COMPARATIVE EVALUATION OF HIGH RESOLUTION MASS SPECTROMETRY ACQUISITION STRATEGIES FOR THE DETECTION AND ANALYSIS OF ILLICIT DRUG SUBSTANCES

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INTRODUCTION

Drug screening is a critical component of successful drug treatment programs worldwide, supporting both compliance monitoring and the detection of illicit substance use. Traditionally, laboratory workflows rely on an initial immunoassay screen followed by confirmatory analysis using LC-MS/MS. More recently, some service providers have streamlined this process by adopting a single targeted LC-MS/MS method, focused on a select group of key analytes. Although both approaches are effective for limited analyte panels, they lack the breadth needed to detect a wider range of drug substances.

This study investigates the performance of two time-of-flight (Tof) acquisition strategies and assesses their ability to meet existing service requirements while expanding analytical capability to enable broad, comprehensive drug screening using the same instrumentation.

Figure 1. The benchtop hybrid quadrupole multi-reflecting time-of-flight mass spectrometer (MS), Xevo™ MRT MS (Waters) coupled with the ACQUITY™ UPLC™ System.

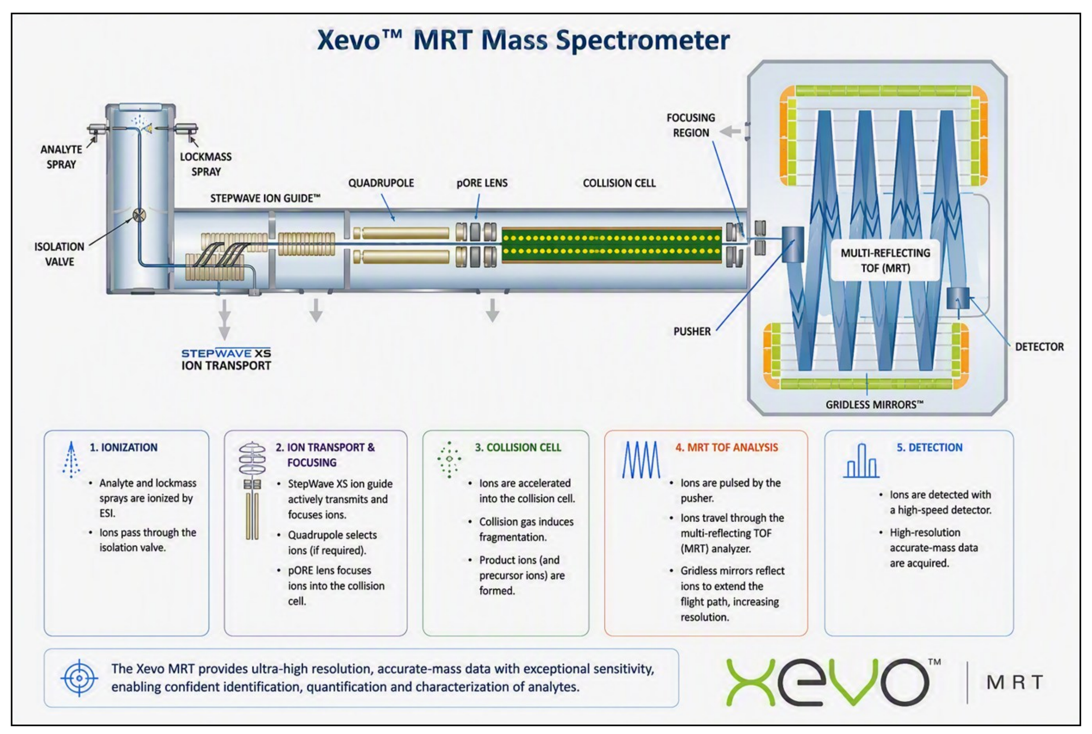

Figure 2. Schematic of Xevo MRT MS. *This image contains content that has been generated, enhanced, or modified using AI technology.*

METHOD

Samples

- The Waters system suitability commercial reference standard was prepared at a final concentration of 25 ng/mL by performing a 1:20 dilution of the supplied 500 ng/mL stock solution.
- Urine calibration samples were prepared by fortifying pooled blank urine with twenty target analytes (Table 1) across a concentration range of 0.01–2500 ng/mL.
- A cohort of twenty anonymized authentic urine samples were analyzed together with a commercial reference urine, ACQ Science DCT –25% UR.
- All urine samples underwent a five fold dilution with 5mM ammonium formate (pH 3), mobile phase A, prior to analysis.

Instrumentation

- Data were acquired using the Waters ACQUITY UPLC I-Class FTN PLUS System coupled with the Xevo MRT MS (Figures 1 and 2).
- Chromatographic separation for both Tof acquisition modes were performed on a Waters ACQUITY HSS C18 Column, employing a 15 minute gradient elution, using 5mM ammonium formate (pH 3) and acetonitrile containing 0.1% formic acid, under ESI+ ionization conditions.[1-3]

Tof-MS^E analysis (Data-Independent analysis)

- The previously described screening method employs a non-targeted acquisition strategy (Tof-MS^E; Figure 3). Because this approach generates a comprehensive, information-rich dataset, all acquired data remain available for subsequent targeted, semi-targeted, and non-targeted ('Discovery') data processing workflows.[1-3]

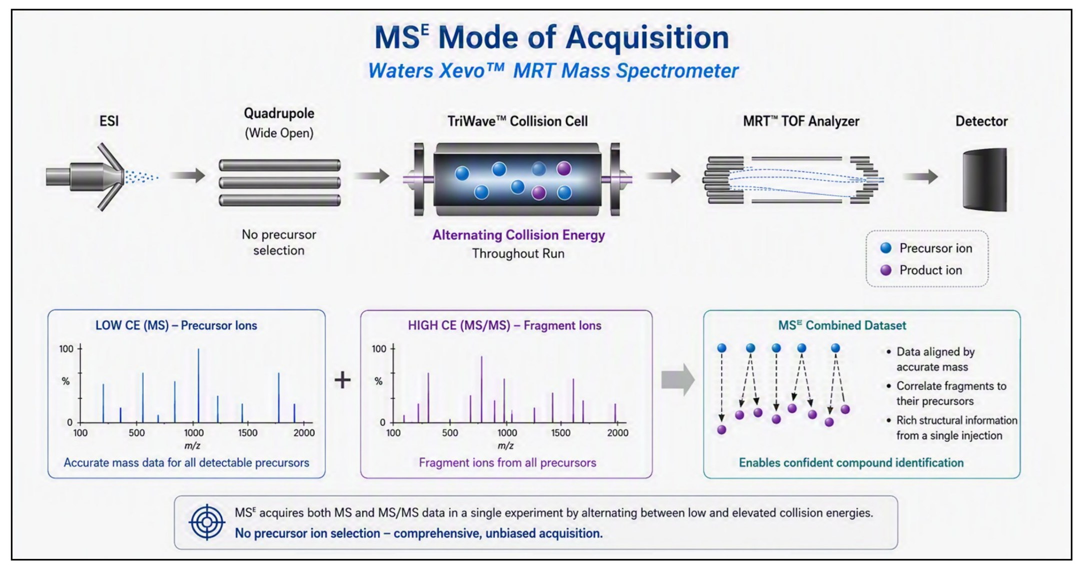

Figure 3: Tof-MS^E is a data-independent acquisition mode utilized on the Xevo MRT MS. During acquisition, the quadrupole is operated in a non-selective mode, transferring all ions into the collision cell. The collision cell alternates rapidly between low energy and a ramped energy. The low energy provides the accurate mass of the precursor ion and the ramped energy (10 to 40 eV) leads to the generation of accurate masses of fragment ions for additional confirmation. This mode of acquisition supports broad spectrum, untargeted toxicological screening. This image contains content that has been generated, enhanced, or modified using AI technology.

Data Processing

- Data processing was performed using the UNIFI™ Application, within the *waters_connect™* Informatics Platform, with compound identification supported by matching against the Waters Toxicology Library, which contains more than 2,100 drugs and metabolites.
- Compound identification relied on concordance between expected retention time (± 0.3 min) and an analyte specific mass spectral signature comprising the exact mass precursor and at least one corresponding fragment ion within a ± 2 ppm tolerance.

Tof-MRM analysis (Targeted Data-Dependent analysis) (Figure 4)

- For this targeted acquisition mode, exact mass fragment ions comprising a quantifier and, where available, a qualifier ion were monitored, with cone voltages and collision energies individually optimized.

Data Processing

- Data processing was performed using MS Quan Application, within the *waters_connect* Informatics Platform. Mass chromatograms for the monitored transitions were generated and peak areas integrated, applying a minimum area threshold of 200 (quantifier and qualifier ions) and a retention time tolerance of ± 0.3 min from the reference retention time.

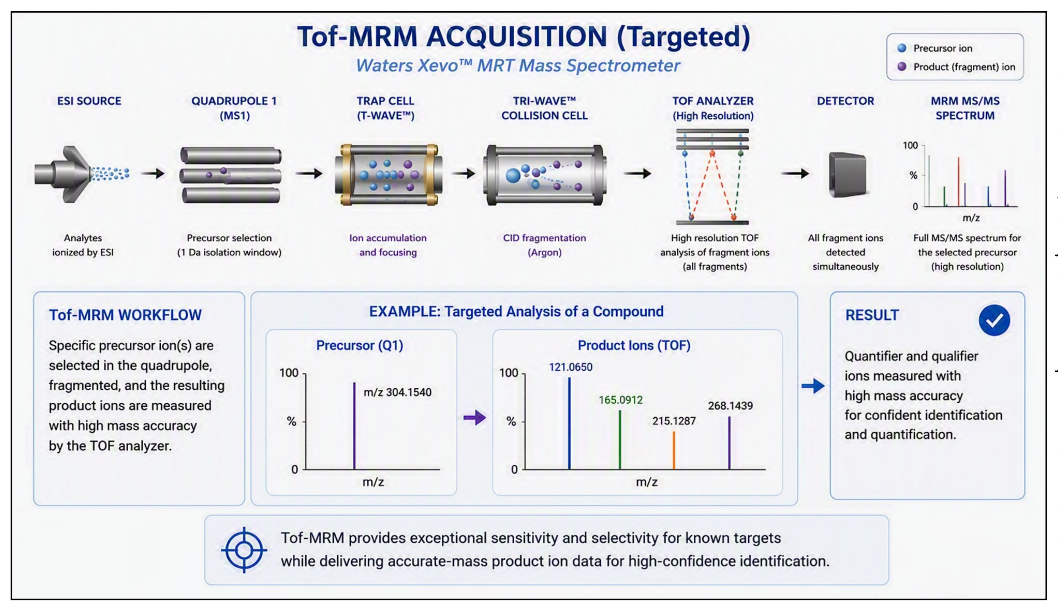

Figure 4: Tof-MRM is a targeted data-dependent acquisition mode utilized on the Xevo MRT MS. This mode uses quadrupole precursor selection to isolate a specific precursor mass, permitting only this mass to enter the collision cell, where it undergoes fragmentation. In this example, the quadrupole is set to isolate the m/z of the green peak. The Tof detector records the accurate masses of all fragment ions. Typically a quantifier and a qualifier ion are monitored, which deliver enhanced sensitivity for quantitative and targeted screening applications. This image contains content that has been generated, enhanced, or modified using AI technology.

RESULTS AND DISCUSSION

- Drug substances were detected based on retention time, the presence of a precursor mass and fragment ions (Tof-MRM and Tof-MS^E), coupled with the power of mass accuracy tolerances (precursor mass ≤2 ppm, Tof-MS^E only) to give absolute confidence in analyte identification.
- The Tof-MRM acquisition mode exhibited substantial enhancement in analytical sensitivity, achieving limits of detection (LOD) spanning 0.05 ng/mL to 0.5 ng/mL in urine spiked samples. In contrast, the broader, non-targeted Tof-MS^E workflow produced LOD ranging from 0.5 ng/mL to 5 ng/mL. Table 2 provides a consolidated summary of the LOD determined for all monitored analytes.

Compounds	
6-Monoacetylmorphine (6-MAM)	Ketamine
Benzoylecgonine	Methamphetamine
Brorphine	Meperidine
Buprenorphine	Methadone
Carfentanil	Mitragynine
Codeine	Spirobrorphine
Dihydrocodeine (DHC)	Spirochlorphine (R6890)
EDDP	Tapentadol
Etonitazene	Temazepam
Fentanyl	Tramadol

Table 1: The twenty target drugs monitored during the Tof-MRM (targeted) acquisition.

LOD (ng/mL)	Tof-MRM	Tof-MS^E
0.05	Carfentanil, EDDP, Fentanyl, Meperidine, Methadone, Tapentadol	-
0.1	DHC, Methamphetamine, Tramadol	-
0.5	6-MAM, Benzoylecgonine, Brorphine, Buprenorphine, Codeine, Etonitazene, Ketamine, Mitragynine, Spirobrorphine, Spirochlorphine, Temazepam	Benzoylecgonine, Carfentanil, EDDP, Fentanyl, Methadone, Tapentadol
1	-	Brorphine
5	-	6-MAM, Buprenorphine, Codeine, DHC, Etonitazene, Ketamine, Meperidine, Methamphetamine, Mitragynine, Spirobrorphine, Spirochlorphine, Temazepam, Tramadol

Table 2. Summary of the LOD, for analytes across the Tof-MRM and Tof-MS^E acquisition modes.

- Figure 5 displays the chromatograms corresponding to the monitored quantifier and qualifier transitions acquired using Tof-MRM at 0.05 ng/mL, accompanied by the calibration profile for urine enriched with carfentanil.
- The two acquisition methods were subsequently applied to a cohort of twenty authentic urine samples, yielding results that demonstrated excellent qualitative concordance. Importantly, the Tof-MS^E acquisition strategy enabled the identification of additional substances outside the predefined targeted panel, encompassing frequently encountered medications, benzodiazepines, antidepressants, and illicit drug substances, which would have otherwise gone unobserved.
- Figure 6 shows the compounds identified by Tof-MS^E, for the commercial reference urine, ACQ Science DCT –25% UR. The substances are present at concentrations between 0.75 ng/mL (Fentanyl) to 225 ng/mL (*e.g.,* Codeine and DHC).

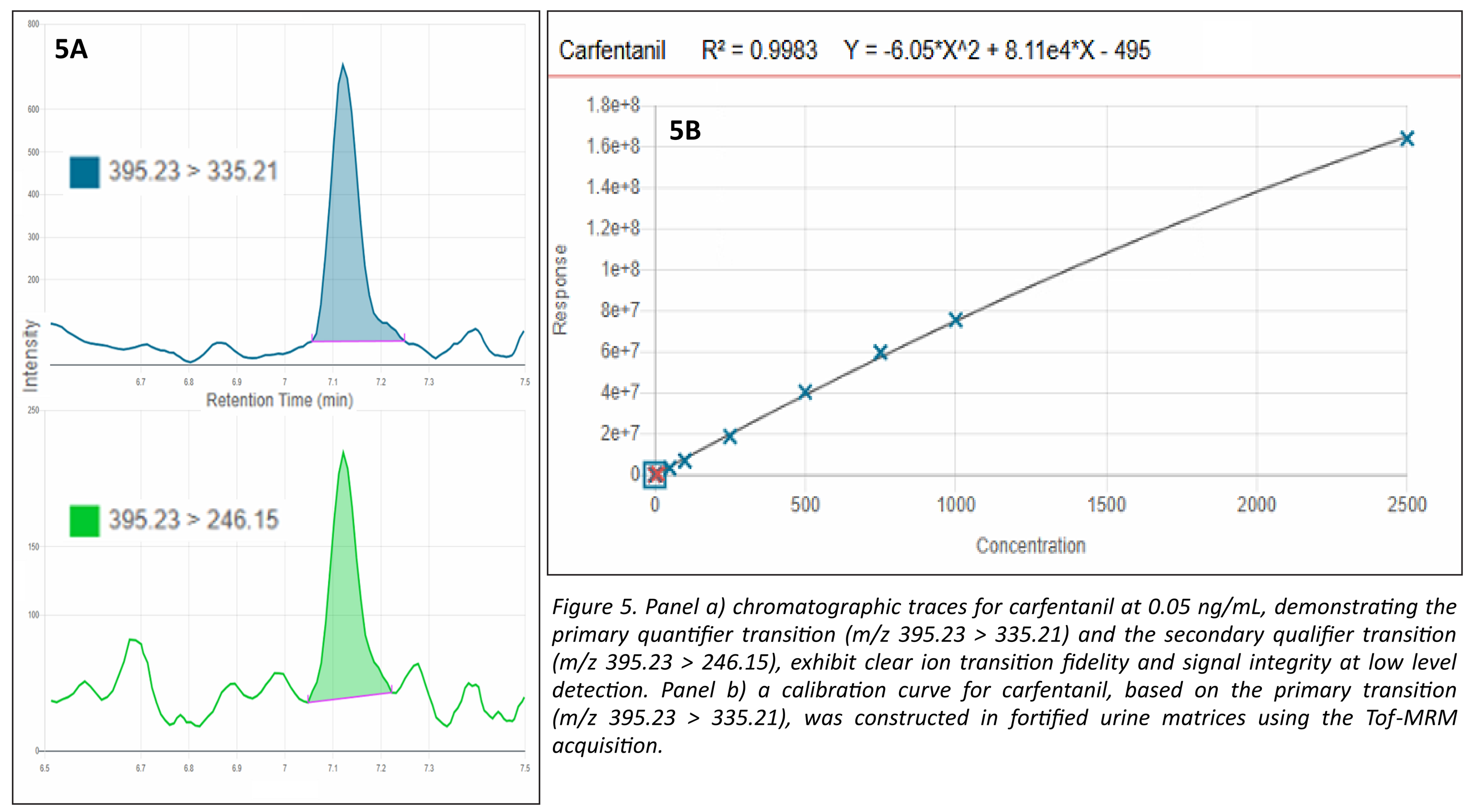

Figure 5. Panel a) chromatographic traces for carfentanil at 0.05 ng/mL, demonstrating the primary quantifier transition (m/z 395.23 > 335.21) and the secondary qualifier transition (m/z 395.23 > 246.15), exhibit clear ion transition fidelity and signal integrity at low level detection. Panel b) a calibration curve for carfentanil, based on the primary transition (m/z 395.23 > 335.21), was constructed in fortified urine matrices using the Tof-MRM acquisition.

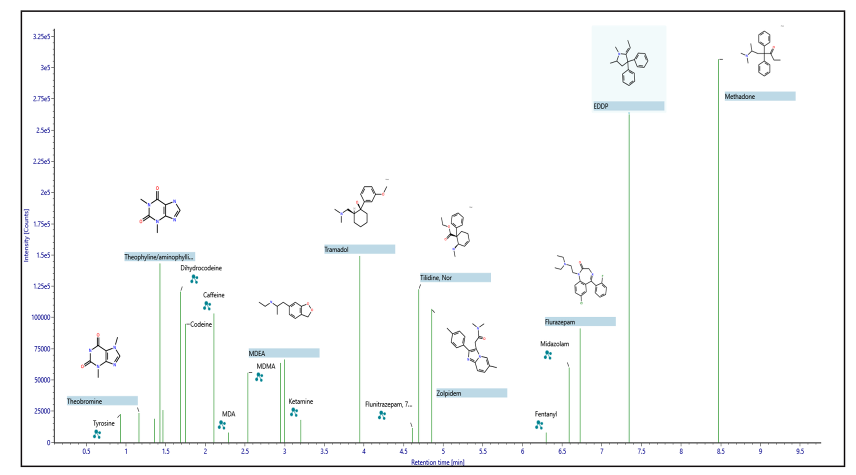

Figure 6. Schematic of the identified compounds using Tof-MS^E, for the commercial reference urine ACQ Science DCT –25% UR. The structures associated with the identified compounds are also displayed (space-permitting).

CONCLUSIONS

Although LC-MS/MS methodologies are a mainstay in contemporary toxicology laboratories due to their exceptional analytical sensitivity, the techniques are intrinsically restricted in the depth of structural and contextual information they can deliver.

Both Tof-MRM (targeted data-dependent analysis) and Tof-MS^E (data-independent analysis) acquisition modes, achieving LOD values of ≤0.5 ng/mL and ≤5 ng/mL, respectively, demonstrated a simple, robust, and highly sensitive analytical approach for urinary drug quantification, delivering sensitivity comparable to conventional tandem-quadrupole methodologies.

Furthermore, incorporation of the complementary Tof-MS^E acquisition, further enhanced overall sample characterization, enabling the identification of additional drug substances beyond the predefined targeted panel. Together, these strategies provide a powerful and comprehensive framework for drug screening and quantitation in complex biological matrices.

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
3. N. S. Mistry, G. Hammond, R. Lieberman, V. Starkie. Forensic Toxicology Data-Independent Analysis Screening Using Xevo MRT Mass Spectrometer Delivering Routine Parts-per-Billion (ppb) Mass Accuracy. Waters Application Note, 720009219, Feb 2026