ELSEVIER

Journal of Chromatography A, 688 (1994) 75–82

JOURNAL OF CHROMATOGRAPHY A

Liquid chromatographic determination of sulfonylurea herbicides in natural waters after automated sample pretreatment using supported liquid membranes

Göran Nilvé[1], Magnus Knutsson, Jan Åke Jönsson*

Department of Analytical Chemistry, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden

First received 15 March 1994; revised manuscript received 23 September 1994

Abstract

Sample pretreatment for the determination of sulfonylurea herbicides in natural water samples is investigated. Both supported liquid membrane (SLM) extraction and solid-phase extraction (SPE) are used for enrichment of spiked samples. The liquid membrane technique is used on-line with liquid chromatography. The liquid chromatographic detection is by UV absorption at 240 nm. Enrichment with SLM gives a more selective extraction than SPE, resulting in much cleaner chromatograms. The detection limits for enrichment of 250-ml samples are 50–100 ng/l for SLM and around 1 μg/l for SPE.

1. Introduction

Sulfonylureas are a relatively new class of herbicides used for control of weed in crops. These herbicides are very potent weed killers and are used in doses that are substantially lower than for conventional herbicides. Since the mid-1970s, when the herbicidal activity of this class of compounds was discovered, the development has been very fast and in May 1989, 375 sulfonylurea herbicide patents had been issued [1]. The low doses used (ca. 4 g/ha; 1 ha = 10^4 m^2) make determinations of these compounds in recipient waters and in soils difficult. Apart from the requirements on the final analytical step (sensitivity and selectivity) the sample preparation step must be capable of large and, if possible, selective enrichments.

Solid-phase extraction (SPE), a technique well known for its large enrichment capacity, has been used for concentration and clean-up of sulfonylurea herbicides in aqueous samples in an off-line mode [2–4]. It is potentially possible to use this technique in an on-line mode as described by several authors [5,6]. This has, however, to our knowledge not been applied to sulfonylurea herbicides. Off-line SPE can be automated using several commercially available robotic instruments (i.e. ASPEC, Gilson).

Liquid–liquid extraction, probably still being the most commonly used sample preparation method for determination of non-volatile organics in aqueous samples, has also been used for sulfonylurea herbicides [7,8]. The general problems encountered when liquid–liquid extraction is used are however quite large and include

* Corresponding author.

[1] Present address: Astra-Draco AB, P.O Box 34, S-221 00 Lund, Sweden.

0021-9673/94/$07.00
SSDI 0021-9673(94)00918-X

factors like: time and labour intensive, emulsion formation, large consumption of organic solvents, evaporation of large volumes of solvents, and not easily automated

Sample preparation by means of liquid membrane extraction is a technique which in essence contains two liquid–liquid extractions in one step, whereby some of the problems mentioned above can be solved [9]. The technique can be used on-line with both gas [10–12] and liquid chromatography [13–15]. The set-up is easily automated and the sample preparation is performed in a closed system, thus minimizing the risks for contamination and losses during the process. As the extraction is made from one aqueous phase (donor) to a second, also aqueous phase (acceptor), further enrichment on a precolumn is possible, before injection into a liquid chromatograph. The supported liquid membrane method provides, in addition to high enrichment factors, also a high degree of clean-up. In complex matrices as urine [11], blood plasma [12,15] and manure [16] it has been demonstrated that chromatograms obtained from samples containing these matrices are very similar to chromatograms from standard solutions in distilled water. The technique can also be used for time integrating field sampling. The total leakage of phenoxy acids into recipient waters has thus been estimated [17,18].

In a previous paper [14] liquid membranes were used for enrichment of two sulfonylureas (metsulfuron methyl and chlorsulfuron) from clean aqueous samples. In the present paper the same approach is used for determinations of four sulfonylureas (thifensulfuron methyl, metsulfuron methyl, chlorsulfuron and tribenuron methyl) in natural water samples. The membrane method is also compared with SPE for the enrichment of the herbicides from these samples.

2. Experimental

2.1. Equipment

The flow system is shown in Fig. 1. Two peristaltic pumps (I) (Minipuls 2; Gilson Medical Electronics, Villiers-le-Bel, France) were used to independently control the flow-rates of the donor and acceptor phases. The pump tubings used in the peristaltic pumps were of the "acid flexible" type (Elkay Products, Shrewsbury, MA, USA). The various parts of the set-up were connected with 0.5 mm I.D. PTFE tubing and Alltech screw fittings. The confluence tees were made of PTFE or Kel-F and the channels meet at a 60° angle. The switching valves were pneumatically actuated four-way Kel-F slider valves (Cheminert; Laboratory Data Control, UK).

The membrane separator (see Fig. 1) consisted of two polyvinylidene difluoride (PVDF) blocks in which channels were machined so that when put together the channels face each other. The channels were 1.5 mm wide, 0.25 mm deep and 250 cm long, giving a volume in each channel of ca. 950 μl. The channels were arranged like Archimedes' spirals. The membrane was placed between the PVDF blocks, separating the donor from the acceptor channel and the set-up was clamped together with six screws.

The liquid membrane support was Fluoropore FG (PTFE membrane, average pore size 0.2 μm, total thickness 175 μm of which 115 μm is polyethylene backing, porosity 0.7; Millipore, Bedford, MA, USA). The liquid membrane was prepared by simply immersing the membrane support in the organic solvent mixture for about 15 min. After installation of the impregnated membrane in the separator both channels were flushed with water to remove excess of the solvent mixture on the surfaces of the membrane.

The chromatographic separations were performed with a Spectra-Physics (San José, CA, USA) SP 8800 liquid chromatographic pump. A pneumatically actuated Valco six-port valve in which the loop was replaced with a 20 mm × 2.1 mm I.D. precolumn (Upchurch Scientific, Oak Harbor, WA, USA) was used for injection. The packing used in the precolumn was pellicular C_{18} packing (Alltech, Deerfield, IL, USA). The analytical column was 150 mm × 2.1 mm I.D. filled with Spherisorb ODS-3 (3-μm particles) (Phase Separations, Queensferry, UK). The chromatographic eluent consisted in the SPE experiments of MeOH–1% aqueous acetic acid (1:1, v/v) and in the liquid membrane experi-

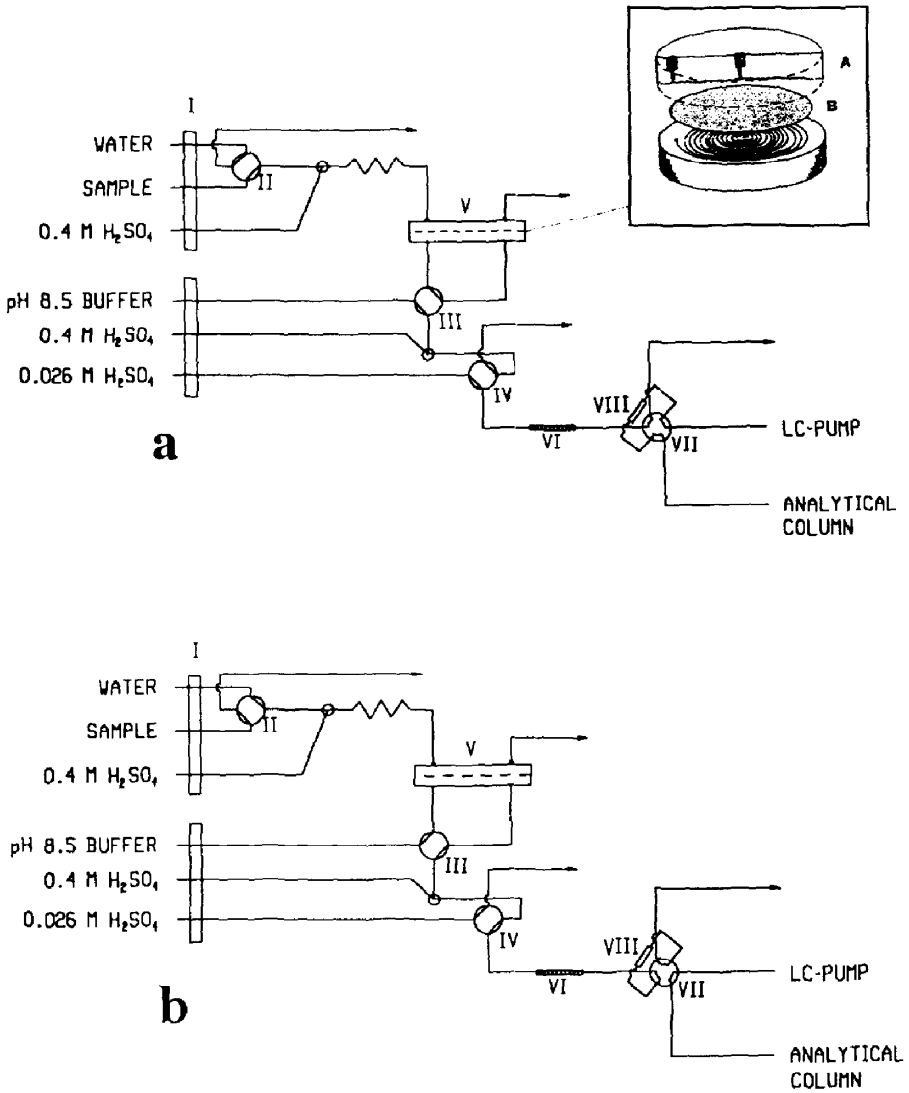


Fig. 1. Experimental setup. (a) Sample enrichment, (b) loading of the enriched sample onto the precolumn. Membrane separator (V): (A) PVDF blocks with grooves like Archimedes' spiral; (B) PTFE membrane with polyethylene backing. For more details on the set-up, see text.

ments of acetonitrile–water adjusted to pH 3 with acetic acid (3:7, v/v). The flow-rates were for the MeOH eluent 0.15 ml/min, and for the acetonitrile 0.2 ml/min.

Spectrophotometric detection was carried out at 240 nm with a LDC Spectromonitor III variable-wavelength UV detector (LDC, Riviera Beach, FL, USA). The chromatographic data were collected and handled with a personal computer (Model V386A; Victor Svenska, Stockholm, Sweden) using the JCL 6000 chromatography data system (Jones Chromatography, Hengoed, UK).

2.2. Operation of the liquid membrane flow system

Operation of the flow system used for liquid membrane sample pretreatment is shown in Fig. 1. Two peristaltic pumps (I) were used to pump

the different solutions. Fig. 1a shows positioning of the valves when the sample enrichment is performed. This can be made simultaneously with the LC analysis of the previous sample. The sample is injected through valve II. The sample volume is determined by the flow-rate through valve II and by the time this valve remains open. After mixing with acid in a mixing coil (100 cm × 0.5 mm I.D.) the acidified sample enters the membrane separation unit (V) on the donor side. The non-ionized solutes are extracted into the liquid membrane and diffuse through the membrane into the aqueous acceptor phase, which is kept stagnant by valve III. The acceptor pH is chosen so that the acidic analytes are ionized. The driving force of analyte mass transfer is thus the concentration gradient of non-ionized analytes between the donor and the acceptor phases. A detailed treatment of the mass transfer process is given elsewhere [19].

In Fig. 1b, loading of the enriched sample on the precolumn is described. Following the switching of valve III the enriched sample plug is mixed with acid in a pearl string mixer (VI), before it is loaded on the precolumn (VIII). After the loading, valve IV is switched to flush the precolumn with diluted acid. This prevents injection of the acceptor phase buffer in the liquid chromatographic system, which has previously been shown to give a large front peak in the chromatogram [11]. Finally, valve VII is switched, introducing the analytes on the column. The extraction efficiencies were calculated by comparison with injected standard solutions.

Typical flow-rates were: donor side: sample (or water) 0.8 ml/min, 0.4 *M* H_2SO_4 0.2 ml/min; acceptor side: pH 8.5 buffer 0.25 ml/min, 0.4 *M* H_2SO_4 0.05 ml/min and 0.026 *M* H_2SO_4 0.25 ml/min. All valves are controlled by the "timed event" facilities in the chromatography data system.

2.3. Solid-phase extraction

The SPEs were basically performed as described by Wells and Michael [2], using Supelclean LC-18 SPE tubes (Supelco, Bellefonte, PA, USA) (6 ml, 0.5 g). To 1 l of water were added 7.5 g KH_2PO_4 and 7.8 g K_2HPO_4 and the pH was then adjusted to 6 with H_2SO_4. The SPE cartridges were activated by flushing with 5 ml MeOH and conditioned with 5 ml water. Samples of 200 ml were extracted at a flow-rate of ca. 3 ml/min. After completed extraction the analytes were eluted with a 5-ml portion of MeOH. The methanol was evaporated to a final volume of < 1 ml and subsequently the volume was adjusted to 1 ml with water. A 20-μl aliquot (unless stated otherwise) of this solution was injected into the liquid chromatograph. The extraction recoveries were calculated by comparison with injected standard solutions.

2.4. Chemicals

Thifensulfuron methyl (98.3%), metsulfuron methyl (99.7%), chlorsulfuron (99.7%) and tribenuron methyl (97.3%) (see Table 1) were all gifts from DuPont, Wilmington, DE, USA. The organic solvents used were *n*-undecane (Merck, Darmstadt, Germany) (analytical-reagent grade) and di-*n*-hexyl ether (Sigma, St. Louis, MO, USA). Apart from tri-*n*-octylphosphine oxide (TOPO) obtained from Fluka (Buchs, Switzerland), all other chemicals came from Merck and were of analytical-reagent grade. Reagent water was purified with a Milli-Q-RO-4 unit (Millipore). Natural water was collected from the Kävlinge river, 10 km north of Lund, southern Sweden.

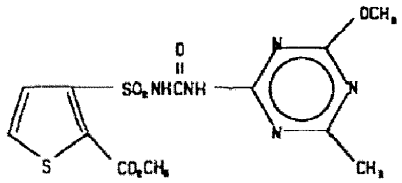
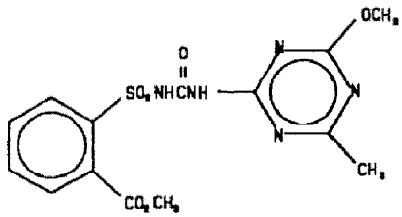
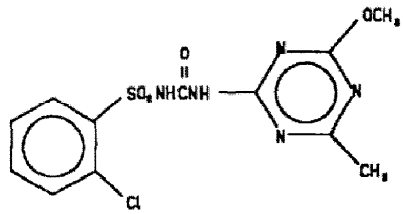
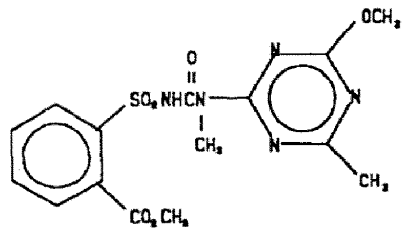
3. Results and discussion

3.1. Solid-phase extraction

The recoveries of the four sulfonylureas (concentrations 10 μg/l), from spiked samples of natural water ($n = 4$), are given in Table 2.

A chromatogram of a 1 μg/l sample enriched by SPE (Fig. 2) shows that the analytes at that concentration are largely masked by interfering compounds. The injection volume was 20 μl. To increase the amount injected, larger volumes (up to 500 μl) of the enriched sample were (by syringe) injected on a small precolumn, 20 mm ×

Table 1
Sulfonylurea herbicides used in this study and their CAS numbers, dissociation constants and structures

Name	CAS No.	pK_a	Structure
Thifensulfuron methyl	79277-27-3	4.0[a]	
Metsulfuron methyl	74223-64-6	3.3[a]	
Chlorsulfuron	64902-72-3	3.6[a]	
Tribenuron methyl	101200-48-0	5.0[b]	

[a]From Ref. [20].
[b]From Ref. [21].

2,1 mm I.D., packed with pellicular C_{18}. The gain in the detection limit achieved by injection of larger volumes on a precolumn is negligible as the background absorption gets very large. Thus the clean-up selectivity of the SPE in this application, using C_{18} type stationary phase, is too low to permit the determination of low concentrations of the sulfonylureas (e.g. below 0.5–1 μg/l). The detection limit is largely governed by the amount of interfering compounds ex-

Table 2
Recoveries for the four sulfonylurea herbicides after SPE of 10 μg/l spiked natural water samples ($n = 4$) (95% confidence intervals)

Compound	Recovery (%)
Thifensulfuron methyl	100 ± 14
Metsulfuron methyl	102 ± 14
Chlorsulfuron	103 ± 13
Tribenuron methyl	103 ± 6

tracted. Wells and Michael [2] did not explicitly state detection limits for sulfonylureas in their paper, but a limit of 1 μg/l was mentioned for a similar compound.

A possible way to increase the selectivity in SPE would be to use an additional clean-up step, e.g. by elution of less-retained interfering compounds with water modified with a small amount of organic solvent [22]. This has not been carried out in this study. It is also possible to use more than one precolumn in series to increase the selectivity. Use of a C_{18} column in series with an ion-exchange column might be a possible route to a more selective enrichment. This has not been reported for sulfonylureas in the literature, but has been used for other pesticides [23].

As the enrichment factor can be made very large with SPE, the detection limit could probably be lowered by use of a more selective detection than UV detection at 240 nm, as described in other papers, namely photoconductivity [24–26] and mass spectrometry [27–30]. For example, Zahnow [4] states a detection limit of 0.2 μg/l of sulfometuron methyl in river water, after SPE enrichment.

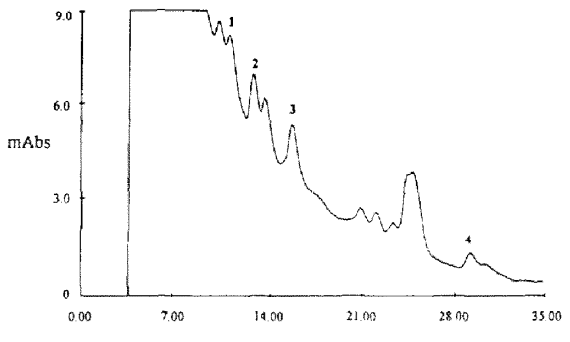


Fig. 2. A 1 μg/l concentration of the sulfonylureas enriched by SPE, 20-μl injection. Peaks: 1 = thifensulfuron methyl; 2 = metsulfuron methyl; 3 = chlorsulfuron; 4 = tribenuron methyl.

3.2. Liquid membrane extraction

The sulfonylureas are extracted from an acidified aqueous donor solution into an organic solvent in the liquid membrane and back extracted into a neutral/alkaline aqueous acceptor phase on the opposite side of the membrane.

The selectivity towards interfering substances is potentially larger when liquid membrane extraction is used for sample pretreatment than in the case of SPE. Only solutes that are non-ionized in the donor phase and ionized in the acceptor phase will be enriched in this system. Compounds which are neutral in both donor and acceptor solutions will simply equilibrate between the phases, while solutes being ionic in the donor will not be extracted into the liquid membrane at all. Furthermore, equilibrated non-ionized species in the acceptor solution may be washed out by pumping a clean solution through the donor channel, resulting in a back extraction of neutral compounds into the donor solution [9].

By changing the polarity of the organic solvent in the membrane, the selectivity may be controlled. Fig. 3 shows chromatograms after enrichment of blank natural water samples using three different solvent mixtures in the liquid membrane.

The least polar solvent used (*n*-undecane–di-*n*-hexyl ether, 1:1) gives the most efficient clean-up. A pure di-*n*-hexyl ether membrane gives a larger front peak in the chromatogram. With TOPO (a modifier that has been used in extractions of phenols and carboxylic acids [31–33]) in the membrane, the selectivity towards other substances in the water, possibly humic substances, is further decreased, reflected by the increased front peak seen after using this membrane.

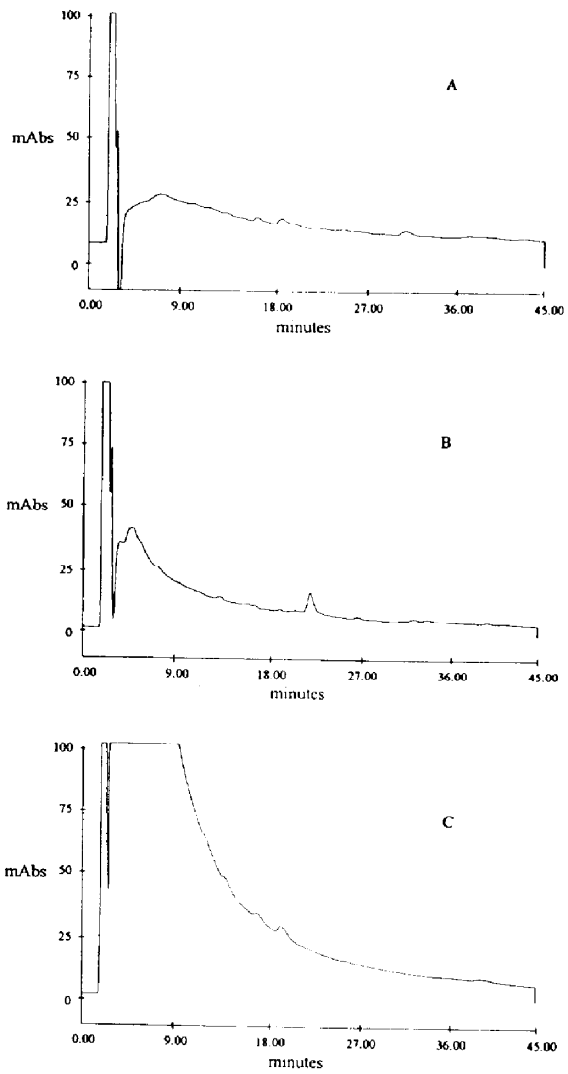


Fig. 3. Supported liquid membrane extraction of blank natural water samples. (A) *n*-Undecane–di-*n*-hexyl ether (1:1), (B) pure di-*n*-hexyl ether, (C) 5% TOPO in di-*n*-hexyl ether.

As the sulfonylureas are rather polar substances themselves, the recoveries in the extraction will also increase when using solvents of increasing polarity. The extraction efficiency for chlorsulfuron increased with a factor of nearly 2 using a di-*n*-hexyl ether compared to the 1:1 mixture [14]. Pure di-*n*-hexyl ether was choosen in the further work as a compromise between selectivity and extraction efficiency.

Table 3
Extraction efficiencies (%) with di-*n*-hexyl ether liquid membrane (concentrations 200, 400, 1000 and 2000 ng/l) ($n = 4$) (95% confidence intervals)

Compound	Extraction efficiency (%)	
	Standard solution	Natural water sample
Thifensulfuron methyl	24 ± 2	19 ± 2
Metsulfuron methyl	35 ± 3	33 ± 4
Chlorsulfuron	50 ± 4	43 ± 3
Tribenuron methyl	49 ± 5	39 ± 4

The extraction efficiencies for the sulfonylureas in standard aqueous solutions and in natural waters are given in Table 3. The R.S.D.s are in all cases in the range 4–7%. This includes both the extraction and determination steps. The extraction efficiencies are independent of the sample concentrations, leading to linear relationships between analytical response and analyte concentrations.

The extraction recoveries are lower in the natural water samples. For all compounds except metsulfuron methyl the difference is statistically significant (0.05 level). A possible explanation is that at the low pH in the donor, there may be some adsorption of the sulfonylureas on particles in the natural water samples. Thus the extraction efficiencies will have to be checked in the samples to be extracted.

The extraction efficiencies in Table 3 are considerable lower than 100%. With a enough low donor flow-rate, efficiencies close to 100% can be obtained on the expense on time. As was discussed elsewhere [9] a faster accumulation of analyte is obtained with high flow-rates, leading to lower, but constant, extraction efficiencies. This mode of operation can be applied when a large sample volume is available.

Fig. 4 shows a chromatogram after enrichment of 240 ml natural water sample containing 0.2 μg/l of the sulfonylureas, showing a higher degree of clean-up than with SPE (cf. Fig. 3). The detection limits are in the order of 50–100 ng/l.

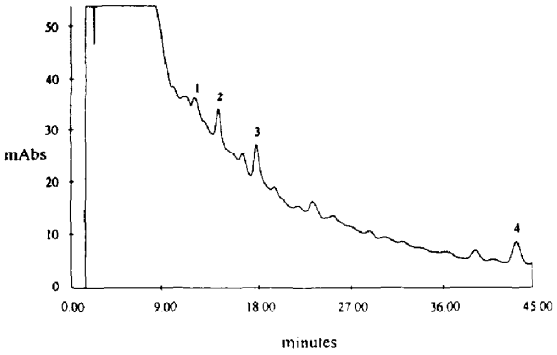


Fig. 4. Chromatogram after supported liquid membrane enrichment of a natural water sample containing 0.2 μg/l of each of the four sulfonylureas. Peaks as in Fig. 2.

4. Conclusions

The supported membrane technique has been shown to give a more selective enrichment of sulfonylurea herbicides than with solid phase extraction, leading to lower detection limits and adequate linearity and repeatability. It is likely that the SPE approach could be improved with some additional effort, for example involving several columns with different polarities. However, the inherent advantage of the supported liquid membrane technique, placing a barrier between the sample and the analytical instrument, remains. The analytes have to actively pass this barrier in order to be measured.

5. Acknowledgement

The financial support of the Swedish Council for Forestry and Agricultural Research is gratefully acknowledged.

References

[1] H.M. Brown, *Pestic. Sci.*, 29 (1990) 263.
[2] M.J.M. Wells and J.L. Michael, *J. Chromatogr. Sci.*, 25 (1987) 345.
[3] E.G. Cotterill, *Pestic. Sci.*, 34 (1992) 291.
[4] E.W. Zahnow, *J. Agric. Food Chem.*, 33 (1985) 479.
[5] R.W. Frei, M.W.F. Nielen and U.A.Th. Brinkman, *Int. J. Environ. Anal. Chem.*, 25 (1986) 3.
[6] M.W.F Nielen, R.W. Frei and U.A.Th. Brinkman, in K. Zech and R.W. Frei (Editors), *Sample Handling and Detection in High-Performance Liquid Chromatography*, Part A, Elsevier, Amsterdam, 1988, Ch. 1, p. 5.
[7] V. Leoni, C. Cremisini, A. Casuccio and A. Gullotti, *Pestic. Sci.*, 31 (1991) 209.
[8] I. Ahmad, *J. Ass. Off. Anal. Chem.*, 70 (1987) 745.
[9] J.Å. Jönsson and L. Mathiasson, *Trends Anal. Chem.*, 11 (1992) 106.
[10] G. Audunsson, *Anal. Chem.*, 58 (1986) 2714.
[11] G. Audunsson, *Anal. Chem.*, 60 (1988) 1340.
[12] B. Lindegård, J.Å. Jönsson and L. Mathiasson, *J. Chromatogr.*, 573 (1992) 191.
[13] G. Nilvé, G. Audunsson and J.Å. Jönsson, *J. Chromatogr.*, 471 (1989) 151.
[14] G. Nilvé and R. Stebbins, *Chromatographia*, 32 (1991) 269.
[15] B. Lindegård, H. Björk, J.Å. Jönsson, L. Mathiasson and A.-M. Olsson, *Anal. Chem.*, in press.
[16] L. Mathiasson, M. Knutsson, G. Bremle and L. Mårtensson, *Swed. J. Agric. Res.*, 21 (1991) 147.
[17] L. Mathiasson, G. Nilvé and B. Ulén, *Int. J. Environ. Anal. Chem.*, 45 (1991) 117.
[18] M. Knutsson, G. Nilvé, L. Mathiasson and J.Å. Jönsson, *J. Agric. Food Chem.*, 40 (1992) 2413.
[19] J.Å. Jönsson, P. Lövkvist, G. Audunsson and G. Nilvé, *Anal. Chim. Acta*, 277 (1993) 9.
[20] E.M. Beyer, M.J. Duffy, J.V. Hay and D.D. Schlueter, in P.C. Kearney and D.D. Kaufman (Editors), *Herbicides: Chemistry, Degradation and Mode of Action*, Vol. 3, Marcel Dekker, New York, 1988, Ch. 3, p. 117.
[21] *DPX L 5300 Herbicide; Technical Data Sheet*, DuPont, Wilmington, DE, 1985.
[22] R.B. Geerdink, C.A.A. Van Balkom and H.-J. Brouwer, *J. Chromatogr.*, 481 (1989) 275.
[23] V. Coquart and M.-C. Hennion, *J. Chromatogr.*, 585 (1991) 67.
[24] E.W. Zahnow, *J. Agric. Food Chem.*, 30 (1982) 854.
[25] R.V. Slates, *J. Agric. Food Chem.*, 31 (1983) 113.
[26] J.L. Prince and R.A. Guinivan, *J. Agric. Food Chem.*, 36 (1988) 63.
[27] A.C. Barefoot and R.W. Reiser, *J. Chromatogr.*, 398 (1987) 217.
[28] A.C. Barefoot, R.W. Reiser and S.A. Cousins, *J. Chromatogr.*, 474 (1989) 39.
[29] A.C. Barefoot and R.W. Reiser, *Biomed. Environ. Mass Spectrom.*, 18 (1989) 77.
[30] R.W. Reiser, A.C. Barefoot, R.F. Dietrich, A.J. Fogiel, W.R. Johnson and M.C. Scott, *J. Chromatogr.*, 554 (1991) 91.
[31] J.D. MacGlashan, J.L. Bixby and C.J. King, *Solv. Extr. Ion Exch.*, 3 (1985) 1.
[32] Y. Kuo and H.P. Gregor, *Sep. Sci. Technol.*, 18 (1983) 421.
[33] Y. Shen, L. Grönberg and J.Å. Jönsson, *Anal. Chim. Acta*, 292 (1994) 31.