K21 The Detection and Quantification of 23 Drugs in Blood and Urine Following Solid Phase Extraction (SPE) Using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS)

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Learning Overview: After attending this presentation, attendees will be able to use the method presented, or develop their own singular method, for both blood and urine analysis of selected amphetamines, antidepressants, anesthetics, opioids, designer, and hallucinogenic drugs.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by offering a method that will provide the ability to analyze different drug groups in one method for both blood and urine matrices.

Background/Introduction: In forensic toxicology, analysis of drugs in biological fluids is performed to determine cause of death, suspected drug use, drug-facilitated crimes, or whether someone was driving under the influence. Determining what analytes are present and the concentration of those compounds in a variety of matrices (e.g., blood, urine, or oral fluid) can be complex. It is therefore necessary to have optimal sample preparation and instrumental conditions that work for all matrices of interests. Determining the best approach can be challenging due to the amount of time and resources to perform expansive evaluations of sample preparation, stationary/mobile phases, LC conditions, and MS operating parameters.

Objective: This project developed and validated an SPE and UPLC-MS/MS method using both human whole blood and urine to identify and quantify selected amphetamines, antidepressants, opioids, anesthetics, designer drugs, and hallucinogenic drugs.

Method: Calibration curves and Quality Controls (QCs) were prepared for SPE in 200µL of either drug-free whole blood or donated urine. All donated samples were collected following approved Institutional Review Board requirements. Analytes were spiked at varying concentrations using certified reference standards. Deuterated internal standards were also spiked at a concentration of 200ng/mL. QCs were prepared at 20ng/mL, 125ng/mL, 450ng/mL, and 950ng/mL. SPE was performed with mixed-mode copolymeric Clean Screen® DAU columns. Samples were reconstituted in 400µL of Millipore® water containing 0.1% formic acid.

The samples were run by UPLC with a 4000 QTRAP® Electrospray Ionization Tandem Mass Spectrometry (ESI/MS/MS) in positive ionization mode. Separation was achieved using a Kinetex® F5 2.6µ 100Å 50mm x 3.0mm column.

Results: Bias, precision, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), calibration model, carryover, and dilution integrity, ion suppression and enhancement, and processed stability validation parameters were assessed. All analytes in both matrices had quadratic fit with 1/x weighting, LOQs were 0.5ng/mL, 5ng/mL or 10ng/mL, and no observed carryover. Calibration ranges of 10ng/mL to1,000ng/mL, 0.5ng/mL to 1,000ng/mL, and 0.5ng/mL–500ng/mL were used. For the 23 drugs in blood, the bias ranged from -18.10% to 11.87%, precision ranged from 0.24% to 14.33%, and recovery in matrix ranged from 85% to 119%. In urine, the bias ranged from -5.40% to 7.67%, precision ranged from 0.09% to 11.99%, and recovery in matrix ranged from 80% to 119%.

Conclusion/Discussion: The method containing 23 drugs, including selected amphetamines, antidepressants, anesthetics, opioids, designer, and hallucinogenic drugs, was successfully validated in both blood and urine using the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for method validation. SPE provided clean extracts for analysis by positive ESI-UPLC-MS/MS for both matrices.

Solid Phase Extraction, Forensic Toxicology, Multi-Drug Analysis