Journal of Chromatography, 349 (1985) 317–322
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 694

GRADIENT PROGRAMMING AND COMBINED GRADIENT–PRESSURE PROGRAMMING IN SUPERCRITICAL FLUID CHROMATOGRAPHY

ANN LISBETH BLILIE and TYGE GREIBROKK*
Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, 0315 Oslo 3 (Norway)

SUMMARY

The properties of flow–pressure gradients, modifier gradients and combined pressure–modifier gradients with supercritical carbon dioxide on reversed-phase columns have been examined. The best combination of high resolution and high detectability of polycyclic aromatic hydrocarbons and of nitrated polycyclic aromatic hydrocarbons was obtained with combined pressure–modifier gradients. Perspectives for more widespread use of modifier gradients in supercritical fluid chromatography have been added in conclusion.

INTRODUCTION

In supercritical fluid chromatography (SFC) the retention of a solute is controlled by the composition of the mobile phase, the density of the mobile phase, the nature of the stationary phase, and the temperature. In analogy with temperature programming in gas chromatography (GC) and gradient elution in high-performance liquid chromatography (HPLC), pressure–density programming has become the standard feature of retention control in SFC. Temperature programming in SFC is less attractive than in GC, since the temperature effect is pressure-dependent. At pressures directly above the critical pressure, the density decreases rapidly with temperature, resulting in increased retention, whereas at higher pressures the density is less affected, resulting in decreased retention[1–3].

One reason for looking into alternatives to pressure–density gradients is the fact that many detector flow-cells are not compatible with the high pressures, and that some instrument parts that are exposed to mechanical wear, *i.e.* injectors, may have a leakage problem. Organic modifiers have been added to supercritical fluids to reduce adsorption and to change selectivity[2–8], but almost always with constant, premixed concentrations in pressurized cylinders. Schmitz *et al.*[9] separated oligomers of styrene and 2-vinylpyridine with a gradient of 10–40% methanol in pentane at 250°C, but unfortunately the silica columns were destroyed because the silica dissolved in the mobile phase. Fluids such as pentane can be pumped and mixed with standard HPLC gradient equipment, but owing to the high critical temperatures, such fluids are not compatible with solutes of low thermal stability and polar solutes with adsorption problems. Such compounds require fluids with low critical temperatures and

TABLE I

IDENTIFICATION OF PEAKS IN THE FIGURES

Peak No.	*Compound*	*Peak No.*	*Compound*
1	Naphthalene	12	1-Nitronaphthalene
2	Fluorene	13	2-Nitrofluorene
3	Anthracene	14	3-Nitrofluoranthene
4	Fluoranthene	15	1-Nitrotriphenylene
5	Pyrene	16	2-Nitrotriphenylene
6	Benz[*a*]anthracene	17	1-Nitrobenzo[*e*]pyrene
7	Chrysene	18	6-Nitrobenzo[*a*]pyrene
8	Benzo[*e*]pyrene	19	9-Nitrodibenz[*a,c*]anthracene
9	Benzo[*a*]pyrene	20	3-Nitrobenzo[*e*]pyrene
10	Dibenz[*a,c*]anthracene	21	7-Nitrobenzo[*ghi*]perylene
11	Benzo[*ghi*]perylene	22	5-Nitrobenzo[*ghi*]perylene

the addition of polar modifiers. Cold carbon dioxide or dinitrogen oxide can be pumped in the liquid state by modified HPLC units, even by reciprocating pumps[10]. If modifiers are to be added in controlled concentrations, a mobile phase delivery based on constant flow becomes more important than the constant-pressure principle traditionally used in SFC. In HPLC, the use of the reciprocating pumps is predominant, owing to their ability to supply instant constant flow and their excellent gradient capabilities. This paper describes the use of reciprocating pumps in constructing flow–pressure gradients, modifier gradients, and combined pressure–modifier gradients for the separation of a group of polycyclic aromatic hydrocarbons (PAHs) and a group of nitro-PAHs (Table I). Both groups contained some components that gave broad asymmetric peaks and some components that were totally retained with supercritical carbon dioxide alone on microbore C_{18} reversed-phase columns.

EXPERIMENTAL

The instrumentation, including modified Waters Model 6000 A pumps, has been described in detail elsewhere[10,11]. These pumps lack the constant-pressure option of more recent reciprocating pumps, but are well suited for applications requiring controlled flow. All built-in tubing ahead of the flow-cell in the Perkin-Elmer LC-55 UV detector was removed in order to reduce bandspreading in the detector. The 8-μl flow-cell, where the windows were tightened with an extra set of springs, could be used at peak pressures of *ca.* 300 bar. The UV absorbance was measured at 254 nm. The CP-Spher C_{18} columns from Chrompack (25 cm × 1.3 mm I.D.) gave a pressure drop across the column of less than 7 bar.

The methanol was of HPLC Grade S quality (Rathburn, Walkerburn, U.K.). The nitro-PAHs have been characterized elsewhere[12–15]. Samples of 1 μl in dichloromethane were injected. Each sample contained 50–400 ng of each compoonent.

RESULTS AND DISCUSSION

When the mobile phase, containing pure carbon dioxide, was modified with

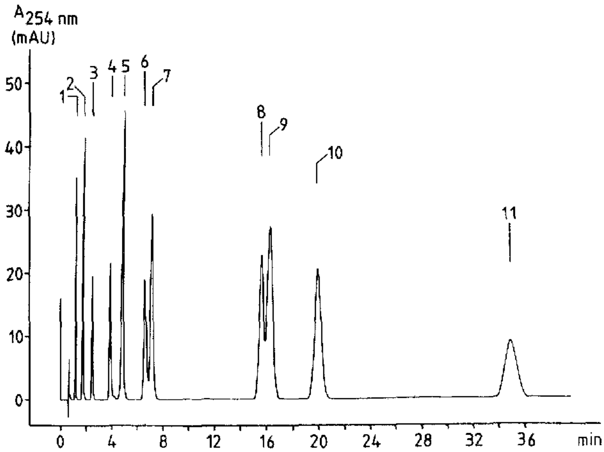


Fig. 1. Isobaric separation of eleven PAHs (see Table I) with a mixture of carbon dioxide (500 μl/min) and methanol (10 μl/min) at 40°C. The inlet pressure was 151 bar and the outlet pressure 145 bar.

2% methanol, all the PAHs (Fig. 1) and all the nitro-PAHs (Fig. 2) were eluted from the columns. Larger amounts of methanol reduced the total elution time, but also compressed the early-eluted components. Isobaric elution at higher pressures had the same effect. The chromatograms in Figs. 1 and 2 are classical examples of the need for a programme that increases the elution strength of the mobile phase during the elution, such as a pressure gradient.

Pressure gradients were constructed as flow gradients. The pressure at the pump was measured as a function of each increment of 0.1 ml/min in the flow-rate. Since all the nitro-PAHs could not be eluted with pure carbon dioxide, methanol was added as a modifier. The flow–pressure gradients shown in Figs. 3 and 4 were constructed by increasing the flow of carbon dioxide, whereas the flow of modifier was kept constant. The relative amount of modifier then actually decreased slowly during the run. The pressures given in the figures were measured at the time of injection and

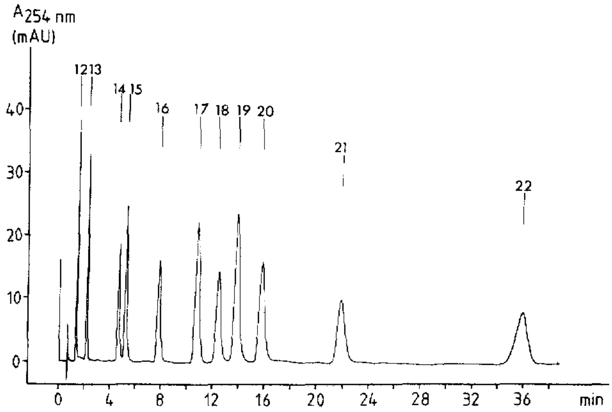
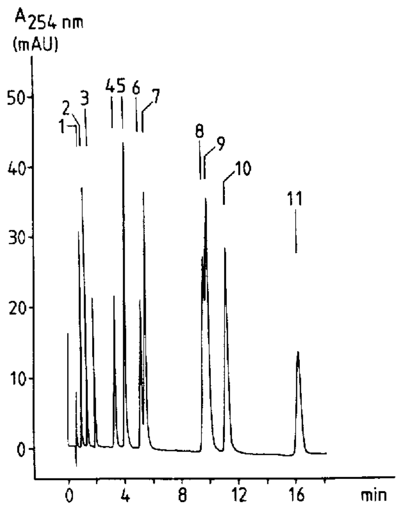


Fig. 2. Isobaric separation of eleven nitro-PAHs (see Table I). Conditions as in Fig. 1.



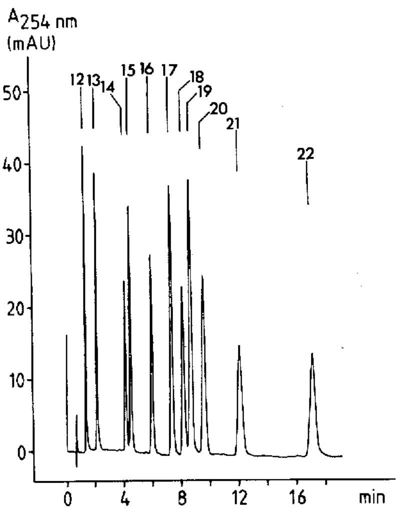
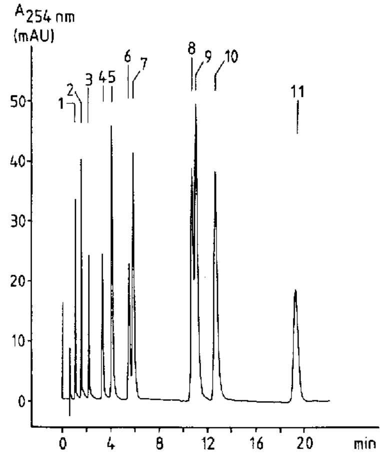


Fig. 3. Flow–pressure gradient separation of eleven PAHs (see Table I). The flow-rate of carbon dioxide was increased from 0.5 to 1.0 ml/min over a 5-min period, while the methanol flow-rate was kept constant at 20 μl/min. The pressure at the pump increased from the initial 150 bar to 270 bar when the last peak was eluted.

Fig. 4. Flow–pressure gradient separation of eleven nitro-PAHs (see Table I). Conditions as in Fig. 3.



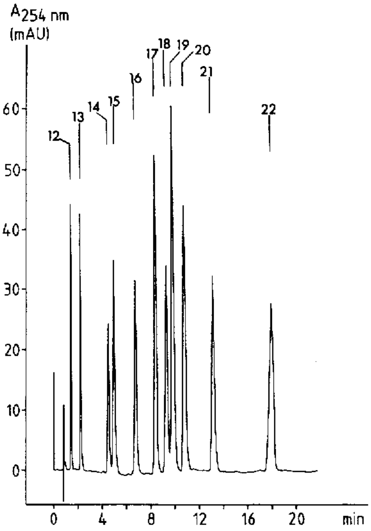
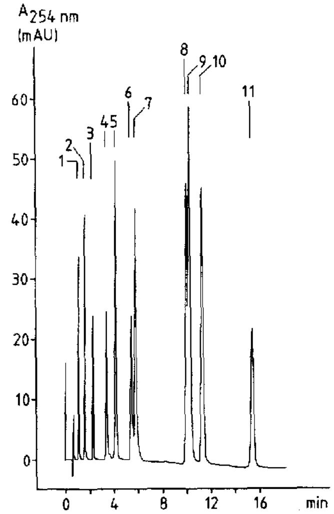


Fig. 5. Modifier gradient separation of eleven PAHs (see Table I). The flow-rate of methanol was increased from 20 to 50 μl/min over a 5-min period, while the flow-rate of carbon dioxide was kept constant at 500 μl/min. Pressure, 155–162 bar.

Fig. 6. Modifier gradient separation of eleven nitro-PAHs (see Table I). The flow-rate of methanol was increased from 15 to 50 μl/min over a 5-min period, while the flow-rate of carbon dioxide was kept constant at 500 μl/min. Pressure, 170–177 bar.



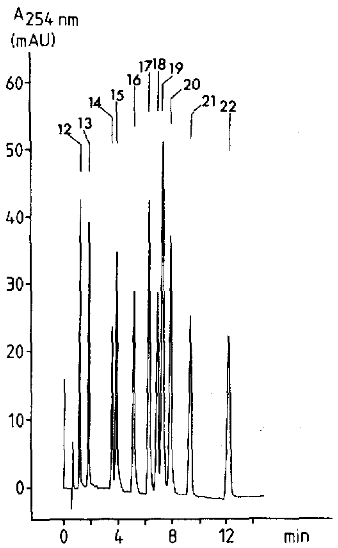


Fig. 7. Combined pressure–modifier gradient separation of eleven PAHs (see Table I). The flow-rate of carbon dioxide was kept constant at 0.5 ml/min for 5 min, then increased to 1 ml/min over a 5-min period. The flow-rate of methanol was increased from 20 to 50 μl/min over the first 5-min period, then kept constant. Pressure, 155–260 bar.

Fig. 8. Combined pressure–modifier gradient separation of eleven nitro-PAHs (see Table I). The flow-rate of carbon dioxide was increased from 0.5 to 1 ml/min over a 5-min period, simultaneously with an increase in flow-rate of methanol from 15 to 50 μl/min. Pressure, 157–267 bar.

when the last peak was eluted. Owing to the relatively large volume of the gas purifier–pulse dampener, the pressure response was slow. Typically, a 5-min flow gradient of 0.5–1 ml/min of carbon dioxide increased the pressure at the pump from 155 to 240 bar after 10 min. With the present narrow-bore columns, the pre-injector volumes must be reduced in order to obtain more rapid pressure increments.

Simple modifier gradients were constructed by linearly increasing the flow of modifier while keeping the flow of carbon dioxide constant. A 1–10% methanol gradient resulted in a slight pressure increase, typically from 180 to 187 bar. With modifier gradients, the resolution and the total elution time of PAHs (Fig. 5) and of nitro-PAHs (Fig. 6) were comparable with what was obtained by flow–pressure gra-

TABLE II

REPRODUCIBILITY OF RETENTION TIMES AND OF PEAK HEIGHTS OF PAHs AND NITRO-PAHs WITH MODIFIER GRADIENTS AND FLOW–PRESSURE GRADIENTS

Gradient	*Coefficient of variation, based on 5–10 measurements*	
	Retention time	*Peak height*
Flow–pressure	2%	2%
Modifier	2%	3%
Combined pressure–modifier	2%	2%

dients. However, the peak heights of the four most retained PAHs increased by 30–40% and of the six most retained nitro-PAHs by 40–140%, compared with the peak heights obtained with flow–pressure gradients.

The combined gradient in Fig. 7 gave the best combination of high resolution and narrow peaks of the higher PAHs. The simultaneous pressure–modifier gradient in Fig. 8 resolved the group of eleven nitro-PAHs as narrow peaks in 13 min.

The short-term (within one day) reproducibility of the different gradients was determined by measuring the coefficioents of variation for early-eluted as well as for late-eluted PAHs and nitro-PAHs. All coefficients of variation were below 2–3% (Table II).

CONCLUSIONS

Pressure programming has been the traditional way of increasing the mobile phase strength during the elution in SFC, and will continue to be used with flame-ionization detectors and with infrared spectrophotometers. With absorption and fluorescence detectors, and probably also with thermoionic detectors, flame photometric detectors and mass spectrometers, there is no apparent reason why modifier gradients cannot be used in addition to pressure–density gradients.

In this paper, the chromatographic properties of modifier gradients, and especially of combined modifier–pressure gradients, have been shown to compete favourably with flow–pressure gradients, at least for two groups of compounds of intermediate polarities. For compounds of higher polarity, the efficacy of modifier gradients should be even greater. With the equipment used in this investigation, modifier gradients and combined modifier–pressure gradients can be constructed as easily as gradients in liquid chromatography. With packed capillary columns and open tubular columns a splitting device ahead of the injector should take care of the requirement for low flow-rates, combined with reproducible gradient formation.

REFERENCES

1 M. Novotný, W. Bertsch and A. Zlatkis, *J. Chromatogr.*, 61 (1971) 17.
2 J. J. Conaway, J. A. Graham and L. B. Rogers, *J. Chromatogr. Sci.*, 16 (1978) 102.
3 P. A. Peaden and M. L. Lee, *J. Liq. Chromatogr.*, 5 (Suppl. 2) (1982) 179.
4 R. E. Jentoft and T. H. Gouw, *J. Polym. Sci., Part B*, 47 (1969) 821.
5 R. E. Jentoft and T. H. Gouw, *Anal. Chem.*, 44 (1972) 681.
6 J. A. Nieman and L. B. Rogers, *Sep. Sci.*, 10 (1975) 517.
7 E. Klesper and W. Hartman, *Eur. Polym. J.*, 14 (1978) 77.
8 D. R. Gere, R. Board and D. Mc Manigill, *Anal. Chem.*, 54 (1982) 736,
9 F. P. Schmitz, H. Hilgers and E. Klesper, *J. Chromatogr.*, 267 (1983) 267.
10 T. Greibrokk, A. L. Blilie, E. J. Johansen and E. Lundanes, *Anal. Chem.*, 56 (1984) 2681.
11 A. L. Blilie and T. Greibrokk, *Anal. Chem.*, 57 (1985) in press.
12 H. Svendsen, H-P. Rønningsen, L. K. Sydnes and T. Greibrokk, *Acta Chem. Scand.*, B37 (1983) 833.
13 E. Johansen, L. K. Sydnes and T. Greibrokk, *Acta Chem. Scand.*, B38 (1984) 309.
14 B. Iversen, L. K. Sydnes and T. Greibrokk, *Acta Chem. Scand.*, B39 (1985) in press.
15 T. Greibrokk, B. Iversen, E. J. Johansen, H.-P. Rønningsen and H. Svendsen, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 671.