Extraction of Pain Management Drugs from Urine using EVOLUTE® CX With the RapidTrace+ Automated SPE Workstation Prior to LC-MS-MS

Introduction

This application note describes the extraction of 21 different drugs in urine which are typically screened for pain management panels using EVOLUTE CX 100 mg/3 mL cartridges and automated using the RapidTrace workstation.

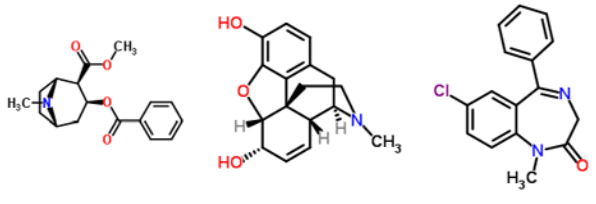

Figure 1. Structures of Cocaine, Morphine and Diazepam

The use of schedule I drugs for patient pain management therapy warrants constant monitoring of therapeutic levels in the patient. Screening patient urine samples for the free drugs is complicated by the metabolism process which converts the free drug to the β-glucuronide form. Patient urine samples can be enzymatically hydrolyzed to cleave glucuronide moiety and produce the free form of drug. The target analytes can them be extracted from the urine matrix using EVOLUTE CX solid phase extraction cartridges.

Analytes

Cocaine, Heroin, Morphine, Codeine, Oxymorphone, Hydromorphone, Oxycodone, Hydrocodone, Methadone, Clonazepam, Diazepam, Flunitrazepam, Oxazepam, Temazepam, Nitrazepam, Alprazolam, Fentanyl, Buprenorphine Meperidine, Naloxone, Naltrexone.

EVOLUTE CX mixed-mode resin-based SPE sorbent extracts a wide range of basic analytes from biological fluids and other aqueous matrices using a generic procedure which minimizes method development time. EVOLUTE CX removes matrix components such as proteins, salts, non-ionizable interferences and phospholipids, delivering cleaner extracts with reproducible recoveries for accurate quantitation.

EVOLUTE CX Procedure

EVOLUTE CX 100 mg/3 mL part number 611-0010-B

Urine Hydrolysis:	Add β-glucuronidase at a concentration of 5000 units/mL to urine and dilute sample 1:1 with 100 mM ammonium acetate (pH 5). Spike the matrix solution with internal standard. Incubate sample as per instructions with enzyme.
Sample Pre-treatment:	Dilute 400 µL of hydrolyzed urine with 400 µL of 0.01N HCL.
Column Conditioning:	Condition column with methanol (1 mL), water (1mL) and 0.01N HCL (1mL) **(F_r = 30mL/min)**
Sample loading:	Load pre-treated urine sample onto column **(F_r = 1mL/min):**
Interference Elution 1:	Wash column with water (2 mL) **(F_r = 5mL/min)**
Interference Elution 2:	Wash column with 0.01N HCl/ Isopropanol (80:20, v/v, 3 mL)
Interference Elution 3:	Wash column with water (2 mL). Dry column for 2 minutes.
Analyte Elution:	Elute drug analytes with Ethyl Acetate: Methanol: Ammonium Hydroxide (73:25:2, v/v, 2 x 1 mL) **(F_r = 1mL/min)**
Post Extraction:	Evaporate to dryness and reconstitute in mobile phase (500 µL)
Additional Information:	F_r = solvent flow rate , 1mL/min =10-12 drops/min

Application Note

RapidTrace Procedure

The SPE steps were loaded into the RapidTrace® software as follows:

No.	Step	Source	Destination	Volume (mL)	Flow (mL/min)
1	Purge-Cannula	Water	Aqueous Waste	6.0	40
2	Condition	Methanol	Organic Waste	1.0	30.0
3	Condition	Water	Aqueous Waste	1.0	30.0
4	Condition	0.01N HCl	Aqueous Waste	1.0	30.0
5	Load	Pre-treated Urine	Biological Waste	1.0	1.0
6	Rinse	Water	Aqueous Waste	2.0	5.0
7	Rinse	0.01N HCl/IPA	Organic Waste	3.0	5.0
8	Rinse	Water	Aqueous Waste	2.0	5.0
9	Dry	————	Time =	2	minutes
10	Collect	Ethyl Actate: MeOH: Amm Hydoxide	Fraction Collection 1	1.0	1.0
11	Collect	Ethyl Actate: MeOH: Amm Hydoxide	Fraction Collection 1	1.0	1.0

Reagent line	Reagent name	Sip speed (mL/min)
1	Water	40
2	Methanol	40
3	0.01N HCl/IPA (80:20)	40
4	0.01N HCl	40
5	Ethyl Acetate:Methanol:Amm Hydroxide (73:25:2)	40

HPLC Conditions

Instrument: Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK)

Column: **Biotage Resolux 200 C_4,** 4.5µm analytical column (150 x 2.1 mm id) (Biotage AB, Uppsala, Sweden)

Mobile Phase: **Solvent A**: 0.1% Ammonium Hydroxide/ 0.01%Formic Acid in Water
Solvent B: 0.01% Formic Acid in Acetonitrile

Gradient:

Step	Time	Flow Rate	%A	%B
1	0.0	500	80	20
2	0.50	500	80	20
3	4.0	500	0	100
4	6.0	500	0	100
5	7.0	500	80	20
6	10.0	500	80	20

Injection Volume: 5 µL

Temperature: 60 °C

Mass Spectrometry Conditions

Instrument: Applied Biosystems/MDS Sciex 4000 Q-Trap triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface for mass analysis.

Ion Source Temperature: 400 °C.

Table 1. MRM Transitions for drugs in positive mode Atmospheric Pressure Ionization (API).

Scan Function	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential
1	Cocaine	304>182	30	30	16
2	Heroin	370>165	30	30	16
3	Morphine	286>165	45	40	16
4	Codeine	300>199	30	40	16
5	Oxymorphone	302>227	30	30	16
6	Hydromorphone	286>185	30	35	16
7	Oxycodone	316>241	45	40	16
8	Hydrocodone	300>199	25	25	16
9	Methadone	310>265	30	30	16
10	Alprozolam	308.8>280.5	30	35	16
11	Clonazepam	315.8>269.8	30	30	16
12	Diazepam	284.9>154	30	30	16
13	Flunitrazepam	313.9>267.9	30	30	16
14	Oxazepam	288>242	30	40	16
15	Temazepam	300.9>255	30	30	16
16	Nitrazepam	282>180	40	40	16
17	Fentanyl	337>188	30	30	16
18	Buprenorphine	468>396	30	60	16
19	Meperidine	248>220	30	30	16
20	Naloxone	328>310	35	30	16
21	Naltrexone	342>323.8	30	40	16

Results

Urine samples (400 µL) were hydrolyzed with β-glucuronidase and spiked with a 21 drug mixture. The final spike concentration was ≤5 ng/mL for all of the analytes except fentanyl which was spiked at concentrations ≤0.5 ng/mL. The samples were incubated for 45 minutes at 37 °C. The samples were further diluted post incubation with the addition of 0.01 N HCl (400 µL).

Figure 2 shows a typical extracted ion chromatogram for the analysis of a 5ng/mL mixed solution of the drug panel in urine and extracted using EVOLUTE® CX cartridges. The target analyte retention times are shown in **Table 2**.

Figure 3 shows a plot of the average % recoveries for the drug panel spiked into human un-hydrolyzed urine at 5ng/mL for all of the drugs except fentanyl which was spiked into matrix at 0.5ng/mL. **Figure 4** shows the average % recoveries for the target analytes spiked at a concentration of 2.5 ng/mL into hydrolyzed urine. The average inter-run recoveries for the target analytes ranged from 75% to 118% with analyte intra-run RSDs <10%.

Results

Table 2. Table of observed retention times for drug screen panel. Targets were separated using the Biotage Resolux C_4 HPLC column.

Analyte	RT (mins)	Analyte	RT (mins)
Cocaine	5.35	Clonazepam	4.57
Heroin	5.75	Diazepam	4.99
Morphine	3.27	Flunitrazepam	4.72
Codeine	4.92	Oxazepam	4.52
Oxymorphone	3.81	Temazepam	4.77
Hydromorphone	4.15	Nitrazepam	4.46
Oxycodone	3.81	Fentanyl	5.51
Hydrocodone	4.92	Buprenorphine	5.93
Methadone	9.14	Meperidine	5.59
Alprazolam	4.75	Naloxone	4.62
		Naltrexone	4.86

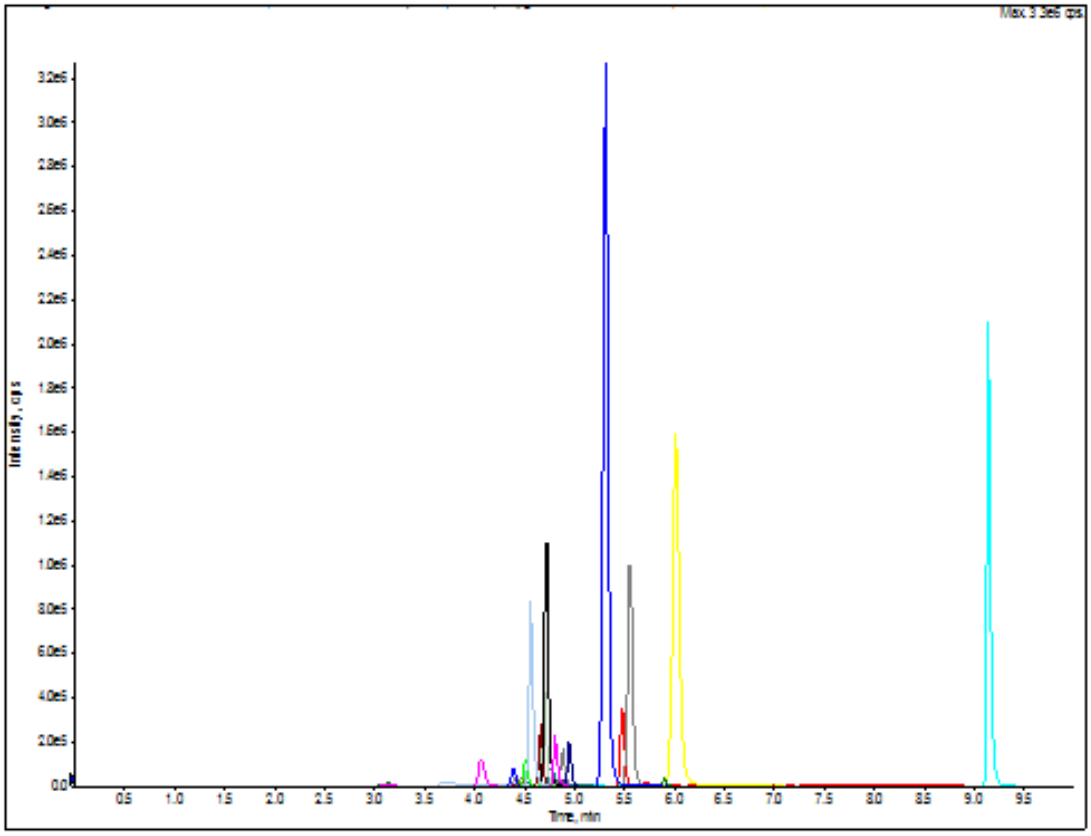

Figure 2. Typical extracted ion chromatogram for drug panel mixture spiked into urine at 5 ng/mL

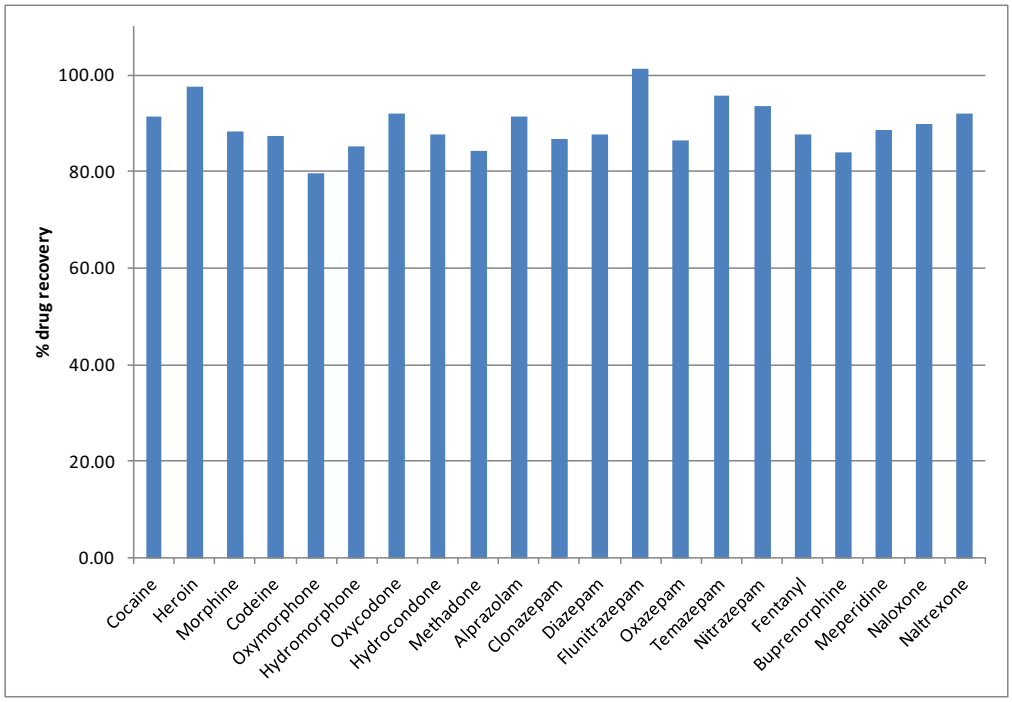

Figure 3. Plot of average % recoveries of drugs spiked into human urine and extracted on Evolute CX 100 mg/3mL cartridge using the Rapid Trace^{+} Automated SPE Workstation. All drugs were spiked at a concentration of 5 ng/mL except fentanyl which was spiked at 0.5 ng/mL.

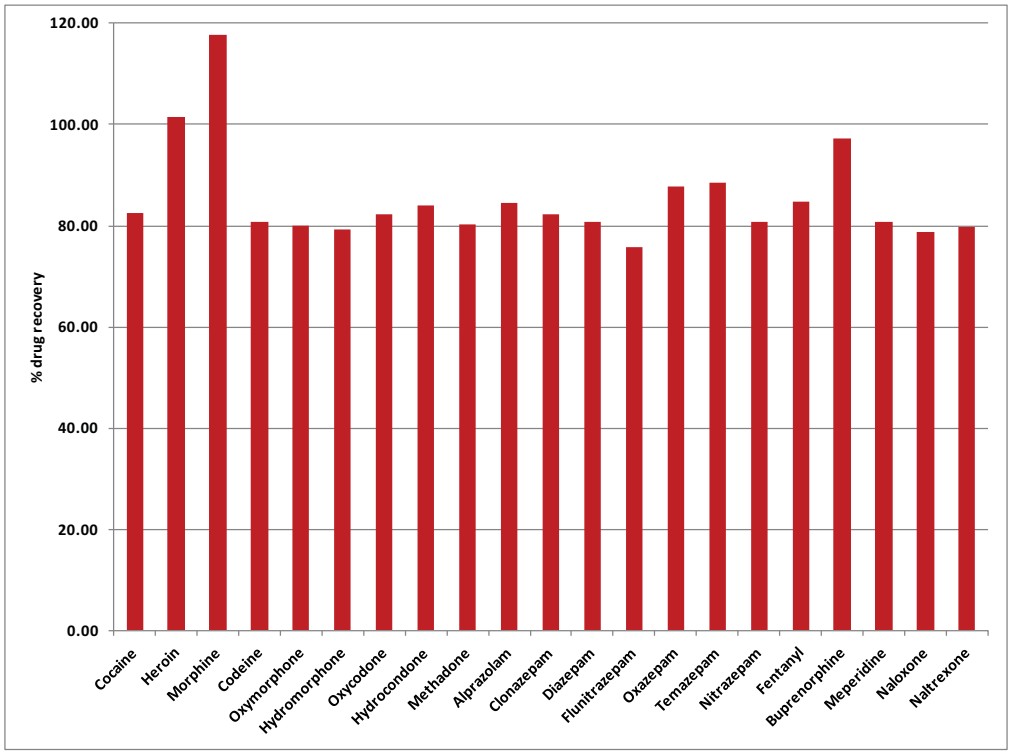

Figure 4. Plot of average % recoveries of drugs spiked into human urine hydrolyzed with β-glucuronidase and extracted on EVOLUTE® CX 3mL cartridges. All drugs were spiked at a concentration of 2.5 ng/mL except fentanyl which was spiked at 0.25 ng/mL.

Ordering information

Part number	Description	Quantity
611-0010-B	EVOLUTE CX, 100mg/ 3mL	50
C50000	RapidTrace+ Workstation, 1 mL and 3 mL	1
C103200	TurboVap LV, ASE, 40/60 mL vial, 110V	1
R2-1521-2045	Resolux 200 HPLC Column C4	1

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Order Fax: +46 18 56 57 05
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EU-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax:+81 3 5627 3121
jp_order@biotage.com
JP-1-pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
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