Organophosphorus Pesticides in Apple Matrix by GC/MS/FPD using an Agilent J&W DB-35ms Ultra Inert GC Column

Application Note

Environmental and Food Safety

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Abstract

The Agilent J&W DB-35ms Ultra Inert (UI) 20 m × 0.18 mm × 0.18 µm column effectively resolved the analytes of interest producing excellent peak shape for even the more problematic organophosphorus (OP) pesticides. The detection limits for most of the pesticides were 15-25 ng/mL. Recovery studies were performed by spiking with a standard solution to achieve the desired concentrations in an apple matrix; 150, 300 and 750 ng/mL GC/MS/SIM and 50, 100, 250 ng/mL FPD in phosphorus mode. Recoveries were >77% for most of the pesticides by GC/MS/SIM and >75% by GC/FPD.

This application note details a quick and effective analytical method for the determination of low ppm and trace level organophosphorus pesticides residues in apple extract. A capillary flow technology (CFT) device was installed post-column to split the effluent between the MSD and FPD, implementing an automated backflush that diminished residual sample carryover and reduced instrument cycle times. This multi-signal configuration allowed for full scan, selective ion monitoring (SIM), and flame photometric detection from a single injection.

A simplified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method provided sufficient sample matrix cleanup while preserving low level analyte detection.

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Introduction

Organophosphorous (OP) pesticides are widely used in the agricultural industry for crop protection. Human toxicities for this class of molecules have shown acute as well as chronic effects from pesticide poisoning. OP pesticides affect the nervous system of insects and mammals by inhibiting an enzyme, acetylcholinesterase, important in helping regulate nerve impulses [1].

Children are considered more susceptible to organophosphate toxicity because their pesticide dose per body weight is larger compared to that of adults [2]. Children also have lower levels of detoxifying enzymes that deactivate OP pesticides, contributing to their vulnerability to pesticide exposure [3,4]. Recent studies have shown a correlation between OP pesticides exposure and an increased risk for attention deficit hyperactivity disorder (ADHD) and other neurodevelopmental deficits in children [3,5,6,7]. Because the main source of exposure for children is through consumption of food containing OP pesticide residues [2,8], analytical testing capable of determining residual pesticides in food samples is critical.

The multiresidue determination of pesticides in fruits and vegetables usually involves an organic extraction of the pesticides from the plant matrix, followed by a cleanup procedure to remove co-extractives and other interferences. Anastassiades et al [9] developed a QuEChERS method for the analysis of pesticide residues in produce. This approach simplifies the traditional, labor intensive extraction and cleanup procedure, while providing a fast, robust, and cost effective method suitable for extracting pesticide residues.

Chromatographically active compounds such as organophosphorus pesticides can adsorb onto active sites in the sample flow path, particularly at trace levels, compromising an analyte's response. These pesticides tend to show peak tailing through interaction with active sites in a chromatographic system. This makes analysis challenging, particularly in difficult sample matrices. Minimizing activity in the GC column is essential to ensure accurate quantitation. Agilent's J&W DB-35ms Ultra Inert (UI) column minimizes column activity so difficult and active analytes can be consistently analyzed at trace levels. The use of the midpolarity DB-35ms UI phase also offers additional selectivity over a nonpolar phase, which can assist in resolving potentially coeluting peaks, or shift a peak of interest away from matrix interferences.

A gas chromatographic system capable of multisignal detection can provide complementary data for identification, confirmation, and quantitation of target analytes from a single injection. This method provides simultaneous detection of organophosphorus pesticides by GC/MS/SIM and FPD in phosphorus mode by splitting the column effluent between the MSD and FPD. The approach chosen here uses a GC/MSD/FPD system to identify and confirm the order of elution for peaks of interest. Once the elution order is established, the chromatographic parameters can easily be transferred to a GC/FPD system. The use of FPD detection without flow splitting is expected to increase sensitivity threefold, further improving lower level detection.

The GC/MS system was also equipped with backflush capability, which shortens instrument cycle time by backflushing late-eluting matrix components through the inlet purge valve. Long bakeout times between injections are avoided by using this technique. Backflushing has the additional benefit of increasing the time intervals for source cleaning by effectively clearing deleterious matrix components from the system [10].

Experimental

An Agilent 7890 GC with an Agilent 5975C MSD equipped with a flame photometric detector and Agilent 7683B automatic liquid sampler were used for this series of experiments. A purged two-way capillary flow technology (CFT) device was used to split the effluent 3:1 to the MSD:FPD. The CFT device also allowed for post-column backflush. Table 1 lists the chromatographic conditions used for these analyses. Table 2 lists the flow path consumable supplies used in these experiments.

Table 1. Chromatographic Conditions

GC/MSD:	Agilent 7890 GC/Agilent 5975C Series GC/MSD
Sampler:	Agilent 7683B automatic liquid sampler, 5.0 µL syringe (Agilent p/n 5181-1273)
CFT Device:	Purged 2-way splitter (Agilent p/n G3180B) Split Ratio 3:1 MSD:FPD
MSD Restrictor:	1.2 m × 0.15 mm id deactivated fused silica tubing
FPD Restrictor:	1.4 m × 0.15 mm id deactivated fused silica tubing
PCM 1:	3.8 psi constant pressure
Inlet:	1 µL splitless; 250 °C, purge flow 60 mL/min at 0.25 min, gas saver on at 2 min 20 mL/min
Column:	Agilent J&W DB-35ms UI 20 m × 0.18 mm × 0.18 µm (Agilent p/n121-3822UI)
Carrier:	Helium, constant pressure 43.5 psi at 95 °C
Oven:	95 °C (1.3 min), 15 °C/min to 125 °C, 5 °C/min to 165 °C, 2.5 °C/min to 195 °C, 20 °C/min to 280 °C (3.75 min)
Postrun Backflush:	5 min at 280 °C, PCM 1 pressure 70 psi during backflush, 2 psi inlet pressure during backflush
MSD:	310 °C transfer line, 310 °C source, 150 °C quad

Table 2. Flow Path Supplies

Vials:	Amber crimp top glass vials (Agilent p/n 5183-4496)
Vial Caps:	Crimp caps (Agilent p/n 5181-1210)
Vial inserts:	250 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5181-1273)
Septum:	Advanced Green (Agilent p/n 5183-4759)
Inlet liner:	Deactivated dual taper Helix liner (Agilent p/n 5188-5398)
Ferrules:	0.4 mm id short; 85/15 vespel/graphite (Agilent p/n 5181-3323)
PCT fittings:	Internal nut (Agilent p/n G2855-20530)
PCT ferrules:	SilTite ferrules, 0.25 mm id (Agilent p/n 5188-5361)
20x magnifier:	20x Magnifier loop (Agilent p/n 430-1020)

Reagents and Chemicals

All reagents and solvents were HPLC or Ultra Resi grade. Acetonitrile (ACN) from Honeywell (Muskegon, MI, USA), toluene from Burdick & Jackson, and acetone from JT Baker was purchased through VWR International (West Chester, PA, USA). The 12-component custom pesticide standard was prepared by Ultra Scientific (N. Kingstown, RI, USA).

Solutions and Standards

The OP pesticide stock standard solution (100 µg/mL of 12 organophoshorus pesticides) was diluted in acetone to yield spiking solutions 1 and 10 µg/mL. A surrogate standard, triphenyl phosphate (TPP), was prepared at concentrations of 1, 15 and 100 µg/mL in toluene. The spiking solutions were used to prepare the calibration curves in the matrix blank extract by appropriate dilution.

Sample Preparation

An organic apple sample was purchased from a local grocery store. The apple was chopped into small cubes and frozen at –80 °C overnight. The samples were then comminuted thoroughly to achieve sample homogeneity. The sample extraction method used the QuEChERS method. Figure 1 illustrates the sample preparation procedure graphically in a flow chart.

Samples containing 15 (± 0.1) grams of apple were weighed into centrifuge tubes. QC samples were spiked with appropriate amount of spiking solutions to yield QC samples with quantitative concentrations relative to the 3:1 split ratio of 150, 300, and 750 ng/mL levels for GC/MS-SIM determination, and 50, 100, and 250 ng/mL by flame photometric detection. Each sample received a 15-mL aliquot of ACN. Two ceramic bars (Agilent p/n 5982-9313) were added to each sample to aid in sample extraction. The samples were vortexed for 1 minute. An original Agilent Bond Elut QuEChERS extraction salt packet (Agilent p/n 5982-5555) containing 6 grams of $MgSO_4$ and 1.5 grams sodium chloride was added to each centrifuge tube.

The capped tubes were shaken on a Geno/Grinder @1500 rpm for 1 minute. The samples were centrifuged at 4000 rpm for 5 min.

An 8 mL aliquot of the upper layer was transferred to an Agilent Bond Elut QuEChERS General Fruits and Vegetables dispersive SPE 15 mL tube (Agilent p/n 5982-5058). The dSPE tube was vortexed for 1 minute and then centrifuged at 4000 rpm for 3 minutes to complete the sample extraction. The extract from the dSPE tube was transferred to a GC vial and analyzed by SIM GC/MS and GC/FPD using the chromatographic conditions listed in Table 1.

Extractions of water and acetonitrile aliquots were prepared in the same manner as the samples and served as reagent blanks.

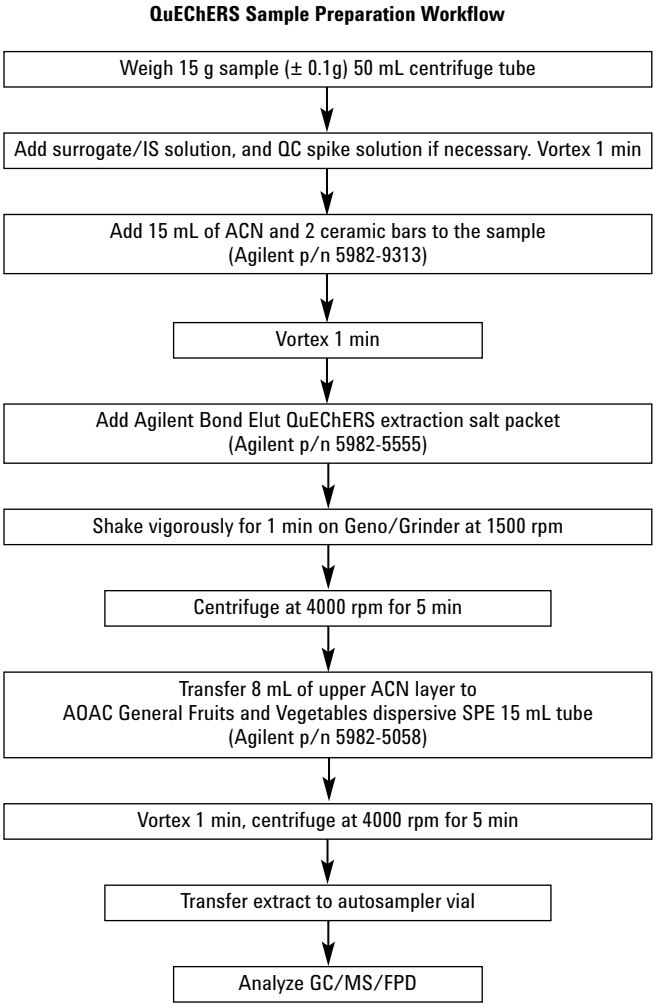

Figure 1. Flow chart of the Agilent QuEChERS extraction procedure for apple samples.

Discussion of Results

The organophosphorus pesticides were resolved on an Agilent J&W DB-35ms UI 20 m × 0.18 mm × 0.18 µm analysis column in about 30 minutes. The 12-component pesticide matrix-matched standard shown in Figure 2 shows good peak shapes for the pesticides in both the GC/MS/SIM and FPD chromatograms. Organophosphorus pesticides, particularly the more polar pesticides can be problematic, often yielding broad peak shapes or excessive tailing making reliable quantitation at low levels difficult. The high level of inertness of the DB-35ms UI column results in better peak shape and decreased sample adsorption allowing lower detection limits. Figure 3 depicts the excellent peak shape seen for four of the more polar OP pesticides (oxydemeton-methyl, methamidophos, mevinphos, acephate) with the DB-35ms UI column.

The performance of the DB-35ms UI high efficiency column yielded excellent linearity and recovery over the calibration range of this study. The linearity of the column as defined by the r^2 values of the pesticide standard curve was ≥0.992 for all the pesticides using both detectors. The individual OP pesticide analyte values are shown in Table 3.

The GC/MS/SIM analysis was able to detect down to the 15–20 ng/mL range for most of the pesticides. A higher SIM signal is necessary to quantify the more volatile pesticides below the 30 ng/mL range due mainly to matrix interferences. Because flame photometric detection in phosphorus mode is selective only for analytes containing phosphorus, it is able to detect low levels of OP pesticides in complex matrices without the matrix interferences. The FPD was able to detect the OP pesticides down to 15 ng/mL with the exception of naled, which could only be detected at higher levels (>25 ng/mL). Naled can undergo debromination, which can have an impact on detection, especially at trace levels. The detection levels for the targeted OP pesticides were well below the US maximum residue levels (MRLs) in an apple matrix, except in the case of chlorpyrifos, which has an MRL of 10 ppb for apples and grapes [11]. Analysis by GC/FPD without flow splitting offers increased sensitivity to monitor the lower levels of detection needed for chlorpyrifos.

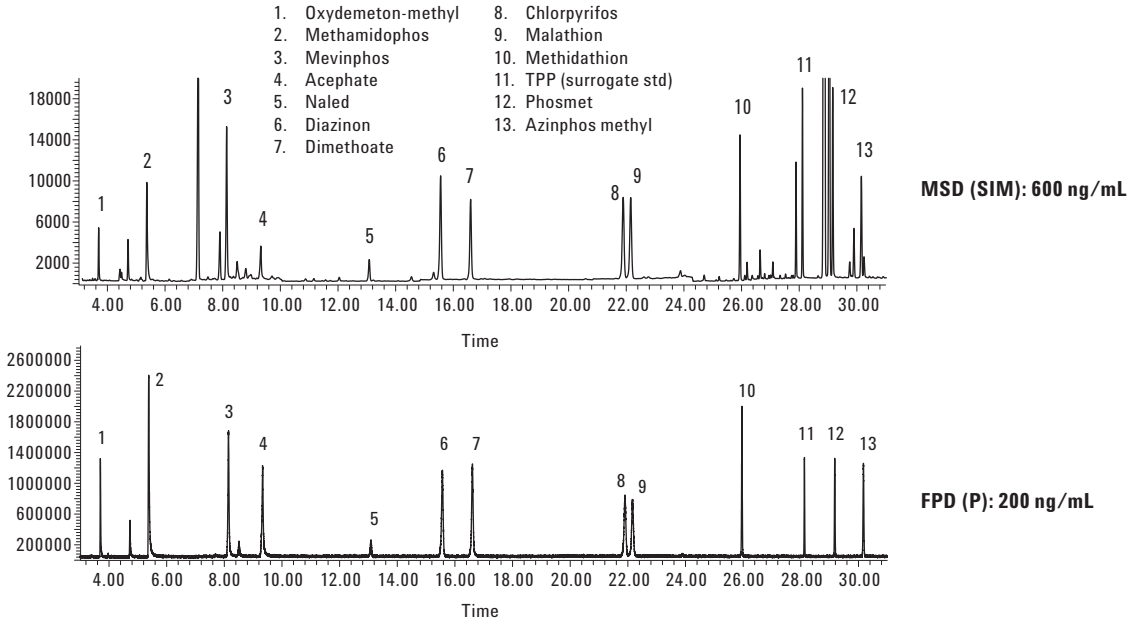

Figure 2. GC/MS-SIM and FPD chromatograms of a matrix matched organophosphorus pesticides standard analyzed on an Agilent J&W DB-35ms UI 20 m × 0.18 mm × 0.18 µm capillary GC column (Agilent p/n 121-3822UI). Chromatographic conditions are listed in Table 1. The effluent split ratio is MSD:FPD = 3:1.

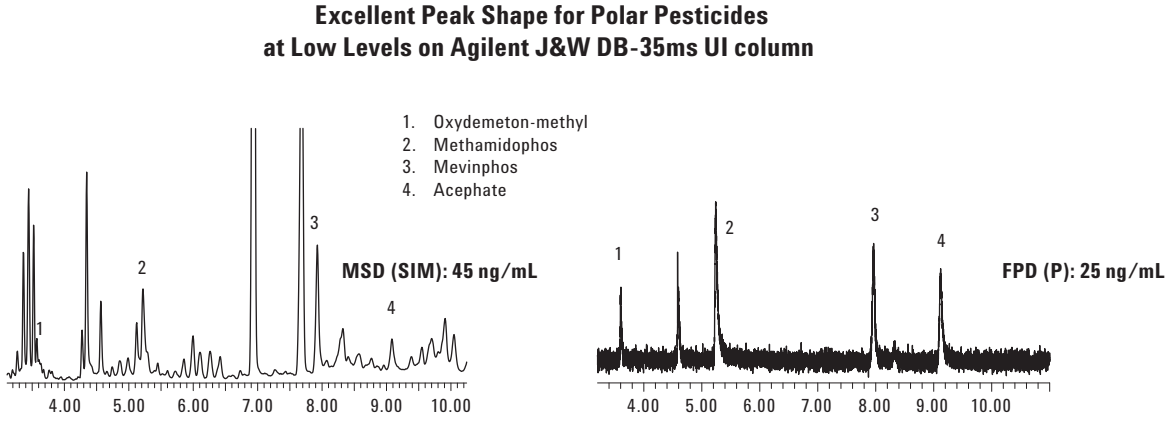

Figure 3. Enlarged section of GC/MS/SIM and FPD chromatograms of the more problematic polar pesticides analyzed on an Agilent J&W DB-35ms UI capillary column (Agilent p/n 121-3822UI). Chromatographic conditions are listed in Table 1. The effluent split ratio is MSD:FPD = 3:1.

Table 3. Correlation Coefficients for the OP Pesticides Calibration Standards Analyzed by GC/MS-SIM and FPD in Phosphorus Mode with a Split Ratio for MSD:FPD = 3:1

Excellent Linearity of OP Pesticides on Agilent J&W DB-35ms UI Column

	MSD	***FPD***
Oxydemeton-methyl	0.994	0.997
Methamidophos	0.997	0.997
Mevinphos	0.997	0.999
Acephate	0.997	0.999
Naled	0.992	0.996
Diazinon	0.996	0.997
Dimethoate	0.997	0.999
Chlorpyrifos	0.997	0.998
Malathion	0.995	0.999
Methidathion	0.996	0.999
TPP	0.999	0.997
Phosmet	0.997	0.999
Azinphos methyl	0.995	0.999

r^2 values for 30, 75, 150, 300, 525, 750, 1500 ppb MSD Calibration Levels
25, 50, 100, 175, 250, 500 ppb FPD Calibration Levels

The extraction process using the QuEChERS method was effective in retaining the OP pesticides in the spiked apple sample and providing sufficient cleanup of the sample matrix for GC/MS analysis. Figure 4 shows the organophosphorus pesticide mix spiked into an apple matrix sample. The matrix was prepared using a QuEChERS sample preparation approach that included extraction/partitioning and dispersive-SPE. A GC/MS/SIM blank matrix trace is shown below the analyte trace to indicate the level of potential matrix interference with the analytes of interest. Peak shapes for the organophosphorus pesticides are still quite sharp and well resolved indicating excellent performance on the DB-35ms UI column in fruit matrix.

Recoveries were determined by GC/MS/SIM at the 150, 300, and 750 ng/mL levels, and 50, 100, and 250 ng/mL using the FPD in phosphorus mode. The recoveries for most of the pesticides were greater than 75% with average RSDs below 10%. Recoveries for the individual OP pesticides are listed in Table 4. Lower recoveries were noted for the more polar pesticides: oxydemeton-methyl, methamidophos, and acephate. One possible explanation is that these polar, highly water soluble pesticides may have been partially lost through incomplete partitioning into the aqueous layer during the extraction step [12].

GC/MS SIM Chromatogram of Apple Extract
Blank Relative to Spiked Sample
after Agilent Bond Elut QuEChERS Extraction and Dispersive SPE

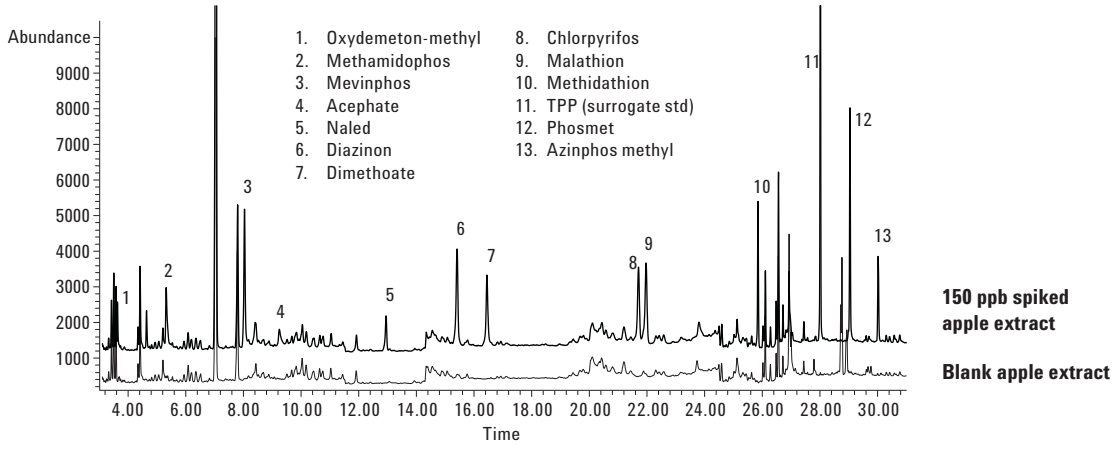

Figure 4. GC/MS/SIM chromatogram of the apple extract blank and a 150 ng/mL spiked apple extract analyzed on an Agilent J&W DB-35ms UI capillary column (Agilent p/n 121-3822UI). Chromatographic conditions are listed in Table 1.

Table 4. Recovery and Repeatability of OP Pesticides in Spiked Apple Matrix with an Agilent J&W DB-35ms UI Column (Agilent p/n 121-3822UI) (continued)

Recovery and Repeatability of OP Pesticides in Spiked Apple Matrix by SIM GC/MS with Agilent J&W DB-35ms UI Column

	150 ng/mL fortified QC		300 ng/mL fortified QC		750 ng/mL fortified QC	
Analysis	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**
Oxydemeton methyl	64.6	5.5	64.0	5.0	61.9	8.2
Methamidophos	68.9	8.5	78.6	3.9	83.8	3.9
Mevinphos	88.7	4.3	93.4	2.6	97.0	3.2
Acephate	77.5	6.4	80.3	5.2	84.3	2.6
Naled	92.9	6.2	87.6	2.4	80.3	5.7
Diazinon	84.5	3.0	89.0	2.7	90.9	3.0
Dimethoate	90.6	2.9	92.9	3.3	96.6	3.6
Chlorpyrifos	87.0	3.7	91.7	3.1	95.6	3.6
Malathion	92.5	3.8	91.9	3.4	97.3	3.6
Methidathion	89.6	4.4	92.1	3.4	99.2	3.7
TPP (surrogate std)	100.6	3.8	101.5	3.1	100.1	3.0
Phosmet	85.9	5.1	86.8	3.4	95.1	3.8
Azinphos methyl	88.6	4.2	84.3	3.5	95.4	3.9

Table 4. Recovery and Repeatability of OP Pesticides in Spiked Apple Matrix with an Agilent J&W DB-35ms UI Column (Agilent p/n121-3822UI)

Recovery and Repeatability of OP Pesticides in Spiked Apple Matrix by GC/FPD with Agilent J&W DB-35ms UI Column

	150 ng/mL fortified QC		300 ng/mL fortified QC		750 ng/mL fortified QC	
Analysis	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**	**% Recovery**	**RSD (n=6)**
Oxydemeton methyl	45.8	8.5	60.6	9.4	62.4	7.7
Methamidophos	63.4	9.2	75.5	5.9	83.7	4.3
Mevinphos	80.5	5.0	90.1	3.5	93.5	4.1
Acephate	64.1	11.1	78.5	7.5	81.3	7.9
Naled	97.2	12.0	87.6	8.0	78.6	8.7
Diazinon	80.1	2.3	86.7	2.9	90.3	3.5
Dimethoate	80.6	7.2	91.0	3.6	93.6	4.6
Chlorpyrifos	80.8	4.7	91.5	5.5	96.6	4.4
Malathion	84.7	4.4	92.9	4.5	96.9	4.8
Methidathion	84.7	7.9	93.9	2.6	96.1	4.1
TPP (surrogate std)	99.6	2.3	99.7	6.0	95.9	3.2
Phosmet	76.6	5.5	89.3	2.6	92.4	4.5
Azinphos methyl	79.4	7.6	88.5	4.5	93.8	3.2

Conclusions

This application note successfully shows a quick and efficient analytical method to monitor low and trace level organophosphorus pesticides residue in apple samples. Splitting the column effluent to both an MSD and FPD facilitated selectivity, identification, and confirmation of OP pesticides from a single injection, thereby increasing laboratory productivity. Using GC/MS in Full Scan mode enabled identification of specific pesticides, while SIM mode offered selectivity and sensitivity for quantitation of the pesticides at trace levels. Confirmation and further specificity was achieved by FPD in phosphorus mode. FPD detection was effective at minimizing matrix interferences enabling lower detection.

The Agilent Bond Elut QuEChERS method for general fruits and vegetables was successful at providing enough sample cleanup to minimize matrix interferences while still maintaining low level analyte detection. The simple QuEChERS extraction method allows for faster sample prep facilitating higher sample throughput. Residual sample matrix carryover is removed through use of backflush, which eliminates the need for a bakeout cycle, significantly reducing analytical run times.

The Agilent J&W DB-35ms UI capillary column resolves the targeted OP pesticides and provides excellent peak shapes for the polar pesticides allowing for more reliable quantitation at low levels. Detection levels for the OP pesticides were at or below the US maximum residue levels (MRLs) for various fruits. Matrix-matched calibration standards yielded regression coefficients $r^2 \geq 0.992$ and recoveries from fortification studies were greater than 75% with an average RSD <10% for both GC/MS/SIM and FPD, further demonstrating the effectiveness of using an Agilent J&W DB-35ms UI column for residual pesticide determination.

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