Extraction of Opiates From Human Urine Using ISOLUTE® SLE+ 96-Well Plates and Columns

Introduction

This application note describes the extraction of a range of opiates from human urine using ISOLUTE SLE+ supported liquid extraction plates (96-well) and columns with LC-MS/MS analysis.

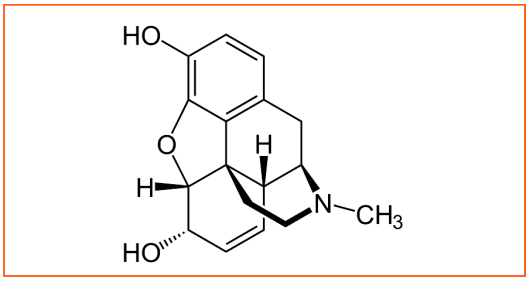

Figure 1. Structure of Morphine

This method describes the use of ISOLUTE SLE+ supported liquid extraction plates (96-well) and 1 mL sample volume columns to extract a range of opiates including opiate treatment analytes (methadone and its metabolite EDDP)) from human urine. This simplified and efficient extraction method has significant analyte recoveries ranging from 70-102% with LOQs as low as 500 pg/mL.

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 time.

Analytes

Morphine, codeine, oxycodone, 6-acetylmorphine, dihydrocodeine, hydromorphone, hydrocodone, oxymorphone. methadone and its metabolite EDDP.

ISOLUTE SLE+ procedure

Sample Pre-treatment: To 1 mL of urine add 100mM ammonium acetate pH5 (950 µL) and β-glucuronidase (50µL) enzyme (5000 u/mL). Hydrolyze with heat in a water bath at 60°C for 2 hours. Cool and add 25% aqueous ammonium hydroxide (10µL) and mix.

ISOLUTE SLE+ 200 Supported Liquid Extraction plate, part number 820-0200-P01

Sample Load: Load pre-treated sample (200 µL) to plate followed by a pulse of vacuum to initiate flow and leave for five minutes.

Analyte Elution: Elute with dichloromethane:isopropanol (95:5, v/v) (1 mL) . Leave to flow under gravity for 5 minutes, then apply short pulse of vacuum.

Post extraction: Evaporate to dryness at room temperature (80 L/min) and reconstitute in 0.1% formic acid (aq) (100 µL).

ISOLUTE SLE + 1 mL sample volume column, part number 820-0140-C

Sample Load: Load pre-treated sample (1 mL) to plate followed by a pulse of vacuum to initiate flow and leave for five minutes.

Analyte Elution: Elute with dichloromethane:isopropanol (95:5, v/v) (2.5 mL). Leave to flow under gravity for 5 minutes, then follow with a further aliquot of dichloromethane:isopropanol (95:5, v/v) (2.5 mL), apply a short pulse of vacuum.

Post extraction: Evaporate to dryness at room temperature (80 L/min) and reconstitute in 0.1% formic acid (aq) (500 µL).

Application Note

Reagents

0.1% Formic acid: Mix 1 mL formic acid (concentration 98%-100%) into 900 mL water, make up to 1 L with water.

Ammonium hydroxide: Mix 25 mL ammonium hydroxide (concentration 28-30%) with water (75 mL.

UPLC Conditions

Instrument: Waters Acquity UPLC interfaced to a Quattro Premier XE triple quadrupole MS using electrospray ionization.

Column: Acquity BEH C18 100 x 2.1mm x 1.7u.

Mobile phase: **A:** 0.1% formic acid (aq) **B:** 0.1% formic acid in methanol.

Flow rate: 0.40 mL/min.

Injection: 15µL (partial loop, needle overfill).

Sample Temperature: 20 °C.

Column Temperature: 40 °C.

Gradient:

Time (min)	% A	% B	Curve
Initial –1.20	80	20	-
1.20 - 2.30	30	70	11
2.30 - 4.00	80	20	11

Mass Spectrometry Conditions

Instrument: Premier XE triple quadrupole mass spectrometer equipped with an electrospray interface for mass analysis.

Source temp: 150 °C.

Desolvation temp: 450 °C.

Table 1. MRM transitions in positive mode for a range of opiates.

Scan function	Compound	MRM transition	Cone voltage	Collision energy (ev)
1	Morphine	286.2 > 201.0	42	25
2	Oxymorphone	302.2 > 198.1	34	37
3	Hydromorphone	286.2 > 185.1	44	29
4	Dihydrocodeine	302.2 > 199.1	42	33
5	Codeine	300.3 > 215.1	42	25
6	Oxycodone	316.2 > 241.2	34	27
7	Hydrocodone	300.2 > 199.1	46	33
8	6-Acetylmorphine	328.1 > 165.1	44	33
9	EDDP	278.2 > 234.2	26	30
10	Methadone	310.2 >265.2	26	15

Results

Figure 2 shows the mass chromatograms for all the extracted opiates and opiate treatment analytes spiked at 4 ng/mL. Figure 3 shows average analyte recoveries for the 96 fixed-well plate method with figure 4 showing the same range on the 1 mL sample volume column. LOQ for 96 fixed-well is 4 ng/mL with an LOD of 1 ng/mL and for the 1 mL column LOQ is 1 ng/mL with an LOD of half that value at 500 pg/mL, RSD were <10% for both formats.

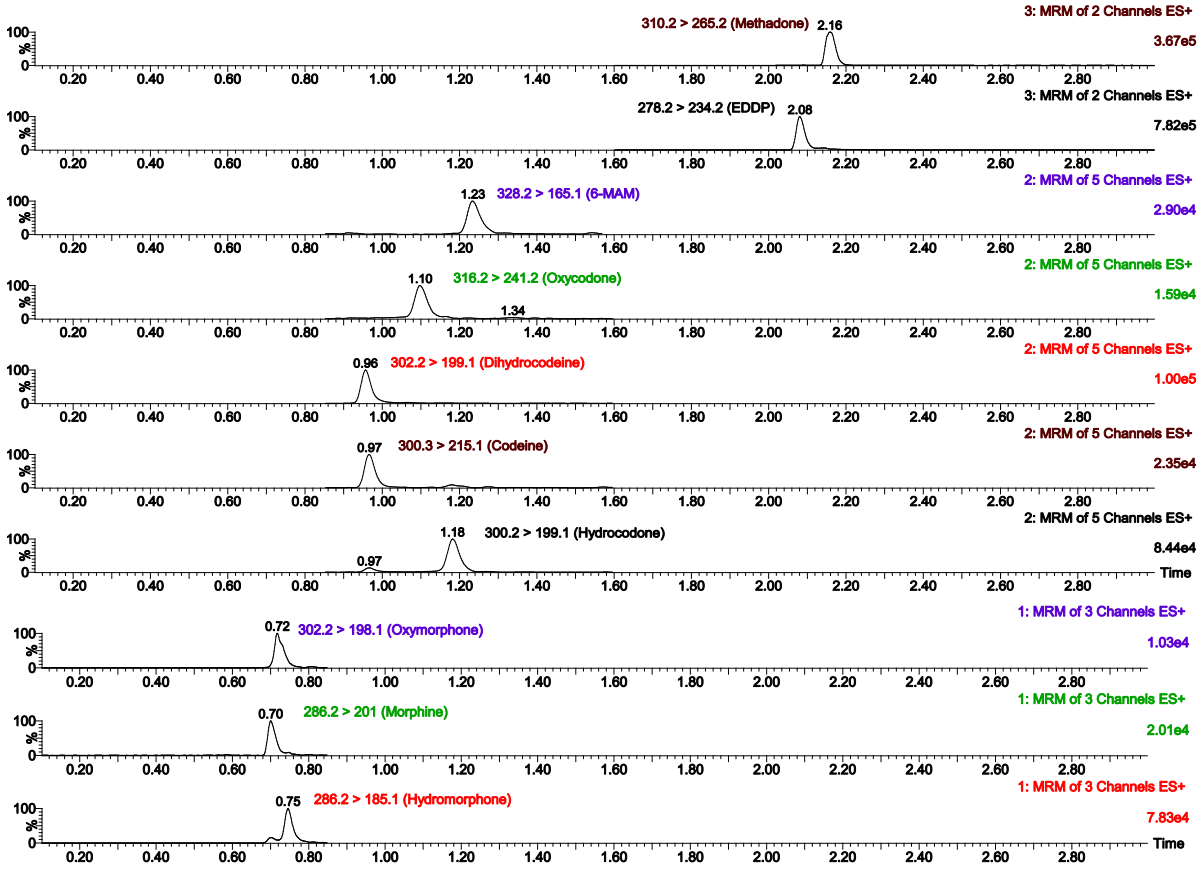

Figure 2. Typical mass chromatograms for all opiate and opiate treatment analytes at 4 ng/mL

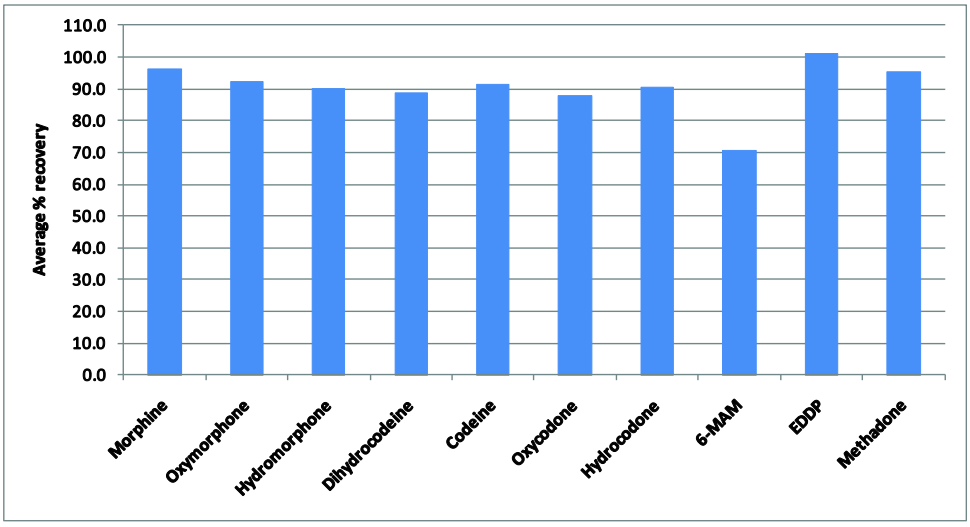

Figure 3. Average analyte recoveries of a range of opiate analytes on the 96 fixed-well plate at 4 ng/mL (n=7).

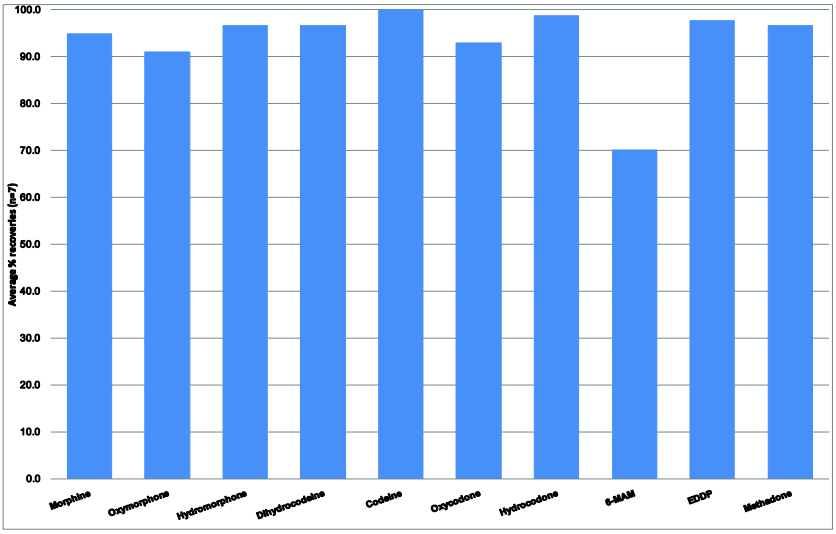

Figure 4. Average analyte recoveries of opiate analytes on 1 mL sample volume columns at 2 ng/mL (n=7)

Ordering information

Part number	Description	Quantity
820-0200-P01	ISOLUTE SLE+ 200 µL supported liquid extraction plate	1
820-0140-C	ISOLUTE SLE + 1 mL sample volume column	30
121-5203	Deep well collection plate 2 mL	50
121-9600	VacMaster-96 Sample processing manifold complete (without vacuum control)	1
SD2-9600-DHS-UK	SPE Dry 96 Dual, 240 V UK	1

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
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