

# *The Chemistry of* **QuEChERS**

**Quick, Easy, Cheap, Effective, Rugged  
and Safe Method for Determining  
Pesticide Residues**



# Fruits and Vegetables



# Milk and Honey



# Tobacco



# Animal Tissues



- Pronounced Catchers
- **Quick, Easy, Cheap, Effective, Rugged & Safe**
- Published in 2003 by Anastassiades et al
- Multiple residue method (MRM) of choice for pesticides analysis
- Used in a full range of agricultural products
  - honey
  - soybeans
  - animal feeds
  - nuts
  - milk
  - dried foods with hydration
  - Animal tissue

# QuEChERS

- Consists of a liquid-liquid micro extraction
- Partitioning and extraction of polar analytes is aided by  $\text{MgSO}_4$
- The preferred solvent is acetonitrile
- Acetonitrile provides extraction of the broadest range of organic compounds without co-extraction of large amounts of lipophilic material and is highly compatible with GC/MS and LC/MS applications showing the fewest interferences
- Followed by dispersive SPE sample clean-up to remove unwanted matrix materials

**20 MINUTES!**

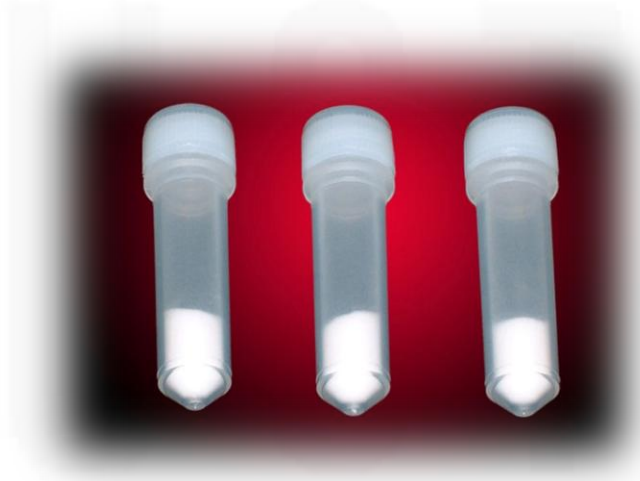
## The Three QuEChERS Methods

- The **original QuEChERS** method. Introduced in 2003, uses sodium chloride to reduce polar interferences
- **AOAC 2007.01**. Uses 1% acetic acid in acetonitrile, sodium acetate as a buffer replacing sodium chloride
- The **European version (EN 15662)** is similar to the AOAC method, except the extraction uses sodium chloride, sodium citrate dihydrate and disodium citrate sesquihydrate instead of sodium acetate. *Acetic acid is not used.*



# The Original Method

1. Weigh 15 g of homogenized (hydrated at least 80%) sample in a 50 mL centrifuge tube
2. Add 15 mL acetonitrile and IS
3. Shake briefly
4. Add 6 g of anhydrous magnesium sulfate and 1 g of sodium chloride
5. Shake by hand for 1 minute
6. Centrifuge at 3700 rpm for 1 minute
7. Transfer a 1 mL aliquot of supernatant to a 2 mL centrifuge tube containing 150 mg anhydrous magnesium sulfate and 50 mg PSA
8. Shake for 30 seconds
9. Centrifuge for 1 minute at 3700 rpm
10. Transfer 0.5 mL aliquot for analysis



1. Weigh 15 g of homogenized (hydrated at least 80%) sample in a 50 mL centrifuge tube
2. Add 15 mL 1% acetic acid in acetonitrile and IS
3. Shake briefly
4. Add 6 g of anhydrous magnesium sulfate and 1.5 g of anhydrous sodium acetate
5. Shake by hand for 1 minute
6. Centrifuge at 3700 rpm for 1 minute
7. Transfer an aliquot of supernatant to a 2 mL centrifuge tube containing 150 mg anhydrous magnesium sulfate and 50 mg PSA
8. Shake for 30 seconds
9. Centrifuge for 1 minute at 3700 rpm
10. Transfer aliquot for analysis and add TPP and either formic acid or toluene/mag. sulfate

# EN 15662

1. Add 10 mLs of ACN to 10 g homogenized/hydrated sample in a 50 ml centrifuge tube
2. Add ISTD
3. Add 4 g MgSO<sub>4</sub>, 1 g NaCl, 1 g Na<sub>3</sub>Citrate·2H<sub>2</sub>O, 0.5 g Na<sub>2</sub>HCitr·1.5H<sub>2</sub>O
4. Shake vigorously for 1 min
5. Centrifuge for 5 minutes at 3000 U/min
6. Transfer 1 ml aliquot of supernatant to a dispersive centrifuge tube containing 25 mg of PSA and 150 mg MgSO<sub>4</sub>, (plus 2.5 or 7.5 mg of GCB to remove pigments)
7. Shake for 30 seconds (5 minutes using GCB)
8. Centrifuge for 5 minutes at 3000 U/min.
9. Preserve with 5% formic acid in ACN.
10. Analyze by LVI/GC/MS or LC/MS/MS

## Advantages of Buffered Methods

- ❑ Extract buffered to protect base sensitive analytes (folpet, dichlorofluanid, chlorothalonil, Pymetrozine, Dicofol, captan, tolyfluanid)
- ❑ Extract protected
  - LC – formic acid (PSA is basic)
  - GC – toluene and magnesium sulfate (prevent thermal breakdown)

## Disadvantage of AOAC Method

- Acetic Acid
  - PSA removes organic acids
- Less cleanup
- Tailing issues on GC

### Additional Clean-up Options

- C18 – removes lipids
- Graphitized carbon black (GCB) – removes pigments (and planar compounds)
- ChloroFiltr<sup>®</sup> – removes chlorophyll
- Dual phase SPE cartridge (GCB/PSA)
  - Removes most pigments with minimum loss of planar analytes using 3:1 acetone toluene

# So, what's the difference?



In December 2008 USDA-ARS-ERRC performed a study comparing :

- Original QuEChERS
- EN 15662
- AOAC 2007.01

Apple-Blueberry Sauce, Peas, and Limes at 50, 250, and 1,000 ng/g  
Levels using Diazinon as the Internal Standard n=36

## Results

With the exception of base sensitive compounds, all three extraction methods gave the same results. Base sensitive compounds gave better recoveries with AOAC 2007.01.



# The Dual Phase Variation

- Matrix plant pigments often interfere with analysis
- To reduce these interferences, graphitized carbon can be added to the dispersive solid-phase clean-up tubes
- Carbon however may result in a loss of planar (polar aromatic) pesticides
- Cleanup of plant pigments with minimum loss of planar pesticides can be accomplished by using a dual-phase cartridge containing GCB and PSA

# Dual Phase Cartridge Clean-Up Procedure

1. Pre-rinse cartridge with 5 mL of toluene
2. Add an aliquot of the supernatant to the cartridge
3. Start collection
4. Elute with 6-12 mL of 3:1 acetone:toluene
5. Concentrate for GC/MS analysis or
6. Concentrate to dryness and reconstitute in mobile phase for LC analysis



1. **Purpose** – remove pigments
2. GCB binds planar analytes such as acephate, bromophos, carbendazim, chlorthiophos, cyprodinil, ethoprop, fonofos, leptophos, methamidophos pyrimethanil, and thiabendazole
3. **More GCB = Lower recovery of planar analytes**

# ChloroFiltr<sup>®</sup>

For the Clean-up of Chlorophyll from QuEChERS Extracts in Acetonitrile

1. QuEChERS spinach extract without clean-up
2. Spinach extract after clean-up with dispersive SPE ChloroFiltr<sup>®</sup> product **ECMPSSGG15CT** containing 900 mg Magnesium sulfate, 300 mg PSA and 150 mg ChloroFiltr in a 15 ml centrifuge tube. **3 ml of extract added to product, shaken and centrifuged.**



## BREAKING IT DOWN



# Acetonitrile

- Extracts the greatest number of analytes with the least number of interferences
- Can be used on GC and LC

# Acetic Acid

- 1% acetic acid in acetonitrile, when combined with **sodium acetate**, prevents base sensitive analytes from breaking down during extraction
- Compromises PSA
- Works best with LC/MS/MS analyses
- Tailing issues with GC

# Sodium citrate dibasic sesquihydrate and sodium citrate tribasic dihydrate

- Buffers extraction to prevent break down of pH sensitive analytes
- Does not require acetic acid
- Does not compromise PSA (primary secondary amine)



# Magnesium sulfate anhydrous

- In extraction, aids in partitioning and improves recoveries of polar analytes
- In clean-up, works as a desiccant



# Sodium Chloride

- Helps to reduce polar co-extractables
- Can reduce recovery of polar analytes

# Primary Secondary Amine (PSA)

- Used in clean-up to reduce levels of organic acids, sterols, some sugars and lipids

## ***Endcapped* C18**

- Used in clean-up to aid PSA in removal of hydrophobic interferants such as lipids

## Graphitized Carbon Black (GCB)

- Used in clean-up to remove pigments and polyphenols
- Will also remove planar analytes



# ChloroFiltr<sup>®</sup>

- A product developed by UCT to remove chlorophyll from acetonitrile extracts using dSPE
- Will not remove planar pesticides\*

\*with the exception of hexachlorobenzene

Do not use with mycotoxins



# Aminopropyl

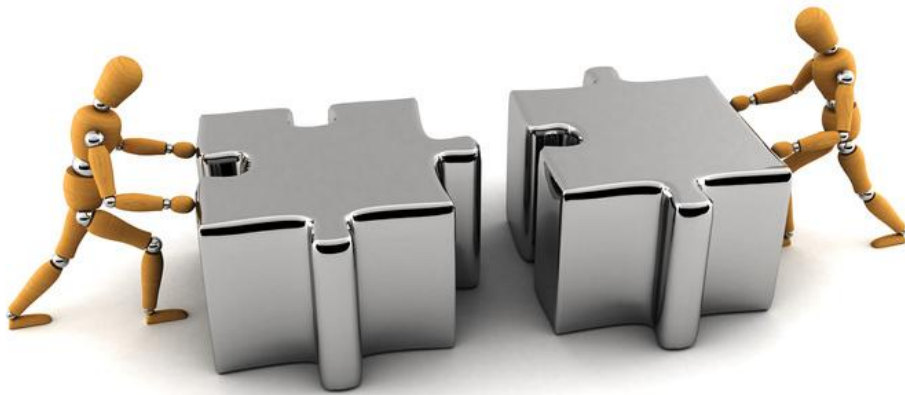
- Similar properties to PSA, but lower exchange capacity
- Less likely to damage base sensitive analytes

# Cartridge or Dispersive Clean-up???

- **Cartridge (not QuEChERS)**
  - Cleaner extracts
  - Takes longer
  - Uses more solvent
  - Requires a manifold and accessories
- **Dispersive (QuEChERS)**
  - Quick
  - Easy
  - Not as clean as a cartridge
  - requires a centrifuge



# PUTTING IT ALL TOGETHER



UCT

# The QuEChERS Method for Pesticide Residues



1) Shake sample with solvent and salts



2) Centrifuge for 1 min



3) Mix a portion with a sorbent



4) Centrifuge for 1 min



5) Analyze Pesticides

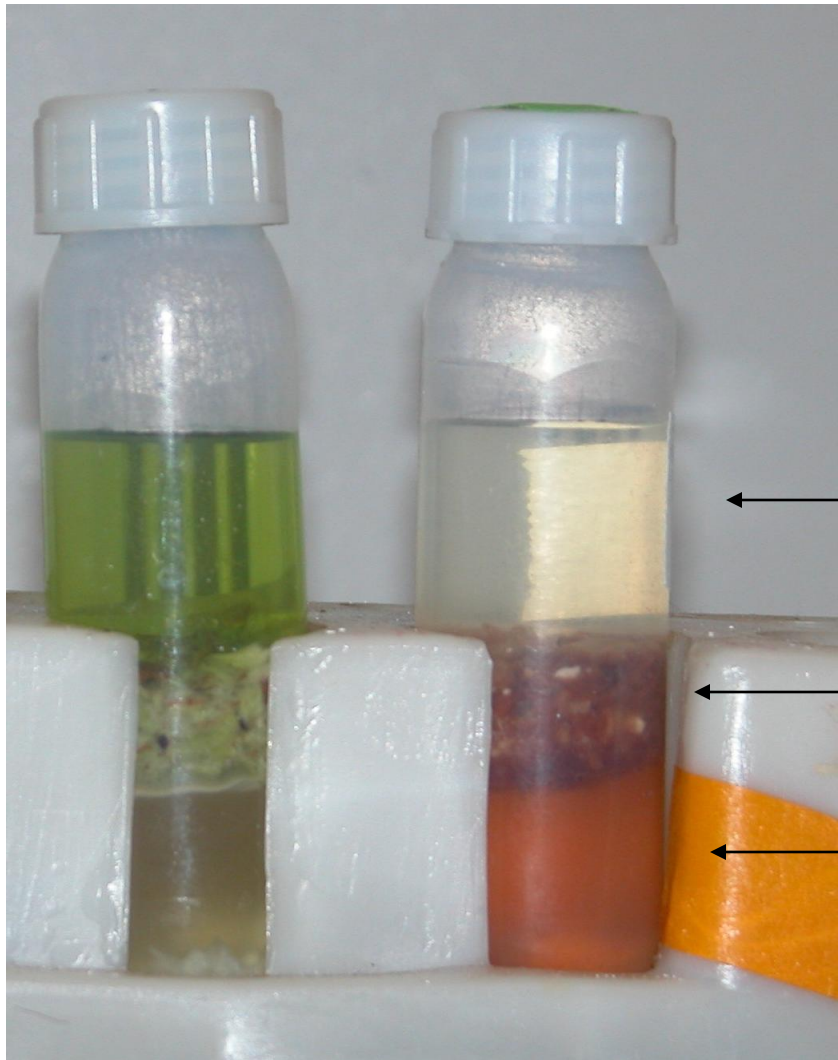
# Add hydrated vegetation to salts and solvent SHAKE



Slide courtesy of Frank Schenck, FDA

# Centrifuge





← MeCN Extract

← Plant Matrix

← Aqueous

Slide courtesy of Frank Schenck, FDA

## Key Steps

1. Hydrate samples to ~ 80% or higher
2. Add solvent prior to adding salts
3. Use internal standard
4. Use matrix matched calibration standards
5. Use buffering when necessary
6. Use analyte protectants (formic acid or toluene)
7. Beware of GCB

# Not QuEChERS?

- It must be **Quick**. GPC clean-up is not quick.
- It must be **Easy**. Any technician can perform.
- It must be **Cheap**. Solvent and glassware are not cheap.
- It must be **Effective**. QuEChERS recovers more analytes than any other approach.
- It must be **Rugged** (of course)
- **Safe** = No halogenated solvents. QuEChERS uses acetonitrile



# Applications Available from UCT

- Acrylamide Using a QuEChERS Approach
- Anthelmintic Drug Residues in Milk
- Cereal Grains by QuEChERS
- Extraction and Analysis of PAHs in Fish, Seafood and other Meat Products
- Pesticides in Fatty Matrices Extraction Procedure
- Extraction of Pesticides from Tomato using the QuEChERS Approach
- Pesticide and PAH Extraction of Grass and Other Leafy Vegetation by QuEChERS using ChloroFiltr Clean-up
- Streamlined Sample Preparation Method for Analysis of Several Antibiotics in Beef Kidney/Juice/Serum
- QuEChERS Extraction and Clean-Up of Pesticides from Olive Oil



- **QuEChERS Multiresidue Pesticide Method for the Determination of Multiple Pesticides in Wines**
- **Flukicides / Anthelmintics in Animal Tissues by QuEChERS**
- **QuEChERS Analysis of Miticides and other Agrochemicals in Honey Bees**
- **Trichothecene Type A & B Mycotoxin Analysis in Wheat and Corn Using the QuEChERS Approach**
- **QuEChERS Pesticide Analysis of Fresh Produce Using GC/MS/MS**

...with more on the way





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