

## How Much Cleanup is Required for the Analysis of Pesticide Residues in Foods?

Frank J. Schenck  
Southeast Regional Laboratory, FDA, Atlanta, GA  
Jon Wong  
Center for Food Safety and Applied Nutrition, FDA, College Park, MD

If a food sample is extracted with acetonitrile ca 0.2% of the sample matrix will be found in the supernatant.

It has been estimated that this extract extract will contain ca.  $10^5$  compounds at a concentration of 10 ppb or greater

## SAMPLE CLEANUP

### Pesticide Analysis Fruits and vegetables: Step #1

Extraction with:  
ACETONITRILE/H<sub>2</sub>O  
or  
ACETONE/H<sub>2</sub>O

### Pesticide Analysis (fruits and vegetables): Step #2

Remove water using:  
NaCl  
or  
NaCl + methylene chloride

## Pesticide Analysis (fruits and vegetables): Step #3

### CLEANUP

(optional)

## Pesticide Analysis (fruits and vegetables): Step #4

### DETERMINATION

GC with ESD or MSD  
LC with fluorescence or MS/MS

## History of Sample Cleanup and Pesticide Analysis?

- 1960's - Nonpolar chlorinated pesticides  
Electron Capture detector

### EXTENSIVE CLEANUP - FLORISIL

- 1975 - Polar and nonpolar pesticides  
Hall detector  
Flame photometric detector

### NO CLEANUP

## History of Sample Cleanup in Pesticide Analysis?

- 1990's - Capillary GC Columns  
Early Ion traps - Luke II

### Extensive SPE CLEANUP

- 2000's - GC/MS & LC/MS/MS

### NO CLEANUP

- 2010 ???

## Sample Cleanup?

"Therefore it is our opinion that all cleanup steps for the retention of matrix components by applying selective adsorption should be omitted because they are no longer necessary."

".....the new dimension of detection sensitivity of modern mass spectral detectors makes them superfluous."

H.-J. Stan (2000)

## Sample Cleanup/or not?

H.-J. Stan (*J Chromatogr. A*, 2000):  
Modified DFG-multimethod S19

1. Acetone extraction
2. Liquid-liquid partition
3. Gel permeation chromatography
4. Injection on GC-MS

## Sample Cleanup/or not?

Jansson et al. (*J. Chromatogr. A*, 2004):  
Swedish Nation Food Administration

1. Ethyl acetate extraction
2. Evaporate to dryness
3. Re-dissolved in methanol
4. Injection on LC-MS/MS

## Sample Cleanup/or not?

Of 88 multiresidue produce  
methods published since the  
year 2000

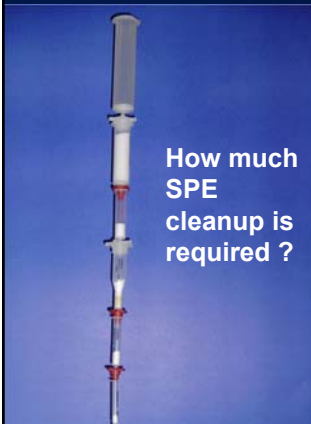
**25% NO CLEANUP**

## Amount of cleanup required may depend on:

**SAMPLE MATRIX**

**DETECTOR**

**Amount of SAMPLE INJECTED**



How much  
SPE  
cleanup is  
required ?

**DETECTORS**  
GC/ECD  
LC/UV  
LC/Fluorescence

GC/Element  
Selective (FPD,  
XSD, ELCD, NPD)

GC/MSD

LC/MS/MS

## How Sample is being Injected?

- LC/MS/MS: 1 mg
- FDA surveillance (GC): 12-15 mg
- FDA Total Diet (GC): 85 mg
- DIOXINS (GC) [1.0 PPT]: Kilograms??

## WHY CLEANUP MAY BE DESIRABLE?

- Lower limits of detection-require more sample
- Matrix ions can interfere with GC/MS analysis!!
- System maintenance!!

## SAMPLE MATRIX CLEANUP

Liquid-Liquid partition

Solid Phase Extraction

Size Exclusion (GPC)

## SPE CLEANUP-MRM

Reverse phase C-18

Strong anion exchange SAX

Weak anion exc./Normal phase NH<sub>2</sub>/PSA

Graphitized carbon black GCB

NOT Al<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub>, Florisil

## Fruit and Vegetable Matrix Components (10<sup>5</sup> compounds) or

LIPIDS  
PIGMENTS  
FATTY ACIDS  
PHENOLS (and other acids)  
STEROLS  
Proteins  
Sugars

## SPE Cleanup of fresh fruit and vegetable sample extracts

Major components:

FATTY ACIDS  
PIGMENTS  
STEROLS

## SPE Cleanup of fresh fruit and vegetable sample extracts

### FATTY ACIDS-

- ions interfere with chromatography (GC/MS-SIM)
- Adsorbed on GC COLUMN

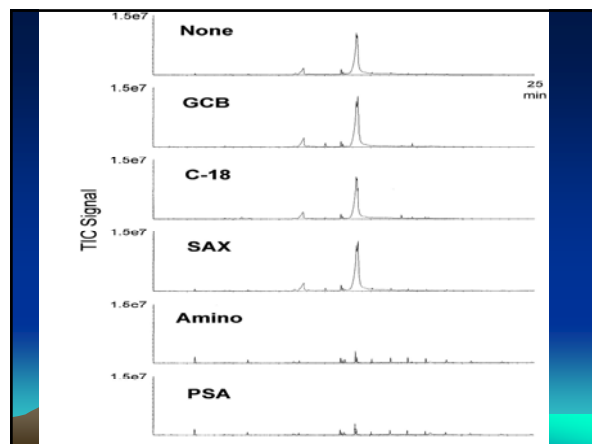
## SPE Cleanup of fresh fruit and vegetable sample extracts

### LESSER PROBLEM:

PIGMENTS-retained on injection liner  
(*may migrate to column*)

STEROLS- late eluting

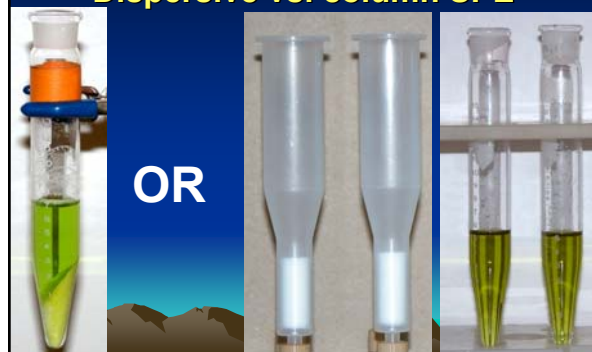
**GC/MSD: Total Ion Chromatograms of fruit and vegetable extracts with no cleanup and various SPE cleanups**



Aminopropyl  $-NH_2$   
Primary-secondary amine PSA

**BASIC** SPE sorbents-  
adsorb: fatty acids, phenols)

**SPE CLEANUP (PSA)**  
Dispersive vs. column SPE



**PSA will not remove green pigments**



**Pigments can be removed by**

**C-18**

**OR:**

**GCB**

(graphitized carbon black)

**columns or dispersive**

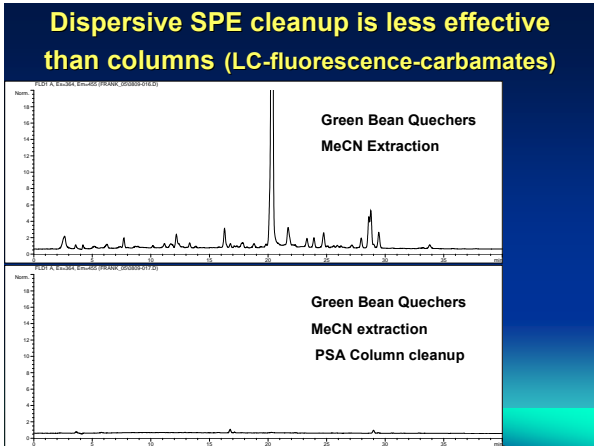
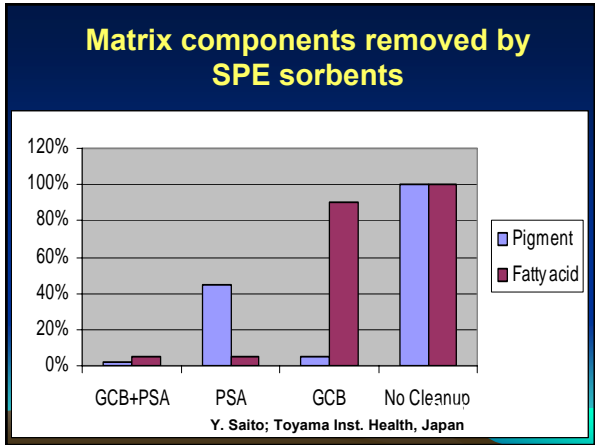
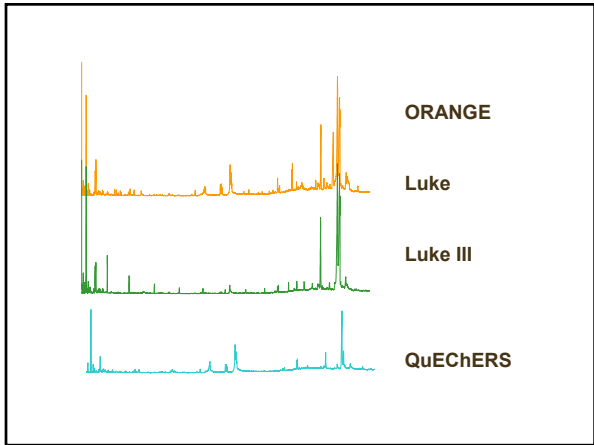
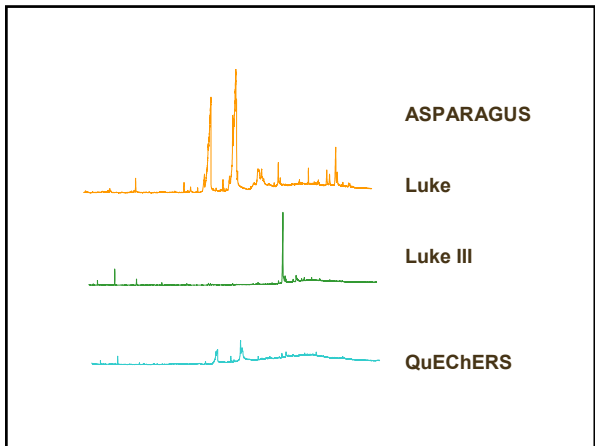
**TANDEM**

**GCB/PSA  
or  
GCB/NH<sub>2</sub>**

**should be ideal QuEChERS Cleanup: GCB/PSA SPE**

# GC/MSD Total ION Chromatograms:

Luke classic-No Cleanup  
Luke-III Extensive cleanup  
QuEChERS- GCB/PSA col.



## GCB/PSA or GCB/NH<sub>2</sub> ELUTION SOLVENT

20-50 mL MeCN/Toluene (3+1) [Fillion et al, 1999; Sheridan & Meola, 2000; Kondo, 2002; Okihashi, 2005; Pang et al., 2006]

20 mL Acetone/hexane (1+1) [Saito et al, 2004]

10-15 mL Acetone/Toluene (3+1) [Schenck et al, 1999, 2000; Podhorniak et al., 1999]

*all give equivalent cleanup with ECD.*

## GCB/PSA or GCB/NH<sub>2</sub> ELUTION SOLVENT

- Certain pesticides, e.g. chlorothalonil and dicofol, in acetonitrile or acetone will be rapidly degraded during PSA or NH<sub>2</sub> SPE (dispersive or column) cleanup
- These same pesticides are stable if PSA or NH<sub>2</sub> SPE (MeCN or acetone)/toluene used for SPE cleanup

## PRODUCE SAMPLES

- LC/MS/MS cleanup may not be necessary
  - Specificity of detector
  - LC Columns
- GC/MS some cleanup required
  - Interfering ions
  - GC columns

## The Future of Pesticide Residue Analysis (10 ppb spikes)

Using Quechers with GCB/PSA dispersive SPE cleanup; extracts (1.0 g/mL) were DILUTED 1:10 (to 0.1 g sample/mL) and analyzed on a UPLC/MS/MS

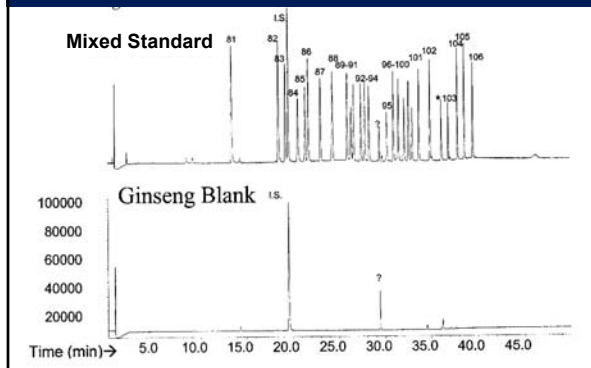
*(J. Wong, CFSAN)*

## HERBAL PRODUCTS

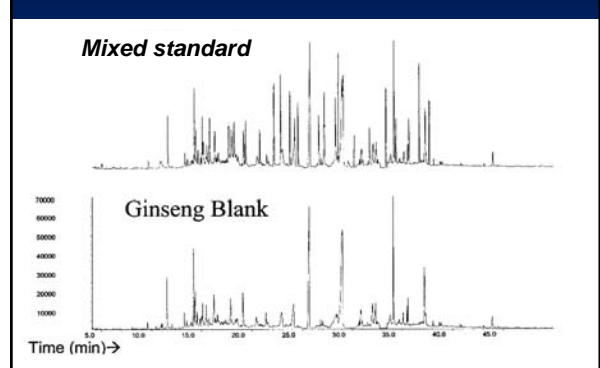
Ginseng  
St. Johns Wort



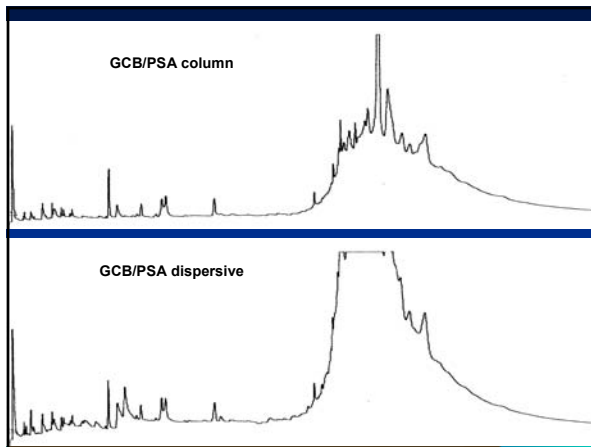
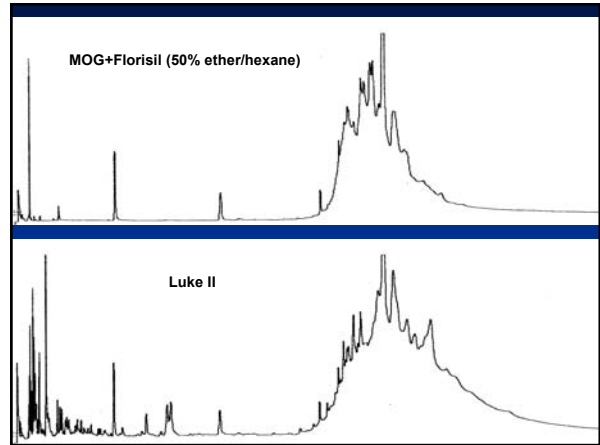
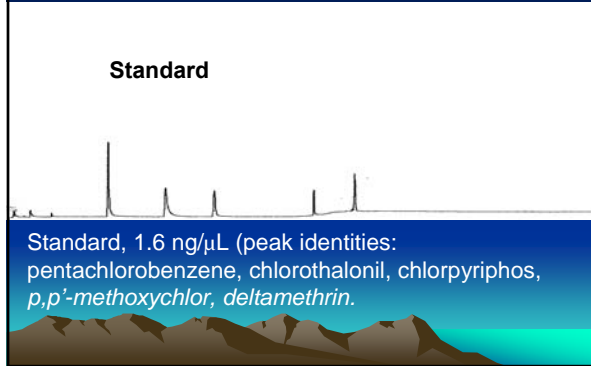
## Ginseng: GC/FPD (1.0 ppm)



## Ginseng: GC/MSD (SIM; 5.0 ppm)

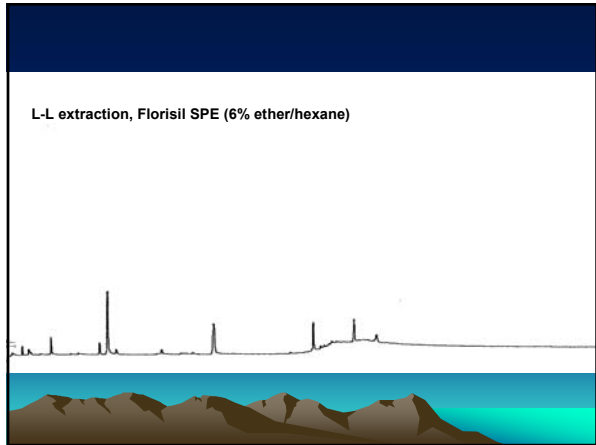
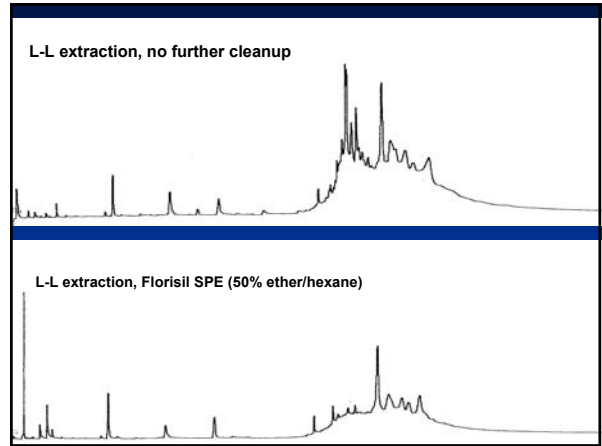
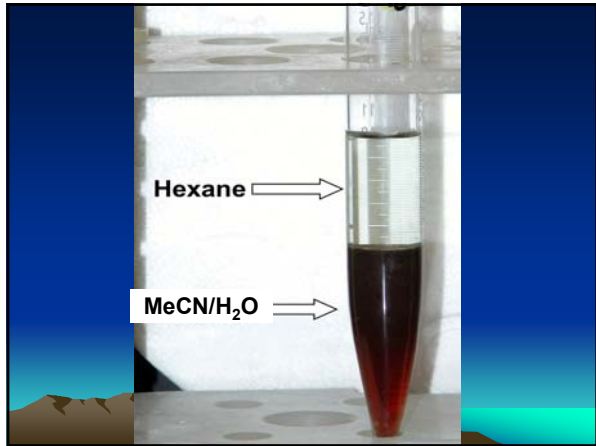


## St. Johns Wort (GC-ECD)



## QuEChERS MeCN extraction; Mini L-L partition

- 1.0 mL MeCN extract
- Add 4.0 mL H<sub>2</sub>O + 5-mL hexane
- Shake
- Transfer top (hexane) phase
- Repeat hexane extraction 2X
- Florisil SPE



### Conclusions-Herbals

- L-L partition with Florisil provides best cleanup
- Only OCs will be recovered
- Unlike produce, OCs (especially early eluting, planar aromatics) are common contaminants found in herbals

