COSMOSIL

Technical Guide for SFC Columns

Technical Note

Supercritical Fluid Chromatography (SFC), which uses a supercritical fluid as the mobile phase, has gained attention in recent years. Compared to HPLC, SFC uses less organic solvent, produces fractions that are easier to purify due to quick solvent evaporation, and exhibits different retention behavior. SFC columns respond differently to changing analysis conditions compared to LC columns. We introduce some common adjustments in this technical note.

Supercritical Fluid Chromatography (SFC)

Supercritical Fluids

In general, substances have solid, liquid, and gas phases. Beyond a certain temperature and pressure, liquid and gas phases become indistinguishable. We call this the supercritical point, and a fluid that has exceeded critical temperature (Tc) and critical pressure (Pc) is called a supercritical fluid (figure 1). Supercritical fluids have viscosity and diffusivity close to gas and solubility close to liquid (figure 2). Carbon dioxide becomes supercritical under relatively easy conditions (Tc: 31°C; Pc: 7.38MPa). Because of this, it has become the standard mobile phase for SFC.

Figure 1: Phase diagram

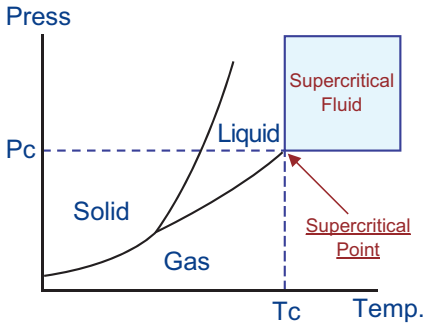


Table 2: Physical properties of gas, liquid, and supercritical fluid (*1)

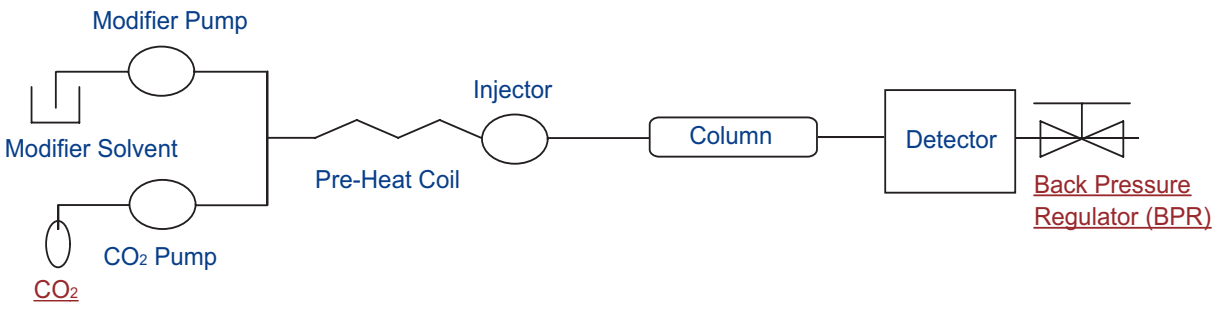
	Density (g/cm^3)	Viscosity (Pa・s)	Diffusion coefficient (cm^2/s)
Gas	$(0.6\sim2.0)\times10^{-3}$	$(1\sim3)\times10^{-5}$	0.1~0.4
Supercritical fluid	0.2~0.9	$(1\sim9)\times10^{-5}$	$(0.2\sim2.0)\times10^{-3}$
Liquid	0.6~1.6	$(0.2\sim3.0)\times10^{-3}$	$(0.2\sim2.0)\times10^{-5}$

(*1) Reference:
Chemical Society of Japan. *Kagaku Binran Kisohen I* (*Handbook of Chemistry* 5th Edition)(Tokyo, Japan: Maruzen); 2004. p. 780 (in Japanese).

SFC Instruments

The basic structure of a supercritical fluid chromatograph is shown in figure 3. The main differences from liquid chromatographs are the carbon dioxide pump and the back pressure regulator.

Figure 3: Structure of a supercritical fluid chromatograph



Influence of Analytical Conditions

Analytical conditions in SFC differ from LC. It is necessary to set appropriate conditions to obtain repeatable analyses. It is generally known that SFC produces similar results to normal phase HPLC. As such, an unmodified silica gel column (SL-II) was used to evaluate the effects of different conditions. Similar effects can be observed in COSMOSIL SFC columns.

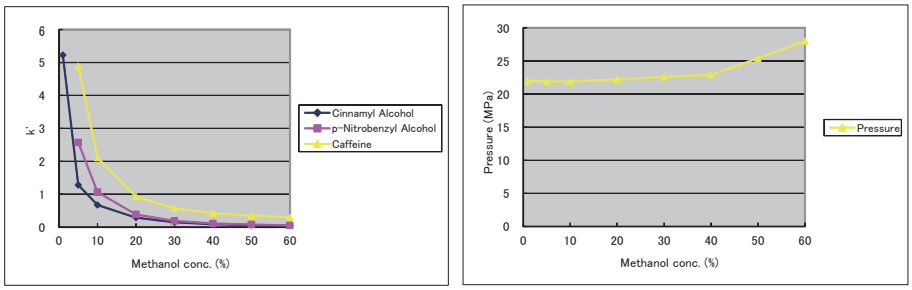
Organic Modifier Concentration

Organic modifiers, such as methanol, are often added to carbon dioxide in SFC mobile phases. In general, retention decreases as more organic solvent is added. This effect can be used to adjust retention time. However, if too much modifier is added, the mobile phase may revert to a non-supercritical state. To avoid this, be careful of changes in pressure and retention behavior.

Effect of Organic Solvent Concentration in Mobile Phase

Column: COSMOSIL 5SL-II
Column size: 4.6mmI.D.-250mm
Mobile phase: A: CO_2
B: Methanol
B conc. **%
Flow rate: 3.0 ml/min
BPR: 10 MPa
Temperature: 40 °C
Detection: UV254nm

Sample: Cinnamyl Alcohol
p-Nitrobenzyl Alcohol
Caffeine



NACALAI TESQUE, INC

Organic Solvent Type

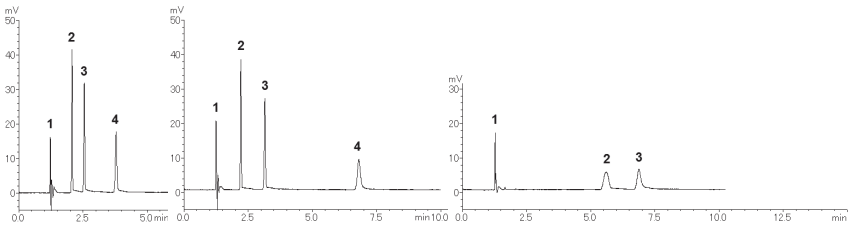
Methanol, ethanol, and acetonitrile are commonly used as organic modifiers. Consider the solubility of the sample and the characteristics of the column when selecting a modifier. Water is not recommended for this purpose.

Effect of Different Organic Modifiers in Mobile Phase

Column: COSMOSIL 5SL-II
Column size: 4.6mmI.D.-250mm
Mobile phase: A: CO_2
B: **
B conc. 10%
Flow rate: 3.0 ml/min
BPR: 10 MPa
Temperature: 40 °C
Detection: UV254nm

Sample: 1; *p*-Xylene
2; Cinnamyl Alcohol
3; *p*-Nitrobenzyl Alcohol
4; Caffeine

Methanol/ CO_2 = 10/90 Ethanol/ CO_2 = 10/90 Acetonitrile/ CO_2 = 10/90



NACALAI TESQUE, INC

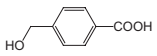
Additives

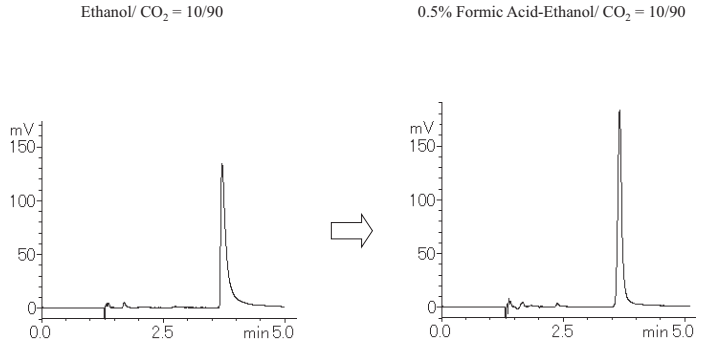
When injecting a dissociative sample, adsorption and peak tailing may occur unless an additive is used. Formic acid and ammonium acetate are commonly used as additives for acidic and basic analytes, respectively.

Effect of Additives in Mobile Phase

Column: COSMOSIL 5SL-II
Column size: 4.6mmI.D.-250mm
Mobile phase: A: CO_2
B: **
B conc. 10%
Flow rate: 3.0 ml/min
BPR: 10 MPa
Temperature: 40 °C
Detection: UV254nm

Sample: *4*-Hydroxymethylbenzoic Acid





NACALAI TESQUE, INC

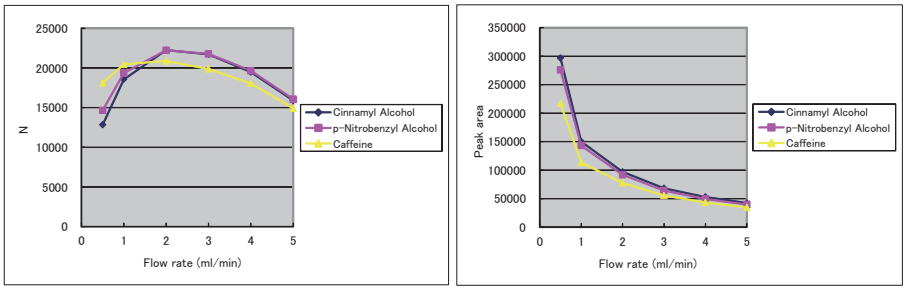
Flow Rate

For a 4.6 mm I.D. column, a flow rate of 3 ml/min is generally recommended. If the flow rate is increased, UV detection sensitivity may decrease.

Effect of Flow Rate

Column: COSMOSIL 5SL-II
Column size: 4.6mmI.D.-250mm
Mobile phase: A: CO_2
B: Methanol
B conc. 10%
Flow rate: ** ml/min
BPR: 10 MPa
Temperature: 40 °C
Detection: UV254nm

Sample: Cinnamyl Alcohol
p-Nitrobenzyl Alcohol
Caffeine



NACALAI TESQUE, INC

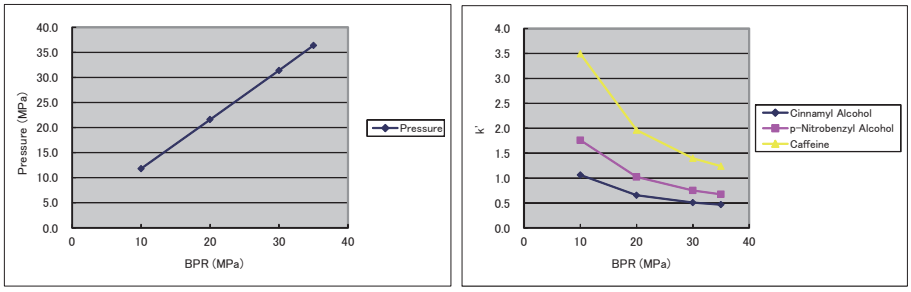
Back Pressure Regulator (BPR)

A back pressure regulator (BPR) is connected at the end of the detector to preserve the supercritical state of the mobile phase within the instrument. If the pressure setting is changed, retention time also shifts. To obtain repeatable results, it is necessary to use consistent BPR conditions. A pressure setting of 10 MPa is appropriate for regular use.

Effect of BPR

Column: COSMOSIL 5SL-II
Column size: 4.6mmI.D.-250mm
Mobile phase: A: CO_2
B: Methanol
B conc. 10%
Flow rate: 3.0 ml/min
BPR: ** MPa
Temperature: 40 °C
Detection: UV254nm

Sample: Cinnamyl Alcohol
p-Nitrobenzyl Alcohol
Caffeine



NACALAI TESQUE, INC

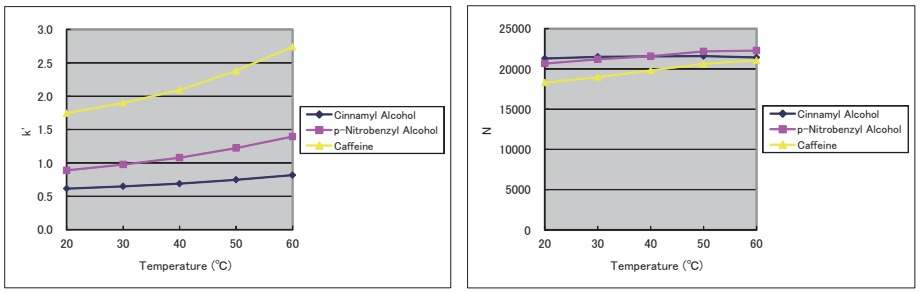
Column Temperature

Increasing the column temperature will generally increase the retention time of the sample. The number of theoretical plates (N) also increases slightly with temperature. If the temperature becomes too low, maintaining the supercritical state becomes difficult, so a temperature of 40°C or greater is appropriate

Effect of Column Temperature

Column: COSMOSIL 5SL-II
Column size: 4.6mmI.D.-250mm
Mobile phase: A: CO_2
B: Methanol
B conc. 10%
Flow rate: 3.0 ml/min
BPR: 10 MPa
Temperature: ** °C
Detection: UV254nm

Sample: Cinnamyl Alcohol
p-Nitrobenzyl Alcohol
Caffeine



NACALAI TESQUE, INC

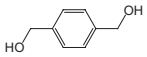
Sample Loading

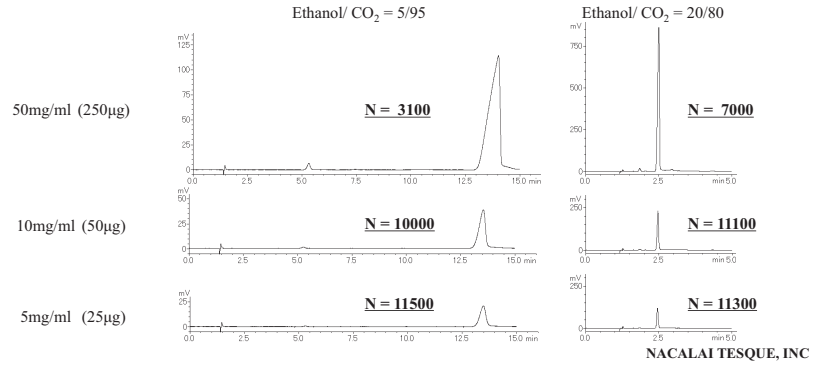
If the sample is poorly soluble in the mobile phase, increasing the amount of sample could result in peak fronting. This can be improved by changing the concentration and/or type of organic solvent to increase sample solubility.

Effect of Sample Loading

Column: COSMOSIL 5SL-II
Column size: 4.6mmI.D.-250mm
Mobile phase: A: CO_2
B: Ethanol
B conc. **%
Flow rate: 3.0 ml/min
BPR: 10 MPa
Temperature: 40 °C
Detection: UV254nm

Sample: *1,4*-Benzenedimethanol (**mg/ml)
Inj.Vol.: 5.0µl





NACALAI TESQUE, INC

For research use only, not intended for diagnostic or drug use.

nacalai tesque
The quality for certainty.

NACALAI TESQUE, INC.
Nijo Karasuma, Nakagyo-ku, Kyoto 604-0855 JAPAN
TEL : +81-(0)75-251-1730
FAX : +81-(0)75-251-1763
Website : www.nacalai.com
E-mail : info.intl@nacalai.com