



K32 Comparison of Data Acquisition Methods for High Resolution Mass Spectrometry (HRMS) Drug Screening

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Learning Overview: After attending this presentation, attendees will understand how HRMS data acquisition can be used to improve strategies for drug detection.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by discussing the relative merits of quadrupole Time-Of-Flight-Mass Spectrometer (qTOF-MS) data acquisition approaches and the challenges associated with the technological shift from traditional immunoassay to HRMS-based drug screening.

According to the most recent census of publicly funded laboratories in the United States, forensic toxicology is now the most outsourced discipline in forensic science. The increased need for outsourcing can be attributed in large part to the proliferation of New Psychoactive Substances (NPS) and the expanded scope of drug testing that is required in antemortem and postmortem toxicology investigations. Over several decades, drug users have shifted from classical drugs of abuse, such as cocaine and marijuana, to new emerging synthetic drugs, including designer benzodiazepines, synthetic cannabinoids, cathinones, fentanyl analogs, and novel opioids. Traditional immunoassay-based screening techniques cannot keep pace with these developments due to the sheer number of drugs, need for adaptation, and limitations in terms of their cross-reactivity.

In order to combat this challenge, some laboratories have transitioned to MS-based screening. HRMS using qTOF-MS is of particular interest because of its sensitivity, specificity, and potential for retrospective data analysis. HRMS allows laboratories to keep abreast of the transitory drug trends and can be more readily adapted to the changing landscape of drug use than traditional immunoassay-based screening. This study focuses on the optimization of data acquisition techniques using Liquid Chromatography (LC) qTOF-MS. Three different acquisition modes were evaluated for this purpose, including TOF MS, All Ions MS, and Auto MS/MS. TOF-MS functions as a traditional time of flight instrument using a precursor ion for detection. All Ions mode MS simultaneously analyzes all precursor ions in addition to fragmenting each precursor at set collision energies, thus providing an additional layer of identification with fragment confirmation. In contrast, Auto MS/MS mode isolates one precursor ion at a time and also fragments each precursor at set collision energies. In addition, the Auto MS/MS mode utilizes an abundance threshold to dictate which precursor ions are fragmented. Using the All Ions approach, all ions that travel through the instrument are fragmented, regardless of abundance. As a consequence, there are relative merits associated with each acquisition mode. The goal of this study was to perform a side-by-side comparison of these techniques using analytes of interest in forensic toxicology. Using certified reference materials, common drugs of abuse and NPS were analyzed using optimized data acquisition methods with an Agilent® 6530 Accurate Mass qTOF-MS. A comparison was conducted for each drug's mass error, target score, and signal-to-noise ratio using each approach. The target score is a weighted value determining how well the mass, isotopic pattern, and retention time of the suspect compound coincide with the target compound.

It was hypothesized that the All Ions data acquisition mode would prove the most robust screening tool since fragmentation occurs for all precursor ions, regardless of abundance. All Ions data acquisition collects and fragments all precursor ions increasing the probability of detecting novel drugs retrospectively as a delay in identification is expected because clandestine production rate surpasses current laboratory identification capabilities. This untargeted approach to screening is highly beneficial in forensic toxicology, given the transient nature of many drug trends. Data-dependent and data-independent acquisition are explored, and the relative merits of these approaches are discussed.

HRMS, NPS, QTOF-MS