Efficient Removal of Beta-Glucuronidase Enzyme for Quantitative Analysis of Therapeutic Drugs and Drugs of Abuse in Urine by LC-MS/MS

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INTRODUCTION

It is widely accepted in urine drug testing (UDT) that enzymatic hydrolysis is the preferred practice to increase target analyte detection limits, minimize cost, and improve clinical utility of the assay. The addition of beta-glucuronidase for hydrolysis of urine samples has been vastly adopted in labs across the globe. Since cutting lab expenses continues to be a focal point in many of today's UDT labs, dilute-and-shoot sample analysis has become more prevalent. However, due to its potential impact on LC-MS/MS robustness, labs must balance the sample dilution factor, analyte sensitivity, and avoid injecting added proteins from the beta-glucuronidase into the system. Large dilutions or protein precipitation with centrifugation are standard protocols in clinical and forensic laboratories alike. Achieving the necessary cutoff levels robustly can be challenging, especially without the luxury of high-end LC-MS/MS instrumentation.

In this presentation, we demonstrate an improved, automated process coupled with a product innovation known as Membrane XTRaction Technology that rapidly removes beta-gluronidase enzyme and other proteins from a urine sample, post hydrolysis. Membrane XTRation Technology is a patent pending technology compromised of a device (Figure 1) that houses a membrane to be used with a high throughput robotic liquid handler. In this study, a proprietary membrane was used for enzyme removal (GlucX). The GlucX product minimizes the required sample dilution factor that is otherwise necessary, improves the limits of detection for all test method analytes, and does not require protein precipitation with off-line centrifugation. This novel, automated method using GlucX removes the protein content from the sample, providing beta-glucuronidase free injection on the LC-MS/MS.

Membrane XTRaction Technology (MXT™)

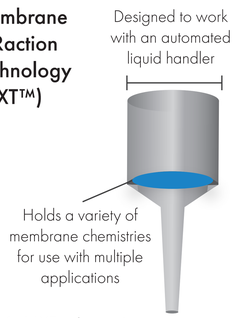


Figure 1. MXT schematic

MATERIALS AND METHODS

Sample Preparation:

Using a Hamilton Nimbus automated liquid handler, a 35µL aliquot of B-One™ buffer stabilized beta-glucuronidase enzyme solution from Kura Biotech was added to 35µL urine sample. This "All-In-One" enzyme was chosen in order to take advantage of the online room temperature incubation. This product makes it possible to hydrolyze urine samples on the deck of a liquid handler. 10µL of internal standard and 35µL of water were added and the sample was incubated at room temperature for 15 minutes. Post hydrolysis, 25µL of 2% formic acid in methanol is added to each sample. This 125µL solution is then dispensed through a GlucX Membrane XTRaction Tip (MX Tip), from DPX Technolgies, passing the solution through a proprietary membrane designed for enzyme removal, as shown in Figure 2 via the method schematic.

At 20% methanol, the solution does not require any additional dilution for LC-MS/MS analysis. The recovery and precision of a variety of therapeutic and abused drugs were monitored. Protein content of beta-glucuronidase was also monitored via gel electrophoresis.

LC-MS/MS Method:

AB Sciex 4000
Shimadzu LC pumps 20AD
CTC autosampler
Mobile Phase A was 0.1% formic acid in Water
Mobile Phase B was Methanol neat
Phenomenex B-phenyl 3 x 50mm
Flow rate: 0.4 mL/min
Starting conditions: 15%B

Time	Flow	%A	%B
0.00	500	85.0	15.0
0.10	500	85.0	15.0
6.00	500	5.0	95.0
8.00	500	5.0	95.0
8.20	500	85.0	15.0
9.50	500	85.0	15.0

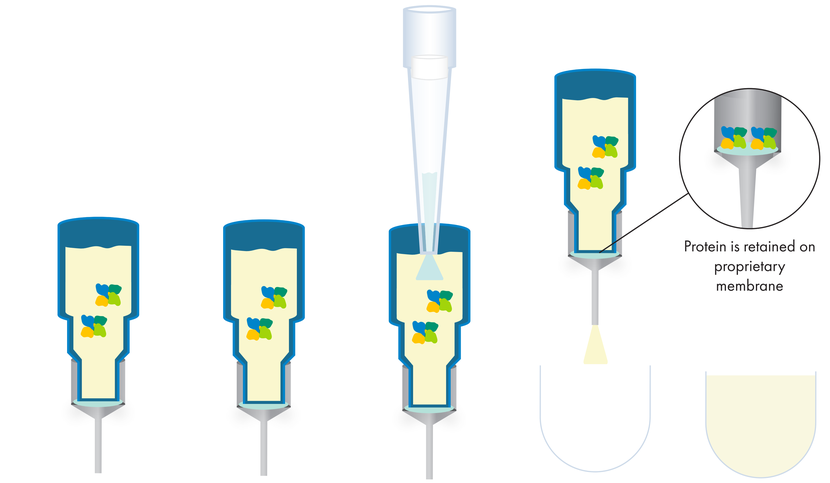
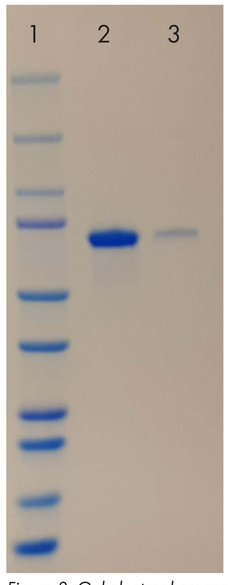


Figure 2. Enzyme removal method schematic

RESULTS AND DISCUSSION

The amount of beta-glucuronidase in the unfiltered sample and the filtered sample was observed via gel electrophoresis as shown in Figure 3. The pictured gel demonstrates greater than 80% removal of the beta-glucuronidase enzyme. The recovery of the analytes monitored, as shown in Figure 4, was found to meet acceptance criteria according to SAMSHA recommendations. Coupled with the minimal required dilution factor compared to traditional dilute-and-shoot protocols, this method provides the sensitivity required for clinical, forensic, and employment drug testing labs.



Run 4-20% Gradient PAGE

Lane Key:
1. Protein Marker
2. Sample
3. Sample Breakthrough

Figure 3. Gel electropherogram

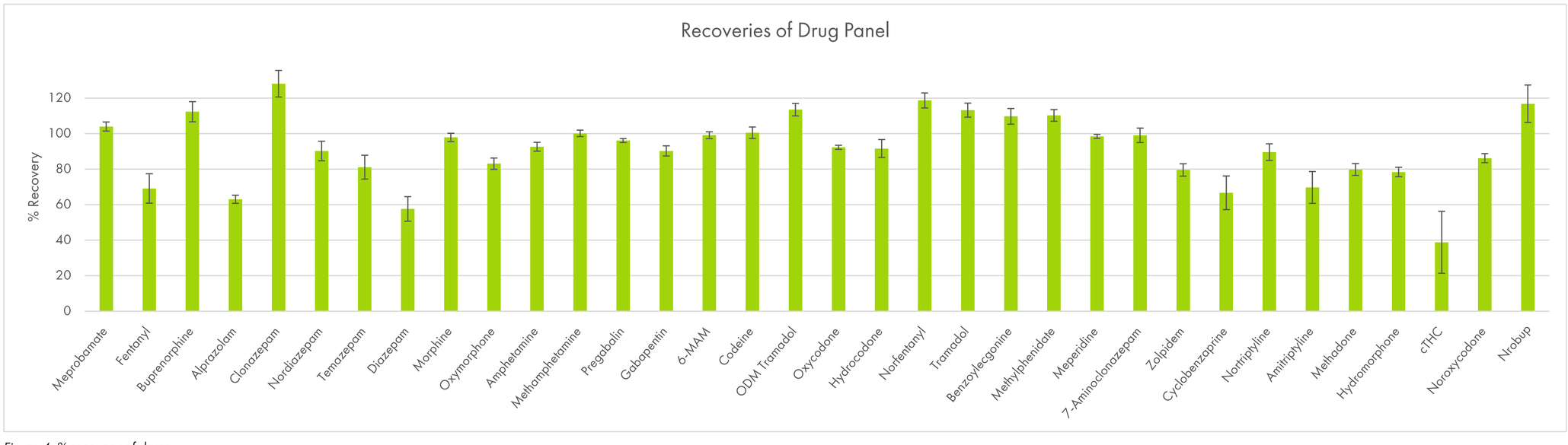


Figure 4. % recovery of drugs