

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

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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 time savings. When combined with a core-shell Kinetex Biphenyl column, absolute 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, mix/vortex for a minute

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

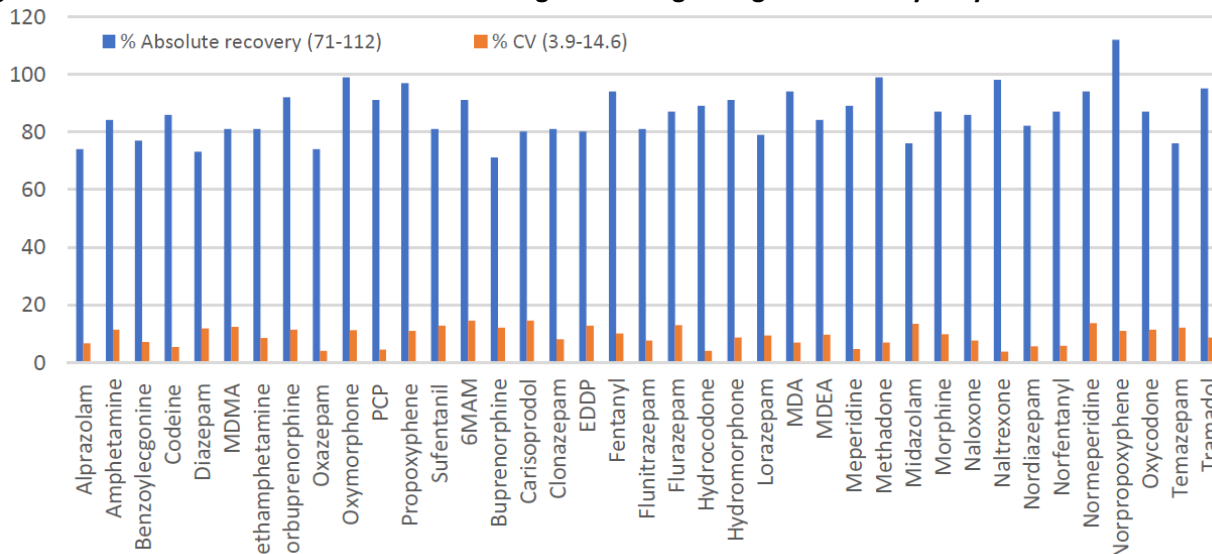


Figure 2. Separation of 39 Pain Panel Analytes Using SPE with In-well Hydrolysis

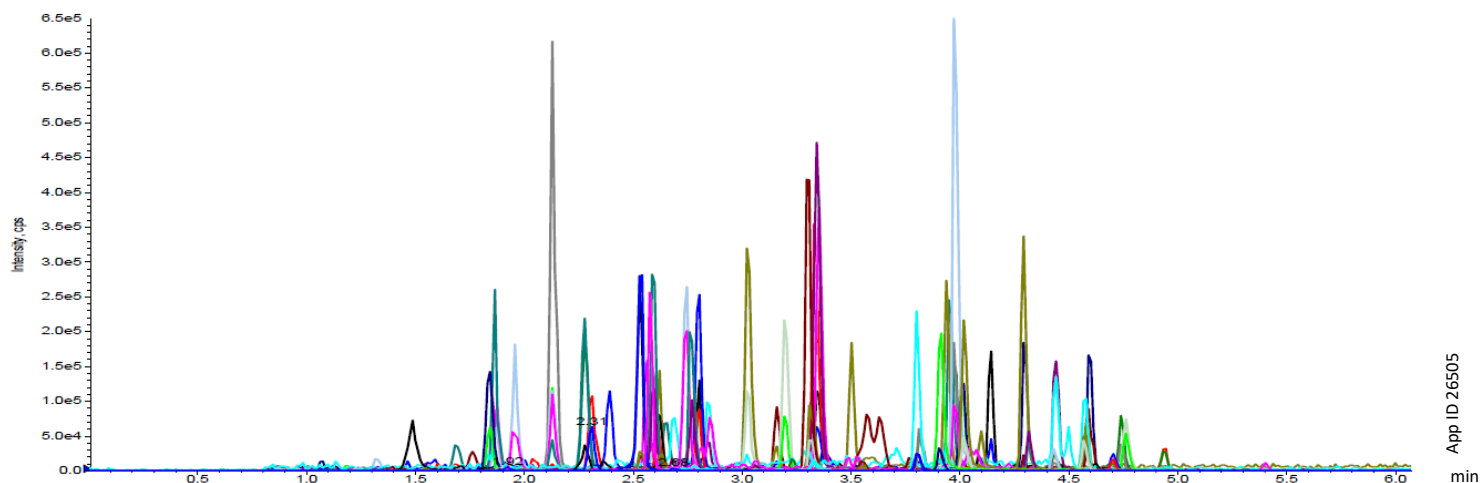


Table 1: Analyte Details for 39 Pain Management Drugs Extracted from Urine

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
20	Flunitrazepam	4.7	314.1	268.2

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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