K35 The Development of a High-Resolution Mass Spectrometry (HRMS) Library and Method Validation for Screening and Confirmation of 800+ Novel Psychoactive Substances (NPS) by Liquid Chromatography/Quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS)

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After attending this presentation, attendees will better understand the development of a compound database and spectral library containing more than 800 NPS, metabolites, and related compounds from a wide variety of drug classes with a particular focus on synthetic cannabinoids and stimulants. In addition, this presentation will demonstrate the use of standard mixtures rather than individual compounds to validate a comprehensive screening/confirmation method for the detection and identification of these NPS in biological fluids using LC/qTOF/MS.

This presentation will impact the forensic science community by presenting a developed HRMS library that can be used to help identify NPS.

NPS are of great interest to forensic toxicology labs due to their potentially high potency and ability to evade detection by many current screening methods. These compounds can also be rapidly developed to avoid current scheduling laws, causing a need to develop comprehensive detection methods covering a wide variety of drug classes.

An Agilent® 1290 Infinity® HPLC system and Agilent® 6530 QTOF-MS with Jet Stream technology Electrospray Ionization (ESI) source was used for this research. A total of 826 compounds to be included in the final method were analyzed using Flow Injection Analysis (FIA) to create an HRMS library with spectral data collected at three collision energies (10eV, 20eV, and 40eV) for each compound. Once the spectral data were collected, all compounds were run through an Agilent® ZORBAX® Rapid Resolution HD Eclipse® Plus C18 column (3.0mm x 100mm; 1.8µm particle size) to obtain retention times. LC was performed with a gradient of 95% A (5mM ammonium formate in HPLC water with 0.1% formic acid) and 5% B (methanol with 0.1% formic acid) from 0min–1min, increasing to 90% B over 1min–9.5min, then held at 90% B for the remainder of the 20min run. All retention times were used to create the final method for validation. The collected HRMS data and retention times were curated into a database/library using the MassHunter™ Personal Computer Database Library (PCDL) Manager software. Each compound entry also contained the following information: compound name, chemical formula, monoisotopic mass, chemical structure, and International Union of Pure and Applied Chemistry (IUPAC) name. Chemspider and Chemical Abstracts Service (CAS) numbers were also included, when available. The developed HRMS library is used to help identify NPS in real-time analysis, as well as to retrospectively search previously collected data.

In order to fully validate the method, calibration curves were created for each drug standard. Completing individual calibration curves for each of the 826 NPS included would be extremely time consuming and inefficient; therefore, an approach using a series of standard calibration mixes was investigated. Validation of the proposed method for 826 compounds involved the creation of 25 mixes containing between 29–37 different compounds each. The compounds selected for each mix were selected so that no compounds had the same retention time and had a minimum of 0.2min between compound peaks in the mix. Seven different calibration levels were chosen for method validation: 1, 2, 5, 10, 20, 50, and 100ng/mL. All calibrators also included an internal standard "supermix" made of 22 deuterated standards representing multiple NPS drug classes. Calibrations were performed with both methanol-based and spiked matrix (urine) mixtures for method optimization. For calibrations completed in urine, a simple "dilute and shoot" approach was used in which a 1:5 dilution was directly injected into the instrument.

To date, individual calibration curves have been created for nine different NPS mixtures representing nearly 300 of the 826 proposed NPS for the final validated method. LC chromatograms were analyzed using MassHunter™ QTOF Quantitation software. This approach was capable of identifying all compounds in each mixture. The results of these experiments clearly demonstrate the value of using standard mixes for method validation in comprehensive toxicological analysis in conjunction with an HRMS library. Work is continuing to create calibration curves for the remaining compounds using mixtures containing a maximum number of compounds to limit the number of mixtures needed for full validation.

LC/qTOF/MS, Novel Psychoactive Substances, Method Validation