

## Effective Sample Preparation and Rapid LC/MS/MS Analysis of a Large Pain Panel Screen using Strata™-X-Drug B Solid Phase Extraction (SPE) and Kinetex® Phenyl-Hexyl Core-Shell Technology HPLC/UHPLC Columns

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Using a single Solid Phase Extraction (SPE) method on a specialized sorbent, 41 pain management drugs from several drug classifications were isolated from urine samples and analyzed by LC/MS/MS in under 5 minutes using a Kinetex Core-Shell Technology HPLC/UHPLC column. The Strata-X-Drug B SPE sorbent was not only able to successfully extract the entire panel of pain management drugs under investigation but it also helped to reduce the amount of time and solvent required for analysis since the sorbent does not require a condition or equilibration step. The Kinetex Core-Shell Technology column further reduced the amount of time required for analysis by separating all 41 compounds in under 5 minutes, without losing sensitivity.

### Introduction

According to a study in 2010, an estimated 34 million people were reported to have used pain relievers nonmedically in their lifetime.<sup>1</sup> With the abuse of pain medications on the rise, it is important that contract laboratories and hospitals have a reliable and efficient means to detect the presence of pain medications within patient samples. The ability to screen for a variety of pain medications at once using a single cleanup method and a quick LC/MS/MS method can result in huge time and solvent savings for contract and hospital laboratories who must process hundreds to thousands of samples per week. For this reason, our work had two goals; 1. Develop an SPE method that could extract 41 pain management drugs from urine samples without sacrificing recovery and 2. Develop an LC/MS/MS method that could be run quickly without sacrificing efficiency or resolution of target drugs. The pain management drugs of interest are listed below in **Table 1** and are listed by compound classification.

**Table 1**  
Compound Classification of 41 Pain Management Drugs

Benzodiazepines	Opiates (Semi-Synthetic)	Opiates (Synthetic)	Drugs of Abuse	Other
α-Hydroxyalprazolam	6-MAM	EDDP	Amphetamine	Carisoprodol
Alprazolam	Buprenorphine	Fentanyl	Benzoylcegonine	Meprobamate
Clonazepam	Codeine	Meperidine	Methamphetamine	Tramadol
Diazepam	Hydrocodone	Methadone	MDMA	
Flunitrazepam	Hydromorphone	Naltrexone	MDA	
Flurazepam	Morphine	Norfentanyl	MDEA	
Lorazepam	Naloxone	Normeperidine	Phencyclidine	
Midazolam	Norbuprenorphine	Norpropoxyphene		
Nordiazepam	Oxycodone	Propoxyphene		
Oxazepam	Oxymorphone	Sufentanil		
Temazepam				

### Materials and Methods

#### Sample Pretreatment

Measure 200 µL urine (calibrator, control) in a 1.5 mL micro centrifuge tube, add 20 µL combined IS (Internal Standard) spiking solution. Vortex 10-15 seconds then add 100 µL 0.1 M Ammonium acetate buffer (pH 4.0) to each tube, mix/vortex another 15 seconds. Add 40 µL β-Glucuronidase solution (100,000 units/mL). Vortex 10-15 seconds.

Incubate for 2 hours in a shaker at 55 °C to complete hydrolysis of the glucuronides.

Add 400 µL of 0.1% Formic acid and vortex for another 60 seconds. Centrifuge the hydrolyzed samples at 21,000 g for 10 minutes (to separate any proteins). Collect supernatant for further cleanup via Solid Phase Extraction (SPE).

#### Solid Phase Extraction (SPE)

<b>Cartridge:</b>	Strata-X-Drug B, 30 mg/3 mL
<b>Part No.:</b>	8B-S128-TBJ
<b>Condition:</b>	NOT REQUIRED
<b>Equilibrate:</b>	NOT REQUIRED
<b>Load:</b>	Pretreated urine sample
<b>Wash 1:</b>	1 mL 0.1 % Formic acid in Water
<b>Wash 2:</b>	1 mL Methanol/Water (30:70)
<b>Dry:</b>	3 to 4 minutes under 10 <sup>-2</sup> Hg vacuum
<b>Elute:</b>	2x 0.5 mL 2 % Ammonium hydroxide in Methanol/Acetonitrile (1:1)
<b>Dry down:</b>	Dry down completely @ 40-45 °C under a stream of nitrogen
<b>Reconstitute:</b>	200 µL 10 mM Ammonium formate/0.1 % Formic acid in Methanol (85:15)

Analysis was performed using an Agilent® 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) coupled with an API 5000™ LC/MS/MS detector (AB SCIEX, Framingham, MA, USA).

<sup>1</sup> National Survey on Drug Use & Health. Substance Abuse and Mental Health Services Administration (SAMHSA). 2010

**LC/MS/MS Conditions**

Column: Kinetex® 2.6 µm Phenyl-Hexyl  
 Dimensions: 50 x 4.6 mm  
 Part No.: 00B-4495-E0  
 Guard: SecurityGuard™ ULTRA Phenyl  
 Guard Part No.: AJ0-8774  
 Mobile Phase: A: 10 mM Ammonium formate  
 B: 0.1 % Formic acid in Methanol  
 Gradient: Time (min) B (%)  
 0 5  
 4 100  
 5 100  
 5.1 5  
 7 5  
 Flow Rate: 0.6 mL/min  
 Injection Volume: 10 µL  
 Temperature: 30 °C  
 Detection: API 5000™ MS/MS, positive polarity

**Gas Parameters**

CUR: 20 psi  
 IS: 5500  
 TEM: 650 °C  
 GS1: 50 psi  
 GS2: 50 psi  
 Ihe: 0n  
 CAD: 7

**MS/MS Parameters**

Scan Function: MRM/SRM  
 Collision Gas: Nitrogen  
 CEM Voltage: 2000  
 Q1 Resolution: High

**Compound MRM Parameters**

Analyte	Q1 (m/z)	Q3 (m/z)
6-MAM 1	328.1	165.2
6-MAM 2	328.1	211.2
Alprazolam 1	309.1	281.1
Alprazolam 2	309.1	205.2
Amphetamine 1	136.1	91.0
Amphetamine 2	136.1	119.0
Benzoyllecgonine 1	290.1	168.1
Benzoyllecgonine 2	290.1	105.0
Buprenorphine 1	468.3	396.2
Buprenorphine 2	468.3	414.3
Carisoprodol 1	261.1	176.2
Carisoprodol 2	261.1	97.2
Clonazepam 1	316.1	270.1
Clonazepam 2	316.1	214.0
Codeine 1	300.2	152.1
Codeine 2	300.2	115.1
Diazepam 1	285.0	193.2
Diazepam 2	285.0	154.1
EDDP 1	278.2	234.2
EDDP 2	278.2	186.2
Fentanyl 1	337.3	105.1
Fentanyl 2	337.3	188.2
Flunitrazepam 1	314.1	268.2
Flunitrazepam 2	314.1	239.2
Flurazepam 1	388.2	315.2
Flurazepam 2	388.2	134.1
Hydrocodone 1	300.2	199.0
Hydrocodone 2	300.2	128.0
Hydromorphone 1	286.1	185.1
Hydromorphone 2	286.1	128.0
Hydroxyalprazolam 1	325.1	297.0
Hydroxyalprazolam 2	325.1	216.1
Lorazepam 1	321.0	275.0
Lorazepam 2	321.0	229.0
MDA 1	180.1	133.0
MDA 2	180.1	105.0
MDEA 1	208.2	163.0
MDEA 2	208.2	105.0
MDMA 1	194.1	105.1
MDMA 2	194.1	163.0
Meperidine 1	248.2	220.2
Meperidine 2	248.2	174.2
Meprobamate 1	219.2	158.1
Meprobamate 2	219.2	97.0
Methadone 1	310.0	265.0
Methadone 2	310.0	105.0
Methamphetamine 1	150.1	91.0
Methamphetamine 2	150.1	119.0

**Compound MRM Parameters (continued)**

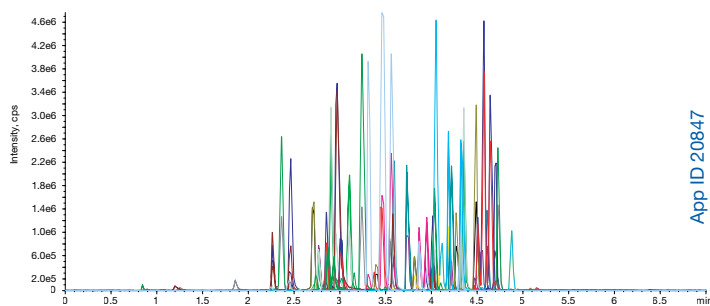
Analyte	Q1 (m/z)	Q3 (m/z)
Midazolam 1	326.1	291.1
Midazolam 2	326.1	249.1
Morphine 1	286.1	152.1
Morphine 2	286.1	165.1
Naloxone 1	328.2	212.0
Naloxone 2	328.2	253.0
Naltrexone 1	342.2	267.1
Naltrexone 2	342.2	212.1
Norbuprenorphine 1	414.3	101.1
Norbuprenorphine 2	414.3	165.2
Nordiazepam 1	271.0	140.0
Nordiazepam 2	271.0	165.1
Norfentanyl 1	233.2	84.1
Norfentanyl 2	233.2	150.1
Normeperidine 1	234.1	160.1
Normeperidine 2	234.1	188.1
Norpropoxyphene 1	308.2	100.1
Norpropoxyphene 2	308.2	143.2
Oxazepam 1	287.0	241.0
Oxazepam 2	287.0	269.1
Oxycodone 1	316.1	241.2
Oxycodone 2	316.1	256.2
Oxymorphone 1	302.1	227.1
Oxymorphone 2	302.1	198.2
PCP 1	244.3	91.1
PCP 2	244.3	159.2
Propoxyphene 1	340.3	266.3
Propoxyphene 2	340.3	91.1
Sufentanil 1	387.2	238.1
Sufentanil 2	387.2	111.1
Temazepam 1	301.1	255.1
Temazepam 2	301.1	177.1
Tramadol 1	264.1	58.1
Tramadol 2	264.1	42.1
6-MAM-d3	331.1	165.0
Amphetamine-d5	141.1	93.0
Benzoyllecgonine-d3	293.1	171.2
Codeine-d6	306.2	152.2
Fentanyl-d5	342.3	105.1
Hydrocodone-d6	306.2	202.1
Hydromorphone-d6	292.1	185.1
Meperidine-d4	252.2	224.1
Methadone-d3	313.2	105.1
Methamphetamine-d5	155.2	92.0
Morphine-d6	292.1	152.0
Nordiazepam-d5	276.1	140.0
Normeperidine-d4	238.2	164.2
Norpropoxyphene-d5	313.2	100.1
PCP-d5	249.3	96.1

**Results and Discussion**

When developing an analytical method for pain management drugs, two goals were set; 1. Develop an SPE method that could extract 41 pain management drugs from urine samples without sacrificing recovery and 2. Develop an LC/MS/MS method that could be run quickly without sacrificing efficiency or resolution of target drugs. To address our first goal, Strata-X-Drug B SPE was selected as the most appropriate extraction sorbent for several reasons; the sorbent is quality control tested and manufactured for the extraction of drugs of abuse and conditioning and equilibration steps were not required. These benefits provided both time and solvent savings when developing our SPE method.

A majority of our targeted pain management drugs are basic in nature, making the strong cation-exchange function of the Strata-X-Drug B sorbent an ideal chemistry for the extraction of these compounds. In addition to the strong cation-exchange function, the sorbent also has a hydrophobic backbone (due to its polymer base) which further helps to retain our target compounds. For these reasons, our method was able to extract all 41 pain management drugs with an accuracy between 84 to 120 % and precision ranging from 2 to 20 %. Accuracy, precision, and linearity data for several of the 41 target drugs is outlined in **Figures 2 and 3**.

**Figure 1**  
LC/MS/MS Separation of 41 Pain Management Drugs Using a Kinetex Core-Shell Technology Phenyl-Hexyl HPLC/UHPLC Column



App ID 20847

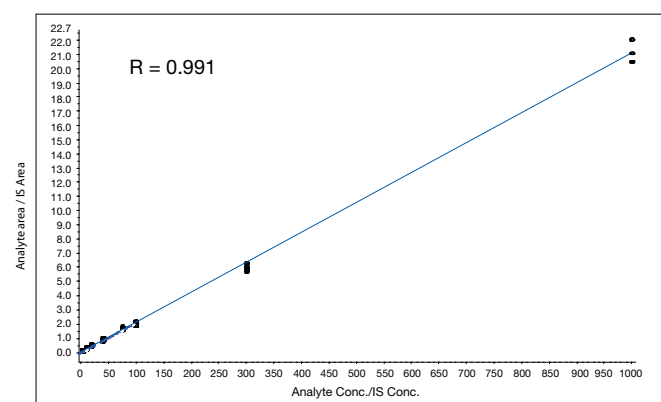
Transition 1 for each analyte is used as the quantifier transition.

Transition 2 for each analyte is used as the qualifier transition.

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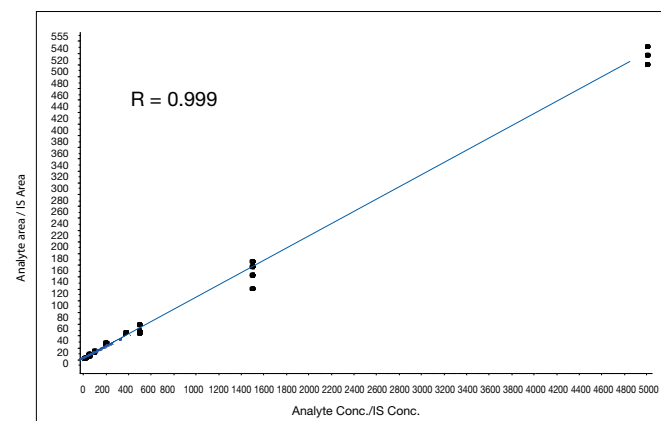
**Figure 2**  
Recovery, Reproducibility, and Linearity of  $\alpha$ -Hydroxyalprazolam

Concentration (ng/mL)	Precision (n = 3)	Accuracy
25	2.388	111.4
200	3.384	102.4



**Figure 3**  
Recovery, Reproducibility, and Linearity of Methamphetamine

Concentration (ng/mL)	Precision (n = 3)	Accuracy
125	2.474	113.5
1000	4.489	110.8



After extraction, we began to develop a rapid yet sensitive LC/MS/MS method. A Kinetex® 2.6  $\mu$ m Phenyl-Hexyl Core-Shell Technology HPLC/UHPLC column was chosen because the core-shell particles produce high efficiencies without the added backpressure that is associated with sub-2  $\mu$ m columns. The Phenyl-Hexyl phase was able to separate compounds based on polarity as well as hydrophobicity, resulting in separation of all 41 compounds in under 5 minutes (**Figure 1**).

## Conclusions

Utilizing a single SPE sorbent and protocol, 41 pain management drugs were successfully extracted from urine samples. After cleanup, a rapid and sensitive LC/MS/MS method was performed which resulted in resolution of all 41 pain management drugs in under 5 minutes. The chemistry of the Strata-X-Drug B SPE sorbent was able to target and retain all 41 drugs, providing excellent recoveries for each drug. In addition to high recoveries of all target drugs, the SPE sorbent does not require a conditioning or equilibration step which significantly saves both time and solvent consumption, particularly when applied to a high throughput study. The Kinetex 2.6  $\mu$ m Core-Shell Technology Phenyl-Hexyl HPLC/UHPLC column further added to our time savings, resulting in a run-time of under 5 minutes. This is due to the unique core-shell design which provides UHPLC results without the increased backpressure.

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## Ordering Information

### Kinetex® Core-Shell HPLC/UHPLC Columns

2.6 $\mu$ m Analytical Columns (mm)				SecurityGuard™ ULTRA Cartridges†
Phases	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Phenyl-Hexyl	00B-4495-E0	00D-4495-E0	00F-4495-E0	AJ0-8774 for 4.6 mm ID

2.6 $\mu$ m Minibore Columns (mm)				SecurityGuard™ ULTRA Cartridges†
Phases	50 x 2.1	100 x 2.1	3/pk	
Phenyl-Hexyl	00B-4495-AN	00D-4495-AN	AJ0-8788	for 2.1 mm ID

†SecurityGuard ULTRA cartridges require holder, Part No. AJ0-9000

### Strata™-X-Drug B SPE

Sorbent Mass	Part No.	Unit
<b>Tube</b>		
10 mg	8B-S128-AAK	1 mL (100/box)
10 mg	8L-S128-AAK†	1 mL (100/box)
30 mg	8B-S128-TAK	1 mL (100/box)
30 mg	8L-S128-TAK†	1 mL (100/box)
30 mg	8B-S128-TBJ	3 mL (50/box)
60 mg	8B-S128-UBJ	3 mL (50/box)
60 mg	8B-S128-UCH	6 mL (30/box)
60 mg	8B-S128-UCL	6 mL (200/bag)
<b>Giga™ Tube</b>		
100 mg	8B-S128-EDG	12 mL (20/box)
<b>96-Well Plate</b>		
10 mg	8E-S128-AGB	2 Plates/Box
30 mg	8E-S128-TGB	2 Plates/Box
60 mg	8E-S128-UGB	2 Plates/Box

† Tab-less tube



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### Complete iMethod Kit\*

Description	Part No.
iMethod Test for the Full Pain Panel V1.2	KH0-9162

\* Kit includes Kinetex 2.6  $\mu$ m Phenyl-Hexyl HPLC/UHPLC column, SecurityGuard ULTRA cartridges and holder, in-line filter, Phenex™ Syringe Filters, Verex™ Vial kit, Sure-Lok™ UHPLC Nut and tightening tool, Sure-Lok Fingertight Nut and wrenches.

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