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Journal of Chromatography A, 754 (1996) 137–144

JOURNAL OF
CHROMATOGRAPHY A

Analysis of pesticides in environmental water samples by solid-phase micro-extraction–high-performance liquid chromatography

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Abstract

Solid-phase micro-extraction (SPME) followed by high-performance liquid chromatography (LC) has been applied to analyze pesticides in water samples. A device interfaces SPME to the LC injection process by solvent extracting analytes from the fiber and then introducing the solvent into the LC injector. LC analysis with UV detection was performed first with a conventional column (4.6 mm I.D.). To enhance efficiency of SPME, three extraction conditions, stirring, temperature and salt concentration, were optimized. Subsequently, semi-microcolumn LC (1.5 mm I.D.) was evaluated for this method, giving lower detection limits and less solvent consumption. Detection limits were within Japanese regulatory limits for drinking water. The SPME–LC method was applied to real-world environmental water samples.

Keywords: Environmental analysis; Water analysis; Extraction methods; Sample preparation; Pesticides

1. Introduction

In water analysis a sample preparation step is often necessary to isolate and concentrate organic compounds of interest from the aqueous matrix. Several sample preparation methods have been developed. Liquid–liquid extraction (LLE) [1,2] and solid-phase extraction (SPE) [3–5] are the most common approaches for non-volatile compounds. These methods, however, are time consuming, have complicated procedures, are difficult to automate, and require a large amount of organic solvent, although SPE uses relatively little solvent. The use of organic solvent is undesirable for environmental and economical reasons, and reducing its consump-

tion has been urged [6]. For volatile compounds the purge-and-trap method is widely used [7,8], but it is expensive and limited to analysis of volatiles only. An ideal sample preparation technique should be simple, inexpensive, efficient, selective, use minimal solvent and be compatible with various analytical instruments [9].

Solid-phase micro-extraction (SPME) has attracted attention as a sample preparation technique with many of these desirable characteristics [10–16]. SPME uses a fused-silica fiber coated with a gas chromatographic (GC) stationary phase to extract analytes from water samples. The fiber is incorporated into a syringe-like device to facilitate handling and portability. The fiber is exposed to a stirred water sample and analytes are absorbed from the aqueous matrix into the coating. After extraction, the

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fiber with absorbed analytes is directly transferred into the injector of a gas chromatograph for thermal desorption, followed by analysis. SPME performs extraction and concentration simultaneously and uses no solvents. Furthermore, the extraction and the sample introduction process can be fully automated using a conventional autosampler [11]. So far, SPME has been applied to analyze many organic compounds, including benzene, toluene, ethyl benzene and xylene isomers (BTEX) [14], polyaromatic hydrocarbons (PAHs) [15], polychlorinated biphenyls (PCBs) [15] and phenol and its derivatives [16] in water.

However, SPME is currently limited to GC as the main analytical separation method after extraction step, and therefore this technique can not be applied to many compounds unsuitable for GC analysis, which are non-volatile or thermally unstable. These compounds have been generally analyzed by high-performance liquid chromatography (LC). To apply SPME to a wider range of compounds Pawliszyn et al. reported a SPME–LC system with a desorption chamber and a six-port valve [17]. In this investigation a different desorption device which interfaces SPME to the LC injection process was developed. In this device, analytes absorbed into the coating are desorbed on a small amount of solvent. This device enables the application of SPME to LC as it maintains the simplicity of the handling.

Pesticides have been widely used in agriculture and their residues contaminate environmental waters, causing an environmental problem. In Japan pesticides on golf courses, often located in valleys, have contaminated rivers at their sources. Methods using LLE [18] or SPE [19] with GC–MS, or direct injection to LC [20,21] have been reported for these environmental water analyses. In this paper the performance of the SPME–LC system developed has been evaluated by analyzing a range of pesticides in environmental water causing concern in Japan. LC analysis with UV detection was done with a conventional column (4.6 mm I.D.) LC or semi-microcolumn (1.5 mm I.D.) LC. Semi-microcolumn LC has higher mass sensitivity, uses less toxic and expensive solvent, and can be coupled with mass spectrometry for better qualitative and quantitative detection [22,23]. The two systems were compared with regards to the pesticides analysis in environmental water samples.

2. Experimental

SPME holder and fiber assemblies for manual sampling were obtained from Supelco (Bellefonte, PA, USA) and used without modification. Polyacrylate coating (85 μm film thickness), which is relatively polar, was used as the stationary phase in SPME. Before measurements a fiber was processed in solvent desorption several times by the desorption device developed until interfering peaks disappeared. The desorption method is described below.

All solvents were reagent grade, purchased from Kishida Chemical (Osaka, Japan) and deionized water was obtained from a Milli-Q water system (Millipore, Tokyo, Japan). For this study 10 pesticides were selected from the many regulated in Japan and are listed with regulatory limits in Table 1. These pesticides were purchased from Wako (Osaka, Japan) except propyzamide, which was purchased from Riedel-de Haën (Hannover, Germany). Each was delivered in methanol and 10 solutions were mixed. Spiking standards were prepared by diluting the mixture in methanol. A water sample was prepared by spiking 15 μl of the spiking standard into 15 ml Milli-Q water in a 20 ml sample vial with cylinder-shaped stir bar (4 \times 6 mm). All measurements were in duplicate or triplicate.

Two LC systems were used. One consists of a Jasco PU-980 pump and UV-970 UV–Vis detector (Tokyo, Japan), Rheodyne 7125 injector (Cotati, CA, USA) with a 20 μl loop and 250 \times 4.6 mm I.D. Superiorex ODS column (Shiseido, Tokyo, Japan), and the flow-rate was 1 ml/min. The other is a

Table 1
Several selected pesticides

Compound	Class	Molecular mass	Regulation (ppb)	
			A	B
Fenobucarb/BPMC	Insecticide	207.3	–	20
Propyzamide	Herbicide	256.1	80	8
Iprofenfos/IBP	Fumigant	288.4	–	8
Isoprothiolane	Insecticide	290.4	400	40
Chlorothalonil/TPN	Fumigant	265.9	400	40
Fenitrothion/MEP	Insecticide	277.2	100	3
Diazinon	Insecticide	304.4	50	5
Thiobencarb	Herbicide	257.8	–	20
Bensulide	Herbicide	397.5	1000	–
EPN	Insecticide	232.3	–	6

A: Guidelines on the usage in a golf course.

B: Environmental standards and the standards of drinking water.

Shiseido Nanospace SI-1, which is constructed with a pump, a UV-Vis detector, a column oven and a degasser, equipped with 250×1.5 mm I.D. Superiorex ODS column, Rheodyne 7125 injector with a 1 μ l loop, the flow-rate was 100 μ l/min and column temperature was controlled at 30°C. In both systems the mobile phase was acetonitrile–water (45:55) and detection was at 220 nm. Data were electronically acquired and processed using BORWIN chromatography software (Jasco) on a personal computer.

3. Results and discussion

Fig. 1 shows a cross section of an SPME device inserted into the SPME-to-LC desorption device. It consists of a stainless-steel tee, connecting fittings, stainless-steel tubing, PTFE tubing and a septum, which are parts normally used for GC or LC. After extraction the SPME device needle, with fiber withdrawn, is inserted into desorption device filled with desorption solvent through PTFE tubing and pierces

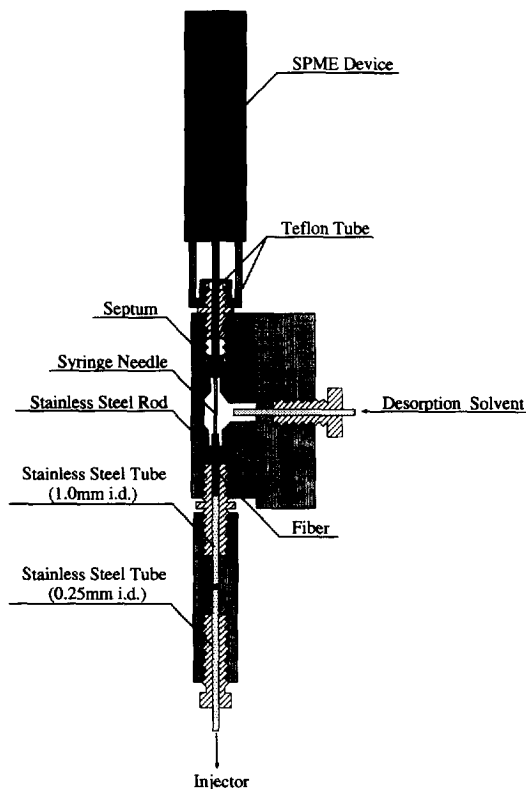


Fig. 1. Schematic of the desorption device used in this study.

the septum, as shown in Fig. 1. The tip of the needle is set in 1.0 mm I.D. stainless-steel tubing, and then pushing down the plunger of the SPME device exposes the fiber to the solvent for the desorption time while analytes absorbed on the fiber partition into the solvent. The solvent containing analytes desorbed from the fiber is carried out into the LC injector by manually flushing a certain amount of solvent using a micro syringe. The volume of the desorption device is approximately 80 μ l and acetonitrile was employed as the desorption solvent in this study. After desorption ends, the fiber is drawn back into the syringe needle and the SPME device is pulled out from the desorption device. The handling manner of the SPME device in SPME-LC using this desorption device is the same as that in SPME-GC.

The acetonitrile containing analytes is injected to the column using a standard LC injector (Fig. 2). When desorption ends, the injector valve is changed to load position and the solvent containing analytes is carried into the LC injector by manually flushing with a micro syringe. As soon as the loop is filled with the acetonitrile containing analytes the valve is changed to inject position, and the acetonitrile in the loop is introduced to the column. In case of conventional column LC, 20 μ l of acetonitrile was flushed into the desorption device, and all 20 μ l of acetonitrile carried out was sent to the column. Not all analytes desorbed from the fiber are introduced to the column to avoid peak broadening.

Preliminary work showed the efficiency of SPME for pesticides with polyacrylate coating was low. Therefore stirring-speed, temperature, and concentration of salt added were optimized. For these optimizations all extractions were 60 min, and desorptions for 30 min. First, the optimum stirring-speed was determined. Fig. 3 shows area counts vs. stirring-speed for four pesticides, indicating the

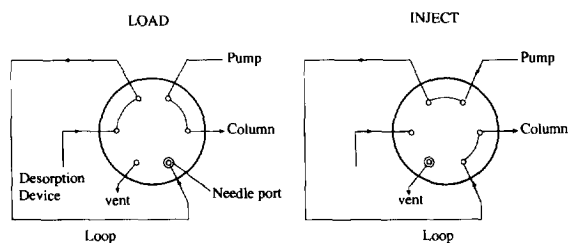


Fig. 2. Schematic of the injection valve.

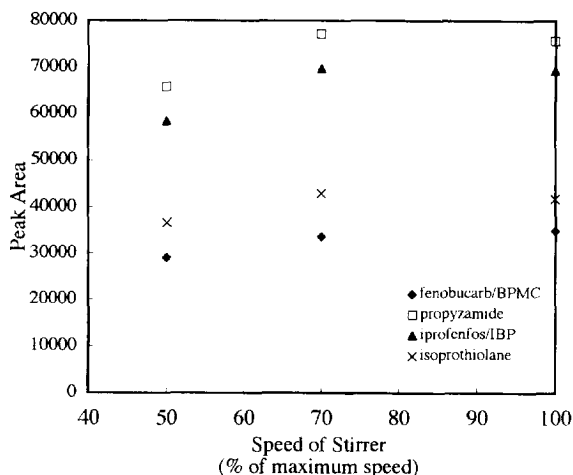


Fig. 3. The effect of stirring-speed on the SPME recovery of pesticides.

saturation at 70% of the full stirring-speed. The same results were obtained for other pesticides studied, although these data are not shown. Therefore the speed of the stirrer was 70% for subsequent experiments.

Second, the extraction temperature was examined. During extraction the vial holding the water sample was set in a water bath controlled at a constant temperature. Results for the 10 pesticides are shown in Fig. 4. Recoveries remarkably increase with temperature due to increase of the rate of diffusion of pesticides. As the increase of recovery on the temperature over 60°C is not observed for fenobucarb/BPMC, fenitrothion/MEP, thiobencarb and bensulide, the extraction temperature was set at 60°C for subsequent experiments.

Salting can enhance extraction of some compounds from water [11]. Fig. 5 shows the effect of sodium chloride concentration on the recovery of pesticides. Some pesticides have maximum recovery at 0.27 g/ml of NaCl concentration, others at 0.13 g/ml, and one at 0 g/ml. NaCl concentration of 0.27 g/ml was arbitrarily selected for subsequent experiments.

SPME is not an exhaustive extraction but an equilibrium between the analyte concentration in the sample solution and that in the coating. Therefore the time required to reach the equilibrium should be

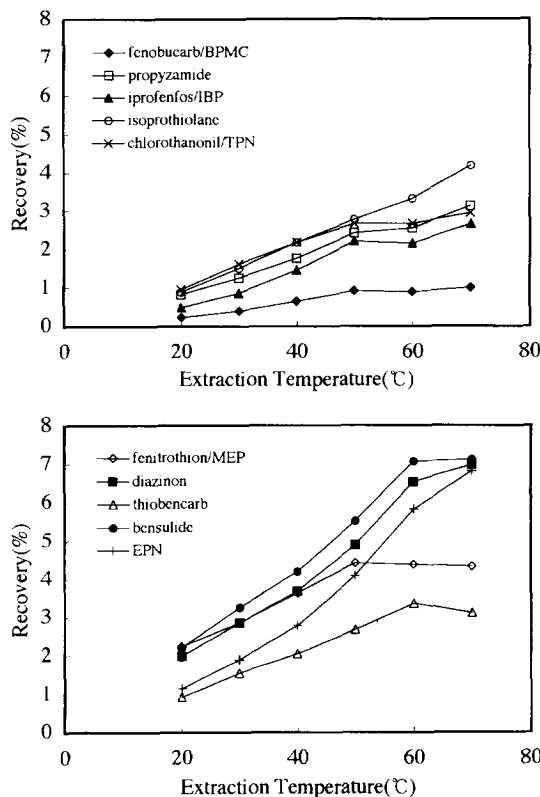


Fig. 4. The effect of extraction temperature on the recovery of pesticides.

examined. Fig. 6 shows the extraction vs. time profiles for the pesticides. So far it has been reported that the shorter extraction time, which is of the order of minutes, is one of the advantages of SPME method. For these pesticides, however, the extraction time of over 180 min was required to reach the equilibrium when polyacrylate coating was used. This problem can be improved to some extent by using the thinner coating, but it is not available at present. Although the equilibrium is not reached at 180 min for isoprothiolane and bensulide, the extraction time was set at 180 min for subsequent experiments for practical convenience.

Fig. 7 shows the desorption vs. time profiles for pesticides. After the LC analysis was finished, the fiber was desorbed again for 30 min without re-exposing to the water sample. The ratio of the amount of pesticides remaining on the fiber after the

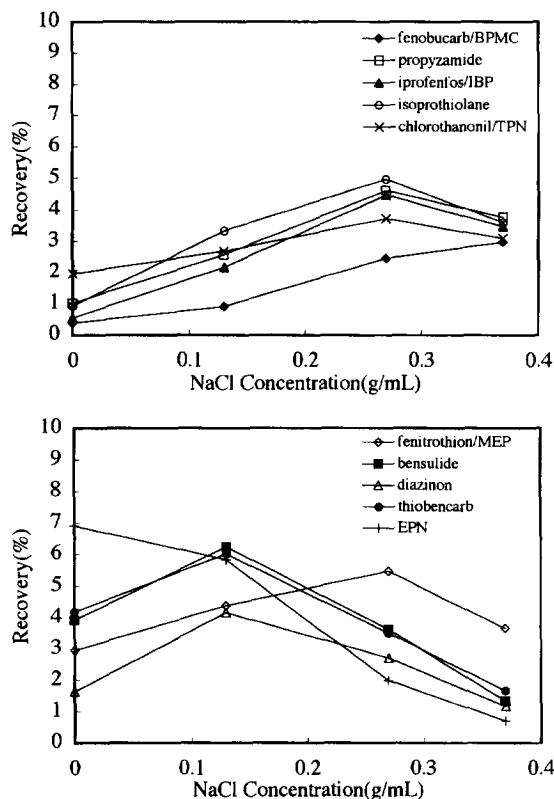


Fig. 5. The effect of NaCl concentration on the recovery of pesticides.

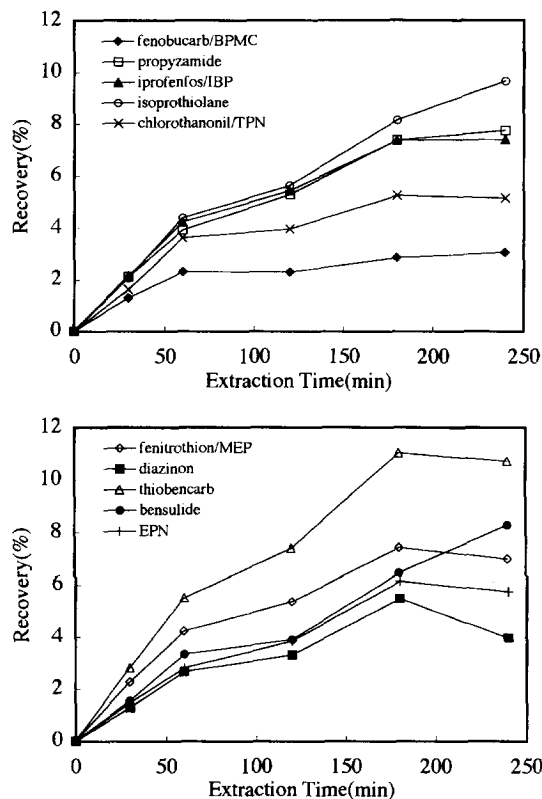


Fig. 6. Extraction vs. time profiles for pesticides. Extraction conditions: stirring-speed 70% of the full power, temperature 60°C, NaCl concentration 0.27 g/ml.

first desorption to the total amount of pesticides absorbed is represented as carryover. For most pesticides, carryover did not decrease after 30 min, although most still had a few percent of carryover. The desorption time was set at 30 min for subsequent experiments.

The fibers lasted for 10 to 20 desorptions, less than in the SPME–GC method. When the fiber is drawn back, the polyacrylate coating is caught inside the syringe needle and comes off the fiber not gradually but at a stroke. Also, the affinity of polyacrylate for pesticides is moderate. More robust and selective coatings are expected to be developed by the manufacturers and would enable a routine analysis.

Table 2 shows detection limits for SPME–conventional column LC performed under the optimized conditions. Progressively diluted concentrations of

water samples were extracted and the detection limits were considered the concentrations that gave a signal-to-noise ratio of 2. These values are lower than the Japanese regulatory limits for a golf course (A), and for environmental and drinking water (B) except for diazinon. Precision was determined by analyzing 5 replicate samples consecutively using the same fiber, and Table 2 shows the relative standard deviation values (R.S.D.) of the peak areas.

The decrease in column diameter brings about several advantages over conventional diameter column [22,23]. Semi-microcolumn LC was expected to be more sensitive than conventional LC despite smaller injection volume. Lower consumption of mobile phase is also desirable because SPME has been developed as a solvent-free technique. Fig. 8 compares chromatograms of the standard solution of

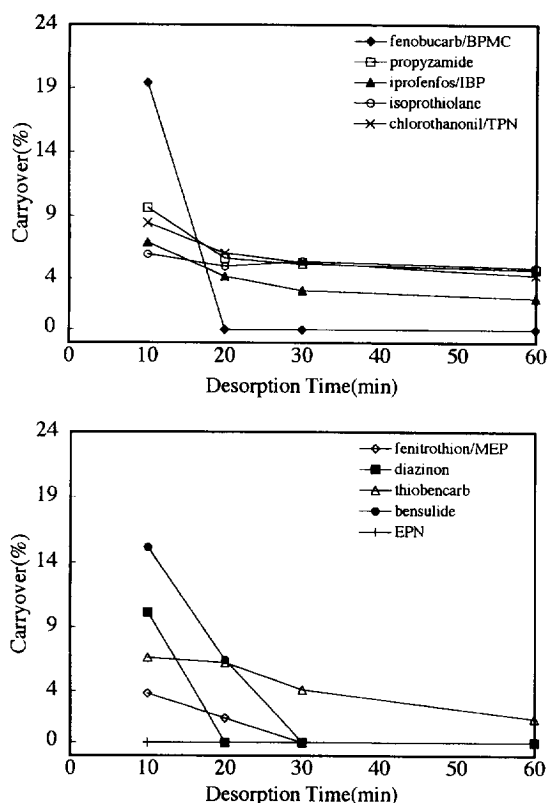


Fig. 7. Desorption time profiles for pesticides. Extraction was performed for 180 min.

pesticides (10 ppm) with a conventional column LC (a) and a semi-microcolumn LC (b), using the same mobile phase composition. Peaks are as high in the chromatogram obtained with the semi-microcolumn as with the conventional column, although twenty times less was injected.

The distribution of pesticides in acetonitrile in the desorption device is suspected of being non-homogeneous. The optimum flushing volume was determined for SPME–semi-microcolumn LC. In this experiment 50 ppb pesticides in water was extracted under optimized conditions, except extraction was for 60 min. After the desorption a certain amount of acetonitrile was flushed into the desorption device using a micro syringe, LC analysis was performed, and the amounts injected into the column were determined for each pesticide. Fig. 9 shows that when 20 μ l of acetonitrile is flushed, the highest recovery is obtained for almost all pesticides. Therefore 20 μ l volume was selected for flushing the acetonitrile solution.

Detection limits and R.S.D. values for pesticides in water using SPME–semi-microcolumn LC are summarized in Table 2. Compared to that by conventional column LC, the detection limit is improved for all pesticides, and for diazinon is within the drinking water regulatory limit. A trend of higher R.S.D. is seen, likely because only 1 μ l out of 20 μ l of

Table 2

Comparison of detection limits and R.S.D. values of peak area based on five replicates for several selected pesticides in environmental water sample using SPME–conventional column (4.6 mm I.D.) LC and SPME–semi-microcolumn (1.5 mm I.D.) LC

Compound	Regulation (ppb)		4.6 mm I.D. column LC		1.5 mm I.D. column	
	A	B	Detection limits (ppb)	R.S.D. (%)	Detection limits (ppb)	R.S.D. (%)
Fenobucarb/BPMC	–	20	8	10.5	5	12.4
Propyzamide	80	8	1.5	5.3	0.5	4.4
Iprofenfos/IBP	–	8	2	9.0	1	13.4
Isoprothiolane	400	40	4.2	5.5	3.8	10.1
Chlorothalonil/TPN	400	40	1.5	5.9	0.5	9.6
Fenitrothion/MEP	100	3	3	7.6	2.7	14.0
Diazinon	50	5	12	9.2	4	6.2
Thiobencarb	–	2	0.5	9.0	0.1	7.1
Bensulide	1000	–	5	6.8	2.8	8.7
EPN	–	6	4.5	7.8	3	15.2

A: Guidelines on usage in a golf course.

B: Environmental standards and the standards of drinking water.

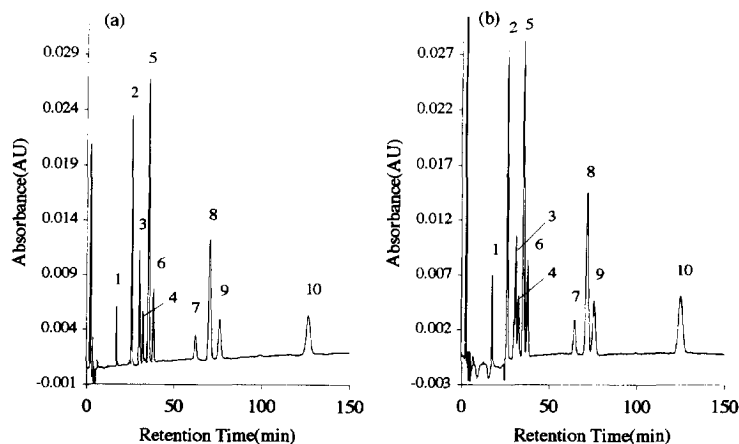


Fig. 8. Chromatograms of the standard solution of pesticides by (a) conventional column LC (4.6 mm I.D.) and (b) semi-microcolumn LC (1.5 mm I.D.). The standard solution is 10 ppm each of pesticide. Injection amount is 20 μ l for (a) and 1 μ l for (b). 1=Fenobucarb/BPMC; 2=propyzamide; 3=iprofenfos/IBP; 4=isoprothiolane; 5=chlorothalonil/TPN; 6=fenitrothion/MEP; 7=diazinon; 8=thiobencarb; 9=bensulide; 10=EPN.

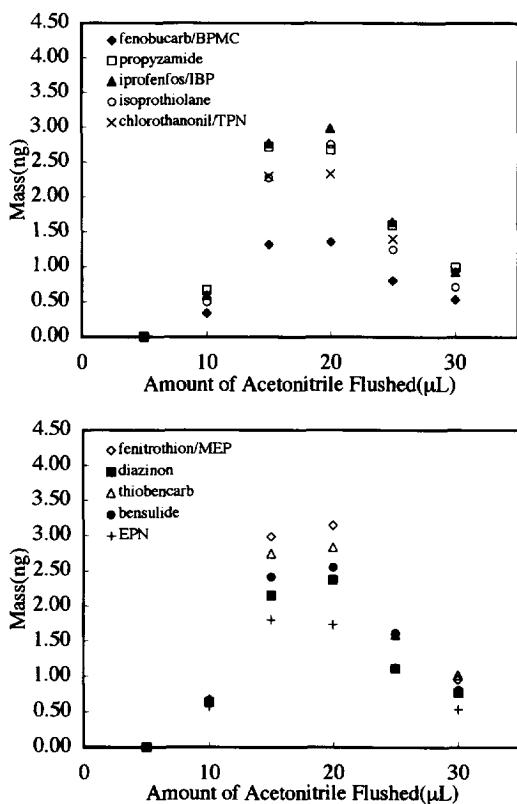


Fig. 9. The effect of the amount of acetonitrile flushed into the desorption device. Extraction for 60 min and desorption for 30 min was carried out.

acetonitrile containing pesticides, manually introduced into the column heightens any variation in the desorption process. The poor precision might be improved by controlling temperature around the desorption device and flushing acetonitrile automatically.

The SPME–semi-microcolumn LC under the optimized conditions was applied to two water samples collected on different days from the same pond in a local golf course. These water samples had considerable suspended matter that stuck to the fiber during extraction. Since the matter could prohibit absorption of analytes, the water samples were filtered using 0.45 μ m pore size DISMIC-1.3HP membrane filter (Advantec, Tokyo, Japan). Fig. 10 shows SPME–semi-microcolumn LC chromatograms of the water samples collected on July 23 (a) and August 30 (b), 1995. In the water sample (a) two pesticides were detected, while in (b) no signals were detected. From the calibration curves for the pesticides on this system, fenitrothion/MEP and thiobencarb in sample (a) were determined 6.6 ppb and 1.2 ppb, respectively. The volume of water sample required for analysis is only 15 ml, much less than for LLE or SPE, and the volume could be further reduced because SPME is equilibrium based. The small sample volume for the SPME–LC method can be very useful when environmental samples are limited, or when analyzing other types of samples such as biological.

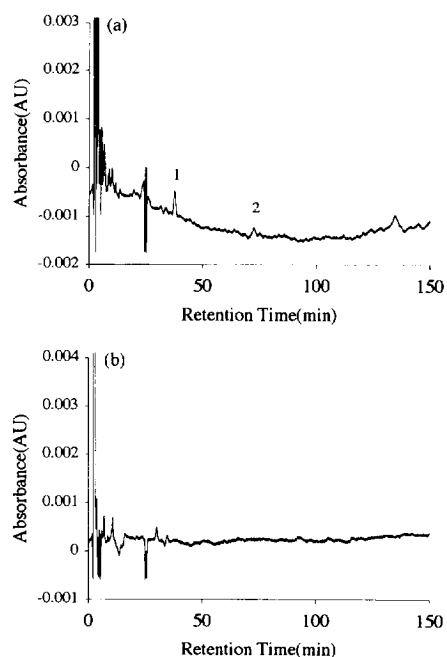


Fig. 10. SPME–semi-microcolumn LC chromatograms of the environmental water samples obtained from a pond in a golf course on (a) 23 July and (b) 30 August, 1995. 1=Fenitrothion/MEP; 2=thiobencarb.

4. Conclusion

The SPME–conventional column LC method developed is applicable to pesticides analysis in environmental water samples. The SPME–semi-microcolumn LC system is more desirable due to enhanced sensitivity and reduced consumption of organic solvents. This method can be used with other detectors such as diode array and mass spectrometer, which can give more qualitative and/or quantitative information. In general, this technique enables SPME analyze compounds unsuitable for GC analysis, including biological, which can be thermally unstable and have small sample volumes.

Acknowledgments

We gratefully acknowledge T. Furukawa and T. Mizoguchi for preparing some of the figures, and the Sumitomo Foundation for financial support.

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