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One step carbon nanotubes-based solid-phase extraction for the gas chromatographic–mass spectrometric multiclass pesticide control in virgin olive oils

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ABSTRACT

This article presents a novel application of carbon nanotubes for the determination of pesticides (chlortoluron, diuron, atrazine, simazine, terbuthylazin-desethyl, dimetoathe, malathion and parathion) in virgin olive oil samples. For this purpose, two carbon nanotubes, multi-walled and carboxylated single-walled, were evaluated, the later being the most appropriate for the aim of the work. The sorbent (30 mg) was packed in 3-mL commercial cartridge and the virgin olive oil samples diluted (20%, v/v) in hexane were passed through it. After a washing step with 3 mL of hexane to remove the sample matrix, the pesticides were eluted with 500 μL of ethyl acetate. In order to achieve lower detection limits, the eluent was evaporated under a nitrogen stream and the residue reconstituted in 50 μL of the same solvent. Aliquots of 2 μL of the extract were directly injected into the GC–MS system for analysis. The low limits of detection achieved, between 1.5 and 3.0 $\mu g\,L^{-1}$, permit the application of the method to control the presence of these pollutants in very restrictive samples such as the ecological virgin olive oil. In addition to the sensitivity enhancement, the solid-phase extraction procedure is rather simple as it involves a single preconcentration–elution step, which allows sample processing in less than 8 min. Moreover, the cartridge can be reused at least 100 times without losing performance. The method was applied to the determination of the pesticides in two monovarietal and one ecologic commercial extra virgin olive oil samples. Two pesticides were detected in each of the monovarietal virgin olive oils while the ecological sample resulted to be a pesticide-free one.

1. Introduction

The increasing production and application of pesticides for agricultural purposes has caused the pollution of the environment (soils, waters and air) involving a serious risk to human and living organisms health by either direct exposure or through residues in foods and drinking waters. In this scenario, alarming levels of pesticides have been reported in air, water or foods, among others [1–3]. For this reason, simplest, reliable methodologies for rapid detection of pesticides are mandatory. Modern trends in analytical chemistry are towards the simplification and miniaturization of sample preparation, as well as the decrease of the organic solvent volume used. In this context, several novel extraction techniques are being developed in order to reduce the analysis step, increase the sample throughput and improve the quality and the sensitivity of analytical methods [4].

Concerning food control analyses, development of sample-treatment procedures for the isolation of pesticides in matrices with relatively high fat content, such as olive oil and olives, is rather complicated [5]. The preparation of these samples for the determination of pesticides by chromatographic techniques requires the complete removal of the high molecular-mass fat to preserve the separation performance of the chromatographic column. For this reason, an additional cleanup step is usually included in these methods. The most used sample-treatment procedures, including liquid–liquid extraction (LLE), gel-permeation chromatography (GPC), solid-phase extraction (SPE), matrix solid-phase dispersion (MSPD) or quick, easy, cheap, effective, rugged and safe (QuEChERS) techniques, have been recently reviewed [6]. One example of MSPD treatment was developed for the quantification of a selected group of the most common pesticides employed in olive grove, but the procedure required a previous liquid–liquid extraction step in the olive oil samples [7]. In other case, a multiresidue method was optimized using a hexane/acetonitrile extraction with a cleanup step by GPC [8] which was followed by gas chromatographic–tandem mass spectrometric determination. A solid-phase microextraction (SPME) approach was also carried out as a screening method

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for organophosphorus insecticides and their metabolites in olive oil samples, polydimethylsiloxane (100 μm) being the fiber that provided the best results [9]. More recently, a supercritical fluid extraction has been developed as an on-line cleanup technique alternative to liquid chromatography for confirmation of paraquat and diquat pesticides in olive oil samples [10].

Concerning SPE methods, multicolumn systems based on different sorption processes have been greatly employed for the cleanup procedure previous to the determination of organophosphorus and organochlorine pesticides in virgin olive oil samples [11,12]. In addition, a multiresidue method based on three LLE procedures was used to study the efficiency of different cleanup systems, being LLE of the olive oil diluted in hexane with acetonitrile followed by an ENVI-Carb SPE cleanup, the method which provided the best results for all target compounds [13]. The same research group has recently optimized the SPE method for pesticides determination in olives and the processing factor in olive oil by comparison of several extraction systems [14].

In the search for reducing the complexity and length of multiple cleanup pre-treatments, the usefulness of carbon nanotubes (CNTs) as SPE sorbent material is being studied in a wide variety of application fields. The most promising results have been provided for the preconcentration and determination of pesticide residues in water samples [15–17]. The objective of this work is to evaluate the potential of CNTs for the extraction of selected pesticides from virgin olive oil samples in a single SPE step combined with gas chromatography–mass spectrometry (GC–MS). For this purpose, factors affecting the SPE procedure have been optimized and discussed. The favorable features obtained in terms of precision, sensibility and recovery demonstrated the applicability of carbon nanotubes for the analysis of fatty matrices prior to the gas chromatographic separation.

2. Experimental

2.1. Standards, reagents and samples

Pesticide analytical standards (viz. chlortoluron, diuron, atrazine, simazine, terbuthylazin-desethyl, dimetoathe, malathion and parathion) were purchased from Riedel-de Häen (Seelze, Germany). Individual stock standards were prepared in acetone (acetonitrile for triazines) at a concentration of 5000 mg L^{-1} and stored at 4 °C. HPLC-grade acetone, acetonitrile, ethyl acetate, hexane, methanol, nitric acid (60%), sulfuric acid (95–98%) and hydrochloric acid (35%) were provided by Panreac (Barcelona, Spain). Individual and cumulative working standard solutions were prepared by appropriate dilution of the stocks in blank virgin olive oil samples.

A pesticide-free virgin olive oil from a Spanish supplier was chosen for optimization tasks and spiked with increasing concentrations of standard solutions for calibration process. In order to validate the proposed method, three different extra virgin olive oils, one from ecologic farming, and two from common monovarietals (arbequina and picual) were analyzed.

Multi-walled carbon nanotubes (MWCNTs), with purity over 95%, and single-walled carbon nanotubes (SWCNTs) with purity of 90% were supplied by Sigma–Aldrich (Madrid, Spain). The outside diameter, internal diameter and length ranged between 20–30 nm, 5–10 nm, and 0.5–200 μm, respectively for MWCNTs. The SWCNTs outside diameter varied between 1 and 2 nm while the length ranged from 0.5 to 2 μm.

2.2. Apparatus

The instrumental setup comprises an HP6890 gas chromatograph equipped with an HP5973 mass spectrometric detector based on a quadrupole analyzer and a photomultiplier detector (Agilent Technologies, Palo Alto, USA). Gas chromatographic separation was achieved on a SLB-5ms fused silica capillary column (30 m × 0.25 mm I.D. and 0.25 μm of film thickness). Chromatographic conditions were as follows: 225 °C, injection temperature; split mode operation with a split ratio 1:10; column initial temperature 60 °C (2 min), raised up to 280 °C at 10 °C/min (2 min). A deactivated pre-column (1 m × 0.25 mm I.D.) of fused silica for retention gap was inserted in the top of the analytical column. Helium (5.0 grade purity, Air Liquide, Sevilla, Spain), regulated by a digital pressure and flow controlled, was used as carrier gas (constant flow 1.0 mL min^{-1}). A solvent delay of 9 min was employed in the optimized method. The temperatures of the mass spectrometer source and quadrupole were maintained at 230 and 150 °C, respectively. The detector operated in full scan mode between 40 and 500 μm and electron impact ionization was used for analyte fragmentation with ionization energy of 70 eV. Total ion current chromatograms were acquired and processed using G1701BA Standalone Data Analysis software (Agilent Technologies, Palo Alto, USA) on a Pentium IV computer that also controlled the whole system.

2.3. Carbon nanotubes cartridge preparation

Multi-walled carbon nanotubes and carboxylated single-walled carbon nanotubes (10–50 mg) packed cartridges were prepared using 3-mL empty PTFE SPE cartridges (Supelco, Madrid, Spain). Polypropylene upper and lower frits were retained at each end of the column to hold the carbon nanotubes packing in place.

Single-walled carbon nanotubes were carboxylated following a previously optimized procedure [18]. Briefly, 100 mg of SWCNTs were added into a glass beaker with 20 mL of H_2SO_4/HNO_3 (3:1 v/v). The mixture was ultrasonicated (50 W, 60 Hz) for 90 min. Afterwards, it was highly diluted with water (2 L) and filtered through 0.45 μm cellulose acetate filter. The obtained residue was then washed with water and treated with 25 mL of 1 M HCl. In this case, the mixture was sonicated only for 45 min. Finally, carboxylated derivates were filtered, washed and dried in air.

2.4. SPE procedure

An accurately weighted amount of 30 mg of c-SWCNTs were packed in to a 3-mL SPE cartridge, firstly preconditioned with acetone (2 mL) and then equilibrated with hexane (2 mL). Subsequently, 3 mL of virgin olive oil:hexane sample solution (20:80 v/v) spiked with the pesticides at appropriate concentration levels were passed through the cartridge for analytes retention on the c-SWCNTs. After that, the cartridge was cleaned with hexane (3 mL) for the removal of the matrix components before elution with ethyl acetate (0.5 mL). The carbon nanotubes sorbent column was washed by passing methanol (3 mL), after which the column was ready for a new analysis. The whole of the procedure was carried out at a flow rate of 1 mL min^{-1}. Finally, the preconcentration of the analytes was improved by means of a dryness step with a gentle N_2 flow and a further re-dissolution of the residue in 50 μL of ethyl acetate.

2.5. Chromatographic procedure

For the optimized GC–MS approach, 2 μL of the organic extract previously obtained by SPE procedure was directly injected into the GC injector and transfer to the chromatographic column by an helium stream (1.0 mL min^{-1}). The separated analytes reached the MS detector and were identified by their retention time. In addition, the mass spectra were compared with those provided by the corresponding standards analyzed using similar proce-

Table 1
Analytical features of the one step SPE procedure for the eight selected pesticides.

Analyte	t_R (min)	m/z Values[a] selected for identification/quantification	Linear range ($\mu g\,L^{-1}$)	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)	R^2	RSD (%) ($n=8$)
Chlortoluron	10.77	**72**, 132, *212*	6–2000	1.8	6.0	0.991	8.7
Diuron	11.49	**72**, 187, *232*	8–2000	2.4	8.0	0.981	7.5
Terbuthylazin-desethyl	16.17	173, **214**, *229*	5–2000	1.5	5.0	0.995	8.8
Dimetoathe	16.73	**87**, 93, *229*	5–2000	1.5	5.0	0.994	3.4
Simazine	16.89	**44**, 186, *201*	8–2000	2.4	8.0	0.987	5.6
Atrazine	16.97	58, **200**, *215*	7–2000	2.1	7.0	0.988	3.6
Malathion	19.09	**125**, *173*, *330*	10–2000	3.0	10.0	0.985	5.8
Parathion	19.40	*97*, ***291***	10–2000	3.0	10.0	0.988	6.0

t_R, retention time; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation. Average olive oil density 0.913 $kg\,L^{-1}$.
[a] m/z Values for base peak and molecular peak are indicated in bold and italic type, respectively. Quantification values are presented as underlined.

dure. For quantification purposes, the peak areas calculated from the total ion current chromatogram were employed. In the case of malathion and parathion, m/z 173 and 97 were used respectively in order to improve the selectivity. Table 1 shows the target extracted m/z corresponding to the ions of each chemical compound used for identification/quantification of the pesticides studied.

3. Results and discussion

Solid-phase extraction is a widely accepted extraction/cleanup technique for the determination of a variety of compounds in liquid samples. It presents very favorable features such as the use of smaller volumes of samples and organic solvents, cleaner extracts, and greater recoveries and preconcentration factors, among others. A wide variety of sorbents are available for this procedure, whose choice depends on the target analytes, sample matrix or potential interferences. In this work, the feasibility of carbon nanotubes as SPE sorbent material for the determination of pesticides in virgin olive oils by a one step procedure is evaluated.

3.1. Optimization of the SPE procedure

In order to achieve an adequate extraction performance by carbon nanotubes as sorbent for SPE, the optimization of several parameters was needed, including the kind of sorbent employed, amount packed into the cartridge, sample volume and dilution (if required), type and volume of eluent, and evaporation–redissolution step for sensitivity improvement.

Regarding the type of sorbent, the capability of multi-walled and single-walled carbon nanotubes was evaluated. In principle, and taking into account the existence of concentric layers of graphene, MWCNTs offer better sorption capabilities than SWCNTs. Notwithstanding this, the presence of different moieties on the nanotubes, such as a COOH group, can modify the interactions analyte-sorbent. For this aim, the MWCNTs sorbent capability was compared with that provided by carboxylated single-walled carbon nanotubes (c-SWCNTs). Fig. 1 compares the results obtained after passing 2 mL of a diluted virgin olive oil (40% v/v in hexane) spiked with the pesticides at a concentration of 1 $\mu g\,mL^{-1}$. As it can be seen, the sorbent capacity of c-SWCNTs was markedly better than that of MWCNTs for all the analytes, which can be ascribed to the additional interaction provided by the carboxylic moiety present in the c-SWCNTs. Next, the influence of the sorbent amount (between 10 and 50 mg) on the analytical signal was evaluated. As can be seen in Fig. 2, the peak area for all analytes increased when increasing the amount of sorbent up to 30 mg with a slight decrease over this value probably as result of a non-quantitative elution of retained analytes. Therefore, 30 mg was selected as optimum.

The analytical methods described for the determination of pesticides in olive oils by SPE usually require the dilution of the fatty

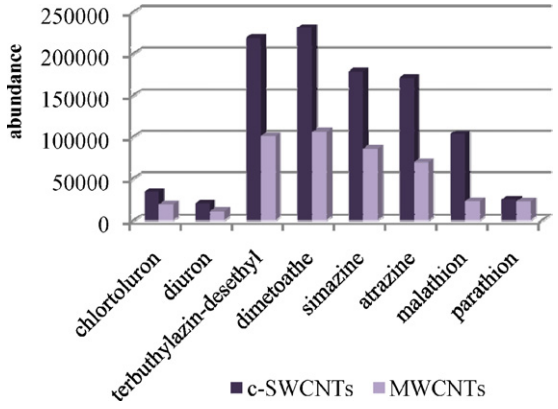

Fig. 1. Comparison of the performance of c-SWCNTs and MWCNTs for the isolation of the selected pesticides from virgin olive oil samples.

sample in a proper solvent in order to decrease the viscosity of the liquid and favor the analyte–sorbent interactions. In this case, hexane was selected among other organic solvents (viz. acetonitrile, methanol, ethyl acetate and dichloromethane). With the aim of determining the optimum percentage of olive oil, different mixtures in hexane were prepared and spiked with the target compounds at a concentration of 1 $\mu g\,mL^{-1}$. Percentages of virgin olive oil higher than 20% made the retention of the analytes on the c-SWCNTs difficult and therefore, it was fixed to study the sample volume that can be passed through the cartridge. Up to 5 mL of diluted olive oil can be preconcentrated without analyte losses. For operating reasons, 3 mL of 20% virgin olive oil in hexane were adopted for further optimization experiments.

Concerning the eluent choice, methanol, acetonitrile and ethyl acetate were the organic solvents evaluated. The two last mentioned offered higher analytical signal, being ethyl acetate slightly better and, therefore, the eluent chosen for the elution of the retained pesticides. Furthermore, the volume passed through the

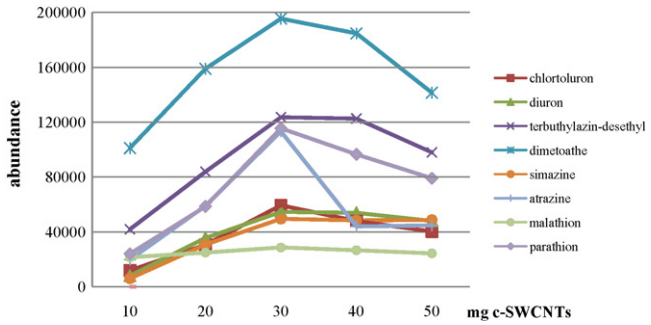

Fig. 2. Influence of the amount of c-SWCNTs packed in the SPE cartridge for the preconcentration of the selected pesticides from virgin olive oil samples.

Table 2
Pesticides concentration found (mean ± SD, $n = 3$) after the analysis of spiked and commercial extra virgin olive oil samples.

Pesticide	MRL (mg kg^{-1})	Spiked samples		Commercial samples		
		100 μg L^{-1}	20 μg L^{-1}	Arbequina	Picual	Ecologic
Chlortoluron	0.05	101 ± 10	21 ± 2	–	–	–
Diuron	0.20	79 ± 9	16 ± 2	+	–	–
Terbuthylazin-desethyl	0.05	105 ± 4	18 ± 1	–	–	–
Dimetoathe	2.00	97 ± 9	18 ± 2	–	–	–
Simazine	0.10	99 ± 9	18 ± 2	–	+	–
Atrazine	0.05	98 ± 7	20 ± 1	–	–	–
Malathion	3.00	99 ± 10	20 ± 2	+	+	–
Parathion	0.05	98 ± 5	21 ± 2	–	–	–

MRL, maximum residue limit; SD, standard deviation; –, not detected; +, detected but not quantified.
Average olive oil density 0.913 kg L^{-1}.

cartridge for the preconcentration step was assayed between 0.2 and 2 mL. The lowest volume required for quantitative elution was 0.5 mL. In order to increase the sensitivity, the ethyl acetate was evaporated under a nitrogen stream and the residue re-dissolved in 50 μL of the same organic solvent.

3.2. Analytical performance of the method

Once the experimental conditions were optimized, the analytical performance of the proposed method was studied in order to evaluate its usefulness for quantitative determination of the eight

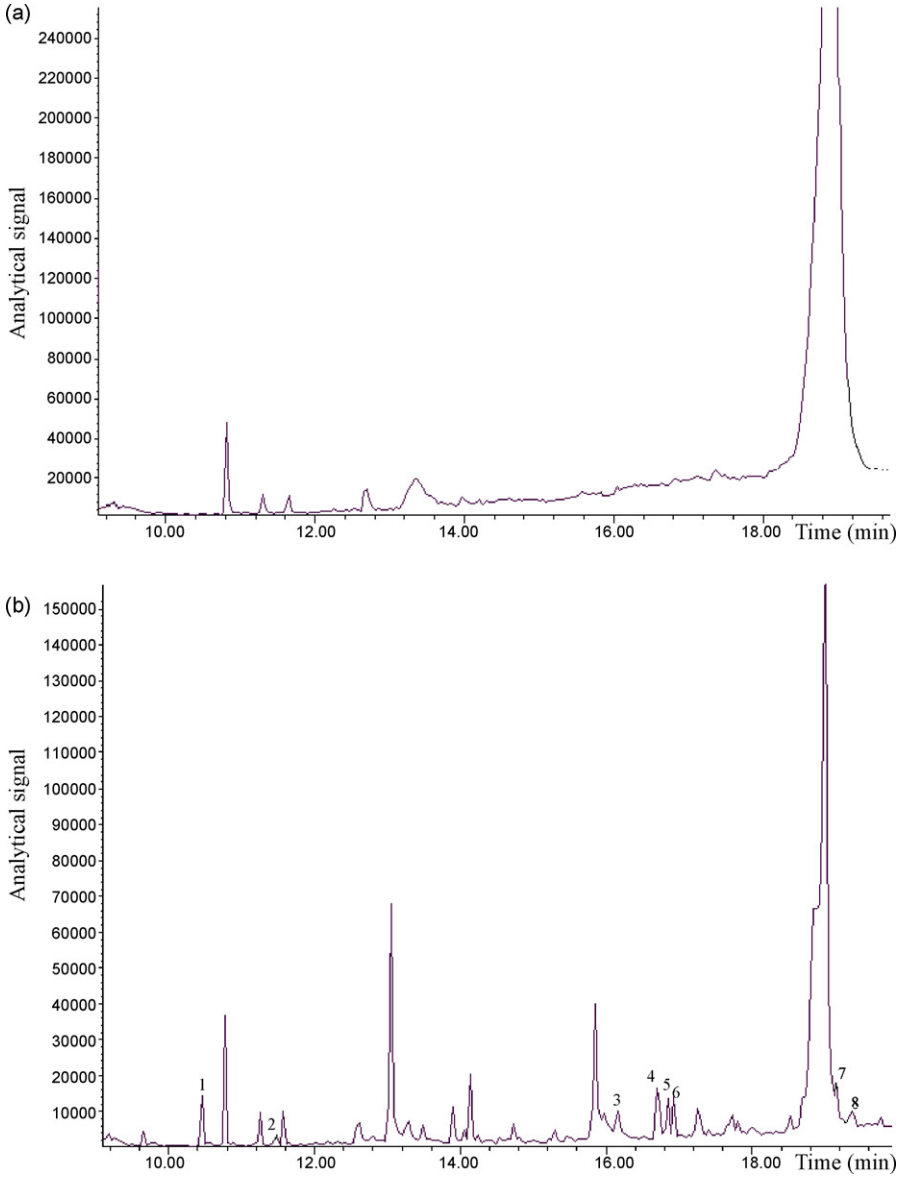

Fig. 3. Typical chromatogram obtained after the analysis of a virgin olive oil sample by the proposed method. (a) Blank virgin olive oil and (b) virgin olive oil spiked with the analytes at a concentration of 20 μg L^{-1}. 1, chlortoluron; 2, diuron; 3, terbuthylazin-desethyl; 4, dimetoathe; 5, simazine; 6, atrazine; 7, malathion; 8, parathion.

pesticides in virgin olive oil samples. For this purpose, a refined olive oil (blank) spiked with increasing concentrations of the analytes, ranging from 5 to 2000 $\mu g\,L^{-1}$ was diluted in hexane (20%, v/v) and 3 mL were preconcentrated to obtain analytical features (viz. reproducibility, limits of detection (LODs), limits of quantification (LOQs), linear ranges and recoveries percentages) which are compiled in Table 1. The samples were analyzed in quintuplicate by the optimized method and calibration curves were generated by plotting peak areas obtained for ten standards versus analyte concentrations for each assayed pesticide. In all cases, the linearity was kept within the concentration range evaluated and the regression coefficients (R^2) were always higher than 0.98.

The LOD for the eight pesticides were calculated as the concentration providing a peak area three times higher than the noise (S/N = 3) and they were in the range of 1.5–3 $\mu g\,L^{-1}$ (see Table 1). Comparing with two different methodologies recently found in the bibliography, which have been applied to the determination of pesticide residues in olive oil samples [19,20], the proposed technique supposes a simplification of the method as it reduces the SPE steps from 2 and 4 to a single one. In addition, the optimized procedure proposed in this article provides LODs, for the same analytes, better than those provided for the previous compared methodologies. These data, together with the LOQs achieved demonstrate the high sensibility of the method for the detection and quantification of the selected more common pesticides in virgin olive oils, being appreciably below the maximum residues levels (MRLs) allowed by the Regulation even for ecologic farming (see Table 2). The precision of the method was evaluated through a repeatability study. For this purpose, eight aliquots of virgin olive oil spiked with the target compounds at a concentration of 100 $\mu g\,L^{-1}$ were analyzed following the optimized process. Relative standard deviation (RSD) was employed to express the repeatability of the method and the results are listed in Table 1, where it can be seen that the RSD was lower than 9% for all the studied analytes. Again, the values of our method are better than those reported in the literature for the same compounds [19,20].

Finally, recovery studies were carried out with a virgin olive oil spiked with the pesticides at two concentration levels, 20 and 100 $\mu g\,L^{-1}$. Spiked samples were extracted and analyzed in triplicate by the optimized procedure. As it can be seen in Table 2, pesticide recoveries ranged between 79 and 105% for the two concentration levels assayed.

3.3. Analysis of commercial virgin olive oil samples

In order to demonstrate the applicability of the c-SWCNTs as SPE sorbent for the determination of pesticides in virgin olive oil matrices, the proposed procedure was applied to three different quality virgin olive oils; two monovarietal extra virgin olive oils (viz. Arbequina and Picual) and one extra virgin olive oil coming from ecological agriculture. As it is shown in Table 2, none of the eight pesticides studied were detected in the ecological extra virgin olive oil, whereas, in the case of monovarietal extra virgin olive oils, both samples resulted to be positive for the presence of two pesticides, respectively. For Arbequina extra virgin olive oil, diuron was found, probably coming from residual application. Simazine was the pesticide detected after the analysis of Picual extra virgin olive oil. In addition, malathion residues were detected in both cases. All compounds were found between LOD and LOQ concentration levels and hence, below the MRLs allowed by the Regulation. Finally, the chromatograms of a pesticides-free extra virgin olive oil (a) and fortified with the analytes at a concentration of 20 $\mu g\,L^{-1}$ (b) are shown in Fig. 3.

4. Conclusions

The development of sample-treatment methodologies for the determination of pesticide residues in matrices with high fat content (such as olives and olive oil) is a demanding task, since even small amounts of co-extracted fat can irreversibly damage the chromatographic column. In addition, there is a clear trend towards the development of miniaturized sample-handling methodologies that consume small amounts of sample and organic solvent volumes. In the present work, a rapid one step methodology for the quantitative extraction of multiclass pesticide residues from virgin olive oils has been reported. c-SWCNTs have shown a great potential as sorbent for the SPE procedure. The 30 mg packed cartridge can be reused for at least 100 samples without losing retention capacity. In light of good linearity, reproducibility and low detection limits obtained by the proposed method, it can be proposed as a feasible low time-consuming sample-treatment approach prior to the gas chromatographic–mass spectrometric determination of multiresidues pesticides in virgin olive oil samples from different sources.

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