

# Analysis of Pesticide Residues in Lettuce and Apple Samples using Agilent Bond Elut QuEChERS AOAC Extraction Kit with Universal Dispersive SPE

## Application Note

Food Safety

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### Abstract

The QuEChERS method was developed in 2003 by scientists at the USDA to easily clean up and prepare food samples for multiclass, multiresidue pesticide analysis. Since then, several changes have improved the effectiveness of the method. This application note describes the use of the AOAC buffered extraction method followed by the use of the Universal Dispersive SPE method for preparing lettuce and apple samples for residue analysis by GC/MS. Twenty-six pesticides of different classes were studied. The experiments were performed using Agilent Bond Elut QuEChERS extraction kits for 15 g samples and dispersive kits for 1 mL sample volumes. The analysis was performed by GC/MS using selective ion monitoring (SIM) mode. Percent recovery and standard deviation data are reported for three different spike concentrations, 25, 100, and 200 ng/g. Excellent linearity and recoveries were noted for all pesticides except the planar pesticide, hexachlorobenzene, which is known to be strongly adsorbed onto graphitized carbon black used in the d-SPE step.



**Agilent Technologies**

## Introduction

Pesticides are commonly used on crops to prevent, destroy, repel, or mitigate pests such as weeds, insects, fungi, and rodents [1]. The majority of foods grown for human consumption are treated with pesticides for this sole purpose. Although some pesticides are more dangerous than others, any pesticide residue on food intended for consumption can be considered a health risk.

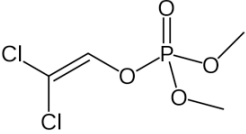
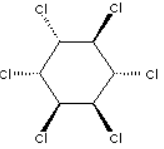
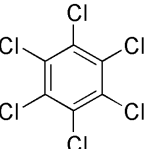
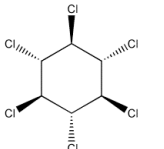
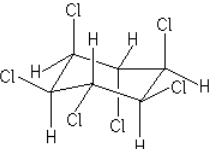
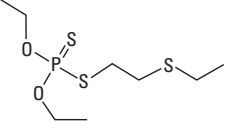
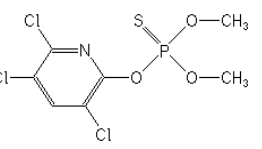
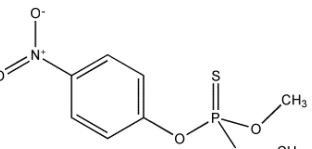
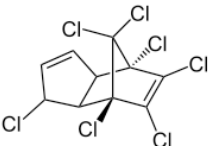
The QuEChERS method was developed in 2003 by scientists at the United States Drug Administration to effectively clean up and prepare fruit and vegetable samples for pesticide residue analysis. This technique, pronounced “catchers”, is a portmanteau for Quick, Easy, Cheap, Effective, Rugged, and Safe and consists of both an extraction and dispersive solid-phase extraction cleanup step [2,3]. The method is commonly used for fruits and vegetables but is being expanded to other matrices such as meat, fish, soil and so on. The initial extraction step of QuEChERS uses the addition of acetonitrile to a finely dispersed sample matrix followed by salting out using  $\text{MgSO}_4$  and other salts. Analytes partition themselves into the separate acetonitrile layer. The extraction step is followed by a dispersive solid phase extraction that uses 150 mg  $\text{MgSO}_4$ , 50 mg PSA (primary-secondary amine), 50 mg C18 (octadecylsilane), 7.5 mg GCB (graphitized carbon black) and has been shown to give superior results [4]. See the Agilent Bond Elut QuEChERS Kit brochure (publication p/n 5990-3562EN) for more information about QuEChERS and suggestions for analyses of different fruits and vegetables.

## Experimental

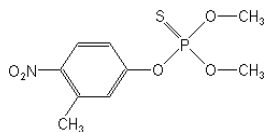
### Materials and Chemicals

All reagents and solvents were HPLC or ACS reagent grade. Acetonitrile was from Burdick and Jackson, Muskegan, MI. Acetic acid was from Sigma-Aldrich (St Louis, MO, USA). A standard pesticide mix (100 ug/mL, Ultra Scientific, N. Kingstown, RI, Table 1) and the internal standard, triphenyl phosphate (500 ug/mL, p/n 5190-0503), were purchased from Agilent Technologies, Inc. Santa Clara, CA. Key supplies used in the analysis (all purchased from Agilent Technologies) included autosampler vials, p/n 5182-0735, autosampler syringe (10  $\mu\text{L}$ ), p/n 9301-0713, helix double taper liners, p/n 5188-5398, GC long life, non-stick septa (11 mm), p/n 5183-4761-100.

Table 1. Pesticide Chemical and Regulatory Information

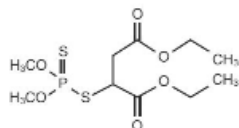
Analyte	Structure	Category
Dichlorvos		Organophosphates
$\alpha$ - BHC		Organochlorine
Hexachlorobenzene		Organochlorine
$\beta$ - BHC		Organochlorine
$\gamma$ -HCH		Organochlorine
Disulfoton		Organophosphate
Chlorpyrifos Methyl		Organophosphate
Methyl Parathion		Organophosphate
Heptachlor		Organochlorine

Fenitrothion



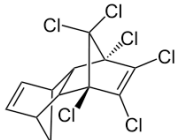
Organophosphate

Malathion



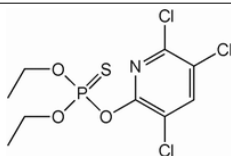
Pyrethroid

Aldrin



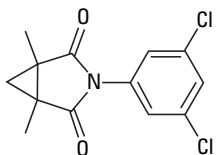
Organochlorine

Chlorpyrifos



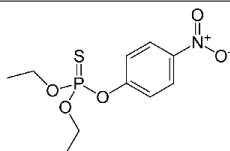
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Procymidone



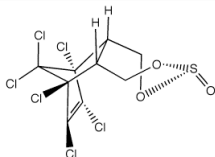
Dicarboximide Fungicide

Parathion



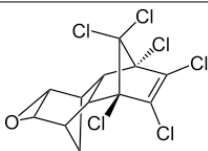
Organophosphate

Endosulfan I



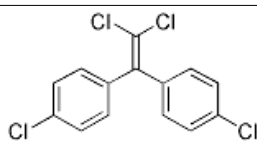
Organochlorine

Dieldrin



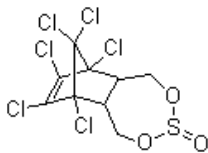
Organochlorine

4,4' - DDE



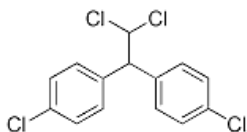
Organochlorine

Endosulfan II



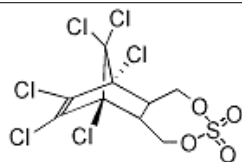
Organochlorine

4,4' - DDD



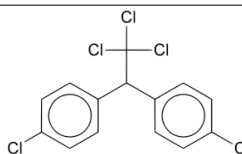
Organochlorine

Endosulfan Sulfate



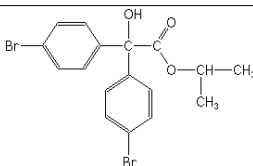
Organochlorine

4,4' - DDT



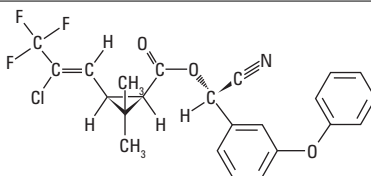
Organochloride

Bromopropylate



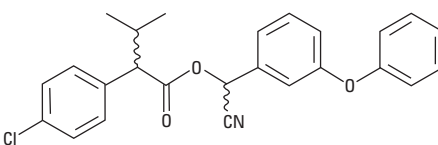
Bridged Diphenyl Acaricide

λ - Cyhalothrin



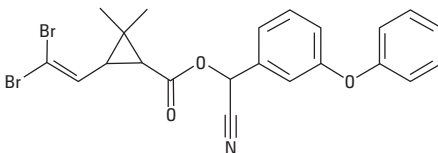
Pyrethroid

Fenvalerate



Pyrethroid

Deltamethrin



Pyrethroid

## Solutions and Standards

A 1% acetic acid solution was prepared by adding 10 mL of acetic acid to 990 mL ACN.

The pesticide and internal standard solutions were stored as purchased at -20 °C. A working solution was prepared with a concentration of 20 µg/mL. Dilutions were made from this solution for use in spiking the fruit samples and for preparation of calibration standards. These solutions were prepared daily. (Table 2)

Table 2. Concentrations of Pesticide Spiked into Homogenized Fruit Samples.

Solution	Low QC	Mid QC	High QC
Solution conc	5 µg/mL	10 µg/mL	20 µg/mL
Volume added	75 µL	150 µL	150 µL
Pesticide conc in apple	25 ng/g	100 ng/g	200 ng/g
Final conc injected	25 ng/mL	100 ng/mL	200 ng/mL

## Construction of Calibration Curve

A five-point calibration curve was created by adding the appropriate amount of the pesticide solution containing all 26 pesticides to the matrix blank extract. The concentrations used for the standard curve were (10 ng/mL, 20 ng/mL, 50 ng/mL, 100 ng/mL and 400 ng/mL). Internal standard solution was added to give a final solution concentration of 100 ng/mL

## Sample Preparation

Certified organic, pesticide-free, romaine lettuce and red delicious apples were purchased at a local grocery. The lettuce was cut into approximately 1 cm strips, and placed in a bag in a -20 °C freezer overnight. The apples were diced into approximately 1/2 cm cubes. The seeds were discarded, but the skin was included. The apple cubes were placed in a plastic bag and frozen at -20 °C overnight. The sample to be homogenized was removed from the freezer at the time of the extraction. A generic grinder (Hamilton Beach model 80344, Picton, Ontario) was used to comminute the sample. The sample was checked to ensure that there were no large pieces or lumps remaining prior to extraction [5].

## QuEChERS Cleanup: Step 1, Extraction

The method was based on the AOAC Method 2007.01 that employs salting out extraction using buffered conditions. Fifteen grams ( $\pm 0.05$  g) of the homogenized sample were placed in a 50 mL centrifuge tube. The appropriate volume of pesticide spiking solution was added. The amount of spiking solution used was kept between 75 µL and 150 µL in all cases. Table 2 lists the resulting concentrations. Internal standard solution in the amount of 75 µL was then added. The sample was vortexed (VWR signature mini vortexer, West Chester, PA) for 1 min, then 15 mL of 1% acetic acid in acetonitrile were added. The sample was shaken vigorously by hand for 30 s, then a salt packet containing 6 g  $\text{MgSO}_4$  and 1.5 g sodium acetate (p/n: 5982-5755) was added. The sample was shaken vigorously by hand for 1 min then centrifuged (Eppendorf 5810R 15amp, Westbury, NY) for 2 min at 3000 rcf.

## QuEChERS Step 2, Dispersive SPE Cleanup

A 1 mL amount of the resultant extract was transferred to a 2 mL centrifuge tube containing 150 mg  $\text{MgSO}_4$ , 50 mg PSA, 50 mg C18, 7.5 mg GCB (p/n: 5982-0028). This was vortexed for 30 s, then centrifuged for 2 mins (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 (Figure 1).

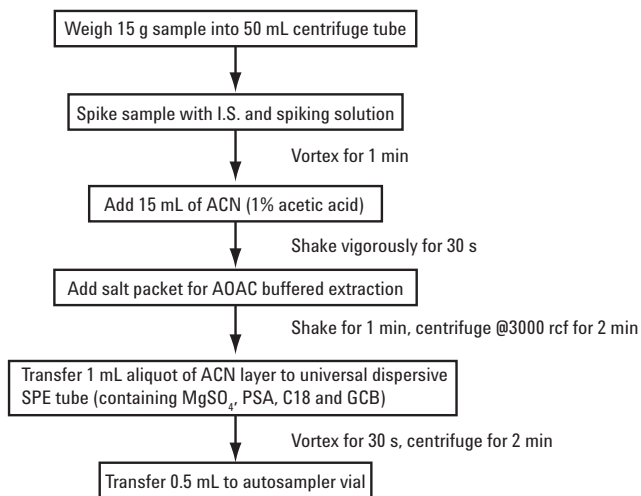


Figure 1. Flowchart for the QuEChERS sample preparation procedure.

## Instrumentation and Instrumental Conditions

Samples were analyzed using an Agilent 7890 GC System with an Agilent 5975C Series GC/MSD (Agilent Technologies Inc., Santa Clara, CA). An Agilent GC/MS method for pesticide analysis [6] was used with some minor modifications [7]. Instrument conditions are listed in Tables 3 and 4.

Table 3. GC MS Instrument Conditions

<b>GC Conditions</b>	
Injection source	Agilent 7683 Automatic Liquid Sampler with 100 sample tray
Inlet	Splitless
Column	Agilent J&W HP-5MS UI, 30 m x 0.250 mm, 0.25 µm film (Agilent Technologies, p/n 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
<b>MS Conditions</b>	
Tune file	Atune.u
Mode	SIM
Source, quad, transfer line temperature	230 °C, 150 °C, 280 °C respectively
Solvent delay	4.00 minutes
Multiplier voltage	Autotune voltage

Table 4. Selective Ion Monitoring (SIM) mode conditions

Peak	Analyte	RT (min)	SIM target ion	SIM qualifying ion	Collection window (min)
1	Dichlorvos	6.89	109.0	185.0	6.0-9
2	α – BHC	14.12	180.9	218.9	13.5–15.0
3	Hexachlorobenzene	14.46	283.8	141.9	13.5–15.0
4	β – BHC	15.35	180.9	218.9	15.0–16.0
5	γ – HCH	15.59	218.9	180.9	15.0–16.0
6	Disulfoton	16.70	88.0	142.0	16.0–18.0
7	Chlorpyrifos Methyl	18.82	285.9	–	18.0–20.0
8	Methyl Parathion	18.82	263.0	–	18.0–20.0
9	Heptachlor	19.08	100.0	271.8	18.0–20.0
10	Fenitrothion	20.29	277.1	66.1	20.0–21.5
11	Aldrin	20.83	66.1	262.9	20.0–21.25
12	Malathion	20.96	173.1	–	20.0–21.25
13	Chlorpyrifos	21.45	97.0	196.9	21.25–23.0
14	Parathion	21.47	291.0	–	21.25–23.0
15	Procymidone	23.83	96.1	283.0	23.0–24.0
16	Endosulfan I	24.45	240.9	194.9	24.0–25.25
17	Dieldrin	25.43	262.9	262.9	25.25–26.0
18	4,4' - DDE	25.48	246.0	318.0	25.25–26.0
19	Endosulfan II	26.49	194.9	236.9	26.0–30.0
20	4,4' - DDD	26.88	235.0	165.1	26.0–30.0
21	Endosulfan Sulfate	27.84	271.8	228.9	26.0–30.0
22	4,4' - DDT	27.99	235.0	165.1	26.0–30.0
I.S.	*TPP	28.61	326.1	325.1	26.0–30.0
23	Bromopropylate	29.42	340.9	183.0	26.0–30.0
24	γ – Cyhalothrin	30.93	181.1	197.1	30.0–38.867
25	Fenvalerate	34.78	125.0	167.1	30.0–38.867
26	Deltamethrin	36.41	181.1	–	30.0–38.867

## Results and Discussion

As shown in Figures 2 and 3, both the apple and lettuce matrix blanks prepared with the buffered AOAC extraction kit followed by the universal dispersive SPE kit show minimal matrix interferences. Extracted ion chromatograms (EIC) were used to calibrate and quantify all compounds. All compounds were free of interferences and gave excellent linearity as shown in Table 5. The peaks corresponding to fenvalerate and deltamethrin needed to be manually integrated at the lower concentrations due to an irregular baseline. This QuEChERS method of sample preparation was proven to be quick, easy and effective for the preparation of both samples. The QuEChERS method cleans up samples well enough for analysis using GC/MS; however, samples will still have impurities. SIM mode was used to improve the sensitivity of the detector for the analytes of interest. The highest abundance ion for each analyte was typically chosen to achieve the best sensitivity. If selectivity was compromised by this choice, another less abundant ion was used. For most of the analytes, a second qualifier ion was also used. The selected ions for each compound and the time segments in which they were monitored are given in Table 4.

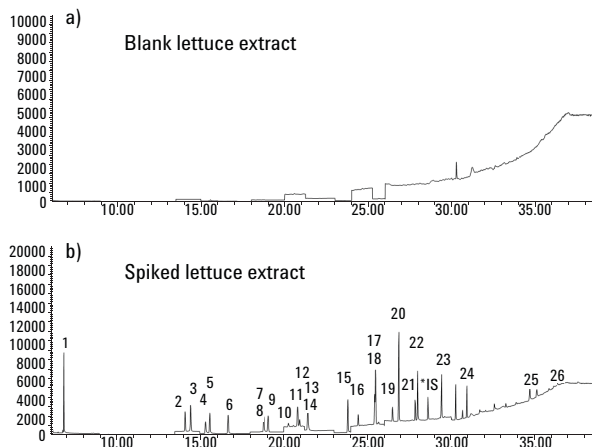


Figure 2. GC/MS of lettuce extracts. (1) blank lettuce extract and (b) spiked lettuce extract after QuEChERS sample preparation. Peak identifications given in Table 4.

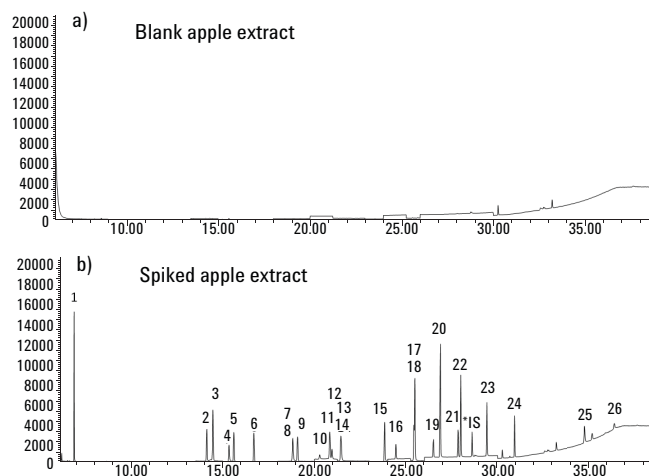


Figure 3. GC/MS of apple extracts (a) blank apple extract and (b) spiked apple extract after QuEChERS sample preparation. Peak identifications given in Table 4.



Table 5. Regression Data for Pesticides

Pesticide	Lettuce		Apple	
	Equation	R <sup>2</sup>	Equation	R <sup>2</sup>
Dichlorvos	$Y=1.763x-0.0609$	0.9997	$Y=2.052x-0.0462$	0.9996
$\alpha$ -BHC	$Y=0.6326x-0.0170$	0.9997	$Y=0.6611x-0.0203$	0.9995
Hexachlorobenzene	$Y=1.734x-0.1226$	0.9988	$Y=1.514x-0.0463$	0.9992
$\beta$ -BHC	$Y=0.3723x-0.0066$	0.9999	$Y=0.4288x-0.0223$	0.9991
$\gamma$ -HCH	$Y=0.4381x+0.1597$	0.9986	$Y=0.4817x-0.1162$	0.9987
Disulfoton	$Y=0.8353x-0.0309$	0.9998	$Y=1.017x-0.0492$	0.9989
Chlorpyrifos Methyl	$Y=0.7770x-0.0197$	0.9998	$Y=0.9424x-0.0414$	0.9995
Methyl Parathion	$Y=0.1118x-0.0035$	0.9977	$Y=0.1545x-0.0061$	0.9986
Heptachlor	$Y=0.5839x-0.0210$	0.9996	$Y=0.5762x-0.0092$	0.9991
Fenitrothion	$Y=0.1113x-0.0064$	0.9978	$Y=0.1442x-0.0001$	0.9978
Aldrin	$Y=0.5632x-0.0004$	0.9999	$Y=0.4842x-0.0022$	0.9990
Malathion	$Y=3854x-0.0214$	0.9993	$Y=0.4687x-0.0250$	0.9987
Chlorpyrifos	$Y=0.7561x-0.0439$	0.9994	$Y=0.7315x-0.0343$	0.9993
Parathion	$Y=0.1066x+0.0035$	0.9996	$Y=0.1368x-0.0093$	0.9988
Procymidone	$Y=1.070x-0.04015$	0.9991	$Y=1.068x-0.0183$	0.9994
Endosulfan I	$Y=0.2002x-0.0013$	0.9994	$Y=0.1854x+0.0028$	0.9986
Dieldrin	$Y=0.2909x-0.0116$	0.9989	$Y=0.2722x-0.0062$	0.9998
4,4' - DDE	$Y=1.857x-0.01246$	0.9987	$Y=1.712x-0.0823$	0.9992
Endosulfan II	$Y=0.1801x-0.0066$	0.9992	$Y=0.1730x-0.0089$	0.9996
4,4' - DDD	$Y=1.928x-0.0660$	0.9997	$Y=1.869x-0.0769$	0.9994
Endosulfan Sulfate	$Y=0.3075x-0.0106$	0.9995	$Y=0.3072x-0.0087$	0.9996
4,4' - DDT	$Y=1.198x-0.1039$	0.9983	$Y=1.242x-0.0584$	0.9993
Bromopropylate	$Y=1.035x-0.0291$	0.9991	$Y=0.9617x-0.0279$	0.9994
$\gamma$ -Cyhalothrin	$Y=0.5308x-0.0228$	0.9995	$Y=0.5997x-0.0272$	0.9992
Fenvalerate	$Y=0.3155x-0.0354$	0.9951	$Y=0.3509x-0.0062$	0.9992
Deltamethrin	$Y=0.1113x+0.0032$	0.9987	$Y=0.1671x-0.0021$	0.9988

Tables 6 and 7 show the recovery and reproducibility for three levels of spiked pesticides in the two matrices studied (25 ng/g, 100 ng/g and 200 ng/g). The results show that Agilent Bond Elut QuEChERS kits offer an effective method of purification for pesticides in these matrices. When used in conjunction with the power of Agilent CG/MS in the SIM mode, this method of sample preparation offers a quick, easy and complete solution to quantify pesticides in fruit matrices.

Table 6. Recovery and Reproducibility of Pesticides in Lettuce (n = 5)

Compound	R.T.	25 ng/g spike		Percent recovery 100 ng/g spike		200 ng/g spike	
		AVG	STD DEV	AVG	STD DEV	AVG	STD DEV
Dichlorvos	6.89	94.4	4.3	95.5	4.9	96.2	3.6
$\alpha$ -BHC	14.12	103.2	1.9	97.2	3.1	95.0	3.9
Hexachlorobenzene	14.46	78.5	4.6	63.3	6.4	59.1	11.3
$\beta$ -BHC	15.35	98.1	8.9	95.8	3.1	97.3	0.5
$\gamma$ -HCH	15.59	139.0	15.1	108.1	9.3	94.3	7.0
Disulfoton	16.70	100.2	3.9	105.7	3.8	100.4	2.1
Chlorpyrifos Methyl	18.82	99.2	2.1	97.8	5.7	98.3	3.7
Methyl Parathion	18.82	100.4	6.4	108.4	9.0	112.7	23.3
Heptachlor	19.08	101.2	4.3	94.7	1.9	98.4	2.2
Fenitrothion	20.29	99.0	9.0	104.7	5.9	104.0	2.7
Aldrin	20.83	89.0	5.2	92.0	2.3	94.6	1.9
Malathion	20.96	98.9	5.2	104.5	7.1	104.6	2.3
Chlorpyrifos	21.45	102.4	3.3	97.9	2.9	99.7	1.6
Parathion	21.47	91.7	8.7	104.2	4.1	103.1	0.4
Procymidone	23.83	104.2	3.2	100.2	3.4	100.9	0.8
Endosulfan I	24.45	94.8	11.4	94.1	1.2	94.5	2.0
Dieldrin	25.43	101.2	2.9	95.2	1.7	95.5	0.8
4,4' - DDE	25.48	102.0	1.5	86.3	1.6	84.5	1.2
Endosulfan II	26.49	83.1	17.7	96.1	3.3	100.0	1.5
4,4' - DDD	26.88	104.1	2.3	102.4	1.6	95.5	7.1
Endosulfan Sulfate	27.84	103.7	4.9	99.9	1.8	100.2	2.5
4,4' - DDT	27.99	106.9	4.7	88.5	5.4	82.5	3.9
Bromopropylate	29.42	96.5	6.2	99.5	1.9	99.3	2.4
$\gamma$ -Cyhalothrin	30.93	107.6	5.4	112.1	2.3	104.0	1.6
Fanvalerate	34.78	102.0	10.4	97.9	7.1	99.1	2.6
Deltamethrin	36.41	90.3	6.8	87.0	6.2	96.2	1.4

Table 7. Recovery and Reproducibility of Pesticides in Apple (n = 5)

Compound	R.T.	25 ng/g spike		Percent recovery 100 ng/g spike		200 ng/g spike	
		AVG	STD DEV	AVG	STD DEV	AVG	STD DEV
Dichlorvos	6.88	88.1	11.4	90.3	3.9	91.6	5.5
$\alpha$ -BHC	14.13	89.6	10.5	89.7	3.0	91.2	4.4
Hexachlorobenzene	14.46	55.5	10.3	46.2	11.7	49.4	13.1
$\beta$ -BHC	15.35	86.5	6.5	86.8	2.9	90.2	1.8
$\gamma$ -HCH	15.60	134.0	15.9	104.3	11.5	98.7	3.2
Disulfoton	16.70	86.9	5.2	87.1	2.5	97.7	4.5
Chlorpyrifos Methyl	18.82	84.4	6.8	83.5	5.2	88.6	3.2
Methyl Parathion	18.81	89.0	3.4	91.9	2.4	99.4	1.2
Heptachlor	19.08	83.3	7.1	84.4	3.3	91.0	4.8
Fenitrothion	20.29	79.3	14.6	87.2	7.3	94.7	4.6
Aldrin	20.83	85.0	9.9	90.2	4.3	95.6	4.5
Malathion	20.95	94.9	6.0	90.6	3.1	99.5	4.5
Chlorpyrifos	21.45	90.1	7.0	87.4	4.5	93.0	3.2
Parathion	21.48	84.6	6.2	84.2	2.5	85.8	4.2
Procymidone	23.83	91.1	5.9	96.7	2.9	98.6	2.4
Endosulfan I	24.45	87.2	9.3	89.8	6.4	90.4	3.6
Dieldrin	25.44	90.0	8.5	87.0	3.1	89.4	3.0
4,4' - DDE	25.48	88.4	5.2	81.0	2.8	83.5	2.3
Endosulfan II	26.50	93.8	7.6	92.2	2.0	93.5	4.9
4,4' - DDD	26.88	91.2	6.6	88.0	3.0	91.9	3.2
Endosulfan Sulfate	27.84	93.5	7.6	92.2	3.7	95.8	3.3
4,4' - DDT	27.99	91.8	4.1	86.2	3.9	88.2	2.2
Bromopropylate	29.42	95.5	6.0	91.3	2.1	92.3	2.4
$\gamma$ -Cyhalothrin	30.93	97.7	6.6	94.7	3.3	103.7	3.2
Fenvalerate	34.79	92.1	4.5	95.9	3.4	99.2	2.9
Deltamethrin	36.39	86.9	10.5	92.5	3.0	98.5	2.5

## Conclusions

A new universal kit was tested for the dispersive SPE cleanup of 26 pesticides in different classes spiked into apple and lettuce samples using the standard Agilent Bond Elut QuEChERS extraction kit for the extraction-partitioning step. The universal d-SPE increases the streamlined approach offered by the QuEChERS methodology. The universal d-SPE should be considered as the d-SPE of choice when implementing the QuEChERS approach for the analysis of compounds or contaminants from products. The universal d-SPE contains a combination of the various SPE materials used in the validated d-SPE step associated with both the AOAC and EN methods at amounts per mL of extract that should offer the cleanest sample for analysis [8]. Part per billion concentrations of these pesticides were measured with excellent linearity and recoveries

(> 90% on the average) with GC/MS in SIM mode. Typical of planar pesticides when using GCB as a d-SPE sorbent, hexachlorobenzene was the only pesticide that gave slightly lower than the average recovery but still showed excellent linearity and acceptable RSD. Three different levels of spiking were studied (25, 100 and 200 ng/g). It is anticipated that other pesticides and other fruits and vegetables can be effectively extracted by these QuEChERS kits and analyzed by GC/MS (or GC/MS/MS) or LC/MS (or LC/MS/MS).

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