



K40 Validation and Comparison of Three Sample Preparation Techniques for Quantitation of Amobarbital, Butalbital, and Phenobarbital in Blood and Urine Using Ultra-Fast Liquid Chromatograph/Tandem Mass Spectrometry (UFLC/MS/MS)

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Learning Overview: After attending this presentation, attendees will be able to choose among the methods developed for the analysis of selected barbiturates in blood and urine.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information on sample preparation methods for the analysis of select barbiturates in various matrices.

Background/Introduction: Barbiturates are a class of drugs that act as Central Nervous System (CNS) depressants. They were vastly used as sedative, hypnotic, anticonvulsant/antiepileptic, and anesthetic agents in the 20th century. Today, only 12 types of barbiturates are still in use for therapeutic purposes. The rest have been greatly replaced by benzodiazepines due to narrow margins of safety. However, barbiturates are still a prevalent choice of abuse among the public and, therefore, are of forensic toxicological importance.

Objective: The objectives of this research study were to validate three different sample preparation methods (Liquid-Liquid Extraction (LLE), Supported-Liquid Extraction (SLE), and Solid-Phase Extractions (SPE)) for the quantitation of three barbiturates (amobarbital, butalbital, and phenobarbital) in blood and urine using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS); and to compare the efficiency and effectiveness between methods for extraction of barbiturates under the laboratory setting at Boston University School of Medicine.

Method: Six-point calibration curve with three quality control samples were extracted using LLE, SLE with Biotage® ISOLUTE® SLE+ cartridge, and SPE with Waters® Oasis™ PRiME Hydrophilic-Lipophilic Balanced (HLB) cartridges. All analyses were conducted using a Waters® XBridge™ C18 LC column (3.5µm particle size; 50mm length; 2.1mm internal diameter) on a Shimadzu® UFLC coupled to a SCIEX™ 4000 QTRAP MS/MS with negative Electrospray Ionization (ESI) mode. Method validation was performed according to the American Academy of Forensic Sciences Standards Board (ASB) standard 036, First Edition 2017. Calibration model, carryover, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), bias and precision, stability, dilution integrity, interferences, ion suppression/enhancement, and recovery were evaluated for quantitative analysis.

Results: A six-point linear calibration model (20-2,000ng/mL) with 1/x weighting was reproducible in all three sample preparation methods for quantifying amobarbital, butalbital, and phenobarbital in blood and urine with r^2 greater than or equal to 0.994. Bias and precision evaluated from three controls throughout the range of the curve were within $\pm 20\%$ and $\pm 20\% CV$, respectively. Neither carryover nor interferences were observed. Detection limits were evaluated down to 5ng/mL depending on the extraction procedure. Samples were able to be diluted up to 50 times prior to instrumental analysis. Samples were stable on autosampler at room temperature up to 72 hours after their initial analysis. Recovery of barbiturates from blood and urine all ranged from 45% to 86%. The effect of ionization suppression or enhancement demonstrated only minimal impact on the validation.

Conclusion/Discussion: This study has successfully validated three sample preparation methods (LLE, SLE, and SPE) for the quantitation of three barbiturates (amobarbital, butalbital, and phenobarbital) in blood and urine using LC/MS/MS. By comparing each method's efficiency and effectiveness, LLE was the best at quantifying barbiturates in blood and SLE was the most suitable method for extracting barbiturates from urine. These findings can be used further for examining the overall reliability and reproducibility of the validated methods in different laboratories. Results obtained can also be used to explore the possibility for streamlining sample preparation in the forensic laboratory.

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Barbiturates, Sample Preparation, LC/MS/MS