Correlation Equation for Retention Factor and Resolution of Ibuprofen in SFC

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Supercritical fluid chromatography (SFC) was considered for separating racemic ibuprofen. The chromatographic column (3.9 × 150 mm) was packed with Kromasil® CHI-TBB, and the mobile phase was supercritical carbon dioxide with modifier of IPA. The experimental variables were the content of IPA, and temperature and pressure of supercritical mobile phase. To determine the separation condition, the empirical equation of retention factor and resolution was proposed. In the case of retention factor, the empirical equation was in the form, $k = a\rho + b/F + c(\rho/F) + d$. The empirical equation for resolution was proposed as a linear form, $R = a\rho + bF + c$.

Key Words : SFC, Retention factor, Resolution, Ibuprofen, Correlation

Introduction

Enantiomers were called chiral compounds, which have the same chemical formula and structure, but they are each other's mirror image. They share the same physical and chemical properties but differ in optical property. The increasing need for pure enantiomer is due to that the pharmaceutical activities of the enantiomers in human body are often different and some times reverse.[1] To prepare high-purity component, separation using chiral stationary phase has been widely used.

Supercritical fluid chromatography (SFC) was demonstrated by Klesper *et al.* in 1962.[2] Supercritical fluids show many advantages: the reduced viscosity results in low pressure drop and allowing high flow rates or long columns, the high solute diffusion coefficients leads to fast mass transfer and high efficiency, the ease of disposal and solute recovery in preparative modes, and the ability to use gas chromatography (GC)-type and liquid chromatography (LC) detectors.[3] In separation of chiral compound, the study using SFC system has been performed, recently. Because various parameters should be considered, the development time of optimum condition requires longer than HPLC. And the relatively few studies of the separation mechanism was reported.[4,5]

The retention of a component in chromatography is the result of a competitive distribution process of the solute between the mobile and stationary phase.[6] Thus, the important parameter for quantification in chromatography is retention factor (k).[7]

In this study, standard sample chosen was racemic ibuprofen. Ibuprofen [(±)-(R.S)-2-(4-isobutylphenyl)propionic acid] (IBU) is a chiral nonsteroidal anti-inflammatory drug widely used for the treatment of several rheumatic and musculoskeletal diseases. Although ibuprofen is used as a racemic mixture, its anti-inflammatory action is mainly associated with the (+)-(S)-enantiomer.[8]

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Several direct and indirect liquid chromatographic analytical methods involving a variety of chiral and achiral phases for resolution of ibuprofen enantiomer have been reviewed.[9] And several supercritical fluid chromatographic methods involving a various chiral stationary phase has been reviewed.[10-17]

The purpose of this study is to develop an optimum SFC separation method for the chiral separation of ibuprofen by investigating the different factors, such as pressure, temperature and the content of IPA in the supercritical fluid mobile phase. And several potential empirical equation of retention factor and resolution will be suggested for the prediction of the parameters.

Experimental Section

Reagents. The standard chemical of R.S-ibuprofen[(±)-(R.S)-2-(4-isobutyl phenyl) propionic acid](the molecular structure shown in Fig. 1) was kindly donated by SAMIL Pham. Co. (Seoul, Korea). The extra-pure grade solvent of IPA used as organic modifier was purchased from J. T. Baker (Phillipsburg, USA). The chromatographic column (3.9 × 150 mm) used in SFC was packed with Kromasil® CHI-TBB (Eka Chemical, Sweden). The coolant was a mixture of water and methanol (Duksan, Korea) (50 : 50, v/v). Water was distilled and deionized before use. Liquid carbon dioxide (99.9%) was purchased from Shin Yang Co. (Seoul, Korea).

Sample Preparation. R.S-ibuprofen was dissolved in IPA and concentration was 10 mg/mL. A constant injection volume (5 μL) was used throughout the experiment.

Apparatus and Method. The SFC system used this study

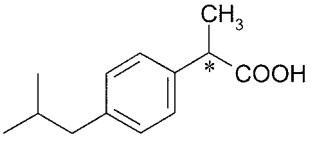

Figure 1. Chemical structure of ibuprofen (*: chiral center).

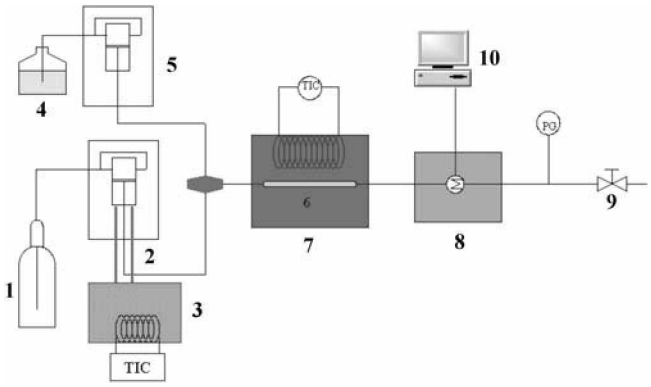

Figure 2. Schematic diagram of supercritical fluid chromatography used in this study. (1: CO_2 gas cylinder, 2: HPLC pump, 3: cooling circulator, 4: organic modifier reservoir, 5: organic modifier pump, 6: column, 7: thermostat, 8: UV detector, 9: back pressure regulator, 10: data acquisition system, PG: pressure gauge, TIC: temperature indicator controller)

was shown Figure 2. It was equipped with a HPLC M930 solvent delivery pump (Younglin, Korea), M729 detector of UV-visible tunable wavelength absorbance (Younglin, Korea) and an injector (0.02 mL sample loop) of Rheodyne(Waters, Milford, MA, USA). The data acquisition system was AutoChro-WIN (ver. 2.0, Younglin, Korea) installed in a PC. To maintain the carbon dioxide as a liquid-state, a jacket was connected to a refrigeration circulator. To maintain a high pressure between column and tubing, the back-pressure regulator (6000 psi, TES-COM) was connected at the outline of a detector, and pressure gage (model 01-0162-F, Millpore) for measuring pressure was used. The column and tubing were kept constant temperature in a thermostat. The content of IPA in carbon dioxide was changed from 3 to 10 (vol %), and the pressure was changed from 100 to 130 bar. The temperature of column and tubing was varied from 38 to 48 °C. The flow rate of mobile phase was 1 mL/min. The UV wavelength was fixed at 220 nm.

Results and Discussion

In the development of separation method by chromatography, there are several factors to be considered such as inner diameter and length of column, the composition of mobile phase, etc. The choice of the column type and stationary phase is dependent on a target material, so the optimum separation condition was mainly to decided to determine the composition of mobile phase.

The retention factor can be calculated according to Eq. (1).

$$k = \frac{V_R - V_M}{V_M} = \frac{t_R - t_M}{t_M} \quad (1)$$

where t_M is the hold-up time, t_R is the retention time, k is retention factor, respectively. The resolution is shown by Eq. (2).

$$R = \frac{t_2 - t_1}{(w_2 + w_1)/2} = \frac{2\Delta t}{w_2 + w_1} \quad (2)$$

where t_{R1} is the retention time of less retained isomer, *i.e* R-enantiomer in this work, t_{R2} is the more retained isomer, *i.e*. S-enantimer, w_1 and w_2 are peak widths.

The separation time and purity of target material must be simultaneously considered. The separation time was adjusted by mobile phase composition and it was commonly expressed as retention factor obtained by Eq. (1). In the purity of target material, it was related to resolution was calculated by Eq. (2). When the resolution was approximately above 1.2, the adjacent peaks were normally separated on the base line. The optimum separation condition was found by the retention factors and the resolutions in various composition of mobile phase.

A. Correlation of Retention Factor. To calculate a retention factor, the hold-up time of column should be measured. The un-retained component such as n-hexane was injected and the injection volume was 0.02 mL. The retention time of n-hexane was 1.65 min. The retention time was experimentally measured. The experimental variables were content of IPA, temperature and pressure of supercritical carbon dioxide. To suggest empirical equation of ibuprofen the retention factors were assumed as the function of the temperature and pressure of supercritical carbon dioxide (represented by density) and the content of IPA. The density of carbon dioxide in this experimental range was listed in Table 1.

Table 1. Density of carbon dioxide in experimental ranges[19] (g/mL)

Pressure (bar) / Temperature (K)	100	110	120	130
311.15	0.6668	0.7096	0.7386	0.7608
313.15	0.6286	0.6835	0.7178	0.7430
315.15	0.5822	0.6542	0.6952	0.7242
317.15	0.5272	0.6212	0.6708	0.7042
319.15	0.4703	0.5842	0.6442	0.6828
321.15	0.4216	0.5439	0.6154	0.6601

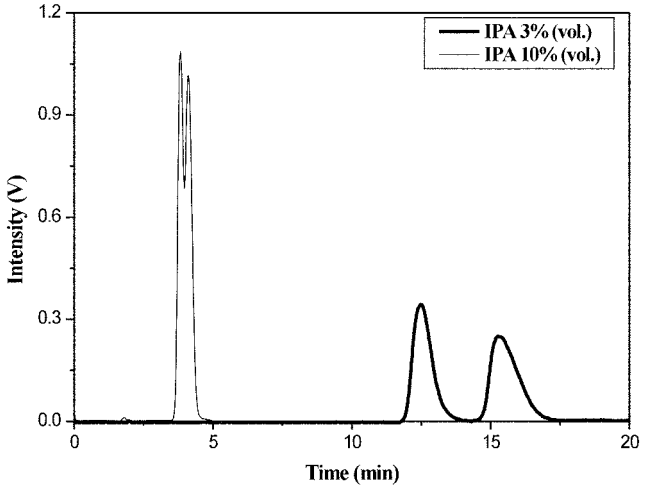

Figure 3. Chromatogram of racemic ibuprofen in mobile phases of different IPA composition. (311.15 K, 100 bar)

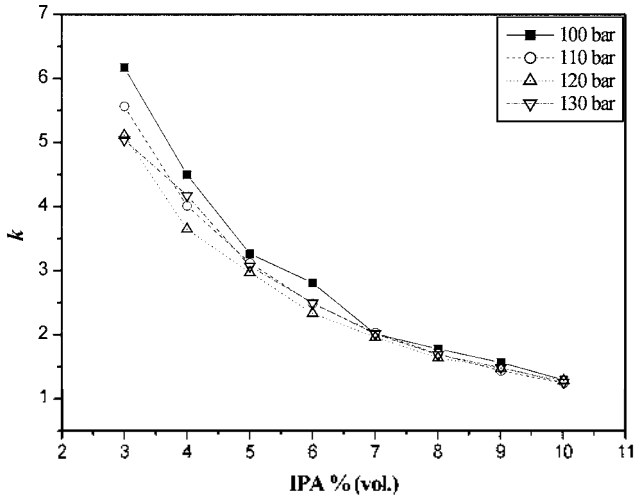

Figure 4. Variation of retention factor of R-ibuprofen with IPA % (vol.) in supercritical carbon dioxide.

While the content of IPA in mobile phase was higher, the retention time was shorter at constant temperature and pressure (refer to Fig. 3). The effect of content of IPA on the retention factor with pressures at constant temperature was shown Figure 4. In the lower content of IPA in mobile phase, the retention factor was a little affected by the pressure, but the effect was negligible in higher content of IPA. Figure 5 showed effect of the density of carbon dioxide on retention factors in mobile phase of 3 and 10% of IPA in mobile phase. In the lower content of IPA (3%), the retention factors decreased with an increment of density of carbon dioxide. But in the higher content of IPA (10%), the retention factor was almost 1.5.

While the interaction between the analyte and the chiral selector bonded on the stationary phase can be attributed to π-π stacking, dipole-dipole or hydrogen bonding. The polarity of the mobile phase can affect the interaction and the differences in enthalpy and entropy between the two isomers and stationary phase, which results in the chiral

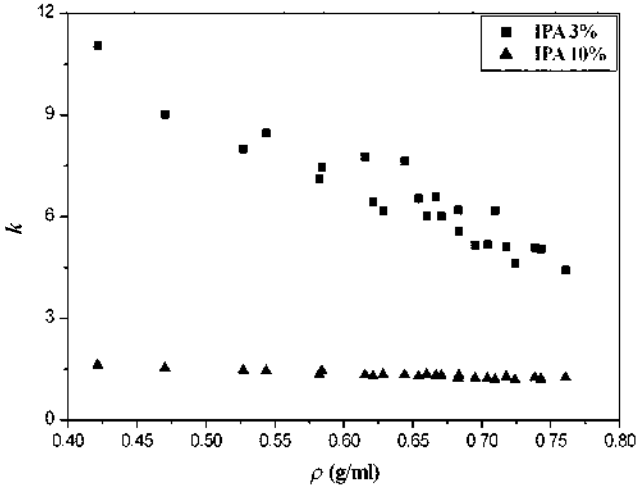

Figure 5. Variation of retention factor of R-ibuprofen with density of supercritical carbon dioxide.

separation of the enantiomers. In the case that interaction between sample and stationary phase is very strong, the retention time mostly may be longer and the peak shape often shows a tailing. To adjust the retention time and peak tailing, organic modifier was added to the mobile phase to alter the polarities and interactions. The retention time was changed with the different operating conditions, so to predict the retention time, empirical equations are considered as a function of experimental variable, which in this work should be the density of carbon dioxide and the content of modifier in the mobile phase. The empirical equation of retention factor was expressed in terms of density of carbon dioxide (ρ) and content of IPA (F) as follows.

$$k = f(\rho, F) \tag{4}$$

When the empirical equation was a linear equation of ρ and F in No. 1 of Table 2, its correlation coefficient was 0.8089. Because the decreasing rate of retention factor in term of content of IPA showed non-linear type, the experimental data and calculated values were not in good agreement. The empirical equation was assumed as a quadratic equation for F and ρ, and the correlation coefficient was 0.9185. Generally, polynomial correlation was adopted when linear equation was not suitable. With increase in order and term, the correlation coefficient approached to 1, but the resulting equation was complicated and it had more parameters estimated.

The reciprocal of F was tried in No. 2 of Table 2, and its correlation coefficient was 0.9289. Because retention factor decreased nonlinearly, it might be described as a reciprocal of F. Thus, higher value of correlation coefficient was obtained. When each term was the second order such as ρ, ρ^2, $1/F$, $1/F^2$, the correlation coefficient was to 0.9304. In this condition, at a lower retention factor, the experimental data and calculated value were in good agreement, but some deviation was observed.

Unfortunately, polynomial correlation was not a good trial equation. As shown in Figure 5, the effect of density of carbon dioxide on the retention factor was greatly changed, the combination term of ρ/F was added to No. 2 of Table 2. Its correlation coefficient was fairly high, 0.9814.

Snyder equation,[18] $\ln k = \ln k_w - SF$ is frequently used to predict the retention factor in RP-HPLC. Similar to Snyder equation, logarithmic relationship was taken as in No. 4 of Table 2, but the form was not so satisfied.

The suitable empirical equation for the retention factor was designated as No. 3 of Table 2. As shown in Figure 6, the experimental data and calculated value were in good

Table 2. Empirical equations of retention factor (R-form)

No.	Empirical equation	r^2
1	$k = -5.5278\,\rho - 0.6758\,F + 10.9282$	0.8089
2	$k = -5.5278\,\rho + 22.3742\,/\,F + 2.5387$	0.9289
3	$k = 6.4790\,\rho + 65.6476\,/\,F - 67.2192\,(\rho/F) - 5.1909$	0.9814
4	$\ln k = -1.4776\,\rho - 0.2205\,F + 3.3244$	0.9243

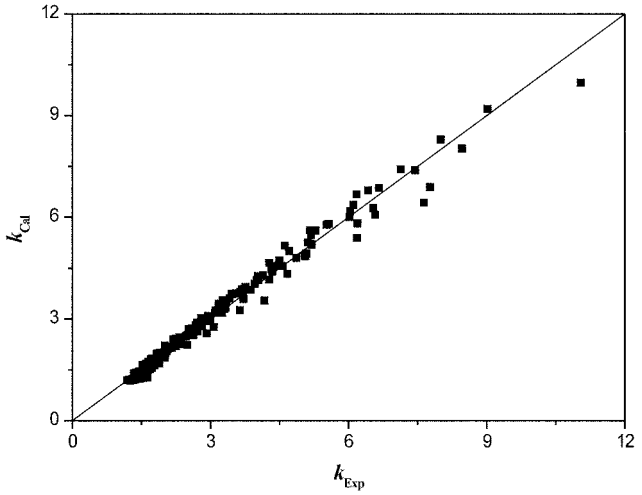

Figure 6. Comparison of experimental and calculated retention factor (Eq. 3).

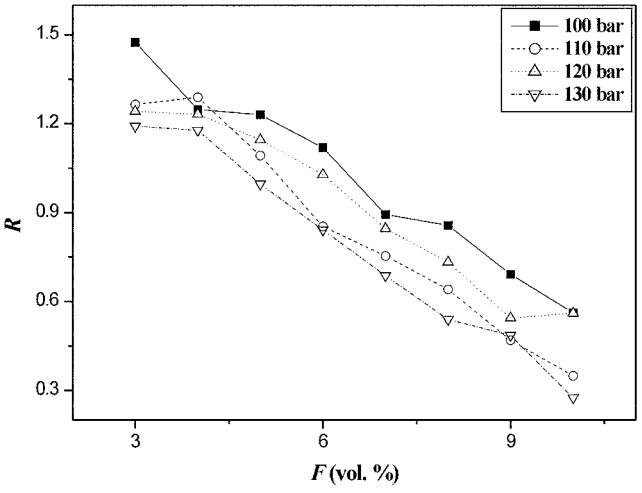

Figure 7. Variation of resolution with IPA % (vol.) in supercritical carbon dioxide.

agreements in the wildly experimental range.

B. Correlation of Resolution. The resolution of two components was calculated by Eq. (2) in experimental range. Figure 7 showed the effect of content of IPA (F) on the resolution. While F increased, resolution was worse, similar to the case of retention factor. Resolution was also affected by the pressure a wide range of F. While the content of IPA in mobile phase was higher, the retention time of racemic ibuprofen was shorter, thus the resolution has lower value. The effect of density of carbon dioxide on the resolution, was shown Figure 8. The resolution was influenced more by the content of IPA than the density of supercritical carbon dioxide. Empirical equation for resolution was proposed in Table 3.

When an empirical equation was linear such as No. 1 of Table 3, the correlation coefficient was relatively high as 0.9170. When a reciprocal term of F was used in No. 2, it was lower. When a quadratic polynomial equation was used, the correlation coefficient was 0.9146. Contrary to the

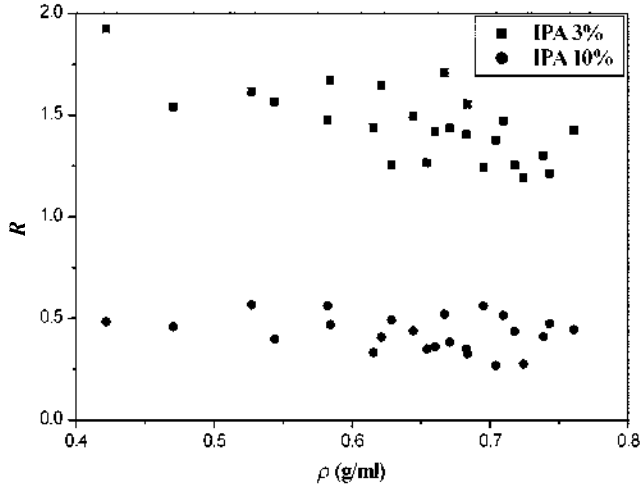

Figure 8. Variation of resolution with density of supercritical carbon dioxide.

Table 3. Empirical equations of resolution

No.	Empirical equation	r^2
1	$R = -0.5487\,\rho - 0.1454\,F + 2.2115$	0.9170
2	$R = -0.5487\,\rho + 4.2881 / F + 0.5002$	0.8464
3	$R = 0.2587\,\rho + 7.1978 / F - 4.5197\,(\rho/F) - 0.0195$	0.8530
4	$\ln R = -0.5874\,\rho - 0.1725\,F + 1.3263$	0.9084

retention factor, the correlation form was not satisfied by No. 3 of Table 3.

The logarithmic resolution was correlated in terms of ρ and F, but the correlation coefficient was 0.9084. Compared to No. 1 of Table 3, disappointedly it was not higher than 0.9170 in No. 1.

Conclusion

The SFC system was utilized for separating racemic ibuprofen. To determine the separation condition, the empirical equation of retention factor and resolution was proposed. In the case of retention time, the empirical equation was in the form, $k = a\,\rho + b/F + c\,(\rho/F) + d$, and the correlation coefficient was 0.9814. The empirical equation for resolution was proposed as a linear form, $R = a\,\rho + b\,F + c$. The correlation coefficient was 0.9170. Resolution was adjusted by the difference in retention factor as well as the peak width of component. Therefore, it is harder to formulate the correlation equation for resolution.

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