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Serially coupled capillary columns supercritical fluid chromatography with midpoint pressure control

Yukio Hirata*, Katsuhito Tsuda, Etsuro Imamura

School of Materials Science, Toyohashi University of Technology, Toyohashi 441-8580, Japan

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Abstract

Two capillary columns of different polarities were coupled in series by means of a coupling restrictor. The pressure of the first column and the midpoint pressure (between the coupling restrictor and the second column) were controlled independently of each other using two pumps. The selectivity of this separation system was highly dependent on the pressure difference and could be continuously changed between those of two columns. The pressure difference could be changed even in course of separation for fine tuning of the selectivity. Several examples were shown to demonstrate the utility of this method.

Keywords: Supercritical fluid chromatography; Capillary column; Serial coupling; Selectivity tuning

1. Introduction

The serially coupled column system has been extensively studied for tuning the selectivity in capillary gas chromatography (GC) [1–5] and recently applied for high speed analysis [6–8]. The simplest configuration is constructed by connecting two capillary columns of different selectivities in series by means of a simple coupling element and the desired selectivity is obtained by adjusting the length of each column. Using double oven allows to tune the selectivity by independent control of the temperature of each column. The sophisticated version allows to control the pressure at the midpoint of the coupled column system and then to tune the selectivity by independent control of the flow rate and/or the temperature of each column. Thus, the selectivity of the separation system can be varied between the extremes of two constituent columns simply by changing the operation conditions.

Since the mobile phase is often confined to carbon dioxide in capillary supercritical fluid chromatography (SFC), the coupled column system may be one of approaches to be studied for controlling the selectivity. Recently, we have reported a simple method of controlling the selectivity of the coupled column system by using a coupling restrictor in capillary SFC, where the contribution of the second column to the selectivity increased with increasing resistance of the coupling restrictor [9]. However, dismantling the columns was required to replace the coupling restrictor, and it was impossible to continuously change the selectivity.

In this study we developed a more flexible coupled column system in capillary SFC. The system included a midpoint pressure control between the coupling restrictor and the second column, and the midpoint pressure was controlled independently of the pressure of the first column. This allowed to continuously change the selectivity of the coupled column system without dismantling the columns.

2. Experimental

2.1. Instrumental setup

The SFC system used in this study is shown in Fig. 1, which was composed of two LC-5A liquid chromatograph

* Corresponding author. Tel.: +81 532 44 6804; fax: +81 532 48 5833.
E-mail address: hirata@chrom.tutms.tut.ac.jp (Y. Hirata).

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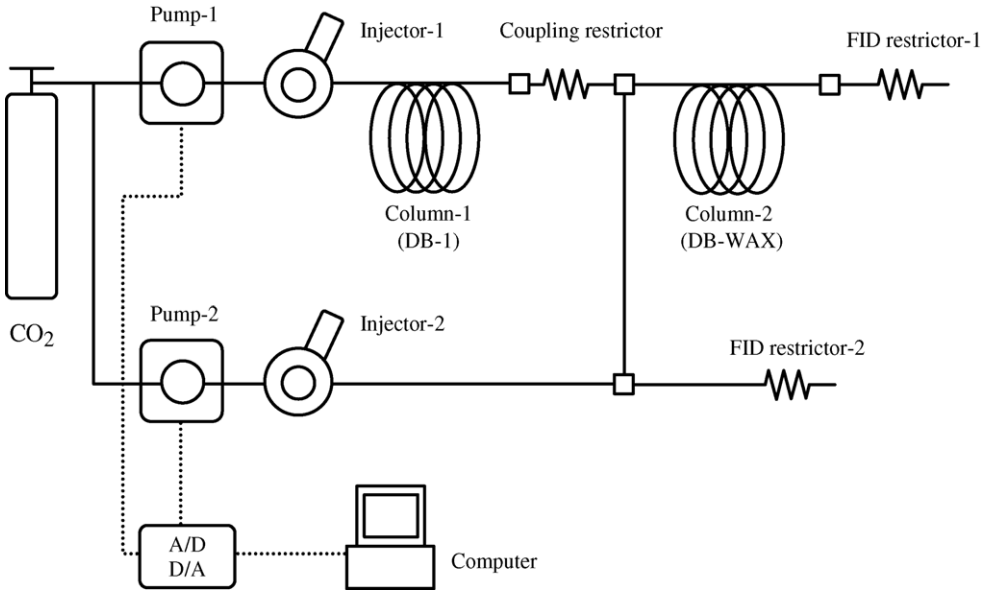

Fig. 1. Schematic diagram of serially coupled capillary columns SFC system with midpoint pressure control.

pumps (Shimadzu, Kyoto, Japan) equipped with a pump head cooler (MC-28T, Netsudenshi Kogyo, Tokyo, Japan), two Rheodyne 7520 injectors (Cotati, CA, USA) with a 0.2 μl rotor, and a GC-14A gas chromatograph (Shimadzu) with a flame ionization detector (FID). An additional FID of same type was attached to the GC oven. Two capillary columns of different polarities from J & W Scientific, DB-1 (column-1, 100% methyl, 10 m × 100 μm i.d., 0.4 μm film thickness) and DB-WAX (column-2, polyethyleneglycol, 10 m × 100 μm i.d., 0.2 μm film thickness) were serially coupled using a coupling restrictor. The coupling restrictor was a fused silica capillary (25 cm × 15 μm i.d.). Two FID restrictors were of integral type, which were prepared from a 30 μm i.d. fused silica capillary. They were prepared so as to give the flow rates of about 1 and 3 ml/min for nitrogen gas at 25 bar for FID restrictor-1 and restrictor-2, respectively. These restrictors and columns were connected by using butt connectors (DKK-TOA, Tokyo, Japan) and a stainless steel capillary (0.1 mm i.d., 0.3 mm o.d., GL Science, Tokyo, Japan). The tee connections were comprised of a homemade joint which was prepared by soldering two stainless steel capillaries into a short stainless steel tubing (0.76 mm i.d., 1.06 mm o.d., Nilaco, Tokyo, Japan) [10].

2.2. Operation conditions and correction of pump pressure

High purity carbon dioxide (Showa-Tansan, Yokkaichi, Japan) was used as mobile phase. The column temperature was 100 °C and the FID temperature 350 °C. Chromatographic data were collected with a personal computer (PC) using a homemade software written by Microsoft Visual Basic ver-5 via a Shimadzu C-R4A integrator. The pressures of two pumps were controlled and monitored with PC using another homemade software via a AD-DA converter (J.J. Joker-E2, Nippon Filcon, Tokyo), which was equipped with two AD and two DA ports. In this study, the pressure of pump-1 was usually programmed in a linear fashion at 5 bar/min after a 10 min isobaric period, and the pressure of pump-2 was programmed so as to give the predetermined pressure difference on the basis of the pressure of pump-1.

Daily correction of pump pressure was performed based on one of pressure sensors of two pumps. Pressures of two pumps were measured at a 50 bar interval in the range of 100–350 bar with one of pressure sensors of two pumps, and they were correlated in a linear relationship with the input signals for two pumps. Then, the input signals for two pumps required to perform a given pressure programming were calculated from the relationships. Calibration of the pressure sensors was done by using a pressure regulator (YR-506, Yamato, Osaka) connected to a nitrogen tank when necessary.

2.3. Samples and data handling

Following several samples were used. A mixture containing *n*-alkanes (C18–C24), fatty acid methyl esters (C16–C22) and *n*-alcohols (C12–C18) was used for the basic study on the selectivity. Other samples were a mixture of fatty acid methyl esters containing saturates (C16, C18 and C20) and unsaturates (C18:1, C18:2 and C18:3), a mixture of polyoxyethylene alkylether oligomers ($R(OCH_2CH_2)_nOH$, R = C12, C14 and C16, *n* (average) = 4) and a mixture as listed

Table 1
List of compounds with varying polarities

Compound number	Compound name
1	Tetradecane
2	Hexadecane
3	Octadecane
4	Eicosan
5	Docosane
6	Octacosane
7	Decyl benzene
8	Dodecylbenzene
9	Dibutyl phthalate
10	Dipentyl phthalate
11	Butyl benzoate
12	Hexyl benzoate
13	*p*-Cresol
14	*p*-Propylphenol
15	Naphthalene
16	Biphenyl
17	Tetradecanol
18	Hexadecanol
19	Methyl palmitate
20	Methyl stearate

in Table 1. All the samples were prepared as dichloromethane solution.

Selectivity of the coupled column system was evaluated by means of the retention indices of fatty acid methyl esters and *n*-alcohols. As the separations in this study were carried out under the pressure programmed mode, retention indices for the solutes were calculated by:

$$I = \frac{t_{RS} - t_{RZ}}{t_{R(Z+1)} - t_{RZ}} 100 + 100Z \quad (1)$$

where t_{RS}, t_{RZ} and $t_{R(Z+1)}$ are the retention times for the solute and for *n*-alkanes with *z* and *z* + 1 carbon atoms, respectively.

3. Results and discussion

3.1. Characteristics of present system and selection of coupling restrictor

In the previous coupled column system where two columns were connected simply by a coupling restrictor, the mobile phase passed all the way through two columns, therefore the mass flow rates of two columns were the same [9]. Since the resistance of the FID restrictor in the system was several times larger than that of the coupling restrictor, the flow rate which passed through two columns was determined virtually by the resistance of the FID restrictor and the pressure difference between the two columns was determined by the resistance of the coupling restrictor. Increase in the resistance of the coupling restrictor lead to the decrease of the second column pressure at the time when the solutes were eluted from the first column in the pressure programmed separations, resulting in increase in the retention on the second column. Thus, the contribution of the second column to the selectivity of the system increased with increasing resistance of the coupling restrictor. Although the method was very simple, the coupling restrictor must be replaced in order to change the selectivity.

In the present system shown in Fig. 1, the flow rate of the first column is determined by the resistance of the coupling restrictor and the pressure difference between the pump-1 and the midpoint, because the pressure drop along the column is negligibly small. The flow rate of the second column is determined by the midpoint pressure and the resistance of the FID restrictor-1. Therefore, the mass flow rates for both columns are not always the same as in the previous simple coupling system. In association with this point, the FID restrictor-2 plays an important role. When the mass flow rate of the first column is higher than that of the second column, the excess is split and vented from the FID restrictor-2, so that a part of sample is detected with FID-2. When the mass flow rate of the first column is lower than that of the second column, the effluent of the first column is made-up with the flow from the pump-2, so that no sample enters FID-2. When both mass flows are the same, the condition should correspond to that of the simple coupling system.

Once a given coupling restrictor is selected, the pressure difference is only parameter to change the flow rate of the first column. Therefore, in order to get proper linear velocity as well as pressure difference, the selection of coupling restrictor is critical. In this study, the mobile phase linear velocity was in a range of 3–5 cm/s in consideration of the analysis time, although it was considerably faster than the optimum [11]. The flow rate of the first column was examined at various pressure differences using various size of coupling restrictors, and the present coupling restrictor (25 cm × 15 μm i.d.) was selected. The mobile phase linear velocity of the second column was regulated to about 3 cm/s by the FID restrictor-1.

3.2. Effect of pressure difference on selectivity

Figs. 2 and 3 show the effect of pressure difference on the separation of a test mixture composed of *n*-alkanes, fatty acid methyl esters and *n*-alcohols. Here, a non-polar column (DB-1) was followed by a polar column (DB-WAX). It is seen that the retentions of polar solutes relative to those of *n*-alkanes or the polarity of the separation system increase with increasing pressure difference. The results indicate that the contribution of the second column to the selectivity of the coupled column system increases with increasing pressure difference. It should be noted that even a small change in the pressure difference results in a drastic changes in resolution as shown in Fig. 3. The retention indices of *n*-tetradecanol are plotted against the pressure difference in Fig. 4. The data for single-column SFC are also plotted for comparison. It is seen that the polarity of the coupled column system almost linearly increases with the pressure difference. Thus, the selectivity

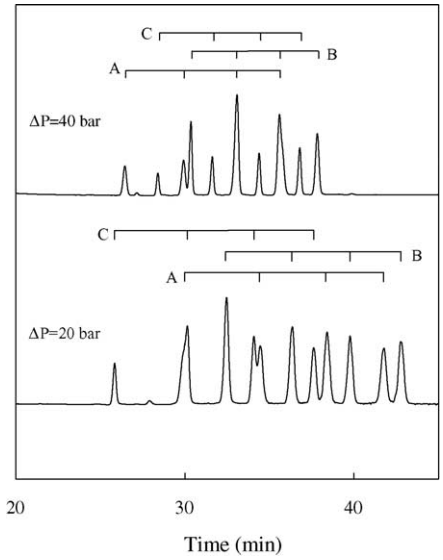

Fig. 2. SFC chromatograms of a test mixture at the pressure difference of 20 and 40 bar. Peaks: A, *n*-alkanes (C18–C24); B, fatty acid methyl esters (C16–C22); C, *n*-alcohols (C12–C18). Conditions: first column, DB-1; second column, DB-WAX; coupling restrictor, 25 cm × 15 μm i.d.; column temperature, 100 °C. Pressure of the first column was programmed at 5 bar/min after a 10 min isobaric period at 100 bar.

of the coupled column system can be easily and continuously changed between those of two columns merely by changing the midpoint pressure.

The reverse configuration, where the polar column precedes the non-polar one, may be utilizable, although the relative retentive power of two columns (or their film thickness) must be considered. If the second column is more retentive than the first one, the solutes will be too strongly retained on the second column, because in the present system the pressure of the second column is always lower than that of the first one.

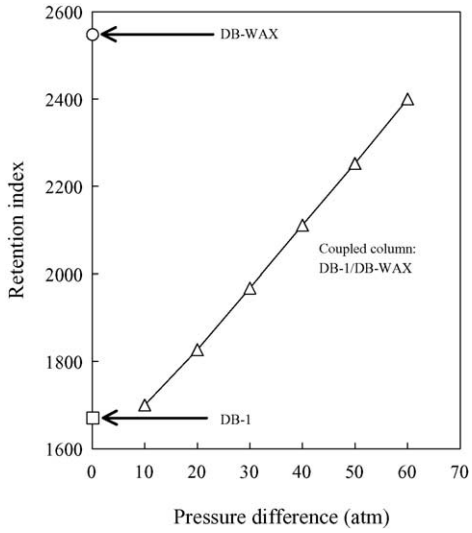

Fig. 4. Plots of the retention indices of *n*-tetradecanol versus pressure difference for the serially coupled column SFC. The data for single-column SFC are also plotted for comparison. Conditions as in Fig. 2.

3.3. Separation of various samples

Fig. 5 shows the separations of a mixture containing saturated and unsaturated fatty acid methyl esters. The relative retention of the unsaturates to the saturates increased with increasing pressure difference and their increasing rate was lager for the solutes with more double bonds, since the polarity of the solutes increases with increasing double bonds.

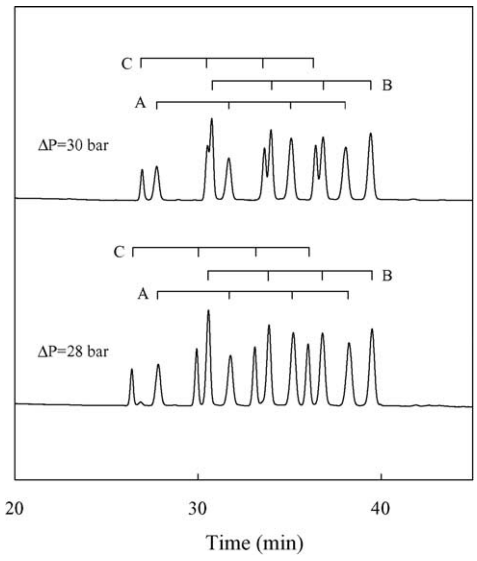

Fig. 3. SFC chromatograms of a test mixture at the pressure difference of 28 and 30 bar. Peaks and conditions as in Fig. 2.

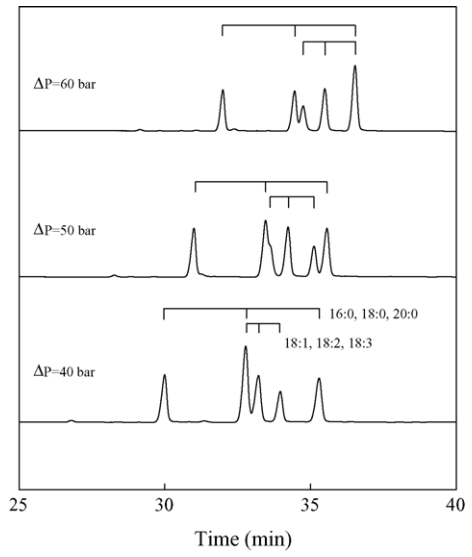

Fig. 5. SFC chromatograms of fatty acid methyl esters. Conditions as in Fig. 2.

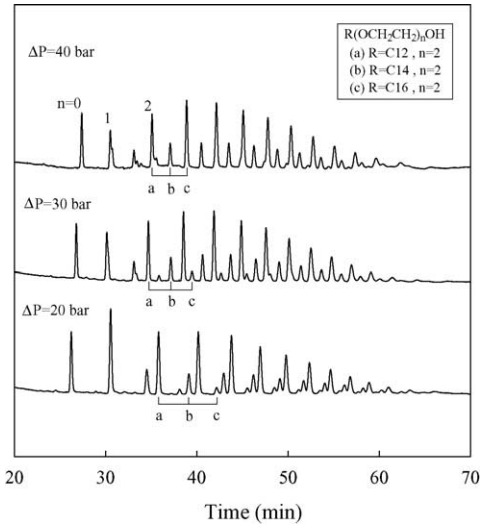

Fig. 6. SFC chromatograms of polyoxyethylene laurylether oligomers. Conditions as in Fig. 2.

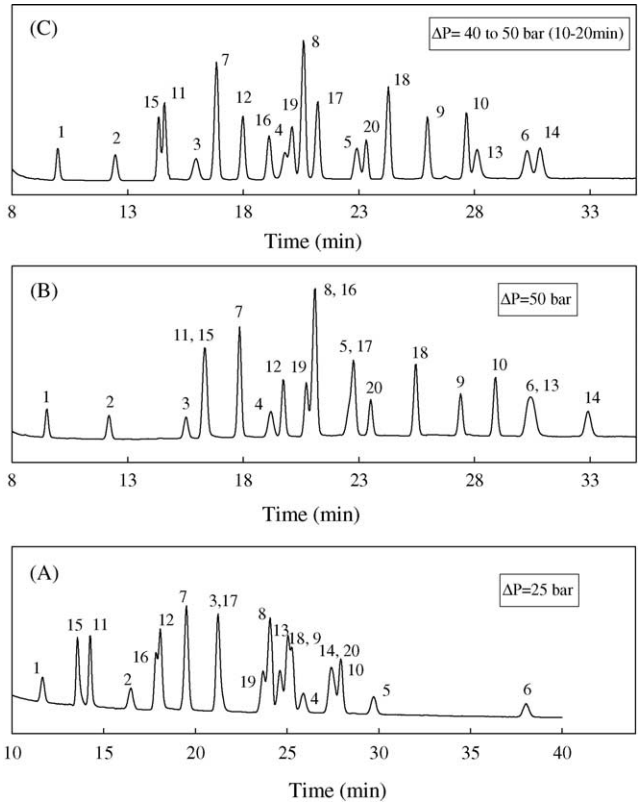

Fig. 7. SFC chromatograms of the mixture listed in Table 1. Pressure of the first column was programmed at 5 bar/min after a 10-min isobaric period at 140 bar. Pressure difference: (A), 25; (B), 50; (C), 40 bar for 10 min, varied to 50 bar in 10 min, then kept at 50 bar. Other conditions as in Fig. 2. Peak assignments as in Table 1.

Fig. 6 shows the separations of polyoxyethylene laurylether oligomers. It is seen that the contribution of oxyethylene unit to the retention increased with increasing pressure difference, compared with ethylene unit. Fig. 7 shows the separations of a relatively complex mixture with a wide range of polarity listed in Table 1. It was difficult to resolve all the solutes under the conditions of constant pressure difference as in Fig. 7A and B, although the selectivity was largely different each other. In Fig. 7C, the pressure difference was initially set at 40 bar, and then gradually increased to 50 bar. In practice the pressure of the first column was always programmed at 5 bar/min, while the midpoint pressure was programmed at 4 bar/min from 10 to 20 min and then at 5 bar/min. This operation allowed the fine tuning of the selectivity resulting in the resolution of most of peaks.

4. Conclusions

The coupled column system with a midpoint pressure control is a flexible method to change the selectivity. With the present system, the selectivity can be easily and continuously changed between those of two columns merely by changing the pressure difference. Various combinations of two columns with varying selectivities should be examined in the future study.

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