

Advanced QuEChERS Method Development: Extending the QuEChERS Approach to a Wide Range of Sample Types and Compounds

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Today's Agenda

- QuEChERS: Brief Overview
- Yohimbine in Botanicals and Dietary Supplements
- QuPPe: Quick Easy Polar Pesticide Method
- Miniaturization of QuEChERS Method
- Enhanced Matrix Removal: EMR-L
- Summary: Take Home Message

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What is QuEChERS (pronounced "*Catchers*")

Quick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe

- Developed jointly by USDA and Food Regulatory Agencies as a sample preparation method for multi-residue analyses
- Simplified extraction and cleanup approaches that reduce use of expensive and/or dangerous solvents
- Primarily for preparing fruits and vegetables for pesticide analysis
- **Rapidly being extended to other food types and compound classes**





What are the Benefits of QuEChERS?

- QuEChERS Approach: Extract +250 compounds at one time
- QuEChERS methodology is non-selective technique, does not remove all the matrix
- Final extract amenable to GC/MS or LC/MS
- **Reduced solvent and labor, increased lab productivity**

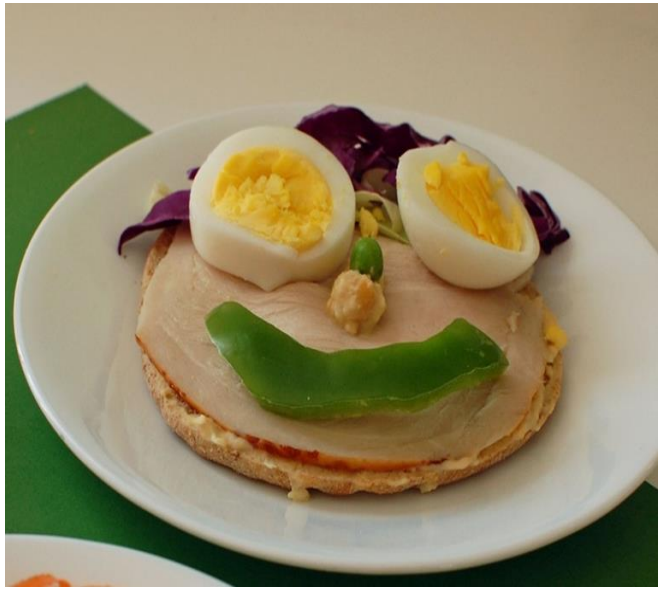
QuEChERS Approach Advantages
30ish minutes to extract multiple samples at once
Minimal solvent usage per sample: 10-15 mL
Chlorinated Solvents: None

If you can weigh, pipette, shake and your lab has a centrifuge, you can perform QuEChERS

Why Choose QuEChERS?

- Lives up to its name
- Flexibility and adaptability
- Works with solids and liquids
- Perfect complement to tandem MS instruments
- Called out in many regulatory methods and norms

QuEChERS
Easy as 1-2-3



Step 1: Salting Out 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

Step 2: Dispersive Solid Phase Extraction (dSPE)

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

Taking QuEChERS to the Next Level – Advanced QuEChERS Approaches & Techniques

The QuEChERS technique for pesticide residues in fruits and vegetables are well-characterized, and accepted

Let's look at these advanced concepts for QuEChERS:

- Characterization and quantitation of adulterants in botanicals and dietary supplements
- Modified approach for very polar pesticides
- Miniaturizing the QuEChERS protocol, application of incurred samples
- Novel matrix removal sorbent: EMR-L

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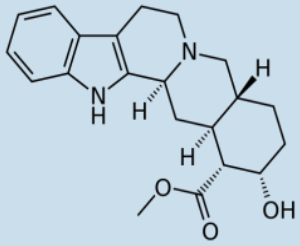
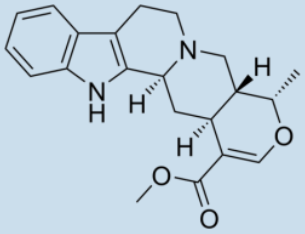
Yohimbine and Analogues in Botanicals and Dietary Supplements

	Yohimbine	Ajmalicine
Molecular Structure		
Molecular Formula	$C_{21}H_{26}N_2O_3$	$C_{21}H_{24}N_2O_3$
[M+H] Measured Mass	355.2019	353.1859

Sample Preparation

Original Method-D&S

- Weigh 1.00 g sample (±0.01g) in 50 mL centrifuge tube
- Add QC spike as necessary and add 10 mL 80% MeOH
- Sonicate 15 min and centrifuge at 5000 rpm for 5 min
- Decant into 25 mL volumetric flask
- Repeat extraction (×2)
- Bring extract to 25 mL with 80% MeOH
- Dilute as necessary (25× – 500,000×)
- Filter into auto-sampler vial with Captiva 0.2 µm PTFE syringe filter
- Sample ready for 6490 LC-QQQ and 6550 LC-Q-TOF

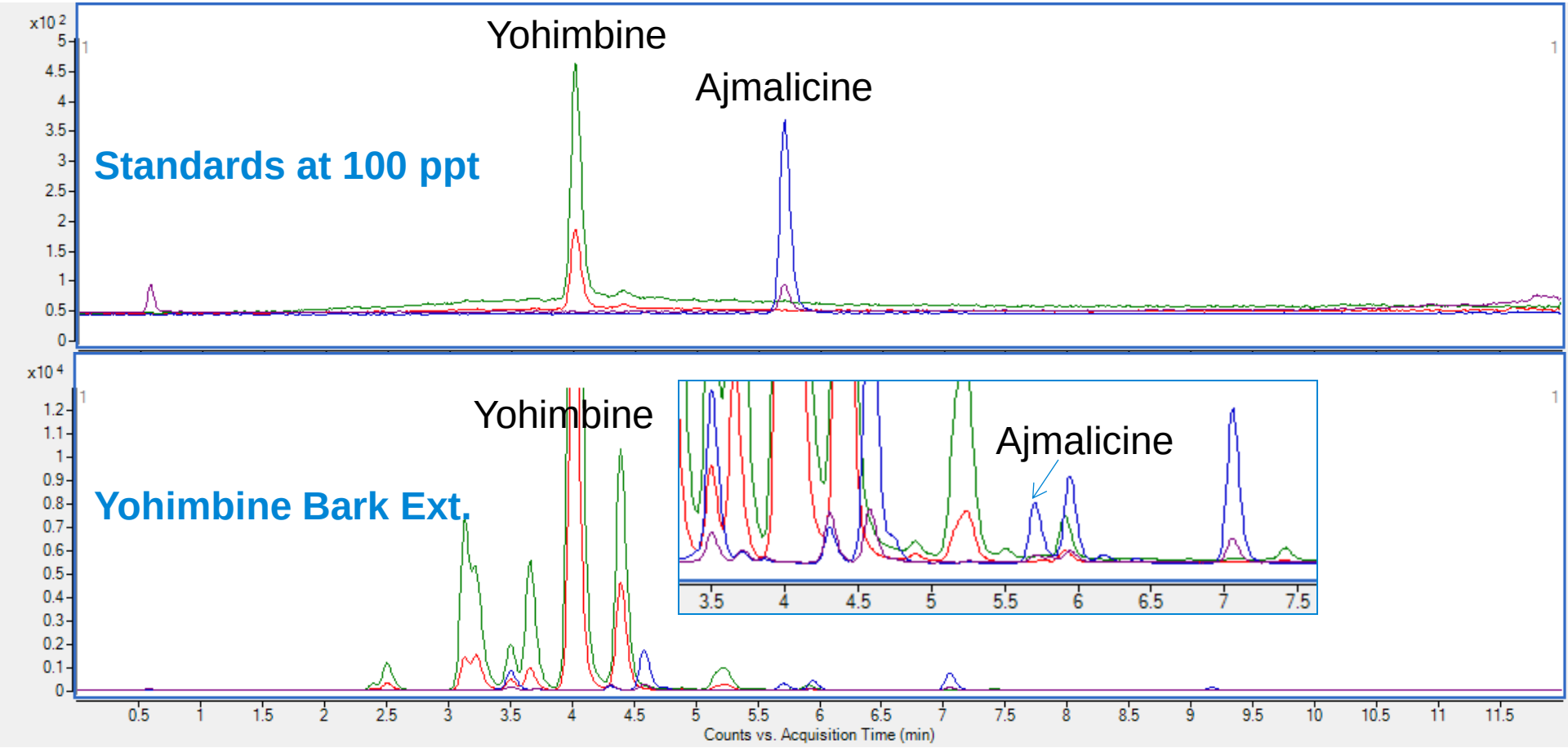
> 90 min from sample to vial
> 25 mL organic solvent/sample

QuEChERS Method

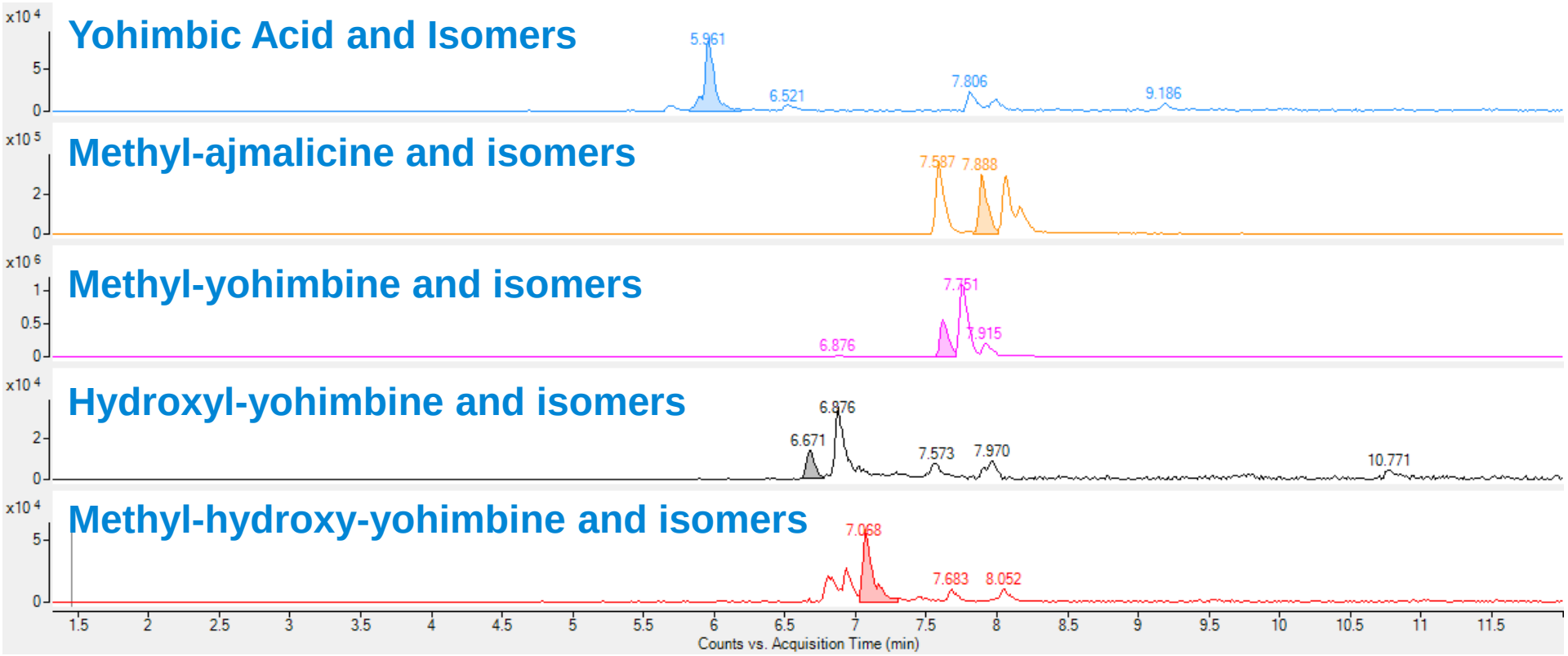
- Weigh 1.00 g sample (±0.01g) in 50 mL centrifuge tube
- Add QC spike as necessary and add 9 mL H_2O
- Add 10 mL acetonitrile and place in SPEX GenoGrinder (GG) for 5 min
- Add QuEChERS Extraction Salts (1 g NaCl, 4 g $MgSO_4$)
- Shake in GG for 1 min, then centrifuge at 5000 rpm for 5 min
- Transfer 1 mL upper layer for dSPE with C18/$MgSO_4$ Mix 1 min, then centrifuge at 7000 rpm for 3 min
- Transfer and dilute as necessary (10× – 500,000×)
- Filter into auto-sampler vial with Captiva 0.2 µm PTFE syringe filter
- Sample ready for 6490 LC-QQQ and 6550 LC-Q-TOF

~ 30 min from sample to vial
< 12 mL organic solvent/sample

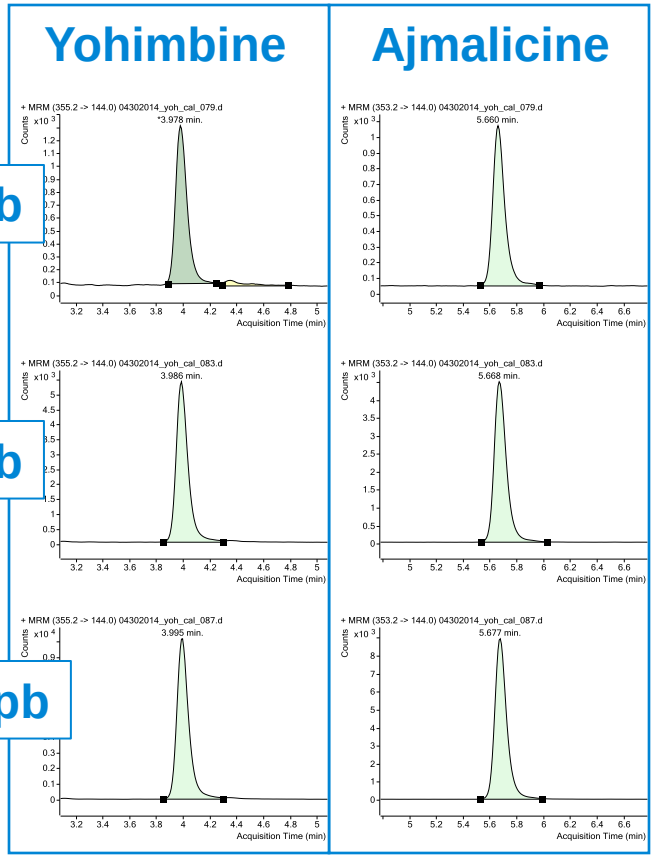
LC-MS/MS Chromatograms



Other Yohimbine Analogues by LC-Q-TOF



Recovery and Reproducibility



Dilute, filter, and shoot

	10 ng/mL Spike		50 ng/mL Spike		100 ng/mL Spike	
Analyte	%Rec.	%RSD	%Rec.	%RSD	%Rec	%RSD
Yohimbine	109.5	4.2	100.9	2.2	95.3	3.5
Ajmalicine	102.3	6.4	94.2	1.6	92.2	1.3

QuEChERS

	10 ng/mL Spike		50 ng/mL Spike		100 ng/mL Spike	
Analyte	%Rec.	%RSD	%Rec.	%RSD	%Rec	%RSD
Yohimbine	103.3	2.0	102.9	2.1	102.6	3.0
Ajmalicine	102.4	2.4	99.7	3.5	100.3	3.6

Incurred Sample Results

Sample	Yohimbine Conc. (µg/mL)	Yohimbine %RSD (n = 3)	Ajmalicine Conc. (µg/mL)	Ajmalicine %RSD (n = 3)	Dilution Factor
Product A	ND	ND	ND	ND	100×
Product B	0.0146	4.7	ND	ND	100×
Product C	1.1795	0.5	0.0321	1.3	100×
Product D	ND	ND	ND	ND	100×
Product E	0.3219	1.5	0.0017	6.5	100×
Product F	72.544	2.4	0.1070	2.8	1000×
Product G	ND	ND	ND	ND	1000×
Product H	9910.8	0.3	74.900	1.1	500000×
Product I	29474	0.4	580.25	0.4	500000×
Product J	17495	0.6	40.172	2.2	500000×
Wild Yohimbine Bark	10752	1.1	59.905	1.6	500000×
Yohimbine Bark Powder	13626	0.3	12.028	2.4	500000×

Lucas et al., Journal of AOAC International, Vol., 98, No. 2, 2015

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QuPPe

Quick Polar Pesticide Method

Overview

- There are certain types of compounds that are not amenable to QuEChERS method (Log K_{ow} < -2)
- Residues are extracted following the addition of acidified methanol
- The mixture is centrifuged, filtered and directly analyzed by LC-MS/MS
- Quantification is performed with isotopically labeled internal standards (analogues of the target analytes), added directly to the sample at the beginning of the procedure

http://www.eurl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf

QuPPe Method

- Weigh 10 g of comminuted sample into 50 mL centrifuge tube
  - If analyzing dry sample amount should be reduced to 5 g
  - Smaller samples may be required for extract-rich commodities or commodities with high water absorbing capacity
- Add 100 uL of isotopically labeled internal standard solution of analytes
- Add 10 mL of acidified methanol (MeOH + 1% FA)
- Close the tube and shake vigorously for 1-2 min
  - The resulting volume of the extract (accounting for natural water content of the sample and miscibility of water/MeOH ~ 20 mL, corresponding to 0.5 g sample/mL extract)
- Centrifuge 2500 g, 5 min
- Transfer filtered aliquot into autosampler vial, LC-MSMS
- d-SPE have been used with animal origin samples, based on matrix; C18, GCB, or polymeric sorbent could be useful

Updated Analyte List Mentioned in the 2015 Version of the QuPPe Method

Ethephon	Amitrole	Streptomycin
HEPA	ETU	Kasugamycin
Glufosinate	PTU	Morpholine
N-Acetyl-glufosinate	Cyromazine	Diethanolamine
MPPA	Trimesium	Triethanolamine
Glyphosate	Daminozide	1,2,4-Triazole
AMPA	Chlormequat	Triazole-alanine
Phosphonic acid	Mepiquat	Triazole-acetic acid
N-Acetyl-AMPA	Difenzoquat	Triazole-lactic acid
Fosetyl-Al	Propamocarb	
Maleic hydrazide	Melamine	
Perchlorate	Diquat	
Chlorate	Paraquat	
Bialaphos	N,N-Dimethylhydrazine	
Cyanuric acid	Nereistoxin	

Updates from Version 7 to 8 in yellow

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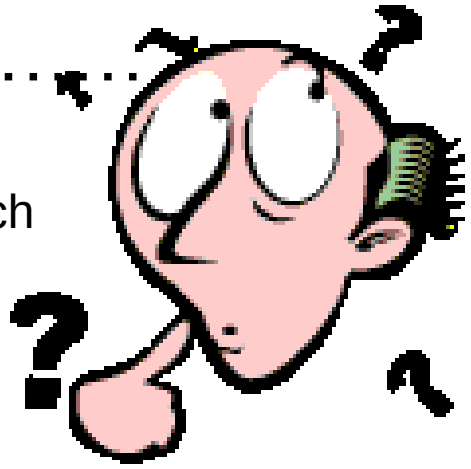
What If?

We miniaturize the analytical approach……



Sample Preparation: Easier to handle, more samples/batch

Less storage space required

Reduction in process time (?)

Reduced salt/sorbent cost

Reduced cost of labeled compounds

Additional labeled compounds for troublesome analytes

Reduced solvent cost and waste

Maintain same sample/salts/solvent ratio

Use existing d-SPE kits

Reduce sample preparation cost!

What If?

We reduce injection volume with 7010 Triple Quadrupole GC/MS

7010 Triple Quadrupole GC/MS: Analysis: High-Efficiency Source

New hardware design

New achievable level of response

Dilute sample prior to injection

Inject less sample only 25% of sample volume

Less matrix injected ≈ prolonged uptime

Sustained performance

Resulting in lower maintenance costs!

Analytical Approach: Miniaturization of QuEChERS

Sample Preparation: **Extraction/Partitioning (AOAC)**

2 g of sample (3 different matrices), 2 ceramic homogenizers

2 mL ACN (1% HAc), vortex

1 g of Agilent Bond Elut AOAC salts

shake, centrifuge

Dispersive SPE

Transfer 1 mL of extract to 2 mL Agilent Bond Elut dSPE (G/FV or P/FV [EN])

vortex, centrifuge

Transfer 250 uL for analysis

Reducing Matrix Effects:

Simplified sample preparation and system maintenance

From smaller injection volumes to scaling-down sample preparation, the benefits of **more ions** are many.

Redefining low-volume injections

Before: 2 µL

NOW: 0.5 µL

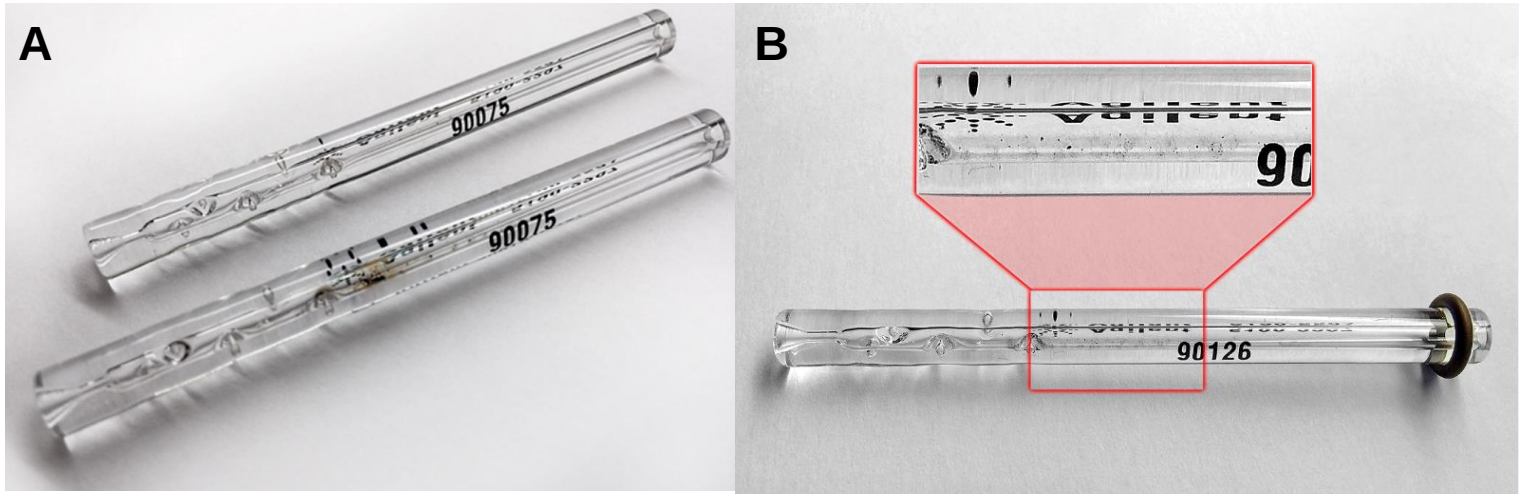
The benefits include:

- **Less frequent liner replacement** for longer consistent response
- **Longer column life,** which reduces maintenance time and costs
- **Faster sample preparation** and less need for sample preconcentration

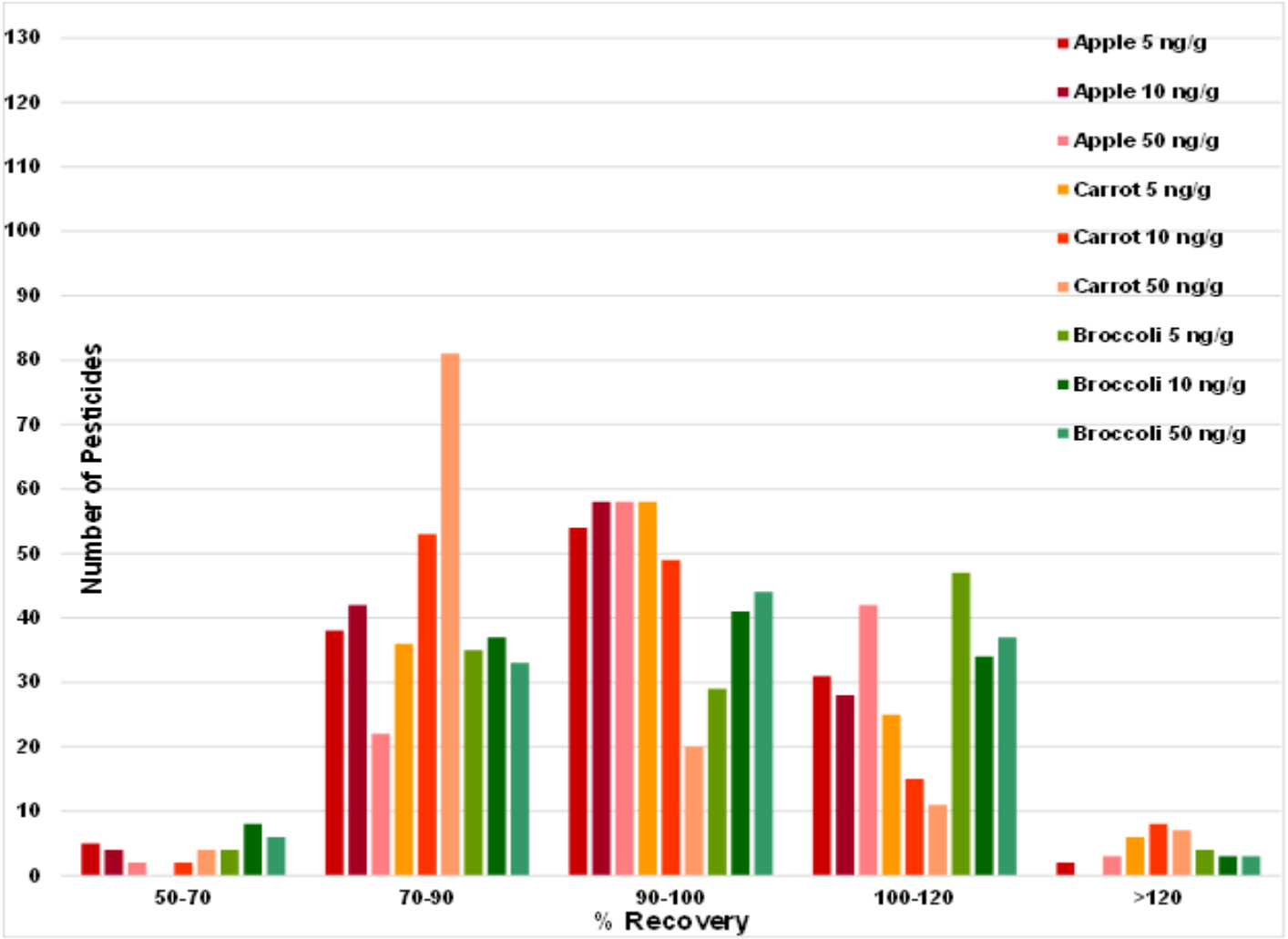
We reduce injection volume with 7010 Triple Quadrupole GC/MS: High Efficiency Source (HES)

Analytical Approach: 7010 Triple Quadrupole GC/MS

- Columns: Agilent J&W Ultra Inert HP-5ms columns: 5 m x 0.25 mm and 15 m x 0.25 mm x 0.25 µm
- Agilent Ultra Inert dimpled liners, UltiMetal plus Flexible Metal ferrules at the PUU, **column backflushing**
- 2.0 µL versus 0.5 µL injection



GC liners: GC liners [same part number] (A-lower liner) residue after 65+ injections of 2 µL; (B) almost free of visible deposits after 200+ injections of 0.5 µL matrix extract.

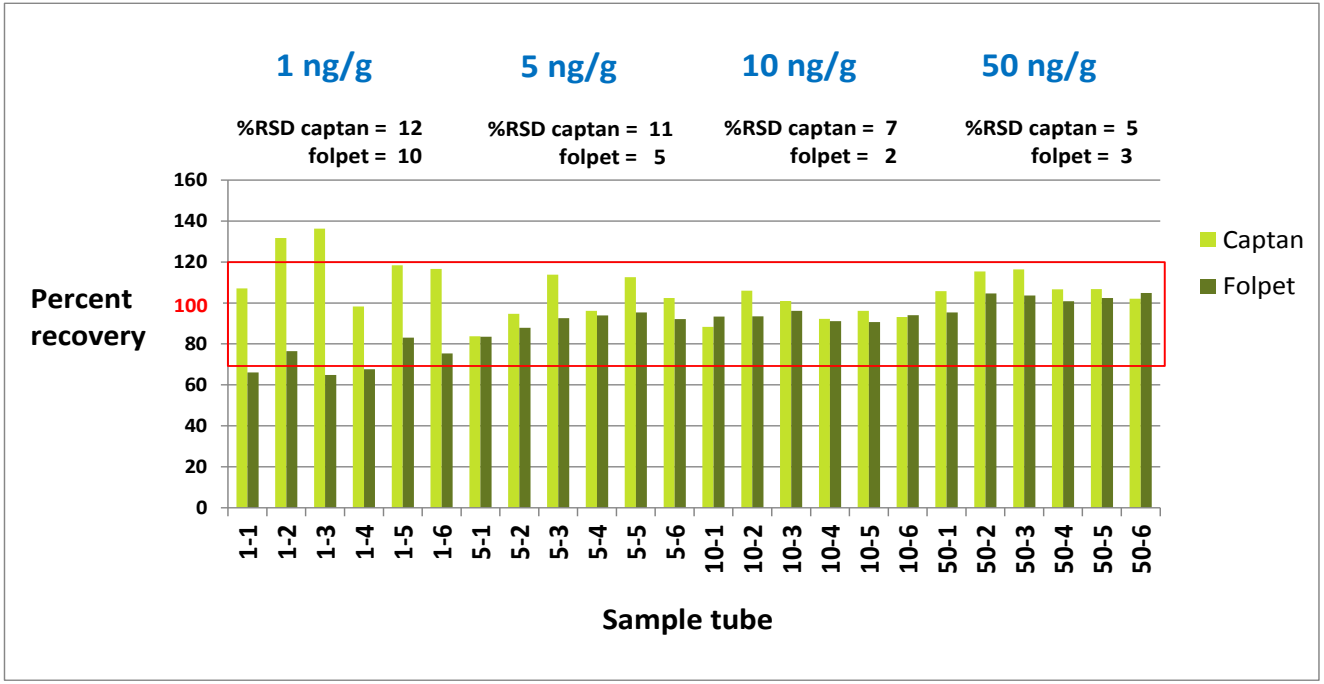


Distribution of recovery for 126 pesticides in apple, carrot and broccoli pre-spiked matrix, using 0.5 µL injection of 5, 10 50 ng/g extracts.

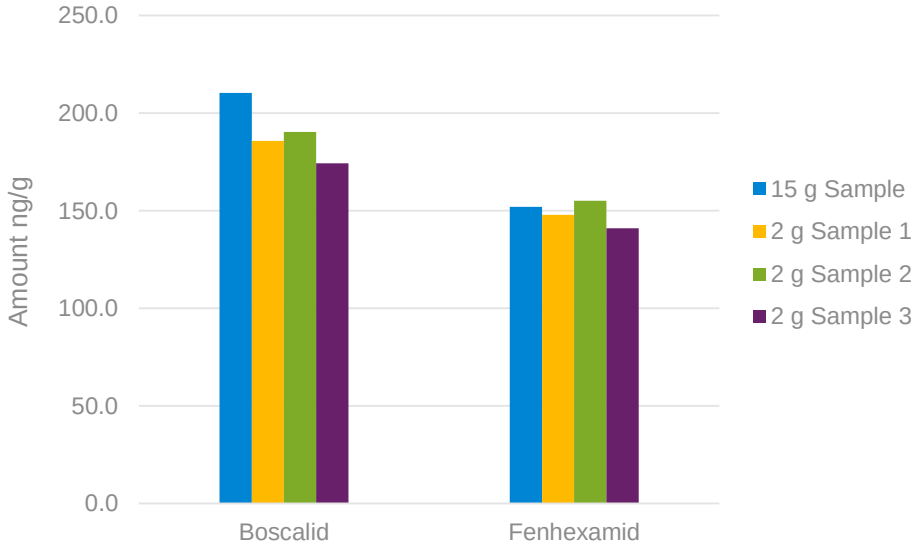
Cost Breakdown and Cost Savings for Sample Preparation with QuEChERS and Mini-QuEChERS Technique

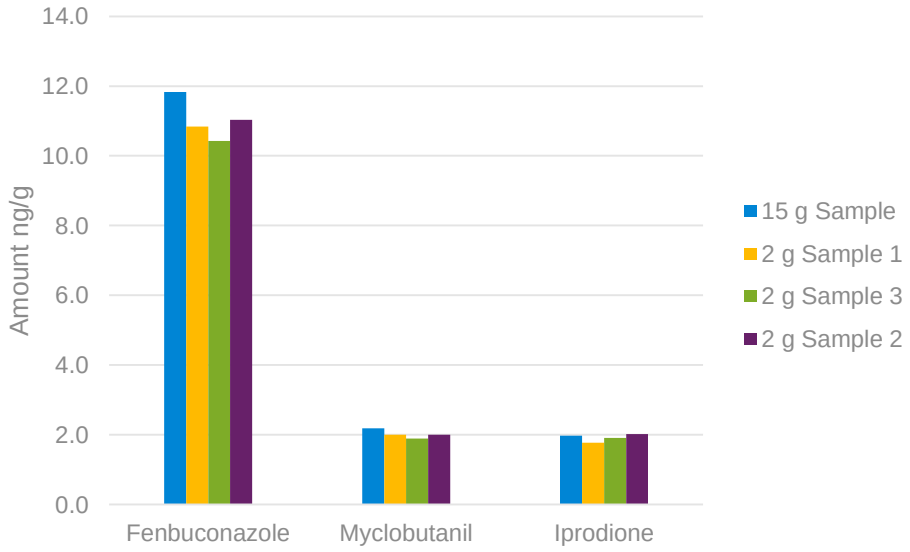
Sample Preparation Cost/Sample	Centrifuge Tube	ACN	Salts	Internal Standards: Captan-d6, Folpet-d4	dSPE General F&V or Universal	Total Cost/Sample	Cost Savings
QuEChERS	$0.43	$1.50	$2.96	$0.30	$1.32/$1.96	$6.51/$7.15	-
Mini-QuEChERS	$0.42	$0.20	$0.80	$0.04	$1.32/$1.96	$2.78/$3.42	**43%/48%**

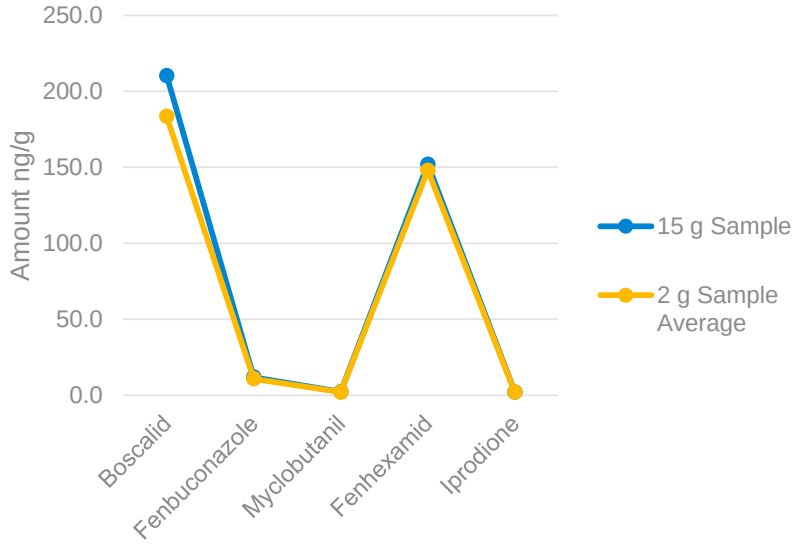
Individual Recoveries of Captan and Folpet Spiked in Celery, 2g sample



Peach Incurred: 15 g and 2 g in triplicate (ng/g)







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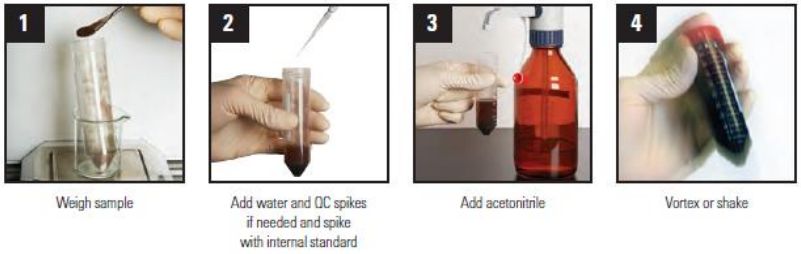
Improving dSPE in QuEChERS

1. Extraction



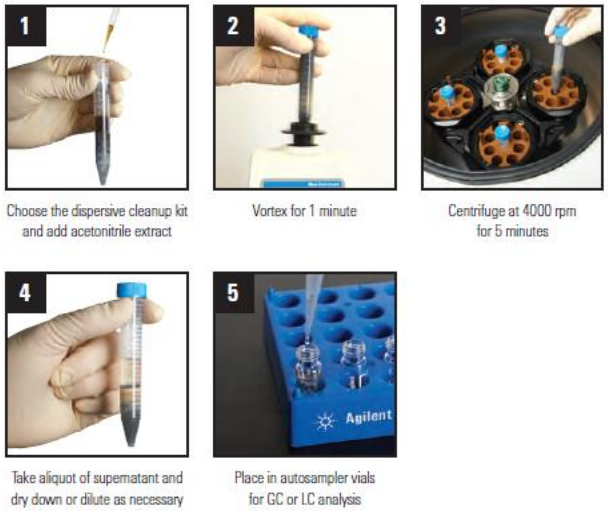
Pros

- 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



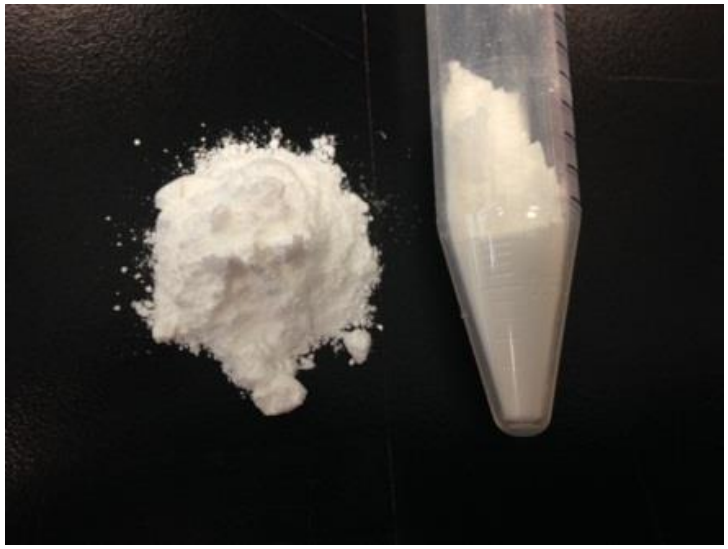
Pros

- Same as extraction

Cons

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

EMR 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*).

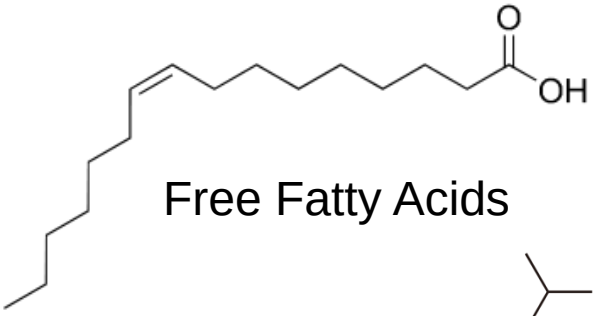
EMR-Lipid Mechanism – Size exclusion and hydrophobic interaction.

… and what does it do?

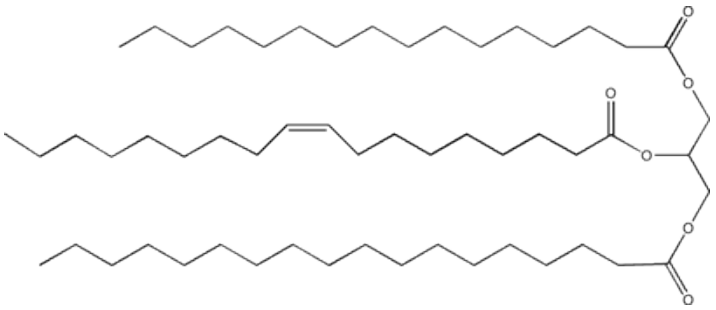
EMR sorbent removes Lipids

What are Lipids?

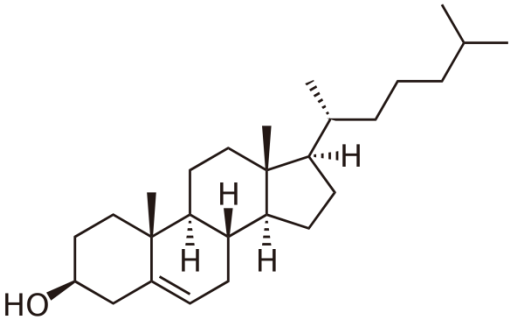
A class of naturally occurring hydrocarbon containing compounds commonly known as fats and oils



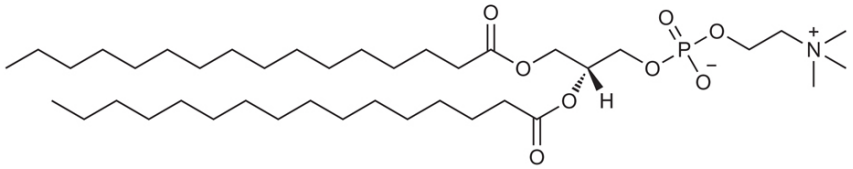
Free Fatty Acids



Triglycerides



Cholesterol



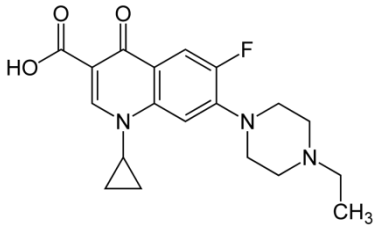
Phospholipids

What Does EMR *NOT* Interact With?

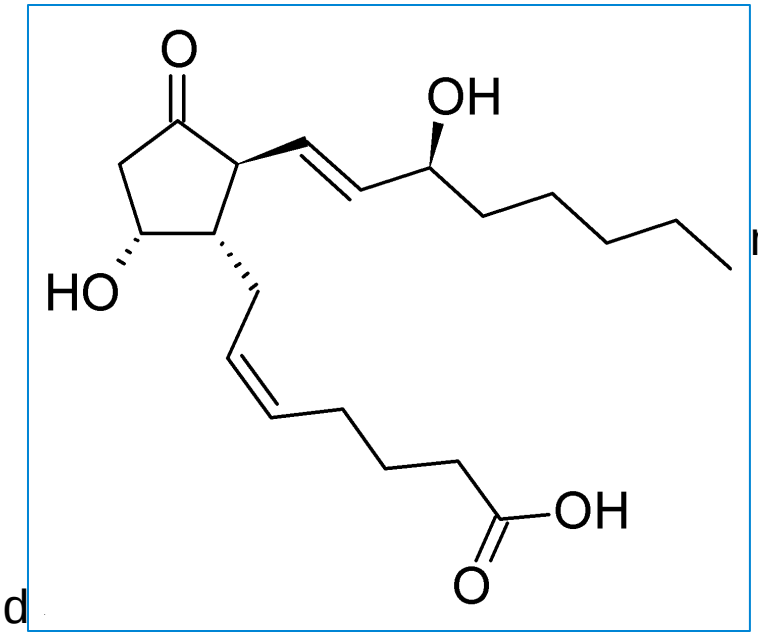
EMR does NOT remove analytes of interest

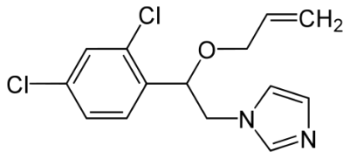
Exceptions?

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

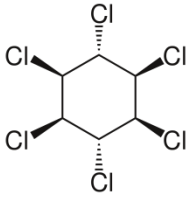


Fluoroquinolones

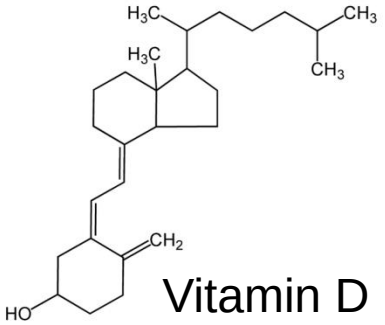




midazole pesticides

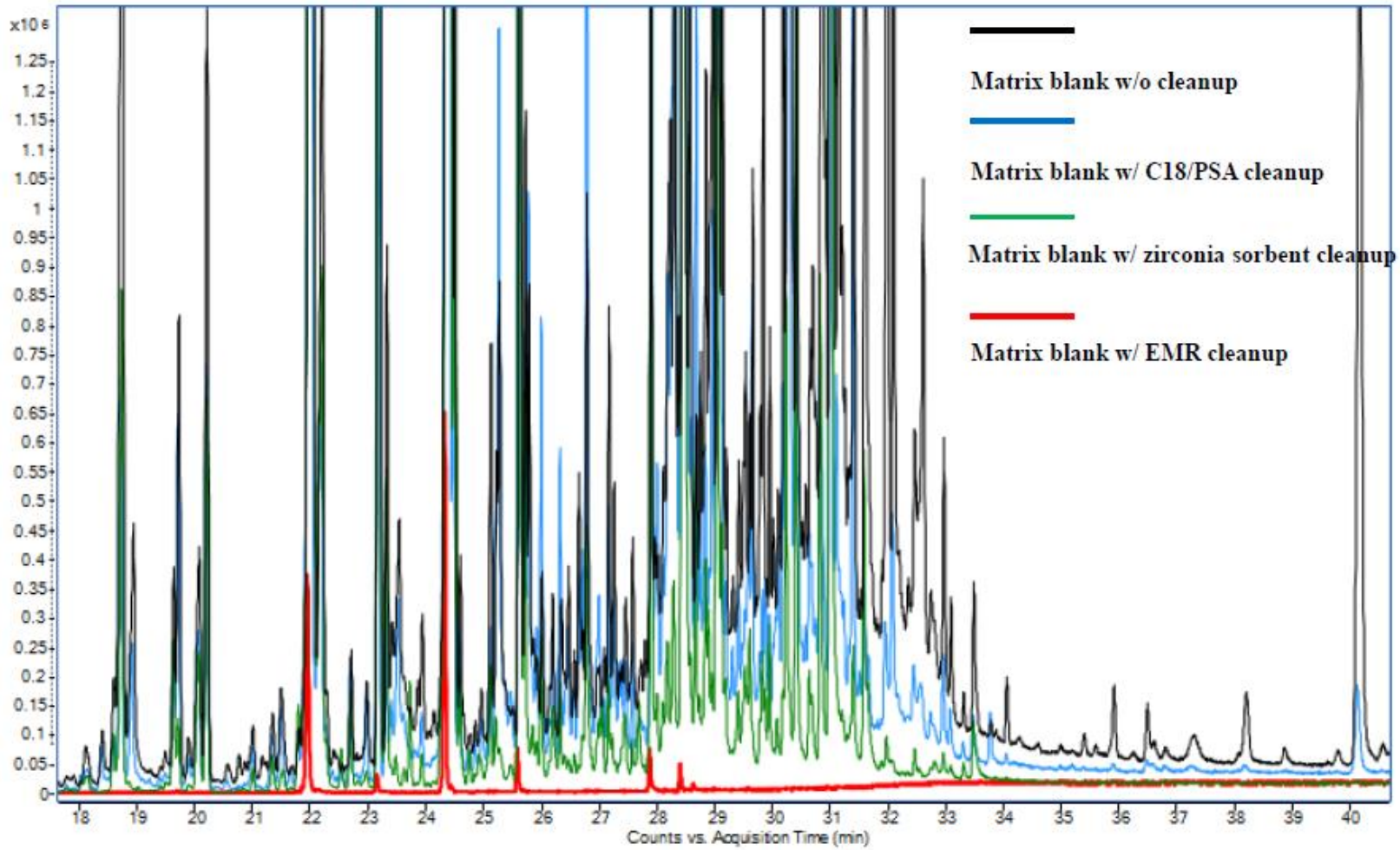


Organochlorine Pesticid



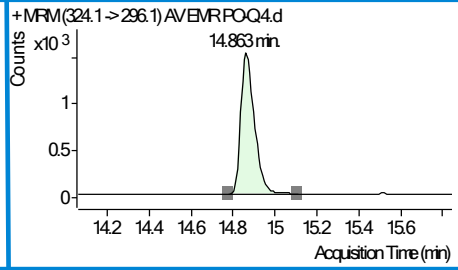
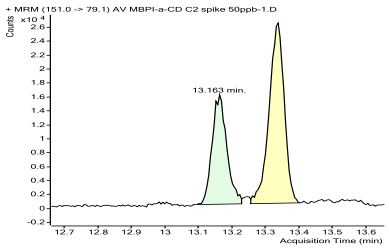
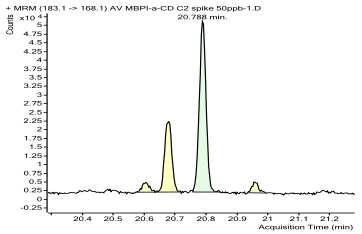
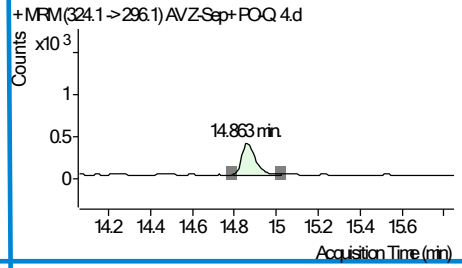
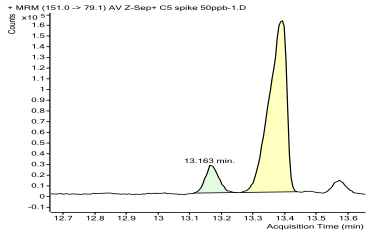
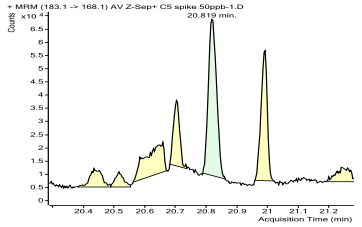
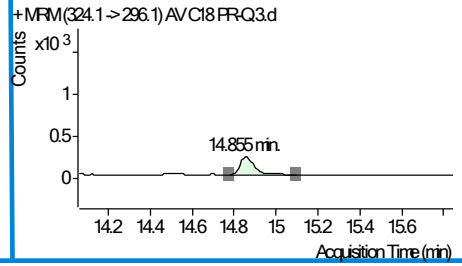
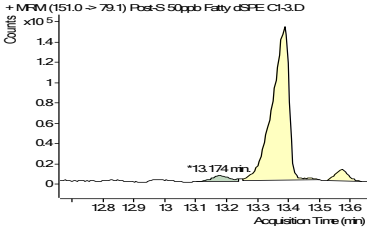
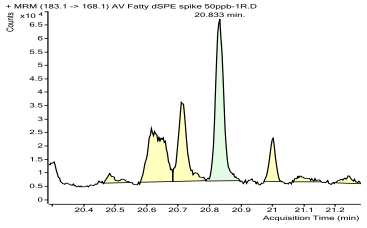
Vitamin D

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

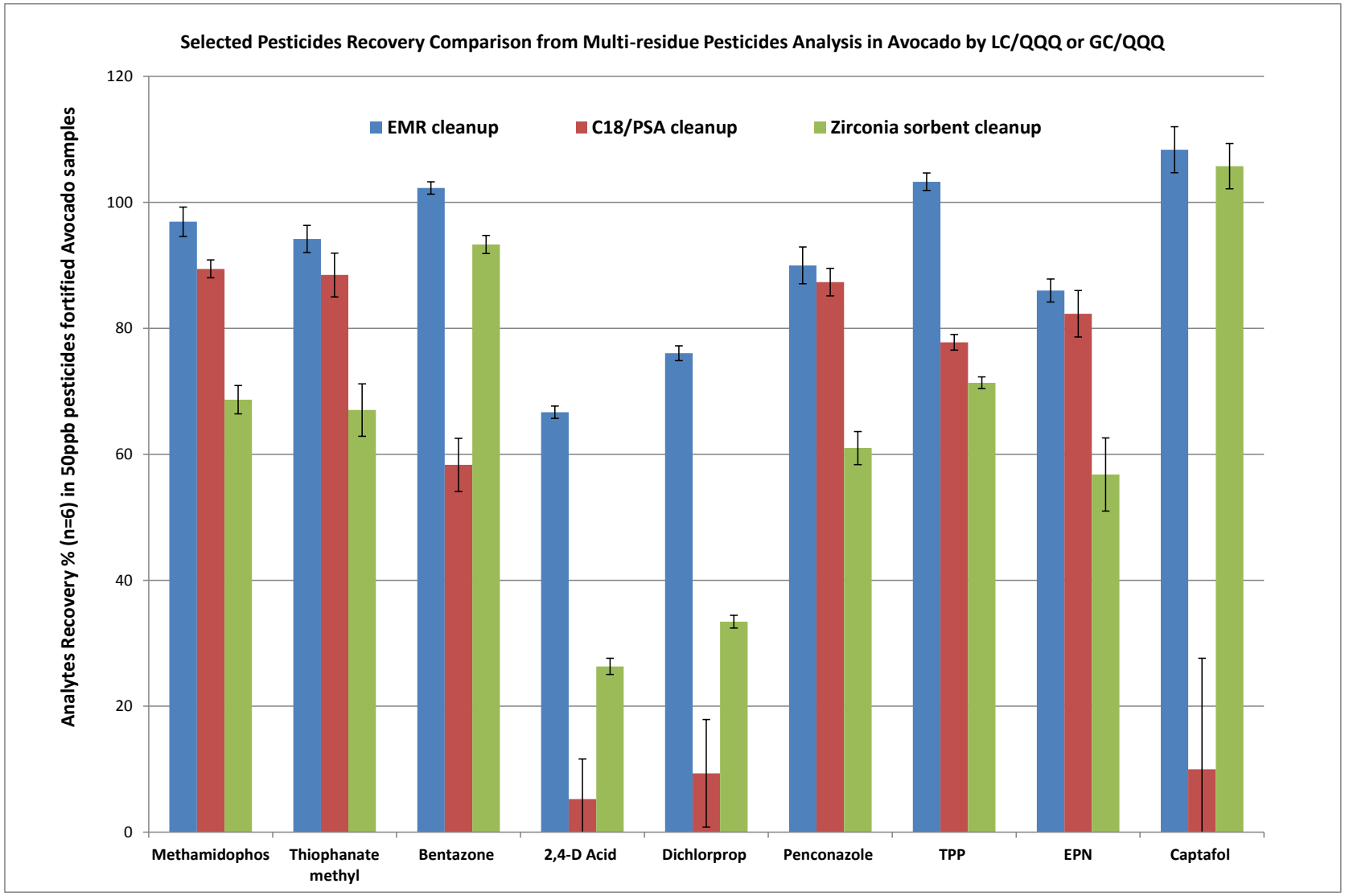


The use of EMR material cleanup provides significantly cleanup chromatographic sample background.

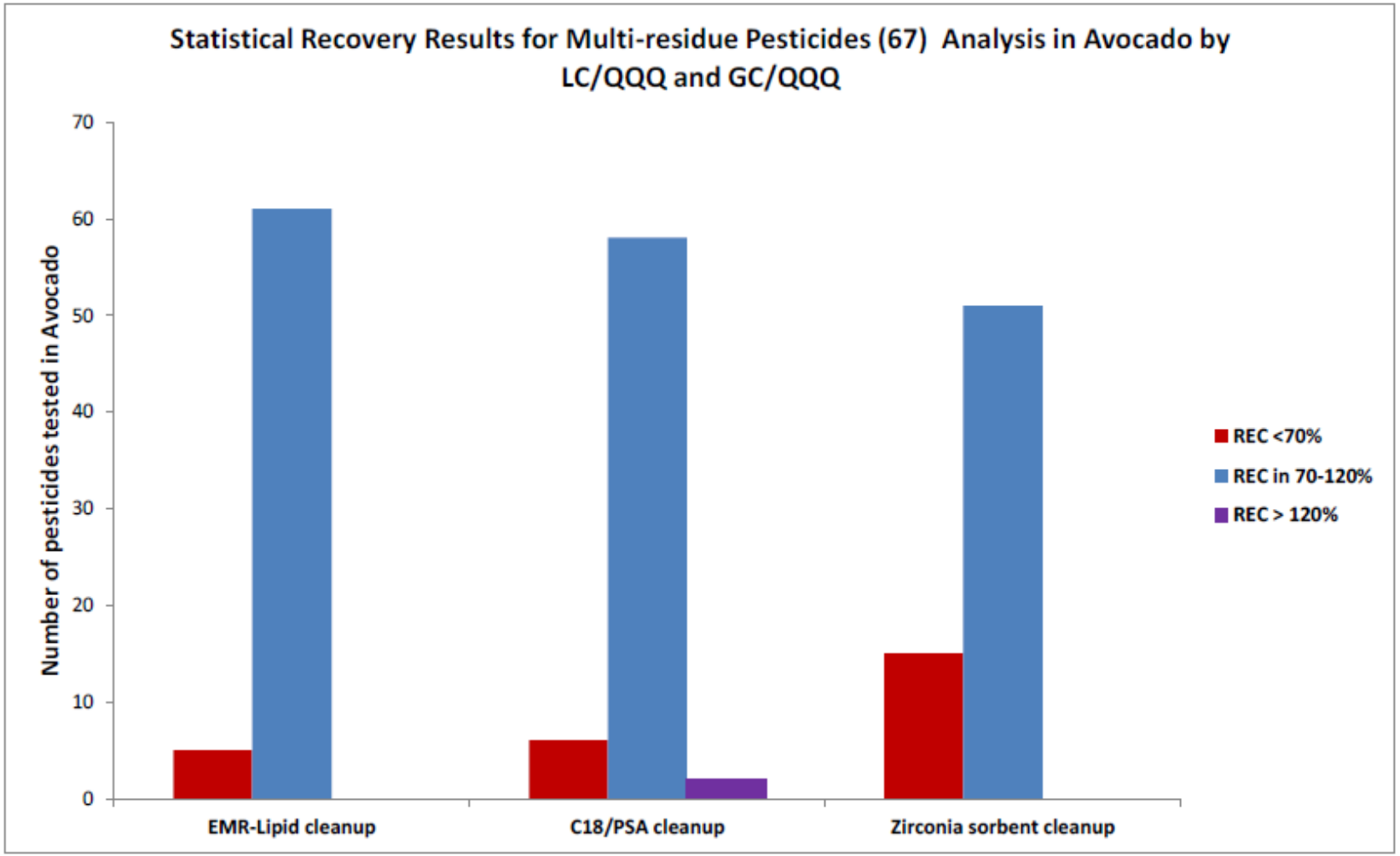
Chromatographic Benefits of Matrix Removal Provided by EMR Cleanup

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			
Zirconia sorbent cleanup			
C18/PSA cleanup			

Selected Problematic Pesticides for Recovery Comparison



Statistical Recovery Results Comparison



Today's Agenda

- QuEChERS: Brief Overview
- Yohimbine in Botanicals and Dietary Supplements
- QuPPe: Quick Easy Polar Pesticide Method
- Miniaturization of QuEChERS Method
- Enhanced Matrix Removal: EMR-L
- **Summary: Take Home Message**

QuEChERS Summary and Take Home Message

- QuEChERS is a flexible, simple, yet powerful tool for preparing a wide variety of food products for analysis by GC and LC-MS/MS
- QuPPE offers an alternative extraction approach for highly polar compounds, where QuEChERS is not amenable
- Reducing sample size is showing great potential and significant reduction in cost/sample, enhanced performance
- QuEChERS reproducibility and ruggedness can be improved with products designed for optimal performance from various matrices
- QuEChERS and EMR-L offers SPE cleanliness with dSPE simplicity and allows a QuEChERS extraction to be employed with high lipid matrices
- Agilent offers the QuEChERS expertise along with the range of products needed for successful QuEChERS sample preparation

Agilent QuEChERS Resources

Easy Product Selection Tool

http://quechersselectiontool.chem.agilent.com/

QuEChERS Poster: *Easy as 1-2-3*

Lit number: 5990-5324EN

QuEChERS Food Application "Notebooks":

Lit number: 5990-4977EN
http://www.chem.agilent.com/Library/brochures/5990-4977EN.pdf

Technical support: Over 50 application notes on QuEChERS:

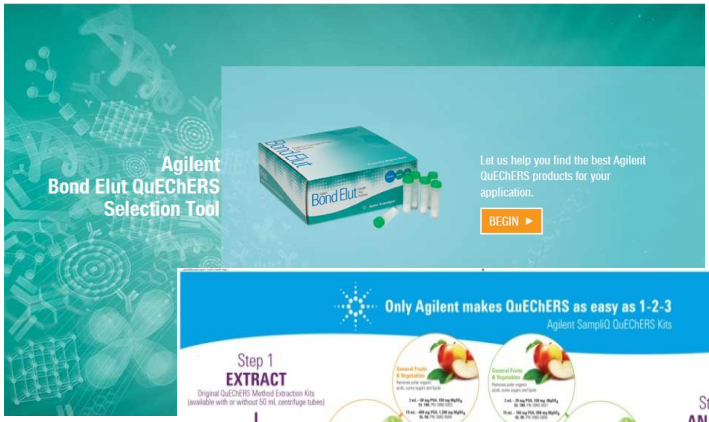
QuEChERS videos: 2 available, Part 1 & 2

www.youtube.com

Sample Preparation Primer

http://www.chem.agilent.com/en-US/promotions/Pages/sampleprepbook.aspx

All available on our website:
www.agilent.com/chem/quechers



Thank You!
Happy Sample Prepping!!!