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Talanta 69 (2006) 669–675
Talanta
www.elsevier.com/locate/talanta

Determination of ethoprop, diazinon, disulfoton and fenthion using dynamic hollow fiber-protected liquid-phase microextraction coupled with gas chromatography–mass spectrometry

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Received 31 August 2005; received in revised form 28 October 2005; accepted 29 October 2005
Available online 1 December 2005

Abstract

A technique for the analysis of organophosphorus pesticides (ethoprop, diazinon, disulfoton, fenthion) in aqueous sample using liquid-phase microextraction (LPME), coupled with gas chromatography–mass spectrometry (GC–MS) was developed. A small section of a hollow fiber inserted into the needle of GC syringe and filled with the 3.5 μl of organic solvent was used to extract pesticides from a 20 ml aqueous sample. The limits of detection (LOD) with the selected ion monitoring (SIM) mode varied from 0.2 to 0.006 μg/l. The calibration curves were linear over three orders of magnitude with $R^2 \geq 0.996$. The relative standard deviations of the analysis (inter- and intra-day) were 5–8%, and the relative recoveries from the lake water sample were greater than 83%. The results were compared with results obtained using solid-phase microextraction (SPME/GC/MS).

Keywords: Pesticides; Ethoprop; Diazinon; Disulfoton; Fenthion; Liquid-phase microextraction; GC–MS; Water analysis

1. Introduction

At present, organophosphorus compounds are one of the most generally used pesticides in agriculture. These pesticides act as cholinesterase inhibitors in insects and mammals, and bring about a non-reversible phosphorylation of esterases in the organisms' central nervous system [1,2]. For environmental and drinking water, the maximum admissible concentration of a single compound established by the European Union (EU) is 0.1 μg/l, and 0.5 μg/l is the maximum allowed for the total concentration of all organophosphorus pesticides [3,4]. Development of an efficient analytical method to detect such contaminants is an important topic for environment protection.

In the past, sample preparation of water pollutants used conventional liquid–liquid extraction (LLE) [5,6] or solid-phase extraction (SPE) [7,8]. However, both techniques are time and labor-consuming and large quantities of solvents are required. Recently, solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) coupled with gas chromatography–mass spectrometric detection have been developed for quantifying trace amounts in aqueous environmental samples [9]. The detection limits of solid-phase microextraction (SPME) that can be achieved lie in the range from μg/l to ng/l [10,11]. But the limitations of coated material on fibers may restrict the applications. SPME fibers are relatively expensive, and are fragile during the extraction process [7,8,12,13]. Sometimes samples involving complex matrices require some level of clean-up before SPME fibers can be used, or methods of protecting the fibers must be developed before the technique can be used [14,15]. Stir bar sorptive extraction (SBSE) is another newly developed method, this involves agitating the sample with a stir bar covered with poly(dimethylsiloxane) (PDMS). Because of the large volume of the coating material [16], detection limits for volatile and semivolatile compounds in the lower ng/l can be achieved, which compares favorably with SPME [17].

Liquid-phase microextraction (LPME) is an attractive alternative which is cheap, rapid and easily automated. In analytical chemistry, the trend is toward simplification and miniaturization of the sample preparation steps, and a decrease in the quantities of organic solvents used. LPME miniaturizes the implementation of liquid–liquid extraction (LLE), in which only microliters of solvents are required to concentrate analytes from aqueous samples rather than the hundreds milliters needed in LLE [18–24].

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doi:10.1016/j.talanta.2005.10.042

In the present work, GC–MS is combined with LPME to determine the analytes listed in US EPA Method 622, which uses conventional LLE to extract pesticides. The proposed opitimized LPME is successfully used to detect ethoprop, diazinon, disulfoton and fenthion in lake water.

2. Experimental

2.1. Chemicals and standards

Ethoprop, diazinon, disulfoton and fenthion were purchased from Riedel-de Haen (Seelze, Germany). Methanol and acetone were obtained from Fisher Scientific (Fair Lawn, NJ). Reagent solvents (cyclohexane, toluene, isooctane, 1-nonanol) were supplied by Fluka (analytical grade). Sodium chloride (Osaka, Japan) and humic acid (technical grade; sodium salt; Aldrich, Milwaukee, WI, USA) were used to prepare the sample solution. Deionized water was prepared with a Milli-Q Millipore (Bedford, MA, USA) purification system. A stock solution of pesticides in acetone was prepared each month with a concentration of 2000 mg/l for each compound and diluted to a concentration of 10 μg/l with distilled water as a working solution every 3 days. Lake water which served as a real sample was obtained from a lake, Khayarhi, in Hsinchu county, Taiwan. All solutions were stored at 4 °C.

2.2. LPME extraction apparatus

The device of hollow fiber-protected LPME is shown in Fig. 1. The Accurel Q 3/2 polypropylene hollow fiber membrane (600 μm i.d., 200-μm wall thickness, 0.2-μm pore size) was purchased from Membrana (Wuppertal, Germany). Generally, hollow fiber-based LPME procedures are based on polypropylene fiber, except a few, which use porous polyvinylidene difluoride [25]. Moreover, polypropylene is highly compatible with a broad range of organic solvents. In addition, with a pore size of approximately 0.2 μm, polypropylene strongly immobilizes the organic solvents, which is most important for ensuring that the organic phase does not leak during extraction [26]. The mechanical stability of the hollow fiber was excellent and the syringe needle can be easily inserted to transfer the acceptor phase into the fiber [27]. Thus, further experiments were done by using polypropylene hollow fiber.

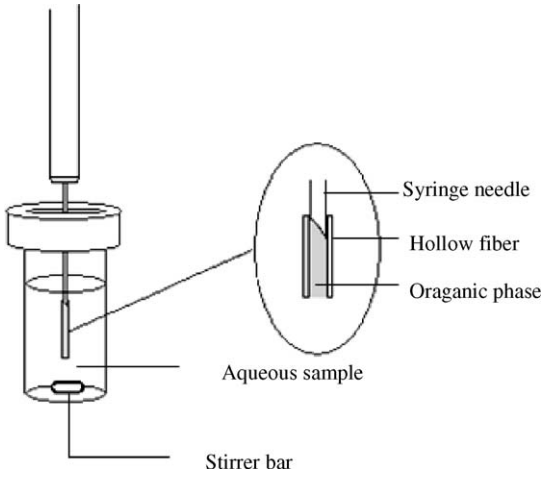


Fig. 1. Device of hollow fiber-protected LPME.

A Model 200 series (KD Scientific Inc., MA, USA) syringe pump was used for automated extraction. A 10 μl SGE syringe (Sydney, Australia) with a cone tip was used to introduce the organic phase, support the hollow fiber, and for injection. Before use, the hollow fiber was cleaned ultrasonically in acetone for 5 min to remove contaminants. The hollow fiber was cut into 1.5 cm lengths manually, and a new fiber was used for each extraction. The experimental steps are as follows: a 1.0 cm × 0.3 cm width stir bar was put into a 20 ml portion sample solution. 3.5 μl of organic solvent (typically cyclohexane) was withdrawn into the syringe, and then 5 μl deionized water; after that, the syringe tip was inserted into the fiber and immersed in the organic solvent for 15 s to impregnate all pores of the fiber. The water in the syringe was injected to flush the hollow fiber to remove excess organic solvent from inside the fiber and 3 μl organic solvent was retained in the syringe. Then, the prepared fiber was removed and immersed into a vial containing 20 ml of the aqueous sample. The syringe was fitted onto the syringe pump. Then, the syringe pump was started at a speed of 0.3 μl/s to push the 3 μl portion of organic solvent into the hollow fiber. The aqueous sample was stirred at 500 rpm at room temperature (25 °C); after 30 min of extraction, the analyte-enriched solvent (2 μl) was injected into the GC–MS.

2.3. GC/ITMS analysis

All analysis were performed using a Saturn 2000 (Walnut Creek, CA, USA) GC–MS and a Model 3800 gas chromatograph through a 1079 injector kept at 280 °C in splitless mode. The GC was equipped with a DB-XLB column (30 mm × 0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Helium (99.995%) was used as the carrier gas at a flow rate of 1.0 ml/min (constant flow). The MS operated at 70 eV with electron ionization. The GC temperature program was as follows: an initial temperature of 50 °C was held for 5 min, and then the temperature was increased at a 40 °C/min ramp to 180 °C, held for 1 min, followed by an increase at 5 °C/min to 220 °C, where the temperature was held for 5 min. Data acquisition in full scan mode is in the range of *m/z* 50–650 to confirm the selection and retention times of the compounds. Selected ion monitoring (SIM) mode (see Table 1) was used for quantification and optimization according to the method illustrated in ref [28].

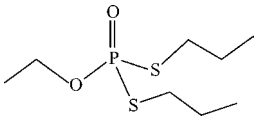
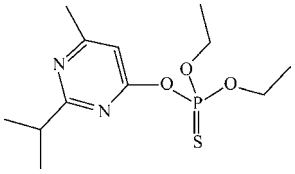
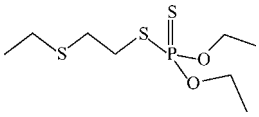
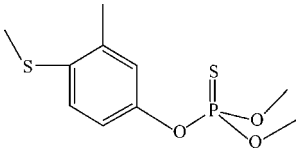
3. Results and discussion

3.1. Basic theory

Liquid-phase microextraction involves the distribution of a solute between two immiscible liquid phases. Most often, a solute is extracted from an aqueous solution into an organic solvent. This can be described as [24]:

$$A_a \leftrightarrow A_o$$

Table 1
The four organophosphorus with their structures, SIM ions, log K_{ow} values and water solubilities

OPP	Structure	Molecular mass	SIM ions	H_2O[a] solubilities (mg/l)	log K_{ow}[a]
Ethoprop		242.30	127, 158, 199	750 (25 °C)	2.14
Diazinon		304.35	137, 199, 304	60 (20 °C)	3.11
Disulfoton		274.40	125, 142, 274	15 (20 °C)	4.02
Fenthion		278.33	109, 125, 278	4.2 (20 °C)	4.8

[a] Obtained from the reference [28].

At equilibrium, the distribution ratio for the analytes in the two-phase system is:

$$K = \frac{C_{o,eq}}{C_{a,eq}}$$

where $C_{o,eq}$ is the equilibrium concentration of analytes in the organic phase and $C_{a,eq}$ is the equilibrium concentration of analytes in the aqueous phase.

According to the mass balance relationship:

$$C_t V_a = C_{o,eq} V_o + C_{a,eq} V_a$$

where C_t is the original concentration of analytes, V_o the volume of the organic solvent and V_a is the volume of the aqueous sample.

In two-phase LPME, dynamic LPME permits the formation of a renewable microfilm within a microsyringe and features the repeated movement of the syringe plunger. This procedure represents the partitioning of analytes between aqueous and organic phase. The organic solvent fills the pores and the inside lumen of the hollow fibers. Surface contact between the organic phase and aqueous phase is increased. Compounds, therefore, partition between the two phases rapidly, and are quickly extracted from the aqueous sample.

3.2. Selection of solvents

The selection of solvent immobilized in the pores of hollow fiber is a significant variable in permitting LPME to achieve the highest enrichment factor. The extraction solvent should be immiscible in water, its polarity should be matched to that of the fiber, and it should be stable during the extraction time [29–32]. Toluene, isooctane, cyclohexane and 1-nonanol were tested in the extraction of 20 ml aqueous samples. Because the target compounds are hydrophobic (ethoprop, diazinon, disulfoton and fenthion have log values of their octanol–water partition coefficients [K_{ow}] (see Table 1) ranging widely from 2.14 to 4.8 [28]) isooctane and cyclohexane exhibited better extraction efficiency (see Fig. 2).

3.3. Extraction time

The impact of extraction time on extraction efficiency was studied. Extraction times of 10, 20, 30, 40 and 50 min (see Fig. 3) were used at a stir rate of 500 rpm with 20 ml, 10 μg/l aqueous samples. As with LLE and SPME, it is not necessary for LPME to reach extraction equilibrium, since this is not an exhaustive extraction but a partitioning between the organic and aqueous phases. For analytes, the longer the exposure time, the higher the extraction efficiency. When the extraction time was longer than

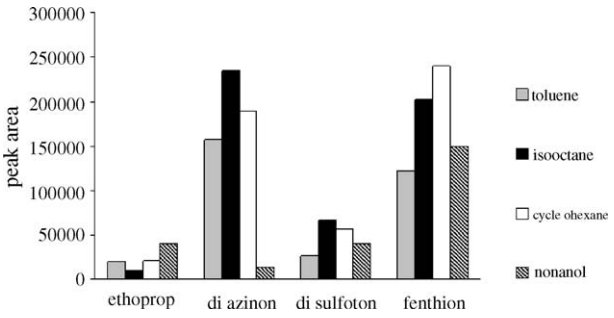


Fig. 2. Selection of the extraction solvent, spiked to 10 μg/l of each of the analytes, 500 rpm, room temperature.

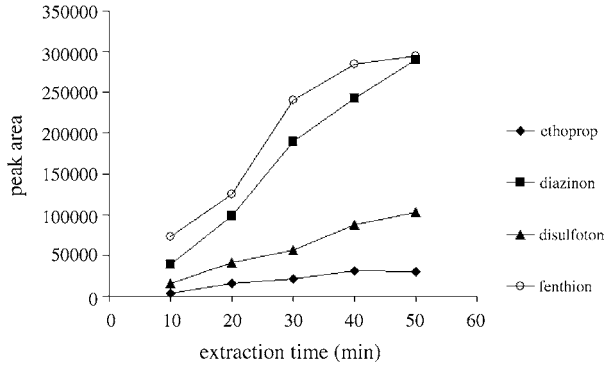


Fig. 3. Effect of the extraction time, cyclohexane as extraction solvent, spiked to 10 μg/l of each of the analytes, 500 rpm, room temperature.

40 min equilibrium was achieved for ethoprop and fenthion; on the contrary, partitioning into the organic phase was still proceeding for diazinon and disulfoton at 50 min. In order to get better sensitivity, an extraction time of 50 min was chosen.

3.4. Rate of agitation

Compared to other microextraction techniques, the mass transfer of the target compounds through the organic solvent residing in the pores of the fiber can be increased, especially for molecules of larger molecular mass by increasing the rate of magnetic stirring [24,31]. Fresh aqueous samples are efficiently brought to the fiber by good agitation. With vigorous agitation a new and fresh interface between aqueous phase and organic phase is provided continuously, so extraction efficiency is enhanced. Stirring rates studied were 0, 300, 500, 700 and 900 rpm (see Fig. 4). Faster stir rates gave rise to higher extraction yields for all compounds. The maximum extraction efficiency was achieved at 700 rpm, which was selected as the optimal stirring rate for LPME.

3.5. Impact of salt

The impact of salt was investigated by adding sodium chloride to 20 ml aqueous samples at 0, 5, 10, 15 and 25% (w/v) (see Fig. 5). For compounds with a lower octane–water partition coefficient, including ethoprop and diazinon, the extraction yield increased up to 15% (w/v) due to the strong salting out effect [30]. The pesticides with higher octanol–water partition coefficients, disufoton and fenthion, showed no significant difference on addition of salt in the concentration range of 0–15% (w/v). A higher concentration of 25% (w/v) of salt decreased the extraction efficiency. In competition with the extraction process is the fact that polar molecules may participate in electrostatic interaction with the salt ions in the solution, thereby reducing their ability to move into the organic extracting phase [24,33–34]. For all compounds, the maximum yields were reached at a salt concentration of 15% (w/v). This concentration was used in studying the effect of other parameters.

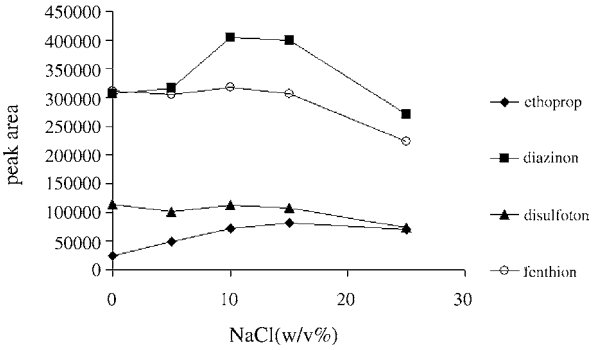


Fig. 5. Effect of sodium chloride, cyclohexane as extraction solvent, spiked to 10 μg/l of each of the analytes, 700 rpm, room temperature.

3.6. Effect of humic acid

The effect of humic acids, common in the general environment, on LPME yield was also investigated. The concentration of humic acid added was varied in the range of 0–100 μg/l. When 10 μg/l of the humic acid was added to the water sample, the extraction efficiency was decreased by 19–23% (see Fig. 6).

3.7. Effect of methanol

The effect of MeOH was also observed by altering its concentration in the aqueous samples between 0 and 25% (v/v) (see Fig. 7). The effect was generally negative for all the pesticides. It was also found that adding more MeOH decreased the extraction efficiency for mixtures [33] of analytes. Especially for fenthion, MeOH had a significant influence; the extraction efficiency decreased by 19% when the concentration of MeOH was only 1% (v/v).

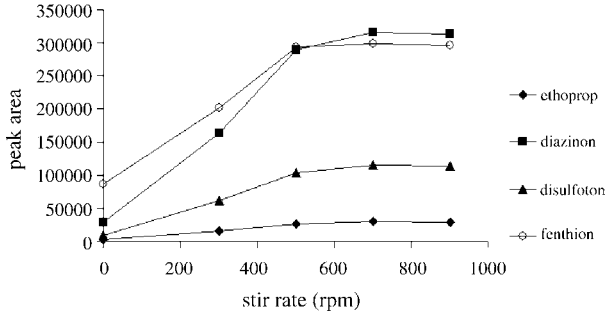


Fig. 4. Effect of agitation rate, cyclohexane as extraction solvent, spiked to 10 μg/l of each of the analytes, room temperature.

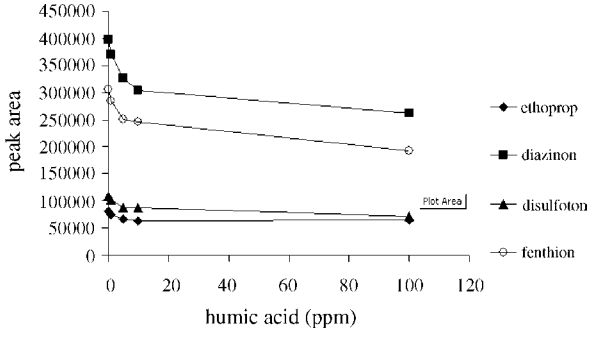


Fig. 6. Effect of humic acid, cyclohexane as extraction solvent, spiked to 10 μg/l of each of the analytes, added 3 g NaCl, 700 rpm, room temperature.

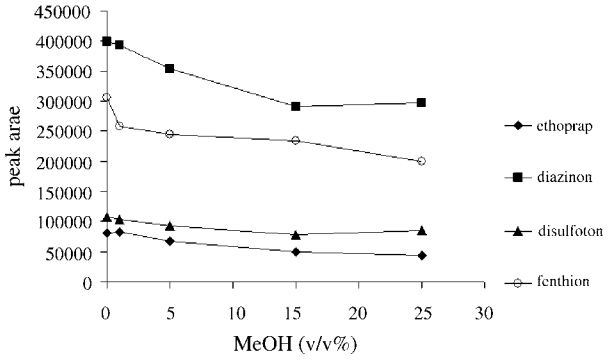


Fig. 7. Effect of MeOH, cyclohexane as extraction solvent, spiked to 10 μg/l of each of the analytes, added 3 g NaCl, 700 rpm, room temperature.

3.8. Method validation

The optimal LPME parameters finally selected were as follows: 20 ml aqueous sample, 3 g NaCl, cyclohexane as extraction solvent, 50 min extraction time, and a stirring rate of 700 rpm. The detection limits, linear dynamic range and the calibration curve are listed in Table 2.

The linear dynamic range was given for each of the analytes. The correlation coefficient of the calibration curve (R^2) was 0.996 or better, which implied that the analytes showed good linearity within these concentration ranges, and allowed the quantification of the agents at concentrations of several μg/l by LPME. Further, the limits of detection differ substantially for the various pesticides. For most of the agents the detection limit is lower than 0.042 μg/l, excluding ethoprop (LOD 0.2 μg/l). The LOD of diazinon is only 0.006 μg/l.

Table 2
Performance of the validation analysis

OPP	LOD[a] (μg/l)	Linear dynamic[b] range (μg/l)	R^2
Ethoprop	0.200	0.5–100	0.999
Diazinon	0.006	0.1–50	0.999
Disulfoton	0.014	0.1–50	0.997
Fenthion	0.042	0.5–50	0.996

[a] LOD is based on a signal-to-noise ratio of 3.
[b] Calibration curve with following concentration: ethoprop 0.5, 1, 5, 10, 50 and 100 μg/l; diazinon 0.1, 0.5, 1, 5, 10 and 50 μg/l; disulfoton: 0.1, 0.5, 1, 5, 10 and 50 μg/l; fention 0.5, 1, 5, 10 and 50 μg/l.

The allowable level for one organophosphorus pesticide is 0.1 μg/l in the European Union (EU) and 0.5 μg/l for the total concentration of these pesticides. The very low detection limits achieved by the method described here can verify organophosphorus pesticide contamination, even though the LOD of ethoprop is somewhat higher than the EU limit. And this method can easily detect the total concentration of mixtures of these pesticides at below the required limit.

3.9. Lake water analysis

To evaluate the extraction method developed on samples made with deionized water, the optimized conditions of analysis

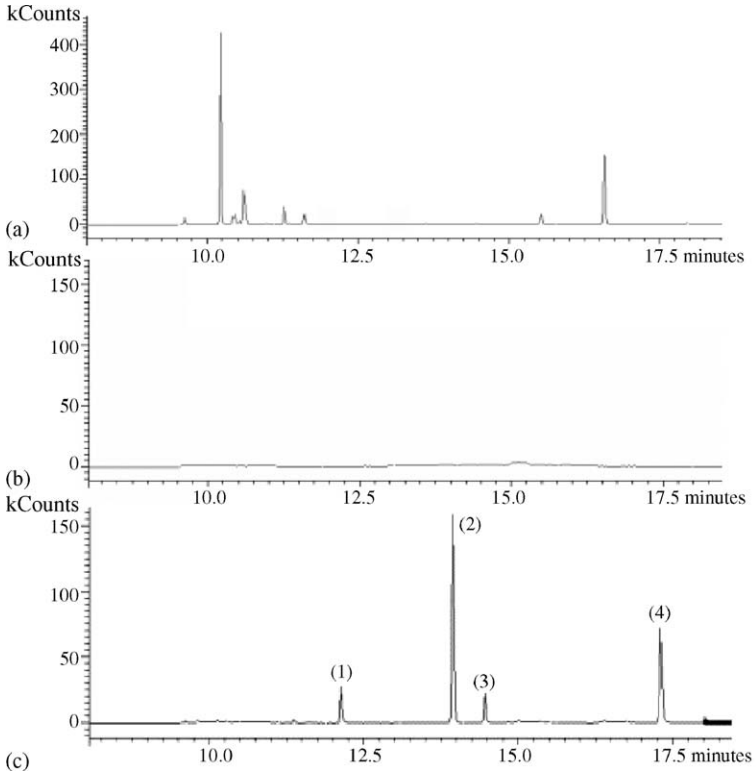


Fig. 8. Chromatogram of the organophosphorus pesticides obtained by LPME under optimized conditions: (a) capillary gas chromatogram of lake water sample. (b) Chromatogram of lake water sample in SIM mode. (c) Spiked at concentrations of 10 μg/l in lake water. Peaks: (1) ethoprop, (2) diazinon, (3) disulfoton and (4) fenthion.

Table 3
Results of the spiked lake water

OPP	Spiked amount (μg/l)	R.S.D.		Lake water	
		Intra-day ($n=5$)	Inter-day ($n=5$)	Absolute recovery (%)	Relative recovery (%)[a]
Ethoprop	10	5.1	5.0	1.78	107.0
Diazinon	10	3.5	7.2	5.17	83.1
Disulfoton	10	7.9	7.9	0.74	85.8
Fenthion	10	6.7	8.0	0.18	93.9

[a] Percentage value obtained considering extraction yields in reagent water as 100%.

were used to investigate a field sample, water from a lake which is surrounded by farms. The lake water was filtered, stored at 4 °C and analyzed within 1 week. Because all target compounds were not found in the field sample, each compound was spiked to a concentration of 10 μg/l in the vial (see Fig. 8). The results of the analyses are listed in Table 3. The precisions of inter- and intra-day are both in the range of 5–8%; the relative recoveries range from 83.1 to 107.0%.

3.10. Comparison of LPME with SPME

SPME holder and fiber assemblies for manual sampling were provided by Supelco (Bellefonte, PA, USA). Polydimethylsiloxane (PDMS, 100 μm) was coated on the fiber. After conditioned in the injector for 3 h at 240 °C, the PDMS fiber was used with the split vent open to remove contaminant that might have caused very high baseline noise and large ghost peaks. Then, the fiber was injected into the GC system until interfering peaks disappeared. During this desorption process, the GC column oven temperature was maintained at 240 °C [35].

Field samples were analyzed using the optimal conditions of LPME, and the precisions and limits of detection were compared with those of SPME/GC/MS [35]. The results are shown in Table 4. It is observed that the LOD found with LPME is comparable to that of SPME. Moreover, the inexpensive and disposable hollow fiber provided some level of clean-up, the small pores on the fibers prevented big and complex components from entering into the fibers [36]. Although SPME and LPME are also fast, solvent-free and non-exhaustive extraction analyses, the coated materials of SPME fibers are not only of limited selection but are also expensive. For LPME, however, a suitable solvent can be chosen easily by considering the different characteristics of the different analytes.

Table 4
Performance of the proposed methods

OPP	LOD (μg/l)		R.S.D.s (%) ($n=3$)	
	SPME[a]	LPME	SPME[a]	LPME
Ethoprop	NS	0.2	–	8.8
Diazinon	0.020	0.006	6	6.6
Disulfoton	0.015	0.014	6	5.0
Fenthion	0.015	0.042	8	6.0

NS: not studied.
[a] The data were obtained from Ref. [35].

4. Conclusion

LPME provides a novel, rapid and easily used technique to extract residues of pesticides from aqueous samples. With optimized parameters, the method needs only 20 ml of sample and 3.5 μl of extraction solvent. The low LOD and low R.S.D. indicate that the technique has great potential and stability for analyzing different field samples with disposable fibers which can decrease matrix influence. The relative recoveries ranged from 83.1 to 107.0%, showing that the extraction process is affected little by the sample matrix. This pretreatment, coupled to a GC–MS provides a powerful and efficient method for identifying and quantitating the target organophosphorus compounds in our environment. The maximum acceptable concentrations set by the European Union for these pesticides can be determined without difficulty.

Acknowledgement

The support of the National Science Council, Republic of China (NSC 94-2113-M-007-017) is acknowledged.

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