Journal of Chromatography, 129 (1976) 53–60

CHROM. 9294

IDENTIFICATION AND DETERMINATION OF PHTHALATE ESTERS IN RIVER WATER BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received March 22nd, 1976)

SUMMARY

The use of three separation modes (normal and reversed-phase adsorption chromatography and gel chromatography) with an ultraviolet absorption detector of variable wavelength provides the basis for a very sensitive method for the determination of phthalate esters in river water. Phthalates in river water were extracted with *n*-hexane and the extract was injected into three chromatographic systems [porous polymer beads–*n*-hexane (at 224 nm); porous polymer beads–methanol (at 224 nm); and polystyrene GPC gel–chloroform (at 243 nm)] without any pre-treatment such as clean-up or concentration. Average concentrations of approximately 45 ppb of di-*n*-butyl phthalate (DBP) and 10 ppb of di-2-ethylhexyl phthalate (DOP) were found in a given river water. Phthalate concentrations as low as 2 ppb could be determined. The limit of determination could be lowered by the use of a concentration process. The absolute detection limit was 2 ng of DOP at 224 nm.

INTRODUCTION

Phthalate ester plasticizers are widely used in synthetic polymers, especially poly(vinyl chloride), and there are indications that phthalates are widely dispersed in the environment. Procedures for the determination of phthalates have been based on the use of gas chromatography (GC). Phthalates in poly(vinyl chloride) have been analyzed using a 7-ft. column containing 10% OV-25 at 260° or a 1.5-m column containing 3% SE-30 at 200° with a flame-ionization detector[1]. The electron-capture detector is more sensitive and specific and is preferred for samples with very low levels of phthalates[2]. A major problem in the analysis of environmental samples is the reduction of background contamination to levels lower than the phthalate content and interference by other organic materials in the samples, so that pre-treatment (clean-up, etc.) of the extracts of the original samples with organic solvents is required[2].

Recent developments in high-performance liquid chromatography (HPLC) are worthy of special attention and several packing materials are being used increasingly for the investigation of the identification and quantification of organic and inorganic materials. A porous polystyrene–divinylbenzene resin appears to be a generally useful and powerful adsorbent for liquid chromatography[3,4]. A polystyrene–divinylbenzene

gel exclusively applied in the gel permeation chromatography of low-molecular-weight compounds has also recently been synthesized and is being used for the separation of oligostyrenes and phthalates[5].

An ultraviolet detector of the the variable-wavelength type, specially designed for HPLC, can selectively detect compounds that show any ultraviolet absorption. The lipophilic organic compounds in environmental samples are extracted with *n*-hexane or methylene chloride and only ultraviolet-active substances in the extracts can be detected using this type of detector. Therefore, the influence of ultraviolet-inactive materials present in the extracts as contaminants is negligible in this instance compared with GC analysis.

This paper reports a procedure for the detection of the very low levels of phthalates in *n*-hexane extracts of river waters in the presence of other organic materials. No pre-treatment or clean-up of the *n*-hexane extracts was required. By simultaneous use of three different LC modes, *viz.*, normal and reversed-phase adsorption chromatography and gel chromatography, the reliable identification of phthalates is possible.

EXPERIMENTAL

Apparatus

The home-made assembly consisting of a pump, a loop injector, columns and a detector was used for LC analysis. The pump was a Nihonbunko (JASCO) FLC-150 syringe-type pump. Injections were made with a Kyowaseimitsu high-pressure six-port valve to which a loop (stainless-steel tube) of the required volume was attached. Detection was effected with a Nihonbunko Model UVIDEC-100 variable-wavelength UV detector.

Columns

Column I. Shodex HP-255 (Showa Denko Co. porous polymer beads) was packed by a high-pressure slurry packing method in our laboratory into a 50 cm × 2 mm I.D. stainless-steel column. This packing material is cross-linked polystyrene and was used for adsorption chromatography.

Column II. Two Shodex A801 packed columns (Showa Denko Co.) of dimensions 500 × 8 mm I.D. (styrene–divinylbenzene resin with an exclusion limit of molecular weight 1000). This packing material was used for gel chromatography.

Eluents

The eluents used with column I were *n*-hexane (system A) and methanol (system B). Chloroform (system C) was used with column II. These solvents were guaranteed-grade reagents and were used after distillation.

Materials

Materials used as standard samples were dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), dihepthyl phthalate (DHP), di-2-ethylhexyl phthalate (DOP), didecyl phthalate (DDP) and dilauryl phthalate (DLP); they were all reagent-grade chemicals obtained from Tokyo Kasei Co. (Tokyo, Japan).

Measurements of chromatograms

The flow-rate in system A was 0.6 ml/min, in system B 1.0 ml/min and in system C 1.5 ml/min. All measurements were performed at room temperature. Elution volumes of phthalates were measured by dissolving them in each eluent to give a *ca.* 0.1% solution, and injecting a volume of 10 μl for systems A and B and 50 μl for system C. The detector was set at 254 nm. Chromatograms of extracts of river water were measured at 224 nm for systems A and B and at 243 nm for system C. A volume of 100 μl was injected in this instance.

Determination of phthalate contents in river water

A calibration graph for the low concentration range of each phthalate was constructed by plotting peak height against the concentration of the standard solution. The concentration of each phthalate in the extract was determined by using this calibration graph.

The concentration of a given phthalate in river water (C) was calculated as follows:

$$C = \text{Concentration of the phthalate in the extract} \times \frac{\text{Volume of } n\text{-hexane taken}}{\text{Volume of river water taken}} \quad (1)$$

Extraction from river water

Suspended particles of all types in river water were filtered by using filter-paper rinsed in *n*-hexane for 24 h before use. A 100-ml volume of this filtrate was then added to the separating funnel, followed by 10 ml of *n*-hexane. After shaking for 10 min on a mechanical shaker, the organic layer was transferred to a 10-ml volumetric flask and the volume was made up to the mark with *n*-hexane. This solution was filtered with 0.5-μm Millipore filter-paper. Sample water was taken from a river in the locality where waste water from an agricultural source flows in.

RESULTS

Typical chromatograms for systems A, B and C are shown in Figs. 1, 2 and 3, respectively. Elution volumes and apparent and corrected theoretical plate numbers (N and N') are given in Table I. N and N' were calculated as follows:

$$N = 5.54 \left(\frac{V_e}{W_{1/2}}\right)^2 \quad (2)$$

$$N' = 5.54 \left(\frac{V_e - V_0}{W_{1/2}}\right)^2 \quad (3)$$

where V_e is the elution volume, V_0 is the void volume or interstitial volume plus dead volume and $W_{1/2}$ is the peak width at half-height, all in units of millilitres.

An example of a chromatogram of the extract of a river water is shown in Fig. 4. The presence of DBP and DOP was observed. The concentrations of phthalates in the extract were 450 ppb* of DBP and 100 ppb of DOP, and their con-

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

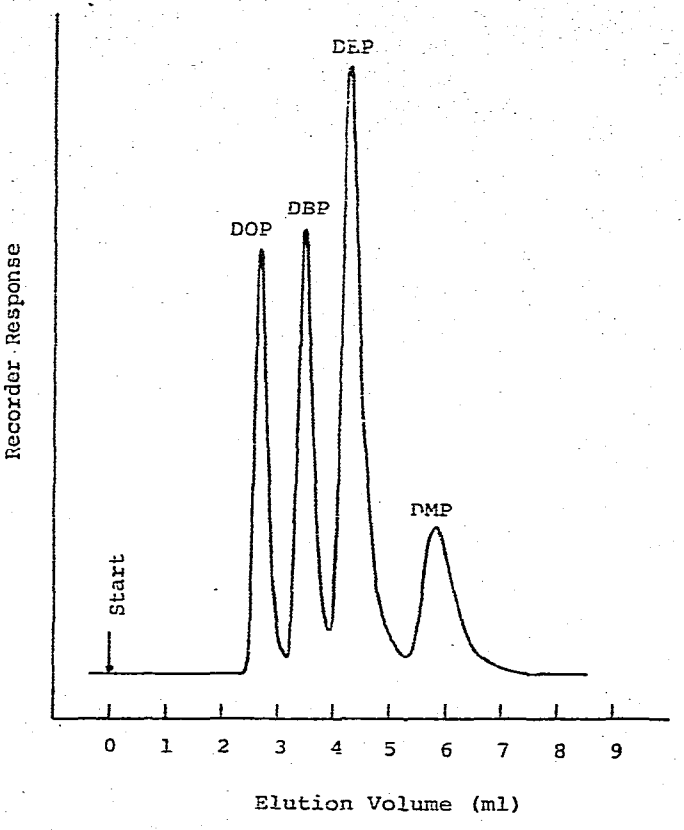

Fig. 1. Chromatogram for system A. Column I, 50 cm × 2 mm I.D., packed with Shodex Polymer Beads HP-255. Eluent, *n*-hexane. Sample volume injected, 10 μl. Flow-rate, 0.6 ml/min. Sample concentration, *ca.* 0.1%. Detector, UV at 254 nm. Attenuation, 0.16 a.u.f.s.

centrations in river water were 45 and 10 ppb, respectively. The first peak in Fig. 4 is contaminant(s) in *n*-hexane.

Chlorinated hydrocarbons and other pesticides that might be present in river water interfere in the GC analysis of the phthalates. Hence a separation of these classes of compounds is required prior to GC analysis, whereas no pre-treatment was required for the LC–UV analysis of the phthalates.

DISCUSSION

Limit of quantitative determination

Phthalate esters have λ_{max} values near 224 nm and the molar absorption coefficient is about ten times that at 254 nm. Calibration graphs for DBP and DOP at 224 nm for system A were linear over the range 20 ppb to 6 ppm. The operating sensitivities were about 13 ng for DBP and 12 ng for DOP, giving 10% of full-scale deflection, if the absorbance was measured at attenuation × 0.01 (0.01 a.u.f.s.)

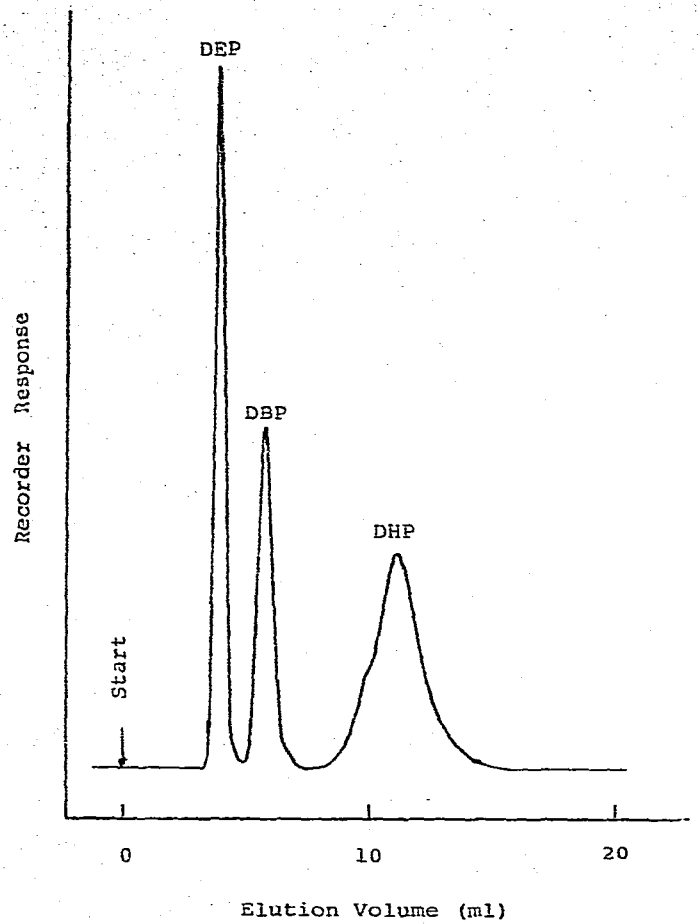

Fig. 2. Chromatogram for system B. Column I, 50 cm × 2 mm I.D., packed with Shodex Polymer Beads HP-255. Eluent, methanol. Sample volume injected, 10 μl. Flow-rate, 1.0 ml/min. Sample concentration, *ca.* 0.1% (DHP 0.2%). Detector, UV at 254 nm. Attenuation, 0.16 a.u.f.s.

and 100 μl of sample solutions were injected. The limit of the determination in the extract was assumed to be 20 ppb (2 ng in this instance) by considering the noise levels. As the volume of *n*-hexane used for extraction was one tenth that of the river water, the limit of determination could be lowered to 2 ppb of phthalates in river water. A concentration procedure can be used in order to decrease this limit further. Similarly, limits of determination in the extract for system B were 20 ppb of DBP and 600 ppb of DOP, and for system C 200 ppb of both DBP and DOP.

Extraction

An aqueous solution containing 0.1 ppm of DBP was prepared for the determination of the extraction efficiency. The shaking times and percentage extracted were as follows: 5 min, 88%; 10 min, 99%; and 20 min, 100%.

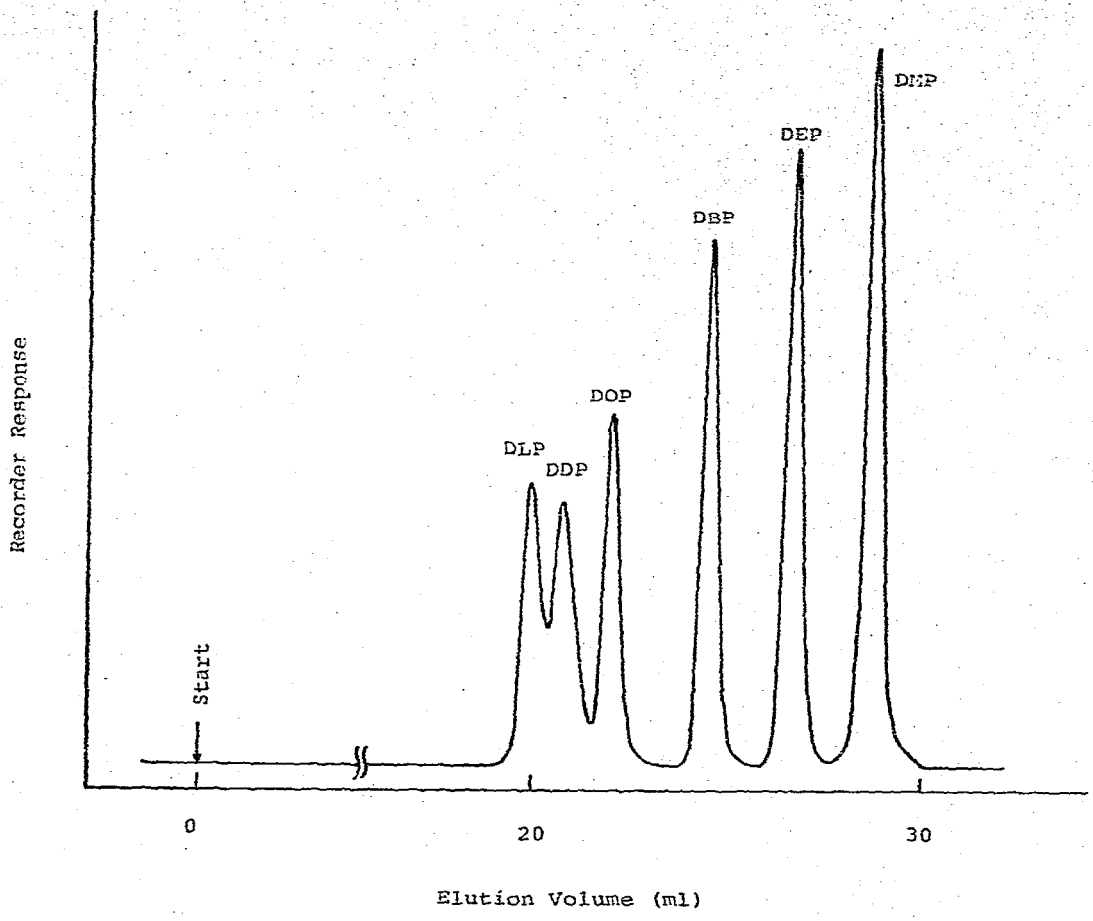

Fig. 3. Chromatogram for system C. Column II, 50 cm × 8 mm I.D., packed with Shodex A801. Eluent, chloroform. Sample volume injected, 50 μl. Flow-rate, 1.5 ml/min. Sample concentration, *ca.* 0.1%. Detector, UV at 254 nm. Attenuation, 0.64 a.u.f.s.

Identification and determination of phthalates in water

Phthalates in the extract of river water shown in Fig. 4 were identified by comparing the chromatogram with that in Fig. 1, and chromatograms for systems B and C were measured and compared with Figs. 2 and 3, respectively. A concentration procedure was needed in order to measure the chromatograms for systems B and C. Phthalates were not detected in the extract of the city water examined, or were below the detection limit (less than 1 ppb).

Phthalates were detected in the extract of a deionized water that had been stored in a polyethylene bottle, with concentrations of 430 ppb of DBP and 8 ppb DOP. Quantitative injection of sample solutions was possible by using a loop injector and internal standards were not required.

TABLE I

SEPARATION PARAMETERS OF PHTHALATE ESTERS FOR SYSTEMS A, B AND C

System	*Phthalate*	*Elution volume (ml)*	*Peak width at half-height (ml)*	*Capacity factor, k'*	*Theoretical plate number, N*	*Corrected plate number, N'*
A	DMP	5.85	0.55	7.3	626	490
	DEP	4.26	0.35	5.1	835	575
	DBP	3.50	0.28	4.0	865	545
	DHP	2.76	0.23	2.9	800	440
	DOP	2.70	0.21	2.86	915	505
	DDP	2.56	0.23	2.66	685	365
	DLP	2.53	0.23	2.62	670	350
	V_0	0.7				
B	DEP	3.9	0.4	4.6	525	355
	DBP	5.6	0.66	7.0	400	305
	DHP	11.0	1.98	14.7	170	150
	V_0	0.7				
C	DMP	28.9	0.44	0.75	23 900	4400
	DEP	26.7	0.44	0.62	20 400	3000
	DBP	24.6	0.44	0.49	17 300	1870
	DHP	22.5	0.48	0.365	12 200	865
	DOP	22.2	0.46	0.345	12 900	850
	DDP	21.1	0.58	0.28	7 330	350
	DLP	19.9	0.55	0.205	7 250	215
	V_0	16.5				

Elution order

The solubility parameters δ, are 9.1 for polystyrene[6] and 7.3, 12.9 and 9.1 for *n*-hexane, methanol and chloroform[7]. If the solubility parameter of cross-linked polystyrene gel is assumed to be the same as that of non-cross-linked polystyrene, then *n*-hexane is less polar than polystyrene gel and phthalates for system A are eluted in a "normal-phase" order of decreasing chain length or increasing polarity. The separation mode for system B is "reversed-phase" adsorption chromatography and phthalates are eluted in order of increasing chain length or decreasing polarity. Adsorption effects cannot occur when chloroform is used as the eluent, because the polarity of chloroform is similar to that of the gels. Steric exclusion effects are predominant for system C, resulting in elution in order of decreasing chain length (molecular weight).

As longer-chain phthalates would tend to bunch up at the dead-volume peak, the separation of DHP, DOP, DDP and DLP by system A is not practical and a longer column is required. Measurements of chromatograms for system B at higher temperatures, *e.g.* 50°, are preferred in order to decrease the elution time and peak width.

Theoretical plate numbers

In spite of smaller capacity factors, the theoretical plate numbers of solutes for system C were higher than those for systems A and B. This is due mainly to the fact that the interstitial volume, V_0, which does not participate in separation, is involved in the calculation of theoretical plate numbers in addition to the large gel volume.

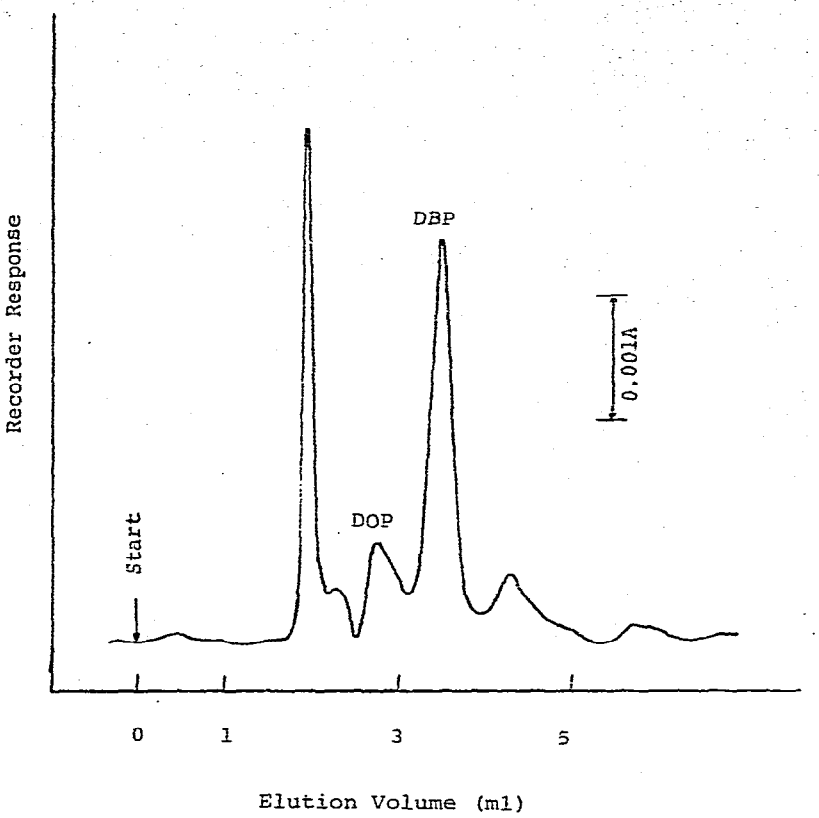

Fig. 4. Chromatogram of an extract of river water measured with system A. Sample volume injected, 100 μl. Detector, UV at 224 nm. Attenuation, 0.01 a.u.f.s.

Corrected plate numbers calculated from eqn. 3 by subtracting the interstitial volume from the observed elution volume were in accordance with values for other systems. Comparison of the separabilities of the three systems is possible by using the corrected plate number.

REFERENCES

1 D. T. Burns, W. P. Hayes and P. Steele, *J. Chromatogr.*, 103 (1975) 241.
2 C. S. Giam, H. S. Chan and G. S. Neff, *Anal. Chem.*, 47 (1975) 2225.
3 M. D. Grieser and D. J. Pietrzyk, *Anal. Chem.*, 45 (1973) 1348.
4 H. Takahagi and S. Seno, *J. Chromatogr.*, 108 (1975) 354.
5 Y. Kato, S. Kido, H. Watanabe, M. Yamamoto and T. Hashimoto. *J. Appl. Polym. Sci.*, 19 (1975) 629.
6 J. Brandrup and E. H. Immergut (Editors), *Polymer Handbook*, Wiley, New York, 1966.
7 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1974.