Available online at www.sciencedirect.com

SCIENCE DIRECT®

Talanta 64 (2004) 887–893

Talanta

www.elsevier.com/locate/talanta

Analysis of triazine in water samples by solid-phase microextraction coupled with high-performance liquid chromatography

Shang-Da Huang*, Hsin-I Huang, Yu-Hsiang Sung

Department of Chemistry, National Tsing Hua University, Hsinchu 30043, Taiwan

Received 21 October 2002; received in revised form 26 March 2004; accepted 26 March 2004
Available online 28 July 2004

Abstract

Solid-phase microextraction (SPME) coupled with high-performance liquid chromatography (HPLC) for the determination of triazine is described. Carbowax/templated resin (CW/TPR, 50 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB, 60 μm), polydimethylsiloxane (PDMS, 100 μm), and polyacrylate (PA, 85 μm) fibers were evaluated for extraction of the triazines. CW/TPR and PDMS/DVB fibers were selected for further study. Several parameters of the extraction and desorption procedure were studied and optimized (such as types of fibers, desorption mode, desorption time, compositions of solvent for desorption, soaking periods and the flow rate during desorption period, extraction time, temperature, pH, and ionic strength of samples). Both CW/TPR and PDMS/DVB fibers are acceptable; a simple calibration-curve method based on simple aqueous standards can be used. The linearity of this method for analyzing standard solution has been investigated over the range 5–1000 ng mL^{-1} for both PDMS/DVB and CW/TPR fibers. All the correlation coefficients in the range 5–1000 ng mL^{-1} were better than 0.995 except Simazine and Atratone by CW/TPR fiber. The R.S.D.s range from 4.4% to 8.8 % (PDMS/DVB fiber) and from 2.4% to 7.2% (CW/TPR fiber). Method-detection limits (MDL) are in the range 1.2–2.6 and 2.8–3.4 ng mL^{-1} for the two fibers. These methods were applied to the determination of trazines in environmental water samples (lake water).

Keywords: Solid-phase microextraction; High-performance liquid chromatography; Triazine

1. Introduction

The analysis of herbicides in natural waters is an area of increasing importance, not only because of their potential toxicity, persistence, and water solubility, but also because of their widespread application. Triazine derivatives are among the most important selective herbicides. They and their degradation products are very toxic and highly resistant and survive many years in the soil [1], water, plants, and animals [2]. In the European Community, the upper limit for the presence of an individual pesticide in drinking water is set at 0.1 ng mL^{-1} and the limit is 0.5 ng mL^{-1} for the total pesticide content [3]. In surface water, these limits are about an order of magnitude higher (1–3 ng mL^{-1}). The US Environmental Protection Agency (EPA) considers the toxicity of the pesticides and has established different limits for each one [3].

Most applications are based on chromatographic determination; gas chromatography (GC) and high-performance liquid chromatography (HPLC) are good options for monitoring triazines in water [1,4–6]. A prior derivatization step is required to analyze some hydroxyl derivatives of triazines by GC [6–9].

Solid-phase microextraction (SPME), a relatively new extraction technique, was introduced by Pawliszyn and Belardi [10]. SPME integrates sampling, extraction, concentration, and sample introduction into several simple processes, and most importantly, it uses no solvent during extraction. Finally, the SPME device based on a reusable microsyringe was commercialized in 1993 by Supelco, together with the coated fibers used for extraction, which were initially polydimethylsiloxane (PDMS) and polyacrylate (PA), and which now include other coatings such as Carbowax-divinybenzene, Carbowax-templated resin, PDMS-divinylbenzene, and Carboxen-PDMS.The applications of SPME on the analysis of triazines were exclusively based on separation and analysis by GC [11–14]. The

---

* Corresponding author. Tel.: +886 3 5721194; fax: +886 3 5736979.
E-mail address: sdhuang@mx.nthu.edu.tw (S.-D. Huang).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.03.063

inter-laboratory validation of SPME–GC for the determination of triazine in water has been carried out [11]. Comparable reproducibility and accuracy were found with the SPME method and the reference methods (purge and trap and static headspace techniques). The detection limits were in the range 4–24 ng L^{-1}. SPME coupled with HPLC has been developed successfully by Chen and Pawliszyn in 1995 [15]. Recently, many applications of SPME/HPLC have appeared in the literature, such as analyte for polyaromatic hydrocarbons, alkylphenol ethoxylate surfactants, proteins, pesticides, corticosteriods, etc. The advantage using HPLC is that compounds with low volatility or thermal labile compounds can be analyzed. The detection limits of this method for triazine analysis using HPLC–UV are generally higher than those obtained with GC–NPD or GC–MS. The coatings on the fiber for SPME are also somewhat limited using HPLC.

In this paper, the analysis of five triazines in water samples by SPME/HPLC was tested. Several parameters of SPME were investigated, such as fiber coating, desorption procedure, the temperature, pH, elutropic strength, and ionic strength of sample solution. Finally, the effect of a different matrix (lake water) was checked using spiked samples.

2. Experimental

2.1. Chemicals and reagents

Analytical-reagent grade Ametryn, Atratone, Prometone, and Simazine were purchased from Riedel-dehaën (Germany); Atrazine was obtained from ChemService (USA). HPLC-grade sodium acetate was obtained from Merck, acetonitrile and methanol were purchased from Tedia, sodium sulfate and sodium chloride were purchased from Showa (Japan). Stock standard solutions were prepared by weighing the triazines and dissolving them in methanol. A working composite standard solution was prepared by combining an aliquot of each stock solution and diluting the mixture with deionized water. Deionized water was purified in a Milli-Q purification system (Millipore). Lake water from National Tsing Hua University served as the environmental sample.

2.2. Equipment

The HPLC system, assembled from modular components (Waters), consisted of a model 600 E pump and a model 486-UV detector. A Millennium workstation (Waters) was utilized to control the system and for acquisition and analysis of data. All separations were carried out on a 4-μm C18 column (15 cm × 3.9 mm, Waters). The mobile phase was acetonitrile–acetate buffer (0.1 M, pH 6) (35/65) and UV detection was at 220 nm.

The SPME fiber assembly and SPME/HPLC interface were purchased from Supelco (Bellefonte, PA). The SPME/HPLC interface consists of a six-port injection valve and a desorption chamber (chamber volume, 200 μL) which replaces the injection loop of a six-port injection system. SPME fibers (from Supelco) coated with Carbowax/templated resin (CW/TPR) (50 μm), PDMS (100 μm), PDMS/DVB (60 μm), and PA (85 μm) were used in this work.

2.3. SPME/HPLC procedure

Each day prior to sample analysis, the fibers are conditioned in the interface with mobile phase for approximately 30 min until they are free from the contaminants (judged from the baseline). After conditioning, the fiber can be used for extraction. Aliquots of 3 mL of standard solutions or real samples were extracted from 4-mL vials sealed with hole caps and Teflon septa which was punctured to provide an aperture to permit passage of the fiber. The depth of immersion was kept constant. The sample solution is stirred with a stiring bar and the stirring speed controlled at 550 ± 10 rpm by a digital/magnetic stirrer (Electrothermal HS 4000/5000). The temperature of the solution was 25 ± 2 °C, unless otherwise specified.

After samples extraction, the SPME fiber was introduced into the desorption chamber under ambient pressure when the injection valve is in the load position. For static desorption, the fiber was soaked in the desorption chamber (full of mobile phase) for several minutes, then the valve was switched to the inject position and the analytes were delivered to the column. For dynamic desorption, the fiber was not soaked in the desorption chamber, and the analytes were directly removed by a moving stream of mobile phase. The flow rate was 0.6 mL min^{-1}.

To minimize the possibility of analyte carry-over, the fiber was held in the desorption chamber for 5 min, and then the fiber was flushed twice with 500 μL portions of mobile phase to clean the fiber.

3. Results and discussion

3.1. Fiber evaluation

Fiber coatings dominate the recoveries of analytes. According to the rule of "like dissolves like", four different fibers were selected for preliminary investigation: PA, PDMS, CW/TPR, and PDMS/DVB fibers. The polar series of these fiber coatings are as follows: polar → PA → CW/TPR → PDMS/DVB → PDMS → nonpolar. The chromatogram by SPME is shown in Fig. 1. Fig. 2 shows the relative extraction efficiencies of these for triazine (expressed by peak areas of each compound). Obviously, PA fiber exhibited the lowest extraction and PDMS/DVB fiber exhibited the best extraction. PDMS/DVB and CW/TPR (the second best) fibers were used for further investigation. Sometimes the fiber with better extraction efficiency may undergo matrix interference.

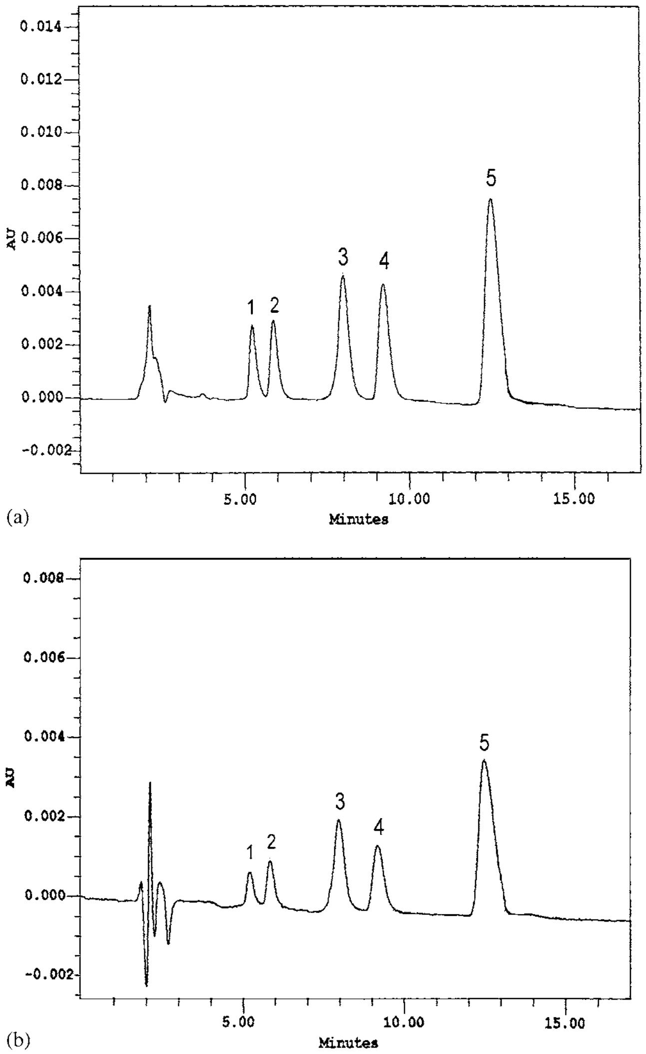

Fig. 1. Chromatograms obtained for SPME extraction of five triazines: (a) with PDMS/DVB fiber; (b) with CW/TPR fiber. Sample volume: 3 mL, concentration: 100 ng mL^{-1}, extraction time: 40 min, static 4 min, flow rate: 0.6 mL min^{-1}. Peak assignment: (1) Simazine; (2) Atratone; (3) Atrazine; (4) Prometone; (5) Ametryn.

3.2. *Extraction/time profile*

SPME is not an exhaustive extraction but an equilibrium process, in which analytes partition between the sample matrix and the coating. The recovery of analytes increase with extraction time, until it reaches equilibrium. Fig. 3 (a) and (b) shows the time profile of extraction for the five triazine using PDMS/DVB and CW/TPR fibers. Equilibration was not reached in the time interval (60 min) using PDMS/DVB fiber. Most of the analytes except Ametryn have almost reached equilibrium after 20 min with CW/TPR fiber. The extraction time of 20 min is a reasonable compromise between a good peak area and an acceptable analysis time for both PDMS/DVB and CW/TPR fibers.

3.3. *Desorption Mode*

After immersion of the fiber (PDMS/DVB and CW/TPR) in the sample solutions for 20 min, the fiber was introduced into the SPME/HPLC interface for desorption. The desorption modes include both a static and a dynamic mode. Table 1 shows the effect of the desorption mode with PDMS/DVB and CW/TPR fibers. For PDMS/DVB fiber, improvements in sensitivity (peak area) of 45%–7% were achieved using the static mode as compared to that for the dynamic mode using mobile phase (mixture of acetonitrile and acetate buffer) as the desorption solvent. For CW/TPR fiber, improvements in sensitivity (peak area) were in the range of 73%–5%, except for atrazine. This indicates that the polar analytes are adsorbed onto the polar fiber strongly, such that the static mode can improve desorption of the polar analytes from the fiber. The static mode was thus used for further study.

3.4. *Compositions of solvent for desorption*

The relative recoveries of triazines with different solvent compositions for desorption are shown in Fig. 4. The mobile phase (a mixture of acetonitrile and acetate buffer, 35/65) is a better solvent to desorb the analytes from the fibers than the other solvents studied. This mobile phase was thus used as the desorption solvent for further investigation.

3.5. *Soaking period*

The soaking time profile for CW/TPR and PDMS/DVB fibers was studied. Ametryn was desorbed more effectively at longer soaking period for both fibers. This indicates that ametryn was adsorbed more strongly on the fibers (which can also be observed from its higher sensitivity), so increasing

Table 1
Peak areas of analytes in the chromatogram[a] showing the effect of desorption mode used for PDMS/DVB and CW/TPR fibers

Compounds	PDMS/DVB			CW/TPR		
	Dynamic mode	Static mode	Gain (%)	Dynamic mode	Static mode	Gain (%)
Simazine	38388	41180	7.3	8168	14146	73.2
Atroatone	38843	43551	12.1	20894	22824	9.2
Atrazine	98498	102600	4.2	64690	52663	−18.6
Prometone	70882	93571	32.0	40022	44476	11.1
Ametryn	148090	214812	45.1	117358	123620	5.3

[a] Concentration: 100 ng mL^{-1}, absorption time: 20 min, desorption mode: dynamic or static 4 min.

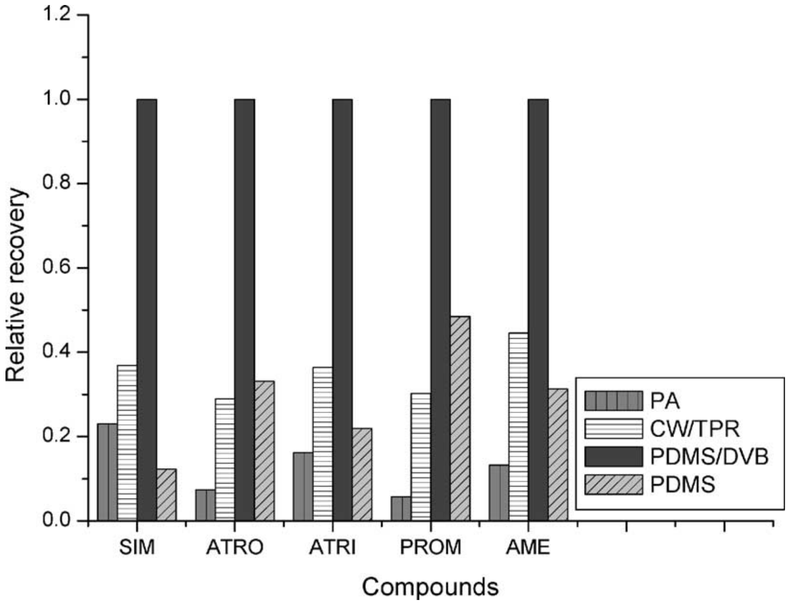

Fig. 2. Relative extraction efficiencies of triazines with various fiber coating. Concentration: 100 ng mL^{-1}, extraction time: 40 min, desorption mode: static 4 min.

soaking time enhances the recovery. Soaking periods of 4 and 3 min were chosen for PDMS/DVB and CW/TPR for further study as these offered somewhat better sensitivity.

3.6. Desorption period

The effects of the desorption period (the period during which the fiber is washed by the mobile phase) were studied. If the desorption rate of analytes is slow, increasing the desorption period can enhance analyte recoveries. It was found that the efficiency of desorption did not vary when the desorption period was varied from 1 to 5 min for both fibers. A desorption period of 2 min was used for further study.

3.7. Flow rate during the desorption period

It was found that no statistical variation in peak area was observed by varying the flow rate during desorption period from 0.2 to 0.6 mL min^{-1}. Band broadening and peak tailing were not observed at the higher flow rate (0.6 mL min^{-1}). A flow rate during the desorption period of 0.6 mL min^{-1} was chosen for both fibers for further study.

3.8. Carry-over

For the runs using PDMS/DVB fiber (sample concentrations, 500 ng ml^{-1}), no carry-over of Atrazine and propazine was found, and the carry-overs of Simazine, Atratone, Desmetryn, Prometone, and Ametryn were 2.6%, 2.6%, 2.3%, 2.1%, and 1.9%, respectively. For the runs using CW/TPR fiber, no carry-over of the analytes was observed, except for Simazine (2.9%) and Ametryn (0.6%). To minimize the possibility of analyte carry-over, the fiber was held in the desorption chamber for 5 min, and then the fiber was flushed twice with 500 μL portions of mobile phase to clean the fiber. All compounds have no carry-over after the cleaning step. However, carry-over in SPME is not such a big concern as in many other methods because SPME is an equilibration method. Carry-over may become a problem, only when the concentration of the analyte in the following sample is so low that the equilibrium concentration in the coating is lower than the concentration caused by carry-over from the previous analysis [16]. When samples of widely differing concentrations are analyzed in sequence, it is recommended that the fiber be flushed twice with 500 μL portions of mobile phase to clean the fiber.

3.9. Effect of pH

The pH of the original sample solutions (100 ng mL^{-1}) was about 5.8. The proper pH ranges are 2–10 and 5–10 (suitable for PDMS/DVB and CW/TPR fibers, respectively). It was found that a pH range of 5–9 does not have a significant effect on the extraction efficiency of triazines using either fiber. When the pH of the solution was adjusted to 3 (for PDMS/DVB fiber), the adsorption of the analytes on the fiber decreased significantly due to the protonation of the weak bases in the acidic solution.

3.10. Effect of extraction-temperature

The temperature of extraction plays an important role on the adsorption of analytes, because it influences the mass transfer rates and the partition coefficients of the analytes. Extraction-temperature profiles for PDMS/DVB and CW/TPR fibers are studied. The peak area of analytes increased with increasing temperature for PDMS/DVB fiber. Note that for an extraction period of 20 min, the adsorption

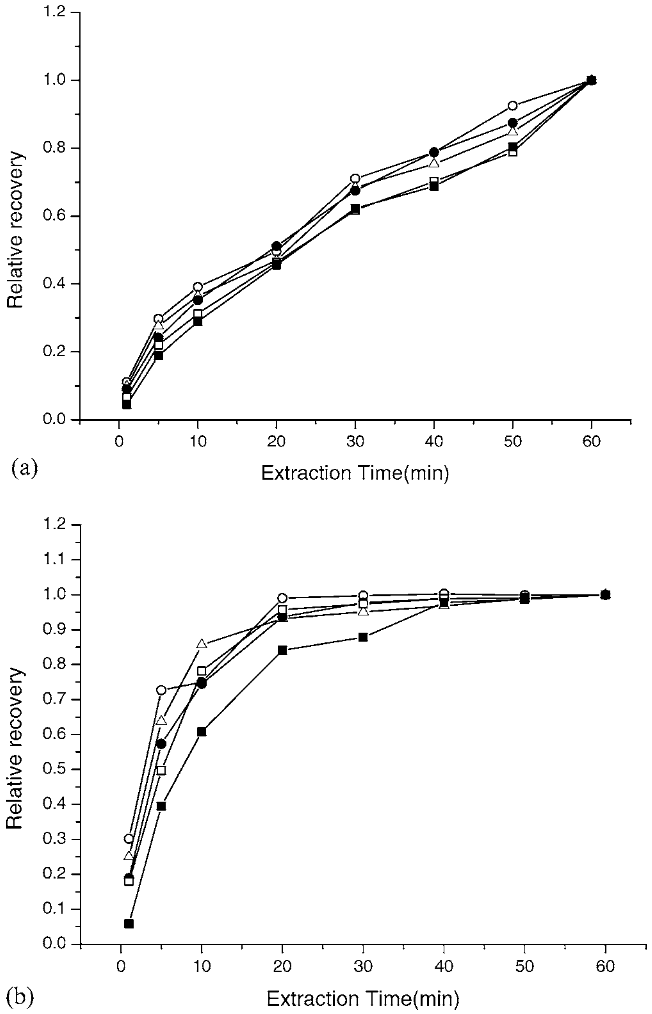

Fig. 3. (a) Extraction time profile for PDMS/DVB fiber; (b) extraction time profile for CW/TPR fiber, concentration: 100 ng mL^{-1}, desorption mode: static 4 min. Peak notation (○) Simazine, (△) Atratone, (●) Atriazine, (□) Prometone, (■) Ametryn.

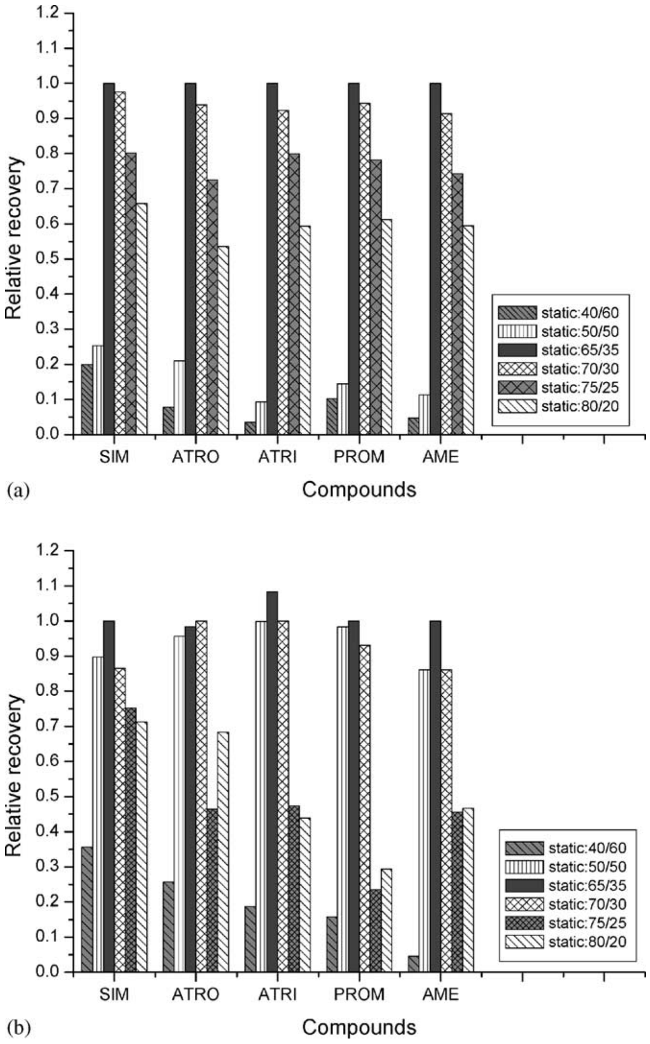

Fig. 4. Relative recoveries of triazines with different compositions of solvent for desorption using (a) PDMS/DVB fiber; (b) CW/TPR fiber. Concentration: 100 ng mL^{-1}, absorption time: 20 min, static 4 min.

of trizines on the PDMS/DVB fiber is far from equilibrium (see Fig. 3), and thus the kinetic effect is the dominant effect. The amount adsorbed increased because the diffusion rate increased with increasing extraction temperature. For CW/TPR fiber, the amount of the analytes adsorbed decreased somewhat with increasing temperature of extraction from 25 to 55 °C. Equilibration is almost reached for an adsorption period of 20 min with CW/TPR fiber; and the decrease in adsorption with increasing temperature is due to the decrease of the distribution constant with increasing temperature. Because adsorption is generally an exothermic process, the amount of analyte adsorbed decreases with increasing temperature [17].

3.11. Effect of ionic strength

The effects of ionic strength on extraction efficiency were studied by preparing standards with Na_2SO_4 at concentrations from 0% to 20% (w/v). Fig. 5 shows the effect of ionic strength on the adsorption of aromatic amines by CW/TPR and PDMS/DVB fibers. The recoveries of the aromatic amines increase with increasing ionic strength of the solutions for both fibers due to a “salting-out effect”. The amounts of trizines adsorbed on the CW/TPR fiber from 20% Na_2SO_4 are 1.2–3.8 times of these adsorbed from water. The corresponding values are in the range of 1.6–3.7 times for PDMS/DVB fiber.

3.12. Detection limits, precision, and linearity

The linearity correlation coefficient (R^2), precision (R.S.D.s), and method detection limits (MDL) are shown in Table 2. The linearity of this method for analyzing standard solution has been investigated over the range 5–1000 ng mL^{-1} for both PDMS/DVB and CW/TPR fibers. All the correlation coefficients in the range 5–1000 ng mL^{-1} were better than 0.995 except Simazine and Atratone by CW/TPR fiber. The precision of this method was investi-

Table 2
Linearity correlation coefficient[a], method-detection limits[b] and relative standard deviation[c] for the analysis of triazines using PDMS/DVB fiber and CW/TPR fiber

Compounds	Linearity correlation coefficient		MDL		R.S.D.s	
	PDMS/DVB	CW/TPR	PDMS/DVB	CW/TPR	PDMS/DVB	CW/TPR
Simazine	0.9908	0.9884	1.50	3.15	8.8	7.2
Atroatone	0.9922	0.9892	2.04	2.79	5.0	6.2
Atrazine	0.9351	0.9956	1.65	3.33	4.4	2.4
Prometone	0.9960	0.9914	1.17	2.85	7.3	7.1
Ametryn	0.9958	0.9954	2.64	3.36	7.2	4.0

[a] Calibration curves with the following concentration: 5, 10, 50, 100, 500, 1000 ng mL^{-1} for both fibers.
[b] Method detection limits are calculated as three times the standard deviation of seven replicate runs.
[c] Date obtained by extraction in five replicates, $n = 5$.

gated for a set of five replicates. The R.S.D.s range from 4.4% to 8.8 % (PDMS/DVB fiber) and from 2.4% to 7.2% (CW/TPR fiber). Method detection limits (MDL) are in the range 1.2–2.6 and 2.8–3.4 ng mL^{-1} for the two fibers which are calculated as three times the standard deviation of seven replicate runs. Note that the sensitivities and the MDL can be improved with the addition of 20% Na_2SO_4 (for both fibers) and/or at higher temperature (55 °C) for PDMS/DVB fiber.

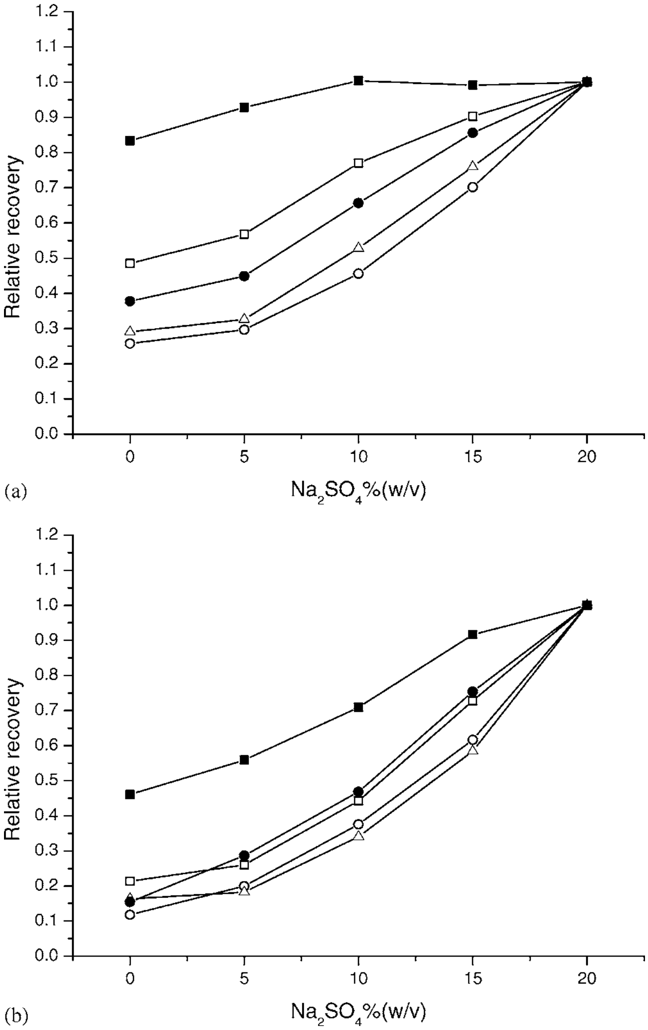

Fig. 5. Effect of ionic strength on adsorption of triazines by (a) PDMS/DVB fiber; (b) CW/TPR fiber. Concentration: 100 ng mL^{-1}, extraction time: 20 min, soaking time: 4 min, desorption time: 2 min (0.6 mL min^{-1}). Peak notation as in Fig. 3.

Table 3
Spike recovery of real sample (lake water)

Compound	Recovery (%)			
	PDMS/DVB		CW/TPR	
	10 (ng mL^{-1})	100 (ng mL^{-1})	10 (ng mL^{-1})	100 (ng mL^{-1})
Simazine	86.5	110.8	92.3	112.9
Atratone	86.8	103.2	87.1	83.0
Atrazine	85.4	99.8	84.4	96.0
Prometon	98.5	95.1	96.3	88.2
Ametryn	90.6	98.2	93.0	91.0

3.13. Matrix effect study

SPME/HPLC was applied to the determination of triazines in lake water to confirm its practicability and feasibility for the analysis of triazines in water samples. No peaks of triazines and interference appeared in the chromatograms using PDMS/DVB and CW/TPR fibers. Different concentrations of the triazines were then spiked into the lake-water to investigate the matrix effect on this method. Table 3 shows the spike recovery of lake-water. The recoveries of these five triazines were little affected by the matrix of lake-water using PDMS/DVB or CW/TPR fibers; and therefore, a calibration curve based on simple aqueous standards can be used for both fibers.

4. Conclusion

SPME coupled with HPLC was successfully applied to the analysis of triazines in water samples. According to the results, appropriate types of fibers and desorption procedures are very important for success with this technology. Both PDMS/DVB fiber and CW/TPR fiber can be used for the determination of these triazines in natural water; a simple calibration-curve method can be used. The detection limits of this method are at the levels of ng mL^{-1}, which is higher than those obtained with GC–NPD or GC–MS. The limit for individual triazine herbicide in European drinking water is 0.1 ng mL^{-1}. The detection limits of this technique (HPLC–UV) are not low enough to determine these herbicides in drinking water. This problem can probably be overcome by using detectors with higher sensitivity (such as an electro-chemical detector or MS). Furthermore, the sensitivity and detection limits can be improved by salting out with Na_2SO_4 and/or by extraction at higher temperature (55 °C) and time.

Acknowledgements

We gratefully acknowledge the support of the National Science Council of the Republic of China (NSC 90-2113-M-007-064).

References

[1] C. Hidalgo, J.V. Sancho, F. Hernández, Anal. Chem. Acta 338 (1997) 223.
[2] V. Pacáková, K. Stulik, M. Prikoda, J. Chromatogr. 442 (1988) 147.
[3] D. Barceló, J. Chromatogr. 643 (1993) 117.
[4] A. Junker-Buchneit, M. Witzenbacher, J. Chromatogr. A 737 (1996) 67.
[5] J. Sherma, Anal. Chem. 67 (1995) 1R.
[6] I. Liska, J. Slobodník, J. Chromatogr. A 733 (1996) 235.
[7] C. Aguilar, F. Borrull, R.M. Marcé, Chromatographia 43 (1996) 592.
[8] V. Gokmen, J. Acar, J. Liq. Chromatogr. Related Technol. 19 (1996) 1917.
[9] H.S. Kim, S.K. Lee, D.W. Lee, J. Liq. Chromatogr. Related Technol. 20 (1997) 871.
[10] R.P. Belardi, J. Pawliszyn, J. Water Pollut. Res. J. Can 24 (1989) 179–191.
[11] R. Ferrari, T. Nilsson, R. Arena, et al., J. Chromatogr. A 795 (1998) 371.
[12] D. Lambropoulou, T. Sakellarides, T. Albanis, Fresenius J. Anal. Chem. 368 (2000) 616.
[13] H. Sabik, R. Jeannot, B. Rondeau, J. Chromatogr. A 885 (2000) 217.
[14] J. Quintana, I. Martí, F. Ventura, J. Chromatogr. A 938 (2001) 3.
[15] J. Chen, J. Pawliszyn, Anal. Chem. 67 (1995) 2530.
[16] J. Gorechi, J. Pawliszyn, J. Anal. Chem. 67 (1995) 3265.
[17] C.L. Arthur, L.M. Killam, K.D. Buchholz, J. Pawliszyn, J. Anal. Chem. 64 (1992) 1960.