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Large Volume GC Injection for the Analysis of Organophosphorus Pesticides in Vegetables Using the Through Oven Transfer Adsorption Desorption (TOTAD) Interface

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A simple, rapid and sensitive multiresidue method has been developed for the determination in vegetables of organophosphorus pesticides commonly used in crop protection. Pesticide residues are extracted from samples with a small amount of ethyl acetate and anhydrous sodium sulfate. No additional concentration and cleanup steps are necessary. Analyses are performed by large volume GC injection using the through oven transfer adsorption desorption (TOTAD) interface. The calculated limits of detection for each pesticide injecting 50 μL of extract and using an NPD are lower than 0.35 μg/kg which is much lower than the maximum residues levels (MRLs) established by European legislation. Repeatability studies yielded a relative standard deviation lower than 10% in all cases. The method was applied to the analysis of eggplant, lettuce, pepper, cucumber, and tomato.

KEYWORDS: Large volume GC injection; TOTAD interface; organophosphorus pesticide residue analysis; vegetables

1. INTRODUCTION

Pesticides are essential in modern agriculture to control pests and to increase harvest productivity; however, due to their potentially dangerous effects on human health, the control of pesticide residue in food is of great importance in order to minimize risk to consumers. Organophosphorus pesticides are widely used in crop protection, and because of their lipophilic properties, residues of the same may accumulate in the human body.

Any analytical method must be fast, easy, inexpensive, and applicable, with slight modifications, to different matrixes. Gas chromatography (GC) is a separation technique widely used in the analysis of pesticide residues because of its high separation power and the variety of sensitive and selective detectors, such as, electron capture detector (ECD), nitrogen−phosphorus detector (NPD), and mass spectrometry, that can be used (*1*−*5*). Liquid chromatography coupled to mass spectrometry (LC−MS) has been used in recent years in the determination pesticides of low volatility or thermolability (*6*, *7*).

Sample preparation is an important step in pesticide residue analyses, which is time consuming and requires the use of large amounts of organic solvents. The pesticides are extracted by different organic solvents such as acetone (*1*), acetonitrile (*8*), or ethyl acetate (*9*) which usually provide high recoveries of pesticides over a wide range of polarity, followed by partitioning by ethyl acetate−cyclohexane (*10*) or dichloromethane−petroleum ether (*11*). A further cleanup step is generally required before chromatographic analysis. Cleanup techniques include gel permeation chromatography (GPC) (*12*), solid-phase extraction (SPE) (*13*), and the use of Florisil, silica, or aluminum oxide liquid chromatography (*14*). These cleanup procedures are time consuming and are susceptible to solute loss and contamination, particularly when operating at trace levels. The use of selective detectors in GC reduces the amount of cleanup necessary for the removal of interfering coextracted components in the analysis. GC−MS systems have arisen as a powerful tool, and many studies have reported the use of GC−MS to analyze pesticide residues in vegetables using either electron impact (EI) or positive chemical ionization (PCI) (*15*).

In the AOAC Official Method 985.22 (*16*), pesticides are extracted with acetone followed by partitioning with dichloromethane−petroleum ether. Solid NaCl is added to saturate the aqueous phase, and the organic phase is dried with Na_2SO_4. An aliquot of concentrated organic phase is injected into the GC for pesticide determination. This method and its many variations (*17*, *18*) are still widely used by pesticide residue monitoring laboratories worldwide.

Supercritical fluid extraction (SFE) is an alternative to the use of organic solvents for extracting pesticides from vegetable (*19*). SFE conditions can be adjusted to provide a more selective

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extraction ability, which furthermore, does not require cleanup steps before GC analysis.

The strict regulations imposed by the European Union (Directive 92/82/CE), with increasingly strict maximum residue levels (MRLs) in recent years has made it necessary to lower the limit of detection reached by multiresidue methods. The use of large volume injection (LVI) techniques is an alternative in this respect. Several injection techniques have been developed that allow the injection of up to several microliters into a capillary GC while maintaining good chromatographic characteristics (*20*). On column injection using a retention gap and working under partially concurrent solvent evaporation is a frequently used technique. Usually, an early solvent vapor exit (SVE) is installed to protect the analytical column and the detector. In this method, a preliminary cleanup is always necessary because an impure extract can contaminate the retention gap and cause analyte adsorption, distorted peak shapes, and even loss of analytes due to catalytic degradation (*21*). The conventional split/splitless injector has been used for the injection of 10 μL of sample in the analysis of pesticides in vegetables (*15*, *22*). Large volume sampling using a programmed temperature vaporization (PTV) with a carbofrit inserted in the liner has also been used (*11*). In such technique, the injector port initial temperature must be maintained at the solvent boiling point while the split vent is open, and after a time, the split vent is closed and the injector is heated to enable the analytes to enter into the column. In this injection technique, the solvent is eliminated as vapor (evaporative mode) via the split line. This PTV operative mode is only recommended for the determination of solutes with high boiling points, because most volatile compounds are partly lost by evaporation with the solvent. The selection of packing material for the PTV liner depends on the volatility and the polarity of the pesticides. Phenylmethylsilicone chemically bonded silica (PMSS) has been used as a new packed material (*23*). The TOTAD interface is a modified PTV injector that allows large volume injection of polar solvents into the capillary GC and the on-line coupling reversed-phase liquid chromatography−gas chromatography (RPLC−GC). The interface has been used to analyze pesticide residues in water by very large volume sampling (*24*), and some methods for analyzing pesticides in water (*25*) and olive oil (*26*, *27*) by RPLC−GC using the TOTAD interface have been previously developed.

In this study the TOTAD interface was used for the analysis of organophosphorus pesticides in different vegetables by injecting large volumes of noncleaned extracts into the GC.

The aim of this work was double: to demonstrate the performance of the TOTAD interface for large volume injection of nonpolar solvent extract and to test a fast and low solvent consuming extraction step which, together, would allow development of a rapid, easy, and sensitive analytical method for organophosphorus pesticides in vegetables

2. EXPERIMENTAL PROCEDURES

2.1. Materials. Vegetables (eggplant, lettuce, pepper, cucumber, and tomato) were purchased from a local market or picked from the field. Tomato field samples were obtained from a plantation located in the province of Toledo (Spain) which was treated with some of the target pesticides. Pesticide standards were obtained from Chem. Service Inc. (West Chester PA, SA). The organophosphorus pesticides used for the experiment were dimethoate, chlorpyrifos, diazinon, fenitrothion, malathion, chlorfenvinphos, methidathion, fenthion, and tetrachlorvinphos. Pesticide-grade ethyl acetate and anhydrous sodium sulfate were obtained from Merck (Darmastad, Germany). Ethyl acetate was used to extract the samples and as mobile phase to push the volume of extract into the TOTAD interface.

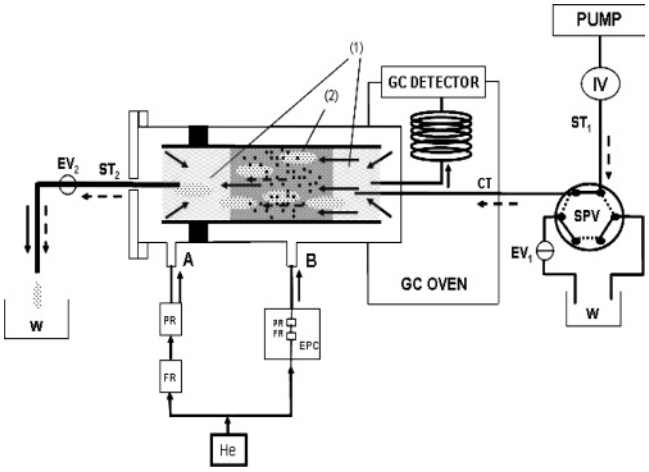

Figure 1. Automated TOTAD interface during the sampling step. Symbols: 1, glass wool; 2, sorbent (Tenax TA); IV, LC manual injection valve; SPV, six-port valve; EV_1 and EV_2, electrovalves 1 and 2; EPC, electronic pressure control; PR, pressure regulator; FR, flow regulator; solid arrows, gas flow; dotted arrows, liquid flow; ST_1, stainless steel tubing, 0.25 mm i.d., to transfer extract from the LC injection valve to the GC; ST_2, stainless steel tubing, 1 mm i.d., to allow the exit of liquids and gases; CT, silica capillary tubing, 0.32 mm i.d.; W, waste; ovals, solvent; dots, analytes.

The methanol used to dissolve the pesticides was HPLC grade from Pestican (LabScan, Dublin, Ireland). A stock solution of 100 mg/L of each pesticide was prepared in methanol and stored at 4 °C. The working pesticide solution (1 mg/L) used for sample fortification was prepared by diluting the stock solution in methanol. The glass liner of the modified PTV (TOTAD interface) was packed with 1 cm of Tenax TA 80-100 mesh (Chrompack, Middelburg, Netherlands) between two plugs of glass wool to keep it in place, then it was conditioned under a helium stream, by heating from 50 to 350 °C at 50 °C/10 min and maintained for 60 min at this final temperature.

2.2. Sample Preparation. A representative portion of vegetable (roughly 200 g) was chopped with a food mixer in order to obtain a homogeneous sample. Then, 2.5 g of chopped sample were weighed and fortified with 250 μL of the stock solution in order to give 10 mg/kg pesticide concentration in the vegetable or with aliquots of the working standard pesticide solution (volume varied form 25 to 250 μL) in order to give pesticide concentrations in the vegetable ranging from 0.01 to 0.1 mg/kg. An amount of 2.5 g of the sample (fortified samples or real samples) was mixed with 5 mL of ethyl acetate and 2 g of anhydrous sodium sulfate. After extraction for 1 min with a high-speed blender, the extract was filtered through a 0.22 μm (Millex-GN SLGN 013 NL) filter.

2.3. Instrumentation. A Konik 4000B gas chromatograph, equipped with a TOTAD interface and FID and NPD was used. The TOTAD interface (U.S. patent 6,402,947 B1, exclusive rights assigned to KONIK-Tech, Sant Cugat del Vallés, Barcelona, Spain) was used for injecting a very large volume of extract into the GC.

For very large volume sampling a manual injection valve (model 7125 Rheodyne, CA) provided with a variable volume loop (20, 40, 50, 60, 80, and 100 μL) was used. A quaternary pump (HP model 1100) was used to push the large volume of extract into the TOTAD interface.

KoniKrom 32 (Konik, Sant Cugat del Vallés, Barcelona) software was used to obtain data from the GC and to automate the process.

2.4. TOTAD Operation Mode. Figure 1 shows a scheme of the TOTAD interface during the sampling step. The operation mode involves the five steps detailed below.

2.4.A. Stabilization. The TOTAD interface and GC oven temperature are stabilized at 100 and 40 °C, respectively. The carrier gas (helium) flow enters in the packed liner both through the oven side (B) and through the opposite side (A) at 500 mL/min. EV_1 is closed and EV_2 opened. The pump is stabilized at the sampling flow.

2.4.B. Sampling. The extract is introduced in the LC manual injection valve. When this valve is switched, the solvent coming from the pump

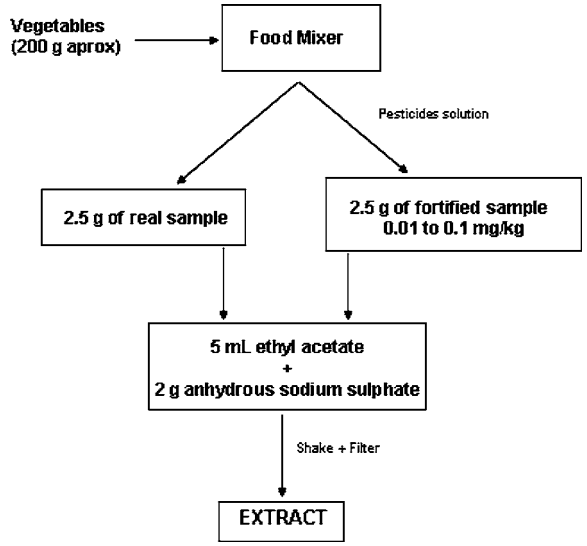

Figure 2. Flow diagram of the steps in the analytical method.

pushes the sample through the stainless steel tube (ST_1 in **Figure 1**) to the six-port valve, which is automatically switched, transferring the large volume of extract to the GC. The solution reaches the glass liner at 0.1 mL/min when an FID was used or 0.05 mL/min when an NPD was used. The sampling time varies with both the injection flow and the volume to be sampled. The helium pushes the solution through the sorbent. Analytes are retained, and solvent is vented to waste through ST_2 tubing.

2.4.C. Remaining Solvent Elimination. The six-port valve is automatically switched so that the solvent coming from the pump is sent to waste. The EV_1 is opened. Helium eliminates the remaining solvent in the liner and pushes the solution remaining in the transfer capillary (CT) to the waste. These conditions are maintained for 2 min to completely eliminate the solvent.

2.4.D. Thermal Desorption. After solvent elimination EV_1 and EV_2 are closed, and helium enters only through the usual gas inlet to reach a PTV injector and to exit only through the GC column. The TOTAD interface is heated to 275 °C and maintained at this temperature for 5 min to achieve the thermal desorption of the retained solutes and their subsequent transfer to the capillary GC column.

2.4.E. Cleaning. After the analysis, the valves and helium flow are changed to the stabilization conditions and the interface is maintained under the helium stream for 5 min at 300 °C. Afterward it is cooled to 100 °C so that step A can begin again.

2.5. GC Conditions. GC separations were carried out on a 5% phenylmethylsilicone fused-silica column (30 m × 0.32 mm i.d., 0.25 μm film thickness) (Quadrex, Weybridge, U.K.) with helium as the carrier gas (flow rate 1.8 mL/min). During the transfer and solvent elimination steps, the oven temperature was kept at 40 °C. The column temperature was maintained at 40 °C for 1 min, programmed to 170 °C at 20 °C/min, then to 210 °C at 3 °C/min, and to 230 °C at 5 °C/min, holding the final temperature for 5 min. The FID or NPD temperature was kept at 250 °C.

3. RESULTS AND DISCUSSION

3.1. Extraction Procedure. The method described in this paper can be used for the determination of organophosphorus pesticides in different vegetables. The method was applied to the analysis of fortified samples of eggplant, cucumber, pepper, lettuce, and tomato. No significant differences were observed within the different vegetables used. The extraction procedure, similar to that used by Agüera et al. (*28*) with slight modification, is a fast and simple extraction step with ethyl acetate and anhydrous sodium sulfate added to improve the extraction of polar pesticides, such as dimethoate. **Figure 2** shows a flow diagram of the steps in the analytical method.

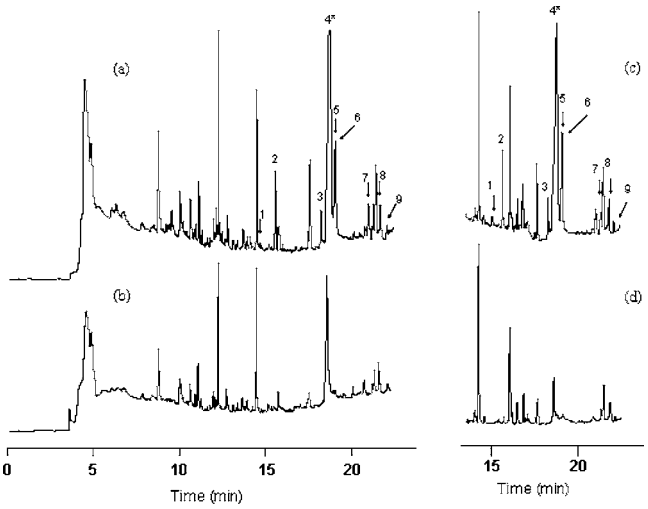

Figure 3. GC chromatograms obtained in the LVI-GC-FID analysis of vegetal samples. On the left the GC chromatograms correspond to (**a**) a tomato sample fortified at 10 mg/kg and (**b**) a blank trace. On the right the GC chromatograms correspond to (**c**) a cucumber sample fortified at 10 mg/kg and (**d**) a blank trace. The peaks identified correspond to the following: 1, dimethoate; 2, diazinon; 3, fenitrothion; 4, malathion plus matrix compounds; 5, fenthion; 6, chlorpyrifos; 7, chlorfenvinphos; 8, methidathion; 9, tetraclorvinphos. In both cases the volume of extract injected was 20 μL. Chromatographic conditions are indicated in the Experimental Procedures.

Most multiresidue methods use a 50−100 g sample, requiring the use of large solvent volumes. The desire to miniaturize analytical methods has made sample diminution increasingly important. Several authors have used smaller samples (*29*, *30*) and concluded that 10 g of sample is acceptable. Lehotay et al. (*31*) showed that a sample as low as 2 g was satisfactory for fortified pesticides in potato. Based on our experience we choose a sample size of 2.5 g which permits meaningful results be obtained, with a very small volume of solvent (5 mL). In most current analytical methods, about 100−250 mL of extractor solvent must be used in order to obtain a volume of concentrated extract higher than 1 mL to obtain the necessary sensitivity if about 1 μL is to be sampled in the GC. The amount of solvent cannot be lower because it is difficult to handle volumes of concentrated extract below 1 mL. However, in the analytical method developed here, only 5 mL of extracting solvent is used because there is no concentration step before sampling. It is not difficult to handle 5 mL of extractor solvent, which explains why such a low amount of solvent and sample can be used in this method.

3.2. Determination of Pesticides Residues. First at all the method was optimized using an FID because of its robustness and because coextracted compounds are also detected. **Figure 3** shows the gas chromatograms of a tomato sample spiked at 10 mg/kg with each pesticide (**Figure 3a**) and a blank sample (**Figure 3b**) when an FID was used. The detection limits (LODs) obtained, calculated as the amount of product giving a signal equal to 5 times the background noise, varied from 0.4 to 2.25 mg/kg (**Table 1**). Although the chromatograms presented in **Figure 3** show some peaks from the matrix, identification of the peaks corresponding to the pesticide could still be carried out without difficulty. Only the peak corresponding to malathion eluted at the same retention time as another peak corresponding to a matrix compound so that this pesticide might give a false positive when an FID is used and no cleanup is carried out. In agreement with Anastassiades et al. (*29*) ethyl acetate provided a lower amount of coextractives than acetone and acetonitrile.

Table 1. Detection Limits (LODs) of the GC-FID Analytical Method When 20 μL of Extract Was Injected, Calculated as the Amount of Product Giving a Signal Equal to 5 Times the Background Noise, and Correlation Coefficients for the Linear Regression of the Absolute Peaks Areas versus Volume Injected (Volumes Varied from 20 to 100 μL)[a]

pesticide	LOD (mg/kg)	R^2
dimethoate	1.85	0.989
diazinon	0.50	0.954
fenitrothion	0.50	0.986
fenthion	0.41	
chlorpyrifos	0.48	
chlorfenvinphos	0.77	0.966
methidathion	0.62	0.993
tetrachlorvinphos	2.25	0.966

[a] Fenthion and chlorpyrifos peaks overlapped when the highest volume was sampled, and so R^2 could not be correctly calculated.

One way in order to avoid this problem could be to carry out the analysis of the extract by on-line RPLC−GC-FID, in which the RPLC acts as a cleanup step. When the on-line RPLC−GC-FID analysis of the extract was tested following the same procedure as used for pesticide residue analysis in olive oil (*26*), some coextracted compounds were eliminated but not all. The improvement obtained with RPLC−GC-FID does not justify the use of what is a more complex and less flexible technique (for instance sample volume cannot easily be increased).

Another alternative to solve this problem could be to use a more selective detector, such as an NPD, as demonstrated below.

The procedure was applied to the analysis of different vegetables (eggplant, lettuce, pepper, and cucumber). The GC chromatograms were very similar to that of the tomato sample, meaning that the matrix effect was in general low and that the proposed method can be applied to different vegetables without modification. **Figure 3** shows also the GC chromatogram obtained in the analysis of a cucumber sample fortified at 10 mg/kg (**Figure 3c**) and a blank sample (**Figure 3d**).

The possibility of increasing the extract volume injected permits lowers LODs. Another option is to use a more sensitive detector, such as an NPD. Both options can be combined. Other authors have analyzed pesticides in vegetables using large volume injection (LVI) with a split/splitless injector but did not recommend the injection of volume larger than 10 μL because there is no improvement in the S/N ratio and frequent maintenance of the GC system (injector, columns) is necessary (*15*). The TOTAD interface, on the other hand, allows larger volumes to be injected, and an extract volume as large as 1 mL has been sampled without problems because solvent vapors do not reach the head of the GC column and because solvent elimination is almost total (*24*). Sensitivity is one of the most important parameters in pesticide residue determination. The LODs obtained injecting 20 μL of the extract were not low enough to monitor pesticide at MRLs, while the injection of larger volumes of extract increased sensitivity. A linear increase of sensitivity with the volume sampled is observed. **Table 1** gives the correlation coefficients of the linearity of the absolute peak areas with the volume injected (volumes varied from 20 to 100 μL).

Once the method had been tested using an FID, the second step was to validate the method using an NPD. In this case, 50 μL of extract was sampled at 0.05 mL/min. Lower detection limits were obtained (**Table 2**), together with better selectivity, as can be observed in **Figure 4**.

Table 2. Relative Standard Deviation (RSD), from the Absolute Peak Areas and from the Retention Time, $n = 3$, of a Tomato Sample Fortified at 0.05 mg/kg for Each Pesticide, for the Whole Analytical Procedure (Extraction and GC Analysis) When an NPD Is Used[a]

pesticide	CV (area)	CV (t_r)	LOD (μg/kg)	R^2
dimethoate	2.4	0.16	0.07	0.996
diazinon	0.3	0.17	0.07	0.977
fenitrothion	4.4	0.20	0.08	0.969
malathion	3.6	0.21	0.07	0.991
fenthion	8.4	0.21	0.06	0.969
chlorpyrifos	2.0	0.20	0.06	0.977
chlorfenvinphos	4.7	0.19	0.10	0.989
methidathion	4.3	0.20	0.15	0.987
tetrachlorvinphos	2.4	0.17	0.34	0.988

[a] Detection limits (LODs) calculated as the amount of product giving a signal equal to 5 times the background noise and correlation coefficients for the linear calibration (R^2).

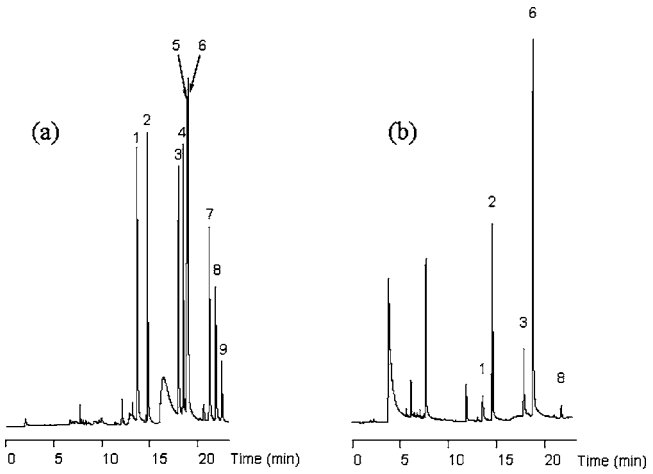

Figure 4. (**a**) GC chromatogram obtained in the LVI-GC-NPD analysis of a tomato sample fortified at 0.05 mg/kg. (**b**) GC chromatogram obtained in the GC-NPD analysis of a real tomato sample containing some pesticide residues. The identification of the peaks is the same as in Figure 3. The volume of extract injected is 50 μL. Chromatographic conditions are indicated in the Experimental Procedures.

3.3. Method Validation. *3.3.A. Linearity.* The linearity of the method was determined for each pesticide using tomato samples fortified in the range from 0.01 to 0.1 mg/kg and considering the absolute peak areas. It must be stressed that no internal standard was necessary. Good linearity was found for all the pesticides with determination coefficients ranging from 0.969 to 0.996 (**Table 2**).

3.3.B. Repeatability. The repeatability of the chromatographic method was determined by performing the analysis of an extract obtained from a tomato sample fortified at 0.05 mg/kg. The same extract was injected three times. The coefficients of variation (CV) for absolute peak areas were lower than 7%, whereas for retention time they ranged from 0.14 to 0.25%. The repeatability for the whole analytical procedure was also determined carrying out the overall procedure (extraction and GC analysis) with the same fortified samples three times. The CV for the retention time and for absolute peak areas is indicated in **Table 2**.

3.3.C. Detection Limits. The LODs of the proposed method using an NPD and injecting an extract volume of 50 μL were determined by considering a value of 5 times the background noise obtained for a blank sample (**Table 2**).

Table 3. Maximum Residue Limits (mg/kg) Established by Spanish Legislation

	eggplant	lettuce	pepper	cucumber	tomato
dimethoate	1.00	1.00	1.00	1.00	1.00
diazinon	0.50	0.02	0.50	0.02	0.50
fenitrothion	0.50	0.50	0.50	0.50	0.50
malathion	3.00	3.00	3.00	3.00	3.00
fenthion	0.05	0.05	0.05	0.05	0.05
chlorpirifos	0.50	0.05	0.50	0.05	0.50
chlorfenvinphos	0.10	0.10	0.10	0.10	0.10
methidathion	0.02	0.02	0.02	0.02	0.02
tetrachlorvinphos	0.05	0.05	0.05	0.05	0.05

3.4. Analysis of Real Samples. The analytical method was applied to the analysis of real tomato samples. One of them was a tomato sample harvested from an experimental plot which was treated, 2 weeks before harvest, with the pesticides with the doses obtained diluting in 100 L of 300 mL of dimethoate 40% weight/volume (w/v); 300 mL of diazinon 60% (w/v); 300 mL of fenitrothion 50% (w/v); 400 mL of chlorpyrifos 48% (w/v), and 300 mL of methidathion 40% (w/v). The tomatoes were harvested in October and were frozen immediately until the analysis was carried out. **Figure 4b** shows the GC chromatogram obtained that contained the following pesticides (μg/kg): dimethoate (13), diazinon (62), fenitrothion (24), chlorpyrifos (73), and methidathion (14). The concentration for all the pesticides was lower than the MRLs established the Spanish legislation (**Table 3**). No residues of the target pesticides were found in other real samples analyzed, purchased in local market.

3.5. Conclusion. The present method based on a rapid extraction with ethyl acetate and large volume injection of the extract into the GC allows the determination of organophosphorus pesticides in different vegetables. The described method reduces the amount of solvent used, minimizes the number of analytical steps necessary, and avoids laborious and time-consuming cleanup steps. The method gives good linearity and repeatability. For all the pesticides, the sensitivity was good enough to ensure a reliable determination at levels much lower than the respective MRLs established by Spanish legislation.

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