A HIGH DUTY CYCLE APPROACH FOR METABOLITE IDENTIFICATION USING ALTERNATING COLLISION ENERGY SCANS MS^E WITH A Q-TOF INSTRUMENT AND DATA MINING ALGORITHMS

Jose Castro-Perez[1], John Shockcor[1], Kate Yu[1], Jane Kirby[2], David G. Temesi[3],*
[1]*Waters Corp., 34 Maple St, Milford, MA, USA*
[2]*Waters MS technologies, Floats Road, Wythenshawe, Manchester, UK*
[3]*AstraZeneca, Alderley Park, UK*

INTRODUCTION

Metabolite identification is very challenging for both *in vitro* and *in vivo* samples. The information obtained from such studies is very important for lead optimization, identification of new leads, and selection of safety species. Typically in metabolite id a different number of analytical strategies are used for both chromatography and mass spectrometry. Usually, the same samples require multiple analyses to obtain the desired information for fragment ions, metabolite confirmation and structure elucidation. In this work, we describe a new method that enables the collection of both parent and fragment information from a single chromatographic run.

Data was acquired using a Waters QTof Premier mass spectrometer (Figure 1). The inlet used for this study was an UPLC™ system.

The data was acquired using two alternating scan functions[1] with the first quadrupole using a wide band mode rf. The first scan function collected information about the intact (5 eV) metabolites and the second scan function used a collision energy ramp to collect fragment ions (15 eV-30 eV). This approach provided a great deal of information, such as metabolite masses, precursor, product ions, and neutral losses. Data visualization and alignment between high and low energy scans was accomplished through a new software tool MetaboLynx MS^E designed to mine both data sets simultaneously. We illustrate this approach using Propanolol incubated with rat hepatocytes at 10 µM level.

By the use of this approach we were able to determine all the metabolites expected and unexpected from the low energy acquisition. Moreover, we were also able to obtain important common product ion information from the high energy scan which allowed a very easy correlation between drug and its metabolites. Neutral loss chromatograms were also generated from the data using exact mass differences between the precursor and fragment ions. Since the data was acquired with no preconceptions on the likely routes of metabolism or precursor ion information, this approach has the potential to be truly comprehensive and universal in its use for *in vivo* and *in vitro* metabolite identification.

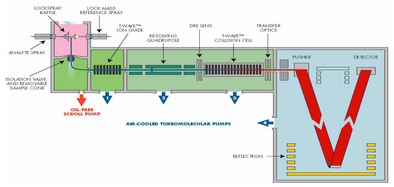

Figure 1. Schematic of QTof Premier

METHODS

Samples

Fresh hepatocytes were prepared from male Sprague-Dawley rats. Propranolol (10 µM) was incubated with hepatocytes. The samples were incubated at 37°C for 0 and 60 minutes gently shaken under an atmosphere of 5% CO2/95% air. The reaction was terminated with ice-cold acetonitrile. After having done that, the samples were centrifuged and the supernatants obtained were analyzed.

LC-MS Methodology

Mass Spectrometer: Q-Tof Premier™
MS scan range: 70-900 Da
Mode of Operation: +/-ve ion mode ESI
V-mode, pDRE (dynamic range enhancement)
Lock Mass: Leucine Enkephalin at 200pg/mL

MS^E Methodology:
The QTof Premier was operated in a parallel data acquisition mode with a wide band RF mode in Q1(Figure 2). Thus, allowing all ions in the collision cell. This resulted in one single injection in which data was collected under one single data file with two functions. These were;
Function 1) Low energy acquisition (5eV) which contained the unfragmented compounds
Function 2) High energy or MS^E acquisition (15eV-30eV ramp) which contained all of the fragmented ions

LC-conditions:
Acquity UPLC™
Acquity BEH C18 Column 100x2.1mm id, 1.7µm
Mobile phase A: 0.1 % formic acid
Mobile phase B: Acetonitrile
Flow rate: 0.6 mL/min
Gradient: 0 min 98% A, 0-10 min 20% A, 10-11 min 0% A, 11-14 98%A
Injection volume: 10 µL

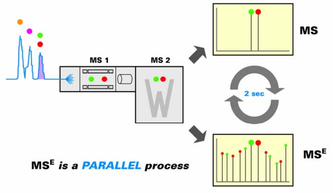

Figure 2. MS^E data acquisition mode with the QTof Premier

Description of software algorithm for data processing -MetaboLynx MS^E - How does it work?

MetaboLynx is a software application manager, which automatically detects putative biotransformations for expected and unexpected metabolites. The application manager automatically runs samples scheduled for analysis by LC/MS and processes the resulting data. Results are reported via a 'Data Browser' that enables the chromatographic and mass spectroscopic evidence that support each automated metabolic assignment to be rapidly reviewed locally or remotely via a secure corporate network.
It operates by comparing and contrasting each metabolised sample with a control sample—although unexpected metabolite searching may still be performed in the absence of a suitable control. Samples from *in vitro* incubations or *in vivo* dosing experiments can be quickly analysed by LC/MS, followed by a multi-dimensional data search which correlates retention time, m/z value, intensity and components from alternative detection technologies (e.g. diode array UV or radiochemical monitoring). Comparison of analyte data with the control sample allows filtering of matrix-related peaks, which would otherwise produce an unmanageable list of false metabolite peaks.
MS^E (Figure 3) can be processed with this software algorithm and will align the data from the low energy and MS^E functions based upon retention times and exact mass alignment

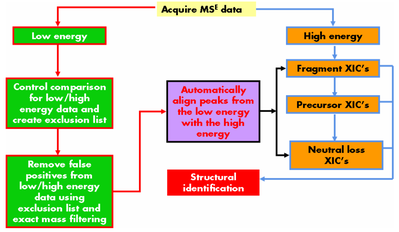

Figure 3. MS^E data acquisition and processing schematics with MetaboLynx

Exact mass data filtering and MetaboLynx MS^E - How does it work?

- It is based on exact mass and mass deficiencies, which are specific to each parent drug compound of interest.
- Each parent drug has a specific number of elements (C,H,N,O, ...) which is known by the chemist.
- Depending on the number of each one of the elements mentioned, the drug of interest will have a very specific mass deficiency. For example in the case of Propranolol, it contains the following elements; $C_{16}H_{21}NO_2$.
- This equates to a monoisotopic protonated mass of 260.1651Da. If we take the propyl group away (N-deisopropyl) then the mass is shifted by –47mDa (Figure 4)
- Therefore, if we were to put a window of around 50 mDa we would be able to detect its N-deisopropyl metabolite and exclude all other entries which fall outside this window

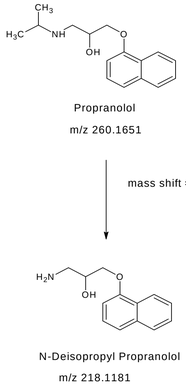

Figure 4. Example for the use of mass defect filtering for Propranolol and one of its metabolites

DISCUSSION

- From Figure 5, it can be observed how the use of mass defect filtering may be used to eliminate false positives
- The elimination of the false positive metabolites is post data processing, which means that if one wants to go back to the processed results to further verify the false positives. It is then possible to do this without loosing the original information
- Another advantage of this approach is the fact that we can set low thresholds and search for low levels and then let the filtering tools such as the mass defect filter and peak area threshold filter to remove the endogenous peaks which may be present in the unexpected metabolite trace

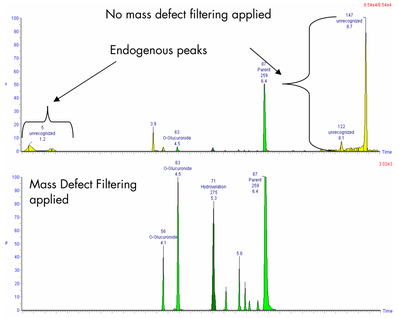

Figure 5. Comparison of combined metabolite trace expected and unexpected metabolites with and without mass defect filtering

- This same mass filter may also be applied to the MS^E data so that cleaner spectra are obtained which will help with the correlation analysis. This is observed in Figure 6

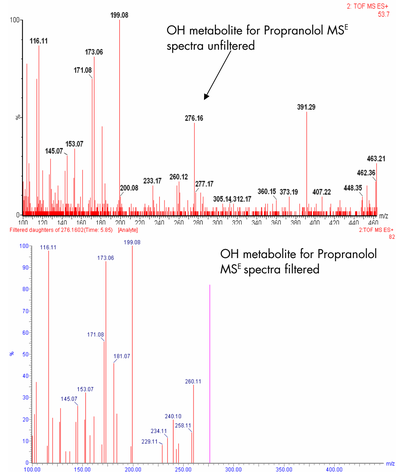

Figure 6. Comparison of MS^E spectra for the hydroxylated metabolite of Propranolol with and without mass defect filtering

- From this analytical strategy, fragment ion, precursor ion and neutral data was generated in the MS^E function
- Precursor ion information is shown (Figure 7) which allows the confirmation of major metabolites found

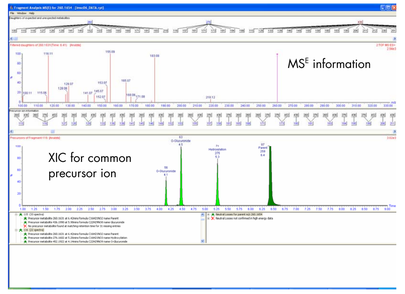

Figure 7. Fragment analysis for the metabolites of Propranolol in vitro

CONCLUSION

- **By this approach we were able to acquire vast amounts of information during the time scale of UPLC**
- **Methodology easy to set-up without any prior knowledge of metabolites**
- **Generation of fragment, precursor and neutral loss information from a single injection**
- **This approach provides a quick 'snapshot' for fragment ion information**
- **It will also help to decide what further MS/MS experiments to carry out**

References

1. Mark Wrona, Kevin Bateman, Russell Mortishire-Smith, Desmond O'Connor; Rapid Commun. Mass Spectrom. 2005; 19: 2597–2602

720001852EN

TO DOWNLOAD A COPY OF THIS POSTER VISIT WWW.WATERS.COM/POSTERS