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Automated multiresidue analysis of pesticides in olive oil by on-line reversed-phase liquid chromatography–gas chromatography using the through oven transfer adsorption–desorption interface

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Abstract

A multiresidue, automated and rapid method for the determination of pesticide residues in olive oil is presented. The method employs the through oven transfer adsorption–desorption interface for the on-line coupling of reversed-phase liquid chromatography and gas chromatography. In this fully automated system, olive oil is directly injected with no sample pre-treatment step other than filtration. Methanol–water is used as eluent in the liquid chromatography pre-separation step. The selected liquid chromatography fraction containing the pesticides is automatically transferred to the gas chromatography. The liquid chromatography column flow during elution is different from the flow during the transfer. Using a flame ionisation detector, pesticide detection limits varied from 0.1 to 0.3 mg/l.

Keywords: Through oven transfer adsorption–desorption interface; Olive oil; Interfaces, LC–GC; Liquid chromatography–gas chromatography; Pesticides

1. Introduction

Olive trees are attacked by several pests and diseases, and many pesticides, mainly organophosphorous, are used in their control. However, these compounds may leave residues which will contaminate the oil. It is clear that fat-soluble pesticides tend to concentrate in the oil.

Multiresidue methods are generally used to determine such pesticide residues since these can detect many pesticides in a single analysis run, obviating the need to analyse a large numbers of samples by automated analytical methods.

The most widely used methods for determining pesticides residues in olive are gas chromatography (GC) methods that require the use of a sample preparation step to separate the pesticides from lipids. This is a crucial step in the analytical procedure since even a small amount of lipids can harm the gas chromatographic injectors, capillary columns and detectors.

Most methods are based on partitioning between hexane or light petroleum and acetonitrile, followed by clean up. These techniques are time-consuming and laborious and require large volumes of both sample and organic solvent. Furthermore, after isolating the analytes, the eluate often has a volume of several millilitres. Consequently, solvent evaporation must to be performed before a small aliquot (1 or 2 μl) is injected into the gas chromatograph [1,2]. During these procedures analytes may be contaminated and lost as the samples are manipulated. Furthermore, automation is not possible. Alternative procedures include solid-phase extraction (SPE) and supercritical fluid extraction (SFE). SPE–GC methods have been used for the determination of pyrethroid insecticides in vegetable oil and butter fat [3] and SFE has been used for separating organochlorine and organophosphorous pesticides from fats [4,5].

Morchio et al. [6] described a method for the determination of organophosphorous insecticides in edible oil,

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injecting oil samples previously diluted 1:1 with acetone directly into a gas chromatograph. This method is described by Grob et al. [7] as injector-internal headspace analysis but peaks usually become broader after several injections, while the selectivity of the column changes.

On-line coupled chromatographic techniques are amongst the most sensitive and selective techniques available for the determination of pesticide residues in complex matrices [8,9]. On-line coupling of liquid chromatography–gas chromatography (LC–GC) combines the effectiveness of sample preparation in the LC step with the high efficiency and sensitivity of GC [10]. Previous works using on-line LC–GC methods for the analysis of pesticide residues in edible oil mainly refer to the use of normal phase in the LC separation step. Grob and Kälin [11] described on-line size exclusion chromatography–gas chromatography (SEC–GC) for the determination of chlorinated pesticides in food containing fat, although the same authors indicated that the amount of triglycerides transferred disturbs the GC system. Coupled SEC–GC methods for organophosphorous pesticide detection in edible oils have also been described [1,12,13]. The use of normal phase in the LC step may cause several problems, which will affect the performance of the LC columns as triglycerides deactivate the silica gel [14]. The use of reversed-phase liquid chromatography (RPLC) in the pre-separation step seems to be an interesting alternative, although the transfer of polar solvent to the gas chromatograph is hindered because of the very large volume of vapour that is produced per unit volume of liquid [15].

Reversed-phase semi-preparative HPLC with acetonitrile as mobile phase has long been used for multipesticide extraction from edible fat and oil prior to gas chromatographic quantitation [16]. However, pesticide analysis by direct coupling of reversed-phase liquid chromatography to gas chromatography (RPLC–GC) is still a hard task, while automation of this chromatographic technique is even more difficult.

Previous works have shown that the use of a programmed temperature vaporiser (PTV) may be a useful alternative, and this has been used for the direct analysis of minor components in edible oils [17–20].

Recently, our research group has developed a new interface, named through oven transfer adsorption–desorption (TOTAD), for the on-line coupling of RPLC–GC. Methods for analysing pesticides in water by RPLC–GC [21,22] and by very large sample volumes have also been developed [23].

The purpose of this work was to develop an automated multiresidue method for the direct analysis of pesticide residues in olive oil by RPLC–GC. To this end, an automated laboratory-built TOTAD interface was constructed and used for on-line coupling of RPLC–GC to allow the full automated analysis without any kind of sample pre-treatment other than a simple filtration step.

2. Experimental

2.1. Materials

Olive oils (extra-virgin and refined olive oil) were purchased from a local market. As pre-treatment prior to RPLC–GC analysis, the oil samples were merely filtered through a 0.22 μm filter (Chromatography Research Supplies Inc.). Pesticide standards were obtained from Chem. Service Inc. (West Chester, PA, USA). The pesticides used for the experiment were: fenitrothion, parathion, diazinon (organophosphorous), lindane (organochlorine), carbaryl (carbamate), atrazine, simazine and terbutryne (triazines). In two separate experiments, each pesticide was added to the olive oil in different concentrations: 50 mg/l in order to determine the LC fraction to be transferred to GC and from 10 to 0.5 mg/l to carry out linear calibration.

Methanol and water (HPLC grade) were obtained from LabScan (Dublin, Ireland). Tenax TA 80–100 mesh (Chrompack, Middelburg, The Netherlands) was used as packing material in the liner of the PTV. The packed liner was conditioned under a helium stream, which was heated from 50 to 250 °C at 50 °C/10 min and maintained for 60 min at this temperature.

2.2. Instrumentation

The analyses were performed using on-line coupled LC–GC equipment. An automated TOTAD interface, US Patent 6,402,947 B1 (exclusive rights assigned to KONIK-Tech, Sant Cugat del Vallés, Barcelona, Spain) was created by substantially modifying a PTV injector (Fig. 1). The TOTAD interface operation mode has been described elsewhere [21,22]. The HPLC system was composed of a manual injection valve (model 7125, Rheodyne, CA, USA) with a 20 μl loop, a quaternary pump (HP model 1100), a column oven (HP model 1100) and a diode-array ultraviolet (UV) detector (Perkin-Elmer model LC 235). The gas chromatograph (Konik model HRGC 4000B) was equipped with a TOTAD interface and a FID detector. The TOTAD interface was placed horizontally on the LHS of the gas chromatograph. EZchrom (Konik, Sant Cugat del Vallés) software was used to obtain data from both LC and GC runs. EZchrom software was also used to automate the process.

2.3. LC conditions

A 50 mm × 4.6 mm i.d. column packed with modified silica (C4, kromasil 100-10, Hichrom, Berks, UK) was used. All analyses were carried out using methanol–water (70:30, v/v) as mobile phase and injecting 20 μl of the filtered olive oil. The LC column was maintained at 45 °C and two different conditions for LC were employed.

The first set of conditions was used to fix the pesticide elution time. In this case, the flow rate was 2 ml/min, and the initial composition of the eluent (methanol–water (70:30,

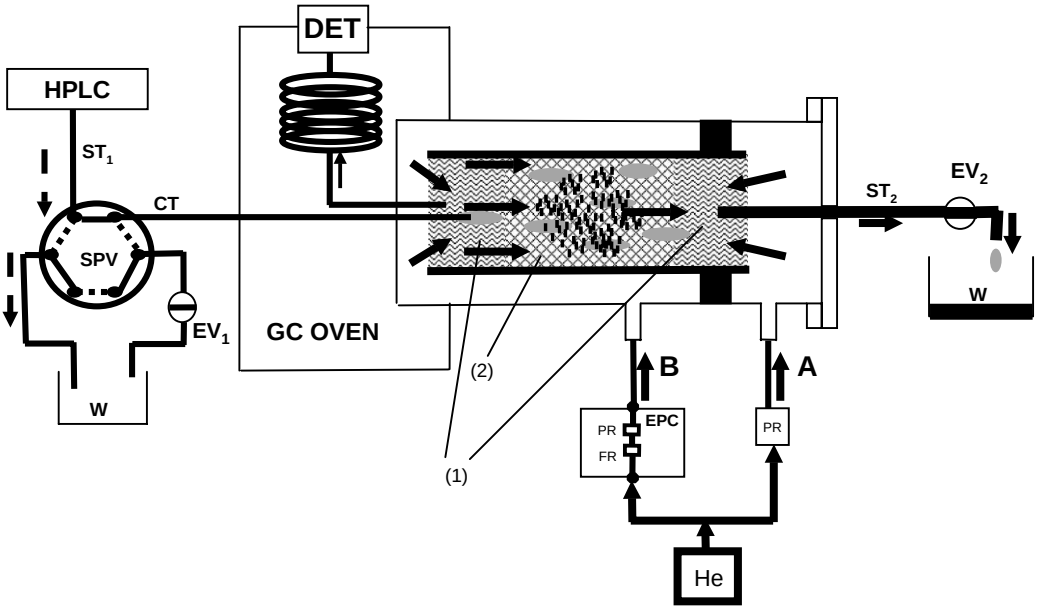

Fig. 1. Automated TOTAD interface represented during the transfer step. Symbols: (1) glass wool; (2) sorbent (Tenax TA); (SPV) six-port valve; (EV_1 and EV_2) electrovalves 1 and 2; (EPC) electronic pressure control; (PR) pressure regulator; (FR) flow regulator; (solid arrows) gas flow; (dotted arrows) liquid flow; (ST_1) stainless steel tubing of 0.25 mm i.d. to transfer eluent from LC to GC; (ST_2) stainless steel tubing of 1 mm i.d. to allow the exit of liquids and gases; (CT) silica capillary tubing between six-port valve and GC; (W) waste; (⬭) solvent; (⁞⁞) analytes.

v/v) was maintained for 3 min and followed by a linear gradient of up to 78% methanol within 3 min. This methanol percentage was kept for 4 min and then again modified to 86% within 2 min and maintained there for 3 min. Finally, the gradient was varied to reach 100% methanol within 4 min. The UV detection was performed at 205 and 255 nm. Twenty microlitres of olive oil containing 50 mg/l of each pesticide was injected, so that their peaks could be monitored with the LC detector, since low pesticide concentrations made their monitoring impossible.

The second set of conditions was used for LC–GC pesticide residue analysis. In these conditions, the LC detector was not used and the LC column was directly connected to the six-port valve by a stainless steel tube (0.25 mm i.d.). The flow rate was 2 ml/min until pesticide elution began (0.4 min), which was then changed in 0.1 min to 0.1 ml/min and maintained at this flow rate during the LC–GC transfer step (from 0.5 to 22.5 min). After the transfer, the flow was raised to 2 ml/min again and the gradient was raised to 100% methanol within 1 min and maintained for 25 min to ensure complete elimination of the retained lipids. The LC chromatogram of Fig. 3 was obtained in these conditions by connecting the LC detector (without transfer to GC).

2.4. LC–GC transfer

A manually operated TOTAD interface for on-line RPLC–GC has been used by our research group in previous studies [21–23]. In the present work, changes in the valves and pneumatics allowed the system to be automated. The manual valves used in the previous system were replaced by electrovalves (EV_1, EV_2 and SPV in Fig. 1). An electronic pressure control (EPC) was used to control helium flow by B, as is shown in Fig. 1. The GC was connected to the LC system via TOTAD interface.

The six-port valve was connected to the GC by silica capillary tubing (62.15 cm length × 0.32 mm i.d., 50 μl internal volume; CT in Fig. 1). The glass-liner was packed with a 1 cm length of Tenax TA between two glass wool plugs.

Initially, the TOTAD interface was stabilised at 100 °C with EV_1 closed and EV_2 open. Helium flow was 1500 ml/min through A and 1500 ml/min through B. The GC oven temperature was maintained at 40 °C. Twenty microlitres of the olive oil was injected into the LC system. At the beginning, the eluent from the HPLC system was sent to waste. At 0.4 min, the front of the pesticide fraction reached the six-port valve, which was automatically switched, transferring the fraction to the GC. The helium pushed the solution through the glass-liner. During the transfer time pesticides were retained by the adsorbent inside the glass-liner and solvent was vented to waste through the ST_2 tubing.

At 22.5 min, the transfer step was completed and the six-port valve was switched and EV_1 opened. The LC eluent was sent to waste, as was the solution in the capillary transfer CT, which was pushed out by the helium. Temperature and helium flows were maintained constant for 1 min to ensure elimination of all the remaining solvent in the glass-liner and the CT tubing. After this time, EV_1 and EV_2 were closed and flow through B was interrupted and the flow through A changed to 1.8 ml/min. Then the TOTAD interface was quickly heated to 250 °C, leading to the thermal desorption of the analytes which were transferred to the GC

column, pushed by the helium. GC analysis was then carried out. At 48.5 min, the GC analysis was completed and EV_2 was opened. The interface was cleaned by maintaining the helium stream at 250 °C for 5 min, after which it was cooled to 100 °C so that another analysis could be carried out.

2.5. GC conditions

Gas chromatographic separations were carried out on a Quadrex (Weybridge, UK) fused-silica column (30 m × 0.32 mm i.d.) coated with 5% phenyl methyl silicone (film thickness 0.25 μm). During the transfer and the solvent elimination steps, the oven temperature was kept at 40 °C. At 23.5 min, the GC analysis began with the column temperature maintained at 40 °C for 3 min. It was then raised to 160 °C at 20 °C/min, then to 240 °C at 4 °C/min. The FID temperature was kept at 250 °C. Helium was used as the carrier gas at a flow rate of 1.8 ml/min.

3. Results and discussion

3.1. LC pre-separation

First at all, the start and end times of the fraction to be transferred from the LC to GC must be selected in the LC chromatogram. Olive oil spiked with the pesticides at 50 mg/l and the first set of conditions described in Section 2.3 were used to this aim. Fig. 2 shows the LC chromatogram obtained. In chromatogram (a), registered at 205 nm, the different fractions of the olive oil constituents eluted after the pesticide can be appreciated. In accordance with Señorans et al. [17], these fractions have been assigned to sterolic, squelene and triglyceride fractions. In chromatogram (b), registered at 255 nm, the pesticide fraction is clearly delimited. It is especially important to establish the correct experimental RPLC conditions in order to ensure that the pesticides do not overlap other majority components of the oil, especially the triglycerides. A satisfactory degree of separation between lipids and pesticides was obtained since lipids, primarily long chain fatty acids and esters, are retained more strongly than pesticides in the LC system. In this way when RPLC is employed, the problem of the triglyceride peak tailing into the pesticide fraction when normal phase is used [7] does not arise. This use of reversed-phase liquid chromatography in the pre-separation step is an interesting alternative to normal phase liquid chromatography.

As can be observed from the LC chromatogram (Fig. 2), pesticides eluted rapidly between 0.4 and 1.5 min. The flow rate in the LC system was 2 ml/min, so that the resulting volume of the fraction containing the pesticides was 2.2 ml, which is the volume that must be transferred from the LC to the GC. As was pointed out in Section 2, when an analysis is carried out, the LC flow must be changed to 0.1 ml/min at the exact moment that pesticide elution begins. This flow is maintained during the transfer step. In order to assure that the transfer window does not change at this lower LC flow rate, an LC chromatogram was obtained in these conditions. Fig. 3 shows the LC chromatogram obtained (a) and a blank trace (b). As can be observed, the fractions containing pesticide is fully included in the fraction transferred. Some components of olive oil co-eluted with the pesticides, as can be observed from the blank trace.

When pesticides residue analysis was carried out LC was employed as a sample preparation step so that, once the pesticides had eluted, the LC fraction was transferred to GC and it was not necessary to end the first set of LC conditions. For the rapid elimination of the lipids retained in the LC column, the methanol percentage of the eluent was raised to 100%. However, it should be stressed that in the first conditions the initial composition of the eluent (methanol–water

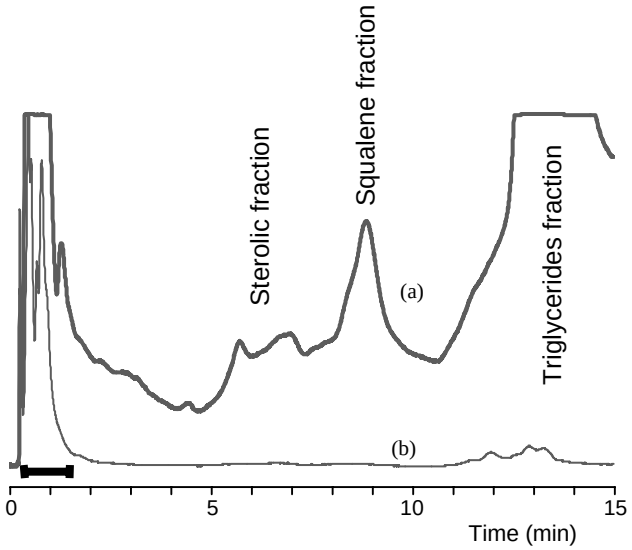

Fig. 2. LC chromatogram of an olive oil spiked with 50 mg/ml of each pesticide obtained in the first set of conditions indicated in Section 2: (a) registered at 205 nm; (b) registered at 255 nm. The thick line between the time axis and the chromatograms indicates the pesticide fraction.

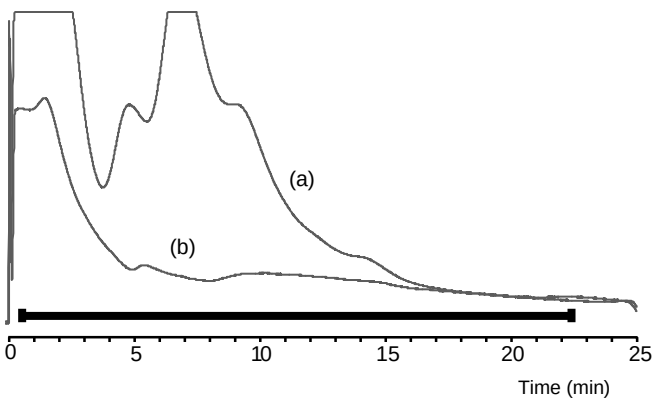

Fig. 3. LC chromatograms obtained in the second set of conditions indicated in Section 2: (a) an olive oil spiked with 50 mg/ml of each pesticide; (b) blank trace. Detection at 255 nm. The thick line between the time axis and the chromatograms indicates the transferred fraction.

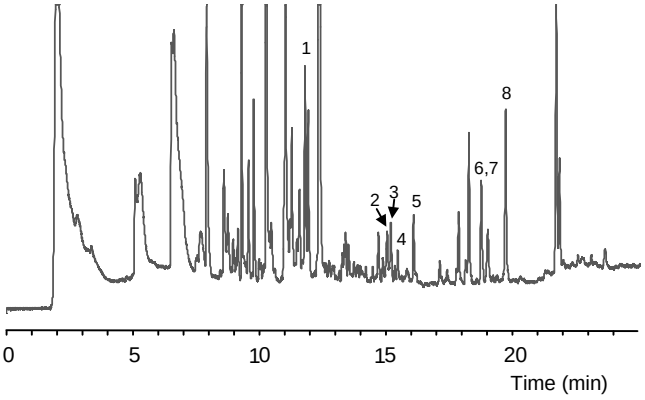

Fig. 4. GC chromatogram of an extra-virgin olive oil sample spiked with 1 mg/ml of each pesticide. Peak identification: (1) carbaryl; (2) atrazine; (3) simazine; (4) lindane; (5) diazinon; (6) fenitrothion; (7) terbutryne; (8) parathion.

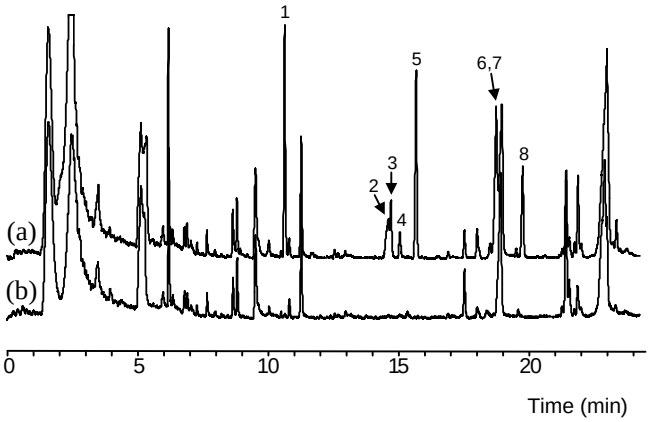

Fig. 5. GC chromatograms of: (a) a refined olive oil sample spiked with 7.5 mg/ml of each pesticide; and (b) blank trace. Peak identification as in Fig. 4.

(70:30, v/v)) was maintained for 3 min, which is longer than the pesticide elution time, so that the mobile phase composition was the same in both LC conditions until pesticide elution finished.

3.2. Transfer and analysis

Pesticide retention and efficient elimination of the extremely large volume of vapour resulting from the methanol–water eluent used as mobile phase in LC was achieved not only in the evaporative mode but also in the solid-phase extraction mode. In the transfer conditions selected, it seems that solvent evaporation plays an important role. Solvent elimination in the evaporative mode is easier if lower introduction speeds are employed, as the requirements for solvent elimination are easily fulfilled if the speed of sample introduction is close to the solvent elimination rate [24]. When the flow rate is decreased, the amount of non-evaporated eluent also decreases and consequently there is a lower loss of analytes. This explains why, in this case, the sensitivity increased as the speed was reduced. For this reason, the flow rate was decreased to 0.1 ml/min during the transfer step. The importance of the injection speed into the PTV in similar evaporative conditions has been pointed out previously [25].

After the transfer step, the eluent in the transfer line is pushed by the helium and discharged, preventing its evaporation and its entrance into the GC column when the oven temperature is raised.

To transfer 2.2 ml at 0.1 ml/min took 22 min. The overall procedure, including LC pre-separation, LC–GC transfer and GC analysis required less than 1 h, a short time compared with the time taken with traditional LLE techniques, which are time-consuming and tedious. Moreover, since the methods is fully automated, it can be used with an autosampler for routine pesticide residue analysis.

To illustrate the potential of the method, Figs. 4 and 5 give the GC chromatograms resulting from the analysis of two different olive oil samples, an extra-virgin (Fig. 4) and a refined oil (Fig. 5). The pesticide peaks are indicated by numbers. Unidentified peaks correspond to components of olive oil which were co-eluted with pesticides in the LC pre-separation step. These components are different for both olive oil samples. A comparison of the chromatograms in Fig. 5 shows that the retention time of components of the olive oil do not match those of the analysed pesticide.

Table 1 shows some parameters related with the quantitative analysis. Considering that the maximum residue levels (MRLs) set by the FAO/WHO Codex Committee for olives and olive oil vary from 10 to 0.5 mg/l [26], the LOD obtained are adequate, although bearing in mind the complexity of the chromatogram, the presence of a pesticide cannot be assured at the LOD concentration. It should be emphasised that a FID was used in order to be able to include in the study pesticides of different chemical natures and sufficiently low LOD were obtained. The use of selective detectors would permit even

Table 1
Detection limit (LOD) calculated as the amount of product giving a signal equal to five times the background noise; recovery, calculated by comparing a splitless injection of a standard solution (1 μl, 100 mg/l each pesticide, four replicates) with the LC–GC analysis of a fortified oil sample (20 μl, 5 mg/l each pesticide, four replicates); correlation coefficient for the linear calibration (R^2) and the relative standard deviation (R.S.D.), $n = 5$, olive oil spiked with 1 mg/l of each pesticide, from the absolute peak areas and from the retention time

Pesticide	LOD (mg/l)	Recovery (%)	R^2	R.S.D.	
				Area	t_r
Carbaryl	0.1	92	0.998	8	0.13
Simazine	0.2	39	0.954	7	0.15
Atrazine	0.2	46	0.970	4	0.13
Lindane	0.3	19	0.995	8	0.11
Diazinon	0.2	34	0.998	9	0.10
Fenitrothion + terbutryne	0.1	57	0.980	8	0.13
Parathion	0.1	33	0.995	6	0.13

lower detection limits, together with better selectivity. In the concentration range tested good linearity was obtained.

The procedure showed good repeatability. It should be pointed out that although the R.S.D. values from the absolute peak areas obtained may be considered slightly high, they correspond to the overall analysis. No variability in the retention time was observed.

Although recoveries are not good, this is not a problem because the repeatability of the procedure permits good linear calibration, so that it is possible to quantify pesticide residues. Another problem usually associated with a poor recovery is sensitivity, although in this case it is sufficient and it can be further improved by using selective detectors.

4. Conclusion

The developed method makes possible the automated multiresidue analysis of different classes of pesticide residues in olive oil in one run, one simple filtration step being required as sample pre-treatment. In routine laboratories, where a large number of samples have to be analysed, automated methods are essential. The TOTAD interface is shown to be highly suitable for the automation of RPLC–GC systems. The described method shows poor recovery, but good repeatability and sensitivity. Accurate quantification is possible.

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