

APPLICATIONS

A Quick β -Glucuronidase Enzyme Removal Procedure Using β -GoneTM β -Glucuronidase Removal for the Analysis of Naltrexone

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Introduction

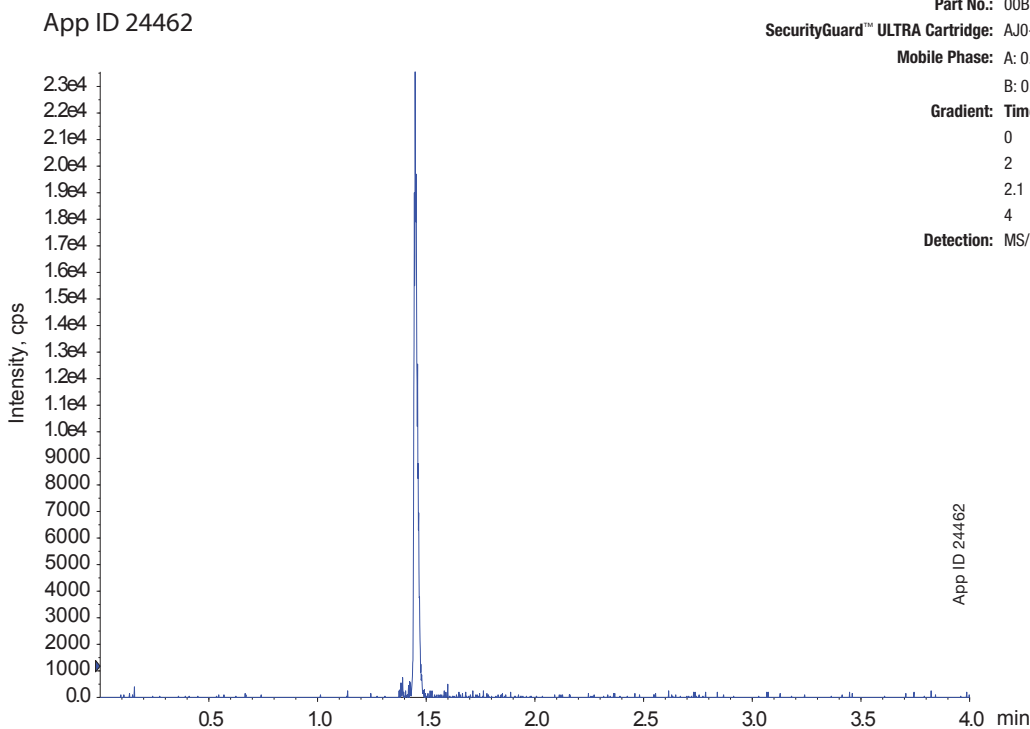
This application describes how β -Gone β -Glucuronidase Removal Products can be used for the analysis of naltrexone from urine that has been hydrolyzed using the β -Glucuronidase Enzyme BG100TM (Kura Biotec SpA). By selectively removing the β -glucuronidase, column lifetime is improved versus a standard "dilute-and-shoot" protocol while achieving exceptional recovery for the analyte of interest.

Materials and Methods

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

Average % Recovery	% CV (n=5)
100	3

Representative XIC for Naltrexone in Urine After Cleanup with β -Gone



Sample Preparation

Enzymatic Hydrolysis

BG100 Enzyme Hydrolysate was prepared as follows:

- 1) Add 10 μ L of analyte spike (1 μ g/mg) to 200 μ L urine
- 2) Dilute with 100 μ L of 0.1 M ammonium acetate buffer and add 40 μ L of Kura BG100 Beta Glucuronidase Solution from Red Abalone, Activity >100KU/mL (Kura Biotec, Part No.: 9001-45-0, Lot No. 4022)
- 3) Add 400 μ L 0.1 % Formic acid

β -Gone Protocol

- 1) Dilute 200 μ L of Urine Hydrolysate with 133 μ L 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 5 μ L for analysis

HPLC Conditions

Columns: Kinetex[®] 2.6 μ m Biphenyl 100Å
Dimensions: 50 x 2.1 mm
Part No.: 00B-4622-AN
SecurityGuardTM ULTRA Cartridge: AJO-8781
Mobile Phase: A: 0.1 % Formic acid in Water
 B: 0.1 % Formic acid in Acetonitrile
Gradient:

Time (min)	% B
0	5
2	100
2.1	5
4	5

Detection: MS/MS (SCIEX API 4000)



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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® 2.6 µm Minibore Columns (mm)

Phases	SecurityGuard™ ULTRA Cartridges [†]				3/pk
	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	
Biphenyl	00A-4622-AN	00B-4622-AN	00D-4622-AN	00F-4622-AN	AJO-9209

for 2.1 mm ID

Kinetex 2.6 µm MidBore™ Columns (mm)

Phases	SecurityGuard™ ULTRA Cartridges [†]			3/pk
	50 x 3.0	100 x 3.0	150 x 3.0	
Biphenyl	00B-4622-YO	00D-4622-YO	00F-4622-YO	AJO-9208

for 3.0 mm ID

[†] SecurityGuard ULTRA Cartridges required holder, Part No.: AJO-9000.



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 dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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



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