K30 Validation of an Assay for Amphetamines in Postmortem Samples Using Supported Liquid Extraction (SLE) and Biotage® Extrahera™ Automation Followed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis

Heidy P. Rivera, BA, Elizabeth, NJ 07202; Kristen M. Bailey, MS, Office of the Chief Medical Examiner, Charleston, WV 25302; Lauren L. Richards-Waugh, PhD, Marshall University Forensic Science Program, Huntington, WV 25701; James C. Kraner, PhD, Office of the Chief Medical Examiner, Charleston, WV 25302*

Learning Overview: After attending this presentation, attendees will have a better understanding of the importance of integrating automation in the forensic toxicology laboratory for the purpose of reduced analyst time and improved assay consistency.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating that the Biotage® Extrahera™ robotic platform for automated drug extraction is capable of efficiently and reliably processing up to 96 samples in a single batch. This method validation demonstrated that the automated method was capable of successfully performing extractions at the intended use level and can be used to replace the current manual extraction for casework involving amphetamines.

The Biotage® Extrahera™ is an automated robotic instrument that can perform an extraction on up to 96 samples, allowing for an increase in time efficiency and minimization of error by increasing pipetting precision and reducing the risk of contamination between samples. The Biotage® Extrahera™ utilizes an interchangeable platform that can be customized for SLE, SPE, Phospholipid Depletion (PLD), and Protein Precipitation (PPT) -based methods.

In this study, an SLE method for amphetamines in postmortem whole blood using the Biotage® Extrahera™ and LC/MS/MS analysis was validated in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology published in 2013. A 200µL aliquot of calibrators, controls, and whole blood samples were manually pipetted onto a Thomas Scientific 1mL, 96-well plate that was loaded onto the Biotage® Extrahera™ where the samples were extracted utilizing a Biotage® Isolute® SLE+ 400µL 96-well plate and a Waters® 2mL 96-well square collection plate. Deuterated analogs of the analytes of interest were used as internal standards. A 100µL aliquot of the eluate was evaporated to dryness and reconstituted on the Biotage® Extrahera™ with 1000µL of Optima-grade water for injection onto a Waters® LC/MS/MS system. Samples were then analyzed by electrospray ionization in positive-ion Multiple Reaction Monitoring mode with optimized collision energy for the precursor ion selected, monitoring two or three transitions for each analyte of interest. The validation plan consisted of studies for various interferences, calibration model, ionization suppression/enhancement, carryover, bias and precision, Limit Of Detection (LOD), and Limit Of Quantitation (LOQ). The analytes of interest were amphetamine, methamphetamine, phentermine, ephedrine/pseudoephedrine, phenylpropanolamine, MDA, and MDMA.

This method produced data that met the acceptance criteria established for the validation. The internal standard, analyte, and matrix interference studies revealed that no deuterated internal standard, commonly encountered non-target analytes, or matrix components produced a signal for any of the target analytes. The method was free from carryover at 3,000ng/mL for each analyte. Ionization suppression or enhancement was less than ±25%. The proper calibration model and weighting function was chosen for each analyte using a Microsoft® Excel® spreadsheet. The method also produced the following quantitative data: the LOD and LOQ for amphetamine, methamphetamine, phentermine, ephedrine, phenylpropanolamine, MDA, and MDMA were 20ng/mL, 10ng/mL, 40ng/mL, 10ng/mL, 10ng/mL, 10ng/mL, and 10ng/mL, respectively. For all compounds, bias and precision were within 20%.

Automation, Amphetamines, LC/MS/MS