

# PROFILING THE MAMMALIAN METABOLOME AND ITS APPLICATION TO TOXICITY PREDICTION USING HIGH TEMPERATURE AND PRESSURE UPLC/MS-OA-TOF

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## INTRODUCTION

The analysis of small molecules in complex biological samples as in, for example, metabolomic or metabolomic studies, where the aim is the non-specific (global) profiling of a very wide range of endogenous metabolites provides a significant analytical challenge for global systems biology. The detection and identification of biomarkers of disease and toxicity represent one of the keys to faster drug development and improved medicines. The identification of many of these biomarkers is often carried out using LC/MS and LC/MS/MS, either alone or in combination with other analytical techniques.

A new approach to obtain parent ion and fragmentation information in UPLC/MS studies of small molecules in complex mixtures is presented using simultaneous acquisition of exact mass at high and low collision energy, MS<sup>E</sup>. The system has been used for the analysis of a model toxin in rat urine. In this example the composition of rat urine was studied and the structure of several endogenous components to be confirmed in one analytical run by the simultaneous acquisition of exact mass precursor and fragment ion data.

LC/MS<sup>14</sup> has been used to provide data to enabling the detection of novel markers via, the generation of elemental composition and fragment ions. Normally obtaining this information requires several experiments with MS experiments used to obtain molecular ions and MS/MS experiments to obtain the fragment ion data. Here the application of a new form of MS and MS/MS data acquisition is described. This new method of UPLC/MS<sup>E</sup> data acquisition uses an intelligent approach where parallel alternating scans are acquired at either low collision or high collision energy to obtain full scan accurate mass fragment, precursor ion and neutral loss information. All of these data are obtained from a single analytical run, with the benefit of both the precursor and product ion data are collected in exact mass mode.

Here we demonstrate how UPLC/MS<sup>E</sup> has been combined with a new novel statistical approach to data analysis to allow the visualization and detection of new novel biomarkers of toxicity.

## METHODS

### UPLC conditions

System: ACQUITY UPLC™  
Column: ACQUITY UPC BEH C<sub>18</sub>  
Mobile Phase: 1.0 x 150 mm, 1.7 µm  
A = 0.1% formic acid,  
B = 95% acetonitrile, 0.1% formic acid  
Gradient: 0-0.5 min 100% A, 0.5-4 min 0-20% B, 4-8 min 0-100% B, 8-9 min 100% B  
Flow rate: 250 µL/min  
Injection Volume: 1 µL  
Column Temp: 90 °C

### MS<sup>E</sup> Data Acquisition

Instrument: Q-ToF Premier™  
Mode: Positive ion electrospray  
Range: 100-850 m/z  
Collection Mode: Centroid  
Cone Voltage: 30 V  
Capillary Voltage: 3200 V  
Collision Energy: 5 eV or 25 eV  
Desolvation gas: 300 L/hr @ 250 °C  
Cone gas: 0 L/hr, cone temp = 120 °C  
Acquisition rate: 0.1 sec with 0.05 inter-scan delay  
LockSpray™: Leucine-enkephalin (m/z 556.2771) at 300 fmol/µL

### Animal Studies

The animals were divided into 3 groups and dosed orally with either vehicle alone (control) low dose (2mg/kg) and high dose (80 mg/kg). Urine was collected from a the Wistar derived strain rates by minimal bladder manipulation. Samples were collected -16, 0, 8, 24, 48, 72 and 96 hrs after dosing. Food (standard rat and mouse diet) and water was available ad libitum prior to sample collection. The samples were stored at -20 °C prior to analysis.

## RESULTS

The data below shows the UPLC/MS separation of rat urine prior to the oral administration of hydrazine. We can see from this data that the peaks are extremely sharp with peak widths in the order of 1 second at the base, Figure 1.

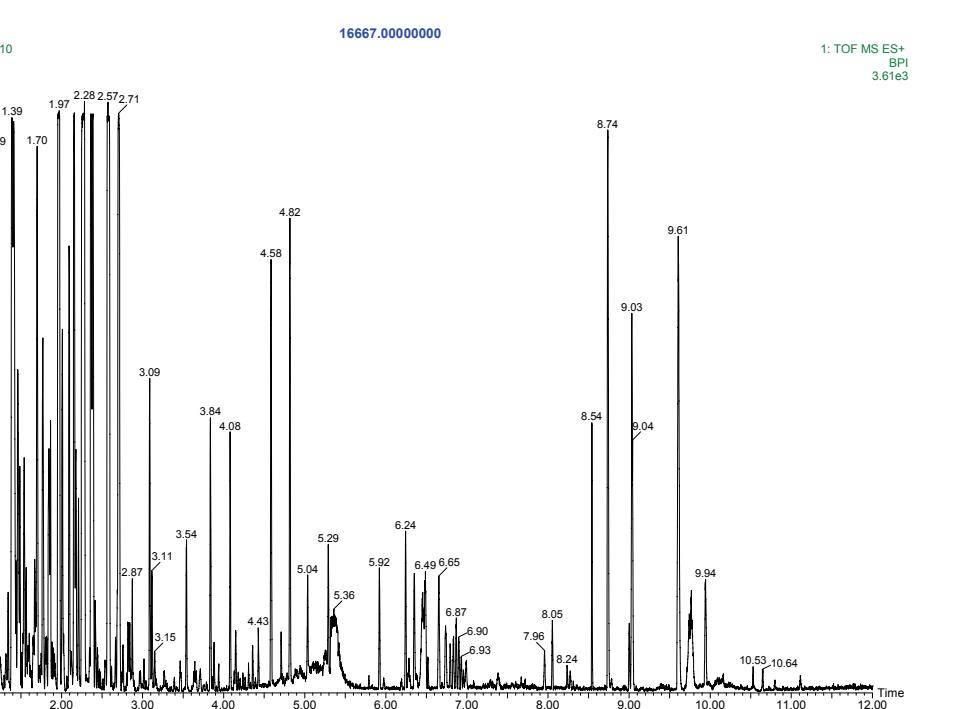


Figure 1. Positive ion TIC of rat urine

Despite the very narrow nature of these the mass spectrometer was still able to collect data at a sufficiently fast rate to obtain good accurate mass data, Figure 2.

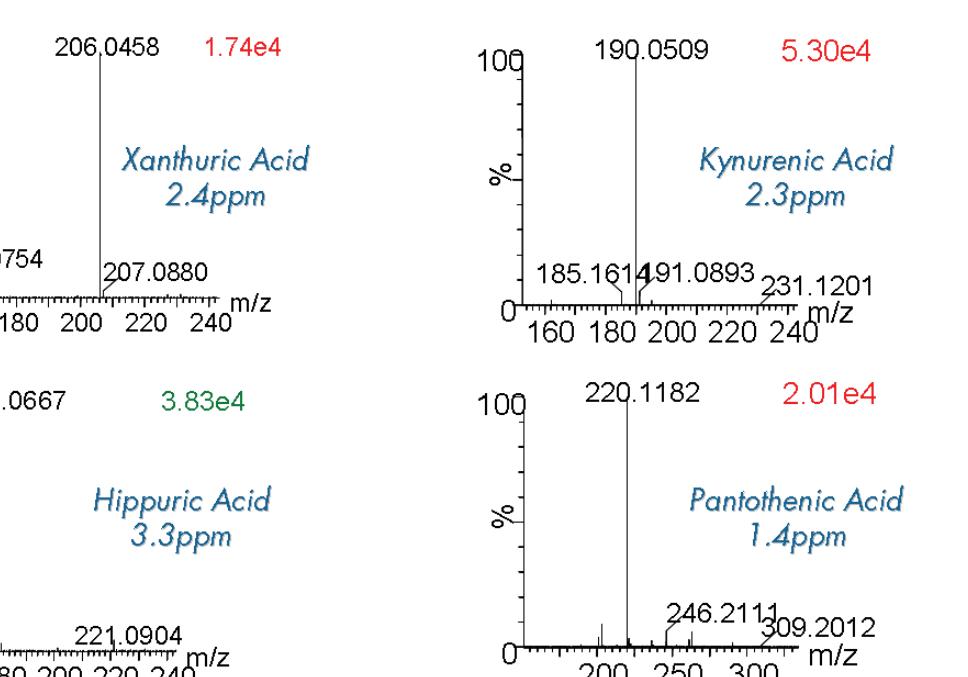


Figure 2. Extracted spectra from urine chromatogram

The ability of the mass spectrometer to collect both low and high collision energy MS data to provide precursor and product ion information is displayed in Figure 3. Here we can see the low and high collision energy data obtained for xanthureic acid in rat urine using MS<sup>E</sup>. Both were obtained under accurate mass spectrum obtained, the data showed excellent mass accuracy.

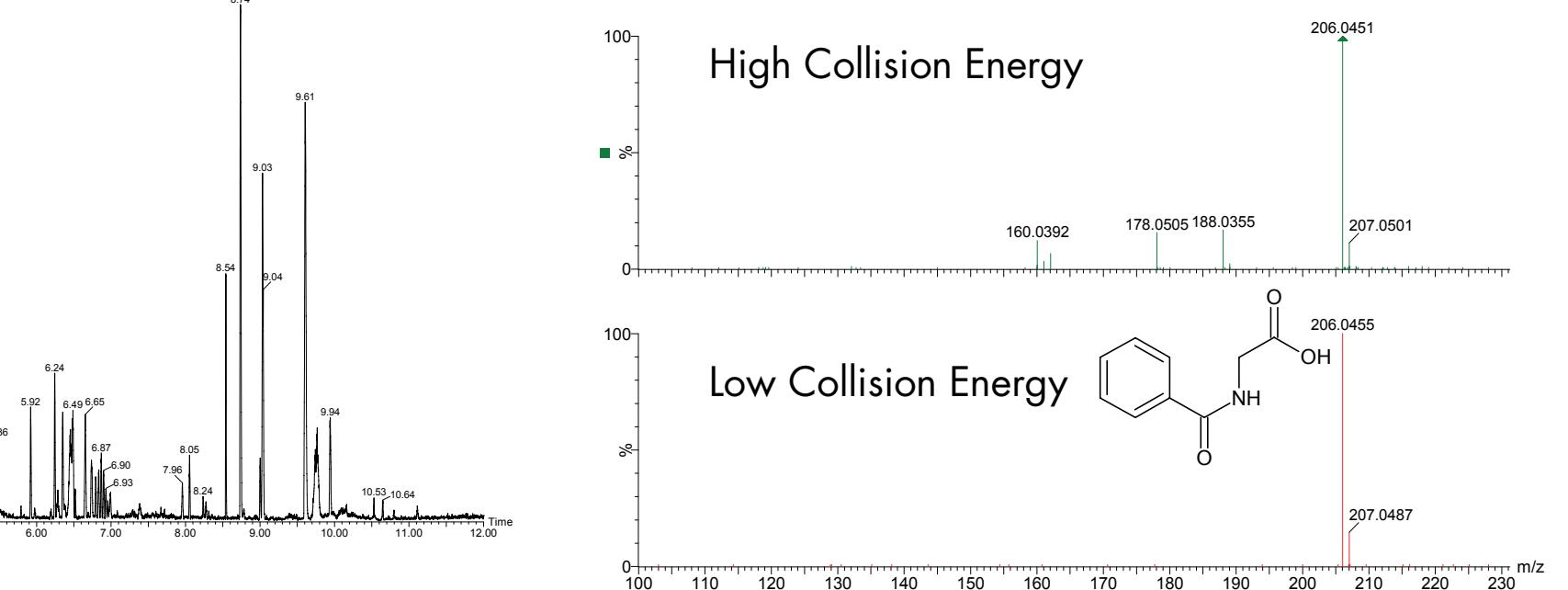


Figure 3. High and low collision spectra of xanthureic acid in rat urine

Previously, to obtain this information it was necessary perform several LC/MS/MS analytical runs followed by data analysis, to fully characterize all of the components of interest in a complex sample. This is very time consuming and labor intensive. MS<sup>E</sup> produces an "information rich" data set which can be mined in many different ways to produce precursor ion, fragment ion, neutral loss and common fragment ion analysis to name but a few. A comparison of the data flow for traditional LC/MS and LCMS/MS and UPLC/MS<sup>E</sup> is given in Figure 4.

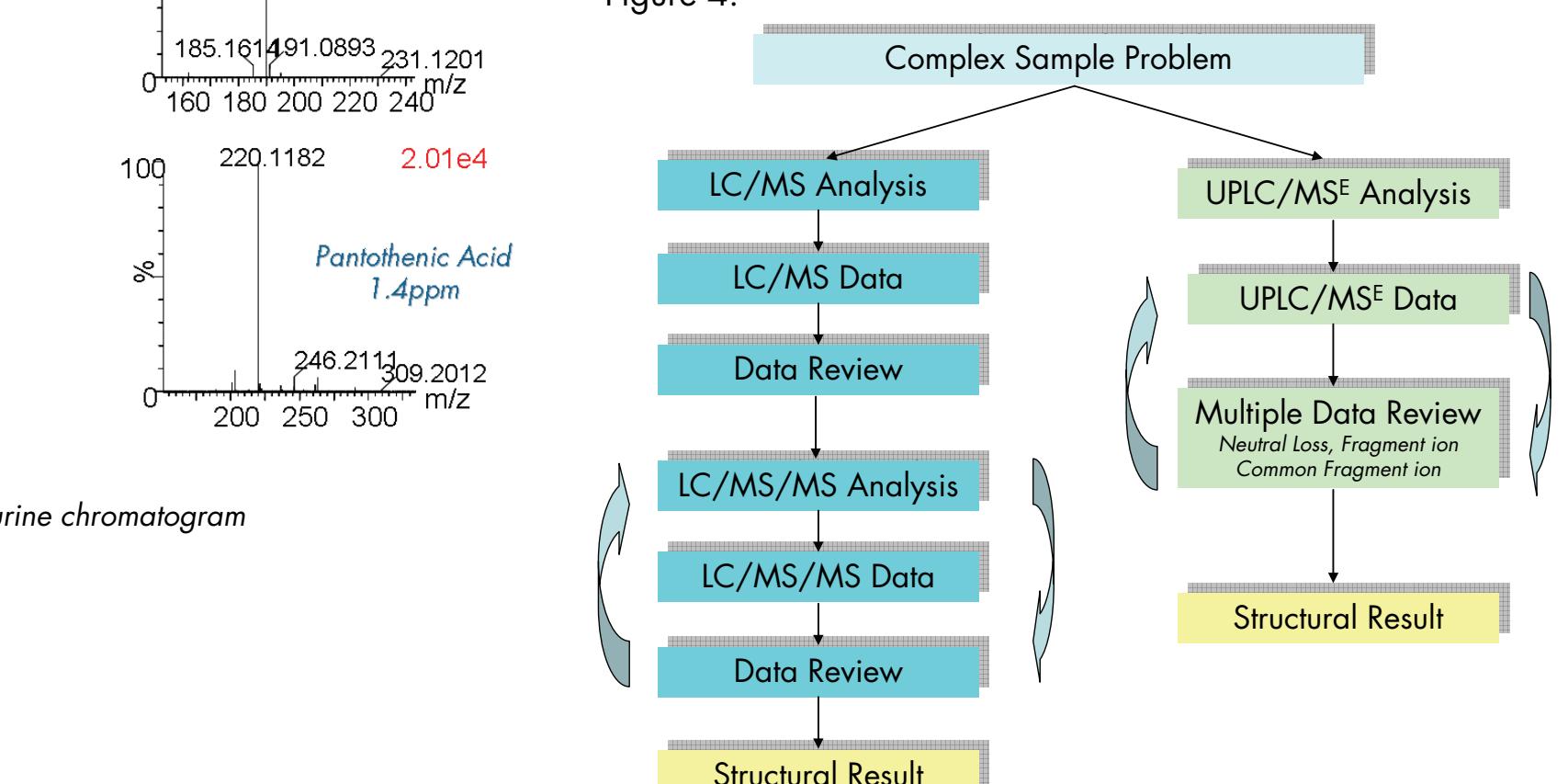


Figure 4. UPLC/MS<sup>E</sup> data flow

This approach to MS data collection was employed for the analysis of urine samples obtained from rats following the oral administration of hydrazine. The data was processed using the Masslynx™ Applications Manager Markerlynx™ and Principal Components Analysis (PCA) to reveal ions contributing to the statistical variation in the data. The UPLC/MSE data was further analysed to reveal the identity of these ions. One of the ions contributing to the separation of the low and high dose samples was identified as m/z = 190. The UPLC/MSE data was analysed to obtain precursor and fragment ion data of this ion, figure 5. This ion was identified as kynurenic acid.

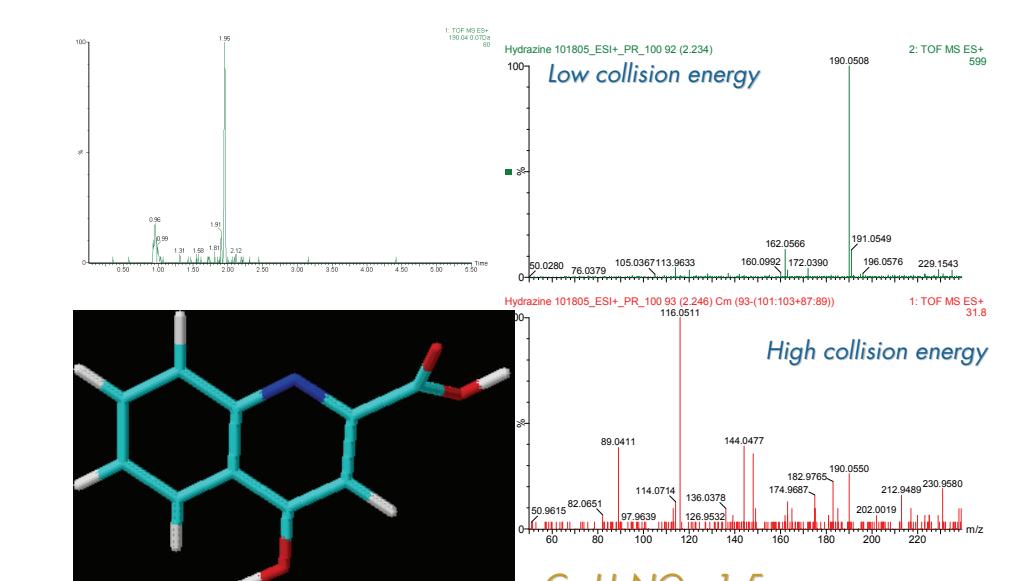


Figure 5. MS<sup>E</sup> analysis of Kynurenic acid

The resulting MS and MS/MS data obtained from the statistical analysis of the data was investigated to reveal potential biomarkers, using their MS elemental composition and MS/MS fragmentation patterns. The resulting ions are displayed below in Table 1 along with their potential identities. Table 1.

m/z	Putative identity	Effect of dosing*	Pathway involvement
402.10	phosphopantethenyl cysteine	↓	coenzyme-A biosynthesis
358.11	L-stercobilinogen fragment	↑	bile pigment excretion
351.19	prostaglandin-E3	↓	anti-inflammatory hormone
311.11	5-ribosylyrophosphate / ribulose-1,5-bisphosphate	↑	purine biosynthesis in liver
290.12	N-succinyl-2-amino-6-oxopimelate	↓	uncertain
265.05	phenylacetylglutamine	↑	nitrogen excretion
260.09	glucose-6-phosphate / glucosamine-6-phosphate	↓	energy metabolism
245.16	biotin	↑	uncertain
219.08	N-acetylserotonin	↑	neurotransmitter
218.11	propionyl carnitine	↑	fatty acid metabolism
217.09	glucuronate-N <sup>+</sup> adduct spermine	↑	detoxification
203.13	190.03	↑	cellular metabolism regulation
190.03	kynurenic acid	↑	neural regulation
164.07	4-hydroxyglutamic acid	↑	arginine & proline metabolism
162.06	2-aminoadipate	↑	lysine catabolism
147.07	2-dehydropantoate	↓	coenzyme-A biosynthesis
132.06	creatine	↓	biomarker of liver dysfunction
90.05	L-alanine / sarcosine	↑	amino acid urea cycle

## CONCLUSION

- The application of UPLC/MS<sup>E</sup> for biomarkers has been demonstrated.**
- Data is collected at a sufficiently high rate to allow for excellent chromatographic peak definition.**
- The low and high collision energy data produce precursor and product ion data allowing component identification.**
- The application UPLC/MS<sup>E</sup> significantly improves analytical throughput reducing the number of experimentally steps.**
- Using this approach it was possible identify several new markers of hepatotoxicity from hydrazine.**

## References

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