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Extraction of Opiates from Whole Blood Using ISOLUTE® SLE+ Prior to GC/MS Analysis

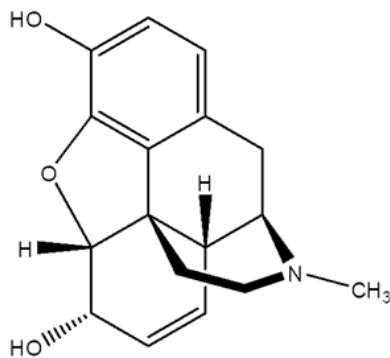


Figure 1. Structure of Morphine

Introduction

This application note describes the extraction of opiate compounds from whole blood, prior to GC/MS analysis. This protocol also allows the simultaneous extraction of various other drugs of abuse classes: amphetamines, barbiturates, benzodiazepines and cocaine

ISOLUTE® SLE+ columns with 1 mL sample capacity are used to extract whole blood samples following a straightforward sample dilution. No protein precipitation or other pre-treatment is required prior to sample loading. The sample preparation procedure delivers clean extracts, good recoveries and RSD values and LLOQs from 50 ng/mL (analyte dependant).

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

Dihydrocodeine, hydrocodone, codeine, hydromorphone, oxycodone, morphine-D₃, morphine, 6-monoacetylmorphine (6-MAM)-D₃, 6-MAM, oxymorphone.

Sample Preparation Procedure

Format:

ISOLUTE® SLE+ 1mL Sample Volume column, part number 820-0140-C

Sample Pre-treatment

To 1 mL of whole blood, add 10 µL of ISTD (total 100 ng/mL). Allow to equilibrate and add 1 mL of 1% ammonium hydroxide (aq). Vortex.

Sample Loading

Load 750 µL of the pre-treated whole blood onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Analyte Extraction

Apply dichloromethane (DCM, 2.5 mL) and allow to flow under gravity for 5 minutes. Collect in an appropriate glass tube.

Apply a second aliquot of DCM* (2.5 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent into the collection vessel.

*Note that MTBE can be used as an alternative extraction solvent if a non-chlorinated option is required. MTBE also suitable for extraction of other analyte classes (amphetamines, benzodiazepines, barbiturates). If simultaneous extraction of cocaine and its BZE metabolite is required, DCM should be used as extraction solvent.

Post Elution and Reconstitution

Evaporate the extract in a stream of air or nitrogen using a TurboVap® LV (ambient, 20 to 40 L/min).

Reconstitute the extracts with ethyl acetate (250 µL) and vortex for 20 seconds before transferring to high recovery GC vials. Evaporate the extract in a stream of air or nitrogen using a Biotage® SPE Dry (40 °C, 20 to 40 L/min).

Reconstitute extracts with ethyl acetate (40 µL) and BSTFA (with 1% t-BDMCS) (40 µL), vortex and heat on a block for 30 minutes at 70 °C to complete derivatization.

GC Conditions

Instrument

Agilent 7890A with QuickSwap

Column

Agilent J&W DB-5, 30 m x 0.25 mm ID x 0.25 µm

Carrier

Helium 1.2 mL/min (constant flow)

Inlet

250 °C, Splitless, purge flow: 50 mL/min at 1.0 min

Injection

1 µL

Wash Solvents

Acetone & ethyl acetate

Oven

Initial temperature 80 °C, hold for 1 minute, ramp 10 °C/min to 273 °C

Post Run

Backflush for 1.6 minutes (2 void volumes)

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	Qual Ion 1	Qual Ion 2
1	Dihydrocodeine	373	146	
2	Hydrocodone	178	371	196
2	Codeine	115	299	242
3	Hydromorphone	357	300	
3	Oxycodone	372	273	212
3	Morphine-D3	432	239	
3	Morphine	429	236	
4	6-MAM-D3	402	343	
4	6-MAM	399	340	
5	Oxymorphone	445	288	

Results

Blank whole blood was spiked at 100 ng/mL for recovery testing; the typical recovery data is shown in **Figure 2**. Both MTBE and DCM protocols gave reproducible data with RSD values <10%.

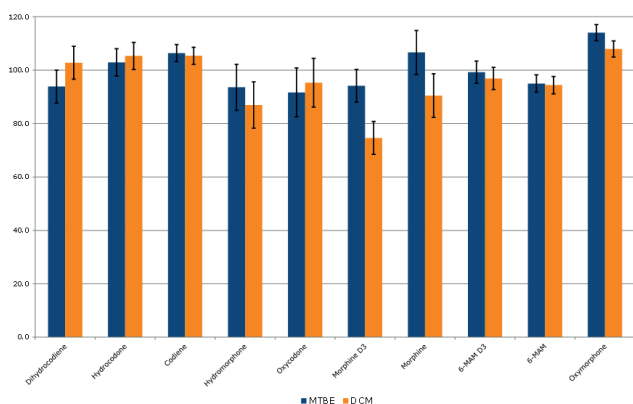


Figure 2. Typical opiate recoveries using MTBE or DCM as extraction solvent.

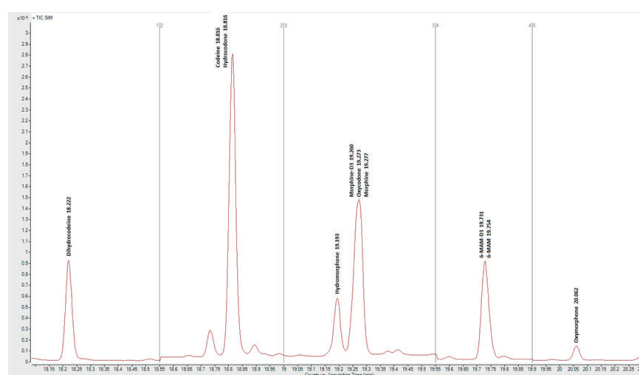


Figure 3. Total Ion Chromatogram of opiates at 100 ng/mL using the DCM extraction protocol.

Calibration Curves

Whole blood was spiked prior to extraction, at concentrations of 10, 20, 50, 75, 100, 200 and 500 ng/mL for each analyte to create calibration curves. Morphine-D₃ and 6-MAM-D₃ were spiked at 100 ng/mL for each level. The curves are shown in **Figure 4**.

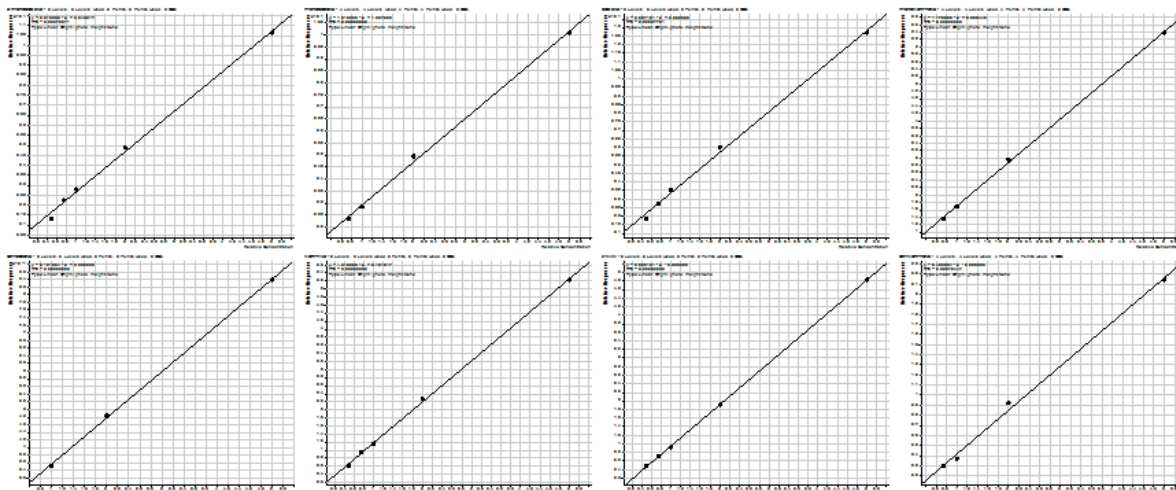


Figure 4. Linearity demonstrating coefficient of determination (r^2) values between 0.9967 and 0.9998 for the opiates using the DCM protocol.

Table 3.
Lower Limits of Quantitation (LLOQ) using ISOLUTE® SLE+ procedure

Analyte	DCM LLOQ (ng/mL)	MTBE LLOQ (ng/mL)
Dihydrocodeine	50	50
Hydrocodone	75	100
Codeine	50	50
Hydromorphone	75	75
Oxycodone	100	100
Morphine	50	50
6-MAM	50	50
Oxymorphone	75	100

Additional Notes

Solvents and reagent preparation:

- » All solvents were HPLC grade.
- » 1% ammonium hydroxide (aq): Add concentrated ammonium hydroxide (28-30%) (1 mL) to HPLC grade water (99 mL).

Column loading: ISOLUTE® SLE+ columns are underloaded (750 μ L sample on a 1 mL capacity column) to avoid breakthrough of whole blood matrix.

Non-chlorinated extraction solvent alternative: MTBE can be used as an alternative extraction solvent if a non-chlorinated option is required. MTBE also suitable for extraction of other analyte classes (amphetamines, benzodiazepines, barbiturates). If simultaneous extraction of cocaine and its BZE metabolite is required, DCM should be used as extraction solvent.

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Column*	30
820-0140-CG	ISOLUTE SLE+ 1 mL Sample Volume Column (tabless)	30
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	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

*ISOLUTE SLE+ 1 mL Sample Volume columns are available in the tabless (or flangeless) format for compatibility with the Biotage® Extrahera™ and other sample processing platforms. Bulk packs are also available, visit www.biotage.com for further information.

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