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DEVELOPMENT OF AN ON-LINE HIGH-PRESSURE PRE-COLUMN CONCENTRATION METHOD FOR MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND ITS APPLICATION TO THE RAPID DETERMINATION OF DIBUTYL PHTHALATE IN WATER

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SUMMARY

An on-line high-pressure pre-column concentration method using switching valves was developed for micro high-performance liquid chromatography, and applied to the high-speed determination of dibutyl phthalate in water.

INTRODUCTION

Very high-speed separations have become of increasing importance in high-performance liquid chromatography (HPLC)[1–4]. High-speed separations are generally achieved using a short column packed with fine particles. Erni[4] classified the speed of HPLC in terms of the eluting time of the non-retained solute, *viz.*, conventional HPLC, very high-speed HPLC and super-speed HPLC. High-speed separations offer several advantages over the conventional methods, *viz.*, decreased time and cost of the analysis, the possibility of analysing unstable species and applicability to kinetic studies.

We have constructed a micro-scale chromatograph that permits high-speed and/or high-pressure operation, and demonstrated some rapid separations at low flow-rates (20–50 μl/min), with a lower consumption of the mobile phase than in conventional HPLC[5].

The pre-column concentration method is a useful pre-treatment in the analysis of real samples by micro HPLC[6–9]. However, previous analyses were carried out with either off-line[6,7,9] or low-pressure on-line pre-column concentration method[8], and they require a long time or involve complicated procedures.

In this work we have developed an on-line high-pressure pre-column concentration method for high-speed analysis in micro HPLC.

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EXPERIMENTAL

Apparatus

The apparatus was constructed from two high-pressure micro pumps, three switching valves, two pre-columns, a separation column and a detector, as shown in Fig. 1. The system with two pre-columns allowed the sample solution to be concentrated in one while the other was used for the analysis, which favoured rapid analysis. One pump was used for feeding the sample solution into the pre-column and the other for feeding the mobile phase. A Familic-300S pump [Jasco (Japan Spectroscopic), Tokyo, Japan] was used in the constant-pressure mode for the former operation, and a 2150 HPLC pump (LKB, Bromma, Sweden) in the constant-flow mode for the latter operation. Two Model 7000 (Rheodyne, Cotati, CA, U.S.A.) and one N6W (Valco Instruments, Houston, TX, U.S.A.) switching valves were used. The former valves (Nos. 1 and 3 in Fig. 1) were fitted with 1/16-in. connecting materials, and the latter valve (No. 2 in Fig. 1) with 1/32-in. connecting materials. The N6W switching valve, which had been developed for micro-scale HPLC, had a low dead volume. In order to minimize sample broadening in the parts between the separation column and the pre-column, narrow-bore fused-silica tubing of 55 μm I.D. and 0.24 mm O.D. (SGE, Melbourne, Australia) was employed as the connecting tubing to the N6W switching valve. Both ends of these fused-silica capillary tubes were ce-

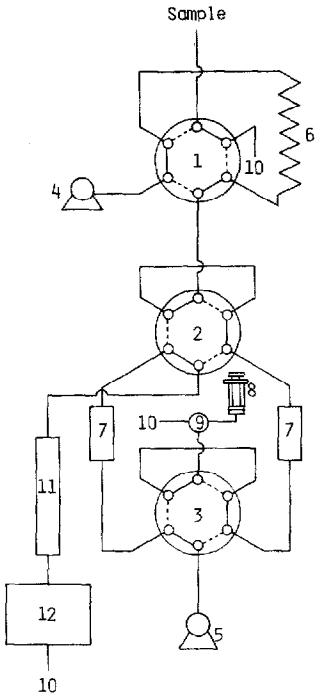

Fig. 1. Diagram of the apparatus. 1 = Switching valve 1 (Rheodyne 7000); 2 = switching valve 2 (Valco N6W); 3 = switching valve 3 (Rheodyne 7000); 4 = pump (Familic-300S); 5 = pump (LKB 2150); 6 = sample loop; 7 = concentration columns; 8 = gas-tight syringe for measurement of sample volume; 9 = three-way tap; 10 = drain; 11 = separation column; 12 = UV detector.

mented into the stainless-steel tubing (0.25 mm I.D. and 0.8 mm O.D.) (Gasukuro Kogyo, Tokyo, Japan) with epoxy resin adhesive, which permitted connections with stainless-steel ferrules.

The structures of the separation and concentration columns are shown in Fig. 2. A 1/32-in. zero dead volume union (Valco Instruments) and a 1/16 × 1/32 in. zero dead volume reducing union (Valco Instruments) were convenient for high-pressure connection with minimal band broadening. The inlet of the fused-silica separation column was glued into the stainless-steel tubing (0.51 mm I.D. and 0.81 mm O.D.) by the method reported previously[5]. On the other hand, the whole length of the fused-silica tubing (0.34 mm I.D. and 0.42 mm O.D.) was glued into the stainless-steel tubing (0.51 mm I.D. and 0.81 mm O.D.) in the case of the concentration column. The length of the pre-column was restricted by unions and nuts and thus the minimum length was 27 mm. These columns withstood pressures higher than 350 bar. ODS SC-01 (5 μm) (Jasco) and Develosil ODS-15/30 (15–30mm) (Nomura Chemical, Seto-shi, Japan) were selected as packing materials for the separation and the concentration column, respectively.

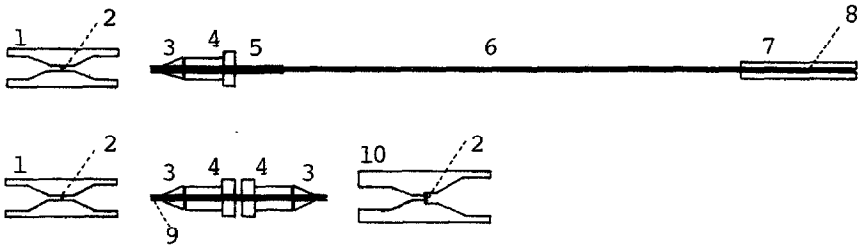

Fig. 2. Structures of the separation column and the pre-column. 1 = 1/32-in. zero dead volume union; 2 = 1/32-in. stainless-steel ferrule; 4 = 1/32-in. nut; 5 = stainless-steel tubing, 0.51 mm I.D. × 0.81 mm O.D.; 6 = fused-silica tubing, 0.34 mm I.D. × 0.42 mm O.D.; 7 = PTFE tubing, 0.25 mm I.D. × 2 mm O.D.; 8 = quartz wool; 9 = 0.34 mm I.D. × 0.81 mm O.D. (see text); 10 = 1/16 × 1/32 in. zero dead volume reducing union.

A UVIDEC-100V UV spectrophotometer (Jasco) was employed as a detector and a time constant of 0.05 sec was selected. The flow cell of the detector was laboratory made and had a detection volume of 0.05 μl. Identification of di-*n*-butyl phthalate (DBP) in water was confirmed by the use of a multi-channel photodiode array detector for micro HPLC[10], in which the separation speed was restricted owing to the slow sampling time (one spectrum per *ca.* 2 sec).

Reagents

DBP was obtained from Tokyo Chemical Industry (Tokyo, Japan). Acetonitrile, HPLC-grade distilled water and ethanol were purchased from Wako (Osaka, Japan). Purified water was purchased from Isekyu (Nagoya, Japan).

Sample preparation

The sample solution was filtered with an Ekicrodisc 13 filter (0.45 μm) (Gelman Sciences Japan, Tokyo, Japan) before injection. The above filter was previously washed with ethanol and then with HPLC-grade distilled water. A 1-ml sample loop

(2 m × 0.8 mm I.D.) was employed and the sample volume was measured with a 1-ml gas-tight syringe.

RESULTS AND DISCUSSION

The on-line high-pressure pre-column concentration system was suitable for the determination of DBP in water. Fig. 3 shows a calibration graph for DBP, with a linear relationship between peak height and concentration up to 20 ppb*. The calibration graph did not pass through the origin, which indicates that the HPLC-grade distilled water used to prepare the sample solution originally contained DBP. The detection limit of DBP was *ca.* 0.5 ppb when the sample volume was 0.7 ml. The detection limit could be improved by increasing the sample volume. It took *ca.* 3 min to pass 0.7 ml of sample solution into the pre-column at 80 bar. Therefore, one separation could be performed every 5 min.

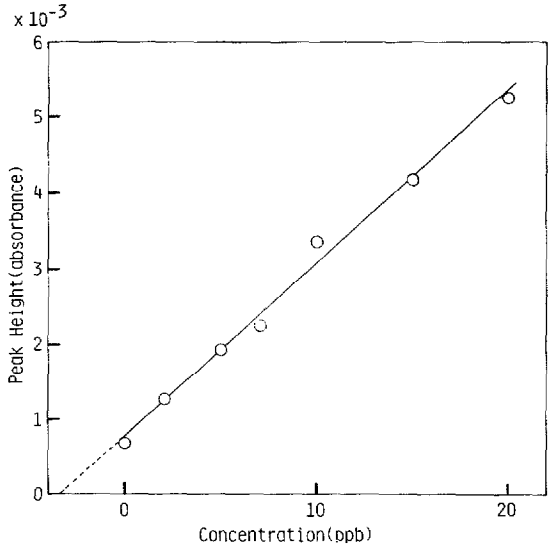

Fig. 3. Relationship between peak height and concentration of DBP. Column: ODS SC-01, 100 × 0.34 mm I.D. Mobile phase: acetonitrile-water (7:3). Pre-column: Develosil ODS-15/30, 27 × 0.34 mm I.D. Flow-rate: 20 μl/min. Sample: DBP in distilled water (0.7 ml). Wavelength of UV detection: 235 nm.

Fig. 4 shows the linear relationship between peak height and sample volume. The sample was laboratory-distilled water that contained *ca.* 20 ppb of DBP. By the use of a larger volume sample loop, the linear range can be extended.

The reproducibility of the method was tested by carrying out twenty chromatographic runs using laboratory-distilled water. When the sample volume was 0.7 ml, the relative standard deviation of the peak height was 5.4%.

Fig. 5 demonstrates separations of DBP using laboratory-distilled and com-

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

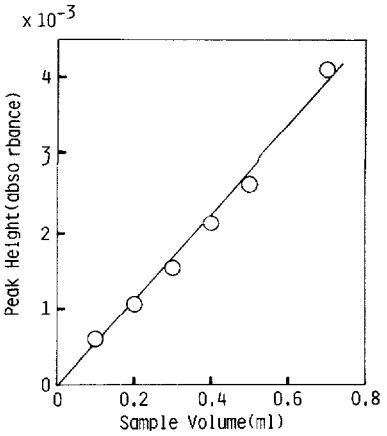

Fig. 4. Relationship between peak height and sample volume. Operating conditions as in Fig. 3 except the sample (laboratory-distilled water).

mercially available HPLC-grade distilled water. The flow-rate of the mobile phase was 20 μl/min, which corresponds to *ca.* 4 ml/min when using a conventional HPLC column (4.6 mm I.D.). The pressure drop across the pre-column and the separation column was 70–90 bar under the conditions shown in Fig. 5. The concentrations of DBP in laboratory-distilled and HPLC-grade distilled water were determined to be 19 and 2.9 ppb, respectively. The separation was achieved in a few minutes.

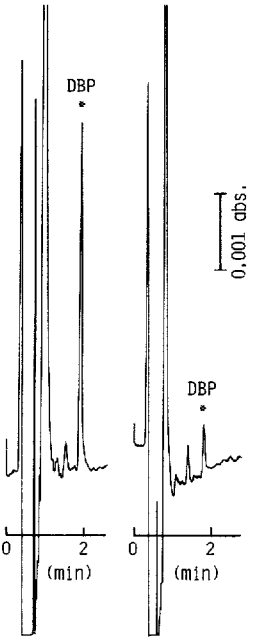

Fig. 5. Separation of DBP in distilled water. Operating conditions as in Fig. 3 except the samples: (A) laboratory-distilled water (0.7 ml); (B) HPLC-grade distilled water (0.7 ml).

Fig. 6 demonstrates the separation of DBP in tap water and commercially available purified water. The concentrations of DBP were determined to be 4.5 ppb in the former and 5.4 ppb in the latter.

DBP in organic solvents could be also determined by diluting organic solvents in distilled water so that the DBP could be effectively concentrated on the pre-column.

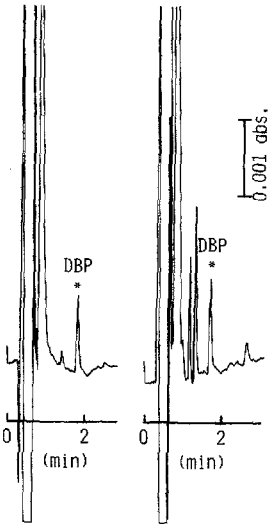

Fig. 6. Separations of DBP in water. Operating conditions as in Fig. 3 except the samples: (A) tap water (0.7 ml); (B) purified water (0.7 ml).

CONCLUSION

DBP in water can be determined very rapidly by fast micro HPLC with the on-line high-pressure pre-column concentration method. The proposed method will be of use for the rapid analysis of various samples that require pre-treatment such as extraction or concentration. It will be possible in the near future for the present system to be operated automatically if automatic switching valves and automatic sample loading system are employed.

REFERENCES

1 R. P. W. Scott, P. Kucera and M. Munroe, *J. Chromatogr.*, 186 (1979) 475.
2 J. L. DiCesare, M. W. Dong and L. S. Ettre, *Chromatographia*, 14 (1981) 257.
3 J. L. DiCesare, M. W. Dong and J. G. Atwood, *J. Chromatogr.*, 217 (1981) 369.
4 F. Erni, *J. Chromatogr.*, 282 (1983) 371.
5 T. Takeuchi, D. Ishii and A. Nakanishi, *J. Chromatogr.*, 285 (1984) 97.
6 D. Ishii, K. Hibi, K. Asai and M. Nagaya, *J. Chromatogr.*, 152 (1978) 341.
7 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 218 (1981) 199.
8 M. Goto, T. Nakamura and D. Ishii, *J. Chromatogr.*, 226 (1981) 33.
9 D. Ishii, S. Murata and T. Takeuchi, *J. Chromatogr.*, 282 (1983) 569.
10 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 288 (1984) 451.