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Direct Analysis of Pesticide Residues in Olive Oil by On-Line Reversed Phase Liquid Chromatography−Gas Chromatography Using an Automated Through Oven Transfer Adsorption Desorption (TOTAD) Interface

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A fully automated on-line reversed phase liquid chromatography−gas chromatography system is described. The system uses a prototype of the automated through oven transfer adsorption desorption interface. The system is demonstrated by presenting a new rapid method for the determination of pesticide residue in olive oil, which is injected directly with no sample pretreatment step other than filtration. Methanol:water is used as the eluent in the LC preseparation step, while the LC fraction containing the pesticide is automatically transferred to the gas chromatograph. Detection limits of pesticides varied from 0.18 to 0.44 mg/L when a flame ionization detector was used. As an example, relative standard deviation and linear calibration are presented for terbutryne.

KEYWORDS: Reversed phase LC-GC; on-line coupling; automated TOTAD interface; pesticide residue analysis; olive oil

1. INTRODUCTION

In chromatographic trace analysis, most of the working time is spent in preparing the sample, which cannot usually be introduced directly into the gas chromatograph (GC). Liquid chromatography (LC) is an alternative to such traditional techniques, and on-line liquid chromatography−gas chromatography (LC-GC) has become a powerful tool for the trace level analysis of complex matrixes. In coupled LC-GC, the specific components of a complex matrix are prefractionated by LC and then transferred on-line to the highly efficient and sensitive GC system for analytical separation. In this way, the LC step replaces the sample preparation steps, including extraction, clean up, and concentration, which are time-consuming, use a large amount of toxic organic solvent, and frequently involve errors and analyte loss.

Automated coupled on-line LC-GC systems have numerous advantages, especially when a large number of samples require analysis. Such a situation frequently arises in food control involving the analysis of toxic components, contaminants, or adulterants, a good example being the analysis of pesticide residues in olive oil. Automated on-line LC-GC eliminates the corresponding manual work, allows complex methods to be performed by nonexperts, and makes analyses more reliable. The technique has been described by a number of authors (*1*−*4*) and used in numerous applications (*5*−*8*), mostly in normal phase, probably because coupling reversed phase liquid chromatography (RPLC) to GC is more complicated than coupling normal phase (*9*, *10*). Nevertheless, in analytical LC, reversed phase predominates. For some samples, reversed phase LC is clearly advantageous because of the range of compounds that can be analyzed. Although several systems have been developed for the LC-GC analysis of pesticide residues in environmental samples and food (*11*−*13*), very few studies exist on reverse phase LC not involving phase switching, i.e., without replacing the water with suitable organic solvent before the GC analysis. Automated direct RPLC-GC has been applied to the analysis of phthalates in water (*14*) and to the analysis of pesticides in red wine (*15*), using a vaporizer/precolumn solvent split/gas discharge interface.

Previous works using on-line LC-GC in the analysis of pesticide residues in edible oil mainly use normal phase (NPLC) in the LC separation step (*11*−*13*). One of the problems in vegetable oil analysis when NPLC is used is the appearance of tailing injection peaks due to the fat present. Reverse phase HPLC with acetonitrile as mobile phase has been used for multipesticide extraction from edible fat and oil (*16*). However, pesticide analysis by direct coupling of RPLC-GC is still difficult. The transfer of polar solvent to the GC is difficult because of the very large volumes of vapor that are produced per unit volume of liquid (*10*). On the other hand, RPLC-GC coupling using a programmed temperature vaporizer (PTV) as

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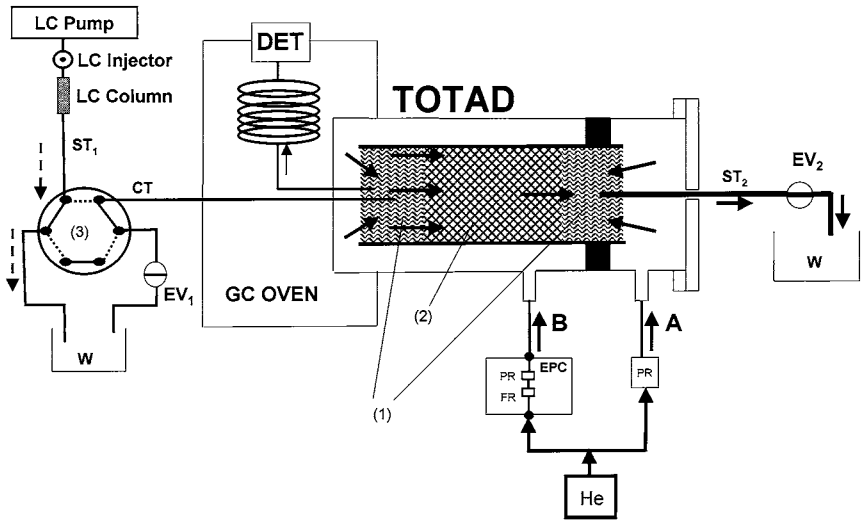

Figure 1. Automated TOTAD interface. Symbols: glass wool (1); sorbent (Tenax TA) (2); six port valve (3); electrovalves 1 and 2 (EV_1 and EV_2); EPC with pressure regulator (PR) and flow regulator (FR); pressure regulator (PR); gas flow (continuous arrows); liquid flow (dotted arrows); stainless steel tubing of dead volume to transfer from LC to GC (ST_1); stainless steel tubing to allow the exit of liquids and gases (ST_2); silica capillary tubing between six port valve and GC (CT); waste (W).

the interface of the system has already demonstrated its usefulness for the analysis of minor components of edible oils (*17*−*20*).

Recently, our research group has developed a new interface named TOTAD (through oven transfer adsorption desorption) for the on-line coupling RPLC-GC. New methods have also been developed to analyze pesticide residues in water, employing the TOTAD interface by RPLC-GC (*21*, *22*) and by very large volume sampling (*23*).

The purpose of this work was to demonstrate the performance of the fully automated TOTAD interface in on-line RPLC-GC by developing a new method for the direct analysis of pesticide residues in olive oil. Several pesticides of different groups were considered and individually analyzed.

An automated laboratory-built TOTAD interface was constructed and used for the on-line coupling of RPLC-GC. In this way, fully automated analyses can be carried out without any kind of sample pretreatment, other than a simple filtration step.

2. EXPERIMENTAL PROCEDURES

2.1. Materials. Olive oils (extra virgin olive oil) were purchased from a local market. As pretreatment 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). The pesticides used for the experiment were as follows: clorpyriphos, fenitrothion, methidathion, parathion (organophosphorus), lindane (organoclorine), carbaryl (carbamate), atrazine, simazine, and terbutryne (triazines). Each pesticide was added to the olive oil at two different concentrations: 50 and 1 mg/L. In the case of terbutryne, concentrations from 0.5 to 10 mg/L were also considered in order to obtain a linear calibration.

Methanol and water high-performance liquid chromatography (HPLC) grade were obtained from LabScan (Dublin, Ireland). Tenax TA 80−100 mesh (Chrompack, Middelburg, Netherlands) was used as packing material in the liner of the PTV. The packed liner was conditioned under a helium stream by heating from 50 to 250 °C in 50 °C steps, holding each step for 10 min. The final temperature of 250 °C was mantained for 60 min.

2.2. Instrumentation. The analyses were performed using on-line coupled LC-GC equipment. An automated TOTAD interface was created by substantially modifying a PTV injector. The TOTAD interface operation mode has been described elsewhere (*21*−*23*). The HPLC system was composed of a manual injection valve (model 7125 Rheodyne, CA) with a 20 μL loop, a quaternary pump (HP model 1100), a column oven (HP model 1100), and a diode array UV detector (Perkin-Elmer model LC 235). The GC (Konik model HRGC 4000B) was equipped with a TOTAD interface and a flame ionization detector (FID). The TOTAD interface was placed horizontally on the left-hand side of the gas chromatograph. EZchrom (Konik, Sant Cugat de Valles, Barcelona) 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, U.K.) was used. All analyses were carried out using methanol/water (70:30; v:v) as the mobile phase and injecting 20 μL of the filtered olive oil. The UV detection was performed at 205 nm. The LC column was maintained at 45 °C, and the flow rate was 2 mL/min until pesticide elution began (this time varied from one pesticide to another) and then changed to 0.1 mL/min within 0.05 min and maintained at this value until the LC-GC transfer step was concluded (this time also varied from one pesticide to another). After the transfer, the flow was changed back to 2 mL/min and the gradient was raised to 100% methanol within 1 min and maintained for at least 20 min to ensure complete elimination of the retained lipids. To ascertain the elution time for each pesticide, 20 μL of olive oil containing 50 mg/L of the pesticide was injected and the LC chromatogram was obtained as described below. The initial composition of the eluent (methanol−water, 70:30 (v/v)) was maintained for 3 min and then reduced to 22% water within 3 min. This percentage of water was maintained for 4 min and then again reduced to 14% within 2 min and maintained for 3 min. Finally, the percentage of water was reduced to 0% within 4 min (LC conditions in **Figure 2**).

2.4. LC-GC Transfer. A manually operated TOTAD interface for on-line RPLC-GC was 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 V_1, V_2, and six port valve used in the previous system (*21*−*23*) were replaced by electrovalves (EV_1, EV_2, and 3 in **Figure 1**). An electronic pressure control (EPC) was used to control helium flow by B, as is shown in **Figure 1**. The GC was connected to the LC system by the automated TOTAD interface and controlled by means the Ezchrom software.

The HPLC detector was used only when the LC fraction to be transferred to the GC was selected. Once this was established, the LC column was directly connected to the six port valve by a stainless steel tube (0.25 mm i.d.).

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 **Figure 1**). The choice of the transfer conditions was based on our previous work concerning different experimental designs (unpublished data). The

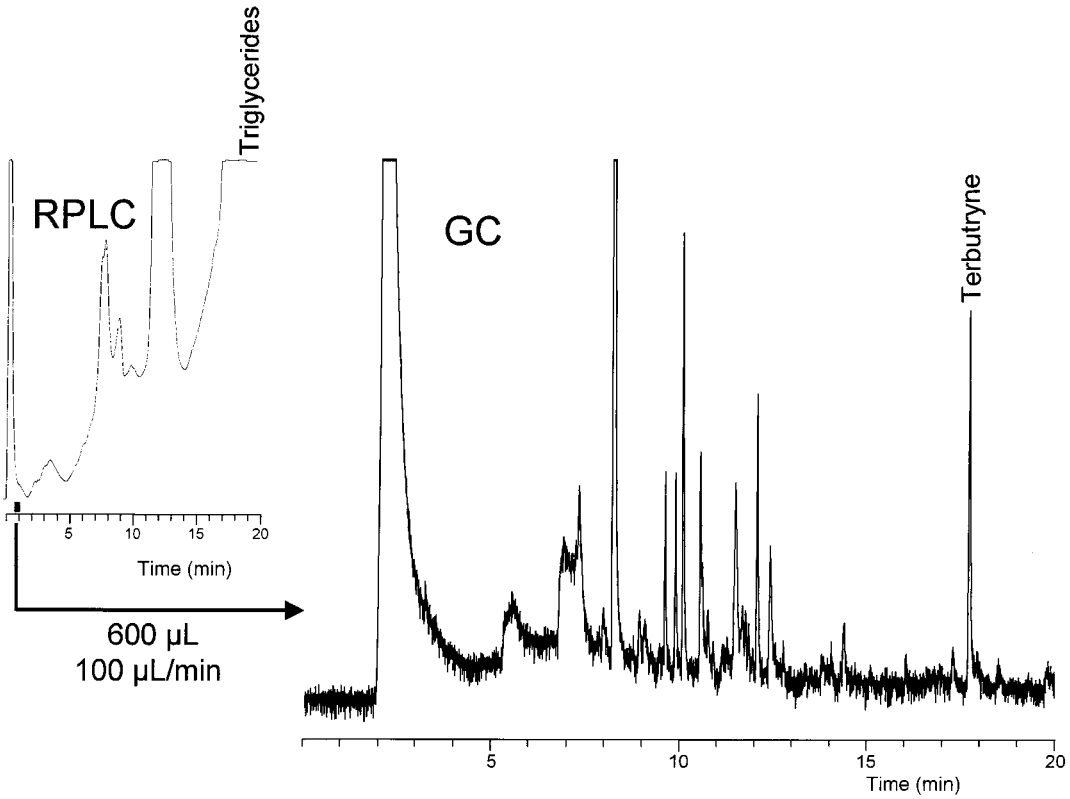

Figure 2. LC and GC chromatograms obtained from the direct LC-GC analysis of an olive oil spiked with 1 mg/L terbutryne. Conditions are indicated in the Experimental Procedures. The thick line situated between the time axis and the chromatogram indicates the LC fraction that has been transferred from LC to GC.

conditions were used as follows: Tenax TA as packing material in the glass liner of the PTV; 1 cm plug length of packing material; 90 °C initial PTV temperature. During the five steps of the TOTAD interface operation, the conditions used were as follows.

2.4.1. Interface Stabilization. Helium flow, 1500 mL/min by A and 1500 mL/min by B (see **Figure 1**). The TOTAD interface temperature stabilized at 90 °C. The GC oven temperature was maintained at 40 °C. The eluent from the HPLC was sent to waste.

2.4.2. Transfer. When the beginning of the fraction of interest reached the six port valve, this was automatically switched and the pump flow changed from 2 to 0.1 mL/min. This flow was maintained until the end of the fraction of interest reached the GC injector.

2.4.3. Remaining Solvent Elimination. Once the transfer step was completed, the six port valve was automatically switched and electrovalve EV_1 was opened. The LC eluent was sent to waste, and the remaining solvent in the CT tube was pushed out by the helium. These conditions were maintained for 1 min in order to eliminate the remaining solvent.

2.4.4. Thermal Desorption. Electrovalves EV_1 and EV_2 were closed. The TOTAD interface was quickly heated to 250 °C and maintained at this temperature for 5 min. After thermal desorption of the analyte, it was transferred to the GC column, pushed by the helium. Then, the GC conditions for the analysis were programmed.

2.4.5. Cleaning. When the GC analysis was finished, electrovalve EV_2 was automatically opened, the TOTAD interface was maintained at 250 °C, and the helium flow at 1500 mL/min. Afterward, it was cooled to 90 °C so that another analysis could be carried out.

2.5. GC Conditions. Gas chromatographic separations were carried out on a Quadrex (Weybridge, U.K.) fused silica column (30 m × 0.32 mm i.d.) coated with 5% phenyl methyl silicone (film thickness 0.25 µm). The column temperature was maintained at 40 °C for 3 min, programmed to 170 °C at 20 °C/min, then to 190 °C at 4 °C/min, and finally to 210 °C at 10 °C/min. This final temperature was maintained for 10 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. During the transfer and solvent elimination steps, the oven temperature was kept at 40 °C.

3. RESULTS AND DISCUSSION

Figure 2 shows the liquid chromatogram obtained by direct injection of 20 µL of olive oil spiked with terbutryne at 1 mg/L and the gas chromatogram resulting from transferring 0.6 mL of eluent into the GC system. The other peaks in **Figure 2** have not been identified. RPLC conditions were established in order to ensure that the pesticides were isolated from the triglycerides of the matrix. In this way, RPLC functions as a sample preparation step. Satisfactory separation between olive oil triglycerides and pesticides was obtained. The triglycerides were more strongly retained than the pesticides in the LC system and so were eluted after them. The problem of the triglyceride peak tailing into the pesticide fraction when NPLC is used (*24*) does not arise in this case, which is a considerable advantage bearing in mind the large size of the triglyceride peak. Hence, the use of RPLC in the preseparation step is an interesting alternative. The initial time and the volume of the fraction to be transferred from LC to GC varied from one pesticide to another (**Table 1**), but all of the used pesticides eluted in the first 2 min and before the triglycerides started eluting.

As can be seen from the GC chromatogram (**Figure 2**), solvent elimination, which is carried out in both the evaporative and the nonevaporative modes, is almost complete. In fact, the packed liner acted as a solid phase extraction cartridge and the analytes were retained in the packing material, while the solvent was pushed through the liner as liquid and vapor by the helium flow.

The importance of the speed with which the sample is injected into the PTV has been pointed out previously (*25*, *26*), a lower speed providing increased sensitivity. It is clear, too, that solvent elimination in the evaporative mode is easier if low introduction speeds are employed. For this reason, the flow rate was decreased to 0.1 mL/min during the transfer step, at which rate

Table 1. Initial Times (T_i), Final Times (T_f), and Volumes (V) for the LC to GC Transfer of the Pesticides and Their Limits of Detection (LOD)

pesticides	T_i (min)	T_f (min)	V (mL)	LOD (mg/L)
carbaryl	0.4	0.6	0.4	0.44
simazine	0.4	0.6	0.4	0.44
methidathion	0.5	1	1	0.28
atrazine	0.53	0.70	0.34	0.34
fenitrothion	0.60	1.10	1	0.18
terbutryne	0.70	1	0.6	0.44
parathion	0.80	1.10	0.6	0.35
lindane	1	2	2	0.40
clorpyriphos	1.35	2	1.3	0.38

the transfer time varied from 3.40 (in the case of atrazine) to 20 min (in the case of lindane). The overall procedure, including LC preseparation, LC-GC transfer, and GC analysis, took from 30 (carbaryl) to 50 min (lindane).

The detection limit for the pesticides analyzed, calculated as the amount of product giving a signal equal to five times the background noise, is given in **Table 1**. Maximum residue levels have been set by the FAO/WHO Codex Committee for several pesticides in olives and olive oil and by the European Union for olives only (*27*). Their limits vary from 10 to 0.5 mg/L, while the detection limit obtained with the system described in this paper was lower than 0.5 mg/L for all of the pesticides used, although a FID detector was used. The use of selective detectors will permit even lower detection limits.

The repeatability of the LC-GC system was determined from five injections of an olive oil spiked with 1 mg/L of terbutryne. The relative standard deviation (RSD) of 9.3% was calculated from the absolute peak areas. It should be emphasized that this RSD value corresponds to the overall analysis, so that it may be affirmed that good repeatability was achieved. No variability was observed in the retention time. When the linearity was tested for terbutryne in a range of 0.5−10 mg/L, good linearity was achieved with a correlation coefficient of 0.998.

Figure 3 shows the gas chromatograms of real olive oil samples made from olives grown in an experimental plot and treated with fenitrothion (**a**) and with clorphyriphos (**b**). The olives were harvested in mid-December, and the oil was extracted immediately in the laboratory. As shown in **Figure 3**, both the fenitrothion and the chlorpyriphos from different samples coeluted in the GC, but because they come from different LC fractions, that is not a problem. The residues found in the oil amounted to 2.1 and 2.8 mg/L for clorpyriphos and fenitrothion, respectively.

The described method permitted the automated analysis of residues from different pesticide groups in a complex matrix such as that represented by olive oil, without the need of a pretreatment step. In laboratories where a large number of samples need to be analyzed, automation is necessary, and the analysis described here demonstrates the usefulness of the automated RPLC-GC system for such a purpose. The TOTAD interface is shown to be suitable for automating RPLC-GC, an advantage that practically eliminates the time-consuming sample preparation step so that the olive oil only had to be filtered and loaded directly into the HPLC. However, new methods that can be used to quantify a relatively large number of pesticides in only one run are also necessary. Now that we have built an automatic TOTAD interface and have demonstrated the ability of the system to analyze pesticide residues in olive oil, experiments are being carried out in our laboratory to develop multiresidue methods with selective detectors.

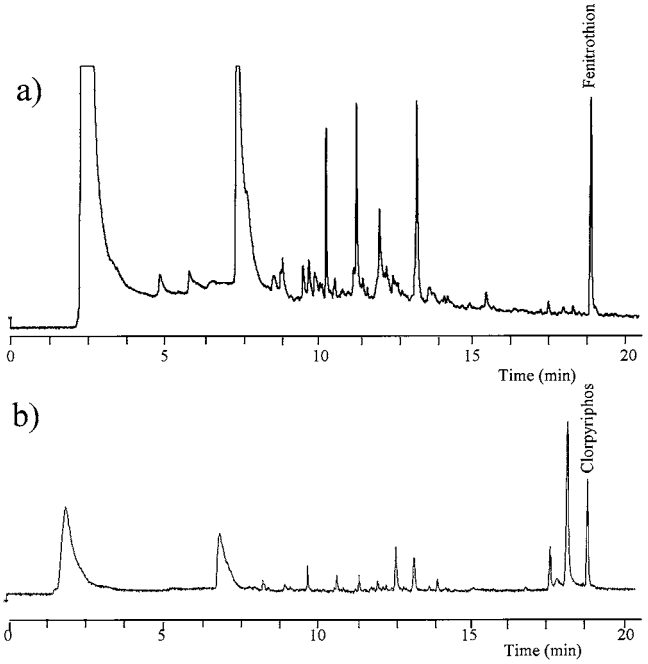

Figure 3. GC chromatograms of real olive oil samples. Conditions are indicated in the Experimental Procedures. Sample **a** corresponds to an olive oil with fenitrothion residue, and sample **b** corresponds to an olive oil with clorpyriphos residue.

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