THOMSON INSTRUMENT COMPANY Solutions ™ At Work

Improved Sample Preparation of Biological Samples Using the Thomson eXtreme|FV® & Analysis by LC-MS/MS

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- Family owned & operated business located in the San Diego area.
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- Products are Made in the U.S.

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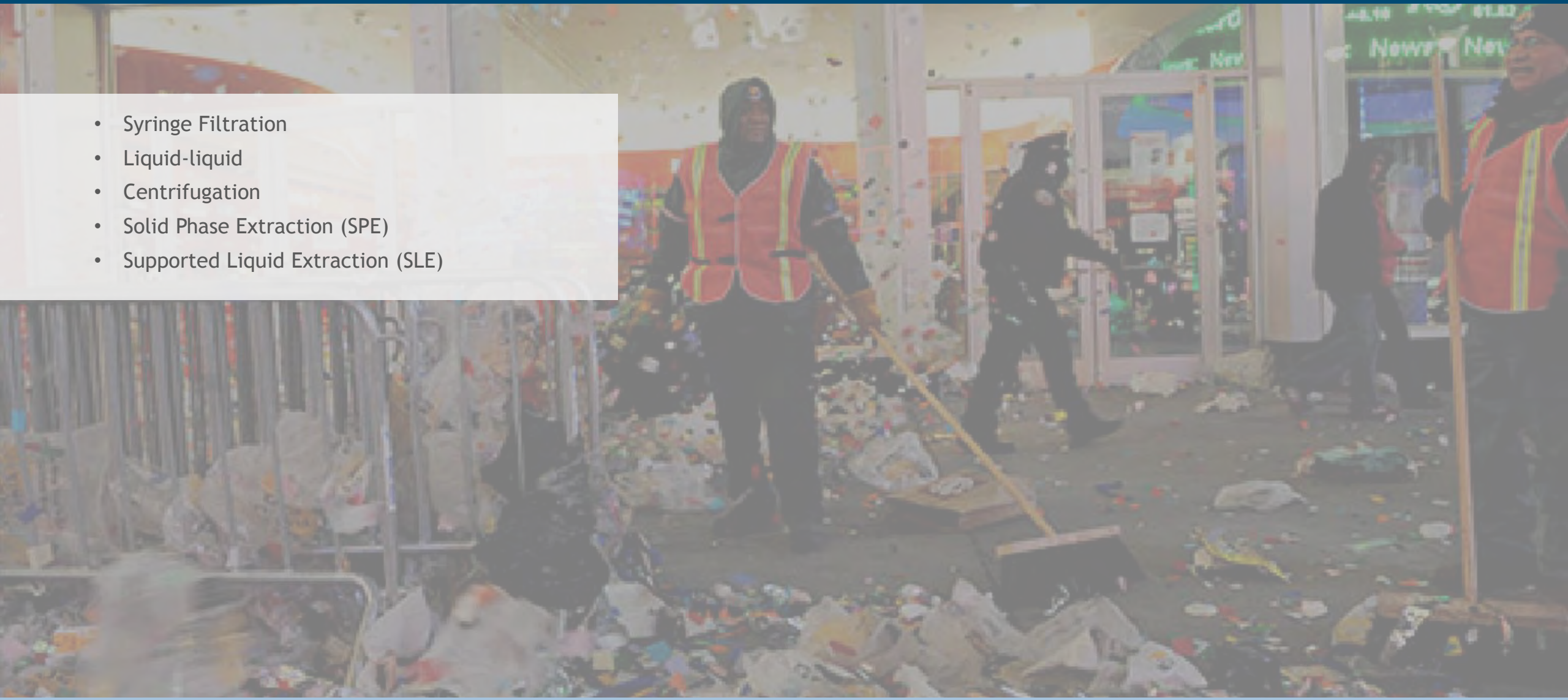
Traditional Sample Clean-up Methods Prior to GC, LC, MS



- Syringe Filtration
- Liquid-liquid
- Centrifugation
- Solid Phase Extraction (SPE)
- Supported Liquid Extraction (SLE)

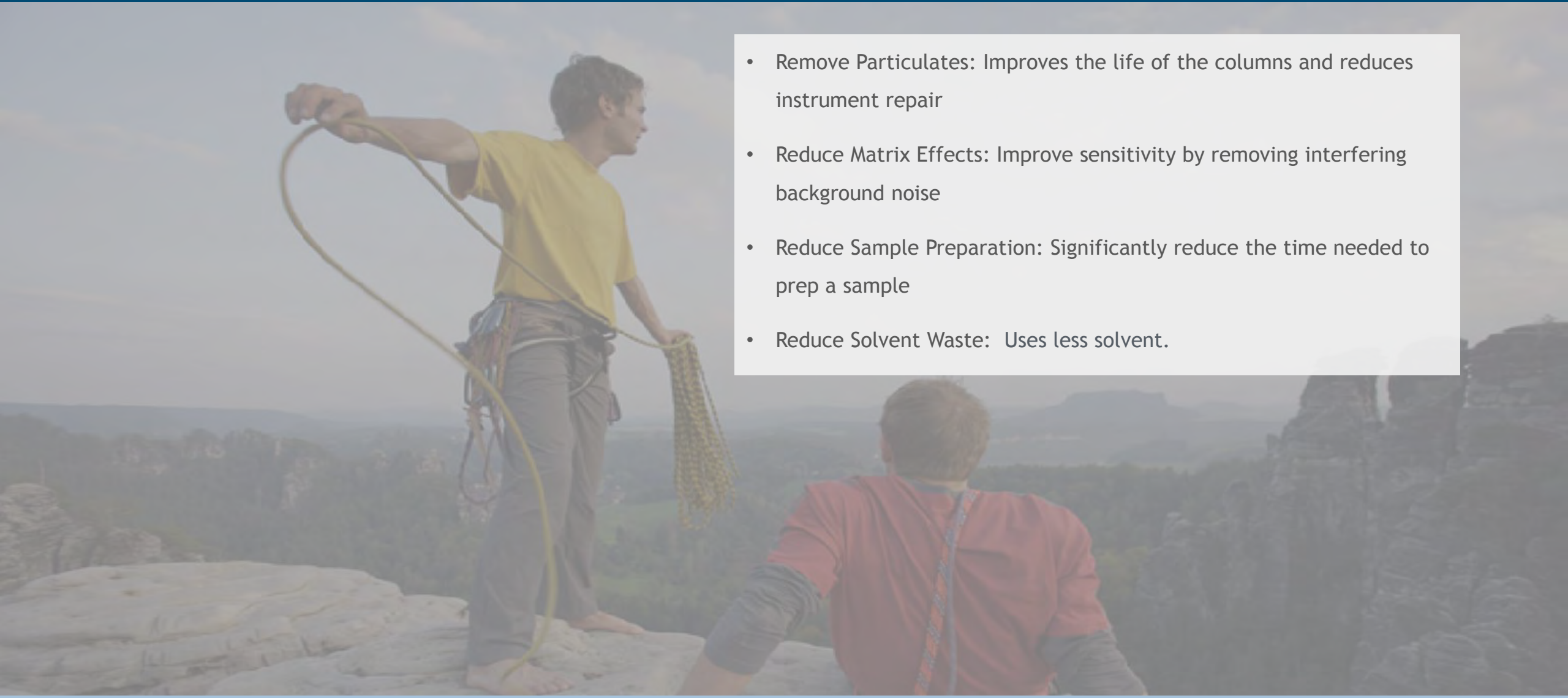
What do we know about these techniques?

- Adversely impact recovery
- Large amounts of solvent/waste
- Produce aerosols
- Require expensive consumables, equipment & space
- Require secondary filtration
- Time consuming

Lab Space Expen$ive

Space Saving Solution

What can we achieve with filter vials?



- Remove Particulates: Improves the life of the columns and reduces instrument repair
- Reduce Matrix Effects: Improve sensitivity by removing interfering background noise
- Reduce Sample Preparation: Significantly reduce the time needed to prep a sample
- Reduce Solvent Waste: Uses less solvent.

Matrix Effects and Ion Suppression: Hidden gems are not obvious in LCMS

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Strong Signal, Noise Lessened, Blue Creature Appears

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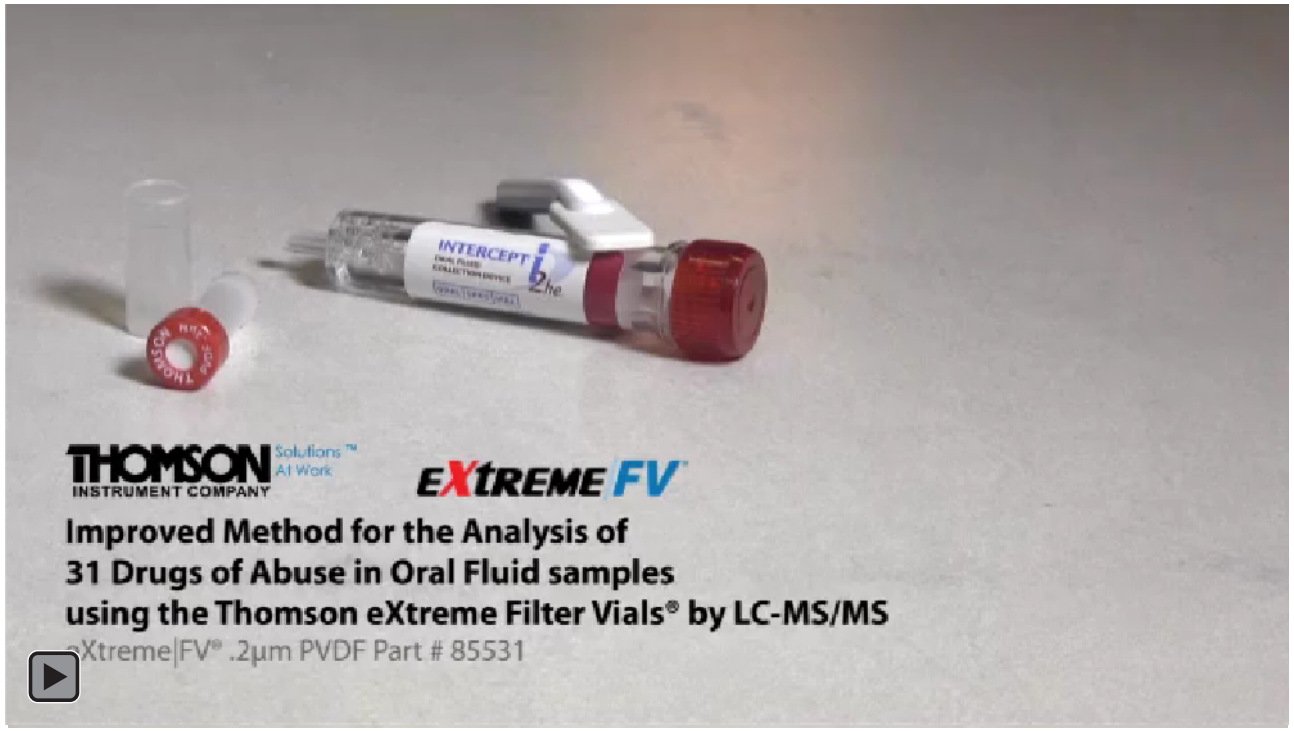
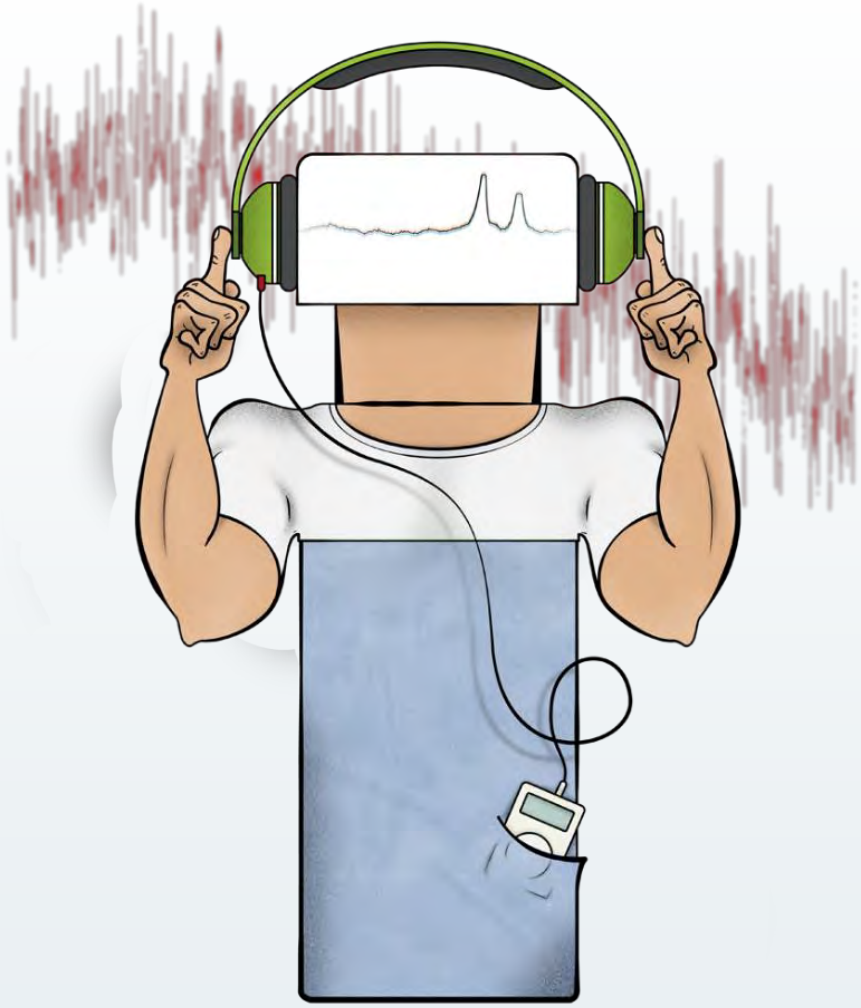

INTERCEPT
THOMSON Solutions™ At Work
INSTRUMENT COMPANY
eXtreme FV
Improved Method for the Analysis of
31 Drugs of Abuse in Oral Fluid samples
using the Thomson eXtreme Filter Vials® by LC-MS/MS
eXtreme|FV® .2µm PVDF Part # 85531


DEPOSIT SAMPLE
PUSH
COMPRESS FILTER VIAL
COMPLETED WITH FILTRATE REMOVED FROM SAMPLE

eXtreme/FV®



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eXtreme|FV® Overview

eXtreme|FV® (Multi-Layered Filtration)

- Multi-layer filtration for viscous samples containing up to 30% solid particulates.
- The filter vial consists of two parts:
  - Filter vial outer shell
  - Plunger which includes a multi-layer filter on one end and a snap cap on the other end.
- Allows for compounds to be separated from the matrix
  - Increase signal-to-noise
  - Improves basline
  - Easier integration
- Can replace the SPE clean up step high levels of particulates were “filtered” by using an SPE step in the method. This method is easily amendable: simply replace the SPE step with a rapid and lower cost eXtreme|FV® step.
- Applications for Thomson eXtreme|FV® include filtration for toxicology analysis in urine, oral fluids and blood; small molecules, cell and cell debris from cell culture; pesticide analysis in food, tissue, soil, and water.

Improved Sample Preparation for the Analysis of 12 Opiates in Urine using the Thomson eXtreme Filter Vials® by LC-MS/MS

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12 Opiates by LC-MS/MS

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

* Additional drugs that were added to the new method

Equipment Highlights

- Shimadzu Prominence HPLC
  - Mobile Phase:
    - A: 0.1% Formic Acid in HPLC Water
    - 0.1% Formic Acid in Methanol
  - Column: Restek Ultra Biphenyl Columns (5µm 50 x 2.1 mm)
- ABI 4500 Mass Spectrometer
- Thomson eXtreme|FV® 0.2µm PVDF (p/n 85531)
- Thomson 48 position Vial Filter Press (p/n 35010)
- Dry Block Heater set at 55°C ± 2°C
- Microcentrifuge

Previous Sample Preparation 8 Drugs

Hydrolysis

1. Allow standards, specimens and controls to come to room temperature.
2. Turn Block Heater on to 37°C±2°C
3. Place the OBASIC.set reagents on the Rapid Trace and purge the lines.
4. Label one 16 x 125 mm screw top tube for each blank, standard, control and client specimen.
5. All samples will be analyzed in the order they are extracted on the Rapid Trace.
6. Prepare a LC Check Standard (equivalent to a Level 1 concentration)
7. To all tubes, add 1.8 mL of pH 5.2 Acetate Buffer.
8. Vortex for 10 seconds.
9. Add 20 µL β-glucuronidase.
10. Cap and vortex for 10 seconds to ensure sample is mixed.
11. Incubate at 37°C±2° for 16 hours.

Improved Sample Preparation 12 Drugs

Hydrolysis

1. Allow standards, specimens and controls to come to room temperature.
2. Label one 1.5 mL Safe-Lock Tube and one Thomson vial for each blank, standard, control and client specimen.
3. Place 300 µL 2% Methanol into the 12 x 75 glass tube for the LC Check.
4. To each 1.5 mL Safe-Lock Tube add 50 µL of Rapid Hydrolysis Buffer.
5. Cap and vortex for 30 seconds using the Eppendorf Mix Mate.
6. Uncap and add 40µL IMCS β-glucuronidase to each tube.
7. Cap and vortex for 30 seconds to ensure sample is mixed.
8. Incubate at 55°C ± 2°C for 30 minutes.
9. Allow tubes to come to room temperature.
10. Microcentrifuge at 14000 rpm for 10 minutes.

Sample Preparation

Sample Prep - 9 drugs

1. Allow tubes to come to room temperature.
2. Label one 13 x 100 mm culture tube and a 12 x 75 mm culture tube for each blank, standard, control and client specimen.
3. Place the OBASIC.set reagents on the Zymark Rapid Trace and purge the reagent lines.
4. To each extraction tube add 3 mL of 50 mM Phosphoric Acid.
5. Vortex for 10 seconds.
6. Centrifuge tubes at 3000 rpm for 10 minutes.
7. Transfer samples into corresponding labeled 13 x 100 mm tubes.
8. The tubes are now ready for automated extraction.
9. After the elution is complete on the Rapid Trace®, remove the racks with the tubes intact.
10. Include LC Check at his point.
11. Dry down extracts/tubes under a gentle stream of nitrogen.
12. Add 400 µL of 10% Methanol.
13. Vortex for 30 seconds.
14. Transfer supernatant using a glass borosilicate pipet to the appropriately labeled autosampler vials. Cap and place vials on autosampler tray. Extracts are ready for LC/MS/MS analysis.

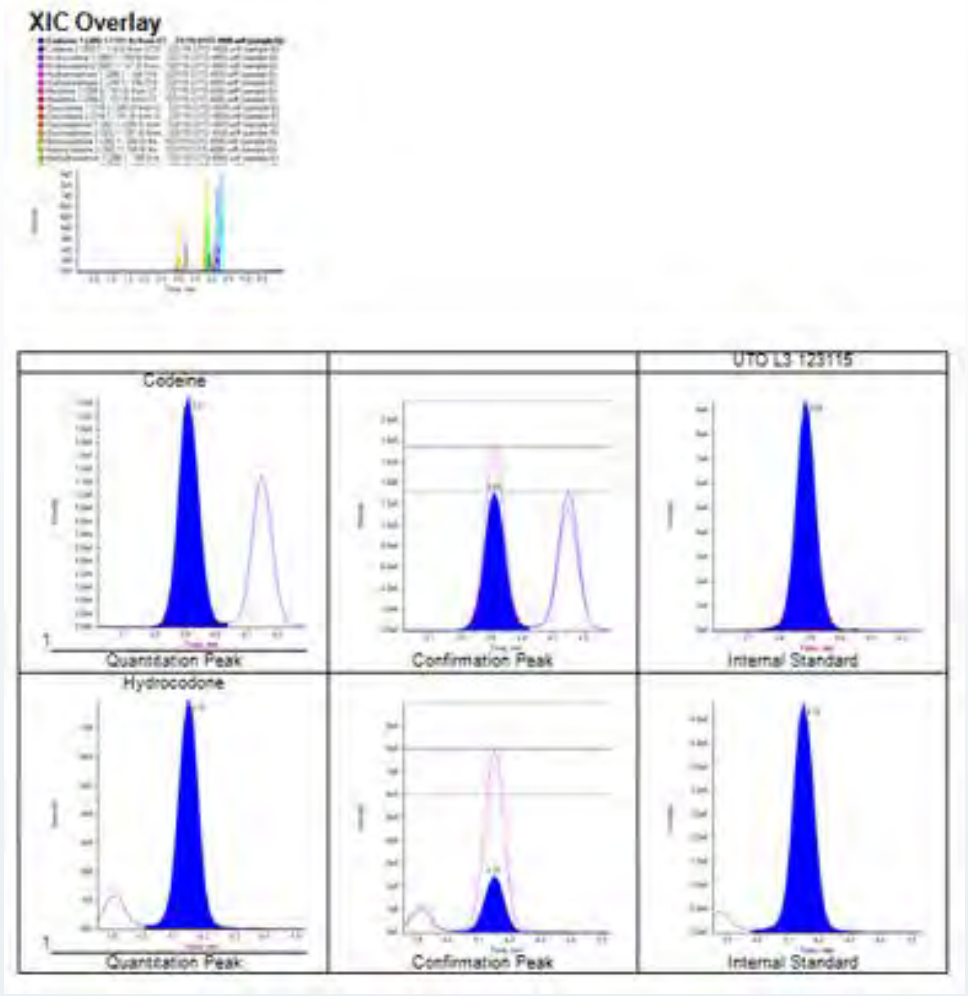
Sample Prep -12 drugs

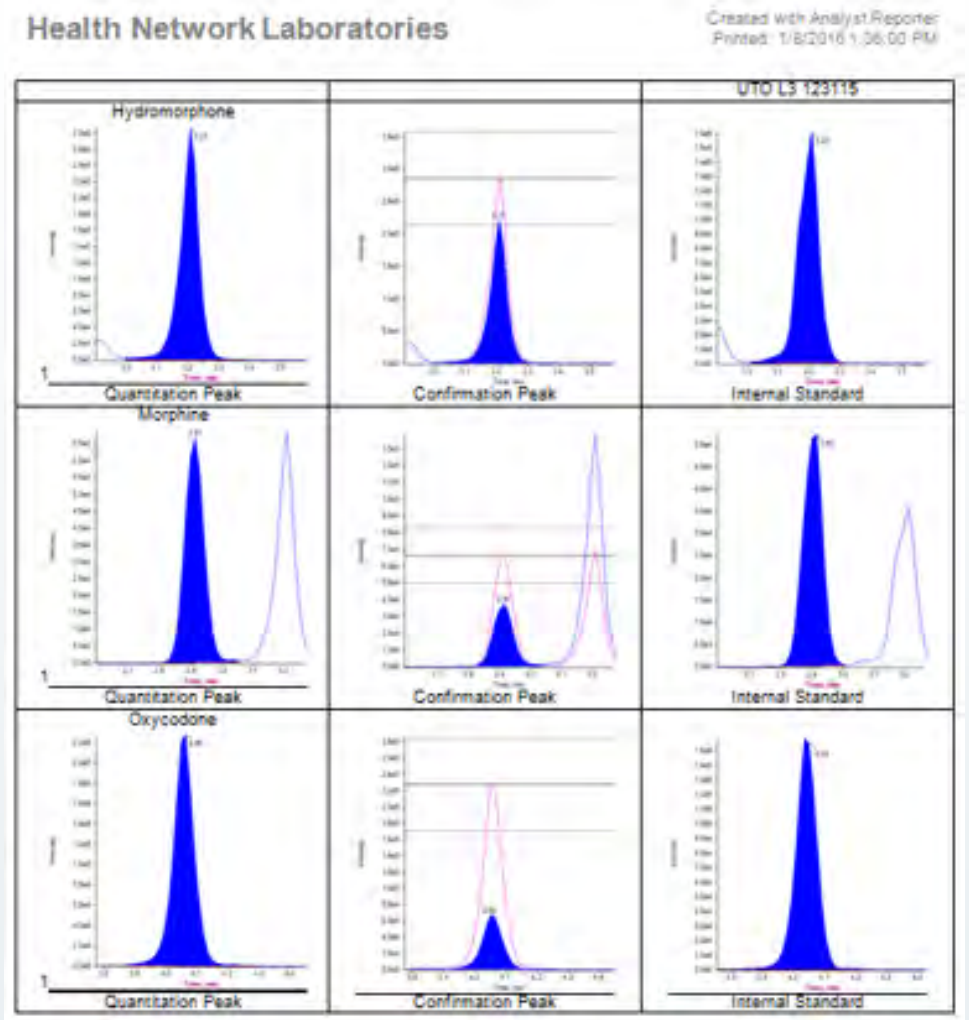
1. Allow tubes to come to room temperature.
2. Add 200 µL of 2% Methanol to each Thomson Vial.
3. Add 100 µL of the hydrolyzed urine sample to its respective Thomson Vial.
4. Place Thomson Filter Plunger on top of Thomson Vial.
5. Press filter plunger down approximately ¼ of the way into each of the Thomson Vials.
6. Vortex for 2 minutes at 1750 rpm using the Eppendorf Mix Mate.
7. Slowly press the filter plunger the rest of the way down using the Thomson 48 position press.
8. Samples are now ready for LC/MS/MS analysis

Results: Final Concentrations of Standards

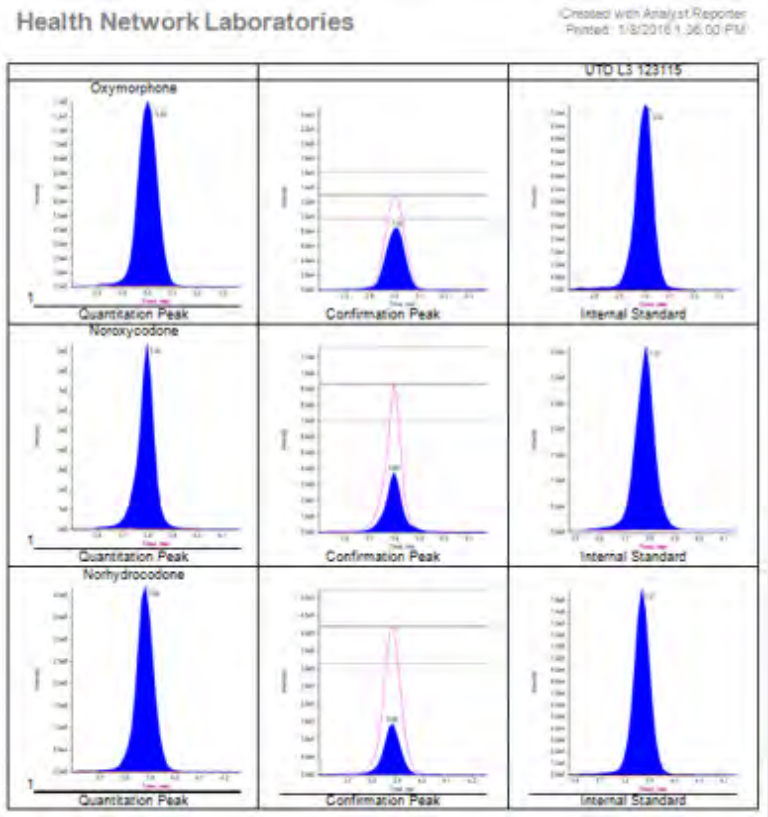
	Final Concentration (ng/mL) Opiates	Final Concentration (ng/mL) 6-MAM
Level 1	50	5
Level 2	200	20
Level 3	1000	50
Level 4	5000	250
Level 5	10000	500
Level 6	20000	1000

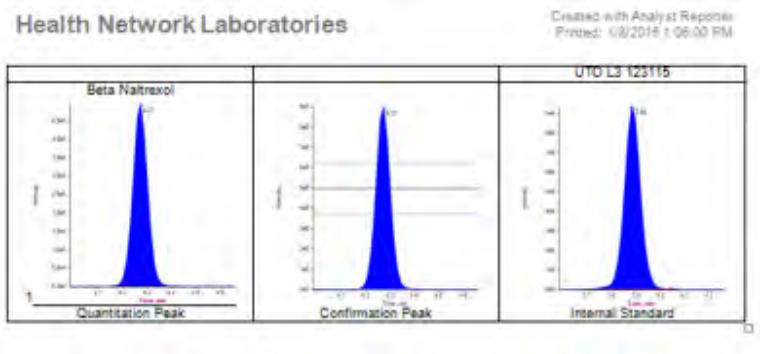
Positive Results

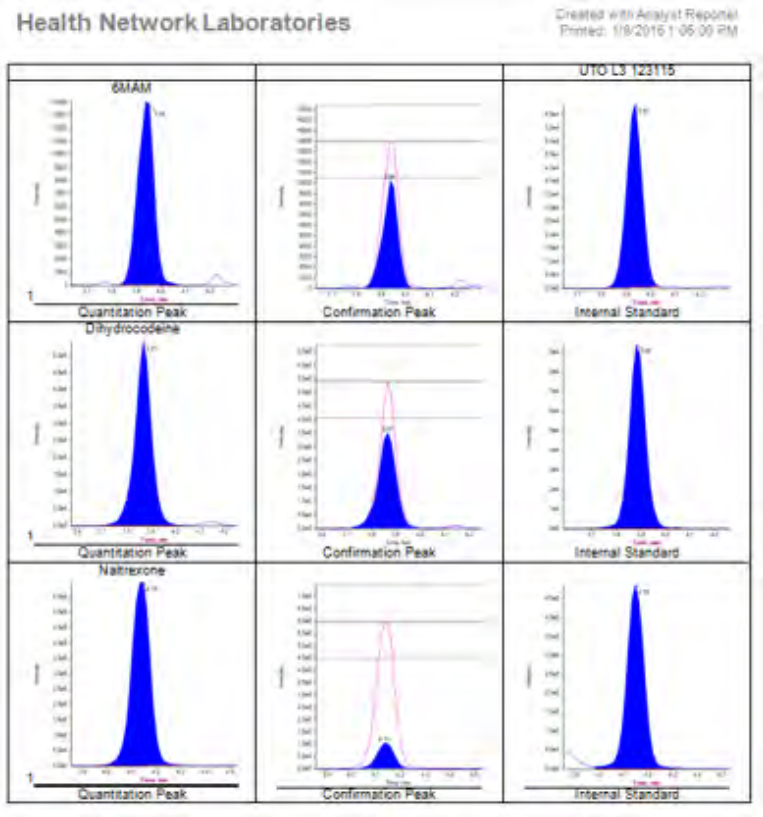




Positive Results Cont.







Conclusion

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Quick and Easy Sample Preparation of Urine for the Analysis of Psychoactive Drugs using the Thomson eXtreme Filter Vials® by LC-MS/MS

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9 Benzos by LC-MS/MS

7-Aminoclonazepam (7AMINO)	Hydroxy-Midazolam (OH-MID)	Oxazepam (OXAZ)
α-hydroxy-Alprazolam (OH-AL)	Lorazepam (LOR)	Temazepam (TEM)
Diazepam (DIAZ)	Nordiazepam (NDIAZ)	Zolpidem (ZOLP)

Previous Sample Preparation 8 Drugs

Hydrolysis

1. Allow standards, specimens and controls to come to room temperature.
2. Turn Block Heater on to 37°C±2°C
3. Place the Benzo.set reagents on the Rapid Trace and purge the lines.
4. Label one 13 x 100 mm screw top tube for each blank, standard, control and client specimen.
5. All samples will be analyzed in the order they are extracted on the Rapid Trace.
6. Prepare a LC Check Standard (equivalent to a Level 1 concentration)
7. To all tubes, add 1 mL of 1.1 M pH 5.2 Acetate Buffer.
9. Vortex for 10 seconds.
10. Add 20 µL ß-glucuronidase.
11. Vortex for 10 seconds to ensure sample is mixed.
12. Incubate at 37°C±2° for 3 hours.
13. Allow tubes to come to room temperature.

Improved Sample Preparation 12 Drugs

Hydrolysis

1. Allow standards, specimens and controls to come to room temperature.
2. Label one 1.5 mL Safe-Lock Tube and one Thomson vial for each blank, standard, control and client specimen.
3. Place 350 µL 40% Methanol into the 12 x 75 glass tube for the LC Check
4. To each 1.5 mL Safe-Lock Tube add 50 µL of Rapid Hydrolysis Buffer.
7. Cap and vortex for 30 seconds using the Eppendorf Mix Mate.
8. Uncap and add 40µL IMCS ß-glucuronidase to each tube.
9. Cap and vortex for 2 minutes to ensure sample is mixed.
10. Uncap and incubate at 55°C ± 2°C for 30 minutes.
11. Allow tubes to come to room temperature.
12. Microcentrifuge at 14000 rpm for 10 minutes.

Sample Preparation

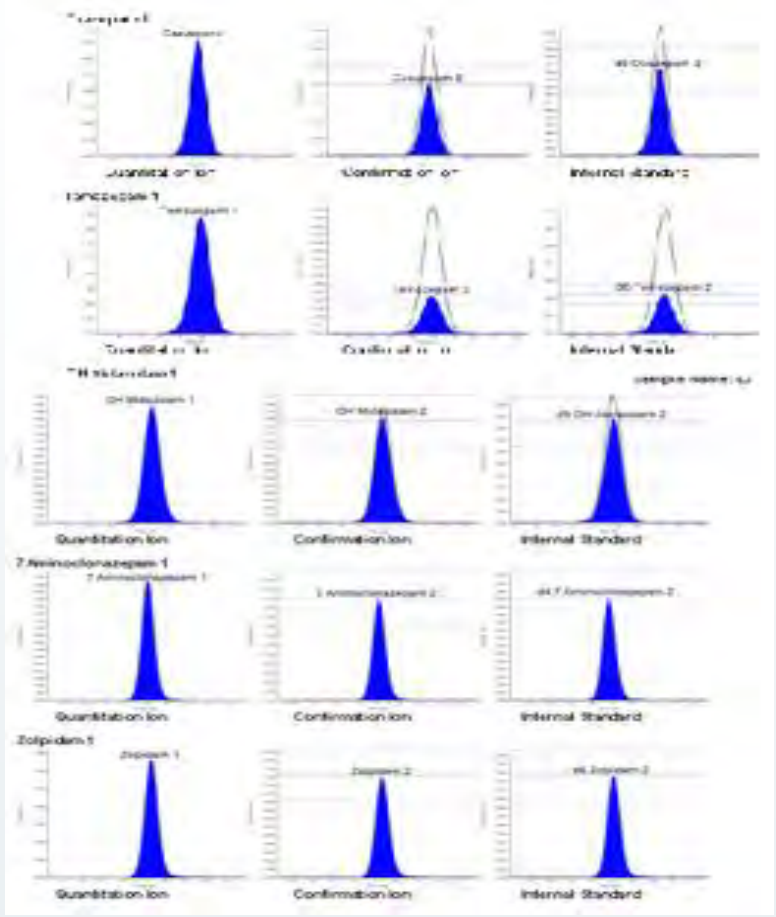
1. Uncap
2. Add 3 mL 0.1 M pH 6.0 Potassium Phosphate Buffer.
3. Place the Benzo.set reagents on the Zymark Rapid Trace and purge the reagent lines.
4. Vortex for 10 seconds.
5. Centrifuge tubes at 3000 rpm for 5 minutes.
6. Transfer samples into corresponding labeled 13 x 100 mm tubes.
7. The tubes are now ready for automated extraction.
8. After the elution is complete on the Rapid Trace®, remove the racks with the tubes intact.
9. Include QC Check at his point.
10. Dry down extracts/tubes under a gentle stream of nitrogen.
11. Add 2mL of 10% Methanol.
12. Vortex for 30 seconds.
13. Transfer supernatant using a glass borosilicate pipet to the appropriately labeled autosampler vials. Cap and place vials on autosampler tray.
14. Extracts are ready for LC/MS/MS analysis.

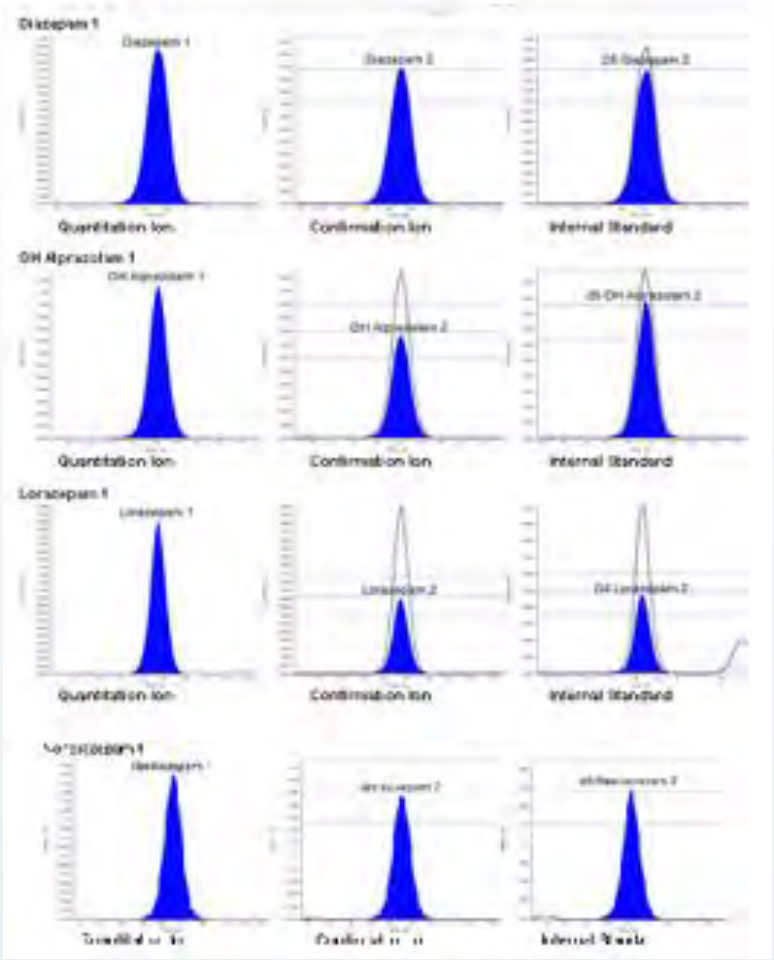
1. Add 300 µL of 40% Methanol to each Thomson Vial.
2. Give each Eppendorf tube a quick vortex and add 50 µL of the hydrolyzed urine sample to its respective Thomson Vial.
3. Place Thomson Filter Plunger on top of Thomson Vial.
4. Press filter plunger down approximately ¼ of the way into each of the Thomson Vials.
5. Vortex for 2 minutes at 1750 rpm 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: Final Concentrations of Standards

	Final Concentration All other analytes (ng/mL)	Final Zolpidem Concentration (ng/mL)
Level 1	75	75
Level 2	300	300
Level 3	1000	500
Level 4	5000	2500
Level 5	10000	5000

Positive Results





Conclusion

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

Nadine Koenig, Crystal Xander, Melanie Stauffer

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Dean Fritch

Analytical Associates, 225 Millwood Drive, East Greenville, PA

Abstract

- Improved sample preparation method allows for the quantitative measurement of the a specialty pain management panel in urine using a simple dilute, filter and shoot sample prep.
- 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.

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

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

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_5R
  - Column: Ultra Biphenyl Column (5µm 50 x 2.1 mm)
- Eppendorf Mix Mate Vortex Mixer
- Thomson eXtreme|FV® 0.2µm PVDF Thomson 48 Position Vial Filter Press

Method:

Flow Rate: 0.5 mL/min

Mobile Phases:

A: 0.1% Formic Acid in Water

B: 0.1% Formic Acid in Methanol

Run Time: 8.5 minutes

Injection Volume: 15µL

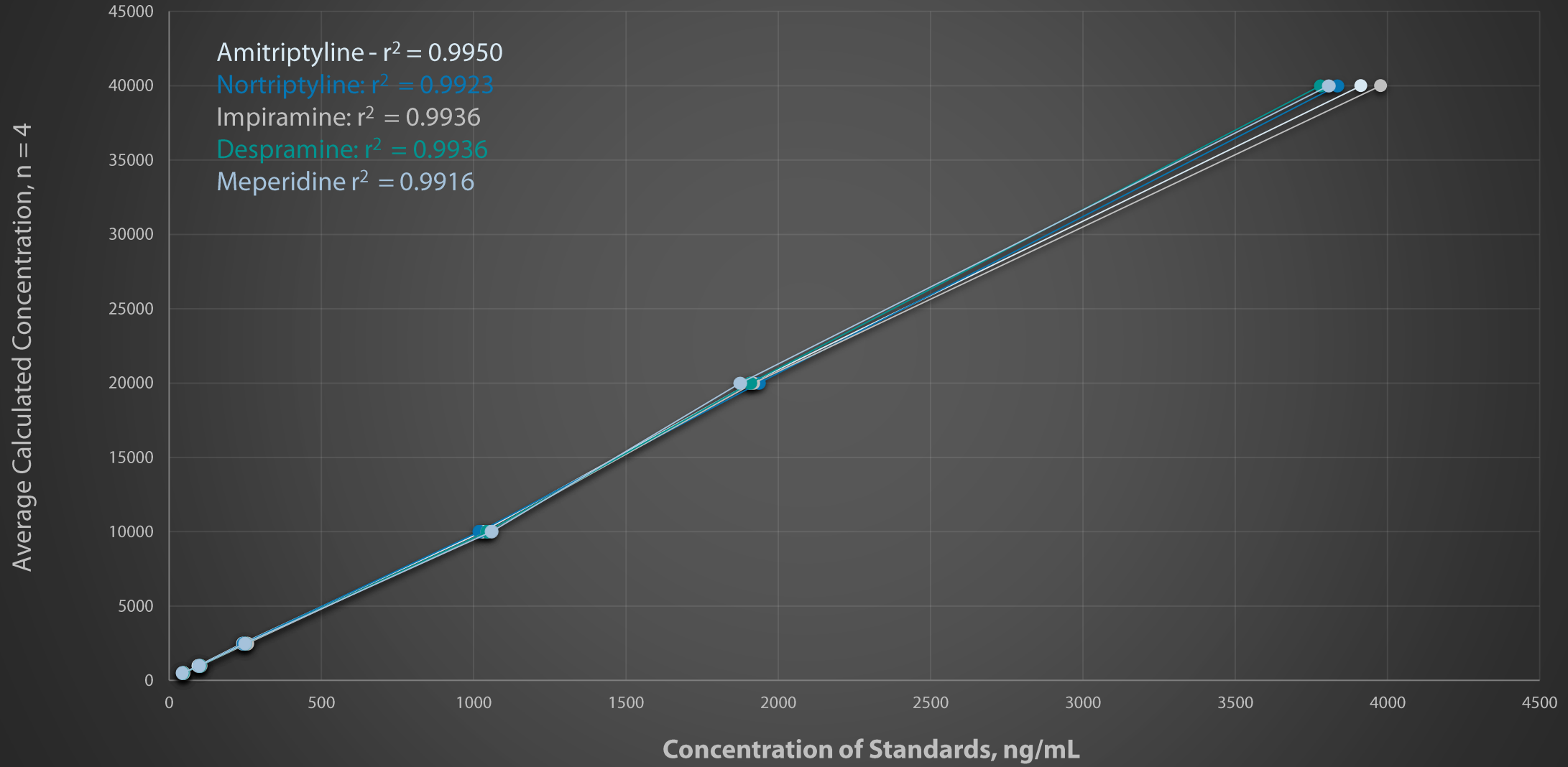
Improved Sample Preparation

- Place 400 µL of 20% MeOH / 80%Water / 0.1% Formic Acid in each of the outer shells of the Thomson Filter Vials
- Add 25µL of Standard/Control/Patient Sample + 10uL of Internal Standard
- Press filter plunger down approximately ¼ of the way into each of the Thomson vials, eXtreme/FV® 0.2µm PVDF
- Vortex for 30-40 seconds
- Slowly press filter plunger the rest of the way down using the 48 Position Filter Vial Press.
- Extracts are ready for LC/MS/MS analysis
- Inject 15µL

Results

- Improved sample preparation method allows for the quantitative measurement of the supplemental pain management drugs in urine
- Method utilizes the Thomson eXtreme|FV® for sample clean-up significantly reducing the cost and time per sample analysis.
- This method was validated for all 17 drugs in the supplemental pain management panel over 3 days.

Calibration Curves - 500ng/mL - 40,000ng/mL



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

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	956226.7	1551280.7	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

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

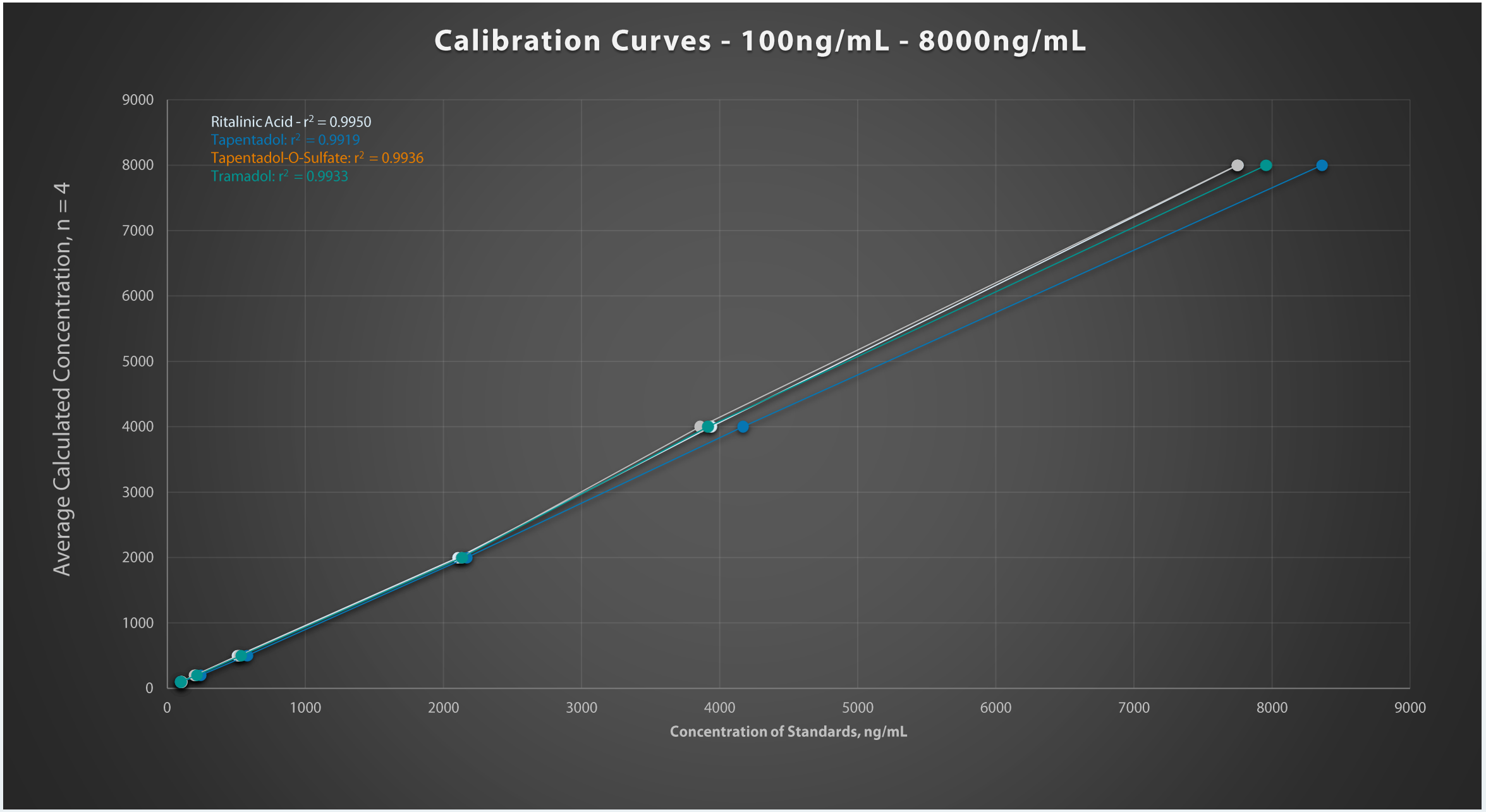
Meperidine Linearity/Carryover					
Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	43.0	10.2	23.8	86.0
Level 2	100	99.4	10.4	10.5	99.4
Level 3	250	249.6	27.3	11.0	99.8
Level 4	1000	1058.3	81.8	7.7	105.8
Level 5	2000	1874.7	19.8	1.1	93.7
Level 6	4000	3806.5	176.1	4.6	95.2
Blank	0				
Correlation Coefficient: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

Calibration Curves - 100ng/mL - 8000ng/mL



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

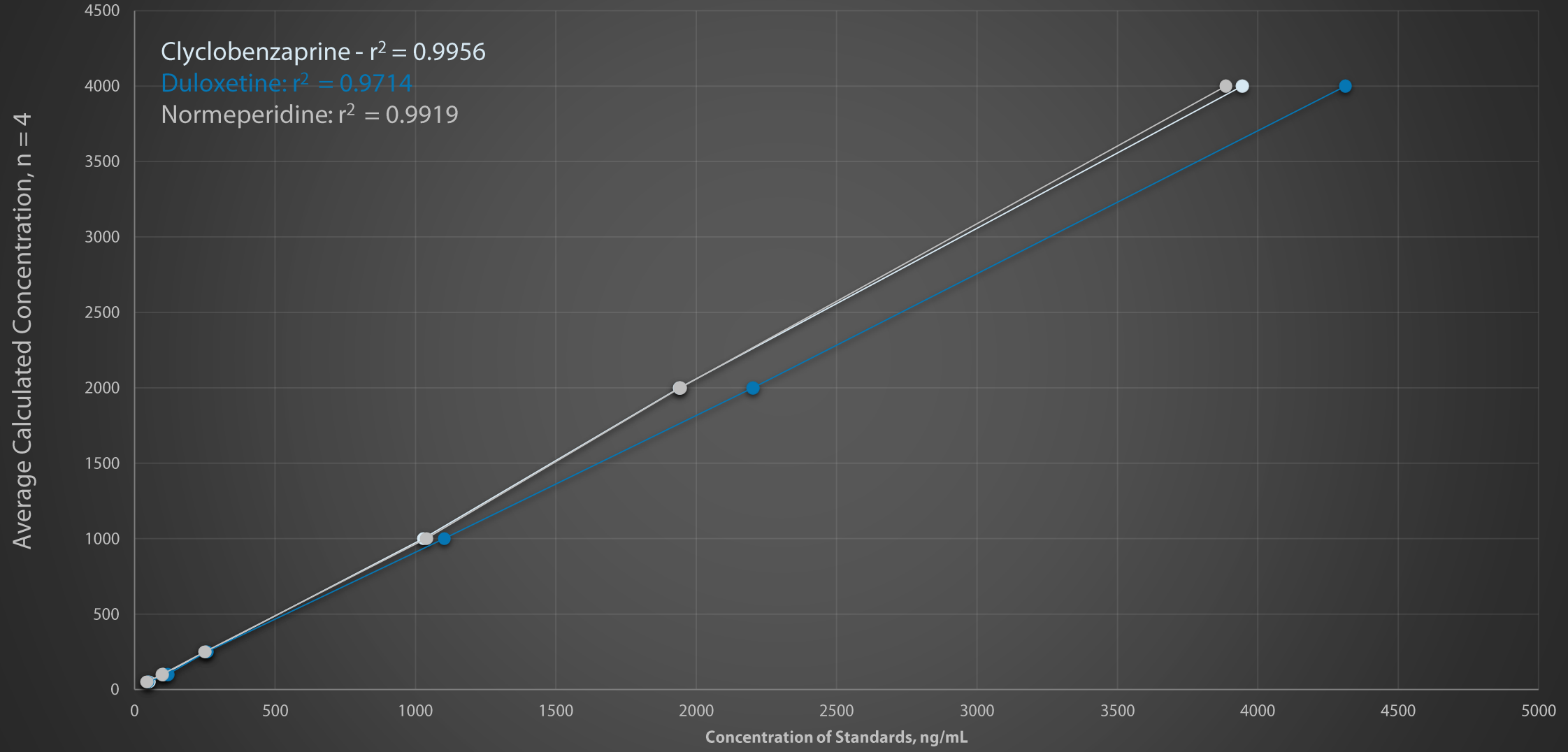
Tramadol Linearity/Carryover					
Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	100	97.3	4.1	4.2	97.3
Level 2	200	214.1	27.0	12.6	107.0
Level 3	500	535.7	64.3	12.0	107.1
Level 4		2134.0	160.6	7.5	106.7
Level 5	4000	3715.5	251.1	6.8	92.9
Level 6	8000	7956.7	453.9	5.7	99.5
Blank	0				
Correlation Coefficient: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

Calibration Curves - 50ng/mL - 4000ng/mL



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

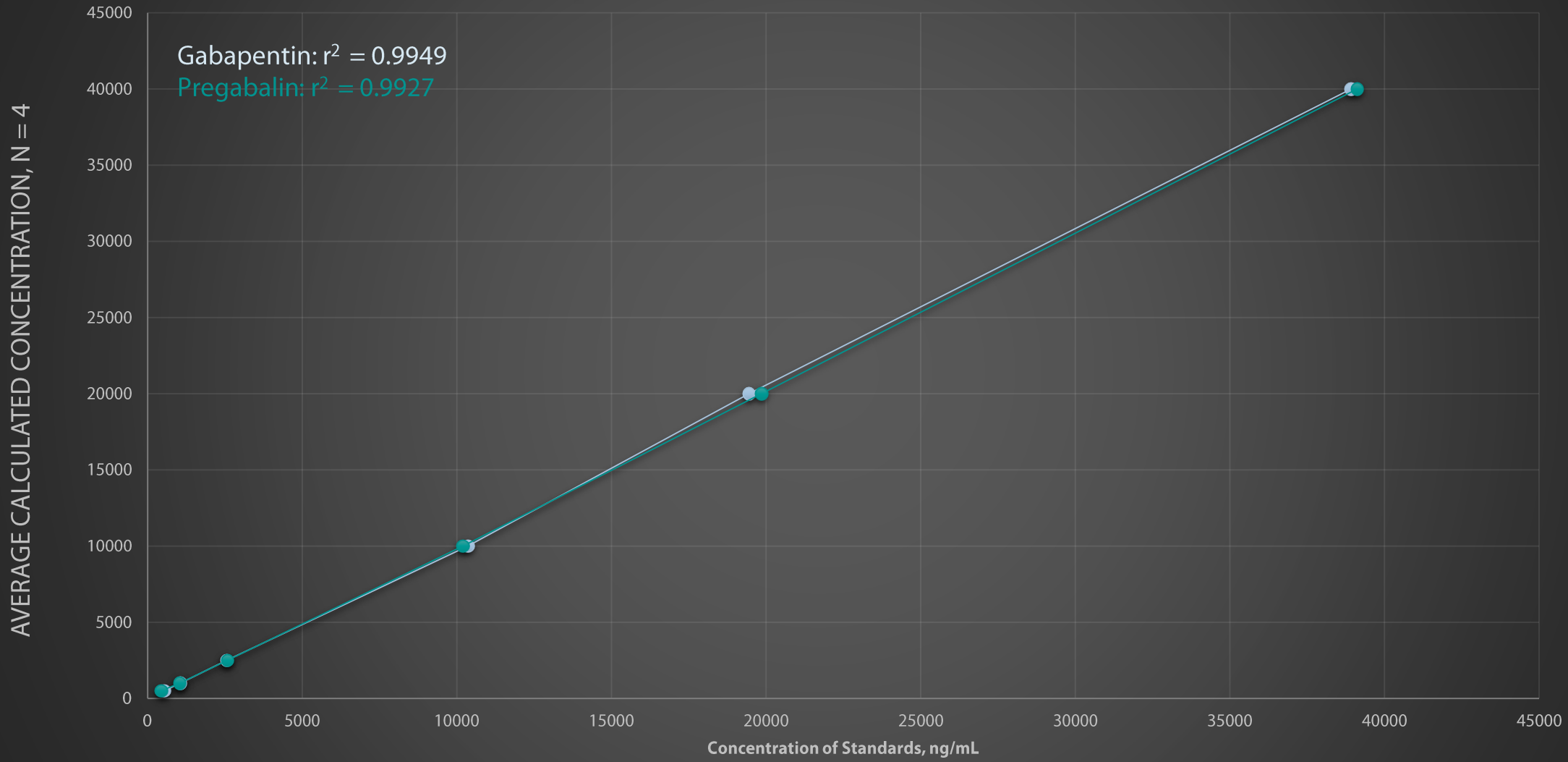
Normeperidine Linearity/Carryover					
Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	50	42.4	7.9	18.6	84.8
Level 2	100	98.7	13.8	14.0	98.7
Level 3	250	250.1	30.3	12.1	100.1
Level 4	1000	1038.6	81.3	7.8	103.9
Level 5	2000	1943.2	113.4	5.8	97.2
Level 6	4000	3885.8	302.7	7.8	97.1
Blank	0				
Correlation Coefficient: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

Calibration Curves - 500ng/mL - 40,000ng/mL



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

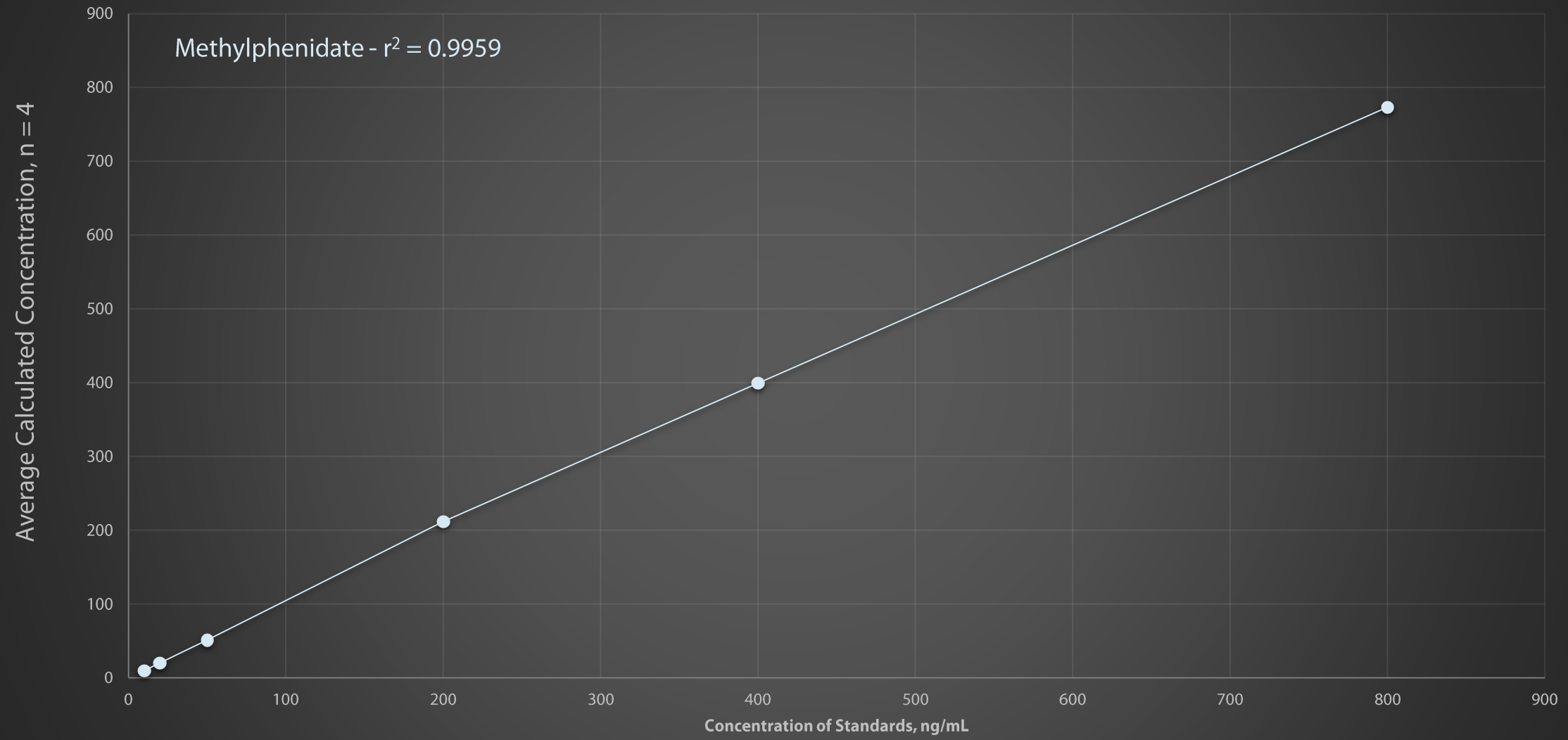
Pregabalin Linearity/Carryover					
Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	500	437.9	88.2	20.1	87.6
Level 2	1000	1037.1	131	12.6	103.7
Level 3	2500	2584.5	309.3	12	103.4
Level 4	10000	11199.9	940.7	8.4	112
Level 5	20000	19850.1	1511.5	7.6	99.3
Level 6	40000	39121.6	1820.6	4.7	97.8
Blank	0				
Correlation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

Calibration Curve - 10ng/mL - 800ng/mL

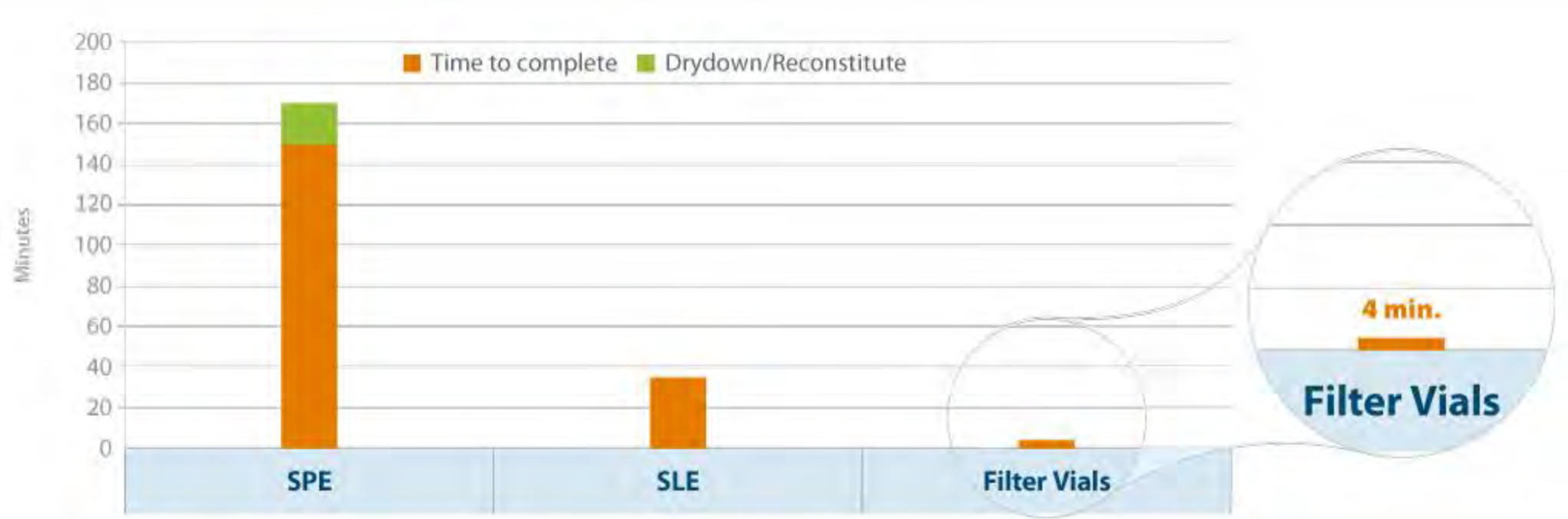


Methylphenidate Linearity/Carryover					
Sample	Conc	Mean	SD	% CV	% Accuracy
Level 1	10	9.8	0.5	5.5	98.0
Level 2	20	20.0	2.1	10.4	99.9
Level 3	50	51.3	4.8	9.3	102.6
Level 4	200	219.8	16.7	7.6	109.9
Level 5	400	390.3	20.2	5.2	97.6
Level 6	800	773.3	29.8	3.9	96.7
Blank	0				
Correlation Coefficient: 0.9959					
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

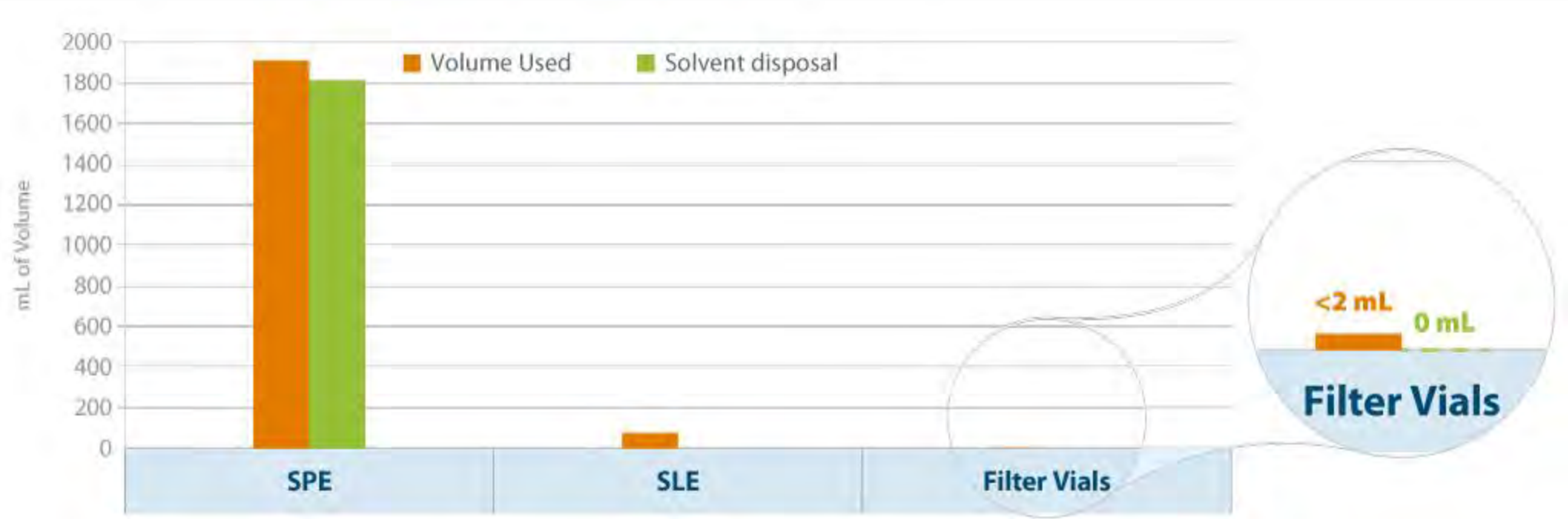
Improved Method Benefits

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**

Time


200
180
160
140
120
100
80
60
40
20
0
Minutes
Time to complete
Drydown/Reconstitute
SPE
SLE
Filter Vials
4 min.
Filter Vials

Solvent Usage & Disposal



Equipment Cost & Maintenance/Annually

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.

Improved Method for the Analysis of 31 Drugs of Abuse/Pain Management Panel in Oral Fluid Samples using the Thomson eXtreme® Filter Vials by LC-MS/MS

Abstract

- Improve the sample preparation for the analysis of drugs of abuse/pain management panels in oral fluids.
- Oral fluid samples were collected with Intercept® i2he™ Oral Fluid Collection Devices.
- The most critical aspects of reliable Oral Fluid 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.
- Thomson eXtreme® Filter Vials (patented) offer multi-layer filtration for viscous samples and samples containing up to 30% solid particulates.

Comparison of Methods

Obsolete Method: 4 drug panel

- Concentration Workstation
- Automated Solid Phase Extraction
- LC-MS/MS

Improved Method: 31 drug panel

- Thomson eXtreme|FV® 0.2µm PVDF
- LC-MS/MS

Oral Fluids Sample Prep

Obsolete Sample Preparation

1. Allow standards, specimens and control to come to room temperature.
2. To appropriately labeled 13 x 100 mm tubes add 3 mL of 50mM Phosphoric Acid.
3. Prepare the 13 x 100 mm tubes for analysis. Standards/Controls/Patient Samples
4. Vortex for 10 seconds.
5. The tubes are now ready for automated extraction using on the Caliper Life Sciences Turbo-Vap® Concentration Workstation
6. After the elution is complete on the Rapid Trace®, remove the racks with the tubes intact.
7. Add 50µL of 1% HCL in Methanol to each tube.
8. Vortex for 15 seconds.
9. The original sample tubes and the used SPEC DAU Columns can be discarded.
10. Take to dryness at 55ºC in the Caliper Life Sciences Turbo-Vap®.
11. Reconstitute samples by adding 1 mL of 10% HPLC Grade Methanol in Water to all tubes.
12. Vortex for 15 seconds.
13. Extracts are ready for LC/MS/MS analysis using the Shimadzu / AB Sciex 3200

Improved Sample Preparation

1. Allow standards, specimens and control to come to room temperature.
2. Add 100 µL of 10% Methanol / Water
3. Add 100 µL of Standard (Intercept i2he Diluent)/ Control/oral fluid sample + 10uL Internal Standard
4. Place Thomson Filter Plunger on top of the Thomson vial, Thomson vials – eXtreme/FV® 0.2µm PVDF, w/Pre-Slit Red Cap (p/n #85531)
5. Press filter plunger down approximately ¼ of the way into each of the Thomson Vial outer shells.
6. Vortex for 10 seconds using the Eppendorf MixMate®.
7. Press Filter plunger the rest of the way down using the Thomson 48 position Vial Filter Press.
8. Extracts are ready for LC/MS/MS analysis using the Shimadzu / AB Sciex 4500

4 drugs were analyzed in the "Obsolete Method"

Benzoylecgonine (BE)
Phencyclindine (PCP)
Methadone (MTHD)
Morphine (MORP)

31 drugs in oral fluid will be analyzed by this "Improved Method":

6-Monoacetylmorphine (6-MAM)	7-Aminoclonazepam (7AMINO)	Alprazolam (ALPR)
Amphetamine (AMPH)	Benzoylecgonine (BE)	Buprenorphine (BUP)
Carisoprodol (CARIS)	Clonazepam (CLONZ)	Cocaine
Codeine (CODE)	Diazepam (DIAZ)	Fentanyl (FENT)
Hydrocodone (HCOD)	Hydromorphone (HMOR)	Lorazepam (LOR)
Meprobamate (MEPRO)	Methadone (MTHD)	Methamphetamine (MAMP)
Methylenedioxyamphetamine (MDA)	Methylenedioxymethamphetamine (MDMA)	Morphine (MORP)
Norbuprenorphine (NBUP)	Nordiazepam (NDIAZ)	Norfentanyl (NFENT)
Oxazepam (OXAZ)	Oxycodone (OCOD)	Oxymorphone (OMOR)
Phencyclindine (PCP)	Temazepam (TEM)	Zolpidem (ZOLP)
α-hydroxy-Alprazolam (OH-AL)		

Ion Suppression & Drug Recovery

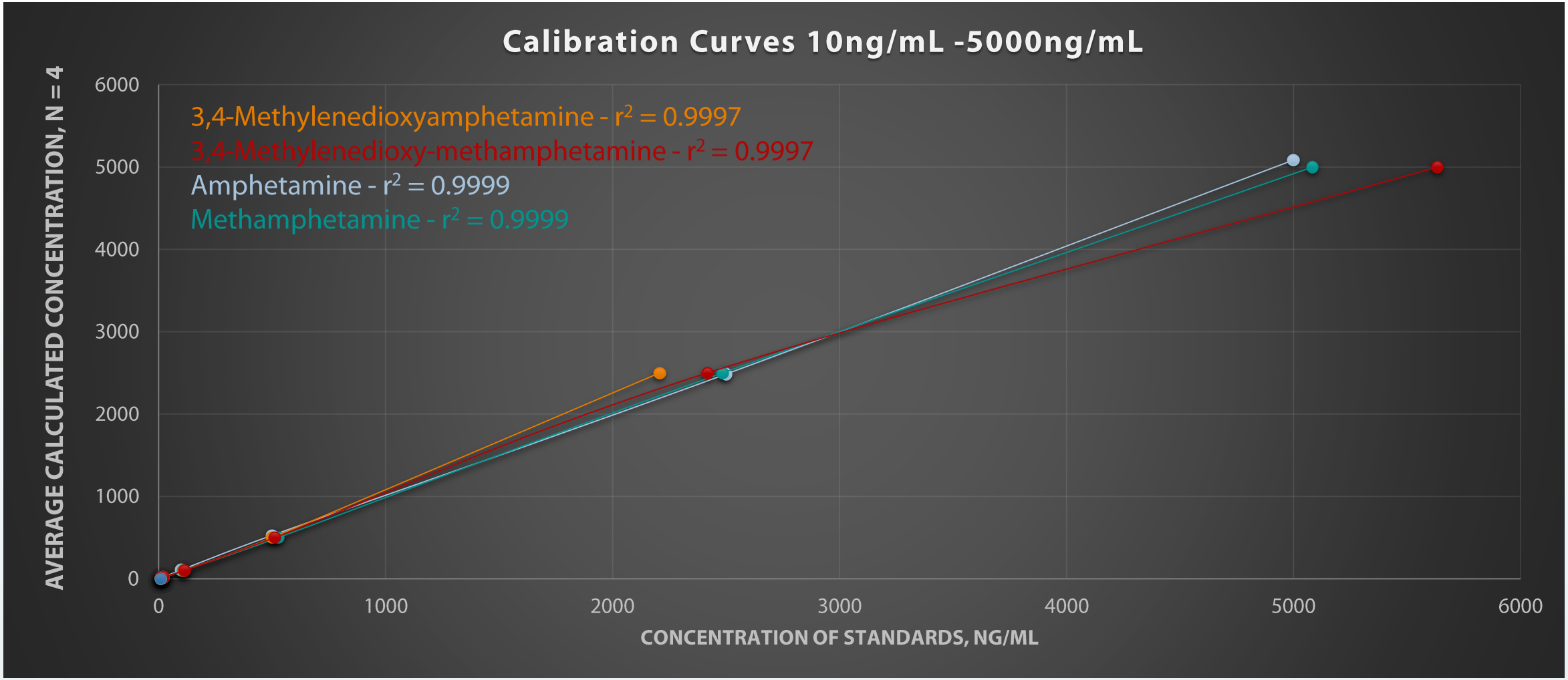
	Ion Suppression (%)		Drug Recovery (% Neat)	
	Collected Sample	Calibrator	Collected Sample	Calibrator
Amphetamine	7	3	70	76
Methamphetamine	3	1	69	52
3,4-Methylenedioxyamphetamine	5	5	79	85
3,4-Methylenedioxy-methamphetamine	4	5	69	73
7-Aminoclonazepam	3	-6	77	80
Clonazepam	-11	0	72	75
Alprazolam	12	0	41	46
OH-Alprazolam	7	-1	66	72
Diazepam	24	10	30	40
Nordiazepam	4	3	47	51
Temazepam	12	-1	40	51
Oxazepam	-3	-4	77	77
Lorazepam	-7	-5	85	86
Zolpidem	11	-2	50	48
Cocaine	7	9	38	45

Ion Suppression & Drug Recovery

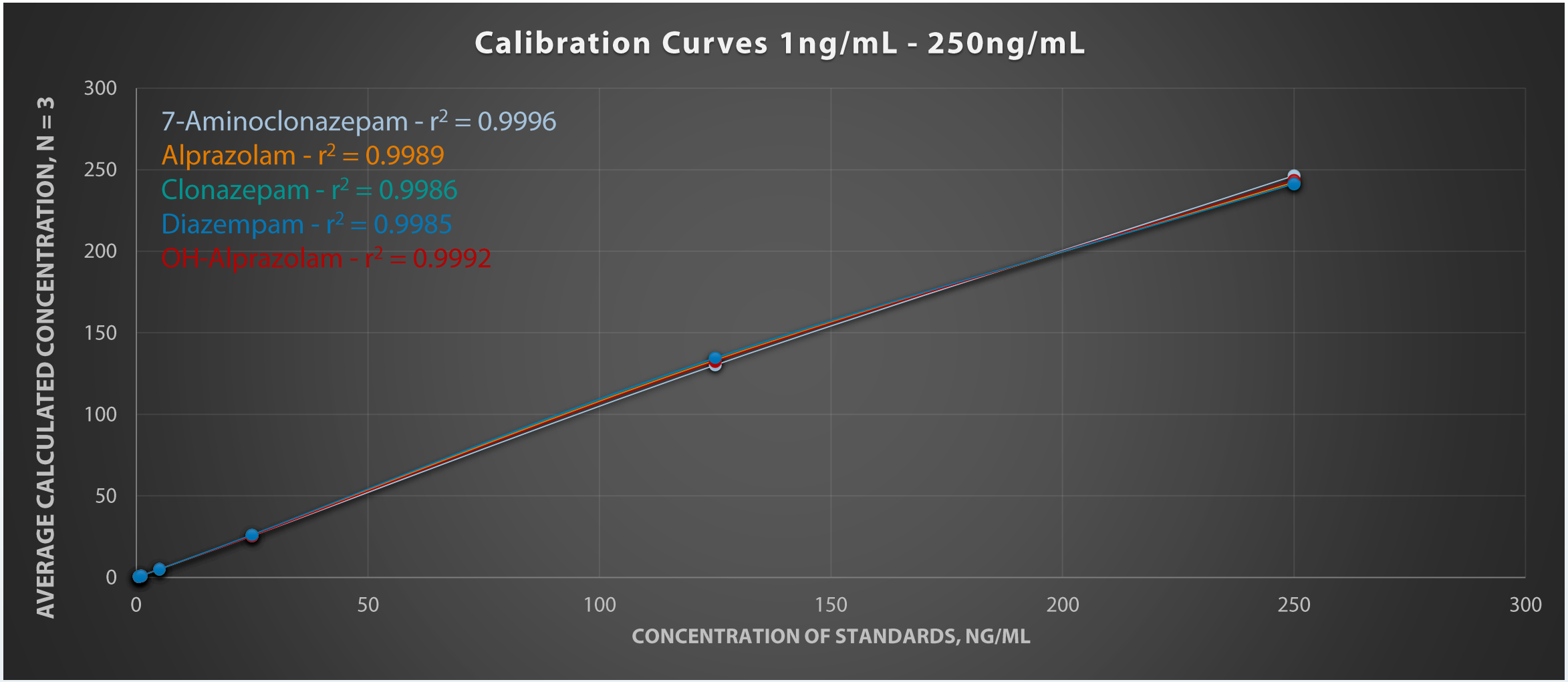
	Ion Suppression (%)		Drug Recovery (% Neat)	
	Collected Sample	Calibrator	Collected Sample	Calibrator
Benzoylecgonine	8	2	78	76
Methadone	31	18	36	36
Codeine	10	5	109	115
Morphine	7	7	83	97
Hydrocodone	8	6	85	94
Hydromorphone	7	6	109	110
Oxycodone	6	-1	92	100
Oxymorphone	6	7	100	103
6-Acetylmorphine	5	2	100	125
Phencyclidine	5	7	47	51
Buprenorphine	3	6	60	76
Norbuprenorphine	5	-1	74	94
Fentanyl	10	2	50	54
Norfentanyl	4	3	86	86
Carisoprodol	-15	-1	70	78

methamphetamine, Amphetamine, Methamphetamine.

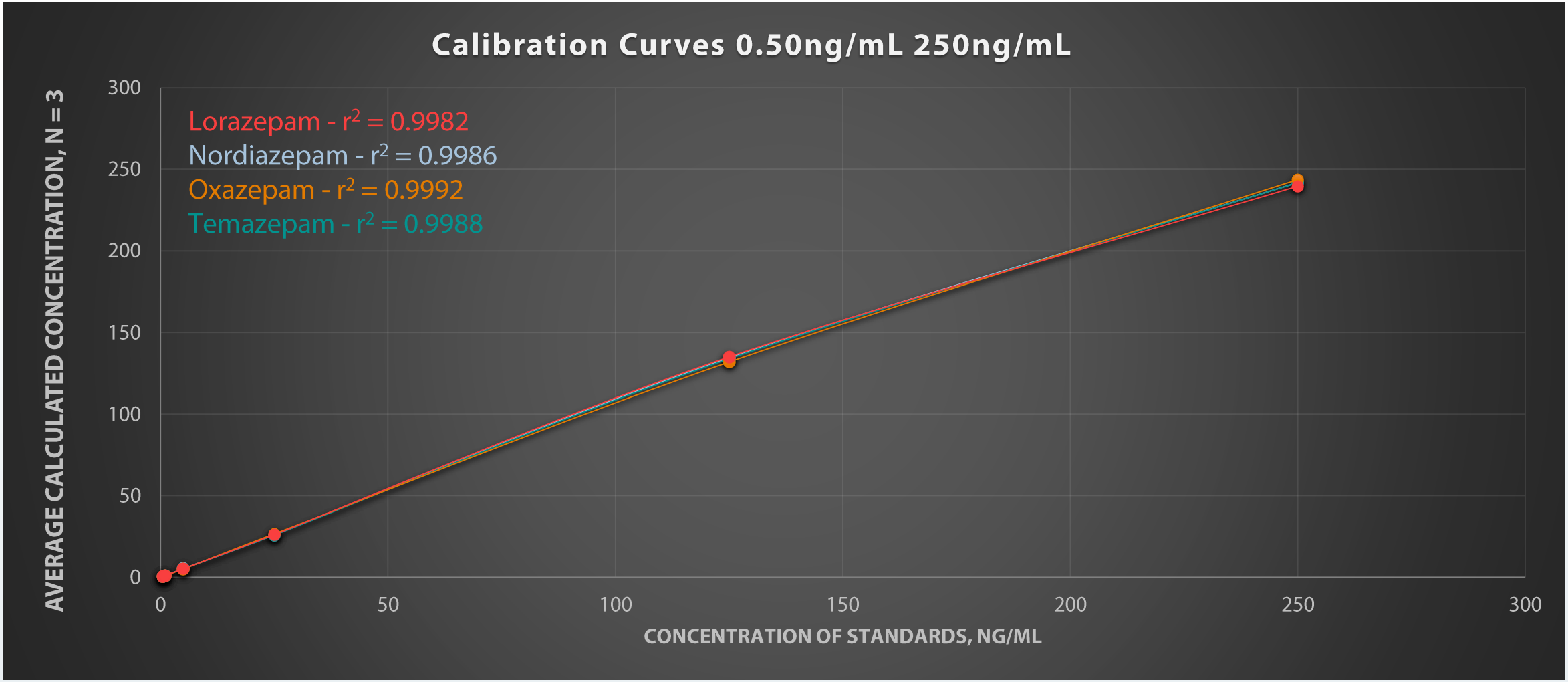
Correlation Coefficients are > 0.99.



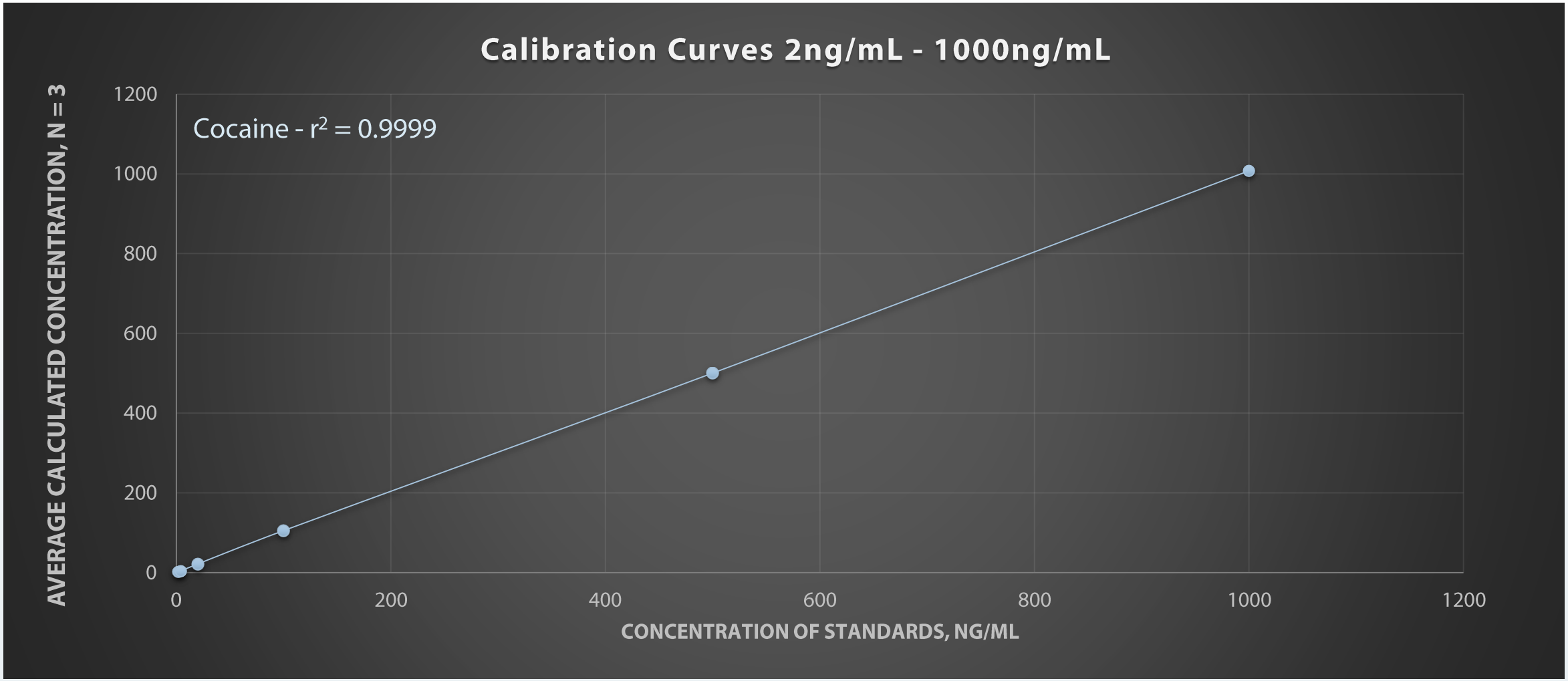
Calibration curves for 7-Aminoclonazepam, Alprazolam, Clonazepam, Diazempam, OH-Alprazolam , . Correlation Coefficients are > 0.99.



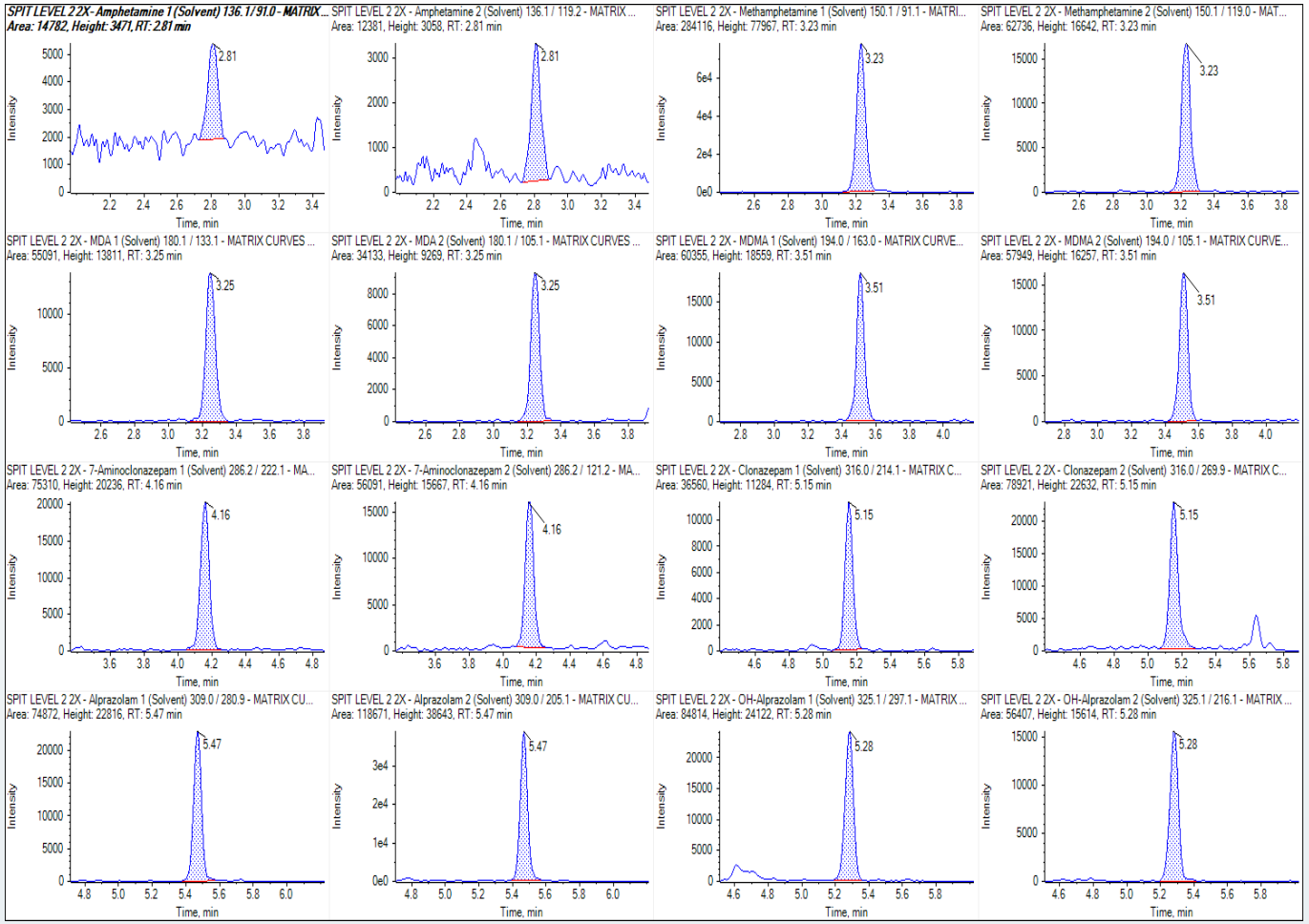
Calibration curves for Lorazepam, Nordiazepam, Oxazepam, Temazepam. Correlation Coefficients are > 0.99.



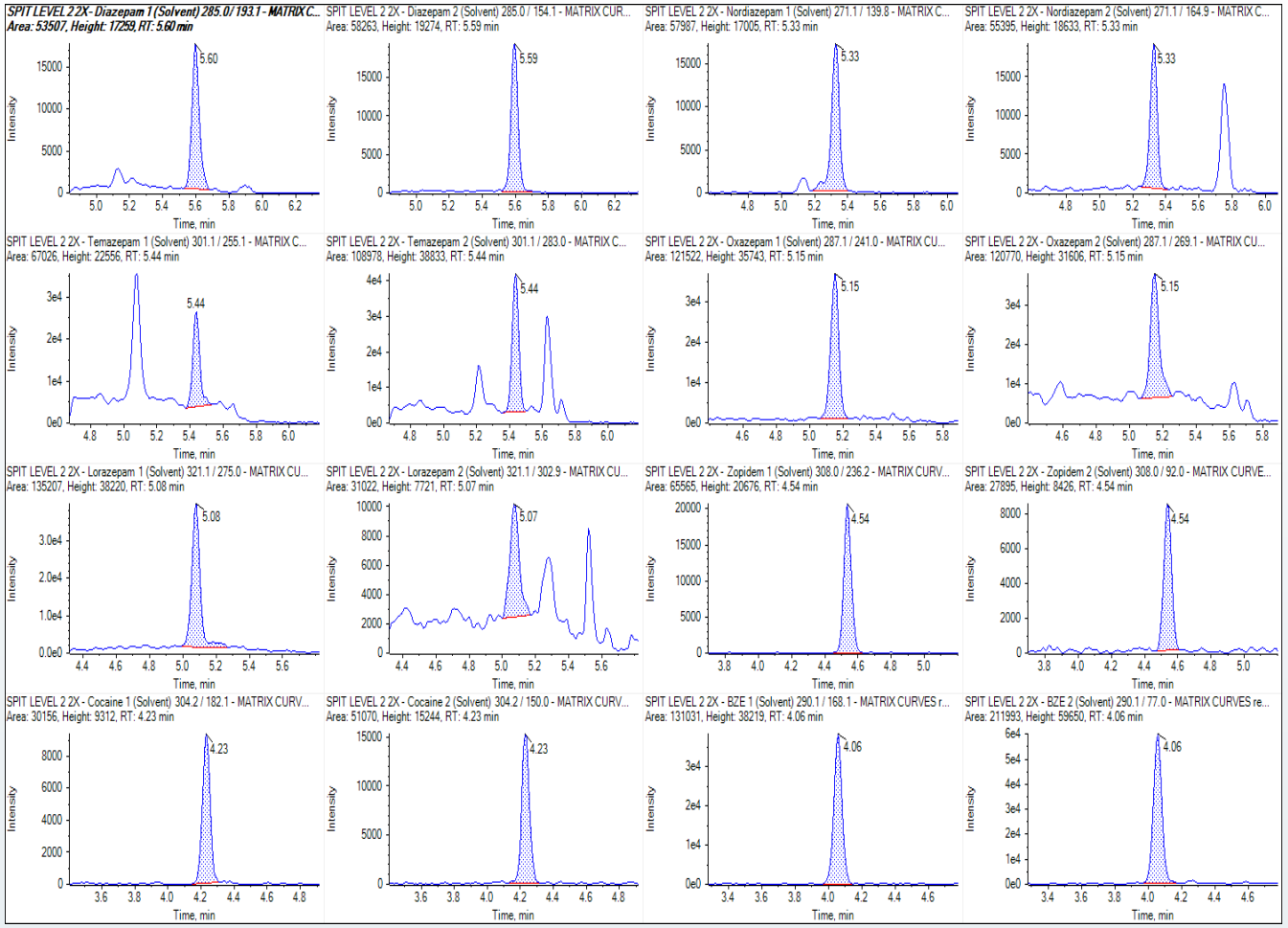
Calibration curve for Cocaine.
Correlation Coefficients are > 0.99



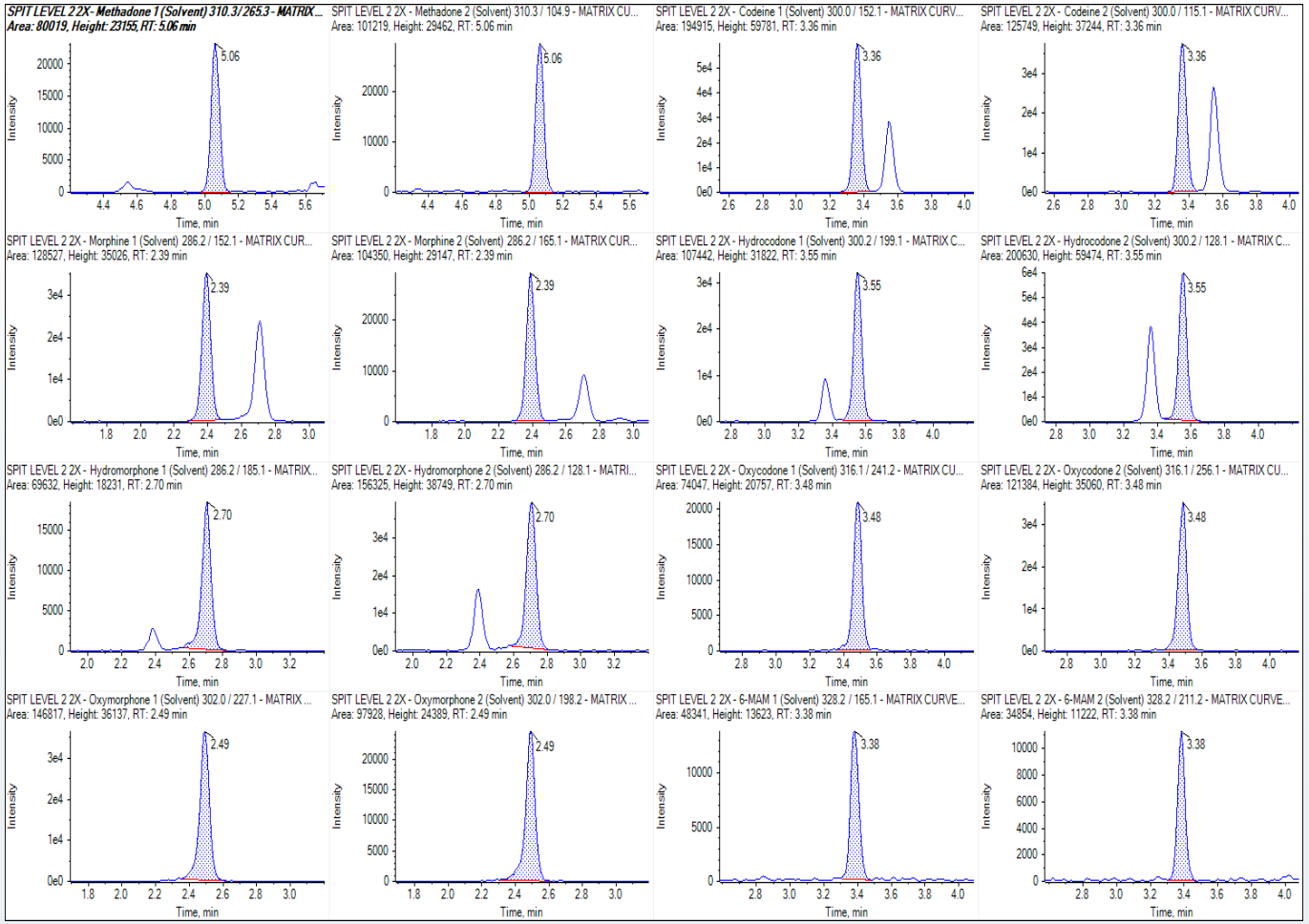
Mass Spectrum - Level 2



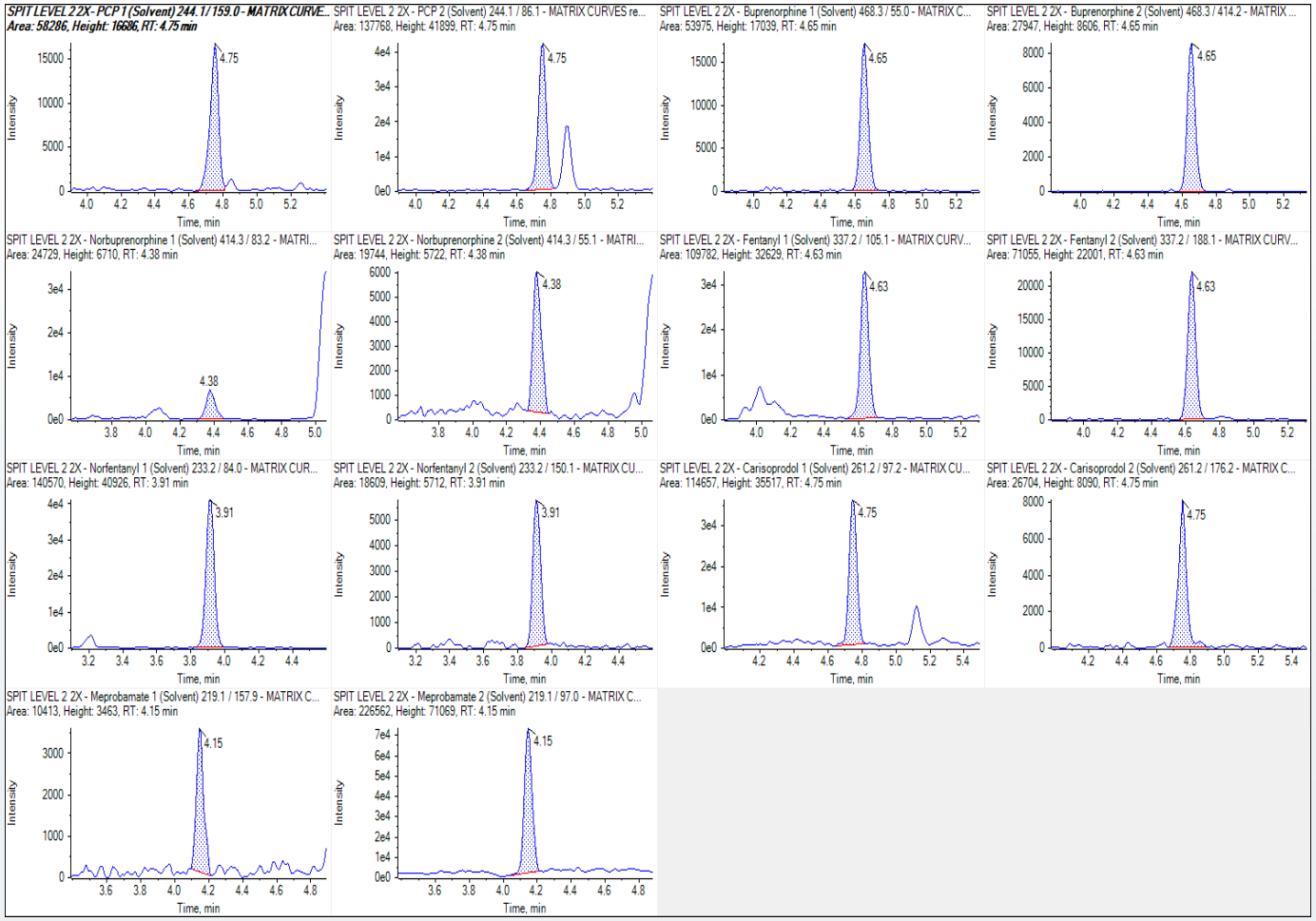
Mass Spectrum - Level 2



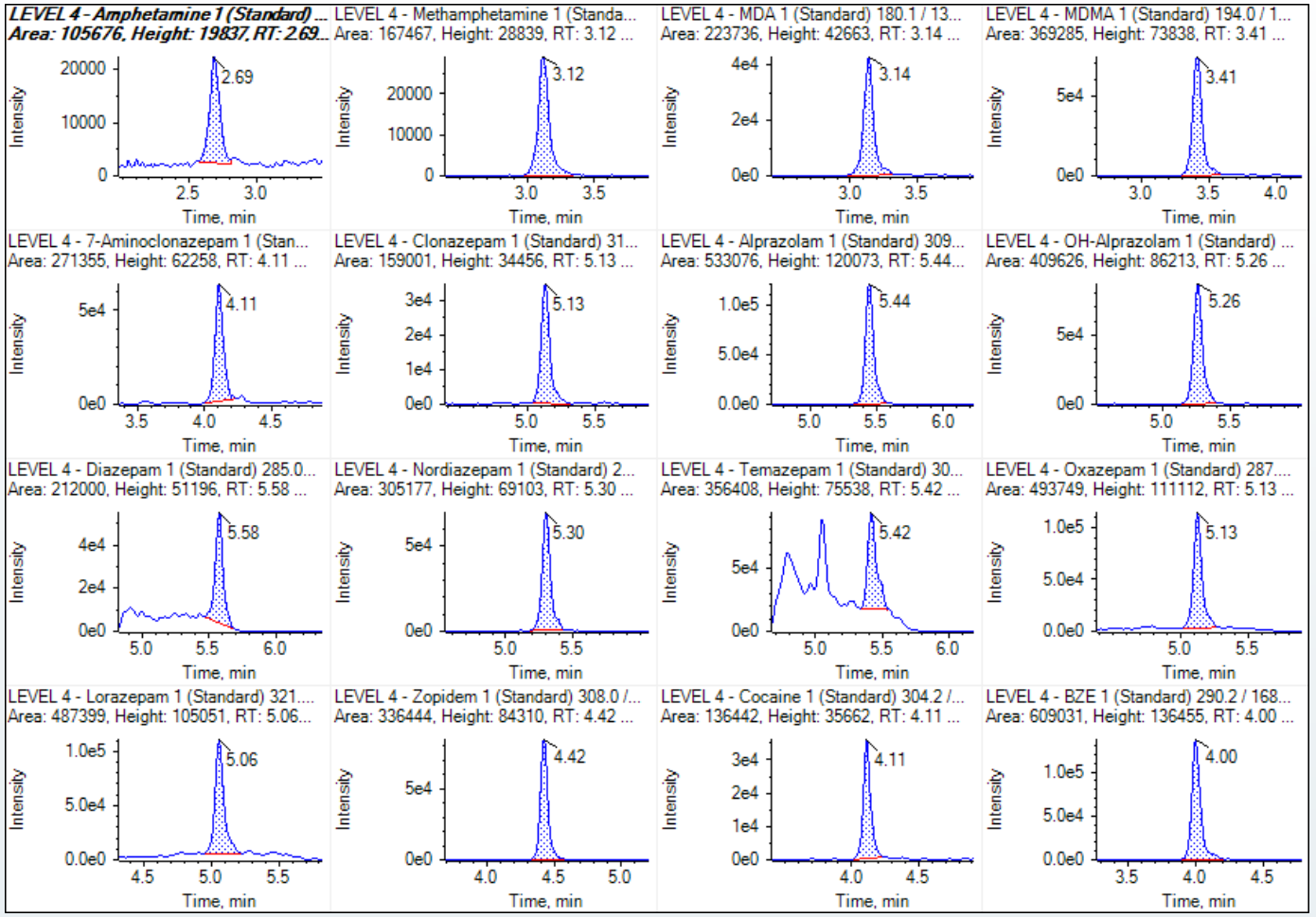
Mass Spectrum - Level 2



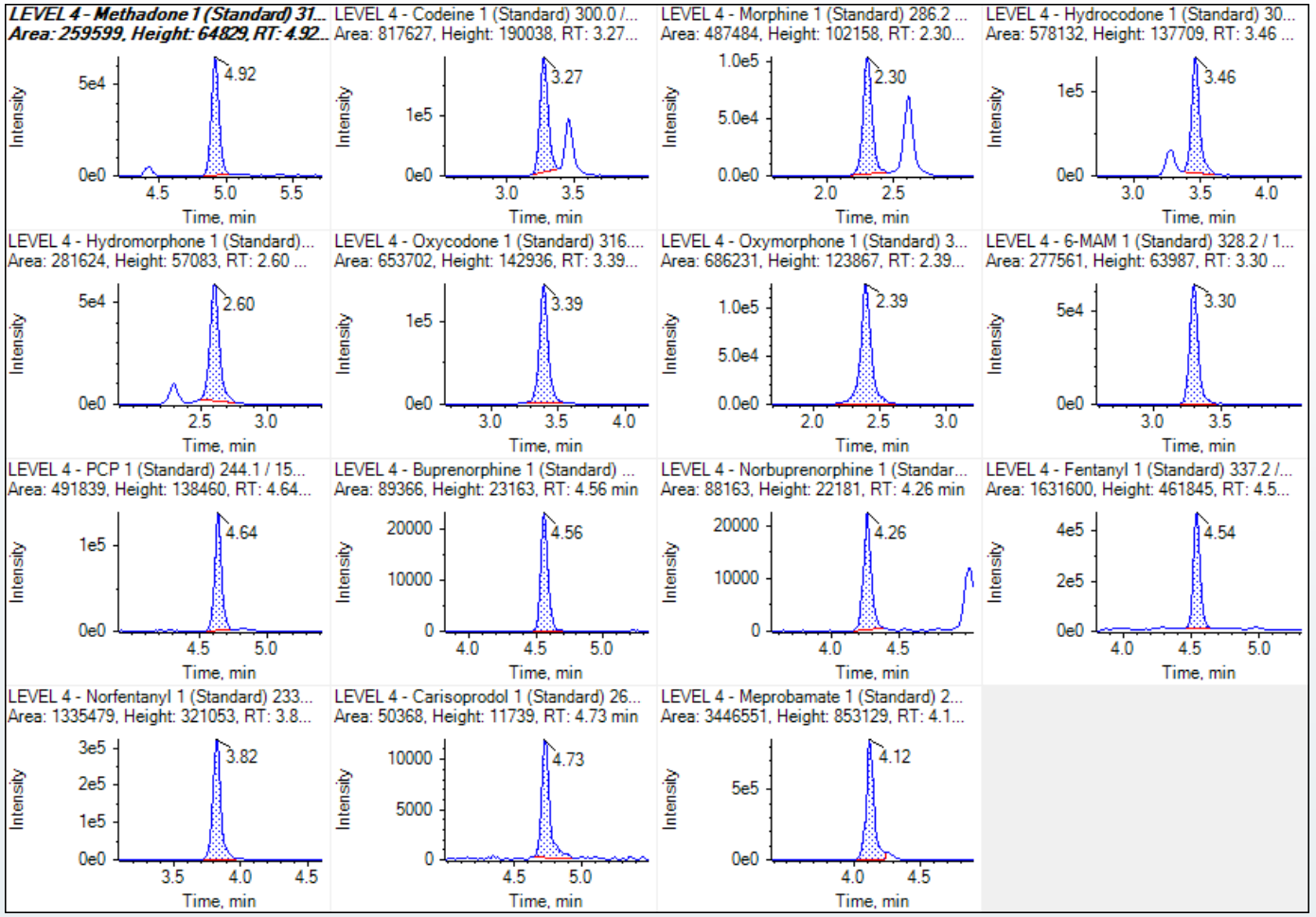
Mass Spectrum - Level 2



Mass Spectrum - Level 4



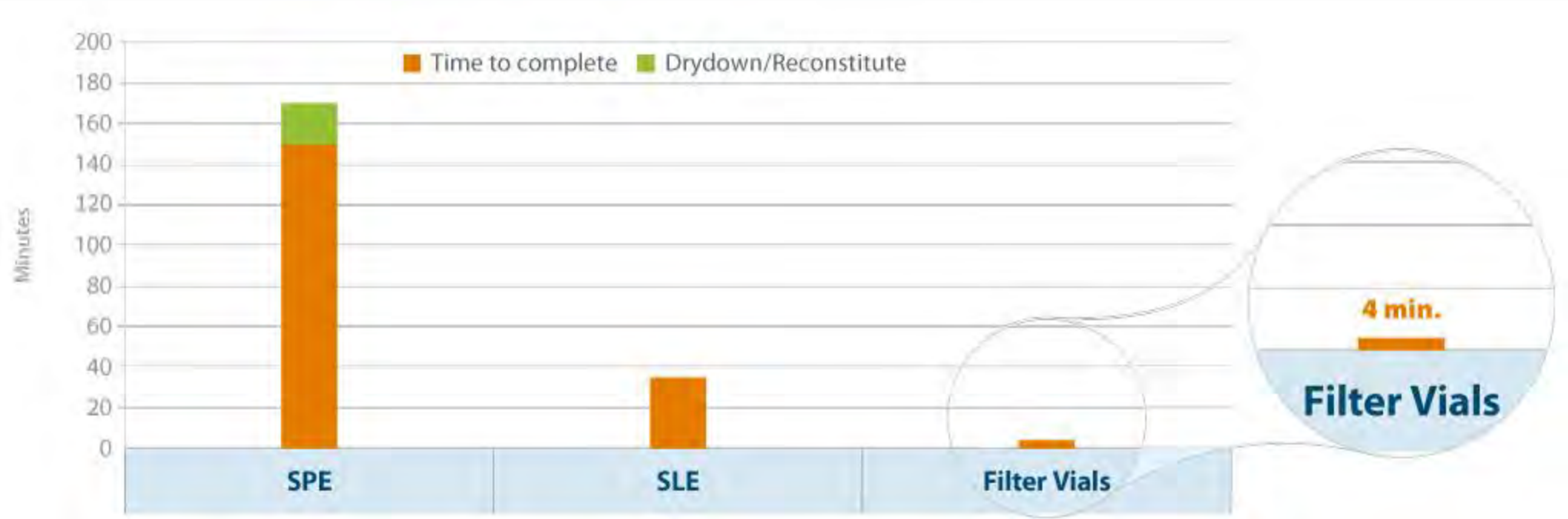
Mass Spectrum - Level 4



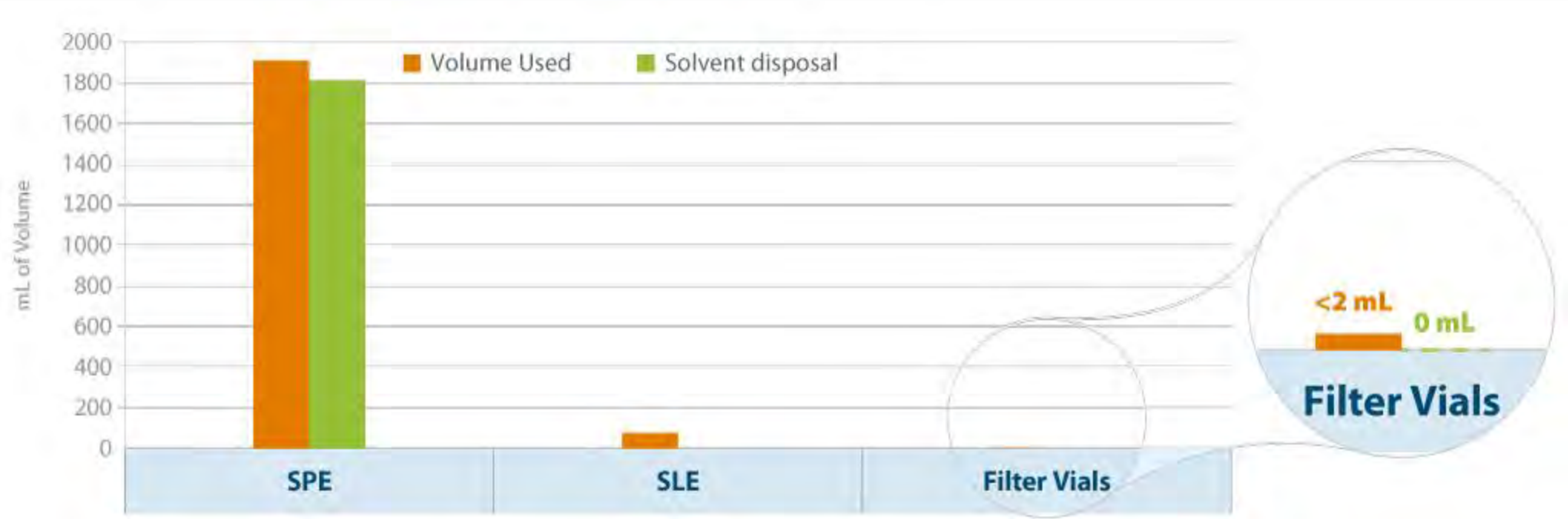
New Method Benefits

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**

Time


200
180
160
140
120
100
80
60
40
20
0
Minutes
Time to complete
Drydown/Reconstitute
SPE
SLE
Filter Vials
4 min.
Filter Vials

Solvent Usage & Disposal



Equipment Cost & Maintenance/Annually

Conclusion

- ✓ Validated method alleviates the need for sample clean-up by SPE or SLE
- ✓ Reduces the amount of equipment required
  - ✓ Reduces solvent usage
  - ✓ Reduces 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® Filter Vials include lower cost, faster sample preparation time, less use and disposal of organic solvents.

American Academy of Forensic Sciences

#2 Advanced Mass Spectrometry (MS) Techniques for Forensic Analysis: What Does the Future Hold?

Monday, February 22 **8:30 a.m. - 12:00 p.m.** **3.0 CE Hours**

Educational Objective(s): After attending this presentation, attendees will be better able to evaluate and select advanced mass spectrometric techniques for solving various analytical problems in forensic science including identification of unknowns, rapid throughput approaches to forensic sample preparation, novel ionization, and fragmentation approaches in hyphenated mass spectrometric techniques.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing attendees to some of the most recent advances in mass spectrometry technology and their potential application to solve challenges in forensic investigations. This workshop has a strong interdisciplinary focus.

Chair:
Sherri L. Kacinko, PhD
Willow Grove, PA

Co-Chair:
Kenyon M. Evans-Nguyen, PhD
Tampa, FL

Faculty:
Adam B. Hall, PhD
Northeastern University
Boston, MA

David M. Schwope, PhD
Aegis Sciences Corporation
Nashville, TN

Jason E. Schaff, PhD
Quantico, VA

Jillian K. Yeakel, MS
Bethlehem, PA

LVT Lehigh Valley Toxicology

Use of Multiplexing and Alternative Sample Preparation Techniques for High Throughput Toxicological Screening

Jill Yeakel

February 2016

Worksop

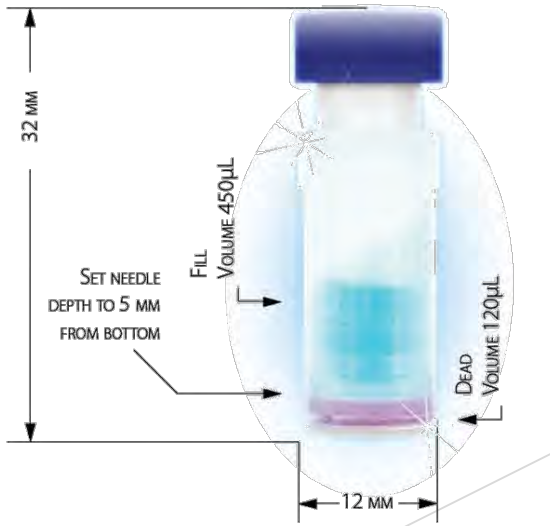
Lehigh Valley Toxicology

Sample Preparation Options

- Extraction:
  - Solid Phase Extraction, Supported Liquid Extraction, Liquid-Liquid Extraction
- Filter Vials
  - Process to dilute and filter urine and oral fluid samples

Thomson Filter Vials

- Shown to reduce matrix interferences for both urine and oral fluid
- Demonstrates adequate analyte recovery
- Simple and efficient method that eliminates solvent waste and other typical extraction consumables



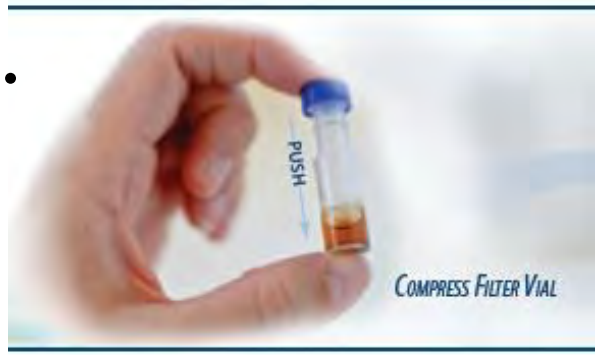
Lehigh Valley Toxicology

http://htslabs.com/downloads/FilterVialFlier.pdf

eXtreme® Filter Vial Method

1.

2.



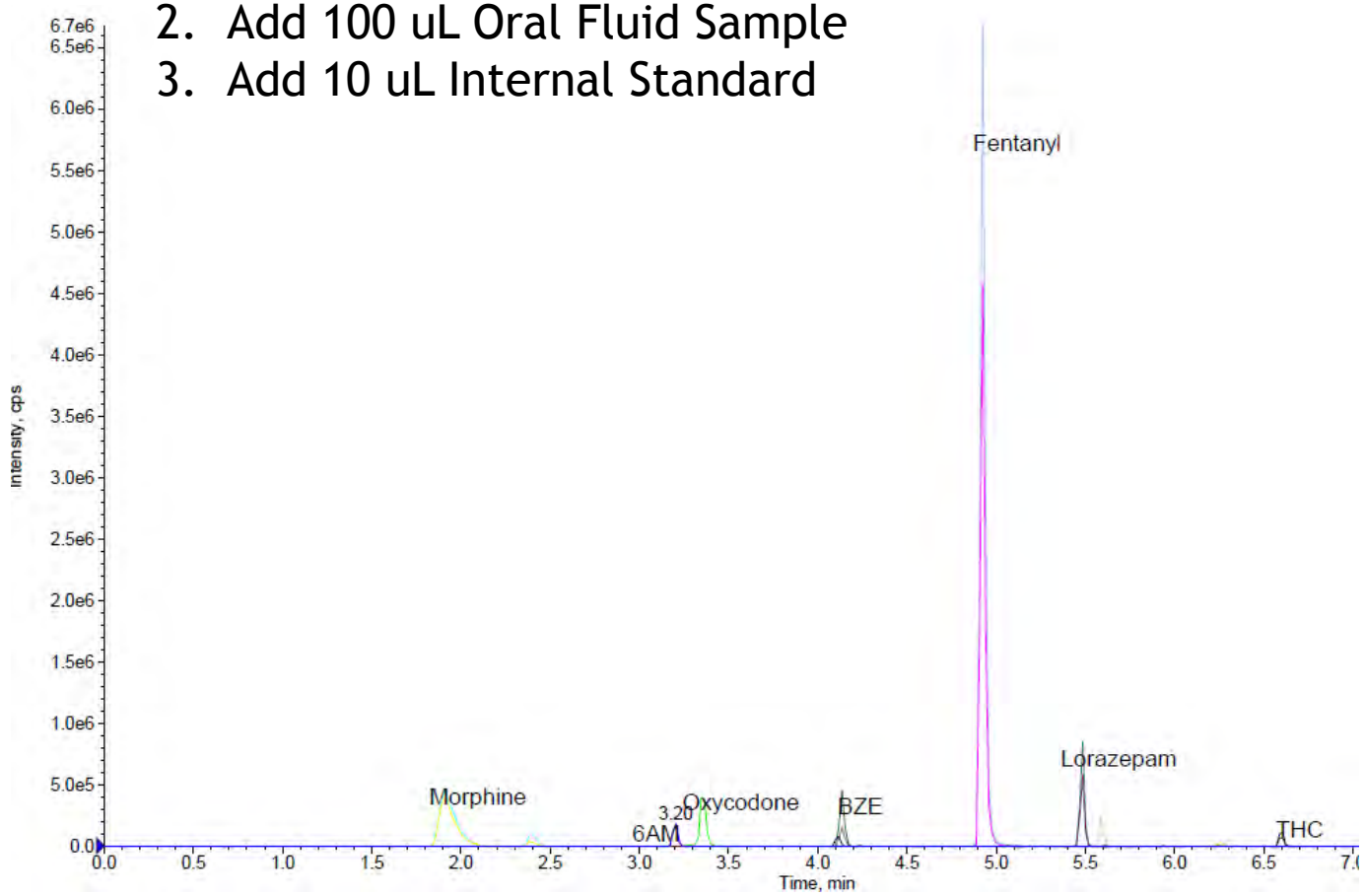
3.

Lehigh Valley Toxicology

http://htslabs.com/downloads/FilterVialFlier.pdf

Extracted Control

1. Add 100 uL of 20% MeOH (0.1% FA):80% H_2O (0.1% FA)
2. Add 100 uL Oral Fluid Sample
3. Add 10 uL Internal Standard

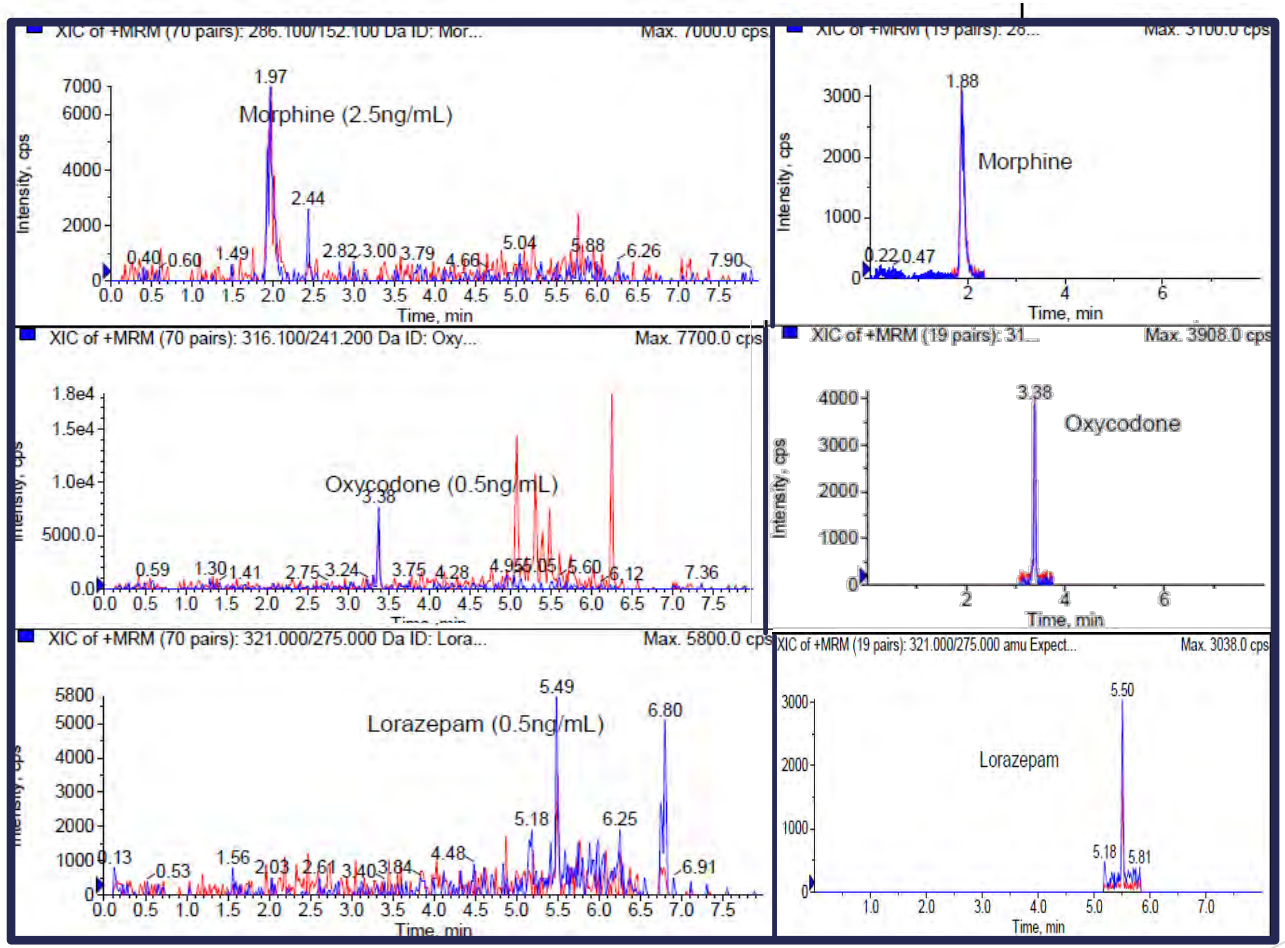


Lehigh Valley Toxicology

Limit of Detection Study

Unscheduled

Scheduled

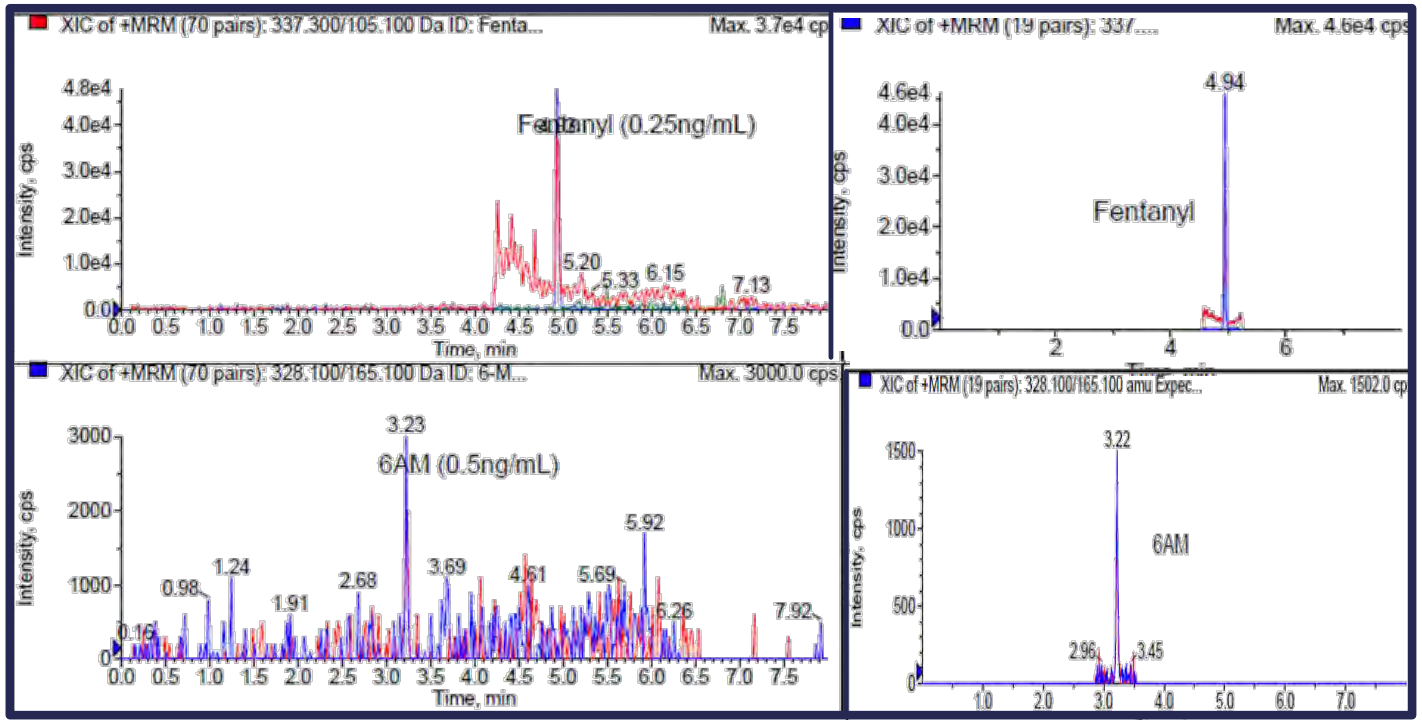


Lehigh Valley Toxicology

Limit of Detection Study

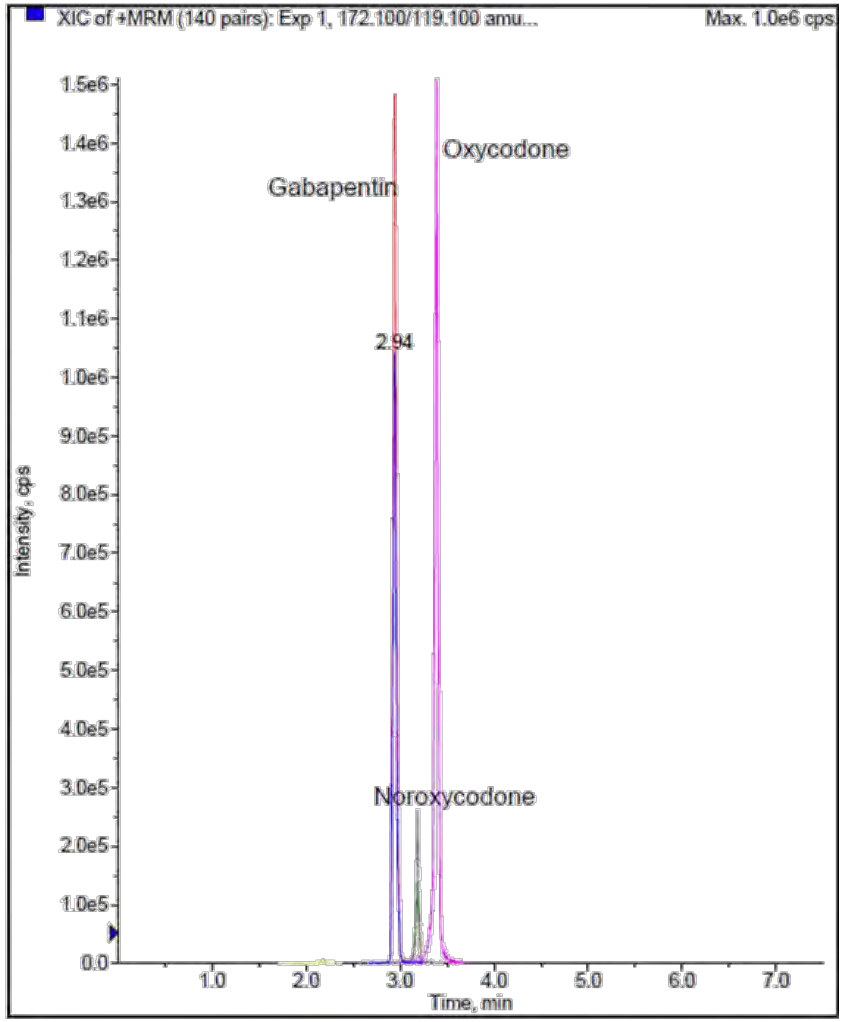
Unscheduled

Scheduled



Lehigh Valley Toxicology

Authentic Oral Fluid Sample



Lehigh Valley Toxicology

*Oral fluid samples were collected with the OraSure Technologies i2he™ Collection Device

Comparison Studies

	SPE	Filter Vial
Number of Samples	48	48
Solvent Used	266.4 mL	4.8 mL
Solvent Waste	168 mL	0 mL
Extraction Time	~2 hours	~12 minutes
Equipment Cost	$127.77**	$120.00

**Does not include labor, extraction setup (manifold, pump, etc), maintenance, waste disposal

LVT Lehigh Valley Toxicology

Filter Vials

- Benefits:
  - Increased efficiency
  - Decreased sample cost
  - Decreased solvent waste
- Drawbacks:
  - Minimal recovery of THC

Thank You!

Any questions please contact:

Jill Yeakel

jyeakel@lvtox.com

Lehigh Valley Toxicology

High Throughput Screening and confirmation of 41 Pain Panel Drugs in Oral Fluid by an Integrated On-Line Extraction UHPLC-MS/MS System

Louis Maljers, Zicheng Yang

Bruker Daltonics Inc., 3500 West Warren Ave, Fremont, CA 94538

Contact: louis.maljers@bruker.com

Introduction

- Here we present a high throughput, cost effective and sensitive procedure for screening and confirmation of Pain Panel Drugs (PPDs)
- Synthetic Saliva using Thomson filter vial for sample preparation and using an integrated On-Line Extraction (OLE)-UHPLC-MS/MS System for sample analysis.
- Lower limit of quantitation (LLOQ) is 0.01-0.2 ng/mL
- Upper limit of quantitation (ULOQ) is 100 ng/mL.
- Linearity regression coefficient R^2 was >0.99.
- Blanks show no interference of the analysis at the LLOQ level.
- Sub ng/mL level PPDs detection with about three orders of dynamic detection range will cover the clinical research needs.

Sample Preparation

- Transfer 200 µL of 60% Methanol/water containing 5 ppb internal standard into Thomson vial.
- Add 200 µL of drug standard in synthetic saliva (Immunalysis Corp.) to the vial and mix.
- Place Thomson Filter Plunger on top of the Thomson vial, Thomson vials-eXtreme/FV 0.2 um PVDF, w/Pre-Slit Red Cap
- Press filter plunger down approximately ¼ of the way into each of the Thomson Vial outer shells.
- Vortex for 10 sec
- Press Filter plunger the rest of the way down using Thomson Vial Filter Press.

Methods

Instruments:

EVOQ Elite triple quadrupole mass spectrometer coupled to a Bruker Integrated On-Line Extraction-UHPLC and CTC Autosampler (see Fig. 1)

LC Parameters:

Trap Column: YMC-Pack Pro ODS-AQ, 3 µm, 10 mm x 3.0 mm I.D.
Mobile Phase C: 0.1%formic acid (FA), 0.05% TFA in water
Equilibration flow: 600µL (3.0 min)
Loading Flow:600 µL
Analytical Column: YMC-Triart pfp, 1.9 µm, 50mm × 2.0 mm (I.D.)
Column Temperature: 40 °C
Injection Volume: 30 µL
Mobile Phase A: 0.1% FA in water
Mobile Phase B: 2 mM Ammonium formate and 0.1% FA in MeOH/Acetonitril=50/50

Gradient:

Time	%A	%B	Flow (µL/min)
0.0	80	20	350
0.2	80	20	350
3.5	5	95	350
3.9	5	95	350
4.0	80	20	350
6.0	80	20	350

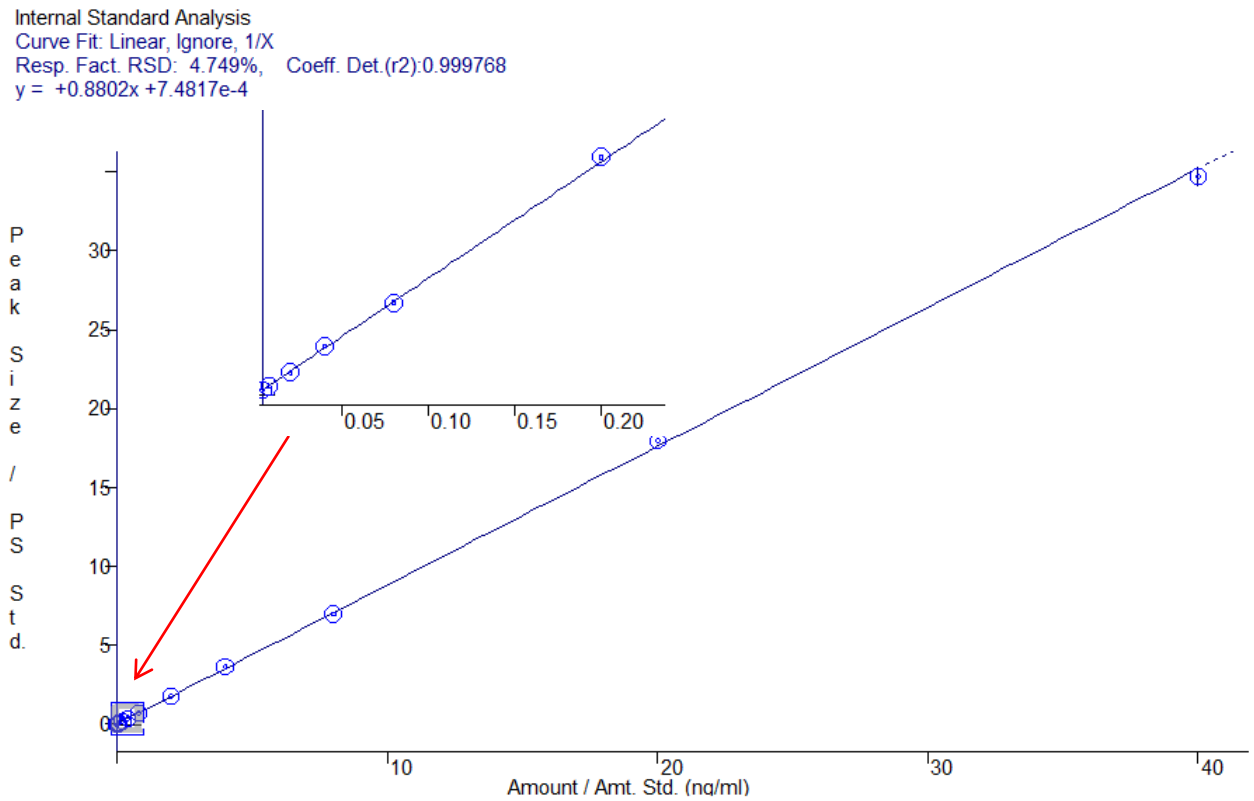
MS Parameters:

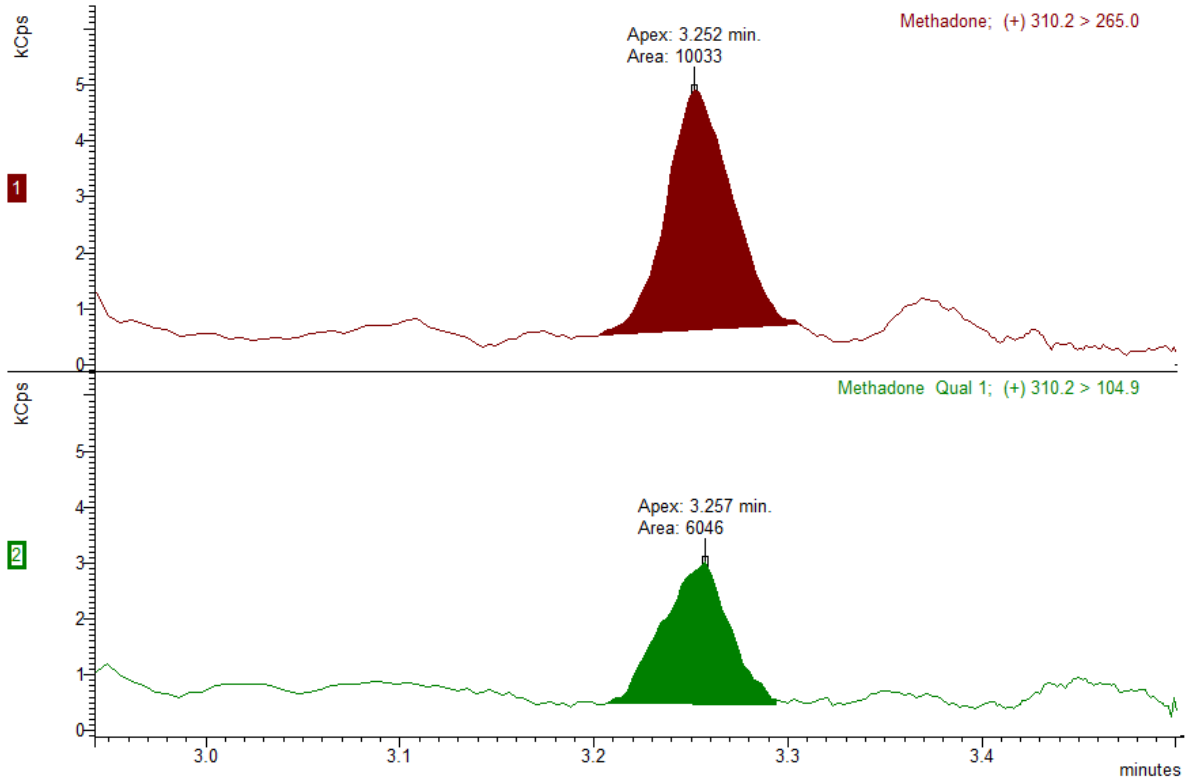
Spray Voltage (ESI positive): 4000 v
Cone Gas Flow: 30 units
Cone Temperature: 350 °C
Heated Probe Gas Flow: 40 units
Heated Probe Temperature: 400 °C
Nebulizer Gas Flow: 65 units
Exhaust Gas: on
q2 pressure: 2.0 mTorr (Argon)

Fig. 1 EVOQ Elite triple quadrupole mass spectrometer coupled to a Bruker integrated On-Line Extraction-UHPLC and CTC Autosampler

Name	Linear Range (ng/mL)	R^2	Response Factor % RSD	Name	Linear Range (ng/mL)	R^2	Response Factor % RSD
6-MAM	0.02-100	0.999	13.3	Meprobamate	0.05-100	0.998	9.1
Alprazolam	0.01-100	1.000	3.5	Methadone	0.01-100	1.000	4.7
Amphetamine	0.02-100	0.999	7.2	Methamphetamine	0.10-100	1.000	8.0
Benzoylecgonine	0.02-100	1.000	10.3	Midazolam	0.01-100	0.999	10.0
Buprenorphine	0.02-100	0.999	8.0	Morphine	0.02-100	1.000	5.0
Carisoprodol	0.05-100	0.999	9.0	Naloxone	0.02-100	0.999	11.2
Clonazepam	0.05-100	1.000	5.7	Naltrexone	0.02-100	1.000	11.0
Codeine	0.02-100	1.000	6.6	Norbuprenorphine	0.20-100	1.000	3.6
Diazepam	0.02-100	0.998	8.1	Nordiazepam	0.02-100	1.000	9.1
EDDP	0.01-100	0.997	6.5	Norfentanyl	0.01-100	1.000	6.1
Fentanyl	0.01-100	1.000	5.0	Normeperidine	0.05-100	0.999	5.8
Flunitrazepam	0.02-100	1.000	5.8	Norpropoxyphene	0.02-100	0.999	8.7
Flurazepam	0.01-100	1.000	2.0	Oxazepam	0.02-100	1.000	12.6
Hydrocodone	0.02-100	0.997	6.3	Oxycodone	0.02-100	0.996	13.8
Hydromorphone	0.02-100	1.000	4.9	Oxymorphone	0.01-100	1.000	4.4
Hydroxyalprazolam	0.02-100	1.000	4.3	PCP	0.01-100	1.000	7.4
Lorazepam	0.10-100	1.000	14.6	Propoxyphene	0.01-100	0.999	4.9
MDA	0.02-100	0.996	9.9	Sufentanil	0.01-100	0.998	9.1
MDEA	0.05-100	0.998	14.4	Temazepam	0.01-100	1.000	6.1
MDMA	0.02-100	1.000	4.3	Tramadol	0.01-100	1.000	6.2
Meperidine	0.02-100	1.000	2.9				

Table 1. 6MAM-d_6, Alprazolam-d_5, Buprenorphine-d_4, Clonazepam-D_4, Codeine-d_6, Fentanyl-d_5, Meperidine-d_4, Methadone-d_3, Morphine-d_6, Norbuprenorphine-d_3, Norfentanyl-d_5, Oxymorphone-d_3, Tramadol ^{13}C-d_3 were used as internal standard for above data.





The curve is plotted as response ratio vs concentration ratio of Methadone/ Methadone-d_3 (Concentration 0.01-100 ng/mL with 2.5ng/mL IS).

The chromatograms are 0.01 ng/mL Methadone in Synthetic Saliva.

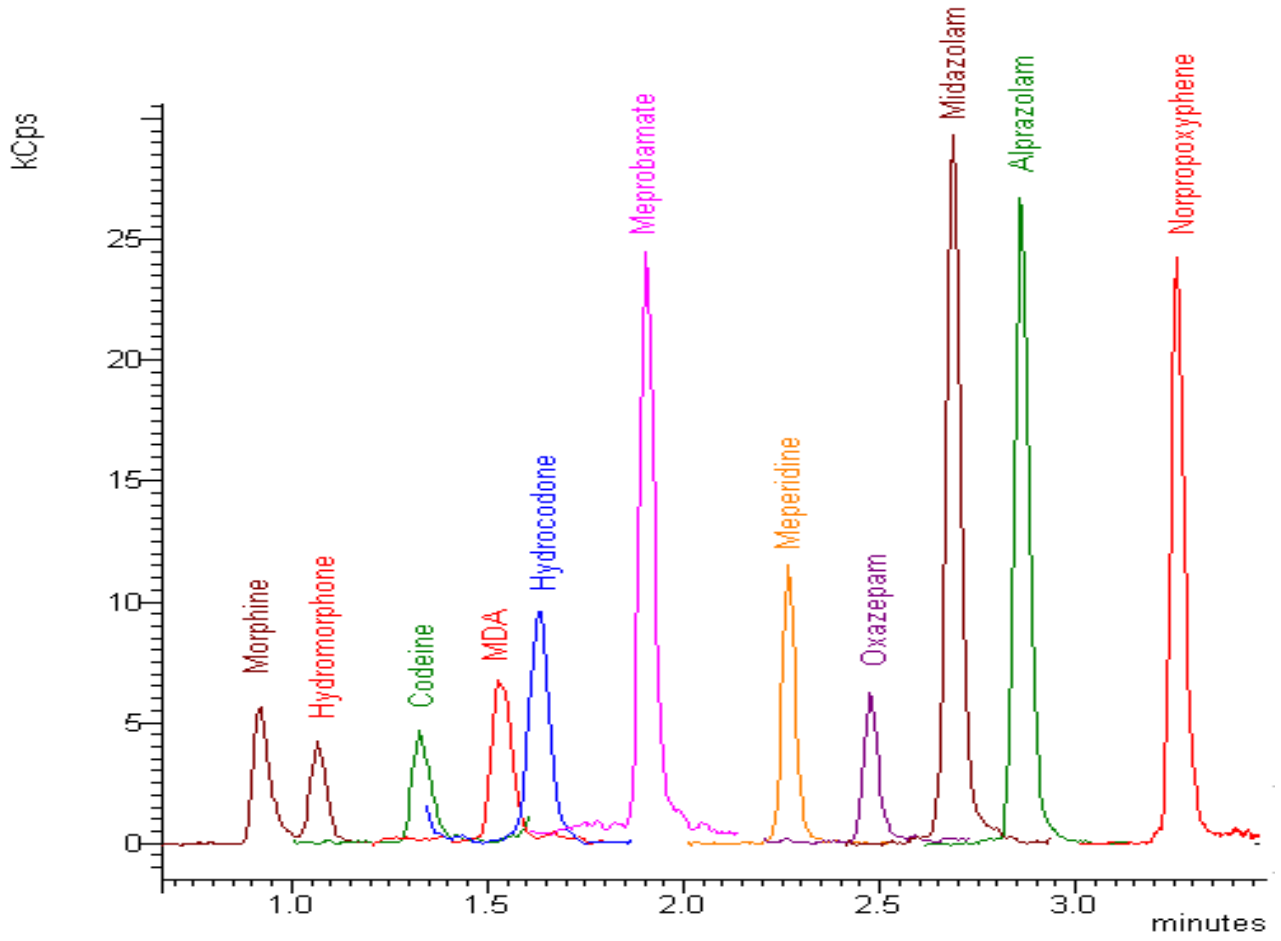


Fig. 3. Selected chromatograms at 0.2 ng/mL PPDs in Synthetic Saliva.

Results & Discussion

The sample preparation time was less than a minute by transferring saliva sample to filter vial and diluting with same volume of 60% methanol/water containing internal standard (IS) followed by mixing and press filtering. Forty one pain drugs were evaluated. Two MRM transitions were used for each compound. The first peak and last peak were eluted at 0.9 minutes and 3.3 minutes, respectively. Thirteen isotope labeled drugs were used as IS that had retention time spreading from 0.9 minutes to 3.27 minutes. The total method run time was 8.5 min including re-equilibration. The time for the entire procedure was less than 10 minutes.

Conclusions

- **Simple** (diluted, filter and shoot), **Fast** (less than 10 min) and **Sensitive** (LOQ at 0.01-0.2 ng/mL)
- Bruker LC/MS/MS coupled with integrated On-Line Extraction-UHPLC is a system of choice for high throughput PPDs analysis for clinical research needs.

Analysis THC in Saliva by EVOQ Elite

Zicheng Yang and Louis Maljers
March 11, 015

LC Conditions

LC Conditions

- Analytical Column: YMC-Pack Pro-C18, 3 µm, 2 x 50 mm
- Trapping column: YMC ODS-AQ (10 µm, 12nm), 3 mm x 10mm
- Mobile Phase A:0.1% Formic acid in water
- Mobile Phase B 2 mM Ammonium formate and 0.1% Formic acid in MeOH/Acetonitril=50/50
- Mobile Phase C:0.1% Formic acid, 0.05%TFA in water
- Injection: 30 µL (with a 100-µL loop)
- Column Temp: 40 °C
- Trap Loading Cycles: 1
- Equilibration Flow: 600 µL/min
- Trap Equilibration Time: 3.0 min
- Loading Flow: 600 µL/min
- Loading Time: 0:30 min
- Extraction Time: 3:20 min

LC Gradient:			
Time min.	Mobile Phase A **(%)**	Mobile Phase B **(%)**	**Flow Rate µL/min.**
0.0	50	50	350
0.1	50	50	350
0.5	25	75	350
2.0	5	95	350
3.5	5	95	350
3.6	50	50	350
6.0	50	50	350

EVOQ Conditions and Selected MRM Transitions

Source parameters	
Source:	HESI
Spray Voltage (Positive)	4000 V
Cone Gas Flow	25
Cone Temperature	350˚ C
Heated Probe Gas Flow	40
Heated Probe Temperature	550˚ C
Nebulizer Gas Flow	65
Exhaust Gas	On

	Name	Retention Time	RT Window	CAS Number	Retention Index	Scan Type	Scan Time (ms)	Polarity
1	THC	3.20	1.00		0	MRM	166.7	Positive
2	THC-d3	3.20	1.00		0	MRM	166.7	Positive

	Precursor	Product	Collision Energy	Q1 Resolution	Custom Res	Q3 Resolution	Custom Res	Scan Time (%)	Qualifier Ion	Qualifier Ratio	Quantifier Ion
1	315.20	193.20	19.00	Custom	1.00	Custom	1.50	50.00%	☐		☑
2	315.20	123.00	31.00	Unit (0.7)		Unit (0.7)		50.00%	☑	34.80%	☐

	Precursor	Product	Collision Energy	Q1 Resolution	Custom Res	Q3 Resolution	Custom Res	Scan Time (%)	Qualifier Ion	Qualifier Ratio	Quantifier Ion
1	318.30	196.00	19.00	Custom	1.00	Custom	1.50	100.00%	☑	99.90%	☑

Sample Prep Procedure

1. Allow standards, specimens and control to equilibrate at room temperature.
2. Transfer 200 µL of 50% Methanol/water containing 10 ppb THC-d_3 (internal standard) into Thomson vial.
3. Add 200 µL of standard in synthetic saliva to the vial and mix.
4. Place Thomson Filter Plunger on top of the Thomson vial, Thomson vials-eXtreme/FV 0.2 um PVDF, w/Pre-Slit Red Cap (p/n #85531)
5. Press filter plunger down approximately ¼ of the way into each of the Thomson Vial outer shells.
6. Vortex for 10 sec
7. Press Filter plunger the rest of the way down using Thomson Vial Filter Press.
8. Extracts are ready for LC/MS/MS analysis

Synthetic Negative Saliva from Immunalysis. Pomona, CA
http://immunalysis.com/

Calibration Solution

LLOQ: 0.05ppb

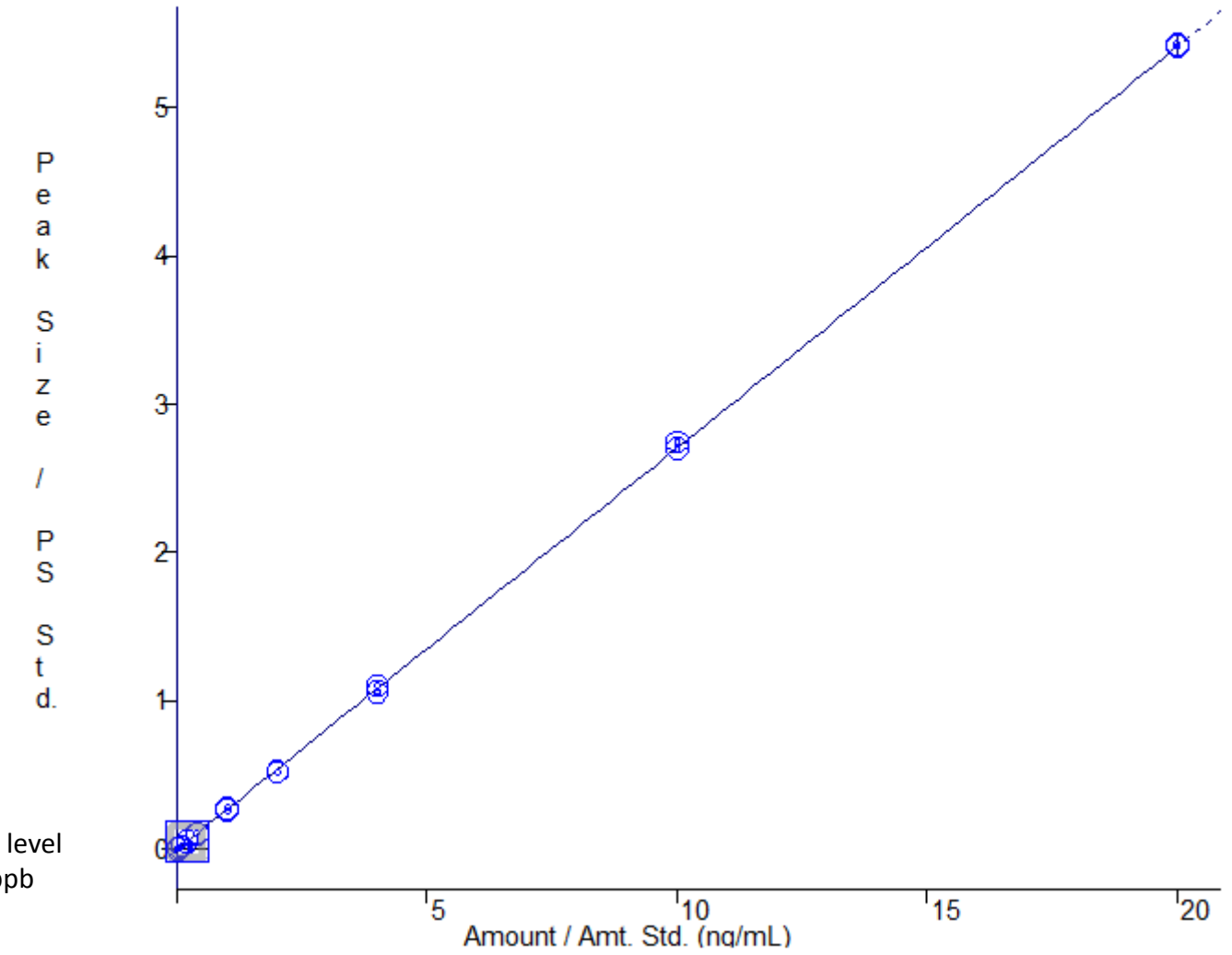
ULOQ: 100ppb

Prep Internal Standard (IS) Solution (10ppb): Transfer 20 uL of 10 ppm THC-d3 into a 20 mL vial containing 19.99 mL of 50% MeOH/water, mix well.

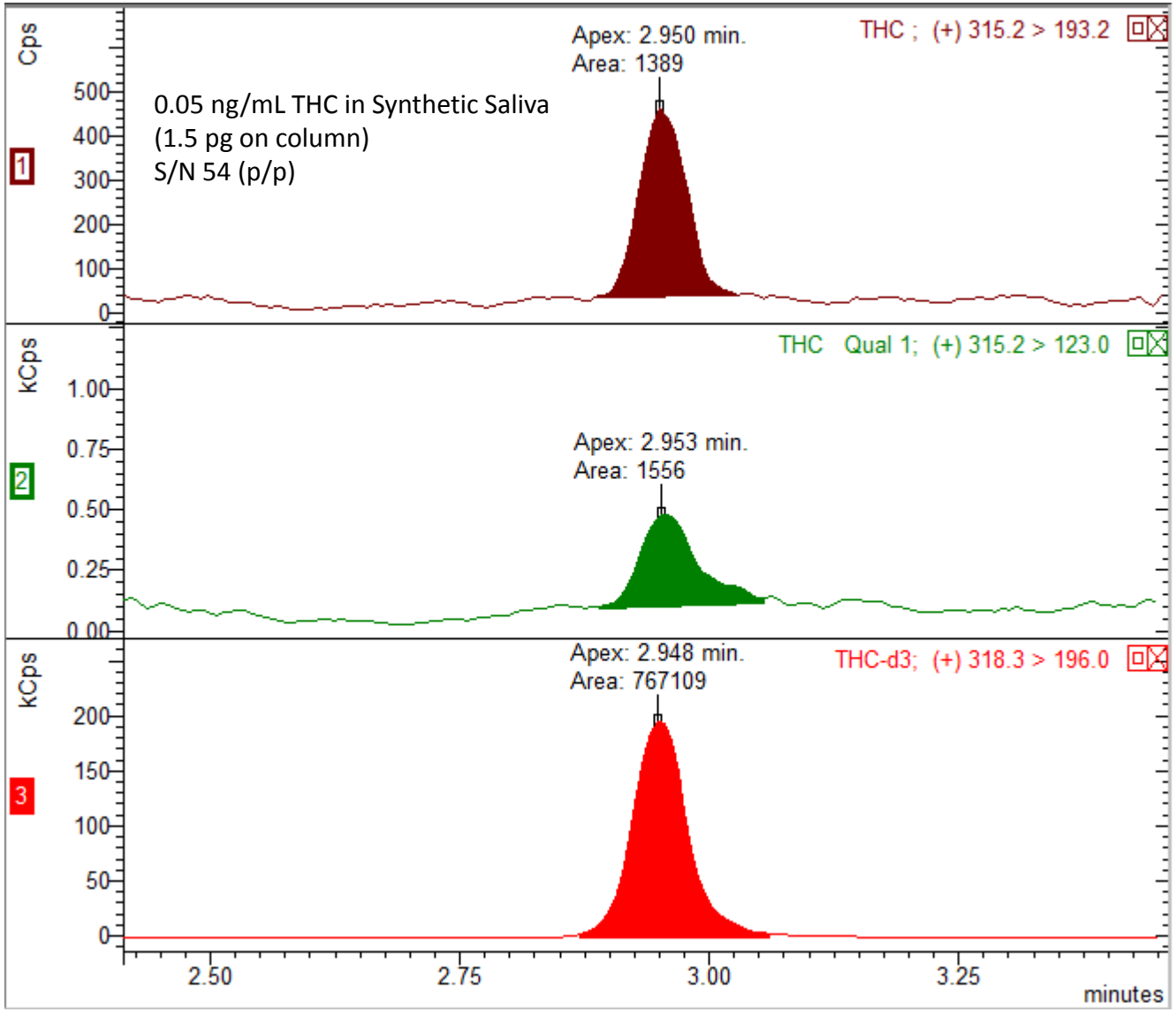
Stocks used for dilution	Stock Conc. (pg/mL)	Volume of Stock used (uL)	uL of Saliva	Final Volume (uL)	Final Conc. (pg/mL, ppt)	Final Concentration
Stocks	100,000	-	-	-	100,000	100ppb
100ppb	100,000	800	800	1600	50,000	50ppb
50ppb	50,000	600	900	1500	20,000	20ppb
100ppb	100,000	100	900	1000	10,000	10ppb
50ppb	50,000	100	900	1000	5,000	5ppb
20ppb	20,000	100	900	1000	2,000	2ppb
10ppb	10,000	100	900	1000	1,000	1ppb
5ppb	5,000	100	900	1000	500	0.5ppb
2ppb	2,000	100	900	1000	200	0.2ppb
1ppb	1,000	100	900	1000	100	0.1ppb
0.5ppb	500	100	900	1000	50	0.05ppb
0.2ppb	200	100	900	1000	20	0.02ppb
0.1ppb	100	100	900	1000	10	0.01ppb

Calibration Curve

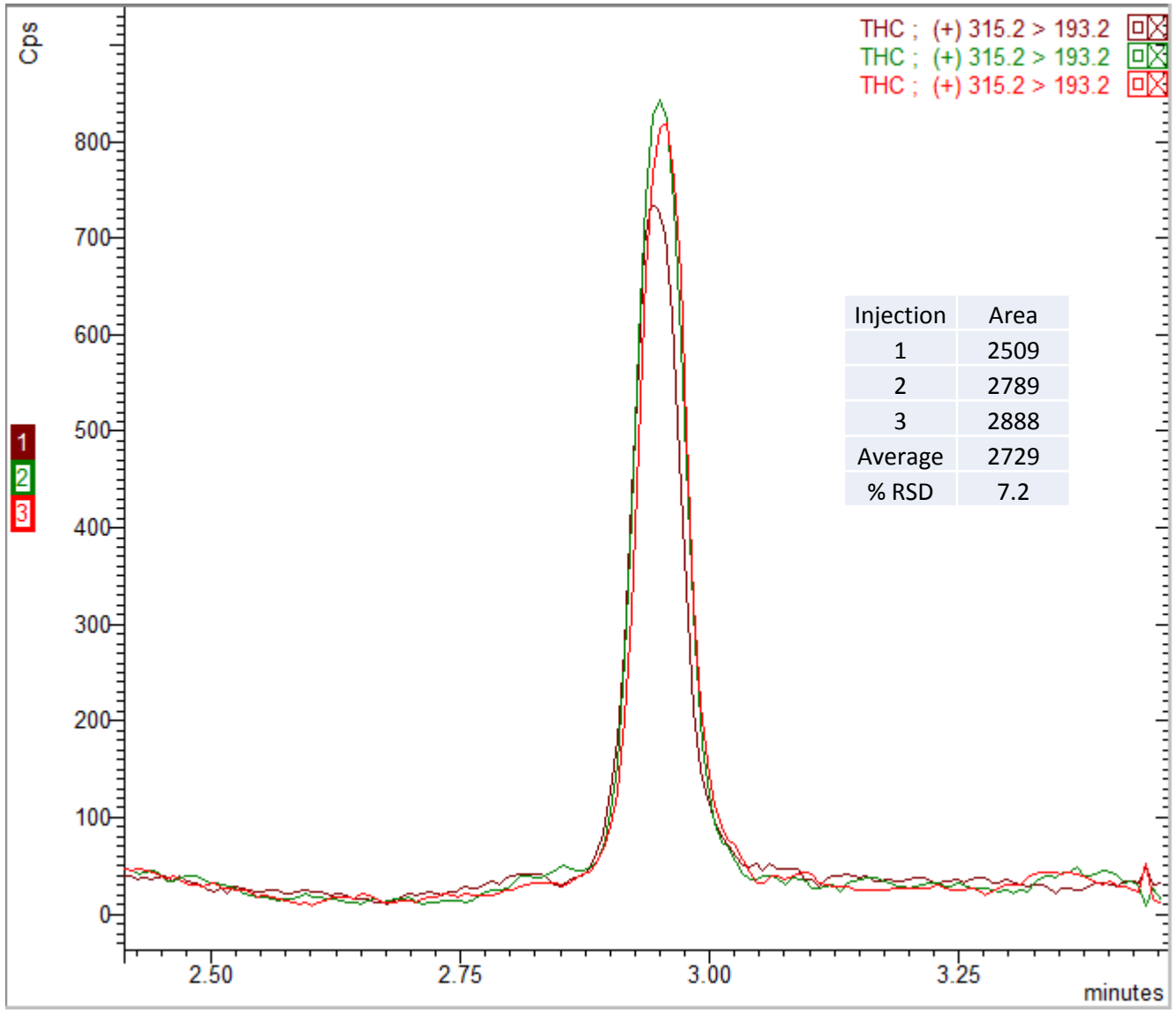
Internal Standard Analysis
Curve Fit: Linear, Ignore, 1/X
Resp. Fact. RSD: 14.30%, Coeff. Det.(r2):0.999945
y = +0.2707x -0.0017



2 injections each level
Range:0.05-100ppb

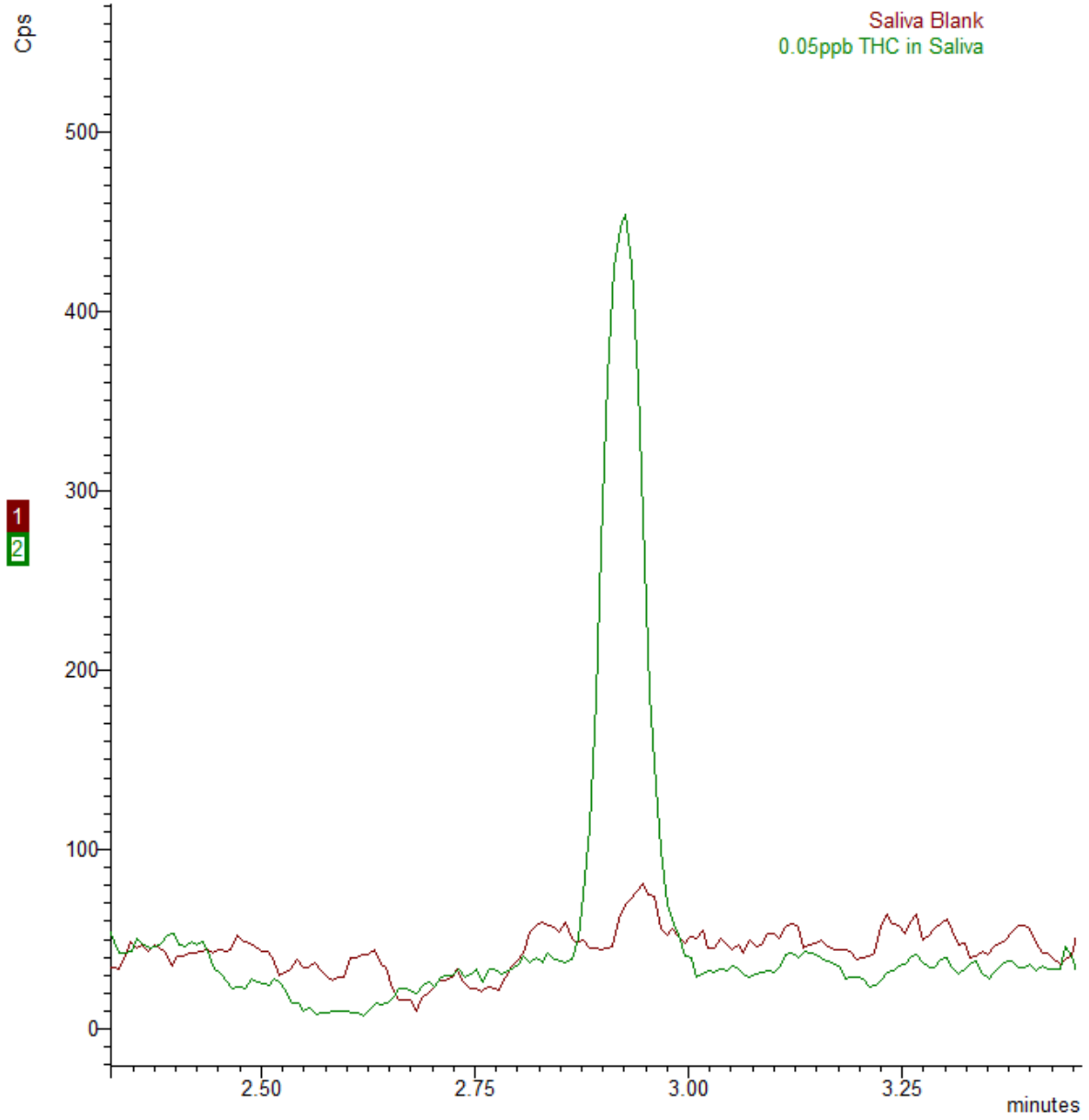

Cps
500
400
300
200
100
0
1
0.05 ng/mL THC in Synthetic Saliva
(1.5 pg on column)
S/N 54 (p/p)
Apex: 2.950 min.
Area: 1389
THC ; (+) 315.2 > 193.2
kCps
1.00
0.75
0.50
0.25
0.00
2
THC Qual 1; (+) 315.2 > 123.0
Apex: 2.953 min.
Area: 1556
kCps
200
150
100
50
0
3
Apex: 2.948 min.
Area: 767109
THC-d3; (+) 318.3 > 196.0
2.50
2.75
3.00
3.25
minutes

Reproducibility at 0.1 ng/mL



Injection	Area
1	2509
2	2789
3	2888
Average	2729
% RSD	7.2

Comparison of Blank and LLOQ



No interference at LOQ level

Acknowledgments

Dean Fritch

Analytical Associates

Zicheng Yang & Louis Maljers

Bruker Daltonics Inc

Nadine Koenig, Crystal Xander, & Melanie Stauffer

Health Network Laboratories

Jill Yeakel

Lehigh Valley Toxicology

Filter Vial Accessories

The Thomson Filter Vial Press enables high solid content and viscous liquids to be easily filtered through vials. Some fermentation cultures that reach over 100 OD or particulate laden samples may require the toggle press.

Filter Vial Toggle Press

Part # 35005

Multi-Use Press

Part # 35015

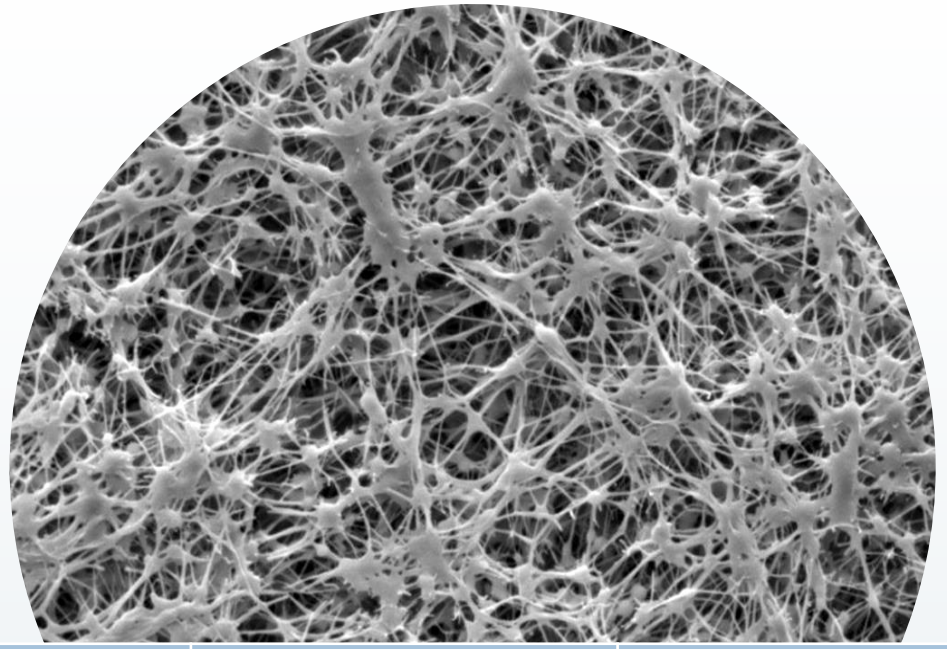
Filter Vial Membrane Material

The recommended membrane for sample filtration is based on the percentage of organic solvent in the sample and the amount of protein binding.

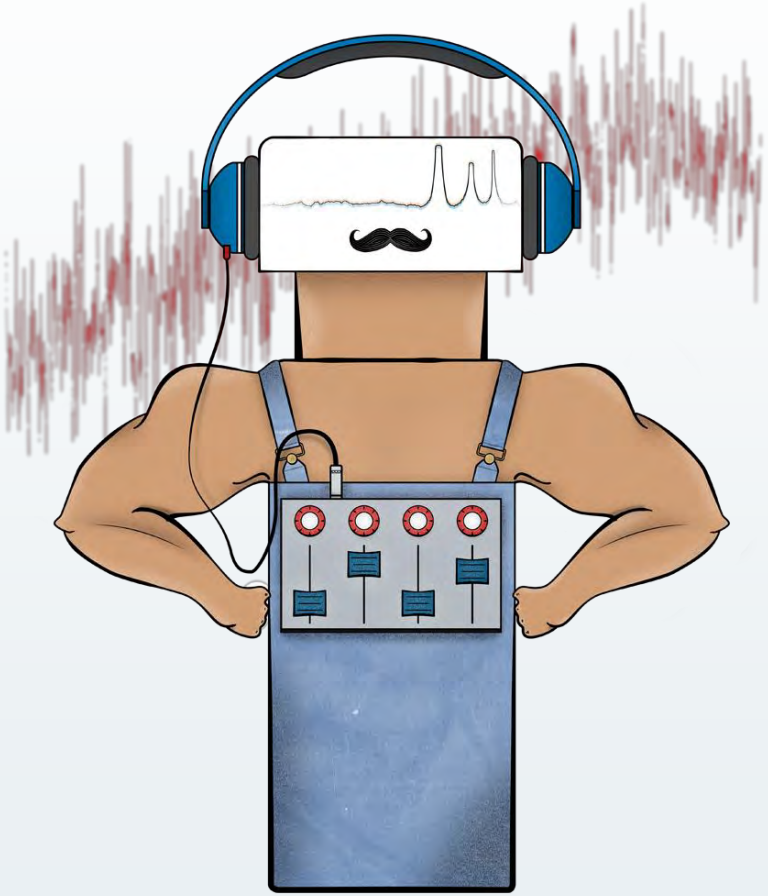
	Aqueous	Organic	Low Protein Binding
PTFE		X	
PVDF	X	X	X
Nylon	X	X	
PES	X	X	X

Filter Vial Membrane Pore Size

The recommended membrane pore size for sample filtration is based on the cell or cell debris content of the sample and the particle size of the packing material in the chromatography column used to analyze the sample. If the sample contains cells or cellular debris, then a 0.2µm pore size membrane is recommended to maintain system sterility.



	Cells or Cell Debris in Sample	Chromatography Column Particle Size <3µm	Chromatography Column Particle Size >3µm
0.2µm Pore Size	X	X	
.45µm Pore Size			X



EXTRACTOR 3D|FV®

htslabs.com | folks@htslabs.com | ☎ 800 541.4792
☎ 760 757.8080 | 760 757.9367

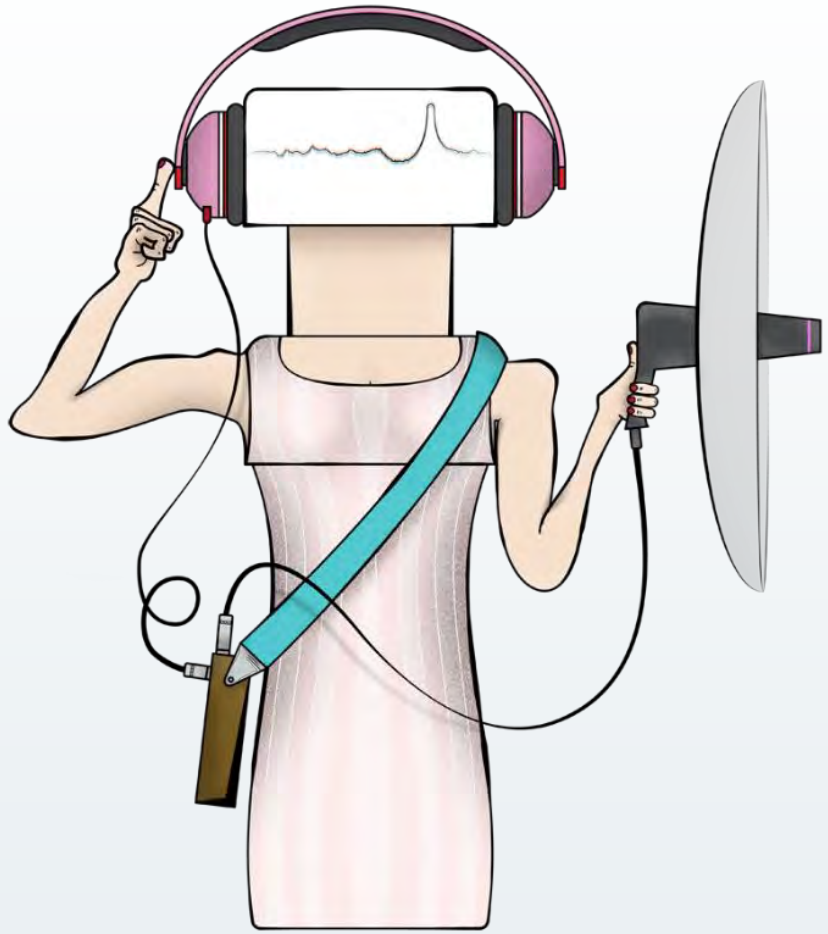
eXtractor3D | FV® Overview

eXtractor3D | FV® (Multi-Mode Filtration)

- Autosampler ready vial
- The filter vial consists of two parts:
  - Filter vial outer shell with mating bottom surface
  - Plunger which includes a filter on one end and a screw cap on the other end.
- Allows for compounds to be separated from the matrix with the addition of resins/sorbents, resulting in both a higher signal-to-noise ratio and peaks that are more differentiated.

eXtractor3D | FV® Filter Vial d-SPE

1. Weigh salts into the filter vial shell
2. Add 0.5mL extract to the filter vial shell
3. Shake then compress filter plunger into the filter vial shell
4. Place the vial into an autosampler tray



nano | Filter Vial™

htslabs.com | folks@htslabs.com | ☎ 800 541.4792
☎ 760 757.8080 | 760 757.9367

nano|Filter Vial® Overview

nano|Filter Vials® (10µL Minimum Volume)

- Low dead volume, allowing as little as 10µL of sample with enough remaining filtrate to make a 2µL injection.
- The filter vial consists of two parts:
  - Filter vial outer shell with mating bottom surface
  - Plunger which includes a filter on one end and a screw cap on the other end.
- Applications include:
  - In-vial evaporation & re-suspension for sample concentration
  - Analysis of enzymes, peptides, DNA, RNA, synthesis reaction intermediates, finished products, and samples in low volumes.

Open Access LCMS - ZQ12 - FRENICA1

File:FRENICA1_ZQ12-1087-1
Notes:DEPTH 2 -10uL
Time:16:18:04
Method:C:\MassLynx\09_LC-C8-BroadRange-ApH.olp

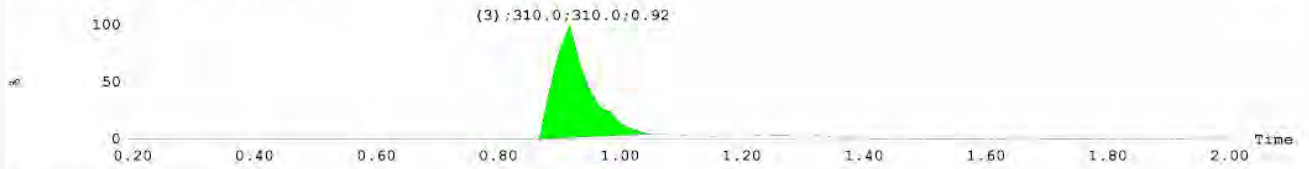
Submitter:FRENICA1
TA-Project:AS
Date:03-Oct-2011
Instrument:ZQ12

ID:MICRO VIAL
Vial:1:44
Sample: 1

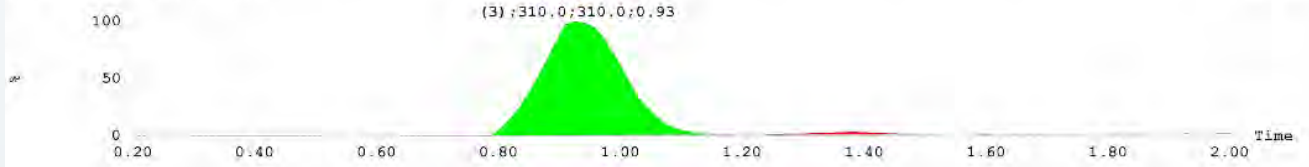
Printed: Mon Oct 03 16:21:39 2011

Sample Report:

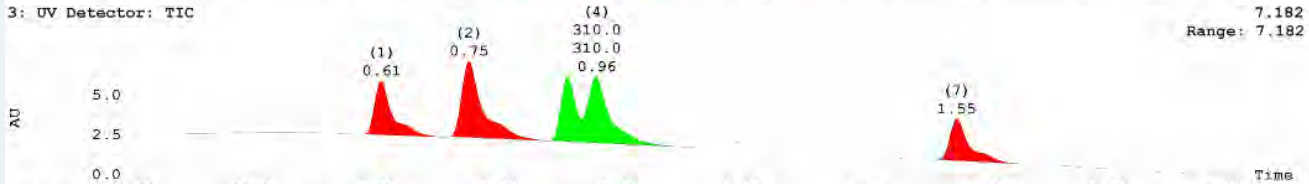
1: MS ES+ :311.008+332.99+352.034 1.2e+007



2: MS ES- :308.992+369.013 9.8e+004



3: UV Detector: TIC 7.182
Range: 7.182



Peak Number	Time	Area %
1	0.61	16.61
2	0.75	27.61
3	0.92	16.36
4	0.96	25.32
7	1.55	14.11


01
TO BE CONTINUED...