K22 Evaluation of Sample Preparation Techniques for the Detection and Quantitation of Benzodiazepines in Human Urine and Whole Blood Using High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC-MS/MS)

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Learning Overview: After attending the presentation, attendees will be able to use the methods developed, or develop their own singular method, for urine and blood analysis of selected benzodiazepines.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information on the analysis of selected benzodiazepines using various matrices and sample preparation methods.

Background/Introduction: Benzodiazepines are a class of drugs generally prescribed for treatment of anxiety, depression, and insomnia. The three most commonly encountered benzodiazepines, alprazolam, diazepam, and clonazepam, reside in the top 25 most frequently identified drugs based on the United States Drug Enforcement Administration's National Forensic Laboratory Information System 2018 Mid-Year Report and therefore remain of forensic toxicological importance.

Objective: The purpose of this research was to evaluate three sample preparation methods, Liquid-Liquid Extraction (LLE), Solid Phase Extraction (SPE), and Supported Liquid Extraction (SLE), for the reliable and accurate identification of six benzodiazepines in human urine and whole blood, including: alprazolam, alpha-hydroxyalprazolam, clonazepam, etizolam, diazepam, and 7-aminoclonazepam. Further, analytical methods were validated using all three of these techniques.

Method: A six-point calibration curve and three Quality Control (QC) samples, each in triplicate, were extracted using SLE with ISOLUTE® cartridges, SPE using Clean Screen® XCEL I cartridges, and LLE. Samples were analyzed on a High-Performance Liquid Chromatograph (HPLC) with a 4000 Q-Trap Electrospray Ionization Tandem Mass Spectrometer (ESI/MS/MS) in positive ionization mode. The method was validated in accordance to the proposed AAFS Standards Board (ASB) Standard 036, First Edition 2018, for quantitative analysis by evaluating calibration model, precision, bias, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), carryover, interferences, and ionization suppression and enhancement.

Results: With this developed method, analysis time totaled nine minutes. A linear dynamic range of 10–1,000ng/mL was used for all analytes. Recovery of all analytes utilizing the SLE sample preparation method for urine and blood ranged from 56.44–87.73 and 26.13–82.87%, respectively; SPE sample preparation method for urine and blood ranged from 36.95–64.5 and 46.66–79.23%, respectively; and LLE sample preparation method for urine and blood ranged from 44.68–143.9 and 36.17–117.61%, respectively. The LOD utilizing SLE for urine and blood ranged from 0.5–1 and 1–5ng/mL, respectively; SPE for urine was 1ng/mL and blood ranged from 1–2ng/mL; and LLE for both urine and blood ranged from 0.5–1ng/mL.

Conclusion/Discussion: The development and validation of these sample preparation and analytical methods demonstrates sensitive, reliable, and reproducible results to identify and quantify six commonly encountered benzodiazepines in human urine and whole blood in rapid time. The use of LLE proved to be efficient; however, sample preparation was laborious and costly. The SLE cartridges used were approximately twice as expensive as SPE, but were the fastest method to prepare samples, whereas SPE consumed a large amount of solvent and took the most amount of time to prepare samples. Given the data discussed above, it is left up to the individual laboratories and analysts to determine which extraction method best suits their needs and current resources.

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Benzodiazepines, Sample Preparation, HPLC-MS/MS

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