Available online at www.sciencedirect.com

SCIENCE DIRECT®

Talanta 65 (2005) 129–134

Talanta

www.elsevier.com/locate/talanta

Determination of simazine in water samples by HPLC after preconcentration with diatomaceous earth

Hideyuki Katsumata [a,*], Aya Fujii [a], Satoshi Kaneco [a], Tohru Suzuki [b], Kiyohisa Ohta [a]

[a] *Department of Chemistry for Materials, Faculty of Engineering, Mie University, Tsu, Mie 514-8507, Japan*
[b] *Environmental Preservation Center, Mie University, Tsu, Mie 514-8507, Japan*

Received 25 March 2004; received in revised form 8 May 2004; accepted 20 May 2004

Abstract

A sensitive and selective batch adsorption method is proposed for the preconcentration and determination of simazine. Simazine was preconcentrated on diatomaceous earth as an adsorbent and then determined by high-performance liquid chromatography (HPLC). Several parameters on the recovery of the analyte were investigated. The experimental results showed that it was possible to obtain quantitative analysis when the solution pH was 2 using 100 mL of validation solution containing 1.5 μg of simazine and 5 mL of ethanol as an eluent. Recovery of simazine was 89.0 ± 1.6% with a relative standard deviation for seven determinations of 1.5% under optimum conditions. The maximum preconcentration factor was 100 for simazine when 500 mL of sample solution volume was used. The linear range of calibration curve was 1–200 ng mL^{-1} with a correlation coefficient of 0.996 and the detection limit (3S/N) was 0.3 ng mL^{-1}. The capacity of the adsorbent was also examined and found to be 1.1 mg g^{-1} for simazine. The proposed method was successfully applied to the determination of simazine in river water with high precision and accuracy.

Keywords: Simazine determination; Preconcentration; Adsorption; Diatomaceous earth; HPLC

1. Introduction

Recently, the environmental pollution, especially marine ecosystem, by pesticides has become a serious problem. Due to their heavy use in agriculture and to their persistence, many of these compounds present in surface and ground waters, and have to be considered a potential risk for marine life as well as for drinking water quality. Siamzine, which is one of triazine herbicides, has an estrogenic activity, i.e. it serves as an environmental endocrine disruptor. Maximum contaminant level goal (MCLG) for simazine has been set at 4 parts per billion (ppb) because the US Environmental Protection Agency (EPA) believes this level of protection would not cause any of the potential health problems. In Japan, the Ministry of the Environment has established a MCL as 3 ng mL^{-1} of simazine in drinking water. Moreover, simazine use is increasingly restricted, for instance, in some European countries is totally banned.

In this manner, high-performance analytical methods are of essential importance for the precise monitoring of trace level simazine in the aquatic environment. Most of the reported methods for triazine determination in water involve separation by high-performance liquid chromatography (HPLC) [1,2] and gas chromatography (GC) [3–5] after liquid–liquid extraction (LLE) or solid phase extraction (SPE) [6,7]. Especially, SPE procedures have provided an efficient new tool for environmental analysis. Until now, a large number of SPE materials are available, for example, modified silica based materials, polymeric phases, and carbon based materials [8–15]. However, these methods are expensive, use high volumes of toxic solvents, tedious and time-consuming. Therefore, it is desired that the development of simple, rapid and low cost method for the analysis of triazines in water samples.

It is considered that the adsorption process is one of the useful methods for the preconcentration of simazine as well as other compounds. To date, the most common commercially used adsorbent has been activated carbon [16,17]. Simazine and atrazine have been preconcentrated from tap and surface water samples by graphitized carbon

---

[*] Corresponding author. Tel.: +81 59 231 9425; fax: +81 59 231 9442/9471/9427.
E-mail address: hidek@chem.mie-u.ac.jp (H. Katsumata).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.05.045

black Carbopack B [18]. However, activated carbon is relatively expensive. Diatomaceous earth, one of the cheapest and most abundantly available adsorbents, is a siliceous, sedimentary rock consisting principally of the fossilized skeletal remains of the diatom, where the silica of the fossilized diatom skeleton resembles opal, or hydrous, silica in composition. Diatomaceous earth has specific physical and chemical properties which make it suitable for a wide range of application. Its low bulk density, high surface area and low thermal conductivity are attributed responsible for its use as a functional filter, thermal insulator and catalyst, as well as a substrate for adsorption and preconcentration of organic compounds.

As described above, SPE procedure is useful for the preconcentration of triazines in water samples. Therefore, it is worthwhile to develop the preconcentration method (SPE-like) for triazines based on the adsorption onto diatomaceous earth as SPE material. This technique, being able to supplement the defects of the conventional SPE method which are expensive and time-consuming, would provide a viable alternative to existing SPE method. In this paper, a batch method was proposed for the preconcentration of simazine in water by using diatomaceous earth as an adsorbent. Simazine was used in this study as one of the representative compounds of triazines. The several parameters affecting the adsorption of simazine onto the adsorbent were evaluated by HPLC, which was able to separate triazines. This proposed method was applied to the determination of simazine in environmental sample (river water).

2. Experimental

2.1. Reagents

Simazine was obtained from Wako Pure Chemical Industries (Osaka, Japan) and was used without further purification (GC grade > 99.0%). Stock standard solution (160 μg mL^{-1}) of simazine was prepared in methanol HPLC grade from Kanto Kagaku (Tokyo, Japan) and stored at $-4\,^{\circ}$C in the dark. Working standard solution was prepared daily by diluting with deionized water. All other organic solvents used were of analytical grade and were used without further purification.

Diatomaceous earth (particle size approximately 45 μm) as an adsorbent was purchased from Wako Pure Chemical Industries. Before use, diatomaceous earth was washed with deionized water and dried in an oven at 100 °C for 24 h.

2.2. Apparatus

Simazine concentration was determined by a Model TRI ROTAR-V HPLC (JASCO, Tokyo, Japan) with a Model UVDEC-100-VI UV spectrophotometer (JASCO). The separation column used was a CHROMSPHER 5POLY C18 REPEAL (150 mm length × 4.5 mm i.d., VARIAN, USA). The mobile phase was acetonitrile/water (1/9) and a flow rate was 1 mL min^{-1}. Injection volume of solution was 20 μL. pH of solution was measured by a Model D-21 digital pH/mV meter equipped with a glass electrode (Horiba Ltd., Kyoto, Japan).

2.3. Preconcentration procedure

The batch procedure was carried out by stirring appropriate amount of diatomaceous earth (20–300 mg) with 100 mL of water spiked with 15 ng mL^{-1} of simazine under different experimental conditions at room temperature for various times (1–30 min). The pH of the solution (1.5–6) was adjusted with HCl or NaOH. Next, the solution was filtered through a 0.20 μm-membrane filter. The retained simazine was eluted from diatomaceous earth with 5 mL ethanol. The analyte in the eluate obtained was determined by HPLC. The absorbance of simazine was monitored at 220 nm. The quantification of simazine was made by comparison of peak height with that of standard. The results obtained were expressed in terms of recovery percentage of simazine and were calculated as follows:

$$\text{Recovery}\ (\%) = \left(\frac{C}{C_{\text{T}}}\right) \times 100$$

where C and C_{T} were simazine concentration after preconcentration found by HPLC and concentration calculated theoretically, respectively. Blank and control experiments were performed in the same manner without simazine or diatomaceous earth.

3. Results and discussion

3.1. Effect of variables on preconcentration of simazine

Effect of diatomaceous earth amount on preconcentration of simazine (15 ng mL^{-1}) was examined in the range of 20–300 mg. The results are shown in Fig. 1. The recovery of simazine increased with increasing the adsorbent amount up to 50 mg, and then reached plateau. About 90% of recovery percentage was obtained upon 50 mg of diatomaceous earth. Therefore, 50 mg of the adsorbent was found to be optimum of the preconcentration method.

A stirring time of the simazine solution affects mass transfer from the solution to the binding sites on diatomaceous earth. For this reason, the retention of simazine on the adsorbent would depend on the stirring time of the simazine solution. The effect of stirring time (1–30 min) on the preconcentration of simazine (15 ng mL^{-1}) in this method was investigated. The result indicates that the recovery percentage slightly increased with increasing stirring time up to 10 min. Therefore, it is considered that the adsorption of simazine on diatomaceous earth was attained equilibrium state for 10 min. A stirring time of 10 min was chosen as optimum.

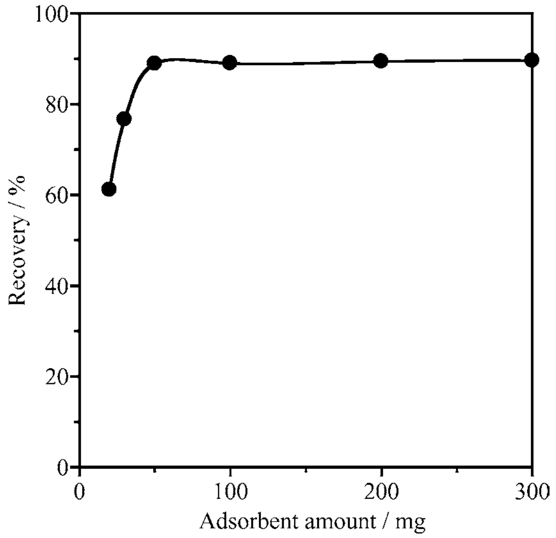

Fig. 1. Effect of diatomaceous earth amount on the recovery of simazine. Simazine solution: 15 ng mL^{-1}, 100 mL; stirring time: 10 min; pH: 2; eluent: ethanol, 5 mL.

The pH of simazine solutions was adjusted to 1.5, 2, 3, 4 and 6. The solutions were then preconcetrated according to the procedure mentioned above. The results are shown in Fig. 2. It was clearly demonstrated that the recovery of simazine was strongly affected by pH. The maximum of recovery was obtained at pH 2 and the value rapidly decreased with increasing pH. Adsorption of atrazine, which has a similar structure to simazine, was also dependent on pH [19]. Since simazine is a weak base ($pK_a = 1.62$), at acidic pHs it is largely in its cationic (protonated) form. This might be indicated that simazine as well as atrazine is adsorbed by ionic groups present on diatomaceous earth. The surface charges of diatomaceous earth may arise from chemical reactions at the surface due to the presence of ionizable functional groups such as –OH, –COOH, –SH and $–NH_3$. The ionization of such groups results in electric charges [20,21]. For example, silanol groups (Si–OH) present on the surface of the diatomaceous earth can gain or lose a proton, resulting in a surface charge that varies with changing pH [22]. At low pH, surface sites are protonated and the surface becomes positively charged ($Si–OH_2^+$). Therefore, it is presumably considered that the ion exchange reaction of cationic (protonated) form of simazine with one proton of protonated silanol groups occurred on the surface of diatomaceous earth at acidic pH. The pH value of simazine solution used for subsequent experiments was 2.

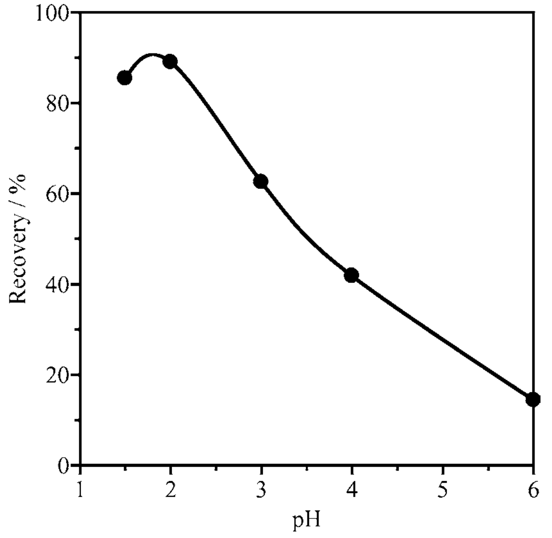

Fig. 2. Effect of pH on the recovery of simazine by diatomaceous earth. Simazine solution: 15 ng mL^{-1}, 100 mL; diatomaceous earth amount: 50 mg; stirring time: 10 min; eluent: ethanol, 5 mL.

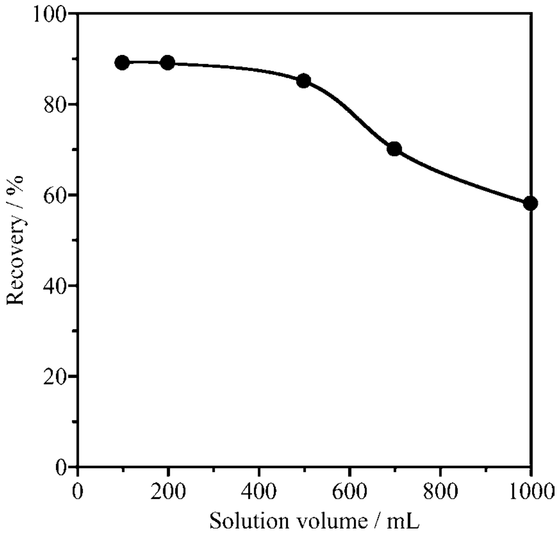

Fig. 3. Effect of simazine solution volume on the recovery of simazine by diatomaceous earth. Simazine amount: 1.5 μg; diatomaceous earth amount: 50 mg; stirring time: 10 min; pH: 2; eluent: ethanol, 5 mL.

In order to determine the maximum applicable sample solution, the effect of changes in the volume of validation solution on the adsorption of simazine was investigated (Fig. 3). By varying the volume of validation solution (100, 200, 500, 700 and 1000 mL) for the fixed amount of adsorbent (50 mg) and simazine (1.5 μg), no change was observed in the recovery of simazine up to a volume of 500 mL. At higher solution volumes, the recovery rapidly decreased with increasing volume of validation solution. This phenomenon would be responsible for a washing effect. Therefore, sample volume up to 500 mL was recommended to use for the preconcentration of unknown sample. These results show that a preconcentration factor was obtained as 100 when we use 500 mL of sample volume.

3.2. Characteristics of the method

For the precision of this method, the optimum conditions described above were used. For this purpose, seven successive adsorption–elution cycles were carried out and simazine was determined in the solution by HPLC. The spiked solution (100 mL) was used to contain 15 ng mL^{-1}

of simazine. The recovery of simazine was 89.0 ± 1.6% with a relative standard deviation for seven determinations of 1.5%. These results are sufficient for preconcentration purposes.

Calibration graph for simazine determination was prepared by using a series of 100 mL simazine solution (initial concentration: 0.3–600 ng mL^{-1}) preconcentrated under the optimum conditions. The liner range for simazine was from 1 to 200 ng mL^{-1} with a correlation coefficient of 0.996. The detection limit (3S/N) was found to be 0.3 ng mL^{-1}. Asperger et al. [2] proposed the on-line SPE-HPLC-MS/MS for the determination of pesticides. The detection limit of this published method was 1.6 pg mL^{-1} for simazine. Although the sensitivity of the present method is inferior to the method of Asperger et al. [2], it would be sufficient because Environmental Standard of Japan for simazine in water samples (ground water and drinking water) is 3 ng mL^{-1}. Furthermore, this proposed method is simple and inexpensive, and use a conventional instrument (HPLC), which are very advantageous.

The saturation adsorption capacity study used was adopted from that recommended by Maquiera et al. [23]. One hundred-milliliter aliquot of a series of concentrations was adjusted to the appropriate pH, then preconcentrated and eluted. The amount of simazine adsorbed at each concentration level was determined from the following equation:

$$C = \frac{cv}{w}$$

where C is the capacity (mg g^{-1}), c the concentration (mg L^{-1}) of simazine eluted, v the volume (L) of elution solution and w is the mass (g) of the adsorbent. The saturation adsorption capacity of diatomaceous earth were calculated from a breakthrough curve by plotting the initial simazine concentration (mg L^{-1}) versus the milligram of simazine adsorbed per gram determined by the above equation. The saturation adsorption capacity was defined as the capacity (C) in where the breakthrough curve reached saturation. The breakthrough curve is shown in Fig. 4. The saturation adsorption capacity of the adsorbent was found to be 1.1 mg g^{-1} for simazine.

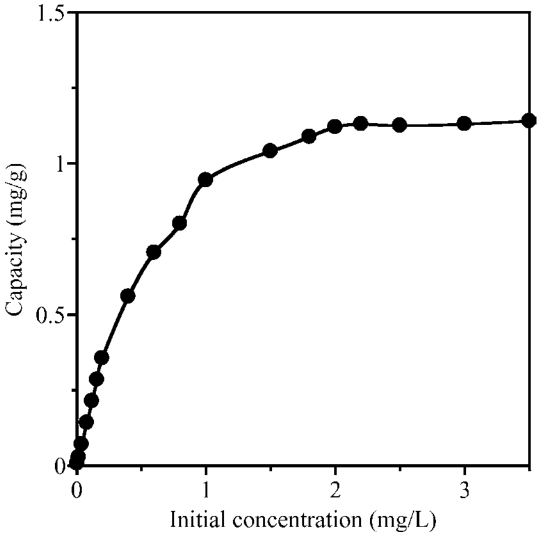

Fig. 4. Breakthrough curve of simazine on diatomaceous earth. Simazine solution volume: 100 mL; diatomaceous earth amount: 50 mg; stirring time: 10 min; pH: 2; eluent: ethanol, 5 mL.

3.3. *Interferences*

Effect of the presence of metal ions on the retention of simazine was investigated because these ions are generally present in water samples, such as river water, seawater and wastewater. For this purpose, the metal ions listed in Table 1 were individually added to 100 mL of solution containing simazine (1.5 μg) and the general procedure was applied. Although the mass ratio of these metal ions to simazine was 50, they did not significantly interfere on the adsorption of simazine.

Effect of humic acid on the adsorption of PAEs was also investigated. It was found that the effect of humic acid could be negligible although the mass ratio of the acids to simazine was 50. The mechanism related to the separation of low molecular weight compounds from humic and fulvic acids was proposed by Martin-Esteban et al. [24]. According to their proposal, these acids have molecular weight of approximately 1 000 000 Da, which is too high to allow the diffusion of the acids across the surface of adsorbent. However, simazine with molecular weights around 200 Da can be easily interact with the binding sites contained in the adsorbent. It is considered that other organic compounds are also contained in water samples, for example pesticides [25], bisphenol A [26] and PCBs [27]. Therefore, effect of atrazine and linuron on the retention of simazine onto diatomaceous earth was examined. Since both pesticides

Table 1
Effect of interferences on recovery of simazine

Ion or compound[a]	Recovery (%)[b]	Relative error (%)
Simazine	89	–
Ca(II)	86	−3
Cd(II)	86	−3
Cr(VI)	86	−3
Cu(II)	91	+2
Fe(III)	83	−6
K(I)	83	−6
Mg(II)	83	−6
Na(I)	88	−1
Pb(II)	83	−6
Zn(II)	88	−1
Humic acid	83	−6
Atrazine	79	−10
	87[c]	−2
Linuron	86	−3

[a] Mass ratio (interference/simazine) = 50.
[b] Average of three determinations.
[c] Atrazine/simazine = 1.

Table 2
Determination of simazine in river water (sample solution 100 mL)

Added (ng mL^{-1})	Found (ng mL^{-1})[a]	Recovery (%)
Anou river		
–	n.d.	–
5.0	5.2 ± 0.3	104
10.0	9.3 ± 0.2	93.0
15.0	14.8 ± 0.2	98.7

n.d.: not detected.
[a] Average of three determinations.

are also herbicides, these compounds would coexist with simazine in environment. As seen in Table 1, linuron did not affect on the retention of simazine although atrazine gave −10% error. It is considered that the recovery of simazine was low because atrazine could adsorb to diatomaceous earth under the similar experimental conditions [19]. From the result of adsorption capacity of diatomaceous earth, the recovery of simazine would be able to be improved in the presence of lower concentration of atrazine. The negative error of atrazine could be negligible when the mass ratio of atrazine to simazine was 1. The two peaks, which were corresponded to simazine and atrazine, appeared in the HPLC chromatogram. Therefore, this method would be able to be applied to the successive determination of triazines in water samples.

3.4. Determination of simazine in river water

The proposed method was applied to the determination of simazine in Anou River water (located in Tsu, Mie, Japan). The preconcentration procedure was carried out as soon as possible after sampling. Simazine was not found in the river water as shown in Table 2.

The river water sample (100 mL) was spiked with 5, 10 and 15 ng mL^{-1} of simazine for evaluating the recoveries. The recovery of spiked-simazine was in the range of 93–104%. The relative standard deviations for spiked sample were in the range of 1.4–5.8% at three replicates. Although river waters are generally present various matrices, the recovery and precision of simazine determination were very satisfactory. Thus, this present method is suitable for the preconcentration and determination of simazine from water samples.

4. Conclusions

A simple and rapid procedure was developed for the determination of simazine in the range 1–200 ng mL^{-1} after the preconcentration with diatomaceous earth as an adsorbent. The adsorption of simazine onto the adsorbent was reached the equilibrium state for 10 min. Simazine was quantitatively recovered with high precision. This method is very economical since only 50 mg of adsorbent is needed and the adsorbent can be easily obtained due to its presence in nature. The maximum preconcentration factor was 100 for simazine when 500 mL of sample solution volume was used. The advantages of this method are more rapidly than LLE and to not use toxic organic solvents as elution solution comparing with SPE method. Furthermore, the proposed method was successfully applied to the determination of simazine in river water. The recovery of spiked simazine in the river water was in the range of 93–104%. In the preconcentration method, it was found that trace simazine in aqueous samples could be determined without interferences of matrix compounds and chemical treatment. This method would be not only applicable to successive analysis of triazines but also to on-line preconcentration. Furthermore, this technique would be able to be adapted to in situ analysis.

Acknowledgements

This work was partly performed at Satellite Venture Business Laboratory (SVBL), Mie University.

References

[1] R. Carabias-Martínez, E. Rodríguez-Gonzalo, M.E. Fernández-Laespada, L. Calvo-Seronero, F.J. Sánchez-San Román, Water Res. 37 (2003) 928.
[2] A. Asperger, J. Efer, T. Koal, W. Engewald, J. Chromatogr. A 960 (2002) 109.
[3] G. Shen, H.K. Lee, J. Chromatogr. A 985 (2003) 167.
[4] C. Gonçalves, M.F. Alpendurada, J. Chromatogr. A 968 (2002) 177.
[5] B. Hauser, P. Popp, E. Kleine-Benne, J. Chromatogr. A 963 (2002) 27.
[6] M.E. León-González, L.V. Pérez-Arribas, L.M.P. Díez, C. Panis, M.P.S. Andrés, Anal. Chim. Acta 445 (2001) 29.
[7] T. Kumazawa, O. Suzuki, J. Chromatogr. B 747 (2000) 241.
[8] C. Schäfer, D. Lubda, J. Chromatogr. A 909 (2000) 73.
[9] H. Sabik, R. Jeannot, B. Rondeau, J. Chromatogr. A 885 (2000) 217.
[10] D. de Almeida Azevedo, S. Lacorte, T. Vinhas, P. Viana, D. Barceló, J. Chromatogr. A 879 (2000) 13.
[11] R. Carabias-Martinéz, E. Rodriguez-Gonzalo, J. Dominguez-Alavez, J. Hernández-Méndez, J. Chromatogr. A 869 (2000) 451.
[12] S. Lacorte, I. Guiffard, D. Fraisse, D. Barceló, Anal. Chem. 72 (2000) 1430.
[13] S. Lacorte, D. Fraisse, D. Barceló, J. Chromatogr. A 857 (1999) 97.
[14] C. Crescenzi, A. Di Corcia, E. Guerriero, R. Samperi, Environ. Sci. Technol. 31 (1997) 479.
[15] A. Di Corcia, C. Crescenzi, E. Guerriero, R. Samperi, Environ. Sci. Technol. 31 (1997) 1658.
[16] F. Orshansky, N. Narkis, Water Res. 31 (1997) 391.
[17] M. Prados, P. Poche, H. Paillard, C. Galley, J. Fr. Hydrol. 23 (1992) 201.
[18] A. Di Corcia, M. Marchetti, R. Samperi, J. Chromatogr. 405 (1987) 357.
[19] K. Agdi, A. Bouaid, A.M. Esteban, P.F. Hernando, A. Azmani, C. Camara, J. Environ. Monit. 2 (2000) 420.
[20] J.S. Noh, J.A. Schwarz, J. Colloid. Interface 130 (1989) 157.

[21] W. Stumm, J.J. Morgan, Aquatic Chemistry, third ed., Wiley, New York, 1995.
[22] M.A. Al-Ghouti, M.A.M. Khraisheh, S.J. Allen, M.N. Ahmad, J. Environ. Manage. 69 (2003) 229.
[23] A. Maquiera, H.A.M. Elmahadi, R. Puchades, Anal. Chem. 66 (1994) 3632.
[24] A. Martin-Esteban, P. Fernández, C. Cámera, Anal. Chem. 69 (1997) 3267.
[25] D. Barceló, in: D. Barceló (Ed.), Environmental Analysis: Techniques, Applications and Quality Assurance, Elsevier, Amsterdam, 1993.
[26] C.A. Staples, P.B. Dorn, G.M. Klecka, S.T. O'Block, D.R. Branson, L.R. Harris, Chemosphere 36 (1998) 2149.
[27] T. Webster, B. Commoner, in: A. Schechter (Ed.), Overview of the Dioxin Debate, Plenum, New York, 1994.