THE IMPACT of PPB MASS ACCURACY UPON BIOTRANSFORMATION PRODUCT IDENTIFICATION USING NEGATIVE ION NON -TARGETED URINARY SCREENING MULTI-REFLECTING TIME-OF-FLIGHT LCMS

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OVERVIEW

- Routine part-per-billion (ppb) mass accuracy for precursor and data independent acquisition (DIA) MS^E fragment ions (754 ppb (RMS) over 24-hours).
- Enhanced metabolite identification confidence.
- Enhanced analyte detection in complex biological matrices using system mass resolving power >200,000 FWHM.
- Uncompromised 10 Hz acquisition duty cycle to retain chromatographic peak fidelity.
- Fine isotope structure (FIS) can provide an additional criterion to enhance identification confidence for small molecule therapeutic drugs and metabolites.

INTRODUCTION

High resolution mass spectrometers (HRMS) such as quadrupole time of flight mass analysers (Q-TOF), have become more prevalent as screening tools for metabolite identification in drug discovery and development, where the constituents of interest are present in complex biological matrices, including urine and blood.

A hybrid quadrupole multi-reflecting time of flight system (Q-MRT) has been used to perform LCMS non-targeted screening to identify pharmaceutical drug xenobiotics in the urine of a healthy volunteer patient. The SELECT SERIES™ MRT (Figure 1) is a state-of-the-art hybrid quadrupole Multi Reflecting Time-of-Flight mass spectrometer (MRT).[1] It provides a unique combination of high resolving power (>200,000 FWHM), and routine ppb mass accuracy, independent of acquisition speed.

Utilising an unbiased data acquisition strategy such as DIA where all precursor and fragment ions are acquired, facilitates a characteristic profile of whole sample composition to be attained. Although DIA MS^E acquisition is not as selective as MS/MS or DDA strategies, the MS^E specificity is enhanced using the MRT system resolving power >200,000 FWHM. High mass resolution enhances ion selectivity of analytes in complex matrices, subsequently providing high mass accuracy and detectability which in turn enhances analyte identification confidence.

A non-targeted urinary screen of a healthy human volunteer has previously been performed, using an LCMS ES^+ (>200,000 FWHM) metabolite identification workflow.[2] Naproxen, carbamazepine, acetaminophen therapeutic drugs and metabolites, were identified. To illustrate the comprehensive benefits of high mass resolution performance a comparative ES^- non-targeted urinary screen is presented.

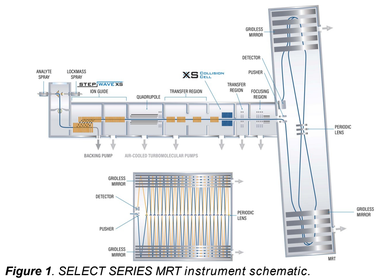

***Figure 1**. SELECT SERIES MRT instrument schematic.*

METHODS

Sample Description
Human urine sample diluted 1:10 (H_2O).
Carbamazepine dosage: 2 x 200 mg tablets.
Acetaminophen dosage: 2 x 500 mg tablets.
Naproxen dosage: 1 x 500 mg tablet.
Sample time points: 0, 2, 4 and 6 hours after medication was administered.

$LCMS^E$ ES^- precursor/fragment ion data acquisition was performed using a Multi Reflecting Time-of-Flight mass spectrometer (>200,000 FWHM). Human urine samples were analysed, using reverse phase separation liquid chromatography (0.1% *v/v* formic acid in H_2O) and (0.1% *v/v* formic acid in acetonitrile)), comprising a 12-minute gradient at a flow rate of 0.5 mL/min, using a C18 (100 mm x 2.1 mm, 1.8 µm) column at 40ºC. Injection volumes 5 µL. Data analysis and visualization: MassLynx™,waters_connect™ 3.1.0.2043 and Tibco Spotfire® 6.0.0 Software (Palo Alto, CA).

RESULTS AND DISCUSSION

A urinary screen of a healthy human volunteer has been performed to identify therapeutic drugs and metabolites, using a metabolite identification workflow. Using $LCMS^E$ ES^+ and $LCMS^E$ ES^- provides a route to comprehensive analysis to characterise knowns and unknowns.

Figure 2 shows an example of an chromatographic complexity obtained using LC-MRT-MS ES^- at 10 Hz for the analysis of the biologically complex human urine sample. Using the extracted mass chromatogram of carbamazepine-N-glucuronide as an example, 19 data points are obtained across a 3.6 second base peak width. The corresponding continuum mass spectrum is shown in Figure 2 (II), where mass resolution >189,000 FWHM was obtained at *m/z* 411. The data illustrates LCMS ES^- DIA analyses can be performed across the mass range, where at low *m/z*, duty cycle is not compromised to retain high mass resolving power and chromatographic fidelity is retained.

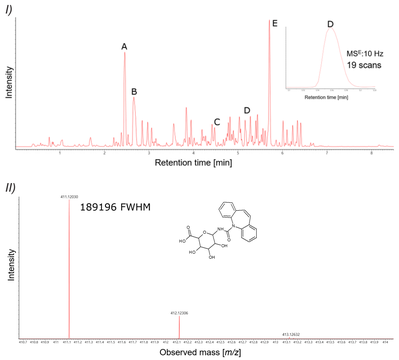

Figure 2. LC-MRT-MS ES^- expanded base peak ion chromatogram for the analysis of therapeutic xenobiotics and metabolites identified in the urine of a healthy volunteer patient. a) acetaminophen glucuronide, b) acetaminophen sulfate, c) carbamazepine-O-glucuronide d) carbamazepine-N-glucuronide and e) naproxen glucuronide. Inset expanded extracted mass chromatogram of carbamazepine-N-glucuronide. II) m/z 411 $[M\text{-}H]^-$ mass spectrum of carbamazepine-N-glucuronide.

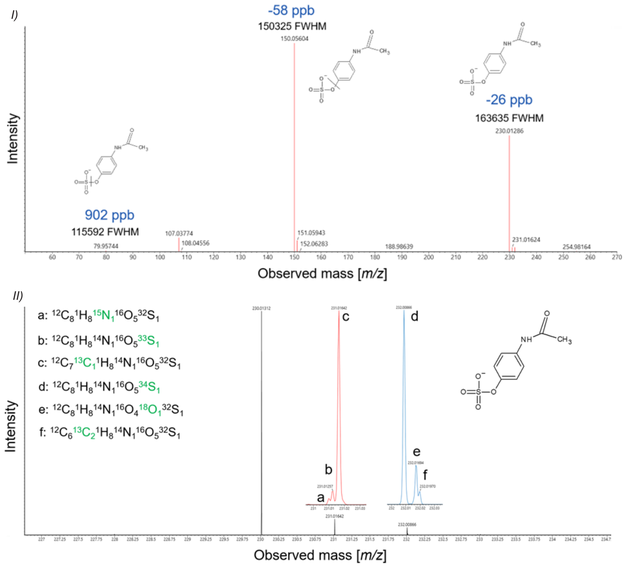

***Figure 3**. I) Precursor and fragment ion spectra obtained for [acetaminophen sulfate -H]⁻. II) [Acetaminophen sulfate -H]⁻ confirmatory fine isotope structure obtained using 10 Hz LC MRT ES^-.*

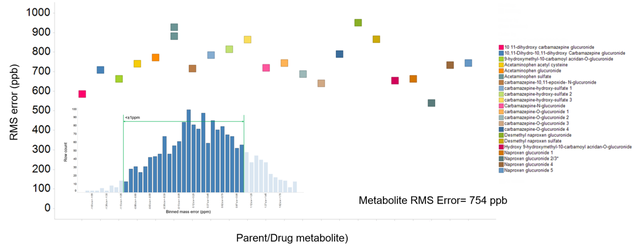

***Figure 4.** MRT LC-MS^E ES^- parent drug and metabolite precursor mass accuracy (RMS) for 1813 detections over 24-hours . (Inset: Mass accuracy frequency distribution over 24-Hours).*

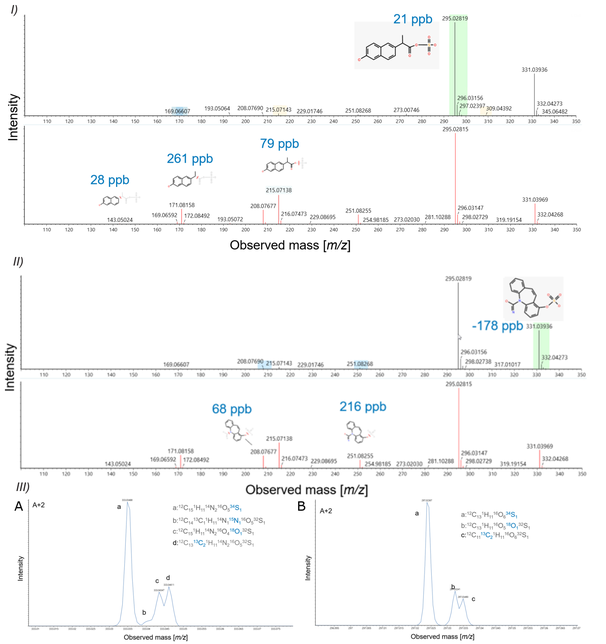

***Figure 5.** LC-MRT-MS ES^- DIA precursor and fragment ion spectra obtained for coeluting I) [desmethyl naproxen sulfate – H]⁻ and II) [carbamazepine-O-sulfate - H]⁻. III) (A) carbamazepine-O-sulphate⁻ and (B) desmethyl naproxen sulfate confirmatory A+2 fine isotope structure obtained using 10 Hz LC MRT ES^-.*

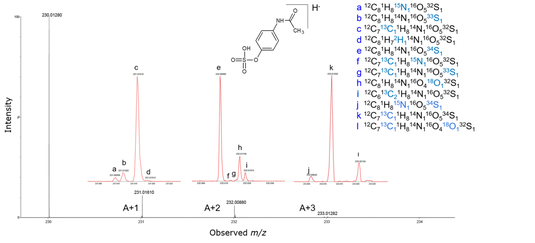

***Figure 6.** Enhanced fine isotope structure observed for acetaminophen-sulfate using 10 Hz LC-MS ES^- REM mode (system resolution >300,000 FWHM).*

Alongside routine precursor/fragment ion ppb mass accuracy, characteristic fine isotope structure also provides an additional identification criterion, as illustrated for the $[M\text{-}H]^-$ A+1 and A+2 isotopes of acetaminophen sulfate (see Figure 3)). Over a 24-hour period a 754 ppb (RMS) error was obtained for the identfied parent drugs and metabolites (Figure 4).

Although not commonly reported, sulfation of 6-O-desmethyl naproxen is observed at t_r 4.75 minutes, with mass accuracy of 21 ppb is illustrated (see Figure 5).[3] The metabolite identification is confirmed with a high degree of confidence, where product ions *m/z* 171 (261 ppb) and *m/z* 143 (28 ppb) have been determined. Additionally characteristic fine isotope structure provides another identification criterion (Figure 5 (III)). In the DIA fragment spectrum illustrated in Figure 5, a peak at *m/z* 331 is also observed, the fine isotope structure attained is also indicative of a species with an elemental composition inclusive of sulfur (Figure 5 (III)). Using a ES^- metabolite identification workflow, it was determined that a phase II sulfonated biotransformation product of carbamazepine had been observed. Such metabolites are also not commonly reported, however at t_r 4.75 minutes the precursor ion *m/z* 331 (68 ppb) and fragment ions (*m/z* 251 (216 ppb)/*m/z* 208 (68 ppb) are observed. The attainment of ppb mass accuracy for precursor and fragment ion spectra provides additional support for proposed identification of a carbamazepine hydroxy sulfate metabolite. From Figure 5 it can be seen, using DIA with sub ppm mass accuracy there is correlation of the respective fragment ions of desmethyl naproxen sulfate and carbamazepine hydroxy sulfate species, illustrating highly specific data has been obtained using a DIA acquisition strategy.

In Figure 6 we illustrate enhanced fine isotope structure observed for acetaminophen-sulfate using *10 Hz LC-MS ES^-* REM mode (system resolution >300,000 FWHM).

CONCLUSION

- LCMS negative ion electrospray at 10 Hz, with a system resolving power of >200,000 FHWM is routine.
- 754 ppb (RMS) level mass accuracy over a 24-hour period enables confident identification of major and minor metabolites therapeutic drugs.
- PPB mass accuracy for DIA precursor ion and fragment ions provides a high degree of specificity for non-targeted analytical acquisition strategies.
- Fine isotope information provides an additional confirmatory criterion to identify knowns and unknown metabolites.
- REM mode enables enhanced fine isotope structure to be acquired in an LCMS time frame (system resolution >300,000 FWHM).
- PPB mass accuracy facilitates implementation of more stringent informatics data processing, reduces false detection rates and provides improved analysis efficiency.

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