

An Evaluation of Physico-Chemical Properties of the Mobile Phase in Supercritical Fluid Chromatography when using Sub-2 μ m Particle Columns

Introduction

In Supercritical Fluid Chromatography (SFC), high speed analysis can be performed easily because the diffusion coefficient of the mobile phase is very high and the viscosity is low.

Recently, the use of columns packed with sub-2 μ m particles has become popular to perform high speed analysis without sacrificing the column efficiency in HPLC, often referred to as Ultra High-Performance Liquid Chromatography (UHPLC). If a column packed with sub-2 μ m particles is used in SFC, Ultra High-Performance Supercritical Fluid Chromatography (UHPSFC) could be performed.

However, in SFC, it is generally believed that the use of a low permeability column such as those packed with sub-2 μ m particles results in poor column efficiency due to a large pressure drop across the column creating a negative density gradient along the length of the column. This may be true if the actual elution conditions are in a supercritical state and close to the critical point.



Jasco UHPSFC System

Keyword: UHPSFC, Sub 2 μ m, UHPLC, HPLC, SFC, Vitamins, Supercritical CO₂ density, Theoretical Plates, Van Deemter Plot

Generally, a mixture of CO₂ and an organic modifier co-solvent, typically an alcohol is used as the mobile phase in SFC. The critical temperature and pressure for such a mixture is generally much higher than that for pure CO₂, and the typical mobile phase used in 'real world' SFC is often not in a supercritical state. Therefore, we have investigated the effect of several parameters such as temperature and pressure for the commonly used mobile phase mixture of CO₂ and methanol and the impact on the H-u curve in UHPSFC.

We first discuss from a theoretical point of view and then evaluate the experimental results by comparing them to the theoretical data. In addition, we evaluated high speed separation of fat-soluble vitamins by UHPSFC.

Physical Properties of CO₂ and Methanol System and Vapor-liquid equilibria for CO₂ and methanol system:

It is important to know the physical properties of the CO₂ and methanol mixture system that is used as a mobile phase.

Figure 1 shows the experimental vapor-liquid equilibria data of CO₂ and methanol mixture system at a temperature of 40 °C by Kodama et al¹⁾. The area enclosed by the measured data points shows the area that the mixture exists as two phases; vapor and liquid. The outside area shows the area that the mixture exists as one phase; liquid or gas or supercritical fluid.

Generally, in SFC a back pressure of 10MPa or higher is applied to the column. At these pressures we can safely say that the mixture system is single phase.

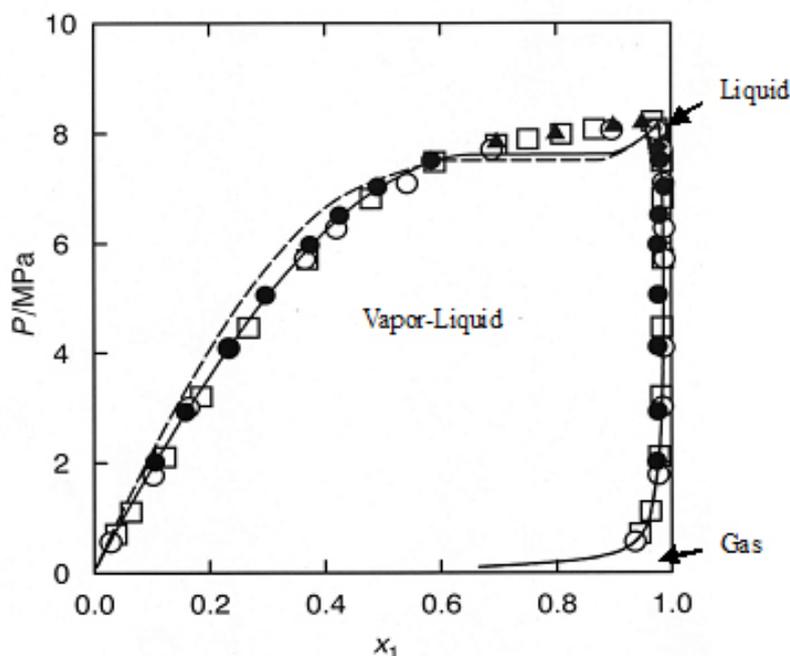


Figure 1. Vapor-liquid equilibria for the CO₂ and Methanol system at 40°C

Calculation of critical temperature and pressure of a mixture of CO₂ and methanol:

The critical temperature and pressure of a mixture of CO₂ and methanol can be calculated from the equations proposed by Cheuh and Prausnitz²⁾. Saito and Nitta³⁾ wrote a convenient computer program for the calculation of various mixtures containing CO₂ and organic solvents and compared the results with experimental data. The Kij value was determined to be 0.2 according to the value proposed by Brunner⁴⁾.

The coefficients, A, B, C, D, and E used in the calculation were obtained from the reference⁵⁾; -0.0076, 0.287, -1.343, 5.443, and -3.038 respectively. The authors used the computer program to calculate the critical parameters.

Figure 2 shows a graph of the calculated critical temperature and pressure against the methanol mole fraction. As shown in this figure, the critical temperature elevates as the methanol mole fraction increases, while the critical pressure exhibits a maximum value at the mole fraction of approximately 0.45.

Table 1 lists several volumetric ratios (90:10, 80:20, 70:30 and 50:50) of CO₂ and methanol and corresponding methanol mole fractions, critical temperatures and pressures for the readers' convenience. Even with a ratio of CO₂ and methanol at 50:50 (considered a modifier rich condition, but often used in SFC) the critical pressure is about 16MPa which is not an unusual pressure. However, the critical temperature is 124°C, which is outside of the normal temperature range used in SFC (a temperature lower than 60°C is generally used). Under these conditions, the mixture mobile phase is in the liquid or subcritical state.

Table 1. Relationship between CO₂ and Methanol volume ratio and the calculated critical temperature and pressure

CO ₂ Volume Ratio	Methanol Volume Ratio	Methanol Mole Fraction	Critical Temperature (°C)	Critical Pressure (MPa)
90	10	0.07	51.13	10.46
80	20	0.12	64.91	12.22
70	30	0.20	85.92	14.37
50	50	0.36	124.37	16.54

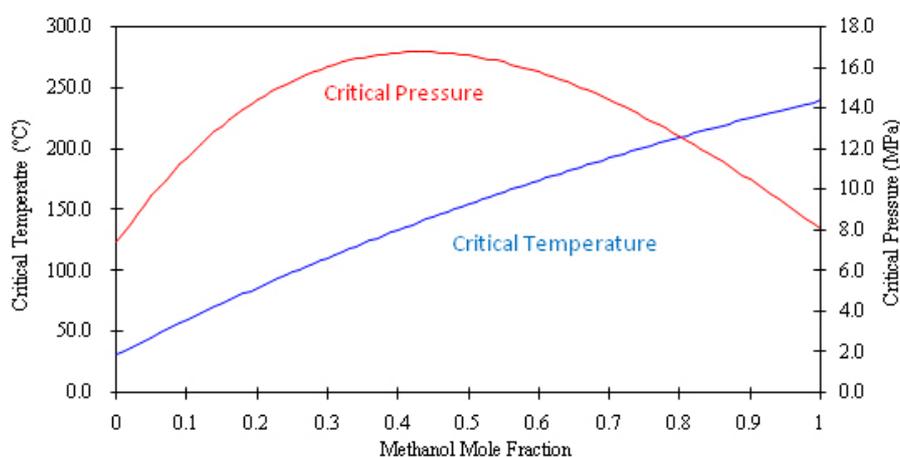


Figure 2 Relationship between the calculated temperature and pressure and the mole fraction of CO₂ and methanol mixture.

Calculation of density of a mixture of CO₂ and methanol:

The density in CO₂ and methanol mixture system can be calculated using the Pseudo cubic perturbed hard-sphere (PPHS) equations proposed by Kato et al⁶). The authors were permitted by Dr. Kato to use his computer program to calculate the density of a mixture of CO₂ and methanol at methanol mole fraction of 0.07. The Kij and lij values have not been measured. Therefore, we assumed these parameters to be zero.

Figure 3 shows the relationship between the density and pressure calculated by the above method. As the pressure becomes higher, the density rapidly increases. This behavior is more significant when the fluid temperature is higher. This shows that the mobile phase state is transferred from gas to supercritical region when the pressure is increased at a higher temperature.

In other words, the chromatographer is changing the chromatographic conditions without knowing from liquid to supercritical to gas chromatography by changing the temperature while the pressure is maintained higher than the mixture critical pressure.

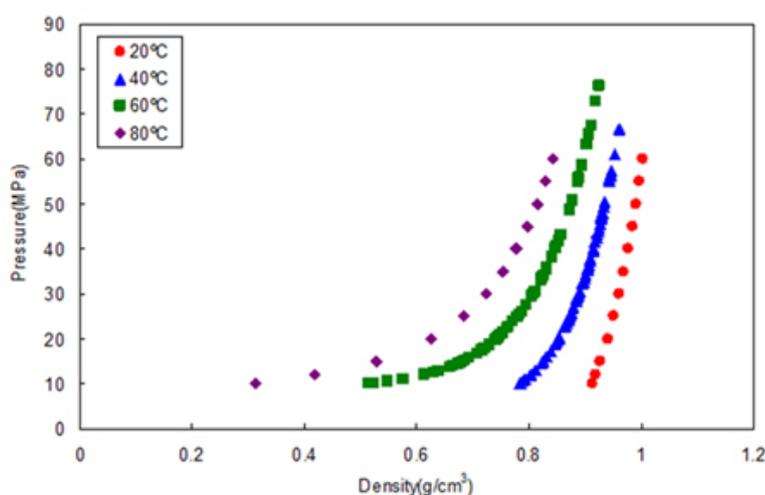


Figure 3. The calculated density of a CO₂ and methanol mixture (mole fraction = 0.07)

Experimental



Figure 4. UHPSFC System

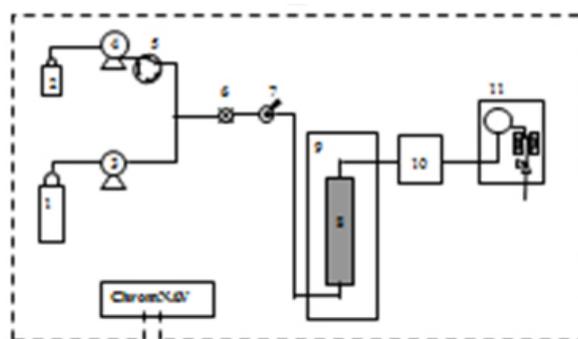


Figure 5. Schematic of the UHPSFC System

- | | | | |
|----|---|-----|---|
| 1. | CO ₂ Cylinder | 7. | X-LC™ 3159AS Auto Sampler |
| 2. | Modifier Solvent | 8. | Column |
| 3. | X-LC™ 3180PU with cooling jacket
CO ₂ Delivery Pump | 9. | CO-2060 Column Oven |
| 4. | X-LC™ 3185PU Modifier Delivery Pump | 10. | X-LC™ 3075 UV Detector
with high pressure cell |
| 5. | X-LC™ 3185HV Switching Valve Unit | 11. | BP-2080 Back Pressure Regulator |
| 6. | MX-2080-32 Mixer | 12. | ChromNAV Chromatography Data Station |

A column, ZORBAX RX-SIL, 1.8 μ m, 2.1 mm ID x 100 mmL and 3.0 mm ID x 100 mmL was purchased from Agilent Technologies, USA.

Carbon dioxide (99.98 %) was supplied by TOYOKO KAGAKU Co., Ltd, Kanagawa, Japan. HPLC-grade methanol used as a modifier solvent; Caffeine, Toluene, and Ergocalciferol (Vitamin D2) were purchased from Wako Pure Chemicals, Osaka, Japan. a, b, d-tocopherol (Vitamin E) were purchased from Eisai, Tokyo, Japan.

Results and Discussion

Van Deemter Equation

The Van Deemter equation is generally used to evaluate the efficiency of the column. The equation is often expressed in a simplified form as equation (1). In SFC, D_M is often more than 10 times higher than that for liquid solvents. Therefore, the H_{min} value can be obtained at a higher u value, offering a higher speed of analysis than that in HPLC.

However, slow mass transfer, still remains within the stationary phase which restricts the efficiency and the speed of the analysis. Thus, a smaller particle size and thinner stationary phase film thickness is preferable. Figure 6 illustrates the contribution of each term in Equation (1) to H and u .

In our experiment, the efficiency of a column packed with sub- $2\mu\text{m}$ particles was measured. H was calculated from the peak of caffeine and u was calculated by using the retention time of toluene as t_0 .

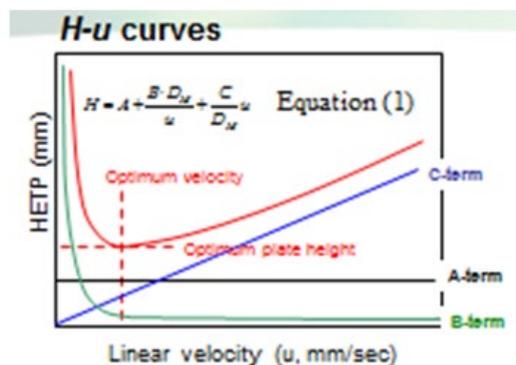


Figure 6. The van Deemter curve showing the relationship of HETP(H) vs. Linear velocity

Figure 7 shows the measured H-u plots for a 2-mm ID column. Unless otherwise noted, the pressures shown in all figures are column outlet pressures applied by the back-pressure regulator. As shown in this figure, H increases in the range of lower linear velocities only when the temperature and pressure is at 60°C and 10MPa, respectively.

Under these conditions, the density is calculated to be 0.5g/cm³ from the PPHS equations, and the chromatography behaves like gas chromatography or traditional supercritical fluid chromatography (pure CO₂ mobile phase at relatively high temperatures); H increases at lower u caused by higher longitudinal diffusion of the sample solute in the mobile phase.

When the density is increased to 0.7g/cm³ or more by elevating the pressure or lowering the temperature, the behavior tends towards liquid chromatography because the solvating power depends on the density. It should be noted that density depends not only on the pressure but also on the temperature.

Figure 8 shows the column pressure drop exhibited in the experiment of H-u plots discussed above. As shown in the figure, when the temperature is high, the column pressure drop is low because both the viscosity and density are low.

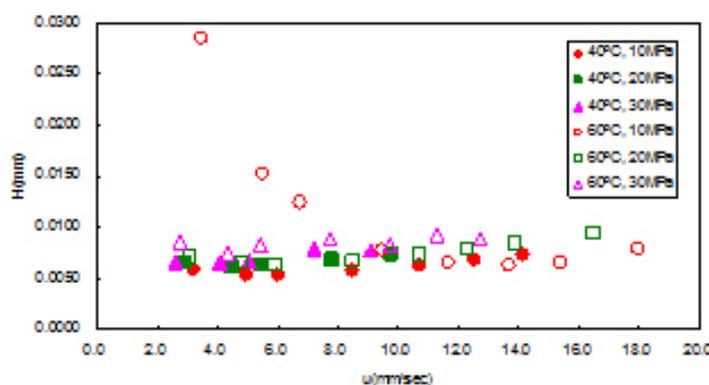


Figure 7. H-u plots for sub 2µm silica column (2-mm ID)

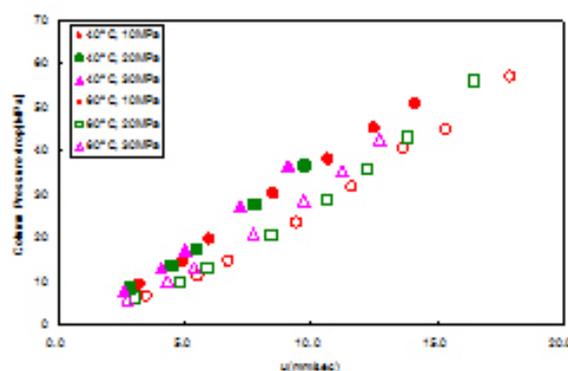


Figure 8. Linear velocity vs. column pressure
The separation conditions are the same as in Figure 7

Separation Conditions

Column:	ZORBAX Rx-SIL (2-mm ID x 100mmL, 1.8µm)
CO ₂ Flow rate:	0.45, 0.72, 0.9, 1.35, 1.8, 2.25, 2.7, 3.6, 4.05, and 4.5mL/min at -10°C
Modifier:	methanol @ flow rates 0.05, 0.08, 0.1, 0.15, 0.2, 0.25, 0.3, 0.45, and 0.5 mL/min
Pressure:	10, 20, and 30MPa
Column Temperature:	40, 60°C
Wavelength:	250nm
Injection Volume:	0.5µL
Sample:	Toluene (5mg/mL) and Caffeine (0.17mg/mL)

The same experiments were performed using a 3-mm ID column. Figure 9 shows the H-u plots and Figure 10 shows the graph of the column pressure drop against the linear velocity. The H-u plots are similar to those for the 2-mm ID column. H was increased in the range of lower linear velocities only when the temperature and pressure are 60°C and 10MPa, respectively.

Figure 10 shows the graph of the column pressure drop against the linear velocity under the same conditions as in Figure 9. As shown in this figure, as the temperature increases, the column pressure decreases for the same reason discussed above. Compared with the 2-mm ID column, the column pressure drop is lower because of the wider column ID.

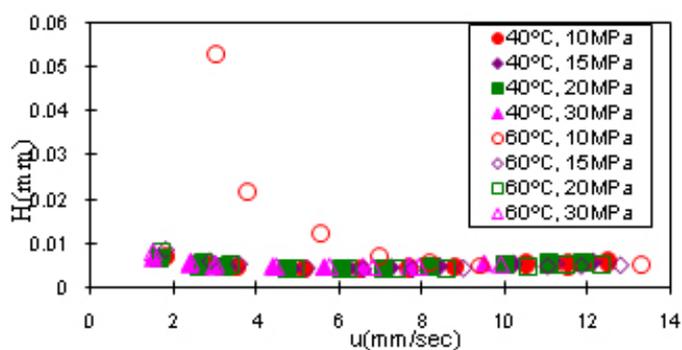


Figure 9. H-u plots on sub 2 μ m silica column (3-mm ID)

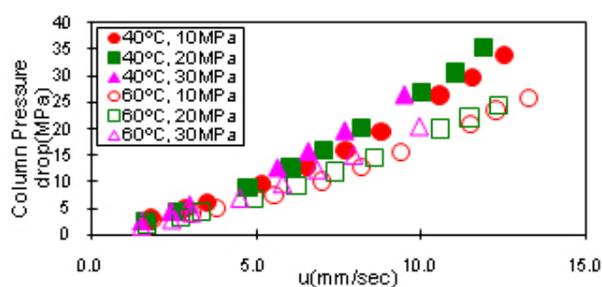


Figure 10. Linear velocity vs. column pressure
The separation conditions are the same as in Figure 9.

Separation Conditions

Column:	ZORBAX Rx-SIL (3-mm ID x 100mmL, 1.8 μ m)
CO ₂ Flow rate:	0.45, 0.72, 0.9, 1.35, 1.8, 2.25, 2.7, 3.6, 4.05, and 4.5mL/min at -10°C
Modifier:	methanol @ flow rates 0.05, 0.08, 0.1, 0.15, 0.2, 0.25, 0.3, 0.45, and 0.5 mL/min
Pressure:	10, 15, 20, and 30MPa
Column Temperature:	40, 60°C
Wavelength:	250nm
Injection Volume:	0.5 μ L
Sample:	Toluene (5mg/mL in MeOH) and Caffeine (0.17mg/mL in MeOH)

H-u plots dependence on temperature

Figure 11 shows the H-u plot depending on temperature. As the temperature was increased, the optimum velocity shifts to the higher velocity region, and H itself becomes smaller. That is because as the temperature increases, the viscosity decreases.

Under the conditions of 60°C and 20MPa, which gave a column pressure drop of ca. 10MPa, the minimum plate height was about 4 μ m, which is very close to the theoretical minimum value, proving that the overall chromatographic system is performing extremely efficiently.

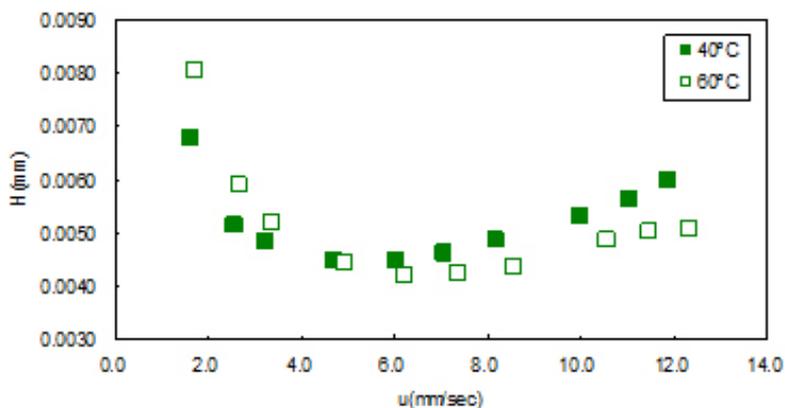


Figure 11. H-u plots depending on the temperature

Separation Conditions

Column:	ZORBAX Rx-SIL (3-mm ID x 100mmL, 1.8 μ m)
CO ₂ Flow rate:	0.45, 0.72, 0.9, 1.35, 1.8, 2.25, 2.7, 3.6, 4.05, and 4.5mL/min at -10°C
Modifier:	methanol @ flow rates 0.05, 0.08, 0.1, 0.15, 0.2, 0.25, 0.3, 0.45, and 0.5 mL/min
Pressure:	20MPa
Column Temperature:	40, 60°C
Wavelength:	250nm
Injection Volume:	0.5 μ L
Sample:	Toluene (5mg/mL in MeOH) and Caffeine (0.17mg/mL in MeOH)

H-u Curve dependence on column ID

Figure 12 A and B compare the H-u plots for the 2-mm and 3-mm ID columns. The optimum velocities were 6mm/sec for both columns. With this velocity the column pressure drop was ca.10MPa for both columns. However, the 3-mm ID column offers lower H than the 2-mm ID column at both 40 and 60°C temperatures.

This result shows that the column pressure drop did not affect H. However, smaller peak volumes generated by the 2-mm ID column could contribute more significantly to extra-column band broadening, resulting in higher H. In any case, it is remarkable that the 3-mm ID column offered the H as low as 4 μ m that is very close to the theoretical minimum value.

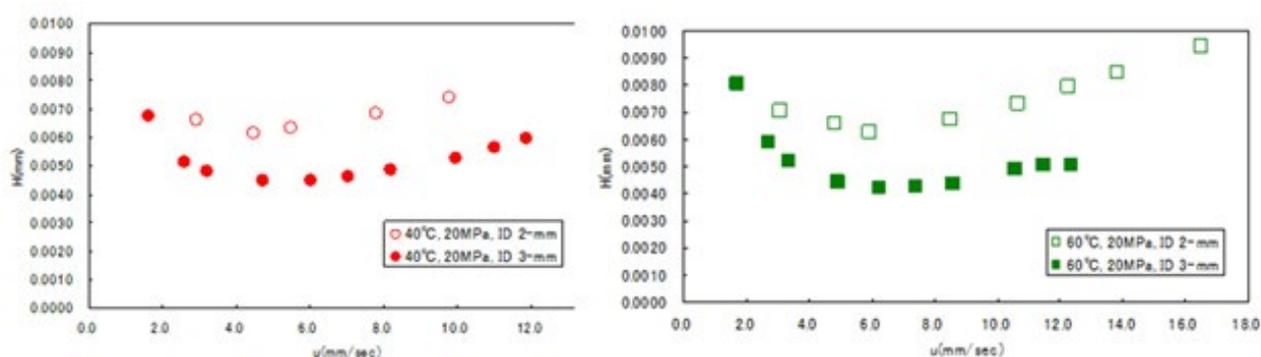


Figure 12. H-u plots on sub 2 μ m silica column; (A) 2-mm ID column, (B) 3-mm ID column

Separation Conditions

Column:	ZORBAX Rx-SIL (2 -and 3-mm ID x 100mmL, 1.8 μ m)
CO ₂ Flow rate:	0.45, 0.72, 0.9, 1.35, 1.8, 2.25, 2.7, 3.6, 4.05, and 4.5mL/min at -10 °C
Modifier:	methanol @ flow rates 0.05, 0.08, 0.1, 0.15, 0.2, 0.25, 0.3, 0.45, and 0.5 mL/min
Pressure:	20MPa
Column Temperature:	40, 60°C
Wavelength:	250nm
Injection Volume:	0.5 μ L
Sample:	Toluene (5mg/mL in MeOH) and Caffeine (0.17mg/mL in MeOH)

Separation of Fat-soluble Vitamins

In order to demonstrate the high performance of the system, which we discussed above, we separated fat-soluble vitamins using the 3-mm ID column packed with sub-2 μ m particles.

Figure 13 successfully shows that four vitamins were separated within only 24 seconds with baseline separation.

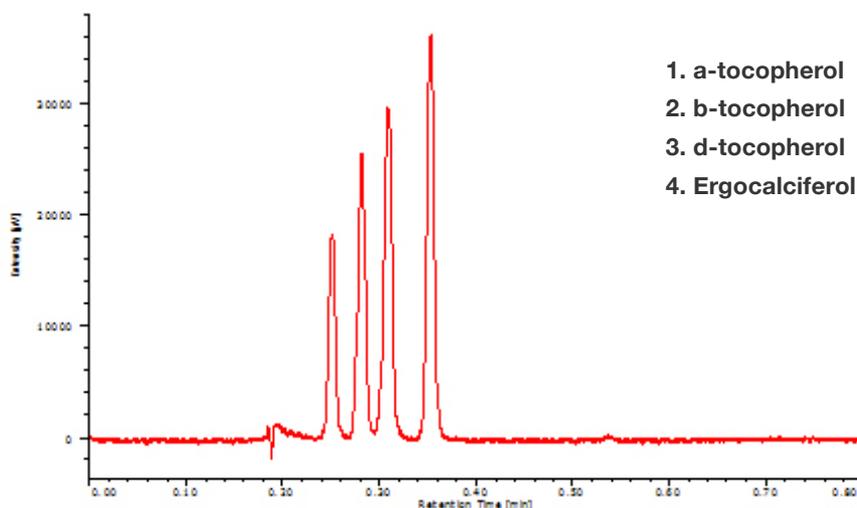


Figure 13. Chromatogram of fat-soluble vitamins

Separation Conditions

Column:	ZORBAX Rx-SIL (3-mm ID x 100mmL, 1.8 μ m)
CO ₂ Flow rate:	3.8mL/min at -10 °C
Modifier:	methanol @ flow rates 0.05, 0.08, 0.1, 0.15, 0.2, 0.25, 0.3, 0.45, and 0.5 mL/min
Pressure:	20MPa
Column Temperature:	40 °C
Wavelength:	3.8mL/min at -10°C
Injection Volume:	0.5 μ L
Sample:	a, b, d-tocopherol, Ergocalciferol (0.14, 0.17, 0.24, 0.18mg/mL in MeOH, respectively)

Conclusions

1. Today's normal SFC conditions with CO₂ containing a relatively high- concentration of organic modifier are often in the liquid or subcritical state.
2. Contrary to the legacy of SFC theory, these conditions do not deteriorate the column efficiency of sub-2 μ m particle columns which give a high column pressure drop.
3. The SFC chromatographer today is often changing the chromatographic conditions without knowing from liquid to supercritical to gas chromatography by changing the temperature while the pressure is kept higher than the mixture critical pressure.
4. It is very important for SFC chromatographers to know the critical parameters of a mobile phase mixture in order to control the elution behavior.
5. Four fat-soluble vitamins were separated within only 24 sec.

Reference

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