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Original Paper

Evaluation of the QuEChERS sample preparation approach for the analysis of pesticide residues in olives

This paper describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for extraction and cleanup of 16 pesticide residues of interest in olives and olive oil. These products contain a high lipid content, which can adversely affect pesticide recoveries and harm traditional chromatographic systems. For extraction, the main factors (oil and water content) were studied and optimized in experiments to maximize pesticide recoveries. Dispersive SPE with different sorbents was also investigated to minimize matrix coextractives and interferences. For analysis, a new automated DSI device was tested in GC-MS to avoid nonvolatile coextractives from contaminating the instrument. LC-MS/MS with positive ESI was used for those pesticides that were difficult to detect by GC-MS. The final method was validated for olives in terms of recoveries, repeatabilities, and reproducibilities using both detection techniques. The results demonstrated that the method achieved acceptable quantitative recoveries of 70–109% with RSDs <20% for DSI-GC-MS and 88–130% with RSDs <10% for LC-MS/MS, and LOQ at or below the regulatory maximum residue limits for the pesticides were achieved.

Keywords: Direct sample introduction / Gas chromatography-mass spectrometry / Liquid chromatography-tandem mass spectrometry / Multiresidue pesticide analysis / Olive oil

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1 Introduction

The olive tree, long employed as a symbol of wisdom and peace, is the oldest known cultivated tree in history [1]. This tree (*Olea europea*) still has a great importance in the socio-economy of the Mediterranean countries. Virgin olive oil obtained from the olive fruits is a product of high quality that gives producers a higher price compared to other cooking oils. The taste and cooking quality have always made olive oil inherently marketable, and in the past few decades, knowledge gained about nutritional health benefits has increased the demand considerably. Studies have shown that virgin olive oil with its high content of monounsaturated fatty acids and antioxidative substances is beneficial to human health [2–3].

The control of diseases and pests in olive trees is one critical factor that increases the number and/or size of olives, and the subsequent yields. The proper use of pesticides also tends to improve the quality of the resulting olive oil. Currently, it is conventional practice for olive growers to apply pesticides to their trees, and thus it is necessary to monitor and control residual levels in olives and olive oils in order to meet regulatory requirements and protect the consumer and the environment. The main purpose for conducting this study was to develop an analytical method to be used in field trials and the determination of harvest intervals for olives after pesticides have been applied.

However, the method should also be applicable in regulatory monitoring programs for table olives and raw olives for olive oil production. Several countries, the European Union (EU), and Codex Alimentarius Commission of the Food and Agriculture Organization (FAO) have established maximum residue limits (MRLs) (called regulatory tolerances in the US) in olives for a large number of pesticides. A partial list can be searched on the internet (http://mrldatabase.com/query.cfm) and appear in Table 1 for several governments. The complete and updated list of >200 MRLs in the EU for pesticide residues in olives also appears on the internet (http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm). It was outside the scope of this study to include all of those ana-

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Table 1. A partial listing of the MRLs of pesticides in olives (http://mrldatabase.com/query.cfm, accessed May 2006). The 1 × Spk refers to the equivalent concentration of the spiking level for the pesticides in experiments

Pesticide	MRL (μg/g)						1 × Spk-GC (μg/g)	1 × Spk-LC (μg/g)
	USA	Codex	EU	Italy	Portugal	Spain		
Omethoate	–	–	–	–	–	–	0.01	0.05
Dimethoate	–	0.5	2	–	2	–	0.01	0.05
Simazine	0.25	–	–	0.1	0.1	0.1	0.10	0.10
Diazinon	1	–	0.02	0.5	–	–	0.02	na
p,*p*′-DDE	–	–	–	–	–	–	0.01	na
Diuron	1	–	–	0.05	0.05	0.2	0.05	0.05
Carbaryl	10	30	5	–	1	1	0.20	0.20
Malathion	–	–	0.5	–	0.5	–	0.10	0.10
Fenthion	–	1	–	–	1	1	0.10	na
Methidathion	0.05	1	1	1	1	1	0.05	0.25
Napropamide	0.1	–	–	–	–	0.05	0.05	na
Oxyfluorfen	0.05	–	–	–	0.01	0.05	0.01	na
Carfentrazone-ethyl	0.1	–	–	–	–	0.05	0.05	na
Phosmet	–	–	–	–	2	–	0.10	0.10
Pyriproxyfen	1	–	–	–	–	0.05	0.05	na
Deltamethrin	–	1	0.1	–	0.1	–	0.10	na

– (not mentioned); na = not added.

lytes in the method, and we simply chose the 16 appearing in Table 1 for our purposes.

1.1 Multiresidue analysis of olives

The development and validation of sensitive and reliable analytical methods for detecting pesticide residues in olives and olive oil are essential for a variety of needs (*e.g.*, monitoring and enforcement, field trials, and the registration of additional pesticides to be used legally on olives). Moreover, it is important for monitoring laboratories to be able to detect other pesticides than just those that have been registered in their country. Olive farmers (organic or conventional) and olive oil producers interested in potential contamination of their products (*e.g.*, spray drift, surface exposures) would also need to screen for a wide range of pesticides.

Several methods have been developed in the past decade to determine multiple pesticide residues in olive oil, but only few of these studies reported results in olives. The majority of the methods are based on HPLC or GC analysis [4]. The sample preparation procedures (extraction and cleanup) for olives and olive oils implicitly need to remove the lipid material from the extracts, which can harm analytical systems or cause signal suppression. Different procedures combining the extraction and cleanup in one step have been reported for these matrixes such as matrix solid-phase dispersion (MSPD) [5], solid-phase microextraction (SPME) [6], or supercritical fluid extraction (SFE) [7]. However, the extraction is frequently done with organic solvents such as petroleum ether saturated with MeCN [5], MeCN saturated with *n*-hexane [8 – 12], or *n*-hexane saturated with MeCN [13, 14] followed by cleanup of the extracts. The most common approach to cleanup involves liquid – liquid partitioning [4], which typically uses large volumes of potentially hazardous solvents and time-consuming manual labor. Alternatively, SPE employing sorbents such as octadecylsilane (C_{18}), Florisil, alumina, and silica gel have been used [15 – 17]. Gel permeation chromatography (GPC), in which molecules are separated according to size, is often applied for cleanup of fatty extracts [12, 18, 19]. This procedure offers a high degree of automation, but it is a rather slow and expensive sequential sample cleanup technique that uses large amounts of potentially hazardous organic solvents.

Recently, Anastassiades *et al.* [20] developed an approach that they dubbed quick, easy, cheap, effective, rugged, and safe (QuEChERS), which involves extraction with MeCN partitioned from the aqueous matrix using anhydrous $MgSO_4$ and NaCl followed by a dispersive-SPE cleanup with $MgSO_4$ and primary secondary amine (PSA). This procedure has been applied with success in several nonfatty (< 2%) and low-fat (2 – 20%) food matrixes, such as milk, egg, and avocado [21].

1.2 Sample injection and analysis

The QuEChERS method (≈ 1 g/mL final extract equivalent conc.) commonly uses GC-MS and LC-MS/MS to cover the wide range of pesticides for analysis. Typical residue monitoring applications require < 10 ng/g LOQ. LC-MS/MS can often achieve ≈5 pg injected LOQ for many pesticides, which affords small injection volumes of MeCN extracts

(*e.g.*, 5 μL) or dilution of the final extracts into the initial mobile phase solvent. GC-MS in SIM mode (or MS/MS) is commonly needed to attain ≈1 pg injected LOQ, and because no solvent evaporation step is conducted prior to the analysis, large-volume injection (LVI) of 10 μL with a programmable temperature vaporizer (PTV) injector is usually employed. Alternately, a solvent exchange and concentration step to toluene can be done to allow traditional split/splitless injection in a hot inlet [22, 23].

In LVI, one drawback is the potential introduction of a greater amount of coextracted matrix material that can contaminate the inlet, column, and MS ion source. Direct sample introduction (DSI) is a novel technique that shows promise to allow LVI, which can lower LOQ while avoiding the transfer of coextracted nonvolatile matrix constituents into the column [24–27]. In DSI, the extract is added to a disposable microvial that has been placed inside an injection liner, which is replaced in the inlet after every injection. The unvolatilized matrix contaminants are removed along with the microvial, and the system remains clean, with reduced need for instrument maintenance. This may also permit injection of dirtier extracts and reduced cleanup needs *versus* traditional GC injection. Due to the activity of the glass surfaces in the liner, microvial, and elsewhere in the system, analyte protectants are especially useful in DSI to improve analyte peak shapes and intensities, as reported previously [20, 27–29].

Initially, DSI was commercially available in a manual format called the ChromatoProbe, which is linked to the Varian 1079 injector [25]. Since then, Atas/GL Science developed an automated DSI device compatible with their Optic PTV, and marketed it as difficult matrix injection (DMI) [26, 27]. Recently, the company has revised their automated approach with a second generation product. Gerstel is also adapting their thermal desorption unit (TDU)/Twister device to also permit automated DSI (Pfannkoch, E. A., Whitecavage, J. A., Stuff, J. R., Gerstel application note 4/2006 (www. gerstel. com)).

The main objective of this current project was to evaluate and possibly adapt the QuEChERS method for application to olives, which contain up to 30% fat, and olive oil (100% lipids) for multiple pesticides residues at and below MRLs established by different countries. An additional goal was to employ and test a new automated DSI device in the GC-MS analysis designed to ease the burden on cleanup during sample preparation, lower LOQ, and extend GC column life.

2 Experimental

2.1 Reagents and solutions

Pesticide analytical standards, all 95% or higher purity, were obtained from National Pesticide Standard Repository of the US Environmental Protection Agency (Fort Meade, MD, USA), Dr. Ehrenstorfer (Augsburg, Germany) and Chemservice (West Chester, PA, USA). Purity-corrected individual pesticide stock solutions (1000–3000 μg/mL) were prepared in MeCN or toluene. Appropriate aliquots of the individual stock solution of each were diluted with MeCN to prepare a mixed stock solution (and 25 × spike solution) for GC-MS experiments (in the ratios of the 1 × Spk-GC column shown in Table 1) containing: 400 μg/mL carbaryl; 200 μg/mL deltamethrin, fenthion, malathion, phosmet, and simazine; 100 μg/mL carfentrazone-ethyl, diuron, methidathion, napropamide, and pyriproxyfen; 40 μg/mL diazinon; and 20 μg/mL *p*,*p*′-dichlorodiphenyldichloroethylene (DDE), dimethoate, omethoate, and oxyfluorfen. For LC-MS/MS experiments, a similar stock solution was prepared in the ratios of the 1 × Spk-LC column (Table 1), which contained: 500 μg/mL methidathion, 400 μg/mL carbaryl; 200 μg/mL malathion, phosmet, and simazine; and 100 μg/mL dimethoate, diuron, and omethoate. Additional spiking solutions of 0.5 ×, 1 ×, and 5 × relative concentrations (see Table 1) for GC-MS and LC-MS/MS were diluted appropriately from these solutions with MeCN for use in experiments. All the solutions were stored at −18°C when not in use.

MeCN and toluene were high purity grade solvents for pesticide residue analysis from Burdick & Jackson (Muskegon, MI, USA) and Sigma (St. Louis, MO, USA), respectively. Spectroscopy grade formic acid (FA) was obtained from Fluka (Neu-Ulm, Germany), ultrapure water came from a Barnstead (Dubuque, IA, USA) water purification system, and anhydrous $MgSO_4$ and NaCl were obtained from United Chemical Technologies (UCT) (Bristol, PA, USA) and Mallinckrodt (Paris, KY, USA), respectively. Dispersive-SPE sorbents for method development experiments included PSA obtained from Varian (Harbor City, CA, USA), C_{18} from J. T. Baker (Phillipsburg, NJ, USA), and graphitized carbon black (GCB) from Supelco (Bellefonte, PA, USA). The GCB was washed with MeCN and dried at 150°C before usage to remove contaminants adsorbed from the air. For method validation experiments, prepackaged PSA/C_{18}/GCB tubes from UCT were used. Ultrahigh purity He for GC-MS and N_2 (liquid nitrogen headspace) for LC-MS/MS and solvent evaporation were obtained from Air Products (Allentown, PA, USA). Analyte protectants were 3-ethoxy-1,2-propanediol, D-sorbitol, L-gulonic acid γ-lactone with 95% or better purity obtained from Sigma and Fluka. A composite stock solution of analytical protectants (10 : 1 : 1 mg/mL of 3-ethoxy-1,2-propanediol, D-sorbitol, L-gulonic acid γ-lactone) was prepared in 7 : 3 water/MeCN as described by Cajka *et al.* [27]. A quality check standard solution of 16 μg/mL triphenylphosphate (TPP) was prepared in MeCN containing 1.6% FA.

2.2 Sample preparation

For olives, extracts were prepared similarly to the original QuEChERS method [20], which entailed the following steps: (i) weigh 10 g of thoroughly homogenized sample into a 50 mL fluoroethylenepropylene (FEP) centrifugation tube; (ii) add 10 mL MeCN using a dispenser; (iii) add 4 g anh. $MgSO_4$ and 1 g NaCl; (iv) shake vigorously for 1 min by hand; and (v) centrifuge the tube at 3450 rcf (relative centrifugal force) for 1 min. Then a dispersive-SPE cleanup was done: (vi) transfer 1 mL of extract to a minicentrifuge tube containing 150 mg anh. $MgSO_4$ + 50 mg PSA + 50 C_{18} + 50 mg GCB; (vii) mix the extract with the sorbent/dessicant for 20 s; (viii) centrifuge the tube at 3450 rcf for 1 min; (ix) transfer 400 μL of extract into an autosampler vial and add 25 μL of TPP solution. This extract was ready for LC-MS-MS analysis. For DSI-GC-MS, 20 μL of the analyte protectant solution was added to all the final extracts and matrix-matched calibration standards (from step ix above). To ensure proper injection volumes with the autosamplers, we transferred the final extracts to low-volume glass inserts added to the autosampler vials (we would use the same vial that contained the extract).

The determination of recovery and repeatability was done using spiked samples that were prepared by adding 125 μL of mixed standard spiking solutions of the pesticides (0.5×, 1×, 5×, and/or 25× in Table 1) to the samples in the tubes. At least three spiking levels of six replicates were chosen. For matrix-matched calibration standards, 5–25 μL of the same spiking solutions used in the experiments were added to the 400 μL aliquots of olive blank final extracts to yield the desired concentrations. Quantitation involved use of the least linear squared calibration plot of the integrated analyte peak areas for the matrix-matched calibration standards to determine spiked and incurred pesticide concentrations in the samples.

For olive oil, a 3 g sample plus 7 g of water was added to the FEP tube and treated as described above for olives. In gravimetric experiments to determine cleanup efficiencies, a Sartorius R160P (Westbury, NY, USA) balance was used. In these experiments, the dispersive-SPE cleanup step was scaled up ten-fold, which entailed mixing 10 mL extract with 1.5 g anh. $MgSO_4$ + 0.5 g of each sorbent tested in a 50 mL FEP tube. After centrifugation, 4 mL extract was transferred to preweighed test tubes and taken to dryness using a Zymark Turbovap LV Evaporator (Hopkinton, MA, USA), and the weight difference was recorded to the 0.1 mg decimal unit.

2.3 Automated DSI-GC-MS analysis

GC-MS was performed using an Agilent (Little Falls, DE, USA) 5890 Series II GC and 5972 MS instrument. Injection was performed by a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland) with the second generation automated DSI accessory (Linex) in combination with an Optic 3 PTV (Atas-GL International BV, Veldhoven, NL). Conditions were similar to the method employed by Cajka *et al.* [27]. Briefly, the injection volume was 10 μL, and other conditions were: 100°C (held 3.5 min with 50:1 split ratio), ramped at 5°C/s to 280°C (splitless period for 3.5 min, then 50:1 split until 9 min, at which point the split flow was 20:1 and the injector temperature was cooled to 150°C). A series of macros was designed using CTC Analytics Cycle Composer to create the DSI method and control the mechanics.

The GC separation was conducted with a Varian VF-5 EZ-guard column (30 m × 0.25 mm id × 0.25 μm film thickness) with an integrated retention gap (5 m × 0.25 mm) at the inlet and an additional 1 m of uncoated capillary at the MS entrance (this avoids possible damage to the stationary phase at the heated transfer line when the inlet is opened to the atmosphere). Helium was used as the carrier gas with a constant flow of 1 mL/min. The oven temperature program (started 3.5 min after sample introduction) was as follows: 80°C held for 3.5 min, ramped to 230°C at 10°C/min, and finally ramped to 300°C at 45°C/min and held for 10 min. The MS transfer line temperature was held at 290°C.

Electron ionization (EI) was used at −70 eV in SIM and full-scan (50–600 *m/z*) modes in different experiments. Agilent Chemstation was used for data acquisition/processing and GC-MS control, and Cycle Composer and Atas Evolution software were used to control the automated DSI process and PTV, respectively. The pesticide analytes in GC-MS consisted of dimethoate, simazine, carbaryl, diazinon, malathion, fenthion, methidathion, napropamide, *p,p′*-DDE, oxyfluorfen, carfentrazone-ethyl, phosmet, pyriproxyfen, and deltamethrin. Table 2 provides the SIM program used for the analysis.

2.4 LC-MS/MS analysis

The method was developed using an Agilent 1100 HPLC (consisting of vacuum degasser, autosampler Model WPALS, and a binary pump) equipped with a Prodigy ODS-3 (150 mm × 3 mm and 5 μm particle size) analytical column coupled to a ODS–C18 (4 mm × 2 mm and 5 μm particle size) guard column, both obtained from Phenomenex (Torrance, CA, USA). Column temperature was maintained at 30°C and injection volume was 5 μL. Mobile phase A was water and B was MeCN, both with 0.1% FA. A gradient program was made as follows: 25% solvent B linear gradient to 100% over the course of the first 5 min, and held for 7 min until 12 min total runtime with constant flow rate of 0.3 mL/min. An 11-min postrun column wash was used after each analysis in order to avoid carry-over. This LC system was connected

Table 2. GC-MS SIM conditions for the monitored pesticides

Pesticide	Start time (min)	t_R (min)	*m/z* (% relative abundance)	
			Quantitation ion	Qualifier ions
Dimethoate	4.5	15.89	87 (100)	125 (45), 93 (54), 58 (19)
Simazine		16.00	201 (78)	173 (41), 186 (51), 158 (25)
Diazinon	16.09	16.18	179 (100)	137 (98), 304 (47), 152 (70)
Diuron		16.52	72 (100)	232 (38), 234 (26), 187 (11)
Carbaryl	17.49	17.70	144 (100)	115 (33), 116 (26), 145 (15)
Malathion		18.03	173 (94)	125 (100), 93 (93),127 (75)
Fenthion	18.1	18.27	278 (100)	125 (37), 109 (33), 79 (19)
Methidathion	19.05	19.29	145 (88)	93 (40), 125 (27), 302 (19)
Napropamide	19.39	19.58	271 (26)	72 (100), 128 (63)
p,*p*′-DDE		19.67	318 (64)	246 (100), 248 (64), 316 (56)
Oxyfluorfen		19.71	361 (38)	252 (100), 300 (35), 280 (14)
Carfentrazone-ethyl	20	20.28	312 (100)	330 (65), 340 (63), 376 (31)
TPP	20.38	20.96	326 (100)	325 (87). 77 (88). 215 (20)
Phosmet		21.17	160 (100)	133 (15), 104 (15), 193 (4)
Pyriproxyfen	21.30	21.50	136 (100)	226 (12), 185 (6)
Deltamethrin	22.8	23.59	253 (85)	181 (100), 251 (44), 152 (20)

Table 3. LC-MS/MS conditions for the monitored pesticides (the quantitiation ion is given in bold text)

Pesticide	Start time (min)	t_R (min)	Precursor ion (*m/z*)	Product ions (*m/z*)
Omethoate	2.5	2.68	214.0	**183.2**, 125.2
Dimethoate	5	6.83	230.0	**199.1**, 125.1
Simazine	7.6	7.98	202.0	**124.2**, 132.2
Carbaryl		8.48	202.2	**145.1**, 127.1
Diuron		8.67	233.1	**72.2**, 160.1
Phosmet	9	9.27	318.0	**160.2**, 133.2
Methidathion		9.28	303.0	**145.1**, 85.1
Malathion		9.64	331.0	**127.2**, 285.2
TPP	9.8	10.18	327.0	**77.2**, 152.0

to an API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Toronto, Canada). The quadrupoles were operated in the ESI positive mode. Optimizations of the mass analyzer parameters were done by infusion of 1 μg/mL analyte solutions at 10 μL/min with a syringe pump (Harvard Apparatus, Holliston, MA, USA) using the autotune function. The N_2 supply pressure for the instrument was 55 psi, and the final MS/MS conditions included: nebulizer gas setting of 14, curtain gas setting of 11, collision gas setting of 12, 4200 V ionspray voltage, 525°C ESI temperature, 100 V focusing potential, 10 V entrance potential, and 0.15 s dwell time. Omethoate, dimethoate, simazine, carbaryl, diuron, phosmet, methidathion, and malathion were analyzed by LC-MS/MS at the conditions shown in Table 3. The most intensive product ion shown in bold in the table from each precursor ion was chosen for quantitation. For identification, the ion ratios for the reference standards and samples had to match in the first and second ion transitions at the proper retention times.

3 Results and discussion

3.1 Extraction and cleanup

Ideally, the extraction step separates all of the analytes from the matrix without any matrix coextractives. Of course, this is very difficult if not impossible in the case of pesticide residue analysis in most of the matrices. Olives have many matrix components that have similar properties as the pesticides of interest, thus traditional solvent extractions are not going to separate these matrix chemicals from the analytes. Removal of matrix interferants becomes especially problematic as the range of physicochemical properties of the analytes broadens, which is the case in this multiclass, multiresidue application using GC and LC analyses.

The United States Department of Agriculture (USDA) Food Composition database gives some information about the composition of canned olives (www.nal.usda.gov/fnic/foodcomp/search/), but this is incomplete and does not list the types of green olives we used for the analysis. Another reference reported table olives to have 60–75% moisture and 10–25% lipids [30]. We used green olives from the Cobrançosa cultivar, which are suitable for olive oil production, not table olives. According to the literature, water content was 46–63% and oil content was 21–31% for the sampled type of olives, depending on the time of harvest [31]. The analyzed olives contained numerous interferants in GC/MS (SIM), which varied from sampling lot to lot. Interestingly, the oil was not too problematic in the extraction, and olive oil with 100% lipids gave few interferences. It is well known that lipids cause serious problems in both GC and LC, but we hypothesized that the DSI approach would reduce or eliminate the transfer of the most nonvolatile lipids to the column in the case of GC, and in LC, a guard column

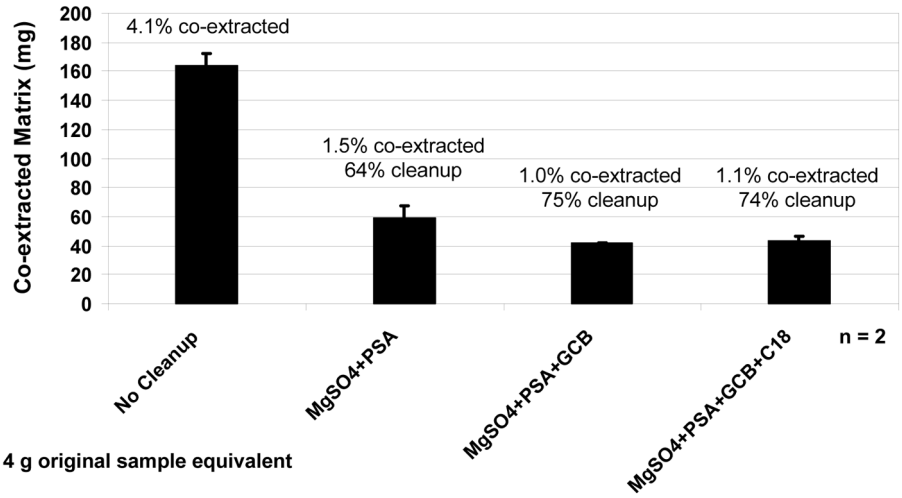

Figure 1. Effectiveness of cleanup of olive extracts using different sorbents in the QuEChERS method.

was used to protect the analytical column from irreversibly retained coextractives in the C_{18}/MeCN system.

Thus, we sought to minimize or eliminate coextraction of lipids, and we chose to use MeCN as the extraction solvent because very little fat partitions into MeCN, and it is compatible with LC [20 – 23]. In the case of the QuEChERS method, the combination of MeCN with anhydrous $MgSO_4$ and NaCl has already been shown to provide high recoveries for many pesticides from different classes [32]. A buffered version of the approach [33] is pending a final approval as an Official Method of AOAC International [23]. Another modified QuEChERS version has also met interlaboratory validation acceptability criteria for regulatory applications in Germany (see www.quechers.com).

Although lipids are not very soluble in MeCN, a small amount of fat is coextracted, so further cleanup is still desirable. Previously, several sorbents (*e.g.*, PSA, C_{18}, GCB) and anh. $MgSO_4$ were evaluated in dispersive-SPE for cleanup of low fatty foods (2 – 20% lipids), and the combination of PSA, C_{18} and anh. $MgSO_4$ was selected in those cases [21]. In this study, PSA, C_{18}, GCB, and $MgSO_4$ were reinvestigated in dispersive-SPE for cleanup of the olive extracts, which have higher lipid content. An interesting facet in the extraction of olive and its oil with MeCN is that the oil layer forms in the centrifuge tube between the aqueous layer at the bottom and MeCN extract on the top. In the cases of avocados, milkfat, soybeans, and eggs, the lipids form an oily film on top of the MeCN due to their lower density. The density of olive oil is reported to be 0.8 – 0.92 g/mL whereas MeCN has a density of 0.782 g/mL at 20°C (www.simetric.co.uk/si_liquids.htm). This difference in the density certainly makes the MeCN extract more accessible and provides greater convenience than if the oil floated.

The effectiveness of QuEChERS extraction and dispersive-SPE in the ability to separate or remove olive matrix components from the extracts is shown in Figs. 1 – 3. For the green olives used in the experiments (olives with different composition yield somewhat different values), 4.1% of the material was coextracted by the QuEChERS method. The dispersive-SPE cleanup using 50 mg PSA sorbent and 150 mg anh. $MgSO_{4\ per}$ mL extract was found to remove 64% of coextractives by weight *versus* no cleanup. When 50 mg GCB was added *per* mL extract for cleanup, the cleanup efficiency increased to 75% by weight. The addition of GCB also worked well from the standpoint of visual appearance as can be surmised from Fig. 2. Moreover, Fig. 3 shows the improvement in the GC-MS full-scan chromatograms of olive extracts that were additionally exposed to GCB.

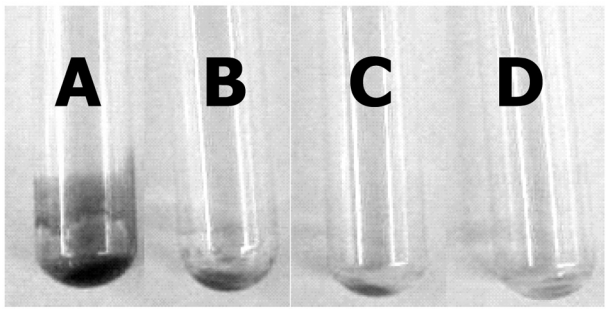

Figure 2. Appearance of evaporated olive extracts (4 g equivalent sample) after dispersive-SPE cleanup using different sorbents: (A) no cleanup; (B) $MgSO_4$ + PSA; (C) $MgSO_4$ + PSA + GCB; (D) $MgSO_4$ + PSA + GCB + C_{18}. This visually depicts the coextracted matrix components remaining in the liner after GC injection.

The effectiveness of GCB for cleanup is well known for pesticide residue analysis, but normally it cannot be used in multiclass, multiresidue applications because GCB strongly retains pesticides with planar ring structures, such as hexachlorobenzene, thiabendazole, and chlorothalonil. However, the chosen list of pesticides for

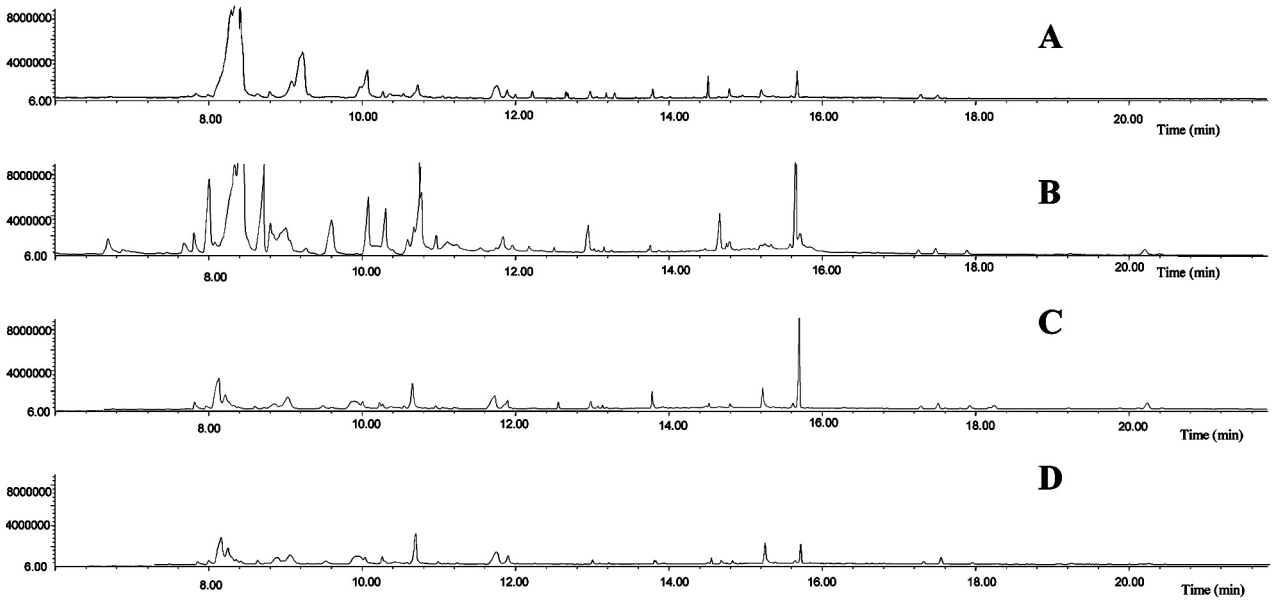

Figure 3. GC-MS full-scan chromatograms (same scale) of QuEChERS olive extracts using different cleanup procedures: (A) $MgSO_4$; (B) $MgSO_4$ + PSA; (C) $MgSO_4$ + PSA + GCB; (D) $MgSO_4$ + PSA + GCB + C_{18}.

olives (Table 1) do not include these types of analytes, thus GCB could be employed to improve cleanup of the extracts for our purposes. Unfortunately, MRLs for olives in the EU exist for hexachlorobenzene, thiabendazole, chlorothalonil, and other planar pesticides (http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm), thus GCB could not be used for their analysis with this method in regulatory monitoring applications.

The additional use of C_{18} did not improve the cleanup efficiency by weight, but C_{18} also did not affect analyte recoveries, and it gave slightly better results in terms of appearance (Fig. 2) and fewer GC-MS interferences (Fig. 3). As the figures demonstrate, the inclusion of all three sorbents plus $MgSO_4$ gave the cleanest extracts. Thus, the final method entailed the use of all the tested sorbents in dispersive-SPE, but still many matrix coextractives remained which made analysis of several of the pesticides a challenge using GC-MS in SIM mode, especially the early-eluting analytes at the lowest concentrations.

3.2 Effect of oil content

Pesticides are known to partition in different ratios in fat *versus* MeCN, roughly correlated with their solubility in water and octanol/water partitioning coefficient ($K_{o/w}$) values [21]. The olives we analyzed contained 20–30% oil and 50–60% water [31] and this oil/water ratio in the sample was studied with respect to coextractives and pesticide recoveries. Olive oil was used to simplify the experiments and avoid olive interferences in GC-MS.

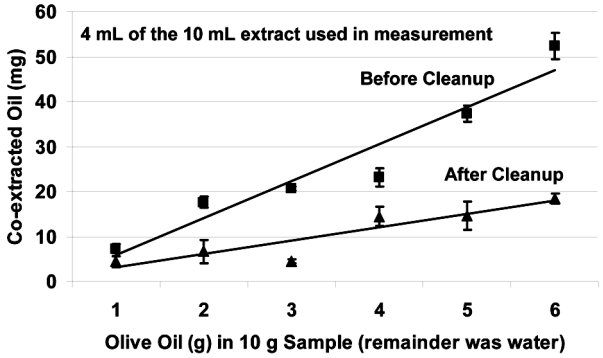

Figure 4. Olive oil coextracted using the QuEChERS method before and after dispersive-SPE cleanup with $MgSO_4$ + PSA + GCB + C_{18} for samples ($n = 2$) with different amounts of oil (plus water was added to give 10 g oil + water). See Section 2 for further details.

Spiked olive oil samples, to which water was added in the FEP tubes to yield 10–60% oil (samples consisted of two phases), were extracted using the QuEChERS method described above including cleanup with dispersive-SPE using PSA, C_{18}, GCB, and anh. $MgSO_4$. As shown in Fig. 4, the amount of coextracted oil in 10 mL MeCN increased proportionally as the oil content in the sample increased. We were expecting that a saturation point would be reached in which no further oil would be extracted in the fixed amount of MeCN, but this was not the case. The differing ratios of water in the sample may have contributed to the result, or densities of the fluids possibly changed more than expected to affect the measurements.

Table 4. Mean recoveries and RSDs obtained with the QuEChERS method for spiked samples (25 × Spk level) with different amounts of oil (plus water to make 10 g sample) and analyzed using GC-MS and LC-MS/MS ($n = 3$). Recoveries >120% or <70% are italicized

	%Recovery (%RSD)						Overall
	1 g	2 g	3 g	4 g	5 g	6 g	
Omethoate[b)]	96 (2)	95 (1)	100 (1)	99 (3)	102 (2)	112 (1)	101 (1)
Dimethoate[a)]	78 (15)	79 (4)	77 (3)	70 (5)	*66* (4)	*63* (8)	72 (12)
Dimethoate[b)]	102 (2)	99 (2)	100 (2)	103 (1)	105 (1)	112 (3)	103 (2)
Simazine[a)]	101 (7)	91 (3)	84 (6)	72 (2)	75 (1)	80 (4)	86 (12)
Simazine[b)]	*131* (1)	*122* (1)	119 (1)	120 (1)	119 (1)	*123* (3)	*122* (1)
Diazinon[a)]	*127* (4)	116 (3)	99 (10)	90 (1)	88 (11)	74 (6)	101(11)
Diuron[b)]	75 (3)	*67* (5)	*68* (9)	75 (1)	86 (3)	93 (1)	77 (4)
Carbaryl[b)]	105 (3)	99 (2)	96 (2)	97 (1)	91 (1)	96 (1)	97 (1)
Malathion[b)]	99 (1)	92 (4)	92 (1)	84 (1)	80 (1)	82 (1)	88 (1)
Fenthion[a)]	97 (5)	89 (4)	84 (9)	70 (2)	79 (1)	70 (5)	82 (15)
Methidathion[a)]	97 (9)	79 (4)	73 (5)	*62* (5)	*55* (3)	*61* (8)	74 (17)
Methidathion[b)]	110 (1)	105 (1)	101 (1)	97 (1)	95 (1)	95 (1)	100 (1)
Napropamide[a)]	78 (15)	74 (1)	75 (9)	*63* (1)	78 (8)	74 (3)	74 (11)
p,*p*′-DDE[a)]	*57* (5)	*41* (1)	*41* (9)	*29* (5)	*27* (5)	*26* (10)	*38* (13)
Oxyfluorfen[a)]	94 (10)	83 (1)	75 (9)	*62* (4)	73 (14)	79 (1)	78 (14)
Carfentrazone-ethyl[a)]	107 (3)	99 (2)	93 (8)	83 (1)	80 (10)	74 (6)	91 (13)
Phosmet[b)]	91 (2)	88 (1)	90 (4)	89 (2)	96 (1)	100 (4)	93 (2)
Pyriproxyfen[a)]	75 (16)	*64* (2)	*56* (10)	*41* (5)	*41* (18)	*33* (7)	*54* (17)
Deltamethrin[a)]	83 (10)	*56* (1)	*51* (10)	*45* (3)	*38* (6)	*45* (14)	*51* (17)

a) DSI-GC-MS.
b) LC-MS/MS.

Independent of the amount of oil in the original sample, 1.8% ± 0.3% before cleanup and 0.8% ± 0.2% after cleanup of the oil was coextracted in the 10 mL MeCN by the QuEChERS method.

Table 4 shows the combined recoveries using GC-MS and LC-MS/MS in the olive oil experiment. High (70–120%) and consistent (<15% RSD) recoveries were achieved in nearly all the cases. Most of the pesticides of interest in olives were polar enough that increasing oil content in the samples did not significantly affect recoveries in LC-MS/MS or even DSI-GC-MS. With a few exceptions, perhaps the analytes showed a 10% decrease in their recoveries *versus* increasing lipid content in DSI-GC-MS, but as anticipated, no effect was observed in LC-MS/MS. The few exceptions in GC-MS were *p*,*p*′-DDE, pyriproxyfen, and deltamethrin, which were the most nonpolar pesticides in the study. Actually, we were surprised that the recoveries were not even lower as fat content increased to 60% in the oil/water sample. Previous experiments with low fatty samples [21] gave steep losses in the recoveries of lipophilic pesticides as fat content increased from 0–10%, and then a leveling at 15% fat. The recoveries followed a similar trend in this case, too, leveling to a minimum of ≈30% for DDE and ≈50% for pyriproxifen and deltamethrin. It would be interesting to compare pesticide partitioning properties with different types of oil and other types of lipids, but that falls outside the scope of this investigation.

Based on the olive oil experiment results, an aliquot of 3 g olive oil was selected for analysis in the method as a compromise to maintain recoveries of lipophilic pesticides to the extent possible in the oil matrix while still achieving adequately low LOQ and minimizing oil matrix influences on the GC-MS and LC-MS/MS analyses. This also approximately corresponded to the oil/water ratio of the olives studied. For a 10 μL injection in DSI-GC-MS, the 30% olive oil sample contained 24 × 8 μg of coextracted oil introduced into the inlet, and 12 × 4 μg in the case of LC-MS/MS (5 μL injection). This was expected to be similar in the case of the olives.

3.3 DSI-GC-MS analysis

Once sample preparation procedures were finalized, some experiments were conducted in order to evaluate the effects of matrix in the analyses. The presence of coextracts (lipids, pigments and nonvolatile molecular mass components) contained even in relatively clean samples can increase the background, change peak responses, and/or decrease the separation efficiency. In an attempt to minimize these effects, we used automated DSI, in which nonvolatile matrix components remained in the disposable microvial [24–27]. At optimized conditions, this serves to keep the injection port, column and MS detector maintenance-free, while still providing low LOQ for potentially dirtier extracts using LVI.

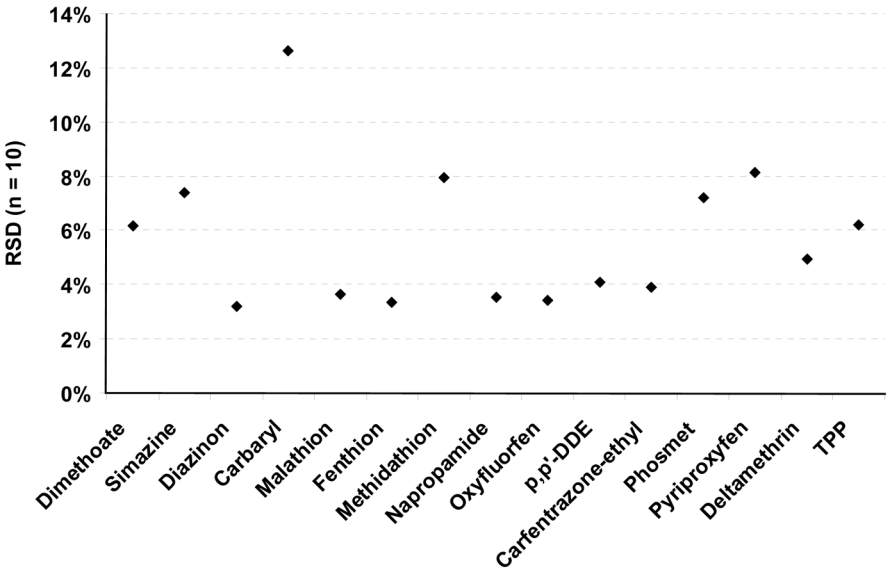

Figure 5. Repeatability (%RSD) of pesticides in standard MeCN solution (25 × Spk equivalent concentrations) plus analyte protectants obtained using automated DSI-C-MS ($n = 10$).

To evaluate the new automated DSI system, analyte standards in MeCN were repetitively injected to measure repeatability in the responses (without use of the internal standard (IS)). Figure 5 shows the acceptability of the DSI approach, with typical RSDs of 4%. Some analytes gave worse repeatabilities than others depending on their peak shapes and chemical nature, and those borderline GC-amenable analytes, such as omethoate and carbaryl, were much better analyzed by LC-MS/MS.

This was the case for those compounds even with analyte protectants added to the extracts. Analyte protectants strongly interact with active sites in the GC system, thus decreasing degradation and/or adsorption and overcoming the matrix-induced chromatographic enhancement effect [20, 27–29]. They are especially useful for relatively polar GC-amenable pesticides such as dimethoate, but LC-MS/MS is even better for determining those analytes.

Despite the high quality of the DSI device in the analysis of standards, performance was not as good for olive extracts. First and foremost, the relatively large quantity of volatile and semi-volatile matrix coextractives often made it difficult to choose interference-free SIM ions for quantitative and qualitative analysis in GC-MS. This was especially problematic for the early-eluting pesticides, as can be surmised from Fig. 3, but the use of LC-MS/MS for those analytes resolved this dilemma.

Olive extracts had worse background than the olive oil extracts, thus chemical matrix interferences were less of an issue for olive oil analysis, but in both cases, an indirect matrix effect occurred, which caused diminished response. This was verified by comparing responses from fortified extracts (matrix-matched standards) with standards in solvent at the same concentrations, both of which contained or did not contain analyte protectants. The reason for this effect was likely due to the thin film of nonvolatile matrix that remained in the microvial after the injected extract was vaporized, as depicted in Fig. 2. This film probably trapped and sealed a fraction of the analytes to the glass surface of the microvial, thus reducing transfer efficiency to the column. This effect had been observed previously [34–36], and dilution of extracts may help reduce this problem, but could increase LOQ. In this study, we simply used the same conditions from a previous study [27] and did not investigate options to overcome the indirect matrix effect. We simply wanted to meet LOQ for the pesticides at the 1 × Spk levels in Table 1, which was achieved despite the suppression effect. Therefore, quantitation of pesticides was performed using matrix-matched calibration standards of fortified blank extracts (olives and olive oil).

3.4 LC-MS/MS analysis

ESI in LC-MS/MS is prone to ion suppression effects due to coeluting matrix peaks, even though the ions seldom interfere in MS/MS [37]. An evaluation of analytical signal was performed in order to assess the matrix effects of olive extracts in the LC-MS/MS method. Comparisons of the signal intensities obtained in solvent-standard solutions with those obtained in matrix-matched standards showed suppression of response for all the compounds studied. Several approaches can be used to overcome this effect [37], and just as in the case of DSI-GC-MS, we chose the option to use matrix-matched calibration standards. The matrix-matched calibration standards compensated for signal suppression of the studied pesticides.

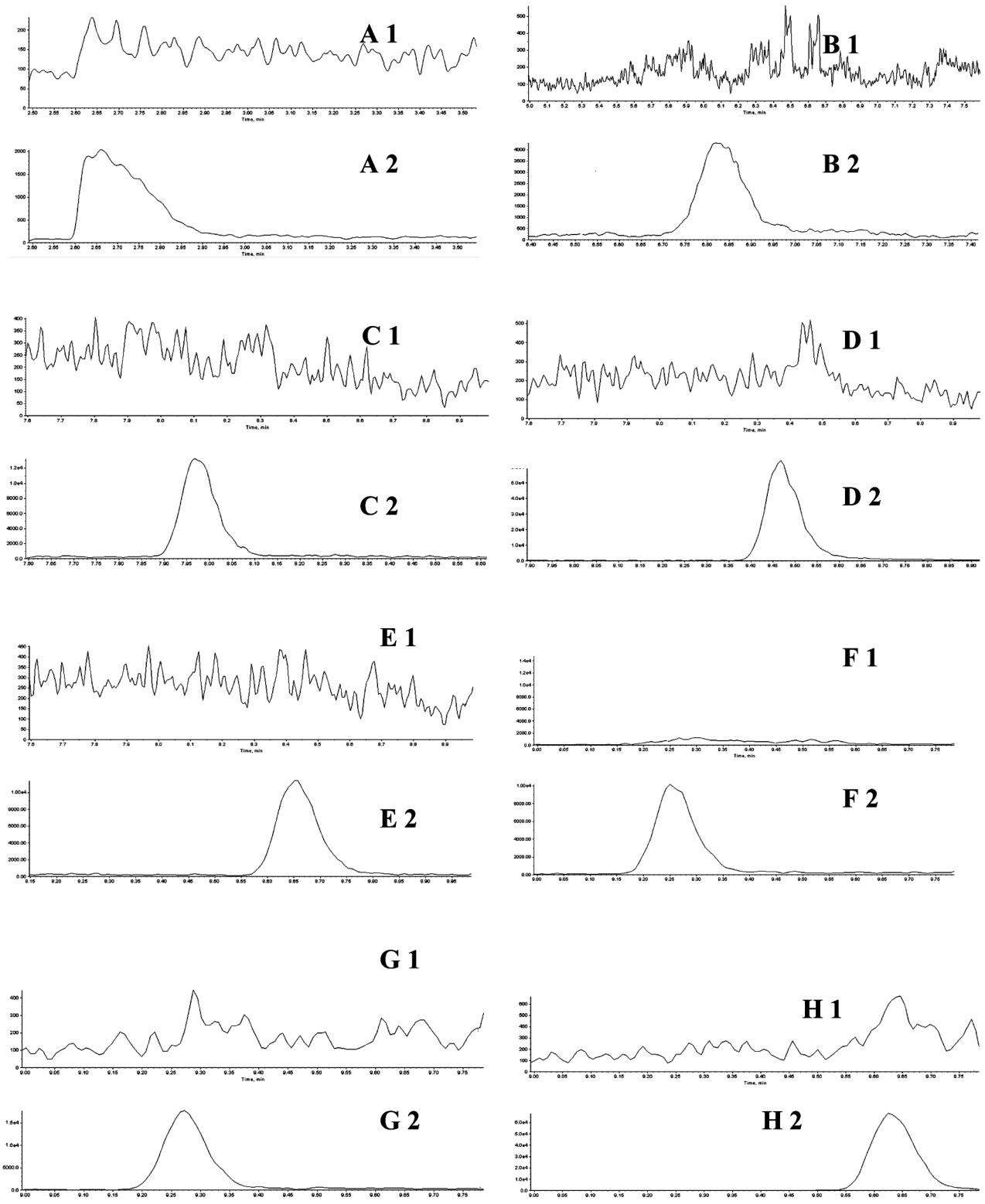

Figure 6. MRM chromatograms of (A) omethoate, (B) dimethoate, (C) simazine, (D) carbaryl, (E) diuron, (F) phosmet, (G) methidathion, and (H) malathion in (1) matrix blank and (2) lowest spiked olives using the QuEChERS method with analysis by LC-MS/MS.

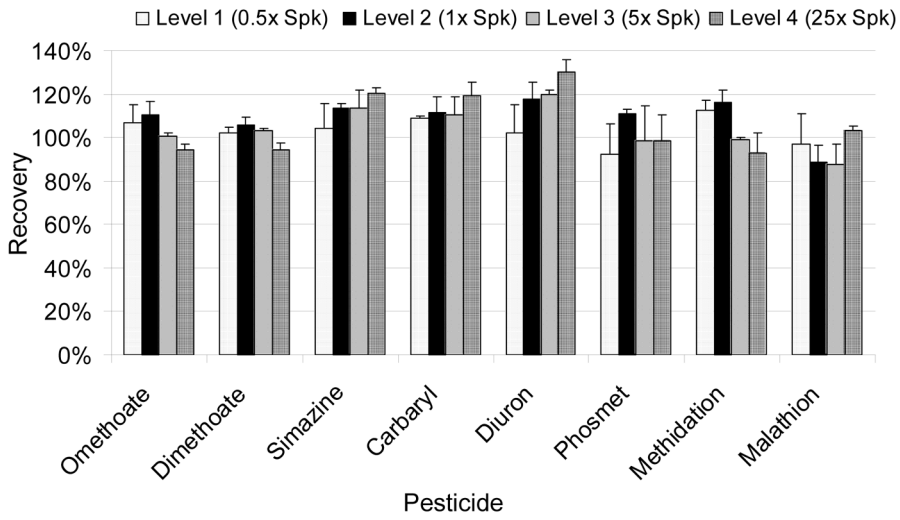

Figure 7. % Recoveries of pesticides in spiked olives at different levels using LC-MS/MS ($n = 6$). Spiking levels are given in Table 1 ($1 \times$ Spk-LC).

Table 5. Mean % recoveries (and %RSDs) obtained with the QuEChERS method for spiked olive samples and analyzed using DSI-GC-MS ($n = 6$). Recoveries >120% or <70% are italicized

Pesticide	$1 \times$ Spk (μg/g)	$1 \times$ Spk	$5 \times$ Spk	$25 \times$ Spk	Overall
Dimethoate	0.01	<LOQ	94 (3)	*69* (5)	72 (10)
Simazine	0.10	<LOQ	71 (2)	96 (4)	84 (16)
Carbaryl	0.20	90 (5)	88 (3)	*69* (9)	82 (13)
Diazinon	0.02	<LOQ	98 (12)	97 (11)	98 (17)
Fenthion	0.10	109 (13)	82 (6)	73 (18)	88 (23)
Methidathion	0.05	<LOQ	70 (3)	86 (12)	78 (15)
Napropamide	0.05	76 (19)	80 (3)	82 (6)	79 (12)
p,*p*′-DDE	0.01	99 (15)	72 (5)	74 (6)	82 (21)
Oxyfluorfen	0.01	100 (7)	77 (5)	80 (5)	86 (14)
Carfentrazone-ethyl	0.05	93 (10)	79 (7)	82 (5)	85 (11)
Phosmet	0.10	<LOQ	96 (16)	88 (17)	92 (19)
Pyriproxyfen	0.05	95 (3)	84 (5)	84 (6)	88 (8)
Deltamethrin	0.10	97 (13)	75 (15)	74 (8)	82 (19)

Another concern in LC-MS/MS is carry-over due to the very low LOD that can be achieved. If precautions were not made, some analytes with the lowest LOD (dimethoate carbaryl, simazine, diuron, and methidation) were observed in solvent blank injections made after high concentration standards. This problem was resolved by making a rapid "system rinse injection" between every sample injection. This also served as an extra sweep of matrix components and maintained a cleaner system. Figure 6 demonstrates the absence of interferences or carry-over in the LC-MS/MS analysis, while still achieving high S/N ratios at the lowest pesticide spiking levels needed for the application to olives.

3.5 Method validation

Once the final sample preparation, DSI-GC-MS, and LC-MS/MS conditions were set, validation experiments were conducted to determine recoveries, repeatabilities within a sample set, and reproducibilities among sample sets at different spiking levels. Employing six replicates, olives were spiked at four different concentrations ($0.5 \times$, $1 \times$, $5 \times$, and $25 \times$ Spk) corresponding to the spiking levels for each pesticide given in Table 1.

In the case of LC-MS/MS (as shown in Fig. 7), nearly all the recoveries fell between 70 and 120% (the typical acceptability criteria for pesticide monitoring). The only exceptions were diuron (128% recovery) and simazine (121%) at the highest spiking levels. This was believed to be a quirk of the matrix-matched calibration curves for those pesticides (nonlinearity at the higher concentrations), and it is noteworthy that this was not a problem for olive oil (Table 4). In terms of repeatabilities and reproducibilities, the RSDs were <15% in all the cases for LC-MS/MS.

The results, given in Table 5, provide evidence that the method achieves acceptable quantitative recoveries of all the pesticides (70–109%) with RSDs <20%, when ana-

lyzed by DSI-GC-MS. The LOQs were lower than the 1 × Spk goals listed in Table 1.

After validation studies were complete, the method was applied to the analysis of 21 olive samples collected in Portugal at the end of the harvest interval incurred with pesticide residues of phosmet, dimethoate, and omethoate. Virgin olive oil from a local store was also analyzed, but no pesticide residues were found. In the case of the incurred olives, the findings demonstrated that the method could be used for residue monitoring of the selected pesticides in olives and olive oil. With the exception of the planar pesticides due to their retention on GCB as previously discussed, the method should be suitable for regulatory monitoring of hundreds of pesticides in olives and olive oil. In the meantime, further analyses are planned using the method in Portugal for investigations to measure the harvest interval for pesticide applications to olive trees.

4 Concluding remarks

Previously, indications with the QuEChERS method were that it would not achieve adequately high recoveries for lipophilic pesticides in high fatty foods. The authors proposed that compensation of known recoveries could be conducted to provide accurate results for those analytes in foods with >20% lipid content. However, the recoveries were still acceptable in olives and olive oil despite the higher lipid content. This may be related to the higher density or other properties of the olive oil than other fats, or it could be due to the rather consistent recoveries for oil content >20% in the samples.

Although the DSI method was not optimized, it was able to achieve the needed LOQ for the application in an easy and rugged approach. After each injection of olive extracts, the removed microvial contained a very noticeable colored film of matrix coextractives that would normally coat the liner and column. A large number of chemical interferences occurred in the SIM chromatograms for the most volatile pesticides, but the use of LC-MS/MS for those analytes solved this problem and additionally gave better quantitative and qualitative results. Moreover, the combination of GC-MS and LC-MS/MS for pesticides that can be detected by both methods gives high confidence in the confirmations for those analytes in real samples. Using this method, an individual would spend only 40 min to prepare a set of 12 extracts, generate <9 mL of MeCN waste *per* sample, expend <$2/sample of materials, and only have FEP tubes to clean for reuse.

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