

APPLICATIONS

Analysis of Opiates from Urine Using Strata[®] Basic Screen Solid Phase Extraction (SPE) Large Reservoir Cartridges (LRC)

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Overview

In this application, SPE is used in combination with LC-MS/MS to analyze a panel of opiates from a urine sample containing β -Glucuronidase. The SPE method uses a 100% organic wash to maximize sample cleanliness, recovery values and relative standard deviation for analytes are shown in **Table 1**. All analytes tested show an absolute recovery of greater than 84% while all standard deviations are less than 10%. A representative TIC for an extracted sample is shown in **Figure 1**.

Materials

Standards were purchased from Cerilliant[®] (Round Rock, TX). Human urine was purchased from BioreclamationIVT[®] (Westbury, NY). All other reagents and chemicals were obtained from Sigma-Aldrich[®].

Experimental Conditions

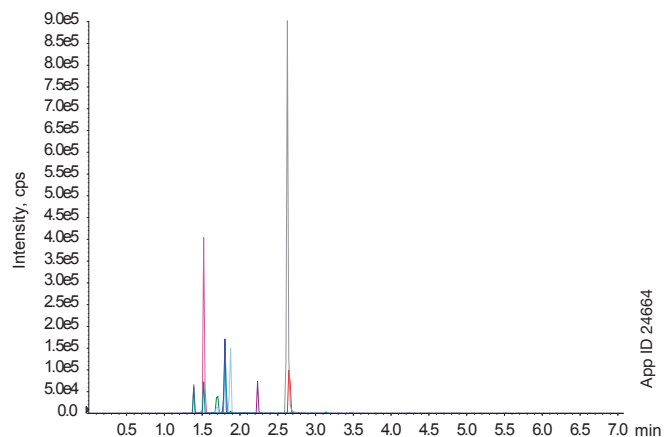
Pre-Treatment: In a single tube, combine 2 mL of urine with 2 mL 0.1% Formic acid in Water. Then add 100 μ L of Cambell β -Glucuronidase Enzyme (Part Number: DR2102) and 10 μ L of 1 μ g/mL Internal Standard and vortex for 3-5 seconds.

* Note these conditions are not optimized for urinary hydrolysis

Table 1.
Absolute Recovery Values

Analyte	% Recovery	% RSD (n=6)
6-MAM	90	7
Buprenorphine	106	6
Codeine	84	10
Fentanyl	105	5
Hydrocodone	79	8
Hydromorphone	98	6
Morphine	107	10
Norbuprenorphine	111	5
Oxycodone	99	5

Figure 1.
TIC of Extracted Analytes



SPE Protocol

- SPE Cartridge:** Strata Basic Screen 200 mg/10 mL LRC
- Part No.:** 8B-S327-FTH
- Condition:** 2.4 mL Methanol
- Equilibrate:** 2.4 mL 0.1% Formic acid in Water
- Load:** 4.1 mL Pre-treated Urine Sample
- Wash 1:** 2.4 mL 0.1% Formic acid in Water
- Wash 2:** 2.4 mL 100% Methanol
- Elute:** 2x 1.2 mL Ethyl acetate/IPA/Ammonium hydroxide (75:20:5)
- Dry:** Vacuum at 10" Hg for 5 minutes
- Reconstitute:** 200 μ L 0.1% Formic acid/Methanol (4:1) with deuterated internal standard

LC-MS/MS Conditions

- Column:** Kinetex[®] 2.6 μ m Biphenyl
- Dimensions:** 50 x 3.0 mm
- Part No.:** 00B-4622-Y0
- Mobile Phase:** A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Acetonitrile
- Gradient:**

Time (min)	% B
0	5
4	100
4.5	100
4.51	5
7.1	5
- Flow Rate:** 0.6 mL/min
- Injection Volume:** 5 μ L
- Detection:** MS/MS (SCIEX API 4000[™]), ESI+

Revision: 0

PHEN-RUO-00020

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APPLICATIONS

Ordering Information

Kinetex[®] Biphenyl Core-Shell LC Columns

Kinetex 2.6 µm MidBore [™] Columns (mm)	SecurityGuard [™] ULTRA Cartridges [†]			
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Biphenyl	00B-4622-YO	00D-4622-YO	00F-4622-YO	AJ0-9208 for 3.0 mm ID

[†] SecurityGuard ULTRA Cartridges required holder, Part No.: AJ0-9000.

Other particle sizes and dimensions are available. Visit www.phenomenex.com/Kinetex for a complete list of available columns.

Strata[®] Basic Screen SPE

Strata Basic Screen			
Format	Sorbent Mass	Part Number	Unit
Large Reservoir Cartridge	200 mg/10 mL	8B-S327-FTH	30/box

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