TN-0127

APPLICATION

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

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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 β-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® 2.6µm 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 133µL urine with 53µL DI Water and add 67µL 0.1 M Ammonium acetate buffer (pH 4). Next, add 27µL Campbell β-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 280µL 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:	350µL 100 mM Sodium acetate buffer (pH 5)
Wash 2:	350µL 30 % Methanol
Dry:	4 minutes at 10" Hg
Elute:	2x 200µL 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:	100µL 0.1 % Formic acid in Methanol/Water (5:95) with internal standard

LC-MS/MS Conditions

Column:	Kinetex 2.6µm Phenyl-Hexyl	
Dimensions:	50 x 3.0mm	
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:	10µL	
Detection:	MS/MS (SCIEX API 4000™), ESI +	

Caption:

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