

K26 The Evaluation of Extraction Parameters for the Analysis of Authentic Hair Reference Material (HRM) in Forensic Hair Testing Using Statistical Design of Experiments (DoE)

Brianna Spear*, Miami, FL 33176; Anthony P. DeCaprio, PhD, Florida International University, Miami, FL 33199

Learning Overview: After attending this presentation, attendees will understand the value of authentic HRM and DoE for evaluating best practice methods in forensic hair testing.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by presenting the most effective parameters for analysis of oxycodone, alprazolam, nordiazepam, and methamphetamine in hair, providing data toward creating standardized procedures for hair testing and understanding the benefits of authentic HRM.

Hypothesis: The hypothesis of this work is that DoE is a valuable approach to evaluating the most effective methods for extraction in forensic hair analysis.

There are many differing opinions regarding the optimal methods for hair analysis, especially regarding the pretreatment parameters. Extraction methods include enzymatic, acid/base, and solvent techniques, each with their own advantages and disadvantages. Other parameters include incubation times, temperatures, and size of the extracted hair particles. To assess pretreatment parameters for drugs of abuse, the statistical technique known as DoE is useful. DoE allows for evaluating both the individual roles and the combinatorial associations between multiple variables and drug extraction efficiency.

Previous research in the laboratory has focused on incorporated HRM, which is prepared in a laboratory at a specified drug concentration, but that does not necessarily reflect the mechanism by which drug incorporates into hair *in vivo*. In addition, using this approach, some drugs are not incorporated into hair at a high enough concentration for use as HRM. In contrast, authentic HRM, which is prepared by mixing hair from drug users to achieve specific drug concentrations, is an effective matrix for drug testing, since drug is incorporated into the hair through natural processes of the body.

Authentic HRM containing the drugs of interest was obtained from a commercial source. Samples of 20mg each were weighed into steel milling jars. The samples underwent extraction parameters determined using a 2³ full factorial DoE matrix. Each sample was randomly assigned specific design points made up of combinations of factors of interest in extraction protocols. These included extraction solvent/sample size ratio (12.5 or 25µL/mg), particle size (pulverized into a powder using a Retsch® MM200 ball mill with chrome-steel milling beads at 3,800rpm for 30s or cut into snippets with scissors), and extraction time (2 or 24h). The samples were extracted using a solvent swelling technique in which the hair was incubated in methanol:acetonitrile:2mM ammonium formate (25:25:50) at 37°C. The samples were then transferred into Eppendorf tubes and centrifuged for 30min. Post-centrifugation, the eluent was subjected to solid phase extraction using a mixed mode C18 + cartridge. An Agilent® 1290/6460 Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) was used for analysis.

Analysis Of Variance (ANOVA) F-tests were performed post-analysis to determine if the parameters were significantly different. The ANOVA F-test indicated that high-level interactions, such as interactions between 2-3 factors (methamphetamine and oxycodone), as well as all interactions (alprazolam), were significant in the extraction of drugs of interest from the hair. This result suggests that studying variables individually and in combination with each other is important in the evaluation of forensic hair analysis methods. A consensus statement was made based on the design points with the highest percent recovery to determine which parameters were most effective for extraction of the drugs of interest. These included pulverizing the hair prior to a 24h extraction in 12.5µg/mg extraction solvent (oxycodone, nordiazepam, and alprazolam) and cutting the hair into snippets prior to a 2h extraction in 12.5µg/mg extraction solvent (methamphetamine). In conclusion, DoE is a valuable approach for determining effective extraction protocols for drugs of interest using authentic HRM.

Forensic Hair Analysis, Statistical Design of Experiments, Authentic Hair Reference Material