THOMSON
Solutions At Work™

series	cap color	membrane	pore size	part #
eXtremeIFV®	●	PVDF	0.2µm	85531

Improved Method for the Analysis of 31 Drugs of Abuse in Oral Fluid samples using the eXtremeIFV® by LC-MS/MS

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Introduction

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 Thomson 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 offer multi-layer filtration for viscous samples and samples containing up to 30% solid particulates.

Improved Method: 31 Drugs

- Eppendorf MixMate®
- Thomson 48 position Vial Filter Press (Part # 35015)

Obsolete Method: 4 drugs

- Caliper Life Sciences Turbo-Vap® Concentration Workstation
- Rapid Trace® Solid Phase Extraction Workstation
- Vortex Mixer

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/Control/oral fluid sample + 10µL Internal Standard.
4. Place Thomson Filter Plunger on top of the Thomson outer shell vial, Thomson vials –eXtremeIFV® 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.

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 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 Shimadzu / AB Sciex 3200.

Results:

The improved method utilizes the Thomson eXtremeIFV®s 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. 1. Table 2 shows the 4 drugs that were analyzed in oral fluid by the obsoleted method. Linearity of the assay, ion suppression and drug recovery for analytes in table 1. were calculated using unextracted standards (neats) 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 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. Final concentrations of the drugs can be seen in table 3.

Table 1. The following 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)		

Table 2. The following analytes were analyzed in the "Obsolete Method"

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

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)
Level 1	10	0.5	5	2
Level 2	20	1	10	4
Level 3	50	2.5	25	10
Level 4	100	5	50	20
Level 5	500	25	250	100
Level 6	2500	125	1250	500
Level 7	5000	250	2500	1000

	PCP THC (ng/mL)	6MAM FENT NFENT (ng/mL)	CARIS MEPRO (ng/mL)	BUP NBUP** (ng/mL)
Level 1	0.25	0.5	20	1
Level 2	0.5	1	40	2
Level 3	1.25	2.5	100	5
Level 4	2.5	5	200	10
Level 5	12.5	25	1000	50
Level 6	62.5	125	5000	250
Level 7	125	250	10000	500

* Cutoff concentration for Amphetamine is 20ng/mL

** Cutoff concentration for Temazepam, Oxazepam, Lorazepam and Buprenorphine are 5ng/mL

All units are in diluted oral fluid concentrations. Multiply results by three to convert to neat oral fluid.

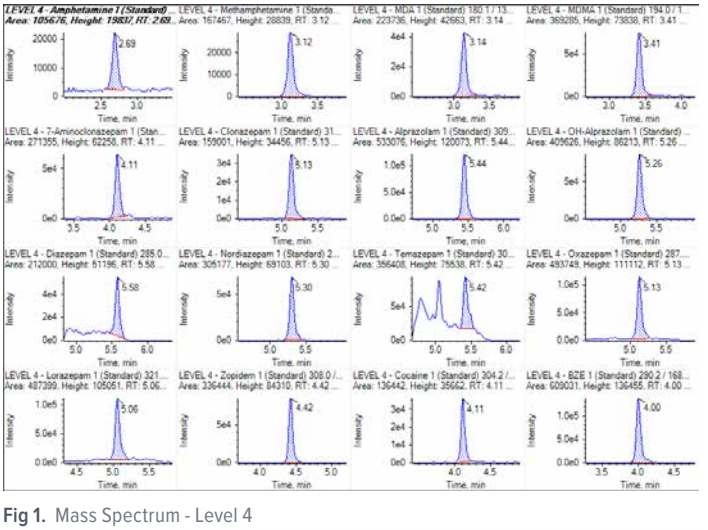

Fig 1. Mass Spectrum - Level 4

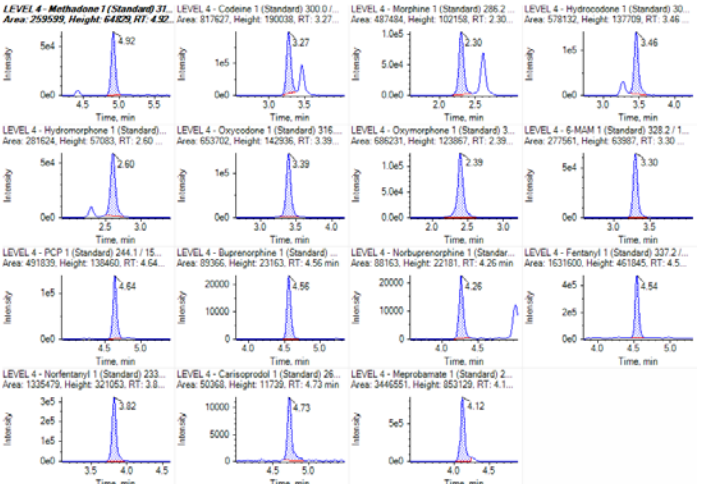

Fig 1. Mass Spectrum - Level 4 (continued)

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 filter vial outer shell,and then pushing the plunger into the outer 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.

For more information please see the full application note at:

http://htslabs.com/downloads/Improved_Method_for_the_Analysis_of_31_Drugs_of_Abuse_in_Oral_Fluid_samples_using_the_Thomson_eXtremeFV_by_LC-MS-MS.pdf