K38 Analysis of Illicit Substances in Urine by Biocompatible Solid-Phase Microextraction (BioSPME) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will have a better understanding of how BioSPME fibers can work as a purification step in extracting drug analytes from biological fluids prior to analysis by LC/MS/MS.

This presentation will impact the forensic science community by providing a simpler and faster extraction alternative to preparing biological samples for analysis using an LC/MS/MS method that is able to simultaneously detect and differentiate illicit substances from varying classes of drugs.

Forensic toxicologists are faced with growing case workloads and demands to produce accurate results within short periods of time. What is even more challenging is the speed at which new substances are being introduced to the black market. New drugs, though they may be similar in structure to commonly recognized drugs, can evade detection by specific techniques like immunoassays, making them difficult or nearly impossible to identify. Moreover, because of the rise in synthetic drug production, crime laboratories face a large backlog and challenge in identifying these illicit substances in a timely and efficient manner using the current extraction procedures.

LC/MS/MS is commonly used by forensic toxicologists for the detection of drug analytes in biofluids. Furthermore, SPME has been used as a solventless purification step prior to LC/MS/MS to avoid complicated or timely extraction procedures. A new BioSPME fiber has been engineered and is being explored for its use in extracting drug analytes from different biological matrices. The BioSPME fiber is stationed within a pipet tippet and is functional in a 96-well format. Each fiber contains either mixed-mode hydrophobic and cation exchange particles or C-18 (reversed-phase) particles to extract drug analytes of interest. The fiber is directly placed into a biological fluid (urine) for extraction, desorbed into solution, dried, and reconstituted for analysis by LC/MS/MS. The method presented here utilized an AB SCIEX™ 3200 Qtrap® triple quadrupole mass spectrometer interfaced with a Shimadzu® LC system. Extracted samples were run in positive-ionization mode using Electrospray Ionization (ESI). Chromatography was performed using an Ultra C18 column (50 x 2.1mm, 3µm). The weak mobile phase was 0.1% (v/v) formic acid in water and the strong mobile phase was 0.1% (v/v) formic acid in acetonitrile. A gradient curve was used over a run time of five minutes per sample. The flow rate was 0.4mL/min., the column temperature was set at 30° Celsius, and the injection volume was 5µL. Drugs from different classes were studied for their extraction efficiency from urine with this novel BioSPME method using both mixed-mode and C-18 BioSPME fiber chemistries. Extraction variables such as pH and extraction time, desorption time and extraction solvents, drying time, and reconstitution solvents were considered in this study.

Seven drugs have been successfully extracted. Extraction recoveries for alprazolam, amphetamine, methamphetamine, cocaine, benzoylecgonine, mephedrone, and MDPV are as follows: 54.4%, 17.8%, 40%, 60%, 18.2%, 41.5%, and 49.5%, respectively. Limit of detection and limit of quantitation data for the same drugs were: 6.5ng/mL and 19.3ng/mL, 12.0ng/mL and 36.5ng/mL, 5.4ng/mL and 16.3ng/mL, 21.5ng/mL and 65.2ng/mL, 7.9ng/mL and 24.0ng/mL, 4.1ng/mL and 12.4ng/mL, and 6.4ng/mL and 19.5ng/mL, respectively. Extraction efficiency ranged from 17% to 60% for the drugs tested.

By utilizing BioSPME's direct sampling capabilities, time-consuming extraction procedures that were previously needed to free drug analytes from biological matrices can be eliminated. Extracted substances are differentiable from one another when analyzed by LC/MS/MS; thus, this method can be used to simultaneously detect multiple substances from a biological matrix. In turn, this method may be used to decrease the burden of lengthy extraction procedures in forensic toxicology laboratories.

Forensic Toxicology, BioSPME, LC/MS/MS

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