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ON-LINE TRACE ENRICHMENT IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING A PRE-COLUMN

H. P. M. VAN VLIET, Th. C. BOOTSMAN, R. W. FREI and U. A. Th. BRINKMAN*
Department of Analytical Chemistry, Free University, De Boelelaan 1083, 1081 HV Amsterdam (The Netherlands)

SUMMARY

The potential of on-line trace enrichment on a pre-column in high-performance liquid chromatography is demonstrated using phthalate esters as model compounds. The esters are concentrated onto a very short, *i.e.*, *ca.* 2 mm long, plug of an apolar chemically bonded stationary phase (5-μm LiChrosorb RP-18). Analysis is performed on LiChrosorb RP-18 with a methanol step gradient as mobile phase; detection is done by UV absorption measurement at 233 nm. Phthalate ester recoveries are 95–100% even after enrichment from sample volumes of 500–1000 ml, with pumping speeds varying between 5 and 25 ml min^{-1}. The single-column and the column-to-column reproducibility of the technique are usually 4–6% (relative standard deviation); band broadening due to the insertion of the pre-column is negligible. As an application, the determination of two esters in tap, distilled, mineral and river water, and in soft drinks, is reported. The trace enrichment of mixtures of polychlorinated biphenyls, and of chloroanilines, is also described.

INTRODUCTION

In recent years the need for sensitive techniques in trace-analytical work and, hence, of low detection limits has been clearly recognised. To quote an example, in high-performance liquid chromatography (HPLC), the distinct advantage of using relatively large, *i.e.*, 100–200 μl, instead of the conventional 10–20-μl injection volumes for the improvement of detection limits (in concentration units) is well documented[1–3]. However, in most chromatographic trace-analytical procedures, a true trace-enrichment, also called pre-concentration, step is unavoidable in order to achieve the necessary concentration of the analytes prior to the detection step.

It is also well known that simple extraction, evaporation and other similar techniques share the disadvantage of high risk of contamination from containers, solvents and laboratory surroundings, and the risk of degradation upon evaporation to dryness, etc. An as alternative, off-line concentration techniques on suitable adsorbents have been adopted, particularly in conjunction with gas chromato-

* To whom correspondence should be addressed.

graphy[4,5], but also with HPLC[6]. However, problems still remain, such as undesired dilution, possible sample contamination and long analysis times.

Therefore, pre-concentration directly on the top of an analytical column prior to the actual chromatography is a distinct step forward. In this technique, conditions are so chosen that initially, *i.e.*, during the enrichment step, the analytes are strongly retained; then a mobile-phase solvent mixture is employed which causes the adsorbed compounds to be eluted with moderate retention times[7–11]. Despite its apparent advantages, this approach can not be recommended for the analysis of complex matrices and/or heavily contaminated samples: in such cases, the performance of the analytical column will rapidly deteriorate. In addition, with conventional columns (10–25 cm), it will not be possible to use the high pumping speeds of 10–20 ml min^{-1} required in order to concentrate analytes from large volumes of very dilute sample solutions within a reasonable time. Therefore, as a further improvement, the use of on-line pre-column technology has recently been suggested by several groups of workers[12–15]. However, they often used relatively long pre-columns, small sample volumes and/or low mobile-phase velocities, which detracts from the usefulness of their work for environmental analysis.

It was the aim of the present investigation to study basic parameters such as pre-column design (length!), particle size of pre-column sorption material and pumping speed of the mobile phase in order to elaborate optimum conditions which will allow the enrichment of traces of contaminants from very large aqueous samples containing realistic, *i.e.*, very low, concentrations of pollutants. The, rather non-polar, phthalate esters were chosen as model compounds for reasons given below. Trace enrichment of chloroanilines was included in our project in order to obtain a general idea of the dependence of pre-column sorption on solute polarity.

MATERIAL AND METHODS

Chemicals

Two phthalate esters, di-*n*-butylphthalate (DBP) and di-(2-ethylhexyl)phthalate (DEHP), were chosen as model compounds. Concentrated solutions of the reagent-grade esters, which were purchased from E. Merck (Darmstadt, G.F.R.), were prepared in methanol ("Analyzed"; J. T. Baker, Phillipsburg, N.J., U.S.A.) and aliquots of these stock solutions were added to large volumes of water to obtain sufficiently dilute phthalate-containing sample solutions; the methanol–water ratio was *ca.* 1:100. Demineralized water was used, that had been purified by a single extraction with hexane (Nanograde; Mallinckrodt, St. Louis, Mo., U.S.A.) in order to remove traces of DEHP.

The commercially available polychlorobiphenyl (PCB) mixtures Aroclor 1254 and 1260, and 4,4′-dichlorobiphenyl, were purchased from Analabs (North Haven, Conn., U.S.A.); 4-mono- and 3,4-dichloroaniline were products from Fluka (Buchs, Switzerland), while *o,p′*-DDT was obtained from Aldrich Europe (Beerse, Belgium). Stock and sample solutions were prepared as described above for the phthalate esters; however, in view of the relatively low retention of the chloroanilines, the methanol–water ratio in their sample solutions was kept at a value of *ca.* 1:1000.

Apparatus

Fig. 1 shows a scheme of the experimental set-up. The system consisted of a Siemens S100 liquid chromatograph equipped with an Orlita MK 00 reciprocating pump and a Zeiss PM2 DLC UV detector set at 233 nm. For the determination of detection limits, either a Perkin-Elmer LC 55 or a Pye Unicam LC 3 UV detector was used instead of the Zeiss instrument. The separation column was a stainless-steel tube (12.5 cm × 4.6 mm I.D.) packed with 5-μm LiChrosorb RP-18 (Merck), while an appropriate methanol–water gradient was used as mobile phase. A Scanivalve 1p./12t. valve with a home-made electronic steering unit was used to generate a reproducible step gradient and to load the sample onto the pre-column. The design of the steering unit was copied from ref. 15, some minor modifications being introduced. The use of a bypass (Pye Unicam, Cambridge, Great Britain) damping system enabled us to restrict the void volume between the Scanivalve valve and the analytical column to *ca.* 2 ml. The pre-column —the construction of which will be described in some detail below— was packed with either 5-μm or 10-μm LiChrosorb RP-18 or a coarse (> 50 μm) C_{18} bonded-phase material used by Waters Assoc. (Milford, Mass., U.S.A.) to fill off-line enrichment columns (Sep-Pak C18 cartridges). All sample solutions and mobile-phase mixtures were degassed for 30 sec *in vacuo*, with a magnetic stirrer. The experiments were carried out at ambient temperature.

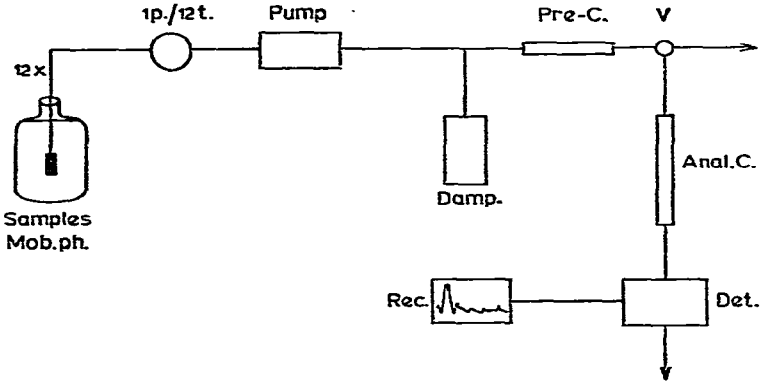

Fig. 1. Diagram of experimental set-up. Degassed sample solutions or mobile-phase mixtures pass through a Scanivalve 1-port/12-tube valve (1p./12t.) using a high-pressure pump (Pump) with bypass damping system (Damp.). Trace enrichment of the analytes occurs on the pre-column (Pre-C.) with the valve (V) in drain position. Separation on the analytical column (Anal. C.) takes place after turning the valve into the on-column position. After elution, the analytes are detected (Det.) and a chromatogram is recorded (Rec.).

Procedure

Routine pre-concentration of samples of up to *ca.* 50 ml was done at a flow-rate of *ca.* 5 ml min^{-1}. With the steering unit at manual override, the effluent was collected with the high-pressure valve in the "drain" position. After the desired sample volume had passed through the pre-column, the valve was turned into the on-column position. Simultaneously, a 75 to 85 to 95% methanol-in-water gradient was started. DBP and DEHP eluted during the 85 and 95% steps, respectively, with typical retention times of *ca.* 6 and 10 min at a mobile-phase flow-rate of 2 ml

min^{-1}. After elution of the DEHP peak, 100% methanol was used as a final column clean-up step. Isocratic elution conditions were chosen if samples had to be analyzed for either DBP or DEHP only.

The above set-up was not suitable for trace enrichment from large sample volumes, since the relatively low maximum flow-rate of the Orlita pump of 5–7 ml min^{-1} led to excessively long analysis times. Therefore, in such cases, either a Perkin-Elmer Series 2 LC or a Haskel MCP 110 pump was used, with which flow-rates of up to 25 ml min^{-1} could be maintained. These high-speed pumps were used for the pre-concentration step only; the actual analysis was invariably carried out with the Orlita pump.

RESULTS AND DISCUSSION

Pre-column design

The pre-column (Fig. 2) consisted of a piece of stainless-steel tubing [45 × 4.6 mm (occasionally, 2.9 mm) I.D.]. Into it a snugly fitting variable-length (*cf.*, below) PTFE rod with an outer diameter of 4.6 (2.9) mm was inserted, which was drilled through with a piece of stainless-steel capillary (1/16 in. O.D., 0.25 mm I.D.). After placing a relatively wide-pore, *i.e.*, 20-μm, frit into position, the pre-column was hand-packed using a micro spatula. To this end, a dense slurry of *ca.* 0.5 g of the stationary phase in 1 ml of methanol was prepared in a small petri dish, allowing most of the solvent to evaporate in order to obtain a slurry of the desired density. The length of the plug of stationary phase was varied by varying the length of the PTFE rod. Finally, after careful smoothing of the surface of the plug of RP-18 material, a Swagelok containing a 0.5- or 2-μm outlet frit was installed. Prior to use, all pre-columns were conditioned for some 5 min at the appropriate, *i.e.*, the highest, flow-rate with methanol.

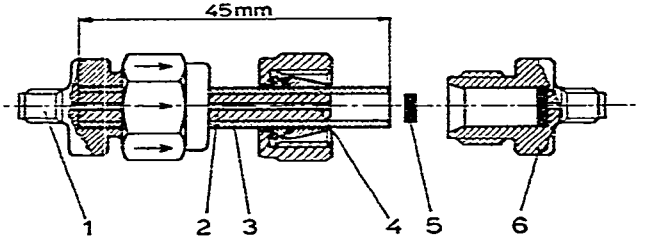

Fig. 2. Pre-column design: 1 = Swagelok, 1/4 × 1/16 in.; 2 = variable-length PTFE rod, 4.6 mm O.D.; 3 = stainless-steel tube, 1/4 in. × 45 mm; 4 = stainless-steel capillary, 1/16 in. O.D., 0.25 mm I.D.; 5 = PTFE or stainless-steel 20-μm frit; 6 = Swagelok, 1/4 × 1/16 in.

The following remarks should be made.

(1) Initially, a plug of quartz wool was used instead of the PTFE rod. This led to the formation of a methanol–water gradient inside the pre-column during the early stage of the 75% methanol-in-water elution step and, hence, to the trace enrichment on the pre-column of some of the DEHP invariably present in the methanol. As a consequence, high blank values were obtained.

(2) Inserting a 20-μm frit in front of the RP-18 plug proved a prerequisite in

order to obtain a uniform flow pattern through the packed column and, thus, a good sample distribution on the bonded-phase material.

(3) Care should be taken to prevent complete evaporation of the methanol during slurry preparation: with "dry-packed" columns irreproducible results were obtained.

Optimization of the trace-enrichment step

In several series of preliminary experiments, calibration curves for DEHP and DBP in the 0.2–6-μg range were produced using pre-columns packed with 5-μm LiChrosorb RP-18 and having lengths ranging from 9 to 1 mm (4.6 mm I.D.). A linear relationship between peak height and amount of phthalate ester was invariably observed, the slope of the various calibration curves being approximately the same. Values of the regression coefficients were typically between 0.9970 and 0.9995 ($n = 7$). As an alternative to a 2 × 4.6 mm I.D. pre-column, a column containing approximately the same amount of RP-18 material, but as a 5 × 2.9 mm I.D. plug, was employed. In the latter case, *ca.* 20% lower peak heights were obtained. Also, packing of the small-bore pre-column proved to be rather laborious, while the pressure-drop was distinctly higher.

In order to diminish the pressure-drop over the pre-column —which amounted to some 15 and 150 bar at 5 and 25 ml min^{-1}, respectively, with a column freshly packed with 5-μm material— 10-μm LiChrosorb RP-18 and a coarse (> 50 μm) C_{18} chemically bonded phase were tested. From the data shown in Table I it is obvious that particle size exerts a profound influence on pre-column performance. Replacing the 5-μm by 10-μm particles led to a slight deterioration of the peak heights, and this was also true as regards standard deviation, which was *ca.* 5 and 10%, respectively, at the $n = 7$–10 level. With both the 5- and 10-μm material, recovery of DEHP was found to be quantitative when concentrating 20 ml of a solution containing *ca.* 1 μg of the phthalate ester. Material larger than 50 μm showed relatively poor performance, the significant decrease in peak height being due to an increased (*ca.* 20%) peak width combined with a low recovery of only 60–70%. No further attempts were made to improve this situation by either increasing the length of the pre-column or decreasing the flow-rate, since this would inevitably cause adverse effects such as additional band broadening or longer analysis times. Instead, 5-μm LiChrosorb RP-18 plugs (length 2 mm) were selected

TABLE I

DEPENDENCE OF PRE-COLUMN PERFORMANCE ON PARTICLE SIZE OF STATIONARY PHASE

Conditions: trace enrichment on a 2 × 4.6 mm I.D. pre-column from *ca.* 20-ml sample volumes containing 50 ng of each phthalate per ml; for further details, see text.

Stationary phase	*Particle size (μm)*	*Peak height (mm/μg)*		*n*
		DBP	*DEHP*	
LiChrosorb RP-18	5	98.3	33.4	9
LiChrosorb RP-18	10	92.3	31.3	6
C_{18} (Sep-Pak)	>50	74.5	15.0	7

for all further work. Band broadening due to the insertion of a pre-column (compared to 10-μl injection) was less than 3%.

In the previous experiments, the flow-rate during the trace-enrichment step was maintained at 5 ml min^{-1}. Increasing this rate to the highest attainable, *i.e.*, 25 ml min^{-1}, did not affect the sorption efficiency of the phthalate esters on the pre-column. This can be seen from the data in Table II; here average values from experiments with 25-, 50- and 100-ml volumes of constant sample concentration are presented. Standard deviations were 10–15% ($n = 9$). Pre-concentration of the collected effluents showed the recovery of DEHP and DBP to be better than 99% in all cases; this was found to be true even after the passage of 500–1000 ml of sample solution.

TABLE II

DEPENDENCE OF PRE-CONCENTRATION EFFICIENCY ON PUMPING SPEED

Conditions: trace enrichment from 25–100-ml sample volumes containing 17 ng of each phthalate per ml; for further details see text.

Pumping speed (ml min^{-1})	*Peak height (mm/µg)*	
	DBP	*DEHP*
5.0	78.7	28.7
12.5	68.0	28.4
25.0	77.5	28.7

In order to assess the column-to-column reproducibility, trace enrichment of DBP was carried out at 5 ml min^{-1}, on four different pre-columns; in addition, with one of these a second series of experiments was performed after re-packing. The results, shown in Table III, are very satisfactory. The largest column-to-column difference is *ca.* 10%, while the standard deviation calculated from the data for all five columns is 4% ($n = 47$). Rather surprisingly, this result hardly differs from the standard deviations calculated for each single column (4–5.5%). Analogous results were obtained in experiments carried out at a flow-rate of 25 ml min^{-1}.

TABLE III

COLUMN-TO-COLUMN REPRODUCIBILITY OF ON-LINE TRACE ENRICHMENT OF DBP

Conditions: trace enrichment from 16-ml sample volumes containing 194 ng DBP per ml at a pumping speed of 5 ml min^{-1} on a 2 × 4.6 mm I.D. pre-column; for further details see text.

Column	*Signal*		*n*
	Peak height (mm/µg)	*rel. S.D. (%)*	
1	49.5	3.9	10
2	47.9	5.3	7
3	45.2	5.4	10
4	49.9	4.7	10
4*	49.2	5.5	10

* After re-packing.

Lastly it was demonstrated that storage of the loaded pre-columns for prolonged periods of time prior to the separation of the phthalate esters on the analytical column did not seriously detract from their performance. For both DEHP and DBP, 0–7% losses were recorded after storage periods of between 18 and 60 h ($n = 10$).

The detection limits (at 233 nm) for DEHP and DBP as obtained in trace-enrichment experiments were 30–40 ng at a signal/peak-to-peak noise ratio of 2:1. These values are in good agreement with those reported by Ishii *et al.*[16].

Application to phthalate esters

In the present investigation, phthalate esters were selected as model compounds, because in a recent project on the determination of phthalate levels in water from the main Dutch rivers serious problems arose, the DEHP blank values being of the same order of magnitude as the levels typically encountered with the water samples[17]. In such cases, the use of an on-line trace-enrichment technique will be most helpful, since pre-concentration is achieved without any sample pre-treatment; that is, contamination hazards are virtually eliminated. Some results obtained with various types of water samples are summarized in Table IV; an HPLC chromatogram recorded for a sample of mineral water is shown in Fig. 3. In addition, we note that, in these studies, the pre-columns could be used for at least 2.5 l of sample solution.

TABLE IV

PHTHALATE ESTER CONTENT OF VARIOUS WATER SAMPLES

Sample	*Pre-concd. volume (ml)*	*Phthalate concn. (ppb)*	
		DEHP	*DBP*
Tapwater*	900	0.55	0.04
	900	0.53	0.05
Demineralized water*	600	0.66	0.44
	600	0.62	0.50
Distilled demineralized water*	500	1.6	0.45
	450	1.6	0.50
Mineral water			
C (plastic bottle)	790	0.23	<0.05
S (glass bottle)	450	0.18	<0.07
A (glass bottle)	800	1.23	0.08
Blank	200	<0.3	<0.1
	500	<0.15	<0.04

* Quoted data refer to experiment performed on different dates.

Concerning the data in Table IV, first, as a rather surprising, but interesting result, we observe that the tapwater in our laboratory consistently displayed low phthalate levels compared to demineralized and, even more so, distilled demineralized water. More importantly, a strong reduction of the phthalate blanks —which with our previous technique[17] were 0.3–1.0 and < 0.1 ppb* for DEHP and

* Throughout this article, the American billion (10^9) is meant.

DBP, respectively— was noted, values of < 0.15 ppb DEHP and < 0.04 ppb DBP now being recorded. For these measurements, water samples purified by a single extraction with hexane, or effluent collected after pre-concentration on a LiChrosorb RP-18 pre-column, were used. The detection limits of the phthalate esters recorded in Table IV are primarily determined by the upper limit of the sample volumes analyzed.

In order to further demonstrate the applicability of the present technique, the migration rate of DEHP and DBP from poly(vinyl chloride) medical foil (DEHP content *ca.* 50%) into demineralized water was studied. Representative results are shown in Table V. Sample volumes were between 225 and 400 ml in all experiments excepting the first, where 950 ml was used. Our results confirm and extend those previously published by Jaeger and Rubin[18] and Persiani and Cukor[19], who report the absence of phthalate esters from buffer solutions stored in poly(vinyl chloride) blood-packaging units at the ppm level (detection limit[19], *ca.* 5 ppm). This result is of some interest, since it is known that stored whole blood can become contaminated with and accumulate up to 500 ppm of phthalate esters.

Lastly, trace enrichment of the notoriously ubiquitous DEHP from sherry and soft drinks, and sea and river water, was studied. With the former type of samples, no serious problem arose, although the occurrence of a broad "solvent" peak occasionally forced us to change the step-gradient profile in order to ensure sufficient resolution between the DEHP and the solvent peak. DEHP levels of 1–20 ppb were typically encountered; that is, trace enrichment could conveniently be carried out with 10–50-ml volumes. In the case of sea and, even more so, river water —where, owing to the relatively low phthalate levels, volumes of 300–1000 ml have to be used— partial clogging of the pre-column was a recurrent phenomenon. Therefore, the use of a freshly packed pre-column for every single experiment is recommended. Under this condition, the present technique can be used successfully, as shown by the detection of low phthalate levels of 0.2 ppb DEHP (gas chromatography, 0–0.2 ppb) and < 0.05 ppb DBP in a water sample from the river Rhine.

Application to other pollutants

As a continuation of the above studies, the pre-concentration technique was successfully applied to the analysis of water samples spiked with 4,4′-dichlorobiphenyl (258 nm), *o,p′*-DDT (220–240 nm) and the PCB mixtures Aroclor 1254 and 1260 (205 nm); detection was done at the wavelengths indicated between parentheses. In all cases, trace enrichment was done on LiChrosorb RP-18, while elution was performed by means of 85% methanol in water. The negligible contribution of the insertion of a pre-column to band broadening, referred to above, is illustrated in Fig. 4A and B. The Aroclor 1260 pre-concentrated from a 16.5-ml aqueous sample shows slightly better resolution than does the Aroclor mixture syringe-injected as a 200-μl solution. With 4,4′-dichlorobiphenyl —which due to its lack of *ortho*-substitution displays a pronounced *k*-band having its maximum at the relatively high wavelength of 258 nm ($\log \varepsilon = 4.3$)[20]— a detection limit of 5–10 ng was obtained. This implies that for such a compound a detection limit of some 20 ppt can be reached using a 300-ml sample volume. The absence of the said compound from sea water at this level was actually demonstrated. Here, it should be added that, according to our experience, detection of trace components enriched from environ-

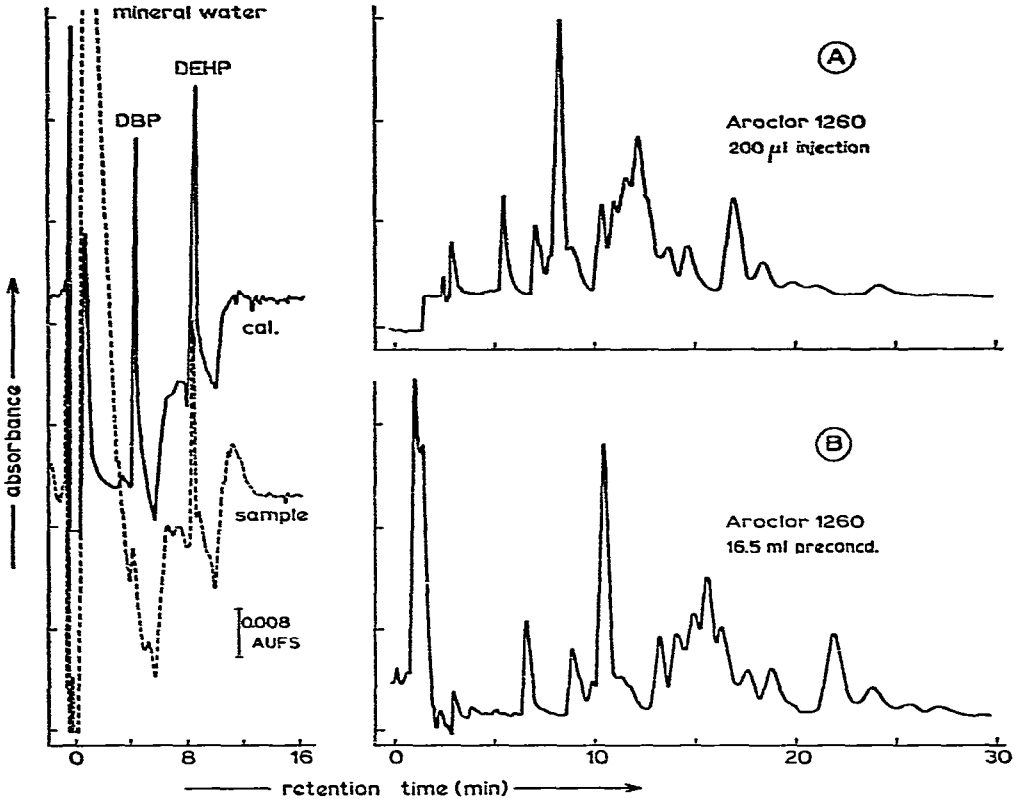

Fig. 3. HPLC chromatogram recorded for a standard solution (——) of DBP and DEHP, and for a sample (– – – –) of mineral water (Type A; *cf.*, Table IV). Conditions: sample solution, 800 ml; 2 × 4.6 mm 5-µm LiChrosorb RP-18 pre-column; loading at pumping speed 25 ml min^{-1}; analysis at 2 ml min^{-1} on a 12.5 cm × 4.6 mm I.D. LiChrosorb RP-18 separation column with 75% (60 sec), 85% (300 sec) and 95% (270 sec) methanol in water as mobile phase; detection at 233 nm (0.08 AUFS).

Fig. 4. Comparison of HPLC chromatograms recorded for equal amounts (*ca.* 20 µg) of Aroclor 1260, using normal 200-µl syringe injection (A), or trace enrichment from a 16.5-ml aqueous sample (B). Mobile phase, 85% methanol in water. Detection at 205 nm. For other details see Fig. 3.

TABLE V

MIGRATION OF PHTHALATE ESTERS FROM POLY(VINYL CHLORIDE) INTO DEMINERALIZED WATER

Condition: demineralized water contacted with 10 g poly(vinyl chloride) per l water.

Contact time (h)	*Pre-concd. volume (ml)*	*Phthalate concn. (ppb)*	
		DEHP	*DBP*
0	925	<0.09	<0.04
1	425	0.2	<0.07
5	425	1.8	0.2
24	375	—	0.5
26	350	2.0	0.3
96	350	3.2	0.5
600	225	2.4	—

mental samples at wavelengths of around 254 instead of at 210–230 nm offers distinct advantages, because the width of the sometimes very broad solvent peak is considerably reduced. For all four (mixtures of) compounds discussed here, recoveries of over 95% were consistently found with up to at least 100-ml sample volumes.

In recent years, increasing attention has been devoted[21,22] to the analysis of nitrogen-containing compounds such as 3,4-di- and 4-monochloroaniline, which are well-known degradation products of urea herbicides. Trace enrichment (at a pumping speed of 1.5 ml min^{-1}) was again done on LiChrosorb RP-18; elution was performed with 45% methanol in water. Detection was carried out at 243 nm; detection limits were *ca.* 25 ng. Preliminary experiments showed that with these sub-

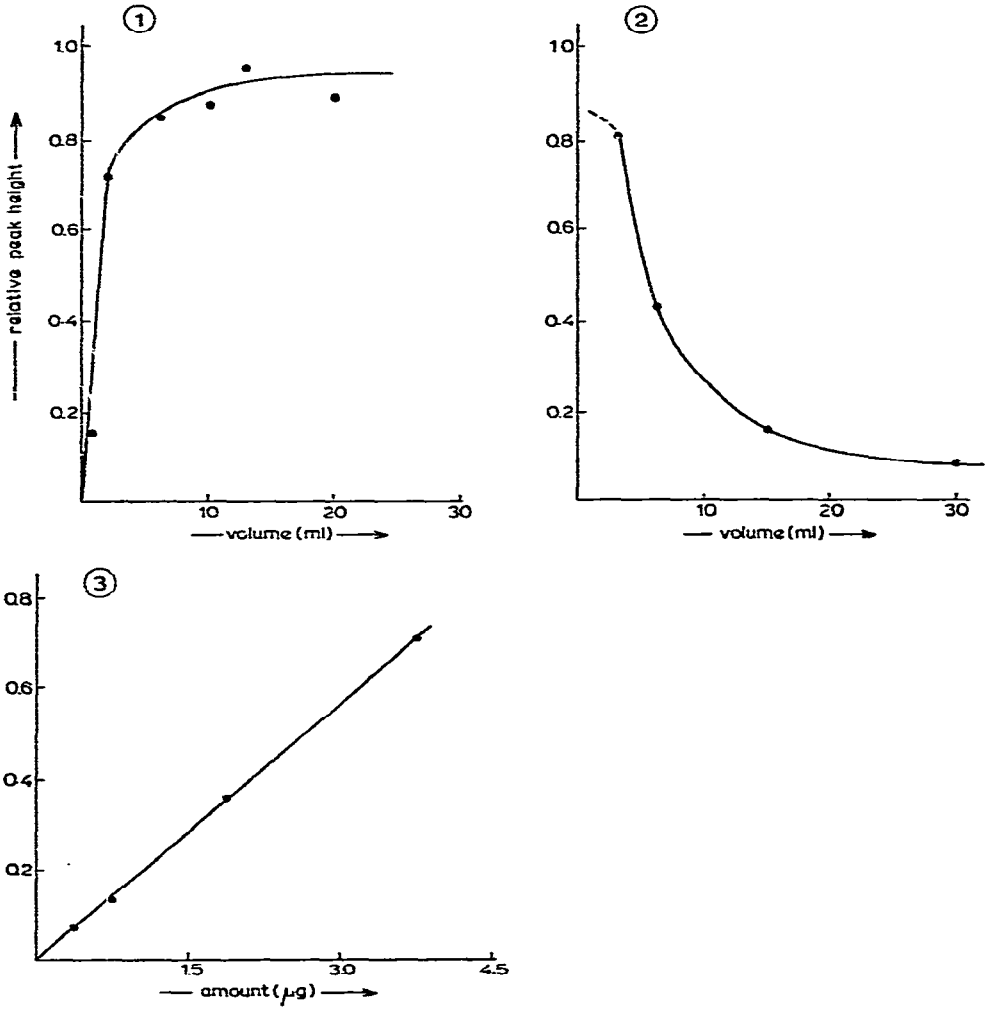

Fig. 5. Trace enrichment of 4-monochloroaniline: 1, from 2–20-ml sample volumes having identical concentrations; 2, from different sample volumes containing equal amounts of the analyte; 3, calibration curve recorded after trace enrichment from 2-ml volumes having different concentrations. For experimental details see Fig. 7.

stituted anilines, which are fairly polar compounds compared to, *e.g.*, PCBs, breakthrough on a pre-column (2 × 4.6 mm I.D.) occurs relatively rapidly (Fig. 5.1); addition of copper(II) salts (complexation) or changing the pH of the mobile phase did not significantly improve the results. From several further series of experiments we conclude that the rapid breakthrough is not caused by overloading of the pre-column but, instead, by the passage of too large a sample volume for trace components displaying relatively small capacity ratios in the system LiChrosorb RP-18–water* (Fig. 5.2). This conclusion is supported by the fact that the disubstituted aniline —which is more strongly retained on LiChrosorb RP-18 than is the monochloroaniline— displays better performance (Fig. 6). Using a 5- instead of a 2-mm long pre-column improved the situation to such an extent that, for 3,4-dichloroaniline, over 90% recovery was consistently obtained with up to at least 10-ml sample volumes containing microgram amounts of the trace component. Here, it should be noted that even trace enrichment from only 10-ml volumes implies a two-orders-of-magnitude improvement compared to results obtained with large conventional, *i.e.*, 100-μl, injections. A chromatogram of a water sample spiked with both chloroanilines under discussion is shown in Fig. 7.

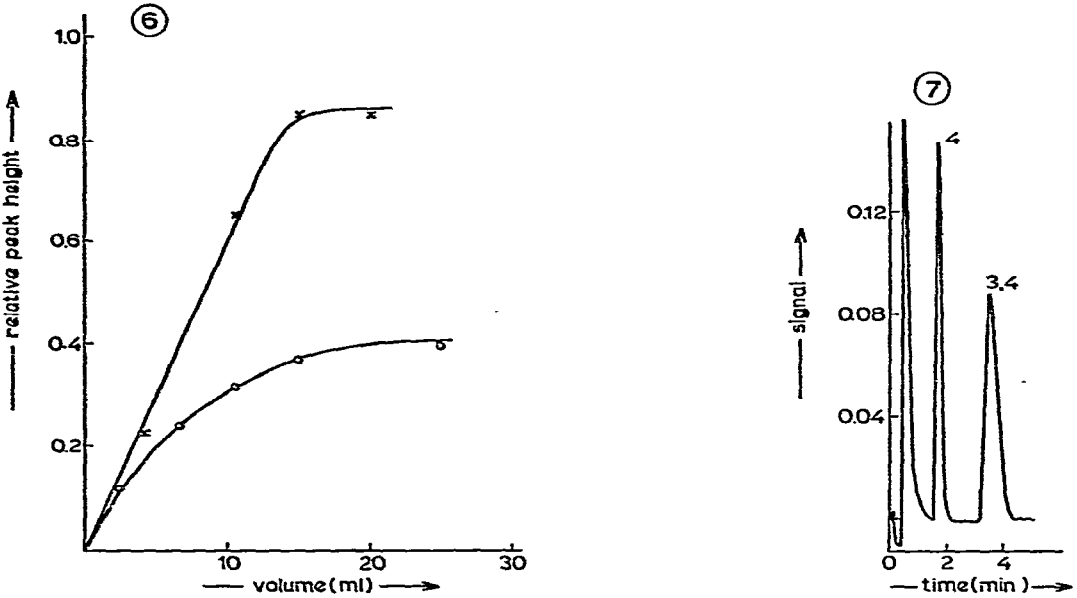

Fig. 6. Trace enrichment of 3,4-dichloroaniline from 2–25-ml volumes having identical concentrations, onto a 2-mm (○) and a 5-mm (×) long pre-column. For experimental details see Fig. 7.

Fig. 7. HPLC chromatogram recorded after trace enrichment of a 3-ml sample containing *ca.* 1.5 μg each of 4-mono- and 3,4-dichloroaniline. Loading speed, 1.5 ml min^{-1}. Stationary phase, LiChrosorb RP-18. Mobile phase, 45% methanol in water. UV detection at 243 nm.

* The retention of the chloroanilines with 45% methanol in water is about equal to that observed for all other compounds tested with 85–90% methanol in water.

CONCLUSIONS

From the above results, we conclude that on-line trace enrichment of non-polar compounds such as the phthalate esters and PCBs from large sample volumes onto a very short plug of an apolar chemically bonded stationary phase can easily be achieved. With such systems, band broadening is of the same order of magnitude as that obtained with conventional 10–20-μl injections (*cf.*, ref. 14) and recovery is quantitative even at the high pumping speed tested. The latter aspect indeed is a promising one: the virtual independence of the sorption efficiency of the flow-rates has now been established for flow-rates which are distinctly higher than those used up till now[12,16,23]. Moreover, the 95–100% recoveries reported in the present study, and also by Ishii *et al.*[16], favourably contrast with those previously published for, *e.g.*, polynuclear aromatic hydrocarbons by May *et al.*[12] (14–92%) and Eisenbeiss *et al.*[23] (75–100%). In summary, trace analysis from 1-l samples can successfully be completed in *ca.* 1 h; also, blank values are much lower than are those obtained with off-line techniques.

The major drawback of the present trace-enrichment technique is the fact that, with environmental samples, a large number of contaminants will often be simultaneously sorbed on, and eluted from, the pre-column. Even setting aside capacity (and clogging) problems, this will inevitably lead to broad solvent peaks and, hence, will seriously interfere with the detection of the trace components of interest. In principle, three alternatives exist to solve this problem: (1) the elaboration of a carefully designed gradient profile to achieve a more or less stepwise elution of the sorbed components from the pre-column; (2) the choice of a more selective stationary phase for the trace-enrichment step; (3) the use of a more selective detection principle, *e.g.*, fluorescence instead of UV-absorption analysis, and/or the introduction of a post-column derivatization technique. The improvement effected by using the last alternative has recently been shown[24–26] by us in the analysis of, *e.g.*, chlorpheniramine and hydroxyatrazine in human urine. Here, detection was done by the post-column addition of a fluorogenic ion-pairing reagent, extraction of the ion-pairs formed with the trace component into a suitable organic solvent and fluorimetric monitoring of this extract. Regarding the problems encountered when working with relatively polar compounds such as anilines and phenols, chemical derivatization or ion-pair formation can be used[27] to lower the polarity and make such compounds more amenable to trace enrichment from large sample volumes. Further work along several lines is in progress.

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