

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



Nadine Koenig¹, Crystal Xander¹, Melanie Stauffer¹, Dean Fritch², Lisa Wanders³, Sam Ellis³
¹ Health Network Laboratories, 794 Roble Road, Allentown, PA 18109
² Analytical Associates, 225 Millwood Drive, East Greenville, PA
³ Thomson Instrument Company, 1121 South Cleveland Street Oceanside, CA 92054



Introduction or 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 |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.

Experimental

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-20A_R
 Column: Ultra Biphenyl Columns (5µm 150 x 2.1 mm) - Restek
 Eppendorf Mix Mate Vortex Mixer
 Thomson eXtreme |FV® 0.2µm PVDF (p/n 85531)
 Thomson 48 position Vial Filter Press (p/n 35010)

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 in each of the outer shells of the Thomson Filter Vials
- Add 25 µL of Standard/Control/Patient Sample + 10 µL of Internal Standard
- Place Thomson Filter Plunger on top of the Thomson vial, Thomson vials – eXtreme |FV® 0.2µm PVDF, w/ Pre-Slit Red Cap #85531.
- Press filter plunger down approximately ¼ 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 15 µL

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.

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

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

Data

Fig. 1. Mass spectrum of the 17 drugs included in the Supplemental Pain Management Panel in Urine.

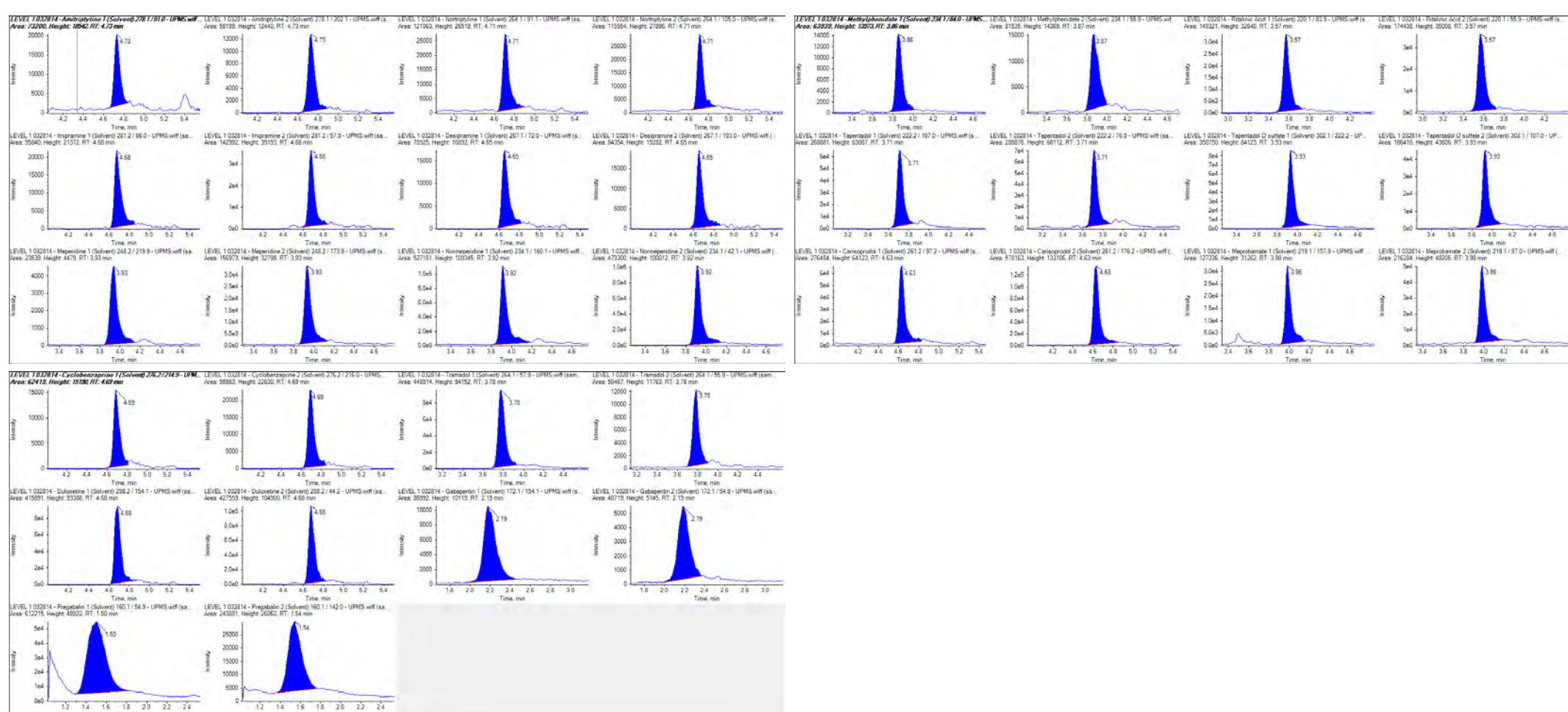
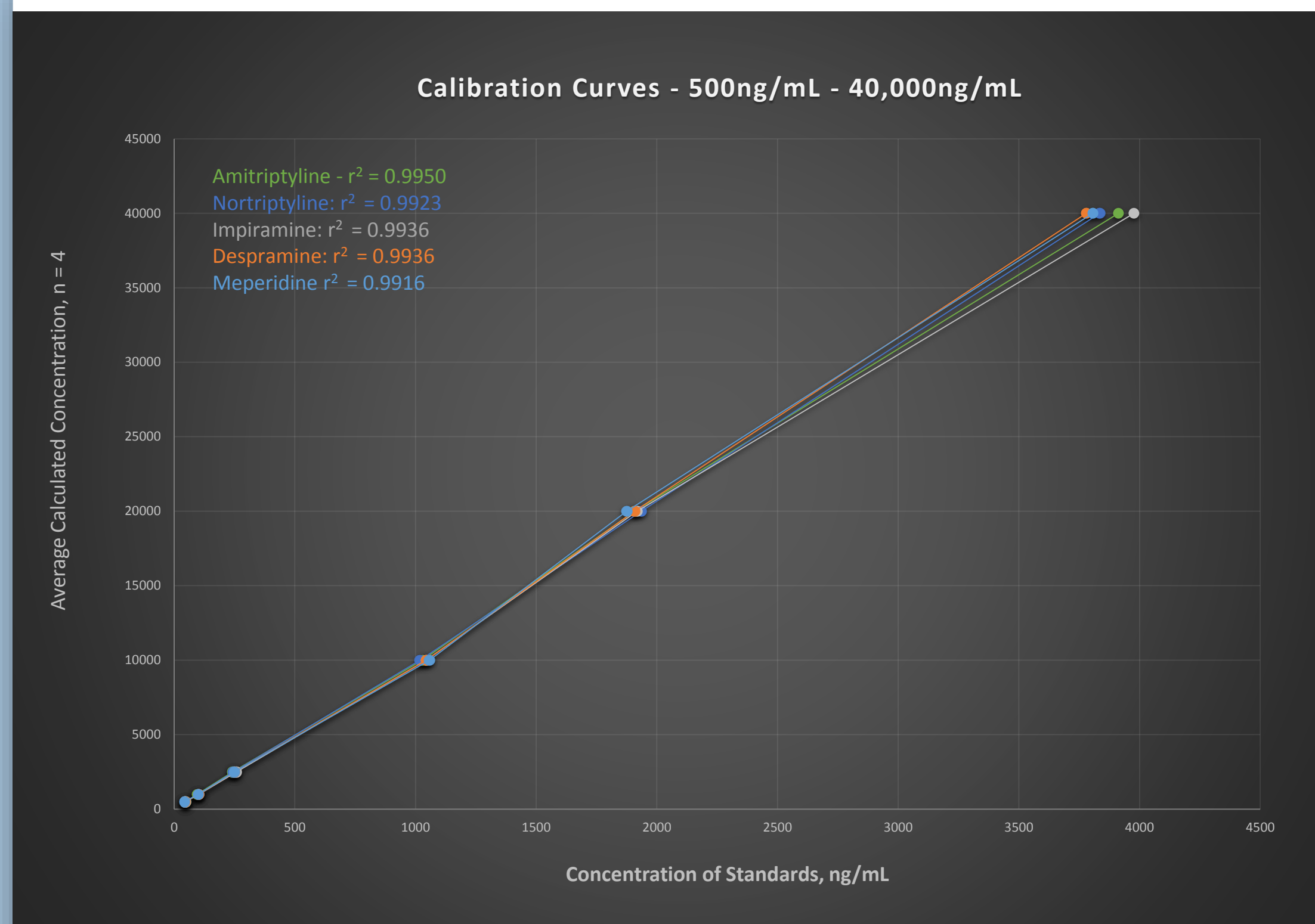


Fig. 2 Calibration curves for Amtriptlyline, Nortriptyline, Imipramine, Desipramine, and Meperidine. Correlation Coefficients are > 0.99.



Amtriptlyline Linearity/Carrier					Nortriptyline Linearity/Carrier					Imipramine Linearity/Carrier							
Sample	Conc	Mean	SD	% CV	% Accuracy	Sample	Conc	Mean	SD	% CV	% Accuracy	Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	47.5	2.3	4.8	95.0	Level 1	50	45.4	9.9	21.9	90.9	Level 1	50	47.3	5.8	12.2	94.6
Level 2	100	96.2	14.5	15.0	96.2	Level 2	100	99.8	10.1	10.2	99.8	Level 2	100	102.6	19.8	19.5	102.6
Level 3	250	241.1	21.0	8.7	96.5	Level 3	250	244.9	29.7	12.1	98.0	Level 3	250	257.7	32.2	10.2	103.1
Level 4	1000	1029.1	70.0	6.8	102.9	Level 4	1000	1018.9	75.2	7.4	101.9	Level 4	1000	1044.2	66.5	6.4	104.4
Level 5	2000	1908.2	138.7	7.3	95.4	Level 5	2000	1935.8	94.1	4.9	96.8	Level 5	2000	1938.2	143.2	7.4	95.9
Level 6	4000	3913.0	193.7	5.0	97.8	Level 6	4000	3835.2	210.7	5.5	95.9	Level 6	4000	3977.4	253.5	6.3	99.4
Blank	0					Blank	0					Blank	0				
Correlation Coefficient: 0.9950					Correlation Coefficient: 0.9923					Correlation Coefficient: 0.9936							
Within Run Precision					Within Run Precision					Within Run Precision							
Sample	Conc	Mean	SD	% CV	% Accuracy	Sample	Conc	Mean	SD	% CV	% Accuracy	Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	50	47.5	2.3	4.8	95.0	LOD/LOQ	50	45.4	9.9	21.9	90.9	LOD/LOQ	50	47.3	5.8	12.2	94.6
Recovery					Recovery					Recovery							
Sample	Mean Extracted	Mean Unextracted	% Recovery			Sample	Mean Extracted	Mean Unextracted	% Recovery			Sample	Mean Extracted	Mean Unextracted	% Recovery		
L1	68919	38502	57.7			L1	187214	25297	57.4			L1	65777	23267.75	20.4		
Ion Suppression					Ion Suppression					Ion Suppression							
Sample	Mean Extracted	Mean Unextracted	% Ion Suppression			Sample	Mean Extracted	Mean Unextracted	% Ion Suppression			Sample	Mean Extracted	Mean Unextracted	% Ion Suppression		
L1 Standard	183276.7	398669.7	59			L1 Standard	240252.7	329319.0	27			L1 Standard	148690.0	328302.3	55		
ISTD	1298615.0	3940545.0	66			ISTD	956226.7	1551280.7	38			ISTD	232738.8	627460.3	61		
Desipramine Linearity/Carrier					Meperidine Linearity/Carrier												
Sample	Conc	Mean	SD	% CV	% Accuracy	Sample	Conc	Mean	SD	% CV	% Accuracy						
Level 1	100	101.1	10.0	9.9	101.1	Level 1	100	101.7	2.6	2.6	101.7						
Level 2	200	201.0	19.5	9.6	101.5	Level 2	200	196.6	29.0	14.8	98.3						
Level 3	500	509.6	55.6	10.9	101.9	Level 3	500	521.3	59.9	11.5	104.3						
Level 4	2000	2205.3	169.4	7.7	110.3	Level 4	2000	2130.9	143.2	6.7	106.5						
Level 5	4000	3938.5	300.7	7.6	98.5	Level 5	4000	3808.5	222.6	5.8	95.5						
Level 6	8000	7750.5	370.2	4.8	96.9	Level 6	8000	7749.0	523.1	6.8	96.9						
Blank	0					Blank	0										
Correlation Coefficient: 0.9936					Correlation Coefficient: 0.9916												
Within Run Precision					Within Run Precision												
Sample	Conc	Mean	SD	% CV	% Accuracy	Sample	Conc	Mean	SD	% CV	% Accuracy						
LOD/LOQ	50	45.2	7.1	15.7	90.3	LOD/LOQ	50	43.0	10.2	23.8	86.0						
Recovery					Recovery												
Sample	Mean Extracted	Mean Unextracted	% Recovery			Sample	Mean Extracted	Mean Unextracted	% Recovery								
L1	115437	187828.5	61.5			L1	33794	55726	60.6								
Ion Suppression					Ion Suppression												
Sample	Mean Extracted	Mean Unextracted	% Ion Suppression			Sample	Mean Extracted	Mean Unextracted	% Ion Suppression								
L1 Standard	118046.7	193366.7	38			L1 Standard	40348.3	58403.0	28								
ISTD	459345.7	7439159.0	38			ISTD	304926.7	4927172.0	38								

Fig. 3 Calibration curves for Ritalinic Acid, Tapentadol, Tapentadol-O-Sulfate, and Tramadol. Correlation Coefficients are > 0.99.

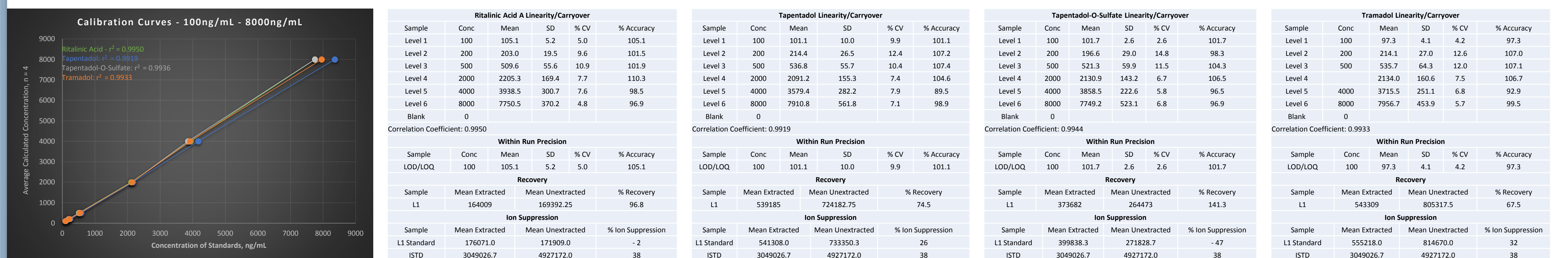


Fig. 4 Calibration curves for Cyclobenzaprine, Duloxetine, Normeperidine. Correlation Coefficients are > 0.99.

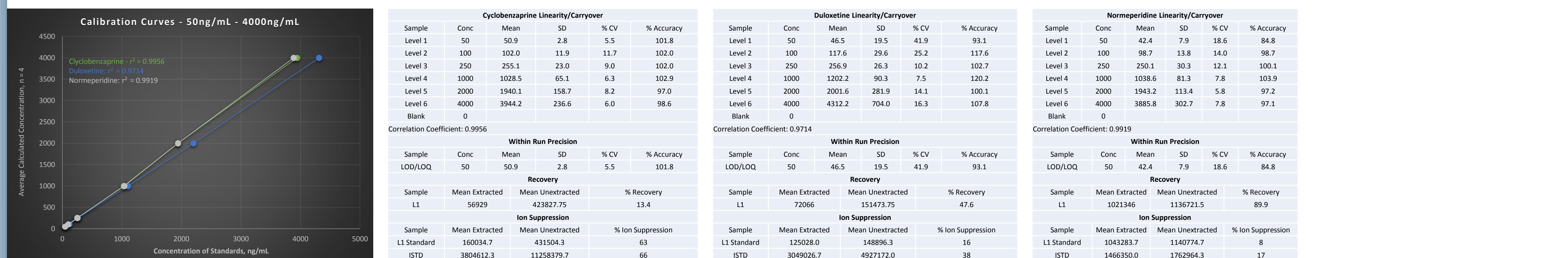
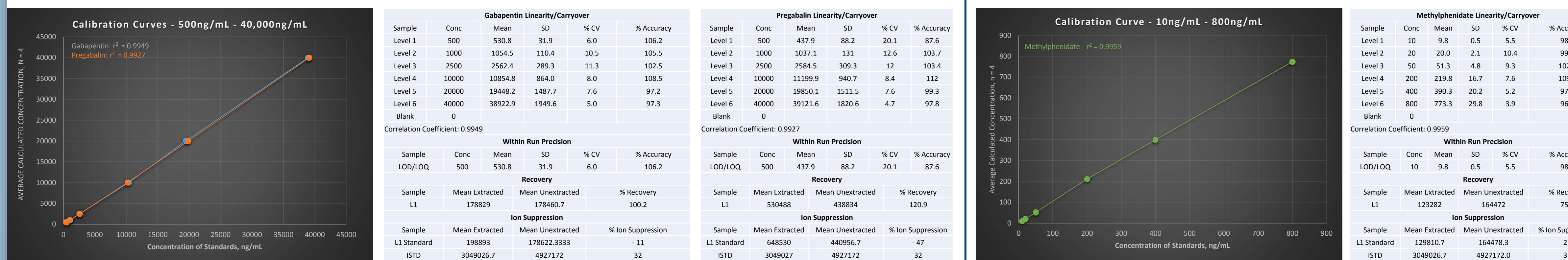


Fig. 5 Calibration curves for Gabapentin and Pregabalin. Correlation Coefficients are > 0.99.



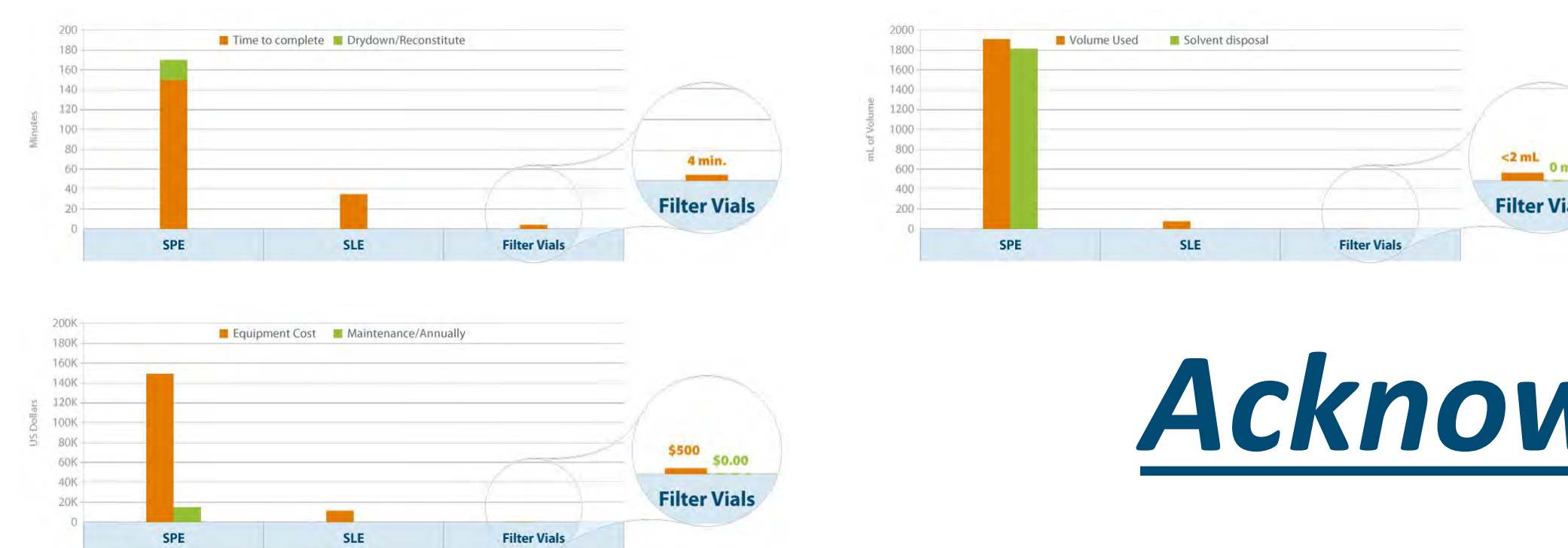
Conclusion

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 eXtreme |FV® include lower cost, faster sample preparation time, less use and disposal of organic solvents.

New Method Benefits

Method	# of Samples	Time to complete	Equipment Cost	Maintenance/Annually	Volume Solvent used	Solvent Disposal
SPE	96	150 min. + 20 min. dry down/reconstitute	~\$150,000.00	\$15,000.00	1920 mL	1824 mL
SLE	96	35 min.	~\$11,400.00	~\$100.00	76.8 mL	0 mL (it gets dried down)
Filter Vial	96	4 min.	\$500.00	\$0.00	< 2 mL	0 mL

*Significant time & money savings because lengthy wash steps are eliminated!



Acknowledgements

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