Journal of Chromatography, 557 (1991) 47–58
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 241

Separation of *trans/cis*- α- and β-carotenes by supercritical fluid chromatography

I. Effects of temperature, pressure and organic modifiers on the retention of carotenes

M.-C. AUBERT, C. R. LEE and A. M. KRSTULOVIĆ*
Synthélabo Recherche (L.E.R.S.), Recherche Analytique et Contrôle Pharmaceutique, 23/25 Avenue Morane Saulnier, 92360 Meudon la Forêt (France)
and
E. LESELLIER, M.-R. PÉCHARD and A. TCHAPLA
LETIAM, Institut Universitaire de Technologie d'Orsay, BP 127, 91403 Orsay Cedex (France)

ABSTRACT

A partial separation of the isomers of α- and β-carotenes has previously been achieved by non-aqueous reversed-phase chromatography on octadecyl silica columns eluted with mixtures of hexane and polar modifiers. We show that improved results are obtained by the use of mobile phases based on liquid or supercritical carbon dioxide. Polar modifiers have similar effects on chromatographic selectivity in the non-aqueous reversed-phase and supercritical fluid chromatography systems, suggesting that the retention mechanism is the same in both cases (predominantly partition). With the carbon dioxide-based mobile phases, it was possible to optimize chromatographic efficiency by varying the temperature and pressure. The use of both binary and ternary mixtures was investigated.

INTRODUCTION

The carotenoids are natural pigments that are responsible for the orange–yellow colour of numerous plant tissues. Carotenes are carotenoid hydrocarbons of which several types are known. The most abundant of these, designated α- and β-carotenes, are the principal forms of provitamin A in the diet of humans. Certain carotenes (notably β-carotene) are anticarcinogenic [1,2].

In fresh plant tissue, all of the double bonds have the *trans* configuration (all-*trans*), which is the most stable thermodynamically [3–5]. Isomerization to *cis* configurations results in a loss of nutritional value [3–5]; the determination of these isomers is necessary for the quality control of fresh foodstuffs and for the evaluation of the effects of food-processing on freshness.

The analysis of carotene isomers is usually carried out by high-performance

0021-9673/91/$03.50

liquid chromatography (HPLC) with either normal-phase [6,7] or reversed-phase [8–10] systems. The simultaneous analysis of the *cis* and *trans* isomers of the α- and β-carotenes has also been achieved with an isocratic non-aqueous reversed-phase NARP) system [10]. In these studies, conventional octadecyl-substituted silica columns were eluted with hexane, to which was added various polar modifiers. Nevertheless, analysis times were long (45 min) and all compounds of interest could not be separated under these conditions.

Supercritical fluids have lower viscosities than liquids and thus solute diffusion coefficients are higher than in conventional solvents; reports of the supercritical fluid extraction [11] and chromatography [12,13] of carotenes have already appeared. Since the solvent polarity of supercritical carbon dioxide is similar to that of hexane, it was a logical step to investigate the use of carbon dioxide for NARP chromatography. The elution power of supercritical (or nearly supercritical) fluids can be varied, not only by the use of solvent modifiers but also by means of changes in temperature and pressure. In this report, we describe the influence of these experimental variables on the separation of carotenes by NARP with high-pressure carbon dioxide as the main constituent of the eluent.

EXPERIMENTAL

Chemicals

The solvents of HPLC grade were purchased either from Prolabo (Paris, France) or Carlo Erba (Milan, Italy). Carbon dioxide (N45 grade, containing $\leqslant 7$ ppm water) was purchased from Alphagaz (Bois d'Arcy, France).

Preparation of samples

Pigments were extracted from carrots (Nantaise variety) with a mixture of hexane, diethyl ether and acetone (50:20:30, v/v/v), as previously described [4]. Dried extracts were stored at −25°C. Before being analysed, the extracts were dissolved in a mixture of methanol and dichloromethane (80:20, v/v) and exposed to ambient light for a few hours. Authentic samples of all-*trans* α- and β-carotenes were purchased from Sigma (St. Louis, MO, USA).

Apparatus

Chromatography was performed using equipment manufactured by Jasco (Tokyo, Japan; supplied by Prolabo). The two pumps (Model 880-PU) were connected for gradient elution by means of a Model 801-SC controller and a Model MX-50 dynamic high-pressure mixing unit. The head of the pump used for carbon dioxide was cooled to −2°C. The mixing unit was connected to a standard injector (Rheodyne, Cotati, CA, USA; Model 7125) fitted with a 20-μl loop, via a pressure-relief valve (Rheodyne; Model 7037). The column was mounted in a thermostatically controlled oven. The UV detector (Model 875-UV) was fitted with the standard high-pressure cell (4 μl; 5 mm path length). Chromatograms were recorded at 340 or 450 nm. The eluent was discharged via an automatic back-pressure regulator (Model 880-81). Chromatograms were recorded using an electronic integrator (Shimadzu, Kyoto, Japan, Model C-R3A; supplied by Roucaire, Vélizy-Villacoublay, France).

The chromatographic columns tested in the course of this study were the fol-

lowing: 5 μm Spheri-5 ODS-5A (250 × 4.6 mm I.D.), Brownlee Labs., Santa Clara, CA, USA; 5 μm Ultrabase UB225 (250 × 4.6 mm I.D.), S.F.C.C., Gagny, France; 5 μm Nucleosil C_{18} (250 × 4.6 mm I.D.) Macherey-Nagel, Düren, Germany; 5 μm Superspher 100 RP-18 (250 × 4.0 mm I.D.), Merck, Darmstadt, Germany; 5 μm LiChrospher 100 RP-18 (200 × 4.0 mm I.D.), Merck; 5 μm LiChrospher 100 RP-18E (200 × 4.0 mm I.D.), Merck; 5 μm ChromTech CT-Sil C_{18} (150 × 4.6 mm I.D.), ChromTech, Norborg, Sweden; and 3-μm Spherisorb ODS-2 (100 × 4.6 mm I.D.), Phase Separations, Deeside, UK.

RESULTS AND DISCUSSION

Effects of variations in pressure and temperature

This part of the study was carried out with a Spheri-5 ODS-5A column that had successfully been used for NARP liquid chromatography [10]. With supercritical fluid chromatography (SFC), the order of elution of the carotenes was the same as with NARP: all-*trans* α-carotene, *cis* isomers of α-carotene, all-*trans* β-carotene, *cis* isomers of β-carotene.

Effect of temperature. When the mobile phase in SFC is carbon dioxide without modifiers, the capacity factor (k') increases in proportion to the temperature (at constant pressure), whether the stationary phase is bare or octadecyl-grafted silica. This effect can most likely be explained by the temperature-dependent decrease in density of the mobile phase [15]. On the other hand, when the carotenes are eluted with carbon dioxide containing 12% methanol, k' decreases with increasing temperatures between 22°C and 55°C (Fig. 1), an effect that is typical of liquid chromatographic separations. It seems that the temperature-dependent increase in solute–solvent interactions more than compensates for the decrease in density.

The resolution between the all-*trans* α- and β-carotenes decreases as the temperature is increased (Fig. 2). Since the selectivity remains constant, the improvement observed on lowering the temperature must be due to the increase in k' or to increased

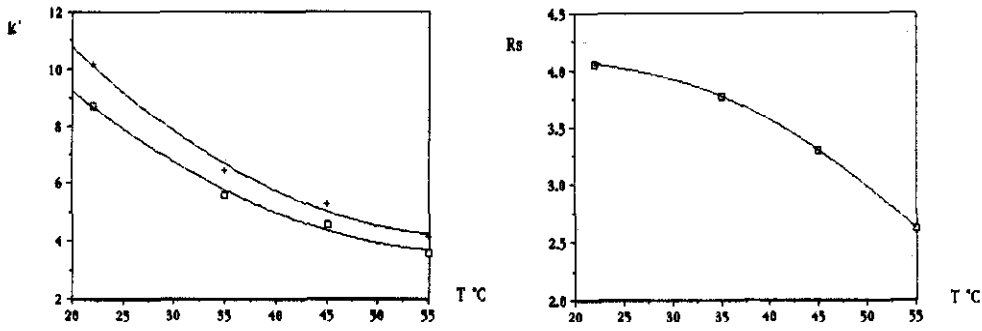

Fig. 1. Dependence of k' values of carotenes on temperature at constant pressure (250 bar, 25 MPa). Column Spheri-5 ODS-5A; mobile phase carbon dioxide–methanol (80:20, v/v); flow-rate 3.0 ml/min; detection 450 nm. Key: □ = all-*trans* α-carotene; + = all-*trans* β-carotene.

Fig. 2. Resolution R_s of all-*trans* α- and β-carotenes as a function of temperature. The chromatographic conditions are indicated in the legend to Fig. 1.

efficiency (theoretical plate number, N). The efficiency is related to the reduced plate height (h) by the equation:

$$N = L/hd_p \tag{1}$$

where L is the column length and d_p the particle size.

The two principal factors determining the reduced plate height are k' and the solute diffusion coefficient (D_m), which appear in terms B and C of eqn. 2:

$$h = A + B/v + Cv \tag{2}$$

where v is the reduced mobile phase velocity.

Term B, which describes the longitudinal diffusion, is generally considered constant and close to 2. Mourier [15] has shown that term C, which describes mass transfer, depends on k' according to eqn. 3:

$$C = k'/30(1 + k') \cdot D_m/D_s \tag{3}$$

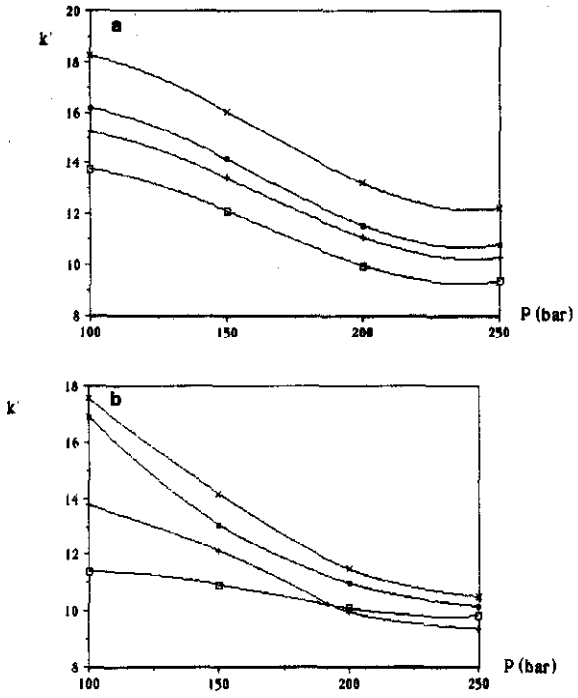

Fig. 3. (a) Dependence of k' values of carotenes on pressure (p) at constant temperature (22°C). The chromatographic conditions were otherwise as indicated in the legend to Fig. 1. Key: □ = all-*trans* α-carotene; + = *cis* α-carotenes; ■ = all-*trans* β-carotene; × = *cis* β-carotenes. (b) Dependence of k' values of carotenes on pressure, with different percentages of organic modifier (methanol). The temperature was 25°C and the other chromatographic conditions were as indicated in the legend to Fig. 1. Key: □ = all-*trans* α-carotene eluted with carbon dioxide–methanol (80:20, v/v); + = *cis* α-carotenes eluted with carbon dioxide–methanol (85:15, v/v); ■ = all-*trans* β-carotene eluted with carbon dioxide–methanol (88:12, v/v); × = *cis* β-carotenes eluted with carbon dioxide–methanol (88:12, v/v).

TABLE I

EFFECT OF PRESSURE ON RESOLUTION (R_s) OF CAROTENES

Pressure (MPa)	Resolution of β/α all-*trans*			Resolution of β *trans*/α *cis*		
	Methanol 20%	Methanol 15%	Methanol 12%	Methanol 20%	Methanol 15%	Methanol 12%
10	4.61	5.17	4.79	1.40	1.91	1.91
15	4.19	4.73	4.73	1.28	1.64	1.75
20	4.31	4.56	4.34	1.31	1.58	1.57
25	3.85	4.29	4.20	1.11	1.47	1.59

Hence C decreases as k' increases, and therefore an increase in k' is associated with increased efficiency. In fact, the increase in retention time should favour increased solute diffusion D_s at the surface of the stationary phase [15], an effect that would lead to a further reduction in the mass transfer term. At carbon dioxide densities greater than 0.6 g/cm^3, D_m can be evaluated from the equation of Wilke and Chang, adapted to SFC [15]:

$$D_m = 7.4 \cdot 10^{-15} \cdot (\psi \cdot M_s)^{0.5}/(\eta V_s)^{0.6} \quad (4)$$

The optimum temperature appears to be between 22 and 25°C; clearly, the mobile phase is subcritical. This is of no consequence, since there is no discontinuity in the physical properties of the fluid at the critical point. In some applications, particularly chiral separations, the best selectivities between isomers are obtained under subcritical conditions [16]. Also, the optimal temperature for NARP separations is between 20 and 25°C [10]; fortunately, this is low enough for the study of thermolabile carotenoids.

Effect of pressure. We studied the effects of varying the mobile phase pressure between 100 and 250 bar (10 and 25 MPa), at 22°C. Increasing the pressure led to decreased capacity factors (Fig. 3), an effect that can be explained by enhanced solubility of the solutes with increasing density of the mobile phase. The capacity factors appear to decrease less rapidly at pressures above 200 bar (20 MPa), probably because of the non-linearity of the P–T curve of carbon dioxide which, at 22°C, becomes progressively less compressible (Fig. 3a). The capacity factor also becomes less pres-

TABLE II

EFFECT OF PRESSURE ON EFFICIENCY (N) CALCULATED FOR ALL-*trans* β-CAROTENE

Methanol (%)	Efficiency (N)			
	10 MPa	15 MPa	20 MPa	25 MPa
15	16550	15900	15400	14300
12	17700	17000	14900	14500
10	17500	15950	15600	15400

sure-dependent as the concentration of methanol is increased (Fig. 3b), indicating that the modifier diminishes the compressibility of the fluid, which becomes almost incompressible at 20% methanol.

The selectivity between the all-*trans* α- and β-carotenes, as well as that between the *cis* and *trans* isomers, is independent of both pressure and temperature (and therefore of the fluid density). The resolution between the different isomers decreases with increasing pressure (Table I), and the separation between the *cis* α-carotenes and all-*trans* β-carotene is adequate only at the lowest pressures that were used. Since the selectivity is constant, the pressure-dependent changes in resolution must be due to changes in efficiency (Table II). Increasing the pressure increases the mobile phase viscosity, thus decreasing the mass transfer term C (eqn. 3) and the diffusion coefficient (eqn. 4). In the present case, when the retention time is increased by decreasing the pressure, the unfavourable effects of the increased diffusion coefficient (term D_m) are more than compensated by a reduction in the peak broadening that is due to the finite rate of solute diffusion at the surface of the stationary phase (term D_s).

In practice, it is preferable to avoid working at pressures at which the fluid is very compressible, as the pressure drop along the column is associated with a density gradient and reduced efficiency. Hence a pressure of 15 MPa was preferred to 10 MPa.

A chromatogram of a carrot extract on a Spheri-5 ODS-5A column, obtained under optimized conditions of pressure and temperature, is shown in Fig. 4. Total analysis time was 16 min, whereas the analysis required 45 min when the same column was used in NARP HPLC [10]. In addition, the SFC analysis revealed a peak (retention time 15.5 min, labelled 4b in Fig. 4) that was not detected by HPLC [10], probably because of its long retention time. This peak absorbs strongly at 340 nm; it is probably due to 9- or 13-*cis* β-carotene.

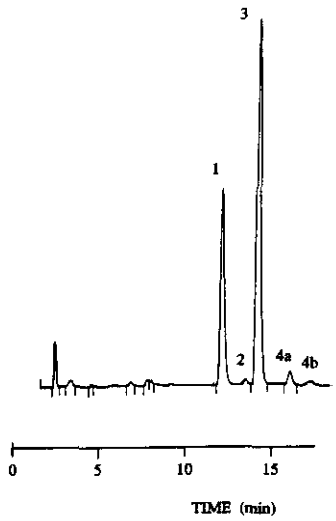

Fig. 4. Separation of the components of a carrot extract on a Spheri-5 ODS-5A column. Peaks: 1 = all-*trans* α-carotene; 2 = *cis* isomers of α-carotene; 3 = all-*trans* β-carotene; 4a = *cis* isomers of β-carotene; 4b = unknown, probably 9- or 13-*cis* β-carotene.

Effects of mobile phase modifiers

Published studies of the effects of modifiers in SFC have generally concerned the chromatography of polar compounds such as phenols [15,17]. Whether the stationary phase is bare or octadecyl silica, the addition of modifiers to supercritical carbon dioxide leads to reduced capacity factors. When the modifier is a polar compound, such as methanol, this reduction is probably due to deactivation of silanol groups and to specific interactions between the solutes and the modifier. However, some reports in the literature [18] do show a direct correlation between solute retention and elution strength of the mobile phase. With carbon dioxide based mobile phases, the effects of apolar modifiers such as hexane are most easily explained by an increase in density of the mobile phase, an effect that is also likely to occur with polar modifiers.

We have studied the effects of a range of solvent modifiers in NARP-SFC of carotenes: methanol, acetonitrile, tetrahydrofuran (THF), dichloromethane and trichlorotrifluoroethane (TTE). Binary or ternary mixtures with carbon dioxide contained 3–20% (v/v) of the modifiers.

Binary mixtures

Each modifier produced a concentration-dependent decrease in the capacity factors of the carotenes (Fig. 5). The effectiveness of the modifiers was not directly correlated with their densities (Table III), which increase in the order: acetonitrile < methanol < THF–methanol < dichloromethane < TTE. The solvent power of these modifiers, at equal concentrations, increases in the order: methanol < acetonitrile < THF–methanol < TTE < dichloromethane. This order correlates neither with the densities nor with the polarities of the modifiers. It appears, therefore, that specific interactions between the solutes and the modifiers are important, particularly in the case of the polar solvent acetonitrile.

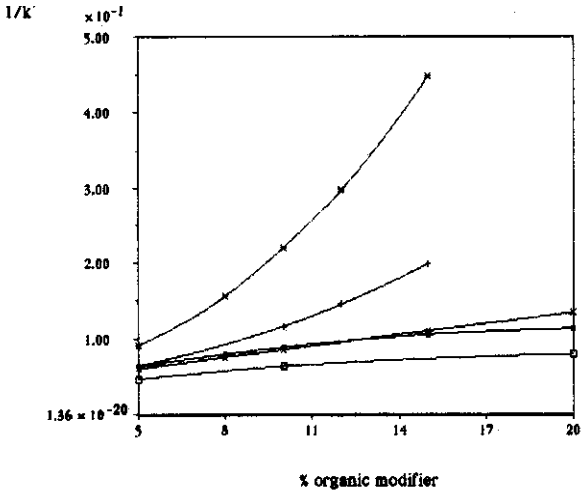

Fig. 5. Variation of $1/k'$ of all-*trans* α-carotene as a function of the concentrations of different modifiers. Chromatographic conditions as in Fig. 1, except that the column was Ultrabase UB225. Key: □ = methanol; * = dichloromethane; ■ = acetonitrile; × THF–methanol; + = trichlorotrifluoroethane.

TABLE III

DENSITIES AND POLARITIES (ACCORDING TO ROHRSCHNEIDER [20]) OF DIFFERENT SOLVENTS USED AS MODIFIERS

	Solvents				
	Acetonitrile	Methanol	THF	Dichloromethane	TTE
Density (g/l)	0.781	0.79	0.88	1.33	1.57
Polarity	5.8	5.1	4.1	3.1	< 1

Minimal retention was obtained with the strongest modifier, dichloromethane, which is the one that is closest to the dipole–dipole interaction pole of Snyder's triangle [19]. THF–methanol was the only modifier that gave a linear plot of $1/k'$ against concentration. With modifiers more polar than this, the line curved slightly downwards, as would be expected where an increase in solvent polarity favours solute–solvent interactions. Conversely, an exponential (upwards) curvature was found for the non-polar modifiers, suggesting that the solubility of the carotenes is enhanced by an amount that is independent of the concentration of modifier. Essentially the opposite effects have been observed for the chromatography of phenols [15].

The retention of the carotenes appears to be uninfluenced by the presence of residual silanol groups, as the addition of 2% water to a methanol–carbon dioxide

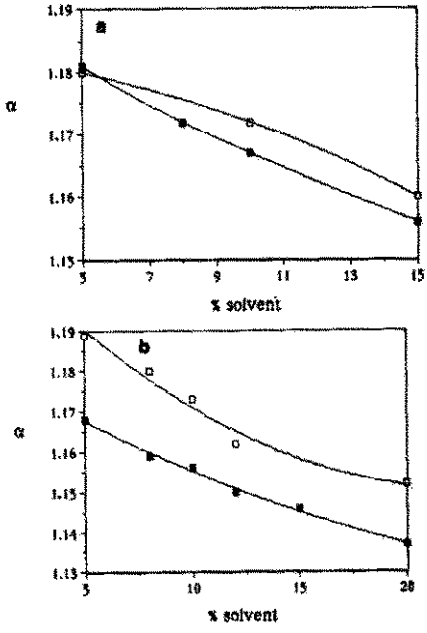

Fig. 6. Variation of the selectivity between the all-*trans* α- and β-carotenes as a function of percentages of the organic modifiers methanol (a) and acetonitrile (b). Key: ■ = Nucleosil C_{18} column; □ = Ultrabase UB225 column. The other chromatographic conditions were as in Fig. 1.

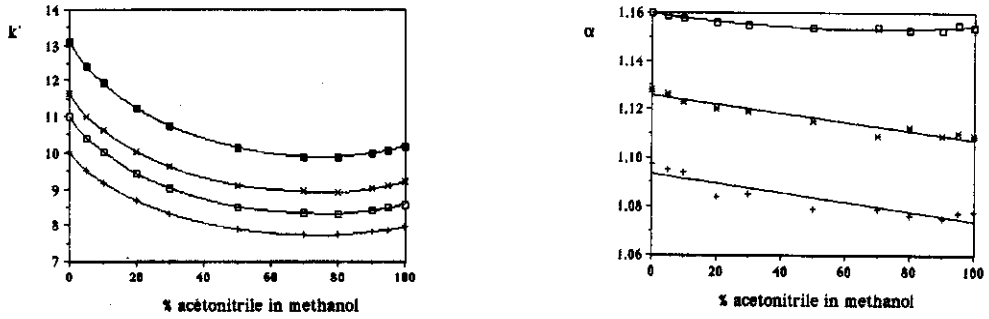

Fig. 7. Variation of k' values of carotenes as a function of the concentration of acetonitrile in methanol, the total concentration of modifier being 15%. Column Ultrabase UB225; pressure 150 bar (15 MPa); temperature 30°C; flow-rate 3.0 ml/min; detection 450 nm. Key: + = all-*trans* α-carotene; □ = *cis* α-carotenes; * = all-*trans* β-carotene; ■ = *cis* β-carotenes.

Fig. 8. Variation of the selectivity between different carotenes as a function of the concentration of acetonitrile in methanol, the total concentration of modifier being 15%. Chromatographic conditions as in Fig. 7. Key: □ = all-*trans* β-carotene/all-*trans* α-carotene; + = *cis* α-carotenes/all-*trans* α-carotene; * = *cis* β-carotenes/all-*trans* β-carotene.

mixture (15:85, v/v) resulted in increased rather than decreased capacity factors. The retention mechanism is by partition, with no adsorptive contribution.

Selectivity between the all-*trans* α- and β-carotenes is affected in a similar manner by methanol (Fig. 6a) and acetonitrile (Fig. 6b). The Ultrabase C_{18} column provided better selectivity than the Nucleosil C_{18} column. In contrast, the selectivity between *trans* and *cis* isomers is unaffected by the modifiers. With increasing percentage of modifier, the selectivity between the α- and β-all-*trans* compounds is diminished (Fig. 6), although it does not fall below 1.15 for 15% modifier.

Ternary mixtures

Analyses were carried out at 22, 25 and 30°C, with the total modifier concentration being maintained constant at 15%. In the first study, the concentration of acetonitrile in methanol was varied between 0 and 100%. The trends in selectivity and retention time were similar at all three temperatures. Although both modifiers have similar densities, they are not equivalent as far as capacity factors are concerned (Fig. 7). Retention diminishes as the methanol–acetonitrile ratio is increased up to about 70%, above which it slightly rises.

This effect, which has already been seen with NARP-HPLC [10], can be ascribed on the one hand to π–π bonding between acetonitrile and the π bonds of the carotenes, and on the other hand to increased solvent polarity (calculated by Rohrschneider's method [20]). It should be noted that acetonitrile is a stronger eluent than methanol in SFC (where the total modifier concentration is 15%), whereas the inverse is true when these solvents are used at 100% concentration in NARP-HPLC [10]. These results described here were reproduced using the following columns: Nucleosil C_{18}, LiChrospher 100 RP-18, LiChrospher 100 RP-18E, Spherisorb ODS-2, ChromTech CT-Sil C_{18}, Superspher 100 RP-18.

The selectivity between the α and β all-*trans* carotenes is independent of the

TABLE IV

SELECTIVITY BETWEEN THE UNIDENTIFIED PEAK AND ALL-*trans* α-CAROTENE AS A FUNCTION OF PERCENTAGE ACETONITRILE

Acetonitrile (%)	Selectivity
20	1.00
30	1.049
50	1.068
70	1.079
90	1.091

methanol–acetonitrile ratio (Fig. 8). However, the selectivity between *trans* and *cis* isomers falls as the proportion of acetonitrile is increased, for both the α- and β-carotenes. This specific effect on the geometric but not on the positional isomers is the reverse of that noted above for binary solvent mixtures. This may be due to differences in topology of the stationary phase between the two cases.

The reduction in selectivity between the *trans* and *cis* isomers produced by acetonitrile was used to advantage in optimizing the separation shown in Fig. 4. Since, with methanol–carbon dioxide, the peaks the least well resolved were those of

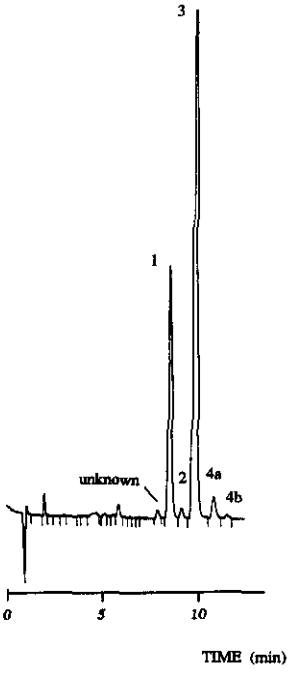

Fig. 9. Separation of the components of a carrot extract on an Ultrabase UB225 column. Mobile phase carbon dioxide–acetonitrile–methanol (85:14.25:0.75, v/v/v); temperature 22°C; pressure 150 bar (15 MPa); flow-rate 3.0 ml/min; detection 450 nm. The peaks are numbered as in Fig. 4. The peak labelled "unknown" is possibly γ- or ζ-carotene (see text).

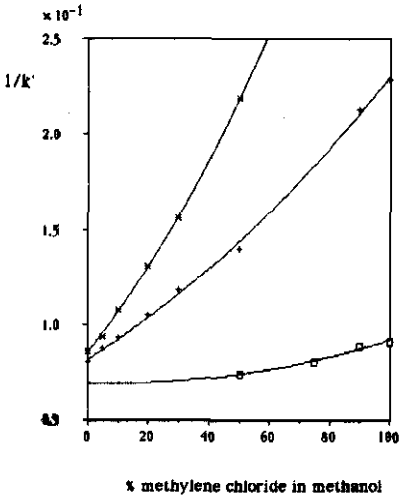

Fig. 10. Variation of $1/k'$ as a function of the percentage of dichloromethane in methanol. Column Ultrabase UB225; pressure 150 bar (15 MPa). The other chromatographic conditions were as indicated in the legend to Fig. 1. Key: □ = total modifier concentration 5%, temperature 22°C; + = total modifier concentration 10%, temperature 30°C; × = total modifier concentration 15%, temperature 22°C.

the *cis* isomers of α-carotene and all-*trans* β-carotene, the separation could be improved by replacing part of the methanol with acetonitrile. This does not cause the *trans* and *cis* isomers to overlap, as there is a good margin between them. Addition of acetonitrile revealed an additional component in the extract of carrots (Table IV), which elutes before all-*trans* α-carotene, and is completely resolved at acetonitrile–methanol ratios greater than 7:3 (Fig. 9). Since the absorbance of this compound at 340 nm is very weak, it is unlikely to be a *cis* isomer. It could be γ- or ζ-carotene, as these two compounds have structures very similar to that of α-carotene.

Some studies were carried out with ternary mixtures of carbon dioxide, methanol and dichloromethane. Total modifier concentrations were 5 and 15% at 22°C, and 10% at 30°C. Increasing the ratio of dichloromethane to methanol led to decreased capacity factors, particularly at high total modifier concentrations (Fig. 10). Plots of $1/k'$ against modifier concentration were exponential, as with the binary mixtures. Unlike acetonitrile, dichloromethane produced a general decrease in selectivities; the α- and β-carotenes were less well separated, as were the *cis* and *trans* isomers. This is probably due to reduced interactions between the solutes and the stationary phase, as all the carotenes have high affinities for this solvent.

CONCLUSIONS

The separation of *cis* and *trans* α- and β-carotenes is improved by the use of SFC techniques in place of HPLC: the analysis time is considerably shortened, and an additional *cis* isomer of β-carotene is resolved.

Pressure- and temperature-induced changes in the density of carbon dioxide affect the chromatographic efficiency, but not the selectivity. Conversely, the selectivity can be varied by the use of eluent modifiers, which do not appear to influence

the efficiency. This effect varies as a function of the type of organic modifier used in both binary and ternary mixtures.

The effects of eluent modifiers on the capacity factors and solubilities of the pigments are similar whether SFC or HPLC is used. This strongly suggests that the retention mechanism(s) are similar in both techniques. The dominant mechanism is partition, with adsorption having little or no rôle.

Ternary mixtures of acetonitrile, methanol and carbon dioxide revealed a presently unidentified compound whose peak otherwise overlapped that of all-*trans* α-carotene. However, the results obtained with this eluent depend on the C_{18} stationary phase that is employed, and they will be discussed in the following paper [21].

ACKNOWLEDGEMENT

The authors wish to acknowledge Jean-Pierre Porziemsky for his technical assistance.

REFERENCES

1 R. C. Moon and L. M. Itri, in M. B. Sporn, A. B. Roberts and D. S. Goodman (Editors), *The Retinoids*, Academic Press, New York, 1984, p. 2.
2 D. E. Ong and F. Chytil, in G. D. Aurbach (Editor), *Vitamins and Hormones*, Academic Press, New York, 1983, p. 103.
3 A. T. Ogunlesi and C. Y. Lee, *Food Chem.*, 4 (1979) 311.
4 J. P. Sweeny and A. C. March, *J. Am. Diet. Assoc.*, 59 (1971) 238.
5 L. A. Chandler and S. J. Schwartz, *J. Agric. Food Chem.*, 36 (1988) 129.
6 K. Tsukida, K. Saiki, T. Takii and Y. Koyana, *J. Chromatogr.*, 245 (1982) 359.
7 M. Vecchi, G. Englert, R. Maurer and U. Meduna, *Helv. Chim. Eng.*, 1 (1981) 264.
8 R. J. Buchway, *J. Liq. Chromatogr.*, 8 (1985) 1527.
9 F. W. Quackenbush, *J. Liq. Chromatogr.*, 10 (1987) 643.
10 E. Lesellier, C. Marty, C. Berset and A. Tchapla, *J. High Chromatogr. Chromatogr. Commun.*, 12 (1989) 447.
11 F. Favati, J. W. King, J. P. Friedrich and K. Eskins, *J. Food Sci.*, 53 (1988) 1532.
12 H. H. Schmitz, W. E. Artz, C. L. Poor, J. M. Dietz and J. W. Erdman, *J. Chromatogr.*, 479 (1989) 261.
13 D. R. Gere, *Hewlett Packard Application Note 800-5*, Hewlett Packard, Avondale, 1983.
14 E. Lesellier, *Thesis*, Paris 7 University, Paris, 1990.
15 P. Mourier, *Thesis*, Paris University, 1986.
16 C. R. Lee, J.-P. Porziemsky, M.-C. Aubert and A. M. Krstulović, *J. Chromatogr.*, 539 (1991) 55.
17 J. G. M. Jansen, P. J. Schoenmakers and C. A. Cramers, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 12 (1989) 645.
18 T. A. Berger and J. F. Deye, *Anal. Chem.*, 62 (1990) 1181.
19 L. R. Snyder, *J. Chromatogr.*, 92 (1974) 223.
20 L. Rohrschneider, *Anal. Chem.*, 45 (1973) 1241.
21 E. Lesellier, M.-R. Péchard, A. Tchapla, M.-C. Aubert, C. R. Lee and A. M. Krstulović, *J. Chromatogr.*, 557 (1991) 59.