(m, 2H), 6.00 (*E*-isomer; 63%) and 6.30 (s, 1H, *Z*-isomer; 37%), 6.95-7.40 (m, 5H); IR ($CDCl_3$) 2940 (vs), 1610, 1440, 970; Mass (m/e, %) 91 (73), 115 (100), 128 (32), 134 (44), 155 (39), 246 (MW, 30).

Compound **3h**. ^{1}H NMR ($CDCl_3$) 0.88 (t, J=6.2 Hz, 6H), 1.29 (bs, 10H), 1.97-2.19 (m, 4H), 2.19 (s, 3H), 2.90-2.98 (m, 2H), 5.11-5.16 (*E*-isomer; 55%) and 5.33-5.55 (m, 2H+1H, *Z*-isomer; 45%).

Supercritical Fluid Chromatographic Separations of Pesticides Employing Methanol Modified CO_2 Mobile Phase

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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 fast analysis time for the analysis of non-volatile or thermally labile mixtures. Some applications of SFC to the separation of pesticides are featured in this paper, along with representative chromatograms. Pesticide analysis has received much attention because of the environmental impact of pesticides and fungicides and the need to monitor their levels and those of their metabolites in complex sample matrices such as foods. GC often is the analytical method of choice because of the availability of sensitive, selective detectors (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 or HPLC for the analysis of pesticides. The SFC analysis of some polar pesticides using mass spectrometry as a detector has previously been reported.[1] Thermally labile carbamate pesticides were also separated by capillary SFC.[2]

The ability to analyze moderately polar compounds with supercritical CO_2 is demonstrated in this paper; however, modifiers must be used. One of the most difficult problems with SFC is how polar substrates can be analyzed. Using the classification scheme of eluents by Synder,[3] carbon dioxide shows a polarity similar to that of hexane. The solvent power of the eluents used in SFC may be enhanced by adding a second eluent, the so-called 'modifier' to the basic mobile phase. Separations are often performed by SFC where the composition of the mobile phase is changed during the run or by adding a modifier before the chromatographic run is started. 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 mixture 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, expecially pump can be eluted slowly during the next run. This may affect the time to achieve chemical equilibrium and cause a corrosion at 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

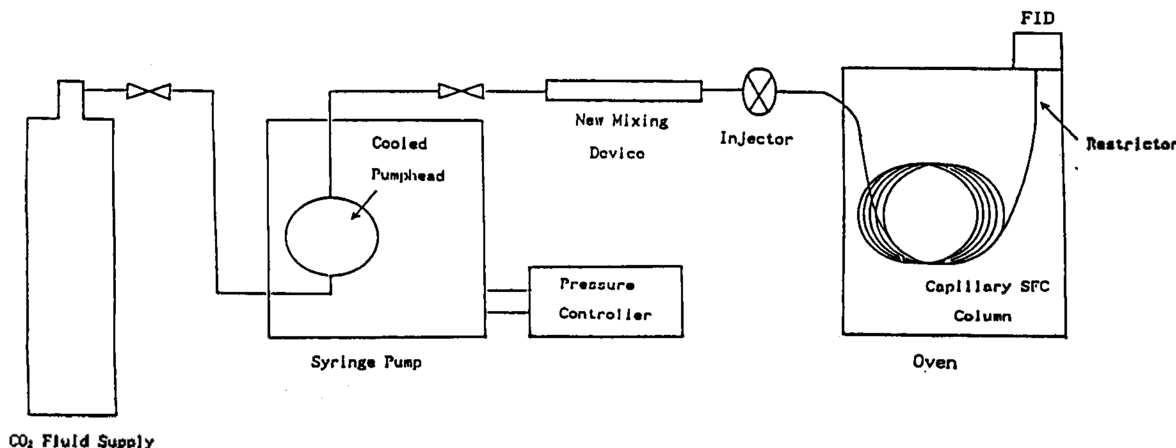

Figure 1. Schematic diagram of the apparatus used for adding a polar modifier to the supercritical fluid mobile phase.

1. Supercritical CO_2 is delivered from the pump to the mixing device (see Figure 2) which is saturated with methanol. The mixing device which has high porous stainless steel filters were used to hold a large amount of methanol. While in the saturator columns,[5-9] methanol is held on the stationary phase by hydrogen bonding, with this device, methanol is held physically inside the small pores of filters. After being saturated with methanol, 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 methanol. When supercritical CO_2 goes through the device, methanol 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, MeOH. 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. The methanol content in the CO_2 mobile phase was 6.8% (V/V) at 23 °C.

An experiment to separate polar samples (pesticides) with this new mobile phase was performed. Figure 3 and 4 are chromatograms for mixtures of pesticides obtained using a mixed mobile phase (supercritical CO_2+methanol). 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, very broad and fused peaks were observed. The addition of a small amount of methanol to supercritical CO_2 improved the sharpness of the peaks. The phenomena are in accord with the results reported by Blilie and Greibrokk.[10] Separation conditions are the follows: CO_2 at 100 °C, Figure 3 was programmed from 150 atm to 400 atm at 6 atm/min, 100 μm ID × 20 m capillary column (SB-biphenyl-30), FID at 300 °C frit restrictor with a initial linear velocity of 5.5 cm/sec. Figure 4 was programmed from 150 atm to 400 atm at 6 atm/min, SB-biphenyl-30 column. The structures of each peaks were

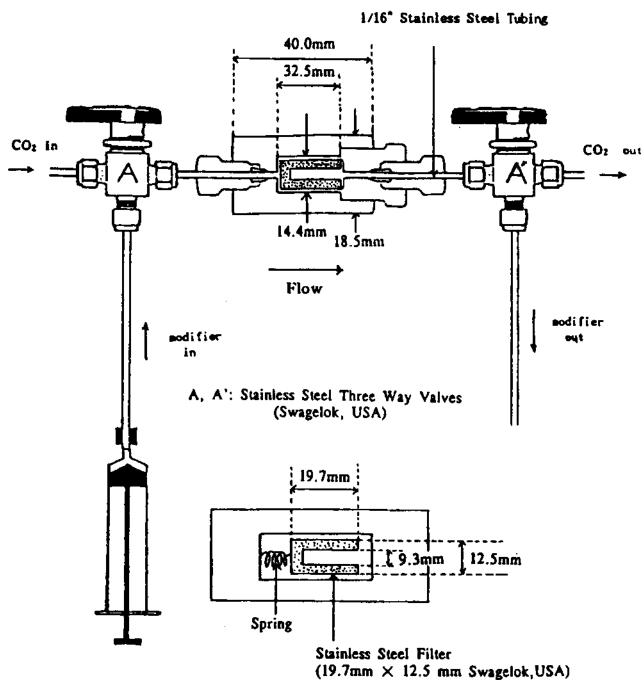

Figure 2. Mixing Device.

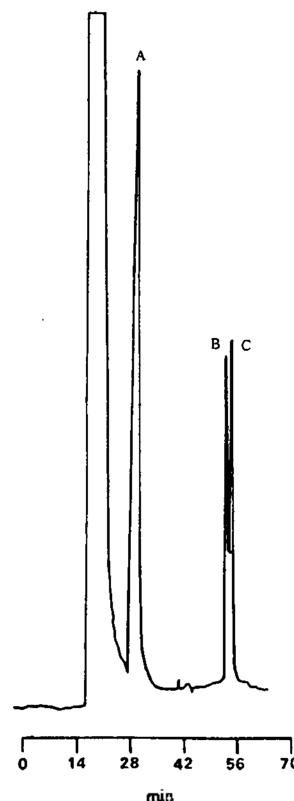

Figure 3. The chromatogram of a mixture of pesticides.

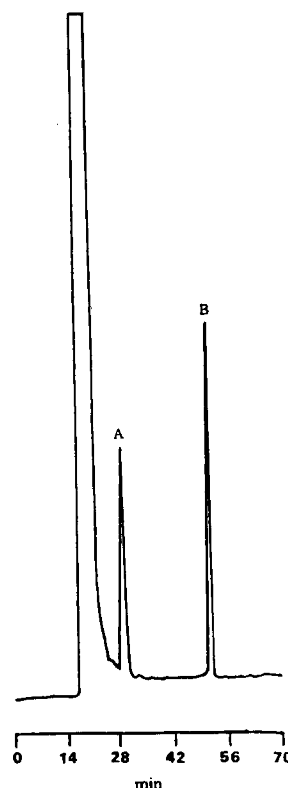

Figure 4. The chromatogram of a mixture of pesticides Peak A; Carbofuran, B; Alanycarb.

Table 1. The Structures of Peak in the Chromatograms

Chromatogram	Peaks	Commercial name	Chemical name	Structure
	A	Methomyl	S-methyl N-(methyl-carbamoyloxy) thioacetimidate	
Figure 3	B	Dimethomorph E	4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl] morpholine	
	C	Dimethomorph Z	4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl] morpholine	
Figure 4	A	Carbofuran	2,3-dihydro-2,2-dimethylbenzofuran -7-yl methylcarbamate	
	B	Alanycarb	Ethyl(Z)-N-benzyl-N-[[methyl(1-methyl thioethylideneamino-oxycarbonyl)amino]thio]-β-alaninate	

shown in Table 1.

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Chemoenzymatic Synthesis of (3R,5R)-3,6-diamino-5-hydroxyhexanoic Acid, the Amino Acid Moiety of (+)-Negamycin

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(+)-Negamycin was isolated from *Streptomyces purpeofuscus* in 1970.[1] It inhibits the growth of Gram-negative and Gram-positive bacteria.[1] (+)-Negamycin shows the misleading of genetic code and the inhibition of protein synthesis.[2] This compound contains two interesting structural features including lysine and hydrazide units with two asymmetric centers which served as an attractive target molecule for the stereoselective synthesis. Modification of the unnatural amino acid moiety of (+)-negamycin showed no antibacterial activity. This result showed that δ-hydroxy-β-amino lysine is a key moiety for the antibiotic activity. This antibiotic has been synthesized in both racemic[3] and optically active forms.[4] Naturally chiral starting materials were used for the total synthesis of (+)-negamycin.

Since the abilities of enzymes as chiral catalysts have been recognized for many years, we planned to control the chiral centers of the amino acid moiety of (+)-negamycin with enzymes. However, the stereochemistry of C(3) of (3R,