

# APPLICATION

## An Optimized Workflow for Drugs of Abuse Testing using Strata<sup>®</sup>-X-Drug B Plus In-Well Hydrolysis SPE

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Sample Preparation

*Matt Brusius is an avid ice hockey player. He likes skating backwards and taking slapshots from the point.*

### Introduction

To help optimize the workflow when working with drugs of abuse in urine for Solid Phase Extraction (SPE), utilizing an in-well urine hydrolysis can save transfer steps, time, and additional lab costs. In this technical note, a simplified method for a  $\beta$ -glucuronidase enzyme hydrolysis coupled with SPE clean-up is investigated to improve productivity and save time. For an enzyme hydrolysis under three hours, the Strata-X-Drug B Plus 96-well plate provides two functions: to serve as the receptacle for enzyme hydrolysis and to carry out the subsequent SPE after hydrolysis is complete. Strata-X-Drug B Plus in-well hydrolysis reduces consumables by eliminating the need for one 96-well collection plate and streamlines the workflow by eliminating the traditionally required transfer step between collection device and SPE 96-well plate. The SPE is a mixed-mode strong cation-exchange sorbent that does not require conditioning or equilibration, providing a simple and efficient three step SPE solution coupled with a Kinetex<sup>®</sup> 2.6  $\mu$ m Phenyl-Hexyl LC column that provides excellent recovery and precision for both neutral and basic drugs of abuse.

### Materials and Methods

#### Sample Pre-Treatment

Hydrolysis Solution Prepared as follows:

Combine 133  $\mu$ L urine with 53  $\mu$ L DI Water and add 67  $\mu$ L 0.1 M Ammonium acetate buffer (pH 4). Next, add 27  $\mu$ L Campbell  $\beta$ -Glucuronidase Enzyme (Part No.: DR2102) and proceed to load hydrolysis solution onto Strata-X-Drug B Plus 96-well plate and incubate at 55 °C for 90 minutes. Upon completion of hydrolysis, dispense 280  $\mu$ L Water into each well and mix for five minutes – ensuring that pH is between 4-6.

### SPE Protocol

- 96-Well Plate:** Strata-X-Drug B Plus, 10 mg/well
- Part No.:** 8E-S128-AGB-P
- Load:** Apply 5" Hg to pull hydrolysis solution through plate
- Wash 1:** 350  $\mu$ L 100 mM Sodium acetate buffer (pH 5)
- Wash 2:** 350  $\mu$ L 30% Methanol
- Dry:** 4 minutes at 10" Hg
- Elute:** 2x 200  $\mu$ L Ethyl acetate/Isopropanol/Ammonium hydroxide (70:20:10)
- Apply:** Vacuum at 5-10" Hg for 10 seconds
- Dry:** Sample under slow stream of Nitrogen at 40 °C
- Reconstitute:** 100  $\mu$ L 0.1% Formic acid in Methanol/Water (5:95) with internal standard

### LC-MS/MS Conditions

- Column:** Kinetex 2.6  $\mu$ m Phenyl-Hexyl
- Dimensions:** 50 x 3.0 mm
- Part No.:** 00B-4495-Y0
- Mobile Phase:** A: 0.1% Formic acid in Water  
B: 0.1% Formic acid in Methanol
- Gradient:**

Time (min)	% B
0	5
4	95
5.5	95
5.51	5
7	5
- Flow Rate:** 0.6 mL/min
- Injection Volume:** 10  $\mu$ L
- Detection:** MS/MS (SCIEX API 4000<sup>™</sup>), ESI +



**Table 1.**  
Percent Recovery for Basic and Neutral Drugs of Abuse

Analyte	% Absolute Recovery	% RSD (n=4)
Amphetamine	99	4
Methamphetamine	108	10
PCP	96	10
Nordiazepam	99	2
Diazepam	98	2
Hydromorphone	98	3
Morphine	107	3
Oxazepam	91	3
Benzoylcegonine	94	13
Hydrocodone	104	5
Codeine	119	10
Temazepam	90	3
Oxycodone	106	8
Lorazepam	99	3
Fentanyl	105	4
Norbuprenorphine	108	3
Buprenorphine	107	3

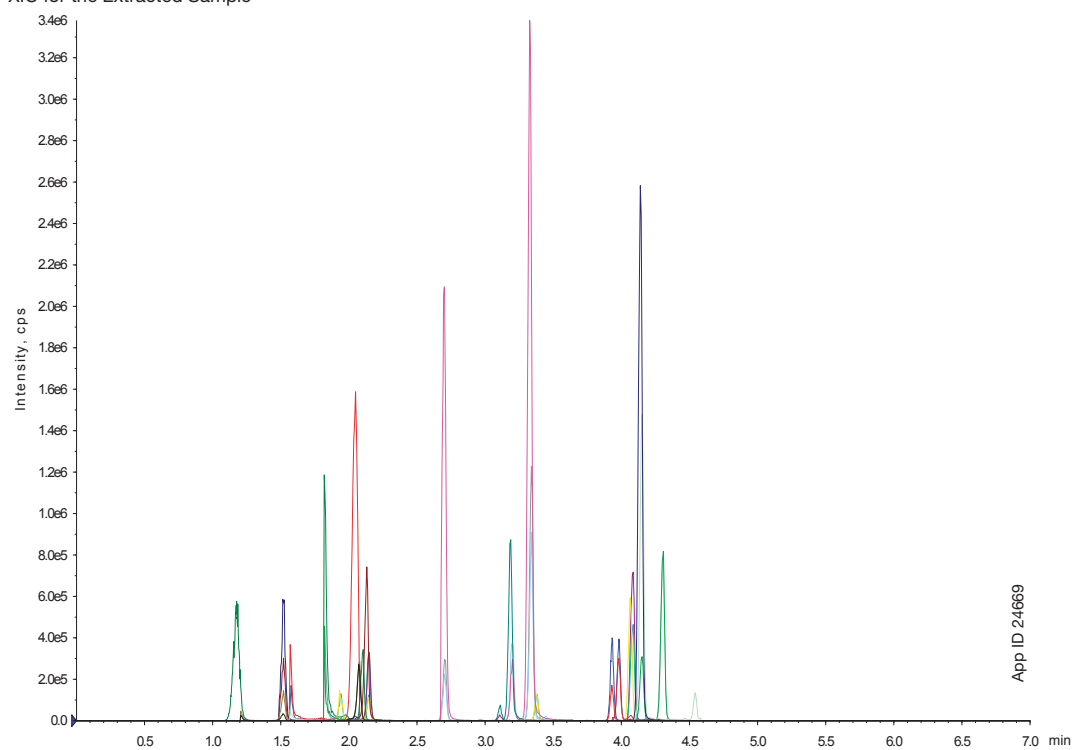
## Results and Discussion

**Table 1** provides the absolute recovery values for all drugs of abuse compounds tested. Each analyte shows a recovery greater than 90 % with all relative standard deviations less than or equal to 13 %. This indicates that the SPE method with the in-well hydrolysis is both extremely accurate and precise. **Figure 1** provides an example XIC for an extracted sample, showing that this method can be used to extract drugs of abuse.

## Conclusion

In this technical note, a streamlined workflow for quantifying drugs of abuse from urine is developed by performing urinary hydrolysis within the wells of the Strata<sup>®</sup>-X-Drug B Plus 96-Well Plates prior to clean-up via solid phase extraction and analysis via LC-MS/MS. This provides an efficient single method for hydrolysis and extraction, which will save time and lab costs.

**Figure 1.**  
XIC for the Extracted Sample



## Ordering Information

### Kinetex<sup>®</sup> Core-Shell LC Columns

Kinetex 2.6 µm MidBore <sup>™</sup> Columns (mm)			SecurityGuard <sup>™</sup> ULTRA Cartridges <sup>†</sup>	
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Phenyl-Hexyl	00B-4495-YO	00D-4495-YO	00F-4495-YO	AJ0-8781 for 3.0 mm ID

<sup>†</sup> SecurityGuard ULTRA Cartridges required holder, Part No.: AJ0-9000.

### Strata<sup>®</sup>-X-Drug B Plus SPE

Sorbent Mass	Part No.	Unit
<b>96-Well Plate</b>		
10 mg	8E-S128-AGB-P	2 Plates/Box
30 mg	8E-S128-TGB-P	2 Plates/Box

### Strata-X-Drug B SPE

Sorbent Mass	Part No.	Unit
<b>Tube</b>		
10 mg	8B-S128-AAK	1 mL (100/box)
30 mg	8B-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<sup>™</sup> 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

### Presston<sup>™</sup> 100 Positive Pressure Manifold

Part No.	Description
AH0-9334	Presston 100 Positive Pressure Manifold, 96-Well Plate
AH0-9342	Presston 100 Positive Pressure Manifold, 1 mL Tube Complete Assembly
AH0-9347	Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly
AH0-9343	Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly

The Presston 100 96-Well Positive Pressure Manifold can also process 1, 3, and 6 mL tubes using the following adapter kits

### Presston 100 Tube Adapter Kits (for AH0-9334)

Part No.	Description
AH0-9344	1 mL Tube Adapter Kit
AH0-9345	3 mL Tube Adapter Kit
AH0-9346	6 mL Tube Adapter Kit



WARRANTY Phenomenex warrants that for a period of 12 months following delivery, the Presston 100 Positive Pressure Manifold you have purchased will perform in accordance with the published specifications and will be free from defects in materials or workmanship. In the event that the Presston 100 Positive Pressure Manifold does not meet this warranty, Phenomenex will repair or replace defective parts. Please visit [www.phenomenex.com/Presston](http://www.phenomenex.com/Presston) for complete warranty information.



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Strata-X is patented by Phenomenex. U.S. Patent No 7,119,145

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