THOMSON Solutions ™ At Work
INSTRUMENT COMPANY

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
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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.

SPE #1

Prepare Sample

1. Add 100 µL oral fluid specimen
2. Add 20 µL internal standard and let sit 10 min
3. Add 300 µL acetic acid
4. Vortex
5. Adjust pH to 4.0 +/- 0.5

Condition column

6. 500 µL Methanol
7. 500 µL DI H_2O
8. Apply sample to SPE #1 column

Wash

9. 500 µL 2% formic acid
10. Dry thoroughly for 5 min
11. Elution:
12. 500 µL methanol:acetonitrile (5% acetic acid)
13. 500 µL methanol:acetonitrile (5% NH_3)
14. Collect eluate at 1-2 mL/min
15. Dry completely at 35°C and reconstitute in 100 µL mobile phase

SPE #2

Prepare Sample
1. Apply 100µL oral fluid specimen
2. Dry thoroughly for 1 min

Wash
3. 1 mL Di H2O
4. 1 mL 1% HCl Solution
5. Dry thoroughly for 5 min

Elution
6. 2 mL Methanol/Ammonium Hydroxide (98:2)
7. Collect eluate at 1-2 mL/min
8. Dry completely at 35°C and reconstitute in 100 µL mobile phase

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 eXtreme Filter Vial. 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®.

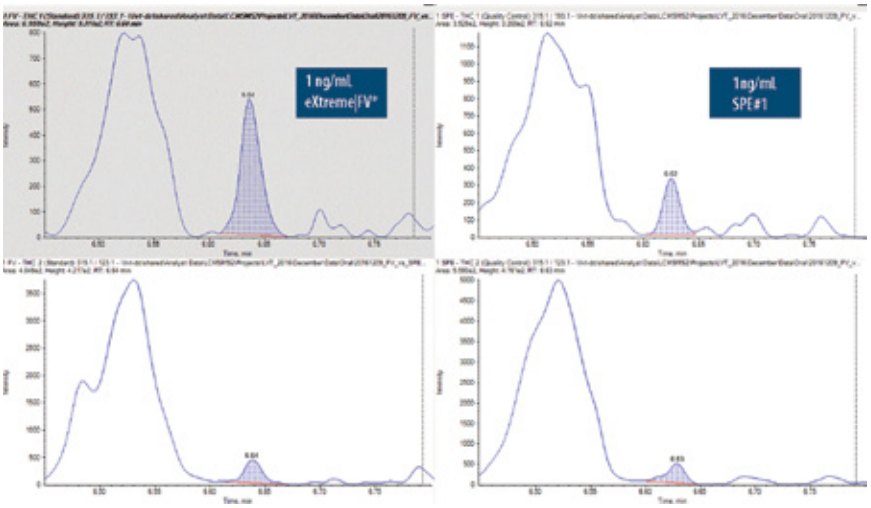

Fig 1. Limit of Detection Study – SPE #1 & eXtreme Filter Vial

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

Step 1

Different Source Temperatures, 450°C - 600°C were evaluated to identify the best temperature for ionization of THC. 550°C provided the best ionization yielding optimum separation and peak height necessary for routine analysis, fig 2.

Step 2

Two analytical columns were tested for best sparation, Biphenyl and C18. The Biphenyl Column is beneficial for increasing retention of early eluters like opiods and to increase the retention of hydrophilic aromatics. The C18 Column is beneficial for the retention of hydrophobic compounds. As you can see in fig 2, The C18 Column gave the best THC peak height and area. But, the next task is to see what the Opiods look like on a C18 Column. figs. 3 & 4 show the decreased signal with the C18 Column as compared to the Biphenyl Column.

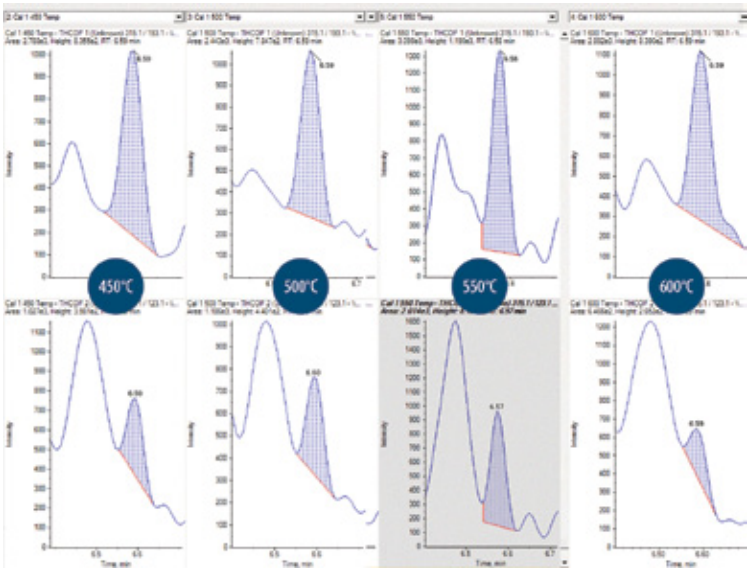

Fig 2. Source Temperature evaluation for THC

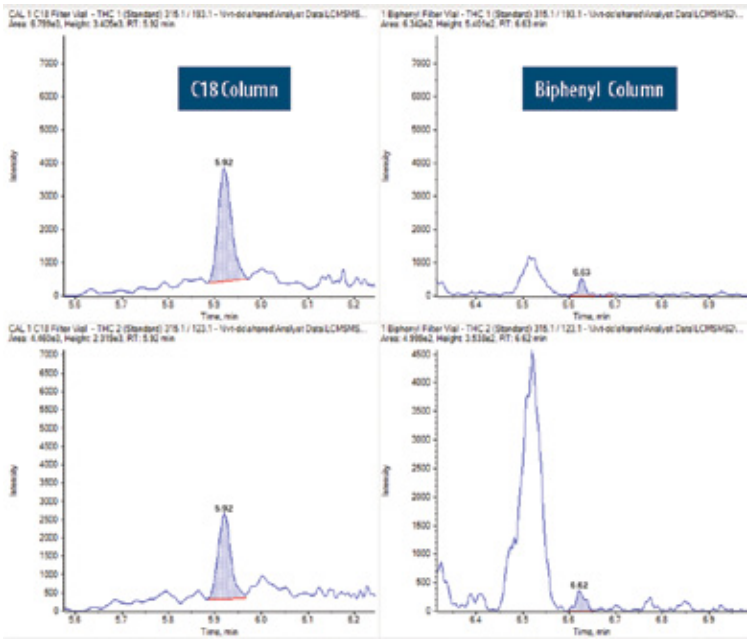

Fig 3. C18 and Biphenyl Column Results for THC.

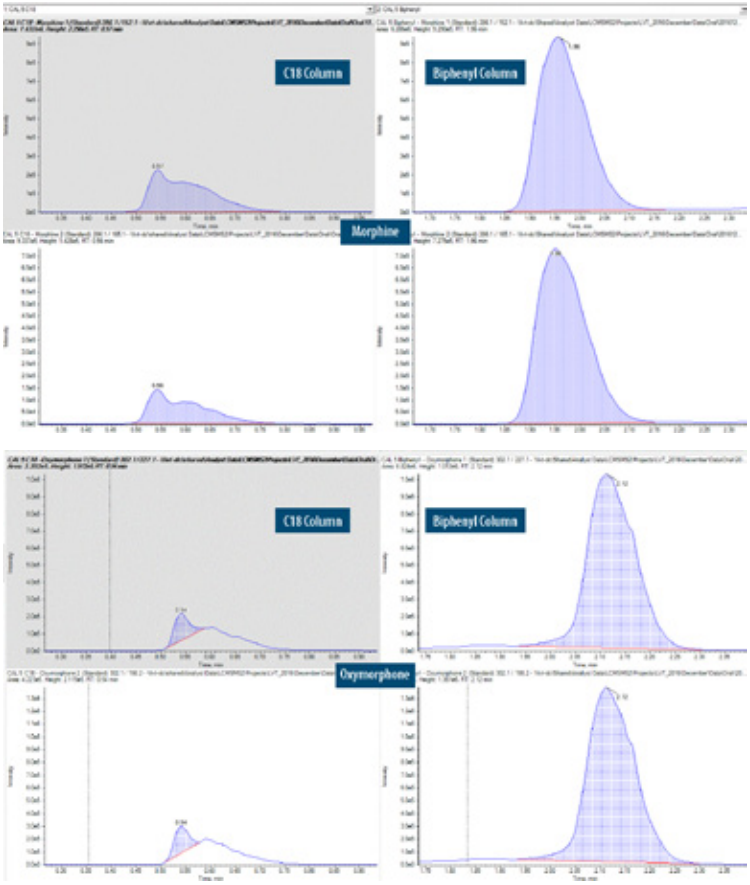

Fig 4. Column comparison for Morphine and Oxymorphone

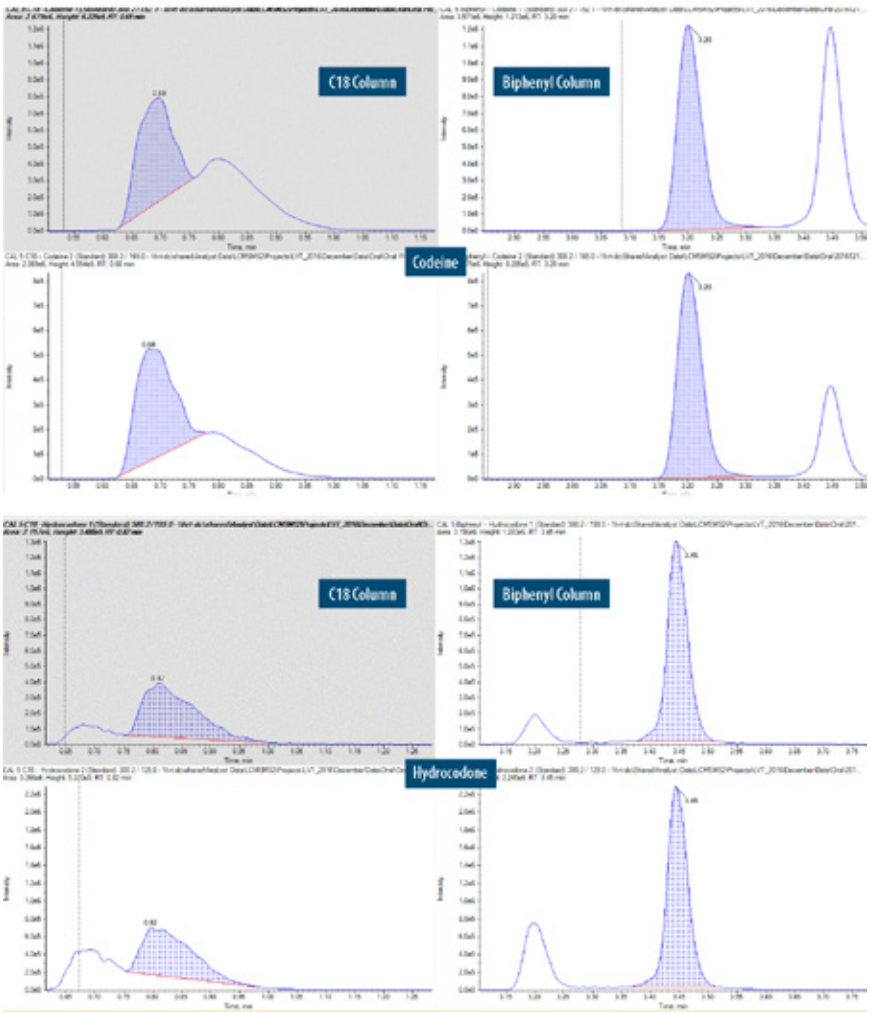

Fig 5. Column comparison of Codeine and Hydracodone

Step 3

Time to optimize the gradient to see if we can improve the results seen with the C18 Column. The organic concentration was increased earlier to improve the Opiods retention on the C18 Column, see fig. 6

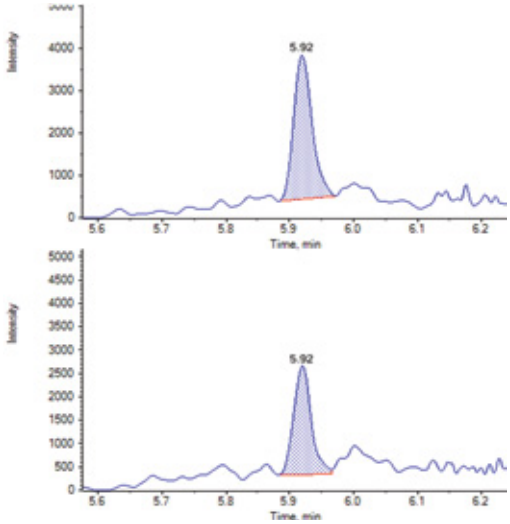

Fig 6. Early gradient improves opioid retention on the C18 column

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

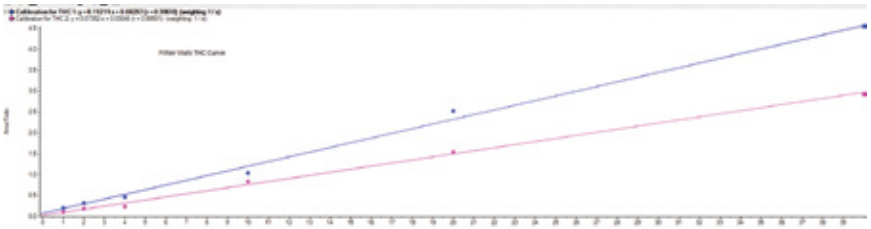

Fig 7. Calibration Curve using the new parameters yields an $r^2 = 0.99$

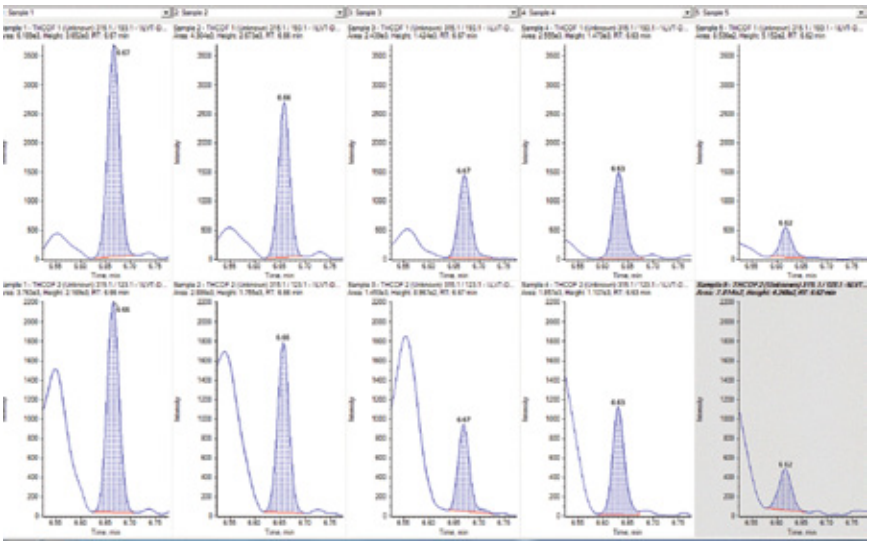

Fig 8. Examples of authentic Oral Fluid sample collected with the OraSure Technologies i2he™ Collection Device

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 eXtreme|FV®, 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 eXtreme|FV® 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