species stated on the label. While it is likely that most consumers want to be confident that they know what they are eating, this issue can have much greater significance for consumers whose diets are restricted for religious reasons. A recent U.K. Food Standards Agency study found that chicken breast, and powders used as water-retaining agents for chicken breast, contained proteins not only originating from poultry but also from pork and/or beef sources. These findings suggest that some chicken products could, potentially, be unsuitable for consumers who, for whatever reason, do not eat pork or beef. This study also highlights the need for a rigorous and reliable testing method to identify the origins of meat products, which will ensure full transparency for consumers and enable food producers to substantiate their label claims. In collaboration with the U.K.'s Food and Environment Research Agency (FERA), Waters®, advanced UltraPerformance Liquid Chromatography® (UPLC) with novel mass spectrometric technology and powerful proteomics-specific software tools enabled the analysis and identification of key peptide markers unique to individual species. After a routine tryptic digest, chicken, beef, pork and fish gelatines were analysed using nanoACQUITY UPLC-Xevo™ QTof MS. The unique MSE functionality of the Xevo QTof quadrupole time-of-flight mass spectrometer allows the analyst to simultaneously acquire both low-energy exact mass precursor ion spectra, and high-energy exact mass product ion spectra. These, information rich, data were then processed using powerful search and quantification algorithms employed in Waters' ProteinLynx™ Global Server (PLGS) v2.4. Incorporated within PLGS, IdentityE software utilises chromatographic retention time, exact precursor and product ion masses and novel "Ion Accounting" informatics to characterise complex protein digest mixtures, allowing reliable and reproducible detection of proteins from their tryptic peptides.

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ADDRESSING CHEMICAL DIVERSITY USING ATMOSPHERIC PRESSURE GC (APGC)

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There has been an increase in the utilisation of liquid chromatography-mass spectrometry (LC/MS) with atmospheric pressure ionization to analyse for a wide range of compounds in recent years although gas chromatography (GC) coupled to MS is still the only option for a significant number of analytes. Atmospheric pressure ionisation has primarily been used to interface MS with LC, but it is also a powerful ionisation method that can be applied to GC. A novel atmospheric pressure ion source (APGC) for tandem quadrupole and quadrupole time-of-flight LC/MS instruments is described, allowing laboratories to switch rapidly between LC

and GC applications to analyse compounds traditionally analysed by dedicated vacuum GC/MS instruments. Ionization of a GC eluent at atmospheric pressure is a softer process, giving molecular weight information for compounds which are extensively fragmented in traditional Electron Ionization (EI). Another key advantage is that GC separations can be optimized as the carrier gas flow and column dimensions are not limited by the pumping capacity of the MS. Atmospheric pressure mass spectra for a selection of compounds will be presented and contrasted with EI spectra. The focus will be some traditionally difficult compounds analysed in food safety and environmental applications.

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SITES OF METABOLIC SUBSTITUTION: DEFINITIVE METABOLITE STRUCTURES DEDUCED USING ION MOBILITY AND MOLECULAR MODELLING

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As an analytical technique, mass spectrometry (MS) cannot separate isomeric species or provide conformational information. Structural information can be gained through tandem MS (MS/MS) techniques. There are classes of substituted molecules, such as hydroxylated metabolites which produce identical MS/MS spectra. This is problematic if the exact position of the hydroxylation is required. Ion-mobility (IM) has the ability to separate isomeric species, such as hydroxylated metabolites, rapidly (msec) based on differences in their collision cross-section (Ω) in the gas-phase, thus providing specific information on ionic configuration and therefore, the position of the hydroxyl moiety. Experiments were performed using a hybrid quadrupole/ion mobility/orthogonal acceleration time-of-flight Synapt G2 HDMS instrument. Ion mobility separation was performed at a pressure of 3.2 mbar nitrogen with a wave velocity of 1100 m/sec and a travelling wave amplitude of 40 V was used. Theoretical Ω values were calculated using MOBCAL and compared to the travelling wave derived Ω values. Three-dimensional metabolite conformations were explored with the "Systematic Search" protocol in MOE. Ten thousand combinations of possible values for variables were explored. All conformations were minimised with the MMFF94 forcefield. The ten conformations with the lowest energy were selected for each metabolite. All three hydroxylated metabolites showed different UPLC chromatographic elution times. However, the MS and MS/MS spectra are all identical, thus hampering identification. The Ω value of the parent compound ondansetron and the three hydroxylated metabolites GR60661, GR63418 and GR90315 were measured by UPLC-IMS-MS. Theoretically derived Ω values for hydroxylated metabolites GR60661, GR63418 and GR90315 are 111.4 Å2, 111.2 Å2 and 109.8 Å2 respectively. Travelling wave ion mobility derived collision cross-section derived values are in very close agreements with the theoretically derived values, thus allowing accurate identification of the hydroxylated metabolites, even those with a collision cross-section difference of less than 1 Å2. No additional structural characterization techniques, such as NMR or X-ray crystallography were required for metabolite structural determination. This is a very time efficient and effective means of identifying drug metabolites directly from biological matrices. Additionally this method can be utilised in a situation where chemically synthesised standards may not be available.

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ANALYSIS OF DRUG RESIDUES IN MILK BY ON-LINE SPE/LC/MS/MS

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World wide countries are under constant vigilance to ensure the health safety of their citizens. In current agricultural practice, raising animals for food depends heavily on the use of drugs, which is essential to the animal's health and for the economics of the industry. Veterinary drugs or residues can enter human food and

increase the risk of ill health in persons who consume products from treated animals. As a consequence, many government agencies have established maximum residue levels (MRLs) for a variety of veterinary drugs in animal tissues, milk and other food products. Unlike drinking water, the analysis of drug residue in food products requires elaborate sample extraction and clean up protocol to minimize matrix effects. However, one major drawback is the high amount of manual labor required to produce a clean extract. A typical extraction protocol usually starts with a sample homogenization in an aqueous or organic buffer. The homogenate is then centrifuged, concentrated and cleaned up with a wide range of extraction techniques, such as liquid-liquid, dispersive or by SPE, just to name a few. With recent advances in turn-key systems, many steps can be automated and provide an immediate benefit, a reduction of manual labor needed to produce a final extract. The coarse extract can then be further refined with an ON-line SPE/LC/MS/MS system. This research will discuss the performance of ON-line SPE/LC/MS/MS for the analysis of drug residues in milk. The milk samples were subjected to a protein precipitation with acetonitrile (1:1 ratio), followed by a centrifugation at 3500 rpm for 15 minutes. The supernatant was collected and diluted with 3 mL of water. The dilution is intended to reduce the organic percentage at 20 % to avoid breakthrough during the loading phase. The entire volume (5mL) was injected onto an ON-line SPE/LC/MS/MS system for enrichment, cleanup and analysis. The results showed good signal-to-noise ratio at low ppb levels.

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PESTICIDE MONITORING TO MEET REGULATORY REQUIREMENTS BY THE DIRECT INJECTION OF DRINKING WATER

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In recent times the use of pesticides, herbicides and fungicides has steadily increased, in an effort to meet global food demands. A percentage of such chemicals applied to crops will, inevitably, end up leaching into the surrounding soil and waterways. Strict environmental monitoring is mandated at a local or federal level, which endeavours to sustain or improve water quality, preserve the environment and protect human health. Tandem quadrupole mass spectrometers provide the ‘gold standard' for ultra trace analysis, offering a combination of high selectivity, high sensitivity and the greatest confidence in results. The performance of some of these instruments has enabled some methods to dispose of sample preparation, improving laboratory productivity, with drinking water samples being stabilised and then directly injected onto the LC/MS/MS instrument. However, difficulties remain when trying to discriminate against matrix components that exhibit similar physiochemical properties. Unawareness of these difficulties in each sample can lead to poor quality results and can impact on a laboratory's performance and reputation. Conventional tandem quadrupole systems when operated in multiple reaction monitoring (MRM) mode do not allow the direct monitoring of the sample matrix during routine high sensitivity determinations. A novel high performance tandem quadrupole instrument coupled with ACQUITY UPLC, along with TargetLynx software data processing, was used to rapidly determine pesticides with the direct injection of 100µL drinking water. All MS parameters were recalled from Quanpedia - a “one stop” database for storing and sharing user-defined acquisition and associated processing methods for the quantification of named compounds. A generic UPLC acidified water/methanol gradient was used, with a total run time of 10 minutes. A unique capability of monitoring the matrix simultaneously, without compromising the performance, during ultra trace multi-residue pesticide analysis was also investigated. Pesticides were successfully determined with high precision at the ng/L level with the ability to detect to pg/L concentrations.

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QUANTITATIVE PHOSPHORYLATION ANALYSIS OF ENDOCYTIC PROTEINS DURING MITOSIS

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Protein phosphorylation is one of the most important post-translational modifications, and plays a key role in regulating signal transduction pathways. We have discovered that endocytic proteins are associated with mitotic progression and completion. Many of these proteins are mitotically phosphorylated suggesting their mitotic function is regulation by phosphorylation. However, all phospho-endocytic proteins and their specific phospho-sites have not been determined. We carried out a global phosphoproteomic approach to identify all phopho-sites in all endocytic proteins and quantitate the change in phosphopeptide levels in Hela S3 cells during mitosis. Lysates from asynchronously growing HeLa S3 cells and HeLa S3 cells in early mitosis (pro-metaphase), and late mitosis (cytokinesis) were enriched for endocytic proteins using a key selection of GST-tagged proteins in a sequential pull-down assay. Each sample was digested on-beads or in-gel, triple dimethylation labeled and TiO_2 purified. Identification and quantitation of phospho- and non-phospho-peptides were performed using an LTQ Velos Orbitrap mass spectrometry. Mascot distiller was used for processing data. Manual verification was also performed for identification of specific phospho-peptides. Quantitative analysis of these phospho-peptides at different stages of the cell cycle revealed that several phospho-sites within a subset endocytic proteins are regulated in a manner analogous to Ser-764 in the endocytic protein, dynamin II, i.e. increased upon mitotic entry, then decreased during cytokinesis. These findings will enable us to understand how the functions of these endocytic proteins are regulated to ensure efficient progression through mitosis and to successful complete cytokinesis.

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ABSORPTION SPECTROSCOPY VIA LASER INDUCED MOBILITY MODIFICATION

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A new method for absorption spectroscopy is being developed. A custom designed ion mobility spectroscopy drift chamber is under construction and will be integrated with a tuneable laser in an attempt to detect absorption of light by looking for differences between laser-on and laser-off arrival time distributions. This apparatus will be used to probe ions that are not responsive to existing methods for absorption spectroscopy.