THOMSON
Solutions At Work™

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

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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 adulterate. 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®.

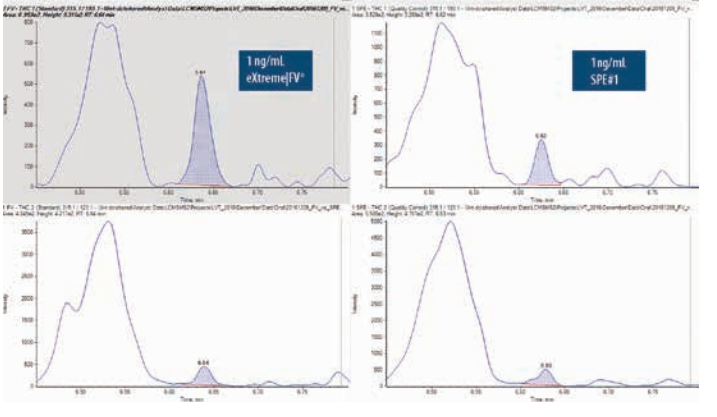

Fig 1. Limit of Detection Study – SPE #1 & eXtreme|FV®

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

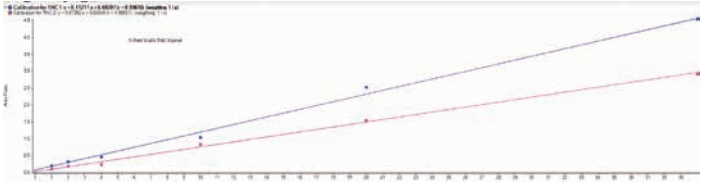

Fig 7. Calibration Curve using the new parameters yields an $r^2 = 0.99$

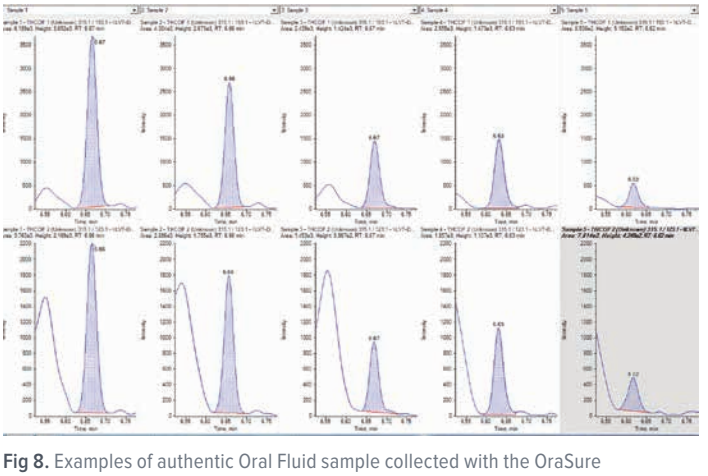

Fig 8. Examples of authentic Oral Fluid sample collected with the OraSure Technologies i2he™ Collection Device

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 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