Journal of Chromatography, 641 (1993) 137–145
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 017

Specific effects of modifiers in subcritical fluid chromatography of carotenoid pigments

E. Lesellier*

LETIAM, IUT d'Orsay, Le Plateau Moulon, B.P. 127, 91403 Orsay Cedex (France)

A.M. Krstulović

Synthelabo Recherche (LERS), 23–25 Avenue Morane Saulnier, 92360 Meudon-la-Forêt (France)

A. Tchapla

LETIAM, IUT d'Orsay, Le Plateau Moulon, B.P. 127, 91403 Orsay Cedex (France)

(First received November 5th, 1992; revised manuscript received February 12th, 1993)

ABSTRACT

The use of subcritical fluid chromatography with filled columns for the analysis of carotenoid pigments affords a threefold reduction in analysis times with respect to HPLC. The addition of modifiers to CO_2 increases the solubility of pigments in the mobile phase. This paper reports the influence of modifiers on the separation of carotenoids in terms of specific interactions, such as hydrogen bonding and $\pi-\pi$ interactions between the solvent and the solute. The understanding of these interactions allowed the optimization of the separation of seven carotenoids and some of their *cis* isomers; this separation required less than 20 min.

INTRODUCTION

Carotenoid pigments are important for human health. Although the provitamin A activity of certain carotenoids is well documented, their potential anticarcinogenic activity is still the subject of intensive research. Further, these compounds are used in foodstuffs as colouring agents (β-carotene, cathaxanthin), and some of their derivatives, such as acitretin, are used for the treatment of skin diseases [1].

Carotenoids are not synthesized *in vivo*; they are introduced into the organism through food intake and are transported by the blood. The main carotenoids that can be detected in blood [2–4] are lutein, zeaxanthin, lycopene, β-crypthoxanthin and α-, β, and γ-carotene.

Some of these compounds contain a hydroxyl or a ketone function on the terminal ring, arising from cyclization or double-bond conformation. These structural properties are of interest, as each compound will not have an identical behaviour in a given chromatographic system. Hence it is important to know these parameters and their possible effects in order to optimize a given separation. These interactions involve both the stationary and the mobile phase. The retention of carotenes in normal-phase chromatography on a polar stationary phase (silica) decreases with increasing number of double bonds and increases with cyclized lateral chains [5]. The trend is the opposite in reversed-phase chromatography with octadecyl-bonded phases. In the

* Corresponding author.

0021-9673/93/$06.00

latter instance, other workers have confirmed the influence of the degree of end-capping of residual silanols on the separation of xanthophylls [6,7]. The effect of polymeric phases on the separation of *trans*/*cis* isomers of different provitamin A activities has also been reported [8–10]. Zakaria *et al.* [11] and Nelis and De Leenheer [12] pointed out improved solubilities of these hydrophobic compounds in non-aqueous reversed-phase (NARP) chromatography. Other researchers have shown the relationship between the retention of xanthophylls and the methanol content of the eluent [6,7].

The simultaneous separation of these compounds, which has been the subject of several studies [13,14], poses two main problems. The first concerns the separation of luteine and zeaxanthine, which both bear a hydroxyl group and which are the least retained among the carotenes. Thus the elution strength of the mobile phase should not be elevated for the separation of these two compounds, which in turn results in excessive retention of other carotenes. The second problem stems from the difficulty in simultaneously separating the *trans* and *cis* isomers. The choice of both the mobile and the stationary phase is of great importance in achieving this separation.

We have already reported on the separation of carotenes by supercritical fluid chromatography (SFC) using filled columns [15]. This method offers the advantage of speed of analysis and efficiency of separation. In addition, it allows one to modulate the solvent strength by means of an appropriate modifier. We have therefore undertaken a systematic study of the influence of modifiers on the separation of carotenes in SubFC, resulting in an optimized separation method.

EXPERIMENTAL

Chemicals

The solvents used were of HPLC grade and were purchased from Prolabo (Paris, France), SDS (Vitry sur Seine, France), Carlo Erba (Milan, Italy) and Merck (Darmstadt, Germany).

Pigment extracts were kindly provided by Hoffmann La Roche (Basle, Switzerland), lycopene, γ-carotene, lutein, zeaxanthin, β-cryptoxanthin and 15-*cis*-β-carotene except for all-*trans*-α- and -β-carotene which were purchased from Sigma (St. Louis, MO, USA).

Carbon dioxide (N45 grade, containing less than 7 ppm of water) was purchased from Alphagaz (Bois d'Arcy, France).

Apparatus

Chromatographic separations were carried out using equipment manufactured by Jasco (Tokyo, Japan). The two pumps (Model 880-PU) were connected to a Séderé pulse damper (Touzart et Matignon, Vitry sur Seine, France). The head of the pump used for carbon dioxide was cooled to −2°C by an F 10c cryostat (Julabo, Seelbach, Germany). The pulse damper was connected to a Model 7125 injection valve fitted with a 20-μl loop (Rheodyne, Cotati, CA, USA).

The chromatographic column was octadecylsilica-based, 5-μm Ultrabase UB 225 (250 × 4.6 mm I.D.) from SFCC–Shandon (Eragny, France). The column was placed in a Crocosil thermostatically controlled oven (Cluzeau, Ste. Foy-la-Grande, France) maintained at 25°C.

Detection was carried out by a UV–Vis detector (Hewlett-Packard Model 1050) with a high-pressure-resistant cell. The detection wavelength was 450 nm. Chromatograms were recorded using a Model CR 6A electronic integrator (Shimadzu, Kyoto, Japan).

RESULTS AND DISCUSSION

Carotenes

The first part of the study was conducted using lycopene and γ-, α- and β-carotenes (all-*trans* and *cis* isomers), the structures of which are shown in Fig. 1. Their elution order in SubFC was found to be the same as in NARP LC [16]. The results concerning the selectivity factors for all-*trans*-α- and -β-carotenes and their isomers has already been discussed [17].

The influence of the type and percentage composition of modifiers in CO_2 under subcritical conditions on the selectivity of pairs of compounds whose separation is difficult, such as

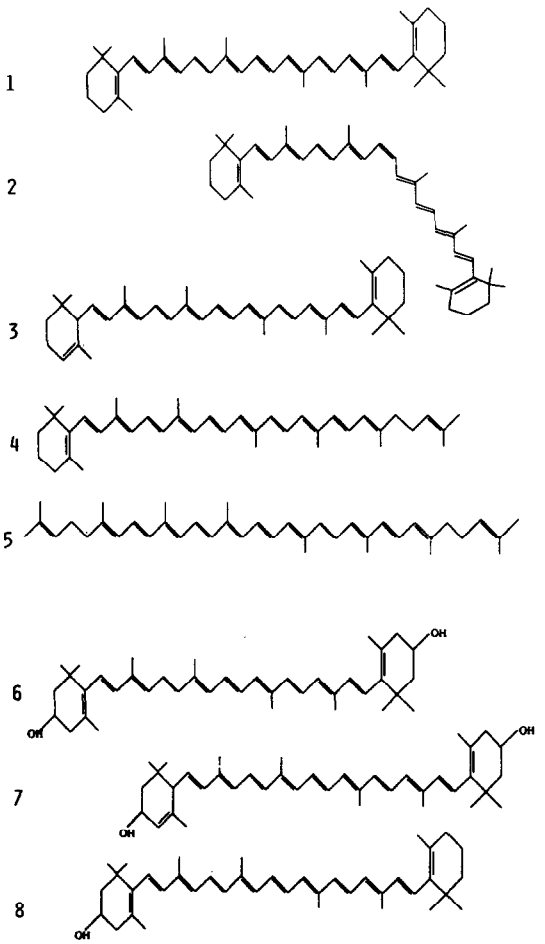

Fig. 1. Structures of carotenoids. 1 = All-*trans*-β-carotene; 2 = 15-*cis*-β-carotene; 3 = all-*trans*-α-carotene; 4 = γ-carotene; 5 = lycopene; 6 = zeaxanthin; 7 = lutein; 8 = β-cryptoxanthin.

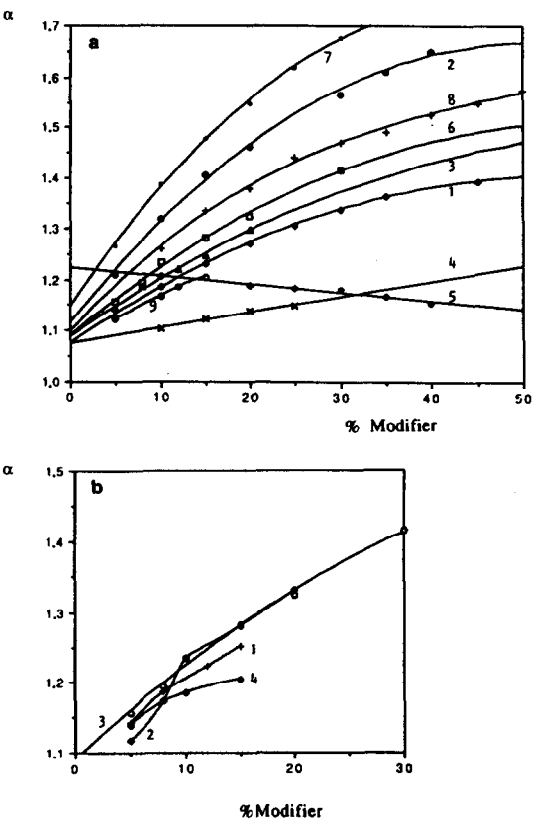

Fig. 2. (a) Variation of the lycopene–α-carotene selectivity as a function of the percentage of the modifier in subcritical fluid chromatography. Flow-rate, 3.0 ml/min; temperature, 25°C; output pressure, 15 MPa. 1 = Acetone; 2 = acetonitrile; 3 = dioxane; 4 = hexane; 5 = methanol; 6 = methylene chloride; 7 = nitromethane; 8 = propionitrile; 9 = tetrahydrofuran. (b) Variation of the lycopene–α-carotene selectivity as a function of the percentage of the chlorinated modifier in subcritical fluid chromatography. Analytical conditions as in (a). 1 = Chloroform; 2 = 1,2-dichloroethane; 3 = methylene chloride; 4 = tetrachloroethylene.

lycopene and α-carotene or γ-carotene and α-carotene, was studied.

Fig. 2a shows the variation of selectivity between lycopene and α-carotene as a function of the percentage of modifier used. The selectivity increases with increasing modifier concentration for most of the compounds studied. This increase is observed for solvents whose effects on the retention of carotenes are not necessarily identical. We have already shown that an increase in the concentration of tetrahydrofuran (THF), acetone or methylene chloride leads to decreased retention of carotenes, whereas with methanol, acetonitrile and nitromethane an initial decrease followed by an increase in retention was observed [17]. The selectivity observed therefore does not depend on the factors that govern retention, such as interfacial tension between the stationary and the mobile phases. On the basis of Hildebrand partial solubility parameters [18] (Table I), the solvents that lead to the largest increase in selectivity between lycopene and α-carotene are those which possess the highest dipole moment (δ_0), *i.e.*, nitromethane ($\delta_0 = 8$), acetonitrile ($\delta_0 = 7$) and methylene chloride ($\delta_0 = 5.5$), followed by a group of solvents whose

TABLE I

PHYSICAL AND CHEMICAL CONSTANTS OF SOME SOLVENTS USED AS MODIFIERS IN SUBCRITICAL FLUID CHROMATOGRAPHY

ε = Dielectric constant; γ = surface tension; δ = Hildebrand solubility parameters: δ_p = dispersion parameter; δ_0 = dipole–dipole parameter; δ_a = proton acceptor parameter; δ_d = proton donor parameter.

Solvent	ε	γ	δ	δ_p	δ_0	δ_a	δ_d
Methanol	32.7	22.5	12.9	6.2	5	7.5	7.5
Acetonitrile	37.5	29.1	11.8	6.5	8	2.5	0
Nitromethane	36	37	11.0	7.3	8	1	0
Ethanol	24.6	22.3	11.2	6.8	4	5	5
Propionitrile	27.2	27.2	10.8	7.5	7	2.7	0
Acetone	21.4	23.3	9.4	6.8	5	2.5	0
1-Propanol	20.1	23.7	10.2	7.2	2.5	4	4
Heptane	1.92	20.8	7.4	7.4	0	0	0
Dioxane	2.2	34.4	9.8	7.8	4	3	0
Tetrahydrofuran	7.6	27.6	9.1	7.6	4	3	0
Methylene chloride	8.9	28.1	9.6	6.4	5.5	0.5	0
Chloroform	4.80	26.5	9.1	8.1	3	0.5	0
1,2-Dichloroethane	10.36	32.2	9.7	8.2	4	0	0
Tetrachloroethylene	2.3	31.2	9.3	9.3	0	0	0

partial solubility parameter is between 4 and 5 (dioxane, acetone and THF). As the main structural difference between these compounds stems from the presence or absence of terminal cyclization of the polyethylene side-chain and the presence of two double bonds in lycopene, one can postulate the existence of dipole–dipole (π–π) interactions between these solvents and the pigments.

The extent of these interactions therefore depends on two factors: the number of double bonds in the molecule and the dipole moment of the solvent. The higher the dipole moment, the higher is the solubility of lycopene.

The difference in relative retention (measured by selectivity) between these compounds will increase with increasing concentration of the solvent in the mobile phase. It should be noted, nevertheless, that an increase in heptane concentration leads to the same effect, although it does not have a permanent dipole moment. One can therefore postulate the effect of the London-type dispersion forces, which could explain why nitromethane causes a larger increase in selectivity than acetonitrile, although their dipole moments expressed in terms of the Hildebrand partial solubility parameters are the same.

Fig. 2b also illustrates the variation in selectivity for chlorinated modifiers, for which it is easier to explain the small fluctuations in behaviour in terms of their inherent properties. The comparison of pairs of solvents confirms the previously evoked hypothesis. Thus, chloroform and 1,2-dichloromethane have similar dispersion forces (8.1 for chloroform and 8.2 for 1,2-dichloromethane), whereas the latter solvent has a larger dipole moment.

The variation of selectivity is larger for 1,2-dichloroethane. The influence of this solvent is comparable to that of methylene chloride, in spite of its lower dipole moment. Here again, the larger dispersion forces in 1,2-dichloroethane could counterbalance this difference. These interactions could also explain why the variation observed with tetrachloroethylene is almost identical with that of chloroform, in spite of the zero dipole moment. In contrast, the addition of methanol to the mobile phase entails a decrease

in selectivity, which diminishes the difference between these compounds needed for the separation.

Fig. 3 shows the behaviour of other solvents with a hydroxyl group. Solvents such as ethanol, 1-propanol, 1-butanol and 1-pentanol have only a limited influence on selectivity compared with other solvents; the selectivity varies only from 1.14 to 1.21. Conversely, one observes an initial increase followed by a decrease for alcohol concentrations close to 20%. It is possible that the proton donor character of these solvents is responsible for the observed decrease in selectivity.

This effect, which should be operative regardless of the methanol concentration owing to its large proton donor character (δ_d), would be operative for other solvents at higher concentrations. This decrease is nevertheless critical for the separation of the pigments under study.

We also studied the evolution of selectivity between γ- and α-carotene (Fig. 4). These two pigments differ only in the additional cyclization at one extreme of the α-carotene molecule. Both compounds have provitamin A activity and their separation is of interest. The selectivity factors between γ- and α-carotene are lower than those between lycopene and α-carotene, as γ-carotene is eluted between them.

The change in selectivity is comparable to the

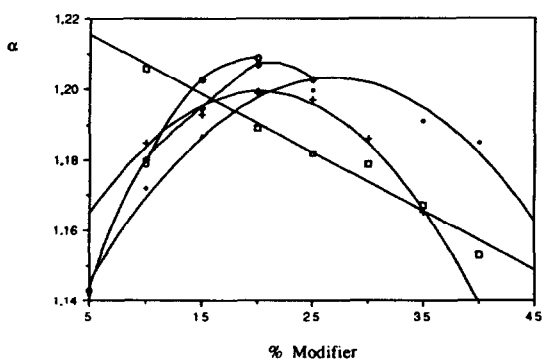

Fig. 3. Variation of the lycopene–α-carotene selectivity as a function of the percentage of the alcoholic modifier in subcritical fluid chromatography. Analytical conditions as in Fig. 2a. ◆ = 1-Butanol; ● = ethanol; □ = methanol; ○ = 1-pentanol; + = 1-propanol.

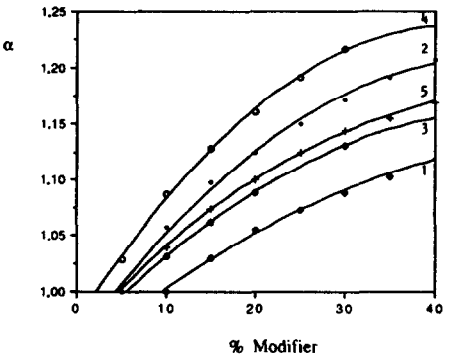

Fig. 4. Variation of the γ-carotene–α-carotene selectivity as a function of the percentage of the modifier in subcritical fluid chromatography. Analytical conditions as in Fig. 2a. 1 = Acetone; 2 = acetonitrile; 3 = methylene chloride; 4 = nitromethane; 5 = propionitrile.

previous one, and can be explained in the same manner, *i.e.*, by the π–π interactions between the double bonds and the solvents, and they are especially operative with solutes bearing an additional double bond. The sequence of solvents in terms of this effect is nitromethane, acetonitrile, propionitrile, methylene chloride and acetone. However, it is not possible to separate these two solutes with the alcohols (methanol, ethanol). The use of these solvents is therefore not advantageous for the separation of complex mixtures of carotenes.

The influence of these solvents is nevertheless very important for the separation of the *cis–trans* compounds. Similarly to the behaviour of the *cis–trans* isomers of α- and β-carotene discussed above, the *cis* isomer of γ-carotene is eluted after the *trans* compound. According to this hypothesis, it seems evident for the all-*trans*-carotenes and, in particular, γ- and α-carotene which have similar chromatographic behaviours, that the larger the extent of this separation, the smaller is the extent of co-elution of the *cis* and *trans* isomers of a given carotene.

By comparing the percentages of solvents needed to obtain identical selectivities, one can see that, for example, 15% nitromethane, 22% acetonitrile, 27% propionitrile, 30% methylene chloride and 45% acetone are required. It is known that for the last three solvents, the

selectivity between the *cis* and *trans* isomers diminishes with increasing percentage of these solvents in the mobile phase.

Two solvents appear to be particularly interesting for these analyses: nitromethane and acetonitrile. Fig. 5 shows the separation of these compounds with a mobile phase containing 15% of nitromethane, which is judged to be the best solvent. Nevertheless, the use of this solvent with a UV absorbance detector is difficult owing to the pronounced baseline noise. Identical behaviour is observed with propionitrile. However, this type of detection is of interest for the analysis of *cis* isomers or retinols.

The use of acetonitrile can also be envisaged, even though a decrease in selectivity is observed for *cis–trans* isomers at higher modifier percentages [17]. Nevertheless, the resolution between the isomers does not diminish as much as one would expect, as the retention of carotenes increases again at acetonitrile concentrations above 20%. In this instance, according to the resolution equation, the increase in the capacity factor k' entails a slight increase in resolution,

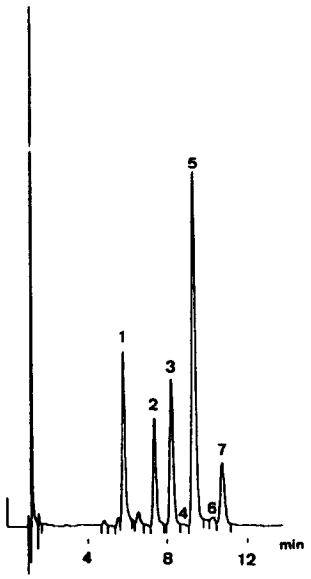

Fig. 5. Subcritical fluid chromatogram of a mixture of carotenes. Peaks; 1 = lycopene; 2 = γ-carotene; 3 = all-*trans*-α-carotene; 4 = *cis*-α-carotene; 5 = all-*trans*-β-carotene; 6 = 9- and/or 13-*cis*-β-carotene; 7 = 15-*cis*-β-carotene. Analytical conditions as in Fig. 2a; mobile phase, nitromethane–CO_2 (15:85, v/v).

which partially counteracts the decrease in selectivity.

Xanthophylls

We also studied the influence of these solvents on the retention and selectivity of lutein and zeaxanthin. These compounds are difficult to elute without an alcohol. Nevertheless, in LC they were separated without the use of alcohols [19,20]. It is possible that the retention of hydroxylated carotenoids is related to the presence of surface silanols. This is confirmed by studies that have shown that end-capping [7] or addition of triethylamine [21] diminished the retention of xanthophylls. In spite of that, these compounds were separated on a non-end-capped column [22]. The addition of alcohol to the mobile phase leads to blocking of residual silanols and thus decreases the interactions between xanthophylls and silanols [6].

An increase in the percentage of an alcohol decreases the extent of retention in SubFC. The selectivity also decreases from 1.2 for 5% methanol to 1.1 for 15% methanol (Fig. 6a). This effect is similar for the five solvents with alcohol functionalities, although it is observed that, for an identical alcohol content, the selectivity between lutein and zeaxanthin increases with increasing length of the solvent alkyl chain. In the presence of an alcohol lutein is eluted before zeaxanthin, which is followed by lycopene, the first carotene eluted. The variation in selectivity between zeaxanthin and lycopene as a function of the nature and percentage of the alcohol modifier is illustrated in Fig. 6a.

These results illustrate that as the retention of xanthophylls decreases rapidly with increasing alcohol content, the selectivity between zeaxanthin and lycopene increases in a pronounced manner. In contrast, the increase in selectivity is inversely proportional to the alkyl chain length of the alcohol, which is in contrast with the selectivity between lutein and zeaxanthin. This is probably due to the relative importance of the hydroxyl group in the case of a homologue with a low mass. Hence a smaller amount of methanol is needed to cover the silanols compared with 1-pentanol. In addition, the solubility of these solutes must be favoured in the same manner,

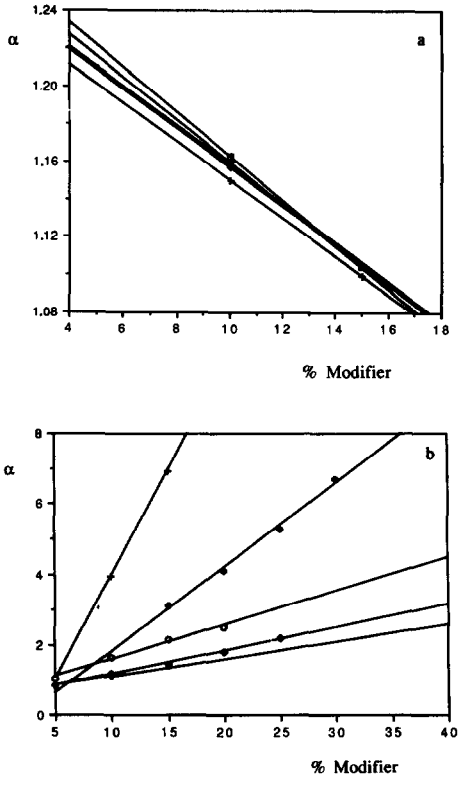

Fig. 6. (a) Variation of the zeaxanthin–lutein selectivity as a function of the percentage of alcohol modifier in subcritical fluid chromatography. Analytical conditions as in Fig. 2a. ◇ = 1-Butanol; ● = ethanol; + = methanol; □ = 1-pentanol; ○ = 1-propanol. (b) Variation of the lycopene–zeaxanthine selectivity as a function of the percentage of alcoholic modifier in subcritical fluid chromatography. Analytical conditions as in Fig. 2a. ◇ = 1-Butanol; ◆ = ethanol; + = methanol; ● = 1-pentanol; ○ = 1-propanol.

therefore leading to faster elution in the presence of these solvents.

In conclusion, it can be said that whereas the presence of an alcohol is needed to elute xanthophylls, an increase in alcohol content is unfavorable for the separation of lutein and zeaxanthin and of γ-carotene and α-carotene. Hence the amount of alcohol should be optimized in order to achieve the best separation.

Simultaneous separation of seven carotenoids

The analyses were carried out by using a mixture of two modifiers [acetonitrile–methanol (95:5, v/v)] and by varying the percentage of the modifier in the mobile phase. Methanol was chosen as it affords high selectivity between lycopene and zeaxanthin.

Fig. 7a shows the variation of the log k' value for the seven carotenoids under consideration. The variation is non-linear and differs depending on the type of compound. This behaviour is typical of solvents with a high dielectric constant [17]. This phenomenon is identical for the four carotenes, while the minimum retention of xanthophylls is displaced towards higher solvent contents. Xanthophylls have higher solubility parameters than carotenes [23], which favours their solubility in the mobile phase. The mini-

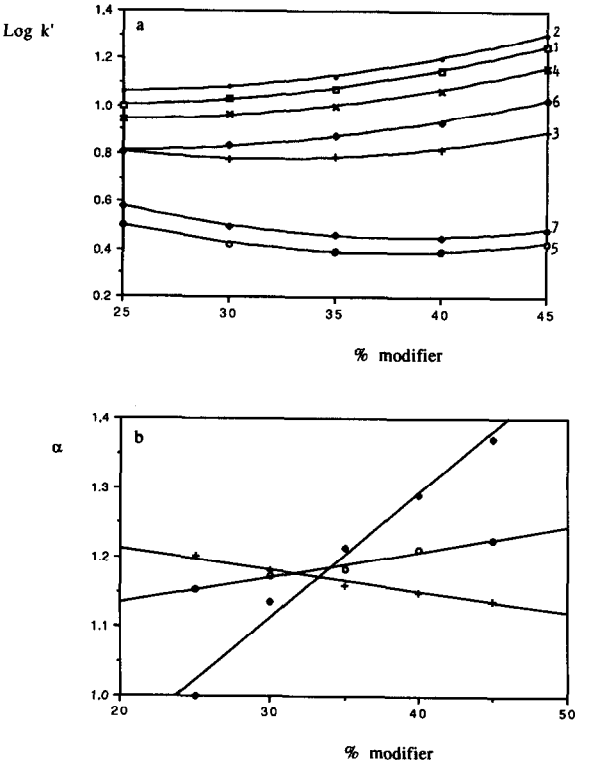

Fig. 7. (a) Variation of the capacity factor of standard carotenoids as a function of percentage of a binary modifier in subcritical chromatography. Analytical conditions as in Fig. 2a. Modifier: acetonitrile–methanol (95:5, v/v). 1 = α-Carotene; 2 = β-carotene; 3 = β-cryptoxanthin; 4 = γ-carotene; 5 = lutein; 6 = lycopene; 7 = zeaxanthin. (b) Variation of the selectivity of some pairs of pigments as a function of the percentage of binary modifier in subcritical fluid chromatography. Analytical conditions as in Fig. 2a. + = Zeaxanthin–lutein; ◆ = lycopene–β-cryptoxanthin; ○ = α-–γ-carotene.

mum retention depends on the number of hydroxyl groups in the molecules: β-cryptoxanthin, which has a hydroxyl group, exhibits minimum retention between those of carotenes and lutein.

When the effects of the dielectric constant of polarity of the solvent become too large for a given compound, the resistance to surface tension or solubilization in the eluent becomes more difficult. Fig. 7a also shows that β-cryptoxanthin is eluted just after lycopene.

Fig. 7b shows the evolution of selectivity of the pairs of compounds whose separation is often difficult with these solvents, *i.e.*, lutein–zeaxanthin, lycopene–β-cryptoxanthin and α-carotene–γ-carotene. These variations are in agreement with those predicted by the preceding study. An increase in the modifier content in the mobile phase entails an increase in selectivity between α- and γ-carotene, related to the overall increase of acetonitrile content, and a decrease in selectivity between lutein and zeaxanthin as the methanol content also increases.

The selectivity between lycopene and β-cryptoxanthin also increases with increasing modifier content, probably owing to favoured elution of a pigment with a hydroxyl group with increasing alcohol concentration. The optimum separation can be achieved with 30–35% modifier. Nevertheless, the chromatograms obtained show that it is preferable to favour the separation between α- and γ-carotene in order to improve the separation of *cis*-γ-carotene and all-*trans*-α-carotene. Hence the best compromise is obtained at 35% (Fig. 8).

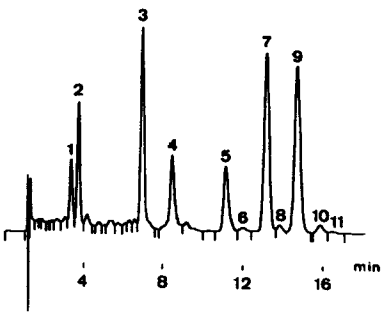

Fig. 8. Subcritical fluid chromatogram of a mixture of carotenoids. Peaks: 1 = lutein; 2 = zeaxanthin; 3 = β-cryptoxanthin; 4 = lycopene; 5 = all-*trans*-γ-carotene; 6 = *cis*-γ-carotene; 7 = all-*trans*-α-carotene; 8 = *cis*-α-carotene; 9 = all-*trans*-β-carotene; 10 = 9- and/or 13-*cis*-carotene; 11 = 15-*cis*-β-carotene. Flow-rate, 3.0 ml/min; temperature, 25°C; output pressure, 15 MPa; mobile phase, acetonitrile–methanol–CO_2 (33.25:1.75:65, v/v/v).

CONCLUSIONS

Subcritical fluid chromatography with conventional columns affords both faster analyses and better utilization of the properties of modifiers than LC. The separation of seven carotenoid pigments and their isomers is obtained in 15 min. This study has demonstrated the particular roles of the solvents examined, which are related to their specific character. The influence of these solvents is comparable to that observed in NARP LC. A better knowledge of the effects exerted by these modifiers permits an easier choice for the separation of the pigments studied. These effects can improve the separation of certain solutes, but also render more difficult the separation of others present in the mixtures of pigments under consideration. Hence a fine adjustment of the percentage of modifier is needed to optimize a complex separation.

For carotenes, nitromethane enables one to obtain a better separation, whereas for the xanthophylls, long-chain alcohols afford a better separation than methanol of lutein and zeaxanthin. The presence of a solvent with a high dipole moment is indispensable for the separation of compounds with different numbers of double bonds; although the presence of an alcohol is needed to elute xanthophylls rapidly, its content should be reduced to a minimum in order to obtain a satisfactory separation of these pigments.

REFERENCES

1 E. Meyer, W.E. Lambert and A.P. De Leenheer, *J. Chromatogr.*, 570 (1991) 149.
2 J.G. Bieri, E.D. Brown and J.C. Smith, Jr., *J. Liq. Chromatogr.*, 8 (1985) 473.
3 F. Khachik, G.R. Beecher and M.B. Goli, *Pure Appl. Chem.*, 63 (1991) 71.
4 T. van Vliet, F. van Schaik, J. van Schoonhoven and J. Schrijver, *J. Chromatogr.*, 553 (1991) 179.

5 S.H. Rhodes, A.G. Netting and B.V. Milborrow, *J. Chromatogr.*, 442 (1988) 412.
6 D.R. Lauren and D.E. McNaughton, *J. Liq. Chromatogr.*, 9 (1986) 2013.
7 Z. Matus and R. Ohmacht, *Chromatographia*, 30 (1990) 318.
8 R.J. Bushway, *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 Resolut. Chromatogr.*, 12 (1989) 447.
11 M. Zakaria, K. Simpson, P.R. Brown and A. Krstulović, *J. Chromatogr.*, 176 (1979) 109.
12 H.J.C.F. Nelis and A.P. De Leenheer, *Anal. Chem.*, 55 (1983) 270.
13 B. Olmedilla, F. Granado, E. Rojas-Hidalgo and I. Blanco, *J. Liq. Chromatogr.*, 13 (1990) 1455.
14 F. Granado, B. Olmedilla, I. Blanco and E. Rojas-Hidalgo, *J. Liq. Chromatogr.*, 14 (1991) 2457.
15 M.-C. Aubert, C.R. Lee, A.M. Krstulović, E. Lesellier, M.R. Péchard and A. Tchapla, *J. Chromatogr.*, 557 (1991) 47.
16 A. Tchapla, S. Heron and E. Lesellier, *Spectra 2000*, 158 (1991) 42.
17 E. Lesellier, A.M. Krstulović and A. Tchapla, *Chromatographia*, 36 (1993) 275.
18 J.H. Hildebrand and R.L. Scott, *The Solubility of Non-Electrolytes*, Dover, New York, 1964.
19 R.K. Juhler and R.P. Cox, *J. Chromatogr.*, 508 (1990) 232.
20 C.A. Bailey and B.H. Chen, *J. Food Sci.*, 54 (1989) 584.
21 H.J.C.F. Nelis, M.M.Z. Van Steenberge, M.F. Lefevere and A.P. De Leenheer, *J. Chromatogr.*, 353 (1986) 295.
22 A.M. Gilmore and H.Y. Yamamoto, *J. Chromatogr.*, 543 (1991) 137.
23 F. Favati, J.W. King, J.P. Friedrich and K. Eskins, *J. Food Sci.*, 53 (1988) 1532.