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Communications

Analysis of Antibacterial Agents Employing Supercritical CO_2 and Modifiers

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In recent years, there has been tremendous growth in research involving supercritical fluid chromatography(SFC). 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. Some applications of SFC to the separation of synthetic antibacterial agents are featured in this paper, along with representative chromatograms. Analysis of antibacterial agents has received much attention because of the protection of domestic animals, productivity movement of domestic animals and the need to monitor their levels and those of their metabolites in complex sample matrices such as foods. GC is often the analytical method of choice because of the availability of sensitivity, selective detector(FPD, NPD, ECD). However, difficulties arise when the solutes cannot be analyzed by GC because of thermal instability. HPLC is not helpful either, because such compounds cannot be detected easily at trace levels by a UV detector or one of the other HPLC detectors. In these cases, SFC is an alternative to GC and HPLC for the analysis of antibacterial agents. However, the most commonly used mobile phases in SFC are all relatively nonpolar fluids. Carbon dioxide, the most widely used fluid, is no more polar than hexane[1]. To bring the SFC technique into routine use, mobile phases that are more polar than the commonly used carbon dioxide are necessary. The polarity range range of solutes that can be separated by SFC can be greatly extended by the addition of polar modifiers to supercritical CO_2[2]. The use of modifiers has been reported by Jentoft and Gouw[3] and by Novotny *et al.*[4]. The latter authors showed that adding 0.1% isopropanol to *n*-pentane as the mobile phase decreases the observed K (Partition coefficient) values for many polynuclear aromatic hydrocarbons by 20-35%. In this paper, we used the mixing device[5] developed in our laboratory in which high porous stainless steel filters (2 μm porosity) were used to generate water-modified carbon dioxide mobile phase. When supercritical CO_2 goes through the mixing device, modifiers held within the small pores of filters can be dissolved in the pressurized supercritical fluids. The solvent power of the eluents used in SFC may be enhanced by adding a modifier to the basic mobile phase[6]. Separations are often performed by SFC where the composition for the mobile phase is changed during the run or by adding a modifier before the chromatographic run is strarted. 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. 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 next run. This may affect the time to achieve chemical equilibrium and cause a corrosion of the pump. Second, many modifiers can diffuse in the laboratory and contaminate the air in the laboratory. To overcome these problems, we designed a new method which is shown in Figure 1[7]. Supercritical CO_2 is delivered from the pump to mixing device which is saturated with water. The mixing device in which has high porous stainless steel filters were used to hold a large amount of water (1.55% v/v). While in the saturator columns[7-9], water is held on the stationary phase by hydrogen bonding, with this device, water is held physically inside small pores of filters. After being saturated with water, the device is placed between a pump and an injector. With this design, supercritical CO_2 is delivered from the pump to the device which is saturated with water. When supercritical CO_2 goes through the device, water held within the small pores of the filters can be dissolved in the pressurized supercritical fluids. Thus nonpolar supercritical CO_2 can have the characteristics of polar mobile phase because it can absorb polar solvent, H_2O. Therefore, after passing the stainless steel filter, supercritical CO_2 is changed to new mobile phase with different polarity, and it is possible to separate polar samples using this new mobile phase.

An experiment to separate polar samples (antibacterial

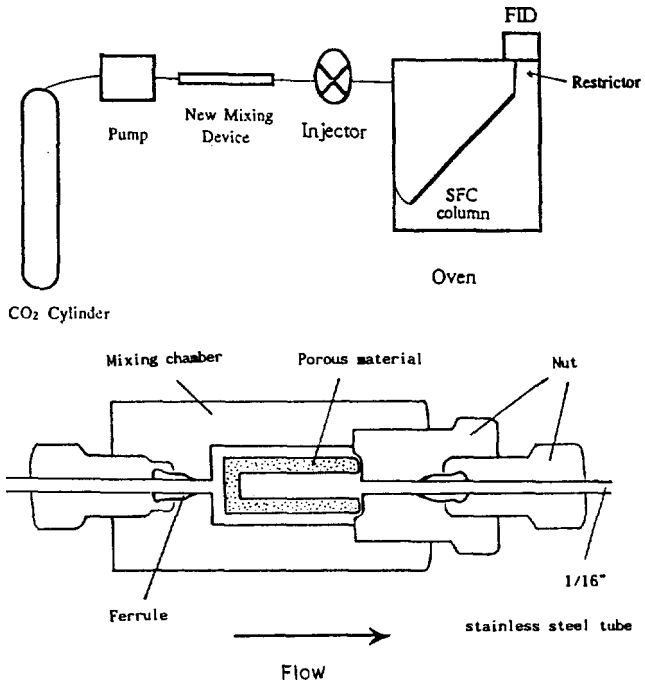

Figure 1. A diagram for adding a polar modifier to supercritical fluid mobile phase (up) and mixing device (down).

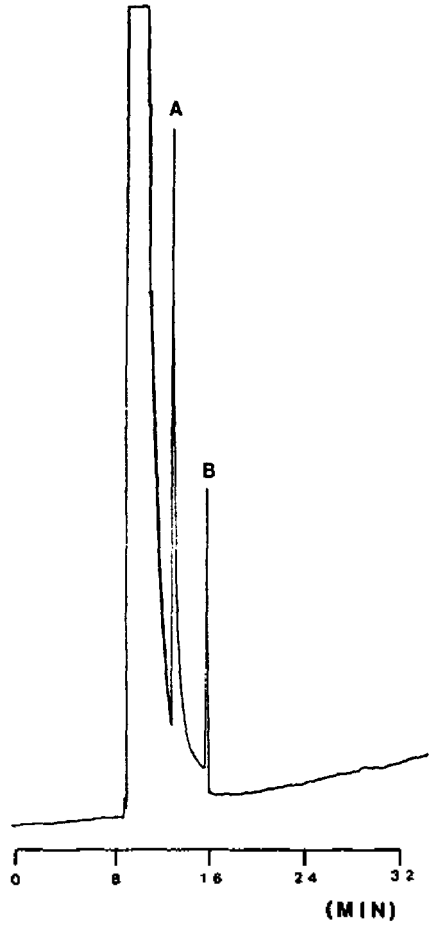

Figure 2. The chromatogram of a mixture of antibacterial agents Peaks A; Zoalene, B; Thiamphenicol.

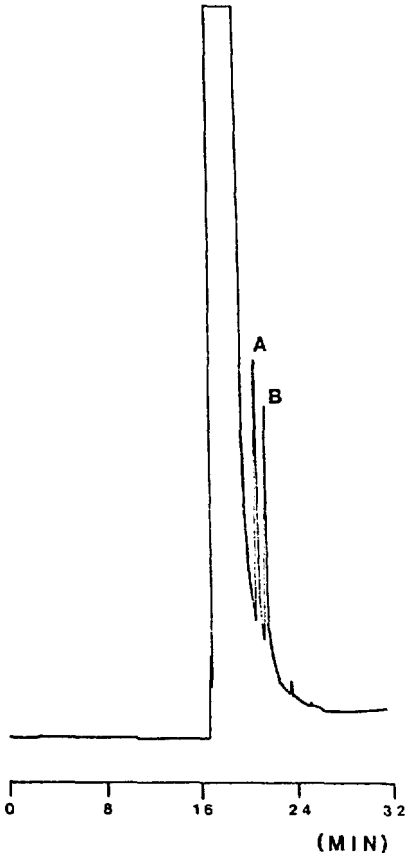

Figure 3. The chromatogram of a mixture of antibacterial agents Peaks A; Nicarbazin, B; Carbadox.

agents) with this new mobile phase was performed. Figure 2 and 3 are chromatograms for mixtures of antibacterial agents obtained using a mixed mobile phase (supercritical CO_2+water). In contrast to the experiment in which only CO_2 was used as mobile phase, excellent separations were obtained. When only CO_2 was used for these samples, unseparated and very broad peaks were observed. The addition of a samll amount of water to supercritical CO_2 improved the peak shapes. The phenomena are in accord with the results reported by Blilie and Greibrokk[10]. Separation conditions are the follows: CO_2 at 110℃, Figure 2 was programmed from 160 atm to 400 atm at 6 atm/min, 100 μm ID×20 m capillary column (SB-biphenyl-30), FID at 300℃ frit restrictor with a initial linear velocity of 5.5 cm/sec. Figure 3 was programmed from 170 atm to 400 atm at 5 atm/min, SB-biphenyl-30 column. The structures of each peaks were shown in Table 1.

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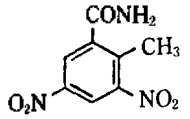
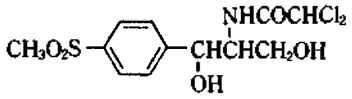
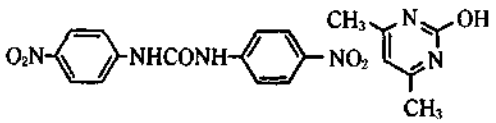
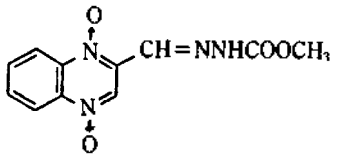
Table 1. The Structures of Peaks in the Chromatograms

Chromatogram	Peaks	Commercial name	Chemical name	Structure
Figure 2	A	Zoalene	2-Methyl-3,5-dinitrobenzamide	
	B	Thiamphenicol	D-threo-2,2-dichloro-N-[β-hydroxy-α-(hydroxymethyl)-*p*-(methylsulfonyl)phenethyl]acetamide	
Figure 3	A	Nicarbazin	N,N'-Bis(4-nitrophenyl)urea, and 4,6-dimethyl-2-(1H)-pyrimidinone (1 : 1)	
	B	Carbadox	2-(2-Quinoxalinylmethylene) hydrazinecarboxylic acid methyl ester N^1, N^4-dioxide	

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Reactivity Study by DSC on the Noncatalyzed Model Curing Reaction of Epoxy Resin with an Unsymmetric Acid Anhydride

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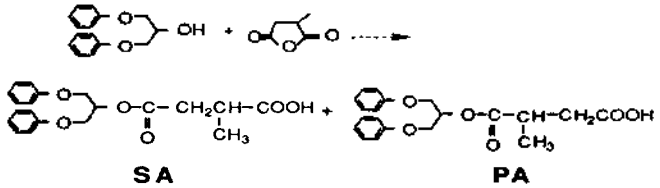
Epoxy resins are certainly an important class of commercical polymers[1] having various applications due to their excellent physical properties revealed when cured. To cure them are used various compounds known as curing agents[2] such as amines, phenols, acids and acid anhydrides. The last generally include phthalic, maleic, succinic, tetrahydro- and hexahydrophthalic and methyl succinic anhydride(MSA) as well as many others. In acid anhydride curing of technical epoxy resins, which are derived mostly from Bisphenol A and epichlorohydrin and hence contain free, secondary hydroxyl groups, it is known[3-8] that the curing reaction is started by the reaction of such a secondary hydroxyl group with the anhydride, to generate a monoester with a free carboxylic acid group, and this acid reacts then with an epoxy group to form another hydroxyl group which, in turn, reacts with another anhydride.

If a symmetric anhydride is reacted with a hydroxyl group, the ester acid formed by the initiation should have the same structure. If MSA is used as an unsymmetric anhydride, the ester acid with two different structures should be formed, of which one is a primary acid and the other is a secondary acid. These two acids could have different reactivity in the next-step reaction with epoxy group.

To make a study on the different reactivity of the two ester acids to be formed in the noncatalyzed initiation reaction of acid anhydride curing of epoxy resins containing hydroxyl groups on their main chains, 3-[(1,3-diphenoxy)-2-propyloxycarbonyl]-butanoic acid(PA) and 2-[(1,3-diphenoxy)-2-propyloxyacetyl]-propanoic acid(SA) were prepared by reaction[9] of MSA with 1,3-diphenoxy-2-propanol at 80℃, 100℃ and 140℃ in 1,3-diphenoxy-propane, as shown in Sheme 1. PA is a primary acid and SA is a secondary acid, both con-



SA PA

Scheme 1.