K58 The Application of Statistical Design of Experiments (DoE) to Assess External Decontamination Methods in Forensic Hair Analysis

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Learning Overview: After attending this presentation, attendees will understand how statistical DoE can help optimize highly variable forensic methodologies, particularly in forensic hair analysis, with the ultimate goal of working toward standardization of these techniques.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing that this work will contribute to a body of research aimed at determining the most effective method(s) for decontaminating hair samples for forensic analysis and subsequently isolating drugs of abuse from these samples.

It was hypothesized that statistical DoE could be used to determine which conditions of a discrete set of levels of defined parameters would result in the most effective removal of amphetamine from the surface of human head hair, deposited by means of external environmental exposure. As a complex solid sample matrix, hair requires pretreatment measures, including decontamination, homogenization, and extraction, to remove the drug from the hair matrix components to allow for analysis. Optimizing pretreatment parameters is essential to accurately identify and quantify drugs present in a hair sample. One of the most challenging issues faced in hair testing analysis and interpretation is the differentiation between a drug that is detected due to superficial deposition onto the surface of the hair and a drug incorporated into hair from intake and systemic distribution. This interpretive challenge necessitates effective removal of the drug on the surface of hair deposited by means of contamination from an individual's environment prior to analysis. The Society of Hair Testing (SoHT) recommends at least one wash step with an aqueous solvent and one with an organic solvent. These guidelines are general, and thus a variety of decontamination methods have been published to date without a systematic comparison of these different techniques.

The major goal of this work was to conduct a comprehensive DoE analysis of decontamination parameters and their effect on the removal of drug from the surface of hair. A 2^4-fractional factorial block design followed by Analysis Of Variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) analysis were used to compare the main factors: wash time, aqueous and organic decontamination solvent identities, and order of wash (i.e., organic wash first vs. aqueous wash first). The blocking factors were the number of sequential washes with both the aqueous and organic solvents. Neat amphetamine powder was superficially applied to blank human head hair. The hair was decontaminated following the 2^4-fractional factorial block design matrix.

A 30mg aliquot of hair was weighed into a stainless steel milling jar with 200µg of amphetamine sulfate. The samples were agitated using a BioSpec® Mini-Beadbeater™ 24 ball mill, without milling beads present. The samples were then washed with the aqueous or the organic solvent first for either 30s or 30min. The aqueous wash was performed by adding 1mL of chromatography-grade water or 1% Sodium Dodecyl Sulfate (SDS) and agitating on an orbital shaker. The organic wash was performed by adding 1mL of 2-propanol or dichloromethane and agitating on an orbital shaker. There were 1–3 washes of the aqueous and organic washes performed, depending on the two blocks of the experimental unit. The hair was then dried for 24h at 40°C in an oven. Once dried, the samples were homogenized using the ball mill with chrome-steel milling beads at 3,800rpm for 30s. The drug was extracted by means of enzymatic hydrolysis of the hair proteins, with a pre-incubation in 12mg/mL aqueous Dithiothreitol (DTT) for 2h at 37°C, followed by incubation with 2mg/mL proteinase K. Extracted samples and wash solutions were purified by spin filter centrifugation (3 KDa MW cut-off) and Solid Phase Extraction (SPE) before Liquid Chromatography/Triple quadrupole/Mass Spectrometry (LC/QqQ/MS) analysis. Three replicate samples for each design point were analyzed.

Analysis of Variance (ANOVA) results indicated that 3- and 4-factor interactions (i.e., the parameters associated with combinations of 3 and 4 parameters under study) were significant. The percent of drug recovered from the hair to which amphetamine was superficially applied was highest, at 46%, for samples that were washed for 30s during each wash step, employed dichloromethane as the organic wash solvent, and were washed with the organic solvent before the aqueous solvent. When expanded to additional drugs, it is expected that this work will contribute to a body of research aimed at determining the most effective method(s) for decontaminating hair samples for forensic analysis and subsequently isolating drugs of abuse from these samples.

Forensic Hair Analysis, External Contamination, Statistical Design of Experiments