A NOVEL APPROACH TO THE IDENTIFICATION OF BIOMARKERS USING THE CROSS CORRELATION OF LOW - HIGH COLLISION ENERGY UPLC/TOF-MS: A METABONOMICS APPROACH

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OVERVIEW

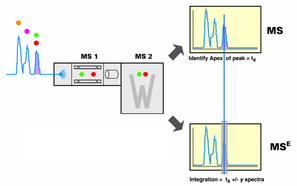
The rapid detection and identification of biomarkers of disease and toxicity is critical for modern drug discovery. In this paper we high collision energy thus both precursor and fragment ions can be acquired simultaneously under exact mass conditions. The precursor illustrate the use of a new novel approach to the acquisition of MS data. LC/MS data was collected with alternating low and high collision energy thus both precursor and product ions were collected simultaneously under exact mass conditions. The precursor and fragment ions were linked using retention time and the mass defect. The data produced was processed using 2D peak integration and multivariate statistics to identify ion of interest and the structures were identified by exact mass and fragmentation patterns.

INTRODUCTION

One of the major scientific challenges of the 21st century will be determining the relationship between the human genome and disease risk. Understanding the relationship between the genome, the proteome and the expressed endogenous metabolites requires the development of ever more powerful analytical chemistry techniques and data interpretation tools. In the Metabonomics arena, this task is normally carried out by LC/MS with exact mass analysis and multivariate statistical analysis.

Ions of interest identified by the multivariate analysis are subsequently reanalyzed by using LC/MS/MS for structural ID. This involves the reanalysis of several samples in order to obtain all of the necessary structural information for the compounds of interest. Traditionally, mass spectrometers lacked the MS/MS duty cycle required to collect product ion scan information for thousands of metabolite ions simultaneously.

In this paper; however, we will describe how the application of the simultaneous acquisition of both precursor and product ion by LC retention time and the application of mass deficiency filters has been applied to this task. The use of this approach removes the need for time consuming reanalysis of valuable samples to obtain MS and MS/MS data. An added benefit is that both the precursor and product ion information is obtained with exact mass.



METHODS

Animal Studies

Urine samples were collected from male obese fa/fa Zucker rats and control rats (BABU, Alderley Park) aged 4 and 20 weeks. The animals were housed in polypropylene cages and allowed free access to water and food. Animals were maintained at room temperature with artificial 12 h dark/12 h light cycles. Samples were stored at -20°C. Prior to analysis the samples were centrifuged at 13,000 rpm and a 100µL aliquot of the supernatant was diluted 1:4 with distilled water and transferred to a total recovery auto-sampler vial for analysis by UPLC-MS.

UPLC Chromatographic Conditions

System:	ACQUITY UPLC™ with PDA detector
Column:	ACQUITY UPC™ BEH 2.1 x 100mm 1.7µm C18
Mobile Phase:	A = 0.1% formic acid, B = 95% acetonitrile, 0.1% formic acid.
Gradient:	0-0.5mins 100% A, 0.5-4 mins 0-20% B, 4-8 min 0-100%B, 8-9mins 100%B, 9.1 mins 100% A.
Flow rate:	500µL/min
Injection Vol:	2µL
Column Temp:	40°C

Mass Spectrometry Conditions

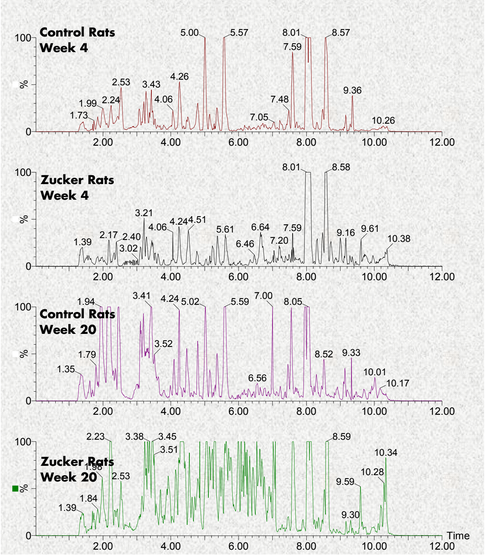
Instrument:	Q-Tof micro™
Mode:	Positive ion electrospray
Range:	100-850 m/z
Collection Mode:	Centroid
Cone Voltage:	30V
Capillary Voltage:	3200V
Collision energy	5eV or 25eV
Desolvation gas:	200L/hr @ 200°C
Cone gas:	0 L/hr @ 120°C
Acquisition rate:	0.1 Sec with 0.05 inter scan delay
LockSpray:	leucine-enkephalin (m/z 556.2771) 300 fmol/µL In 50/50 MeOH/H_2O @ flow rate 30µL/min, frequency 5s.

RESULTS & DISCUSSION

Chromatography

The Zucker rat urines showed a slight difference in their chromatographic profile on week 4 compared to the control animal urine however by week 20 this difference is quite marked, Figure 1.

Figure 1: TIC of Control and Zuckers Rat Urine on WK 4 & 20



We can see from this data that the urinary profile of the Zucker rats becomes increasingly complex on week 20 whereas the control animal urine changes to a lesser degree. The MS data generated two separate channels of data, one at low collision energy and one at high collision energy. These two separate sets of data were processed independently, Figures 2 and then combined Figure 3 by PCA using MarkerLynx™ application manager. We can see in Figure 2 that the Zucker and control animals are clearly separated, with the early Zucker rats clustering with the control animals and then moving further away from the control rats with increasing age. The ions identified as contributing significantly to the variance in the data are highlighted in Table 1. For comparison purposes the low collision energy ions of interest were subjected to analysis by conventional MS/MS. The data in Table 1shows that the resulting MS/MS ions were in fact the ions that contributed most in the high collision energy MS PCA analysis.

Figure 2: Principal Components Analysis Scores & Loadings Plot of Low Collision Energy Data

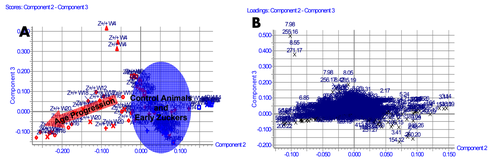


Figure 3: Principal Components Analysis Scores and Loadings Plot of Low and High Collision Energy Data

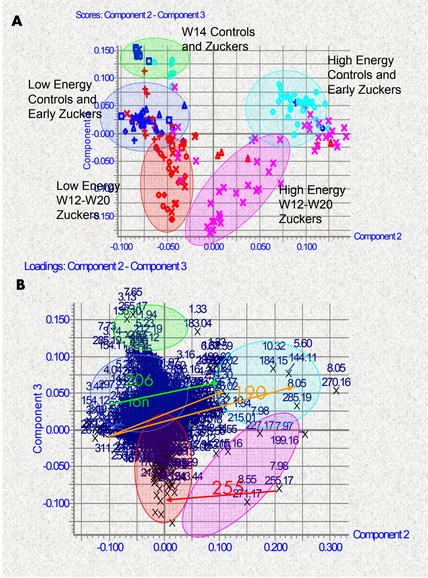


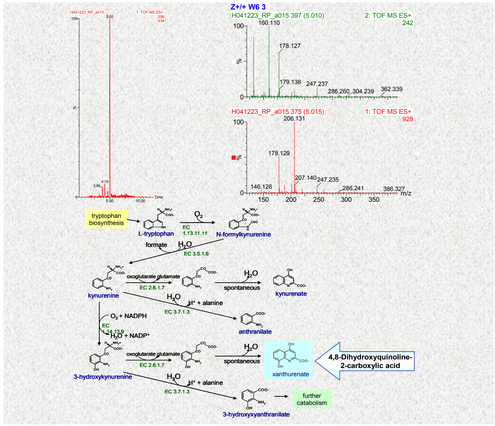
Table 1: Summary of Linked Low and High Energy Ions

Major Marker ions from low collision energy data	Fragment ions from LC/MS/MS analysis	Major Marker ions from high collision energy data
297.16	132.08	132.08
190.05	162.06, 144.04	144.06
206.06	178.08,160.09, 132.07	178.08, 160.09, 132.06
674.83	144.97	160.05

In the loadings plot we can see not only the molecular ions but also the fragment ions clearly identified. In order to link the related precursor ion to the corresponding product ions we can use not only the ions retention times but also the mass deficiency. Thus if we look at the m/z = 206.131ion, the proposed related fragment ions are 178.127, and 161.110, each of these ions has a mass defect within 20mDa of the precursor ion. Thus we can use this information to identify/confirm linked precursor and product ions in the analysis of the whole data set without the need to resort to time consuming MS/MS analysis.

This m/z = 206 ion was determined to be xanthurate and is part of the tryptophan catabolism pathway, probably associated with gut microflora metabolism, Figure 4.

Figure 4: LC/MS of xanthurate and metabolic pathway of tryptophan



CONCLUSIONS

- **UPLC / MS combined with PCA was able to clearly distinguish between control and diseased (Zucker) animals.**
- **MS and MS/MS data acquired in the same run—no need for time consuming reanalysis of precious samples.**
- **Both precursor and product ions acquired with exact mass.**
- **Precursor and product ion data was linked by use of retention time and mass defect.**
- **From the data it was possible to visualize age related changes in the Zucker over the 20 week period.**

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