

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

Derick Lucas, PhD
Agilent Technologies

Today's Agenda

Introduction

EMR-Lipid - Enhanced Matrix Removal

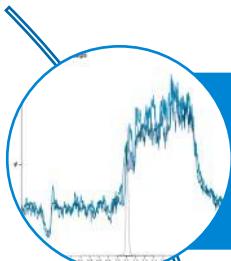
- Background, workflows, and results

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



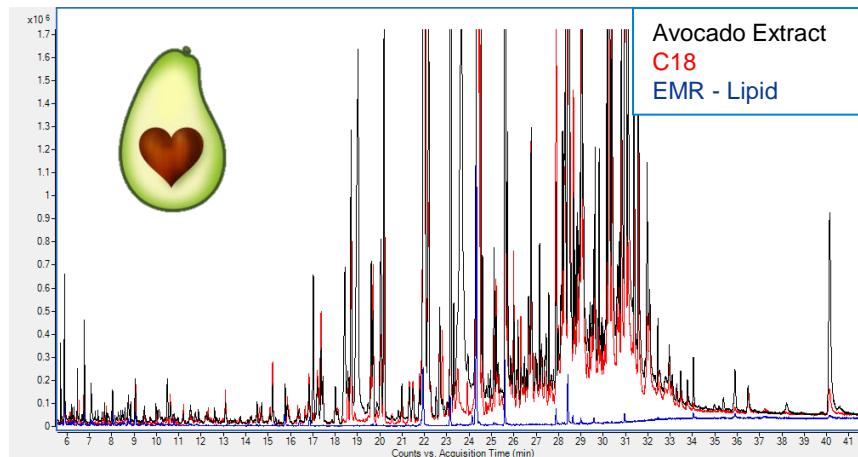
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EMR-Lipid: In The Beginning

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



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EMR Product offering



Extraction Tube

EMR-Polish (p/n 5982-0101)

EMR-Lipid (p/n 5982-1010)

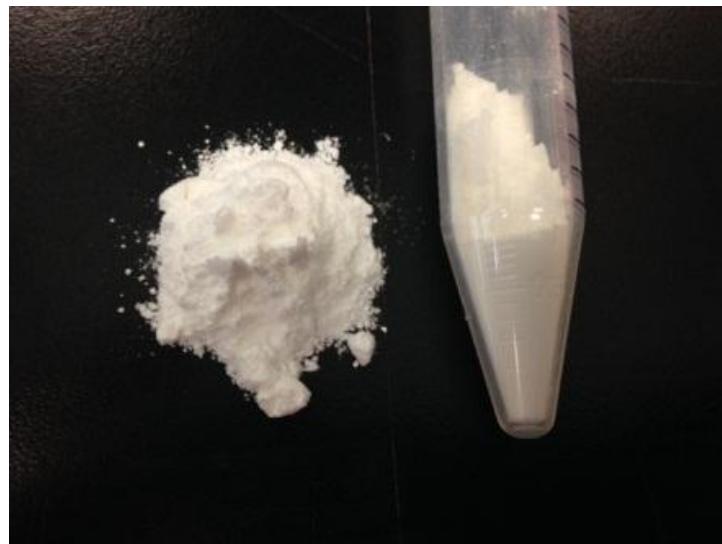
EMR fits into current sample preparation workflows



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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.



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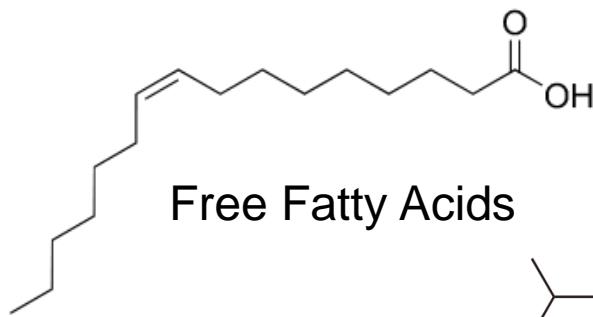
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... and what does it do?

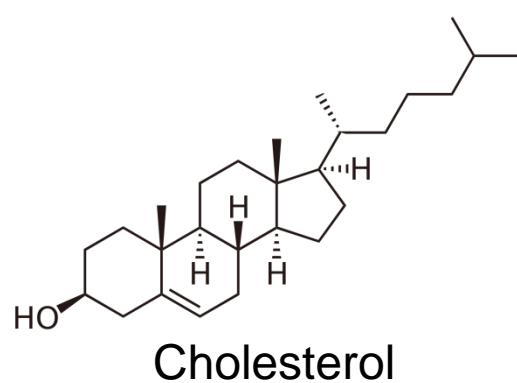
EMR sorbent removes Lipids

What are Lipids?

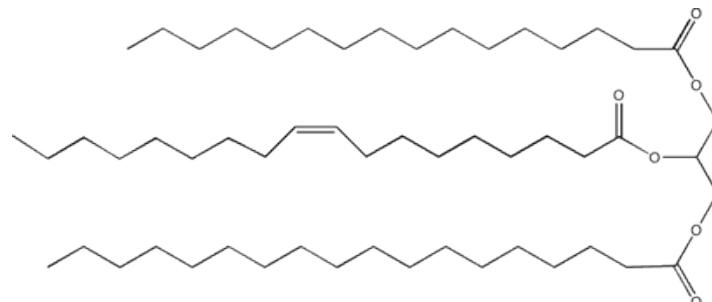
A class of naturally occurring hydrocarbon containing compounds commonly known as fats and oils



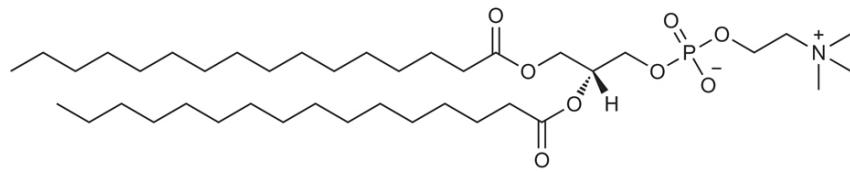
Free Fatty Acids



Cholesterol



Triglycerides



Phospholipids

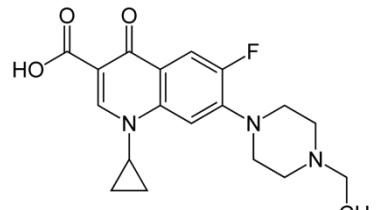


What Does EMR NOT Interact With?

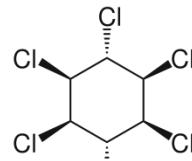
EMR does NOT remove analytes of interest

Exceptions?

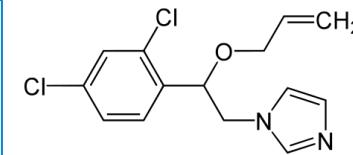
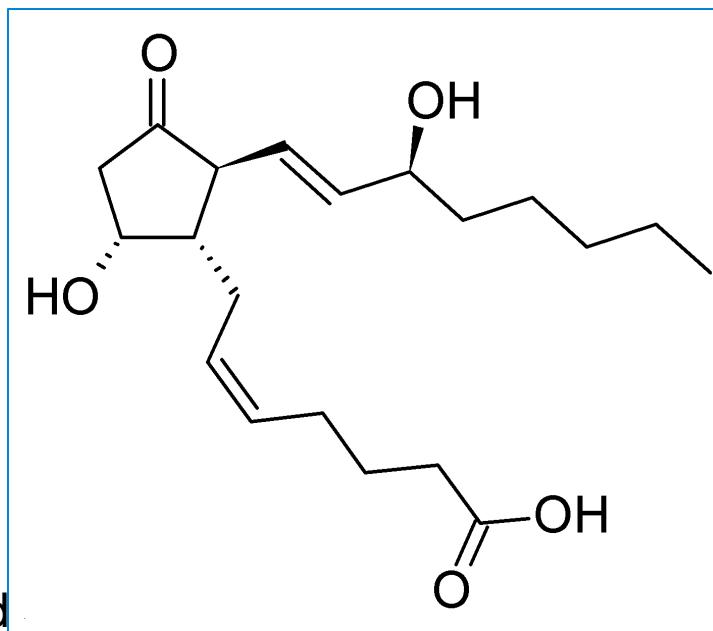
Compounds containing long aliphatic functional groups (e.g. prostaglandins)



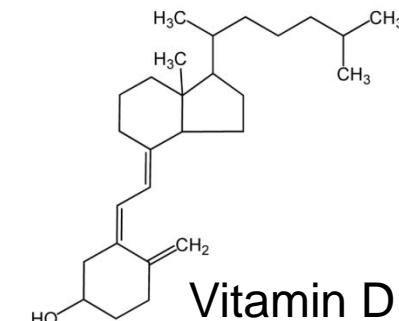
Fluoroquinolones



Organochlorine Pesticid



midazole pesticides



Vitamin D



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EMR Protocols for Applications



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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.)

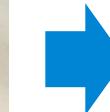
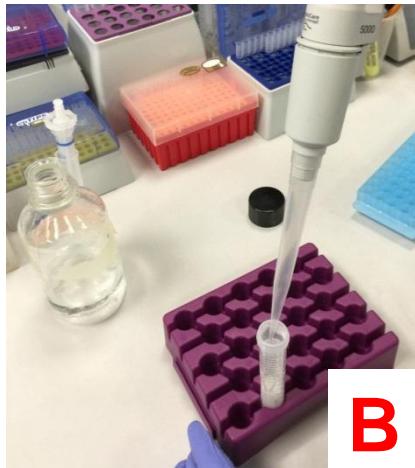
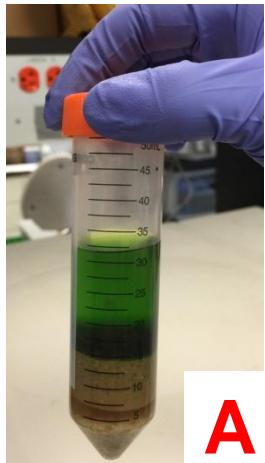
EMR Applications: - PAHs in Salmon,
- Veterinary Drugs in Bovine Liver



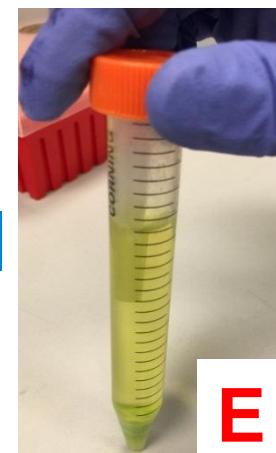
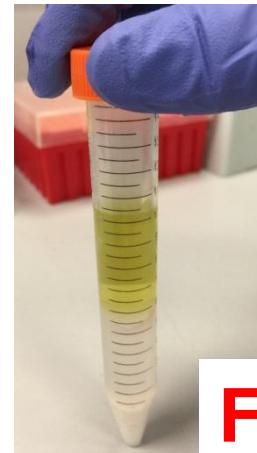
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EMR – Lipid dSPE Cleanup



- 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)**
- F. Polish (MgSO₄/NaCl)**



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ANNA 2016

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...

PAH Analysis in Salmon with Enhanced Matrix Removal

Auth. Derick Agile

Multiresidue Analysis of Pesticides in Avocado with Agilent Bond Elut EMR—Lipid by LC/MS/MS

Auth. Limian Agile

Multiresidue Analysis of Pesticides in Avocado with Agilent Bond Elut EMR—Lipid by GC/MS/MS

Auth. Limian Agile

Multiresidue Analysis of Veterinary Drugs in Bovine Liver by LC/MS/MS

Agilent Bond Elut QuEChERS Enhanced Matrix Removal—Lipid

Auth. Limian Agile

Multiresidue Analysis of Veterinary Drugs in Bovine Liver by GC/MS/MS

Agilent Bond Elut QuEChERS Enhanced Matrix Removal—Lipid

Application Note

Food Testing and Agriculture

Abstract

Authors

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Pesticides in Avocado



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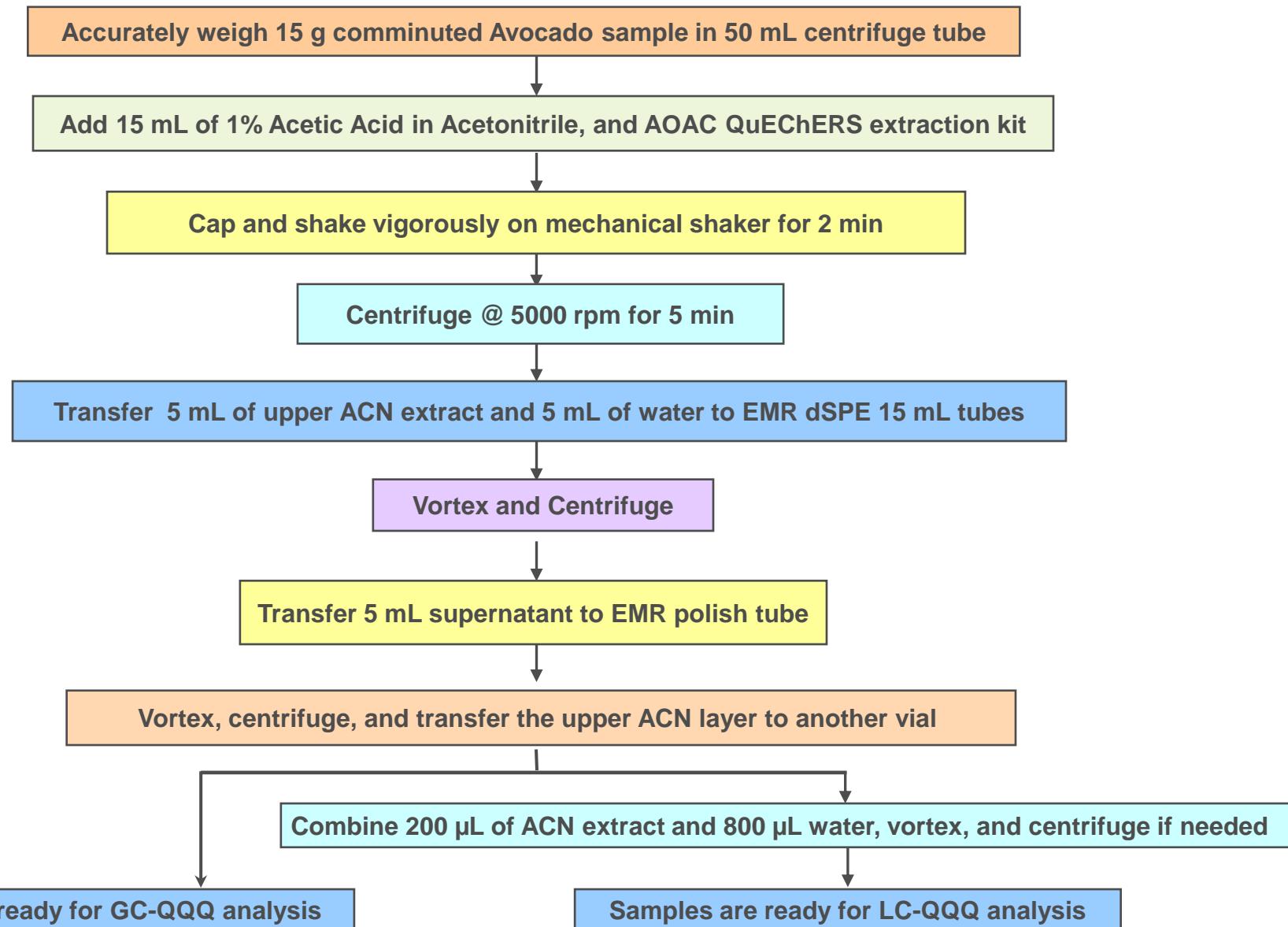
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67 Pesticides Analysis in Avocado by LC and GC-QQQ

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					
Diazinon				Omethoate				Terbutylazine					
Chlorpyriphos methyl				Dimethoate				Carbofuran					
Coumaphos				Phosmet				Methiocarb					
Trichlorfon				Carbaryl	Carbamate	Fungicide	LC-MS	Chlorpropham					
Lindane				Propoxur				Propham					
Aldrin				Dichlofuanid				Monuron					
Endrin				Tolylfluanid				Chlorotoluron					
DDT				Carbendazim				Diuron					
Endosulfan sulfate	Organochlorine	Insecticide		Thiabendazole	Benzimidazole	Insecticide	LC-MS	Fluometuron	Urea	Herbicide			
Methoxychlor				Thiophanate methyl				Isoproturon					
2-Phenylphenol				Cyprodinil				Metobromuron					
Atrazine				Imidacloprid				Siduron					
Bupirimimate				Pymetrozine				Linuron					
Chlorothanil				Imazalil	Fungicide	Insecticide	LC-MS	Neburon					
Captan				Penconazole				2,4-D Acid	Chlorophenoxy acid	Insecticide			
Folpet	Phthalimide	Fungicide		Aminocarb				Didchl orprop					
Captafol				Oxamyl				Metazachlor					
Iprodione				Methomyl				Bentazon					
Procymidone				Aldicarb				Malathion					
Permethrin				Fenuron	Carbamate	Herbicide	OP	EPN					
Deltmethrin				Metoxuron				Tepp-A					
Pyraclostrobin								Monocrotophos					
Ethalfluralin													



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



Comparison of Avocado Co-extractives by Weight

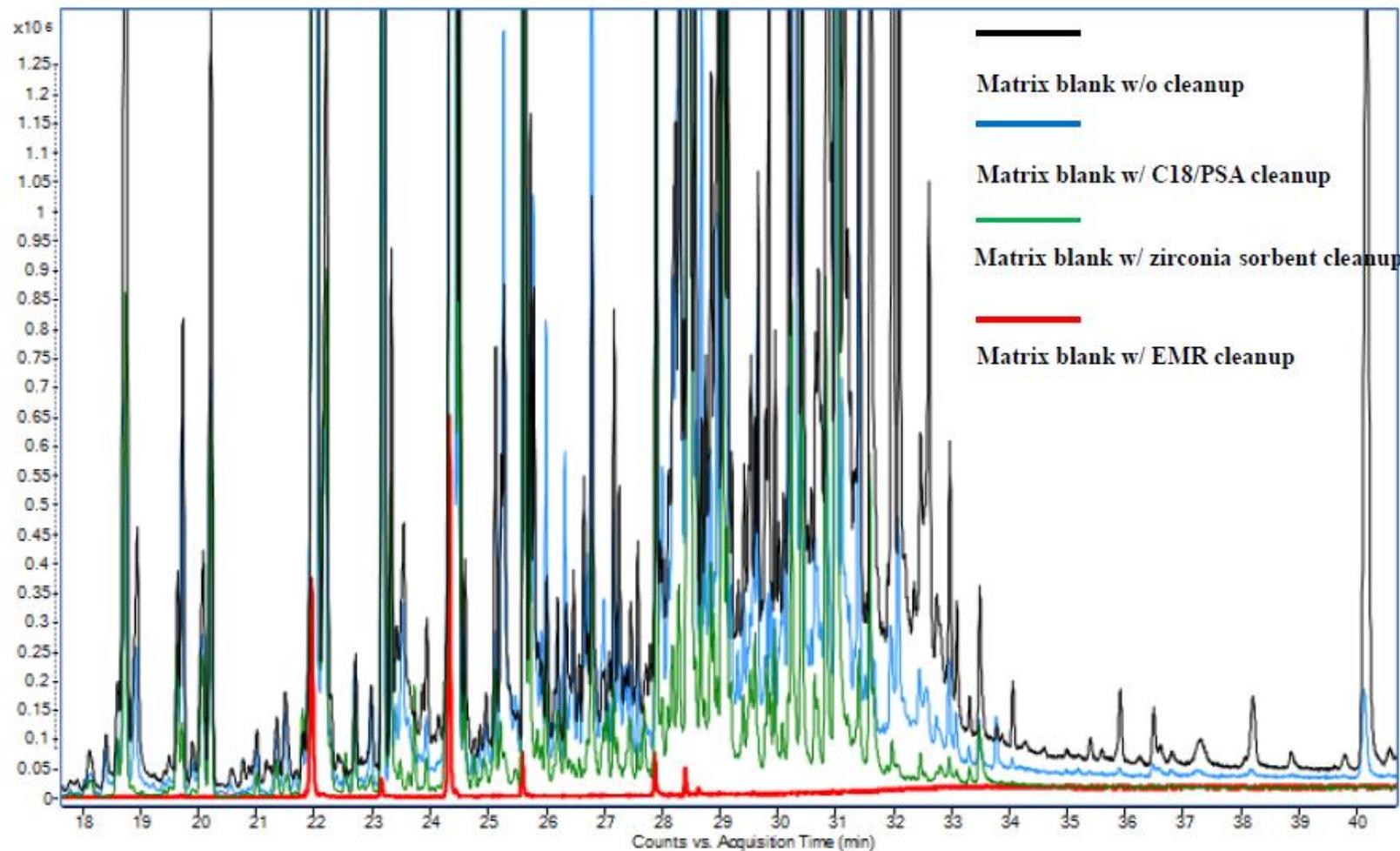
Cleanup	Amount of co-extractives (mg, n= 3)	Amount of co-extractives removed by cleanup (mg, n = 3)	% of matrix co-extractives removed by cleanup
No further cleanup	14.7	--	
C18/PSA Cleanup	9.5	5.2	35.4
EMR-Lipid Cleanup	4.2	10.5	71.4
Zirconia sorbent Cleanup	7.0	7.7	52.4

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

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



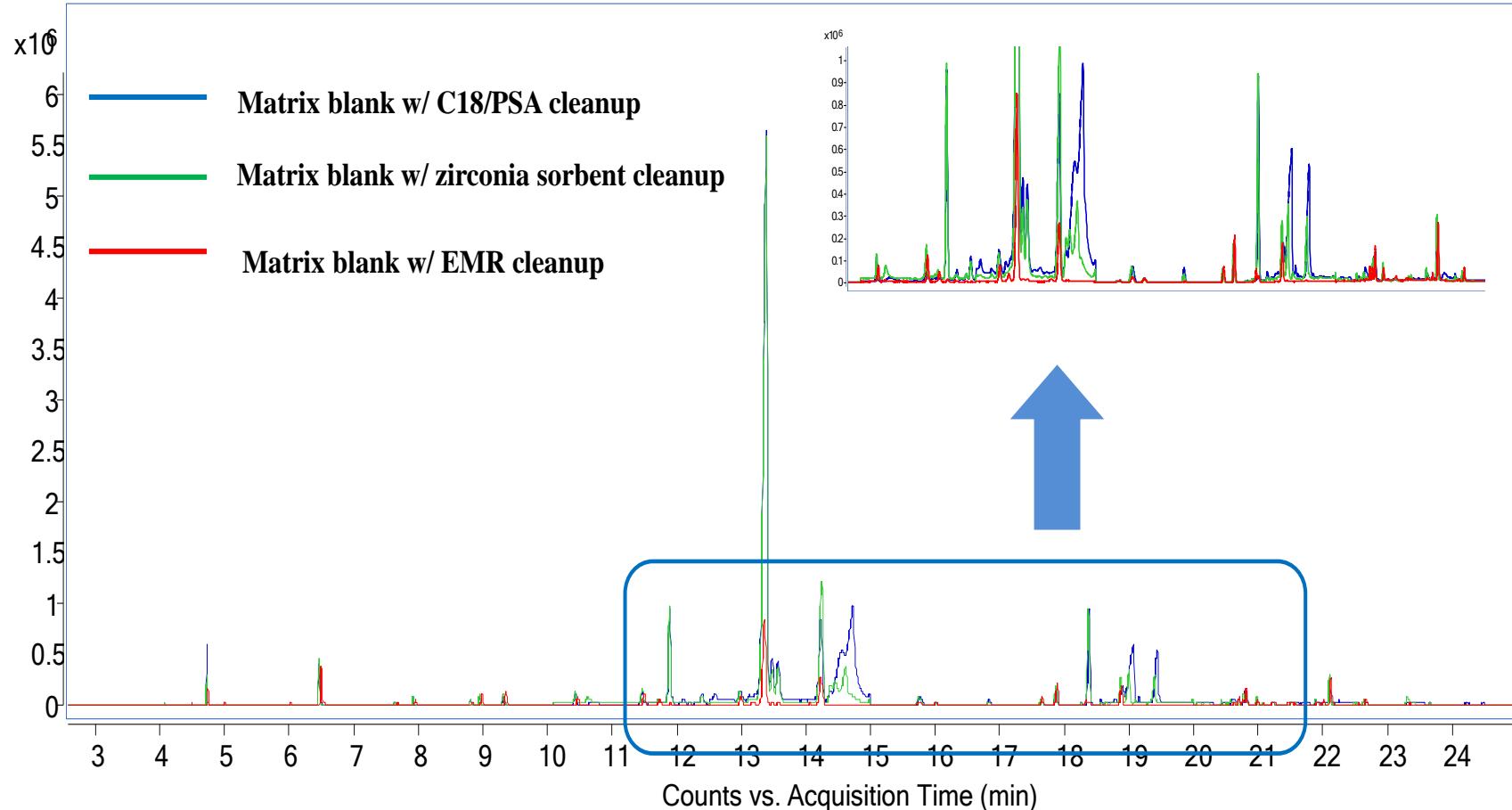
The use of EMR material cleanup provides significantly cleanup chromatographic sample background.



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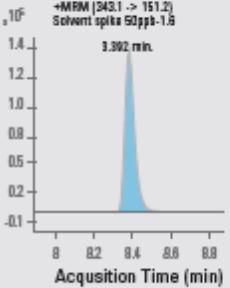
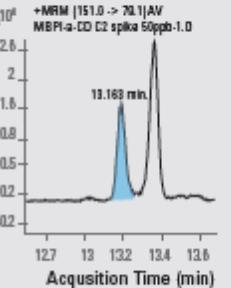
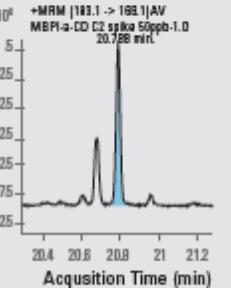
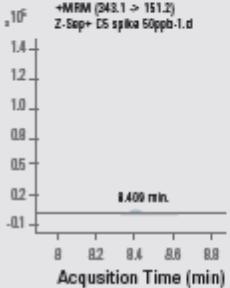
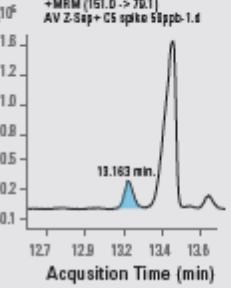
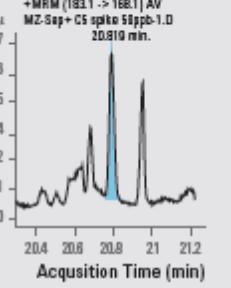
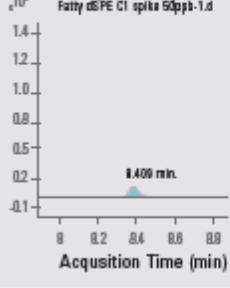
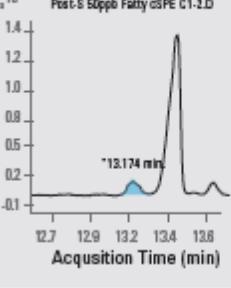
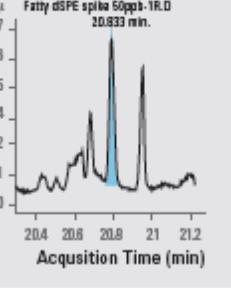
Comparison of GC/MS/MS MRM Chromatogram for Matrix Background



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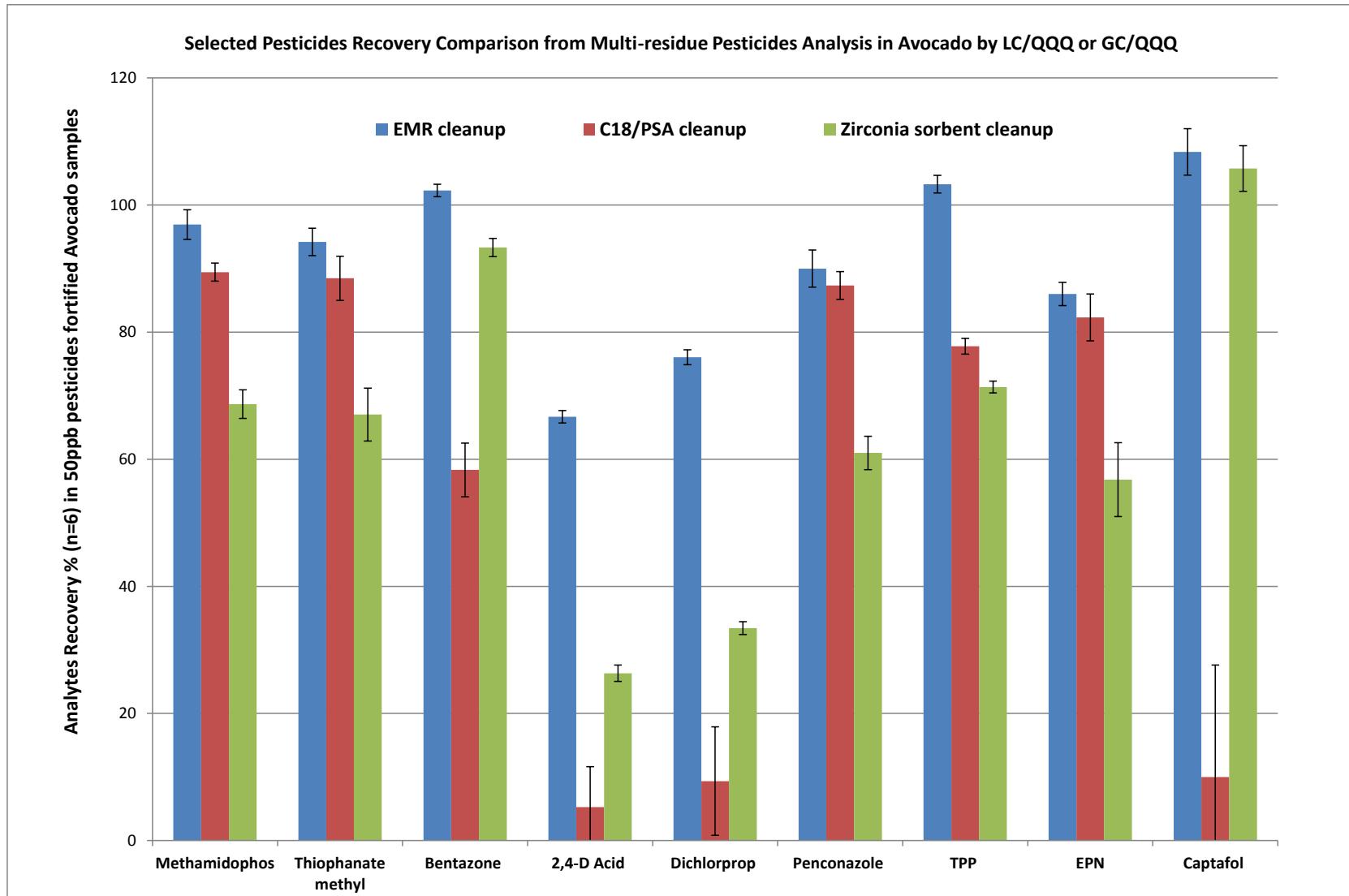
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Chromatographic Benefits of Matrix Removal Provided by EMR Cleanup

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
	 <p>+MRM (243.1 -> 151.2) Solvent spike 50ppb-1.0 3.392 min.</p>	 <p>+MRM (151.0 -> 70.1) AV MBP-a-CD C2 spike 50ppb-1.0 13.168 min. 13.183 min.</p>	 <p>+MRM (183.1 -> 168.1) AV MBP-a-CD C2 spike 50ppb-1.0 20.788 min.</p>
	CEMR Cleanup		
Zirconia Cleanup	 <p>+MRM (243.1 -> 151.2) Z-Sep+ C5 spike 50ppb-1.0 8.400 min.</p>	 <p>+MRM (151.0 -> 70.1) AV Z-Sep+ C5 spike 50ppb-1.0 13.163 min. 13.183 min.</p>	 <p>+MRM (183.1 -> 168.1) AV Z-Sep+ C5 spike 50ppb-1.0 20.819 min.</p>
Fatty dSPE cleanup	 <p>+MRM (243.1 -> 151.2) Fatty dSPE C1 spike 50ppb-1.0 8.400 min.</p>	 <p>+MRM (151.0 -> 70.1) AV Post-C1 50ppb Fatty dSPE C1-2.0 13.174 min. 13.183 min.</p>	 <p>+MRM (183.1 -> 168.1) AV Fatty dSPE spike 50ppb-1.0 20.833 min.</p>

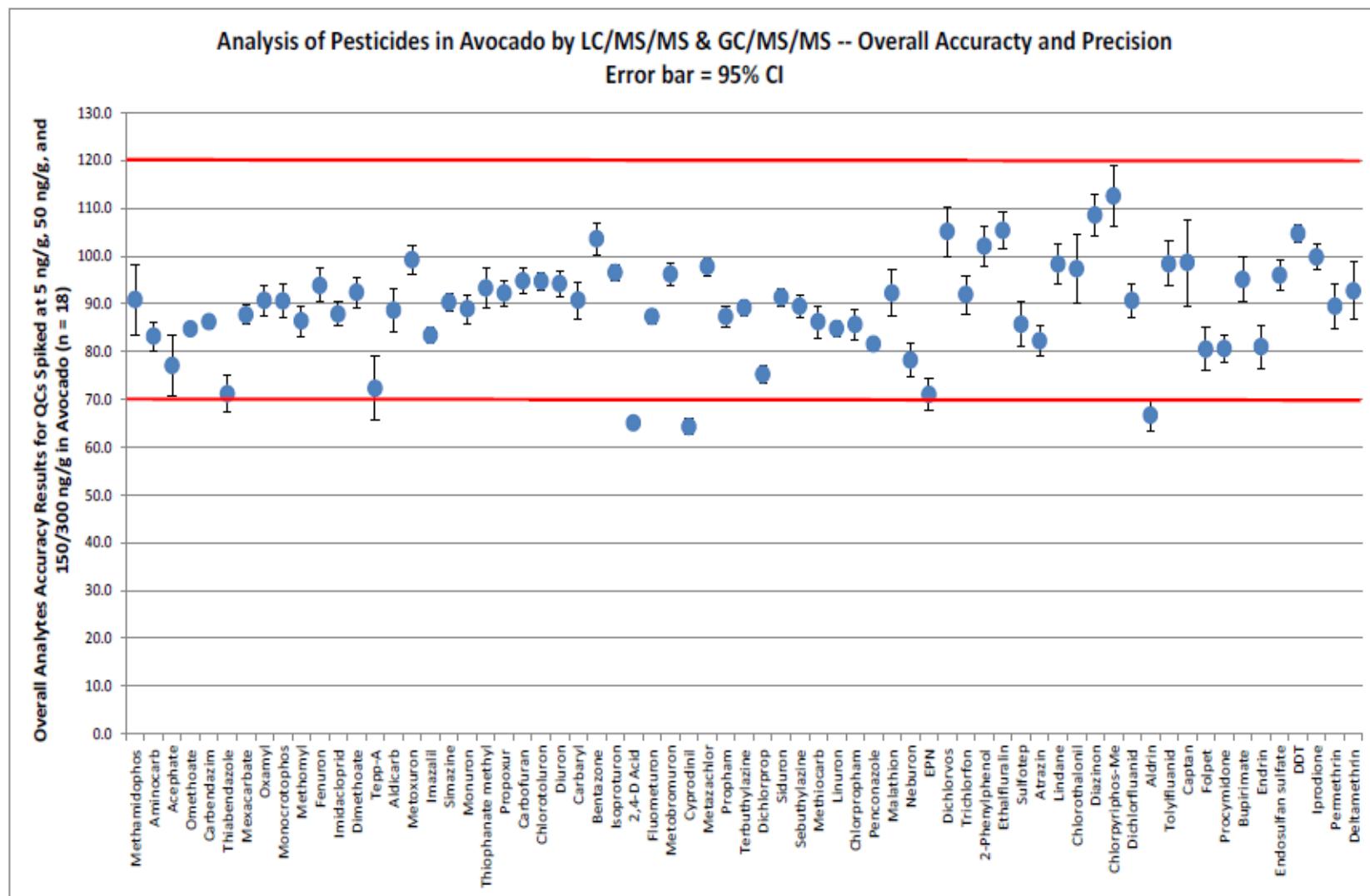


Selected Problematic Pesticides for Recovery Comparison



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Method Accuracy and Precision



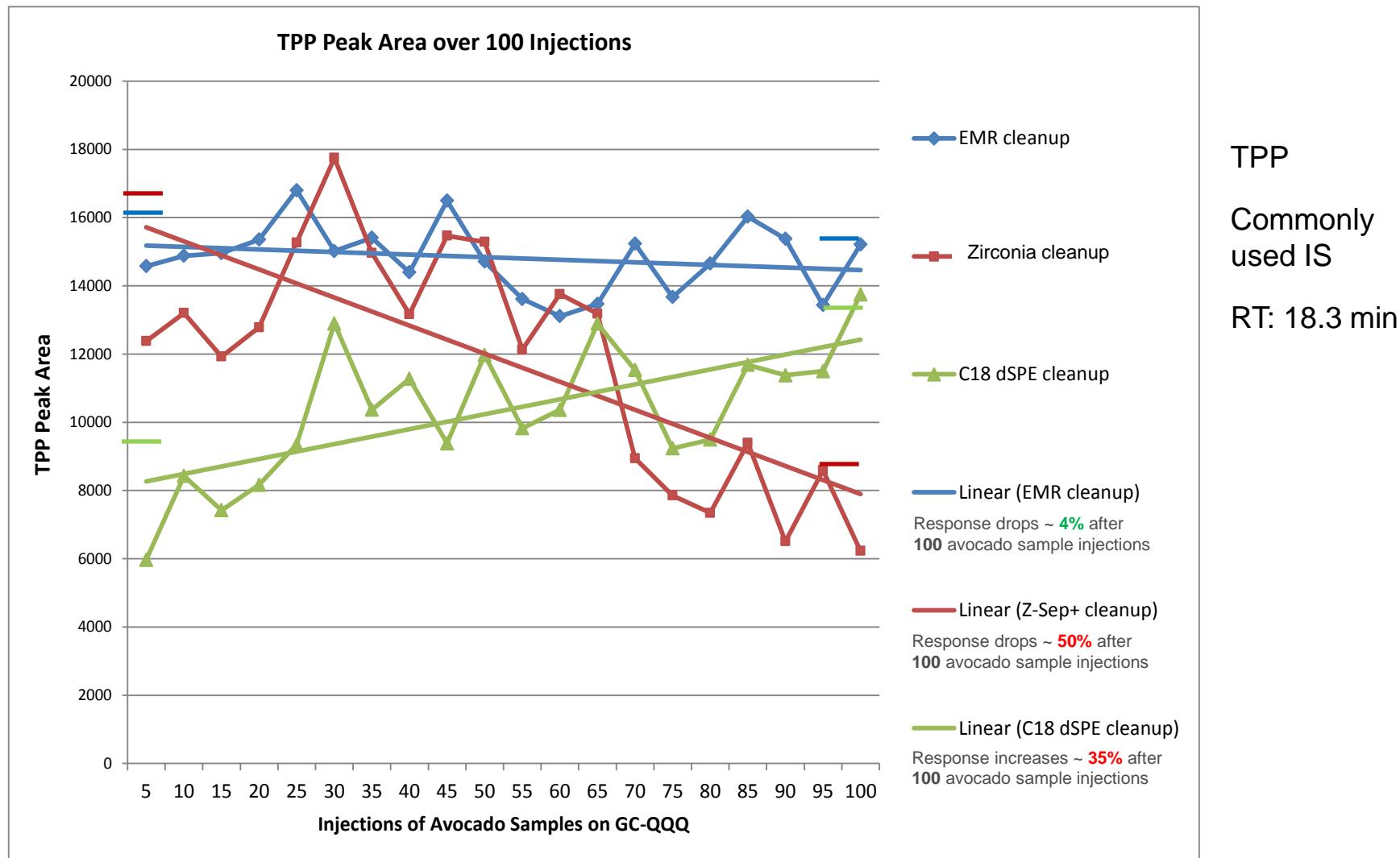
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Analytes Responses Reproducibility on GC/MS/MS over 100 Injections of Avocado Samples

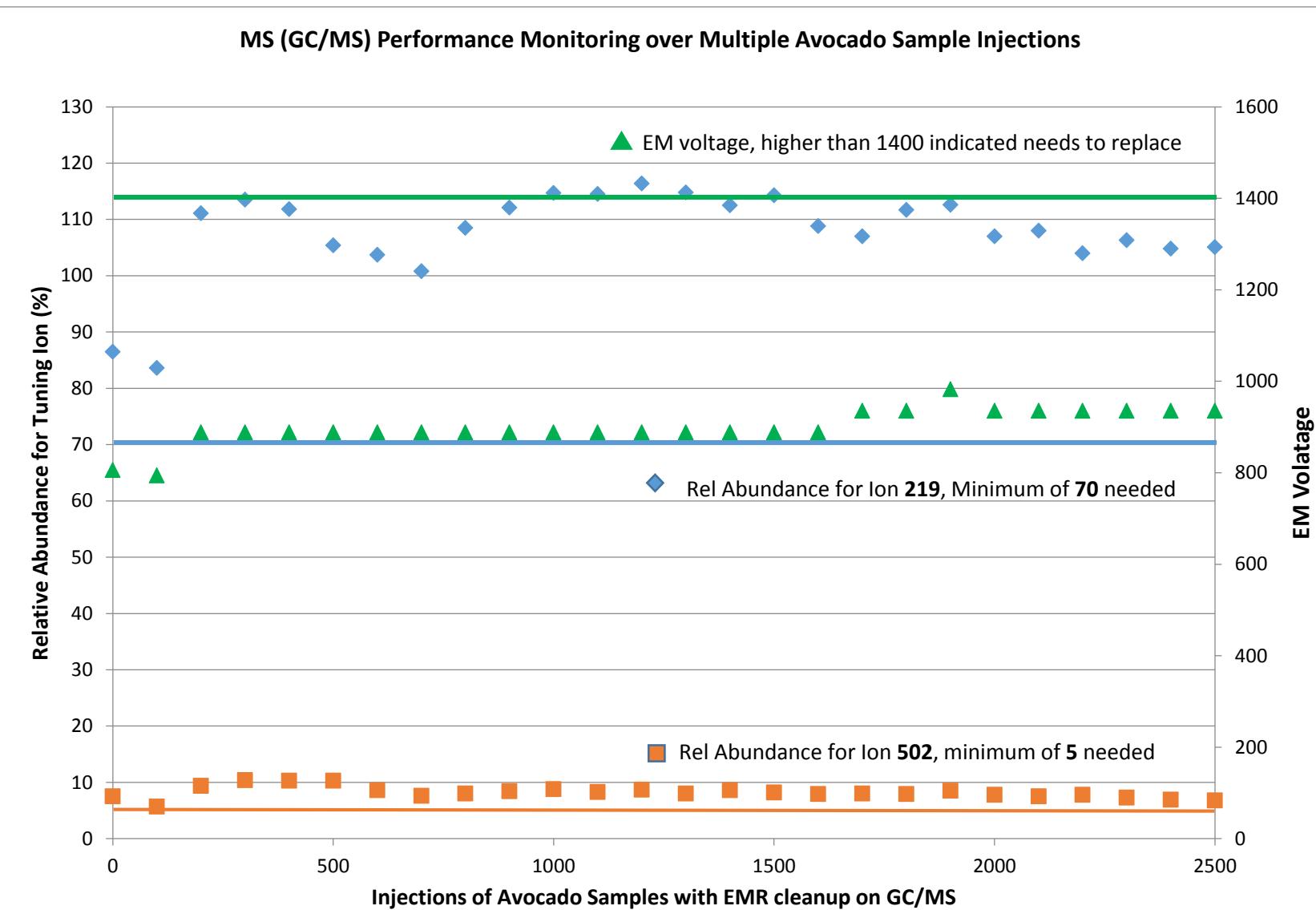
Pesticides	Analytes RSD over 100 injections on GC/QQQ (n = 20)		
	EMR Cleanup	C18/PSA cleanup	Zirconia sorbent cleanup
Dichlorvos	6.2	10.5	16.8
2-Phenylphenol	7.0	13.6	19.5
Ethalfluralin	12.4	18.8	32.0
Sulfotep	7.1	11.8	17.2
Atrazin	6.8	12.2	19.1
Lindane	8.5	10.8	20.0
Chlorothalonil	12.5	11.7	37.4
Diazinon	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



MS Source Critical Tuning Parameters



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Veterinary Drugs in Bovine Liver



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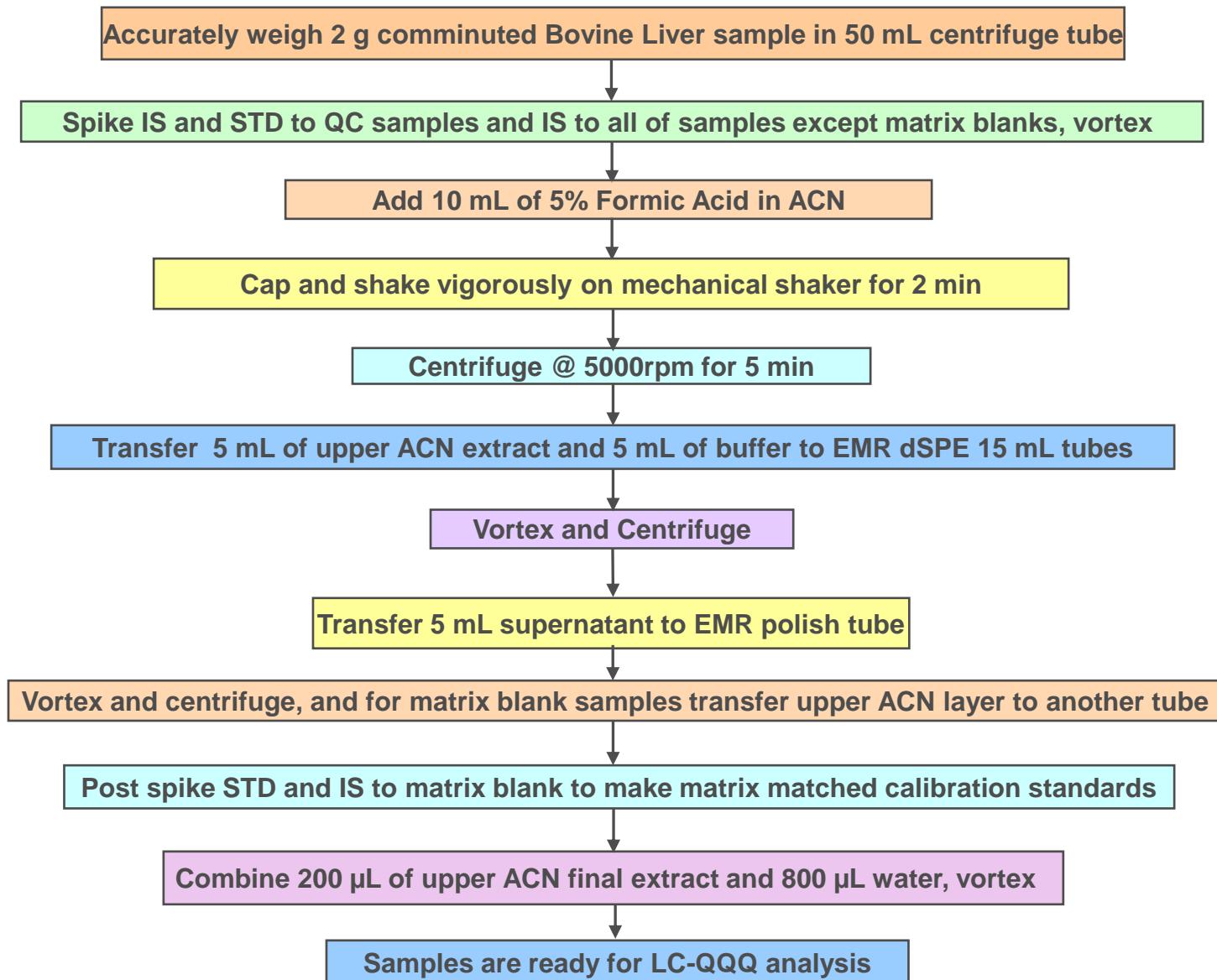
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Vet Drugs List for Evaluation

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	Oxytetracycline	
Florfenicol	Phenicol	2-Thiouracil	Thyreostat	Doxycycline	Tetracycline
Chloramphenicol		Metronidazole-OH	Nitroimidazole	Chlortetracycline	
Sulfamethizole	Sulfonamide	Fenbendazole	Anthelmintic	Acepromazine	
Sulfamethoxypridazine		Lavamisole		Chlorpromazine	Tranquilizer
Lincomycin	Lincosamide	Morantel		Ketoprofen	
Ciprofloxacin	Fluoroquinolone	Bithionol		Cefazolin	Cephalosporin
Norfloxacin		Clorsulon	Flukicide	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

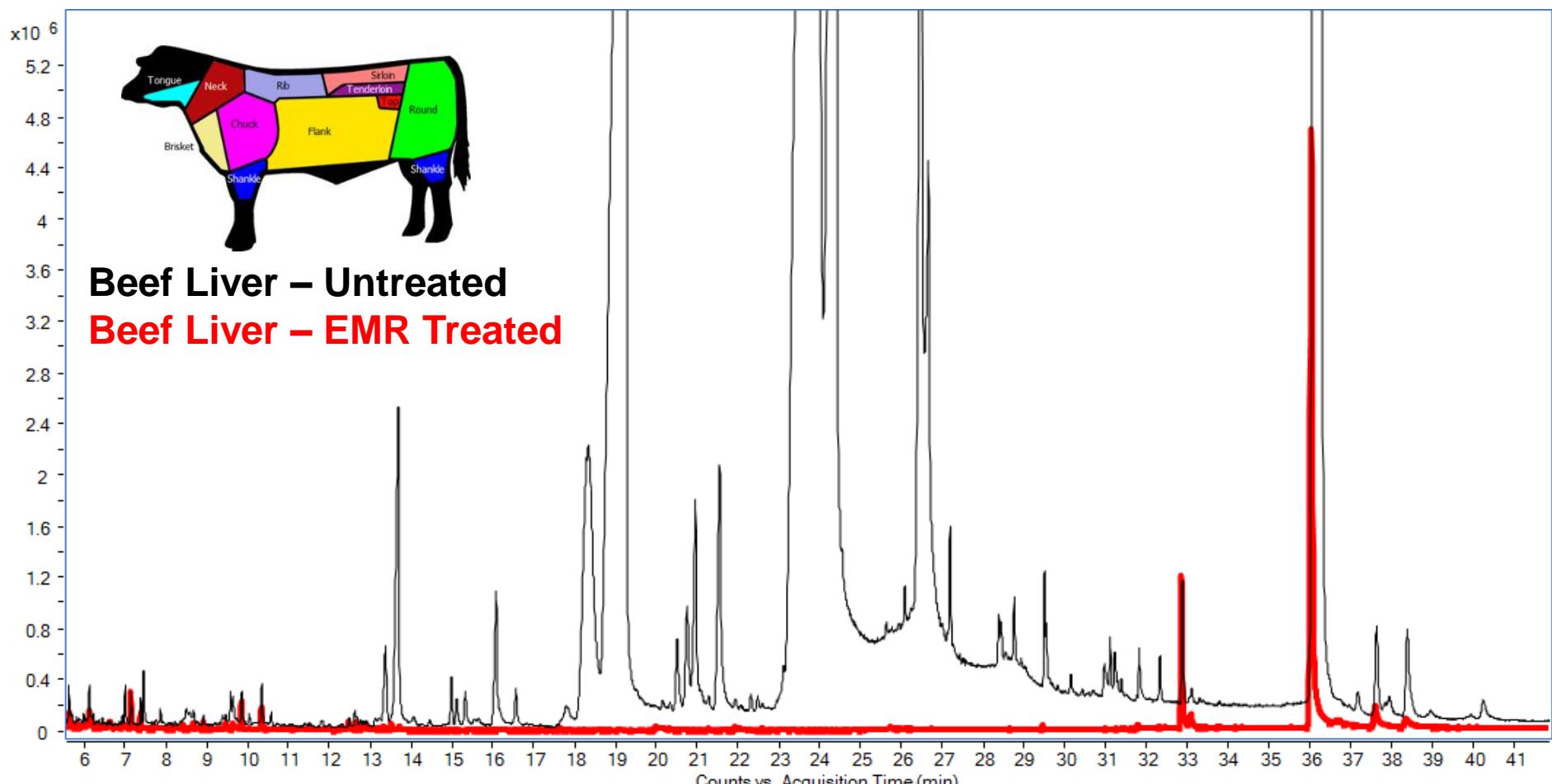
Cleanup	Amount of co-extractives (mg, n= 3)	% of matrix co-extractives removed by cleanup
No further cleanup	12.1	--
EMR dSPE	5.3	56.2
Zirconia dSPE	6.0	50.4
C18 dSPE	7.8	35.5

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

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



Another difficult matrix



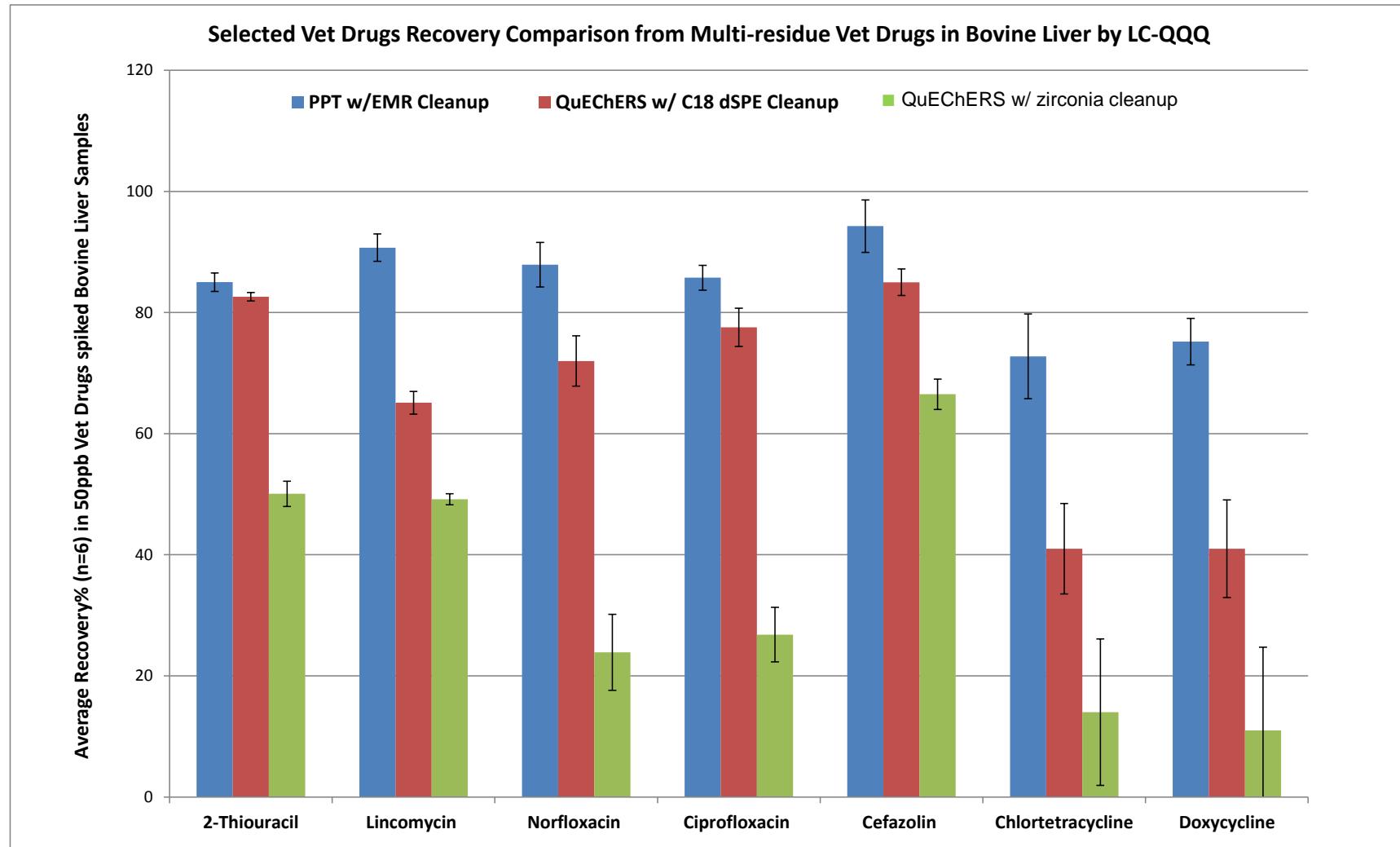
GC/MS data



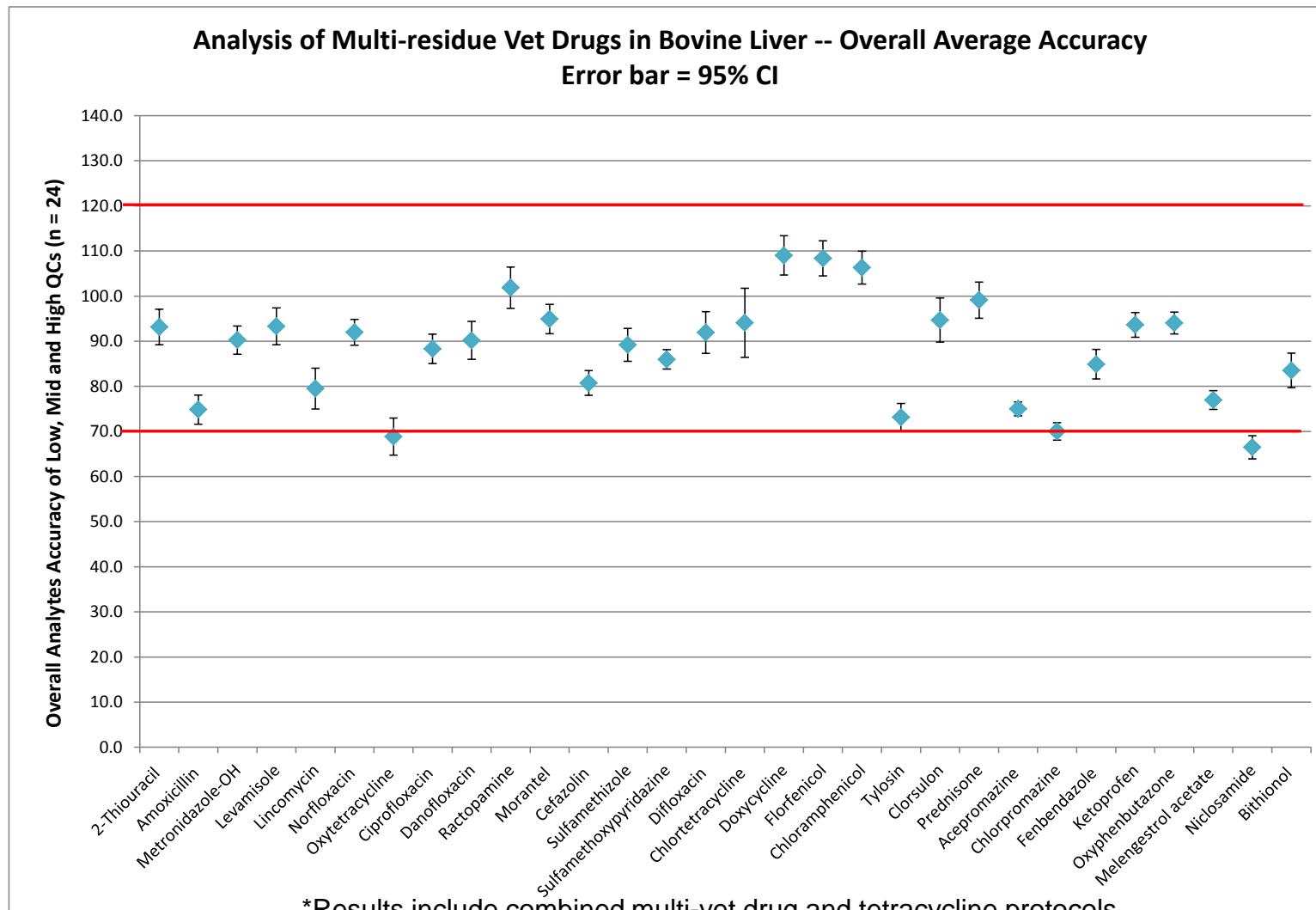
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Results for Multiple Vet Drugs Residue Analysis in Bovine Liver (Selected individual analytes comparison chart)

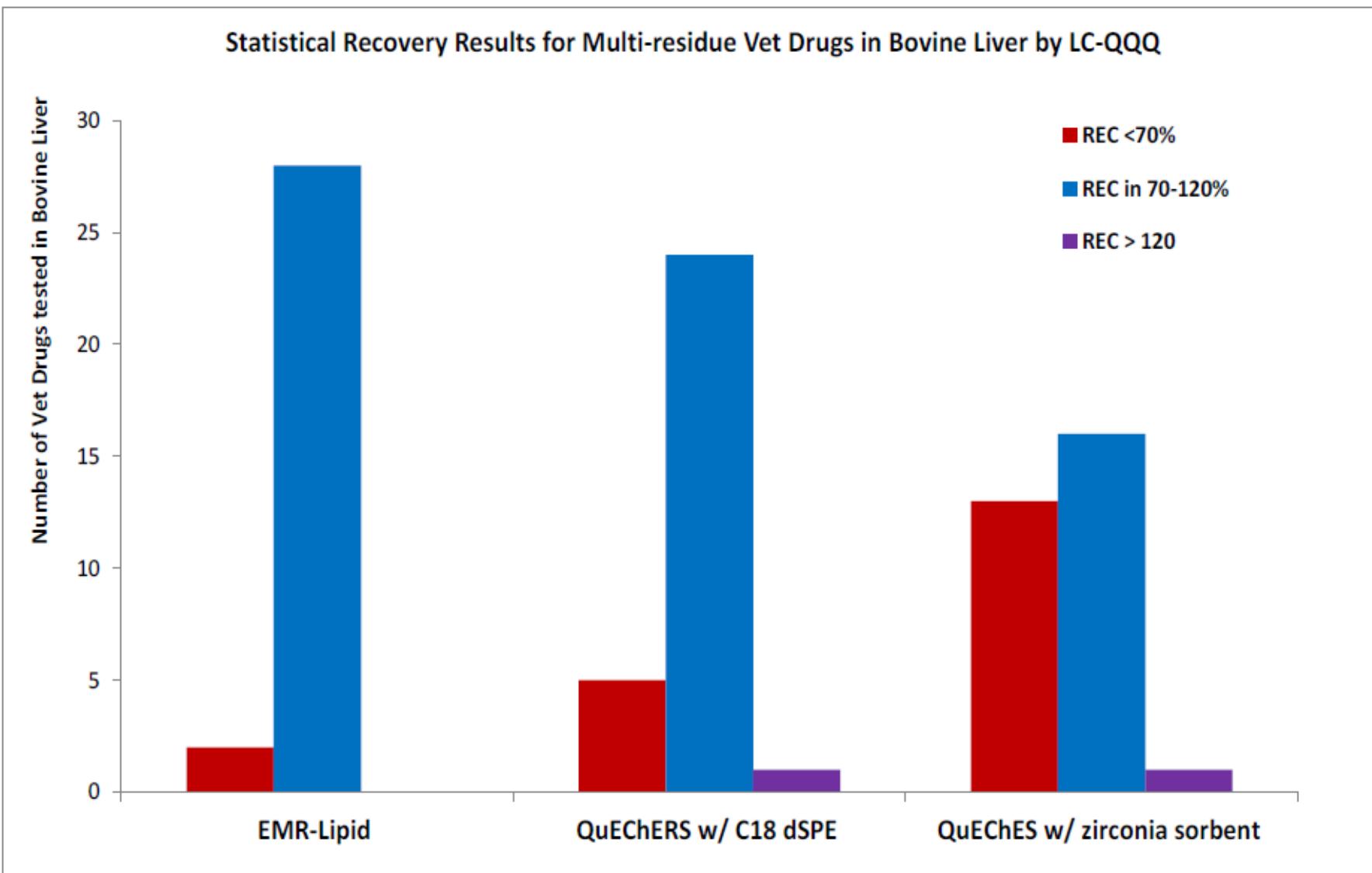


Results for Multiple Vet Drugs Residue Analysis in Bovine Liver



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Results for Multiple Vet Drugs Residue Analysis in Bovine Liver



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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

Agilent Technologies

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Megan Marr

Phil Stremple

Supply Chain

William Hudson

Scott Choi



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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...

PAH Analysis in Salmon with Enhanced Matrix Removal

Auth
Derick
Agile

Multiresidue Analysis of Pesticides in Avocado with Agilent Bond Elut EMR—Lipid by LC/MS/MS

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Multiresidue Analysis of Pesticides in Avocado with Agilent Bond Elut EMR—Lipid by GC/MS/MS

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Multiresidue Analysis of Veterinary Drugs in Bovine Liver by LC/MS/MS

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Multiresidue Analysis of Veterinary Drugs in Bovine Liver by GC/MS/MS

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Application Note

Food Testing and Agriculture

Abstract

Agilent Bond Elut QuEChERS Enhanced Matrix Removal-Lipid (EMR-Lipid) is the next generation of sample preparation product, and is available for convenient dispersive solid phase extraction (dSPE). The material is highly selective towards coextracted matrix, especially from fatty samples (fat content > 5%) without negatively impacting analyte recovery. This study demonstrates the application of this novel product for the analysis of 30 representative veterinary drugs in bovine liver. The procedure involves a rapid and efficient protein precipitation extraction by acidiified acetonitrile, followed by the use of EMR—Lipid dSPE and a polish kit for further cleanup. The amount of matrix removed by the EMR-Lipid protocol was determined by the weight of coextractives and postcolumn infusion experiments. Compared to other matrix cleanup products, EMR—Lipid dSPE provides more effective matrix removal and better analyte recoveries. The optimized EMR-Lipid method delivers superior cleanliness, and excellent accuracy and precision for all 30 veterinary drug compounds at all levels, providing fast, robust, and effective analysis of high-fat samples.

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Appendix

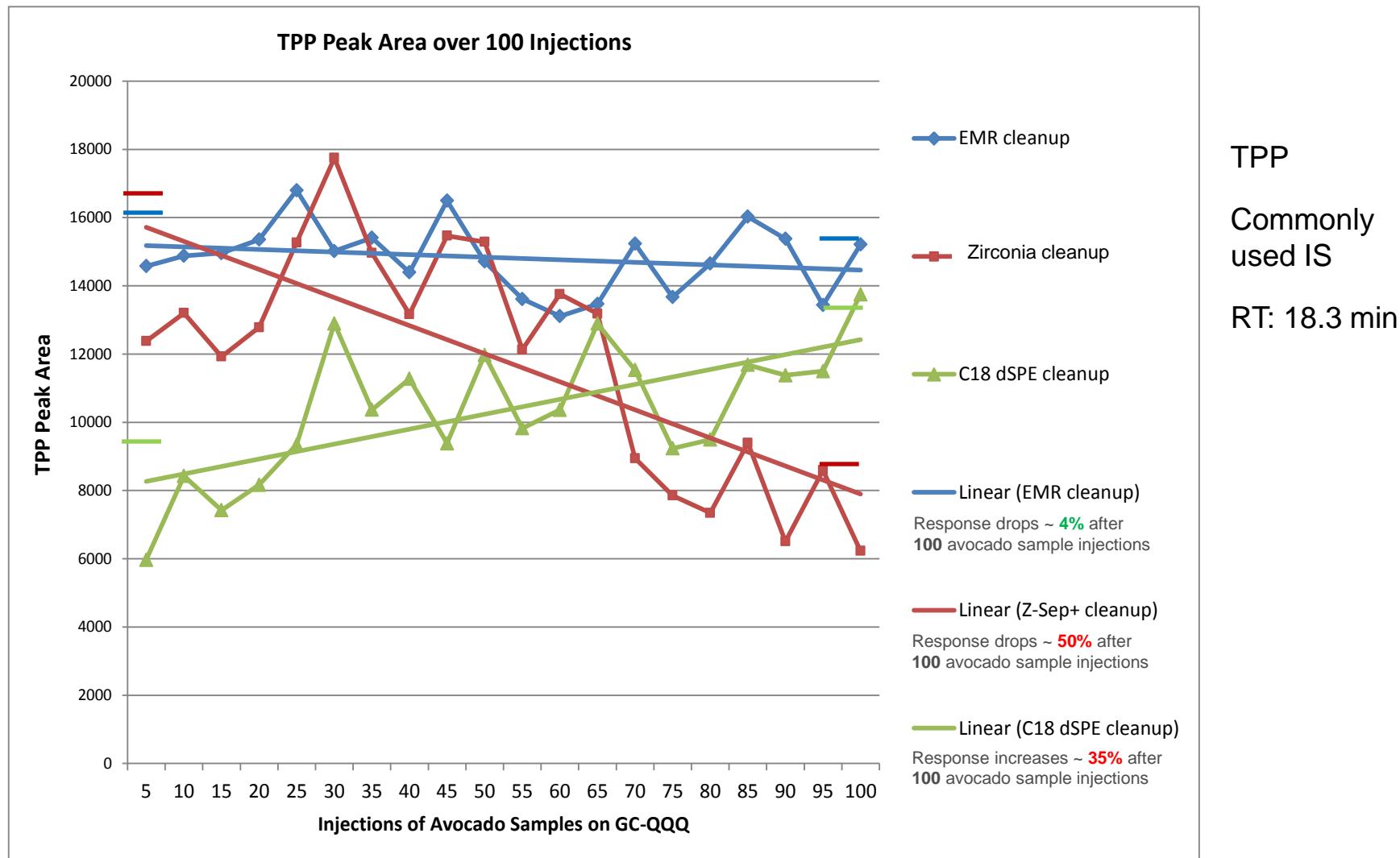


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NACRW 2015

November 15

Comparison of Analytes Response Consistency over Multiple Avocado Sample Injections



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Improving dSPE in QuEChERS

1. Extraction



Weigh sample



Add water and QC spikes if needed and spike with internal standard



Add acetonitrile



Vortex or shake



Add salt packet



Shake 1 minute



Centrifuge at 4000 rpm for 5 minutes



Phase separation of acetonitrile and aqueous layer

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



Choose the dispersive cleanup kit and add acetonitrile extract



Vortex for 1 minute



Centrifuge at 4000 rpm for 5 minutes



Take aliquot of supernatant and dry down or dilute as necessary



Place in autosampler vials for GC or LC analysis

Pros

- Same as extraction

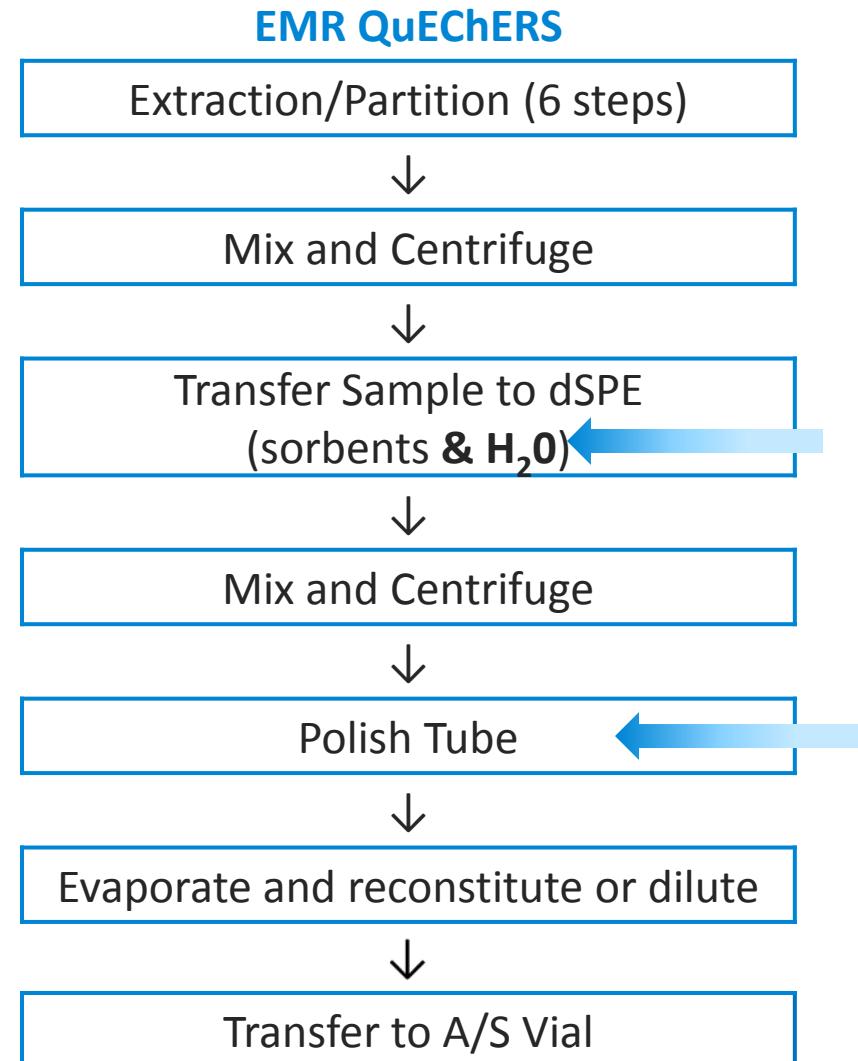
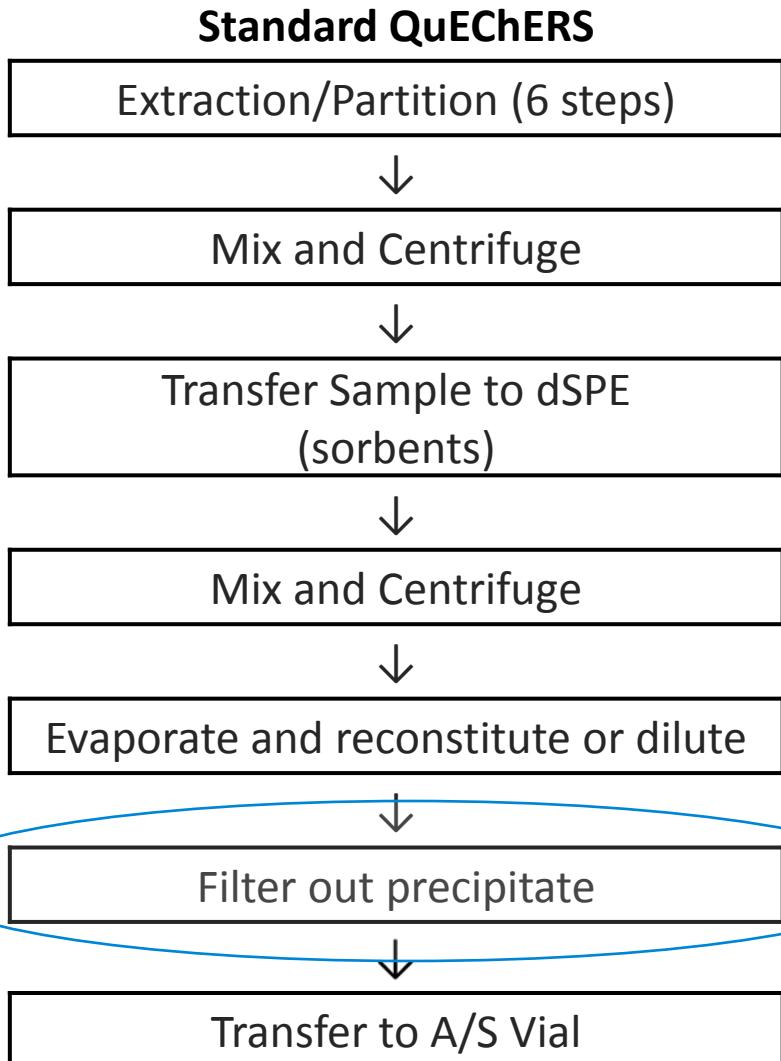
Cons

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

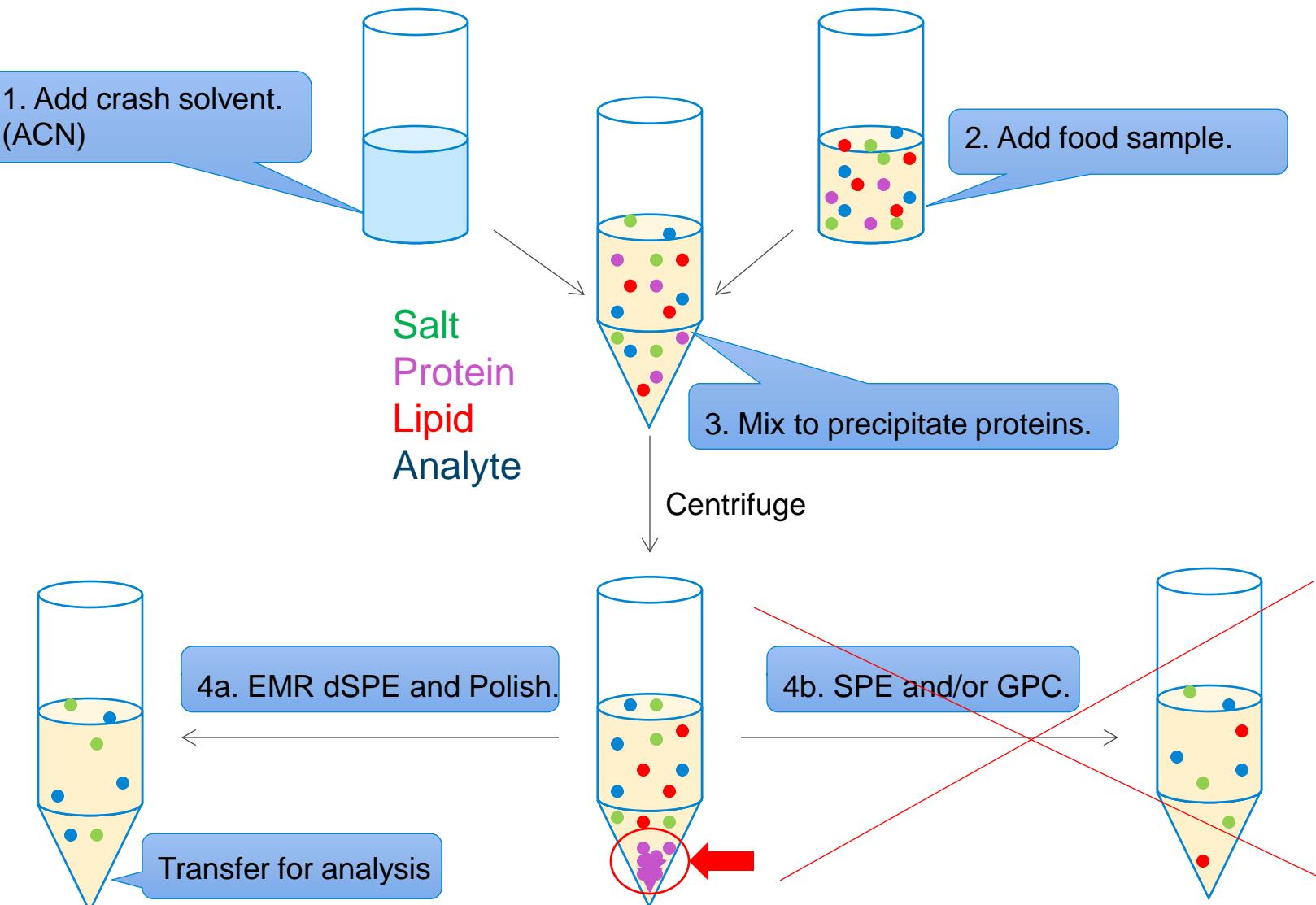


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Traditional QuEChERS versus QuEChERS EMR-Lipid



Improving Liquid Extraction Workflows



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Traditional versus EMR-Lipid Liquid Extraction

Liquid Extraction with SPE/GPC

Add Sample and Acetonitrile



Mix and Centrifuge



Sample Pretreatment



SPE and/or GPC



Evaporate and reconstitute or dilute



Filter out precipitate



Transfer to A/S Vial

EMR Liquid Extraction

Add Sample and Acetonitrile



Mix and Centrifuge



Transfer Sample and H₂O to EMR dSPE



Mix and Centrifuge



Polish Tube



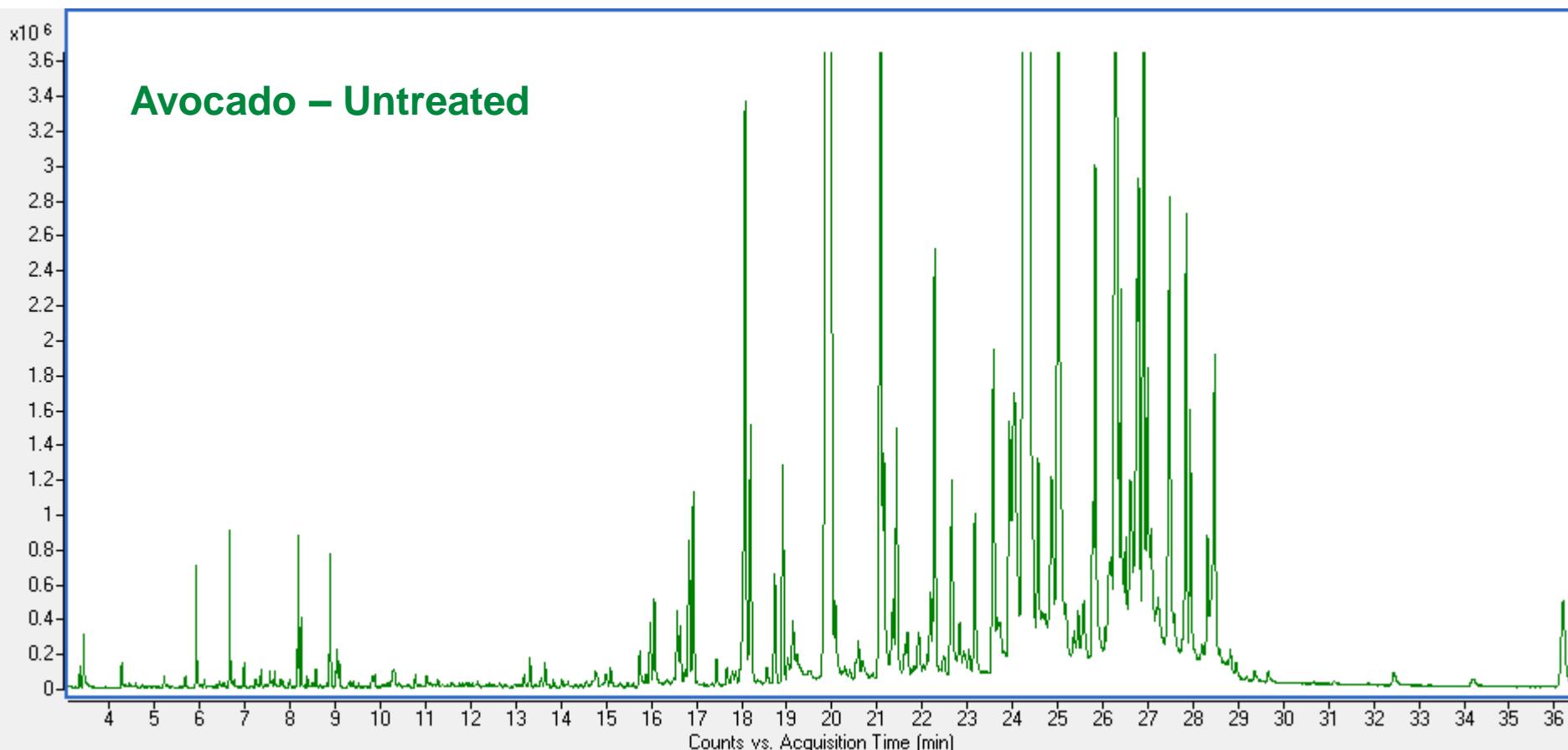
Evaporate and reconstitute or dilute



Transfer to A/S Vial



GC-MS Fullscan Avocado

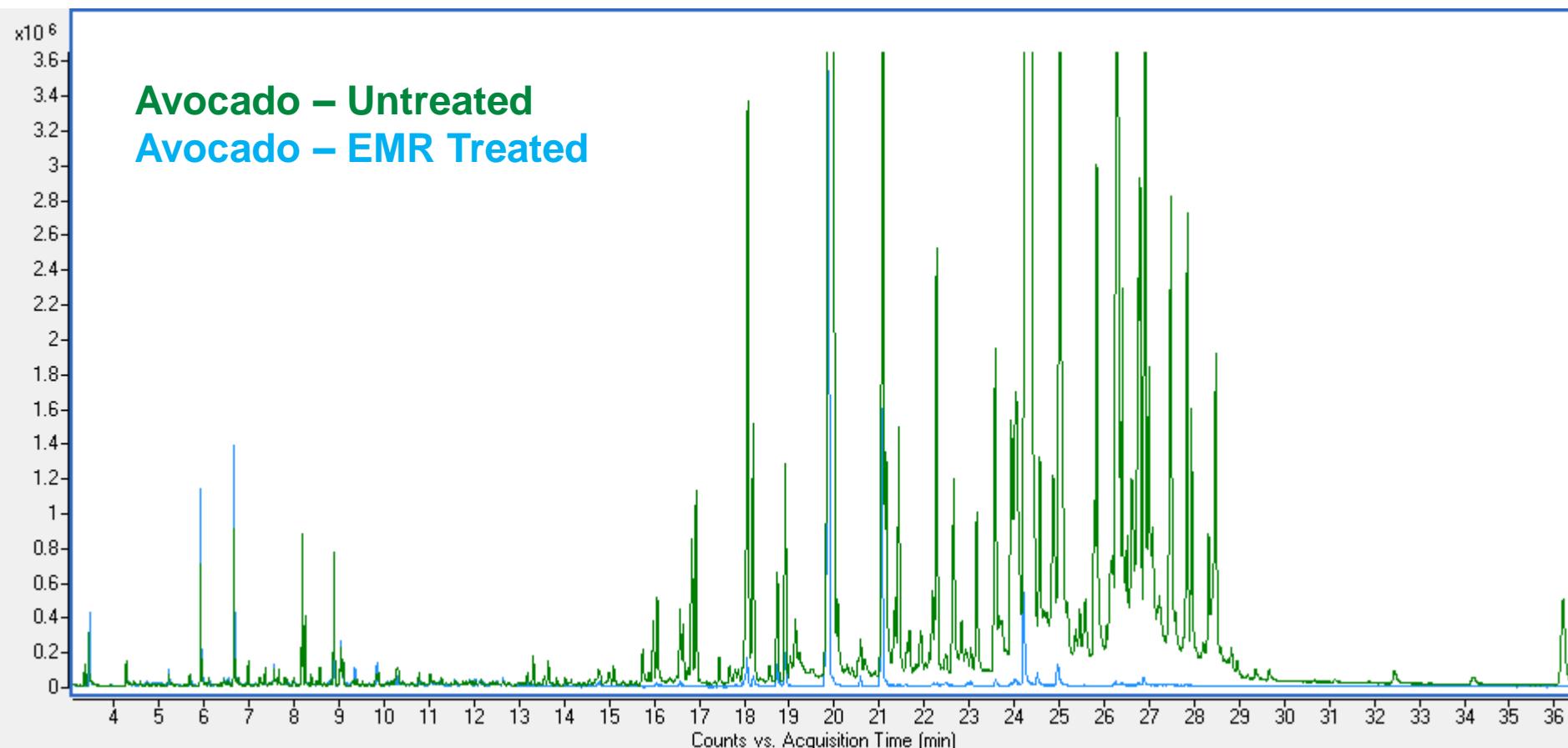


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November 15

GC-MS Fullscan Avocado

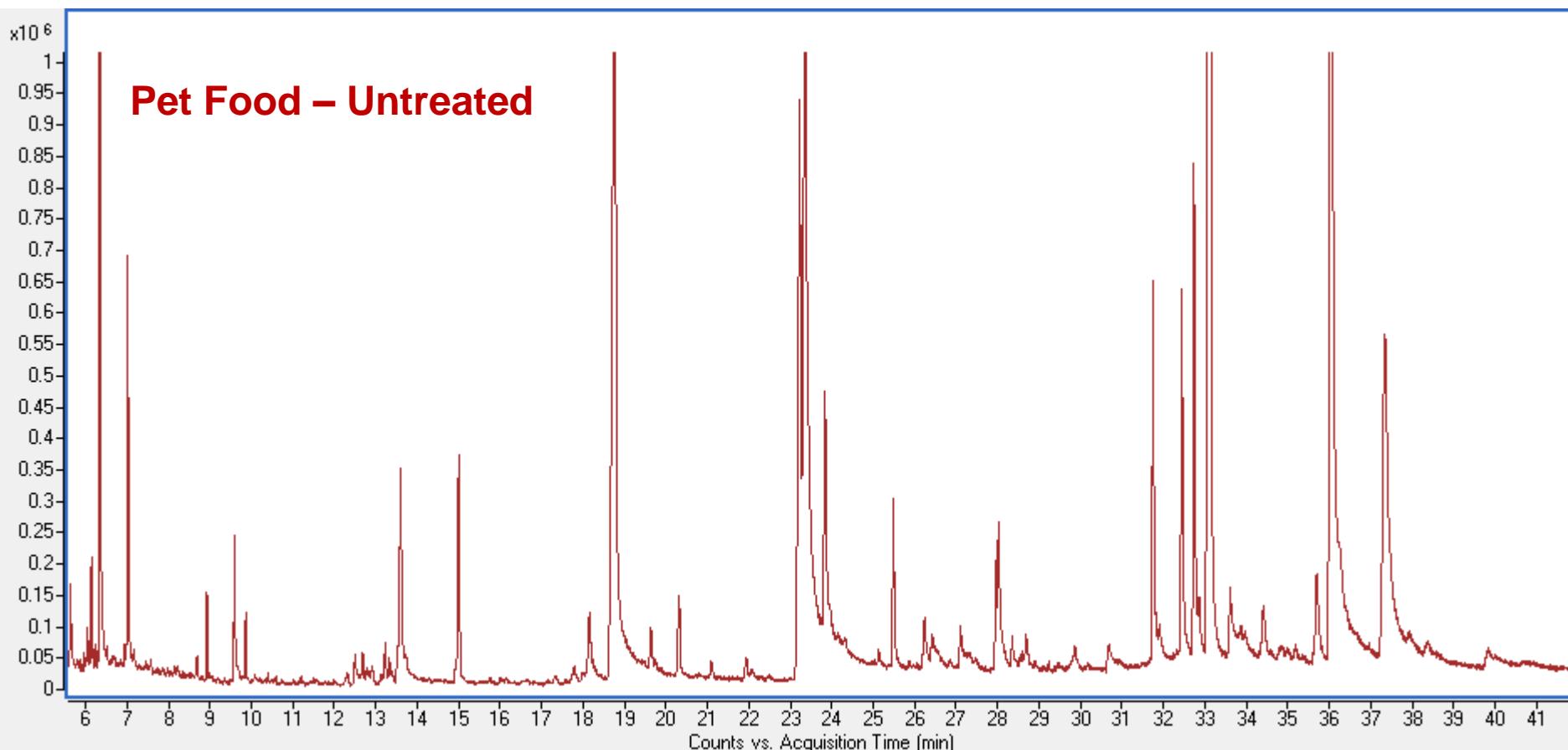


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GC-MS Fullscan Pet Food

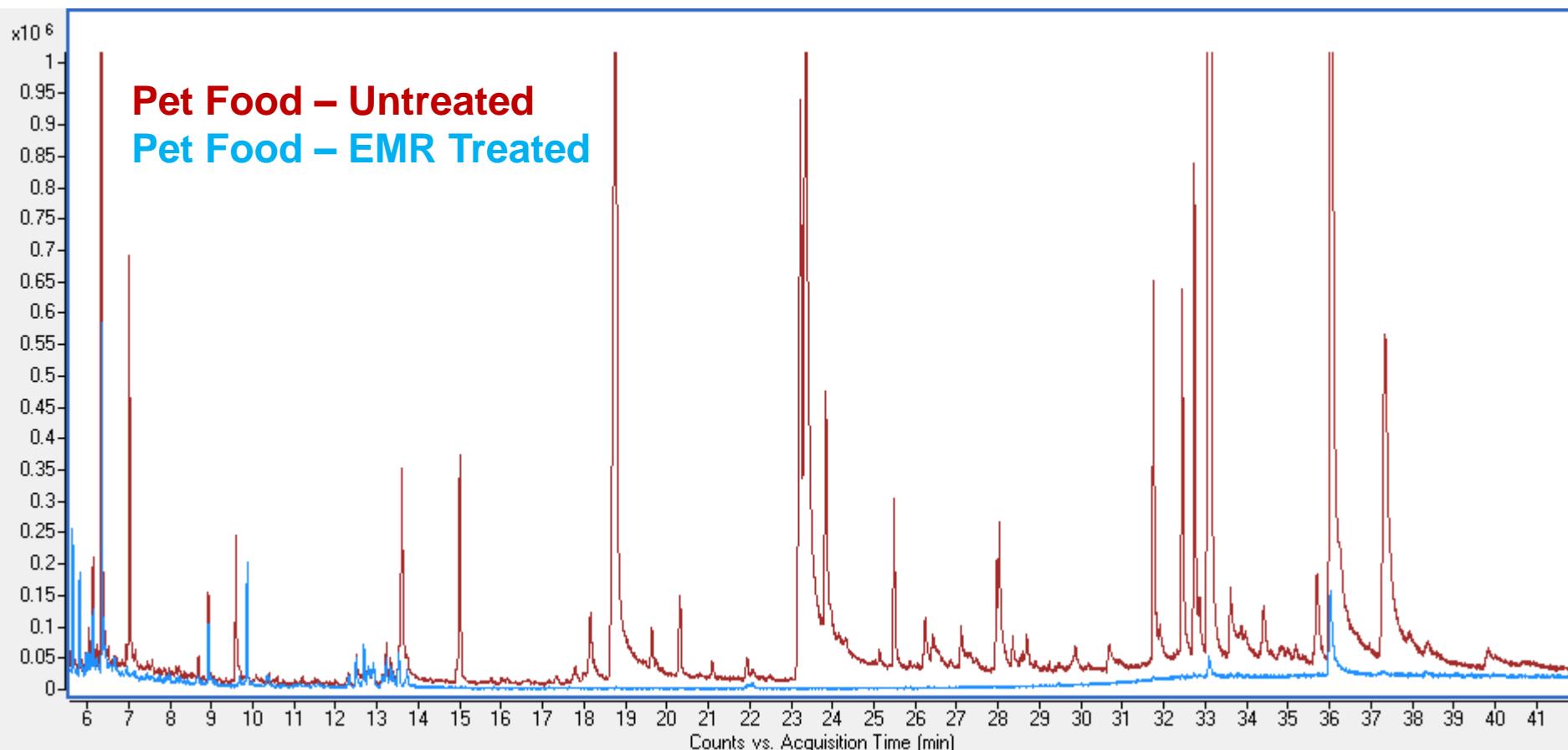


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GC-MS Fullscan Pet Food

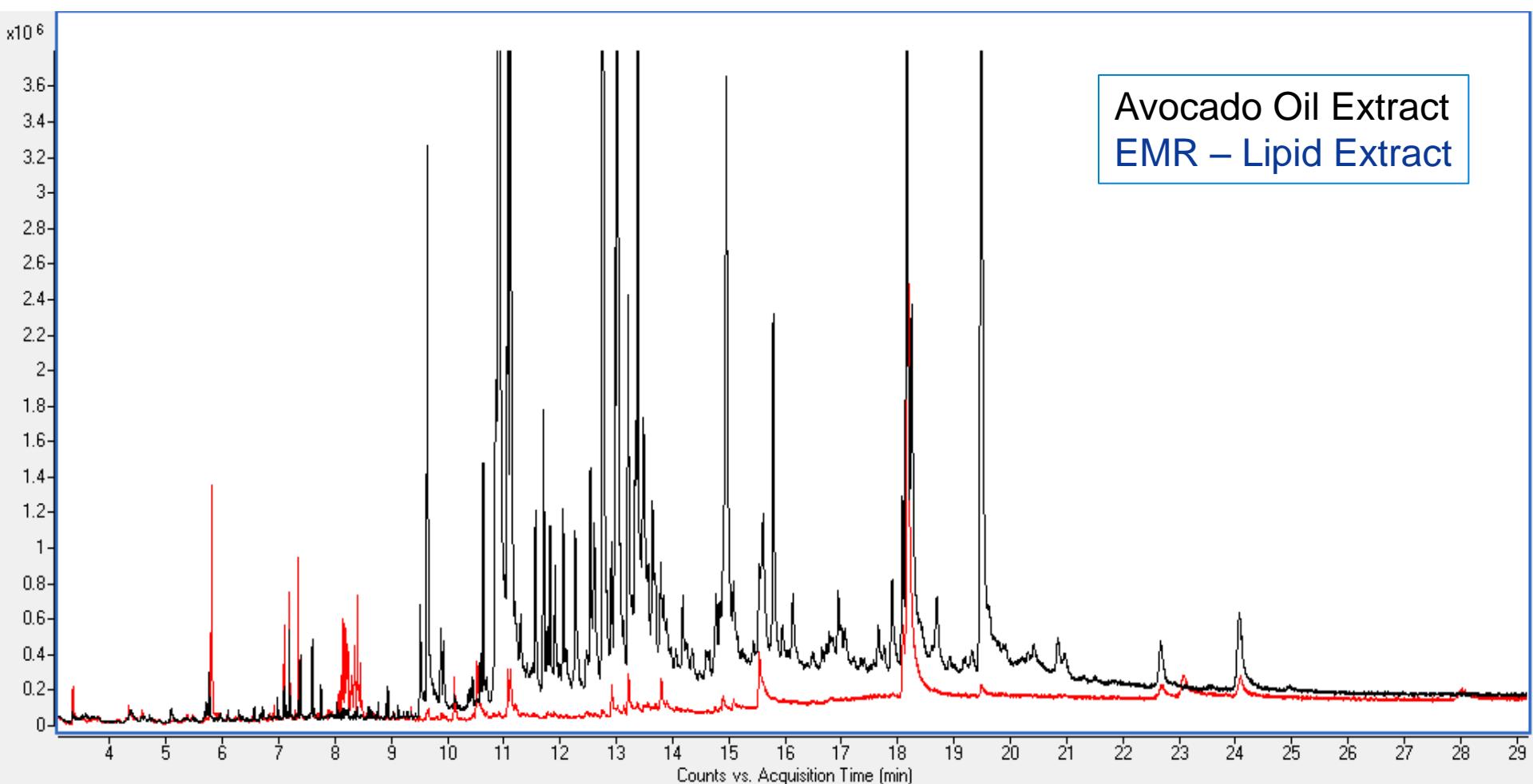


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GC-MS Full Scan– Avocado Oil

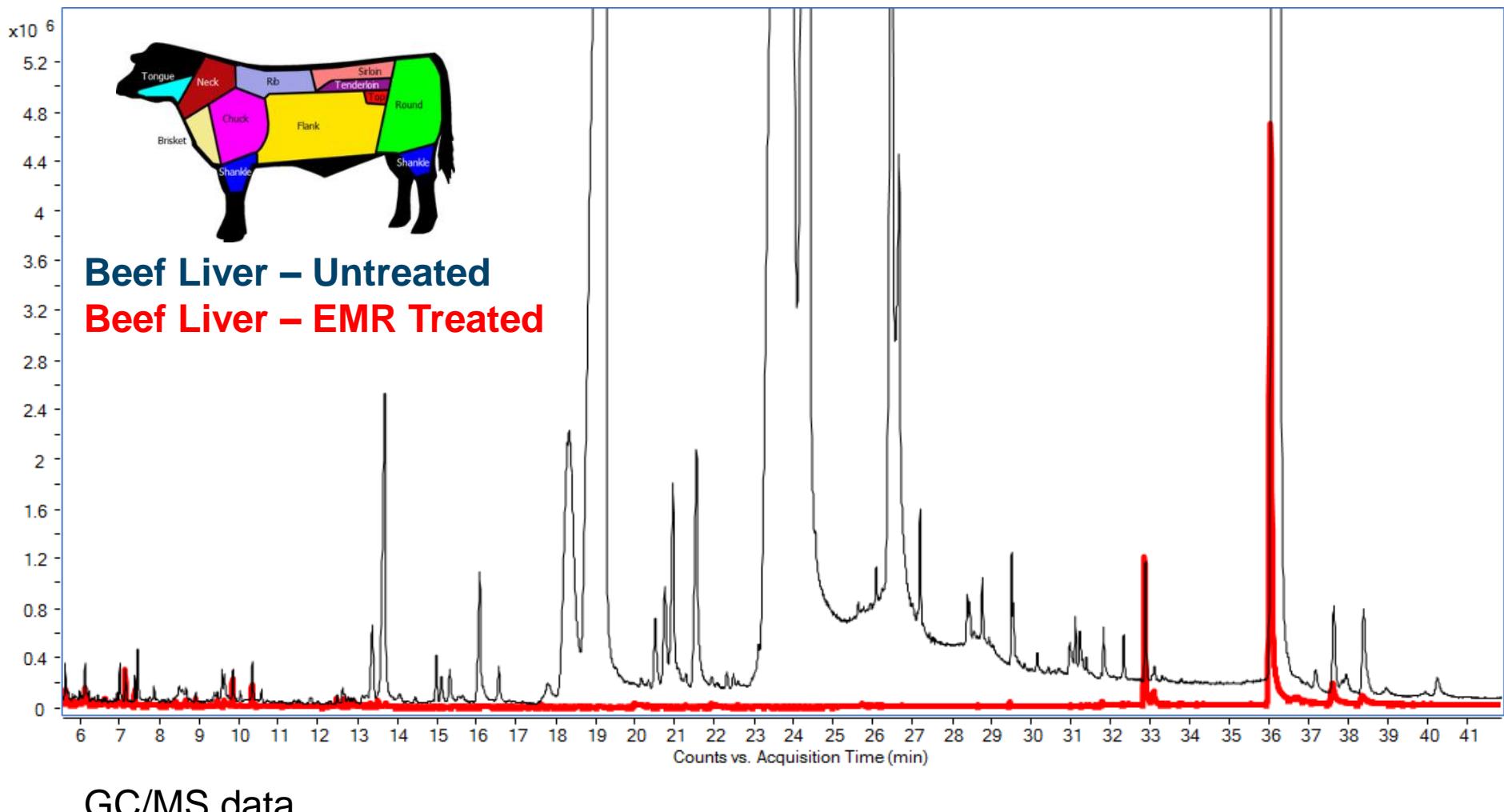


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Another difficult matrix



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PAHs in Salmon



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Target Analytes

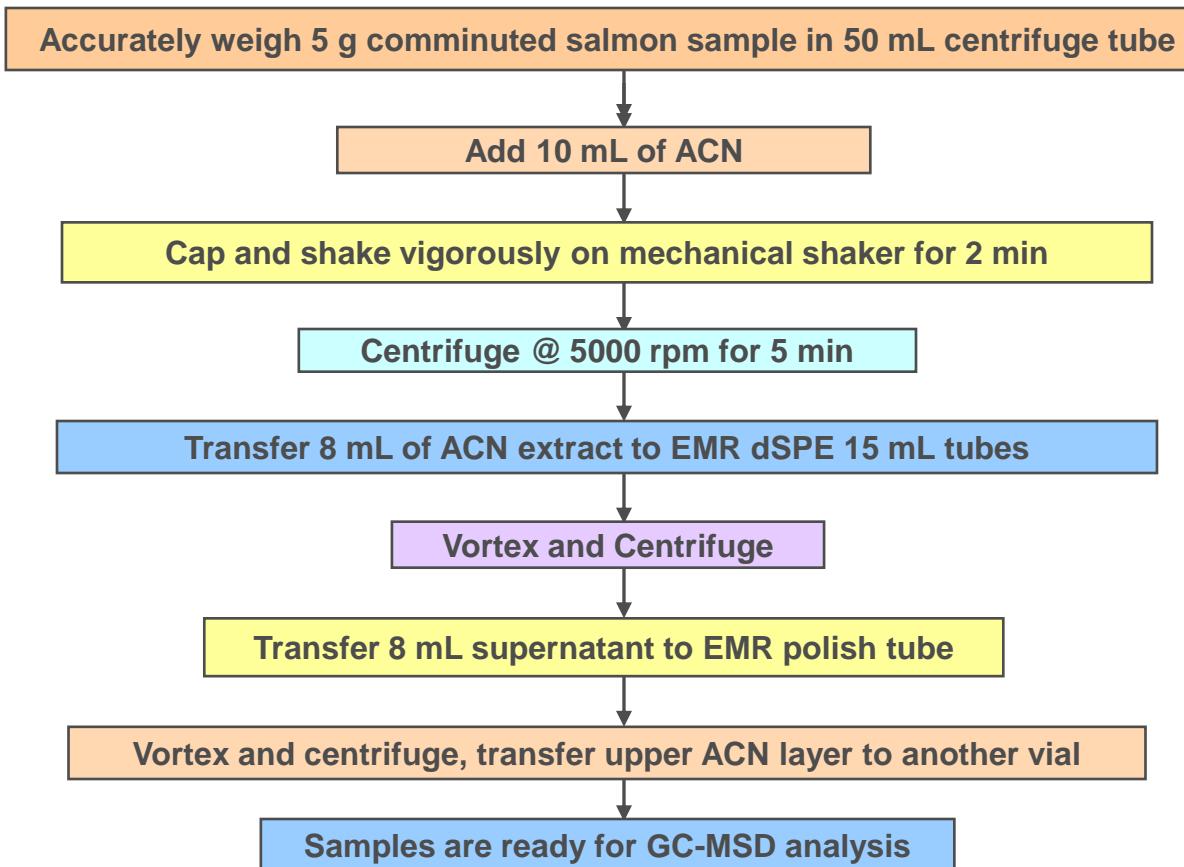
Compound	GC-MS (SIM)	
	RT	Target Ion
Naphthalene	3.89	128.0
Acenaphthalene	5.37	152.0
Fluorene	6.05	166.0
Phenanthrene	7.25	178.0
Anthracene	7.34	178.0
Pyrene	10.31	202.0
Benz[a]anthracene	13.83	228.0
Chrysene	13.93	228.0
Benzo[b]fluoranthene	16.99	252.0
Benzo[k]fluoranthene	17.08	252.0
Benzo[a]pyrene	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



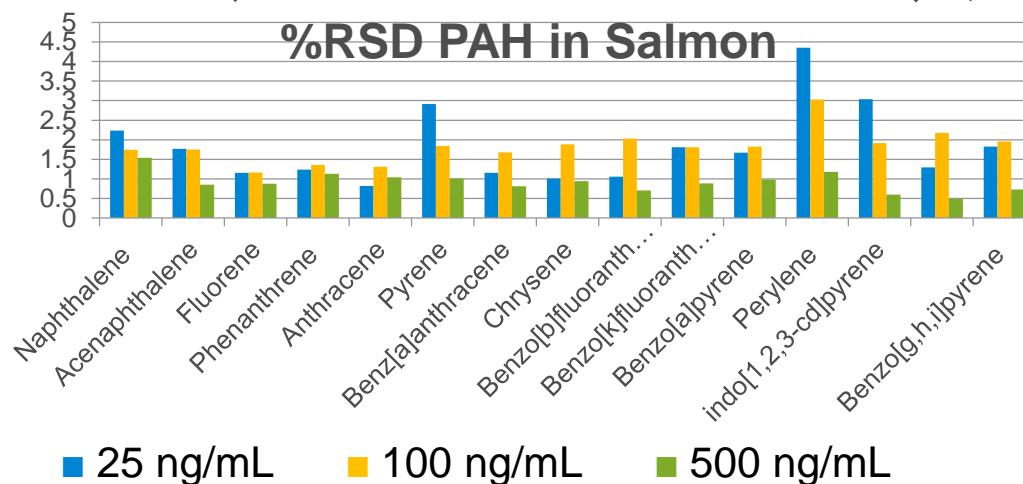
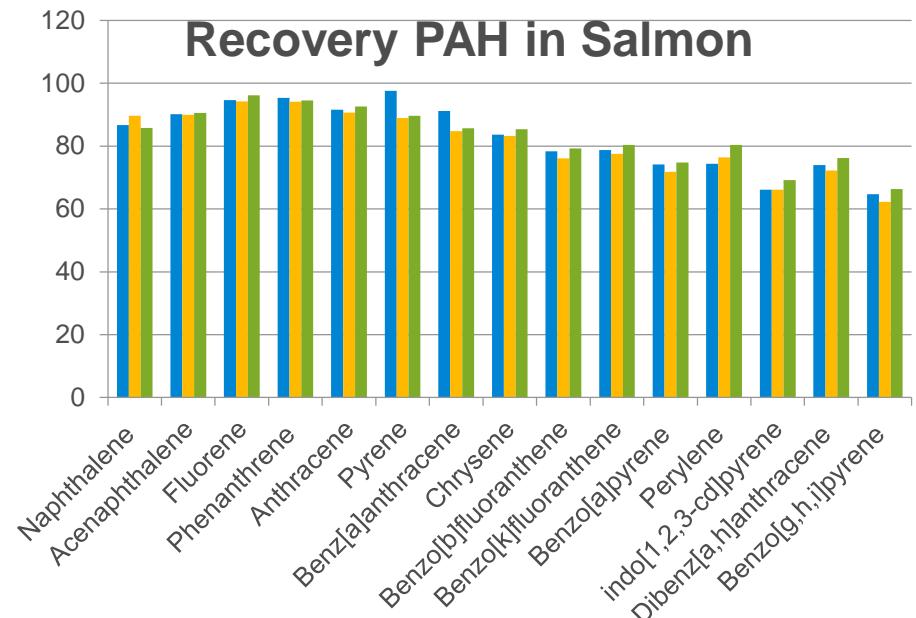
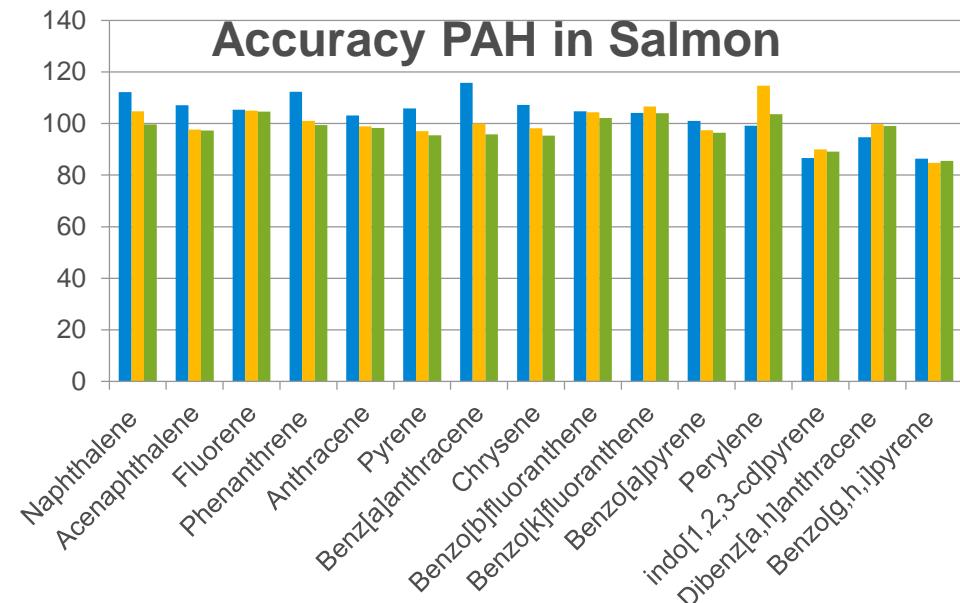
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EMR Modified Protocol for Analysis of PAHs in Salmon



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EMR – New Procedure Results



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