ELSEVIER

Journal of Chromatography A, 737 (1996) 93–99

JOURNAL OF CHROMATOGRAPHY A

Determination and stability of pesticides in freeze-dried water samples by automated on-line solid-phase extraction followed by liquid chromatography with diode-array detection

I. Ferrer, D. Barceló*

Department of Environmental Chemistry, CID–CSIC, c/Jordi Girona 18–26, 08034 Barcelona, Spain

Abstract

The determination of selected pesticides, atrazine, simazine, carbaryl, propanil, linuron and fenamiphos, was carried out in freeze-dried water using automated on-line solid-phase extraction (Prospekt) followed by liquid chromatography with diode-array detection. The concentration of the pesticides in the water varied from 1.2 to 20.5 μg/l after reconstitution of the freeze-dried water sample. A stability study was undertaken using freeze-dried water for a period of 1.5 months at three different storage temperatures (4, −20 and 20°C). It was shown that the degradation of fenamiphos, linuron and simazine observed at 4 and 20°C was correlated with various parameters such as Henry's law constant, hydrolysis, photolysis and temperature.

Keywords: Environmental analysis; Water analysis; Pesticides

1. Introduction

The stability of different contaminants in water during transport and storage is useful information as regards quality assurance parameters. Acidification with hydrochloric acid effectively prevented the degradation of volatile compounds and pesticides, thus allowing sample storage [1–3]. The use of solid-phase-extraction (SPE) material is an alternative to storage of the original matrix [4,5]. Recent studies on stability have been carried out using Empore disks compared with their storage in water at 4°C. Relationships were established between storage stability with the soil organic partition coefficient (K_{oc}) values and the solubility of the different pesticides. Degradation of pesticides was attributed to either hydrolysis or microbial breakdown [6–10].

Other methods for stabilization have been reported, e.g., freeze-drying with the addition of glycine [11], which permitted the stabilization of fenamiphos and parathion for 1 month, but the system was not effective for fenitrothion and tetrachlorvinphos.

In view of the current need to monitor pesticides in environmental water matrices, we examined the stability of various pesticides under different storage conditions following freeze-drying. Compound selection was based on the importance of these compounds in the European Community [12]. The use of automated SPE precolumns has been reported for the determination of pesticides in water samples and it is currently used in the Rhine monitoring pro-

* Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)01340-7

gramme and other European programmes [13–15].

The specific objectives of this work were (i) to determine the different pesticides in freeze-dried water by on-line automated SPE and to compare the results with an interlaboratory exercise and (ii) to establish the storage stability of selected pesticides from European priority lists stored in freeze-dried water for 1.5 months at three different temperatures. It was also intended to improve the quality assurance parameters in relation to sample handling, transport and storage of pesticides in environmental water analysis and to apply the present approach to on-going monitoring programmes.

This work follows our previous research on the determination and degradation of pesticides in environmental water samples [11,13,14,16,17].

2. Experimental

2.1. Chemicals

HPLC-grade solvents, acetonitrile, methanol and water, and also hydrochloric acid were purchased from Merck (Darmstadt, Germany). Purified water was prepared by ultrafiltration with a Milli-Q system (Millipore, Bedford, MA, USA). Pesticide standards were obtained from Promochem (Wesel, Germany). Stock standard solutions of 500 μg/ml were prepared by weighing the solutes and dissolving them in acetonitrile. A stock solution of 2 μg/ml was used to spike Milli-Q water at the μg/l level for the construction of calibration graphs.

2.2. Apparatus

LC analyses were performed with a Waters 600-MS solvent-delivery unit with a 20-μl injection loop and a Waters Model 996 photodiode-array detector (Waters–Millipore, Milford, MA, USA). The analytical column was 25 cm $\times$ 4.6 mm I.D. packed with 5-μm octylsilica gel (Shandon). Trace enrichment was performed on an automated SPE system (Prospekt). It consisted of a cartridge exchange module, a solvent-delivery unit (SDU) (Spark, Emmen, Netherlands) and a low-pressure six-port valve, which was connected to the gradient pumps. Water samples were preconcentrated on 10 mm $\times$ 2 mm I.D. Prospekt disposable precolumns (Spark) prepacked with 40-μm C_{18} (Baker, Deventer, Netherlands). For more details, see Refs. [13] and [14].

2.3. Freeze-dried water sample preparation

A 150-l sample of drinking water was spiked with atrazine, simazine, carbaryl, propanil, linuron and fenamiphos to give final concentrations of 1–40 μg/l depending on the pesticide. A control blank was prepared by freeze-drying 40 ml of the same drinking water but without any addition of pesticides. After freeze-drying, the residue of each batch was homogenized for 2 h. Thirty amber glass bottles with PTFE inserts were filled with ca. 2.5 g of residue. The remaining material was rehomogenized for 10 min and an additional batch of 30 bottles were filled. These bottles were subsequently used in the interlaboratory exercise and also served for the control of the stability of the samples throughout this exercise. For more details of this procedure, see Ref. [11].

2.4. Reconstitution of freeze-dried water samples

The lyophilized water samples containing pesticides were reconstituted by dissolving the solid in 0.5 l of Milli-Q water. Addition of HCl was necessary to complete the final dissolution of the sample. The samples and the standard solutions were prefiltered using 0.45-μm PTFE fibre-glass filters (Millipore, Bedford, MA, USA). The initial sample was divided into three parts: one sample was analysed in the first week after receiving the lyophilized sample from the European Community laboratory in Geel (Belgium), the second was stored at 4°C and the third was kept at room temperature. After 1.5 months the second and the third samples were analysed to investigate the stability.

2.5. Sample extraction

In the first stage, 10 ml of water sample were preconcentrated in C_{18} cartridges. The extraction procedure was as follows: the cartridge was washed with 10 ml of acetonitrile, 10 ml of methanol and 10 ml of HPLC-grade water. Subsequently, the water sample was passed through the cartridge at a flow-rate of 2 ml/min and then the clean-up was performed by passing 6 ml of HPLC-grade water.

For the stability study, 20 ml of water sample were preconcentrated in order to make easier the quantification of some compounds such as fenamiphos, which are at the lowest concentration levels, and also for the determination of possible metabolites. Moreover, in the preconditioning of the cartridge the use of methanol was eliminated because an unexpected interference (peak b) in all the chromatograms was observed (see Fig. 1).

2.6. LC analysis

After the preconcentration step, desorption was carried out by coupling the precolumn on-line with the analytical column and starting the gradient. The acetonitrole–water gradient conditions were as follows: from 20% linearly to 40% acetonitrile in 20 min, to 100% acetonitrile in 20 min and then back to the initial conditions in 5 min, at a flow-rate of 1 ml/min (see Figs. 1 and 2).

2.7. Calibration graphs

Calibration graphs were constructed after spiking 100 ml of Milli-Q water with different volumes of the stock standard solution in order to reach the range established by the intercalibration study (from 1 to 40 μg/l). A sample volume of 10 ml of the spiked Milli-Q water was percolated through C_{18} cartridges.

Once the exact concentration of the selected compounds in the lyophilized sample was known (from 1 to 20 μg/l), more accurate calibration graphs were constructed in this range for the stability study after 1.5 months. In this case the sample volume percolated was of 20 ml, as indicated before.

3. Results and discussion

3.1. Analytical performance

Complete separation of all compounds was achieved with the above gradient conditions, as can be seen in Fig. 1. In this case the interference (peak b) due to the conditioning of the cartridge with methanol is clear. In Figs. 2 and 3, the conditioning of the cartridge was carried out only with acetonitrile and water, so that the interfering peak did not appear.

Table 1 shows the calibration equations for the pesticides studied in the intercalibration together with the wavelength at which the quantification was performed. The correlation coefficients for

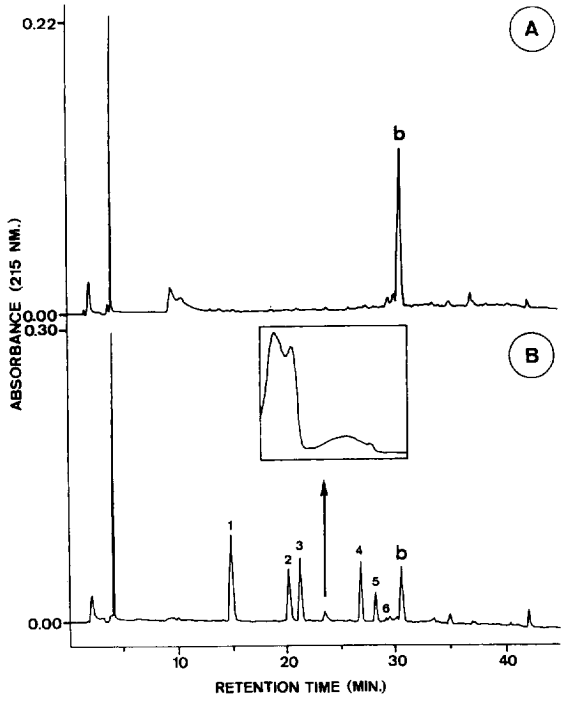

Fig. 1. LC–DAD traces obtained after preconcentration of (A) 10 ml of blank water sample in a C_{18} cartridge and (B) 10 ml of water sample in a C_{18} cartridge. Peaks: 1 = simazine; 2 = atrazine; 3 = carbaryl; 4 = propanil; 5 = linuron; 6 = fenamiphos; b = interferent. For gradient conditions, see text.

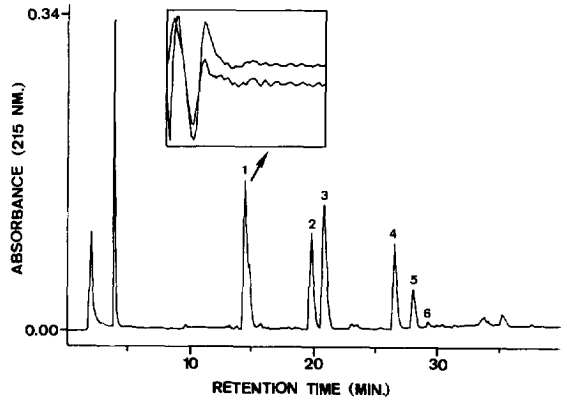

Fig. 2. LC–DAD trace obtained after preconcentration of 20 ml of the freeze-dried sample stored at 4°C in a C_{18} cartridge. Gradient conditions as in Fig. 1.

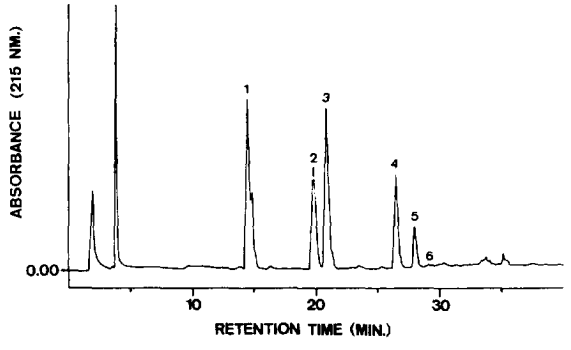

Fig. 3. LC–DAD trace obtained after preconcentration of 20 ml of the freeze-dried sample stored at room temperature in a C_{18} cartridge. Gradient conditions as in Fig. 1.

all the pesticides are over 0.99, indicating good linearity over the range of interest. As reported before [18], calibration equations are not very different when constructed using LC-grade water or drinking water. For this reason, correct results can be obtained for a non-spiked drinking water sample using the calibration equation obtained with a spiked solution made with LC-grade water. Table 2 shows the calibration equations obtained in the stability study (preconcentrating 20 ml of Milli-Q water).

One advantage of automation in an on-line preconcentration is that more reproducible results are expected, provided that the precolumns are packed with the same amount of sorbent and with the same efficiency. The relative standard deviation (R.S.D.) is always between 1 and 6%, which are similar to those reported by Lacorte and Barceló [14].

3.2. Intercalibration study

Fig. 1 shows the chromatograms obtained with diode-array detection (DAD) at 215 nm for one of the lyophilized samples and the blank. Table 3 gives the mean concentrations for each compound in three successive determinations and the mean for all laboratories. All compounds were quantified at 215 nm, except fenamiphos, which was quantified at 205 nm owing to its low concentration in the sample and its higher absorbance at this wavelength. All concentrations were between the expected values given by the intercalibration study. Problems with the quantification of fenamiphos have been encountered by many laboratories. As shown in previous work [13], fenamiphos is rapidly degraded in water. Further, there was the problem regarding to the low concentration of this compound in the sam-

Table 1
Calibration equations (1–40 μg/l) after preconcentration of 10 ml of spiked Milli-Q water in C_{18} percolumns

Compound	Wavelength (nm)	Equation[a]	r^2
Simazine	215	$y = 79570x + 28444$	0.9987
Atrazine	215	$y = 76818x + 10180$	0.9986
Carbaryl	215	$y = 166981x + 57554$	0.9983
Propanil	215	$y = 67707x + 8875$	0.9984
Linuron	215	$y = 65329x + 12381$	0.9984
Fenamiphos	205	$y = 3.9987x + 2080$	0.9992

[a] y = Absorbance (A.U.); x = concentration (μg/l).

Table 2
Calibration equations (1–20 μg/l) after preconcentration of 20 ml of spiked Milli-Q water in C_{18} percolumns

Compound	Wavelength (nm)	Equation[a]	r^2
Simazine	215	$y = 159826x + 20301$	0.9988
Atrazine	215	$y = 155877x + 11013$	0.9998
Carbaryl	215	$y = 342115x + 45700$	0.9999
Propanil	215	$y = 137744x + 3702$	0.9999
Linuron	215	$y = 131778x + 4340$	0.9999
Fenamiphos	205	$y = 81223x + 120$	0.9999

y = Absorbance (A.U.); x = concentration (μg/l).

ple. Such a low concentration makes it subject to more interferences when performing the quantification. The on-line methodology permits a rapid analysis between the concentration step and the LC analysis, so that no problems regarding degradation of fenamiphos arose in this case. Nevertheless, problems were encountered owing to its low concentration in the lyophilized sample.

3.3. Stability study

Figs. 2 and 3 show the chromatograms obtained at 215 nm for the freeze-dried sample stored at 4°C and at room temperature (20°C), respectively, for 1.5 months.

Table 4 shows the ratios, R_T, of the mean values of the three measurements on samples stored for 1.5 months at 4 and 20°C against the mean value for three analyses of the first freeze-dried sample stored at −20°C. Table 4 also gives the uncertainty, U_T, calculated from the R.S.D. (s) of each set of three measurements:

$$U_T = (s_T^2 + s_{-20°C}^2)^{1/2} R_T \quad (1)$$

Henry's law constants

One of the key issues in the disappearance of pesticides is the Henry's law constant value (HLC). Although simazine has a lower vapour pressure (VP) than atrazine (0.003 versus 0.04 mPa), the parameter to take into consideration in the experiments involving air–water interfaces is HLC, which can be used to determine the evaporation rates of chemicals from water and soil.

Table 3
Mean concentrations ($n = 3$) of the selected pesticides and the mean concentration for all laboratories

Compound	Peak No.[a]	Concentration (μg/g)[b]	
		Mean ± S.D.	Laboratory mean + S.D.
Simazine	1	20.57 ± 0.53	17.3 ± 4.0
Atrazine	2	12.26 ± 0.28	11.4 ± 1.5
Carbaryl	3	6.25 ± 0.14	9.4 ± 2.4
Propanil	4	12.15 ± 0.17	11.7 ± 1.3
Linuron	5	5.13 ± 0.22	5.5 ± 0.9
Fenamiphos	6	1.16 ± 0.01	1.2 ± 0.5

[a] See Fig. 1.
[b] Concentrations are in μg/g of freeze-dried water (solid powder).

Table 4
Ratios (R_T) of the mean values of three measurements on samples stored at 4 and 20°C for 1.5 months to the mean value of three determinations made on the initial freeze-dried sample stored at −20°C, and uncertainties (U_T) determined from the R.S.D. of each set of three measurements according to Eq. 1

Compound	R_T		U_T	
	4°C	20°C	4°C	20°C
Simazine	0.81	0.79	0.05	0.02
Atrazine	1.01	1.06	0.02	0.03
Carbaryl	1.20	1.37	0.03	0.03
Propanil	1.11	0.94	0.01	0.01
Linuron	0.99	0.84	0.05	0.04
Fenamiphos	1.33	0.18	0.03	0.01

The ratio VP/WS (WS = water solubility) gives an excellent estimate of HLC and it can be used to evaluate the possibility of the evaporation rate in a more accurate way than VP. In this respect, when calculating the HLC it can be noted that simazine has slightly higher HLC than atrazine (0.00034 versus 0.00029 Pa m^3/mol), which indicates the greater tendency of simazine to volatilize. This is probably also related to their structures. It has been shown that the isopropyl groups in atrazine show a lower photodecomposition rate than other shorter substituents, e.g., ethyl in simazine [19]. The results for simazine agree with previous results that showed that after storage for 3 months, only 60% of the compound remained in the freeze-dried extract [11].

Hydrolysis

Carbaryl degradation can be explained by hydrolysis to 1-naphthol (see spectrum of Fig. 1). Probably during transport, when the samples arrived at the laboratory some water was present and the concentration of carbaryl was lower. After drying, water was eliminated and the concentration of carbaryl increased. This is in line with previous work [20] that showed that 40% of carbaryl degraded to 1-naphthol due to photolysis in only 30 min.

Photolysis

The formation of fenamiphos sulfoxide is caused by the easy hydrolysis and instability of fenamiphos in water. Fenamiphos has a half-life of 1.8 days [13]. The high solubility of fenamiphos in water (700 mg/l, as can be seen in Table 5) favoured the formation of fenamiphos sulfoxide, although the water sample was acidified in this respect. Fenamiphos is very sensitive to photolysis [13]. In a previous study using SPE cartridges, fenamiphos also degraded [21]. As fenamiphos sulfoxide and simazine co-eluted, the presence of the former was confirmed by the second derivative of the spectra at two different points of the double peak (see Fig. 2). In Fig. 2, the minimum of the absorbance is different at the two points, confirming the presence of two different compounds. This overlapping was also confirmed by co-elution of authentic standards of simazine and fenamiphos sulfoxide at similar concentrations to those in real samples. After the LC analysis it was observed that a double peak appeared and the spectrum corresponding to this peak was the same as for the real freeze-dried water sample.

Table 5
Characteristics of the pesticides studied

Compound	Vapour pressure (mPa)	Solubility (mg/l)
Simazine	0.003	5
Atrazine	0.04	30
Carbaryl	0.3	40
Propanil	3.60	268
Linuron	1.40	75
Fenamiphos	0.13	700

Temperature

As can be seen in Table 4, the stability of fenamiphos is very dependent on the temperature of storage. At 20°C it is totally degraded to fenamiphos sulfoxide, but at 4°C it is stable. The stabilities of simazine and linuron are also dependent on temperature, as shown by the R_T values at 4 and 20°C. This is in line with studies that showed a drastic loss of analyte for organophosphorus pesticides on increasing the storage temperature [21].

4. Conclusions

The determination of atrazine, simazine, carbaryl, propanil, linuron and fenamiphos in freeze-dried water at the 1.2–20.5 μg/l level was feasible by reconstitution of the freeze-dried water and preconcentration of 10–20 ml of water using Prospekt SPE–LC–DAD. The results of an interlaboratory study showed that good values were obtained with this automated system as compared with those reported by other laboratories using conventional extraction methods, mainly off-line SPE followed by GC or LC. The stability study indicated that atrazine, carbaryl and propanil are stable over a period of 1.5 months at 4 and 20°C, whereas simazine and linuron suffered losses of 16–20% after this

storage period. Fenamiphos suffered the highest losses and only ca. 20% of the compound remained after 1.5 months of storage at 20°C. Degradation was attributed to the high Henry's law constant (simazine), photolysis and hydrolysis (fenamiphos) and temperature (linuron and fenamiphos).

Further studies are needed using different methods, e.g., SPE cartridges, to overcome the stability problem when storing water samples containing pesticides.

Acknowledgements

This work was supported by the Commission of the European Communities, Environment and Climate Programme 1994–98 (ENV4-CT95-0066), and CICYT (AMB95-0075-CO3).

References

[1] M.P. Maskareinec, L.H. Johnson, S.K. Holladay, R.L. Moody, Ch.K. Bayne and R.A. Jenkins, Environ. Sci. Technol., 24 (1990) 1665–1670.
[2] D. Barceló, J. Chromatogr., 643 (1993) 117–143.
[3] D.J. Munch and Ch.P. Frebis, Environ. Sci. Technol., 26 (1992) 921–925.
[4] D.R. Green and D. Le Pape, Anal. Chem., 59 (1987) 699–703.
[5] D. Barceló, S. Chiron, S. Lacorte, E. Martinez, J.S. Salau and M.C. Hennion, Trends Anal. Chem., 13 (1994) 352–361.
[6] K. Berkane, G.E. Caissie and V.N. Mallet, J. Chromatogr., 139 (1977) 386–390.
[7] S.A. Senseman, T.L. Lavy and J.D. Mattice, Anal. Chem., 67 (1995) 3064–3068.
[8] S.A. Senseman, T.L. Lavy, J.D. Mattice, B.M. Myers and B.W. Skulman, Environ. Sci. Technol., 27 (1993) 516–519.
[9] W.G. Johnson, T.L. Lavy and S.A. Senseman, J. Environ. Qual., 23 (1994) 1027–1031.
[10] C. Crescenzi, A. Di Corcia, M.D. Madbouly and R. Samperi, Environ. Sci. Technol., 29 (1995) 2185–2190.
[11] D. Barceló, W.A. House, E.A. Maier and B. Griepink, Intern. J. Environ. Anal. Chem., 57 (1994) 237–254.
[12] M. Fielding, D. Barceló, A. Helweg, S. Galassi, L. Torstensson, P. van Zoonen, R. Wolter and G. Angeletti, Pesticides in Ground and Drinking Water, Water Pollution Research Report 27, Commission of the European Communities, Brussels, 1992, pp. 1–136.
[13] S. Lacorte, S.B. Lartigues, Ph. Garrigues and D. Barceló, Environ. Sci. Technol., 29 (1995) 431–438.
[14] S. Lacorte and D. Barceló, Anal. Chim. Acta, 296 (1994) 223–234.
[15] U.A.Th. Brinkman, Environ. Sci. Technol., 29 (1995) 79A–84A.
[16] S. Lacorte and D. Barceló, Environ. Sci. Technol., 28 (1994) 1159–1163.
[17] S. Lacorte, C. Molina and D. Barceló, Anal. Chim. Acta, 281 (1993) 71–84.
[18] V. Pichon and M.C. Hennion, J. Chromatogr. A, 665 (1994) 265–281.
[19] D. Barceló, G. Durand, N. de Bertrand and J. Albaigés, Sci. Total Environ., 132 (1993) 283–296.
[20] N. de Bertrand and D. Barceló, Anal. Chim. Acta, 254 (1991) 235–244.
[21] S. Lacorte, N. Ehresmann and D. Barceló, Environ. Sci. Technol., 29 (1995) 2834–2841.