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Note

Reversed-phase adsorption of phthalate esters from aqueous solutions and their gradient elution using a high-performance liquid chromatograph

AKIRA OTSUKI

National Institute for Environmental Studies, Division of Chemistry and Physics, Yatabemachi, Tsuku-bagun, Ibaraki 300-21 (Japan)

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Phthalate esters have been used as plasticizers in a variety of films and plastics for the last 30 years, but it has only been within recent years that their migration into human tissues[1–4] and their increasing occurrence in the environment[5–9] have been reported. The widespread use of these compounds and their presence in the environment have resulted in the development of chromatographic methods[10], such as gas chromatography[11–13] and mass fragmentography[6,8,14], for their determination after solvent extraction. In the application of these methods to environmental water samples, clean-up and concentration by a factor of 10–1000 are necessary and artificially high values may be obtained owing to contamination during the analysis because relatively high concentrations of phthalate esters are often found in distilled water, solvents[15–17] and the laboratory air[18].

With the development of bonded stationary phases[19], liquid–solid chromatography currently offers the greatest possibilities for the separation of a wide variety of organic compounds, with the use of high-performance liquid chromatography and solvent programming techniques. In an attempt to develop a method for the determination of phthalate esters in water by adsorption chromatography which simultaneously involves concentration and separation, the high-performance liquid chromatographic behaviour of phthalate esters in water using an octadecyltrichlorosilane bonded stationary phase has been examined.

EXPERIMENTAL

Apparatus

A Model ALC 202 liquid chromatograph equipped with two 6000A pumps, a Model 660 solvent programmer and a Model 440 UV detector operating at 254 nm (Waters Assoc., Milford, Mass., U.S.A.) was used. A 30 cm × 4 mm I.D. μBondapak C-18 column was used, which has a monomolecular layer of octadecyltrichlorosilane chemically bonded to Porasil beads with an average particle size of 10 μm (Waters Assoc.). Gradient elution was started at 100% water and continued to 100% methanol in a hyperbolic manner. "Curve 2" on the Waters programmer was selected; the time setting for the programme was 10 or 15 min.

Reagents

Nanograde methanol (Mallinckrodt, St. Louis, Mo., U.S.A.), reagent-grade di-2-ethylhexyl, di-*n*-butyl and di-*n*-heptyl phthalate (Wako, Osaka, Japan) and practical-grade di-*n*-pentyl and di-*n*-nonyl phthalate (Eastman-Kodak, Rochester, N.Y., U.S.A.) were used without further purification. Redistilled water was prepared by distillation followed by alkaline permanganate distillation. Although frontal redistilled water was discarded (about 100–500 ml), it gave some small peaks on the liquid chromatogram when a blank run was carried out. Standard solutions were prepared by dissolving each phthalate ester in methanol to give concentrations of 0.2–0.3 μg/μl.

Procedure A

While redistilled water was pumped through the column at 3 ml/min, 1–5 μl of the standard solution was injected through a U6K universal injector (Waters Assoc.) and all phthalate esters were retained on the packed materials. A gradient was started after the flow-rate had been changed to 2 ml/min. When the mobile phase composition became 50:50 water–methanol, the gradient was held for 5–50 min (usually 10 min), then the gradient was continued further to 100% methanol. The column regeneration effected between two successive runs was always carried out under the same conditions[20]; the regeneration time was 5 min and the gradient mode was "Curve 2" on the Waters programmer.

Procedure B

After sample water containing 0.2–2 μg of each phthalate ester had passed through the column at 3 ml/min, the column was washed with about 20 ml of redistilled water, then the gradient elution was started as described above.

In the present study, procedure A was mostly used in order to obtain basic information on the reversed-phase adsorption chromatography of phthalate esters in aqueous solution. All experiments were conducted at ambient temperature.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of the separation of phthalate esters by gradient elution with a "holding" process. After holding the mobile phase composition of 50:50 water–methanol for 10 min, the esters were eluted by the further gradient in the order di-*n*-butyl, di-*n*-pentyl, di-*n*-hexyl, di-2-ethylhexyl and di-*n*-nonyl phthalate, indicating that the retention times were directly related to number of alkyl carbon atoms in the ester groups of the phthalates. The elution order when using the usual gradient without the holding process was the same as that shown in Fig. 1, which was the reverse of that in a normal phase separation[21]. A linear relationship was found between the retention times and number of linear alkyl carbon atoms in the ester groups of the phthalate, as shown in Fig. 2. These results confirm Kikta and Grushka's conclusion[22] that compounds which contain a significant alkyl side-chain are likely to interact with alkyl-bonded stationary phase, and suggest that the separation mechanism in this reversed-phase adsorption chromatography may involve the interaction of affinity between the bonded alkyl stationary phase and the length of the alkyl chain in
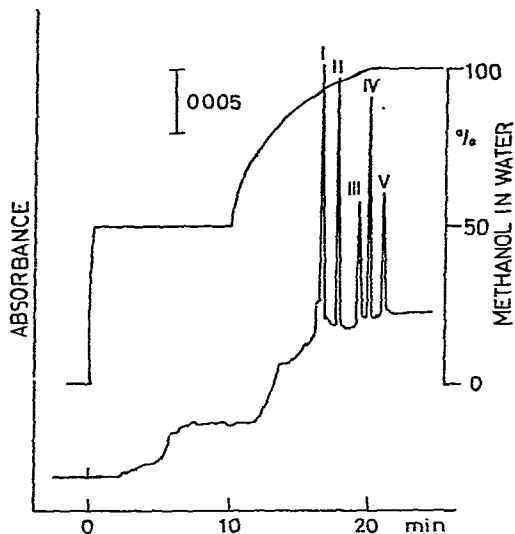
Fig. 1. Typical chromatogram of a mixture of phthalate esters obtained by gradient elution with a 10-min holding process. Flow-rate, 2 ml/min; setting time for programme, 10 min. Peaks: I = di-*n*-butyl; II = di-*n*-pentyl; III = di-*n*-hexyl; IV = di-2-ethylhexyl; V = di-*n*-nonyl phthalate.

the ester group of each phthalate with solubilities of each phthalate ester for the mobile phase.

Locke[23] demonstrated that in a reversed-phase system the retention order within a class of compounds is solely a function of solubilities of the solutes in the mobile phase. Kikta and Grushka[22] also reported that as the solubility of phenones in the mobile phase decreases with increase in the length of the alkyl side-chain, the retention time increases. The present results using phthalate esters seem to be consistent with their results, and also suggest that the importance of the interaction of the benzene ring of phthalates with a bonded alkyl stationary phase and aromatic ester groups of phthalic acid with the residual silanol group of a siliceous surface is extremely reduced in this system.
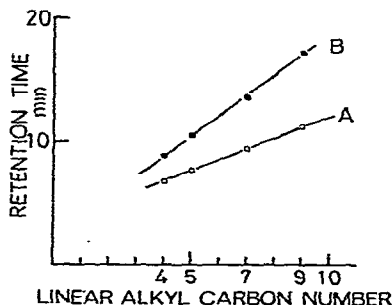

Fig. 2. Relationship between retention time and number of linear alkyl carbon atoms in the ester groups of phthalates. A, Gradient elution with a 10-min holding process, the retention time being measured from the starting point of the further gradient; B, gradient elution without the holding process, the setting time for the programme being 20 min.

In the application of the present method to environmental water samples, a reason for selecting hyperbolic solvent programming and for introducing a holding process during the gradient was to elute organic compounds to be adsorbed from natural water samples in the early stage of the gradient in order to avoid overlapping of the peaks of the other adsorbed compounds with those of the phthalate esters eluted in the later stage.

The effects of the composition of the mobile phase in holding on the apparent retention times from the starting point of the further gradient are shown in Figs. 3 and 4. In the mobile phase of water–methanol composition 50:50, there was little effect on the retention times for a holding time of 15 min at 2 ml/min. On the other hand, when the water–methanol composition 40:60 was used, the apparent retention time of di-n-butyl phthalate was reduced with an increase in the holding time, indicating that di-n-butyl phthalate will be eluted during the holding process when the holding time is longer than 25 min. This effect was confirmed by the other experiments. These results seem to support the mechanism of reversed-phase liquid–solid chromatography proposed by Telepchak[24] that involves mainly reversed-phase adsorption on an inert surface rather than liquid–liquid partition such that the various solutes are distributed differently between the bonded alkyl stationary phase and the mobile phase. These results also suggest that the affinity between the di-n-butyl group of phthalate esters and the octadecyl group of the bonded stationary phase may be approximately equal to that between di-n-butyl phthalate and the mobile phase consisting of water–methanol (50:50), and also indicate that if procedure B is applied to the analysis of environmental water samples, a mobile phase composition of 50:50 water–methanol should be used in the holding process. In preliminary experiments, most organic compounds adsorbed from filtered natural waters and having an absorbance near 254 nm were found to be eluted during a holding process of about 10 min when using a mobile phase composition of 50:50 water–methanol.

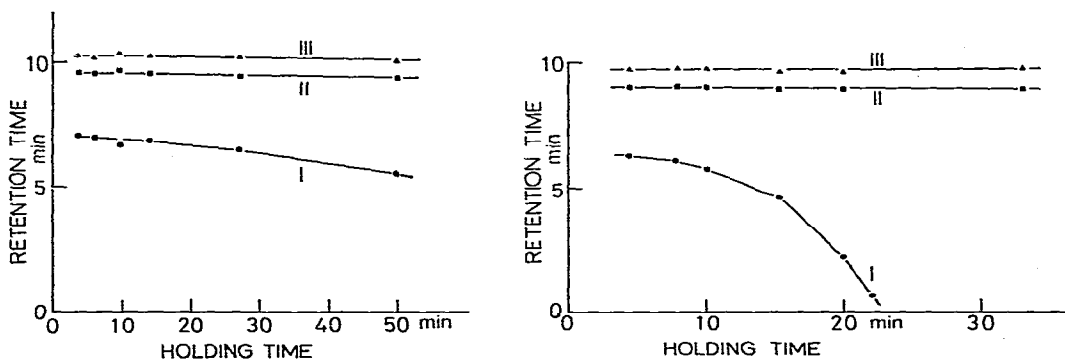To test the applicability of this reversed-phase adsorption chromatography

Fig. 3. Effect of holding time on apparent retention time for a mobile phase composition of 50:50 water–methanol. Flow-rate, 2 ml/min; gradient elution with 10-min holding process; setting time, 10 min. I = Di-n-butyl; II = di-n-hexyl; III = di-2-ethylhexyl phthalate. The retention time was taken from the starting point of the further gradient.

Fig. 4. Effect of holding time on apparent retention time for a mobile phase composition of 40:60 water–methanol. Conditions as in Fig. 3. I = Di-n-butyl; II = di-n-hexyl; III = di-2-ethylhexyl phthalate.

in quantitative studies, calibration graphs were constructed from a plot of peak height *versus* amount injected according to procedure A. Linear relationships were found for all of the phthalate esters studied, as shown in Fig. 5. These calibration graphs indicate a limit of detection of 0.1 μg with a full-scale range of 0.05 absorbance unit. However, recovery experiments on redistilled and filtered natural waters showed that recoveries obtained by procedure B do not always agree with those obtained by procedure A at concentrations above about 5 ppb, with the exception of di-*n*-butyl and di-*n*-pentyl phthalate. The cause of this discrepancy may be attributed to the decrease in the solubilities in water of phthalate esters with longer alkyl chains than di-*n*-pentyl.
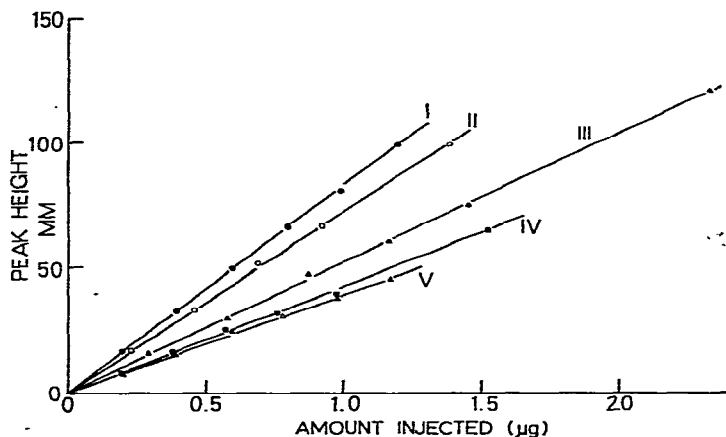

Fig. 5. Calibration graphs for gradient elution with a 10-min holding process. I = Di-*n*-butyl; II = di-*n*-pentyl; III = di-*n*-hexyl; IV = di-2-ethylhexyl; V = di-*n*-nonyl phthalate.

Further investigations are needed in order to be able to apply procedure B in the analysis of environmental water samples.

In summary, phthalate esters in water were found to be completely adsorbed on octadecyltrichlorosilane bonded beads and their elution order in 100% water to 100% methanol gradient system was directly related to the number of alkyl carbon atoms in the ester groups of phthalates. A linear relationship was found between retention times and the number of linear alkyl carbon atoms in the ester groups of phthalates. The present study indicated that the separation mechanism in this reversed-phase adsorption chromatography may be due to the interaction of affinity between octadecyl groups of the bonded stationary phase and the length of the alkyl chain in the ester group of each phthalate with solubilities of each phthalate ester for the mobile phase.

ACKNOWLEDGEMENT

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