Forensic Toxicology Data-Independent Analysis Screening Using Xevo™ MRT Mass Spectrometer Routine Parts-per-Billion (ppb) Mass Accuracy

Authors: ***Nayan. S. Mistry*** *and Michael McCullagh*
Affiliations: Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX, UK

INTRODUCTION

- **Laboratories are frequently required to perform broad screening techniques on complex biological matrices, to identify drug substances, and other toxicants in a quick and efficient timeframe. As well as accurate results and rapid turnaround times, laboratories are constantly under pressure to seek options that are cost effective and environmentally sustainable.**
- **In recent years, broadband data-independent analysis (DIA) and post-acquisition targeted processing, has fast become the preferred method applied for screening of forensic samples. Currently, the analytical strategy uses high-resolution mass spectrometry (Xevo G3 QTof Mass Spectrometer) to facilitate the collection of an unbiased dataset, providing a complete profile of the sample.**
- **Here, we demonstrate a step change enhancement of DIA specificity, through use of routine parts-per-billion (ppb) mass accuracy, achieved using the Xevo MRT Mass Spectrometer (MS), with the use of a previously characterized commercial standard and anonymized authentic urine samples, illustrating the enhanced analyte selectivity, improved mass accuracy and increasing confidence in identification.**

Figure 1. Xevo MRT MS coupled with the ACQUITY™ UPLC™ System

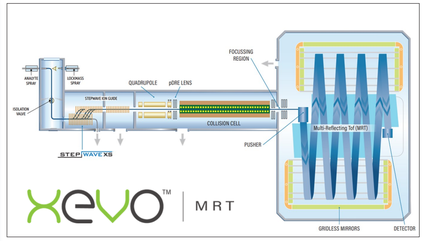

Figure 2. Schematic of Xevo MRT MS

METHOD

Samples

- System suitability test (SST) mix, a commercial standard, prepared at 25 ng/mL with 5mM ammonium formate pH 3 (mobile phase A).
- Ten anonymized authentic urine samples diluted 1:10 with mobile phase A.

Instrumentation

- Data were acquired using the Waters ACQUITY UPLC I-Class FTN PLUS System coupled with the Xevo MRT MS (Figures 1 and 2). The Forensic Toxicology Screening solution (MS^E mode), in ESI+ ionization mode, utilizing a 15-minute gradient elution[1,2] (Figure 3) was the mode of acquisition.

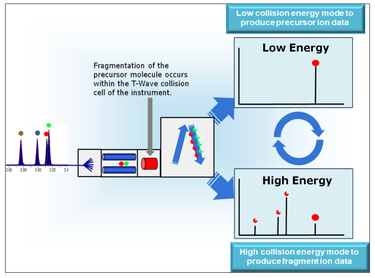

Figure 3. Schematic representing DIA (MS^E) mode of acquisition

Data Processing

- Data were acquired and processed using the waters_connect™ Informatics package. The Forensic Toxicology Screening solution also incorporates a comprehensive library for more than 2000 drugs and metabolites with reference retention times and exact masses of the precursor mass and fragment ions.

RESULTS AND DISCUSSION

- Drug substances (illicit and non-illicit) were detected based on retention time, the presence of a precursor mass and fragment ions coupled with the power of mass accuracy tolerances (precursor mass ≤2 ppm and fragment ions ≤0.2 mDa) to give absolute confidence in analyte identification.
- Across the ten anonymized authentic urine samples, excellent mass accuracies were achieved for the compounds identified following a targeted analysis.
- Figures 4 and 5 are frequency distribution plots displaying the overall mass accuracies obtained for the precursor and fragment ions. Figure 4 displays the frequency distribution plot illustrating the binned precursor mass error (ppm) for 160 identified substances (*m/z* 122—478), that were detected in the ten anonymized authentic urine samples. A root mean square (RMS) of 571 ppb was attained, achieving a 94% detection rate within 1 ppm.
- Figure 5 displays the frequency distribution plots illustrating the binned mass error (mDa and ppb) for 92 fragment ions (*m/z* 80—268), obtained for 23 randomly selected substances that were identified for the 160 detections, achieving a 96% detection rate within 1 ppm.
- Figure 6 provides an example where a false positive identification was prevented. Panel 6A shows Anabasine as the 'Best Match', based on retention time (±0.35 min) tolerance, and mass accuracy tolerances of ≤2 ppm (precursor mass) and ≤2 mDa (fragment ions). Fragment ion *m/z* 131.07162 with a mass accuracy of 1.5 mDa (highlighted), potentially a false positive identification. Reducing the fragment ion tolerance to ≤0.2 mDa removes this fragment detection, thus reducing the number of detections to 3 out of 4 fragments for Anabasine. Nicotine, previously an alternative assignment, now becomes the 'Best Match', with all its expected fragments ions less than 0.2 mDa, panel 6B.
- Parts-per-billion (ppb) mass accuracy reduces workload and provides opportunity for less experienced analysts to work independently and with confidence.
- Estimated cost to the NHS to perform a single identification confirmation assay in-house, can be approximated at £100, in addition to consumables, coroner fees, etc.
- Analyses costs escalate when samples are outsourced, *e.g.*, couriers fees, etc. This can potentially increase the sample reporting time to 6-weeks or more, causing an unnecessary wait for the bereaved.

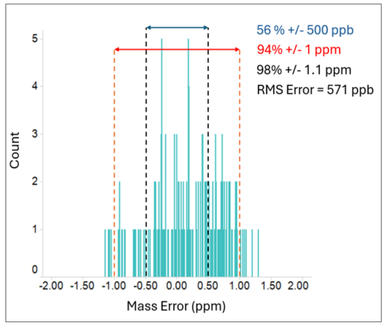

Figure 4. Frequency distribution plot of the binned precursor ions mass error for 160 detections, attaining an RMS of 571 ppb and achieving a 94% detection rate within 1 ppm

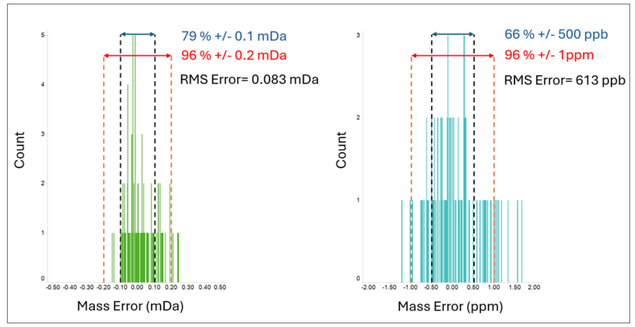

Figure 5. Frequency distribution plot of the binned mass error for 92 fragment ions identified, achieving a detection rate of 96% with 1 ppm

References

1. N. S. Mistry, L. J. Calton, J. Cooper. The Utility of MSE for Toxicology Screening with waters_connect and the Xevo G3 QTof Mass Spectrometer. Waters Application Note, 720008045, Sept 2023
2. N. S. Mistry, L. J. Calton, J. Cooper. The Ultimate Forensic Toxicology Screening Companion—Xevo G3 QTof. Waters Application Note, 720008033, Sept 2023

Disclaimer: I am an employee of Waters Corporation and any opinions that I state, are my own.

6A

6B

Figure 6. Example where a false positive identification was avoided. Panel 6A shows Anabasine as the 'Best Match', based on retention time (±0.35 min) tolerance, and mass accuracy tolerances of 2 ppm (precursor mass) and ≤2 mDa (fragment ions). Fragment ion m/z 131.07162 could be a false positive identification. Fragment ion tolerance reduced to ≤0.2 mDa. Nicotine, previously an alternative assignment, now becomes the 'Best Match', with all expected fragments ions present, less than 0.2 mDa, panel 6B

CONCLUSIONS

- **This study has demonstrated the power of ppb mass accuracy, producing high specificity and selectivity for the detection of low *m/z* analytes in complex biological matrices.**
- **PPB specificity facilitates the game changing application of stringent data processing tolerances for the precursor (≤2 ppm, processed with ≤1 ppm workflow filter) and ≤0.2 mDa for the fragment ions, to increase analyte identification confidence and improve analysis efficiency.**
- **This is pivotal in achieving efficiency, by reducing false positive identifications, resulting in more efficient data review; reducing the number of samples selected for confirmation analysis, thus reducing unnecessary expenditure leading to a positive impact upon environmental sustainability.**