Extraction of Amphetamines and Analogues from Hair Using ISOLUTE® SLE+ Prior to GC/MS Analysis

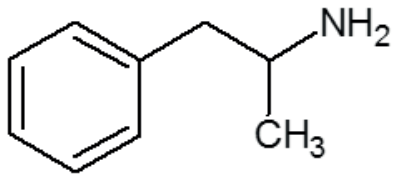

Figure 1. Structure of amphetamine.

Introduction

This application note describes the extraction of amphetamine and amphetamine-style compounds from hair matrix prior to GC/MS analysis following bead homogenization.

This application note describes an effective and efficient ISOLUTE® SLE+ protocol optimized for 1 mL capacity formats. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 72% with RSDs lower than 10% for all analytes.

In addition, this note describes how to achieve quantitation of all analytes down to 0.2 ng/mg of hair. This complies with the LOQ values for laboratories involved in amphetamine testing, as suggested by the Society of Hair Testing (SoHT).

ISOLUTE SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation.

Analytes

Amphetamine, Methamphetamine, MDA, MDMA, MDEA, Amphetamine-D_5 (internal standard).

Sample Preparation Procedure

Format

ISOLUTE® SLE+ 1 mL Sample Volume Columns, part number 820-0140-C.

Sample Pre-treatment

Transfer 20 mg of hair into a 2 mL Lysera tube containing five 2.8 mm ceramic beads. Add methanol (1 mL) and internal standard solution (20 µL) before sealing the tube tightly. Homogenize the sample using the Biotage® Lysera 24 for four cycles of 3 minutes at 5.3 m/s with a dwell time of 0.2 minutes. Centrifuge the sample for 10 minutes at 13,300 rpm.

Add 20 µL of ethylene glycol to a clean glass tube and transfer 0.7 mL of supernatant sample to this new tube prior to evaporation at 20 °C . The glycol prevents full evaporation and assists against the loss of amphetamine and methamphetamine.

Reconstitute the dried sample in 0.1% ammonium hydroxide (aq) (850 µL) – see notes for preparation.

Sample Loading

Load the pre-treated hair extract (0.8 mL) onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Analyte Extraction

Apply MTBE (3 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of MTBE (3 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure to pull through any remaining extraction solvent. (5–10 seconds).

Collect in an appropriate glass tube with 100 µL HCl in methanol (0.05 M – see notes for preparation). This acts to stabilize free-base analytes in the solvent prior to evaporation.

Post Elution and Reconstitution

Dry the extract in a stream of air or nitrogen using a Biotage® SPE Dry at ambient temperature, 20 to 40 L/min, or a TurboVap® at ambient temperature, 1.5 L/min, for 20 minutes.

Reconstitute dried samples with 50 µL ethyl acetate and 50 µL pentafluoropropionic anhydride (PFPA). Vortex-mix and place on a heating block for 15 minutes at 50 °C for optimum amphetamine results.

Evaporate the extract in a stream of air or nitrogen using a SPE Dry (ambient room temperature, 20 to 40 L/min).

Reconstitute extracts with 25 µL ethyl acetate and vortex.

Biotage®

GC Conditions

Instrument
Agilent 7890A with QuickSwap

Column
Restek RXi-5ms, 30 m x 0.25 mm ID x 0.25 µm)

Carrier
Helium 1.2 mL/min (constant flow)

Inlet
250 °C, Splitless, purge flow: 50 mL/min at 1.0 min

Injection
2 µL

Wash Solvents
Methanol and ethyl acetate

Oven
Initial temperature 60 °C

Ramp 25 °C/min to 260 °C

Post Run
Backflush for 1.6 minutes (2 void volumes)

Transfer Line
280 °C

Mass Spectrometry Conditions

Instrument
Agilent 5975C

Source
230 °C

Quadrupole
150 °C

MSD Mode
SIM

SIM Parameters

Table 1. Ions acquired in the Selected Ion Monitoring (SIM) mode.

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion
1	Amphetamine-D_5	194	96	123
1	Amphetamine	190	91	118
2	Methamphetamine	204	118	160
3	MDA	162	135	
4	MDMA	162	204	
4	MDEA	218	162	

Results

This optimized ISOLUTE® SLE+ protocol demonstrated analyte recoveries greater than 70% as shown in **Figure 2**. RSDs were below 10% for all analytes.

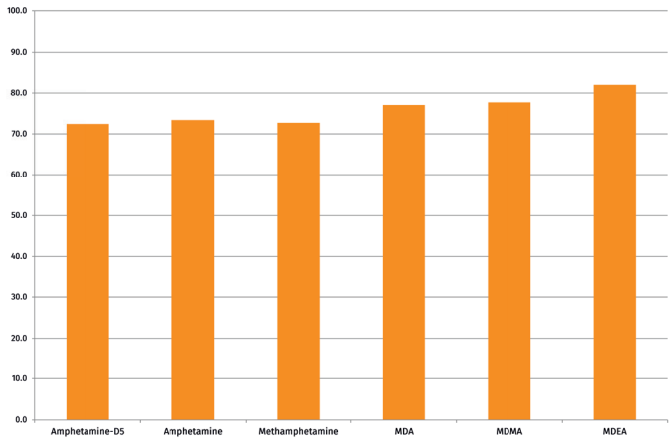

Figure 2. Typical analyte % extraction recoveries (n=7) using the ISOLUTE® SLE+ Protocol.

Biotage®

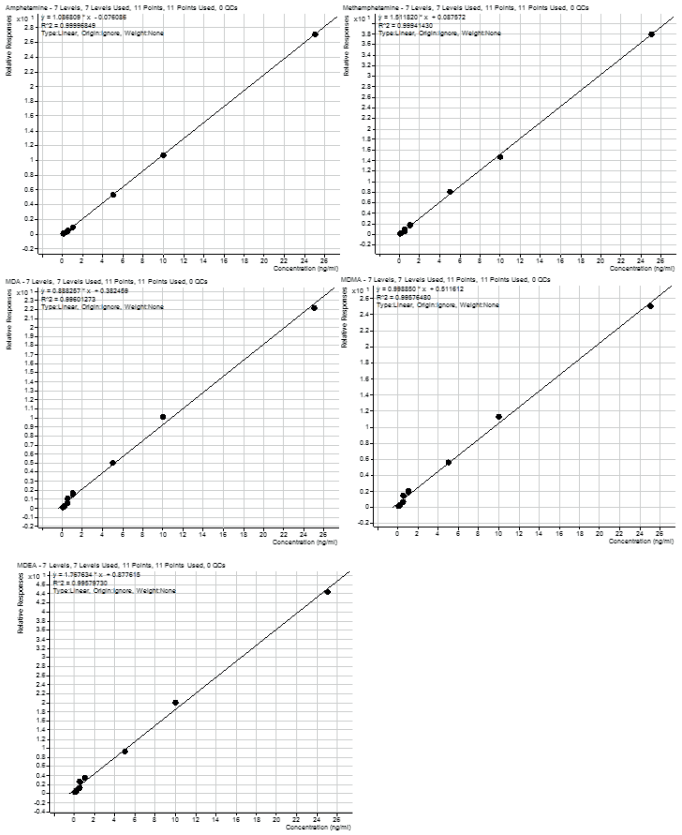

Figure 3. Calibration curves for extracted levels of spiked homogenized hair matrix, using 1 mL ISOLUTE® SLE+ format. Concentrations are 0.1, 0.2, 0.5, 1, 5, 10 and 25 ng/mg showing r^2 values of 0.9957 to 0.9999.

Additional Information

Reagent Preparation

0.1% ammonium hydroxide is prepared by adding 50 µL concentrated ammonium hydroxide to 49.95 mL of HPLC grade water. Concentrated stock used to modify pH prior to extraction is commercially available 28–30%.

0.05 M HCl in methanol is prepared by adding 50 µL concentrated hydrochloric acid to 11.95 mL of HPLC grade methanol. Concentrated stock used to modify pH prior to extraction is commercially available 12M.

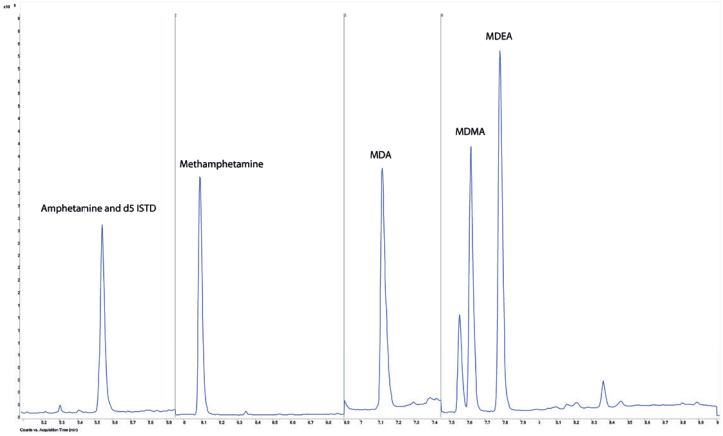

Figure 4. Representative chromatography for application analytes spiked at 75 ng/mg.

Table 2. Lower Limits of Quantitation (LLOQ) using the optimized ISOLUTE ® SLE+ procedure.

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

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Column	30
19-060	Biotage® Lysera*	1
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
SD-9600-DHS-EU	Biotage® SPE Dry 96 Sample Evaporator 220/240V	1
SD-9600-DHS-NA	Biotage® SPE Dry 96 Sample Evaporator 100/120V	1
415000	TurboVap® LV	1

*Biotage® Lysera is available in North America, Europe and China only

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com
cn-1-pointsupport@biotage.com

KOREA
Tel: + 82 31 706 8500
Fax:+ 82 31 706 8510
korea_info@biotage.com
kr-1-pointsupport@biotage.com

Distributors in other regions are listed on www.biotage.com

Part Number: AN885.V.1

Biotage®