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# Extraction of Cocaine and Metabolites from Whole Blood Using ISOLUTE® SLE+ Prior to GC/MS Analysis

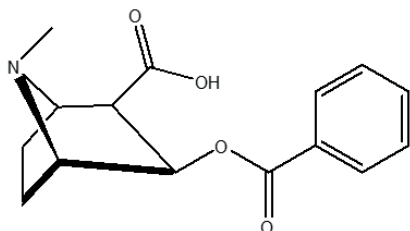


Figure 1. Structure of Benzoyllecgonine

## Introduction

This application note describes the extraction of cocaine and major metabolites from whole blood, prior to GC/MS analysis. This protocol also allows the simultaneous extraction of various other drugs of abuse classes: amphetamines, barbiturates, benzodiazepines and opiates

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 20 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

Anhydroecgonine methyl ester (AEME), Ecgonine (EME), Cocaine, Cocaethylene, Benzoyllecgonine-D3, (BZE-D3), Benzoyllecgonine (BZE)

## 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 dichloromethane (DCM, 2.5 mL) and allow to flow under gravity for 5 minutes. Collect in an appropriate glass tube.

Apply a second aliquot of DCM (2.5 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent into the collection vessel.

### 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 (40 °C, 20 to 40 L/min).

Reconstitute extracts with ethyl acetate (40 µL) and BSTFA (with 1% t-BDMCS) (40 µL), vortex and heat for 30 minutes at 70 °C to complete derivatization.

## 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

260 °C, Splitless, purge flow: 50 mL/min at 1.0 min

### Injection

2 µL

### Wash Solvents

Acetone & Ethyl acetate

### Oven

Initial temperature 60 °C, hold for 1 minute, ramp 50 °C/min to 200 °C, hold for 1.5 minutes, ramp 10 °C/min to 250°C.

### 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	AEME	152	181	
2	EME	82	96	
3	Cocaine	94	82	
4	Cocaethylene	196	82	94
4	BZE-D <sub>3</sub>	85	243	
4	BZE	82	240	

## Results

Blank whole blood was spiked at 100 ng/mL for recovery testing. Reproducible data was observed from the typical recovery data with RSD values <10% as shown in Figure 2.

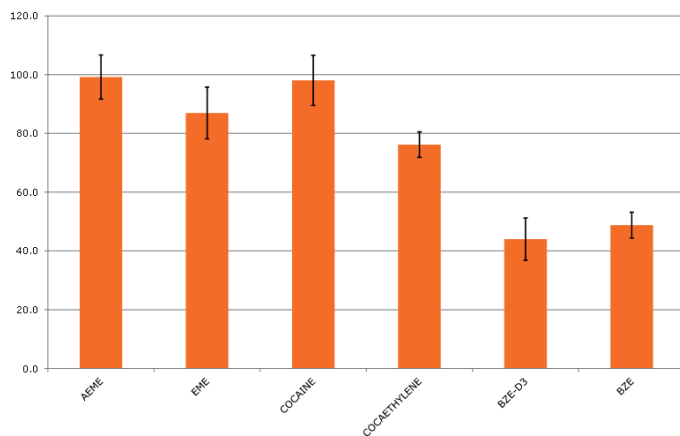


Figure 2. Typical recoveries for cocaine and metabolites.

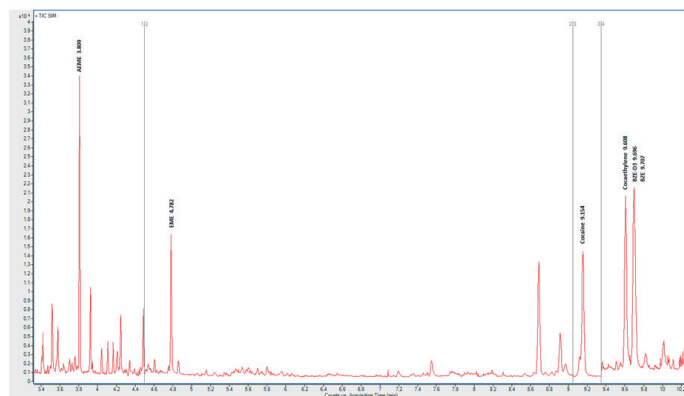
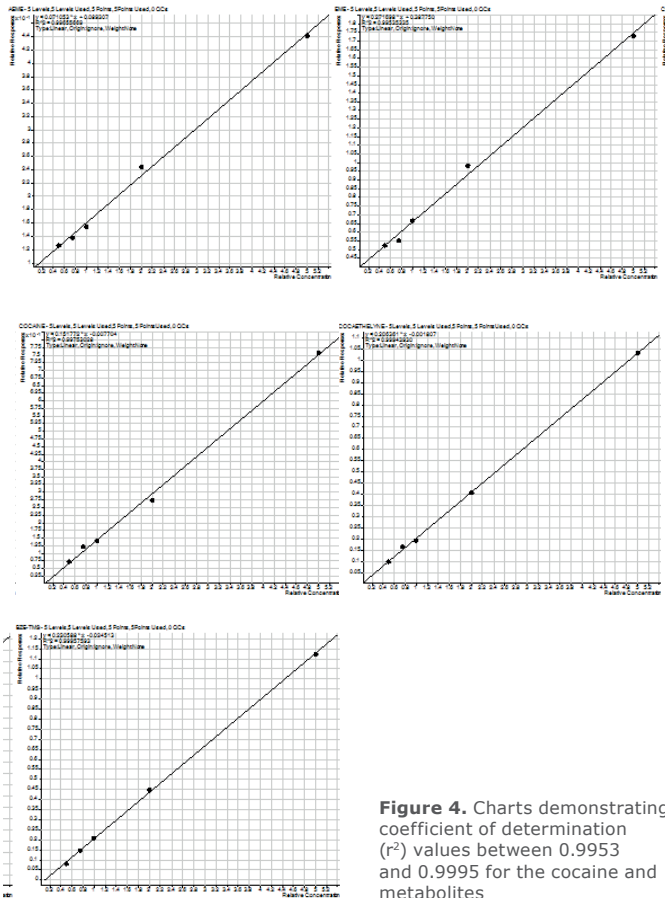


Figure 2. Total Ion Chromatogram of cocaine and metabolites at 100 ng/mL.

## 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 calibration curves. BZE-D3 was 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 0.9953 and 0.9995 for the cocaine and metabolites

**Table 3.**

Lower Limits of Quantitation (LLOQ) using ISOLUTE® SLE+ procedure

Drug Analyte	DCM LLOQ (ng/mL)
AEME	20
EME	50
Cocaine	50
Cocaethylene	50
BZE	50

## 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).

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

### Simultaneous extraction of other drug classes:

This protocol allows the simultaneous extraction of amphetamines, barbiturates, benzodiazepines and opiates.

## 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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