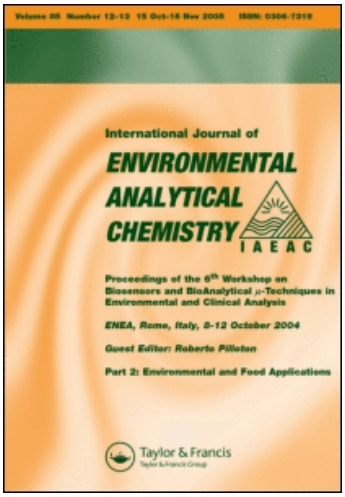

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Optimization of headspace solid-phase microextraction conditions for the determination of organophosphorus insecticides in olive oil

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OPTIMIZATION OF HEADSPACE SOLID-PHASE MICROEXTRACTION CONDITIONS FOR THE DETERMINATION OF ORGANOPHOSPHORUS INSECTICIDES IN OLIVE OIL

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A method based on headspace solid-phase microextraction (HS-SPME) technique followed by gas chromatography with flame thermionic detection was developed for the determination of seven organophosphorus (OP) insecticide residues in olive oil samples. The extraction capacity of four fiber coatings, polyacrylate (PA 85 μm), poly(dimethylsiloxane) (PDMS 100 μm), carbowax-divinylbenzene (CW-DVB 65 μm) and poly(dimethylsiloxane)divinylbenzene (PDMS-DVB 65 μm), have been studied and compared. The method was developed using spiked olive oil samples in the concentration range 0.025 to 0.5 mg/L. The PDMS 100-μm fiber showed good extraction efficiency for the target compounds. An increase in the extraction efficiency of OP insecticides was observed when the parameters affecting the HS-SPME process such as temperature, extraction time, salt additives and stirring rate were optimized. Good linearity was observed for the compounds in the tested concentration range. The limits of detection were between 0.005 and 0.01 μg/L.

Keywords: Olive oil analysis; OP insecticides; HS-SPME; Gas chromatography

INTRODUCTION

Pesticide residue analysis in food is nowadays a priority objective in pesticide research in order to get an extensive evaluation of food quality and avoid possible risks to human health. Organophosphorus (OP) insecticides are widely used in agriculture to combat a large number of pests in a great variety of crops. The utilization of these pesticides is favored over their more persistent organochlorine counterparts because of their ability to degrade more readily in the environment. OP insecticides demonstrate rather low environmental persistence but high toxicity. As a consequence, the determination of OP insecticide residues in crops has been strictly regulated by governments in all countries, with two basic aims, namely, to detect the presence of forbidden pesticides on a particular commodity and to determine whether the concentrations of the pesticides used exceed their maximum residue limits (MRLs) [1, 2].

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To achieve a practical and reliable method for the determination of pesticide residues in complex matrices such as food samples, several sample-preparation methods have been developed including liquid–liquid extraction (LLE) [3], supercritical fluid extraction (SFE) [4], solid-phase extraction (SPE) [5, 6] and solid-phase microextraction (SPME), [7, 8]. However, in LLE and SPE large quantities of solvent waste are generated, multiple operation steps are needed, preconcentration of the extract prior to analysis is required and interfering compounds are more likely to be coextracted. On the other hand, headspace sampling and purge-and-trap methods are simpler, less laborious, faster and solvent-free techniques. Nevertheless, these methods have some disadvantages, such as the risk of cross-contamination and leaks, and the use of high flow rates that can sometimes be incompatible with on-line operation.

SPME constitutes a convenient alternative to other commonly used extraction methods because it integrates sampling, extraction, concentration and sample introduction into a single step without the use of solvents. This technique is of increasing interest in the field of pesticide residue analysis and has been applied for the determination of several classes of pesticides in aqueous media [9, 10], or in other sample matrices [11–13]. Results of the analysis showed than SPME was an accurate and rapid method for sample preparation and analysis in several matrices. However, several disadvantages related to fiber stability and sensitivity have been pointed out [14]. Sometimes, the small sample volume used in accordance with the matrix of the sample may affect the HS-SPME precision, eliminating the advantages of this technique.

Recently, headspace SPME (HS-SPME) has also been used to determine pesticides in water [15] and biological fluid samples [16,17]. Compared with direct SPME, headspace SPME can shorten the time of extraction significantly because of the faster diffusion rate of the analytes in the gaseous phase than in the liquid phase [18]. Because the fiber is not in contact with the sample, matrix effects can be reduced, enhancing the life expectancy of the fiber. Furthermore, HS-SPME eliminates the possibility of introducing trace-level water caused by the wick effect in direct SPME [19].

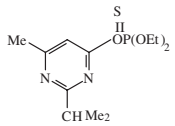
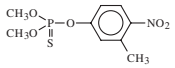
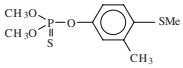
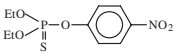
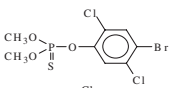
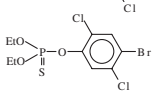
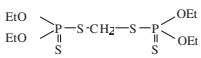
The determination of pesticides in food samples by SPME has received only limited attention. Most of the SPME work to date has focused on liquid samples, such as wine [13, 20, 21] and honey samples, which are usually analyzed after dilution with water [22, 23]. Applications of HS-SPME for the analysis of OP insecticides in olive oil have not been well documented. The aim of the present study is to investigate the applicability of HS-SPME for the determination of OP insecticides in olive oil samples. Parameters such as fiber type, temperature, extraction time, stirring rate and salt content, were optimized. Seven insecticides, diazinon, fenitrothion, fenthion, ethyl parathion, methyl bromophos, ethyl bromophos and ethion were selected because of their widespread use in agriculture in Greece [24] and other Mediterranean countries [25] (Table I). The analysis was performed by gas chromatography with flame thermionic detection (FTD).

EXPERIMENTAL

Reagents and Standards

Pesticide standards (diazinon, fenitrothion, fenthion, ethyl parathion, methyl bromophos, ethyl bromophos and ethion) were purchased from Riedel-de Haën (Seelze, Germany). All pesticide standards were 98–99% purity. Methanol was purchased

TABLE I Chemical structure and some physicochemical properties of the selected insecticides

Insecticide	*Chemical structure*	*Molecular weight*	*Water solubility* (mg/L)	*Vapor pressure*[a] (mPa)	*Henry's constants*[b] (m^3 atm/mol)	*Log* K_{ow}[c]
Diazinon		304.3	40	8	0.60×10^{-6}	3.30
Fenitrothion		277.2	30	18	1.64×10^{-6}	3.40
Fenthion		278.3	55	4	0.20×10^{-6}	4.09
Ethyl parathion		291.3	24	5	0.60×10^{-6}	3.76
Methyl bromophos		366.1	40	17	1.54×10^{-6}	4.88
Ethyl bromophos		394.0	2	6.1[d]	11.87×10^{-6}	5.68
Ethion		384.5	1	0.2[e]	0.76×10^{-6}	5.07

[a]Values are taken from [26], and refer to 20°C unless marked otherwise; [b]Values are taken from [30]; [c]Values are taken from [26]; [d]At 30°C; [e]At 25°C.

from Pestiscan (Labscan, Ltd., Dublin, Ireland). Anhydrous sodium sulfate was purchased from Merck (Darmstadt, Germany). Stock standard solutions of 1000 μg/L of each compound were prepared in methanol. Working standard solutions of analyzed insecticides at concentration levels 50, 100 and 250 mg/L were prepared by diluting the stock solutions with methanol. Olive oil solutions were prepared by spiking the olive oil with an appropriate amount of the working solutions to yield concentrations ranging from 0.025 to 0.5 mg/L. In this way, a methanol content of $<0.1\%$ v/v was present in the olive oil samples, thus not affecting the extraction performance. Each concentration level was run in triplicate under optimum extraction conditions. The samples were analyzed by GC-FTD to obtain calibration curves and the linear range of the method.

SPME Fibers

SPME holder and fiber assemblies for manual sampling were provided from Supelco (Bellefonte, PA) and used without modification. The fiber coatings assayed were as follows: polyacrylate (PA 85 μm), poly(dimethylsiloxane) (PDMS 100 μm), carbowax-divinylbenzene (CW-DVB 65 μm) and poly(dimethylsiloxane)–divinylbenzene (PDMS-DVB 65 μm). Before measurements, the fibers were conditioned in the injector for 3 h at 240°C, with the split vent open and PA fiber was conditioned overnight under the same conditions, to fully remove any contaminant that might cause high baseline noise and large ghost peaks. Then the fiber was repeatedly injected into the GC until

interfering peaks disappeared. During this desorption process the GC column oven temperature was maintained at 240°C.

Headspace Solid-phase Microextraction Procedure

Olive oil samples for spiking were selected from the products of Preveza Agricultural Union (N.W. Greece). The HS-SPME extractions after optimization were performed by placing 5 mL of spiked olive oil into 10 mL crimp-top headspace vials, capped with PTFE-gray butyl-coated septa. The samples were heated by supporting them with a clamp in a water bath on top of a hot-plate stirrer. After 10 min, the needle of the SPME device pierced the septum of the vial and the fiber was immersed in the headspace of the sample for 60 min, 1 cm above the spiked olive oil which was kept at (75°C ± 1°C). Magnetic stirring with a 0.8-cm PTFE-coated stir bar was used to agitate the sample at 960 rpm. After extraction, the fiber was inserted into the hot injector of the GC system for analysis.

Gas Chromatographic Conditions

Chromatographic analysis was performed using a Shimadzu 14A capillary gas chromatograph with flame thermionic detector (FTD) at 250°C. The DB-1 30 m × 0.32 mm i.d. column used contained dimethylpolysiloxane (J & W Scientific, Folsom, CA). The temperature was programmed as follows: initial temperature was kept at 150°C for 2 min, increased to 200°C, at 5°C/min, held for 8 min, then raised to 210°C at 1°C/min and held for 2 min. The temperature was finally increased to 270°C at 20°C/min and held for 4 min. The injection temperature was 240°C. Helium was used as the carrier (1.5 mL/min) and make-up gases (40 mL/min) The detector gases were hydrogen and air, and their flow rates were regulated at 4 and 120 mL/min, respectively. The SPME fiber was desorbed for 5 min in the GC split/splitless injection port, held at 240°C. The injection port was in splitless mode, the splitter opening after 2 min.

RESULTS AND DISCUSSION

Optimization of Headspace Solid-phase Microextraction

The different parameters that influence the partition of analytes between the headspace and the solution (fiber type, extraction time, temperature, ionic strength, stirring rate) were optimized by analyzing spiked olive oil samples containing 1 μg/L of target compounds in the FTD system.

The choice of an appropriate coating is essential for the HS-SPME method. Four types of coating were investigated: 100-μm PDMS, 85-μm PA, 65-μm PDMS/DVB and 65-μm CW-DVB fibers. Distilled water samples were spiked with a concentration of 1 μg/L of the target analytes and analyzed by HS-SPME (three replicates). The FTD response for the HS-SPME injection was calculated from the FTD calibration curve generated with injections of standard solvent [27] (Fig. 1). The PDMS 100-μm coating exhibited the highest extraction efficiency for most of the analytes and was selected for the subsequent experiments.

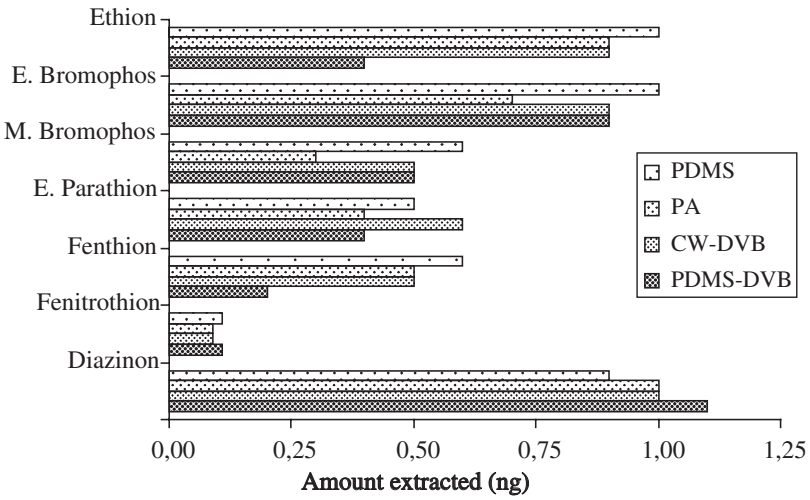


FIGURE 1 Amount extracted (ng) by four types of SPME coatings at concentration level 1-μg/L.

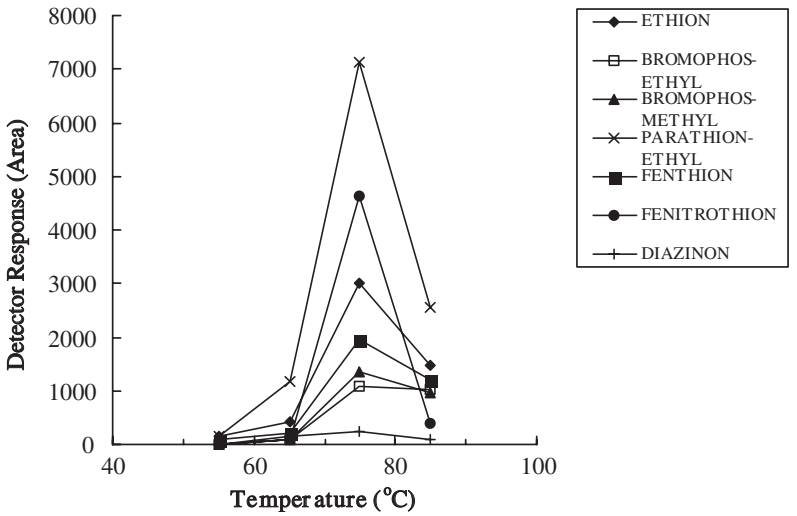


FIGURE 2 Influence of temperature on detector response area, by using a PDMS 100-μm fiber for the selected insecticides at concentration level of 1 μg/L.

The effect of temperature on the extraction process of insecticides from the olive oil samples was examined over a range 25–85°C. HS-SPME was carried out for 60 min. The obtained results have shown that increasing the temperature influences the extraction of analytes (Fig. 2). The decreased extraction performance at higher temperatures can be attributed to the exothermic nature of the adsorption step as well as to enhanced hydrolysis of organophosphorus insecticides at elevated temperature [28]. The temperature of 75°C was optimal and was selected for the subsequent experiments.

The time required to reach equilibrium between the stationary phase and the sample headspace was also determined. The olive oil samples spiked with 1 μg/L of target

compounds were exposed for times ranged from 15 to 120 min at 75°C (Fig. 3). An extraction time of 45 min was selected as a compromise between analyte response and time of analysis.

The effect of ionic strength on extraction efficiency was evaluated by analyzing the amount of insecticides extracted in olive oil samples containing 5 and 10% (w/v) of sodium sulfate (Na_2SO_4). The addition of salt to the olive oil samples reduced the recovery of all analytes (Fig. 4). The decreased extraction performance after addition of the salt can be attributed to the lipophilic nature of the organophosphorus insecticides, and the matrix of the sample (olive oil). Therefore Na_2SO_4 was not added in all subsequent experiments.

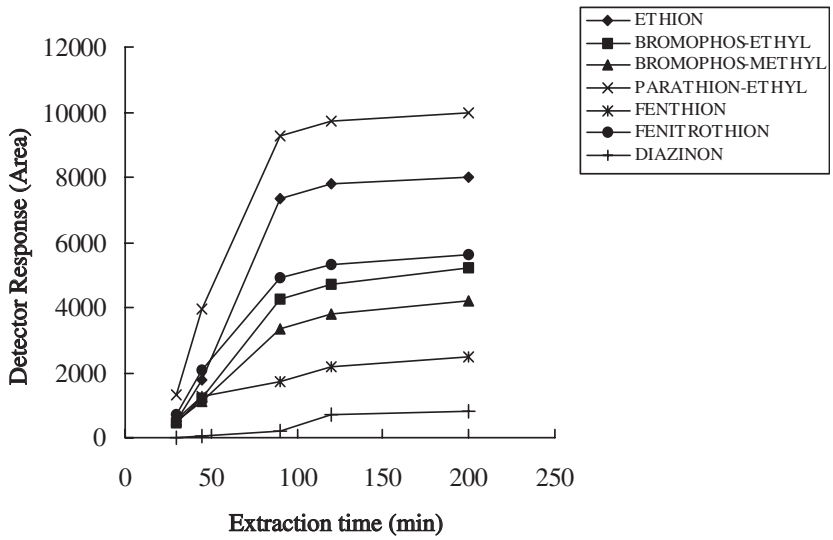


FIGURE 3 Influence of adsorption time on detector response area, by using a PDMS 100-μm fiber for the selected insecticides at concentration level of 1 μg/L.

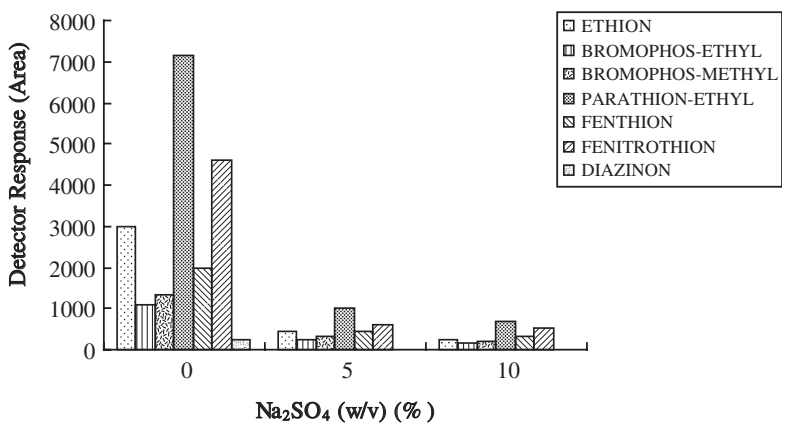


FIGURE 4 Influence of sodium sulfate on detector response area, by using a PDMS 100-μm fiber for the selected insecticides at concentration level of 1 μg/L.

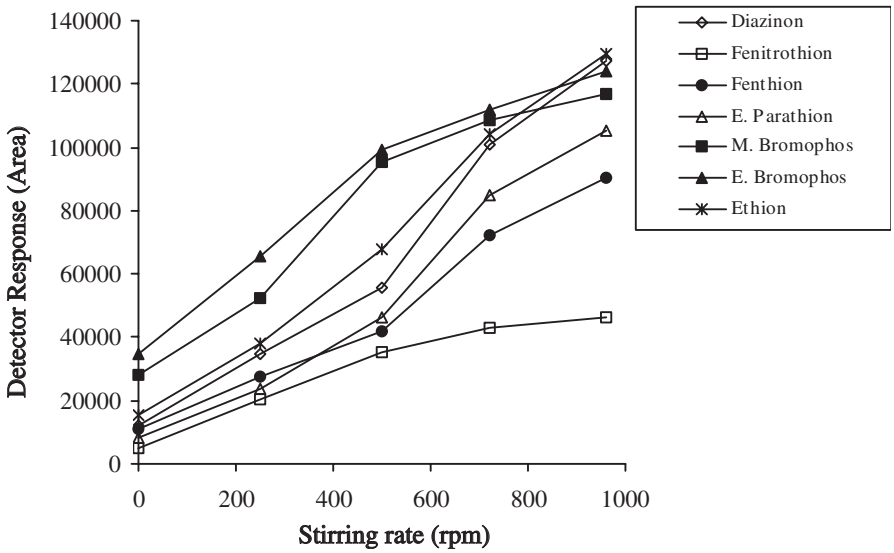


FIGURE 5 Influence of stirring rate (rpm) on detector response area, by using a PDMS 100-μm fiber for the selected insecticides at concentration level of 1 μg/L.

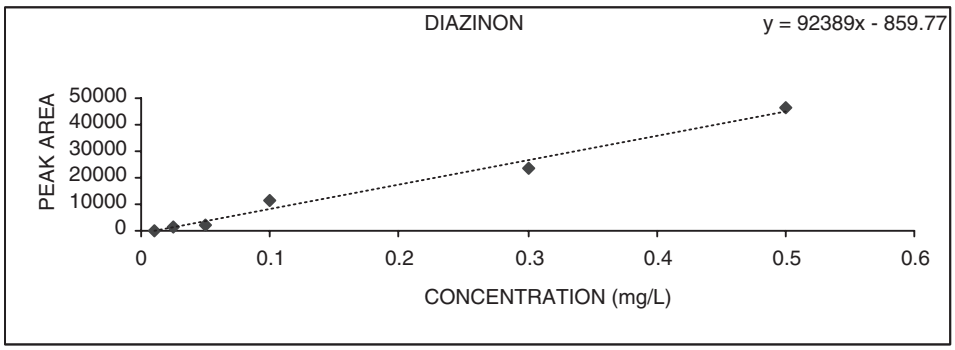
Sample agitation enhances the extraction, especially for higher molecular mass analytes. Three replicate analyses were taken at four different stirring rates: 0 (static case), 260, 480 and 960 rpm (Fig. 5). Faster stirring rates were avoided as they resulted in worse agitation due to stirring bar vibrations. The optimum stirring rate was observed at 960 rpm and was used in all subsequent experiments.

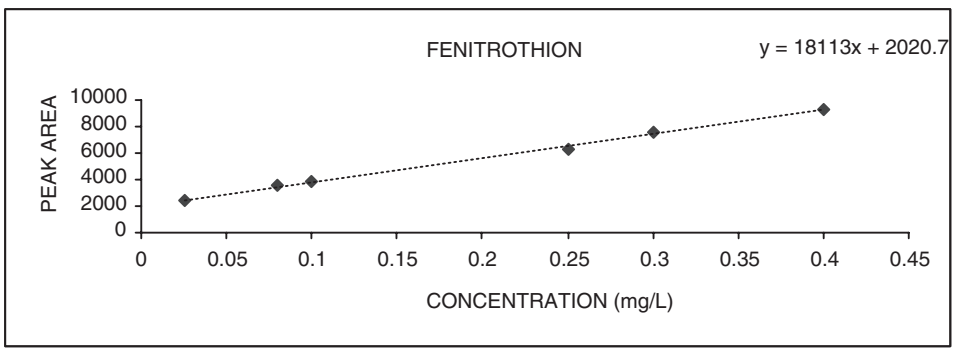
Sampling from the headspace reduced the interaction between the sample matrix and the fiber. Thus, the coating was protected and its lifetime was increased. However, fiber destruction after extensive use could not be avoided. This parameter affects analysis reproducibility and clearly suggests that any routine use of the HS-SPME approach especially for complex matrices such as olive oil should include frequent calibration runs after several uses. The PDMS 100-μm fiber was proven to be effective after almost 100 runs for the analysis of OP insecticides in olive oil, corresponding to the fiber life for environmental water samples using the HS-SPME mode [15].

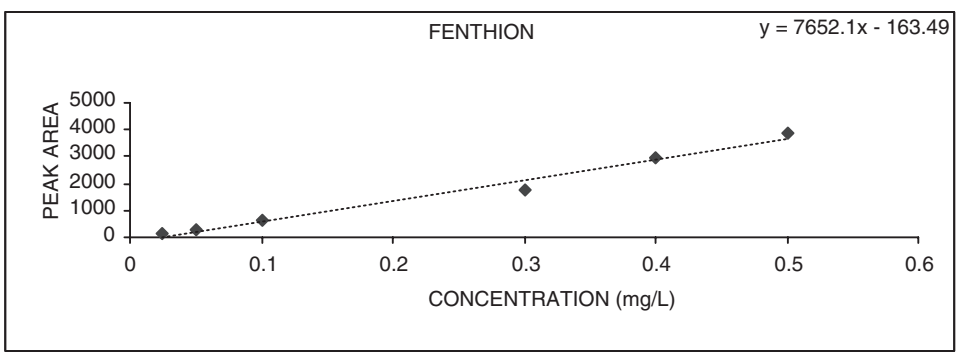
Analytical Characteristics

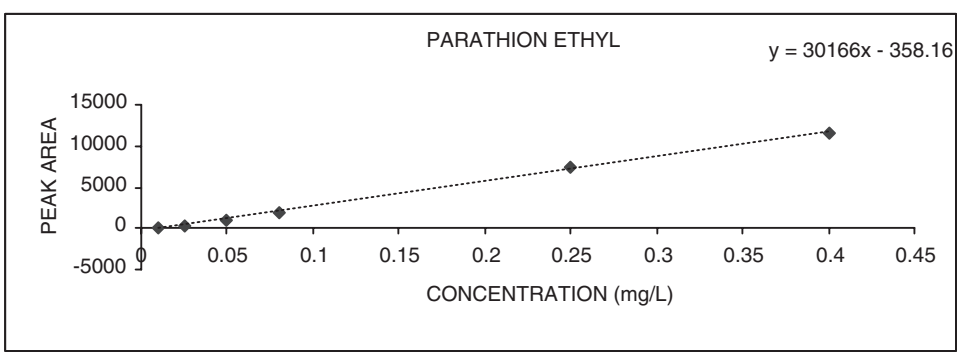
Once the preliminary investigations were completed and extraction parameters (extraction time, temperature, salt addition and stirring rate) were optimized (in the FTD system), the feasibility of the HS-SPME method was investigated with regard to the linearity and limit of detection for olive oil samples with a GC-FTD instrument.

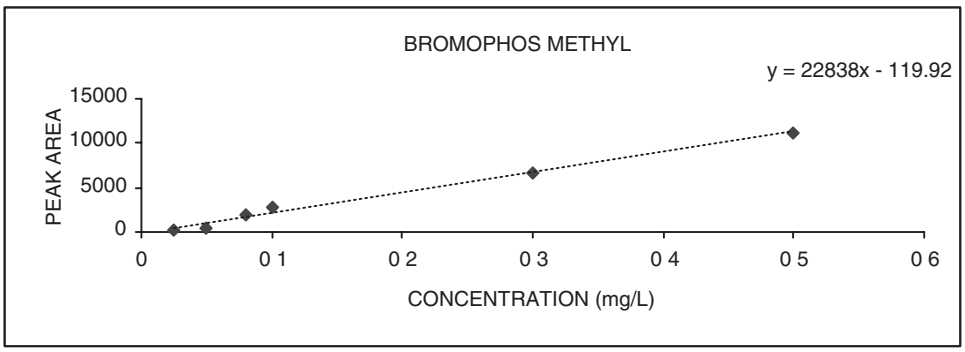
The linearity of the method was investigated by plotting the measured detector response over a series of concentration levels between 0.025 and 0.5 mg/L. Each solution was run in triplicate. Peak areas for each compound were plotted against the actual concentration to obtain calibration curves for each analyte. The obtained results have shown linear regression with correlation coefficients between 0.980 and 0.999 with the FTD detector (Table II and Figure 6).

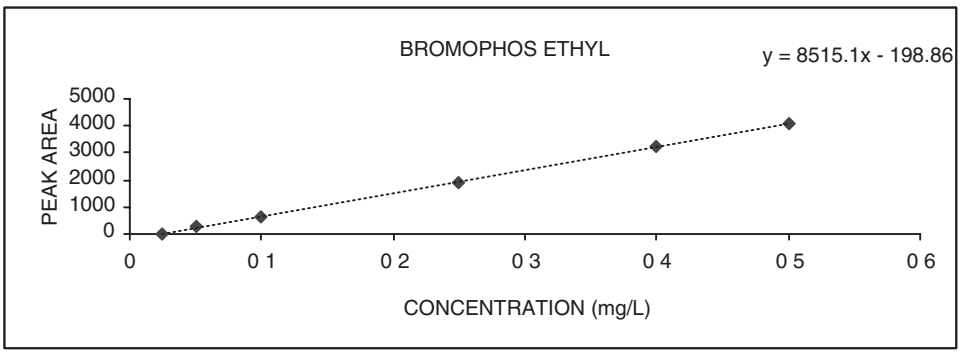












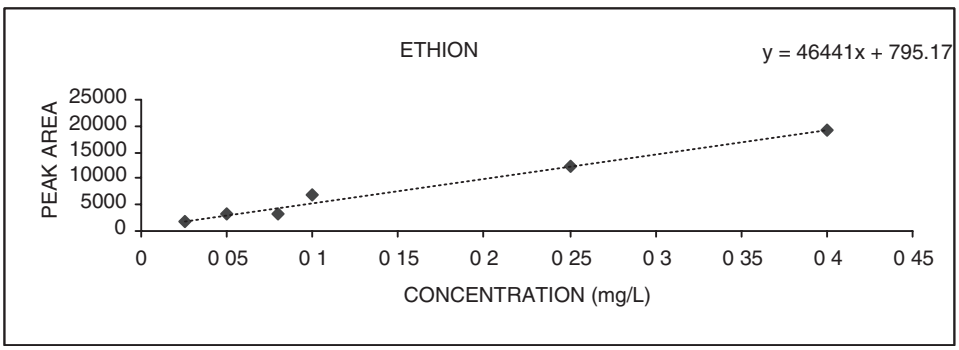


FIGURE 6 Calibration of the selected insecticides for the concentration ranges 0.025–0.5 mg/L.

TABLE II Analytical date for the seven insecticides

Insecticide	t_R[a] (min)	*Linearity*	*LOD*[b] (mg/L)	*RSD*[c] (%)
Diazinon	11.735	0.987	0.005	9
Fenitrothion	13.033	0.996	0.01	12
Fenthion	13.975	0.980	0.005	14
Ethyl parathion	14.107	0.999	0.01	19
Methyl bromophos	14.633	0.990	0.005	11
Ethyl bromophos	17.495	0.999	0.01	8
Ethion	26.285	0.910	0.005	7

[a]t_R = Retention time; [b]LOD = Limit of detection; [c]RSD = Relative standard deviation.

Limits of detection (LOD) were estimated on the basis of 3 : 1 signal-to-noise ratios obtained with standards containing the compounds of interest at low concentration levels. SPME analysis was performed as described under calibration procedures with GC-FTD. The average signal-to-noise ratio of three measurements was used to calculate the LOD. The method allowed detection of the insecticides in olive oil samples at concentrations < 0.01 mg/L with the GC-FTD instrument (Table II). Thus, the limits of maximum residues (LMR) required by European and international regulations for the majority of products can be verified without difficulty [29].

CONCLUSIONS

A method for the extraction and analysis of priority organophosphorus insecticide residues from olive oil was developed. Optimization of the parameters affecting the method sensitivity should be carefully developed in order to permit substantial increases in the amount extracted of most analytes and to improve the limit of detection. This methodology has the advantages of simplicity, shorter duration and not needing a cleanup step for the sample. Finally, it has been used routinely in combination with GC-FTD for screening analysis of complex matrices such as olive oil samples.

Although many aspects of the application of HS-SPME for analyzing insecticide residues in complex matrices still have to be investigated, this extraction technique should make it a valuable tool for the food analysis of insecticides or other classes of pesticides in the future.

Acknowledgements

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