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

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

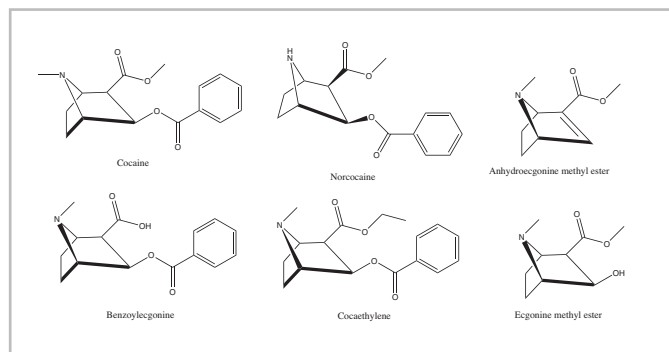


Figure 1. Cocaine structures

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 volumes of either 400 μ L or 1 mL. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 80% with RSDs of <10% for all analytes.

Analytes

Cocaine, AEME, EME, Benzoylecgonine, Cocaethylene and Norcocaine

Sample Preparation Procedure

Sample Pre-treatment Dilute pre-treated urine (1 mL) with 0.1% ammonium hydroxide (1 mL). Spike BZE-d₃ internal standard and vortex mix thoroughly.

ISOLUTE SLE+ 400 μ L Sample Volume Columns, Part Number 820-0055-B

Sample Loading: Load the pre-treated urine (400 μ 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 the pre-treated urine (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 Biotage® SPE Dry Sample Concentrator System (40°C, 20 to 40 L/min) or TurboVap® (1.5 bar at 40 °C for 1 hr). Upon dryness, add 50 μ L ethyl acetate and 50 μ 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 °C, for 20 minutes. Remove vial from the block and allow to cool.

GC Conditions

Instrument:	Agilent 7890A
Column:	SGE capillary column; BPX5, 30 m x 0.25 mm ID x 0.25 µm
Carrier:	Helium 1.2 mL/min (constant flow)
Inlet:	250 °C, Split (ratio 20:1), 24 mL/min, septum purge flow: 3 mL/min
Injection:	1 µL Wash solvents: ethyl acetate and dichloromethane/isopropanol (95:5, v/v)
Oven:	Initial temperature 100 °C Ramp 4 °C/min to 190 °C Ramp 100 °C/min to 250 °C Hold temperature for 4 min
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 st Qual Ion	2 nd Qual Ion	3 rd Qual Ion
1	AEME	152	153	166	122
2	EME	96	97	82	83
3	Cocaine	94	82	83	105
4	Benzoyllecgonine-d ₃ TMS	243	364	N/A	N/A
4	Benzoyllecgonine 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 82–105% as shown in **Figure 2**. RSDs were below 8% for all analytes.

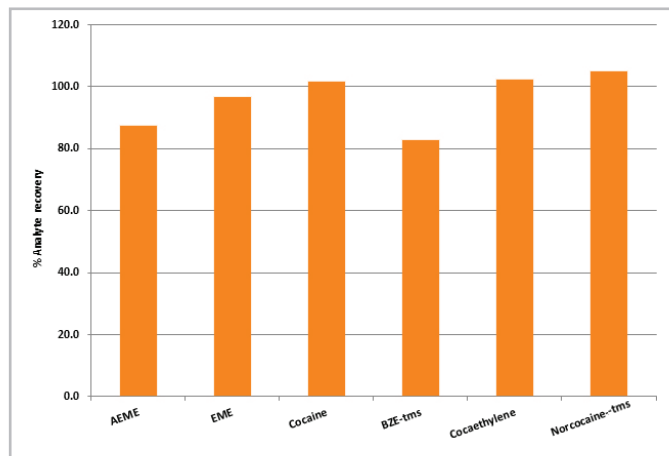


Figure 2. Typical analyte % recoveries for extracted cocaine and metabolites from urine (n=7) using the ISOLUTE® SLE+ protocol

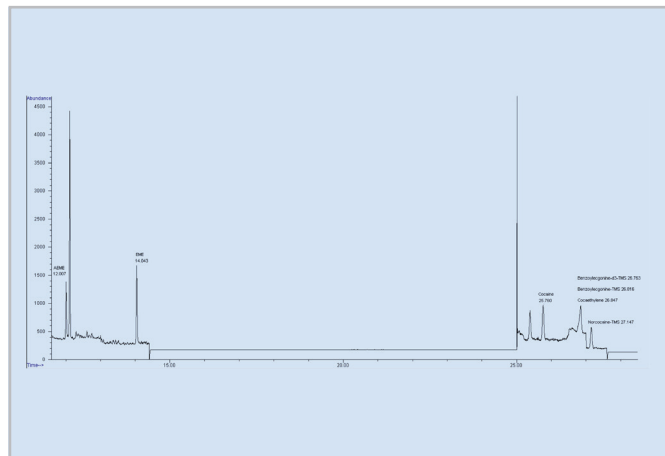


Figure 3. GC-MS chromatography for cocaine and metabolites from urine spiked at 100 ng/mL

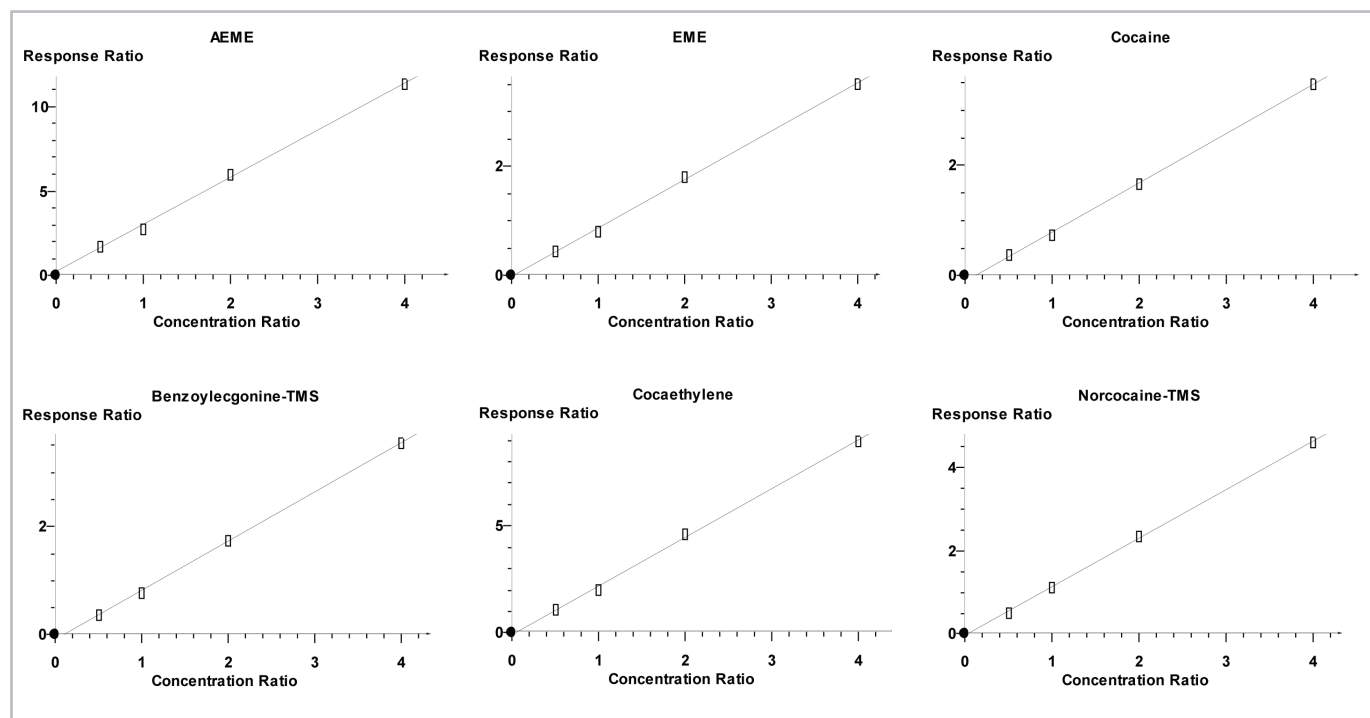


Figure 4. Calibration curves for the application analytes showing r^2 values ranging from 0.9978 to 0.9997

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

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 columns

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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