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SEPARATION OF ISOTOPIC MOLECULES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The separation of the isotopic molecules C_6H_6–C_6D_6 and C_7H_8–C_7D_8 is reported. Reversed-phase liquid chromatography was used with a non-polar column and water–methanol mixtures as solvent, and separation factors higher than in gas chromatography were obtained. The operating conditions are examined and a procedure for optimizing the resolution between the two isotopic peaks is described.

INTRODUCTION

Many isotopic molecules partially or completely substituted with deuterium atoms have been separated by gas chromatography. By using high-efficiency capillary columns or long packed columns, separations have been obtained for many isotopic hydrocarbons by either gas–solid or gas–liquid chromatography. With the large number of theoretical plates given by these columns, it has been possible to separate isotopic compounds also even when the differences in the separation factors are very small[1-5].

In this paper, we report some examples of separations of isotopes by high-performance liquid chromatography, in order to demonstrate that this technique also can be employed successfully in this field.

We investigated the separations of benzene–deuterobenzene and toluene–deuterotoluene, which had previously been achieved by gas chromatography, in order to compare the results obtained.

EXPERIMENTAL

We used reversed-phase liquid chromatography by employing a non-polar stationary phase and a very polar solvent. An octadecylsilane (ODS) Zipax column DuPont, Wilmington, Del., U.S.A.) 1 m × 1.2 mm I.D., packed with a chemically bonded phase of 30-μm silica particles, was used. Measurements were carried out with a DuPont Model 830 liquid chromatograph equipped with a UV detector (254 nm), and with different compositions of the mobile phase (water–methanol) in the temperature range 20–50 °C.

The deuterated compounds were purchased from Merck (Darmstadt, G.F.R.)

and a 1 % solution in methanol of the deuterated compound, alone or mixed with the corresponding non-deuterated compound, was injected into the liquid chromatograph. The dead volume was measured from the small peak observed for methanol, assuming that under the conditions used it is not retained.

RESULTS AND DISCUSSION

Figs. 1 and 2 show examples of the chromatograms obtained and the separations achieved with $C_6H_6-C_6D_6$ and $C_7H_8-C_7D_8$. The deuterated molecules were eluted before the corresponding non-deuterated molecules, as was also observed in gas chromatography.

The resolution (R) between two adjacent peak, in this instance for two isotopic molecules, according to the relationship

$$R = \frac{\sqrt{n}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{K'}{K' + 1} \right) \tag{1}$$

depends on three parameters: the number of theoretical plates of the column (n), the separation factor (ratio of the corrected retention volumes, α) and the capacity ratio (K'). The effect of these parameters will be discussed separately.

Tables I and II report the experimental values of these parameters together with other chromatographic characteristics.

The separation factor depends on the column temperature and on the types of the stationary and mobile phases employed. The best α values are obtained, for both of the isotopic systems studied, with a non-polar stationary phase and a very polar solvent. As shown in Tables I and II, there is little effect due to the temperature or small variations in the concentration of methanol in water.

By comparing these α values with those previously obtained by gas chromatography, a "reversed" isotopic effect is observed in both instances. In gas chromatography also, the best α values are obtained with a non-polar stationary phase (squalane). Increasing the polarity of the liquid phase results in a decrease in α.

In gas chromatography, a "normal" isotopic effect ca be observed only at very low temperatures; for example, with CH_4-CD_4 at $-120°C$, the deuterated compound is eluted first, but at $-180°C$ the elution order is reversed.
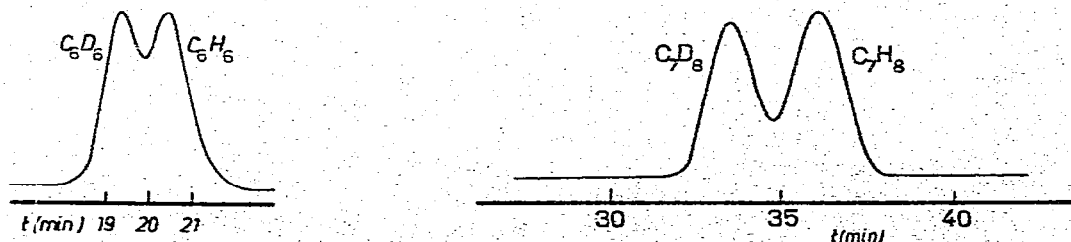

Fig. 1. Separation of C_6H_6 and C_6D_6. Column, Zipax ODS, 1 m × 1.2 mm I.D.; $T = 20°C$; solvent, water.

Fig. 2. Separation of C_7H_8 and C_7D_8. Column, Zipax ODS, 1 m × 1.2 mm I.D.; $T = 20°C$; solvent, water–methanol (9:1).

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS FOR THE SEPARATION OF C_6H_6–C_6D_6

T (°C)	Solvent	Flow-rate (ml/min)	t_R' (min) (C_6H_6)	α	K'	n	H (mm)	R
50	H_2O	0.83	5.30	1.070	2.15	2490	0.40	0.45
20	H_2O	0.93	5.98	1.072	2.76	1840	0.54	0.43
20	H_2O	0.48	11.10	1.077	2.55	2150	0.46	0.52
20	H_2O–CH_3OH (95:5)	0.80	5.60	1.068	2.54	1730	0.58	0.33
20	H_2O–CH_3OH (95:5)	0.45	10.50	1.072	2.52	2640	0.38	0.44

In liquid chromatography, where it is possible to use two liquid phases of very different polarities, higher separation factors are obtained; as shown in Table I for C_6H_6–C_6D_6 $\alpha = 1.070$, whereas in gas chromatography with a squalane column at 20°C $\alpha = 1.059$. As shown in Table II, for C_7H_8–C_7D_8 in liquid chromatography $\alpha = 1.10$, whereas in gas chromatography $\alpha = 1.059$. Consequently, by using high-performance liquid chromatography, a required separation can be achieved with a lower number of theoretical plates in a shorter time. The capacity ratio, K', is greatly affected by the solvent composition and also by the column temperature. By increasing the concentration of methanol in water or increasing the temperature, K' decreases (see Tables I and II). By selecting the appropriate values for these parameters, it is possible to obtain any required value. From eqn. 1, it follows that the resolution increases as K' increases but also the analysis time increases. This fact will be examined later for the optimization procedure.

The resolution increases with $\sqrt{n}$, and there are three main ways to increase n: by increasing the column length (L), decreasing the mobile phase velocity ($\bar{u}$) or decreasing the particle diameter, according to the relationship:

$$n = \frac{L}{H} = \frac{L}{A + C\bar{u}}$$

TABLE II

CHROMATOGRAPHIC CHARACTERISTICS FOR THE SEPARATION OF C_7H_8–C_7D_8

Solvent: water–methanol (9:1).

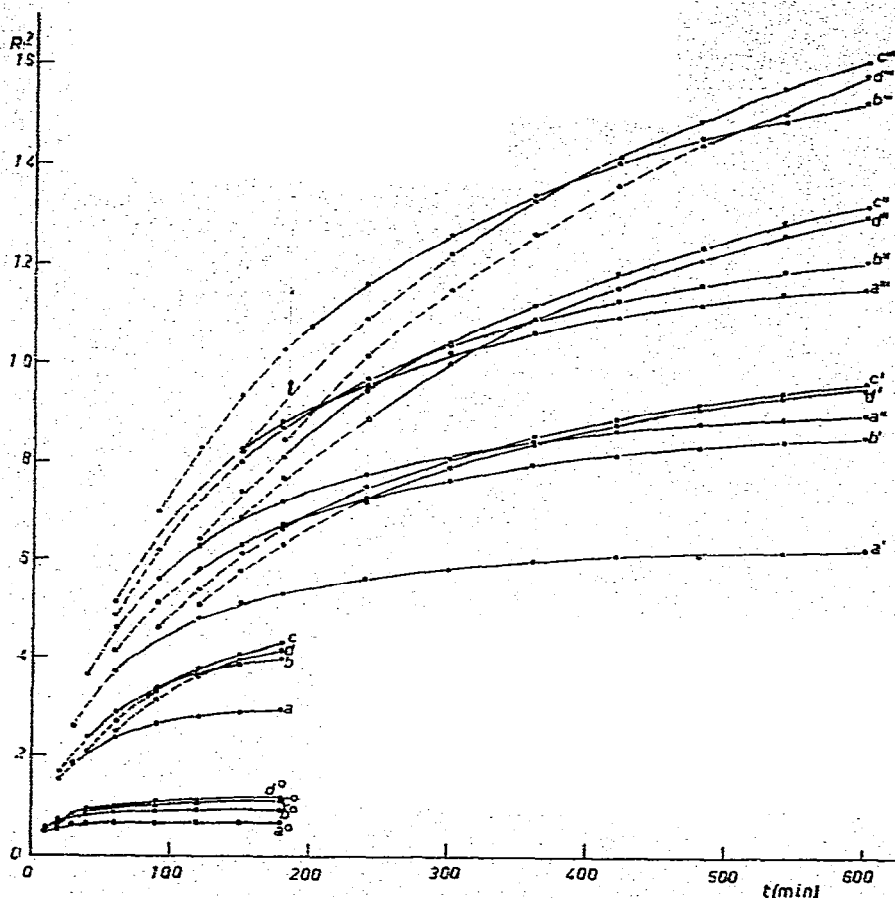| T (°C)	Flow-rate (ml/min)	t_R' (min) (C_7H_8)	α	K'	n	H (mm)	R
20	0.42	20.30	1.10	6.30	2080	0.48	0.76
20	0.59	14.51	1.10	6.25	1620	0.62	0.68
20	0.79	10.82	1.097	6.26	1500	0.67	0.67
20	1.00	8.67	1.097	5.72	1340	0.75	0.61
50	0.22	29.60	1.090	4.69	2950	0.34	0.80
50	0.40	14.00	1.090	4.22	2330	0.43	0.73
50	0.60	9.60	1.088	4.30	2100	0.47	0.63
50	0.77	8.22	1.092	4.34	1620	0.62	0.60
50	0.98	6.12	1.092	4.23	1560	0.64	0.59

Fig. 3. Plots of resolution *versus* analysis time. Curves: a, b, c, d, for $K' = 2, 4, 8$ and 10, respectively. Superscripts: none, °, ′, ″, ‴, for $L = 1, 5, 10, 15$ and 20 cycles, respectively.

The relationship $H = A + C\bar{u}$ was verified in the range of operating conditions considered. The experimental values for the constants A and C, are respectively, 0.42 nm and 0.021 sec^{-1} at 20 °C, and 0.28 mm and 0.027 sec^{-1} at 50 °C.

An increase in the column length can be effected either by connecting two or more columns in series or by using a recycling procedure through the same column. In both instances there is an increase in the analysis time, but only in the first instance is there also an increase in the inlet pressure.

Optimal conditions

An procedure for optimizing the resolution can be derived in terms of analysis time and pressure drop. Eqn. 1 can be expressed in the following form:

$$R^2 = \frac{1}{16} \cdot \frac{L}{A + C\left[\frac{L}{t}(K' + 1)\right]} \left(\frac{\alpha - 1}{\alpha}\right)^2 \left(\frac{K'}{K' + 1}\right)^2 \qquad (2)$$

where, for a given column, the resolution is a function of the analysis time (t), K'' and α.

Fig. 3 shows the plots of R^2 versus t for C_7H_8–C_7D_8 and with the values of the constants A and C at 50°C reported above. A family of curves is obtained by inserting various values of K' and the corresponding α values in eqn. 2. For a fixed column length ($L = 1$ m), the curves for $K' = 2, 4, 8$ and 10 (curves a°, b°, c° and d°) are shown. Other families of curves are obtained increasing the column length by recycling (1, 5, 10, 15 and 20 cycles). The broken lines to inlet pressures of >200 atm, which is the maximum value available with our instrument.

From these curves, it appears that for K' in the range 2–4, the best resolutions are obtained in a shorter analysis time; a further increase in K' does not improve the separation much, but only increases the analysis time.

From these curves, it is possible to establish the optimal operating conditions for obtaining a required resolution from a column. For instance, if $R^2 = 4$ is required, it can be seen from Fig. 3 that this value can be reached in 70 min with $K' = 2$ and 10 cycles of 7 min each. The mobile phase composition (concentration of methanol in water) should be adjusted so as to give this value of K' at 50°C.

The mobile phase velocity is calculated from the relationship

$$\bar{u} = \frac{L}{t}(K' + 1)$$

which in this example gives a value of $\bar{u} = 7.14$ mm/sec.

With columns of different particle diameter, other plots similar to Fig. 3 can be obtained by inserting in eqn. 2 the new values for L and for the constants A and C obtained from the plot of h versus $\bar{u}$.

CONCLUSION

These examples indicate that high-performance liquid chromatography should not only be competitive with gas chromatography for the separation of isotopic molecules, but in many instances better separations should be possible in a shorter analysis time. Different groups of isotopic systems can be explored, extending the field of application to non-volatile or unstable compounds that cannot be examined by gas chromatography, and with the possibility of isolating pure isotopic molecules on a preparative scale.

ACKNOWLEDGEMENT

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