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.

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, neat CO₂ 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 CO₂ are necessary. Polar mobile phases such as NH₃ exhibit useful properties, 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 CO₂ changes the polarity of the mobile phase and also leads to a deactivation of the column packing material.

Janssen and co-workers explained the effects of adding polar modifiers to supercritical fluid CO₂ 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 CO₂ 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.

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 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 which is usually a silica column saturated with polar modifiers. In our laboratory, µ-Porasil column and highly porous stainless-steel filters 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 CO₂ can not be changed easily because the principle of mixing is based on the saturation.

In this paper, a newly developed mixing device 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 CO₂ 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. report that at CO₂/methanol mixtures at 50 °C UV absorbance maxima shifts for 2-nitroanisole. 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, especially pump can be eluted slowly during the analysis of fatty acids.
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 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 CO2/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 CO2. The overall schematic diagram of the modifier addition system using new mixing device is shown in Figure 2.

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 CO2 mobile phase and (B) 1.0% water modified supercritical CO2 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 CO2. This is because the solvent strength of pure supercritical CO2 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 CO2 reduced the retention and improved the peak shapes. The phenomena are in accord with the results reported by Blilie and Greibrokk. 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 antibiotics were separated using water modified supercritical fluid mobile phase. When only CO2 was used for the separations of these compounds, very broad, fused peaks and long retentions were observed. The results were expressed in min.

Table 1. Analytical results of retention times of heptanoic acid, octanoic acid, nonanoic acid, lauric acid using water modified supercritical CO2.

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<tr>
<th>Sample ion</th>
<th>Retention time (min±SD)</th>
<th>RSD (%)</th>
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<tbody>
<tr>
<td>heptanoic acid</td>
<td>7.44±0.12</td>
<td>1.6</td>
</tr>
<tr>
<td>octanoic acid</td>
<td>8.49±0.15</td>
<td>1.8</td>
</tr>
<tr>
<td>nonanoic acid</td>
<td>10.20±0.19</td>
<td>1.9</td>
</tr>
<tr>
<td>lauric acid</td>
<td>11.85±0.22</td>
<td>1.9</td>
</tr>
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a mean value of six independent replicates (n=6), expressed in min. b SD, standard deviation. c RSD, relative standard deviation.
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 CO₂, we performed an experiment to separate Triton X-100. Figure 5(A), 5(B) were run with neat CO₂ and 2.0% water in CO₂ respectively. Adding water in CO₂ mobile phase permits more oligomers to be eluted in shorter analysis times.

Acknowledgment. This investigation was supported by a grant from Korean Science and Engineering Foundation (KOSEF, 96-0501-07-01-3). This work was also supported partially by the Ministry of Education (BSRI-97-3425).

References