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CONSECUTIVE GRADIENTS IN CHROMATOGRAPHY

GRADIENTS UNDER SUB- AND SUPERCRITICAL CONDITIONS INCLUDING COMPOSITION GRADIENTS AND MULTIPLE GRADIENTS

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SUMMARY

Supercritical-fluid chromatography offers the possibility of using gradients of pressure, temperature or eluent composition, which allows the "tuning" of the chromatographic conditions to a specific separation. The gradients may be combined by using them either simultaneously or consecutively. Starting from the subcritical gaseous region, separations are presented in which two simultaneous and several consecutive gradients of the three individual gradient types have been combined. Thereby, the influence of the absolute levels and of the ramps of pressure, temperature and composition on the chromatographic performance is demonstrated. Possibilities for increasing or decreasing the speed of the chromatographic separations and increasing the resolution are demonstrated.

INTRODUCTION

In addition to the well established techniques of gas chromatography (GC) and liquid chromatography (LC), supercritical-fluid chromotagraphy (SFC) is being increasingly used as an alternative approach. By definition, a supercritical fluid used as the mobile phase in SFC is a medium that is above its critical temperature, T_c, and above its critical pressure, p_c, *i.e.*, which is a dense gas. The properties of such dense gases are intermediate between those of gases and liquids. Depending on the applied temperature and pressure, the behaviour can be more gas-like or more liquid-like.

The intermediate position of dense gases gives rise to a variety of gradient techniques that are useful for SFC. Similarly to GC, gradients of increasing temperature at, *e.g.*, constant density or pressure are applicable for enhancing solute transport through the separation column. Similarly to LC, gradients of eluent composition can be applied[1]. A unique property of SFC is that the density of the mobile phase and thereby its elution strength can be increased by means of a pressure gradient or by decreasing the temperature by a temperature gradient at constant pressure[2].

The sign (positive or negative) of the temperature gradient at constant pressure to be employed in SFC depends to some extent on the transport mechanism of the

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analyte[3,4]. For instance, for analytes that have a high vapour pressure under vacuum, a positive temperature programme in the supercritical-fluid region will probably be more effective for elution than for analytes whose transport depends solely on solvation, given comparable solvation of the analytes by the dense gas. Moreover, multiple simultaneous gradients have been found to be useful for effecting high-resolution separations[1–3,5–9].

The intermediate position of SFC between GC and LC may be shown if a separation is started in the subcritical gaseous region and terminated under subcritical liquid conditions[10], as has been demonstrated by "circling" the critical point of the mobile phase under isocratic conditions by use of consecutive temperature and pressure programmes[11–13]. The interconnection between GC, SFC and LC was elaborated upon further in this study by using a series of consecutive gradients including composition gradients. In addition, the usefulness of combining consecutive and simultaneous gradients is demonstrated. Thereby, emphasis is placed on general features rather than on the optimal compromise between analysis time and resolution.

EXPERIMENTAL

The chromatographic equipment consisted of a Model 1084 B high-performance liquid chromatograph from Hewlett-Packard (Waldbronn, F.R.G.) modified for use in SFC as described previously[14], a Fractovap 4200 GC oven from Carlo Erba (Hofheim/Ts., F.R.G.) and a Tescom 26-3220-24-004 back-pressure valve from WSM (Eching, F.R.G.).

Temperature gradients were obtained using a Carlo Erba LT 430 temperature programmer. Pressure gradients were obtained by closing continuously the back-pressure valve. Gradients of eluent composition were programmed by means of the flow control device of the 1084 B instrument. The feed rate of the pumps was always 1 ml/min (liquid). UV detection was performed at 254 nm.

The stationary phase was LiChrosorb Si 100 (10μm), which was slurry packed into a stainless-steel column (25 cm × 4.5 mm I.D.) using a procedure described previously[10,15]. Before use the stationary phase was permanently modified by in-column treatment with 1,4-dioxane[15]. The mobile phase components (pentane and dioxane) were distilled and degassed before use.

The sample consisted of PS 800 polystyrene standard (from Pressure Chemical, distributed by Pfannenschmidt, Hamburg, F.R.G.) or an aromatic test mixture (naphthalene, anthracene, pyrene and chrysene) which was mixed with the PS 800. The sample solvent was hexane.

RESULTS

One advantage of SFC is the availability and usefulness of gradients of either pressure, temperature and eluent composition, which makes it possible to adjust precisely the chromatographic conditions to a given separation problem. Some of the possible combinations of the three gradients are discussed here.

In Fig. 1 a schematic outline of the first programme sequence is shown. It starts in the subcritical, low-pressure gaseous state with a temperature gradient (1), which leads to an end temperature above the critical temperature which is, however, still

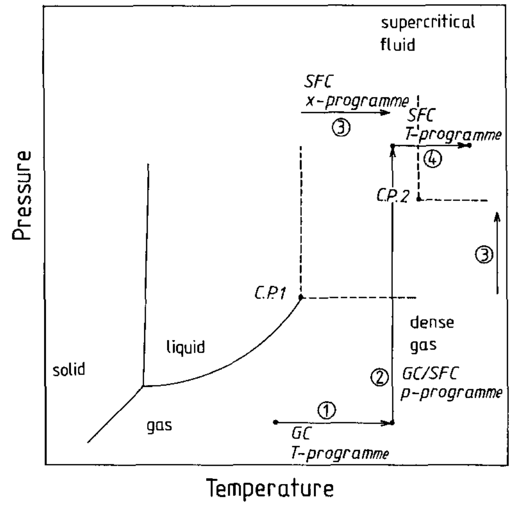

Fig. 1. Schematic representation of a sequence of consecutive gradients consisting of temperature, pressure and eluent composition gradients, the latter being superimposed by a simultaneous positive temperature gradient.

subcritical with respect to pressure. During this part of the programme sequence, elution is controlled predominantly by volatility, *i.e.*, by the vapour pressure of the analytes. Subsequently, a pressure gradient (2) is applied, leading to an end value above the critical pressure, *i.e.*, to the supercritical state. The increasing pressure causes an increase in mobile phase density, bringing about a more intense interaction between the mobile phase and the solutes and improved elution by way of solvation, a typical feature of SFC.

Finally, a composition gradient (*x* programme) (3) is applied after termination of the pressure gradient (2). By adding a component of higher solvating ability to the eluent, the retention is decreased owing to an increased interaction between the mobile phase and solute molecules, which depends on the chemical structure of the added component. The change in the mobile phase composition leads to a shift of the critical point of the mobile phase. This is indicated in Fig. 1 by the different positions of C.P. 1 (critical point for the initial mobile phase composition) and C.P. 2 (critical point for the mobile phase composition at the end of the composition programme). If the end temperature of programme 1 is below the critical temperature of the mobile phase at the end of the composition gradient (3), then the mobile phase will be a subcritical liquid at the end of the programme sequence. This effect is prevented, keeping the mobile phase in the supercritical state, if a temperature programme (4) is run simultaneously with the composition programme (3).

The separation of a polystyrene sample according to the gradient sequence in Fig. 1 is shown in Fig. 2. The pertinent chromatographic data are listed in Table I. Elution starts with a mobile phase of pentane–1,4-dioxane (95:5, v/v) at 10 bar and 190°C. The starting values were chosen as to achieve the elution of some of the low-molecular-weight oligomer species in the low-pressure gaseous state. During the first gradient, where the elution process is like that in GC, only the first two oligomers of the analyte mixture are eluted. Such a temperature gradient at a low pressure can

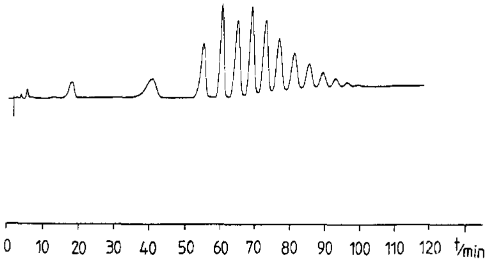

Fig. 2. Chromatographic separation according to the scheme in Fig. 1. Separation of PS 800. T_1 = 190–260°C; p = 10–70 bar; x = 5–60%. On the x programme is superimposed a temperature programme in two linear sections, T_2 = 260–290°C and T_3 = 290–300°C. For more detailed conditions see Table I and Experimental.

TABLE I

DATA FOR THE GRADIENTS UTILIZED TO OBTAIN THE CHROMATOGRAMS WITH PENTANE–1,4-DIOXANE AS THE MOBILE PHASE

T = Temperature (°C), either initial and final values or constant values; programming of T is linear with time. p = pressure at column exit (bar), either initial and final values or constant values; programming is linear. x = eluent composition. [% dioxane (v/v)], either initial and final values or constant values; programming is linear with respect to Q_B, where $Q_B = P_B/(1 - P_B)$ and P_B is the volume fraction of the mobile phase component B (1,4-dioxane). Hence the volume fraction itself is programmed non-linearly with time. t = time (min); all times are counted from the start of the chromatogram.

Chromatogram in Fig.	*T (°C)*	*p (bar)*	*x (% of second component)*	*t (min)*
2	190–260	10	5	0–30
	260	10–70	5	30–60
		70	5–60	60–140
	260–290	70		60–80
	290–300	70		80–140
4a	220–270	10–50	5	0–40
	270	50	5–60	40–120
4b	220–285	10–50	5	0–40
	285	50	5–60	40–120
4c	220–300	10–50	5	0–40
	300	50	5–60	40–120
6a	220–300	10–70	5	0–40
	300	70	5–60	40–120
6b	220–300	10–60	5	0–40
	300	60	5–60	40–120
6c	220–300	10–50	5	0–40
	300	50	5–60	40–120
8a	220–250	10–60	5	0–40
	250–300	60	5–60	40–120
8b	220–270	10–50	5	0–40
	270–300	50	5–60	40–120
9	180–260	10–60	5	0–40
		60	5–60	40–120
	260–290	60		40–60
	290–300	60		60–120

be of interest for the separation of samples that contain components with considerable vapour pressures, which are suitable for the GC mode.

During the subsequent pressure gradient (2), elution is greatly accelerated once a certain pressure level has been reached. If the chromatographic conditions are kept constant at the end of the pressure programme, exponentially increasing elution times would result for the remainder of the sample which has not yet been eluted, a typical feature of homologous series[16]. To compensate for this effect, a composition gradient (3) is applied subsequently. The temperature gradient (4) run in parallel with the composition gradient (3) keeps the mobile phase in the supercritical state despite the changing composition.

A different programme sequence is shown in Fig. 3. Here, the separation starts in the gaseous state at a temperature above the critical temperature of the eluent. A simultaneous (coupled) pressure–temperature (1) programme leads to the supercritical state. This yields a reasonably fast elution of the first components of the sample. Three different gradients, A, B, and C, are shown which all end at the same pressure but at different temperatures. The simultaneous p,T programmes are followed by a composition programme (2), in order to compensate for the increasing distances between the peaks of the higher members of the homologous series, this spreading occurring when the p,T programme has ended. During the composition programme a transition of the eluent from the supercritical to the subcritical gaseous state takes place for programmes B and C, whereas for programme A even a transition of the mobile phase from the gaseous to the liquid may occur. This is indicated when calculating the critical temperature of a pentane–1,4-dioxane mixture containing 60% (v/v) of dioxane[17].

The chromatograms corresponding to the scheme of gradients in Fig. 3 are shown in Fig. 4a–c. The numerical data for the gradients are given in Table I. Each of

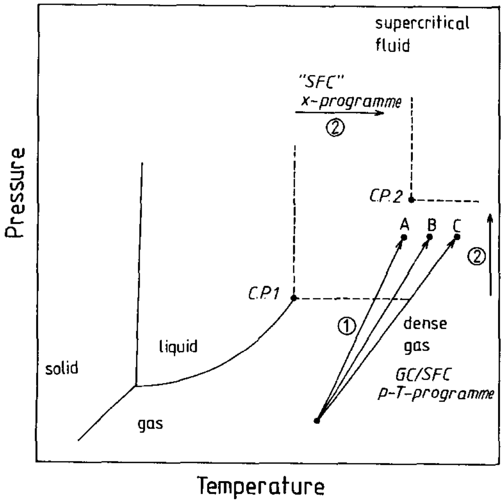

Fig. 3. Schematic representation of a sequence of gradients consisting of a simultaneous pressure–temperature programme (1) and a subsequent composition programme (2). Three gradient sequences have been run which differ in the end temperature of the simultaneous pressure–temperature programme A, B, and C.

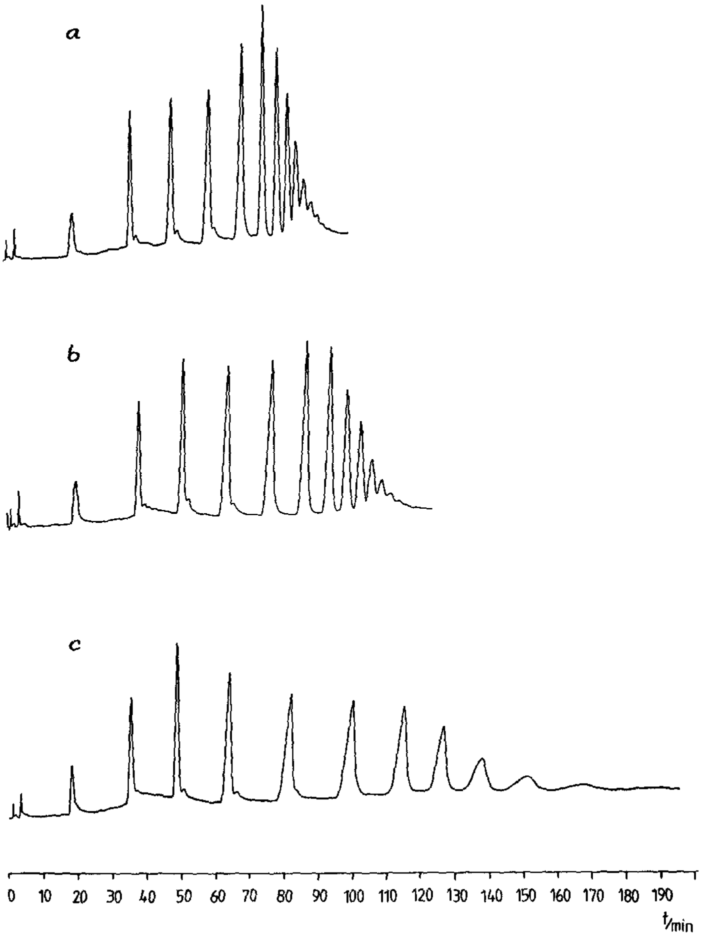

Fig. 4. ·Chromatographic separations according to the scheme in Fig. 3. Separation of PS 800. p = 10–50 bar; x = 5–60%. (a) T = 220–270°C; (b) T = 220–285°C; (c) T = 220–300°C. See Table I and Experimental.·

the chromatograms follows the same pressure programme from 10 to 50 bar and the same composition programme from 5 to 60%. The ramp of the temperature gradients, however, increases from chromatogram a to c, ending at 270, 285 and 300°C, respectively, each starting at 220°C. During the simultaneous pressure–temperature gradients hardly any difference can be seen between the three chromatograms, three peaks appearing in each. The subsequent composition gradient speeds up the elution of the remaining less volatile and less soluble components. Because the same composition gradient has been used for the three p,T programmes, the effect of the end temperature of the p,T programme becomes obvious. Whereas at an end temperature of 270°C elution is very rapid and resolution is only moderate during the composition programme, elution is much retarded at 300°C and the resolution is improved. Comparing the number of peaks detectable in the three chromatograms, fifteen oligomeric species can be counted in Fig. 4a and b, whereas only twelve peaks and a much longer analysis time are apparent in Fig. 4c. This retardation of elution at the higher temperature is due to the lower density of the mobile phase and, thereby, to the decreasing interaction between the analytes and the mobile phase. For chromatogram c, the interaction is even too weak to elute all components of the sample.

In the programme series in Figs. 3 and 4 it was the influence of temperature on the chromatographic separation that was discussed. In Fig. 5, a sequence of programmes is shown by means of which the effect of pressure can be demonstrated. Again the separation is started with a coupled pressure–temperature programme (1), this time ending at the same temperature but at three different end pressures (programmes A, B and C). After the end pressures have been reached, a composition gradient follows (2). For programmes A and B, the pressure and temperature are high enough to keep the eluent in the supercritical state during the whole range of the composition gradient. In C the conditions become subcritical during the composition gradient inasmuch as a pressure lower than the critical pressure leads to a subcritical dense gaseous state.

Fig. 6 shows the chromatograms corresponding to the scheme in Fig. 5. As seen in Table I, the temperature and composition programmes are the same for all three runs. The simultaneous pressure gradients, however, become steeper on going from c to a (Table I). The increased pressure ramp and level result in definite acceleration of elution going on from trace c to a. Whereas at the lowest pressure only twelve peaks are detected (c), there are fifteen peaks in the second chromatogram (b) and seventeen in the third (a). The steeper pressure gradient shows its influence at the beginning of the separation: the higher the pressure level, the faster is the elution of the first peaks. The subsequent composition gradient is matched well with the preceding p,T gradient in that the distances between the later peaks are kept approximately the same. For the composition gradient the effects of an increased pressure level are accelerated elution

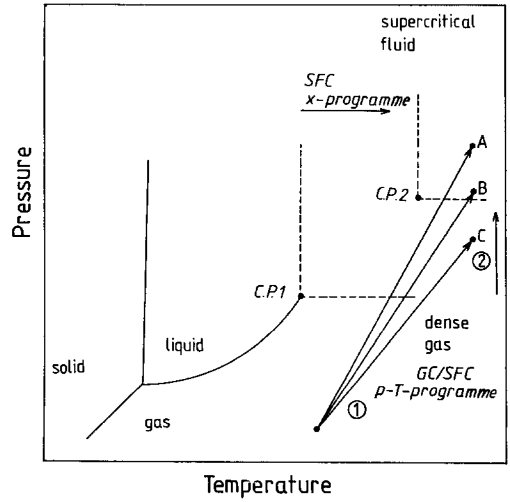

Fig. 5. Schematic representation of a sequence of gradients of simultaneous pressure–temperature programming (1) and subsequent composition programming (2) at different final pressures (A, B and C).

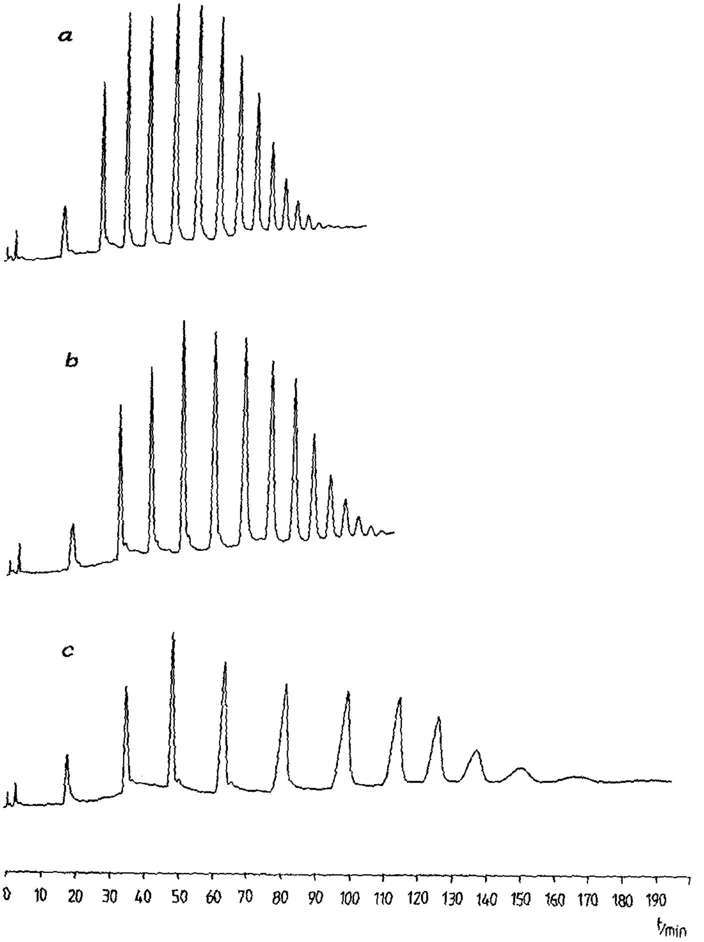

Fig. 6. Chromatographic separations according to the scheme in Fig. 5. Separation of PS 800. T = 220–300°C (a–c); x = 5–60% (a–c); p = (a) 10–70 bar, (b) 10–60 bar and (c) 10–50 bar. See Table I and Experimental.

and smaller peak widths. Hence, variation of the pressure level provides an effective means of optimizing the conditions for the composition gradient.

The scheme in Fig. 7 shows another possibility for working with coupled gradients. As in the two preceding series a pressure–temperature gradient is applied to proceed from the gaseous to the supercritical state, and then a composition gradient follows, the latter paralleled by a positive temperature gradient. The two programme sequences A and B can be regarded as a combination of the sequences shown in Figs. 1, 3 and 5. Combining the pressure and temperature gradients (p,T programme) corresponds to Figs. 3 and 5, and the final combination of a composition gradient with a temperature gradient (x,T programme) corresponds to Fig. 1. The two programme sequences shown in Fig. 7 differ in that for programme sequence A the mobile phase is supercritical during the entire x programme whereas a transition to

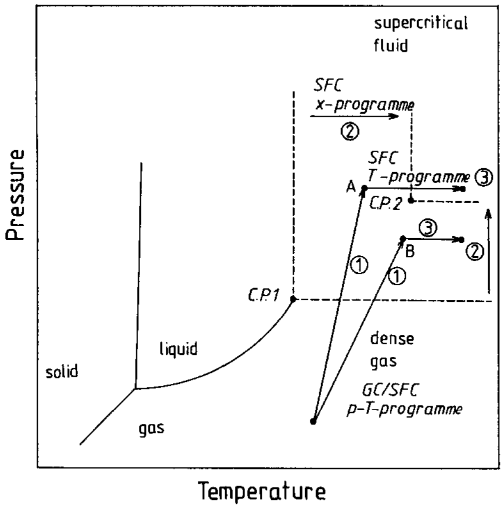

Fig. 7. Schematic representations of sequential programmes as in Figs. 3 and 5, except for combined composition (2)–temperature (3) programming.

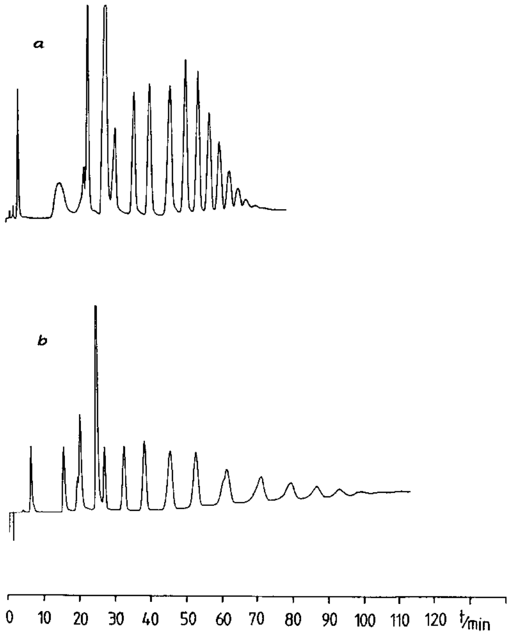

Fig. 8. Chromatographic separations according to the scheme in Fig. 7. (a) p = 10–60 bar, T_1 = 220–250°C, x = 5–60%, T_2 = 250–300°C; (b) p = 10–50 bar, T_1 = 220–270°C, x = 5–60%, T_2 = 270–300°C. Elution order: styrene oligomer with n = 1, naphthalene, anthracene, n = 2, pyrene, chrysene, n = 3 to n = 14. See Table I and Experimental.

the subcritical gaseous state occurs during the x programme in sequence B. The latter transition is due to the pressure being below p_c for part of the composition range of the binary mobile phase.

The chromatograms in Figs. 8 and 9, which were run according to the programming sequences in Fig. 7, show separations of a polystyrene sample to which a mixture of naphthalene, anthracene, pyrene and chrysene was added in order to give a larger number of volatile components in the sample. Fig. 8a shows a fast separation of the sample. As with the chromatogram in Fig. 8b, partial resolution of pyrene and the styrene oligomer species with $n = 2$ and also partial resolution of chrysene and the oligomer species with $n = 3$ are seen. Whereas fourteen oligomers can be detected in Fig. 8a, only twelve are resolved in Fig. 8b. The separation in Fig. 8b shows a significantly increased elution time in comparison with Fig. 8a, particularly in the second part of the chromatogram. This is caused by two effects acting in the same direction; the first is the lower pressure level compared with Fig. 8a, and the second is the more steeply ramped temperature gradient that takes the eluent more quickly to temperatures where lower density and slower elution prevail. During the whole separation the temperature level is higher in Fig. 8b than in Fig. 8a. The high temperature also brings about considerable peak broadening, the reason for which is not clear. Possibly isomeric species are partly separated, which makes the peaks look broader.

A similar effect of temperature is observed for the chromatograms in Figs. 6c and 8b. Compared with the chromatogram in Fig. 6, which was run with the same pressure programme, the same composition programme and to the same end temperature, elution is faster in Fig. 8b. This is due to the slower temperature increase in Fig. 8b, so that during the whole separation, except at the very end of the programme sequence, a lower temperature prevails.

Fig. 9 demonstrates a variation of the chromatogram in Fig. 8a. With the p and the x programmes being the same, the separation in Fig. 9 was started at a lower temperature (1) and the temperature gradient (3) was subdivided into two steps, a faster and a slower one (*cf.*, Table I). Based on previously published data for pentane–dioxane[17,18], this temperature gradient, which runs simultaneously with the composition programme (2), was chosen so as to match conditions of high resolution. Such combined x–T gradients have been reported to yield improved resolution compared with isothermal composition gradients[3,6,19]. In fact, a higher resolution is obtained towards the end of the chromatogram. However, the analysis time is longer and the peaks are broader. The broadening of the peaks may again be partly due to isomer separation.

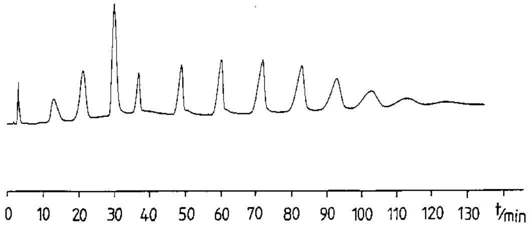

Fig. 9. Chromatographic separation according to the scheme in Fig. 7. p = 10–60 bar; T_1 = 180–260°C; x = 5–60%; T_2 = 260–290°C; T_3 = 290–300°C. See Table I and Experimental.

CONCLUSION

Chromatographic separations that utilize the *p–T–x* properties of supercritical fluids offer unique possibilities, *e.g.*, the option of applying gradients of pressure, temperature and eluent composition. As demonstrated here, SFC can be used as a link to either GC or LC, enabling the chromatographer both to extend and to interconnect the three techniques.

Consecutive and/or simultaneous gradients can be of value in order to "tune" chromatographic conditions to a given analytical problem. If a separation starts under subcritical gaseous conditions, volatile components of a given sample can be separated under high-resolution, GC-like conditions, followed by separation of the remainder of the sample with increasing temperature and pressure until supercritical conditions are reached. Thereby the temperature does not have to be raised as high as in high-temperature GC. Consecutively, composition gradients can be utilized to elute sample components of higher molar mass. The composition gradients may also be combined with simultaneous temperature gradients.

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