Fast quantitative Forensic Analysis of THC and its Metabolites in Biological Samples using Captiva EMR-Lipid and LC/MSMS

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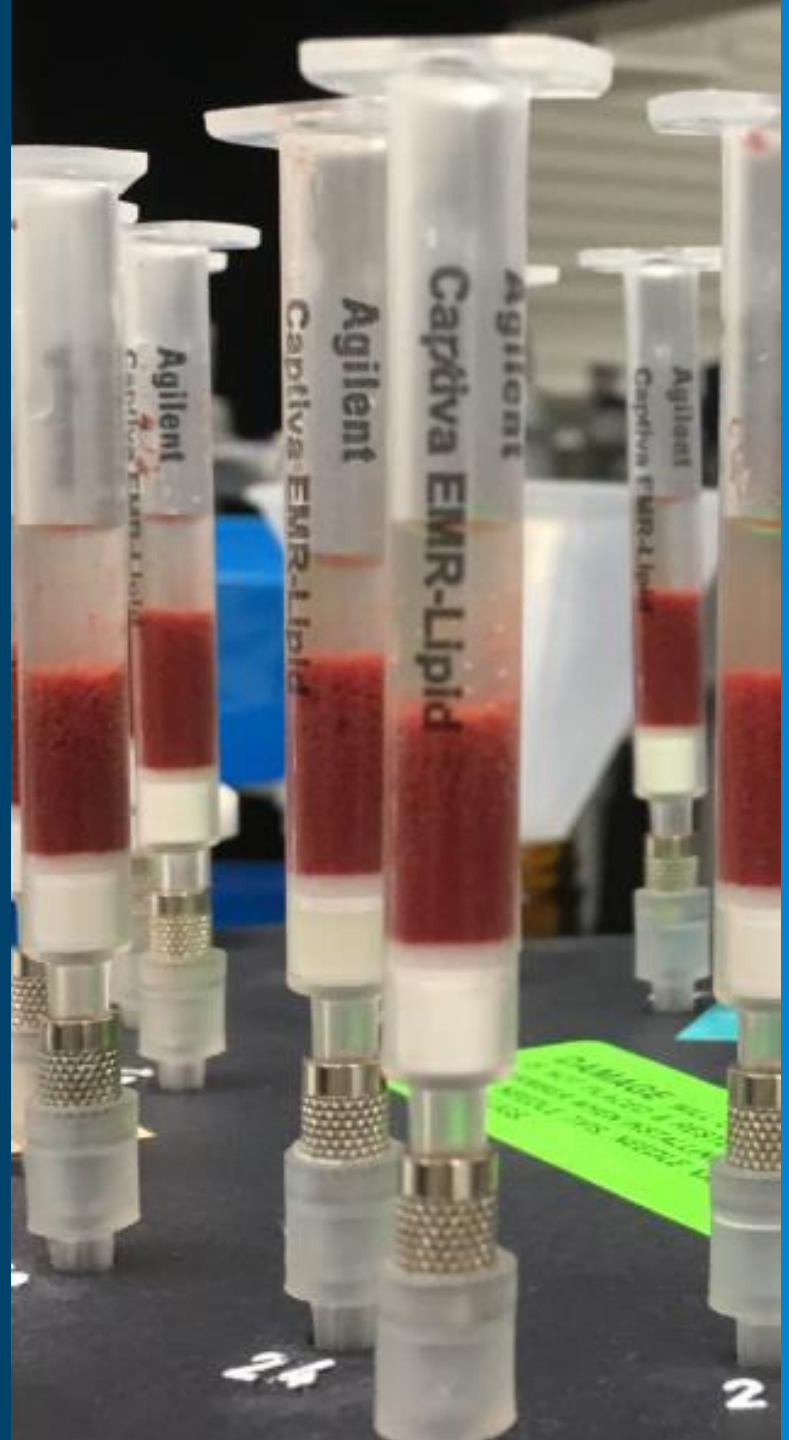
Sample prep Application Scientist

For Forensic Use



Agilent

Trusted Answers

Types of ``Interferences`` in Biological Samples

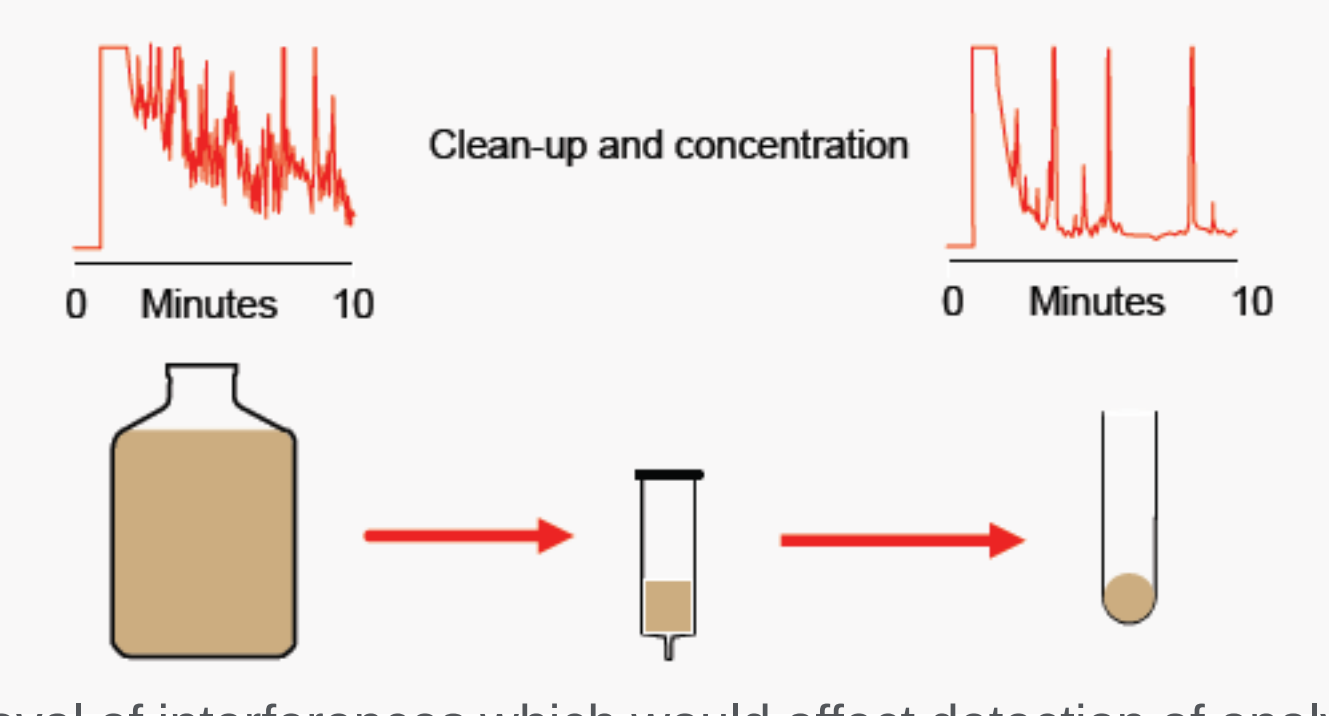
Major causes of matrix effects:

- Salts – generally elute early in the run
- Proteins – most prominent interference
- Lipids, phospholipids, and lysophosphatidylcholines – difficult to remove
- Surfactants, dosing agents, excipients
- Phthalates and plasticizers from plasticware

Effects of Endogenous Interferences

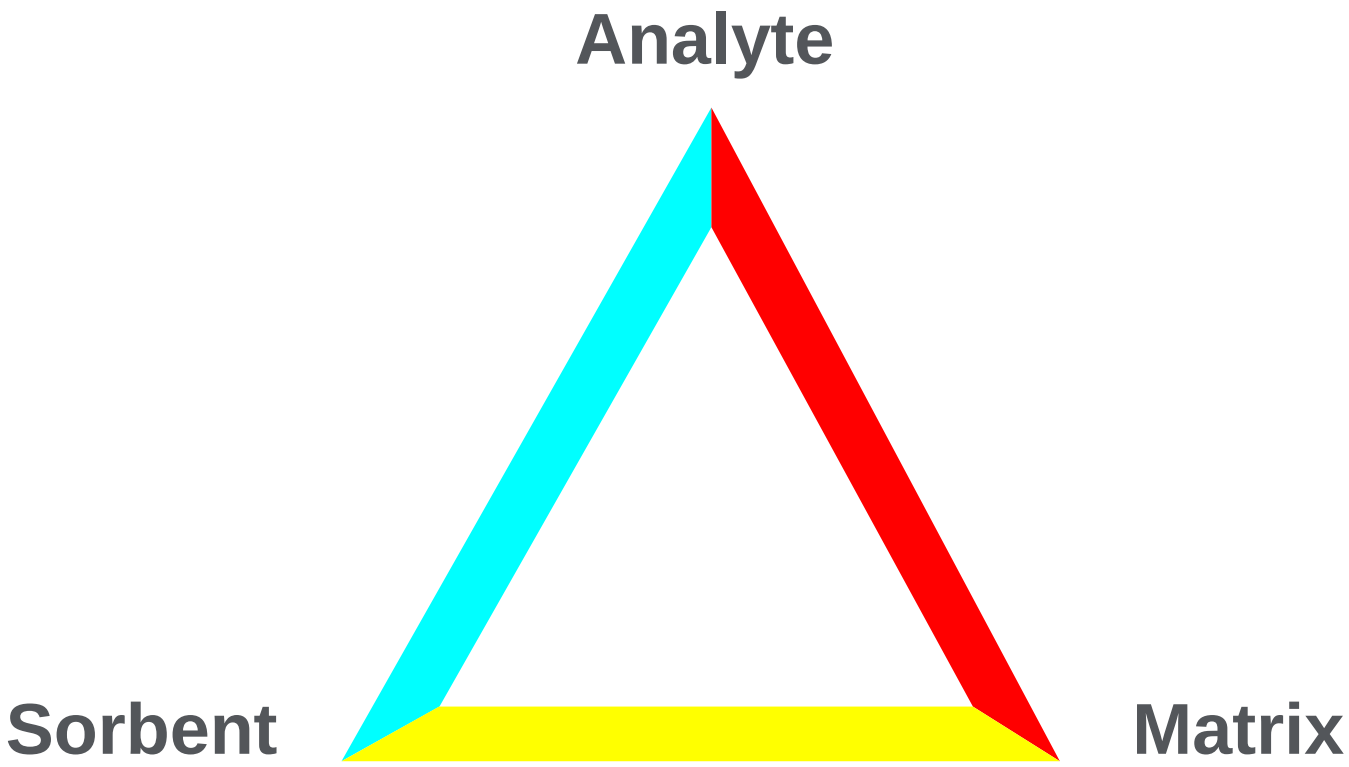
- Poor Chromatography
- Mechanical Issues (particulates, blockages)
- LC Column Lifetime Issues
- Carry Over
- Ion Suppression
- Overall Loss in Analytical Sensitivity
- Increase in Sample Run Time/Cost

Objectives of Sample Preparation prior to LC or GC



- Removal of interferences which would affect detection of analyte
- Removal of interferences that would affect instrument or column lifetime
- Concentration of an analyte to a detectable concentration
- Solvent Switching into an analytically more compatible solvent

Interrelationship in sample extractions

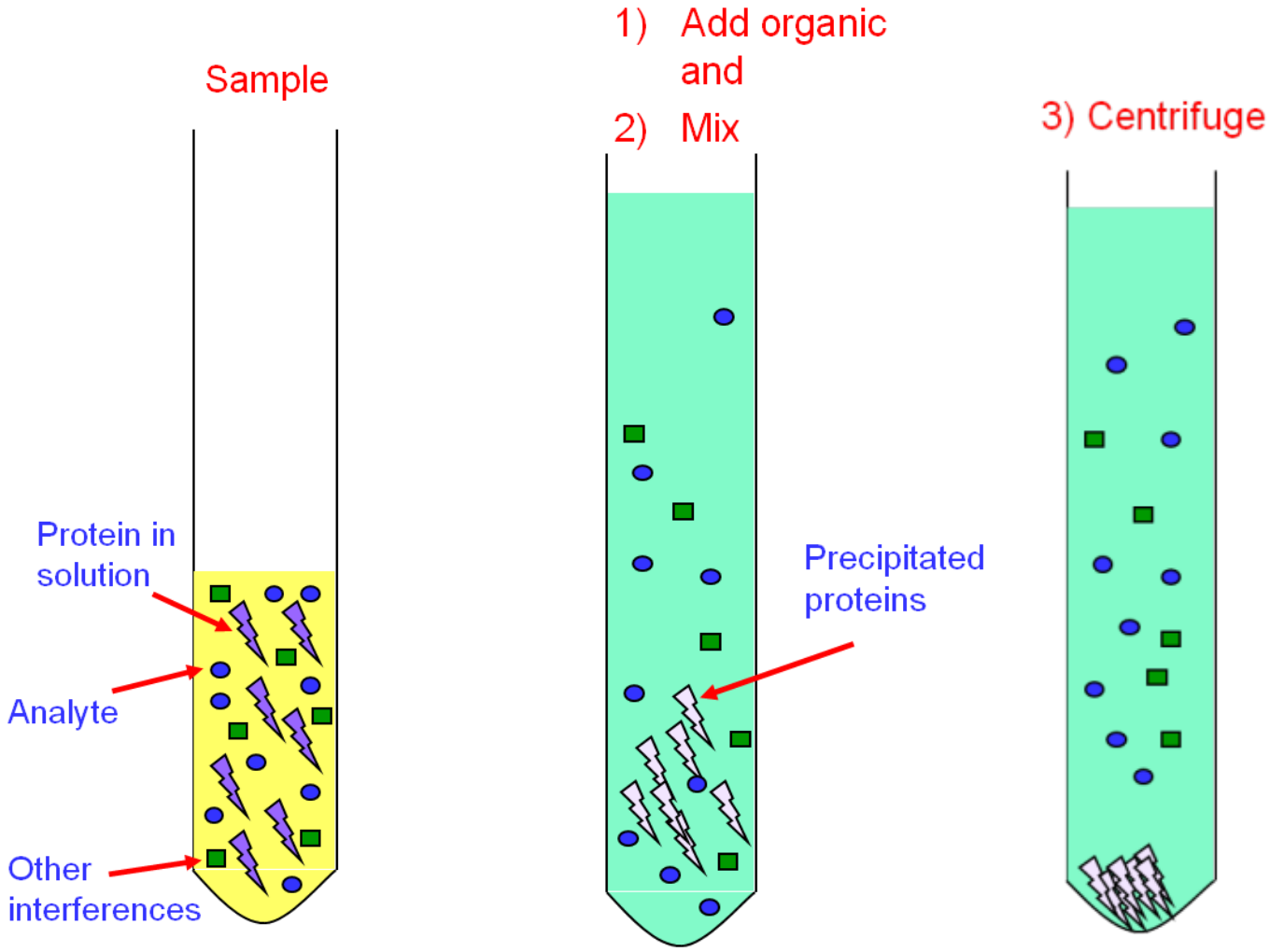


With news instruments - more Sample Preparation Techniques can be used

Matrix removal VS analyte extraction

	More Specific ← **Instrument Separation and Detection Specificity** ← *Less Specific*								
	Less Specific → ***Sample Preparation Specificity*** → *More Specific*								
Sample Prep Technique / **Interference Removed**	**Dilute & Shoot**	**Filtration**	**Liquid/Liquid Extractions**	**Supported Liquid Extractions (SLE)**	**Dried Matrix Spotting**	**Precipitation**	**QuEChERS**	**Lipid Removal 'Hybrid' Filtration**	**Solid Phase Extraction**
Lipids	No	No	No	Some	No	No	Yes	Yes	Yes
Oligomeric Surfactants	No	No	No	No	No	No	No	Yes	Yes
Particulates	No	Yes	No	Some	No	Yes	Yes	Yes	Yes
Pigments	No	No	No	Some	No	No	Yes	No	Yes
Polar Organic Acids	No	No	Yes	Yes	No	No	Yes	No	
Proteins	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Salts	No	No	Yes	Yes	No	No	No	No	Yes
Suggested Agilent Product	Agilent Autosampler Vials	Captiva Syringe Filters		Chem Elut		Captiva	Bond Elut QuEChERS	Captiva EMR LIPIDS	Bond Elut Silica and Polymeric SPE
Agilent Captiva Filtration Products are recommended for use with any LC or LC-MS method									

Traditional protein Precipitation : Lipids are not removed

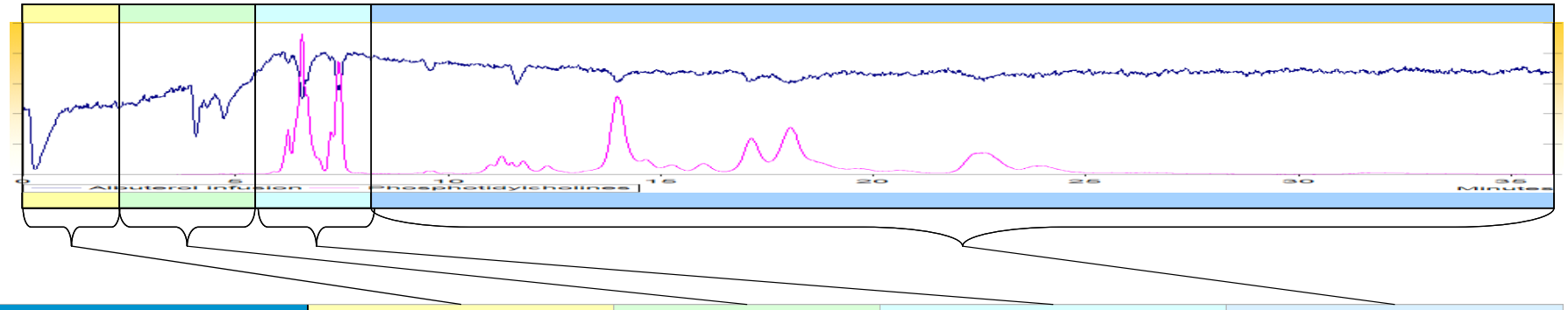


4) Remove supernatant

5) Analyze supernatant, often after dry down and resuspension

Ion Suppression Regions by LCMSMS after protein crash

Protein precipitation sample
PCI with procainamide



Interference type	Salt/Polar ionics	Proteins/ Peptides	Lyso-phosphatidylcholines	Lipids and other hydrophobics
Typical Elution Conditions (C18 column)	At or near void with < 20% organic	10's of column volumes at 40% - 70% organic	10's of column volumes at 70% - 90% organic	10's to 100's of column volumes at > 90% organic
Short term effect (single injection)	Significant ion-suppression	Significant ion-suppression	Significant ion-suppression	Some ion suppression, however, usually retained on LC column)
Long term effect (multiple injections)	Unknown	Unknown	Decreased sensitivity, Increased variability	Decreased sensitivity, Increased variability
Likely long term causes	Ion source contamination	Ion source contamination	Ion source contamination, Some column build-up	Ion source contamination, Column build-up

Similar for Liquid-liquid extraction !!! (LLE and SLE)

Effects of Endogenous Interferences

- Poor Chromatography
- Mechanical Issues (particulates, blockages)
- LC Column Lifetime Issues
- Carry Over
- Ion Suppression
- Overall Loss in Analytical Sensitivity
- Increase in Sample Run Time/Cost

Making laborious work in the Forensic lab easier with optimized sample prep

Is that possible?

1. Faster, simpler, and cleaner
2. Less sample handling and transfers
3. Good recoveries and minimal matrix effects
4. Linear, accurate and precise results for all analytes
5. Cleaner eluents with removal of over 99% of phospholipids compared to PPT and LLE

CAPTIVA EMR – LIPIDS

ENHANCED MATRIX REMOVAL-LIPIDS

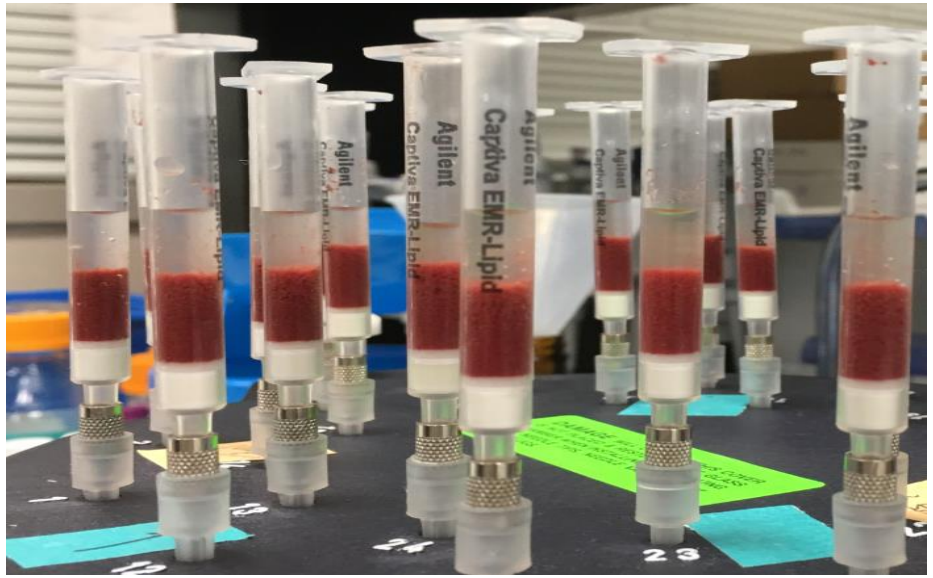
A short overview of the technology

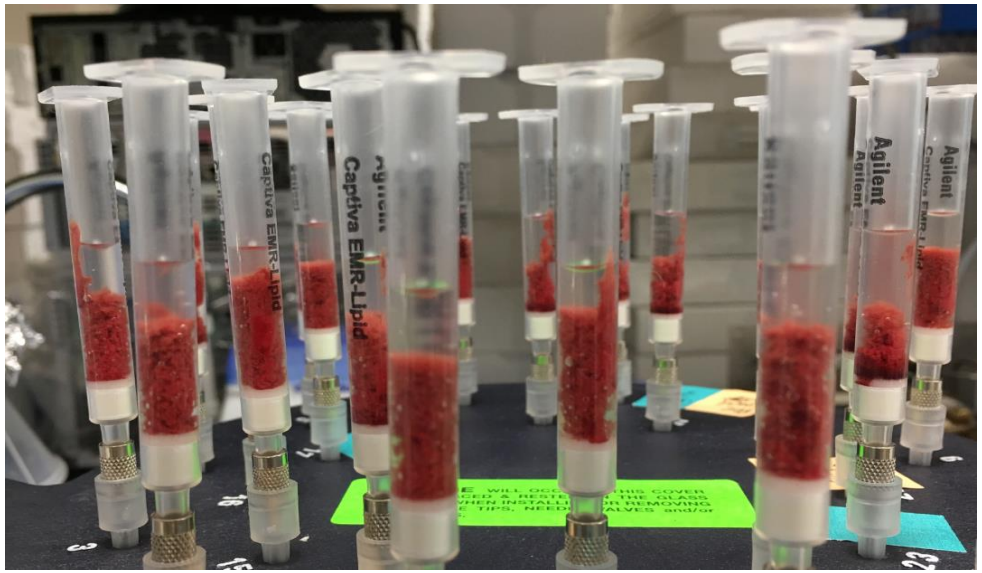
Captiva EMR-Lipid

- Product developed in response to requests from overloaded crime labs
- Simple pass through format
- Non-drip cartridge format for in well ppt (*in situ*)
- Unique cartridge/well construction minimizes clogging – and **ensures protein and lipid removal** (no cloudy samples)
- Good analyte recoveries

Captiva EMR-Lipid

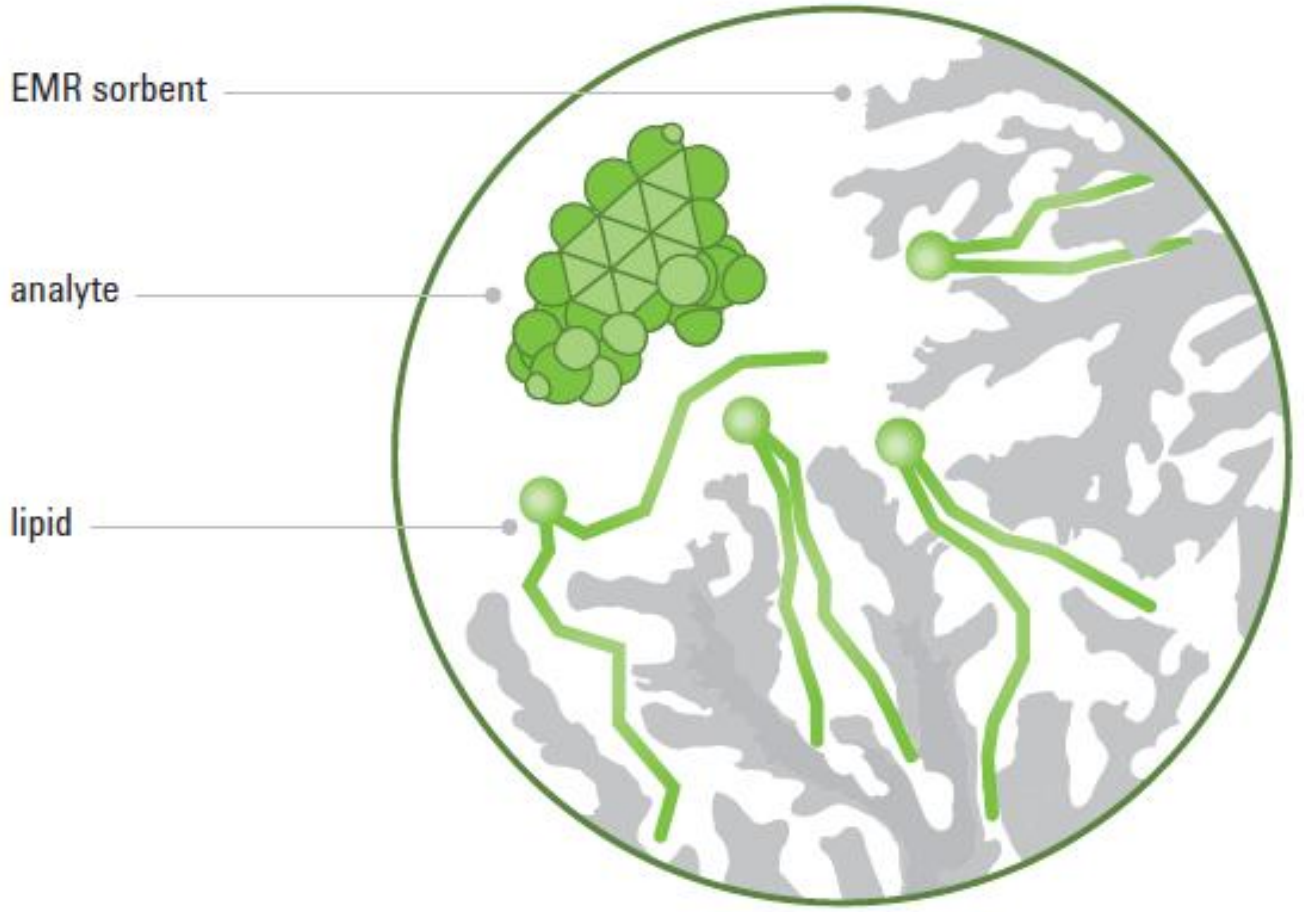
Simple one step filtration





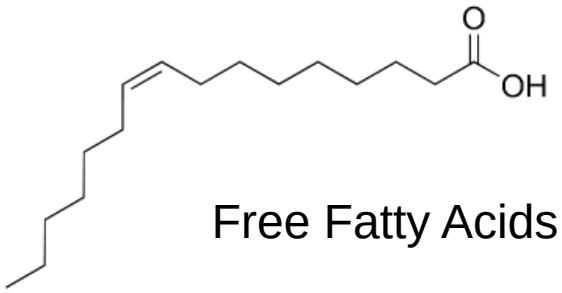
EMR sorbent technology effectively traps lipids through two mechanisms:

- ✓ **Size exclusion** – Unbranched hydrocarbon chains (lipids) enter the sorbent; bulky analytes do not
- ✓ **Sorbent chemistry** – Lipid chains that enter the sorbent are trapped by hydrophobic interactions

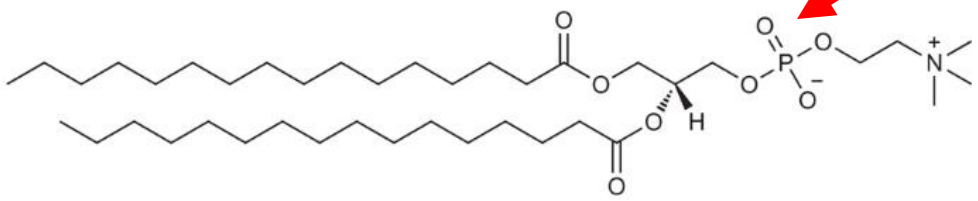


Lipids

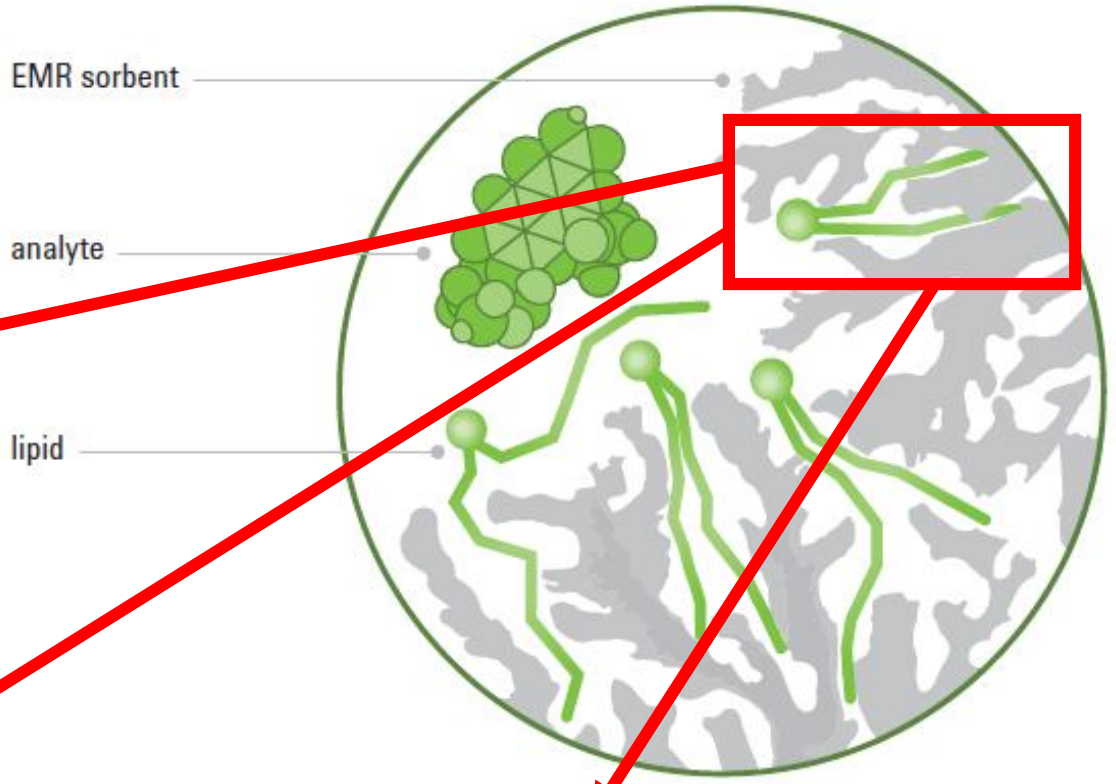
Lipids - A class of naturally occurring hydrocarbon containing compounds commonly known as fats and oils

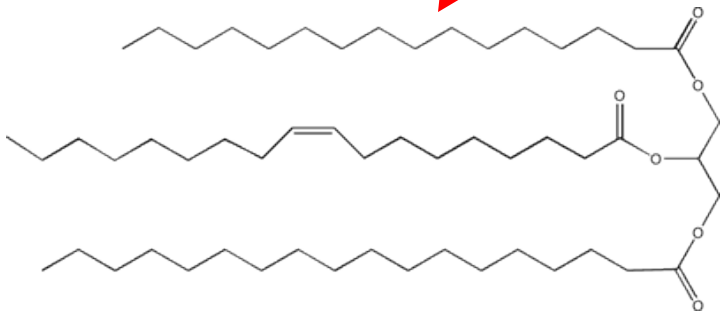


Free Fatty Acids



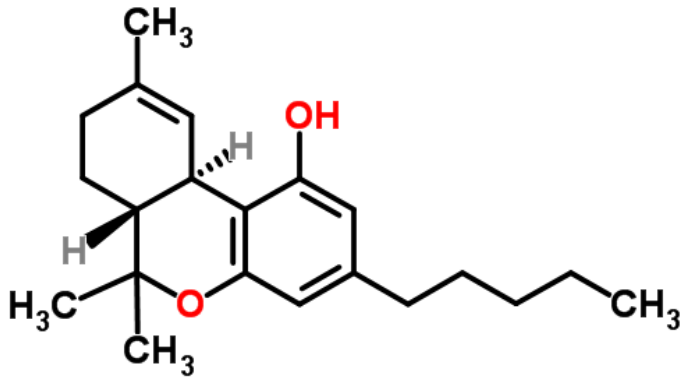
Phospholipids



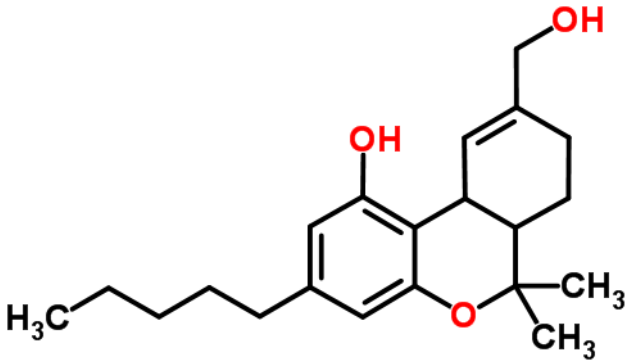


Triglycerides

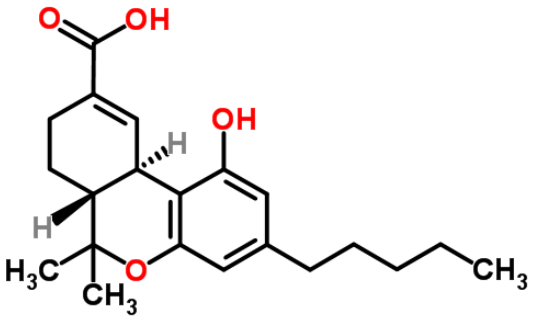
Analytes



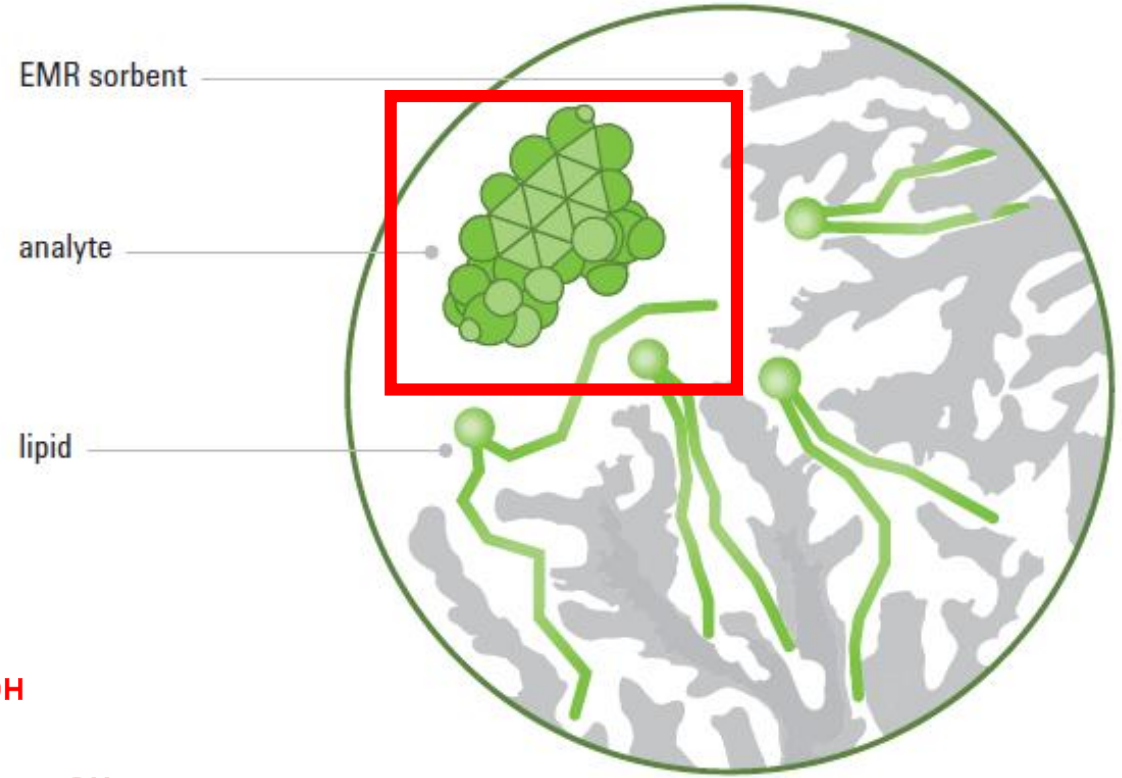
THC



11-Hydroxy-THC

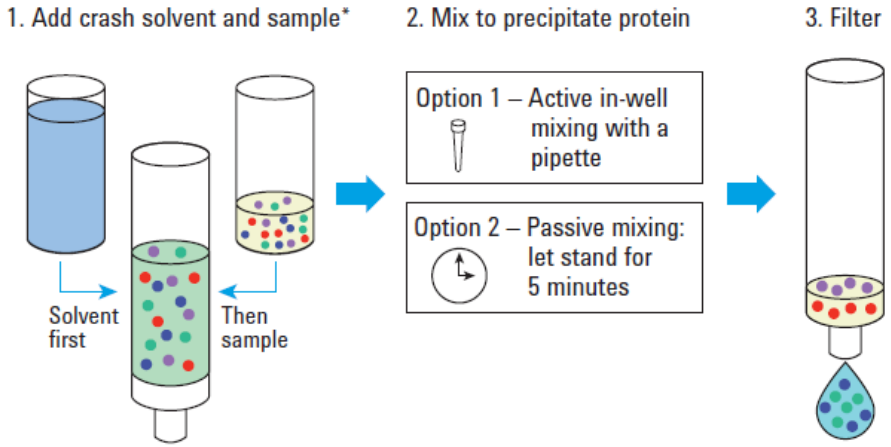


11-Nor-9-Carboxy-THC



Captiva EMR-Lipid General Protocol

Operating instructions and tips for Agilent Captiva EMR—Lipid 96-Well Plate and 1 mL Cartridge Products



** Alternatively, protein precipitation (Steps 1 and 2) can be performed off-line (Option 3), at which point the sample can be transferred to step 3.*

● Salts ● Proteins ● Lipids ● Analyte

Captiva EMR=Lipid 1 mL: 5190-1002

User Tips

Protein precipitation workflow	
Product configuration	96-well plate or 1 mL cartridge
Sample size	Between 20–200 µL
Sample treatment	Crash solvent ratio: between 3:1 and 5:1 ACN + 1 % formic acid to sample. Most commonly 3:1 and 4:1. If total volume is less than 500 µL, add additional 4:1 ACN:H_2O to reach a minimum volume of 500 µL. ACN is preferable to MeOH to maximize protein precipitation and avoid gelation.
Sample addition order	1) Crash solvent 2) Sample
Mixing	Option 1: Active in-well mixing. For in-well protein precipitation, pipette mixing (preferably using wide bore pipette tips) is recommended for 3 to 5 aspiration/dispense cycles. Option 2: Passive mixing. Let stand for 5 minutes to allow for complete protein precipitation to occur. Option 3: Protein precipitation and mixing can be performed in a separate tube, centrifuged, and subsequently transferred to the Captiva EMR—Lipid well/cartridge.
Pass-through filtration and cleanup	Vacuum between 2–5 in Hg initiates flow. Positive pressure (3–4 psi) is also acceptable. **For optimal lipid removal, a controlled flow rate of one drop every 3–5 seconds is highly recommended.** After elution, apply higher vacuum or positive pressure to ensure maximum sample recovery. Flow rate is dependent on sample type, age, and mixing. An alternative approach to vacuum and positive pressure is centrifugation. For 96-well plates, 500–800 rpm for a minimum of 10 minutes is recommended. Centrifugation speed and time are dependent on the sample volume and matrix.

MRM transitions

11 Phospholipids

(*m/z*) Precursor Ion	(*m/z*) Product Ion	Collision Energy (eV)
808.4	184.4	30
806.4	184.4	30
786.4	184.4	30
784.4	184.4	30
760.4	184.4	30
758.4	184.4	30
704.4	184.4	30
524.4	184.4	30
522.4	184.4	30
520.4	184.4	30
496.4	184.4	30

Procedure: Phospholipid Removal Evaluation on Blood:

Protein Precipitation:

Add 500 µL of **COLD** ACN 1% formic acid into a test tube

Add 100 µL of blank whole blood

Vortex on a Heidolph Multi Reax® at 800-1000 rpm, 5 minutes

Centrifuge at 5000 rpm, 5 min

Evaporate and reconstitute 100 µL MeOH (0.1%FA), vortex

Pipette to an autosampler vial for analysis

Captiva EMR-Lipid:

Add 500 µL of **COLD** ACN 1% formic acid to Captiva EMR-Lipid 1 mL cartridge

Add 100 µL of blank whole blood

In-well mixing

Pull low vacuum, 3.5-4 psi

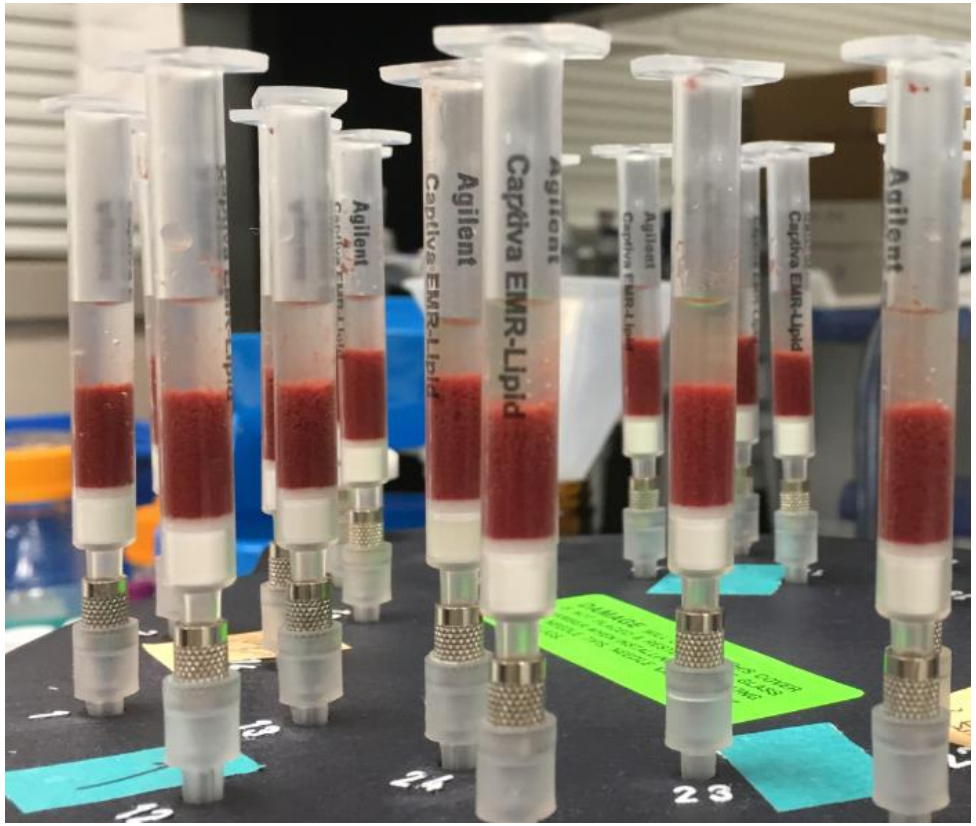
Add 200 uL of **COLD**1:4 H2O:ACN

Pull vacuum until all volume is through cartridge, then increase to 11-13 psi to pull remaining solvent through

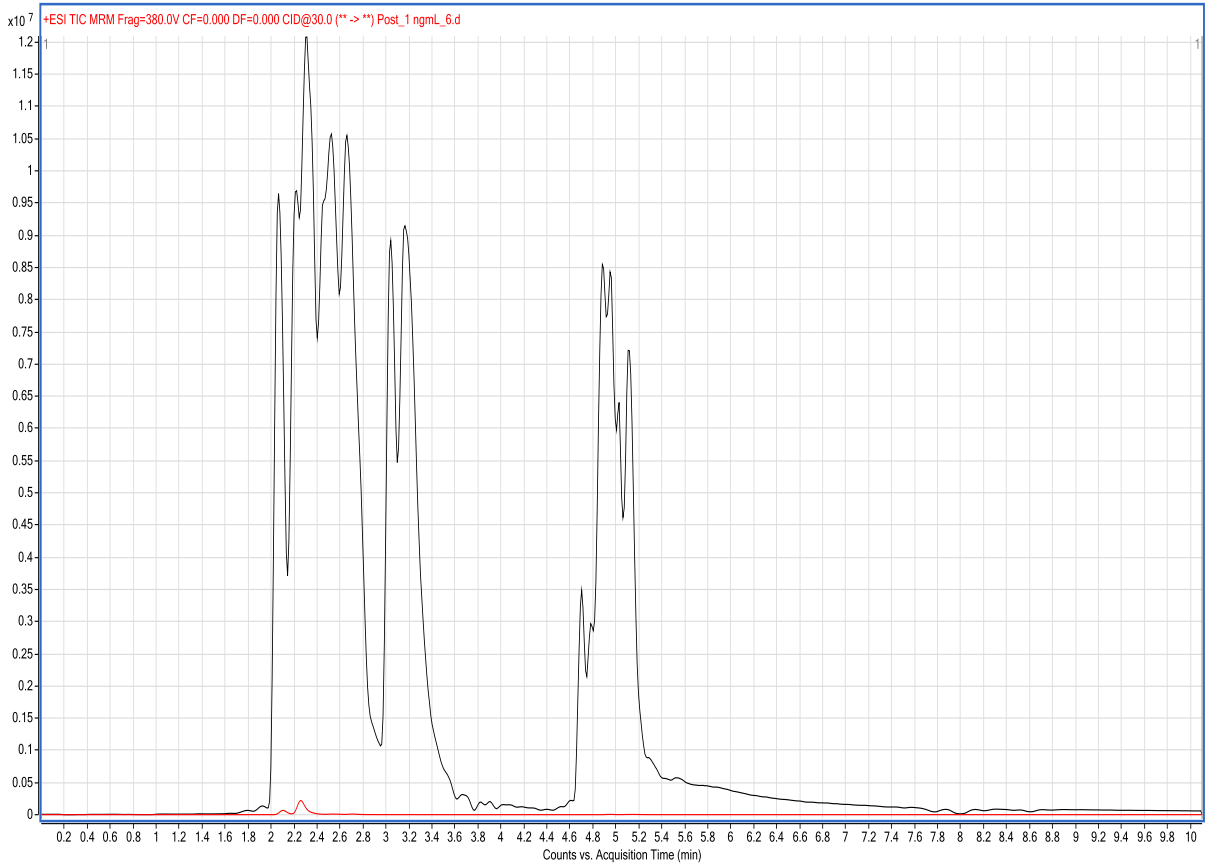
Evaporate, recon 100 uL MeOH (0.1%FA)

Captiva EMR-Lipid Process

Blood mixing, 5min wait, then filter



Phospholipid Removal: Captiva EMR-Lipid versus Protein PPT



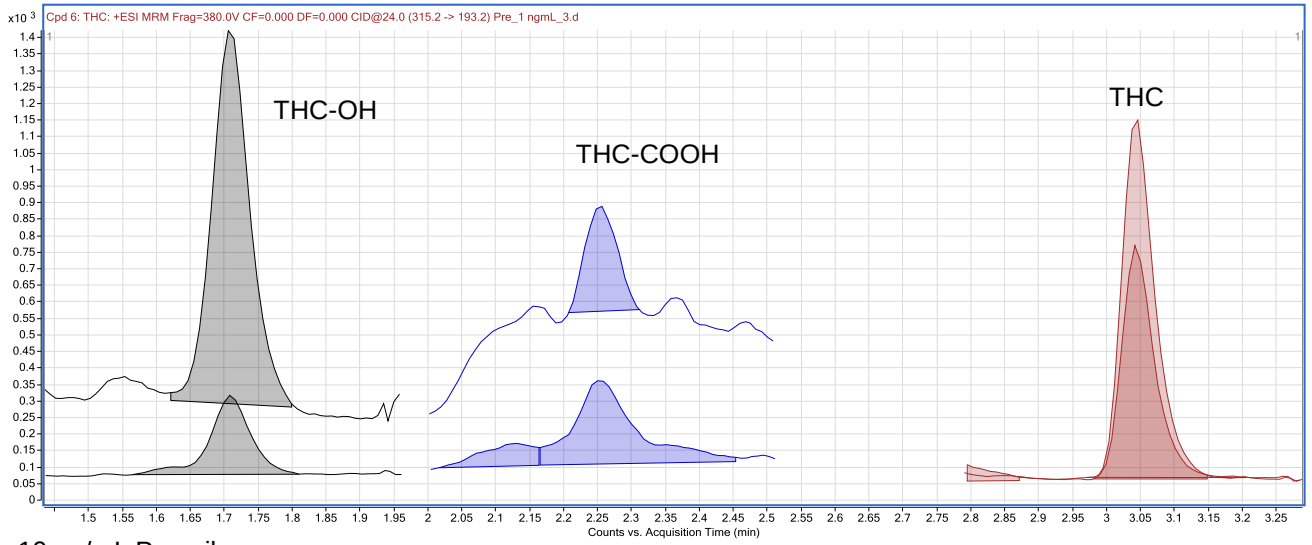
11 MRM Phospholipid transitions monitored product ion 184.0, 10 min run t time

THC and its Metabolites in Plasma:

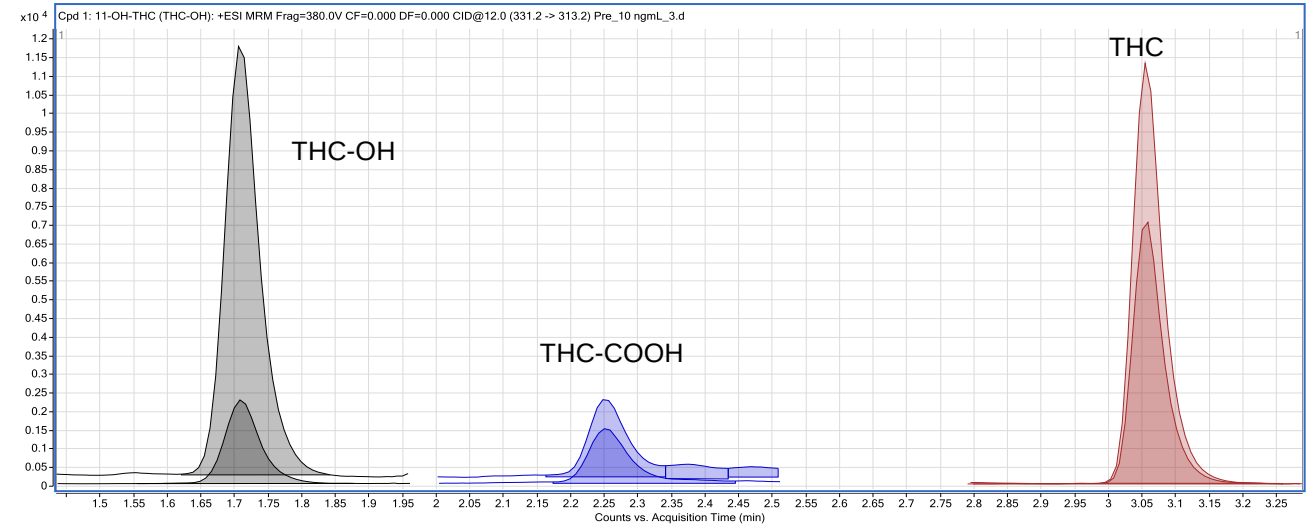
Protocol

- Add 500 uL of ACN (1%FA) to Captiva EMR-Lipid 1 mL cartridge
- Add 100 uL of human plasma
- Mix in well
- Pull vacuum 1.5-3 psi
- Add 200 uL of 1:4 H2O:ACN
- Pull vacuum until all volume is through cartridge, then increase to 11-13 psi to pull remaining solvent through
- Evap, recon 100 uL MeOH (0.1%FA)
- Inject 5 uL + 10 uL water for dilution

1 ng/mL Prespike

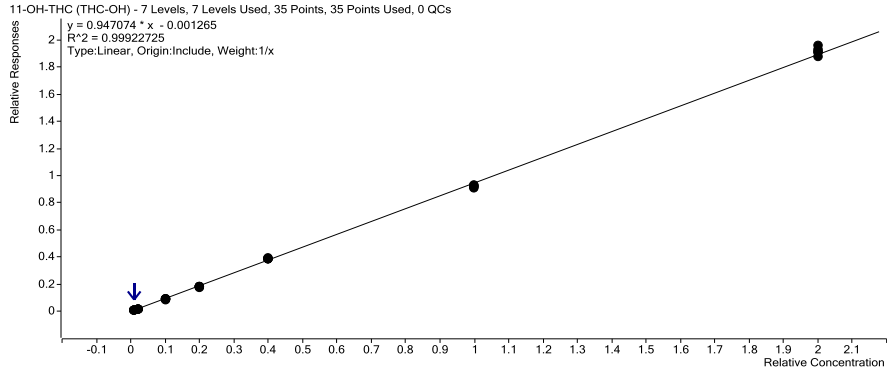


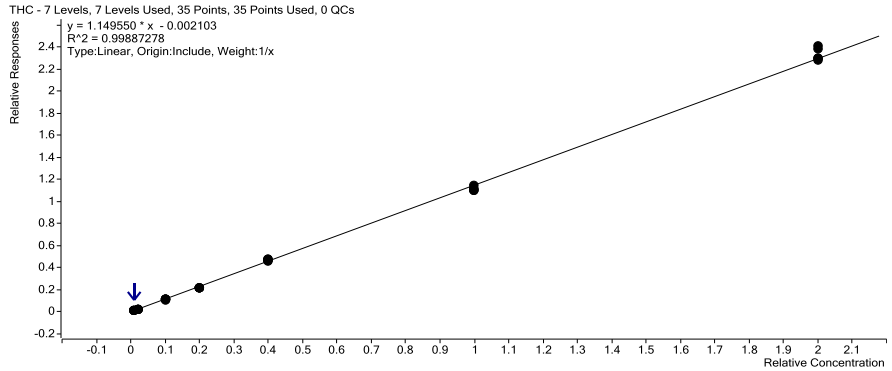
10 ng/mL Prespike

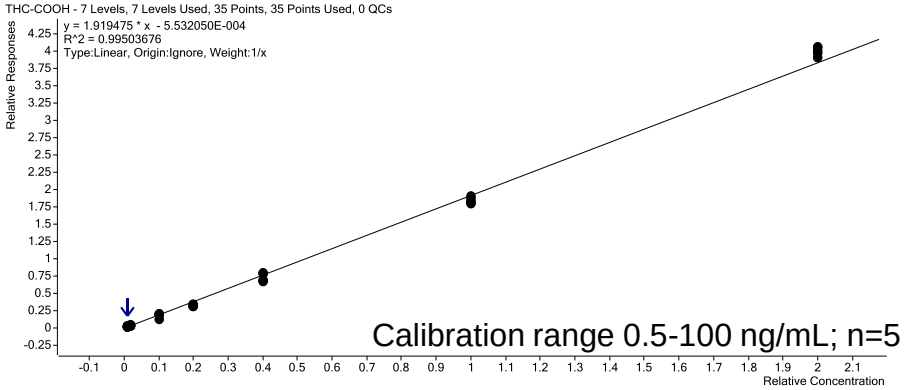


Different scaling

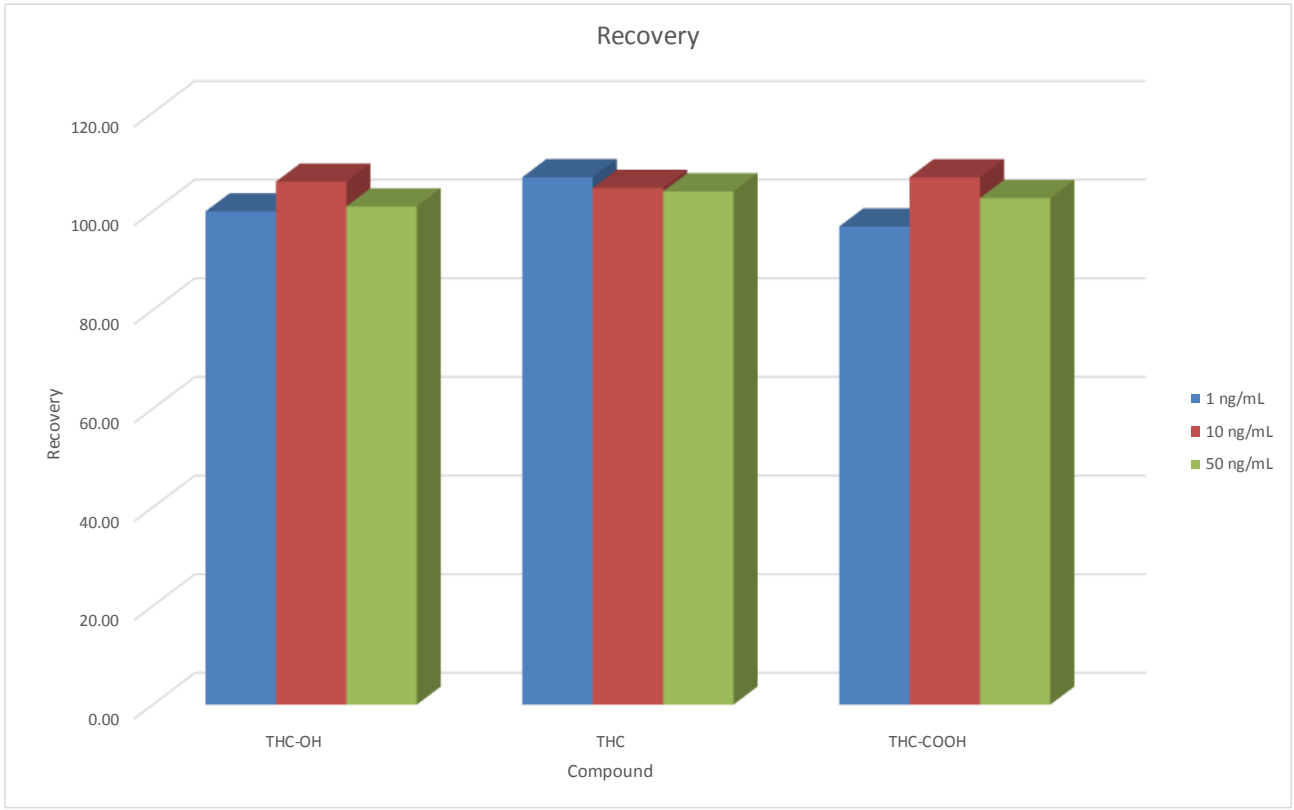
Accuracy and Precision of THC and its Metabolites in Plasma: Day 1







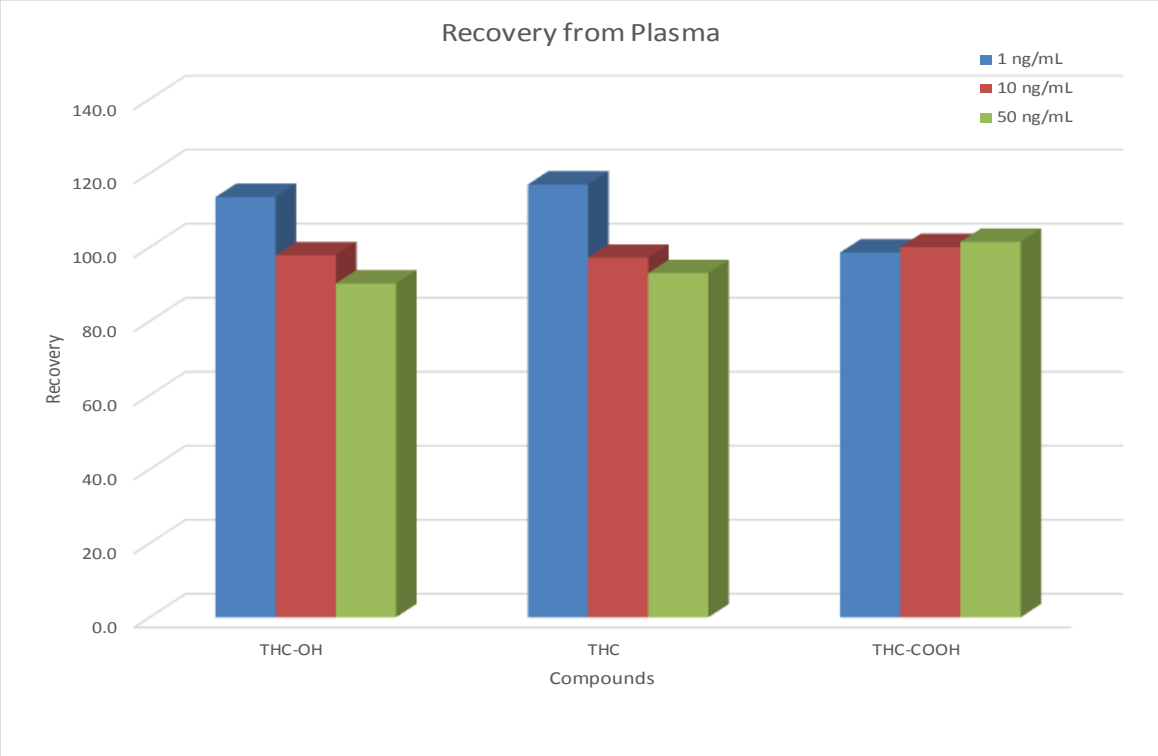
Calibration range 0.5-100 ng/mL; n=5

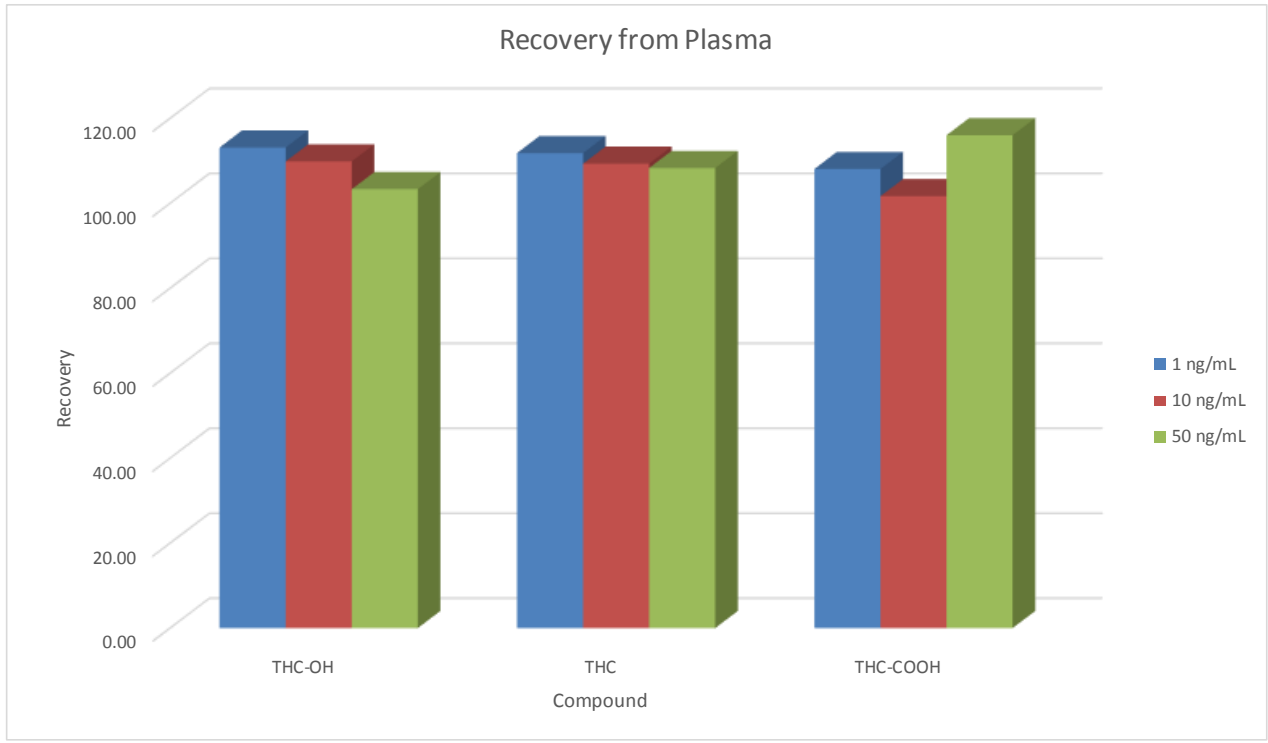


Compound	1 ng/mL		10 ng/mL		50 ng/mL	
	Recovery	%RSD	Recovery	%RSD	Recovery	%RSD
THC-OH	100	7.6	106	1.4	101	1.4
THC	107	1.2	105	3.2	104	3.2
THC-COOH	97	5.6	107	4.2	103	4.2

n=7

Accuracy and Precision of THC and its Metabolites in Plasma: Day 2&3





Compound	1 ng/mL		10 ng/mL		50 ng/mL	
	Recovery	%RSD	Recovery	%RSD	Recovery	%RSD
THC-OH	113.6	6.0	97.8	1.4	90.0	4.5
THC	116.9	2.7	97.2	31.8	93.0	3.0
THC-COOH	98.6	7.1	100.1	6.7	101.4	4.1

Compound	1 ng/mL		10 ng/mL		50 ng/mL	
	Recovery	%RSD	Recovery	%RSD	Recovery	%RSD
THC-OH	113.1	5.7	109.9	2.5	103.3	2.6
THC	111.8	2.5	109.3	3.6	108.3	0.8
THC-COOH	108.1	10.2	101.7	1.2	116.1	6.3

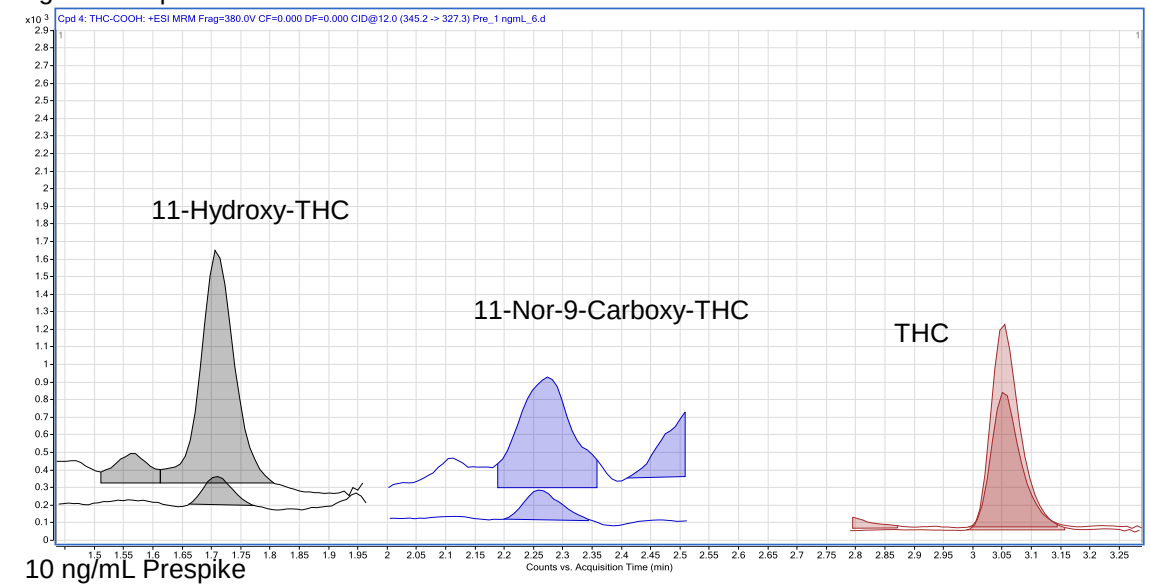
THC and its Metabolites in Whole Blood:

Protocol

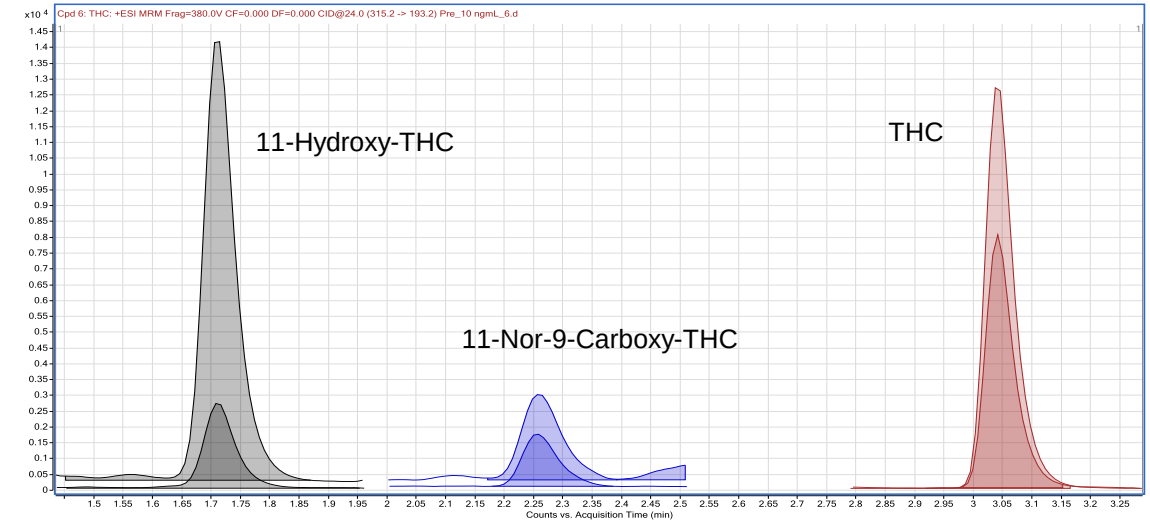
- Add 500 uL of **COLD*** 15:85 Methanol:ACN to Captiva EMR-Lipid 1 mL cartridge
- Add 100 uL of human whole blood
- Mix in well with disposable glass pipette or allow 5-7 min for passive mixing
- Pull vacuum 3.5-4 psi
- Add 200 uL of **COLD**1:4 H2O:ACN
- Pull vacuum until all volume is through cartridge, then increase to 11-13 psi to pull remaining solvent through
- Evaporate, recon 100 uL MeOH (0.1%FA)
- Inject 5 uL + 10 uL water for dilution

* Cold 15:85 Methanol:ACN was stored in -20 degree freezer and placed in frozen container while in use

1 ng/mL Prespike

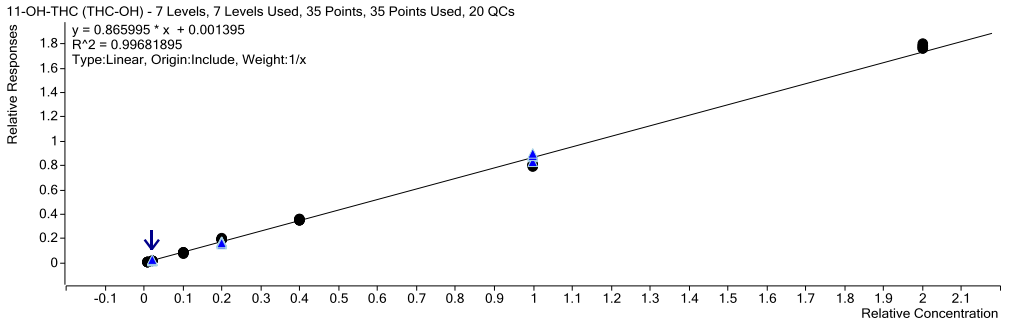


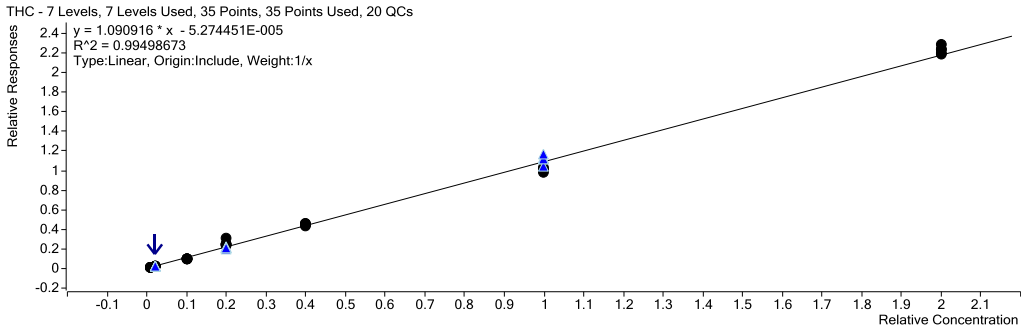
10 ng/mL Prespike

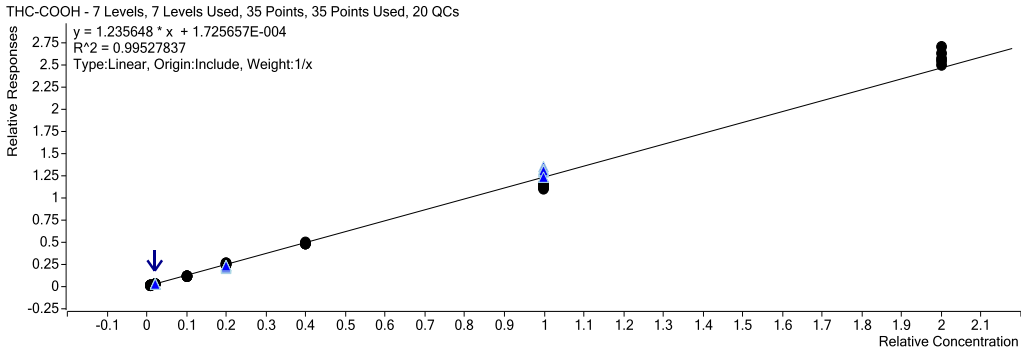


Different scaling

Accuracy and Precision of THC and its Metabolites in Whole Blood: Day 1







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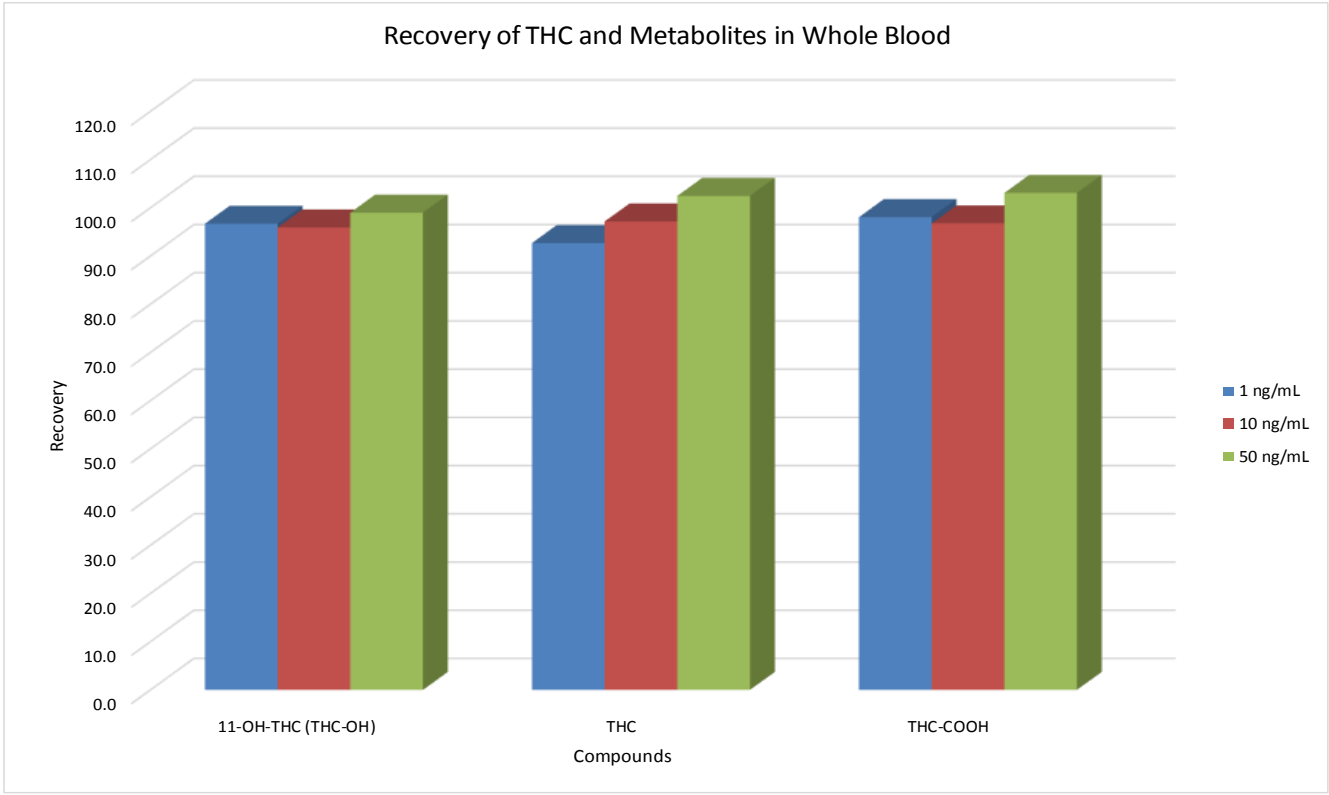
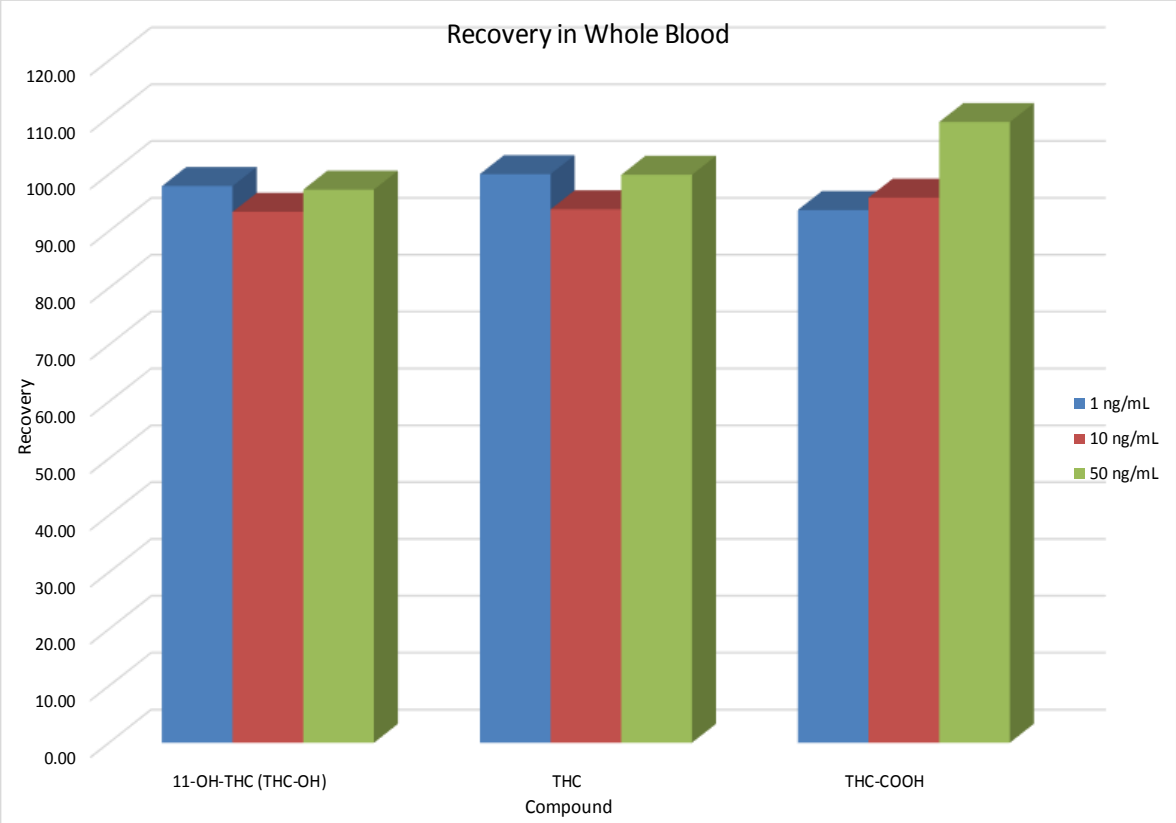
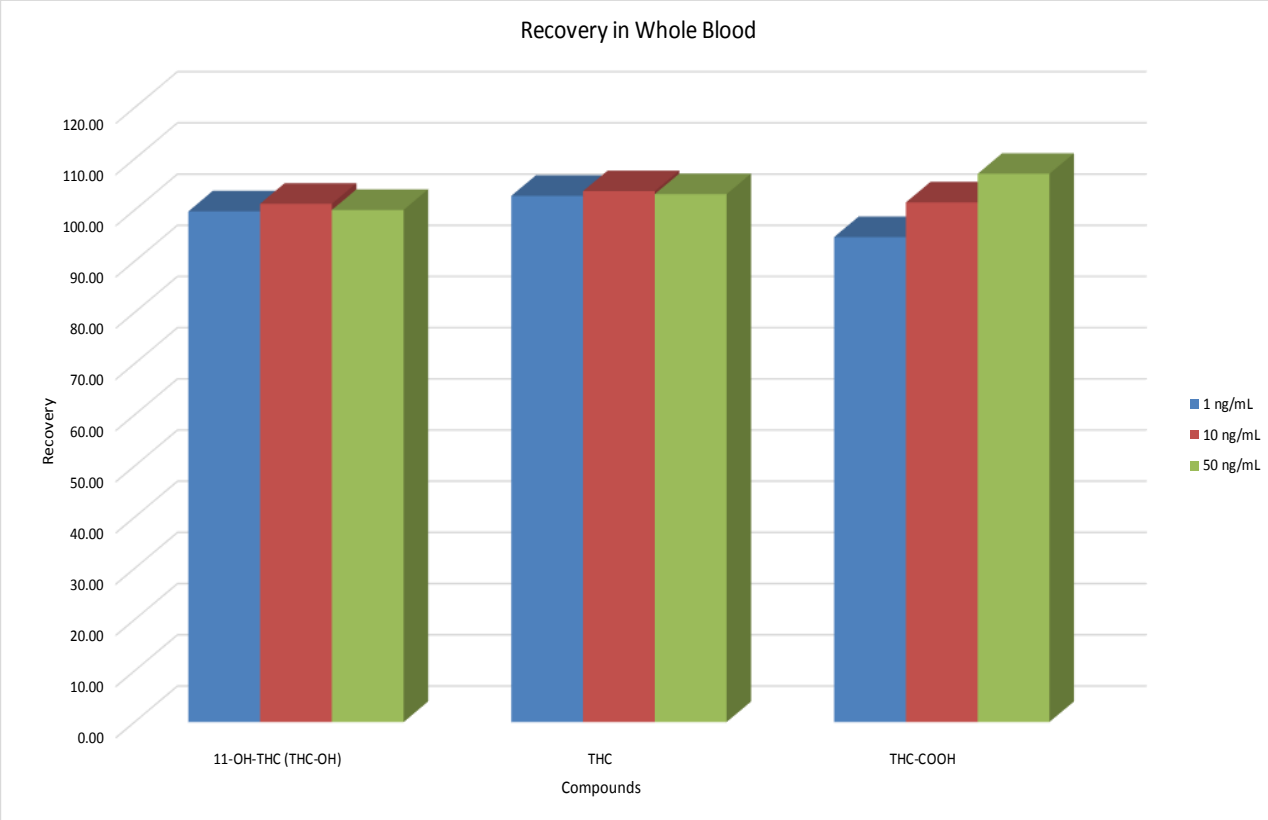


Compound	1 ng/mL		10 ng/mL		50 ng/mL	
	Recovery	%RSD	Recovery	%RSD	Recovery	%RSD
THC-OH	96.7	11.5	95.9	3.5	99.0	2.4
THC	92.7	6.2	97.2	2.8	102.5	3.5
THC-COOH	98.1	9.2	96.8	3.7	103.1	3.6

n=7

Accuracy and Precision of THC and its Metabolites in Whole Blood: Day 2 & 3





Compound	1 ng/mL		10 ng/mL		50 ng/mL	
	Recovery	%RSD	Recovery	%RSD	Recovery	%RSD
THC-OH	97.9	7.5	93.4	2.6	97.3	2.3
THC	100.0	2.5	93.8	4.2	99.9	2.4
THC-COOH	93.7	6.9	95.9	3.5	109.2	2.9

n=7

Compound	1 ng/mL		10 ng/mL		50 ng/mL	
	Recovery	%RSD	Recovery	%RSD	Recovery	%RSD
THC-OH	99.8	8.1	101.2	3.4	100.0	6.0
THC	102.8	2.4	103.7	3.8	103.1	3.8
THC-COOH	94.7	6.4	101.5	2.4	107.1	3.4

n=7

Forensic drug panel in human serum using Captiva EMR-Lipid

``Diverse``drugs panel with good responses

Acid, bases and neutrals – Polar and non-polars

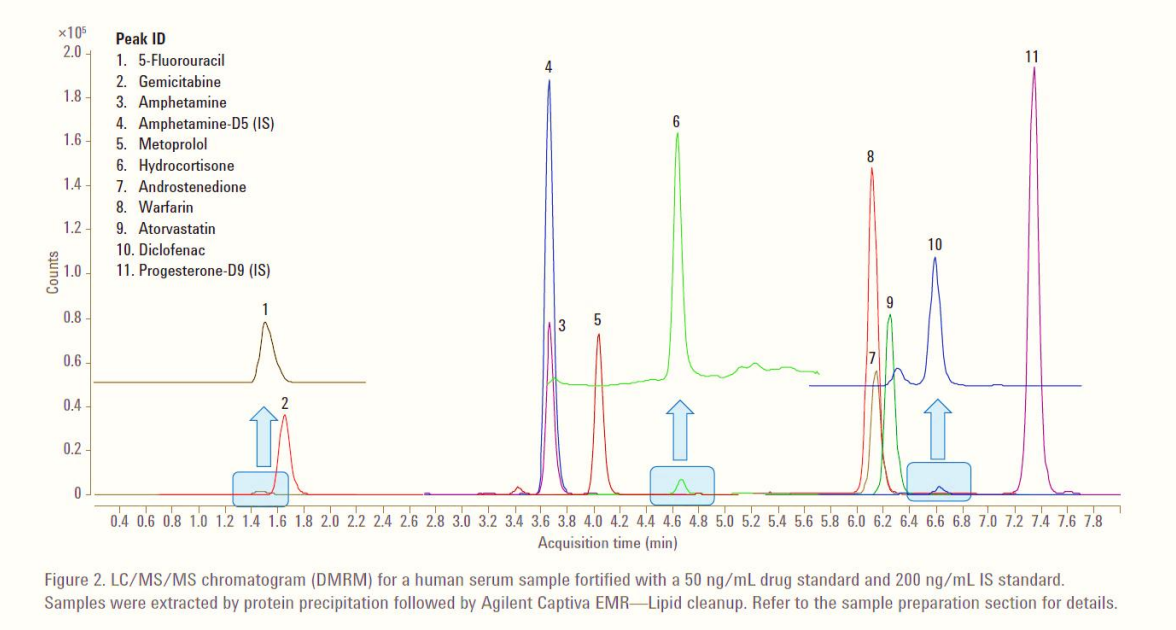


Figure 2. LC/MS/MS chromatogram (DMRM) for a human serum sample fortified with a 50 ng/mL drug standard and 200 ng/mL IS standard. Samples were extracted by protein precipitation followed by Agilent Captiva EMR—Lipid cleanup. Refer to the sample preparation section for details.

Less ion suppression using Captiva EMR

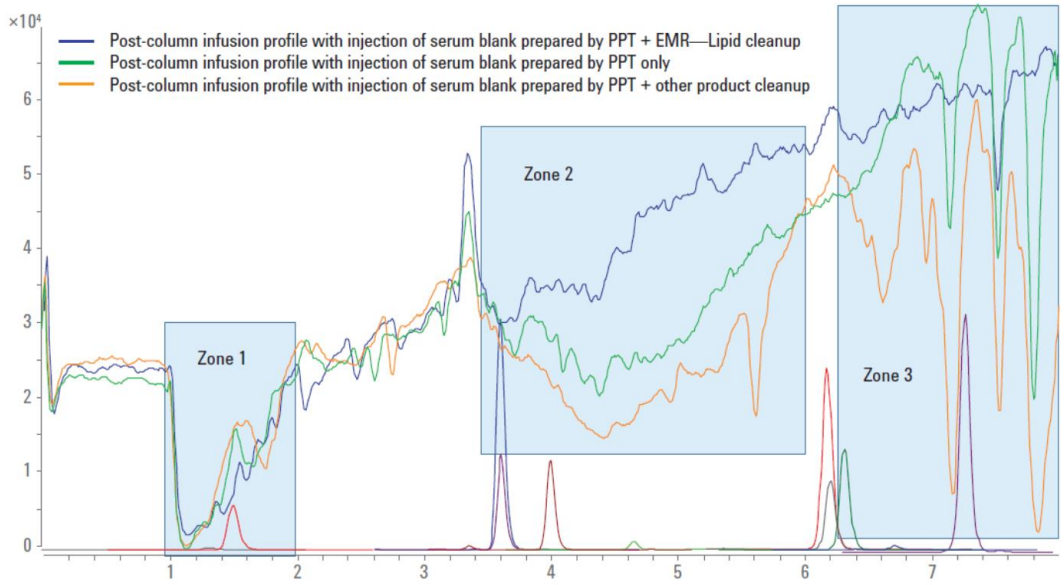


Figure 6. Standard post column infusion profiles comparison and demonstration of matrix ion suppression effect on target analytes.

Who should use Captiva EMR-Lipid?

- Any lab working with serum, plasma samples (human or animal)
  - Clinical **research** labs, large contract labs
  - Pharma / DMPK samples, big pharma, CROs, research labs
  - Coroners/Medical Examiners/Forensics Labs
  - Veterinary research labs

- Working with compounds that co-elute with lipids (mid to late eluters)
  - Steroids
  - Hormones
  - THC
  - Fat soluble vitamins (D, E, A, K)
  - Immunosuppressants
  - Per/Polyfluoroalkyl Substances (PFASs)

What is the value?

1. Simple and fast
2. Better data and better analytical method robustness
3. Analyze large forensic drug panels (acids, basic drugs, neutral, polar et non polar analytes)

Aknowledgments

Dr. Joni Stevens

Dr. Peter Stone

THANK YOU !!

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