HPLC Application

ID No.: **20847**

Pain Panel using Strata-X-Drug B and Kinetex Phenyl-Hexyl 2.6u 50x4.6mm

Column: Kinetex® 2.6 µm Phenyl-Hexyl 100 Å, LC Column 50 x 4.6 mm, Ea
Dimensions: 50 x 4.6 mm ID
Order No: 00B-4495-E0
Elution Type: Gradient
Eluent A: 10mM ammonium formate
Eluent B: 0.1% Formic acid/methanol

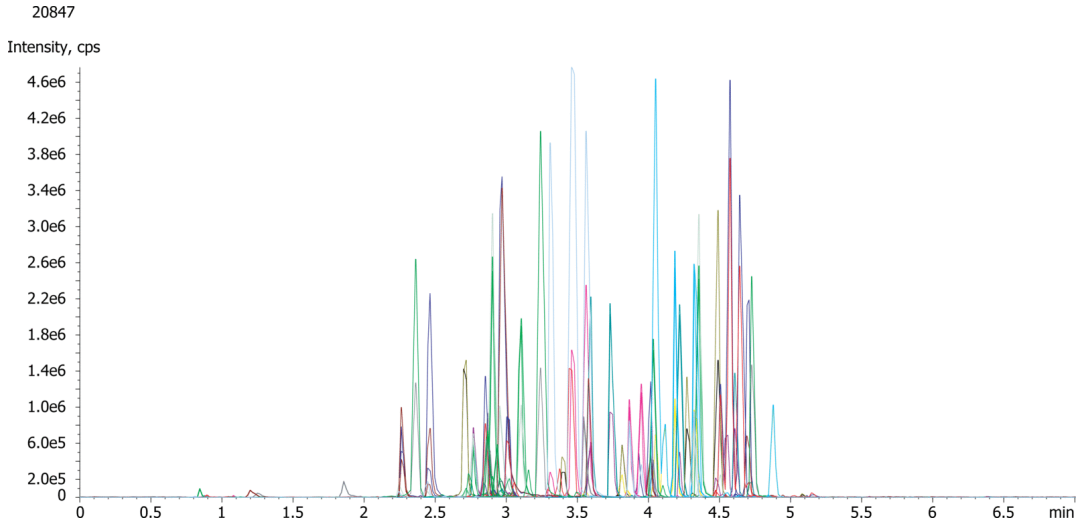
Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	95	5
2	4	0	100
3	5	0	100
4	5.1	95	5
5	7	95	5

Flow Rate: 0.6 mL/min
Col. Temp.: ambient
Detection: Mass Spectrometer (MS) @ amu (ambient)
Detector Info: <a target="_blank" href="https://sciex.com/products/mass-spectrometers?utm_campaign=2019%20application%20search&utm_source=phenomenex&utm_medium=referral">SCIEX<,
Analyst Note: MS/MS Parameters

Detection: API 5000 MS/MS, TurboIonspray, positive polarity

CAD Gas: 7; GS1, GS2 = 50; curtain gas: 20, ESI spray voltage: 5500; Temp: 650 deg C



Products used in this application:

For more information contact your Phenomenex Representative at support@phenomenex.com

Phenomenex products are available worldwide.

Pain Panel using Strata-X-Drug B and Kinetex Phenyl-Hexyl 2.6u 50x4.6mm

ANALYTES:

1 Morphine
2 Morphine-d6
3 Hydromorphone
4 Hydromorphone-d6
5 Naloxone
6 Codeine
7 Oxymorphone
8 Naltrexone
9 Amphetamine
10 Amphetamine-d5
11 6-MAM
12 Oxycodone
13 Hydrocodone
14 Hydrocodone-d6
15 Methamphetamine
16 MDA
17 MDMA
18 MDEA
19 Norfentanyl
20 Tramadol
21 Benzoylecgonine
22 Benzoylecgonine-d3
23 Meperidine
24 Meperidine-d4
25 Normeperidine
26 Normeperidine-d4
27 Meprobamate
28 Norbuprenorphine
29 PCP
30 PCP-D5
31 Fentanyl
32 Fentanyl-d5
33 EDDP
34 Flurazepam
35 Norpropoxyphene
36 Norpropoxyphene-d5
37 Sufentanil
38 Buprenorphine
39 Propoxyphene
40 Carisoprodol
41 Methadone
42 Methadone-d3
43 Midazolam
44 Lorazepam
45 Clonazepam
46 Hydroxyalprazolam
47 Oxazepam
48 Flunitrazepam
49 Alprazolam
50 Temazepam
51 Nordiazepam
52 Nordiazepam-d5
53 Diazepam
54 6-MAM-d3
55 Methamphetamine-d5

Sample Preparation Details

for **HPLC** Application ID No.: **20847**

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PRODUCT DESCRIPTION:

Strata™-X-Drug B 33 µm Polymeric Strong Cation, 30 mg / 3 mL, Tubes , 50/Pk

Order No.: 8B-S128-TBJ

SOLID PHASE EXTRACTION (SPE) PRODCEDURE:

Note: The solvent volumes shown below are for a 30 mg bed mass.
The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Sample Hydrolysis: Measure 200uL Urine (calibrator, control) in a 1.5mL micro centrifuge tube, add 20 uL combined IS (Internal Standard) spiking solution.
Vortex 10-15 secs followed by adding 100uL 0.1M pH 4.0 ammonium acetate buffer to each tube, mix/vortex another 15 secs.
Add 40uL
beta-Glucoronidase solution (100,000 units/mL).
Vortex 10-15 secs.
Incubate for 2 hours in a shaker at 55o C to complete hydrolysis of the glucuronides.
Add 400ul of 0.1% FA (formic acid) followed by vortexing for another 60 secs.
Centrifuge the hydrolyzed samples at appx 21000 g for 10 mins (to separate any proteins). Collect Supernatant

Wash:

Dry:

3 to 4 mins at high vacuum (10" of Hg)

Elute:

Final Prep and Analysis:

Load: Samples from hydrolysis Using b-Glucoronidase as below
To 200 µL Urine (calibrator, control) in a 1.5 mL micro centrifuge tube, add 20 µL combined IS

Inject: 10 µL on HPLC Mass Spectrometer (MS) @ amu (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Morphine	25			115	
2 Morphine-d6	500				
3 Hydromorphone	25			110	
4 Hydromorphone-d6	500				
5 Naloxone	25			103	
6 Codeine	25			111	
7 Oxymorphone	25			107	
8 Naltrexone	25			108	
9 Amphetamine	125			99	
10 Amphetamine-d5	2500				
11 6-MAM	2.5			99	
12 Oxycodone	25			113	
13 Hydrocodone	25			113	
14 Hydrocodone-d6	312				
15 Methamphetamine	125			113	
16 MDA	62.5			99	

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ANALYTES (cont.):	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
17 MDMA	62.5			102	
18 MDEA	62.5			104	
19 Norfentanyl	2.5			112	
20 Tramadol	25			96	
21 Benzoylecgonine	37.5			100	
22 Benzoylecgonine-d3	750				
23 Meperidine	250			95	
24 Meperidine-d4	3000				
25 Normeperidine	25			108	
26 Normeperidine-d4	500				
27 Meprobamate	50			108	
28 Norbuprenorphine	25			93	
29 PCP	6.25			120	
30 PCP-D5	125				
31 Fentanyl	0.75			110	
32 Fentanyl-d5	100				
33 EDDP	25			113	
34 Flurazepam	25			117	
35 Norpropoxyphene	37.5			103	
36 Norpropoxyphene-d5	750				
37 Sufentanil	0.75			84	
38 Buprenorphine	25			100	
39 Propoxyphene	37.5			98	
40 Carisoprodol	50			112	
41 Methadone	25			101	
42 Methadone-d3	500				
43 Midazolam	25			114	
44 Lorazepam	25			102	
45 Clonazepam	25			117	
46 Hydroxyalprazolam	25			111	
47 Oxazepam	25			99	
48 Flunitrazepam	25			98	
49 Alprazolam	25			101	
50 Temazepam	25			104	
51 Nordiazepam	25			110	
52 Nordiazepam-d5	500				
53 Diazepam	25			114	
54 6-MAM-d3	500				
55 Methamphetamine-d5	2500				

Note: *This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals. Call your local Phenomenex Representative for assistance in method development and optimization techniques.*

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