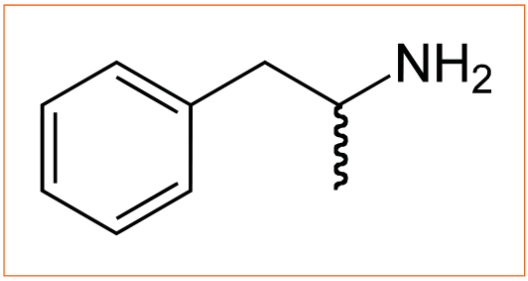
Extraction of Amphetamines From Urine Using ISOLUTE® SLE+ Columns

Introduction

This application note describes the extraction of amphetamines from urine using ISOLUTE SLE+ columns with LC-MS/MS analysis.



This method describes the use of ISOLUTE SLE+ in column format for the extraction of a range of amphetamines. This method is effective for sample volumes of 500µL with analyte recoveries > 95%. For smaller volume (100 µL) samples, see AN742.

Figure 1. Structure of Amphetamine

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 time.

Analytes

Ephedrine, amphetamine, methamphetamine, MDMA, MDA, MDEA.

ISOLUTE SLE+ procedure

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

Sample Pre-treatment: Dilute urine (500µL) 1:1 with 0.5 M ammonium hydroxide (500 µL)

Sample Load: Load pre-treated sample (1 mL) to column followed by a pulse of vacuum to initiate flow and leave for five minutes. Add 0.05 M HCl/Methanol (100uL) to each collection plate well.*

Analyte Elution: Elute with dichloromethane (2.5 mL). Leave to flow under gravity for 5 minutes then apply a second aliquot of dichloromethane (2.5 mL) followed by a short pulse of vacuum.

Post extraction: Evaporate to dryness and reconstitute in 500 µL 0.1% formic acid (aq) in water/ methanol (90/10, v/v).

Additional information: *During evaporation, amphetamines in the free base form suffer major losses. As a result it was necessary to convert these to a stable HCl form at low pH using 0.05M HCl/Methanol (100uL) in the collection plate. All samples were processed and dried down using the Vacmaster and SPE Dry respectively.

Application Note

UPLC Conditions

Instrument: Waters Acquity UPLC interfaced to a Quattro Premier XE triple quadrupole MS using electrospray ionization.

Column: Acquity BEH C18 100 x 2.1mm x 1.7u.

Mobile phase: Isocratic, 0.1% formic acid aq/0.1% formic acid in methanol (80/20, v/v).

Flow rate: 0.43 mL/min.

Temperature: 40 °C.

Mass Spectrometry Conditions

Source temp: 150 °C.

Desolvation temp: 450 °C.

Collision cell pressure: 3.58 e^{-3} mbar.

Table 1. MRM transitions for a range of amphetamines.

Scan function	Compound	MRM transition	Cone voltage	Collision energy (eV)
1	Ephedrine	166.1 > 133.0	20	19
2	Amphetamine	136.0 > 118.9	16	9
	Methamphetamine	150.0 > 90.9	22	17
	MDA	180.1 > 105.0	16	23
	MDMA	194.1 > 163.0	20	13
3	MDEA	208.2 > 163.0	22	13

Results

Figure 2 shows the total ion chromatogram for all amphetamine analytes detected using this methodology whilst recoveries of all analytes were > 95% at 2 ng/mL using the 1 mL column method as shown in figure 3.

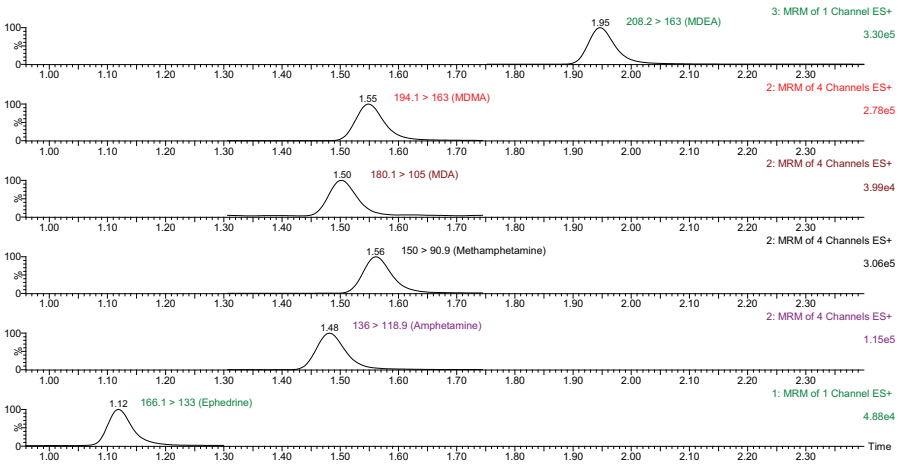


Figure 2. Analyte % recoveries of amphetamines using 1 mL SLE+ methodology at 2 ng/mL

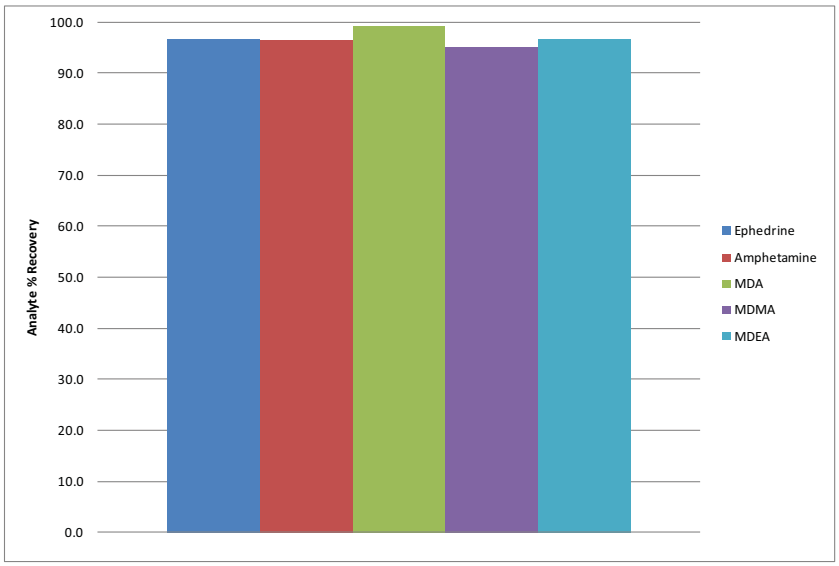


Figure 3. Analyte % recoveries of amphetamines using 1 mL SLE+ methodology at 2 ng/mL (n=7)

Ordering information

Part number	Description	Quantity
820-0140-C	ISOLUTE SLE+ 1 mL sample	30
121-2016	VacMaster-20 Sample processing manifold complete with 16 mm	1
SD2-9600-DHS-UK	SPE Dry 96 Dual, 240 V UK	1

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