Extraction of a Range of Opiates and Metabolites From Human Urine Using ISOLUTE® SLE+ Columns prior to GC-MS Analysis

Introduction

This application note describes the supported liquid extraction clean-up of a wide range of opiates and metabolites from urine prior to quantitative GC-MS analysis.

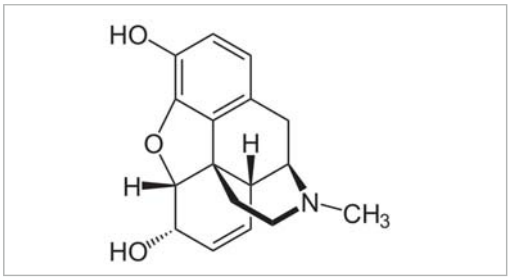

Figure 1. Structure of Morphine

This application note demonstrates an effective and efficient supported liquid extraction protocol for the clean-up and concentration of a range of forensically significant opiates and their metabolites.

Analyte recoveries achieved using this method ranged from 70-99% with RSDs below 5% for all analytes.

ISOLUTE SLE+ Supported Liquid Extraction 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 time.

Analytes

Dihydrocodeine, Oxycodone, Oxymorphone, Codeine, Morphine, Hydrocodone, Hydromorphone and 6-monoacetylmorphine (6-MAM).

Sample Preparation Procedure

Sample pre-treatment: Dilute urine (1 mL) with 100 mM ammonium acetate pH 5 (950 µL) and β-Glucuronidase enzyme (~4500 AU) (50 µL). Hydrolyze with heat in a water bath at 60 °C for 2 hours. Cool and add 25% ammonium hydroxide in water (10µL) and vortex.

(i) If urine volume is more than 1 mL, an extra 50 µL of enzyme is required for every extra 1 mL of urine to achieve required activity.

(ii) To achieve desired pH after hydrolysis, an extra 10 µL of 25% ammonium hydroxide in water is required for every extra 1 mL of urine.

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

Sample loading: Load the pre-treated sample (1 mL) onto the column and apply a pulse of vacuum (VacMaster 20 Sample Processing Manifold, **121-2016**) or positive pressure (PRESSURE+ 48 Positive Pressure Manifold **PPM-48**) to initiate flow. Allow the sample to adsorb for 5 minutes.

Analyte extraction: Apply dichloromethane:isopropanol (95:5, v/v) (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a second aliquot of dichloromethane:isopropanol (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: Evaporate the extract to dryness (40 ºC). Reconstitute in ethyl acetate (500 µL) and transfer to high recovery glass vial. Evaporate the extract to dryness (40 ºC). Derivatize with BSTFA:TMCS (99:1, v/v) (20 µL) and dichloromethane/Isopropanol (95:5, v/v) (20 µL). Seal with non-split caps and heat in a heating block at 70 ºC for 30 minutes.

GC Conditions

Instrument: Agilent 7890AC GC

Column: SGE capillary column; 30 m x 0.25 mm ID-BPX5 x 0.25 µm,

Carrier: Helium 1.2 mL/min (constant flow),

Inlet: 250 °C, Split (ratio 10:1), 12 mL/min,

Septum purge flow: 3 mL/min,

Injection: 1 µL, wash solvents: acetone and ethyl acetate,

Oven: 80 °C initial , 10 °C/min to 280 °C then 50 °C/min to 330 °C, hold 5 min,

Transfer Line: 280 °C

Mass Spectrometry Conditions

Instrument: Agilent 5975C MSD

Source: 230 °C

Quadrupole: 150 °C

Solvent Delay: 17 min

MSD mode: SIM

SIM Groups: 1 – 17.00 min to 19.20 min

2 – 19.20 min to 19.50 min

3 – 19.50 min to 19.78 min

4 – 19.78 min to 20.10 min

5 – 20.10 min to 22.00 min

Table 1. SIM Parameters

SIM Group	Analyte	Quant Ion	1st Qual Ion	2nd Qual Ion	Dwell (ms)
1	dihydrocodeine-TMS	146.1	373.3	-	75
1	oxycodone	315.2	230.1	-	75
1	oxymorphone-diTMS	373.2	288.1	-	75
2	codeine-TMS	371.3	178.1	196.1	75
3	morphine-d3-diTMS	432.2	239.1	417.2	40
3	morphine-diTMS	429.3	414.2	236.1	40
3	hydrocodone	299.1	242.1	115.0	40
4	hydromorphone-TMS	357.2	300.1	243.1	75
5	6-MAM-d3-TMS	402.3	343.2	290.1	50
5	6-MAM-TMS	399.2	340.2	-	50

Results

This SLE+ protocol demonstrates analyte recoveries ranges from 70-99% as shown in figure 2. RSDs were all below 5% for all analytes. Robustness testing was carried out across three days using three different sources of urine.

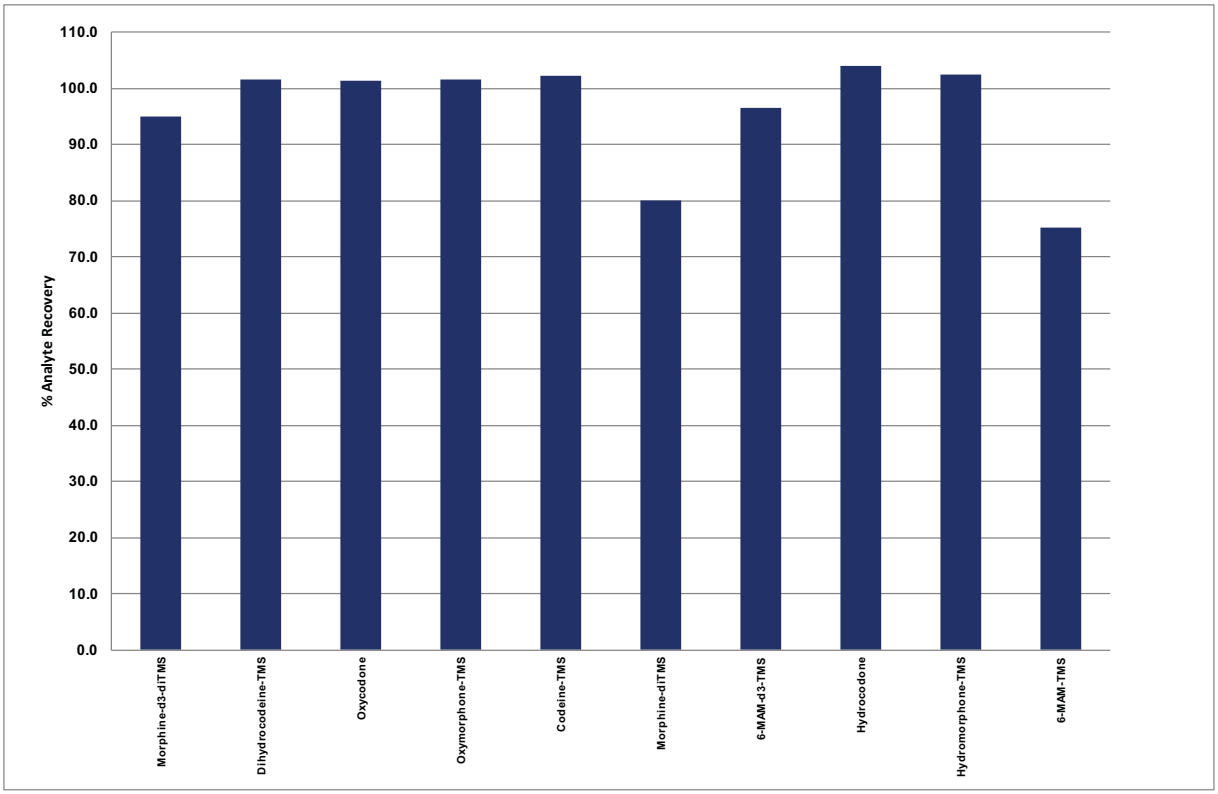

Figure 2. Typical analyte % recoveries for a range of extracted Opiates (n=7) using the ISOLUTE SLE+ protocol

Typical extractions showed limits of quantitation ranging from 2-200 ng/ml dependent upon analyte and required detection limit as shown in table 2. Figure 3 shows the chromatogram for the full range of extracted opiates zoomed in at a concentration range of 1000ng/mL.

Table 2. Limits of Quantitation for extracted opiates using the SLE+ protocol

Analyte	LOQ (ng/mL)
Dihydrocodeine	40
Oxycodone	200
Oxymorphone	200
Codeine	40
Morphine	100
Hydrocodone	200
Hydromorphone	200
6-monoacetylmorphine (6-MAM)	2

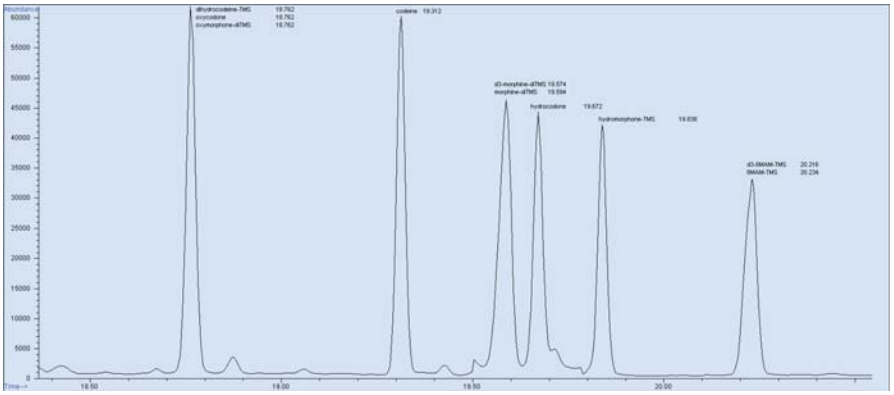

Figure 3. Zoomed chromatogram showing extracted opiate analytes at 1000 ng/mL

Ordering information

Part number	Description	Quantity
820-0140-C	ISOLUTE SLE+ 1 mL Sample Volume column	30
PPM-48	PRESSURE+48 Positive Pressure Manifold	1
121-2016	VacMaster 20 Sample Processing Manifold	1

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