

What you don't see...
...CAN hurt you



Sample Prep for Today's
Analytical World

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Today's Agenda

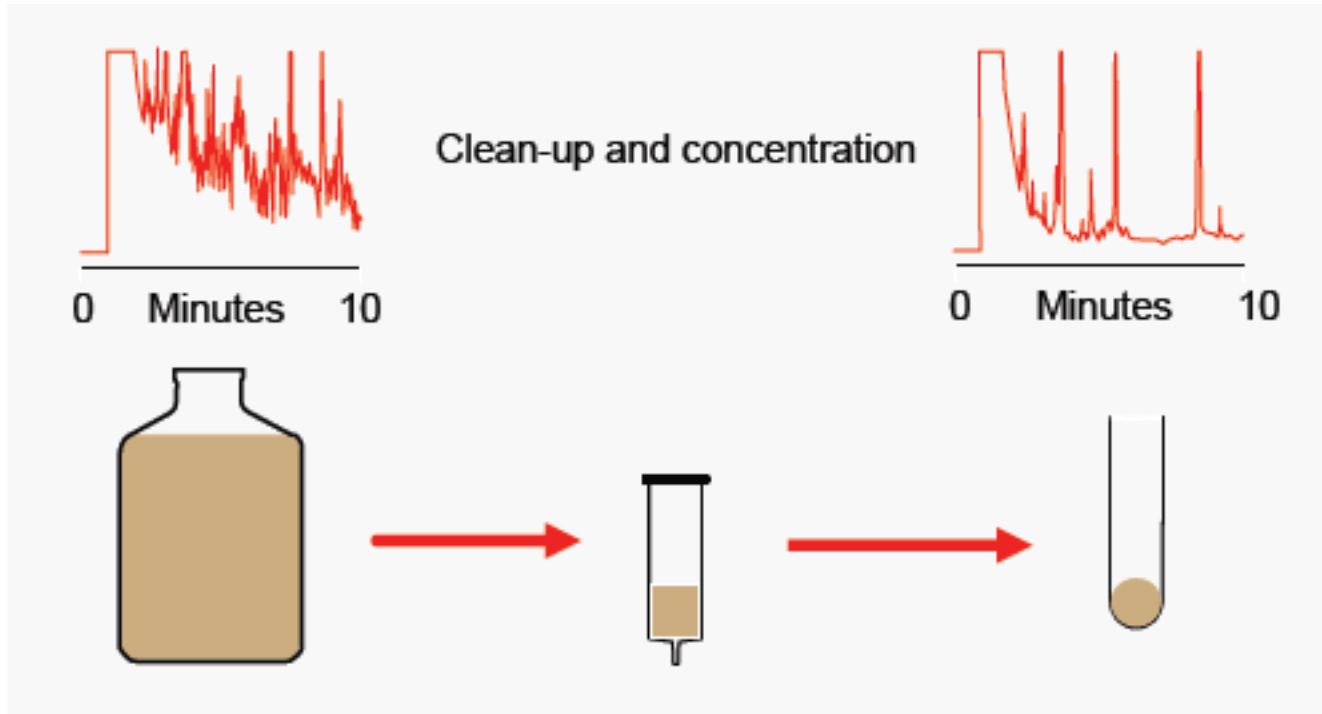
Introduction

Addressing difficult samples

1. Polar analytes
2. High fat samples
3. How to get rid of interfering terpenes

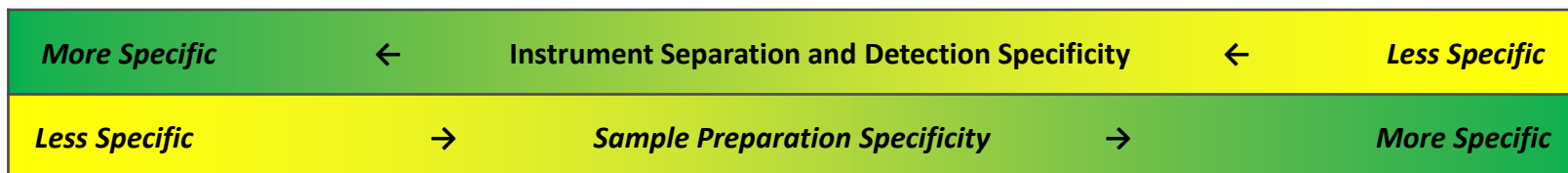
Summary and Conclusions

Objectives of Sample Preparation



- 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

With news instruments - more Sample Preparation Techniques can be used



Sample Prep Technique Interference Removed	Instrument Separation and Detection Specificity ←								
	← Sample Preparation Specificity →								
	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 ND	Bond Elut QuEChERS	Captiva ND LIPIDS	Bond Elut Silica and Polymeric SPE

Agilent Captiva Filtration Products are recommended for use with any LC or LC-MS method

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Summary and Conclusions

Polar analytes often require advanced solid phase extraction (SPE)

- Analyte is too polar for acetonitrile extraction
- Filtration is not clean enough
- Ion exchange can be used to filter polar interferences away
- We then often need an SPE phase that can bind a wide range of polar analytes that are basic, neutral and acidic

Bond Elut Packed Bed Phases – Which one!

Non-polar

C18, C8, C2, C1

C18 variations in carbon load and endcapping

EnvirElut

CH – cyclohexyl

CN-E – endcapped cyano

PH – phenyl

ENV, LMS, PPL, Focus, Nexus

Bond Elut Plexa

Polar

PSA - primary and secondary amine

NH2 - aminopropyl

CN-U –unendcapped cyano

DEA - diethylaminopropyl

Diol

Si - silica

Cation Exchange

SCX – benzenesulfonic acid

PRS – propylsulfonic acid

CBA – carboxylic acid

Reversible Covalent

PBA – phenylboronic acid

Specialty Phases

AccuCAT

Atrazine

Etc..

Anion Exchange

SAX – quaternary amine

PSA – primary and secondary amine

NH2 – aminopropyl

DEA – diethylaminopropyl

Mixed mode IEX/NP

Certify – SCX/C8

Certify II – SAX/C8

Plexa PCX

Plexa PAX

Alumina – aluminum oxide

Florisil – magnesium-silica

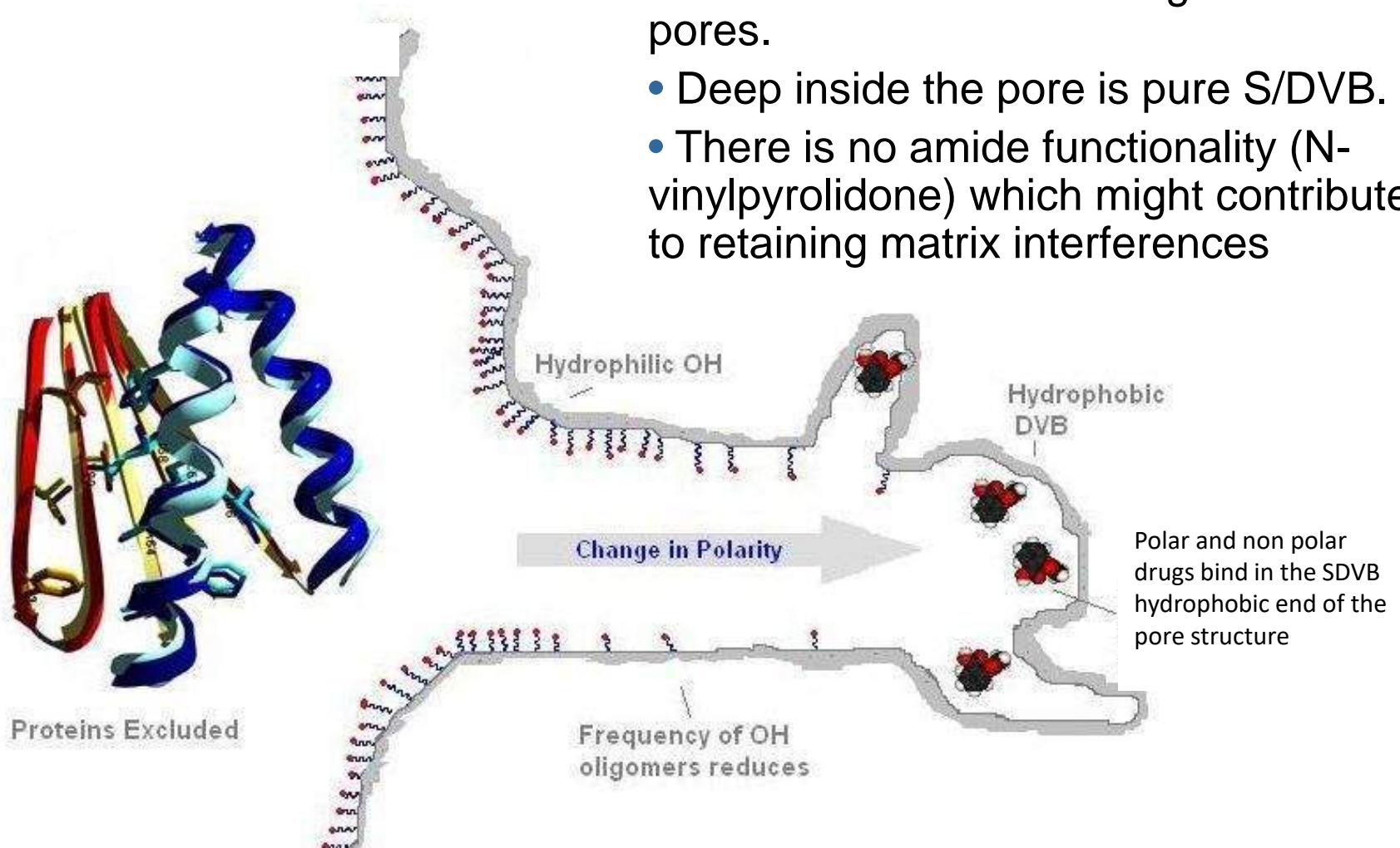
Carbon

Carbon/NH2



Bond Elut Plexa - A Different Type of Polymeric Sorbent

- The hydroxylated ligands exist on the surface and in the outer regions of the pores.
- Deep inside the pore is pure S/DVB.
- There is no amide functionality (N-vinylpyrrolidone) which might contribute to retaining matrix interferences



Plexa Recoveries Versus HLB – good results with a wide range of compounds

Analyte	Plexa	HLB	pKa	LogP
Albuterol	97.9	115.4	5.9	1.3
Atenolol	97.0	94.0	4.2	4.2
Loratadine	71.0	49.0	5.7	1.5
Metoprolol	92.0	74.0	5.7	1.5
Naltrexone	85.7	13.0	4.9	5.2
Pravastatin	85.0	59.0	4.9	5.2
Propranolol	55.0	35.0	4.9	5.2
Zolpidem	93.0	96.8	9.9	3.4

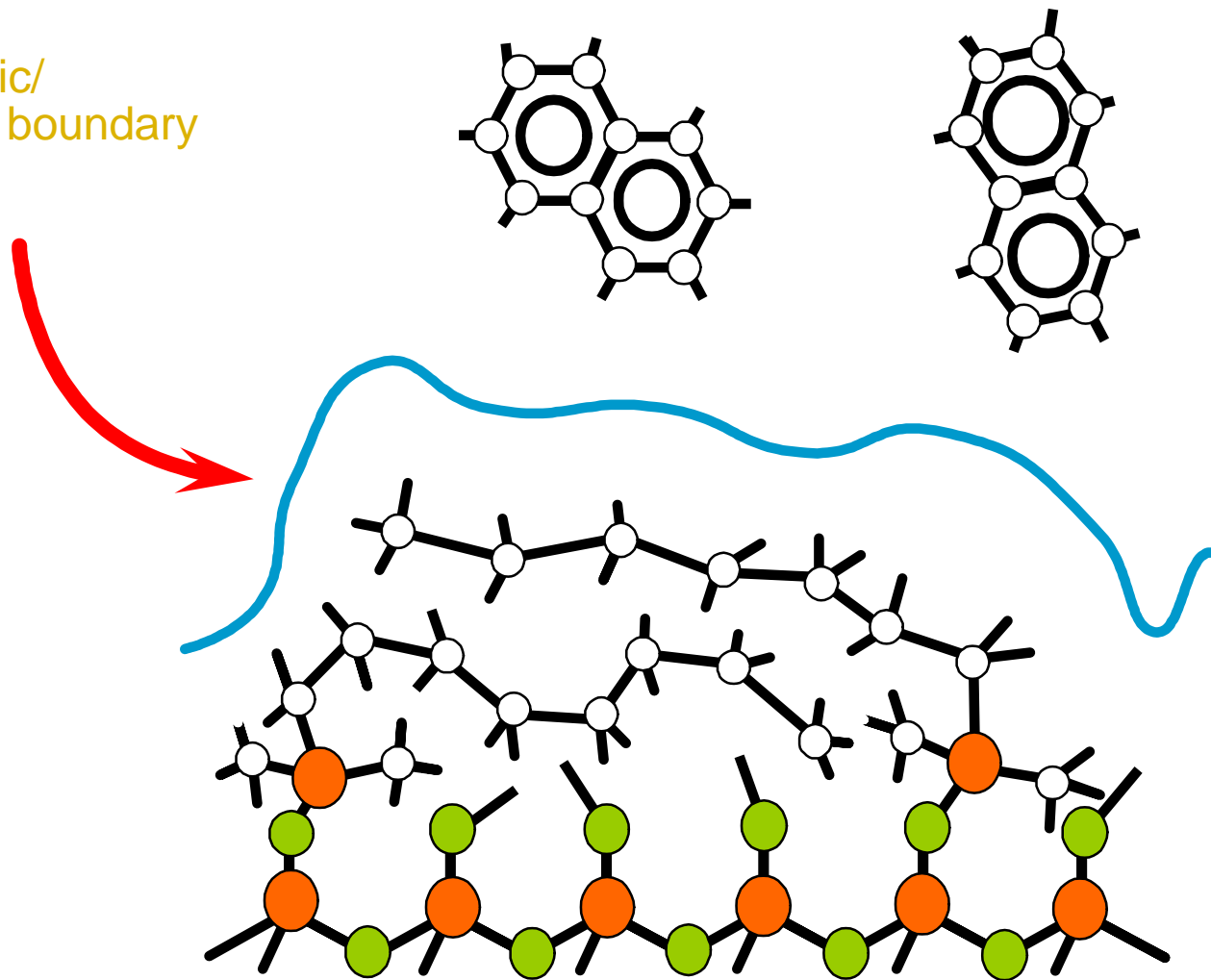
Plexa gives equivalent or better absolute recoveries than Oasis HLB for a wide range of pKa and logP compounds

Conditions: Absolute Recovery from human plasma

Basic load conditions, n=6, 200 ng/ml

Sorbent Conditioning - Why Do It?

Hydrophobic/
hydrophilic boundary



Drying a silica-based Cartridge (ex: C18)

Over drying / lack of conditioning before sample application can contribute to

- low recoveries
- poor flow
- irreproducibility (high RSDs)

Examples on how polymeric SPE perform

Bond Elut Plexa / Phenols from Drinking Water

Bond Elut™ Plexa™

Efficient Preparation of Phenols in Drinking Water

Advantage Statement: Varian's Bond Elut Plexa solid phase extraction (SPE) column has a very high extraction capacity for analytes and efficient removal of contaminants, making it ideal for extraction of phenols from drinking water. Plexa features a unique structure never before seen in standard styrene divinyl benzene polymer SPE columns, whereby polarity is separated between a hydrophilic exterior and hydrophobic core (Figure 1).

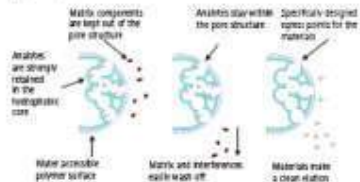


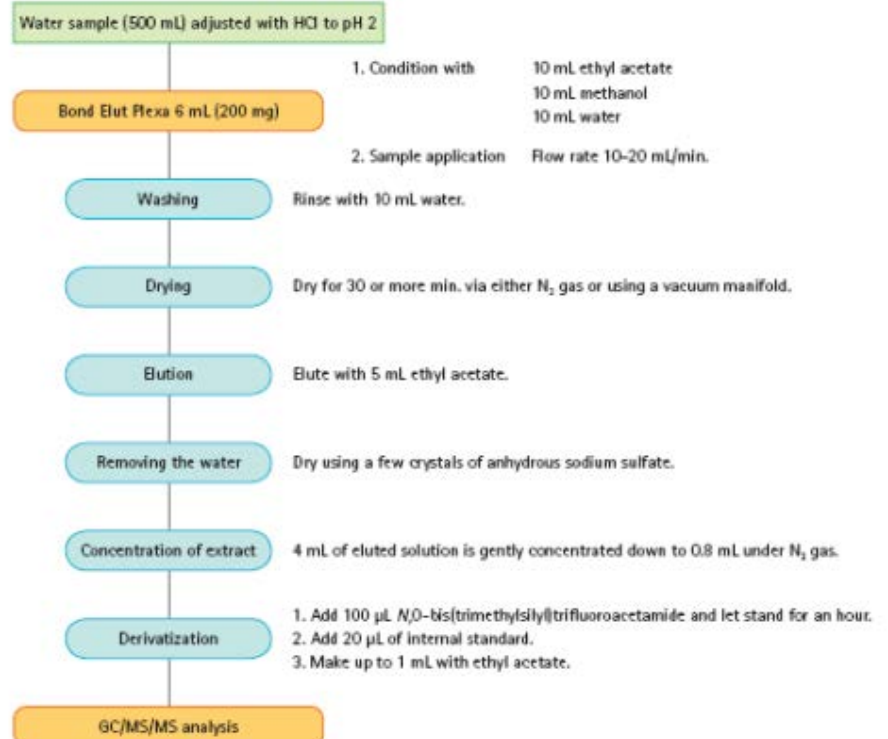
Figure 1. Varian Bond Elut Plexa retention, washing and elution mechanisms.



Figures 4 and 5 show a comparison of extraction for one of the analytes in the phenol class, between a styrene divinyl benzene *N*-vinyl pyrrolidone SPE and Varian Bond Elut Plexa.



Sample preparation method of phenols in drinking water using Varian Bond Elut Plexa (200 mg)



Bond Elut PPL - EPA 528 Phenols from Drinking Water

Method 528 provides procedures for the determination of phenols in finished drinking water. The method may be applicable to untreated source waters but has not been evaluated for these uses. The method is applicable to a variety of phenols that are efficiently partitioned from the water sample into a modified PSDB SPE sorbent (Varian's PPL) and is sufficiently volatile and thermally stable for gas chromatography.

Extraction is performed by passing a 1L water sample through a SPE cartridge with 500mg modified PSDB (PPL). The phenols are eluted with methylene chloride.

ANALYTE	CAS NUMBER
phenol	108-95-2
2-chlorophenol	95-57-8
2-methylphenol (o-cresol)	95-48-7
2-nitrophenol	88-75-5
2,4-dimethylphenol	105-67-9
2,4-dichlorophenol	120-83-2
4-chloro-3-methylphenol	59-50-7
2,4,6-trichlorophenol	88-06-2
2,4-dinitrophenol	51-28-5
4-nitrophenol	93951-79-2
2-methyl-4,6-dinitrophenol	534-52-1
pentachlorophenol	87-86-5

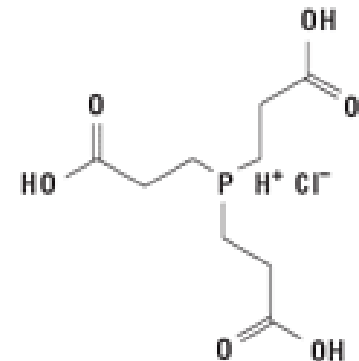
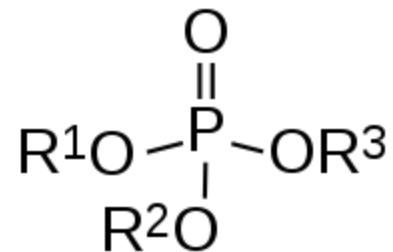
Determination of Organophosphates in Lake Water: Bond Elut PPL

SI-02094

Julia Regnery, Goethe-Universität Frankfurt, Institut für Atmosphäre und Umwelt, AG-Umweltanalytik, 60438 Frankfurt, Germany, Elisabeth Korte, Varian Deutschland GmbH

Chlorinated organophosphates are used as flame retardants in insulation foams, paints, coatings, plastics, and textiles while the non-chlorinated ones are mostly used as plasticizers. The compounds have been detected in air, sediment and soil, sewage sludge, streams and lakes. Particularly the chlorinated organophosphates are known to persist in the aquatic environment.

- **Because of the low concentration of the analytes in the water sample an enrichment step is necessary.**



TCEP·HCl
M.W. 286.65

SPE Conditions

Cartridge: Bond Elut PPL, 100 mg sorbent in 1 mL cartridge

Condition: 1 mL methanol, 1 mL methanol / acetonitrile (1/1)

Apply 1.5 -2.5 L water sample

Dry the cartridge using nitrogen

Elution with 3 x 333 μ L methanol / acetonitrile (1/1)

Results

The simple clean-up and enrichment with SPE has the advantage that up to 20 water samples can be extracted simultaneously without using complex apparatus. Bond Elut PPL has been proven to be a robust sorbent with high capacity for the extraction of polar and medium polar analytes.

Table 1. Recoveries and LODs of organophosphates; extracted from the water sample with SPE.

Analyte	Recovery (%)	LOD (ng/L)	Quantifying ion m/z
Tris(1-chloro-2-propyl)-phosphate (TCPP)	91	1	99
Tris(2-chloroethyl)-phosphate (TCEP)	95	2	63
Tris(1,3-dichloro-2-propyl)-phosphate (TDCP)	99	1	75
Tri-n-butylphosphate (TnBP)	89	1	99
Tri-isobutylphosphate (TiBP)	85	2	99
Tris(2-butoxyethyl)-phosphate (TBEP)	93	3	125

Conclusion

Novel polymeric SPE phases are recommended:

- 1. They have double the binding capacity over silica**
- 2. They can bind a wide range of polar analytes**
- 3. They don't decondition/dry out on the SPE manifold**
- 4. They provide more robust methods**

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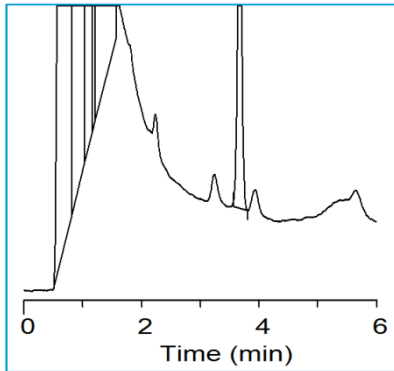
3. How to get rid of interfering terpenes

Summary and Conclusions

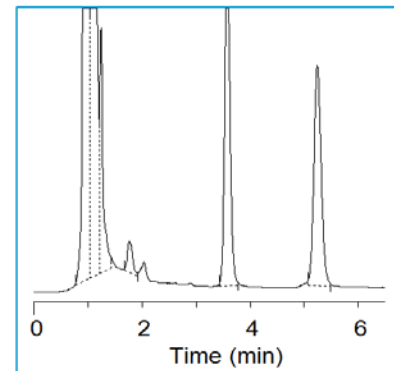
Remove interferences...

- To acquire desired sensitivity/selectivity
- To reduce contamination/carryover issues
- Use of sensitive and expensive instruments: Protect your investment!!!

Pesticides in Avocado without SP



Pesticides in Avocado with SP



Lipids

Matrices and Approximate Total Lipid Content

<2%

4-12%

>12%

Low

Med

High

Spinach (0%)	Soy Milk (4%)	Nut Butters (16%)
Strawberry (0%)	Beef Liver (4%)	Avocado (21%)
Onion (0%)	Pork Liver (4%)	Salmon (27%)
Paprika (1%)	Corn (4%)	Soy Oil (100%)
Cumin (2%)	Trout (8%)	Avocado Oil (100%)
Rice (2%)	Chocolate (8%)	Canola Oil (100%)
Hops (2%)	Canned Pet Food (~10%)	Coconut Oil (100%)
Tilapia (2%)	Cow's Milk (5%)	Catfish (12%)
Sea Bass (3%)		Carp (12%)
Wheat (3%)	Plasma or whole blood (10 – 20 %)	

Current Procedures for Lipid Removal

Method	How Lipids are removed	Weakness
Dilute and Shoot	No lipid removal, only dilution	No lipid removal
Protein Precipitation	PPT followed by centrifugation	Insufficient lipid removal
	PPT followed by filtration with or without sorbent	Insufficient lipid removal; low analyte recovery
QuEChERS	PSA/C18 sorbent (dSPE)	Not selective; insufficient lipid removal; analyte loss
	Zr-containing sorbent	Low total lipid capacity; analyte recovery
	Freeze sample	Time needed; loss of analyte
SPE/SLE	Load and elute	Time needed; solvent usage; extensive method development
SEC/GPC	Chromatographic separation	Uses copious amounts of solvent and time; capital expense

EMR-Lipid

Enhanced Matrix Removal

EMR: As easy to use as QuEChERS; as clean as SPE

EMR Product offering



Extraction Tube

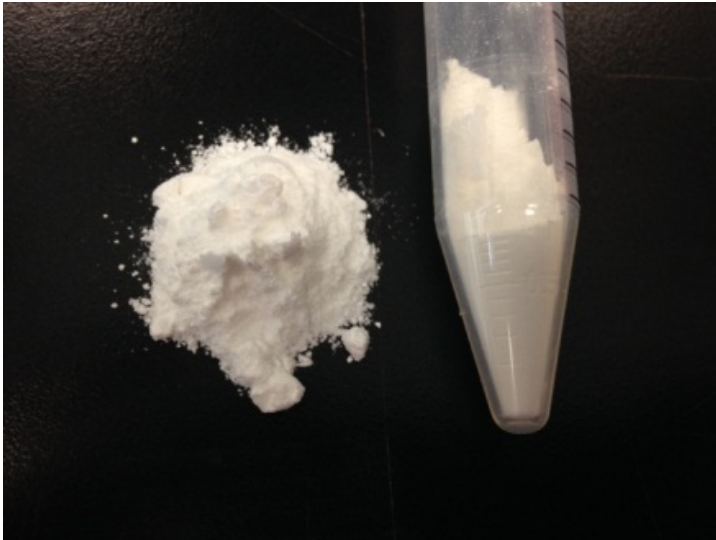


EMR-Polish (p/n 5982-0102)

EMR-Lipid (p/n 5982-1010)

EMR fits into current sample preparation workflows

EMR Sorbent - What is it?



1.0 g EMR in 15 mL tube

When “activated” by water...

- The materials **selective hydrophobic interactions** increase.
- **Suspension of nano particles** (high surface area).
- Rapidly **interacts with straight chain, “lipid-like” functional groups.**

Centrifugation preferably used to separate precipitate from solution (*not filtration*).

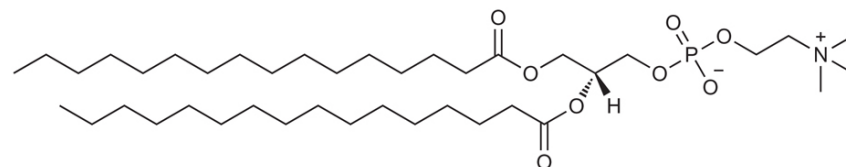
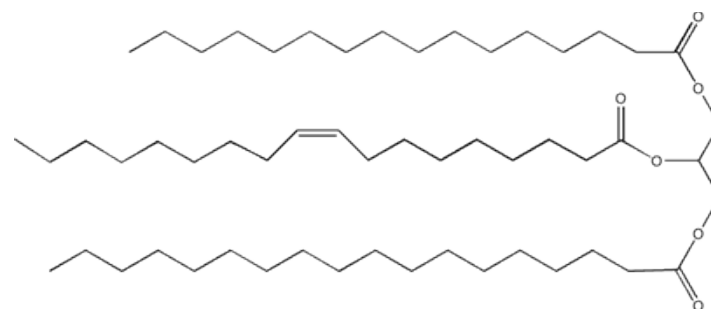
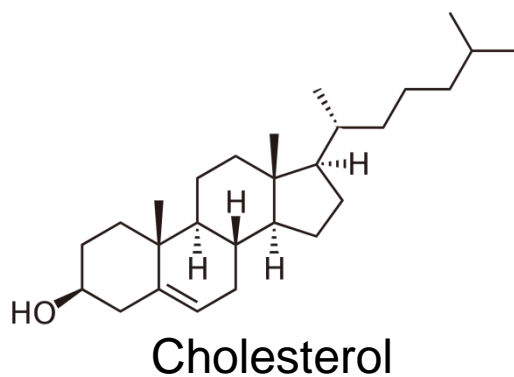
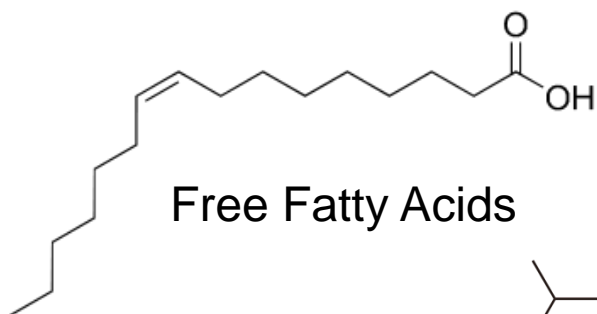
EMR-Lipid Mechanism – Size exclusion and hydrophobic interaction.

... and what does it do?

EMR sorbent removes Lipids

What are Lipids?

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

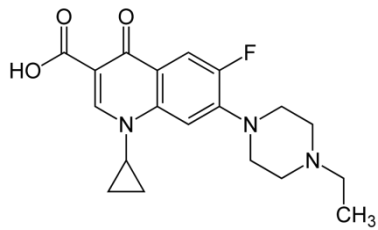


What Does EMR *NOT* Interact With?

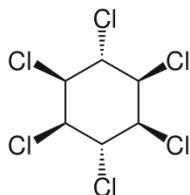
EMR does **NOT** remove analytes of interest

Exceptions?

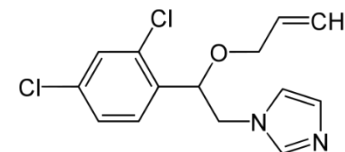
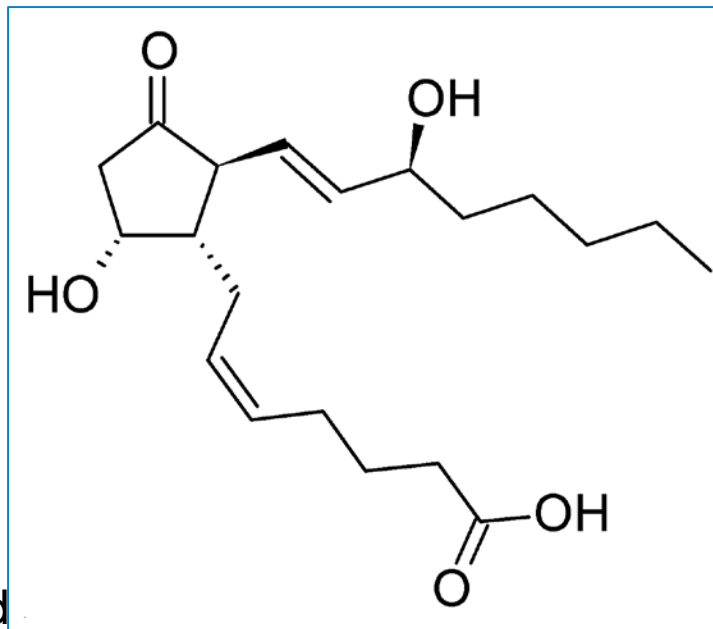
Compounds containing long aliphatic functional groups (e.g. prostaglandins)



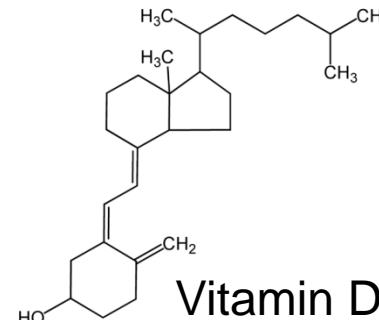
Fluoroquinolones



Organochlorine Pesticide



midazole pesticides



Vitamin D



EMR Fits into Existing Workflows

QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe)

- Easy-to-use sample preparation for food testing, solid samples (e.g. vegetables, fruits, meat, seafood, etc.)

EMR Applications: - Pesticide Residues in Avocado,

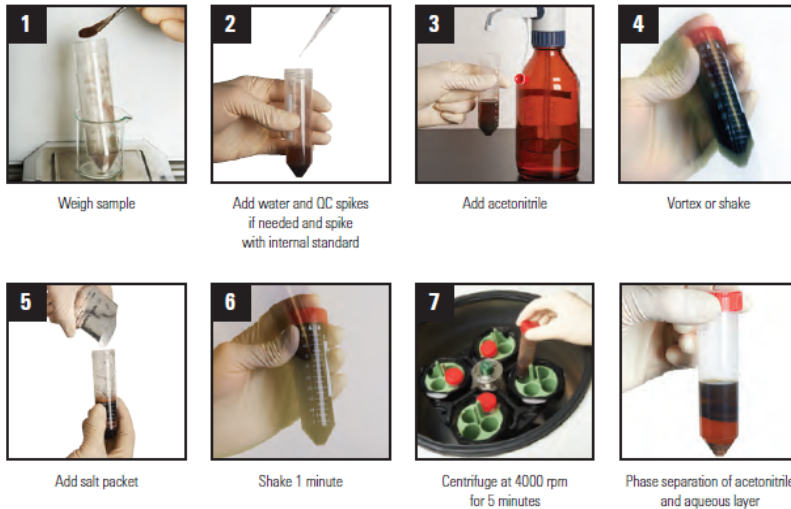
Modified Liquid Extraction (Protein Precipitation)

- Proteins are removed by a “crash” step prior to injection or cleanup (e.g. milk, meat, seafood, etc.)

EMR Applications: - PAHs in Salmon,
- Veterinary Drugs in Bovine Liver

Improving dSPE in QuEChERS

1. Extraction



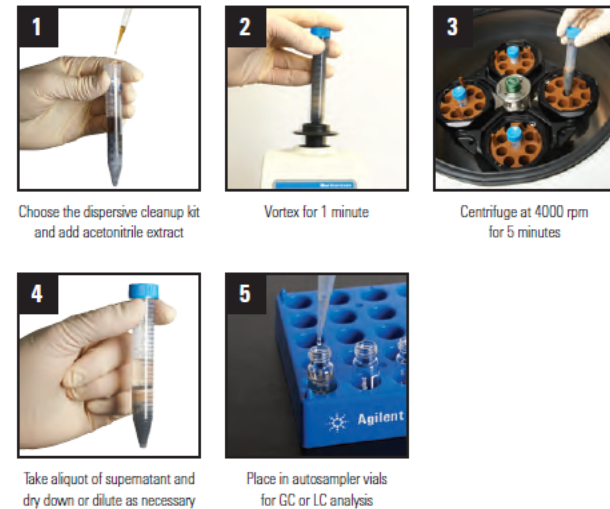
Pros

- Fast and inexpensive
- Takes minimal experience
- Doesn't require special equipment
- Accommodates multiple matrices
- Accommodates large analyte groups

Cons

- Large amount of coextractives

2. Dispersive SPE



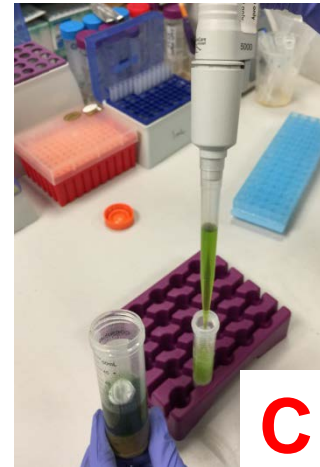
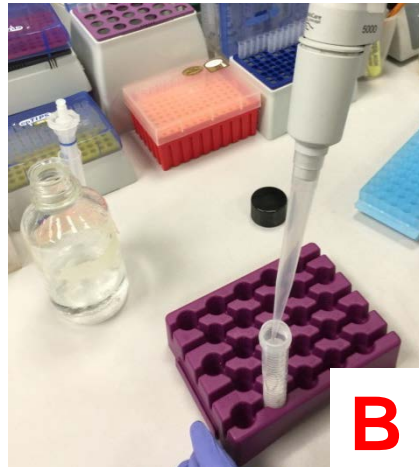
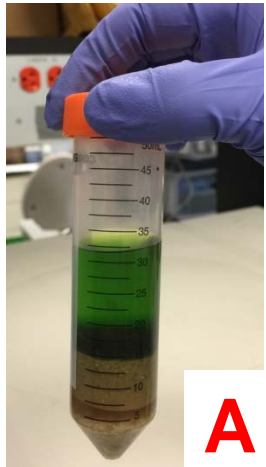
Pros

- Same as extraction

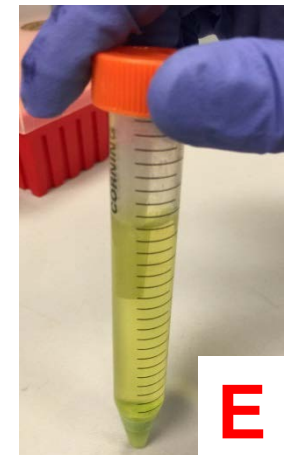
Cons

- Minimal cleanup provided
- Can remove analytes
- Lipids are challenging to remove selectively

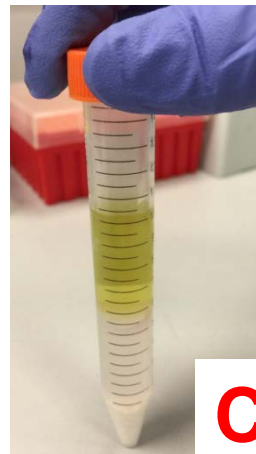
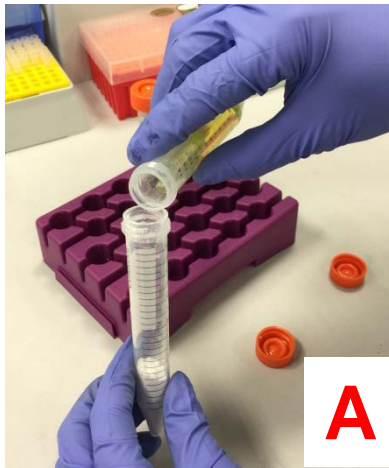
EMR – Lipid – dSPE Cleanup



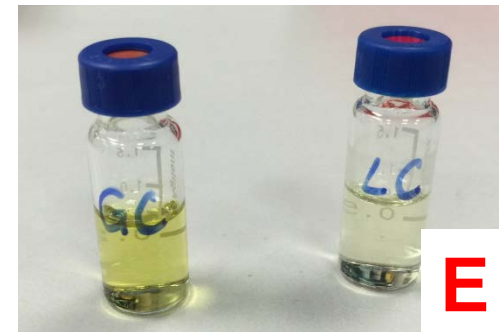
- A. QuEChERS or Liquid Extract**
- B. Add H₂O to EMR tube (“activation”)**
- C. Transfer extract**
- D. Vortex and centrifuge**
- E. Supernatant (1:1; extract: H₂O)**



EMR – Polish – ACN/H₂O Phase Separation



- A. Add supernatant to EMR – Polish tube**
- B. Vortex immediately**
- C. Phase separation after centrifuge**
- D. Transfer upper layer for analysis**
- E. Final samples split for GC and LC analysis**
- F. Extra dry step recommended prior to GC**

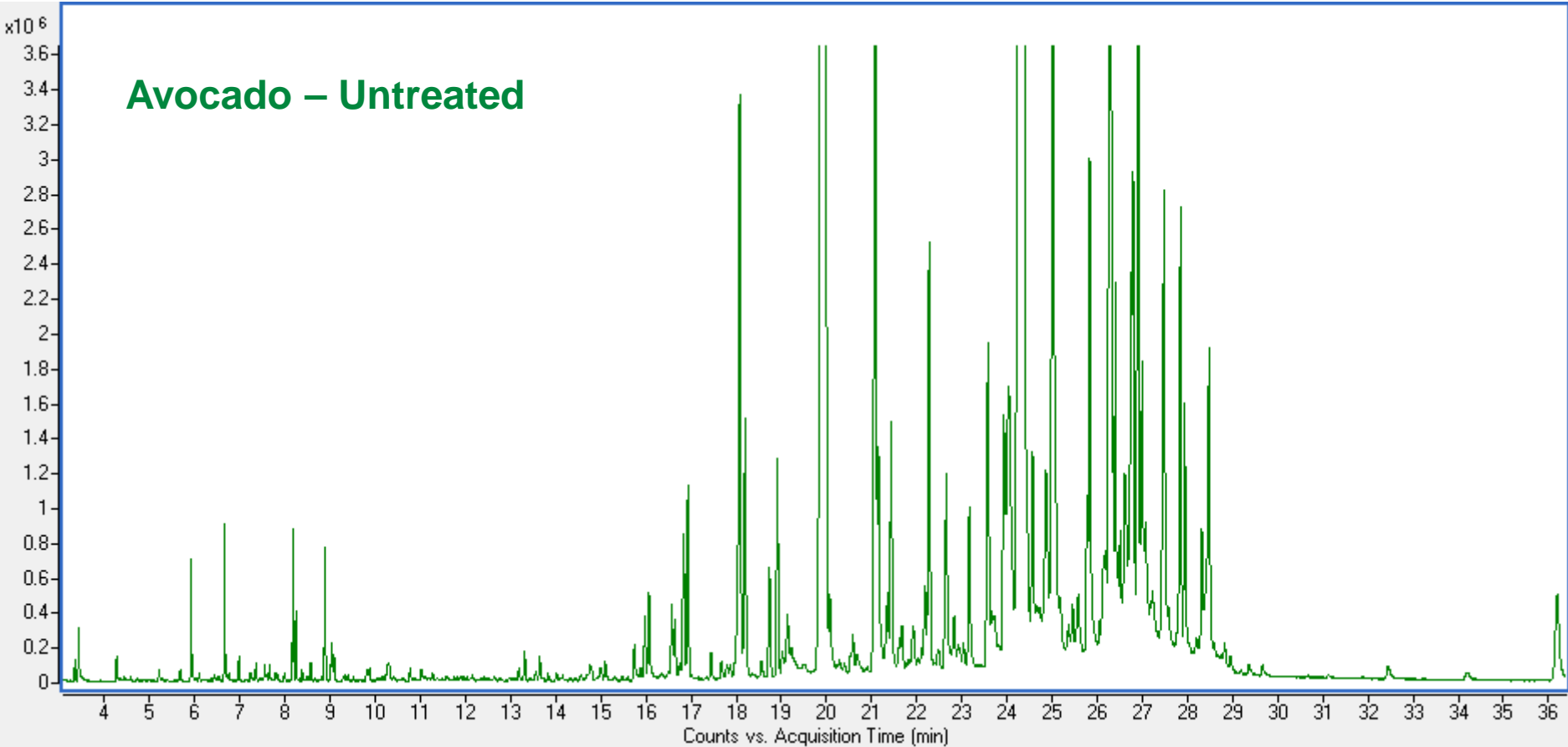


EMR Workflow

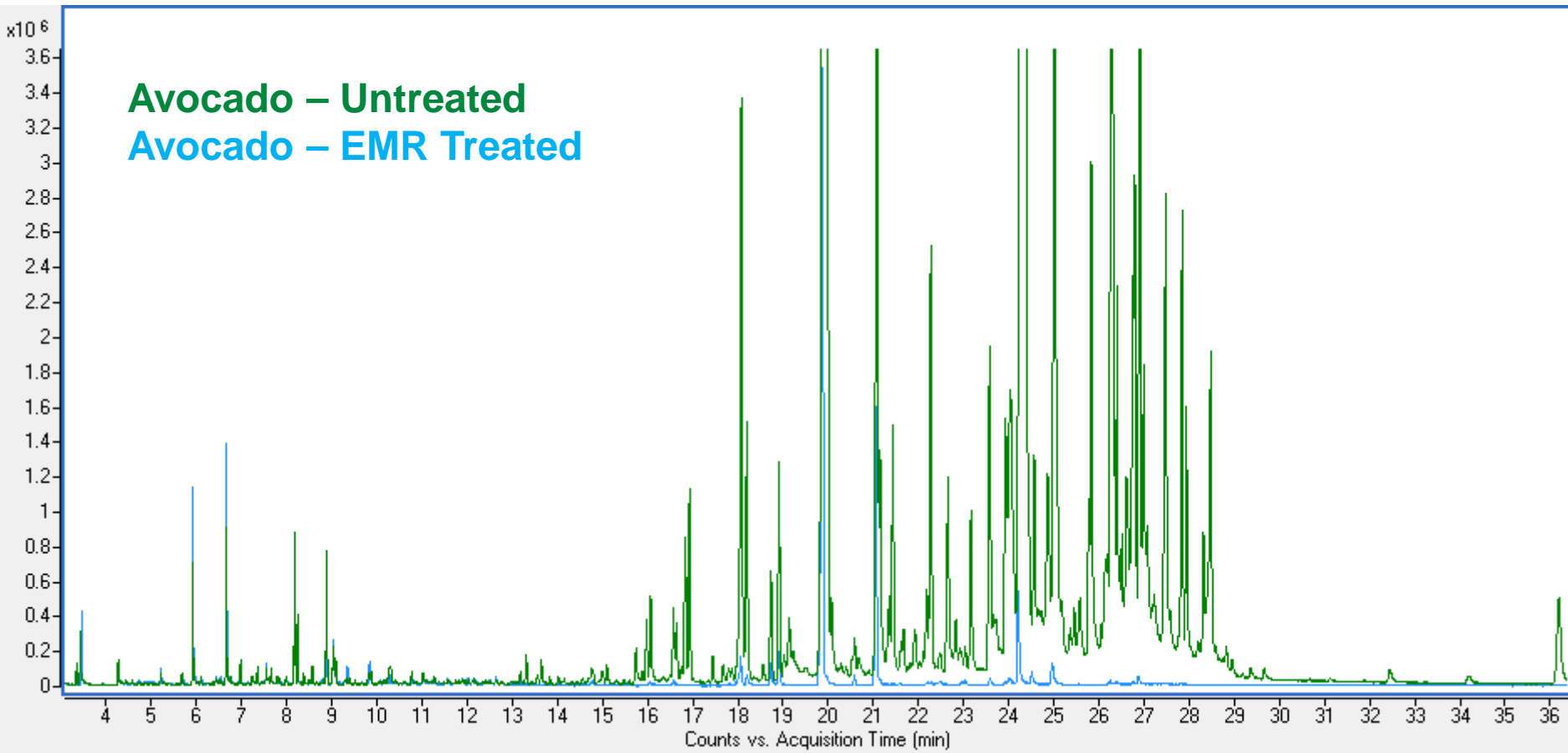


In acetonitrile
Or acetone

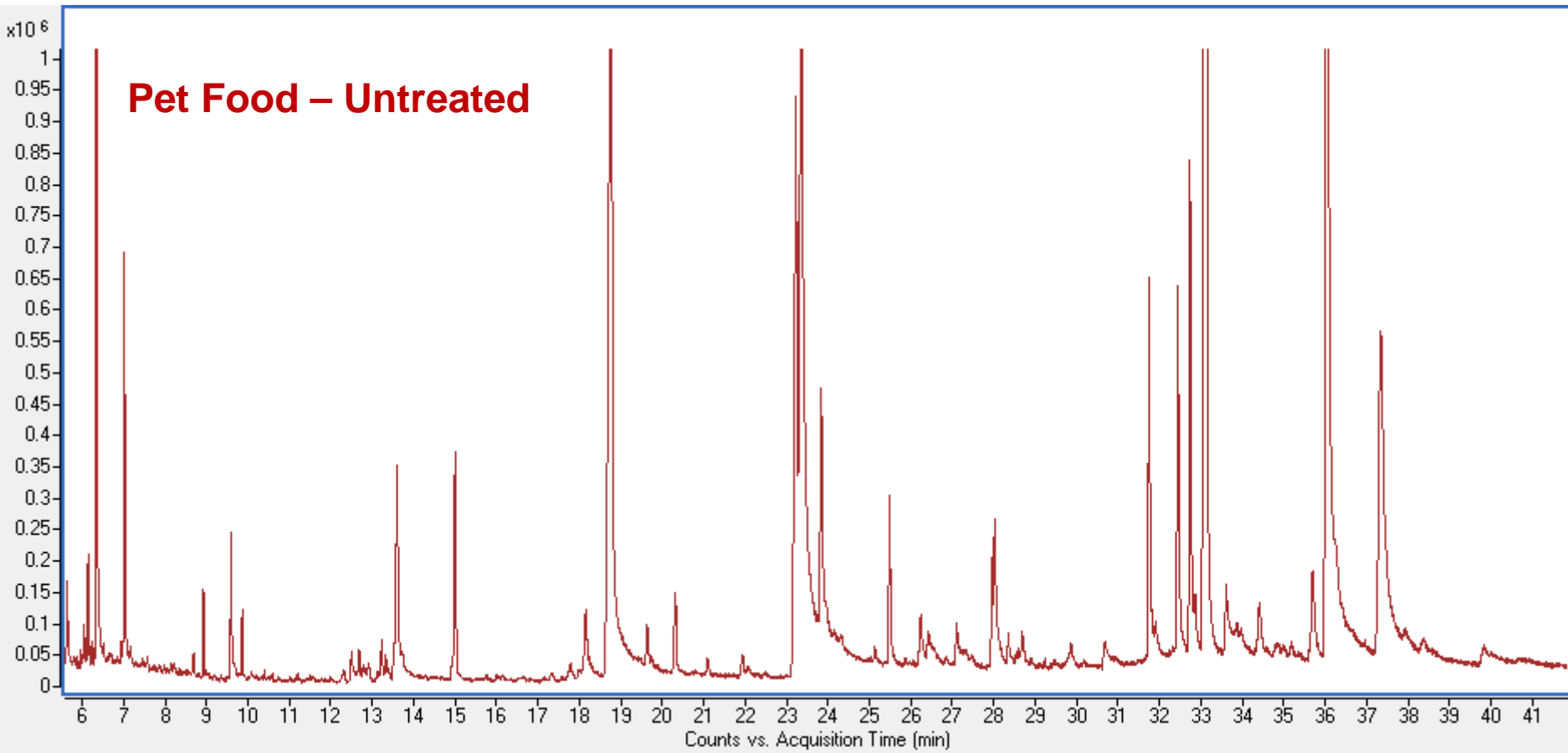
GC-MS Fullscan Avocado



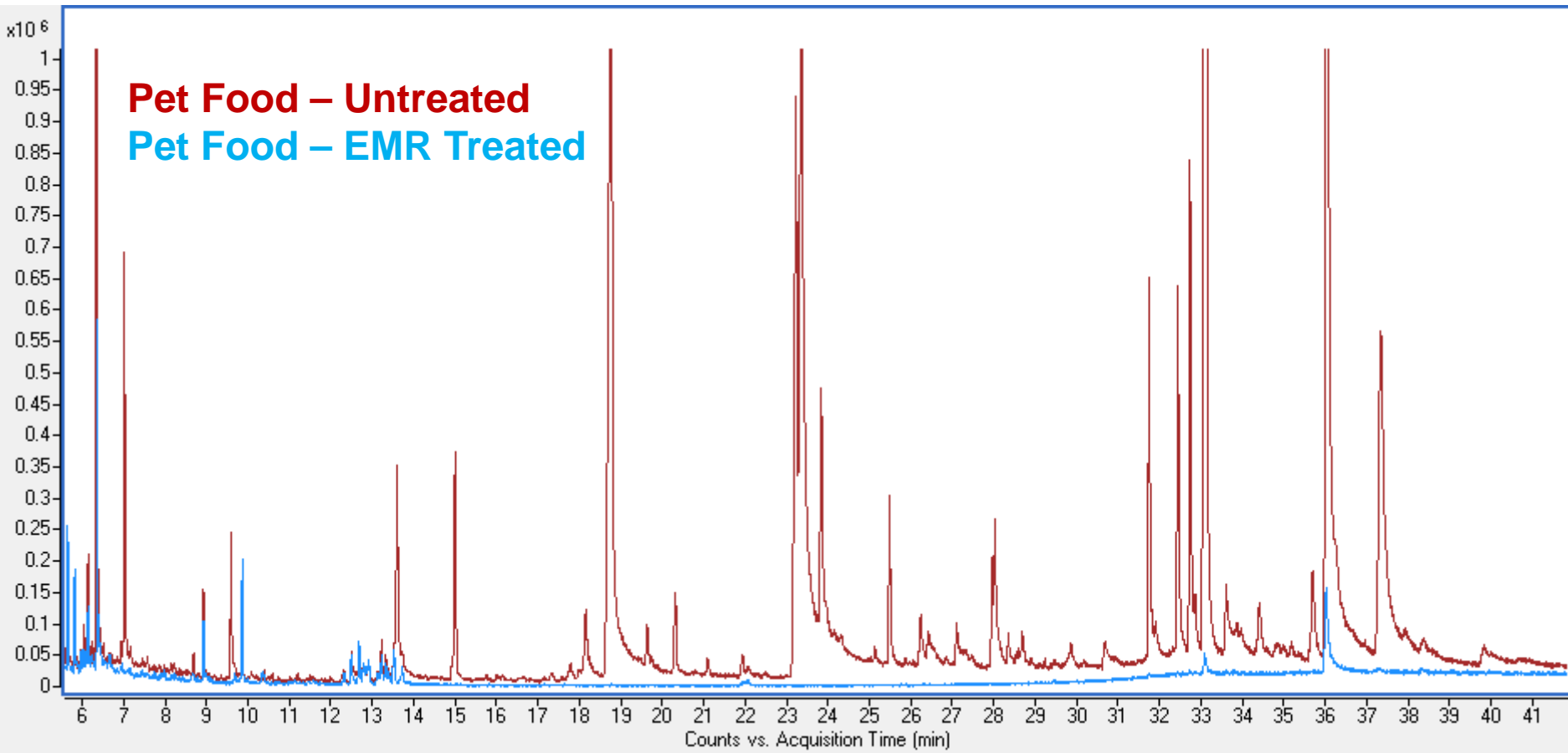
GC-MS Fullscan Avocado



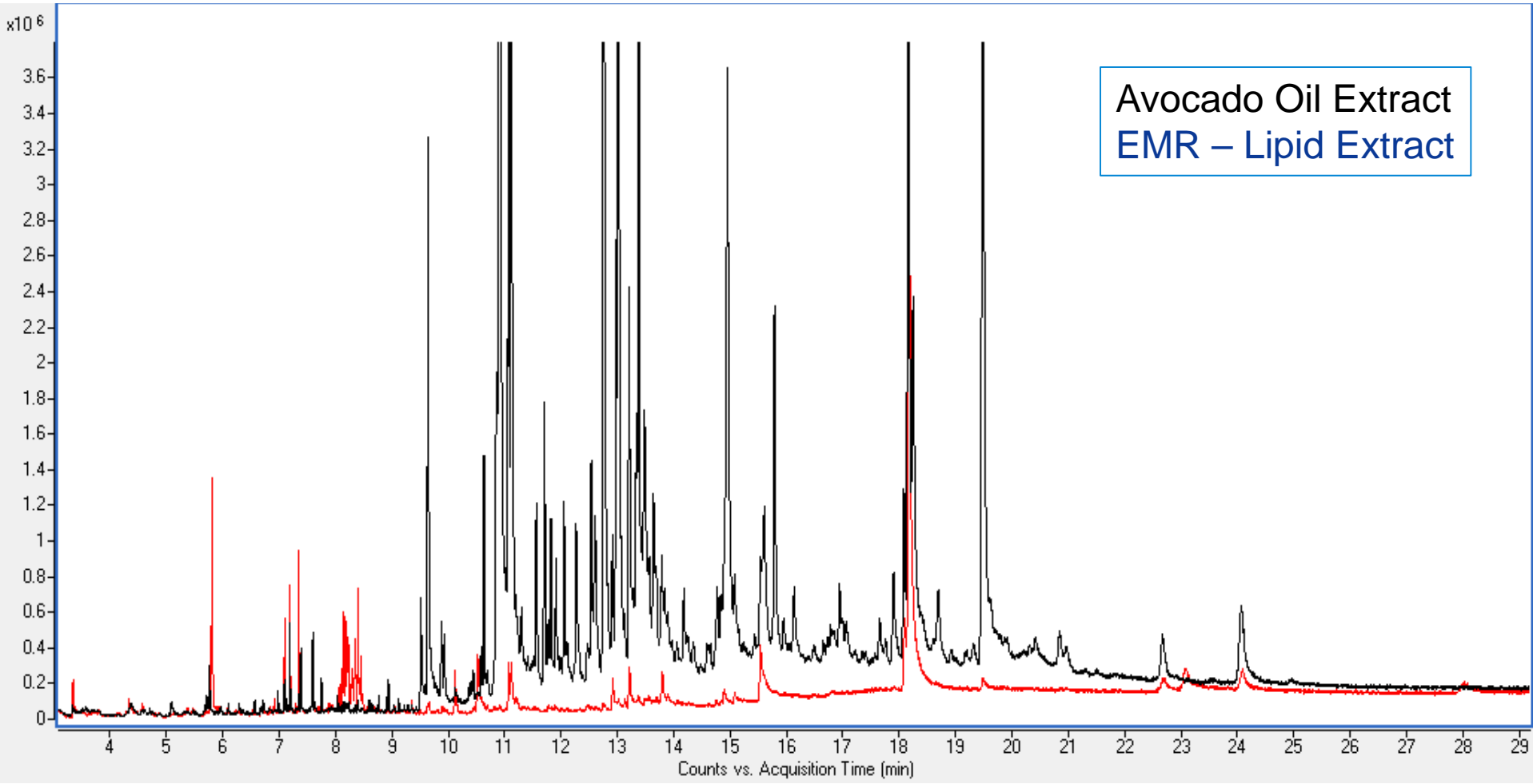
GC-MS Fullscan Pet Food



GC-MS Fullscan Pet Food

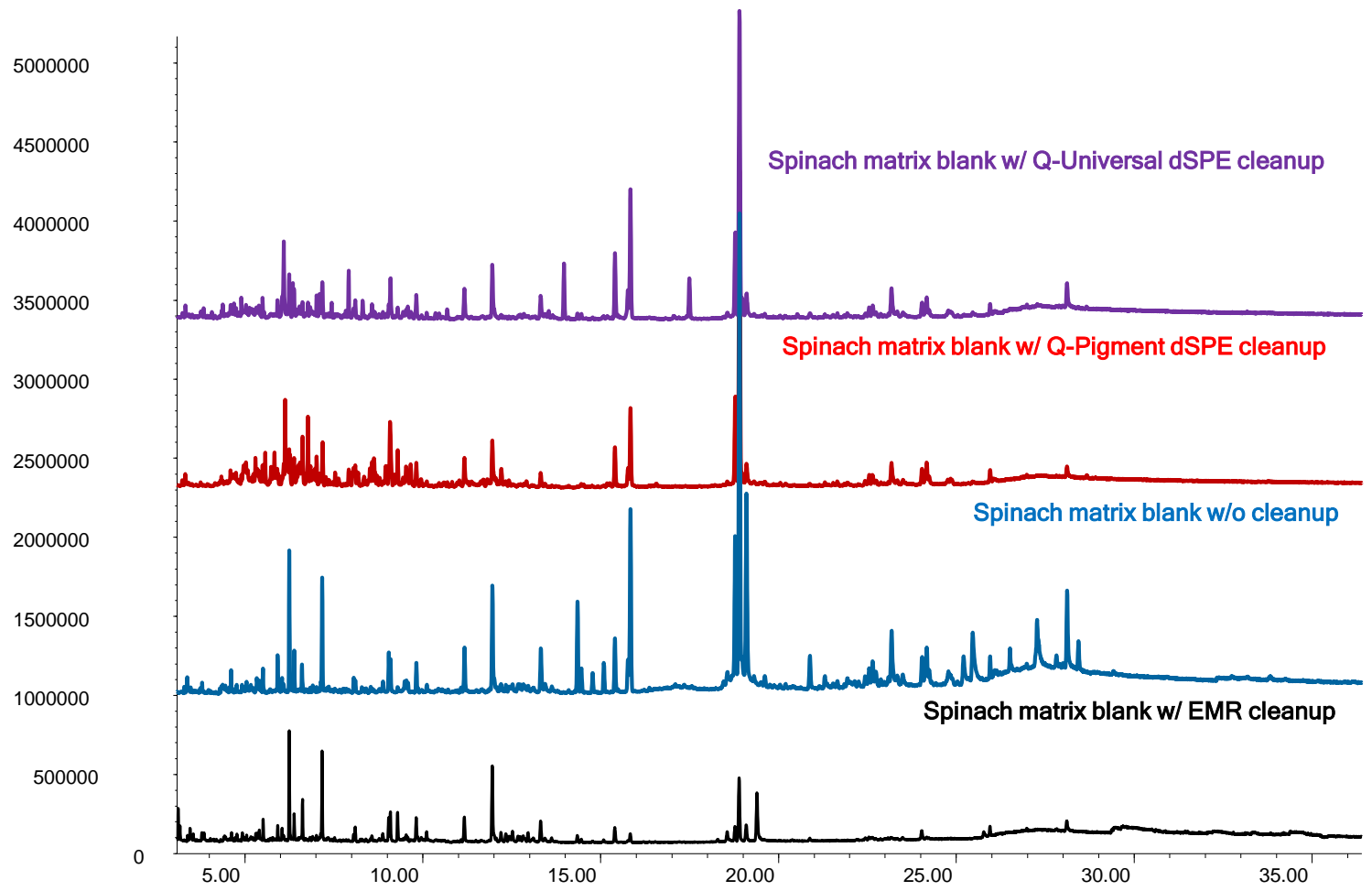


GC-MS Full Scan- Avocado Oil



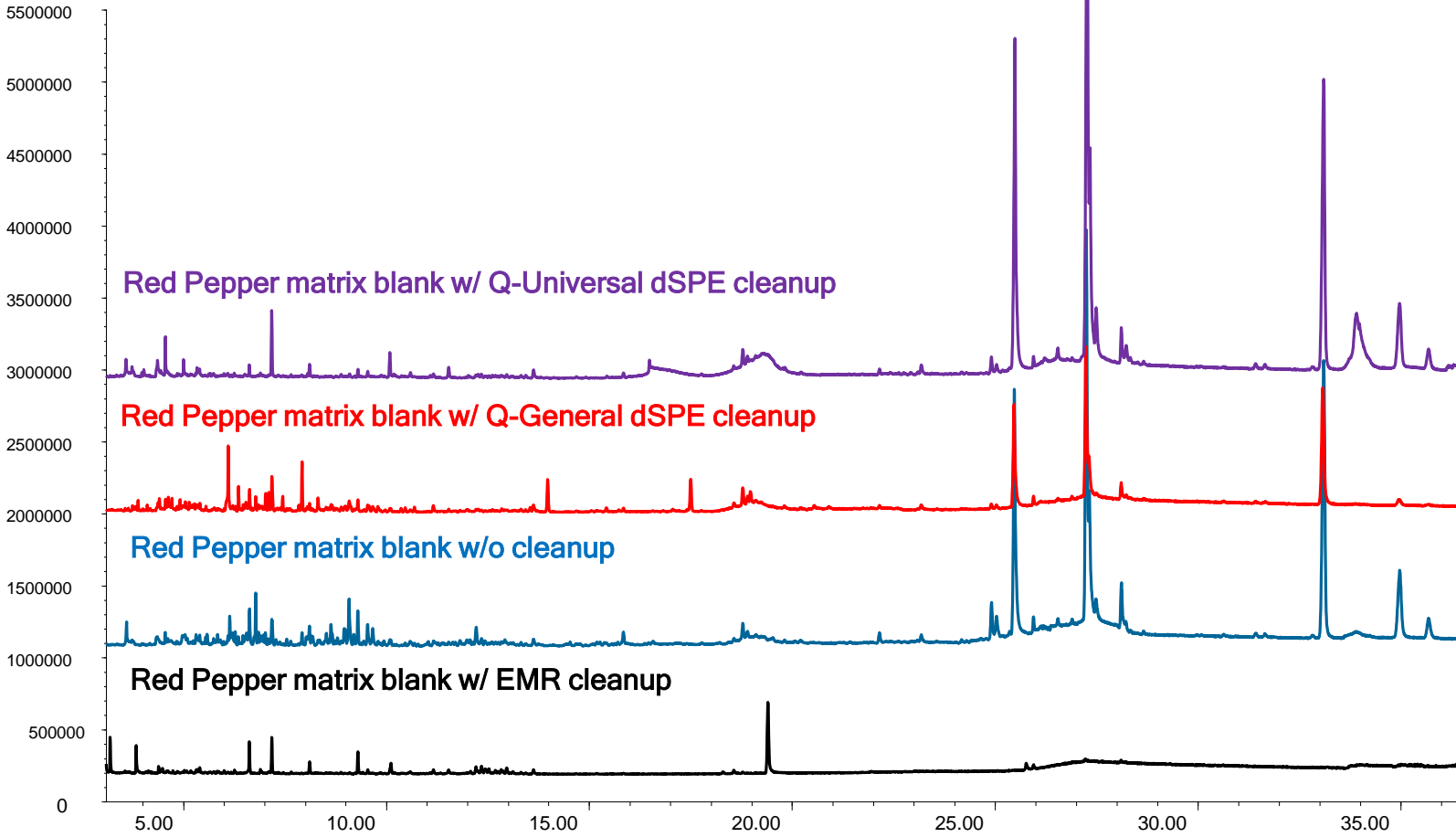
GC-MS Spinach Fullscan

Abundance



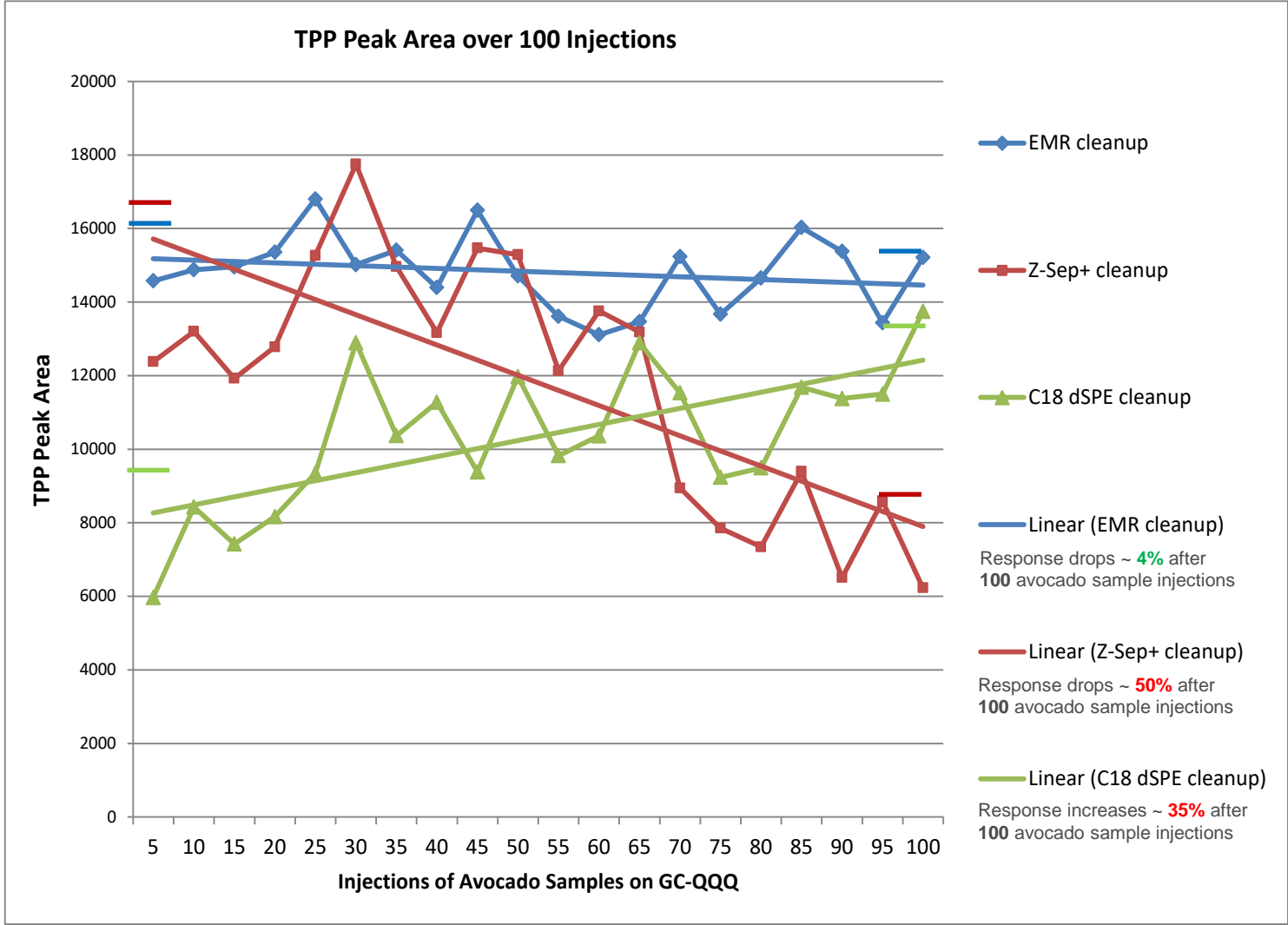
Time-->

GC-MS Red Pepper Fullscan



Benefits to Instrumental Flowpath

Comparison of Analytes Response Consistency over Multiple Avocado Sample Injections

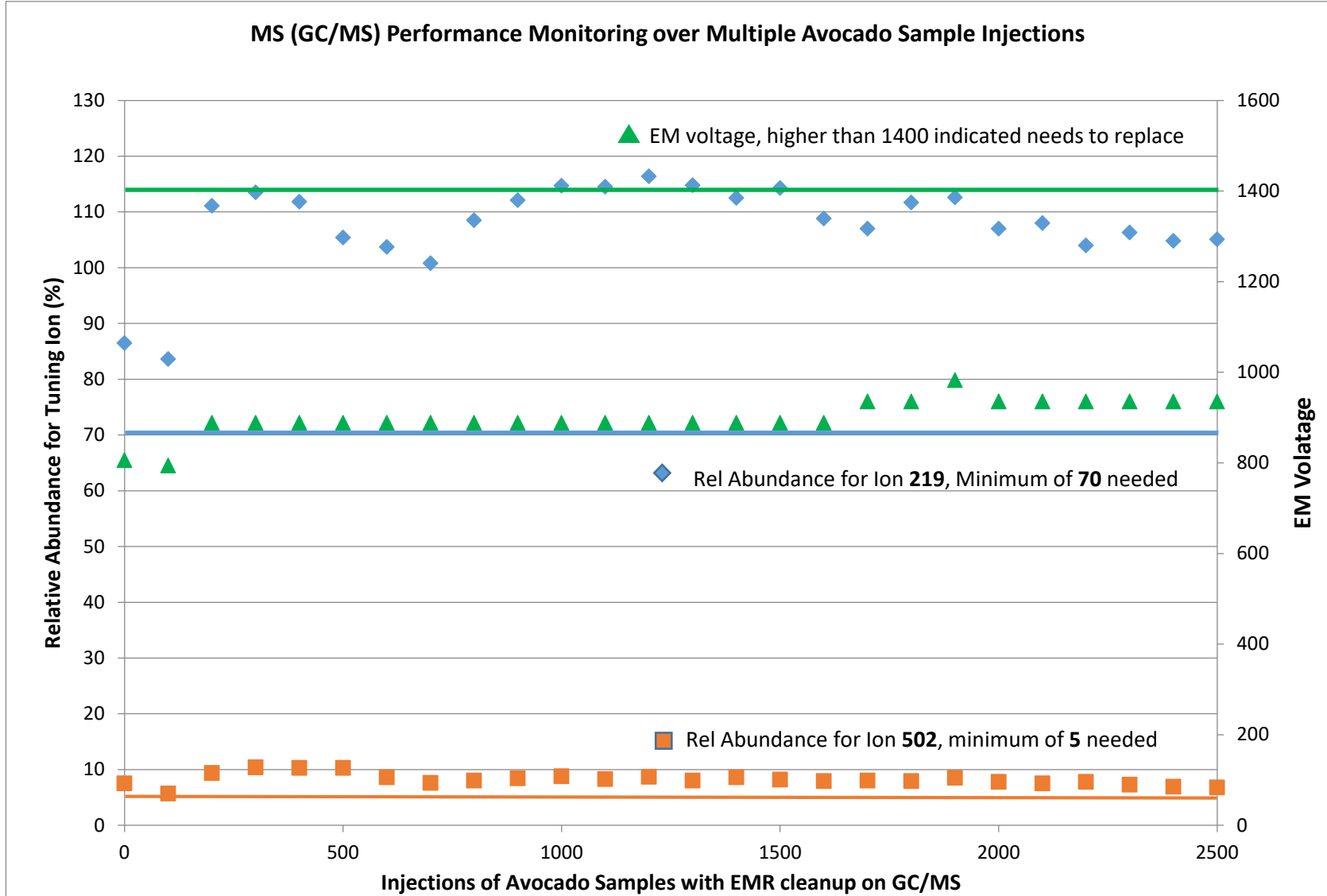


TPP
 Commonly used IS
 RT: 18.3 min

Analytes Responses Reproducibility on GC/MS/MS over 100 Injections of Avocado Samples

Pesticides	Analytes RSD over 100 injections on GC/QQQ (n = 20)			RSD over 50 injections on GC/QQQ (n = 10)		
	EMR Cleanup	C18/PSA cleanup	Zirconia sorbent cleanup	EMR Cleanup	C18/PSA cleanup	Zirconia sorbent cleanup
Dichlorvos	6.2	10.5	16.8	2.2	9.4	6.3
2-Phenylphenol	7.0	13.6	19.5	5.0	12.4	8.4
Ethalfuralin	12.4	18.8	32.0	5.8	10.3	7.9
Sulfotep	7.1	11.8	17.2	3.1	6.4	10.8
Atrazin	6.8	12.2	19.1	3.2	12.2	5.2
Lindane	8.5	10.8	20.0	4.6	10.9	5.1
Chlorothalonil	12.5	11.7	37.4	8.0	12.9	11.0
Diazinon	6.6	11.7	16.9	4.4	10.5	5.6
Chlorpyriphos-methyl	8.4	8.9	14.9	3.8	8.6	6.6
Dichlorfluanid	11.7	9.0	25.9	5.4	9.9	5.5
Aldrin	9.8	19.3	25.7	8.6	19.3	7.1
Tolyfluanid	10.5	6.6	17.8	4.2	6.9	6.6
Captan	29.9	51.9	47.1	11.1	24.9	21.7
Procymidone	6.8	14.3	22.5	5.6	13.8	4.8
Bupirimate	6.8	10.4	20.7	7.6	11.0	6.2
Endrin	8.3	12.6	24.1	5.9	13.8	5.4
Endosulfan sulfate	8.5	12.1	22.4	5.3	12.7	6.4
DDT	21.6	22.4	42.6	6.4	12.0	11.8
Iprodione	11.0	10.7	40.0	8.2	10.9	16.3
Permethrin	6.8	11.8	18.8	5.2	11.2	8.6
Parathion ethyl-D10 (IS)	11.8	7.2	13.0	4.7	6.8	7.0
TPP (IS)	9.1	19.9	28.3	9.0	22.5	12.8

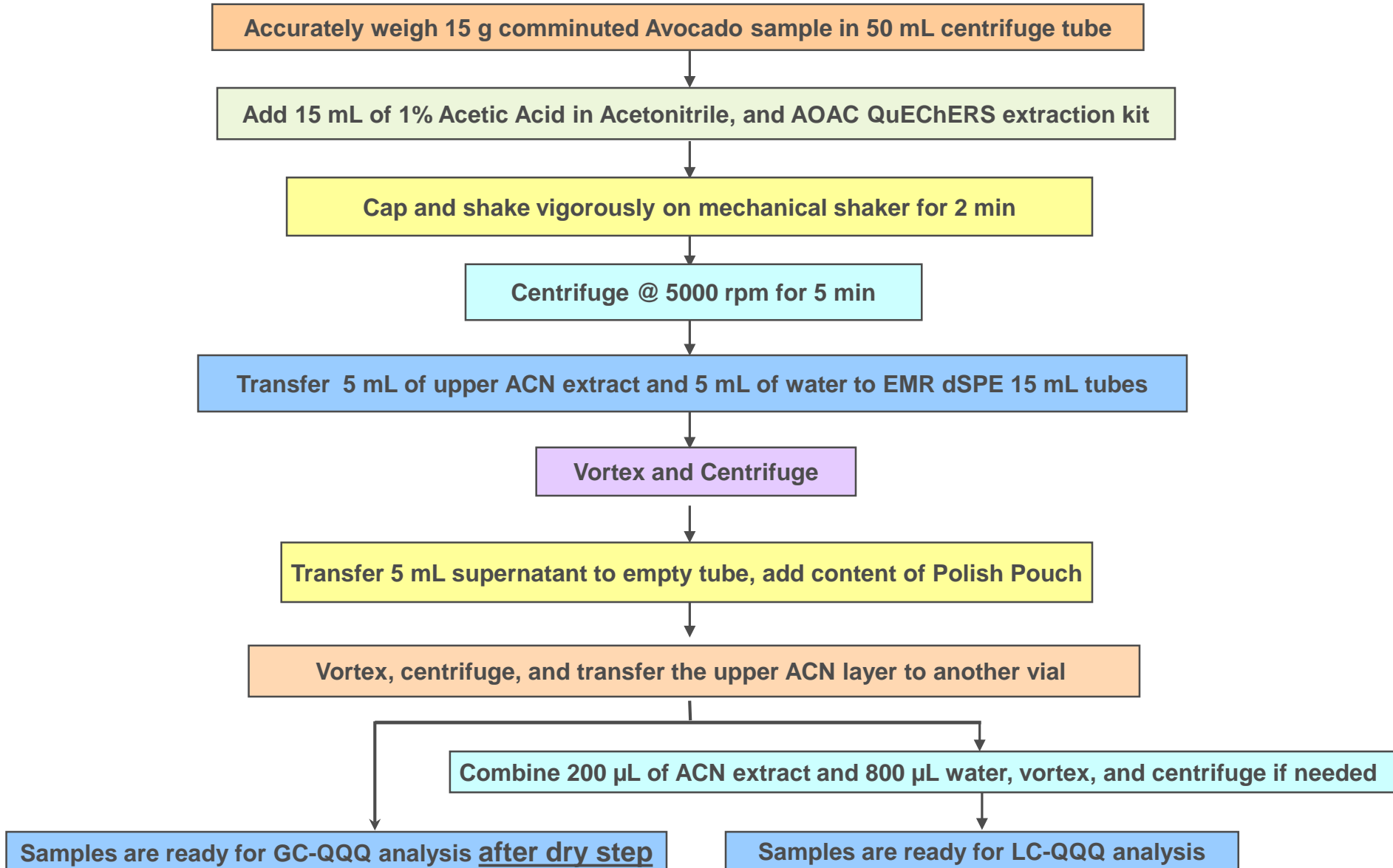
MS Source Critical Tuning Parameters



67 Pesticides Analysis in Avocado by LC and GC-QQQ

Representative Pesticide	Chemical Class	Pesticide Group	Detection Technique	Representative Pesticide	Chemical Class	Pesticide Group	Detection Technique	Representative Pesticide	Chemical Class	Pesticide Group	Detection Technique			
Dichlorvos	Organophosphate	Herbicide	GC-MS	Methamidophos	Organophosphate	Insecticide	LC-MS	Simazine	Triazine	Herbicide	LC-MS			
Sulfotep				Acephate						Sebuthylazine		Algaecide		
Diazinon				Omethoate						Terbuthylazine				
Chlorpyrifos methyl				Dimethoate					Carbofuran	Carbamate		Insecticide		
Coumaphos				Phosmet					Methiocarb					
Trichlorfon				Carbaryl				Carbamate	Chlorpropham					
Lindane		Organochlorine		Insecticide				Propoxur	Sulphamide	Fungicide			Propham	Urea
Aldrin					Dichlofluanid			Monuron						
Endrin					Tolyfluanid			Chlorotoluron						
DDT					Carbendazim	Benzimidazole		Diuron						
Endosulfan sulfate					Thiabendazole			Fluometuron						
Methoxychlor					Thiophanate methyl			Isoproturon						
2-Phenylphenol	Phenol				Fungicide	Cyprodinil		Anilinopyrimidine					Metobromuron	
Atrazine	Triazine	Herbicide		Imidacloprid	Neonicotinoid	Insecticide		Siduron	Chlorophenoxy acid					
Bupirimate	Pyrimidinol	Fungicide		Pymetrozine	Pyridine					Linuron				
Chlorothanil	Chloronitrile			Imazalil	Imidazole	Fungicide		Neburon						
Captan	Phthalimide			Penconazole	Triazole					2,4-D Acid				
Folpet					Aminocarb	Carbamate		Insecticide		Dichlorprop				
Captafol					Oxamyl							Metazachlor	Chloracetanilide	
Iprodione	Dicarboximide			Methomyl						Bentazon		Unclassified		
Procymidone					Aldicarb					Malathion		OP	Insecticide	
Permethrin	Pyrethroid	Insecticide	Fenuron	Urea	Herbicide									
Deltamethrin				Metoxuron										
Pyraclostrobin	Strobilurin	Fungicide					Monocrotophos							
Ethalfuralin	Dinitroaniline	Herbicide												

QuEChERS-EMR Protocol for Multi-residue Analysis of Pesticides in Avocado



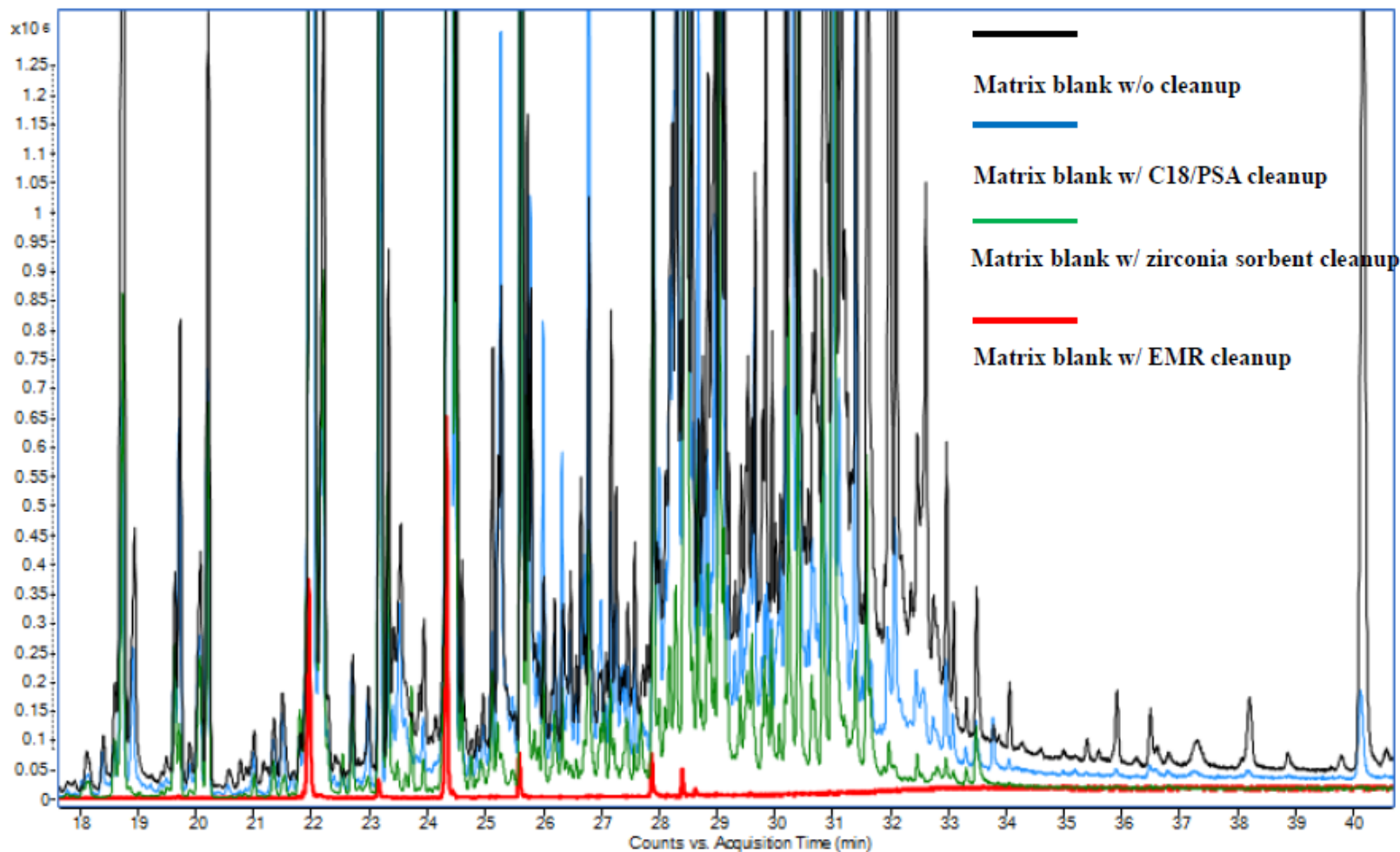
Comparison of Avocado Co-extractives by Weight

Cleanup	Amount of co-extractives (mg, n= 3)	Amount of co-extractives removed by cleanup (mg, n = 3)	% of matrix co-extractives removed by cleanup
No further cleanup	14.7	--	
C18/PSA Cleanup	9.5	5.2	35.4
EMR-Lipid Cleanup	4.2	10.5	71.4
Zirconia sorbent Cleanup	7.0	7.7	52.4

$$\% \text{ Matrix Co-extractives Removed by Cleanup} = \frac{\text{Amount of Co-extractives Removed after Cleanup}}{\text{Amount of Co-extractives without Cleanup}} \times 100\%$$

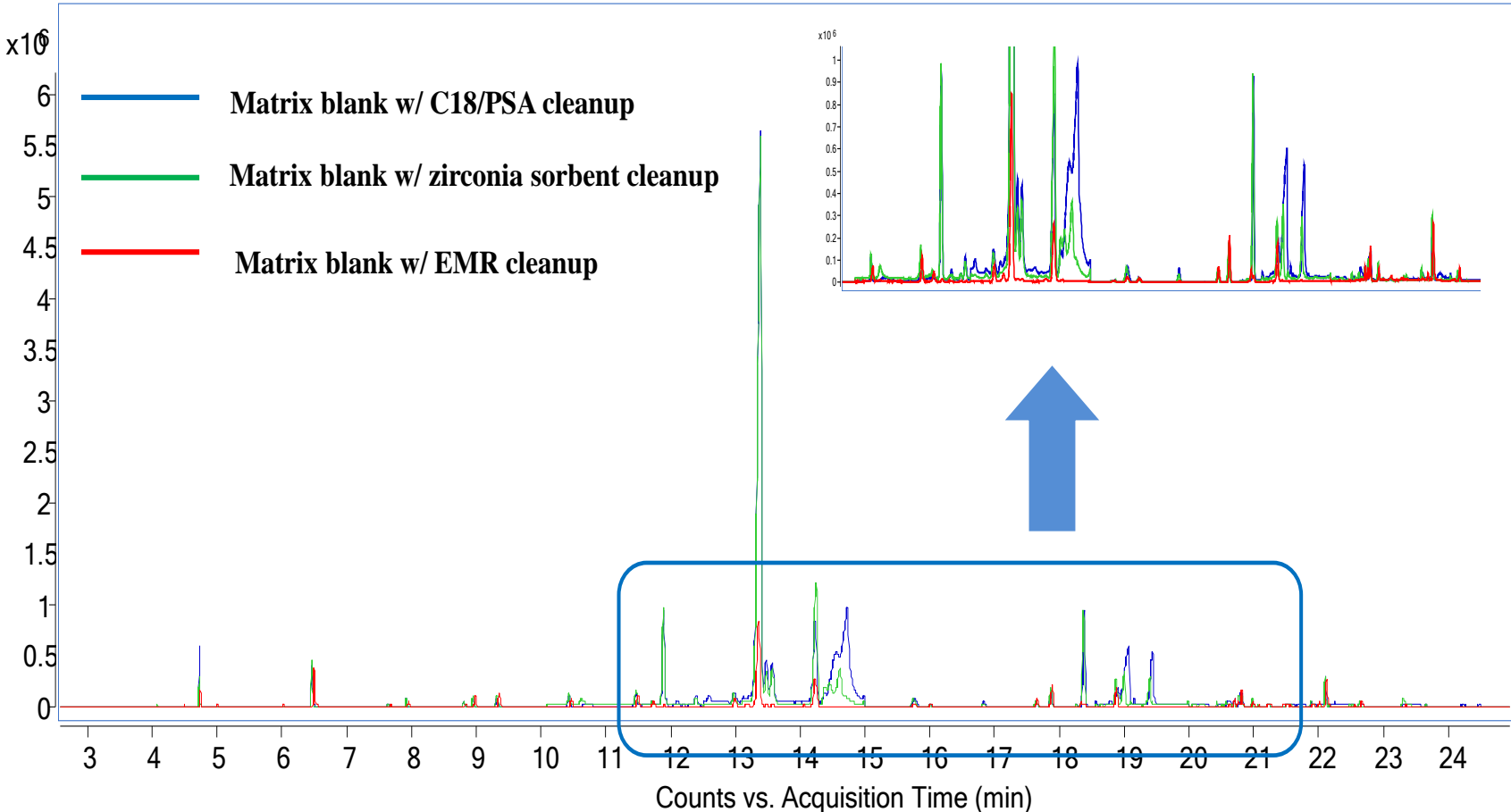
The use of EMR material cleanup removes extra 20-30% of Avocado co-extractives in comparison to traditional QuEChERS and/or competitor's cleanup

Comparison of GC/MS Full-scan Chromatogram for Matrix Background

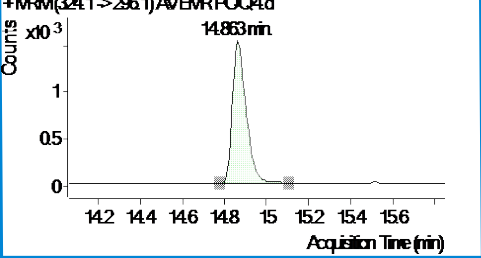
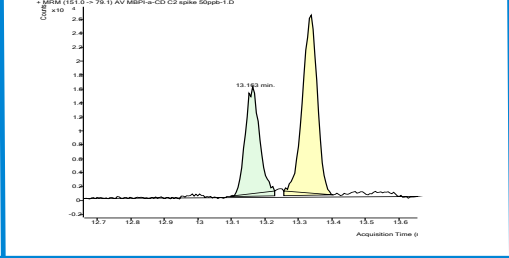
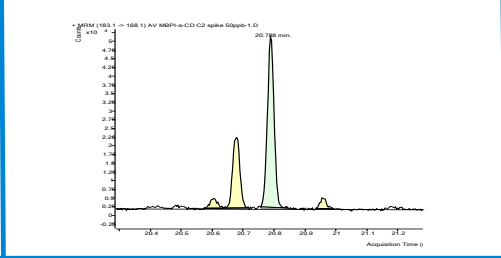
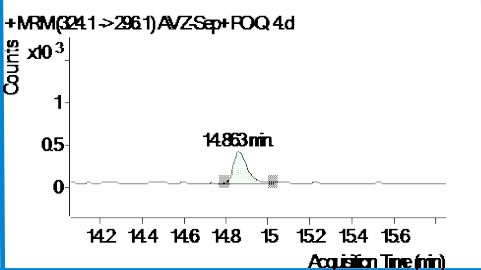
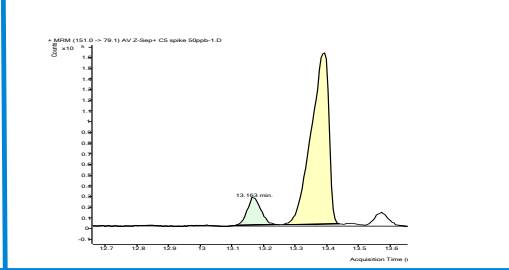
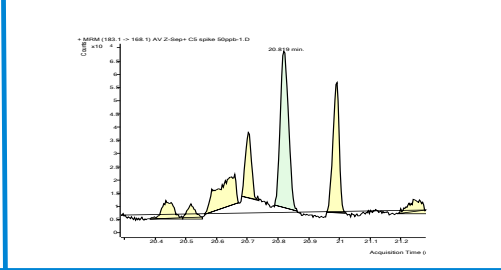
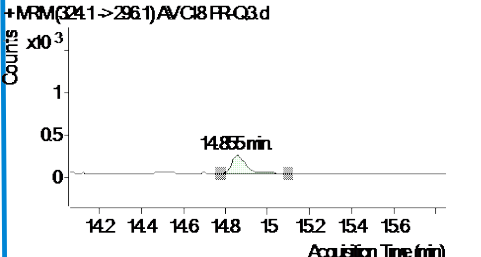
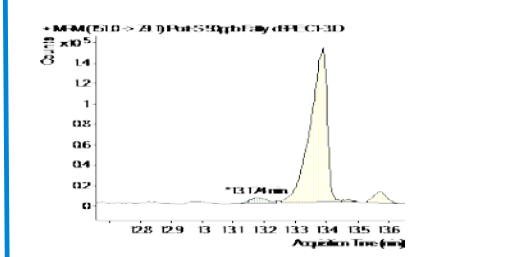
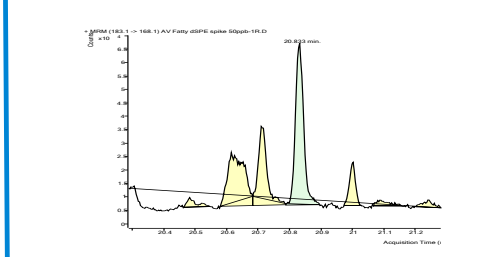


The use of EMR material cleanup provides significantly cleanup chromatographic sample background.

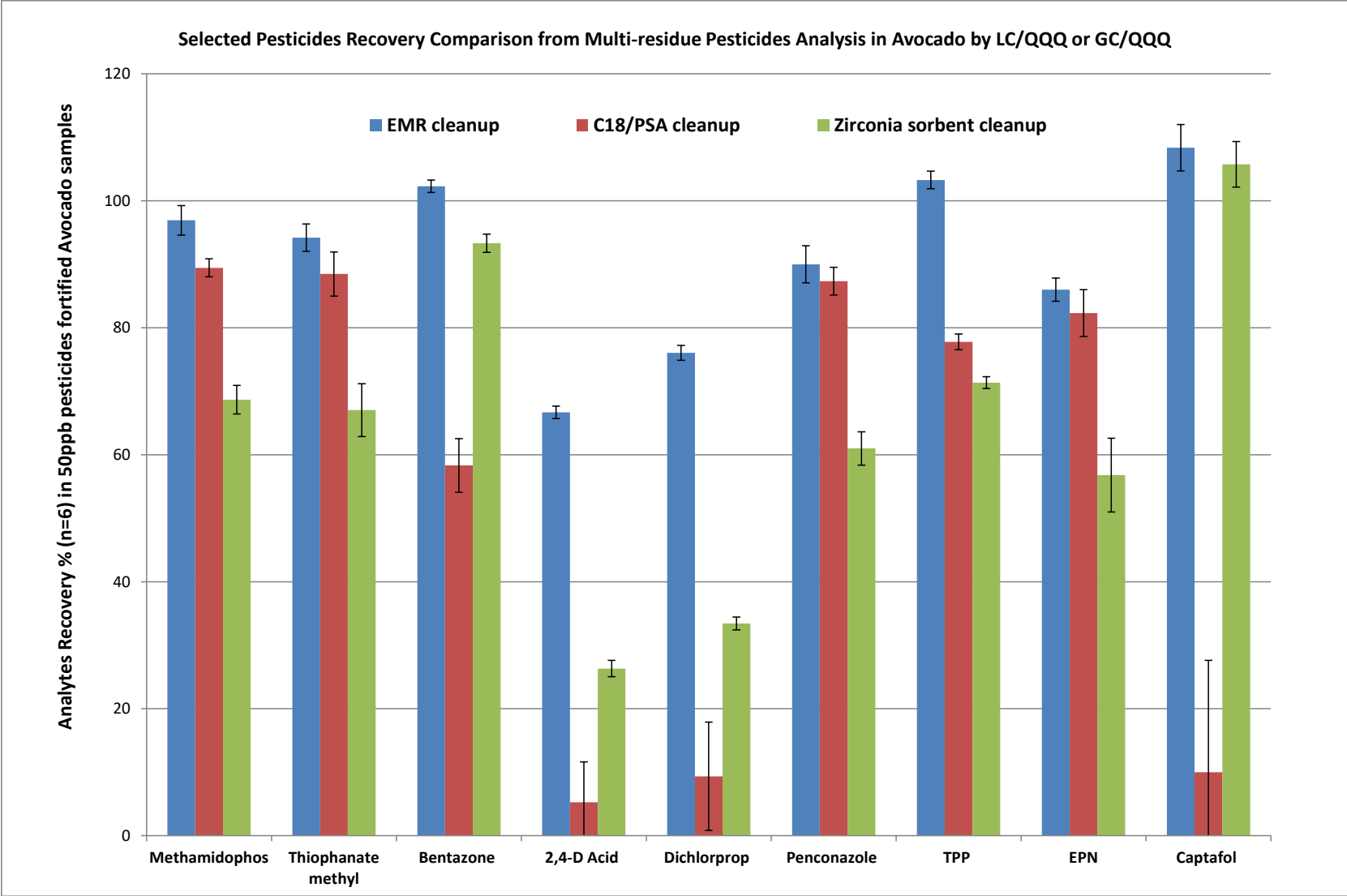
Comparison of GC/MS/MS MRM Chromatogram for Matrix Background



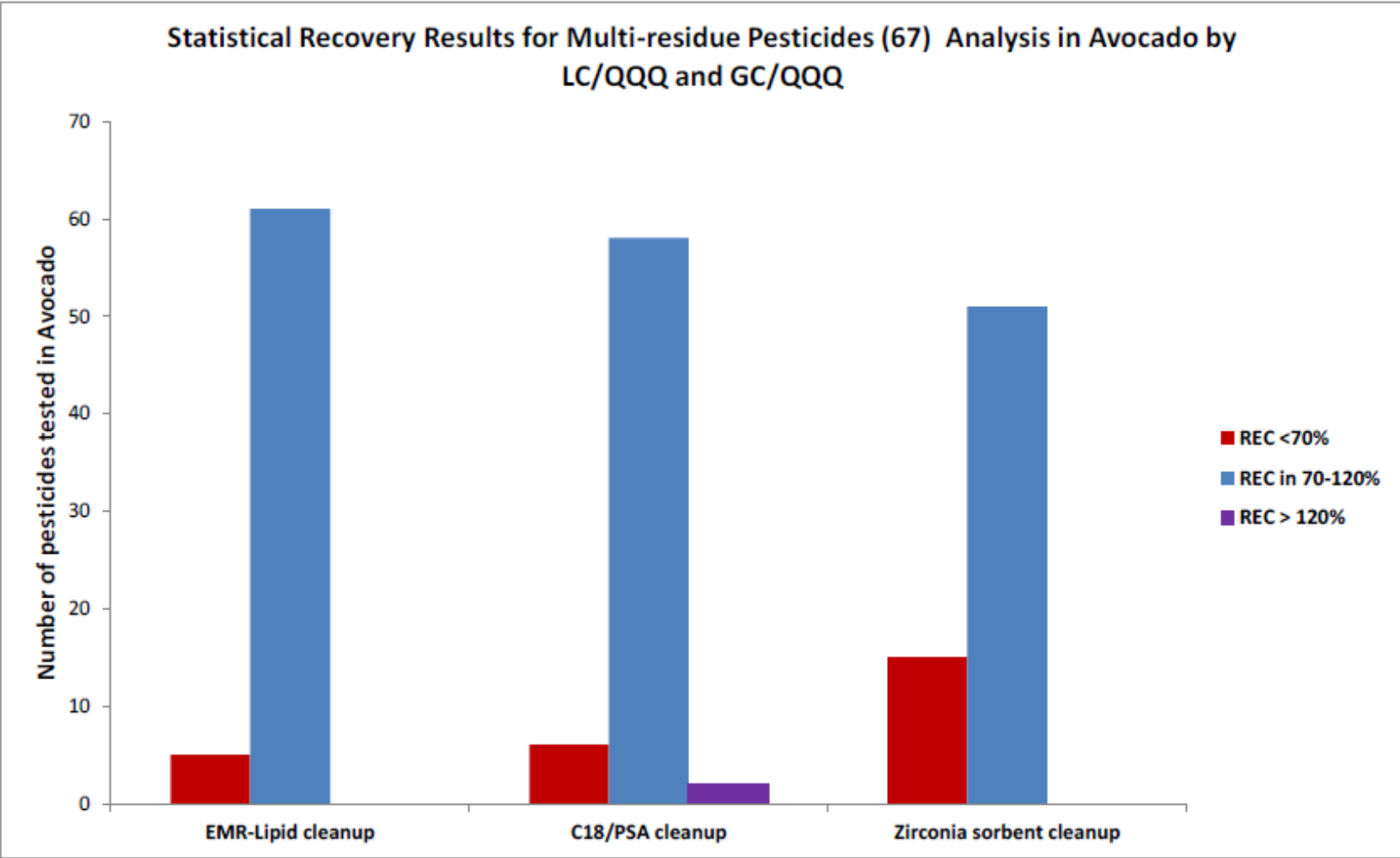
Chromatographic Benefits of Matrix Removal Provided by EMR Cleanup

Benefit	Reduced matrix suppression	Improved S/N ratio	Less interferences for accurate integration
Example	EPN in Avocado on LC-QQQ	Captan in Avocado on GC-QQQ	Permethrin in Avocado on GC-QQQ
EMR-Lipid cleanup	<p>+MRM(324.1 → 296.1)AV/EMRFOQ4d</p> 	<p>+MRM(151.0 → 79.1)AV/MBP+CD/CD/epine/50ppb-1.D</p> 	<p>+MRM(183.1 → 168.1)AV/MBP+CD/CD/epine/50ppb-1.D</p> 
Zirconia sorbent cleanup	<p>+MRM(324.1 → 296.1)AV/ZSp+FOQ 4d</p> 	<p>+MRM(151.0 → 79.1)AV/2-Dep+CD/epine/50ppb-1.D</p> 	<p>+MRM(183.1 → 168.1)AV/2-Dep+CD/epine/50ppb-1.D</p> 
C18/PSA cleanup	<p>+MRM(324.1 → 296.1)AV/C18/PSA/Q3d</p> 	<p>+MRM(151.0 → 79.1)AV/2-Dep+CD/epine/50ppb-1.D</p> 	<p>+MRM(183.1 → 168.1)AV/2-Dep+CD/epine/50ppb-1.D</p> 

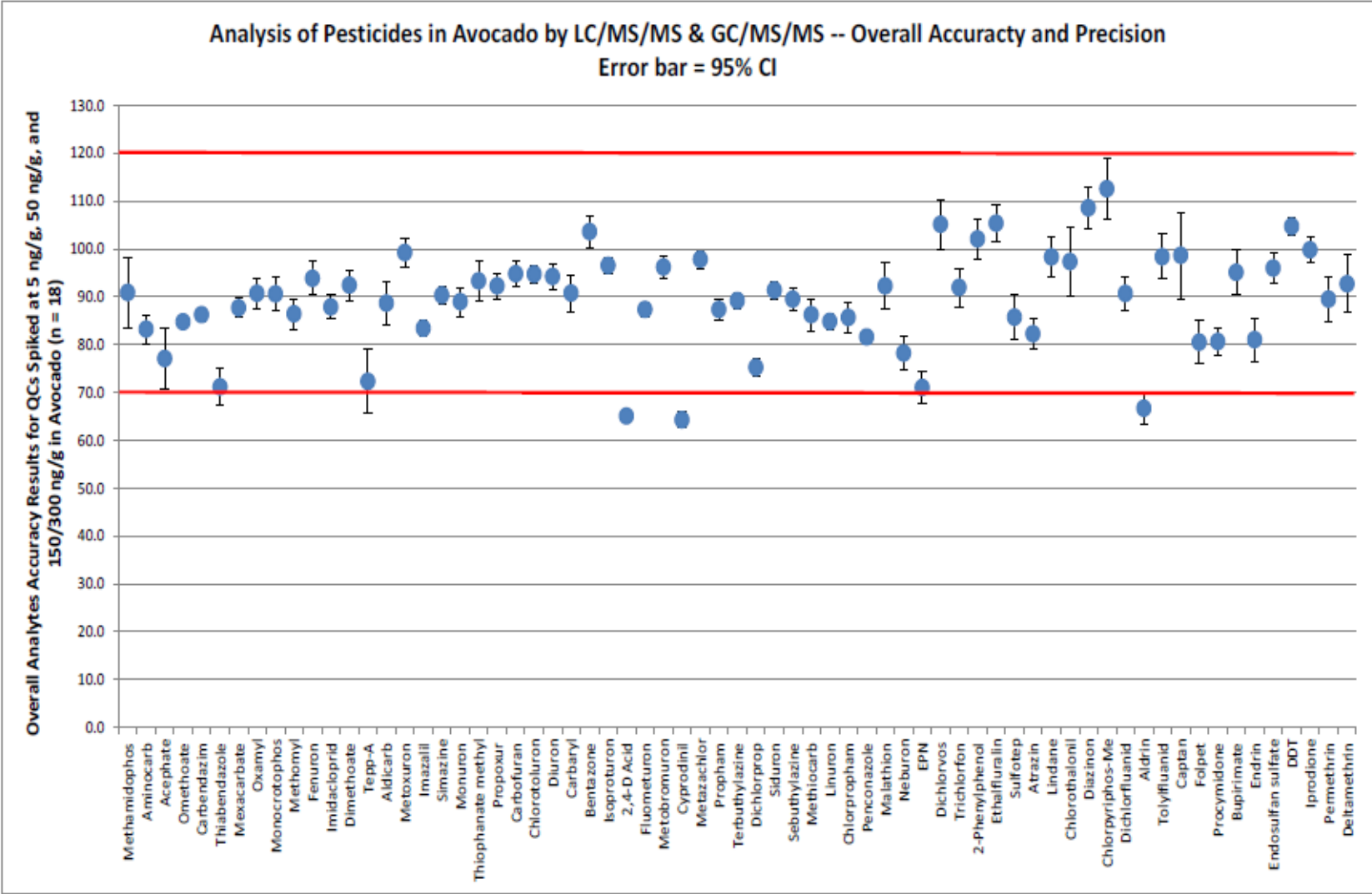
Selected Problematic Pesticides for Recovery Comparison



Statistical Recovery Results Comparison



Method Accuracy and Precision



Summary and Conclusion

- EMR-Lipid provides the **most complete lipid removal** of any sorbent on the market.
- **Achieve SPE cleanliness with dSPE simplicity.**
 - **EMR is a one size fits all sorbent** for a variety of sample types and analytes.
- Key applications were validated with EMR and demonstrate **better recovery, better precision, and decreased matrix impact** to the instrument and results.

Today's Agenda

Introduction

Addressing difficult samples

1. Polar analytes
2. High fat samples
- 3. How to get rid of interfering terpenes**

Summary and Conclusions

Constituents of Cannabis and Hop: Complex

- **Nitrogenous compounds (27 known)**

- Amino acids (18),
- Proteins (3)
- Glycoproteins (6)
- Enzymes (2)

- **Sugars and related compounds (34)**

- Hydrocarbons (50)
- Simple alcohols (7)

- Aldehydes (13)

- Ketones (13)

- Simple acids (21)

- **Fatty acids (22)**

- Simple esters (12)

- Lactones (1)

- Steroids (11)

- **Terpenes (120)**

- **Non-cannabinoid phenols (25)**

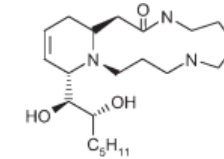
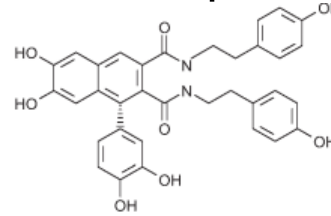
- **Cannabinoids (66)**

- **Flavonoids (21)**

- Vitamins (1) [Vitamin A]

- Pigments (2)

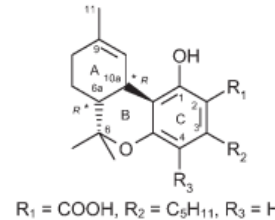
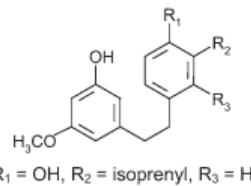
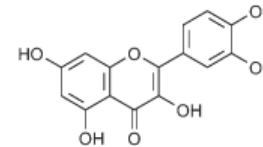
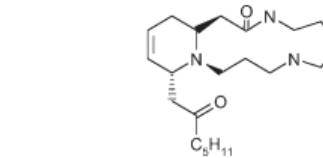
- Elements (9).



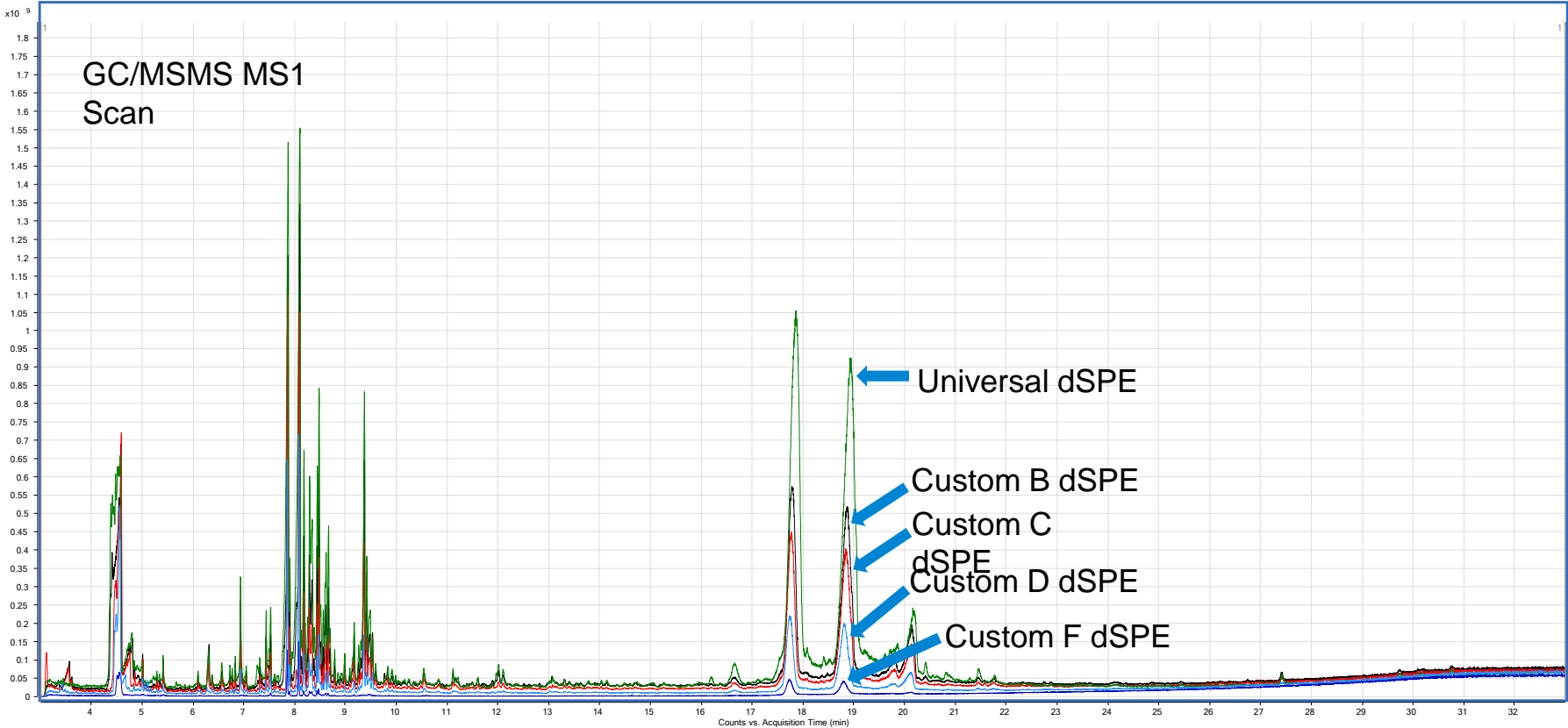
Structure of Terpenes



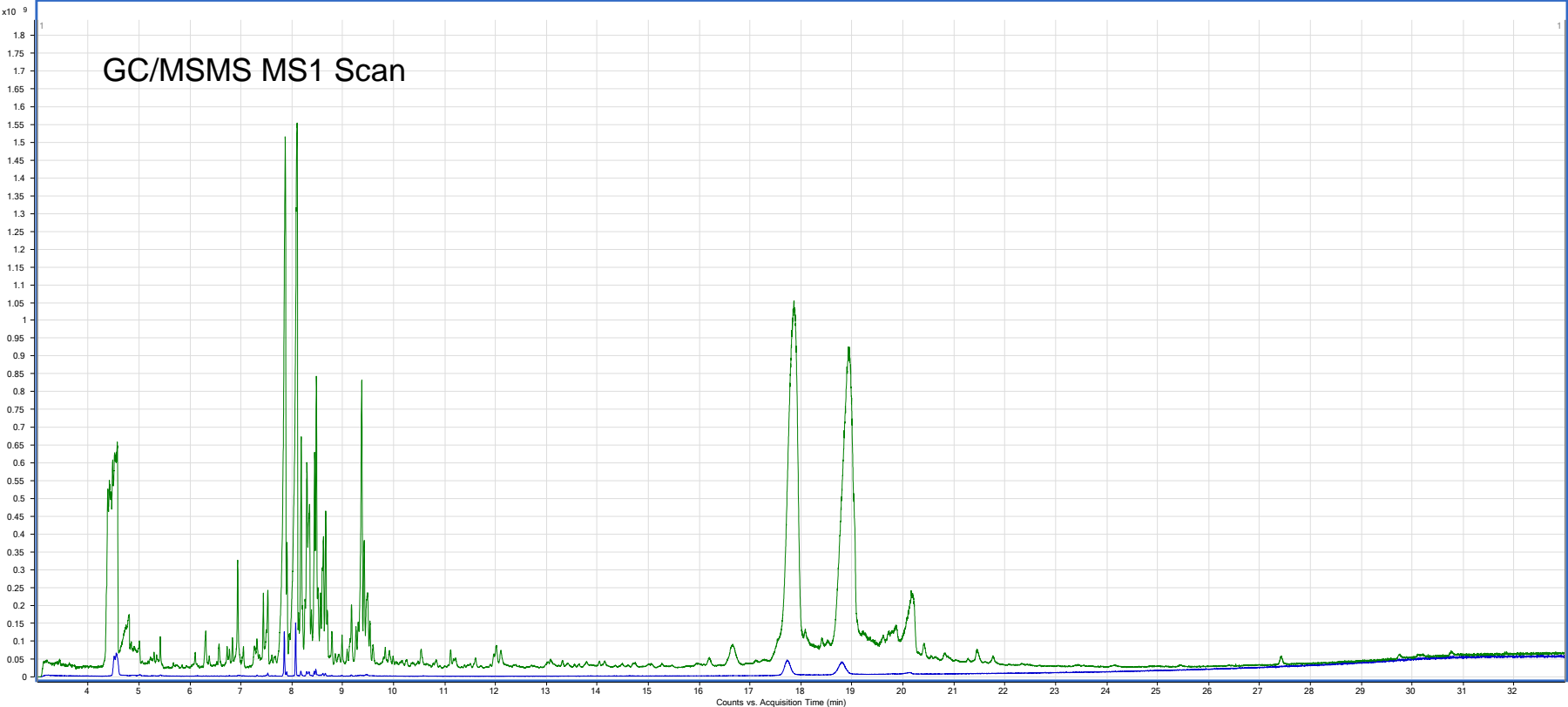
- Terpenes are composed of two or more isoprene units.
- The isoprene units will maintain its isopentyl, usually with modification of the isoprene double bonds.



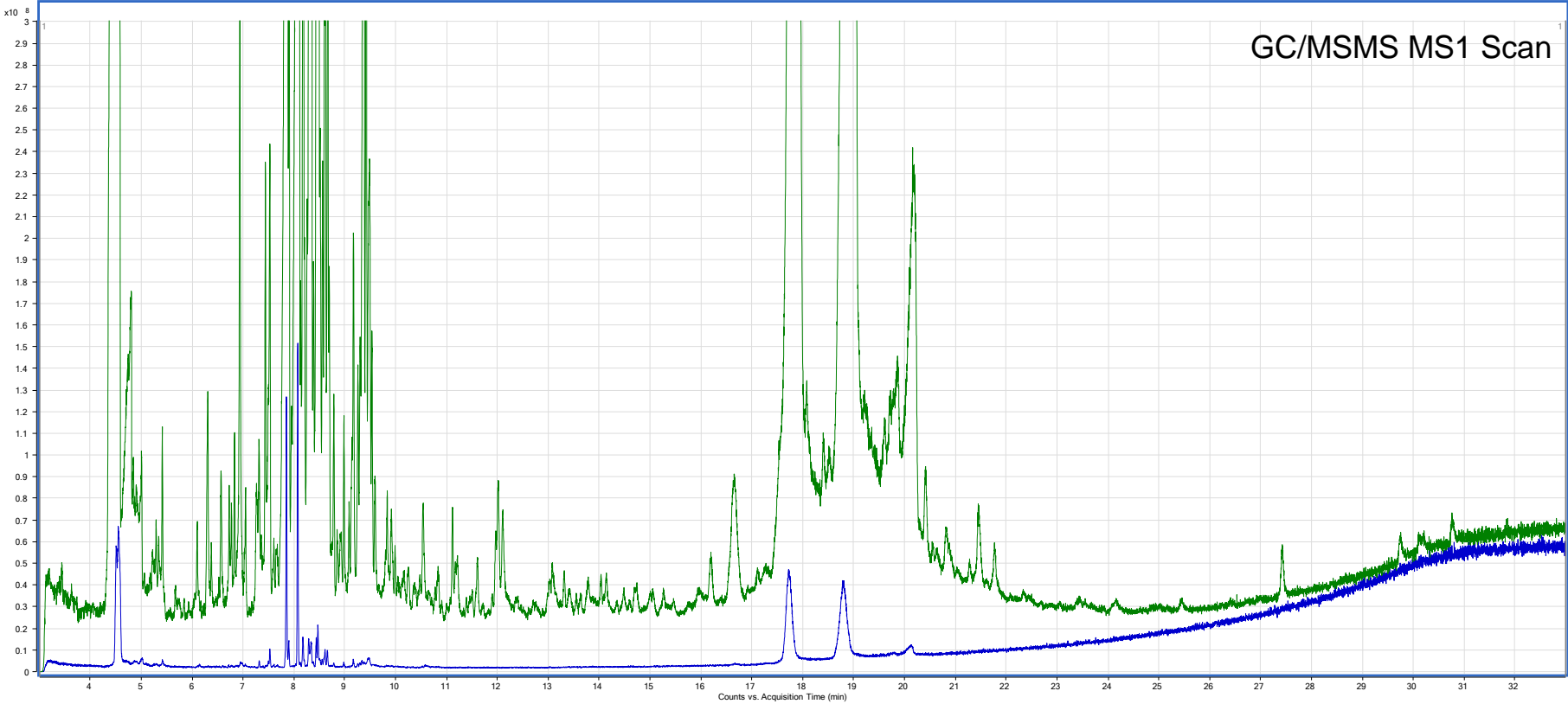
QuEChERS AOAC Extraction/Partitioning with Custom dSPE versus Universal dSPE: Hops



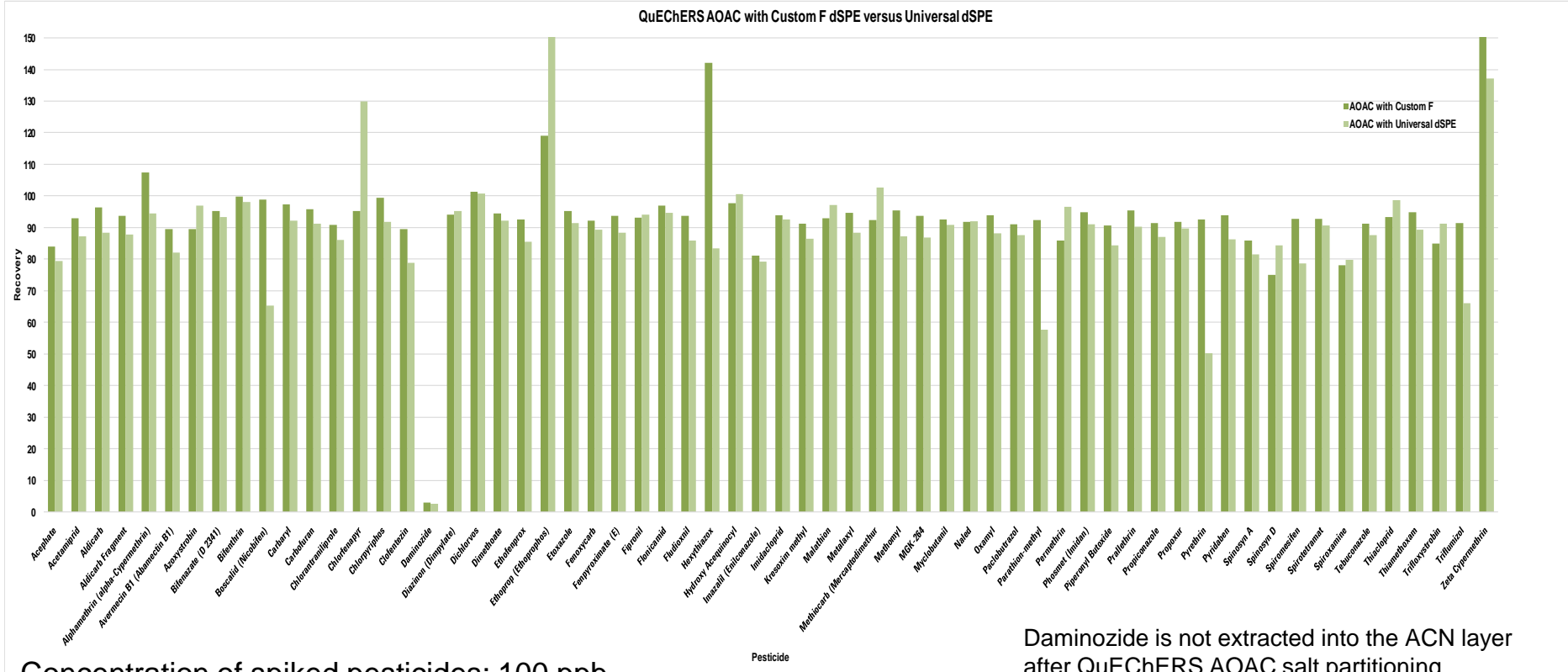
QuEChERS AOAC Extraction/Partitioning with Custom F dSPE versus Universal dSPE: Hops



QuEChERS AOAC Extraction/Partitioning with Custom F dSPE versus Universal dSPE: Hops



Pesticide Recovery after QuEChERS AOAC with Custom F dSPE or Universal dSPE: Hops



Concentration of spiked pesticides: 100 ppb

Conclusion

Addressing difficult samples

1. Polar analytes : Explore new polymeric SPE phases
2. High fat samples: Consider EMR Lipids as a dSPE
3. How to get rid of interfering terpenes: stay tuned 😊



Thank You!