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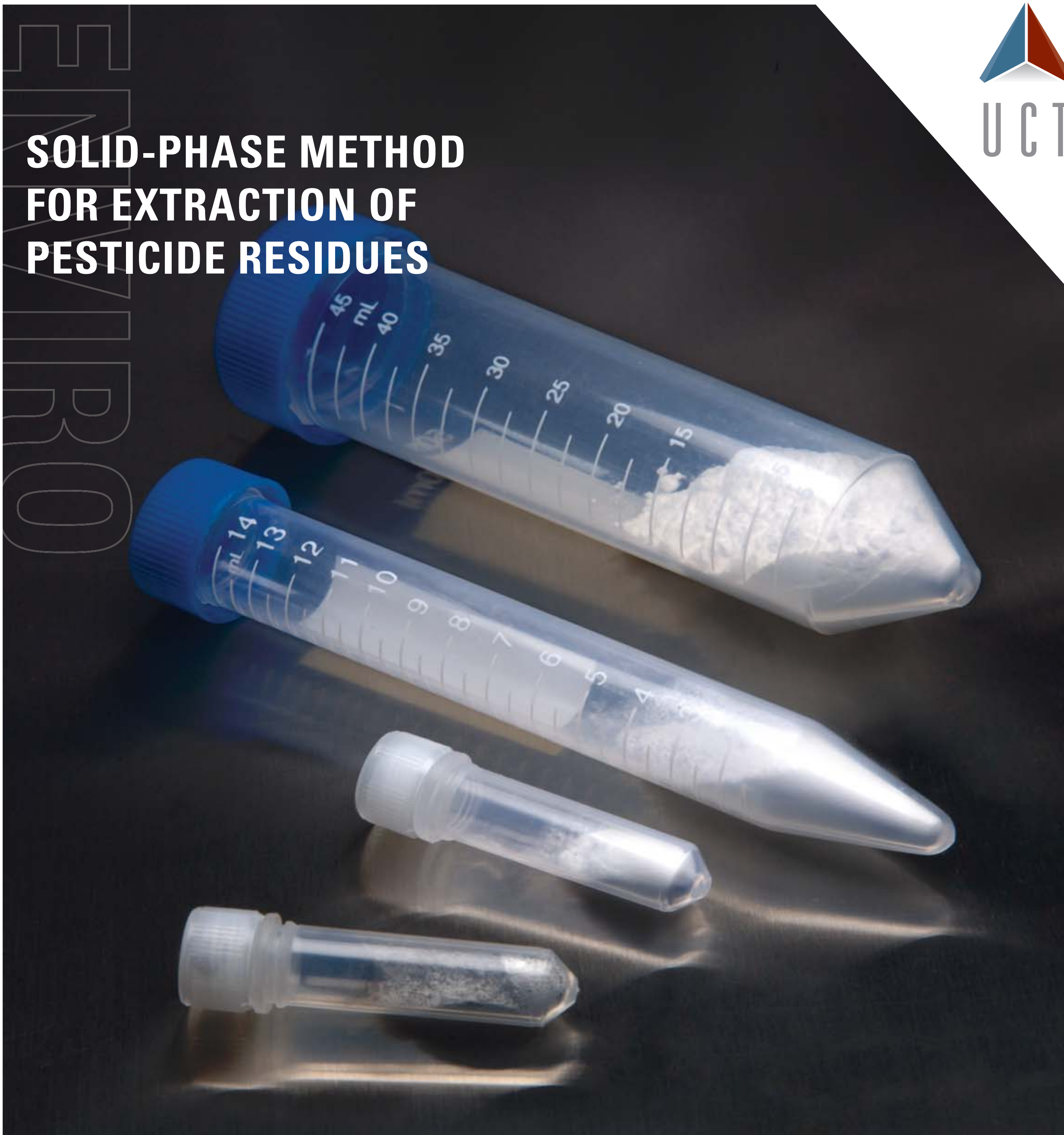
SOLID-PHASE METHOD FOR EXTRACTION OF PESTICIDE RESIDUES



QuEChERS

INFORMATIONAL BOOKLET

QUICK, EASY, CHEAP, EFFECTIVE, RUGGED AND SAFE

ENVIRO

QuEChERS, the Multiresidue Method of Choice

QuEChERS (pronounced Catchers), an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe, is a sample preparation and clean-up technique for the analysis of multiple pesticide residues in high moisture food samples. Since the development and publication of the method by Anastassiades and Lehotay, et al in 2003,**QuEChERS** has been gaining significant popularity. It is the method of choice for food analysis because it combines several steps and extends the range of pesticides recovered over older, more tedious extraction methods. The method has undergone various modifications and enhancements over the years since its first introduction. These have been designed to improve recovery for specific types of pesticides. Although Schenck and Vega published a clean-up method in 2001 prior to the introduction of **QuEChERS**, its techniques may be incorporated in the current method enhancing **QuEChERS** utility.

While primarily used for the analysis of fruits and vegetables, the **QuEChERS** method is also finding utility as the sample preparation method of choice for a full range of food products such as honey, nut meats, soybeans, animal feeds, foliage and other foods as well. Organic acids, plant pigments and other potential contaminants are removed during the cleanup process yielding cleaner chromatograms. The method offers the advantages of high recoveries, accurate results, high sample throughput and low non-chlorinated solvent usage. This reduces reagent costs and staff exposure to hazardous solvents. Additionally, glassware usage and labor costs are reduced since sample requirements are small and less bench space is required. The broad utility and ease of use makes the method an excellent choice for residue analysis.

The Need for QuEChERS

Consensus has been growing within the scientific community that small doses of pesticides and other chemicals can have adverse health effect on humans and animals. In the last few years, pesticide residues in foods have become a major consumer safety issue since application of chemical pesticides for food products is widely used. Also, as large quantities of fruits and vegetables are now imported, concerns have arisen as to their safety versus those grown domestically. To address these concerns, regulatory agencies have resorted to the use of various analytical methods to monitor these food stocks increasing both the scope of residue analysis and the number of samples analyzed.

The analysis of pesticide residues in food and environmental samples has been practiced for over 40 years by laboratories throughout the world. The method of extracting pesticide residues from food samples and preparing them for analysis is a time consuming, expensive, and labor intensive process. To address this problem, new multiresidue methods such as the **QuEChERS** method have been developed to accommodate the increase in sample loads. This new multiresidue method has yielded an increase in laboratory throughput while also improving analytical sensitivity. Improved throughput has been accomplished primarily by enhanced sample cleanup products that reduce potential interferences to yield cleaner chromatograms and reduced potential instrument downtime.

Multiresidue methods cover a broad scope of pesticides (see Appendix I) and offer the advantages of being cost-effective, rapid, sensitive, and sufficiently accurate for regulatory purposes. The **QuEChERS** method streamlines analysis and makes it easier and less expensive for analytical chemists to examine high moisture foods where water content may present problems with extraction of pesticides. Even dried vegetation can be rehydrated prior to extraction to facilitate the use of the **QuEChERS** method.

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How Does QuEChERS Work?

QuEChERS is known as a multiclass, multiresidue method (MRM) for analysis of pesticides from high water content (80-95%) matrices. Multi-residue pesticide analysis of food and environmental samples can be problematic due to the wide range of chemical properties encountered with pesticide residues. Also, 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 now published as AOAC method 2007.01 **"Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate"** consists essentially of a liquid-liquid micro extraction. This is followed by sample cleanup to remove unwanted matrix materials that interfere with chromatographic analysis. After shaking a sample with acetonitrile (MeCN) buffered with sodium acetate, partitioning is aided by the addition of $MgSO_4$. The preferred solvent is acetonitrile because it has been shown to provide extraction of the broadest range of organic compounds without co-extraction of large amounts of lipophilic material. Although other solvents can be employed depending upon the residues to be extracted, acetonitrile is highly compatible with GC/MS and LC/MS applications showing the fewest interferences.

Some modifications to the original **QuEChERS** method have been introduced to ensure efficient extraction of pH dependent compounds (e.g. phenoxyalcanoic acids), to minimize degradation of susceptible compounds (e.g. base and acid labile pesticides) and to expand the spectrum of food matrices amenable by the method. Buffering with citrate salts has been introduced in the first extraction/partitioning step 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 using a primary-secondary amine (PSA) sorbent. Acidic pesticides are directly analyzed from the raw extract before PSA cleanup. In another modification introduced by Schenck, graphitized carbon black (GCB) is used to remove plant pigments.

Currently there are three variations of the **QuEChERS** method being used in the United States

1) **The original QuEChERS method.** Introduced in 2003, this method used sodium chloride to enhance extraction.
2) **Dispersive AOAC 2007.01.** Uses sodium acetate as a buffer replacing sodium chloride.
3) **The dual phase column:** This method variation introduces the use of PSA and GCB 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).

***Examples of planar (polar aromatic) pesticides which may be removed by graphitic carbon:** chlorothalonil, coumaphos, hexachlorobenzene, thiabendazoleterbufos, quintozene

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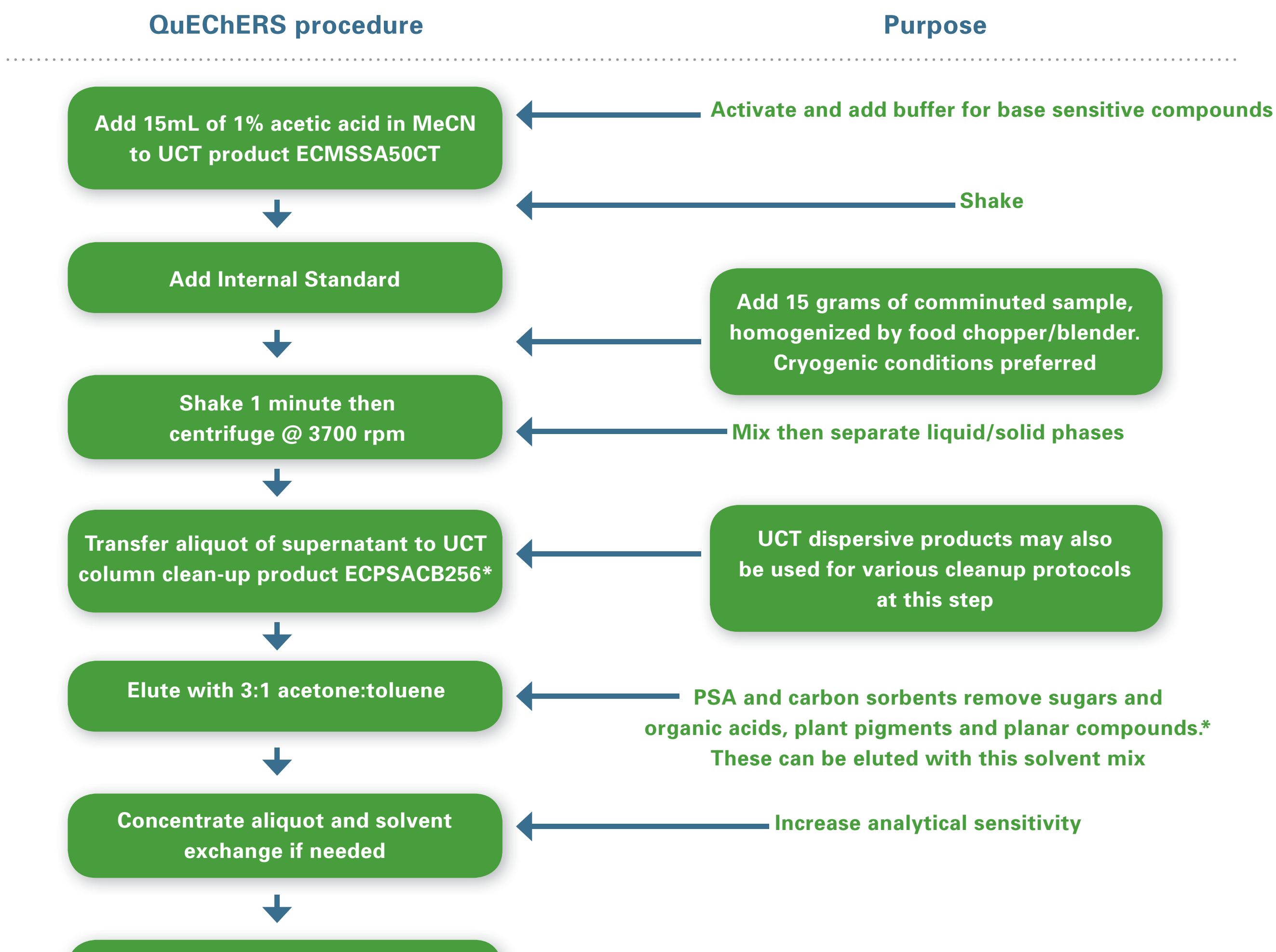
Dry commodities such as cereals, dried fruits, tobacco or teas require the addition of water prior to extraction to weaken interactions of pesticides with the matrix and to ensure adequate partitioning. Even commodities with high lipid content such as avocados or other high oil load plants can be analyzed by this method. However, due to a partitioning to the lipid phase, highly non-polar pesticides may give relatively low yet consistent recoveries of up to 70%. These co-extracted lipids can be reduced by a freezing-out step or the use of a C18 cleanup step.

The **QuEChERS** method gives at least fourfold lower material costs with significantly greater sample throughput per analyst than traditional methods. By combination of several different steps there is less chance for the introduction of error at each step. A polypropylene (PP) tube is the only consumable item required eliminating all glassware used in conventional methods. Furthermore, less than 10 mL of solvent waste is generated, much less than the 75-450 mL generated by other methods. Key to the new approach is the re-discovery of a rapid procedure called dispersive solid-phase extraction. This technique quickly removes residual moisture with magnesium sulfate. Other potential interferences are reduced by employing a primary-secondary amine sorbent to capture acidic components.

How is QuEChERS Conducted?

Procedural steps in the QuEChERS analysis for base sensitive compounds can be outlined in the schematic representation below:



*ECPSACB6 or ECPSACB506 may be used instead for different sorbent quantities

QuEChERS Methods

Dispersive Methods

AOAC 2007.01 in brief

(if base sensitive compounds are present)

1. To 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 1 minute
6. Centrifuge for 1 minute at 3700 rpm
7. Add an aliquot of the supernatant to the appropriate dispersive clean-up product (See **Product List and Use Description** below for selection) UCT CUMPSCB2CT, CUMPS2CT, CUMPSC18CT, ECMPSCB15CT, or ECMPSC1815CT
8. Shake for 1 minute
9. Centrifuge for 1 minute at 3700 rpm
10. Analyze extract

For compounds that are not base sensitive the following procedure provides a cleaner extract. This procedure is also necessary for acid labile compounds.

1. To 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 dispersive solid-phase cleanup tube: UCT CUMPSCB2CT, CUMPS2CT, CUMPSC18CT, ECMPSCB15CT, or ECMPSC1815CT (See Product List and Use Description below)
8. Shake for 1 minute
9. Centrifuge for 1 minute at 3700 rpm
10. Analyze extract

Matrix plant pigments often interfere with analysis. To reduce these interferences, graphitized carbon can be added to the dispersive solid-phase clean-up tubes. However, the use of carbon 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 (elution for planar [polar aromatic] compounds)

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

Cartridge product selection used for this analysis: UCT ECPSACB6, ECPSACB256 or ECPSACB506 depending upon sorbent mass required.

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Effect of Solvent Volume on Extraction

In a study designed to evaluate the effect of sample mass to solvent ratio (1:1 & 2:1MeCN) on recovery in a spiked fruit sample, Table 1 shows that the most polar pesticides did not partition into the MeCN phase as readily with the use of a lower solvent volume. However recovery still remained above 75% which is suitable for most analytical purposes. This indicates that the use of a minimum quantity of solvent for increased sensitivity will still yield good recovery values.

Table 1
Average Recovery (%) of Selected Pesticides from a 10 gram Fruit Sample

Pesticide	MeCN, 5 mL	MeCN, 10 mL
Dichlorvos	95	96
Methamidophos*	76	95
Mevinphos	96	100
Acephate*	84	99
o-Phenylphenol	94	94
Omethoate*	85	100
Diazinon	95	99
Chlorothalonil	94	95
Metalaxyl	94	100
Carbaryl	93	99
Dichlofluanid	97	97
Captan	97	100
Thiobendazole*	88	99
Folpet	92	94
Imazalil	92	102

*most polar   Anastassiades & Lohotay, J of AOAC International, Vol., 86, 2003

UCT Products Reduce Contamination Often Found in Laboratory Preparations

Many laboratories assemble their own clean-up products for the **QuEChERS** analysis. However contamination may be inadvertently introduced in the final extract complicating analyte peak identification. In a study conducted at the USDA ARS Eastern Regional Research Center, commercially prepared **QuEChERS** products were compared to those products prepared in the USDA lab. Bulk anhydrous magnesium sulfate, PSA and endcapped C18 sorbents provided by UCT, were assembled in the USDA lab then compared to UCT manufactured products using the same lot of bulk sorbents. The ratio of magnesium sulfate, PSA and C18 was 3:1:1 for this test. The clean-up products were evaluated on extracts of milk, honey and soybean and the efficacy of clean-up was determined by GC/MS analysis. Comparison of the extracts was made by counting the number of GC peaks above the threshold. Results clearly showed that the commercially prepared product provided superior clean-up to the product prepared in the lab. This result was confirmed in all three matrices. The extra peaks observed in the lab prepared product were probably caused by contamination from within the lab environment. The UCT assembled products were prepared under controlled manufacturing conditions that eliminated potential contamination typically encountered in lab environments. These results, coupled with obvious time and labor savings for assembly, indicate that **QuEChERS** products preassembled at UCT are preferable to products made "in-house".

The results of these tests are summarized in Table 2. Chromatograms are shown for honey.

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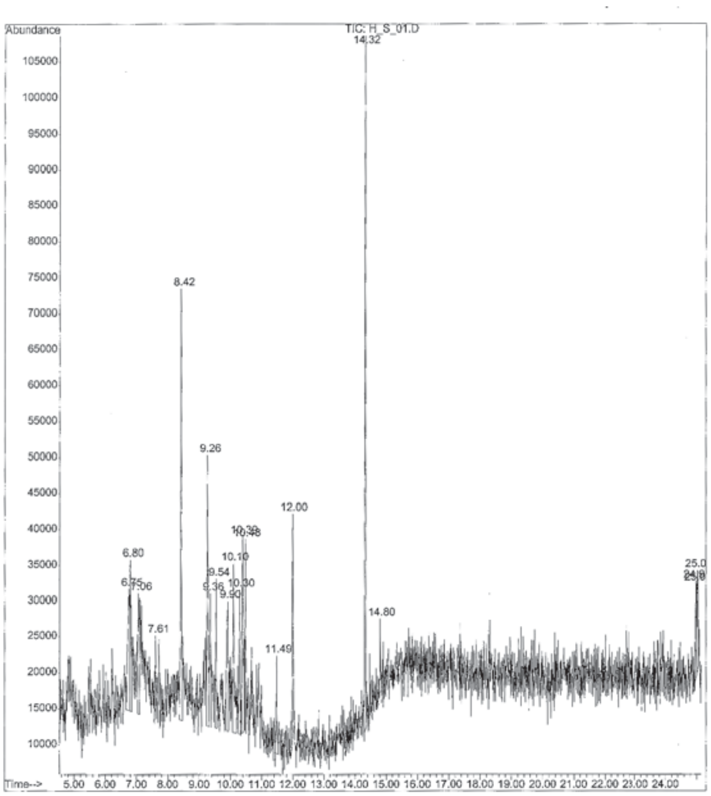
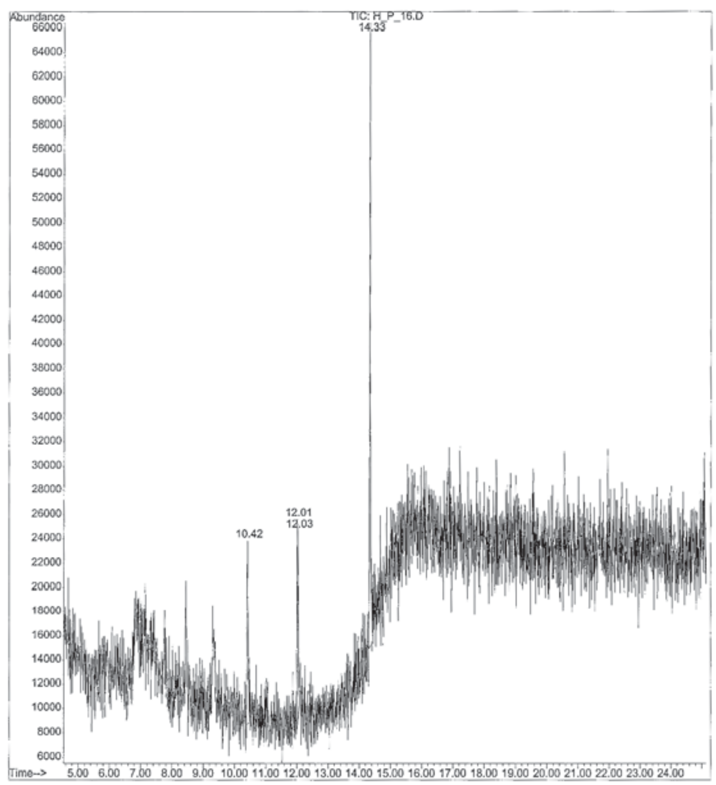
Chromatograms

Honey Extracted with "In-House" Product	Honey Extract Cleaned with UCT Products





The chromatogram on the left is representative of those obtained when clean-up materials were prepared in-house. The chromatogram on the right was obtained using UCT prepackaged **QuEChERS** products and shows a significant reduction in background interferences. This difference in background interference is thought to be due to sorption of contaminants from the laboratory environment.

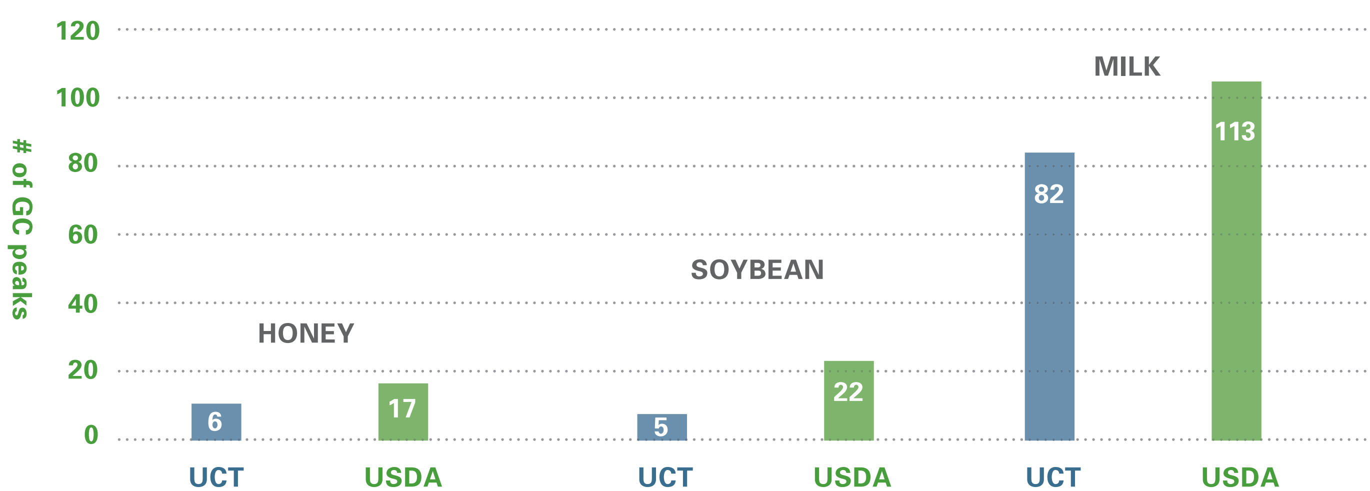
Chromatograms for soybean and milk products showed similar improved clean-up when using UCT manufactured vs. "in-house" prepared products.

Table 2
Total Number of Peaks as Seen in a GC Chromatogram

Data indicate that the use of UCT prepared products results in cleaner chromatograms

Matrix	HONEY		SOYBEAN		MILK	
	# of peaks	# of peaks	# of peaks	# of peaks	# of peaks	# of peaks
Replicate	UCT	USDA	UCT	USDA	UCT	USDA
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

Summary Comparison of Sample Cleanliness



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Why use UCT SPE QuEChERS products?

- Save valuable laboratory time in preparation
  - Reduced variability due to consistent product and rigorous quality control
- Cleaner extracts from cleaner products
- Extraneous GC peak counts are significantly lower using UCT prepared **QuEChERS** product
- Variability of GC peak counts on replicate samples were significantly lower using UCT **QuEChERS** products
- Dual layer columns are packaged in Mylar to eliminate potential sorbent contamination
- $MgSO_4$ is specially treated in a muffle furnace to remove organic contaminates typically encountered with in-house preparation

UCT provides a variety of solid-phase QuEChERS clean-up products that contain the proper sorbents for optimum extraction, clean-up and separation of analytes from complex matrices

UCT Products Used in the Micro Extraction Step

Cartridge product ECMSSA50CT is a 50 mL extraction tube that contains 6 grams of anhydrous magnesium sulfate and 1.5 grams of anhydrous sodium acetate. It complies with AOAC Method 2007.01. It is designed to allow the extraction of "base sensitive" compounds such as chlorothalonil, dichlofluanid, tolyfluanid, folpet, captafol, and captan from non-acidic matrices.

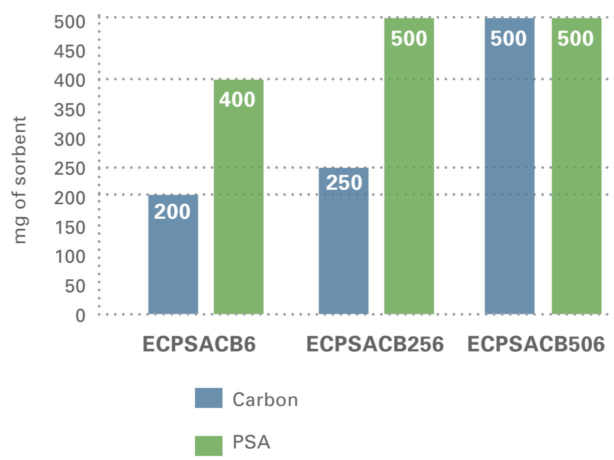
Cartridge product ECMSSC50CT is a 50 mL extraction tube contains 4 grams of anhydrous magnesium sulfate and 1 gram of sodium chloride. It is designed for use where base sensitive compounds are not present or are not of analytical interest. Eliminating the buffer allows a cleaner extract, and the sodium chloride aids in the extraction of the analytes.

Cartridge product EUMIV50CT is a 50 mL extraction tube that contains 6 grams anhydrous magnesium sulfate, 1.5 grams of sodium chloride, 1.5g sodium citrate dihydrate, and 0.75 grams of sodium citrate sesquihydrate.

UCT Cartridge Products Used for Sample Clean-Up

Several cartridge products are offered for use in sample clean-up. UCT provides a variety of **QuEChERS** products in SPE cartridge format which include PSA and GCB. These sorbents are used to remove various polar organic acids, polar pigments, some sugars and fatty acid co-extractables from **QuEChERS** extracts. These sorbents may be additionally combined with C18 for the removal of fatty plant lipids and sterols. Graphitized carbon black is used to remove sterols and pigments such as chlorophyll. Magnesium sulfate or other salts are used to enhance extraction as well as the removal of water and the partitioning of residues into the solvent phase. Because carbon has a strong affinity to retain planar molecules, Schenck et al have reported that the use of a 3:1 acetone:toluene solvent blend performed well at eluting these compounds from carbon sorbents. Bulk sorbents are also available from the UCT catalog.

Sorbent Mass Comparison by Cartridge Product



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Products List and Use Description

Part Number	Contents and Use Description
	Micro 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 polypropylene centrifuge tube with 4g anhydrous Magnesium Sulfate,1.0 g Sodium Chloride.** This product is used for the extraction of pesticide residues using the QuEChERS method. It is preferred over ECMSSA50CT when base sensitive compounds are not an issue. The addition of sodium chloride improves extraction efficiency.
EUMIV50CT	**50 mL polypropylene centrifuge tube with 6 grams of magnesium sulfate anhydrous, 1.5g of sodium chloride, 1.5g of sodium citrate dihydrate, and 750 mg of disodium citrate sesquihydrate.** This product is the European version of ECMSSA50CT and is used for the extraction of pesticide residues.
	Dispersive Products
CUMPSCB2CT	**2mL micro-centrifuge tubes with 150mg Anhydrous Magnesium Sulfate, 50mg PSA & 50mg Carbon.** A dispersive SPE product for removing polar organic acids, some sugars and lipids which may 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.

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Products List and Use Description

Dispersive Products

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, some sugars and lipids. Designed for use with a 2 mL aliquot of supernatant.

ECMPSCB15CT — **15mL centrifuge tubes with 900mg Anhydrous Magnesium Sulfate, 300mg PSA & 150mg carbon.**

A dispersive SPE product for removing polar organic acids, some 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

ECPSACB6 — **6mL columns with 200mg Graphitized Carbon on top, 400mg PSA on bottom, separated by a Teflon frit.***

Used in the Schenck 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. When in doubt use ECPSACB256.

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. When in doubt use ECPSACB256.

*Products available with polyethylene or teflon frits. Your choice will depend on your application and price requirements.

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Appendix I

List of possible pesticide analytes that have been shown to yield >90% (or >70 %*) recoveries using the QuEChERS method. GC-amenable pesticides are capitalized; those preferentially analyzed by LC/MS-MS are not capitalized; those that can be analyzed by either technique are underlined.**

Pesticide Analytes

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 sulfone
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
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penconazole
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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

**from "Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Approach for Determining Pesticide Residues", Steven J. Lehotay, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center; 600 East Mermaid Lane; Wyndmoor, Pennsylvania 19038; USA

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