THOMSON Solutions ™ At Work
INSTRUMENT COMPANY

series	cap color	membrane	pore size	part #
eXtreme|FV®		PVDF	0.2µm	85531

Improved Method for the Analysis of a Pain Management Supplemental Panel in Urine using the eXtreme|FV® by LC-MS/MS

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Introduction

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 Thompson eXtreme|FV®, 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, SLE 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|FV® (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.

Equipment

- ABI 4500 Mass Spectrometer
- Shimadzu Prominence HPLC equipped with:
  - Autosampler: SIL-20AC HT
  - Pumps A, B: LC-20AD
  - Communication Bus Module: CBM-20A
  - Column Oven: CTO-20A
  - Degasser: DGU-20A5R
  - Column: Ultra Biphenyl Columns (5µm 50 x 2.1 mm) - Restek
  - Flow Rate: 0.5 mL/min
  - Injection Volume: 15µL
  - Mobile Phases:
    - A: 0.1% Formic Acid in HPLC Water
    - B: 0.1% Formic Acid in Methanol
- Eppendorf Mix Mate
- Thomson eXtreme|FV® 0.2µm PVDF (p/n 85531)
- Thomson 48 position Vial Filter Press (p/n 35015)

Sample Preparation

Improved Sample Preparation

1. Place 400 µL of 20% MeOH / 80%Water / 0.1% Formic Acid in each of the outer shells of the Thomson Filter Vials
2. Add 25µL of Standard/Control/Patient Sample + 10uL of Internal Standard
3. Place Thomson Filter Plunger on top of the Thomson vial, Thomson vials –eXtreme/FV® 0.2µm PVDF, w/Pre-Slit Red Cap #85531.
4. Press filter plunger down approximately ¼ of the way into each of the Thomson vials.
5. Vortex for 30-40 seconds
6. Slowly press filter plunger the rest of the way down using the Vial Filter Press.
7. Extracts are ready for LC/MS/MS analysis using the Shimadzu / ABI 4500
8. Inject 15µL

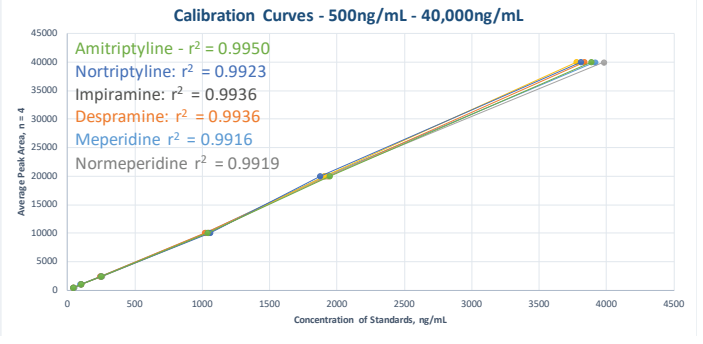
Results

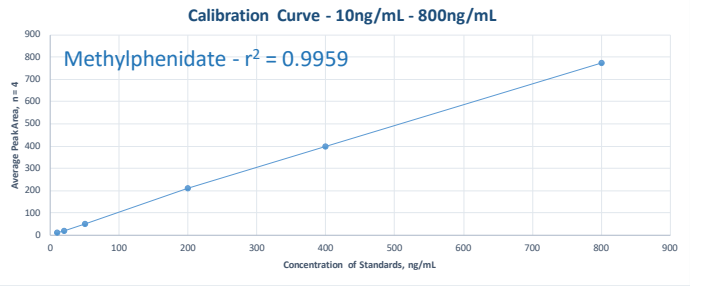
This improved sample preparation method allows for the quantitative measurement of the following pain management drugs in urine, Table 1. The improved method utilizes the Thomson eXtreme|FV® for sample clean-up significantly reducing the cost and time of per sample analysis. This method was validated for all 17 drugs in the supplemental pain management panel over 3 days. See Table 1 for the complete list of drugs in the panel. The 6 point calibration curve for Gabapentin in urine on Day 1 can be seen in fig 1. The R^2 was > 0.99. LC-MS/MS spectrum of the 17 drugs of interest in Table 1 can be seen in Fig 1.

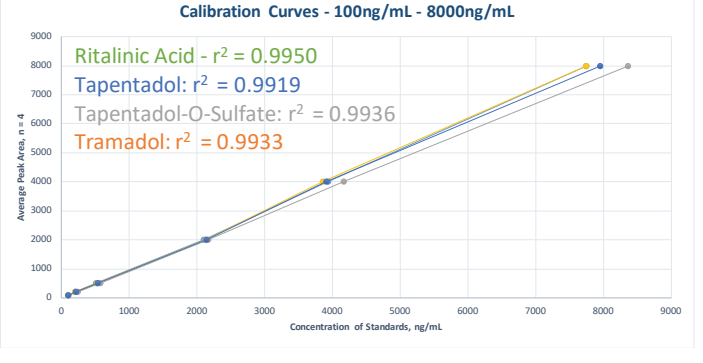
Table 1. Drugs analyzed as part of the Pain Management Supplemental Panel in urine.

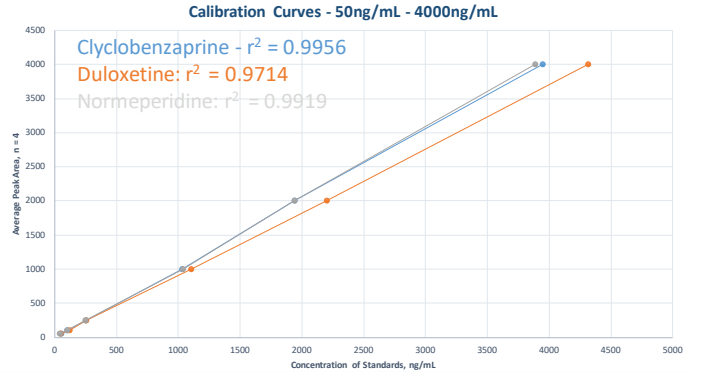
Tapentadol-O-Sulfate	Amitriptyline	Desipramine
Methylphenidate	Nortriptyline	Meperidine
Meprobamate	Carisoprodol	Tapentadol
Normeperidine	Imipramine	Pregabalin
Cyclobenzaprine	Gabapentin	Tramadol
Ritalinic Acid	Duloxetine	

Fig 1. 6 point calibration curve in urine.









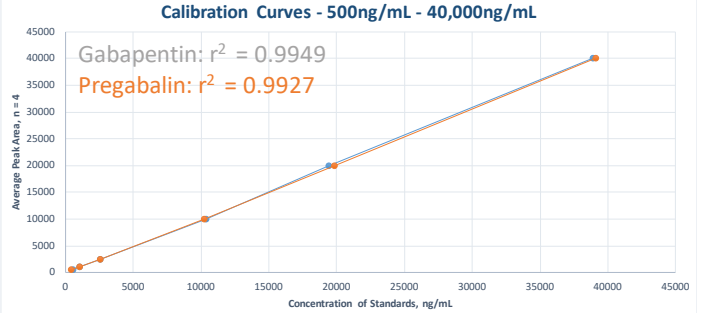
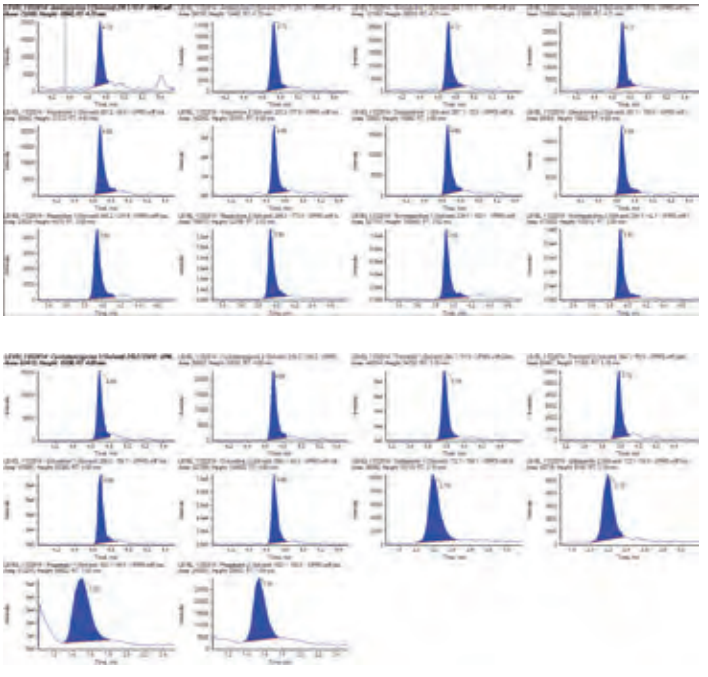


Fig 2. Mass spectrum of the 17 drugs included in the Supplemental Pain Management Panel in Urine.



Conclusion

This validated method alleviates the need for sample clean-up by SPE or SLE thereby reducing the amount of equipment required, 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 eXtremeIFV® include lower cost, faster sample preparation time, less use and disposal of organic solvents.

Extraction time for 96 samples may be reduced from 4 hours to 20 minutes

Time savings using eXtremeIFV®