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 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.

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_3R
- 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)

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 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 + 10uL 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 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	Cuclomenzaprine	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.

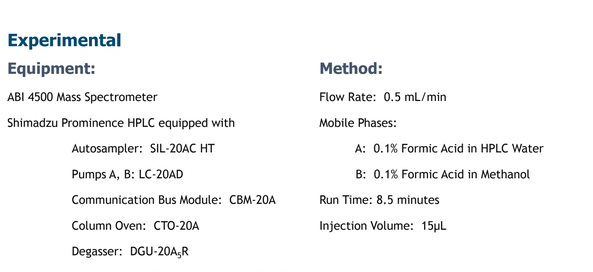
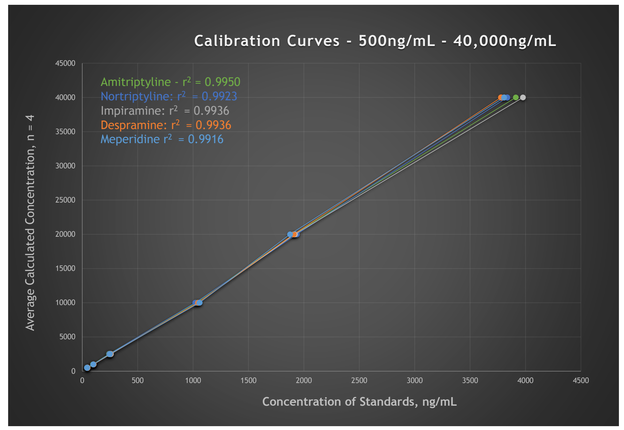


Fig. 2 Calibration curves for Amitriptyline, Nortriptyline, Impiramine, Despramine, and Meperidine. Correlation Coefficients are > 0.99.



Amitriptyline - Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	47.5	2.3	4.8	95.0
Level 2	100	96.2	14.5	15.0	96.2
Level 3	250	241.1	21.0	8.7	96.5
Level 4	1000	1029.1	70.0	6.8	102.9
Level 5	2000	1908.2	138.7	7.3	95.4
Level 6	4000	3913.0	193.7	5.0	97.8
Blank	0				

Correlation Coefficient: 0.9950

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	50	47.5	2.3	4.8	95.0

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	68913	389402	17.7

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	163276.7	398669.7	59
ISTD	1339615.0	3940545.0	66

Desipramine Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	45.2	7.1	15.7	90.3
Level 2	100	101.7	11.3	11.1	101.7
Level 3	250	250.9	21.8	8.7	100.4
Level 4	1000	1044.5	83.0	7.9	104.5
Level 5	2000	1907.2	131.4	6.9	95.4
Level 6	4000	3779.8	288.5	7.6	94.5
Blank	0				

Correlation Coefficient: 0.9936

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	50	45.2	7.1	15.7	90.3

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	115437	187828.5	61.5

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	138644.7	191306.7	28
ISTD	4593416.7	7439159.0	38

Nortriptyline Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	45.4	9.9	21.9	90.9
Level 2	100	99.8	10.1	10.2	99.8
Level 3	250	244.9	29.7	12.1	98.0
Level 4	1000	1018.9	75.2	7.4	101.9
Level 5	2000	1935.8	94.1	4.9	96.8
Level 6	4000	3835.2	210.7	5.5	95.9
Blank	0				

Correlation Coefficient: 0.9923

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	50	45.4	9.9	21.9	90.9

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	187214	325997	57.4

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	240252.7	329319.0	27
ISTD	864794.7	1454589.7	59

Meperidine Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	43.0	10.2	23.8	86.0
Level 2	100	99.4	10.4	10.5	99.4
Level 3	250	249.6	27.3	11.0	99.8
Level 4	1000	1058.3	81.8	7.7	105.8
Level 5	2000	1874.7	19.8	1.1	93.7
Level 6	4000	3806.5	176.1	4.6	95.2
Blank	0				

Correlation Coefficient: 0.9916

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	50	43.0	10.2	23.8	86.0

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	33794	55726	60.6

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	40364.3	55803.0	28
ISTD	3049026.7	4927172.0	38

Imipramine Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	47.3	5.8	12.2	94.6
Level 2	100	102.6	13.8	13.5	102.6
Level 3	250	257.7	26.2	10.2	103.1
Level 4	1000	1044.2	66.5	6.4	104.4
Level 5	2000	1918.2	141.2	7.4	95.9
Level 6	4000	3977.4	251.5	6.3	99.4
Blank	0				

Correlation Coefficient: 0.9936

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	50	47.3	5.8	12.2	94.6

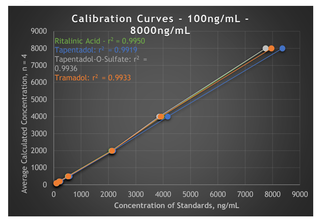
Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	65777	323067.75	20.4

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	148690.0	328302.3	55
ISTD	2327338.3	6274660.3	63

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



Ritalinic Acid A Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	100	105.1	5.2	5.0	105.1
Level 2	200	203.0	19.5	9.6	101.5
Level 3	500	509.6	55.6	10.9	101.9
Level 4	2000	2205.3	169.4	7.7	110.3
Level 5	4000	3938.5	300.7	7.6	98.5
Level 6	8000	7750.5	370.2	4.8	96.9
Blank	0				

Correlation Coefficient: 0.9950

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	100	105.1	5.2	5.0	105.1

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	164009	169392.25	96.8

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	176071.0	171909.0	- 2
ISTD	3049026.7	4927172.0	38

Tapentadol Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	100	101.1	10.0	9.9	101.1
Level 2	200	214.4	26.5	12.4	107.2
Level 3	500	536.8	55.7	10.4	107.4
Level 4	2000	2091.2	155.3	7.4	104.6
Level 5	4000	3579.4	282.2	7.9	89.5
Level 6	8000	7910.8	561.8	7.1	98.9
Blank	0				

Correlation Coefficient: 0.9919

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	100	101.1	10.0	9.9	101.1

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	539185	724182.75	74.5

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	541308.0	733350.3	26
ISTD	3049026.7	4927172.0	38

Tapentadol-O-Sulfate Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	100	101.7	2.6	2.6	101.7
Level 2	200	196.6	29.0	14.8	98.3
Level 3	500	521.3	59.9	11.5	104.3
Level 4	2000	2130.9	143.2	6.7	106.5
Level 5	4000	3858.5	222.6	5.8	96.5
Level 6	8000	7749.2	523.1	6.8	96.9
Blank	0				

Correlation Coefficient: 0.9944

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	100	101.7	2.6	2.6	101.7

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	373682	264473	141.3

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	399838.3	271828.7	- 47
ISTD	3049026.7	4927172.0	38

Tramadol Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	100	97.3	4.1	4.2	97.3
Level 2	200	214.1	27.0	12.6	107.0
Level 3	500	535.7	64.3	12.0	107.1
Level 4		2134.0	160.6	7.5	106.7
Level 5	4000	3715.5	251.1	6.8	92.9
Level 6	8000	7956.7	453.9	5.7	99.5
Blank	0				

Correlation Coefficient: 0.9933

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	100	97.3	4.1	4.2	97.3

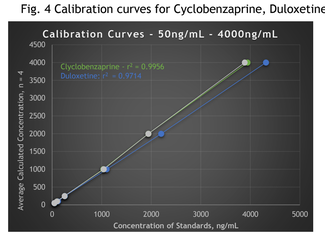
Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	543309	805317.5	67.5

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	555218.0	814670.0	32
ISTD	3049026.7	4927172.0	38

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



Cyclobenzaprine Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	50.9	2.8	5.5	101.8
Level 2	100	102.0	11.9	11.7	102.0
Level 3	250	255.1	23.0	9.0	102.0
Level 4	1000	1028.5	65.1	6.3	102.9
Level 5	2000	1940.1	158.7	8.2	97.0
Level 6	4000	3944.2	236.6	6.0	98.6
Blank	0				

Correlation Coefficient: 0.9956

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	50	50.9	2.8	5.5	101.8

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	56929	423827.75	13.4

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	160034.7	431504.3	63
ISTD	3804612.3	11258379.7	66

Duloxetine Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	46.5	19.5	41.9	93.1
Level 2	100	117.6	29.6	25.2	117.6
Level 3	250	256.9	26.3	10.2	102.7
Level 4	1000	1202.2	90.3	7.5	120.2
Level 5	2000	2001.6	281.9	14.1	100.1
Level 6	4000	4312.2	704.0	16.3	107.8
Blank	0				

Correlation Coefficient: 0.9714

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	50	46.5	19.5	41.9	93.1

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	72066	151473.75	47.6

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	125028.0	148896.3	16
ISTD	3049026.7	4927172.0	38

Normeperidine Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	42.4	7.9	18.6	84.8
Level 2	100	98.7	13.8	14.0	98.7
Level 3	250	250.1	30.3	12.1	100.1
Level 4	1000	1038.6	81.3	7.8	103.9
Level 5	2000	1943.2	113.4	5.8	97.2
Level 6	4000	3885.8	302.7	7.8	97.1
Blank	0				

Correlation Coefficient: 0.9919

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	50	42.4	7.9	18.6	84.8

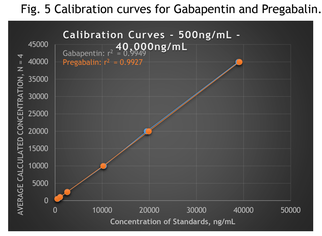
Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	1021346	1136721.5	89.9

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	1043283.7	1140774.7	8
ISTD	1466350.0	1762964.3	17

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



Gabapentin Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	500	530.8	31.9	6.0	106.2
Level 2	1000	1054.5	110.4	10.5	105.5
Level 3	2500	2562.4	289.3	11.3	102.5
Level 4	10000	10854.8	864.0	8.0	108.5
Level 5	20000	19448.2	1487.7	7.6	97.2
Level 6	40000	38922.9	1949.6	5.0	97.3
Blank	0				

Correlation Coefficient: 0.9949

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	500	530.8	31.9	6.0	106.2

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	178829	178460.7	100.2

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	198893	178622.3333	- 11
ISTD	3049026.7	4927172	32

Pregabalin Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	500	437.9	88.2	20.1	87.6
Level 2	1000	1037.1	131	12.6	103.7
Level 3	2500	2584.5	309.3	12	103.4
Level 4	10000	11199.9	940.7	8.4	112
Level 5	20000	19850.1	1511.5	7.6	99.3
Level 6	40000	39121.6	1820.6	4.7	97.8
Blank	0				

Correlation Coefficient: 0.9927

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	500	437.9	88.2	20.1	87.6

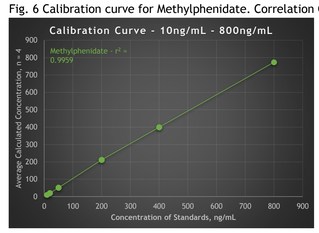
Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	530488	438834	120.9

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	648530	440956.7	- 47
ISTD	3049027	4927172	32

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



Methylphenidate Linearity/Carryover

Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	10	9.8	0.5	5.5	98.0
Level 2	20	20.0	2.1	10.4	99.9
Level 3	50	51.3	4.8	9.3	102.6
Level 4	200	219.8	16.7	7.6	109.9
Level 5	400	390.3	20.2	5.2	97.6
Level 6	800	773.3	29.8	3.9	96.7
Blank	0				

Correlation Coefficient: 0.9959

Within Run Precision

Sample	Conc	Mean	SD	% CV	% Accuracy
LOD/LOQ	10	9.8	0.5	5.5	98.0

Recovery

Sample	Mean Extracted	Mean Unextracted	% Recovery
L1	123282	164472	75.0

Ion Suppression

Sample	Mean Extracted	Mean Unextracted	% Ion Suppression
L1 Standard	129810.7	164478.3	21
ISTD	3049026.7	4927172.0	32

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.