**Improved Method for the Analysis of a Pain Management Supplemental Panel in Urine using the Thomson eXtreme Filter Vials® by LC-MS/MS**

**Abstract**

This improved sample preparation method allows for the quantitative measurement of the following pain management drugs in urine. The urine samples were diluted and filtered using Thomson eXtreme® filter vials, followed by LC/MS/MS analysis. The most critical aspects of reliable urine analysis are the reduction of interferences from the sample matrix and analyte recovery. Traditionally, SPE, SiC, and centrifugation have been used to reduce matrix interference prior to MS analysis; however, these techniques are time-consuming, adversely impact recovery, require expensive consumables, lab equipment, and use large amounts of solvent. Thomson eXtreme® filter vials (patented) offer multi-layer filtration for viscous samples and samples containing up to 30% solid particulates. The filter vial consists of two parts: a filter vial outer shell and a plunger, which includes the multi-layer filter on one end and a vial cap on the other end.

**Experimental**

**Equipment:**
- ABI 4500 QTRAP Mass Spectrometer
- Shimadzu Prominence HPLC equipped with:
  - Autosampler: SIL-20AC HT
  - Pumps: LC-20AD
  - Communication Bus Module: CBM-20A
  - Columns: CTO-20A
  - Degasser: DGU-20A, R
- Columns: Ultra Biphenyl Column (5μm SD x 3.5 mm) - Analytical
  - Ultra Biphenyl Column (5μm SD x 2.1 mm) - Analyte
- Eppendorf Micropure Vortex Mixer
- HSV-48 position Vial Filter Press (p/n 35010-00)

**Method:**
- Flow Rate: 0.5 mL/min
- Mobile Phases:
  A: 0.1% Formic Acid in HPLC Water
  B: 0.1% Formic Acid in Methanol
- Run Time: 8.5 minutes
- Injection Volume: 15µL

**Improved Sample Preparation**

- Place 400 µL of 20% MeOH / 80% Water / 0.1% Formic Acid each in the outer shells of the Thomson Filter Vial.
- Add 25 µL of Standard/Control/Patient Sample + 15µL of Internal Standard.
- Place Thomson Filter Plunger on top of the Thomson vial. Thomson vials - eXtreme® 0.2µm PVDF, w/Pre-SK Red Cap #35031.
- Press filter plunger down approximately ⅓ of the way into each of the Thomson vials.
- Vortex for 30-40 seconds.
- Slowly press filter plunger the rest of the way down using the Vial Filter Press.
- Extracts are ready for LC/MS/MS analysis using the Shimadzu / ABI 4500.
- Inject 15µL.

**Results**

This improved sample preparation method allows for the quantitative measurement of the following pain management drugs in urine. Table 1. The improved method utilizes the Thomson eXtreme® filter vials for clean-up significantly reducing the cost and time of per sample analysis. This method was validated for all 17 drugs in the supplemental pain management panel over 3 days.

**Table 1. Drugs analyzed as part of the Pain Management Supplemental Panel in urine.**

<table>
<thead>
<tr>
<th>Aminophylline</th>
<th>Cyclobenzaprine</th>
<th>Desipramine</th>
<th>Diphenylpropionic Acid</th>
<th>Tramadol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norbuphine</td>
<td>Dextroamphetamine</td>
<td>Meprobamate</td>
<td>Pregabalin</td>
<td></td>
</tr>
<tr>
<td>Carisoprodol</td>
<td>Gabapentin</td>
<td>Norpropbene</td>
<td>Tapentadol</td>
<td></td>
</tr>
<tr>
<td>Mecamylate</td>
<td>Imipramine</td>
<td>Methylphenidate</td>
<td>Tapentadol-O-Sulfate</td>
<td></td>
</tr>
</tbody>
</table>

**Data**

Fig. 1. Calibration curves for Amitriptyline, Gabapentin, and Tapentadol. Correlation Coefficients are > 0.99.

**Conclusion**

This validated method alleviates the need for sample clean-up by SPE or SLE. This reduces the amount of equipment needed, solvent usage, and sample preparation time. Samples are filtered by pipetting the sample into the filter vial shell, inserting the plunger into the shell, and then pushing the plunger into the shell. The filtration process from sample pipetting to autosampler ready only requires 15 seconds. Benefits to the use of Thomson eXtreme® vials include lower cost, faster sample preparation time, less use and disposal of organic solvents.

**New Method Benefits**

- **Sample Pre-treatment:**
  - Eliminates need for SPE or SLE
- **Equipment:**
  - Lower cost, faster sample preparation time
- **Solvents:**
  - Less use and disposal of organic solvents

**Acknowledgements**

Health Network Laboratories - Nadine Koenig, Crystal Kander, and Melaine Stauffer

Analytical Associates - Dean Fritch

**Disclaimer**

Thomson Instrument Company is not affiliated with Health Network Laboratories®, Analytical Associates, Eppendorf®, or their products.