

# Extraction of Cocaine and Metabolites from Hydrolyzed Urine Using ISOLUTE® SLE+ Prior to GC-MS Analysis

This application note describes the extraction of cocaine and a range of cocaine metabolites from hydrolyzed human urine prior to GC-MS.

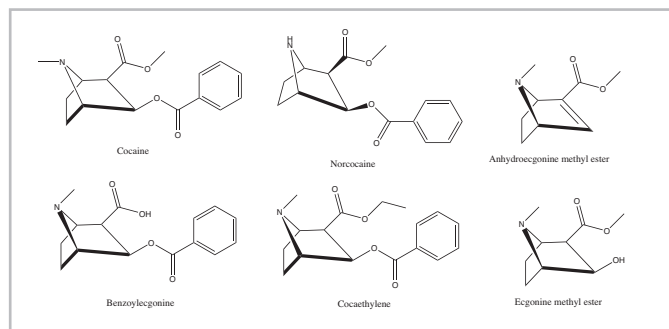


Figure 1. Cocaine structures

## Introduction

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.

This application note describes effective and efficient ISOLUTE SLE+ protocols optimized for sample loading volumes of either 400  $\mu$ L or 1 mL. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 83% with RSDs of <7% for all analytes.

## Analytes

Cocaine, AEME, EME, Benzoylecgonine, Cocaethylene and Norcocaine

## Sample Preparation Procedure

**Sample Pre-treatment** Dilute urine (1 mL) with 100 mM ammonium acetate buffer at pH 5 (950  $\mu$ L) and  $\beta$ -Glucuronidase enzyme (50  $\mu$ L, equivalent to approximately 4500 U/mL of urine). Spike BZE- $d_3$  internal standard. Hydrolyze with heat in a water bath at 60  $^{\circ}$ C for 2 hours. Cool and add 10  $\mu$ L concentrated ammonium hydroxide (28-30 % stock (aq)). Vortex mix thoroughly.

### ISOLUTE SLE+ 400 $\mu$ L Sample Volume Columns, Part Number 820-0055-B

**Sample Loading:** Load pre-treated sample (400  $\mu$ L total volume) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

**Analyte Extraction:** Apply dichloromethane/isopropanol (95:5, v/v, 1 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of dichloromethane/isopropanol (95:5, v/v, 1 mL) and allow to flow for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

### ISOLUTE SLE+ 1 mL Sample Volume Columns, Part Number 820-0140-C

**Sample Loading:** Load pre-treated sample (1 mL total volume) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

**Analyte Extraction:** Apply dichloromethane/isopropanol (95:5, v/v, 2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of dichloromethane/isopropanol (95:5, v/v, 2.5 mL) and allow to flow for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

**Post Elution and Reconstitution:** Dry the extract in a stream of air or nitrogen using a SPE Dry (30  $^{\circ}$ C, 20 to 40 L/min) or TurboVap (1.5 bar at 40  $^{\circ}$ C for 1 hr). Upon dryness, add 50  $\mu$ L ethyl acetate and 50  $\mu$ L BSTFA:TMCS (99:1). Vortex for 20 seconds and transfer to a high recovery glass vial and cap with a non-split cap. Heat vial in a heating block set to 70  $^{\circ}$ C, for 20 minutes. Remove vial from the block and allow to cool.

## GC Conditions

<b>Instrument:</b>	Agilent 7890A
<b>Column:</b>	SGE capillary column; BPX5, 30 m x 0.25 mm ID x 0.25 µm
<b>Carrier:</b>	Helium 1.2 mL/min (constant flow)
<b>Inlet:</b>	250 °C, Split (ratio 20:1), 24 mL/min, septum purge flow: 3 mL/min
<b>Injection:</b>	1 µL  Wash solvents: ethyl acetate and dichloromethane/isopropanol (95:5, v/v)
<b>Oven:</b>	Initial temperature 100 °C  Ramp 4 °C/min to 190 °C  Ramp 100 °C/min to 250 °C  Hold temperature for 4 min
<b>Transfer Line:</b>	280 °C

## MS Conditions

<b>Instrument:</b>	Agilent 5975C
<b>Source:</b>	230 °C
<b>Quadrupole:</b>	150 °C
<b>MSD mode</b>	SIM

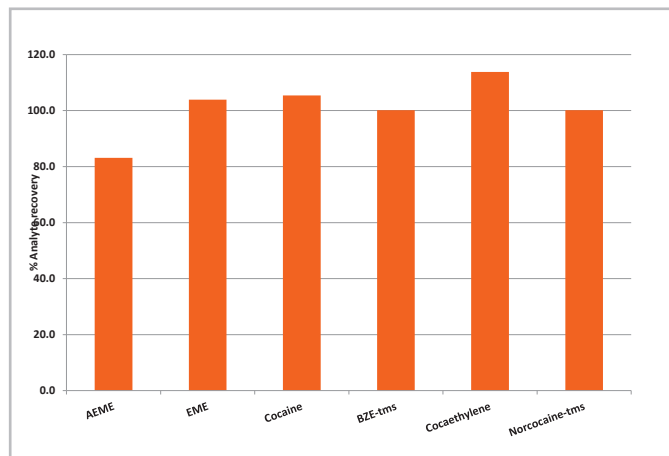
## SIM Parameters

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

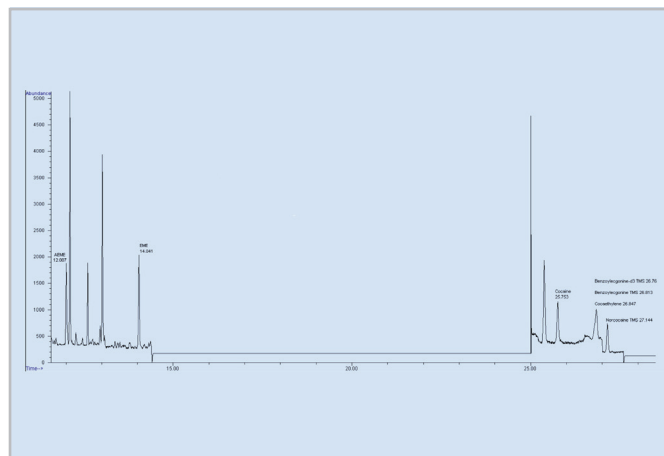
SIM Group	Analyte	Target (Quant) Ion	1 <sup>st</sup> Qual Ion	2 <sup>nd</sup> Qual Ion	3 <sup>rd</sup> Qual Ion
1	AEME	152	153	166	122
2	EME	96	97	82	83
3	Cocaine	94	82	83	105
4	Benzoylecgonine-d <sub>3</sub> TMS	243	364	N/A	N/A
4	Benzoylecgonine TMS	240	361	256	N/A
4	Cocaethylene	196	317	272	N/A
5	Norcocaine TMS	140	240	105	N/A

## Results

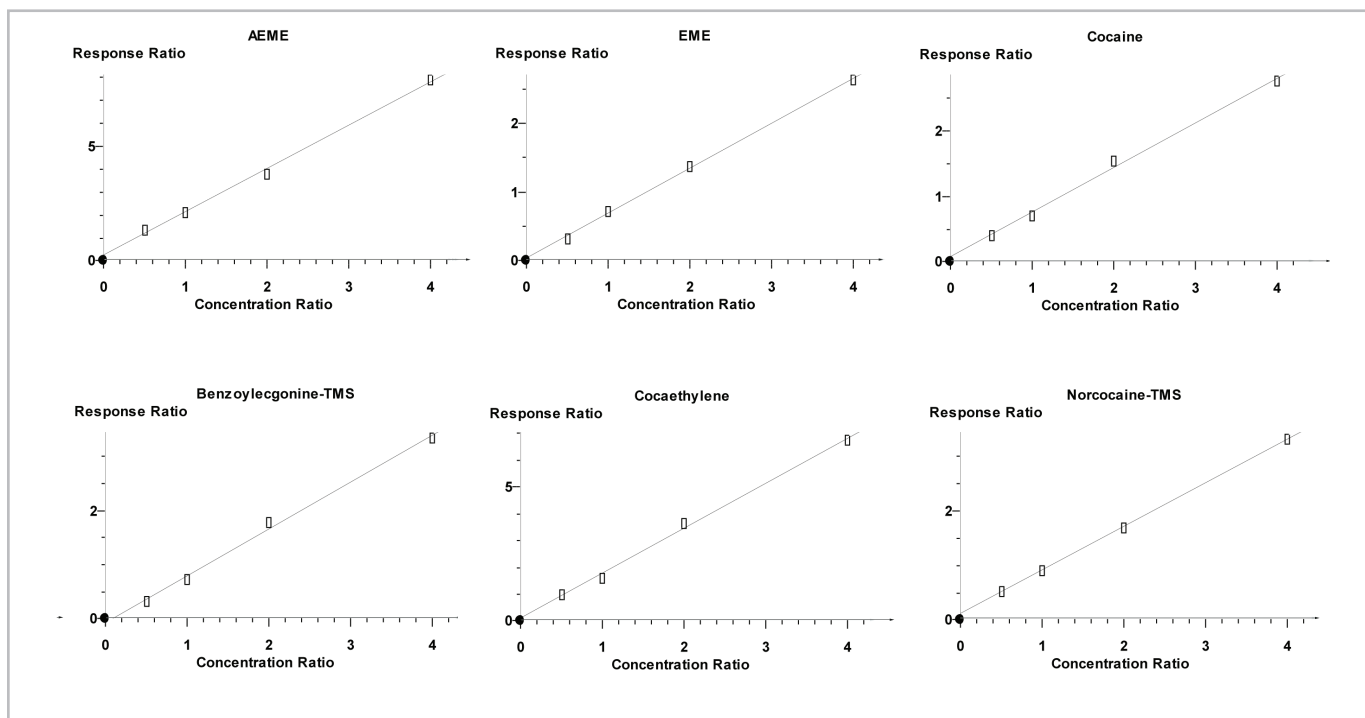
This ISOLUTE® SLE+ protocol demonstrates analyte recoveries ranging from 83–113% as shown in **Figure 2**. RSDs were below 10% for all analytes.



**Figure 2.** Typical analyte % recoveries for extracted cocaine and metabolites from urine (n=7) using the ISOLUTE® SLE+ protocol



**Figure 3.** GC-MS chromatography for cocaine and metabolites from hydrolyzed urine spiked at 100 ng/mL



**Figure 4.** Calibration curves for the application analytes showing  $r^2$  values ranging from 0.9953 to 0.9988

**Table 2.** Limits of Quantitation using the ISOLUTE® SLE+ 400 µL sample volume

Analyte	Extracted LOQ
AEME	20 ng/mL
EME	20 ng/mL
Cocaine	20 ng/mL
Benzoylecgonine	20 ng/mL
Cocaethylene	20 ng/mL
Norcocaine	20 ng/mL

**Table 3.** Limits of Quantitation using the ISOLUTE® SLE+ 1 mL sample volume

Analyte	Extracted LOQ
AEME	10 ng/mL
EME	10 ng/mL
Cocaine	10 ng/mL
Benzoylecgonine	10 ng/mL
Cocaethylene	10 ng/mL
Norcocaine	10 ng/mL

## Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Column	50
820-0140-C	ISOLUTE® SLE+ 1 mL Supported Liquid Extraction Column	30
121-9600	Biotage® VacMaster™-96 Sample Processing Manifold	1
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold 48 position	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
C103263	TurboVap® 96 100/120 VAC	1
C103264	TurboVap® 96 1220/240 VAC	1

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