Extraction of Amphetamines and Metabolites from Urine (including Elimination of Sympathomimetic Amine Interferences) Using ISOLUTE® SLE+ Prior to GC/MS Analysis

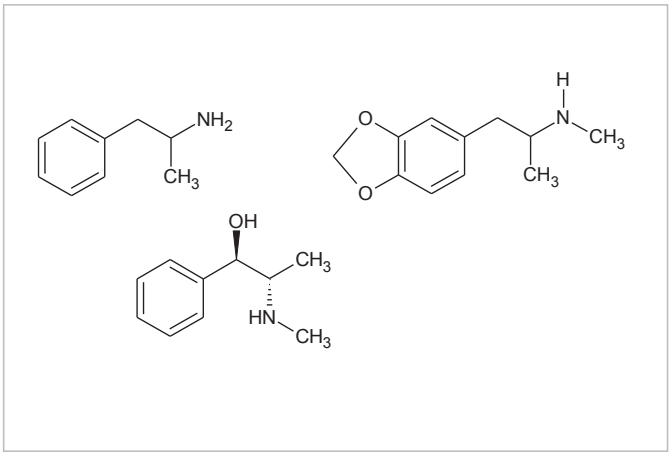

Figure 1. Structures of Amphetamine, MDMA and Ephedrine

Introduction

This application note describes the extraction of a range of amphetamines and metabolites from urine using supported liquid extraction and subsequent analysis by GC/MS. Prior to extraction, a simple oxidation step is performed to eliminate sympathomimetic compounds such as ephedrine and pseudoephedrine so they do not interfere with quantitation of methamphetamine.

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 –D5, Amphetamine, Methamphetamine, MDMA, MDA, MDEA, Ephedrine, Pseudoephedrine

Sample Preparation Procedure

Sample pre-treatment: To urine (2 mL), add phosphate buffer (pH 6, 0.8M, 1 mL) and vortex. Add sodium periodate (0.3M, 1 mL). Heat for 15 minutes at 60 °C. Allow to cool, add concentrated ammonium hydroxide (85 µL) and vortex.

Format: **ISOLUTE® SLE+ 1 mL Sample Volume columns, part number 820-0140-C**

Sample Loading: Load 1 mL of the pre-treated urine mixture 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 dichloromethane/isopropanol, (95/5, v/v, 2.5 mL) and allow to flow under gravity for 5 minutes into tubes. Apply a further aliquot of DCM/IPA, (95/5, v/v, 2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

Post Elution, Derivatization and Reconstitution: Remove tubes from the elution rack and add 1% HCl in methanol (100 µL) to each tube. This devolatilizes the analytes and helps to prevent losses on evaporation.

Dry the extract in a stream of air or nitrogen at ambient temperature using a SPE Dry (20 to 40 L/min) or TurboVap (1.0 bar) for 30 mins.

Add HFBA (100 µL) and ethyl acetate (100 µL) and vortex for 10 seconds. Transfer to a high recovery vial, cap and incubate at 75 °C for 15 minutes. Cool and then evaporate the HFBA at room temperature. On dryness, reconstitute each vial with ethyl acetate (100 µL). Cap and vortex for 10 seconds.

GC Conditions

Instrument:	Agilent 7890A with QuickSwap
Column:	Agilent J&W DB-5 30 m x 0.25 mm ID x 0.25 µm
Carrier	Helium 1.2 mL/min
Inlet:	175 °C, Splitless, purge flow: 50 mL/min at 1.0 min
Injection:	1 µL
Wash solvents:	Ethyl acetate
Oven:	Initial temperature 50 °C, hold for 1.0 minute
	Ramp 20 °C/min to 275 °C
Transfer Line:	280 °C

MS Conditions

Instrument:	Agilent 5975C
Source:	230 °C
Quadrupole:	150 °C
MSD mode:	SIM

SIM Parameters

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

SIM Group	Analyte	Quantifier Ion	Quantifier Ion
1	Amphetamine-D5	244	123
1	Amphetamine	240	118
2	Methamphetamine	254	210
3	MDA	162	135
4	MDMA	162	210
4	MDEA	268	240

and the two compounds that potentially cause interference to methamphetamine

2	Pseudoephedrine	254	210
2	Ephedrine	254	210

Results

The oxidation protocols prior to the SLE procedure demonstrated complete removal of the ephedrine and pseudoephedrine from urine. This is shown on page 3 in **Figure 2**. The top chromatogram is a urine specimen spiked with the interferents **after** oxidation (demonstrating retention times), the bottom chromatogram illustrates urine spiked with the interferents **before** oxidation (and subsequently removed in the oxidation process). In SIM window 2 it can be seen that ephedrine and pseudoephedrine are completely removed from the sample. (Concentration of all analytes is 100 ng/mL.)

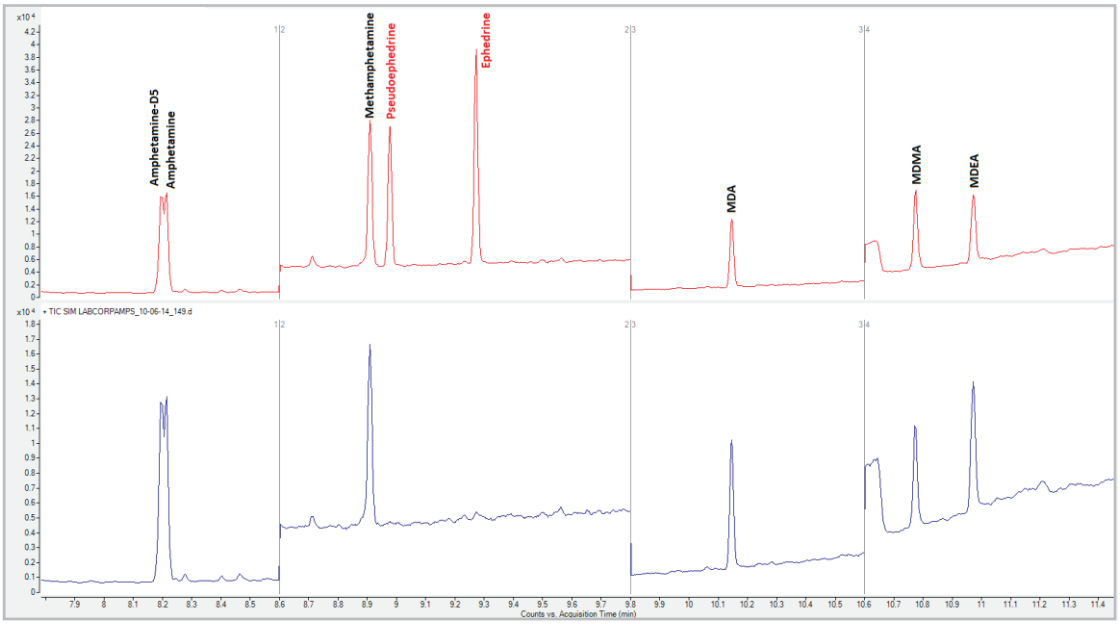

Figure 2. Chromatography of all analytes at 100 ng/mL. Analytes spiked after oxidation (top) and spiked before oxidation (bottom).

This experiment was repeated at a pseudoephedrine / ephedrine concentration of 2.5 µg/mL with the remainder of the analytes at 100 ng/mL in urine. Similar results were demonstrated with total removal of the interferents without any adverse effects on the amphetamines.

Figure 3 shows the linearity of the optimized method on ISOLUTE® SLE+ columns. The coefficient of determination was determined to be greater than 0.997 for all analytes across concentration values, 5, 10, 20, 50, 100, 200 and 500 ng/mL. **Table 2** shows the lower limit of quantitation for each analyte using this protocol on 1 mL capacity columns.

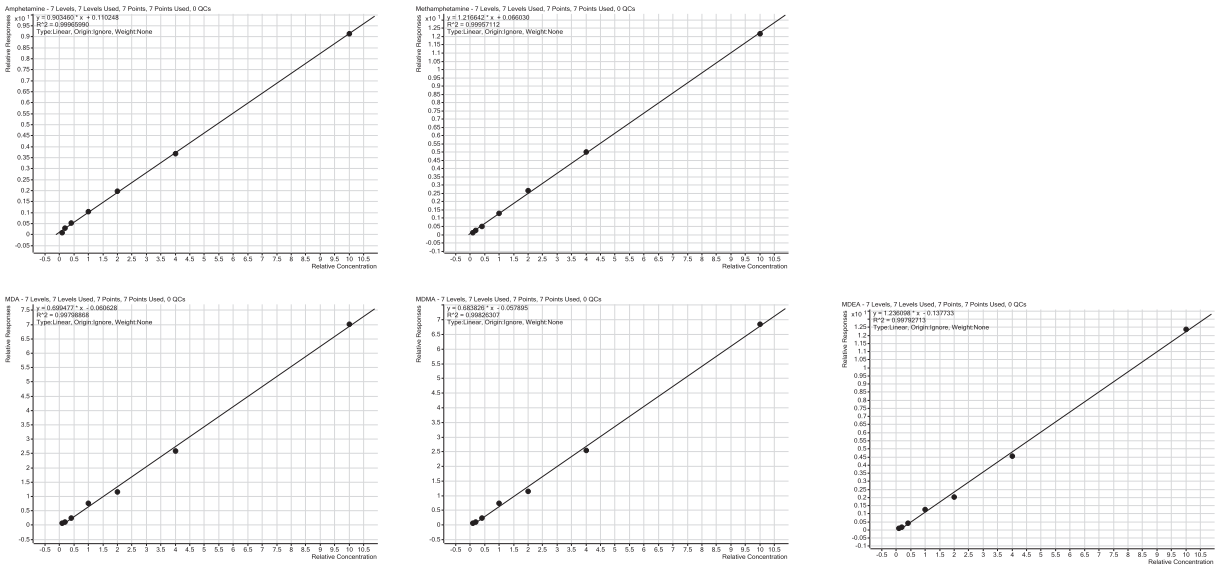

Figure 3. Calibration curves for extracted levels of spiked urine, using ISOLUTE SLE+ protocol (1 mL capacity column format).

Table 2. Lower limits of quantitation (LLOQ) for each amphetamine or metabolite using this optimized approach.

Analyte	LLOQ with 500 µL urine (ng/mL)
Amphetamine	5
Methamphetamine	5
MDA	5
MDMA	5
MDEA	5

Additional Information

1% HCl in methanol was prepared by adding 400 µL concentrated HCL (commercially available 37%) to 39.6 mL HPLC grade methanol.

0.8M potassium phosphate buffer was prepared by weighing 54.436g monopotassium phosphate and adding to 500 mL HPLC grade water. The pH was adjusted to 6 with ammonium hydroxide.

0.3M sodium periodate was prepared by weighing 6.4167g sodium periodate and adding to 100 mL HPLC grade water.

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume columns	30
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1
C103198	TurboVap® LV, 100/120V	1
C103199	TurboVap® LV, 220/240V	1

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