



EMR – Lipid: Enhanced Matrix Removal for Fatty Samples

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Today's Agenda

Introduction

EMR-Lipid - Enhanced Matrix Removal

- Background, workflows, and results

Applications

- Pesticides in avocado
- Veterinary drugs in beef liver
- PAHs in salmon

Summary and Conclusions

The challenges and goals of most sample analyses:

Accurate, consistent data

Keep the instruments running

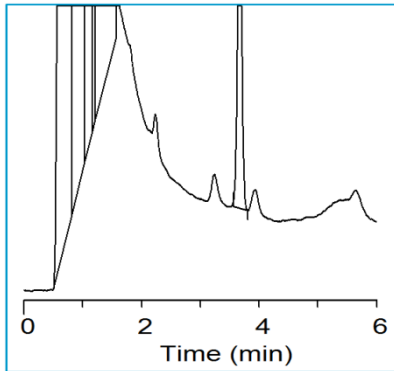
Process as many samples for as little cost as possible

Remove interferences

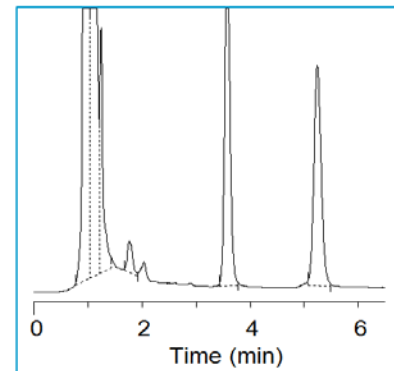
Remove interferences...

- 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

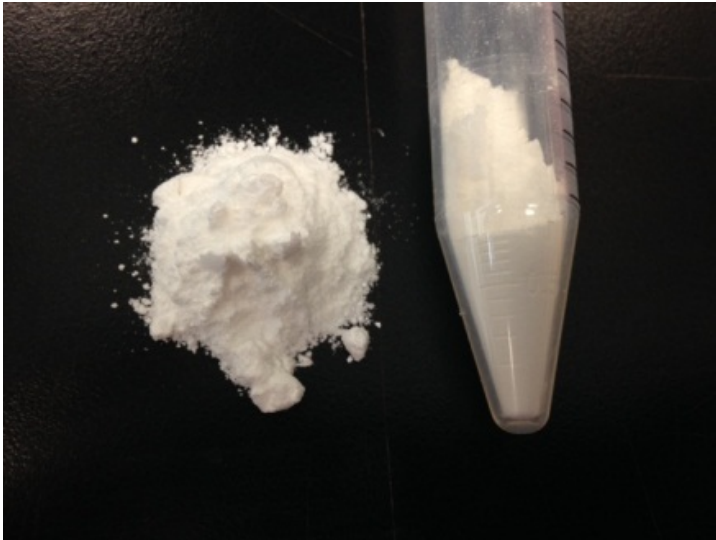


EMR-Polish (p/n 5982-0101)

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*).

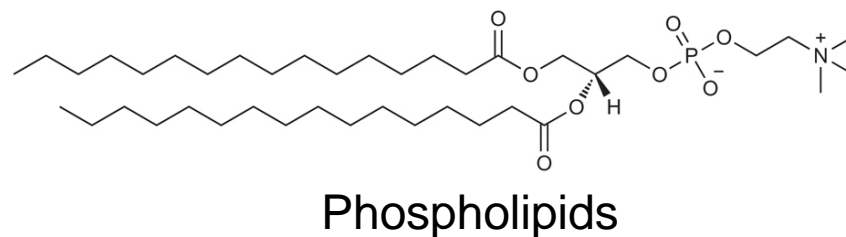
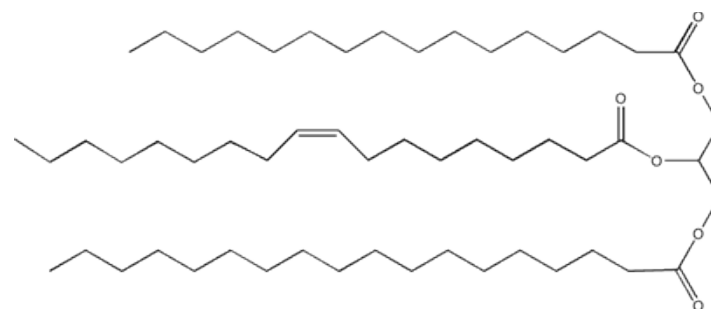
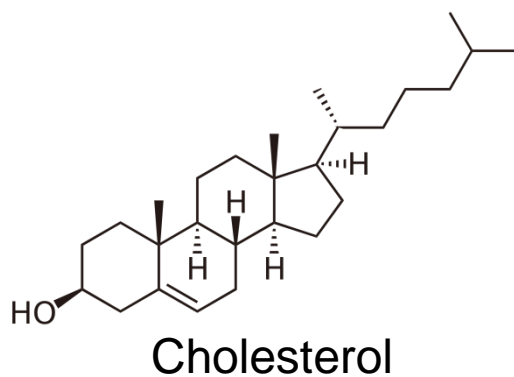
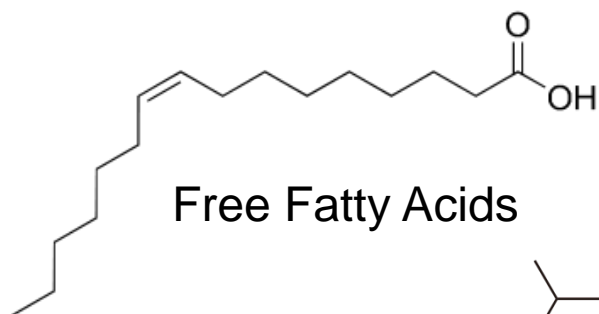
EMR-Lipid Mechanism – Size exclusion and hydrophobic interaction.

... 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

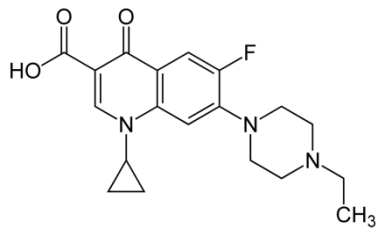


What Does EMR *NOT* Interact With?

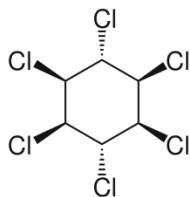
EMR does **NOT** remove analytes of interest

Exceptions?

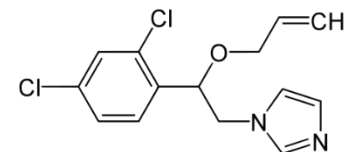
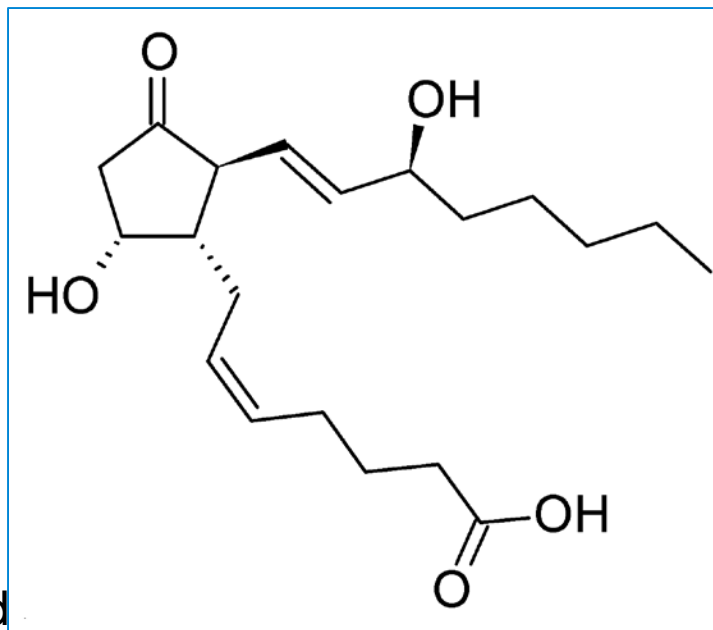
Compounds containing long aliphatic functional groups (e.g. prostaglandins)



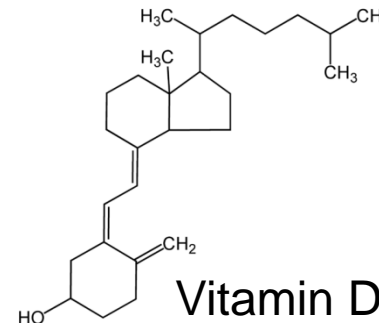
Fluoroquinolones



Organochlorine Pesticide



midazole pesticides



Vitamin D

EMR Protocols for Applications

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,

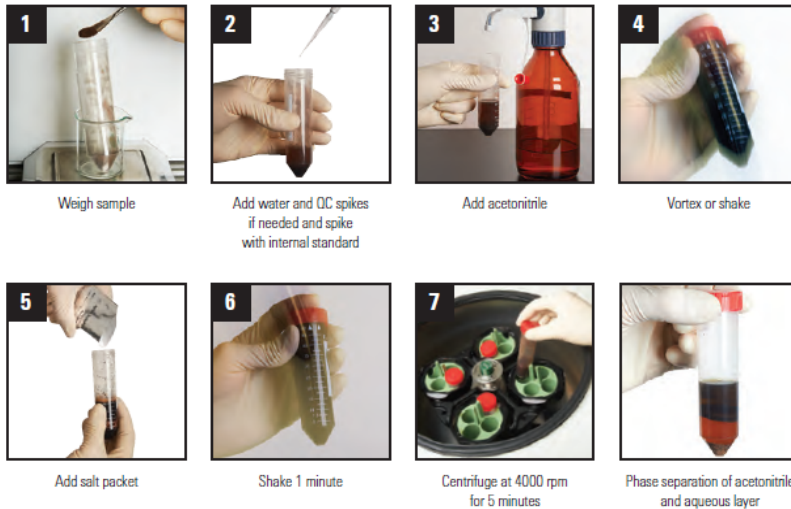
Modified Liquid Extraction (Protein Precipitation)

- 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



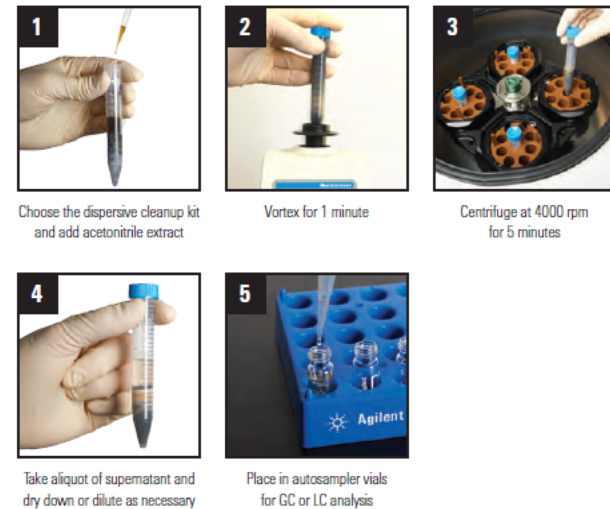
Pros

- 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

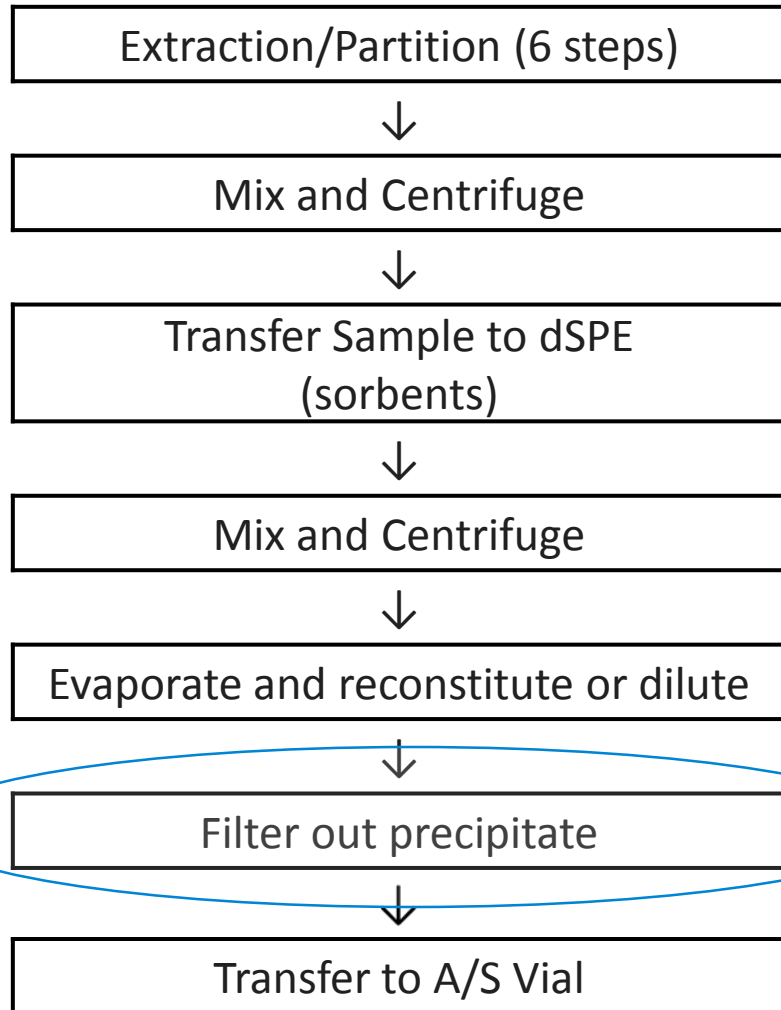
- Same as extraction

Cons

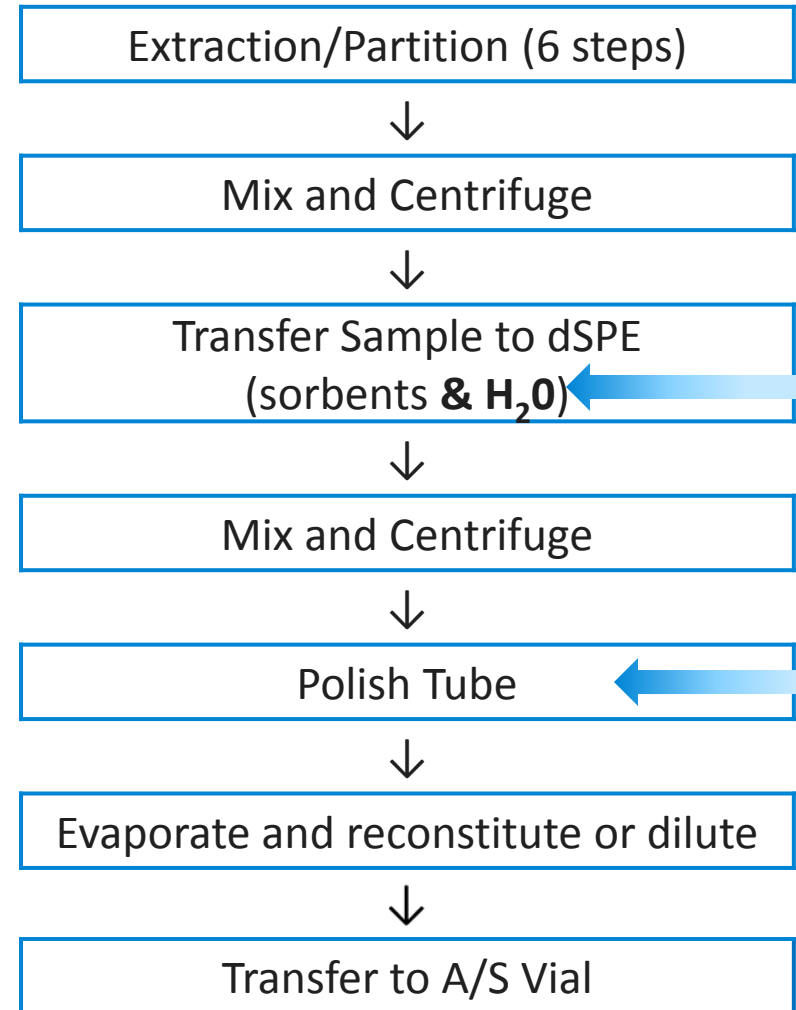
- Minimal cleanup provided
- Can remove analytes
- Lipids are challenging to remove selectively

Traditional QuEChERS versus QuEChERS EMR-Lipid

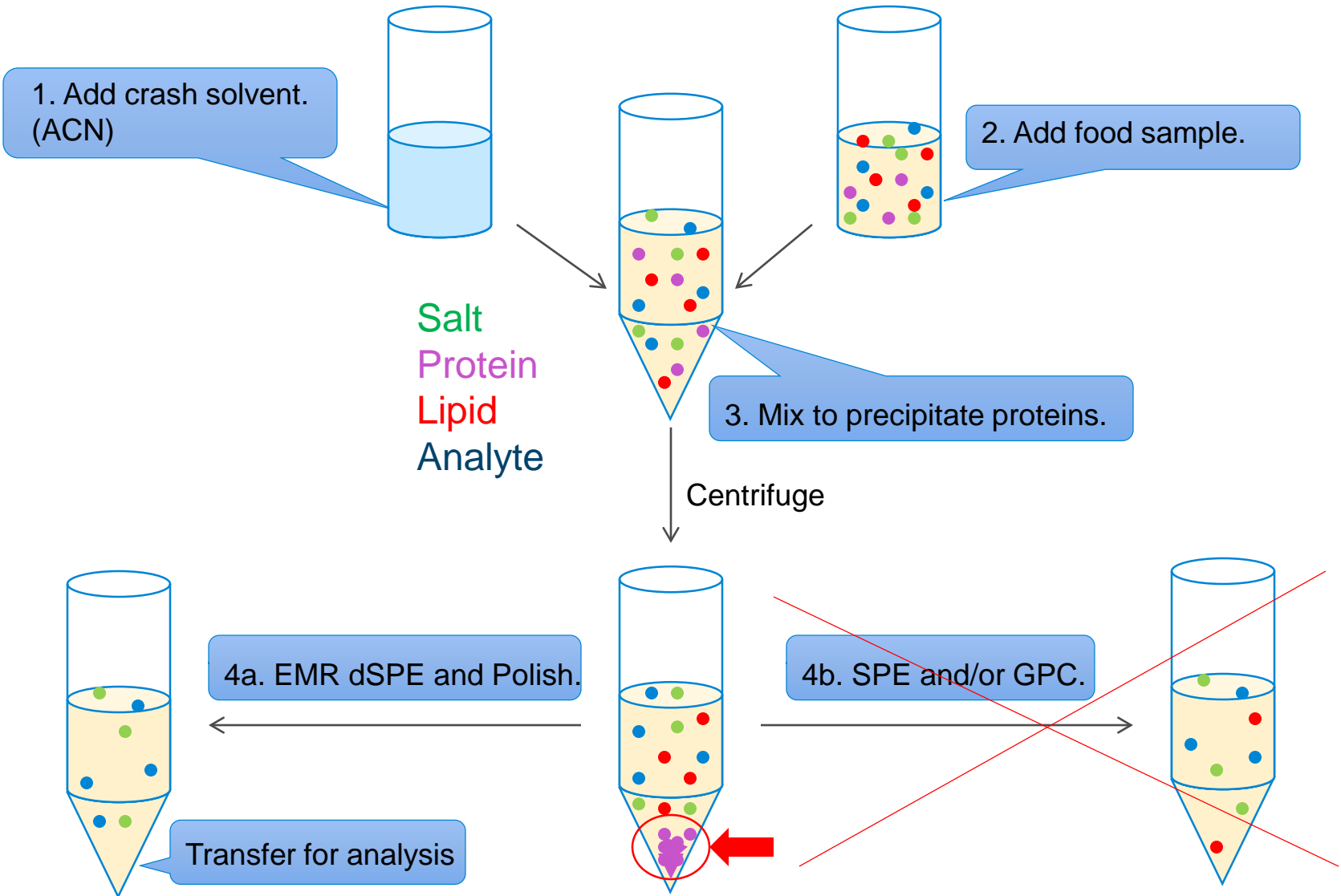
Standard QuEChERS



EMR QuEChERS

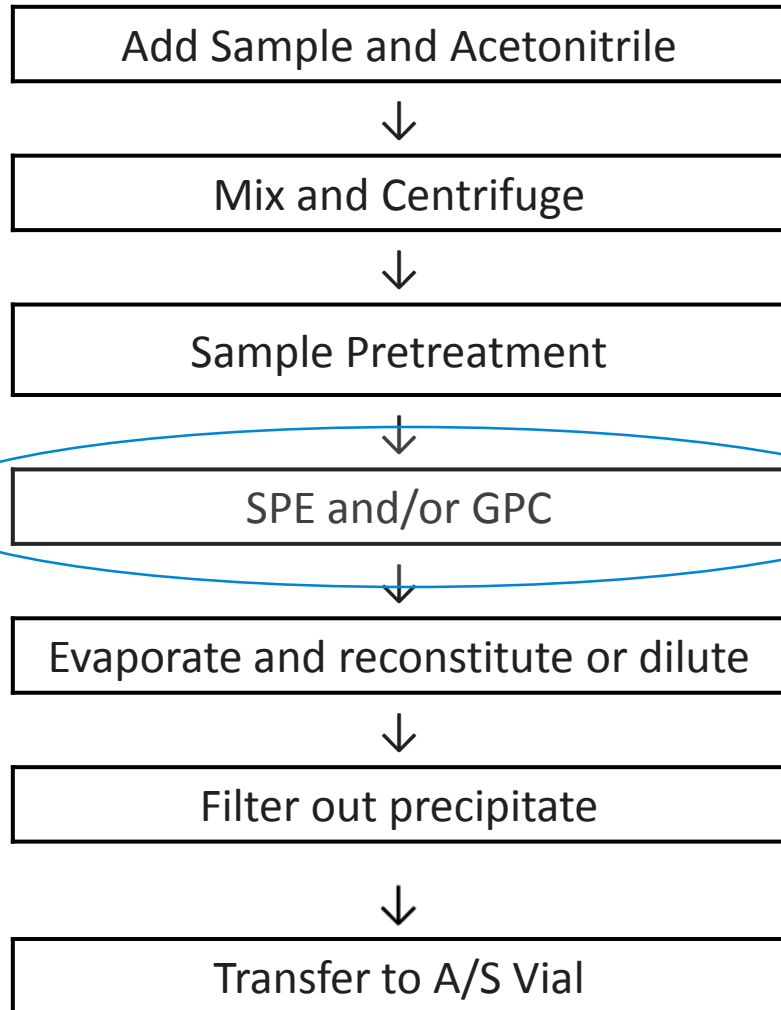


Improving Liquid Extraction Workflows

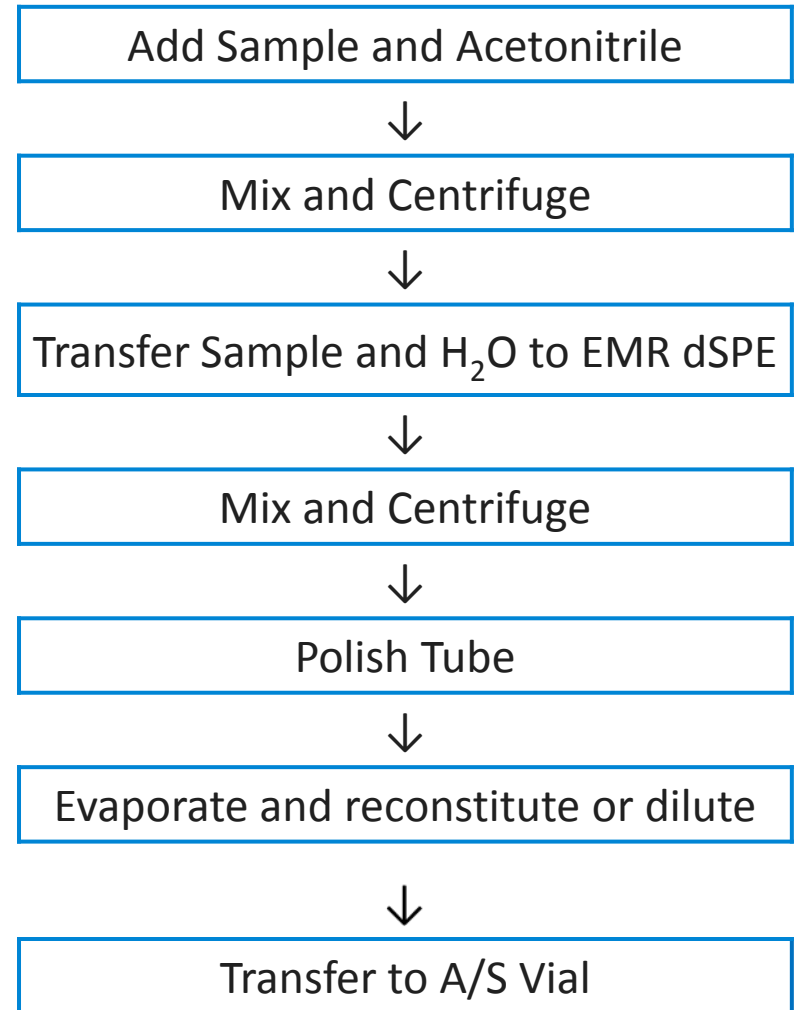


Traditional versus EMR-Lipid Liquid Extraction

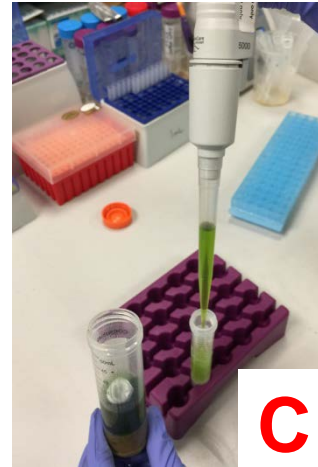
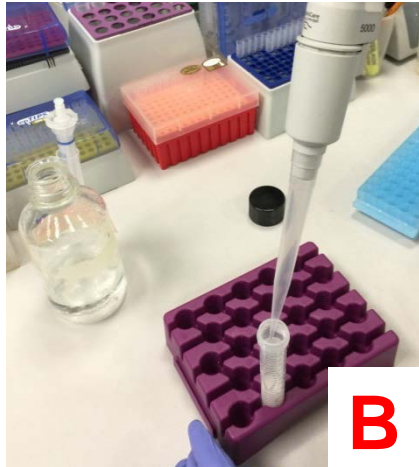
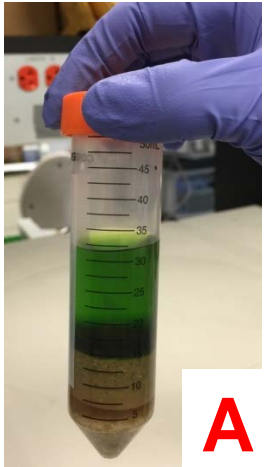
Liquid Extraction with SPE/GPC



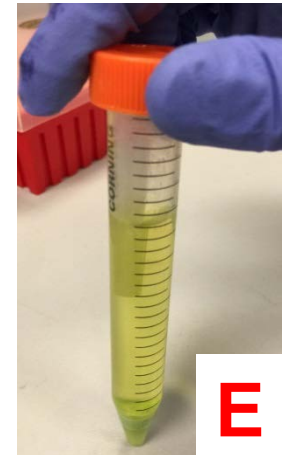
EMR Liquid Extraction



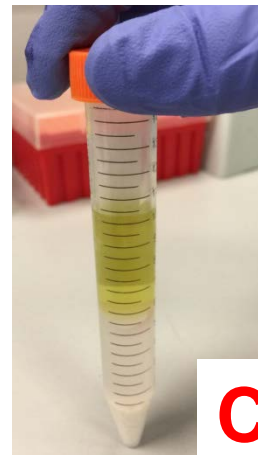
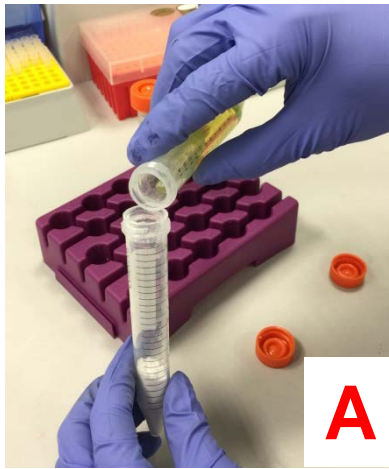
EMR – Lipid – dSPE Cleanup



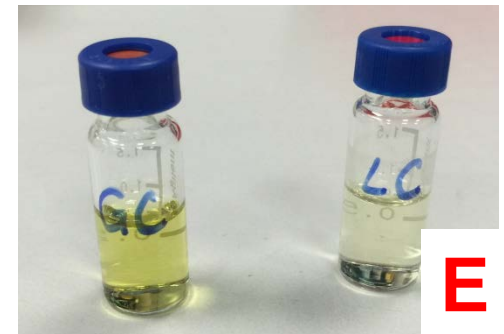
- A. QuEChERS or Liquid Extract**
- B. Add H₂O to EMR tube (“activation”)**
- C. Transfer extract**
- D. Vortex and centrifuge**
- E. Supernatant (1:1; extract: H₂O)**



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**



Tips for Success: EMR-Lipid Protocols

Extraction Solvent

- ***Acetonitrile** – preferred for broad extraction and water miscibility.
- **Acetone** – also amenable to EMR workflow.
- **Do not use...**
 - *Water immiscible solvents* (e.g. hexane, ethyl acetate, DCM) – no interaction without initial miscibility.
 - *Alcohols* – can dissolve salts, EMR, and give poor partitioning.

Tips for Success: EMR-Lipid Protocols

dSPE “Activation”

- **Addition of water for dSPE**

- Increases hydrophobic interaction between EMR-Lipid and matrix components.

- **“Activation Strength”**

- **1 to 1; extract/water (recommended)** – Excellent matrix removal and analyte recovery.
- **1 to >1; extract/water** – marginal increase in matrix removal, lower hydrophobic analyte recovery.
- **1 to <1; extract/water** – decrease matrix removal, improve hydrophobic analyte recovery.

Tips for Success: EMR-Lipid Protocols

Polishing Step (Partition)

- Contains 2.0 g NaCl/MgSO₄ (1:4; same as QuEChERS)
- Applied *AFTER* EMR-Lipid dSPE for phase separation

Purpose

- *Remove unwanted water* from “activation”
- *Removes dissolved solids*
- *Improves recoveries* for non-polar compounds

Additional Information

- Use the Polish salts after EMR-Lipid
- Mix immediately – avoid clumping
- Customer may chose bulk salts

Separate Product but **HIGHLY RECOMMENDED** for optimal results

Tips for Success with EMR-Lipid

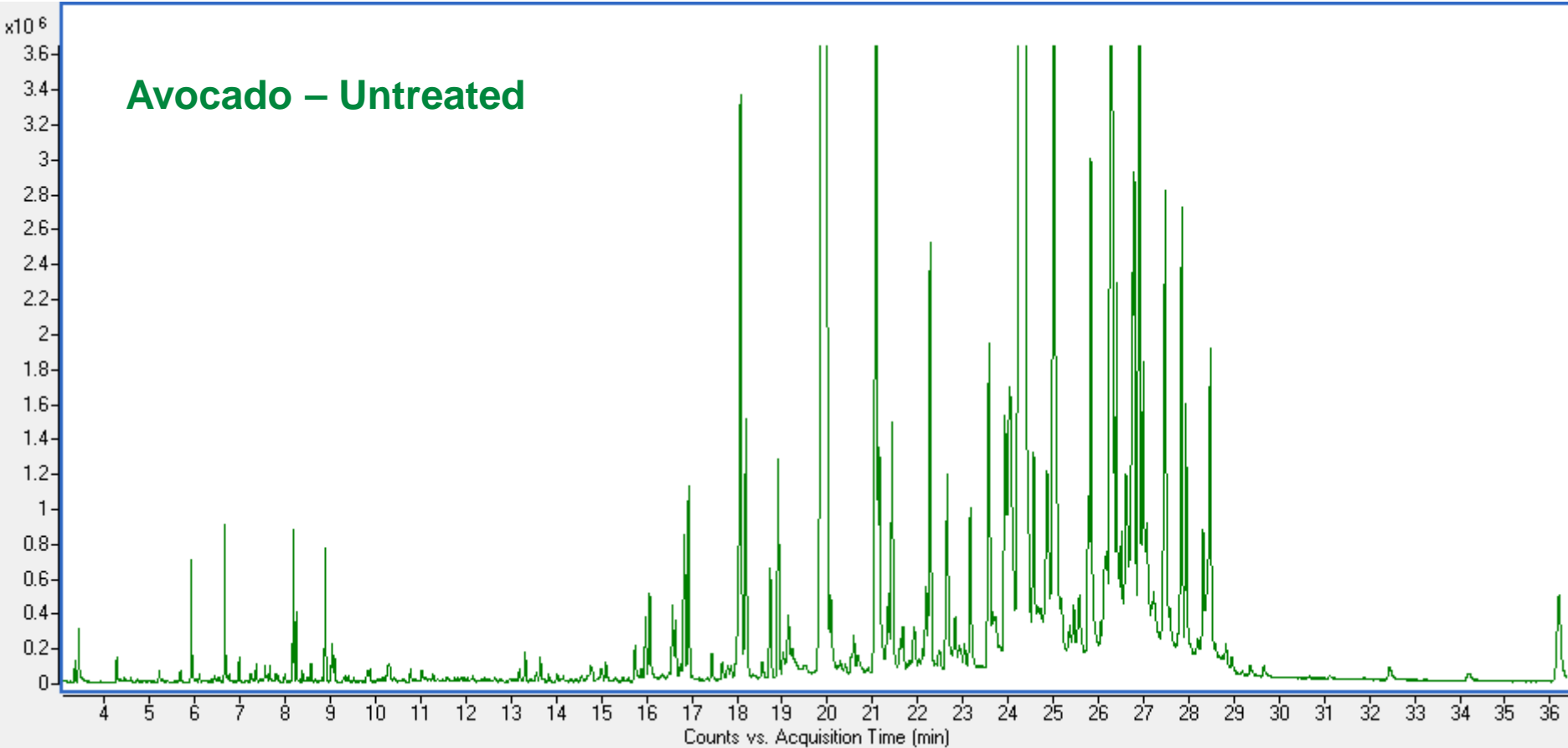
Recommend Centrifugation *NOT* Filtration

- Filter membranes put EMR Samples **at Risk for Analyte Retention**
 - Analytes with properties that risk retention on **hydrophobic membranes**
 - Less matrix leaves **analytes “susceptible”** for retention
 - If filtration must be used, **choose a hydrophilic membrane** such as regenerated cellulose.

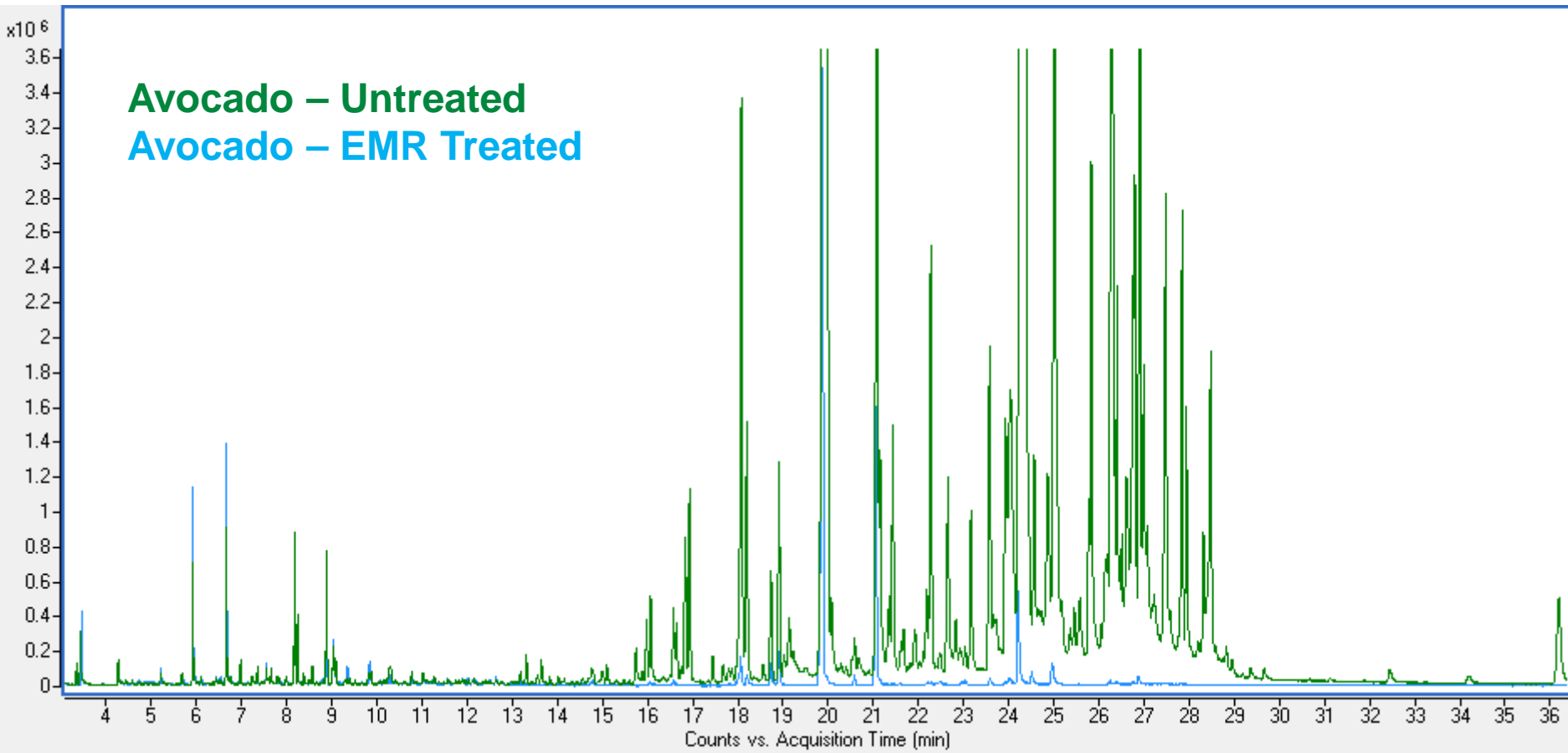
Recommend Immediate Mixing

- *Avoid Clumping; Immediately vortex* extract/water in the EMR dSPE tube and EMR Polish tube.

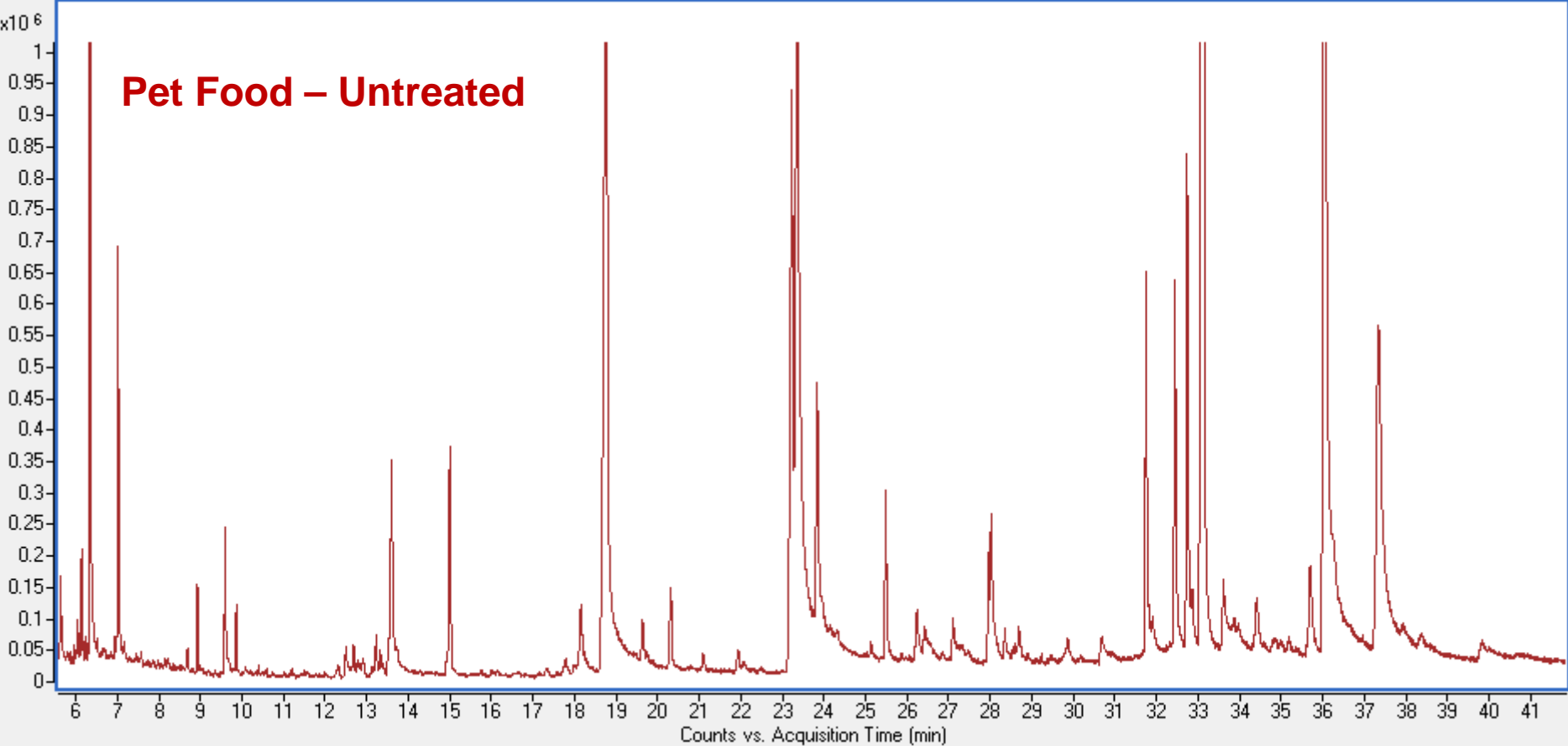
GC-MS Fullscan Avocado



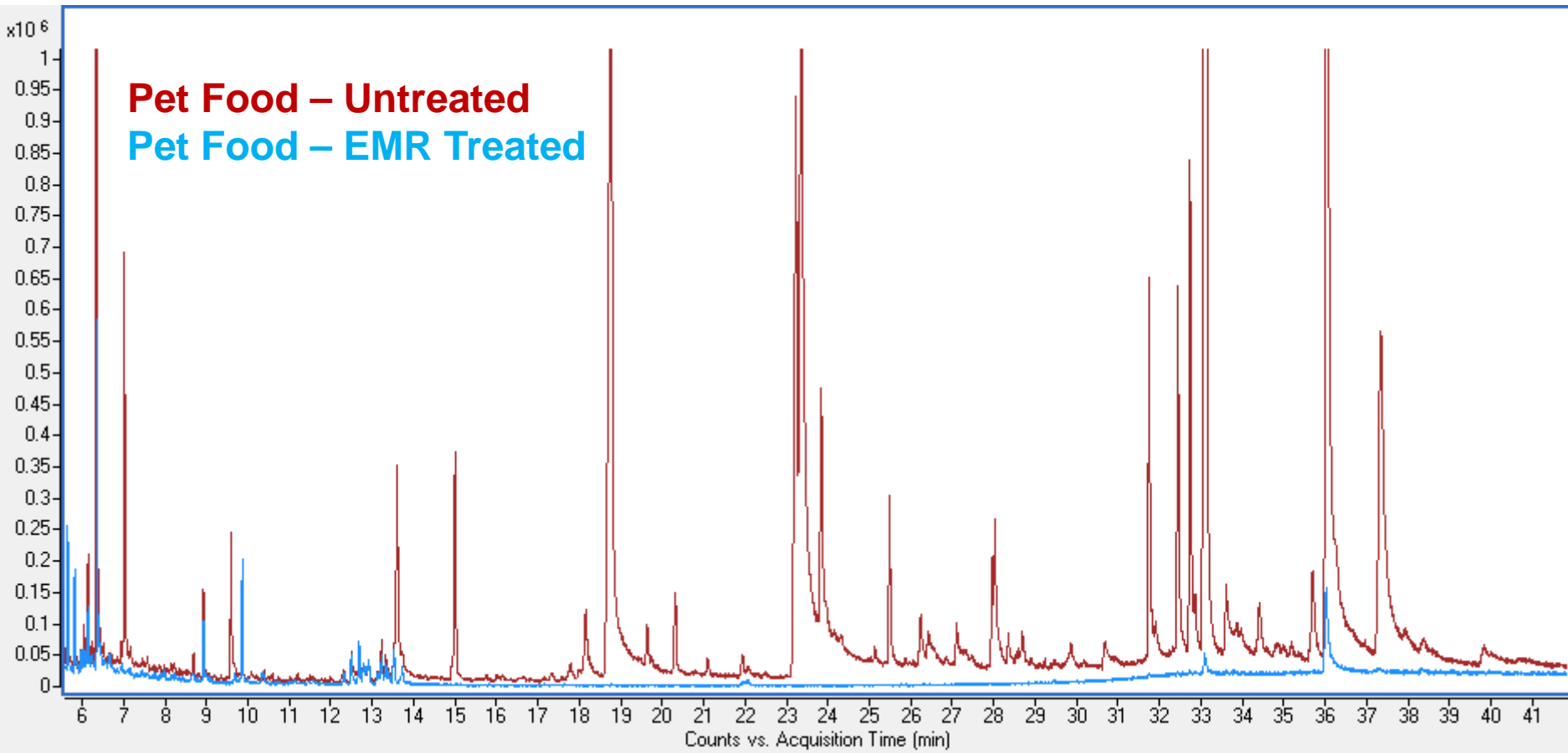
GC-MS Fullscan Avocado



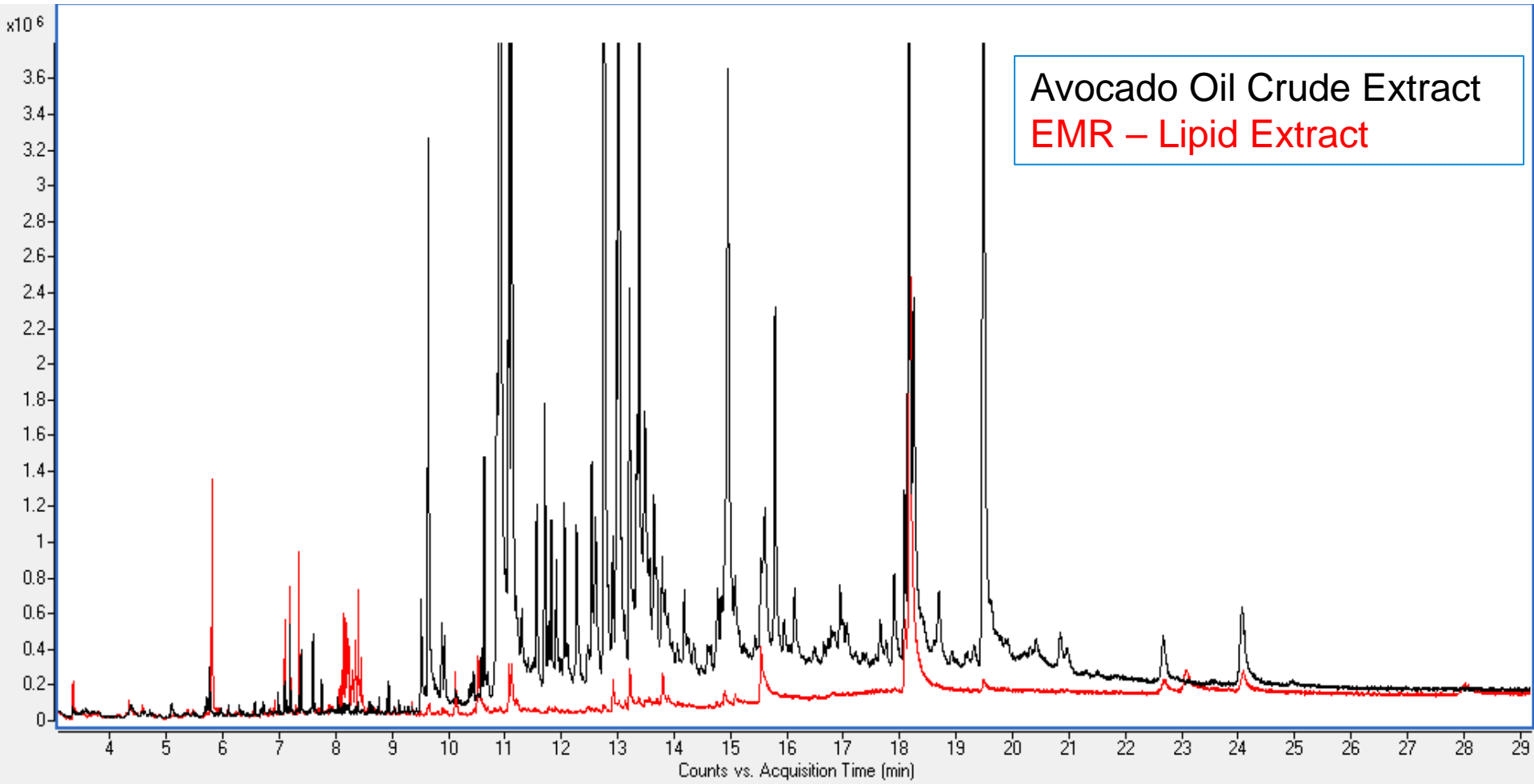
GC-MS Fullscan Pet Food



GC-MS Fullscan Pet Food

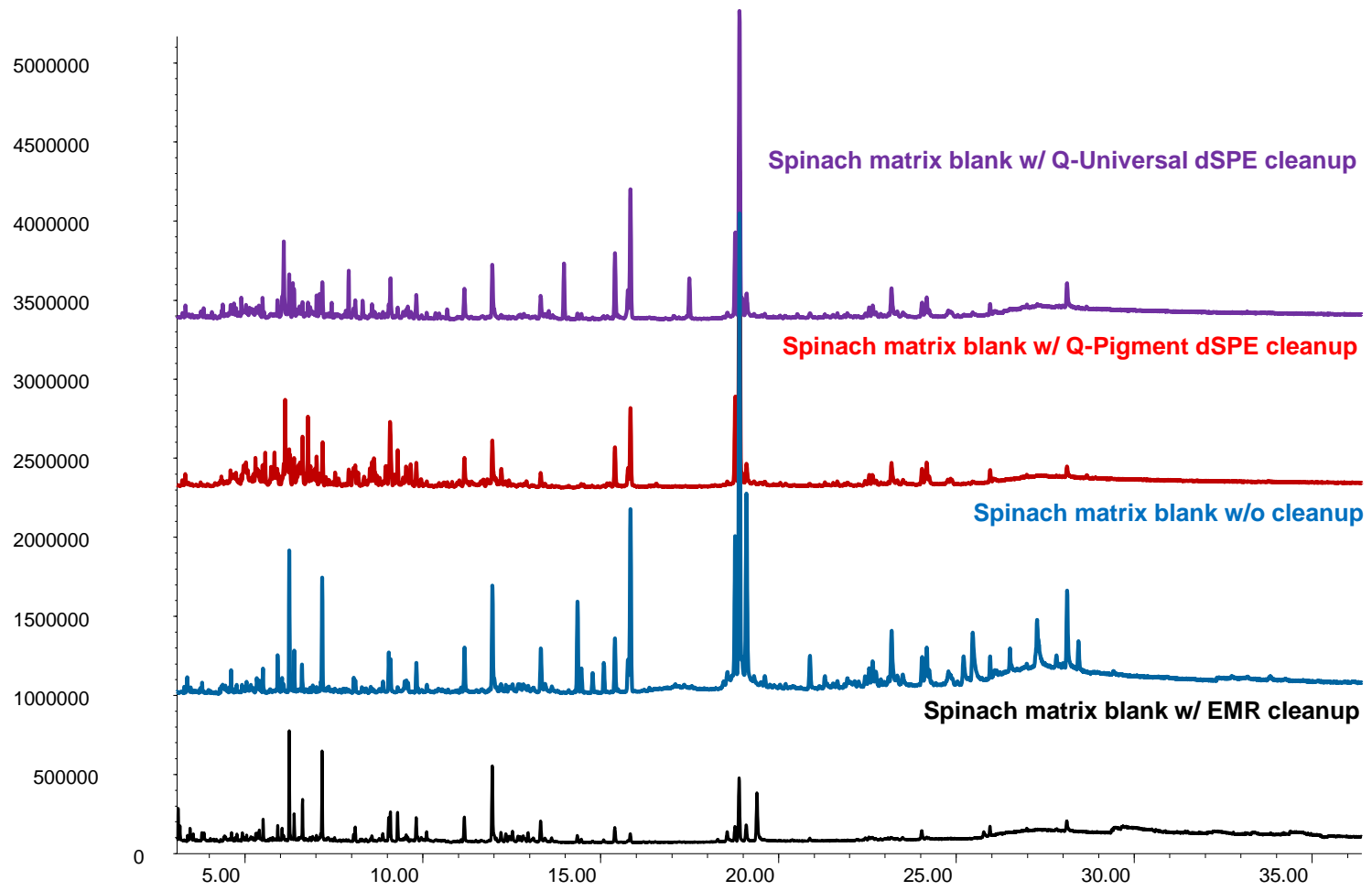


GC-MS Full Scan- Avocado Oil



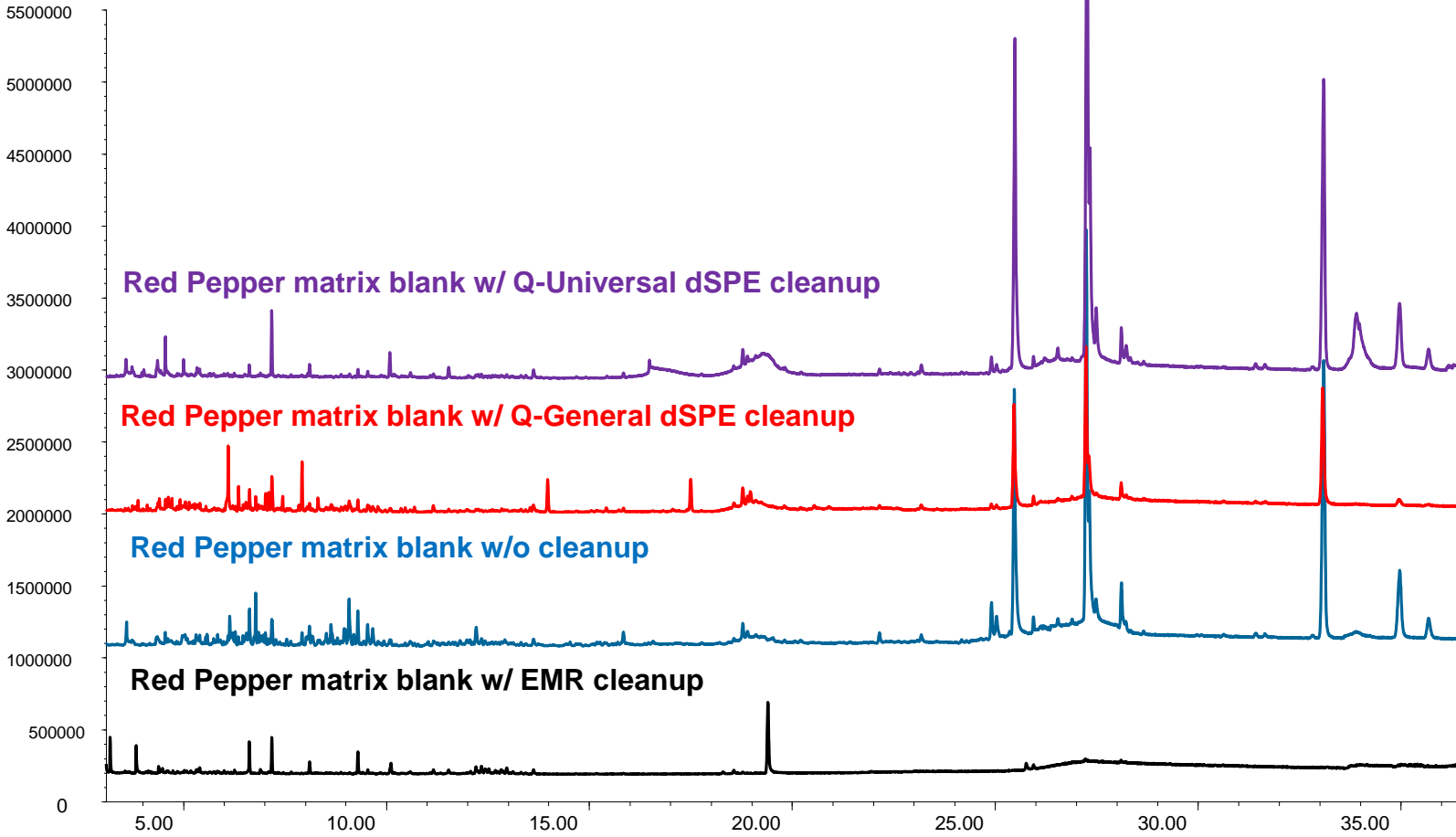
GC-MS Spinach Fullscan

Abundance



Time-->

GC-MS Red Pepper Fullscan



Suggested Workflow for Multi-Residue Analysis

- **Sample Preparation:** EMR-Lipid with QuEChERS/Modified Liquid Extraction workflow
- **System:** Agilent LC or GC MS/MS (6400, 6500, 5977, 7000, 7010, 7200 series)
- **Columns:**
 - LC: Agilent Poroshell 120 EC-C18, 2.7 μ m
 - LC: ZORBAX RRHD Eclipse Plus C18, 1.8 μ m
 - GC: Agilent J&W DB-5ms Ultra Inert
- Agilent MassHunter Software
- Experimental setup: Matrix-matched Calibration

Matrix Matched Calibration

What is matrix matched calibration?

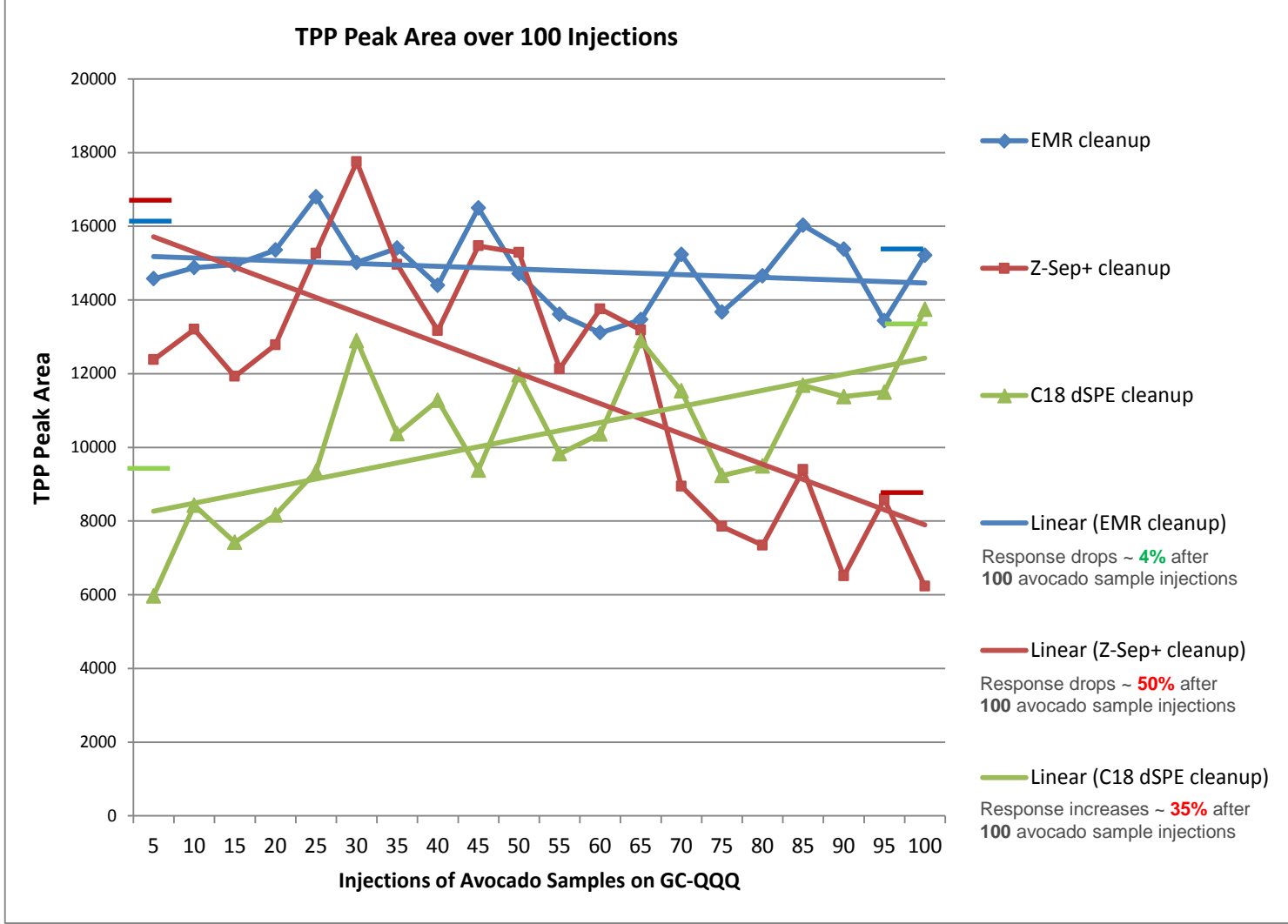
- A sample blank is added to final calibration standards to **correct any ion enhancement/suppression due to co-extracted matrix**.
 - Example: 950 μL blank extract + 25 μL working standard + 25 μL internal standard = 1000 μL matrix matched calibration standard

Matrix Matched Calibration and EMR-Lipid

- We **HIGHLY RECOMMEND** the use of matrix matched calibration standards for complex food samples.
 - Small amounts of matrix can cause poor results.

Benefits to Instrumental Flowpath

Comparison of Analytes Response Consistency over Multiple Avocado Sample Injections

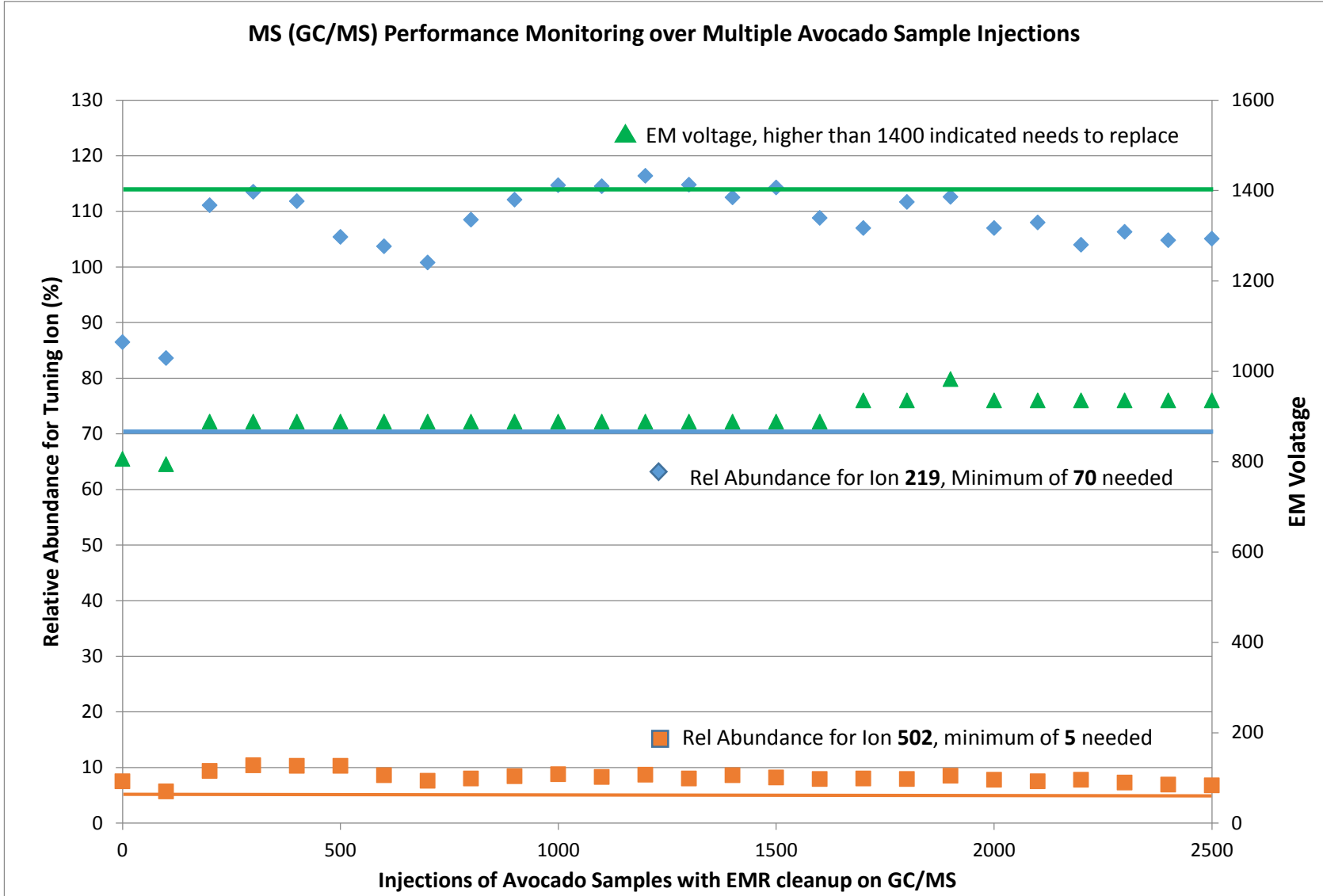


TPP
 Commonly used IS
 RT: 18.3 min

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

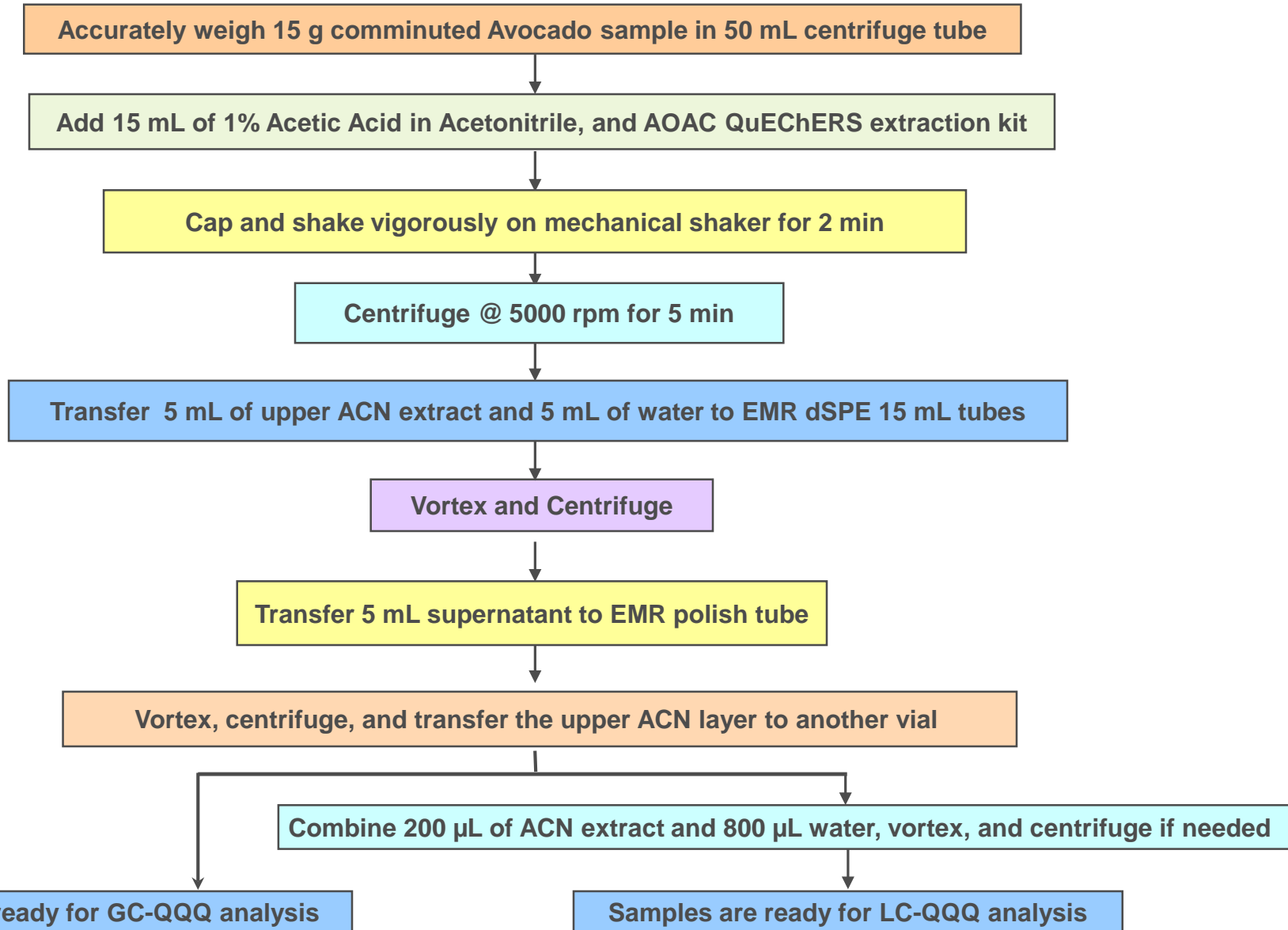


Pesticides in Avocado

72 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		LC-MS	Propham	Urea	Herbicide
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					
Permethrin	Pyrethroid	Insecticide	Fenuron	Urea	Herbicide	EPN									
Deltmethrin				Metoxuron			Tepp-A								
Pyraclostrobin	Strobilurin	Fungicide				Monocrotophos									
Ethalfuralin	Dinitroaniline	Herbicide													

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



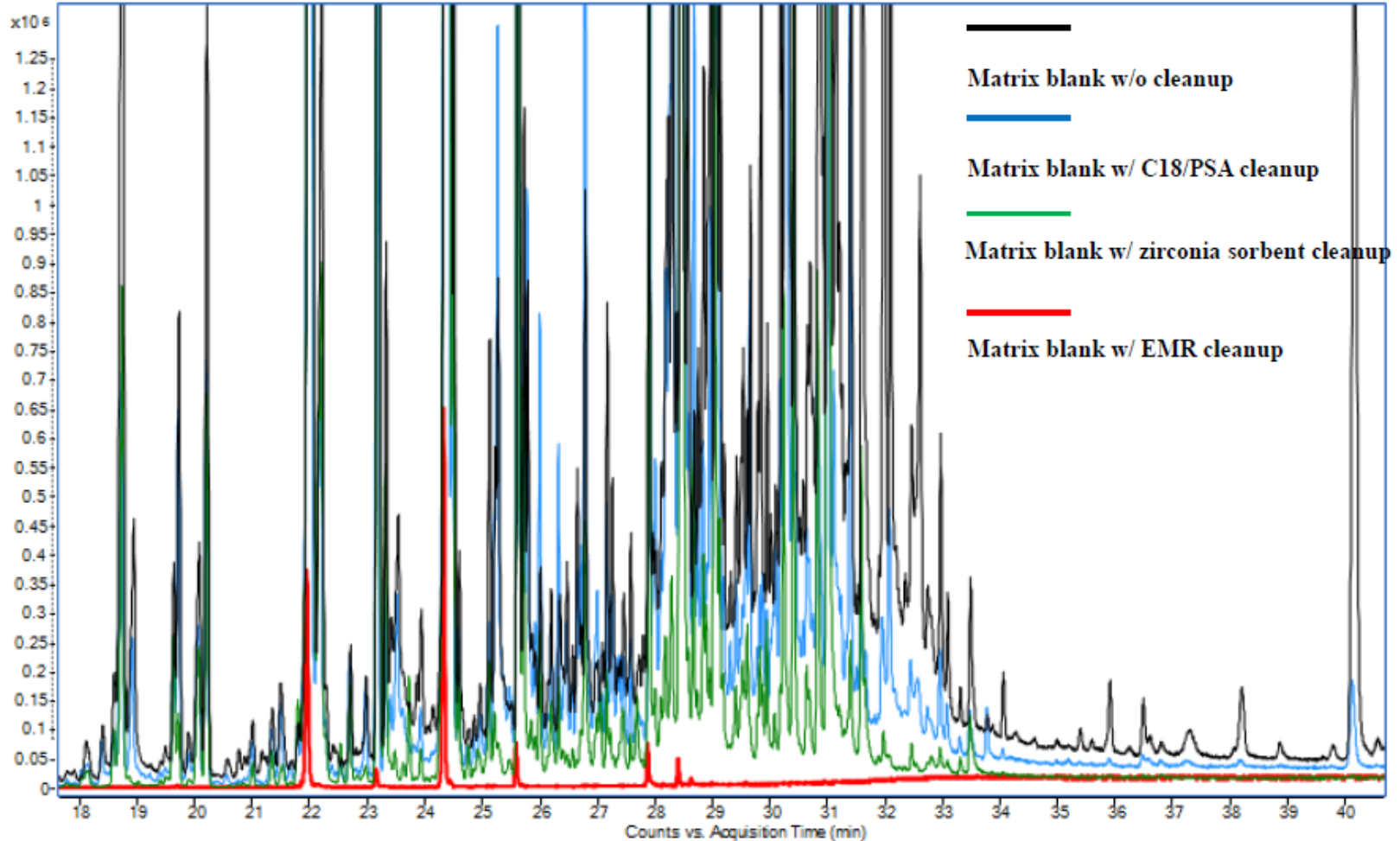
Comparison of Avocado Co-extractives by Weight

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

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

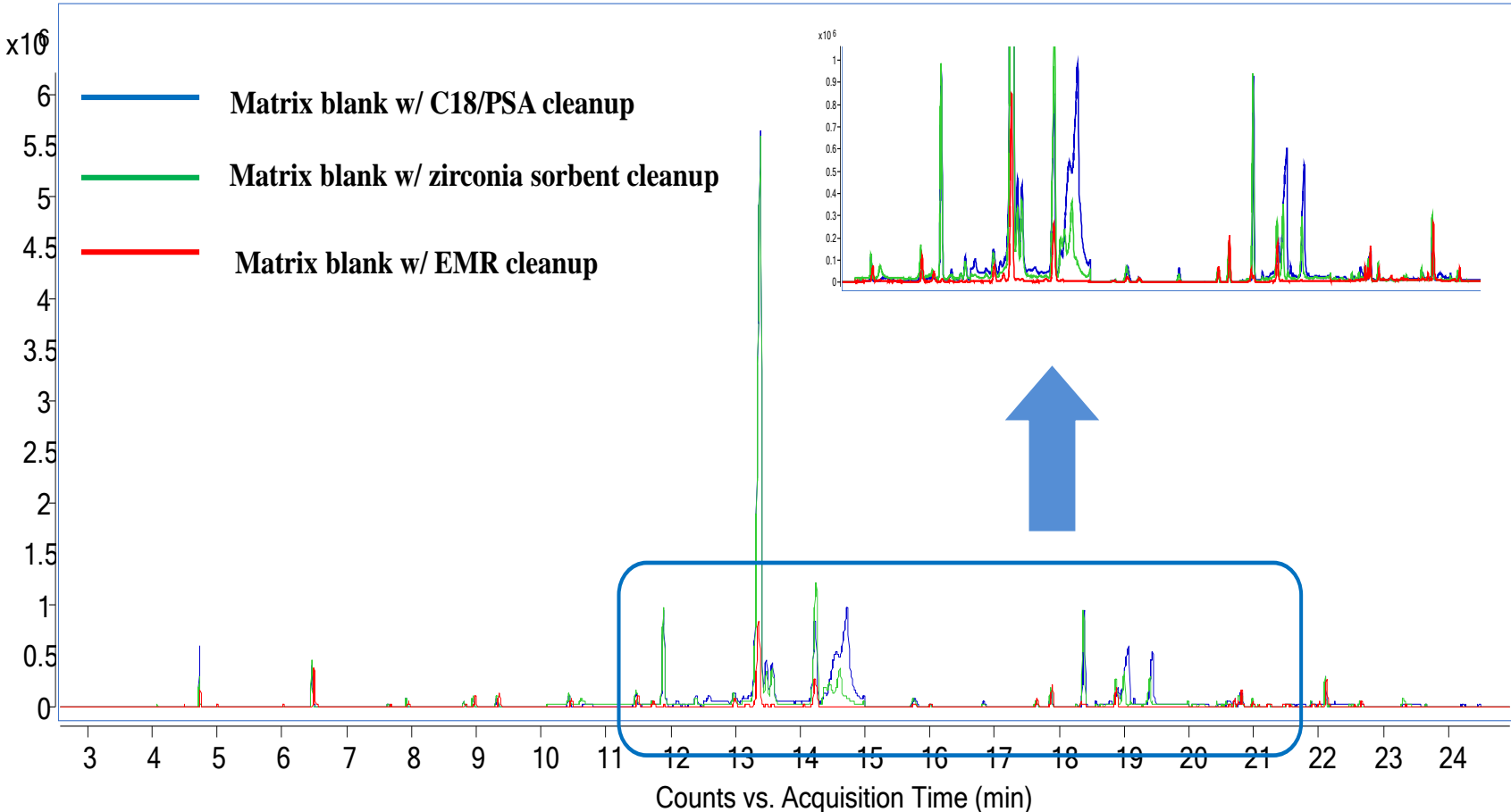
The use of EMR material cleanup removes extra 20-30% of Avocado co-extractives in comparison to traditional QuEChERS and/or competitor's cleanup

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

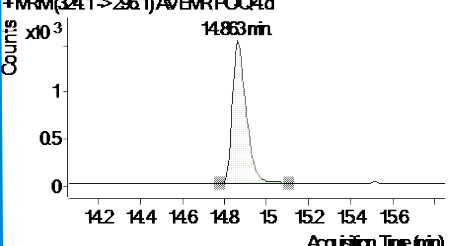
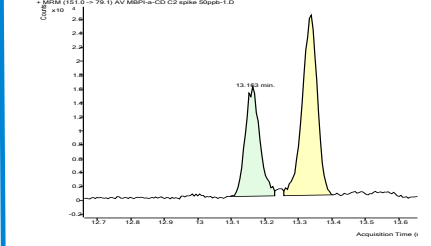
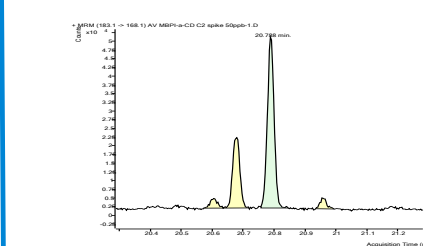
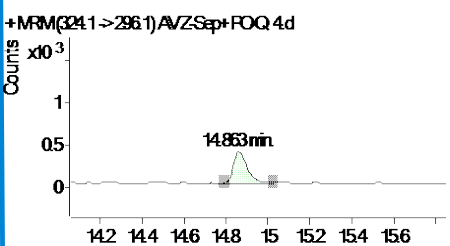
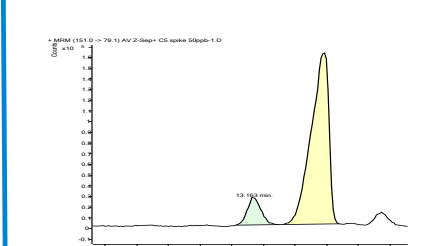
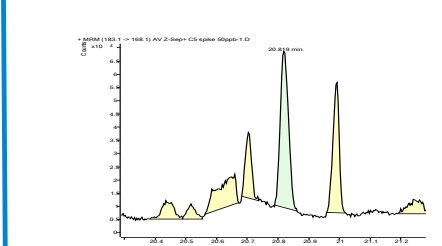
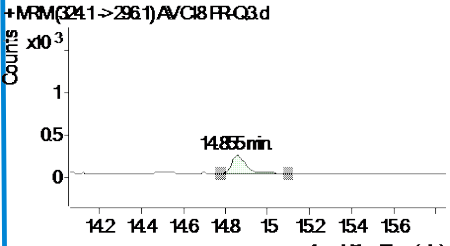
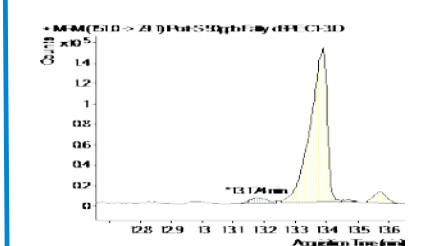
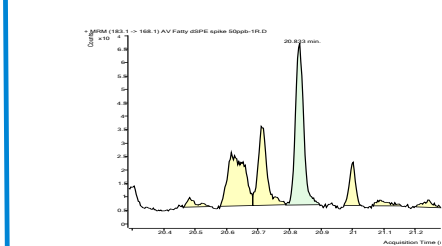


The use of EMR material cleanup provides significantly cleanup chromatographic sample background.

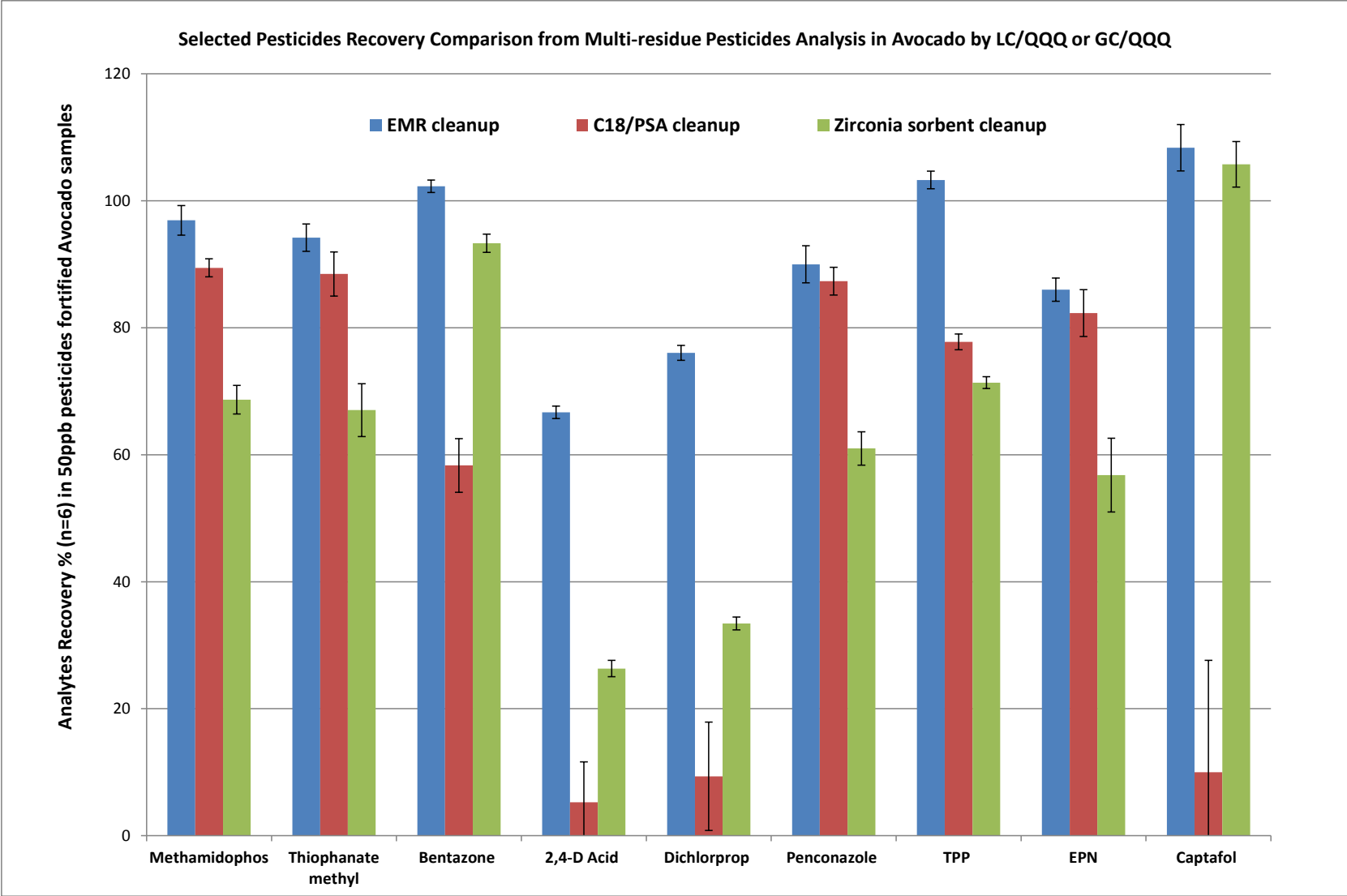
Comparison of GC/MS/MS MRM Chromatogram for Matrix Background



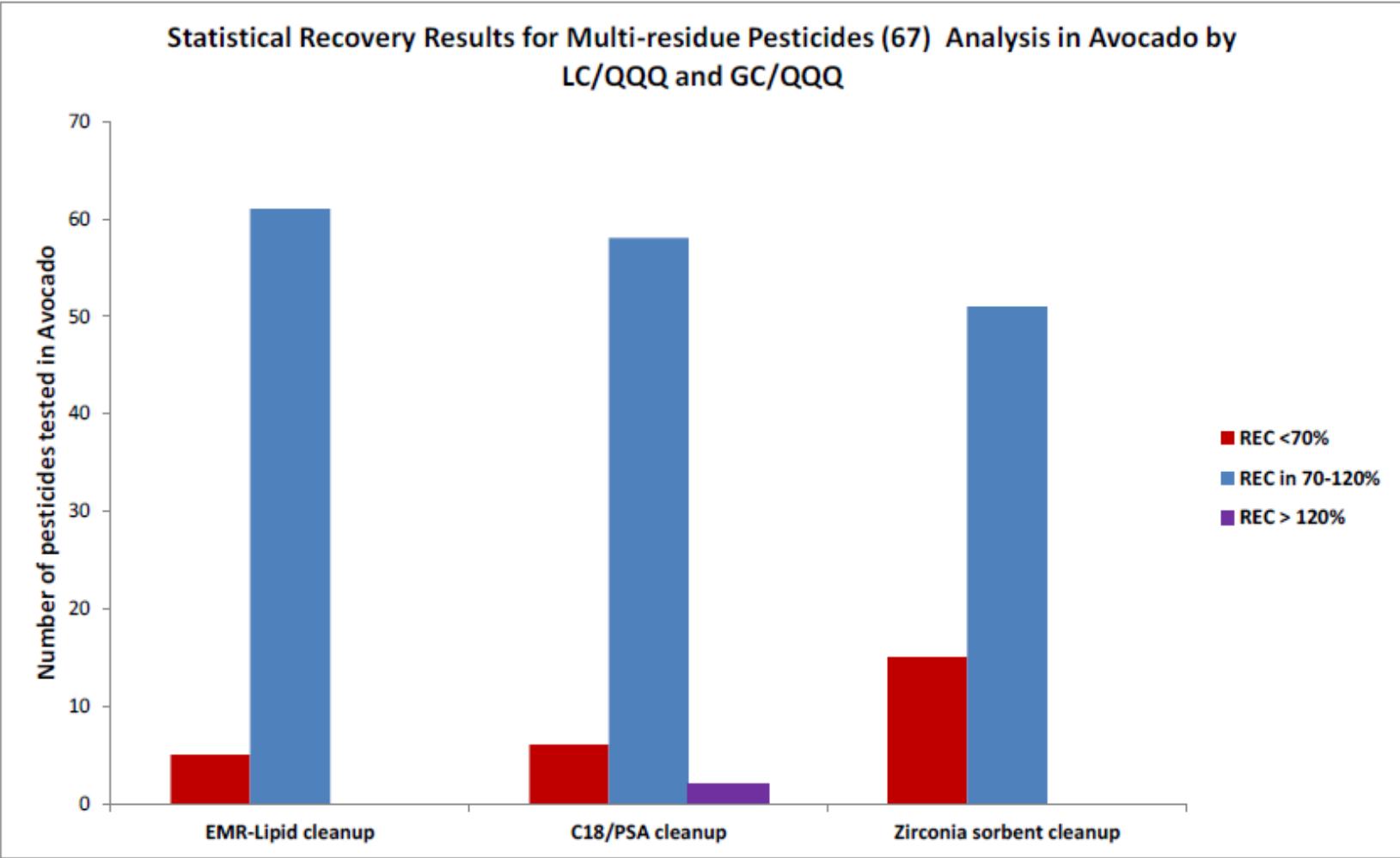
Chromatographic Benefits of Matrix Removal Provided by EMR Cleanup

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+e-CD/C2 epine 50ppb-1.D</p> 	<p>+MRM(183.1 → 168.1)AV/MBP+e-CD/C2 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+e-CD epine 50ppb-1.D</p> 	<p>+MRM(183.1 → 168.1)AV/2-Dep+e-CD epine 50ppb-1.D</p> 
C18/PSA cleanup	<p>+MRM(324.1 → 296.1)AV/C18FRQ3d</p> 	<p>+MRM(151.0 → 79.1)AV/2-Dep+e-CD epine 50ppb-1.D</p> 	<p>+MRM(183.1 → 168.1)AV/Fatty acid epine 50ppb-1.D</p> 

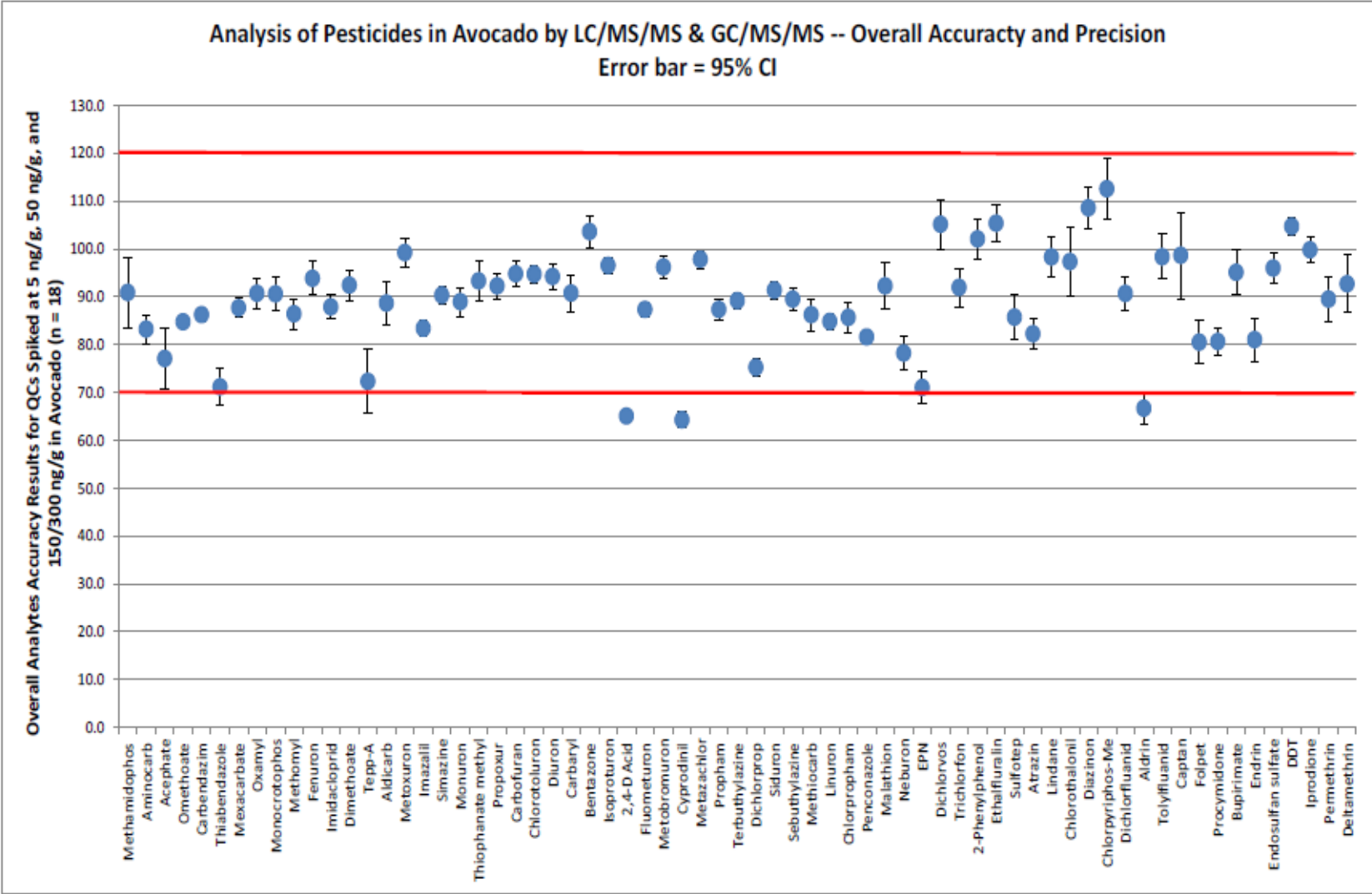
Selected Problematic Pesticides for Recovery Comparison



Statistical Recovery Results Comparison



Method Accuracy and Precision

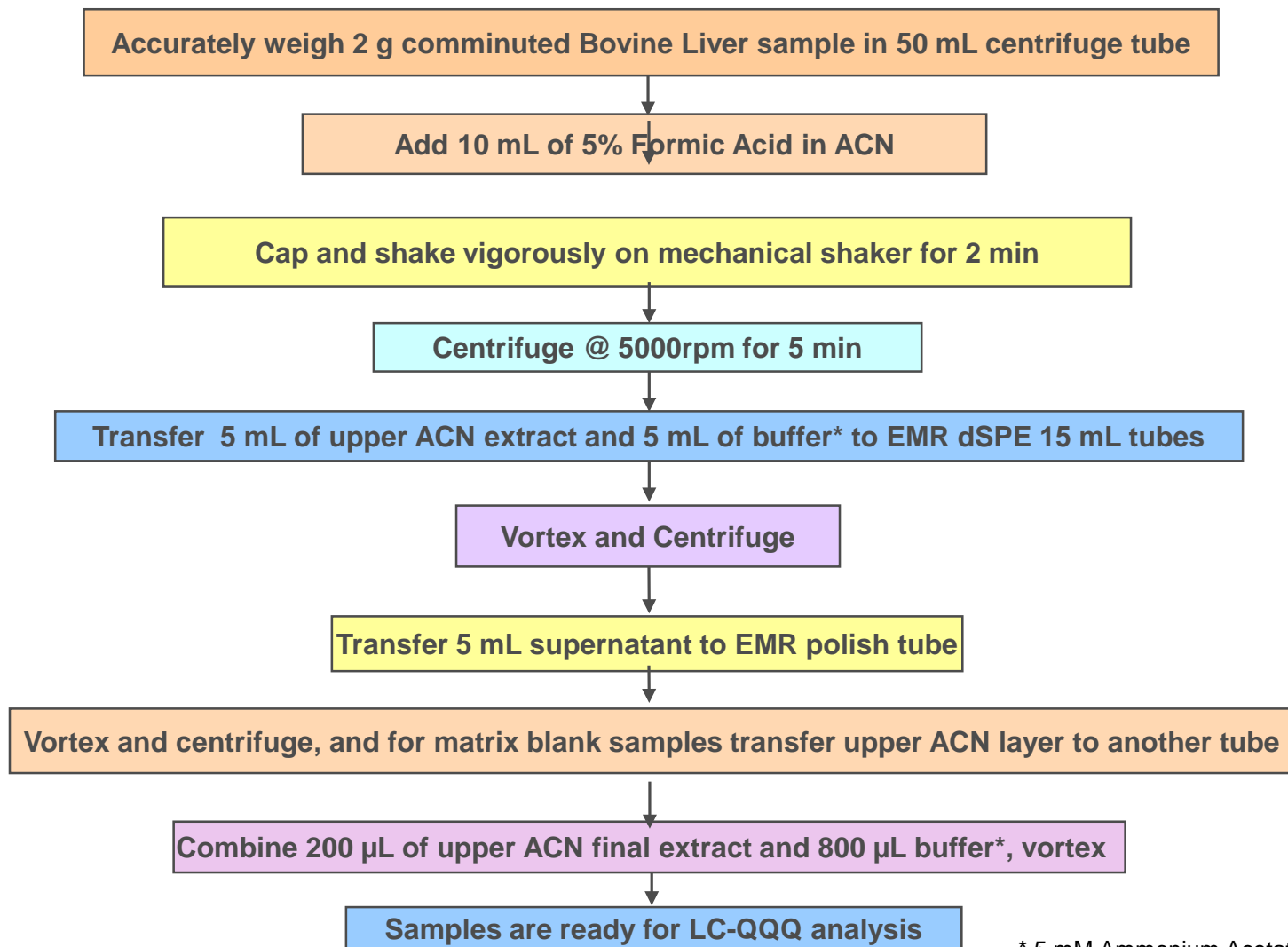


Veterinary Drugs in Bovine Liver

Representative Vet Drugs for Evaluation

Representative Vet Drug	Drug Class	Representative Vet Drug	Drug Class	Representative Vet Drug	Drug Class
Amoxicillin	β-Lactam	Pednisone	Corticosteroid	Tylosin	Macrolide
Difloxacin		Oxyphenylbutazone	NSAID	Oxytetracine	Tetracycline
Florfenicol	Phenicol	2-Thiouracil	Thyreostat	Doxycycline	
Chloramphenicol		Metronidazole-OH	Nitroimidazole	Chlortetracycline	
Sulfamethizole	Sulfonamide	Fenbendazole	Anthelmintic	Acepromazine	Tranquilizer
Sulfamethoxypridazine		Lavamisole		Chlorpromazine	
Lincomycin	Lincosamide	Morantel		Ketoprofen	
Ciprofloxacin	Fluoroquinolone	Bithionol	Flukicide	Cefazolin	Cephalosporin
Norfloxacin		Clorsulon		Melengesterol	other
Danofloxacin		Niclosamide		Ractopamine	β-Agonist

EMR-Lipid Protocol for Multi-residue Analysis of Vet Drugs in Bovine Liver



* 5 mM Ammonium Acetate, pH 5.0

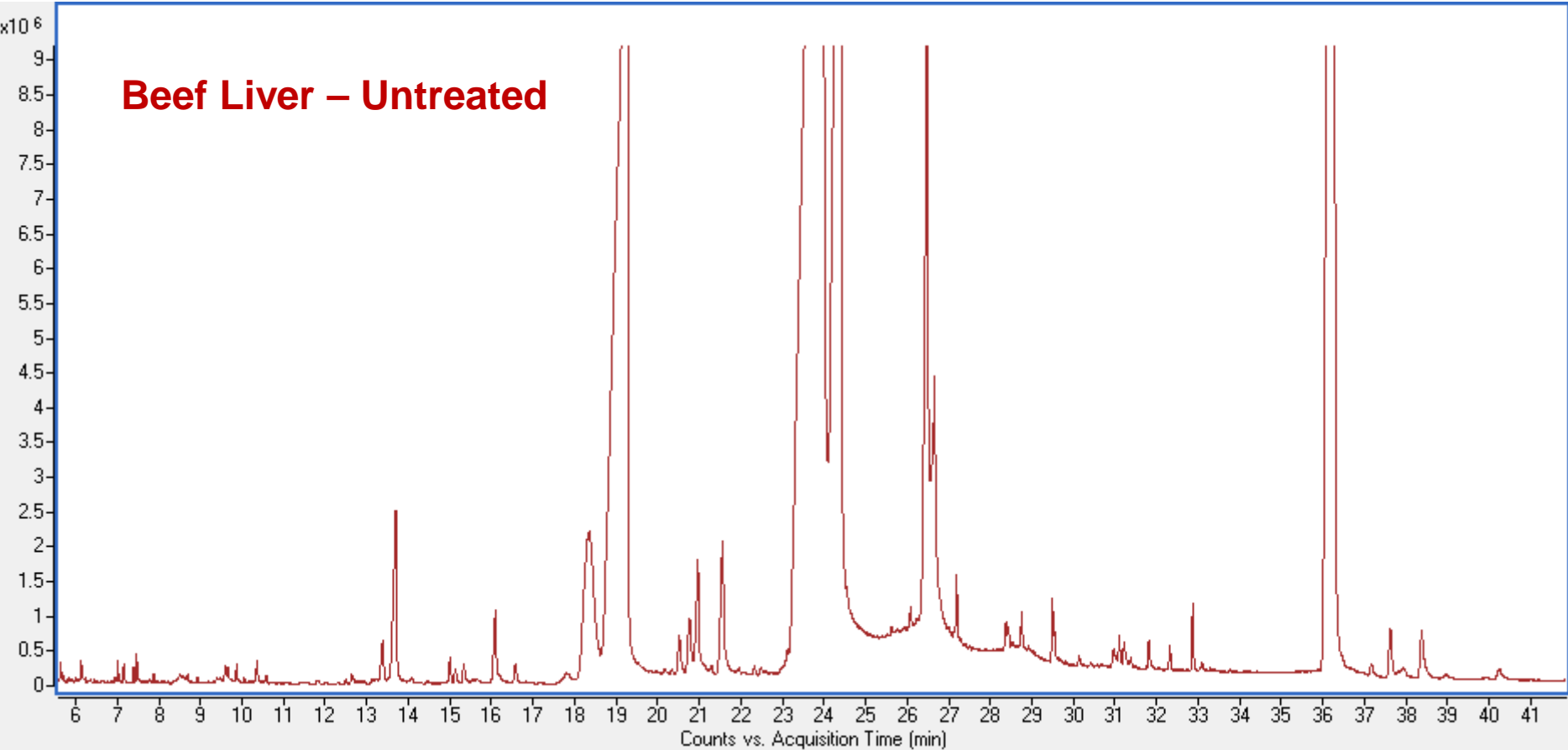
Bovine Liver Matrix Co-Extractives Comparison by Weight

Cleanup	Amount of co-extractives (mg, n= 3)	% of matrix co-extractives removed by cleanup
No further cleanup	12.1	--
EMR dSPE	5.3	56.2
Zirconia dSPE	6.0	50.4
C18 dSPE	7.8	35.5

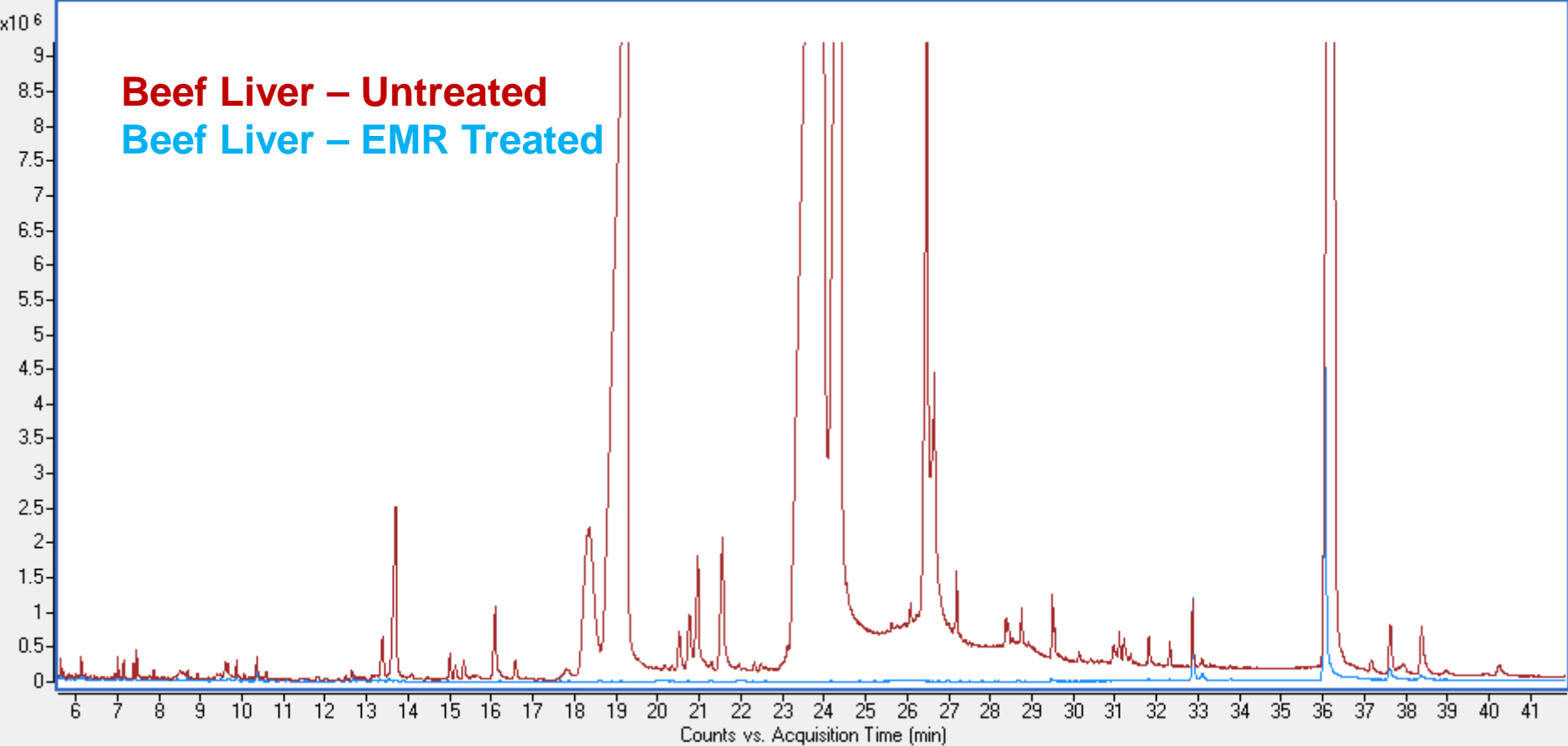
$$\% \text{ Matrix Co-extractives Removed by Cleanup} = \frac{\text{Amount of Co-extractives Removed after Cleanup}}{\text{Amount of Co-extractives without Cleanup}} \times 100\%$$

The use of EMR material cleanup removes extra 20% of liver co-extractives in comparison to tradition QuEChERS cleanup.

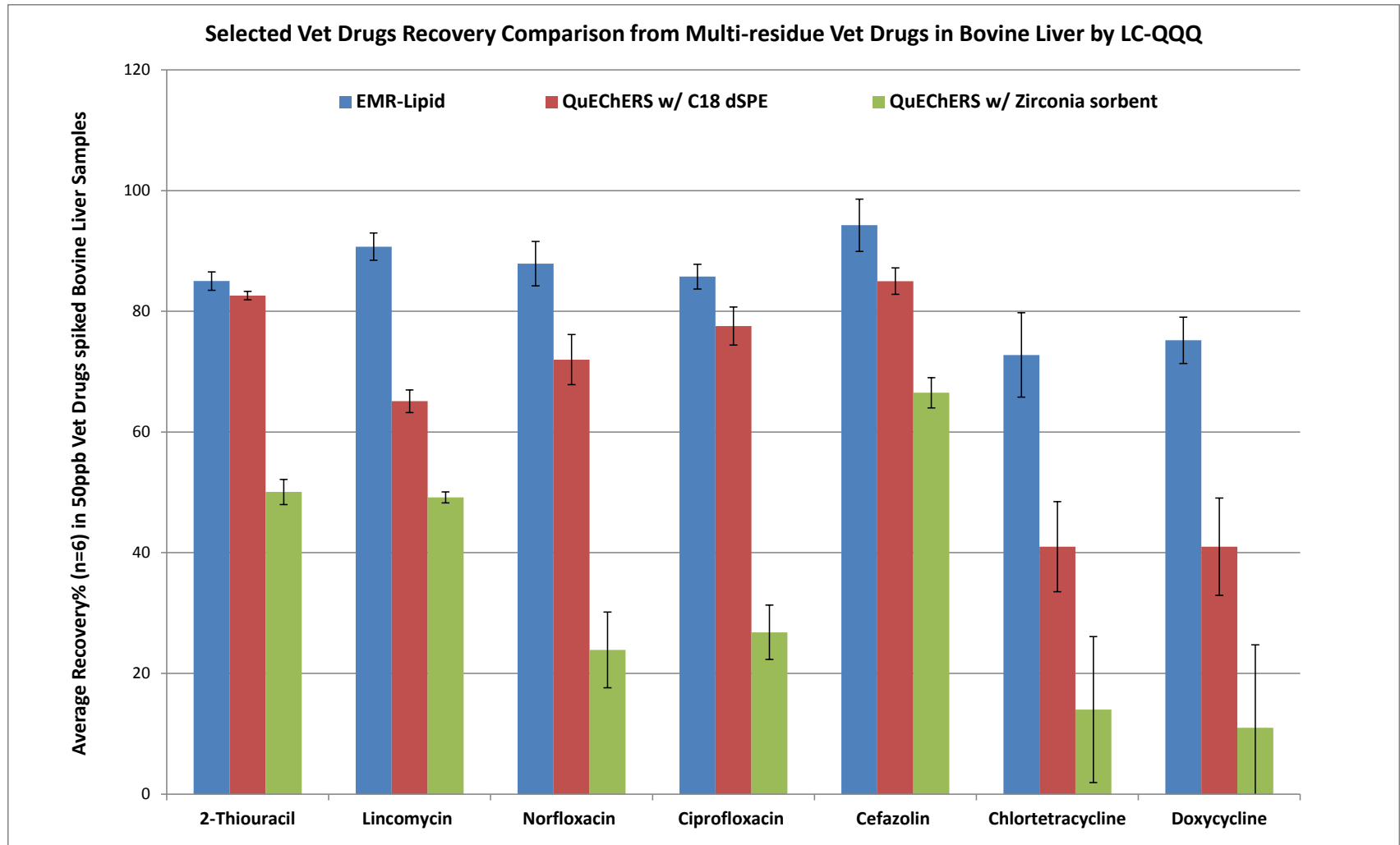
GC-MS Fullscan Beef Liver



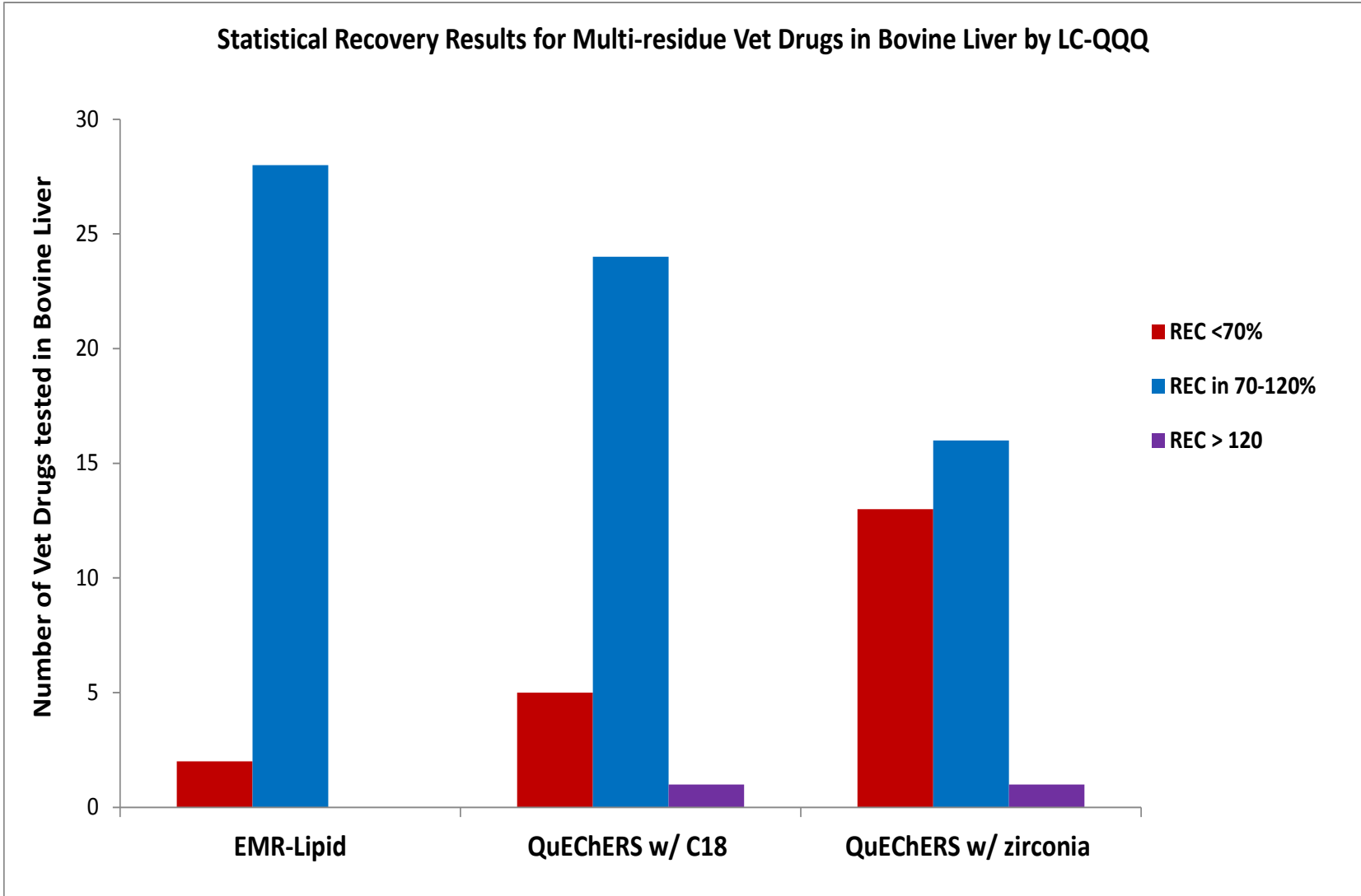
GC-MS Fullscan Beef Liver



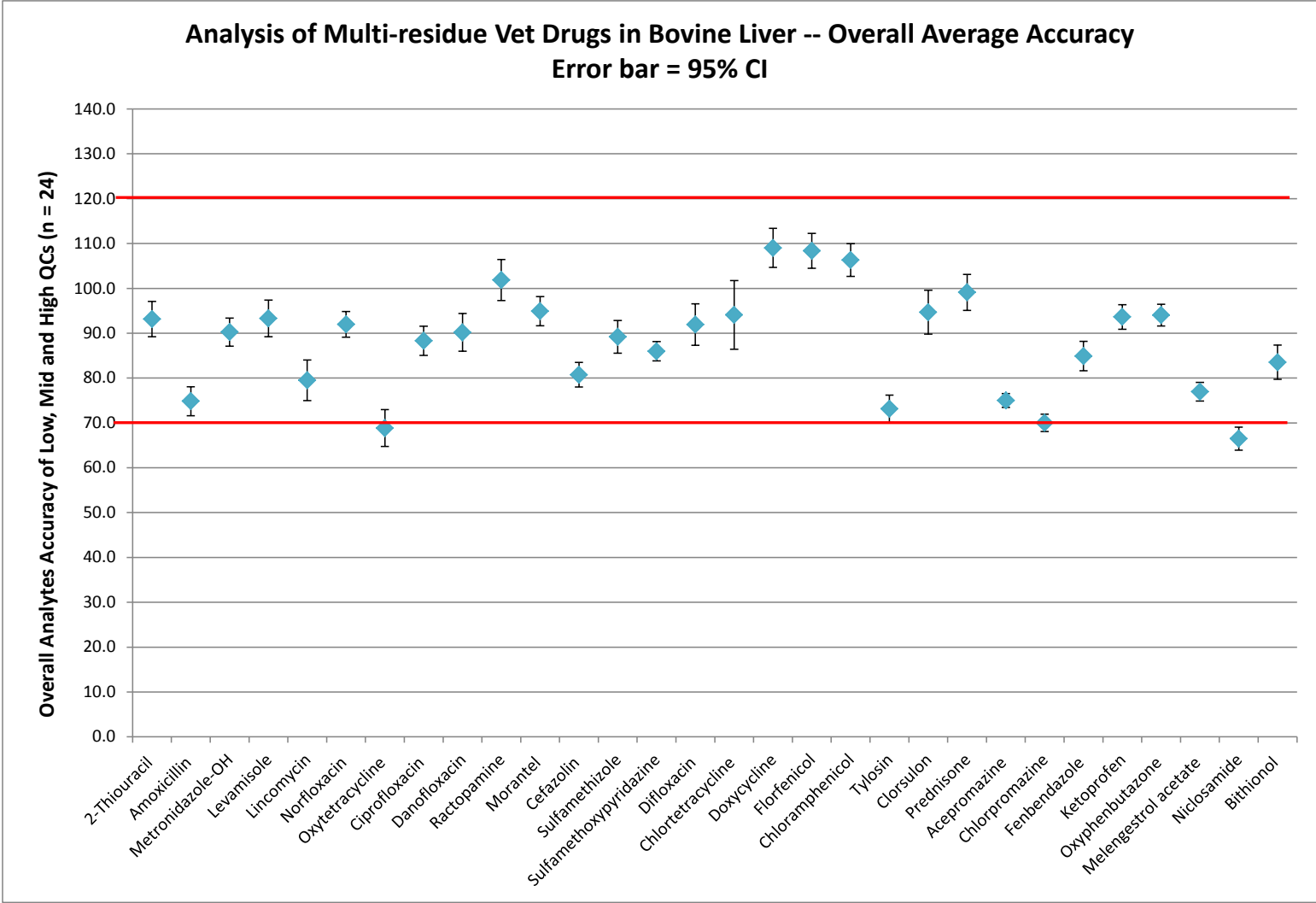
Selected Problematic Vet Drugs for Recovery Comparison



Statistical Recovery Comparison for Vet Drugs in Bovine Liver



Method Accuracy and Precision



*Results include combined multi-vet drug and tetracycline protocols

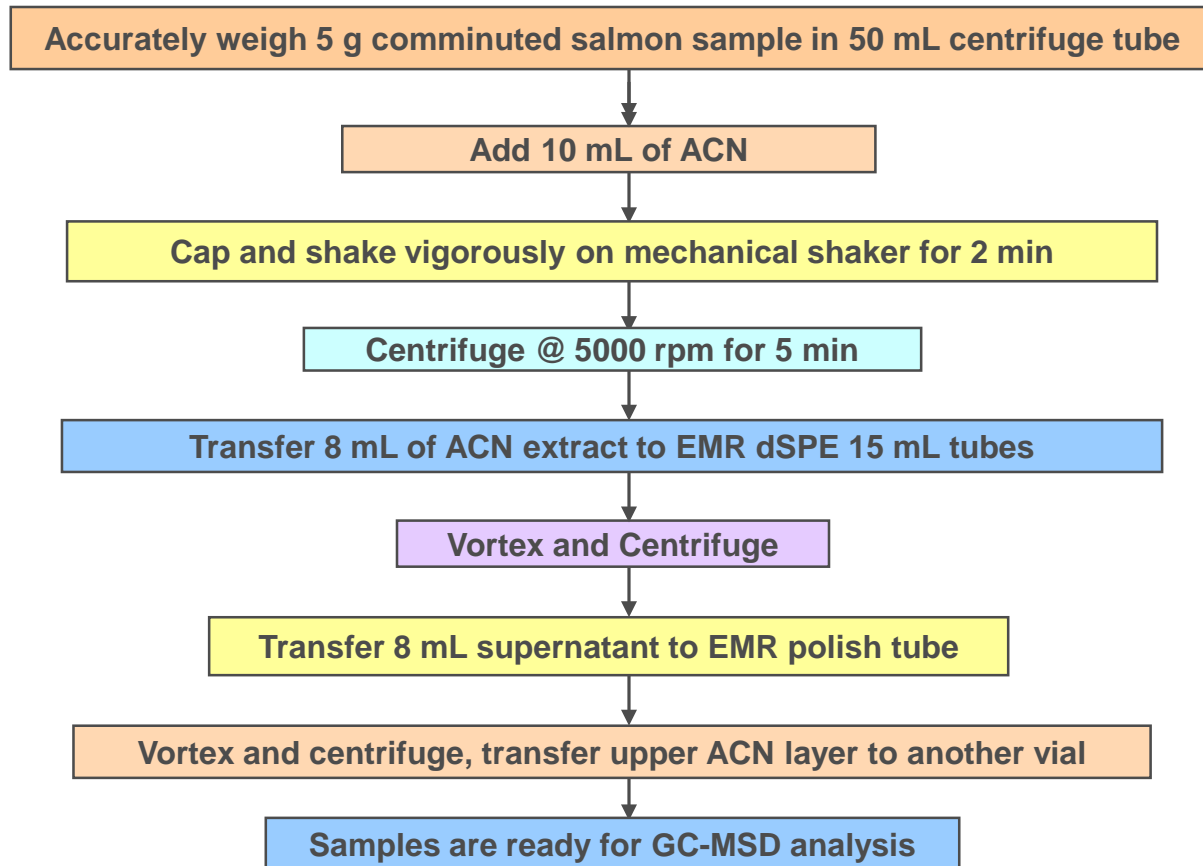
PAHs in Salmon

Target Analytes

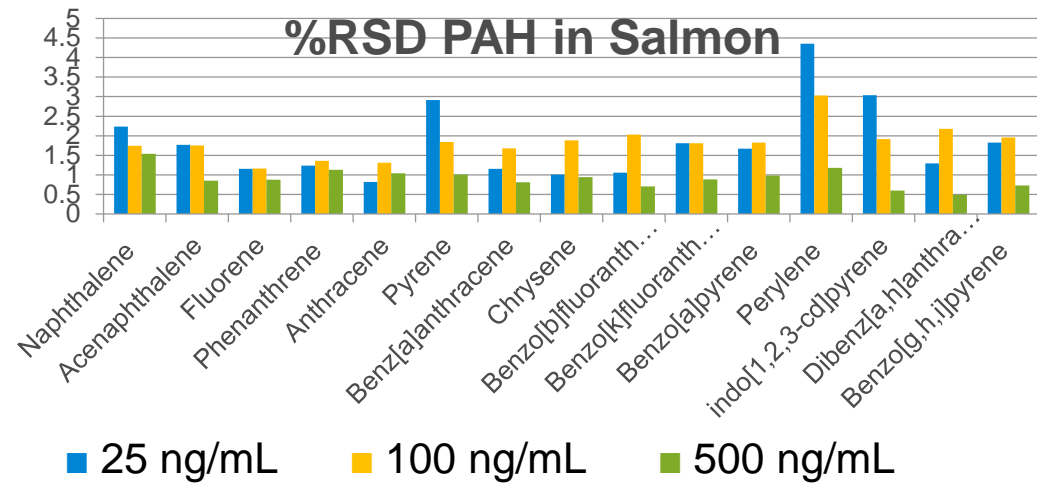
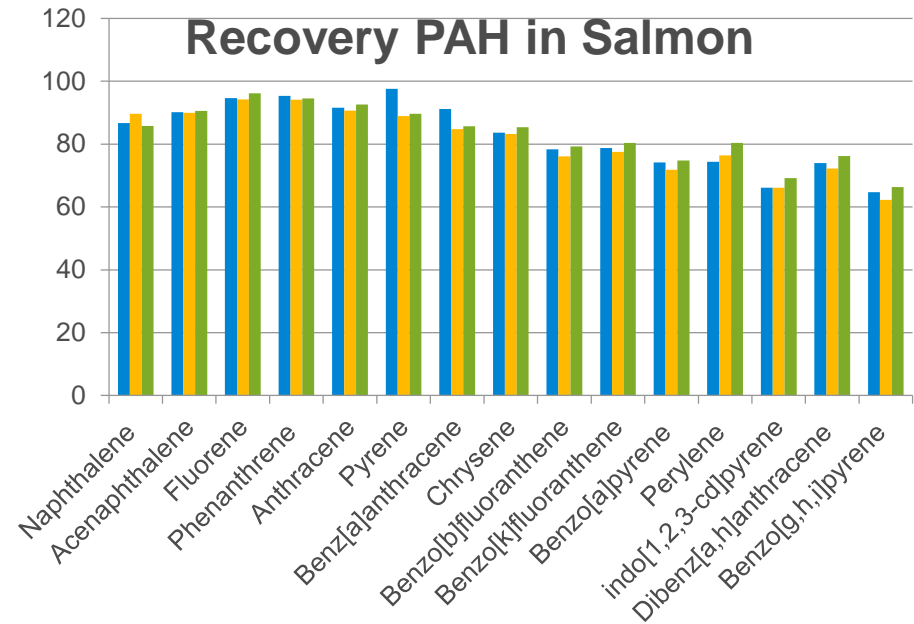
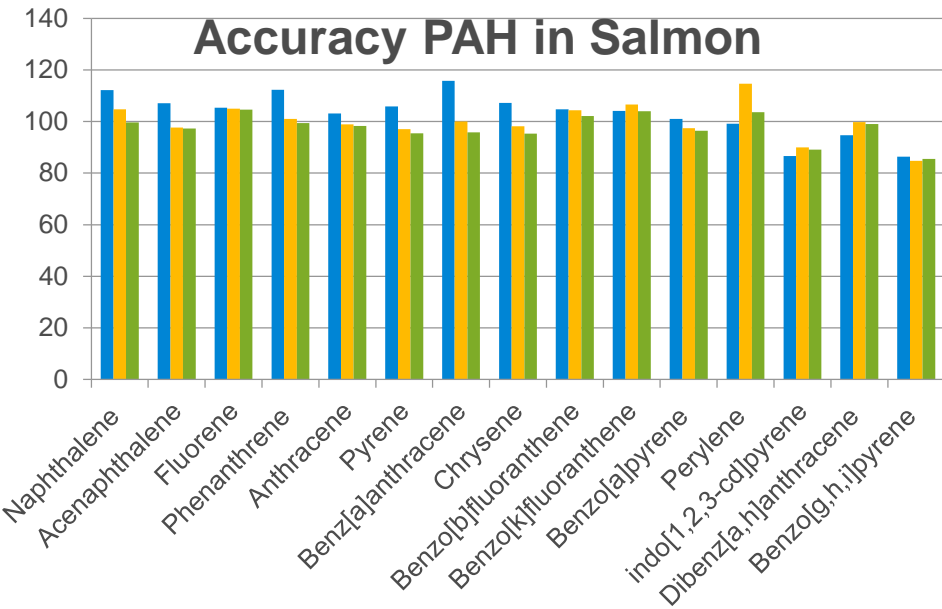
Compound	GC-MS (SIM)	
	RT	Target Ion
Naphthalene	3.89	128.0
Acenaphthalene	5.37	152.0
Fluorene	6.05	166.0
Phenanthrene	7.25	178.0
Anthracene	7.34	178.0
Pyrene	10.31	202.0
Benz[a]anthracene	13.83	228.0
Chrysene	13.93	228.0
Benzo[b]fluoranthene	16.99	252.0
Benzo[k]fluoranthene	17.08	252.0
Benzo[a]pyrene	17.85	252.0
Perylene	18.09	252.0
Indo[1,2,3-cd]pyrene	20.72	276.0
Dibenz[a,h]anthracene	20.87	278.0
Benzo[g,h,i]pyrene	21.29	276.0
Naphthalene-d8	3.87	136.0
Acenaphthalene-d10	5.52	162.0
Phenanthrene-d10	7.22	188.0
Chrysene-d12	13.86	240.0
Perylene-d12	18.03	264.0

Color coded according to internal standard being used

EMR Modified Protocol for Analysis of PAHs in Salmon



EMR – New Procedure Results



■ 25 ng/mL
 ■ 100 ng/mL
 ■ 500 ng/mL

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.
 - EMR fits easily into existing workflows including QuEChERS and modified liquid extraction.
- Key applications were validated with EMR and demonstrate **better recovery, better precision, and decreased matrix impact** to the instrument and results.
- **Less** re-runs, **Less** time manual data integration, **Less** time/cost unexpected instrument maintenance (matrix)
- **More** efficient and productive laboratory = ***reducing cost/sample!***