

Analysis of Pesticide Residues in Apple by GC/MS using Agilent Bond Elut QuEChERS Kits for Pre-injection Cleanup

Application Note

Food Safety

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Abstract

The QuEChERS method, which stands for Quick, Easy, Cheap, Effective, Rugged and Safe was developed in 2003 by scientists at the USDA. This method was created to easily clean up and prepare food samples for multi-class, multi-residue pesticide analysis. This application note describes the use of the original, non-buffered QuEChERS method to prepare apple samples for residue analysis by gas chromatography/mass spectrometry (GC/MS). Fifteen pesticides of different classes were studied. The experiments were done using Agilent Bond Elut QuEChERS extraction kits for 10-g samples and dispersive kits for 1-mL sample volumes. The analysis was done by GC/MS using selective ion monitoring (SIM) mode. The limit of quantitation for all the pesticides studied was 10 ng/g in apple using this method. At 200 ng/g, the recoveries ranged from 89% to 102%, and at 10 ng/g, the recoveries ranged from 72% to 103%. The relative standard deviations associated with these recoveries were less than 11% in all cases.



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Introduction

Pesticides, which include herbicides, fungicides and other pest-control chemicals, have long been a part of agriculture. While their use can be widespread and beneficial, pesticides can also be harmful to both humans and animals. Because of this, pesticide handling is monitored by several agencies, including the United States Environmental Protection Agency (EPA) [1]. Of concern to the general population is the maximum residue levels (MRL) for pesticide in food items.

In 2003, scientists at the USDA developed a method for the quick and easy cleanup of food samples for pesticide analysis.[2] This method was given the acronym QuEChERS, which stands for Quick, Easy, Cheap, Effective, Rugged and Safe. The method has since been modified for other analyses. The method for this analysis incorporates a simple acetonitrile/water extraction facilitated by the addition of $MgSO_4$, which salts out water from the sample and includes a liquid/liquid extraction with these two solvents. The extraction step is followed by a dispersive solid phase extraction that combines

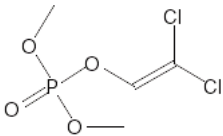
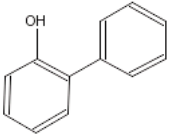
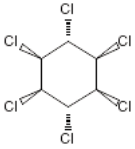
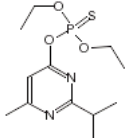
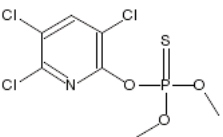
both a primary secondary amine (PSA) and anhydrous $MgSO_4$ to remove fatty acids and reduce the remaining water in the extract respectively. See the Agilent Bond Elut QuEChERS Kit brochure (publication 5990-3562EN) or www.agilent.com/chem/quechers for more information about QuEChERS and suggestions for analyses of different fruits and vegetables.

Experimental

Reagents and Chemicals

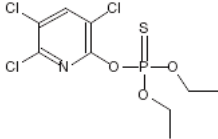
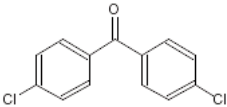
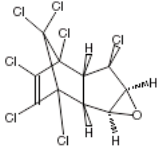
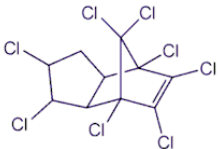
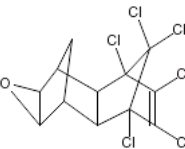
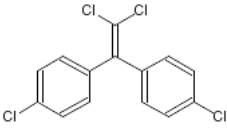
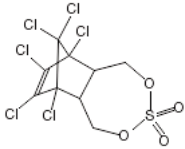
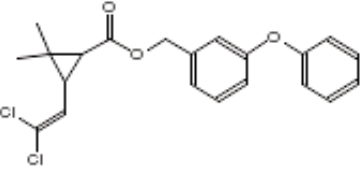
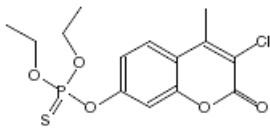
Water (EMD Chemicals, Gibbstown, NJ) and acetonitrile (Burdick and Jackson, Muskegan, MI) were HPLC grade. The pesticides were all analytical grade. Dichlorvos (98.9%), and diazinone (99.5%) were purchased from Ultra Scientific (Kingstown, RI). Coumaphos was purchased from Honeywell Riedel De Haen (Seelze, Germany). All other pesticides were purchased from Chem Service (West Chester, PA). See Table 1 for the chemical and regulatory information for the pesticides used in this study. [3-5] The internal standard, triphenyl phosphate (TPP) was purchased from Sigma-Aldrich (St. Louis, MO).

Table 1. Pesticide Chemical and Regulatory Information.

Analyte	Structure	Category	Log P	pKa	MRLs in apple
Dichlorvos		Organophosphate	1.9	NA	10
σ -phenylphenol		Phenol	3.18	9.4	20
Lindane		Organochlorine	3.69	NA	10
Diazinone		Organophosphate	3.69	2.6	100
Chlorpyrifosmethyl		Organophosphate	4.00	NA	500

(Continued)

Table 1. Pesticide Chemical and Regulatory Information.

Analyte	Structure	Category	Log P	pKa	MRLs in apple
Chlorpyrifos		Organophosphate	4.55	NA	100
dichlorobenzophenone		Organochlorine	4.44	NA	500
Heptachlor-epoxide		Organochlorine	5.83	NA	10
Chlordane		Cyclodiene organochlorine	2.78	NA	20
Dieldrin		Chlorinated hydrocarbon	3.7	NA	10
DDE		organochlorine	6.55	NA	50
Endosulfan sulfate		Organochlorine	3.13	NA	50
Permethrins		Pyrethroid	6.1	NA	50
Coumaphos		Organophosphate	3.86	NA	100

Solutions

Individual stock solutions of the pesticides (2 mg/mL) were prepared in methanol and stored at -20 °C. From these, the High-QC solution which was 20 µg/mL for all the pesticides was prepared in 50:50 acetonitrile/water. The internal standard was 20 µg/mL triphenyl phosphate in acetonitrile.

The High-QC solution was used to prepare all other spiking solutions. A mid range spiking solution (Mid-QC) with a concentration of 5 µg/mL was prepared in 50:50 acetonitrile/water. A low range spiking solution (Low-QC) with a concentration of 1 µg/mL was prepared in 50:50 acetonitrile/water.

Calibration Curve

A 2.5 µg/mL standard working solution was prepared using the High-QC solution. A six-point calibration curve (10, 20, 50, 100, 250 and 400 ng/mL) was created by adding the appropriate volume of this 2.5 µg/mL solution to the matrix blank extract. Internal standard solution was added to have a final concentration of 100 ng/mL.

Sample Preparation

Certified organic, pesticide-free red delicious apples were purchased at a local grocery. Approximately 3 pounds of apples were diced into approximately 1-cm cubes. The seeds were discarded, but the skin was included. The cubes were placed in a plastic bag and frozen at -20 °C overnight. For the first 5 hours in the freezer, the samples were massaged to prevent them from freezing together. When ready to perform the extraction, the amount of sample required was removed from the freezer. A coffee grinder (Mr. Coffee 2.3-oz coffee grinder, Shelton, CT) was used to comminute the sample. If necessary, dry ice may be added to aid this. The sample was checked to ensure that there were no large pieces or lumps remaining prior to extraction. [6].

QuEChERS Cleanup

Step 1 Extraction

The Bond Elut Original QuEChERS Method (non-buffered) Extraction Kit, for use with 10g samples (p/n 5982-5550) was used for the extraction step. A 10-g (± 0.05 g) amount of the homogenized apple sample was placed in a 50-mL centrifuge tube. 100 µL of the appropriate spiking solution was added. The sample was vortexed (VWR vortex mixer model K-550-G, West Chester, PA) for 1 min, then 10 mL of acetonitrile were

added. The sample was shaken vigorously for 1 min, then 4 g of MgSO₄ and 1 g of NaCl from the extraction salt packet (p/n 5982-5550) were added. The sample was vortexed for 1 min. A 100-µL amount of internal standard solution was added, then the sample was centrifuged (Eppendorf 5810R 15 amp, Westbury, NY) for 5 min at 5000 rpm. See Figure 1.

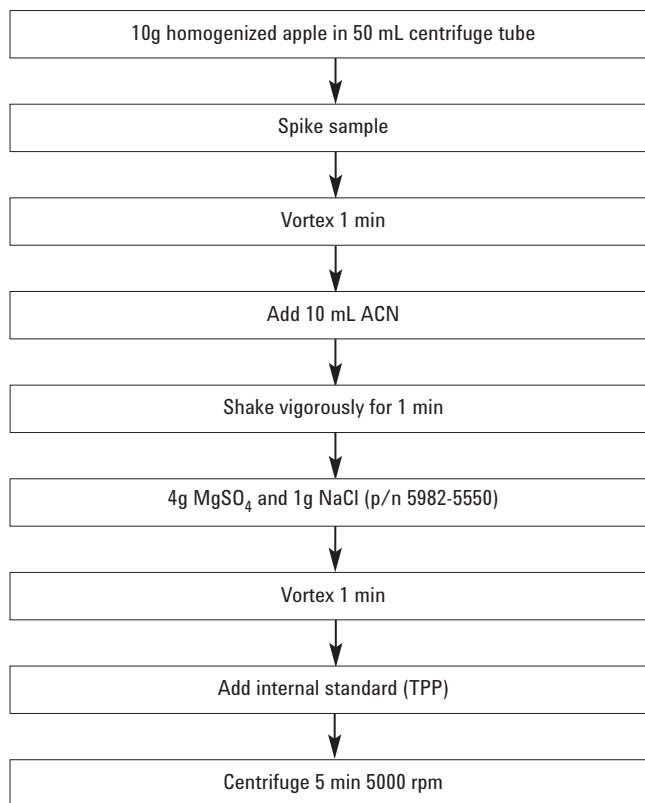


Figure 1. Extraction using Bond Elut QuEChERS kit.

Step 2 Dispersive SPE cleanup

The Bond Elut QuEChERS Dispersive Kit for General Fruits and Vegetables was used for dispersive SPE cleanup (p/n 5982-5022). This kit removes polar organic acids, some sugars and lipids. One milliliter of the resultant solution was transferred to a 2-mL centrifuge tube containing 50 mg of PSA and 150 mg of MgSO₄. This was vortexed for 30 sec, then centrifuged for 5 min (VWR micro-centrifuge model 235 B, West Chester, PA). A 0.5-mL amount of the resulting extract was transferred to a sample vial to be analyzed by GC/MS. See Figure 2.

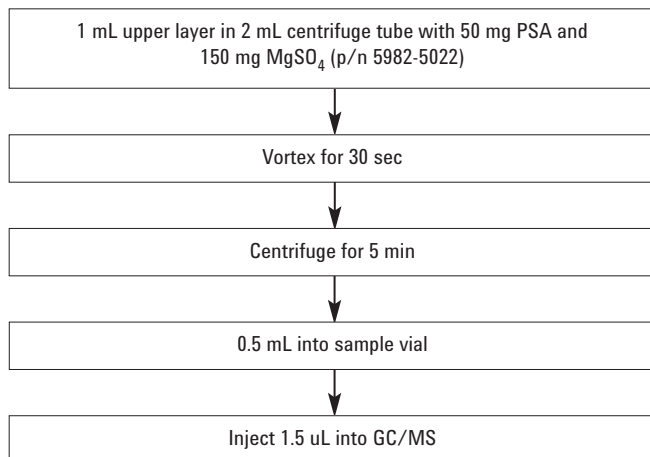


Figure 2. Dispersive SPE using Bond Elut QuEChERS kit.

Instrument Conditions

Samples were analyzed using an Agilent 7890A GC system with an Agilent 5975C Series GC/MSD (Agilent Technologies Inc., Santa Clara, CA). An Agilent GC/MS method for pesticide analysis was used with some minor modifications. (7) See Tables 2 and 3 for instrument conditions.

Table 2. GCMS instrument conditions

GC conditions	
Injection source	Manual
Inlet	Splitless
Column	Agilent J&W HP-5ms Ultra Inert, 30 m × 0.250 mm, 0.25 µm film (PN: 190915-433UI)
Carrier Gas	Helium in constant flow mode
Oven Temperature Program	70 °C (2 min) 25 °C/min to 150 °C (0 min) 3 °C/min to 200 °C (0 min) 8 °C/min to 280 °C (7 min)
Injection volume	1 µL
MS Conditions	
Tune File	Atune.u
Mode:	SIM
Source temperature	230 °C
Quad temperature	150 °C
Transfer line temperature	280 °C
Solvent Delay	4.00 minutes
Multiplier Voltage	Autotune voltage

Table 3. Selective Ion Monitoring (SIM) mode conditions.

Peak	Analyte	RT (min)	SIM target	SIM qualifier	Collection window (min)
1	Dichlorvos	6.9	185.00	109.50	4.0–9.0
2	σ-phenylphenol	10.4	170.10	169.10	9.0–14.0
3	Lindane	15.7	180.90	182.90	14.0–16.0
4	Diazinone	16.6	137.10	179.10	16.0–18.0
5	Methyl-chlorpyrifos	18.9	285.90	287.90	18.0–21.0
6	Chlorpyrifos	21.5	196.90	–	21.0–22.0
7	Dichlorobenzophenone	21.5	139.00	–	21.0–22.0
8	Heptachlor-epoxide	23.0	352.90	354.90	22.0–23.6
9	γ-chlordane	24.0	372.90	374.90	23.6–24.3
10	α-chlordane	24.6	372.90	374.90	24.3–25.0
11	Dieldrin	25.4	79.10	–	25.0–27.0
12	DDE	25.5	246.00	317.90	25.0–27.0
13	Endosulfan Sulfate	27.9	271.80	273.80	27.0–28.0
	I.S. TPP	28.6	325.1	326.1	28.0–29.5
14	Permethrin	32.1	183.10	–	29.5–38.0
15	Coumaphos	32.2	96.90	109.00	29.5–38.0

Results and Discussion

As shown in Figure 3b, the apple matrix blank sample had only a few peaks spread across the experimental collection times for the pesticides using the chosen GC/MS method. In the spiked sample (3a), all compounds except coumaphos were free of interferences and gave good linearity as shown in Table 4.

The peak corresponding to coumaphos was difficult to integrate in some samples due to an irregular baseline, which is a possible reason for poor linearity. The QuEChERS method of sample preparation was proven to be quick, easy and effective for this type of analysis. When using the QuEChERS method, samples may still have some impurities that can show up in the chromatograms. In order to achieve the best sensitivity for the analytes of interest, SIM mode was used. Sensitivity for the pesticides was greatly increased by selecting ions corresponding to the analytes of interest to be monitored during different segments of the experiment. In most cases, the highest abundance ion for each analyte was chosen to give the best sensitivity. However, in some cases where selectivity was compromised by this choice, another less abundant ion was used for quantitation. For most of the analytes, a second qualifier ion was also used. The selected ions for each compound and the time segments during which they were monitored are given in Table 3.

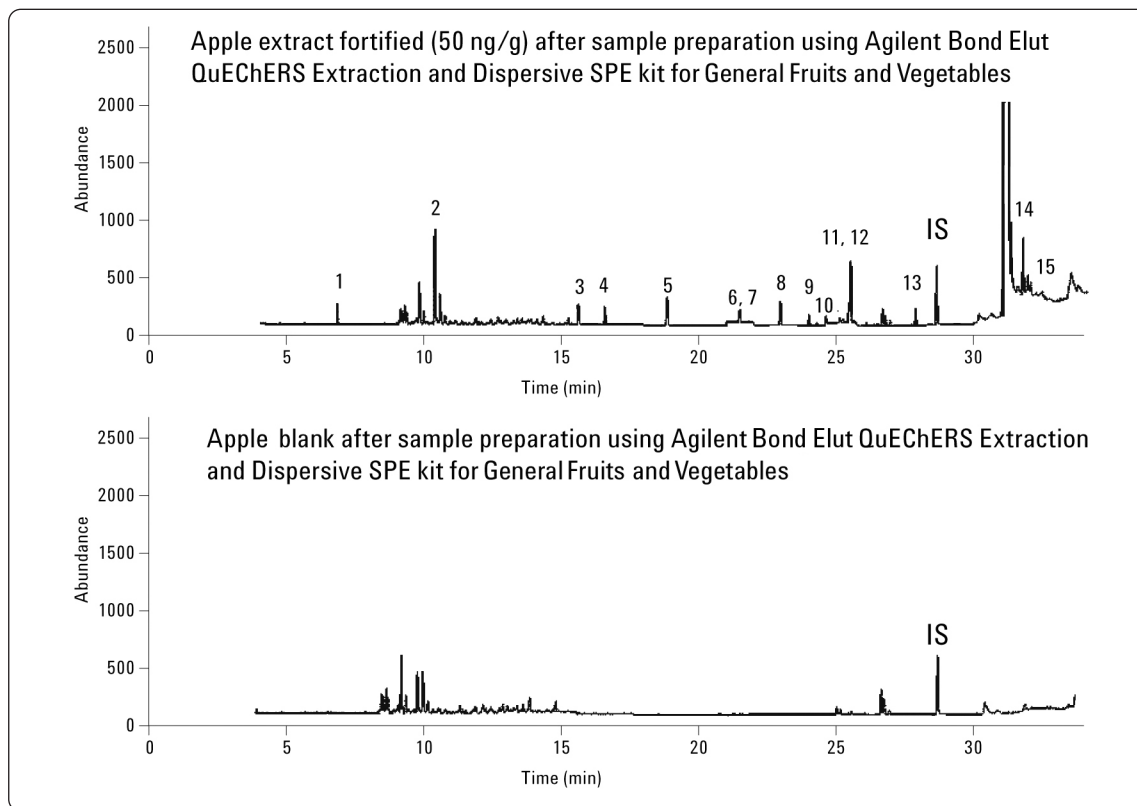


Figure 3. GC/MS chromatograms of apple extract. Peak identifications in Table 3.

Table 4. Regression data for pesticides

Pesticide	Regression Equation	R ²
Dichlorvos	$y = 0.1243x - 0.01141$	0.9965
σ -phenylphenol	$y = 0.6885x - 0.03763$	0.9965
Lindane	$y = 0.1719x - 0.02280$	0.9967
Diazinone	$y = 0.1811x - 0.02608$	0.9945
Methyl-chlorpyrifos	$y = 0.3242x - 0.05026$	0.9943
Chlorpyrifos	$y = 0.1459x - 0.02455$	0.9916
Dichlorobenzophenone	$y = 0.1573x - 0.01840$	0.9937
Heptachlor-epoxide	$y = 0.1995x - 0.02828$	0.9906
γ -chlordane	$y = 0.07058x - 0.005587$	0.9917
α -chlordane	$y = 0.05601x - 0.001840$	0.9927
Dieldrin	$y = 0.2091x - 0.02544$	0.9923
DDE	$y = 0.4609x - 0.05950$	0.9901
Endosulfan Sulfate	$y = 0.1262x - 0.01675$	0.9897
Permethrin	$y = 0.1327x + 0.03232$	0.9889
Coumaphos	$y = 0.06985x + 0.01864$	0.9543

Table 5. Recovery and reproducibility of pesticides in apple using the original QuEChERS method (n=4).

Pesticide	High-QC 200ng/g		Mid-QC 50ng/g		Low-QC 10ng/g	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Dichlorvos	99.4	2.8	96.7	10.8	102.8	5.0
σ -phenylphenol	89.5	6.3	79.6	6.8	92.0	6.1
Lindane	92.6	4.2	88.5	9.7	97.9	2.0
Diazinone	102.1	4.4	98.8	5.5	90.5	9.1
Methyl-chlorpyrifos	98.5	3.1	90.0	4.3	88.7	7.1
Chlorpyrifos	100.2	1.2	95.6	4.0	93.5	6.5
Dichlorobenzophenone	99.4	0.6	89.1	6.4	90.3	5.0
Heptachlor-epoxide	95.4	3.9	85.6	5.4	87.0	3.2
γ -chlordane	95.9	2.0	90.0	6.8	92.3	3.5
α -chlordane	93.5	2.6	85.8	6.9	95.5	4.7
Dieldrin	99.9	1.8	93.6	5.3	99.4	4.2
DDE	92.7	1.9	87.1	5.7	94.5	4.2
Endosulfan Sulfate	99.5	2.3	90.8	2.8	97.8	2.3
Permethrin	97.6	2.1	93.0	3.4	100.7	4.8
Coumaphos	96.6	3.0	79.6	3.5	72.5	4.5

Table 5 shows the recovery and reproducibility for each pesticide in the apple matrix spiked at three different concentrations (200 ng/g, 50 ng/g and 10 ng/g).

Conclusions

The results show that Agilent Bond Elut QuEChERS kits offer an effective method of purification for pesticides in an apple matrix. The impurities remaining after the extraction and dispersive steps were minimal. When used in conjunction with the power of GC/MS in the SIM mode, this method of sample preparation offers a quick, easy and complete solution to quantitate pesticides in fruit matrices.

Acknowledgements

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