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## MODIFICATION OF CONVENTIONAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY EQUIPMENT FOR USE IN CARBON DIOXIDE SUPERCRITICAL FLUID CHROMATOGRAPHY

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### SUMMARY

Rapid and simple modifications of conventional high-performance liquid chromatography equipment enabled use of the system for carbon dioxide supercritical fluid chromatography. No permanent alterations were required, and the system could be used interchangeably for conventional or supercritical fluid chromatography. The system described was capable of operating in four elution modes: (1) isocratic–isobaric; (2) solvent gradient; (3) flow gradient; (4) combined solvent–flow gradient. Each elution mode was successfully used for the separation of a polynuclear aromatic hydrocarbon mixture. Effects of system parameters, such as pressure, temperature, and flow-rate were also examined.

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### INTRODUCTION

The use of supercritical fluids as solvents was first reported by Hannay and Hogarth<sup>1</sup> and their use as a chromatographic mobile phase initially reported by Klesper *et al.*<sup>2</sup>. The recent literature contains numerous reports on the use of supercritical fluid chromatography (SFC) for a wide variety of applications<sup>3–10</sup>. For the individual desiring a more complete discussion of SFC concepts, reviews are available in the literature<sup>11,12</sup>.

The current report discusses the construction of a carbon dioxide SFC system by modifying standard high-performance liquid chromatography (HPLC) equipment. Operation in isocratic as well as various gradient modes is described. Examples of separations obtained by use of these SFC techniques with microparticulate reversed-phase columns are given.

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## EXPERIMENTAL

*Apparatus*

Two Series 10 pumps, a Series 10 LC system controller, an LC-100 oven, and a LC-85B variable-wavelength detector (Perkin-Elmer, Norwalk, CT, U.S.A.) were employed in the SFC system. Sample introduction was accomplished by a 7125S injection valve (Rheodyne, Cotati, CA, U.S.A.). Pressure was monitored by a 0–6000 p.s.i. in-line pressure gauge (Supelco, Bellefonte, PA, U.S.A.). The system back pressure was maintained and adjusted by a Model 7037 adjustable spring-loaded diaphragm valve (Rheodyne) and a Swagelok needle metering valve (Crawford Fitting, Solon, OH, U.S.A.). The column used in the study was 8.3 cm × 4.6 mm and packed with 3- $\mu$ m C<sub>18</sub> material (Perkin-Elmer). A Haake FE circulating pump (American Scientific Products, Bedford, MA, U.S.A.) was used to circulate coolant over the pump head.

*Apparatus modifications*

Schematic diagrams of the assembled SFC system are presented in Figs. 1 and 2. To prevent cavitation of the carbon dioxide during the refill cycle and to ensure efficient and reproducible flow-rates of the supercritical carbon dioxide mobile phase, the head of the carbon dioxide pump was cooled to 0°C. The cooling was accomplished by circulating ice water through a heat exchanger plate in thermal contact with the pump head. The exchanger was a hollow copper plate, approximately 0.25 in. thick and of the same diameter as the pump head. Inlet and outlet ports on the exchanger permitted the circulation of the ice water coolant. The exchanger plate was secured to the pump head by the head retaining screws, which were passed through appropriately placed holes in solid areas of the exchanger plate. Foam insulation was placed over the modified head assembly to increase the efficiency of the cooling process.

The injection valve was mounted in a hole drilled into the side of the LC-100 oven. It was placed so that the body of the injector was inside the oven, while only the grip and needle port were outside the heated oven enclosure.

A coil of 6 ft. × 0.049 in. I.D. (1/16 in. O.D.) stainless-steel tubing was secured inside the oven and used to connect the injection valve to the pump. A minimal

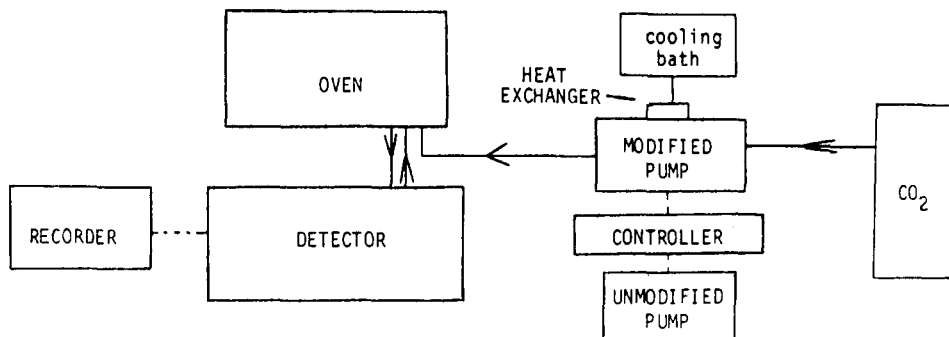


Fig. 1. Schematic diagram of modified carbon dioxide SFC system.

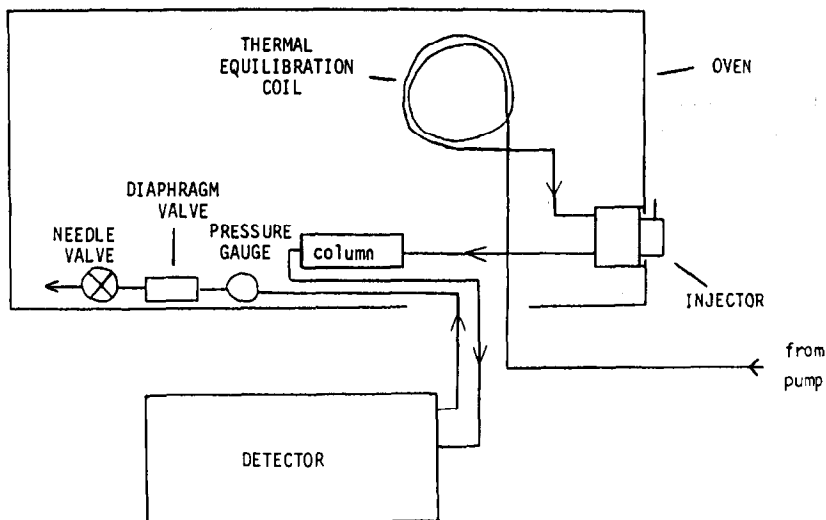


Fig. 2. Expanded schematic diagram of hardware contained inside oven compartment.

length of the tubing was placed outside the oven to establish the pump connection. The purpose of the coil was to allow the liquid carbon dioxide to obtain the supercritical state (pressure and temperature) prior to entrance into the injector and column.

A minimal length of tubing was used to connect the column outlet to the detector. Foam insulation was placed around the connecting tube to prevent cooling of the supercritical mobile phase as it passed out of the oven enclosure and into the detector. The detector outlet line was also insulated and passed back into the oven compartment. Heating of the outlet line was necessary to prevent icing and blockage of the line due to cooling caused by the expansion of the carbon dioxide upon pressure reduction at the detector outlet.

To monitor and provide a variable source of back pressure in the system, several valves were used. Connected directly to the detector outlet line was a 0–6000 p.s.i. in-line pressure gauge to permit monitoring of the outlet pressure  $P_0$ . The outlet line of the pressure gauge was attached to an adjustable spring-loaded diaphragm valve. The diaphragm valve was used to adjust the system back-pressure to the desired value. The vent line of the diaphragm valve was left open to release excess pressure. The outlet line of the diaphragm valve was connected to a metering needle valve. The needle valve was used to make minor adjustments in the system back-pressure when necessary. The assembly of the pressure gauge and pressure adjustment valves was located within the oven compartment to prevent icing of the tubing upon expansion of the carbon dioxide. The inlet pressure of the system,  $P_i$ , was monitored by the pump pressure gauge.

### Chemicals

Test solutes were available through Aldrich (Milwaukee, WI, U.S.A.). Liquefied carbon dioxide (bone dry grade) was supplied from a 50-lb tank equipped with a siphon (eductor) tube (Cryodyne, Danbury, CT, U.S.A.). Use of a siphon tube was

necessary to provide liquified, rather than gaseous, carbon dioxide to the modified pump.

### *Procedures*

*Pump start-up.* The pump head was initially chilled by the circulating ice bath for a period of 10–15 min. At the end of the initial cool-down period, the valve of the carbon dioxide tank was opened and a flow-rate of 9.0 ml/min entered into the pump. After a period of 2–3 min, the pump pressure ( $P_i$ ) obtained a value of 3000 p.s.i. The set flow-rate was then decreased to the desired value and allowed to come to equilibrium for approximately 2 min. In addition, the oven had previously been equilibrated at the desired operating temperature.

*Isocratic-isobaric system.* Following the pump start-up procedure, the pressure adjustment valves were used to set the system back-pressure ( $P_o$ ) to the desired value. The flow and backpressure were held constant during the separation process.

Sample injection was performed in the conventional manner. However, we recommend removal of the syringe before rotating the injection valve from the Inject position back to the Load position. If the syringe is not removed, supercritical carbon dioxide, trapped in the sample loop, will vaporize upon release of pressure during the valve rotation. This results in the rapid ejection of the plunger from the syringe barrel.

*Flow gradient system.* The modified carbon dioxide pump was connected as "Pump A" to the system controller. The pump was chilled and pressurized, as described in the previous section. After system pressurization was achieved, the system controller was used to control the flow-rate. The flow on the controller was set at 9.9 ml/min. The appropriate "Initial %A" was selected to obtain the desired initial set flow-rate. (Example: a setting of 15% A gave a flow-rate of 1.5 ml/min). The appropriate "Final %A" was also entered to produce the desired final set flow-rate. Finally, the length of the gradient time was selected. When the desired initial set flow-rate was established, the system pressure and temperature were adjusted to the values of choice. Sample injection was then made and the gradient initiated. As the %A value increased over the course of the gradient, the flow-rate of carbon dioxide also increased. The result was the formation of a flow gradient for elution of the sample components.

*Solvent gradient system.* The solvent gradient system required the use of two Series 10 pumps and the system controller. The modified carbon dioxide pump was attached to the controller as "Pump B" and the remaining unmodified pump was attached as "Pump A". Pump A delivered the organic modifier to the mobile phase as the stronger solvent. Plumbing and operation of the system was identical to that for normal HPLC gradient elution.

In this study, acetonitrile was successfully used as the mobile phase modifier, at concentrations up to 30%. Some baseline disruption did occur at higher acetonitrile concentrations. Generally 20 min at a set flow-rate of 1.5 ml/min were required to re-equilibrate the column after the completion of a solvent gradient separation.

*Solvent-flow gradient system.* The solvent-flow gradient mode combines the principles of both solvent programming and flow programming. The system requires the use of two pumps and the system controller. The modified carbon dioxide pump was not connected to the controller, but allowed to operate independently. The

second pump was used to deliver organic modifier to the mobile phase, and was attached as "Pump A" to the system controller. The outlet of the carbon dioxide pump was attached to the mixing coil of Pump A. The combined flow stream then passed on to the remainder of the SFC system.

The carbon dioxide pump was chilled and the system was pressurized as previously described. The desired carbon dioxide set flow-rate was then selected. The flow on the controller was set at 9.9 ml/min. The appropriate "Initial %A" and "Final %A" values were entered to obtain the desired initial and final flow-rates of organic modifier. The selection of initial and final percentages of solvent A was identical to the procedure used in the flow gradient elution. Finally, the gradient time was entered into the system controller. Adjustments of system controller parameters did not affect the set flow-rate of the carbon dioxide, since the modified carbon dioxide pump was operated independently of the system controller.

Upon sample injection, the gradient was initiated. Throughout gradient elution, the carbon dioxide set flow-rate remained constant. However the amount of organic modifier added to the mobile phase was increased. Thus both the total flow-rate of the mobile phase as well as its eluting strength were increased during the gradient elution.

## RESULTS AND DISCUSSION

### *General discussion*

The heat exchanger for the pump head is a very simple and inexpensive means of converting existing HPLC equipment into a SFC system, capable of operating with supercritical carbon dioxide as the mobile phase. The system described is simple and requires no exceptional precautions or procedures for operation or maintenance. The start-up time (power-up until first injection) was typically 20 min.

Perhaps the weakest point in this system was the method used to chill the pump head. The system used a circulating water bath to pump ice water through the heat exchanger on the pump head. Such an approach worked satisfactorily, but it was necessary to replace the ice in the bath every hour to maintain a relatively constant bath temperature (*i.e.* head temperature) of approximately 1°C. If the ice was

TABLE I  
EFFECT OF PUMP HEAD TEMPERATURE ON RETENTION TIME

Test components: 1 = toluene; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = terphenyl; 6 = pyrene; 7 = chrysene.

Head temp. (°C)	Retention time (min)						
	1	2	3	4	5	6	7
1	1.6	2.4	3.4	4.6	5.6	8.8	13.0
9	2.2	3.1	4.6	6.4	7.8	12.2	18.6
13	2.6	3.7	5.4	7.6	9.2	14.7	22.6
18	3.2	4.5	6.6	8.6	10.7	18.8	29.4
1	1.7	2.5	3.7	5.0	6.0	9.2	13.4

not replenished often enough, the head began to warm. An increase in the temperature of the pump head was found to affect the pumping efficiency and in turn the quality and reproducibility of the separation. The effect of pump-head temperature on retention time is summarized in Table I for a test mixture composed of 7 polynuclear aromatic hydrocarbons (PAHs). As indicated by the results in the table, constant pump head temperature is essential. Constant temperature could most easily be maintained by use of a refrigerated circulating bath.

Another weak point of the initially developed system was the repeated rupturing of the pulse dampner diaphragm in the modified carbon dioxide pump. Inspection of the ruptured diaphragms suggested that under the elevated pressure the carbon dioxide may have been permeating the diaphragm and dissolving in the liquid contained within the dampner unit. Over a period of time the amount of dissolved carbon dioxide increased, thus increasing the internal pressure of the dampner unit until the diaphragm ruptured.

To eliminate the problem, the internal pulse dampner was replaced with a 10-ft. length of coiled 0.010 in. I.D. stainless-steel tubing. Very similar baseline noise levels were produced with both types of dampners. With the internal dampner out of the flow path, the pump pressure gauge was deactivated. Therefore, insertion of an in-line pressure gauge at the pump outlet was necessary to monitor the column inlet pressure ( $P_i$ ).

The flow-rate of the mobile phase was observed to have the expected effects on the chromatographic results. Increased flow-rates resulted in faster elution and narrower peaks. Table II lists data demonstrating the effects of the set flow-rate on the retention times of the components of the PAH mixture separated by carbon dioxide SFC. The data obtained indicate that the use of flow programming may be a practical way to decrease the length of time required to obtain a desired separation.

The increased flow-rates were also expected to influence the observed column efficiency. Height-equivalent-to-a-theoretical-plate (HETP) values for five baseline-resolved peaks in the PAH mixture are given in Table III. The results indicate that the optimum flow-rate was in the range of 3–7 ml/min for most components.

Although increased flow can be used to decrease the retention times of components, it does not in itself increase the elution strength of the supercritical mobile

TABLE II  
EFFECT OF SET FLOW-RATE ON RETENTION TIME

Test components: 1 = toluene; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = terphenyl; 6 = pyrene; 7 = chrysene.

Set flow (ml/min)	Retention time (min)						
	1	2	3	4	5	6	7
1.5	1.5	2.2	3.2	4.5	5.3	8.2	12.0
3.5	0.85	1.2	1.7	2.3	2.8	4.3	6.3
5.5	0.70	0.95	1.3	1.8	2.1	3.2	4.7
7.5	0.55	0.75	1.0	1.4	1.6	2.5	3.5
9.5	0.45	0.60	0.85	1.2	1.4	2.2	3.0

TABLE III  
EFFECT OF SET FLOW ON EFFICIENCY

Test components: 1 = fluorene; 2 = anthracene; 3 = terphenyl; 4 = pyrene; 5 = chrysene.

Set flow (ml/min)	HETP (mm/plate)				
	1	2	3	4	5
1.5	0.0309	0.0452	0.0327	0.0344	0.0251
3.5	0.0539	0.0309	0.0208	0.0292	0.0243
5.5	0.0480	0.0246	0.0262	0.0208	0.0203
7.5	0.0390	0.0442	0.0329	0.0239	0.0275
9.5	0.0518	0.0585	0.0430	0.0309	0.0188

phase. However, mobile phase pressure-density increases caused by increased flow-rates may increase the elution strength of the supercritical mobile phase and increase the solubility of an analyte in the mobile phase. Such an increase in mobile phase solvation strength should be viewed as a secondary effect of the increased mobile phase flow-rate.

At constant pressure, the density and hence elution strength of a supercritical fluid will decrease as the temperature is increased. Therefore, elevated temperatures result in increased retention of sample components. Such behavior is in contrast to that typically observed in conventional HPLC. As a result, at constant pressure the solubility of an analyte in a supercritical fluid is greatest at temperatures near the critical temperature. To illustrate the effect of oven temperature on solute retention, retention data of fluorene as a function of column temperature are presented in Table IV. The data indicate that the oven-mobile phase temperature is an important factor in the separation parameters.

At constant temperature an increase in pressure will increase the density, and hence solvating strength, of the supercritical mobile phase. Ideally, there should be

TABLE IV  
EFFECT OF MOBILE PHASE TEMPERATURE ON RETENTION TIME

Test compound: fluorene.

Temp. ( $^{\circ}$ C)	$t_R$ (min)
35	3.0
40	3.2
45	3.6
50	3.8
55	4.2
60	5.2
65	7.0
70	11.0
75	11.9
80	16.6
85	17.8

TABLE V  
EFFECT OF SYSTEM PRESSURE ON RETENTION TIME

Test components: 1 = toluene; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = terphenyl; 6 = pyrene; 7 = chrysene.

Pressure $P_i/P_o$ (p.s.i.)	Retention time (min)						
	1	2	3	4	5	6	7
2000/1900	1.4	2.1	3.3	4.7	5.9	9.1	14.1
2450/2300	1.6	2.2	3.3	4.4	5.3	8.2	12.2
2800/2650	1.6	2.2	3.1	4.1	4.8	7.4	10.6
3200/2975	1.8	2.3	3.2	4.2	4.7	7.2	10.0
3600/3375	1.8	2.3	3.1	4.0	4.6	7.0	9.7
4000/3725	2.1	2.7	3.6	4.4	5.6	7.6	10.3

no pressure drop across the column, so as to maintain constant solvating strength of the supercritical mobile phase throughout the length of the column. Absence of a pressure drop is an idealized situation and is not attainable in practice. However, by use of short packed columns or open tubular columns, the pressure drop can be minimized. Therefore, use of these types of columns is advantageous in SFC applications. Data concerning the effects of system pressure on retention times are shown in Table V. All separations were obtained at the same set flow-rate. The system pressure was adjusted by use of the valve assembly at the detector outlet. Late-eluted peaks displayed the most marked decrease in retention times as the pressure was increased. Early-eluted peaks also decreased in retention times as the pressure was increased, and then actually started to be retained longer as the pressure was further increased. Such behavior suggests that there may be some optimum pressure for achieving maximum solubility of various solutes, but further study along these lines is required before any firm conclusions may be drawn.

#### Isocratic-isobaric separations

The column used provided a maximum of 4100 plates for chrysene at 5.5 ml/min. This value was compared with a plate count of 5200 for *tert.*-butylbenzene obtained under standard column test conditions [2.0 ml/min of acetonitrile-water (65:35)]. However, without obtaining a plate count for chrysene under the optimum reversed-phase conditions, no direct quantitative comparison of system efficiencies can be made. Such a comparison was not performed in the current study.

The instrumental bandwidth of the SFC system was determined to be 112  $\mu$ l when a 8- $\mu$ l detector flow cell was used. To minimize the dispersion further to a level compatible with very short columns (3 cm), the flow cell may be replaced with a 1.4- $\mu$ l cell. Use of the smaller flow cell resulted in reduction of the instrumental bandwidth to 33  $\mu$ l.

Figs. 3 and 4 illustrate the resolution and separation times obtained for various mixtures by use of the isocratic-isobaric mode described above. Fig. 3 shows the isocratic-isobaric SFC separation of five alkylbenzenes. The separation of a seven-component PAH mixture is illustrated in Fig. 4.



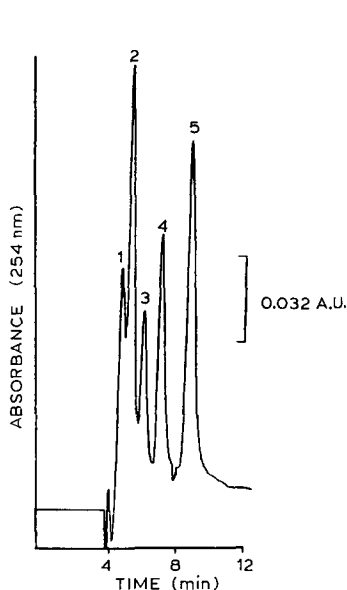


Fig. 3. Isocratic-isobaric carbon dioxide SFC separation of alkyl benzenes. Mobile phase: supercritical carbon dioxide. Set flow-rate: 1.0 ml/min. System pressure:  $P_i = 2200$  p.s.i.;  $P_o = 2000$  p.s.i. Temperature:  $35^\circ\text{C}$ . Column:  $3.3\text{ cm} \times 4.6\text{ mm}$ ,  $3\text{-}\mu\text{m C}_{18}$ . Detection: 254 nm, 0.32 a.u.f.s. Injection volume:  $5\ \mu\text{l}$ . Peaks: 1 = ethylbenzene; 2 = *n*-butylbenzene; 3 = *n*-hexylbenzene; 4 = *n*-octylbenzene; 5 = *n*-decylbenzene.

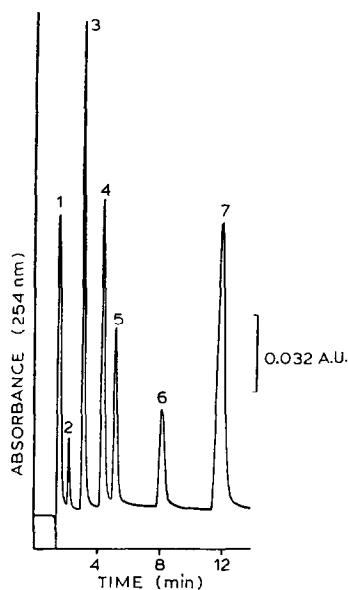


Fig. 4. Isocratic-isobaric carbon dioxide SFC separation of a PAH mixture. Mobile phase: supercritical carbon dioxide. Set flow-rate: 1.5 ml/min. System pressure:  $P_i = 2600$  p.s.i.;  $P_o = 2300$  p.s.i. Temperature:  $35^\circ\text{C}$ . Column:  $8.3\text{ cm} \times 4.6\text{ mm}$ ,  $3\text{-}\mu\text{m C}_{18}$ . Detection: 254 nm, 0.32 a.u.f.s. Injection amount: 70 ng each component. Peaks: 1 = toluene; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = terphenyl; 6 = pyrene; 7 = chrysene.

### Gradient separations

Solvent gradient SFC is not a new technique, but most experimenters have used a second tank of carbon dioxide, doped with the organic modifier, as the strong solvent<sup>5</sup>. Our work demonstrates that the organic modifier can be introduced directly into the carbon dioxide mobile phase by use of a second pump. Such an approach greatly simplifies SFC solvent programming and can be easily accomplished with the equipment described in this paper.

The solvent gradient SFC separation of a PAH mixture, obtained with acetonitrile as the organic modifier, is shown in Fig. 5. The separation was achieved in approximately 75% of the time required for the isocratic-isobaric separation in Fig. 4, with some loss in resolution of anthracene and terphenyl. Also the peak shapes of later eluted bands were greatly narrowed, resulting in the improvement of minimum detection limits by solvent gradient elution. The separation time was approximately 1.5 times that required for a similar separation obtained by conventional reversed-phase HPLC<sup>13</sup>. Addition of an organic modifier to the supercritical mobile phase may allow manipulation of solvent-solute interactions to obtain a desired separation not otherwise possible.

Flow programming permit is a simple way to decrease the separation time.

Fig. 6 depicts the flow gradient SFC separation of a seven-component PAH mixture. In comparison to the isocratic-isobaric separation in Fig. 4, the gradient decreased the separation time by a factor of three while still maintaining adequate resolution. The separation time was approximately 80% of that required for a similar separation by conventional reversed-phase methodology<sup>13</sup>. Flow programming provided a stable baseline and good peak shapes. Additionally, later-eluted peaks were significantly sharpened and increased in height, thus yielding lower minimum detection limits than isocratic-isobaric separations.

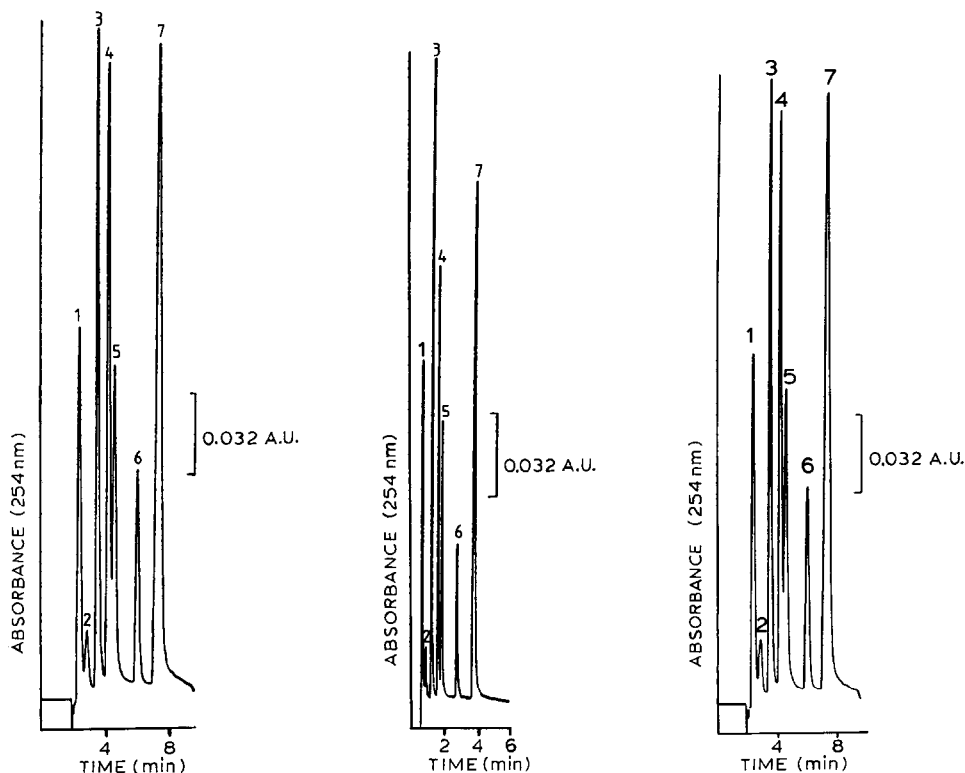


Fig. 5. Solvent gradient SFC separation of PAH mixture. Mobile phase: (A) acetonitrile; (B) carbon dioxide. Gradient: 4–19% A in 15 min (linear). Set flow-rate: 2.0 ml/min. System pressure:  $P_i = 3000$  p.s.i.;  $P_o = 2500$  p.s.i. Temperature: 35°C. Column: 8.3 cm  $\times$  4.6 mm, 3- $\mu$ m  $C_{18}$ . Detection: 254 nm; 0.32 a.u.f.s. Injection amount: 70 ng each component. Peaks: 1 = toluene; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = terphenyl; 6 = pyrene; 7 = chrysene.

Fig. 6. Flow gradient carbon dioxide SFC separation of PAH mixture. Mobile phase: carbon dioxide. Flow gradient: 5.0–8.9 ml/min over 5 min (linear). System pressure: initial  $P_i = 3000$  p.s.i. Final  $P_i = 3100$  p.s.i. Initial  $P_o = 2700$  p.s.i. Final  $P_o = 2750$  p.s.i. Temperature: 35°C. Column: 8.3 cm  $\times$  4.6 mm, 3- $\mu$ m  $C_{18}$ . Detection: 254 nm; 0.32 a.u.f.s. Injection amount: 70 ng each component. Peaks: 1 = toluene; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = terphenyl; 6 = pyrene; 7 = chrysene.

Fig. 7. Combined solvent-flow SFC separation of PAH mixture. Mobile phase: (A) acetonitrile; (B) carbon dioxide. Flow-rates: solvent A, 0–3.5 ml/min over 7 min; solvent B, 1.5 ml/min. Total flow gradient: 1.5–5.0 ml/min over 7 min. Temperature: 35°C. Column: 8.3 cm  $\times$  4.6 mm, 3- $\mu$ m  $C_{18}$ . Detection: 254 nm; 0.32 a.u.f.s. Injection amount: 70 ng each component. Peaks: 1 = toluene; 2 = naphthalene; 3 = fluorene; 4 = anthracene; 5 = terphenyl; 6 = pyrene; 7 = chrysene.

The combined solvent-flow gradient is a unique approach. It combines the essentials of solvent programming and flow programming. Combination of the two principles may result in better and faster separations than those obtained by either solvent or flow programming. The chromatogram in Fig. 7 is the SFC solvent-flow gradient separation of a PAH mixture. The gradient separation was achieved in approximately 30% of the time required for the isocratic-isobaric separation in Fig. 4 and provided much sharper peaks, resulting in improved minimum detection limits. The separation time was approximately 80% of that required for a similar separation by conventional HPLC techniques<sup>13</sup>.

We have also examined the use of pressure gradients to increase the solvating strength of the supercritical mobile phase.

However the application of pressure gradients to the system described in this paper proved unsuccessful. The maximum pressure limit of 4000 p.s.i. for the detector flow cell prevented the use of pressure gradients over a sufficiently wide range to be of practical value.

## CONCLUSIONS

Our study has shown that carbon dioxide SFC is easily carried out on modified HPLC equipment, yielding acceptable separations. Through simple and inexpensive modifications to existing HPLC equipment, a laboratory can expand its capabilities to include both carbon dioxide SFC and conventional HPLC.

Various gradient elution techniques were shown to be successful in reducing the separation time and improving the detector response. For solvent gradient elution the organic modifier was introduced directly into the carbon dioxide mobile phase, thus simplifying the procedure. Combination of solvent- and flow-gradient principles resulted in the development of solvent-flow programming, which may add greater flexibility and selectivity to SFC methodologies.

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