Extraction of Cocaine and Metabolites using Resin-based Mixed-mode Cation Exchange SPE with LC-MS/MS Analysis

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Introduction

Cocaine is one of the most widely abused illicit drugs available and not confined to any particular socio-economic class. Available in various forms, it is highly addictive, however, instantaneous euphoric effects has led to huge popularity. This widespread misuse has led to the necessity of rapid and reliable methods for analysis and quantitation from various matrices. EVOLUTE® CX is a resin-based mixed-mode strong cation exchange SPE sorbent designed for the extraction of basic drugs. The dual retention mechanism of hydrophobic interaction and strong cation exchange enables a rigorous interference wash regime resulting in cleaner final extracts.
This poster describes the use of EVOLUTE® CX for the extraction of cocaine and its major metabolites (structures shown in ***Figure 1***.) from various biological matrices.

Experimental

Reagents

Cocaine, norcocaine, benzoylecgonine, ecgonine methyl ester, anhydroecgonine methyl ester and cocaethylene were purchased from Lipomed (Kinesis distributor, Cambs. UK). Ammonium acetate, ammouium hydroxide, formic and acetic acids were purchased from Sigma Chemical Co. (Poole, UK). Blank plasma was obtained through the Welsh Blood Service (Pontyclun, UK). Urine was donated from a healthy human volunteer. Blood was purchased from Sera Laboratories International (West Sussex, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK) and Milli-Q water used throughout.

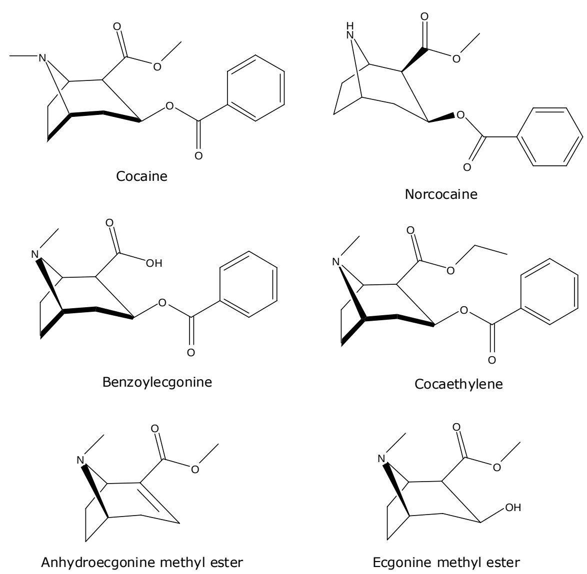

Figure 1. *Cocaine and metabolite structures.*

Modified Sample Pre-treatment (protocol 2): Matrix (100 µL) diluted 1:3 (v/v) with 1% formic acid (aq).

Whole Blood Cell Lysis: Sonication for 10 minutes in buffer followed by centrifugation at 11,000 rpm for 10 minutes. Cellular debris (pellet) was discarded.

SPE Column: EVOLUTE® CX 25 mg 96-Well (P/N 601-0025-P01)

Generic Extraction Protocol (1).
Column Conditioning: Methanol (1 mL)
Column Equilibration: 50 mM ammonium acetate buffer at pH 6 (1 mL)
Sample Loading: Pre-treated matrix (400 µL)
Interference Elution 1: 50 mM ammonium acetate buffer at pH 6 (1 mL)
Interference Elution 2: Methanol (1 mL)
Analyte Elution: 5% NH_4OH:Methanol (1 mL)

Optimized Extraction Protocol (3).
Column Conditioning: Methanol (1 mL)
Column Equilibration: 50 mM ammonium acetate buffer at pH 6 (1 mL)
Sample Loading: Pre-treated matrix (400 µL)
Interference Elution 1: 50 mM ammonium acetate buffer at pH 6 (1 mL)
Interference Elution 2: 2% formic acid aq (v/v, 1 mL)
Interference Elution 3: Methanol (1 mL)
Analyte Elution: 5% NH_4OH:Methanol (1 mL)

Post Extraction: Extracts were evaporated to dryness and reconstituted in 500 µL of 80:20 (v/v) H_2O/MeOH for subsequent LC-MS/MS analysis.

HPLC Conditons

Instrument: Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA).
Column: Zorbax Eclipse XDB C18 3.5 µm analytical column (Agilent Technologies, Berkshire, UK). 100 x 2.1 mm id.
Guard Column: C8 guard column (Agilent Technologies, Berkshire, UK).
Mobile Phase: 0.1% NH_4OH aq and 0.1% NH_4OH/MeOH at a flow rate of 0.25 mL/min.
Gradient: The gradient conditions were set to 90% 0.1% NH_4OH aq and 10% 0.1% NH_4OH:MeOH incorporating a step gradient to 100% 0.1% NH_4OH/MeOH after 0.5 minutes. Initial starting conditions were resumed at 5.1 minutes.
Injection Volume: 10 µL
Temperature: Ambient

Mass Spectrometry

Instrument: Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive ions were acquired in the multiple reaction monitoring mode (MRM).

Desolvation Temperature: 350 °C
Ion Source Temperature: 100 °C
Collision Gas Pressure: 2.6×10^{-3} mbar

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Results

Figures 1-3. show the recovery charts for extraction of cocaine and metabolites from plasma, urine and whole blood, respectively. Three extraction protocols are detailed based on the standard generic method. Protocol 1 did not involve a 2% formic acid wash step. Protocol 2 used modified pre-treatment (1:3 matrix:1% formic acid instead of ammonium acetate buffer) and protocol 3, the optimized extraction protocol as detailed in the experimental section. Whole blood was not processed using the modified pre-treatment protocol due to oxidation effects on the blood from the acidified buffer.

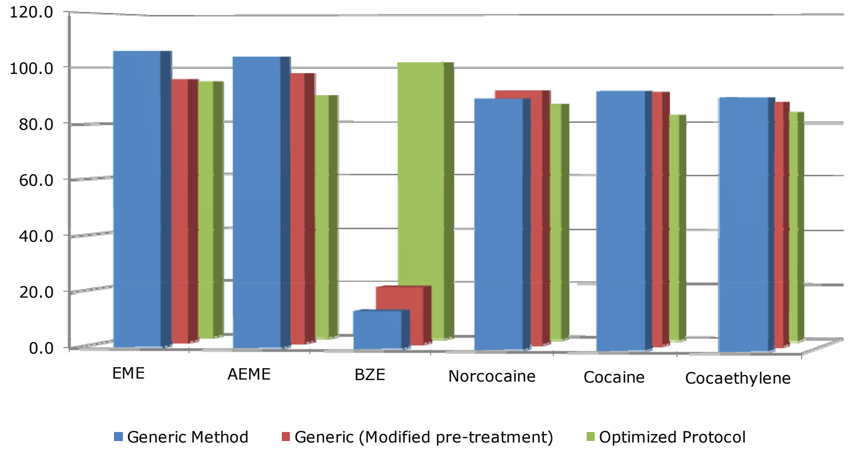

***Figure 1**. Plasma extraction recovery profile of cocaine and metabolites*

For three matrices, recoveries greater than 80% were
The generic method resulted in recoveries greater than 80% for all analytes except benzoylecgonine, which demonstrated recoveries < 30%. Using the modified pre-treatment protocol (method 2) similar recoveries were apparent. Using the optimized extraction protocol (method 3) BZE recoveries > 90% were observed.

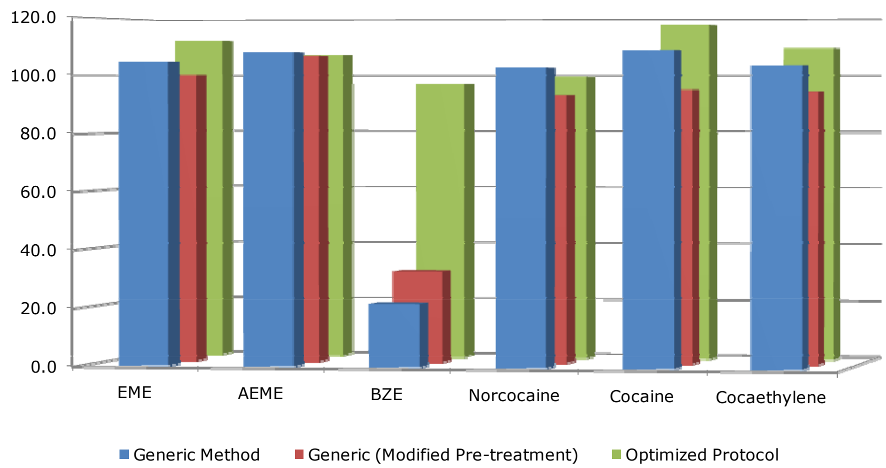

***Figure 2**. Urine extraction recovery profile of cocaine and metabolites*

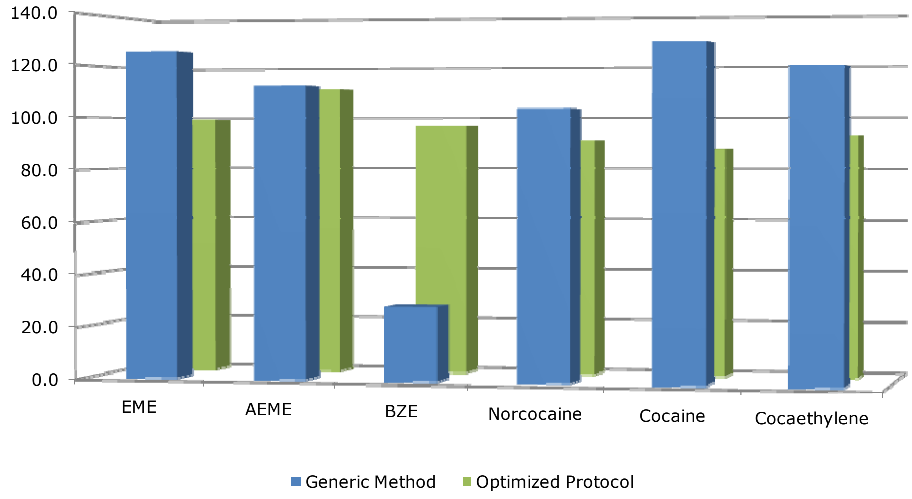

***Figure 3**. Whole Blood extraction recovery profile of cocaine and metabolites*

Conclusions

- Whole blood was not processed using the modified pre-treatment protocol as the 1% formic acid oxidized the iron resulting in difficulty in processing and deterioration of extract cleanliness.
- The generic and modified pre-treatment protocols yielded recoveries >80% for all analytes apart from benzoylecgonine.
- BZE has two ionizable functionalities and under pH 6 conditions does not have substantial cation exchange interaction with the sorbent, resulting in breakthrough and low recoveries. By adding an extra wash step of 2% formic acid the amine functionality is ionized and retains through an ionic mechanism.
- Recoveries > 80% with corresponding RSDs < 10% were observed for all matrices using the optimized protocol.
- This poster demonstrates the application of EVOLUTE CX to the extraction of cocaine and metbolites from various biological matrices using an optimized SPE protocol.

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