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Dikma QuEChERS

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OVERVIEW

1. Dikma QuEChERS

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QuEChERS

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

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Fruits and Vegetables

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Milk and Honey

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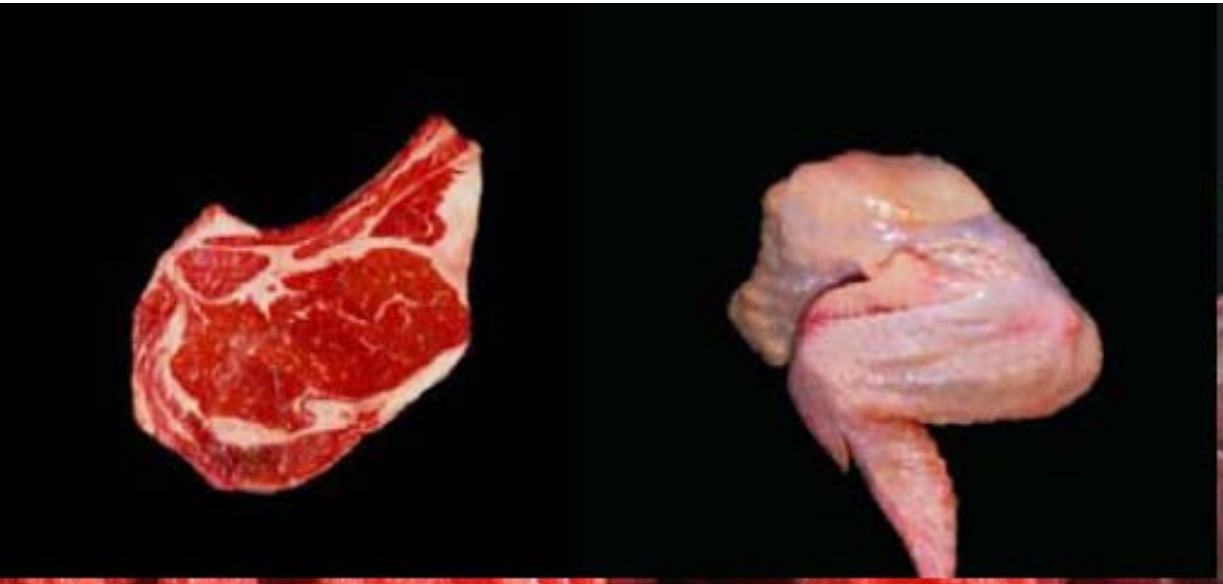
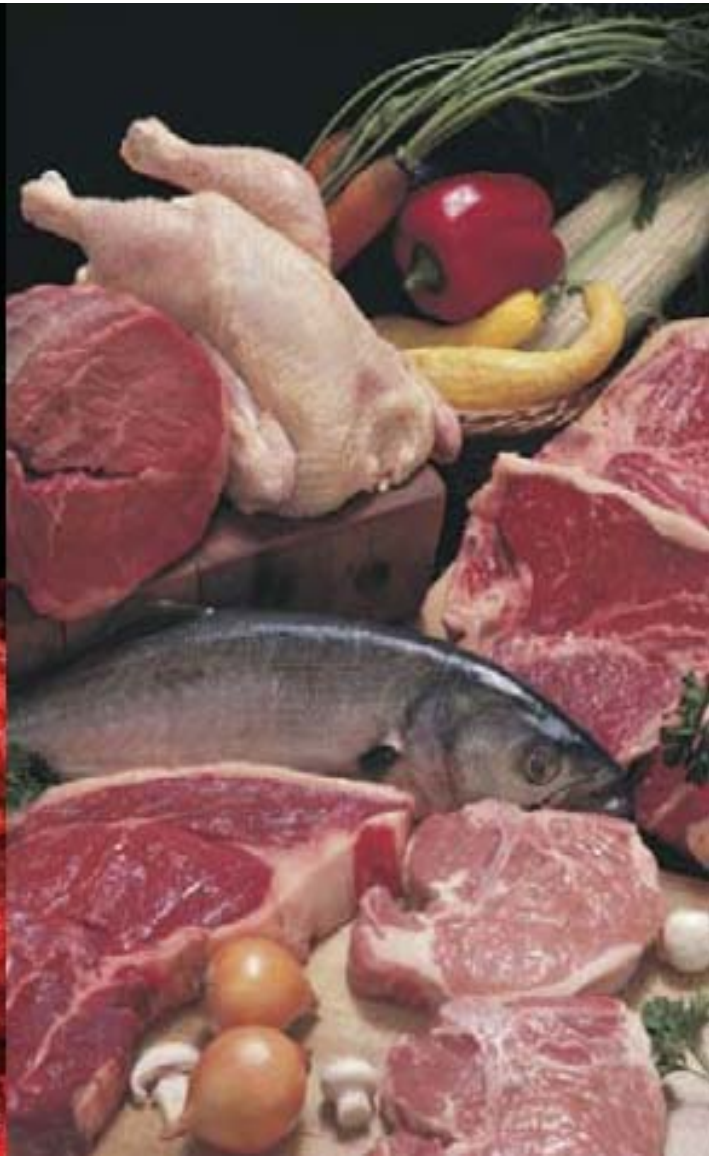
Animal Tissues





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Tobacco

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What Makes Traditional Multiresidue Methods Inefficient?

- Complicated
- Laborious
- Time consuming
- Require high amount of solvents
- Expensive

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Ideal Multiresidue Method

- Fast and easy to perform
- A minimum amount of chemicals
- Good selectivity to avoid complicated cleanup procedures
- Cover sufficiently broad spectrum of analytes

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Benefits of QuEChERS Method

- High recoveries
- Accurate results
- High sample throughput
- Low non-chlorinated solvent usage
- Reduce reagent costs and staff exposure to hazardous solvents
- Reduce glassware usage & labor costs
- Broad utility & ease of use

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QuEChERS

- **Primarily for analysis of pesticides from hydrated matrices (80-95% water content)**
- **Multi-residue pesticide analysis of food and environmental samples can be problematic due to the wide range of chemical properties encountered with pesticide residues**
- **The complex sample matrix may contain abundant quantities of chlorophyll, lipids, sterols and other components that can interfere with good sample analysis**
- **Use of the QuEChERS method reduces these problems**

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QuEChERS Method

- Consists of a liquid-liquid micro extraction
- Partitioning and extraction of polar analytes is aided by $MgSO_4$
- The preferred solvent is acetonitrile
- Acetonitrile provides extraction of the broadest range of organic compounds without co-extraction of large amount 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

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Modifications to the Original Method

- **Some modifications to the original QuEChERS method have been introduced to ensure efficient extraction of pH dependent compounds and to minimize degradation of base and acid labile pesticides**

- **Buffering with citrate salts has been introduced in the micro extraction to adjust the pH to a compromise value of 5 to 5.5, where most acid and base labile pesticides are sufficiently stabilized. To improve stability of base-labile compounds in the sample extracts, a small amount of formic acid is added to the final extract after cleanup**

- **Acidic pesticides are directly analyzed from the raw extract before PSA cleanup since they would be adsorbed and not released by the sorbent**

- **In another modification introduced by Schenck, a graphitized carbon black (GCB) PSA cartridge is used to remove plant pigments without the loss of planar compounds**

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Four QuEChERS Methods

- **The orginal QuEChERS method introduced in 2003, uses NaCl to enhance extraction (reduce polar interferences)**
- **Dispersive AOAC 2007.01 uses 1% acetic acid in acetonitrile, sodium acetate as a buffer replacing sodium chloride**
- **The dual phase column – this method variation introduces the use of PSA and GCB cartridges to remove high levels of chlorophyll and plant sterols in the final extract without the loss of planar pesticides (polar aromatics) using a 3:1 aectone:toluene solvent mixture**
- **The European version (EN 15662) is similar to the AOAC method, except the extraction uses NaCl, sodium citrate dihydrate and disodium citrate sesquihydrate instead of NaOAc. *Acetic acid is not used***

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Original QuEChERS

extraction

10 g sample + 10 mL MeCN

add internal standard

add 4 g $MgSO_4$ + 1 g NaCl
shake vigorously for 1 min

centrifuge for 5 min

dispersive SPE clean-up

1 mL of the upper layer
+ 25 mg PSA + 150 mg $MgSO_4$
mix for 30 s
centrifuge for 1 min

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Buffered QuEChERS (AOAC 2007.01)

extraction

15 g sample + 15 mL 1% HOAc in MeCN

add internal standard

add 6 g $MgSO_4$ + 1.5 g NaOAc
shake vigorously for 1 min

centrifuge for 5 min

dispersive SPE clean-up

1 mL of the upper layer
+ 50 mg PSA + 150 mg $MgSO_4$
(+ 50 mg C_{18})
mix for 30 s
centrifuge for 1 min

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Buffered QuEChERS (EN 15662)

extraction

10 g sample + 10 mL MeCN

add internal standard

add 4 g $MgSO_4$ + 1 g NaCl
+ 1 g $Na_3Citr \cdot 2H_2O$ + 0.5 g $Na_2HCitr \cdot 1.5H_2O$
(+ 0.6 mL 5N NaOH for lemons, limes etc.)
shake vigorously for 1 min

centrifuge for 5 min

dispersive SPE clean-up

1 mL of the upper layer
+ 25 mg PSA + 150 mg $MgSO_4$
(+ 2.5 or 7.5 mg GCB for matrices with a high content of carotenoids and chlorophyll)
mix for 30 s, centrifuge for 1 min

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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 cleanup 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 PSA and GCB

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Dual Phase Cartridge Clean-up Procedure

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

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Other Variations – additional cleanup options

- C18 – removes lipids
- Graphitized carbon black (GCB) – removes pigments (and planar compounds)
- ChloroFiltr® - removes chlorophyll

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QuEChERS

BREAKING IT DOWN

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Acetonitrile

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

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Acetic Acid

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

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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)**

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Anhydrous Magnesium Sulfate

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

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Sodium Chloride

- **Help to reduce polar co-extractables**
- **Can reduce recovery of polar analytes**

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PSA

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

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Endcapped C18

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

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Aminopropyl

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

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Graphitized Carbon Black (GCB)

- **Used in clean-up to remove pigments and polyphenols**
- **GCB binds planar analytes such as acephate, bromophos, carbendazim, chlorthiophos, cyprodinil, ethoprop, fonofos, leptophos, methamidophos, pyrimethanil, and thiabendazole and will remove these planar analytes**
- **More GCB = Lower recovery of planar analytes**

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Advantages of Buffered Methods

- **Buffered extract to protect base sensitive analytes such as folpet, dichlorofluanid, chlorothalonil, pymetrozine, dicofol, captan, tolyfluanid**

- **Extract protected**
  - LC – formic acid (PSA is basic)
  - GC – toluene and magnesium sulfate (prevent thermal breakdown)

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Disadvantage of AOAC Method

- Acetic acid – PSA removes organic acids
- Less clean-up
- Tailing issues on GC

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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

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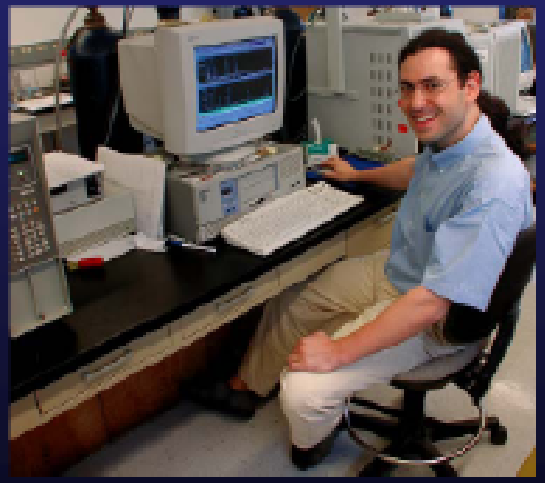
PUTTING IT ALL TOGETHER

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



5) Analyze Pesticides

4) Centrifuge for 1 min

Courtesy of Dr. Steven Lehotay, USDA-ARS

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Add hydrated vegetation to salts & solvent

Shake

Courtesy of Dr. Frank Schenck, USFDA

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Centrifuge

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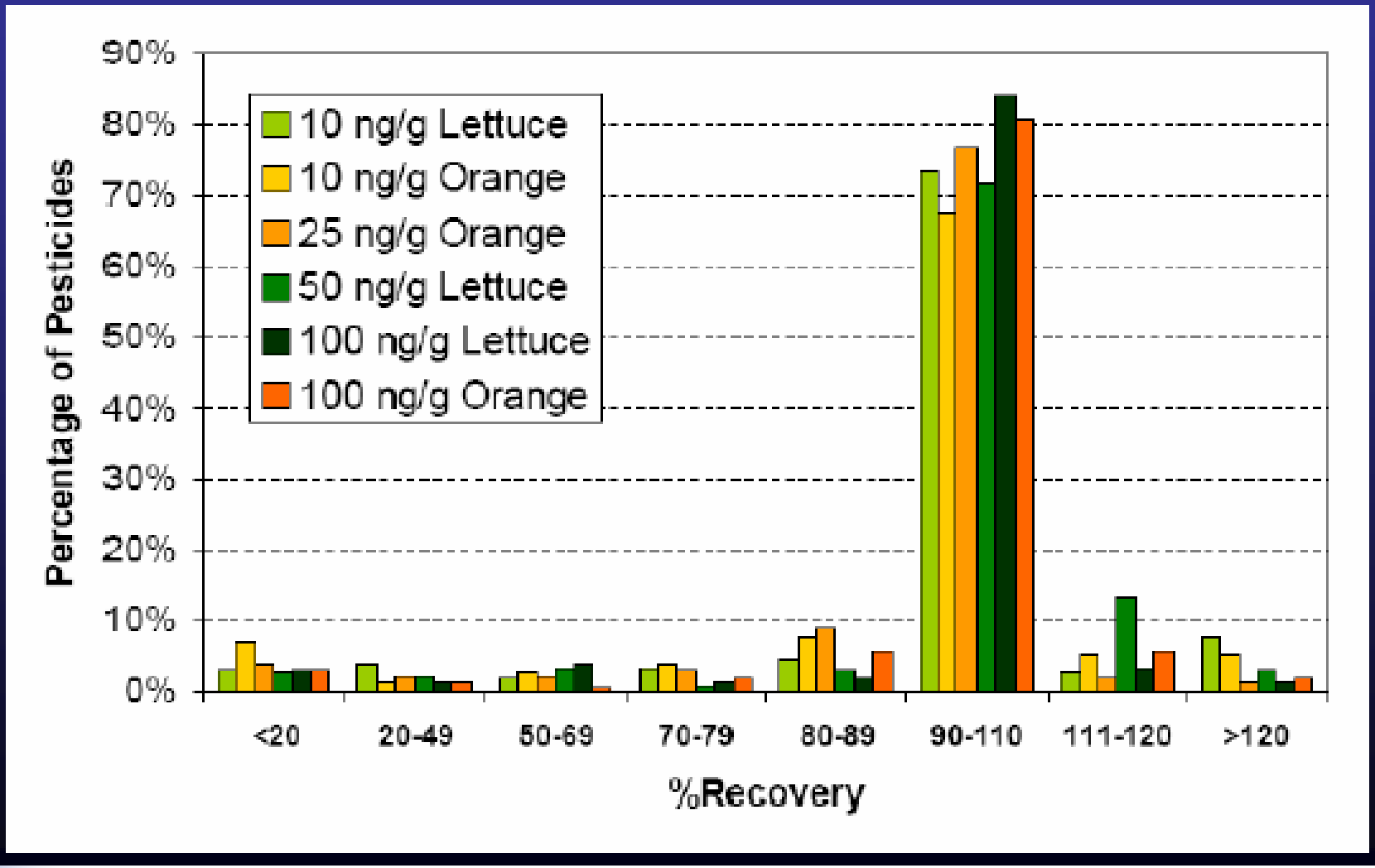
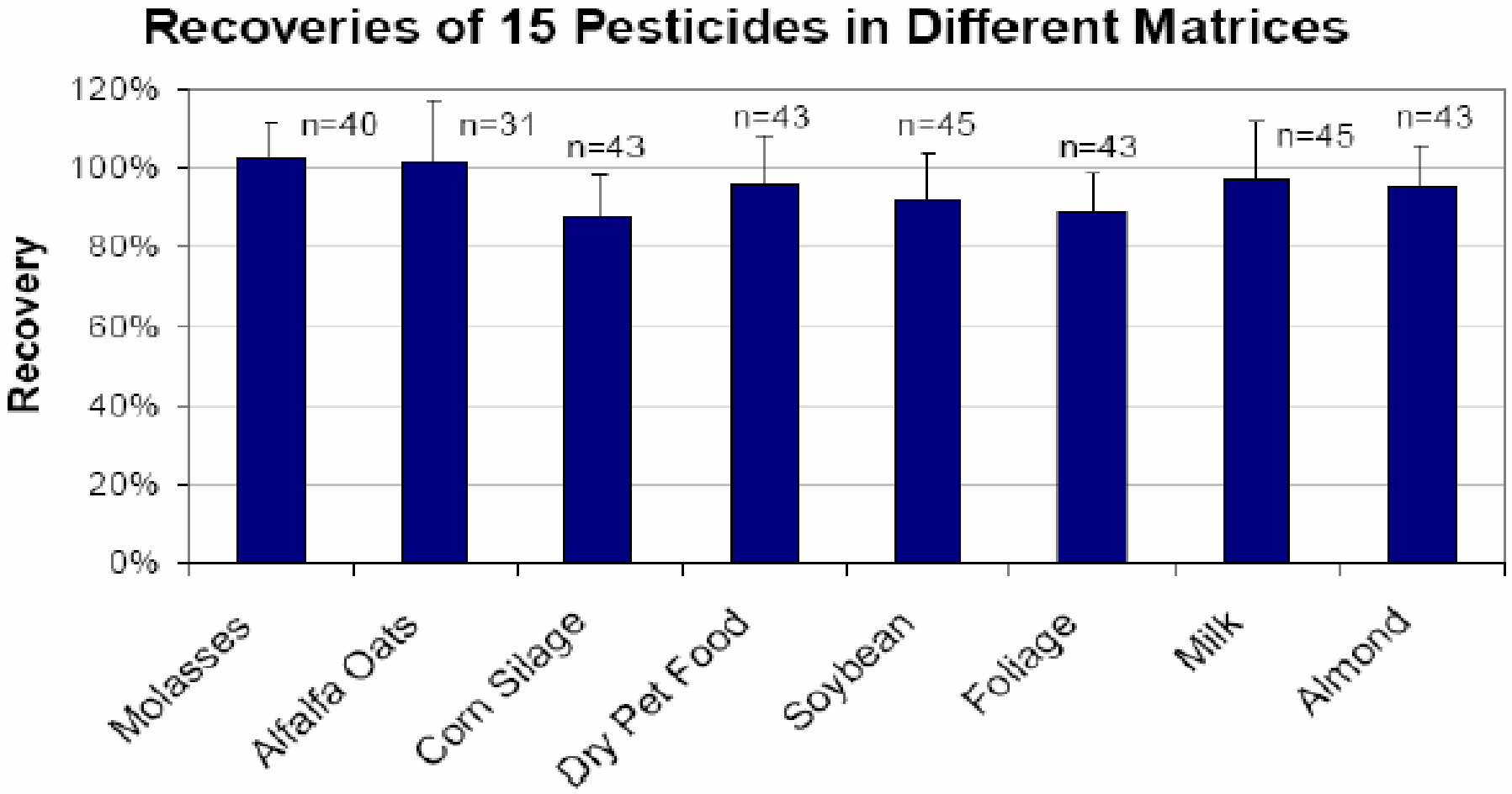
Recoveries in the QuEChERS method

229 pesticides analyzed by GC-MS and LC-MS/MS



Courtesy of Dr. Steven Lehotay, USDA-ARS

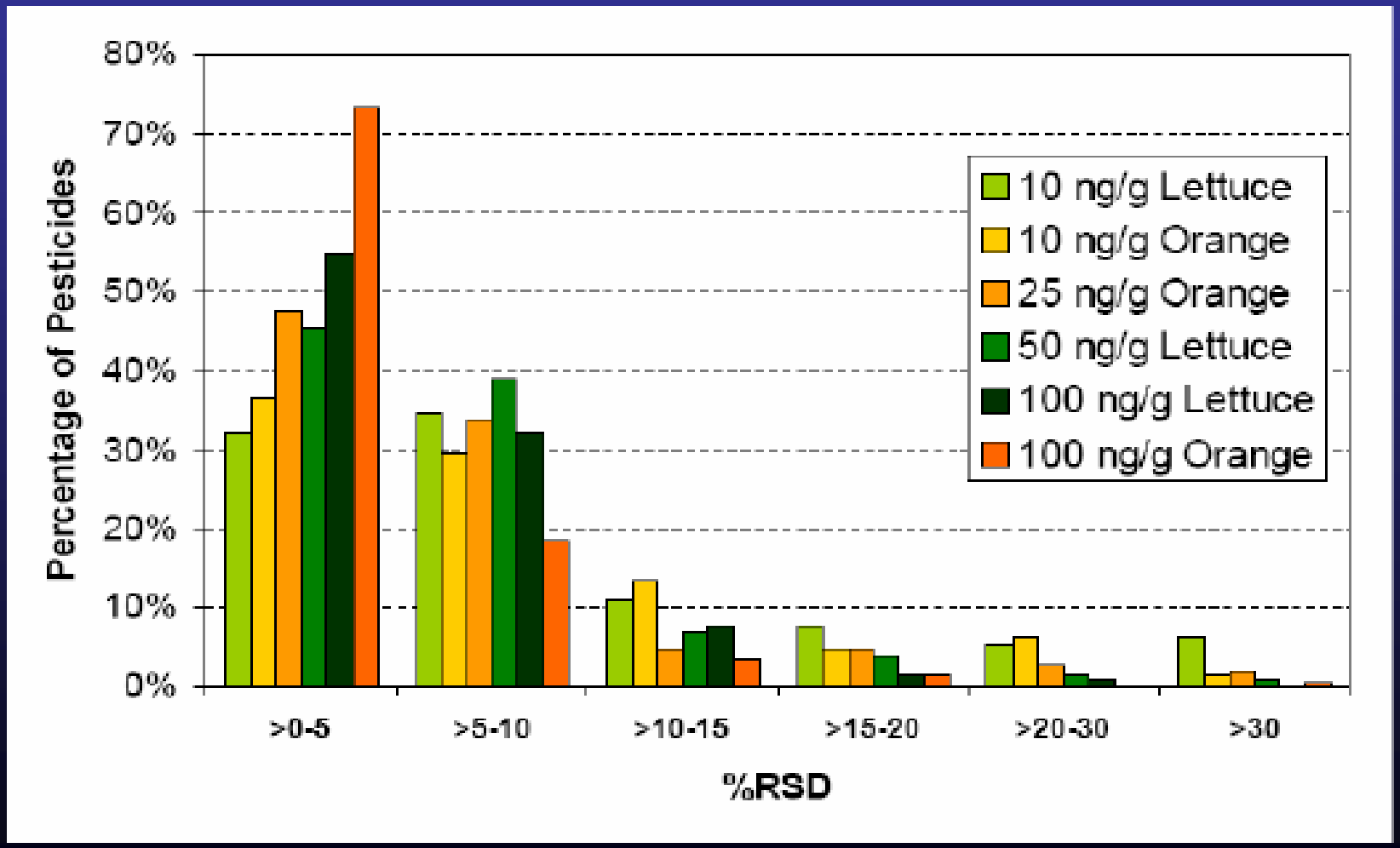


No differences were found vs. matrix for individual pesticides or concentration

Courtesy of Dr. Steven Lehotay, USDA-ARS

Repeatability in the QuEChERS method

229 pesticides analyzed by GC-MS and LC-MS/MS



Courtesy of Dr. Steven Lehotay, USDA-ARS

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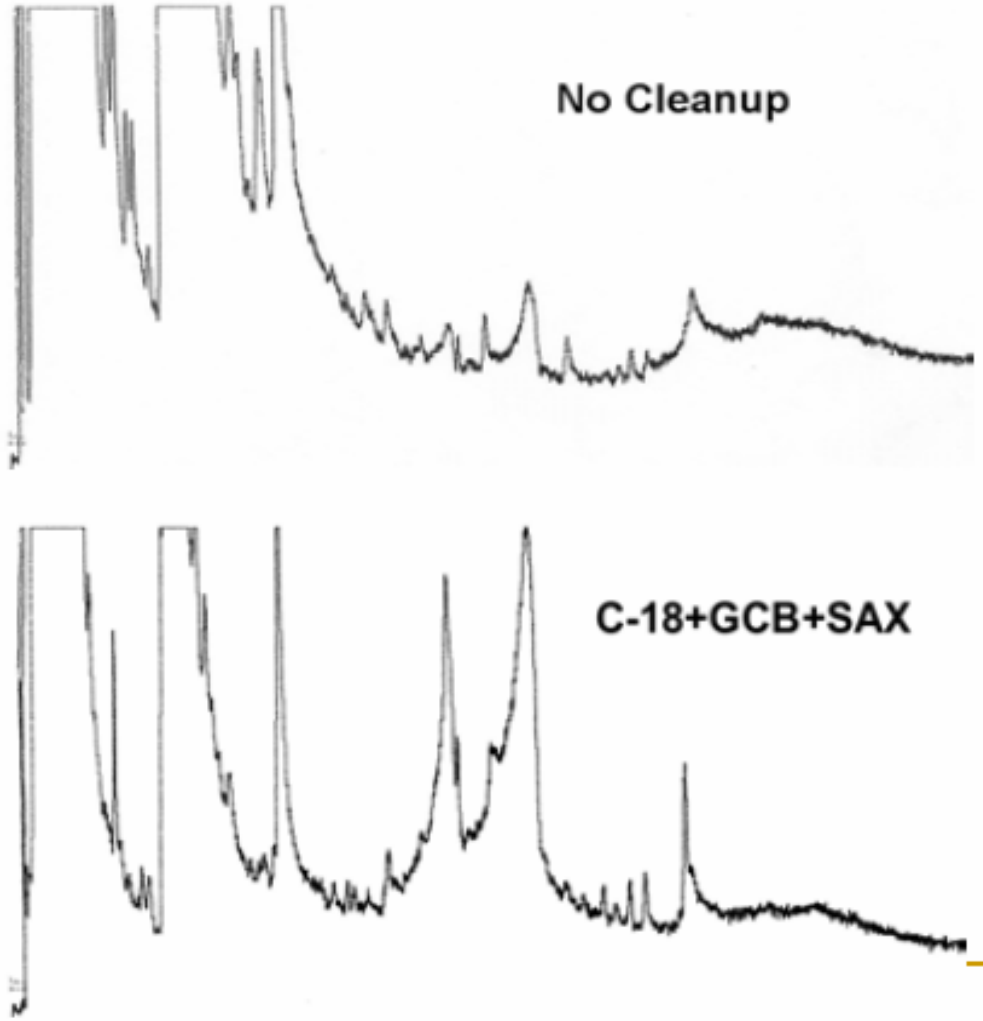
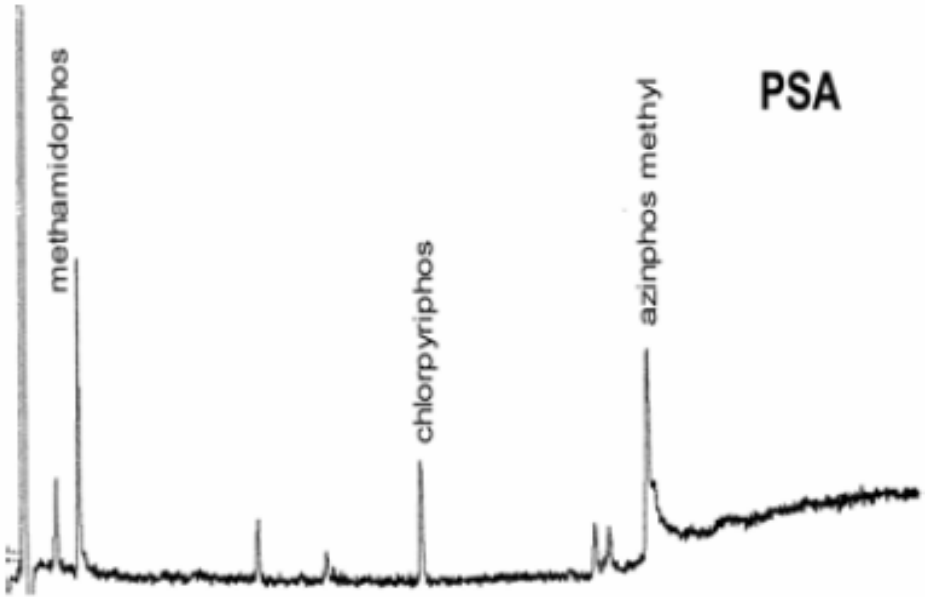
Why PSA Cleanup – GC/FPD asparagus extract





Schenck et al. (2002), J.Sep.Sci. 85, 883
LIBs # 4262, 4282

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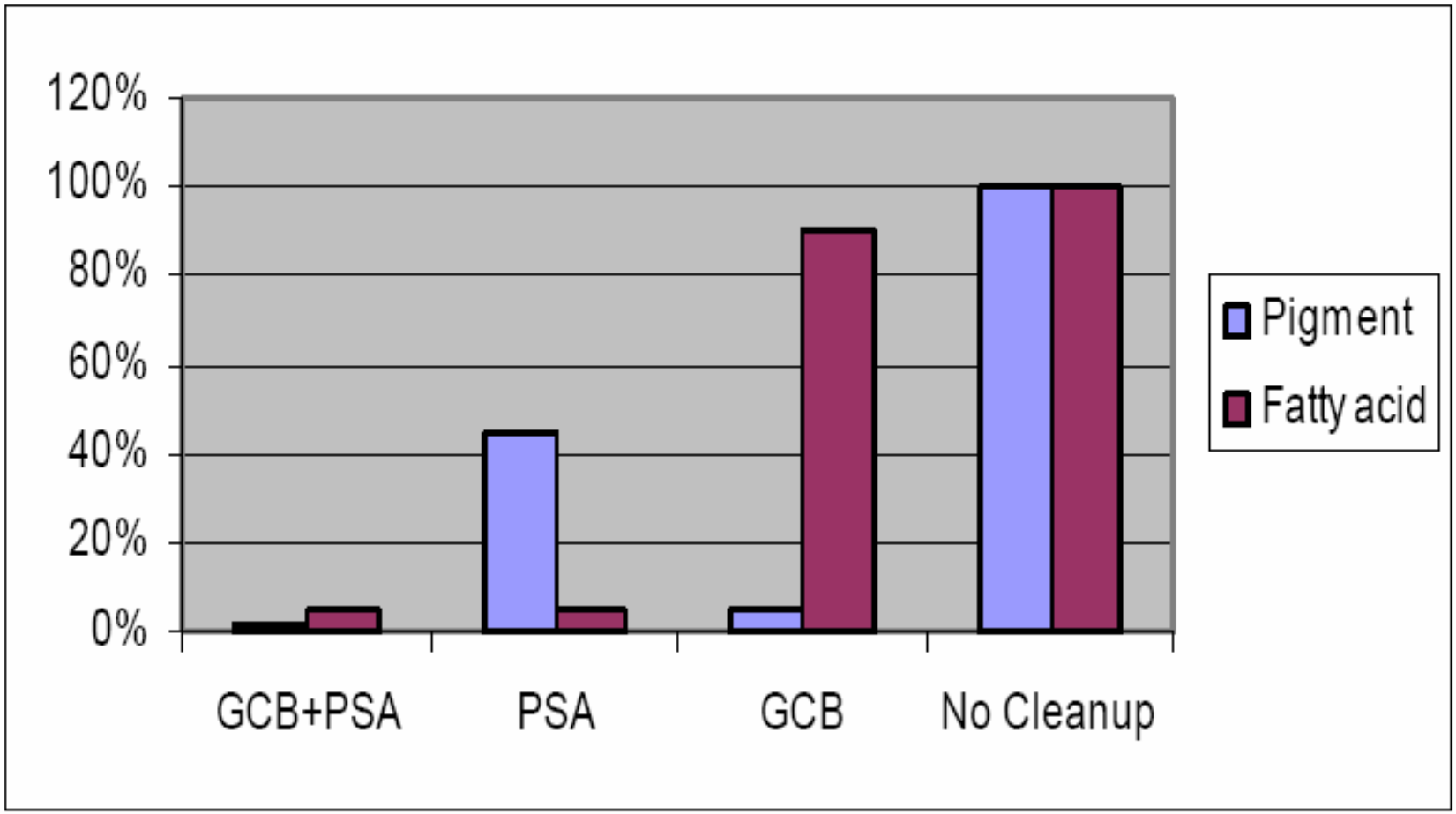
Matrix Components Removed by SPE Sorbents



Saito et al (2004)

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Pesticide Analytes

GC amenable pesticides are capitalized. LC/MS/MS pesticides are not capitalized. Analytes that can be analyzed under either instrument are underlined

acephate*	acetamiprid	Acrinathrin	aldicarb	aldicarb sulfone
aldicarb sulfoxide	Aldrin	azaconazole	azamethiphos	azinphos-methyl
azoxystrobin	Bifenthrin	bitertanol	Bromopropylate	bromuconazole
Bupirimate	buprofezin	butocarboxim	butocarboxim sulfone	butocarboxim sulfoxide
Cadusafos	carbaryl	carbendazim	carbofuran	3-hydroxy-carbofuran
chlorbromuron	(α-, γ-)Chlordane	(α-,β-Chlorfenvinphos	Chlorpropham	Chlorpyrifos
Chlorpyrifos-methyl	Chlorthaldimethyl	Chlorothalonil*	Chlozolinate	clofentezine
Coumaphos	cycloxydim*	(λ-)Cyhalothrin	cymoxanil	Cypermethrin
cyproconazole	cyprodinil	(2,4'-4,4'-)DDE	(2,4'-4,4'-)DDT	Deltamethrin
demeton	demeton-O-sulfoxide	demeton-S-methyl	demeton-S-methyl sulfone	desmedipham
Diazinon	dichlofluanid*	Dichlorobenzophenone	dichlorvos	diclobutrazole
Dicloran	dicrotophos	Dieldrin	Diethofencarb	difenoconazole
Diflufenican	dimethoate	dimethomorph	diniconazole	Diphenyl
Diphenylamine	disulfoton	disulfoton sulfone	diuron	dmsa
dmst	dodemorph	α- Endosulfan	β-Endosulfan	Endosulfan sulfate

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ethiofencarb sulfoxide	Ethion	ethirimol	Ethoprophos	etofenprox
Etridiazole	Famoxadone	fenamiphos	fenamiphos sulfone	Fenarimol
Fenazaquin	fenbuconazole	fenhexamid*	Fenithrothion	fenoxycarb
Fenpiclonil	Fenpropathrin	Fenpropidine	fenpropimorph	fenpyroximate
Fenthion	fenthion sulfoxide	Fenvalerate	florasulam*	Flucythrinate I & II
Fludioxonil	flufenacet	Flufenconazole	flusilazole	Flutolanil
Fluvalinate	Fonophos	fosthiazate	Furalaxyl	furathiocarb
furmecyclox	Heptachlor	Heptachlor epoxide	Heptenophos	Hexachlorobenzene
hexaconazole	hexythiazox	imazalil	imidacloprid	Iprodione
iprovalicarb	isoprothiolane	isoxathion	kresoxim-methyl	Lindane
linuron	Malathion	malathion oxon	Mecarbam	mephosfolan
Mepronil	Metalaxyl	metconazole	methamidophos*	Methidathion
methiocarb	methiocarb sulfone*	methiocarb sulfoxide	methomyl	methomyl-oxime
metobromuron	metoxuron	Mepanipyrim	Mevinphos	monocrotophos
monolinuron	myclobutanil	nuarimol	Ofurace	omethoate
oxadixyl	oxamyl	oxamyl-oxime	oxydemeton-methyl	paclobutrazole

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Parathion	Parathion-methyl	penconazole	pencycuron	*cis*- Permethrin
trans-Permethrin	phenmedipham	*o*-Phenylphenol	Phorate	phorate sulfone
Phosalone	Phosmet	Phosmet-oxon	phosphamidon	Phthalimide
picoxystrobin	Piperonyl butoxide	pirimicarb	pirimicarb-desmethyl	Pirimiphos-methyl
prochloraz	Procymidone	profenofos	Prometryn	Propargite
Propham	propiconazole	propoxur	Propyzamide	Prothiofos
pymetrozine*	Pyrazophos	pyridaben	pyridaphenthion	pyrifenox
pyrimethanil	Pyriproxyfen	Quinalphos	Quinoxyfen	Quintozene
sethoxydim*	spinosad	spiroxamine	tebuconazole	tebufenozide
Tebufenpyrad	tetraconazole	Tetradifon	Tetrahydrophthalimide	Terbufos
Terbufos sulfone	thiabendazole	thiacloprid	thiamethoxam	thiodicarb
thiofanox	thiofanox sulfone	thiofanox sulfoxide	thiometon	thiometon sulfone
thiometon sulfoxide	thiophanate-methyl	Tolclofos-methyl	tolylfluanid*	triadimefon
triadimenol	Triazophos	trichlorfon	tricyclazole	tridemorph
trifloxystrobin	trifluminazole	Trifluralin	Triphenylphosphate	vamidothion
vamidothion sulfone	vamidothion sulfoxide	Vinclozolin		

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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 buffer when necessary
6. Use analyte protectants (formic acid or toluene)
7. Beware of GCB


Thank You!