DISCOVERY AND IDENTIFICATION OF PRECURSOR IONS TO PRE-DEFINED PRODUCT AND NEUTRAL LOSS EXACT MASSES BY HPLC-CID-MS FOR METABOLISM STUDIES

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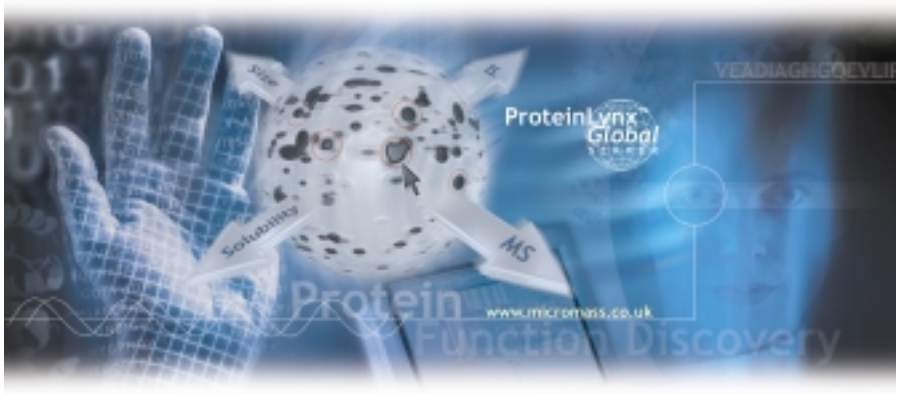

GC-MS

LC-MS

LC-MS-MS

MALDI-MS

ICP-MS

LC-ICP-MS

IR-MS

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Overview

Precursor ion and neutral loss acquisitions are commonly used in drug metabolism studies for the selective detection of drug related compounds. For example, neutral loss acquisitions may be used to
search for common losses produced from metabolites formed by sulfate or glucuronide conjugation.
These modes of acquisition have previously only been possible, within a chromatographic time frame,using tandem quadrupole mass spectrometers. This paper presents data from the in vivo metabolism ofdiclofenac (*o*-(2,6-dichlorophenyl)aminophenylacetic acid), a commonly prescribed non-steroidal anti-inflammatory drug (NSAID), acquired using a novel acquisition mode on a quadrupole-time of flight hybrid mass spectrometer (Q-TOF). This mode allows the discovery and identification of precursor ions based upon the detection of pre-defined product ion and neutral loss exact masses.

Introduction

Often in metabolism studies the use of neutral loss acquisitions proves to be a very valuable tool for selective searching for common losses like glucuronides and sulfates. Previously, this work has been carried out with triple quadrupoles using nominal masses. The advantages of the hybrid quadrupole-time of flight mass spectrometer are the speed with which candidate precursor ions may be identified and corresponding MS/MS spectra acquired, and the selectivity that can be achieved by defining exact masses for both product ions and neutral losses. This enables the method to be used with HPLC, and also enables survey functions to be carried out with a very high level of specificity; thus eliminating matrix related interference that may occur when analysing complex biological matrices.

Precursor ion discovery is achieved by operating the Q-TOF using a survey function with the quadrupole operating in broad bandpass mode and the collision cell pressurised with argon. Alternate low-energy and high-energy (CID) spectra of the column eluent are acquired with real-time lock mass correction being used for exact mass measurement. After the acquisition of each low/high energy pair the data may be interrogated automatically to search for pre-defined product ions or pre-defined neutral losses that are present above a set threshold and within a mass tolerance window of typically +/- 10mDa.

If a pre-defined product ion is detected in the high-energy spectrum then MS/MS is automatically performed on any candidate precursor ions identified in the low energy spectrum.

For neutral loss detection, if sequential high and low energy spectra contain ions separated by the pre-defined mass difference then the MS/MS product ion spectrum will be automatically acquired for the precursor ion. The system then returns to the alternating low and high-energy survey scan mode. This cycle may be carried out within the time frame of a chromatographic peak.

Method

A dose of 200mg/kg diclofenac was administered to a rat via i.p. A urine sample was collected over the time period 8-24 hours post-dose and the sample was diluted 1:4 in water prior to injecting 5uL directly onto an HPLC column without prior extraction.

HPLC system:	Waters Alliance HT 2790 Separations Module	
Column:	Waters Xterra MS C18 3.5µmm, 2.1 x 100mm	
Mobile phase A:	10mM aqueous ammonium formate + 0.1% formic acid	
Mobile phase B:	10mM ammonium formate in 90:10 acetonitrile: water + 0.1% formic acid	
Flow rate:	0.2ml/min	
Gradient:	0 mins	98%A 2%B
	2mins	98%A 2%B
	10mins	80%A 20%B
	20mins	30%A 70%B
	25mins	0%A 100%B
	26mins	98%A 2%B
	30mins	98%A 2%B
MS:	Micromass Q-Tof Ultima	
Ion mode:	Electrospray, negative ion	
Low energy survey:	5eV	
High energy survey:	40eV	
MS/MS gas cell:	25eV (argon)	
Lock mass:	Sulphadimethoxine $(M-H)^-$ at 309.0658	

Schematics of Q-Tof Ultima API W and Lock Spray

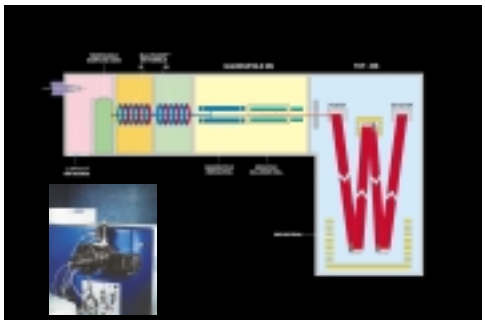

Figure 1

Proposed Metabolic Pathway for Major Metabolites of Diclofenac

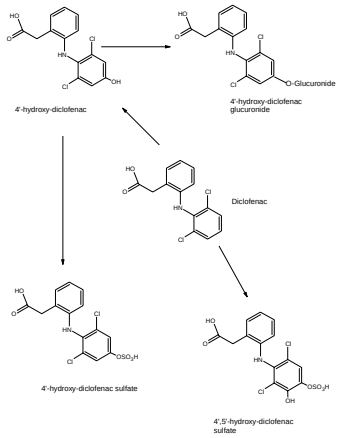

Figure 2

Results

Precursor Ion Discovery

The precursor ion scan mode searches for all ions that decompose to give a common diagnostic fragment (**Figure 3**). In this case the fragment of interest is the glucuronide ion at m/z 175.0243.

Figure 3

Figure 4 shows the TIC chromatograms for the low and high energy survey scans as well as the chromatogram for all the MS/MS precursor ions triggered by the detection of the m/z 175 glucuronide ion.

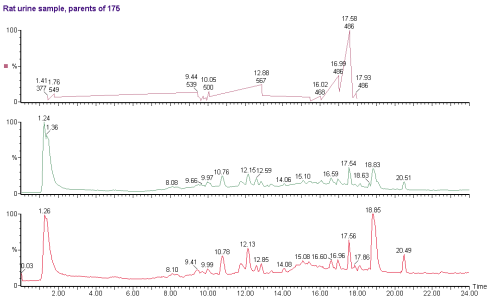

Figure 4

Figure 5 shows the low and high energy scans and the resulting MS/MS product ion spectrum from m/z 486.04 eluting at 17.58minutes. The advantage the Q-Tof has over a triple quadrupole is that both the survey scans and MS/MS spectra are acquired at high resolution and use of LockSpray facilitates exact mass selection of the ion of interest. The elemental composition report shows that all the major ions have exact mass measurements within 5ppm of the expected mass.

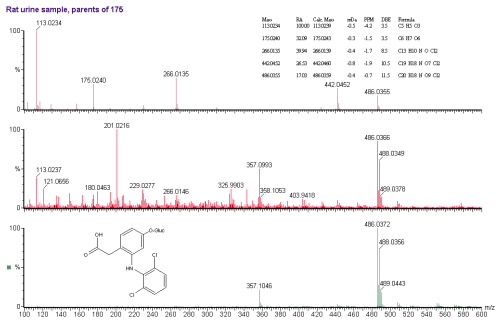

Figure 5

Figure 6 shows the m/z 175 region of the high energy spectrum expanded to show a potentially interfering isobaric ion at m/z 174.9571from the matrix (67.2mDa difference from the glucuronide ion) which could not be resolved on a triple quadrupole. The resolution shown is approx. 14,000 FWHM.

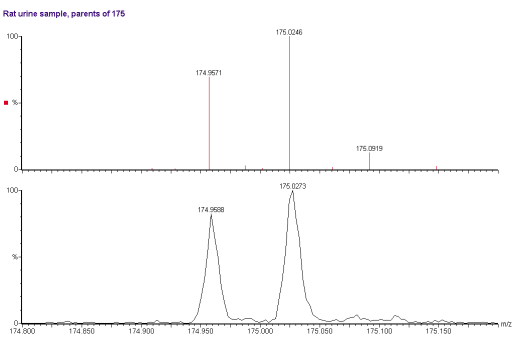

Figure 6

Exact Neutral Losses

Exact neutral loss scanning was used to monitor for the presence of both sulfate and glucuronide conjugated metabolites within one acquisition by searching for the accurate neutral loss of 79.9568 and 176.0321 respectively (**Figure 7**).

Figure 7

Figure 8 shows TIC chromatograms from the low and high energy survey scans and the chromatogram produced by the switching to MS/MS triggered by the detection of the exact neutral loss of either a sulfate or glucuronide. If the neutral loss is not detected in the MS/MS product ion spectrum the instrument immediately switches back to the low energy survey scan mode.

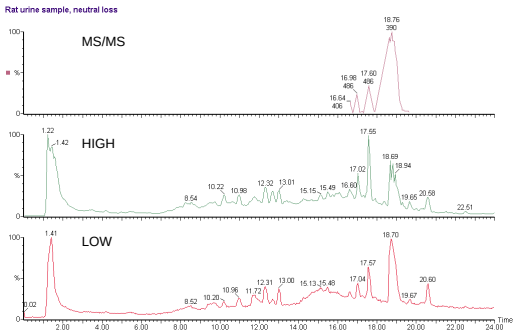

Figure 8

Figure 9 shows the low and high energy survey scan spectra which resulted in the instrument switching to acquire the MS/MS product ion spectrum from the glucuronide conjugate eluting at 17.0 minutes. The 176.0321 ($C_6H_8O_6$) neutral loss can be clearly seen between the $(M-H)^-$ ion at 486.0357 and the fragment at m/z 310.0045 in the high energy survey scan.

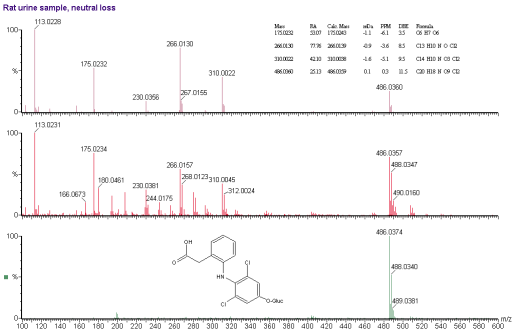

Figure 9

An example of the neutral loss of a sulfate moiety (79.9568) from the 4′-hydroxy sulphate metabolite (m/z 389.9603) eluting at 18.7 minutes and resulting in a fragment ion at m/z 310.0060 can be observed in **Figure 10**.
The MS/MS spectrum was submitted to ACD software for spectral interpretation (**Figure 11**).

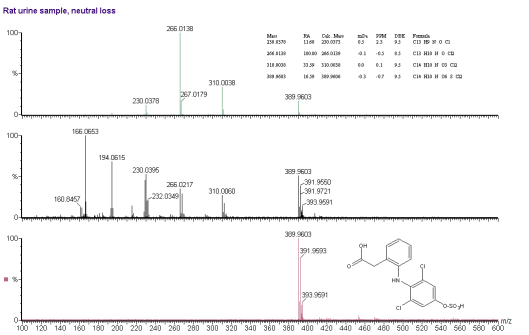

Figure 10

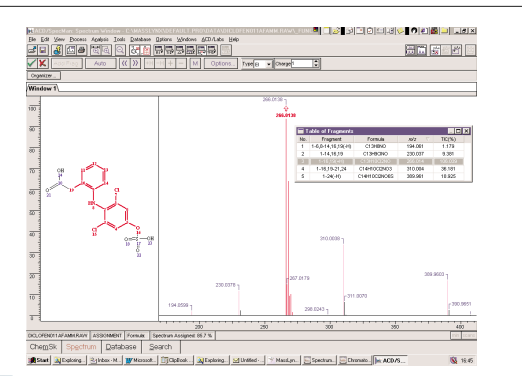

Figure 11

Conclusion

We have shown a novel method for the automated discovery and detection of precursor ions and neutral losses within chromatography time frames using the accurate mass and high resolution capabilities of a quadrupole -time of flight mass spectrometer.

Metabolites containing sulfate and glucuronide conjugates were found by searching for their accurate neutral loss of 79.9568 and 176.0321 respectively with a tolerance window of +/- 10mDa. The glucuronide conjugates were also identified using precursor scanning mode by searching for the fragment at m/z 175.0243. The mass accuracies found for the major metabolites were better than 5ppm.

These low level metabolites were easily detected and identified in the high level of endogenous materials associated with urine samples with no prior clean up and with much higher sensitivity than a scanning quadrupole instrument. The exact mass product ion spectra generated can greatly aid structural elucidation and identification of unknown metabolites.

References

In-vivo Metabolite Identification of Diclofenac in Rat Urine using Capillary LC coupled with a Hybrid Quadrupole Time of Flight Spectrometer. Jose Castro-Perez, Chris Hughes, Steve Preece, Olivia Corcoran and Jeremy K. Nicholson. 17th Montreux Symposium 2000.

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