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On-line combination of automated micro liquid–liquid extraction and capillary gas chromatography for the determination of pesticides in water

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ABSTRACT

The determination of pesticides in water is often based on liquid–liquid extractions combined with concentration by evaporating the organic solvent followed by analysis with capillary GC. The use of selective detection such as thermionic detection (NPD) or flame photometric detection (FPD) makes the use of additional clean-up unnecessary in many instances. To obtain detection limits in the sub-ppb range with these detectors, typically the equivalent of approximately 1 ml of sample is injected. Hence, micro-extraction techniques, transferring the pesticide content of 1 ml of aqueous sample to a capillary GC are feasible. In this study, micro liquid–liquid extraction with methyl *tert.*-butyl ether was combined with GC–FPD in a fully automated set-up, using GC sample introduction volumes of 500 μl, which were transferred via an on-column interface equipped with an early vapour exit. The organophosphorus pesticides diazinon, chlorpyriphos-methyl, malathion, chlorpyriphos-ethyl, chlorfenvinphos-*cis*, bromophos and azinphos-ethyl were determined in pond water spiked at the 0.5 μg/l level. In most cases recoveries were over 70%, while the detection limit allowed quantification at the level of the EC maximum residue limits for water intended for human consumption (0.1 μg/l). This communication demonstrates the practicality of an on-line micro liquid–liquid extraction procedure which eliminates the need to use a phase separator, resulting in a set-up robust also in the hands of relatively inexperienced personnel.

INTRODUCTION

Recent developments in LC–GC coupling open new ways for on-line sample handling in capillary GC. In particular, the solvent evaporation LC–GC interfaces equipped with an early vapour exit developed by Grob and co-workers [1,2], which allow the introduction of almost any volume of solvent in a gas chromatograph, are extremely powerful.

The use of sample enrichment on non-polar solid phases coupled with LC–GC-type large-volume injections is described by Noroozian *et al.* [3] and more recently by Vreuls *et al.* [4]. Other approaches to automated sample enrichment coupled to gas chromatography are dis-

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cussed by Zlatkis [5] and Kaiser and Rieder [6], who described the extraction of analytes into the stationary phase film of the GC column. Major drawbacks of these approaches concerned low extraction efficiency (slow diffusion process) and poor reconcentration before on-line GC analysis (low phase ratio).

This paper deals with liquid–liquid extraction techniques coupled to capillary GC. Today, the gas chromatographic analysis of pesticides in aqueous environmental samples is focused on nitrogen- and phosphorus-containing pesticides, which are either deemed to reach the groundwater due to their mobility or found in surface water due to their extensive use. Detection with the readily available selective thermionic (NPD) and flame-photometric (FPD) detection systems is relatively simple. Owing to the selectivity of these detectors clean-up procedures by using, for example, adsorption chromatography can usually be omitted. In LC–GC this means that it is not necessary to use the LC part in a LC–GC system for clean-up. Standard procedures for the determination of nitrogen and phosphorus pesticides involve liquid–liquid extraction or solid-phase extraction of large sample volumes, typically 500–1000 ml, with appropriately volatile extraction solvents. In these procedures the extracts are concentrated down to a few millilitres, of which 1 or 2 μl are injected splitless or on-column into a capillary GC, thus introducing only about 0.1% of the original sample. LC–GC technology offers the possibility to inject larger samples, of the order of 1 ml, into a GC system. This gives the opportunity to combine the use of micro-extraction techniques and GC analysis. The comparison of conventional and micro-extraction techniques presented in Table I clearly illustrates the attractiveness of the latter approach.

Preference for either a liquid–liquid or a solid-phase extraction is primarily determined by the nature of the environmental problem under consideration. In wastewater and surface water analysis one is usually interested in the total sample, including pesticides adsorbed on particulate matter; the same is true for rain water if the total deposition is to be estimated. In groundwater analysis, however, one is usually interested in the liquid phase of the sample only. If one is interested in the contents of the total sample, liquid–liquid extraction is to be preferred, since one can handle the total sample without filtration.

TABLE I

COMPARISON OF CONVENTIONAL LIQUID–LIQUID EXTRACTION AND MICRO LIQUID–LIQUID EXTRACTION TECHNIQUES

Conventional extraction	*Micro extraction*
1000 ml water → 1 ml extract	1 ml water → 1 ml extract
1 μg/l → 1 μg/ml	1 ng/ml → 1 ng/ml
1 μl injection → 1 ng	1 ml injection → 1 ng

The disadvantages of conventional liquid–liquid extraction are: (i) the low sample throughput due to the laboriousness of first use, and then evaporating hundreds of millilitres of organic solvent and (ii) the waste problem created by the use of these amounts of organic solvent. If liquid–liquid extraction is preferred it is therefore highly attractive to use miniaturized extraction procedures which, in addition, are more easily automated.

Liquid–liquid extraction using segmented flow systems followed by flow injection-type phase separation coupled on-line with capillary GC has been utilized for chlorinated pesticides [7], aromatic hydrocarbons [8] and halocarbons [9], and for chlorinated anilines and carboxylic acids using a phase-transfer derivatization [10]. A similar approach was used for interfacing reversed-phase LC with capillary GC by on-line extraction of the analytes from the aqueous LC eluent into solvents of lower polarity [11].

Modern LC autosamplers are able to perform operations such as reagent addition, solvent mixing, collection and liquid–liquid extraction. This paper describes the application of such a sampler for micro liquid–liquid extraction coupled on-line with an LC–GC interface, thus providing the automated sample handling of aqueous environmental samples whilst eliminating the insertion of a phase separator.

EXPERIMENTAL

Chemicals

Pesticides with a purity of >99% were purchased from Promochem (Wesel, Germany). Stock solutions were prepared in acetone (Promochem, nanograde). Dilute solutions for direct LC–GC analysis were prepared in *n*-pentane (Baker resianalysed grade), methyl *tert.*-butyl ether (MTBE) (Baker HPLC grade) or dichloromethane (Promochem, nanograde). MTBE used in the LC pump was degassed ultrasonically under light vacuum each day.

Equipment

In order to perform automated micro liquid–liquid extractions the original Dualchrom 3000 LC–GC system was modified. The LC–GC equipment consisted of a Dualchrom 3000 HPLC–HRGC system from Carlo Erba Strumentazione (Milan, Italy) equipped with a Model 232 Bio autosampler from Gilson (Villiers-le-Bel, France), in combination with a Model 401 dilutor from Gilson equipped with a 5.0-ml syringe and 3.0-ml PTFE transfer tubing for solvent delivery and sample manipulation (Fig. 1). Since it is not necessary to use an LC column, the UV–Vis LC detector of the Dualchrom 3000 was also removed.

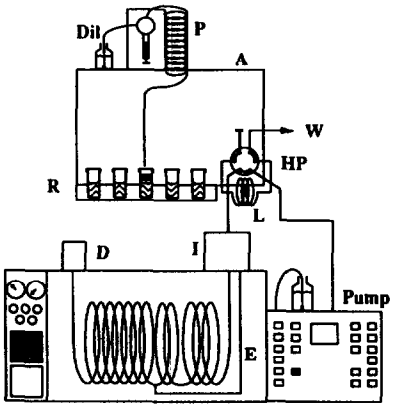

Fig. 1. Schematic representation of the equipment used for automated micro liquid–liquid extractions. A = Autosampler; Dil = dilutor; P = PTFE transfer tubing; R = rack with samples; HP = high-pressure six-way valve; L = 500-μl storage loop; W = waste; I = on-column interface; E = early vapour exit; D = flame photometric detector.

For sample introduction the on-column interface equipped with an early vapour exit using partially concurrent solvent evaporation of the Dualchrom was utilized.

Solvent evaporation was performed in a 6 m × 0.53 mm I.D. phenyl-silyl deactivated retention gap obtained from Gimex (Düren, Germany) connected via a pressfit connection to a 3 m × 0.32 mm I.D. DB-5 retaining precolumn with a film thickness of 0.25 μm (J&W Scientific, Folsom, CA, USA). The GC separation was achieved on a 22 m × 0.32 mm I.D. DB-5 capillary column with a film thickness of 0.25 μm obtained from J&W Scientific, which was coupled to the retaining precolumn by means of a three-way pressfit. The third exit of the three-way pressfit was connected to a 0.4 m × 0.32 I.D. fused-silica capillary connected to the early vapour exit. The oven temperature was programmed as follows: 65°C for 10 min, 20°C/min to 150°C, 5°C/min to 260°C, 30 min hold at 260°C. The helium inlet pressure was set at 100 kPa.

Detection was performed with a Model 700 flame photometric detector from Carlo Erba equipped with two photomultiplier tubes for phosphorus and sulphur detection, using filters of 526 nm and 394 nm, respectively. The detector body temperature was 180°C and the detector base temperature 300°C. The volumetric flow-rates of the flame gases hydrogen and air were set at 90 ml/min and 140 ml/min, respectively. A volumetric flow-rate of 22 ml/min helium was used as make-up gas.

Spiked samples were prepared from stock standard solutions in acetone, ensuring an acetone content of less than 1% in the final solution. All calibrations were performed by large-volume injections following essentially the same procedure as for the extracts of the samples.

RESULTS AND DISCUSSION

Instrumental set-up

The commercially available Dualchrom equipment provides two options for sample intro-

duction: the loop-type interface and the on-column interface. In this study the use of the on-column interface was preferred since this injection technique is more versatile with respect to the more volatile compounds, a group of compounds that is certainly relevant for the development of future applications.

Choice of extraction solvent

The selection of extractants used in micro-extraction techniques is based on the applicability of the solvent in the interface introduction in combination with its properties for the extraction of moderately polar pesticides from water. The GC introduction technique requires a low boiling solvent; the application of the technique to polar pesticides requires a relatively polar organic solvent. Technically a solvent with a density lower than water should be preferred in order to transfer the organic layer efficiently to the GC column. In early experiments diethyl ether was tested as extraction solvent. However, this resulted in difficulties with the handling of the liquid in the PTFE transfer coil of the autosampler (Fig. 1) due to evaporation of the solvent in the aspirator tubing. In order to prevent cross-contamination, an airplug is aspirated between diethyl ether and the solvent present in the remainder of the dilutor system. Apparently, the combination of the airplug with the evaporation of diethyl ether inside the transfer tubing causes overpressure in the PTFE transfer coil. Hence, diethylether is partially lost during the extraction procedure. MTBE was tested in the same procedure without the problems caused by undesired solvent loss during the manipulations performed by the autosampler. Apparently, the application of very volatile solvents in combination with solvent manipulation by an autosampler should be avoided due to solvent loss and solvent evaporation in the sample vial in which the extraction is performed. At this moment, the application of volatile solvents seems to be limited to closed on-line extraction systems in combination with a phase separator. With regard to automation, solvents with a density lower than water are manipulated by the autosampler more easily, without the risk of aspirating water which is situated below the extraction solvent.

Micro liquid–liquid extraction

De Ruiter *et al.* [12] used autosamplers for their micro liquid–liquid extractions. They described an extraction of phenolic steroids that were derivatized by a phase transfer-catalysed dansylation in a two-phase system consisting of an aqueous solution and dichloromethane or chloroform. By aspirating the mixture repeatedly in the coiled PTFE transfer capillary of the sampler, they created a segmented flow in which efficient extraction took place. In the quoted paper, the introduction of the segmented aqueous/organic mixtures into the capillary caused cross-contamination; therefore rinsing with acetone and water was necessary. To prevent this problem we performed the extraction procedure by aspirating the organic phase only.

Micro liquid–liquid extraction was carried out using 4-ml autosampler vials closed with a cap containing a PTFE inlay to prevent solvent evaporation during the process. These vials contained 1.5 ml of the water sample, to which the dilutor added 1.5 ml of MTBE. Automated liquid–liquid extraction was carried out by letting the dilutor aspirate —at the correct needle depth— 1.0 ml of solvent at a flow-rate of 100 μl/s. After raising the needle to a level of 4 ml, dispensing was performed at a flow-rate of 1600 μl/s, forcing the organic solvent into the water phase and thus extracting the pesticides. After 1 min —the time needed to separate the two immiscible phases— the procedure was repeated six times.

Optimization of GC introduction

After extraction, 1.0 ml of the organic fraction was injected through a 500-μl storage loop situated at the six-way valve of the autosampler (Fig. 1). The extract was transported from the loop to the on-column interface by means of the LC pump of the Dualchrom. In order to compensate for the volume of the transfer lines an additional 100 μl of MTBE were introduced, yielding a total injection volume of 600 μl of MTBE to be transferred into the GC column. Sample introduction took place over 3 min at a

flow-rate of 200 μl/min, using an oven temperature of 65°C and a helium pressure of 100 kPa. The required closure time of the early vapour exit was determined by igniting the solvent vapours leaving the exit tube.

Solvent vapours arrived after 14 s, counting from the moment the GC introduction commenced, which can be considered as the dead time of the retention gap and retaining precolumn. Flame extinction after GC introduction was observed after 267 s, closure of the early vapour exit was set at 282 s, a delay of 15 s after completion of solvent evaporation. Hence, the time needed for the evaporation of 600 μl of MTBE can be estimated correcting of the total sample introduction time (267 s) for the dead time of the retention gap/solvent vapour exit system (14 s), yielding an evaporation time of 253 s. The corresponding evaporation rate can be calculated by division of the volume introduced in GC (600 μl) by the evaporation time (253 s), which resulted in an evaporation rate of 142 μl/min MTBE, which is in agreement with the evaporation rate for MTBE found by Schmarr *et al.* [2]. The introduction rate (200 μl/min) leaves approximately 175 μl to be evaporated after completion of the sample transfer. It can be concluded that the flooded zone is rather high in comparison with those reported by other authors [2]. Prediction of the allowable flooded zone is complicated, because it depends on the wettability of the retention gap surface. The relatively low surface tension of ethers in combination with the retention gap used in this study partially explains the large flooded zone that can be handled. It should be mentioned here that too large a flooded zone should result in irregular flames during the experiments for the determination of the evaporation rate. However, no such effect was observed.

Application of automated micro liquid–liquid extraction

After stabilization of the LC–GC–FPD system, 4-ml vials containing aliquots of the pond-water samples were placed into a rack of the autosampler. Automated micro liquid–liquid extraction was performed by the autosampler, which applied the extraction procedure discussed above. Six-fold repetition of this procedure gave plateau conditions for the analyte recovery for all seven organophosphorus pesticides tested. Table II shows an event schedule of the whole procedure of automated micro liquid–liquid extraction coupled to GC–FPD.

Table III shows that the total on-line extraction–GC–FPD system showed good performance at the sub-μg/l level, with bromophos being a notable exception. The reason for this anomalous behaviour is as yet unknown.

Fig. 2 shows a chromatogram obtained for a 1.5-ml pond-water sample using the procedure described above. Detection was performed by means of dual-FPD detection; that is, chromatographic traces in the P- and S-mode are recorded simultaneously. Unfortunately, however, even with the Model 700 FPD system, the S-mode is not sensitive enough to detect sub-μg/l levels of the pesticides in the small sample volumes used.

Micro liquid–liquid extraction coupled on-line to a GC system is attractive for volatile pesticides, since losses due to evaporation can be minimized because one uses a closed system during the evaporation step. In order to test the applicability of this set-up to volatile pesticides, dichlorvos (DDVP), a volatile organophosphorus pesticide, was used as a model compound. The application of MTBE as solvent to introduce DDVP into GC turned out to be unsuitable: no peak appeared in the chromatogram, probably because of co-evaporation of the pesticide with the evaporating MTBE despite the use of an on-column interface.

As regards the selection of an alternative extractant, a distinctly polar solvent is required to extract the polar DDVP efficiently from water samples. Besides, because of GC introduction, the selected solvent should be low boiling. Dichloromethane appears to be the only sufficiently pure solvent available to meet these requirements. Unfortunately, the density of dichloromethane is higher than water, hence, the organic phase is situated below the aqueous sample phase. Therefore the organic phase has to be transferred through the aqueous phase, introducing a source of contamination, or even droplets of water.

Preliminary experiments applying liquid–liq-

TABLE II

EVENT SCHEDULE OF THE AUTOMATED LIQUID–LIQUID EXTRACTION OF WATER SAMPLES COUPLED ON-LINE TO GC–FPD

Step	Time (min:s)	Event(s) that take(s) place
1	0:00	LC autosampler starts extraction procedure, rinsing of the PTFE transfer tubing
2	0:30	Addition of 1.5 ml of MTBE to sample vial (first extraction), stabilization of two mixed phases
3	1:30	Aspiration of 1.0 ml of MTBE from sample vial
4	1:40	Dispensing of 1.0 ml of aspirated MTBE into sample vial (second extraction) and stabilization of two mixed phases
5	2:40	Aspiration of 1.0 ml of MTBE from sample vial
6	2:50	Dispension of 1.0 ml of aspirated MTBE into sample vial (third extraction) and stabilization of two mixed phases
7	3:50	Aspiration of 1.0 ml of MTBE from sample vial
8	4:00	Dispension of 1.0 ml of aspirated MTBE into sample vial (fourth extraction) and stabilization of two mixed phases
9	5:00	Aspiration of 1.0 ml of MTBE from sample vial
10	5:10	Dispension of 1.0 ml of aspirated MTBE into sample vial (fifth extraction) and stabilization of two mixed phases
11	6:10	Aspiration of 1.0 ml of MTBE from sample vial
12	6:20	Dispensing of 1.0 ml of aspirated MTBE into sample vial (sixth extraction) and stabilization of two mixed phases
13	7:20	Transfer of 1.0 ml of MTBE extract of the water sample to a 500-μl storage loop
14	7:30	Start of GC introduction of the MTBE extract and start of temperature programme
15	10:30	End of GC transfer of the MTBE extract
16	11:57	End of solvent evaporation
17	12:12	Closure of the solvent vapour exit
18	61:23	End of temperature programme, GC oven starts cooling.
19	68:00	GC oven stabilizes on introduction temperature while LC autosampler starts extraction cycle from step 1

TABLE III

TRACE-LEVEL DETERMINATION OF ORGANOPHOSPORUS PESTICIDES IN POND WATER USING ON-LINE MICRO LIQUID–LIQUID EXTRACTION–GC–FPD

No.	Compound	Spiking level (μg/l)	Recovery[a]					LOD[b] (μg/l)
			Extr. 1	Extr. 2	Extr. 3	Mean (%)	R.S.D. (%)	
1	Diazinon	0.50	107	98	102	102	5	0.02
2	Chlorpyriphos-methyl	0.56	85	74	80	80	5	0.01
3	Malathion	0.44	75	72	76	74	2	0.03
4	Chlorpyriphos-ethyl	0.65	75	67	73	72	4	0.02
5	Chlorfenvinphos-*cis*	0.43	105	80	97	94	13	0.09
6	Bromophos	0.43	44	42	43	43	1	0.05
7	Azinphos-ethyl	0.53	113	92	109	105	11	0.02

[a] Recovery values for the three separate extractions are given, as well as the mean recovery and the relative standard deviation (R.S.D.).

[b] Limit of determination defined as a signal-to-noise ratio of 3.

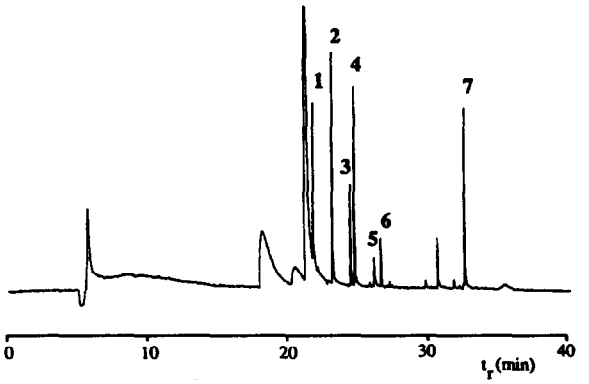

Fig. 2. Automated liquid–liquid extraction coupled on-line to GC–FPD analysis (P-mode; att. 16) of a pond-water sample spiked with seven organophosphorus pesticides at 0.5 μg/l, using MTBE as extraction solvent. For peak assignment, see Table II.

uid extraction with dichloromethane were performed: 1.5 ml of water sample were transferred into a vial of 4 ml, after which 1.5 ml of dichloromethane were added. This vial was shaken for 30 s, and subsequently 600 μl of the organic extract were transferred into an autosampler vial, which was placed in the autosampler. The dichloromethane extract was introduced into the GC system by means of an on-column interface at an oven temperature of 50°C using a dichlormethane flow-rate of 150 μl/min.

Water spiked with DDVP at the 0.4 μg/l level resulted in a chromatogram with a large solvent peak tailing up to a retention time of 20 min, probably caused by strong phase soaking of the stationary phase in the retaining precolumn, which resulted in a very broad DDVP peak.

Apparently, for the extraction of very volatile pesticides other extraction solvents are needed, because the use of dichloromethane can cause damage to the introduction system and the quartz windows of the FPD system. Also, soot formation inside the flame detector can occur. An additional drawback of dichloromethane is that its applicability for NPD is limited [11].

CONCLUSIONS

Micro-extraction techniques offer distinct advantages over conventional-size liquid–liquid extractions because of the ease of on-line coupling to LC–GC interfaces (and their automation), the increased sample throughput and the distinctly lower organic solvent consumption.

The present communication demonstrates the practicality of a simple micro liquid–liquid extraction procedure which eliminates the need to use a phase separator. This certainly makes the set-up robust even in the hands of relatively inexperienced personnel. As an example, a number of organophosphorus pesticides are determined in pond water at the 0.1–1 μg/l level.

Problems are still encountered when highly volatile and polar analytes (and organic solvents) have to be used. Future research will involve a more detailed study of the compatibility of a wide range of organic solvents with the proposed technique.

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