K38 Supported Liquid Extraction (SLE) as a New Technique for the Clean-Up of Hair Extracts Containing Drugs of Abuse

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After attending this presentation, attendees will understand a novel method for the clean-up of hair extracts in order to detect drugs of abuse using SLE.

This presentation will impact the forensic science community by introducing a faster and more efficient method for detecting drugs in the hair matrix.

Hair analysis has been regarded as a complementary matrix to blood and urine in forensic cases as it is a very stable matrix, thus allowing for a detection window that is days to years post-exposure. Analyzing hair for the presence of drugs of abuse consists of many steps, initially starting with sampling and cutting of the hair into small segments, which is followed by decontamination, extraction, and clean-up of the hair extract prior to qualitative and quantitative analysis. Drug residues in hair have previously been reported at trace levels, which progressively degrade by oxidation and/or hydrolysis, so the optimization of analyte extraction and clean-up steps are critical components to ensure the quality of method performance.

The objective of this research was to apply a reduced workflow preparation technology to a range of basic drugs extracted from hair. SLE has been used for the determination of drugs in conventional matrices, such as blood and urine, both of which showed high recovery. In the SLE process, the sample is pH adjusted, then loaded in an aqueous phase to ensure the analytes of interest load as neutral compounds. To date, no peer-reviewed literature discussing the application of this technique in hair has been published.

In this study, Biotage® ISOLUTE® SLE+ was compared to traditional Solid Phase Extraction (SPE) for the determination of cocaine, methadone, opiates, and amphetamines using control samples. These samples were prepared by spiking washed drug-free hair in deionized water and Dichloromethane (DCM) prior to spiking with the drugs (methamphetamine, amphetamine, morphine, 6-monoacetylmorphine, codeine, methadone, cocaine, and benzoylecgonine) at a concentration of 0.5ng/mg and 2ng/mg.

Validation of the SLE method was carried out according to the Scientific Working Group for Toxicology guidelines using the control hair samples, which were weighed (20mg) and, together with standards and negative controls, were tested according to specific procedure. One mL of phosphate buffer pH=7.4 and 50µL of β-glucuronidase solution was added to controls containing amphetamines. Samples were sonicated for one hour at 40°C and incubated for one hour. Prior to extraction, 100µL of 10% ammonium hydroxide was added. In controls containing opiates and methadone, 1mL of 0.1M hydrochloric acid was added before sonication for one hour at 40°C and overnight incubation at the same temperature. Before extraction, 100µl of 5% ammonium hydroxide was added to the supernatant. For controls with cocaine, 1mL of methanol was added and samples were sonicated for one hour at 40°C and incubated over night at 40°C. Before extraction, 100µL of 5% ammonium hydroxide was added to the supernatant. Deuterated internal standards for all drugs were used. A set of split samples was extracted by both SPE and SLE+ in order to compare results. Before applying samples (1mL) onto ISOLUTE® SLE+ cartridges, samples were pH adjusted to pH>10 in aqueous environment in buffer. Prior to evaporation, 100µL of 1mg/mL tartaric acid in ethyl acetate was added to samples containing amphetamine. The extracts were then evaporated and derivatized using pentafluoropropionic acid (PFPA):ethyl acetate (2:1) for amphetamines and BSTFA + 1%TCMS for other drugs. The samples were analyzed using Gas Chromatography Mass Spectrometry (GC/MS) in selected ion monitoring mode.

In order to verify the SLE+ method, hair samples from rats, containing amphetamine and methamphetamine, and human hair from forensic and clinical cases were tested in similar fashion. SLE+ was determined to be a suitable alternative to SPE for the quantitation of cocaine, benzodiazepines, methadone, morphine, codeine, and 6-monoacetylmorphine in hair. Amphetamines and methamphetamines were detected in rat hair at concentration ranging from <0.1ng/mg to >5ng/mg with comparable results between the two clean-up methods. Similarly, a wide range of concentrations (from lower than low limit of quantitation to higher than upper limit of quantitation) were determined in human hair samples.

SLE+ provides better efficiency and less solvent waste than SPE and is a suitable clean-up method for hair analysis.

SLE, SPE, Hair