

Extraction of Cocaine and Metabolites from Biological Fluids using EVOLUTE CX

Introduction

This procedure is recommended for the extraction of cocaine and its principle metabolites (norcocaine, benzoylecgonine, ecgonine methyl ester, anhydroecgonine methyl ester and cocaethylene) from whole blood, plasma and urine using polymeric mixed-mode cation exchange SPE columns.

EVOLUTE CX mixed-mode resin-based SPE sorbent extracts a wide range of basic analytes from biological fluids and other aqueous matrices using a generic procedure which minimizes method development time. EVOLUTE CX removes matrix components such as proteins, salts, non-ionizable interferences and phospholipids, delivering cleaner extracts with reproducible recoveries for accurate quantitation.

Sample Preparation Procedure

EVOLUTE CX Configuration

25 mg 96-fixed-well plate (part number 601-0025-P01)

EVOLUTE CX Procedure

Sample:	Plasma and Urine: Dilute sample (100 μ L) with ammonium acetate buffer (1:3, v/v, 50 mM, pH 6). Whole blood: Lyse red blood cells by sonication in buffer (10 mins) followed by centrifugation at 11,000 rpm for 10 minutes. Discard cellular debris (pellet). Treat as plasma and urine.
Column Conditioning:	Condition column with methanol (1 mL)
Column Equilibration:	Equilibrate column with ammonium acetate (50 mM, pH 6, 1 mL)
Sample Loading:	Load pre-treated sample (400 μ L) at a flow rate of 1 mL/min
Interference Elution 1:	Remove polar and ionic interferences with ammonium acetate buffer (50 mM, pH 6, 1 mL)
Interference Elution 2:	Rinse column with aqueous formic acid (2% v/v, 1 mL)
Interference Elution 3:	Remove non-polar interferences with methanol (1 mL)
Analyte Elution:	Elute cocaine and metabolites with methanol containing ammonium hydroxide (95:5, v/v, 1 mL).
Post Extraction:	Evaporate to dryness and reconstitute in water/methanol (80:20, v/v, 500 μ L) prior to analysis.

Analytical Procedure

HPLC Conditions

Instrument:	Waters 2795 Liquid Handling System
Column:	Agilent Zorbax Eclipse XDB C18 3.5 μ m
Guard Column:	Agilent C8 guard column
Mobile Phase A:	Aqueous ammonium hydroxide (0.1% v/v)
Mobile Phase B:	Methanol:ammonium hydroxide (99.9:0.1, v/v)
Flow rate:	0.25 mL/min

Time	%A	%B
0.0	90	10
0.5	90	10
5.0	10	90
5.1	90	10

Injection Volume:	10 μ L
Temperature:	Ambient

Mass Spectrometry Conditions

Instrument:	Waters Ultima Pt triple quadrupole mass spectrometer equipped with an electrospray interface
Desolvation Temperature:	350 °C
Ion Source Temperature:	100 °C
Collision Gas Pressure:	2.46 x 10 ⁻³ mbar

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

MRM Transitions

Analyte	Transitions
Ecgonine methyl ester	200>182
Anhydroecgonine methyl ester	182>90.9
Benzoylecgonine	290.1>168
Norcocaine	290>135.9
Cocaine	304.2>182
Cocaethylene	318.1>196

Typical results:

Table 1. below shows analyte recoveries obtained from whole blood using the methodology described in the application note. Recoveries for all analytes from both plasma and urine were >80%, with %RSD < 10%.

Table 1. Recovery of Cocaine and metabolites from whole blood

Analyte	Recovery	%RSD
Ecgonine methyl ester	99.1%	7.1
Anhydroecgonine methyl ester	111.2%	5.4
Benzoylecgonine	96.9%	2.4
Norcocaine	91.0%	3.0
Cocaine	87.8%	5.5
Cocaethylene	93.1%	4.5

Table 2. Recovery of Cocaine and metabolites from urine

Analyte	Recovery	%RSD
Ecgonine methyl ester	112.3%	3.9
Anhydroecgonine methyl ester	107.2%	6.4
Benzoylecgonine	96.9%	2.4
Norcocaine	99.5%	5.6
Cocaine	117.7%	4.2
Cocaethylene	109.4%	3.0

Table 3. Recovery of Cocaine and metabolites from plasma

Analyte	Recovery	%RSD
Ecgonine methyl ester	94.8%	3.0
Anhydroecgonine methyl ester	90.0%	5.7
Benzoylecgonine	101.9%	2.3
Norcocaine	86.7%	2.2
Cocaine	82.7%	4.0
Cocaethylene	83.8%	3.6

References

This application note is based on the poster 'Extraction of cocaine and metabolites using resin-based mixed-mode cation exchange SPE and analysis with LC-MS/MS', L Williams et al, presented at SOFT, Oklahoma City, October 19th -23rd 2009.

NORTH AMERICA

Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
press (4) at the auto attendant
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
1-pointsupport@biotage.com

EUROPE

Main Office: +46 18 56 5900
Fax: +46 18 59 1922
Order Tel: +46 18 56 57 10
Order Fax: +46 18 56 57 05
order@eu.biotage.com

Japan

Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com

Distributors

Please visit our Web site at
www.biotage.com
for contact details.