Addition of Water in Carbon Dioxide Mobile Phase for Supercritical Fluid Chromatography

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Supercritical fluid chromatography (SFC) may be defined as a form of chromatography in which the mobile phase is subjected to pressures and temperatures near or above the critical point for the purpose of enhancing the mobile phase solvating power.[1]

During its relatively short existence, supercritical fluid chromatography (SFC) has become an attractive alternative to GC and LC in certain industrially important applications. SFC gives the advantage of high efficiency and allows the analysis of non-volatile or thermally labile mixtures.

Carbon dioxide is widely used as a primary mobile phase in SFC because of its low critical temperature, low toxicity, chemical inertness and nonflammability. However, because of its nonpolar nature,[2,3] neat CO_2 is limited in its ability to elute polar compounds.

Therefore, a major objective of research into SFC has been directed towards increasing the range of solute polarity that can be handled by the technique. To bring the SFC technique into routine use, mobile phases that are more polar than the commonly used CO_2 are necessary. Polar mobile phases such as NH_3 exhibit useful properties,[4] but a more practical way to extend the range of compounds separable by SFC is the use of mixed mobile phases. The addition of modifiers (generally organic solvents) to supercritical CO_2 changes the polarity of the mobile phase and also leads to a deactivation of the column packing material.[5]

Janssen and co-workers[6] explained the effects of adding polar modifiers to supercritical fluid CO_2 mobile phase in three different ways: (A) increasing mobile phase polarity, (B) increasing mobile phase density, (C) deactivation of active sites on the surface of the stationary phase. In capillary SFC, most separations are carried out with pure CO_2 because of its compatibility with a flame-ionization detector (FID); indeed, except for formic acid and water the addition of any common modifier precludes the use of an FID.[7] Although it is desirable to use FID in SFC, only water and formic acid produce acceptably low background noise and enable the use of this universal detector. Water and formic acid have been suggested by some investigators as very useful modifiers in packed column SFC[8,9] because they significantly improve the separation of some polar compounds.

One of the simple and effective ways for the addition of modifiers to supercritical fluid mobile phase reported in the literature is to use a saturator column[10] which is usually a silica column saturated with polar modifiers.

In our laboratory, μ-Porasil column[11] and highly porous stainless-steel filters[12,13] which are saturated with polar modifiers have been used successfully as a modifier mixing device. One problem with these kinds of saturation type mixing devices is that the amount of polar modifiers dissolved in supercritical fluid CO_2 can not be changed easily because the principle of mixing is based on the saturation.

In this paper, a newly developed mixing device[14] was used to demonstrate the effects of addition of water in carbon dioxide mobile phase. The new mixing device in which small mixing chamber with magnetic bar was used to generate water-modified carbon dioxide mobile phase was applied for the separations of free fatty acids.

For the analysis of fatty acids, GC often is not the method of choice because of the low volatility of fatty acids. HPLC is not helpful either, because free fatty acids cannot be detected easily by a UV detector or one of the other HPLC detectors. In this case, SFC is an alternative to GC or HPLC for the analysis of fatty acids.

The ability to analyze fairly polar compounds with supercritical CO_2 is demonstrated in this paper; however, a modifier must be used. The influence on the retention behaviour of adding a modifier depends on the nature of the substrate, the stationary phase, and on the modifier itself. Yonker *et al.*,[4] report that at CO_2/methanol mixtures at 50 °C UV absorbance maxima shifts for 2-nitroamisole. When dealing with the use of modifiers, it should be mentioned that some problems arise. First, a binary mixture of eluents can contaminate the instrument. The modifier remaining in a injector, tubing, expecially pump can be eluted slowly during the

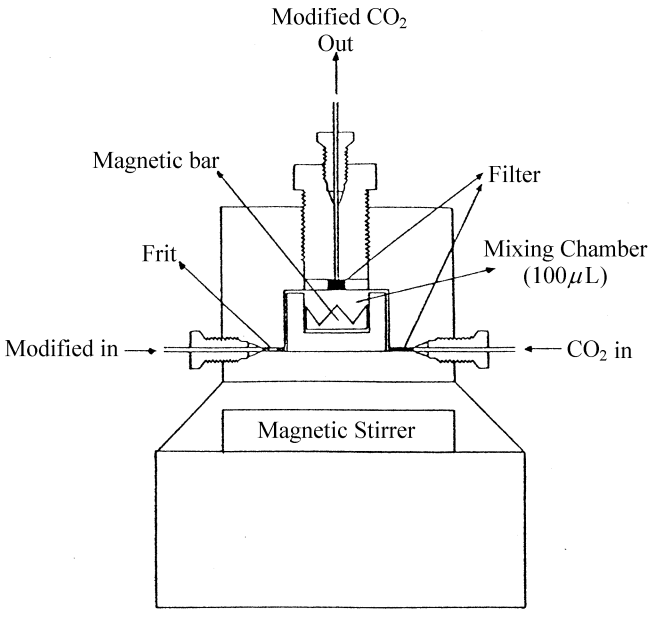

Figure 1. Mixing Device.

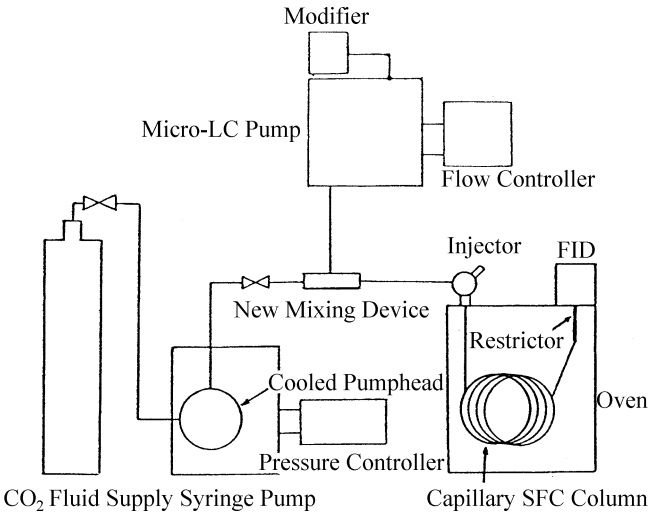

Figure 2. Schematic diagram of on-line modifier addition system using new mixing device.

next run. This may affect the time to achieve chemical equilibrium and cause a corrosion of the pump. Second, saturator type mixing devices can not control the amount of modifier added to the mobile phase. To overcome these problems, we designed a new device[14] which is shown in Figure 1. In Figure 1, a small volume of mixing chamber (100 μL) and a magnetic bar were used to allow rapid equilibration of the CO_2/water mixture. A frit (2 cm×0.3 mm i.d.) was also utilized to inject a small amount of water into the mixing chamber. Using the described modifier mixing device, 0.2%-1.2% water mixture could be generated without problems. The amount of water added can be controlled by changing the flow rate of Micro-LC pump and the pressure of supercritical CO_2. The overall schematic diagram of the modifier addition system using new mixing device is shown in Figure 2.

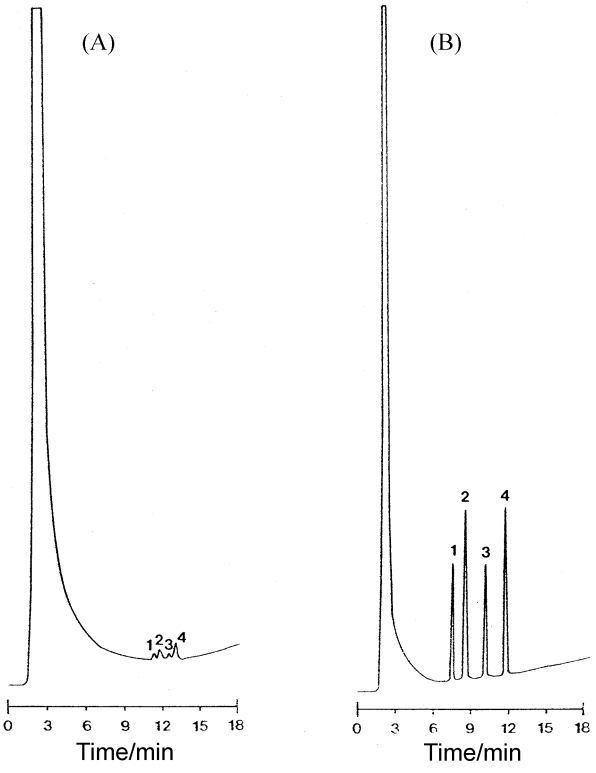

Figure 3. Chromatograms of mixtures of fatty acids using (A) pure supercritical CO_2 mobile phase and (B) 1.0% water modified supercritical CO_2 mobile phase. Peak 1) Hepanoic acid, 2) Octanoic acid, 3) Nonanoic acid, 4) Lauric acid. Separation conditions: 200 atm, 3 atm/min increase, 100 °C, 10m×50 i.d. SB-methyl-100 polydimethylsiloxane column, FID at 300 °C.

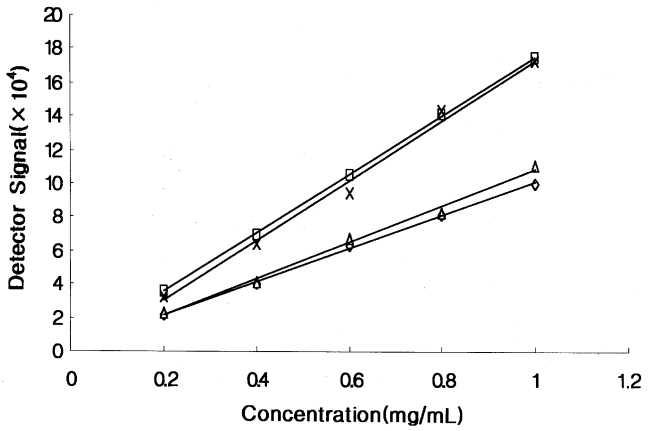

Figure 4. Calibration curves for the quantitative analysis of heptanoic acid (◇), octanoic acid (□), nonanoic acid (△), and lauric acid (×).

An experiment to separate polar samples (free fatty acids) with the new mixing device was performed. Figure 3(A) and Figure 3(B) are chromatograms for mixtures of four fatty acids using (A) pure supercritical CO_2 mobile phase and (B) 1.0% water modified supercritical CO_2 mobile phase.

In contrast to the chromatogram of Figure 3(A), excellent separations (see Figure 3(B)) were obtained when 1.0% water was added to supercritical CO_2. This is because the solvent strength of pure supercritical CO_2 is not sufficient for the elution of polar compounds, such as, fatty acids. In Figure 3(B), we could observe that the addition of a small amount of water to supercritical CO_2 reduced the retention and improved the peak shapes. The phenomena are in accord with the results reported by Blilie and Greibrokk.[5] For the four fatty acids, the calibration curves (Figure 4) and the reproducibility data (Table 1) for the retentions were obtained. In Figure 4, a mixture of sulfonamide antibacterials were separated using water modified supercritical fluid mobile phase. When only CO_2 was used for the separations of these compounds, very broad, fused peaks and long reten-

Table 1. Analytical results of retention times of heptanoic acid, octanoic acid, nonanoic acid, lauric acid using water modified supercritical CO_2

Sample ion	Retention time min[a]±SD[b]	RSD[c] (%)
heptanoic acid	7.44±0.12	1.6
octanoic acid	8.49±0.15	1.8
nonanoic acid	10.20±0.19	1.9
lauric acid	11.85±0.22	1.9

[a] mean value of six independent replicates (n=6), expressed in min. [b] SD, standard deviation. [c] RSD, relative standard deviation.

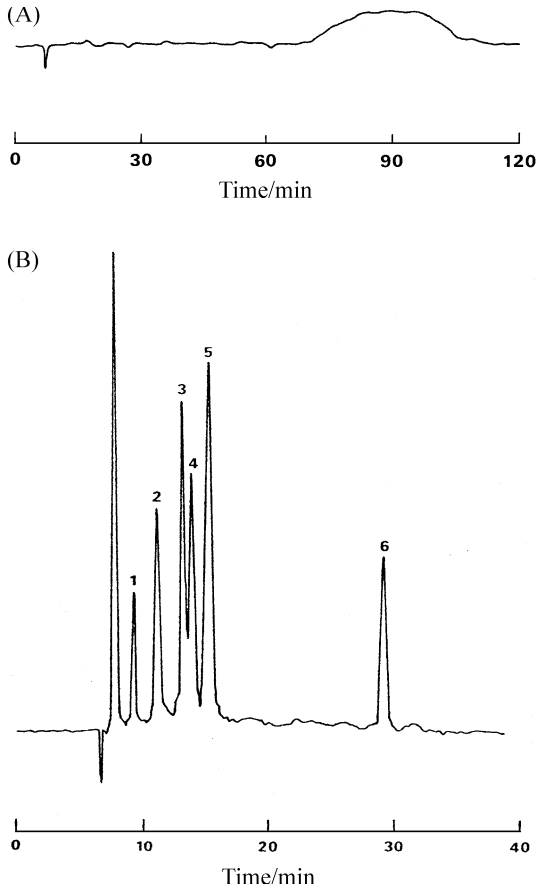

Figure 5. Chromatograms of mixtures of sulfonamide antibacterials using (A) pure supercritical CO_2 mobile phase and (B) water modified supercritical CO_2 mobile phase. Peak 1) Sulfadiazine, 2) Sulfamerazine, 3) Sulfamethazine, 4) Sulfamonomethoxine, 5) Sulfadimethoxine, 6) Sulfaquinoxaline. Separation conditions: 260 atm, 50 °C, 4.6×150 mm i.d. SFCpak Crest column, 5 μm ODS particles, Flow rate; 1 mL/min (CO_2)+0.1 mL/min (water), UV at 270 nm.

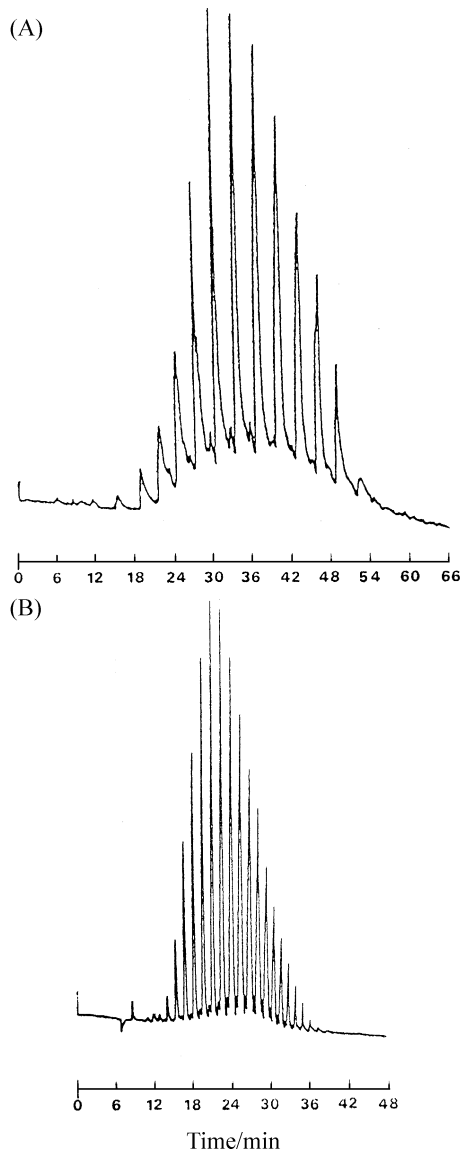

Figure 6. Chromatograms of Triton X-100 using (A) pure supercritical CO_2 mobile phase and (B) 2.0% water modified supercritical CO_2 mobile phase. Separation conditions: 180 atm, 2.5 atm/min increase, 100 °C, 1.1m×200 μm i.d. packed capillary column, 5 μm C_{18} particles.

tion time (about 90 min) were observed (see Figure 4(A)). To show the effect of mixed mobile phases on assisting the solvating ability of CO_2, we performed an experiment to separate Triton X-100. Figure 5(A), 5(B) were run with neat CO_2 and 2.0% water in CO_2 respectively. Adding water in CO_2 mobile phase permits more oligomers to be eluted in shorter analysis times.

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