

PESTICIDE RESIDUE ANALYSIS: Where have we been? Where are we going?

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EARLY PESTICIDES

- Sulfur
- Nicotine sulfate
- Pyrethrum, rotenone



ORGANOCHLORINE INSECTICIDES

- *p,p'*-Dichloro-Diphenyl-Trichloroethane
- γ -Hexachlorocyclohexane
(γ -BHC or Lindane)
- Chlordane
- Dieldrin



Organochlorine Insecticides



1960's-Analysis of Organochlorine Insecticide Residues in foods:

- ORGANOCHLORINES ARE:**
- Nonpolar- lipophilic
 - Heat stable-
 - Amenable to GC analysis
using element selective
detectors



1960's-Analysis of Organochlorine
Insecticide Residues in foods:
MULTIRESIDUE METHODS

Mills, P.A. et al. (1959) *J. Assoc. Off. Agric.
Chem.* 42, 734-740

Mills, P.A., Onley, J.H., & Gaither, R.A. (1963)
J. Assoc. Off. Agric. Chem. 46, 186-191

AOAC and PAM

Extraction of (nonpolar lipophilic)
Organochlorine Insecticide
Residues from foods:
FATTY FOODS-FISH

- EXTRACTION WITH
PETROLEUM ETHER
(USP-"Solvent Hexane")
- Liquid-liquid partition with
acetonitrile (4 X)



Extraction of (nonpolar lipophilic)
Organochlorine Insecticide
Residues from foods:
FATTY FOODS-MILK

EXTRACTION WITH:
PETROLEUM ETHER,
ETHYL ETHER,
ETHANOL*
POTASSIUM OXALATE*

*REQUIRED TO DISRUPT THE FAT GLOBULE
C.Y.W. Ang & L.R. Dugan (1973) *J. AOAC*, 56, 718-720



Extraction of (nonpolar lipophilic)
Organochlorine Insecticide
Residues from
**NON-FATTY HIGH MOISTURE
FOODS-PRODUCE**

Extraction with **POLAR**
solvents:
ACETONITRILE*

* Chlorinated hydrocarbon insecticides
deposited in surfactant lipids of the plant
(phospholipids, sulfolipids, glycolipids)
Mumma, R.O., et al. (1966) *Science* 152, 530-
531



Extraction of (nonpolar lipophilic)
Organochlorine Insecticide
Residues from
**NON-FATTY LOW MOISTURE FOODS-
GRAINS**

EXTRACTION WITH
POLAR SOLVENTS:
ACETONITRILE/H₂O

Bertuzzi, P.F. et al. (1967) *J. Assoc. Off. Anal.
Chem.*, 50, 623-627.



**Cleanup of organochlorines in the
acetonitrile extract**

LIQUID-LIQUID EXTRACTION

MeCN + H₂O ↔ **Hexane**
polar compounds ↔ pesticides



followed by

Florisil column cleanup

ORGANOCHLORINE Insecticides

- Extremely persistent
- Endocrine disrupters
- Environmental effects (birds)
- Not allowed in the USA
- Still widely used-**CHEAP**



Electron Capture Detector

RELATIVE SENSITIVITY

10^5 polyhalogens (HCB, PCBs)

10^4 trichlorides; **anhydrides**

10^3 dichlorides

10^2 **alcohols, ketones, amines, monosaccharides**



FDA Total Diet Study (n=2000)

Pesticide	Freq	Avg. ppb
DDE, p,p'-	24%	2.0
Malathion	17%	8.0
Chlorpyrifos methyl	15%	5.0
Endosulfan sulfate	13%	4.0
Dieldrin	12%	1.0
Endosulfan II	8%	2.0
Chlorpyrifos	7%	3.0
Thiabendazole	7%	57.0
Endosulfan I	7%	2.0
Chlorpropham	6%	13.8

ORGANOPHOSPHORUS INSECTICIDES (1970's)

- Acetylcholinesterase Inhibitors (nerve agents)
- Wide range of toxicity
- **POLAR!!**



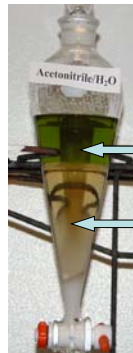
CLEANUP OF **POLAR** ORGANOPHOSPHATES IN AN ACETONITRILE EXTRACT?



Florisil® column cleanup



Acetonitrile extract- Salting Out (green beans)




← ACETONITRILE (7% H₂O)

← AQUEOUS

Schenck et al. (2002) J. AOAC Int. 85, 1177-1180

Acetone extract- Salting Out



ACETONE (18% H₂O)

AQUEOUS

Schenck et al. (2002) J. AOAC Int. 85, 1177-1180

Acetone extract- Salting Out
ACETONE (18% H₂O)

- Add MeCl₂, PE & NaCl (Luke)
- Add EtOAc + c-Hexane
- Add NaCl+Fructose+MgSO₄ &
 - azeotrope (LIB 4178)
 - second salt out (FEDSO Fast easy Double Salt out)

Luke et al. (1981) J. Assoc. Off. Anal. Chem. 64, 1187-1195
 Specht et al. (1995) Fresenius J. Ana. Chem. 353, 183-190
 Luke et al. FDA LIB 4178
 Fairchild et al. FDA LIB (in press)

Ethyl Acetate extract-
Salting Out??


- EtOAc and H₂O are immiscible*: DO NOT NEED SALT for phase separation *(7.9% H₂O soluble in EtOAc)
- Na₂SO₄ is added to force polar compounds into organic phase

Jansson et al. (2004) J. Chromatogr. A 1023, 93-104

What is the ideal extraction solvent?

ACETONITRILE:


- EASY TO SALT OUT
- Difficult to evaporate
- Not compatible with Hall, NPD
- Compatible with LC/MS



What is the ideal extractoion solvent?

ACETONE:


- Difficult TO SALT OUT
- Easy evaporate



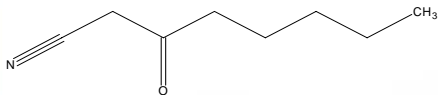
What is the ideal extraction solvent?

Ethyl Acetate

- Immiscible with H₂O
- Very clean extracts
- Will extract LIPIDS (GPC cleanup commonly used)



"Schenck-oleum"



- Combination petroleum ether, acetone and acetonitrile
- Ideal BP =
- Analytes lost: none



Extraction solvents used by Govt. Regulatory Agencies

- FDA-acetone
- CA CDFA-acetonitrile
- FL FDOACS-acetonitrile
- Canada-acetonitrile
- Sweden-ethyl acetate
- Netherlands-acetone
- China-acetonitrile



SAMPLE CLEANUP-POLAR OP's

1970's-1980's
Packed column GC
Selective detectors

NO CLEANUP



SPE Cleanup for pesticides

- CDFA (1991) C18 cleanup of MeCN extracts (Lee et al. (1991) *Frezenius J Anal Chem.* 339, 376-383;
- FDA (1993-Luke II) C18+ SAX+PSA cleanup of acetone extracts (Cairns et al. (1993) *Rapid Commun. Mass Spectrom.* 7, 1070-1076; PAM 302E7C6



SPE cleanup of sample matrix

- C-18 Fats, waxes, pigments
 - GCB Pigments, sterols
 - PSA, NH₂ Fatty acids, other acids
- Proteins
Sugars (aqueous phase)

SPE CLEANUP

FRUITS & VEGETABLES

FATTY ACIDS

PSA or NH₂ SPE



SPE CLEANUP Fruits & Vegetables

<u>FATTY ACIDS:</u>	<u>mg/100 g</u>
Lima beans	420 mg
Grapes	102 mg
Apples	120 mg

- > 1000 ppm fatty acids
- Interfering ions-MSD detectors
- Contribute to matrix effects

Pesticide Analysis Today and in the Future



Some xenobiotic residues may
present a serious health risk



Thus far this is **NOT** the case with
pesticides

Pesticide residue analysis:

- ENFORCE LAWS
- ASSURE CONSUMERS
THAT THE FOOD IS
SAFE TO EAT
- PROVIDE RISK
ASSESSMENT DATA



>1100 chemicals registered as
pesticides.

WE SHOULD SCREEN FOR:

- greatest number of
pesticides
- Lowest cost



May 6, 2005

National Standard of P.R.China GB/T 19648-2005 Simultaneous Determination of 446 Pesticides Residues in Fruits and Vegetables by Three-Cartridge Solid Phase Extraction/GC-MS and LC-MS-MS

Guo-Fang Pang, Chun-Lin Fan, Jin-Jie Zhang, Yong-Ming Liu, Yan-Zhong Cao, Xue-Min Li, Zeng-Yin Li, Yan-Ping Wu and Tong-Tong Guo

Qinhuangdao Entry-Exit Inspection and Quarantine Bureau, 39 Haibin Rd, Qinhuangdao, Hebei, PC 066002, People's Republic of China. Fax: +86-335-3407608, Email: gfpang@heinfo.net.

***Scope

This standard specifies the method for determination by GC-MS and LC-MS-MS of 446 pesticides residues in fruits and vegetables.

This standard is applicable to the determination of the 446 pesticides residues in fruits and vegetables, such as apple, pear, orange, banana, grape, pineapple, kiwi, cabbage, tomato, cucumber, green pepper, spinach,

Pesticide Analysis-FDA

- GC/MSD-
organohalogens
- GC/MSD-
organonitrogens
- GC/MSD & GC/FPD
organophosphates



Pesticide Analysis: FUTURE

LC/MS/MS



Why LC-MS/MS?

Increasing
number of polar
heat labile
pesticides

Less sample
cleanup
required

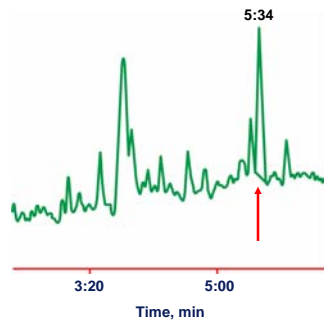


Single Quadrupole
Mass Spectrometry (SIM)

vs.

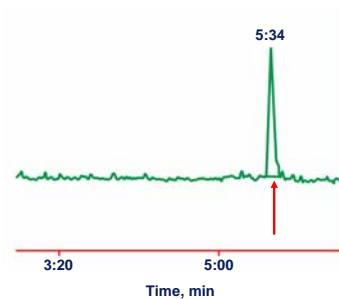
Triple Quadrupole
Mass Spectrometry (MS/MS)

1 ppb Imazethapyr (Pursuit) in Soil-
LC/MS



Slide provided by Alex Krynskiy

1 ppb Imazethapyr (Pursuit) in Soil
Using LC/MS/MS m/z 290>86



Slide provided by Alex Krynskiy

LC-MS/MS vs. GC/MS

1: [Mass Spectrom Rev.](#) 2006 Nov-Dec;25(6):838-65.

Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS/MS?

[Alder L.](#), [Greulich K.](#), [Kempe G.](#), [Vieth B.](#)

Federal Institute for Risk Assessment, Residue Analysis Unit, Thielallee 88-92, 14195 Berlin, Germany. l.adler@bfr.bund.de

Survey of 88 multiresidue methods published since the year 2000

>20% use LC-MS/MS with **NO CLEANUP***

* other than removal of coextracted water



How much SPE cleanup is required ?

Too much sample cleanup:

• Can be expensive

• Can result in loss of some pesticides

Still: some cleanup is desirable

INSTRUMENT MAINTENANCE

MATRIX EFFECTS



412 ANASTASIADES ET AL. JOURNAL OF AOAC INTERNATIONAL VOL. 88, NO. 2, 2005

RESIDUES AND TRACE ELEMENTS

Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce

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A simple, fast, and inexpensive method for the determination of pesticide residues in fruits and vegetables is introduced. The procedure involves initial single-phase extraction of 10 g sample with 10 mL acetonitrile, followed by liquid-liquid partitioning formed by addition of 4 g anhydrous MgSO₄ plus 1 g NaCl. Removal of residual water and cleanup are performed simultaneously by us-

Pesticide residue analysis of food and environmental samples has been performed in numerous government and private laboratories throughout the world for approximately 40 years. However, the methods used for analysis of common pesticides are far from ideal. Some residue monitoring laboratories still use methods developed 30 years ago when analytical needs were less demanding, solvent usage was less of an issue, extended analysis time and manual labor were the

QuEChERS:
15 grams of produce
15.0-mL of MeCN

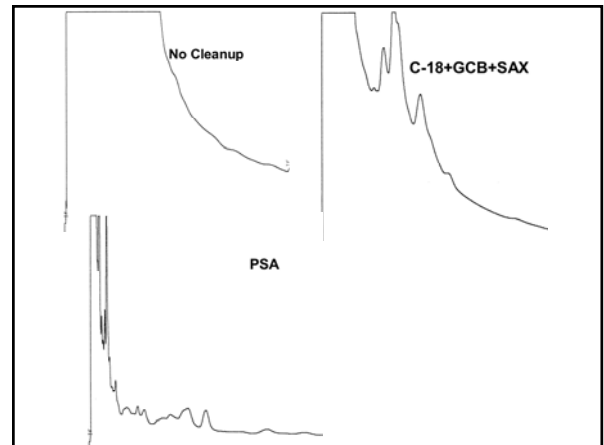


Add $MgSO_4$ and NaCl
SHAKE & Centrifuge



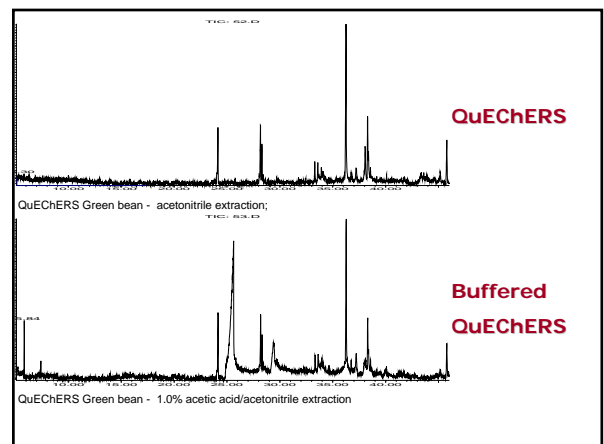
MeCN Extract
Plant Matrix
Aqueous

CLEANUP: Dispersive SPE - PSA

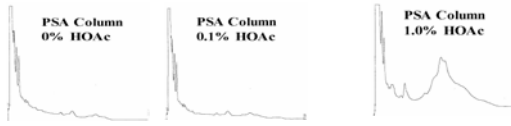


QuEChERS vs. "Buffered QuEChERS"

- PSA – loss of "base sensitive" pesticides.
- Buffered QuEChERS –
(AOAC Official method 2007.01)
1% acetic/acid/MeCN
sodium acetate + $MgSO_4$



QuEChERS: Green bean ECD



- Cleaner extract when using acetonitrile rather than 1% acetic acid-acetonitrile

- What about base sensitive pesticides?

QuEChERS

- Buffered QuEChERS – resulted in less cleanup
- This may not be a problem, depends on instrumentation used and LOQs desired
- GC/MSD vs. LC-MS/MS



Dispersive SPE:
QuEChERS MeCN (no acid) extract:

+ Toluene + PSA + GCB + MgSO₄

Wong et al. (2007) J. Agric. Food Chem. 55, 1117-1128



QuEChERS-GCB/PSA cleanup

- Toluene “protects” base sensitive pesticides from degradation by PSA
- Toluene is ideal “injection solvent” for GC/MSD due to low expansion volume



Even better cleanup required?

“Modified QuEChERS”

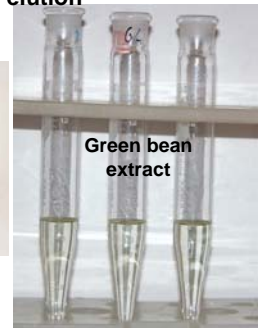
- buffered QuEChERS
- PSA SPE columns
- acetone/toluene elution

• Schenck et al. 2007 (submitted)



Even better cleanup required?

“Modified QuEChERS”, using GCB/PSA SPE COLUMNS-acetone/toluene elution



Schenck et al. (2007) Unpublished data

Cleanup & LOQ/LOD

How low should (can) we go?

- **Parts per million**
- Parts per billion
- Parts per trillion
- Parts per quadrillion
- Molecules



- *10 ppb is EC limit for pesticides in baby foods for infants and young children*

- *1.0 ppb used in some risk assessment studies (FDA Total Diet Study, EPA)*



Current Literature-GC/MSD

SPE cleanup using:
PSA, NH₂ or GCB/PSA
columns

LOQ = ~10 ppb



LC-MS/MS

Luke method with
"no cleanup*"

Hienstra & deKok (2007) J Chromatogr. A 1154, 3-25

~10 ppb

*other than separation of
coextracted H₂O



LC-MS/MS

LC-MS

LC-fluorescence:

CARBAMATES

Luke method with
NH₂ SPE cleanup

Podhorniak et al. 2004 J.AOAC Int. 87, 1237-1251

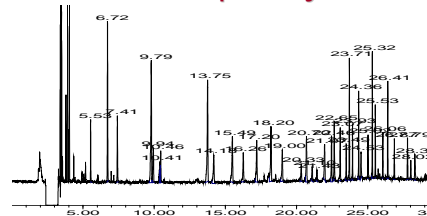
1.0 ppb

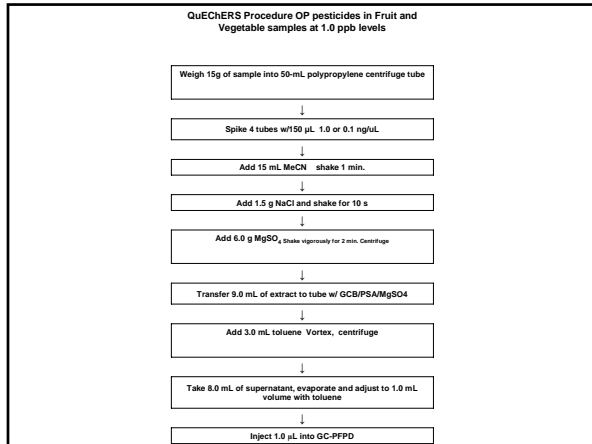


Personal experience

Risk assessment study (Emory Univ.): OPs in
children's foods (1.0 ppb-LOQ PFPD)
QuEChERS with dispersive GCB/PSA:

>24 samples/day.



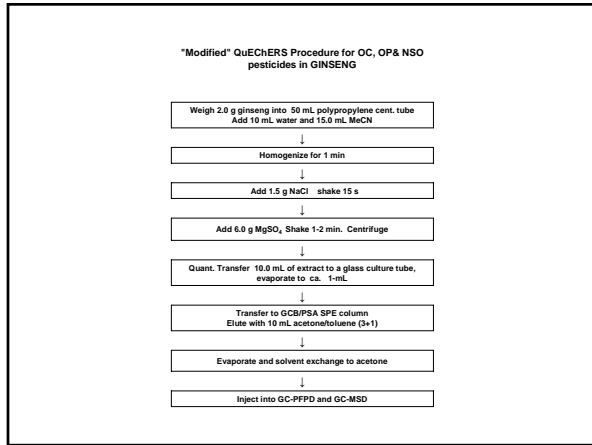


Personal experience: OC and OP pesticides in ginseng

Modified QuEChERS using GCB/PSA columns

- Extract by blending with MeCN + H₂O
- Salt-out with NaCl & MgSO₄
- GCB/PSA column cleanup

Results- Cleanup (on ECD) equivalent to MOG with 15% ether/Florisil cleanup and FEDSO with C-18 + SAX/PSA cleanup




Personal Opinion: QuEChERS

LC-MS/MS –

- extract with 1% HOAc/MeCN
- salt-out with NaOAc & MgSO₄
- dispersive PSA cleanup of MeCN extract


GC/MS –

- extract with MeCN
- salt-out with NaCl & MgSO₄
- GCB/PSA: Dispersive or column cleanup (with 25% toluene)



FUTURE TRENDS* LC-MS/MS

- UPLC
- Triple Quadrupole
- TOF
- Linear ion trap
- Q-TOF
- ??



FUTURE TRENDS* LC-MS/MS

* "It's tough to make predictions, especially about the future." *Yogi Berra*



REFERENCE

Schenck, F.J. & Wong J.W.
(2007 in press), Chapter 6,
"Pesticides in Foods of
Vegetal Origin" in Analysis
of Pesticides in Food and
Environmental Samples:
J.L. Tadeo Lluch (Ed), CRC
Press



QUESTIONS?



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