

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

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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

Method:

Flow Rate: 0.5 mL/min

Shimadzu Prominence HPLC equipped with

Mobile Phases:

Autosampler: SIL-20AC HT A: 0.1% Formic Acid in HPLC Water

Pumps A, B: LC-20AD B: 0.1% Formic Acid in Methanol

Communication Bus Module: CBM-20A Run Time: 8.5 minutes

Column Oven: CTO-20A Injection Volume: 15µL

Degasser: DGU-20A_R

Column: Ultra Biphenyl Columns (5µm 50 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)

Improved Sample Preparation Post Hydrolysis

- Place 400 µL of 20% MeOH / 80% Water / 0.1% Formic Acid in each of the outer shells of the Thomson Filter Vials
- Add 25µL of Standard/Control/Patient Sample + 10µL of Internal Standard
- Place Thomson Filter Plunger on top of the Thomson vial, Thomson vials -eXtreme|FV[®] 0.2µm PVDF, w/ Pre-Slit Rep 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 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.

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

Amitriptyline	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.

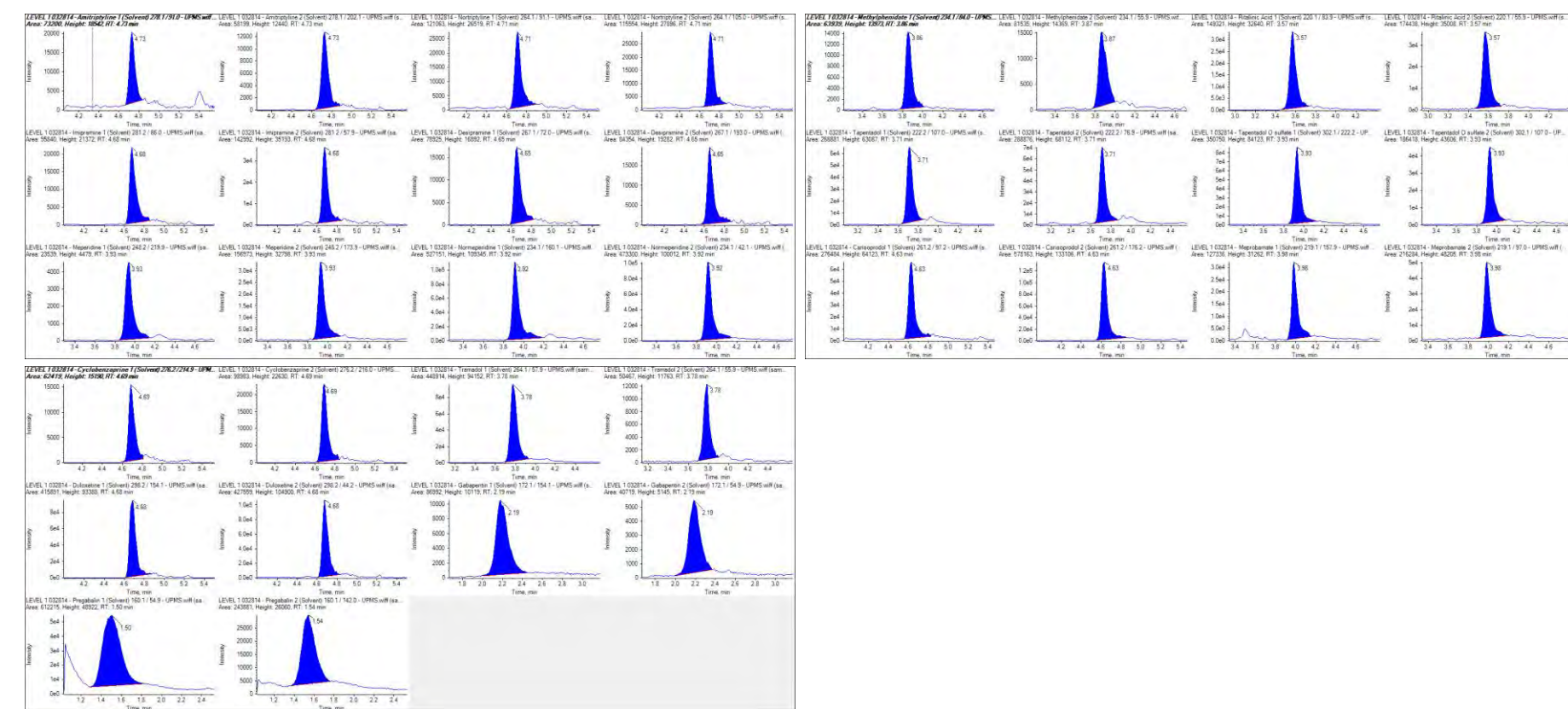
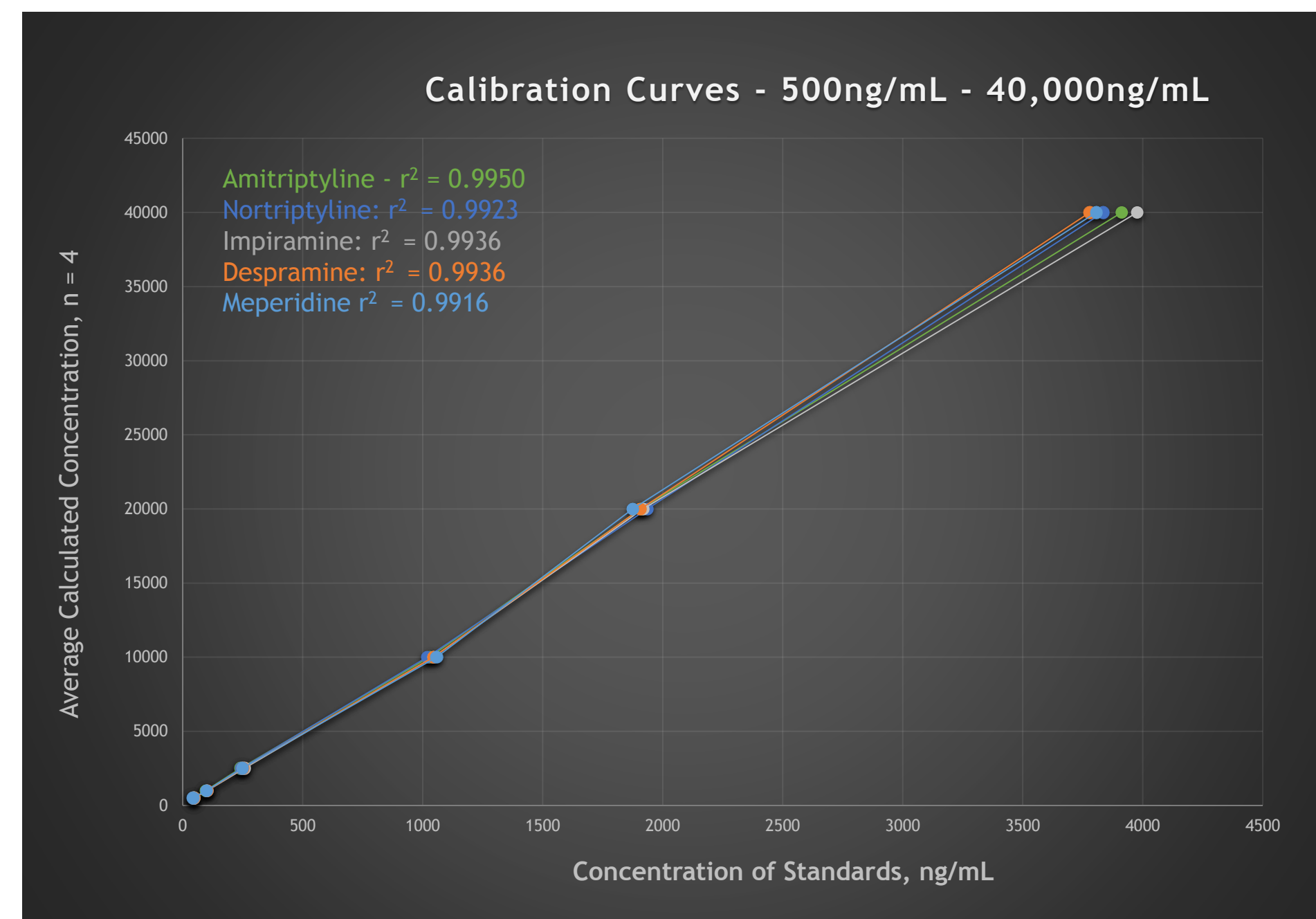


Fig. 2 Calibration curves for Amitriptyline, Nortriptyline, Imipramine, Desipramine, and Meperidine. Correlation Coefficients are > 0.99.



Amitriptyline - Linearity/Carryover						Nortriptyline Linearity/Carryover						Imipramine Linearity/Carryover							
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	95.9	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	13.8	13.5	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	26.2	10.2	103.1		
Level 4	1000	1020.1	70.0	6.8	102.9	Level 4	1000	1018.9	75.2	7.4	101.9	Level 4	1000	1044.2	46.5	4.4	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	1918.2	141.2	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	251.5	6.3	99.4		
Blank	0	0	0	0	0	Blank	0	0	0	0	0	Blank	0	0	0	0	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	95.9	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	Sample	Mean Extracted	Mean Unextracted	% Recovery	Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	6893	38402	17.7	L1	18724	32597	57.4	L1	65777	323067.5	20.4	L1	65777	323067.5	20.4	L1	65777	323067.5	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	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	395919.0	27	L1 Standard	146090.0	328303.3	55	L1 Standard	146090.0	328303.3	55	L1 Standard	146090.0	328303.3	55
L1 STD	1339615.0	3940450.0	66	L1 STD	1339615.0	3940450.0	66	L1 STD	1339615.0	3940450.0	66	L1 STD	1339615.0	3940450.0	66	L1 STD	1339615.0	3940450.0	66
Desipramine Linearity/Carryover						Desipramine Linearity/Carryover						Desipramine Linearity/Carryover							
Sample	Conc	Mean	SD	% CV	% Accuracy	Sample	Conc	Mean	SD	% CV	% Accuracy	Sample	Conc	Mean	SD	% CV	% Accuracy		
Level 1	50	45.2	7.1	15.7	90.3	Level 1	50	43.0	10.2	23.8	86.0	Level 1	50	43.0	10.2	23.8	86.0		
Level 2	100	101.7	11.3	11.1	101.7	Level 2	100	99.4	10.4	10.5	99.4	Level 2	100	99.4	10.4	10.5	99.4		
Level 3	250	250.9	21.8	8.7	100.4	Level 3	250	249.6	27.3	11.0	99.8	Level 3	250	249.6	27.3	11.0	99.8		
Level 4	1000	1044.5	83.0	7.9	104.5	Level 4	1000	1058.3	81.8	7.7	105.8	Level 4	1000	1058.3	81.8	7.7	105.8		
Level 5	2000	1907.2	131.4	6.9	95.4	Level 5	2000	1874.7	19.8	1.1	93.7	Level 5	2000	1874.7	19.8	1.1	93.7		
Level 6	4000	3779.8	288.5	7.6	94.5	Level 6	4000	3806.5	176.1	4.6	95.2	Level 6	4000	3806.5	176.1	4.6	95.2		
Blank	0	0	0	0	0	Blank	0	0	0	0	0	Blank	0	0	0	0	0		
Correlation Coefficient: 0.9926						Correlation Coefficient: 0.9916						Correlation Coefficient: 0.9916							
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	45.2	7.1	15.7	90.3	LOD/LOQ	50	43.0	10.2	23.8	86.0	LOD/LOQ	50	43.0	10.2	23.8	86.0		
Recovery						Recovery						Recovery							
Sample	Mean Extracted	Mean Unextracted	% Recovery	Sample	Mean Extracted	Mean Unextracted	% Recovery	Sample	Mean Extracted	Mean Unextracted	% Recovery	Sample	Mean Extracted	Mean Unextracted	% Recovery	Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	11547	187828.5	61.5	L1	11547	187828.5	61.5	L1	11547	187828.5	61.5	L1	11547	187828.5	61.5	L1	11547	187828.5	61.5
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	Sample	Mean Extracted	Mean Unextracted	% Ion Suppression	Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	178671.0	171909.0	-2	L1 Standard	178671.0	171909.0	-2	L1 Standard	178671.0	171909.0	-2	L1 Standard	178671.0	171909.0	-2	L1 Standard	178671.0	171909.0	-2
L1 STD	3049026.7	4927172.0	38	L1 STD	3049026.7	4927172.0	38	L1 STD	3049026.7	4927172.0	38	L1 STD	3049026.7	4927172.0	38	L1 STD	3049026.7	4927172.0	38

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

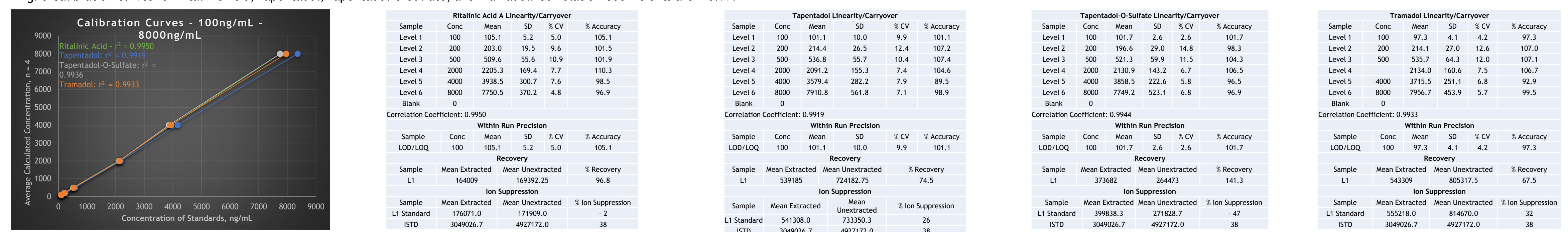


Fig. 4 Calibration curves for Cyclobenzaprine, Duloxetine, Normeperidine. Correlation Coefficients are > 0.99.

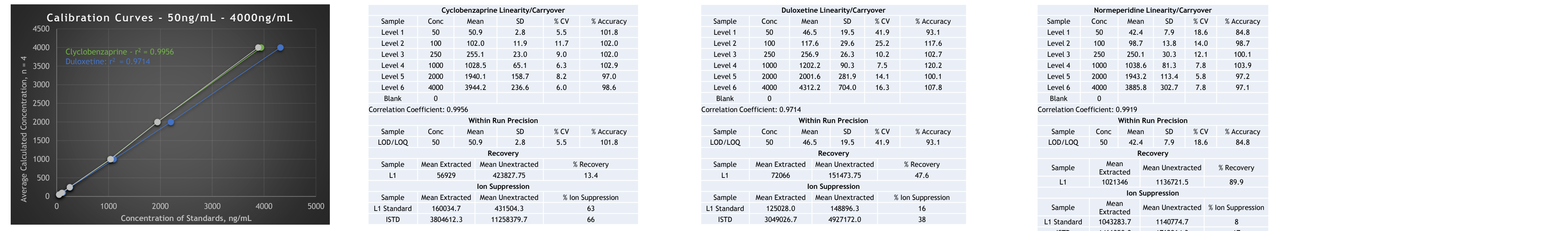


Fig. 5 Calibration curves for Gabapentin and Pregabalin. Correlation Coefficients are > 0.99.

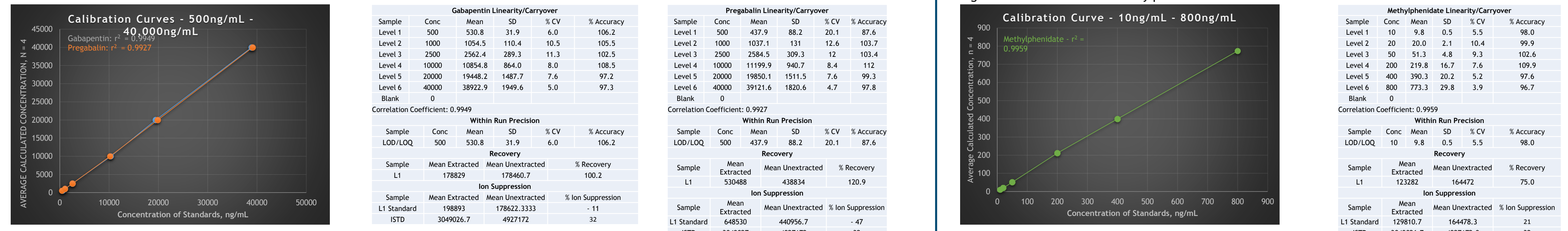
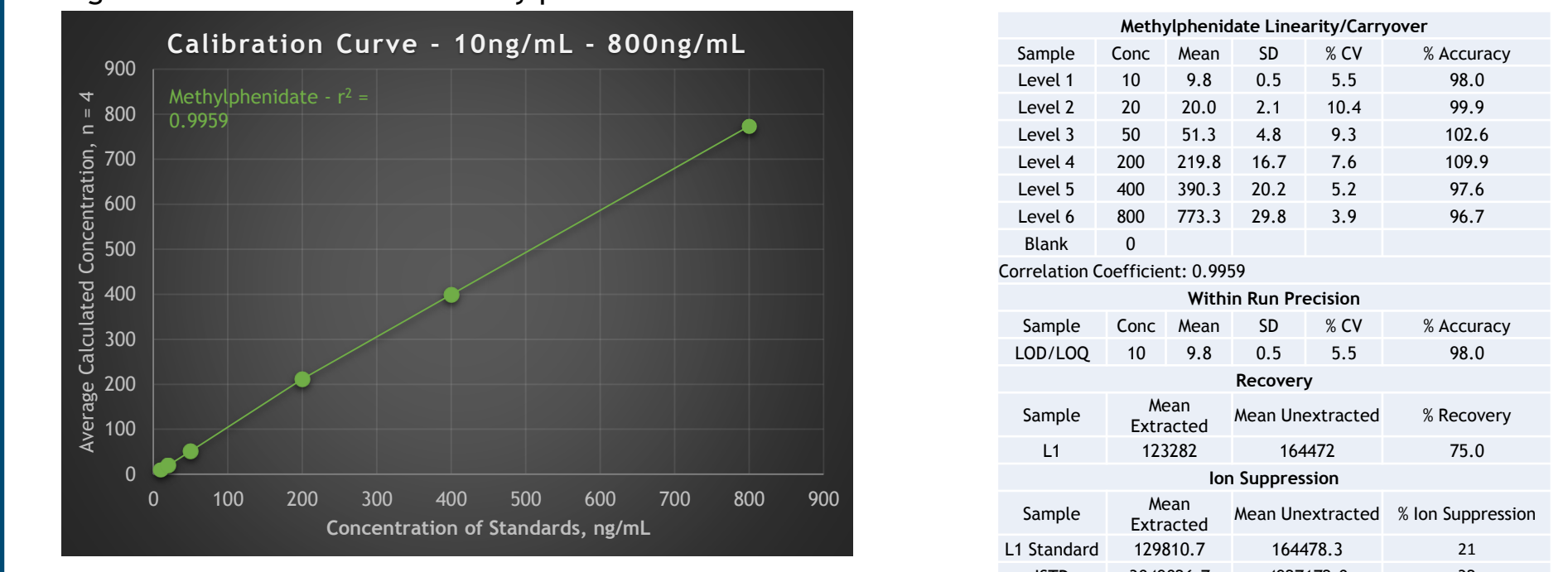


Fig. 6 Calibration curve for Methylphenidate. Correlation Coefficient is > 0.99



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.

