

series	cap color	membrane	pore size	part #
eXtremelFV®		PVDF	0.2µm	85531

Sample Preparation for the Analysis of 12 Opiates in Urine using the eXtremelFV® by LC-MS/MS

Nadine Koenig¹, Crystal Xander¹, Melanie Stauffer¹, Dean Fritch²

¹Health Network Laboratories, Allentown, PA.

²Analytical Associates, Inc., East Greenville, PA.

Introduction

This improved sample preparation method allows for the quantitative measurement of Opioids in urine. Opioids are highly addictive and affects nearly 5 million people in the U.S. Opioids include naturally occurring Opiates, semi-synthetic opioids derived from morphine and synthetic opioids are analgesic alkaloids found naturally in *Papaver somniferum*, poppy plant. The urine samples are hydrolyzed, then prepared using the eXtremelFV®, 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. eXtremelFV®, were compared to an existing SPE sample preparation method to reduce the sample matrix causing interference prior to analysis. SPE is time consuming, adversely impacts recovery, uses large amounts of solvent and are expensive. The improved sample preparation method using the Thomson eXtremelFV® allows for the analysis of 12 Opioid Panel in urine.

Experimental

Equipment

- Thomson eXtremelFV® 0.2µm PVDF (p/n 85531)
- Thomson 48 position Vial Filter Press (Part # 35015)
- Eppendorf MixMate®
- Vortex Mixer
- Dry Block Heater set at 55°C ± 2°C
- Microcentrifuge
- AB Sciex 4500 Mass Spectrometer
- Shimadzu Prominence HPLC
 - Column: Restek Ultra Biphenyl Columns (5µm, 50 x 2.1 mm)
 - Mobile Phases:
 - A: 0.1% Formic Acid in HPLC Water
 - B: 0.1% Formic Acid in Methanol
 - Flow Rate: 0.5 mL/min
 - Run Time: 8.5 minutes
 - Injection Volume: 15µL

Sample Preparation

Table 1. Drugs analyzed in this Opiate Panel

6-Monoacetylmorphine	Hydrocodone	Norhydrocodone
β-Naltrexone	Hydromorphone	Noroxycodone
Codeine	Morphine	Oxycodone
Dihydrocodeine/Hydrocodol	Naltrexone	Oxymorphone

Improved Sample Preparation

1. Add 200 µL of 2% Methanol to each Thomson Vial.
2. Add 100 µL of the hydrolyzed urine sample to its respective Thomson Vial (see htslabs.com for hydrolysis steps used in this method)
3. Place Thomson Filter Plunger on top of Thomson Vial.
4. Press filter plunger down approximately ¼ of the way into each of the Thomson Vials.
5. Vortex for 2 minutes at 1750rpm using the Eppendorf Mix Mate.
6. Slowly press the filter plunger the rest of the way down using the Thomson 48 position press.
7. Samples are now ready for LC/MS/MS analysis

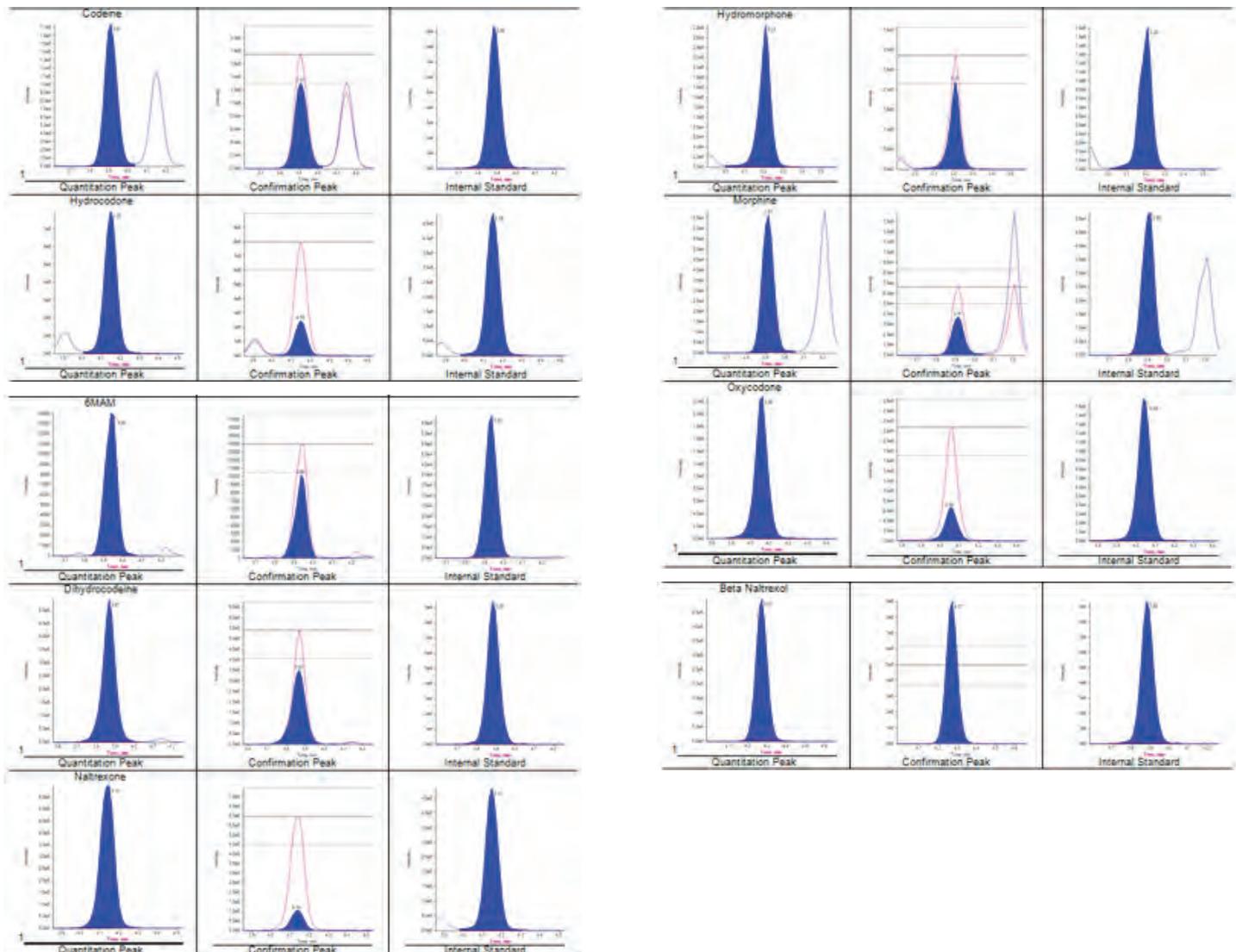
Results

The improved method utilizes the Thomson eXtreme Filter Vials for sample clean-up significantly reducing the cost and time of per sample analysis. This method was validated for all the analytes in Table 1. Mass spectrum of all the analytes in Table 1 can be seen in Fig. 1. Table 2 shows the validated concentrations used to generate a 6 point calibration curve. Linearity of the assay for the drugs listed in Table 1. Unextracted standards (neats) were run along with 3 different negative patient samples, extracted and spiked with standard and internal standard post extraction at the cutoff concentration to access ion suppression and drug recovery. To calculate drug recovery, the mean area counts of the extracted samples was compared to the mean area counts of the unextracted samples. To determine ion suppression, the mean concentration of the extracted samples was compared to the mean concentration of the post-extracted samples.

Table 2. Final concentrations for the various analytes

Level	Final Concentration (ng/mL) Opiates	Final Concentration (ng/mL) 6-MAM
Level 1	50	5
Level 2	200	20
Level 3	1000	50
Level 4	5000	250
Level 5	10000	500
Level 6	20000	1000

Fig 1. Mass Spectrum of Positive Results

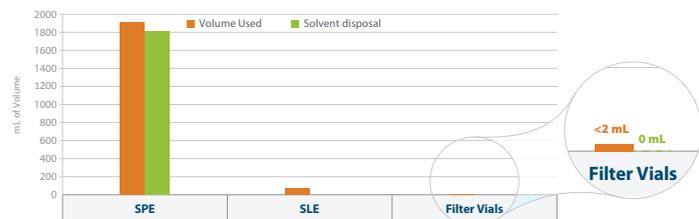


Conclusion

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 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® Filter Vials include lower cost, faster sample preparation time, less use and disposal of organic solvents.

Solvent waste generated is reduced from 1.8L to 0mL; when extracting 96 samples

Significantly less solvent used



Thomson Instrument Company is not affiliated with Health Network Laboratories®, Analytical Associates, Eppendorf®, IMCS - Integrated Micro- Chromatography System, Zymark or their products