DATA INDEPENDENT ANALYSIS EVOLUTION: EXPLORING THE USE OF HIGH RESOLVING POWER MULTI-REFLECTING TIME-of-FLIGHT MASS SPECTROMETRY FOR METABOLITE IDENTIFICATION

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OVERVIEW

- Uncompromised chromatographic peak fidelity using 10 Hz LCMS^E ES$^+$ using system resolving power >200,000 FWHM.
- Routine Multi Reflecting Time-of-Flight (MRT) part-per-billion (ppb) mass accuracy for precursor and data independent acquistion (DIA) fragment ions in complex sample analysis.
- 549 ppb ppb (RMS) level mass accuracy over a 24-hour period has enabled confident and efficient identification of metabolites.
- Fine isotope structure (FIS), ion selectivity and ppb mass accuracy facilitate identification of unknowns.
- Transformative mass measurement resulting from greater mass resolving power affords the opportunity to improve informatics output and moreover study efficiency, in the drug discovery and development process.

INTRODUCTION

Data independent acquistion (DIA) to identify small molecule drug metabolites, has been used to assess the LCMS accurate mass measurement specificity attained using the SELECT SERIES™ MRT (Figure 1), 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.

Using unbiased non-targeted “wide-scope” data acquisition, thousands of detections can be made in a single analysis. High mass resolving power enhances ion selectivity and subsequently the detection of analytes in complex matrices, providing high mass accuracy which enhances analyte identification confidence and facilitates use of more stringent data tolerances during retrospective targeted data analysis. The attained precursor/fragment ion ppb mass accuracy can be utilised to improve identification confidence in research involving small molecules, such as metabolite identification.

Metabolite identification is an important part of the drug development process where the metabolic fate of a drug molecule is investigated. This requires mass spectrometry techniques with high specificity for structural elucidation. A urinary screen of a healthy human volunteer was undertaken to identify therapeutic drugs and metabolites. A metabolite identification workflow using LCMS (system resolution >200,000 FWHM) has been implemented.

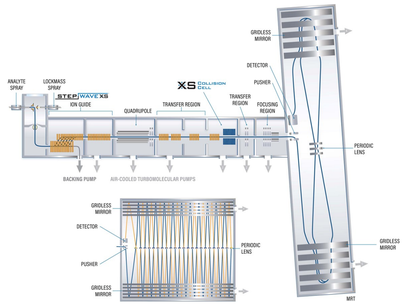

***Figure 1**. SELECT SERIES MRT instrument schematic.*

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 C_{18} (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

To explore the impact of high-resolution mass spectrometry (HRMS) mass accuracy upon small molecule identification, LCMS urinary screening of a healthy volunteer patient has been performed at time course points of 0, 2, 4 and 6 hours post dose.[2] Figure 2 shows high resolution DIA can be performed across the mass range, where at low *m/z,* duty cycle is not compromised for high mass resolution. Chromatographic fidelity is retained and ppb mass accurracy facilitates routine identification of parent drug and metabolites.

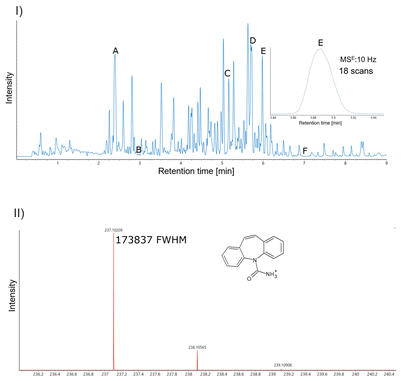

***Figure 2.** LC-MRT-MS ES$^+$ base peak ion chromatogram, for the analysis of therapeutic xenobiotics and metabolites identified in the urine of a healthy volunteer patient. I) a) acetaminophen glucuronide, b) acetaminophen, c) carbamazepine-N-glucuronide, d) naproxen glucuronide, e) carbamazepine and f) naproxen. Inset expanded extracted mass chromatogram of carbamazepine. II) m/z 237 [M+H]$^+$ high resolution mass spectrum of carbamazepine.*

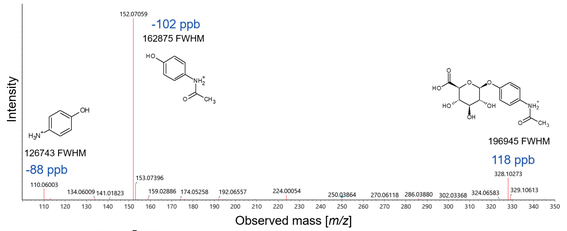

***Figure 3.** LC-MRT-MS^E ES$^+$ precursor and fragment ion spectra obtained for [acetaminophen glucuronide +H]$^+$.*

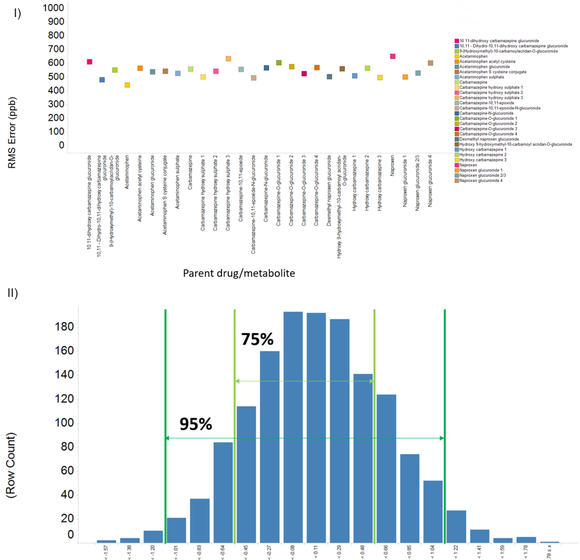

***Figure 4. I)** LC-MRT-MS^E ES$^+$ parent drug and metabolite precursor mass accuracy (RMS). II) Mass accuracy frequency distribution over 24-Hours.*

***Table 1**. Impact of accurate mass on biotransformation false detection rate determined using a metabolite identification workflow at system* resolving power *> 200,000 FWHM for carbamazepine 4-hour time course point.*

	Applied Processing Mass Accuracy Tolerance				
	Precursor 5ppm	Precursor 5ppm	Precursor 5ppm	Precursor 1ppm	Precursor 1ppm
	Fragment 2 mDa	Fragment 1 mDa	Fragment 0.2 mDa	Fragment 0.2 mDa	Fragment 0.1 mDa
	t, 0.1 parent	t, 0.1 parent	t, 0.1 parent	t, 0.1 parent	t, 0.1 parent
Detections	200	200	200	142	142
Detections with theoretical Fragments	196	191	181	144	138
Detections with expected Fragments	140	140	133	112	98

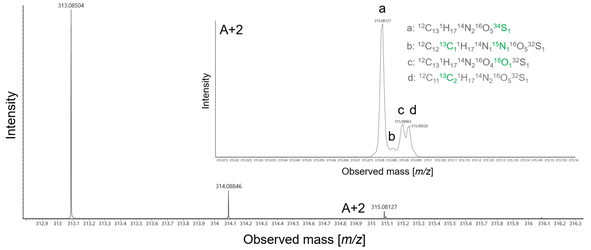

***Figure 5**. Fine isotope structure (FIS) observed for acetaminophen-acetyl-S-cysteine conjugate system resolution >200,000 FWHM.*

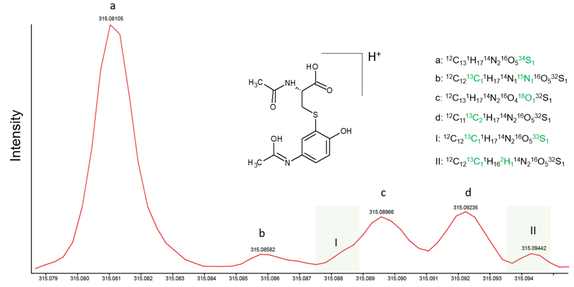

***Figure 6**. Enhanced fine isotope structure observed for acetaminophen-acetyl-S-cysteine conjugate using REM mode system resolution >300,000 FWHM.*

Confidence in therapeutic drug xenobiotics and metabolite identification, is further enhanced where DIA fragment ions are generated using MS^E and mass resolved from matrix interferences (see Figure 3). An example of LC-MRT-MS^E ES$^+$ precursor and fragment ion spectra obtained for [acetaminophen glucuronide + H]$^+$ acquired at 10 Hz is presented with measurement error between -88 ppb and 118 ppb.

Over a 24-hour period a 549 ppb (RMS) error was obtained for the identfied parent drugs and metabolites (Figure 4). Figure 4 shows 75% of identifications had a mass measurement error of < 500 ppb and 95% < 1ppm. The impact of mass accuracy can be harnessed using post processing filters and is illustrated in Table 1 for the carbamazepine 4 hr time point. Post processing mass accuracy tolerance thresholds applied to precursor and fragment ions, simultaneously enhance identification confidence, and reduce the false detection rate.

System mass resolving power >200,000 FWHM, also enables FIS to be accessed within a LCMS timeframe, FIS also provides an additional identification criterion to confirm identfication or aid identfication of unknowns. FIS for acetaminophen-acetyl-S-cysteine conjugation in shown in Figure 5. Further LCMS FIS investigations have been performed using resolution enhanced mode (REM), (>300,000 FWHM) and illsutrated in Figure 6.

CONCLUSION

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

References

1. Cooper-Shepherd D, Wildgoose J, Kozlov B, Johnson W.J, Tyldesley-Worster R, Palmer M, Hoyes J, McCullagh M, Jones E, Tonge R, Marsden-Edwards E, Nixon P, Verenchikov A, and Langridge J. Novel Hybrid Quadrupole-Multireflecting Time-of-Flight Mass Spectrometry System. Journal of the American Society for Mass Spectrometry 2023 34 (2), 264-272.

2. McCullagh M, Palmer M, Eatough D, Marsden-Edwards E, Langridge J, Cooper-Shepherd D. Exploring the Impact of Part Per Billion Mass Accuracy for Metabolite Identification using Multi Reflecting Time-Of-Flight MS with UPLC Part A. . 720007896, April 2023.

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