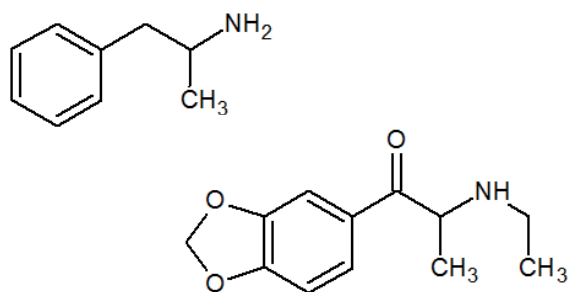


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# Extraction of Amphetamine and Designer Amphetamine Analogues from Whole Blood Using ISOLUTE® SLE+ Prior to GC/MS Analysis



**Figure 1.** Structures of Amphetamine and Ethylone

## Introduction

This application note describes the extraction of a range of amphetamine-type compounds from whole blood, prior to GC/MS analysis. A protocol that allows the simultaneous extraction of various other drugs of abuse classes: barbiturates, benzodiazepines, cocaine and opiates, is also evaluated.

ISOLUTE® SLE+ columns with 1 mL sample capacity are used to extract whole blood samples following a straightforward sample dilution. No protein precipitation or other pre-treatment is required prior to sample loading. The sample preparation procedure delivers clean extracts, good recoveries and RSD values and LLOQs from 10 ng/mL (analyte dependant).

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, Amphetamine-D5, Methamphetamine, Methcathinone, Mephedrone, 5-APB, 6-APB, MDA, pMMA, Methedrone, BZP, TFMP, MDMA, MDEA, Methylone, Butylone, Ethylone, Ethylone-D5, 2C-B, mCPP, MDPV, Naphyrone

## Sample Preparation Procedure

### Format:

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

### Sample Pre-treatment

To 1 mL of whole blood, add 10 µL of ISTD (total 100 ng/mL). Allow to equilibrate and add 1 mL of 1% ammonium hydroxide (aq). Vortex.

### Sample Loading

Load 750 µL of the pre-treated whole blood 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\* (2.5 mL) and allow to flow under gravity for 5 minutes. Collect in an appropriate glass tube containing HCl in methanol (0.2 M, 100 µL). This acts to stabilize free-base analytes in the solvent prior to evaporation.

Apply a second aliquot of MTBE\* (2.5 mL) and allow to flow under gravity for 5 minutes. Collect as before. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

\*Note that dichloromethane (DCM) can be used as an alternative extraction solvent if simultaneous extraction of other analyte classes (barbiturates, benzodiazepines, opiates and cocaine including the BZE metabolite) is required

### Post Elution and Reconstitution

Evaporate the extract in a stream of air or nitrogen using a TurboVap® LV (ambient, 20 to 40 L/min).

Reconstitute the extracts with ethyl acetate (250 µL) and vortex for 20 seconds before transferring to high recovery GC vials. 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 ethyl acetate (25 µL) and pentafluoropropionic anhydride (PFPA) (25 µL). Vortex mix then heat on a block for 20 minutes at 70 °C to complete derivatization.

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 ethyl acetate (50 µL) and vortex.

## 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 (constant flow)

### Inlet

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

### Injection

2 µL

### Wash Solvents

Acetone & Ethyl acetate

### Oven

Initial temperature 25 °C/min to 350 °C, hold for 0.4 minutes

### Post Run

Backflush for 1.6 minutes (2 void volumes)

### Transfer Line

280 °C



## MS Conditions

### Instrument

Agilent 5975C

### Source

230 °C

### Quadrupole

150 °C

### MSD mode

SIM

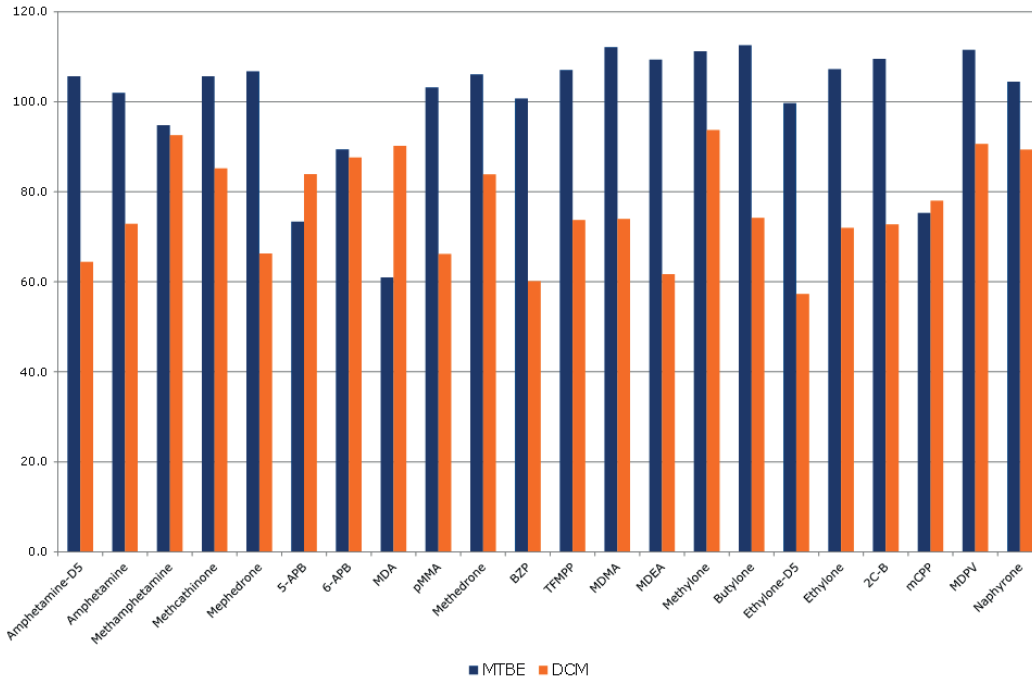
### SIM Parameters

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

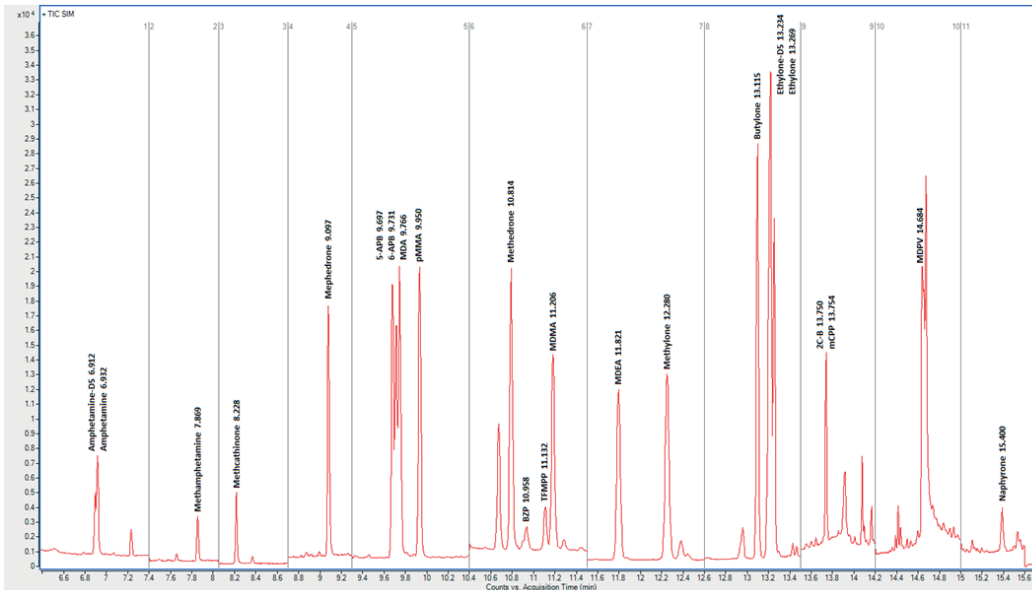
SIM Group	Analyte	Target (Quant) Ion	1 <sup>st</sup> Qual Ion	2 <sup>nd</sup> Qual Ion
1	Amphetamine-D5	194	122	
1	Amphetamine	190	118	
2	Methamphetamine	204	160	119
3	Methcathinone	105	204	160
4	Mephedrone	119	91	204
5	5-APB	131	158	
5	6-APB	158	131	
5	MDA	135	325	162
5	pMMA	121	148	
6	Methedrone	135	204	119
6	BZP	231	175	
6	TFMPP	172	200	
6	MDMA	204	162	135
7	MDEA	218	162	135
7	Methylone	149	204	160
8	Butylone	149	218	121
8	Ethylone-D5	223	191	
8	Ethylone	218	149	190
9	2C-B	242	229	
9	mCPP	195	139	
10	MDPV	126	149	121
11	Naphyrone	126	155	

## Results

Blank whole blood was spiked at 100 ng/mL for recovery testing; typical recovery data is shown in **Figure 4**. Both MTBE and DCM protocols gave reproducible data with RSD values <10% for barbiturates, benzodiazepines and opiates.



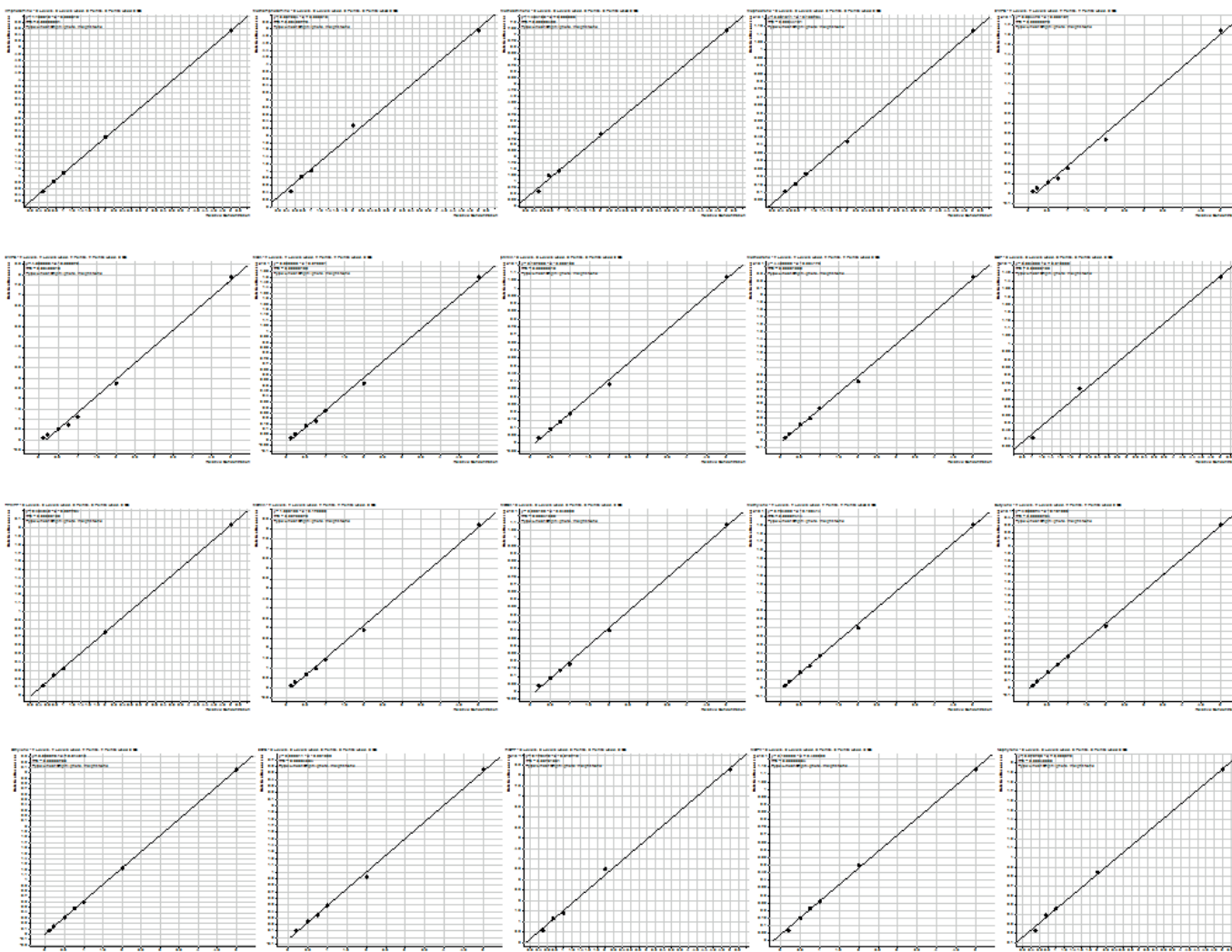
**Figure 2.** Typical amphetamine/analogue recoveries using MTBE or DCM extraction solvent.



**Figure 3.** Total Ion Chromatogram of amphetamines and amphetamine type analytes at 100 ng/mL using the MTBE extraction protocol.

## Calibration Curves

Whole blood was spiked prior to extraction at concentrations of 10, 20, 50, 75, 100, 200 and 500 ng/mL for each analyte to create calibrators. The internal standards were spiked at 100 ng/mL for each level. The curves are shown in **Figure 4**.



**Figure 4.** Charts demonstrating coefficient of determination ( $r^2$ ) values between 0.9939 and 0.9999 for the amphetamine analytes using the MTBE extraction protocol.

**Table 3.**  
Lower Limits of Quantitation (LLOQ) using the ISOLUTE® SLE+ procedure described

Analyte	LLOQ (ng/mL) MTBE Elution	LLOQ (ng/mL) DCM Elution
Amphetamine	50	50
Methamphetamine	50	50
Methcathinone	50	50
Mephedrone	50	50
5-APB	10	10
6-APB	10	10
MDA	10	20
pMMA	20	20
Methedrone	10	20
BZP	100	100
TFMPP	50	20
MDMA	10	10
MDEA	20	20
Methylone	10	10
Butylone	<10	<10
Ethylone	10	10
2C-B	20	20
mCPP	50	50
MDPV	20	20
Naphyrone	50	20

## Additional Notes

### Solvents and reagent preparation:

- » All solvents were HPLC grade.
- » 1% ammonium hydroxide (aq): Add concentrated ammonium hydroxide (28–30%) (1 mL) to HPLC grade water (99 mL).
- » 0.2M HCl in methanol: Add concentrated HCl solution (37%) (200 µL) to methanol (11.8 mL).

**Column loading:** ISOLUTE® SLE+ columns are underloaded (750 µL sample on a 1 mL capacity column) to avoid breakthrough of whole blood matrix.

**Simultaneous extraction of other drug classes:** MTBE is the optimum solvent for amphetamine extraction. However if simultaneous extraction of cocaine and its BZE metabolite is required, dichloromethane (DCM) should be used. DCM will also allow the simultaneous extraction of barbiturates, benzodiazepines and opiates. Comparable recoveries, RSDs, LLOQs and linearity for amphetamines are observed with both solvents.

## Ordering Information

Part Number	Description	Quantity
<b>820-0140-C</b>	ISOLUTE® SLE+ 1 mL Sample Volume Column*	30
<b>820-0140-CG</b>	ISOLUTE SLE+ 1 mL Sample Volume Column (tablets)	30
<b>PPM-48</b>	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
<b>SD-9600-DHS-EU</b>	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
<b>SD-9600-DHS-NA</b>	Biotage® SPE Dry Sample Concentrator System 100/120 V	1

\*ISOLUTE SLE+ 1 mL Sample Volume columns are available in the tablets (or flangeless) format for compatibility with the Biotage® Extrahera™ and other sample processing platforms. Bulk packs are also available, visit [www.biotage.com](http://www.biotage.com) for further information.

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