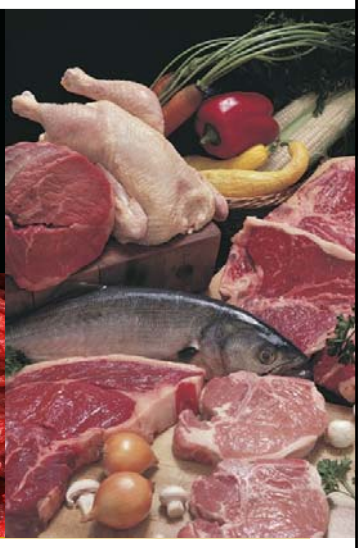
QuEChERS

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

Fruits and Vegetables

Milk and Honey

Meat



Tobacco

QuEChERS

- Multiclass, multiresidue method (MRM)
- Primarily for the analysis of pesticides from hydrated (80-95% water content) matrices
- Multi-residue pesticide analysis of food and environmental samples can be problematic
- 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

The QuEChERS Method

- Consists of a liquid-liquid micro extraction
- Partitioning 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 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!

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

Currently there are four variations of the QuEChERS method being used in western countries

•The original QuEChERS method. Introduced in 2003, uses sodium chloride to enhance extraction

•AOAC 2007.01. Uses sodium acetate as a buffer replacing sodium chloride and 1% acetic acid in acetonitrile

•The dual phase variation: This method variation introduces the use of a PSA and GCB cartridge to remove high levels of chlorophyll and plant sterols in the final extract without the loss of planar pesticides (polar aromatics) using an acetone:toluene solvent blend (3:1).

•The European version is similar to the AOAC method, except the extraction uses sodium chloride, sodium citrate dihydrate and disodium citrate sesquihydrate instead of sodium acetate.

Original QuEChERS

1. To **UCT** product **ECMSSC50CT** containing 6 grams anhydrous magnesium sulfate and 1.0 gram of sodium chloride in a 50 mL polypropylene centrifuge tube, add 15 ml of acetonitrile
2. Shake to mix contents
3. Add surrogate or internal standards if desired
4. Add 15 grams homogenized hydrated sample to the centrifuge tube
5. Shake for 1 minute
6. Centrifuge for 1 minute at 3700 rpm
7. Add a aliquot of the supernatant to the appropriate **UCT** dispersive solid-phase cleanup tube: (**CUMPSCB2CT, CUMPS2CT, CUMPSC18CT, ECMPSCB15CT, or ECMPSC1815CT**)
8. Shake for 1 minute
9. Centrifuge for 1 minute at 3700 rpm
10. Analyze extract

AOAC 2007.01

1. To **UCT** product **ECMSSA50CT** containing 6 grams anhydrous magnesium sulfate and 1.5 grams of anhydrous sodium acetate in a 50 mL polypropylene centrifuge tube, add 15 ml of 1% acetic acid in acetonitrile
2. Shake to mix contents
3. Add surrogate or internal standards if desired
4. Add 15 grams homogenized hydrated sample to the centrifuge tube
5. Shake for I minute
6. Centrifuge for 1 minute
7. Add an aliquot of the supernatant to the appropriate **UCT** dispersive clean-up tube: **(CUMPSCB2CT, CUMPS2CT, CUMPSC18CT, ECMPSCB15CT, or ECMPSC1815CT**)
8. Shake for 1 minute
9. Centrifuge for 1 minute at 3700 rpm
10. Analyze extract

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 without loss of planar pesticides can be accomplished by using the **UCT** 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
7. Cartridge product selection used for this analysis: ECPSACB6, **ECPSACB256** or ECPSACB506 depending upon sorbent mass required

Available Options from UCT

PART NUMBER	DESCRIPTION
CUMPSCB2CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA & 50mg Carbon
CUMPS2CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA
CUMPSC18CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA & 50mg endcapped C18
ECMPSCB15CT	15mL centrifuge tubes with 900mg Anhydrous Magnesium Sulfate, 300mg PSA & 150mg Carbon
ECMPSC1815CT	15mL centrifuge tubes with 900mg Anhydrous Magnesium Sulfate, 300mg PSA & 150mg endcapped C18
ECPSACB6	6mL columns with 400mg PSA on bottom, 200mg Graphitized Carbon on top, separated by a Teflon frit*
ECPSACB256	6mL columns with 250mg Graphitized Carbon on top, 500mg PSA on the bottom, separated with a Teflon frit*
ECPSACB506	6mL columns with 500mg Graphitized Carbon on top, 500mg PSA on the bottom, separated with a Teflon frit*
ECMSSA50CT	50mL PP centrifuge tube with 6g Anhydrous Magnesium Sulfate, 1.5g Anhydrous Sodium Acetate
ECMSSC50CT	50mL PP centrifuge tube with 4g Anhydrous Magnesium Sulfate, 1g Sodium Chloride
EUMIV50CT	50 mL PP centrifuge tube with 6g of magnesium sulfate anhydrous, 1.5g of sodium chloride, 1.5g of sodium citrate dihydrate, And 750 mg of disodium citrate sesquihydrate

Dispersive Products

Part Number	Contents and Use Description
CUMPSCB2CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA & 50mg Carbon

A dispersive SPE product for removing pigments, polar organic acids, sugars and lipids. Will cause some loss of planar pesticides. Designed for use with a 2 mL aliquot of supernatant.

CUMPS2CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA

A dispersive SPE product for removing polar organic acids, some sugars and lipids. Designed for use with a 2 mL aliquot of supernatant.

CUMPSC18CT	2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA & 50mg endcapped C18

A dispersive SPE product for removing polar organic acids, sterols, sugars and lipids. Designed for use with a 2 mL aliquot of supernatant.

Dispersive Products Continued

Part Number	Contents and Use Description
ECMPSCB15CT	15mL centrifuge tubes with 900mg Anhydrous Magnesium Sulfate, 300mg PSA & 150mg carbon

A dispersive SPE product for removing pigments, polar organic acids, sugars and lipids. This product will cause the loss of planar pesticides. Designed for use with a 10 mL aliquot of supernatant.

ECMPSC1815CT	15mL centrifuge tubes with 900mg Anhydrous Magnesium Sulfate, 300mg PSA & 150mg endcapped C18

A dispersive SPE product for removing polar organic acids, sterols, some sugars and lipids from a 10 mL aliquot.

Cartridge Products

Part Number	Contents and Use Description
ECPSACB6	6mL columns with 200mg Graphitized Carbon on top, 400mg PSA on bottom, separated by a Teflon frit

Used in the dual phase variation of QuEChERS, this product removes pigments, polar organic acids, some sugars and lipids from an aliquot of extract.

ECPSACB256	6mL columns with 250mg Graphitized Carbon on top, 500mg PSA on the bottom, separated with a Teflon frit

Used for the same application as ECPSACB6 but with a different quantity of sorbents.

ECPSACB506	6mL columns with 500mg Graphitized Carbon on top, 500mg PSA on the bottom, separated with a Teflon frit*

Used for the same application as ECPSACB6 but with a different quantity of sorbents.

Extraction Products

ECMSSA50CT	50mL PP centrifuge tube with 6g Anhydrous Magnesium Sulfate, 1.5g Anhydrous Sodium Acetate

This product is listed in the AOAC for the extraction of pesticide residues using the QuEChERS method. It's primarily designed to preserve base sensitive compounds such as fungicides during extraction.

ECMSSC50CT	50mL PP centrifuge tube with 4g anhydrous Magnesium Sulfate, 1.0 g Sodium Chloride

This product is used for the extraction of pesticide residues when base sensitive compounds are not an issue. The addition of sodium chloride improves extraction efficiency. This product also results in a cleaner extract.

EUMIV50CT	50 mL polypropylene centrifuge tube with 6 g of anhydrous magnesium sulfate, 1.5 g of sodium chloride and 750 mg of disodium citrate sesquihydrate.

This product is the European version of ECMSSA50CT. It contains both a buffer and sodium chloride.

- Slide courtesy of Dr. Steven Lehotay, USDA-ARS

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

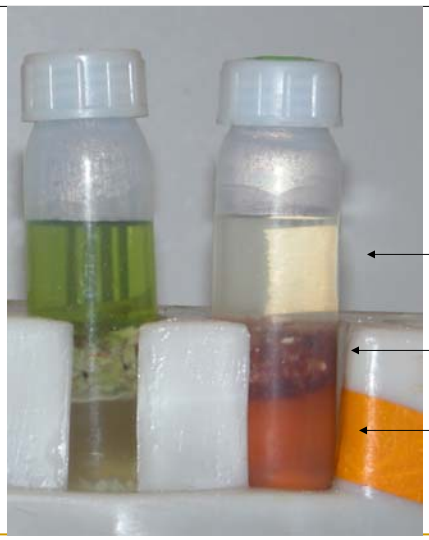
4) Centrifuge for 1 min

5) Analyze Pesticides

Add hydrated vegetation to salts and solvent SHAKE

Slide courtesy of Frank Schenck, FDA

Centrifuge

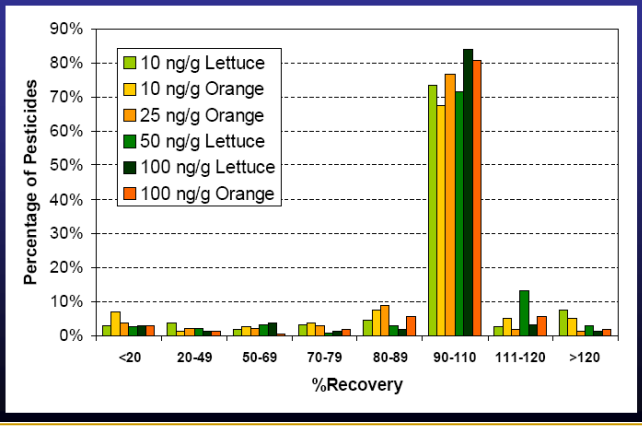


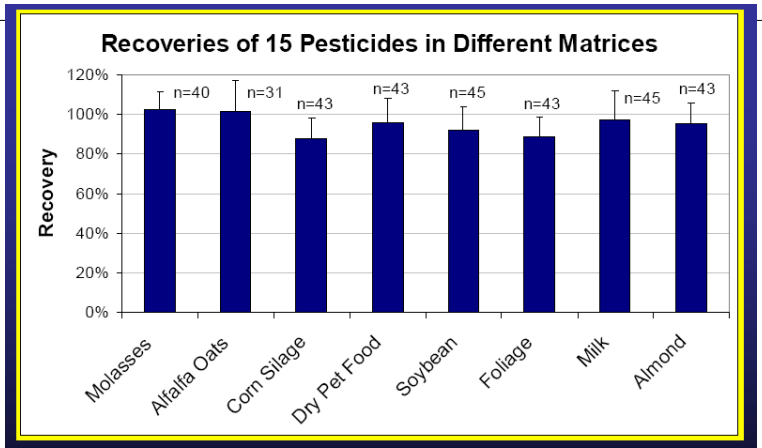
Slide courtesy of Frank Schenck, FDA

- Slides courtesy of Dr. Steven Lehotay, USDA-ARS

Recoveries in the QuEChERS method

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

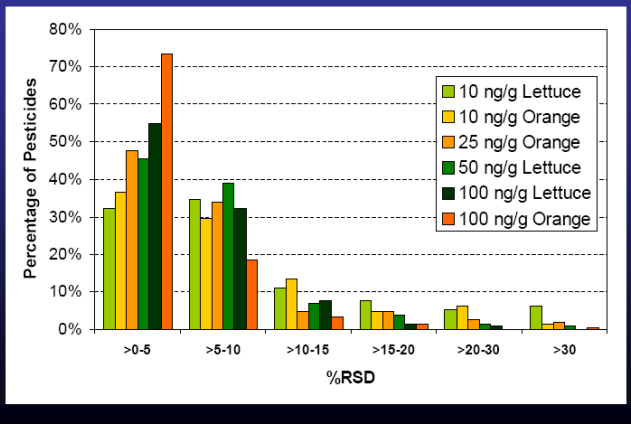




No differences were found vs. matrix for individual pesticides or concentration

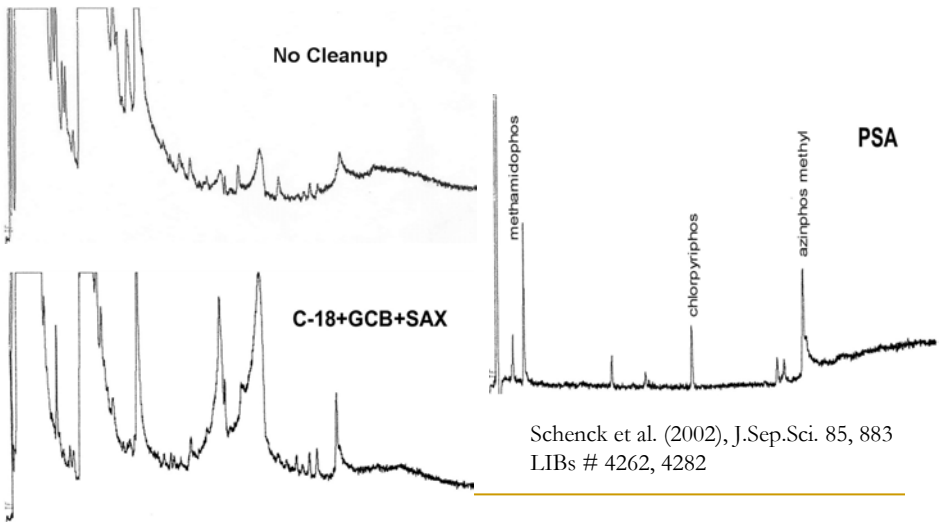
Repeatability in the QuEChERS method

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



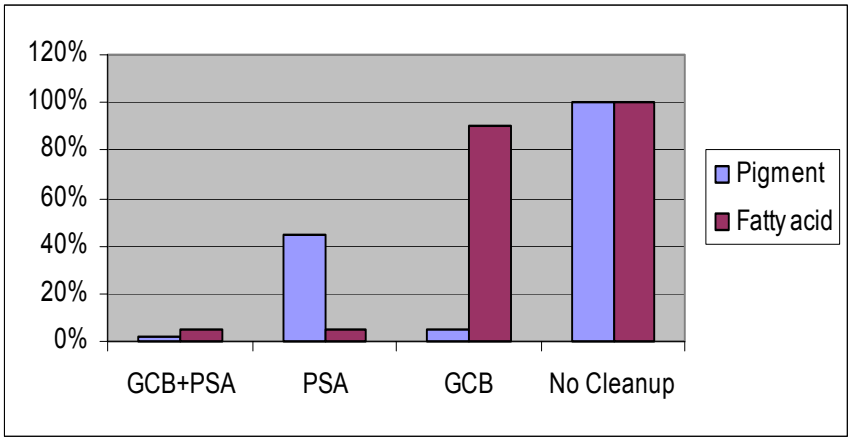
Why PSA CLEANUP

(GC/FPD - Asparagus Extract)



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

Matrix components removed by SPE sorbents



Saito et al (2004)

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
EPN	epoxiconazole	Esfenvalerate	etaconazole	ethiofencarb

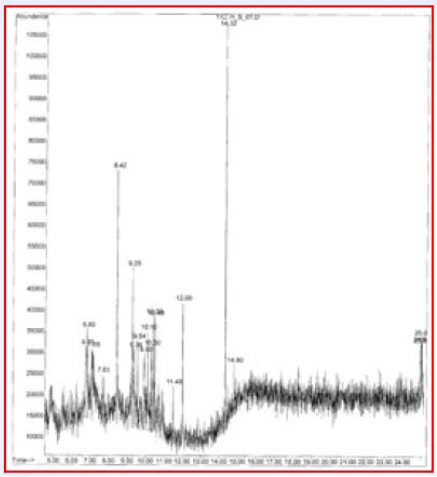
ethiofencarb sulfoxide	Ethion	ethirimol	Ethoprophos	etofenprox
Etridiazole	Famoxadone	fenamiphos	fenamiphos sulfone	Fenarimol
Fenazaquin	fenbuconazole	fenhexamid*	Fenithrothion	fenoxycarb
Fenpiclonil	Fenpropathrin	Fenpropidine	fenpropimorph	fenpyroximate
Fenthion	fenthion sulfoxide	Fenvalerate	florasulam*	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

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		

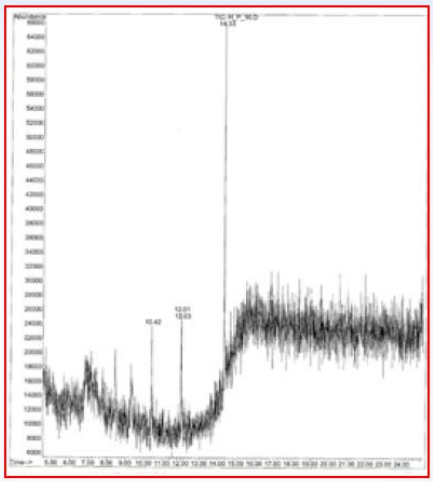
Why buy it from UCT?

Since many laboratories assemble their own clean-up products for this analysis, a comparison study was conducted at the USDA ARS Eastern Regional Research Center to determine if commercially prepared QuEChERS products could be as effective as products prepared in the users lab. Bulk magnesium sulfate anhydrous, primary secondary amine (PSA) and endcapped C18 were purchased from a commercial source and compared to the commercial product assembled using the same lot of bulk sorbents. The ratio of magnesium sulfate, PSA and C18 was 3:1:1. The clean-up products were tested on extracts of milk, honey and soybean. Efficacy of clean-up was determined by GC/MS analysis and compared the number of peaks above threshold values. Results clearly showed that the commercially prepared product provided fewer interferences than the product prepared in the lab in all three matrices. The extra peaks observed in the lab prepared product were probably caused by contamination from the lab environment. The commercially assembled product was prepared under controlled conditions minimizing potential contamination. These results, coupled with the obvious time and labor savings for assembly, indicate that commercially available QuEChERS products are preferable to products made "in-house".

Honey Extract Cleaned with "In House" Product



Honey Extract Cleaned with Commercial Product

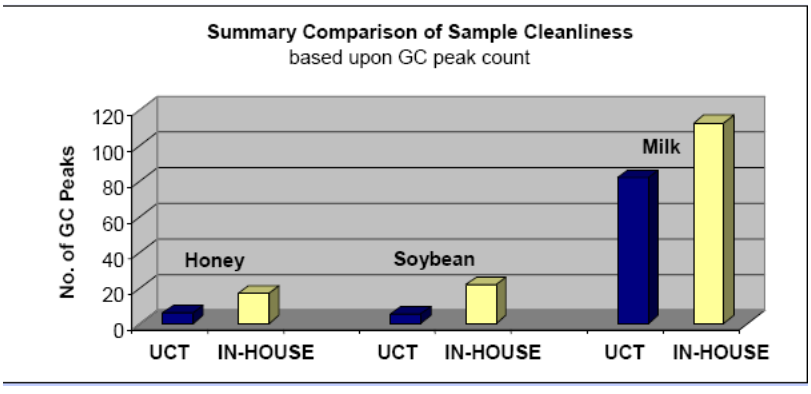


Chromatograms for soybean and milk products showed similar improved clean-up when using commercial vs. "in-house" prepared products.

GC Peak Count

Above Threshold Value

Matrix	HONEY		SOYBEAN		MILK	
	UCT	IN-HOUSE	UCT	IN-HOUSE	UCT	IN-HOUSE
Replicate	# of peaks	# of peaks	# of peaks	# of peaks	# of peaks	# of peaks
1	7	20	7	17	43	91
2	9	12	8	15	49	103
3	7	21	5	20	52	108
4	8	24	2	12	43	121
5	5	18	6	8	46	117
6	5	22	2	13	45	104
7	8	8	7	11	49	89
8	4	13	4	10	103	117
9	5	18	4	7	107	127
10	8	12	3	9	106	127
11	6	15	2	31	116	120
12	6	12	8	28	126	118
13	6	19	6	35	104	119
14	6	21	4	51	106	108
15	5	20	4	43	100	118
16	4	14	7	43	109	113
Average	**6**	**17**	**5**	**22**	**81**	**113**



Conclusions

- GC peak counts were significantly lower with the commercial QuEChERS product, indicating cleaner extracts
- Variability of peak counts on replicate samples were significantly lower using commercial QuEChERS products
- Savings in preparation costs and reduced product variability when using commercial QuEChERS products

References

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S.J. Lehotay, "Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Approach for Determining Pesticide Residues" in Methods in Biotechnology, Vol. 19, Pesticide Protocols, Martinez Vidal, J.L. and Garrido Frenich, A. (Eds.) Humana Press, Totowa, NJ, 2005, pp. 239-261.

F. J. Schenck, Victor Vega, "The Elution/Retention Characteristics of Pesticides on Graphitized Carbon and Amino Solid Phase Extraction Columns" Southeastern Regional Laboratory Pesticides; April 2001 Laboratory Information Bulletion

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F.J. Schenck and J.E. Hobbs, "Evaluation of the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) approach to pesticide residue analysis" Bull. Environ. Contam. Toxicol., 73, 24-30 (2004)

Current Trends in QuEChERS Research

UCT

Multiresidue analytical method using dispersive solid-phase extraction and gas chromatography/ion trap mass spectrometry to determine pharmaceuticals in whole blood

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UCT

QuEChERS for Anthelmintics

New Method for the Analysis of Anthelmintics in Animal Tissues

Brian Kinsella[1,2,3], Steven Lehotay[3], Katerina Mastovska[3], Alan Lightfield[3], Martin Danaher[1], Ambrose Furey[2]

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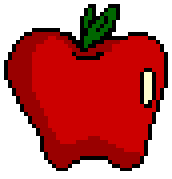


1. Add 10 mL acetonitrile to **UCT** product **ECMSSC50CT**
2. Shake
3. Add 10 g. hydrated sample
4. Add internal standard (Cyprodinil + 2,4D)
5. Shake for 1 minute
6. Centrifuge for 5 minutes
7. Clean-up by adding 1 mL of supernatant to a 2 mL centrifuge containing 50 mg **UCT EEC18** sorbent and 150 mg **UCT $MgSO_4$**
8. Mix for 1 minute
9. Centrifuge for 1 minute

Analysis

- Place 0.5 mL into auto sampler vial
- Add QC spike (TPP)
- Inject onto LC-MS/MS (ESI+ and ESI-)

UCT

39 Flukicides/Anthelmintics

ESI +

Triphenyl Phosphate (IS)
Cyprodinil (QC spike)

Abamectin
Doramectin
Emamectin
Eprinomectin
Moxidectin
Ivermectin
Selamectin

Dichlorvos
Coumaphos
Coumaphos-Oxon
Haloxon
Morantel
Levamisole

Albendazole
Albendazole-Sulfoxide
Albendazole-Sulfone
Albendazole-Amino-Sulfone
Cambendazole
Flubendazole
Amino-Flubendazole
Hydroxy-Flubendazole
Fenbendazole
Fenbendazole-Sulfone
Oxfendazole
Mebendazole
Amino-Mebendazole
Hydroxy-Mebendazole
Oxibendazole
Thiabendazole
5-Hydroxy-Thiabendazole
Triclabendazole
Triclabendazole-Sulfoxide

ESI –

2,4-D (IS)

Bithionol
Clorsulon
Closantel
Niclosamide
Nitroxynil
Oxyclozanide
Rafoxanide
Triclabendazole

Slide adapted from Brian Kinsella, Teagasc

UCT

Removal of Co-extractives

Percentage of Co-extractives Removed

	PSA	C18	PSA+C18
Liver	81%	36%	89%
Milk	10%	73%	90%

- C_{18} removes lipophilic compounds
- PSA removes organic acids

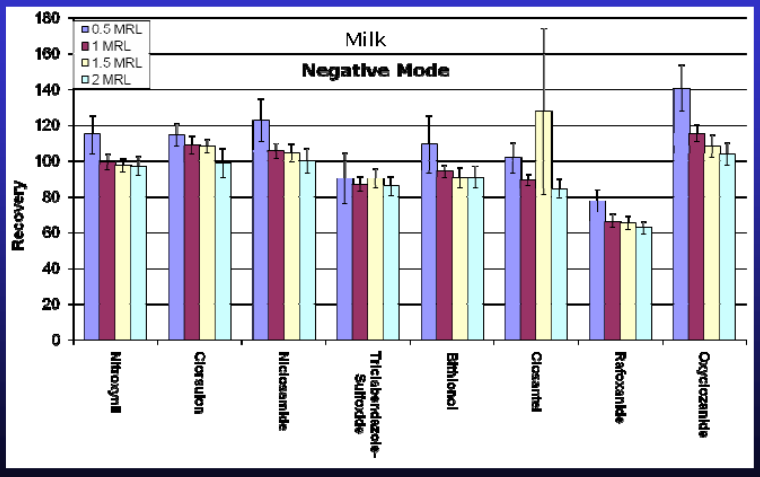
- PSA + C_{18} removed highest % of co-extractives
- But PSA + C_{18} also removed analytes

- C_{18} alone provides sufficient clean-up and gives high recoveries

Slide adapted from Brian Kinsella, Teagasc

UCT

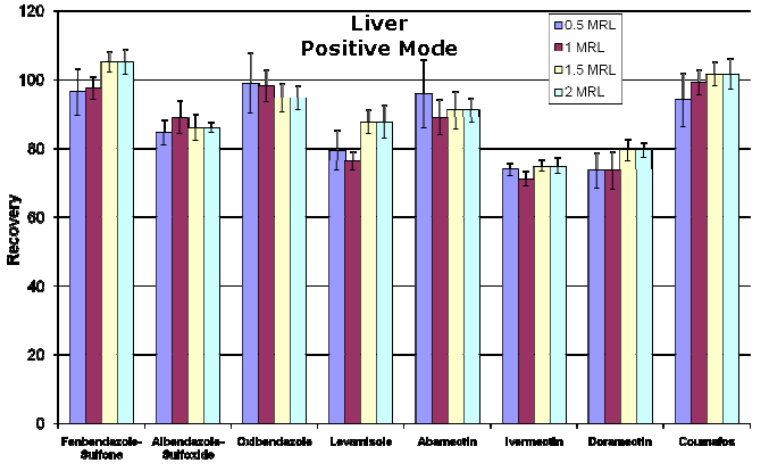
Recovery Experiments



Slide adapted from Brian Kinsella, Teagasc

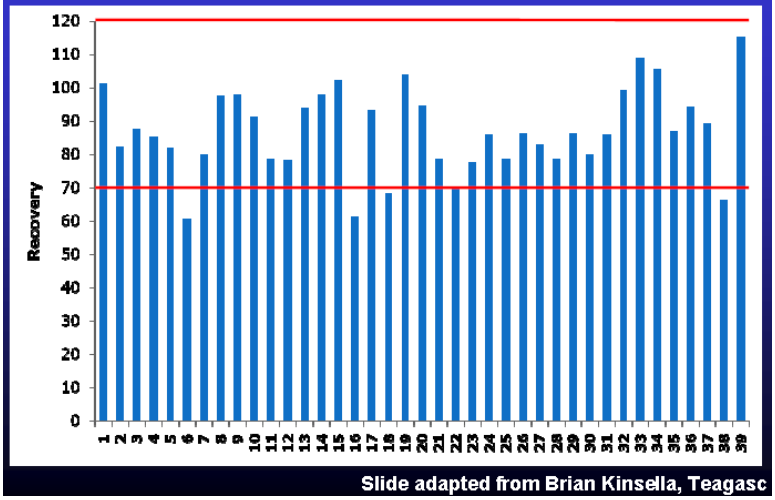
UCT

Recovery Experiments



Slide adapted from Brian Kinsella, Teagasc

Recoveries of the 39 Analytes

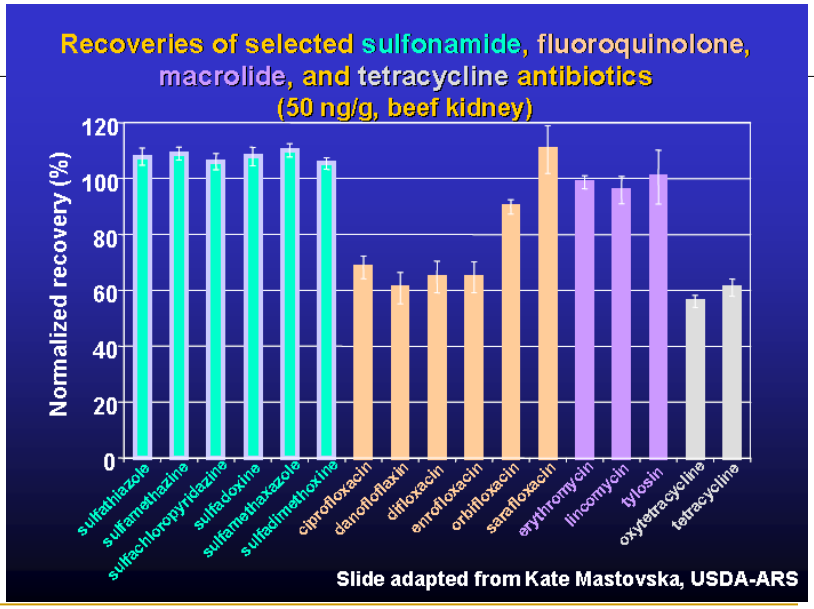


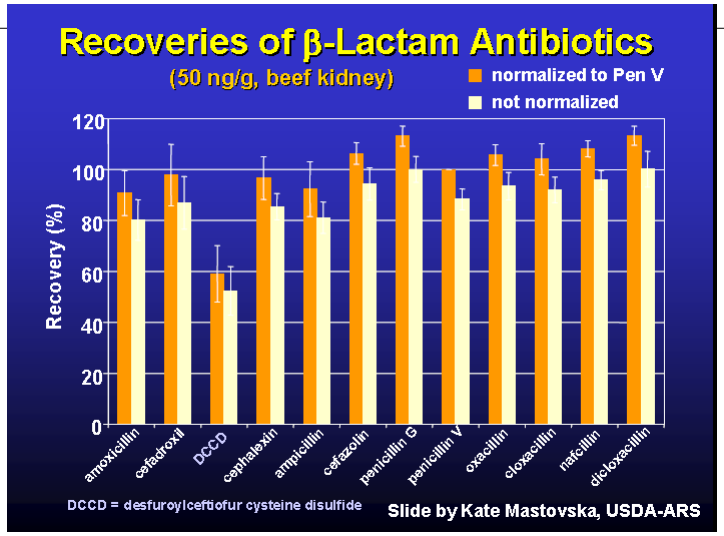
Slide adapted from Brian Kinsella, Teagasc

Vet. Drug Analysis in Animal Tissue

Streamlined sample preparation method for analysis of many antibiotics in beef kidney/juice or serum

•Weigh out 1 g of homogenized beef kidney sample, or kidney juice or serum in a 50 mL FEP (fluorinated ethylene propylene) tube (a disposable polypropylene Corning tube can be used instead).
•Add 100 µL of 1 µg/mL composite internal standard solution of 13C-sulfamethazine (serving for volume changes compensation), penicillin-V and cefadroxil (a penicillin and cephalosporin, respectively, serving for method performance control) in water.
•Add 2 mL water and 8 mL acetonitrile.
•Vortex briefly, shake for 5 min.
•Centrifuge at 3450 rcf for 5 min.
•Decant the supernatant into a 50 mL tube with 500 mg ***UCT EEC18*** **bulk sorbent**.
•Vortex briefly, shake for 30 s.
•Centrifuge at 3450 rcf for 1 min.
•Place 5 mL aliquot of the supernatant into a graduated tube.
•Evaporate down to < 1 mL.
•Make up the volume to 1 mL with water.
•Transfer the extract into Whatman Uni-Prep vials (PVDF, 0.45 µm) and compress the plunger to filter the extracts, which are then ready for LC-MS/MS analysis.





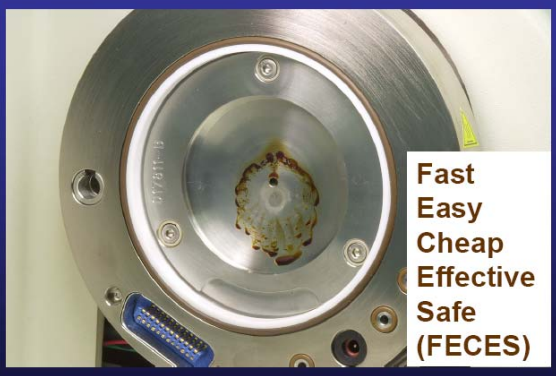
LC-MS/MS results for 121 veterinary drugs in kidney juice

spiked at 10, 25, 50, and 100 ng/mL
n = 16 (1 spike each day for 16 days)

80-120% avg recov (10-20% RSD):	35 analytes
70-120% avg recov (21-30% RSD):	52
50-69% avg recov (20-30% RSD):	9
55-130% avg recov (31-42% RSD):	17
20-35% avg recov (30-45% RSD):	6
Good results but degradation issue:	2

(cephapirin => desacetyl cephapirin)

QuEChERS without Rugged is:



Fast
Easy
Cheap
Effective
Safe
(FECES)

Curtain plate after injection of 25 samples with extracts from raisins without cleanup

Picture from Lutz Alder, BfR, Germany

Don't Suffer in Silence!
This man's job is to help you.

Contact him with any questions or problems.

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