K5 An Assessment of the Incorporation of Amphetamine and Diazepam Into Human Head Hair for the Preparation of Hair Reference Material

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After attending this presentation, attendees will better understand the process of preparing Hair Reference Material (HRM) with incorporated xenobiotic substances.

This presentation will impact the forensic science community by contributing to a body of research targeted at better understanding the interactions between drugs of abuse with different physiochemical properties and hair that would be considered during routine analysis in forensic laboratories.

HRM is essential for the development and validation of methodologies used in forensic hair analysis. At present, HRM containing selected xenobiotic substances is only available on a limited basis. In addition, most xenobiotic substances relevant to forensic casework are not available as incorporated drug standards in human head hair. Additionally, little is known about the incorporation process that occurs when human head hair is soaked in a xenobiotic-containing buffer solution. This body of work demonstrates the intentional incorporation of Amphetamine (AMP) and Diazepam (DZP) into human head hair for the development of "in-house" HRM by exploring the relationship between time and the concentrations of drug detected in the incorporation buffer solution, daily hair extracts, and solutions used to wash daily hair aliquots. The hypothesis was that, over time, the concentration of xenobiotic detected will decrease in the buffer solution, increase in the hair extracts, and remain relatively constant in the wash solutions.

Purchased human head hair was soaked in 1X Phosphate-Buffered Saline (PBS) spiked with either AMP or DZP at 800pg/mL for five days (120h). Each day, aliquots of hair and spiked PBS solution were taken to monitor incorporation. The hair aliquots were washed using both organic (2-propanol) and aqueous (1X PBS) solvents, then pulverized using a Retsch® MM200 ball mill. Subsequently, the pulverized hair was incubated in a mixture of organic and aqueous solvents (methanol:cetonitrile: 2mM ammonium formate in water; 1:1:2) for 18h to extract incorporated drug from the matrix. After incubation, the samples were centrifuged to separate the hair particulates from the solvent mixture containing recovered drug. The resulting solution was subjected to online Solid-Phase Extraction (SPE) cleanup and Liquid Chromatography/Triple Quadrupole/Tandem Mass Spectrometry (LC/QqQ-MS/MS) analysis. Mass Spectrometry (MS) analysis was performed on the spiked PBS solutions, extracted samples, and wash solutions. A 1µL injection of each sample was introduced to an Agilent® 1290 Infinity Flexible Cube to perform online SPE. A reversed-phase LC column (Agilent® ZORBAX® Rapid Resolution High-Definition Eclipse Plus C18, 2.1 X 50mm, 1.8µm) was used as the analytical column on an Agilent® 1290 Infinity® HPLC system. A gradient elution was used over 8min using 5mM ammonium formate in water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B). Analysis was performed with positive mode Electrospray Ionization (ESI) on an Agilent® 6460 QqQ-MS instrument.

Quantitative results demonstrated the successful incorporation of AMP and DZP into blank human head hair. Contrary to the hypothesis, the amount of drug extracted from the hair aliquots was not significantly different between the 24h and 120h time points for either drug. Final incorporated drug levels were approximately five-fold higher for AMP than DZP. The organic wash did not remove a significant quantity of AMP, but did remove DZP from the surface of the hair. The three aqueous washes each removed AMP from the hair surface, with decreasing concentration in each wash. In contrast, the organic wash and the first of the aqueous washes removed DZP, while subsequent aqueous washes did not remove any additional drug. These results suggest that the maximum transfer of drug from the incorporation buffer into hair occurs within the first 24h of incubation for both drugs. In addition, AMP may be more prone to contaminating the surface of the hair or it may be more loosely bound to the hair matrix than DZP. The differences between incorporation, decontamination, and extraction of these two drugs may be attributed to differences in their physiochemical properties.

Hair Analysis, Hair Reference Material, Incorporation of Xenobiotics

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