

In-well Hydrolysis and Clean-up of 39 Pain Management Drugs using Strata®-X-Drug B Plus SPE and Analysis with a Kinetex® Biphenyl Column

Shahana Huq
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

Overview

In this application, Strata-X-Drug B Plus Solid Phase Extraction (SPE) is used for an easy clean up solution for the urine sample matrix. It uses an in-well beta-glucuronidase hydrolysis to save time and transfer steps. The SPE method does not require conditioning or equilibrations steps for additional times savings. When combined with a core-shell Kinetex Biphenyl column, recoveries are between 71-112% and good separation of analytes.

Sample Preparation Protocol

Sample Pretreatment: Combine 200 µL urine sample spiked with 40 µL internal standard (500 ng/mL), 60 µL hydrolysis buffer, 20 µL of IMCSzyme® RT enzyme (Part No.:04-RTB-030), on the Strata-X-Drug B Plus 30 mg plate (Part No.: [8E-S128-TGB-P](#)). Incubate at room temperature for 15 minutes.

Load: Add 200 µL 0.1 % Formic acid in Water to the plate

Wash 1: 1 mL of 0.1 % Formic acid in Water

Wash 2: 1 mL of Water/Methanol (70:30)

Dry: 5 minutes at high vacuum (15-20" Hg)

Elute: 2x 0.5 mL Ethyl acetate/Isopropanol/Ammonium hydroxide (7:2:1)

Dry Down: Under gentle stream of Nitrogen at 40-45°C

Reconstitute: 200 µL initial mobile phase

LC Conditions

Column: Kinetex 2.6 µm 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 Methanol

Gradient:	Time	%B
	0	15
	3.5	95
	5	95
	5.01	15
	7	15

Flow Rate: 0.5 mL/min

Temperature: Ambient

Injection: 5 µL

Detection: SCIEX® 4500 MS/MS (ESI+)

System: Agilent® Technologies 1200 Series

Figure 1. % Recoveries and CVs for 39 Pain Management Drugs using an In-well Hydrolysis SPE Product

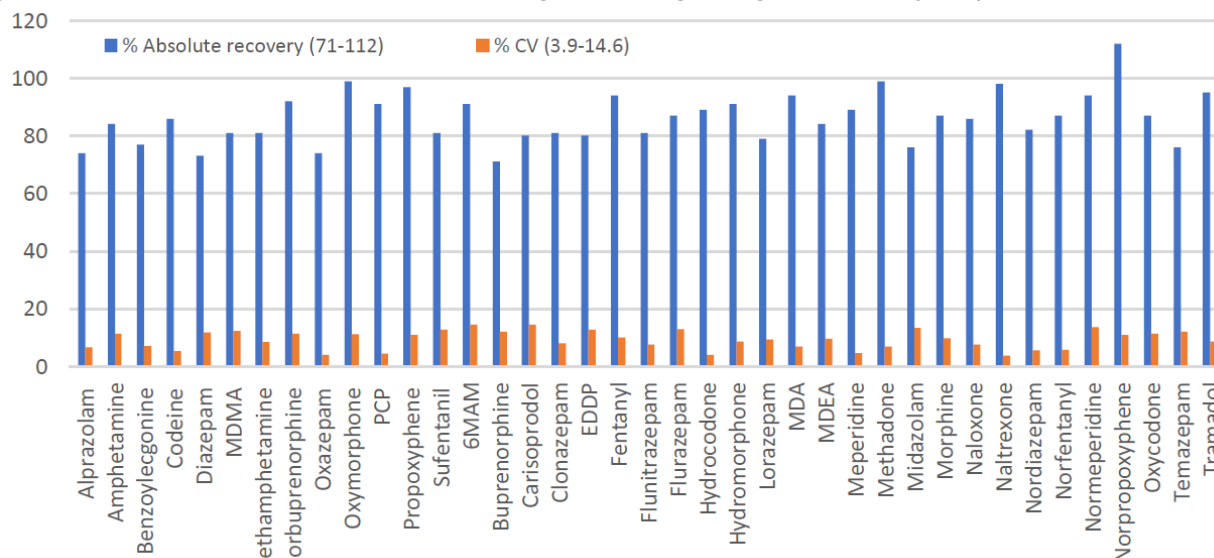


Figure 2. Separation of 39 Pain Panel Analytes Using SPE with In-well Hydrolysis

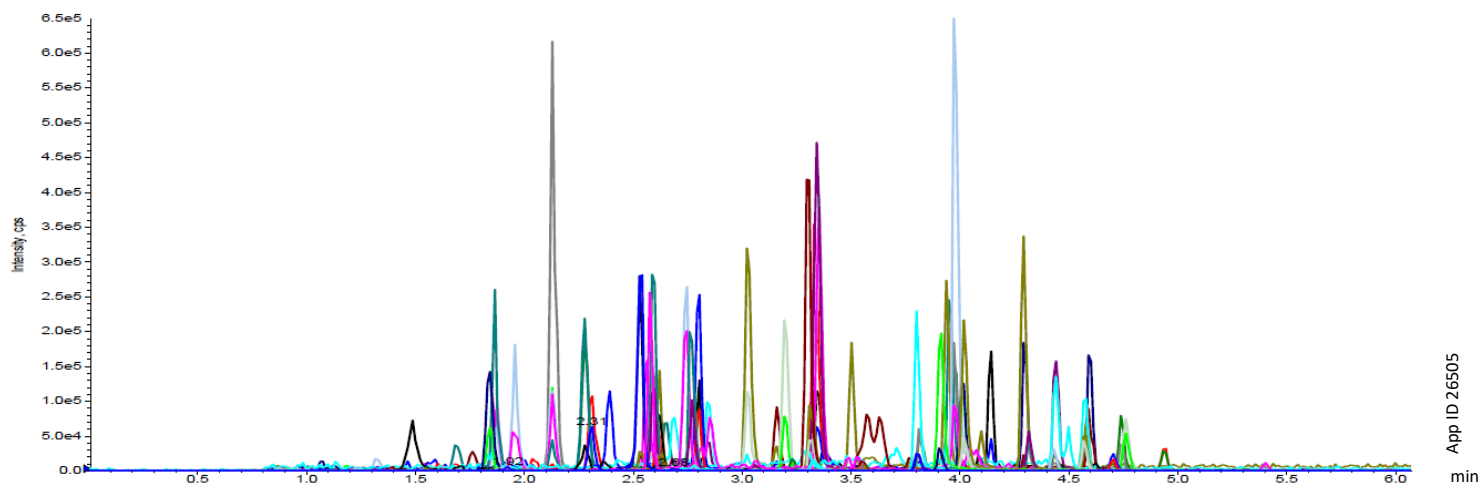


Table 1: Analyte Details for 39 Pain Management Drugs Extracted from Urine

Peak ID	Analyte Name	RT (min)	Q1	Q3
1	Alprazolam	4.8	309.1	281.1
2	Amphetamine	2.3	136.1	91.1
3	Benzoyllecgonine	3.3	290.1	168.1
4	Codeine	2.6	300.2	152.1
5	Diazepam	4.9	285	193.2
6	MDMA	2.9	194.1	105.1
7	Methamphetamine	2.6	150.1	91
8	Norbuprenorphine	3.6	414.3	83.2
9	Oxazepam	4.4	287	241
10	Oxymorphone	2	302.1	227
11	PCP	4	244.3	91
12	Propoxyphene	4	340.3	266.3
13	Sufentanil	4.1	387.2	238.1
14	6MAM	2.57	328.1	165.1
15	Buprenorphine	3.9	468.3	55.2
16	Carisoprodol	3.9	261.1	176.2
17	Clonazepam	4.4	316.1	270.1
18	EDDP	4.2	278.2	234.2
19	Fentanyl	3.9	337.3	105.1

Peak ID	Analyte Name	RT (min)	Q1	Q3
21	Flurazepam	4	388.2	315.2
22	Hydrocodone	2.8	300.2	199
23	Hydromorphone	2.1	286.1	185.1
24	Lorazepam	4.3	321	275
25	MDA	2.7	180.1	133
26	MDEA	3	208.2	163
27	Meperidine	3.4	248.2	220.2
28	Methadone	4.4	310	265
29	Midazolam	4.1	326.1	291.1
30	Morphine	1.9	286.1	152.1
31	Naloxone	2.56	328.2	212
32	Naltrexone	2.8	342.2	267.1
33	Nordiazepam	4.64	271	140
34	Norfentanyl	3.2	233.2	84.1
35	Normeperidine	3.4	234.1	160.1
36	Norpropoxyphene	4.1	308.2	100.1
37	Oxycodone	2.8	316.1	241.2
38	Temazepam	4.7	301.1	255.1
39	Tramadol	3.2	264.1	58.1



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Australia

t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
info@phenomenex.com

China

t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic

t: +420 272 017 077
cz-info@phenomenex.com

Denmark

t: +45 4824 8048
nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong

t: +852 6012 8162
hkinfo@phenomenex.com

India

t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy

t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway

t: +47 810 02 005
nordicinfo@phenomenex.com

Poland

t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal

t: +351 221 450 488
ptinfo@phenomenex.com

Singapore

t: +65 800-852-3944
sginfo@phenomenex.com

Slovakia

t: +420 272 017 077
sk-info@phenomenex.com

Spain

t: +34 91-413-8613
espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland

t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan

t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand

t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
info@phenomenex.com

🌐 **All other countries/regions**
Corporate Office USA
t: +1 (310) 212-0555
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