K40 The Effect of Sample Preparation Techniques on Matrix Effects and Absolute Recovery of Opiates in Liver Tissue Using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS): Part 1

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After attending this presentation, attendees will better understand the effectiveness of the various sample preparation techniques for the extraction of opiates from liver tissue in order to determine which method may be suitable for their own implementation.

This presentation will impact the forensic science community by increasing knowledge regarding sample preparation techniques for the forensic pathology and postmortem toxicology communities. Many sample preparation techniques are primarily designed for the extraction of drugs from blood or urine, and the adoption of these techniques for difficult matrices, such as liver tissue, has occurred without complete understanding of the effects of the matrix on the analysis of the analyte(s) of interest.

In this presentation, an evaluation of the effect of sample preparation techniques on matrix effects and absolute recovery of opiates in liver tissue will be presented. In postmortem toxicology, the concentration of drugs in the blood is often used to assist in the determination of the cause and the manner of death; however, central cavity drug blood concentrations can be unreliable because of the phenomenon of postmortem redistribution. This can be combated by evaluating liver concentrations, as drug concentration in liver is fairly stable after death. While liver is a valuable tissue for postmortem toxicology, the protein, fat, and phospholipid components of the matrix can interfere with analysis, and thus the drug must be isolated from the matrix prior to analysis. If proper and effective sample preparation and clean-up are not performed, matrix effects, such as ion enhancement or suppression, can hinder analysis and affect recovery of the drug. To limit matrix effects, it is necessary that the preparation technique used has the ability to extract the analyte as completely as possible while limiting the extraction of any interfering compounds.

There are many different approaches to sample preparation for drug extraction. The three traditional types of techniques are Solid-Phase Extraction (SPE), Liquid-Liquid Extraction (LLE), and filtration. A growing number of simple and rapid sample preparation techniques have become commercially available in recent years. These new techniques are commonly based on the traditional techniques but have added features to improve the extraction process. While these newer techniques have the ability to make sample preparation both easier and faster, there are still limitations. A majority of the user guides and technical notes for these new products focus on either blood or urine sample matrices. There is limited published data regarding tissue matrices, such as liver. For these techniques to be effectively used for liver samples, the matrix effects, absolute recovery, and process efficiency for extractions from liver must be evaluated.

These sample preparation techniques were evaluated for matrix effects and recovery by extracting opiates from homogenized liver tissue. Liver tissue was homogenized in saline at a ratio of 1:4. Homogenates were fortified with six opiates, at two concentrations (n=6), and their respective isotopic derivatives. The opiates analyzed were codeine, hydrocodone, hydromorphone, morphine, oxycodone, and oxymorphone. Three sets of samples were analyzed: neat, fortified before, and fortified after. Sample preparation was performed following manufacturer's guidelines (Waters® Oasis™ PriME HLB cartridge, Biotage® ISOLUTE® SLE+, and Biotage® ISOLUTE® PLD+) and using a laboratory validated LLE technique. Samples were analyzed using a previously validated UPLC-MS/MS method.

Results varied greatly between the methods evaluated. For Waters® Oasis™ PriME HLB, the observed matrix effects were between -35% and +5%, and recoveries were between 100% and 122%. For Biotage® ISOLUTE® SLE+, the observed matrix effects were between -18% and 0%, and recoveries were between 86% and 109%, with the exception of morphine, which had recoveries between 30% and 32%. For Biotage® ISOLUTE® PLD+, the observed matrix effects were between -16% and +50%, and recoveries were between 55% and 94%. For LLE, the observed matrix effects were between -59% to -37%, and recoveries were between 39% and 82%.

Liver is a difficult matrix to analyze. Sample preparation is not as simple as it is for blood or urine. It was observed that not all sample preparation techniques are effective or reliable for the extraction of opiates from liver tissue. Of the techniques evaluated, the Biotage® ISOLUTE® SLE+ was more effective at removing matrix effects and improved recovery.

Opiates, Liver, Sample Preparation

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