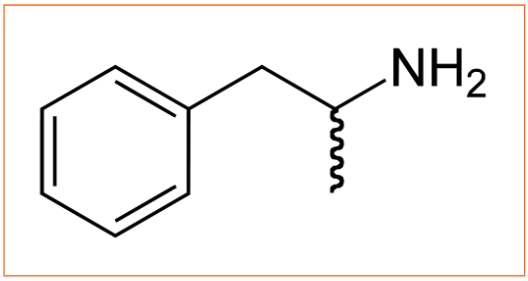
Extraction of Amphetamines From Urine Using ISOLUTE® SLE+ 96-Well Plates

Introduction

This application note describes the extraction of amphetamines from urine using ISOLUTE SLE+ 96 fixed-well plates with LC-MS/MS analysis.



This method describes the use of ISOLUTE SLE+ in 96 well plate format for the extraction of a range of amphetamines. This procedure is effective with sample volumes of 100 µL for analyte recoveries > 90%. For larger volume (500 µL) samples, see AN746.

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+ 200 Supported Liquid Extraction Plate, part number 820-0200-P01.

Sample Pre-treatment: Dilute urine (100 µL) 1:1 with 0.5 M ammonium hydroxide (100 µL).

Sample Load: Load pre-treated sample (200 µL) to plate 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 (1 mL). Leave to flow under gravity for 5 minutes then apply a second aliquot of dichloromethane (1 mL) followed by a short pulse of vacuum.

Post extraction: Evaporate to dryness and reconstitute in 200 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
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 > 90% at an LLOQ of 500 pg/mL using the standard SLE+ 200 plate method as shown in figure 3.

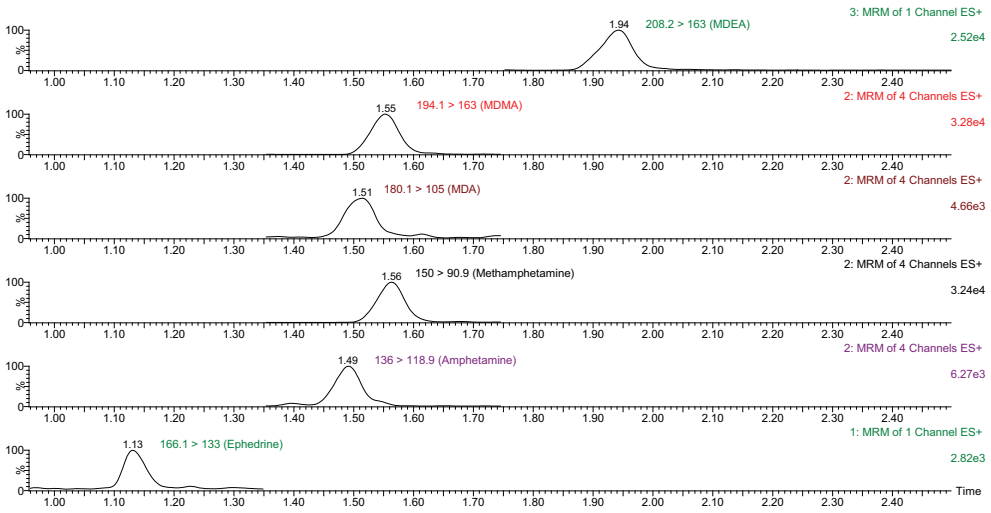


Figure 2. Total ion chromatogram for all amphetamine analytes at the LLOQ level of 500 pg/mL

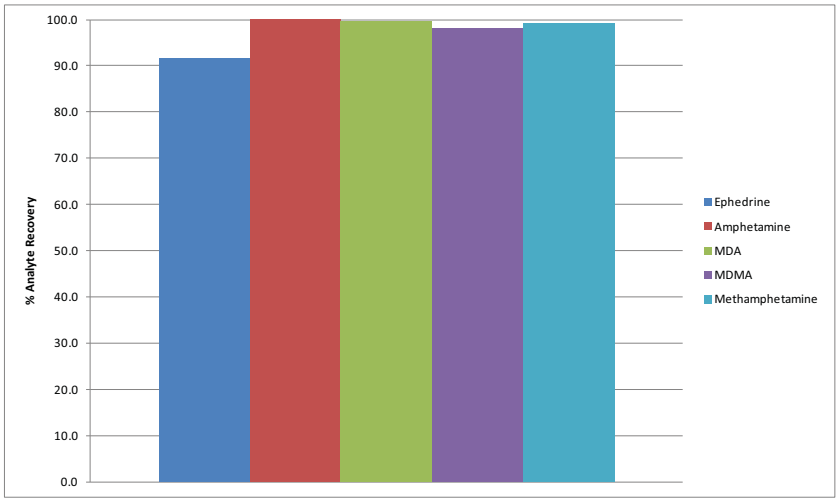


Figure 3. Analyte % recoveries of amphetamines using SLE+ 200 methodology at 500 pg/mL (n=7)

Ordering information

Part number	Description	Quantity
820-0200-P01	ISOLUTE SLE+ 200 µL supported liquid extraction plate	1
121-9600	VacMaster-96 Sample processing manifold complete (without Vacuum control)	1
SD2-9600-DHS-UK	SPE Dry 96 Dual, 240 V UK	1

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