# Analysis of Pesticide Residues in Apple Using Agilent Bond Elut QuEChERS EN Kits by GC/MS

Application Note

Food Safety

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## Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation approach described in the European Committee (EN) for extraction and cleanup of 17 GC-amenable multiple pesticide class residues in apple. The method involves initial extraction in an aqueous/acetonitrile system, an extraction/partitioning step after the addition of salt, and a cleanup step using dispersive solid phase extraction (dispersive SPE). The two different dispersive SPE cleanup approaches (1 mL and 6 mL aliquot volumes) were evaluated simultaneously after sample extraction. The target pesticides in the apple extracts were then analyzed by gas chromatography/mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode. The method was validated in terms of recovery and reproducibility. The limit of quantitation (LOQ) for pesticides in apple is 10 ng/g. This application employed Agilent's Bond Elut QuEChERS kit and produced results well below the maximum residue limits (MRLs) for all the pesticides screened. The spiked levels for the recovery experiments were 10, 50, and 200 ng/g. Recoveries ranged between 68 and 112% (86.0% on average), with RSD below 15% (4.7% on average).

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## Introduction

The QuEChERS method for pesticide analysis was first introduced by USDA scientists in 2003. [1] The method was modified to address some problematic pesticides by including a buffered extraction system. [2] The EN method 15662:2007 is a European variation to the QuEChERS method. [3, 4] The method uses acetonitrile extraction, followed by the salting out of water from the sample using anhydrous magnesium sulfate ($MgSO_4$), NaCl and buffering citrate salts to induce liquid-liquid partitioning. A dispersive solid phase extraction (dispersive SPE) is conducted for cleanup using a combination of primary secondary amine (PSA) to remove fatty acids among other components and anhydrous $MgSO_4$ to reduce the remaining water in the extract. After mixing and centrifugation, the upper layer is ready for analysis.

Although the EN and AOAC are similar methods, they do have several differences. First, the extraction buffered system in the EN method uses sodium chloride, sodium citrate and disodium citrate sesquihidrate instead of sodium acetate in the AOAC extraction step. Second, in the dispersive SPE step, the EN method uses 25 mg PSA per mL of extract rather than 50 mg PSA per mL of extract as stated in the AOAC method.

Gas Chromatography/Mass Spectrometry (GC/MS) has been widely used in pesticide analysis for many years. Many pesticides are volatile or semi-volatile, which makes them GC-amenable compounds. Previously, we evaluated the performance of Agilent's Bond Elut EN buffered extraction kit and Bond Elut EN dispersive SPE kits for the analysis of polar pesticides in apple using LC/MS/MS for detection and quantification. [5] In this study, the performance of the Bond Elut EN Buffered Extraction kit (p/n 5982-5650) and Bond Elut EN dispersive SPE kit for General Fruits and Vegetables (p/n 5982-5021 and 5982-5056) was evaluated for the extraction of volatile and semi-volatile pesticides. Analysis was performed by GC/MS. Seventeen GC-amenable pesticides were selected which represent multiple classes, including non-polar organochlorine pesticides (OCs), certain organophosphorus pesticides (OPs) and organonitrogen pesticides (ONs). The MRLs of these pesticides are a function of both the pesticide class and food matrix and have been set at 10 ng/g or higher. Table 1 shows the chemical and regulatory information for these pesticides in apple.

## Experimental

### Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. Acetonitrile (ACN), methanol (MeOH) were from Honeywell (Muskegon, MI, USA). Formic acid (FA) was from Fluka (Sleinheim, Germany). The pesticide standards and internal standard (triphenyl phosphate, TPP) were purchased from Sigma-Aldrich (St Louis, MO, USA), Chem Service (West Chester, PA, USA), or Ultra Scientific (North Kingstown, RI, USA).

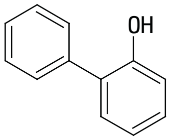
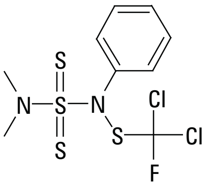
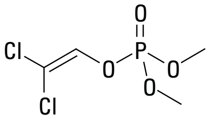
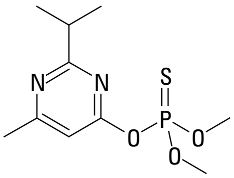
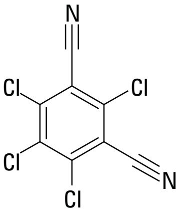
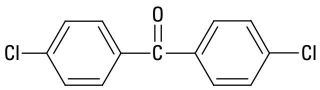
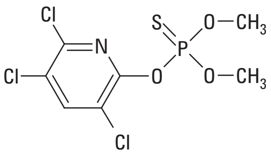
### Solutions and Standards

Standards and internal standard (IS) stock solutions (2 mg/mL) were made in MeOH, respectively, and stored at –20 °C. Three QC spiking solutions of 1, 5 and 20 µg/mL were made fresh daily in 1:1 ACN/H2O (0.1% FA). A 2.5 µg/mL standard solution (17 pesticides) in ACN (0.1% FA) was used to prepare the calibration curves in the matrix blank extract by appropriate dilution. A 10 µg/mL amount of TPP spiking solution in 1:1 ACN/$H_2O$ (0.1% FA) was used as the internal spiking standard (IS).

### Equipment and Materials

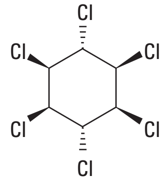
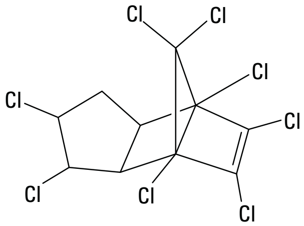
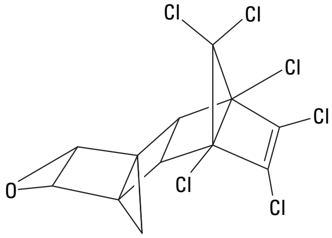
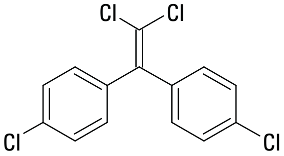
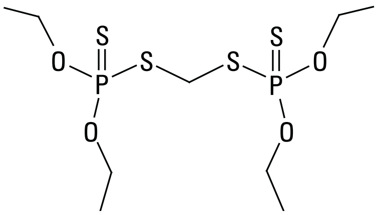
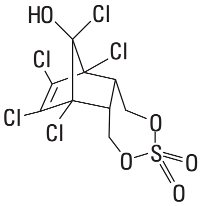
- Agilent Gas Chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA).
- Agilent 5975C Mass Spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA).
- Agilent Bond Elut QuEChERS EN Extraction kit, p/n 5982-5650 (Agilent Technologies Inc., Wilmington, DE, USA).
- Agilent Bond Elut QuEChERS EN dispersive SPE kits for General Fruits and Vegetables, p/n 5982-5021 and 5982-5056 (Agilent Technologies Inc., Wilmington, DE, USA).
- CentraCL3R Centrifuge (Thermo IEC, MA, USA)
- Bottle top dispenser (VWR, So Painfield, NJ, USA)
- Eppendorf microcentrifuge (Brinkmann Instruments, Westbury, NY, USA)
- Grinder (St Joseph, MI USA)

*Table 1. Pesticides Chemical and Regulatory Information [6–9]*

| Name | Category | Log P | pKa | Structure | MRLs in apple (ng/g)* |
|---|---|---|---|---|---|
| σ-Phenylphenol | Phenol | 3.18 | 9.4 |  | 20 |
| Dichlofluanid | Sulphamide | 3.7 | NA |  | 5000 |
| Dichlorvos | Organophosphate | 1.9 | NA |  | 10 |
| Diazinon | Organophosphate | 3.69 | 2.6 |  | 100 |
| Chlorothalonil | Chloronitrile | 2.94 | NA |  | 10 |
| Dichlorobenzophenone | Organochlorine | 4.44 | NA |  | 500 |
| Chlorpyrifosmethyl | Organophosphate | 4.00 | NA |  | 500 |

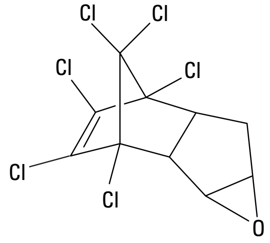
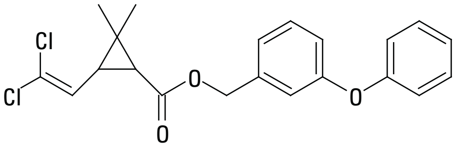
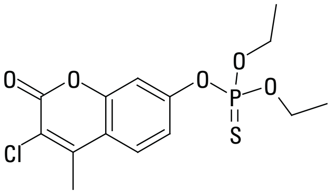
(Continued)

*Table 1. Pesticides Chemical and Regulatory Information [6–9]*

| **Name** | **Class** | **Log P** | **pKa** | **Structure** | **MRLs in apple (ng/g)*** |
|---|---|---|---|---|---|
| Lindane | Organochlorine | 3.69 | NA |  | 10 |
| Chlordane | Cyclodiene organochlorine | 2.78 | NA |  | 20 |
| Dieldrin | Chlorinated hydrocarbon | 3.7 | NA |  | 10 |
| DDE | Organochlorine | 6.55 | NA |  | 50 |
| Ethion | Organophosphate | 5.07 | NA |  | 300 |
| Endosulfan sulfate | Organochlorine | 3.13 | NA |  | 50 |

(Continued)

*Table 1. Pesticides Chemical and Regulatory Information [6–9]*

| Name | Class | Log P | pKa | Structure | MRLs in apple (ng/g)* |
|---|---|---|---|---|---|
| Heptachlor epoxide | Organochlorine | 5.83 | NA |  | 10 |
| Permethrins | Pyrethroid | 6.1 | NA |  | 50 |
| Coumaphos | Organothiophosphate | 3.86 | NA |  | 100 |

*The MRLs numbers list in the table are for apple or lowest level in other fruit and vegetables. They could be higher in different commodities.

## Instrument Condition

An Agilent GC/MS method for pesticides analysis was used for this study. [10]

**GC conditions**

| | |
|---|---|
| Inlet | Splitless |
| Inlet liner | Helix double taper, deactivated (p/n 5188-5398) |
| Carrier gas | Helium |
| Inlet pressure | 20.18 psi (constant pressure mode) during run<br>1.0 psi during backflush |
| Inlet temperature | 250 °C |
| Injection volume | 1.0 µL |
| Purge flow to split vent | 30 mL/min at 0.75 min |
| Oven temperature program | 70 °C (1 min), 50 °C/min to 150 °C (0 min), 6 °C /min to 200 °C (0 min), 16 °C/min to 280 °C (6 min) |
| Post run | 3 min |
| Capillary flow technology | Purged Ultimate Union (p/n G3186B) – used for backflushing the analytical column and inlet. |
| Aux EPC gas | Helium plumbed to Purged Ultimate Union |
| Aux EPC pressure | 4.0 psi during run, 80.0 psi during backflush |
| Column | Agilent J&W HP-5ms Ultra Inert 15 m × 0.25 mm × 0.25 µm (p/n 19091S-431UI) |
| Connections | Between inlet and Purged Ultimate Union (p/n G3186B) |
| Restrictor | 65 cm × 0.15 mm × 0.15 µm DB-5MS Ultra Inert. |
| Connections | Between the Purged Ultimate Union and the MSD |

**MS conditions**

| | |
|---|---|
| Tune file | Atune.u |
| Mode | SIM (refer to Table 2 for settings in detail) |
| Source, quad, transfer line temperatures | 230 °C, 150 °C and 280 °C respectively, |
| Solvent delay | 2.30 min |
| Multiplier voltage | Autotune voltage |

*Table 2. Instrument Acquisition Data Used for the Analysis of 17 Pesticides by GC/MS.*

| Analyte | SIM | Collection window (min) | RT (min) |
|---|---|---|---|
| (1) Dichlorvos | 184.9 | 2.3 – 4.0 | 2.88 |
| (2) σ-Phenylphenol | 170.1, 169.1 | 4.0 – 5.0 | 4.35 |
| (3) Lindane | 180.9, 182.9 | 5.0 – 6.9 | 6.67 |
| (4) Diazinon | 137.1, 179.1 | 6.9 – 7.7 | 7.19 |
| (5) Chlorothalonil | 265.9, 263.9 | 14.65 – 16.0 | 14.8 |
| (6) Chlorpyrifos-methyl | 285.9, 287.9 | 7.7 – 8.6 | 8.25 |
| (7) Dichlofluanid | 123, 167.0 | 8.6 – 9.35 | 9.16 |
| (8) Dichlorobenzophenone | 139, 249.9 | 18.8 – 20.5 | 19.2 |
| (9) Heptachlor epoxide | 352.8, 354.8 | 10.0 – 10.4 | 10.31 |
| (10) γ-Chlordane | 372.8, 374.8 | 10.85 – 11.6 | 10.97 |
| (11) DDE | 245.9, 317.9 | 10.85 – 11.6 | 11.21 |
| (12) α-Chlordane | 372.374.8 | 10.85 – 11.6 | 11.50 |
| (13) Dieldrin | 262.9, 264.9 | 11.0 – 12.3 | 11.89 |
| (14) Ethion | 230.9 | 12.3 – 13.6 | 12.97 |
| (15) Endosulfan sulfate | 273.8 | 12.3 – 13.6 | 13.35 |
| TPP (IS) | 325.1, 326.1 | 13.6 – 15.0 | 13.84 |
| (16) Permethrin | 183.1 | 15.0 – 23.0 | 15.69, 15.79 |
| (17) Coumaphos | 362.0, 225.9 | 15.0 – 23.0 | 15.83 |

## Sample preparation

*Sample comminution*

Organically grown, pesticide free apples were purchased from a local grocery store. Approximately three pounds of apples were chopped into small, bean sized cubes. Skin was included, but the core was discarded. The chopped apple cubes were then placed into a clean plastic bag and frozen at –20 °C overnight. The bag was massaged occasionally to make sure the cubes remained separate. The following day, only the required amount of frozen apple cubes was removed and thoroughly blended. Dry ice was added while comminuting, when possible. Samples were comminuted thoroughly to get the best sample homogeneity. It was verified that no pieces of apple were visible in the final sample.

*Extraction/Partitioning*

A 10 g (± 0.1 g) amount of previously homogenized sample was placed into a 50 mL centrifuge tube. QC samples were fortified with 100 µL of appropriate QC spiking solution. 100 µL of IS spiking solution (10 µg/mL of TPP) was added to all the samples except the control blank to yield a 100 ng/g concentration in the samples. Tubes were capped and vortexed for 1 min. A 10 mL aliquot of ACN was added to each tube using the dispenser. Tubes were capped and shaken by hand for 1 min. An Agilent Bond Elut QuEChERS EN extraction salt packet (p/n 5982-5650), containing 4 g anhydrous $MgSO_4$, 1 g NaCl, 1 g $Na_3$Citrate, and 0.5 g $Na_2H$ Citrate sesquihydrate, was added directly to each tube. The salt bag was massaged carefully to loosen any clumped salts before pouring. No powders were left in the threads or rims of the tubes. Tubes were sealed tightly and shaken vigorously for 1 min by hand to ensure that the solvent interacted well with the entire sample and crystalline agglomerates were broken up sufficiently. Sample pH was checked and 5M NaOH solution was used to adjust the pH to 5–5.5, if necessary. Sample tubes were centrifuged at 4000 rpm for 5 min.

*Dispersive SPE Cleanup*

A 1 mL aliquot of upper ACN layer was transferred into Agilent Bond Elut QuEChERS EN dispersive SPE 2 mL tube (p/n 5982-5021); or a 6 mL of aliquot was transferred into Agilent Bond Elut QuEChERS EN dispersive SPE 15 mL tube (p/n 5982-5056). The 2 mL tube contained 25 mg of PSA and 150 mg of anhydrous $MgSO_4$; while the 15 mL tube contained 150 mg of PSA and 900 mg of anhydrous $MgSO_4$. The tubes were capped tightly and vortexed for 1 min. The 2 mL tubes were centrifuged with a micro-centrifuge at 13,000 rpm for 2 min, and the 15 mL tubes in a standard centrifuge at 4000 rpm for 5 min. A 500 µL portion of the extract was transferred into an autosampler vial and 25 µL of 1% FA in ACN was added immediately.

Figure 1 shows the flow chart for the QuEChERS EN sample extraction procedure.

## Results and Discussion

Using the Bond Elut QuEChERS kits, the entire procedure is fast, easy, and offers time and labor savings, while ensuring consistency. An analyst can process 40-50 samples in just a few hours. Agilent's Bond Elut extraction salts are uniquely prepared in an anhydrous package. The addition of a food sample with a high content of water directly to the salts creates an exothermic reaction, which can affect analyte recoveries, especially for volatile pesticides. The unique Bond Elut anhydrous salts packet allows salt addition **AFTER** the addition of organic solvent to the sample, as specified in the original QuEChERS methodology.

Our previous study demonstrated good performance of Agilent's Bond Elut QuEChERS EN kits on the extraction of a broad variety of semi-polar to polar pesticides analyzed by LC/MS/MS. [5] It is also advantageous to evaluate the performance of the EN kit for the analysis of volatile and semi-volatile pesticides using GC/MS, since these classes of pesticides have been widely used for many years. The selectivity of GC/MS (SIM mode) is not as powerful as that of LC/MS/MS (MRM mode). Furthermore, the final QuEChERS prepared samples still contain some food matrix impurities, which can be observed in the GC/MS chromatogram of blank apple extract. Therefore, it is critical to carefully choose the selected ions of each compound for monitoring when setting up the SIM method. In general, the most abundant ions were selected in order to achieve the best sensitivity; however in a few instances the sensitivity was compromised to obtain better selectivity by using more unique but less abundant ions.

Another potential issue with the use of GC/MS for the analysis of QuEChERS samples is the contamination of the ionization source and deterioration of the GC column. QuEChERS food samples usually still contain high-boiling indigenous impurities, which can accumulate on the head of the column, causing peak tailing and retention time shift. Over time, these impurities can migrate to the mass spectrometer (MS) source, causing contamination of the source. Decreased sensitivity and peak shape distortion, especially for the semi-polar compounds, were observed when additional QuEChERS samples were injected into the GC/MS system. Therefore, column backflushing was employed to increase column life as well as preserve the MS source. Agilent's capillary flow technology makes column backflushing routine [10–12]. Several different capillary flow devices can be used for this purpose.

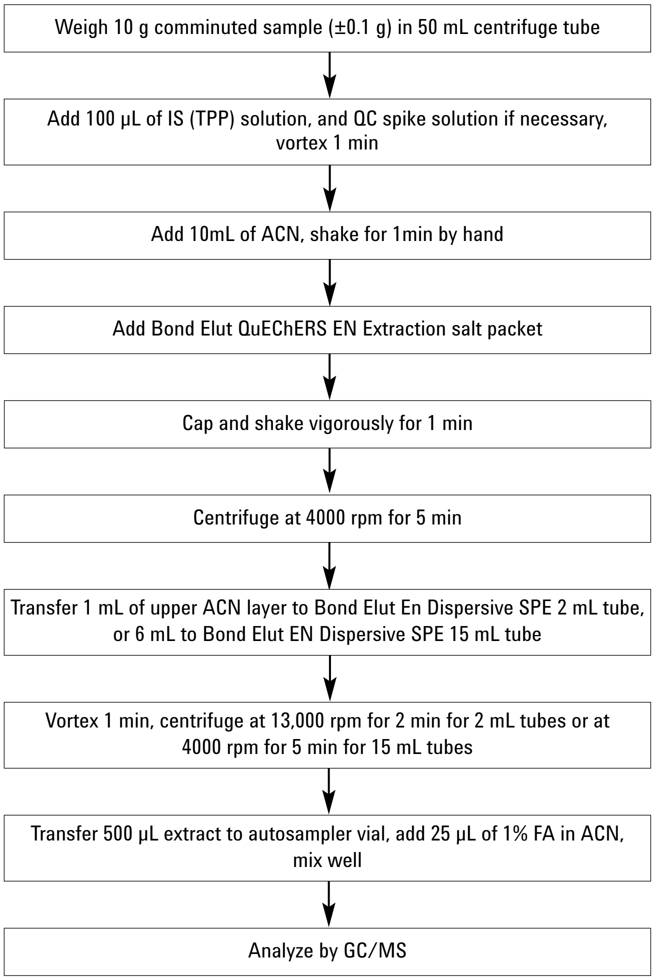


*Figure 1. Flow chart of the Agilent Bond Elut QuEChERS EN extraction procedure.*

In this study, the GC/MS system used a Purged Ultimate Union. The analytical column was connected to the capillary flow device. A short restrictor (65 cm × 0.15 mm × 0.15 µm of DB-5ms Ultra Inert column) was used to couple the capillary flow device to the mass spectrometer. In a previous application note [10], there are figures showing the backflush system, that was used in this study.

Figure 2(a, b) shows the chromatograms of a blank apple extract and a 50 ng/g fortified apple extract. As shown in Figure 2a, interference peaks are found in the blank chromatogram; fortunately most pesticides are free of co-eluting interferences. There was an interference eluting at a retention time very close to that of $\sigma$-phenylphenol (peak 2), and cannot be differentiated for integration. The average response of this interference in the blank extract was 215 (n=4), while the average response of $\alpha$-phenylphenol in the LOQ (10 ng/g)

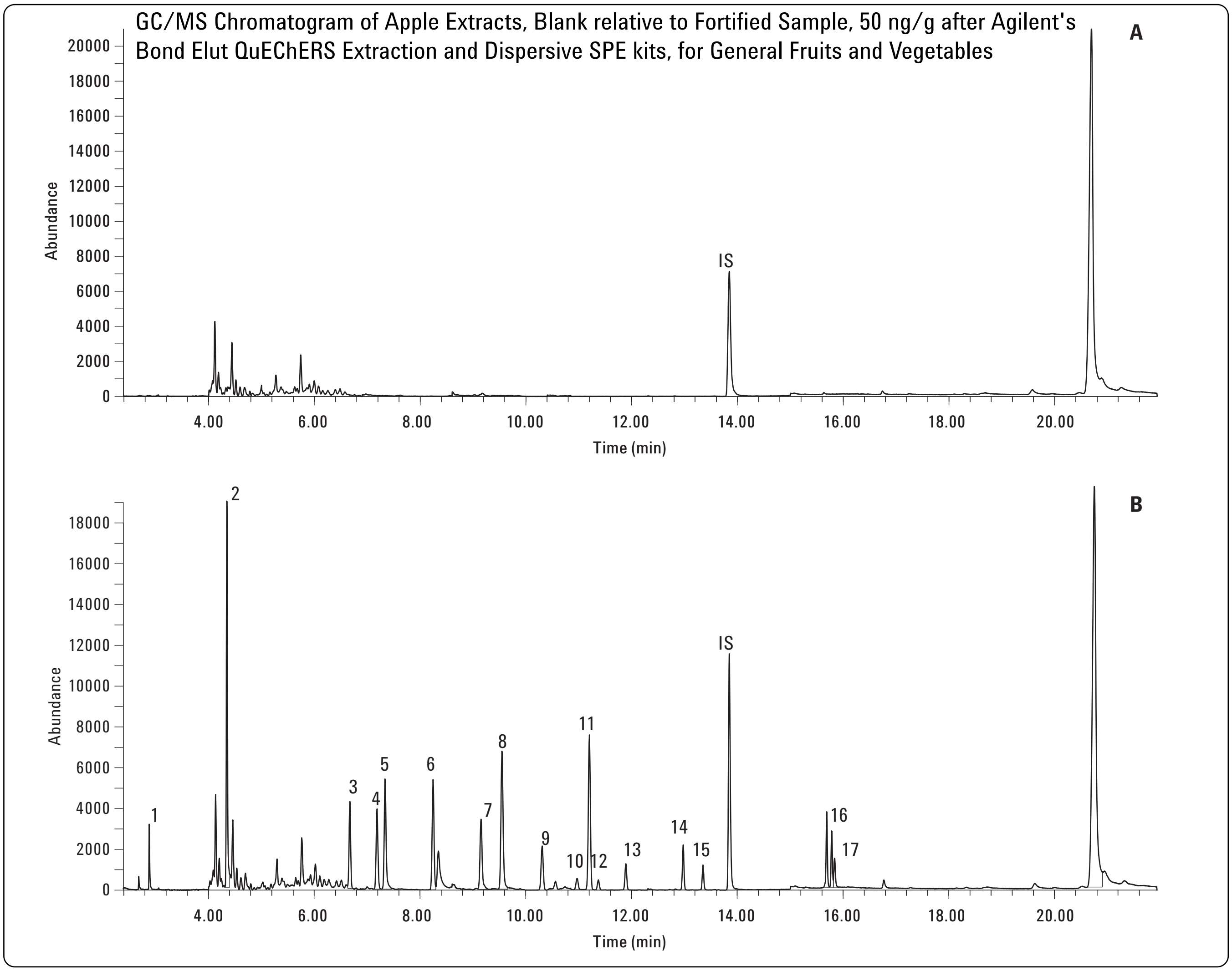


*Figure 2. GC/MS chromatogram of apple extract. (A) apple extract blank; (B) 50ng/g fortified apple extract. Peak Identification: 1. Dichlorvos, 2. $\sigma$-Phenylphenol, 3. Lindane, 4. Diazinon, 5. Chlorothalonil, 6. Chlorpyrifos-methyl, 7. Dichlofluanid, 8. Dichlorobenzophenone, 9. Heptachlor epoxide, 10. $\gamma$-Chlordane, 11. DDE, 12. $\alpha$-Chlordane, 13. Dieldrin, 14. Ethion, 15. Endosulfan sulfate, 16, Permethrin, 17. Coumaphos. IS. Triphenyl phosphate (TPP)*

was 3196 (n=12). The interference response was less than 20% of the response of the $\sigma$-phenylphenol peak at the LOQ (10 ng/g) sample. Therefore the selectivity was considered acceptable for this compound.

## Linearity and Limit of Quantification (LOQ)

The linear calibration range for all of the pesticides was 0–400 ng/g. Two different dispersive SPE volumes (1 mL and 6 mL) were used for evaluation and comparison; therefore, two calibration curves were generated from matrix blanks prepared from each size. Calibration curves were made at levels of 10, 20, 50, 100, 250, and 400 ng/g. The TPP was the internal standard (IS) at 100 ng/g in all cases. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). Table 1 shows that the 10 ng/g quantification limits LOQ (10 ng/g or 10 ppb) established for the pesticides are substantially lower than many MRLs for the pesticides in fruit and vegetables. The regression fit used for the calibration curves was the average response factor. Table 3 shows the linear term and RF relative standard deviation (%) for both 1 mL and 6 mL dispersive SPE samples. The RF relative SD is an important parameter for the evaluation of the linearity of calibration. In general, the smaller the value the better linearity of the curve, and it is usually acceptable for less than

*Table 3. Linearity of 17 Pesticides in Apple Extract*

| | 1 mL dispersive SPE | | 6 mL dispersive SPE | |
|---|---|---|---|---|
| **Analytes** | **Linear Term** | **RF Rel Std Dev (%)** | **Linear Term** | **RF Rel Std Dev (%)** |
| Dichlorvos | 4.53e–001 | 7.9 | 5.52e–001 | 8.6 |
| σ-Phenylphenol | 2.41e+000 | 7.5 | 2.82e+000 | 9.3 |
| Lindane | 6.79e–001 | 11.5 | 8.09e–001 | 9.2 |
| Diazinon | 8.35e–001 | 15.0 | 9.32e–001 | 13.6 |
| Chlorothalonil | 1.39e+000 | 14.1 | 1.69e+000 | 14.1 |
| Chlorpyrifos-mehyl | 1.32e+000 | 14.7 | 1.31e+000 | 16.5 |
| Dichlofluanid | 1.03e+000 | 11.7 | 1.29e+000 | 12.9 |
| Dichlorobenzophenone | 6.08e–001 | 10.0 | 7.13e–001 | 10.4 |
| Heptachlor epoxide | 5.41e–001 | 12.4 | 5.58e–001 | 12.3 |
| γ-Chlordane | 1.77e–001 | 9.3 | 1.83e–001 | 9.1 |
| DDE | 2.44e+000 | 10.7 | 2.67e+000 | 9.5 |
| α-Chlordane | 1.34e–001 | 10.0 | 1.38e–001 | 9.4 |
| Dieldrin | 2.85e–001 | 9.8 | 3.09e–001 | 6.9 |
| Ethion | 7.06e–001 | 27.8 | 7.30e–001 | 27.9 |
| Endosulfan sulfate | 2.95e–001 | 11.2 | 3.29e–001 | 11.5 |
| Permethrin | 8.73e–001 | 11.8 | 8.20e–001 | 17.6 |
| Coumaphos | 2.36e–001 | 19.0 | 2.16e–001 | 28.7 |

20% RF relative SD. The data in Table 3 shows that the linearity of the calibration curve for most analytes is perfect with less than a 15% of RF relative SD value. However, the two standard curves of Ethion (1 mL and 6 mL) and one standard curve of Coumaphos generated RF relative SD values higher than 20%, possibly due to the matrix effect.

## Recovery and Reproducibility

The recovery and reproducibility were evaluated by spiking pesticides standards in comminuted apple sample at levels of 10, 50 and 200 ng/g. These QC samples were quantitated against the matrix spiked calibration curve. The analysis was performed in replicates of six (n=6) at each level. The recovery and reproducibility (shown as % RSD) data for 1 mL and 6 mL dispersive SPE sample are shown in Table 4 and 5, respectively. It can be seen from the results that all of the pesticides give good recoveries (average of 84.7% for 1 mL and 87.2% for 6 mL) and precision (average of 4.3% RSD for 1 mL and 5.1% RSD for 6 mL). Compared to the results of these pesticides extracted with AOAC QuEChERS method [13], the EN QuEChERS method gives slightly lower recovery (recovery 5–6% lower on average) but similar precision (RSD 4–5% on average for both methods). Variance may be possible due to a different buffering system and solvent volume used in the first extraction step.

*Table 4. Recovery and Repeatability of Pesticides in Fortified Apple With Agilent's Bond Elut Dispersive SPE Tube, 2 mL (p/n 5982-5021); Recovery 84.7%, RSD 4.3% (avg)*

| | **10 ng/g fortified QC** | | **50 ng/g fortified QC** | | **200 ng/g fortified QC** | |
|---|---|---|---|---|---|---|
| **Analytes** | **Recovery** | **RSD (n=6)** | **Recovery** | **RSD (n=6)** | **Recovery** | **RSD (n=6)** |
| Dichlorvos | 97.6 | 5.1 | 90.8 | 6.2 | 81.0 | 6.9 |
| σ-Phenylphenol | 94.4 | 5.5 | 83.1 | 6.6 | 76.3 | 5.2 |
| Lindane | 87.4 | 4.9 | 80.0 | 6.1 | 73.3 | 3.8 |
| Diazinon | 83.6 | 5.6 | 79.6 | 5.5 | 69.6 | 5.0 |
| Chlorothalonil | 68.3 | 4.9 | 71.8 | 5.8 | 69.6 | 5.0 |
| Chlorpyrifos-mehyl | 79.3 | 4.5 | 80.7 | 4.8 | 83.1 | 3.0 |
| Dichlofluanid | 91.8 | 5.6 | 85.8 | 6.9 | 65.2 | 4.7 |
| Dichlorobenzo phenone | 83.9 | 6.4 | 83.0 | 4.8 | 80.0 | 3.1 |
| Heptachlor epoxide | 80.0 | 4.7 | 82.9 | 5.0 | 81.4 | 1.7 |
| γ-Chlordane | 79.6 | 4.3 | 80.5 | 5.4 | 78.3 | 1.6 |
| DDE | 80.5 | 3.2 | 80.3 | 5.1 | 76.8 | 1.2 |
| α-Chlordane | 84.8 | 3.3 | 83.1 | 4.7 | 78.6 | 1.3 |
| Dieldrin | 83.4 | 3.1 | 80.5 | 4.3 | 76.2 | 1.1 |
| Ethion | 97.7 | 4.4 | 104.9 | 4.8 | 91.7 | 1.4 |
| Endosulfan sulfate | 93.2 | 5.4 | 88.5 | 4.5 | 87.9 | 1.1 |
| Permethrin | 88.8 | 6.2 | 93.9 | 4.6 | 104.3 | 0.7 |
| Coumaphos | 101.4 | 4.7 | 111.9 | 3.9 | 111.2 | 1.6 |

*Table 5. Recovery and Repeatability of Pesticides in Fortified Apple With Agilent's Bond Elut Dispersive SPE Tube, 15 ML (p/n 5982-5056), Recovery 87.2%, RSD 5.1% (avg)*

| Analytes | 10 ng/g fortified QC<br>Recovery | RSD (n=6) | 50 ng/g fortified QC<br>Recovery | RSD (n=6) | 200 ng/g fortified QC<br>Recovery | RSD (n=6) |
|---|---|---|---|---|---|---|
| Dichlorvos | 99.4 | 8.2 | 90.9 | 2.6 | 85.7 | 4.4 |
| σ-Phenylphenol | 76.9 | 8.9 | 81.6 | 1.6 | 82.0 | 3.6 |
| Lindane | 87.8 | 7.0 | 88.9 | 2.7 | 86.3 | 2.7 |
| Diazinon | 87.0 | 8.3 | 86.6 | 1.8 | 89.3 | 2.7 |
| Chlorothalonil | 71.7 | 11.1 | 77.9 | 1.8 | 75.9 | 3.8 |
| Chlorpyrifos-mehyl | 77.7 | 9.8 | 82.7 | 2.3 | 86.7 | 2.2 |
| Dichlofluanid | 80.0 | 7.8 | 86.5 | 6.1 | 76.6 | 5.1 |
| Dichlorobenzo phenone | 86.2 | 6.1 | 87.6 | 2.4 | 85.7 | 1.5 |
| Heptachlor epoxide | 82.6 | 5.7 | 86.7 | 2.8 | 85.9 | 1.9 |
| γ-Chlordane | 89.6 | 7.1 | 85.1 | 2.9 | 83.6 | 2.1 |
| DDE | 91.9 | 5.5 | 88.7 | 3.5 | 83.8 | 1.8 |
| α-Chlordane | 90.3 | 4.4 | 88.0 | 3.1 | 84.0 | 1.3 |
| Dieldrin | 93.6 | 7.3 | 88.1 | 4.3 | 83.2 | 1.6 |
| Ethion | 81.0 | 6.7 | 94.2 | 4.0 | 91.1 | 1.7 |
| Endosulfan sulfate | 96.4 | 5.3 | 91.2 | 3.9 | 89.8 | 1.2 |
| Permethrin | 89.3 | 5.6 | 95.5 | 3.9 | 108.9 | 1.3 |
| Coumaphos | 89.0 | 10.8 | 90.7 | 6.6 | 97.1 | 2.0 |

Figure 3 shows the recovery and precision results for 1 mL dispersive SPE and 6 mL dispersive SPE. The two different dispersive SPE clean-ups were performed by transferring 1 mL or 6 mL of ACN extract from the same sample following the extraction step. In order to simplify the comparison, the average recovery and precision of three fortification concentrations were used for all of the pesticides. The results of each dispersive SPE clean-up appeared to be independent of volume used. Both approaches provided similar efficient sample clean-up and generated relatively equivalent results.

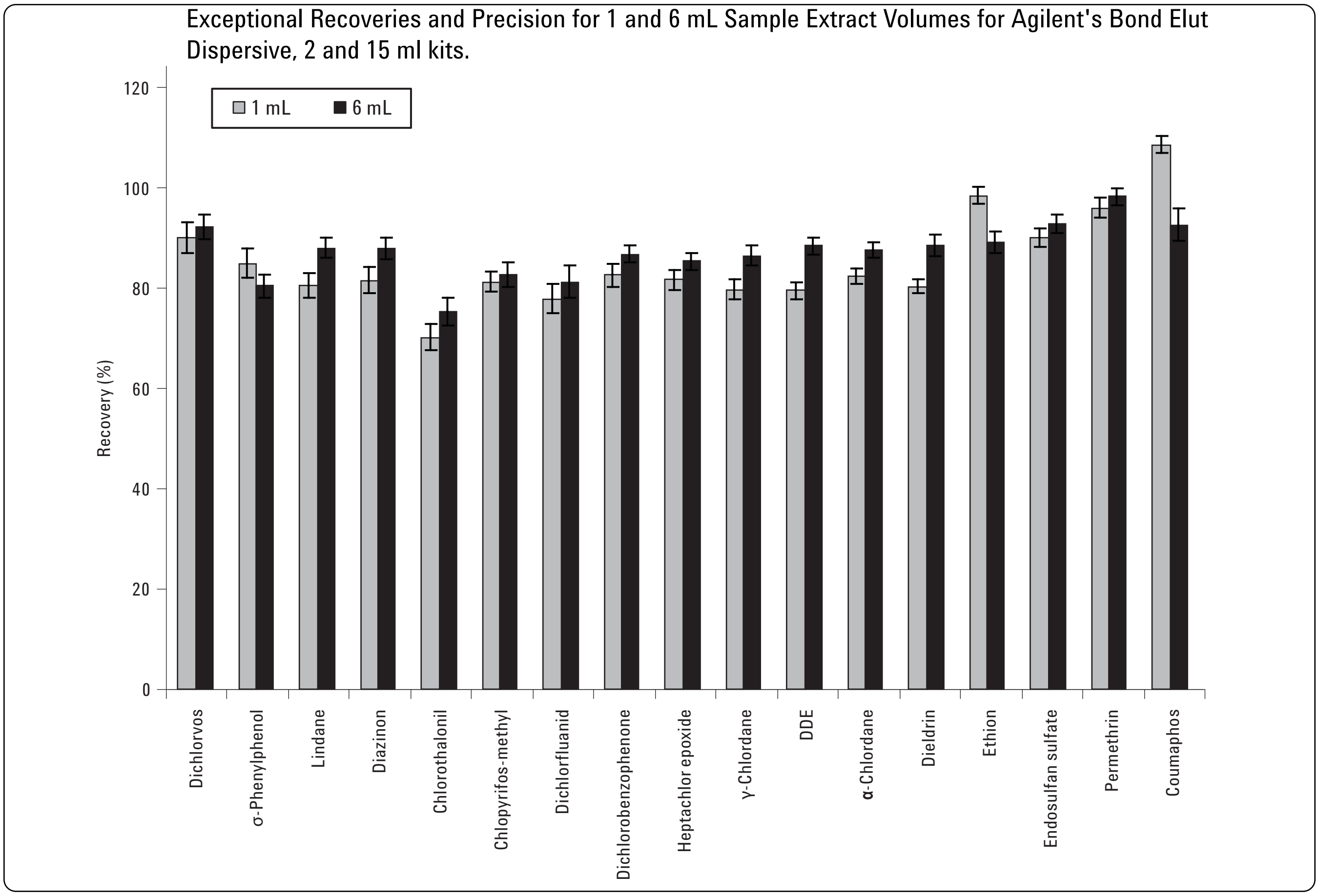


*Figure 3. The recovery and precision results of 1 and 6 mL sample volumes employing Agilent's Bond Elut Dispersive SPE, 2 and 15 mL kits, respectively.*

## Conclusions

Agilent's Bond Elut QuEChERS EN Extraction and Dispersive SPE kits for General Fruits and Vegetables provide a simple, fast and effective method for the purification and enrichment of representative volatile to semi-volatile pesticides in apple. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multi-class, multi-residue pesticide determination in apple. The impurities and matrix effects from apple did not interfere with the quantitation of target compounds. The LOQs of the pesticides were lower than regulated MRLs in apple. Since the selected pesticides represented a broad variety of different classes and properties, the Agilent Bond Elut QuEChERS EN Extraction and Dispersive SPE kits for General Fruits and Vegetables is an excellent choice for other pesticides in similar food matricies

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