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

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**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 build up 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-YO
**Mobile Phase:**
- A: 0.1% Formic acid in Water
- B: 0.1% Formic acid in Methanol
**Flow Rate:** 0.7 mL/min
**Gradient:**
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>10</td>
</tr>
<tr>
<td>1.00</td>
<td>10</td>
</tr>
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<td>4.00</td>
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<td>5.00</td>
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</tr>
<tr>
<td>5.01</td>
<td>10</td>
</tr>
<tr>
<td>6.00</td>
<td>10</td>
</tr>
</tbody>
</table>
**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.**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Average Recovery %</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoylecgonine</td>
<td>109</td>
<td>3</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>93</td>
<td>6</td>
</tr>
<tr>
<td>Codeine</td>
<td>109</td>
<td>4</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>79</td>
<td>5</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>106</td>
<td>3</td>
</tr>
<tr>
<td>Norbuprenorphine</td>
<td>109</td>
<td>5</td>
</tr>
<tr>
<td>PCP</td>
<td>102</td>
<td>3</td>
</tr>
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</table>
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**

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>8B-S139-TAK</td>
<td>1 mL Tubes, Recombinant Enzyme</td>
<td>100/Box</td>
</tr>
<tr>
<td>8B-S322-D4K</td>
<td>1 mL Tubes, Non-Recombinant Enzyme</td>
<td>100/Box</td>
</tr>
<tr>
<td>8E-S139-TOA</td>
<td>96-Well Plate, Recombinant Enzyme</td>
<td>1/Box</td>
</tr>
<tr>
<td>8E-S322-DGA</td>
<td>96-Well Plate, Non-Recombinant Enzyme</td>
<td>1/Box</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phases</th>
<th>SecurityGuard™ ULTRA Cartridges</th>
<th>Unit</th>
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<tbody>
<tr>
<td>Biphenyl</td>
<td>00A-4622-AN</td>
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<tr>
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<td>00B-4622-AN</td>
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<tr>
<td></td>
<td>00D-4622-AN</td>
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<tr>
<td></td>
<td>00F-4622-AN</td>
<td>AJ0-9209</td>
</tr>
</tbody>
</table>

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

**Kinetex 2.6 μm Minibore Columns (mm)**

<table>
<thead>
<tr>
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</tr>
<tr>
<td></td>
<td>00F-4622-AN</td>
<td>AJ0-9209</td>
</tr>
</tbody>
</table>

*Phases indicate ID size.*

**Kinetex 2.6 μm MidBore™ Columns (mm)**

<table>
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<th>Phases</th>
<th>SecurityGuard™ ULTRA Cartridges</th>
<th>Unit</th>
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</thead>
<tbody>
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<td>Biphenyl</td>
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</tr>
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<td>00D-4622-YO</td>
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<tr>
<td></td>
<td>00F-4622-YO</td>
<td>AJ0-9208</td>
</tr>
</tbody>
</table>

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

Vacuum Manifolds

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Description</th>
<th>Unit</th>
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</thead>
<tbody>
<tr>
<td>AH0-6023</td>
<td>12-Position Vacuum Manifold Set, complete assembly</td>
<td>ea</td>
</tr>
<tr>
<td>AH0-6024</td>
<td>24-Position Vacuum Manifold Set, complete assembly</td>
<td>ea</td>
</tr>
</tbody>
</table>

* 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**

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>AH0-9334</td>
<td>Presston 100 Positive Pressure Manifold, 96-Well Plate</td>
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<tr>
<td>AH0-9342</td>
<td>Presston 100 Positive Pressure Manifold, 1 mL Tube Complete Assembly</td>
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<tr>
<td>AH0-9347</td>
<td>Presston 100 Positive Pressure Manifold, 3 mL Tube Complete Assembly</td>
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<tr>
<td>AH0-9343</td>
<td>Presston 100 Positive Pressure Manifold, 6 mL Tube Complete Assembly</td>
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**The Presston 100 96-Well Positive Pressure Manifold can also process 1, 3, and 6 mL tubes using the following adapter kits**

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>AH0-9344</td>
<td>1 mL Tube Adapter Kit</td>
</tr>
<tr>
<td>AH0-9345</td>
<td>3 mL Tube Adapter Kit</td>
</tr>
<tr>
<td>AH0-9346</td>
<td>6 mL Tube Adapter Kit</td>
</tr>
</tbody>
</table>

**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/Preston](http://www.phenomenex.com/Preston) for complete warranty information.
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