UPLC with the SELECT SERIES™ MRT for Metabolite Identification

INTRODUCTION

The SELECT SERIES MRT (Figure 1) is a state-of-the-art hybrid quadrupole Multi Reflecting Time-of-Flight mass spectrometer. It provides a unique combination of ultra-high (200,000 FWHM) resolution, and routine part-per-billion mass accuracy, independent of acquisition speed. This performance results in high quality mass spectrometry data for non-targeted screening workflows such as forensic toxicology and metabolite identification, where the constituents of interest are present in complex biological matrices such as blood and urine.

Metabolite identification is a critical part of the drug development process where the metabolic fate of a drug molecule is investigated. This enables critical biotransformation information to be determined and requires mass spectrometry techniques with high specificity for structural elucidation. Here we describe the use of an UPLC with a SELECT SERIES MRT, shown in Figure 1, for the LC-MS analysis of drug metabolites.

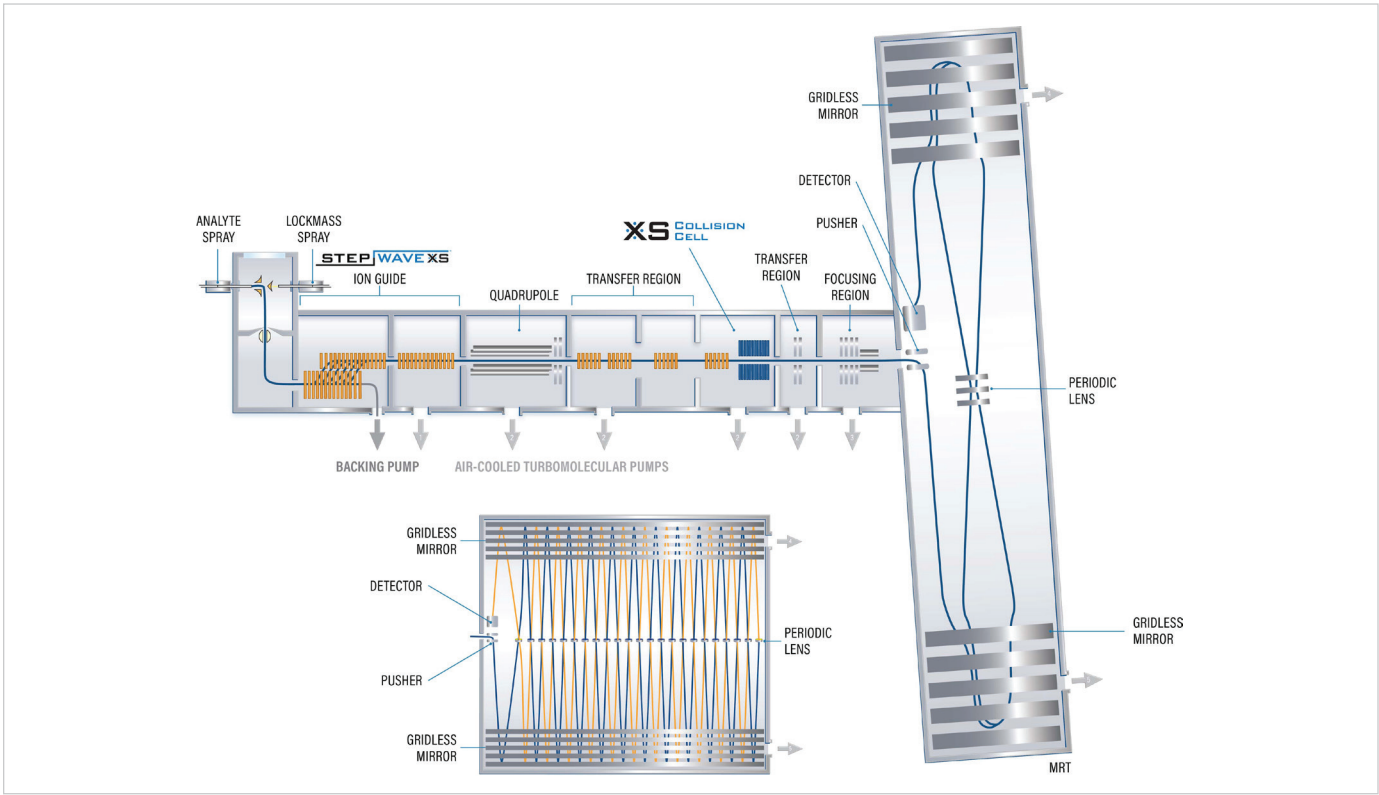

Figure 1. SELECT SERIES MRT instrument schematic.

EXPERIMENTAL CONDITIONS

Sample	Human urine sample diluted 10:1 (H_2O) spiked with SST mix: 1 pg/µL to 1000 pg/µL Acetaminophen, sulphaguanidine, caffeine, sulphadimethoxine, terfenadine, verapamil, val-tyr-val, leucine enkephalin and reserpine Human urine sample diluted 10:1 (H_2O) Carbamazepine Dosage: 2 x 200 mg tablets Acetaminophen Dosage: 2 x 500 mg tablets Naproxen Dosage: 1 x 500 mg tablets Sample taken 6 hrs after medication was administered

LC Conditions	
LC System	Waters ACQUITY UPLC™ I-Class chromatograph
Column	ACQUITY UPLC HSS T3 C_{18} (100 mm x 2.1 mm, 1.8 µm) column
Column temperature	40 °C
Sample temperature	4 °C
Injection volume	5 µL
Flow rate	0.5 mL/min
Mobile phase A	Water (containing 0.1% formic acid v/v)
Mobile phase B	Acetonitrile (containing 0.1% formic acid v/v)

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
0	0.5 mL/min	99.0	1	initial
1	0.5 mL/min	99.0	1	6
3	0.5 mL/min	85	15	6
6	0.5 mL/min	50	50	6
9	0.5 mL/min	5	95	6
10	0.5 mL/min	5	95	6
10.1	0.5 mL/min	99	1	6
12	0.5 mL/min	99	1	6

MS Conditions	
Acquisition	ES^+
Capillary voltage	0.5 kV
Desolvation temperature	550 °C
Source temperature	150 °C
Cone	20 V
Mass range	*m/z* 50 – 2400
Acquisition rate	10 Hz
Acquisition / Processing Software	MassLynx™ v4.2 SCN1024 and waters_connect™ 1.94.0.53

RESULTS

Five patient samples were analysed, acetaminophen, naproxen and carbamazepine pharmaceutical xenobiotics have been identified. Ninety percent of acetaminophen is metabolized in the liver to sulfate and glucuronide conjugates at therapeutic doses, they are excreted in the urine, identification is illustrated in Figure 2, which shows an example of an MRT-LC-MS ES+ chromatographic integrity obtained at 10 Hz for the analysis of a human urine sample in a metabolite identification workflow. An example of mass measurement error <70 ppb is presented in Figure 3 for the fragment ion spectrum obtained for acetaminophen glucuronide.

In humans the nonsteroidal anti-inflammatory drug naproxen is oxidised to 6-0- desmethylnaproxen and both parent drug and metabolite are conjugated as acyl glucuronides. In Figure 4, mass measurement error <200 ppb is shown for the fragment ion spectrum obtained for naproxen glucuronide. Carbamazepine is an anticonvulsant medication used primarily in the treatment of epilepsy and neuropathic pain, mass measurement error obtained for carbamazepine/epoxidized carbamazepine are presented in Table 1. Examples of single mass measurements and mass accuracy observed, for the parent drugs detected, metabolites and product ions are listed in Table 1. Overall combining precursor and product ion identifications for the examples shown RMS error= 491 ppb (N=82) has been obtained.

Spiking a system suitability test (SST) solution which incorporates acetaminophen into urine matrix, enabled the MRT-LC-MS^E ES+ linear performance to be illustrated (Figure 5), correlation coefficients R^2>0.99 were obtained for all SST constituents. For SST solvent standard solutions and spiked into urine matrix RMS mass errors <500 ppb have been achieved and are presented in Figure 6.

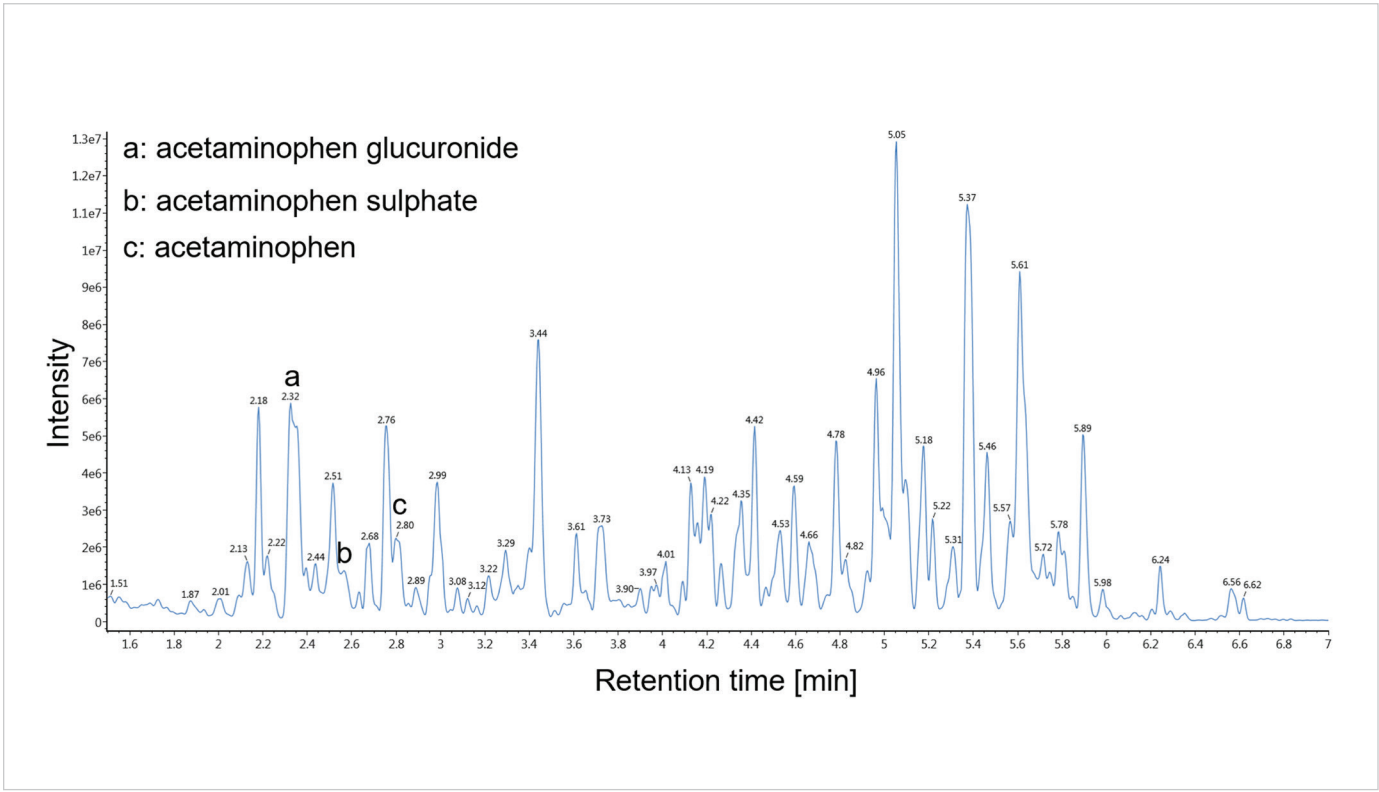

Figure 2. MRT-LC-MS ES+ expanded base peak ion chromatogram for the analysis of acetaminophen metabolites identified in the urine of a healthy volunteer patient.

Table 1. Table illustrating mass measurement errors for precursor ion and fragment ions for parent drugs acetaminophen, naproxen, carbamazepine, and example metabolites identified in human urine, using MRT-LC-MS^E ES+ at 0.1 second acquisition rate (N=1 mass measurement error).

Parent/Metabolite Name	Parent/ Metabolite Elemental Composition	Expected *m/z*	Observed *m/z* Mass Error (ppb)	Fragment Elemental Composition	Expected Fragment Ion *m/z*	Fragment Ion Observed *m/z* Mass Error (ppb)
Acetaminophen	$C_8H_{10}NO_2$	152.07060	152.07066 **(386)**	C_6H_8NO	110.06004	110.06006 **(220)**
Acetaminophen sulphate	$C_8H_{10}NO_5S$	232.02742	232.02748 **(307)**	$C_8H_9NO^2$ C_6H_8NO	152.07060 110.06004	152.07065 **(318)** 110.06004 **(38)**
Acetaminophen glucuronide	$C_{14}H_{18}NO_8$	328.10269	328.10250 **(618)**	$C_8H_9NO_2$ C_6H_8NO	152.07060 110.06004	152.07052 **(536)** 110.05995 **(83)**
Naproxen	$C_{14}H_{15}O_3$	231.10157	231.10162 **(196)**	$C_{13}H_{13}O$ $C_{12}H_{10}O$ $C_{12}H_{10}$ $C_{12}H_9$	185.09600 170.07262 154.07770 153.06988	185.09610 **(50)** 170.07265 **(190)** 154.07776 **(360)** 153.06992 **(280)**
Naproxen glucuronide	$C_{20}H_{22}O_9Na$	429.11560	429.11572 **(-276)**	$C_{20}H_{21}O_8$ $C_{14}H_{12}O_2$ $C^{13}H_{13}O$	389.12309 212.08320 185.09606	389.12309 **(-17)** 212.08320 **(105)** 185.09606 **(-195)**
Carbamazepine	$C_{15}H_{12}N_2O$	237.10224	237.10226 **(67)**	$C_{14}H_{12}N$	194.09643	194.09645 **(102)**
Carbamazepine 10,11 epoxide	$C_{15}H_{12}N_2O_2$	253.09715	253.09715 **(60)**	$C_{14}H_{12}NO$	210.09134	210.09131 **(-163)**

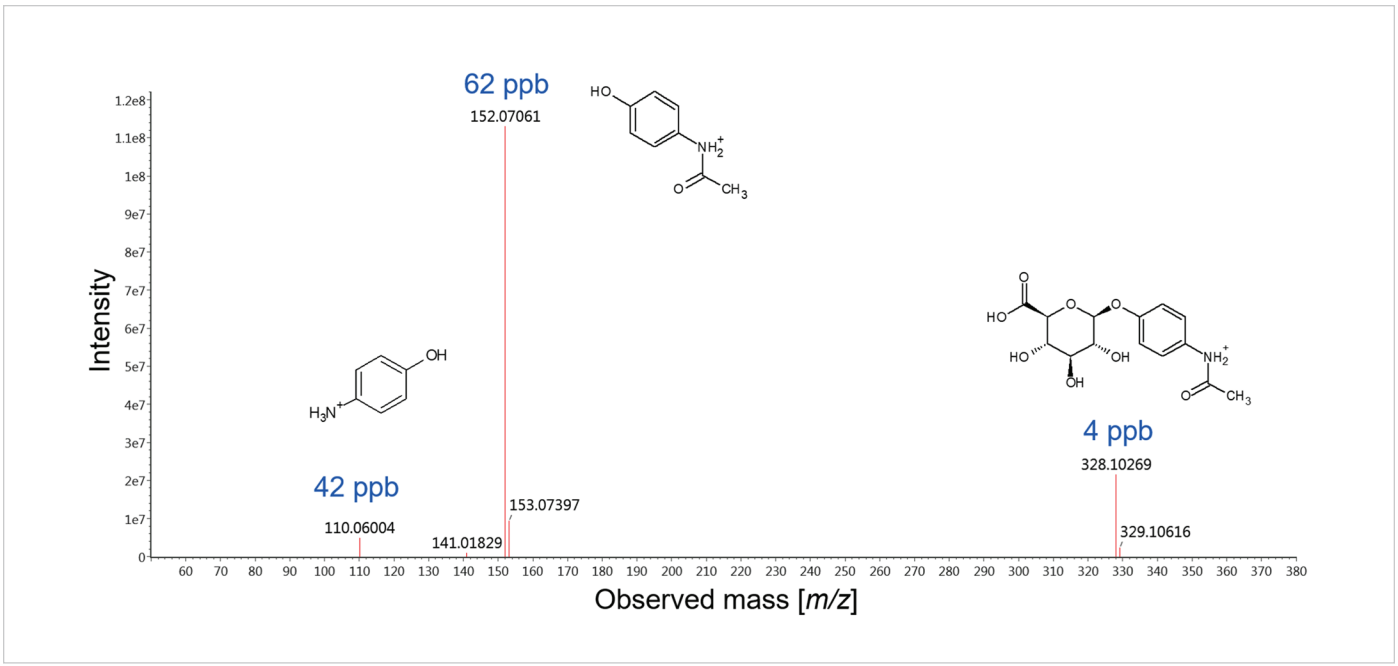

Figure 3. MRT-LC-MS^E ES+ fragment ion spectrum obtained for metabolite acetaminophen glucuronide identified in human urine.

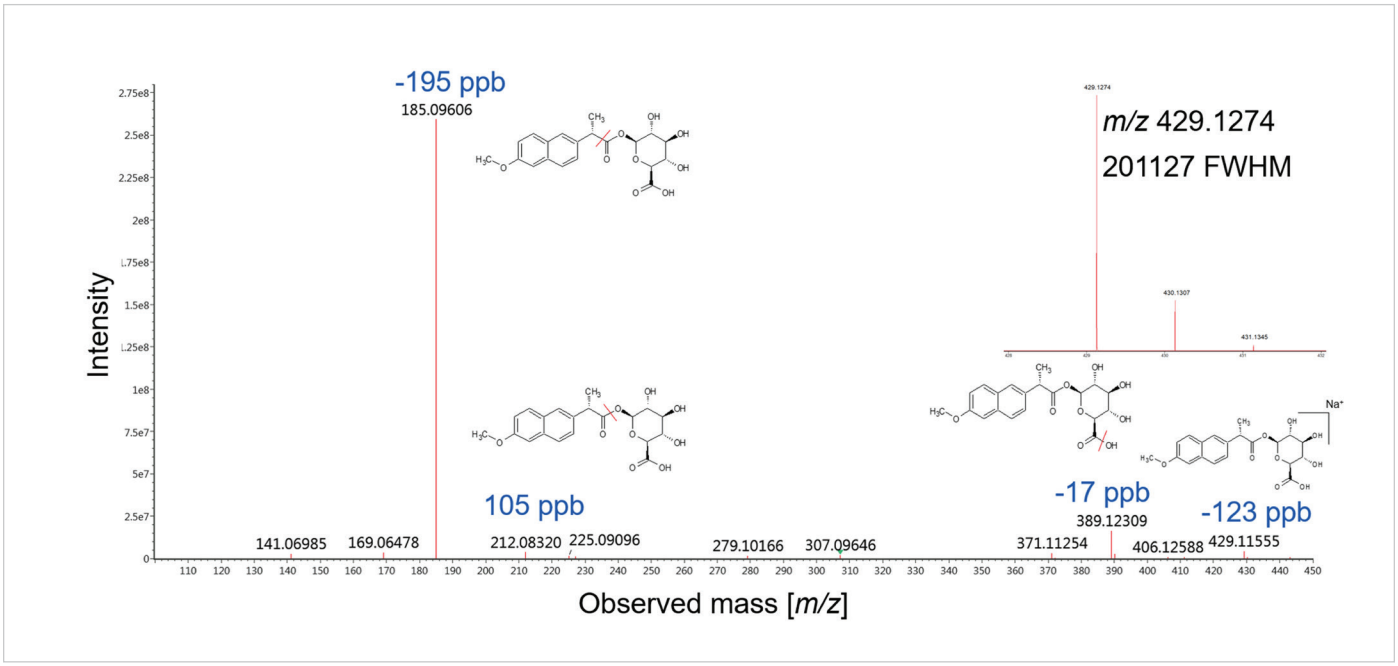

Figure 4. MRT-LC-MS^E ES+ fragment ion spectrum obtained for metabolite naproxen glucuronide identified in human urine, with continuum mass resolution inset.

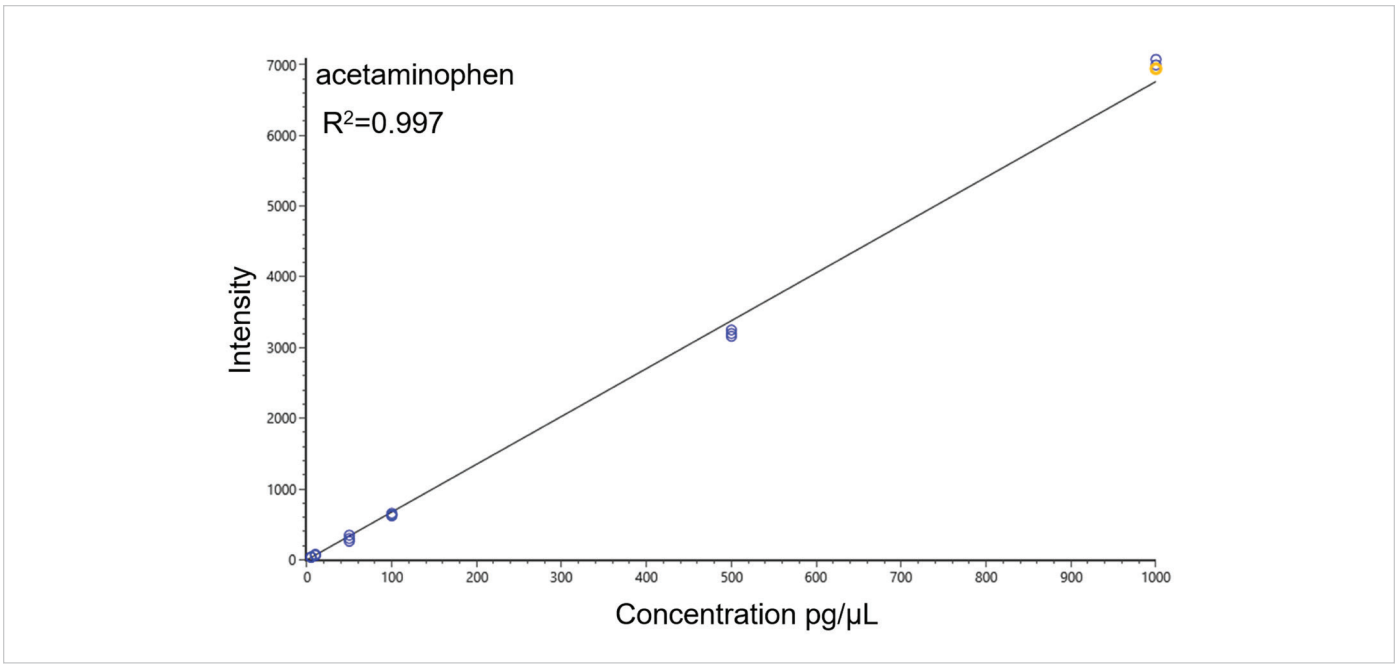

Figure 5. MRT-LC-MS^E ES+ linearity obtained for acetaminophen spiked into human urine biological matrix.

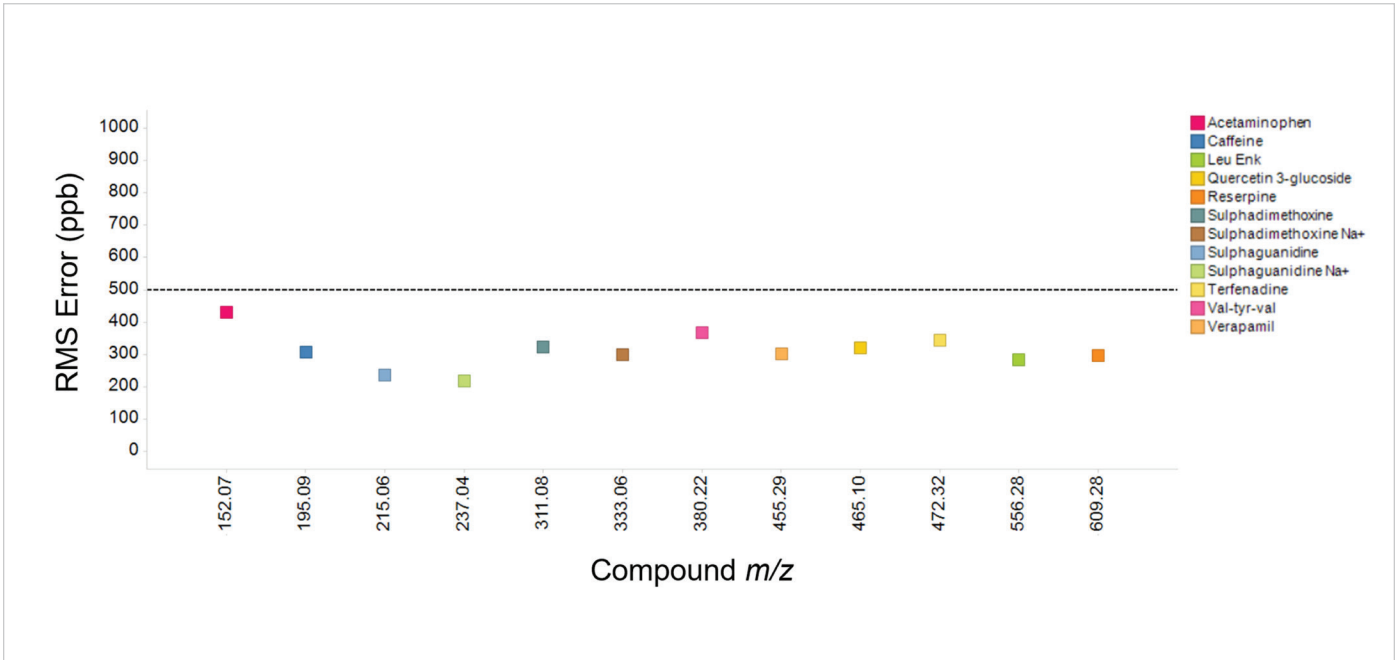

Figure 6. Plot of RMS mass error (ppb) obtained for SST test mix solvent standards and SST mix spiked into human urine biological matrix. All mass measurements were <500 ppb.

SUMMARY

The resolving power of the SELECT SERIES MRT enables pharmaceutical xenobiotics to be routinely identified in complex human urine matrix. Exogenous and endogenous isobaric species are resolved, with ppb mass accuracies obtained for both precursor and fragment ions facilitating accurate and time efficient identification.

A symbiotic relationship exists between data quality and the power of informatics solutions. Transformative MRT-LC-MS ES+ input affords the opportunity to transform informatics output, and analysis efficiency. Combining routine MRT-LC-MS performance with the Waters Metabolite Identification Application Solution within waters_connect, biotransformation scientists are provided with the most comprehensive high end performance analytical tools to identify and characterize metabolites, providing unparalleled ease and efficiency to facilitate a routine metabolite identification workflow.

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