ELSEVIER

Journal of Chromatography A, 750 (1996) 161–174

JOURNAL OF CHROMATOGRAPHY A

Automated at-line solid-phase extraction–gas chromatographic analysis of micropollutants in water using the PrepStation

Th. Hankemeier*, P.C. Steketee, J.J. Vreuls, U.A.Th. Brinkman

Free University, Department of Analytical Chemistry, De Boelelaan 1083, 1081 HV, Amsterdam, Netherlands

Abstract

A fully automated at-line solid-phase extraction–gas chromatography procedure has been developed for the analysis of aqueous samples using the PrepStation. The sample extract is transferred from the sample preparation module to the gas chromatograph via an autosampler vial. With flame-ionization detection, limits of determination ($S/N=10$) of 0.05–0.13 μg/l were obtained for the analysis of HPLC-grade water when modifying the PrepStation by: (i) increasing the sample volume to 50 ml, (ii) increasing the injection volume up to 50 μl, and (iii) decreasing the desorption volume to 300 μl. The HP autosampler had to be modified to enable the automated "at-once" on-column injection of up to 50 μl of sample extract. The amount of packing material in the original cartridge had to be reduced to effect the decrease of the desorption volume. The total set-up did not require any further optimization after having set up the method once. The analytical characteristics of the organonitrogen and organophosphorus test analytes, i.e. recoveries (typically 75–105%), repeatability (2–8%) and linearity (0.09–3.0 μg/l) were satisfactory. The potential of the system was demonstrated by determining triazines and organophosphorus pesticides in river Rhine water at the 0.6 μg/l level using flame-ionization and mass-selective detection. No practical problems were observed during the analysis of more than 100 river water samples.

Keywords: Water analysis; Sample preparation; Automation; Environmental analysis; Pestide; Pesticides; Triazines; Organophosphorus compounds; Large volume injection

1. Introduction

The determination of micropollutants in environmental samples is generally achieved by means of chromatographic analysis. Sample preparation is usually necessary prior to the actual determination step because of the complexity of the samples and the low determination levels which have to be achieved. Unfortunately, sample preparation, which is usually done off-line, and often manually, is generally time consuming [1]. For the determination of micropollutants in water samples by means of gas chromatography (GC), solid-phase (SPE) or liquid–liquid extraction (LLE) are primarily used to transfer the analytes from the water into an organic solvent prior to analysis. Obviously, integration of sample preparation and method of analysis into one set-up is desirable. This can be achieved by on-line or at-line coupling. In on-line interfacing the extract obtained after sample preparation is directly and completely transferred to the chromatographic system. In recent years several on-line solid-phase extraction (SPE)–GC systems have been developed and utilized [2–5]. When using an on-column interface for the transfer of 50–100 μl of desorption solvent, a critical param-

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00556-0

eter is the total amount of solvent transferred to the GC; changes in the dead volume of the SPE cartridge have to be considered. In an at-line approach the sample extract is transferred from the sample preparation module to the chromatographic system via, for example, an autosampler vial, from which an aliquot (constant volume!) is injected, and no optimization is necessary. The at-line (and also the on-line) set-up offers advantages such as good precision and low contamination due to the closed nature of the system. In addition, the amount of organic solvent used is small, sample throughput is increased and it becomes relatively easy to achieve automation which will help to reduce the cost per analysis. Additionally, the at-line approach allows the injection of (part of) the extract into another GC system with a different detection mode, the addition of an internal standard, the injection of organic extracts or standard solutions for calibration next to the analysis of aqueous samples, and at-line analyte derivatization prior to injection.

To achieve good analyte detectability with an at-line system, it is necessary to analyze a substantial portion (equivalent to the original sample) in the final GC run. Typical sample extract volumes obtained with miniaturized SPE procedures are 100–1000 μl. Today, in GC, the injection of sample volumes up to 100 μl is increasingly being used to enhance sensitivity, e.g. by using an on-column interface [6–8]. Recently, Hewlett-Packard introduced their so-called PrepStation, which comprises an SPE module for analyte enrichment, desorption with an organic solvent, and a loading/transfer-to-GC module. In the integrated system, the concentrated sample extracts are transported at-line in a vial via a robotic arm from the SPE unit to the autosampler of the GC system, and, finally, an aliquot is injected into this system [9].

The goal of this study was to develop a fully automated PrepStation–GC method for the at-line SPE–GC determination of micropollutants in 1–50 ml of aqueous samples at the 0.1–1 μg/l level. The total set-up should be simple and require essentially no optimization. Modification of the commercial system, if required to achieve our aims, was part of the plans. Several triazines and organophosphorus pesticides were selected as test compounds.

2. Experimental

2.1. Chemicals

HPLC-grade water (J.T. Baker, Deventer, Netherlands) was used for the condition and clean-up steps. Ethyl acetate and methanol (both analytical-reagent grade, J.T. Baker) were distilled before use. Chloroform (analytical-reagent grade) for Soxhlet extraction of the septa and cartridges was purchased from J.T. Baker. The ten organophosphorus pesticides (OPPs) and six triazines were purchased from Riedel-de Haën (Seelze, Germany). Stock solutions of the OPPs and triazines were stored in the dark at 4°C. Water samples were spiked just before analysis. 4,4′-Difluorobiphenyl (Aldrich, Axel, Netherlands) or N,N-dibenzylaniline (ICN, Costa Mesa, CA, USA) was used as the internal standard. River water samples were filtered through 0.45-μm membrane filters (Schleicher and Schüll, Dassel, Germany).

2.2. Set-up of PrepStation–GC system

A schematic of the PrepStation–GC system is shown in Fig. 1. The whole set-up consists of a HP 7686 PrepStation with SPE module (Hewlett-Pac-

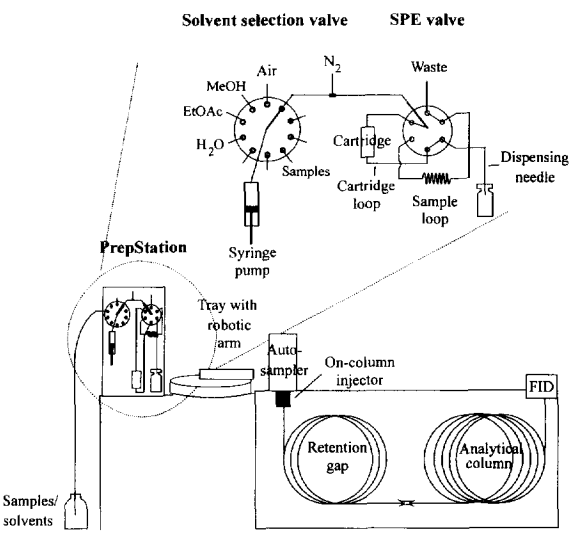

Fig. 1. Set-up of the PrepStation–GC–FID system. Abbreviations: EtOAc, ethyl acetate; H_2O, water; MeOH, methanol; N_2, nitrogen.

kard, Waldbronn, Germany), a (modified) HP 7673 autosampler with a HP 7673B autosampler tray and a HP 5890 Series II gas chromatograph with electronic pressure control, an on-column injector and a flame-ionization detection (FID) system. The PrepStation itself consists of a 10 port solvent selection valve, a 2.5 ml syringe pump, a nitrogen 3 port valve, an SPE valve and three drop-off positions, one for an SPE cartridge and two for autosampler vials.

A total of eight samples and solvents can be positioned at the selection valve; in our case, there were five samples next to HPLC-grade water, methanol and ethyl acetate. A solvent or sample is pumped through the nitrogen purge valve to the SPE valve by the syringe pump. The SPE valve allows one to choose different flow paths which are a combination of the sample loop, the SPE cartridge loop and the waste or dispensing needle as outlet (see Fig. 2 for flow paths used in the SPE procedure to be discussed below). The SPE cartridge is sealed by two sealing probes at the top and bottom within the cartridge loop. The SPE cartridge and the autosampler vial are transported by a robotic arm from the sample tray to the drop-off positions of the PrepStation (or vice versa). For an exchange of the SPE cartridge and/or the autosampler vial, the top sealing probe of the SPE cartridge and the dispensing needle are lifted. The needle height of the dispensing needle within the autosampler vial can be programmed. The whole

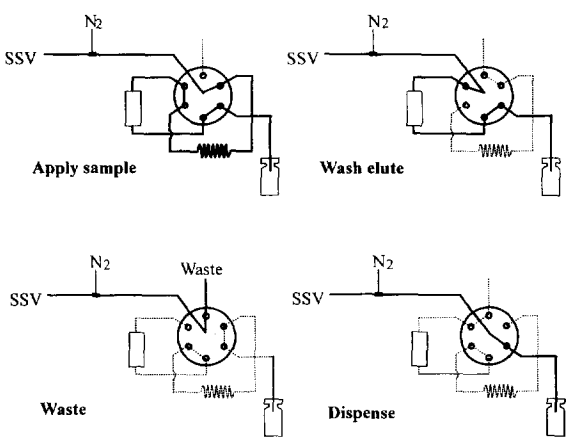

Fig. 2. Flow paths of the PrepStation used during the SPE procedure. Abbreviations: N_2, nitrogen; SSV, solvent selection valve.

flow system or a part of it can be cleaned with solvent by lowering the dispensing needle into the needle rinser in such a way that also the outside of the dispensing needle is cleaned.

In the present study, a HP G1296A Mixer/Bar Code Reader was used to mix solutions; it was mounted next to the autosampler tray. The drop-off position for an autosampler vial for preparation processes such as heating and evaporation was not used. The SPE cartridge was packed in-house with 20 μm, 100 Å PLRP-S, a polystyrene-divinylbenzene copolymer (Polymer Laboratories, Church Stretton, UK). To this end, the commercial SPE cartridge filled with 100 mg C_{18}-bonded silica, was disassembled, repacked and closed with special home-made tools. Before repacking, the disassembled SPE cartridge was cleaned by 6 h Soxhlet extraction with chloroform to reduce the amount of plasticizers present in the cartridge material that can be extracted by the desorption solvent. To decrease the internal volume of 200 μl of the commercial 14.3×4.2 mm I.D. SPE cartridge, a 17.1×2.0 mm I.D. insert made of PTFE was introduced into the cartridge. Two 1.6-mm-thick frits were inserted at both ends to keep the stationary phase in place. This modification resulted in an internal volume of only 44 μl. The frits and the insert were also Soxhlet extracted prior to use as described above. A carbon trap (20–40 mesh Carbotrap C, Supelco, Bellefonte, PA, USA) was inserted before the nitrogen purge valve to purify the nitrogen.

On-column injections were done into a 0.53 mm I.D. diphenyltetramethyldisilazane-deactivated retention gap (BGB Analytik, Zürich, Switzerland). The length of the retention gap was 0.7 m for a 5-μl injection, 3 m for a 25-μl injection and 6 m for a 50-μl injection. The retention gap was connected to the GC column (SPB 5, 25 m×0.32 mm I.D., 0.25-μm film; Supelco) by means of a press-fit connector. Injection was done at 70°C, and this temperature was kept for 2, 3.5 or 6 min for a 5-, 25- or 50-μl injection, respectively. The temperature was programmed to 280°C at 10°C/min and finally held at 280°C for 5 min. The pressure was 90 kPa and was programmed to provide a constant flow. The temperature of the on-column injector was always kept 3°C above the oven temperature, and the temperature

of the FID was 300°C. For the 25- and 50-μl injections the HP autosampler was equipped with a new EPROM (Hewlett-Packard, Wilmington, DE, USA) to enable the programming of a pre- and post-injection delay time of 4 s and 6 s, respectively. The slow injection speed was chosen, but the injection was still finished in less than 2 s.

One software package controls both the PrepStation and the GC system. This allows simultaneous sample preparation of the next, and GC analysis of the previous, sample.

2.3. At-line SPE–GC procedure

The procedure of an at-line SPE–GC analysis is summarized in Table 1. After rinsing the total system with methanol, the cartridge is conditioned with 10 ml of ethyl acetate and 10 ml of HPLC-grade water. As one 2.5-ml syringe pump is used for all solvents, the pump has to be flushed with methanol between the changeover from ethyl acetate to water. After preflushing of the sample tubing and the pump with 10 ml of sample, 50 ml of sample are sampled through the SPE cartridge. After flushing of the syringe with water, clean-up is effected with 5 ml of HPLC-grade water. Next, the cartridge is dried for 30 min with nitrogen at ambient temperature. Initially, the water left in the tubing between the solvent selection valve and the nitrogen purge valve was removed before 30 min of drying by pumping air to waste with the syringe pump. However, this step appeared not to be necessary and was therefore omitted.

After flushing the syringe pump with methanol and ethyl acetate to remove all water, the analytes are desorbed with ethyl acetate into an autosampler vial; 10 μl of internal standard are added from a vial to the autosampler vial containing the extract. The contents of the latter vial are mixed using the Mixer/Bar Code Reader and, finally, delivered to the GC autosampler for injection. During the whole procedure, i.e. from the conditioning of the cartridge to the desorption, the cartridge remained in the drop-off position of the PrepStation. Sample preparation took about 90 min.

2.4. GC–MS analysis

GC analyses using mass selective (MS) detection were done with a HP 5890 Series II GC with on-column injector, electronic pressure control and a HP 5972 MSD detector. Manual 50-μl "at-once" injections were done within 2 s into a 6 m×0.53 mm I.D. diphenyltetramethyldisilazane-deactivated reten-

Table 1
Procedure of at-line SPE–GC analysis of aqueous samples with PrepStation

Step	Solvent [a]	Flow path [b]	Volume (ml)	Flow-rate (ml/min)
Rinsing of whole system	MeOH	Apply sample	7.5	10
Conditioning of cartridge	EtOAc	Wash elute	7.5	5
Priming syringe	MeOH	Waste	7.5	10
Conditioning of cartridge	H_2O	Apply sample	7.5	5
Preflushing with sample	Sample	Waste	10	10
Sample enrichment	Sample	Wash elute	50	5
Priming syringe	H_2O	Waste	5	10
Clean-up	H_2O	Wash elute	5	5
Drying of cartridge for 30 min		Apply sample		
Priming syringe	MeOH	Waste	7.5	10
Priming syringe	EtOAc	Waste	5	10
Desorption of cartridge to vial [c]	EtOAc	Wash elute	0.9	0.2
Addition of internal standard to extract	Standard (vial)	Dispense	0.01	0.1
Mixing of extract				
Injection into GC by autosampler				

[a] Abbreviations: MeOH, methanol; EtOAc, ethyl acetate.
[b] For flow paths, see Fig. 2.
[c] A small laboratory-made cartridge with an internal volume of 44 μl was used.

tion gap (BGB Analytik). The syringe was removed 4 s after the end of the injection. The retention gap was connected via a press-fit connector to a 1.5 m × 0.25 mm I.D. section of the GC column (HP5MS, 0.25-μm film, Hewlett-Packard), which served as a retaining precolumn. An early solvent vapour exit was inserted between the retaining precolumn and the GC column (HP5MS, 28 m×0.25 mm I.D., 0.25-μm film) to remove most of the solvent vapour, as described earlier [3]. A 0.32 mm I.D. fused-silica capillary was used to connect the press-fit T-splitter (BGB Analytik) between the retaining precolumn and the GC column with the solvent vapour exit. The laboratory-made solvent vapour exit was installed on the top of the split/splitless injector and kept at 150°C to prevent solvent condensation. The solvent vapour exit was closed just before the last μl of solvent were evaporated. The GC oven temperature was kept at 75°C for 3 min, and then increased to 280°C at 20°C/min, and kept at 280°C for 5 min. The initial head pressure was 90 kPa and was programmed to provide a constant flow. Ionization was achieved by electron impact, and ions with m/z 47–335 were monitored at 1.5 scans/s. The electron multiplier voltage was set at 1800 V.

3. Results and discussion

3.1. Development of at-line SPE–GC procedure

The set-up of the SPE module of the PrepStation (cf. Fig. 1) resembles an on-line SPE–GC system apart from the transfer module to the GC. The size of the precolumn (14.3×4.2 mm I.D.) is 2–6 times larger than those typically used in on-line SPE–GC approaches. In on-line SPE–GC [2–4], the SPE procedure for the determination of micropollutants in aqueous samples involves (i) preconditioning of the SPE cartridge with ethyl acetate and water, (ii) sampling, (iii) clean-up with water, (iv) drying of the SPE cartridge with nitrogen, and (v) desorption with ethyl acetate. Contrary to on-line SPE–GC, in at-line SPE–GC after addition of an internal standard, an aliquot of the extract is injected into the GC system by the autosampler.

A polystyrene-polyvinylbenzene copolymer, PLRP-S, was preferred as the packing material for the SPE cartridge because of its (i) high break-through volumes for more polar analytes, and (ii) a shorter drying time necessary to remove water compared to silica-based materials. As only commercial SPE cartridges containing silica-based material were available for the PrepStation, it was necessary to use laboratory-packed cartridges. Ethyl acetate was chosen as the desorption solvent, because it desorbs analytes covering a wide range of polarity from PLRP-S-packed SPE cartridges [10]. A drying time of 30 min with a nitrogen pressure of 270 kPa was necessary to remove all water from the SPE cartridge. This is a critical aspect because no traces of water can be tolerated in the extract to be injected into the GC. The efficient removal of water was checked by transferring ethyl acetate through the dried cartridge into hexane [11]. No formation of small water droplets or opaque colouring was observed. FID detection was preferred for method development as it is a universal detection mode and its sensitivity is comparable with that of full-scan mass-selective detection.

When the PrepStation was introduced on the market, it was intended for samples taken from 1.8 ml autosampler vials with 5 μl injections out of the extract finally obtained. Therefore, in a first experiment intended to explore the potential of the instrument, a 1.5-ml HPLC-grade water sample was analyzed which had been spiked at the 170 μg/l level with four triazines, atrazine, trietazine, terbutryn and cyanazine. As the dead volume between the SPE cartridge and the tip of the PrepStation needle is about 0.25 ml and the internal volume of the SPE cartridge 200 μl, the analytes had to be desorbed with 600 μl of ethyl acetate. A 5-μl aliquot was injected via the autosampler into the GC–FID. All four triazines showed up with recoveries of 90–95% (see Fig. 3C below). The R.S.D. values were 1–3% ($n=6$), and the determination limit ($S/N=10$) was 130 pg injected into the GC or 15–30 μg/l for a 1.5 ml HPLC-grade water sample.

Since the determination of micropollutants in surface and drinking water is required at the 0.1–1 μg/l level, the analyte detectability of the whole SPE–GC procedure obviously had to be improved. This required three steps: (i) increase the sample volume, (ii) inject a larger aliquot of the extract, and

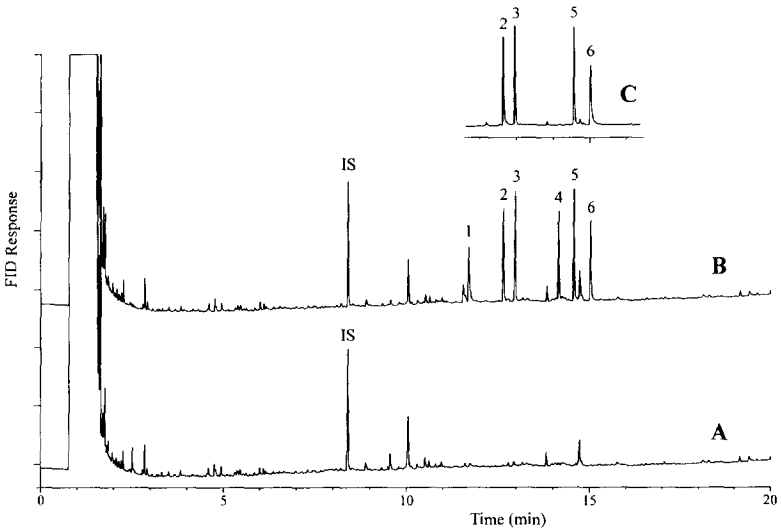

Fig. 3. PrepStation–GC–FID chromatogram obtained after preconcentration of 50 ml of: (A) river Rhine water, and (B) river Rhine water spiked at the 5 μg/l level with six triazines. The insert (C) shows a PrepStation–GC–FID chromatogram of a 1.5 ml HPLC-grade water sample spiked at the 170 μg/l level with four triazines. In all cases, 5 μl out of the 600 μl of organic extract were injected. Peak assignment: IS, internal standard (4,4′-difluorobiphenyl); 1, desethylatrazine; 2, atrazine; 3, trietazine; 4, simetryn; 5, terbutryn; 6, cyanazine.

(iii) decrease the desorption volume. Increasing the sample volume to 50 ml was a viable approach, because for most analytes no breakthrough will occur with the cartridge in use. Ideally, this 30-fold increase in sample volume should lead to a determination limit of 0.4–0.9 μg/l. By increasing the injected volume of the extract from 5 to 50 μl, the determination limit can be improved another 10-fold to below 0.1 μg/l, i.e. 0.04–0.09 μg/l. Obviously, in this calculation it is assumed that the recoveries and the absolute detection limit will remain constant, i.e. that the noise will not increase. Finally, a two-fold decrease of the desorption volume allows a further two-fold improvement of the determination limit, or, alternatively, a two-fold reduction of the sample size or injection volume (e.g. 25 μl instead of 50 μl).

As regards the practical implementation of the above suggestions, the autosampler will require some modification to increase the injection volume to 25 or 50 μl, as the original autosampler is designed only for the injection of up to 5 μl. A reduction of the desorption volume will be possible by decreasing the size of the SPE cartridge and, thus, the amount of stationary phase.

Table 2
Recoveries, repeatability ($n=6$) and limits of determination ($S/N=10$) for at-line SPE–GC–FID analysis of 50 ml of HPLC-water spiked at 5 μg/l level; 5 μl out of 600 μl of organic extract were injected

Analytes	Recovery (%)	R.S.D. (%)	Determination limit (μg/l)
Desethylatrazine	91	2	1.0
Atrazine	94	3	0.4
Trietazine	95	2	0.4
Simetryn	91	3	0.7
Terbutryn	94	2	0.4
Cyanazine	93	6	0.8

3.1.1. Increase of sample volume

Sampling of volumes larger than 1.5 ml has to be done via the solvent selection valve of the PrepStation and the syringe pump. Six triazines were now used as test compounds instead of four. Fig. 3A and B indicate that the determination limit is 1 μg/l or better, when analysing 50 ml of river Rhine water (sampled at Lobith, Netherlands; August 2, 1994). This increase of sample volume did not lead to a breakthrough of all six triazines, the recoveries were 91–95% (Table 2). Comparable peak areas were obtained as for the analysis of a 1.5-ml sample (Fig.

3C), in which the same absolute amounts of four triazines were preconcentrated. For the analysis of 50 ml of HPLC-grade water the analyte detectability showed the expected 30-fold improvement compared with a 1.5-ml sample, i.e. from 15–30 μg/l to 0.4–1.0 μg/l ($S/N=10$). 5 μl were injected out of the 600 μl of organic extract. When using non-selective FID detection, the determination limit for river water appeared to be 0.6–1 μg/l due to the presence of interfering compounds (Fig. 3A and B). Repeatability was good, with R.S.D. values of 2–6% ($n=6$) (Table 2).

OPPs

Next to the analysis of the medium polar triazines, the trace-level determination of the OPPs which cover a wider polarity range is of distinct importance in environmental analysis. When analysing a 50 ml HPLC-grade water sample spiked at the 5 μg/l level, five out of the ten OPPs showed recoveries of 92–96% (method 1; Table 3). The recoveries were somewhat lower for fenchlorphos, pyrazophos and coumaphos (81–85%), and significantly lower for bromophos-ethyl and ethion (66–67%). The recovery decreased with decreasing polarity of the analytes, which implies that this is due to adsorption on the walls of the tubing between sample bottle and sample valve [3]. Actually, the losses due to the well-known adsorption correlated rather well with the octanol–water partition coefficients, which reflect the hydrophobicity of the analytes (Table 3).

Three options were tested to prevent the losses due to adsorption. Preflushing of the tubing with sample up to the sample valve before the sampling step increased the recoveries of the more apolar OPPs 1–5% (method 2; Table 3). The tubing between the sample bottle and the solvent selection valve had been shortened as much as possible, but this did not have a significant influence on the recoveries. Flushing the syringe and the tubing between the solvent selection valve and the sample valve with 5 ml of methanol–water (30:70, v/v) after the sampling step gave an even better result (1–12% increase of recoveries) (method 3; Table 3). Recoveries over 90% could be achieved for all OPPs by adding 30% (v/v) of methanol to the sample solution prior to sampling (method 4; Table 3). However, as is to be expected, the addition of an organic modifier to the

Table 3
Recoveries of selected OPPs and triazines using four methods [a] to overcome analyte loss due to adsorption

Compound	log K_{ow} [b]	Recovery (%) using four methods			
		1	2	3	4
	Preflush volume (ml)	0	25	0	0
	MeOH (%, sample)	0	0	0	30
	MeOH (%, clean-up)	0	0	30	0
OPPs					
Sulfotep		96	92	91	91
Diazinon	3.3 [c]	93	94	94	99
Fenchlorphos	4.9 [c]	83	85	87	95
Parathion-ethyl	3.8 [c]	92	92	93	94
Bromophos-ethyl	5.9 [c]	66	67	72	92
Tetrachlorvinphos	3.5 [c]	97	97	96	95
Ethion	5.1 [c]	67	72	79	95
Triazophos	3.6 [c]	93	93	93	97
Pyrazophos		81	83	91	91
Coumaphos		85	87	92	95
Triazines					
Desethylatrazine	1.5 [c]	84			0
Atrazine	2.7 [d]	94			95
Cyanazine	2.2 [d]	92			71

[a] Sample: 50 ml HPLC-water spiked at 5 μg/l level, 5 μl out of 600 μl of organic extract injected.
[b] K_{ow}=octanol–water coefficient.
[c,d] Values taken from [15] and [16], respectively.

sample decreased the breakthrough volumes which had adverse effects especially for the more polar analytes: for a 50 ml sample and with the cartridge in use, cyanazine showed a recovery of only 71%, and desethylatrazine was completely lost. Obviously, if apolar analytes have to be determined in one run next to rather polar ones, no modifier should be added to the sample and preflushing the tubing with sample prior to sampling probably is the best option. Admittedly, the recoveries of the more apolar analytes then inevitably will be somewhat lower — but they will still be quite reproducible, as is demonstrated by the results reported in Table 5 below.

3.1.2. Increase of injection volume

The HP autosampler is in principle designed for on-column injections of up to 5 μl only. Such an injection is done in less than 2 s. However, this is also the case when injecting a larger volume, e.g. 25 or 50 μl. Therefore, the latter type of injection is referred to as "at-once", i.e. only a small part of the sample solvent is evaporated during the injection, while the major part is spread as a film in the retention gap and evaporated after the injection. The retention gap has to be long enough to prevent the solvent film from reaching the analytical column, otherwise distorted peaks will be obtained [12]. A 3 m-long or 6 m-long retention gap was used when injecting 25 or 50 μl, respectively, as the flooded zone of ethyl acetate for a DPTMDS-deactivated retention gap with 0.53 mm I.D. is about 11 cm/μl [10,13].

Two aspects appeared to be critical for large-volume injections with the HP autosampler and were, therefore, studied: (i) the occurrence of solvent backflush into the injector during injection or after injection during withdrawal of the syringe, and (ii) the repeatability of the large-volume injections. To study the former aspect, 50 μl injections were made manually. The syringe was withdrawn either immediately after the injection, as is done by the unmodified HP autosampler, or with a post-injection delay time of 6 s between the end of injection and withdrawal of the syringe. Solvent backflush, which caused a tailing solvent peak and a noisy baseline of the FID chromatogram, was observed in the former case, whereas with a post-injection delay of 6 s, no solvent backflush was observed for more than 50 injections. The solvent backflush is, in all probability, due to solvent which is left between the syringe and the retention gap with large-volume injections, and which is pulled back into the injector if the syringe is withdrawn immediately after the injection [14]. When a post-injection delay is introduced, this solvent is pushed further into the retention gap by the carrier gas. Satisfactory R.S.D. values of less than 2% ($n=10$) were obtained for an alkane standard in ethyl acetate (Table 4). However, it is not possible to program any post-injection delay time with the original HP autosampler, because the syringe is immediately withdrawn after the injection. A post-injection delay time could only be programmed using an autosampler with a modified EPROM, which was obtained from Hewlett Packard. With this autosampler and a post-injection delay time, no solvent backflush was observed (Fig. 4).

One further remark should be made. A small gas bubble of approximately 5 μl was left in the syringe after filling the syringe for a 50-μl injection. Even though the size of the gas bubble was found to be reproducible, its formation was thought not to be convenient. Further study showed that the use of a syringe needle with an internal diameter of 0.27 mm I.D. instead of 0.09 mm I.D. nearly completely eliminated the formation of a bubble. It should be added that the PTFE-tipped plunger of the syringe should be always at least slightly wet, that is, preflushing with sample or solvent during the autosampler procedure is recommended. During the rest

Table 4
Comparison of repeatability of large-volume injections [a] with autosampler

Alkane	R.S.D. (%, $n=10$) for injection volume of:	
	40 μl (manual)	50 μl (autosampler [b])
C_9	2.0	0.8
C_{10}	2.0	0.4
C_{11}	2.0	0.8
C_{12}	2.0	0.3
C_{13}	2.0	0.3
C_{14}	1.5	0.3
C_{15}	0.5	0.4

[a] Post-injection delay time, 6 s.
[b] Autosampler with modified EPROM and injection needle with 0.27 mm I.D.

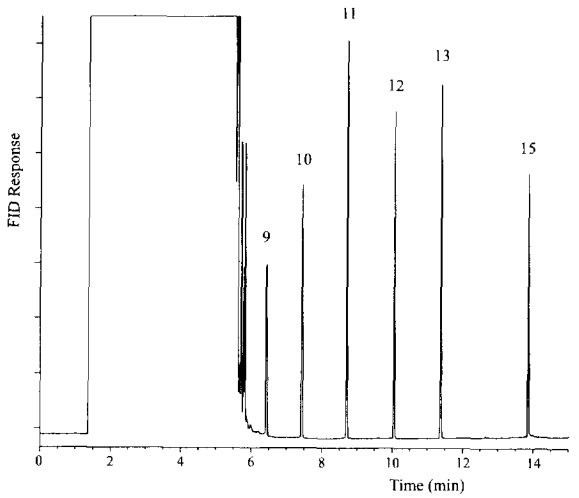

Fig. 4. GC–FID chromatogram of a 50-μl injection of a solution of *n*-alkanes in ethyl acetate using the autosampler. A post-injection delay time of 6 s was used. The eleventh injection is shown. Peak assignment: the numbers indicate the number of carbon atoms.

of the study, only syringes with wide-bore needles were used. The repeatability for 50-μl injections was good with R.S.D. values around 0.5% ($n=10$) (Table 4).

Unfortunately, when injecting a larger aliquot of the extract into the GC system, e.g. 50 μl out of 600 μl, peaks of compounds extracted from either the SPE cartridge or the septum of the autosampler vial, increasingly showed up in the chromatogram. As an example, Fig. 5 shows GC–FID chromatograms obtained for 50-μl injections out of 600 μl of ethyl acetate. In Fig. 5B, the solvent passed the dispensing needle of the PrepStation which pierced the septum. Obviously during the piercing some material of the septum remained at the end of the needle, which in its turn produced peaks in the chromatogram. As a result, the increase in analyte detectability expected on the basis of the increase of the injection volume could not be achieved completely. Various types of septa from other manufacturers were also tested, but with none of them a good blank chromatogram was obtained. The blank problem could be drastically decreased by 6-h Soxhlet extraction of the septa with chloroform (Fig. 5A).

In Fig. 5D, the solvent was transferred through the untreated SPE cartridge into a septum-less autosampler vial prior to injection. The interfering groups of peaks showing up in the chromatograms are mainly due to plasticizers present in the polymeric constituents of the SPE cartridge. As a consequence, increase of the injection volume did not result in the expected increase of analyte detectability. 6 h Soxhlet extraction was successfully used to clean the cartridge prior to use, which resulted in a good blank

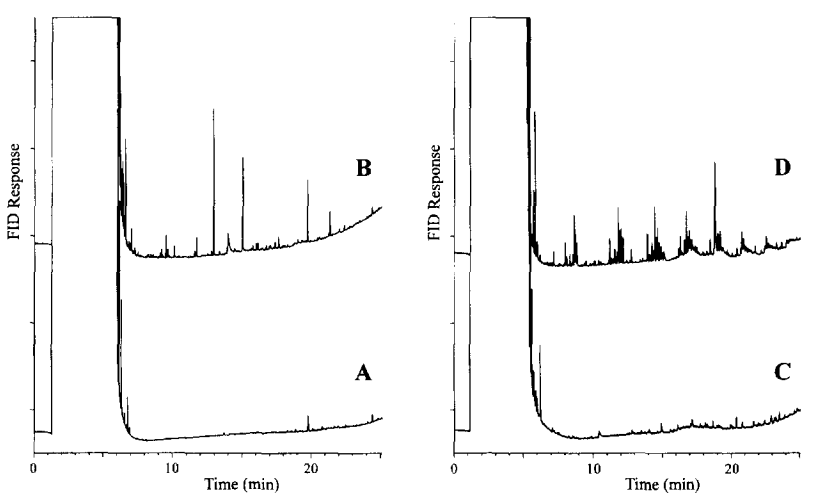

Fig. 5. PrepStation–GC–FID chromatograms obtained by transferring 600 μl of ethyl acetate by means of the PrepStation to an autosampler vial through a septum, (A) with, and (B) without precleaning, and through an empty SPE cartridge, (C) with, and (D) without precleaning. In the latter two instances, there was no septum on the autosampler vial. 50 μl were injected into the GC–FID system.

chromatogram (Fig. 5C). The cartridge used for this test was an empty one and it was disassembled for the Soxhlet extraction.

With pre-cleaned septa and cartridges going from 5 to 50 μl injections typically effected a 5–10-fold increase in analyte detectability.

3.1.3. Decrease of desorption volume

The desorption volume was determined for the larger, commercial cartridge (internal volume of 200 μl) and the smaller home-made one (44 μl). 50 ml of HPLC-grade water samples spiked at 25 μg/l with the test mixture of OPPs were analyzed, and 5 μl of the extract injected.

When using the larger, commercial cartridge, almost all OPPs were recovered quantitatively with 200 μl of ethyl acetate. However, compounds as apolar as bromophos-ethyl, required 400 μl. In practice, 600 μl of solvent were used to be on the safe side. With the smaller home-made cartridge, 150 μl of ethyl acetate were sufficient to desorb all OPPs quantitatively, only bromophos-ethyl required 200 μl of ethyl acetate; in practice, 300 μl were used. Since the desorption volume with the smaller cartridge was two-fold lower, this cartridge was preferred for further work, as it allows a two-fold decrease of the injection volume or, in the ideal case, a two-fold improvement of the determination limit.

3.1.4. Final at-line SPE–GC procedure

Several further parameters of the at-line SPE–GC procedure had to be optimized using the smaller home-made cartridge, i.e. the preflushing volume and the sampling and desorption speed. The main criteria were high recoveries and short analysis times, the former one being considered more important. The smaller home-made cartridge was used. A 0.6 μg/l test mixture of triazines and OPPs (same analytes as in Table 5 below) was analyzed and 25 μl of the extract injected.

The preflushing volume was varied from 5 to 20 ml at a flow-rate of 5 ml/min. At the 0.6 μg/l level preflushing had a somewhat larger effect than at the 5 μg/l level discussed above. The recoveries of the more apolar OPPs, bromophos-ethyl, pyrazophos and coumaphos, increased about 10% to 58%, 71% and 88%, respectively, when increasing the preflushing volume from 5 to 10 ml; a further increase to 20 ml did not cause a significant improvement any more. A preflushing volume of 10 ml was, therefore, selected for all further work.

Table 5
Analytical characteristics of at-line SPE–GC–FID of HPLC-grade water spiked at 0.6 μg/l level; 25 μl out of 300 μl organic extract injected

Analyte	Recovery (%)	R.S.D. (n=6) (%)	Linearity [a] R^2	LOD [b] (μg/l)
Desethylatrazine	87	5	0.9971	0.3
Sulfotep	86	3	0.9978	0.1
Atrazine	97	2	0.9995	0.1
Trietazine	105	3	0.9998	0.05
Diazinon	97	2	0.9980	0.05
Simetryn	101	3	0.9995	0.1
Terbutryn	91	2	0.9996	0.05
Cyanazine	90	3	0.9999	0.1
Bromophos-ethyl	56	4	0.9954	0.1
Tetrachlorvinphos	103	4	0.9951	0.2
Pyrazophos	70	5	0.9987	0.1
Coumaphos	78	6	0.9963	0.1

[a] Concentration range: 0.09–3 μg/l, six data points.
[b] Calculated from sample spiked at 0.09 μg/l level, LOD=limit of determination (S/N=10).

Varying the sampling flow-rate from 1 to 10 ml/min did not influence the recoveries of the test compounds. A flow-rate of 5 ml/min was used to prevent back-pressure due to the cartridge becoming too high.

A desorption flow-rate of 100–200 μl/min did not influence the recoveries. Actually, no effects were observed for flow-rates up to 800 μl/min for any test compound except bromophos-ethyl. For this analyte, the recovery decreased some 20% upon going from 200 to 800 μl/min. A flow-rate of 200 μl/min was, therefore, selected.

The time required for the total sample preparation procedure of the PrepStation was about 90 min. This comprised a sampling time of 20 min and a drying time of 30 min; the other steps of the procedure took about 20 min. The difference between the 70 min so calculated and the actual SPE time is due to the fact that some operations of the PrepStation are rather slow, e.g. positioning and transport of vials or the cartridge, lifting the sealing probe of the SPE cartridge and the dispensing needle for exchange of the cartridge and/or vial or flushing of the dispensing needle. During the PrepStation procedure the

analysis of the previous extract plus an injection of a standard solution were performed.

3.2. Performance of at-line SPE–GC

The performance of the at-line SPE–GC procedure was tested by analysing 50 ml HPLC-grade water samples spiked with 0.6 μg/l of several triazines and OPPs (Fig. 6). 25 rather than 50 μl out of the 300 μl extract were injected. The repeatability was good for all analytes with R.S.D. values of 2–8% (Table 5). A 25-μl on-column standard injection was used as the reference for the calculation of the recoveries. Slight variations of the extract volume were compensated for by the use of an internal standard, which was added to the extract before injection.

The recoveries were above 85% for nine out of the twelve test compounds. The somewhat lower results for pyrazophos and coumaphos (70–80%) and especially bromophos-ethyl (56%) can be explained on the basis of their hydrophobicity (cf. above). The linearity over the relevant range of 0.09–3 μg/l was satisfactory for all tested analytes (see Table 5). The analyte detectability of the total procedure was studied by analysing 50 ml of HPLC-grade water spiked at the 0.09 μg/l level. The limits of determination ($S/N=10$) were between 0.05 and 0.1 μg/l for all analytes except tetrachlorvinphos (0.2 μg/l) and desethylatrazine (0.3 μg/l) (Table 5). The determination limit of desethylatrazine is higher because this compound is easily adsorbed on the walls of the retention gap, when the deactivation layer is partly destroyed. The improvement of the determination limit found experimentally is similar to that calculated: analyte detectability has been improved from 15–30 μg/l to 0.05–0.2 μg/l upon a 30-fold sample volume increase, a five-fold increase of the injection volume and a two-fold decrease of the desorption volume.

3.3. Application: analysis of river water

The potential of the at-line SPE–GC system was demonstrated by determining OPPs in 50 ml of river Meuse water (sampled at Eysden, Netherlands; September 6, 1994), spiked at the 0.5 μg/l level. In this instance, a large SPE cartridge was used and a 50-μl aliquot out of the 600-μl extract injected into the GC–FID system. All OPPs except bromophos-ethyl (57%) and ethion (69%) showed recoveries of 90% or better. Although the detection of the test analytes is obviously hampered by the presence of many interfering compounds in the surface water sample — which all show up in FID detection — the limits

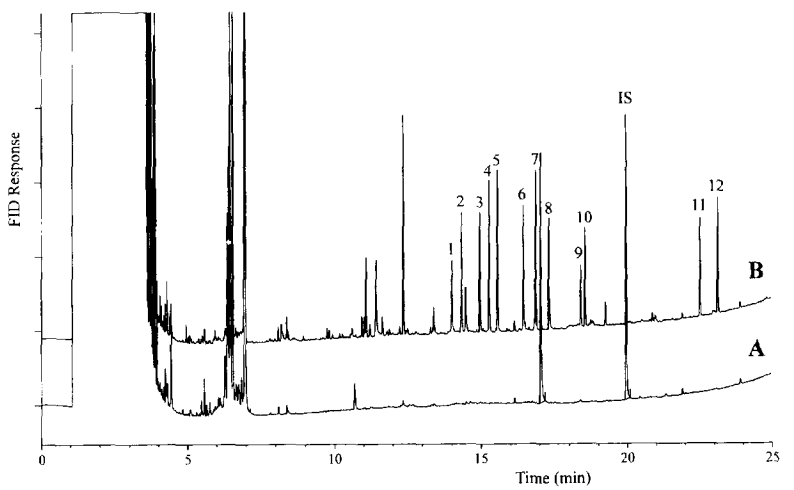

Fig. 6. PrepStation–GC–FID chromatogram obtained after preconcentration of 50 ml HPLC-grade water, (A) without, and (B) with spiking at the 0.6 μg/l level. 25 μl out of the 300 μl extract were injected. Peak assignment: IS, internal standard (N,N-dibenzylaniline); 1, desethylatrazine; 2, sulfotep; 3, atrazine; 4, trietazine; 5, diazinon; 6, simetryn; 7, terbutryn; 8, cyanazine; 9, bromophos-ethyl; 10, tetrachlorvinphos; 11, pyrazophos; 12, coumaphos.

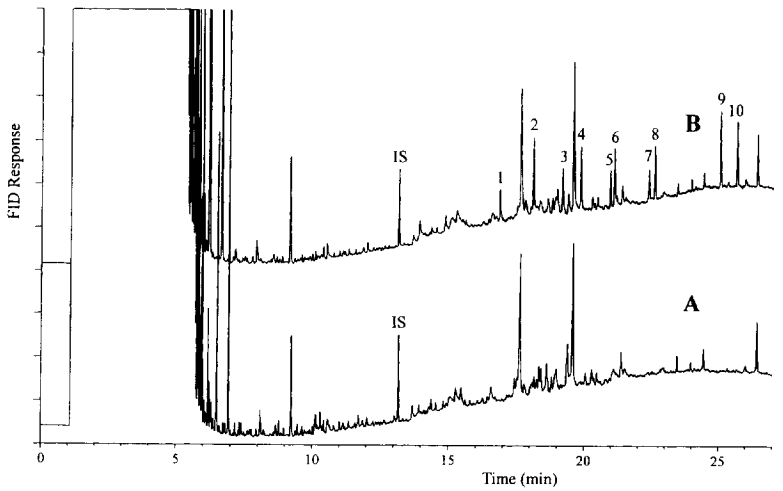

Fig. 7. PrepStation–GC–FID chromatogram of 50 ml river Meuse water, (A) without, and (B) with spiking at the 0.5 μg/l level with OPPs. 50 μl out of the 600 μl extract were injected. Peak assignment: IS, internal standard (4,4′-difluorobiphenyl); 1, sulfotep; 2, diazinon; 3, fenchlorphos; 4, parathion-ethyl; 5, bromophos-ethyl; 6, tetrachlorvinphos; 7, ethion; 8, triazophos; 9, pyrazophos; 10; coumaphos.

of determination ($S/N=10$) typically were 0.2–0.7 μg/l (Fig. 7). None of the spiked OPPs were found to be present in the samples at this level. Compared to the limits of determination for the analysis of HPLC-grade water of 0.05–0.2 μg/l, an approximately five-fold loss of sensitivity is found, as is to be expected for real-life work using FID detection.

Obviously, a more selective detector is preferred for the determination of micropollutants in river water at a level of around 0.5 μg/l or below. One example is the analysis of 50 ml of river Meuse water (sampled at Eysden, The Netherlands; September 6, 1994) spiked with 0.5 μg/l of several triazines. 50 μl out of the 600-μl extract were injected into a GC–MS system. Again it is clear that the compounds present in the river water will start to interfere with the determination of the test analytes in the full-scan chromatogram at around the 0.1–0.3 μg/l level (Fig. 8A and B). When using the reconstructed ion chromatogram of a characteristic mass of each analyte instead, the determination limits improve about ten-fold. As an example, the reconstructed ion chromatograms of ion $m/z=200$ for the spiked and non-spiked sample are shown in Fig. 8C and D, respectively. From these chromatograms the limits of determination for atrazine and trietazine can be calculated to be approximately 0.03 μg/l, and the presence of atrazine in the river water sample is clearly revealed (concentration, 0.07 μg/l; Fig. 8C). The presence of atrazine could be confirmed by the presence of all four characteristic diagnostic ions of atrazine ($m/z=200$, 202, 215 and 217).

No experimental problems with the PrepStation–GC were observed during the analysis of more than 100 river water samples.

4. Conclusions

A fully automated at-line SPE–GC–FID procedure was developed for the determination of micropollutants in aqueous samples using the PrepStation. The system combines several advantages of on-line and off-line SPE–GC: an integrated and closed system is used and, because only an aliquot, although a large one, is injected, the remaining extract is available for another GC analysis. Once the SPE–GC procedure has been developed, no reoptimization is necessary, e.g. with regard to compensation of slight changes of the dead volume of the cartridge or of the evaporation rate. The obvious disadvantage of the commercial unit is that the typical determination limits required for environmental analysis of 0.1–1 μg/l can not be obtained. Therefore, the sample volume was increased to 50

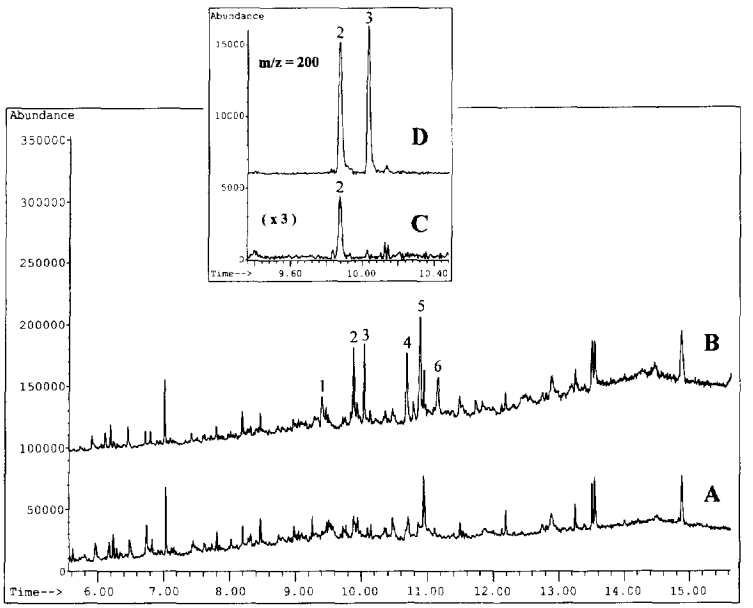

Fig. 8. Full-scan PrepStation–GC–MS chromatogram of 50 ml river Meuse water, (A) without, and (B) with spiking at the 0.5 μg/l level. 50 μl out of the 600 μl extract were injected. The insert shows the reconstructed ion chromatogram of *m/z* 200, (C) without, and (D) with spiking at the 0.5 μg/l level. The reconstructed ion chromatogram of the non-spiked water sample is blown up by a factor of 3. Peak assignment: 1, desethylatrazine; 2, atrazine; 3, trietazine; 4, simetryn; 5, terbutryn; 6, cyanazine.

ml, the injection volume was increased to 25–50 μl and the extract volume decreased to 300 μl. This means that, per injection into the GC, up to 15–20% of the mass of the analytes in the aqueous sample are transferred to the GC. For an increase of the injection volume the HP autosampler had to be adapted such that a post-injection delay time could be used. The injection of volumes up to 50 μl was "at-once" and required no further optimization, except for the use of a retention gap of up to 6 m length. No (usually critical) evaporation of a part of the extract was necessary.

The analytical data of the PrepStation–GC–FID were satisfactory. To quote an aspect of primary importance, the determination limits ($S/N=10$) for a series of well-known pesticides were between 0.05–0.2 μg/l for the analysis of HPLC-grade. As was to be expected, with river water these values increased, viz. to 0.2–0.7 μg/l. Still, these are below the threshold value of 1 μg/l commonly used as the alert level for surface water. Much-improved performance can be obtained with a selective detector, and preliminary work using a PrepStation–GC–MS indeed showed determination limits of 0.02–0.04 μg/l for the determination of the same analytes in river water.

The modified at-line SPE–GC system with its rather robust set-up and ease of operation offers a promising route to determine, and identify, micropollutants in aqueous samples at the sub-μg/l level using moderate sample volumes only. Future research will be focused on the determination of micropollutants at low levels using primarily MS detection. An attempt will be made to improve the set-up in such a way that no precleaning of the cartridges and/or septa of the autosampler vials will be necessary any more.

Acknowledgments

The authors thank the European Union for their support to Th. Hankemeier via a Human Capital and Mobility grant (No. EV5V-CT-93-5225). The Rhine Basin Program (Amsterdam/Waldbronn) is acknowledged for providing the PrepStation.

References

[1] I. Liska, J. Chromatogr. A, 655 (1993) 163.
[2] A.J.H. Louter, U.A.Th. Brinkman and R.T. Ghijsen, J. Microcol. Sep., 5 (1993) 3031.
[3] Th. Hankemeier, A.J.H. Louter, F.D. Rinkema and U.A.Th. Brinkman, Chromatographia, 40 (1995) 119.
[4] A.J.H. Louter, C.A. van Beekevelt, P. Cid Monañes, J. Slobodník, J.J. Vreuls and U.A.Th. Brinkman, J. Chromatogr. A, 725 (1996) 67.
[5] H.G.J. Mol, H.-G. Janssen, C.A. Cramers, J.J. Vreuls, U.A.Th. Brinkman, J. Chromatogr. A, 703 (1995) 277.
[6] K. Grob, in W. Bertsch, W.G. Jennings and R.E. Kaiser (Editors), On-Column Injection in Capillary GC, Hüthig, Heidelberg, 1987.
[7] F. Munari, P.A. Colombo, P. Magni, G. Zilioli, S. Trestianu and K. Grob, J. Microcol. Sep., 7 (1995) 403.
[8] S. Ramalho, Th. Hankemeier, M. de Jong, U.A.Th. Brinkman and J.J. Vreuls, J. Microcol. Sep., 7 (1995) 383.
[9] K.D. Fogelman, Lab. Robotics Automation, 5 (1993) 103.
[10] J.J. Vreuls, W.J.G.M. Cuppen, G.J. de Jong and U.A.Th. Brinkman, J. High Resolut. Chromatogr., 13 (1990) 157.
[11] J.J. Vreuls, R.T. Ghijsen, G.J. de Jong and U.A.Th. Brinkman, J. Chromatogr., 625 (1992) 237.
[12] K. Grob Jr., G. Karrer and M.-L. Riekkola, J. Chromatogr., 334 (1985) 129.
[13] K. Grob, in W. Bertsch, W.G. Jennings and P. Sandra (Editors), On-line Coupled LC–GC, Hüthig, Heidelberg, 1991, pp. 209–211.
[14] K. Grob Jr., J. Chromatogr., 283 (1984) 21.
[15] A. Noble, J. Chromatogr., 642 (1993) 3.
[16] D. Brown and E. Flagg, J. Environ. Qual., 10 (1981) 382.