ELSEVIER

Journal of Chromatography A, 955 (2002) 183–189

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Determination of sulfonylurea herbicides by continuous-flow liquid membrane extraction on-line coupled with high-performance liquid chromatography

Jing-bo Chao[a,b], Jing-fu Liu[a], Mei-juan Wen[b], Jie-min Liu[a], Ya-qi Cai[a], Gui-bin Jiang[a,*]

[a]*Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China*

[b]*Department of Chemistry, Beijing University of Science and Technology, Beijing 100083, China*

Received 25 July 2001; received in revised form 29 January 2002; accepted 31 January 2002

Abstract

On-line coupling continuous-flow liquid membrane extraction (CFLME) with HPLC, a novel automatic system was developed for the determination of sulfonylurea herbicides in water. After an automatic trace-enrichment process by CFLME, which is the combination of continuous flow liquid–liquid extraction and support liquid membrane (SLM) extraction, the target analytes were concentrated in 50 μl of 0.2 *M* Na_2CO_3–$NaHCO_3$ (pH 10.0) buffer. The concentrated sample solutions were injected directly onto a C_{18} analytical column with a valve, and detected at 240 nm with a diode array detector. Metsulfuron methyl (MSM), and DPX-A 7881 were baseline separated with a mobile phase consisting of methanol and 67 m*M* KH_2PO_4–Na_2HPO_4 (pH 5.91) buffer (45+55, v+v) at a flow-rate of 1.0 ml min^{-1}. With an enrichment time of 10 min and enrichment sample volume of 20 ml, the enrichment factors and detection limits are 100 and 0.05 μg l^{-1} for MSM, and 96 and 0.1 μg l^{-1} for DPX-A 7881, respectively. The linear range and precision (RSD) are 0.1–50 μg l^{-1} and 7.0% for MSM, and 0.2–50 μg l^{-1} and 9.2% for DPX-A 7881, respectively. This proposed method was applied to determine MSM and DPX-A 7881 in seawater, tap water, and bottled mineral water with spiked recoveries in the range of 83–95% for MSM and 88–100% for DPX-A 7881, respectively.

Keywords: Continuous-flow liquid membrane extraction; Trace enrichment; Sulfonylureas; Pesticides

1. Introduction

Sulfonylurea herbicides are widely used for controlling weeds in crops. They are potent and highly active weed killers, which were introduced as a class of herbicides in the 1980s [1]. As they are very specific to the target organisms, sulfonylurea herbicides are used in doses that are 100–1000 times smaller than for conventional herbicides (ca. 2 g/ha) [2]. From the chemical point of view, they are thermal labile and weakly acidic compounds that breakdown rapidly in the environment.

The low use rate and their extensively decomposition present a challenge for the determination of these herbicides in recipient waters. Clean-up and enrichment before analysis are necessary and become a crucial step for determination of these compounds. A number of sample pretreatment methods for the enrichment of sulfonylurea herbicides have been reported.

Liquid–liquid extraction (LLE), a classic tech-

*Corresponding author. Fax: +86-10-6292-3563.

E-mail address: gbjiang@mail.rcees.ac.cn (G.-b. Jiang).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00109-7

nique of sample pretreatment, has been used to enrich sulfonylurea herbicides [3]. Many efforts have been made to overcome the drawbacks of LLE, such as emulsion formation and large organic solvent consumption.

Solid phase extraction (SPE) has large enrichment capacity and is widely used for the extraction of sulfonylurea herbicides from various aqueous samples [1,4–9]. Although in these procedures, however, SPE was usually carried out off-line, on-line operation does not create any problems, as has frequently been reported for related phenylureas [10,11]. Nevertheless, further study of an alternative procedure is of interest.

Supported liquid membrane (SLM) extraction has been used for the trace-enrichment of sulfonylurea herbicides and coupled with liquid chromatography on-line [2,12,13]. Briefly, a polytetrafluoroethylene (PTFE) membrane impregnated with an organic solvent (di-*n*-hexyl ether) is mounted between two flat blocks, in which grooves are machined, to form an acceptor channel and a donor channel. Samples were acidified and introduced into the donor channel where the sulfonylurea herbicides are transported through the membrane and are trapped in the acceptor. After a certain time, typically 30 min, the acceptor with the enriched sulfonylurea herbicides was removed onto a pre-column to be enriched for a second time. Then, the analytes were injected onto the analytical column for separation. With an enrichment sample volume of 250 ml and an enrichment time of 5 h, the detection limits obtained were 50–100 ng l^{-1} [12].

The advantages of SLM are that it provides high enrichment factors and a high degree of clean-up, low consumption of organic solvents, and can be conveniently coupled on-line with chromatographic and spectroscopic instruments [14]. To obtain a stable SLM system, the organic solvent used as liquid membrane should be non-polar and low volatility. On the other hand, however, polar organic solvents are preferable to obtain high enrichment rates. This gives a dilemma in the choice of organic solvents, and *n*-undecane, di-*n*-hexyl ether, or tri-*n*-octyl phosphate were commonly adopted as a compromise.

In our previous work [15], a novel aqueous–organic–aqueous extraction technique that we termed continuous-flow liquid membrane extraction (CFLME) was developed for trace enrichment. CFLME is the combination of continuous-flow liquid–liquid extraction (CFLLE) and supported liquid membrane (SLM) extraction. In this CFLME procedure, the analyte is first transformed to a neutral molecular phase and is extracted into the organic phase in the CFLLE step, then it is transported onto the organic liquid membrane that formed on the surface of the micro porous membrane of the SLM equipment. Finally, it diffuses through the liquid membrane and is back-extracted into the acceptor as an ionic state to prevent its re-entering into the membrane. If the acceptor remains stagnant, the analyte can be effectively trapped and thus enriched in the acceptor.

CFLME can overcome some drawbacks of the SLM. As organic solvents are continuously flowing in the system, it avoids the danger of membrane breakthrough thus the liquid membrane is always stable. On the other hand, as polar and volatile organic solvents can be used as liquid membrane, this method provides a wide range of selecting polar organic solvents that may give higher enrichment rates than the conventional SLM extraction.

The objective of this work was to develop a simple and automatic system, by on-line coupling of CFLME with high-performance liquid chromatography, for the determination of sulfonylurea herbicides. With an enriched sample volume of 20 ml and an enrichment time of 10 min, this proposed method gave a detection limit of 0.05–0.1 μg l^{-1} for the two kinds of sulfonylurea herbicides studied.

2. Experimental

2.1. Apparatus and materials

A schematic diagram of the system is shown in Fig. 1. P1 and P2, the two peristaltic pumps of the FIA 5020 Analyzer (Tecator, Sweden), are employed to deliver aqueous solutions. Organic solvents (O) were pumped by a piston pump (P3) of a FI-3000 flow injection analyzer (SNK, Japan). A six-port valve (V1) was adopted to manually control the entrance of the mixture of sample solution, reagent and organic solvent, into the donor of the SLM

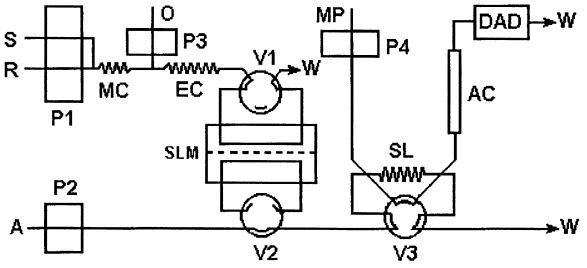

Fig. 1. Schematic diagram of the experimental set-up. See text for detail. The flow-rates were: sample (S), 2.0 ml min^{-1}; H_2SO_4 (R), 0.4 ml min^{-1}; dichloromethane (O), 0.05 ml min^{-1}; 0.2 *M* Na_2CO_3–$NaHCO_3$ (pH 10.0) buffer (A), 0.8 ml min^{-1}; mobile phase (MP), 1.0 ml min^{-1}.

device. Another six-port valve (V2, equipped in the FIA 5020 Analyzer) was used to switch the acceptor to enter the acceptor channel of the SLM device. A 7725 injector (Rheodyne, USA) equipped with a laboratory-made PEEK sample loop (60 μl) was used as sample injector. The mixing coil (MC, 30 cm×0.5 mm I.D.) and extraction coil (EC, 240 cm×0.5 mm I.D.) were made of PTFE tubing and the T-shaped three-way connectors were also made from PTFE.

The custom-made SLM device consisted of a PTFE holder, PTFE membrane and aluminum backer. The PTFE holder was machined from two blocks of Teflon by cutting two grooves on the opposite faces of the two blocks. The grooves have identical dimensions of 0.5 mm deep, 2.0 mm wide and 50 mm long. The PTFE membrane was clamped tightly and evenly between the planar surfaces of the blocks by six screws. Thus, two channels (the acceptor channel and the donor channel) with the same volume of 50 μl were obtained. To make the device more rigid, each PTFE block was backed by an aluminum block. The membranes used as support for the organic liquid film are Fluoropore FG PTFE membrane (average pore size 0.2 μm, porosity 0.7; Millipore, Bedford, MA, USA).

The liquid chromatographic separation was performed with a system that consisted of an SCL-10Avp system controller (Shimadzu, Japan), an LC-10ATvp pump (Shimadzu, Japan), and an SPD-M10Avp diode array detector (DAD, Shimadzu, Japan) set at 240 nm wavelength for detection. Data acquisition and processing were accomplished with a Class-VP Workstation (Shimadzu, Japan). The analytical column was a 150 mm×6.0 mm I.D. C_{18} column (Shim-pack CLC-ODS, 5-μm particles).

2.2. Operation of the system

The whole procedure of system operation consisted of three steps. In the sample enrichment procedure as shown in Fig. 1, sample solution (S) and diluted sulfuric acid (R) were introduced into the system by P1, and mixed in MC to transform the sulfonylurea herbicides into neutral form. Then the mixture was segmented with dichloromethane (O) delivered by P3. Analytes were extracted into dichloromethane in EC and the segments of aqueous and organic solvent were introduced into the donor side of the SLM by switching V1 at sampling position. V2 was switched at the bypass position while the sample was introduced into the SLM device, so the acceptor solution, delivered by P2, remained stagnant in the acceptor channel and the analytes were trapped in it. When the analytes were enriched in the acceptor, the mobile phase was directly delivered into the analytical column.

After a certain time, typically 10 min, V1 was switched to the bypass position and V2 was switched to the injection position to introduce the analytes that had been enriched in the acceptor channel to the injector V3 setting at loading position. Fifteen seconds later, P2 stopped automatically to insure the analytes injected were trapped in the sampling loop (SL) completely.

The final step is the injection step. When P2 stopped, V3 was switched to the injection position to introduce the analytes onto the analytical column for separation and finally detected by the DAD detector.

While the analytes were being separated on the analytical column, the next enrichment could be processed simultaneously.

2.3. Chemicals

Sulfonylurea herbicide standards, metsulfuron methyl (MSM, 98.5%) and ethametsulfuron (DPX-A 7881, 90%), were obtained from Tianjin Pesticides Factory (Tianjin, China). Stock solutions of MSM and DPX-A 7881 were prepared by dissolving 5 mg of standards in 50 ml of 10% CH_3OH and pure

CH_3OH, respectively. Working solutions were obtained daily by appropriately diluting the stock solutions with water. Standard stock solutions were stored at 4 °C. Methanol (Guarantee Grade), dichloromethane, acetic acid, sulfuric acid, anhydrous sodium acetate, anhydrous sodium carbonate, sodium hydrogen carbonate, sodium hydrogen, and di-sodium hydrogen phosphate were purchased from Beijing Chemicals Corporation and were analytical grade except when specified. De-ionized water was used throughout.

2.4. Sample collection

Tap water was collected from the laboratory after flow of about 5 min in order to eliminate the sediment and gas pockets; two kinds of bottled drinking mineral water were purchased from the market with different brands; seawater was collected from Bering Sea at the Arctic Pole. Before enrichment, tap water and seawater were filtered with a 0.45-μm Micropore membrane. The mobile phase was also filtered with a 0.45-μm Micropore membrane.

3. Results and discussion

3.1. Optimization of the chromatographic step

For identification and optimization of the retention time of the two analytes, single standard or mixed standard solution, diluted with acceptor, of 100 μg l^{-1} of MSM and DPX-A 7881 was directly injected onto the analytical column. Good separation was achieved using a mobile phase consisting of methanol and 67 m*M* KH_2PO_4–Na_2HPO_4 (pH 5.91) buffer (45+55, v+v) at a flow-rate of 1.0 ml min^{-1}, and detecting at 240 nm. The two analytes were eluted in 12 min and the retention times of MSM and DPX-A 7881 were about 5.4 and 11.1 min, respectively. Then, the CFLME enrichment unit was coupled on-line with the chromatography separation system. Experiments showed that there were no significant differences for the retention times of the two analytes.

Typical chromatograms of the mixed standard solution of 0.2 μg l^{-1} MSM and 0.4 μg l^{-1} DPX-A 7881, bottled mineral water blank, and the bottled mineral water spiked with 0.2 μg l^{-1} MSM and 0.4 μg l^{-1} DPX-A 7881 are shown in Fig. 2.

3.2. Parameters for CFLME

In our previous study [15], some parameters for CFLME of sulfonylurea herbicides, including flow-rates and coil length of the flow system, concentration of sulfuric acid, physical parameters of PTFE membrane, and kinds of organic solvents used as liquid membrane, were optimized and results are shown in Fig. 1.

3.3. Recoveries of analytes in real samples

Sulfonylurea herbicides, which are weak acids, are extracted into the organic phase as neutral molecular form and back-extracted into the acceptor in this proposed CFLME procedure. Thus, target analytes were first transformed to neutral molecular form by acidifying samples to diffuse through the liquid membrane, and then trapped as the ionic state to prevent its re-entering into the membrane using alkaline acceptor. Therefore, the pH of the acceptor has a crucial effect on the efficient trapping of analytes.

In our previous study [15], the influence of the acceptor pH was studied using standard solutions and 0.1 *M* Na_2HPO_4–NaOH buffer (pH 12.0) was selected as optimum. When real samples such as tap water or natural waters were analyzed however, the coexisting substances such as humic acid will be extracted together with the target analytes, which resulted in the decrease of acceptor pH and thus influences the recoveries of sulfonylurea herbicides. Therefore, it is necessary to study the influence of buffer (acceptor) concentration and pH on the recovery of sulfonylurea herbicides in real samples.

Considering that a low concentration of acceptor is beneficial for the analytical column, 0.025 *M* Na_2HPO_4–NaOH buffer (pH 12.0) was used as acceptor at first in this study. Unfortunately, when the samples such as tap water, mineral water and seawater that were spiked with 10 μg l^{-1} of MSM and 20 μg l^{-1} of DPX-A 7881 standard solutions were analyzed, the recoveries of these two analytes

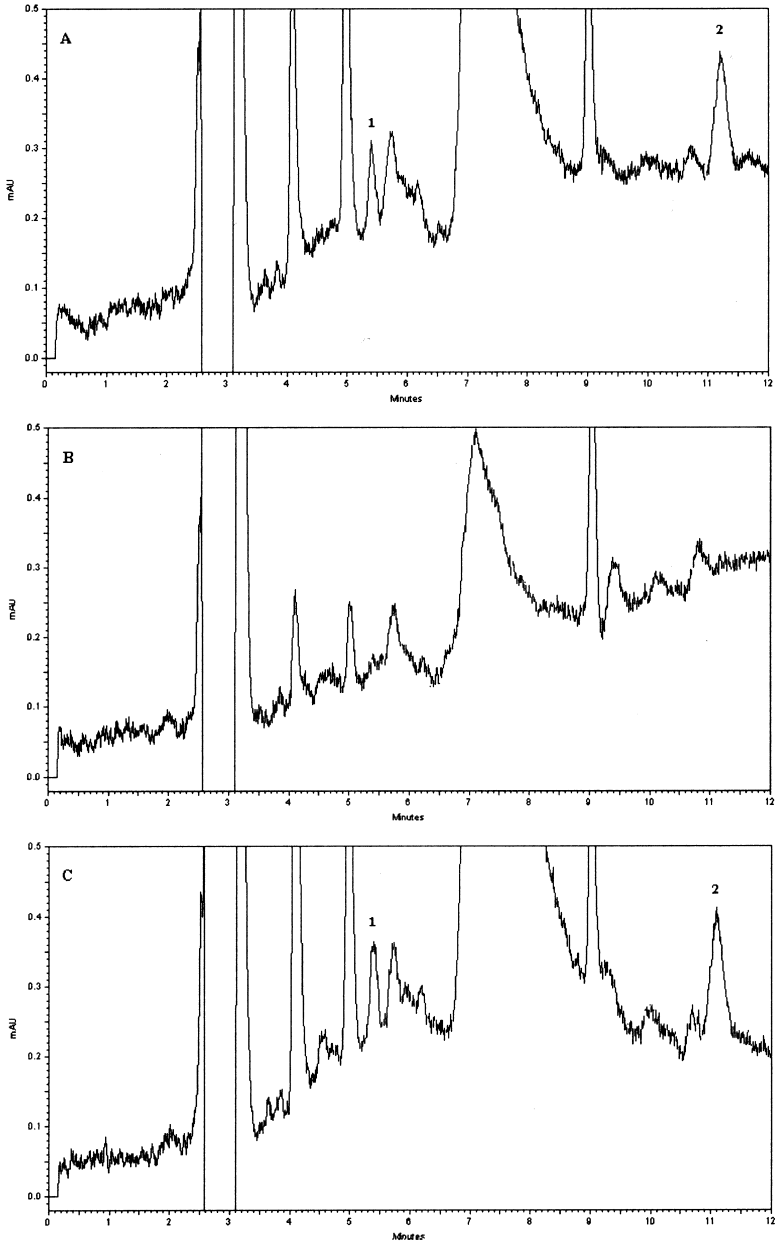

Fig. 2. Typical chromatograms obtained by the proposed method with an enrichment time of 10 min. (A) Standard solution consisting of 0.2 μg l^{-1} MSM and 0.4 μg l^{-1} DPX-A 7881. (B) Bottled mineral water blank. (C) Bottled mineral water spiked with 0.2 μg l^{-1} MSM and 0.4 μg l^{-1} DPX-A 7881. Peaks identified as: 1, MSM; 2, DPX-A 7881.

were much lower than expected (42% of MSM and 5% of DPX-A 7881 for spiked tap water). We suspected the capacity of the buffer was not large enough, so the concentration of the acceptor buffer was increased to 0.1 *M*. However, the recoveries of these two analytes remained almost unchanged.

Knutsson et al. [16] also observed this phenomenon when they determined chlorinated phenols in

natural water. They proposed that the sample matrix, probably humic substances, reduced the pH in the acceptor. But in this study, the situation must be different as the humic material in tap water is so low that it should not significantly influence the recoveries of the analytes. Thus, we suspected that it might be the large amount of CO_2 present that reduced the recoveries of analytes in tap water. Further experiments showed that when 0.02 *M* Na_2CO_3 was added into a mixed standard solution of 10 μg l^{-1} of MSM and 20 μg l^{-1} of DPX-A 7881, their recoveries were 29 and 3%, respectively. This is because Na_2CO_3 present in the sample solution mixed with H_2SO_4 in the donor stream will produce CO_2, which diffuses through the liquid membrane and is trapped in the acceptor. Thus, the pH of the acceptor decreased which resulted in reduced recoveries.

To quantitatively recover the analytes spiked in tap water, other solutions such as 0.1 *M* NaOH, $NH_3 \cdot H_2O$–NH_4Cl buffer (pH 10.0), and 0.2 *M* Na_2CO_3–$NaHCO_3$ buffer solution (pH 10.0) were tried as acceptors. Results showed that while the analytes cannot be enriched when 0.1 *M* NaOH and $NH_3 \cdot H_2O$–NH_4Cl buffer (pH 10.0) were used as acceptors, good recoveries for both MSM and DPX-A 7881 were obtained when 0.2 *M* Na_2CO_3–$NaHCO_3$ buffer solution (pH 10.0) was used. This is probably because the solubility of CO_2 in Na_2CO_3–$NaHCO_3$ buffer solution is much lower than in other acceptor solutions, thus the decrease in acceptor pH resulting from the dissolving of CO_2 is much smaller. Therefore, pH 10.0 Na_2CO_3–$NaHCO_3$ was adopted as the acceptor in the following studies.

3.4. Characteristics of the proposed method

Some characteristics of the proposed method such as linear range, regression equations, correlation coefficients, detection limits and enrichment factors, were investigated by performing 10-min enrichment of standard solutions and results are shown in Table 1.

Regression equations and correlation coefficients were obtained by determining nine MSM standards covering the linear range of 0.1–50 μg l^{-1} and seven DPX-A 7881 standards covering the linear range of 0.2–50 μg l^{-1}, respectively. The precision (RSD) of the system was measured by repeated enrichment of standard solution containing 0.5 μg l^{-1} MSM and DPX-A 7881. To determine the enrichment factors of the proposed method for these two kinds of herbicides, calibration curves without the CFLME enrichment step for these two analytes were prepared by direct injection of a series of mixed standard solutions diluted with acceptor onto the analytical column. Then, a mixed standard solution with known concentration of analytes (C_0) was analyzed with the CFLME enrichment step and the calculated concentrations (C) were obtained from the calibration curves. The enrichment factors for these two analytes is equal to the ratios of these two concentrations, C/C_0.

As shown in Table 1, the enrichment factors with an enrichment time of 10 min, determined with a standard solution of 0.5 μg l^{-1} MSM and DPX-A 7881, were 100 and 96 for MSM and DPX-A 7881, respectively. Extending the enrichment time to 30 min, the enrichment factors obtained for MSM and

Table 1
Some parameters of the proposed method (determined with an enrichment time of 10 min)

	MSM	DPX-A 7881
Linear range (μg l^{-1})	0.1–50	0.2–50
Regression equation[a]	$A=9462.7C+602.5$	$A=7997.4C+819.9$
Correlation coefficient	$R^2=0.9970$	$R^2=0.9960$
Detection limit (μg l^{-1})	0.05	0.1
Precision (RSD, $n=5$)	7.0	9.2
Enrichment factor	100	96
Solvent consumption (ml)	0.5	0.5
Sample consumption (ml)	20	20

[a] Area (A) and concentration (C, μg l^{-1}) are related.

Table 2
Recoveries (mean±SD, $n=5$) of water samples spiked with 0.2 μg l^{-1} MSM and 0.4 μg l^{-1} DPX-A 7881 determined by the proposed method

Sample	MSM	DPX-A 7881
Bottled mineral water 1	95±3	100±4
Bottled mineral water 2	83±8	88±14
Tap water	95±9	92±15
Sea water	89±14	88±21

DPX-A 7881 were 170 and 120, respectively, which were not as large as expected. This is probably due to the acceptor pH decrease with the increase in enrichment time, resulting from the co-extraction of acid compounds in sample solutions, which gives rise to the inefficient trapping of the target analytes.

3.5. Application of the method for aqueous samples

In order to validate the proposed method, five aqueous samples including seawater, tap water and bottled mineral water were analyzed and the recoveries were determined by spiking the samples with 0.2 μg l^{-1} MSM and 0.4 μg l^{-1} DPX-A 7881. Results shown in Table 2 indicate that the content of MSM and DPX-A 7881 in these four samples were all under the detection limits and the recoveries of MSM and DPX-A 7881 were in the range of 83–95 and 88–100%, respectively. It must be admitted, however, that the standard deviations were rather high in most instances.

4. Conclusions

This work demonstrated the feasibility for determination of sulfonylurea herbicides at low μg l^{-1} levels in water samples by on-line coupling of the novel CFLME sample enrichment technique with HPLC. Because polar solvent, dichloromethane can be used as liquid membrane in this proposed procedure, an enrichment factor of about 100 was obtained with an enrichment time of 10 min. On the other hand, the proposed system is stable as the organic solvents used as liquid membrane were continuously introduced into the system at a very low flow-rate, which overcomes the danger of membrane breakthrough. The proposed method is automatic and rapid. With the exception of the analysis of the first sample which took 22 min, the determination of the following samples takes only 12 min, because while a sample is being separated and detected, the enrichment of the following sample can be carried out at the same time. Compared to the existing SLM-HPLC method, this proposed procedure has the advantages of higher system stability, higher enrichment factor with shorter analytical time, and similar detection limits.

Acknowledgements

This work was financed by the National Natural Science Foundation of China (20177026 and 29825114) and the Chinese Academy of Sciences (KZCX2-414).

References

[1] A.D. Corcia, C. Crescenzi, R. Samperi, L. Scappaticcio, Anal. Chem. 69 (1997) 2819.
[2] G. Nilvé, R. Stebbins, Chromatographia 32 (1991) 269.
[3] I. Ahmad, J. Assoc. Off. Anal. Chem. 70 (1987) 745.
[4] R.W. Reiser, A.C. Barefoot, R.F. Dietrich, A.J. Fogiel, W.R. Johnson, M.T. Scott, J. Chromatogr. 554 (1991) 91.
[5] M. Rodriguez, D.B. Orescan, Anal. Chem. 70 (1998) 2710.
[6] E.W. Zahnow, J. Agric. Food Chem. 33 (1985) 479.
[7] G.C. Galletti, A. Bonetti, G. Dinelli, J. Chromatogr. A 692 (1995) 27.
[8] M.J.M. Wells, J.L. Michael, J. Chromatogr. Sci. 25 (1987) 345.
[9] B. Køppen, N.H. Spliid, J. Chromatogr. A 803 (1998) 157.
[10] H. Bagheri, E.R. Brouwer, R.T. Ghijsen, U.A.Th. Brinkman, Analusis 220 (1992) 475.
[11] A.C. Hogenboom, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 794 (1998) 201.
[12] G. Nilvé, M. Knutsson, J.A. Jönsson, J. Chromatogr. A 688 (1994) 75.
[13] M. Knutsson, G. Nilvé, L. Mathiasson, J.A. Jönsson, J. Agric. Food Chem. 40 (1992) 2413.
[14] J.A. Jönsson, L. Mathiasson, J. Chromatogr. A 902 (2000) 205.
[15] J. Liu, J. Chao, G. Jiang, Anal. Chim. Acta, 2002, in press.
[16] M. Knutsson, L. Mathiasson, J.A. Jönsson, Chromatographia 42 (1996) 165.