K36 Comparison of Manual Protein Precipitation and Automated Protein Precipitation Using DPX Low Porosity Tips in Blood, Urine, and Tissues

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Learning Overview: After attending this presentation, attendees will understand the benefits of automating their extractions using low porosity tips in place of traditional protein precipitation methods.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating a novel automated option for protein precipitation of various common toxicological matrices.

Historically, protein precipitation is a commonly used manual clean-up process in many toxicological drug assays for blood and tissue samples. Often referred to as "protein crash," this initial step rapidly removes numerous biological interferences prior to a more thorough downstream sample clean up. Traditionally, an organic solvent, most commonly acetonitrile or methanol, is added to denature the sample and initiate protein precipitation. This is followed by a quick vortex step and centrifugation prior to decanting the supernatant for further extraction, if needed.

The widespread use and robustness of protein precipitation makes it an ideal process to automate, especially in laboratories with large case loads. Recently, DPX Technologies has presented an automated method for the quantitation of testosterone in serum using Low Porosity Filtration Tips (LPFT). This tip-based method uses a quick (less than two minutes) and effective, patent-pending, Tip-on-Tip (ToT) technology that automates protein precipitation and filtration to isolate the supernatant. Using the DPX tips, one can eliminate the need for manual vortex mixing, centrifugation, and supernatant transfer.

This study is a comparison of the DPX ToT method on an INTEGRA Biosciences VIAFLO using LPFT to the Orange County Crime Lab's Scientific Working Group for Forensic Toxicology (SWGTOX) -validated method that uses a traditional protein precipitation process. The validated method analyzes for 23 drugs, including benzodiazepines, antihistamines, and sedative hypnotics, in antemortem blood, postmortem blood, urine, brain homogenate, liver homogenate, and stomach content homogenates. Brain and liver homogenates were diluted anywhere from 2x to 12x with water, and stomach content homogenates were diluted between 10x and 500x with water prior to treatment.

After completing protein precipitation, both techniques followed the validated extraction procedure. Both precipitation methods, which included standards, controls, and case samples, were injected on a Waters® ACQUITY® UPLC with a Waters® BEH C18 column (1.7µm, 2.1 x 100mm) and XeVo TQ-S to compare the results.

Various concentrations and matrices were analyzed using both techniques to determine if the ToT method using the LPFT correlated to the SWGTOX-validated method. Same-day studies between the two techniques indicated that the LPFT works just as well as the more traditional protein crash method. For antemortem and postmortem blood specimens, the percent difference between the two methods ranged between 5.8% and 8.5%. Significantly, the ToT method was able to handle not only blood and urine but also tissue homogenate samples at various pre-extraction dilutions. Varying diluted brain, liver, and stomach content specimens had % differences for the two methods of 8.3%, 4.0%, and 4.7%, respectively. Importantly, precision studies were successful using the ToT method on a concentrated liver homogenate with percent differences of 2.5% and 7.9% for alprazolam and hydroxyzine, respectively. These initial investigative results demonstrate that protein precipitation can be automated for SWGTOX-validated forensic applications for Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) analysis. Significantly, adaptation of an automated crash method for forensic laboratories offers several unique advantages, including reducing the hands-on time requirement for the analyst, minimizing potential human errors, and further negating the long-term health effects of repeated pipetting.

Protein Precipitation, Sample Preparation, Benzodiazepines