APPLICATIONS

Improved Sensitivity of Hydrolyzed Urine Samples Using β-Gone™ β-Glucuronidase Removal Products

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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

During metabolism, drugs are tagged with a glucuronic acid that helps change the polarity of the drug compound and aids in absorption into the kidneys. When the drugs exit the body through urine, they are still in their glucuronide form and before chromatographic analysis can occur, the glucuronide bond must be cleaved through hydrolysis. Enzymatic hydrolysis, using β-glucuronidase, is preferred over acid hydrolysis because the bond is cleaved without introducing harsh solvents into the sample. Now the sample contains drug compounds and residual β-glucuronidase enzyme, which if the enzyme is not removed can precipitate out in the LC column during the run. The column's selectivity and lifetime is negatively affected and can result in buildup in the mass spectrometer (MS). "Dilute-and-shoot" is a common method that is used to prepare hydrolyzed urine samples for LC/MS analysis. This method can cause issues with the sensitivity because it dilutes the sample 10x up to 30x before injection onto the column. In this application note, we will demonstrate how using β-Gone β-Glucuronidase Removal Products helps to improve sensitivity in comparison to the "dilute-and-shoot" method. Focusing on two notoriously low responding compounds, norbuprenorphine and buprenorphine, the differences in methods are displayed.

Experimental Conditions

All reagents and solvents were HPLC or analytical grade. Analyses were performed using an API 4000™ LC/MS/MS (SCIEX, Framingham, MA)

Sample Preparation:

Prepare Urine Hydrolysate as follows:

1) Add 10 µL of analyte spike (1 µg/mL) to 200 µL of urine
2) Dilute with 100 µL of 0.1 M ammonium acetate buffer
3) Add 40 µL of Campbell Science β-Glucuronidase Enzyme Solution (Part No.: DR2102)
4) Add 400 µL of 0.1 % formic acid in water to mixture and vortex for 15 seconds

β-Gone Protocol:

1) Dilute 200 µL of Urine Hydrolysate with 133 µL of 0.1 % Formic acid in Methanol
2) Load diluted sample onto β-Gone 96-Well Plate (Part No.: 8E-S322-DGA) and apply 2-5 psi using a positive pressure manifold or a vacuum manifold
3) Collect eluent and inject 10 µL for analysis

Dilute-and-Shoot Protocol:

1) Transfer 100 µL of Urine Hydrolysate to vial
2) Dilute sample by adding 900 µL of 0.1 % Formic acid in Water
3) Vortex and inject 10 µL for analysis

HPLC Conditions

Column: Kinetex® 2.6 µm Biphenyl
Dimensions: 50 x 3.0mm
Part No.: 00B-4622-Y0
Mobile Phase: A: 0.1% Formic acid in Water
B: 0.1% Formic acid in Methanol
Flow Rate: 0.7 mL/min
Gradient:

Time (min)	% B
0.01	10
1.00	10
4.00	100
5.00	100
5.01	10
6.00	10

Detection: MS/MS (API 4000, SCIEX)

Results and Discussion

In **Figure 1** the signal response for buprenorphine is shown for a sample that has been diluted 10x (blue peak) and one that has been filtered through the β-Gone 96-Well Plate (red peak).

Figure 2 shows the same comparison for norbuprenorphine. In both cases, β-Gone indicates a gain in sensitivity almost 10x more than the dilute-and-shoot prepared sample.

In addition, **Table 1** shows the average recovery values (n=8) using β-Gone for a large panel of drug compounds. All % CVs are below 6% and most recoveries are near 100%.

Conclusion

This work shows that by utilizing β-Gone β-Glucuronidase Removal Products, laboratories can expect to significantly improve sensitivity while adding little variability to the results (%CV) in comparison to dilute-and-shoot protocols.

Table 1.

Analyte	Average Recovery %	% CV
Benzoylecognine	109	3
Buprenorphine	93	6
Codeine	109	4
Lorazepam	79	5
Methamphetamine	106	3
Norbuprenorphine	109	5
PCP	102	3

Figure 1.
Buprenorphine: β-Gone™ vs Dilute-and-Shoot

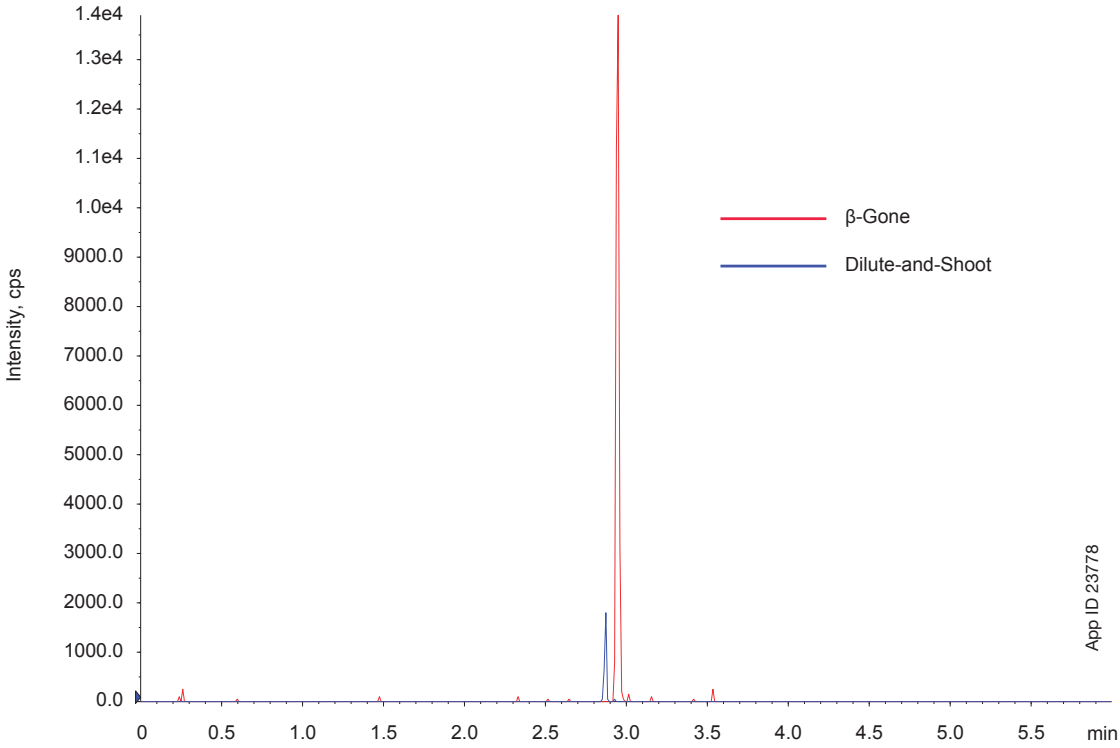
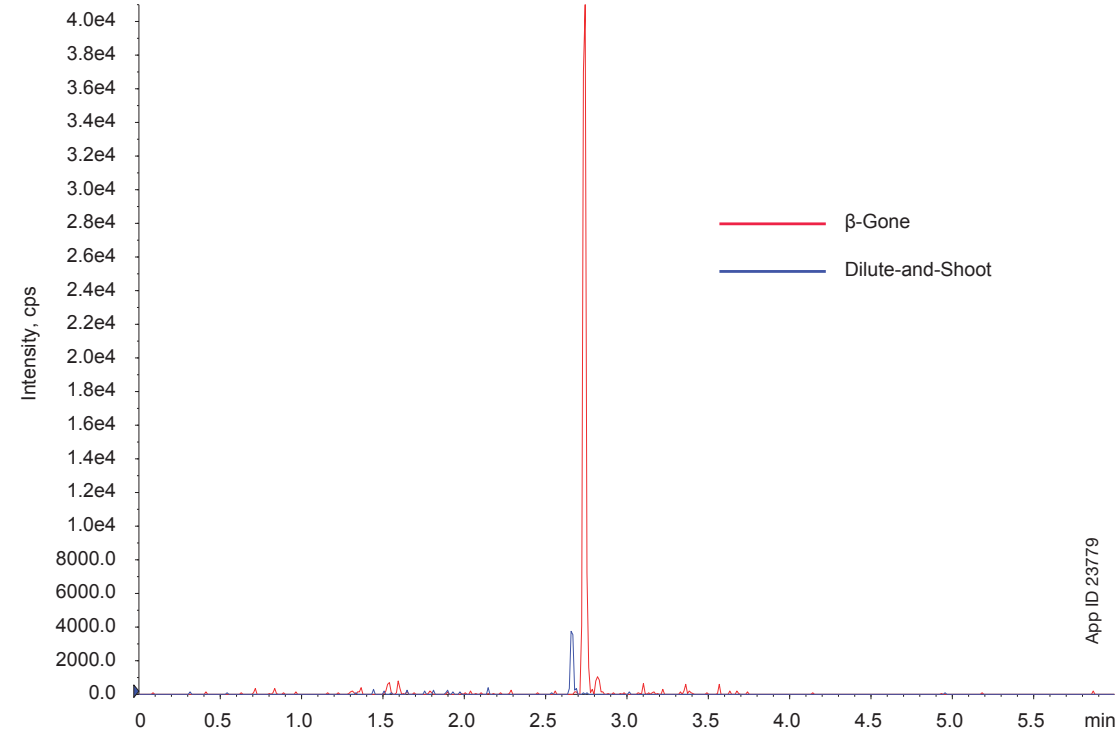


Figure 2.
Norbuprenorphine: β-Gone vs Dilute-and-Shoot



Ordering Information

β-Gone™ β-Glucuronidase Removal Products

Part No.	Description	Unit
8B-S139-TAK	1 mL Tubes, Recombinant Enzyme	100/Box
8B-S322-DAK	1 mL Tubes, Non-Recombinant Enzyme	100/Box
8E-S139-TGA	96-Well Plate, Recombinant Enzyme	1/Box
8E-S322-DGA	96-Well Plate, Non-Recombinant Enzyme	1/Box

Kinetex 2.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00A-4622-AN	00B-4622-AN	00D-4622-AN	00F-4622-AN	AJ0-9209 for 2.1 mm ID

‡ SecurityGuard ULTRA Cartridges required holder, Part No.: AJ0-9000.

Kinetex 2.6 µm MidBore™ Columns (mm)				SecurityGuard™ ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Biphenyl	00B-4622-Y0	00D-4622-Y0	00F-4622-Y0	AJ0-9208 for 3.0 mm ID

‡ SecurityGuard ULTRA Cartridges required holder, Part No.: AJ0-9000.

Vacuum Manifolds

Part No.	Description	Unit
12–Position Vacuum Manifold for Tubes*		
AH0-6023	12-Position Vacuum Manifold Set, complete assembly	ea
24–Position Vacuum Manifold for Tubes*		
AH0-6024	24-Position Vacuum Manifold Set, complete assembly	ea
96-Well Plate Manifold		
AH0-8950	96-Well Plate Manifold, Universal with vacuum gauge	ea

* Manifolds include: Vacuum-tight glass chamber, vacuum gauge assembly, polypropylene lid with gasket, male and female luers and yellow end plugs, stopcock valves, collection rack assemblies, polypropylene needles, lid support legs. Waste container included with 12-position manifold.

Presston™ 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 for complete warranty information.

APPLICATIONS

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