Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1069 (2005) 281–285
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Analysis of herbicides in water using temperature-responsive chromatography and an aqueous mobile phase

Eri Ayano [a, b], Yuji Okada [b], Chikako Sakamoto [b], Hideko Kanazawa [b, *], Teruo Okano [c], Masanori Ando [a], Tetsuji Nishimura [a]

[a] *Division of Environmental Chemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*
[b] *Department of Physical Pharmaceutical Chemistry, Kyoritsu University of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan*
[c] *Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1 Kawadacho, Shinjyuku-ku, Tokyo 162-8666, Japan*

Received 16 July 2004; received in revised form 26 January 2005; accepted 31 January 2005

Abstract

A simple and rapid method has been developed for herbicides in water using temperature-responsive liquid chromatography (LC) and a column packed with poly(*N*-isopropylacrylamide) (PNIPAAm), a polymer anchored on the stationary-phase surface of modified silica. PNIPAAm reversibly changes its hydrophilic/hydrophobic properties in water in response to temperature. The method was used to determine five sulfonylurea and three urea herbicides. Separation was achieved with a 10 mM ammonium acetate (pH 3.0) isocratic aqueous mobile phase, and by changing the column temperature. The analytes were extracted from water by off-line solid-phase extraction (SPE) with an *N*-vinyl-pyrrolidone polymer cartridge. The average recoveries of the eight herbicides from spiked pure water, tap water and river water were 70–130% with relative standard deviations (RSDs) of <10%. The limits of quantitation (LOQ) of the eight herbicides were between 1 and 4 $\mu g\,l^{-1}$.

Keywords: Poly(*N*-isopropylacrylamide); LC; Temperature-responsive chromatography; Sulfonylurea herbicides; Urea herbicides

1. Introduction

Herbicides are used in rice paddies, golf courses, and other types of fields. They are transported by aquifers in groundwater and are widely distributed in the environment. Sulfonylurea herbicides are labile, weakly acidic compounds. Sulfonylurea and urea herbicides are used at lower concentrations, and are more rapidly degraded in soil than older herbicides. Therefore, parts-per-billion concentrations of these herbicides are to be expected in the water supply. These herbicides have been analyzed in water by liquid chromatography (LC) with UV detection [1,2], capillary electrophoresis with UV [3], LC with mass spectrometry (MS) [4,5], immunoassay [6], bioassay [7] and radio immunoassay [1].

Recently, various polymers have been developed which change their structure in response to surrounding conditions, such as the pH, electric field, and temperature. Such polymers have been widely utilized in drug delivery systems [8], cell culture dishes [9], cell sheets [10] and bioconjugates [11]. Poly(*N*-isopropylacrylamide) (PNIPAAm) is one of these; it exhibits a thermally reversible phase transition in response to temperature changes across a lower critical solution temperature (LCST) of 32 °C in aqueous solution [12]. In water, the polymer chains of PNIPAAm hydrate and expand below this LCST, while they dehydrate to form a compact conformation above it. We previously reported a considerable and reversible change in the hydrophilic/hydrophobic properties of PNIPAAm-grafted surfaces in response to a change in temperature. Taking advantage of this characteristic, we developed an LC column packed with PNIPAAm to selectively separate analytes by controlling the external column temperature [13–17].

* Corresponding author. Tel.: +81 3 5400 2657; fax: +81 3 5400 1378.
E-mail address: kanazawa-hd@kyoritsu-ph.ac.jp (H. Kanazawa).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.01.100

Temperature-responsive chromatography is a method with little load on the environment, because no organic solvent is used in the mobile phase. Urea herbicides in environmental water have been widely studied by Hogenboom and coworkers [2,18,19] and very rapid analyses were made by using a single short column for both SPE and analytical separation. However, there are fewer reports on sulfonylurea herbicides [5]. The aim of this study was to achieve the separation of both groups of herbicides by temperature-responsive LC with an aqueous mobile phase.

2. Experimental

2.1. Chemicals

Analytical-grade standards of bensulfuron-methyl (99.7%), imazosulfuron (99.7%), pyrazosulfuron-ethyl (99.9%), halosulfuron-methyl (100%), siduron (98.9%), daimuron (100.0%) and diuron (100.0%) were purchased from Wako Pure Chemical Industries, Osaka, Japan. Analytical-grade flazasulfuron (99.9%) was purchased from Hayashi Pure Chemical Industries, Osaka, Japan. The structures of these herbicides are shown in Fig. 1. *N*-isopropylacrylamide (NIPAAm) was kindly provided by KOHJIN, Tokyo, Japan and was purified by recrystallization from *n*-hexane. 3-mercaptopropionic acid (MPA), 2,2′-azobisisobutyronitrile (AIBN), *N*,*N*-dimethylformamide (DMF), ethyl acetate, 1,4-dioxane, *N*,*N*′-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide, HPLC-grade tetrahydrofran (THF) and ammonium acetate were purchased from Wako Pure Chemical Industries. Aminopropyl silica beads (average diameter, 5 μm; pore size, 120 Å) were purchased from Nishio Industries, Tokyo, Japan. The pure water used for sample preparation and the LC mobile phase was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The synthesis of PNIPAAm and a modification of aminopropyl silica with the NIPAAm polymer were carried out by radical polymerization, as previously reported [13,20].

2.2. Temperature-responsive LC

A PNIPAAm-grafted silica beads were packed into a stainless-steel column (150 mm × 4.6 mm i.d.). LC was carried out on an Agilent 1100 series (Agilent, Waldbronn, Germany) instrument equipped with a UV detector and a Rheodyne Model 7750 injector. The column oven was a product of Shodex AO-30C (Showa Denko, Tokyo, Japan). The mobile phase was 10 mM ammonium acetate (pH 3.0). The thermoresponsive elution behavior of the herbicides was monitored at 240 nm at a flow rate of 1.0 ml min^{-1} at various temperatures. The injection volume was 20 μl.

2.3. Preparation of standard solutions

Stock solutions (1000 mg l^{-1}) of each analytical standard were prepared in THF. Next, working standard mixtures were prepared by diluting each herbicide stock solution with THF. These stock solutions were stored at 4 °C. Standard solutions

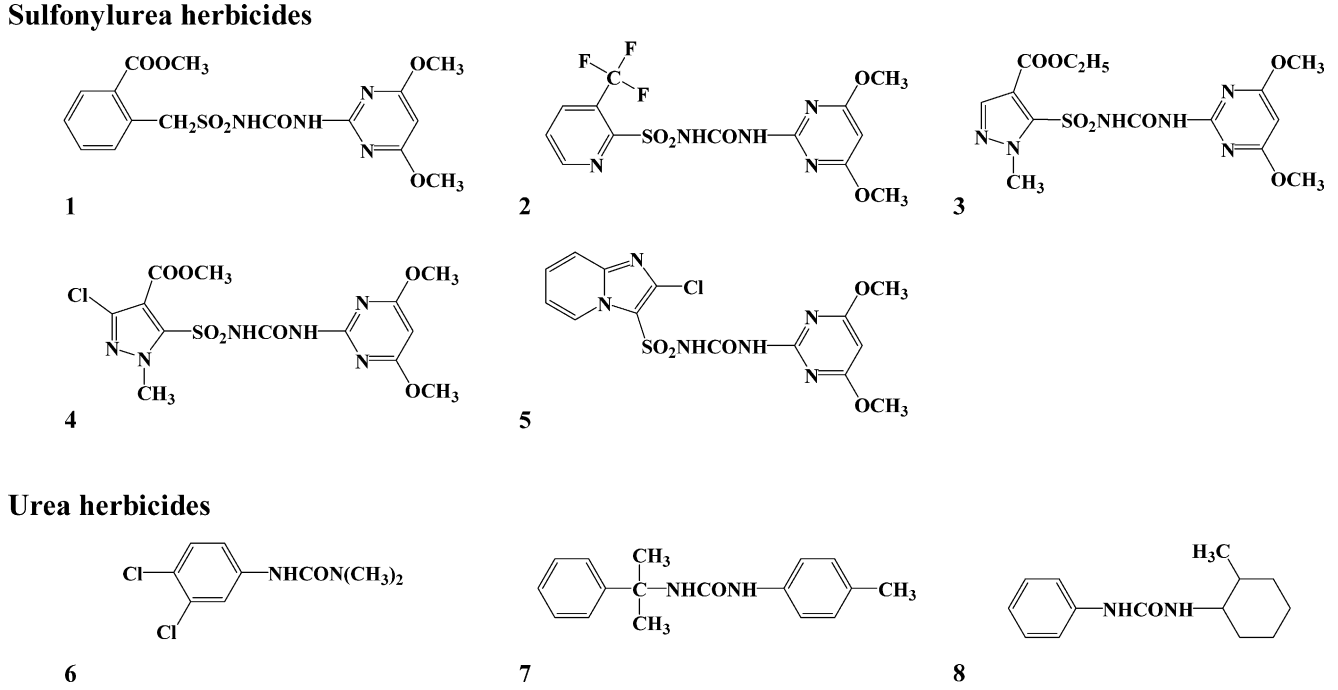

Fig. 1. Structures and common names of the eight herbicides. 1, bensulfuron-methyl; 2, flazasulfuron; 3, pyrazosulfuron-ethyl; 4, halosulfuron-methyl; 5, imazosulfuron; 6, diuron; 7, daimuron; and 8, siduron.

were prepared by diluting the stock solution with THF. The standard solutions were used for calibration plots and spiking of the water samples.

2.4. Water samples

Three types of water were analyzed: pure water, tap water and river water. The tap water was from a tap in the laboratory. L(+)-Ascorbic acid sodium salt (Wako Pure Chemical Industries) was added to the tap water at 0.005% (w/v), which eliminated chlorine that could react with and degrade some of the compounds of interest. The river water was collected from the Tama River near Tokyo; it was filtered through a glass-fiber filter before use.

2.5. Analytical methods

For recovery studies, three water samples (0.5 l each) were spiked with 1 ml of 2 mg l^{-1} (except for 0.5 mg l^{-1} diuron and daimuron) of the composite standard. Then, the spiked water samples were passed through a SPE cartridge to extract the analytes [5]. SPE was performed with cartridges prepacked with *N*-vinyl-pyrrolidone polymer resin (Oasis HLB Plus Extraction Cartridges) from Waters (Milford, MA, USA). The SPE cartridges were equilibrated with 5 ml of methanol and then 5 ml of pure water. The water samples were extracted at a 10 ml min^{-1} flow rate. Then, the cartridges were washed with 10 ml of pure water at a 5 ml min^{-1} flow rate and dried with air passed through the cartridge for 40 min. The herbicides were eluted from the cartridges with 3 ml of methanol at a speed of 1–2 drops s^{-1}. After evaporating the samples to near-dryness under a gentle nitrogen stream, the materials were dissolved to a final volume of 1.0 ml in THF.

3. Results and discussion

3.1. Sulfonylurea herbicides

Sulfonylurea herbicides were separated based on their temperature-controlled hydrophilic/hydrophobic properties by using an LC system connected to a column packed with PNIPAAm-modified silica beads. Fig. 2(a) shows van't Hoff plots for sulfonylurea herbicides separated using a PNIPAAm-modified column in 10 mM ammonium acetate (pH 3.0). The linearity in the van't Hoff plots is commonly observed for commercially available reversed-phase columns under standard chromatographic conditions. On the PNIPAAm-modified column, however, a deviation from linearity was found between ln *k* values and the reciprocal temperature (1/*T*). Interestingly, the slope of the van't Hoff plots of each analyte on the PNIPAAm-modified column changed markedly at the LCST boundary (Fig. 2 (a)). This corresponds to a phase transition of the polymer modified on the surface. Typical chromatograms for the standards of the five sulfonylurea herbicides using the PNIPAAm-modified column at 10 and 50 °C are shown in Fig. 3.

The log *P* values of these herbicides are given in Table 1. log *P* values were calculated by the CAChe system (Fujitsu, Japan). We reported in previous papers that the order of separation on a temperature-responsive-polymer-modified column depends on the hydrophobicities, corresponding to increasing log *P* values [13]. In this study, the retention time of the strongly hydrophobic imazosulfuron was remarkably increased, compared with four other sulfonylurea herbicides. When trying to separate the same herbicides on an ODS column using an aqueous/organic solvent, the three peaks of bensulfuron-methyl, flazasulfuron and imazosulfuron overlapped, and the two peaks of pyrazosulfuron-ethyl

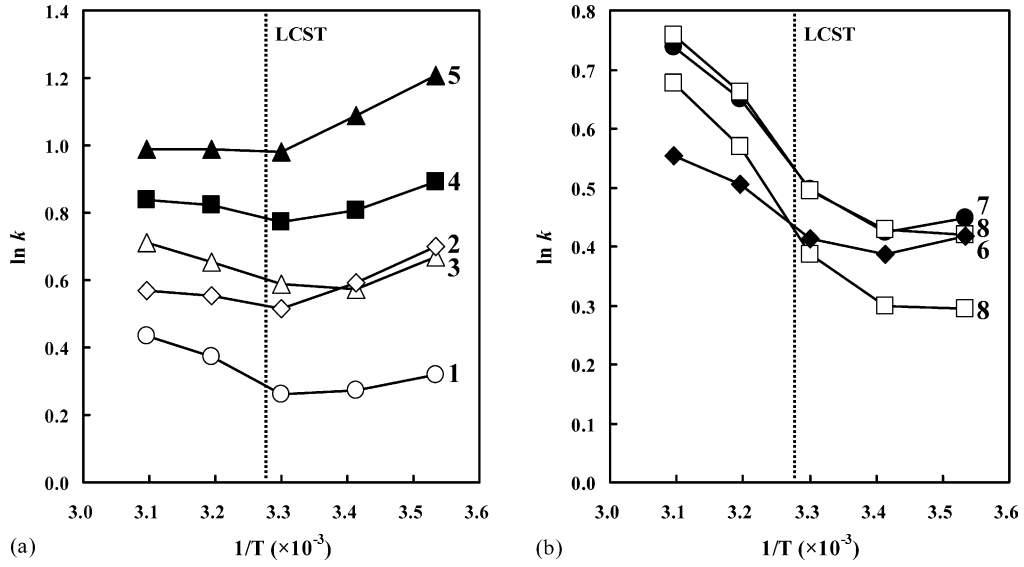

Fig. 2. van't Hoff plots of (a) sulfonylurea and (b) urea herbicides. For LC conditions, see Section 2. For peak numbers, see Fig. 1.

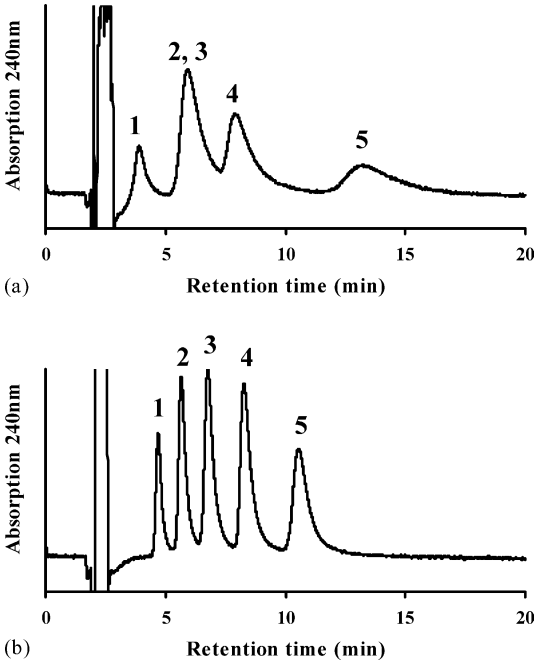

Fig. 3. LC–UV of standards using a PNIPAAm-modified silica column at (a) 10 °C and (b) 50 °C. For LC conditions, see Section 2. For peak numbers, see Fig. 1.

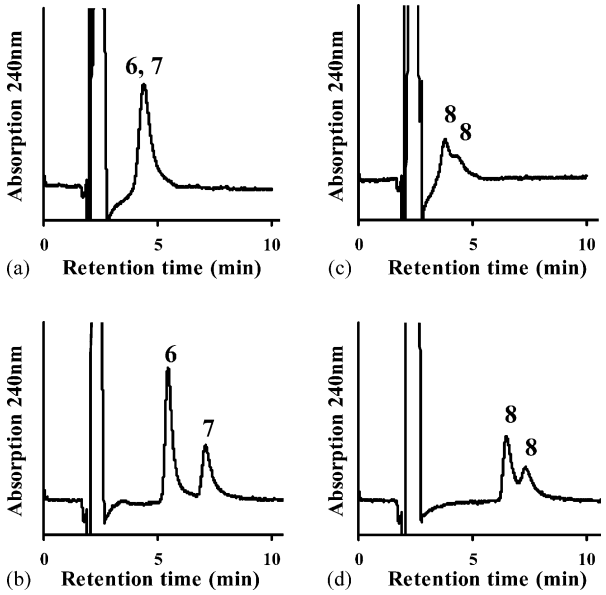

Fig. 4. LC–UV of standards using PNIPAAm-modified silica column at (a) and (c) 10 °C, and (b) and (d) 50 °C. For LC conditions, see Section 2. For peak numbers, see Fig. 1.

and halosulfuron-methyl also overlapped (data not shown). In contrast, upon raising the column temperature of the temperature-responsive system, these five sulfonylurea herbicides could be separated from each other with an aqueous mobile phase.

In this study, the mobile phase was adjusted to pH 3 which was lower than the pK_a values of these herbicides, bensulfuron-methyl (pK_a 5.2), flazasulfuron (pK_a 4.37) and imazosulfuron (pK_a 4.0), in order to suppress their ionization and effect their interaction with the surface of the stationary phase. With increasing temperature, the temperature-responsive surface of the stationary phase changed from hydrophilic to hydrophobic, the retention time increased as a result of hydrophobic interaction, and the separation of the five sulfonylurea herbicides markedly improved.

Table 1
Calibration, LOD and log *P* data for the eight herbicides

Compound	Calibration equation[a]	R^2	LOD (mg l^{-1})	log *P*
Bensulfuron-methyl	$y = 12.493x + 0.6557$	1.000	0.5	1.49
Flazasulfuron	$y = 9.8272x - 0.5951$	0.998	0.5	1.93
Pyrazosulfuron-ethyl	$y = 8.976x - 1.1398$	0.997	0.5	0.66
Halosulfuron-methyl	$y = 12.011x - 1.3876$	0.998	0.5	1.21
Imazosulfuron	$y = 16.043x - 0.951$	1.000	0.5	2.15
Diuron	$y = 20.209x + 0.6761$	0.996	0.5	2.15
Daimuron	$y = 11.74x - 0.2518$	0.995	0.2	3.61
Siduron	$y = 13.661x - 0.2925$	0.999	0.2	2.86

[a] y = area; x = concentration (mg l^{-1}).

3.2. Urea herbicides

The urea herbicides were separated using conditions similar to those for the sulfonylurea herbicides. Fig. 2(b) shows van't Hoff plots for urea herbicides using a PNIPAAm-modified column. For urea herbicides, the ln *k* values increased markedly above the LCST (or lower 1/*T* values), indicating a hydrophobic interaction between the analyte molecules and the hydrophobized stationary phase surface of the column. The difference in retention behavior of the sulfonylurea and urea herbicides reflects differences in their physicochemical properties. Typical chromatograms for the standards of the two urea herbicides, and siduron using the PNIPAAm-modified column at 10 and 50 °C are shown in Fig. 4. Siduron gave two peaks corresponding to its cis/trans isomers. The retention times of urea herbicides also increased with the log *P* values. An increase in the retention times with increasing temperature was clearly observed.

3.3. Analytical performance

The calibration plots of all eight herbicides using temperature-responsive LC at 50 °C were linear. The concentrations range of the five sulfonylurea herbicides were 0.2–10 mg l^{-1} (six data points in triplicate), those of diuron and daimuron were 0.2–2.0 mg l^{-1} (four data points in triplicate), and those of siduron were 0.5–10.0 mg l^{-1} (five data points in triplicate). In all cases, the R^2 values were at least 0.995 (Table 1). Because siduron has two isomers, the area of the two isomer peaks was calculated and summed to give the total amount of siduron. The LODs of the eight herbicides were 0.2–0.5 mg l^{-1} (Table 1).

Table 2
Performance data for extracting five sulfonylureas and three ureas from pure water, tap water and river water

Compound	Pure water			Tap water			River water		
	Recovery[a] (%)	RSD (%)	LOQ ($\mu g\,l^{-1}$)	Recovery[a] (%)	RSD (%)	LOQ ($\mu g\,l^{-1}$)	Recovery[a] (%)	RSD (%)	LOQ ($\mu g\,l^{-1}$)
Bensulfuron-methyl	91	3.6	4	94	2.2	1	88	6.4	4
Flazasulfuron	90	1.9	1	86	1.7	1	72	9.7	4
Pyrazosulfuron-ethyl	93	1.6	1	98	2.5	1	100	5.0	4
Halosulfuron-methyl	90	2.7	1	98	1.1	1	97	4.5	4
Imazosulfuron	86	1.8	1	98	1.8	1	89	6.7	4
Diuron	91	4.5	1	84	6.8	1	97	4.5	1
Daimuron	127	2.8	1	100	5.3	1	94	6.0	1
Siduron	93	2.5	1	87	3.2	4	100	6.0	4

[a] Mean values from three individual samples.

3.4. Application

Water samples were prepared by adding $4\,\mu g\,l^{-1}$ (final concentration) of all herbicides, except for diuron and daimuron, which were added at a final concentration of $1\,\mu g\,l^{-1}$ to pure water, tap water, or river water. Then, 0.5 l of each sample was concentrated 500-fold by SPE. Using temperature-responsive chromatography, these eight herbicides were detected with acceptable recoveries and precisions (70–130% and relative standard deviation, RSD $\leq$10%, respectively) (Table 2).

4. Conclusions

Temperature-responsive LC with an aqueous solution without organic solvents as mobile phase can be used to determine sulfonylurea and urea herbicides. Combined with off-line SPE, trace levels of the herbicide can be quantified in real-life samples.

In temperature-responsive LC, analyte behavior is controlled merely by the temperature, without any changes in the mobile-phase composition.

Acknowledgement

This study was supported by a grant from the Ministry of Health, Labour and Welfare of Japan.

References

[1] E.W. Zahnow, J. Agric. Food Chem. 33 (1985) 479.
[2] A.C. Hogenboom, U.K. Malmqvist, K. Nolkrantz, J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A 759 (1997) 55.
[3] G. Dinelli, A. Vicari, A. Bonetti, P. Catizone, J. Agric. Food Chem. 45 (1997) 1940.
[4] N. Wang, W.L. Budde, Anal. Chem. 73 (2001) 997.
[5] E. Ayano, H. Kanazawa, M. Ando, T. Nishimura, Anal. Chim. Acta 507 (2004) 211.
[6] J.F. Brady, J. Turner, D.H. Skinner, J. Agric. Food Chem. 43 (1995) 2542.
[7] S.L. Sunderland, P.W. Santelmann, T.A. Baughmann, Weed Sci. 39 (1991) 296.
[8] Y.H. Bae, T. Okano, S.W. Kim, J. Polym. Sci. Polym. Phys. 28 (1990) 923.
[9] T. Okano, N. Yamada, H. Sakai, Y. Sakurai, J. Biomed. Mater. Res. 27 (1993) 1243.
[10] T. Shimizu, M. Yamato, A. Kikuchi, T. Okano, Tissue Eng. 7 (2001) 141.
[11] M. Matsukata, T. Aoki, K. Sanui, N. Ogata, A. Kikuchi, Y. Sakurai, T. Okano, Bioconjugate Chem. 7 (1996) 96.
[12] M. Heskins, J.E. Guillet, E. James, J. Macromol. Sci. Chem. A2 (1968) 1441.
[13] H. Kanazawa, K. Yamamoto, Y. Matsushima, Y. Takai, A. Kikuchi, Y. Sakurai, T. Okano, Anal. Chem. 68 (1996) 100.
[14] H. Kanazawa, T. Sunamoto, E. Ayano, Y. Matsushima, A. Kikuchi, T. Okano, Anal. Sci. 18 (2002) 45.
[15] K. Yamamoto, H. Kanazawa, Y. Matsushima, K. Oikawa, A. Kikuchi, Y. Sakurai, T. Okano, Environ. Sci. 7 (2000) 47.
[16] H. Kanazawa, T. Sunamoto, Y. Matsushima, A. Kikuchi, T. Okano, Anal. Chem. 72 (2000) 5961.
[17] C. Sakamoto, Y. Okada, H. Kanazawa, E. Ayano, T. Nishimura, M. Ando, A. Kikuchi, T. Okano, J. Chromatogr. A 1030 (2004) 247.
[18] A.C. Hogenboom, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 794 (1998) 201.
[19] A.C. Hogenboom, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 841 (1999) 33.
[20] K. Yamamoto, H. Kanazawa, Y. Matsushima, N. Takai, A. Kikuchi, T. Okano, Chromatography 209 (2000) 21.