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



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Abstract

The goal of this study was to improve the sample preparation for the analysis of drugs of abuse/pain management panels in oral fluids. The oral fluid samples were collected with Intercept[®] i2he[™] Oral Fluid Collection Devices. The diluted oral fluid samples were filtered using Thompson Filter Vials, followed by LC/MS/MS analysis. 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. However, these techniques are time consuming, adversely impact recovery, require expensive consumables and equipment and use large amounts of solvent. Thomson eXtreme[®] Filter Vials (patented) offer multi-layer filtration for viscous samples and samples containing up to 30% solid particulates.

Obsolete Method: 4 drugs

AB Sciex 3200 Mass Spectrometer

• Pumps A & B: LC-20AD

• Column Oven: CTO-20AC

Mobile Phases

Flow rate: 0.5 mL/min

Injection Volume = 30µl

• Degasser: DGU-20A₅

• Morphine and BZE

• PCP and Methadone

• Autosampler: SIL-20AC HT

Shimadzu Prominence HPLC

Vortex Mixer

Result

6-Monoacetylmorphine (6-MAM)

Methylenedioxyamphetamine (MDA)

Amphetamine (AMPI

Carisoprodol (CARIS)

Hydrocodone (HCOD) Meprobamate (MEPRO)

Norbuprenorphine (NBUP)

α-hydroxy-Alprazolam (OH-AL)

Oxazepam (OXAZ)

Phencyclindine (PCP)

Codeine (CODE)

The improved method utilizes the Thomson eXtreme Filter Vials for sample clean-up significantly reducing the cost and time of per sample analysis. This method was validated for all the analytes in Table 1. Mass spectrum of all the analytes in Table 1 can be seen in Fig. 5. Table 2 shows the 4 drugs that were analyzed in oral fluid by the obsoleted method. Table 3 shows the transitions used to validate the improved method. Linearity of the assay for the drugs listed in Table 1 is displayed in Table 4. Table 5 shows ion suppression and drug recovery for analytes in table 1. Unextracted standards (neats) were run along with 3 different negative patient samples, extracted and spiked with standard and internal standard post extraction at the cutoff concentration to access ion suppression and drug recovery. To

Fig 5. Mass Spectrum - Level 2



Experimental

Equipment

Improved Method: 31 drugs

- Orasure Technologies Intercept[®] i2he[™] Oral Fluid Collection Device (p/n 3001-2673)
- Orasure Technologies Intercept i2he Diluent (p/n 1001-0384)
- Thomson eXtreme | FV[®] 0.2μm PVDF (p/n 85531)
- Thomson 48 position Vial Filter Press (p/n 35010)
- Thomson 48 position Vial Filter Press (Part # 35010)
- Eppendorf MixMate[®]
- AB Sciex 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-20A₅R
- 31 Analytes, see Table 1:

- Column: Restek Ultra Biphenyl Columns
 - (5µm, 50 x 2.1 mm)
 - Mobile Phases: • A: 0.1% Formic Acid in HPLC
 - Water

calculate drug recovery, the mean area counts of the extracted samples was compared to the mean area counts of the unextracted samples. To determine ion suppression, the mean concentration of the extracted samples was compared to the mean concentration of the post-extracted samples.

Table 1. The following 31 drugs in oral fluid will be analyzed by this "Improved Method":

Oxycodone (OCOD)

Temazepam (TEM)

	Table 2. The following analytes wthe "Obsolete Method"	ere analyzed in
	Benzoylecgonine (BE)	

	7-Aminoclonazepam (7AMINO)	Alprazolam (ALPR)		Benzo
	Benzoylecgonine (BE)	Buprenorphine (BUP)		Phen
	Clonazepam (CLONZ)	Cocaine		Meth
	Diazepam (DIAZ)	Fentanyl (FENT)		Weth
	Hydromorphone (HMOR)	Lorazepam (LOR)		Morp
	Methadone (MTHD)	Methamphetamine (MAMP)		
A)	Methylenedioxymethamphetamine (MDMA)	Morphine (MORP)		
	Nordiazepam (NDIAZ)	Norfentanyl (NFENT)		
			1	

Oxymorphone (OMOR)

Zolpidem (ZOLP)

cyclindine (PCP) adone (MTHD) hine (MORP)

Fig. 2 Calibration curves for 7-Aminoclonazepam, Alprazolam, Clonazepam, Diazempam, OH-Alprazolam

250

300

Correlation Coefficients are > 0.99.

Table 3. Final concentrations for the various analytes are as follows

	AMPH* MAMP MDA MDMA (ng/mL)	7-AMINO CLONZ ALPR OH-AL DIAZ NDIAZ TEM** OXAZ** LOR** ZOLP (ng/mL)	CODE MORP HCOD HMOR OCOD OMOR MTHD (ng/mL)	COKE BZE (ng/mL)	PCP THC (ng/mL)	6MAM FENT NFENT (ng/mL)	CARIS MEPRO (ng/mL)	BUP NBUP** (ng/mL)		
evel 1	10	0.5	5	2	0.25	0.5	20	1		
_evel 2	20	1	10	4	0.5	1	40	2		
_evel 3	50	2.5	25	10	1.25	2.5	100	5		
_evel 4	100	5	50	20	2.5	5	200	10		
evel 5	500	25	250	100	12.5	25	1000	50		
_evel 6	2500	125	1250	500	62.5	125	5000	250		
_evel 7	5000	250	2500	1000	125	250	10000	500		
Cutoff concent ** Cutoff conce All units are in c	Cutoff concentration for Amphetamine is 20ng/mL * Cutoff concentration for Temazepam, Oxazepam, Lorazepam and Norbuprenorphine are 5ng/mL Il units are in diluted oral fluid concentrations. Multiply results by three to convert to neat oral fluid.									

Table 4. Ion Suppression and 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	
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	

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128527. Height: 35026. FT: 2.39 min	Area: 104350, Height: 29147, RT: 2.39 min	Area: 107442, Height: 31822, RT: 3.55 min	Area: 200630, Height 59474, RT: 3.55 min	Prod. 27/22, Telgin, or 10, 11, 10,00 mm	/ 600 1 6 120 /		
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632, Height, 18231, RT: 2.70 min	Area: 156325, Height: 38749, RT: 2.70 min	Area: 74047 Height: 20757. HT: 3.48 min	Area: 121384, Height: 35060, RT: 3,48 min	404 1 3.91	8100 J	1 1275	8000 1 4.75
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		- Ann		36 38 40 42 44 46 48	36 38 40 42 44 46 48		

Fig 6. Mass Spectrum - Level 4

EPEL 4 - Apabetasiner / Standard _ LEVEL - Melangebranner (Standard 2013), EVEL 4 - Mill 1: Standard 311, 12, EVEL 4 - Melander 317, LEVEL 4 - Melande Time, min Time, Inne. mn Inn $\frac{4}{9} = \frac{4}{264} + \frac{5}{65} + \frac{5}{60} + \frac{5}{56} + \frac{6}{60} + \frac{5}{56} + \frac{5}{56}$ $1045 \frac{1}{9} \frac{1}{9000} \frac{1}{45} \frac{1}{50} \frac{1}{50} \frac{1}{50} \frac{1}{40} \frac{1}{45} \frac{1}{50} \frac{1}{50} \frac{1}{40} \frac{1}$

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

New Method Benefits

Faustine and Cost Maintenance / Annually Values Column trend Column Dispace

• B: 0.1% Formic Acid in
Methanol
Flow Rate: 0.5 mL/min
Injection Volume: 15µL
Run-Time: 8 minutes

• A: 0.1% Formic Acid in HPLC Grade Water • B. 0.1% Formic Acid in HPLC Grade Methanol Flow rate: 0.3 mL/min Injection Volume: 30µl

 \circ Column: Restek Allure Biphenyl (5µm 50 x 2.1mm)

• Caliper Life Sciences Turbo-Vap[®] Concentration Workstation

Column: Restek Ultra Biphenyl (5μm, 50 x 2.1mm)

• A: 0.1% Formic Acid in HPLC Grade Water

Mobile Phases (Isocratic 10% Water in Methanol):

• B. 0.1% Formic Acid in HPLC Grade Methanol

Rapid Trace[®] Solid Phase Extraction Workstation

Communication Bus Module: CBM-20A

Sample Preparation

	Improved Sample Preparation:	(Obsolete Sample Preparation:
1.	Allow standards, specimens and control to come	1.	Allow standards, specimens and control to come to
	to room temperature.		room temperature.
2.	Add 100 µL of 10% Methanol / Water	2.	To appropriately labeled 13 x 100 mm tubes add 3 mL
3.	Add 100 µL of Standard (Intercept i2he Diluent)/		of 50mM Phosphoric Acid.
	Control/oral fluid sample + 10uL Internal Standard	3.	Prepare the 13 x 100 mm tubes for analysis.
4.	Place Thomson Filter Plunger on top of the		Standards/Controls/Patient Samples
	Thomson vial, Thomson vials –eXtreme/FV [®]	4.	Vortex for 10 seconds.
	0.2µm PVDF, w/Pre-Slit Red Cap (p/n #85531)	5.	The tubes are now ready for automated extraction
5.	Press filter plunger down approximately ¼ of the		using on the Caliper Life Sciences Turbo-Vap®
	way into each of the Thomson Vial outer shells.		Concentration Workstation
6.	Vortex for 10 seconds using the Eppendorf	6.	After the elution is complete on the Rapid Trace [®] ,
	MixMate [®] .		remove the racks with the tubes intact.
7.	Press Filter plunger the rest of the way down	7.	Add 50μ L of 1% HCL in Methanol to each tube.
	using the Thomson 48 position Vial Filter Press.	8.	Vortex for 15 seconds.
8.	Extracts are ready for LC/MS/MS analysis using the	9.	The original sample tubes and the used SPEC DAU
Sh	imadzu / AB Sciex 4500		Columns can be discarded.
		10	. Take to dryness at 55°C 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

7.	Add 50 μ L of 1% HCL in Methanol to each to
8.	Vortex for 15 seconds.
9.	The original sample tubes and the used SPI
	Columns can be discarded.
10.	Take to dryness at 55°C in the Caliper Life S
	Turbo-Vap [®] .
11.	Reconstitute samples by adding 1 mL of 10
	Grade Methanol in Water to all tubes.
12.	Vortex for 15 seconds.
13.	Extracts are ready for LC/MS/MS analysis u
	Shimadzu / AB Sciex 3200

Fig. 1 Calibration curves for 3,4-Methylenedioxyamphetamine, 3,4-Methylenedioxy-methamphetamine, Amphetamine, Methamphetamine . Correlation Coefficients are > 0.99.



Fig. 3 Calibration curves for Lorazepam, Nordiazepam, Oxazepam, Temazepam. Correlation Coefficients are > 0.99. Fig. 4 Calibration curve for Cocaine. Correlation Coefficients are > 0.99

	Calibration Curves 0.50ng/mL 250ng/mL	Calibration Curves 2ng/mL - 1000ng/mL
ε 300 ν 250	Lorazepam - $r^2 = 0.9982$ Nordiazepam - $r^2 = 0.9986$	Cocaine - r ² = 0.9999
CENTRAI	$Temazepam - r^2 = 0.9988$	008 CENTRAT
		800 9 9

weinoa	# Of Samples	Time to complete	Equipment Cost	waintenance/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









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Disclaimer



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