APPLICATION

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

Using a Step by Step Dilution to Improve β-Gone™ β-Glucuronidase Removal Methods

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Overview

The standard protocol for β-Gone β-Glucuronidase Removal Products suggests diluting the sample to a final concentration of 40 % methanol prior to filtration. In this technical note we will explore how using a smaller dilution with a smaller percentage of methanol in a step by step approach, as opposed to diluting the solution all at one time, can yield acceptable results for most comprehensive drug research panel suites.

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

IMCSzyme® Hydrolysate Mix was prepared as follows:

1. Add 10 µL of analyte spike (1 µg/mg) to 140 µL of urine
2. Dilute with 80 µL of IMCS buffer (IMCS Part No.: 04-EZRHB-20) and add 30 µL IMCS Enzyme (IMCS Part No.: 04-EIF-010) then vortex for 15 seconds
3. Proceed to β-Glucuronidase Removal Protocols

β-Glucuronidase Removal

β-Gone Recombinant Enzyme – 20 % Methanol Step by Step Addition

1. Dilute 200 µL urine hydrolysate and load onto the Recombinant β-Gone 96-Well Plate (Phenomenex Part No.: 8E-S139-TGA) then apply 2-5" Hg vacuum for one minute
2. Add 67 µL of 1 % formic acid in methanol and apply 5" Hg vacuum for one minute.
3. Add 67 µL of 1 % formic acid in water and apply 5" Hg vacuum for one minute
4. Collect pooled eluent and inject 10 µL

Results and Discussion

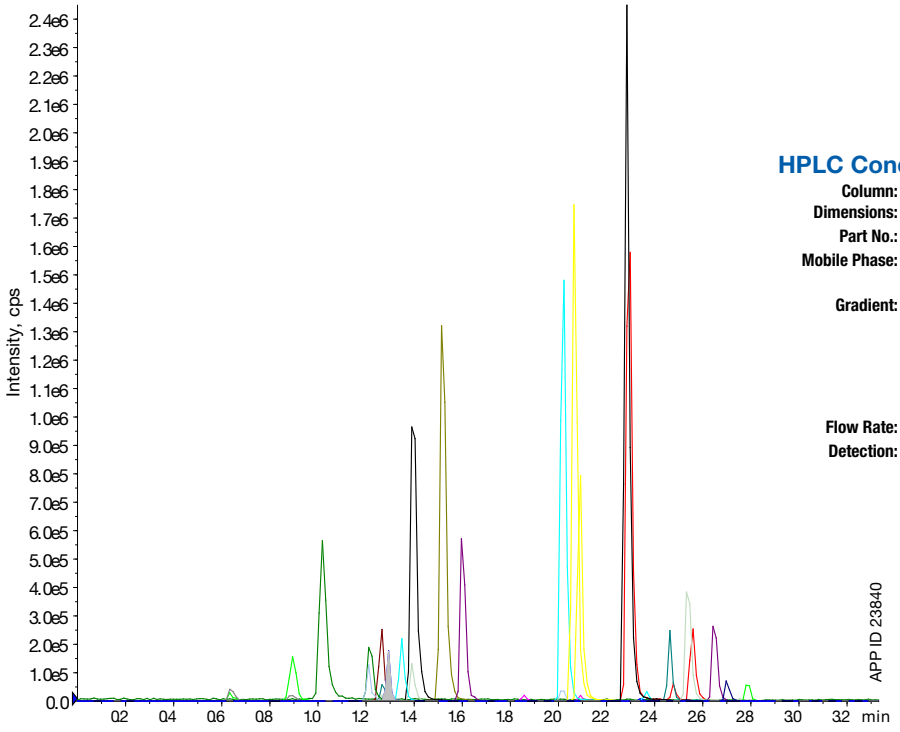
Table 1 provides absolute recovery values of the samples prepared as described in the "Materials and Methods" section. The undiluted urine hydrolysate is loaded directly onto the well plate and diluted by 67 µL of 0.1% formic acid in methanol. The dilution is then repeated with 67 µL of 0.1% formic acid in water. The order of the dilution is critical and the methanolic mixture must be added first to effectively disrupt hydrophobic interaction which improves recovery and reproducibility for more non-polar species (i.e. THC-COOH). The secondary elution of 0.1% formic acid in water is required to ensure that the entire bed is sufficiently saturated. 133 µL is equal to approximately 4 bed volumes of the 30 mg sorbent bedmass, which is the established practical minimum volume for wash and elution conditions in traditional Solid Phase Extraction (SPE).

Data not shown in this experiment also suggests that the same protocol can be used for the Non-Recombinant version of β-Gone, but requires a secondary elution of 117 µL of 0.1% formic acid in water to fully saturate the larger bed mass. This results in a 33% absolute recovery of THC-COOH, but with a CV of 9% (n=8).

Table 1.
Recovery for Recombinant β-Gone™ using a step by step dilution

Analyte	Average % Recovery	%CV (N=8)
6-MAM	105	4
Alprazolam	97	6
Buprenorphine	76	13
Codeine	91	8
Diazepam	88	11
Fentanyl	88	7
Flurazepam 1	96	6
Hydrocodone	83	8
Hydromorphone	107	16
MDMA	89	8
Methadone	95	9
Methamphetamine	105	5
Morphine	83	17
Naloxone	105	10
Norbuprenorphine	106	10
Nordiazepam	101	7
Norfentanyl	106	9
Oxycodone	97	11
PCP	91	11
Temazepam	88	9
THC-COOH	55	8

Figure 1:
Chromatogram of β-Gone with step by step dilution



HPLC Conditions

Column: Kinetex® 2.6 µm Biphenyl MercuryMS™ Cartridge
Dimensions: 20 x 2.0 mm
Part No.: 00M-4622-B0-CE
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol
Gradient:

Time (min)	B (%)
0	20
2	100
2.5	100
2.51	20
4	20

Flow Rate: 0.7 mL/min
Detection: MS/MS (API 4000™, SCIEX)

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
8E-S322-TUK	2 mL Centrifuge Tubes, Recombinant and Non-Recombinant Enzyme	100/Box

LC/MS Cartridges		
2.6 µm	Phase	20 x 2.0 mm
Kinetex	Biphenyl	00M-4622-B0-CE

Standard Cartridge Holders

Part No.	Description
CH0-5846	10 mm standard holder
CH0-5845	20 mm standard holder

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.

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guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and demensions, return the products with comparative data within 45 days for a FULL REFUND.

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