URINARY FORENSIC TOXICOLOGY DATA INDEPENDENT ANALYSIS SCREENING: USING HIGH RESOLVING POWER MULTI-REFLECTING TIME-OF-FLIGHT MASS SPECTROMETRY

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OVERVIEW

- **Identification of drugs of abuse, prescribed agents and other toxicants using** data independent acquisition with Multi Reflecting Time-of-Flight mass spectrometry and ppb precursor and product ion mass accuracy
- **Transformative mass measurement resulting from greater mass resolving power affords the opportunity to improve algorithmic results interpretation and meet the analytical challenge of identifying knowns and unknowns within the constantly evolving drug landscape**
- **Application of stringent data processing parameters, 2 ppm precursor and 0.2 mDa product ion tolerances**
- **Enhancement of DIA performance with ppb mass accuracy for precursor and fragment ions**
- **Improved analysis efficiency through enhanced identification confidence and reduced false detection rates**

INTRODUCTION

Laboratories are frequently required to screen complex biological samples to identify drugs of abuse, prescribed agents and other toxicants. The constant emergence of new psychoactive substances poses a significant analytical challenge. High resolution mass spectrometry *(*HRMS) analysis is increasingly used for toxicological screening.

Data independent acquisition (DIA) has been previously applied for non-targeted screening of forensic samples [1-3]. Broadband DIA (MS^E) HRMS analysis of unrestricted and unbiased datasets, using Time-of-Flight technology, provides complete sample profiles, providing a precursor and product ion record that can be retrospectively probed using non-targeted and targeted workflows. Comparison with large libraries comprising elemental formulae, retention time (t_r) and fragment ion information, are essential to provide specificity and selectivity in identification, improving both efficiency and reducing false detection rates.

Here, a SELECT SERIES™ MRT, a hybrid quadrupole Multi Reflecting Time-of-Flight (MRT) mass spectrometer, shown in Figure 1, was applied for the analysis of anonymised authentic human urine samples. Further enhancements of DIA specificity through the use of > 200,000 FWHM mass resolving power, combined with screening and data management informatics tools, will be demonstrated.

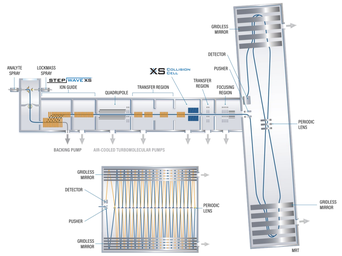

Figure 1. SELECT SERIES MRT instrument schematic.

METHODS

Sample description

Forensic Toxicology QC System Suitability Test (SST) Mix spiked at a fixed level (250 pg/µL) and serially diluted (2.5 - 500 pg/µl) in water, and anonymized authentic human urine samples diluted 1:10 (water).

Compound Library

Waters Forensic Toxicology Library (1975 entries).

LC conditions

Chromatographic separation was achieved using an ACQUITY™ UPLC™ I-Class Premier system and an ACQUITY UPLC HSS C18, 150 mm × 2.1 mm, 1.8 µm column. A reversed-phase gradient was used for chromatographic LC separation, comprising mobile phase A (5 mM aqueous NH_4HCO_2, pH 3) and mobile phase B (0.1% *v/v* formic acid in acetonitrile). Gradient: 0 - 0.5 min (87% A), 10 min (50% A), 10.75 - 12.25 min (5% A), 12.5 - 15 min (87% A). Flow rate: 0.4 mL/min; column temperature: 50ºC; injection volumes: 5 µL.

MS conditions

Acquisition/polarity:	ESI+
Capillary voltage:	0.8 kV
Desolvation temperature:	500 ºC
Source temperature:	120 ºC
Cone voltage:	20 V
Collision energy ramp:	15 - 40 eV
Mass range:	50 - 2400 *m/z*
MS^E acquisition rate:	10 Hz

RESULTS AND DISCUSSION

Data analysis for a series of anonymised authentic human urine samples was performed using the workflow illustration shown in Figure 2. Data were compared with the Forensic Toxicology Library, based on t_r, precursor ion and fragment ion accurate mass data for 1975 toxicologically relevant analytes, including illicit, pesticides, prescription drugs and over the counter (OTC) medications.

Figure 2. HRMS toxicology DIA screening workflow.

System performance was assessed using SST samples. The data were processed using ± 0.35 min t_r and ± 2 ppm precursor mass accuracy tolerances, respectively, and the presence of at least 1 diagnostic product ion within a mass tolerance of 0.2 mDa. For the SST Mix samples, an RMS mass error of 522 ppb was obtained for the single concentration level standard, and for the dilution series, the RMS mass accuracy errors are shown in Figure 3. All target analytes were identified, confirming the stringent mass accuracy data processing parameters could be adopted. An example of routine ppb mass accuracy performance is shown for the clozapine MS^E fragment ion spectrum in Figure 4, illustrating mass resolution 130,000 FWHM for the *m/z* 84 fragment. ion

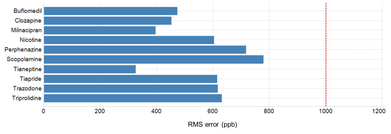

Figure 3. RMS precursor ion mass accuracy SST Mix dilution series samples over all concentration levels. Dashed red line = 1 ppm match tolerance reference.

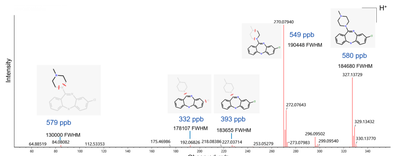

Figure 4. LC-MRT-MS^E ESI+ fragment ion spectrum obtained for SST mix constituent clozapine ($[M+H]^+$ m/z 327.13710).

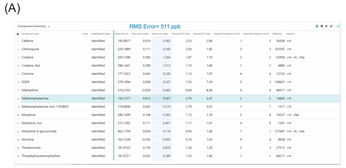

Figure 5. (A) Component summary illustrating illicit, prescription, OTC and dietary compounds identified in anonymised human urine sample '103', (B) Methamphetamine enhanced MS^E precursor and fragment ion spectrum, and (C) Methadone enhanced MS^E precursor and fragment ion spectrum.

The same tolerance criteria were applied for authentic urine samples and compared against the toxicology library entries. Identifications included illicit drugs and prescribed medications, shown in Figure 5, together with their metabolites, as well as dietary and endogenous constituents. In sample '103', oxycodone was excluded as a false detection. Methamphetamine was identified with a mass measurement error of 87 ppb and methadone with -83 ppb. Drug metabolites have also been identified, as well as compounds resulting from dietary consequence. An overall RMS mass measurement error of 511 ppb has been obtained for these compounds.

Routine ppb mass accuracy provides increased confidence to determine positive identifications. Repeat analysis of sample '51' confirmed the identification of recreational polydrug use, including illicit, prescription and OTC drugs. The combination of small molecule drugs identified within authentic samples is overview in Figure 6. The variety of identified drug classes, emphasises the analytical challenge and illustrates why unbiased DIA is required. A total of 14 'drug' compounds, 7 drug metabolites and endogenous urine matrix species were identified. In addition to nicotine, caffeine, and corresponding metabolites were detected.

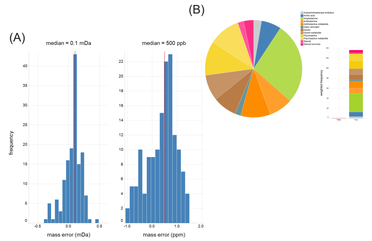

Figure 6. (A) Frequency distribution of mass measurement error for analyte identifications determined to be present in sample '51'. Dashed red lines = media. (B) Drug class distribution and weighted frequency of tentative observed true/false detections.

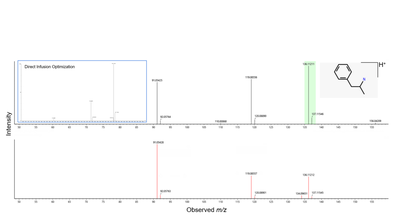

Figure 7. Amphetamine enhanced LC-MRT-MS^E ESI+ precursor and product ion spectrum and low temperature direct infusion analysis ESI+ (inset).

Specific investigations were performed on amphetamine (*m/z* 136.11207), methamphetamine (*m/z* 150.12773), pseudoephedrine (*m/z* 166.12264), and MDA (*m/z* 180.10191), which may be susceptible to labile fragmentation . The results shown Figure 5 suggest that minimal labile fragmentation was observed for some of the compounds of interest. Using direct analysis infusion, instrument parameters were optimised to reduce labile fragmentation. At low source temperature (100ºC), it can be seen, shown in Figure 7, that for amphetamine acquired under chromatographic conditions, the precursor ion spectrum, *m/z* 136, forms the base peak ion. However, using analysis conditions with lower source temperature, substantially reduced fragmentation.

CONCLUSION

- **High mass resolving power enhances ion selectivity and subsequently the detection of analytes in complex matrices**
- **SELECT SERIES MRT instrument routine ppb mass accuracy performance generates high quality mass spectrometry data, facilitating unequivocal determination of analyte elemental compositions using non-targeted screening workflows**
- **The enhanced mass accuracy specificity can be utilised to improve identification confidence in analytical research involving small drug molecules, in an everchanging drug landscape**
- **Data interpretation informatics is a key element to fully maximising all available information from the dataset, since a symbiotic relationship exists between data quality and informatics functionality**
- **Stringent data processing tolerances can be applied with confidence to improve analysis efficiency**
- **Illicit drugs were identified in all samples using retention time, and precursor ion and product ions ppb mass accuracy**
- **Additionally, all samples were positive for other recreational drug substances or OTCs**

References

1. G. Rosano, Michelle Wood, Kenneth Ihenetu, Thomas A. Swift, Drug Screening in Medical Examiner Casework by High-Resolution Mass Spectrometry (UPLC–MS^E-TOF), *J Anal Toxicol*, 37 (8), 2013, 580–593.
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