EMR – Lipid: Next Generation Sample Preparation Materials for Selective Matrix Removal

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Today's Agenda

Introduction

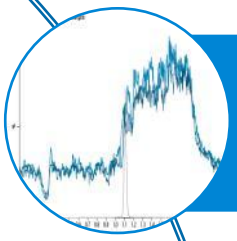
EMR-Lipid - Enhanced Matrix Removal

- Background, workflows, and results

Applications

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

Summary and Conclusions



Remove interferences 1

Accurate and consistent data 2

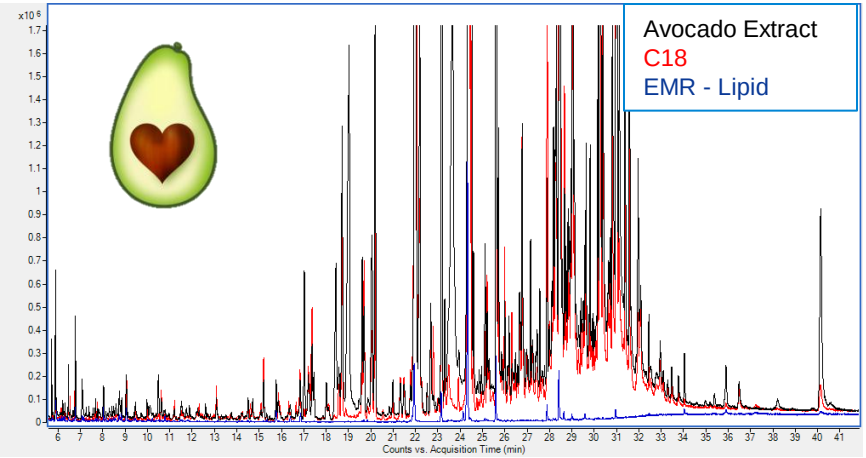
Keep the instruments running 3

Process as many samples for as little cost as possible 4

EMR-Lipid: In The Beginning

Voice of Customer (VOC):

- Strategic Advisory Board - Collection of customers from government, academia, and food industry.
- Identified simplified and selective lipid removal as the number one challenge in sample preparation for the food industry.
- *Although chemists have learned to live with and work around these issues, they can benefit from better lipid / matrix removal.*



EMR-Lipid

Enhanced Matrix Removal

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

EMR Product offering

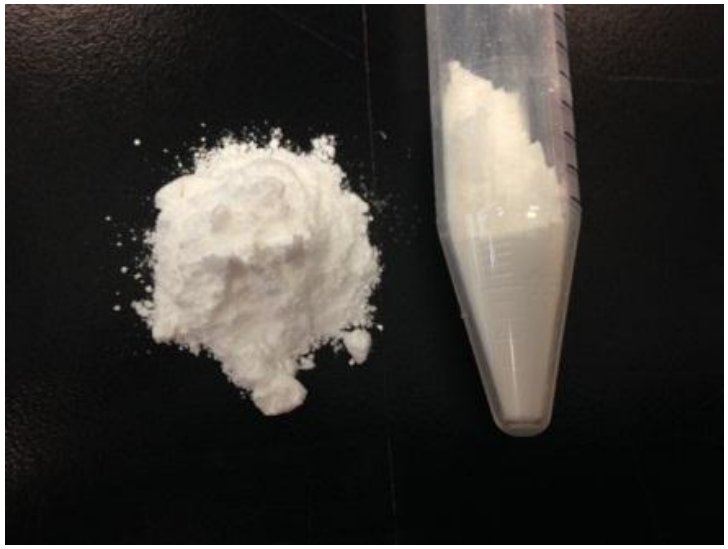
Extraction Tube

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.
- Rapidly interacts with straight chain, "lipid-like" functional groups.

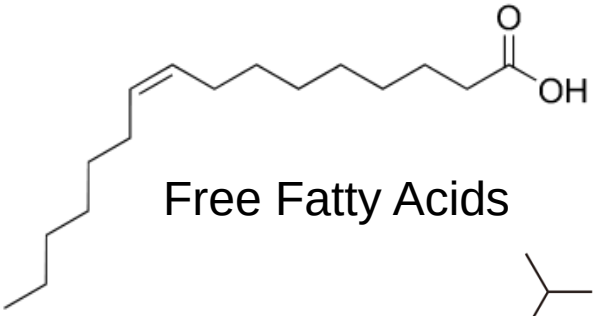
EMR-Lipid Mechanism – Size exclusion and hydrophobic interaction.

… and what does it do?

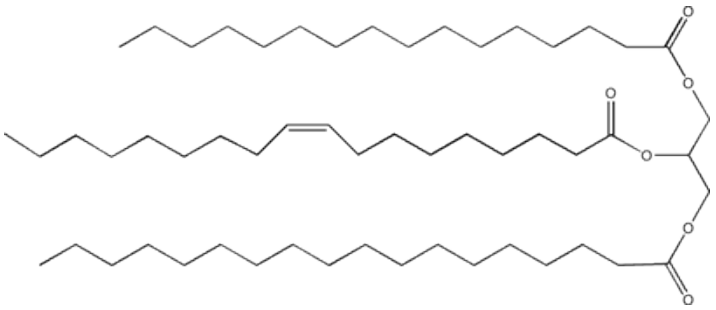
EMR sorbent removes Lipids

What are Lipids?

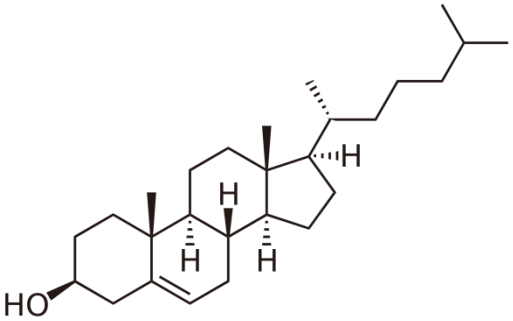
A class of naturally occurring hydrocarbon containing compounds commonly known as fats and oils



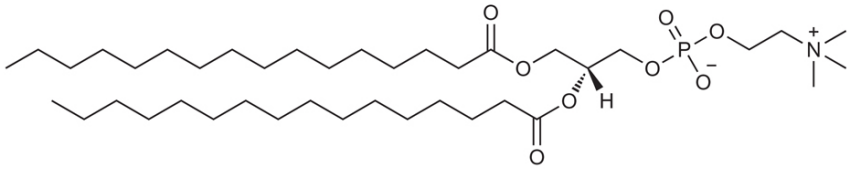
Free Fatty Acids



Triglycerides



Cholesterol



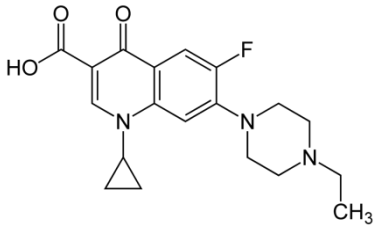
Phospholipids

What Does EMR *NOT* Interact With?

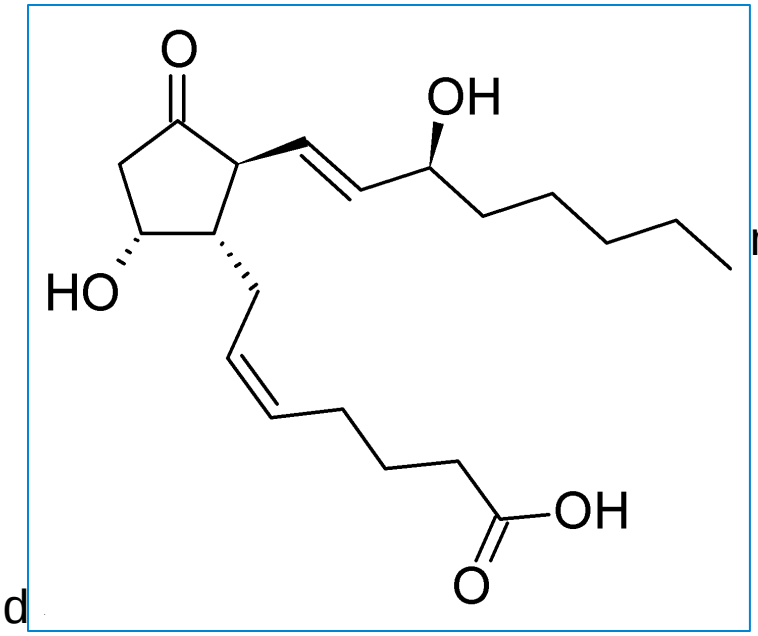
EMR does NOT remove analytes of interest

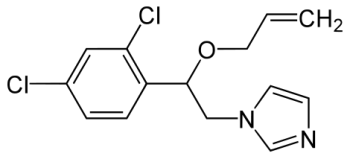
Exceptions?

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

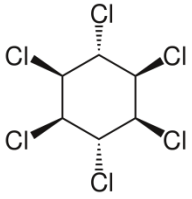


Fluoroquinolones

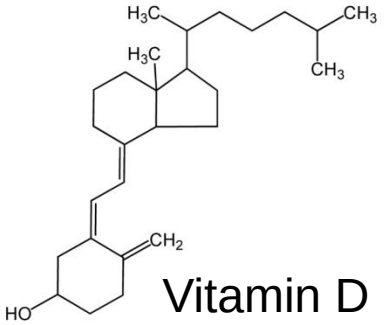




midazole pesticides



Organochlorine Pesticid



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.)

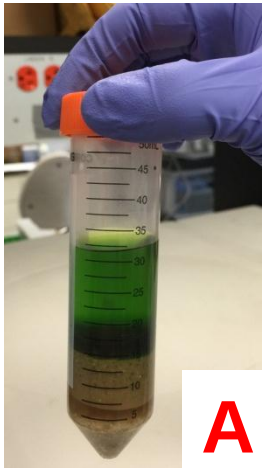
  ***EMR Applications*: -** Pesticide Residues in Avocado,

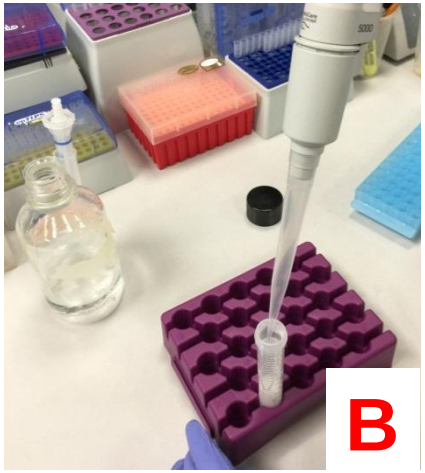
Liquid Extraction (Protein Precipitation)

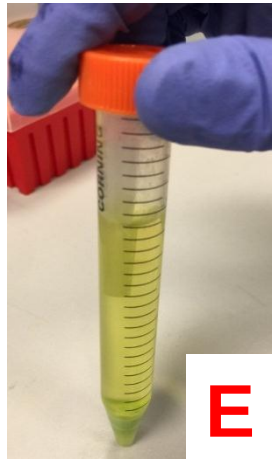
- Proteins are removed by a “crash” step prior to injection or cleanup (e.g. milk, meat, seafood, etc.)

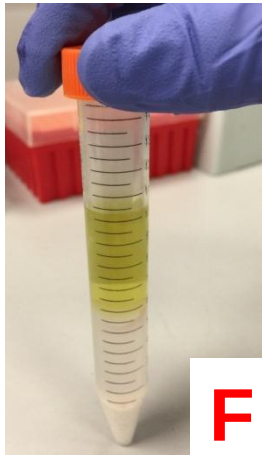
  ***EMR Applications*: -** PAHs in Salmon,
  - Veterinary Drugs in Bovine Liver

EMR – Lipid dSPE Cleanup









A. QuEChERS or Liquid Extract
B. Add H_2O to EMR tube ("activation")
C. Transfer extract
D. Vortex and centrifuge
E. Supernatant (1:1; extract: H_2O)
F. Polish ($MgSO_4$/NaCl)

EMR-Lipid Launch Applications

Launch Applications:

- *Veterinary Drugs in Beef Liver*

  5991-6098EN

- *Pesticides in Avocado (LC-MS/MS)*

  5991-6096EN

- *Pesticides in Avocado (GC-MS/MS)*

  5991-6097EN

- *PAHs in Salmon*

  5991-6088EN

More coming soon…

Pesticides in Avocado

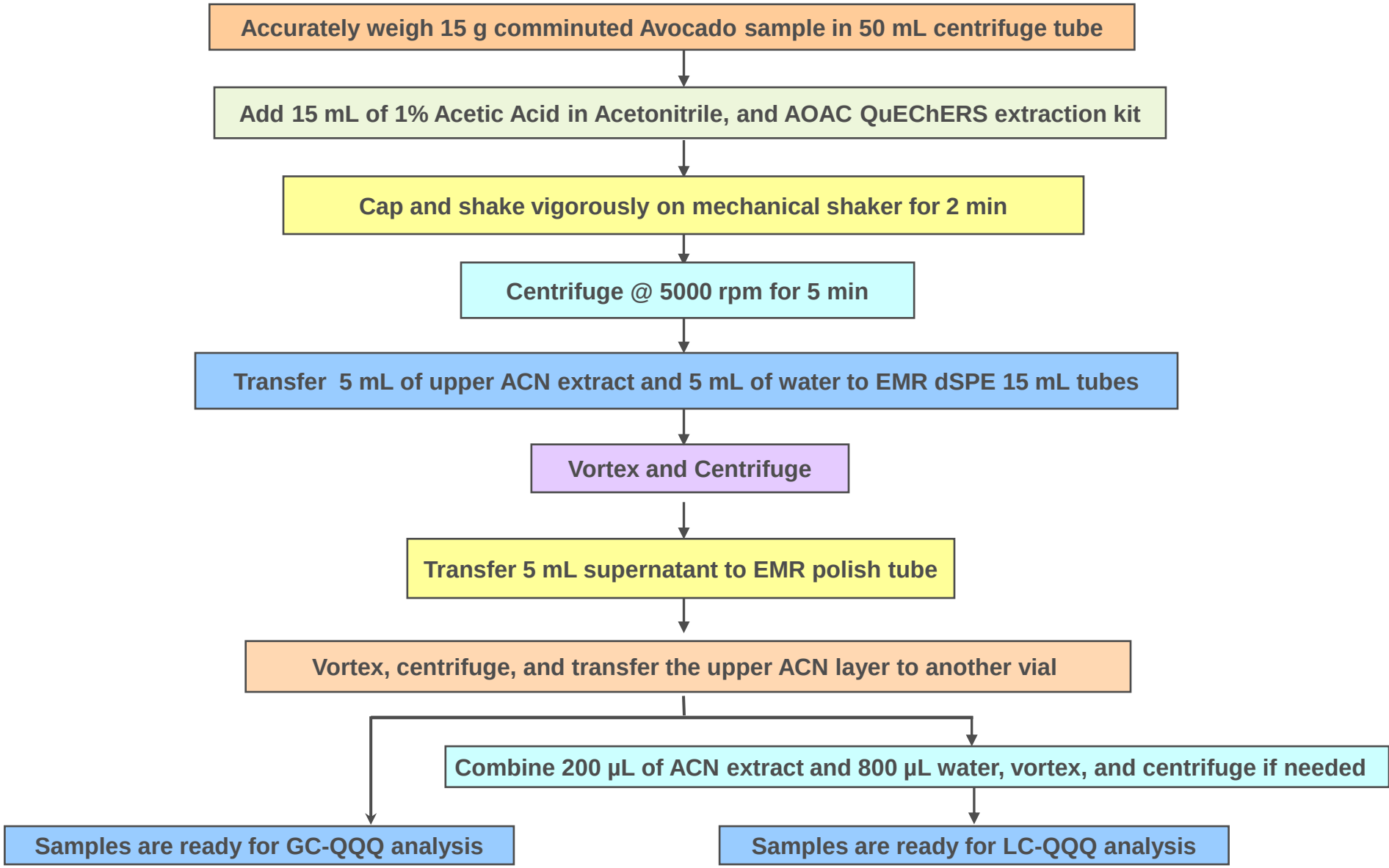
67 Pesticides Analysis in Avocado by LC and GC-QQQ

Representative Pesticide	Chemical Class	Pesticide Group	Detection Technique
Dichlorvos	Organophosphate	Herbicide	GC-MS
Sulfotep		Insecticide	
Diazinon			
Chlorpyriphos methyl			
Coumaphos			
Trichlorfon			
Lindane	Organochlorine		
Aldrin			
Endrin			
DDT			
Endosulfan sulfate			
Methoxychlor			
2-Phenylphenol	Phenol	Fungicide	
Atrazine	Triazine	Herbicide	
Bupirimate	Pyrimidinol	Fungicide	
Chlorothanil	Chloronitrile		
Captan	Phthalimide		
Folpet			
Captafol			
Iprodione	Dicarboximide		
Procymidone			
Permethrin	Pyrethriod	Insecticide	
Deltmethrin			
Pyraclostrobin	Strobilurin	Fungicide	
Ethalfluralin	Dinitroaniline	Herbicide	

Representative Pesticide	Chemical Class	Pesticide Group	Detection Technique
Methamidophos	Organophosphate	Insecticide	LC-MS
Acephate			
Omethoate			
Dimethoate			
Phosmet			
Carbaryl	Carbamate		
Propoxur			
Dichlofluanid	Sulphamide	Fungicide	
Tolylfluanid			
Carbendazim	Benzimidazole		
Thiabendazole			
Thiophanate methyl			
Cyprodinil	Anilinopyrimidine		
Imidacloprid	Neonicotinoid	Insecticide	
Pymetrozine	Pyridine		
Imazalil	Imidazole	Fungicide	
Penconazole	Triazole		
Aminocarb	Carbamate	Insecticide	
Oxamyl			
Methomyl			
Aldicarb			
Fenuron	Urea	Herbicide	
Metoxuron			

Representative Pesticide	Chemical Class	Pesticide Group	Detection Technique
Simazine	Triazine	Herbicide	LC-MS
Sebuthylazine			
Terbuthylazine		Algaecide	
Carbofuran	Carbamate	Insecticide	
Methiocarb			
Chlorpropham		Herbicide	
Propham			
Monuron	Urea		
Chlorotoluron			
Diuron			
Fluometuron			
Isoproturon			
Metobromuron			
Siduron			
Linuron			
Neburon			
2,4-D Acid	Chlorophenoxy acid		
Dichlorprop			
Metazachlor	Chloracetanilide		
Bentazon	Unclassified		
Malathion	OP	Insecticide	
EPN			
Tepp-A			
Monocrotophos			

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



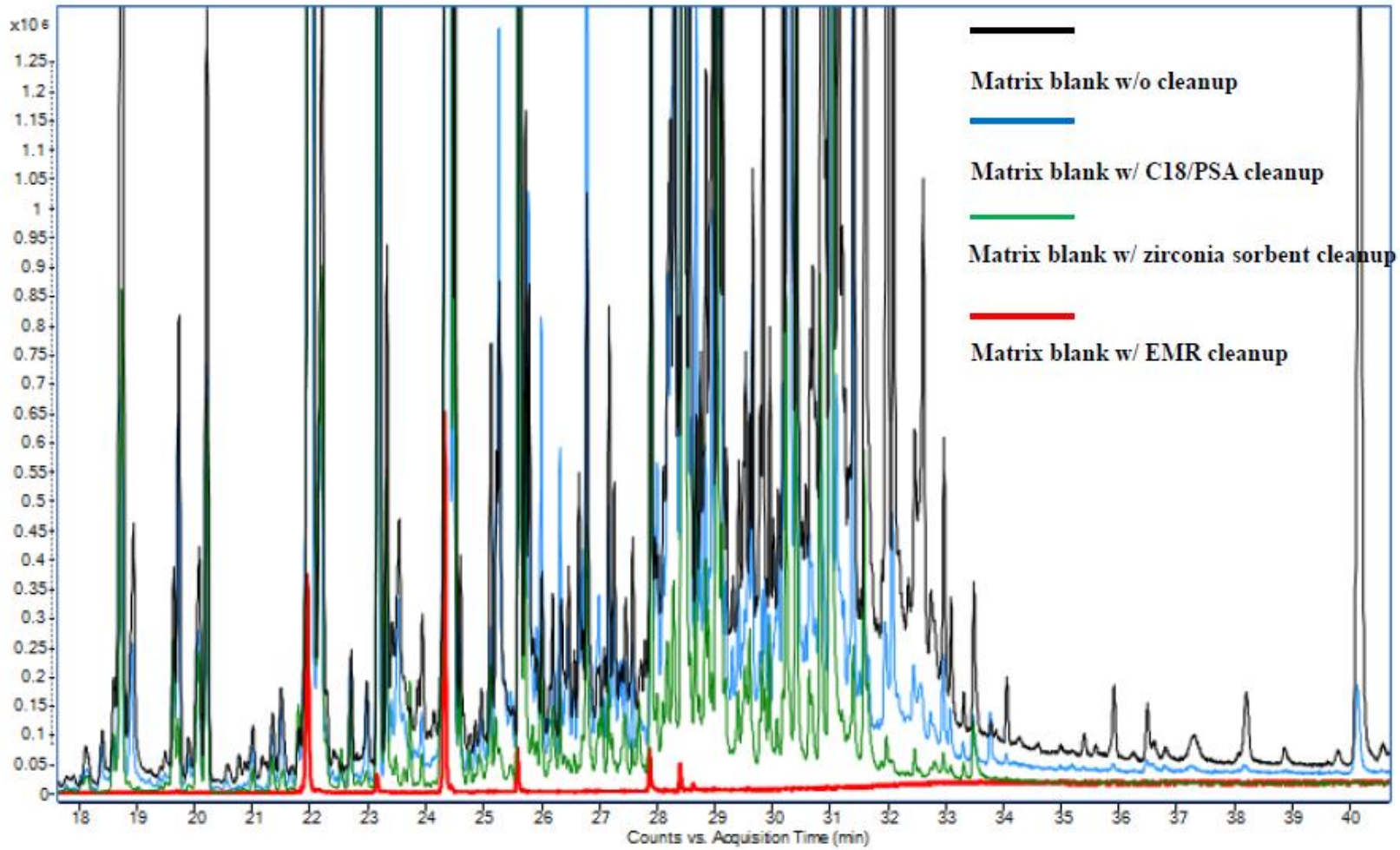
Comparison of Avocado Co-extractives by Weight

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

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

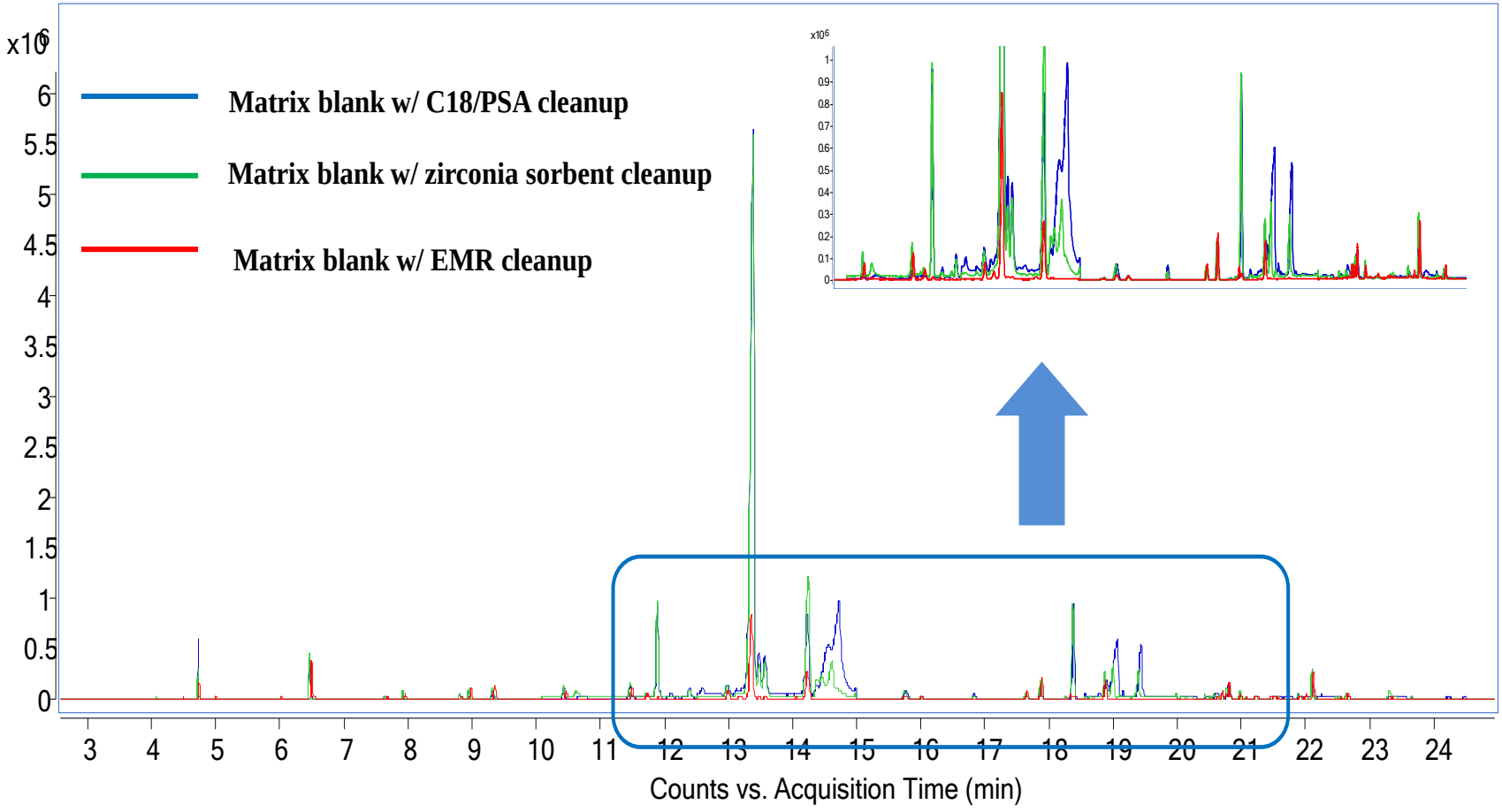
The use of EMR material cleanup removes extra 20-30% of Avocado co-extractives in comparison to traditional QuEChERS and/or competitor's cleanup

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

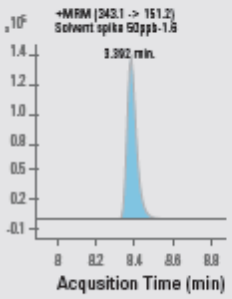
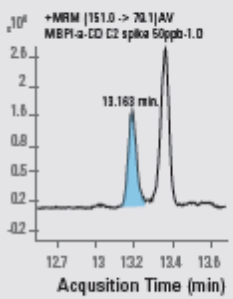
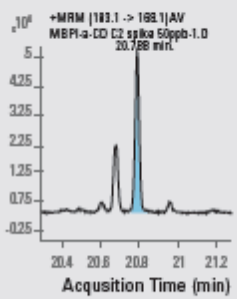
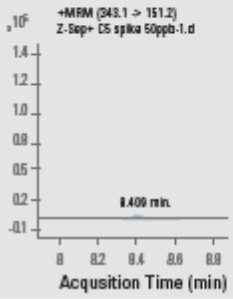
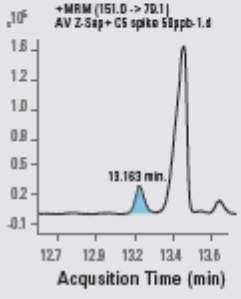
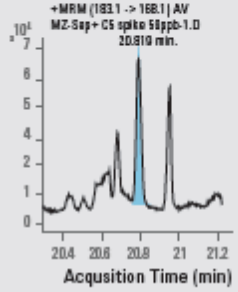
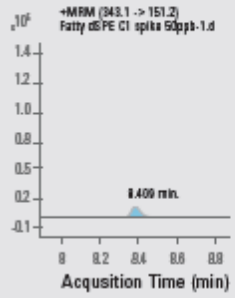
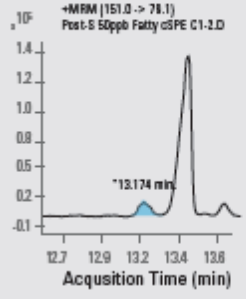
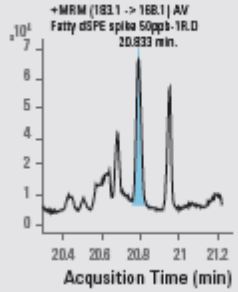


The use of EMR material cleanup provides significantly cleanup chromatographic sample background.

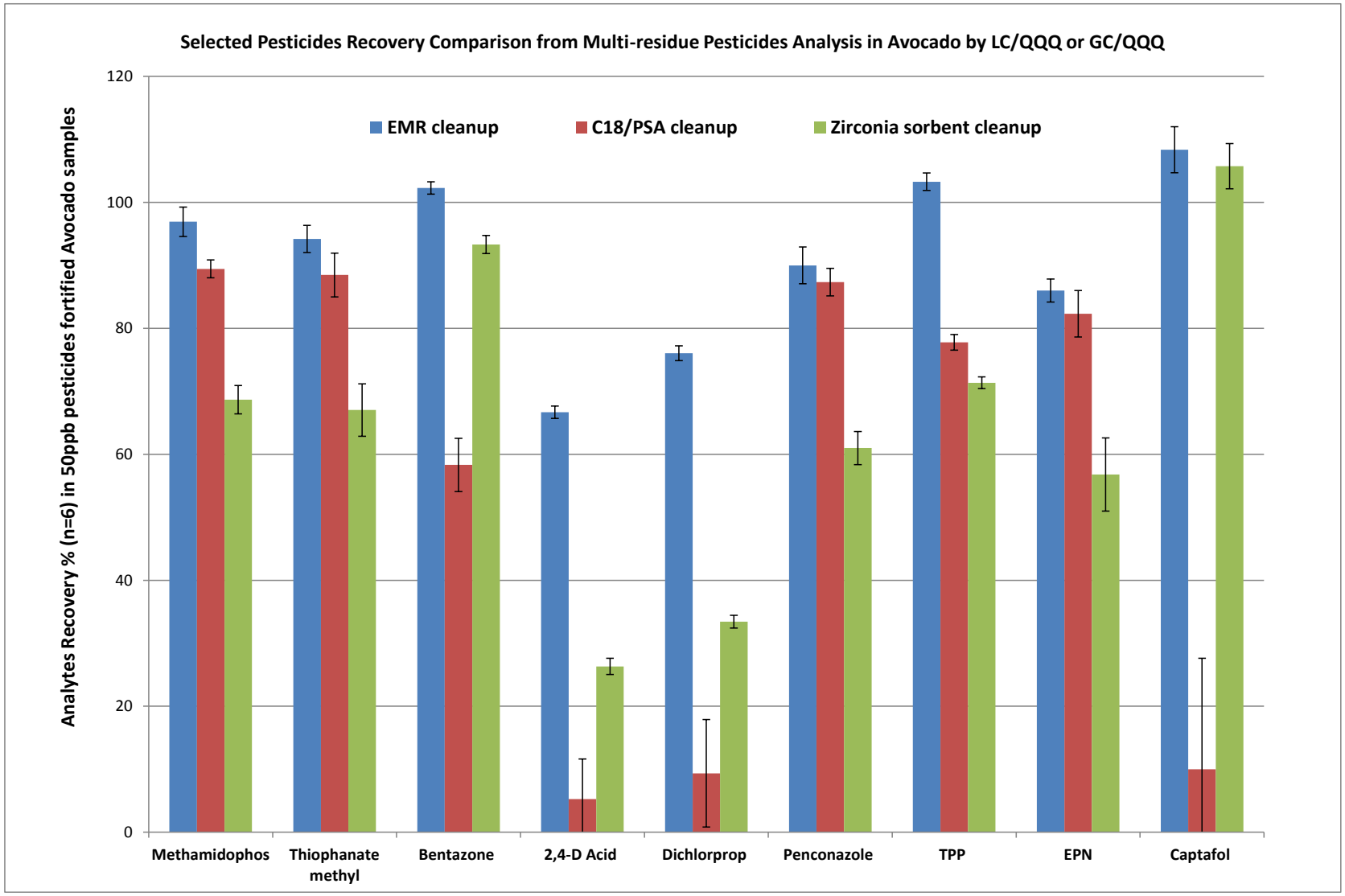
Comparison of GC/MS/MS MRM Chromatogram for Matrix Background



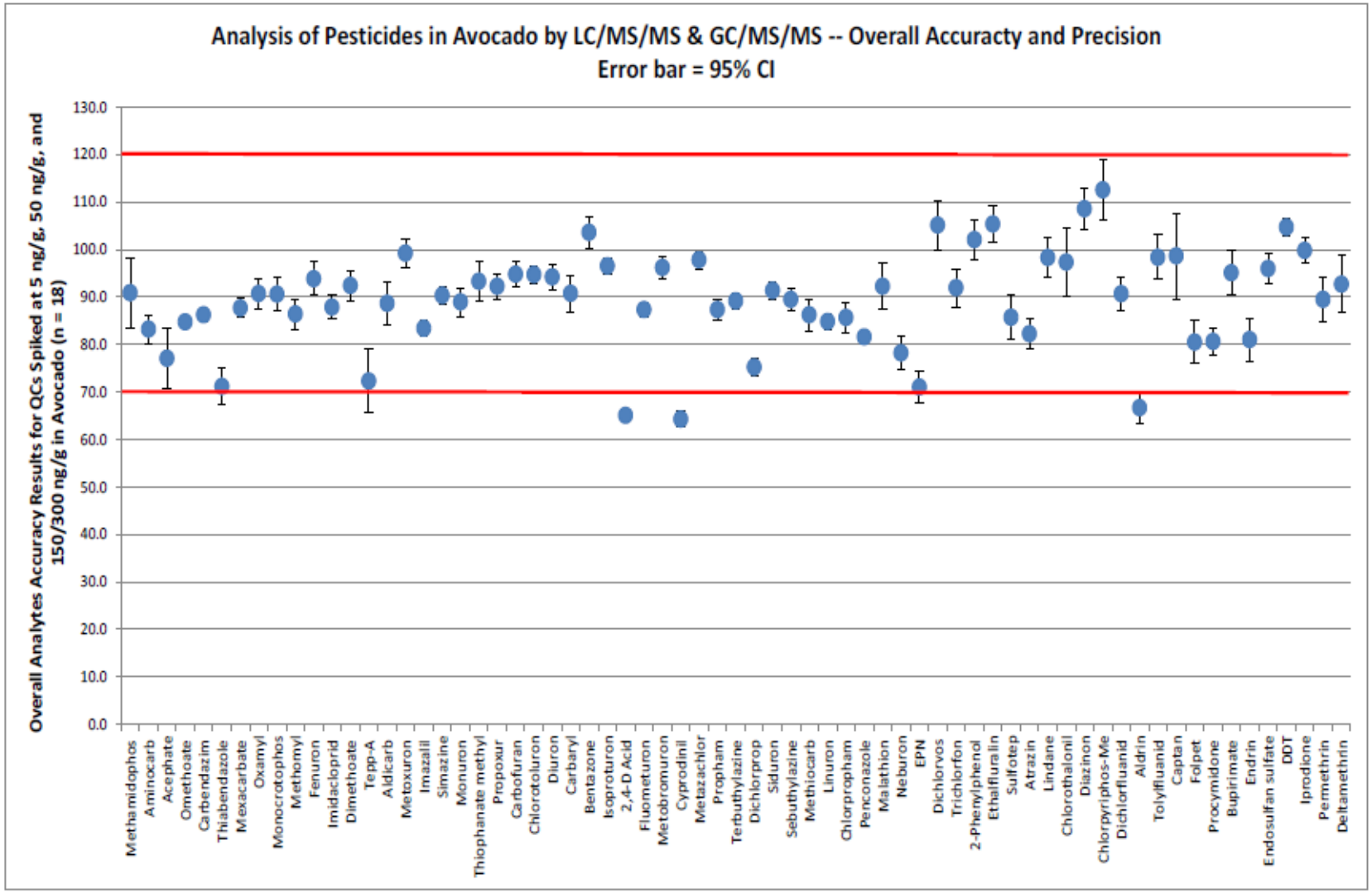
Chromatographic Benefits of Matrix Removal Provided by EMR Cleanup

BENEFIT	REDUCED MATRIX SUPPRESSION	IMPROVED S/N RATIO	LESS INTERFERENCES FOR ACCURATE INTEGRATION
Example	Thiophanate methyl in Avocado on LC-QQQ	Captan in Avocado on GC-QQQ	Permethrin in Avocado on GC-QQQ
CEMR Cleanup			
Zirconia Cleanup			
Fatty dSPE cleanup			

Selected Problematic Pesticides for Recovery Comparison



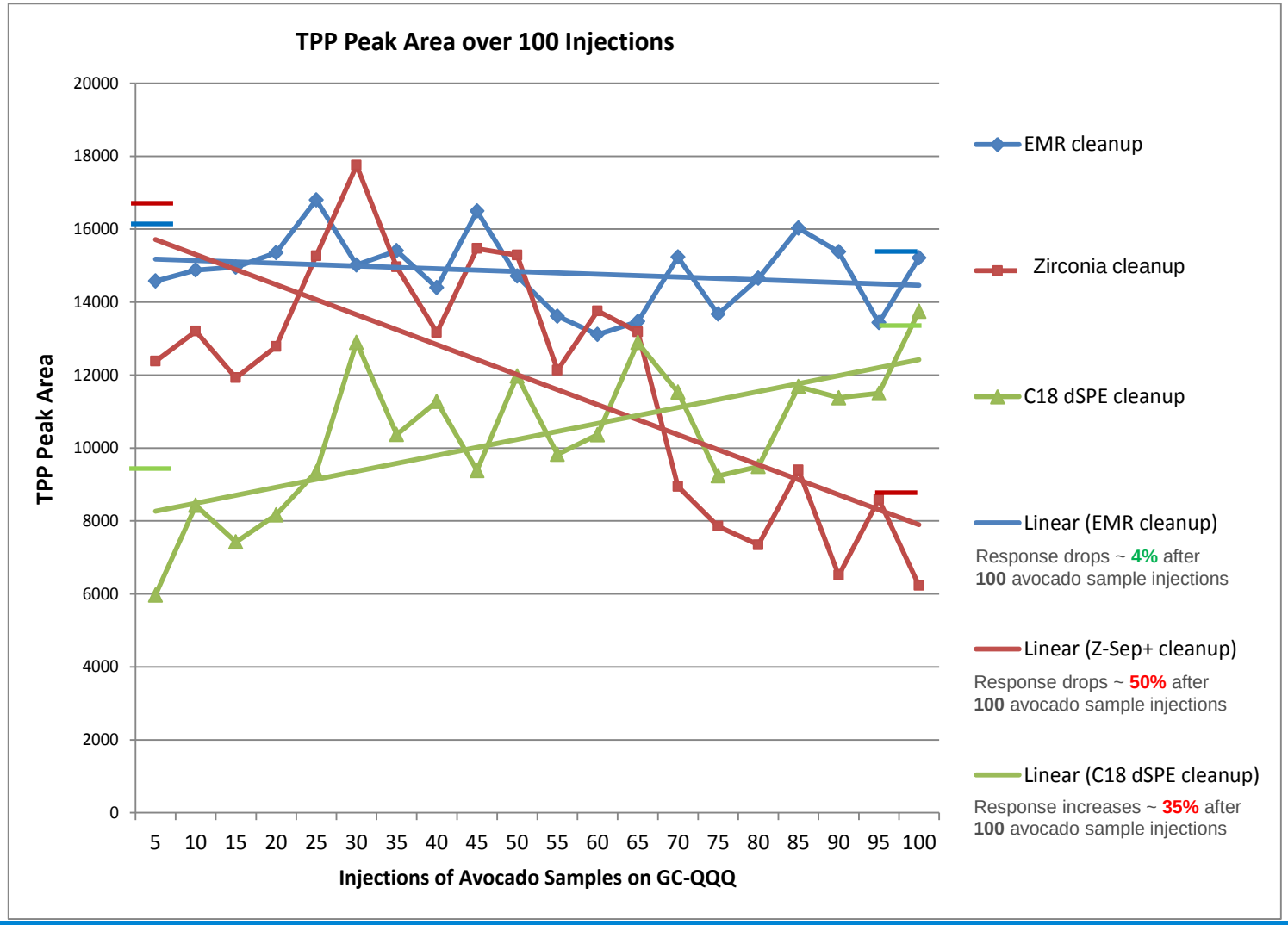
Method Accuracy and Precision



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

Pesticides	Analytes RSD over 100 injections on GC/QQQ (n = 20)		
	EMR Cleanup	C18/PSA cleanup	Zirconia sorbent cleanup
Dichlorvos	6.2	10.5	16.8
2-Phenylphenol	7.0	13.6	19.5
Ethalfluralin	12.4	18.8	32.0
Sulfotep	7.1	11.8	17.2
Atrazin	6.8	12.2	19.1
Lindane	8.5	10.8	20.0
Chlorothalonil	12.5	11.7	37.4
Diazinon	6.6	11.7	16.9
Chlorpyriphos-methyl	8.4	8.9	14.9
Dichlorfluanid	11.7	9.0	25.9
Aldrin	9.8	19.3	25.7
Tolylfluanid	10.5	6.6	17.8
Captan	29.9	51.9	47.1
Procymidone	6.8	14.3	22.5
Bupirimate	6.8	10.4	20.7
Endrin	8.3	12.6	24.1
Endosulfan sulfate	8.5	12.1	22.4
DDT	21.6	22.4	42.6
Iprodione	11.0	10.7	40.0
Permethrin	6.8	11.8	18.8
Parathion ethyl-D10 (IS)	11.8	7.2	13.0
TPP (IS)	9.1	19.9	28.3

Comparison of Analytes Response Consistency over Multiple Avocado Sample Injections

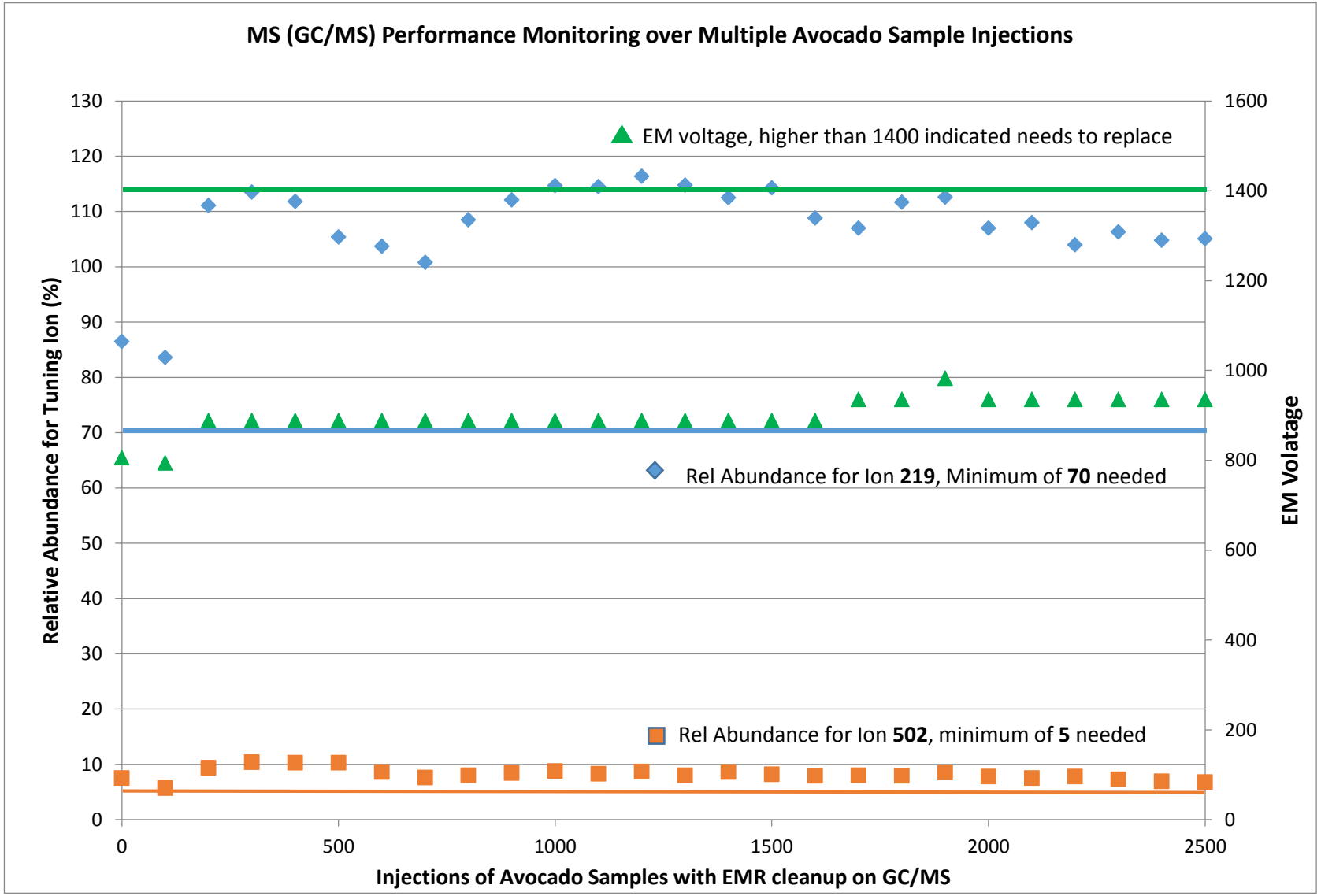


TPP

Commonly used IS

RT: 18.3 min

MS Source Critical Tuning Parameters

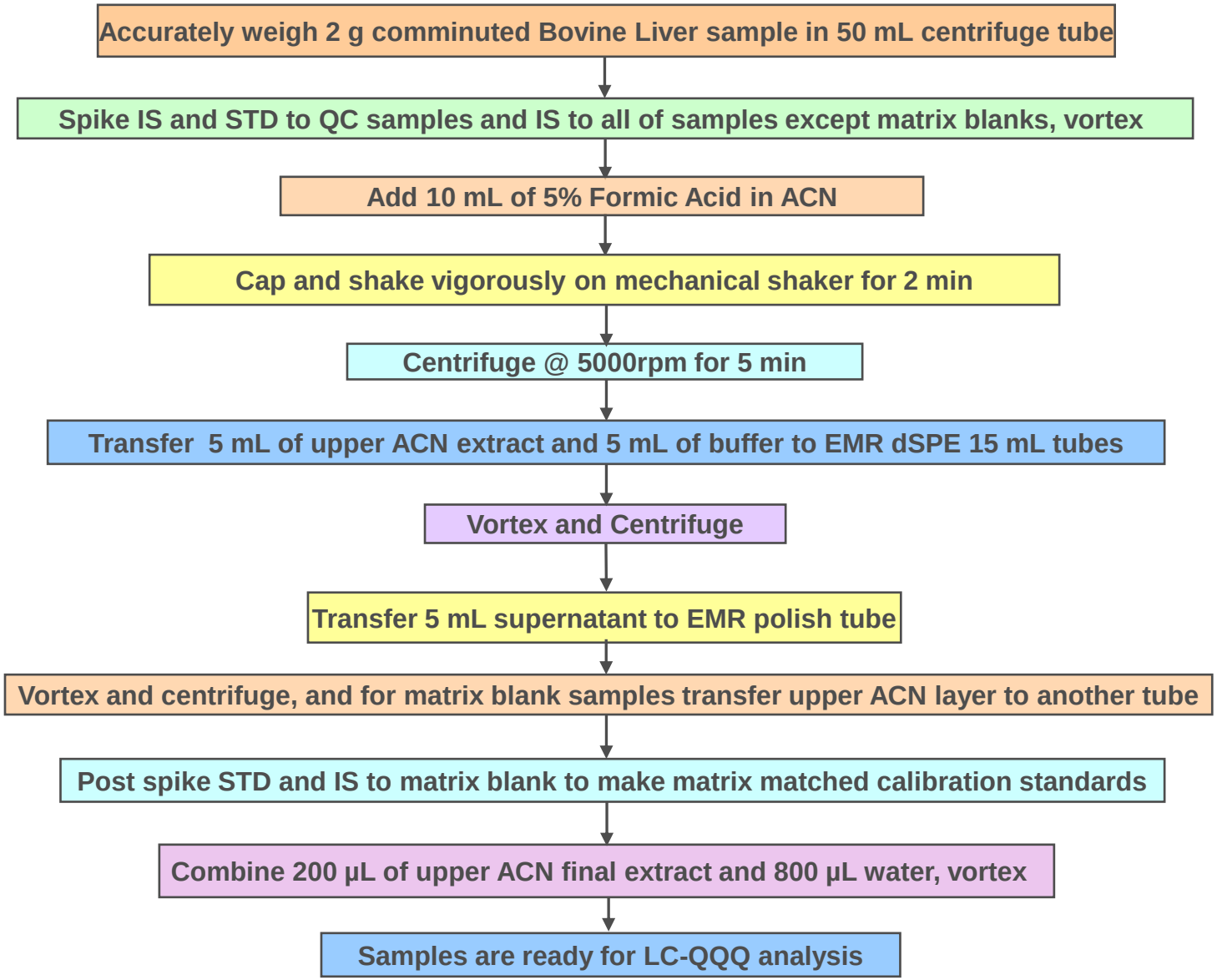


Veterinary Drugs in Bovine Liver

Vet Drugs List 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	Oxytetracline	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

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



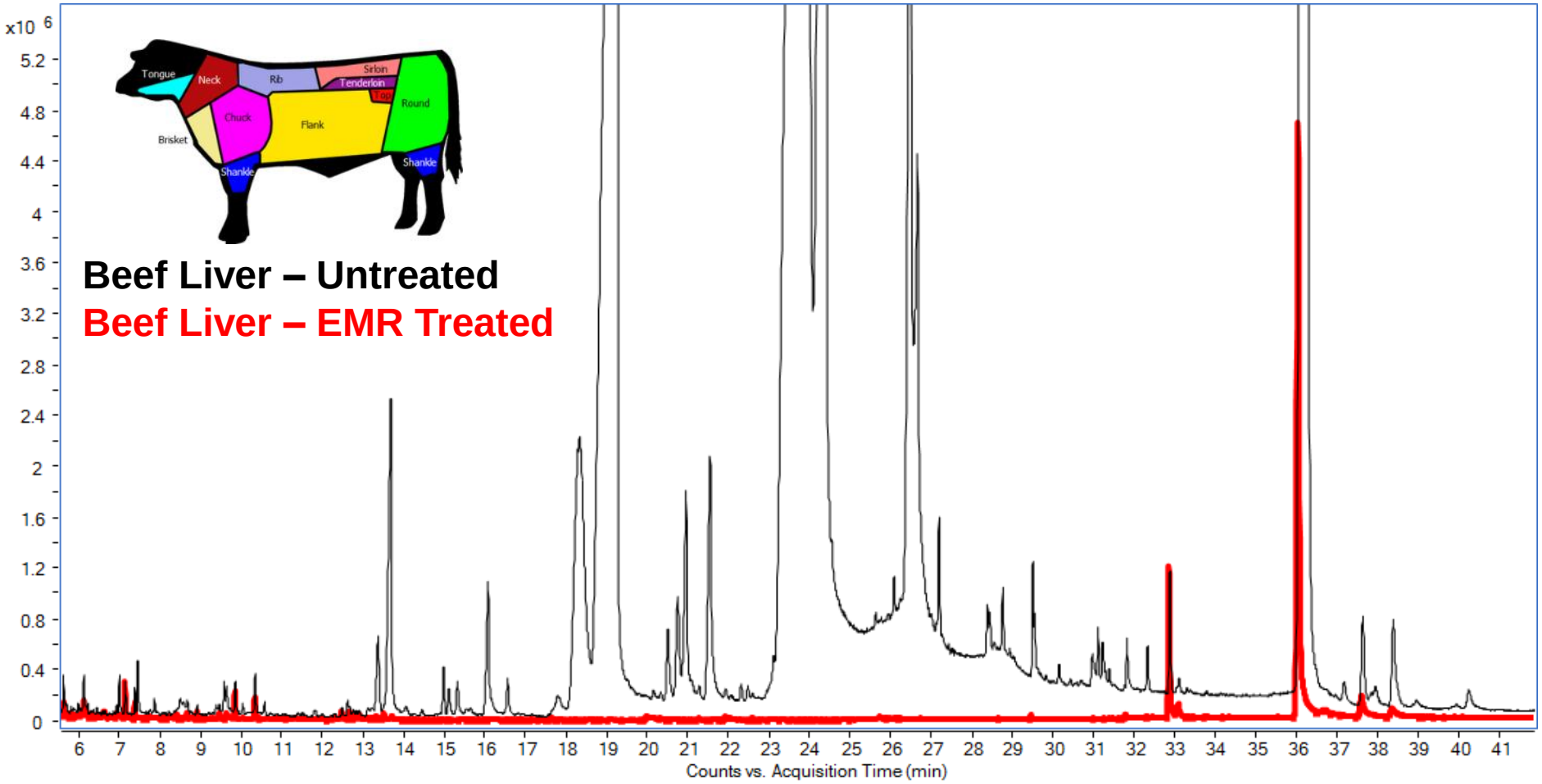
Part II – Multiple Vet Drugs Residue Analysis in Bovine Liver

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

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

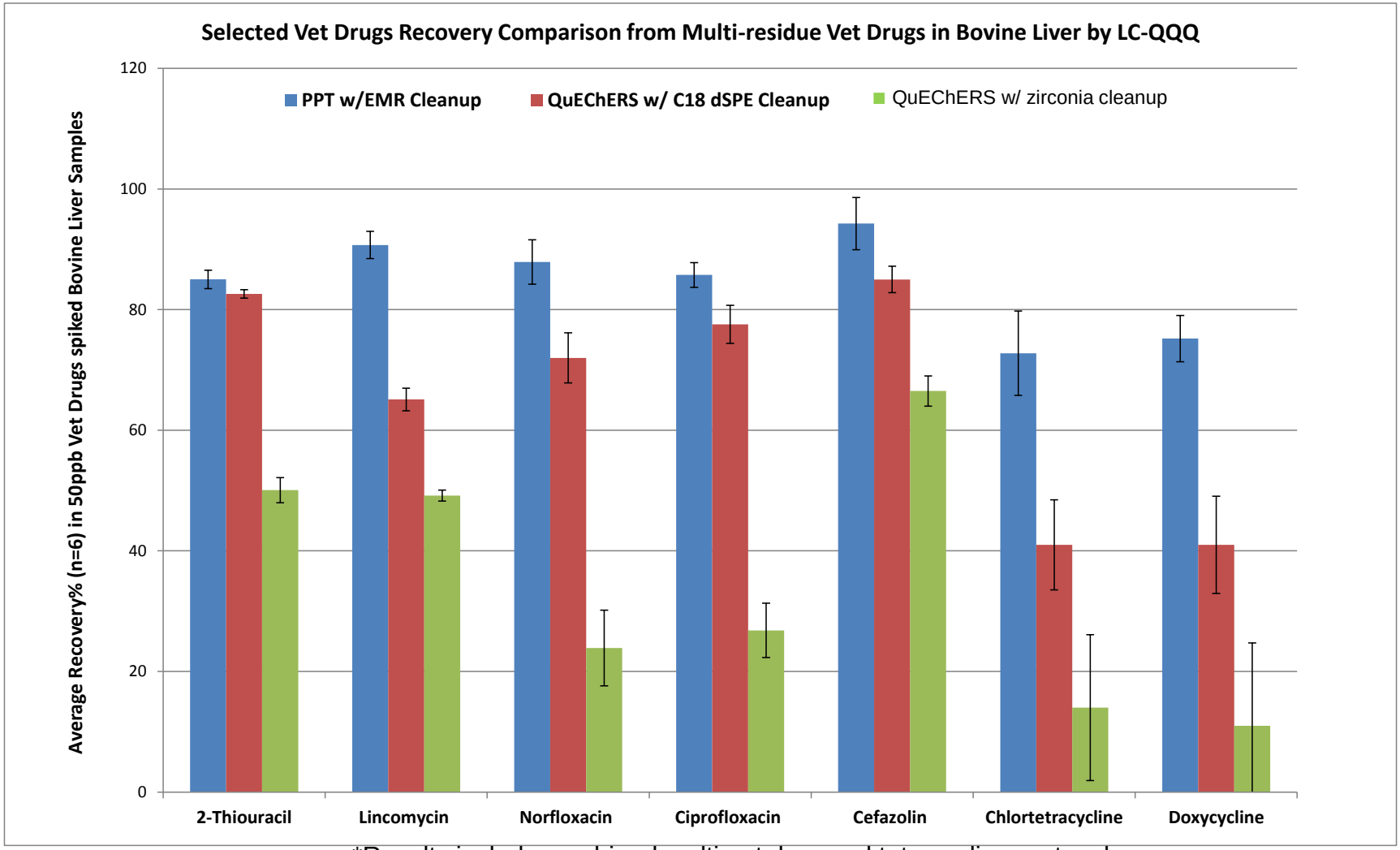
The use of EMR material cleanup removes extra 20% of liver co-extractives in comparison to tradition QuEChERS cleanup.

Another difficult matrix



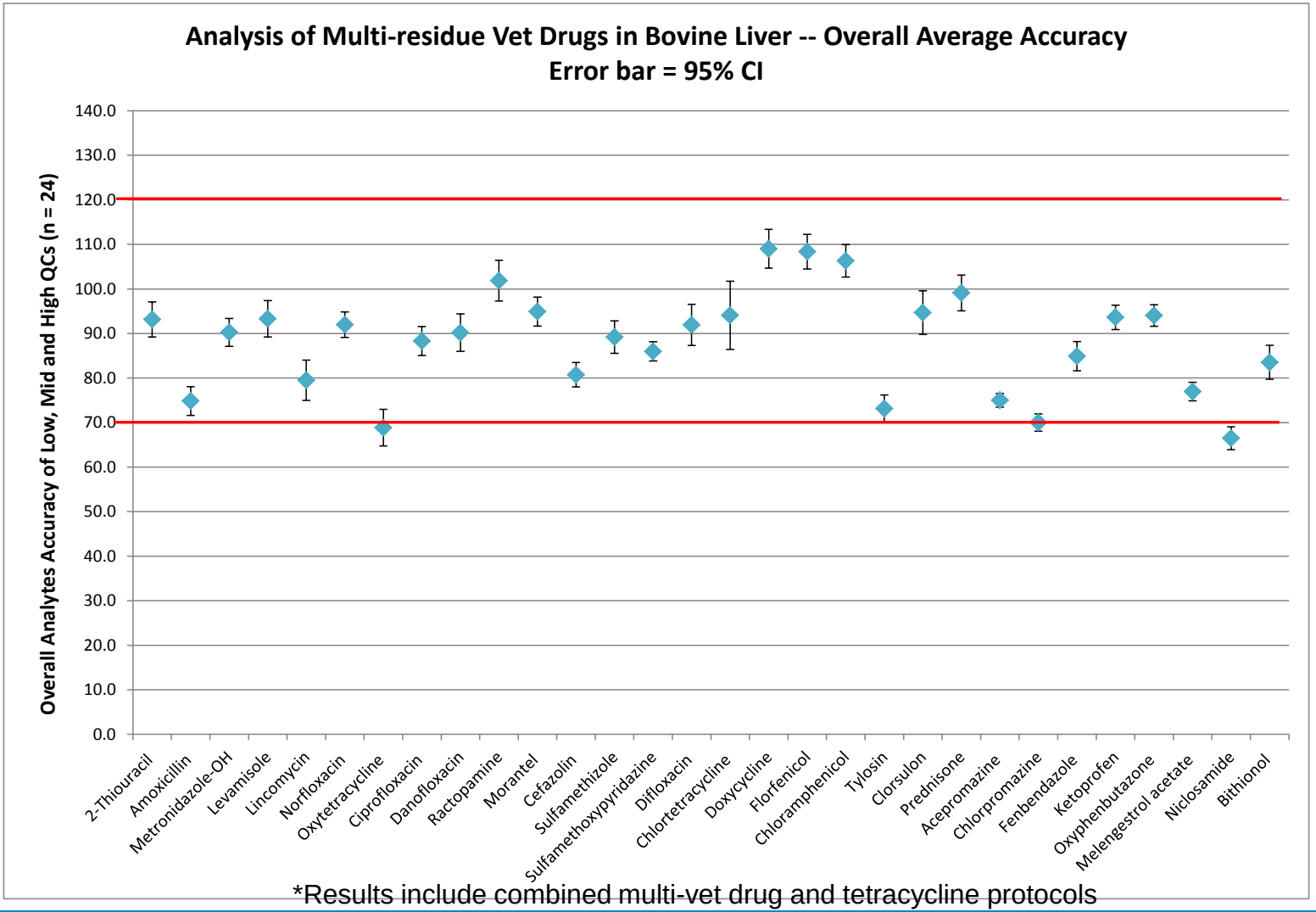
GC/MS data

Results for Multiple Vet Drugs Residue Analysis in Bovine Liver (Selected individual analytes comparison chart)



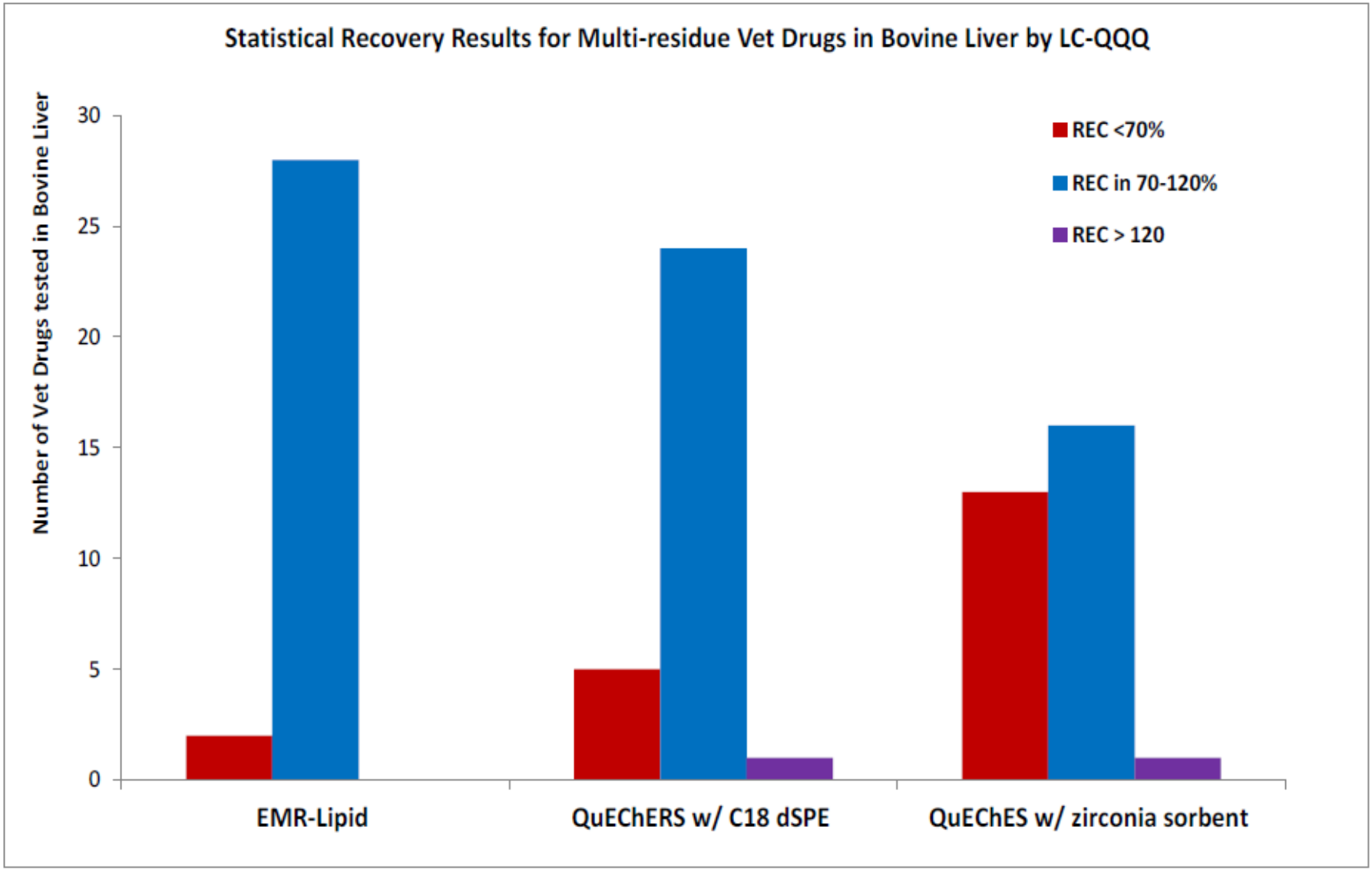
*Results include combined multi-vet drug and tetracycline protocols

Results for Multiple Vet Drugs Residue Analysis in Bovine Liver



*Results include combined multi-vet drug and tetracycline protocols

Results for Multiple Vet Drugs Residue Analysis in Bovine Liver



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 and analyte types.
- Key applications were validated with EMR and demonstrate better recovery, better precision, and decreased matrix impact to the instrument and results.
- EMR is a drop-in dSPE for existing workflows including QuEChERS and protein precipitation (liquid extraction).

Acknowledgements

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William Hudson

Scott Choi

Visit www.Agilent.com for more information

Launch Applications:

- *Veterinary Drugs in Beef Liver*

  5991-6098EN

- *Pesticides in Avocado (LC-MS/MS)*

  5991-6096EN

- *Pesticides in Avocado (GC-MS/MS)*

  5991-6097EN

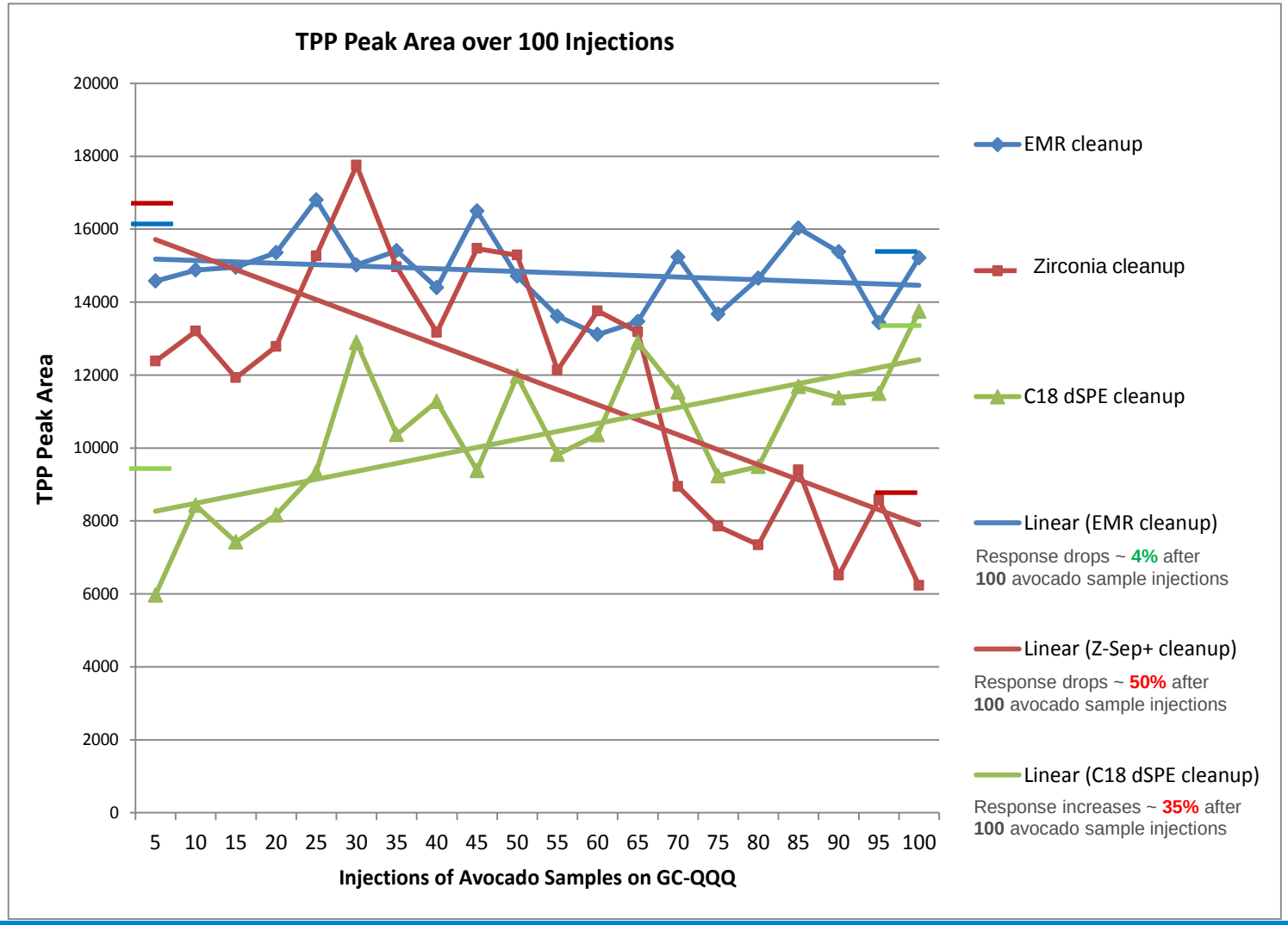
- *PAHs in Salmon*

  5991-6088EN

More coming soon…

Appendix

Comparison of Analytes Response Consistency over Multiple Avocado Sample Injections



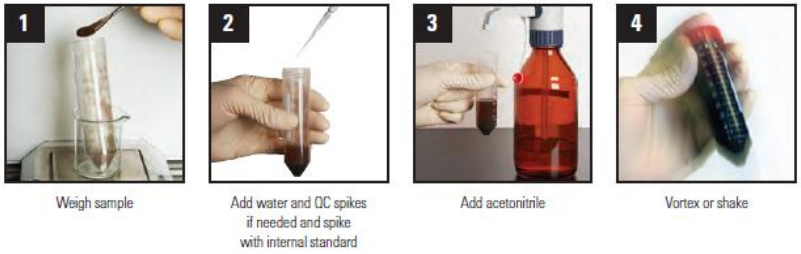
TPP

Commonly used IS

RT: 18.3 min

Improving dSPE in QuEChERS

1. Extraction



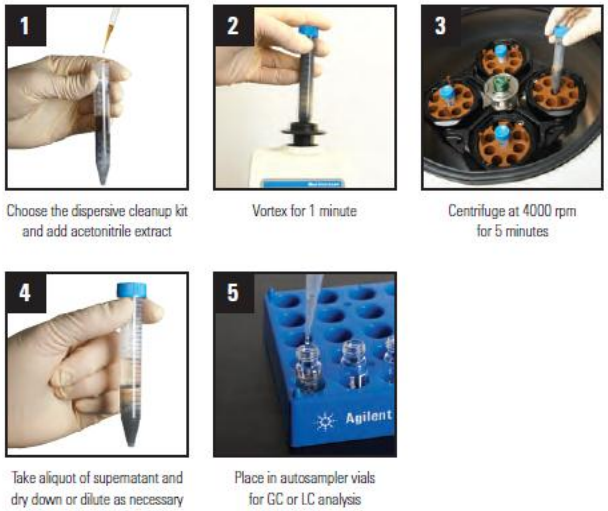
Pros

- 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



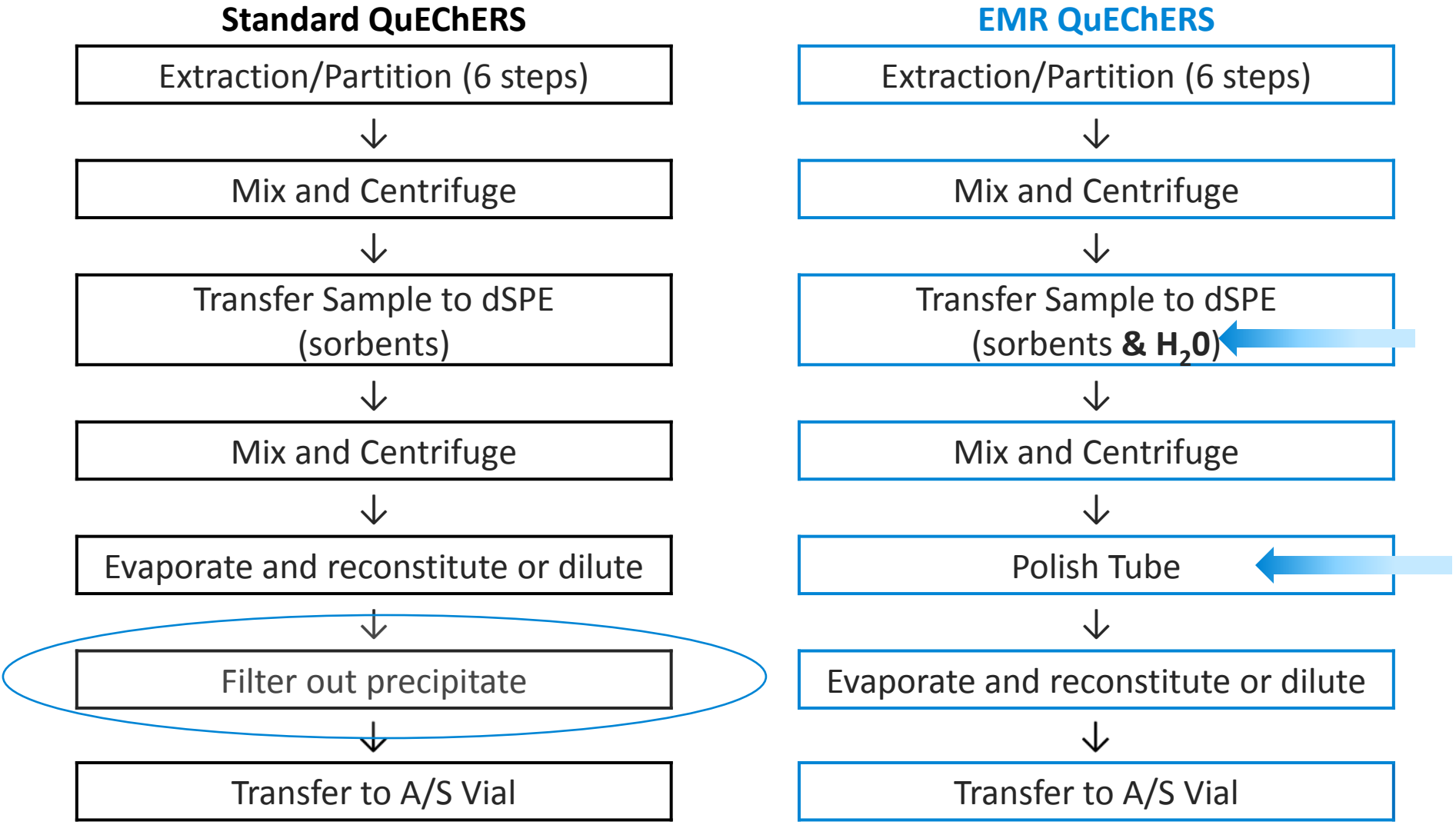
Pros

- Same as extraction

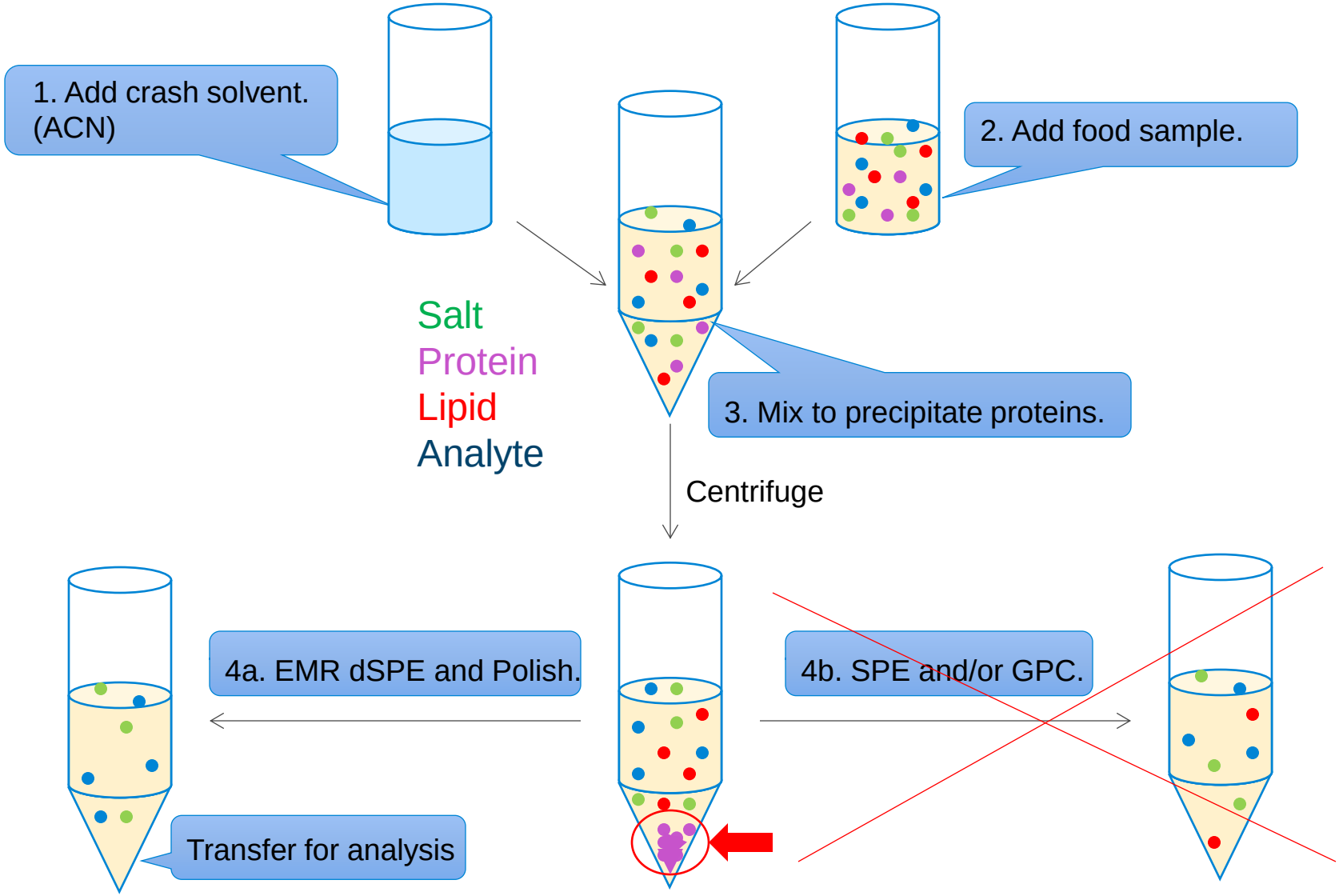
Cons

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

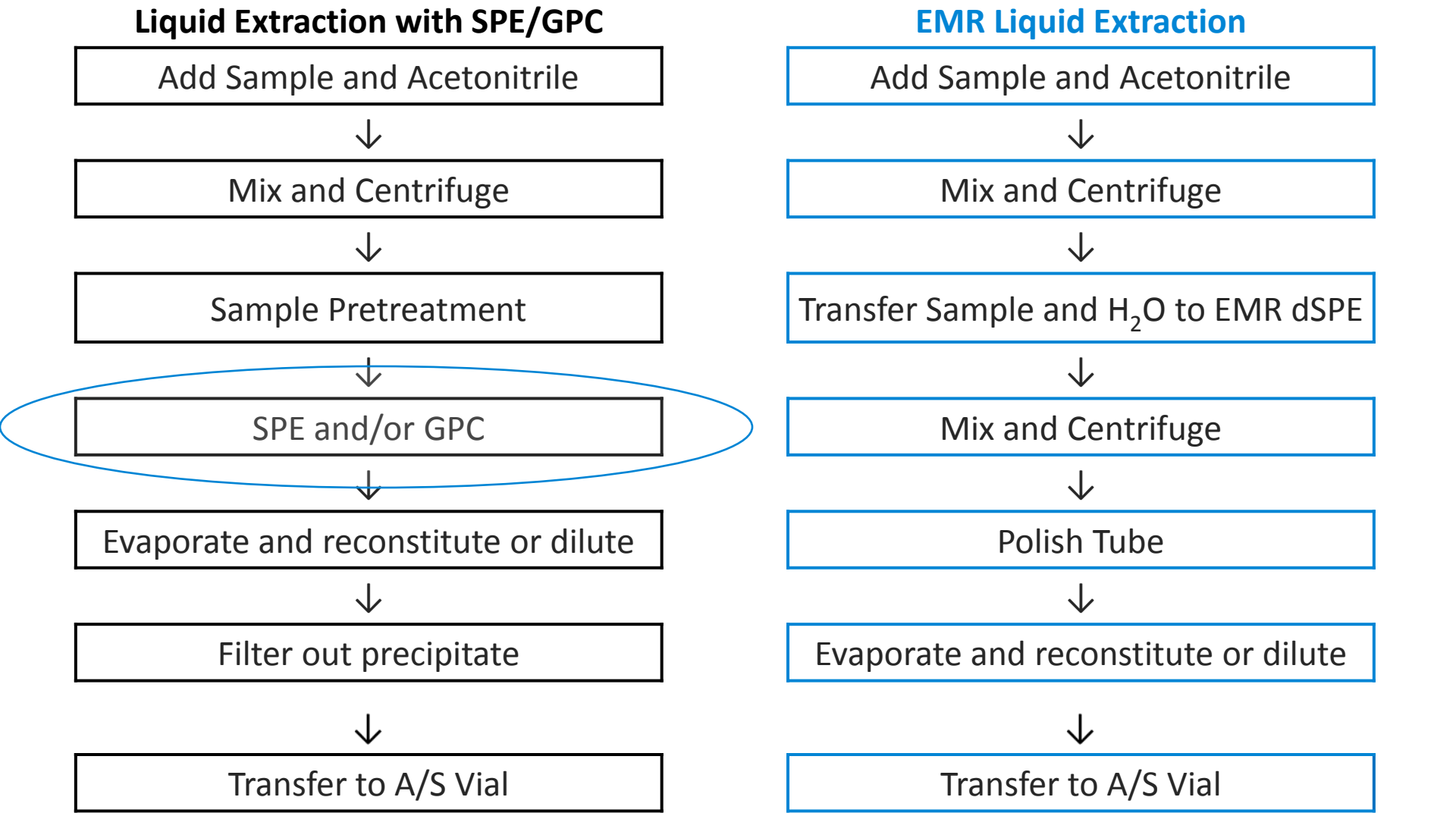
Traditional QuEChERS versus QuEChERS EMR-Lipid



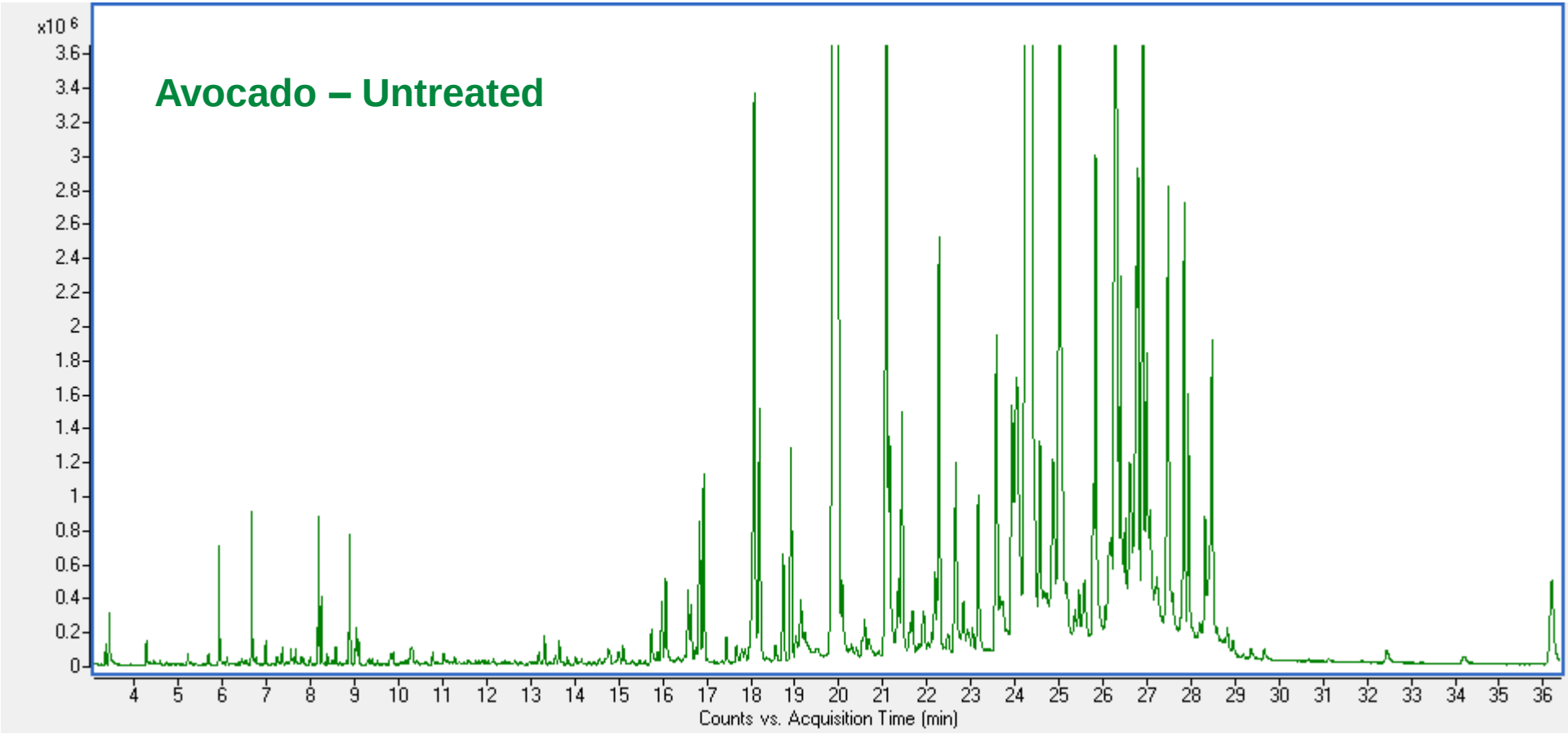
Improving Liquid Extraction Workflows



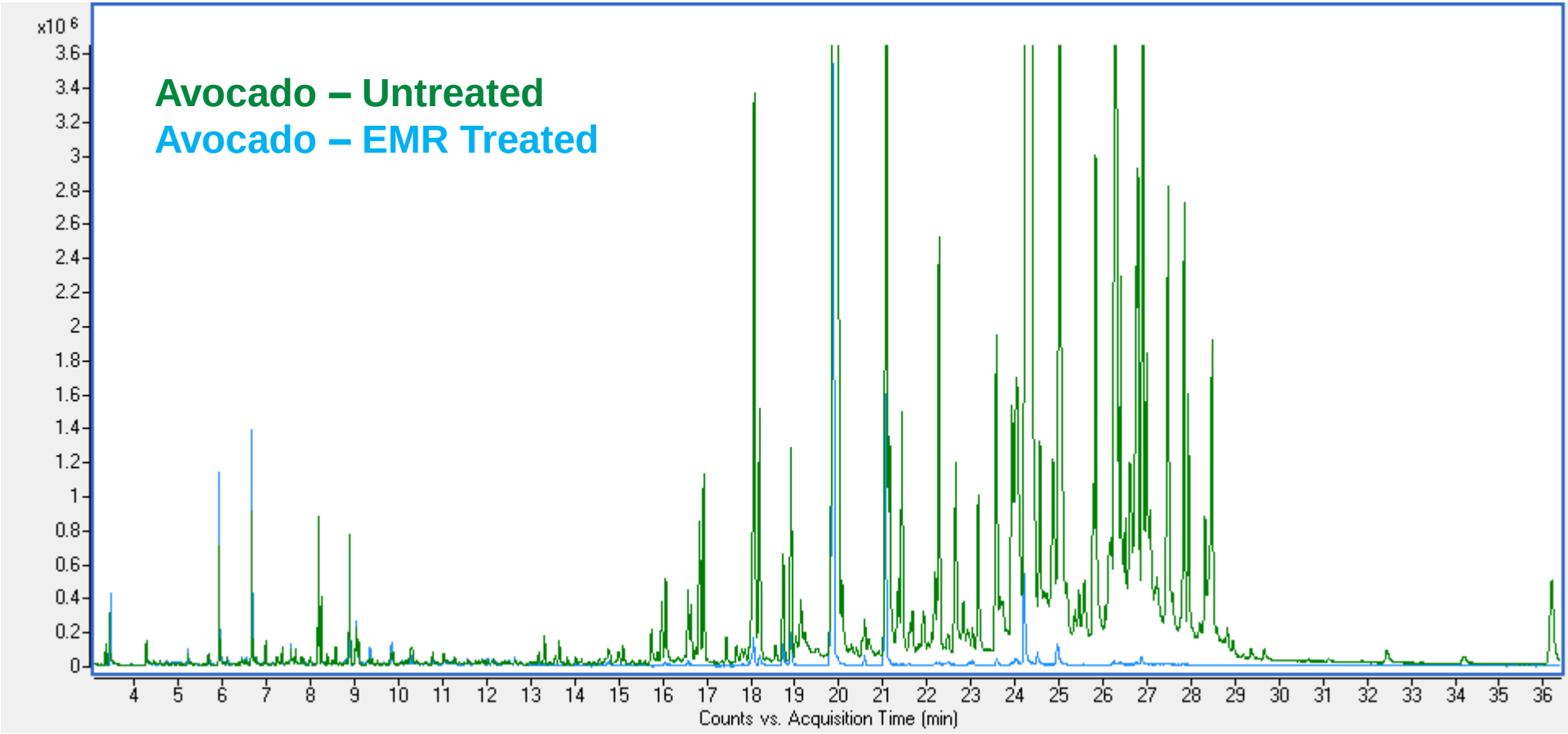
Traditional versus EMR-Lipid Liquid Extraction



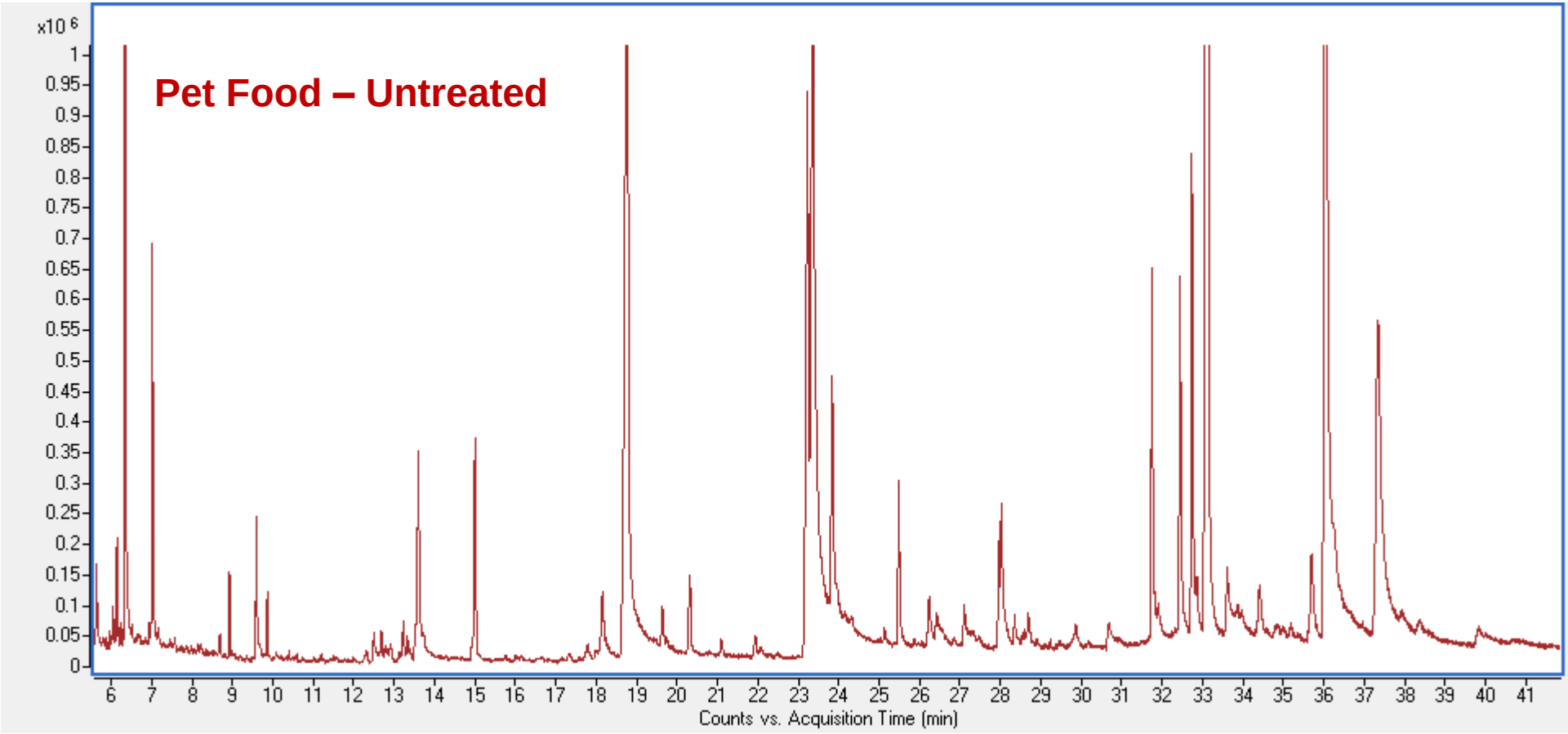
GC-MS Fullscan Avocado



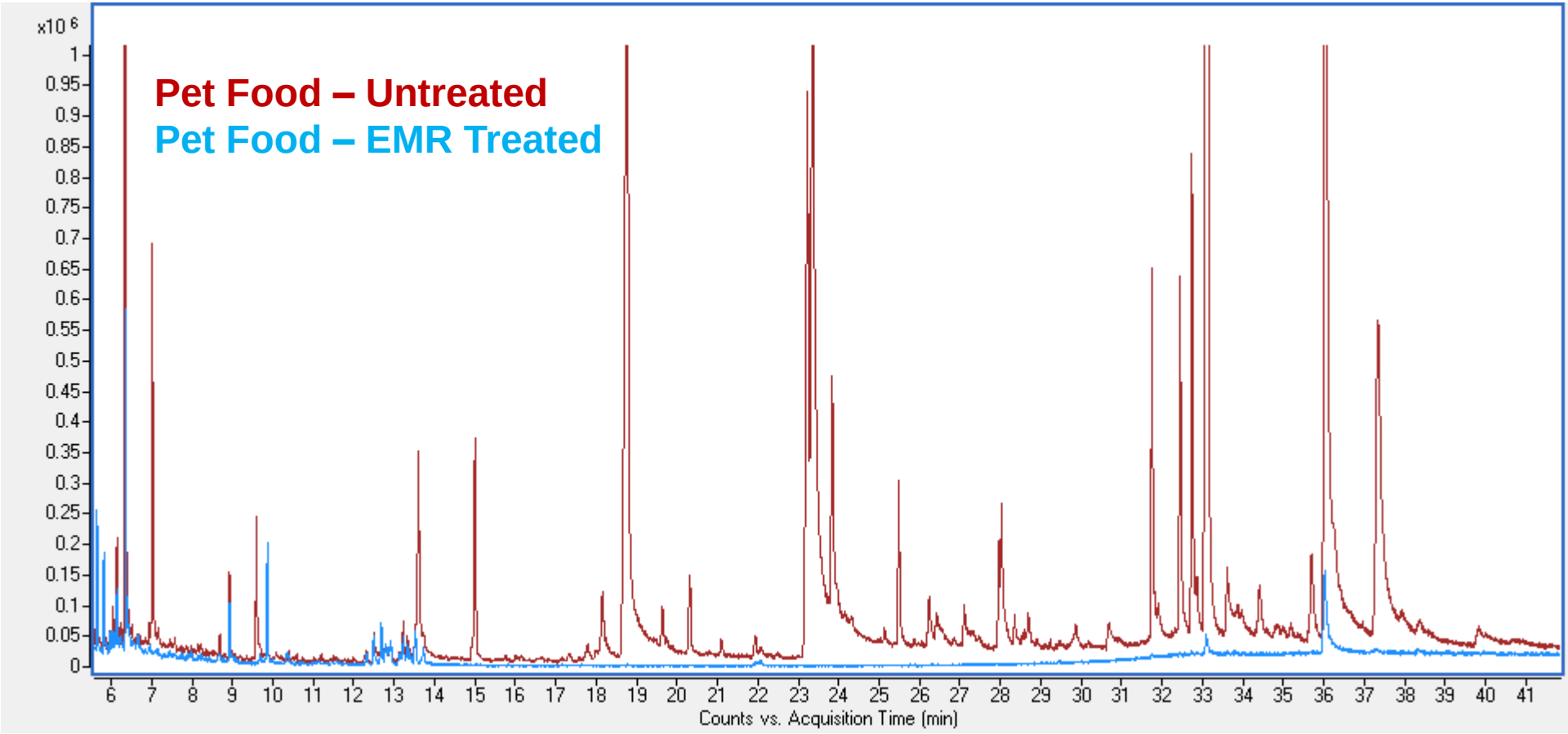
GC-MS Fullscan Avocado



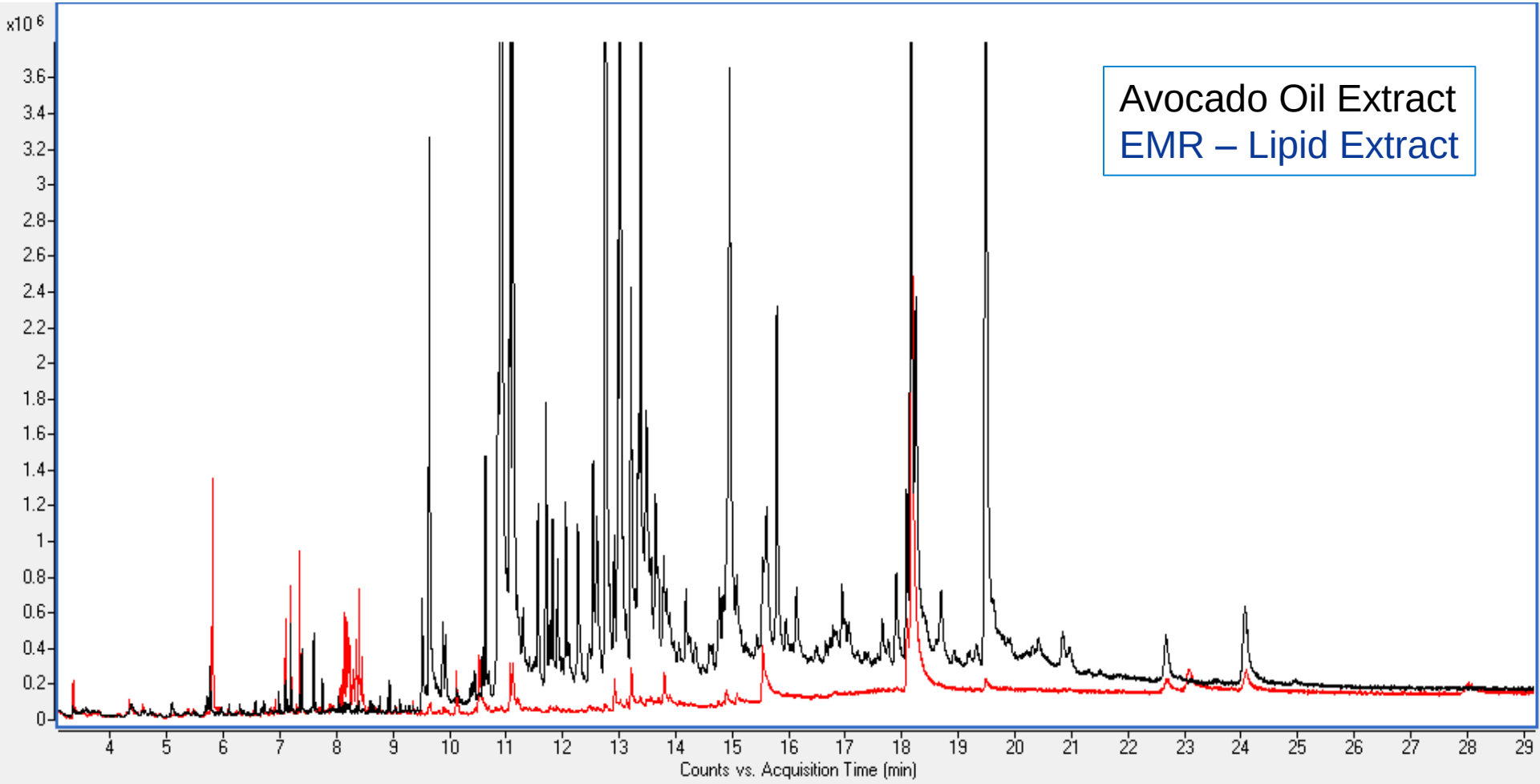
GC-MS Fullscan Pet Food



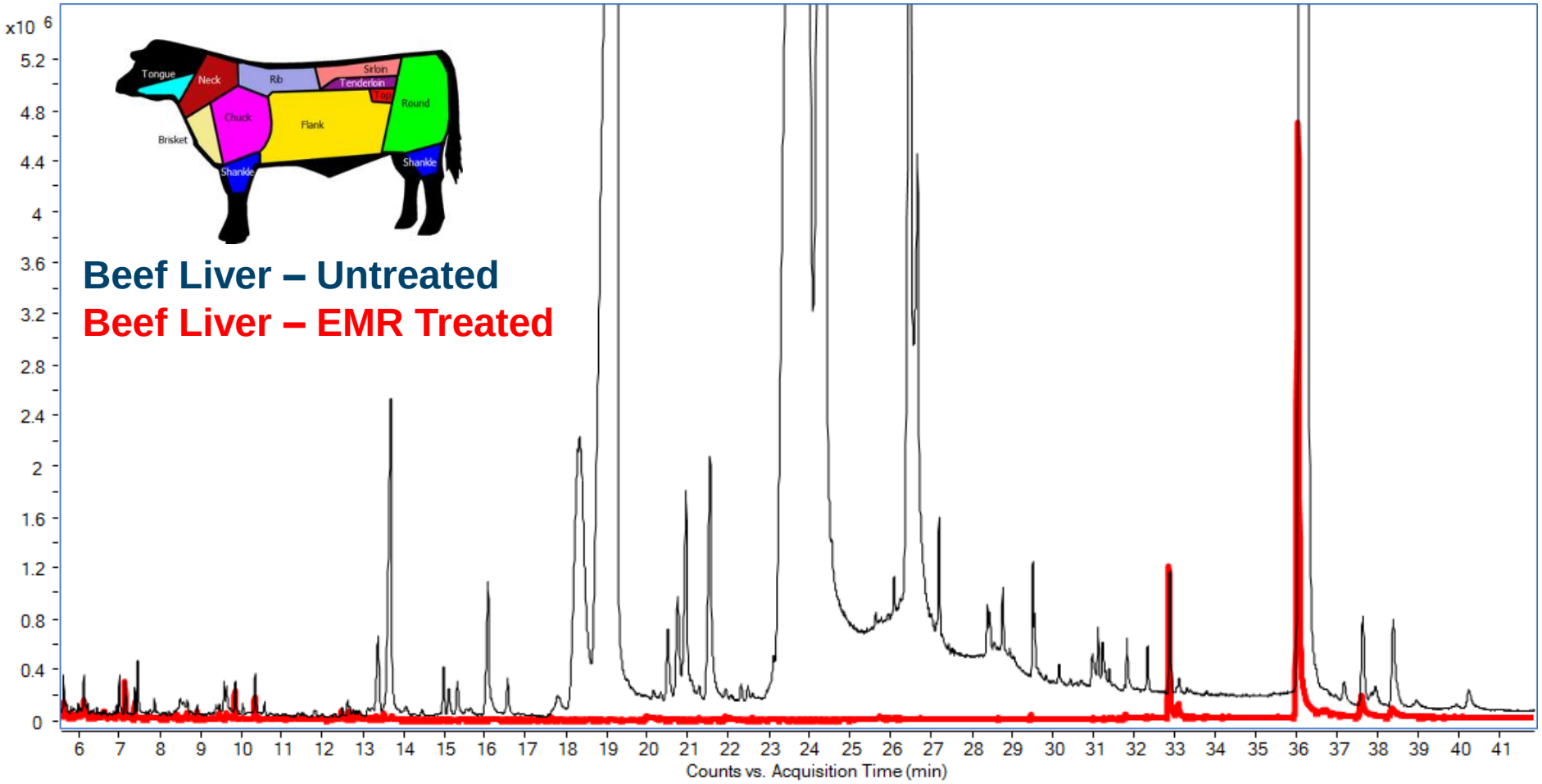
GC-MS Fullscan Pet Food



GC-MS Full Scan– Avocado Oil



Another difficult matrix



GC/MS data

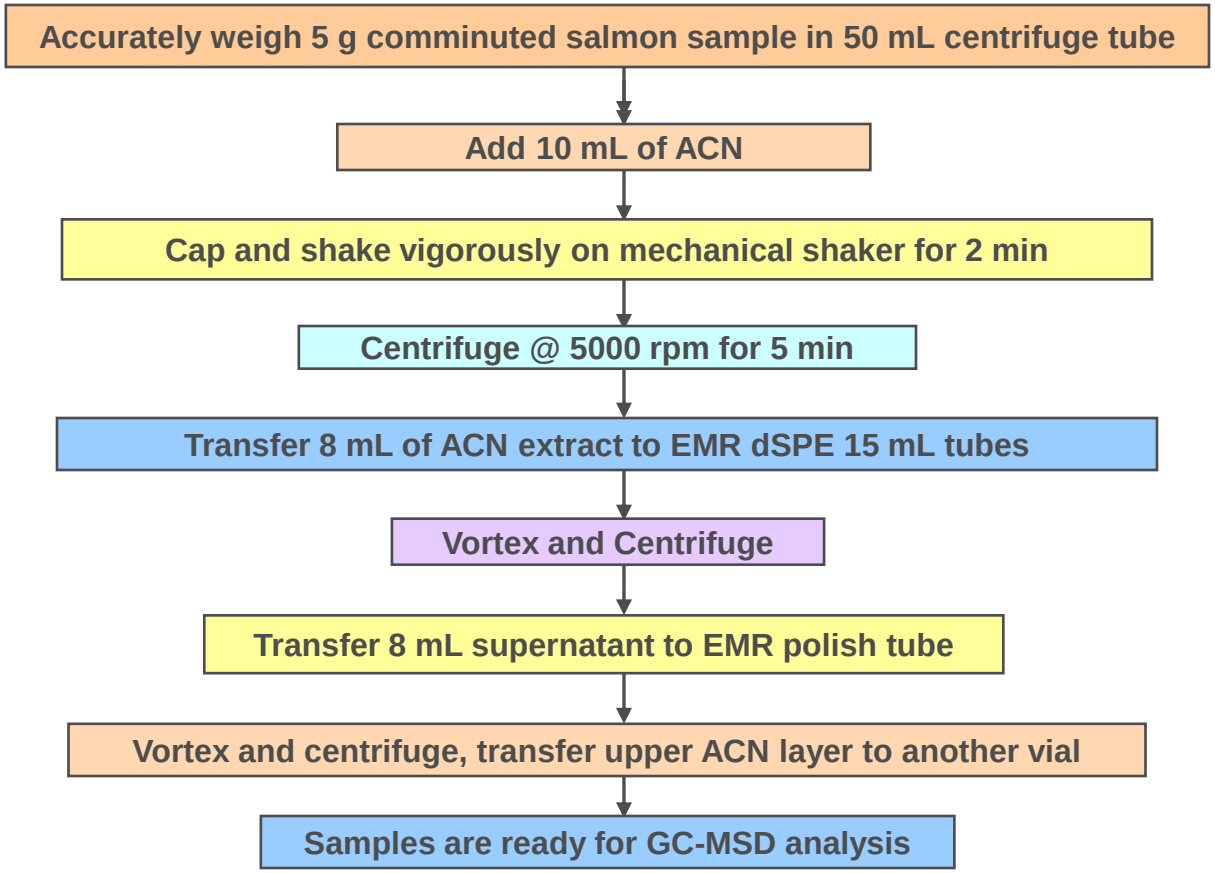
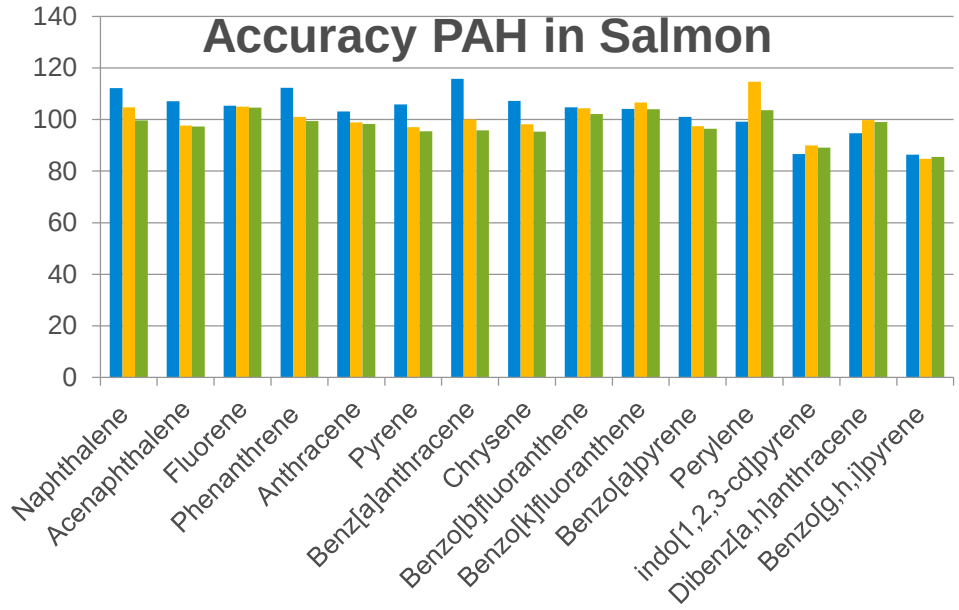
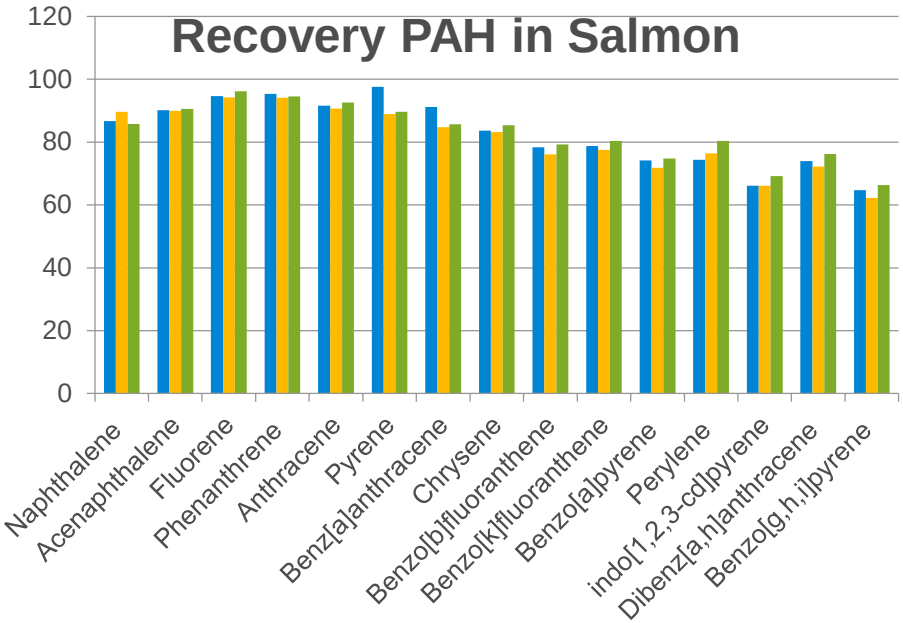
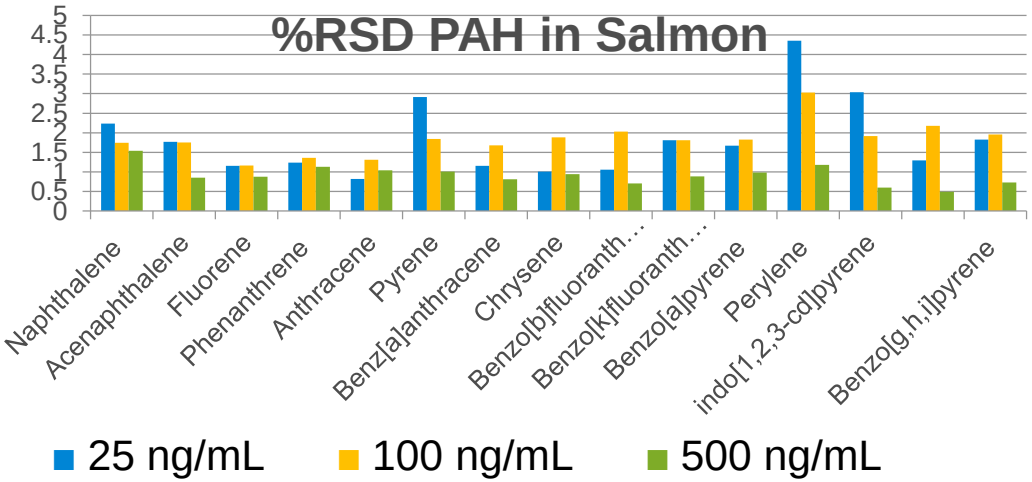
PAHs in Salmon

Target Analytes

	GC-MS (SIM)	
Compound	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