ANALYSIS OF POLAR COMPOUNDS BY SUPERCRITICAL FLUID CHROMATOGRAPHY

by

Swati H. Shah

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APPROVED:

Larry T. Taylor, Chairman

D.G.I. Kingston

G.L. Long

H.M. McNair

J.K. Palmer

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Committee Chairman: Larry T. Taylor
Department of Chemistry

(ABSTRACT)

The analysis of polar compounds has been studied by SFC using 100% CO_2 or methanol modified CO_2 as the mobile phase. Both microbore packed and capillary columns are employed to separate the mixtures of steroids and agricultural compounds of various chemical classes such as amides, sulfonamides and ureas. The highly deactivated and crosslinked stationary phases used for both packed and capillary columns afford the elution of polar analytes with 100% CO_2 which in turn makes the on-line FT-IR detection of these analytes feasible. The flow cell interface is employed which provides very low detection limits. Spectra with high signal to noise ratio are obtained for the analytes with real time data acquisition.

Some polar and structurally similar triazine herbicides are separated using a gradient mobile phase and a rapid separation of all the components with complete resolution is achieved. The effect of flow rate, column outlet pressure and the temperature on resolution is also studied for these analytes.

The performance of microbore packed and capillary columns is compared using polar and nonvolatile solutes and the retention and resolution offered by both the columns are also compared. Several van Deemter plots are generated at various constant operating densities and temperatures. Also, the effect of density and temperature on efficiency and resolution is studied for capillary column with split and splitless injection techniques. Several conclusions regarding the favorable operating conditions in each case can be drawn based on the results obtained here.

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1.0 Chapter 1 : Introduction and Literature Review

1.1 Introduction

Supercritical fluid chromatography (SFC) has become increasingly popular as an analytical technique over last decade. The properties of the supercritical fluid (SF) are intermediate between those of gases and liquids and hence the chromatographic properties of the supercritical fluids are also intermediary. SFC bridges the gap between gas chromatography (GC) and high performance liquid chromatography (HPLC) and the current applications illustrate that it is complementary to both these methods. It can be employed to analyze thermally labile, nonvolatile and high molecular weight compounds where GC cannot be applied. It can be coupled with most of the GC and HPLC detectors including sensitive and universal detectors such as the flame ionization detector (FID). Also, the efficiency per unit time is higher in SFC than in HPLC owing to the higher diffusivity and the lower viscosity of the SF mobile phase. These important physical properties of the SF are compared to those of gases and liquids in Table 1.

The lower viscosity of the mobile phase leads to a lower pressure drop across the column which makes the use of longer columns possible yielding high efficiency per column. As the density of the fluid is directly related to its solvating power, varying the density of the fluid, gives

Table 1. Physical Properties of Gas, Supercritical Fluid and Liquid.

Phase	Physical Property of the Phase		
	Density	Diffusion	Viscosity
	($gm\ cm^{-3}$)	($cm^2\ sec^{-1}$)	($gm\ cm^{-1}\ sec^{-1}$)
Gas @1 atm, 21°C	10^{-3}	10^{-1}	10^{-4}
Supercritical Fluid	0.3 - 0.8	10^{-3} - 10^{-4}	10^{-4} - 10^{-3}
Liquid	1	$<10^{-5}$	10^{-2}

a range of solvating powers to attain the desired chromatographic separation. The analyte, as in HPLC, interacts with both the stationary and the mobile phases, therefore, a higher selectivity is achieved as compared to GC. The methods development in SFC is easier than in HPLC. Also, interfacing to information rich detectors such as Mass spectrometer (MS) and Fourier Transform Infrared (FT-IR) spectrometer is easier with SFC than with HPLC. Because of the ease of solvent elimination, the preparative scale work is also easier with SFC than with HPLC.

SFC, however, has its limitations due to the mobile phases available and the present artifacts in instrumentation. Most of the protonated compounds possess very high critical pressure and/or critical temperature. Due to their more accessible critical parameters, n-alkanes, freons and CO_2 are most commonly used as the mobile phases in SFC but the nonpolar nature of these mobile phases limit their applications to nonpolar or moderately polar analytes. Both packed and capillary columns have been successfully used in SFC but more deactivated stationary phases, especially for packed columns, are desired which would minimize or eliminate the adsorption of the polar analytes on the stationary phase which would lead to improved chromatographic performance. Research involving improvements in instrumentation (e.g. injection devices) is also required for SFC to be used as a routine analytical technique.

1.2 Research Objective

The application of SFC to compounds which are difficult to analyze by GC has been of prime interest to the researchers. HPLC offers high selectivity but the analyte needs to possess a chromophore for it to be detectable by a sensitive ultra-violet (UV) detector. The MS and FT-IR interfaces to HPLC are complicated and the most commonly used mobile phases cause interferences for the hyphenated techniques.

The major goals of this research effort were directed towards:

1. Methods development for analysis of moderately polar compounds eluting with 100% CO_2 from packed and capillary columns.

2. On-line FT-IR detection of the analytes for identification purposes.

3. Comparison of on-line SFC/FT-IR analysis with on-line HPLC/FT-IR analysis for the same analytes.

4. Comparison of packed and capillary columns for column efficiency, retention and resolution achieved under the operating experimental parameters.

5. Separation of structurally similar analytes using gradient mobile phases in SFC.

Separation of a mixture of steroids on cyanopropyl bonded stationary phase using HPLC and SFC modes is described in chapter two. An analytical scale column is used with liquid and supercritical mobile phases and the efficiency offered by the column under different modes is compared. Also, the spectra attained in two mobile phase media are compared to examine the subtle differences in spectra, if any. SFC separations on packed and capillary columns are compared at two temperatures using pressure gradient and the efficiency, selectivity and resolution offered by the columns in each case is evaluated. On-line FT-IR detection is performed with both the separation modes.

Further applications of the flow cell interface for FT-IR detection are presented in chapter three. Mixtures of agricultural compounds of different classes are separated on packed or capillary columns with 100% CO_2 mobile phase. Full infrared spectrum of each analyte is achieved in the chromatographic time frame.

Chapter four deals with SFC separation of triazine herbicides using a gradient mobile phase where the flow of methanol is gradually increased in CO_2 and a rapid separation of these structurally related compounds is attained.

An attempt is made to compare the performance of packed and capillary columns in chapter five. The normally used experimental parameters are chosen for each type of column and van Deemter plots at various constant densities and temperatures are generated. Both split and splitless injection devices are used with a capillary column and different results obtained are presented. The effects of density and temperature on retention and resolution of the analytes under study are also studied here.

1.3 Literature Review

The major classes of compounds of interest such as steroids, drugs of abuse, pharmaceutical products, agricultural and environmental compounds, food products etc. are polar and nonvolatile therefore GC cannot be applied to the analysis of these samples without derivatizing the analytes. However, GC has been used for some pesticides,[1-3] derivatized drugs[4-6] and derivatized sugars.[7] HPLC has been a very popular technique for the analysis of polar, nonvolatile and high molecular weight compounds. Both reversed phase and normal phase HPLC methods have been widely used for the analysis of samples such as food products,[8,9] drugs[10-12] and pesticides.[13,14]

In recent years, numerous articles have appeared on the instrumental development in SFC[15] including injection devices,[16-18] detection methods[19,20] and restrictor fabrication.[21-23] Several technological challenges for injection improvement, column preparation, development of novel and stable stationary phases and restrictor reproducibility pose obstacles to the full growth of SFC as a routine analytical technique.[24] The potential of various detectors such as MS, FT-IR, thermionic detector (TID) and inductively coupled plasma (ICP) interfaced to SFC has been demonstrated for specific applications.[25-29] Novel stationary phases have been synthesized and have been reported for the analysis of structurally similar compounds like aromatic hydrocarbons and for the separation of enantiomers.[30-33] The most commonly used mobile

phase, CO_2 is nonpolar and hence exhibits limited solubility to most of the polar compounds. The mobile phase modifiers such as alcohols, dimethylsulfoxide, propylene carbonate, methylene chloride and tetrahydrofuran have been investigated with packed columns and the solvatochromic polarity of different modifiers was studied.[34-36] Herbicides of moderate polarity and molecular weight range were analyzed employing packed and capillary columns with and without using modifiers respectively.[37] Capillary columns have a highly deactivated surface which minimizes or completely eliminates the solute adsorption on the stationary phase and hence do not usually require the mobile phase modifiers. Packed columns, on the other hand, do have some residual silanol sites on the stationary phase and therefore need mobile phase modifiers for elution of some polar compounds. The use of a mobile phase of high polarity such as ammonia has also been demonstrated for polycyclic aromatics[38] but the toxicity and the reactive nature of ammonia limit its use as a mobile phase in SFC.

Various classes of polar compounds such as steroids, drugs of abuse, pharmaceuticals, pesticides etc. have been analyzed by both packed and capillary columns. Rapid analysis of several drugs of abuse was carried out on a 2.1 mm i.d. packed column using methanol modified CO_2 as the mobile phase. Two detectors, UV and MS, were used in series and the total ion chromatograms obtained were used for the positive identification of the compounds.[39] Derivatives of oligo and polysaccharides containing upto 18 glucose units were resolved on a capillary column and a degree of polymerization (DP) was assigned to the sugar derivatives. A silylated polyglucose with a DP = 18 and molecular weight of 7000 was eluted with a 100% CO_2 mobile phase.[40] Several derivatives of monosaccharides were analyzed on packed column with 100% CO_2 and were detected by FT-IR.[41] A mixture of thermally labile azo dyes was separated on a capillary column a using an FID.[42] A highly deactivated 25% biphenyl capillary column was used for the elution of a derivatized polar penicillin antibiotic.[43]

Similar columns were used for analysis of fatty acids and triglycerides[44,45] and lipids.[46] Several mixtures of free fatty acids were separated based on their carbon number on an analytical scale column using on-line FT-IR detection.[47] Capillary column SFC has been reported for the analysis of surfactants,[20] polyols[48] and alkaloids.[49] A mixture of an anti-malarial drug and related com-

pounds was separated on a packed column using 0.3% formic acid in CO_2 as the mobile phase.[50] Mixtures of steroids, barbiturates and tetrahydrocannabiol with its metabolites were analyzed on capillary columns.[51] Both packed and capillary columns have been used for separation of several ecdysteroids where a modifier was needed to elute the steroids from the analytical scale packed column.[52] Two mixtures of steroids and opiates were analyzed on a capillary column under isobaric isoconfertic conditions using SFC and GC. The ion mobility spectra were obtained in each case and comparable results were achieved by both these techniques.[53] An analytical scale column required 25% methanol modified CO_2 for the elution of several ecdysteroids and the mass spectrum of each analyte was obtained using the thermospray interface.[54] SFC/MS and SFC/MS/MS analysis of steroids was performed by Hurst[55] and both the chemical ionization (CI) and electron impact (EI) spectra were generated to gain structural information of the analytes. The analysis of several lipids and sterols has been reported by several workers using a N_2O mobile phase under isoconfertic conditions[42] and using MS detection with CO_2 mobile phase.[56,57] A packed capillary column with alcohol modified n-hexane mobile phase was used to separate mixtures of both fat soluble and water soluble vitamins and MS was used for their detection.[58]

The widespread use of pesticides in agriculture and horticulture involves the direct application of these compounds to the environment. This class of compounds is therefore, of both agricultural and environmental interest. Most of the pesticides are polar and thermally labile, so GC can not be employed for their direct analysis and SFC has been widely used for their analysis. A mixture of five pesticides and herbicides was separated on a capillary column with a multi-channel UV detector and a full UV spectrum of each solute was achieved.[59] Several rotendicides were separated on an analytical scale column with 15% methanol modified CO_2 . The analysis was completed in about five minutes and both CI and EI mass spectra were achieved for the solutes.[60] Nine pesticides and metabolites were separated on a capillary column in about 25 minutes. These temperature sensitive carbamates with no chromophores were detected by FID and a high sensitivity was achieved.[61] MS has been successfully applied to the SFC analysis of the pesticides since it provides very low detection limits and also leads to

positive identification of the analytes. Mixtures of carbamates and organophosphorous pesticides have been analyzed by both packed and capillary columns and the mass spectra are achieved with detection limits in the range of nanogram (ng).[62-68] Flame Ionization Detector (FID) is the most commonly used detector in SFC with both microbore and capillary columns as it gives a universal response to all the organic compounds. Several reports have appeared where FID was used for the detection of samples such as ergot alkaloids[69] and pesticides.[70-71] Various other classes of compounds such as fatty acids,[72] pharmaceuticals and fat soluble vitamins[73] and pyrethrins[74] have been analyzed using a 100% CO_2 mobile phase. Metalloporphyrins have been separated on an analytical scale packed column[75] and on capillary column[76] using alcohol modified CO_2 as the mobile phase. Other metal containing compounds have been separated on various different columns of 4.6 mm i.d. using modifiers.[77]

It is desirable to couple SFC to an information rich detector such as MS or FT-IR which would provide the structural information about the sample. Both these detectors have been successfully interfaced to SFC. However, the interfacing of FT-IR is constrained due to the absorbance of most of the mobile phases used in mid-IR region. Also, FT-IR is relatively less sensitive compared to the other commonly used detectors. There are two general approaches, namely, the solvent elimination and the flow cell interface for coupling FT-IR to SFC. Each of these methods offer certain advantages over the other method. The solvent elimination technique, which provides transmission or reflectance spectra, exhibits no interferences from the mobile phase. A hard copy of the separation is achieved in this case and a large number of scans can be collected, if needed, to achieve higher sensitivity. Detection limits in the nanogram range are quite common with this interface. The flow cell interface, on the other hand, is easier and simpler to work with and offers a technique where data is collected in real time and the transmission spectrum of the analyte can be stored versus time. Also, the volatile samples can be analyzed using this method while they may be lost during the solvent evaporation in the solvent elimination method. Numerous applications of both types of interfaces have appeared in literature for a variety of compounds.[78-93] Two articles by Jinno[94] and by Taylor[95] review and compare both types of interfaces.

Supercritical fluid CO_2 absorbs strongly in two regions between 3475-3850 cm^{-1} and 2040-2575 cm^{-1} in the mid-IR region. These two regions therefore, are not available while using the on-line flow cell interface. Also, the IR absorbance of CO_2 increases with an increase in pressure. Pressure programming is very common in SFC so the use of CO_2 causes a baseline drift with on-line FT-IR detection. This artifact is removed by using Gram-Schmidt orthogonalization with an augmented basis vector set to compensate this baseline drift with pressure programming.[96] The flow cell interface commercially available from Nicolet Instruments (Madison, WI) is reported to provide detection limits in nanogram range for caffeine[97] and for methyl palmitate[98] when capillary columns were used in each case with 100% CO_2 mobile phase. Griffiths and co-workers[99] recently reported a minimum identification limit of 1.4 ng for indole-3- acetic acid with solvent elimination which involves the condensation of the eluants in a small area onto a moving substrate. The FT-IR interface thus provides detection limits comparable to the MS detection in addition to the full IR spectrum of the analyte. This aids in positive identification of the analytes along with the structural information.

The literature available on SFC presents the applications of this method to the analysis of nonpolar and moderately polar compounds.An attempt is made here to develop the methods for analysis of polar and nonvolatile analytes on both packed and capillary columns using 100% CO_2 mobile phase. A flow cell approach is employed to perform on-line FT-IR detection and complete infrared spectra of the analytes are attained in real time. When use of modifier is required to achieve chromatographic separation, a gradient mobile phase is employed which results in shorter analysis times and sharper peaks. Finally, data are presented to study the controversial topic of the performance of packed vs capillary columns in SFC. Since SFC is mainly applied to analyze polar and nonvolatile compounds, these experiments are carried out with polar and nonvolatile probe solutes.

2.0 Chapter 2 : NP-HPLC and SFC Separation of Steroids with FT-IR Detection

2.1 Introduction

Adrenocorticosterones are an important class of steroidal hormones which are synthesized by the cortex of adrenal glands. They influence a large number of physiological organs and in turn the metabolism of the body. The analysis of these steroids in biological fluids and in pharmaceutical preparations is very important for both diagnostic purposes and for quality control.

For past few years, hyphenated techniques have become increasingly popular. Various spectroscopic methods such as mass spectrometry (MS), inductively coupled plasma (ICP), nuclear magnetic resonance (NMR) and Fourier Transform infrared (FT-IR) have been coupled with varying degrees of success to different chromatographic techniques such as GC and HPLC. Supercritical fluid chromatography (SFC) has emerged as a separation method with great potential as its complements both GC and HPLC. HPLC has been the popular method for analysis

of steroids employing both normal phase and reversed phase modes since GC cannot be applied directly to these nonvolatile and thermally labile analytes.[100–104]

SFC has also been applied to the steroid analysis.[42,51,98] A variable or fixed wavelength ultraviolet (UV) detector is used most commonly for HPLC while flame ionization detector (FID) is the most popular detector used with SFC. Although both these detectors provide good sensitivity and can be considered to give fairly universal response, both these are essentially nonspecific detectors. The information rich detectors such as MS and FT-IR are desired for positive identification of the compounds. While interest in SFC/MS is justifiably keen, the problem of mass transfer, insufficient ionization and variable conditions during density programming need to be further addressed. In like manner, FT-IR detection has suffered from similar types of problems albeit with considerably less initial financial investment.

Both HPLC and SFC have been successfully interfaced with FT-IR for analysis of a wide range of compounds, both off-line and on-line. Similar approaches to interfacing have been employed for both chromatographic techniques because the most commonly used mobile phases in both the modes are not completely transparent in mid-IR region, thereby offering reduced IR transparency. The off-line detection requires elimination of the solvents from the chromatographic effluent before it is subjected to spectroscopic detection.[27,79,90] The on-line detection involves a flow cell interface enabling the real time data acquisition.[47,84,105] Merits and demerits of both these approaches have been discussed in several review articles[94,95,108] which cover different aspects of these two techniques.

Several flow cell designs have been reported for SFC/ FT-IR work. High pressure CaF_2[78] and ZnS[83] flow cells have been developed for capillary columns. Two lightpipe flow cells for interfacing with packed columns have been reported.[80] One of these cells (5 mm, 4 μL) had ZnSe windows and was gold coated to give maximum IR reflectivity; while the other flow cell (10 mm, 8 μL) had CaF_2 windows and was constructed to give a less tortuous path than the 5 mm pathlength cell for the column effluent to prevent any loss of chromatographic resolution in the flow cell. Injected minimum detectable quantities in these studies were several hundred nanograms for highly absorbing analytes. In addition the chromatography here was limited in

that constant supercritical CO_2 conditions had to be employed since the infrared absorption spectrum of CO_2 changes with density.

Recently, Wieboldt and Hanna[96] have presented a method for removing undesirable features in chromatograms generated from FT-IR data by using Gram-Schmidt orthogonalization. The procedure involves adding vectors containing the undesired information to the basis set and recalculating the chromatogram with the augmented basis set. The technique allows density programming with minimal baseline drift and detection of minor chromatographic peaks with no alteration of the original spectral data. Employing a prototype of high pressure flow cell (5 mm, 1.4 μL), the on-line detection of four chromatographed (capillary column) pesticides has been demonstrated.[29]

In this study we employ the flow cell approach as it allows real time data acquisition and is mechanistically simpler than the solvent elimination approach. An in-house manufactured flow cell for HPLC and the prototype of a commercially available SFC/FT-IR interface were used to study the separation and identification of a mixture of steroids. Three columns of entirely different dimensions, but of similar stationary phase were used to compare the separation and resolution of the steroid mixture. An analytical scale column packed with conventional cyanopropyl bonded silica and a microbore scale column packed with a crosslinked cyanopropyl bonded phase silica *DELTABOND™* were compared using the same mobile phase in HPLC mode. Similarly, the packed microbore column previously described and a capillary column coated with 25% cyano-25% phenyl-50% methylpolysiloxane were used to develop the steroid separation with 100% supercritical CO_2. The employment of the same microbore column for the steroid separation using both a liquid and a supercritical fluid mobile phases should provide a valuable comparison of column efficiency and the resolving power between the two chromatographic modes. Moreover, the use of a small bore packed column and an open tubular capillary column both with essentially the same stationary and mobile phases should give yet another interesting comparison.

2.2 *Experimental*

2.2.1 Normal Phase HPLC

A Perkin-Elmer (Norwalk, CT) model Series 10 HPLC equipped with a fixed wavelength (254 nm) UV detector with a 10 μL flow cell was used for development of separations on the analytical scale and microbore columns. For these experiments, a 0.5 μL sample was injected onto the column using a Rheodyne (Cotati, CA) injection valve. A 250 x 4.6 mm Spherisorb (Alltech, Deerfield, IL) cyanopropyl column (5 μm particle size) and a *DELTABOND™* cyanopropyl bonded phase silica (5 μm particles) microbore column 100 x 1.0 mm i.d. (Keystone Scientific, Bellefonte, PA) were used to develop the steroid separation. The same mobile phase of 0.2% CD_3OD in $CDCl_3$ was used for both the columns. Both $CDCl_3$ and CD_3OD which had 99.8 atom % D and 99.5 atom % D, respectively, were purchased from Aldrich Chemical Co.(Milwaukee, WI).

2.2.2 SFC

The same microbore column described earlier for the HPLC experiment was used to develop the separation of the steroid mixture using 100% CO_2 (Scott Specialty Gases, Plumsteadville, PA) as the mobile phase. A Suprex (Pittsburgh, PA) model 200A supercritical fluid chromatograph attached with an auxiliary flame ionization detector was used for all separations. A tapered restrictor manufactured in-house from a 50 μm i.d.capillary tubing was employed for both types of columns. A 10 m long SB-Cyanopropyl-25 bonded fused silica capillary column (Lee Scientific Co., Salt Lake City, UT) of 100 μm i.d. and 0.25 μm film thickness was

used. Different pressure ramps were used to develop the separations on the packed and capillary columns at an oven temperature of 60 °C and FID temperature of 375 °C.

The steroid separation was also achieved at 100 °C. For packed column, similar pressure ramps were employed at both the temperature; while for capillary column, different pressure gradients were used at each temperature to achieve a comparable separation. A 0.1 μL injection loop (Valco Instruments, Houston, TX) was used to introduce the sample onto the column. A direct injection was employed for the packed column, while a split (1:1) injection was employed for the capillary column.

The detection limit studies for the SFC/FT-IR flow cell interface were performed on the same cyanopropyl capillary column at 60 °C . The pressure in this case was programmed from 100 atm to 175 atm in 15 minutes and then to 400 atm in next 5 minutes. Caffeine was the substance of choice due to its high absorbance in the IR region. An injection loop of 0.1 μL and a split ratio of 1:1 was employed for this experiment.

2.2.3 FT-IR

All infrared spectra were obtained in real time at 8 cm^{-1} resolution by collecting 1 file/sec. Four scans were collected per sample file and eight scans were collected per background file. The interferograms in each file were ratioed against a mobile phase background files. Standard GC/FT-IR and SFC/FT-IR software (Nicolet Instruments, Madison, WI) were used to collect and manipulate the spectrum of each component by HPLC and SFC separations, respectively. A narrow band (MCT-A) detector was used for both chromatographic modes. For HPLC an in-house manufactured flow cell of 0.45 mm effective pathlength and 0.33 μL cell volume with CaF_2 windows was used. A 620 beam condenser (Spectra Tech, Stamford, CT) was used to focus (6X) the beam onto the flow cell. For SFC, a prototype of an SFC/FT-IR interface (Nicolet Instruments, Madison, WI) was used which employs a highly polished stainless steel flow cell of 1.4 μL cell volume, 5 mm pathlength and 0.6 mm i.d.. The condensed (6:1) infrared beam was

passed through the flow cell onto the MCT-A detector. The flow cell temperature was maintained at 33 °C.

2.3 Results and discussion

The major objective of this research effort was to demonstrate a new on-line SFC/FT-IR interface and to study the separation of the same analytes on columns of different dimensions but containing similar stationary phase by both liquid and supercritical fluid mobile phases.

The micro cell volume and negligible connecting tube volume prevented the loss of chromatographic resolution and band broadening. Zero dead volume butt connectors were used to connect both the column to the inlet transfer line and the post-detector restrictor to the cell outlet. Thin capillaries of 25 μm i.d. were used for all interface connections. The column effluent, typically passed to the FT-IR flow cell from the SFC oven, then back to the oven, and finally, to an auxiliary FID detector.

A study to determine the injected minimum detectable quantity (IMDQ) was performed with the current interface. Strongly absorbing caffeine with a characteristic carbonyl stretch was the analyte chosen for this study. Caffeine eluted with 100% CO_2 from the SB-cyanopropyl-25 column in approximately 17.5 minutes with a k' of 0.97. A wide range of quantities of caffeine (125 ng, 50 ng, 12.5 ng, 5 ng and 2.5 ng) was injected and the absorbance of the intense carbonyl peak was correlated with the amount injected.

A Gram-Schmidt Reconstruction (GSR) was obtained for each injection from which twelve files were coadded across the caffeine peak to acquire the IR spectrum of greatest signal to noise ratio. A representative GSR of 12.5 ng of injected caffeine is shown in Figure 1. The lowest detection limit previously reported for an SFC/FT-IR interface was by the solvent elimination technique[79] where a detection limit (approximately 60 scans) of about 11 ng for

acenaphthaquinone was achieved. A detection limit of 270 ng was reported by Taylor et al.[84] for acetophenone using a less than optimized flow cell interface.

A plot of carbonyl peak absorbance vs the injected amount shown in Figure 2 indicates linearity over several orders of magnitude with a correlation factor of 0.998. A signal to noise (peak to peak + 50 cm^{-1} from the reference peak) of greater than three was achieved for as low as 2.5 ng injected as illustrated in Figure 3. The low IMDQ achieved here can be attributed to various factors, such as improved optics, smaller flow cell volume, better match of detector area and illuminated area, sharper chromatographic peak and better baseline correlation concerning data manipulation.

This study of comparison of the HPLC and SFC separations was divided into four experiments, each with a different column and/or mobile phase. A steroid mixture containing five components served as the model mixture. For the HPLC/FT-IR interface an in-house manufactured flow cell was used while for SFC/FT-IR interface, the flow cell employed for IMDQ study was used. The cell dimensions, the cell volumes, the illuminated areas of the cells and other optics were different for both these interfaces, therefore, a direct quantitative comparison of sensitivity can not be made here. The main objective of this research work was to qualitatively compare the separation of a mixture of steroids using a cyanopropyl stationary phase with both a liquid and a supercritical fluid mobile phase. In addition, a comparison of packed and capillary columns for this model mixture was possible under different operating conditions. On-line FT-IR detection was performed in each case to show the potential of this technique for positive identification of the analyte and to determine if subtle spectral differences exist between spectra obtained in the liquid phase vs supercritical fluid phase.

In the first HPLC experiment, an analytical scale column was used. A fixed wavelength UV detector (254 nm) was employed to develop the separation as shown in Figure 4. An isocratic separation of five steroids was achieved using 0.2% CD_3OD in $CDCl_3$ as the mobile phase. The use of 100% $CDCl_3$ as a mobile phase did not yield a satisfactory separation, consequently, a low percentage of modifier (CD_3OD) was added to the mobile phase. Deuterated solvents were preferred as mobile phases over their protonated counterparts since they offer

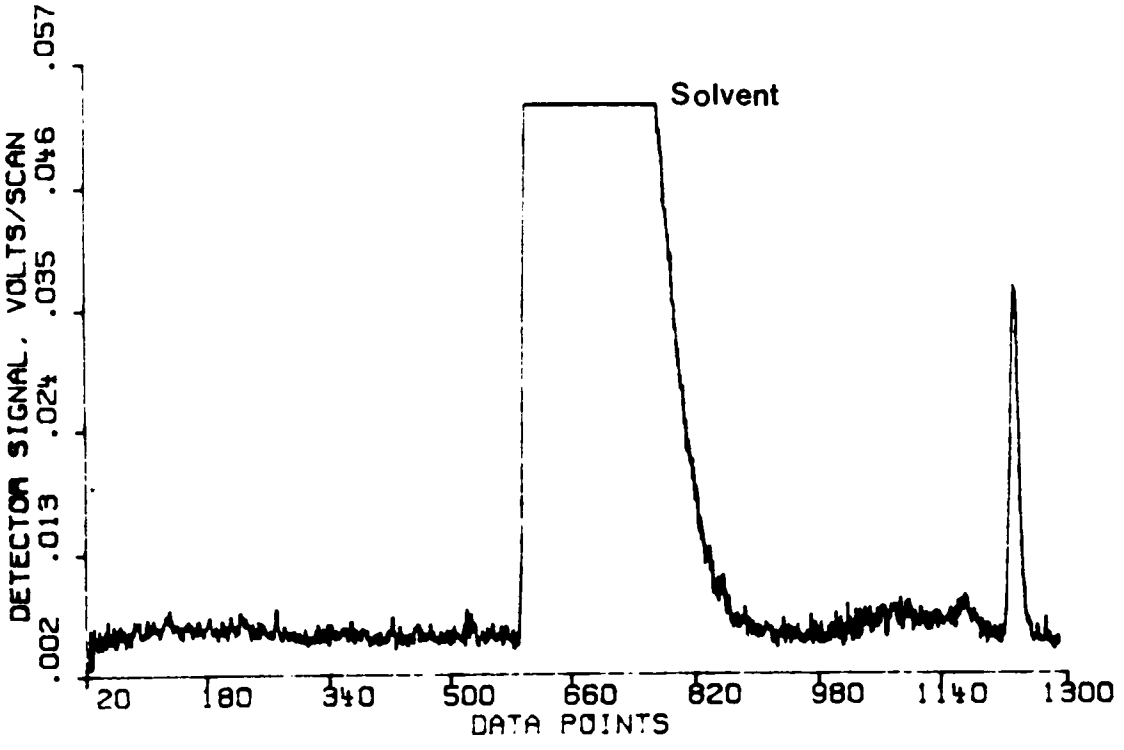


Figure 1. **GSR of caffeine:** Separation performed on SB-cyanopropyl-25 capillary column (10 m x 100 μm, i.d.) at 60 °C with 100% CO_2 using linear pressure programming (100-175 atm/15 min., 175-400 atm/5 min.). Solvent = Methylene Chloride.

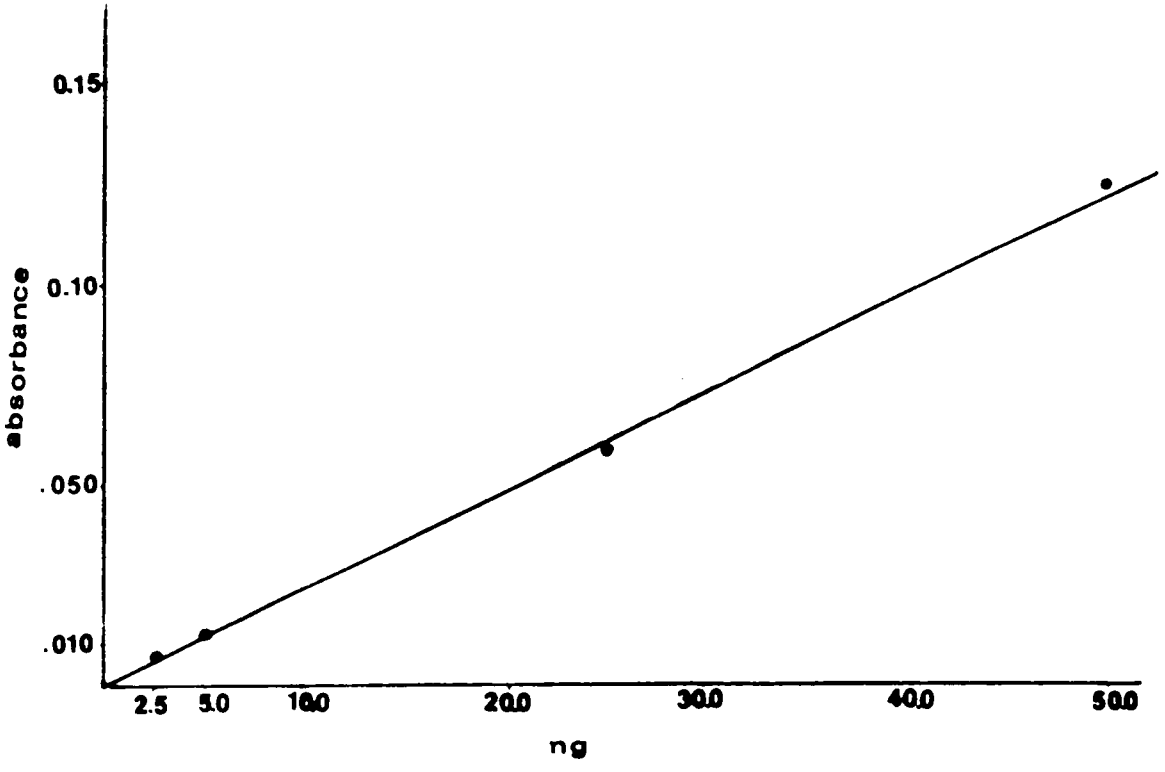


Figure 2. Plot of amount injected vs absorbance of caffeine carbonyl peak.

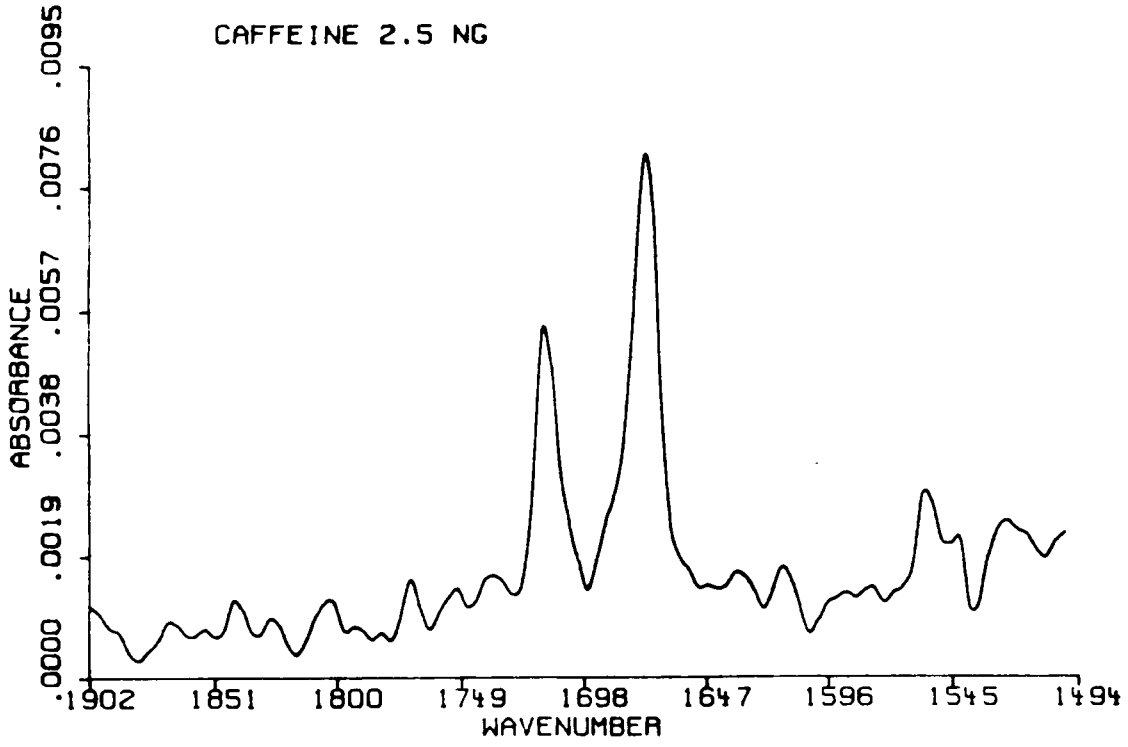


Figure 3. On-line SFC/FT-IR spectrum of caffeine (2.5 ng injected): 12 coadded files, 4 scans/file, 1 file/sec.

spectral advantages for an HPLC/FT-IR interface.[107] Specifically, IR absorption for deuterated solvents is shifted to lower wavenumbers as compared to their protonated counterparts thereby allowing FT-IR detection of the analytes in a region which otherwise would have been blocked by solvent absorption.

The same separation as previously described was performed with FT-IR detection and the GSR is shown in Figure 5. The column eluent directly passes to the flow cell from the column and real time data are acquired during the experiment. The use of the modifier does not seriously reduce the solvent IR window any further than that of pure $CDCl_3$. All five steroids used for this study exhibited a unique carbonyl stretching vibrational mode.

The steroids eluted in the order: progesterone (P), 17-hydroxy- progesterone (17-OHP), testosterone (T), corticosterone (C) and 11- deoxycortisol (11-DC). The order of elution can be related to the number of hydroxyl groups present on the steroid molecule. Progesterone with two carbonyl but no hydroxyl groups elutes first, followed by 17-OHP and testosterone with one hydroxyl group each. A tertiary hydroxyl group is characteristic of 17-OHP and no doubt accounts for its earlier elution than testosterone which has a less hindered secondary hydroxyl group. The last two steroids (C and 11-DC) have two carbonyl and two hydroxyl groups, one of which is a primary functionality in each case. The other hydroxyl group is secondary in the case of corticosterone and tertiary in the case of 11-deoxycortisol. All five components were completely resolved in in about 10 minutes by normal phase HPLC.

The next step was to carry out the same separation on a microbore column packed with a cyanopropyl bonded silica phase and the identical mobile phase. The separation was attempted using a range of constant flow rates (50-100 μL /min.), but no separation of these components was achieved with this mobile phase, even though all individual components eluted from the column when injected separately. The highly deactivated nature of the crosslinked *DELTABOND™* cyanopropyl stationary phase utilized here compared to the conventional cyanopropyl phase employed with the analytical scale column apparently rendered the column too inactive to offer selective retention to the components of the mixture with our chosen HPLC mobile phase. Spectroscopic resolution was expected at the leading and the tailing edges of

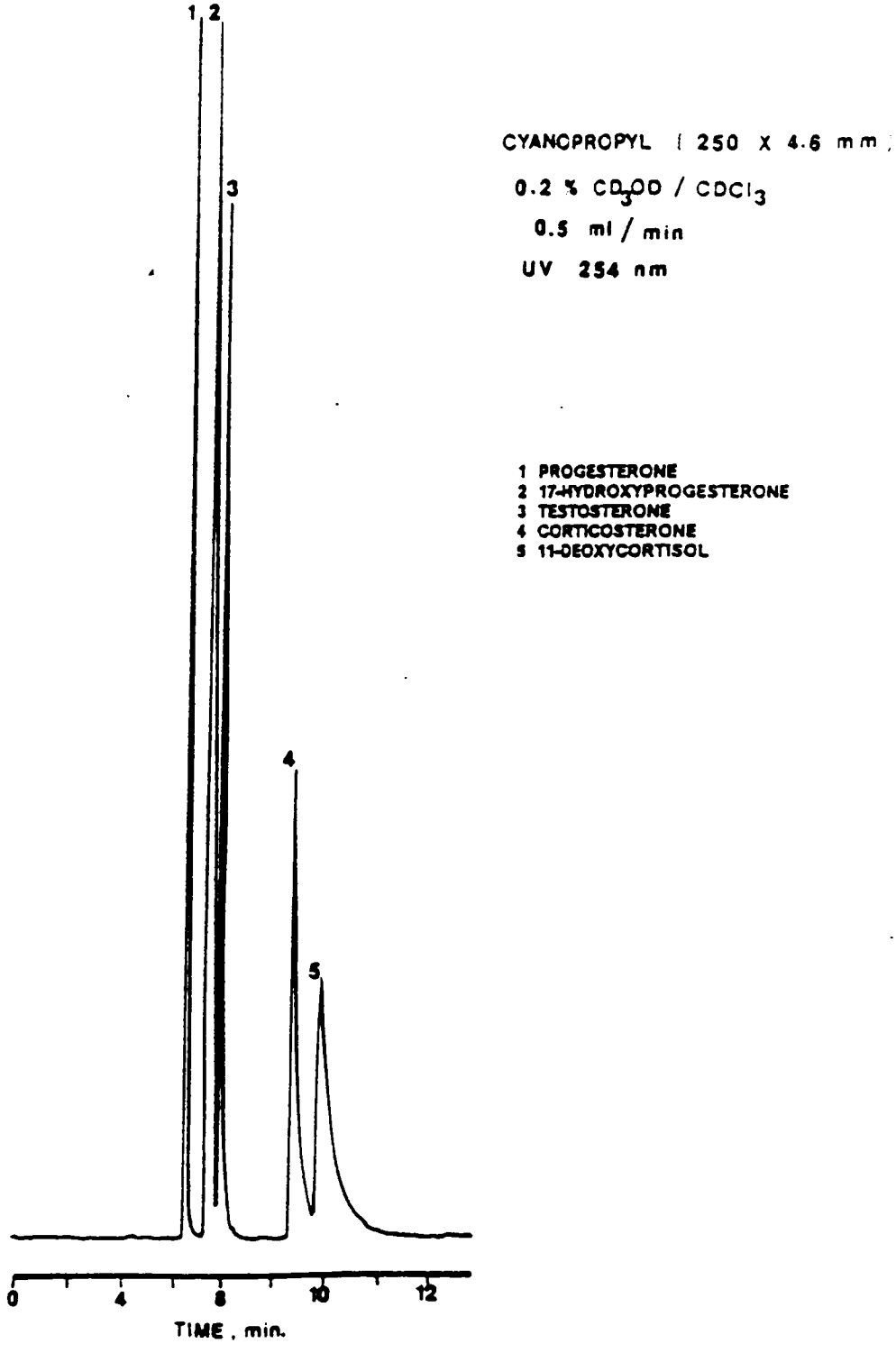


Figure 4. Separation of steroid mixture with UV detection: Conditions: Column: Alltech cyanopropyl bonded 250 x 4.6 mm i.d. silica (5 μm particle size); Mobile Phase: 0.2% CD_3OD in $CDCl_3$, Flow Rate: 0.5 mL/min.

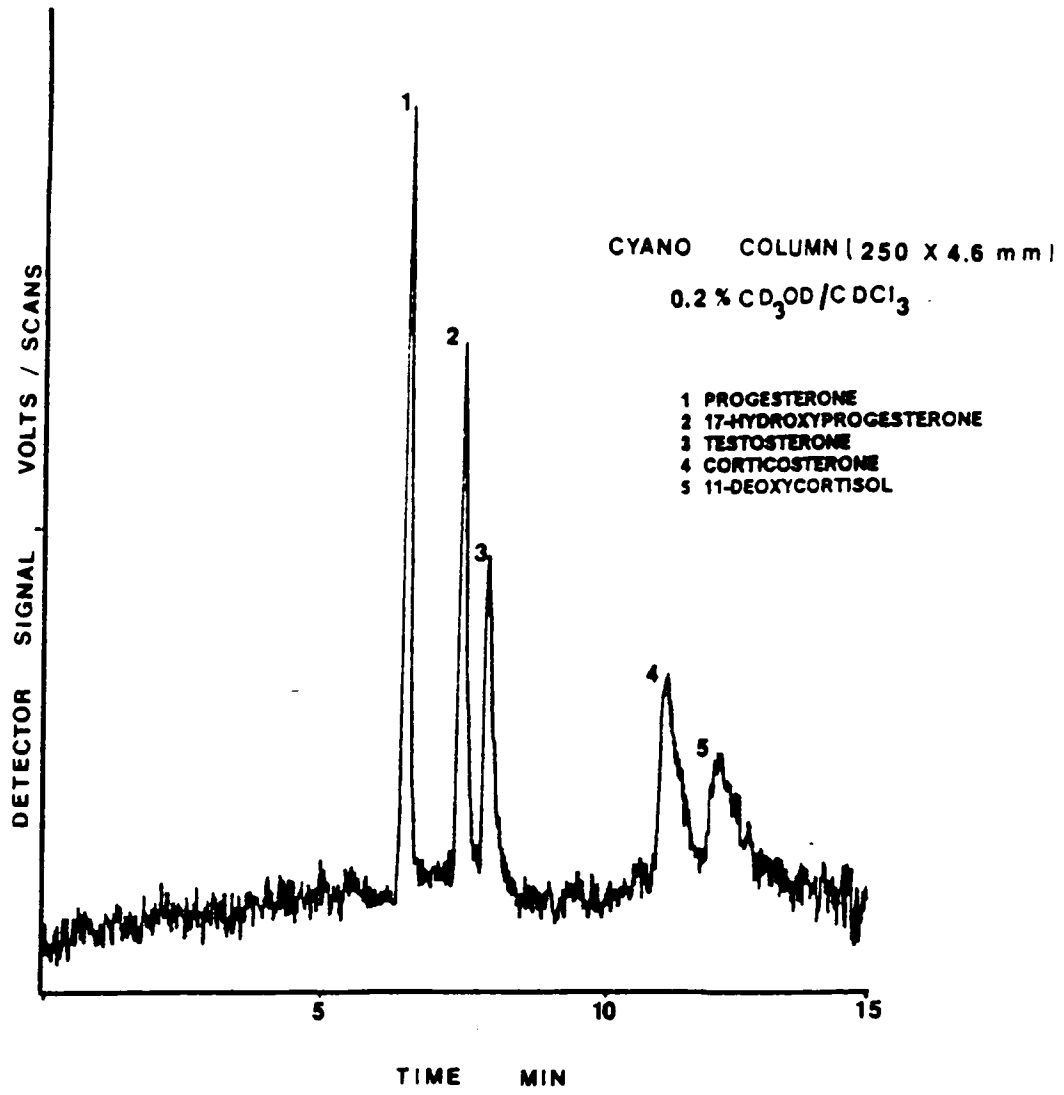


Figure 5. GSR of the steroid mixture after HPLC separation.

the single broad IR peak, but due to the apparent coelution of the five components, very little difference in the spectra was observed.

The *DELTABOND™* microbore column was then used with supercritical fluid CO_2 as a mobile phase. This solvent offers differential solubilities to the steroids and hence pressure programming yields their separation with 100% CO_2. Baseline resolution for progesterone, testosterone and 17-hydroxyprogesterone was achieved while the steroids containing two hydroxyl groups were not baseline resolved. An auxiliary FID was used in series with the FT-IR detector. The chromatograms obtained using two different detectors compared well as seen in Figure 6.

Baseline resolution was achieved for all five components of the mixture when the same separation was tried on a much longer cyanopropyl capillary column with 100% CO_2. The analysis time was about 30 minutes in this case which was about twice the analysis time of the packed column separation. The order of elution was the same as in the case of the packed column separation. Chromatograms obtained with sequential FT-IR and FID detection were very similar as shown in Figure 7. In spite of the extra transfer line connecting the two detectors, peak widths were comparable for the two chromatograms.

As in the case of HPLC, progesterone eluted first followed by the two steroids with one hydroxyl group, followed by the two steroids with two hydroxyl groups each. The order of elution for the monohydroxy steroids and for dihydroxy steroids was reversed in SFC. Testosterone eluted earlier than 17-OHP and 11-DC eluted prior to corticosterone. The reversal of the elution order among the steroids with one and two hydroxyl groups could be attributed to their differential solubilities in supercritical CO_2 as opposed to different degrees of interaction by these steroids with the stationary phase in HPLC.[108] Stahl and Quirin showed that the threshold pressure of supercritical CO_2 necessary to effect extraction increased as the number of hydroxyl and carbonyl groups on the tetracyclic steroid structure increased.

The IR spectra of each steroid in both the liquid and supercritical phases match very well. Due to complete absorption by CO_2, two regions from 3850-3475 cm^{-1} and 2575-2050 cm^{-1} were obscured employing the flow cell method. The spectrum of each separated steroid in both

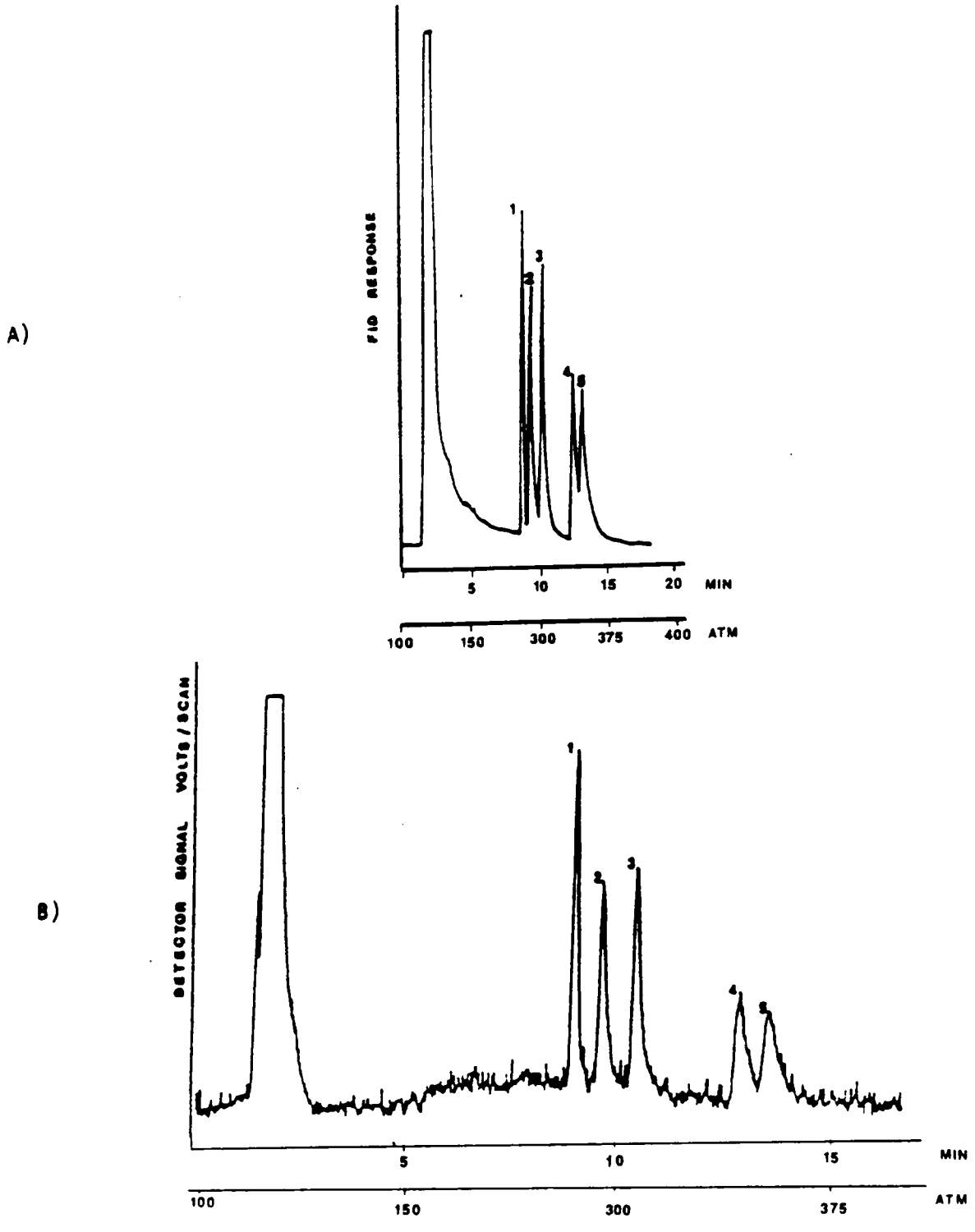


Figure 6. **SFC separation of steroid mixture on a packed column:** (a) FID detection (b) FT-IR detection.

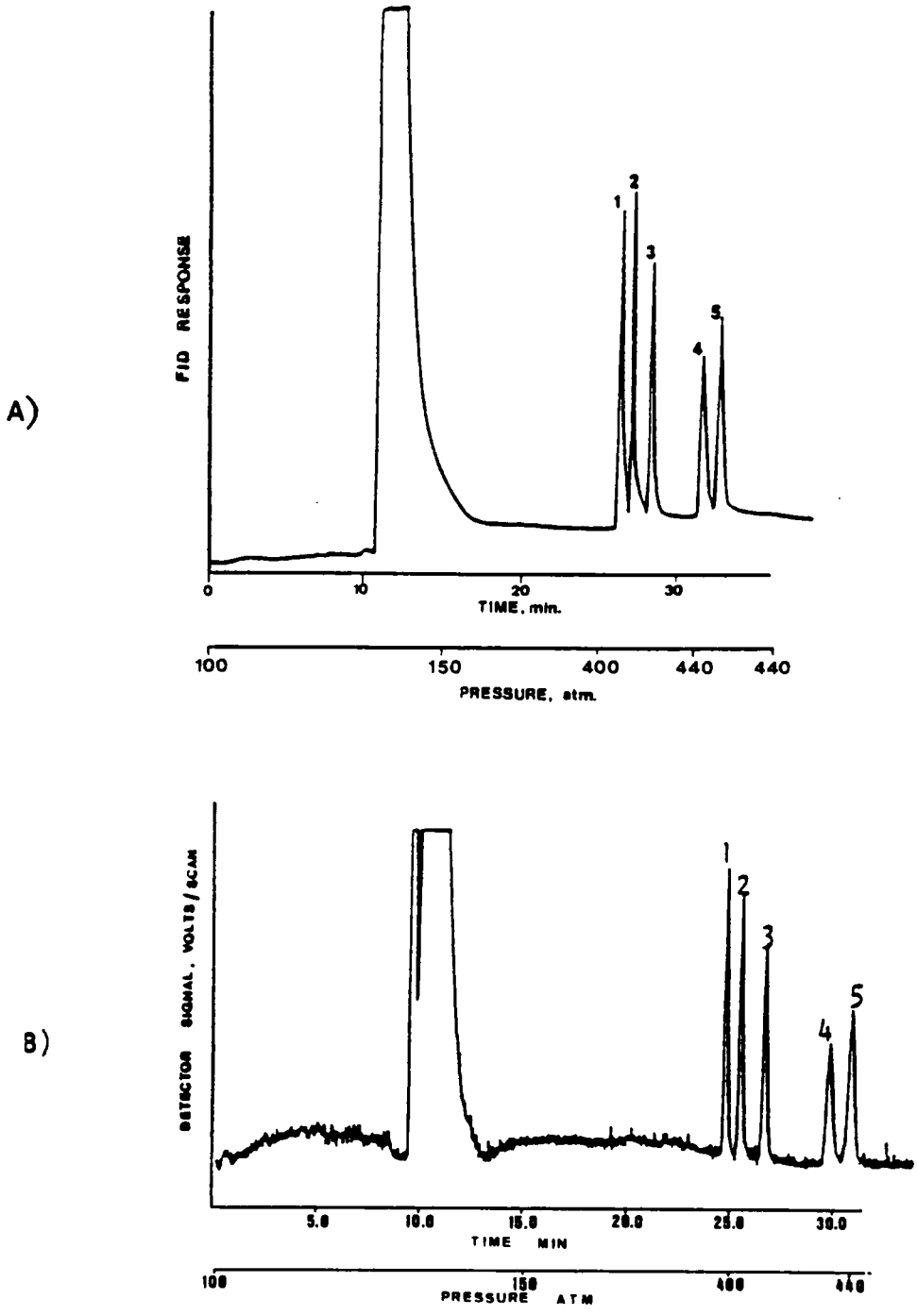


Figure 7. **SFC separation of steroid mixture on a capillary column:** (a) FID detection (b) FT-IR detection.

phases is shown in Figure 8- Figure 12. A high signal to noise ratio is achieved in both the phases. The carbonyl stretching frequencies for all the steroids in both the phases are listed in Table 2. All the steroids except testosterone possess two carbonyl groups. The conjugated carbonyl stretching frequency occurs at a substantially lower wavenumber (around 1665 cm^{-1}) as compared to the nonconjugated carbonyl stretching frequency (around 1705 cm^{-1}). The spectrum of testosterone shows only one carbonyl band at 1663 cm^{-1} owing to the conjugated carbonyl group while the spectra of all other steroids show two carbonyl stretches around 1663 and 1705 cm^{-1} in liquid medium. It was observed that the carbonyl bands shift slightly to higher wavenumbers in the supercritical medium as compared to the liquid medium. Carbonyl groups may participate in hydrogen bonding with the more polar liquid mobile phase, thus absorbing at a lower wavenumber. Hydrogen bonding of the steroids with the nonpolar supercritical medium should be less favorable. The liquid phase spectra also show hydroxyl stretching modes while these can not be seen in the supercritical phase spectra. The use of deuterosolvents also results in the partial exchange of hydrogen for deuterium which yields both O-H and O-D bands around 3620 cm^{-1} and 2650 cm^{-1}, respectively. Corticosterone and 11-deoxycortisol show two O-H and O-D bands due to the presence of two hydroxyl groups. Other major peaks around 2945 cm^{-1} and 3060 cm^{-1} arise from aliphatic C-H stretching. These band positions are not shifted in the two media under study as they do not participate in hydrogen bonding.

SFC and HPLC compare well for the separation and resolution of the steroids except for the difference in elution order. Unfortunately, a direct comparison can not be made between these two separations even though the particle size of the packing material was identical. The bonding chemistry both to the particle and to the silica base differed. Packing efficiencies between the 4.6 mm i.d. and 1.0 mm i.d. columns were also not similar. Furthermore, pressure programming was employed in the SFC case while isocratic/isobaric parameters were characteristic of the HPLC experiment. In order to gain a more valid comparison, an SFC separation of the steroids was developed with the 4.6 mm i.d. column on a 1082B Hewlett Packard (Avondale, PA). The components failed to elute with 100% CO_2 under isobaric conditions probably because the analytical scale column was too active. Employing 1.2% methanol-modified

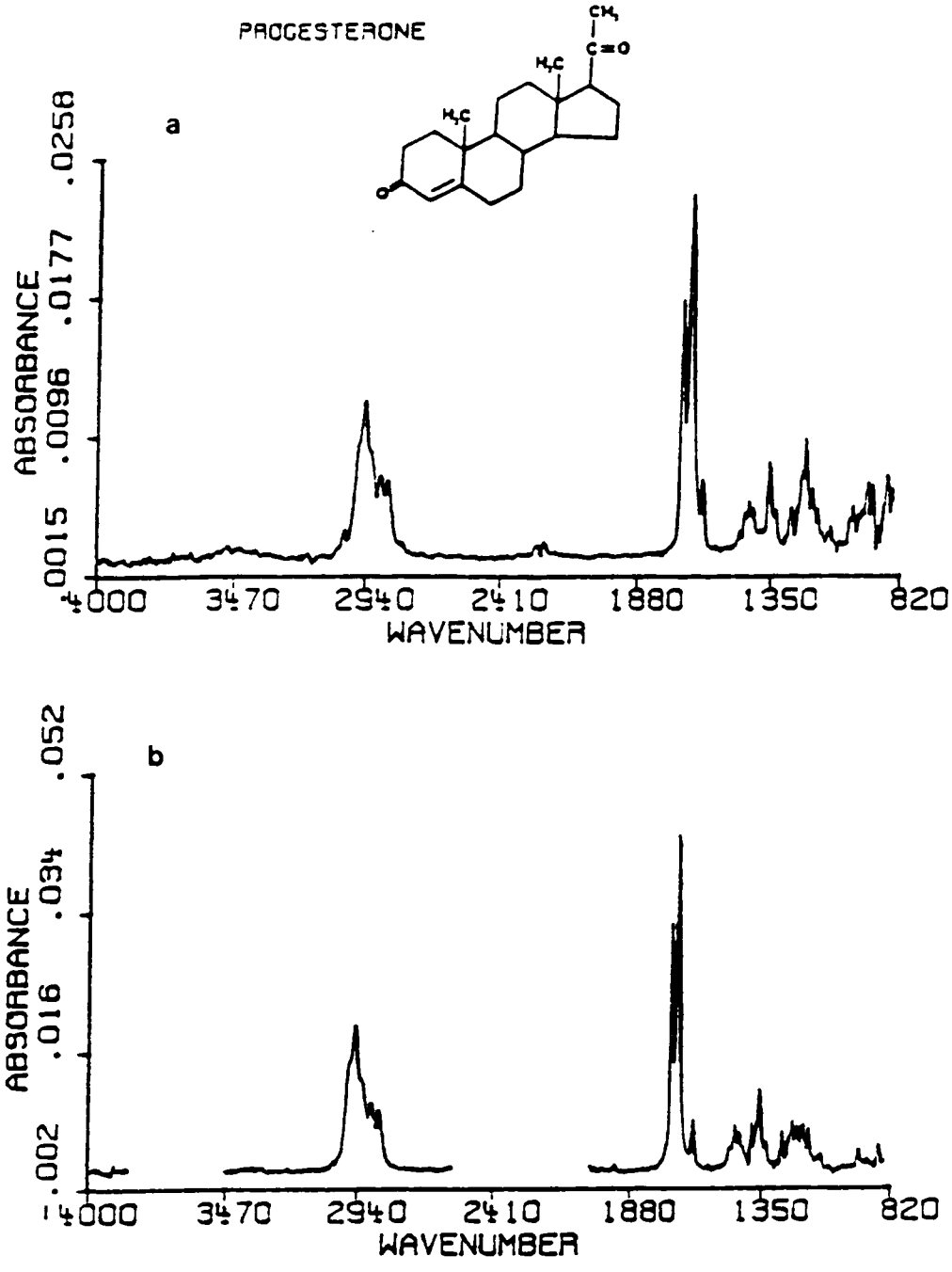


Figure 8. On-line FT-IR spectrum of progesterone: (a) Liquid medium (b) Supercritical fluid medium.

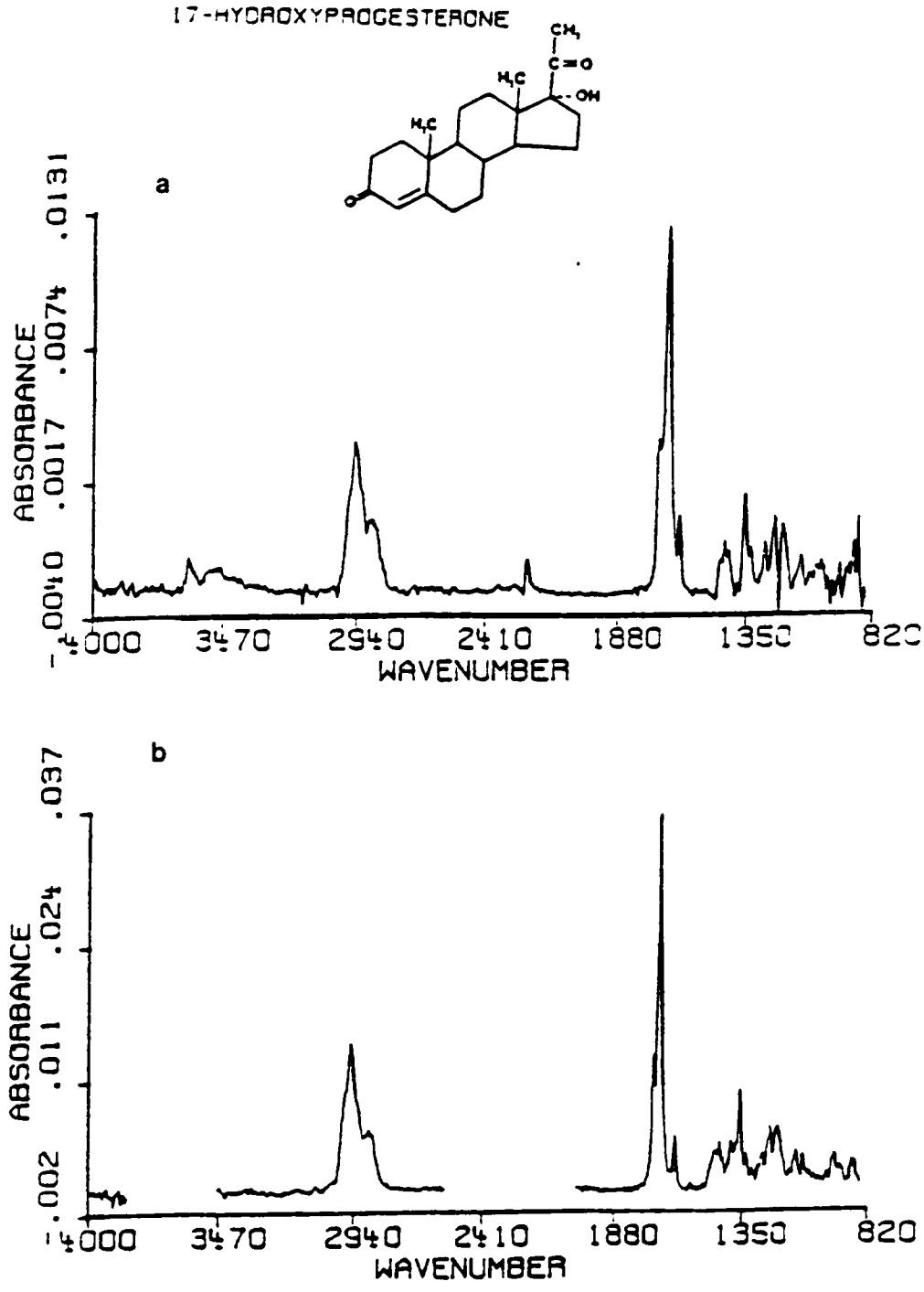


Figure 9. On-line FT-IR spectrum of 17-hydroxyprogesterone: (a) Liquid medium (b) Supercritical fluid medium.

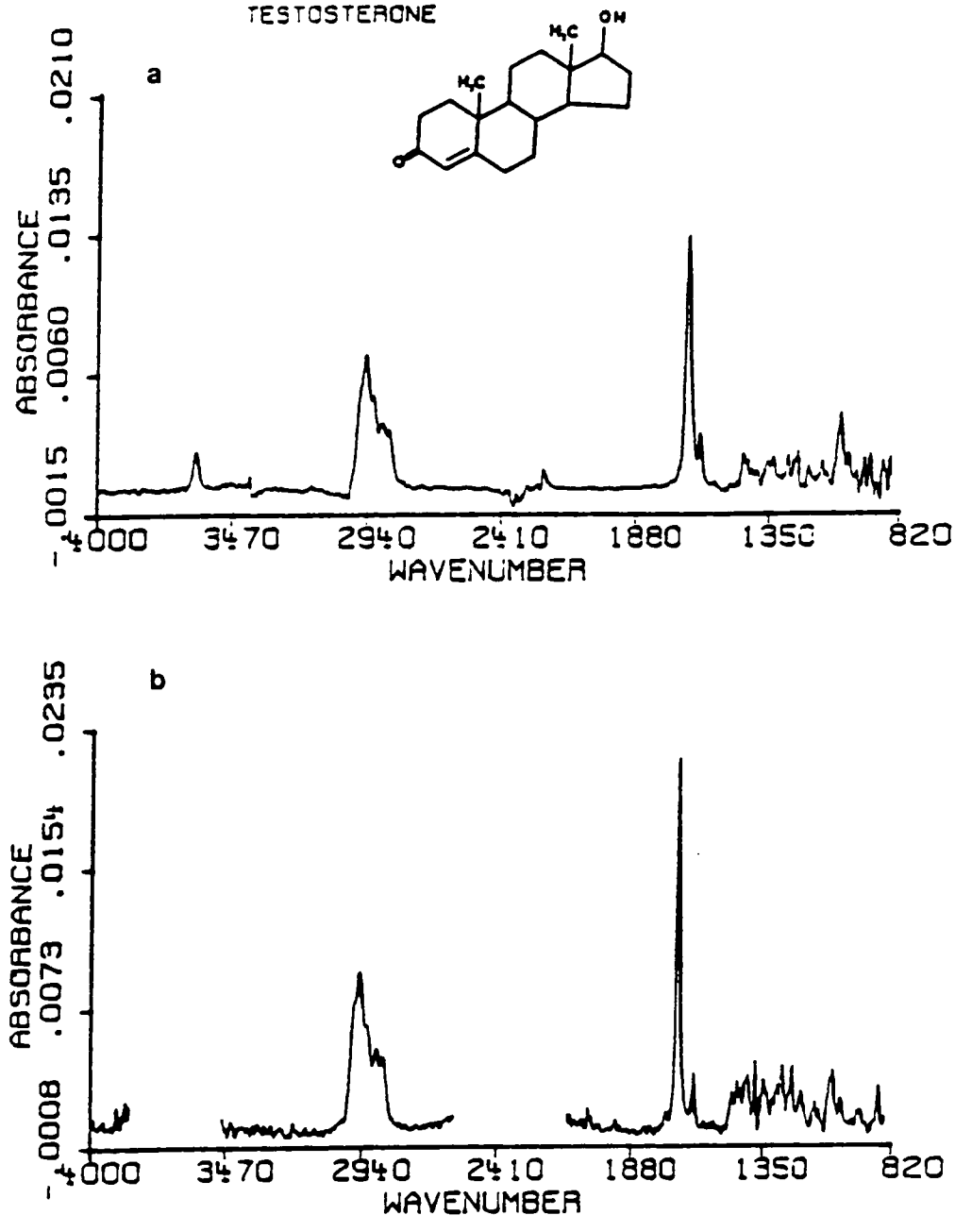


Figure 10. On-line FT-IR spectrum of testosterone: (a) Liquid medium (b) Supercritical fluid medium.

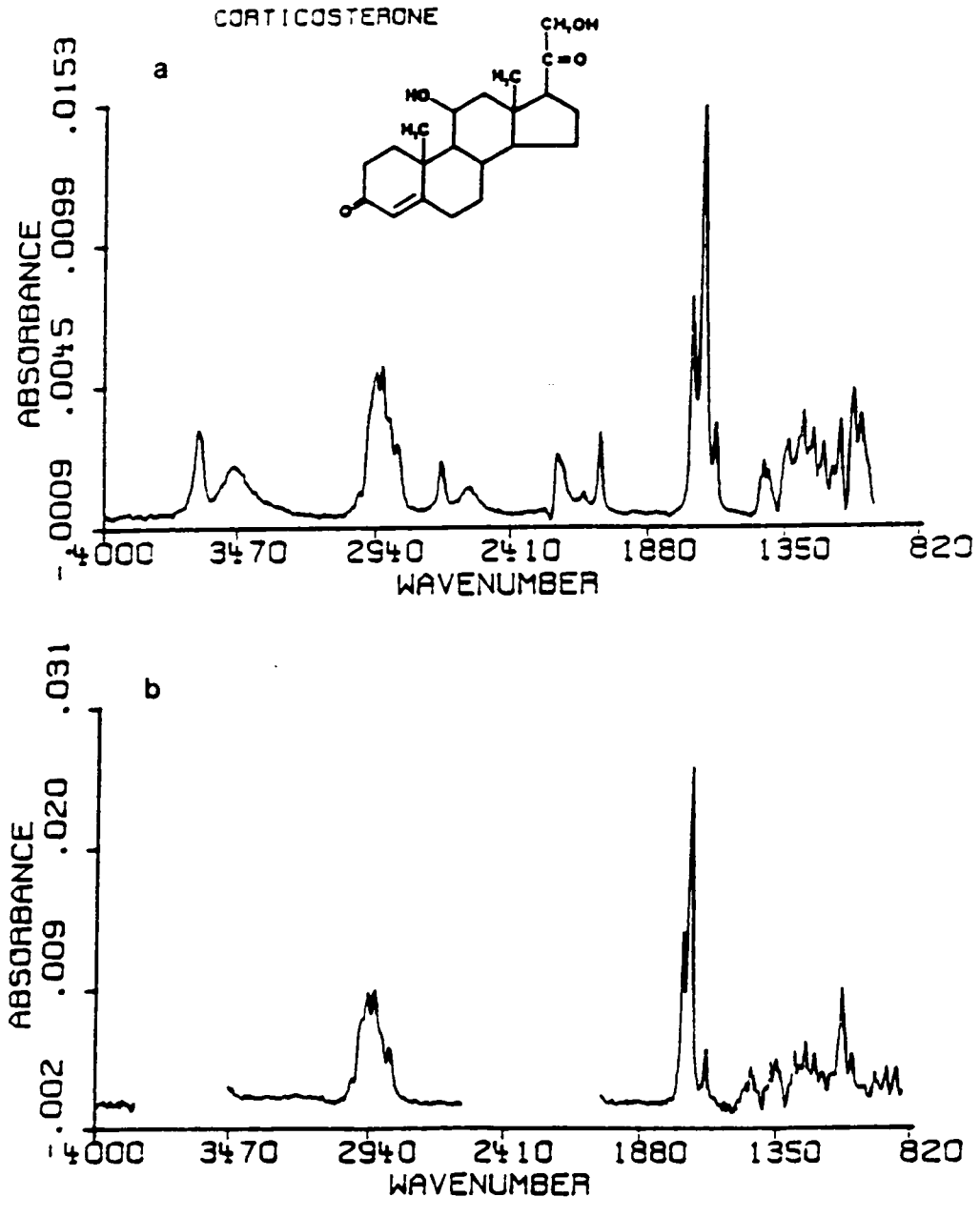


Figure 11. On-line FT-IR spectrum of corticosterone: (a) Liquid medium (b) Supercritical fluid medium.

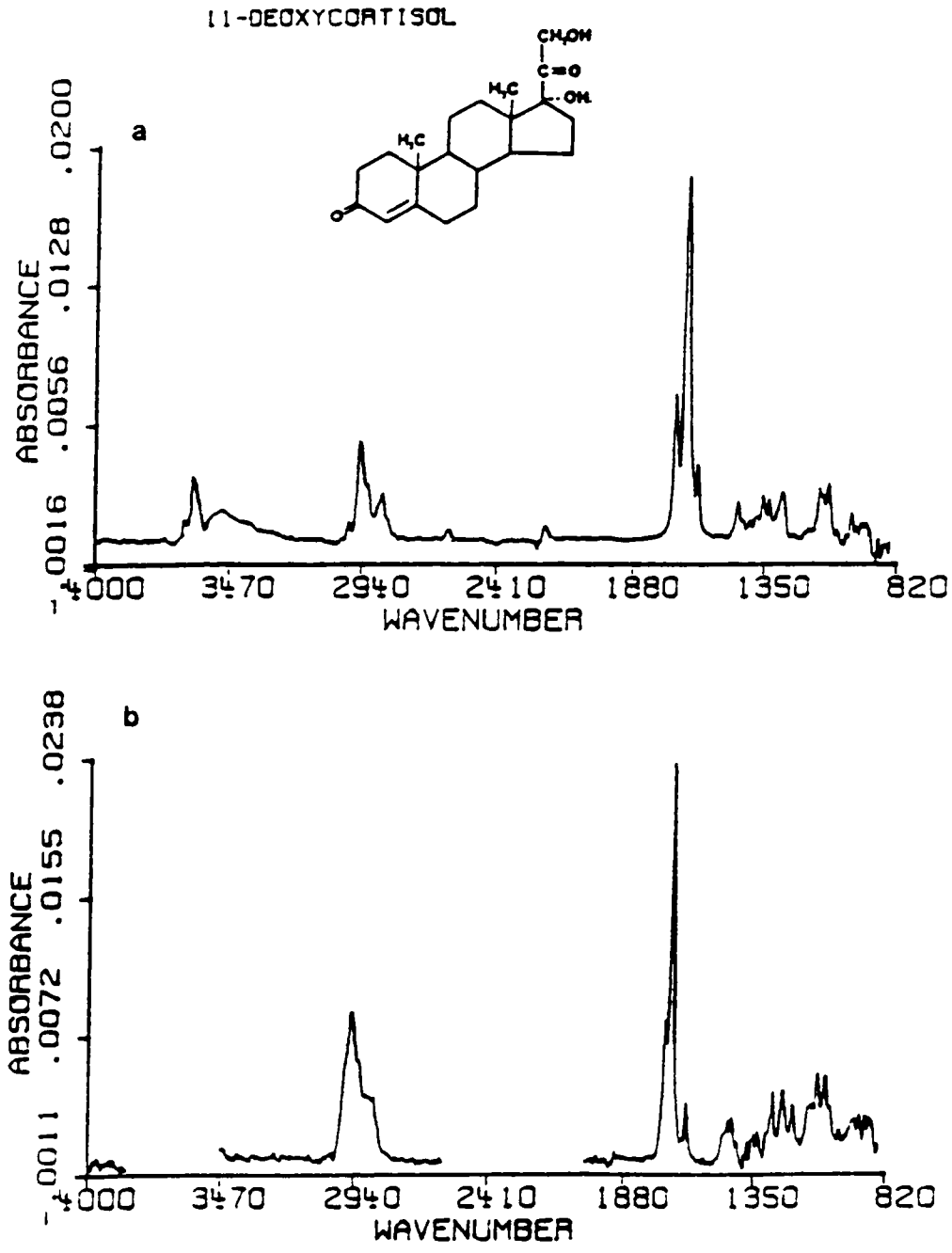


Figure 12. **On-line FT-IR spectrum of 11-deoxycortisol:** (a) Liquid medium (b) Supercritical fluid medium.

Table 2.

Carbonyl Stretching Frequencies (cm^{-1}) for Steroids

Component	Liquid Phase	Supercritical Phase
Progesterone	1699 1662	1711 1684
17-hydroxy-progesterone	1703 1662	1713 1683
Testosterone	1663	1682
Corticosterone	1706 1663	1712 1682
11-Deoxycortisol	1708 1663	1713 1684

CO_2, however, gave a satisfactory isobaric separation. The order of elution was same as in the other two SFC separations as shown in Figure 13. The testosterone peak was chosen for estimating the column efficiency with HPLC and SFC modes. Table 3 shows the efficiency and selectivity comparison for these two modes of separation.

Each separation was accomplished in such a way as to achieve comparable capacity factors. The temperature was ambient and 40 °C for the HPLC and SFC separations, respectively. The concentration of the mixture and injection volumes were identical. While SFC is generally thought to provide more efficient separations per unit time compared to HPLC , one recognizes from Table 3 that at essentially fixed time the efficiency of the column appears to be better in the HPLC mode than in the SFC mode. A firm explanation of this phenomenon is not readily apparent. Little work has been done regarding extra column variances in SFC and therein may lie the explanation for this difference. On the other hand, neither experiment is carried out at optimum linear velocity which is approximately 1.5 mm/sec for HPLC and 4.0 mm/sec for SFC. For these experiments the estimates of linear velocity were 3.3 mm/sec for HPLC and 3.7 mm/sec for the SFC mode. Furthermore, the activity of the column with CD_3OD / $CDCl_3$ versus CH_3OH/ CO_2 as the mobile phases may be different. Concerning the resolution of testosterone and 17-hydroxyprogesterone, SFC affords better selectivity than HPLC with this particular cyanopropyl column.

Currently the goal of most SFC separations is to employ pressure (density) gradients with 100% CO_2 as the mobile phase in order to accommodate as many detectors as possible and to achieve a separation in a reasonable amount of time. Column efficiency calculations while using pressure programming are rather misleading, but an attempt was made to investigate the performance of both the 1.0 mm and 100 μm i.d. columns under operating conditions used in each case. The separation was carried out on both columns at 60 °C (see Figures 6 and 7) and at 100 °C (see Figure 14) using pressure gradients so as to achieve comparable separation on the two two columns.

The same pressure gradient could be used for the packed column at two temperatures while, different pressure gradients were necessary for the capillary column. Based on the pressure

Table 3. Comparison of Efficiency and Resolution for HPLC and SFC.

Comparison of Efficiency and Resolution Employing Identical Packed Columns Under HPLC and SFC Conditions

Separation Mode	Plates[a]	k'	R[b]	HETP (mm)
NP-HPLC 0.5% CD_3OD/ 99.5% $CDCl_3$	18,500	5.5	1.14	0.014
SFC-Isobaric 1.2% CH_3OH/CO_2	11,400	4.9	2.10	0.022

[a] Analyte = Testosterone; 250 x 4.6 mm, 5 µm: Spherisorb Cyanopropyl

[b] Testosterone/17-hydroxyprogesterone

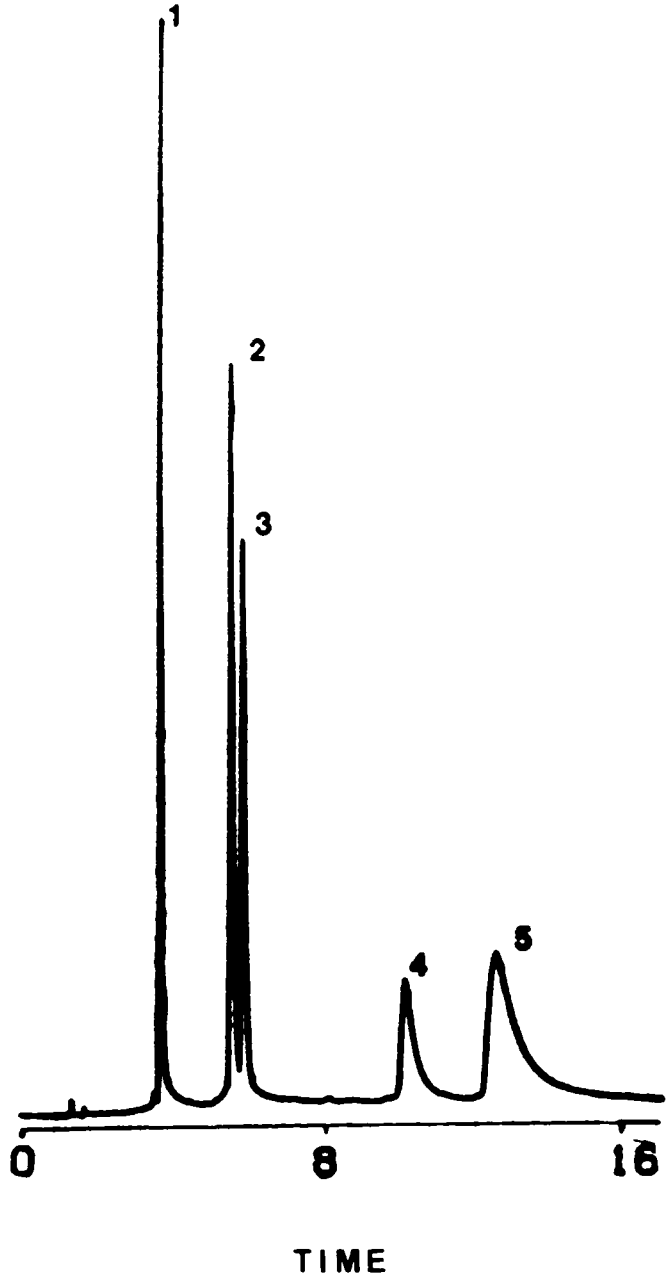


Figure 13. SFC Separation of steroid mixture on an analytical scale column: Conditions: Column: Alltech cyanopropyl bonded 250 x 4.6 mm i.d. silica (5 μm particle size); Mobile Phase: 1.2% $CH_3OH/\ CO_2$ at 40 °C, Flow Rate: 2.0 mL/min., Column Outlet Pressure: 4200 psi.

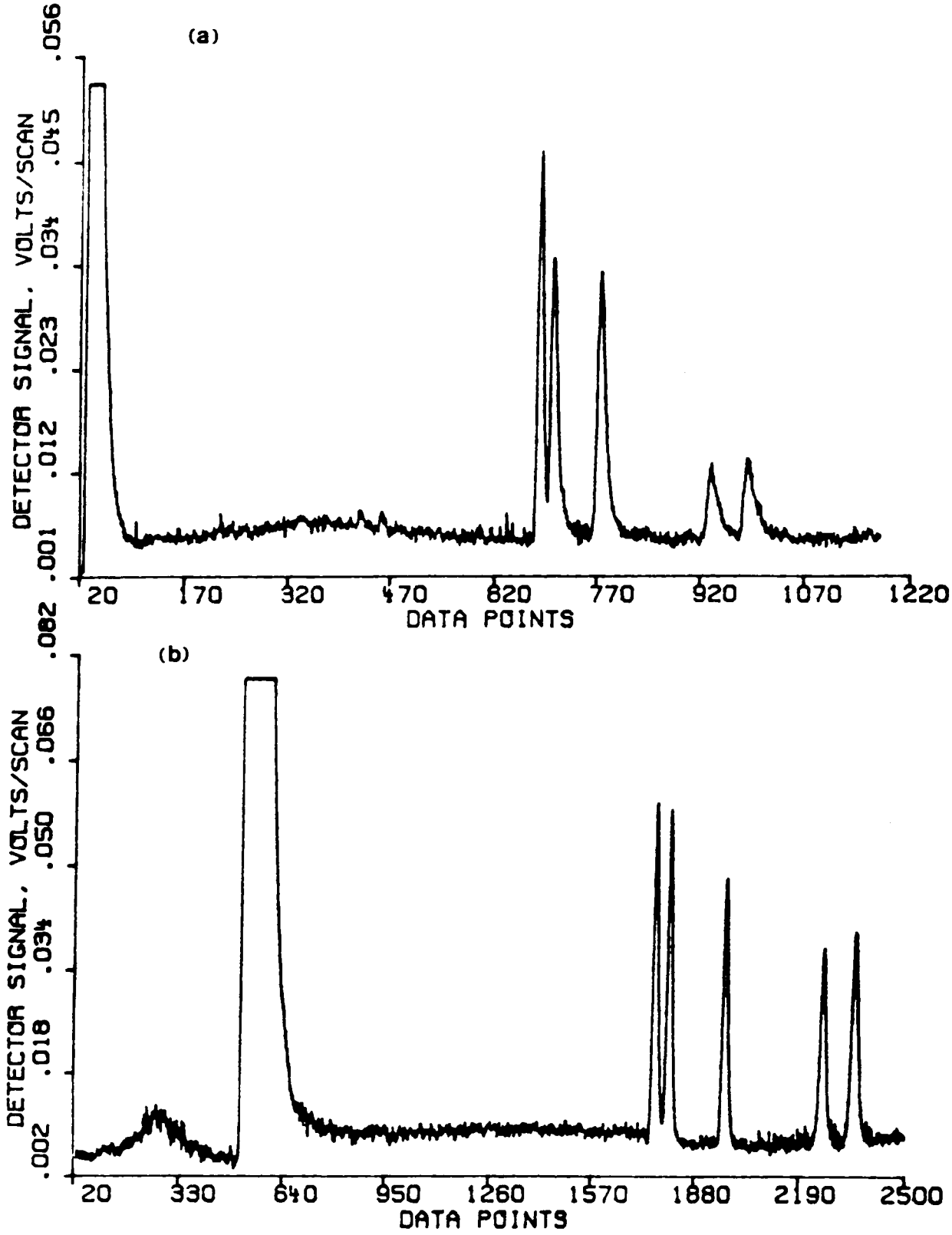


Figure 14. SFC separation of steroid mixture at 100 c: (a) Packed column (b) Capillary column.

programming rate, the pressure (and hence the density) required to elute testosterone in each case was calculated. Table 4 lists the pertinent data at both 60 °C and 100 °C.
Naturally the capillary column offers the greater number of apparent plates since it is 100 times longer. The mismatch in apparent plates, however, is just as great on a per meter basis but in the opposite sense. The capillary column requires a higher pressure (and higher density) than does the 1.0 mm column (0.88 vs 0.83 g/mL) for comparable resolution of testosterone/17-OHP at 60 °C. Even though both columns are termed cyanopropyl, the two bonded phases are not identical. The open tubular column is 25% cyano-25% phenyl-50% methylpolysiloxane; whereas, the *DELTABOND™* column is a highly crosslinked 100% cyanopropyl phase. The relative amounts of each phase per column is unknown.

It is interesting to compare the efficiency these two columns for the elution of testosterone at different temperatures (see Table 4). At a higher temperature with approximately the same pressure programming, elution should require a longer time since the density of the CO_2 mobile phase will be less. This prediction would most likely hold if the vapor pressure of the analyte is low as is expected to be the case for testosterone. As Table 4 attests, the capacity factor for both the columns is increased (i.e. 4.8 to 7.5 for packed column and 1.4 to 2.4 for capillary column) about 60%. An intriguing point here, however, is the observation that the column efficiency of the packed column is higher at the lower density while the capillary column offered a higher efficiency at a higher density. This observation is in agreement with recent fundamental isoconfertic studies performed by our group[109] with high boiling amides on a packed and a capillary column. It was shown in this study that at lower density, a packed column offers higher efficiency while, for a capillary column higher efficiency can be achieved at a higher density. As in the current study, comparable resolution was achieved for a pair of steroids with each column at both temperatures. These data also agree with our earlier conclusion that a shorter (10 cm) packed column operating near the optimum linear velocity and a longer (10 m) capillary column operating at linear velocities much greater than optimum offer apparently comparable resolving power for relatively simple mixtures of nonvolatile polar analytes. For more complex mixtures no doubt the capillary column should afford some significant advan-

Table 4. Comparison of Packed and capillary columns.

Column Efficiency/Selectivity Comparison[a]

Column	Temp.	Apparent Plates/ Column[a]	Apparent Plates/ Meter[c]	Elution Density (g/mL)	k'	R[b]
DELTABOND™ Cyanopropyl	60°C	8,350	83,500	0.83	4.8	2.35
100 mm X 1.0 mm, 5 µm	100°C	12,400	124,000	0.67	7.5	
SB-Cyanopropyl-25	60°C	55,000	5,500	0.88	1.4	2.33
10 m X 50 µm	100°C	49,500	4,950	0.68	2.4	

[a] Analyte = Testosterone, 100%, CO_2
[b] Testosterone/17-hydroxyprogesterone
[c] Apparent efficiency, non-isobaric (non-isoconfertic)

tages. Clearly, more carefully designed experiments should be performed in order to test the generality of this notion.

3.0 Chapter 3 : SFC/FT-IR of Agricultural Compounds

3.1 Introduction

The agriculture and the horticulture industries use pesticides and herbicides extensively. Since these compounds are directly applied to the environment, their analysis is of great importance from both an agricultural and environmental point of view. Pesticides belong to various different chemical classes which include carbamates, amides, triazines, ureas, uracils, phenoxycarboxylic acids and organophosphorous compounds. Several amides and ureas also act as precursors to sulfonylurea herbicides.

High performance liquid chromatography (HPLC) has been used for the analysis of amides and ureas because the nonvolatile and thermally labile nature of these compounds limit the use of gas chromatography (GC) for their analysis. For example, several sulfonamides were separated on an ACT-1 column using a gradient mobile phase containing water, acetonitrile and tetrabutylammonium bromide with detection at 254 nm.[110] The residue analysis of phenylurea herbicides has been performed on a reversed phase column after preconcentration of the trace

amounts of herbicides was carried out using a solid-phase extraction system. Both ultraviolet (UV) and electrochemical detections were performed for this mixture.[111–113] Urea herbicides have been analyzed using a column switching technique.[114] A group of phenylurea herbicides has also been detected by electron capture and UV detectors after their elution from a reversed phase column.[115] Since the nonvolatile ion-pairing reagents present in the mobile phase interfered with their detection, a post-column extraction of the solutes in this case was necessary. Coupling of packed capillary liquid chromatography to a mass selective detector has been recently reported for urea and sulfonylurea herbicides.[116] Specifically a mixture of urea herbicide and its metabolites and a mixture of sulfonylureas were separated on an octadecyl fused silica packed capillary column of 250 μm i.d. using a binary mixture of water and acetonitrile. Mass spectra were obtained using a moving belt interface. Several urea derivatives have been further analyzed by HPLC and detected at ppb level using electron capture detector.[117,118]

Supercritical fluid chromatography (SFC) offers several advantages over HPLC such as: faster analysis, higher efficiency per unit time, more rapid methods development and ability to use both GC and HPLC detectors. In the past SFC has been partially successful for amide and urea analysis using both packed and capillary columns. The amide study involved two polar high molecular weight polymer additives which were separated on a methylpolysiloxane capillary column individually using both CO_2 and N_2O as the mobile phases. Two packed columns were tried for these separations but the analytes were not eluted with 100% CO_2 mobile phase.[119] Several substituted sulfonamide antibiotics were separated on a capillary column coated with 30% biphenyl stationary phase using 8% isopropanol modified CO_2 as the mobile phase. The separation of the same mixture with modifier was also attempted on both a *DELTABOND™* cyanopropyl and on an octadecyl packed column. The detection with both the columns was performed by measuring UV absorbance of the eluted analytes. Both packed and capillary column separations provided poor resolution. The use of 25% cyanopropyl stationary phase on a capillary column eluted all five antibiotics with the same mobile phase; however, peak tailing was evident even in this case.[120] In another study, five sulfonamides were separated on an analytical scale silica packed column with 15% methanol-modified CO2. The separation

was achieved in about five minutes at a high operating pressure and a higher flow rate employed here as opposed to the previous study. Also, two alkaloids containing hydroxyl, carboxyl and amide functionalities were separated on an analytical scale amino column using 20% methoxyethanol modified CO_2 as the mobile phase. Both sulfonamide and alkaloid mixtures were detected by UV and mass spectrometry (MS).[69]

Several thermally labile "urea-derived" herbicides and fungicides have been analyzed using a combination of HPLC and both packed and capillary SFC. These compounds were eluted with 100% CO_2 from a capillary column while a packed column required 4-8% methanol modifier. Lower detection limits, faster analysis times and greater reproducibility were achieved on the packed column as compared to the capillary columns.[37] In another study, a mixture of urea and carbamates was separated on a microbore octadecyl column. The use of 100% CO_2 resulted in broad and tailing peaks with several components of the mixture unresolved while, addition of 1% methanol to CO_2 resulted in an improved peak shape for all the compounds. MS detection was able to identify each component including the poorly resolved or unresolved peaks of the herbicides.[62] Several agricultural compounds have been analyzed by SFC with the use of different modifiers. Various mixtures of compounds such as benzamides, sulfonamides and ureas were separated on packed columns and their retention behavior was studied using different modifiers. A relationship was established between the modifier to be used and the functional group present on the analyte molecule. This type of study is important since it involves various pesticide precursors and it can be used as a guideline while analyzing the actual pesticides.[121]

In this study an attempt was made to develop the separation of various pesticide precursors using 100% CO_2 in order that Fourier Transform Infrared (FT-IR) detection could be applied for identification purposes. SFC has been successfully interfaced with FT-IR both on-line and off-line.[79,94,95] Employing a prototype flow cell interface a detection limit of 2.5 ng (injected) was achieved for caffeine.[97] With 100% CO_2, FT-IR detection of these analytes provides most of the IR spectrum of each eluted component. If concentrations of alcoholic modifier exceed 0.1%, considerable IR information is lost due to extensive mobile phase absorbance. The research is

divided into three experiments based upon the chemical classes in each mixture: six ureas, six benzamides and anilides, nine benzamides and sulfonamides. The anilides and positional isomers of benzamides and sulfonamides were separated on a capillary column while the ureas were separated on a packed column.

3.2 Experimental

3.2.1 Urea Mixture

A Suprex (Pittsburgh, PA) model 200A supercritical fluid chromatograph was used for this experiment. A *DELTABOND™* cyanopropyl bonded silica column of 10 cm length, 1.0 mm i.d. and 5 μm particle size (Keystone Scientific Inc., Bellefonte, PA) was used to develop the separation. An oven temperature of 100 °C was maintained constant during the pressure programmed separation. A tapered in-house manufactured restrictor was used for this experiment. FT-IR and flame ionization detector (FID) detection were sequentially used; FT-IR (33 °C), FID (350 °C). A Valco injector of internal loop of 0.1 μL (Valco Instruments, Houston, TX) was used to introduce the sample onto the column. The mixture of ureas was prepared in pesticide grade methylene chloride at a concentration of 3 mg/mL. The same separation was tried on a Hewlett Packard 1082B supercritical fluid chromatograph using a 250 mm x 4.6 mm Spherisorb (Alltech Associates, Deerfield, IL) conventional cyanopropyl column (5 μm particle size) at 40 °C and 4200 psi inlet pressure. Previously mixed methanol modified (2%) CO_2 (Scott Specialty Gases, Plumsteadville, PA) was required at a flow rate of 3 mL/min for this experiment. UV detection at 254 nm was utilized. An injection of 1.0 μL (200 ng/μL/component) onto this column was employed.

3.2.2 Benzamide and anilide mixture

A Lee Scientific (Salt Lake City, UT) model 501 supercritical fluid chromatograph was used for this separation. A 10 m x 100 μm i.d. SB-cyanopropyl-25 fused silica capillary column (Lee Scientific, Salt Lake City, UT) with film thickness of 0.25 μm and a frit restrictor (50 μm) were employed. Oven temperature was maintained constant at 100 °C. Detection was sequential FT-IR/FID. The mixture was prepared in 1:1 HPLC grade methanol and pesticide grade methylene chloride, 3 mg/mL/component. A splitless injection of 0.1 μL was employed.

3.2.3 Benzamide and sulfonamide mixture

The same Lee Scientific instrument, model 501, was used for the separation of positional isomers of benzamides and sulfonamides. Two SB-cyanopropyl-25 fused silica columns of 10 m x 100 μm i.d. and 20 m x 50 μm i.d., respectively were initially used but with little success. The separation, however, could be successfully achieved on a prototype Naphthylethylbenzamide chiral column (Lee Scientific Co., Salt Lake City, UT) of 5 m length, 100 μm i.d. and 0.25 μm film thickness. An oven temperature of 100 °C was again employed for the separation. A frit restrictor of 50 μm (Lee Scientific Co., Salt Lake City, UT) was used to maintain the system pressure. Injection and detection were as described above. This mixture was prepared in HPLC grade methanol, 3 mg/mL/component.

All compounds were donated by Dr. M. E. McNally from the agricultural Division of E. I. DuPont Co., Wilmington, DE.

3.2.4 FT-IR

All SFC/FT-IR data were collected with a Nicolet (Madison, WI) 5SXC FT-IR spectrometer equipped with a high pressure flow cell of 0.6 mm i.d., 5.0 mm pathlength and 1.4 μL cell volume. All spectra were obtained at 8 cm^{-1} resolution. The temperature of the stainless steel flow cell was maintained at 33 °C. The condensed IR beam was passed through the flow cell and then focused on an MCT detector. Data were acquired by collecting 1 file/sec in real time. Four scans were collected per sample file and eight scans were collected per background file. The Nicolet software was used to collect and process the data.

3.3 Results and Discussion

The principal goal of this work was to develop the separation of individual mixtures containing (a) ureas, (b) benzamides and anilides and (c) positional isomers of benzamides and sulfonamides and to detect them by on-line FT-IR. With this goal it was necessary to effect separations with 100% CO_2 as the mobile phase since the presence of most modifiers in concentrations greater than 0.1% absorb a large fraction of incident IR radiation.

3.3.1 Ureas

Nearly baseline resolution was achieved for the six components in less than fifteen minutes with pressure programming as shown in Figure 15. The utilization of the highly deactivated *DELTABOND™* cyanopropyl stationary phase afforded elution of these materials with 100% CO_2 even though these analytes are not expected to exhibit high solubility in supercritical

CO_2.[122] Peak assignments are based upon both retention time comparisons with neat injections and IR spectra matching. The IR spectra of all these solutes exhibit a high signal to noise ratio as in Figure 16 even though a complete chromatographic resolution of the first two components was not achieved. The amide carbonyl stretching band is present in each spectrum below 1700 cm^{-1}. Except for the first eluting compound, all five ureas have N-H functionality which can be seen around 3475 cm^{-1}. Discontinuities in each spectrum correspond to CO_2 absorption. Spectra with excellent signal to noise were obtained considering that only 300 ng/component were injected onto the column. The identification of these compounds no doubt could be accomplished if 30 ng/component had been injected. The order of elution cannot be rationalized based upon a single factor. The availability of an N-H functionality may be important since the first eluting component contains no N-H, the sixth component exhibits two N-H 's and those in between have one N-H. On the other hand the elution order may be following the order of decreasing solubility in supercritical CO_2. For example, tertiary amides such as N,N-dimethylacetamide and N,N-diethylacetamide have been reported to be miscible in CO_2 at 25 °C and approximately 1000 psi., whereas acetamide is only 1% (w/w) soluble and urea is insoluble under the same conditions. Given these guidelines it seems reasonable that N,N'-diphenylurea should elute last since it would be expected to be the least soluble in the mobile phase and require the greatest elution density.

The same separation was carried out on a conventional analytical scale column. The active sites, free silanol groups, present on the stationary phase rendered this column very active. The elution of none of these analytes could be achieved with 100% CO_2. The use of a polar modifier, 2% methanol-modified CO_2, was necessary in order to achieve complete separation of all the components as seen in Figure 17. The elution order was the same as in case of the microbore *DELTABOND™* column but the analysis time was reduced to about half. For this separation all six components were baseline resolved.

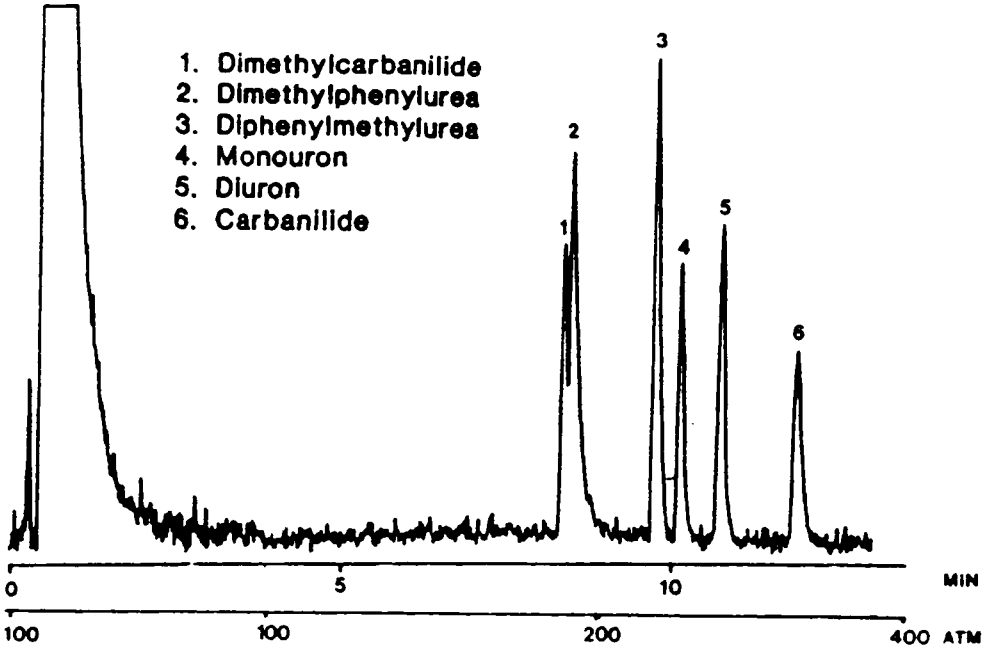


Figure 15. GSR of the urea mixture: Conditions: Column: 100 mm x 1.0 mm i.d. *DELTABOND™* cyanopropyl bonded silica column (5 μm Particle size); Mobile Phase: 100% CO_2 at 100 °C.

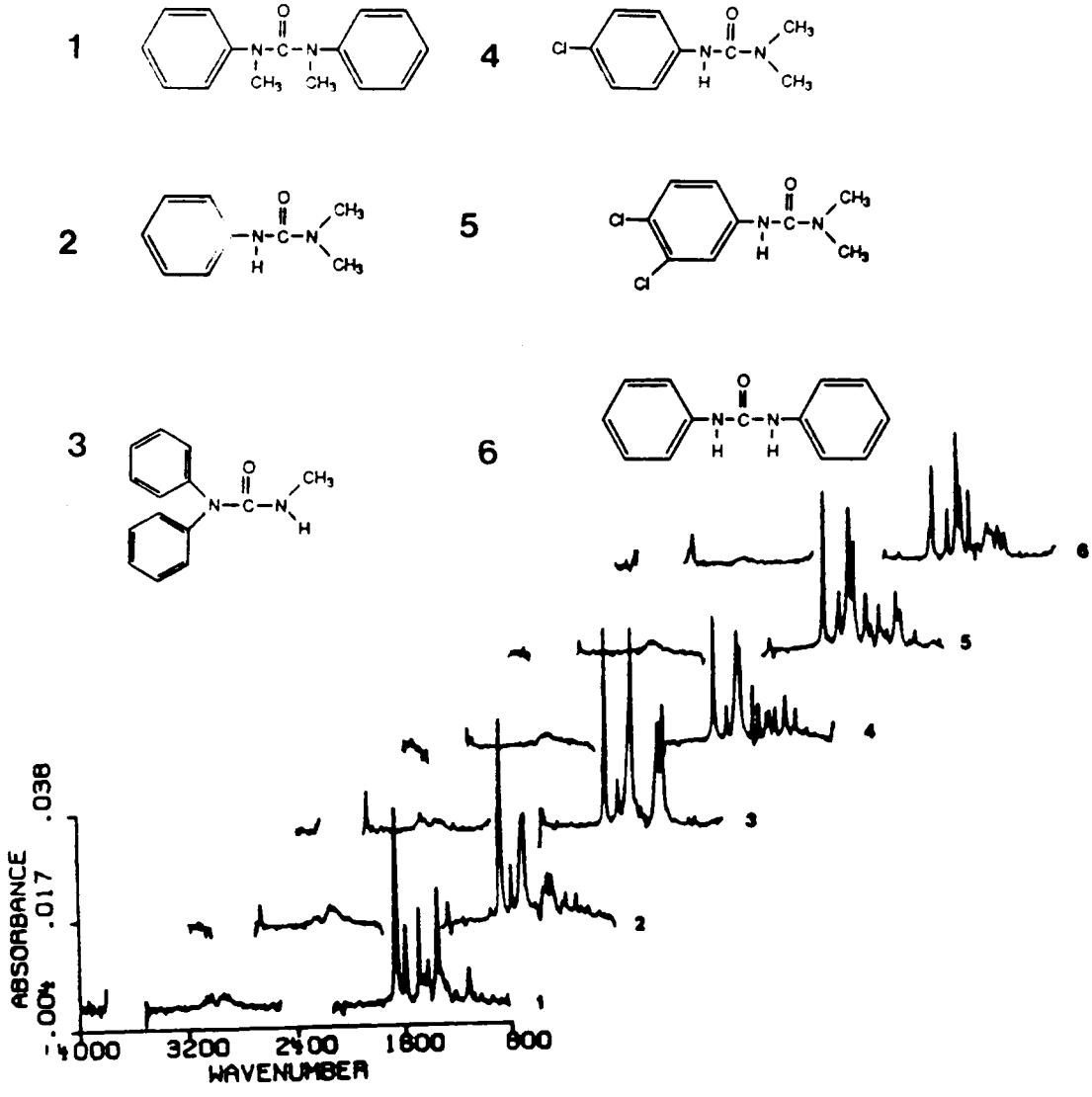


Figure 16. Stacked spectra of individual urea.

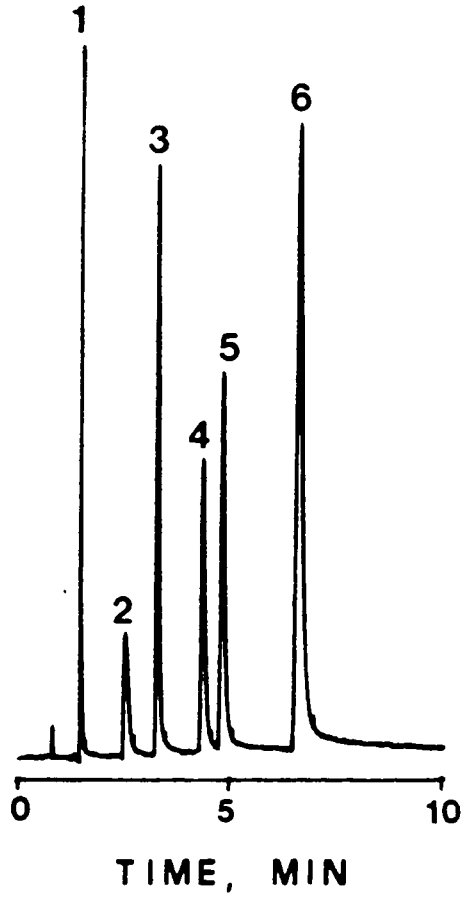


Figure 17. Separation of the urea mixture on analytical scale column.: Conditions: Column: 250 mm x 4.6 mm i.d. Alltech cyanopropyl bonded silica column (5 μm Particle size); Mobile Phase: 2% methanol/CO_2 at 40 °C. Flow Rate: 3 mL/min.

3.3.2 Benzamides and Anilides

The mixture of substituted benzamides and acetanilides was separated on a 25% cyanopropyl-25% phenyl-50% methyl polysiloxane stationary phase as shown in Figure 18. Here again the order of elution may reflect differences in mobile phase solubility. Certainly the trend in basicity of these amides can not be used to justify the retention pattern. For example the benzamides should be less basic than acetanilides; yet, the latter consistently elutes before the former in both the unsubstituted and the chloro-substituted case. Furthermore, no trend is observed for primary, secondary etc. amines as was noted for the urea mixture.

The on-line spectrum of each analyte was achieved at 8 cm^{-1} resolution. Each spectrum shows a high signal to noise ratio (Figure 19). The N-H stretching vibrational mode for anilides is observed around 3455 cm^{-1}; while, that for benzamides appears at a slightly lower wavenumber (3420 cm^{-1}). The N-H stretch for the methyl substituted benzamide appears at the highest wavenumber (3485 cm^{-1}). The carbonyl amide stretching band appears between 1680-1710 cm^{-1} for these compounds. The nitro substituted compound exhibits symmetric and assymetric O-N-O stretching bands around 1450 and 1550 cm^{-1}. The chloro substituted analytes show a C-Cl stretch around 875 cm^{-1}. It would appear that given the high quality spectra obtained here identification of benzamides and acetanilides in a complex matrix by this technique is highly feasible.

3.3.3 Benzamides and Sulfonamides

A mixture of nine positional isomers of benzamides and sulfonamides was separated using 100% CO_2 as the mobile phase. The separation was tried on a packed cyanopropyl *DELTABOND™* packed column but the polar compounds such as sulfonamides did not elute with 100% CO_2. The separation was attempted on a 20 m long column containing 25%

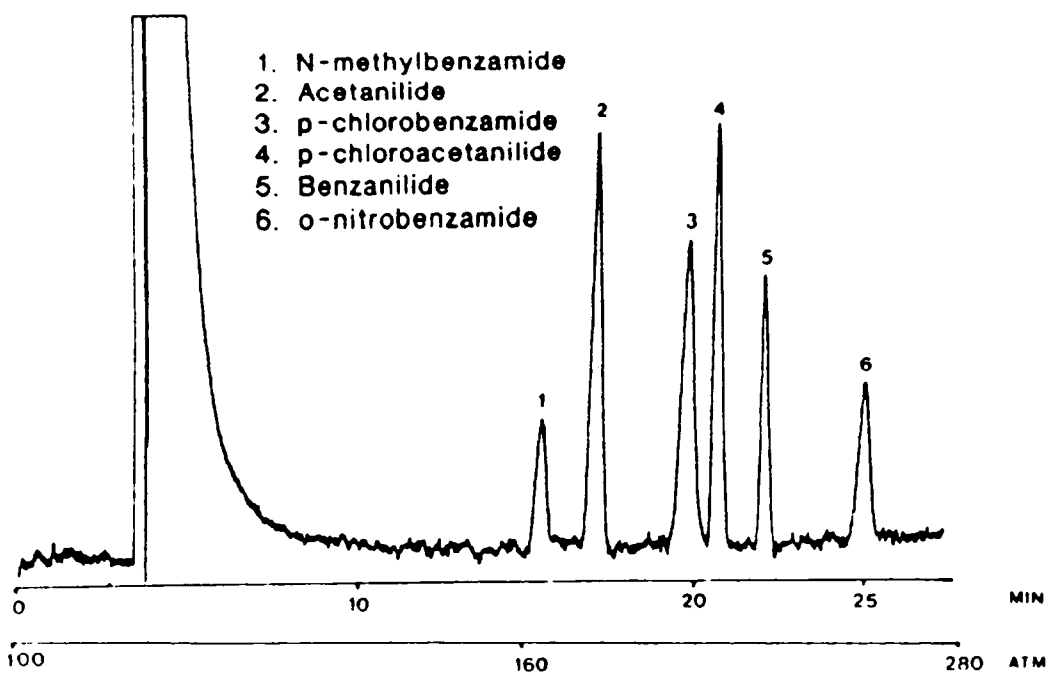


Figure 18. GSR of the mixture of benzamides and anilides.: Conditions: Column: 10 m x 100 μm i.d., SB- cyanopropyl-25 capillary (0.25 μm Film thickness); Mobile Phase: 100% CO_2 at 100 °C.

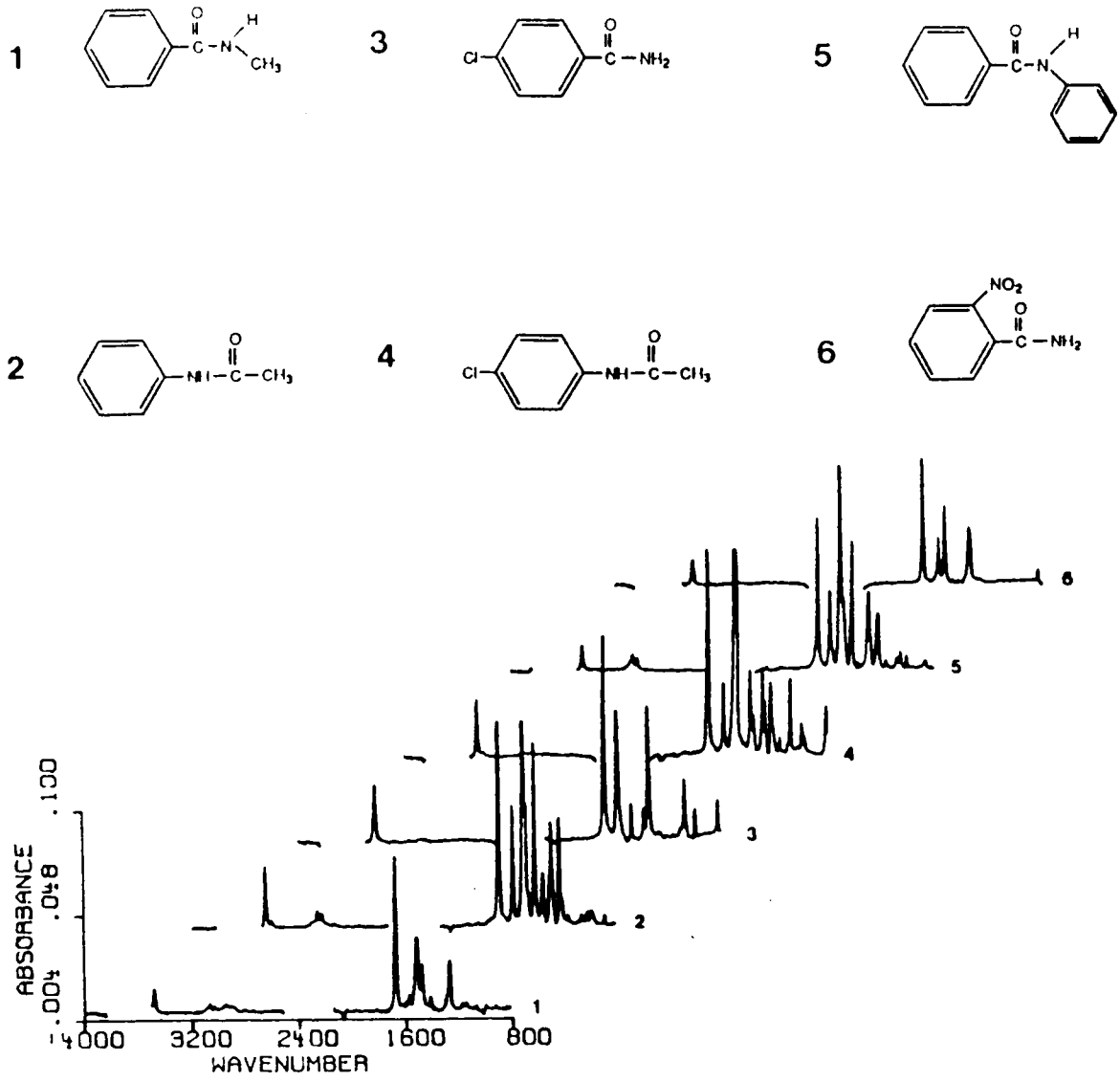


Figure 19. Stacked spectra of individual benzamide and anilide.

cyanopropyl-25% phenyl-50% methyl polysiloxane stationary phase but all the isomers were not resolved on this stationary phase. An increased column length did not offer efficiency high enough to separate all the isomers. The three isomers, p-chlorobenzenesulfonamide, m-nitrobenzamide and o-nitrobenzamide coeluted even under isoconfertic condition at several temperature that were tried. An amide stationary phase which became available (i.e. the prototype chiral column) was successful in separating the various isomers as shown in Figure 20. The synthesis of this novel stationary phase is described elsewhere[33] and its application to the analysis of derivatized amino acid isomers has been reported. The naphthylethylbenzamide functionality is similar to a Pirkle 1-A type column which has been used for chiral HPLC separations. While the 25% cyanopropyl phase allowed elution of each component in this mixture, the amide stationary phase apparently offered better selectivity. The elution pattern is therefore not solely determined by the differences in solubility but partitioning of the amide analytes into the amide stationary phase is a factor. A complete resolution was achieved for almost all the components. Both FID and FT-IR separations were comparable and no loss in resolution was noticed in the FID trace.

The nitro substituted isomers are retained less than their chloro substituted counterparts. Also, the benzenesulfonamides exhibit a stronger interaction than the benzamides. Among the ortho, meta and para positional isomers, the para isomer appears to interact the strongest with the stationary phase probably due to the fact that this isomer can more optimally align itself with the stationary phase thereby resulting in a strong interaction. The stacked spectra of all the components of the mixture are shown in Figure 21. As before spectra exhibit a high signal to noise ratio. Two N-H stretching bands (symmetric and asymmetric) corresponding to a primary amide are seen in the spectrum around 3450 cm^{-1}. The failure to see this doublet with benzamides is probably due to the fact that one component of this stretching mode appears in the totally obscured region. The amide carbonyl stretching band is seen around 1700 cm^{-1} in the spectra of all the benzamides while, all the sulfonamides exhibit characteristic bands around 1350 and 1150 cm^{-1}. The chloro substituted analytes show a C-Cl stretch around 875 cm^{-1} and

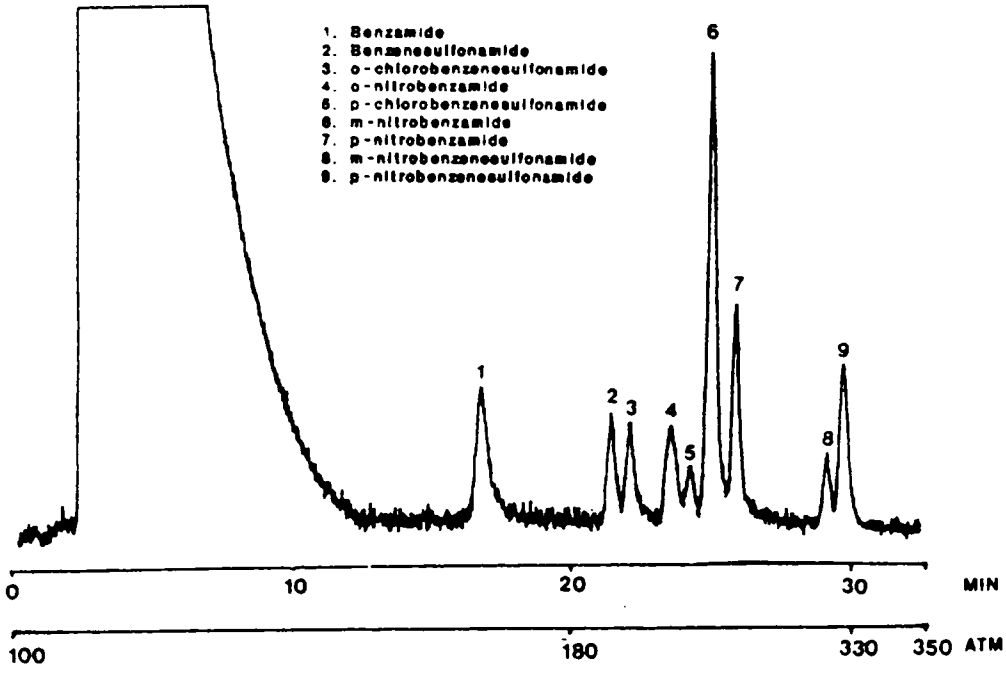


Figure 20. GSR of the mixture of benzamides and sulfonamides.: Conditions: Column: 5 m x 100 μm i.d., Naphthylethylbenzamide capillary (0.25 μm Film thickness); Mobile Phase: 100% CO_2 at 100 °C.

the nitro substituted analytes exhibit the asymmetric and the symmetric O-N-O stretches around 1550 and 1350 cm^{-1}, respectively.[123]

In conclusion, the separation of ureas, benzamides and sulfonamides can be easily performed employing 100% CO_2 as the mobile phase provided deactivated polar stationary phases are utilized. In the absence of a polar modifier, on-line FT-IR detection can be readily applied for the identification of these high boiling analytes in real time. Based upon the excellent IR spectra obtained with these model compounds in the supercritical medium at sub-microgram levels, extension of this technology to more complex matrices in both a qualitative and a quantitative sense appears feasible.

The packed column SFC method described here is unique in the sense that all the methods reported for the analysis of compounds of agricultural and environmental interest involve the use of modifiers for their elution from packed columns. Both packed and capillary column SFC methods shows the feasibility of elution of the compounds of moderate to high polarity with 100% CO_2 as the mobile phase so that on-line FT-IR detection can be performed for positive identification of the analytes.

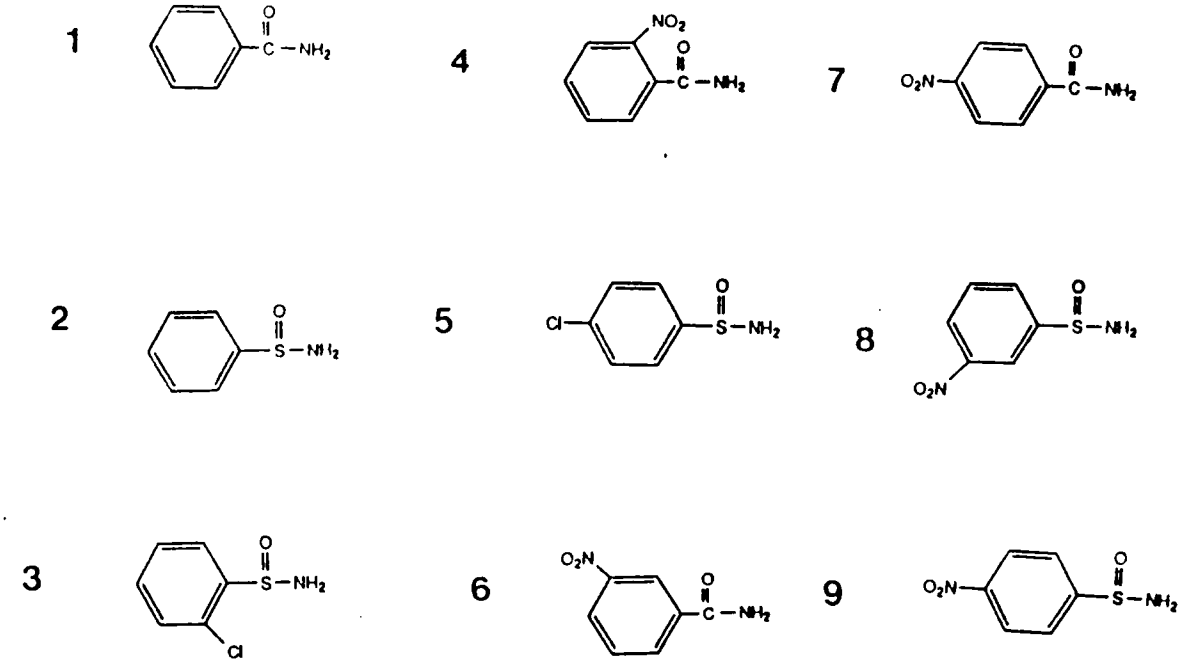


Figure 21. Stacked spectra of individual benzamide and sulfonamide.

4.0 Chapter 4 : Analysis of Triazine Herbicides by Gradient Mobile Phase SFC

4.1 Introduction

Triazines and triazoles are widely used as herbicides and fungicides. Several methods have been developed for the analysis of triazines[124–128] by both GC and HPLC. GC with flame based detectors and coupled with spectroscopic detection have been employed for the volatile components of triazines.[127–134] HPLC methods on the other hand have been applied to the analysis of high molecular weight and thermally labile herbicides and metabolites.[135–140] Ultraviolet (UV) and mass spectrometric (MS) detection with both positive and negative ion modes have been used for triazine detection[137135] although positive ion thermospray MS detection appears to be more common for LC effluent containing triazines.[137,138,140]

SFC has gained popularity for analysis of various classes of compounds of industrial interest, specifically SFC/MS has been employed to analyze pesticides with a basic triazine structure. In this regard six triazine derivatives have been separated on an analytical scale packed column with methanol modified CO_2 and the mass spectrum of each solute was

achieved with a thermospray interface.[141] In another study a triazole fungicide metabolite was separated by SFC on a capillary column with electron capture detection.[142]

None of the above mixtures of herbicides contained both triazine- and triazole- based compounds, or very polar derivatives of either class of compounds. Our recent research has been directed to the analysis of a mixture of various triazine and triazole derivatives, Figure 22. The nonvolatile nature of these specific triazoles and triazines restrict their analysis by GC. Since SFC offers certain advantages such as, faster analysis and higher resolution per unit time over HPLC, SFC has been applied to the analysis of this mixture. Since several highly polar components of our mixture did not elute with 100% CO_2 from either packed or capillary columns, a modified gradient mobile phase has been employed for their elution from an analytical scale packed column. The effect of increasing percent of polar modifier in SFC on the elution and retention of fat soluble vitamins has been reported[143] by Board et al. A mixture of 24 derivatized amino acids has also been separated using a gradient mobile phase of CO_2 and methanol containing a base, tetramethylammonium hydroxide. Nearly complete resolution of 22 derivatives was achieved in 15 minutes.[144] We describe herein the separation of our mixture of herbicides, with a CO_2-methanol gradient.

4.2 Experimental

A Hewlett Packard (Avondale, PA) 1082B liquid chromatograph modified for supercritical fluids was used to deliver CO_2 (Scott Specialty Gases, Plumsteadville, PA) to the system. This instrument was equipped with an HP 79875 variable wavelength ultraviolet detector. A Suprex (Pittsburgh, PA) model 200A micro LC syringe pump was used to deliver methanol (Fisher Scientific, Fairlawn, NJ). Methanol and CO_2 were dynamically introduced in a T-mixing chamber (Lee Co., West Brook, CT) and the mixed mobile phase was then passed on to the column. A back pressure regulator was used to maintain system pressure. A *DELTABOND™* (Keystone

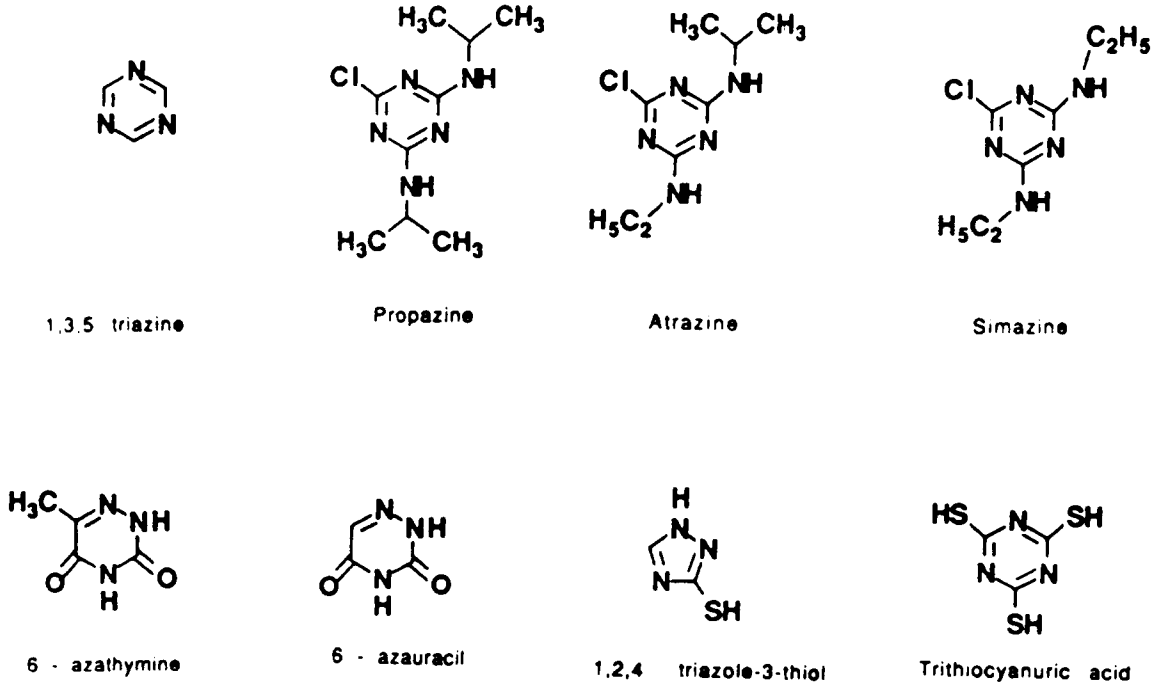


Figure 22. Structures of triazine and triazole derivatives.

Scientific Inc., Bellefonte, PA) crosslinked cyanopropyl bonded silica column of 25 cm length, 4.6 mm i.d. and 5 μm particle size was used to develop the separation. All triazine and triazole based samples were purchased from Aldrich Chemicals Co., Inc. (Milwaukee, WI). The injection solution had a concentration of 200 ng/μL of each component prepared in HPLC grade methanol.An injection volume of 1.0 μL was employed.

4.3 Results and Discussion

The dual pump system employed in this study (shown in Figure 23.) is similar to that described previously.[144] A separation of the eight component mixture was unsuccessfully tried on several analytical scale conventional columns packed with different stationary phases such as amino, cyano and octadecyl under isocratic conditions. Various concentrations of methanol and different temperatures were attempted to develop the separation. The complete separation of all the components in the mixture was however achieved only using gradient elution. An oven temperature of 60 °C and a flow rate of 2 mL/min of CO_2 on a highly deactivated *DELTABOND™* cyanopropyl column with an outlet pressure of 4000 psi was employed. The percent methanol was increased to about 33% by the end of the chromatographic run as shown in Figure 24with an analysis time of less than six minutes. Unsubstituted sym-triazine (1,3,5-triazine) elutes first followed by the chloro substituted sym-triazines. Among these substituted triazines, the one with two propyl groups elutes before the analyte with one propyl and one ethyl followed by the analyte with two ethyl groups. The two asym-triazines (1,2,4-triazines) elute next followed by the two, thiol containing compounds. Throughout the separation the flow of CO_2 was maintained constant while the flow of methanol was gradually increased. The baseline shifted slightly when the amount of methanol was increased beyond 33%.

The same separation was then carried out under exactly identical conditions but at a flow rate of 4 mL/min, Figure 25. The flow of methanol in this case was also increased by a factor

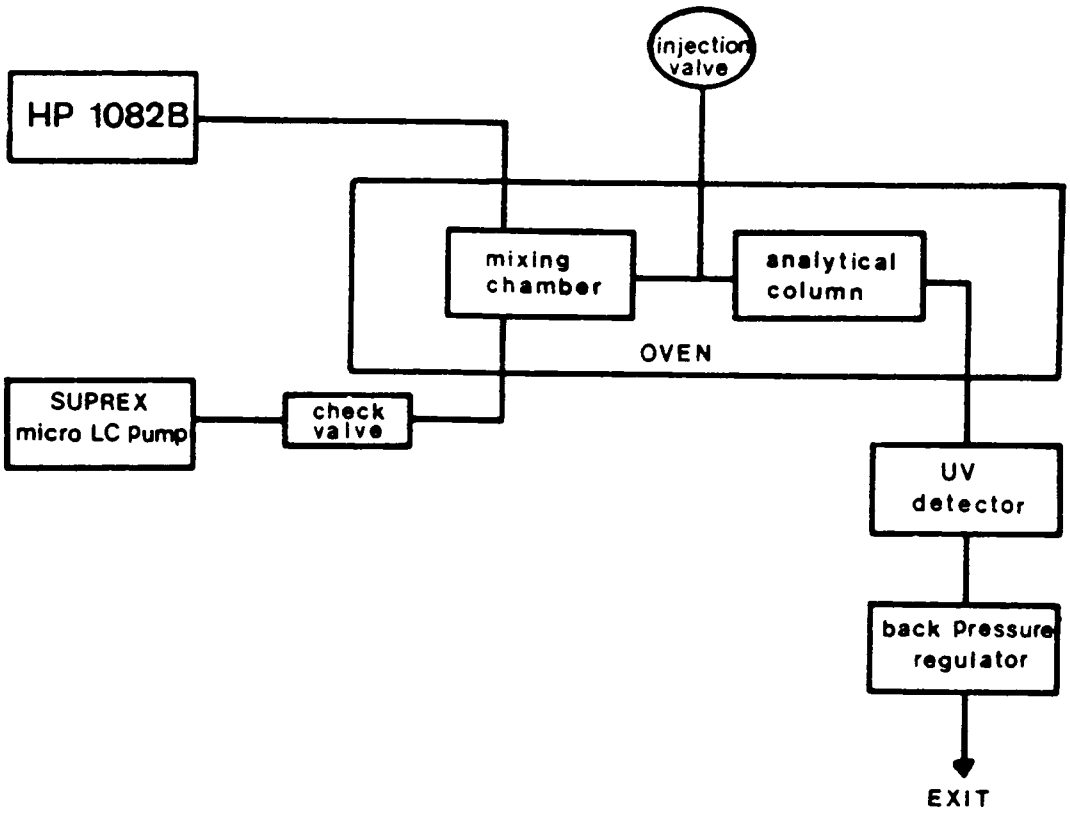


Figure 23. Instrumentation for mobile phase gradient SFC.

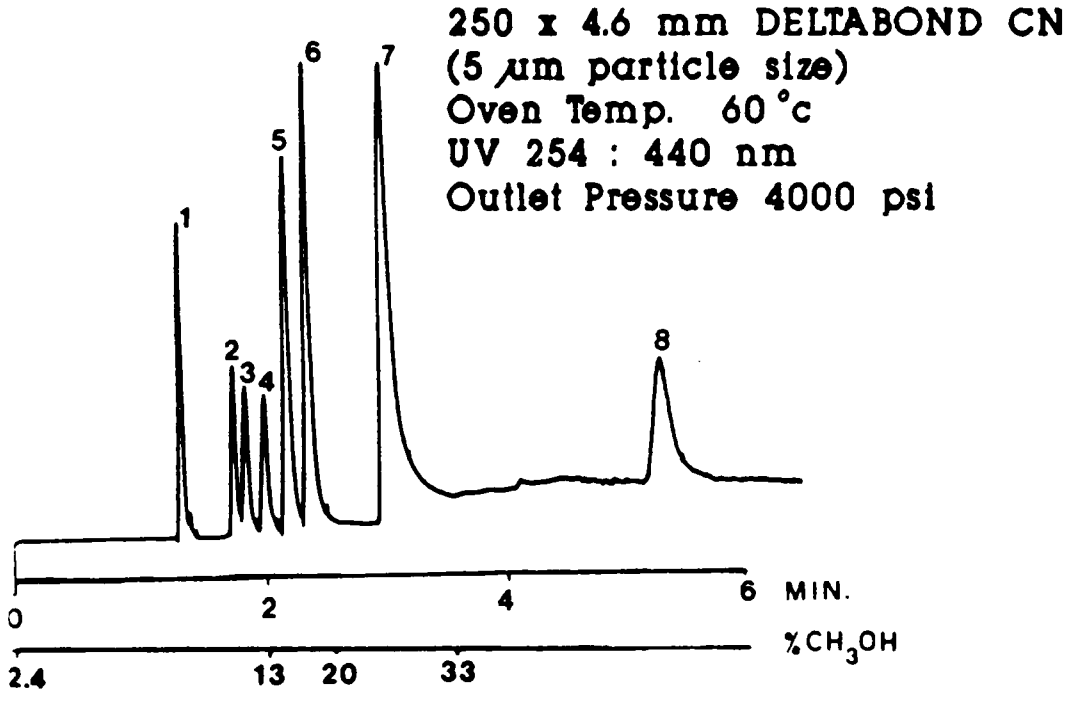


Figure 24. Separation of triazine mixture at a flow rate of 2 mL/min.: See Figure 22 for peak identification.

of two to achieve the same gradient as in the previous case. Both separations are quite comparable. The resolution between the closely related compounds, namely propazine, atrazine and simazine, is slightly lost at a higher flow rate but the total analysis time is reduced by a factor of two. The peak shape for component 8 has deteriorated somewhat at the higher flow rate.

The next goal was to develop the same separation at a different temperature and/or a different pressure and study the effect of the changed parameters on resolution. Initially the experiment was performed at the same outlet pressure of 4000 psi as before but at a temperature of 100 °C. The separation at the elevated temperature (2 mL/min) was very similar to that achieved at the lower temperature as seen in Figure 26. Peak shapes and retention times were almost identical in both the cases. The increase in temperature probably does not change the density and the solvating power of the methanol modified mobile phase significantly thereby having little effect on the separation. Next, the outlet pressure was reduced to 2000 psi with the same gradient and the separation was carried out at 60 °C (Figure 27). Most of the components are retained longer due to reduced density of the mobile phase, yet the resolution between peaks is comparable to previously described separations. It is important to note that mobile phase even with 2.4% of methanol in the initial stages of the separation is in the subcritical phase. The sym and asym triazines in this case required higher percent (20 %) of methanol for elution in this case. The elution of the most polar component of the mixture, trithiocyanuric acid, is little affected by the changes in both temperature and pressure.

The addition of base, tetramethylammonium hydroxide, to methanol was found to have a great effect on the gradient elution of acidic and basic PTH amino acids as reported previously by Berger et al.. The same base was added to methanol (0.001 M) and the separation of triazines was attempted. The addition of base in this case was found to have a slightly negative effect on the elution in this case. Hexanol instead of methanol was also tried for the triazine separation. The kinetics of mixing of CO_2 and hexanol was however found to be very slow with our instrumental setup. The failure to achieve a homogeneously mixed mobile phase mani-

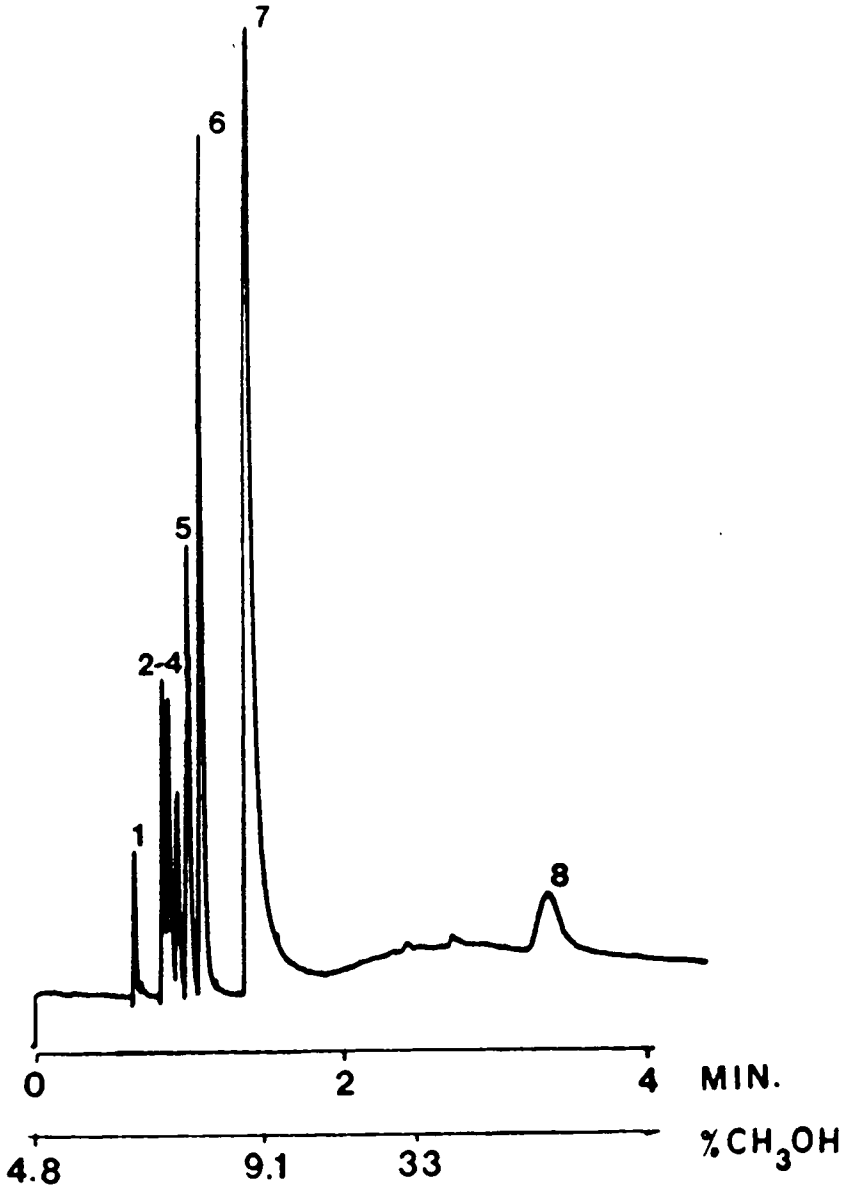


Figure 25. Separation of triazine mixture at a flow rate of 4 mL/min.: See Figure 22 for peak identification and Figure 24 for experimental conditions.

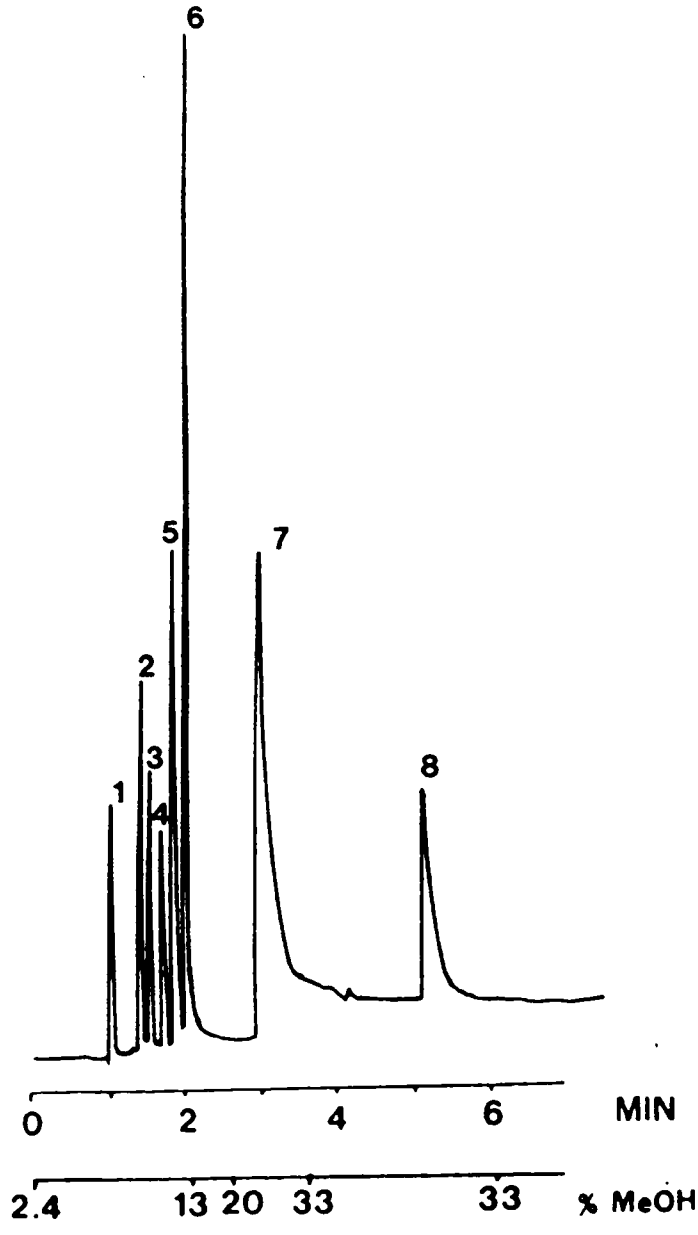


Figure 26. **Separation of triazine mixture at 4000 psi outlet pressure and 100 c:** Peak identification and experimental parameters same as in Figure 24.

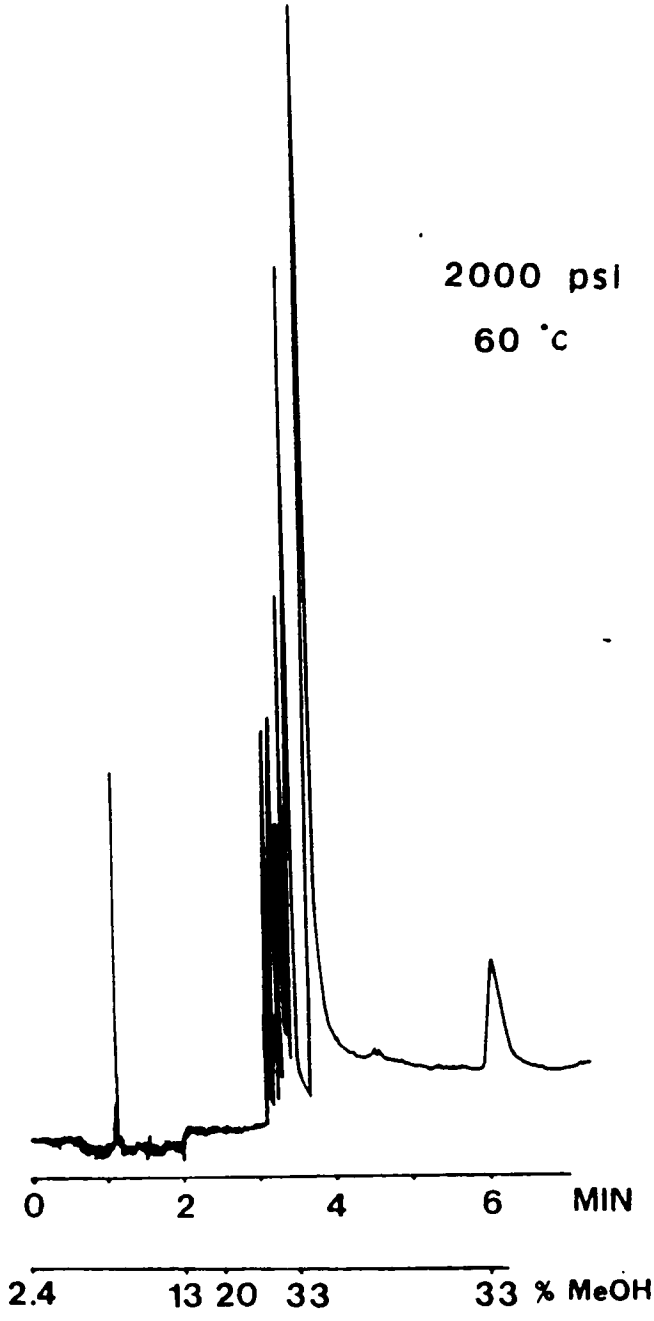


Figure 27. Separation of triazine mixture at 2000 psi outlet pressure and 60 c: Peak identification and experimental parameters same as in Figure 24.

fested itself in a very unstable and noisy baseline probably due to air bubbles introduced into the flow cell.

In summary it can be concluded from these experiments that little apparent loss in resolution occurs with a 100% increase in flow rate. Further if a high pressure is maintained, the separation under subcritical conditions is quite comparable to the separation achieved under supercritical conditions.

Little chromatography literature is available for asym-triazine and triazole derivatives. Most of these compounds are high boiling, therefore GC cannot be used for their analysis. High temperature GC has been employed for analysis of sym-triazine derivatives. Both normal phase and reversed phase HPLC methods have been used to analyze these herbicides. SFC, on the other hand, offers certain advantages, such as high solute diffusivity and high resolution per unit time, over HPLC and hence can be employed for analysis of these polar herbicides using gradient elution as shown above. HPLC and gradient elution SFC are comparable in terms of resolution and analysis times. Gradient elution, where higher percent of polar modifier is used, bridges SFC and HPLC and leads to faster analysis. Also, higher mobile phase flow rates reduce the analysis time drastically, thereby making this technique suitable for routine analysis.

5.0 Chapter 5 : Efficiency of Packed and Capillary Columns

5.1 Introduction

Supercritical fluid chromatography (SFC) has become increasingly popular as an analytical technique for past couple of years, mainly because of its potential for the analysis of non-volatile, thermally labile and high molecular weight compounds. The favorable properties of a supercritical fluid, such as its higher density as compared to a gas and higher diffusivity and lower viscosity as compared to that of a liquid, make it a mobile phase with desirable chromatographic properties.[145] SFC can be applied to the compounds where gas chromatography (GC) cannot be confidently used. SFC offers higher efficiency per unit time than high performance liquid chromatography (HPLC) and is compatible with the commonly used GC and HPLC detectors. SFC is also equally amenable to both packed and capillary columns.

While many workers have extolled the virtues of both types of columns for SFC, experimental comparisons are generally lacking, wherein typical operating conditions and identical parameters (where feasible) are employed. Schwartz et al., however, have made an excellent

comparison of these two types of columns based on theoretical considerations.[146] Figure 28 shows theoretically generated van Deemter curves from this study for both packed and capillary columns. Three molecules (A, B, C) of different molecular weight were chosen as models to generate these plots. The smallest molecule (A) was assumed to have molecular weight of 78 amu; the intermediate molecule (B) with molecular weight of 500 amu and the largest molecule (C) with a molecular weight of 2000 amu. A capacity factor of 2 was assumed for all three molecules on both types of columns. To achieve the same capacity factor, a low density of 0.28 g/mL, an intermediate density of 0.54 g/mL and a high density of 0.79 g/mL were assumed to be required to elute the small-intermediate-large molecules, respectively. It is evident from the plots that there is an optimum mobile phase linear velocity where the efficiency of the column is the greatest. At linear velocities higher than optimum, the efficiency of the column decreases. This effect of higher linear velocity on column efficiency was predicted to be more pronounced for larger molecules due to their higher elution density than for smaller molecules. Also, for the same typical molecule, a capillary column of internal diameter of 50 μm was suggested to show more dramatic increase in plate height (HETP) at increased linear velocities while a packed column was predicted to be more tolerant of increases in linear velocity of the mobile phase. This implies that higher working linear velocities can be used for a packed column without significant loss of efficiency, which ultimately leads to faster analysis times provided sufficient resolution is available. For both types of columns, the highest density gave a decreased column efficiency for most linear velocities. In terms of total column efficiency, naturally capillary columns can be made longer and give a greater number of total plates than a packed column even at higher linear velocities.

Several researchers have studied the effect of various experimental parameters on (a) column efficiency, (b) resolution achieved for two analytes and (c) retention of the analyte for a chosen column. Sie et. al. studied the retention of alkanes using gaseous and supercritical fluid n-pentane as the mobile phase and it was discovered that the supercritical fluid was obviously a better solvent than its gaseous counterpart and much lower k′ values were obtained under supercritical conditions.[147,148] Also, the authors compared efficiency of a packed column

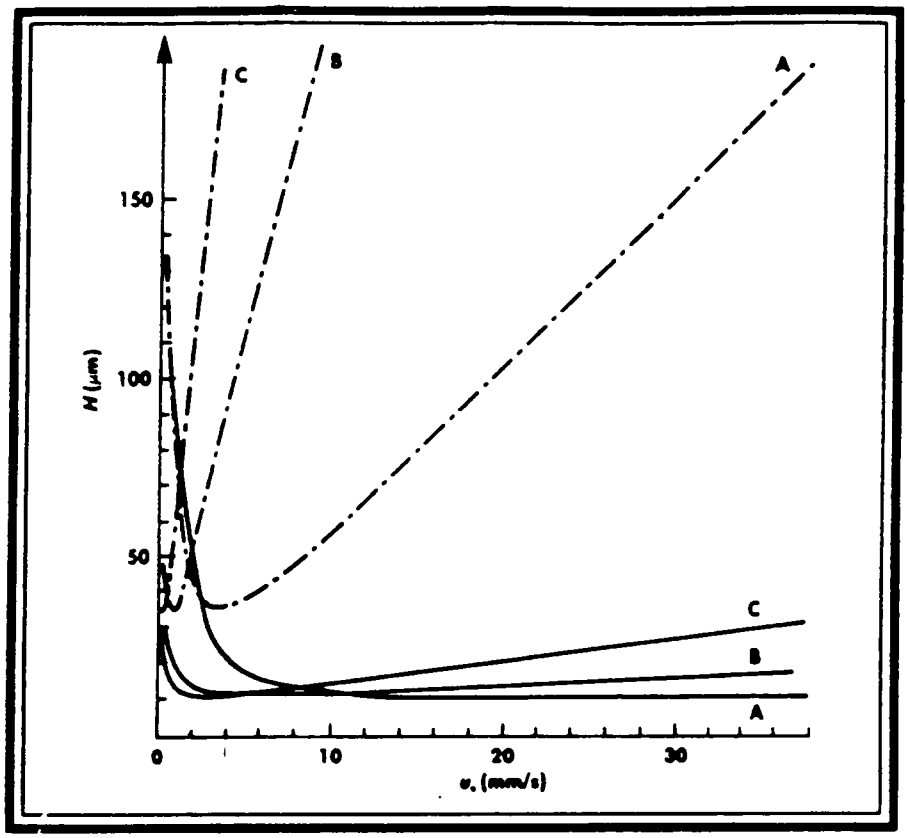


Figure 28. Theoretical van Deemter plots for packed and capillary columns.: (From Ref. 146).

(6 mm i.d.) with liquid and supercritical n-pentane. The higher efficiency achieved using the supercritical fluid mobile phase was attributed to the lower viscosity and higher diffusivity of the supercritical fluid mobile phase as compared to the liquid mobile phase. Mathematical treatment of the influence of capillary column internal diameter, stationary phase film thickness and injector - detector - connecting tubing volumes has been reported by Peaden and Lee.[149] The fundamentals of capillary column performance and van Deemter plots for alkanes using n-butane mobile phase have been published by Novotny et. al..[150,151] They used uncoated columns to obtain fundamental information on diffusion coefficient for a non-retained solute. Capillary columns of different radii were compared to show that the column with a lower internal diameter offers higher efficiency. For a coated column, the efficiency was shown to decrease as the molecular weight and hence the solute retention increased when hydrocarbons were used as analytes.[152]

Randall compared van Deemter curves obtained experimentally with packed and capillary columns using hydrocarbon analytes[153] and 100% CO_2 as the mobile phase. The curves matched well to those predicted based on theoretical calculations.[148] Randall also showed that capillary SFC and GC offer comparable column efficiencies at optimum linear velocity but high linear velocities could be employed for GC as compared to SFC. Packed column SFC was also stated to afford high efficiency at higher linear velocities than those velocities commonly employed for capillary SFC. A capillary column was in addition stated to offer higher efficiency for a smaller analyte molecule as compared to a larger analyte molecule. Gere et. al. obtained van Deemter plots for analytical scale columns packed with different particle size material using hydrocarbon analytes. It was illustrated that higher column efficiency and increased optimum linear velocity are realized as the particle diameter decreases.[154] A modified van Deemter equation has been proposed to show the dependance of reduced plate height (h) on reduced linear velocity with an analytical scale packed column using aromatic hydrocarbons as the analytes.[155] The modified equation accounts for the density drop in the column which was stated to lead to peak broadening and can be used when the compressibility of the mobile phase cannot be neglected.

From this brief review, it appears that the literature available on the fundamental studies of column performance in SFC deals with nonpolar and/or volatile solutes as the analytes. Since SFC is more uniquely applicable to the analysis of polar and nonvolatile compounds where GC can not always be applied, fundamental studies in SFC using polar analytes seemed appropriate. In our laboratory we have extended this technique to analytes such as sugars, steroids, pharmaceuticals and compounds of agricultural and environmental interest. A study involving efficiency, retention and resolving power of both packed and capillary columns was undertaken and results obtained are presented here.

5.2 *Experimental*

5.2.1 Instrumentation

A Suprex (Pittsburgh, PA) model 200A supercritical fluid chromatograph with flame ionization detector (FID) was used for both packed and capillary columns. The mobile phase was 100% CO_2 (Scott Specialty Gases, Plumsteadville, PA). Samples were injected onto each column through a 100 nL internal loop injection valve (Valco Instruments, Houston, TX).

A 100 x 1.0 mm *DELTABOND™* cyanopropyl column (Keystone Scientific, Bellefonte, PA) packed with 5 μm particle size material and a capillary column of SB-cyanopropyl-25 (i.e. 25% cyanopropyl- 25% phenyl- 50% methyl polysiloxane) fused silica (10 m x 100 μm) with a film thickness of 0.25 μm (Lee Scientific, Salt Lake City, UT) was used for this study. The FID temperature was maintained at 375 °C. Acetanilide and 4-chloroacetanilide both with boiling points greater than 300 °C were purchased from Sigma Chemicals (St. Louis, MO). A mixture of 2 mg/mL of each component was prepared in pesticide grade methylene chloride (J. T. Baker, Phillipsburg, NJ).

All the experiments for both packed and capillary columns were carried out under isoconfertic and isothermal conditions. The linear velocity was varied by using a different pulled tapered restrictor in the case of packed column or by progressively cutting a frit restrictor in the case of capillary column. The density of the mobile phase was varied at a constant temperature of 60 °C from 0.5 g/mL to 0.85 g/mL. Identical experiments were then repeated at three other constant temperatures of 80 °C, 100 °C and 120 °C. Each injection was made in triplicate.

5.2.2 Calculation of efficiency

The most commonly used method for manual calculation from a chromatogram is that via triangulation (e.g. peak width at half-height times the peak height). This method, however, does not give very satisfactory results in case of tailing peaks[158] The "height/area ratio" is one of several methods suggested for measurement in this case. According to this method the efficiency of the column can be calculated as follows:

$$N = 2\pi(\frac{h}{A})^2 t_R^2$$

where, N is the plate number of the column, h and A are the height and area of the peak, respectively, and t_R is the uncorrected retention time of the analyte. Since this method also accounts for peak tailing, a more realistic number for the total plate count of the column is obtained. The height/area ratio and the retention time of the peak were obtained from the integrator (Hewlett Packard 3390A) used for this experiment. The height equivalent to theoretical plate (HETP) was calculated as H = L/N where, L, the length of the column is in mm.

5.3 Results and Discussion

Several points have guided us in this study. First, we wanted to employ as similar stationary phase (cyanopropyl) and column deactivation as possible with the same mobile (100% CO_2) phase. Second, analytes were desired which are highly polar and where the possibility of a mixed separation mode (e.g. GC-like/LC-like) is not likely. Amides boiling above 300 °C seemed to satisfy this requirement. Third, isobaric and isothermal separations were mandatory, even though peaks were sometimes broad and retention times lengthy. The commonly used restrictor (tapered-packed, frit-capillary) with each column was utilized. Finally, we attempted to employ column parameters which were fairly typical in each case.

The efficiency of each column was calculated at various linear velocities of the mobile phase. A van Deemter plot of H vs linear velocity (μ) was next obtained at each constant density under various isothermal conditions. The nature of the van Deemter curves obtained, can be rationalized and explained based on chromatographic theory. Hawkes[157] has introduced several modifications to the original van Deemter equation

$$H = A + B/\mu + C\mu$$

where A is the multipath effect term, B is the longitudinal diffusion term and C is the resistance to mass transfer term. The multipath model indicates that a variety of multipaths must spread out the molecules and increase H. Since this effect is caused by diffusion, this term is flow- dependent and therefore can be included in the C term as follows :

$$H = \frac{B}{\mu} + (C_s + C_m)\mu$$

where, C_s is the resistance to mass transfer in the stationary phase and C_m is the resistance to mass transfer in the mobile phase.[158]

Figure 29 illustrates how each of these terms probably contributes to HETP. The longitudinal diffusion term, B, is a function of the solute diffusivity in the mobile phase D_m and the linear velocity μ as follows :

$$B = \frac{2\gamma D_m}{\mu}$$

where γ = obstruction factor. At fast mobile phase linear velocity, this term is not very significant since its contribution to HETP is minimal.

The effect of an increase in density on the the contribution of the B term to HETP can be explained as follows. As the density of the mobile phase increases at a fixed temperature, the viscosity of the mobile phase also increases. Since the linear velocity is directly proportional to the density with a fixed restrictor, a higher linear velocity is afforded at elevated densities. The overall effect, therefore, of an increase in linear velocity and a decrease in solute diffusivity created by an increased density leads to a decreased HETP. Consequently, the B term contribution to HETP should diminish at elevated density.

The next term of the van Deemter equation is the C_s term which can be expressed as

$$C_s = q \frac{k'}{(k'+1)^2} \frac{d_f^2}{D_L} \mu$$

q = constant, k' = capacity factor, d_f = average stationary phase film thickness, D_L = solute diffusivity in the stationary phase. The resistance to mass transfer in the stationary phase, C_s is affected by solute retention, solute diffusivity in the stationary phase and film thickness of the stationary phase. Assuming, there is no swelling of the stationary phase[159] over the experimental parameters utilized (density: 0.5 g/mL to 0.85 g/mL and temperature: 60 °C to 120 °C), changes in d_f and D_L will be insignificant and their contribution to C_s can be ignored. The important factor in addition to μ as far as the C_s term is concerned is then the capacity factor. As density increases, solute is retained less by the column and lower capacity factors are obtained. The term $\frac{k'}{(k'+1)^2}$ is directly proportional to k' for values k' < 1 while this term varies inversely

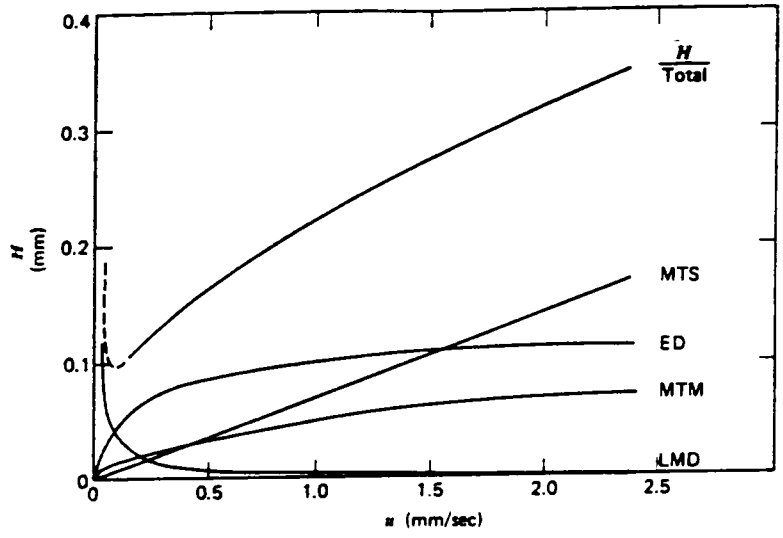


Figure 29. Typical plot of the rate equation for LC..(From Ref. 158).

with k′ when k′ > 1. This suggests that the contribution of the C_s term to HETP will depend upon whether the values of k′ are less than or greater than 1. For most of the data obtained using capillary columns, k′ < 1 were achieved therefore, C_s and hence HETP will decrease with an increase in mobile phase density. For data obtained using a packed column, k′> 1 were achieved therefore, as the density increases, C_s and HETP increase. Thus the C_s term is affected differently by increases in density for packed and capillary columns. It should, however, be noted that regardless of the capacity factor value, the term $\frac{k'}{(k'+1)^2}$ changes very little with changes in k′. From this argument low density would be expected to enhance efficiency with packed columns, whereas high density would favor increased efficiency with capillary columns. In our study with direct injection in both cases, this situation appeared to hold, vide infra. This result is surprising since it contradicts earlier predictions.

The last term of the van Deemter equation, C_m is the resistance to mass transfer in the mobile phase and it is expressed as

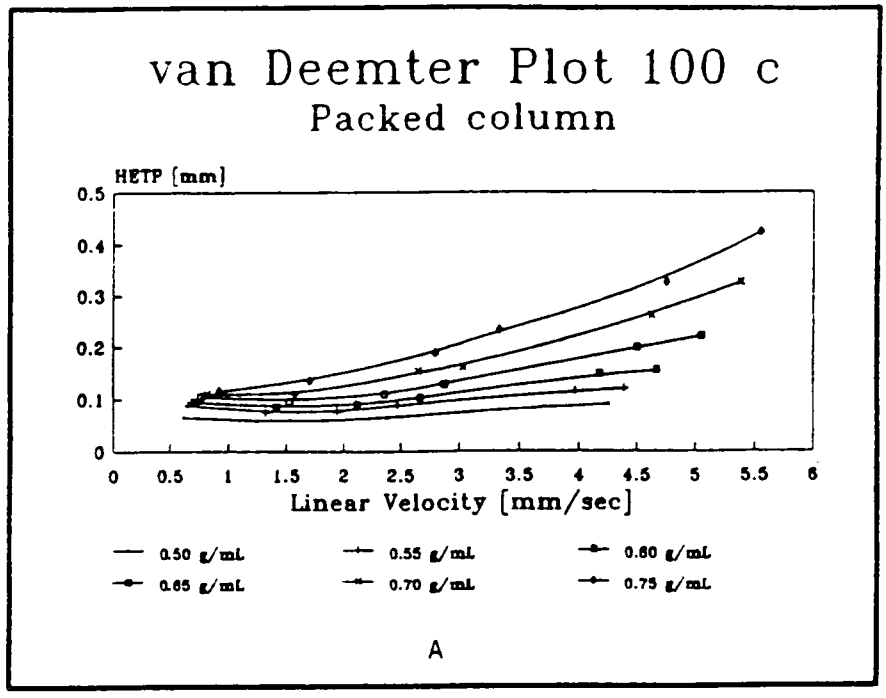
$$C_m = \frac{f_n(d_p^2, d_c^2, end)}{D_m}\mu$$

where d_p = particle diameter of the column packing, d_c = column internal diameter, D_m = solute diffusivity in the mobile phase, end = zone spreading in the injection system and in the connecting tubing. The resistance to mass transfer in the mobile phase, C_m is a function of both particle diameter or column inner diameter and injection system zone spreading. The compressibility factor and the solute diffusivity in the mobile phase also affect the efficiency of the column. Under our experimental conditions (i.e. changes in linear velocity and density) all terms except D_m will be unimportant as far as their contribution to the C_m term is concerned. D_m should decrease as the density and the viscosity of the mobile phase increase. In addition, the linear velocity should increase with an increase in density. This suggests that the contribution of the C_m term to HETP will increase at elevated densities. Thus, a higher column efficiency can be expected at a lower density for both columns as far as the effect of the C_m term on HETP is concerned.

Plots of HETP vs μ for packed and capillary columns at a constant temperature of 100 °C and various constant densities are shown in Figure 30. Five different linear velocities and six densities were employed with the capillary column whereas, six linear velocities and six densities were used for the packed column. The combined contribution of B, C_s and C_m terms should determine the shape of the van Deemter curve. For a packed column faster operating flow rates are employed therefore the contribution of the B term is believed to be insignificant as compared to the C_s and C_m terms. For both C_s and C_m terms, a higher efficiency is predicted at a lower density. The van Deemter curves are shown for densities ranging from 0.5 g/mL to 0.75 g/mL. As the density increases, it is observed that HETP does in fact increase when the temperature is maintained constant, Figure 30a. At a fixed lower density an increase in linear velocity leads to practically no loss in column efficiency while at a fixed higher density, decreased packed column efficiency is found when linear velocity is increased.

For the capillary column, an exactly opposite effect of density on column efficiency is observed. Surprisingly, this is contrary to what other researchers have found with less polar analytes. An examination of Figure 30b reveals this to be the case for the six densities examined. Naturally with 5 μm particles the packed column demonstrated a lower HETP than the capillary column with 100 μm inner diameter. The van Deemter plots at 60 °C, 80 °C and 120 °C (shown in the appendix) exhibit similar trends for both types of columns.

The effect of temperature on column efficiency under an isoconfertic (0.7 g/mL) condition is shown in Figure 31. The shape of the curves can be explained based on similar arguments as in the case of increasing density. Increasing temperature increases solute diffusivity in the mobile phase, thereby, causing the B term to increase and if dominate should result in lower column efficiency. Our data suggest that this is the case for packed columns. The situation appears to be reversed for the capillary column (i.e. the highest temperature yields the lowest HETP). The differences in the way that column efficiency depends on both density at fixed temperature and on temperature at fixed density for the two columns was puzzling especially since it goes against previous notions. Further the minima seen in plots for the capillary are not easily rationalized.



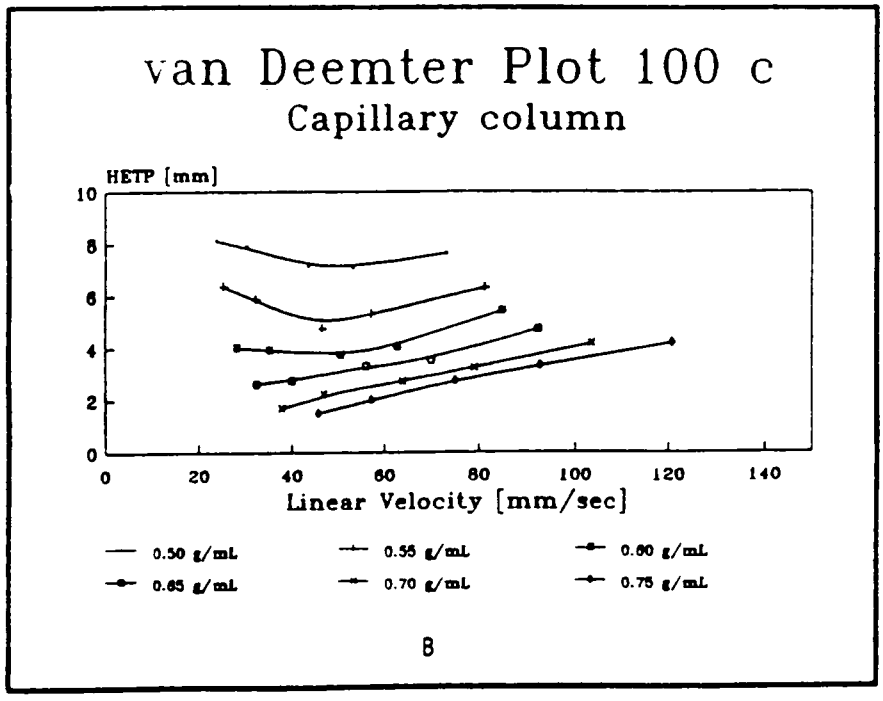
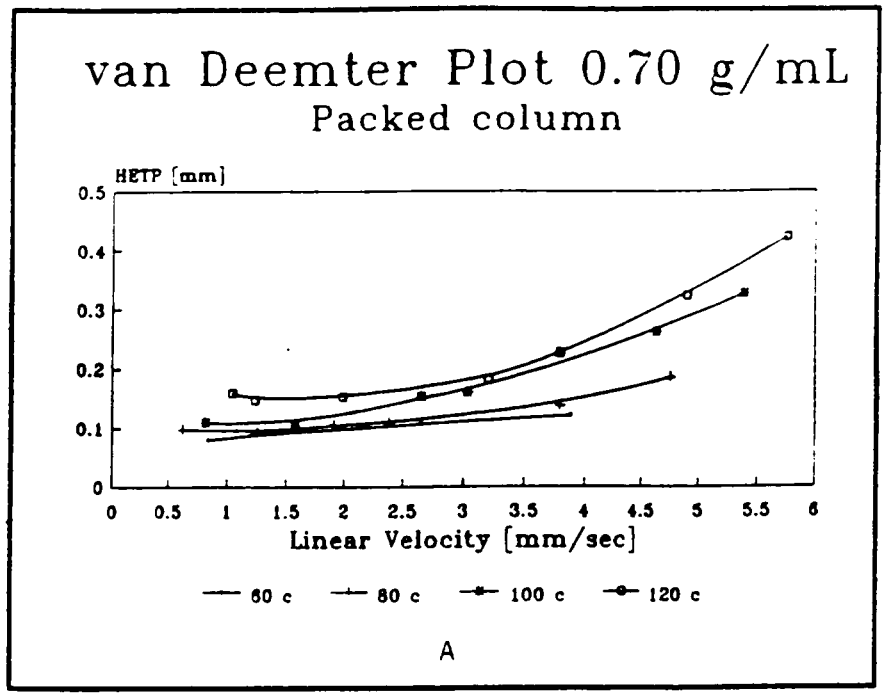


Figure 30. **van Deemter plots at different densities at 100 c** (a) Packed column (b) Capillary column.



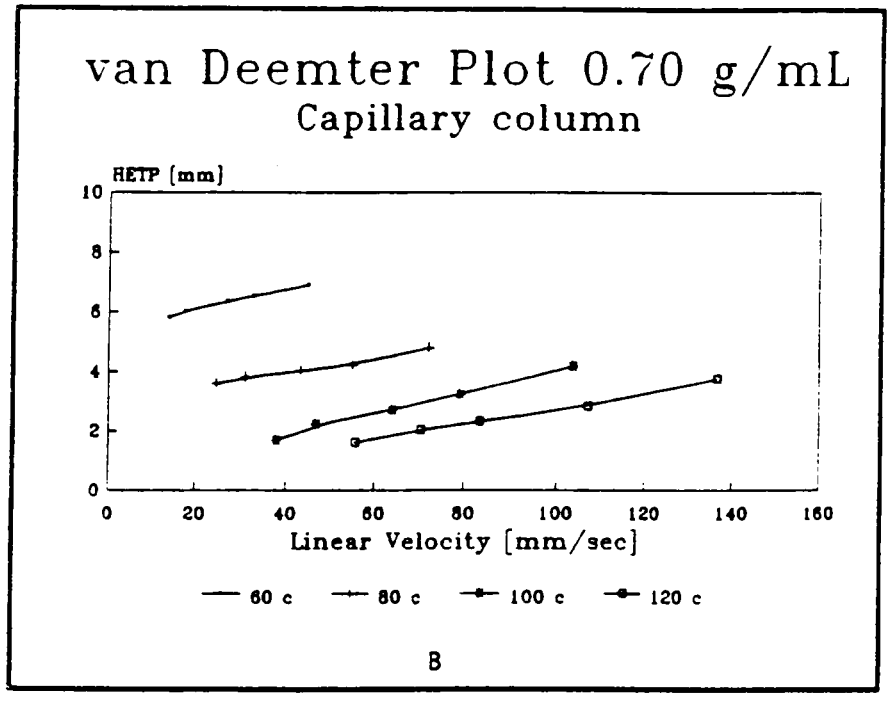


Figure 31. van Deemter plots at different temperatures at 0.7 g/mL. (a) Packed column (b) Capillary column.

Overloading of the capillary column with 200 ng of injected (100 nL injection volume) amount was suspected to account for this anomaly and hence a study of the effect of injected analyte mass on the column efficiency was performed as shown in Figure 32. Surprisingly, no significant change was observed in the column efficiency at a fixed temperature of 100 °C and a fixed density of 0.55 g/mL, when the injected amount of solute was increased from 12.5 ng to 200 ng with an injection volume of 100 nL (no split). It should be pointed out that at no time did analyte peaks demonstrate features (fronting) that would suggest column overload. Zone spreading in the injector was next thought to be significant and hence it cannot be neglected. Based on theoretical calculations, a 100 nL solvent plug could cause a significant increase in HETP. A similar study therefore of HETP vs injected amount using a 1:10 split ratio was performed. The results showed that column efficiency significantly increased with column loading when a more narrow solvent plug of about 10 nL was introduced onto the column. A plot of HETP vs injected amount shows a continuous increase in HETP when the injected amount was increased from 10 ng to 100 ng, Figure 32. At an injected amount of 100 ng, both split and splitless injections offered comparable column efficiency.

From these results it became necessary to repeat the study of the effect of density and temperature on column efficiency using a split injection technique. An intermediate linear velocity from the van Deemter plots generated with splitless injections was chosen for this study with a split ratio of 1:10. At four different temperatures the effect of density on column efficiency was studied and the data (Table 5) were obtained with triplicate injections to calculate the column efficiency. At a fixed temperature, higher column efficiency was now achieved at a lower density than at a higher density. Similar comparisons at two different temperatures showed that at a fixed density, a higher column efficiency is achieved at a lower temperature than at a higher temperature as indicated in Table 6. These data show trends similar to those observed in the case of the packed column. Under the ideal situation, when the columns are not overloaded and a smaller liquid plug is introduced on the column, both packed and capillary columns offer higher efficiency at a lower density and at a lower temperature. At higher temperature and/or higher density, peak tailing for these polar analytes was found to be greatly reduced which ul-

timately led to an increased column efficiency. However, at lower linear velocity and lower density, there was no significant change in peak tailing. Also, overloading of the column and a large liquid plug lead to increased peak widths, thereby increasing HETP instead of further decreasing HETP even at lower linear velocities. Thus, this "false" increase in HETP observed at lower linear velocity and lower density can be attributed to the direct injection technique employed. The minimum observed in the van Deemter plot for the capillary column therefore should not be mistaken for the optimum linear velocity.

The effect of an increase in both density and temperature on the capacity factor of 4-chloroacetanilide retention was studied with direct injection technique. Figure 33 shows plots of log k′ vs increasing density for different temperatures. Since k′ and density are related logarithmically, log k′ plotted against density shows a linear relationship. At a fixed temperature, retention decreases as density increases for both packed and capillary columns. At higher density, the linear velocity of the mobile phase increases thereby eluting the solute faster from the column. Also, at elevated density, the solute is more soluble in the mobile phase. At higher temperatures and at fixed density, the linear velocity rises (t_0 decreases) and the solubility of the solute in the mobile phase increases thereby decreasing the capacity factor.[160] It should be remembered that all the data shown in each figure were generated with one restrictor at different densities and different temperatures. The statistical T-test was performed on these data. It was confirmed from these tests that each point was statistically different. The influence of an increase in both density and temperature on solute retention is similar to the data obtained previously by several workers.[161–164]

Figure 34 illustrates how the resolution achieved on these columns is affected by changes in density and temperature. As expected, the resolution which is defined as

$$R = \frac{2\Delta t_R}{w_1 + w_2}$$

(where, w_1 and w_2 are the peak widths at the base), decreases with an increase in temperature at a fixed density and with an increase in density at a fixed temperature for both types of col-

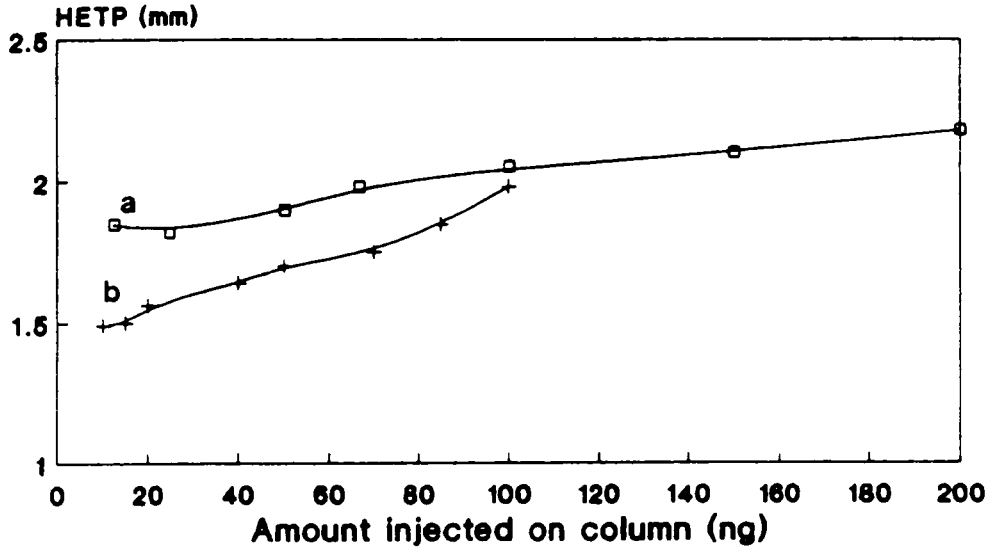


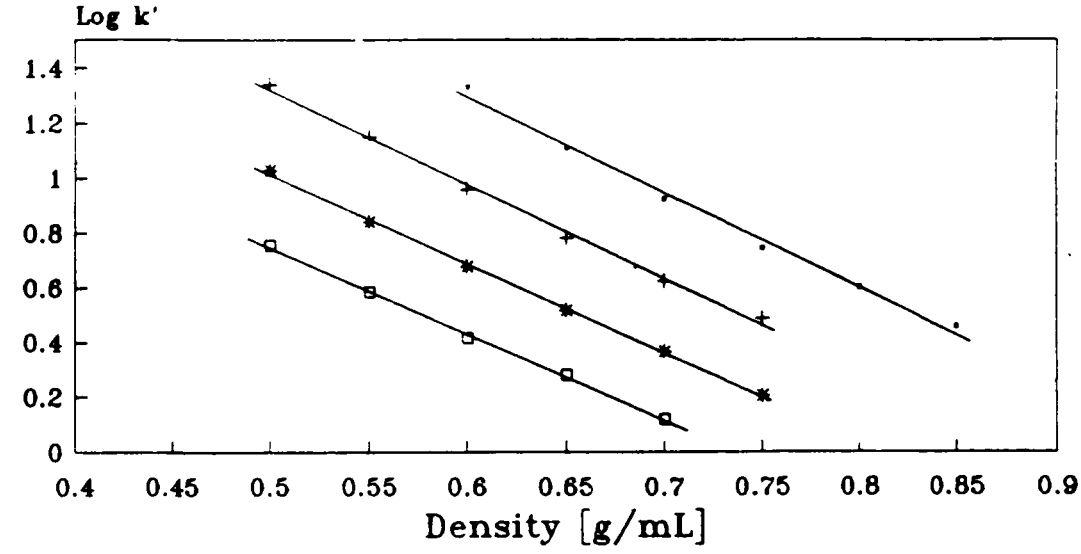
Figure 32. HETP vs Amount Injected at 0.55 g/mL and 100 c: (a) Direct injection technique (b) Split injection technique.

Table 5. Density effects on column efficiency with split injection.

Temperature (°c)	Density (g/mL)	HETP (mm)
60	0.65	1.78
	0.80	1.84
80	0.65	1.90
	0.80	2.04
100	0.55	1.53
	0.70	1.92
120	0.55	1.73
	0.70	2.53

Table 6. Temperature effects on column efficiency with split injection.

Density (g/mL)	Temperature (°c)	HETP (mm)
0.65	60	1.78
	80	1.90
0.80	60	1.84
	80	2.04
0.55	100	1.53
	120	1.73
0.70	100	1.92
	120	2.53



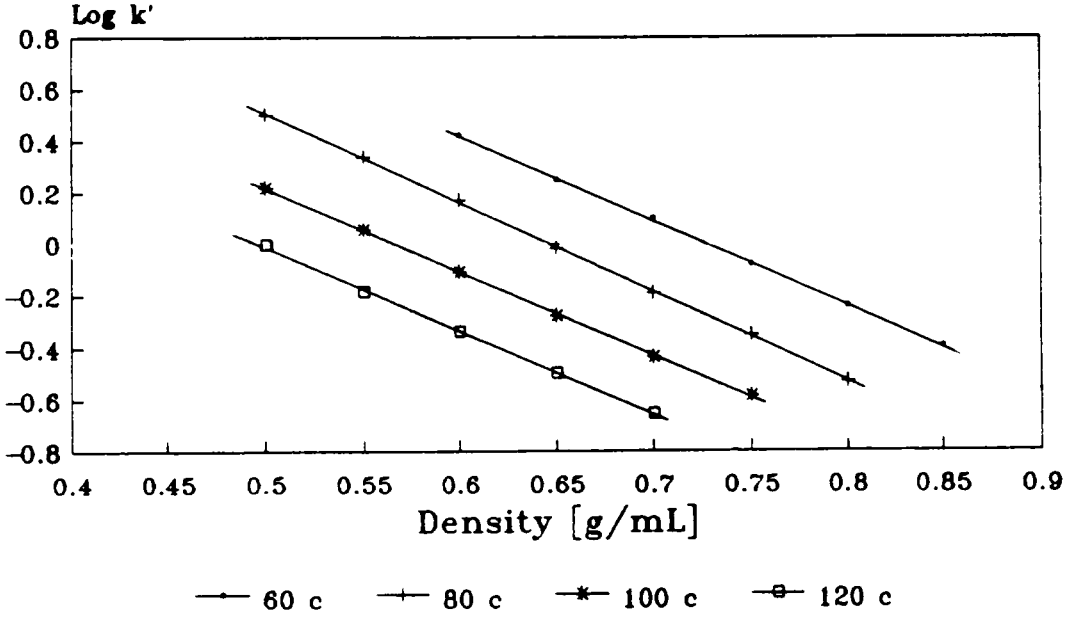
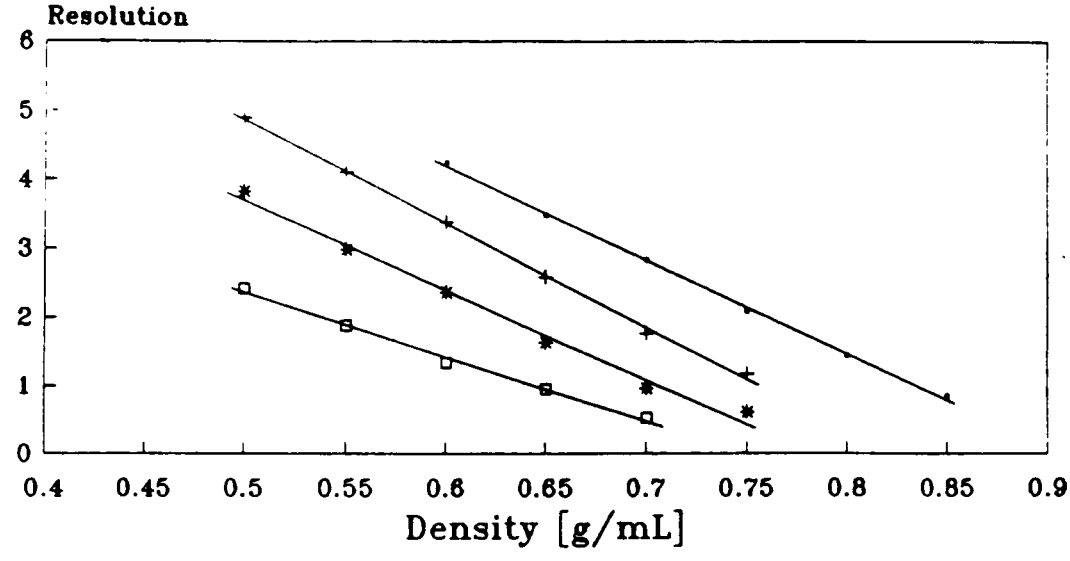


Figure 33. Plots of log k' vs density at different temperatures. (a) Packed column (b) Capillary column.

umns. Again, with the help of T-test each point on these curves was shown to be statistically different.

At elevated temperature and/or density, the solubility of both the solutes in the mobile phase should increase. The faster elution of both the solutes decreases the difference in their retention times. Also, at higher density and higher temperature the solute zone movement in the mobile phase is faster due to higher linear velocity. At higher density, the viscosity of the mobile phase increases which may also lead to the band broadening. Thus, decreased t_R and increased peak widths lead to decreased resolution. Better resolution for both types of columns can be achieved at both a lower temperature and a lower mobile phase density. This observation is supported by data obtained previously.[147,148] It is noteworthy that under the typical chromatographic conditions employed in this study, resolution of these two analytes for these two columns is very comparable when a direct injection technique was employed with capillary columns. When a split injection technique was used, a similar trend as with direct injection was observed. Resolution was seen to decrease with an increase in temperature and/or density. But, the resolution achieved with split injection was substantially higher than that achieved with direct injection. At elevated temperatures and densities, due to increased peak widths, a lower resolution was obtained with direct injection. the narrow solute bands attained with split injection gave a higher column efficiency. The column efficiency and the resolution achieved are directly related and hence a higher resolution was achieved.

Based on the data obtained for both types of columns, several conclusions can be drawn. The van Deemter plots generated at various densities and temperatures illustrate that packed columns offer higher column efficiency at lower temperature and lower density while capillary column appears to offer higher efficiency at a higher temperature and higher density when direct injection is employed and the column is overloaded with the analyte. However, capillary column shows the same trend as that of packed column when split injection is used to introduce the sample onto the column. The plug of solvent introduced on the column affects the column performance and hence the column efficiency. Direct injection is commonly not employed with capillary columns; the adverse effects of the same are demonstrated here. The data comparing



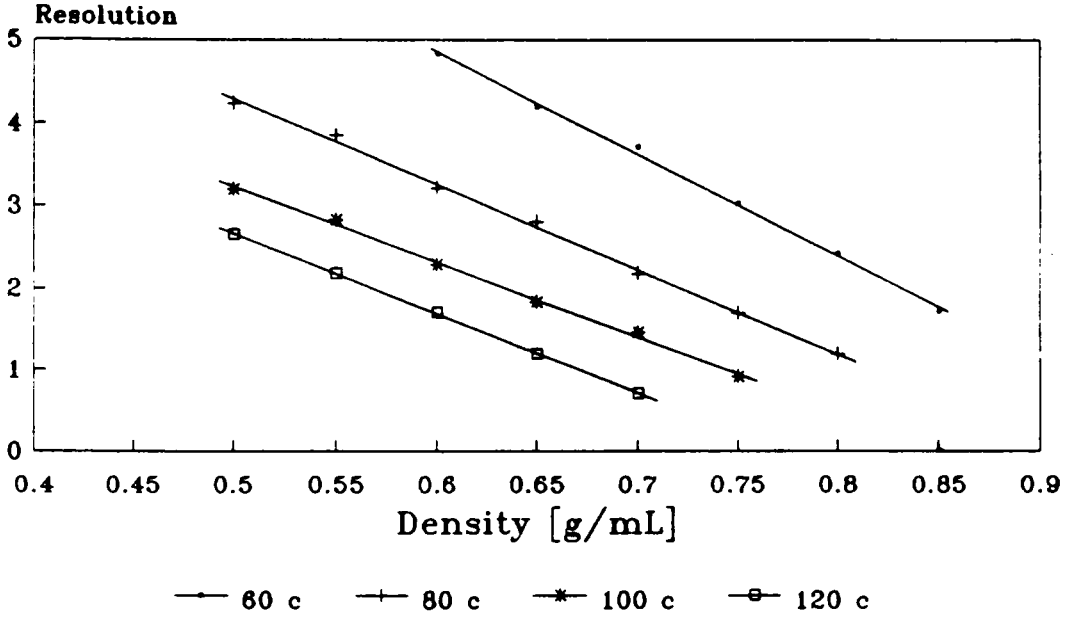


Figure 34. Plots of resolution vs density at different temperatures. (a) Packed column (b) Capillary column.

the split and the direct injection illustrate the deleterious effects of a larger solvent plug and increased analyte loading on the column efficiency. The data and results presented here for packed column are, however, in good agreement with those obtained by Gere[154] and Mourier et al.[155] illustrating that at higher density, due to decreased capacity factors, packed columns show a decreased column efficiency.

Fundamental studies of this kind provide an insight into the theoretical aspects of SFC and could act as a guideline to optimize experimental parameters to be chosen for the analysis of polar compounds by SFC. However, one should be careful in selecting the operating conditions for each type of column. Packed column appears to allow more experimental flexibility while with capillary column, exact optimum experimental parameters need to be employed to achieve the maximum column efficiency. This type of study can be performed by taking different approaches. The experimental conditions could be changed for each type of column so as to achieve the same capacity factor for a solute, as was assumed by Schwartz, and the comparisons be made. On the other hand, the experiments could be carried out employing exactly the same parameters to study the column performance as was done in this case. Both retention and resolution are seen to decrease at higher density and/or higher temperature. The efficiency and hence the resolution are better for a capillary column when split injection as opposed to direct injection is employed.

6.0 Chapter 6 : Conclusion

The principal goal of this research work has been to develop SFC methods to analyze polar compounds. Due to the favorable physical properties of the supercritical mobile phase, SFC offers certain advantages over both GC and HPLC. SFC can be applied to the analysis of polar, nonvolatile and thermally labile compounds of industrial interest. Both packed and capillary columns have been used to separate model mixtures of polar compounds such as steroids and agricultural compounds. On-line FT-IR detection can also be performed when 100% CO_2 is used as the mobile phase. Fundamental studies involving efficiency, retention and resolution were also performed to evaluate packed and capillary columns.

Chapter two covers several aspects of on-line FT-IR detection with both HPLC and SFC. It was demonstrated that the flow cell approach provides very low detection limits which are comparable to the MS detection. An analytical scale column was used under HPLC and SFC conditions for the analysis of steroids and it was observed that a higher efficiency was achieved for the same column when liquid mobile phase was employed as opposed to a supercritical fluid mobile phase. Comparisons of packed and capillary columns at different temperatures using pressure gradient for analysis of the same steroid mixture showed that the packed column offers greater number of apparent theoretical plates per meter than the capillary column. Also, the capillary column offered higher efficiency at a higher operating density while the packed

column was more efficient at a lower density. The resolution achieved on both types of columns was, however, quite comparable. The feasibility of elution of polar agricultural compounds such as amides, sulfonamides and ureas with 100% CO_2 was demonstrated in chapter three. The on-line FT-IR detection provided the spectra with a very high signal to noise ratio. The major peaks of the spectrum which correspond to different functional groups of the analyte, could be used for positive identification and even for quantitation purposes. The use of analytical scale column with modified CO_2 mobile phase reduces the analysis time greatly and complete resolution with sharp peaks is achieved.

The application of gradient mobile for analysis of polar and structurally related compounds was described in chapter four. The increasing amount of polar modifier in the mobile phase elutes very polar compounds from the column which would be difficult to achieve by using only density gradient. Several triazines and triazole derivatives were separated on an analytical scale column with a dual pump system. The flow, and hence the amount of methanol were gradually increased in the mobile phase to afford the separation of a mixture of eight components in less about six minutes. The higher flow of CO_2 was found to have no detrimental effect on the separation, but on the other hand, it led to shorter analysis times. Also, at high pressure, the subcritical conditions showed no major difference on the separation.

An attempt was made to compare packed and capillary columns under normal operating conditions for both types of columns using polar and nonvolatile analytes in chapter five. The packed and capillary columns were found to offer higher efficiency under different experimental conditions. Higher temperature and higher density seemed to favor the capillary column while the packed column was found to be more efficient at lower temperature and lower density. The retention of the solute decreased at elevated temperature and at elevated density for both types of columns. Comparable resolution was achieved on a shorter packed and a longer capillary columns. When split injection technique was used with capillary column, the results regarding the efficiency were identical to that of the packed column. Since the efficiency of a column is a function of various experimental parameters, a more thorough and carefully designed study needs to be performed to be able to compare the two types of columns.

7.0 References

1 R. Barcarolo, E.Tealdo, C. Tutta, *J.of HRC & CC, 11, 539 (1988).*

2 D.F.Gurka, L.D.Betowski, T.L.Jones, S.M.Pyle, R.Titus, J.M.Ballard, Y.Tonteur, W.Neiderhurt *J. Chromatogr. Sci., 26, 301 (1988).*

3 D.F.Gurka, R.Titus, *Anal. Chem., 58, 2189 (1986).*

4 R. Adatia, M. Cooke, *Chromatographia, 25, 598 (1988).*

5 M.Korn, R. Wodarz, K.Drysch, F.W.Schmahl, *J. HRC & CC, 11, 313 (1988).*

6 G.P.Cartoni, M.Ciardi, A.Giarrusso, F.Rosati, *J. HRC & CC, 11, 528 (1988).*

7 H.Karlsson, C. Hansson *J. HRC & CC, 11, 820 (1988).*

8 L.R. Hogge, D.W.Reed, E.W.Underhill, *J.Chromatogr.Sci., 26, 348 (1988).*

9 J.H.Ng, B. Tan, *J.Chromatogr.Sci., 26, 463 (1988).*

10 N.D.Danielson, M.A.Targove, B. E. Miller, *J.Chromatogr.Sci., 26, 362 (1988).*

11 M.Leclercq, M.Fouillit, G.Panteix, R.Guinet, *J.Chromatogr., 337, 423 (1985).*

12 R.J.Bopp, J.H.Kennedy, *LC-GC Magazine, 6, 514 (1988).*

13 D.Barcelo', *LC-GC Magazine, 6, 324 (1988).*

14 W.H.McFadden, S.A.Lammert, *J.Chromatogr., 385, 201 (1987).*

15 P.Peaden, J.C.Fjeldsted, M.L.Lee, S.R.Springston, M.Novotny, *Anal. Chem., 54, 1090 (1982).*

16 B.E.Richter, D.E.Knowles, M.R.Anderson, N.L.Porter, E.R.Campbell, D.W.Later, *J. HRC & CC, 11, 29 (1988).*

17 A.F.Bushke, B.E.Berg, O.Gyllenhaal, T. Greibrokk, *J. HRC & CC, 11, 16 (1988).*

18 Y.Hirata, F.Nakata, M.Horihata, *J. HRC & CC, 11, 81 (1988).*

[19] M.Novotny, *J. HRC & CC, 9, 137 (1986).*

[20] T.C.Chester *J.Chromatogr., 299, 424 (1984).*

[21] M.W.Raynor, K.D.Bartle, I.L.Davies, *J. HRC & CC, 11, 289 (1988).*

[22] S.Green, W.Bertsch, *J. HRC & CC, 11, 414 (1988).*

[23] E.J.Guthrie, H.E.Schwartz, *J.Chromatogr. Sci., 24, 239 (1986).*

[24] M.L.Lee, K.E.Markides, *J. HRC & CC, 9, 652 (1986).*

[25] J.W.Olesik, S.V.Olesik, *Anal.Chem., 59,796 (1987).*

[26] W.R.West, M.L.Lee, *J. HRC & CC, 9, 161 (1986).*

[27] S.L.Pentoney,Jr., K.H.Shafer, P.R.Griffiths, *J.Chromatogr. Sci., 24, 230 (1986).*

[28] R.D.Smith, H.R.Udseth, *Anal.Chem., 55, 2266 (1983).*

[29] D.W.Later, D.J.Bornhop, E.D.Lee, J.D.Henion, R.C.Wieboldt *LC-GC Magazine, 5, 808 (1987).*

[30] S.Rokushika, K.P.Naikwadi, A.L.Jadhav, H.Hatano, *Chromatographia, 22, 209 (1986).*

[31] H-C.K.Chang,K.E.Markides, J.S.Bradshaw, M.L.Lee, *J.Chromatogr.Sci., 26, 280 (1988).*

[32] W.Roder, F.J.Ruffing, G.Schomburg, W.H.Pirkle, *J. HRC & CC, 11, 665 (1988).*

[33] J.S.Bradshaw, S.K.Aggarwal, C.A.Rouse,B.J.Tarbet, K.E.Markides, M.L.Lee, *J.Chromatogr., 405, 169 (1987).*

[34] J.M.Levy, W.M.Ritchey, *J.Chromatogr.Sci., 24, 242 (1986).*

[35] J.M.Levy, W.M.Ritchey, *J. HRC & CC, 8, 503 (1985).*

[36] J.M.Levy, W.M.Ritchey, *J. HRC & CC, 10, 493 (1987).*

[37] J.R.Wheeler, M.E.McNally, *J.Chromatogr., 410, 343 (1987).*

[38] J.C.Kuei, K.E.Markides, M.L.Lee, *J. HRC & CC, 10, 257 (1987).*

[39] J.B.Crowther, J.D.Henion, *Anal.Chem., 57, 2711 (1985).*

[40] T.L.Chester, D.P.Innis, *J. HRC & CC, 9, 209 (1986).*

[41] E.M.Calvey, L.T.Taylor, J.K.Palmer, *J. HRC & CC, 11, 739 (1988).*

[42] J.C.Fjeldsted, R.C.Kong, M.L.Lee, *J.Chromatogr., 279, 449 (1983).*

[43] K.E.Markides, S.M.Fields, M.L.Lee, *J.Chromatogr.Sci., 24, 254 (1986).*

[44] S.M.Fields, M.L.Lee, *J.Chromatogr., 349, 305 (1985).*

[45] M.Proot, P.Sandra, E.Geeraert, *J. HRC & CC, 9, 189 (1986).*

[46] S.J.DeLuca, K.J.Voorhees, T.A.Langworthy, G.Holzer, *J. HRC & CC, 9, 182 (1986).*

[47] J.W.Hellgeth, J.W.Jordan, L.T.Taylor, M.Ashraf-Khorassani, *J.Chromatogr.Sci., 24, 183 (1986).*

[48] B.E.Richter *J. HRC & CC, 8, 297 (1985).*

[49] G.Holzer, L.H.Zalkow, C.F.Asibal, *J.Chromatogr., 400, 317 (1987).*

[50] S.K.Yarboro, D.R.Gere, *Chromatography, 49 (1987).*

[51] D.W.Later, B.E.Richter, D.E.Knowles, M.R.Anderson, *J. Chromatogr. Sci., 24, 249 (1986).*

[52] M.W.Raynor, J.P.Kithinji, I.K.Barker, K.D.Bartle, I.D.Wilson, *J. Chromatogr., 436, 497 (1988).*

[53] R.L.Eatherton, M.A. Morrissey, H.H.Hills, *Anal. Chem., 60, 2240 (1988).*

[54] E.D.Morgan, S.J.Murphy, D.E.Games, I.C.Mylchreest, *J. Chromatogr., 441, 165 (1988)*

[55] J.Hurst, *TSA Report, 2 (1988).*

[56] G.Holzer, S.DeLuca, K.J.Voorhees, *J. HRC & CC, 8, 528 (1985).*

[57] S.D.Zuagg, S.J.DeLuca, G.U.Holzer, K.J.Voorhees, *J. HRC & CC, 10, 100 (1987).*

[58] K.Matsumoto, S.Tsuge, Y.Hirata, *Chromatographia, 21, 617 (1986).*

[59] J.E.France, K.J.Voorhees, *J. HRC & CC, 11, 692 (1988).*

[60] D.E.Games, *Laboratory Practice, Feb. (1987).*

[61] D.W.Later, B.E.Richter, W.D.Felix, M.R.Anderson, D.E.Knowles, *American Laboratory, Aug., 108 (1987).*

[62] R.D.Smith, H.R.Udseth, *Anal. Chem., 59, 13 (1988).*

[63] D.A.Laude, S.L.Pentoney,Jr., P.R.Griffiths, C.L.Wilkins, *Anal. Chem., 59, 2283 (1987).*

[64] R.D.Smith, B.W.Wright, C.R.Yonker *Anal. Chem., 60, 1323A (1988).*

[65] H.T.Kalinoski, R.D.Smith, *Anal. Chem., 60, 529 (1988).*

[66] H.T.Kalinoski, H.R.Udseth, B.W.Wright, R.D.Smith, *J. Chromatogr., 400, 307 (1987).*

[67] B.W.Wright, H.T.Kalinoski, H.R.Udseth, R.D.Smith, *J. HRC & CC, 9, 145 (1986).*

[68] R.D.Smith, H.R.Udseth, H.T.Kalinoski, *Anal. Chem., 56, 2973 (1984).*

[69] A.J.Berry, D.E.Games, J.R.Perkins, *J. Chromatogr., 363, 147 (1986).*

[70] B.W.Wright, R.D.Smith, *J. HRC & CC, 8, 8 (1985).*

[71] B.W.Wright, R.D.Smith, *J. HRC & CC, 9, 73 (1986).*

[72] T.Greibrokk, B.E.Berg, A.L.Blilie, J.Doehl, A.Barefoot, E. Lundanes, *J. Chromatogr., 394, 429 (1987).*

[73] C.M.White, D.R.Gere, D.Boyer, F.Pacholec, L.K.Wong, *J. HRC & CC, 11, 94 (1988).*

[74] R.C.Wieboldt, K.D.Kempfert, D.W.Later, E.R.Campbell, *J. HRC & CC, 12, (1988).*

[75] M.Ashraf-Khorassani, L.T.Taylor, *J.Chromatogr.Sci., Submitted (1989).*

[76] S.M.Fields, K.E.Markides,M.L.Lee, *J. HRC & CC, 11, 25 (1988).*

[77] M.Ashraf-Khorassani, L.T.Taylor, *Anal. Chem., 59, 2077 (1987).*

[78] K.H.Shafer, P.R.Griffiths, *Anal. Chem., 55, 1939 (1983).*

[79] K.H.Shafer, S.L.Pentoney,Jr., P.R.Griffiths, *Anal. Chem., 58, 58 (1986).*

[80] J.W.Jordan, L.T.taylor, *J. Chromatogr. Sci., 24, 82 (1986).*

[81] P.Maurin, M.Caude, H.Richard, R. Rossett, *J. Chromatogr., 407, 87 (1987).*

[82] P.Maurin, M.Caude, H.Richard, R. Rossett, *Chromatographia, 21, 523 (1986).*

[83] S.V.Olesik, S.B.French, M.Novotny, *Chromatographia, 18, 489 (1984).*

[84] C.C.Jhonson, J.W.Jordan, L.T.Taylor, D.W.Vidrine, *Chromatographia, 20, 717 (1985).*

[85] S.B.French, M.Novotny, *Anal. Chem., 58, 164 (1986).*

[86] C.Fujimoto, T.Oosuka, K.Jinno, *Anal. Chim. Acta, 178, 159 (1985).*

[87] J.W.Hellgeth, L.T.Taylor, *J. Chromatogr. Sci., 24, 519 (1986).*

[88] P.R.Griffiths, S.L.Pentoney,Jr., A.Giorgetti, K.H.Shafer, *Anal. Chem., 58, 1349A (1986)*

[89] C.C.Jhonson, L.T.Taylor, *Anal. Chem., 56, 2642 (1984).*

[90] C.Fujimoto, Y.Hirata, K.Jinno, *J. Chromatogr., 332, 47 (1985).*

[91] M.W.Raynor, K.D.Bartle, I.L.Davies, A.Williams, A.A.Clifford, J.M.Chambers, B.W.Cook, *Anal. Chem., 60, 427 (1988).*

[92] M.W.Raynor, I.L.Davies, K.D.Bartle, A.Williams, J.M.Chambers, B.W.Cook, *European Chromatogr. News, 1, 19 (1987).*

[93] R.C.Wieboldt, J.A.Smith, *ACS Symp. Series, 366, 229 (1988).*

[94] K.Jinno, *Chromatographia, 23, 55 (1987).*

[95] L.T.Taylor, E.M.Calvey, *Chemical Reviews, Submitted (1989).*

[96] R.C.Wieboldt, D.A.Hanna, *Anal. Chem., 59, 1225 (1987).*

[97] S.H.Shah, M.Ashraf-Khorassani, L.T.Taylor, *Chromatographia, 25, 632 (1988).*

98 R.C.Wieboldt, G.A.Adams, D.W.Later, *Anal. Chem., Submitted (1988).*

99 P.R.Griffiths, S.L.Pentoney, G.L.Pariente, K.L.Norton, *Mikrochimica Acta. (1988).*

100 M.W.Capp, M.H. Simonian, *Anal. Biochem., 147, 374 (1985).*

101 M.D.Smith, "Steroid Analysis by HPLC", Chap. 4, Ed. by M.P.Kautsky, Marcel Dekker, Inc. (1981).

102 C.H.L.Shackelton, *J. Chromatogr., 379, 91 (1986).*

103 J.W.Honour, "HPLC of Small Molecules", Chap.7, Ed. by C.K.Lim, Oxford IRL Press Limited (1986).

104 E.Stoner, S.Loche, A.Mirth, M.I.New, *J. Chromatogr., 374, 358 (1986).*

105 J.W.Jordan, L.T.Taylor, *Chromatographia, 21, 3 (1986).*

106 L.T.Taylor, *J. Chromatogr. Sci., 23, 265 (1985).*

107 C.Fujimoto, G.Vematsu, K.Jinno, *Chromatographia, 20, 112 (1985).*

108 E.Stahl, K.W.Quirin, *Fluid Phase Equilibria, 10, 269 (1983).*

109 S.Shah, L.T.Taylor, *Anal. Chem., Submitted (1989).*

110 G.Marshall, *Interaction Chemicals, Inc., Private communication.*

111 M.W.F.Nielen, G.Koomen, R.W.Frei, U.A.Th.Brinkman, *J. Liq. Chromatogr., 8, 315 (1985).*

112 R.W.Frei, M.W.F.Nielen, U.A.Th.Brinkman, *Intern. J. Environ. Anal. Chem., 25, 3 (1986).*

113 F.A.Maris, R.B.Geerdink, R.W.Frei, U.A.Th.Brinkman, *J. Chromatogr., 323, 113 (1985).*

114 C.E.Goewie, E.A.Hogendoorn, *J. Chromatogr., 410, 211 (1987).*

115 F.A.Maris, M.Nijenhuis, R.W.Frei, G.J.de Jong, U.A.Th.Brinkman, *Chromatographia, 22, 235 (1986).*

116 A.C.Barefoot, R.W.Reiser, *J. Chromatogr., 398, 217 (1987).*

117 U.A.Th.Brinkman, A. Dekok, R.W.Geerdink, *J. Chromatogr., 283, 113 (1984).*

118 F.A.Maris, R.B.Geerdink, R.Van Delft, U.A.Th.Brinkman, *Bull. Environ. Contam. Toxicol., 35, 711 (1985).*

119 J.Doehl, A.Farbrot, T.Greibrokk, B.Iversen, *J. Chromatogr., 392, 175 (1987).*

120 S.Schmidt, L.G.Blomberg, E.R.Campbell, *Chromatographia, 22, 775 (1988).*

121 M.E.McNally, J.R.Wheeler, W.R.Melander, *LC-GC Magazine, 6, 816 (1988).*

122 A.W.Francis, *J. Phys. Chem., 58, 1099 (1954).*

[123] R.M.Silverstein, G.C.Bassler, T.C.Morrill, *"Spectroscopic Identification of Organic Compounds", Chap. 3 John Wiley and Sons (1981).*

[124] M.Popl, Z.Voznakova, V.Tatar, *J. Chromatogr. Sci., 21, 39 (1983).*

[125] D.E.Bradway, R.F.Mosemann, *J. Agri. Food Chem., 30, 244 (1982).*

[126] H-B.Lee, A.S.Y.Chau, *J. Assoc. Off. Anal. Chem., 66, 1322 (1983).*

[127] Y.Xu, W.Lorenz, P.Pfister, M.Bahadir, F.Korte, *Fresenius Z. Anal. Chem., 325, 377 (1986).*

[128] H.Jork, B.Roth, *J. Chromatogr., 144, 39 (1977).*

[129] H.Roseboom, H.A.Herbold, *J. Chromatogr., 202, 431 (1980).*

[130] P.A.Leclerq, U.Pacalova', *J. Chromatogr., 178, 193 (1979).*

[131] R.Bailey, G. LeBel, J.F.Lawrence, *J. Chromatogr., 161, 251 (1978).*

[132] P.C.Bardalaye, W.B.Wheeler, *Intern. J. Environ. Anal. Chem., 25, 105 (1986).*

[133] W.C.Budde, J.W.Eichelberger., *"Organics Analysis using Gas Chromatography/Mass Spectrometry", Ann Arbor science: Ann Arbor, MI (1979).*

[134] D.F.Gurka, L.D.Betowski, *Anal. Chem., 54, 1819 (1982).*

[135] C.E.Parker, C.A.Haney, D.J.Harvan, J.R.Hass, *J. Chromatogr., 242, 77 (1982).*

[136] P.Dufek, V.Pacakova', V.Tesarova', *J. Chromatogr., 191, 115 (1980).*

[137] R.D.Voyksner, W.H.McFadden,S.A.Lammert, *"Applications of New Mass Spectrometry Techniques in Pesticide Chemistry", J.D.Rosen, Ed.,John Wiley and Sons, New York, 1987, Chap. 17.*

[138] R.D.Voyksner, J.T.Bursey, E.D.Pellizzari, *Finnigan MAT Newsletter, Number 205, San Jose', California (1984).*

[139] A.Di Corcia, M.Marchetti, R.Samperi, *J. Chromatogr., 405, 357 (1987).*

[140] L.M.Shalaby, *Biomed. Mass Spectrom., 12, 262 (1985).*

[141] A.J.Berry, D.E.Games, I.C.Mylchreest, J.R.Perkins, S.Pleasance, *J. HRC & CC, 11, 61 (1988).*

[142] S.Kennedy, R.J.Wall, *LC-GC Magazine, 6, 930 (1988).*

[143] R.Board, D.McManigill, H.Weaver, D.R.Gere, *Publication no. 43-5953-1647, p. 17, Hewlett Packard Co., Avondale, PA (1982).*

[144] T.A.Berger, J.F.Deye, M.Ashraf-Khorassani, L.T.Taylor, *J. Chromatogr. Sci., Accepted for publication.*

[145] D.R.Gere, *Science, 222, 259 (1983).*

[146] H.Schwartz, P.J.Barthel, S.E.Moring, H.H.Laner, *LC-GC Magazine, 5, 490 (1987).*

147 S.T.Sie, W.Van Beersum, G.W.A.Rijinders, *Separ.Sci., 1, 459 (1966).*

148 S.T.Sie, G.W.A.Rijinders, *Separ.Sci., 2, 729 (1967).*

149 P.A.Peaden, M.L.Lee, *J. Chromatogr., 259, 1 (1982).*

150 M.Novotny, S.R.Springston, *J. Chromatogr., 279, 417 (1983).*

151 S.R.Springston, M.Novotny, *Anal. Chem., 56, 1762 (1984).*

152 S.R.Springston, M.Novotny, *Anal. Chem., 58, 2698 (1986).*

153 R.Randall, *ACS Symp. Ser., 250, 135 (1984).*

154 D.R.Gere, R.Board, D.McManigill, *Anal. Chem., 54, 736 (1982).*

155 P.A.Mourier, M.H.Claude, M.H.Rosset, *Chromatographia, 23, 21 (1987).*

156 B.A.Bidlingmeyer, F.V.Warren,Jr., *Anal. Chem., 56, 1583A (1984).*

157 S.J.Hawkes, *J. Chem. Ed., 60, 393 (1983).*

158 James A. Miller, *"Chromatography: Concepts and Contrasts", Chap. 1, John Wiley & Sons, New York, 1988.*

159 M.Novotny, P.David, *J. HRC & CC, 9, 647, 1986.*

160 P.A.Peaden, M.L.Lee, *J. Liq. Chromatogr., 5, 179, 1982.*

161 F.P.Schmitz, E.Klesper, *J. HRC & CC, 10, 519, 1987.*

162 T.L.Chester, D.P.innis, *J. HRC & CC, 8, 561 (1985).*

163 U.van Wasen, G.M.Scheider, *Chromatographia, 8, 274 (1975).*

164 A.Wilsch, R.Feist, G.M.Scheider, *Fluid Phase Equil., 10, 299 (1983).*

Appendix A.

This appendix presents the results obtained from the experiments similar to those described in chapter five with different experimental parameters. Several van Deemter plots were generated at temperatures of 60 °C, 80 °C and 120 °C. The results achieved under isoconfertic and isothermal conditions at all the studied temperatures are identical to those presented in chapter five. The van Deemter plots for packed and capillary columns exhibit the trends similar to that at 100 °C as shown in Figure 35- Figure 37. Also, the plots of log k′ vs density (Figure 38- Figure 46) and resolution vs density (Figure 47- Figure 55) show similar trends in each case for both types of columns. The data presented here were generated with a splitless injection technique using a different restrictor and hence a different linear velocity for each plot for both types of columns. The data in figures 38 and 47 were generated at the slowest linear velocity for packed column. The linear velocity was progressively increased to achieve the data presented in next couple of plots; the data shown in figures 42 and 51 having generated at the highest linear velocities. Similarly, the linear velocity was the slowest for data shown in figures 43 and 52 for capillary columns and the fastest for data in figures 46 and 55.

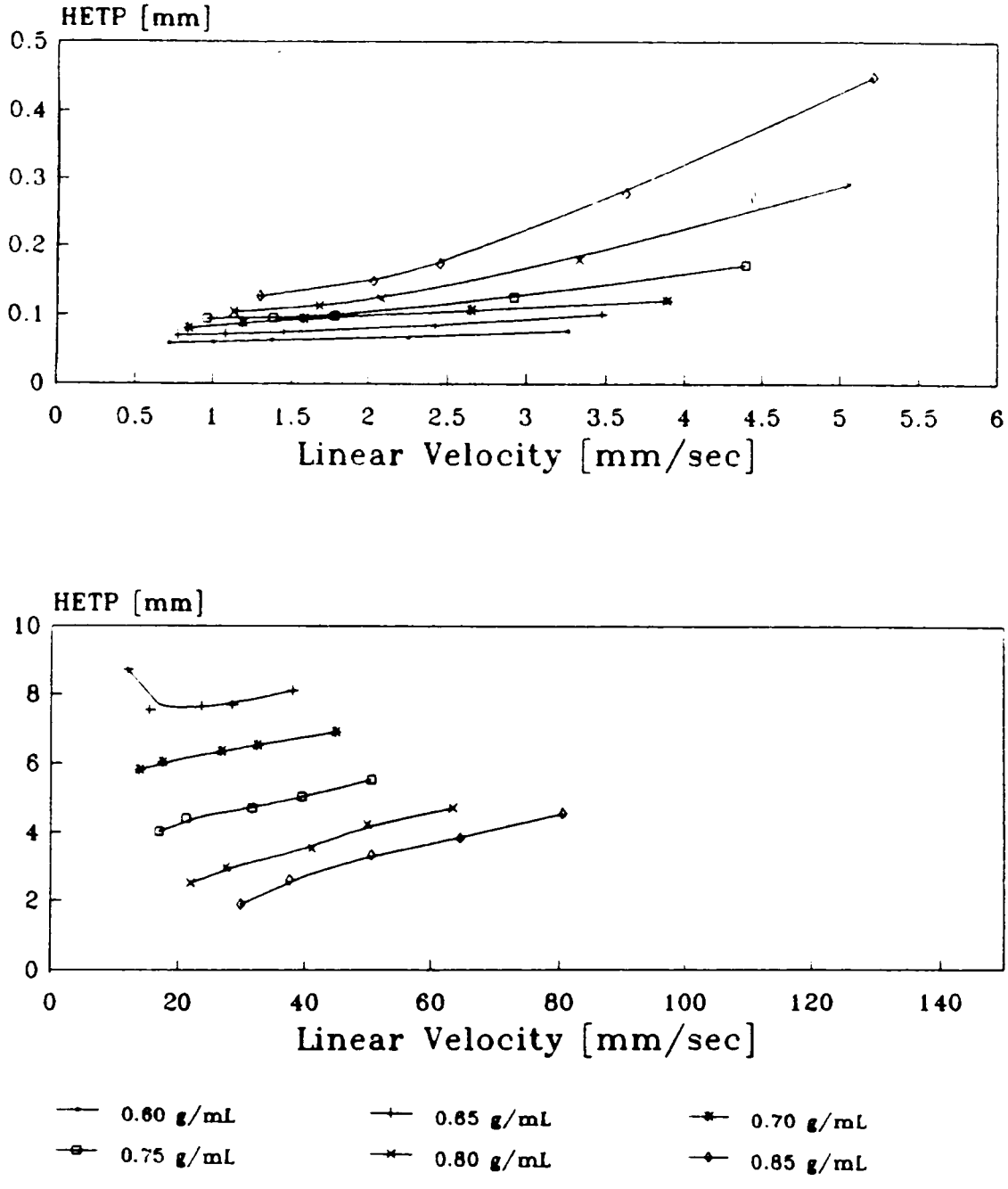


Figure 35. van Deemter Plots at 60 °c. (a) Packed column (b) Capillary column.

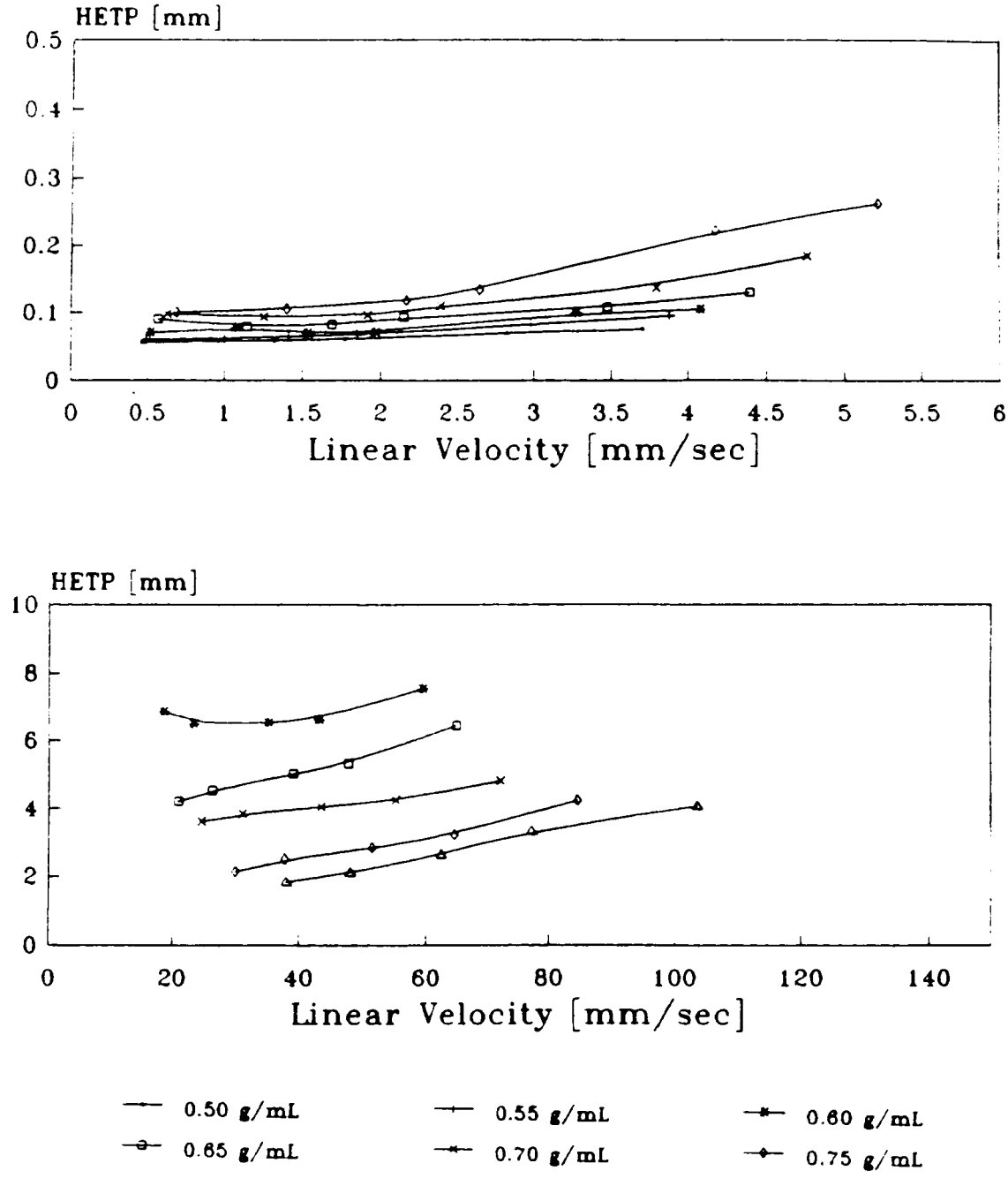


Figure 36. van Deemter Plots at 80°c. (a) Packed column (b) Capillary column.

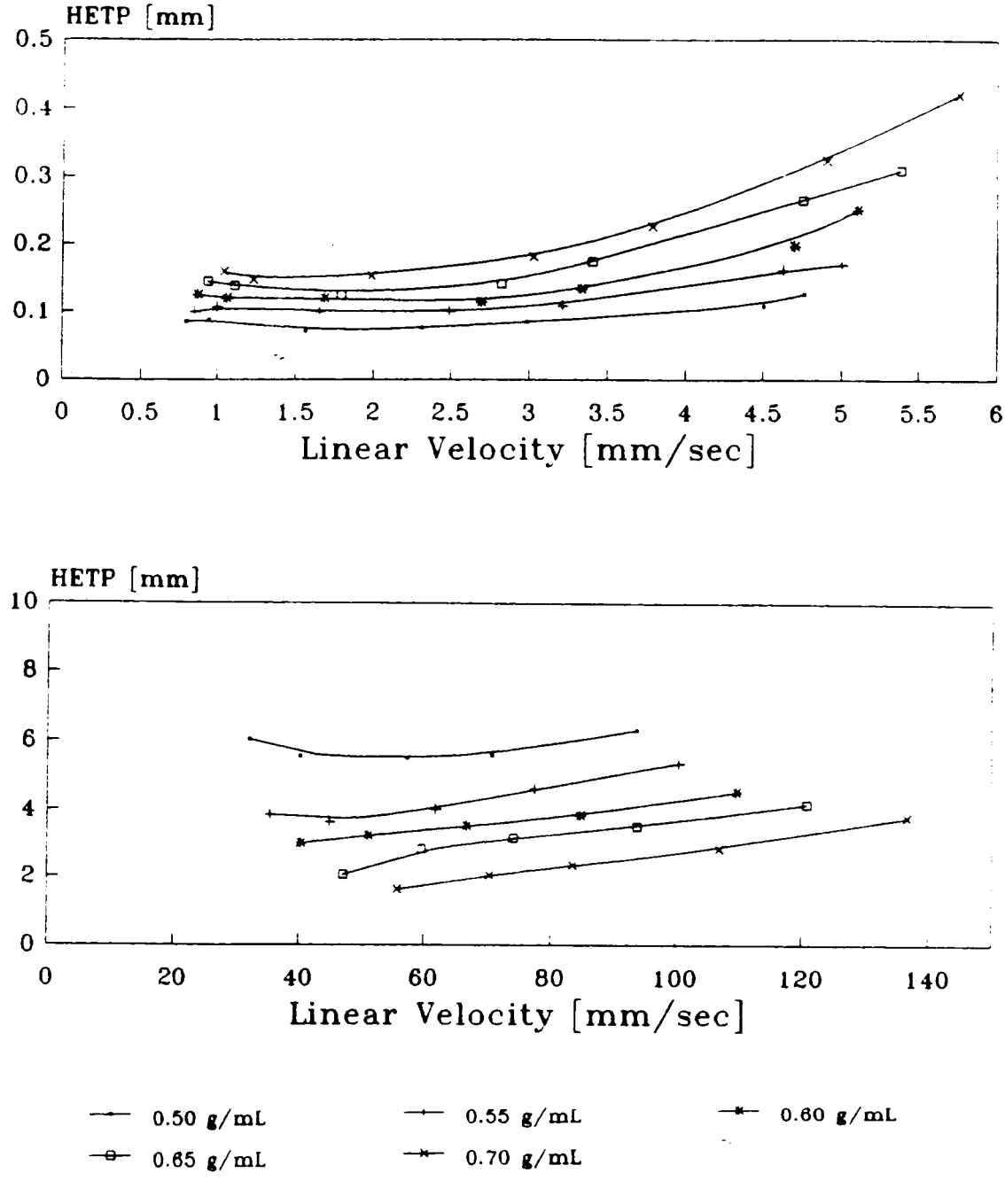


Figure 37. van Deemter Plots at 120°c. (a) Packed column (b) Capillary column.

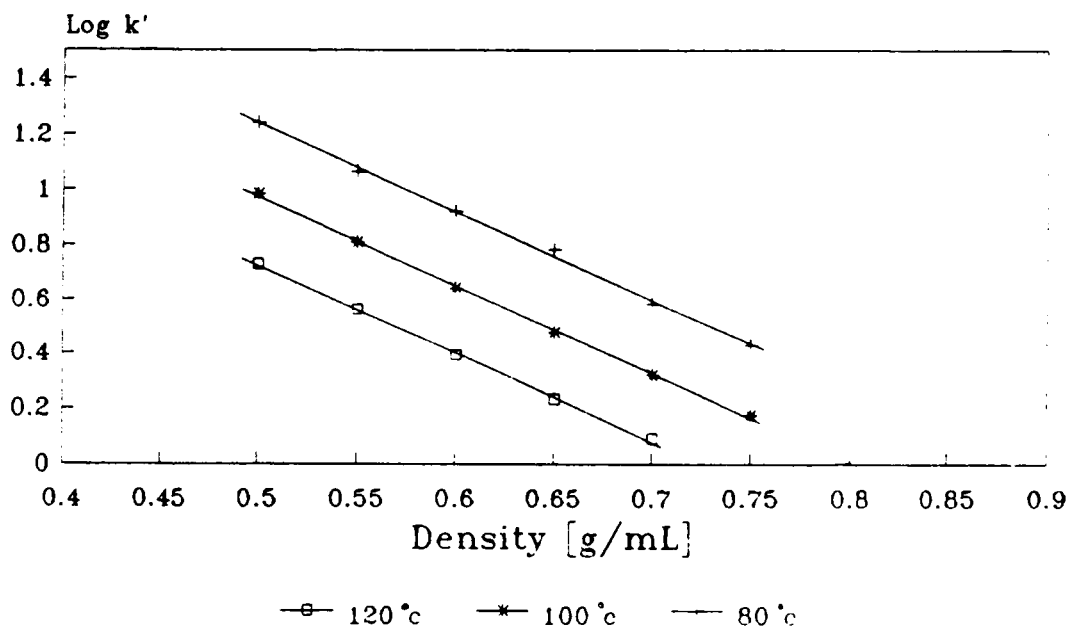


Figure 38. Plots of log k' vs density - packed column

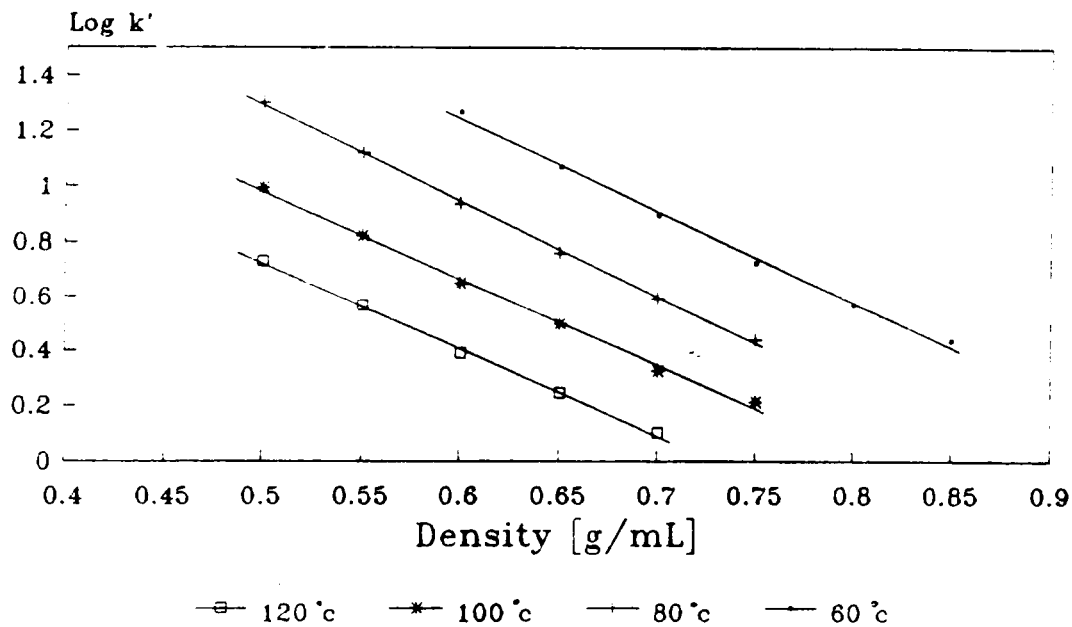


Figure 39. Plots of log k' vs density-packed column.

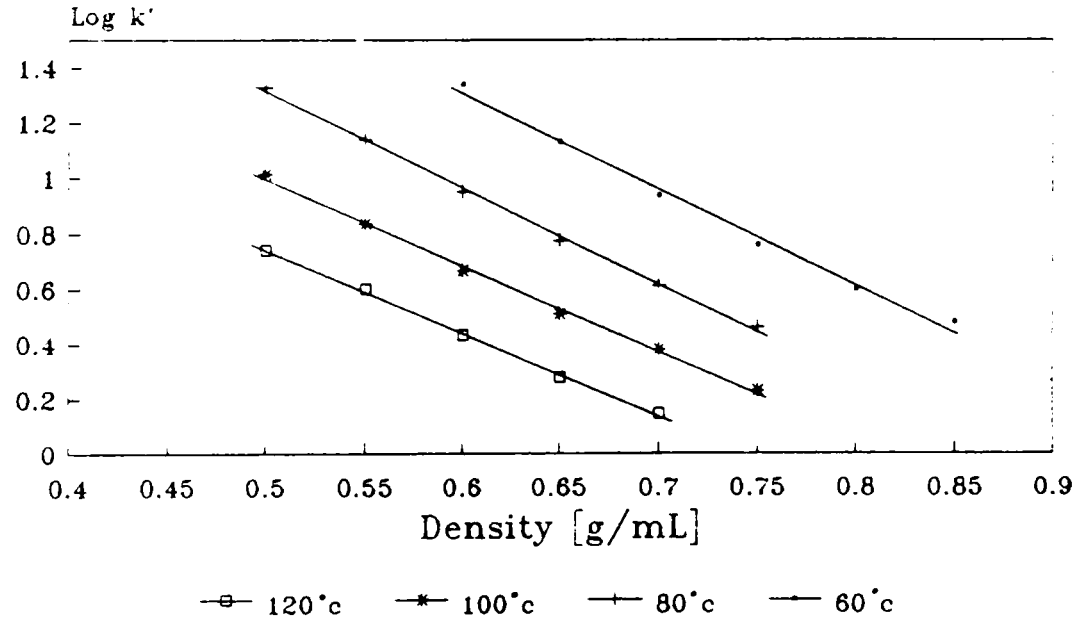


Figure 40. Plots of log k' vs density-packed column.

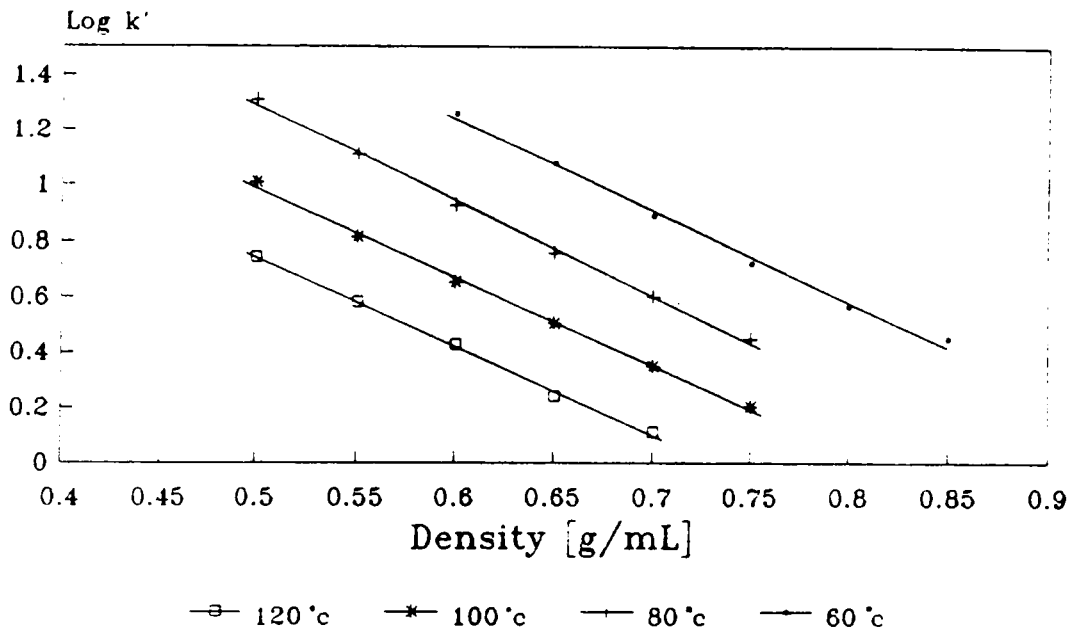


Figure 41. Plots of log k' vs density-packed column.

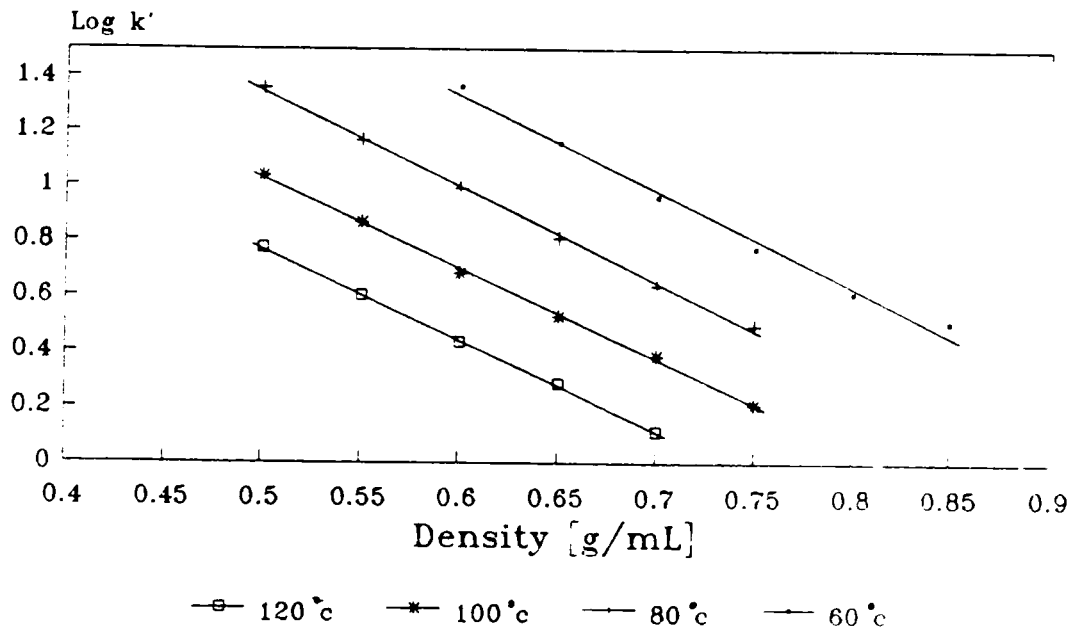


Figure 42. Plots of log k' vs density-packed column.

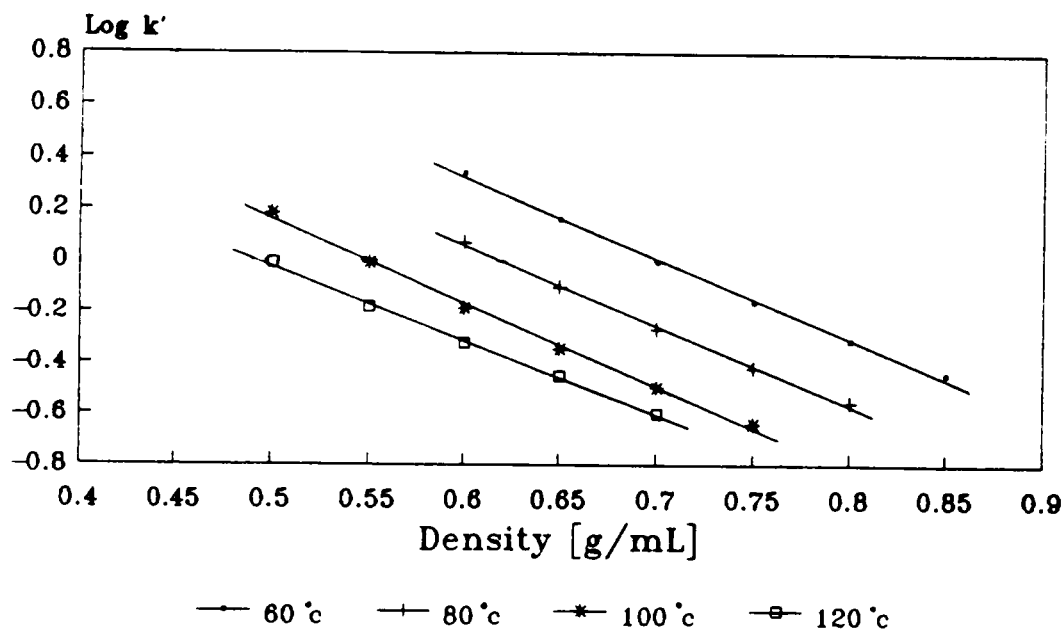


Figure 43. Plots of log k' vs density-capillary column.

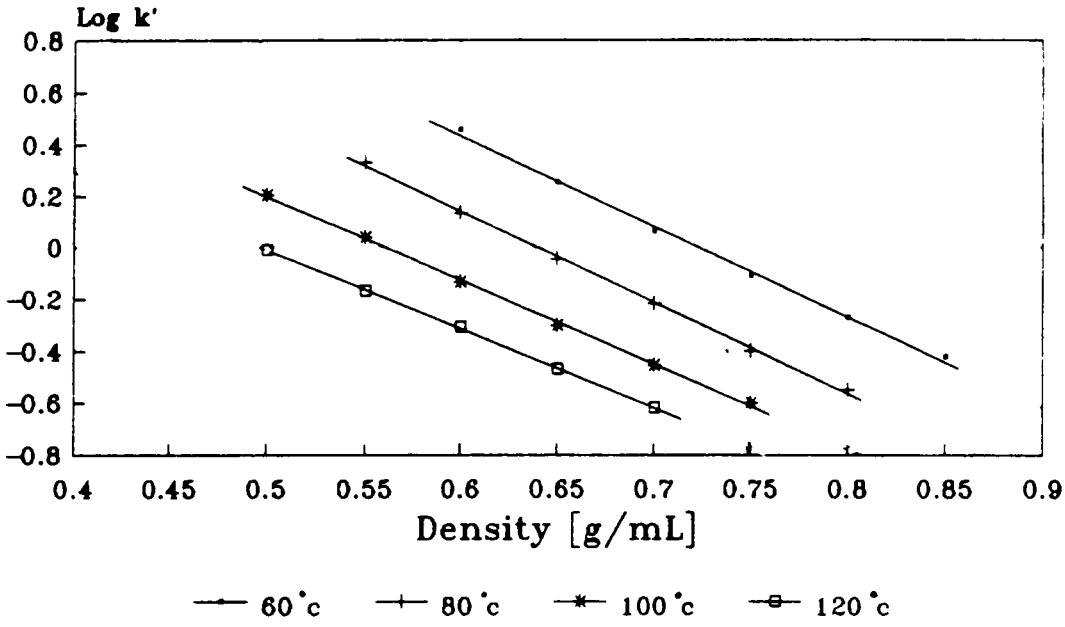


Figure 44. Plots of log k' vs density-capillary column.

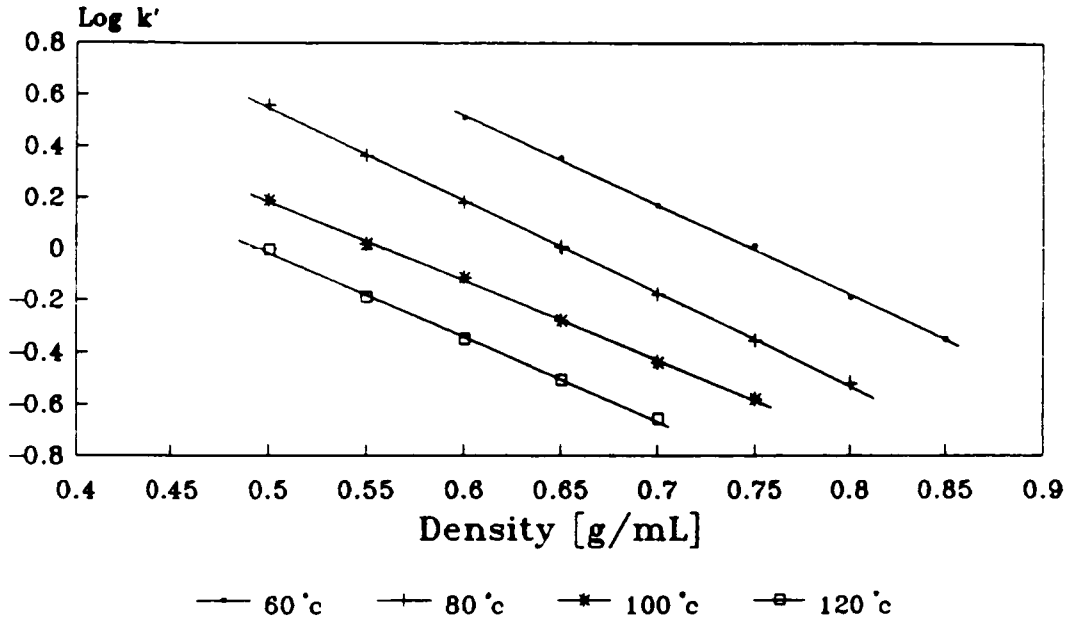


Figure 45. Plots of log k' vs density-capillary column.

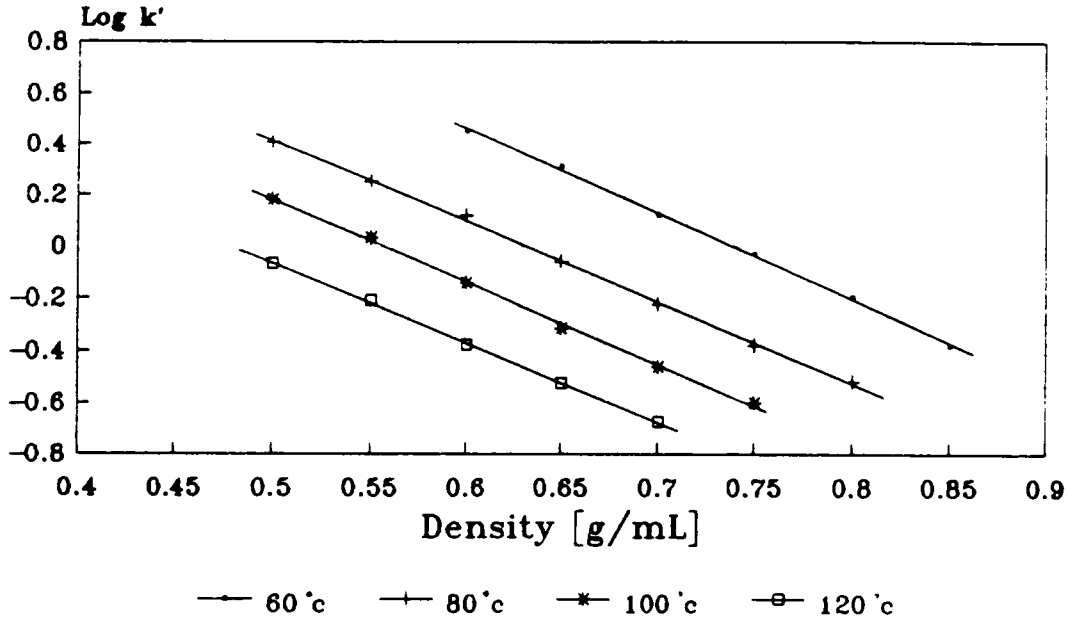


Figure 46. Plots of log k' vs density-capillary column.

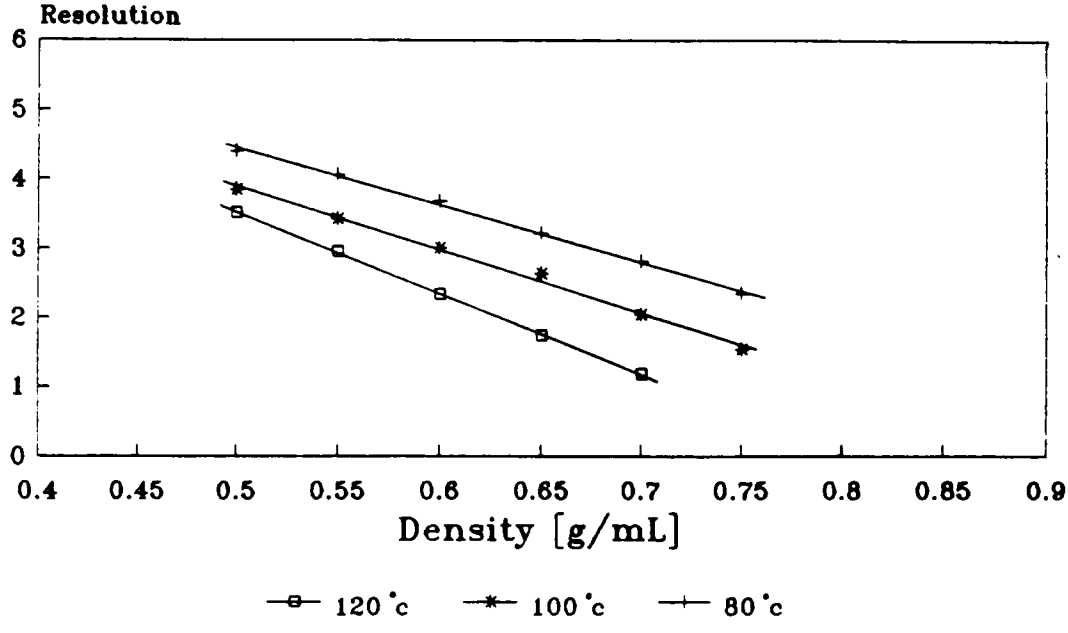


Figure 47. Plots of Resolution vs density-packed column.

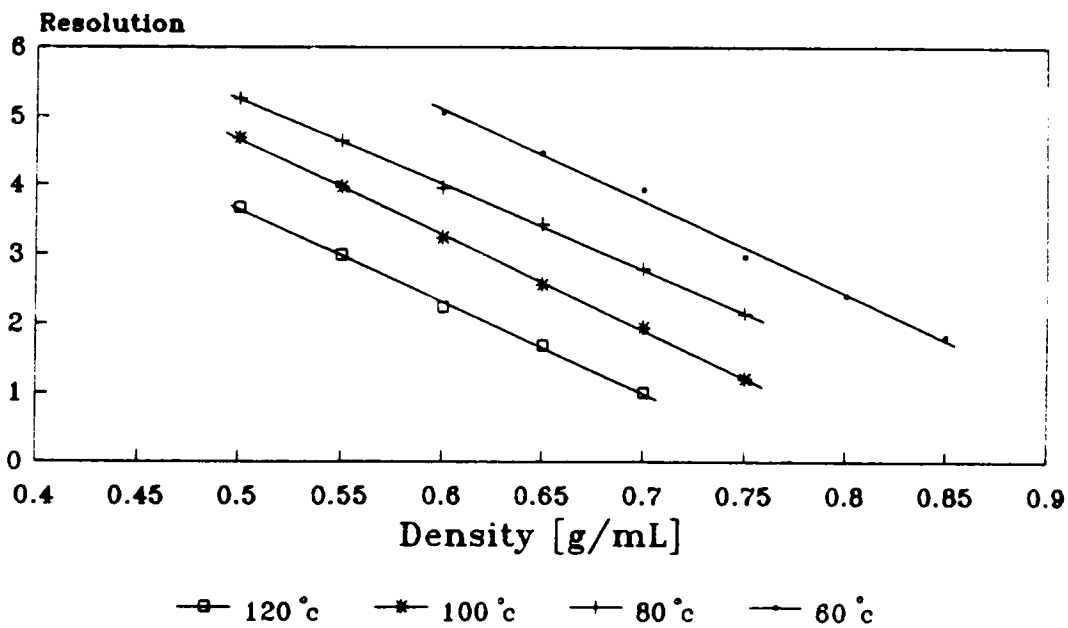


Figure 48. Plots of Resolution vs density-packed column.

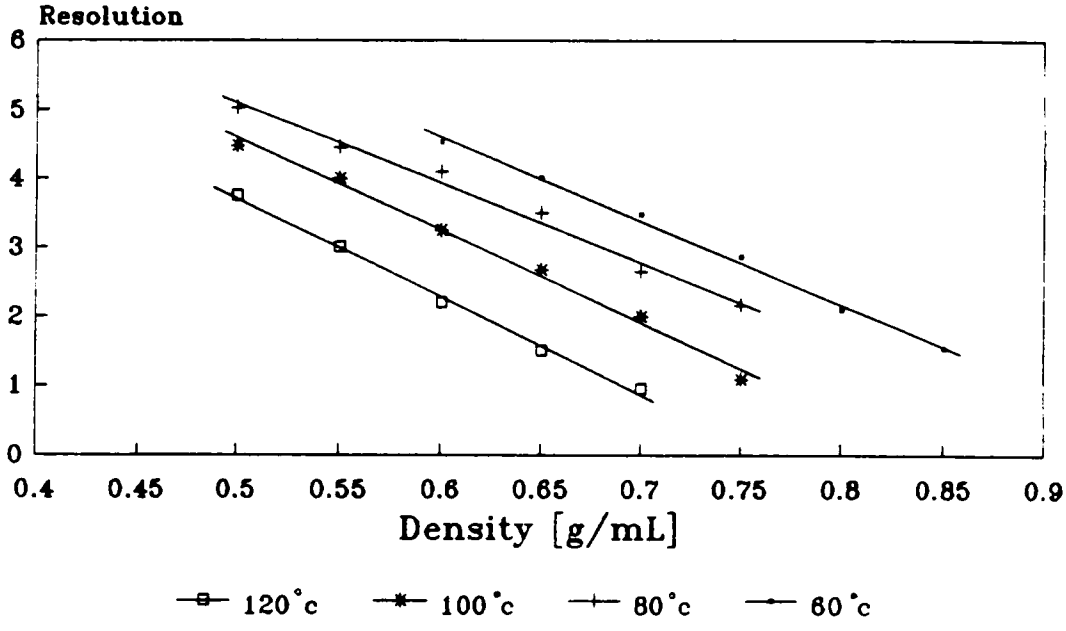


Figure 49. Plots of Resolution vs density-packed column.

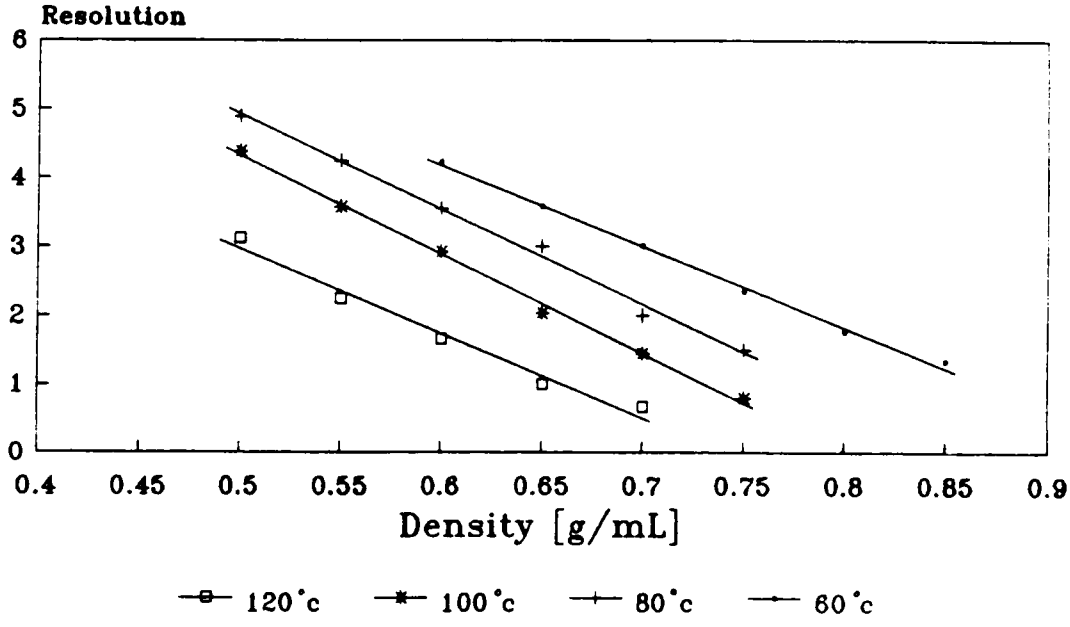


Figure 50. Plots of Resolution vs density-packed column.

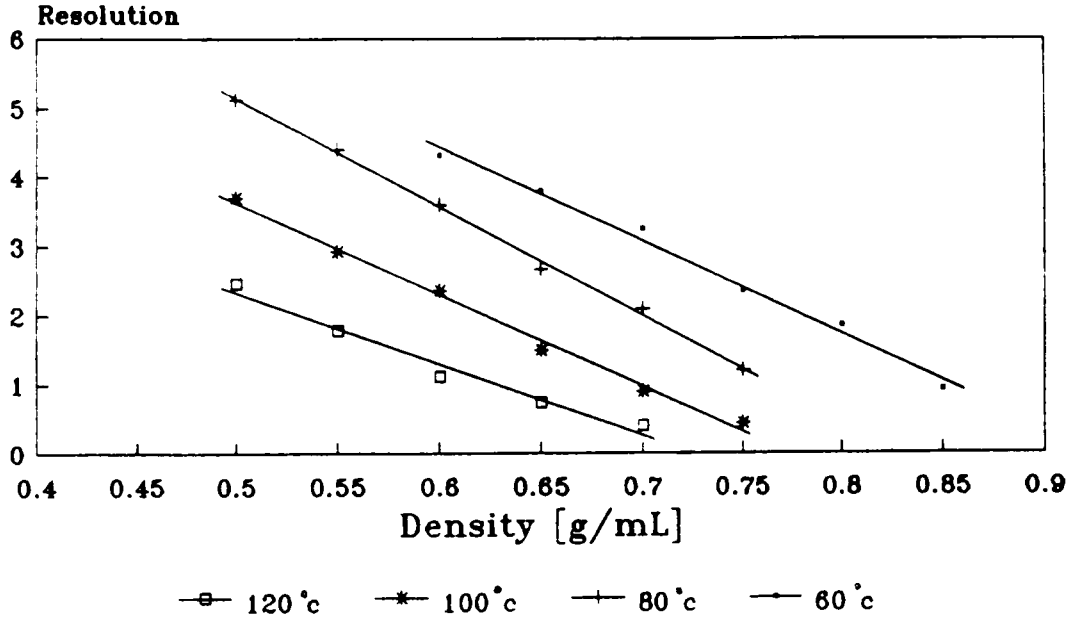


Figure 51. Plots of Resolution vs density-packed column.

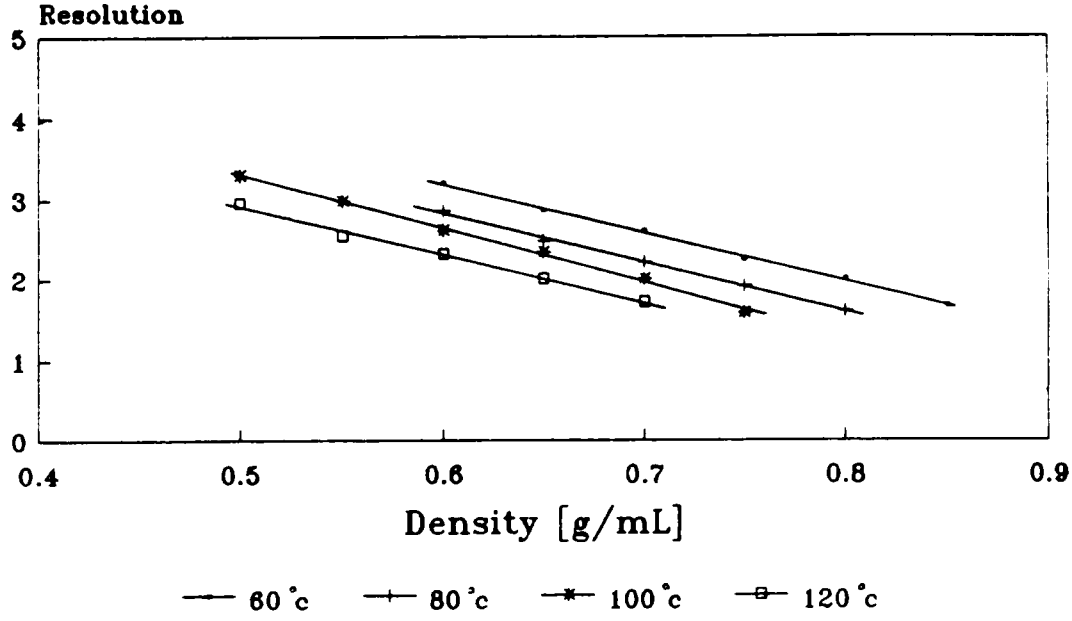


Figure 52. Plots of Resolution vs density-capillary column.

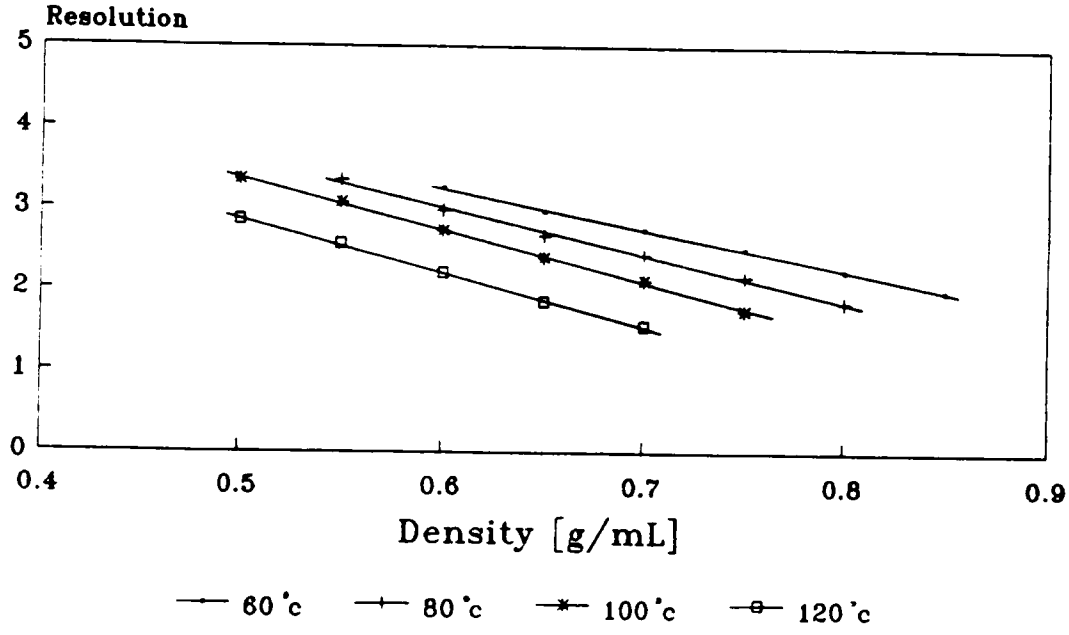


Figure 53. Plots of Resolution vs density-capillary column.

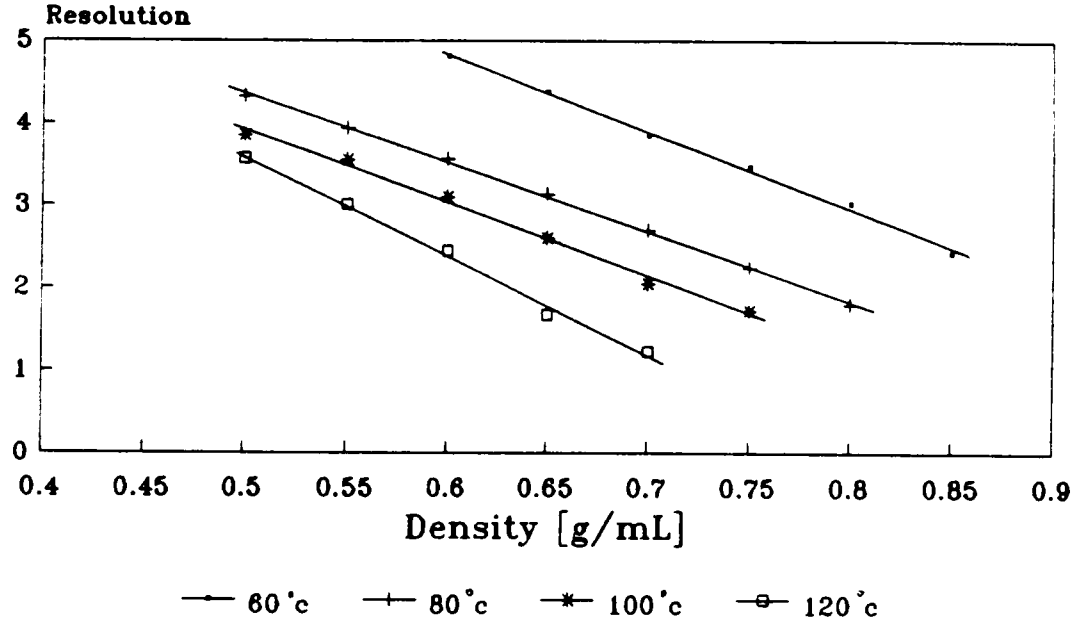


Figure 54. Plots of Resolution vs density-capillary column.

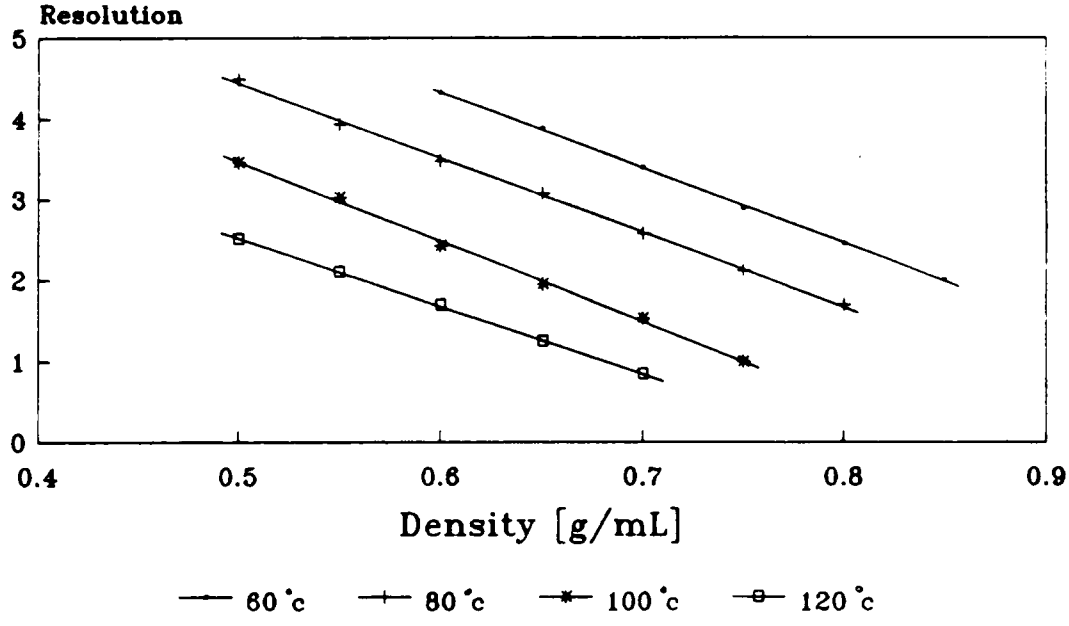


Figure 55. Plots of Resolution vs density-capillary column.

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