## B15 A Method Development for Analyzing 17 Benzodiazepines and Metabolites Used in Crimes Using Solid Phase Extraction-Tandem Mass Spectrometry

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**Learning Overview:** The goal of this presentation is to explain the results of a research project on developing an analytical method for detection, identification, and quantification of drugs used in crimes, at very low concentrations, using Solid Phase Extraction coupled to Liquid Chromatography/Tandem Mass Spectrometry (SPE-LC/MS/MS), which can be used in analyzing these drugs in biological samples, such as urine and blood.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a newly developed analytical method for detection, identification, and quantification of drugs used in crimes at very low concentrations in biological samples such as urine and blood. Victims of Drug Facilitated Crimes (DFCs) often report the crime hours or even days after they have happened, which adds to the challenge of detecting the drugs in the biological samples by the currently used analytical methods in forensic toxicology laboratories. The developed method can help detect the drugs at lower concentrations than possible with the current methods, which can extend the time the drugs can be detected in victims' urine or blood samples, giving them a better chance to prove the case.

Benzodiazepines are a class of antianxiety drugs, including drugs such as flunitrazepam, alprazolam, and diazepam. They are also known as predator or date-rape drugs used in DFCs—crimes that include robbery, the maltreatment of the elderly and children, as well as rape and other sexual assaults. Identification of these drugs, or their metabolites, in biological specimens such as the urine, blood, saliva, and hair of victims is commonly proof of exposure to the drug.

Like other psychoactive drugs used in DFCs, benzodiazepines are highly potent and have short half-lives. The combination of potency and short half-life makes the time window for when the drug is still detectable in biological samples very small. Victims of DFCs usually experience short-term memory loss and often report the crime days after they have happened, which adds to the challenge of detecting the drugs in the biological samples by the currently used analytical methods in forensic toxicology laboratories. The goal of the current project is to develop a method for detection, identification, and quantification of 17 benzodiazepines at sub-ppb concentrations in aqueous solutions and apply it to the analysis of these drugs and their metabolites in biological samples such as urine and blood.

In this study, an SPE-LC/MS/MS) method was successfully optimized. A Supel™-Select HLB 54183-U 200mg cartridge was selected for SPE. Washing and conditioning of the packing was completed using 12mL of methanol and 2mL of ultra-pure water, respectively. After sample introduction, the analytes of interest were eluted using 5mL of a 50:50 mixture of methanol and acetonitrile. The elutant was evaporated using rotary evaporation and, upon dryness, was reconstituted using 1mL of 0.01M ammonium acetate. The reconstituted sample was then subjected to LC using C18 HPLC column (particle size: 1.9µm and length: 20cm) with gradient elution with a mixture of acetonitrile and 0.01M ammonium acetate buffer solution. The elution started with 100% 0.01-M ammonium acetate and was gradually decreased to 10%, while acetonitrile was increased to 90% and held for half a minute. Then 0.01-M ammonium acetate was increased back to 100% and held for four minutes. For MS detection, a Selected Ion Monitoring (SIM) method was started at minute 3.00 of analysis until minute 8.00. Using the optimized SPE and LC/MS/MS method, this study determined the Limits Of Detection (LODs) for 17 analytes to be in the range of 0.01 to 1.8ng/ml. The optimized method was applied to analyze the drugs in the spiked urine samples and will be applied to spiked blood samples.

**Benzodiazepines, Drug Facilitated Crimes (DFCs), SPE-LC/MS/MS**