QuEChERS 101: The Basics and Beyond

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Outline

- **Background information: sample preparation**
    - LLE, and SPE
- **Background information: QuEChERS**
  - Why, what, where and when
- **QuEChERS Procedure**
  - Extraction/partitioning
  - Dispersive SPE
  - Simplicity of the sample preparation process
  - Analysis LC/MS/MS, GC/MS, and GC/MS/MS
- **QuEChERS modifications**
    - Low water content products
    - Very polar compounds

– **QuEChERS: Thinking out of the sample preparation box**

Sample Preparation

- LLE: liquid liquid extraction

Advantages

Inorganic salts easily removed

Short Method development time

Low Cost

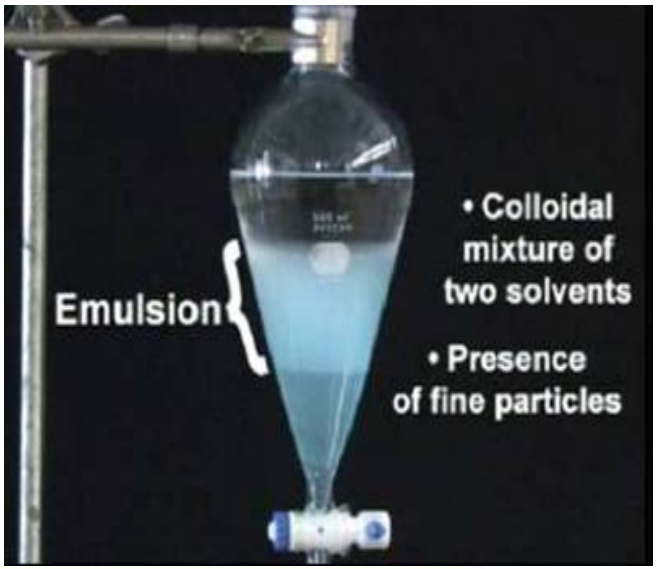
Disadvantages

Labor intensive

Large volumes of organics

Difficult to automate

Emulsion formation



Sample Preparation

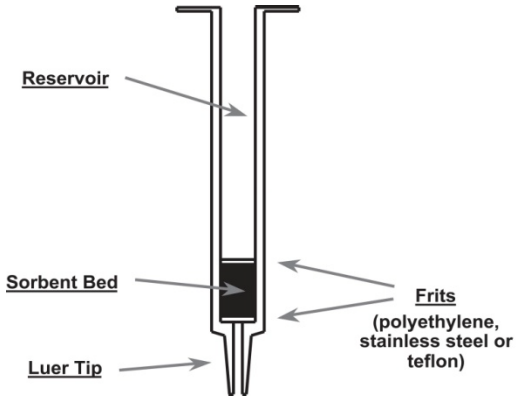
- SPE: Solid phase extraction

Advantages

Very selective
Effective with variety of matrix
Concentration effect
High recoveries
High reproducibility

Disadvantages

Greater complexity/difficult to master
Lengthy method development
Costly
Many choices



Sample Preparation

- Combinations of sample preparation techniques
  - LLE with SPE
  - GPC with SPE
  - Soxhlet extraction with SPE

•Increase the sample preparation time and possible error

QuEChERS
(Pronounced *"catchers"*)

- **Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, and **S**afe
- Portmanteau: blend of 2 or more words
- ***www.quechers.com***

- Introduced in 2003: M. Anastassiades, S.J. Lehotay, D. Stajnbaher, and F.J. Schenck, J. AOAC Int 86 (2003) 412
- Validated in 2005, with subsequent modification in 2007
    - AOAC 2007.01 and European Method EN 15662

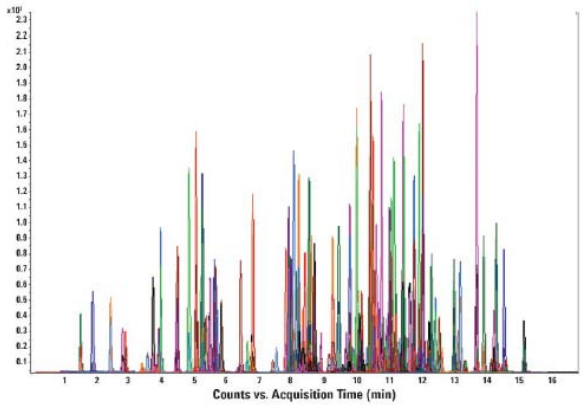
- Streamlined approach that makes it easier and less expensive to examine pesticide residues in food

QuEChERS

- Alternative to existing methods: LLE, SPE, GPC
- QuEChERS is still a very young; being adopted worldwide
- Detector availability: MS and MS/MS (selective and sensitive)
- Automated solution for QuEChERS on the market today: ChemSpeed, Gerstel, and Anatune
- QuEChERS process substantially decreases cost per sample

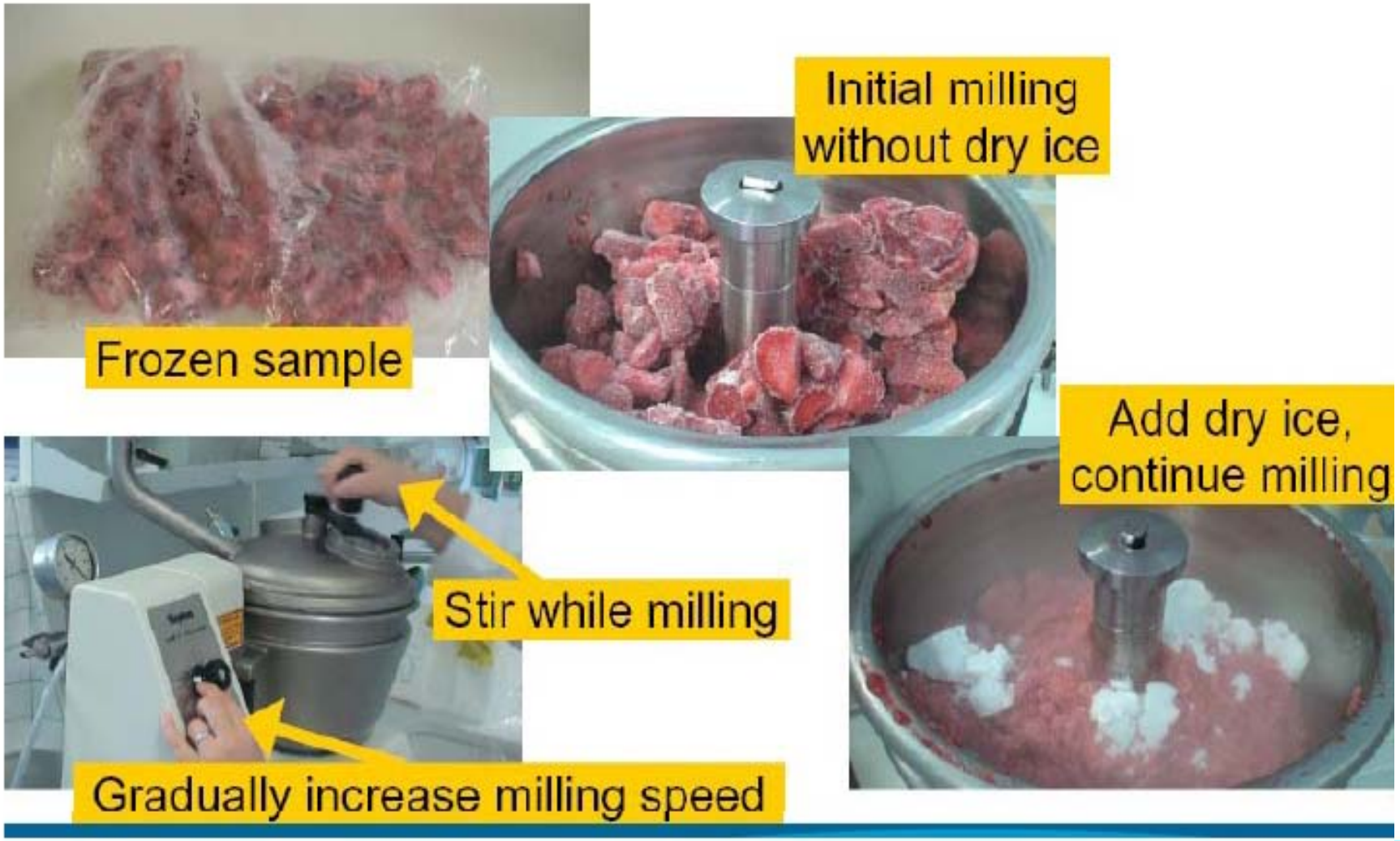
	Luke method, traditional SPE, or GPC	QuEChERS	QuEChERS Benefits!
Estimated Time to process 6 samples (min)	120	20	6 x faster
Solvent Used (mL) per sample	90 mL	10-15mL	9 x less solvent
Chlorinated Waste (mL)	30 mL	none	safer, greener, less costly
Glassware/ specialized equipment	Clean Separatory funnels, water bath, 200mL containers, evaporator, etc.	None	No additional supplies needed

We Can't Do This……



We Can Do This……QuEChERS

QuEChERS: Prepping the Sample for Processing



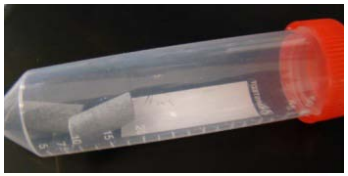
QuEChERS: Prepping the Sample for Processing

- Representative sampling
- Homogenizing the sample
  - Uniform size
  - Uniform distribution
  - High surface area

QuEChERS Procedure:

3 Steps: 1.Extraction
2.Dispersive
3.Analysis

1. Add homogenized sample to tube,
   Add 2 ceramic homogenizers
2. Add ACN, vortex
4. Add Extraction packet
   Shake, Shake, Shake!
6. Centrifuge
8. Transfer to dispersive-SPE
9. Vortex, Centrifuge
11. Analyze



Improvements to the Extraction of Samples

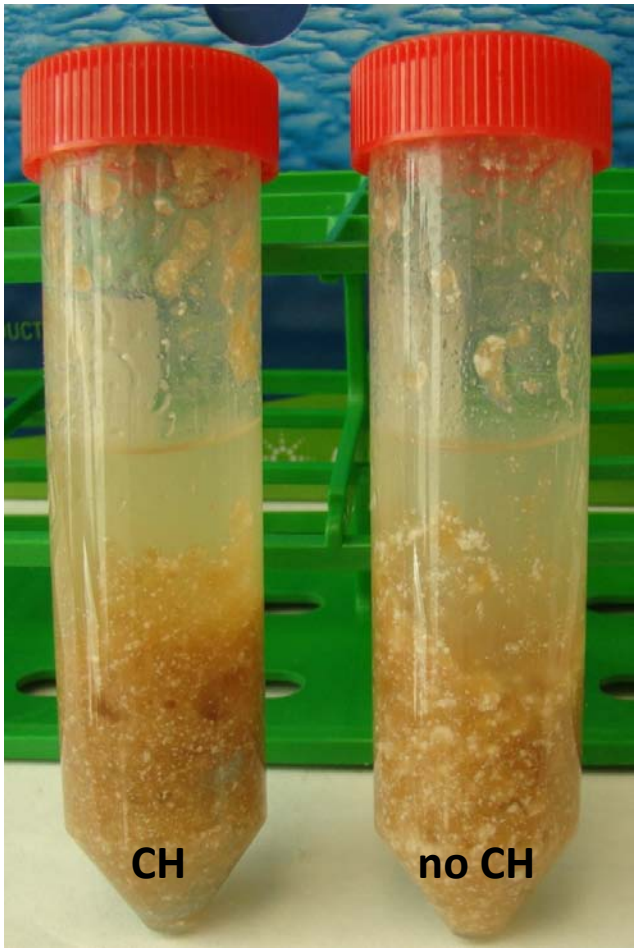
- ***Step 1: Extraction salts with comminuted fruit/vegetable***
  - Consistency in shaking, everyone shakes differently
  - Variability in QuEChERS applications, recovery and RSDs

–SampliQ Ceramic Homogenizers

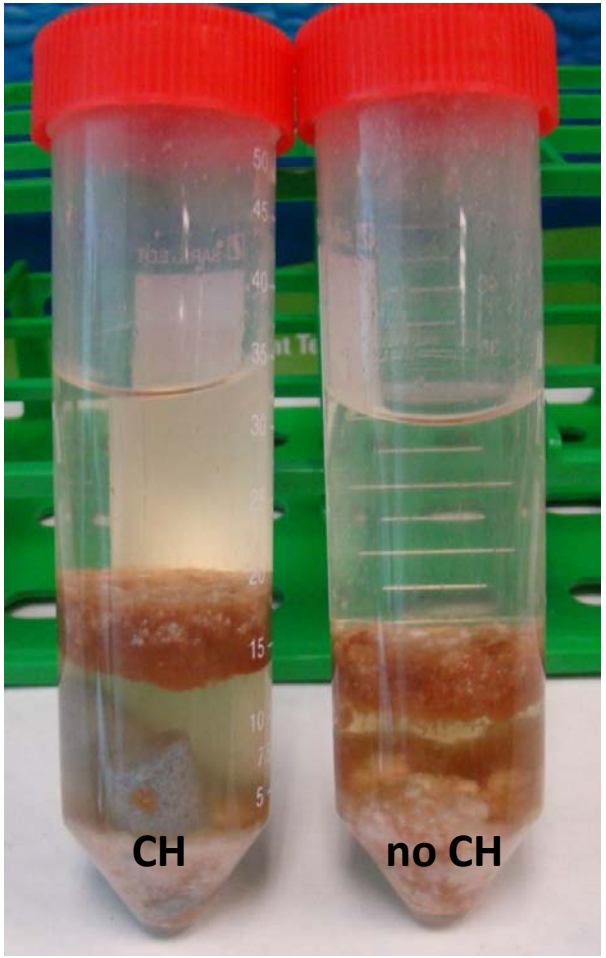
- **Reduces shaking time from 1 minute to <20 seconds!**
- Consistent extraction of the sample with the salts
- Breaks up salt agglomerates
- Facilitates homogenization with angle cut
- Increase recovery/reduce RSDs of pesticides from sample
- 3 different sizes: 50 mL extraction tube and 2 dispersive tubes 15 and 2 mL

QuEChERS Extraction: With and Without Ceramic Homogenizers

Before Centrifuge

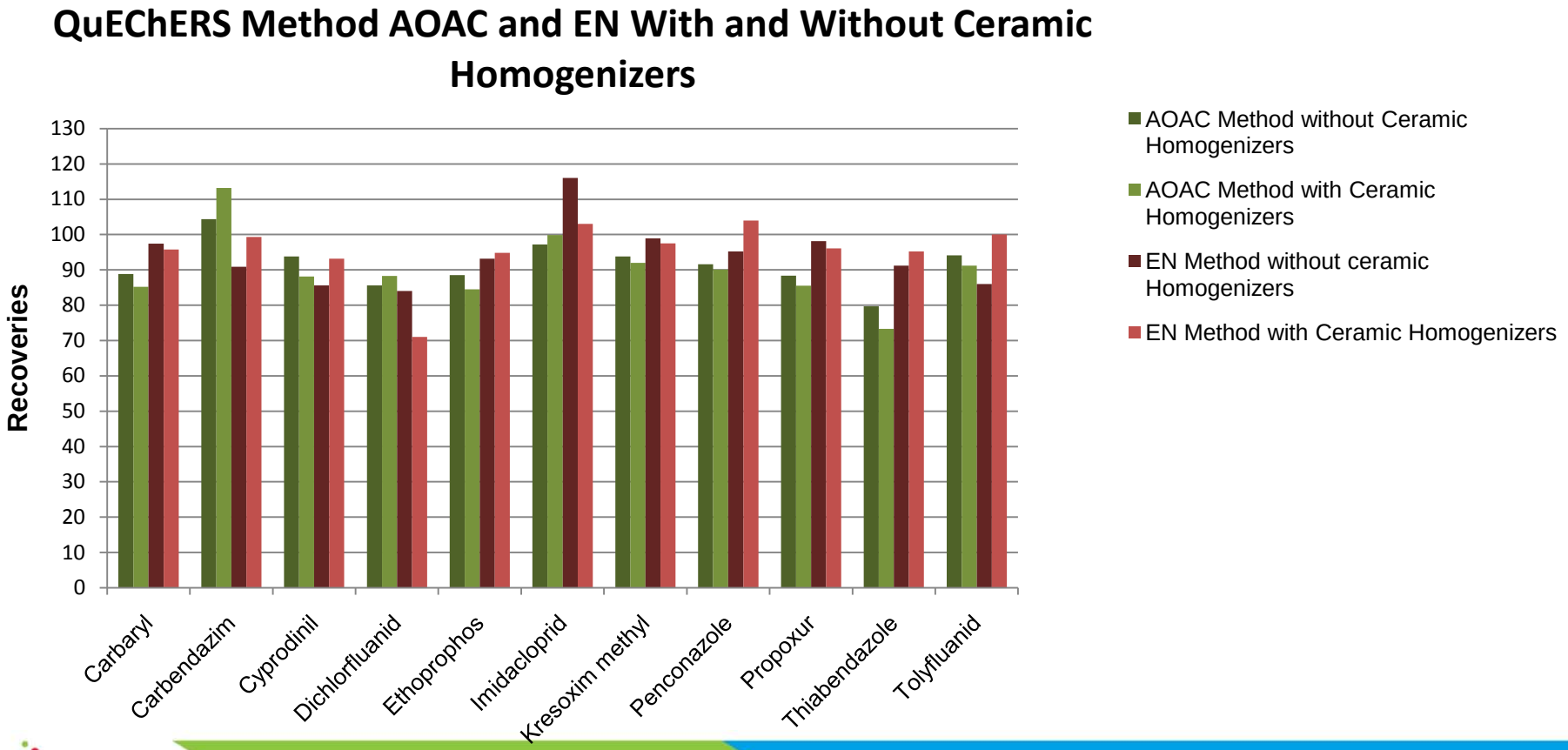


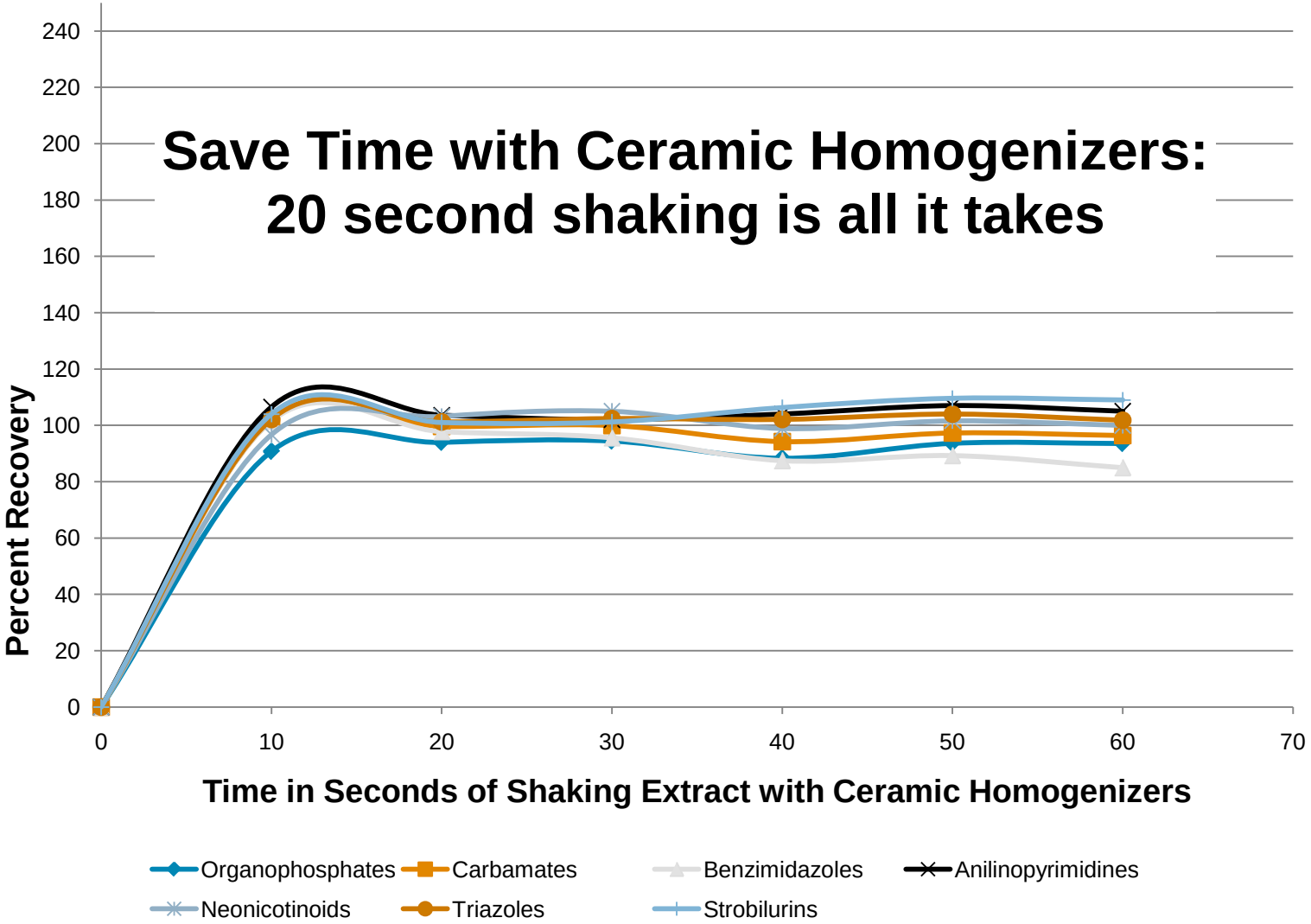
After Centrifuge



Ceramic Homogenizers

- Inert ceramic material
- No loss in pesticide recovery
- Consistent recovery with RSD





Pesticides used in study: Acephate, Carbaryl, Carbendazim, Cyprodinil, Imidacloprid, Imazalil, Methamidophos, Penconazole, Propoxur, Pymetrozine, Thiabendazole, Thiophanate-methyl, Ethoprophos, Kresoxim-methyl ; Apple matrix

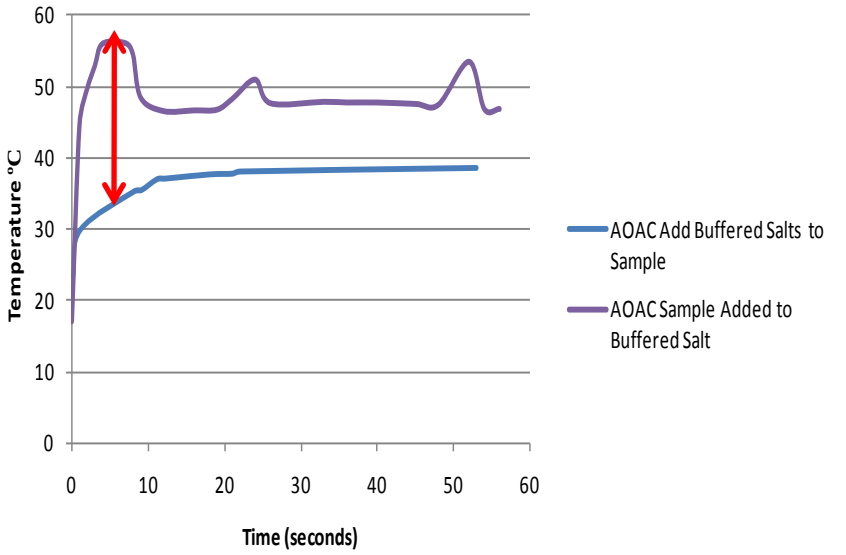
Extraction Kit Packaging

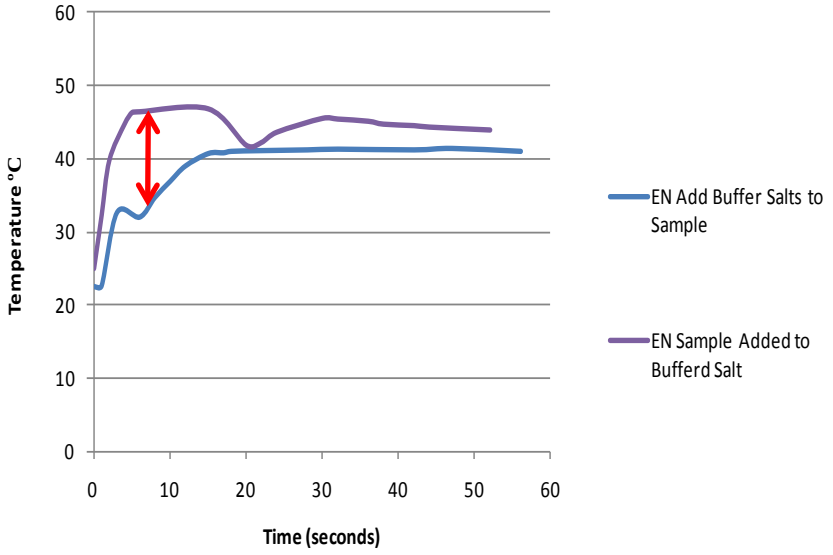
- Water and UV resistant packaging 3 layer material
- Automated packing of the salts
- Salts packed under nitrogen
- Pourable
- Minimal clumping of salts
- Ingredients listed on packet

Packaging salts separately allows customers to add sample before adding salts

- Prevents exothermic reaction
- Prevents degradation of sample
- Ensures maximum recoveries

Temperature Graphs from AOAC and EN Extraction Kits

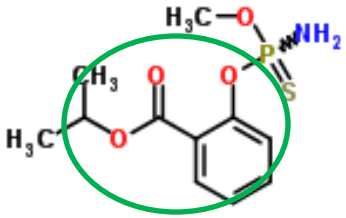




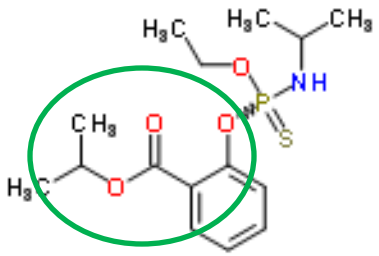
Represent the thermal data acquired from the AOAC and EN extraction kits. A substantial increase in temperature is observed (increase of approx 25 °C for the AOAC method and approx 10 °C for the EN method) when the produce sample is added to the extraction salt in the 50 mL PP tube versus when the extraction salt from an anhydrous pouch is added to the produce sample

Pesticides used to Evaluate the Effect of Temperature on Recovery

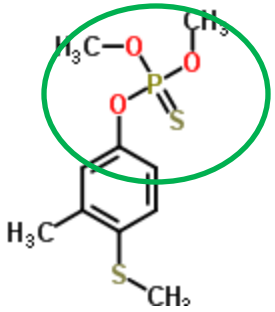
The following three pesticides were used to evaluate the thermal effects that are prevalent when the produce is added to the extraction salts



Isocarbophos



Isofenphos

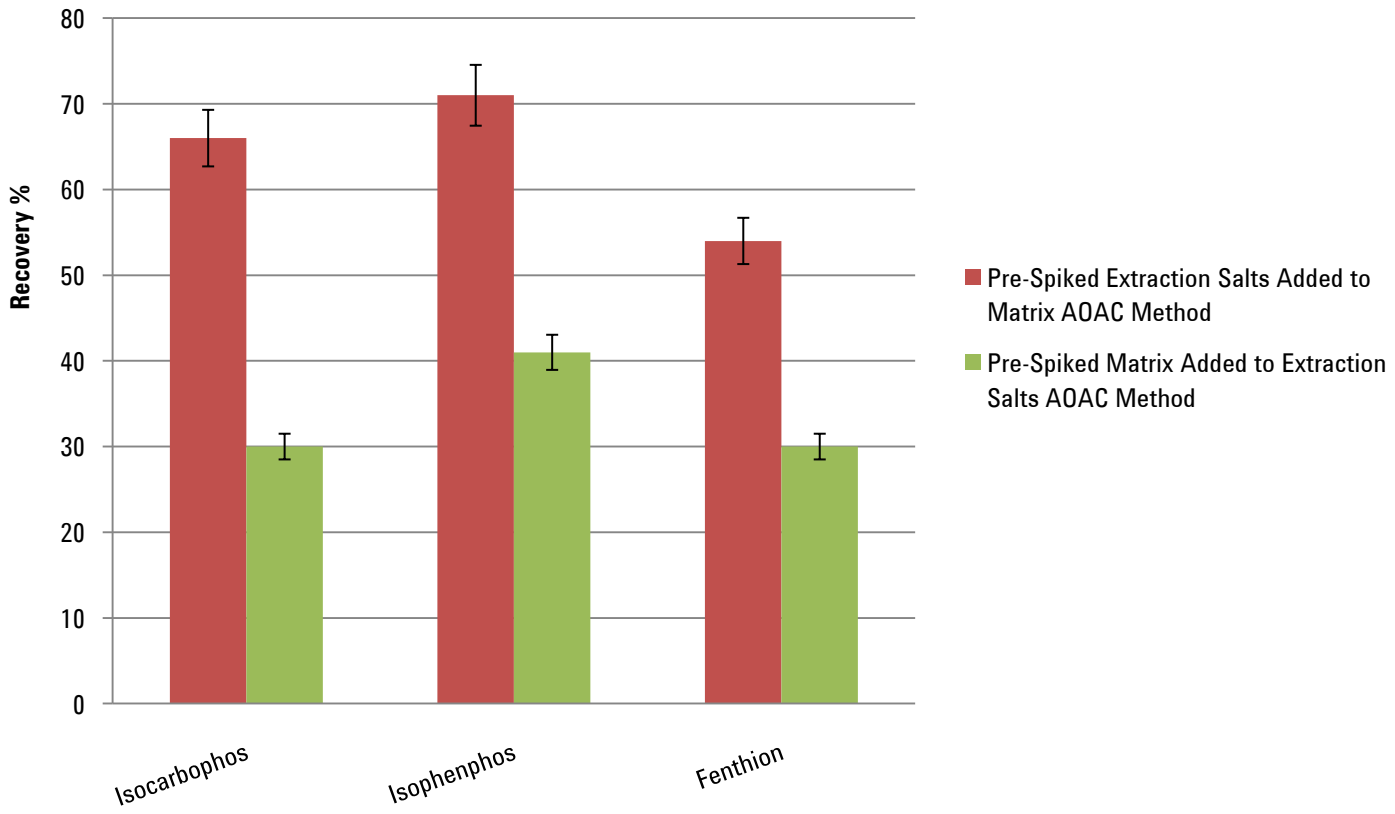


Fenthion

The 3 pesticides were chosen based on their labile chemical structures

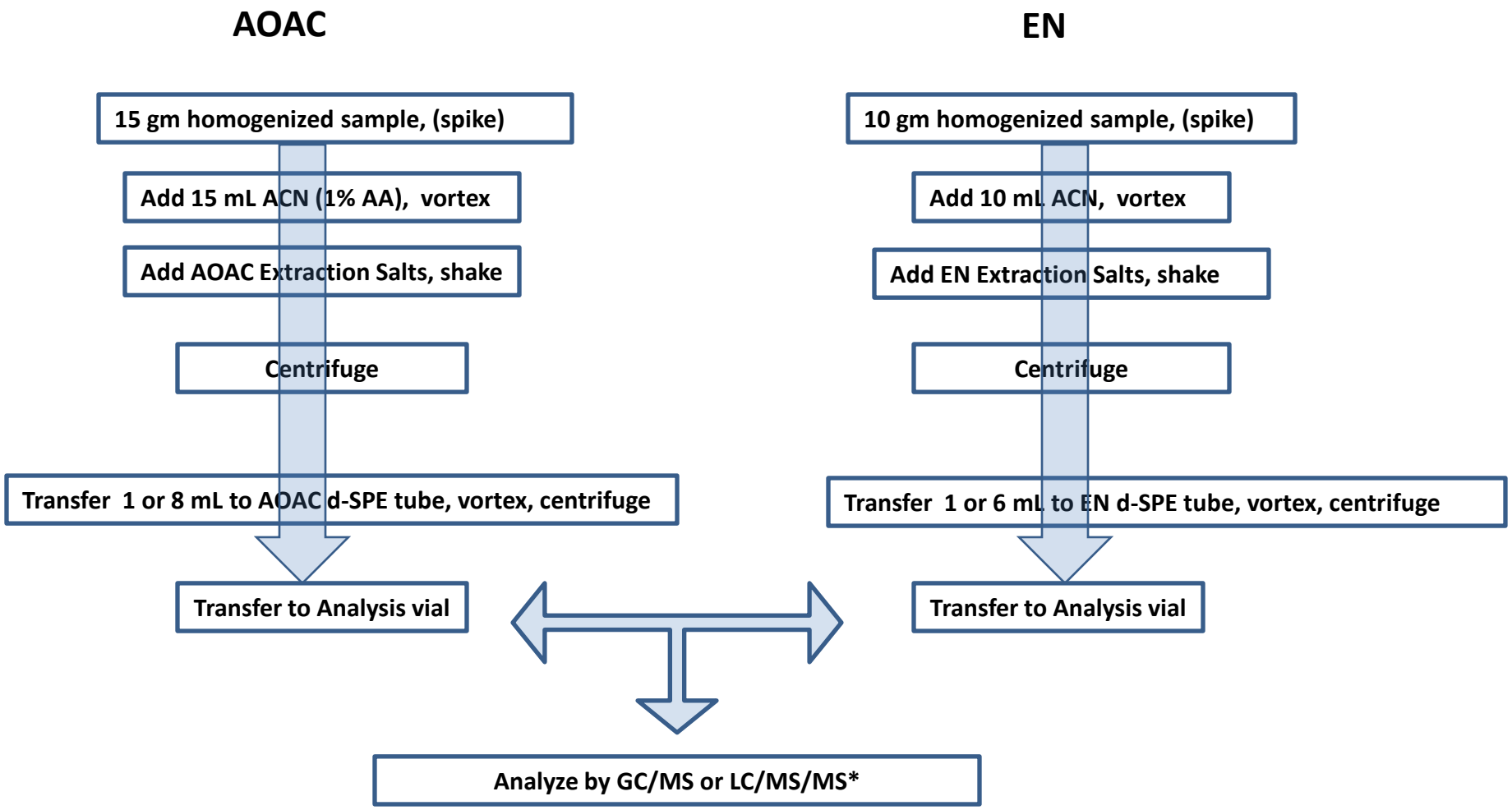
Analysis was performed on a Agilent 6410 LC/MS/MS

Effect on Recovery



Negative effect adding the homogenized sample to the salt has on recovery during the QuEChERS extraction step, similar results for AOAC and EN extraction salts

QuEChERS Extraction Flow Chart



* Requires a dilution prior to analysis

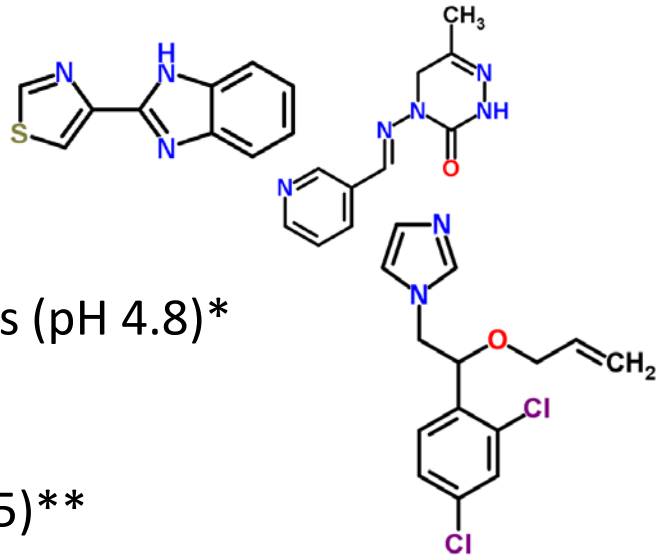
3 Types of Extraction Salts Used: First Step How to Choose?

- Original QuEChERS method (unbuffered)
  - 4 or 6 g $MgSO_4$, 1 or 1.5 g NaCl
- AOAC method 2007.01 (AOAC)
  - 6 g $MgSO_4$, 1.5 g Na Acetate
- EN method 15662 (CEN)
  - 4 g $MgSO_4$, 1 g NaCl 1 g NaCitrate, 0.5 g disodium citrate sesquihydrate

Historical Perspective: Extraction Salts

- Unbuffered method first published in 2003
- 2 validated versions
  - AOAC official method 2007.01
  - EN official method 15662
    - ***www.quechers.com***
- All 3 methods give excellent results: average 98% recoveries with 10% RSDs
- Unbuffered method have a negative effect on few pH-dependent pesticides

Ionic Strength: Salt Buffering



- AOAC buffered method
  - Relatively strong acetate buffering conditions (pH 4.8)*
- EN buffered method
  - Weaker citrate buffering conditions (pH 5-5.5)**
- Bother versions went through extensive interlaboratory trials ~ 50,000 – 100,000 data points
- Buffering at pH 5 during extraction offers optimum balance to achieve acceptable high recoveries (> 70%) for certain pH-dependent pesticides (e.g. pymetrozine, imazalil, thiabendazole)

*S.J. Lehotay, K. Mastovska, A.R. Lightfield, J. AOAC Int. 88 (2005), 615-629 & 60A

** M. Anastassiades, E. Scherbaum, B. Tasdelen, D. Stajnbaher, in: H. Ohkawa, H. Miyagawa, P. W. Lee (Eds.), Crop Protection, Public Health, Environmental Safety, Wiley-VCH, Weinheim, Germany, 2007, p.439.

Three Methods: How To Choose

- Work equally well
- Matrix co-extractions
    - Evaluate
        - Matrix effects on quantification
        - Chemical noise from matrix

Matrix Effects:

- *S. Lehotay: J. Chromatography A 1217 (2010), 2548-2560*
  - "Slight differences in color and color intensity: AOAC versus EN"
  - Not possible to determine that one extract was actually "cleaner" than another by visual appearance

1) Determine by gravimetric measurements
2) Chromatography
3) Determination of matrix effects on quantitation, ion enhancement/suppression

- Extract: Apple-blueberry sauce: unbuffered = 0.23%
  citrate buffered = 0.17%
  acetate buffered = 0.13%

" Analytical methods are like toothbrushes, everybody uses their own"

QuEChERS approach is very FLEXIBLE

- Template for Modifications
- Depend on analyte properties
- Matrix composition
- Instrumentation
- Analytical techniques

QuEChERS: Rule of Thumb

*Use matrix match calibration standards

Extent of matrix effects can be measured

- Compare calibration standards of same concentration in solvent to those in matrix extracts (~ 16%)
- The matrix can negatively or positively effect individual compounds

*Extraction Choice

- AOAC versus EN versus Original method
  - identify the compounds of interest
  - pH stability
  - matrix co-extractants

Step 2: Choose the dispersive-SPE (d-SPE) based on matrix and analytes

- $MgSO_4$ is found in all dispersive kits, removal of remaining water
- PSA* for removal of organic acids
- C18 for removal of fat, and lipids
- GCB* for removal of pigment, chlorophyll

- dispersive-SPE (d-SPE)

  C18 had greater impact than PSA for the matrix cleanup in both EN and AOAC method; improving performance, without negatively effecting recoveries

►Universal d-SPE (dispersive-SPE)◄

- 50 mg PSA, 50 mg C18, 7.5 mg GCB, 150 mg $MgSO_4$ (2 mL)*
- 400 mg PSA, 400 mg C18, 60 mg GCB, 1200 mg $MgSO_4$ (15 mL)*

- Cleanest extract, for all matrices without unacceptably affecting recoveries even for structurally planar pesticides

(*S. Lehotay: J. Chromatography A 1217 (2010), 2548-2560)*

*Available through Agilent

* PSA = Primary Secondary Amines GCB = Graphitized Carbon Black

QuEChERS Modifications:

- Low water content products
  - fish, meats, grains, rice, spices, oils
  - add water to initial homogenized sample
  - facilitates the partitioning and compounds of interest into the ACN layer

- Very Polar Compounds
  - Modifications with the extraction solvent
  - Addition of methanol facilities partitioning of polar compounds into the organic layer

- Oily Matrixes
  - Addition of hexane to remove fat, discard hexane

- GC/MS matrix issues
  - Serial d-SPE to add additional clean-up without recovery loss

Instruments Used in the Analysis

- LC/UV/FLD
  - Based on compounds being analyzed
- LC/QQQ
  - Selectivity and sensitivity
- GC/MS and GC/MS/MS
  - Selectivity and sensitivity
  - Back flush strongly suggested
  - Large volume injections
  - Solvent exchange: Not the first choice, but an option

QuEChERS: Current Food Research Areas

- Food Contamination
  - Agrochemical
    - chemicals used in agriculture practices and animal husbandry
    - pesticides, plant growth regulators, veterinary drugs, bovine somatotropin (rBST)
  - Environmental
    - Chemicals are in the environment where food is grown, harvested, transported, stored, packaged, processed, and consumed
      - PAH, PCB, dioxins, PBDE
      - Mycotoxins, toxins

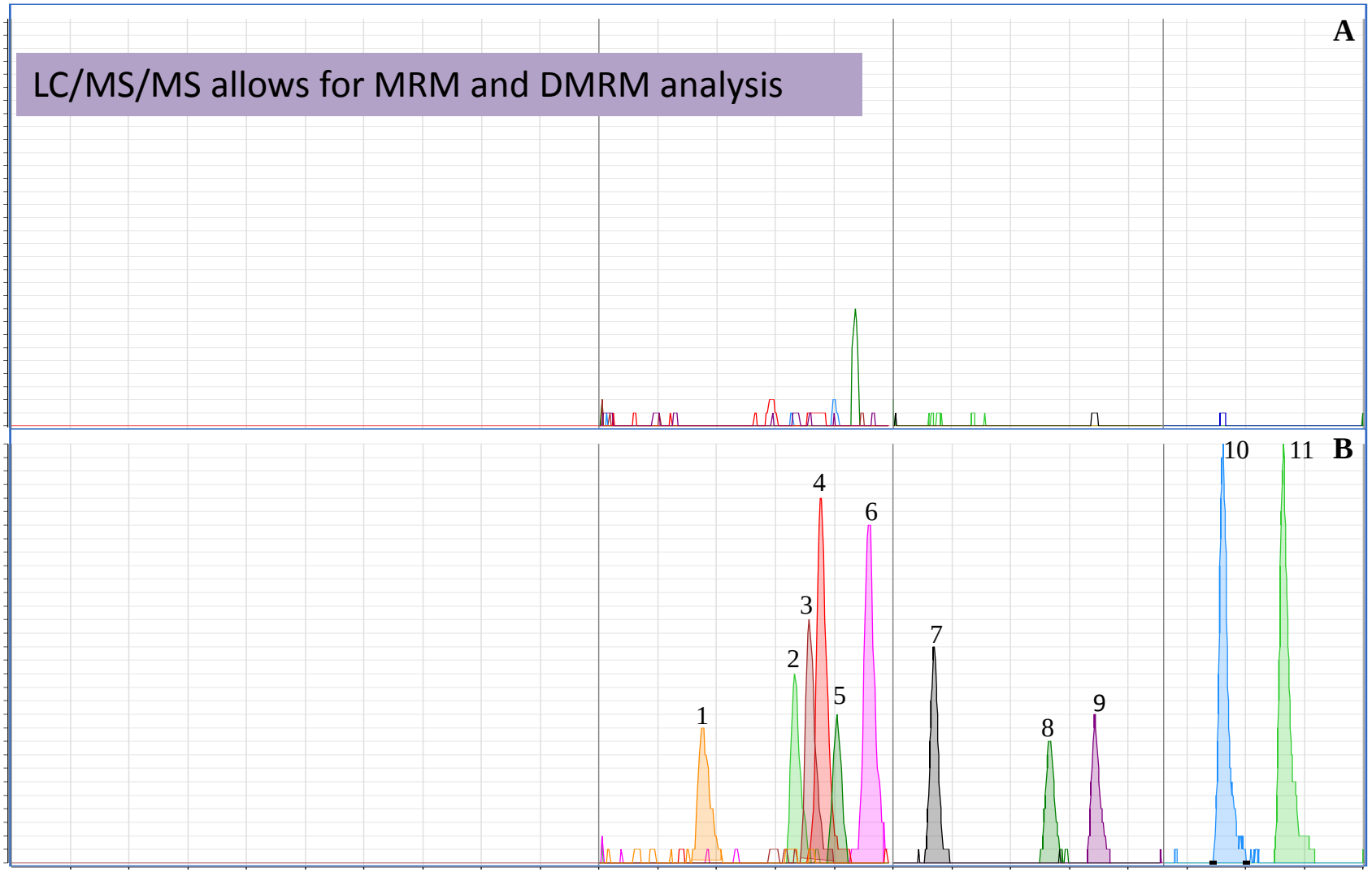
QuEChERS Applications: Beyond Produce

Application	Literature
Veterinary Drugs (antibiotics) in animal tissues	5990-5085EN, 5990-5086EN, 5990-5395EN
PAHs in Fish	5990-5441EN, 5990-6668EN
Pesticides in Olive Oil	5990-5553EN
PAH's in Soil	5990-5452EN, 5990-6324EN
Acrylamides in Fried Food and Oil	5900-5940EN, 5990-5988EN
Pesticides in Baby Food	5990-5028EN
Pesticides in Green Tea	5990-6400EN
PCBs in Fish and Fish Oil Supplements	5990-6236EN
Universal d-SPE	5990-6558EN
Hormones in Shrimp	5990-6589EN

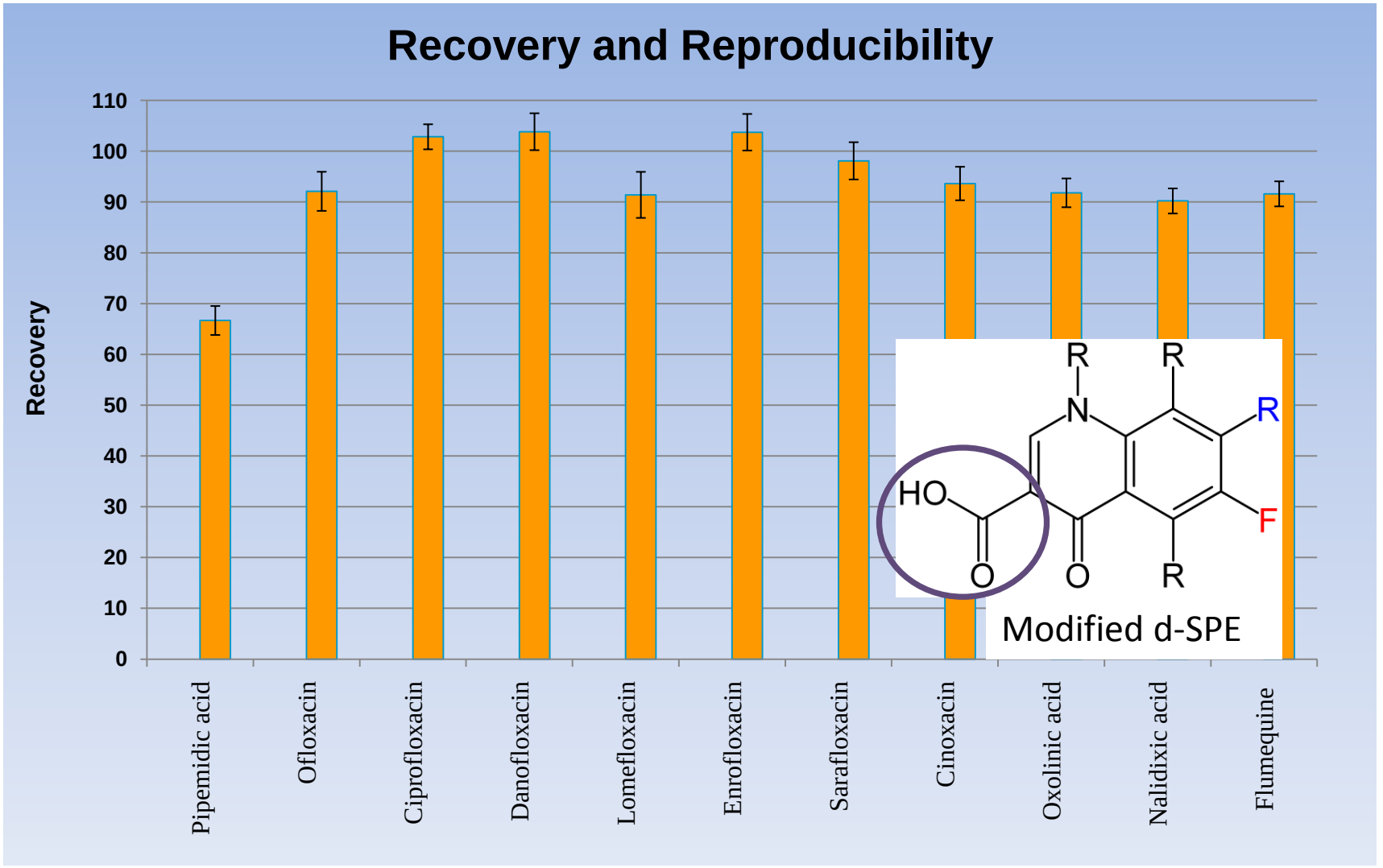
Learn more at www.agilent.com/chem/SampliQ

QuEChERS: Extraction Matrices and Analysis Examples

LC/MS/MS allows for MRM and DMRM analysis

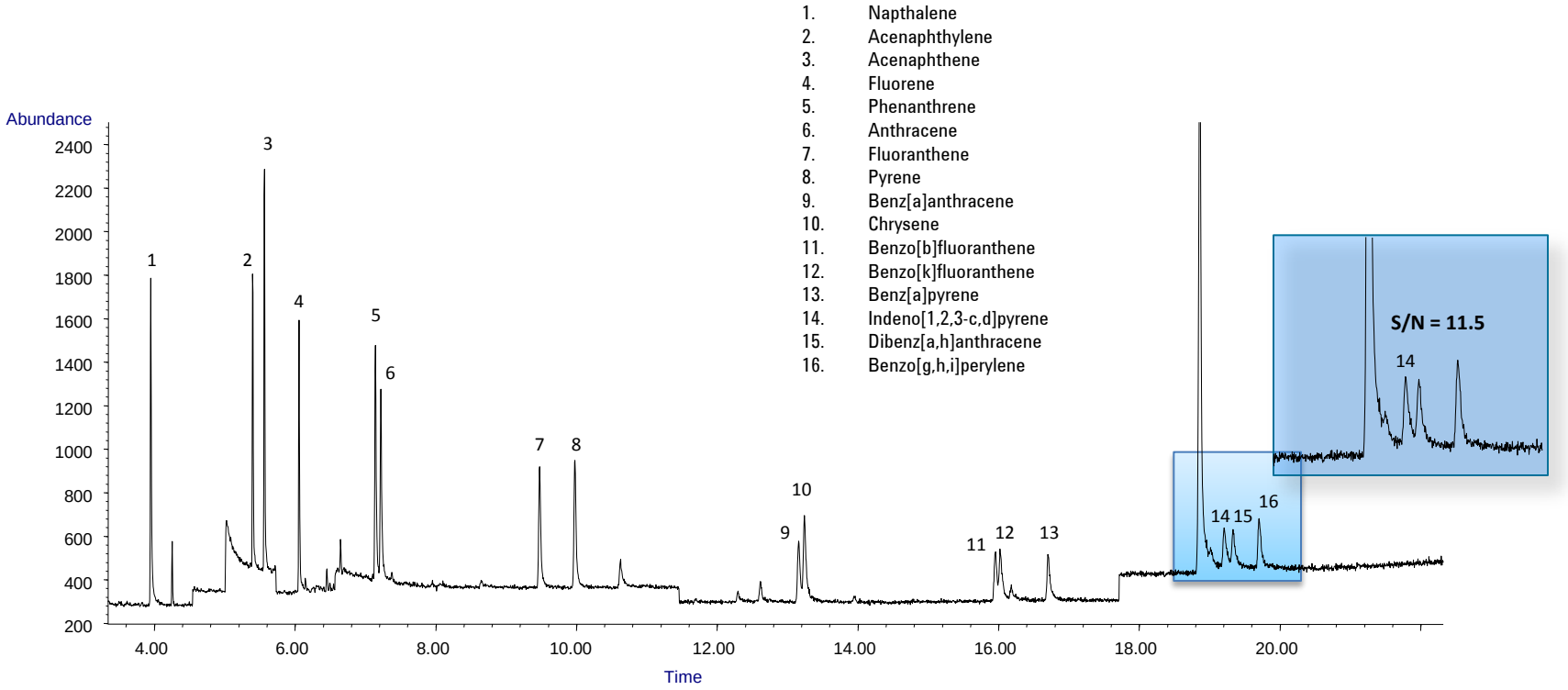


LC/MS/MS Chromatograms of A) liver blank extract, and B) 5 ng/g fortified liver extract (LOQ). Peaks identification: 1. Pipemidic acid, 2. Ofloxacin, 3. Ciprofloxacin, 4. Danofloxacin, 5. Lomefloxacin, 6. Enrofloxacin, 7. Sarafloxacin, 8. Cinoxacin, 9., Oxolinic acid, 10. Nalidixic acid, 11. Flumequine



Recoveries for the 11 Quinolones from Bovine Liver: d-SPE with C18 and $MgSO_4$, no PSA

Excellent Signal to Noise Ratios at Trace Levels



GC/MS SIM Chromatogram of 10 ppb PAHs spiked in fish matrix blank extract

GC/MSD:	7890/5975B with purged ultimate union
Column:	**DB-5ms UI 20 m 0.18 mm 0.18 µm**
Restrictor :	Siltek 0.7m x 0.15mm ID (Col 2)
MMInlet:	0.5µL, 320°C, splitless , purge flow 50mL/min at 0.8min gas saver 30mL/min at 2min
Carrier:	Helium, 1.7mL/min cnst flow col 1 PCM 1=3.8 psi cnst pressure, col 2=3.8ml/min flow @ 50°C
Oven:	50°C (0.4 min) to 195°C (25°C/min) hold 1.5 min, 8°C/min to 265°C 20°C/min to 315°C (1.25 min) *Postrun backflush 7 min @320°C*
MSD:	Transfer line 340°C Source 340°C Quad 150°C

Recovery and Repeatability of PAHs in Fortified Red Snapper Fish with Agilent J&W DB-5ms UI column

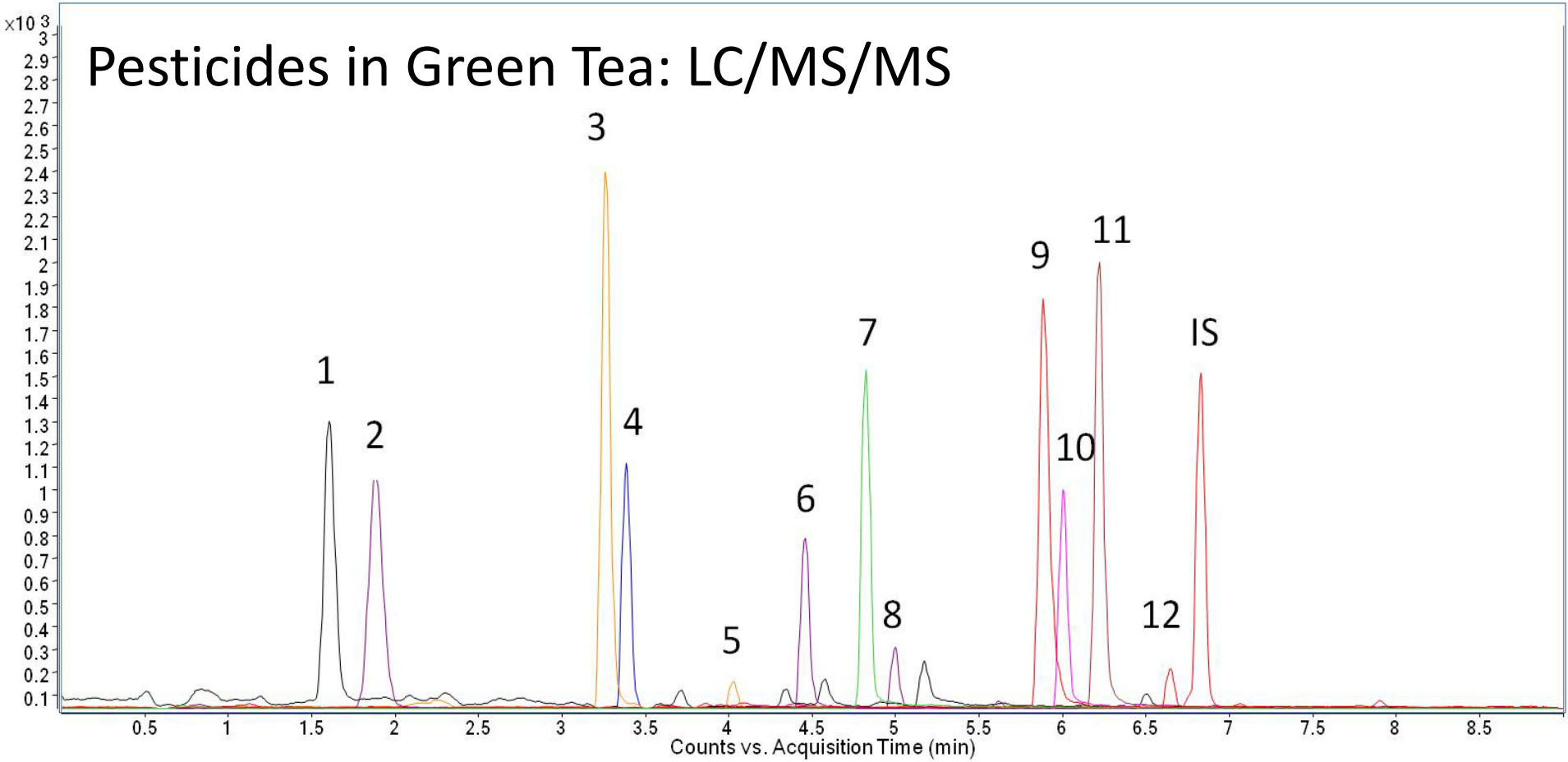
Analytes	25 ng/mL fortified QC		250 ng/mL fortified QC		500 ng/mL fortified QC	
	%Recovery	RSD (n=6)	%Recovery	RSD (n=6)	%Recovery	RSD (n=6)
Naphthalene	80.35	3.29	96.77	4.23	98.64	1.88
Acenaphthylene	95.28	2.30	103.36	2.80	101.02	2.27
Acenaphthalene	92.28	2.51	101.18	2.87	100.69	2.34
Fluorene	95.98	2.99	105.94	2.82	105.00	1.28
Phenanthrene	100.51	3.46	104.93	2.71	103.25	1.70
Anthracene	107.38	3.51	105.95	3.45	105.38	1.74
Fluoranthene	113.27	3.87	105.76	3.33	103.64	1.81
Pyrene	113.55	3.51	103.99	3.24	102.29	1.94
Benz[a]anthracene	129.79	3.41	101.45	3.91	100.61	3.24
Chrysene	116.75	4.01	98.55	4.17	95.95	5.61
Benzo[b]fluoranthene	131.20	3.70	98.77	4.08	98.08	3.24
Benzo[k]fluoranthene	139.45	2.52	99.13	3.98	95.31	4.54
Benzo[a]pyrene	125.30	3.68	95.33	3.89	96.82	1.80
Indeno[1,2,3-cd]pyrene	119.51	3.47	94.57	3.23	93.71	2.55
Dibenz[a,h]anthracene	126.35	3.54	98.55	3.50	98.85	2.24
Benzo[g,h,i]perylene	114.91	4.93	97.30	3.37	95.63	1.83

FDA: PAH in Selected Seafood and Surfactant in Seafood

http://www.fda.gov/downloads/ScienceResearch/UCM220209.pdf

http://www.prnewswire.com/news-releases/noaa-and-fda-announce-chemical-test-for-dispersant-in-gulf-seafood-106323493.html

Pesticides in Green Tea: LC/MS/MS



MRM chromatograms of 50 ng/g fortified sample processed by EN method. Peak identification: 1. Acephate, 2. Pymetrozine, 3. Carbendazim, 4. Thiabendazole, 5. Imidacloprid, 6. Imazalil, 7. Propoxur, 8. Carbaryl, 9. Cyprodinil, 10. Ethoprophos, 11. Penconazole, 12. Kresoxim-methyl, IS: TPP

Recovery and Reproducibility of Pesticides in Fortified Green Tea with QuEChERS

Analytes	10 ng/g fortified QC		50 ng/g fortified QC		250 ng/g fortified QC	
	Recovery	RSD (n=6)	Recovery	RSD (n=6)	Recovery	RSD (n=6
Acephate	80.5%	5.4%	91.7%	2.9%	88.9%	8.2%
Pymetrozine	43.1%	3.0%	42.2%	3.4%	43.4%	9.8%
Carbendazim	114.6%	11.6%	97.6%	2.0%	105.0%	6.2%
Thiabendazole	98.1%	6.9%	90.4%	2.4%	81.7%	5.8%
Imidacloprid	104.3%	11.7%	108.6%	2.5%	93.9%	7.9%
Imazalil	97.5%	4.4%	87.8%	5.6%	92.4%	4.6%
Propoxur	98.1%	2.4%	110.2%	1.7%	107.8%	3.9%
Carbaryl	89.7%	11.4%	104.9%	3.3%	108.1%	5.2%
Cyprodinil	84.9%	2.1%	92.5%	3.7%	93.9%	5.5%
Ethoprophos	103.4%	3.1%	111.2%	3.2%	104.9%	5.7%
Penconazole	108.7%	2.9%	94.3%	4.5%	89.8%	3.3%
Kresim-methyl	105.7%	12.4%	96.4%	2.5%	99.2%	5.5%

Agilent QuEChERS Options, Information and Kits

Easy Product Selection: QuEChERS Kits Identified by method and matrix

Lit number: 5990-3562EN

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

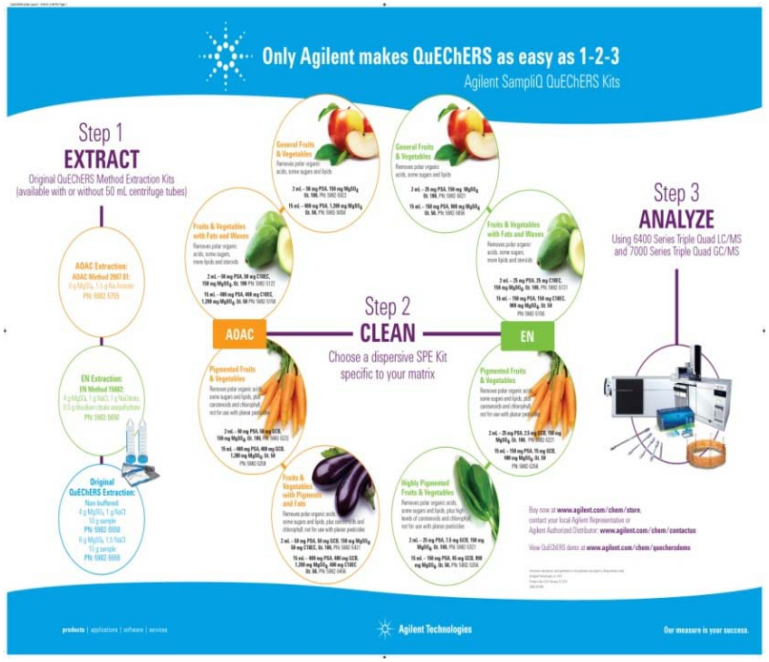
Lit number: 5990-5324EN

QuEChERS Food Application "Notebooks":

Lit number: 5990-4977EN

Technical support: Over 33 application notes on QuEChERS

– All available on our website: www.agilent.com/sampliQ



Conclusion: QuEChERS Advancements

- ***QuEChERS methodology easy as 1-2-3!***
- ***"Just enough sample preparation"***
- ***Minimal sample preparation expertise required: "If you can pipette and shake you can do QuEChERS"***
- ***Excellent results***
- ***Green and very cost effective technique***