Optimisation of Supported Liquid Extraction for the Analysis of Amphetamines in Hair

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Introduction

Hair is a fundamental matrix for drug analysis showing historical use due to increased compound stability over prolonged periods of time compared to other more traditional matrices. A single exposure to a drug can be detected within hair, allowing potential doping to be detected. Usually, analytes are extensively metabolized once they enter the body so it can be difficult to establish the parent compound. Within hair, parent drugs are usually present in high concentrations, allowing easy identification, making it a complementary technique to traditional blood and urine analysis.

A method that simultaneously pulverizes and extracts target analytes from hair matrix is called micropulverised extraction (MPE). Compared to other extraction methods, MPE has been used to reduce the sample preparation time for hair analysis significantly. Although this technique remains relatively unknown, it is a significant alternative to other more time consuming methods for pulverization and extraction.

Clean-up is an important part of sample preparation for hair analysis. An alternative to traditional LLE is supported liquid extraction (SLE) that is based on the same principles as LLE – the use of two immiscible solvents to exchange analytes. SLE offers several advantages over LLE; there is no emulsion formation, it produces a more efficient exchange and is in a cartridge form that is quick and simple to use. The SLE process involves only three stages – load, wait, elute.

Experimental

Reagents

Amphetamine-d_5 – used as internal standard, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxy-N-ethylamphetamine (MDEA) were purchased from LGC Standards (Teddington, UK). Methanol, dichloromethane (DCM), isopropyl alcohol (IPA), tert-butyl methyl ester (MTBE), ammonium hydroxide (NH_4OH, 28-30%), hydrochloric acid (37 %), ethyl acetate, ethylene glycol and pentafluoropropionic anhydride (PFPA) were purchased from Sigma Aldrich (Dorset, UK) and all were HPLC grade. Water (18.2 MΩ.cm) was drawn fresh daily from a Direct-Q 5 water purifier (Merck Millipore, Watford, UK).

Sample Preparation

ISOLUTE® SLE+ Procedure (*Figure 1.*)

Columns: ISOLUTE® SLE+ 1 mL capacity 'C' columns; 820-0140-C.

Matrix Preparation:

Weigh 20 mg of hair and deposit in 2 mL Biotage® Lysera tubes containing 2.8 mm ceramic beads. Add 1.0 mL of methanol and ISTD at 1 ng/mg. Cap and load the tubes into the Lysera instrument.

Micropulverisation (MPE) Procedure:

Program the Lysera: Four cycles at 5.3 m/sec for 3 minutes with a 0.2 dwell.
Transfer the Lysera tubes into a micro centrifuge to operate at 13,300 rpm for 10 minutes.

Preconcentration Procedure:

700 µL of supernatant was transferred into a test tube containing 20 µL ethylene glycol. The mixture was evaporated using a TurboVap LV at 20 °C with a flow rate increasing from 1.6 L/min to 5.0 L/min. The use of ethylene glycol avoided complete evaporation and potential analyte losses due to volatility. Evaporated samples were reconstituted in 850 µL 0.1% aqueous ammonium hydroxide and vortexed.

Sample Application:

800 µL of reconstituted extract was applied to the columns.

Analyte Extraction:

2 x 3 mL aliquots of MTBE. Each aliquot was allowed to flow under gravity for 5 minutes and collected in an appropriate glass tube containing 100 µL HCl in methanol (0.05 M). A pulse of positive pressure for 10-20 seconds was applied to completely remove the final aliquot.

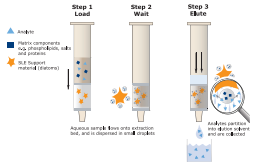


***Figure 1**. Schematic of ISOLUTE® SLE+ Supported Liquid Extraction Procedure.*

Post Extraction:

The extracts were evaporated to dryness at ambient temperature, reconstituted with 50 µL ethyl acetate and 50 µL PFPA prior to vortex mixing, capping and heating for 15 minutes at 50 °C on a heat block. The samples were evaporated for a final time at ambient temperature and reconstituted with 25 µL ethyl acetate.

GC/MS Conditions

GC: 7890A GC with QuickSwap (Agilent Technologies Inc.)
Column: Restek Rxi-5ms, 30m x 0.25mm DF= 0.25 µm
Carrier Gas: Helium 1.2 mL/min (constant flow)
Inlet: Splitless, Temp: 250 °C

Injection volume: 2 µL
Oven: Initial: 40 °C, ramp 25 °C/min to 350 °C, hold for 0.4 min.
Backflush: 2 void volumes (1.6 min)
Transfer Line: 280 °C
MS: 5975C MSD (Agilent Technologies Inc.).
Source Temperature: 230 °C
Quadrupole Temperature: 150 °C
Monitored Ions: EI signals were acquired using selected ion monitoring (SIM) mode. See Biotage.com application notes section for monitored ions for each analyte.

Results

MPE was optimised from a method developed by Di Rago et al.[1]. Optimisation was performed to ensure full homogenisation and drug extraction within a selected time period. The Lysera instrument facilitated this by allowing fast sample pulverisation of solid hair whilst simultaneously extracting amphetamines from the matrix. Hair samples were prepared using masses of 20 - 50 mg to determine maximum matrix/solvent proportions. Final homogenisation was achieved using 2 mL tubes, 2.8 mm ceramic beads for MPE and 1 mL of methanol as the extraction solvent. Methanol was selected as it is a universal extraction solvent for drugs of abuse and has been shown to be an effective extraction in previous MPE methods developed. ***Figure 2.*** shows recovery data for samples spiked before and after homogenisation demonstrating extraction efficiency of the MPE method.

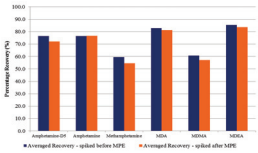


***Figure 2**. Recovery data for spiked hair samples before and after MPE.*

Figure 3. demonstrates the effect on recovery of incorporating high pH pre-treatment in the aqueous load. The highest recoveries were obtained using 0.1% NH_4OH in the pre-treatment. Traditional methodology uses 0.2M HCl to prevent evaporative losses of the analytes – most critically amphetamine and methamphetamine. Once pH pre-treatment was elevated, increased extraction residue was observed. This residue was not seen at neutral pH conditions thus attributed to salt formation between NH_4OH and HCl contents. Lowering the HCl concentration to 0.05M resulted in improved cleanliness.

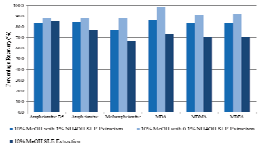


***Figure 3.** Effect of NH_4OH on recovery.*

Figure 4. demonstrates analyte recoveries using either 95:5 DCM:IPA or MTBE as the water immiscible extraction solvents. Both produced clean extracts and high recoveries. However, overall signal to noise determination resulted in lower baselines returned for MTBE extraction. As a result this was used as the final extraction protocol.

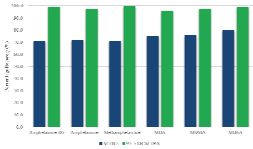
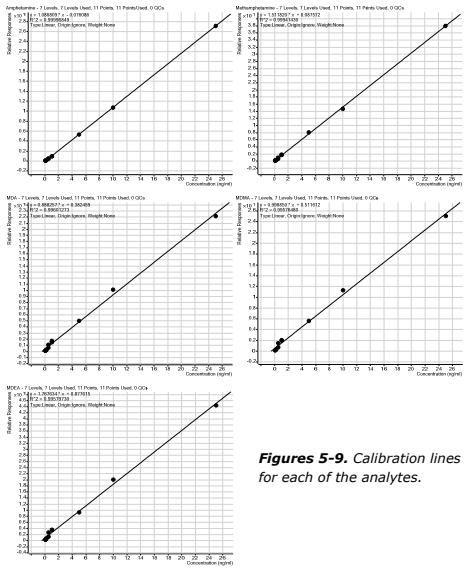


***Figure 4.** Recovery profile chart for elution solvents.*

Calibration lines were constructed from 0.1 – 25 ng/mg. ***Figures 5-9.*** illustrate typical calibration curves demonstrating excellent linearity and coefficient of determination (r^2) greater than 0.99 for each analyte.



***Figures 5-9.** Calibration lines for each of the analytes.*

The calibration lines incorporate and extend lower than the cut off requirements for determination of chronic drug abuse recommended by the Society of Hair Testing. However method LOQs based on S/N ratios of 10:1 correspond to the cut off levels, summarized in ***Table 1.***

***Table 1.** Analyte LOQ values.*

Drug Analyte	LOQ (ng/mg)	SoHT (ng/mg)
Amphetamine	0.2	0.2
Methamphetamine	0.2	0.2
MDA	0.2	0.2
MDMA	0.2	0.2
MDEA	0.2	0.2

Figure 10. demonstrates representative chromatography from an extracted sample spiked at 75 ng/mL.

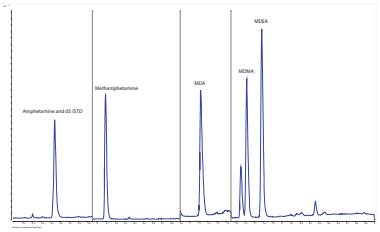


***Figure 10.** Representative chromatography for analytes spiked at 75 ng/mL.*

Conclusion

- Spiked hair samples (n=24) were simultaneously homogenized and drugs extracted within 12 minutes using the Biotage® Lysera 24 system.
- ISOLUTE® SLE+ enables a fast, reliable protocol to extract amphetamines from hair matrix.
- Limits of quantitation set by the Society of Hair Testing for amphetamines are achievable with the optimum method (0.2 ng/mg).
- This poster illustrates multiple benefits to laboratory workflow saving both worker hours and consumable costs.

References

- M. Di Rago, T. Vo, J. Fernandez, K. Crump, V. Staikos, L. Glowacki, M. Gerostamoulos, Ultra-Rapid Targeted Analysis of 63 Drugs in Hair by LC-MS/MS, In: *Society of Forensic Toxicologists*, 16th-21st October 2016, Dallas, Texas, 2016, S32.