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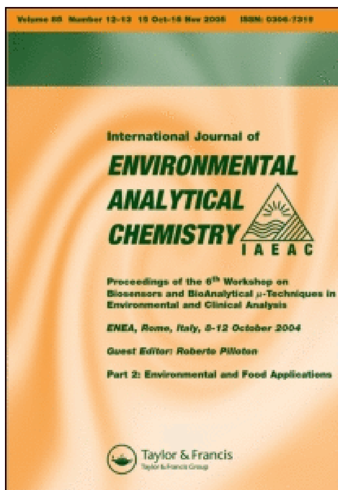

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Sample Clean-Up and Fractionation of Organophosphorus Pesticide Residues in Mussels Using Normal-Phase LC

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SAMPLE CLEAN-UP AND FRACTIONATION OF ORGANOPHOSPHORUS PESTICIDE RESIDUES IN MUSSELS USING NORMAL-PHASE LC

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A method has been developed for the sample clean-up of organophosphorus pesticides (OPPs) in mussels prior to gas chromatography analysis. The system applied uses a HPLC pump and a silicagel column A diode array detector is used to monitor, on line, the elution of the fat present in the mussel soft tissue that was extracted with acetonitrile:acetone and redissolved in hexane.

Polar and moderately polar pesticides were fractionated and separated from fats using hexane:ethyl acetate 95/5, v/v as mobile phase. The most polar pesticides (chlorfenvinphos, phosmet and dimethoate) were eluted from the column using ethyl acetate. For the elution of non polar pesticides (chlorpyrifos, phorate and fonofos) in fat free fractions it was necessary to use hexane as mobile phase switching on to hexane:ethyl acetate 99/1 (v/v) (4 ml) after 4 min of elution. Quantitative measurement of fat content in collected fractions from LC system was carried out by colorimetry in order to know the efficiency of the clean-up and the amount of fat injected in the GC system. Fat free fractions containing pesticides obtained after LC clean-up can be injected directly, without further clean-up steps or solvent exchange, in the GC system.

The limits of detection for the organophosphorus pesticides investigated varied between 1 and 40 ng/g using mass selective detector and between 0.1 and 2 ng/g using nitrogen-phosphorus selective detector. Recoveries of the overall procedure, including extraction and clean-up, were obtained at 400 and 40 ng/g levels and they varied between 89 ± 9% and 105 ± 4%.

Keywords: Normal phase LC; clean-up; fat; organophosphorus pesticides; marine organisms

INTRODUCTION

A recent report directed by the FAO,[1] dealing with the assessment of the state of pollution of the Mediterranean Sea by organophosphorus compounds, shows

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the need of precautionary actions. This study indicates the environmental risks of organophosphorus pesticides (OPPs) to the marine environment, as they could be very toxic and harmful to marine life. As an example, the organophosphorus pesticides chlorpyrifos, chlorfenvinphos and malathion have been detected in surface coastal waters [2] and fènitrothion, methyl-parathion and malathion have been detected in marme organisms [3] from Mediterranean Spanish coast. Therefore, fast and reliable methods are needed for the determination of organophosphorus pesticide residues in marine organisms for environmental monitoring.

Marine organisms contain a variety of lipids that can be divided into neutral lipids (cholesterol and its esters, mono-, di- and triacylglycerids) and polar lipids (phospholipids). [4, 5] Lipids have to be separated from the analytes of interest because severely hamper the final gas chromatographic determination.

The major difficulty in the analysis of OPP residues in marine organisms concerns the wide polarity range for both the OPPs and lipids present in the matrix. Therefore, in order to obtain sample extracts amenable to gas chromatography (GC), appropriate sample clean-up is of extreme importance to avoid contamination of the GC system.

Official Methods of analysis for several organochlorine and organophosphorus compounds in fat-containing samples, including fish, use adsorption chromatography and/or liquid-liquid partitioning as clean-up technique. [6–8] Currently, laborious and time consuming clean-up procedures are still used for OPPs analysis in foods. [9–12] Although permissible levels of OPPs in fat foods ranged from 10 to 50 ng/g in European Union (Directive 93/57/CEE), environmental analysis require lower limits of detection for monitoring pesticide residues.

Previous works have shown that the application of liquid-liquid partitioning using acetonitrile:hexane for the purification of mussel extracts is not effective, remaining large amounts of fats that make difficult the analysis of the extracts, besides rendering low recoveries for apolar pesticides. [13]

Adsorption column chromatography has been widely applied for clean-up purposes in fatty samples. It is based on a polarity fractionation using either Florisil, silica and magnesia Silicagel has been widely used as sorbent for clean-up purposes [13–16] usually as an additional system after a first clean-up by liquid-liquid partition or by GPC. The adsorption column chromatography over silicagel improved the efficiency of the liquid-liquid partition in the purification of mussel samples, achieving limits of detection of a few ng/g for selected OPPs.

However, an appreciable amount of fats still remained in the extracts after purification due to the wide range of polarities of both matrix lipids and OPPs. [13]

Gel permeation chromatography (GPC) has also been applied to OPPs determination in fatty matrices. Important advantages of this technique are that it can be automated simply and has a large scope for pesticide residue analysis, since it

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merely separates the macro molecular matrix compounds from all possible analytes. The most important drawbacks of GPC are the coelution of small interfering molecules with analytes and the tailing of the triacylglycerids due to adsorption to LC valves in the GPC system. [17] Due to these problems, small amounts of fat will be present in final pesticide fraction, and further clean-up step over silica is usually needed.[18]

High performance liquid chromatography (HPLC) techniques have been applied successfully to organochlorine and organophosphorus compounds determination in fats, oils and butterfat. Gillespie and Walters[19] applied HPLC silicagel columns for the fractionation of organochlorine, organophosphorus and PCBs in butterfat. The high efficiency in fat elimination, high speed of elution, low solvent consumption and short clean-up time are considered to be the main advantages of the method. Moreover, HPLC silicagel columns have load capacity higher than that of conventional columns.

Hogendoorn et al. [20] published a method for the automated clean-up of organochlorine pesticides (OCPs) and PCBs in fatty samples based on normal phase HPLC column switching technique. Normal phase HPLC has also been successfully applied to the analysis of chlorpyrifos, methyl-chlorpyrifos and metabolites in mussels. [21]

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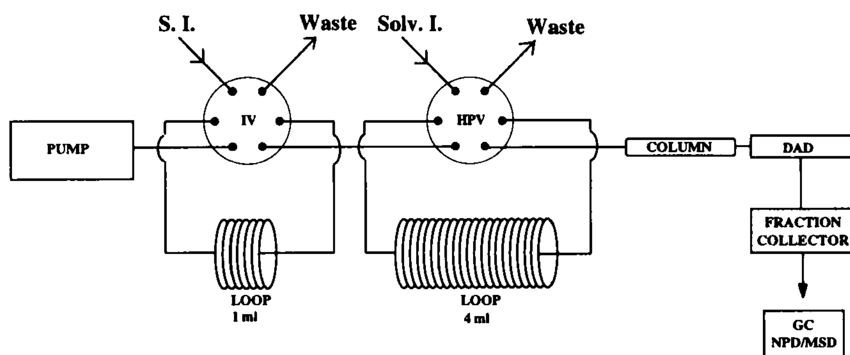

SI : Sample Injection
Solv. I: Solvent Injection
IV: Injection Valve
HPV: High Pressure Valve
DAD: Diode array detector

FIGURE 1 Liquid chromatography system used in this work

Several authors have also used reverse phase HPLC for fatty samples clean-up[22–24] and for biological matrices [25, 26] previous to analytical determi-

nation of OCPs, OPPs and derivatives. Some problems related to dirty samples have been pointed out using this technique making necessary a further clean-up step by adsorption chromatography on Florisil [23].

In this paper a method is described using a LC-system for the sample clean-up and fractionation of ten organophosphorus pesticides (phorate, fonofos, chlorpyrifos, methyl-parathion, fenitrothion, malathion, methidathion, chlorfenvinphos, phosmet and dimethoate) in mussels prior to GC determination. The main aim has been to improve the performance of existing clean-up procedures for the analysis of organophosphorus pesticides in marine organisms by the use of normal-phase LC. The efficiency of the purification has been checked by determining the fat content in the LC fractions by a colorimetric method, and also by on-line monitoring the clean-up process using diode array detection.

EXPERIMENTAL

Reagents

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Reference materials from *Dr S. Ehrenstorfer* (Promochem, Wesel, Germany) with a purity >93–99% were used for the preparation of standards of organophosphorus pesticides (phorate, fonofos, chlorpyrifos, methyl-parathion, fenitrothion, malathion, methidathion, chlorfenvinphos, phosmet and dimethoate) in n-hexane. Ethyl acetate, acetonitrile and acetone for residue pesticide analysis were purchased from Scharlau. Anhydrous sodium sulfate was obtained from Baker (Deventer, Holland) and was dried for 18 hours at 300°C before use. Celite was purchase from Merck (Darmstadt, Germany).

LC instrumentation

A schematic representation of the LC system is shown in Figure 1. The LC system used in the clean-up procedure consisted of an injection valve Rheodyne (Cotati, CA, USA) equipped with a 1.0 ml loop. A Spectroflow 400 (Applied Biosystems, Ramsey, NJ, USA) was used for the delivery of the n-hexane and/or hexane-ethyl acetate mixtures used as mobile phase at a flow of 1.0 ml/min. A 6 way high-pressure valve from Valco Instruments (Houston, TX, USA) equipped with a loop of 4 ml, electronically controlled, was used for the incorporation of modifier solvents to the mobile phase. A 2140 Rapid Spectral Detector from LKB (Bromma, Sweden) was used for monitoring the fat elution pattern on-line, and a Model 2212 Helirac from LKB was used as fraction collector. LC clean-up

was performed using a 4 μm 150 × 3.9 mm ID LC column packed with Silica Novapack (Waters, Milford, MS, USA).

GC instrumentation

GC-NPD analysis was performed on a Hewlett-Packard 5890 series II (Avondale, USA) with nitrogen-phosphorus detector, equipped with a HP 7673 autosampler. On-column injections of 2 μl were performed on a fused silica HP Ultra 2 capillary column coated with cross linked 5% phenyl methyl-silicone with a length of 25 m × 0.25 mm ID and a film thickness of 0.33 μm. Helium was used as carrier gas at a flow of 0.5 ml/min as well as make up gas at a flow of 30 ml/min. The oven temperature was programmed as follows: 90°C during 1 min, at 30°C/min to 180°C, at 4°C/min to 270°C with a final hold for 20 min. This instrument was used for quantitative analysis, which was carried out by means of an external standard method.

GC-MSD analysis was performed on a Hewlett-Packard 5890 series II which was equipped with a HP 7673 autosampler and a MSD 5971 mass selective detector. Splitless injections of 3 μl were performed into a fused silica Ultra 2 capillary column coated with crosslinked 5% phenyl methyl silicone with a length of 12 m × 0.25 mm ID and a film thickness of 0.33 μm. Helium was applied as carrier gas at a flow of 0.7 ml/min. The oven temperature was programmed as follows: 90°C during 1 min, at 10°C/min to 140°C, at 5°C/min to 270°C with a final hold for 10 min.

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Mass detector characteristics were as follows: Electron impact (70 eV) mode with electron multiplier voltage of 1700; temperature of transfer line was 280°C. Analysis were carried out in selected ion monitoring mode using the single ions listed in Table I. This system was used for confirmatory purposes.

Preparation of samples

Mussels *(Mytilus galloprovincialis)* were collected alive, directly from the sea (western Mediterranean coast) and stored at – 20°C. The mussels were thawed at room temperature and the soft tissue was removed from the shell before analysis.

Extraction

The wet mussel tissue (10 g) was homogenized with anhydrous sodium sulphate and 2 g of Celite. A mixture of acetonitrile:acetone (90:10; v/v) (150 ml) was added and mixed in a high speed blender (8000 rpm) during 3 min by means of an Ultraturrax® T 25 (Janke and Kunkel, Germany).

TABLE I Selected ions for the single monitoring mode

Pesticide	*Selected ions*
Phorate	260–97
Fonofos	246–109
Chlorpyrifos	278–125
Methyl-parathion	263–125
Fenitrothion	277–125
Malathion	173–125
Methidathion	302–145–85
Chlorfenvinphos	199–314
Phosmet	160
Dimethoate	125–93

After two times filtration (firstly with gentle vacuum and secondly by gravity) the extract was concentrated in a Kuderna-Danish to ca. 5 ml, and evaporated to dryness under a gentle stream of nitrogen at 40°C. The final residue was dissolved in 2 ml of n-hexane.

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Determination of the fat

The fat content in the mussel extract and in the LC-fractions was determined by gravimetry and by a colorimetric method for total lipids [27], respectively. The method used for total lipids determination is based on the sulpho-phosphovainilline reactivity. Triacylglycerids calibration solution (Sigma, St Louis, Missouri, USA) was used as standard.

Clean-up procedure

Depending on the polarity of the organophosphorus pesticides, which have to be analyzed separately, two different procedures are recommended:

Method A: For the clean-up of methyl-parathion, fenitrothion, malathion, methidathion, chlorfenvinphos, phosmet and dimethoate, use a mobile phase containing a mixture of hexane-ethyl acetate (95:5; v/v). Inject an aliquot of 1 ml hexane mussel extract onto the silica LC column and collect the 2-ml LC fractions 4 (6–8 minutes), 6 (10–12 min) and 7 (12–14 min). After elution of the fatty matrix, the most polar OPPs elute, with 4 ml of ethyl acetate after switch on to ethyl acetate at 16 min.

Method B: For the clean-up of chlorpyrifos, phorate and fonofos, use n-hexane as mobile phase. Inject an aliquot of 1 ml hexane mussel extract onto the silica

LC column. Collect the fraction from 6 to 8 min after switch on to hexane:ethyl acetate (99:1, v/v) at 4 min.

In both methods, the collected fractions are analyzed by GC-NPD and GC-MSD.

Regeneration of the column after each clean-up cycle was performed by means of two flush steps with 4 ml ethyl acetate each when method B is applied (minutes 12 and 18) and only one in method A (minute 23).

RESULTS AND DISCUSSION

Choice of target analytes

In order to examine the scope of the method in combination with the matrix composition ten organophosphorus pesticides (phorate dimethoate, fonofos, methyl-parathion, fenitrothion, malathion, chlorpyriphos, chlorfenvinphos, methidathion and phosmet) were selected representing a wide polarity range. Previous works on fractionation of some of these compounds by means of gravity-controlled adsorption column chromatography over silicagel have been carried out by Hernández et al.[13]

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Optimization of clean-up

Optimization of clean-up procedures was carried out studying separately the elution pattern of pesticide standards in hexane (analyzing the LC fractions by GC) and the elution pattern of lipids present in hexanic mussel extracts. In the last case, the use of DAD ($\lambda = 280$) allowed us to monitor on line the elution of fats, obtaining semiquantitative information on fat content of the LC-fractions, which was also determined by colorimetry. [27] The optimized procedures were subsequently confirmed by application to hexanic mussel extracts spiked with pesticides.

Earlier studies on the LC clean-up of fatty samples [20, 28] were concerned with organochlorine pesticides and PCBs, that eluted before the fat matrix. However, the target OPPs studied in this work eluted before and after the matrix peak thus requiring a more thorough optimization of the LC conditions.

Preliminary experiments using n-hexane as mobile phase showed that only apolar pesticides (phorate, fonofos and chlorpyrifos) eluted at reasonable time. The use of a mobile-phase 2.5% ethyl acetate in n-hexane showed an elution-volume of about 60 ml (flow 1 ml/min) for the moderately polar compounds. The

majority of the mussel matrix (neutral lipids) eluted within the first 10 min of mobile phase, although unfortunately the remainder of the matrix (phospholipids) eluted in several fractions from 10 to 60 min. This elution pattern of the mussel matrix produced problems due to both overlap with the pesticides investigated and a large clean-up time.

The use of 5% ethyl acetate in n-hexane showed the fat compressed in the first 10 ml. Phorate, fonofos and chlorpyrifos eluted in a 2-ml fraction between 2–4 min (F2) containing 3.5 mg of fat, methyl-parathion and fenitrothion co-eluted with ca. 0.5 mg of fats in F4 (6–8 min), finally, malathion and methidathion eluted in fat free fractions (F6 and F7) (Figure 2). Using these LC conditions with hexane-ethyl acetate (95:5, v/v), elution of the remaining three OPPs (chlorfenvinphos, fosmet and dimethoate) was not feasible in a small elution volume.

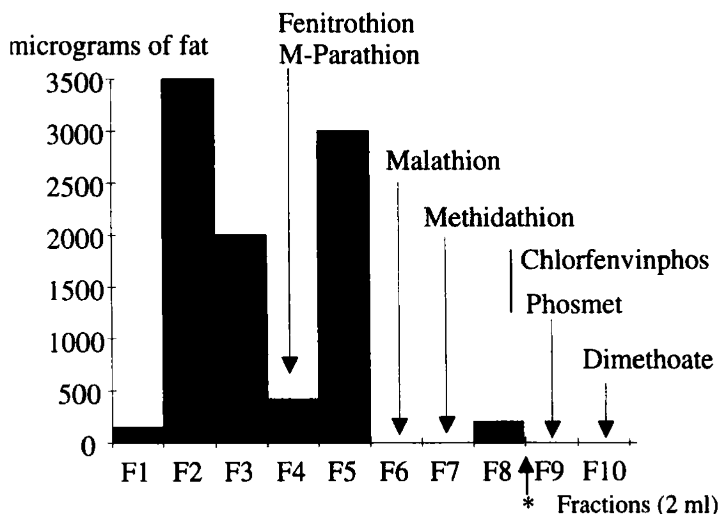

FIGURE 2 Elution patterns of lipids and moderately and polar pesticides after silicagel liquid chromatography using hexane:ethyl acetate 95/5, v/v as mobile phase. (*) after 16 min, 4 ml of ethyl acetate are injected *(Method a)*

The LC system developed allows to performance step gradients with high reproducibility due to the storage-loop for modifier solvents is sited just before the column, so diffusion processes are avoided between modifiers and mobile phase. Elution of the most polar compounds was achieved by activation of the valve equipped with a storage-loop which contained 4 ml ethyl acetate and which was actuated after an elution volume of 16 ml, obtaining chlorfenvinphos, fosmet and dimethoate between 16–20 min (Figure 2). As it can be seen, comparing

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Figure 2 and 3, the DAD chromatograms were very similar to the quantitative elution profile of fats obtained by colorimetric determination. As ethyl acetate, used as modifier solvent, presents UV-absorbance at 280 nm, it was necessary the performance of a blank injecting 1-ml hexane in the LC system in order to discriminate the signal due to fat elution (Figure 3). Therefore, the DAD allowed us to obtain semi-quantitative information on elution of fats and to monitor on line the clean-up process.

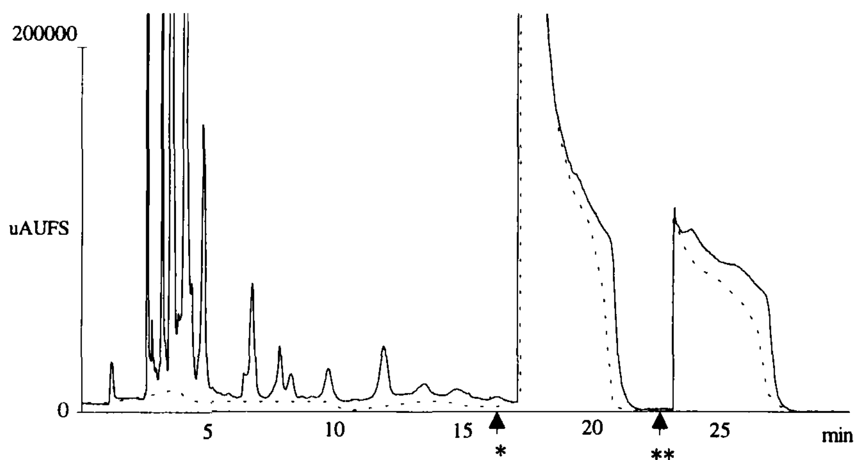

FIGURE 3 DAD chromatogram (λ = 280 nm) of a mussel extract obtained by silicagel liquid chromatography using hexane:ethyl acetate 95/5, v/v as mobile phase. (*, **) injections of 4 ml of ethyl acetate---blank performed injecting 1-ml hexane in the LC system

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Using this elution procedure with hexane-ethyl acetate 95/5 (v/v), it was observed a wide yellow fraction between 40 and 50 min which was produced by mussel pigments because no fat was detected in this fraction.

Due to the wide range of polarity of the selected pesticides, it was necessary to split the 10 OPPs in two groups in order to achieve the elution of all the pesticides in fat free fractions with a short clean-up time.

Polar organophosphorus pesticides

As shown in the preliminary experiments, to elute the most polar pesticides using 5% ethyl acetate in n-hexane as mobile phase it was necessary to activate the valve filled with 4 ml ethyl acetate at 16 min, obtaining the three compounds in the fraction between 16–20 min. This procedure (indicated as *Method a* in the

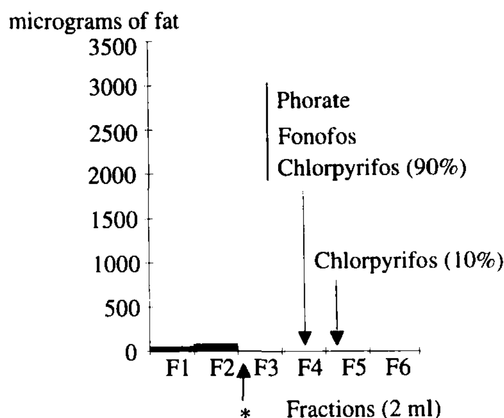

FIGURE 4 Elution patterns of lipids and non polar pesticides after silicagel liquid chromatography using hexane as mobile phase. (*) after 4 min, 4 ml of hexane:ethyl:acetate 99/1, v/v are injected *(Method b)*

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General Procedure) was selected for the determination of polar pesticides in the next experiences.

In Figure 2 it is shown the behaviour of the pesticides studied applying this procedure and the elution pattern of the lipids contained in the mussel extract. As it can be seen, this procedure *(Method a)* allows the fractionation of most OPPs (malathion, methidathion, chlorfenvinphos, phosmet and dimethoate) analyzed in fat free fractions. Methyl-parathion and fenithrotion elute in the forth 2-ml fraction together with 575 μg fat. This amount of fat is close to the maximum allowable that can be transferred without any problem when an on-column introduction technique is applied, as 575 ng fat were introduced in the GC system in a 2-μl injection [29].

Non polar organophosphorus pesticides

The non-polar pesticides (chlorpyrifos, phorate and fonofos) eluted in these conditions in the second 2-ml fraction together with 3.5 mg fat which was considered excessively high to be injected in the GC. In this way, the clean-up for these three non-polar pesticides was more problematic, being necessary to perform additional work in order to separate these pesticides in fat free fractions.

After several attempts, the use of hexane as mobile phase, activating the valve filled with 4 ml hexane-ethyl acetate 99/1 (v/v) after 4 min led to satisfactory results *(Method b)*. As it can be seen in Figure 4 chlorpyrifos, phorate and fono-

fos elute in the forth 2-ml fraction free of fat. This procedure *(Method b)* allows, in this way, the clean-up of the sample for the analysis of these three non-polar OPPs.

The LC system applied in this work showed high efficiency in the purification of mussel extracts, improving the results of the method based on gravity-controlled adsorption column chromatography [13]. In the gravity method, chlorfenvinphos, chlorpyrifos, phosmet and methidathion were obtained in a 8-ml fraction coeluting with 4.5 mg of fat, and dimethoate in a 5-ml fraction free of fat. After evaporation of the first fraction to 1-ml, the amount of fat introduced in the GC system in a 2-μl splitless injection was estimated in 9 μg. Although that procedure improved the efficiency of the classical liquid-liquid partition clean-up using acetonitrile-hexane, the fat present in the fractions injected in the GC system caused deterioration in the capillary GC column after 1 month of work, approximately. However, the application of the LC clean-up developed in the present paper allowed us to introduce only 0.6 μg fat as maximum in a 2-μl on-column injection, and the capillary GC column did not show any appreciable deterioration after 3 months of routine use. As an example Figures 5 and 6 show NPD chromatograms corresponding to mussel tissue spiked with pesticide standards at a level of 40 ng/g. It can be observed the cleanness of chromatograms, even at ng/g level.

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Analytical results

The high reproducibility of elution patterns for both pesticides and fats allowed us to maintain the same elution procedures after two months of routine use. Therefore, regeneration of silicagel columns by means of 4 ml ethyl acetate flushes seems to be enough efficient to maintain the column in good conditions. It is of extreme importance to inject completely dry extracts in the LC system, avoiding the presence of water in the mussel extracts by adding sodium sulphate to the samples as indicated in *Extraction.*

Table II shows the recoveries obtained after application of the overall analytical procedure -including extraction of sample, LC clean-up and GC analysis- to mussel samples spiked at 400 and 40 ng/g. In all cases, the results obtained were considered satisfactory except for methyl-parathion whose relative standard deviation was high.

Developed methods allowed the detection of the OPPs in mussel matrix at optimum level for environmental monitoring (Table II). Obviously, lower limits of detection can easily be obtained by concentrating the 2-ml fractions to smaller volumes or by injecting volumes larger than 2 μl. Limits of detection of the procedures were calculated at a signal-noise ratio of three from chromatograms

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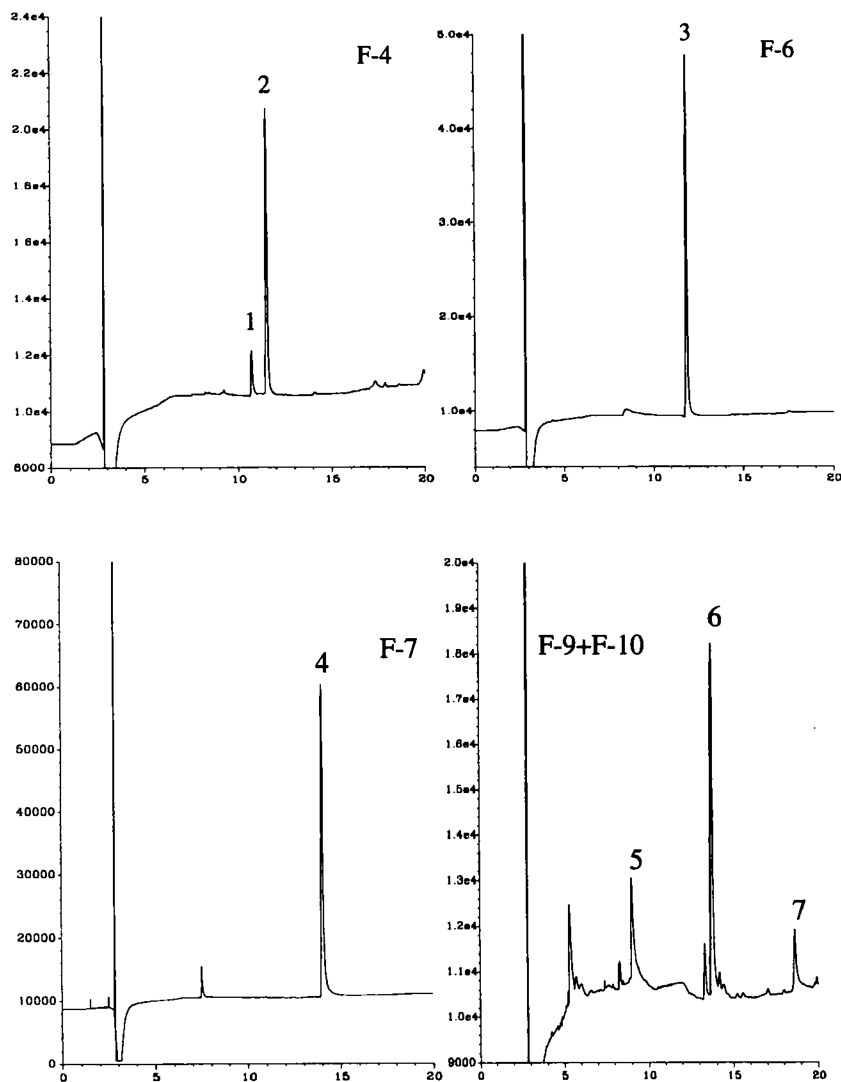

FIGURE 5 NPD Chromatograms corresponding to mussel tissue spiked with polar pesticides at 40 ng/g level *(Method a)* methyl-parathion (1), fenitrothion (2), malathion (3), methidathion (4), dimetoato (5), chlorfenvinphos (6) and phosmet (7)

obtained for samples spiked at 4 ng/g *(Method a)* and 0.4 ng/g *(Method b)* using NPD, and for samples spiked at 40 ng/g using MSD. As an example, Figures 7 and 8 show the NPD chromatograms used to calculate the limits of detection.

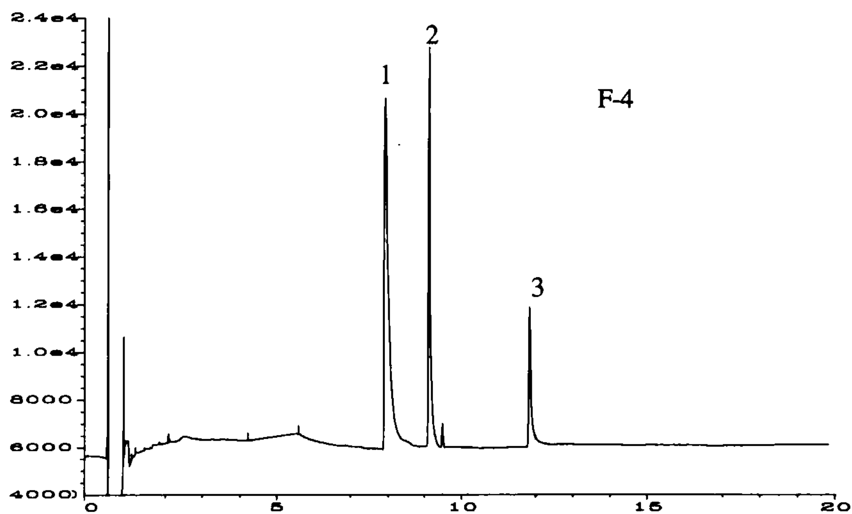

FIGURE 6 NPD Chromatogram corresponding to mussel tissue spiked with non-polar pesticides at 40 ng/g level (*Method b*), phorate (1), fonofos (2) y chlorpyrifos (3)

TABLE II Recoveries (average and relative standard deviation for n = 4) after application of the overall procedure to mussel samples spiked at 400 and 40 ng/g. Limits of detection (LD)

	400 ng/g	*40 ng/g*	*LD ng/g*	
			NPD	*MSD*
Phorate	82(4)	101(8)	0.1	25
Fonofos	94(8)	96(9)	0.1	8
Chlorpyrifos	90(6)	89(7)	0.3	20
Methyl-Parathion	76(10)	92(24)	2	10
Fenitrothion	89(9)	95(7)	1	20
Malathion	82(7)	85(7)	0.4	30
Methidathion	102(5)	89(8)	0.4	40
Chlorfenvinphos	102(7)	92(5)	0.4	3
Phosmet	87(10)	89(5)	2	40
Dimethoate	105(4)	91(13)	2	20

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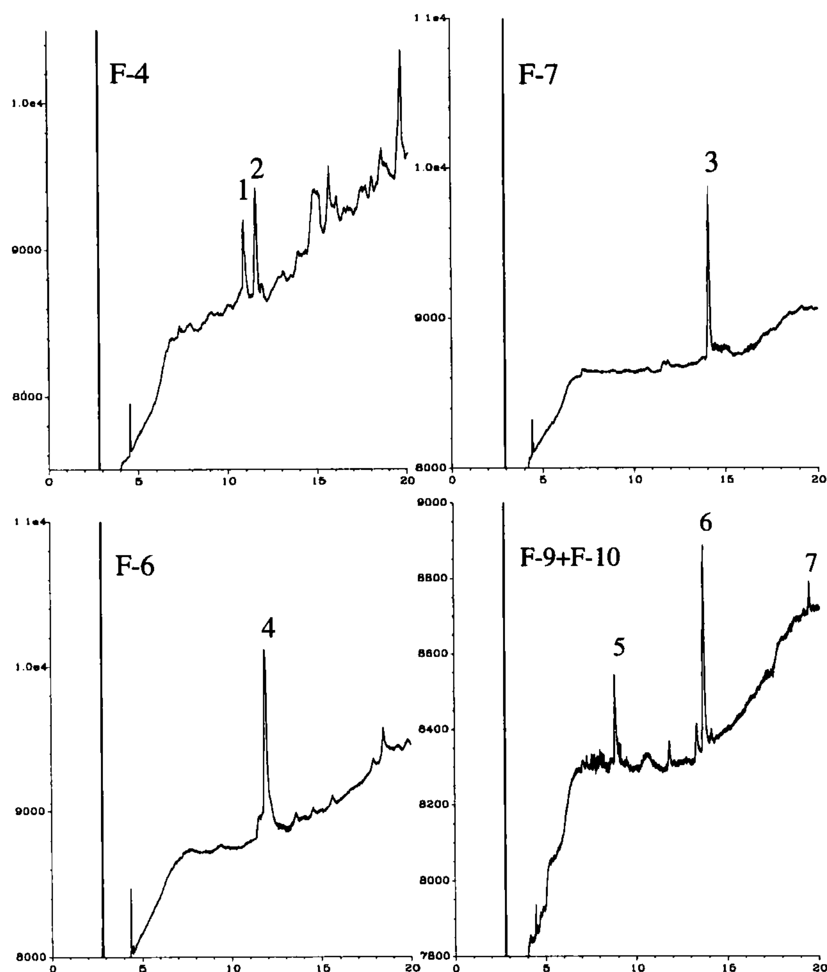

FIGURE 7 NPD Chromatogram corresponding to mussel tissue spiked with polar pesticides at 4 ng/g level *(Method a)*. methyl-parathion (1), fenitrothion (2), malathion (3), methidathion (4), dimethoate (5), chlorfenvinphos (6) y phosmet (7)

Conclusions

The method proposed in this paper allows the efficient clean-up of mussel extracts by LC prior to gas chromatography determination. It has several advantages in comparison with conventional gravity-controlled chromatography, the main being its higher efficiency for the elimination of fats. The clean-up process

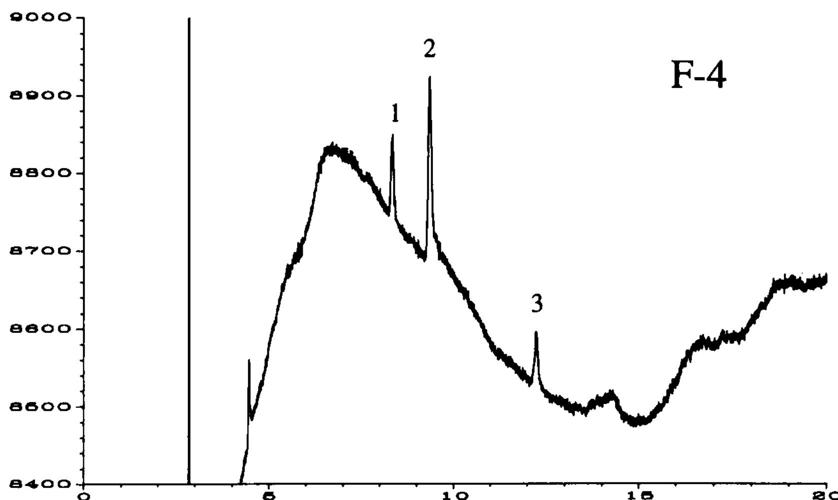

FIGURE 8 NPD Chromatogram corresponding to mussel tissue spiked with non-polar pesticides at 0.4 ng/g level *(Method b)*. phorate (1), fonofos (2) y chlorpyrifos (3)

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can be monitored on-line by means of DAD, thus rendering a fast and reliable optimization of the system. The clean-up time varies from 8 min for the non polar OPPs up to 20 min for the rest, allowing the transfer of fractions collected directly (without further clean-up steps or solvent exchange) from the LC to the GC system. Besides, the procedure described can be easily automated, and it could be very useful for LC-GC hyphenation. Finally, for confirmatory purposes, the method has potential as a bidimensional chromatography.

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References

[1] UNEP/FAO/WHO/IAEA, *Assessment of the state of pollution of the Mediterranean sea by organophosphorus compounds.* M.A.P. Technical Reports Series, n° 58, Athens (1991).
[2] F. Hemández, R. Serrano, M.C. Miralles and N. Font, *Chromatographia,* **42,** 151–158 (1996).
[3] D. Barceló, C. Porte, J. Cid and J. Albaigés, *Intern. J. Environ. Anal. Chem.,* **38,** 199–209 (1990).

[4] W.S. Hoar, *General Comparative Physiology*, 3rd Ed, Prentice Hall, New Jersey (1983).
[5] R. Serrano, Ph.D. Thesis, *Efecto de la manipulación ambiental y hormonal sobre la calidad de la progenie en lubina (Dicentrarchus labrax L)*, Servicio de Publicaciones. Universidad de Valencia, Valencia, Spain (1991).
[6] Manual of Pesticide Residue Analysis, vol 1, (H.P. Their and H. Zeumer Eds), DFG Pesticide Comission of Germany. Weinheim, Germany (1987).
[7] P.A. Greve, *Analytical methods for residues of pesticides in foodstuffs*, 5th ed., Rijkinstituut voor Volksgezondheid en Milieuhygiëne, Bilthoven, The Netherlands (1988).
[8] *Official Methods of Analysis*, AOAC, 15th, Arlington, VA (1990)
[9] M. Miyahara, Y. Okada, H. Takeda, G. Aoki, A. Kobayashi and Y. Saito, *J. Agric. Food Chem.*, **42**, 2795–2802 (1994).
[10] R. Nakagawa, H. Hirakawa and T. Hori *J. Assoc. Off. Anal. Chem.*, **78**, 921–929 (1995).
[11] W. Dejonckheere, W. Steurbaut, S. Drieghe, R. Verstraeten and H. Braeckman, *J. Assoc. Off. Anal. Chem.*, **79**, 97–110 (1996).
[12] S. Dogheim, E-Z. Mohamed, S.A.G. Alla, S. El-Saied, S.Y. Emel, A.M. Mohsen and S.M. Fahmy, *J. Assoc. Off. Anal. Chem.*, **79**, 99–111 (1996).
[13] F. Hernández, R. Serrano, J. Beltrán, and F.J. López, *J. Assoc. Off. Anal. Chem.*, **79**, 123 (1996).
[14] M.L. Hopper and K.K. Griffitt, *J. Assoc. Off. Anal. Chem.* **70**, 724–726 (1987).
[15] P.J. Marquis, R.L. Hanson, M.L. Larsen, W.M. De Vita, B.C. Butterworth and D.W. Kuehl, *Chemosphere*, **29**, 509–521 (1994).
[16] L. Alder and B. Vieth, *Fresenius J. Anal. Chem.*, **354**, 81–92 (1996)
[17] K. Grob and I. Kalin, *J. High Res. Chrom.*, **14**, 1–13 (1991).
[18] W. Specht and M. Tillkes, *Fresenius Z. Anal. Chem.*, **322**, 443–448 (1985).
[19] A.M. Gillespie and S.M. Walters, *J. Liq. Chromatogr.*, **9**, 2111–2141 (1986).
[20] E.A. Hogendoom, G.R. van der Hoff and P. van Zoonen, *J. High Res. Chrom.*, **12**, 784–789 (1989).
[21] R. Serrano, F.J. López, A. Roig-Navarro and F. Hernández, *J. Chromatogr. A.* **778**, 151–160 (1997).
[22] D. Barceló, *Biomed. Environ. Mass Spectrom.*, **17**, 363–369 (1988).
[23] A.M. Gillespie and S.M. Walters, *J. Liq. Chromatogr.*, **12**, 1687–1703 (1989).
[24] H. Hyvönen, T. Auvinen, M-L. Riekkola and K. Himberg, *J. Microcol. Sep.*, **4**, 123–127 (1992).
[25] I.A. Mostert and K.A. Ramsteiner, *J. Chromatogr.*, **477**, 359–365 (1989).
[26] M.J.W. Chang, C.Y. Lin, L.W. Lo and R.S. Lin, *Bull. Environ. Contam. Toxicol.*, **56**, 367–374 (1996).
[27] N. Zöllner, and A. Kersch, Z. *Ges. Exp. Med. Bd.*, **133s**, 545- (1962).
[28] G.R van der.Hoff, F. Pelusio, U.A.Th. Brinkman, R.A. Bauman and P. van Zoonen J. *Chromatogr. A.*, **719**, 59–67 (1996).
[29] K. Grob, I. Kalin and A. Artho, *J. High Res. Chrom.*, **14**, 373–376 (1991).

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