Organophosphorus Residues in Olive Oil by GC/FPD with Agilent J&W DB-35ms Ultra Inert

Application Note

Environmental and Food Safety

Author

Doris Smith and Ken Lynam
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19809-1610
USA

Abstract

This application note details a quick and effective analytical method for the determination of low ppm and trace level organophosphorus (OP) pesticide residues in an olive oil extract. The Agilent J&W DB-35ms Ultra Inert (UI) 30 m × 0.25 mm, 0.25 µm column resolved the pesticides of interest in less than 16 minutes, yielding excellent peak shape for even the more problematic OP pesticides. Gas chromatographic analysis was performed using a Flame Photometric Detector (FPD) in phosphorus mode for quantitation with confirmation by GC/MS. The detection limits for most of the pesticides were 10–15 ng/mL. Pesticide recoveries at three fortification levels (20, 100, and 500 ng/mL) ranged from 63% to 110% with RSDs < 9%.

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

A capillary flow technology (CFT) device was installed post column to split the effluent between the MSD and FPD and implement an automated backflush to diminish residual sample carryover and reduce instrument cycle times.

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Introduction

The Mediterranean diet has long been considered to provide many health benefits ranging from a decrease in cardiovascular disease and hypertension to protection against certain cancers.[1,2] Olive oil is the primary source of lipids for the Mediterranean diet. On average, 4 kg of olives is needed to produce 1 kg of olive oil, resulting in concentrated levels of residual pesticides in the olive oil.[3] Because of its high consumption rate, the occurrence of toxic pesticide residues in olive oil presents a significant health concern.

Many common insecticides used in the olive grove industry belong to the organophosphorous (OP) class. Sixteen OP pesticides were targeted for monitoring because of their extensive use in olive tree pest protection. Human toxicities for organophosphorous pesticides 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.[4]

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. A sample preparation extraction procedure was used based on the evaluation of the QuEChERS approach for the analysis of pesticide residues in the high lipid olive oil matrix.[5] This approach simplifies the traditional, labor intensive extraction and cleanup procedure, while providing just enough sample matrix cleanup for pesticide residues analysis.

Chromatographically active compounds such as organophosphorus pesticides can adsorb onto active sites in the sample flow path, particularly at trace levels, compromising an analytes' response. These pesticides tend to show peak tailing due to interactions 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. The Agilent J&W DB-35ms Ultra Inert (UI) column minimizes column activity and so difficult and active analytes are consistently analyzed at trace levels. The use of the mid-polarity DB-35ms UI phase offers additional selectivity over a non-polar phase, which can assist in resolving potentially co-eluting peaks, or shift a peak of interest away from matrix interferences.

An additional potential source of activity in the sample path is the GC inlet. In residue analyses, repeated injections of matrix samples can lead to a gradual accumulation of nonvolatile matrix components in the inlet liner and column head, producing active sites and the need for maintenance.[6] This matrix-induced effect can impact peak shape, response, and retention times. Agilent's Ultra Inert liner with wool minimizes liner activity and helps prevent matrix component buildup at the inlet base and column head by trapping the nonvolatiles on the deactivated wool.

Another recognized matrix effect is referred to as matrix-induced signal enhancement effect. [6] This effect is seen in the improved peak shape and signal of affected analytes when injected in-matrix versus non-matrix. This enhancement is a consequence of sample matrix components acting as protectants, reducing thermal degradation and masking active sites within the injector. Organophosphorus pesticides containing P = O bonds, such as methamidophos, acephate, omethoate, etc., often benefit from this matrix effect, yielding a higher response for the analyte in-matrix than in matrix-free standards, which can lead to inaccurate recoveries for fortified samples.[6,7]

Anastassiades et al. [8] suggested the use of analyte protectants to help minimize the errors caused by matrix-induced signal enhancements.[7] These analyte protectants (APs) are compounds that can be added to matrix extracts to protect the susceptible analytes from degradative interactions. A wide variety of compounds were evaluated as viable APs by Anastassiades et al.[8] Based on the results of that study, L-glulonic acid γ-lactone (gulonolactone), was chosen as the analyte protectant for this application.

A gas chromatographic system capable of multi-signal detection can provide complementary data for identification, confirmation, and quantitation of target analytes from a single injection. This method enables simultaneous detection of organophosphorus pesticides by GC/MS-SIM and FPD in phosphorus mode by splitting the column effluent 1:1 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.

The GC/MS system was also equipped with backflush capability. This capability enables faster instrument cycle time by back flushing late eluting matrix components back through the inlet purge valve. Long bake- out times between injections are avoided by using this technique. Backflushing has the added benefit of extending the time intervals between source cleaning by keeping low volatility components away from the source.[9]

Experimental

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

Table 1. Chromatographic Conditions

GC/MSD:	Agilent 7890/5975C
Sampler:	Agilent 7683B, 5.0 µL syringe (p/n 5181-1273)
CFT device:	Purged 2-way splitter (p/n G3180B) Split Ratio 1:1 MSD:FPD
MSD restrictor:	1.43 m × 0.18 mm id deactivated fused silica tubing (p/n 160-2615-10)
FPD restrictor:	0.53 m × 0.18 mm id deactivated fused silica tubing (p/n 160-2615-10)
Aux EPC:	3.8 psi constant pressure
Inlet:	2 µL splitless; 250 °C, Purge flow 60 mL/min at 0.25 min, gas saver on at 2 min 20 mL/min
Column:	DB-35ms UI 30 m × 0.25 mm × 0.25 µm (p/n 122-3832UI)
Carrier:	Helium, constant pressure 28.85 psi at 95 °C
Oven:	95 °C (0.5 min), 25 °C/min to 210 °C, 10 °C/min to 250 °C (0.5 min), 20 °C/min to 290 °C (4.5 min)
Postrun backflush:	7.5 min at 290 °C, Aux EPC pressure 54 psi during backflush, 2 psi inlet pressure during backflush
MSD:	300 °C transfer line, 300 °C source, 150 °C quad
FPD:	230 °C, Hydrogen 75 mL/min, Air 100 mL/min, Carrier + makeup (N_2) 60 mL/min

Table 2. Flow Path Supplies

Vials:	Amber crimp top glass vials (p/n 5183-4496)
Vial Caps:	Crimp caps (p/n 5181-1210)
Vial inserts:	250 µL glass/polymer feet (p/n 5181-8872)
Syringe:	5 µL (p/n 5181-1273)
Septum:	Advanced Green (p/n 5183-4759)
Inlet liner:	Ultra Inert single taper splitless liner with wool (p/n 5190-2293)
Ferrules:	0.4 mm id short; 85/15 vespel/graphite (p/n 5181-3323)
PCT fittings:	Internal nut (p/n G2855-20530)
PCT ferrules:	SilTite ferrules, 0.25 mm id (p/n 5188-5361)
20x magnifier:	20x Magnifier loop (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 were purchased through VWR International (West Chester, PA, USA). The neat pesticide standards were purchased from Chem Service, Inc. (West Chester, PA, USA), gulonolactone from Aldrich (St. Louis, MO), and triphenyl phosphate from Alfa Aesar (Ward Hill, MA).

Solutions and Standards

The individual OP pesticide standards were prepared in acetone to yield neat solutions at a 1–2 mg/mL concentration. These neat solutions were then used to prepare a 50 µg/mL stock standard in acetone. A 1 µg/mL and 5 µg/mL spiking solutions were prepared from the stock standard. A surrogate standard, triphenyl phosphate (TPP), was prepared at concentrations of 1, 15, and 100 µg/mL in toluene. An analyte protectant solution was prepared by dissolving the neat gulonolactone in a minimum amount of water and appropriate amount of ACN to yield a 50 mg/mL concentration. The pesticide and surrogate standard spiking solutions were used to prepare the calibration curves in the matrix blank extract by appropriate dilution. The appropriate amount of gulonolactone solution was added to the calibration standards to yield a 0.5 mg/mL concentration in each standard.

Sample Preparation

A sample of extra virgin olive oil was purchased from a local grocery store. The sample extraction method utilized a modified QuEChERS approach. Figure 1 illustrates the sample preparation procedure graphically.

Samples containing 3.00 g (± 0.05 g) olive oil were weighed into centrifuge tubes. Two ceramic homogenizer bars (p/n 5982-9313) were added to each sample to aid in sample extraction. Quality control samples were spiked with appropriate amounts of spiking solutions to yield QC samples with quantitative concentrations of 20, 100, and 500 ng/mL. Each sample received a 7 mL aliquot of cold reagent-grade water and was vortexed 1 minute. A 10 mL aliquot of ACN was added to the tube. The samples were vortexed for 1 minute. An Agilent original QuEChERS extraction salt packet (p/n 5982-5550) containing 4 g $MgSO_4$ and 1 g sodium chloride was added to each centrifuge tube. The capped tubes were shaken on a mechanical shaker at 1500 rpm for 1 minute. The samples were centrifuged at 4000 rpm for 5 minutes.

An 8 mL aliquot of the upper layer was transferred to an Agilent QuEChERS AOAC dispersive SPE 15 mL tube for fatty samples (p/n 5982-5158). The dSPE tube was vortexed for 1 minute and then centrifuged at 4000 rpm for 5 minutes. Approximately 5.5 mL of the extract was then transferred to a second fatty sample dispersive SPE 15 mL tube and the vortex and centrifuge procedure repeated to complete the sample extraction. The extract from the second dSPE tube was transferred to a vial and the appropriate amount of gulonolactone solution was added to yield 0.5 mg/mL gulonolactone. The extract was then analyzed by GC/MS/FPD using the chromatographic conditions in Table 1.

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

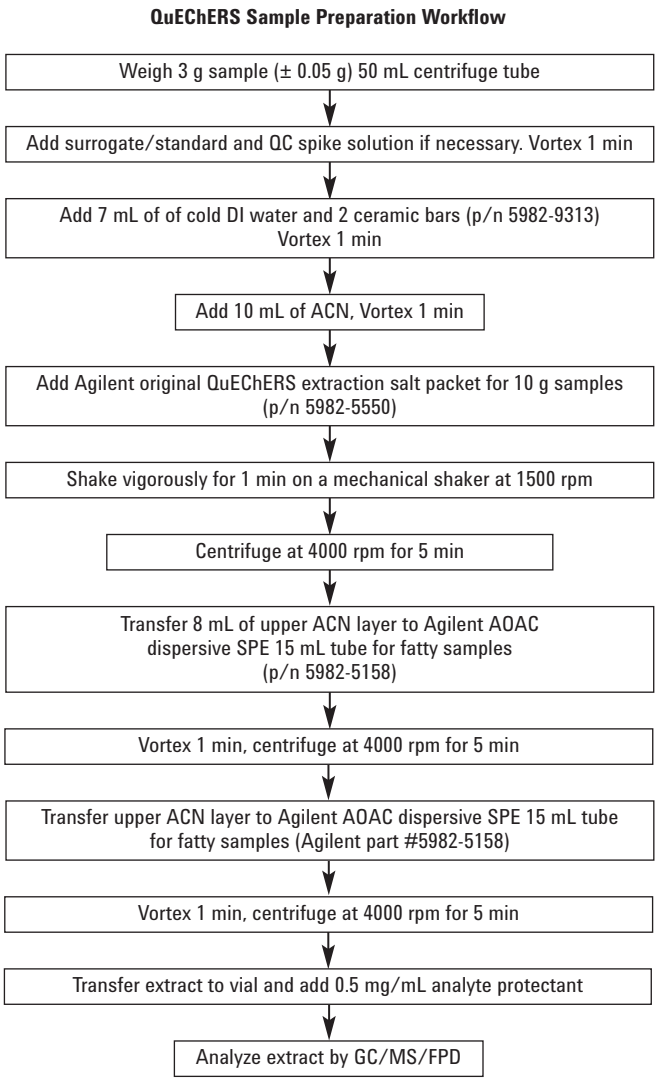

Figure 1. Flow chart for the QuEChERS sample preparation procedure for pesticides in olive oil.

Results and Discussion

The 16 targeted organophosphorus pesticides were resolved on the Agilent J&W DB-35ms UI 30 m × 0.25 mm, 0.25 µm analysis column in less than 16 minutes. The pesticide matrix-matched standard in the Figure 2 chromatogram exhibits good separation and peak shape for all of the pesticides. 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 results in better peak shape and decreased sample adsorption on active sites within the column enabling lower detection limits. Figure 3 depicts the excellent peak shape at 15 ppb for the four polar OP pesticides with the DB-35ms UI column.

Methamidophos, acephate, omethoate, and dimethoate, in particular, are known to be challenging to analyze by gas chromatography. These pesticides all contain a P = O bond that has the potential for hydrogen bond formation, making them more susceptible to matrix-induced effects. The analyte protectant used in this analysis, gulonolactone, effectively reduced matrix related effects and improved the analyte response. This improvement can be seen in Figure 4, which compares a 500 ng/mL standard with and without added gulonolactone.

Since flame photometric detection in phosphorus mode is selective only to analytes containing phosphorus, it is able to detect low levels of OP pesticides in complex matrices such as olive oil with minimal matrix interferences. Excellent signal to noise ratios were seen at trace levels, indicating a high level of sensitivity. Figure 5 shows the signal-to-noise ratio for chlopyrifos at 10 ng/mL. The FPD was able to detect OP pesticides down to 10 ng/mL with the exception of omethoate, diazinon, azinphos-methyl, and azinphos-ethyl, which were detected at a slightly higher limit of detection of 15 ng/mL. The detection levels for the targeted OP pesticides were within the maximum residue levels (MRLs) range of 0.01–2 mg/kg established by the US, EU, and Codex Alimentarius for pesticide residues in olives.[10,11,12] To date, few MRLs are reported for the residues in olive oil, and thus the MRLs in olives is used as a guideline. Because the level of pesticide residues in olives is expected to be concentrated in the oil, the MRLs in olive oil may be higher than in olives.

Sample preparation using the QuEChERS approach was effective in retaining the OP pesticides in the spiked oil sample and providing sufficient cleanup of the sample matrix for GC analysis. Figure 6 shows an olive oil sample which was fortified with the organophosphorus pesticide mix and prepared using QuEChERS.

Resolution of 16 Organophosphorus Pesticides with an Agilent J&W DB-35ms UI Column

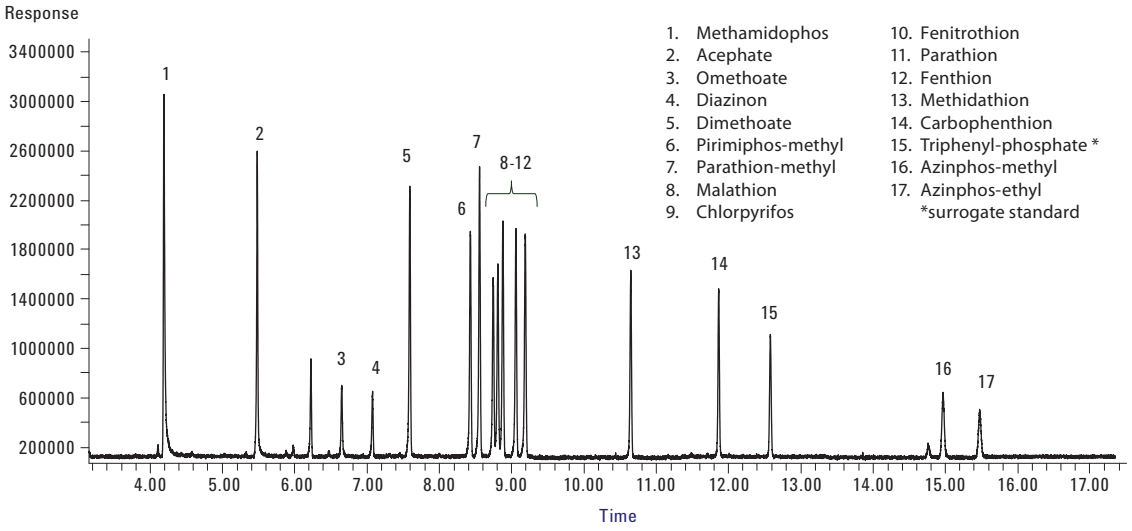

Figure 2. GC/FPD chromatogram of a 100 ng/mL matrix-matched organophosphorus pesticide standard with analyte protectant analyzed on an Agilent J&W DB-35ms UI 30 m × 0.25 mm, 0.25 µm capillary GC column (p/n 122-3832UI). Chromatographic conditions are listed in Table 1.

Excellent Peak Shape for Polar OP Pesticides on Agilent J&W DB-35ms UI Column

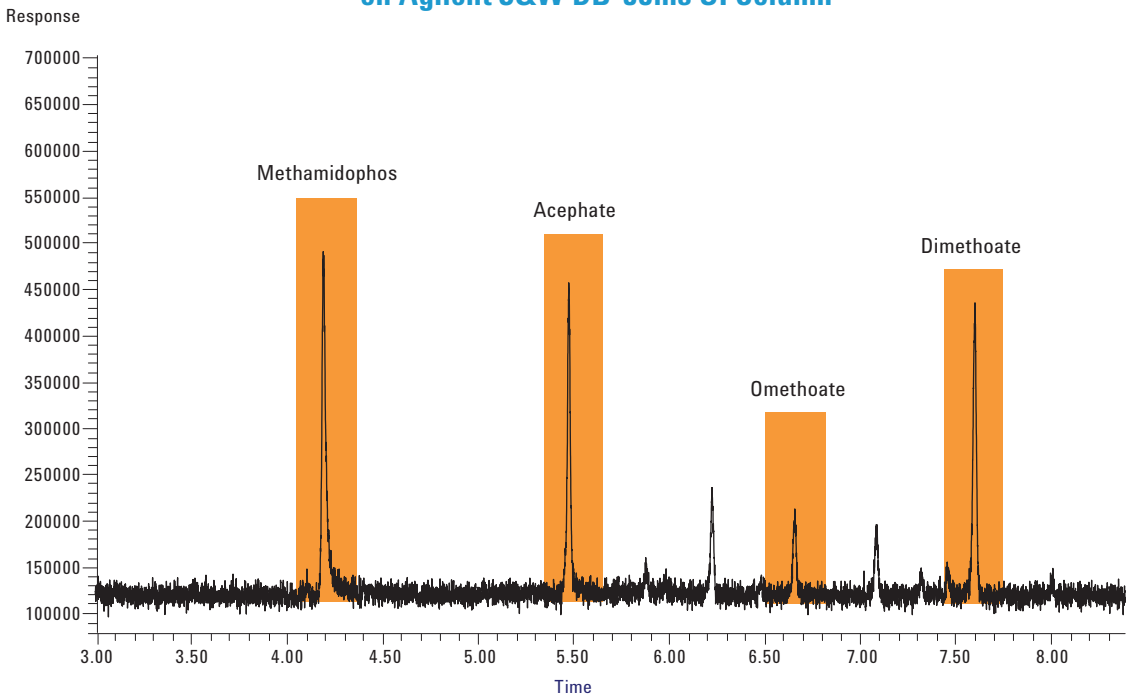

Figure 3. Enlarged section of the GC/FPD chromatogram of a 15 ng/mL matrix-matched pesticide standard with analyte protectant analyzed on an Agilent J&W DB-35ms UI capillary column (p/n 122-3832UI). Chromatographic conditions are listed in Table 1.

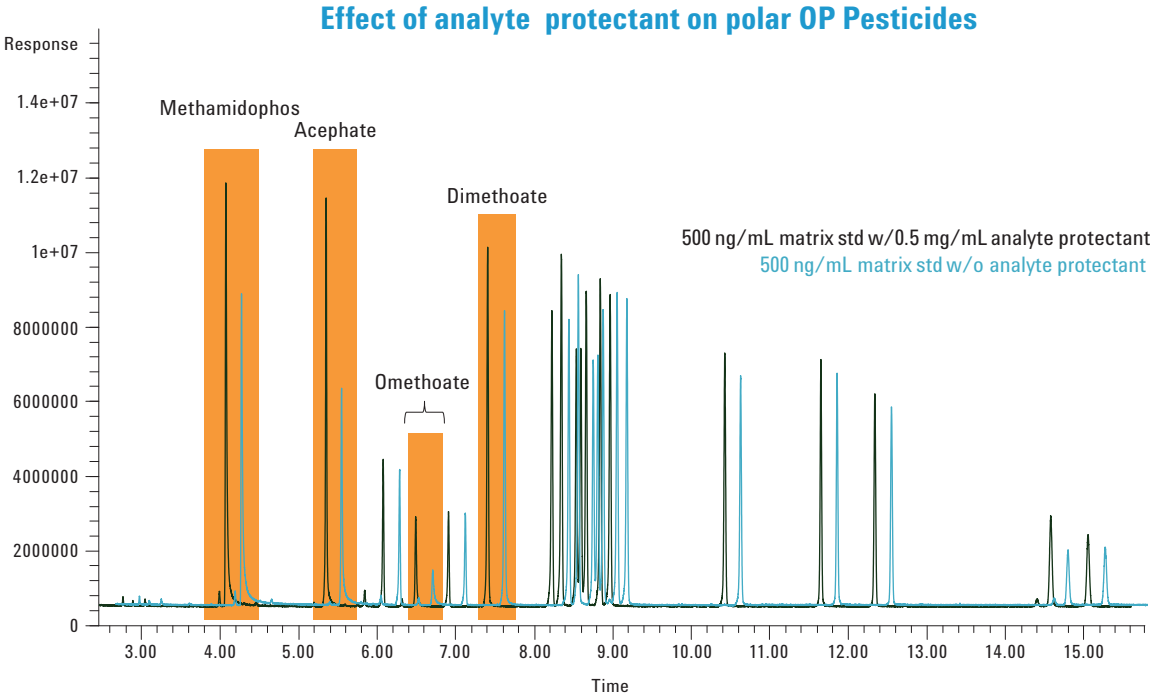

Figure 4. GC/FPD chromatograms of a 500 ng/mL matrix-matched organophosphorus pesticides standard with and without analyte protectant analyzed on an Agilent J&W DB-35ms UI 30 m × 0.25 mm, 0.25 µm capillary GC column (p/n 122-3832UI). The standard without protectant is time offset for better illustration. Chromatographic conditions are listed in Table 1.

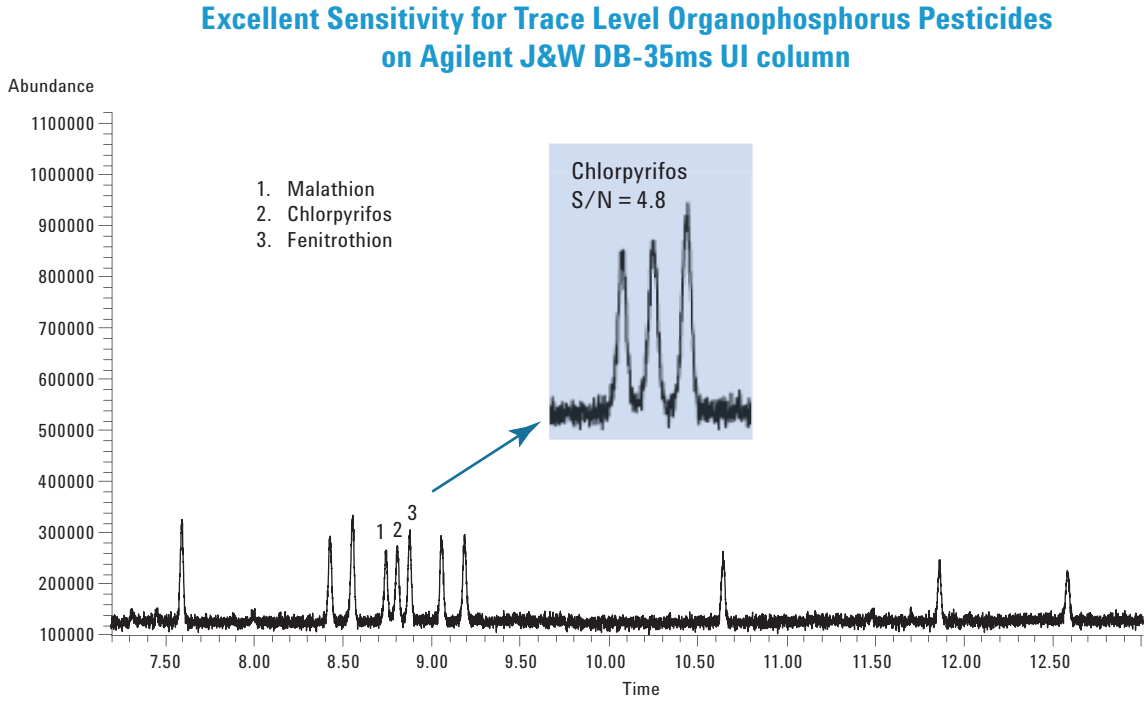

Figure 5. Enlarged section of a GC/FPD chromatogram of a 10 ng/mL matrix-matched pesticide standard with analyte protectant analyzed on an Agilent J&W DB-35ms UI capillary column (p/n 122-3832UI). Chromatographic conditions are listed in Table 1.

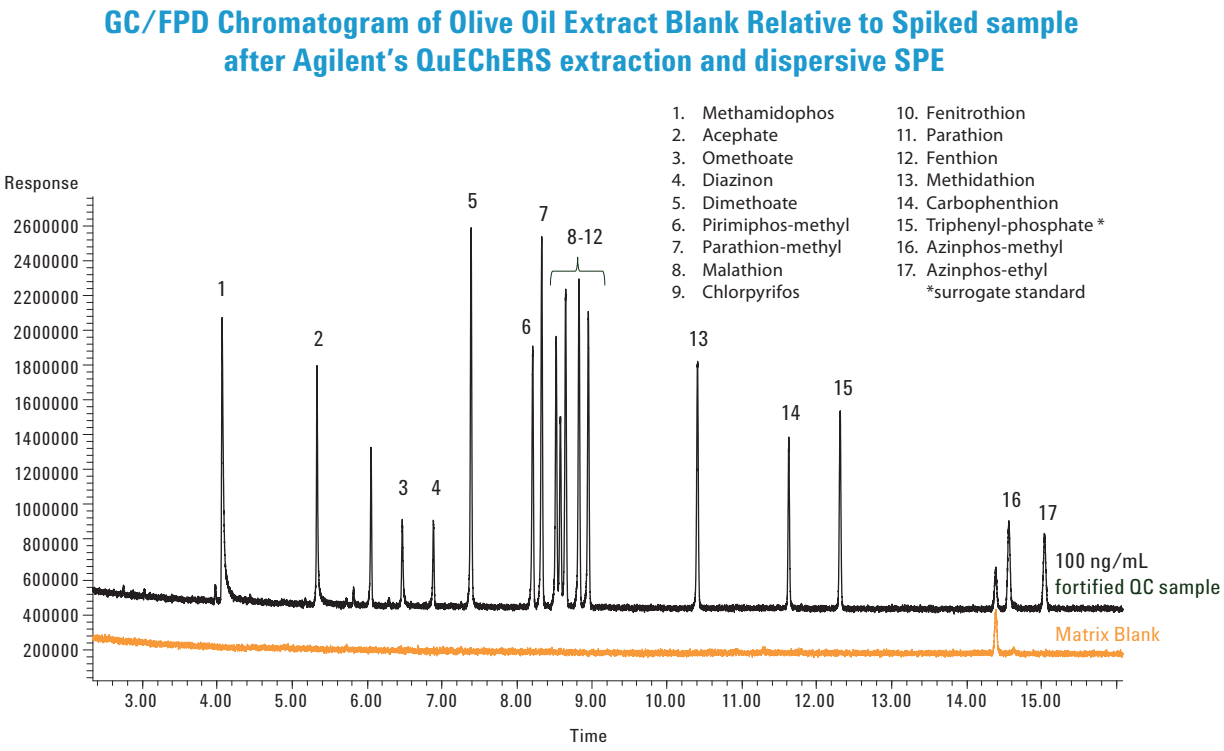

Figure 6. GC/FPD chromatogram of the olive oil extract blank and a 100 ng/mL fortified olive oil extract both with analyte protectant analyzed on an Agilent J&W DB-35ms UI capillary column (p/n 122-3832UI). Chromatographic conditions are listed in Table 1.

A 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 an olive oil matrix.

A nonlinear response for an analyte can be indicative of breakdown or adsorption of the compound on the column. The performance of the DB-35 ms UI column yielded excellent linearity over the calibration range of this study. The linearity of the column as defined by the R^2 values of the calibration standard curve was ≥ 0.999 for all the pesticides studied. The individual OP pesticide analyte values are shown in Table 3.

Recoveries were determined by GC/FPD at the 20, 100, and 500 ng/mL levels. The recoveries of the pesticides were greater than 70% with RSDs below 10% except in the case of acephate, which was slightly lower with an average recovery of 66%. Recoveries for the individual OP pesticides are listed in Table 4.

Table 3. Correlation Coefficients for the OP Pesticide Matrix-Matched Calibration Standards with Analyte Protectant Analyzed by GC/FPD

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

	R^2
Methamidophos	0.9997
Acephate	0.9997
Omethoate*	0.9996
Diazinon*	0.9992
Dimethoate	0.9995
Pirimiphos-methyl	0.9996
Parathion-methyl	0.9995
Malathion	0.9995
Chlorpyrifos	0.9996
Fenitrothion	0.9995
Parathion	0.9995
Fenthion	0.9994
Methidathion	0.9994
Carbophenothion	0.9993
Triphenyl phosphate	0.9995
Azinphos methyl*	0.9995
Azinphos ethyl*	0.9990

R^2 values for 10, 15, 25, 50, 100, 250, 500 ppb Calibration Levels
*Calibrated at 15, 25, 50, 250, and 500 ppb

Table 4. Recovery and Repeatability of OP Pesticides in Spiked Olive Oil Matrix with an Agilent J&W DB-35ms UI Column (p/n 122-3832UI)

Recoveries and Repeatability of OP Pesticides on Agilent J&W DB-35ms UI Column

	1 x Spike		5 x Spike		25 x Spike	
Analytes	**20 ng/mL % Recovery**	**Fortified QC RSD (n=6)**	**100 ng/mL % Recovery**	**Fortified QC RSD (n=6)**	**500 ng/mL % Recovery**	**Fortified QC RSD (n=6)**
Methamidophos	82.1	4.2	77.5	2.2	79.3	1.2
Acephate	70.9	3.6	64.1	3.2	62.7	1.9
Omethoate	71.3	8.8	87.0	3.2	92.5	3.1
Diazinon	73.8	6.6	93.8	4.1	98.4	1.0
Dimethoate	97.2	3.9	101.6	3.0	107.2	1.7
Pirimiphos-methyl	79.2	3.7	81.6	2.2	86.0	1.2
Parathion-methyl	93.2	6.0	92.4	2.7	97.2	1.8
Malathion	102.8	5.1	104.5	2.5	109.3	1.1
Chlorpyrifos	72.9	4.9	72.4	1.6	76.1	1.2
Fenitrothion	94.9	4.9	95.9	3.1	101.1	1.5
Parathion	104.2	7.0	104.8	2.8	110.6	1.2
Fenthion	90.2	6.1	92.7	2.3	96.4	1.2
Methidathion	99.5	5.6	100.6	3.2	106.5	1.3
Carbophenothion	75.8	4.2	72.9	3.0	75.6	1.4
Triphenyl phosphate*	108.8	1.2	106.4	2.5	115.6	1.3
Azinphos methyl	91.5	4.7	97.1	3.2	100.7	2.8
Azinphos ethyl	81.7	6.1	101.8	4.0	110.7	1.3

*surrogate standard

Conclusions

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 in olive oil are at or below the EU, Codex, and US maximum residue levels (MRLs) for olives. Matrix-matched calibration standards yield regression coefficients $R^2 \geq 0.999$ and recoveries of fortification studies were 63% to 107% with an average RSD < 9%, further demonstrating the effectiveness of using the Agilent J&W DB-35ms UI columns for residual pesticide determination.

This trial successfully demonstrates a quick and efficient analytical method to monitor low and trace level organophosphorus pesticide residues in olive oil samples. By splitting the column effluent between the MSD and FPD, selectivity, identification, and confirmation of OP pesticides from a single injection are achieved, thereby increasing laboratory productivity. GC/MS-SIM provides selectivity and confirmation, while further specificity and quantitation is achieved by FPD in phosphorus mode.

The Agilent QuEChERS approach is successful at providing just enough sample clean-up 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.

Acknowledgements

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