2091 Rutherford Rd., Suite 150
Carlsbad, CA 92008
info@htslabs.com
800 541.4792
760 757.8080
htslabs.com

TIC-PL-082-205 Rev. B

Filter Vial Applications

Toxicology & Forensics

A Little About Thomson

SOLUTIONS AT WORK™

Thomson sells innovative single-use Solutions At Work™, our mission is to provide technical expertise while partnering with our customers to deliver practical scientific innovations enabling scientific advancements in pharmaceutical, biotech, environmental/food, toxicology/forensics, and contract manufacturing industries.

Open to Collaboration

INNOVATIVE PRODUCT LINE

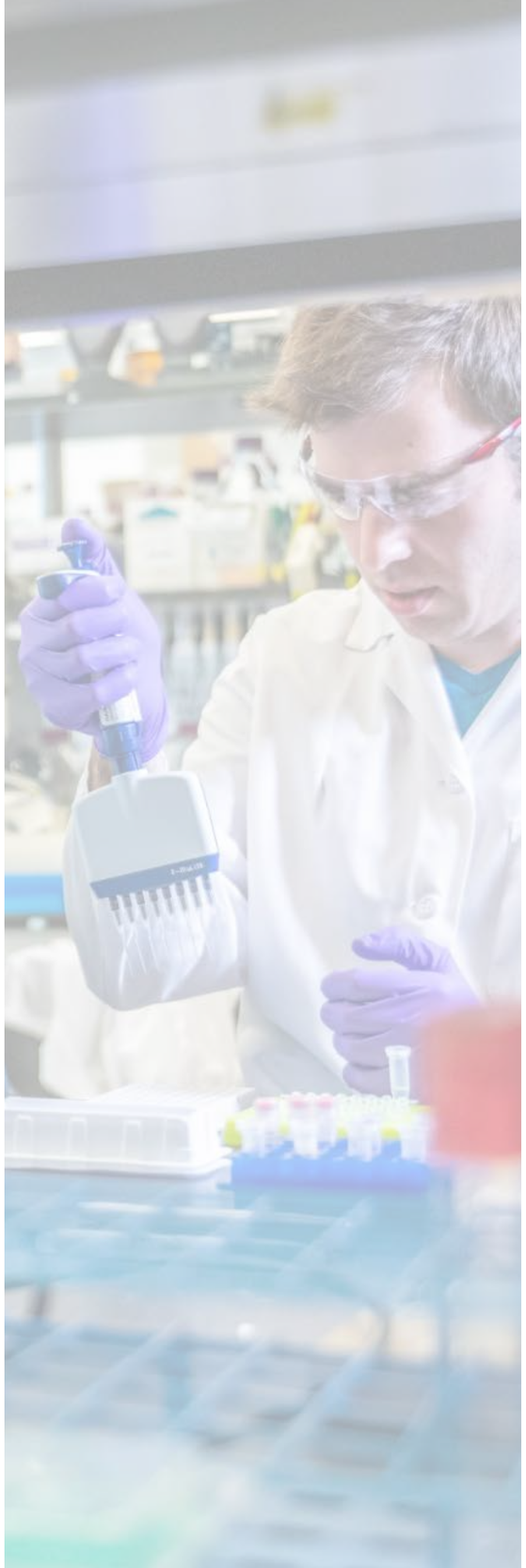
Scientists around the world are discovering new ways to use Thomson Filter Vials. Whether testing pharmaceuticals, performing toxicology, or testing for drugs of abuse Thomson Filter Vials have proven to be indispensable tools for sample prep when using HPLC, GC, LC-MS, or GC-MS, methodologies.

Thomson offers a full line of shake flasks and accessories with above-average yields and higher working volumes, designed specifically for insect/mammalian, or microbial/*E. coli* cells based on an understanding and experience of lab operations.

Our well-plate catalog continues to grow and provide the highest quality plates, ready for robotics, cell culture, synthesis, or analysis.

SINGLE StEP® Empty Columns are ready for the addition of sorbents or resins depending on the application.

If you have unique needs or need a new product please reach out to us. We look forward to collaborating with you.



Filter Vial Applications

Toxicology & Forensics

About Filter Vials

Oral Fluid

Urine

Cannabis

An Introduction to Filter Vials

Thomson Filter Vials are a single system which replaces HPLC Vials, HPLC Caps, Syringes, & Syringe Filters for the filtration of samples. In 15 seconds, Thomson Filter Vials filter samples in an autosampler-ready vial.

Key Features

- Same Size as a standard HPLC Vial and will fit easily into any standard HPLC vial machine or tray
- PTFE, PVDF, PES and Nylon membranes are available depending on the percentage of organic solvent in the sample and the amount of protein binding
- Pore sizes of either 0.2µm or 0.45µm will provide the perfect degree of filtration needed from viscous to clarified samples
- Versatility is built into Thomson's line of Filter Vials. Whether your samples are low volume or viscous or particulate-laden or contain a high volatility organic solvent Thomson has a Filter Vial to fit your needs

Syringe Filter Built In

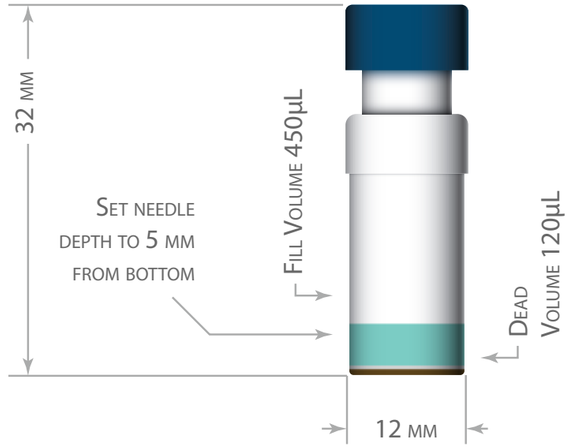
Equivalent to A Syringe Filter Built Into Your HPLC Vial

Filter Vials are equivalent to a syringe filter built into your HPLC vial. Even samples that appear clear to the eye potentially have particulates that can clog the machine, causing down time and costly maintenance. Filter Vials increase productivity by eliminating a transfer step required when using a syringe filter.

How Filter Vials Work

Similar to How A French Press Works...

Similar to how a french press (cafetière à piston) works, Filter Vials filter particulates out of the sample with similar membranes used in syringe filters. The pressing of the plunger into the shell vial forces the sample up through a filter to separate the particulates from the sample to be analyzed. Thomson has several filter membranes and pore sizes to choose from making the Filter Vial a versatile tool in the lab.



Easy As 1, 2, ... Done!

In Two Steps

1. Deposit 450µL of sample into shell vial
2. Insert plunger into the outer shell & press

15 Seconds

In two steps and 15 seconds you can have filtered sample for analysis. If you need to filter more than one sample, the Toggle Press (up to 5) or Multi-Use Press (up to 48) can be used.

You can prepare a particulate free sample in less time than it takes to open the syringe packaging and add a syringe filter.

Filter Vial Membrane

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.

Which to use?

- **0.2µm Pore Size**
  - Cells or Cell Debris in Sample
  - Chromatography Column Particle Size <3µm
- **0.45µm Pore Size**
  - Chromatography Column Particle Size >3µm

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.

Compatibility

For chemical or compound compatibility with our Filter Vials & membranes see the Chemical Compatibility Index & Compound Compatibility Index in our Technical Library.

	Aqueous	>50% Organic	Low Protein Binding
PTFE		●	
PVDF	●		●
Nylon	●	●	
PES	●		●

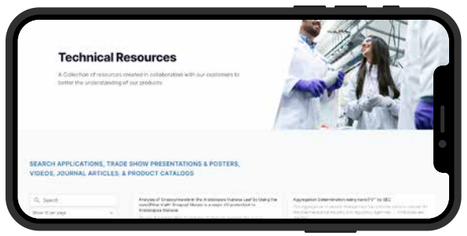
What Applications Can the Filter Vial be Used For?

With Thomson's family of Filter Vials and membranes available to you, finding ways to replace cumbersome and expensive syringe filters in the lab is easy. Here are just some of the documented applications you can use Filter Vials for in your lab today. See our Technical Library at htslabs.com to see a full list of applications. We work hard with small and large companies to produce proven protocols and methods for our products. If you find a use for Filter Vials in your workflow we would love to hear about it.

	nanoIFilter Vial®	StandardIFilter Vial	Low EvapIFilter Vial	eXtremeIFV®
10µL-250µL	●			
450µL		●	●	●
UPLC Compatible	●	●	●	●
GCMS Compatible	●		●	
30% Particulates				●
Viscous				●
Replacement for SPE				●
General Liquids < 10% particulates	●	●	●	●
Cell Fermentation	●			●
Particulate Removal	●	●	●	●
Automation Compatible	●	●	●	●
Small Molecules	●	●	●	●
Food & Supplements		●	●	●
Toxicology	●	●	●	●
Pesticides	●			●
Environmental	●	●	●	●

Thomson's Technical Library

You can find application notes, videos and more information on our products by visiting our website at **htslabs.com.**



What do Filter Vials Replace in the Lab?

Thomson Filter Vials simplify general filtration by replacing syringes & syringe filters, microcentrifuge spin columns, and/or liquid-liquid extractions.

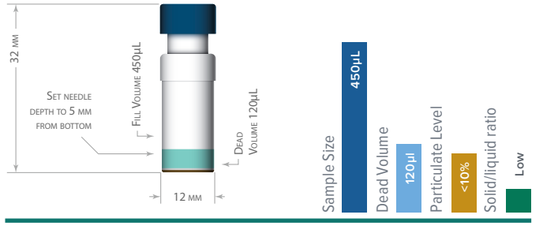
Applications for Thomson Filter Vials include all sample types to be analyzed by HPLC, UHPLC, LC-MS, and GC-MS.

A Comparison of the Filter Vial Types

Filter Vial

Standard For Most Samples

Max Fill Vol. **450µL**
Dead Vol. **120µL**



Key Features

- General purpose filtration
- <10% particulates
- Pre-slit septum

Replaces in the lab

- Syringe Filters
- Syringes
- HPLC Vials/Caps

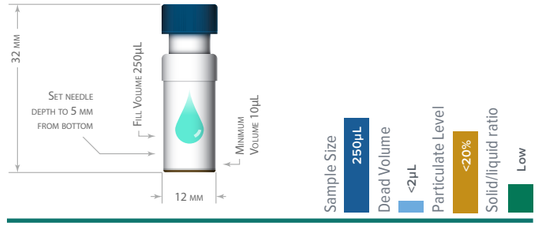
Applications

- 120µL-450µL
- General Liquids < 10% particulates
- Particulate Removal
- Automation Compatible
- Small Molecules
- Food & Supplements
- Toxicology
- Environmental

nano|Filter Vial

When Every µL Counts

Max Fill Vol. **250µL**
Min Fill Vol. **10µL (for 2µL injection)**



Key Features

- 10µL sample for 2µL injection
- Available with pre-slit or non-slit septum

Replaces in the lab

- Centrifugation & Spin Filters
- Small Volume Syringe Filters
- Syringes
- High Recovery Vials/Caps
- Inserts with HPLC Vials/Caps

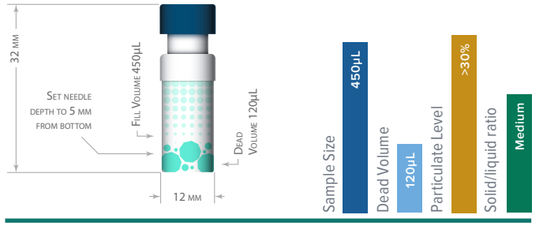
Applications

- 10µL-250µL
- General Liquids < 10% particulates
- Cell Fermentation
- Particulate Removal
- Automation Compatible
- Small Molecules
- Toxicology
- Pesticides
- Environmental

eXtreme|FV

Multi-Layered Filtration

Max Fill Vol. **450µL**
Dead Vol. **120µL**



Key Features

- Used for Particulate Laden Samples
- Contains a Depth Pre-Filter
- Pre-slit septum

Replaces in the lab

- Syringe Filters
- Syringes
- HPLC Vials/Caps

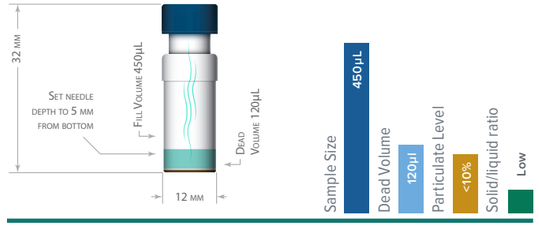
Applications

- 120µL-450µL
- ≤ 30% Particulates
- Viscous
- Replacement for SPE
- Cell Fermentation
- Particulate Removal
- Automation Compatible
- Small Molecules
- Food & Supplements
- Toxicology
- Pesticides
- Environmental

Low Evap|Filter Vial

Standard For Most Samples

Max Fill Vol. **450µL**
Dead Vol. **120µL**



Key Features

- General purpose filtration
- Non-split septum
- <10% particulates
- Evaporation rate <0.4% over 24-hour

Replaces in the lab

- Syringe Filters
- Syringes
- HPLC Vials/Caps

Applications

- 120µL-450µL
- General Liquids < 10% particulates
- Particulate Removal
- Automation Compatible
- Small Molecules
- Food & Supplements
- Toxicology
- Environmental

Plasticizers content in Filter Vials Compared to Syringe Filters

Testing by Takeda Pharmaceutical Company Limited® UPLC - ELSD

Introduction

Thomson Filter Vials are manufactured without the use of plasticizers or mold release agents, making them LC/MS clean. Testing with ELSD, PDA, and MS detection by Takeda Pharmaceutical showed no leaching from Thomson Standard Filter Vial with a 0.45µm, PTFE membrane compared to significant leaching from Millipore Millex-FH® Filter, 0.45µm, hydrophobic PTFE, 4mm. Method: A. Water B. ACN 45-90% with 0.05% TFA Ballistic Gradient over 1.4 minutes using Waters® Acquity® UPLC Thomson Filter Vial (patented) Part # 34440 Filter Vial 0.45µm hydrophobic PTFE, w/ Pre-Slit Cap Millipore Syringe Filter Part #:SLFHR04NL Millex-FH® Filter, 0.45µm, hydrophobic PTFE, 4mm, non-sterile.

Method:

A. Water
B. ACN 45-90% with 0.05% TFA

Ballistic Gradient over 1.4 minutes using Waters® Acquity® UPLC

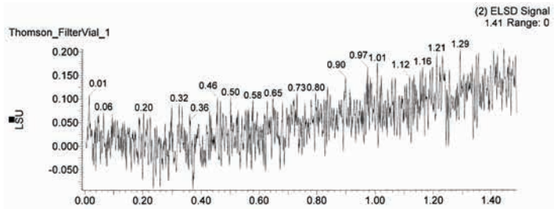
Thomson Standard Filter Vial

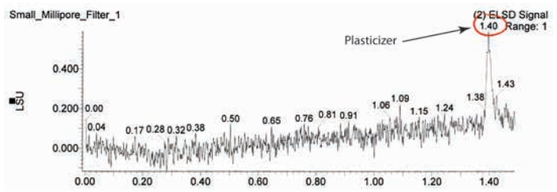
0.45µm hydrophobic PTFE, w/ Pre-Slit Cap
Part#: 34440

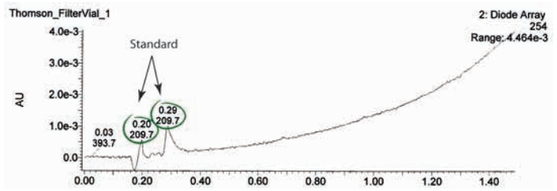
Millipore Syringe Filter

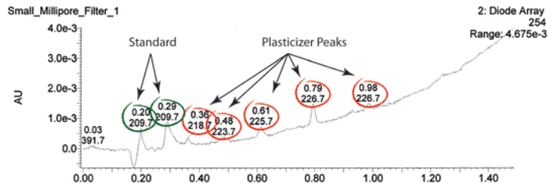
Millex-FH® Filter, 0.45µm, hydrophobic PTFE, 4mm, non-sterile.
Part #: SLFHR04NL

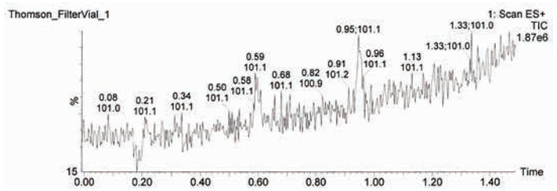
Plasticizers

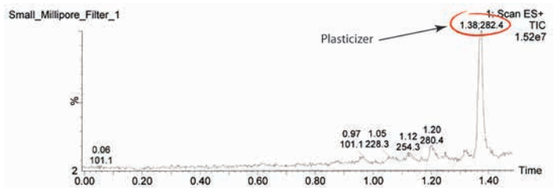












Thomson is not affiliated with Takeda Pharmaceutical Company®, Millipore®, Waters® or their products

Increase Signal-to-Noise Ratio with eXtremeIFV® for More Targeted & Accurate Peaks

Matrix Effects & Ion Suppression:

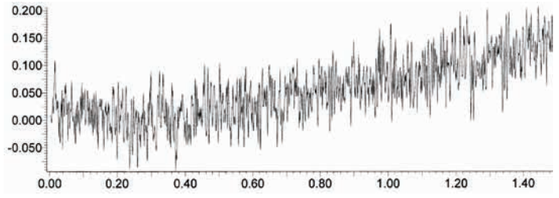
Analytes are obscured by the matrix like the octopus in this photo is difficult to find among its surroundings.

Strong Signal; Noise Lessened:

By adding compounds to the eXtremeIFV® the signal to noise ratio is increased allowing you to find the analyte with ease.

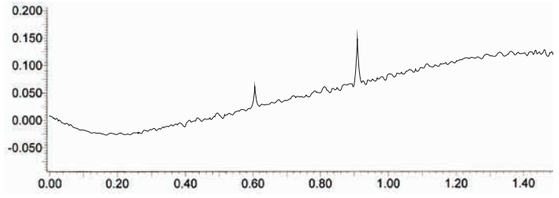
Low Signal to Noise Ratio

Difficult to find analyte in the matrix



High Signal to Noise Ratio

In this example the addition of C-18 to eXtremeIFV® with your sample binds excess compounds to C-18 and the Matrix clears up allowing you to see analyte peaks



Octopus images courtesy Jukin Video

High Viscosity Presses

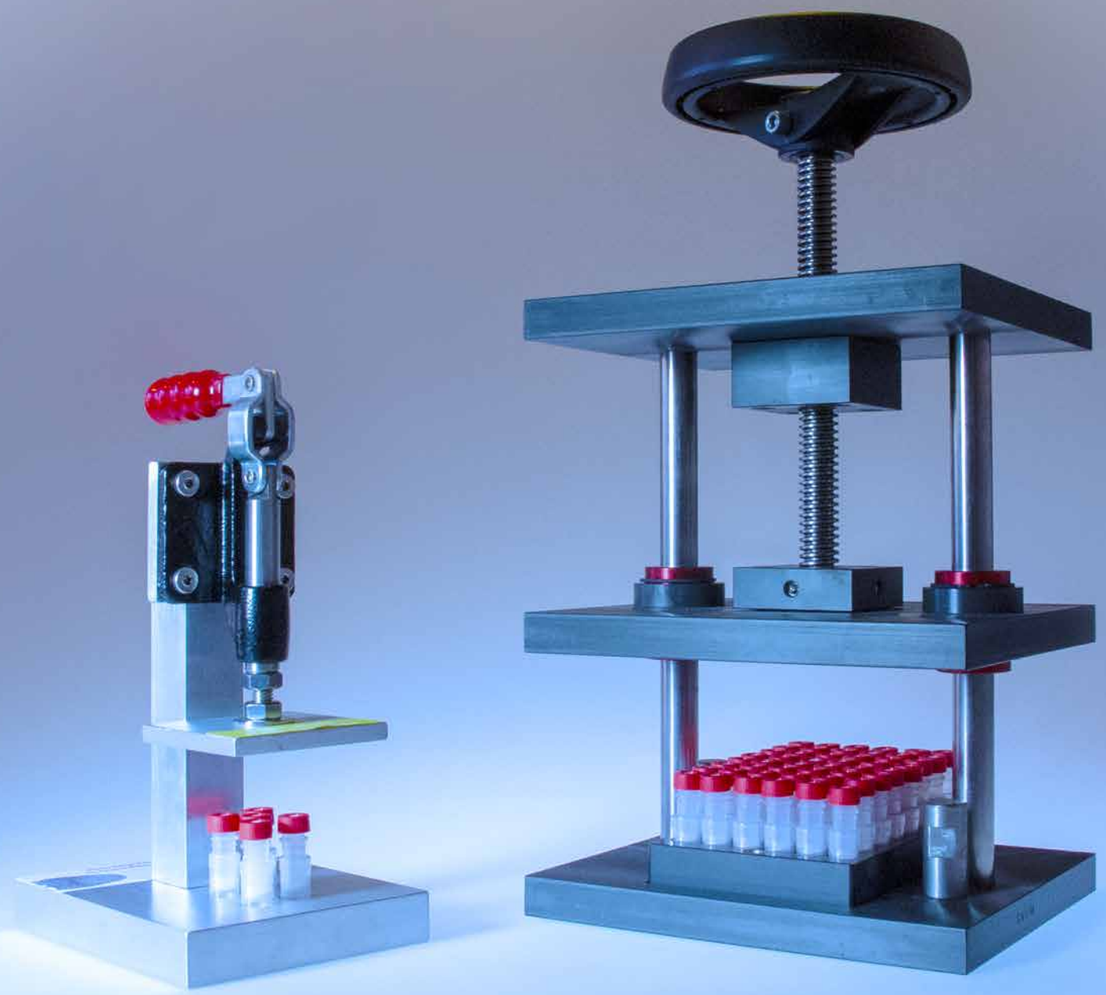
The Thomson Filter Vial Press enables high solid content and viscous liquids to be easily filtered through vials. Some fermentation cultures that reach 100OD or particulate laden samples may require the toggle press.

Toggle Press

- Press up to 5 autosampler ready Filter Vials
- Allows for consistency and ergonomic concerns
- Small footprint; sits on bench top
- Works with all Thomson Filter Vials

Multi-Use Press

- Presses up to 48 Autosampler Ready Filter Vials at a time
- Works with 48 position block; block fits some autosamplers
- 48 position block can be transferred to a robot for automation
- Easily Automate Filter Vial Pressing
- Works with all Thomson Filter Vials



htslabs.com info@htslabs.com 800 541.4792 760 757.8080

Chemical Compatibility

	Housing Materials	Filter Membrane			
	Polypropylene	PTFE	PVDF	PES	NYLON
Acetic Acid (glacial) | *acid, organic*	TST	R	R	R	NR
Acetone | *ketone*	R	R	NR	GNR	R
Acetonitrile (ACN) | *nitrile*	R	R	LTD	NR	R
Alconox, 1% | *surfactant/detergent*	ND	TST	TST	ND	TST
Ammonium Hydroxide | *caustic*	TST	GR	R	NR	TST
Ammonium Sulfate (saturated) | *salt, aqueous solution*	R	GR	NR	ND	R
Amyl Acetate | *ester*	TST	R	R	GR	TST
Amyl Alcohol | *alcohol*	R	R	R	GR	TST
Benzene | *HC, aromatic*	NR	—	—	—	—
Benzyl Alcohol | *HC aromatic/alcohol*	NR	—	—	—	—
Boric Acid (aqueous solution) | *acid, inorganic*	R	GR	TST	GR	R
Butyl Acetate | *ester*	TST	GR	TST	GNR	R
Butyl Alcohol | *alcohol*	R	GR	R	GR	R
Carbon Tetrachloride | *HC, halogenated*	NR	—	—	—	—
Cellosolve (Ethyl) | *glycol ether*	R	GR	ND	GR	R
CHAPS (aqueous solution) | *surfactant/detergent*	ND	TST	ND	ND	TST
Chloroform | *HC, halogenated*	NR	—	—	—	—
Cyclohexanone | *ketone*	NR	—	—	—	—
Diethyl Pyrocarbonate, 0.2% | *carboxylic anhydride*	ND	ND	TST	ND	ND
Dimethyl Sulfoxide (DMSO) | *sulfoxide*	R	R	NR	NR	R
Dimethylacetamide | *amide*	R	GR	NR	NR	NR
Dimethylformamide | *amide*	R	GR	NR	ND	R
Dioxane | *ether*	R	GR	R	ND	R
Ethers | *ether*	NR	—	—	—	—
Ethyl Acetate | *ester*	TST	R	R	GNR	R
Ethyl Alcohol | *alcohol*	R	R	R	GR	TST
Ethylene Glycol | *glycol*	R	R	R	GR	R
Formaldehyde | *aldehyde*	R	R	R	ND	R
Formic Acid, 50% | *acid, organic*	R	GR	R	ND	NR
Freon (TF or PCA) | *HC, halogenated*	R	GR	R	ND	R
Gasoline | *HC*	NR	—	—	—	—
Glycerine (Glycerol) | *glycol*	R	GR	R	GR	R
Guanidine Hydrochloride, 6M | *salt, aqueous solution*	ND	GR	ND	ND	ND
Guanidine Thiocyanate, 5M | *salt, aqueous solution*	ND	GR	ND	ND	ND
Helium | *gas*	R	R	TST	ND	R
Hexane | *HC, aliphatic*	NR	—	—	—	—
Hydrochloric Acid, 1N (HCL) | *acid, inorganic*	GR	R	R	GR	GR
Hydrochloric Acid, 6N (HCL) | *acid, inorganic*	TST	R	TST	GR	TST
Hydrochloric Acid, conc. (HCL) | *acid, inorganic*	NR	—	—	—	—
Hydrofluoric Acid | *acid, inorganic*	NR	—	—	—	—
Hydrogen | *gas*	R	R	R	ND	R
Hydrogen Peroxide, 3% | *peroxide*	R	R	R	ND	R
Hydrogen Peroxide, 30% | *peroxide*	TST	R	R	ND	TST
Hydrogen Peroxide, 90% | *peroxide*	R	R	R	ND	NR
HYPO (aqueous solution) | *salt, aqueous solution*	R	GR	R	ND	R
Isobutyl Alcohol | *alcohol*	R	R	R	GR	TST

R = Recommended | **GR** = Generally Recommended | **NR** = Not Recommended | **GNR** = Generally Not Recommended
LTD = Limited Recommendation | **TST** = Testing Recommended | **ND** = No Data Presently Available | — = Not Recommended, polypropylene is **NR**

	Housing Materials	Filter Membrane			
	Polypropylene	PTFE	PVDF	PES	NYLON
Isopropyl Acetate | *ester*	TST	R	R	GNR	R
Isopropyl Alcohol | *alcohol*	R	R	R	GR	TST
Kerosene | *HC*	TST	LTD	R	GR	R
Lactic Acid, 50% | *acid, organic/alcohol*	R	GR	TST	ND	TST
Lubrol PX (aqueous solution) | *surfactant/detergent*	ND	TST	ND	ND	ND
Methyl Ethyl Ketone (MEK) | *ketone*	R	R	NR	GNR	R
Mercaptoethanol, 0.1M | *alcohol/mercaptan*	ND	ND	ND	ND	ND
Methyl Acetate | *ester*	TST	R	NR	GNR	R
Methyl Alcohol | *alcohol*	R	R	R	GR	TST
Methylene Chloride | *HC, halogenated*	NR	—	—	—	—
Methyl Isobutyl Ketone (MIBK) | *ketone*	NR	—	—	—	—
Mineral Spirits | *HC*	NR	—	—	—	—
Nitric Acid, 6N | *acid, inorganic*	TST	R	R	R	NR
Nitric Acid (concentrated) | *acid, inorganic*	NR	—	—	—	—
Nitrobenzene | *HC, aromatic*	NR	—	—	—	—
Nitrogen | *gas*	ND	R	R	ND	R
Nonidet-P40 (aqueous solution) | *surfactant/detergent*	ND	ND	ND	ND	ND
Ozone | *gas*	NR	—	—	—	—
Paraldehyde | *aldehyde*	TST	GR	TST	ND	R
Pentane | *HC, aliphatic*	NR	—	—	—	—
Petroleum Ether | *ether*	ND	GR	R	ND	R
Phenol (aqueous solution) | *phenol*	NR	—	—	—	—
Potassium Hydroxide, 3N | *caustic*	R	R	R	ND	R
Pyridine | *amine*	R	GR	NR	NR	TST
Silicone Oils | *silicone*	R	GR	R	ND	R
Sodium Carbonate (aqueous solu-tion) | *salt, aqueous solution*	R	R	R	ND	TST
Water (Brine) | *salt, aqueous solution*	R	R	R	ND	R
Sodium Chloride (aqueous solution) | *salt, aqueous solution*	R	R	R	ND	R
Sodium Dodecyl Sulfate | *surfactant/detergent*	ND	ND	ND	ND	ND
Sodium Hydroxide, 3N | *caustic*	R	R	R	R	R
Sodium Hydroxide (concentrated) | *caustic*	R	R	R	R	NR
Sulfuric Acid (concentrated) | *acid, inorganic*	NR	—	—	—	—
Tetrahydrofuran (THF) | *ether*	NR	—	—	—	—
Toluene | *HC, aromatic*	NR	—	—	—	—
TCA (aqueous solution) | *acid, organic*	R	GR	R	ND	TST
Trichloroethane | *HC, halogenated*	NR	—	—	—	—
Trichloroethylene | *HC, halogenated*	NR	—	—	—	—
Tween 20® (aqueous solution) | *surfactant/detergent*	ND	R	TST	ND	TST
Urea, 8M | *salt, aqueous solution*	R	GR	R	ND	R
Xylene | *HC, aromatic*	NR	—	—	—	—

R = Recommended | **GR** = Generally Recommended | **NR** = Not Recommended | **GNR** = Generally Not Recommended
LTD = Limited Recommendation | **TST** = Testing Recommended | **ND** = No Data Presently Available | — = Not Recommended, polypropylene is **NR**

Compound Compatibility

	Recommended Filter Membrane				
	PVDF	PES	PTFE	PES	PVDF
	0.2 µm	0.2 µm	0.2 µm	0.45 µm	0.45 µm
5-Fluorouracil			●		
(18F) Fluoromisondazole, Misiomidazole	●				
Acebutolol		●			
Acetylsalicylic acid		●			
Alpha1-Proteinase Inhibitor (Human)					●
Alprenolol		●			
Amiloride		●			
Amphotericin B for Injection USP					●
Atenolol		●			
Azathioprine				●	●
Azodicarbonamide		●			
Bleomycin Sulfate			●		
Caffeine		●			
Cetirizine				●	●
Chlorothiazide		●			
Chloramphenicol		●			
Cimetidine		●			
Ciprofloxacin		●			
Cisplatin, Cisplatin Injection			●		
Cyclosporine A	●				
Cytarabine			●		
Daunorubicin			●		
DE-310		●			
Diclofenac					●
Enalapril		●			
Ethionamide			●		
Factor IX Complex Heat-Treated					●
Gatifloxacin				●	●
Hydrochlorothiazide		●			
Ibuprofen				●	●
Iosniazid			●		
isonicotinic acid			●		
Ketamine		●			
Las 35917					●
Levofloxacin				●	●
Lomefloxacin				●	●
Methyl Gag; NSC-32946			●		
Metoprolol		●			
Mitomycin			●		
Morphazinamide			●		
Nadolol		●			
Nicotinic acid			●		
Paclitaxel	●				
p-Aminobenzoic acid (PABA)					●
p-aminosalicylic acid			●		
Pefloxacin				●	●
Pentoxifylline (PTX)	●				
Phenytoin					●

	Recommended Filter Membrane				
	PVDF	PES	PTFE	PES	PVDF
	0.2 µm	0.2 µm	0.2 µm	0.45 µm	0.45 µm
Pyrazinamide			●		
Pyrimethamine				●	●
Ranitidine		●			
Rifampicin				●	●
Sabeluzole					●
Streptokinase					●
Sulfadozine					●
Sulphasalazine		●			
Sulpiride		●			
Terbutaline		●			
Thiotepa Parenteral Sterile			●		
Timolol		●			
Tobramycin Vincristine Sulfate			●		
Tranexamic acid		●			
Triamcinolone Acetonide		●			
Triazinate; NSC-139105			●		
Tropicamide				●	
Vinblastine Sulfate			●		

Part Numbers

Standard|Filter Vial Snap Cap

Membrane	PTFE	PTFE	PVDF	PVDF	NYLON	NYLON	PES
Pore Size	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm
Cap Color	green	blue	red	yellow	black	pink	grey
Cap Style	snap-cap	snap-cap	snap-cap	snap-cap	snap-cap	snap-cap	snap-cap
Septum	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit
Fill Vol.	450µL	450µL	450µL	450µL	450µL	450µL	450µL
Dead Vol.	120µL	120µL	120µL	120µL	120µL	120µL	120µL
Part #	35530	35540	35531	35541	35538	35539	35535
Qty/Case	200 & 500	200 & 500	200 & 500	200 & 500	200 & 500	200 & 500	200 & 500

Standard|Filter Vial Screw Cap

Membrane	PTFE	PTFE	PVDF	PVDF	NYLON	NYLON	PES
Pore Size	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm
Cap Color	green	blue	red	yellow	black	pink	grey
Cap Style	screw cap	screw cap	screw cap	screw cap	screw cap	screw cap	screw cap
Septum	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit
Fill Vol.	450µL	450µL	450µL	450µL	450µL	450µL	450µL
Dead Vol.	120µL	120µL	120µL	120µL	120µL	120µL	120µL
Part #	34430	34440	34431	34441	34438	34439	34435
Qty/Case	100	100	100	100	100	100	100

eXtremeIFV® Snap Cap

Membrane	PTFE	PTFE	PVDF	PVDF	NYLON	NYLON	PES
Pore Size	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm
Cap Color	green	blue	red	yellow	black	pink	grey
Cap Style	snap-cap	snap-cap	snap-cap	snap-cap	snap-cap	snap-cap	snap-cap
Septum	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit
Fill Vol.	450µL	450µL	450µL	450µL	450µL	450µL	450µL
Dead Vol.	120µL	120µL	120µL	120µL	120µL	120µL	120µL
Part #	85530	85540	85531	85541	85538	85539	85535
Qty/Case	200 & 500	200 & 500	200 & 500	200 & 500	200 & 500	200 & 500	200 & 500

eXtremeIFV® Screw Cap

Membrane	PTFE	PTFE	PVDF	PVDF	NYLON	NYLON	PES
Pore Size	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm
Cap Color	green	blue	red	yellow	black	pink	grey
Cap Style	screw cap	screw cap	screw cap	screw cap	screw cap	screw cap	screw cap
Septum	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit
Fill Vol.	450µL	450µL	450µL	450µL	450µL	450µL	450µL
Dead Vol.	120µL	120µL	120µL	120µL	120µL	120µL	120µL
Part #	84430	84440	84431	84441	84438	84439	84435
Qty/Case	100	100	100	100	100	100	100

Low Evap|Filter Vial

Membrane	PTFE	PTFE	PVDF	PVDF	NYLON	NYLON	PES
Pore Size	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm
Cap Color	green	blue	red	yellow	black	pink	grey
Cap Style	screw cap	screw cap	screw cap	screw cap	screw cap	screw cap	screw-cap
Septum	non-slit	non-slit	non-slit	non-slit	non-slit	non-slit	non-slit
Fill Vol.	450µL	450µL	450µL	450µL	450µL	450µL	450µL
Dead Vol.	120µL	120µL	120µL	120µL	120µL	120µL	120µL
Part #	64430	64440	64431	64441	64438	64439	64435
Qty/Case	100	100	100	100	100	100	100

nanoIFilter Vial® Non-Slit

Membrane	PTFE	PTFE	PVDF	PVDF	NYLON	NYLON	PTFE	PES
Pore Size	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm	0.2µm	0.2µm	0.2µm
Cap Color	green	blue	red	yellow	black	black	green	grey
Cap Style	screw cap	screw cap	screw cap	screw cap	screw cap	screw cap	screw cap	screw cap
Septum	non-slit	non-slit	non-slit	non-slit	non-slit	non-slit SIL I PP	non-slit PTFE I SIL I PTFE	non-slit
Fill Vol.	250µL	250µL	250µL	250µL	250µL	250µL	250µL	250µL
Dead Vol.	8µL	8µL	8µL	8µL	8µL	8µL	8µL	8µL
Part #	15530	15540	15531	15541	15538	14638	14930	15535
Qty/Case	200 & 500	200 & 500	200 & 500	200 & 500	200 & 500	100	100	200 & 500

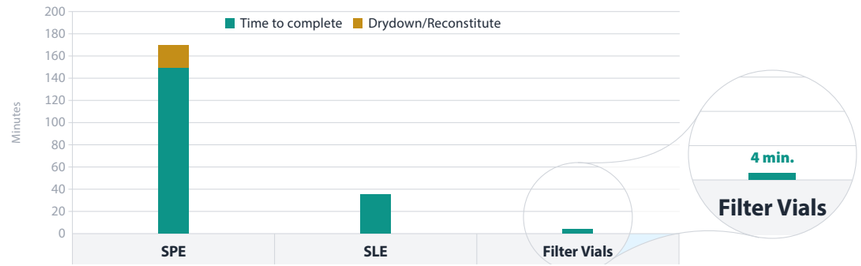
nanoIFilter Vial® Pre-Slit

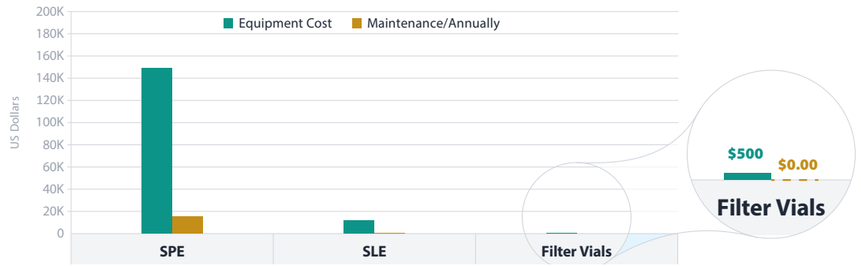
Membrane	PTFE	PTFE	PVDF	PVDF	NYLON	PES
Pore Size	0.2µm	0.45µm	0.2µm	0.45µm	0.2µm	0.2µm
Cap Color	green	blue	red	yellow	black	grey
Cap Style	screw cap	screw cap	screw cap	screw cap	screw cap	screw cap
Septum	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit	pre-slit
Fill Vol.	250µL	250µL	250µL	250µL	250µL	250µL
Dead Vol.	8µL	8µL	8µL	8µL	8µL	8µL
Part #	25530	25540	25531	25541	25538	25535
Qty/Case	200 & 500	200 & 500	200 & 500	200 & 500	200 & 500	200 & 500

High Viscosity Filter Vial Presses

Press	**Description**	**Capacity**	**Qty**	**Part #**
Toggle Press	5 Position for Autosampler Ready Filter Vials	5	1	35005
Multi-Use Press	48 Position for Autosampler Ready Filter Vials	48	1	35015

Time & Cost Savings with Filter Vials





Time savings using eXtremeIFV®

Time savings using the eXtremeIFV®

Traditional sample preparation methods, SPE (Solid Phase Extraction) and SLE (Supported Liquid Extraction) require multiple steps making them time consuming. The Thomson Filter Vials streamline sample preparation minimizing the number of steps required. Simply add the sample and diluent to the outer shell; depress the plunger and the sample is ready for analysis.

- Filter a sample in < 15 seconds
- Autosampler compatible vial
- Prevents costly repairs & instrument downtime

Significant cost savings compared to automated SPE or SLE

Significant cost savings compared to automated SPE or SLE

For sample clean-up, the Thomson Filter Vials requires minimum steps and processing time. Significant cost savings are achieved by the Filter Vials replacing the SPE/SLE system, evaporators SPE/SLE cartridges, solvent waste HPLC vials & caps.

- Less equipment
- Less maintenance
- Less waste

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

Detection of THC in Oral Fluid: The Bane of a Toxicologist's Existence

Jill Yeakel
Lehigh Valley Toxicology
MSACL 2017 Oral Presentation
For the full application note visit htslabs.com/tech/?id=170

Introduction

It is critical that samples collected in a clinical setting meet the requirments for compliance or drug monitoring. Urine samples can be difficult to obtain in patients with medical conditions, elderly, and drug addicts. Urine samples have a long detection window but require large measurable volume and are easily adulturated. While Oral Fluids, have a shorter detection window, the sample is easily collected with minimal invasion of privacy and the collection can be observed making it difficult to adulturate. This shorter window with Oral Fluids, in most cases allows for confirmation of recent ingestion, active drug versus metabolites.

Method

Several factors were considered when developing and optimizing this method.

- Factors affecting analyte detection
- Pharmacokinetics
- Oral Fluid has a pH range ~5.6-8
- Analyte properties – lipophilicity, pKa, protein binding

In Table 1 are the analytes/drugs choosen to be included in this panel because they are lipid soluble, unionized and unbound. We will focus on the detection of THC and what was needed to achieve good recovery and reproducibility including sample preparation, column choice, and Mass Spec settings.

Table 1. The following drugs to be included in this Oral Fluid Panel.

6-Acetylmorphine	Fentanyl	Norsertraline
7-Aminoclonazepam	Fluoxetine	Nortriptyline
α-Hydroxyalprazolam	Hydrocodone	Norvenlafaxine
Alprazolam	Hydromorphone	Oxazepam
Amitriptyline	Lorazepam	Oxycodone
Amphetamine	1-(3-Chlorophenyl) piperazine	Oxymorphone
Benzoylecgonine	MDMA	Phencyclidine (PCP)
Buprenorphine	Meprobamate	Sertraline
Carisoprodol	Methadone	Tapentadol
Citalopram	Methamphetamine	Temazepam
Cocaine	Morphine	Δ9-Tetrahydrocannabinol (THC)
Codeine	Norbuprenorphine	Tramadol
Clonazepam	Nordiazepam	Trazodone
Cyclobenzaprine	Norfentanyl	Venlafaxine
Diazepam	Norfluoxetine	

Sample Preparation Optimization

Three methods for sample preparation were evaluated, 2 different Solid Phase Extraction (SPE) Cartridges and the eXtremeIFV®, 0.2µm PVDF, p/n 85531.

eXtremeIFV®, 0.2µm PVDF

Prepare Sample

1. Add 100 µL curve diluent
2. Add 20 µL internal standard
3. Add 100 µL oral fluid specimen
4. Depress the plunger

A limit of detection study was done at 1, 5, 10ng/mL for SPE #1, SPE #2 and eXtremeIFV®. SPE #1 yielded a lower basline than SPE #2 but still low recovery (~600 area) as compared to the eXtremeIFV®. The eXtremeIFV® has a larger quantitation ion, more disernable from noise and higher peak height at 1ng/mL, fig. 1. We will move forward to the next step of optimization with the eXtremeIFV®.

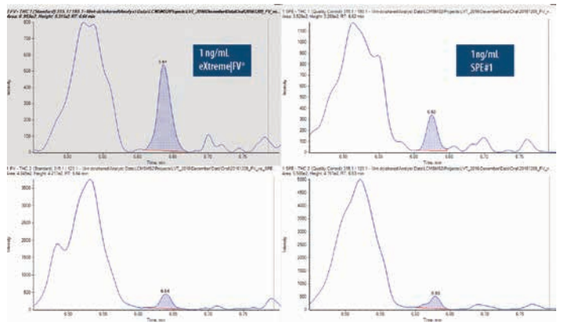


Fig 1. Limit of Detection Study – SPE #1 & eXtremeIFV®

Analytical Method Development

To ensure good reproducible quantification and identification of THC, the LC and MS/MS parameters were optimized:

LC Parameters

- Column
- Gradient

MS/MS Parameters

- Source
- Ions, CE, CXP & DP

Final Analytical Method

Sample Preparation

eXtremeIFV®, 0.2µm PVDF

1. Add 100 µL curve diluent
2. Add 20 µL internal standard
3. Add 100 µL oral fluid specimen
4. Depress the plunger

LC Parameters

- **Column:** C18
- **Gradient:**

Time (min)	%B
0.2	20
0.3	95
1.5	95
1.6	20
2.2	20

MS Parameters

- **Curtain Gas:** 40 psi
- **Ion Spray Voltage:** 4000 V
- **Source Temp:** 550°C
- **Ion Source Gas 1:** 60psi
- **Ion Source Gas 2:** 50psi

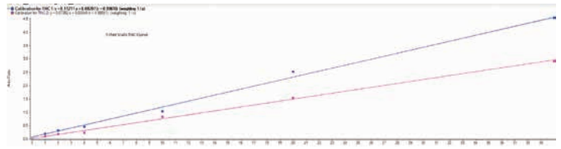


Fig 7. Calibration Curve using the new parameters yields an $r^2 = 0.99$

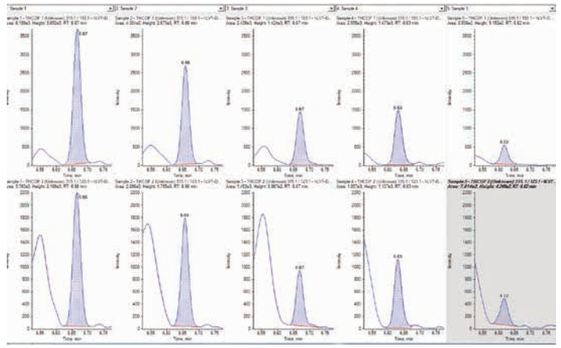


Fig 8. Examples of authentic Oral Fluid sample collected with the OraSure Technologies i2he™ Collection Device

Conclusion

Oral Fluids are easily and rapidly obtained, minimal invasion of privacy, difficult to adulterate, short detection window indicates recent ingestions, active drug vs. metabolite in most cases. The eXtremeIFV®, p/n 85531 allow for the samples to be 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 eXtremeIFV® include lower cost, faster sample preparation time, less use and disposal of organic solvents, see Table 2.

Benefits

- Increased efficiency
- Decreased sample cost
- Decreased solvent waste

Table 2. 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
Supply Cost	$127.77**	$103.68

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

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

eXtremeIFV® Extraction for the Detection of Fentanyl & Analogues in Oral Fluid Samples

Stevi Hooper[1], Jill Yeakel[1], Lisa Wanders[2]
[1]Lehigh Valley Toxicology, Bethlehem, PA [2]Thomson Solutions At Work™, Oceanside, CA

Presented at MSACL 2018

Introduction

Use of oral fluid (saliva) in toxicology has been increasing within recent years due to its non-invasive, low cost effectiveness in recent drug use detection. There is little opportunity for adulteration of the sample and a large volume is not required for collection and analysis. Liquid chromatography tandem mass spectrometry (LC-MS/MS) has proven to be a useful tool in the analysis of oral fluid due to its ability to run highly sensitive assays. The necessity for a sensitive assay is especially important in the detection of an analyte and its analogues that are administered in low concentrations. Detection of fentanyl and its analogues such as furanyl fentanyl and sufentanil, have become important due to the increasing widespread use of illicit fentanyl formulations in heroin and counterfeit opioid tablet formulations. Suppliers of illicit heroin are developing fentanyl analogues and including them as cutting agents in the final product due to their high potency and relatively low production cost. As a result, development of a method to detect these compounds has become vital to many toxicology labs. Thomson eXtremeIFV®s provide a simple and efficient extraction technique that has demonstrated adequate analyte recovery, reduced matrix interferences from a simple dilute-and-shoot method and the elimination of solvent waste and other consumables. This project specifically explores the efficacy of these vials in extracting a range of fentanyl analogues in oral fluid specimens.

Sample Preparation

1. Oral fluid sample collected using OraSure Intercept I2he collection device
   A. Place swab under tongue and hold until tab changes from white to blue (approximately 3-4 minutes and for a maximum of 15 mins)
   B. Slide swab into collection vial and screw on cap
2. Break tab on end of collection vial, and place into test tube
3. Centrifuge samples to pull diluted sample into test tube
4. Aliquot 100µL sample, calibrator or control into eXtremeIFV® outer shells
5. Add 100µL of mobile phase to outer shell
6. Add 20µL of internal standard to outer shell
7. Place plunger filter into the outer shell and press slowly and firmly until cap is secured in place
8. Vortex samples and inspect samples to assure no bubbles are present
9. Place sample onto instrumentation to be analyzed via LC-MS/MS

Method

Samples were analyzed using a Shimadzu liquid chromatograph and ABSciex Triple Quad mass spectrometer. The developed method injects 12 µL of sample onto a Kinetex® Biphenyl LC column. The sample is chromatographically separated at a flow rate of 0.7 mL/min using the following gradient in table 1.

Table 1. LC-MS/MS gradient
Mobile Phase A: 0.1 % Formic Acid in Water
Mobile Phase B: 0.1% Formic Acid in Methanol

Time (min)	Mobile Phase B (%)
0	20
0.2	20
0.5	50
1.5	95
3.0	95
3.1	20
5	20

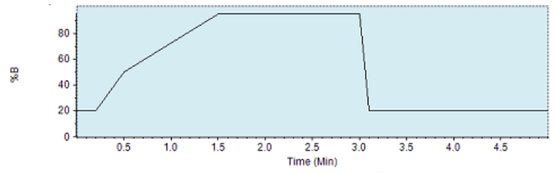


Fig 1. LC-MS/MS gradient

Results

Adequate chromatographic separation of all tested analytes was achieved while still attaining optimal sensitivity. Calibration range was established between 0.5 ng/mL to 20 ng/mL for each analyte. Controls sufficiently passed quantitatively and qualitatively within established ranges of targeted values (1.5 and 15 ng/mL respectively). To obtain the undiluted concentration of analyte in the sample, values were multiplied by a factor of three.

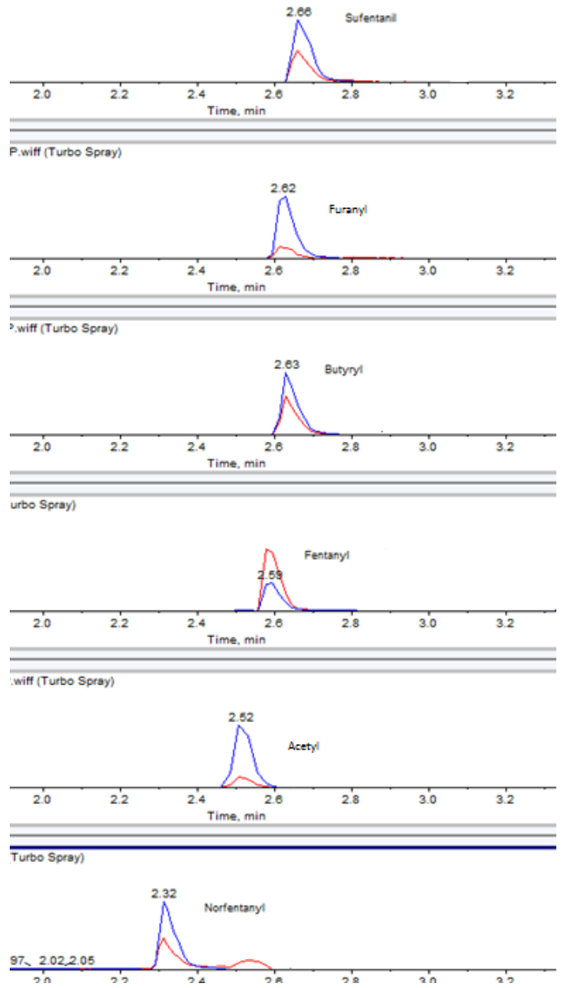


Fig 2. Chromatogram of a calibrator containing all analytes

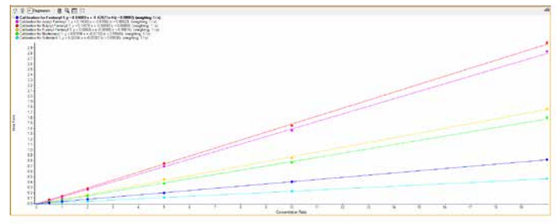


Fig 3. Calibration curves for each of the analyzed fentanyl compounds

Furanyl fentanyl appeared to have an interference peak present during method development. Analysis of solely the oral fluid diluent compared to the infusion data of furanyl fentanyl showed the presence of a co-eluting peak. Altering the MRM transition of the qualifier ion appeared to eliminate this issue.

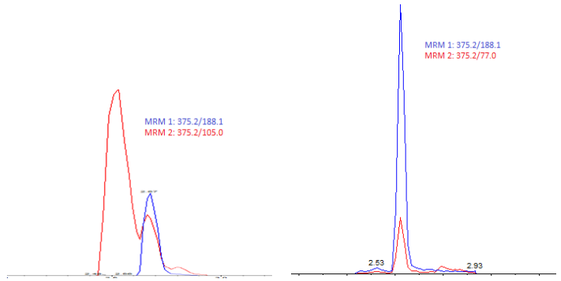


Fig 3-4. Chromatogram of Furanyl Fentanyl with co-eluting peak (left). Furanyl Fentanyl after altering MRM transition (right)

Of the 100 authentic oral fluid samples analyzed, 4 were positive for fentanyl at or above the established cutoff of 0.5 ng/mL. The specimens were collected from both pain management and addiction clinic patients to target a population with a higher incidence of fentanyl and illicit drug use.

Conclusion

A simple, rapid, and accurate comprehensive method was developed for the detection of these 6 analytes in oral fluid samples. In method development, one compound appeared susceptible to matrix effects from the oral fluid diluent utilized as the negative buffer. According to literature and infusion data for furanyl fentanyl, the MRM transition of 375.2/105.0 is commonly recommended due to its high intensity. This transition proved to have an interference peak present in the oral fluid diluent causing chromatographic resolution issues. This interfering peak was eliminated upon the change of the transition to 375.2/77.0. Additional research may be required to determine if this interference exists solely with the Intercept i2he Oral Fluid Diluent or amongst all buffers used in oral fluid assays. While only four samples had levels of fentanyl above the current limit of quantitation (LOQ), fentanyl and acetyl fentanyl were detected in additional samples below this established LOQ. The LOQ required for clinical relevance was unknown based on the lack of literature resources available containing information on fentanyl analogues and their concentrations in oral fluid. This indicates that additional studies need to be performed to determine the lowest achievable LOQ. The developed method utilizing the eXtremeIFV® proved successful in extracting and detecting fentanyl and five of its analogues present in oral fluid with a high level of sensitivity and accuracy.

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

Nadine Koenig[2], Crystal Xander[2], Melanie Stauffer[2], Dean Fritch[3]
[2]Health Network Laboratories, Allentown, PA
[3]OraSure Technologies, Inc,. Bethlehem, PA
Presented at MSACL 2015

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)
Methylenedioxyamphet-amine (MDA)	Methylenedioxymetham-phetamine (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.

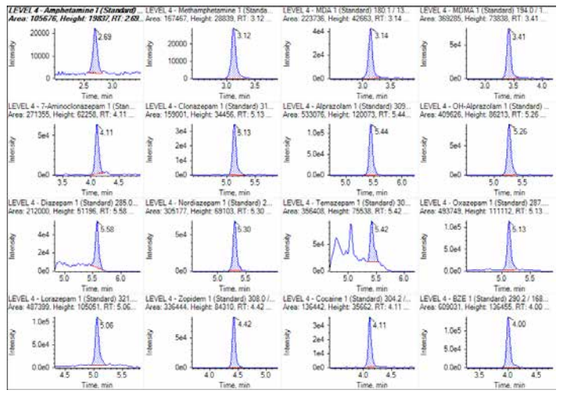


Fig 1. Mass Spectrum - Level 4

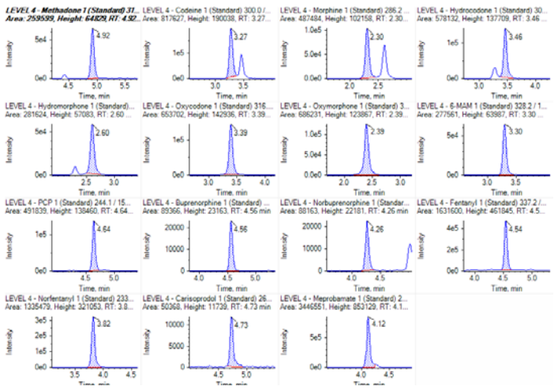


Fig 1. Mass Spectrum - Level 4 (continued)

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

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

Cost Effective Dilute and shoot Approach For Determination of Illicit Drugs in Oral Fluids Using LC-MS/MS

Kavinda De Silva, Mariko Nakano, Siobhan McKenney-Hara
Molecular Testing Labs, Vancouver, WA
Presented at MSCAL 2016

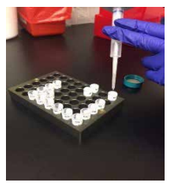
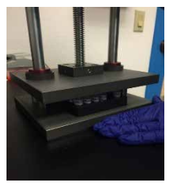
Introduction

Due to a recent increase in the demand of oral fluid analysis, many challenges have been set forth in developing robust and cost effective assays for determination of illicit drugs. Forensic testing on oral fluids has been increasingly appreciated due to reduction in time, simplicity of collection and reduction of adulteration and substitution. Thus, we developed a simplified and robust assay using filtering vials.

Demand for alternative matrices for drug testing has increased in the recent years. Even though urine, blood and hair have been utilized as the most common specimen, oral fluid is a more promising matrix for forensic testing. The use of oral fluid as an alternate matrix has a variety of advantages more so than disadvantages due to less pathogenicity and easier accessibility. In addition, oral fluid sample collection is an easy and non-invasive techniques and reduces the chances for sample substitutions or adulteration. Oral fluid analysis in the field of toxicology has had enormous growth recently. The techniques and instrumentations have evolved to meet the growing demands. Early analytical methods for oral fluid testing were developed primary based on gas chromatography - mass spectrometry (GC-MS or GC-MS/MS). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as the preferred analytical instrument in recent years. This assay demonstrates an easy and cost effective method to analyze illicit drugs in an oral fluid matrix.

Method and Materials

Sample preparation was developed with minimum and easy steps that did not involve the traditional and time consuming clean ups (e.g., SPE columns). Standards and samples were diluted in methanol – water diluent fortified with internal standards.These diluted samples were filtered by 0.2µm eXtremeIFV® (Thomson).


Analytes were separated with a Phenomenex® Biphenyl 1.7µm column on SCIEX 6500 QQQ coupled with Shimadzu 30 HLPC.The total run time was 6.5 minutes with a simple gradient utilizing 0.1% formic acid in water as mobile phase A and 0.1% formic acid in methanol as mobile phase B. The LC-MS/MS method was validated according to the CLIA guidelines.

Results

We were able to achieve three orders of magnitude in linear dynamic range. Table 1 shows the linear ranges and LOQ of all the analytes. The % coefficient of variation (%CV) was less than 20% and the coefficient of determination (R2) for all the analytes were also greater than 0.990. As depicted in Table 2, the day-to-day precision was determined with the low quality control (LQC) and high quality control (HQC). The % coefficient of variation for all the analytes were less than 10%. Interferences were evaluated using the analytes shown in Table 3. No interference was observed with assay

Table 1. Linearity

			Calibrator	
Transition name	LOQ (ng/mL)	Linear range (ng/mL)	%CV	R value
6-MAM 1	1	1-300	< 8.7	0.99580
6-MAM 2	1	1-300	< 16.9	0.99371
Amphetamine 1	5	5-1500	< 6.5	0.99719
Amphetamine 2	5	5-1500	< 7.5	0.99699
Benzoylecgonine 1	1	1-300	< 9.6	0.99691
Benzoylecgonine 2	1	1-300	< 13.7	0.99058
MDA 1	1	1-300	< 17.3	0.99285
MDA 2	1	1-300	< 12.9	0.99109
MDMA 1	10	10-3000	< 5.6	0.99516
MDMA 2	10	10-3000	< 7.5	0.99406
Methamphetamine 1	5	5-1500	< 11.3	0.99314
Methamphetamine 2	5	5-1500	< 12.2	0.99344
Oxycodone 1	2.5	2.5-750	< 7.0	0.99698
Oxycodone 2	2.5	2.5-750	< 13.5	0.99601
Oxymorphone 1	2.5	2.5-750	< 12.8	0.99297
Oxymorphone 2	2.5	2.5-750	< 13.9	0.99251
Phencyclidine 1	1	1-300	< 13.3	0.99359
Phencyclidine 2	1	1-300	< 13.3	0.99352
THC 1	5	5-1500	< 9.0	0.99533
THC 2	5	5-1500	< 12.7	0.99479

Table 2. Day-to-Day Precision

Transition name		% CV
6-MAM	HQC	4.9
6-MAM	LQC	6.1
Amphetamine	HQC	1.7
Amphetamine	LQC	4.4
Benzoylecgonine	HQC	3.4
Benzoylecgonine	LQC	6.3
MDA	HQC	4.9
MDA	LQC	8.6
MDMA	HQC	2.8
MDMA	LQC	1.9
Methamphetamine	HQC	2.3
Methamphetamine	LQC	3.8
Oxycodone	HQC	4.1
Oxycodone	LQC	5.3
Oxymorphone	HQC	7.4
Oxymorphone	LQC	7.8
Phencyclidine	HQC	6.3
Phencyclidine	LQC	8.8
THC	HQC	6.7
THC	LQC	8

Table 3. Interference Compounds

Interference Compounds	
Acetaminophen	Caffeine
CPAM	Ibuprofen
Naproxen	Pseudoephedrine
Trazodone	Tizanidine
Salicilic Acid	Venlafaxine
Diphenhydramine	Lisinopril
Dextromethorphan	Hydromorphone
Hydrocodone	Naloxone

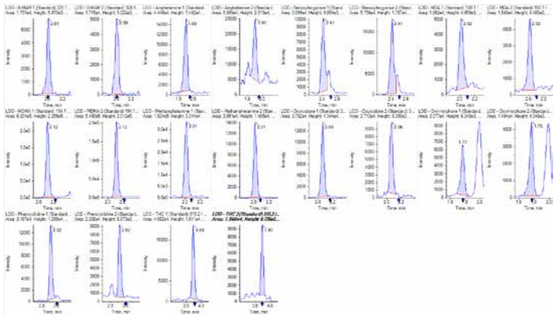


Fig 1. Chromatograms of the LOQ Standards

Table 4. Analyte Recoveries after with filter vials

Analyte Name	% Recovery
6-MAM	110
Amphetamine	106
Benzoylecgonine	106
MDA	106
MDMA	103
Methamphetamine	102
Oxycodone	107
Oxymorphone	102
Phencyclidine	87
THC	105

Analyte recoveries at LQC concentrations were compared in HPLC vials against filtered samples. Table 4 shows the percent recovery of each analyte. The recoveries for all the analytes were in a range of between 87%-110%.

Conclusion

We were able to develop a robust, simple and easy assay to determine illicit drugs in oral fluids. We were also able to cut the cost greater than half compared to the traditional sample preparation techniques, as this assay remarkably reduced the sample preparation time, the necessity of extra equipment (e.g. SPE system, evaporators) and drastic reduction of solvent uses. Further cost reductions could be achieved by automating the sample preparation.

References

Dams, Riet, Robin E. Choo, Willy E. Lambert, HendreeJones, and Marilyn A. Huestis. "Oral Fluid as an Alternative Matrix

to Monitor Opiate and Cocaine Use in Substance-abuse Treatment Patients." Drug and Alcohol Dependence 87.2-3 (2007): 258-67.

Heltsley, R., A. Depriest, D. L. Black, T. Robert, L. Marshall, V. M. Meadors, Y. H. Caplan, and E. J. Cone. "Oral Fluid Drug Testing of Chronic Pain Patients. I. Positive Prevalence Rates of Licit and Illicit Drugs." Journal of Analytical Toxicology 35.8 (2011): 529-40.

Koenig, N., Xander, C., et al. Improved method for the analysis of 31 drugs of abuse/pain management panel in oral fluid samples using the Thomson eXtremeIFV® by LC-MS/MS. (provided by OraSure).

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

eXtremeIFV® Extraction for the Detection of 11 Antidepressants in Oral Fluid Samples

Sarah Muller[1], Jill Yeakel1, Lisa Wanders[2]
[1]Lehigh Valley Toxicology, Bethlehem, PA
[2]Thomson Solutions At Work™, Oceanside, CA
Presented at SOFT 2016

Introduction

The use of oral fluid has recently become more prevalent in drug testing laboratories. The benefits of using oral fluid as a biological matrix include the ability to detect recent drug use, ease of collection, and the collection process can be observed to prevent adulteration of the sample. Many laboratories currently use solid phase extraction techniques to detect drugs and metabolites in oral fluid, however this extraction technique is laborious, expensive, and time consuming. A new, efficient technique has been introduced which optimizes the extraction process by reducing waste and amount of time spent extracting samples. Thomson eXtremeIFV®s provide a simple and efficient extraction technique that has demonstrated adequate analyte recovery, reduced matrix interferences and the elimination of solvent waste and other consumables. This project specifically explores the efficacy of these vials in extracting a wide range of antidepressants in oral fluid specimens.

Method:

1. Oral fluid sample collected using OraSure Intercept I2he collection device.
   A. Place swab under tongue and hold until tab changes from white to a blue (approximately 3-4 minutes and for a maximum of 15 mins).
   B. Slide swab into collection vial and screw on cap.
2. Break tab on end of collection vial, and place into test tube.
3. Centrifuge samples to pull diluted sample into test tube.
4. Aliquot 100 µL sample, calibrator or control into eXtremeIFV® shells.
5. Add 100 µL of mobile phase to outer shell vial.
6. Add 20 µL of internal standard to outer shell vial.
7. Place plunger filter into outer shell vial and press slowly and firmly until cap is secured in place.
8. Vortex samples and inspect samples to assure no bubbles are present.
9. Place sample onto instrumentation to be analyzed via LC-MS/MS.

Results:

Table 1 lists the 11 Antidepressants and retention times in Oral Fluid Samples. Fig 1 shows adequate chromatographic separation of all tested analytes was achieved while still attaining optimal sensitivity. Fig 2 Calibration range was established between 5 ng/mL to 200 ng/mL for each analyte. Controls sufficiently passed quantitatively and qualitatively within established ranges of targeted values (15 and 150 ng/mL respectively). To obtain the undiluted concentration of analyte in the sample, values were multiplied by a factor of three.

135 patient samples were analyzed, 38 were positive for antidepressants and their metabolites, see Table 2. These results were consistent with the provided medication lists. Samples were also simultaneously analyzed for opioids, benzodiazepines, barbiturates and drugs of abuse, see Fig 3.

Table 1. Antidepressants that were validated in this method.

Analyte	Retention Time
Citalopram	4.80
Amitriptyline	5.40
Nortriptyline	5.40
Fluoxetine	5.00
Norfluoxetine	5.00
Sertraline	5.60
Norsertraline	5.60
Venlafaxine	4.50
Desvenlafaxine	3.60
Trazodone	4.90
mCPP	4.10

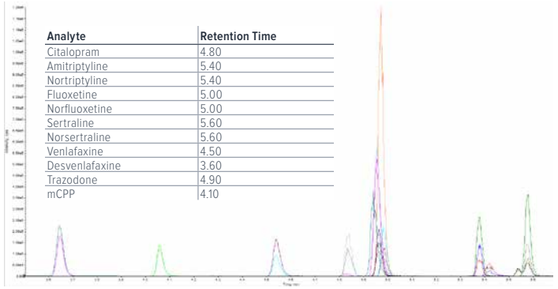


Fig 1. Chromatographic separation of the antidepressants in the method.

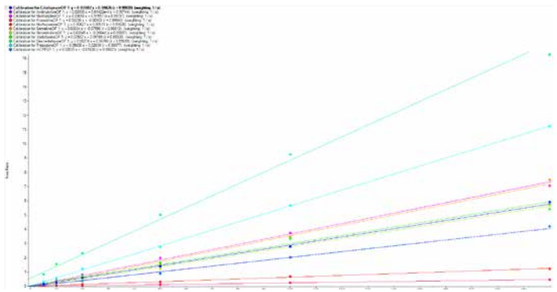


Fig 2. Calibration Curves.

Table 2. Positive results found in 135 Oral Fluid patient samples

Analyte/ Metabolite	Positives
Citalopram	8
Trazodone	7
mCPP	5
Sertraline	2
Norsertaline	3
Venlafaxine	5
Desvenlafaxine	4
Amitriptyline	1
Nortriptyline	1
Fluoxetine	1
Norfluoxetine	1

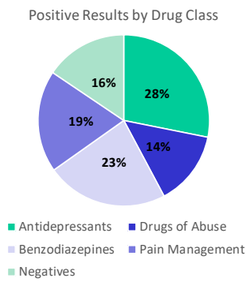


Fig 3. Positive results in Patient Oral Fluid Samples by Drug Class.

Conclusion:

The developed method utilizing the eXtremeIFV's® proved successful in extracting and detecting antidepressants and metabolites present in oral fluid with a high level of sensitivity and accuracy. A simple, rapid, and accurate comprehensive method was developed for the detection of 48 drugs in oral fluid samples.

Notes:

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

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
Presented at MSACL 2015

Introduction

Saliva test is one of the easiest, cost-effective and most accurate ways to measure the presence of drugs in the body. Collecting saliva sample is relatively non-invasive, easier to procure and reduced risk of sample adulteration. However, saliva matrix display much lower levels of drug compounds compared to urine samples, making the need to test at lower cut-off levels more important. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a technique of choice for both screening and confirmation lower levels because it is sensitive, specific, and accurate.

Solid Phase Extraction (SPE) is widely used for sample clean up before LC-MS/MS analysis. It is costly and time consuming. Here we present a high throughput, cost effective and sensitive procedure for screening and confirmation of Pain Panel Drugs (PPDs) in Synthetic Saliva using Thomson eXtremeIFV® for sample preparation and using an integrated On-Line Extraction (OLE)-UHPLC-MS/MS System for sample analysis. The lower limit of quantitation (LLOQ) was 0.01-0.2 ng/mL and upper limit of quantitation (ULOQ) was 100 ng/mL. The linearity regression coefficient R2 was >0.99. The blanks show no interference of the analysis at the LLOQ level. The 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 outer shell vial.
- Add 200 µL of drug standard in synthetic saliva (Immunalysis Corp., p/n NOFC-0500) to the vial and mix.
- Place Thomson Filter Plunger on top of the Thomson outer shell vial, eXtremeIFV® 0.2µm PVDF, w/Pre-Slit Red Cap (P/N 85531).
- Press filter plunger down approximately ¼ of the way into each of the outer shell vials.
- Vortex for 10 sec.
- Press filter plunger the rest of the way down the outer shell vial using the Thomson Filter Vial Toggle Press (P/N 35005).

Methods

Instruments:

EVOQ Elite triple quadrupole mass spectrometer coupled to a Bruker Integrated On-Line Extraction-UHPLC and CTC Autosampler

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/Acetonitrile=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)

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 were used as internal standard for above data.

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

Name	Linear Range (ng/mL)	R^2	Response Factor % RSD
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

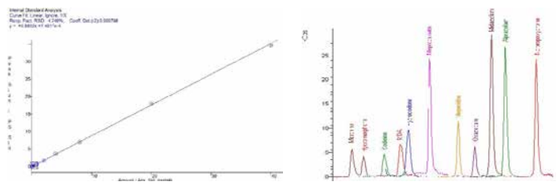


Fig 1. The curve on the left was plotted as response ratio vs concentration ratio of Methadone/Methadone-d3(Concentration 0.01-100 ng/mL with 2.5ng/mL IS). The chromatograms on the right was 0.01 ng/mL Methadone in Synthetic Saliva.

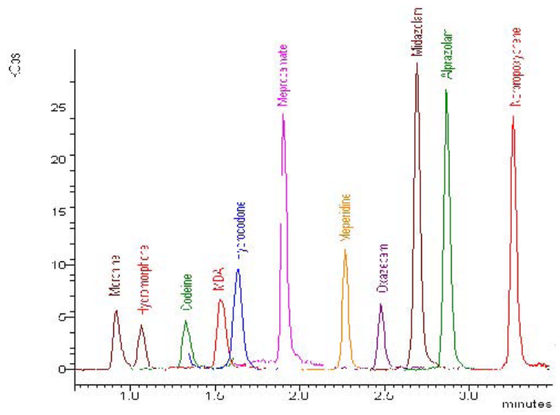


Fig 2. Selected chromatograms at 0.2 ng/mL PPDs in Synthetic Saliva.

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.

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

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

Nadine Koenig[1], Crystal Xander[1], Melanie Stauffe[1], Dean Fritch[2].
[1]Health Network Laboratories, Allentown, PA.
[2]Analytical Associates, East Greenville, PA
Presented at MSACL 2015

Introduction

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 eXtremeIFV®, 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 eXtremeIFV® 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.

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-20A5R
  - Column: Ultra Biphenyl Columns (5µm 50 x 2.1 mm) - Restek
  - Flow Rate: 0.5 mL/min
  - Injection Volume: 15µL
  - Mobile Phases:
    - A: 0.1% Formic Acid in HPLC Water
    - B: 0.1% Formic Acid in Methanol
- Eppendorf Mix Mate
- Thomson eXtremeIFV® 0.2µm PVDF (P/N 85531)
- Thomson 48 position Vial Filter Press (P/N 35015)

Improved Sample Preparation

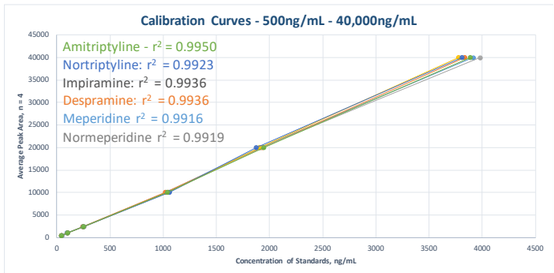
1. Place 400 µL of 20% MeOH / 80%Water / 0.1% Formic Acid in each of the outer shells of the Thomson Filter Vials
2. Add 25µL of Standard/Control/Patient Sample + 10uL of Internal Standard
3. Place Thomson Filter Plunger on top of the Thomson vial, Thomson vials –eXtreme/FV® 0.2µm PVDF, w/Pre-Slit Red Cap #85531.
4. Press filter plunger down approximately ¼ of the way into each of the Thomson outer shell vials.
5. Vortex for 30-40 seconds.
6. Slowly press filter plunger the rest of the way down using the Vial Filter Press.
7. Extracts are ready for LC-MS/MS analysis using the Shimadzu / ABI 4500.
8. Inject 15µL.

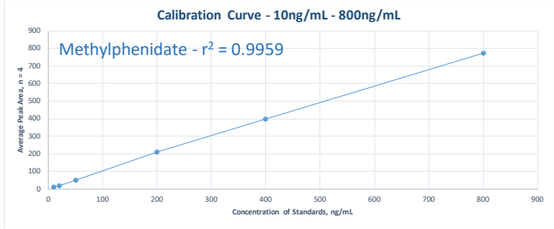
Results

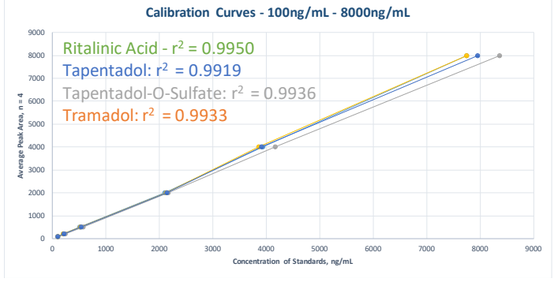
This improved sample preparation method allows for the quantitative measurement of the following pain management drugs in urine, Table 1. The improved method utilizes the Thomson eXtremeIFV® for sample clean-up significantly reducing the cost and time of per sample analysis. This method was validated for all 17 drugs in the supplemental pain management panel over 3 days. See Table 1 for the complete list of drugs in the panel. The 6 point calibration curve for Gabapentin in urine on Day 1 can be seen in fig 1. The R^2 was > 0.99. LC-MS/MS spectrum of the 17 drugs of interest in Table 1 can be seen in Fig 1.

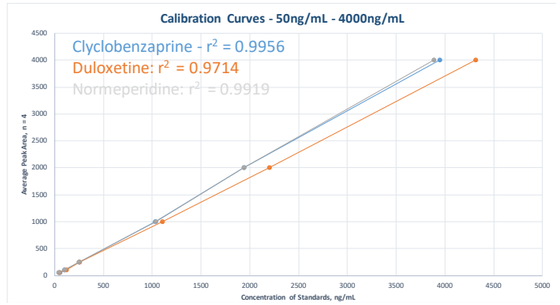
Table 1. Drugs analyzed as part of the Pain Management Supplemental Panel in urine.

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









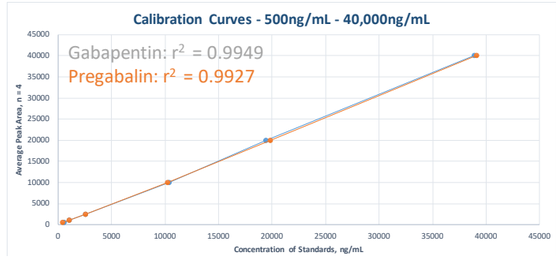


Fig 1. 6 point calibration curve in urine.

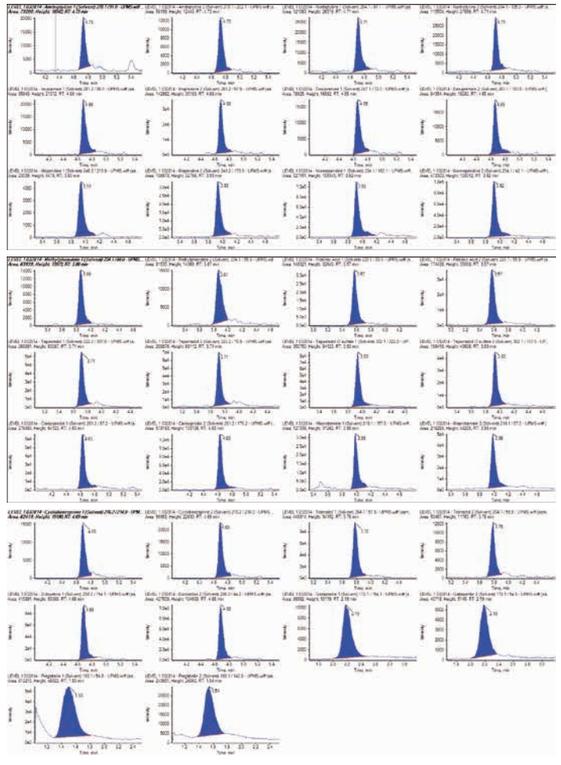


Fig 2. Mass spectrum of the 17 drugs included in the Supplemental Pain Management Panel in Urine.

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 eXtremeIFV® include lower cost, faster sample preparation time, less use and disposal of organic solvents.

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

t eXtremeIFV® by LC-MS/MS

Nadine Koenig[1], Crystal Xander[1], Melanie Stauffer[1], Dean Fritch[2]
[1]Health Network Laboratories, Allentown, PA.
[2]Analytical Asociates, Inc,. East Greenville, PA.

Introduction

This improved sample preparation method allows for the quantitative measurement of Opioids in urine. Opioids are highly addictive and affects nearly 5 million people in the U.S. Opioids include naturally occurring Opiates, semi-synthetic opioids derived from morphine and synthetic opioids are analgesic alkaloids found naturally in *Papaver somniferum*, poppy plant. The urine samples are hydrolyzed, then prepared using the eXtremeIFV®, 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. eXtremeIFV®, were compared to an existing SPE sample preparation method to reduce the sample matrix causing interference prior to analysis. SPE is time consuming, adversely impacts recovery, uses large amounts of solvent and are expensive. The improved sample preparation method using the Thomson eXtremeIFV® allows for the analysis of 12 Opioid Panel in urine.

Equipment

- Thomson eXtremeIFV® 0.2µm PVDF (P/N 85531)
- Thomson 48 position Vial Filter Press (P/N 35015)
- Eppendorf MixMate®
- Vortex Mixer
- Dry Block Heater set at 55°C ± 2°C
- Microcentrifuge
- AB Sciex 4500 Mass Spectrometer
- Shimadzu Prominence HPLC
  - Column: Restek Ultra Biphenyl Columns (5µm, 50 x 2.1 mm)
  - Mobile Phases:
    - A: 0.1% Formic Acid in HPLC Water
    - B: 0.1% Formic Acid in Methanol
  - Flow Rate: 0.5 mL/min
  - Run Time: 8.5 minutes
  - Injection Volume: 15µL

Analytes

Table 1. Drugs analyzed in this Opiate Panel

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

Improved Sample Preparation

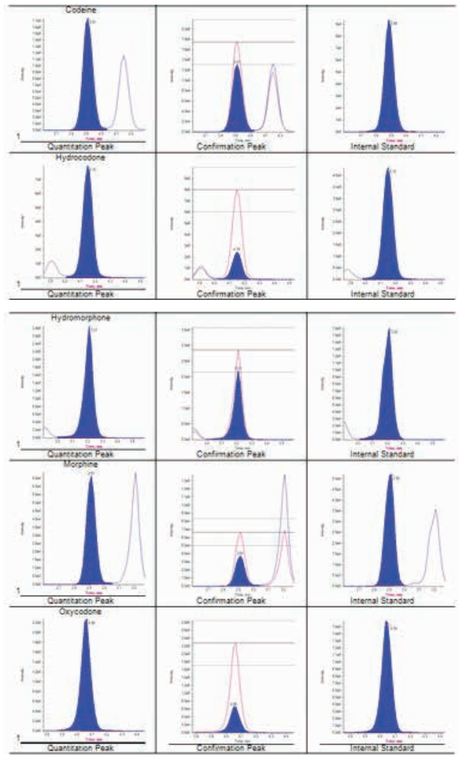
1. Add 200 µL of 2% Methanol to each Thomson Vial.
2. Add 100 µL of the hydrolyzed urine sample to its respective Thomson Vial (see htslabs.com for hydrolysis steps used in this method).
3. Place Thomson Filter Plunger on top of Thomson Vial.
4. Press filter plunger down approximately ¼ of the way into each of the Thomson Vials.
5. Vortex for 2 minutes at 1750rpm 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

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 validated concentrations used to generate a 6 point calibration curve. Linearity of the assay for the drugs listed 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 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 2. Final concentrations for the various analytes

Level	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



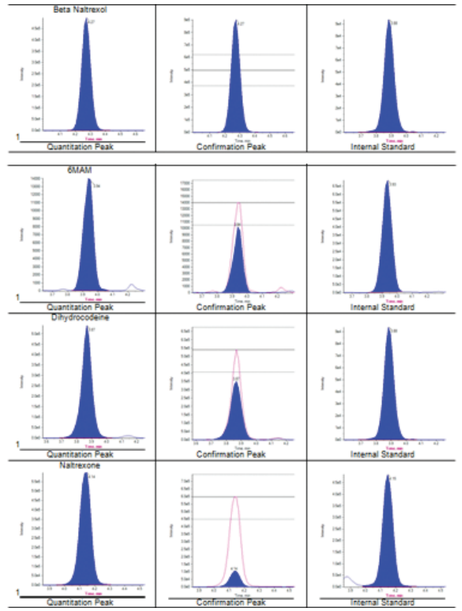


Fig 1. Mass Spectrum of Positive Results

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

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

Automated Hydrolysis and Sample Preparation for the Analysis of 12 Opiates in Urine using the Thomson eXtremeIFV®s by LC-MS/MS

Presented at MSACL 2017

Nadine Koenig[1], Crystal Xander[1], Melanie Stauffer[1], Dean Fritch[2],

[1] Health Network Laboratories, 794 Roble Road, Allentown, PA 18109

[2] Analytical Associates, 225 Millwood Drive, East Greenville, PA

Introduction

This improved sample preparation method allows for the quantitative measurement of Opioids in urine. Opioids are highly addictive and affects nearly 5 million people in the U.S. Opioids include naturally occurring Opiates, semi-synthetic opioids derived from morphine and synthetic opioids are analgesic alkaloids found naturally in Papaver somniferum, poppy plant. The use of hydrolysis in the analysis of natural and synthetic opiates in urine has become standard practice in forensic toxicology. Many laboratories currently use solid phase extraction or solid liquid extraction techniques in the sample preparation of urine for the analysis for opiates. This improved automated sample preparation method evaluates the robustness for the quantitative measurement of opiates in urine without the need for SPE/SLE thereby reducing pipetting errors. The sample preparation of incurred urine, controls, standards and internal standard additions as well as the hydrolysis step are performed by a liquid handler. The Thomson eXtremeIFV®s provide a simple and efficient extraction technique that has demonstrated adequate analyte recovery, reduced matrix interferences and the reduction of solvent and consumable waste.

Analytes

Table 1. Drugs analyzed in this Opiate Panel

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

Equipment / Reagents

- Hamilton Automated Liquid Handler
- ABI 4500 Mass Spectrometer
- Shimadzu Prominence HPLC
  - Flow Rate: 0.5 mL/min
  - Run Time: 8.5 minutes
  - Injection Volume: 15µL
  - Mobile Phases:
    - A: 0.1% Formic Acid in HPLC Water
    - B: 0.1% Formic Acid in Methanol
- Column: Restek Ultra Biphenyl Columns (5µm 50 x 2.1 mm)
- Thomson eXtremeIFV® 0.2µm PVDF (P/N 85531)
- Thomson 48 position Vial Filter Press (P/N 35010)
- ß-Glucuronidase - IMCSzyme™, genetically engineered beta-glucuronidase, p/n #04-E1F-010

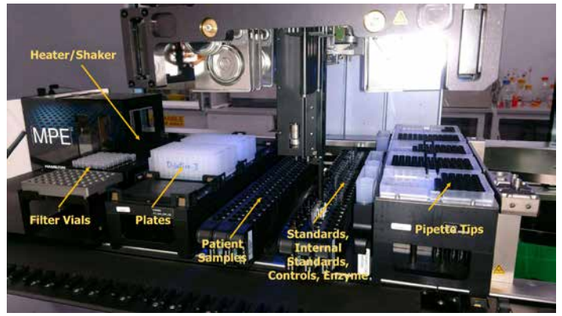
Method:

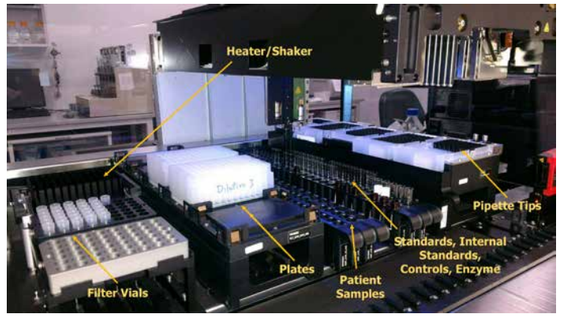
The method created for the Hamilton Liquid Handler will replace manual steps currently performed by lab personnel. The method includes the pipetting of blanks, patient samples (dilution if necessary), controls, standards, internal standard and enzyme from 12x75mm glass tubes into a 96-Well plate for the hydrolysis step. The final step in the robotic method is the transfer of the hydrolyzed urine samples to a 48 position plate containing the outer shell vial of the eXtremeIFV®s. The plungers are than added to the outer shell vials of the eXtremeIFV® and the plate is transferred to the Multi-Press. The samples are analyzed by LC-MS/MS.

The opiates analyzed in this method include Codeine, Oxycodone, Dihydrocodeine/Hydrocodol, Hydrocodone, Hydromorphone, 6-Monoacetylmorphine, Morphine, Noroxycodone, ß-Naltrexone, Naltrexone, Norhydrocodone, Oxycodone, and Oxymorphone. Stock solutions for each analyte and internal standards are made in methanol. These stock solutions are diluted in negative urine by the liquid handler to generate working solutions for a 6-point calibration curve, generate controls and add internal standards to specimens. Limit of Detection (LOD) and Limit of Quantification (LOQ) were compared to the existing validated method for opiate analysis by LC-MS/MS. The 6-point standard curve for 6-Monoacetylmorphine includes the LOQ, Level 1 concentration of 5ng/mL with upper limit, Level 6 concentration of 1000ng/mL. For all the other analytes the LOQ, Level 1 concentration of 50ng/mL with upper limit, Level 6 concentration of 20000ng/mL.

Liquid Handler / Sample Prep

- To ensure proper tracking, the liquid handler reads barcode on the sample.
- 12 x 75mm glass culture tubes are used for: Urine Standards, LC Checks, Controls, Internal Standard and Enzyme.
- Eight channel pipette head is utilized for adding standards, internal standards and enzyme to each of the 12x75mm culture tubes to create blanks, Standard Curve, patient samples and controls.
- All samples are transferred to a 96-Well plate and the plate is transferred to the heater/shaker unit.
  - Vortexed for 2 minutes.
  - Hydrolyzes for 30 minutes at 55° +/- 2°C.
- Upon completion the plate is removed from the heater/shaker and returned to the deck for a 10 minute cool down.
- During cool down, 2% Methanol is added to each eXtremeIFV® outer shell.
- All hydrolyzed samples are transferred to the eXtremeIFV® outer shell.
- The rack containing the eXtremeIFV® outer shells is removed from the deck of the robot and the plungers are added ¼ of the way down.
  - The rack containing the eXtremeIFV®s is vortexed for 5 minutes.
- The plungers are completely depressed using the Thomson Multi-Press.
- Samples are ready for analysis by LC-MS/MS.



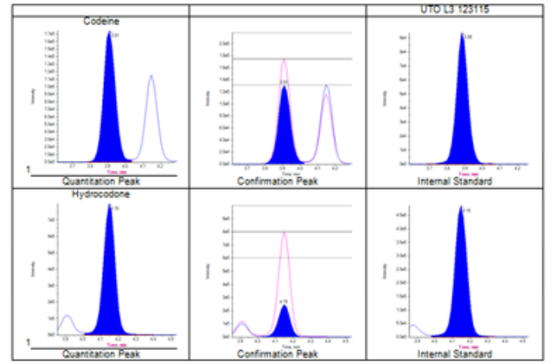


Results

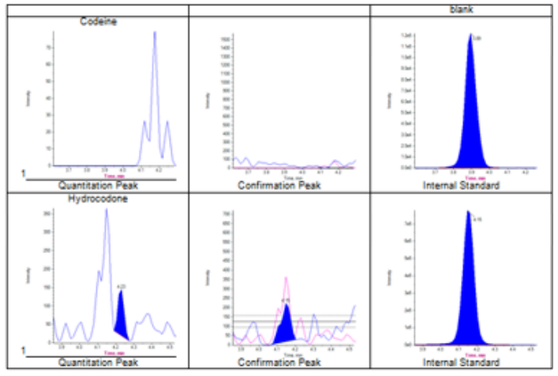
Final concentrations for the various analytes are as follows:

	Final Concentration: Opiates (ng/mL)	Final Concentration: 6-MAM (ng/mL)
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



Negative Results



Conclusion

This validated automated method reduces the risk of contamination and alleviates the risk of human pipetting errors during specimen transfer steps. Samples are diluted, receive Internal Standards, hydrolyzed and dispensed into the vial going onto the LC-MS/MS. All sample/specimen containers are barcoded and tracked throughout the process significantly reducing the need for repeat sampling.

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

Quick and Easy Sample Preparation of Urine for the Analysis of Psychoactive Drugs using the eXtremeIFV® by LC-MS/MS

Nadine Koenig[1], Crystal Xander[1], Melanie Stauffer[1], Dean Fritch[2]
[1]Health Network Laboratories, Allentown, PA.
[2]Analytical Asociates, Inc,. East Greenville, PA.
Presented at MSACL 2016

Introduction

This improved sample preparation method allows for the quantitative measurement of Benzodiazepines in urine. Benzodiazepines are psychoactive drugs that enhance the effect of the neurotransmitter GABA at the GABAA receptor. The urine samples were prepared using the eXtremeIFV®, 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. eXtremeIFV®, were compared to SPE for sample preparation to reduce the sample matrix causing interference prior to analysis. SPE is time consuming, adversely impacts recovery, uses large amounts of solvent and are expensive. The improved sample preparation method using the Thomson eXtremeIFV® allows for the analysis of 9 Benzodiazepines.

Equipment

- Thomson eXtremeIFV® 0.2µm PVDF (P/N 85531)
- Thomson 48 position Vial Filter Press (P/N 35015)
- Eppendorf MixMate®
- Vortex Mixer
- Dry Block Heater set at 55°C ± 2°C
- Microcentrifuge
- AB Sciex 4500 Mass Spectrometer
- Shimadzu Prominence HPLC
  - Column: Restek Ultra Biphenyl Columns (5µm, 50 x 2.1 mm)
    - Mobile Phases:
      - A: 0.1% Formic Acid in HPLC Water
      - B: 0.1% Formic Acid in Methanol
    - Flow Rate: 0.5 mL/min
    - Run Time: 8.5 minutes
    - Injection Volume: 15µL

Analytes

Table 1. Drugs analyzed in this Benzodiazpine Panel

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

Improved Sample Preparation

1. Add 300 µL of 40% Methanol to each Thomson Vial.
2. Add 50 µL of hydrolyzed urine sample to its respective Thomson Vial (see htslabs.com for hydrolysis method used).
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

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 validated concentrations used to generate a 6 point calibration curve. Linearity of the assay for the drugs listed 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 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 2. Final concentrations for the various analytes

Levels	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

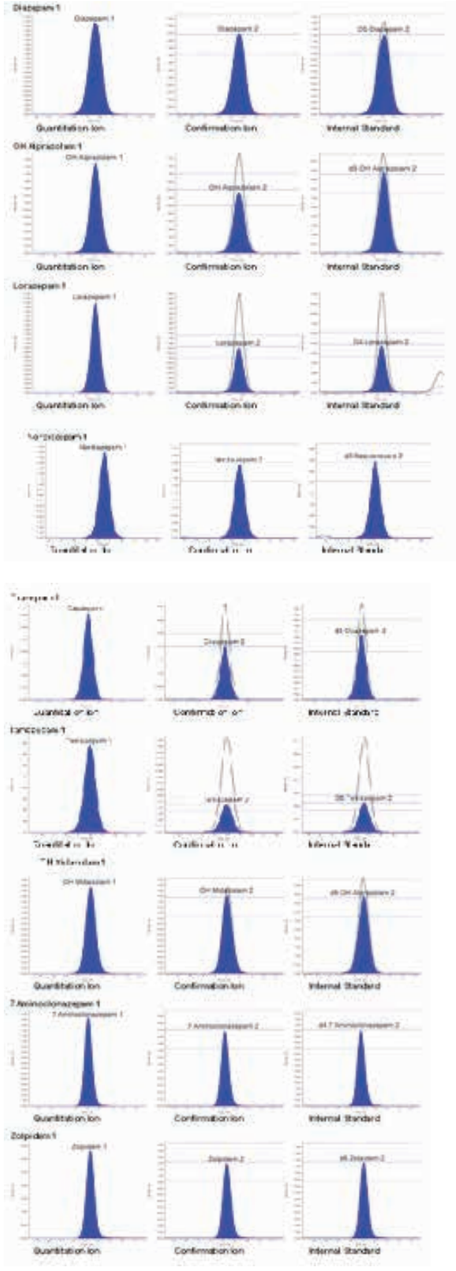


Fig 1. Mass Spectrum of Positive Results

Conclusion

Alleviate the need to use and dispose of Hexane, Glacial Acetic Acid, Potassium Hydroxide.

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

Improved Sample Preparation Methods for Athlete Doping Analysis of Common Compounds in Urine by LCMS

Dr. Catrin Goebel[2] , Lisa Wanders[1], Sam Ellis[1]

[1]Thomson Solutions At Work™

[2]Australian Sports Drug Testing Laboratory in the National Measurement Institute Department of Industry

Abstract

Anti-doping testing by urine analysis requires fast and robust screening methods with repeatable sample preparation. Since, every sample has to be screened, methods are designed to be sufficiently sensitive and specific to identify all suspect samples. One must be careful to minimize false suspects. Ensuring samples are spiked with internal standards accordingly will help verify that samples are being extracted and tested correctly and with accurate uniformity.

The Australian Sports Drug Testing Laboratory, our collaborators, have invested time in determining a limited number of comprehensive screening methods. These methods, using Thomson's eXtremeIFV®s , comply with the World Anti-Doping Agency's (WADA) Prohibited List.

In exploring new methods labs have looked at both detection and sample prep as routes to quicker and more accurate analysis. Liquid chromatography coupled with mass spectrometry detection is prevalent, superseding many of the gas chromatographic coupled with mass spectrometry methods because of the simpler sample preparation. Specifically, the anti-doping testing shown below consisted of sample preparation without the initial use of cumbersome traditional SPE methods, and instead consisted of the comparison of filtration techniques. Filter plates versus Thomson eXtremeIFV®s were tested to determine which product allowed for a method of simple and quick urine analysis while complying with the WADA's guidelines.

Experiment

The experiments were performed at the National Measurement Institute (Australia) in the Sports Drug Testing Laboratory.

The 11.8 minute run time for the instrumental analysis meets the requirements of the WADA Technical Document- Minimum Required Performance Level (TD2013MRPL). This document details the analysis of a large number of analytes from the classes on the WADA Prohibited List, while meeting sensitivity requirements. The analytes included compounds in the following classes anabolic agents, B2-agonists, hormone antagonists and modulators, diuretics, stimulants, narcotics, glucocorticoids, B-blockers, etc.

Full Method

A comparison between sample preparation using filter plates sourced from several different manufactures, and Thomson eXtremeIFV®s PVDF 0.2µm (85531-500) was conducted. The preparation with the Thomson eXtremeIFV®s were automated using a Tecan robotics platform for liquid dispensing in the Thomson 48 position rack (#35010-RACK), and 48 position press (#35015).

Direct Urine Preparation

1. Label each eXtremeIFV® with sample/quality control sample information.
2. Pipette 200 µL of each sample into labeled eXtremeIFV®.
3. Add 200 µL of the Mefruside Internal Standard (300 ng/mL in 0.5% formic acid) to each filter vial cup.
4. Place the eXtremeIFV® tops onto each vial and press shut.

LCHRMS System

UPLC coupled to High Resolution Mass Spectrometry with an electrospray source in full scan mode. Data acquisition in both positive and negative polarity modes within a single 11.8 min chromatographic run.

- Column: C18, 2.1mm × 50mm, 1.7µm
- Column Temperature: 30 °C
- Flow rate: 300µL/min
- Mobile Phase:
  - A: 0.3% aqueous Formic Acid in Water
  - B: 0.3% Formic Acid in Acetonitrile
- Gradient:

Time	A%	B%
0.00	95	5
0.50	95	5
3.50	80	20
5.50	75	25
7.00	43	57
8.00	10	90
8.60	10	90
8.80	95	5

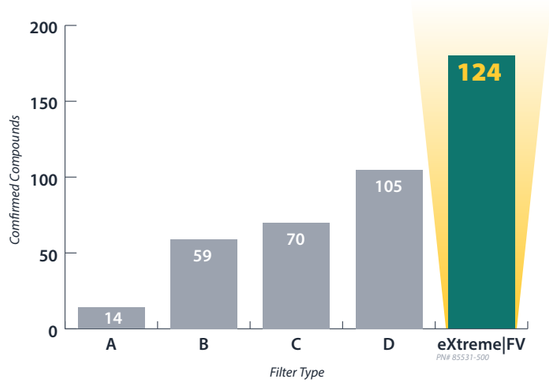
- Injection volume: 10µL
- Sample tray temperature: 18°C
- Column Temperature: 30°C
- Method run time: 11.8 minutes
- Gas: UHP Nitrogen

Conclusions

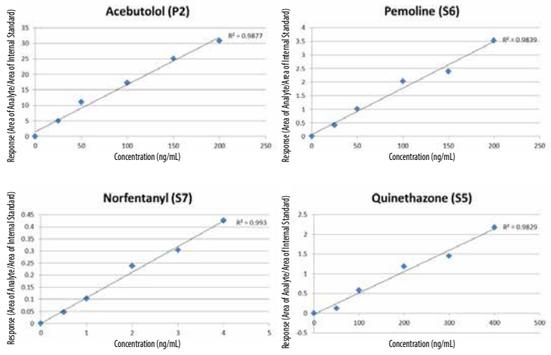
The Thomson eXtremeIFV®s PVDF 0.2 µm (85531-500) performed the best in compound extraction and identification while allowing the end user to follow the WADA validated method. The elimination of SPE steps from laboratory methods is a large time saver, and enables urine-direct-injection solely using Thomson eXtremeIFV®s for filtration. Together the Thomson 48 position Filter Vial Press and automation enabled 48 position rack equaled timing of filter plate methodology but provided the best extraction and identification of all filter types. A total of 180 compounds can be identified through the screening analysis with the Thomson eXtremeIFV®s PVDF 0.2µm (85531-500).

The method presented is being used for the analysis of athlete's urine samples for banned substances at the Australian Sports Drug Testing Laboratory.

Comparison of Filter Types



Linearity of The Analysis Method Was Assessed Over a Range From 25% To 200% Of MRPL With R2 Generally Being Greater Than 0.98



124 Confermed Compounds

5-Hydroxyindapamide
Bisdesmethylsibutramine
Desmethylsibutramine
Exemestane
Mefruside (+)
Mefruside (-)
D3-epitestosterone glucuronide
D3-epitestosteronea
AICAR
GW1516
Atenolol
Bisoprolol
Esmolol
Metipranolol
Nadolol
Nadoxolol
Oxprenolol
Clenbuterol
Gestrinone
Methyldienolone
Methyltrienolone
Metribolone
Tetrahydrogestrinone
Tibolone
Zilpaterol
3'-Hydroxystanozolol
4'-Hydroxystanozolol
Bambuterol
Formoterol
Salbutamol
Salmeterol
Terbutaline
Andarine
Exemestane metabolite
Aminoglutethimide
Raloxifene
Fulvestrant
GW1516 (501516)
Methazolamide
Piretanide
Quinethazone
Spironolactone
Trichlormethiazide
Acetazolamide
Althiazide
Amiloride
Bendroflumethiazide
Benzthiazide
Bumetanide
Canrenone
Chlorexolone
Chlorothiazide
Chlorthalidone
Clopamide
Probenecid
Cyclopenthiazide
Cyclothiazide
Dichlorphenamide
Epitizide
Eplenerone
Etacrynic acid (frag?)
Furosemide
Hydrochlorothiazide
Mefruside metabolite 2
Indapamide
Metolazone
Polythiazide
Torasemide
Triamterene
Xipamide
Caffeine
Cis-4-Methylaminorex
Cotinine (Nicotine metab)
MBDB
Methoxyamphetamine
Methylenedioxyethylamphetamine
Adrafinil
Amiphenazole
Amphetamine
Benzoylecgonine
Benzylpiperazine
Carphedon
Cathine
Crotethamide
Cyclazodone
Ephedrine
Phenylpropanolamine
Pseudoepherine
Etamivan
Etilefrine
Fenetylline
Hydroxy mesocarb
Isometheptene
MDA
MDMA
Methylphenidate
Modafinil
Modafinil Acid (metabolite)
Nikethamide
Oxilofrine
Pemoline
Pentetrazol
Phenmetrazine
Pholedrine
p-Hydroxy amphetamine
Ritalinic Acid
nor-Selegiline
Methylecgonine
Codeine
Hydromorphone
Morphine
JWH018 N-(5-hydroxypentyl) metabolite
JWH073 N-butanoic acid metabolite
Budesonide
Cortisol
Cortisone
Flumethasone
Fluticasone propionate metabolite
Methylprednisolone
16a-OH-Prednisolone
Prednisolone
Sildenafil
Tadalafil
Vardenafil

Acknowledgments

We would like to thank Dr. Catrin Goebel, Director, of Australian Sports Drug Testing Laboratory in the National Measurement Institute, Department of Industry (a WADA accredited laboratory in Australia) for her extensive testing. Dr. Goebel is also an Executive member of World Association of Anti-Doping Scientist.

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

Clinical Urine Mega Method by LC-MS/MS

Nadine Koenig[1], Crystal Xander[1], Melanie Stauffer1, Dean Fritch[2]
[1] Health Network Laboratories, 794 Roble Road, Allentown, PA 18109
[2] Analytical Associates, 225 Millwood Drive, East Greenville, PA
Presented at MSACL 2019

Introduction

This improved sample preparation method allows for the quantitative measurement of over 60 drugs of different classes in urine for clinical purposes. Drugs of abuse include naturally occurring, semi-synthetic and synthetic drugs. The use of hydrolysis in the analysis of natural and synthetic drugs in urine has become standard practice in toxicology labs. Many laboratories currently use solid phase extraction or solid liquid extraction techniques in the sample preparation of urine. This method quantitatively measures multiple drugs of different classes in urine for clinical purposes. This method is known as the CLUMM (Clinical Urine Mega Method) and run on the Sciex 4500 using the Phenomenex Phenyl-hexyl Kinetex analytical column. The samples are hydrolyzed, then prepared using a dilute and filter technique followed by LC-MS/MS analysis.

Amphetamine	Codeine
Meperidine	Nortriptyline
Methamphetamine	Morphine
Normeperidine	Duloxetine
MDA	6 MAM
Methadone	Ketamine
MDMA	Hydrocodone
EDDP	Norketamine
Gabapentin	Hydromorphone
Mitragynine	Methylphenidate
Pregabalin	Norhydrocodone
7-Hydroxymitragynine	Ritalinic Acid
2-Hydroxyethylflurazepam	Dihydrocodeine
Tapentadol	Zolpidem
7 Aminoclonazepam	Oxycodone
N-Desmethyl Tapentadol	Carboxyzolpidem
aOH-Alprazolam	Oxymorphone
Tramadol	THC-COOH
Diazepam	Noroxycodone
O-desmethyltramadol	Nicotine
Nordiazepam	Buprenorphine
Carisoprodol	Cotinine
Oxazepam	Norbuprenorphine
Meprobamate	3-OH-Cotinine
Temazepam	Fentanyl
Cyclobenzaprine	Butalbital
aOH-midazolam	Norfentanyl
Benzoylecgonine	Pentobarbital (qualitative only)
Lorazepam	Acetylfentanyl
PCP	Phenobarbital (qualitative only)
Secobarbital (qualitative only)	

Equipment

- Sciex 4500 LC-MS/MS System
- Phenomenex Phenyl-hexyl Kinetex analytical 100A 50 x 4.6 mm column
- Eppendorf Mix Mate
- Thomson eXtremeIFV®s, 0.2µm

Sample Preparation

A. Urine Specimens are 1.5mL and are kept refrigerated. Allow standards, specimens and controls to come to room temperature. Turn Block Heater on to 55ºC±2ºC. Label one 1.5 mL Safe-Lock Tube and one Thomson vial for each blank, standard, control and client specimen. For samples falling outside the calibration range, make appropriate dilutions using Negative Urine and record on the run sheet. The goal is to prevent mass spectral distortion (failing ion ratios) that occurs in a sample that is too concentrated while keeping the concentration of the diluted sample above the cutoff (or a least the limit of quantitation).

NOTE: The maximum dilution allowed for this analysis is 1:20. This dilution is for all analytes with the exception of THC. Perform this dilution in a separate 12x75 mm glass tube. Place 950 µL of Negative Urine into the tube using the 200-1000 µL and add 50µL of sample requiring dilution into the same tube. Vortex for 20-30 seconds.

For the LC Check, place 400 µL of 2% Methanol into a 12 x 75 mm glass culture tube. Add 20 µL of working IS and 1 µL of Cutoff Calibrator Spiking Standard A and 1 µL of Cutoff Calibrator Spiking Standard B. Vortex and transfer to an autosampler vial with insert. To each 1.5 mL Safe-Lock Tube add 90 µL of Rapid Hydrolysis Mixture. Prepare 1.5 mL Safe-Lock Tubes for analysis. Cap and vortex for 5 minutes at 850 rpm using the Eppendorf Mix Mate. Incubate at 55ºC±2ºC for 30 minutes uncapped. Allow tubes to come to room temperature.

Add 200 µL of 2% Methanol to each Thomson outer shell vial. Give each Eppendorf tube a quick vortex and add 200 µL of the hydrolyzed urine sample to its respective Thomson outer shell vial. Place Thomson Filter Plunger on top of Thomson outer shell vial. Press filter plunger down approximately ¼ of the way into each of the Thomson outer shell vial. Vortex for 5 minutes at 1750 rpm using the Eppendorf Mix Mate.

B. Add 200 µL of 2% Methanol to each Thomson outer shell vial. Briefly vortex each sample tube. 200 µL of the hydrolyzed urine sample should be added to its respective Thomson outer shell vial. Place Thomson Filter Plunger on top of Thomson outer shell vial. Press filter plunger down approximately ¼ of the way into each of the Thomson outer shell vials. Vortex for 5 minutes at 1750 rpm using the Eppendorf Mix Mate.

Results

Final concentrations (ng/mL) including linearity for the various analytes including controls can be found in Table 1.

Validation of any method must include evaluation of interfering substances/co-eluting peaks. There may be unknown substances in certain specimens which co-elute with the analyte or the internal standard and may cause low recovery or cause ion ratios to fail. Seven analyte mixes, were evaluated for interference. The analytes in table 1 had % accuracies exceeding 60-140% when spiked into the low control. There are unknown substances that interfere with Barbiturates*. Examples of mass spectrum of some of the analytes can be seen in Fig. 1-8.

*Note: If any of these analytes appears positive in any patient sample they will be reflexed and repeated by an appropriate alternate method.

Table 1. Concentrations of the various analytes

Analyte	Level 1 (LOD/LOQ/CUTOFF CONCENTRATION)	Level 2	Level 3	Level 4	Level 5 (LINEARITY)	Low Control	High Control
Buprenorphine	5	10	25	100	250	10	150
Carisoprodol	100	200	500	2000	5000	200	3000
Fentanyl	1	2	5	20	50	2	30
Meprobamate	100	200	500	2000	5000	200	3000
Norbuprenorphine	5	10	25	100	250	10	150
Norfentanyl	1	2	5	20	50	2	30
O-desmethyltramadol	100	200	500	2000	5000	200	3000
Tramadol	100	200	500	2000	5000	200	3000

For more information, see the full application note at https://htslabs.com/technical/urine-mega-method

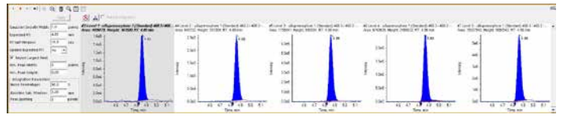


Fig 1. Buprenorphine

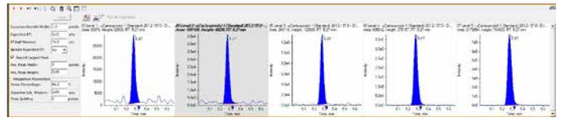


Fig 2. Carisoprodol

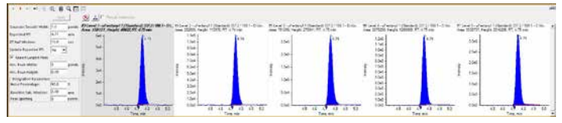


Fig 3. Fentanyl

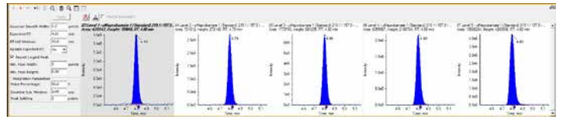


Fig 4. Meprobamate

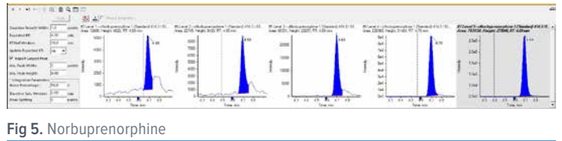


Fig 5. Norbuprenorphine

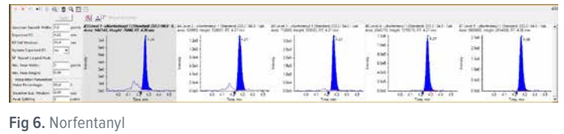


Fig 6. Norfentanyl

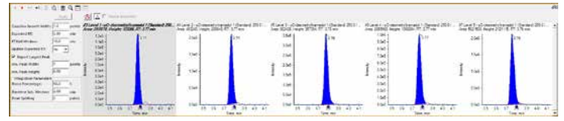


Fig 7. O-desmethyltramadol

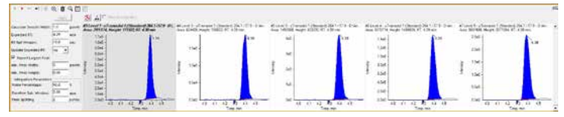


Fig 8. Tramadol

Conclusion

This method quantitatively measures multiple drugs of different classes in urine for clinical purposes. This method is known as the CLUMM (Clinical Urine Mega Method). This new improved method allows for a large sample panel, reduces sample prep time, limits transfer steps, improves column life, and reduces instrument downtime.

series	cap color	membrane	pore size	part #
Standard Filter Vial	●	PTFE	0.2µm	34430

Time saving sample prep for the analysis of 54 pesticide & aflatoxin residues in Cannabis by LC-MS/MS

Presented at NACRW 2017

Kavinda De Silva[1], Tami Nguyen[1]

[1] Molecular Testing Labs, Vancouver, WA 98684

Introduction

Pesticide analysis of cannabis leaves and finished goods is becoming increasingly important as many states are legalizing it for medicinal and recreational purposes. Dosing methods include smoking/vaporizing and edibles but cannabis is still a Schedule 1 illegal drug and therefore have no FDA testing guidelines. Trace levels of pesticides can be incurred during cultivation or inhaled from dried pesticides on the cannabis. This study evaluates the sample preparation aspect for LC-MS/MS analysis of a 50+ analyte panel of pesticides, fungicides and aflatoxins. QuEChERS was used to extract the analytes from the cannabis flowers, followed by centrifugation and Thomson Standard Filter Vial for sample clean-up.

Equipment:

- Sciex 6500 QQQ Mass Spectrometer
- Shimadzu LC-30AD Pumps
- Run Time: 15 minutes
- Flow Rate: 0.5 mL/min
- Injection Volume: 12 µL
- Column: Kinetex C18, 5µm, 3mm x 150mm
- Mobile Phase A: 0.1% FA in Water
- Mobile Phase B: 5mM Ammonium Formate, 0.1% Formic Acid in MeOH
- Centrifuge
- Thomson Standard|FV 0.2µm PTFE (P/N 34430)*
- Thomson 48 position Vial Filter Press (P/N 35015)

*For some autosamplers it is important to adjust the needle depth of your autosampler when using Thomson filter vials to improve the reproducibility of injections.

Sample Preparation of Cannabis Flowers

1. Weigh out 0.25g of the flower into a 50mL conical.
2. Add 7g of QuEChERS.
3. Add 15mL of 1% Acetic Acid in Acetonitrile.
4. Vortex for 30 minutes.
5. Centrifuge for 5 minutes.
6. Transfer 400µL into the outer shell of (P/N 35015).
7. Add 4µL of ISTD.
8. Partially depress the plunger and vortex.
9. Ready to analyze.

Results

20+ compounds were extracted from cannabis flower with excellent recoveries utilizing a modified QueChERS method. The linear range for all the aflatoxins and ochratoxins are 0.5-50ng/mL; while the other analytes are 1.0-100ng/mL. Excellent linearity (see Table 2) and good recovery was achieved for all the compounds.

Table 1. Shows the LOQ, linear range, % CV, r2 and accuracy for each analyte

Analyte	LOQ (ng/mL)	Linear Range (ng/mL)	% CV	r2 Value	% Accuracy
Abamectin Group 1	1	1- 100	< 14.6	0.9932	93.4 - 105.5
Abamectin Group 2	1	1- 100	<25.4	0.98806	93.6 - 103.4
AFLATOXIN B2 1	0.5	0.5 - 50	<3.3	0.99837	93.7 - 105.7
AFLATOXIN B2 2	0.5	0.5 - 50	<4.9	0.99833	94.0 - 104.6
AFLATOXIN G2 1	0.5	0.5 - 50	<5.0	0.99829	93.1 - 105.2
AFLATOXIN G2 2	0.5	0.5 - 50	<5.4	0.9983	93.7 - 104.9
AFLATOXIN B1 1	0.5	0.5 - 50	<3.9	0.99805	92.2 - 105.9
AFLATOXIN B1 2	0.5	0.5 - 50	<4.0	0.99789	92.0 - 106.4
AFLATOXIN G1 1	0.5	0.5 - 50	<4.2	0.99853	94.1 - 104.6
AFLATOXIN G1 2	0.5	0.5 - 50	<4.5	0.99827	93.8 - 105.1
Bifenthrin 1	1	1- 100	<7.9	0.99699	92.6 - 105.6
Bifenthrin 2	1	1- 100	<6.2	0.99704	92.8 - 105.3
Chlormequat 1	1	1- 100	<1.4	0.99593	87.3 - 111.0
Chlormequat 2	1	1- 100	<4.5	0.99512	86.6 - 111.3
Daminozide 1	1	1- 100	<1.9	0.96303	66.0 - 131.6
Daminozide 2	1	1- 100	<4.5	0.99512	65.5 - 131.7
Dichlorvos 1	1	1- 100	<7.2	0.99369	86.0 - 112.4
Dichlorvos 2	1	1- 100	<7.2	0.99371	86.1 - 112.8
Imidacloprid 1	1	1- 100	<4.9	0.99904	97.4 - 101.3
Imidacloprid 2	1	1- 100	<5.5	0.99887	97.5 - 101.6
Malathion A 1	1	1- 100	<4.3	0.99574	86.9 - 108.7
Malathion A 2	1	1- 100	<3.7	0.99416	84.5 - 111.4
Myclobutanil 1	1	1- 100	<3.5	0.99808	91.6 - 105.2
Myclobutanil 2	1	1- 100	<4.8	0.99773	91.0 - 106.2
OCHRATOXIN A 1	0.5	0.5 - 50	<8.6	0.97237	67.4 - 120.0
OCHRATOXIN A 2	0.5	0.5 - 50	<18.5	0.96764	67.2 - 121.2
Paclobutrazol 1	1	1- 100	<5.7	0.99481	86.6 - 109.5
Paclobutrazol 2	1	1- 100	<3.8	0.99469	85.6 - 109.6
Permethrin, cis- 1	1	1- 100	<6.6	0.99813	95.5 - 103.2
Permethrin, cis- 2	1	1- 100	<6.5	0.99782	93.6 - 102.8
Permethrin, trans- 1	1	1- 100	<8.1	0.99723	92.9 - 102.9
Permethrin, trans- 2	1	1- 100	<7.3	0.99694	91.8 - 105.2
Piperonyl butoxide 1	1	1- 100	<8.4	0.99523	93.2 - 106.3
Piperonyl butoxide 2	1	1- 100	<8.9	0.99526	93.1 - 106.3
Propiconazole 1	1	1- 100	<3.8	0.99759	90.1 - 105.4
Propiconazole 2	1	1- 100	<2.8	0.99722	89.6 - 106.7
Pyrethrins Cinerin I 1	1	1- 100	<13.0	0.99779	98.6 - 101.9
Pyrethrins Cinerin I 2	1	1- 100	<20.5	0.99494	96.4 - 103.3
Pyrethrins Cinerin II 1	1	1- 100	<8.3	0.99651	90.3 - 105.5
Pyrethrins Cinerin II 2	1	1- 100	<12.7	0.99351	88.2 - 110.2
Pyrethrins Jasmolin I 1	1	1- 100	<12.9	0.99702	94.6 - 103.7
Pyrethrins Jasmolin I 2	1	1- 100	<21.5	0.99449	96.2 - 103.5
Pyrethrins Jasmolin II 1	1	1- 100	<22.7	0.99355	93.8 - 103.3
Pyrethrins Jasmolin II 2	1	1- 100	<10.0	0.99751	94.5 - 103.7
Pyrethrins Pyrethrin I 1	1	1- 100	<17.6	0.99626	97.4 - 101.7
Pyrethrins Pyrethrin I 2	1	1- 100	<5.0	0.99906	96.4 - 102.4
Pyrethrins Pyrethrin II 1	1	1- 100	<3.2	0.99853	92.9 - 104.2
Pyrethrins Pyrethrin II 2	1	1- 100	<38.3	0.98319	91.9 - 106.9
Spinosyn A 1	1	1- 100	<4.0	0.99913	95.2 - 102
Spinosyn A 2	1	1- 100	<3.2	0.99931	96.1 - 103.0
Spinosyn D 1	1	1- 100	<3.9	0.99897	94.9 - 103.2
Spinosyn D 2	1	1- 100	<5.4	0.9987	94.8 - 103.4
Spiromesifen 1	1	1- 100	<16.6	0.99223	95.8 - 105.0
Spiromesifen 2	1	1- 100	<13.8	0.99457	95.4 - 104.1
Uniconazole 1	1	1- 100	<4.7	0.99774	91.1 - 104.8
Uniconazole 2	1	1- 100	<8.0	0.99667	89.5 - 105.5

Conclusion

Using a modified QuEChERS approach on difficult matrices allows for many compounds to be included in multiresidue pesticide screens that would have otherwise been excluded due to matrix suppression or false negative results. This modified QuEChERS – Filter Vial method saves time, reduces solvent waste and cost over the traditional approach, QuEChERS – SPE. This validated method for the compounds in Table 2 has good linearity and recovery without having to use more expensive time consuming clean-up techniques. This approach is an extremely cost effective way to ensure problem analytes on difficult matrices can be included in a screen. The Thomson Standard Filter vials save time and money when replacing SPE and traditional syringe filtration techniques.

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

eXtremeIFV® for sample prep prior to the analysis of cannabinoids by HPLC-UV

Introduction

Analysis of cannabinoids in marijuana flower, hemp and finished goods is becoming increasingly important as many states are legalizing it for medicinal and recreational purposes. Dosing methods include smoking/vaporizing and edibles but cannabis is still a Schedule 1 illegal drug and therefore have no FDA testing guidelines. This study evaluates streamlining the sample preparation aspect for HPLC-UV analysis of a panel of cannbinoids. The following analytes were used:

Cannabinol (CBN)
Cannabidivarinic acid (CBDVA)
Cannabinolic acid (CBNA)
Cannabidivarin (CBDV)
Δ9-Tetrahydrocannabinol (Δ9-THC)
Cannabidiolic acid (CBDA)
Δ8-Tetrahydrocannabinol (Δ8-THC)
Cannabigerolic acid (CBGA)
Cannabicyclol (CBL)
Cannabigerol (CBG)
Cannabichromene (CBC)
Cannabidiol (CBD)
Tetrahydrocannabinolic acid A (THCA-A)
Tetrahydrocannabivarin (THCV)
Cannabichromenic acid (CBCA)
Tetrahydrocannabivarinic acid (THCVA)

Equipment

HPLC: Shimadzu Prominance
UV/VIS: 228nm

Column: Raptor ARC, 150 mm x 4.6 mm ID
Column Temperature: 30 °C
Flow Rate: 1.5mL/min

Mobile Phase:

A: 25%: Water, 5 mM ammonium formate, 0.1% Formic Acid
B: 75%: Acetonitrile, 0.1% Formic Acid

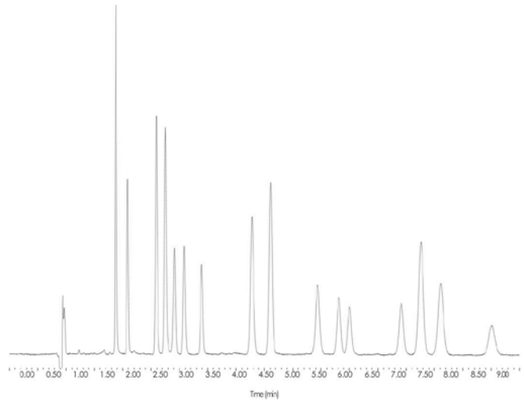
Sample Preparation

- Place 200µL of sample into the outer shell of the eXtremeIFV®, 0.2µm PVDF
- Add 200µL of 25:75 (Water:Methanol)
- Partially depress the plunger
- Vortex the sample
- Depress the plunger completely

Results

16 cannabinoids are baseline resolved using the eXtremeIFV® for sample prep and a isocratic HPLC method.

Peaks #	Analyte	Time (min)
1	Cannabidivarinic acid (CBDVA)	1.877
2	Cannabidivarin (CBDV)	2.86
3	Cannabidiolic acid (CBDA)	2.592
4	Cannabigerolic acid (CBGA)	2.75
5	Cannabigerol (CBG)	2.912
6	Cannabidiol (CBD)	3.48
7	Tetrahydrocannabivarin (THCV)	3.391
8	Tetrahydrocannabivarinic acid (THCVA)	4.279
9	Cannabinol (CBN)	4.609
10	Cannabinolic acid (CBNA)	5.437
11	Δ9-Tetrahydrocannabinol (Δ9-THC)	5.815
12	Δ8-Tetrahydrocannabinol (Δ8-THC)	6.2
13	Cannabicyclol (CBL)	6.916
14	Cannabichromene (CBC)	7.263
15	Tetrahydrocannabinolic acid A (THCA-A)	7.612
16	Cannabichromenic acid (CBCA)	8.51



Conclusion

The HPLC method fully resolves 16 major and minor cannabinoids. Simple quick sample prep using the eXtremeIFV® allows for the baseline separation of the analytes ensuring positive identification and accurate quantitation of the cannabinoids. With <10 seconds per sample and a fast 9-minute analysis, all compounds were resolved making this method suitable for high-throughput cannabis testing labs.

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

THC analysis in candy using the eXtremeIFV® for sample prep

Introduction

What are the challenges faced by analytical labs working with edibles? Measuring the chemical contents and accuately labelling edible products has been a challenge to the cannabis industry. A recent study published by the Journal of the American Medical Society (JAMA) regarding cannabinoid mislabeling in edible medical cannabis products, Dr. Ryan Vandrey of Johns Hopkins School of Medicine looked at 75 products from 47 separate brands purchased at medical dispensaries. Items included baked goods, beverages, and chocolate/candy. Their criteria for selection included those items with a specifically-stated cannabinoid content level. The results, indicated only 17% of edibles tested were “accurately” labeled. The results indicated a +/- 10% range of the stated THC content for beverages and baked goods while baked goods where off by +/- 25%. This could lead to over and under usage which could represent a safety concern. We looked at streamlining the sample prep and analysis of THC in candy.

Equipment

HPLC: Shimadzu Prominence
UV/VIS: 228nm
Column: Raptor ARC-18, 150 mm x 4.6 mm ID
Column Temperature: 30 °C
Flow Rate: 1.0mL/min
Mobile Phase:

A: 25%: Water, 5 mM ammonium formate, 0.1% Formic Acid
B: 75%: Acetonitrile, 0.1% Formic Acid

Sample Preparation

A. Chocolate

1. 2 g of cold chocolate was weighed into a 50 mL centrifuge tube.
2. Bring up to a total volume of 40 mL with cold IPA.
3. Sonicate at 40 °C for 5 minutes followed by gentle mixing by hand.
4. Allow the lipids to precipitate. If necessary, store in a -20 °C freezer for 30 minutes.
5. Vortex briefly.
6. Centrifuge at 3000 rpm for 5 minutes.
7. Transfer the supernatant to a 20mL a graduated cylinder and diluted 10-fold in 25:75 Water:Methanol Vortex briefly.
8. Filter using an eXtremeIFV®, 0.2µm PVDF.

B. Hard Candy

1. 1g of ground hard candy was weighed into a 50mL centrifuge tube.
2. Add 5mL of HPLC grade water.
3. Vortex until the candy is completely dissolved.
4. Bring up to a total volume of 40 mL with cold IPA.
5. Vortex for 30 seconds.
6. Centrifuge at 3000rpm for 5 minutes.Transfer the supernatant to a 20mL a graduated cylinder and diluted 10-fold in 25:75 Water:Methanol Vortex briefly.
7. Filter using an eXtremeIFV®, 0.2µm PVDF.

Results

Nicely resolved THC peak allows for the simple quantification of THC in chocolate, Fig 1 and hard candy, Fig 2.

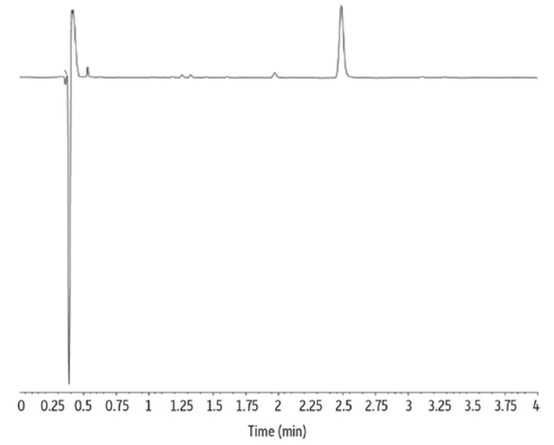


Fig 1. THC peak in chocolate, 2.49 minutes Δ9-Tetrahydrocannabinol (Δ9-THC)

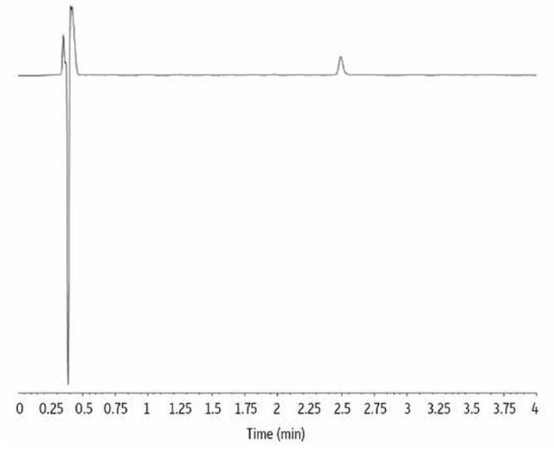


Fig 2. THC peak in hard candy, 2.49 minutes Δ9-Tetrahydrocannabinol (Δ9-THC)

Conclusion

Accurate THC analysis is possible using a streamlined approach to sample prep. Sample prep and analysis for the chocolate utilizing cold organic solvent for complete crash of the lipids and final clean-up using the eXtremeIFV® is < 1 hour per sample. Sample prep and analysis for the hard crushed candy and final clean-up using the eXtremeIFV® is < 20 minutes per sample.