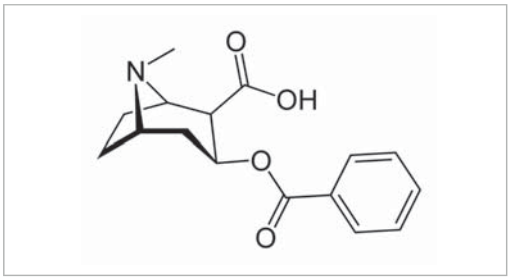
Extraction of Cocaine and Metabolites From Hydrolysed Urine Using ISOLUTE SLE+ prior to LC-MS/MS Analysis

Introduction

This application note describes the extraction of cocaine and a full range of cocaine metabolites from hydrolysed human urine prior to analysis and quantitation by LC-MS/MS



This application note describes effective and efficient ISOLUTE SLE+ protocols optimized for sample (hydrolysed urine) volumes of either 200 µL or 1 mL. Procedures for high throughput (96-well plate) and column format are included.

The simple sample preparation procedure delivers clean extracts and analyte recoveries of 82-100% with RSDs of <5% for all analytes. Sub ng/mL LOQs can be achieved.

Figure 1. Structure of Benzoylecgonine

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

Cocaine, Norcocaine, Cocaethylene, Benzoylecgonine, Ecgonine Methyl Ester (EME) and Anhydroecgonine Methyl Ester (AEME).

Sample Preparation Procedure

Sample pre-treatment: Dilute urine (1 mL) with 100mM ammonium acetate buffer at pH5 (950µL) and β-Glucuronidase enzyme (50µL, equivalent to approximately 4500U/mL of urine). Spike ISTD (10µL of BZE-d3; total concentration 50ng/mL). Hydrolyse with heat in a water bath at 60°C for 2 hours. Cool and add concentrated ammonium hydroxide (28-30% stock) aq (10µL). Vortex mix thoroughly.

ISOLUTE SLE+ 200 µL Supported Liquid Extraction Plate, part number 820-0200-P01

Sample loading: Load the hydrolysed urine (200 µL) onto the plate and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Analyte extraction: Apply DCM/IPA (95:5, v/v) (1 mL) and allow to flow under gravity for 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 hydrolysed urine (1 mL) 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 DCM/IPA (95:5, v/v) (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of DCM/IPA (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-extraction: Add 50mM HCl in MeOH (100 µL) to stabilize AEME and EME during evaporation. Evaporate to dryness at 40 °C.

Reconstitution:

820-0200-P01: Water/Methanol (90/10, v/v) (200 µL)

820-0140-C: Water/Methanol (90/10, v/v) (500 µL)

UPLC Conditions

Instrument: Waters Acquity UPLC fitted with 20 µL loop

Column: Waters UPLC BEH C18 (1.7 µm, 100 x 2.1 mm id)

Sample temperature: 20 °C

Column temperature: 40 °C

Injection volume: 10 µL (partial loop with overfill)

Mobile phase: A= 0.1% ammonium hydroxide (aq)

B= 0.1% ammonium hydroxide/MeOH

Gradient:

Time (min)	%A	%B	Curve
0	60	40	-
2	10	90	11
3	60	40	6

Mass Spectrometry Conditions

Instrument: Premier XE triple quadrupole mass spectrometer equipped with an electrospray interface for mass analysis.

Desolvation temperature: 450 °C

Ion source temperature: 150 °C

Table 1. Positive ions acquired in the multiple reaction monitoring (MRM) mode

Analyte	Transition	Dwell Time (s)	Cone voltage (V)	Collision energy (eV)
EME	200 > 81.9 QUANT 200 > 182.0 QUAL	0.07	30	23 17
BZE	290.1 > 168.0 QUANT 290.1 > 149.9 QUAL	0.07	30	18 22
BZE-d3 ISTD	293.1 > 171.0	0.07	30	18
AEME	182.0 > 122.0 QUANT 182.0 > 90.9 QUAL	0.2	30	19 21
Norcocaine	290.1 > 135.9 QUANT 290.1 > 107.9 QUAL	0.05	30	22 26
Cocaine	304.2 > 182.0 QUANT 304.2 > 149.9 QUAL	0.05	30	20 25
Cocaethylene	318.1 > 196.0 QUANT 318.1 > 149.9 QUAL	0.05	30	19 23

Results

This SLE+ protocol demonstrates analyte recoveries ranges from 82-100% as shown in figure 2. RSDs were below 5% for all analytes. Figure 3 shows an example calibration curve for the cocaine metabolite, benzoylecgonine. Figure 4 shows the MRM transitions, both quantification and qualification ions, for analytes extracted using the supported liquid extraction protocol.

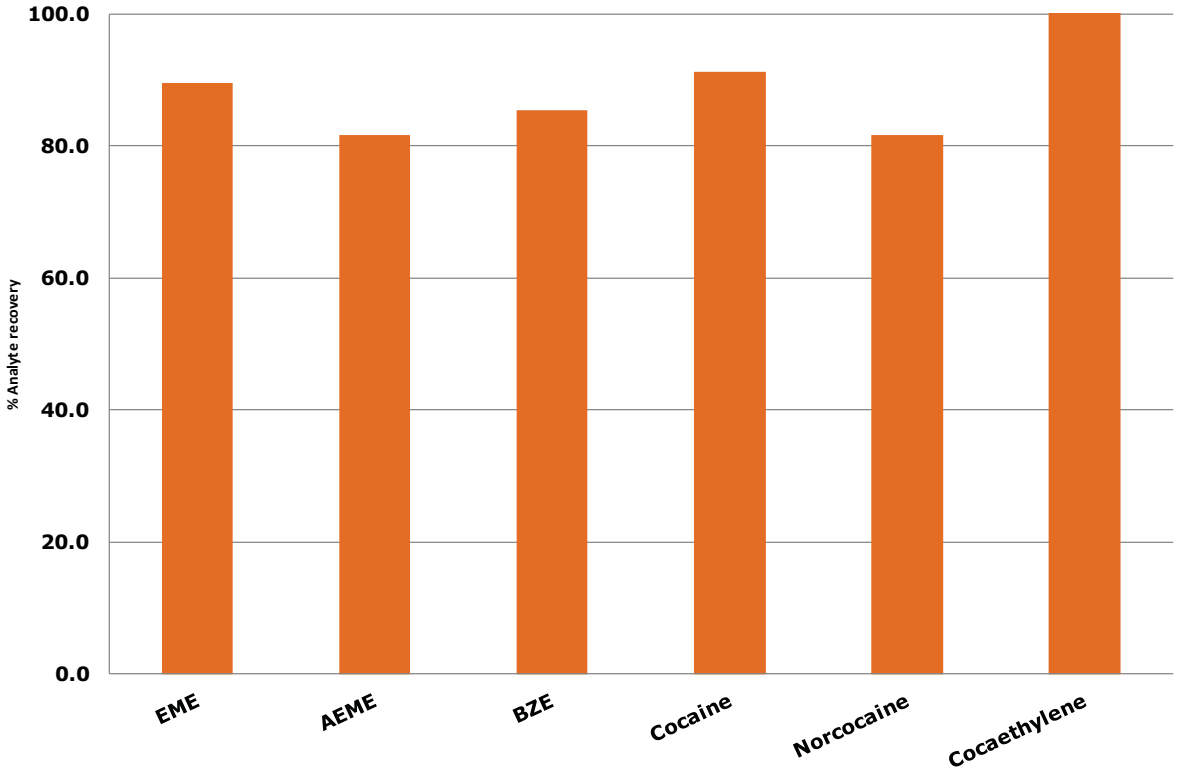


Figure 2. Typical analyte % recoveries for a extracted Cocaine and metabolites (n=7) using the ISOLUTE SLE+ protocol

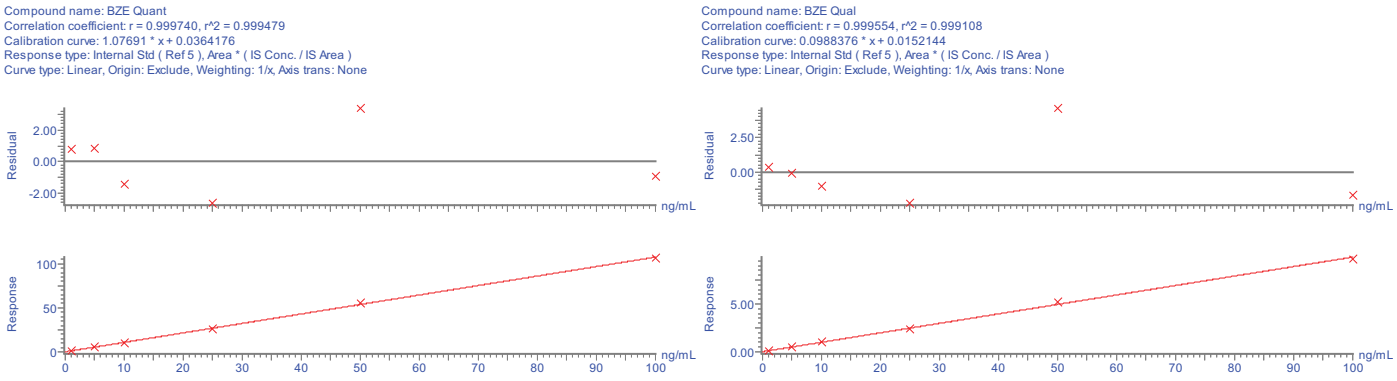


Figure 3. Example calibration curve for the cocaine metabolite Benzoylecgonine

Table 2. Estimated Limits of Quantitation for all analytes using the ISOLUTE SLE+ 1 mL sample volume column

Analyte	Estimated LOQ
EME	< 200 pg/mL
BZE	< 200 pg/mL
AEME	< 400 pg/mL
Norcocaine	< 800 pg/mL, restricted by qualifier ion
Cocaine	< 50 pg/mL
Cocaethylene	< 100 pg/mL

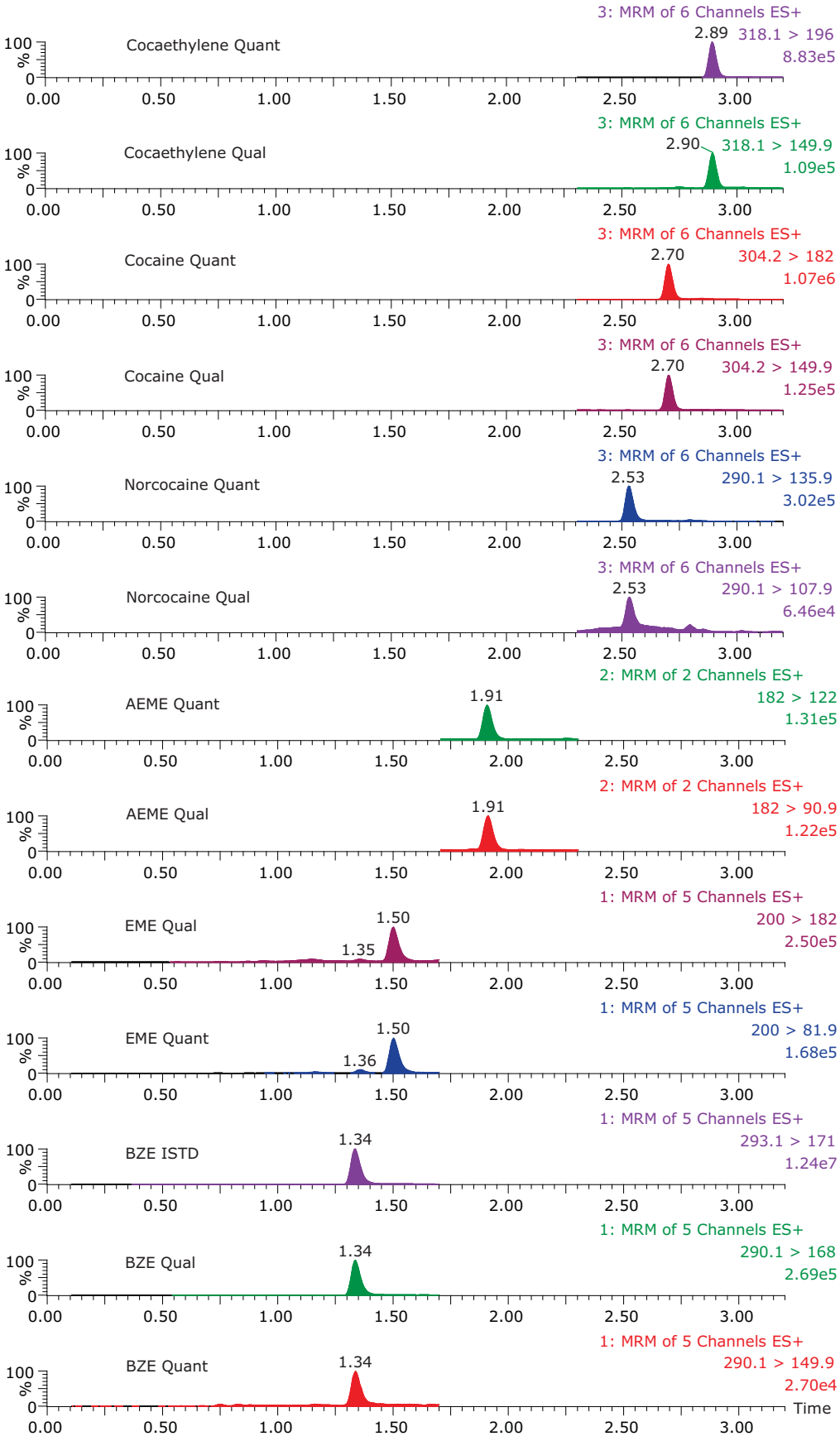


Figure 4. MRM transitions for Cocaine and metabolites at 1 ng/mL

Ordering information

Part number	Description	Quantity
820-0200-P01	ISOLUTE SLE+ 200 Supported Liquid Extraction Plate	1
820-0140-C	ISOLUTE SLE+ 1 mL Sample Volume columns	30
820-0140-CG	ISOLUTE SLE+ 1 mL Sample Volume columns (tabless for use with PPM-48)	30
PPM-96	PRESSURE+96 Positive Pressure Manifold 96 Well	1
PPM-48	PRESSURE+48 Positive Pressure Manifold 48 position	1
121-9600	VacMaster 96 Sample Processing Manifold	1
121-1016	VacMaster-10 Sample Processing Manifold	1
121-2016	VacMaster-20 Sample Processing Manifold	1
SD-9600-DHS-UK	SPE Dry 96 240 V	1

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