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 routine data independent acquistion (DIA) with Multi Reflecting Time-of-Flight (MRT) mass spectrometry precursor and fragment ion part-per-billion (ppb) mass accuracy.
- Transformative mass measurement resulting from greater mass resolving power affords the opportunity to improve informatics output and meet the analytical challenge of identifying knowns and unknowns where the drug landscape is constantly evolving.
- Application of more stringent data processing parameters, 2 ppm precursor ion and 0.2 mDa fragment ion tolerance.
- Enhancement of DIA MS^E 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 perform broad screening techniques on 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 *e.g.,* Time-of-flight (TOF) analysis, is increasingly used for toxicological screening. Broadband data independent acquisition (DIA) has been previously applied for non-targeted screening of forensic samples. The high-resolution mass spectrometry technology (HRMS (20,000 FWHM)) was used for the acquisition of unrestricted and unbiased datasets, thus providing a complete profile of the samples, which include precursor and fragment ion information that can be used for non-targeted and targeted workflows.[1-3] DIA was performed using MS^E mode, which is a full scan acquisition method, alternating between a low and high energy to provide information for the intact precursor and fragment ions. The nature of this technique allows for retrospective examination of the data. Comparison with large libraries comprising elemental formulae, retention time (t_r) and high energy fragment ion information, are essential to provide specificity and selectivity in identification, improving efficiency and reducing false detection rates.

In this study we use the SELECT SERIES™ MRT (Figure 1) a state-of-the-art hybrid quadrupole Multi Reflecting Time-of-Flight mass spectrometer for the analysis of anonymised authentic human urine samples. Here we demonstrate further enhancements of DIA specificity, through use of a high mass resolving power (>200,000 FWHM).

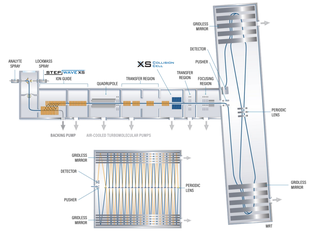


***Figure 1**: SELECT SERIES MRT instrument schematic.*

METHODS

Sample description

Forensic Toxicology QC System Suitability Test Mix (SST, 10-component mix)

Authentic human urine samples diluted 1:10 (water)

Library: Waters Forensic Toxicology Library (ESI+, 1975 entries)

LC chromatographic separation was achieved using Waters™ ACQUITY™ UPLC™ I-Class Premier chromatograph and Waters ACQUITY UPLC HSS C18 column (150 mm × 2.1 mm, 1.8 µm). A reversed-phase gradient was used, comprising Mobile Phase A (5 mM aqueous ammonium formate buffer adjusted to pH 3 with formic acid) and Mobile Phase B (0.1% *v/v* formic acid in acetonitrile)). Gradient: 0 min (87% A), 0.5 min (87% A), 10.0 min (50% A), 10.75 min (5% A), 12.25 min (5% A), 12.50 min (87% A), 15.0 min (87% A). Flow rate: 0.4 mL/min. Column Temperature: 50ºC. Injection volumes 5 µL.

MS Conditions

Acquisition:	ES+
Capillary voltage:	0.8 kV
Desolvation temperature:	500 ºC
Source temperature:	120 ºC
Cone:	20V
Collision energy ramp:	15-40 eV
Mass Range:	*m/z* 50–2400
MS^E Acquisition rate:	10 Hz
Data analysis and visualization:	MassLynx™ 4.2 SCN1026 and waters connect™ 3.1.0.243 Tibco Spotfire® 6.0.0 Software (Palo Alto, CA).

RESULTS AND DISCUSSION

Data analysis for a series of anonymised authentic human urine samples was performed. Data were compared with the Waters 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.

Prior to screening the authentic human urine samples, the system performance was assessed using a SST mix. Data were processed using a retention tolerance (t_r) of ±0.35 min, precursor ion mass accuracy tolerance of ±2ppm, and the presence of at least 1 diagnostic fragment ion with a mass tolerance of 0.2 mDa. For the SST mix (250 pg/µL) mass error (RMS) of 522 ppb was obtained and for the dilution series (2.5pg/µL to 500 pg/µl), the mass accuracy RMS errors for the SST mix constituents are shown in Figure 2.

All SST mix analytes were identified, when compared with the library, confirming the stringent mass accuracy data processing parameters could be adopted, providing greater specificity for precursor and fragment ions identification, in turn helping to mitigate false detections.

For complex analyses, the mass resolution that can be achieved using the Q-MRT allows matrix interferences and analytes of interest to be distinguished and typically results in ppb mass accuracy. An example of routine ppb mass accuracy performance is shown for the clozapine MS^E fragment ion spectrum (see Figure 3), mass resolution 130000 FWHM is illustrated for the *m/z* 84 fragment.

The same stringent tolerance criteria were also applied when processing the authentic urine samples and were compared with the toxicology library. Identifications made, included illicit drugs and prescribed medications, together with their metabolites, as well as dietary and endogenous constituents. As an example, in sample "103", oxycodone has been excluded as a false detection. Methamphetamine has been identified with a mass measurement error of 87 ppb and methadone, -83 ppb (see Figure 4). Drug metabolites have also been identified, aswell as compounds resulting from dietary consequence, an overall mass measurement error of 511 ppb (RMS) has been obtained for these compounds, providing confidence that true identifications have been made.

In combination with software tools, routine ppb mass accuracy can provide enhanced confidence and a time efficient route to determine positive identifications, resulting from recreational drug use. In the case of sample "51", repeat analyses have confirmed the identification of polydrug use, including illicit, prescription and OTC drugs. The combination of small molecule drugs identified, is described in Figure 5. 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.

During our research, specific investigations were performed, to assess 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 because of energy imparted from the ion source/transfer ion optics of mass spectrometers. Figure 4 shows for methamphetamine, minimal labile fragmentation was observed. Using direct analysis infusion, instrument parameters were optimised to reduce labile fragmentation. At low temperature (100ºC), it can be seen that in the case of amphetamine, under LCMS conditions, for the precursor ion spectrum, *m/z* 136, forms the base peak ion, however using analysis conditions with lower source temperature substantially reduces labile fragmentation (see Figure 6).

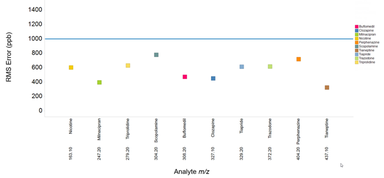
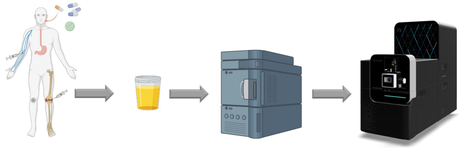


***Figure 2**. LC-MRT-MS^E ES+ precursor ion mass accuracy ppb (RMS) for SST mix.*



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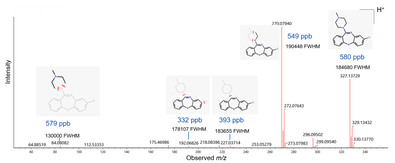
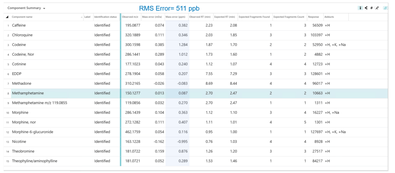


***Figure 3**. LC-MRT-MS^E ES+ fragment ion spectrum obtained for SST mix constituent clozapine ([M+H]$^+$ *m/z* 327. 13710).*



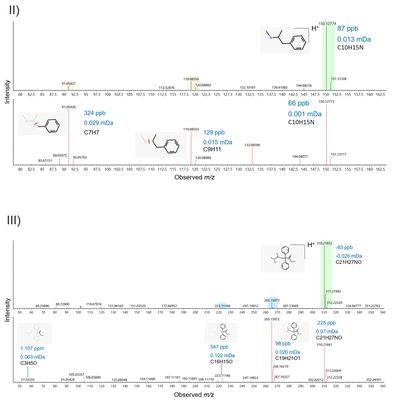
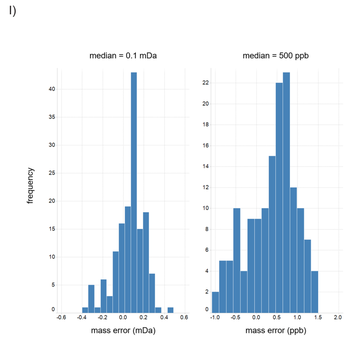


***Figure 4**. I) Component summary illustrating illicit, prescription, OTC and dietary compounds identified in anonymised human urine "sample 103". II) Methamphetamine enhanced MS^E precursor and fragment ion spectrum. III) Methadone enhanced MS^E precursor and fragment ion spectrum.*



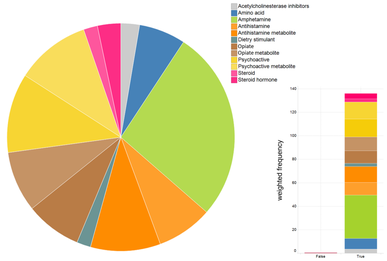


***Figure 5**. I) Frequency distribution of mass measurement error for analyte identifications determined to be present in "Sample 51". II) Distribution of "drug class" and plot of weighted frequency of observed true/false detections.*

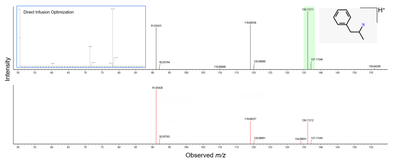


***Figure 6.** Amphetamine enhanced LCMS^E ES+ precursor and fragment ion spectrum, with low temperature direct infusion analysis ES+ (inset).*

CONCLUSION

- A 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.
- Software tools are a key element to fully maximising all available information from the dataset, 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 fragment ions with ppb mass accuracy.
- All samples were also positive for other recreational drug substances or OTC's.

References

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