Comparative Evaluation of High Resolution Mass Spec Acquisition Strategies for the Detection and Analysis of Illicit Drug Substances

Nayan. S. Mistry

Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX. UK.

Background:

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. In recent years, 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 various 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.

Methods:

Quantitative analysis was carried out for 20 drug substances, including amphetamines, benzodiazepines, benzoylecgonine, etonitazene, opiates, and sedatives, using the benchtop hybrid quadrupole multi-reflecting time-of-flight mass spectrometer (MS), the Xevo™ MRT MS (Waters™). Data were acquired using both Tof-MS^E (data-independent type) and Tof-MRM (data-dependent type) acquisition modes. Pooled blank urine samples were fortified with the target analytes across a concentration range of 0.01 to 1000 ng/mL, followed by a simple 1:5 dilution step prior to LC-MS analysis. Data were additionally collected for the commercial reference material, the Waters system suitability mixture (SSM) at 25 ng/mL, alongside 20 anonymised authentic urine samples (diluted 1:5). Tof-MS^E data were acquired using the Waters Forensic Toxicology Screening solution, in ESI+ ionisation mode, with a collision energy ramp of 10-40 eV. Samples were analysed using reversed phase chromatography comprising a 15-minute gradient elution with a C_{18} column, maintained at 50°C. Tof-MRM data were acquired using the Waters Forensic Toxicology Screening solution chromatography only. For each analyte, two exact-mass product ions (a quantifier and a qualifier) were monitored, with cone voltages and collision energies individually optimised.

Results:

In this investigation, the Tof-MRM acquisition mode, demonstrated a marked enhancement in analytical sensitivity, achieving limits of detection of 0.5 ng/mL (or better) for all compounds, compared with Tof-MS^E (5 ng/mL). This superior performance emphasizes the sensitivity and robustness of the Tof-MRM approach, highlighting its capacity to reliably quantify analytes with greater precision and confidence on a high-resolution mass spectrometer. The methods were subsequently applied to a set of authentic urine samples (n = 20), and the results showed strong quantitative agreement. 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.

Conclusions:

Both data-independent and data-dependent Tof acquisition modes demonstrated a simple, robust and highly sensitive approach for quantifying drug substances in urine, delivering sensitivity comparable to traditional tandem quadrupole methods. 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.