APPLICATION

Analyzing β-Gone™ β-Glucuronidase Removal Centrifuge Tube Recovery and Clean Up

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Introduction

This techincal note describes how β-Gone β-Glucuronidase Removal 2 mL Centrifuge Tubes can be used for the analysis of most comprehensive drugs research panel suites prepared from a urine hydrolysate. By selectively removing the β-glucuronidase, column lifetime is improved versus a standard "dilute-and-shoot" protocol while achieving exceptional recovery for all classes of compounds.

Materials and Methods

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

Sample Preparation

Enzymatic Hydrolysis

Campbell Enzyme Hydrolysate was prepared as follows:

1) Add 10 µL of analyte spike (1 µg/mg) to 200 µL urine

2) Dilute with 100 µL of 0.1 M ammonium acetate buffer and add 40 µL of Campbell Science β-Glucuronidase Enzyme Solution (Campbell Part No.: DR2102)

3) Add 400 µL 0.1 % formic acid in water. Proceed to β-Gone Centrifuge Tube Protocol.

IMCSzyme® Hydrolysate Mix was prepared as follows:

1) Add 10 µL of analyte spike (1 µg/mg) to 140 µL urine

2) Dilute with 80 µL of IMCS buffer (IMCS Part No.: 04-EZ-RHB-20) and add 30 µL of IMCS Enzyme (IMCS Part No.:04-E1F-010). Proceed to β-Gone Centrifuge Tube Protocol.

β-Gone Centrifuge Tube Protocol

1) Tap tube prior to use to consolidate sorbent

2) Combine 200 µL of urine hydrolysate with 133 µL of 0.1 % formic acid in methanol

3) Load the diluted sample into the centrifuge tube

4) Cap and invert the tube 10 times. Vortex for 30 seconds

5) Centrifuge tube at 14000 rpm for 10 min (16000 xg RCF)

6) Collect supernatant for analysis

HPLC Conditions

Column: Kinetex® 2.6 µm Phenyl Hexyl 100Å
Dimensions: 50 x 3.0 mm ID
Part No.: 00B-4495-Y0
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol

Flow Rate: 0. 6 mL/min
Gradient:

Time (min)	% B
0	5
4	95
5.5	95
5.51	5
7	5

Table 1. Recovery Values for IMCS and Campbell Enzyme Prepared Samples

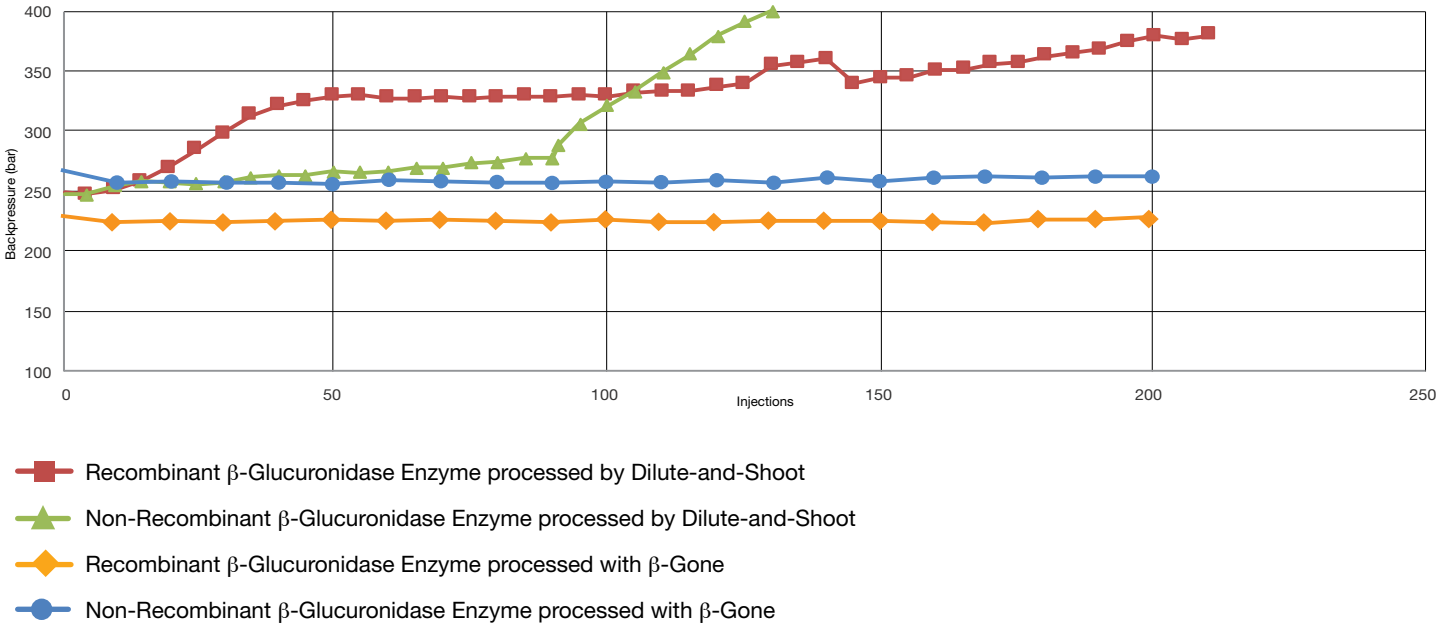
	IMCSzyme Prepared Samples		Campbell Enzyme Prepared Samples	
Analyte	Average % Recovery	%CV (n=4)	Average % Recovery	%CV (n=4)
6-MAM	108	10	105	5
7-Aminoclonazepam	108	5	109	6
α-Hydroxyalprazolam	105	5	100	3
Alprazolam	106	5	103	7
Amitriptyline	104	7	102	3
Amphetamine	105	2	102	4
Benzoylecgonine	110	4	98	4
Buprenorphine	100	2	103	5
Carisoprodol	110	5	98	5
Citalopram	104	5	104	2
Codeine	103	9	100	6
Cotinine	112	9	99	2
Diazepam	103	6	99	2
EDDP	104	4	105	4
Fentanyl	106	6	101	3
Fluoxetine	101	5	104	3
Gabapentin	112	6	98	3
Hydrocodone	110	8	103	5
Hydromorphone	110	10	106	3
Imipramine	103	5	101	2
Lorazepam	102	2	97	4
MDA	111	7	93	3
MDEA	109	6	98	3
MDMA	110	5	96	2
Meperidine	102	5	103	0
Meprobamate	106	4	104	4
Methadone	105	7	103	3
Methamphetamine	106	6	102	1
Methylphenidate	95	6	101	2
Morphine	115	8	101	6
Naloxone	112	13	100	7
Norbuprenorphine	106	4	114	6
Nordiazepam	106	6	100	4
Norfentanyl	97	4	109	5
Norhydrocodone	108	8	102	4
Noroxycodone	99	10	99	7
Nortriptyline	105	6	102	2
O-Desmethyltramadol	105	4	100	3
Oxazepam	103	7	97	7
Oxycodone	111	5	103	7
Oxymorphone	108	8	104	1
Paroxetine	102	7	107	3
PCP	102	6	102	2
Pregabalin	133	15	92	1
Ritalinic Acid	106	7	97	1
Tapentadol	102	6	94	2
Temazepam	105	6	98	3
THC-COOH	100	7	96	4
Tramadol	100	5	104	4
Zolpidem	109	5	108	4
Zolpidem4carboxy	106	4	103	7

Results and Discussion

Table 1 shows the average % recoveries and % CVs for the comprehensive drugs research panel suite for both the IMCS (recombinant enzyme) and the Campbell (non-recombinant/natural enzyme) solutions. In general, all compounds show an absolute recovery above 90%, and only pregabalin prepared with IMCS shows a % CV of 15 or greater, all other compounds are less than or equal to a % CV of 10.

Figure 1 monitors the change in backpressure over the course of 200 20 µL injections between a urine sample that is prepared by dilution and one that is prepared with the β-Gone Centrifuge Tubes. The samples prepared with the β-Gone Centrifuge Tubes maintain a constant backpressure over the span of these injections while the urine samples that were prepared by "dilute-and-shoot" trend toward a higher backpressure, and in the case of the natural enzyme, pressure out before the completion of 200 injections. This shows that the sample is cleaner after being subjected to the β-Gone Centrifuge Tubes and could help improve column lifetime versus the standard "dilute-and-shoot" protocol.

Figure 1.
Column Lifetime: β-Gone Centrifuge Tubes vs. Dilute-and-Shoot



Conclusion:

By using the β-Gone β-Glucuronidase 2 mL Centrifuge Tubes, one can obtain acceptable recoveries for a large drugs of abuse suite, while improving column lifetime in comparison to a dilute-and-shoot procedure.

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
8N-S323-TUK	2 mL Centrifuge Tubes, Recombinant and Non-Recombinant Enzyme	100/Box

Kinetex® Core-Shell LC Columns

2.6 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Phenyl-Hexyl	00B-4495-Y0	00D-4495-Y0	00F-4495-Y0	AJ0-8781 for 3.0 mm ID

‡ SecurityGuard ULTRA Cartridges require 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	Unit
AH0-9334	Presston 100 Positive Pressure Manifold, 96-Well Plate	ea
AH0-9342	Presston 100 Positive Pressure Manifold, 1 mL Tube Complete Assembly	ea
AH0-9347	Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly	ea
AH0-9343	Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly	ea

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	Unit
AH0-9344	1 mL Tube Adapter Kit	ea
AH0-9345	3 mL Tube Adapter Kit	ea
AH0-9346	6 mL Tube Adapter Kit	ea

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.

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