Extraction of a Range of Amphetamines and Metabolites From Human Urine Using ISOLUTE® SLE+ Columns prior to GC-MS Analysis

Introduction

This application note describes the supported liquid extraction clean-up of a range of amphetamines and metabolites from urine prior to quantitative GC-MS analysis.

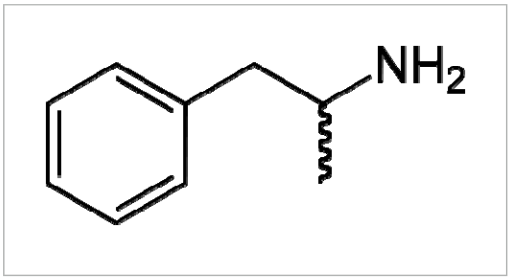

Figure 1. Structure of Amphetamine

This methodology has been designed to give an effective and efficient supported liquid extraction protocol for the clean-up and concentration of a range of forensically significant amphetamines followed by derivatization to optimize for GC-MS analysis.

Analyte recoveries achieved using this method ranged from 99-104% with RSDs below 10% for all analytes.

ISOLUTE SLE+ Supported Liquid Extraction 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 time.

Analytes

Amphetamine, Amphetamine-d5, Methamphetamine, MDA, MDMA, MDEA

Sample Preparation Procedure

ISOLUTE SLE+ 1 mL Sample Volume column, part number 820-0140-C

Sample pre-treatment: Dilute urine (1 mL) with 15 mM ammonium hydroxide (1 mL).

Sample loading: Load the pre-treated sample (1 mL) onto the column and apply a pulse of vacuum (VacMaster 20 Sample Processing Manifold, **121-2016**) or positive pressure (PRESSURE+ 48 Positive Pressure Manifold **PPM-48**) to initiate flow. Allow the sample to adsorb for 5 minutes.

Analyte extraction: Apply ethyl acetate (4 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent, collecting into a glass culture tube containing 0.2 M hydrochloric acid (100 µL) to add stability during evaporation.

Post-extraction: Evaporate the extract to dryness (ambient temperature). Add pentafluoropropionic acid anhydride (PFPA) (50 µL) and ethyl acetate (50 µL) for derivatization. Vortex for 30 seconds, transfer to a high recovery glass vial and cap with a non-split cap. Heat vial in a heating block (70 ºC) for 20 minutes. Remove vial and allow to cool. Evaporate the mixture to dryness (ambient temperature). Reconstitute in dichloromethane:isopropanol (95:5, v/v) (100 µL). Cap with a non-split cap and vortex for 30 seconds.

GC Conditions

Instrument: Agilent 7890A GC

Column: SGE capillary column; 30 m x 0.25 mm ID-BPX5 x 0.25 µm

Carrier: Helium 1.2 mL/min (constant flow)

Inlet: 250 °C, Split (ratio 20:1), 24 mL/min

Septum purge flow: 3 mL/min

Injection: 1 µL, wash solvents: ethyl acetate and DCM:IPA (95:5, v/v)

Oven: 75 °C initial hold for 1 minute, 20 °C/min to 185 °C then 60 °C/min to 275 °C, hold 0.5 min

Transfer Line: 280 °C

Mass Spectrometry Conditions

Instrument: Agilent 5975C MSD

Source: 230 °C

Quadrupole: 150 °C

MSD mode: SIM

Table 1. SIM Parameters

SIM Group	Analyte	Quant Ion	1st Qual Ion	2nd Qual Ion	Dwell (ms)
1	Amphetamine-d5	194.1	123.1	122.1	25
1	Amphetamine	190.0	118.1	91.1	25
2	Methamphetamine	204.0	160.0	118.1	40
3	MDA	135.1	162.1	-	50
4	MDMA	162.1	135.1	204.0	40
5	MDEA	218.0	162.0	190.0	40

Results

This ISOLUTE SLE+ protocol demonstrates analyte recoveries ranges from 99-104% as shown in figure 3 (page 3) with RSDs below 10% for all analytes. Robustness testing was carried out across three days using three different sources of urine. Figure 2 shows the chromatogram for the full range of extracted amphetamines zoomed in at a concentration range of 250ng/mL.

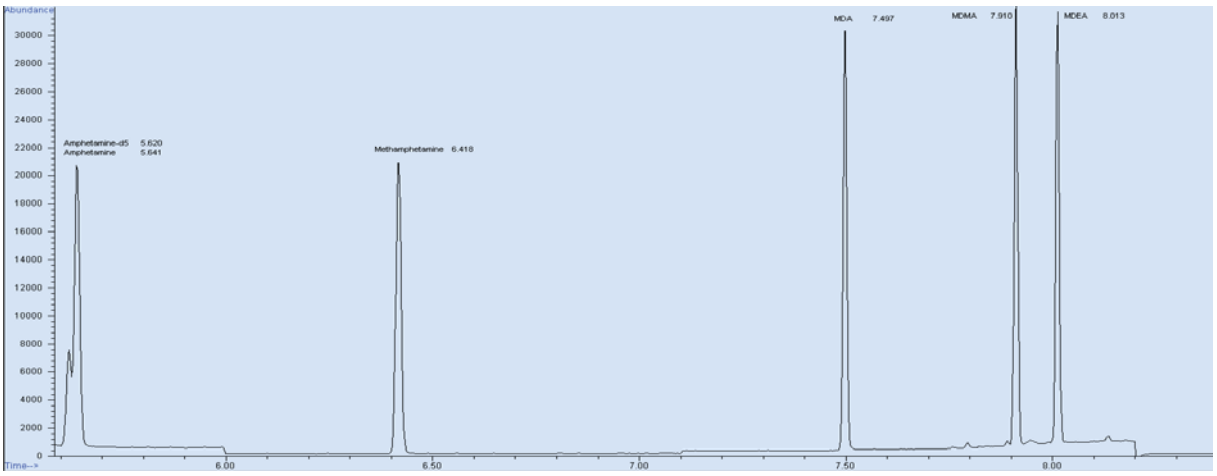

Figure 2. Zoomed chromatogram showing extracted amphetamine analytes at 250 ng/mL

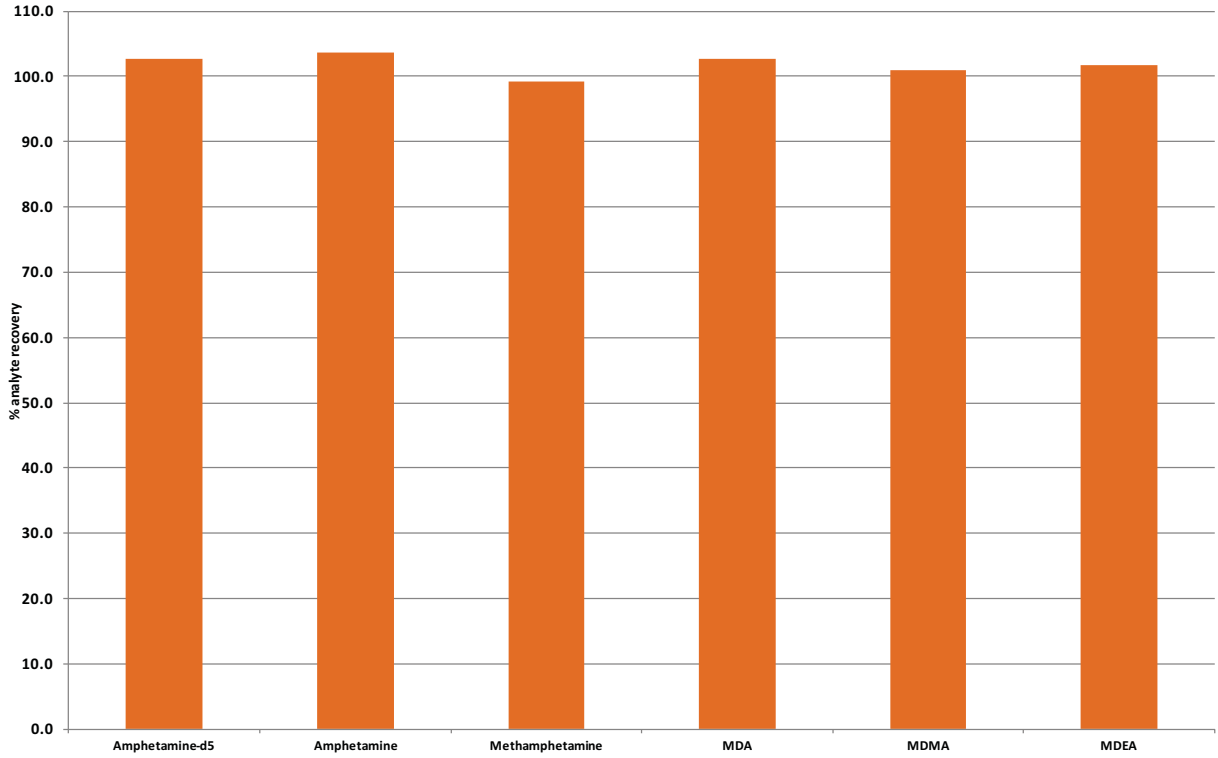

Figure 3. Typical analyte % recoveries for a range of extracted amphetamines (n=7) using the ISOLUTE SLE+ protocol

Typical extractions showed limits of quantitation ranging from 5-25 ng/mL dependent upon analyte and required detection limit as shown in table 2. Figure 4 shows the calibration curves for amphetamine and methamphetamine , demonstrating linearity over the range from 5-250 ng/mL.

Table 2. Limits of Quantitation for extracted opiates using the ISOLUTE SLE+ protocol

Analyte	LOQ (ng/mL)
Amphetamine-d5	10
Amphetamine	10
Methamphetamine	5
MDA	25
MDMA	25
MDEA	25

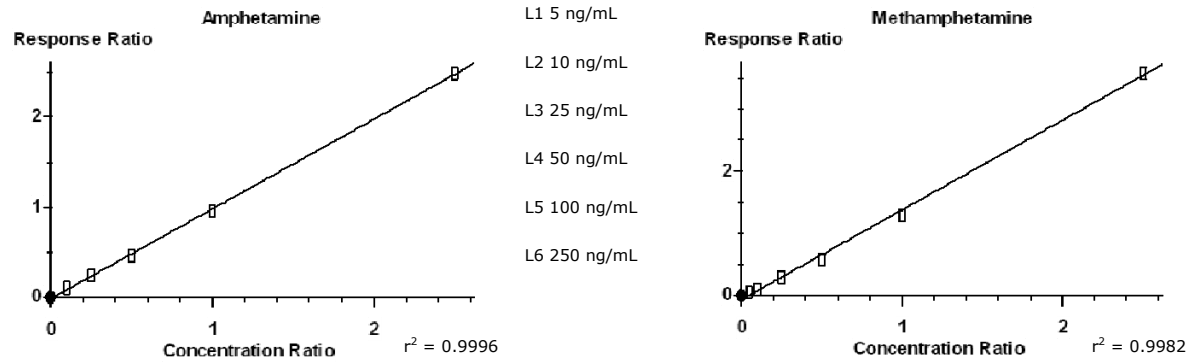

Figure 4. Calibration curves for amphetamine and methamphetamine over the range of 5-250 ng/mL

Ordering information

Part number	Description	Quantity
820-0140-C	ISOLUTE SLE+ 1 mL Sample Volume column	30
PPM-48	PRESSURE+48 Positive Pressure Manifold	1
121-2016	VacMaster 20 Sample Processing	1

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