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Tip-on-Tip™ Urine Filtration

HIGHLIGHTS: Reduced Hands-On Time, High Reproducibility, High Throughput, Seamless Integration

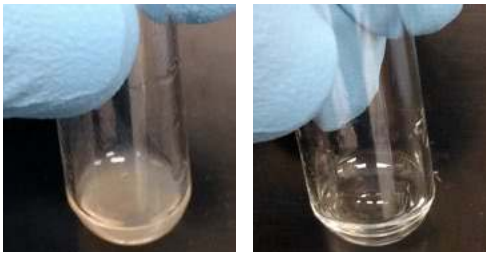
PURPOSE AND OBJECTIVE

- Demonstrate the utility of DPX Low Porosity Filtration Tip for β-glucuronidase and particulate removal prior to analysis
- Fast method that will help extend LC column life and reduce instrument maintenance
- Improve robustness of dilute and shoot methods resulting in minimal loss of drug
- Process up to 96 samples in under 5 minutes replacing labor intensive filtration plates or centrifugation steps

METHOD

A.

B.



C.

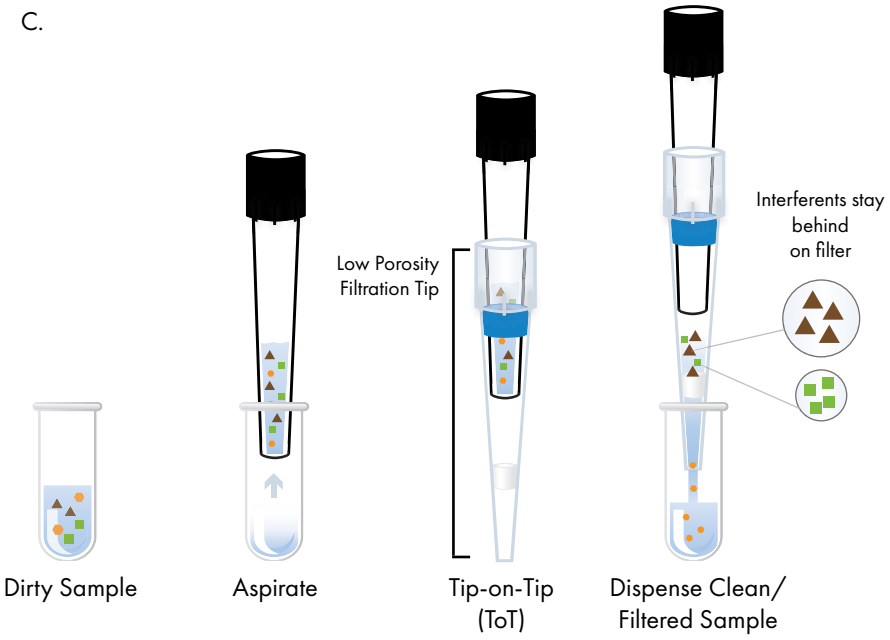


Figure 1.
A. All sample preparation was performed on a Nimbus96 as shown here.
B. Crude β-glucuronidase urine sample before and after ToT filtration
C. Schematic represents ToT filtration workflow. The sample was first diluted with MeOH. The filtrate was diluted with water prior to LC-MS/MS analysis.

ToT technology was utilized for filtration of post-hydrolysis urine samples using the DPX Low Porosity (< 1 mm) Filtration Tip. The ToT Filtration method involves aspirating the post-hydrolysis urine sample, diluting with methanol (ratios vary), attaching the filtration tip, and then dispensing the solution through the filter into a clean well plate. Most labs will require a dilution with water to an appropriate final percentage of methanol. Dilute and shoot methods usually incorporate some filtration or centrifugation to eliminate potential clogging of the LC column. ToT filtration provides an automated high-throughput alternate to laborious filtration plates and centrifugation steps. Further, it provides additional removal of β-glucuronidase while minimizing losses of commonly analyzed compounds, like therapeutic and abused drugs.

This method was evaluated for β-glucuronidase removal, filtrate volume reproducibility and analyte recovery.

RESULTS AND DISCUSSION

β-glucuronidase removal

Two commercially available b-glucuronidase enzymes were evaluated using a combination of Bradford Assay and UPLC-DAD. Varying amounts of methanol were added to the sample to enhance the β-glucuronidase removal. A visual representation of the crude b-glucuronidase urine sample before and after ToT Filtration is shown (Figure 1. B). The other purified β-glucuronidase removal is shown in Figure 2. Figure 2 highlights the chromatographic peak intensity decreasing with increasing amounts of methanol at the three wavelengths monitored, 215 nm, 280 nm, and 254 nm. Table 1 summarizes the study with average percent removal calculated by peak area relative to the control shown in Figure 2. As little as a 1:1 dilution factor with methanol results in greater than 75% β-glucuronidase removal. Increasing the methanol percentage pushes the recovery to over 90%.

Filtrate Volume Reproducibility

The dead volume inherent to the Low Porosity Filtration tip and the variability of the filtrate volume were evaluated by taking 200 µL 1:1 methanol:water through the ToT method and analyzing each well by weight. The conversions of those weights to volumes are shown in Figure 2. The average filtrate volume was 176 µL, equating to a Low Porosity Filtration Tip dead volume of 24 µL. Further, the percent coefficient of variation (%CV) of the volumes across the well plate was 1.9%.

	1	2	3	4	5	6	7	8	9	10	11	12
A	175	178	177	181	179	180	177	175	172	177	177	169
B	179	170	176	179	178	172	175	168	177	183	173	171
C	182	176	181	172	181	176	184	175	182	178	181	172
D	177	175	179	177	174	175	179	171	172	178	174	172
E	179	174	183	171	178	178	175	175	176	173	179	172
F	177	180	177	176	167	180	172	174	174	178	173	174
G	178	178	172	176	174	176	174	172	174	182	178	175
H	178	181	178	176	173	176	177	179	176	178	174	177

Figure 3. Filtrate volume variances across a 96-well plate.

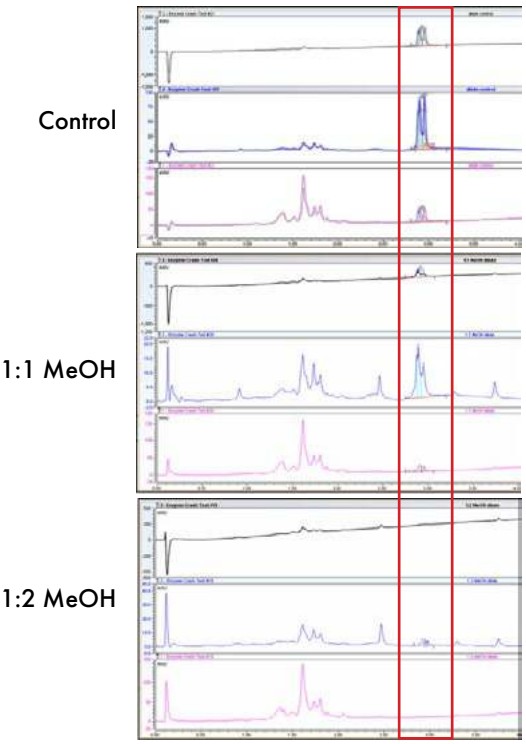


Figure 2. Urine filtration β-glucuronidase removal

Average % Removal			
	215 nm	280 nm	254 nm
1:3 MeOH	96	94	90
1:2 MeOH	100	97	100
1:1 MeOH	76	77	78

Table 1. Comparison of β-glucuronidase removal with varying methanol ratios at 3 wavelengths.

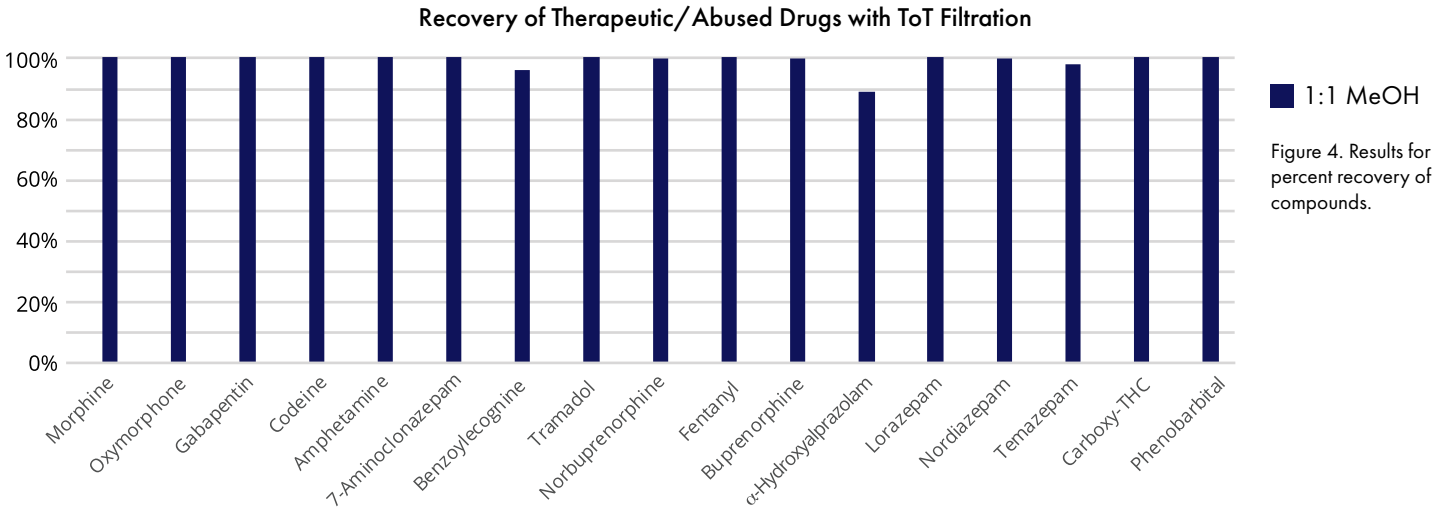


Figure 4. Results for percent recovery of compounds.

Common Analyte Recovery

Lastly, common analytes of interest in urine (therapeutic/abused drugs) were evaluated for recovery during the ToT method. The results for the recovery of the compounds in 1:1 methanol:water are shown in Figure 4. The filtrate was diluted with water prior to analysis with LC-MS/MS. Any increase in methanol content prior to filtration will only help to boost recoveries and β-glucuronidase removal.