Enhanced Duty Cycle on a Hybrid Quadrupole oa-TOF Instrument to Improve the Limit of Detection for Reactive Metabolite Screening

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INTRODUCTION

Reactive metabolite screening in drug discovery is an integral part of the early screening process as it helps to detect possible NCE's which may undergo bioactivation. Such behaviour can pose a threat to drug programs and is widely accepted to be a mechanism for xenobiotic-induced toxicity. Having the capability to detect reactive electrophiles earlier on allows pharmaceutical companies to re-optimise the compound in question and minimize the risk of toxicity at the development stage.

Typically, the detection of reactive intermediates are carried out by typical LC/MS-MS experiments by using a combinations of precursor ion (PI) and neutral loss (NL) acquisitions with tandem quadrupole Mass Spectrometry[1,2]. Even though this technique proves to be selective because it is targeted towards specific losses or precursor ions such as in the case of glutathione (GSH) trapping assays. For NL in positive ion mode, it is common to search for the loss of the pyroglutamic acid (m/z 129), for aliphatic and benzylic thioethers monitor the loss of (m/z 147). For PI monitor the ion at m/z 272 in negative ion corresponding to the γ glutamyl-dehydroanalyl-glycine. ESI +ve ion mode will require multiple NL experiments to obtain maximum coverage for all types of NCE's due to the low duty cycle of scanning instruments. This may hinder detection of low level GSH adducts.

The other significant but important factor is that most of the GSH adducts may manifest as the doubly charged species $(M+2H)^{+2}$ which does not fragment during CID to provide the appropriate NL of interest but giving rise to singly charged species. ESI –ve ion acquisition may overcome some of the issues relating to doubly charge formations and can become a more universal technique especially for GSH screening. With this in mind, we have designed a novel approach to allow us to obtain a highly sensitive methodology for GSH screening which could be further extended to other trapping assays such as cyano trapping or semicarbazide trapping.

We describe a method that enables the collection of both parent and fragment information from a single injection using a hybrid quadrupole / oa-TOF instrument in negative electrospray ion mode. Acquisition of data used two functions interleaved such that the first acquisition function collected information about the intact GSH metabolites and the second acquisition function collected fragment ions with an enhanced duty cycle operation for both acquisition modes allowing for an increased signal for diagnostic fragments of interest that denoted the presence of the GSH adduct in both low and high energy acquisitions.

METHODS

Samples

Nefazodone was incubated with human liver microsomes at 10 µM at 37 °C, in a solution of 50 mM Tris buffer adjusted to pH 7.4 containing the NADPDH regenerating system. GSH was added at a concentration of 5mM to the microsomal incubation. The reaction was terminated after 60 minute with 1 volumes of cold acetonitrile to 1 volume of sample. Then, the sample was centrifuged at 13,000 rpm for 15 minutes and the supernatant supernatant was injected directly to the UPLC-TOF-MS system for analysis.

LC-MS Methodology

LC-conditions:
LC System : Waters Acquity UPLC™
Column: Acquity HSS T3 C18 Column 100x2.1mm id, 1.7µm
Column Temperature: 45 °C
Mobile phase A: 5mM Ammonium Acetate pH 5; B: Acetonitrile.
Flow rate: 0.6 mL/min
Gradient: 95%A – 25% A in 5 mins, ramp to 25-5%A in 1 min before returning to 95% A for re-equilibration
Injection volume: 10 µL

MS-conditions:
Mass Spectrometer: Synapt HDMS™
MS scan range: 50-900 Da
Mode of Operation: –ion mode ESI
Lock Mass: Leucine Enkephalin at 200pg/mL

MS^E Methodology[3,4]:

The Synapt HDMS™ was operated in a parallel data acquisition mode with a wide band RF mode in Q1(Figure 1). Thus, allowing all ions enter into the collision cell without pre-filtering. This resulted in one single injection in which data was collected under one single data file with two functions. Function (1) Low energy acquisition (5eV) which contained the intact compounds and Function (2) High energy or MS^E acquisition Trap (10eV-25eV ramp) and Transfer (20eV) which contained all of the fragmented ions

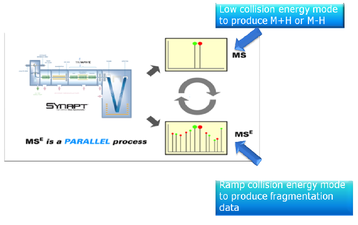

Figure 2. Schematic showing the MS^E methodology

Enhanced (Duty Cycle) EDC Methodology:

In this mode of operation the duty cycle of the mass spectrometer is increased by pre-defining the masses of interest. Thus providing significant increase in sensitivity and limits of detection. This is achieved by utilizing the T-Wave collision cell technology to shape the ion beam into discrete ion packets. These ion packets are then released from the collision cell at certain time intervals with the pusher of the oa-TOF, synchronized to operate as the ion of interest enters the extraction region (Figure 2). EDC may be operated in full scan MS or MS/MS mode

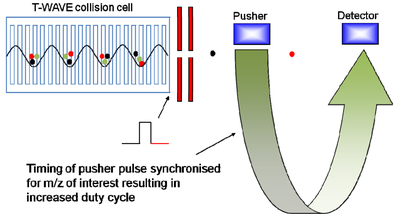

Figure 2. Schematic showing the EDC mode

Processing software

- MetaboLynx was used for the Enhanced Duty Cycle MS^E peak detection of putative metabolites
- MassFragment was used for the structure elucidation step

GSH reactive metabolite screening methods by LC-MS / MS

GSH 'Trapping' [1,2]
- Typical in-vitro incubation in microsomes forcing the reaction to form GSH 'adducts'

For positive ion mode;
- Monitor the loss of the pyroglutamic acid moiety m/z 129 (Figure 4)
- Monitor the loss of GSH m/z 307 for aliphatic and benzylic thioethers
- Monitor the loss of glutamic acid m/z 147 for thioesters

For negative ion mode
- Monitor the precursor ion at m/z 272 arising from the γ glutamyl-dehydroanalyl-glycine. Other GSH fragments may also be used (Figure 3)

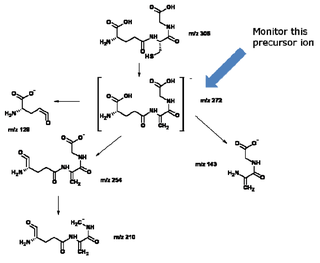

Figure 3. Negative ion ESI-CID fragmentation pathway for GSH

RESULTS

- The advantage of MS^E is that we obtain parallel information from the low and high energy in one single injection in an unbiased way. Below is an example for one of the Nefazodone O+ GSH adducts (Figure 4)

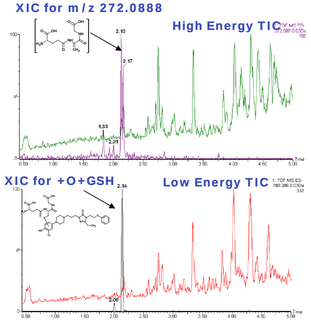

Figure 4. Low and high energy data for Nefazodone in –ve ion mode

- Even though we were searching for the specific diagnostic precursor ion of γ glutamyl-dehydroanalyl-glycine m/z 272 in negative ion. It is also possible to search for the corresponding neutral losses in positive ion. Because we see **'all of the fragment ion spectra'** on the high energy it is possible to search for all losses or precursor ions of interest

- The spectra for the high and low energy is obtained in a parallel fashion (Figure 5)
- In this example the mass at m/z 789.2798 from the low energy spectrum corresponds to the O+GSH metabolite of Nefazodone
- From the same injection the high energy spectra for the same mass at m/z 789.2789 gives rise to the diagnostic fragment ion of interest which will help us to localize the GSH adduct and then confirm it in the low energy trace with exact mass

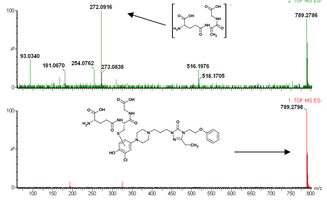

Figure 5. Low and high energy spectra in –ve ion for the +O+GSH metabolite of Nefazodone

- The corresponding information was interrogated automatically with MetaboLynx
- MetaboLynx was set up specifically to search for GSH adducts
- This resulted in the detection of 7 GSH adducts (Figure 6)

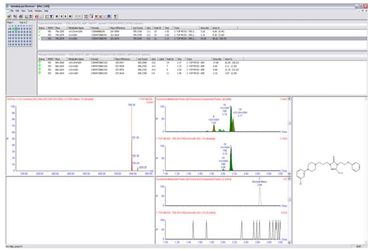

Figure 6. MetaboLynx browser showing all 7 GSH adducts for Nefazodone in the low energy mode

- Once the metabolites were detected in the low energy, this was followed by confirmation and aligment with the high energy data by monitoring the fragment ion at m/z 272 (γ glutamyl-dehydroanalyl-glycine) (Figure 7)

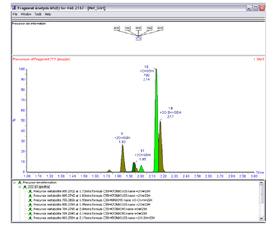

Figure 7. High energy data for all GSH adducts of Nefazodone showing the precursor ion information for m/z 272 (γ glutamyl-dehydroanalyl-glycine)

- The use of EDC function (Figure 8) in the low and high energy acquisition allowed us to obtain a much higher signal increase up to x 5 higher than the normal mode of operation. Since in most cases GSH tend to be at lower levels this enhances the likelihood of detection.

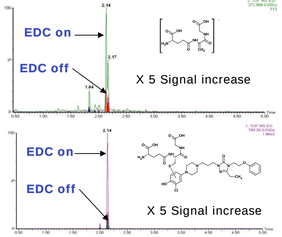

Figure 8. EDC on/off comparison for the +O+GSH metabolite and the fragment ion γ glutamyl-dehydroanalyl-glycine

CONCLUSION

- **The selectivity and superior sensitivity of this method using MS^E and EDC in a parallel fashion makes this analytical strategy very powerful for the detection of GSH adducts**
- **MetaboLynx automates the process of GSH detection and interpretation**

References

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3. Wrona, Mark, Mauriala, Timo, Bateman, Kevin P., Mortishire-Smith, Russell J., O'Connor, Desmond. *Rapid Communications in Mass Spectrometry* 19:18 (2005): 2597-602.
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