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COMBINATION OF AUTOMATED PRE-COLUMN TRACE ENRICHMENT AND ON-LINE EVAPORATION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

By means of a high-performance liquid chromatograph connected to a fully automated sample-handling unit which combines pre-column enrichment and evaporation of water using a rotary vane pump, it is possible to introduce aqueous samples into an adsorption liquid chromatographic system. It was demonstrated that difficult separations on silica which failed in the presence of water can be achieved without any significant band broadening. For concentrated samples in organic solvents a second injection valve directly in front of the separation column was employed. By using direct injection and enrichment in an integrated analysis, recoveries can be determined.

INTRODUCTION

Various research groups have shown that by using automated on-line sample-enrichment techniques for aqueous samples in high-performance liquid chromatography (HPLC) the detection limits (in concentration units) can be reduced by several orders of magnitude[1–10]. However, in all of these studies the sample-enrichment technique was combined either with reversed-phase HPLC or with normal phase HPLC using modified silica, and aqueous eluents[7] in order to avoid interference in the separation step from the water on the enrichment column. This is certainly a limitation when substances in complex aqueous samples have to be identified by comparison of chromatographic parameters, and when it is desirable to confirm the identity by a second analysis under different HPLC conditions. By using reversed-phase and adsorption chromatography the correctness of any identification may possibly be enhanced.

Very often adsorption HPLC has a superior separating capability for resolving isomers[11]. In order to achieve separations with less polar solvents on silica gel it is necessary to transfer the substances from water to an organic solvent by means of a separating funnel or off-line extraction cartridges. Thus, there was a need to develop an on-line sample-handling technique which included the removal of water after the enrichment step. In order to do so we modified an HPLC device with alternating

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pre-columns[8] by adding two multiposition valves and a rotary vane pump. For calibration purposes and the determination of recovery rates, the device was equipped with a second sampler, or alternatively an injection valve for concentrated samples in organic solvents.

The aim of this investigation was to study chromatographic effects due to the pressure drop after switching from an evacuated pre-column to the separation column, and to optimize basic parameters concerning the design of the pre-column and duration of the evaporation process. The rather non-polar organochlorine insecticide DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], its degradation products, a newly developed pyrethroide insecticide[12] and six aromatic hydrocarbons were chosen as model compounds.

EXPERIMENTAL

Chemicals

HPLC grade *n*-hexane, acetonitrile and dioxane were purchased from Merck (Darmstadt, F.R.G.). For the analysis of DDT and its degradation products, *n*-hexane was dried over alumina. Water was purified using a water purification system from Millipore (Bedford, MA, U.S.A.).

Technical grade nitrogen (99.6%), the purging gas, was supplied by Messer Griessheim (Düsseldorf, F.R.G.) and employed after on-line cleaning with charcoal.

DDT and its degradation products were purchased from Riedel-de Haën (Seelze, F.R.G.). With the exception of fluoranthene (Ega, Steinheim, F.R.G.), the aromatic hydrocarbons were obtained from Merck. The chemicals were used as received.

The isomers of the pyrethroide insecticide were separated and purified by preparative chromatography from technical FCR 1272 (Bayer AG, Wuppertal, F.R.G.).

The test solutions used for the determination of recovery efficiencies are given in Table I.

Apparatus

Fig. 1 shows a scheme of the liquid chromatograph with on-line sample-handling device. In addition to the usual HPLC set-up comprising a liquid chromatograph Vista 5040 combined with a Vista data system (Varian, Palo Alto, CA, U.S.A.), an Uvikon variable wavelength detector LC 720 (Kontron, Zürich, Switzerland) connected to a recorder Servogor 210 D (Goerz Electro, Wien, Austria) and an autosampler MSI 660 with a 20-μl sample loop (Kontron) or alternatively an injection valve Rheodyne 7125 (Rheodyne, Berkely, CA, U.S.A.) with a 20-μl loop, there is an enrichment unit with switching valves Rheodyne 7010 as described[8] and an evaporation unit with multiposition valves Valco SF 6 (Valco, Houston, TX, U.S.A.). Valves 1a and 1b, as well as 2a and 2b, operate in pairs, and are switched simultaneously. The vacuum in the evaporation unit is generated by a rotary vane pump D 8A (Leybold-Heraeus, Köln, F.R.G.). Diluted aqueous samples are inserted using autosampler A (Model MSI 660, Kontron) equipped with a gas-tight 10-ml syringe (Hamilton, Bonaduz, Switzerland) and a 4-ml sample loop. The sample is delivered to the enrichment column by means of water pump B which is a double-piston constant-volume pump (Model 600; Gynkotek, München, F.R.G.). This pump is also

TABLE I

TEST SOLUTIONS

Solution	*Solvent*	*Concentration*
Pyrethroide isomers 1–4		
(calibration solution)	*n*-Hexane	*ca.* 5 mg/l
(diluted solution)	Water–acetonitrile (80:20, v/v)	*ca.* 2 μg/l
Organochlorine compounds		
(calibration solution)	*n*-Hexane–dioxane (98:2, v/v)	
1 = 4,4′-DDE		*ca.* 9 mg/l
2 = 2,4′-DDT		*ca.* 13 mg/l
3 = 4,4′-DDT		*ca.* 17 mg/l
4 = 2,4′-DDD		*ca.* 10 mg/l
5 = 4,4′-DDD		*ca.* 8 mg/l
(diluted solutions, proportion of acetonitrile varied)	Water–acetonitrile (99:1, 90:10, 80:20, 70:30, v/v)	1: *ca.* 2 μg/l 2: *ca.* 3 μg/l 3: *ca.* 4 μg/l 4: *ca.* 2.5 μg/l 5: *ca.* 2 μg/l
Aromatic hydrocarbons		
(calibration solution)	*n*-Hexane–dioxane (99:1, v/v)	
1 = Naphthalene		*ca.* 16 mg/l
2 = Biphenyl		*ca.* 3.9 mg/l
3 = Anthracene		*ca.* 1.1 mg/l
4 = Fluoranthene		*ca.* 6.3 mg/l
5 = *o*-Terphenyl		*ca.* 9.2 mg/l
6 = *p*-Terphenyl		*ca.* 6.6 mg/l
(diluted solution for 3.8 ml sample volume, proportion of acetonitrile varied)	Water–acetonitrile (99:1, 90:10, 80:20, 70:30, 60:40, 50:50, v/v)	1: *ca.* 100 μg/l 2: *ca.* 20 μg/l 3: *ca.* 7 μg/l 4: *ca.* 40 μg/l 5: *ca.* 60 μg/l 6: *ca.* 40 μg/l
(diluted solution for 50 ml sample volume, proportion of acetonitrile varied as above)	Water–acetonitrile	1: *ca.* 8 μg/l 2: *ca.* 2 μg/l 3: *ca.* 0.6 μg/l 4: *ca.* 3 μg/l 5: *ca.* 5 μg/l 6: *ca.* 3 μg/l

used when highly diluted samples have to be analysed and sample volumes between 5 and 500 ml are inserted using a multiposition valve Valco SD 16. The water pump A for sample clean-up is an Orlita oscillating displacement pump (Model DMPSK 15/7; Orlita, Giessen, F.R.G.).

The air-actuated switching valves Valco SF 6, SD 16 and Rheodyne 7010, the autosamplers and water pump B are controlled by the Vista data system.

Three types of enrichment columns were tested:

(1) Brownlee cartridge with holder (distributor: Kontron), 30 × 4.6 mm, packed with 10-μm ODS

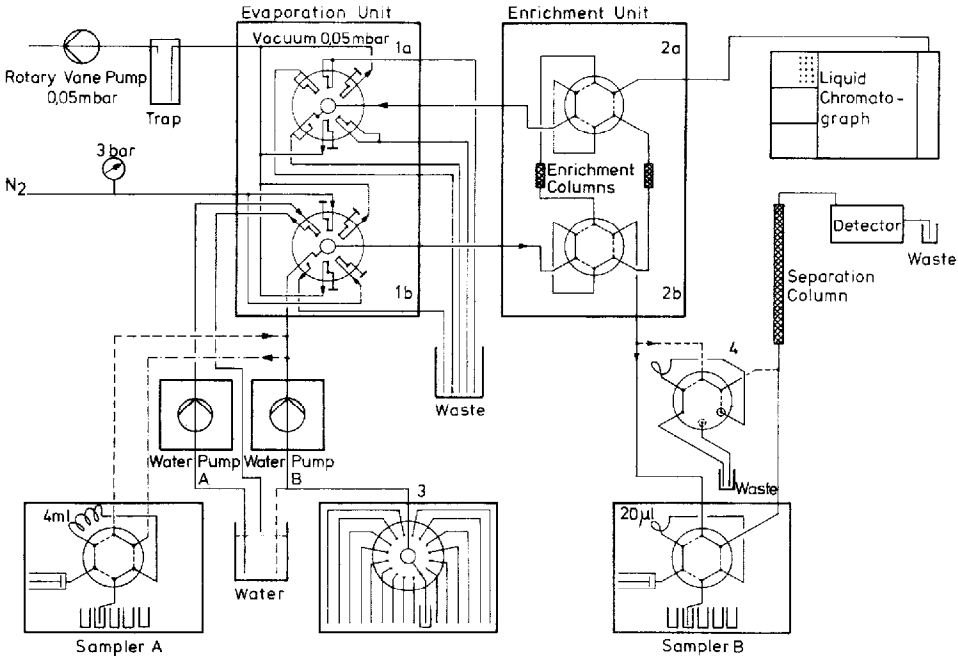

Fig. 1. Scheme of the liquid chromatograph with a device for combined pre-column trace enrichment and on-line evaporation. 1a, 1b = Multiposition valve Valco SF 6; 2a, 2b = valve Rheodyne 7010; 3 = multiposition valve Valco SD 16; 4 = valve Rheodyne 7125. – – – –, Optional connections.

(2) Home-made column, 40 × 4.6 mm, with Gyrolok fittings 4 RU 1 LC special (distributor: Hoke, Frankfurt, F.R.G.), dry-packed with Perisorb RP 18, 30–40 µm (Merck)

(3) Bischoff cartridge with holder, 10 × 4.6 mm, packed with 5-µm Hypersil ODS (Bischoff, Leonberg, F.R.G.).

Two versions of the working cycle of the analytical system are summarized in Table II. With autosampler A (version 2a), up to 60 samples with a sample volume of 4 ml can be analysed. Using the valve SD 16 for highly diluted samples (version 2b), a maximum of 16 sample flasks may be connected to the valve by means of PTFE tubes (1.6 × 0.8 mm I.D.). The air in the tubes is removed by purging the pump while the valve SD 16 passes all 16 postions and valve 1b is in the bypass position. Any stream not connected to the common valve outlet flows through an individual outlet, so that there are five bypass positions.

In version 2b the sample volume is controlled by the flow-rate of the constant-volume pump and the time interval in which the valves 1a + 1b are open for the sample stream. Before the sample stream is pumped to the enrichment unit the pump and the tubes are washed by the sample stream for 3 min.

After the sample has been pumped to the enrichment column the water is removed by purging with nitrogen, and evaporation under vacuum.

The exact volumes of the sample loops of the autosamplers A and B were determined photometrically by injecting aqueous nitrobenzene solutions into mea-

TABLE II

WORKING CYCLE OF THE ANALYTICAL SYSTEM

Time (min)	*Phase of the working cycle*		*Sequence of switching steps*
	First enrichment column	*Second enrichment column*	
With autosampler A for sample volumes of 4 ml			
0.1		Elution	Water pump B on: valves 1a + 1b (vacuum); valves 2a + 2b inject to load position or reverse, autosampler A (loop filling)
0.2	Nitrogen purge		Step valves 1a + 1b (N_2 stream)
1.2	Evacuation		Step valves 1a + 1b (Vacuum on)
2.2	Water purge		Step valves 1a + 1b (stream of pump B)
3.5	Sample enrichment and water purge		Valve in autosampler A (injection)
8.5	End of enrichment		Water pump B off; autosampler A to next sample position
12*	Stand-by position		Autosampler B (loop filling)
14.2		Elution of calibration sample	Valve in autosampler B (injection)
20			Autosampler B to next sample position
22			Step valves 1a + 1b
24	Nitrogen purge		Step valves 1a + 1b (N_2 stream)
26	Evacuation		Step valves 1a + 1b (vacuum)
30 + *ca.* 3**	Start of the next cycle; elution of second sample	N_2 purge, vacuum, loading of third sample	Valves 2a + 2b load to inject position or reverse
With SD 16 valve and water pump (sample volume ≫ 4 ml)			
0.1		Elution	Valve SD 16 step; water pump B on; valves 1a + 1b (vacuum);valves 2a + 2b inject to load position or reverse
0.2	Nitrogen purge		Step valves 1a + 1b (N_2 stream)
1.2	Evacuation		Step valves 1a + 1b (vacuum on)
3	Enrichment		Step valves 1a + 1b (sample stream)
20***			Autosampler B (loop filling)
22.2***		Elution of calibration sample	Autosampler B (injection)
28			Autosampler B to next sample position
53	End of enrichment; water purge		Step valves 1a + 1b (water stream)
54			Water pump B off
55	Nitrogen purge		Step valves 1a + 1b (N_2 stream)
57	Evacuation		Step valves 1a + 1b (vacuum)
60 + *ca.* 3**	Start of the next cycle; elution of second sample	N_2 purge, vacuum, loading of third sample	Valves 2a + 2b load to inject position or reverse

* Times may vary depending on retention time of substances.

** Depending on time necessary for print-out the duration of a working cycle may vary slightly.

*** Time may vary depending on sample volume and flow-rate of water pump B.

suring flasks. After adjustment to volume, the absorbances of the solutions were measured at 254 nm. The volumes were calculated as 24.3 ± 0.2 μl (autosampler B) and 3.80 ± 0.02 ml (autosampler A) by dividing the absorbance of the diluted solution by that of the original solution and multiplying by the volume of the measuring flask.

RESULTS AND DISCUSSION

A prerequisite for the development of an adsorption HPLC method for the direct analysis of aqueous samples is the complete removal of water from the sample in order to avoid changes in mobile-phase water content. The retention is extremely sensitive to small changes in water content when the saturation of adsorbent sites on the silica surface is very low. Thus, the efficiency of the method must be demonstrated with dried non-polar mobile phases.

Some preliminary experiments with aqueous samples of FCR 1272 using *n*-hexane–dioxane (985:15, v/v) as mobile phase gave some hints as to a suitable concept of an analytical system. It was found that the design of the enrichment column is very important to the removal of water. The column should be as small as possible, and the access to the packing should be relatively unrestrained. Cartridges from Brownlee were not satisfactory as the frits tended to become clogged by particles which were abraded during the enrichment process. A home-made column dry-packed with Perisorb was quite effective for sample enrichment but the interparticle volume filled with water after the enrichment process was too large. The commercial

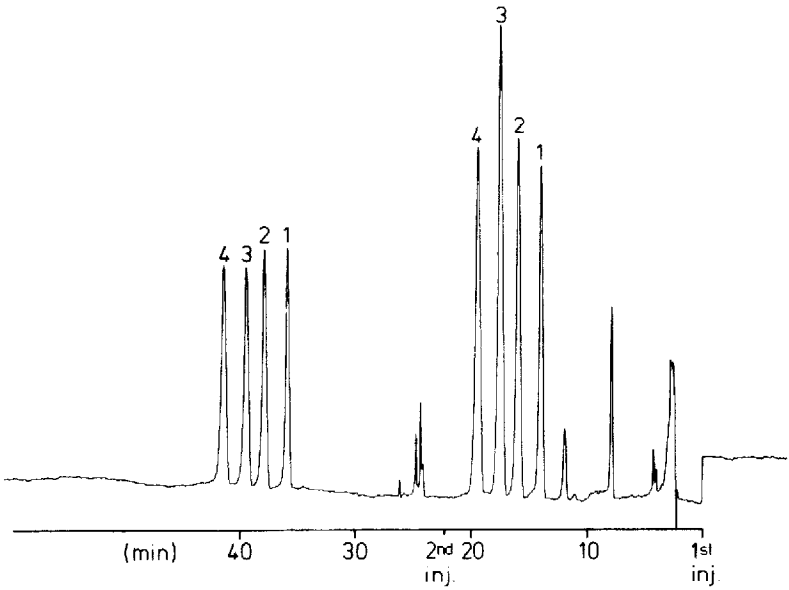

Fig. 2. Chromatogram of pyrethroide isomers with double injection. First injection of enriched sample; sample volume, 85 ml; on-line evaporation; temperature of enrichment column, 30°C. Second injection of calibration sample; sample volume, 24.3 μl; column, LiChrosorb Si 60, 5 μm, 250 × 4 mm (Merck); mobile phase, *n*-hexane–dioxane (985:15, v/v); flow-rate, 1.5 ml/min; temperature, 30°C; UV detection, 220 nm. Peaks: 1–4 = pyrethroide isomers 1–4.

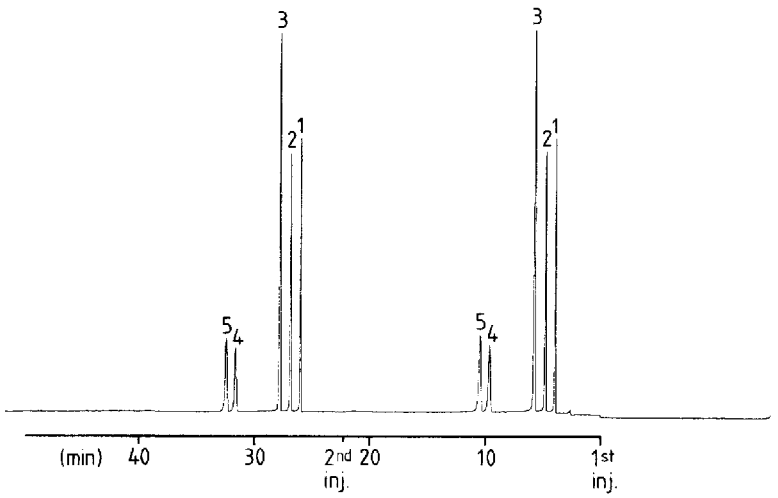

Fig. 3. Chromatogram of DDT and its degradation products with double injection. First injection of enriched sample; sample volume, 100 ml; on-line evaporation; temperature of enrichment column, 60°C. Second injection of calibration sample; sample volume, 24.3 µl; column, LiChrospher Si 100, 5 µm, 250 × 4 mm (Merck); mobile phase, *n*-hexane, fow-rate 1.5 ml/min; temperature, 35°C; UV detection, 238 nm. Peaks: 1 = 4,4'-DDE; 2 = 2,4'-DDT; 3 = 4,4'-DDT; 4 = 2,4'-DDD; 5 = 4,4'-DDD.

cartridges from Bischoff were most suitable. They are small, and the filter element consisting of two stainless-steel sieves and two glass-fiber filters in the centre does not obstruct the removal of water.

The desiccation is most rapid as a two-step process. First, the main portion of water in the tubing, and in the interparticle volume, is ejected by purging for 1 min with nitrogen. Residual water is then evaporated under vacuum at 0.1 mbar. The evaporation proved to be most effective when both ends of the enrichment column were connected to the vacuum tube. After *ca.* 5 min the water content absorbed on the silica cannot be reduced any further as is demonstrated by comparing the retention times of two consecutive chromatographic analyses in which the sample desiccation parameters are identical. If extremely non-polar eluents, *e.g.*, *n*-hexane dried over alumina, are used, the evaporation process must be supported by maintaining the enrichment columns at 60°C by means of a heating coil.

The device shown in Fig. 1 is suitable for on-line evaporation with direct determination of recovery in an integrated analysis. Figs. 2 and 3 show double chromatograms of pyrethroide isomers, and a mixture of DDT and its degradation products. There is neither a measurable band broadening, nor any significant shift in retention times when both injections are compared. The pressure drop after switching the evacuated enrichment column over to the eluent does not influence the separation.

The recovery of the pyrethroides, DDT and its degradation products amounted to 95–105%. A prerequisite for values as high as these is stabilization of the solutions by adding 20% of acetonitrile. It is well established that solutions of substances of low solubility are unstable due to adsorptive losses from the solution to the surface of the containers[1]. Furthermore, it is uncertain whether the normal injection technique for the preparation of solutions of sparingly soluble substances is suitable for the generation of genuine solutions. If a small volume of a concentrated solution in

TABLE III

EFFECT OF ACETONITRILE ON RECOVERY OF DDT AND ITS DEGRADATION PRODUCTS

Concentrations of test solutions as in Table I, sample enrichment condition as in Table II (SD 16 valve) and HPLC Conditions as in Fig. 3.

Acetonitrile (%, v/v)	*Recovery (%)*				
	4,4'-DDE	*2,4'-DDT*	*4,4'-DDT*	*2,4'-DDD*	*4,4'-DDD*
1	18	17	17	25	26
10	65	69	68	95	91
20	99	100	100	104	104
30	99	96	99	93	95
40	38	31	32	15	15

an organic solvent miscible with water is injected into a large volume of water there is every likelihood of producing colloidal, micellar or particulate forms. The column-enrichment technique is not suitable for detecting these forms, as is demonstrated in Table III in the case of organochlorine compounds. Unless there is a solvent concentration between 20 and 30% (v/v) in the sample the recovery rates are low.

Some investigators attributed this effect to the poor wettability of the packing[2]. However, while using the same enrichment column one very often gets higher recoveries with solutions prepared by the column elution method[13] than with those prepared by the injection technique.

If the enrichment technique is applied to substances less hydrophobic than the

TABLE IV

EFFECT OF ACETONITRILE ON RECOVERY OF AROMATIC HYDROCARBONS AT TWO CONCENTRATION LEVELS

Concentrations of test solutions as in Table I; concentration of the 50-ml sample is approximately one order of magnitude lower than the concentration of the 3.8-ml sample. Enrichment conditions as in Fig. 2a (sample volume 3.8 ml), or else Fig. 2b (sample volume 50 ml). HPLC conditions same as in Fig. 3 except UV detection at 254 nm.

Acetonitrile (%, v/v)	*Sample volume (ml)*	*Recovery (%)*					
		Naphthalene	*Biphenyl*	*Anthracene*	*Fluoranthene*	*o-Terphenyl*	*p-Terphenyl*
1	3.8	63	54	37	44	41	29
1	50	89	86	56	61	57	26
10	3.8	64	67	57	66	65	39
10	50	69	104	89	94	100	76
20	3.8	69	77	71	81	76	58
20	50	32	65	81	101	97	99
30	3.8	50	78	86	105	93	94
30	50	15	27	42	48	99	103
40	3.8	33	60	64	81	83	97
40	50	2	6	9	11	20	27
50	3.8	19	29	36	47	62	65
50	50	<1	<1	<1	2	2	2

above mentioned substances breakthrough may occur, all the more so as the enrichment column is relatively short. Table IV summarizes the retention efficiency for aromatic hydrocarbons at two concentration levels as a function of the solvent concentration in the sample. The results show that the recovery efficiencies are not satisfactory for compounds less hydrophobic than fluoranthene. The solvent concentration in the sample necessary for optimum recovery is dependent on the hydrohobicity of the substance and on sample volume. A larger sample volume which corresponds with lower substance concentrations must contain less modifying solvent than a small sample volume.

It is self-explanatory that there are losses in volatile substances when using the on-line evaporation technique. To ensure the accuracy of the results it is necessary to check the applicability of the method by measuring the recovery efficiency.

CONCLUSIONS

The above examples show that by using on-line evaporation it is possible to combine pre-column enrichment with adsorption chromatography. The elimination of water is adequate for chromatographic separations provided the enrichment columns are small, and connections between the enrichment column and valves are as short as possible. Even with non-polar solvents there is practically no shift in retention time when chromatograms of directly injected samples and aqueous samples are compared. The small shift due to the pressure drop after connecting the enrichment column and separation column is negligible.

It is obvious that the described method could simplify the sample-handling procedures for environmental samples when adsorption chromatography is necessary. Volatile solvent contaminants which lead to large solvent peaks with ordinary enrichment techniques are partly removed during the on-line evaporation so that the method could be applied as a clean-up step not only in adsorption chromatography but also in reversed-phase chromatography.

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