



ELSEVIER

Journal of Chromatography A, 866 (2000) 79–85

JOURNAL OF  
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

# Application of pressurized liquid extraction followed by gas chromatography–mass spectrometry to determine 4-nonylphenols in sediments

Wang-Hsien Ding\*, John C.H. Fann

*Department of Chemistry, National Central University, Chung-Li 32054, Taiwan*

Received 9 July 1999; received in revised form 30 September 1999; accepted 30 September 1999

## Abstract

A time- and solvent-saving method, pressurized liquid extraction (PLE), to extract 4-nonylphenol (4-NP) in sediment was developed. The effects of various operational parameters (i.e., temperature, pressure, etc.) for the quantitative extraction of 4-NP by PLE were investigated. The analytes were then identified and quantitated by a large-volume injection GC–MS technique. The 4-NP can be completely extracted by methanol at 100°C and 100 atm combined with 15 min static and then 10 min dynamic extraction steps (1 atm=101 325 Pa). Recovery of 4-NP in spiked blank kaolin samples was 98% with 5% RSD. The degrees of recovery of 4-NP in the spiked sediment samples from a reservoir and a polluted river were 111% with 4% RSD and 106% with 5% RSD, respectively. The perfect applicability of PLE for 4-NP was determined after testing it with spiked and aged samples. The extraction efficiency of the PLE was compared with conventional Soxhlet and bath ultrasonication extraction methods using the spiked sediment samples. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Pressurized liquid extraction; Extraction methods; Sediments; Environmental analysis; Nonylphenol

## 1. Introduction

Nonylphenol polyethoxylates (NPEOs) form one of the major classes of nonionic surfactants used in Taiwan and over  $48 \cdot 10^6$  kg were produced in 1996 [1]. These surfactants are used in an extensive variety of industrial, household and commercial applications. The biodegraded products of NPEOs, such as nonylphenol (NP) and shortened ethoxy-NPEO metabolites, are more lipophilic than their

parent compounds [2]. Therefore, they tend to absorb and accumulate on sediments, which could become a secondary contamination source for river water when the sediment resuspends at high water flows. There is a significantly higher concentration of NPEO-type residues in Taiwanese rivers than in other countries because of Taiwan's deficient municipal wastewater treatment [3,4]. Due to the estrogenic activity of 4-NP [5,6] and persistence in the aquatic environment [7–9], it is vital to develop a convenient analytical technique to study the occurrence and fate of these pollutants in the aquatic environment. Although surfactant residues in wastewater directly discharged into the aquatic environment is a significant source of surface water contamination through-

\*Corresponding author. Tel.: +886-3-4227-151; fax: +886-3-4227-664.

E-mail address: wding@cc.ncu.edu.tw (W.-H. Ding)

out Taiwan and entire Asia-Pacific area due to deficient wastewater treatment, the distribution and concentrations of 4-NP in the river sediments has yet to be investigated.

The newly developed pressurized liquid extraction (PLE) [10] technique has been found to be a better alternative to the conventional extraction methods (i.e., Soxhlet and ultrasonication) for the extraction of environmental samples because it offers great reductions in solvent use coupled with faster sample processing times. PLE achieves rapid extraction with small volumes of organic solvents at elevated temperature (up to 200°C) and high pressure (up to 150 atm, 1 atm=101 325 Pa) to maintain the solvent in the liquid state. Raising the temperature increases the diffusion rates, solubility and mass transfer, therefore, the viscosity and surface tension of the solvents are less than at room temperature. These changes facilitate better contact of the analytes with the solvent and enhance the extraction. High pressure helps to force the solvent into the pores of the sample matrix to keep the solvent in the liquid state at operating temperature. In addition, only the extraction time and temperature have to be optimized since the solvent choice for PLE can be the same as that used in the Soxhlet extraction. Recently, Kreisselmeier and Durbeck used CO<sub>2</sub> supercritical fluid extraction (SFE) combined with a high percentage of methanol as an organic modifier to extract alkylphenols, alkylphenol polyethoxylates (APEOs) and linear alkylbenzene sulfonates (LASs) residues in the sediments [11]. According to their results, the presence of methanol and a maximum pressure up to 450 atm were essential for the complete extraction of APEOs. However, due to the strong matrix interaction of aged spiked samples, the degree of recovery was reduced by approximately 40%.

This study attempts to develop a 'pure' PLE method (without supercritical CO<sub>2</sub>) for the quick extraction of 4-NP from sediment sample in order to reduce the time and solvent consumption. The temperature, pressure, static/dynamic extraction times and solvent flow-rate were investigated to assess the effect of experimental conditions on the performance of PLE to extract 4-NP from solid samples. The conventional Soxhlet and bath ultrasonication extraction methods were employed as comparisons.

## 2. Experimental

### 2.1. Chemicals and reagents

Unless specified otherwise, all high purity chemicals and solvents were purchased from Aldrich (Milwaukee, WI, USA), Tedia (Fairfield, OH, USA) and Merck (Darmstadt, Germany), and were used without further purification. 4-Nonylphenol (85%, technical grade) and 4-(*tert.*-octyl)phenol (4-t-OP, as surrogate, 97%) were purchased from Aldrich. The finest powder of blank kaolin soil was purchased from Riedel-de Hën (Seelze, Germany).

### 2.2. Sample collection and preparation

Sediment samples (containing 2.0% of total organic matter) from a surfactant polluted river were collected from Lao-Jie River at Chung-Li City (Taiwan). In this city, untreated municipal wastewater is discharged directly into the river. Blank sediment samples (containing 1.5% of total organic matter) were collected from a reservoir in the mountains of Tao-Yuan County (Taiwan). Sediments were collected using a grab, homogenized by stirring and were stored in stainless steel containers. The sediment pore water was removed by centrifugation upon arrival in the laboratory. The solid samples were freeze-dried and kept at -25°C until analysis. For the analysis, the sample was thawed at room temperature, ground in a mortar and pestle, and sieved through a 30-mesh screen in order to enhance the extractability of the analytes.

The recovery experiments were performed using the spiked samples. Known amounts of 4-t-OP and 4-NP, dissolved in 100 µl of methanol were carefully distributed on top of the samples by a glass syringe. The samples were then mixed by tumbling for 30 min. The spiked samples were then stored in a tightly closed brown glass bottle at room temperature for 24 h, and made ready for spiked experiments. The aged spiked samples from the reservoir sediment were prepared as described by Kreisselmeier and Durbeck [11]. A 50-g portion of the reservoir sediment was spiked with known amounts of 4-t-OP and 4-NP, the sample was mixed by tumbling, and

then stored in a tightly closed brown glass bottle at room temperature for 45 days in the dark.

### 2.3. Pressurized liquid extraction

The pump used was an ISCO Model 260D syringe pump (ISCO, Lincoln, NE, USA). Methanol was used as the extraction solvent in all cases. The pump was operated in the constant pressure mode to supply methanol to the extraction cell through a 1/16-in. O.D.×0.020 in. I.D. (1 in.=2.54 cm) stainless steel tubing that included a 4-m preheating coil. The extraction cell and preheating coil were placed inside of an oven (Super CO-150 from Enshine, Taiwan). SFE extractor vessels (10 ml) from Supelco (Bellefonte, PA, USA) were used as extraction cells for all extractions. The extraction cell was mounted vertically in the oven with the methanol flowing from top to bottom in order to sweep the extracted analytes in the cell. The outlet of the vessel was connected by stainless steel tubing to a Swagelok stainless steel needle valve (Swagelok, Solon, OH, USA) and connected to a fused-silica flow restrictor (30 cm×50 μm I.D., Supelco) to provide a flow-rate of ~0.5 ml/min.

To extract 4-NP residues from sediments, 5 g of solid sample (kaolin or sediment prepared as described in Section 2.2) was added into 10 ml extractor vessels. The volume of the extractor vessel was made up with pre-cleaned glass beads and filled with methanol. The extractor vessel was closed and fixed into the oven. The extraction was performed at 100°C with 100 or 150 atm at static extraction for 15 min, followed by dynamic extraction at 100°C/100 atm, 100°C/150 atm, or 150°C/100 atm, respectively (see Results and discussion). The extraction was followed by dynamic processes of three 10-min periods with subsequent elution, while each extract was collected in separate vials (10, 20 and 30 min) to optimize the dynamic step. The concentrations of the analytes in each period of extraction were determined and summed up to set as 100%.

After the extraction, the PLE extracts were cleaned by deactivated alumina and eluted by methanol as described by Ahel and Giger [12]. The eluents were evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved in 100 μl of

chloroform containing 20 ng/μl of [<sup>2</sup>H<sub>12</sub>]chrysene as an internal standard and, thus made ready for GC–MS analysis.

### 2.4. Soxhlet extraction and bath ultrasonication extraction

The sediment samples from surfactant polluted river were used to compare the extractions.

#### 2.4.1. Soxhlet extraction

The spiked samples (5 g) were mixed with the same amount of sodium sulfate and extracted using 150 ml of methanol for 24 h at the approximate rate of 6–8 cycle/h in a Soxhlet extraction apparatus. The extracts were concentrated by rotary evaporation to 1–2 ml under vacuum, and then transferred to a 5-ml vial, where they were evaporated to dryness under a gentle stream of nitrogen.

#### 2.4.2. Bath ultrasonication extraction

Extractions were performed with a Branson ultrasonic cleaner (Branson 5210, Branson Ultrasonic, Danbury, UT, USA). Portions of 5 g of spiked sediment samples mixed with the same amount of sodium sulfate were extracted three times with 50 ml of methanol for 30 min each at room temperature. Following each extraction, the outstanding methanol was decanted and the combined extracts were concentrated by rotary evaporation to 1–2 ml under vacuum. The concentrated extracts were then transferred to a 5-ml vial, where they were evaporated to dryness under a gentle stream of nitrogen.

The residues for these two extraction methods were redissolved in 100 μl of chloroform containing 20 ng/μl of [<sup>2</sup>H<sub>12</sub>]chrysene as an internal standard, to prepare them for the GC–MS analysis.

### 2.5. GC–MS analysis and quantitation

Analyses were performed on a Varian 3400CX gas chromatograph directly connected to a Saturn 2000 ion-trap mass spectrometer (Varian, USA). Large-volume samples were introduced with a direct sample introduction (DSI) device (or ChromatoProbe, from Varian, USA) and a temperature-programmed

injector (Varian 1078 injector port) with 3.4 mm I.D. liner, as described by Ding and coworkers [3,4]. Briefly, 20  $\mu\text{l}$  of sample extraction was introduced into a DSI micro sample vial. The vial was placed into the probe's vial holder, and then pushed into the heated zone of the injection-port. The injector was held at 80°C for 4 min, then rapidly heated to 300°C, and held for another 40 min. A DB-5MS capillary column (30 m $\times$ 0.25 mm I.D., 0.25  $\mu\text{m}$  film, from J&W, USA) connected to 2 m of deactivated fused-silica per column, was used. After the injector temperature had reached 300°C, the GC temperature program began as follows: 100°C for 3 min, followed by a 7°C/min ramp to 300°C, and held for 7 min. The transfer line was set at 280°C. Full scan electron impact (EI) ionization data were acquired under the following conditions: mass range 50–550  $m/z$ , scan time 1 s, manifold temperature 120°C, emission current 10  $\mu\text{A}$ , automatic gain control (AGC) target 25 000 (represents as the target total ion current value).

The characteristic ions of 4-NP were produced by benzylic cleavages of  $[\text{M}-85]^+$  and  $[\text{M}-71]^+$ , corresponding to the ions of  $m/z$  135 and 149, as described in our earlier works [3,4]. The quantitation of 4-NP was calculated from the five-level calibration curve (or average response factor) covering the range 20 ng/ $\mu\text{l}$  to 250 ng/ $\mu\text{l}$ , each divided by the fixed concentration of  $[\text{}^2\text{H}_{12}]$ chrysene (20 ng/ $\mu\text{l}$ ) as an internal standard. The response factors were calculated by comparing the sum of the total peak areas of two EI selected characteristic ion chromatograms at  $m/z$  135+149 to the peak area of  $[\text{}^2\text{H}_{12}]$ chrysene. The precision of the curve, as indicated by the RSD of the response factors, was 2.2%.

### 3. Results and discussion

#### 3.1. 4-NP extraction by PLE

The extraction parameters were optimized using 4-t-OP and 4-NP spiked blank kaolin samples. The initial PLE extraction conditions were set at 100°C and 100 atm with methanol utilized as the extraction solvent because these conditions previously allowed for the successful extraction of phenols from soil

[13] and NPEO residues from sediment [14] by PLE with various solvent combinations, and methanol was used as solvent successfully extraction of 4-NP from solid samples by Soxhlet [15,16]. Although PLE can be done in static, dynamic or combination modes (static then dynamic extraction), the combination mode was most commonly employed since it reduces the extraction time and obtains the better extraction efficiency. In this study, the extractions were performed in a combination mode: 15-min static extraction and then 10–30 min dynamic extraction. The dynamic extraction recoveries determined at various combinations of pressure and temperature are depicted in Fig. 1a. The dynamic extraction curves were constructed by collecting an extract every 10 min and analyzing each extract separately. The total recovery of 4-NP from kaolin was faster at 100 atm and 100 or 150°C after 10 min of dynamic extraction as illustrated in Fig. 1a. The

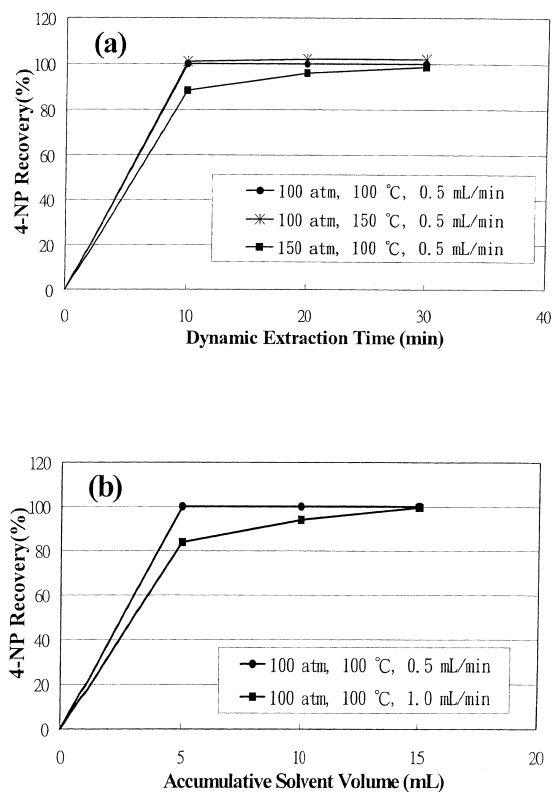


Fig. 1. Effect of (a) pressure/temperature and (b) flow-rate on the recovery of spiked 4-NP from blank kaolin samples.

increasing pressure induced a change of the matrix surface resulting in a reduced extraction efficiency of the analytes as revealed by a comparison of the extraction obtained using condition of 150 atm and 100°C, which required 30 min to achieve maximum recovery [11,17].

The extraction efficiency was not influenced by the flow-rate of supercritical CO<sub>2</sub> when CO<sub>2</sub>-SFE was used to extract 4-NP from sludge samples [18]. However, it took longer to achieve maximum recovery when the flow-rate of methanol increased to 1.0 ml/min (Fig. 1b), probably because the faster flow-rate reduced the extraction efficiency by reducing the contact time between the solvent and analytes. Herein, all the extractions were performed at 100 atm and 100°C with methanol as the extraction solvent, flow-rate 0.5 ml/min, static extraction 15 min, and dynamic extraction 10 min.

### 3.2. Method quantitation limits and precision

The quantitation limits of the combined PLE extraction method and large-volume injection GC–MS technique were determined by spiked samples from reservoir sediment extracted under optimal conditions. The quantitation limits of the method for 4-t-OP and 4-NP were 0.005 and 0.01 µg/g, respectively, defined at a signal to noise (*S/N*) ratio of 10. Seven replicate spiked samples (spiked concentration 20 µg/g) in two types of solid samples were extracted in order to determine the precision of the PLE extraction process for 4-NP. The precision of the method, as indicated by the relative standard deviation (RSD), was 5 and 4% for kaolin and reservoir sediment, respectively.

### 3.3. Recovery study and application to environmental samples

The recovery from PLE was evaluated by a spiked known amounts of 4-t-OP (as surrogate) and 4-NP in various solid samples. Seven replicate 5 g kaolin samples were each spiked to obtain the final concentrations of 2 µg/g of 4-t-OP and 20 µg/g of 4-NP. The average recoveries of 4-t-OP and 4-NP were 100% with 7% RSD and 98% with 5% RSD, respectively. Seven replicate 5-g sediment samples

Table 1  
Background concentration of 4-NP, and recovery results of 4-t-OP and 4-NP spiked in different sediments<sup>a</sup>

Sample	4-NP	4-t-OP (surrogate)
Kaolin soil		
Spiked recovery (%)	98 (5%)	100 (7%)
Reservoir sediment		
Background conc. (µg/g)	N.d. <sup>b</sup>	
Spiked recovery (%)	111 (4%)	103 (3%)
Polluted river sediment		
Background conc. (µg/g)	18 (5%)	
Spiked recovery (%)	106% (5%)	75 (2%)

<sup>a</sup> Relative standard deviation (RSD) is given in parentheses, for kaolin soil and reservoir sediment *n*=7; for polluted river sediment *n*=3.

<sup>b</sup> N.d., not detected at the method quantitation limit, 0.01 µg/g.

from a reservoir and three replicate 5-g sediment samples from a polluted river were used to investigate the recoveries of 4-t-OP and 4-NP from the environmental samples. Table 1 summarizes the average percentage recoveries of 4-t-OP and 4-NP in the environmental samples, as well as their estimated average background concentrations. Recovery of 4-t-OP and 4-NP ranged from 75 to 111% with RSD ranging from 2 to 7%. The quantitation of 4-NP was calculated from the five-level calibration curve (or average response factor) covering the range 20–250 ng/µl, each divided by the fixed concentration of [<sup>2</sup>H<sub>12</sub>]chrysene (20 ng/µl) as an internal standard. Fig. 2 depicts the selected EI characteristic ion chromatograms of 4-NP, and their corresponding mass spectra detected in a sediment sample from a surfactant polluted river.

Since spiked analytes were typically extracted more efficiently and rapidly than native analytes as discussed previously [19,20], the analytes might be diffused into matrix particles in aged samples or native materials due to growing influence of analyte–matrix interaction. The comparison of the degree of recovery of the recently spiked (24-h) and aged samples (45-day) were also investigated. The average recovery of 4-t-OP and 4-NP were 98% (2% RSD, *n*=3) and 104% (1% RSD, *n*=3), respectively. These results demonstrate that despite the longer interaction time between the analyte and the matrix, the PLE extraction efficiency was not significantly

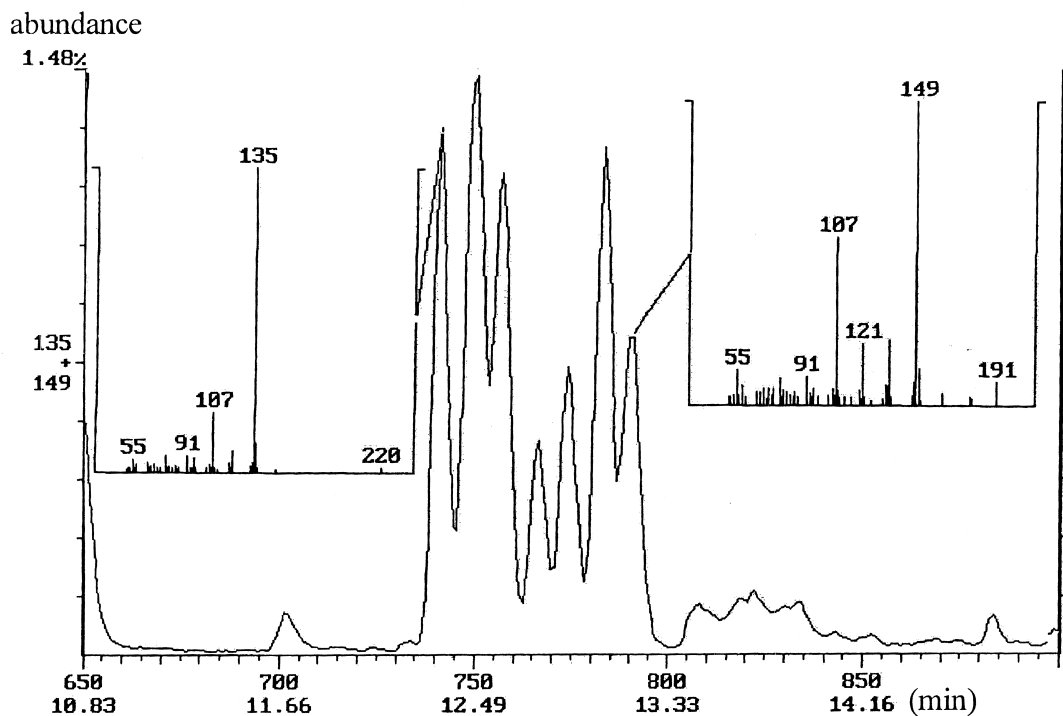


Fig. 2. Selected characteristic mass chromatograms of 4-NP and their corresponding mass spectra detected in a sediment sample from a surfactant polluted river.

affected when methanol was used as the extraction solvent.

### 3.4. Method comparison

A comparison of the three methods reveals that the extraction time of PLE was 30 min, bath ultrasonication took 1.5 h, and Soxhlet 24 h per extraction. Solvent consumption in PLE was 10 ml, but 150 ml in both bath ultrasonication and Soxhlet. The degree of recoveries of spiked sediment samples by PLE, Soxhlet extraction and bath ultrasonication methods were 106% (5% RSD,  $n=3$ ), 85% (6% RSD,  $n=3$ ) and 82% (5% RSD,  $n=3$ ), respectively. The concentrations of 4-NP extracted by these three methods were 18, 7 and 11  $\mu\text{g/g}$ , respectively. These results indicate that PLE technique is more effective in extracting 4-NP from the sediment samples with significant time and solvent saving. In addition, the risk of analytes losses is greatly reduced in PLE

technique since the small amount of solvent is used and the concentration step is much shortened.

## 4. Conclusion

The analytical procedure developed herein demonstrated that the PLE and large-volume injection GC-MS methods are reliable, sensitive and offer a convenient analytical technique for trace determination of 4-NP residues in complex sediment samples. The PLE is the preferable technique to extract various organic pollutants (polar to nonpolar) from solid samples if the equipment costs are not taken into account. The major advantages of PLE are short extraction time (30 min) and a small amount of solvent consumption with various solvents choice. The survey of 4-NP in sediment samples is currently being studied across Taiwan in order to understand the fate and influence of 4-NP in untreated waste-

water directly discharged into the aquatic environment.

### Acknowledgements

This research was supported by a grant from the National Science Council of Taiwan, contract No. NSC 88-2113-M-008-002. The authors would like to thank Professor. Lee, Jiunn-Fwu's laboratory (Graduate Institute of Environmental Engineering, National Central University, Taiwan) for sediment preparation.

### References

- [1] C.R. Shih, *Chem. Technol.* 5 (1997) 112.
- [2] M. Ahel, W. Giger, *Chemosphere* 26 (1993) 1471.
- [3] W.H. Ding, S.H. Tzing, *J. Chromatogr. A* 824 (1998) 79.
- [4] W.H. Ding, S.H. Tzing, J.H. Lo, *Chemosphere* 38 (1999) 2597.
- [5] S. Jobling, D. Sheahan, S.A. Hoare, J.P. Sumpter, *Environ. Toxicol. Chem.* 15 (1996) 194.
- [6] S. Jobling, J.P. Sumpter, *Aquat. Toxicol.* 27 (1993) 361.
- [7] J.A. Field, R.L. Reed, *Environ. Sci. Technol.* 30 (1996) 3544.
- [8] W.H. Ding, Y. Fujita, R. Aeschmann, M. Reinhard, *Fresenius J. Anal. Chem* 48 (1996) 354.
- [9] A. Di Corcia, A. Costantino, C. Crescenzi, E. Marinoni, R. Samperi, *Environ. Sci. Technol.* 32 (1998) 2401.
- [10] B.E. Richter, B.A. Jones, J.L. Ezzell, N.L. Porter, N. Avdalovic, C. Pohl, *Anal. Chem.* 68 (1996) 1033.
- [11] A. Kreisselmeier, H.W. Durbeck, *J. Chromatogr. A* 775 (1997) 187.
- [12] M. Ahel, W. Giger, *Anal. Chem.* 57 (1985) 2584.
- [13] J.A. Fisher, M.J. Scarlett, A.D. Stott, *Environ. Sci. Tech.* 31 (1997) 1120.
- [14] D.Y. Shang, R.W. MacDonald, M.G. Ikonou, *Environ. Sci. Tech.* 33 (1997) 1366.
- [15] A. Marcomini, W. Giger, *Anal. Chem.* 59 (1987) 1709.
- [16] A. Marcomini, F. Cecchi, A. Sfriso, *Environ. Technol.* 12 (1991) 1047.
- [17] T.M. Fahmy, M.E. Paulaitis, D.M. Johnson, M.E.P. McNally, *Anal. Chem.* 65 (1993) 1462.
- [18] J.G. Lin, R. Arunkumar, C.H. Liu, *J. Chromatogr. A* 840 (1999) 71.
- [19] V. Camel, A. Tambute, M. Cande, *J. Chromatogr. A* 693 (1995) 101.
- [20] M.D. Burford, S.B. Hawthorne, D.J. Miller, *Anal. Chem.* 65 (1993) 1497.