GC-µECD Analysis and Confirmation of Contract Laboratory Protocol Pesticides in Olive Oil

Application Note

Food Safety and Environmental Markets

Authors

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Abstract

An olive oil sample obtained from a local grocery store is analyzed for 20 contract laboratory protocol (CLP) pesticides. A QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) with dispersive solid phase extraction (dSPE) procedure cleaned the sample prior to analysis. A dual µECD and dual capillary GC column approach accomplished simultaneous primary and confirmatory analysis. The primary column, an Agilent J&W DB-35ms 30 m × 0.25 mm × 0.25 µm and a confirmatory column, an Agilent J&W DB-XLB 30 m × 0.25 mm × 0.50 µm effectively resolved all 20 CLP pesticides. An unpurged two-way capillary flow technology splitter divided the flow from a single injection port to the two analytical GC columns. Endosulfan sulfate and endosulfan 1 were present in the olive oil sample.

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Introduction

Lower cholesterol and reduced cancer risk are among the reported beneficial health effects obtained from the antioxidants and monounsaturated fats found in olive oil. The potential health benefits have bolstered the popularity of using olive oil as a cooking oil, salad dressing, or direct replacement in baking for other traditional food oils. This rise in olive oil's popularity has led to higher demand and production levels.

Higher olive oil production levels increase the potential for olive crop destruction by pests. Therefore the industry is more reliant on the use of pesticides, leading to increased regulation of pesticide use [1]. For example, food safety concerns have led to tighter government regulation of pesticide residues found in foods such as olive oil. Production of olive oil has a tendency to concentrate pesticide residues in the oil because it takes four kilograms of olives to make one kilogram of olive oil [2]. Olive oil producers need inexpensive, robust analytical methods for necessary monitoring and testing.

In previous studies of pesticides residue analysis in olive oil, the extraction procedures varied from liquid-liquid followed by solid phase extraction (SPE), gel permeation chromatography (GPC), and more recently to the use of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) with dispersive solid phase extraction (dSPE) [3-4]. QuEChERS and dSPE are convenient ways to clean up sample matrixes sufficiently to remove chromatographic interferences, while retaining sensitivity for analytes of interest. In this olive oil example, the high boiling lipid portion of the sample is removed along with its potential for carryover and interference with peaks of interest.

A dual column, dual µECD system with an Agilent J&W DB-35ms 30 m × 0.25 mm × 0.25 µm primary analysis column and an Agilent J&W DB-XLB 30 m × 0.25 mm × 0.50 µm confirmatory column was used to separate the CLP pesticides in olive oil [5-6]. Continuous improvements and stringent process control with respect to column activity make this column pair a particularly good choice for analysis of active analytes such as pesticides.

The GC was also fitted with an unpurged two-way splitter capillary flow technology (CFT) device. This device allows the operator to disassemble and service the inlet, the inlet transfer line or either of the analytical columns individually. System maintenance and troubleshooting are faster with reusable connections to the CFT.

Experimental

Standard Preparation

CLP pesticide (PPM-808C-1) and surrogate standards (ISM-320-1) were purchased from Ultra Scientific, 250 Smith St, N. Kingstown, RI. The CLP pesticide standard solution was prepared by diluting the standard to 8000 ng/mL with 2,2,4-Trimethylpentane (Ultra-Resi grade from VWR International, West Chester, PA). The 2000 ng/ml stock surrogate solution was made by diluting the surrogate standard with 2,2,4-Trimethylpentane. Working solutions of 4, 8, 80 and 800 ng/mL for the CLP pesticides and 1, 2, 20, and 200 ng/mL of the surrogates were used for spiking and calibration samples. 2,2,4-Trimethylpentane was used as a solvent blank and syringe wash solvent.

Sample Preparation

A sample of extra virgin olive oil was purchased at a local grocery store. The sample extraction method used the QuEChERS method followed by dSPE. Figure 1 illustrates the sample preparation procedure graphically in a flow chart.

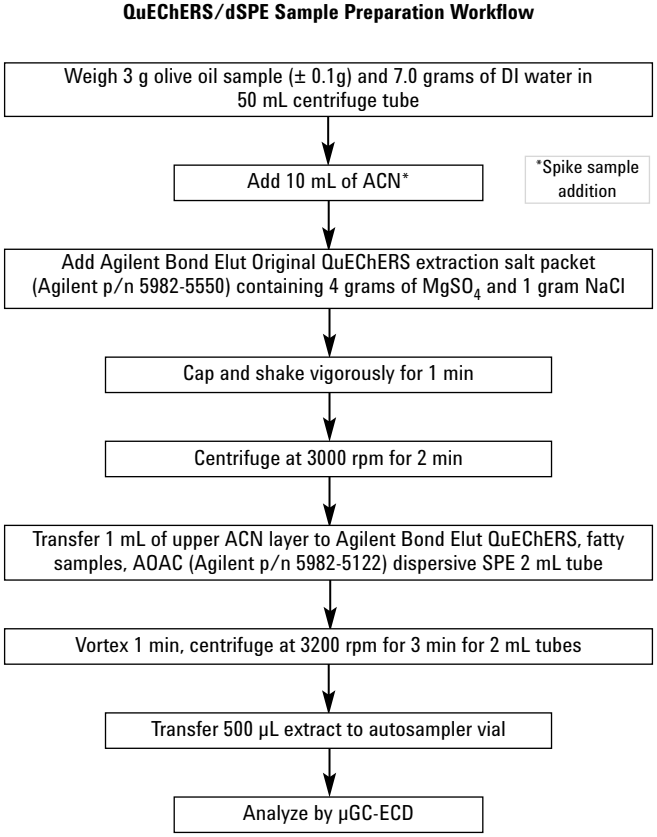

Figure 1: Flow chart of the Agilent Bond Elut QuEChERS original extraction procedure for olive oil sample.

A 3.0-g olive oil aliquot and 7.0-g aliquot of deionized water were weighed into a centrifuge tube. The sample, spiked sample and blank each received 10-mL aliquots of acetonitrile (HPLC grade from VWR International). An Agilent Bond Elut QuEChERS extraction salt packet (Agilent p/n 5982-5550) containing 4 g of $MgSO_4$ and 1 g NaCl was added to each centrifuge tube. The capped tubes were shaken for 1 min by hand and then 1 min on a mechanical shaker. The samples were centrifuged at 3000 rpm for 2 min.

A 1-mL aliquot of the upper layer was transferred into an Agilent Bond Elut QuEChERS, fatty samples, AOAC (Agilent p/n 5982-5122) dispersive SPE 2 mL tube. The dSPE tube was vortexed for 1 min and then centrifuged at 3200 rpm for 3 min to complete the sample extraction. The liquid from the dSPE tube was transferred to a GC vial and run on the GC-µECD using the chromatographic conditions listed in Table 1 and Table 2.

Table 1. Chromatographic Conditions

GC/Dual µECD:	7890A equipped with dual µECD detection and a 7873B auto sampler
CFT Device:	2-way unpurged splitter capillary flow technology (Agilent p/n G3181B)
Column 1:	DB-35 ms 30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-3832)
Column 2:	DB-XLB 30 m × 0.25 mm × 0.50 µm (Agilent p/n 122-1236)
Carrier Gas:	Hydrogen 56 cm/sec
Oven:	110 °C (1.4 min), 21 °C/min to 285 °C (1 min), 30 °C/min to 300 °C (2 min)
Injection:	1µL, 250 °C splitless, purge 50 mL/min at 0.3 min, gas saver 50 mL/min on at 2 min
Dual µ-ECD:	350 °C, N_2 makeup; constant column + makeup = 30 mL/min

Table 2 Flow Path Supplies

Vials:	Amber screw top glass vials (Agilent p/n 5183-2072)
Vial Caps:	Screw caps (Agilent p/n 5182-0723)
Vial inserts:	100 µL glass/polymer feet (Agilent p/n 5181-8872)
Syringe:	5 µL (Agilent p/n 5183-4729)
Septum:	Advanced green (Agilent p/n 5183-4759)
Inlet Seal:	Gold plated inlet seal (Agilent p/n 5188-5367)
Inlet liners:	Dual taper direct connect linear (Agilent p/n G1544-80700)
Ferrules:	0.4 mm id short; 85/15 vespel/graphite (Agilent p/n 5181-3323)
CFT fittings:	Internal nut (Agilent p/n G2855-20530)
CFT ferrules:	SilTite ferrules, 0.25 mm id (Agilent p/n 5188-5361)
20x magnifier :	20x Magnifier loop (Agilent p/n 430-1020)

To produce the spiked sample, a 3-mL aliquot of the 80 ng/mL CLP standard solution was added to the olive oil and water mixture, before the Bond Elut QuEChERS original extraction salt packet addition. Extractions of water and acetonitrile aliquots in the same manner as the samples and the spiked sample served as reagent blanks.

Discussion of Results

The CLP pesticides and surrogates standards were resolved on the Agilent J&W DB-35ms 30 m × 0.25 mm × 0.25 µm primary analysis column in less than 12 min. Figure 2 show the separation of a 4.0 ng/mL CLP standard solution with the surrogate standards added at a concentration of 1.0 ng/mL. Peak numbers in the chromatogram label the peaks of interest and a compound key is included with the figure. Figure 3 shows a chromatogram of the same 4 ng/mL CLP standard (0.5 ng/mL surrogate standard) injection on the Agilent J&W DB-XLB 30 m × 0.25 mm × 0.50 µm confirmatory analysis column. Although peaks 10 and 11 (α-chlordane and endosulfan 1) are not completely resolved, the separation is suitable for confirming the presence of these analytes when observed on the primary analysis (DB-35ms) column.

The performance of the dual column set yielded acceptable linearity, limits of detection (LOD), and quantitative (LOQ) in accordance with current EU guidelines for these analytes. The linearity of the column set as defined by the R^2 values of the CLP pesticide standard curve ranged from 0.994-0.999. Individual pesticide values are shown in Table 3. The LOD (S/N = 3) and LOQ (S/N = 10) were determined through close examination of an expanded section of chromatograms on each column relative to a known concentration peak in the lowest standard. Figure 4A shows this comparison for the tetrachloro-m-xylene peak on the primary analysis DB-35ms column and Figure 4B shows the same comparison on the DB-XLB confirmation column. The average LOD (S/N = 3) and LOQ (S/N =10) across both columns using the lowest concentration (0.5 ng/mL) of the standard CLP pesticides was 0.3 ng/mL and 1.0 ng/mL respectively. The dual column set of a primary analytical column and a confirmatory column on one instrument allows simultaneous confirmation of the presence of the CLP pesticides. The single injection, dual column approach saves instrument and analyst time and offers the analyst an alternative to GC/MS screening for olive oil samples.

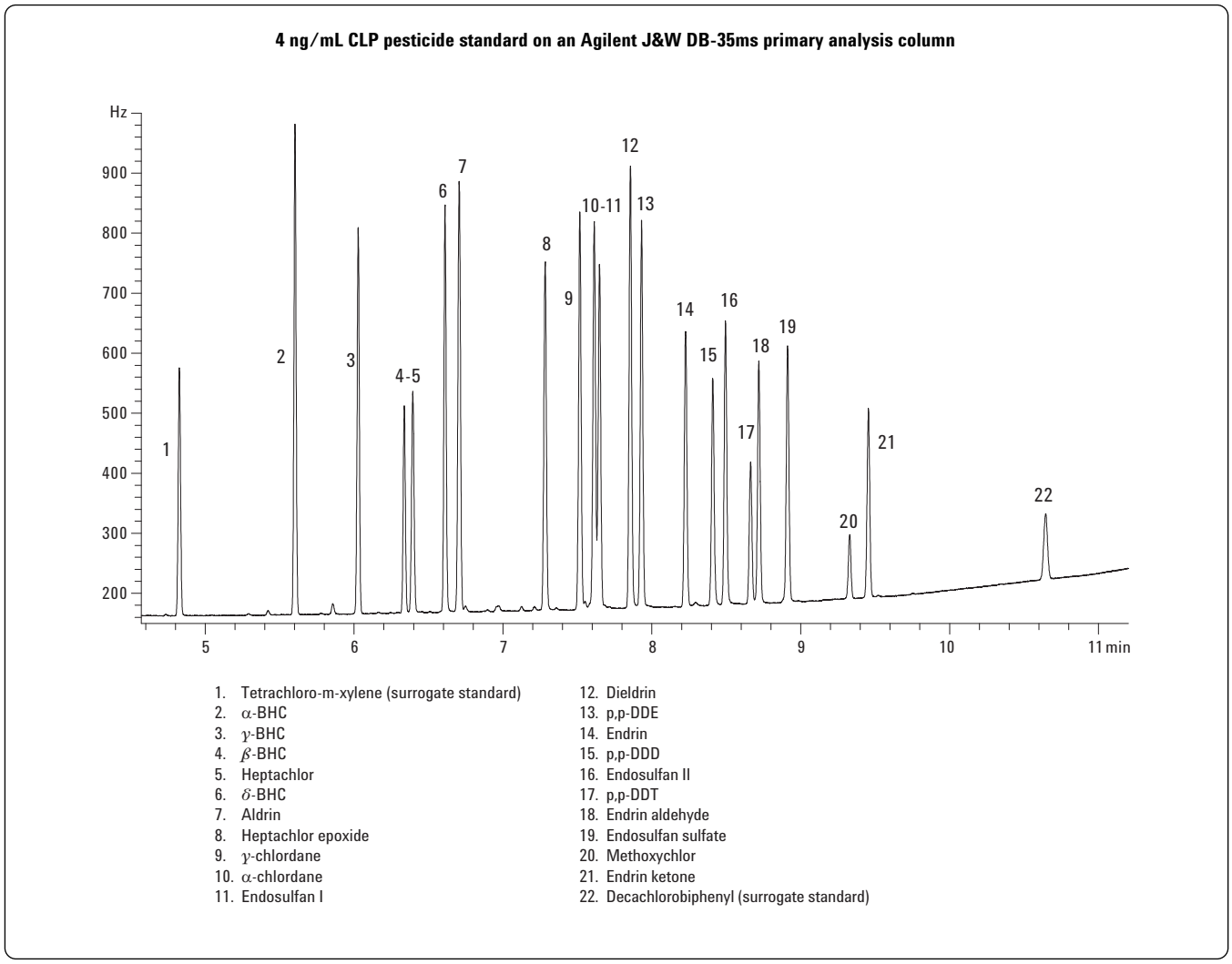

Figure 2. GC-μECD chromatogram of 4 ng/mL standard of CLP pesticides and surrogates standard analyzed on an Agilent J&W DB-35ms, 30 m × 0.25 mm × 0.25 μm column.

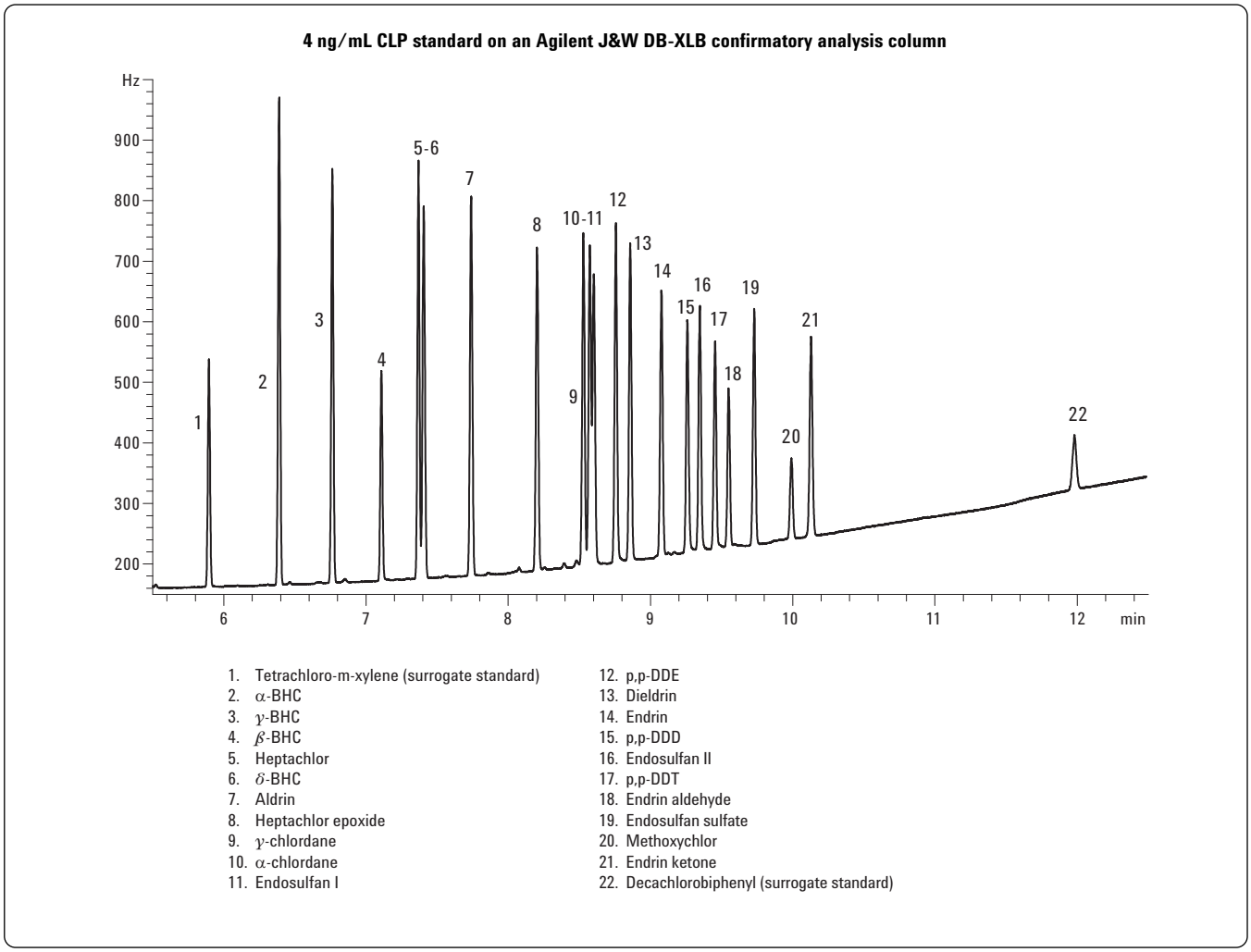

Figure 3. GC-μECD chromatogram of 4 ng/mL standard of CLP pesticides and surrogates standard analyzed on an Agilent J&W DB-XLB, 30 m × 0.25 mm × 0.50 μm column.

Table 3. Calibration Curve - Calibration Standards of 4, 8, 10, 20, and 40 ng/mL Were Prepared in 2,4-Trimethylpentane.

	DB-35 ms	DB-XLB
Compounds	r^2	r^2
α-BHC	0.998	0.998
γ-BHC	0.998	0.998
β-BHC	0.997	0.998
Heptachlor	0.998	0.998
δ-BHC	0.997	0.995
Aldrin	0.997	0.998
Heptachlor epoxide	0.998	0.998
γ-chlordane	0.998	0.997
α-chlordane	0.998	0.996
Endosulfan I	0.997	0.996
p,p-DDE	0.998	0.998
Dieldrin	0.997	0.998
Endrin	0.995	0.997
Endosulfan II	0.999	0.998
Endrin Aldhyde	0.998	0.995
p,p-DDD	0.998	0.998
p,p-DDT	0.999	0.998
Endosulfan sulfate	0.995	0.998
Methoxychlor	0.998	0.996
Endrin Ketone	0.998	0.994

A dual column µECD system resolved the 20 CLP pesticides in the spiked olive oil sample. The primary analytical column DB-35ms separated the 20 CLP pesticides with minor matrix interference in a sample of olive oil spiked with an 80 ng/mL CLP pesticide and surrogate standard. There are several olive oil matrix peaks observed in the spiked samples, including one large peak, that fortunately do not co-elute with the CLP pesticides. Figure 5 clearly shows the separation of all 20 CLP pesticides in an olive oil matrix. In Figure 6, the DB-XLB confirms the presence and separation of the CLP pesticides in the spiked olive oil sample.

Figures 7 and 9 show the overlaid chromatograms on the DB-35ms primary analysis column of the spiked and native olive oil sample with peaks for endosulfan sulfate and endosulfan 1 labeled. Figures 8 and 10 show the overlaid chromatograms on the DB-XLB confirmatory column with peaks for endosulfan sulfate and endosulfan 1 labeled. Chromatogram overlays of the native and spiked olive oil samples identify and confirm endosulfan sulfate and endosulfan 1 as pesticide residues in the olive oil sample. Concentrations from the CLP calibration standard curves were 23.1 ng/mL for endosulfan sulfate and 7.1 ng/mL for endosulfan 1.

The extraction process using the QuEChERS followed by dispersive SPE was effective in retaining the pesticides in the spiked olive oil sample as well as cleaning up the sample matrix for GC-µECD analysis. The fact that endosulfan sulfate and endosulfan 1 were present and detected at low levels in the olive oil sample investigated underscores this point.

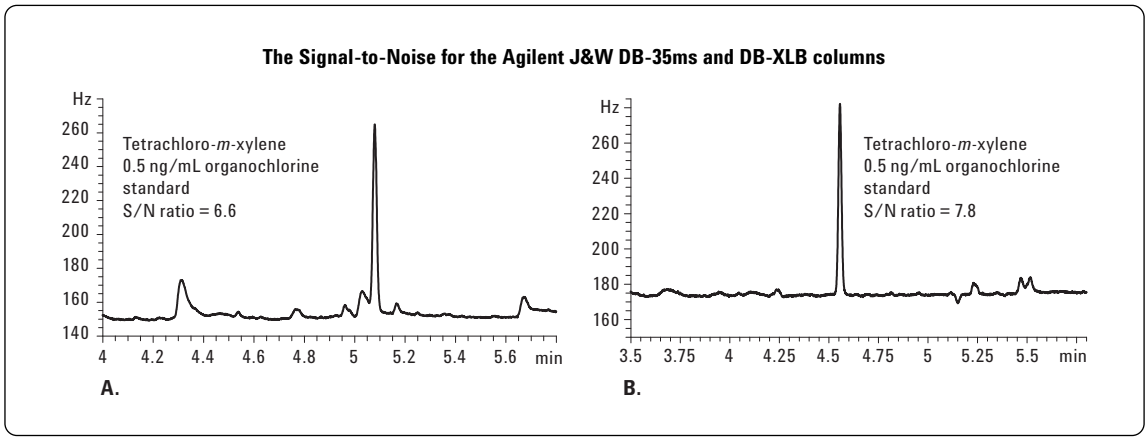

Figure 4. GC-µECD chromatogram of 0.5 ng/mL standard of CLP pesticides and surrogates standard. A.) The S/N on the Agilent J&W DB-35ms, 30 m × 0.25 mm × 0.25 µm column is 6.6. B.) The S/N on an Agilent J&W DB-XLB, 30 m × 0.25 mm × 0.50 µm column is 7.8.

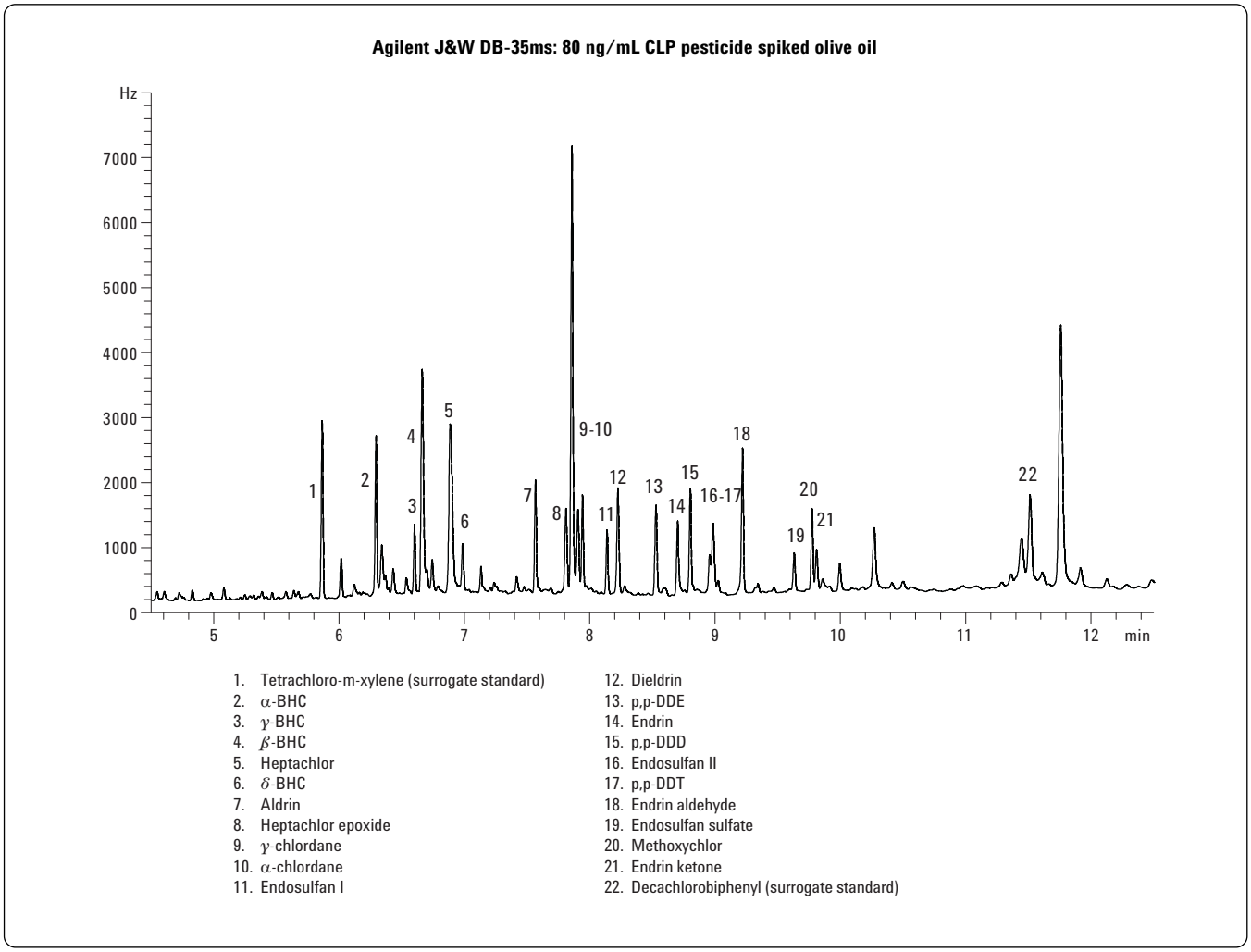

Figure 5. GC-µECD chromatogram of olive oil sample spiked with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-35ms 30 m × 0.25 mm × 0.25 µm column.

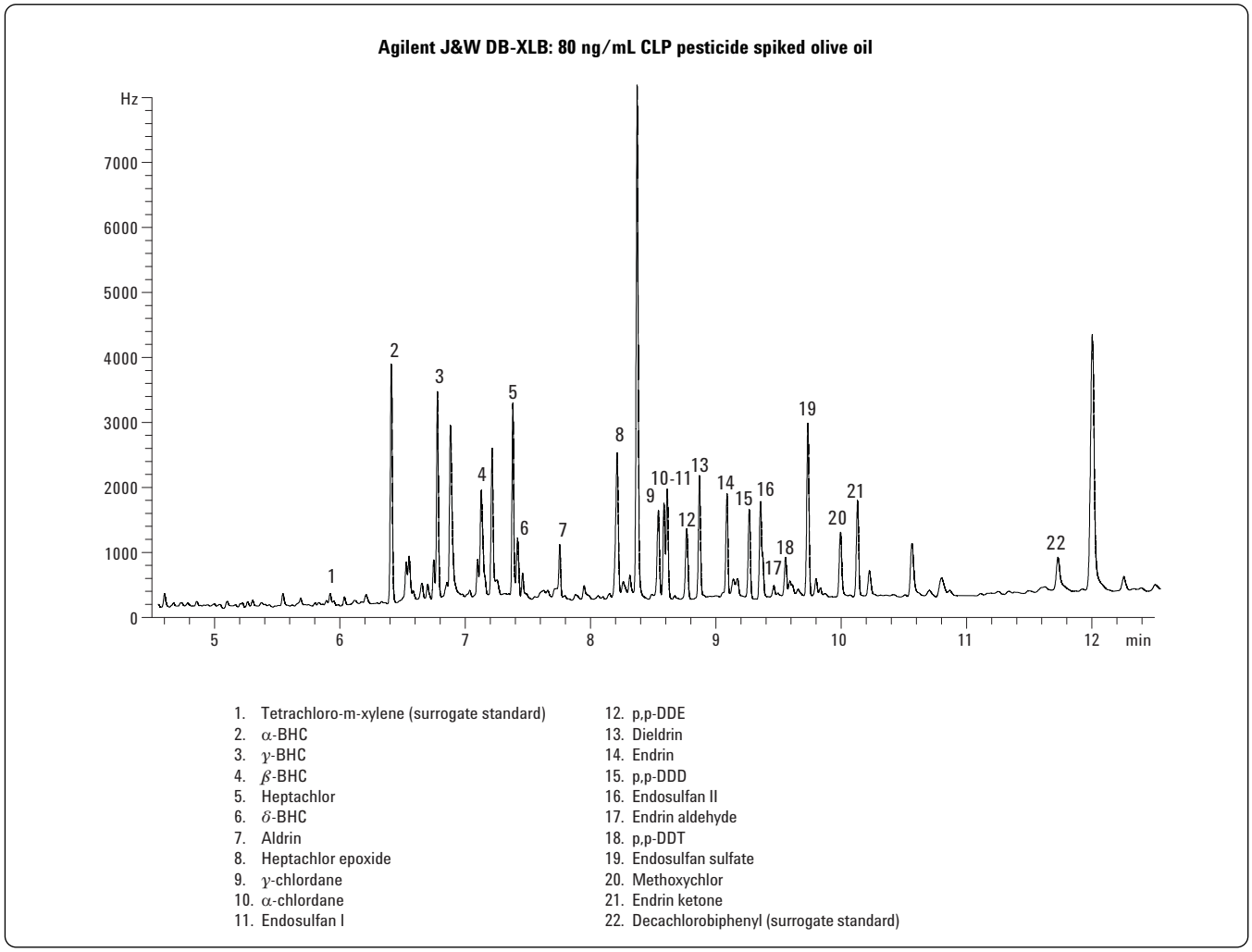

Figure 6. GC-µECD chromatogram of olive oil sample spiked with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-XLB 30 m × 0.25 mm × 0.25 µm column.

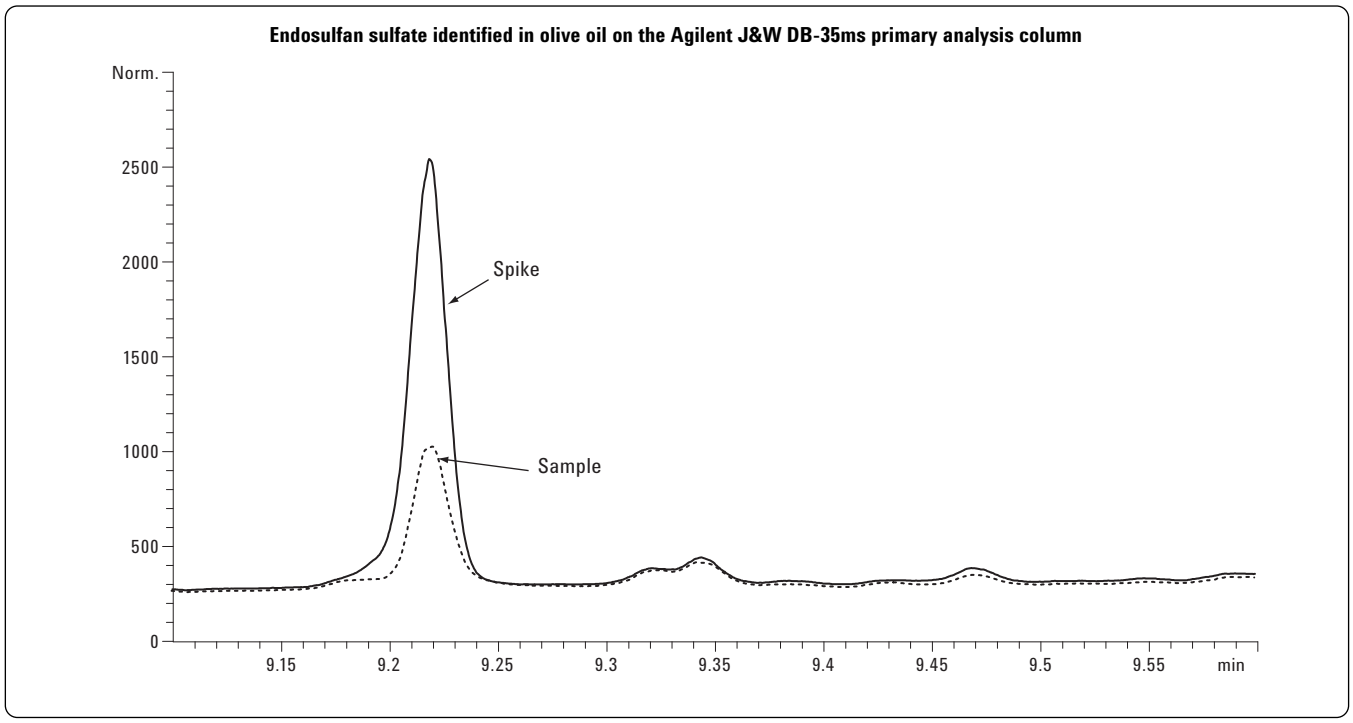

Figure 7. GC-µECD chromatogram of olive oil sample overlaid with olive oil sample spike with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-35ms, 30 m × 0.25 mm × 0.25 µm column. This overlay offers more evidence of the presence of endosulfan sulfates on the Agilent J&W DB-35ms column.

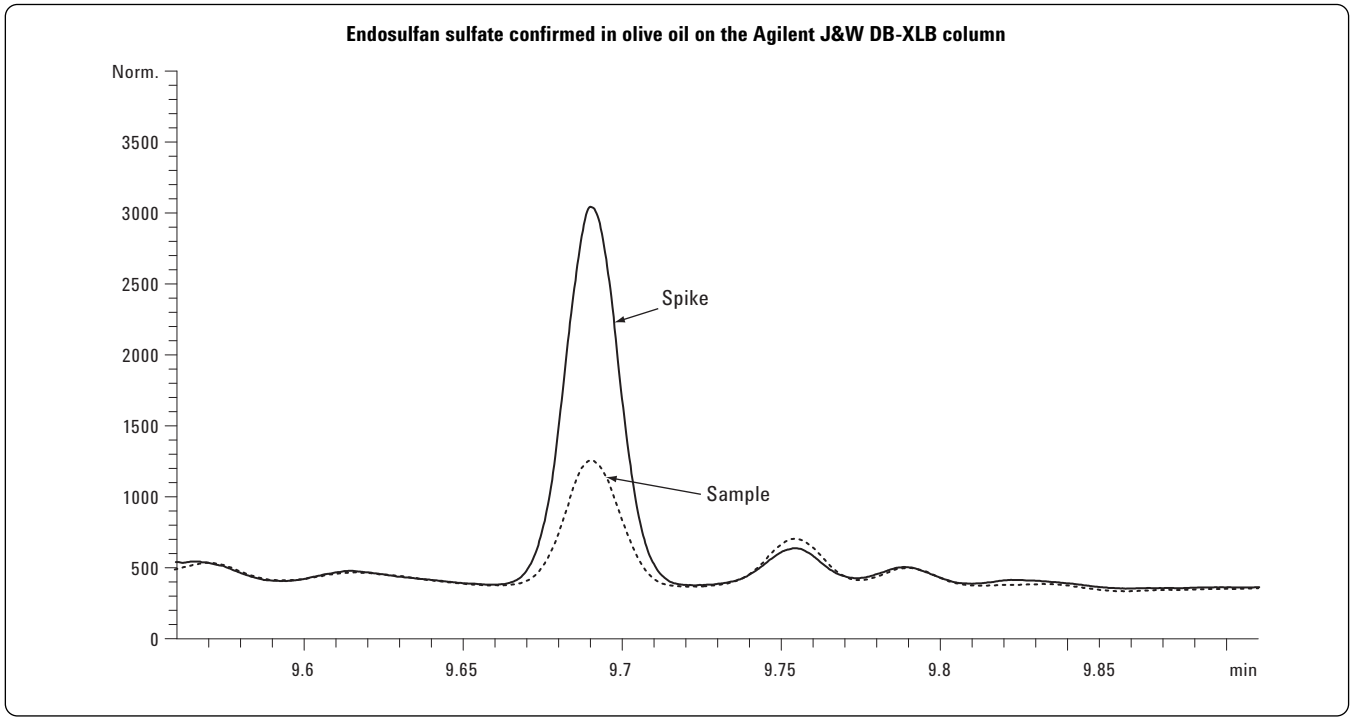

Figure 8. GC-µECD chromatogram of olive oil sample overlaid with olive oil sample spike with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-XLB, 30 m × 0.25 mm × 0.50 µm column. This overlay offers more evidence of endosulfan sulfates presence on the Agilent J&W DB-XLB column.

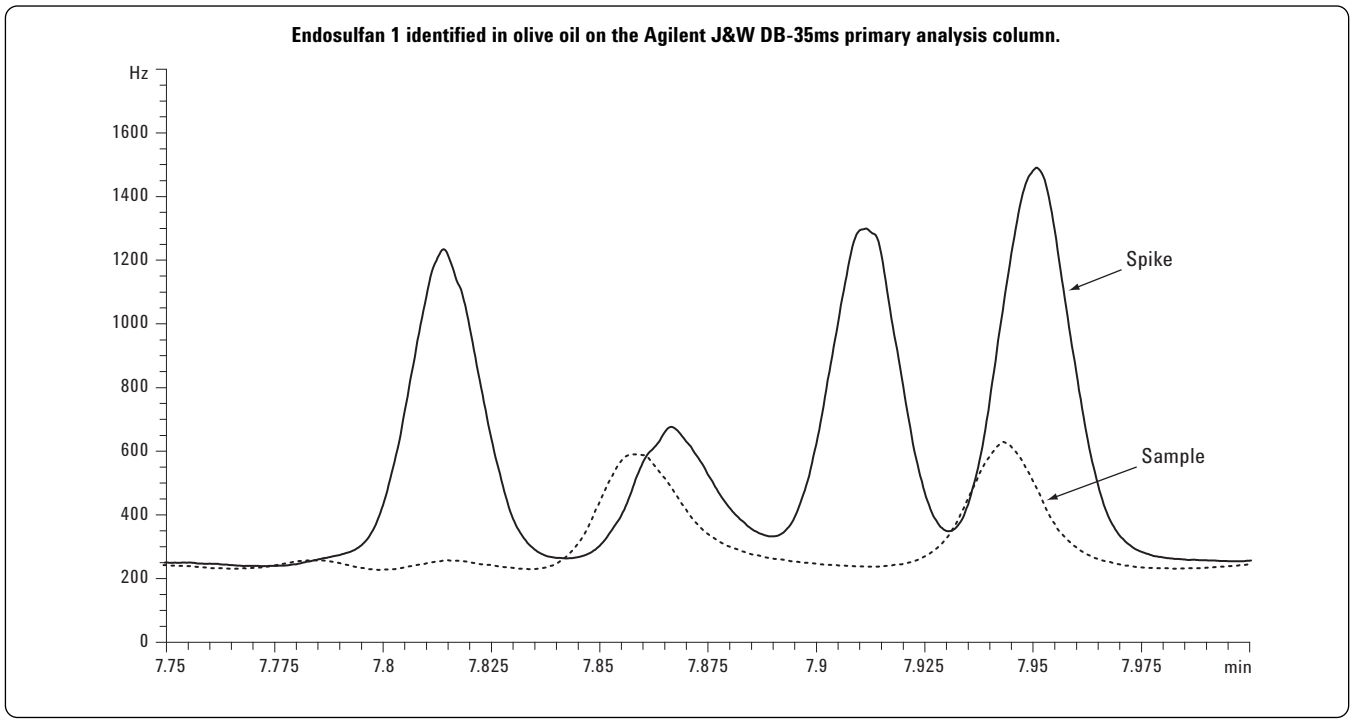

Figure 9. GC-µECD chromatogram of olive oil sample overlaid with olive oil sample spike with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-35ms, 30 m × 0.25 mm × 0.25 µm column. This overlay offers more evidence of endosulfan 1 presence on the Agilent J&W DB-35ms column.

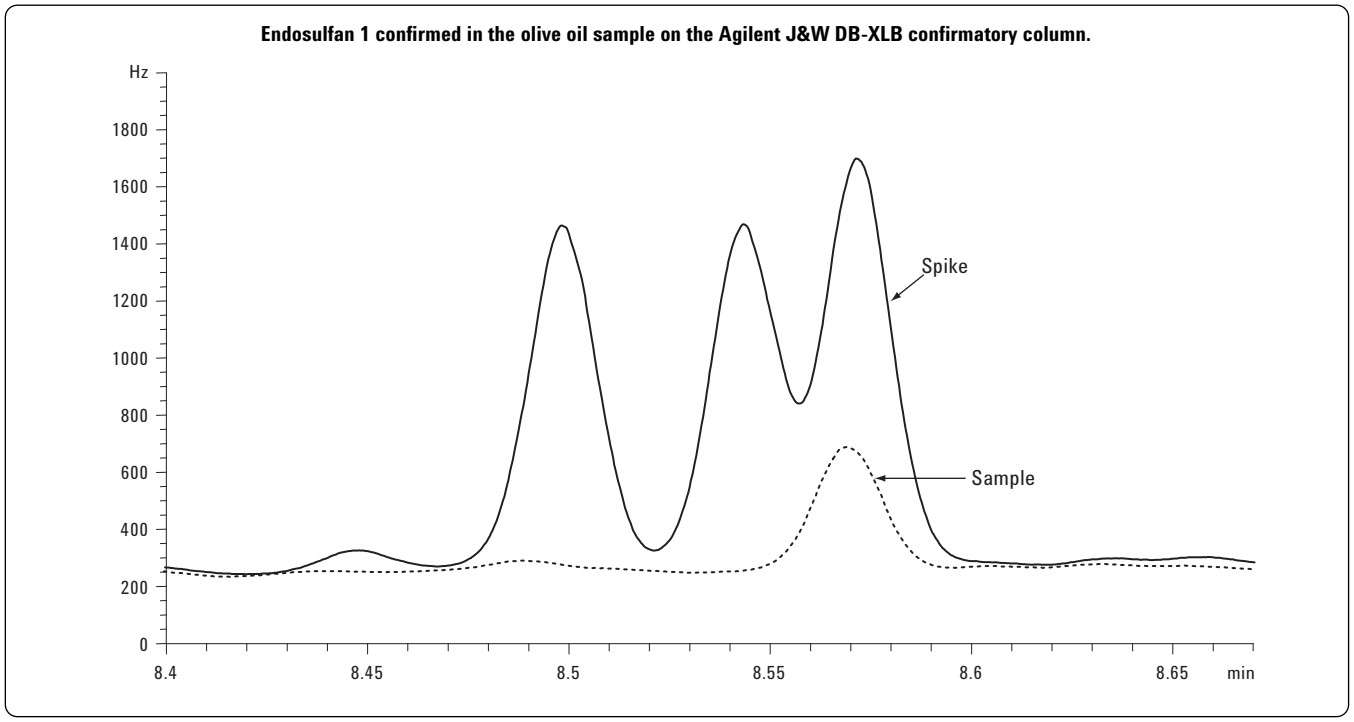

Figure 10. GC-µECD chromatogram of olive oil sample overlaid with olive oil sample spike with 80 ng/mL of CLP pesticides and 20 ng/mL surrogates standard analyzed on an Agilent J&W DB-XLB, 30 m × 0.25 mm × 0.50 µm column. This overlay offers more evidence of endosulfan 1 presence on the Agilent J&W DB-XLB column.

Conclusions

The dual column set of an Agilent J&W DB-35ms primary analytical column and an Agilent J&W DB-XLB confirmatory column on one instrument allows simultaneous identification and confirmation of the presence of the CLP pesticides. The DB-35ms primary analysis and a DB-XLB confirmatory column with dual µ-ECD detection were effective at analyzing 20 CLP pesticides in an olive oil matrix following sample matrix cleanup. The single injection, dual column approach improves productivity by saving instrument and analyst time. Continuous improvements and stringent process control with respect to column activity make the DB-35ms and DB-XLB column pair an excellent choice for analysis of active analytes such as pesticides.

This note successfully shows a robust, inexpensive analytical method to monitor CLP pesticides in olive oil suitable to address food safety concerns. This method demonstrates the feasibility of using a dual column µECD approach for routine olive oil screening as an alternative to GC/MS.

QuEChERS followed by dSPE were effective at providing just enough sample cleanup to avoid matrix interferences while still maintaining low-level analyte detection. The performance of the dual column set DB-35ms and DB-XLB on the GC-µECD had excellent linearity over the range of concentrations studied with R^2 values between 0.994 and 0.999 for the individual pesticides. The limits of detection (LOD) quantitative (LOQ) for this analysis were 0.3 ng/mL and 1.0 ng/mL, demonstrating low level detection capability. QuEChERS followed by dSPE also provided a fast and effective way to prepare the sample.

The primary analysis DB-35ms column identified endosulfan sulfate and endosulfan 1 in the olive oil sample. The DB-XLB confirmatory column confirmed that endosulfan sulfate and endosulfan 1 were present in the olive oil sample. Overlaid chromatograms of pesticide spiked and native olive oil samples on both columns provided additional evidence confirming the presence of these two pesticides through a process of pattern recognition. The evidence proves that these two pesticides were present in this grocery store olive oil.

The fact that a single randomly selected commercial olive oil sample came back positive for two pesticide residues indicates residues are present in these oils. Clearly, there is a need to monitor olive oil for pesticide residues through tests such as the dual column dual GC-µECD analysis described in this note.

References

1. E. Amvrazi and T. Albanis, "Pesticides Residue Assessment in Different Types of Olive Oil and Preliminary Exposure Assessment of Greek Consumers to the Pesticides Residues Detected," Food Chem 2009, 113, 253-261.
2. E. Amvrazi and T. Albanis, "Multiresidue Method for Determination of 35 Pesticides in Virgin Olive Oil by Using Liquid-Liquid Extraction Techiques Coupled with Solid-Phase Extraction Clean Up and GC with NPD and ECD" *J. Agric. Food chem*. 2006, 54, 9642.
3. J. Garcia-Reyes, C. Ferrer, et al., "Determination of Pesticide Residues in Olive Oil and Olives," Trends in Analytical Chemistry, 2007, 26, 239-251.
4. S. Cunha, S. Lehotay, K. Mastovska, et al., "Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olive," *J. Sep. Sci* 2007, 30, 620-632.
5. D. Smith and K. Lynam, "Rapid Contract Laboratory Program (CLP) Pesticides Analysis with 0.32 mm ID Capillary GC Columns Utilizing Hydrogen Carrier Gas," Agilent Technologies publication 5990-4351EN, August 12, 2009.
6. C. George, "A Complete Solution for Chlorinated Pesticides and Herbicides Using DB-35ms and DB-XLB Columns," Agilent Technologies publication 5988-4971EN, December 17, 2001.

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