

Detection of THC in oral fluid: the bane of a **toxicologist's existence**

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January 2017



Lehigh Valley Toxicology

TIC-PL-082-271

Disclosure/Disclaimer

The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the author.



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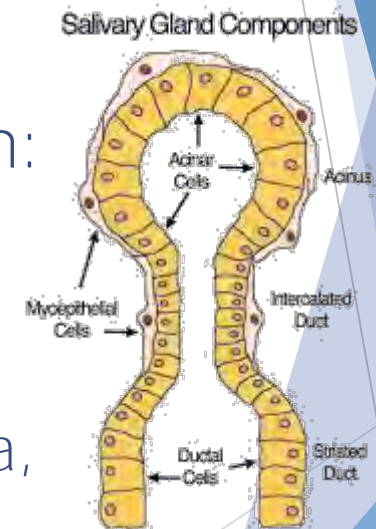
Specimen Type

- ▶ Samples are collected in a clinical setting to ensure compliance or for drug monitoring
- ▶ Sample Types:
 - ▶ Urine
 - ▶ Difficult in patients with medical conditions, easily adulterated, typically large measurable volume, long detection window
 - ▶ Oral Fluid
 - ▶ Ease of collection, observed collection, assumed sample volume (diluent), smaller detection window



Oral Fluid

- ▶ Drugs move into saliva via simple diffusion across cell membranes
- ▶ Saliva is composed mostly of water in addition to mucin, amylase and other proteins and enzymes
- ▶ Factors affecting analyte detection:
 - ▶ Pharmacokinetics
 - ▶ Oral fluid pH (~5.6-8)
 - ▶ Analyte properties (lipophilicity, pKa, protein binding)



THC in Oral Fluid

- ▶ Excreted in only small amounts into saliva because
 - ▶ Low ingestion concentrations
 - ▶ Weakly acidic nature (pKa 9.5)
 - ▶ Highly plasma protein bound (97-99%)
- ▶ Low saliva:plasma ratio of ~0.01
- ▶ Target limit of detection: 3 ng/mL



Analysis of THC in Oral Fluid

- ▶ Sample Preparation

 - ▶ Extraction:

 - ▶ Solid Phase Extraction, Supported Liquid Extraction, Liquid-Liquid Extraction

 - ▶ Filter Vials

 - ▶ Process to dilute and filter urine and oral fluid samples

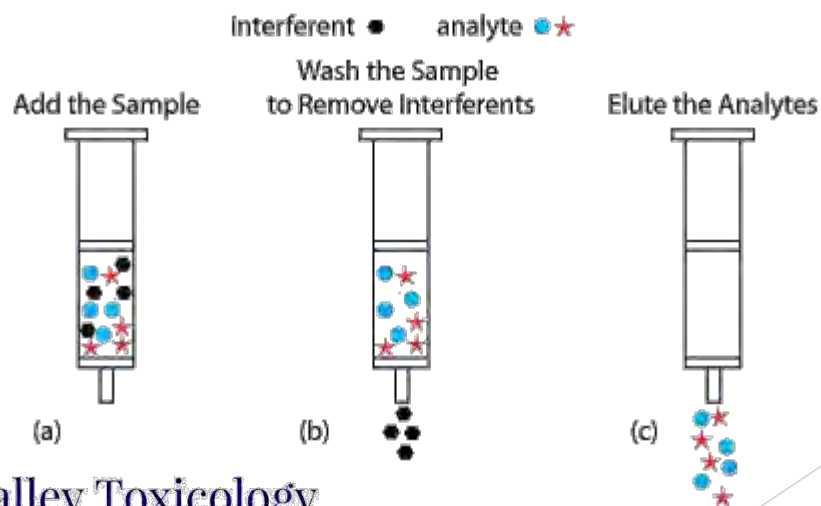
- ▶ Analytical Detection

 - ▶ LC-MS/MS



Solid Phase Extraction

- ▶ Bind and Elute Technology
 - ▶ Column is conditioned
 - ▶ Sample binds to sorbent
 - ▶ Wash unwanted constituents and interferents
 - ▶ Elute analyte using cation exchange



Solid Phase Extraction #1

- ▶ Prepare Sample:
 - ▶ Add 100 uL oral fluid specimen
 - ▶ Add 20 uL internal standard and let sit 10 min
 - ▶ Add 300 uL acetic acid
 - ▶ Vortex
 - ▶ Adjust pH to 4.0 +/- 0.5

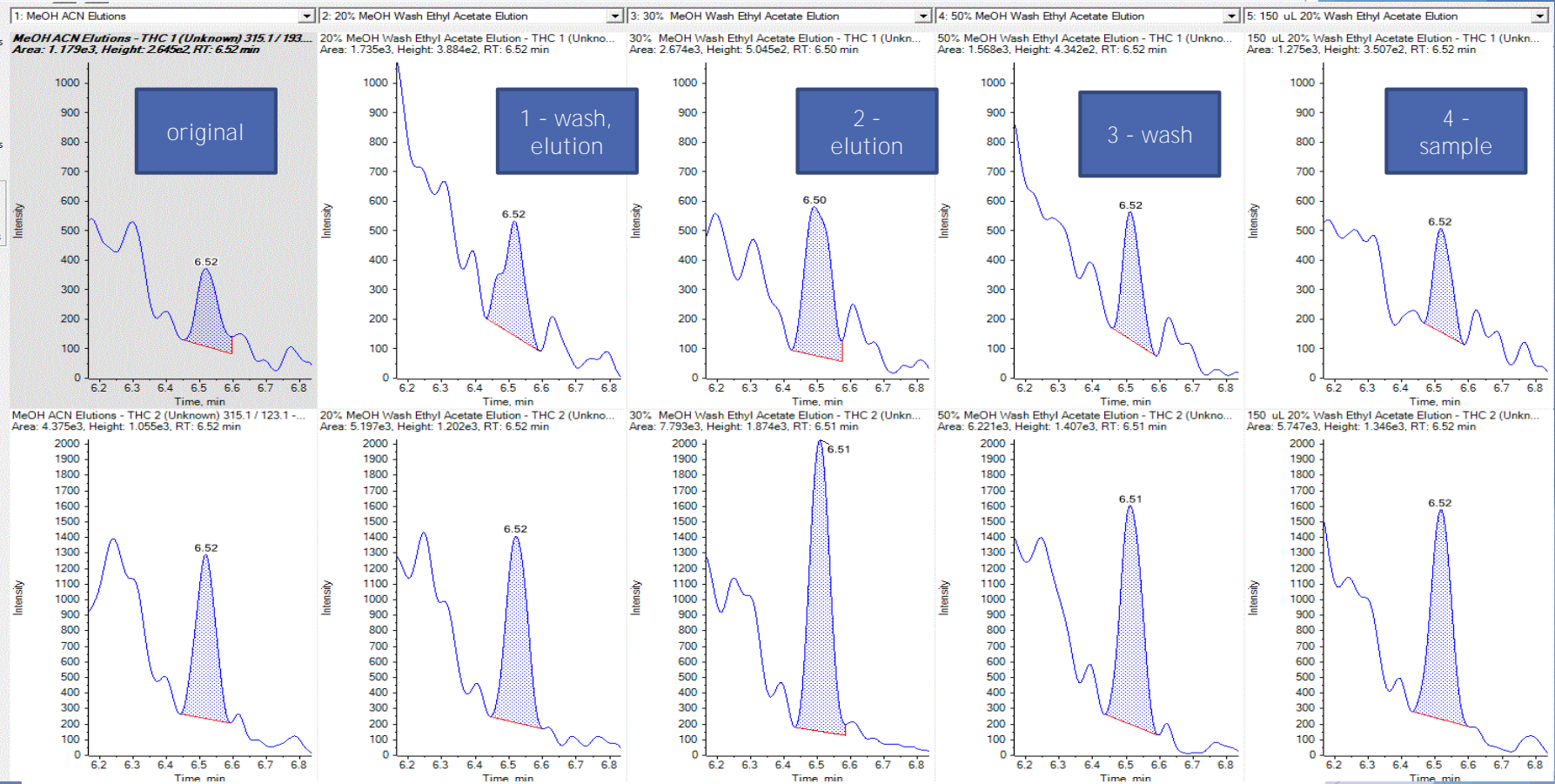


Solid Phase Extraction #1

- ▶ Condition column:
 - ▶ 500 uL Methanol
 - ▶ 500 uL DI H₂O
- ▶ Apply sample to Agilent Plexa PCX column
- ▶ Wash:
 - ▶ 500 uL 2% formic acid
 - ▶ Dry thoroughly for 5 min
- ▶ Elution:
 - ▶ 500 uL methanol:acetonitrile (5% acetic acid)
 - ▶ 500 uL methanol:acetonitrile (5% NH₃)
 - ▶ Collect eluate at 1-2 mL/min
- ▶ Dry completely at 35°C and reconstitute in 100 uL mobile phase



SPE Method Variations



1- Wash: 500 uL 20% Methanol; Elution: 500 uL 50:50 Methanol:Acetonitrile (5% NH₄OH)

2- Elution: 500 uL 50:50 Methanol:Ethyl Acetate (5% NH₄OH)

3- Wash: 500 uL 50% Methanol

4- Prepare Sample: Add 150 uL oral fluid specimen, Add 450 uL acetic acid



Solid Phase Extraction #2

- ▶ Prepare Sample:
 - ▶ Add 100 uL oral fluid specimen
 - ▶ Add 20 uL internal standard and let sit 10 min
 - ▶ Add 800 uL of 100mM Phosphate buffer (pH 6)
 - ▶ Vortex
 - ▶ Adjust pH to 6.0 +/- 0.5 with 100 mM monobasic or dibasic sodium phosphate



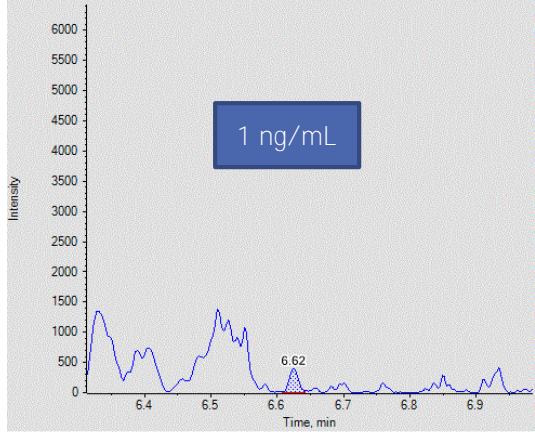
Solid Phase Extraction #2

- ▶ Apply sample to Clean Screen XCEL I column
 - ▶ Dry thoroughly for 1 min
- ▶ Wash:
 - ▶ 1 mL Di H₂O
 - ▶ 1 mL 1% HCl Solution
 - ▶ Dry thoroughly for 5 min
- ▶ Elution:
 - ▶ 2 mL Methanol/Ammonium Hydroxide (98:2)
 - ▶ Collect eluate at 1-2 mL/min
- ▶ Dry completely at 35°C and reconstitute in 100 uL mobile phase

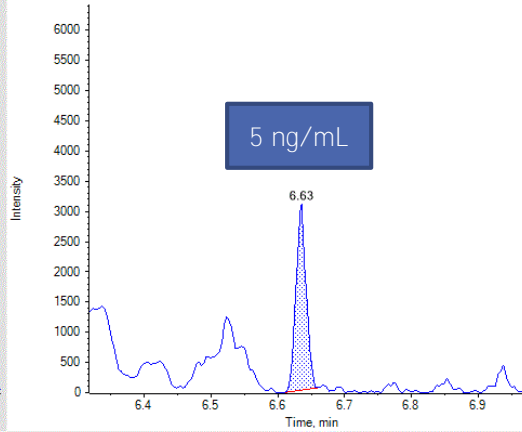


Limit of Detection Study - SPE

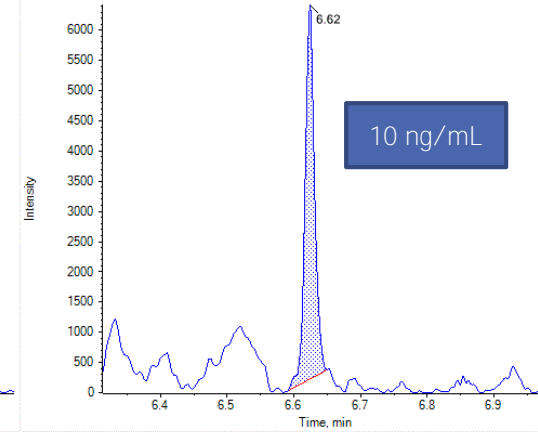
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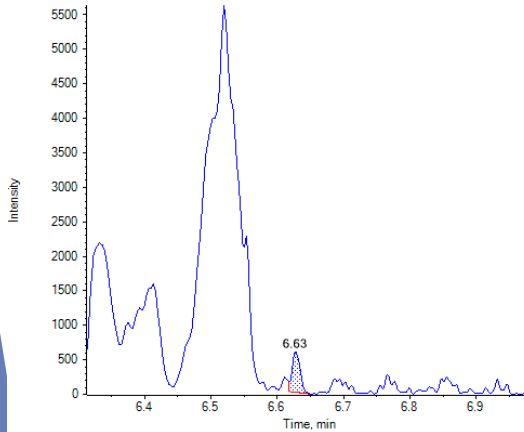
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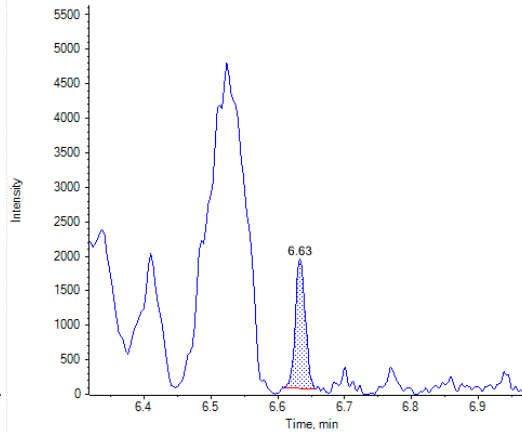
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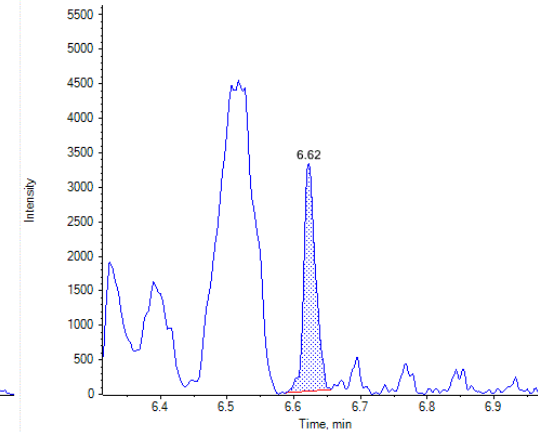
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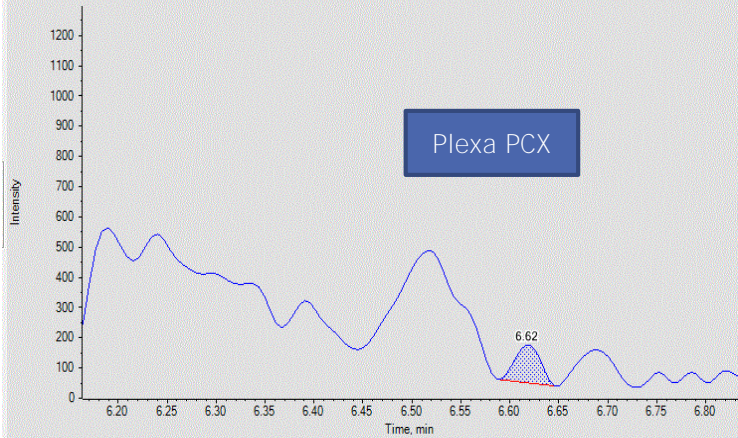


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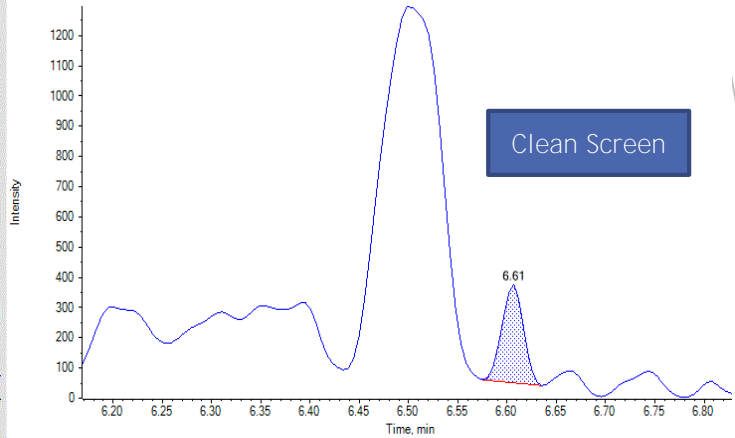


SPE Comparison

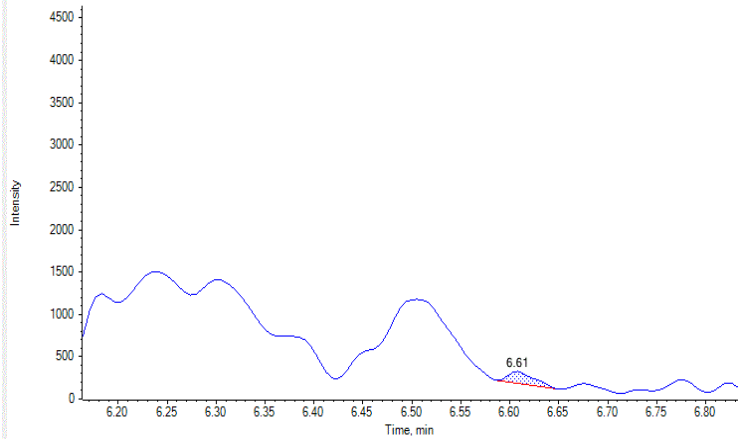
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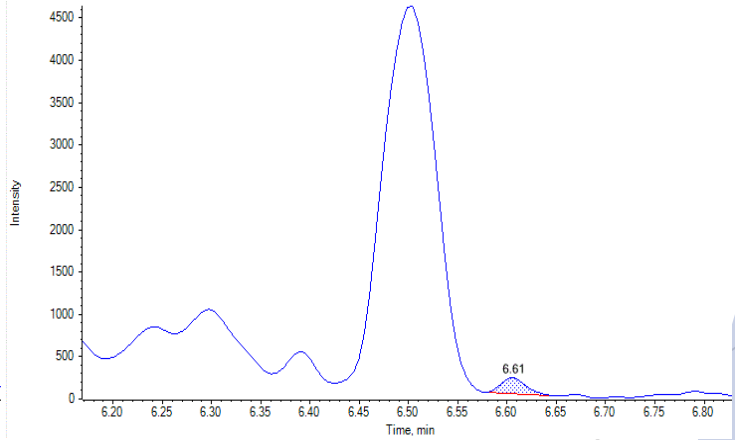
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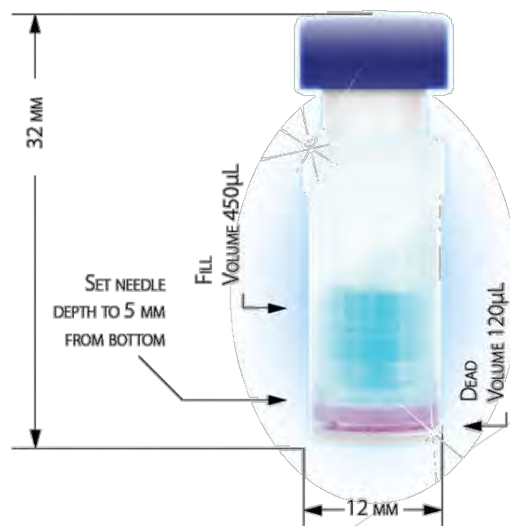


UCT - THCOF 2 (Unknown) 315.1 / 123.1 - \\LVT-DC\Shared\Analyst Data\LCMSMS2\Projects\LVT_2016\June\Data\Oral Fluid SPE\20160629.w...
Area: 3.312e2, Height: 1.910e2, RT: 6.61 min



Thomson Filter Vials

- ▶ Shown to reduce matrix interferences for both urine and oral fluid
- ▶ Demonstrates adequate analyte recovery
- ▶ Simple and efficient method that eliminates solvent waste and other typical extraction consumables



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eXtreme® Filter Vial Method

1.



- ▶ Add 100 uL curve diluent
- ▶ Add 20 uL internal standard
- ▶ Add 100 uL sample

2.



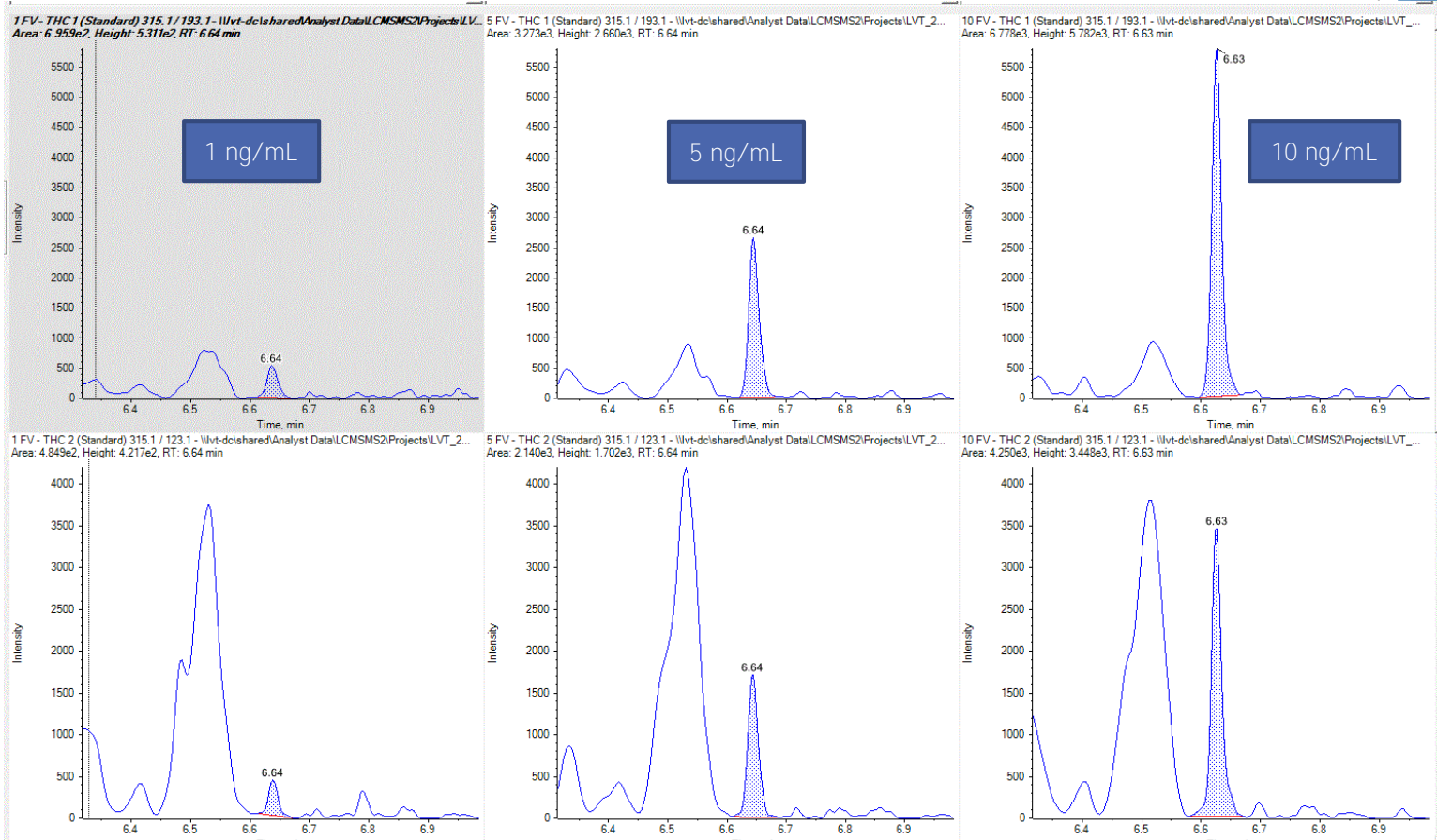
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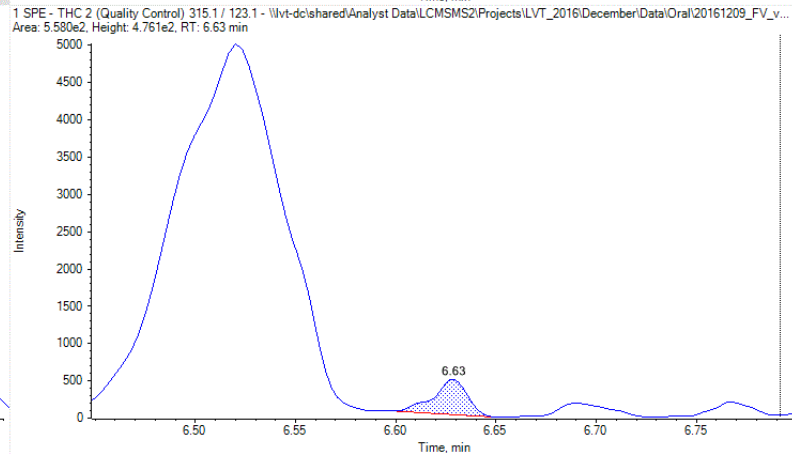
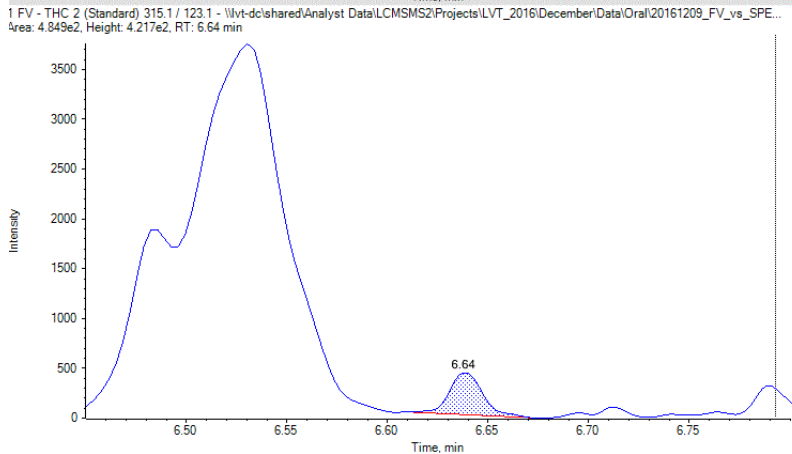
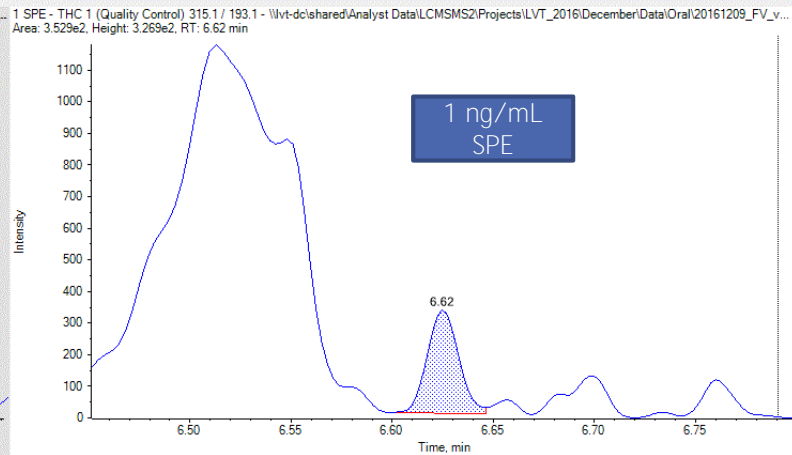
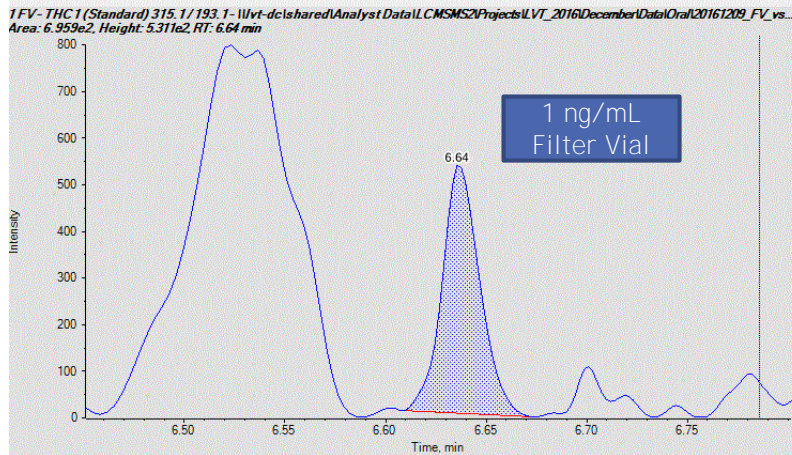
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<http://htslabs.com/downloads/FilterVialFlier.pdf>

Limit of Detection Study - FV



Comparison of Extraction Method



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



Analytical Method

- ▶ MS/MS Parameters:
 - ▶ Source Parameters
 - ▶ Ions, CE, CXP, DP

- ▶ LC Parameters:
 - ▶ Column
 - ▶ Gradient



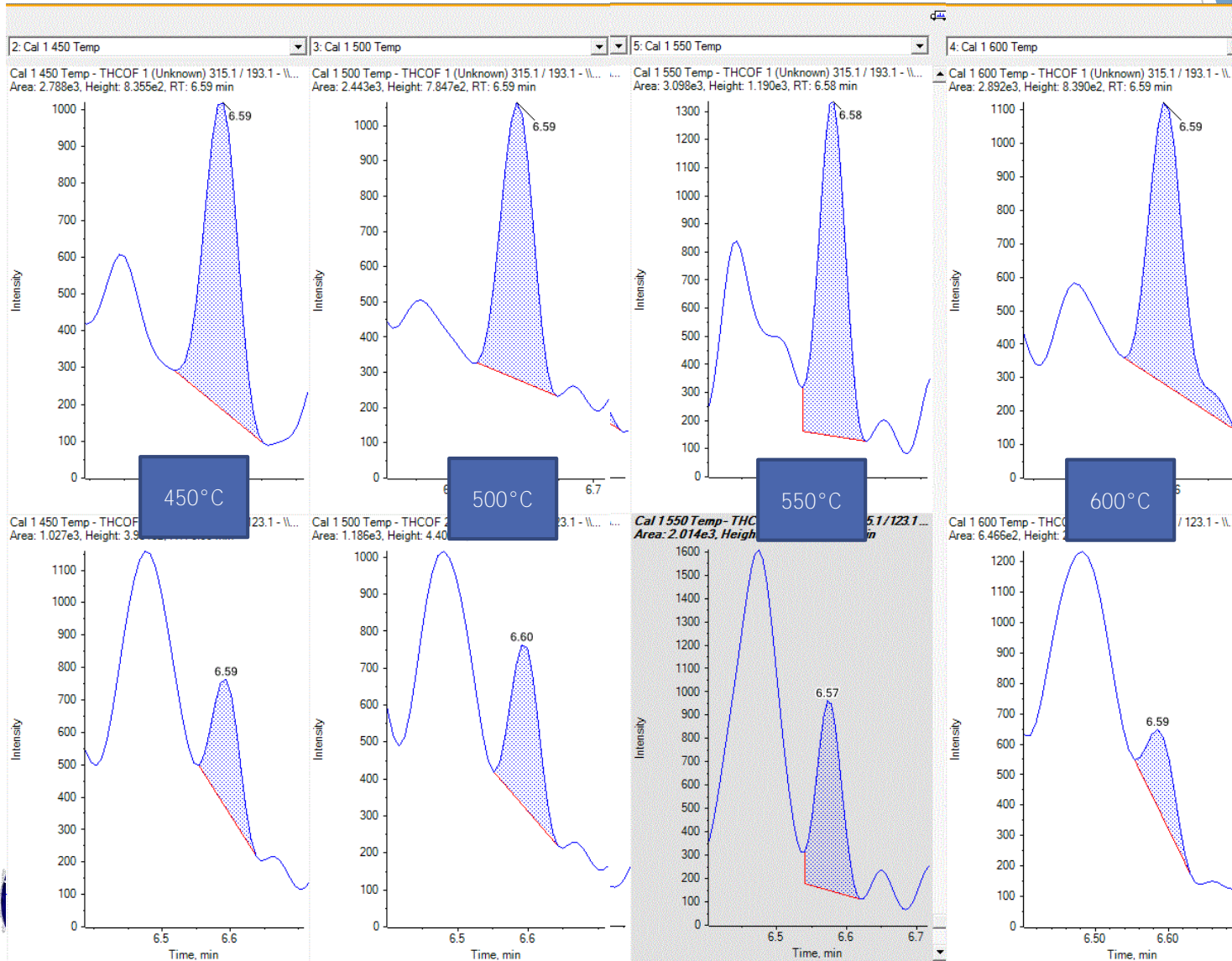
MS/MS

1. Curtain Gas: 40
2. Ion Spray Voltage: 4000
3. Source Temperature: 550°C
4. Ion Source Gas 1: 60
5. Ion Source Gas 2: 50

▶ CAD gas: 9

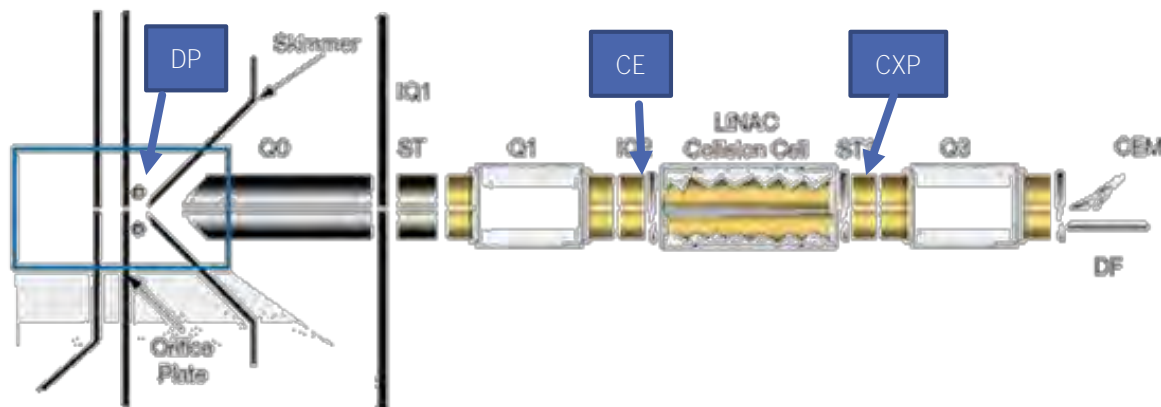


Source Temperature

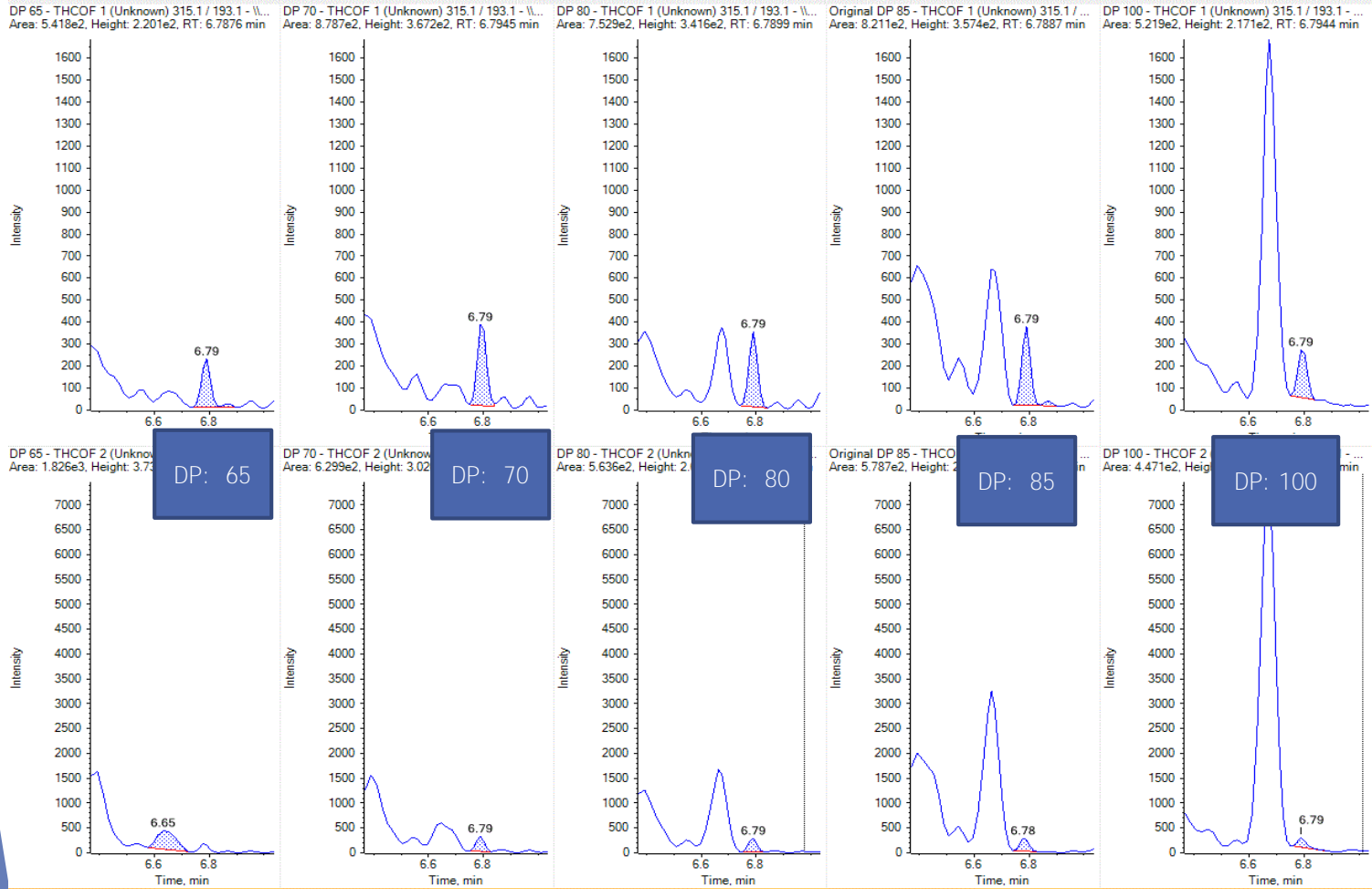


MS/MS

	Quantifier Ion	Qualifier Ion
Q1/Q3	315.1 / 193.1	315.1 / 123.1
DP	70	70
CE	30	41
CXP	6	8



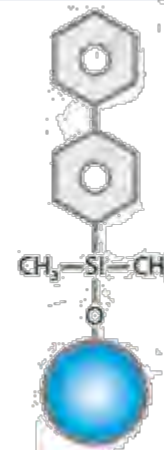
Declustering Potential



Liquid Chromatograph

- ▶ Biphenyl Column

- ▶ Beneficial for increasing retention of early eluters (opioids)



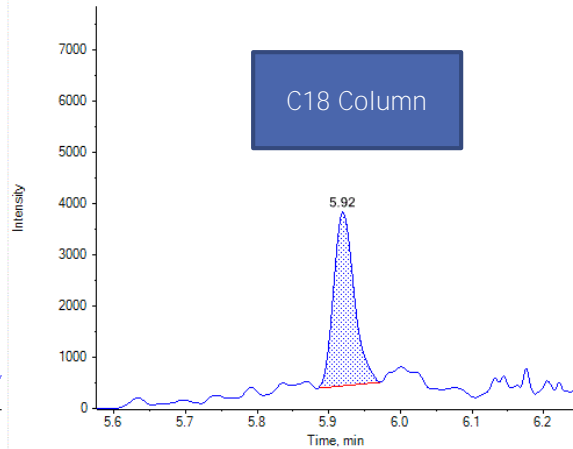
- ▶ C18 Column

- ▶ Beneficial for retention of hydrophobic compounds

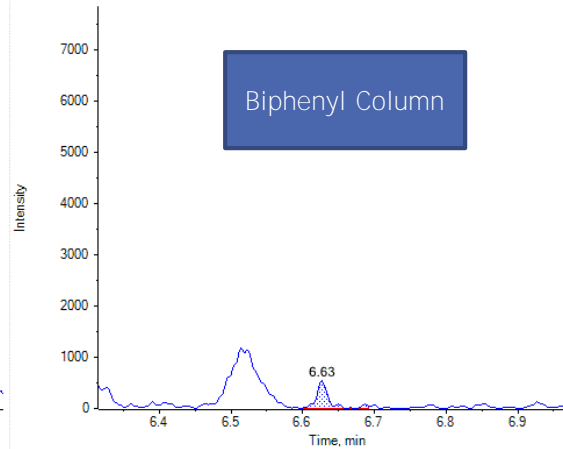


Column

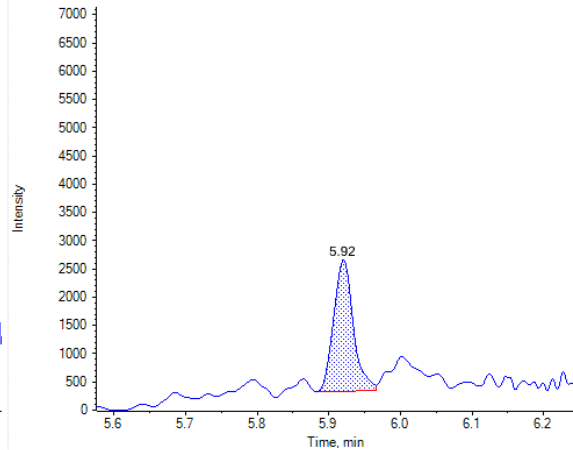
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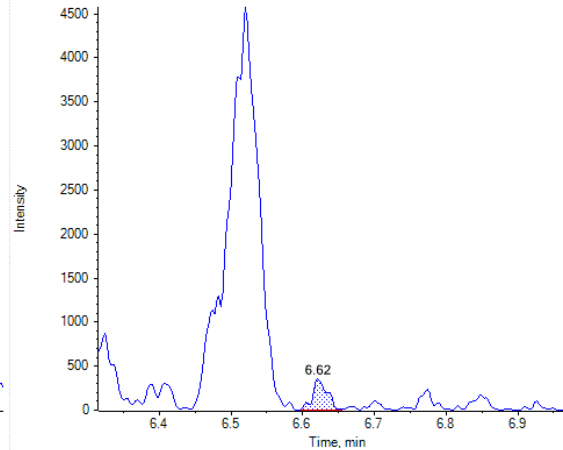
1 Biphenyl Filter Vial - THC 1 (Standard) 315.1 / 193.1 - \\vlt-dc\shared\Analyst Data\LCMSMS2...
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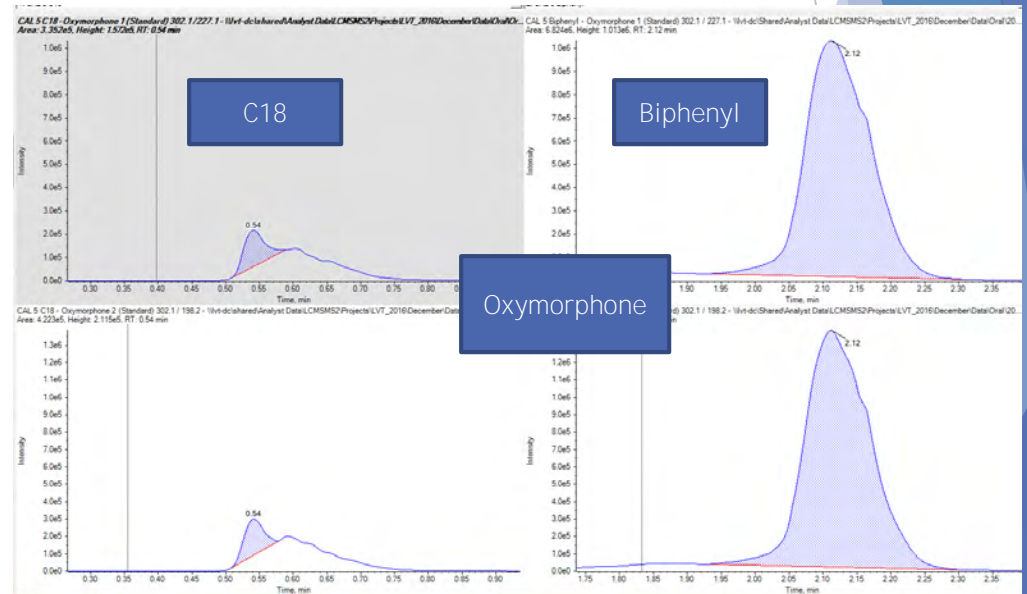
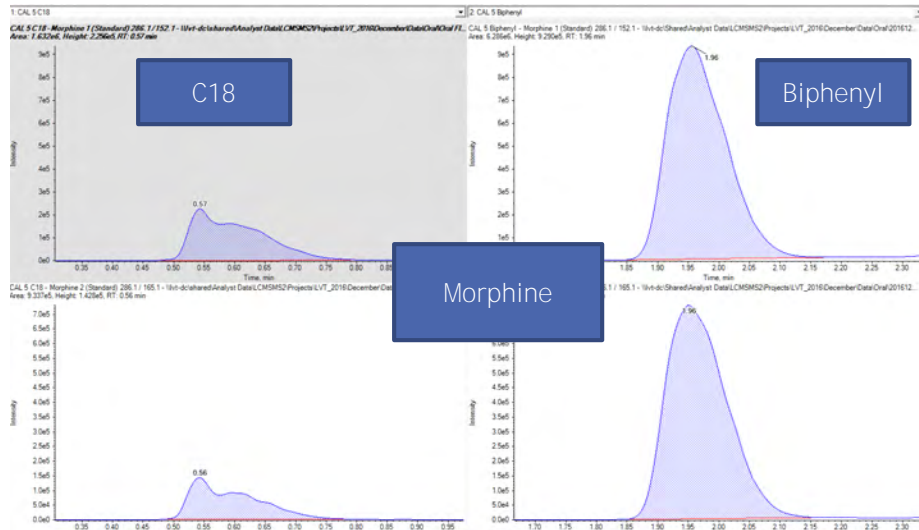
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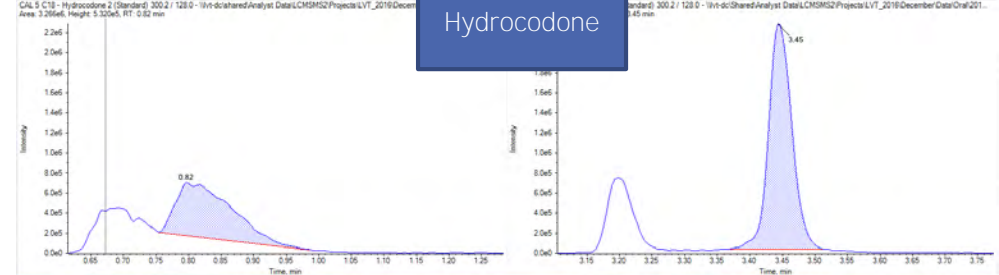
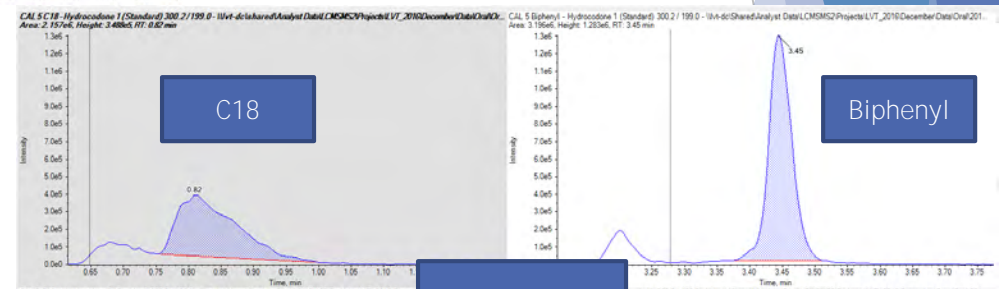
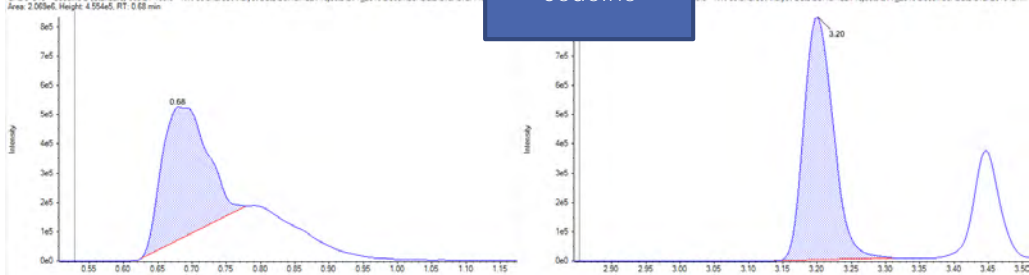
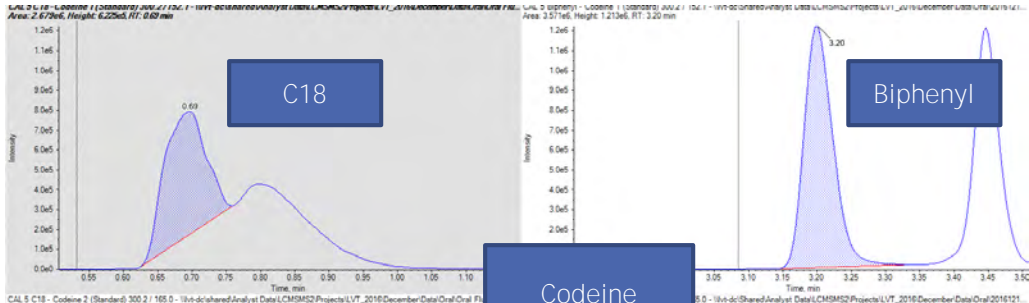
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Area: 4.999e2, Height: 3.538e2, RT: 6.62 min



Opioids on C18 vs. Biphenyl

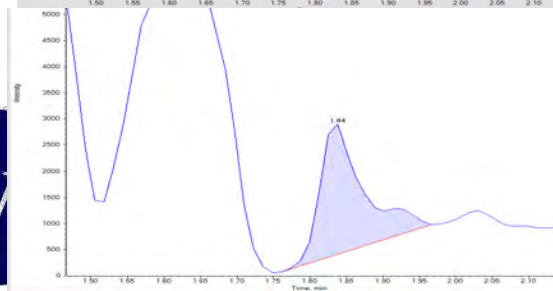
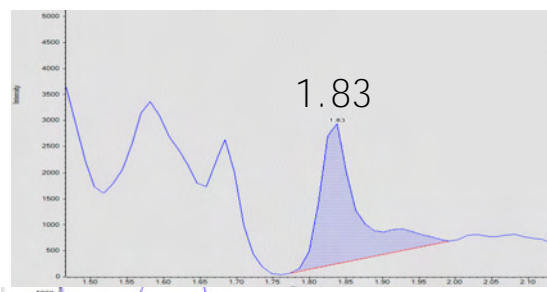
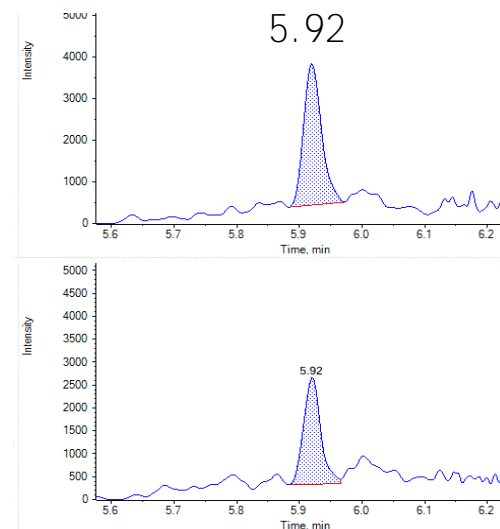


Opioids on C18 vs. Biphenyl



Gradient Alteration

Time (min)	%B
0.5	20
2.6	55
4.9	95
6.5	95
6.7	20
8.0	20



LV

ogy

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

Final Analytical Method

▶ LC Parameters:

- ▶ C18 Column
- ▶ Gradient:

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

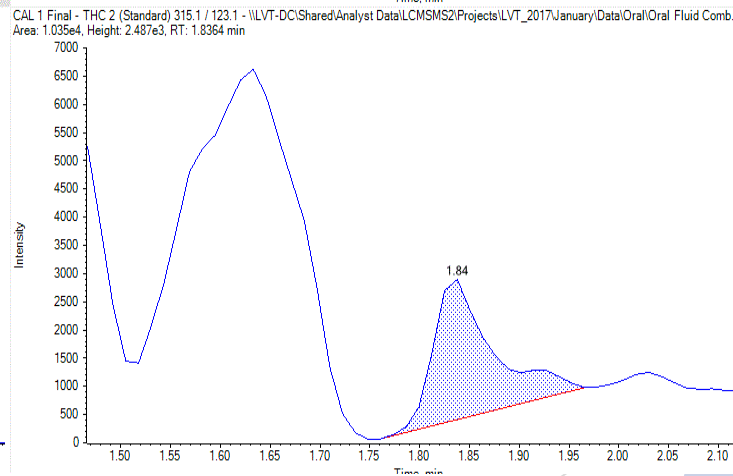
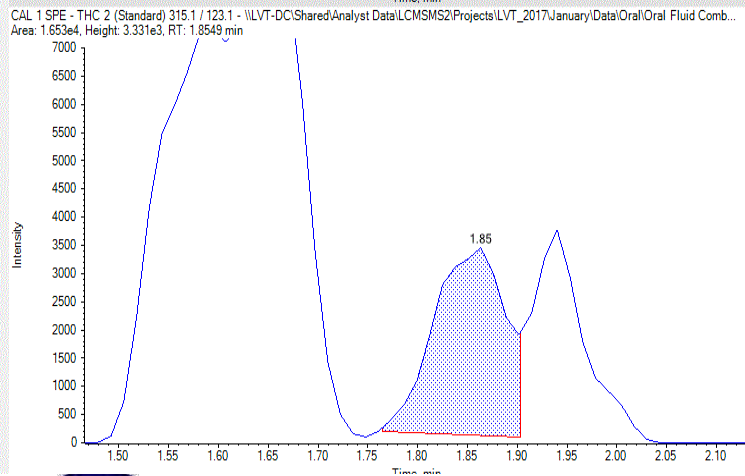
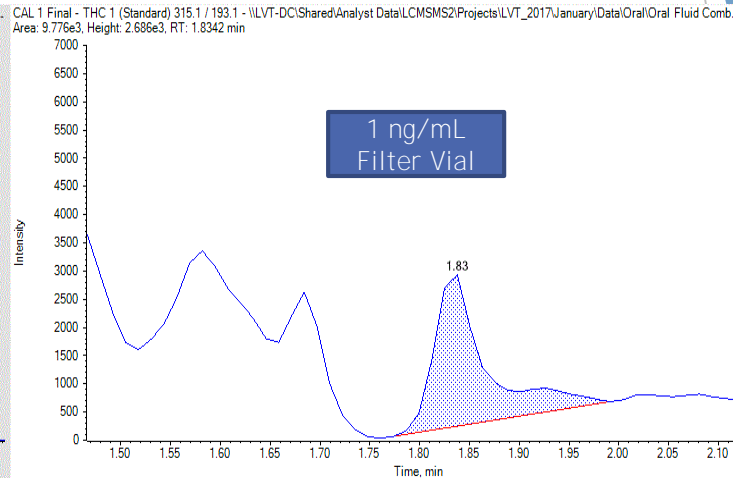
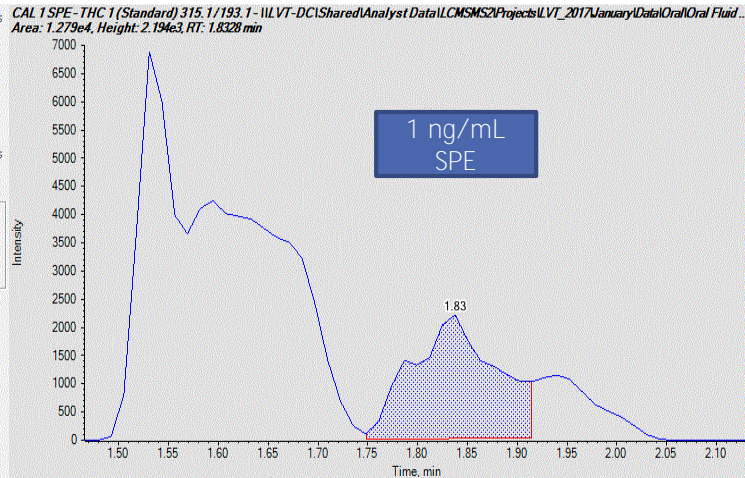
▶ MS Parameters:

	Quant Ion	Qual Ion
Q1/Q3	315.1 / 193.1	315.1 / 123.1
DP	70	70
CE	30	41
CXP	6	8

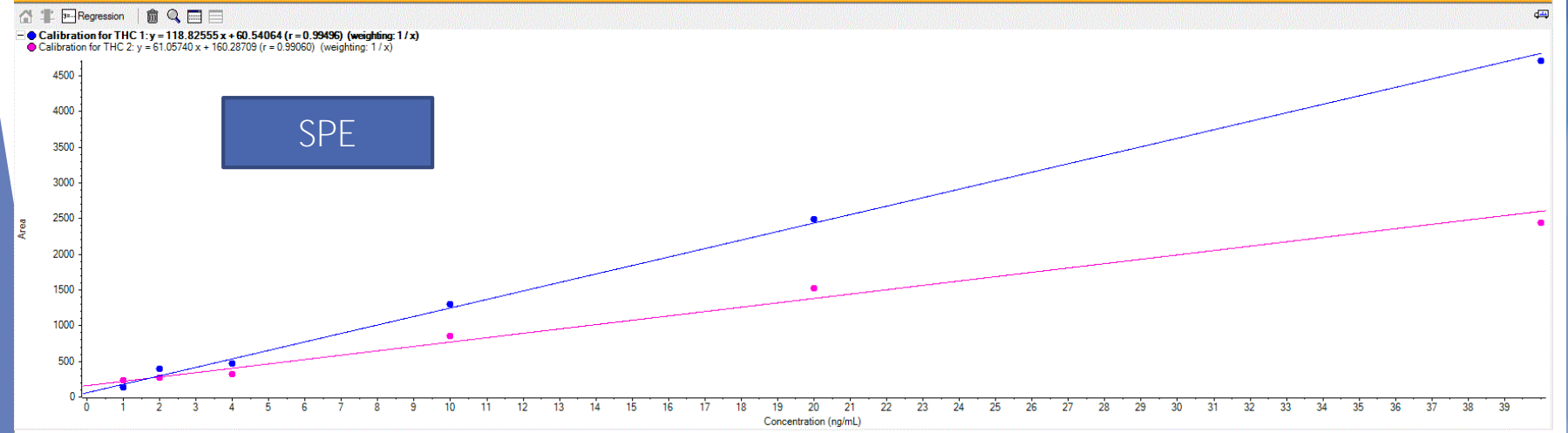
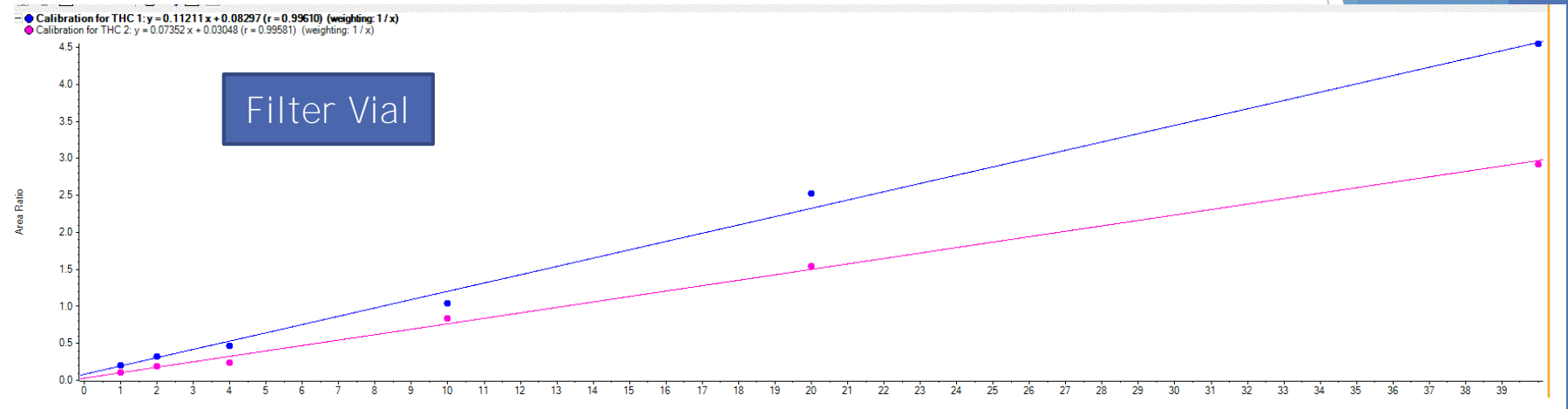
Curtain Gas: 40
Ion Spray Voltage: 4000
Source Temp: 550 °C
Ion Source Gas 1: 60
Ion Source Gas 2: 50



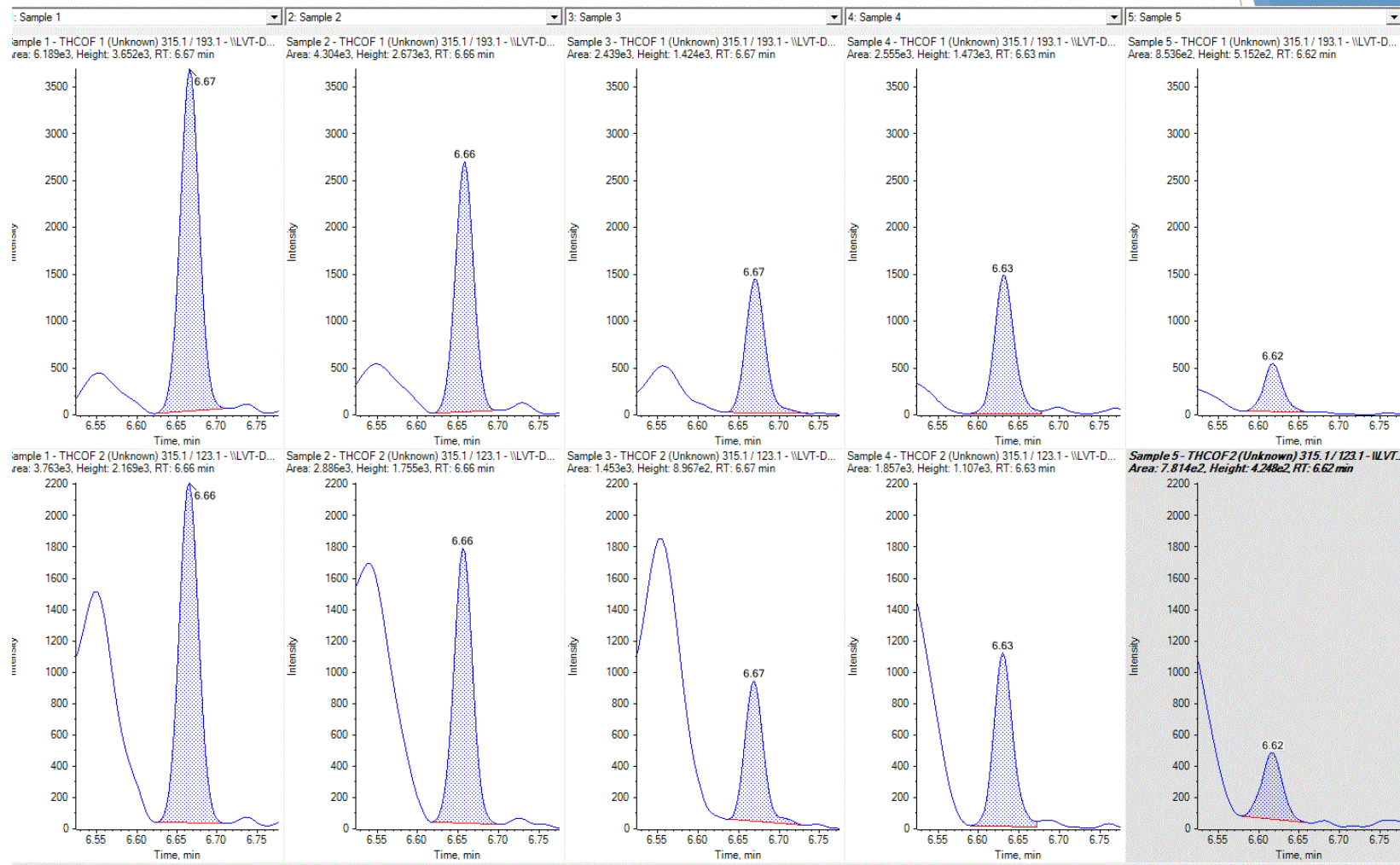
Comparison of Extraction Method - Updated Parameters



Calibration Curve Comparison



Authentic Oral Fluid Samples



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*Oral fluid samples were collected with the OraSure Technologies i2he™ Collection Device

Thank You!

Acknowledgements:

Lisa Wanders, Thomson Instrument Company

Jess Helwig, Lehigh Valley Toxicology

Sarah Muller, Lehigh Valley Toxicology

Any questions please contact:

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