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Liquid–liquid–liquid microextraction of nitrophenols with a hollow fiber membrane prior to capillary liquid chromatography

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Abstract

A simple liquid–liquid–liquid microextraction device utilizing a 2 cm×0.6 mm I.D. hollow fiber membrane was used to preconcentrate nitrophenols from water sample prior to capillary liquid chromatography (cLC) analysis. The extraction procedure was induced by the pH difference inside and outside the hollow fiber. The donor phase outside the hollow fiber was adjusted to pH~1 with HCl; the acceptor phase was NaOH solution used at various concentrations. Organic solvent was immobilized into the pores of the hollow fiber. With stirring, the neutral nitrophenols outside the fiber were extracted into the organic solvent, then back extracted into 2 μl of basic acceptor solution inside the fiber. The acceptor phase was then withdrawn into a microsyringe and injected into the cLC system directly. This technique used a low-cost disposable extraction ''device'' and is very convenient to operate. Up to 380-fold enrichment of analytes could be achieved. This procedure could also serve as a sample clean-up step because large molecules and basic compounds were not extracted into the acceptor phase. The RSD ($n=6$) was less than 6.2%, while the linear calibration range was from 1 to 200 μg/ml with $r>0.998$. The procedure was applied to the analysis of seawater.

Keywords: Liquid–liquid–liquid microextraction; Extraction methods; Hollow-fiber membranes; Water analysis; Environmental analysis; Nitrophenols

1. Introduction

Phenols and substituted phenols are important pollutants in water because of their wide use in many industrial processes such as the manufacture of plastics, dyes, drugs, antioxidants and pesticides [1]. Nitrophenols are formed photochemically in the atmosphere from vehicle exhausts [2]. They are very toxic and have a diverse effect on the taste and odor of drinking water at low concentrations [3,4], so they are environmentally of particular interest and concern. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are the most common analytical techniques used for the determination of phenols [5–8]. In GC, derivatization is needed to analyze phenols in order to avoid peak tailing [6,7,9]. Compared with GC, HPLC is a good alternative technique, in which isocratic or gradient elution can be used to separate these compounds.

Capillary liquid chromatography (cLC) has assumed greater applicability in recent years, and has been used for environmental analysis [10,11] due to its high resolution, small sample volume requirement and comparatively better analyte detectability than conventional LC. San Andres et al. [12] used cLC to analyze phenols in water. A preconcentration step is

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always necessary prior to LC due to the low concentration of phenols in water [12].

Liquid–liquid extraction (LLE) is still used as the standard preconcentration step for the determination of phenols in water [13,14]. Recently, much research has been done in the way of solid-phase extraction (SPE) [15,16] and solid-phase microextraction [17] of phenols from water. However, due to their acidity and polarity, nitrophenols have much lower affinity for non-polar sorbents and their breakthrough volume is small [16]; thus, it is difficult to use SPE for nitrophenols directly.

Recently three-phase microextraction was developed to extract ionizable and chargeable compounds from different aqueous samples. Ma and Cantwell [18,19] used an unsupported liquid organic membrane to separate two aqueous phases, donor phase and acceptor phase. The pH of donor phase was adjusted to basic and the acceptor phase was acidic. An ionizable compound, mephentermine, was extracted from the donor phase to the organic phase, then back extracted to the acceptor phase. The same basic principle was used by Pálmarsdóttir et al. [20]. In the latter study, a hollow fiber was used to support the liquid membrane. Hollow fiber membrane has proved useful for enrichment of ionizable and charged species, giving a high degree of clean-up and enrichment of various analytes in different samples [21]. Pedersen-Bjergaard and co-workers [22–24] extracted some drugs from 2.5-ml aqueous samples through a thin film of organic solvent immobilized inside the pores of a polypropylene hollow fiber tubing and finally into a 25-μl acceptor solution inside the hollow fiber. This method was successfully coupled with capillary electrophoresis (CE) and HPLC.

In the present work, a simple liquid–liquid–liquid microextraction (LLLME) device was set up to enrich nitrophenols from water using polypropylene hollow fibers as the membrane. Different aspects of the extraction procedure such as the kinds of organic solvent for the immobilization; compositions of the acceptor and donor phase, the extraction time, and magnetic stirring speed were investigated. In addition, this method was validated for quantitative purposes and applied to seawater analysis by cLC.

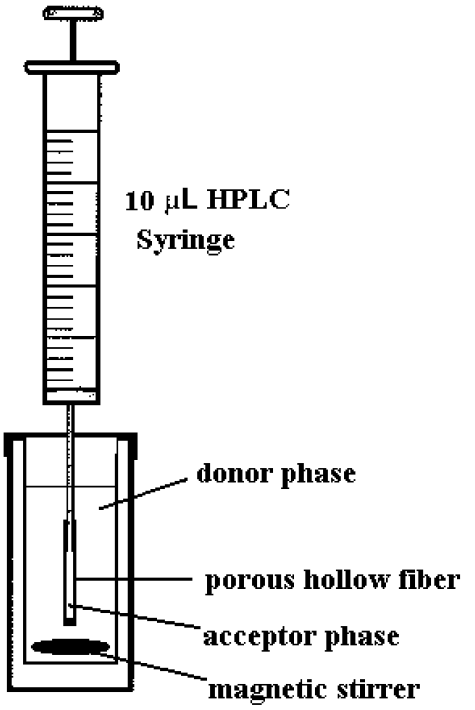

Fig. 1. Schematic of the LLLME extraction device (not to scale).

2. Experimental

2.1. Liquid–liquid–liquid microextraction

The experimental set-up is illustrated in Fig. 1. The sample solution was placed in a 4-ml sample vial. A 10-μl HPLC syringe (Hamilton, Australia) of 0.8 mm O.D. was used to introduce acceptor phase, support the hollow fiber and also acted as an injection syringe at the same time. Because the extraction units should be compatible with both aqueous solutions and a broad range of organic solvents, polypropylene was selected as the material for the porous hollow fiber. One end of the Q3/2 Accurel polypropylene hollow fiber (Membrana, Wuppertal, Germany) was flame-sealed. The total length of the fiber was 2.0 cm. The inner diameter of the hollow fiber was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.64 μm.

Extractions were performed according to the following procedure: a 2.5-ml aliquot of sample solution (to which varying concentrations of HCl were

added) was added to the vial, and a 12×4 mm magnetic bar was placed into the solution to ensure efficient stirring during the extraction. A hollow fiber, with a sealed end, was sonicated for 2 min in acetone to remove any contaminants in the fiber. It was removed from acetone, and the solvent was allowed to evaporate completely. A 2-μl volume of acceptor phase (to which varying concentrations of NaOH were added) was withdrawn using a syringe. The syringe was then inserted into the hollow fiber, and the acceptor solution was introduced into the fiber. The fiber was then immersed in the organic solvent for 10 s for impregnation. The organic solvent filled the pores of the hollow fiber wall. After impregnation, the fiber (together with the syringe) was put into the donor phase. Magnetic stirring utilizing a MR3001K hotplate (Heidolph, Kelheim, Germany) was used during the extraction. After extraction, the syringe-fiber assembly was taken out of the solution. A 1-μl volume of acceptor solution was withdrawn from the fiber, and then injected into the cLC system.

2.2. cLC system

Chromatography was carried out with a Shimadzu LC-9A pump (Shimadzu, Tokyo, Japan), a Shimadzu C-R6A integrator and an Applied Biosystems 785A (Foster City, CA, USA) variable-wavelength UV–Vis programmable absorbance detector (operated at 235 nm) equipped with an LC Packings (San Francisco, CA, USA) ''UZ'' capillary flowcell (8 mm× 75 μm I.D.×280 μm O.D.). An LC Packings Acurate flow-rate processor (with a split ratio 1:70) was connected to the LC-9A pump to generate an eluent of 3–4 μl/min flow-rate. An LC Packings capillary C_{18} column (15 cm×300 μm I.D., 3 μm particle size) column was used for separation. The injection volume was 100 nl. The mobile phase was acetonitrile–water (40:60) with 75 m*M* sodium acetate (NaAc)–glacial acetic acid (HAc).

2.3. Reagents and standards

HPLC-grade acetonitrile was bought from EM Science (Gibbstown, NJ, USA); NaAc, HAc, 1-octanol, isooctane and *n*-hexane were bought from Merck (Darmstadt, Germany). Nonane, octanone, hexane, butyl acetate and methylene chloride were supplied by Fisher (NJ, USA). Ultrapure water was produced on a Nanopure system (Barnsted, Dubuque, IA, USA). 2,4-Dinitrophenol (2,4-DN, pK_a 4.09), 2-nitrophenol (2-N, pK_a 7.22), 3-nitrophenol (3-N, pK_a 8.36), 3,4-dinitrophenol (3,4-DN, pK_a 5.42) and 4-nitrophenol (4-N, pK_a 7.15) were supplied by Supelco (Bellefonte, PA, USA). They were dissolved in methanol at 1 mg/ml concentration as stock solutions. Mixtures containing each nitrophenol at different concentrations in 0.1 *M* NaOH were prepared from the stock solution and used as working solutions. A 500-ml surface seawater sample was collected and filtered through a 0.45-μm membrane before use.

3. Results and discussion

3.1. Ion-pair effect in the mobile phase on the separation

Generally, due to the high efficiency of the cLC column, the five nitrophenols in water could be baseline separated isocratically using acetonitrile–water (40:60) as mobile phase. Under this condition, most of the nitrophenols were eluted out in the neutral states. However, the acceptor solution was made up of NaOH at varying concentrations. The nitrophenols exist mainly as ions in the basic matrix. Thus when the seawater extract was injected into the column directly, the chromatograms were completely different from that of the standard mixture in deionized water. In order to address this issue, some NaAc–HAc buffer solution (pH 3.5) was added to the mobile phase. The buffer in the mobile phase neutralizes the NaOH at the injection point due to its buffering capacity, and the ionic nitrophenols in the extract revert to the neutral state, so the difference between samples in different matrix is significantly eliminated. The effect of concentration of the buffer on the separation is shown in Fig. 2. With no buffer in the mobile phase, the five nitrophenols were eluted out almost at the same time with NaOH; as the concentration of buffer in the mobile phase was increased, the chromatogram of nitrophenols in the

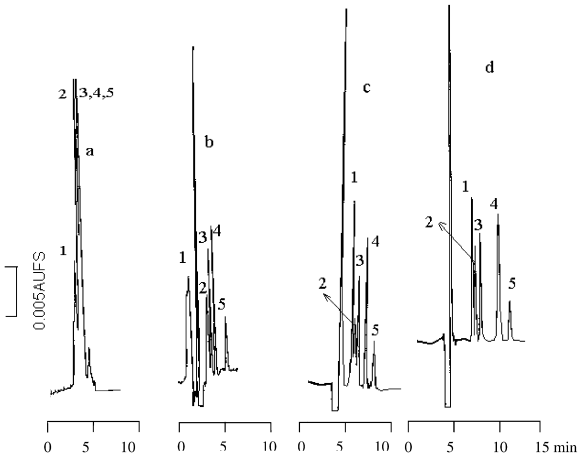

Fig. 2. The effect of concentration (C) of buffer (NaAc–HAc, pH 3.50) in the mobile phase on the separation. Mobile phase: MeCN–water (40:60); (a) C=0 mM; (b) C=5 mM; (c) C=50 mM; (d) C=75 mM; peaks: 1=2,4-DN; 2=4-N; 3=3-N; 4=3,4-N; 5=2-N.

NaOH matrix became more and more similar to that in water, and the resolutions improved, as shown in Table 1. When the concentration of buffer was increased to 75 mM, the chromatograms of the standards in 0.1 M NaOH and in water were identical. Thus, 75 mM buffer in acetonitrile–water (40:60) (pH 3.50) was used as the mobile phase.

3.2. The effect of organic solvent

The type of solvent immobilized within the pores of the hollow fiber is of high importance in order to achieve efficient analyte preconcentration. Several kinds of organic solvents, 1-octanol, isooctane, nonane, octanone, hexane, butyl acetate and methylene chloride were selected to study their effect on enrichment. When butyl acetate and methylene chloride were used, no compounds could be enriched, these may be because of their relative solubility in water. Isooctane and nonane could only extract 2,4-dinitrophenol and 2-nitrophenol; *n*-hexane could extract 2,4-dinitrophenol and 2-nitrophenol and 3-nitrophenol. Only 1-octanol could extract all five nitrophenols and the enrichment factor (EF) was relatively higher than any other organic solvent studied. This is probably due to its relatively higher polarity and its greater affinity to the nitrophenols. On the basis of the immobilization experiments, 1-octanol was selected for the rest of the study.

Table 1
Effect of buffer concentration on the resolutions of the nitrophenols

Resolution	C=0 mM	C=5 mM	C=50 mM	C=75 mM
$R_{1,2}$	0.24	2.94	0.43	0.70
$R_{2,3}$	0.59	1.08	1.18	1.40
$R_{3,4}$	0.00	1.05	1.70	4.16

C is the concentration of NaAc–Hac buffer in the mobile phase. The resolutions of the first three pairs of peaks, which are most difficult to separate, are displayed. (1) 2,4-DN; (2) 4-N; (3) 3-N; (4) 3,4-N.

3.3. Compositions of donor and acceptor phase

The compositions of donor and acceptor phases are very important parameters that effect the efficiency in LLLME. With the hollow fiber impregnated with 1-octanol, a series of experiments were conducted to optimize the compositions leading to pH changes of both the donor and acceptor solutions. For all of the experiments, the microextraction was accomplished for 20 min at 400 rpm stirring speed with HCl in the donor phase and NaOH in the acceptor phase. For the donor phase, the concentration of HCl was varied between 0.01 and 1.0 M; at the same time, the concentration of NaOH also varied between 0.01 and 1.0 M. The results are shown in Table 2.

On the whole, the EF was greater when 0.5 M NaOH was used in the acceptor phase because the higher concentration NaOH has bigger absolute buffer capacity within the small volume of acceptor solution. 1.0 M HCl in the donor phase and 0.5 M NaOH in the acceptor phase provided the highest enrichment factor for nitrophenols except for 2,4-DN. However 0.5 M NaOH is too basic for the capillary C_{18} column. Also, a lower concentration of NaOH, e.g., 0.1 M NaOH, was suitable for the extraction of 2,4-DN due to its low pK_a value (lowest of the pK_a values of all the five nitrophenols). For both stability and practical reasons, 0.1 M NaOH was selected as the optimum concentration. NaCl was added to the donor solution to study the possibility of salting-out effect. No significant in-

Table 2
Effect of compositions of donor and acceptor phases on the enrichment factor

		Enrichment factor			
		0.01 *M* NaOH	0.1 *M* NaOH	0.5 *M* NaOH	1.0 *M* NaOH
0.01 *M* HCl	2,4-DN	82.0	32.2	51.5	50.9
	4-N	58.5	49.5	50.9	61.6
	3-N	59.2	45.4	100	79.7
	3,4-DN	137	57.9	97.8	99.1
	2-N	68.3	57.5	82.8	78.4
0.1 *M* HCl	2,4-DN	82.7	80.5	83.2	50.0
	4-N	39.3	102	114	68.4
	3-N	27.8	103	125	82.9
	3,4-DN	80.0	129	115	108
	2-N	46.6	126	121	64.8
0.5 *M* HCl	2,4-DN	62.8	67.5	57.0	58.5
	4-N	29.3	69.2	121	87.8
	3-N	28.4	68.7	133	113
	3,4-DN	35.6	28.4	122	115
	2-N	30.4	32.5	124	86.8
1.0 *M* HCl	2,4-DN	25.2	27.3	59.0	50.1
	4-N	31.2	30.8	134	70.0
	3-N	33.4	31.4	152	100
	3,4-DN	38.3	35.3	137	88.9
	2-N	29.8	21.5	184	88.0

Extraction conditions: concentration of each standard 10 ng/ml; organic solvent for impregnation: 1-octanol; extraction time 20 min; extraction stirring speed 400 rpm.

crease in enrichment was achieved when 5%, 10% and 20% NaCl was added.

3.4. Effect of extraction time

Mass transfer is a time-dependent process, so the function of extraction time was studied here. The extraction experiment was performed on a standard mixture solution in 0.1 *M* HCl (10 ng/ml of each nitrophenol). The acceptor phase was 0.1 *M* NaOH, the impregnation solvent was 1-octanol, and the stirrer speed was fixed at 400 rpm. The result is shown in Fig. 3. Because the extraction time affected the five nitrophenols similarly, only the data for 3-N are illustrated in the figure for simplicity. The enrichment factor increased rapidly with the extraction time before 50 min, but very slowly after 50 min. Based on this, 50 min was selected as the optimum extraction time. Although the extraction time was relatively long, a large number (10–15) of samples may be extracted simultaneously due to the simplicity and the low cost of the extraction device.

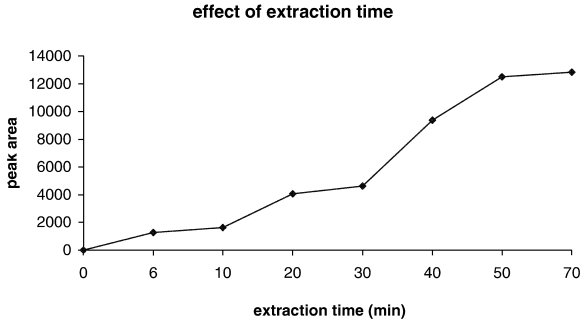

Fig. 3. The effect of extraction time on analyte enrichment using 3-N as an example. Conditions: 1-octanol as the impregnation solvent; 0.1 *M* HCl in the donor phase and 0.1 *M* NaOH in the acceptor phase; stirring speed 400 rpm.

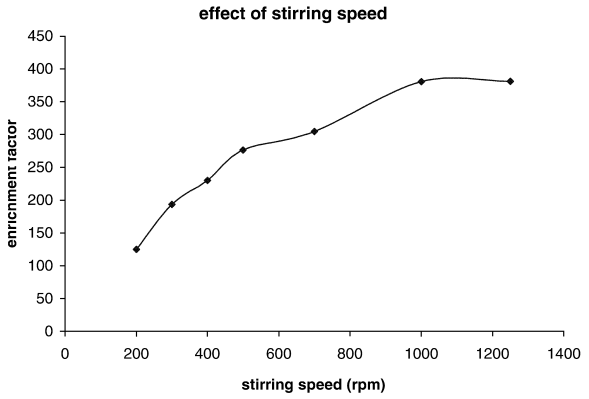

Fig. 4. The influence of magnetic stirring speed using 3,4-DN as an example. Conditions: 1-octanol as the impregnation solvent; 0.1 *M* HCl in the donor phase and 0.1 *M* NaOH in the acceptor phase; extraction time 50 min.

3.5. Effect of stirring speed

Magnetic stirring was used to facilitate the mass transfer process and the extraction efficiency. The stirring speed was also optimized for the extraction. The extraction experiment was performed on the aforementioned standard mixture solution under the optimum conditions mentioned above. Fig. 4 illustrates the effect of extraction stirring speed on the enrichment factor using 3,4-DN as an example. The enrichment factor increased with the stirring speed and leveled out from 1000 rpm. From this, LLLME with a hollow fiber as a supporter is superior to LLLME with a liquid drop at the end of needle [19], during which the drop is liable to be lost under great agitation. The acceptor solution was contained and protected by the hollow fiber. The operation is easier to handle and can tolerate high speed agitation. The latter factor helps to improve the stability and repeatability of the extraction.

3.6. Extraction efficiency

On the basis of the experiments discussed above, optimum LLLME of nitrophenols was obtained by utilizing a 2 cm porous hollow fiber immobilized with 1-octanol, an acceptor solution of 0.1 *M* HCl, a donor solution of 0.1 *M* NaOH, under a stirrer speed 1000 rpm and with an extraction time of 50 min. Under these optimum extraction conditions, the enrichment factor could be high as 380-fold, as shown in Table 3.

3.7. Quantitative aspects

To evaluate the practical applicability of the proposed LLLME, repeatability, linearity and limits of detection were investigated using the optimum conditions. The performance of this method is shown in Table 3. It can be seen that the RSD was smaller than 6.2% based on the peak areas for six replicates. The linearity was evaluated within the range 1–200 ng/ml. Each analyte exhibited good linearity with regression coefficient, $r>0.9986$. The limits of detection (LODs) for the nitrophenols were calculated at a signal-to-noise ratio of 3. The wide linear range combined with the low detection limit suggests a high potential for monitoring the nitrophenols in water samples.

Table 3
Performance of LLLME

Compound	Enrichment factor	RSD (%) ($n=6$)	Linear range (ng/ml)	LOD (ng/ml)	Recovery (%)	
					2 ng/ml[a]	10 ng/ml[a]
2,4-DN	236	6.23	1.0–200	0.5	91.2	100.2
4-N	339	2.03	1.0–200	0.5	93.2	99.8
3-N	325	3.58	1.0–200	0.5	92.5	98.9
3,4-DN	380	1.28	1.0–200	0.5	95.3	100.5
2-N	360	6.01	2.0–200	1.0	90.8	98.6

LLLME conditions: 0.1 *M* HCl in donor phase, 0.1 *M* NaOH in acceptor phase, extraction time 50 min; extraction stirring speed 1000 rpm.

[a] The final concentration of each analyte after spiking in seawater.

3.8. Real-world water sample analysis

To evaluate this LLLME technique in the real world, a surface seawater sample was analyzed using the above method. The concentration of HCl in the seawater sample (30 ml) was made up to 0.1 *M* using concentrated HCl. A 2.5-ml volume of this acidified solution was placed in the sample vial and was extracted using 0.1 *M* NaOH for 50 min. As illustrated in Fig. 5a, no target nitrophenols could be found in the original seawater sample extract (although several other peaks were present, probably due to other phenolic compounds or organic acids present in seawater). The seawater sample was then spiked with nitrophenols such that one sample contained 2 ng/ml each of the analyte, and the other contained 10 ng/ml of the analytes; the respective extracts generated the chromatogram shown in Fig. 5b. The recoveries from both spiked sample were >90%, as shown in Table 3. It is clear that this method is applicable to real word aqueous sample.

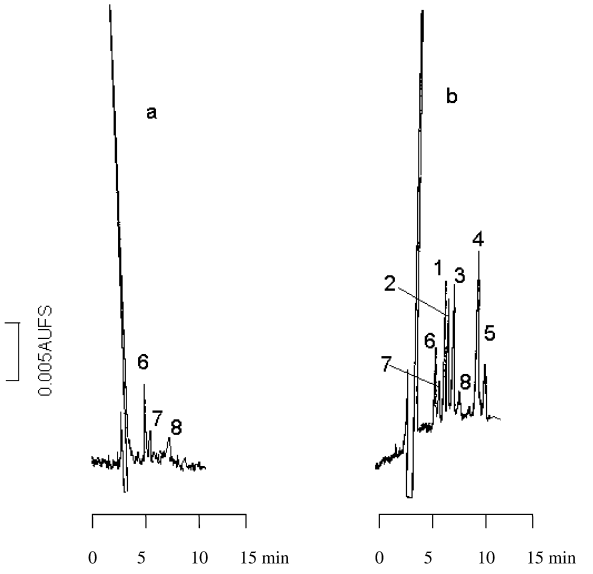

Fig. 5. Capillary liquid chromatograms of seawater sample after extraction. (a) Extract from 2.5 ml seawater sample; (b) extract from seawater sample after spiking (final concentration 2 ng/ml of each analyte). Conditions: 1-octanol as the impregnation solvent; 0.1 *M* HCl in the donor phase and 0.1 *M* NaOH in the acceptor phase; extract for 50 min with stirring speed 1000 rpm. Peaks: 1=2,4-DN; 2=4-N; 3=3-N; 4=3,4-N; 5=2-N; 6, 7, 8= possible phenolic compounds or organic acids extracted from seawater sample.

4. Conclusion

This study has demonstrated the high performance of LLLME of nitrophenols from water samples utilizing a porous polypropylene fiber. Compared to the device set up by Pedersen-Bjergaard and Rasmussen [22], only one syringe was needed, and the volume of acceptor phase was decreased to 2 μl. Up to 380-fold enrichment factor and effective sample clean-up was obtained. Due to the simplicity and the low cost of the extraction device, the hollow fiber can be discarded after each extraction to avoid carryover and cross-contamination. This serves to maintain high reproducibility and repeatability. The whole operation is very convenient to handle because the acceptor phase was contained and protected by the hollow fiber. We have shown this to be an effective method to enrich nitrophenols from water samples prior to cLC analysis.

Acknowledgements

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References

[1] J.W. Moore, S. Ramamoorthy, Organic Chemical in Natural Waters; Applied Monitoring and Impact Assessment, Springer, New York, 1984.
[2] J. Tremp, P. Mattrel, S. Fingler, W. Giger, Water Air Soil Pollut. 68 (1993) 113.
[3] P.J. Vuorinen, Chemosphere 14 (1985) 1729.
[4] P.A. Reanlini, J. Chromatogr. Sci. 19 (1981) 124.
[5] M.R. Lee, Y.C. Yeh, W.S. Hsiang, B.H. Hwang, J. Chromatogr. A 806 (1998) 317.
[6] T. Heberer, H. Stan, Anal. Chim. Acta 341 (1997) 21.
[7] D. Jahr, Chromatographia 47 (1998) 49.
[8] K.K. Chee, M.K. Wong, H.K. Lee, Mikrochim. Acta 126 (1997) 97.
[9] I. Rodriguez, M.C. Mejuto, M.H. Bollaín, R. Cela, J. Chromatogr. A 786 (1997) 285.
[10] W.P. Liu, H.K. Lee, Talanta 45 (1998) 631.

[11] A. Cappiello, G. Famiglini, A. Berloni, J. Chromatogr. A 768 (1997) 215.
[12] M.P. San Andres, M.E. Leon-Gonzalez, L.V. Perez-Arribas, L.M. Polo-Diez, J. High Resolut. Chromatogr. 23 (2000) 367.
[13] I. Turnes, I. Rodríguez, C.M. García, R. Cela, J. Chromatogr. A 743 (1996) 283.
[14] H. Lee, T.E. Peart, R.L. Hong-You, J. Chromatogr. 636 (1993) 263.
[15] S. Fingler, V. Drevenkar, Z. Vasilic, Mikrochim. Acta 11 (1987) 163.
[16] M.T. Galceran, O. Jáuregui, Anal. Chim. Acta 304 (1995) 75.
[17] M.P. Llompart, B. Blanco, R. Cela, J. Microcol. Sep. 12 (2000) 25.
[18] M.H. Ma, F.F. Cantwell, Anal. Chem. 70 (1998) 3912.
[19] M.H. Ma, F.F. Cantwell, Anal. Chem. 71 (1999) 388.
[20] S. Pálmarsdóttir, E. Thordarson, L.E. Edhom, J.Å. Jönsson, L. Mathiasson, Anal. Chem. 69 (1997) 1732.
[21] Y.Z. Luo, J. Pawliszyn, Anal. Chem. 72 (2000) 1058.
[22] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[23] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H.G. Ugland, T. Gronhaug, J. Chromatogr. A 873 (2000) 3.
[24] S. Pedersen-Bjergaard, K.E. Rasmussen, Electrophoresis 21 (2000) 579.