

# Drugs of Abuse Extraction from Whole Blood using Supported Liquid Extraction (SLE) and Extrahera Automation Prior to UPLC-MS/MS Analysis

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## Introduction

Whole blood continues to be a valuable tool in forensic toxicology for the immediate and near-term detection of illicit drugs, and in cases where no other sample is available. Screening drugs of abuse can be complicated due to the wide variation of functional groups associated with different analyte classes. Most extraction techniques cannot extract all analytes using a single procedure without using non-optimal extraction protocols resulting in compromised extract cleanliness. Supported liquid extraction allows for the simultaneous analysis of cross functional analytes in a single extraction protocol without forfeiting extract cleanliness. This poster discusses the potential for a single extraction protocol for various drugs of abuse classes prior to UPLC-MS/MS analysis.

## Experimental

### Reagents

Drug standards were purchased from LGC Standards (Teddington, UK). Ammonium hydroxide, ammonium acetate, formic acid, hydrochloric acid were purchased from Sigma-Aldrich (Dorset, UK). Blank whole blood was purchased from Sera Labs International (Sussex, UK). All solvents were HPLC grade from Honeywell UK and Milli-Q (Merck Millipore, Germany) water was used throughout.

### Sample Preparation

ISOLUTE<sup>®</sup> SLE+ Procedure (Figure 1.)

Columns: ISOLUTE<sup>®</sup> SLE+ 1 mL capacity 'C' columns; 820-0140-C.

#### Matrix Pre-treatment:

500  $\mu$ L whole blood was mixed with 10  $\mu$ L internal standard solution and allowed to bind for 1 hour. 500  $\mu$ L 0.1% NH<sub>4</sub>OH (aq) was added prior to vortex mixing.

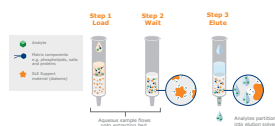
#### Sample Application:

750  $\mu$ L of pre-treated whole blood was applied to columns and flow was initiated with positive pressure at 5 psi (3-5 seconds).

#### Analyte Extraction:

DCM/IPA (95/5, v/v, 2.5 mL) was applied and allowed to flow under gravity for 5 minutes. MTBE (2.5 mL) was applied and allowed to flow under gravity for 5 minutes, followed by a further MTBE aliquot (2.5 mL). A pulse of positive pressure at 10 psi (10-20 seconds) allowed complete removal of the final aliquot.

**Figure 1.** Schematic of ISOLUTE<sup>®</sup> SLE+ Supported Liquid Extraction Procedure.



#### Post Extraction:

Extracts were evaporated at 40 °C in the presence of 100  $\mu$ L of 50 mM HCl in MeOH in order to avoid loss of amphetamines.

### Manual Sample Preparation

All extraction protocols were developed using a semi-automated 48 position positive pressure manifold.

### Biotage<sup>®</sup> Extrahera<sup>™</sup> Automated Sample Preparation Platform

The optimized extraction protocols were transferred to an automated sample preparation platform, equipped with an 8 channel pipetting head and positive pressure processing functionality. The system is interconvertible between 4 and 8 channel pipetting into 24 (6 x 4 arrangement) columns or 96-well plates, respectively. The Extrahera<sup>™</sup> platform is shown in Figure 2.



**Figure 2.** Biotage<sup>®</sup> Extrahera<sup>™</sup> automated sample preparation platform.

### LC/MS Conditions

**Instrument:** Waters Acquity UPLC interfaced via electrospray ionization to a Quattro Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK). Positive ions were acquired in the multiple reaction monitoring (MRM) mode. **Column:** Restek Raptor<sup>™</sup> Biphenyl 2.7  $\mu$ m (100 x 2.1 mm) with EXP guard cartridge (Thames Restek UK Ltd., Saunderton, UK.) **Mobile Phase A:** 2 mM Ammonium Acetate (aq) 0.1% formic acid **Mobile Phase B:** 2 mM Ammonium Acetate (MeOH), 0.1% formic acid **Flow Rate:** 0.4 mL/min

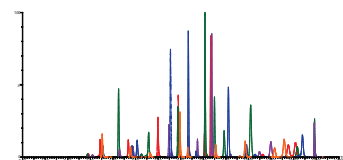
**Gradient and MRM transitions:** Details on Biotage.com

**Dissolution Temp:** 450 °C

**Ion Source Temp:** 150 °C

## Results

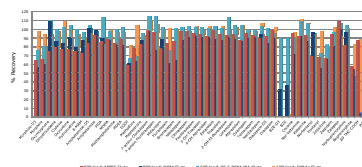
The chromatography as delivered using the Restek Raptor<sup>™</sup> Biphenyl core shell HPLC column is shown in Figure 3.



**Figure 3.** Representative chromatogram.

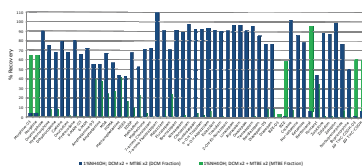
Method development was performed pre-treating whole blood 1:1 with various aqueous buffers for pH control and extraction using the ISOLUTE<sup>®</sup> SLE+ 1 mL sample volume format. Sample loading experiments demonstrated sub-optimal extract cleanliness when volumes in excess of 750  $\mu$ L were used. When moving to a 400  $\mu$ L column for extraction the loading volume scaled to 300  $\mu$ L. Previous investigation has shown NH<sub>4</sub>OH (aq) to be an effective pre-treatment for the extraction of basic drugs of abuse. However, a significant objective in this work was to achieve concomitant extraction of the THC-COOH metabolite.

**Figure 4.** demonstrates analyte recovery testing when pre-treating whole blood with 1% NH<sub>4</sub>OH aq solution and screening various elution solvents. Noticeably BZE performs better using DCM elution solvent combinations. However, THC-COOH requires extraction with MTBE or EtOAc.



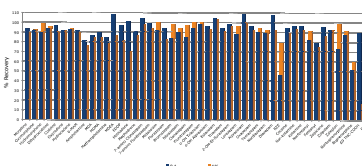
**Figure 4.** Recovery chart comparing elution solvent screening.

Due to the necessity for different extraction conditions we evaluated elution using multiple solvents. **Figure 5.** demonstrates subsequent extraction using DCM followed by MTBE. Both fractions were analysed separately. The disparity in the recovery of certain analytes illustrates the necessity for multiple solvents for broad panel drug screening.



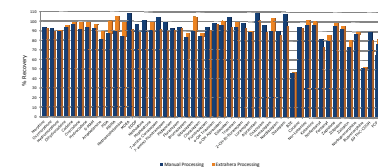
**Figure 5.** Recovery chart pre-treatment using 1% NH<sub>4</sub>OH and extraction with 2x DCM followed by 2x MTBE, in isolated fractions.

At 1% concentration, while the majority of analytes partition very well into the extraction solvents, THC-COOH recovery is inadequate. **Figure 6.** demonstrates that lowering the NH<sub>4</sub>OH (aq) to 0.1% provided significant improvement to THC-COOH with minimal recovery and sensitivity tradeoff to other analytes.



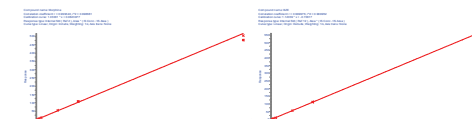
**Figure 6.** Recovery chart comparing 0.1% and 1% NH<sub>4</sub>OH pre-treatment.

Final elution combination was optimized to 1 aliquot of 95/5 DCM/IPA followed by two MTBE aliquots. The final method was adapted to the Extrahera sample preparation platform. **Figure 7.** demonstrate recovery profiles comparing manual and automated processing. The majority of analytes are greater than 70% for both processing methods, with the exception of BZE and buprenorphine. Lower values for these two analytes did not impact sensitivity at the lowest concentration levels. RSDs were below 10% for all analytes with the Extrahera demonstrating lower values for most analytes.



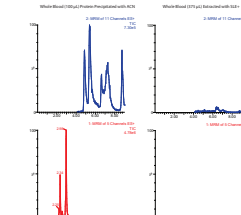
**Figure 7.** Recovery chart comparing manual processing with the Extrahera automated platform.

Calibration curves were constructed from 1-500 ng/mL. **Figures 8 and 9.** demonstrate calibration curves for morphine and BZE following manual and Extrahera processing respectively. Quadratic function was observed at high concentrations for a number of analytes. However, dilution and internal standards helped and ultimately demonstrated excellent linearity and coefficients of determination ( $r^2 > 0.99$ ) for all analytes. All LOQ values were 200pg/mL or below with the exception of THC-COOH (5 ng/mL).



**Figures 8-9.** Calibration curves for morphine (manual processing) and BZE (automated processing) respectively.

Extract cleanliness in terms of phospholipid removal was also investigated. **Figure 10.** demonstrates phospholipid content in extracted samples. The left trace corresponds to 100  $\mu$ L of whole blood precipitated with acetonitrile, compared to 375  $\mu$ L of whole blood extracted using the final SLE+ extraction protocol on the right.



**Figure 10.** TICs showing phospholipid content in the final extracts.

## Conclusions

- » This poster demonstrates a simple extraction protocol capable of recovering a broad range of drugs of abuse from whole blood while delivering excellent extract cleanliness and low limits of quantitation.
- » We also demonstrate the performance of a novel sample preparation system capable of processing 24-column or 96-well formats.