Fresenius J Anal Chem (1999) 363 : 680–685

ORIGINAL PAPER

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Determination of polar pesticides in soil by solid phase microextraction coupled to high-performance liquid chromatography-electrospray/mass spectrometry

Received: 17 December 1997 / Revised: 30 October 1998 / Accepted: 10 November 1998

Abstract The determination of carbamate and triazine pesticides from soil leachates and slurries was investigated using solid phase microextraction (SPME) coupled to high-performance liquid chromatography-electrospray/mass spectrometry (HPLC-ESI/MS). SPME was carried out using fibres with a newly developed 50 µm Carbowax/template coating which are suitable for relatively polar analytes. These fibers exhibit precisions better than 10% RSD, and are resistant against high contents of organic solvents during desorption. The technique shows a high sampling frequency resulting in an increasing sample throughput.

Introduction

The increasing application of polar pesticides in agriculture and household as well as the better known environmentally hazardous risks of these substances lead to a great variety of methods applied for their determination, mainly chromatographic ones [1–14]. Despite of highly sensitive detection methods sample enrichment procedures are often required to measure residues at a target concentration of 100 ng/L set in the Drinking Water Regulations of the European Commission [15]. To improve the sensitivity of pesticide determination in aqueous solution solid phase extraction (SPE) is often applied as a preconcentration method that can be used off- and on-line with HPLC-API/MS (atmospheric pressure ionization –API) [16, 17].

New strategies have been developed to reduce the laboratory resources and especially time for sample extraction and preparation. Solid phase microextraction (SPME) with fibre coatings as poly(dimethylsiloxane) (PDMS) and polyacrylate (PA) were developed for GC [18–24]. For coupling with HPLC a special SPME-HPLC-interface was designed [25, 26]. This unique sample preparation method can be carried out fully automated [27, 28]. A new generation of fibres with a high affinity for polar compounds based on Carbowax coatings exhibit extraction yields and a reversibility of the adsorption/desorption mechanism that makes this fibre material suitable for LC applications.

Besides a continuous monitoring of ground and drinking water the determination of pesticides in contaminated soils is applicable. In order to avoid time and solvent consuming preparation of soil samples with the usual several steps of liquid-liquid extraction and liquid chromatographic clean-up, SPME coupled to ESI/MS was examined as determination method for pesticides. For the analysis of complex environmental samples the selectivity was further increased by using hyphenated techniques, i.e., LC-MS.

SPME using new Carbowax based films was applied for the determination of heavy polluted soils at a former chemical production plant near Bitterfeld in former East Germany.

Experimental

Materials and methods

The reference pesticides purchased from Supelco (Deisenhofen, Germany) and Chem Service (West Chester, PA, USA) are listed in Table 1. They were of ≥ 98% purity and used as received. All other chemicals (solvents and salts) were received from Merck (Darmstadt, Germany) and used without further purification.

90 g air-dried soil from a waste disposal of a former chemical production plant near Bitterfeld were leached with 900 mL water adjusted at pH 4 with 1 M HNO_3. The mixture was kept in suspension by shaking for 24 h and then 4 mL of the eluate were sampled by SPME. Slurries made from 200 mg air-dried soil and 4 mL of water (containing 1 g NaCl) were adjusted to pH 4 (1 M HNO_3) and directly sampled by SPME.

For GC-MS screening 900 mL of the leachate were extracted three times with 100 mL of toluene, then dried and evaporated to a final volume of 1 mL.

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Table 1 Reference pesticides and detection limits

Reference substance	Trademark	Target ion $[M + H]^+$	Limits of detection ng/mL	Application as
4-Dimethylamino-m-tolylmethylcarbamate	Aminocarb	209	8–9	I
4-Amino-benzosulfonyl-methylcarbamate	Asulam	228	1–2	H
4-Chlorobut-2-ynyl-3-chlorophenylcarbamate	Barban	272	50	H
N-(3-Chlorophenyl)-isopropylcarbamate	Chlorpropham	213	0.5	H
2-Methylthio-propionaldehyde-O-(methylcarbamoyl)-oxime	Methomyl	185 (M + Na)	8	I
Methyl-N,N′-dimethyl-N-](methylcarbamoyl)oxy]-1-thio-oxamitate	Oxamyl	242	5	I
5-Isopropyl-3-methylphenyl-N-methylcarbamate	Promecarb	230 (M + Na)	5	I
Isopropyl-N-phenylcarbamate	Propham	202 (M + Na)	10	H
2,3-Dihydro-2,2-dimethyl-benzofurane-7-yl-N-methylcarbamate	Carbofuran	222	0.5	I
2-Chloro-4,6-bis-ethylamino-s-triazine	Simazine	202	0.5–1	H
2-Chloro-4-isopropylamino-6-ethylamino-s-triazine	Atrazine	216	0.3–0.5	H
2-Chloro-4,6-bis-(isopropylamino)-s-triazine	Propazine	230	0.3–0.5	H
2-Methylthio-4,6-bis-propylamino-s-triazine	Prometryn	242	0.1-0.5	H

I = Insecticide H = Herbicide

In comparison, 10 g of the original soil were spiked with 10 ng/g (3-(3,4-dichlorophenyl)-1,1-dimethylurea (Diuron, Promochem, Wesel, Germany) and extracted for 10 min using a Dionex ASE™ 200 Accelerated Solvent Extractor (ASE) and acetone/CH_2Cl_2 (1:1 v/v) at a pressure of 14.3 Pa and a temperature of 100 °C.

Standards and calibration

Stock solutions of 1 mg/mL of the individual pesticides in methanol were prepared. These are the basis for a series of water as well as leachate standard mixtures were obtained.

The reproducibility (n = 3) of the method was examined for CW/DVB and two CW/TPR-fibres using 4 mL of leachate solutions spiked with simazine, propazine, atrazine and prometryn. For quantitation of the pesticide residues in real soil leachates standard addition was applied. The determination by GC-MS was achieved by using external standard calibration curves.

SPME

Carbowax/Divinylbenzene (CW/DVB, 65 µm thickness) and Carbowax/Template Resin (CW/TPR, 50 µm thickness) were used for the most SPME experiments and an 85 µm polyacrylate (PA) coated fibre (all from Supelco, Deisenhofen, Germany) for comparison purposes. The fibres were conditioned prior to use in methanol at room temperature for 1 h. Before SPME sampling 1 g NaCl was added to each sample solution (4 mL). During the extraction the solution was stirred at 1000 rpm by magnetic stirring. The extracted analytes were immediately transferred to the HPLC column via an SPME interface [25]. The interface uses a regular six-port-valve, a Rheodyne 7725 Model, where the injection loop is replaced by a tee piece. The attached PEEK tubing mainly forms the desorption chamber with a total volume of 9 µL. While the Rheodyne injection valve was in the LOAD position 50 µL of methanol were injected via the syringe inlet to guarantee a complete flushing with the desorption solvent. Immediately, the SPME device was inserted and sealed. After 5 min the valve was switched to the INJECT position while the fibre remained in the interface for additional 2 min to complete the desorption. After removing the fibre from the interface it was flushed five times with 100 µL methanol. Carryover was examined after analysis.

Instrumentation

The HPLC-MS instrument consists of a solvent delivery system from Thermo Separation (Egelsbach, Germany) connected via a Supelcosil LC-18 column (150 × 2.1 mm ID, 5 µm, Supelco, Deisenhofen, Germany) to a UV-detector and a single quadrupol mass spectrometer Finnigan SSQ 7000 (San José, USA) equipped with a standard electrospray ionisation interface (ESI). Gradient elution at a flow rate of 0.2 mL/min with water (A) and methanol (B) as solvents was applied: linear from 100% A to 100% B within 2 min; after 18 min linear to 50% of both A and B to 22 min and returning to 100% A to 30 min. ESI/MS data and UV-signals at 225 nm were used for detection.

For selected ion monitoring (SIM) the target ions listed in Table 1 were used (scan rate 3 amu/s, span 0.3 amu). The main ESI parameters were: spray voltage 4.5 kV, capillary temperature 200 °C, manifold temperature 70 °C, N_2 at a pressure of 380 kPa as sheath gas and a CID offset of 10 V to reduce abundant cluster ions.

The mass spectrometer was tuned and calibrated with a mixture of 5 pmol/µL apomyoglobin and 20 pmol/µL of a peptide, MRFA (Sigma, Deisenhofen, Germany and Finnigan MAT, Bremen, Germany) both dissolved in methanol/water (1:1 v/v, 0.1% of acetic acid).

Corresponding GC-MS analysis were performed at an HP-GC-MSD "Hewlett Packard 5980, series II" (Waldbronn, Germany) equipped with an HP-5/MS capillary (30 m × 0.25 mm ID, film thickness 0.25 µm). The following GC-program was used: 50 °C at 3 min, then with 7 K/min to 270 °C, hold for 15 min. A transfer-line temperature of 280 °C was applied. Electron impact (70 eV) at an ion source temperature of 180 °C and full scan analysis (50 to 500 amu) was used as screening procedure. The triazines were detected by SIM (target ions = molecular ions).

Results and discussion

A series of experiments was carried out to select the most suitable fibre coating for the SPME-HPLC-MS determination of carbamate and triazine pesticides. The performance of commercially available PA, CW/DVB, and CW/TPR fibres, all designated for the extraction of more polar compounds was studied. The PDMS/DVB was not tested because first investigations showed poor extraction yields for some carbamates, like methomyl, oxamyl and carbo-

furan [29]. All Carbowax coatings tested showed sufficient extraction yields and inter-fibre precision between 1.6 and 12% (n = 5, CW/DVB) for the analysis of selected pesticides (oxamyl, methomyl, carbofuran, aminocarb, promecarb) at ng/g levels. The CW/TPR coating showed roughly 60% of the extraction yield obtained by the CW/DVB coated fibre. However, the CW/TPR fibre could be used repeatedly achieving consistent results (minimum of 50 extraction cycles), which reflects the higher ruggedness compared to CW/DVB coatings with only 10–15 extraction cycles. The long term stability of the CW/TPR coating compensated for, in general, slightly lower capacity. PA coated fibres were not suitable for this application due to their low selectivity against carbamate pesticides.

Unfortunately, compounds with a high affinity to the Carbowax coating, like the triazines and some carbamates (carbofuran, aminocarb and promecarb) investigated showed a high carryover, between 11 and 20%. A washing step of 10 min using pure methanol overcame carryover effects as blanks proved.

Extraction – time profiles

In order to consider the matrix influence the extraction-time profile was obtained with the original leachate spiked with selected herbicides. The obtained extraction time profiles in Fig. 1 showed that with exception of prometryne all compounds reach the maximum extraction yield within 30 min. Since the prometryne graph indicated a continuous increase in extraction yield, 60 min were used as the default sampling time being a reasonable compromise between sensitivity and precision within an acceptable time.

In general, the limit of detection achieved was below 10 μg/L (see Table 1). Using the 50 μm CW/TPR fibres and 1 h exposure time the precision of the method was < 10% RSD (n = 4) determined for four triazine herbicides in soil leachates.

An extension of the sampling time to 18 h led in all cases to a slight decrease of the extraction yield for all investigated analytes. This observation could be confirmed by repeated experiments with the leachate as well as with pure water (plus NaCl), where the optimum extraction times of all analytes were shortened by about 5 min. These results were in good agreement with previously published data for SPME of phenols [30]. However, the effect of decreasing extraction yields at longer extraction times (18 h) has been maintained and seems to be a general phenomenon in SPME because numerous papers reported about the same effects, too [31–36]. Different proposals have been discussed to explain the loss of analytes e.g. through a septum leak or a loss by adsorption of analytes on the silicon vial cap or onto the coating supporting silica rod of the fibre or by degradation and interactions of analytes during the extraction or non-equilibrium conditions within the coating and the boundary layer [31–36]. Consequently, there are many effects potentially affecting the extraction yield in a complex manner if the exposure time exceeds the analytical frame. Most frequently, effects not exclusively related to the SPME step affect the overall performance.

The matrix influence was examined by comparing the extraction yields of the triazines from pure water and from leachate solution containing 77% aromatic, 16% aliphatic and 7% chloroorganic compounds and pesticides. The high correlation of the SPME recoveries obtained between both experiments showed, that the influence of the accompanying matrix on the SPME results was insignificant. The extraction yields obtained from pure water and from the leachate differed only between 3 and 8% and were within the error of measurement.

Quantitation in soil leachates

The calibration curves created from the original matrix by standard addition showed a small linear range between lower ng/mL and 100 ng/mL concentrations. The UV-signal indicated the same small linear range of the calibration curve as the ESI-signal, however LODs were in equal range or sometimes slightly higher. Detection limits of 1.5 ng/mL were obtained for compounds like propham or chloropham. Most compounds show LODs of 0.5 to 10 ng/mL (Table 1). In the case of strong loaded wastewater a UV-detection of the pesticides was aggravated due to chromatographic interference of accompanying matrix compounds (Fig. 2). Thus, in principle, a simultaneous UV-detection of unpolar compounds like PAHs and API-MS for polar substances was convenient, but required a compromise in optimization of appropriate SPME-HPLC conditions for both substance classes.

Comparison of extraction methods

Previous monitoring of soil extracts from our sampling site near Bitterfeld revealed several organic pollutants [37] like phosphorus and chlorine containing pesticides and herbicides as potential risks for ground water contamination.

Investigations on the mobility of pollutants in soil/water systems often require a batch-slurry procedure [38] with subsequent sample preparation and analysis steps [39]. SPME combines many sample preparation and enrichment steps in a single procedure and when coupled to API-MS it can be used as a multiresidue method for samples with complex matrices. To demonstrate the capability of SPME-HPLC-ESI-MS, triazines were determined by different sampling and detection techniques. They were directly extracted from a soil slurry as well as a leachate of this soil using SPME(CW/TPR)-HPLC-ESI-MS. In comparison a toluene extract of the same leachate as well as an ASE extract [40] of the original soil were analyzed by GC-MS. The results of these different experiments are summarized in Fig. 3.

The GC-MS analysis of the leachate extract showed lower triazine amounts than those obtained by SPME of

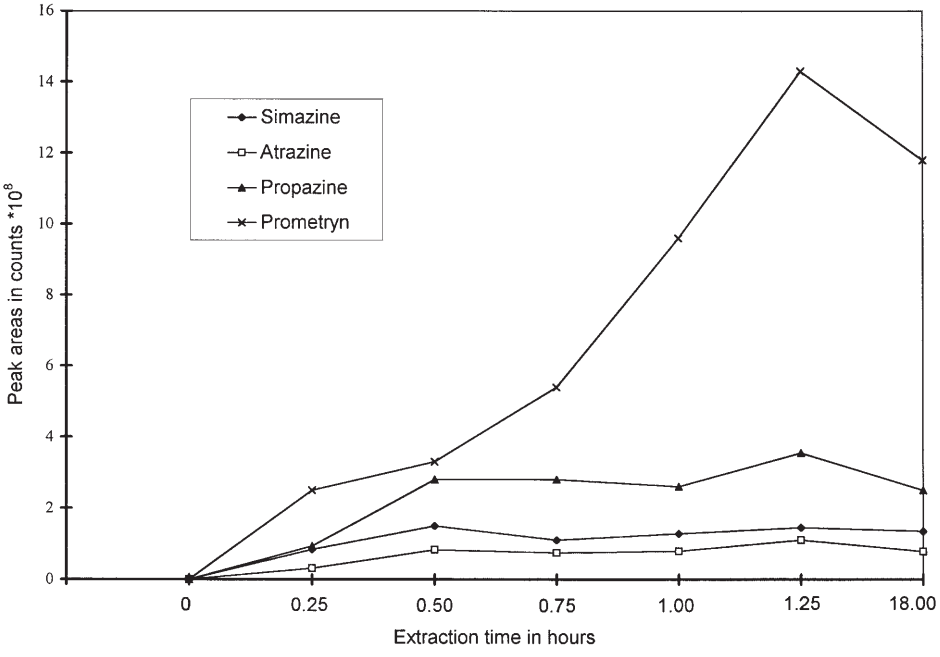

Fig. 1 Extraction time profiles of selected pesticides using a CW/TPR fibre

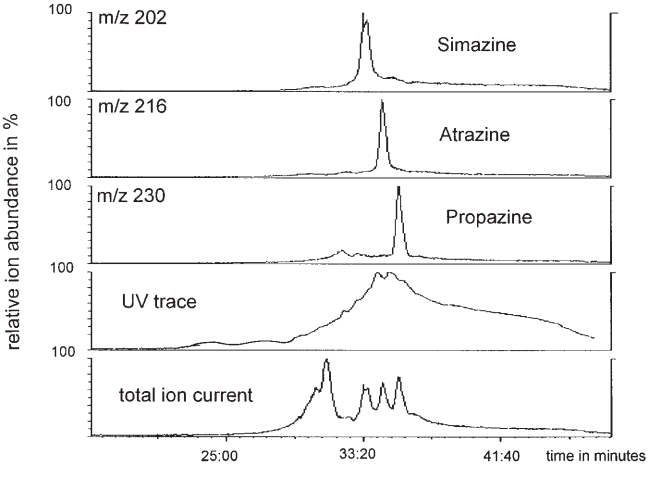

Fig. 2 ESI/MS trace chromatograms and UV-trace of 4 mL of the original soil leachate obtained by SPME-HPLC

the leachate. Even the GC-MS results of the leachate extract showed only traces of atrazine and propazine. The leachate procedure seemed to discriminate special analytes most likely related to different sorption behavior of the pesticides. However, the yields of simazine and prometryn obtained by SPME-HPLC-MS of the aqueous leachate fitted well with GC-MS results of the leachate extract.

SPME of 200 mg soil/water slurry resulted in lower amounts of extracted triazines than the corresponding SPME sampling from the leachate. Preconditioning of the same slurry, i.e., stirring for 110 min, improved the extraction yields by a factor of 1.4. The desorption of the analytes from the soil into the aqueous phase seemed to be the limiting step for this extraction procedure. A 130 min precondition time was determined optimum when identical results were obtained for the SPME of the leachate.

The composition of soil, especially the content of organic matter, is a major factor which determines the soil/water partition of contaminants and their extractability by water. Studies on pesticide behavior in different soil classes using SPME-GC proved, that strong analyte/soil interactions drastically reduced the recovery of pesticides [41–43].

If the total amount of analytes in a soil had to be determined, a detailed characterization of the soil matrix and texture is necessary to select an appropriate sample extraction and analysis procedure. In general, organic solvent extraction (Soxhlet or ASE) is most successful in determining the total amount of pesticides (Fig. 3) but required an additional time and substance consuming sample preparation. The pesticides contained in the soil were determined by GC-MS as well as HPLC with an average recovery of 90% (for diuron as an internal standard, $n = 2$). Among the triazines, mainly chlorinated pesticides like γ- and β-hexachlorocyclohexane, DDE, DDT and tin-organic compounds were identified as main contaminants. The concentration of the triazines detected by GC-MS differed by factors between 3 and 10 from the SPME-HPLC-MS results obtained from the leachate (Fig. 3) which was obviously caused by the higher extraction power of organic solvents compared to the water used in the leaching study. The differences observed reflect the different water solubility of the triazines (simazine 3–6 mg/L, atrazine 33 mg/L, propazine 3–8 mg/L and prometryn 30–40 mg/L, all at 20–25 °C) [44].

The HPLC method did not detect any carbamates, neither in the leachate nor in the soil samples. The absence of carbamates could be possibly caused by rapid microbial degradation in the soil matrix which is a well known process investigated for carbofuran, aldicarb or carbaryl [45, 46]. On the other hand, the water solubility of the carbamates results in a high mobility and it was assumed

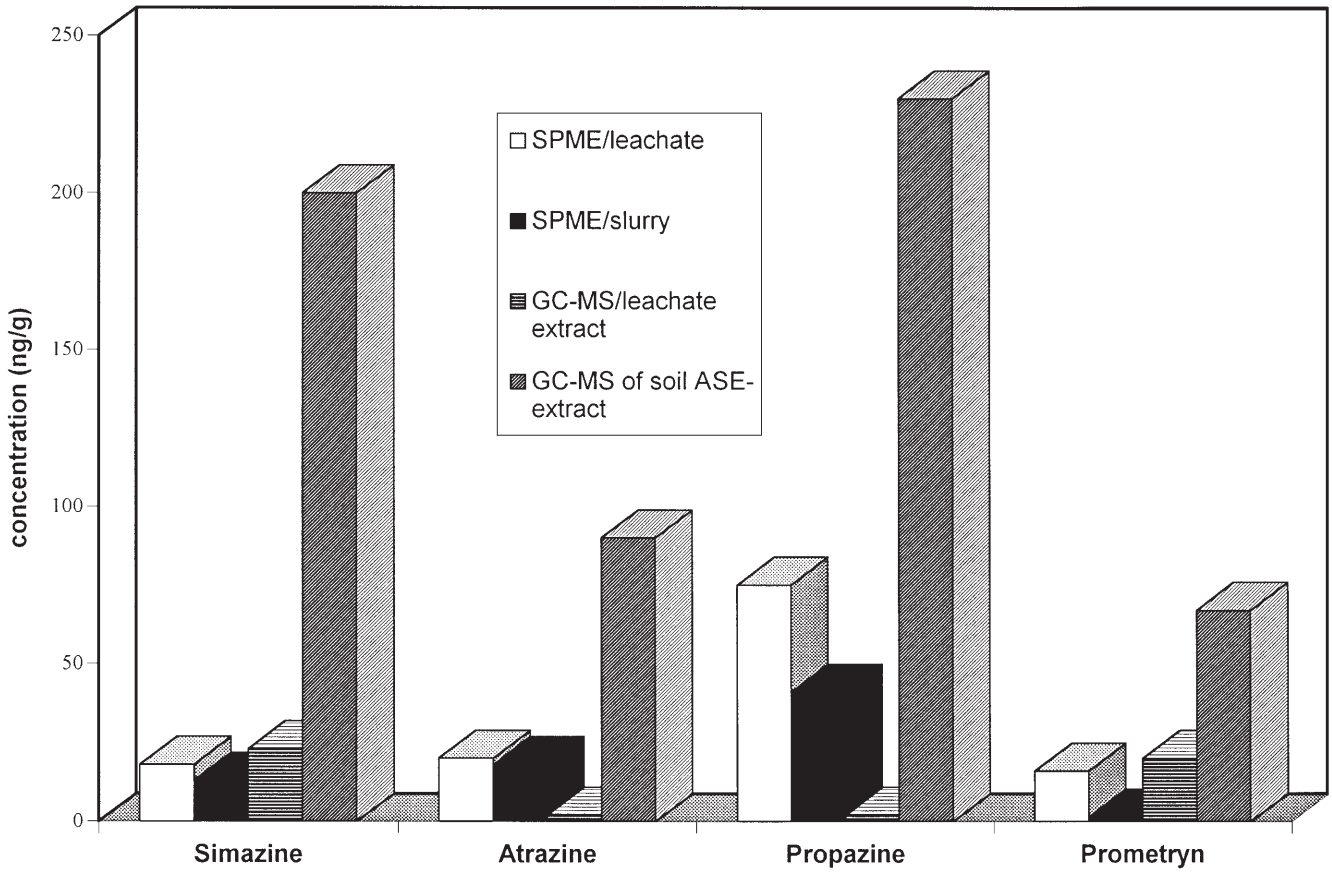

Fig. 3 Comparison of triazine amounts determined by different sample preparation methods

that in the last five years the largest amounts were dissolved by rain and surface runoff water and removed from the their original exposure spot.

Conclusions

SPME in combination with HPLC-ESI-MS proved to be a suitable method for the determination of polar pesticides from water. Furthermore this method allows the detection of pesticides leached from soil where the leaching conditions and the soil class are sensitive parameters. Especially for risk assessment regarding the environmental mobility of pesticide contaminants the presented method seems valuable. Consequently, SPME provides a fast and easy tool to monitor organic compounds in aqueous matrices. Compared to quantitative extraction methods it shows less interference from matrix compounds, especially in combination with MS (SIM) detection. Even matrix compounds present at high concentrations with a high affinity to the SPME fibre are only extracted at their partition concentration which is far less compared to quantitative recoveries. SPME helps to generate fast and precise data from soil leaching experiments which are necessary for risk assessment. In addition, the method provides excellent conditions to run the entire extraction on-site in the field. The fibres can be easily shipped to the lab and labile compounds are already enriched in the field. The potential for an easy to handle field portable method is one of the major advantages of SPME methods [47].

Acknowledgements The authors are grateful to the Foundation for Canadian-German-Scientific Corporation for supporting the work. Thank is also given to Mrs. Büttner and Dr. Paschke (Dept. of Chem. Ecotoxicology) for sampling of the soils and preparing the soil leachates.

References

1. Mattina MJI, Huang LQ (1989) Org Mass Spectrom 24:360
2. Climent MJ, Miranda MA (1996) J Chromatogr A 738:225
3. Lin HY, Voyksner RD (1993) Anal Chem 65:451
4. Volmer D, Levsen K, Wünsch G (1994) J Chromatogr A 660: 231
5. Volmer D, Preiss A, Levsen K, Wünsch G (1993) J Chromatogr A 647:235
6. Geerdink RB, Berg PJ, Kienhuis PGM, Niessen WMA, Brinkman UATh (1996) Int J Environ Anal Chem 64:265
7. Charrêteur C, Colin R, Morin D, Peron JJ, Madec CL (1996) Analusis 24:203
8. Slobodník J, Jager ME, Hoeckstra-Oussoren JF, Honing M, van Baar BLM, Brinkman UATh (1997) J Mass Spectrom 32:43
9. Barceló D (1993) J Chromatogr 643:117
10. Slobodnik J, van Baar BLM, Brinkman UATh (1995) J Chromatogr A 703:81
11. Spliid NH, Køppen B (1996) J Chromatogr A 736:105
12. Schroeder HFr (1997) Environ Monit Assess 44:503
13. Volmer DA, Volmer DL, Wilkes JG (1996) LC GC 14:216

14. Molina C, Honing M, Barceló D (1994) Anal Chem 66:4444
15. EEC Drinking Water Guidelines, 80/779/EEC, EEC No. L229/11–29, EEC, Brussels, 1980
16. Slobodník J, Hogenboom AC, Vreuls JJ, Rontree JA, van Baar BLM, Niessen WMA, Brinkman UATh (1996) J Chromatogr A 741:59
17. Crescenzi C, DiCorcia A, Guerriero E, Samperi R (1997) Environ Sci Technol 31:479
18. Pawliszyn J (1997) Solid Phase Microextraction-Theory and Practice, Wiley-VCH, 1997
19. Arthur CL, Killam LM Buchholz KD, Pawliszyn JB (1992) Anal Chem 64:1960
20. Boyd-Boland A, Magdic S, Pawliszyn JB (1996) Analyst 121:929
21. Young R, Lopezavila V, Beckert WF (1996) J High Resol Chromatogr 19:247
22. Magdic S, Boyd-Boland A, Jinno K, Pawliszyn JB (1996) J Chromatogr A 736:219
23. Eisert R, Levsen K (1995) Fresenius J Anal Chem 351:555
24. Eisert R, Levsen K (1995) J Am Soc Mass Spectrom 6:1119
25. Chen J, Pawliszyn J (1995) Anal Chem 67:2530
26. Boyd-Boland AA, Pawliszyn JB (1996) Anal Chem 68:1521
27. Eisert R, Pawliszyn J (1997) J Chromatogr A 776:293
28. Eisert R, Pawliszyn J (1997) Anal Chem 69:3140
29. Application Note 121, SUPELCO (1997)
30. Möder M, Schrader S, Franck U, Popp P (1997) Fresenius J Anal Chem 357:326
31. Johansen SS, Pawliszyn J (1996) J High Resol Chromatogr 19:627
32. Page BD, Lacroix G (1997) J Chromatogr A 757:173
33. Valor I, Molto JC, Apraiz D, Font G (1997) J Chromatogr A 767:195
34. Tutschku S, Mothes S, Wennrich R (1996) Fresenius J Anal Chem 354:587
35. Nilsson T, Pelusio F, Montanarelle L, Larsen B, Fachetti S, Madsen JO (1995) J High Resol Chromatogr 18:617
36. Paschke A, Popp P (1998) In: Pawliszyn J (ed) Application of SPME (in press)
37. Popp P, Kalbitz K, Oppermann G (1994) J Chromatogr 687: 133
38. Busche U, Hirner AV (1997) Acta Hydrochim Hydrobiol 25: 248
39. German standard methods for the determination of water, waste water and sludge, DIN 38 407, part II (1993), p 20
40. U.S. EPA Office of Solid Waste and Emergency Response, Pressurized Fluid Extraction (PFE), U.S. EPA Method 3545. In: SW-846 Laboratory Manual-Update III, promulgated May 29, 1997
41. Bengtsson S, Berglöf T, Sjöqvist T (1996) J Agric Food Chem 44:2260
42. Koskinen W, Harper, SS (1990) In: Cheng HH (ed) Pesticides in the Soil Environment: Process, Impacts, and Modelling, Soil Science Society of America Book Series 2, Madison, WI, p 51
43. Barriuso E, Koskinen W, Sorenson B (1992) Sci Total Environ 123/124:333
44. CIBA GEIGY Corporation-Toxicology Data (1989) In: ARS Pesticide Properties Database, Remote Sensing & Modeling Laboratory (RS & ML)
45. Harris CR, Chapman RA, Harris C, Tu CM (1984) Environ Sci Health Part B 19:1
46. Chaudhry GR (1994) Biological Degradation and Bioremediation of Toxic Chemicals, Chapman & Hall, London, p 198
47. Shirey R, Mani V, Mindrup R (1998) Am Environ Lab 1/2:21