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

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Today's Agenda

- QuEChERS: Brief Overview
- Analyte Protectants and GC Techniques
- Method Modifications with Acidic Matrices
- Pesticide Analysis from Chicken
- Pesticides in Herbal Tea
- Preservatives in Pet Food
- Summary: Take Home Message

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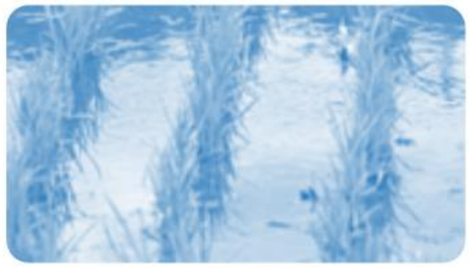
What is QuEChERS (pronounced "*Catchers*")

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**

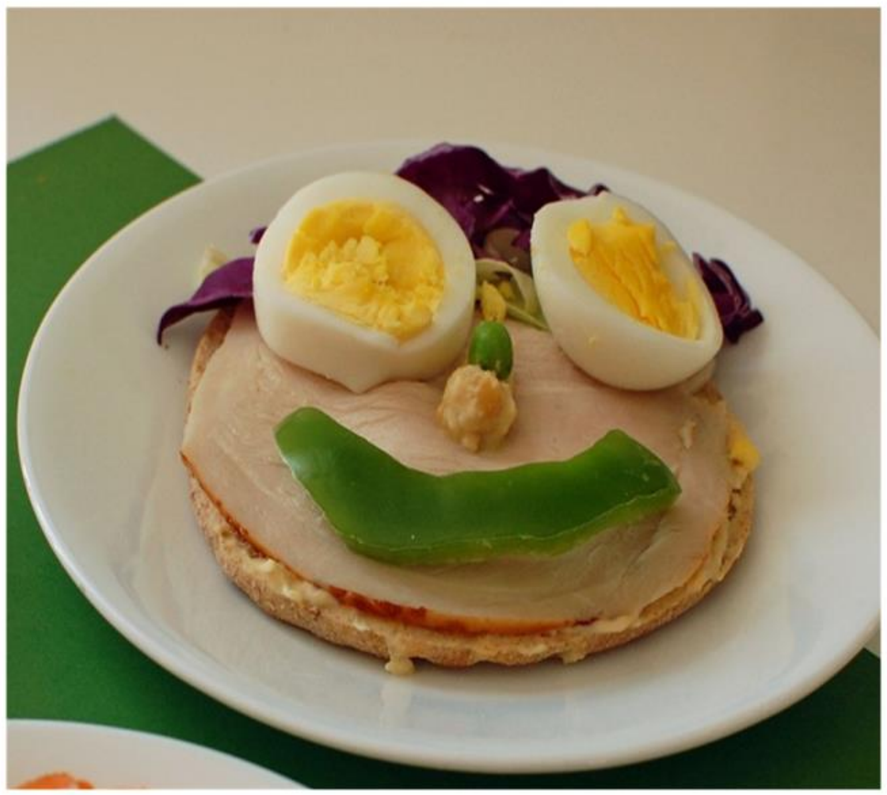
QuEChERS Approach Advantages
30ish minutes to extract multiple samples at once
Minimal solvent usage per sample: 10-15 mL
Chlorinated Solvents: None

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

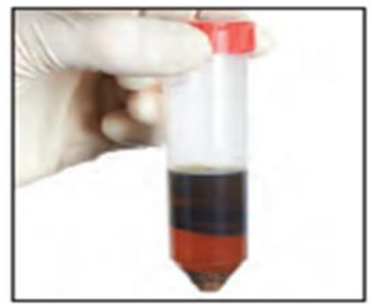
Add acetonitrile

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

Today, we'll look at these advanced concepts for QuEChERS:

- Improving the analytical technique to enhance results
- Overview of QuEChERS applied to alternate food types and target analytes
- A method development approach to applying QuEChERS to different food types

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What are Analyte Protectants (AP)

- Compound mix added to sample extracts and calibration standards to deactivate liner and column during every injection (GC)
- Strongly interact with active sites (free silanol groups and active sites created by nonvolatile matrix deposits) in the inlet and column
- Protect susceptible analytes against adsorption or degradation on active sites
- Suitable combination of AP should be selected for given set of analytes to cover their volatility range, decrease matrix effects, improve method ruggedness
- Mixture of compounds, e.g. L-gulonolactone/D-sorbitol
- Analytes are inexpensive, nontoxic, and safe for routine GC/MS systems

Analyte protectants are an inexpensive but effective way to improve and stabilize your GC-MS and GC-MSMS analyses

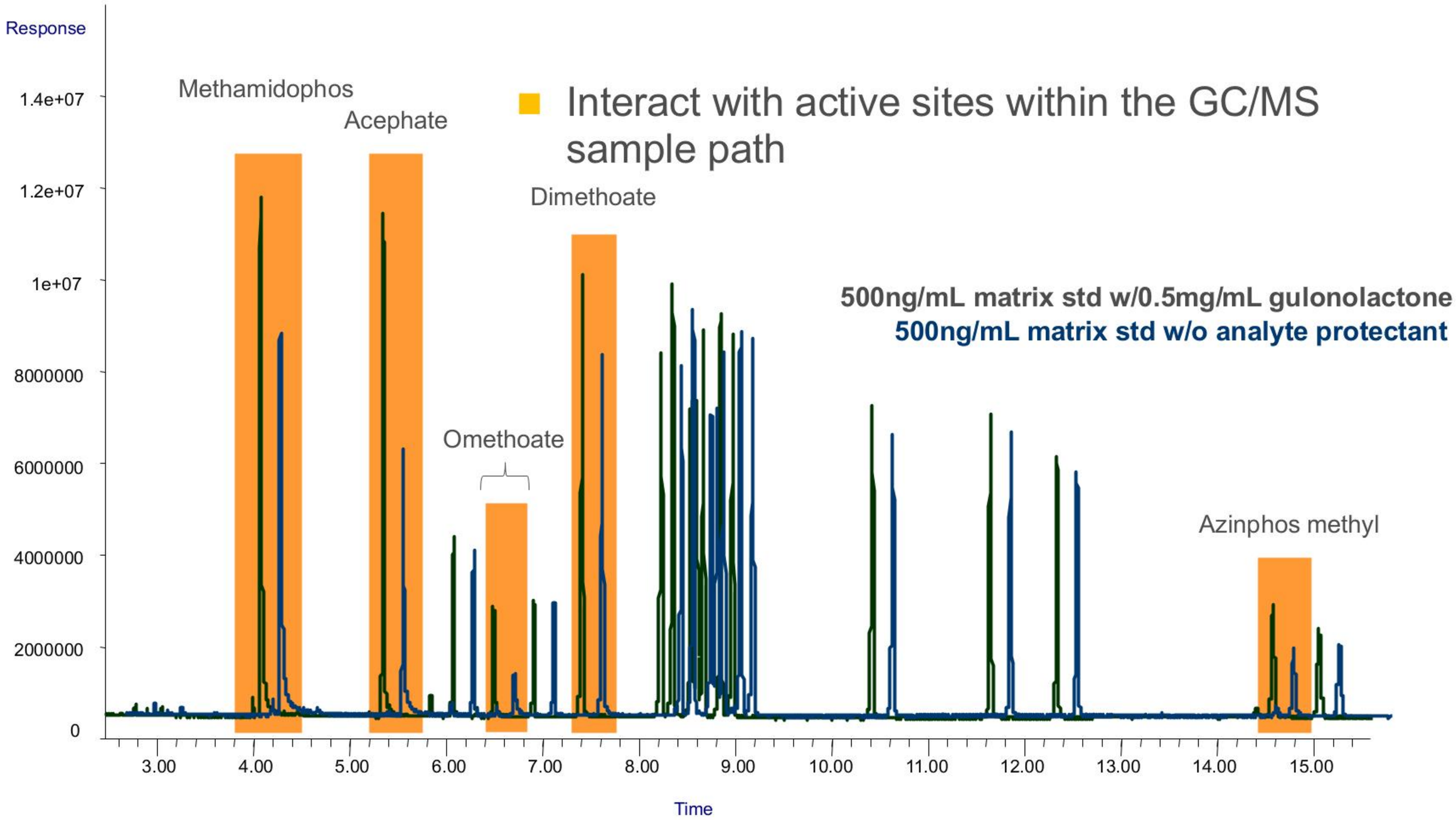
Compound	CAS No.	pesticide elution region (min)
3-ethoxy-1,2-propanediol (ethylglycerol)	1874−62−0	<10
1,2,3,4-butanetetrol (*meso*-erythritol)	149−32−6	<10
4,6-*O*-ethylidene-α-D-glucopyranose	13224−99−2	10−13
caffeine	58−08−2	12−13
L-gulonic acid γ-lactone (gulonolactone)	1128−23−0	8−15
poly(ethylene glycol) (PEG) 300	25322−68−3	8−20[a]
triglycerol	20411−31−8	15−20
D-sorbitol (D-glucitol)	50−70−4	14−20

[a] Scattered coverage.

Compounds evaluated as analyte protectants and approximate pesticide elution regions for which they are the most effective

K. Mastovska, S. Lehotay, M. Anastassiades, Anal. Chem., 2005, 77, 8129-8137.

FPD Chromatogram Showing the use of Analyte Protectants



- Interact with active sites within the GC/MS sample path

500ng/mL matrix std w/0.5mg/mL gulonolactone
500ng/mL matrix std w/o analyte protectant

Ultra Inert Liner/DB-35ms UI

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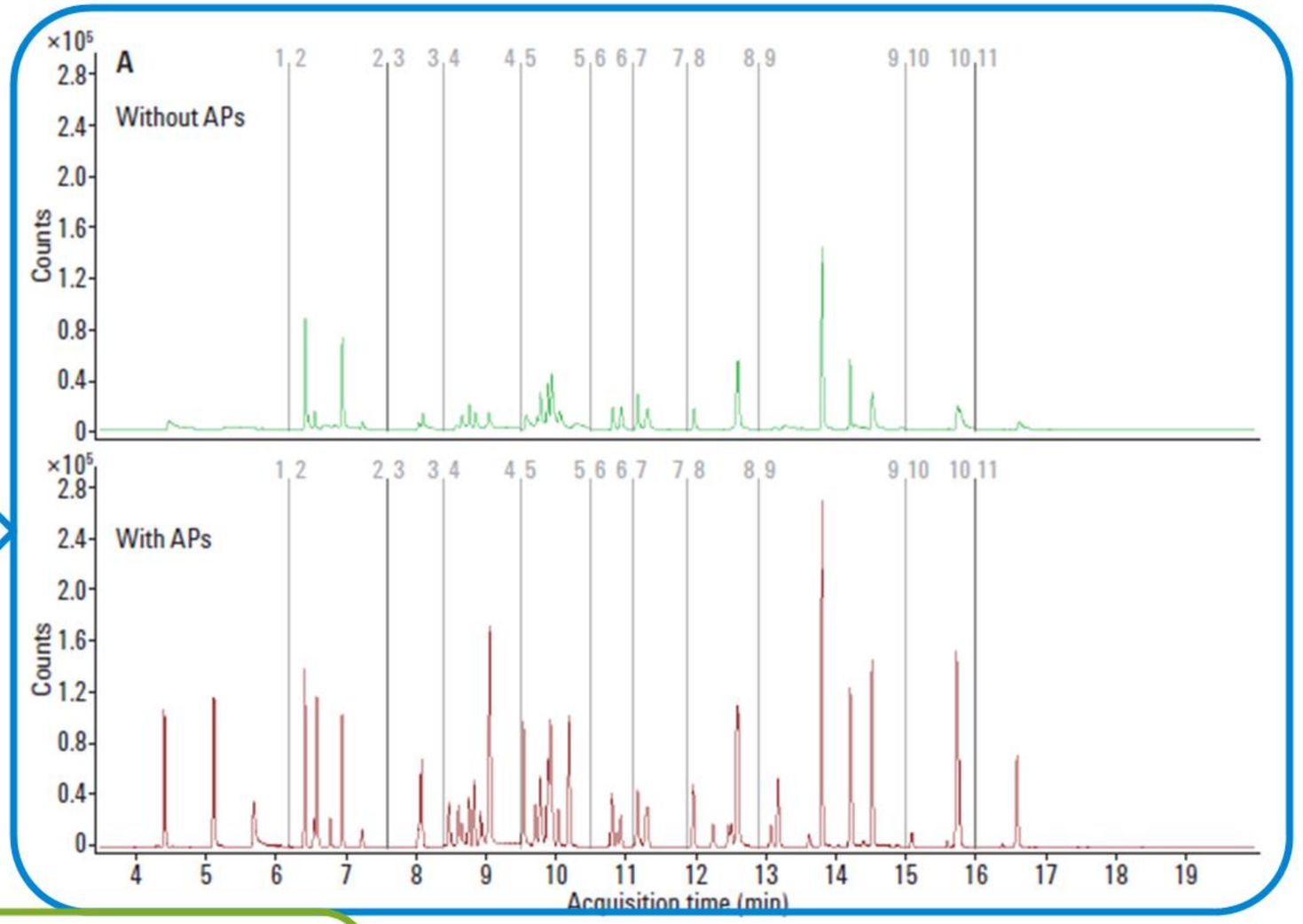
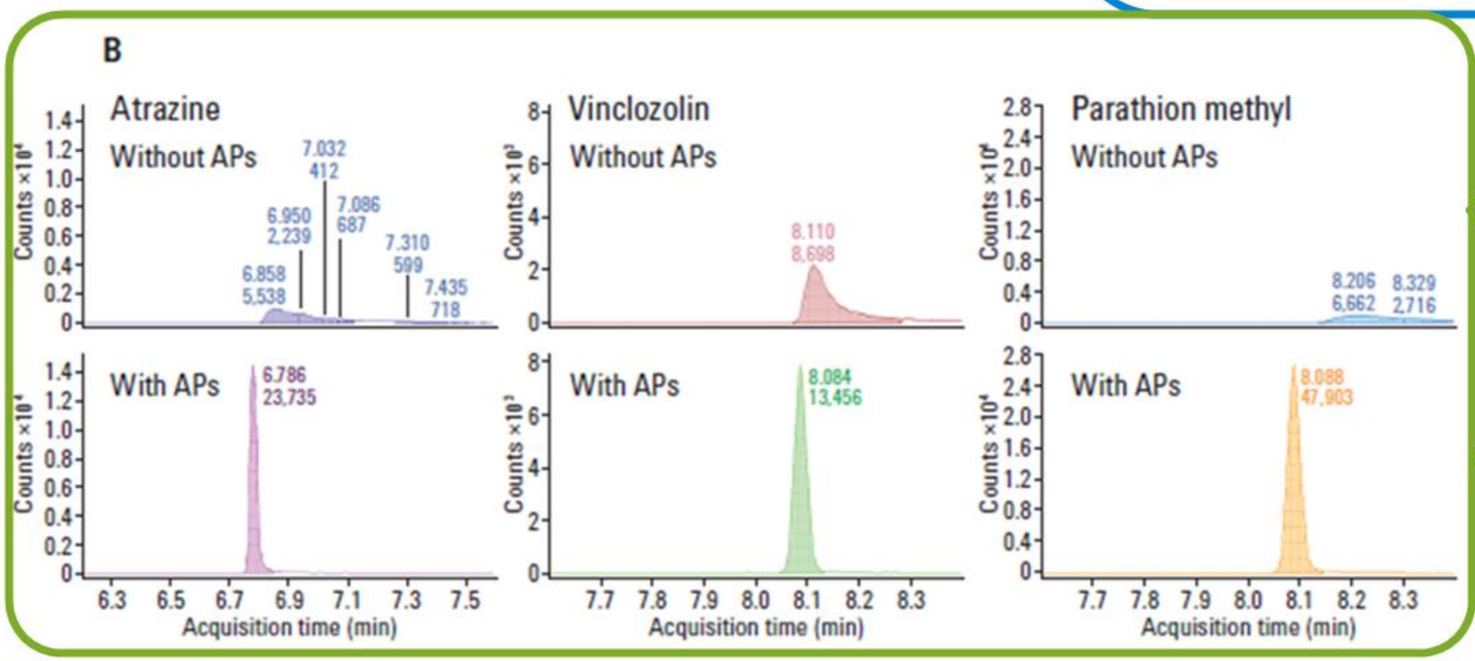
Effects of Analyte protectants in Pesticide Analysis by GC/MS/MS

A. TIC comparison between samples with and without analyte protectants





B. EIC of selected MRMs without analyte protectants – 100 ppb mix

B. EIC of selected MRMs with analyte protectants – 100 ppb mix

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Quantitation results comparison for samples spiked in strawberry and spinach using Agilent IFP with and without AP

	Strawberry						Spinach					
	Agilent IFP			Agilent IFP with AP			Agilent IFP			Agilent IFP with AP		
Pesticide	R^2	Average recovery (%)	%RSD n = 18	R^2	Average recovery (%)	%RSD n = 18	R^2	Average recovery (%)	%RSD n = 18	R^2	Average recovery (%)	%RSD n = 18
Dichlorvos	0.9993	95.6	5.4	0.9996	96.3	2.8	0.9963	90.6	3.4	0.9971	91.2	2.4
Methamidophos	0.9951	88.8	9.6	0.9992	94.2	3.9	0.9978	88.6	11.5	0.9969	86.1	9.4
Acephate	0.9980	84.3	23.5	0.9997	94.9	8.8	0.9920	83.0	23.2	0.9976	84.7	22.6
Mevinphos	0.9997	92.6	5.7	0.9988	95.7	2.5	0.9985	92.1	3.3	0.9996	93.0	2.5
Omethoate	0.9960	79.7	35.3	0.9974	96.1	13.0	0.9952	80.6	26.2	0.9925	79.9	28.1
o-Phenylphenol	0.9986	94.0	3.6	0.9913	93.1	1.7	0.9994	91.6	1.4	0.9993	91.5	3.1
Atrazine	0.9996	93.7	5.5	0.9990	94.1	2.7	0.9993	91.5	1.9	0.9993	94.6	1.7
Dimethoate	0.9994	89.8	9.9	0.9991	96.2	2.1	0.9997	87.7	7.3	0.9985	87.6	10.1
Lindane	0.9999	93.0	5.7	0.9990	93.9	1.9	0.9996	88.1	9.7	0.9986	86.3	8.8
Chlorothalonil	0.9991	93.5	8.9	0.9989	95.5	4.2	0.9990	88.5	13.6	0.9984	85.2	20.1
Carbaryl	0.9988	92.2	13.4	0.9964	96.4	6.2	0.9947	91.4	16.2	0.9944	91.4	24.3
Chlorpyrifos methyl	0.9995	93.2	3.7	0.9944	94.9	2.0	0.9992	92.3	1.9	0.9992	92.6	2.5
Tolclofos methyl	0.9996	92.9	4.2	0.9993	95.4	1.5	0.9992	92.3	1.4	0.9994	93.9	0.7
Vinclozolin	0.9999	93.7	3.6	0.9987	95.7	2.0	0.9991	91.6	1.7	0.9992	95.7	2.3
Aldrin	0.9985	91.7	4.4	0.9988	93.5	2.6	0.9982	91.8	2.6	0.9974	96.0	2.3
Dichlofluanid	0.9991	91.4	5.4	0.9987	92.3	2.6	0.9986	84.0	15.2	0.9985	80.9	16.5
Dichlorobenzophenone	0.9995	93.6	4.7	0.9976	95.5	2.0	0.9987	91.7	1.4	0.9997	93.7	1.2
Malathion	0.9981	94.5	2.7	0.9988	95.6	1.3	0.9959	90.7	2.4	0.9995	92.0	3.4
Pirimiphos ethyl	0.9958	90.7	4.0	0.9985	94.7	1.6	0.9970	92.0	1.5	0.9973	93.4	3.5
Procymidone	0.9988	91.4	2.9	0.9993	94.3	1.5	0.9988	92.0	1.1	0.9996	93.6	3.5
Tolyfluanid	0.9992	91.5	5.2	0.9991	93.9	2.9	0.9990	82.8	14.3	0.9994	80.9	1.7
Endrin	0.9969	90.0	4.0	0.9961	93.3	4.1	0.9991	85.1	8.0	0.9965	87.8	8.4
Ethion	0.9959	96.3	5.4	0.9967	95.1	1.6	0.9963	92.7	1.3	0.9991	92.4	1.0
DDT	0.9991	87.7	10.2	0.9954	92.3	13.9	0.9993	72.4	34.1	0.9983	72.5	33.1
Endosulfan sulfate*	N/A	N/A	N/A	N/A	N/A	N/A	0.9986	87.6	9.5	0.9989	87.7	10.2
Endrin ketone	0.9971	92.4	2.1	0.9886	92.0	3.1	0.9938	77.3	21.6	0.9883	73.5	23.7
Iprodione	0.9993	93.4	2.6	0.9975	94.3	2.8	0.9986	90.1	15.0	0.9981	89.4	17.0
Phosmet	0.9957	90.2	5.1	0.9956	92.5	3.8	0.9984	79.2	18.0	0.9977	80.2	23.2
Phosalone	0.9972	94.2	1.8	0.9969	93.7	1.9	0.9992	86.5	5.9	0.9972	87.3	9.7
Coumaphos	0.9905	90.5	3.4	0.9980	88.4	3.7	0.9984	83.4	6.5	0.9993	85.3	10.6
Permethrin	0.9995	90.2	3.1	0.9980	90.5	1.9	0.9981	89.5	1.5	0.9948	94.3	1.9
Deltamethrin	0.9974	94.3	4.7	0.9986	97.9	4.2	0.9957	88.9	7.3	0.9960	89.6	15.0

* Endosulfan sulfate was detected in strawberry matrix blank, thus was not quantifiable.

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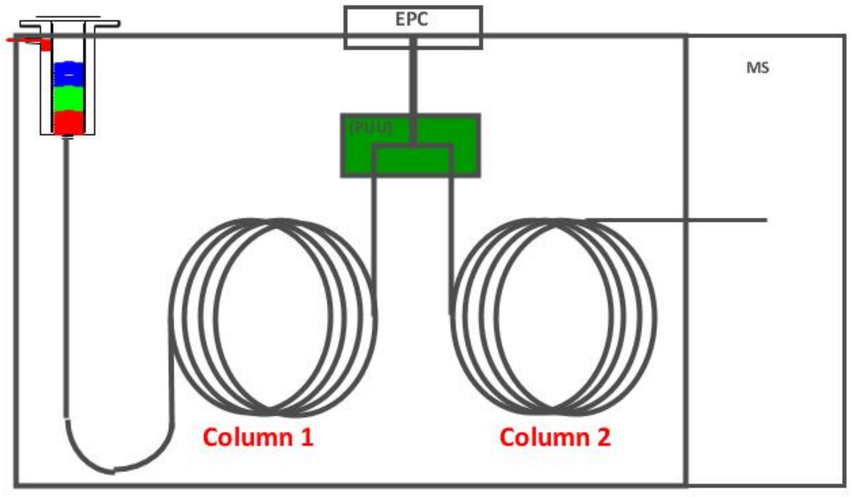
Additional GC Techniques

- Issue: Potential contamination of the GC column by less-volatile matrix components:
  - Affect method ruggedness by causing decrease in the analyte signal gradually
  - Peak broadening
  - R_t shift

✓ Solution: Column backflush eliminates the less volatile matrix components from GC column by reversing the column flow

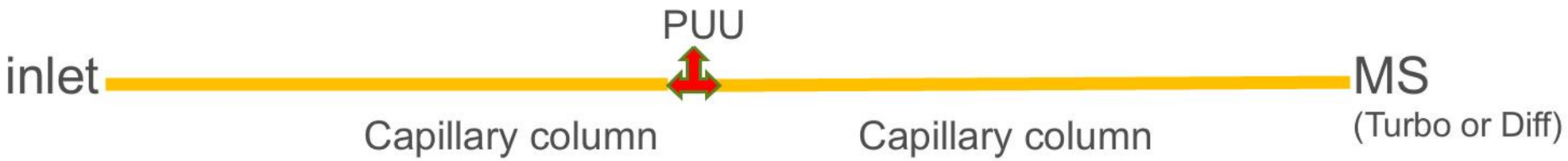
The Purged Ultimate Union (PUU) configurations



Vocabulary

Post-column

PCT: Mid-column



Under Evaluation

- "Sandwich Injection Technique"
  - Allows for different matrix calibrations curves to be made by injecting 1 set per calibration standards with sandwich injection of matrix blank
  - Saves significant bench work to prepare matrix-matched calibration standards, increasing consistency and decreasing potential errors caused by preparation mistakes
- 3-layer sandwich injection: total volume of 1.1 µL, clean standard not in matrix
  - 0.5 µL of clean standard + 0.5 µL matrix blank + 0.1 µL solvent blank

- 2-layer sandwich injection: total volume of 1.1 µL, matrix sample
  - 0.5 µL of matrix sample + 0.6 µL solvent blank

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QuEChERS Method Optimization – Multi-residue Pesticide Analysis in Juice Concentrates

A GC/MS/MS multiple reaction monitoring method was developed on the Agilent 7890A/7000 GC Triple Quadrupole Mass Spectrometer system(GC/QQQ) for 84 pesticide residues in lemon and orange juice concentrates provided by a world-wide beverage company.

Sample Preparation process follows the QuEChERS EN extraction with modifications to account for pH and sample volume.

Results: Lemon juice concentrate was acidic (pH = 2) and treatment with NaOH gave better results for several pesticides. Analyte protectants (AP) were required to obtain good peak shapes by GC-MS. The method recoveries for the majority of the compounds were between 70-120% with RSD below 10%.

40,000 Foot View of the Project

- Challenges in Lemon Juice Concentrate
  - Extreme pH condition → pH is below 2 and adjustment is needed
  - High sugar content → different buffering capacity EN vs. AOAC
  - Unknown concentration factor → sample amount is optimized to 4 g
  - Sample type → Not conventional water-containing fruits & vegetables. It is liquid.

Schematic Workflow of Optimized QuEChERS Application for Lemon and Orange Juice Concentrates

Optimized QuEChERS Parameters

- Sample amount
- pH variation
- AP (analyte protectant)
- Extraction (AOAC, EN) and Dispersive SPE (fruits & vegetables, fatty samples)

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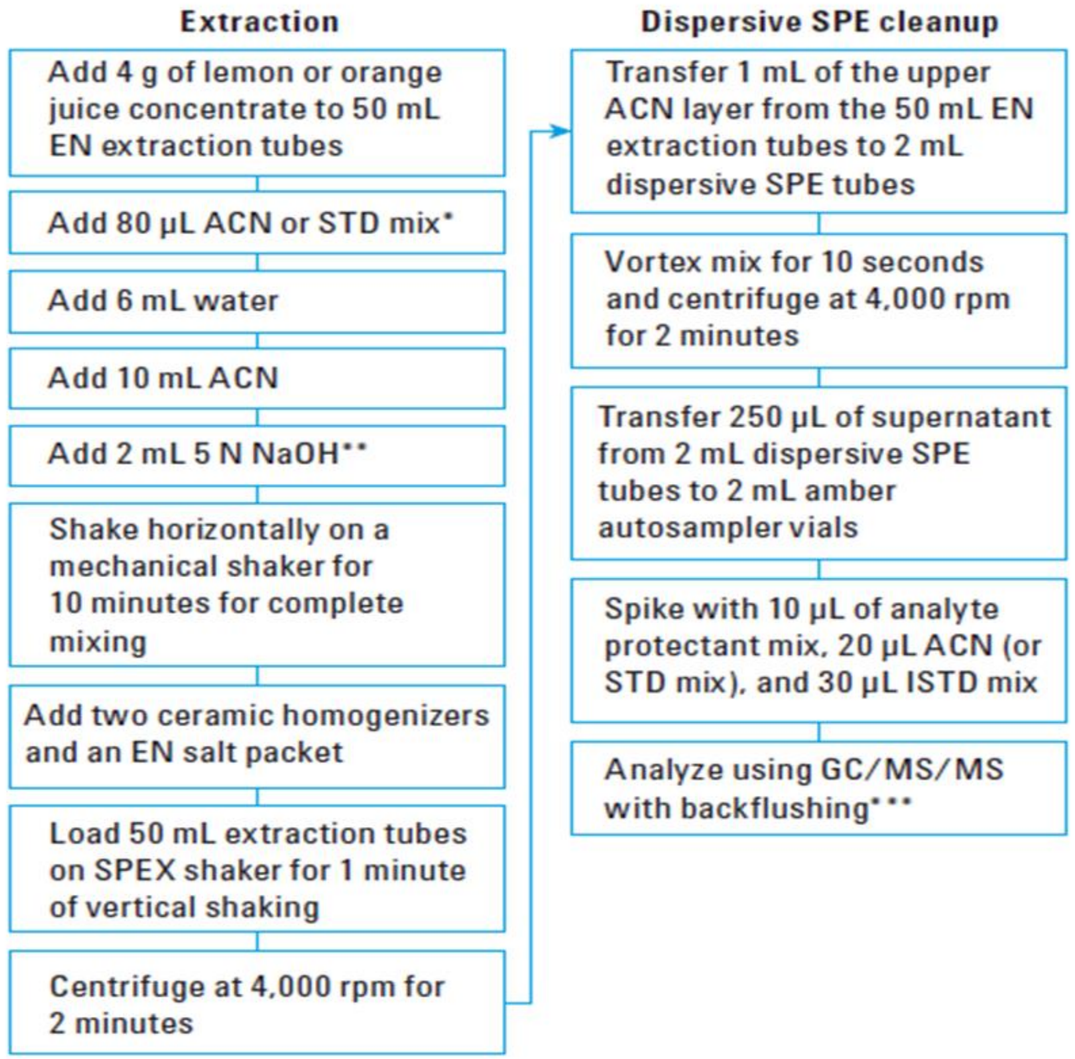
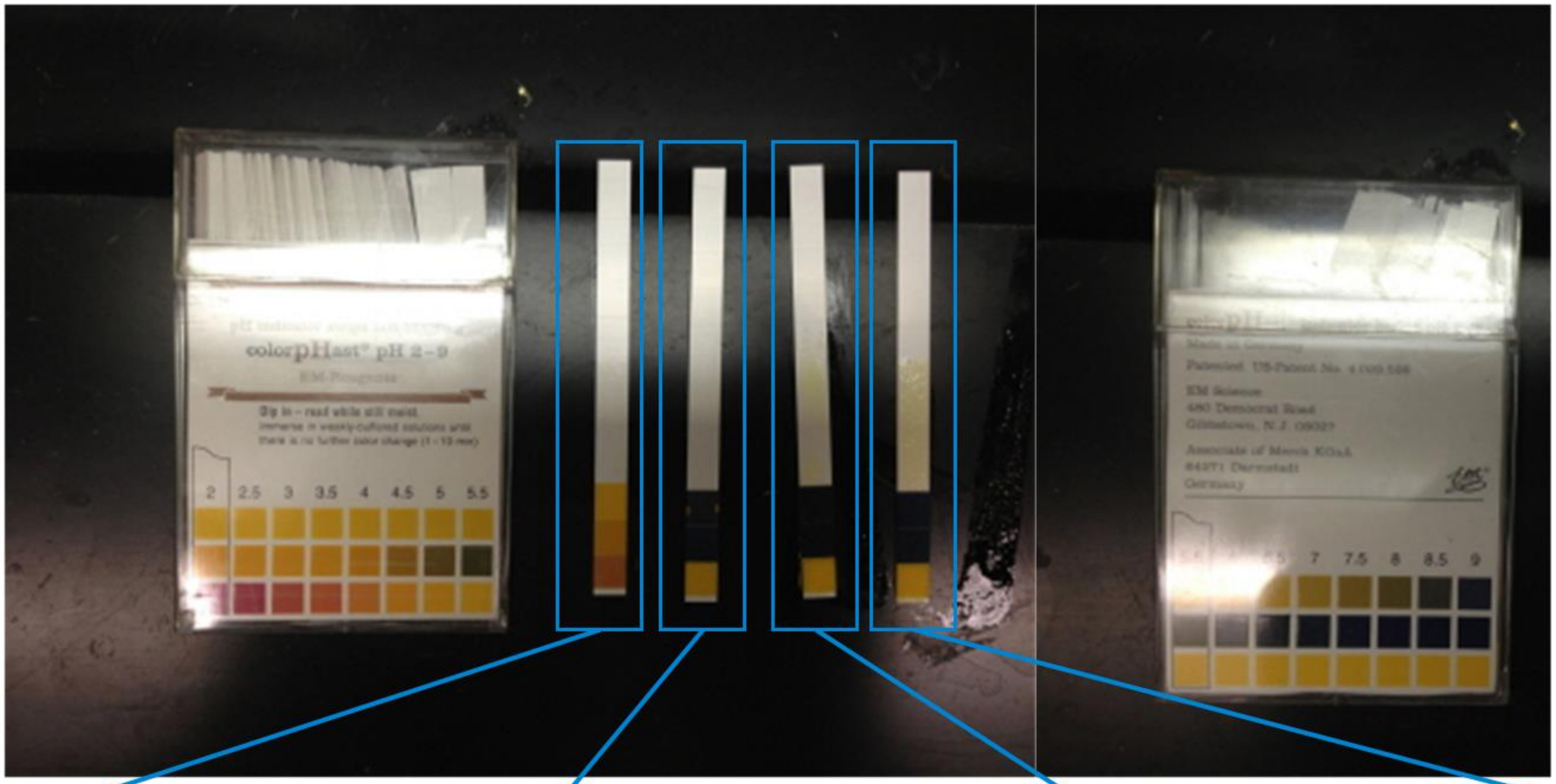


* Add 80 µL ACN for matrix-matched standard calibrators and 80 µL STD mix at appropriate concentration levels for recovery samples.

** After optimization of pH values, 2 mL and 0 mL 5 N NaOH solution were used for lemon juice and orange juice, respectively.

*** Backflushing is recommended to improve throughput and minimize system contamination by excluding high boiling matrix components in each run.

QuEChERS Optimization – pH Variation



0 mL 5 N NaOH

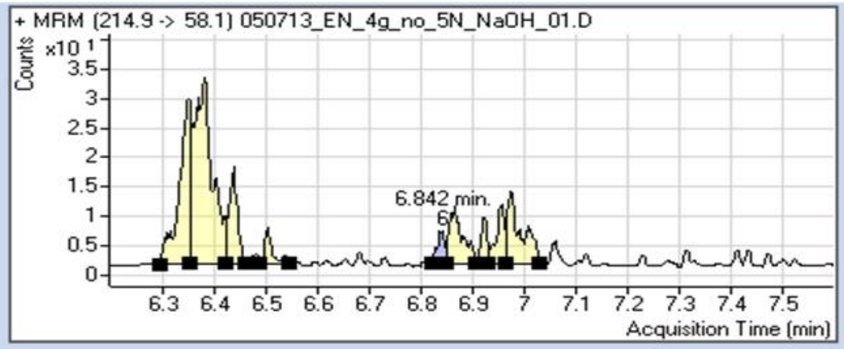
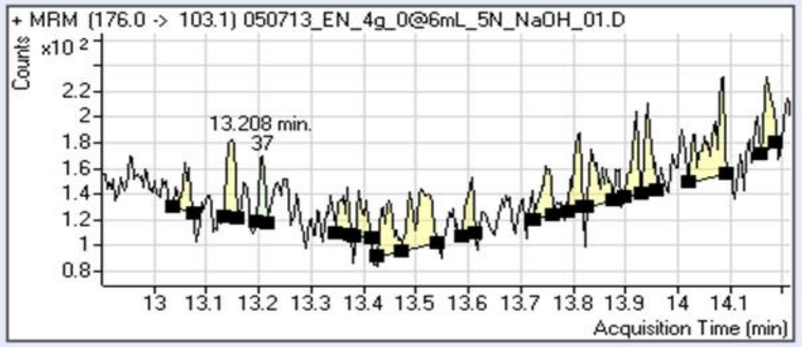
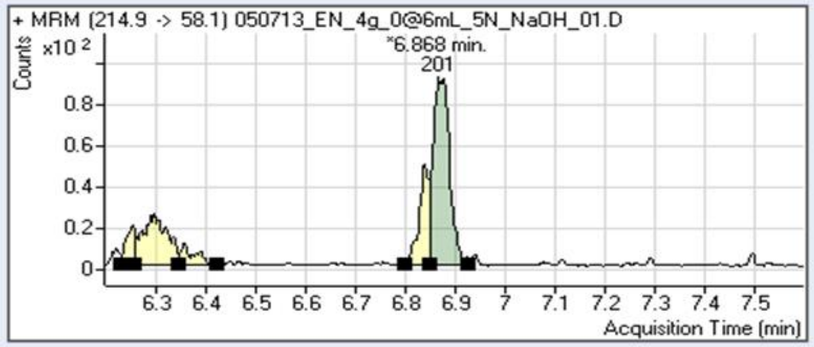
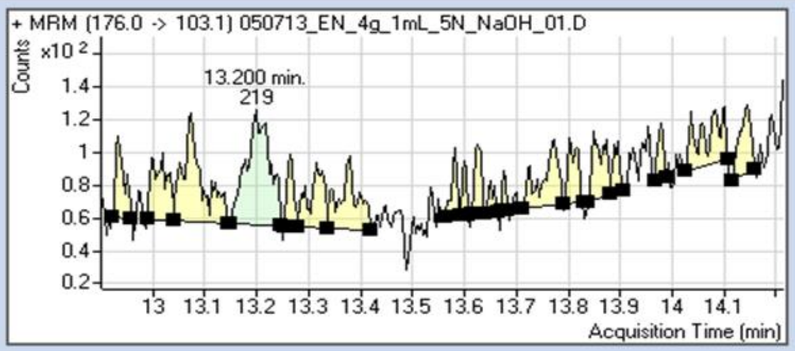
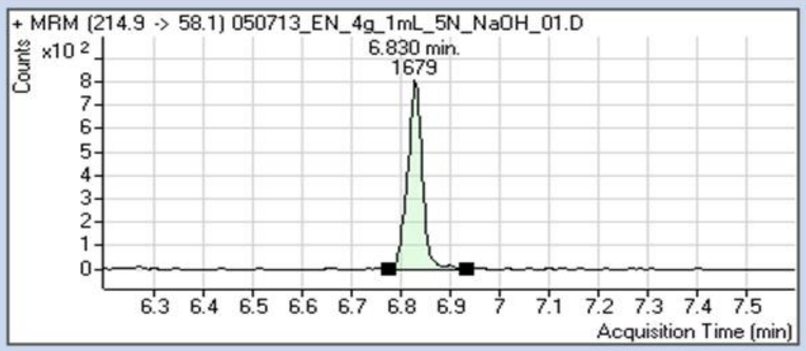
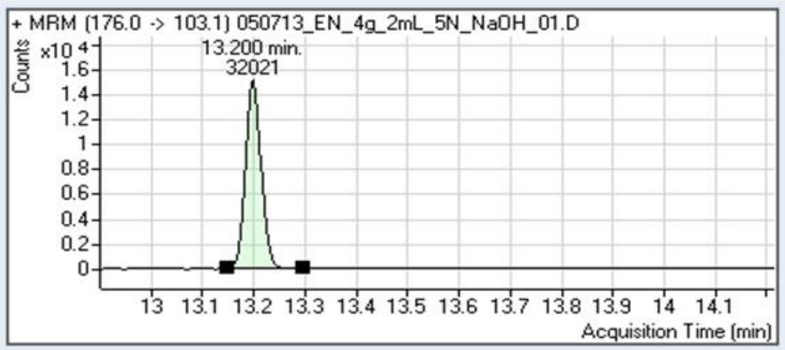
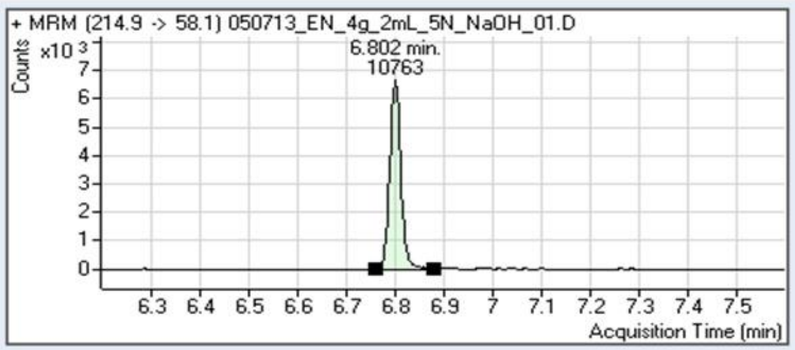
0.6 mL 5 N NaOH

1 mL 5 N NaOH

2 mL 5 N NaOH

Small amount of 5 N NaOH changed the pH rapidly to basic environment.

Improving Peak Shape with pH

	Piperonyl Butoxide	Atrazine
0 mL 5 N NaOH		
0.6 mL 5 N NaOH		
1 mL 5 N NaOH		
2 mL 5 N NaOH		

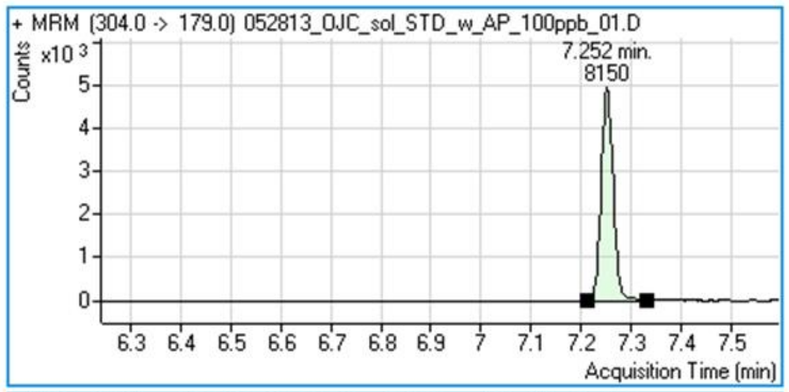
QuEChERS Optimization – AP (Analyte Protectant)

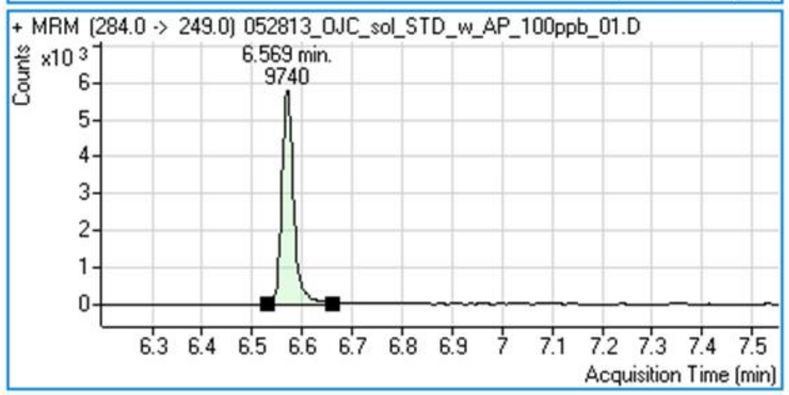
Diazinon

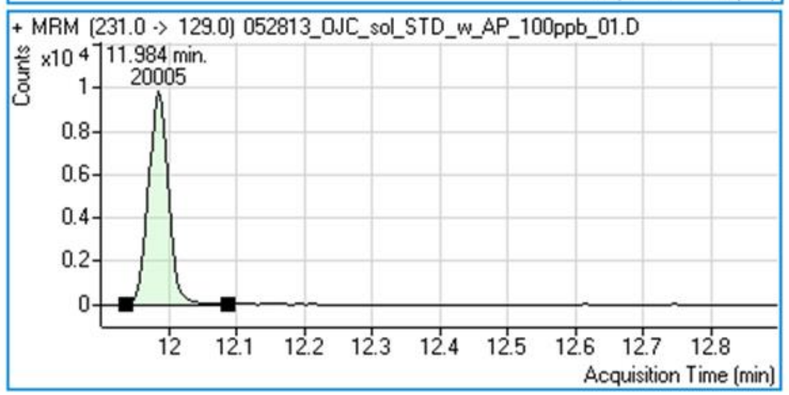
Hexachlorobenzene

Ethion

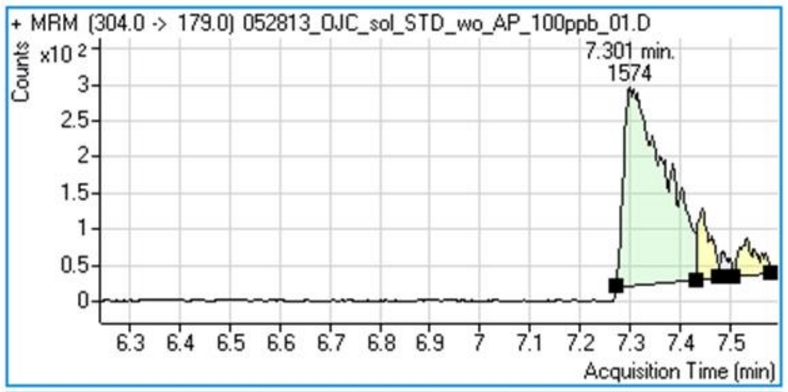
with AP

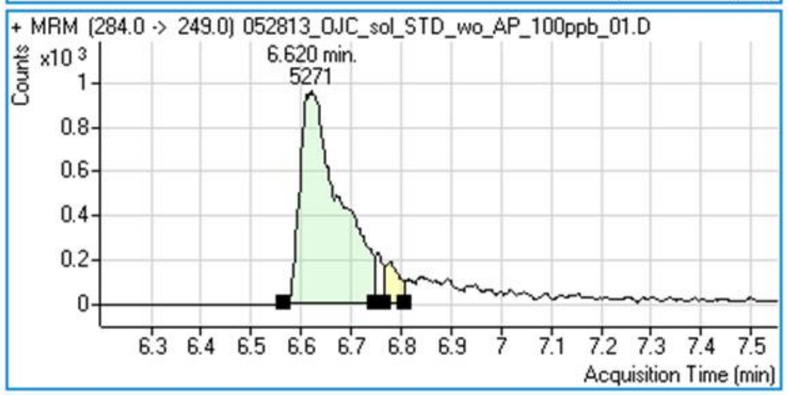






without AP





APs are a must in multi-residue pesticide analysis

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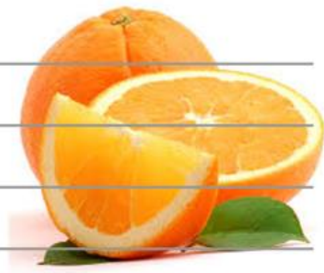
Precision & Accuracy for Lemon Juice Conc.

CAL range: *2 – 200 ppb*, *n=6* for %REC and %RSD for 5,10, 50, 100 ppb/ each compound

Precision & Accuracy for Orange Juice Conc.

CAL range: 2 – 200 ppb, n=6 for %REC and %RSD for 5,10, 50, 100ppb each compound



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QuEChERS Method – Pesticides in Chicken using GC-Tandem MS

A GC/MS/MS multiple reaction monitoring method was developed on the Agilent 7890/7000 GC Triple Quadrupole Mass Spectrometer system(GC/QQQ) for 54 pesticide residues in chicken.

Sample Preparation process follows the QuEChERS AOAC extraction. Two dispersive cleanup options were evaluated.

Results: Chicken samples were analyzed to verify the calibration coefficient, recovery and reproducibility. The detection limit of most compounds is down to 5 µg/kg (5 ppb). The method recoveries for all of the compounds were between 70-120% with RSD below 15%.

Sample Preparation

Weigh 7.5 g chicken muscle sample (±0.1g) in 50 mL centrifuge tube and add 7.5 mL water

↓

Add Internal Standard (TPP) solution, and QC spike solution if necessary, vortex 1 min

↓

Add 15mL of ACN containing 1% AcOH

↓

Add Bond Elut AOAC QuEChERS Extraction salt packet

↓

Cap and shake vigorously for 30 seconds

↓

Centrifuge @ 4000rpm and 4 ℃for 5 min

↓

Transfer 8 mL of upper ACN layer to Bond Elut Dispersive-SPE* 15 mL tube

↓

Vortex 1min, centrifuge @ 4000 rpm and 4 ℃ for 5 min

↓

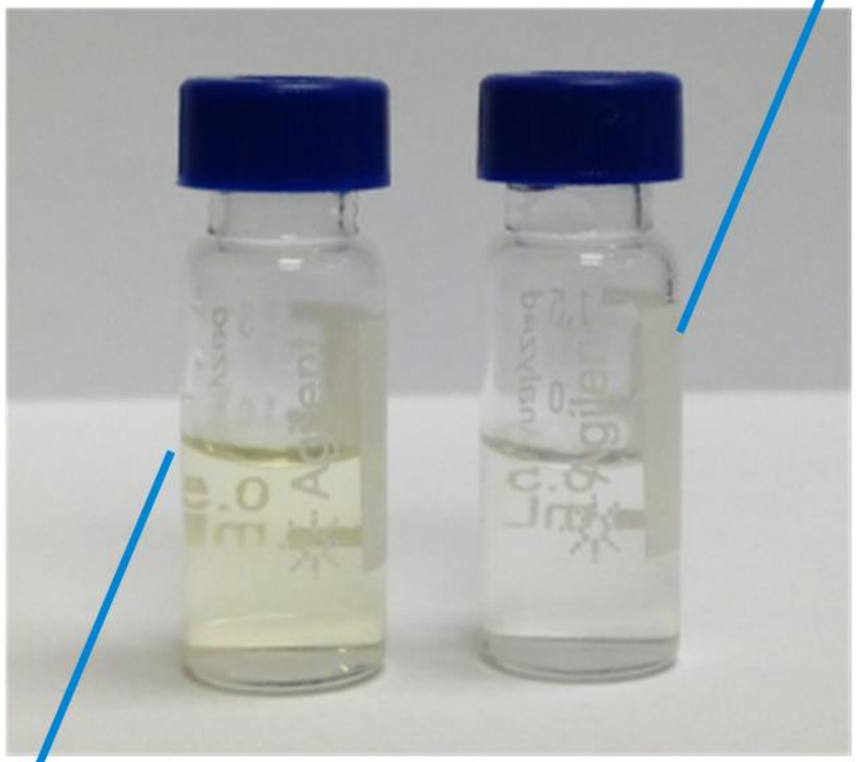
Transfer 4 mL extract to tube and dry under nitrogen

↓

Reconstitute with 1 mL of ACN and analysis with GC-QQQ

* Two different Dispersive-SPE kits were used.
Bond Elut QuEChERS Dispersive Kit for other food methods, 5982-4956CH
Bond Elut QuEChERs Dispersive Kit for all food types, 5982-0029CH

Picture of the Solutions after Sample Preparation

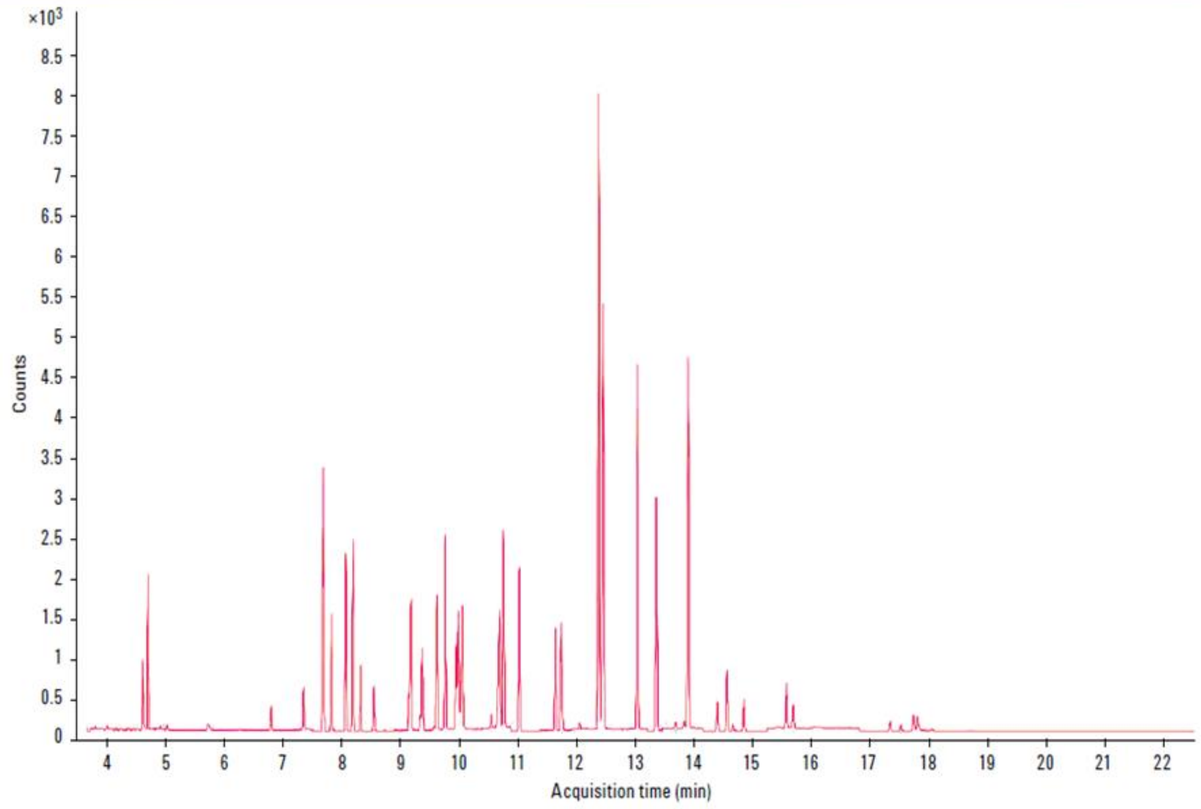


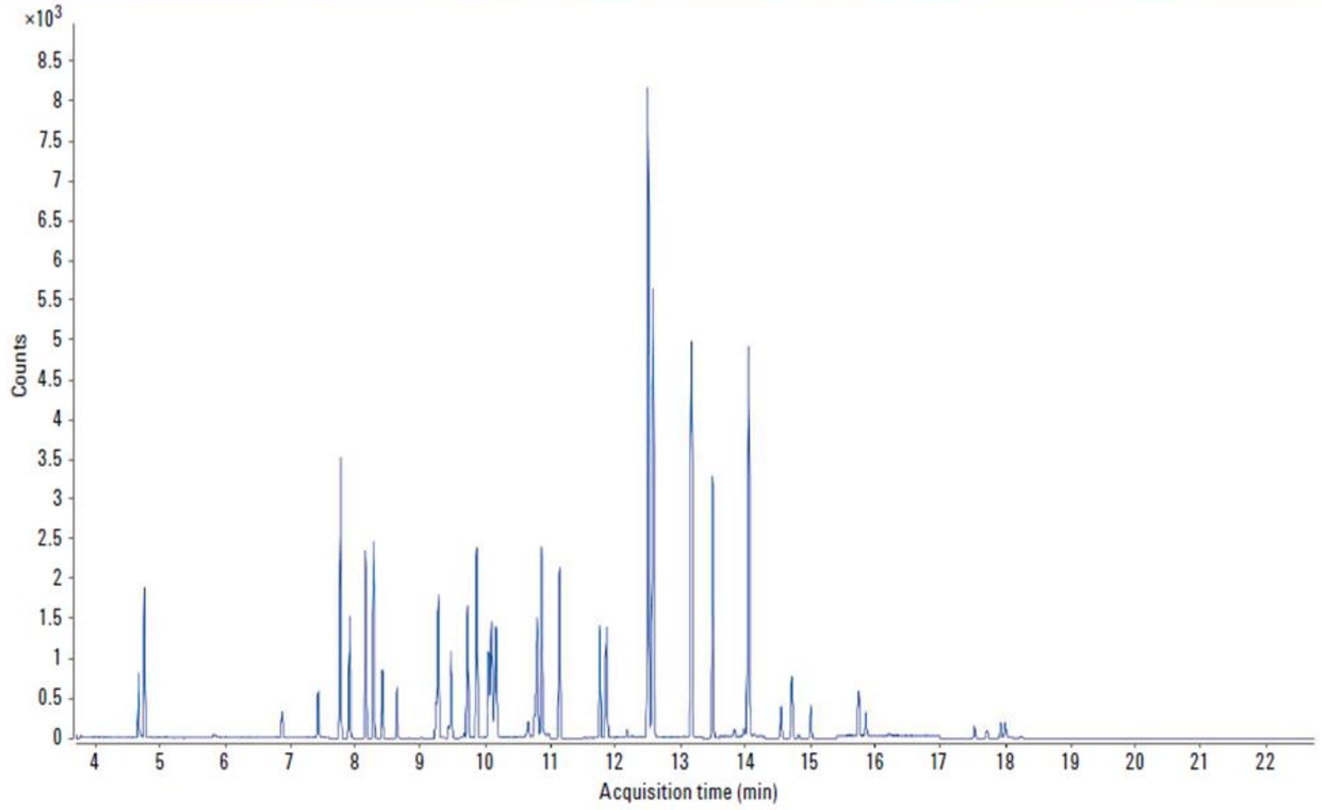
Clean up with Bond Elut QuEChERS Dispersive Kit for all food types

400 mg PSA, 400 mg C18, 45 mg GCB, 1200 mg MgSO4

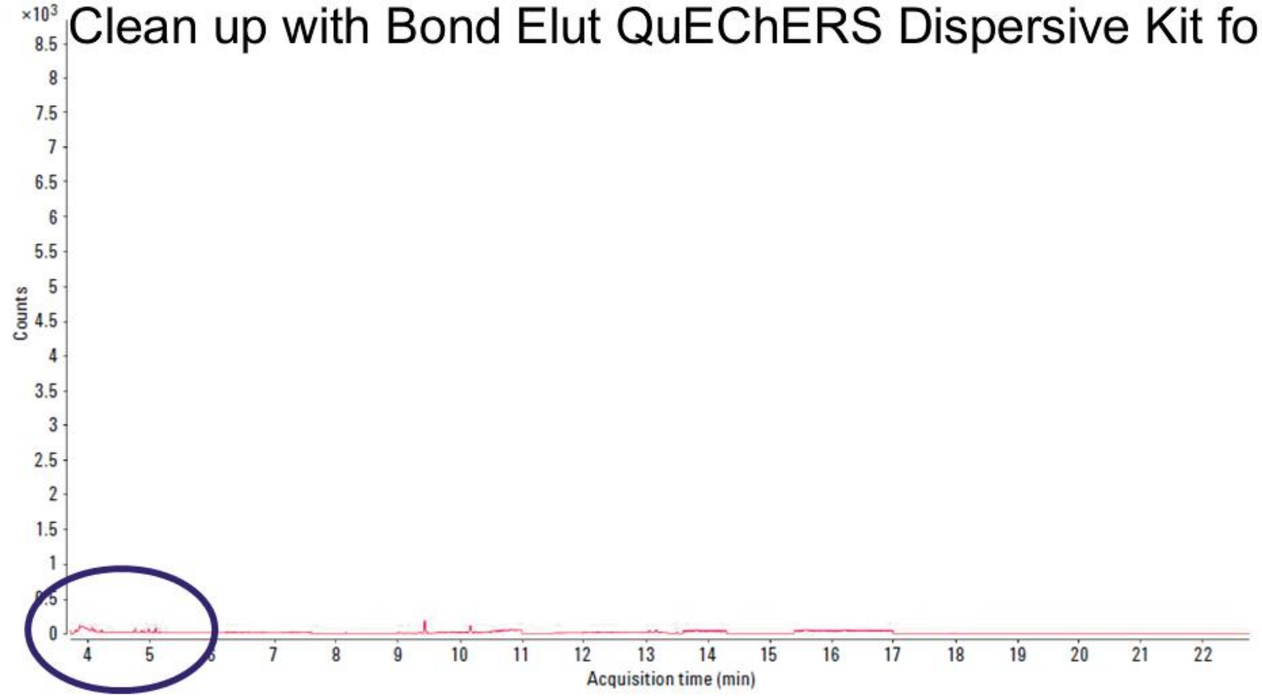
Clean up with Bond Elut QuEChERS Dispersive Kit for other food methods

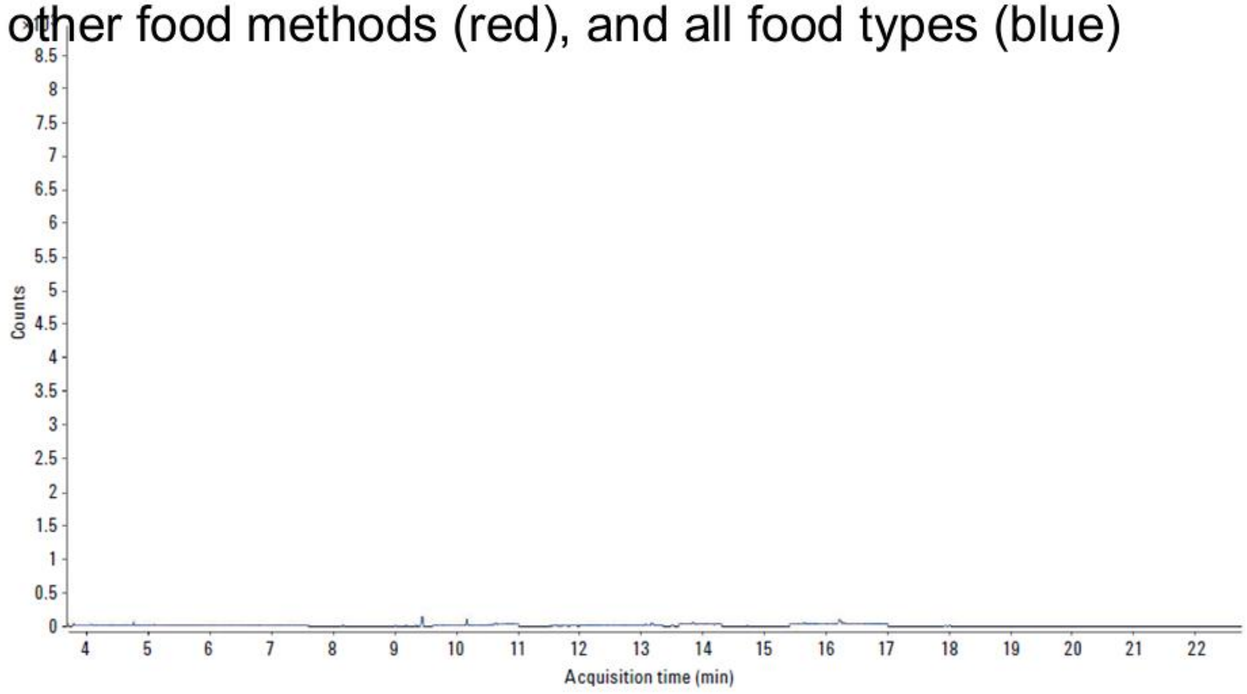
150 mg C18, 900 mg MgSO4





TIC of spike 10 ug/kg pesticides in chicken muscle with Bond Elut QuEChERS AOAC kits.
Clean up with Bond Elut QuEChERS Dispersive Kit for other food methods (red), and all food types (blue)

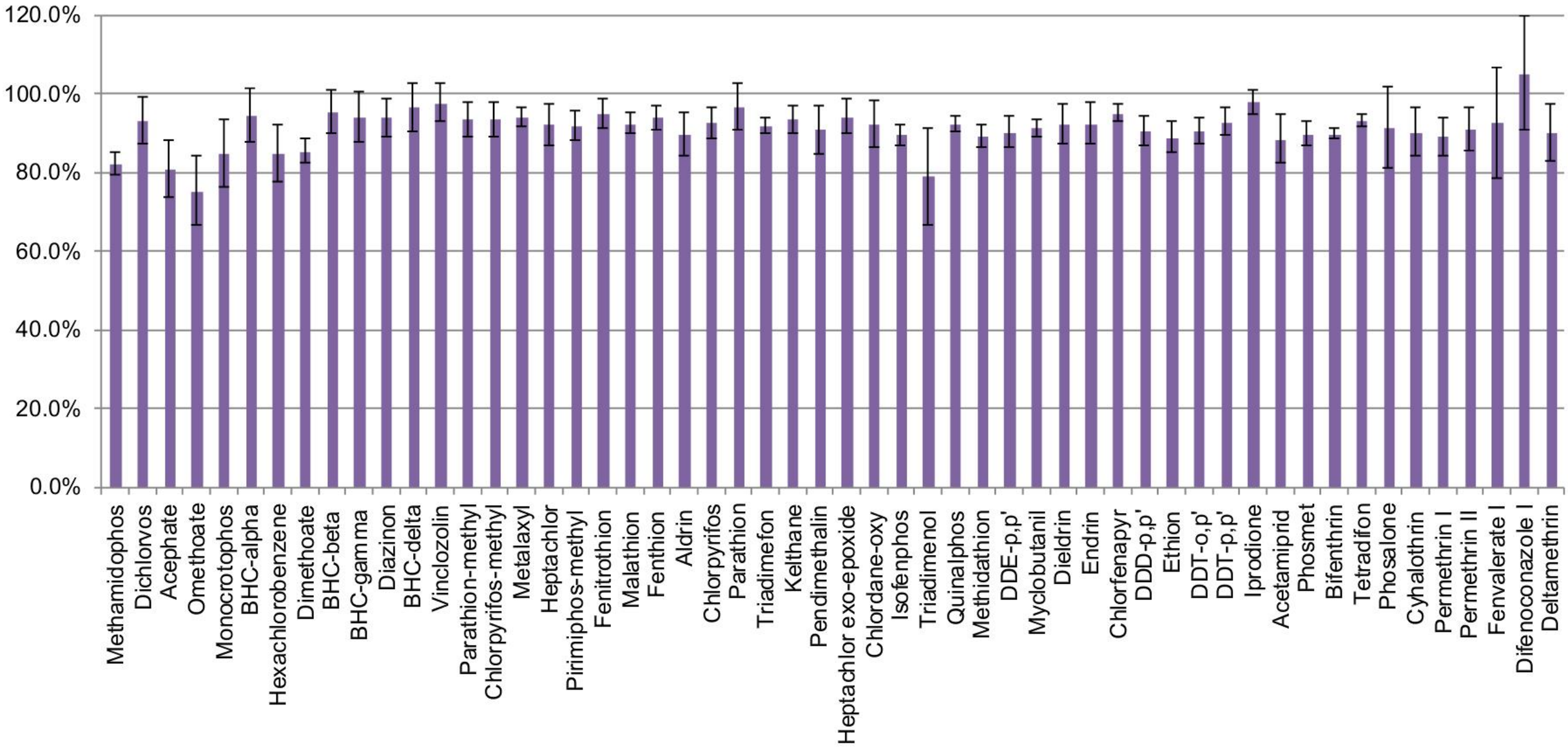




TIC of chicken muscle sample blank with Bond Elut QuEChERS AOAC kits.
Clean up with Bond Elut QuEChERS Dispersive Kit for other food types (red), and all food types (blue)

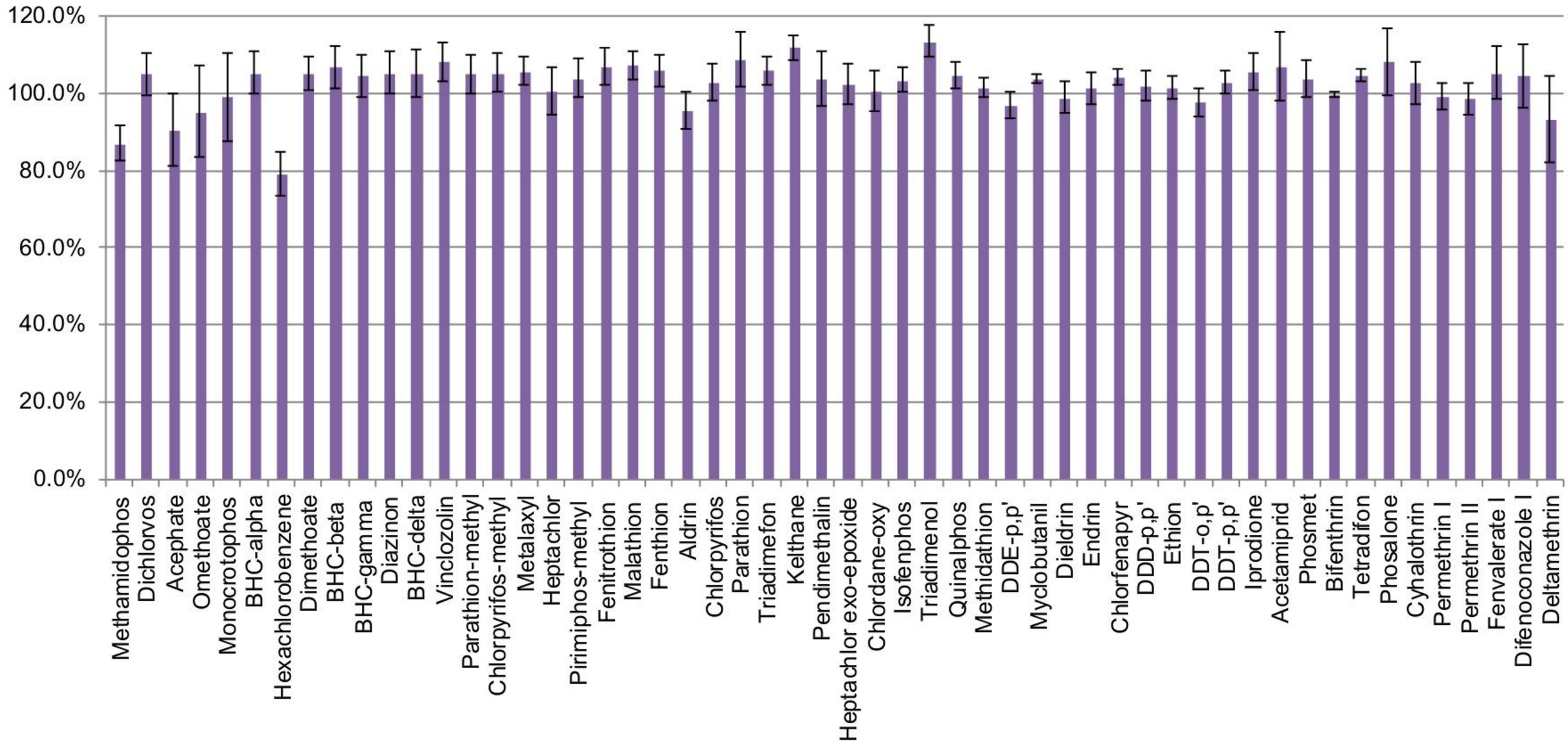
5991-2182EN

Results – Recoveries, Continued



Recoveries for the pesticides of 50 µg/kg spiked in chicken muscle sample with QuEChERS and clean up with Bond Elut Dispersive Kit for other food methods.

Results – Recoveries



Recoveries for the pesticides of 50 µg/kg spiked in chicken muscle sample with QuEChERS and clean up with Bond Elut Dispersive Kit for all food types.

Today's Agenda

- QuEChERS: Brief Overview
- Analyte Protectants and GC techniques
- Method Modifications with Acidic Matrices
- Pesticide Analysis from Chicken
- **Pesticides in Herbal Tea**
- Preservatives in Pet Food
- Summary: Take Home Message

QuEChERS Method – Multi-residue analysis of Pesticides in Herbal Tea

An LC/MS/MS multiple reaction monitoring method was developed on the Agilent 1290/6410 LC Triple Quadrupole Mass Spectrometer system(LC/QQQ) for 176 pesticide residues in herbal green and black teas.

Sample Preparation process follows the QuEChERS original 10 g extraction. Several dispersive cleanup sorbent options were evaluated.

Results: Tea samples were analyzed to verify the calibration coefficient, recovery and reproducibility. Planar pesticide recoveries are affected by carbon sorbent mass and type. The method recoveries for the majority of the compounds were between 70-120% with RSD below 15%.

Sample Preparation

Weigh 1.00 g dried tea (±0.01g) in 50 mL centrifuge tube and spike as necessary

↓

Add 10 mL Water and vortex 1 min

↓

Add 10 mL of ACN containing I.S. (50 ppb dimethoate-d6)

↓

Cap and shake vigorously for 60 seconds

↓

Add Bond Elut Original QuEChERS Extraction salt packet

↓

Cap and shake vigorously for 60 seconds

↓

Centrifuge @ 4000rpm for 5 min

↓

Transfer 5 mL of upper ACN layer to Dispersive-SPE, 15 mL tube

↓

Vortex 1 min, centrifuge @ 4000 rpm for 5 min

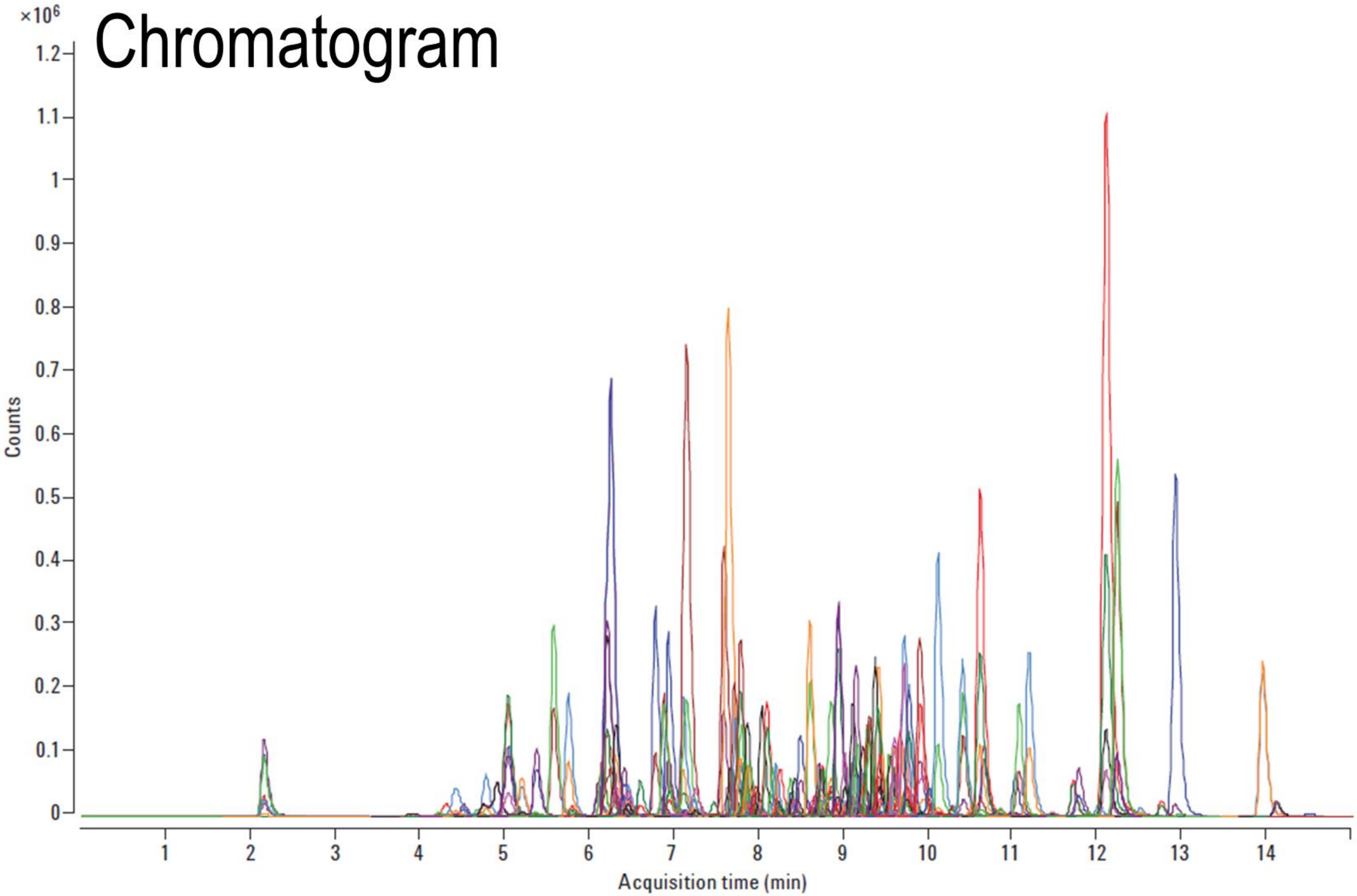
↓

Transfer 2 mL extract to tube and dry under nitrogen

↓

Reconstitute with 1 mL of 70/30 Water/ ACN, syringe filter and analysis by LC-QQQ

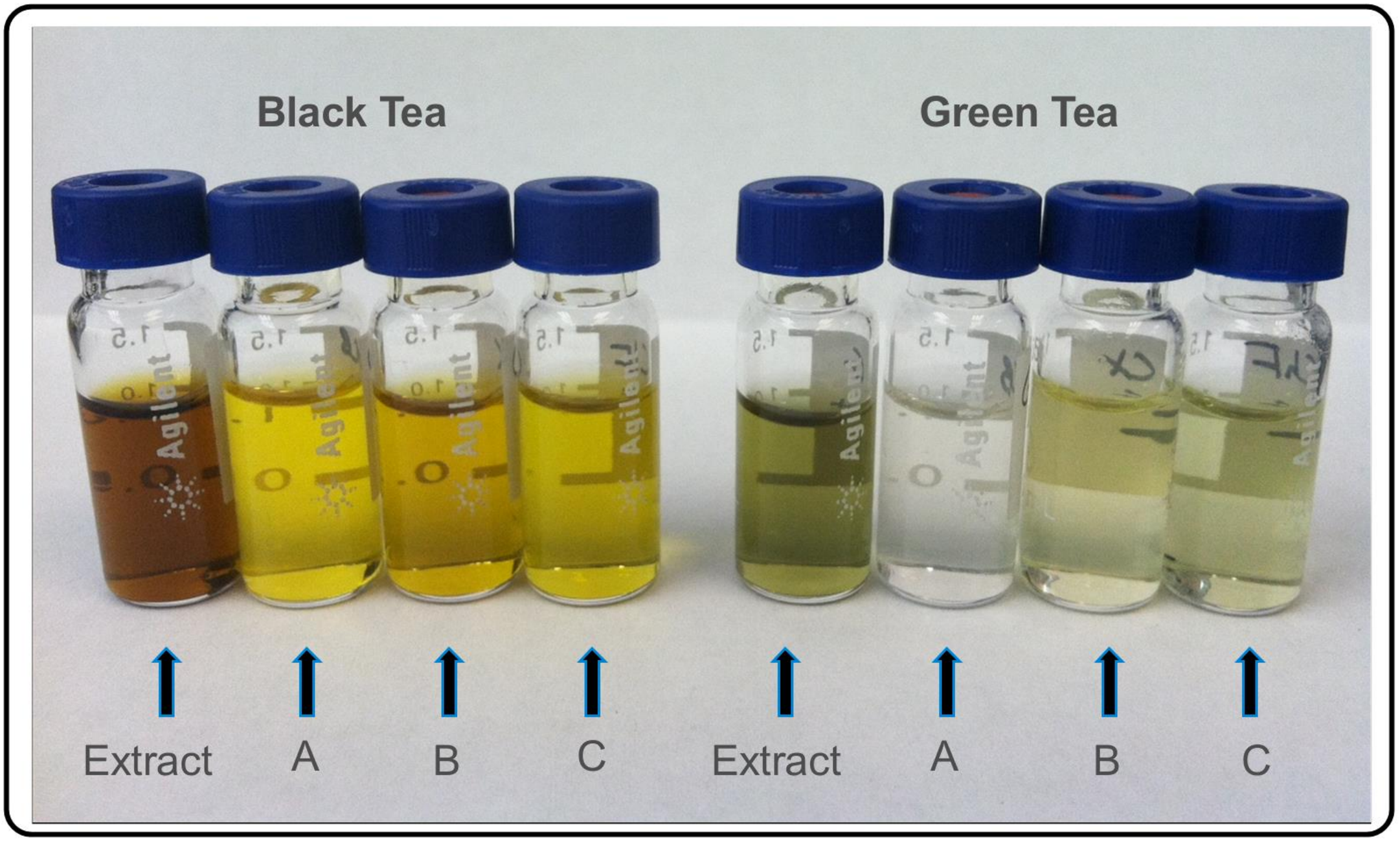
Chromatogram



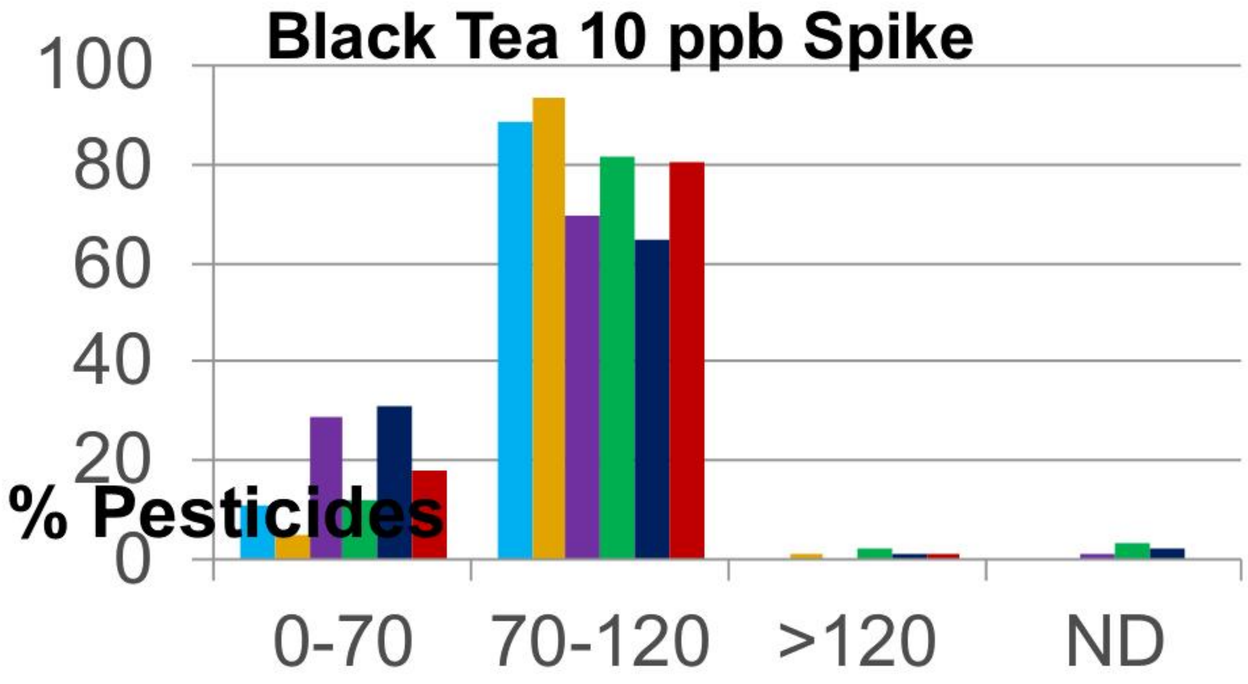
MRM chromatogram overlay of 100 ppb spiked pesticide mixture in black tea for the 176 pesticides analyzed

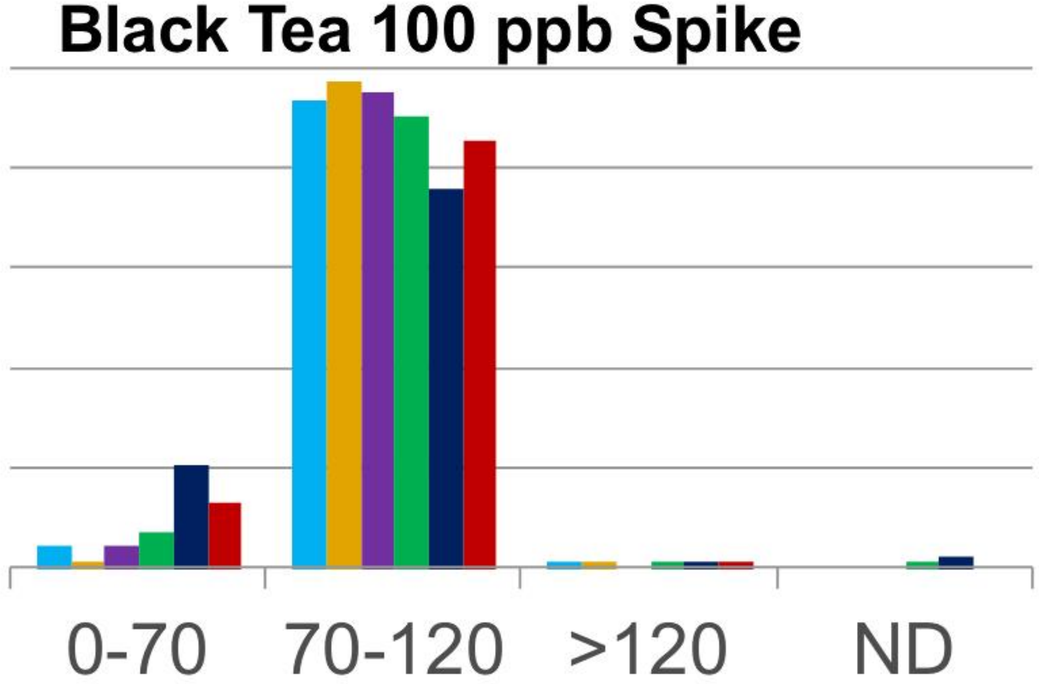
5991-3728EN

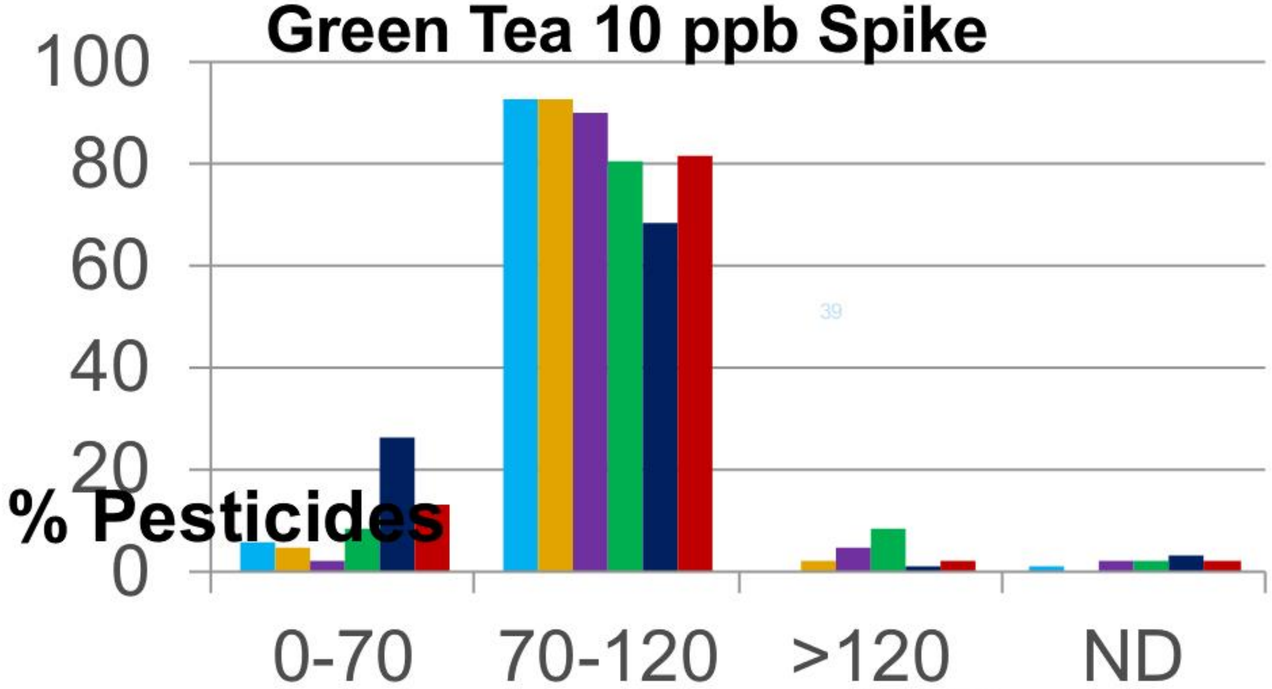
Green and Black Tea dSPE Cleanup

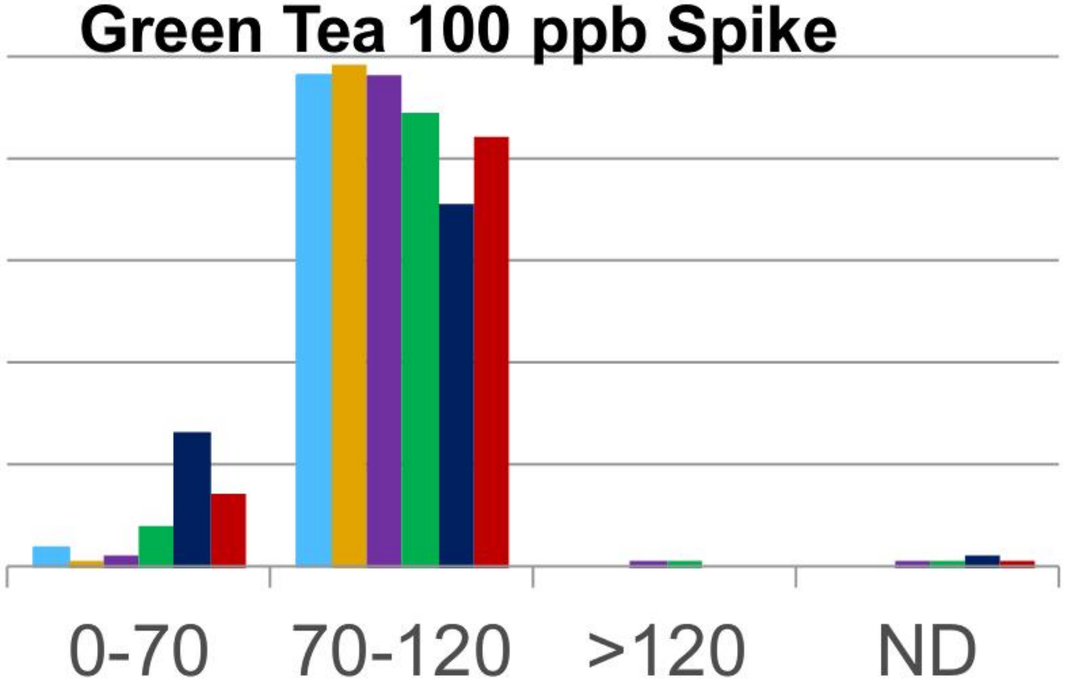


A = GCB, B = Alternative Carbon Sorbent, C = Chlorophyll Specific Sorbent





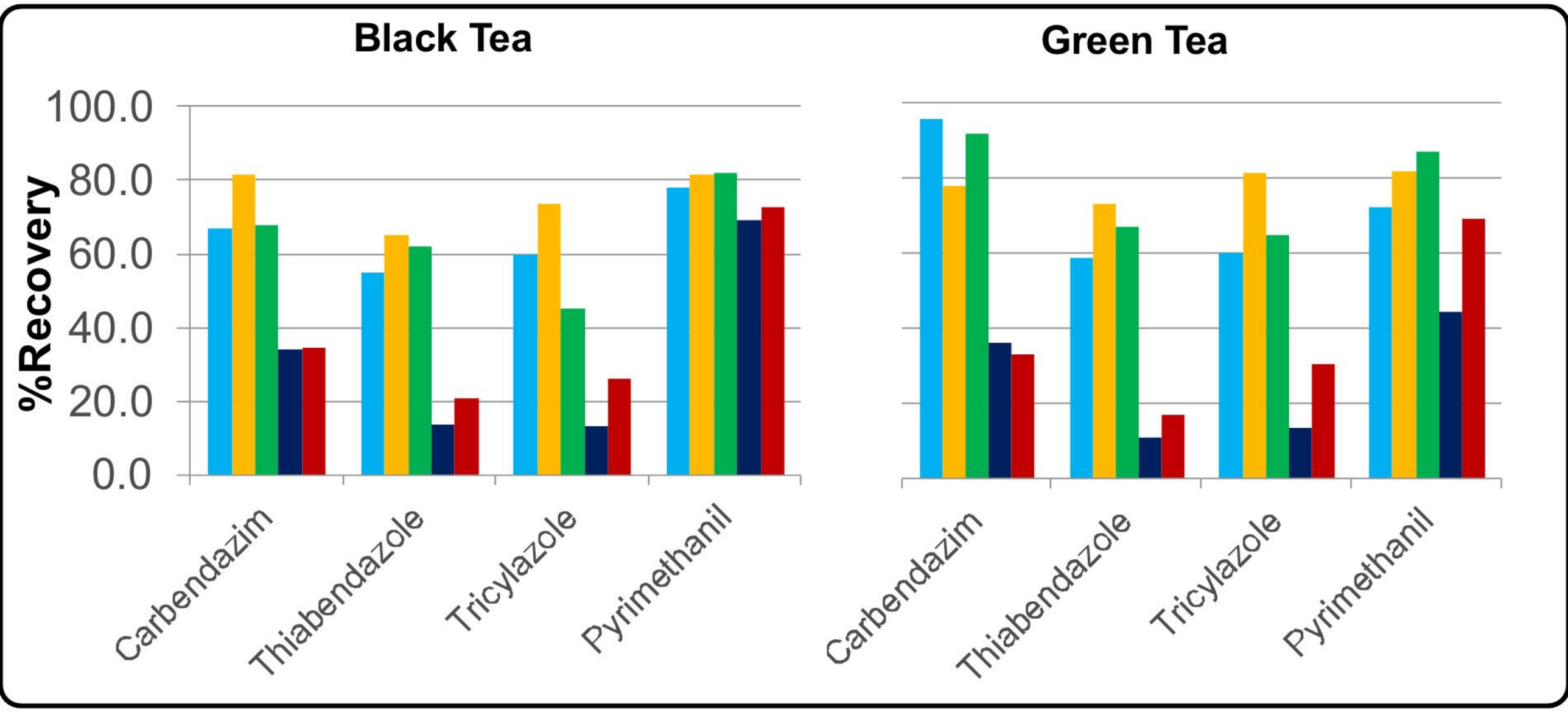




% Recovery

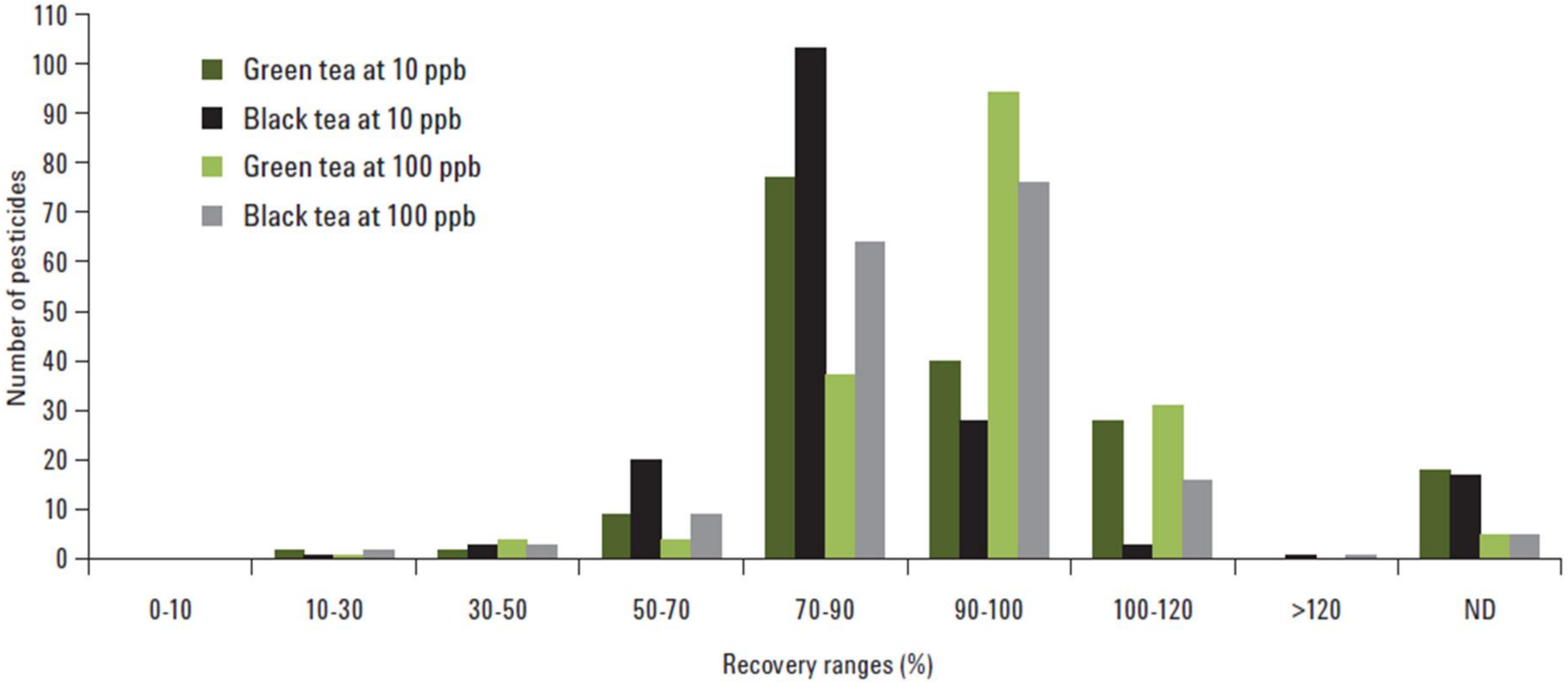
GCB (45 mg) Alternative carbon sorbent (45 mg) No carbon Chlorophyll specific, polymeric sorbent (150 mg) GCB (400 mg) Alternative carbon (400 mg)

Comparison of Recoveries for Planar Pesticides



GCB (45 mg) Alternative carbon sorbent (45 mg) Chlorophyll specific, polymeric sorbent (150 mg) GCB (400 mg) Alternative carbon (400 mg)

Plot of Recovery Ranges for 176 Pesticides



For green tea samples, 82% of the pesticide recoveries at 10 ppb, 92% at 100 ppb were between 70-120%
For black tea samples, 76% of the pesticide recoveries at 10 ppb and 88% at 100 ppb were between 70 and 120 %

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QuEChERS Application Examples: Preservatives in Pet Food

Looked at BHA, BHT and ethoxyquin in pet food: antioxidants, levels allowed by FDA 150 ppm

Used QuEChERS sample prep approach to preparing pet foods for LC-MS/MS analysis

Developed LC-MS/MS method

Results:

- ✓ Incurred sample analysis proved suitability of the technique
- ✓ Difficult to find pet food samples that would be free of preservatives
- ✓ Method optimization was required

We will get back to the method optimization in a moment

LC-MS/MS Conditions

- LC conditions:
  - Poroshell 120 SB-C18, 2.7 µm, 2.1 x 50 mm
    - Mobile phase A: Water (0.1% FA)
    - Mobile Phase B: ACN (0.1% FA)
    - Time 0 25% B
      0.1 25% B
      4.0 90% B
      4.5 90% B
      4.6 90% B
- MS Settings:

Compound	MRM Channels (m/z)	Dwell Vol	Fragmentor (V)	CE (V)
Ethoxyquin TS1 EMV 300	218 > 174 218 > 160	6 6	30 30	27 31
BHA TS3 EMV 1000	181.1 > 166 181.1 > 138	100 20	89 89	11 19
BHT TS4 EMV 0	221.2 > 165 221.2 > 41.1	6 6	84 84	8 27
Propyl Paraben IS* TS2 EMV 500	181 > 139 181 > 120.9	20 20	57 57	3 15

QuEChERS Method Development Approaches

- Evaluate extraction salt mixtures
- Evaluate dSPE cleanup mixtures

We will use the pet food example to highlight method development and optimization approaches

Extraction Procedure: General QuEChERS Method

- Add 2 g pet food, either dry or wet, to a 50 mL centrifuge tube
- Add 2 ceramic homogenizers (to help facilitate extraction)
- Add 8 mL water, vortex 1 min at 2500 rpm
- Add 10 mL ACN, vortex 1 min at 2500 rpm
- Add QuEChERS extraction salts
- Shake vigorously for 1 min, or vertical shake at 1000 rpm
- Centrifuge at 5000 rpm, 5 min

Optimization of QuEChERS Procedure: Pet Food Extraction

- Three variations of the QuEChERS extraction salts were investigated
  - Non-buffered: 4 g $MgSO_4$, 1 g NaCl
  - AOAC: 6 g $MgSO_4$, 1.5 g NaAc
  - EN: 4 g $MgSO_4$, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate

TIP!

Use one dSPE mixture and keep this part the same for the extraction salt optimization

QuEChERS dSPE Method Optimization Summary

Use one dSPE type with three salt types to identify the best combination for the application

Method Development Products – Fruits & Vegetables

dSPE Mix 1

- EN Extraction Salts 5982-5650CH
- Original 5982-5550CH
- AOAC 2007.01 5982-5755CH

Method Development Process


Select Representative Sample


Homogenize


Weigh


Mix with salts, solvent


Centrifuge


Transfer aliquots of supernatant to each of the dSPE tubes


Mix


Centrifuge


Transfer supernatant to vials

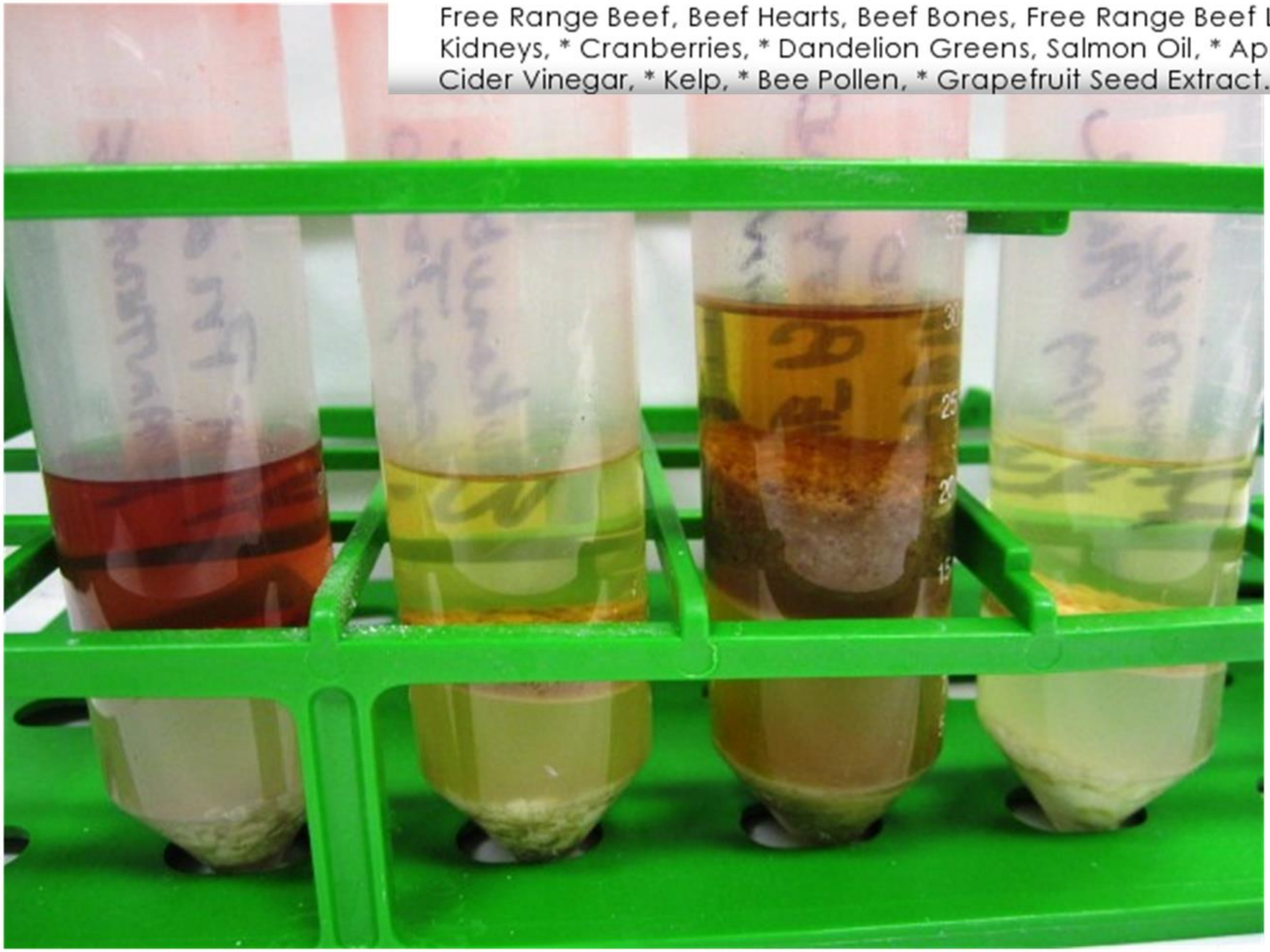

Analyze


Identify best extraction salts

Various Pet Foods After QuEChERS Extraction (Step 1)

beefbatch sliders:

Free Range Beef, Beef Hearts, Beef Bones, Free Range Beef Liver & Kidneys, * Cranberries, * Dandelion Greens, Salmon Oil, * Apple Cider Vinegar, * Kelp, * Bee Pollen, * Grapefruit Seed Extract.



Optimization of QuEChERS Procedure: Pet Food Cleanup

- Four variations of the d-SPE material were investigated
  - 25 mg PSA, 150 mg $MgSO_4$
  - 25 mg C18, 150 mg $MgSO_4$
  - 25 mg PSA, 25 mg C18, 150 mg $MgSO_4$
  - 25 mg PSA, 2.5 mg GCB, 150 mg $MgSO_4$

TIP!

Use the extraction salt mix identified in the first experiment and keep this part the same

QuEChERS dSPE Method Optimization Summary

Use one extraction tube and 4 dSPE tubes to identify the best combination for the application

Method Development Products

EN Extraction Salts
5982-5650CH

- dSPE Mix 1 5982-5021CH
- dSPE Mix 2 5982-4921CH
- dSPE Mix 3 5982-5121CH
- dSPE Mix 4 5982-5221CH

Method Development Process


Select Representative Sample


Homogenize


Weigh


Mix with salts, solvent


Centrifuge


Transfer aliquots of supernatant to each of the dSPE tubes


Mix


Centrifuge

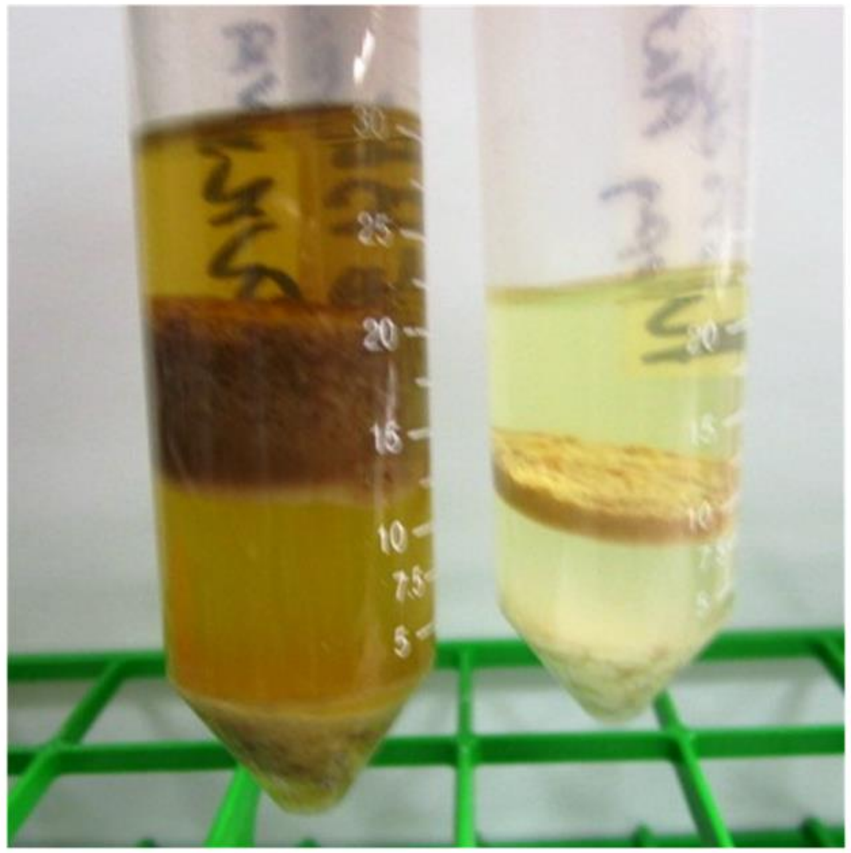

Transfer supernatant to vials


Analyze

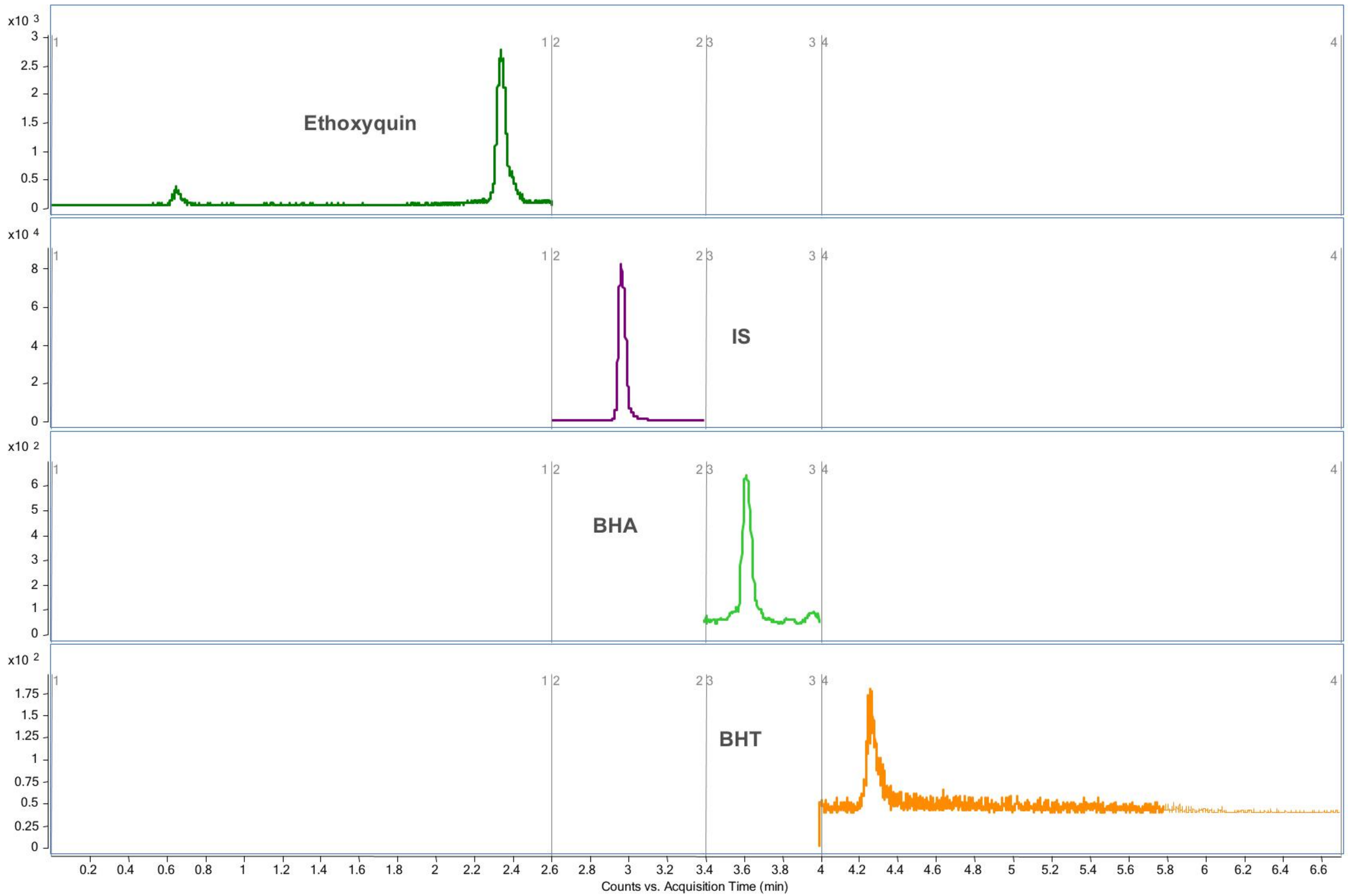

Identify best dSPE sorbent

d-SPE (Step 2)

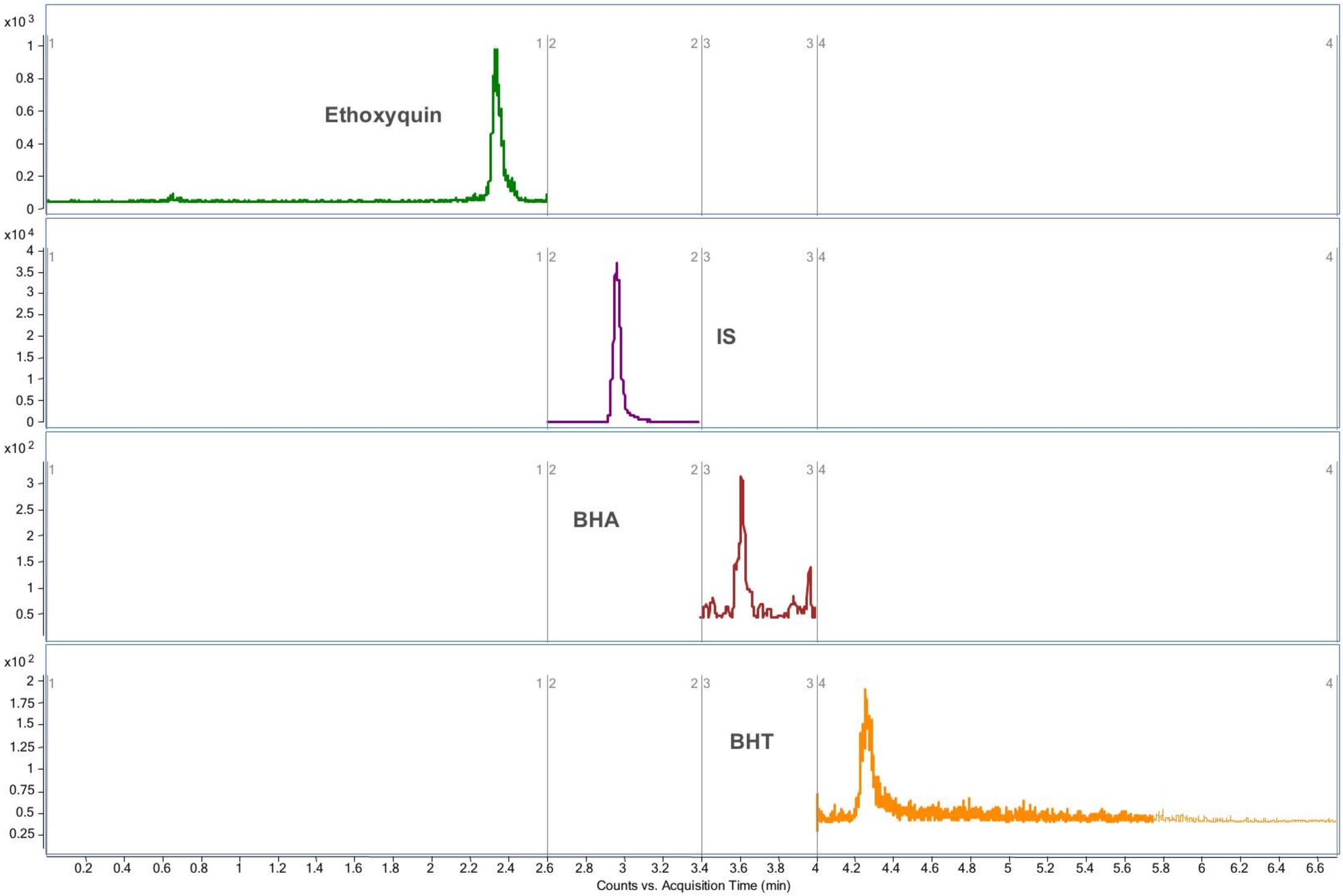
- Transfer 6 mL to 15 mL d-SPE tube
- Vortex 1 min at 2500 rpm
- Centrifuge at 5000 rpm for 5 min
- Transfer 1 mL to tube for evaporation
- Reconstitute in 1 mL ACN:water (1:4)
- Filter sample with 0.45 µm PP
- Analyze by LC-MS/MS



400 ppb Post-Spiked Sample



400 ppb Pre-Spiked Sample



Pet Food QuEChERS Optimization Results

Extraction:

Use the EN extraction salts: 4 g $MgSO_4$, 1 g NaCl, 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate (PN 5982-5650CH)

Cleanup:

d-SPE: 25 mg PSA, 2.5 mg GCB, 150 mg $MgSO_4$ (PN 5982-5221CH)

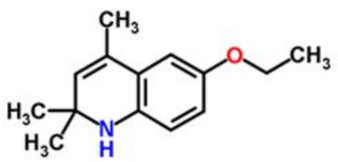
In a minimal number of steps and experiments, the ideal match of extraction and dSPE processes can be determined

Results for Spike Samples

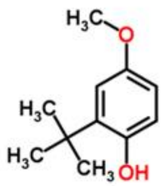
Compound	400 ppb Recovery (%)	400 ppb RSD (%)	600 ppb Recovery (%)	600 ppb RSD (%)	800 ppb Recovery (%)	800 ppb RSD (%)
Ethoxyquin	64	4	57	6.5	55	5.4
BHA	101	1.5	100	3.6	100	1.0
BHT	103	7.6	110	6.5	130	6.9

Levels may seem high to MRLs of pesticides in human food products but in pet food, FDA allows up to 150 ppm of antioxidants

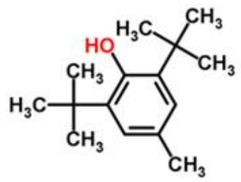
Based on the parameters we've already discussed, what might you suggest to possibly improve recovery of Ethoxyquin?



Ethoxyquin
Log P: 4.1
pKa: 4.56

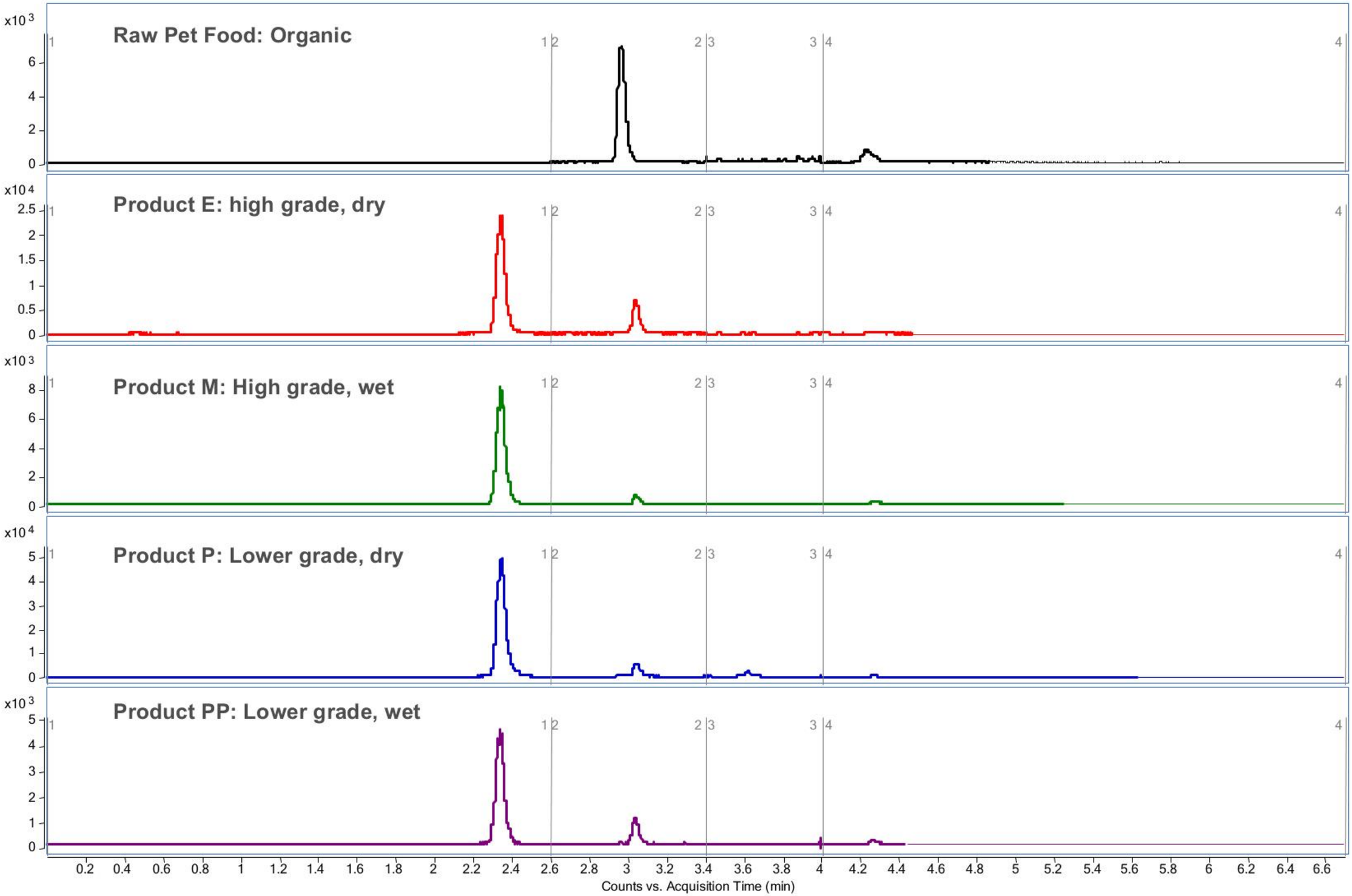


Butylated hydroxyanisole (BHA)
Log P: 3.09
pKa: 8.9



Butylated hydroxytoluene (BHT)
Log P: 5.32
pKa: 12.23

Incurred Samples



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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
- QuEChERS reproducibility and ruggedness can be improved with products designed for optimal performance from various matrices
- Don’t forget to take a 40,000 foot view of the sample this will allow you to see characteristics of the sample that will need to be addressed
- A systematic and straightforward approach to method development and optimization is easy with pre-packaged kits and bulk sorbents
- 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:

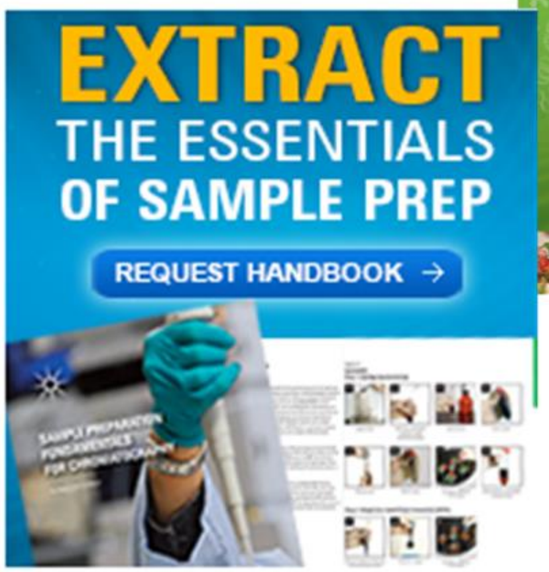
QuEChERS videos: 2 available, Part 1 & 2

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



Acknowledgements

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Thank You for Your Time and Attention!

Questions