Supported Liquid Extraction as a New Technique for the Clean-up of Hair Extracts Containing Drug of Abuse

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Introduction

Hair

Hair 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.

Supported liquid extraction

Supported liquid extraction (SLE) has been successfully used for the determination of drugs in conventional matrices, such as blood and urine. In the SLE process the sample is pH adjusted and then loaded in an aqueous phase, to ensure the analytes of interest load as a neutral compounds. The aqueous phase is immobilized on an inert diatomaceous earth based support material and the water immiscible organic phase flows through the support eluting the compounds of interest. As a result recoveries are often higher, with better reproducibility and cleaner extracts within 15 minutes.

Experimental

Biotage ISOLUTE® SLE+ (with positive pressure) was compared to traditional SPE method for clean-up of drug extracts containing morphine, codeine, 6-acetylmorphine (6-AM), methadone, cocaine, benzoylecgonine (BZE).

Control samples were prepared by spiking washed drug free hair with the drugs at a nominal concentrations of 0.5ng/mg and 2ng/mg.

For each testing two batches were prepared, one clean-up by SLE+, and the other by SPE.

Each batch consisted of weighed (20mg) low and high controls in duplicates, standards and negative controls. All samples contained deuterated internal standards (IS).

Extraction: An acidic extraction (1ml of 0.1M HCl) was applied for opiates and methadone, while a methanolic extraction (1ml of methanol) was used for cocaine. Samples were then sonicated for 1 hour at 40^{0}C and incubated overnight at the same temperature.

Clean-up:

- ***SPE*** - Clean Screen Columns were washed with methanol, deionized water and pH6 0.1M phosphate buffer. The supernatant was transferred to the columns, allowed to pass completely, and washed with deionized water, 0.1M HCl, and methanol. Columns were dried under full vacuum for 5 minutes and samples were eluted with DCM:IPA:NH_3 (78:20:2).
- ***SLE+*** - Before clean-up, 100µL of 5% ammonium hydroxide was added to the supernatant . Each sample was transferred on the INSOLUTE® SLE+ columns, and after five minutes eluted twice with 2.5ml of DCM:IPA (95:5).

Derivatization: Extracts were evaporated and derivatized using BSTFA+1%TCMS

Analytical Method

- Analysis was carried out on a Agilent 5700 MSD GC/MS fitted with DB-5ms UI (Agilent) capillary column (15m x 0.25mm i.d. x 0.25µm film thickness).
- Helium was used as a carrier gas at a flow rate of 1.2mL/min.
- Temperature was programmed from 80^{0}C to 320^{0}C over 10.8 minutes.
- The injection port was operated in splitless mode at 250^{0}C.
- The MS was operated in Full Scan mode from 70 to 450m/z and SIM mode.

SPE v SLE Opioids

Clean-up of hair samples using SPE:

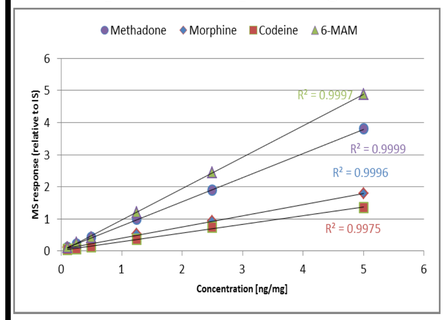

Figure 1. Calibration curve for opioids after clean-up by SPE method

Table 1. Average Control Concentrations for SPE Method		
Drug	**Low [0.5ng/mg] (n=4)**	**High [2ng/mg] (n=4)**
Methadone	0.42 ±0.10	1.75 ±0.11
Morphine	0.34 ±0.05	1.44 ±0.06
Codeine	0.30 ±0.02	1.22 ±0.06
6-AM	0.29 ±0.05	1.49 ±0.08

Clean-up of hair samples using SLE:

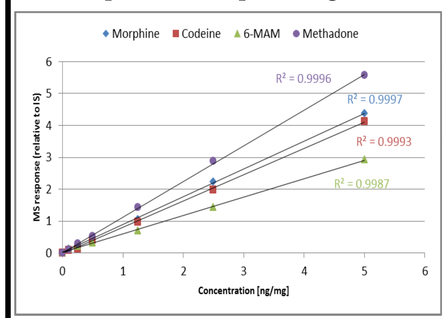

Figure 2. Calibration curve for opioids after clean-up by SLE method

Table 2. Average Control Concentrations for SLE Method		
Drug	**Low [0.5ng/mg] (n=4)**	**High [2ng/mg] (n=4)**
Methadone	0.39 ±0.01	1.52 ±0.06
Morphine	0.38 ±0.05	1.61 ±0.09
Codeine	0.39 ±0.01	1.93 ±0.05
6-AM	0.37 ±0.05	1.48 ±0.06

In general, obtained results from the SPE and SLE methods are comparable. Less bias was observed after SLE for low and high controls for codeine and morphine, and low controls for 6-AM. However, less bias was observed for methadone using SPE methods.

Acknowledgments

The authors would like to thank Biotage for providing ISOLUTE® SLE+ cartridges and the Center for Forensic Science Research and Education for the laboratory space and resources, which allowed for completion of the this project.

SPE v SLE Cocaine

Clean-up of hair samples using SPE:

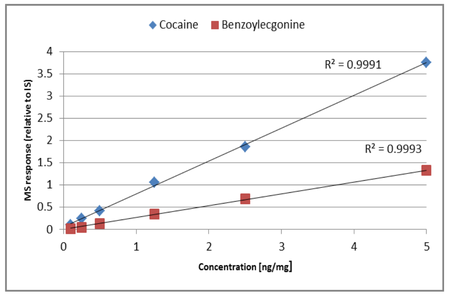

Figure 3. Calibration curve for cocaine and benzoylecgonine after clean-up by SPE method

Table 3. Average Control Concentrations for SPE Method		
Drug	**Low [0.5ng/mg] (n=4)**	**High [2ng/mg] (n=4)**
Cocaine	0.43 ±0.03	1.84 ±0.03
BZE	0.46 ±0.01	1.98 ±0.05

Clean-up of hair samples using SLE:

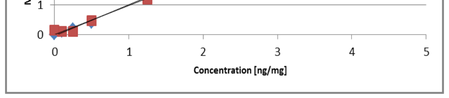

Figure 4. Calibration curve for cocaine and benzoylecgonine after clean-up by SPE method

Table 4. Average Control Concentrations for SPE Method		
Drug	**Low [0.5ng/mg] (n=4)**	**High [2ng/mg] (n=4)**
Cocaine	0.48 ±0.02	1.74 ±0.10
BZE	0.45 ±0.04	1.73 ±0.19

Obtained results by the two methods are comparable with almost the same values for low controls.

Conclusion

SLE was determined to be a suitable alternative clean-up method for hair extracts containing drugs of abuse compared to traditional SPE methods. It can be seen in the results of this study that the SLE method of extraction provided better extraction efficiency than SPE for hair samples containing opiates and cocaine including its major metabolite.

The biggest advantage of using the SLE procedure is a simplistic approach of three step, load-wait-elute, procedure (Figure 5) that not only saves time, but also provides clean extracts with minimal solvent waste. Faster turn around time and decreased volume of solvents used could prove to be more cost effective than other extraction techniques. In future research, different extraction parameters for SLE will be evaluated in order to improve recovery.

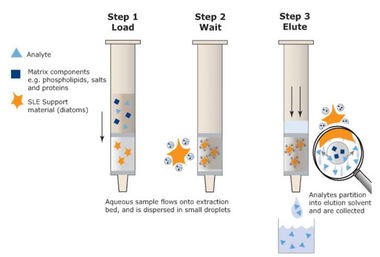

Figure 5. SLE mechanism. ISOLUTE® SLE+ USER GUIDE, Biotage 2013