

Reversed- and normal-phase separations by high-temperature open-tubular column liquid chromatography

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ABSTRACT

The selectivity and stability of some commercial reversed-phase open-tubular columns were studied under high-temperature conditions. Differently substituted chlorobenzenes were used as test compounds. Graphs of $\ln k'$ versus the number of chlorine atoms revealed the reversed-phase nature of the separations at elevated column temperature. Normal-phase separation of some aromatic hydroxy compounds on a cyanopropyl capillary column was also examined.

INTRODUCTION

In a previous paper [1], high-temperature open-tubular column liquid chromatography (HT-OTCLC) was proposed as a new approach to high efficiency in liquid chromatography. A total efficiency of over 10^6 theoretical plates was obtained on commercial 50 μm I.D. column.

Compared with liquid chromatography carried out at room temperature and on capillary columns with small bore (I.D. < 10 μm) [2–5], HT-OTCLC is advantageous in several respects. First, the applicability of commercially available 50–100 μm I.D. columns makes the construction of the OTCLC systems easier and difficulties in handling small-bore columns are circumvented. Second, the detection of the eluted solutes is less of a problem, as on-column UV detection is feasible for columns of I.D. \approx 50 μm . For small-bore columns, applications have been limited to some solutes that can only be detected electrochemically [6] or with fluorescence [7].

Owing to the reduced viscosity of the mobile phase at elevated temperatures, long columns (up to 20 m) can be used to generate high total plate num-

bers. Not only can a high total efficiency be obtained on long columns, but also a larger sample volume (up to 30 nl) can be injected.

Some problems arise with use of high column temperature. Commercial capillary columns, designed for application in supercritical fluid chromatography, showed limited stability at elevated temperatures when tested in our study. Further, the use of a thick film coating may be necessary in order to have retention at temperatures between 150 and 200°C. Unfortunately, however, thicker films are not always available with commercial 50 μm I.D. columns.

In this study, our objective was to separate test mixtures by reversed- and normal-phase capillary liquid chromatography at high temperatures and to examine the selectivity of different stationary phases at temperatures up to 200°C.

EXPERIMENTAL

The experimental set-up has been detailed elsewhere [1]. SB-Biphenyl-30, SB-Octyl-50, SB-Methyl-100 and SE-54 capillary columns were obtained from Lee Scientific (Salt Lake City, UT, USA), a DB-225 column from J&W Scientific (Folsom, CA, USA) and an SE-54 column from Macherey-Nagel

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(Oensingen, Switzerland). All columns had I.D. 50 μm and a film thickness of 0.25 μm . Bare fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA).

Acetonitrile, methanol and hexane were of HPLC grade from Rathburn (Walkerburn, UK). Synthetic test mixtures were prepared from the pure solutes obtained from Aldrich-Chemie (Steinheim, Germany) or Fluka (Buchs, Switzerland). The elution time of tropolone was used as the column dead time (t_0).

RESULTS AND DISCUSSION

Reversed-phase separation

Commonly used reversed-phase materials in column liquid chromatography are chemically bonded octyl and octadecane phases. On these column materials the retention mechanism is mainly based on hydrophobic interaction. The retention mechanism has not been studied in OTCLC at column temperatures up to 200°C. It is reasonable to expect that the reversed-phase nature of the separation will not change under HT-OTCLC conditions. To confirm this, we studied changes in retention with increase in the number of chlorine atoms in the homologous series of chlorobenzenes. Higher halogen substitution increases the hydrophobic properties of organic molecules. The dependence of $\log k'$ (k' = capacity factor) of various chlorobenzene solutes on the number of chlorine atoms at different column temperatures and different concentrations of organic solvent (percentage of acetonitrile) in the mobile phase is depicted in Figs. 1 and 2, respectively. In both instances $\log k'$ increased linearly with increase in the number of chlorine atoms. This, together with the fact that the retention increased when concentration of acetonitrile decreased from 60 to 40%, indicates the reversed-phase nature of the separations.

In the temperature range 150–200°C, a 10% change in concentration of acetonitrile in the mobile phase produced a twofold change in k' for chlorobenzenes with $k' > 0.1$. This was in agreement with published data for chloronaphthalene [8] and alkyl phthalate solutes [9] on reversed-phase packing materials at lower column temperatures. Also, a temperature change of 50°C was needed to produce a 50% change in retention. According to the two

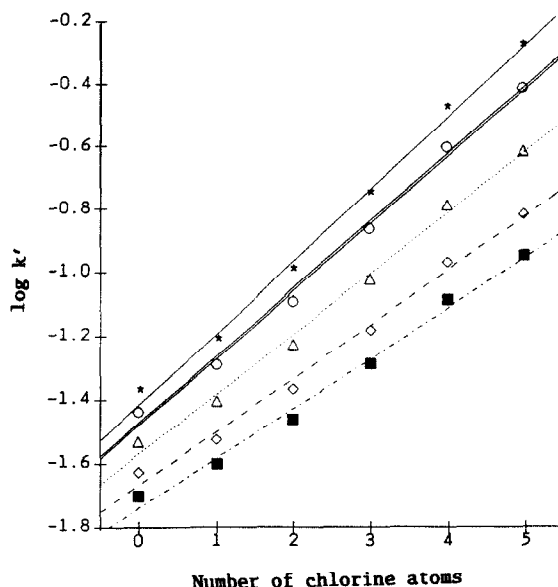


Fig. 1. Plots $\log k'$ vs. number of chlorine atoms at different temperatures on an SB-Octyl-50 column (9.5 m \times 50 μm I.D.). Mobile phase, acetonitrile-water (50:50); test compounds, benzene, chlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, 1,2,4,5-tetrachlorobenzene, pentachlorobenzene. Temperature: \star = 100; \circ = 120; \triangle = 150; \diamond = 180; \blacksquare = 200°C.

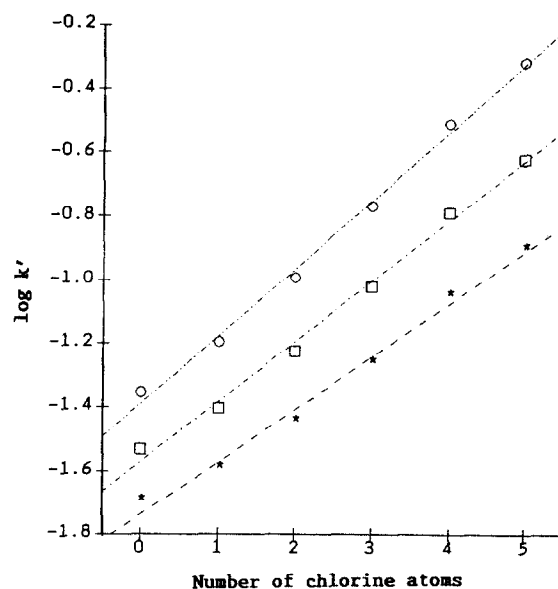


Fig. 2. Plots of $\log k'$ vs. number of chlorine atoms at different concentrations of acetonitrile (\star = 60%; \square = 50%; \circ = 40%). Column, SB-Octyl-50 (9.5 m \times 50 μm I.D.); column temperature, 150°C; test compounds as in Fig. 1.

approximations, a 1% increase in acetonitrile concentration has the same effect as a 5°C increase in column temperature in controlling solute retention. As shown in Fig. 3, equivalent separations were obtained at 150°C with 50% acetonitrile and at 200°C with 40% acetonitrile. A notable difference between the two separations was a higher column efficiency at 200°C. The plate numbers for benzene and pentachlorobenzene were 269 300 and 120 300, respec-

tively, at 150°C and 351 500 and 185 400, respectively, at 200°C. Further, a temperature increase led to a 50% higher column efficiency for more retained solute. This indicates that retention can be controlled either by the amount of organic solvent in the mobile phase or by column temperature.

The selectivities of four different reversed-phase columns, SB-Biphenyl-30, SB-Octyl-50, SB-Methyl-100 and SE-54, were compared. A mixture of

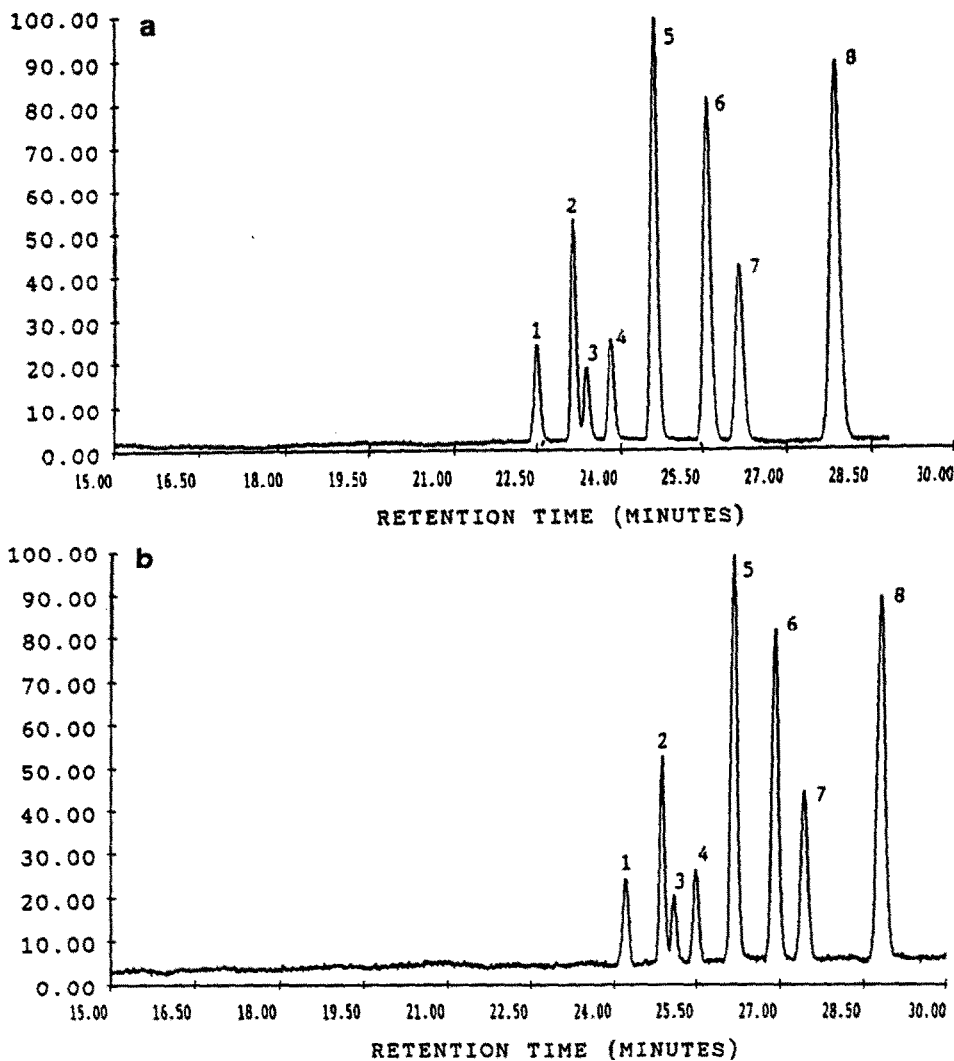


Fig. 3. (a) Separation of chlorobenzenes: 1 = tropolone; 2 = benzene; 3 = chlorobenzene; 4 = 1,4-dichlorobenzene; 5 = 1,2,4-trichlorobenzene; 6 = 1,3,5-trichlorobenzene; 7 = 1,2,4,5-tetrachlorobenzene; 8 = pentachlorobenzene. Column, SB-Octyl-50 (9.5 m \times 50 μ m I.D.); column temperature, 150°C; mobile phase, acetonitrile-water (50:50); flow-rate, 0.8 μ l min⁻¹. (b) As (a) except column temperature 200°C and mobile phase acetonitrile-water (40:60).

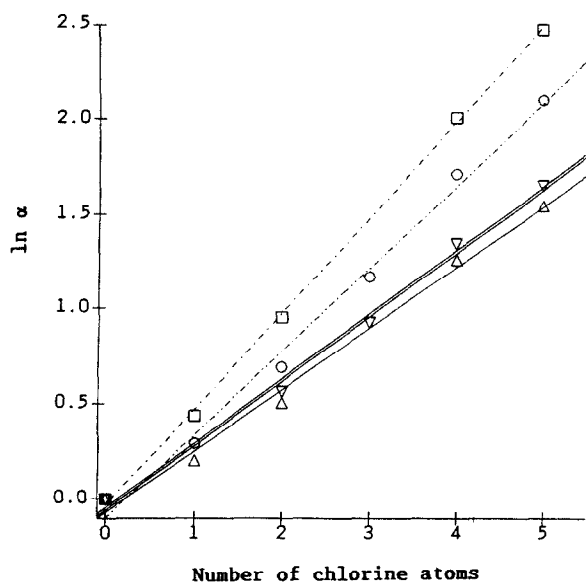


Fig. 4. Selectivity of reversed-phase columns. Column temperature, 150°C; mobile phase, acetonitrile–water (50:50); test compounds as in Fig. 1. Columns: \square = biphenyl; \circ = octyl; \triangle = methyl; ∇ = SE-54.

chlorobenzenes was used to study the selectivity of these columns. Retentions were measured with the same mobile phase composition [acetonitrile–water

(1:1)] and column temperature (150°C). For each column, plots of $\ln \alpha$ vs. number of chlorine atoms were made (Fig. 4), where $\alpha = k'/k'_0$, k' being the capacity factor of the individual chlorobenzene k'_0 the capacity factor of benzene. A linear relationship between $\ln \alpha$ and the number of chlorine atoms indicates a hydrophobic retention mechanism for all the solutes and stationary phases tested. The slope of the curve can be used as a measure of the column selectivity. A steeper slope indicates a stronger reversed-phase character and stronger hydrophobic interaction. For the test compounds, the biphenyl column showed the highest and the methyl column the lowest selectivity. The methyl and SE-54 columns contain mainly methylpolysiloxane (SE-54 contains only 5% phenyl groups) and are expected to show similar selectivities. In fact, their lines almost overlap. The contribution of the 5% phenyl groups to solute retention was reflected in a steeper slope on the line for the SE-54 phase. It should be noted that the selectivity and efficiency of all the applied columns deteriorated with time.

The lifetime of the reversed-phase columns at 200°C was notably short owing to an accelerated dissolution of the siliceous matrix in the aqueous mobile phase. With dissolution of silica base, the stationary phase was washed off by the mobile phase. The washed-off material accumulated along

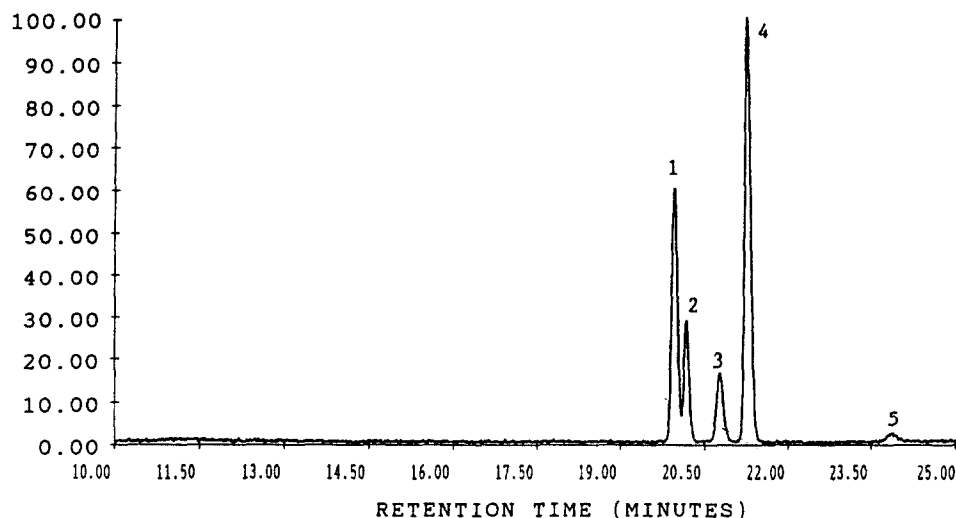


Fig. 5. Separation of aromatic hydroxy compounds. Column, DB-225 (10 m \times 50 μ m I.D.); column temperature, 100°C; mobile phase, *n*-hexane; flow-rate, 0.8 μ l min^{-1} . Peaks: 1 = 2,6-di-*tert*-butyl-4-methylphenol; 2 = 2,6-dimethylphenol; 3 = 1,1'-bi-2-naphthol; 4 = 1-naphthol; 5 = unknown.

the column, producing a non-uniform film thickness and reducing the column efficiency. It must be pointed out that very high column temperatures (e.g., 200°C) are not always needed. At lower column temperatures, in the vicinity of 150°C, the columns tested showed satisfactory stability.

Normal-phase separation

In normal-phase separation the column lifetime is expected to be less of a problem as the mobile

phase does not contain water. Fig. 5 depicts the separation of four aromatic hydroxy compounds by normal-phase OTCLC at elevated temperatures. The separation was achieved on a DB-225 column (polysiloxane backbone with 25% cyanopropyl, 25% phenyl and 50% methyl groups) with hexane as mobile phase and a column temperature of 100°C. As expected, a pure untreated fused-silica column shows selectivity towards aromatic hydroxy compounds. Fig. 6a and b display chromatograms

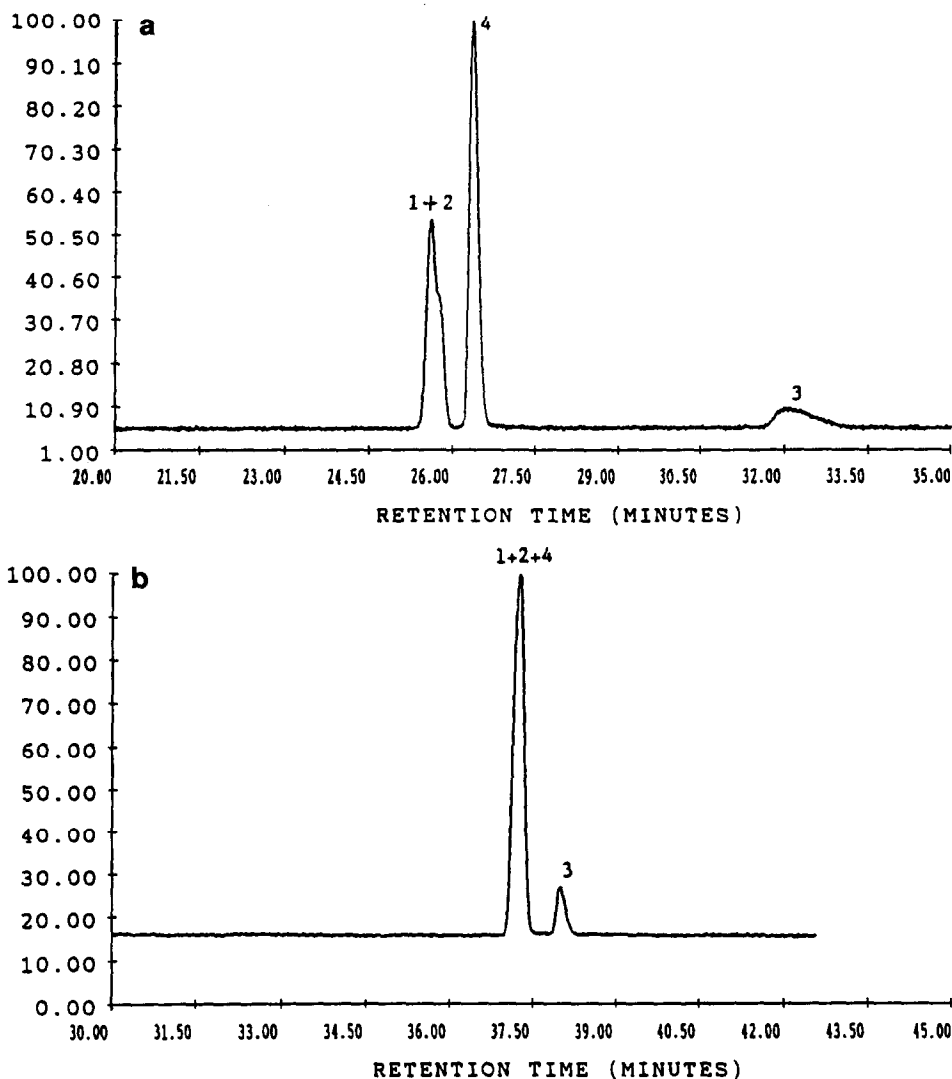


Fig. 6. Separation of aromatic hydroxy compounds on a bare fused-silica column (19.3 m \times 50 μ m I.D.). (a) Column temperature, 22°C; mobile phase, *n*-hexane; flow-rate, 1.4 μ l min⁻¹. (b) Column temperature, 100°C; mobile phase, *n*-hexane; flow-rate, 1.0 μ l min⁻¹. Peak numbers as in Fig. 5.

of the test compounds on pure fused silica at 22 and at 100°C, respectively. There was serious peak tailing at 22°C, but a nearly Gaussian peak at 100°C for 1,1'-bi-2-naphthol was observed. Tailing at room temperature caused by strong solute adsorption on the silanol groups decreased at elevated temperature. On the DB-225 column, 1,1'-bi-2-naphthol eluted before α -naphthol, whereas the opposite elution order was observed on pure fused silica. Reversal of the peak elution order indicates different selectivities on these two stationary phases.

CONCLUSIONS

The retention mechanism for substituted chlorobenzenes on the tested reversed-phase columns in the temperature range 150–200°C was based on hydrophobic interactions. Because of the short column lifetime at 200°C, commercially available columns cannot be recommended for routine application. Much has to be done in developing stable reversed-phase columns for use at temperatures above 150°C. Considering the vast choice of non-

polar elution solvents in normal-phase LC and improved peak symmetry (reduced solute adsorption) at elevated temperatures, in the future more attention should be paid to normal-phase separations in HT-OCTLC.

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