

ADVANCES IN AUTOMATED METABOLITE IDENTIFICATION USING DE-CLUSTERING AND DATA-DIRECTED MS/MS EXPERIMENTS WITH AN ORTHOGONAL TIME OF FLIGHT MASS-SPECTROMETER

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Within the Pharmaceutical and Biotechnology industry LC-MS and LC-MS-MS with atmospheric pressure ionisation is widely used for both quantitative and qualitative bioanalysis in different biological fluids for DMPK studies. With instrumentation continually improving, and sample throughput capability increasing due to technology which using parallel analysis such as MUX, sample analysis is no longer the bottleneck. The major obstacle is becoming the ability to rapidly process the acquired data in order to extract the required information. This is apparent when looking at low level metabolites, which are difficult to see in the TIC and some data manipulation is necessary to identify these putative metabolites. A software application manager (MetaboLynx) has been previously reported (ref 1) which allows the automated processing of LC-MS data to detect and report expected and unknown metabolites. This automates the routine and labor-intensive of extracting metabolite peaks from complex chromatograms and presents the results in a reduced data set for rapid review. It will also compare a metabolism sample with a matrix control sample to eliminate some of the peaks due to endogenous components. As an additional filter to help distinguish drug-related compounds from endogenous peaks, the software can automatically set up a subsequent MS/MS acquisition and compare the resulting product ion spectra from the parent drug and the putative metabolites to determine if they are related. MetaboLynx has now been further developed to allow improved filtering and more rapid generation of the required data. The mass accuracy of the orthogonal time-of-flight data has been used to enhance filtering of the data by the use of an improved algorithm for comparing the matrix control with metabolised samples and also in pulling specific isotope patterns from LC-MS data. Results of the processing of data from the metabolism of bromoaniline will be shown where filtering the data on the basis of the bromine isotope ratio and the accurate mass difference between the isotopes gives confident assignment of bromoaniline metabolites. Improved throughput has been achieved by incorporating data directed MS/MS acquisitions with automated processing to generate both MS and MS/MS from a single acquisition. Results will be presented showing data generated by this approach.

Ref 1. Pittsburgh Conference, Orlando, March 7-12 1999, no313.
