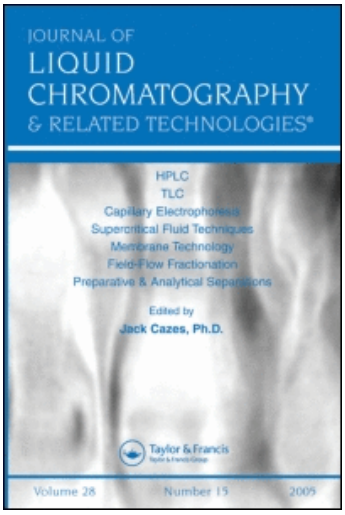

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

An improved HPLC Method for the Separation of Fourteen Carotenoids, Including 15-/13- and 9-CIS-β-Carotene Isomers, Phytoene and Phytofluene

F. Granado[a]; B. Olmedilla[a]; I. Blanco[a]; E. Rojas-hidalgo[a]
[a] Nutrition Service Clínica Puerta de Hierro, Madrid, Spain

To cite this Article Granado, F. , Olmedilla, B. , Blanco, I. and Rojas-hidalgo, E.(1991) 'An improved HPLC Method for the Separation of Fourteen Carotenoids, Including 15-/13- and 9-CIS-β-Carotene Isomers, Phytoene and Phytofluene', Journal of Liquid Chromatography & Related Technologies, 14: 13, 2457 — 2475
To link to this Article: DOI: 10.1080/01483919108049333
URL: http://dx.doi.org/10.1080/01483919108049333

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(13), 2457-2475 (1991)

AN IMPROVED HPLC METHOD FOR THE SEPARATION OF FOURTEEN CAROTENOIDS, INCLUDING 15-/13- AND 9-CIS-β-CAROTENE ISOMERS, PHYTOENE AND PHYTOFLUENE

F. GRANADO, B. OLMEDILLA,
I. BLANCO, AND E. ROJAS-HIDALGO
Nutrition Service
Clínica Puerta de Hierro
c/ San Martín de Porres, 4
28035 Madrid, Spain

ABSTRACT

The isocratic separation of 14 carotenoids, as well as retinol, retinyl acetate, retinyl palmitate, α-tocopherol and tocopherol acetate, is accomplished in 12 minutes, using a Spheri-5-ODS column and acetonitrile:dichloromethane:methanol (70:20:10) as mobile phase, with two-channel, programmable multiwavelength detection. The carotenoids separated are as follows: lutein/zeaxanthin, canthaxanthin, β-apo-8'carotenal, β-cryptoxanthin, echinenone, lycopene, γ-carotene, α-carotene, β-carotene, 9-cis-β-carotene, 15-cis-/13-cis-β-carotene, phytoene and phytofluene. The separation of lutein and zeaxanthin is obtained simply by changing the mobile phase to acetonitrile:methanol (85:15).

The separation of the cis-isomers of β-carotene is maintained, in spite of changes in the proportions and even after 250 hours of analysis. This chromatographic separation is not dependent on temperature (within a range of 15-37°C), on the injection solvent (THF, EtOH or the mobile phase) or flow rate (in a range of 1.3-2.5 ml/min).

The identification of these compounds has been assayed in both vegetable samples and serum.

INTRODUCTION

The continual advances in carotenoid analysis by chromatography are affording the separation of an increasing number of carotenoids, as well as several isomeric forms, in standards, vegetable samples and serum (1-15).

The accurate determination of qualitative and quantitative data for these classes of compounds, including the amounts of various isomers of individual carotenoids in foods and serum, is important for several reasons, among which should be considered their anticarcinogenic potential, the vitamin A activity of different compounds, their antioxidant capacity and colorant properties.

Although cis-isomerism is a widely recognized property of the carotenoids, only a few of them occur naturally in cis-form. The presence of these cis-isomers has been reported by different authors both in processed foods (1,8,16) and in serum (10,15,17).

For the purpose of broadening and improving the system of chromatographic separation of ten carotenoids previously reported by us (13), we have achieved, after different assays, the separation of several cis-isomers of trans-β-carotene (9-cis-β-carotene and 13-cis- or 15-cis-β-carotene), efficiently and continuously for 250 hours of column life, as well as that of phytoene and phytofluene.

MATERIALS

All-trans-β-carotene, lycopene, all-trans-α-carotene, all-trans-retinol, retinyl acetate, retinyl palmitate, d-α-tocopherol and d-α-tocopherol acetate standards were obtained from Sigma Chemical Co. (St.

Louis, Mo.). 15-cis-β-carotene, 9-cis-β-carotene, 13-cis-β-carotene, phytoene, γ-carotene, canthaxanthin, β-cryptoxanthin, echinenone, lutein and zeaxanthin were gifts from Hoffmann-La Roche (Basel, Switzerland). β-apo-8'carotenal was obtained from Fluka Chemie AG (Switzerland). Stabilized tetrahydrofuran (THF), hydroxybutyl-toluene (BHT) and dichloromethane were purchased from Carlo Erba (Spain). Methanol, ethanol, n-hexane and acetonitrile were supplied by Merck (Spain).

METHODS

Preparation of standards

Stock solutions of each carotenoid were prepared by dissolving 2-5 mg of the compound in 50 ml of THF. Standards were stored at -20°C, under nitrogen atmosphere and protected from light. These solutions were further diluted to provide working standards.

Concentrations were calculated on the basis of the published absorptivity value (E 1% 1 cm) in hexane, ethanol or petroleum ether (18). Values used, and the wavelength maxima, were: phytoene, 1250 at 285 nm; β-carotene, 2592 at 453 nm; lutein, 2550 at 445 nm; canthaxanthin, 2200 at 466 nm; β-apo-8'carotenal, 2640 at 457 nm; β-cryptoxanthin, 2386 at 452 nm; echinenone, 2158 at 458 nm; lycopene, 3450 at 472 nm; zeaxanthin, 2540 at 450 nm; α-carotene, 2800 at 444 nm; γ-carotene, 3100 at 462 nm; and 15-cis-β-carotene in hexane was 2340 at 450 nm (data from Hoffmann-La Roche, Basel). The concentrations of 9-cis-β-carotene and 13-cis-β-carotene was not spectrophotometrically corrected as

the absorptivity value was unknown. The concentrations of the stock solutions were recalibrated monthly.

Liquid chromatographic system

High performance liquid chromatography was carried out in an ALC/GPC chromatograph (model 201, Waters Associates, Milford, Mass.) equipped with a model 6000 A pump, dual reciprocating piston heads, model U6K septumless injector and programmable multiwavelength detector (model 490 E, Waters Associates). The detector signals were recorded on a M730 data module (Waters Associates) and an Omniscribe recorder.

The chromatographic conditions were as follows: a 5 μm Spheri-5-ODS, 220 x 4.6 mm column (Brownlee Labs, Kontron Analytic) with a guard column of Aquapore ODS type RP-18, 15 x 3.2 mm, 7 μm. Solvent, acetonitrile: :dichloromethane:methanol (70:20:10); flow rate, 1.8 ml/min.

To separate lutein and zeaxanthin, the mobile phase was changed to acetonitrile:methanol (85:15) (13).

The detection of the different compounds was performed at 450 nm (carotenoids), 450 and 340 nm (cis-β-carotenes), 370 nm (phytofluene), 286 nm (phytoene), 294 nm (tocopherol and tocopherol acetate), 325 nm (retinyl palmitate) and 313 nm (retinol and retinyl acetate). The identification of the peaks for the different samples was based on retention times, maxplot, 340/436 nm ratio, Q-ratio (absorbance at maximum/absorbance at cis-peak) and stop-flow scan at the top of the peak.

RESULTS AND DISCUSSION

By using a Spheri-5-ODS column (polyfunctional) rather than the Spheri-5-RP-18 column (monofunctional),

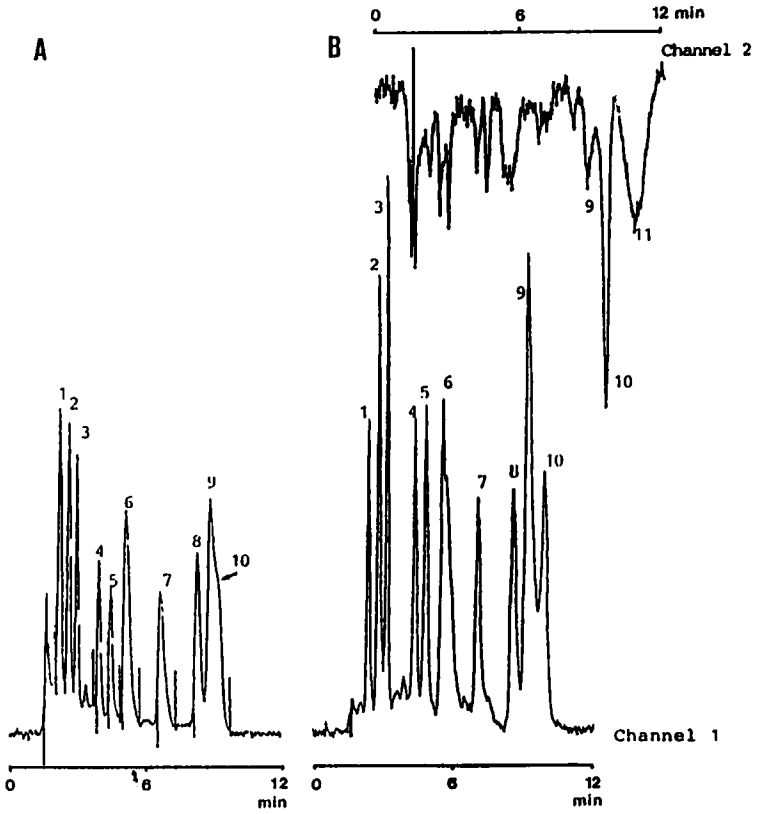

FIGURE 1. Separation of carotenoid standards: a) on Speri-5-RP-18; and b) on Spheri-5-ODS. Mobile phase: acetonitrile:dichloromethane:methanol (70:20:10). Flow rate: 1.8 ml/min.
Channel 1, detection at 450 nm (x0.01 AUFS); channel 2, detection at 340 nm and 286 nm (at minute 10) (x0.004 AUFS).
Peak identification: 1, lutein; 2, canthaxanthin; 3, β-apo-8'-carotenal; 4, β-cryptoxanthin; 5, echinenone; 6, lycopene; 7, γ-carotene; 8, α-carotene; 9, β-carotene; 10, 15-cis-β-carotene; and 11, phytoene.

with which we had carried out the separation of ten carotenoids reported previously (fig. 1a) (13), we have extended the analytical separation of carotenoids to the identification of 13-cis o 15-cis-β-carotene and phytoene (fig. 1b), as well as 9-cis-β-carotene and phytofluene.

The chromatographic constants for the carotenoids studied in a Spheri-5-ODS column (Brownlee) are given in Table 1. When these constants are compared with those obtained for the same carotenoids (except 15-cis-β-carotene) in a Spheri-5-RP-18 column (Kontron Analytic) (13), at the same flow rate and using the same mobile phase (acetonitrile:dichloromethane:methanol) (70::20:10), we can observe a marked increase in the resolution (R), which becomes more evident for the apolar carotenoids, as well as a decrease in the separation factor (α), which becomes more pronounced as the polarity of these compounds diminishes.

The selectivity and resolution for the mixture of β-carotene and 15-cis-β-carotene were assessed periodically over the course of more than 1000 injections (approximately 250 hours) (figure 2).

The chromatographic constants for phytoene are not included in Table 1, although this substance appears in figure 1b, due to the anomalous width of the peak. Nonetheless, the absorption spectrum for this standard corresponds to that reported in the referenced literature (12,18). Nor were the chromatographic constants calculated for phytofluene as there is no available standard.

With respect to 9-cis and 13-cis-β-carotene, received during the preparation of this manuscript, preliminary studies were performed to assay them chromatographically, together with all-trans-β-carotene and 15-cis-β-carotene. Figure 3 shows the chromatographic

TABLE 1.-

Chromatographic constants of Spheri-5-ODS (Brownlee) High Performance Liquid Chromatographic Column (at 23 ºC), using acetonitrile / dichloromethane / methanol (70/20/10) as mobile phase and a flow rate of 1.8 ml/min. Chromatographic constants after aprox.350 injections.

	Lutein	Canta-xanthin	3-apo-8' carotenal	8-crypto-xanthin	Echinenona	Lycopene	γ-carotene	α-carotene	β-carotene	15-cis-β-carot.	K'	N
					Separation factor (α)							
Lutein	—	1.83	2.50	4.17	5.00	6.34	8.51	10.85	12.01	13.18	0.43	711
Cantaxanthin	1.67	—	1.36	2.27	2.73	3.46	4.64	5.91	6.55	7.19	0.79	1111
β-apo-8'carot.	3.00	1.33	—	1.67	2.00	2.53	3.40	4.33	4.80	5.27	1.07	1495
β-cryptoxanthin	5.94	4.38	3.13	—	1.20	1.52	2.04	2.60	2.88	3.16	1.79	2105
Echinenona	7.50	5.94	4.69	1.47	—	1.26	1.70	2.17	2.40	2.64	2.14	2680
Lycopene	10.66	9.00	7.67	4.06	2.50	—	1.34	1.71	1.89	2.08	2.71	4807
γ-carotene	12.85	11.43	10.29	7.03	5.68	3.71	—	1.27	1.41	1.55	3.64	4225
α-carotene	16.86	15.43	14.29	10.81	9.46	7.71	3.50	—	1.11	1.22	4.64	6241
β-carotene	16.50	15.25	14.25	11.19	10.00	8.50	4.67	1.56	—	1.10	5.14	4733
15-cis-β-carot.	20.86	19.42	18.25	14.59	13.24	11.71	7.00	3.50	1.56	—	5.64	8649
					Resolution (R)							

Separation factor (α)=K'_2 / K'_1; W= peak width; Resolution (R) = $V_2-V_1/\frac{1}{2}(W_2+W_1)$; V=retention volume; V_o= void volume;
Capacity factor (K') = $V-V_o/V_o$; Number of theoretical plates (N) = $16(V/W)^2$

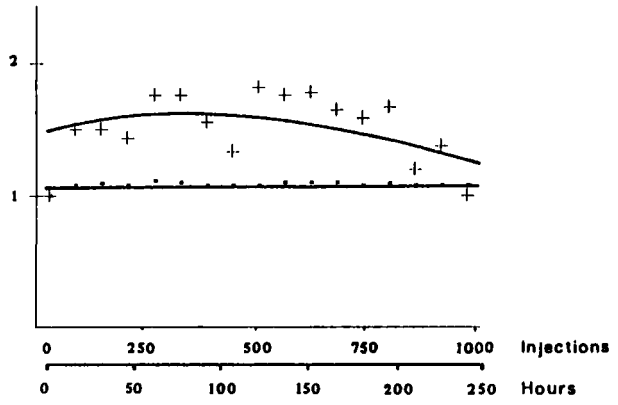

FIGURE 2. Evolution of the chromatographic parameters, selectivity ( ) and resolution (+) for a mixture of β-carotene and 15-cis-β-carotene, on a spheri-5-ODS column.

separation of all-trans-β-carotene from its isomers, 9-cis and 13-cis or 15-cis (the latter two isomers co-elute) using a new Spheri-5-ODS column. In a column that has has been in use for more than 250 hours, this separation is maintained. The isomers 9-cis and 13-cis-β-carotene are injected together with β-carotene in different proportions. The monitoring of channel 2 at a wavelength of 340 nm is useful as it permits a better separation of the isomer peaks at certain analysis concentrations.

The selectivity and resolution of 9-cis and 13-cis-β-carotene with respect to β-carotene is summarized in Table 2. These parameters were calculated using a proportion of β-carotene:9-cis-β-carotene:13-cis-β-carotene of 1:0.6:1.6, which are the optimum conditions for the separation of these peaks (figure 3c).

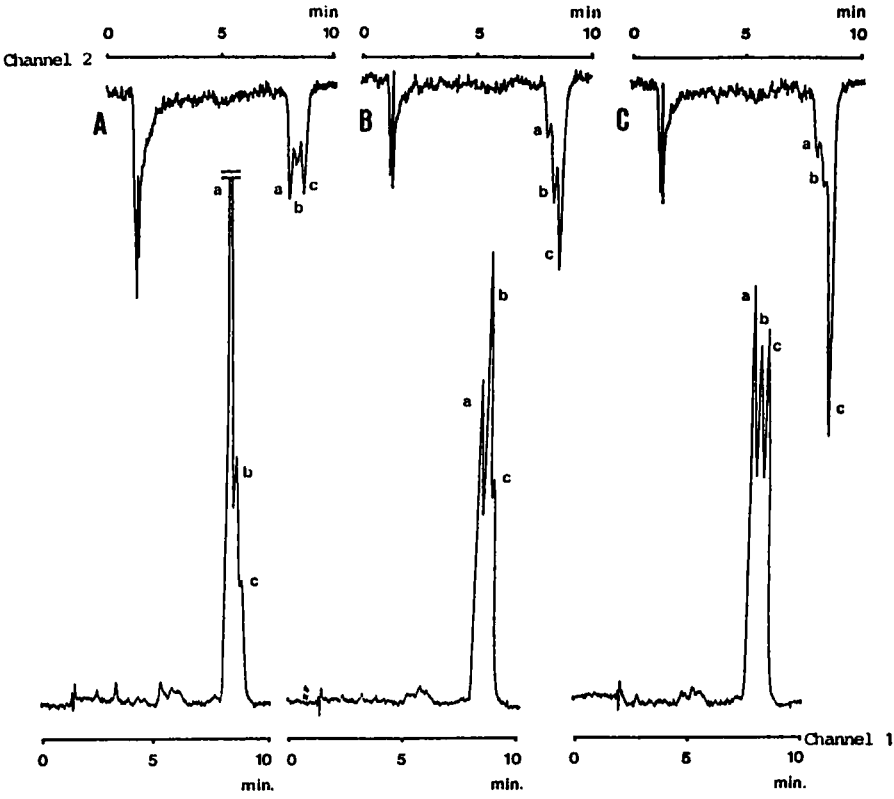

FIGURE 3. Separation of a mixture of all-trans-β-carotene (a), 9-cis-β-carotene (b) and 13-cis-β-carotene (c) in different proportions (see text) on a Spheri-5-ODS column. Channel 1, detection at 450 nm (x0.02 AUFS); channel 2, detection at 340 nm (x0.008 AUFS).

More complete chromatographic studies were carried out with β-carotene and 15-cis-β-carotene. Solutions were prepared using different proportions, in which β-carotene represented from 6 to 95%. The correlation coefficient was 0.960 - 0.999 for 15-cis-β-carotene and 0.900 - 0.999 for β-carotene, after applying several

TABLE 2

	β-carotene	9-cis-β-carotene	13-cis-β-carotene
	Selectivity (α)		
β-carotene	—	1.05	1.10
9-cis-β-carotene	0.65	—	1.04
13-cis-β-carotene	1.03	0.45	—
	Resolution (R)		

calibration curves and with attenuations ranging from x 0.003 to 0.05.

Figure 4 shows the chromatograms corresponding to the separation of β-carotene and 15-cis-β-carotene in the following proportions: 1.3:1 (figure 4a); 0.24:1 (figure 4b); and 10.7:1 (figure 4c). When the proportion of 15-cis-β-carotene is less than 5%, the correlation coefficient for the cis-isomer diminishes, although the separation of the two compounds is maintained. Channel 2 of these analyses was monitored using windowplot (at a ratio of 340:436 nm and in a range of 0.20 - 5), revealing a single peak corresponding to 15-cis-β-carotene (ratio 0.52), the height of which is proportional to the amount of the isomer present in the mixture.

A good resolution of the mixture of β-carotene and 15-cis-β-carotene is obtained whether THF, ethanol or the mobile phase is used as the injection solvent. The chromatographic separation, at the proportions assayed

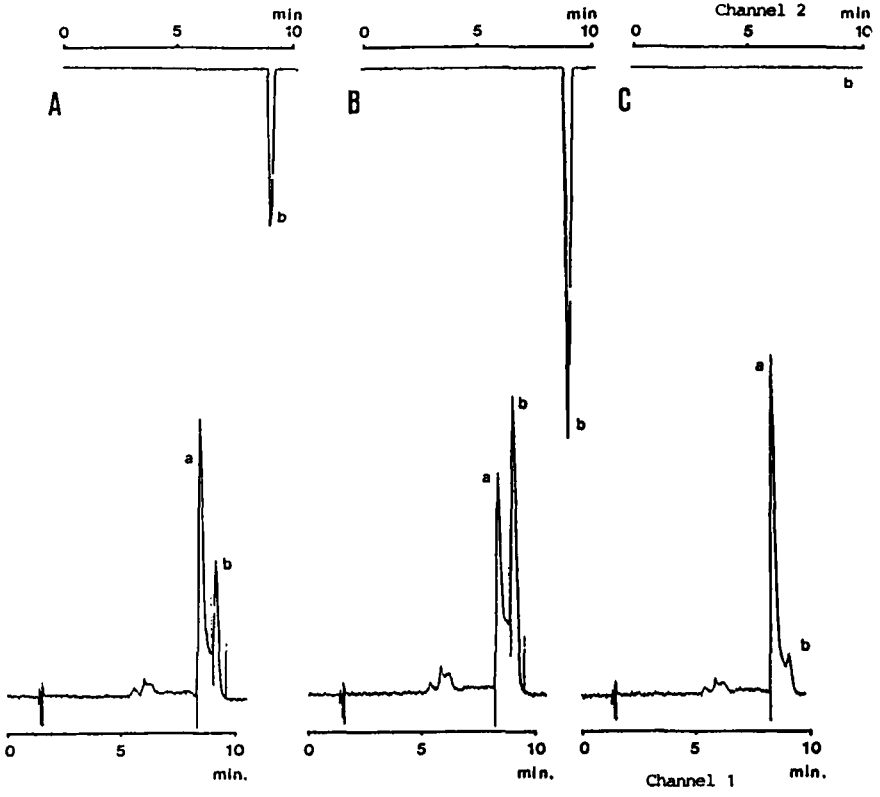

FIGURE 4. Separation of all-trans-β-carotene (a) and 15-cis-β-carotene (b) in mixtures at different proportions (see text) on a Spheri-5-ODS column. Channel 1, detection at 450 nm; channel 2, windowplot (ratio 340/436 nm; range 0.2-5).

here, does not depend on the column temperature (within a range of 15 to 37°C), although the best resolution and most suitable retention time are obtained at 23°C.

When the injection volume surpasses 25 μl, the resolution of β-carotene and 15-cis-β-carotene depends on their proportions in the mixture. Thus, when the concentration of β-carotene is greater than that of

15-cis, they do not separate at a detection setting of 450 nm, but do separate when read at 340 nm.

The variation coefficient for the mixtures of β-carotene and 15-cis-β-carotene injected within the same day was 2.5% for 15-cis-β-carotene at 340 nm and 1.9% at 450 nm, and 7.2% for β-carotene at 450 nm. The variation coefficient between days for this mixture was not determined because of the risk of spontaneous isomerizations in the mixture being studied (14).

The detection limit of the different carotenoids has been determined by calculating the mean of those obtained in a new Spheri-5-ODS column and in another after approximately 200 hours of use. The detection limits for standards injected into 5 μl of THF were: 0.1 ng of canthaxanthin and β-apo-8'-carotenal; 0.2 ng of lutein; 0.3 ng of β-cryptoxanthin, echinenone, γ-carotene, α-carotene and β-carotene. 15-cis-β-carotene and lycopene were detectable from 0.7 and 0.8 ng, respectively, while phytoene was detectable from 2.4 ng on. The detection limits for 9-cis-β-carotene and 13-cis-β-carotene could not be calculated because neither the absorptivity values nor amounts sufficient for their determination were available. This parameter is not included for phytofluene either because of a lack of the standard.

The presence of cis-isomers both in standards, mostly in trans- form, and in samples can be detected by ratio monitoring (6,19). In the case of β-carotene standards, monitoring the ratio at 340/436 nm, we obtained values under 0.20 for all-trans-β-carotene and 0.52-0.56 for 15-cis-β-carotene. For the isomers 13-cis-β-carotene and 9-cis-β-carotene, the ratio values were 0.45-0.50 and 0.31-0.36, respectively. Using the Q-ratio, the values obtained were very similar to those reported by Quackenbush (6), that is, 2.4-2.6 for 13-cis-β-carotene, 7.5-8 for 9-cis-β-carotene, over 12 for

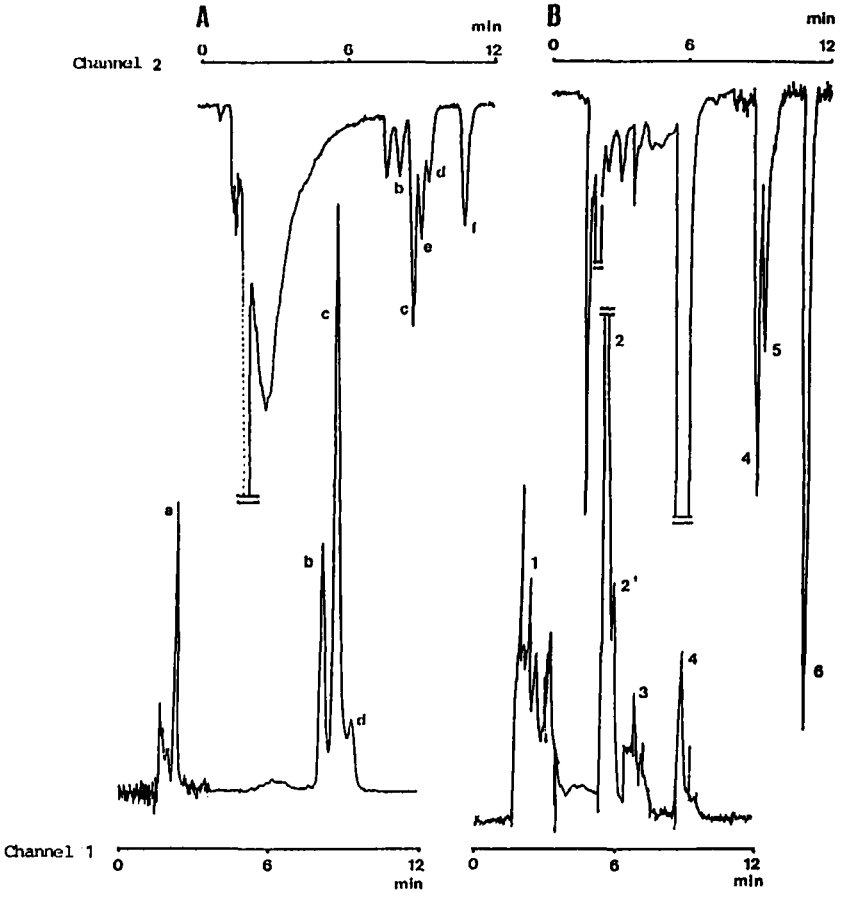

FIGURE 5. A) Chromatogram of cooked carrot extract. Channel 1, detection at 450 nm (x0.003, at 3 min x0.05 AUFS); channel 2, detection maxplot (340 - 370 - 286 - 450 nm) (x0.02 AUFS).
Peak identification: a, lutein; b, α-carotene; c, β-carotene; d, 13-cis-β-carotene; e, phytofluene; and f, phytoene.
B) Chromatogram of raw tomato ("pear" variety) extract. Channel 1, detection at 450 nm (x0.01, at 3 min x0.05, at 6 min x0.003, at 7.30 min x0.01 AUFS); channel 2, detection maxplot (340 - 370 - 286 - 450 nm) (x0.02, at 7.30 min x0.004 AUFS).
Peak identification: 1, lutein; 2, lycopene; 2', cis-isomer of lycopene; 3, γ-carotene; 4, β-carotene; 5, phytofluene; and 6, phytoene.

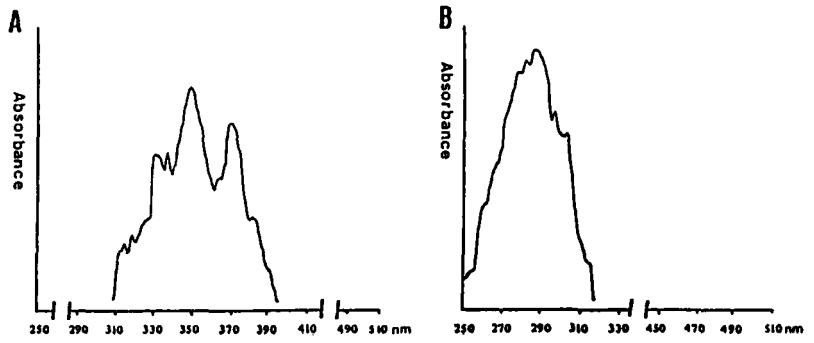

FIGURE 6. Stop-flow scan of A) phytofluene, and B) phytoene in the HPLC mobile phase, under the conditions described in the text.

all-trans-β-carotene and less than 2 for 15-cis-β-carotene.

This chromatographic system was applied to the analysis of vegetable and serum samples. Figure 5a depicts a chromatogram of an extract of cooked carrot; in channel 1 are detected, at 450 nm, the presence of lutein (the absence of zeaxanthin was confirmed when acetonitrile:methanol (85:15) was employed as the mobile phase), α-carotene, β-carotene and a peak identified as 13-cis-β-carotene (on the basis of the retention time, 340/436 ratio of 0.46 and Q-ratio of 2.30). In channel 2, monitored at 340-370-286-450 nm: α and β carotene are detected, a peak tentatively identified as phytofluene (with maximum absorption at 350 nm, the scan is shown in figure 6a); the peak identified as 13-cis-β-carotene; and phytoene (with absorbance at 286 nm; the stop-flow scan of the latter appears in Figure 6b).

Figure 5b corresponds to the chromatogram of an extract of raw tomato ("pear" variety). In channel 1, monitored at 450 nm, lutein, lycopene (and a lycopene

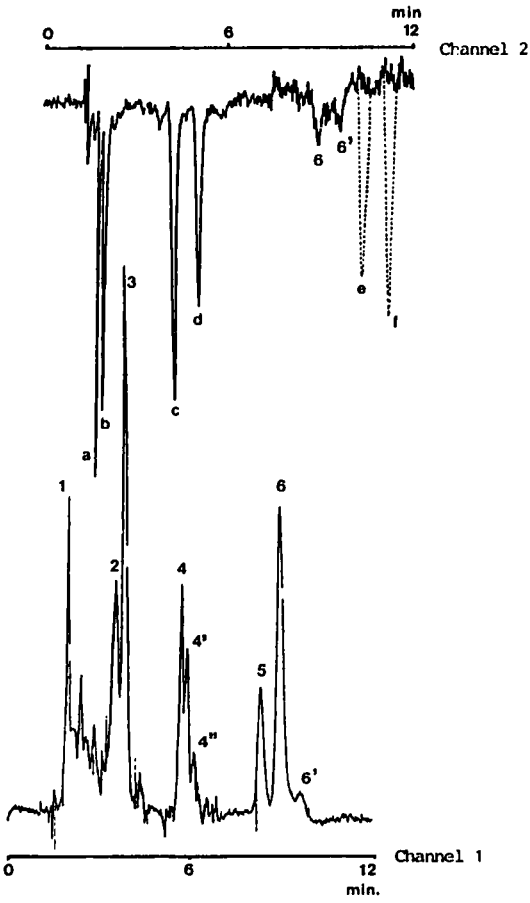

FIGURE 7. Chromatogram of serum extract from a control subject. Channel 1, detection at 450 nm (x0.01, at 3 min x0.003, at 6.5 min x0.01 AUFS); channel 2, detection at 313 nm (x0.01 AUFS, retinol and retinyl acetate), 294 nm (x0.01 AUFS, tocopherol and tocopherol acetate), 325 nm (x0.003 AUFS, retinyl palmitate). Peak identification: 1, lutein + zeaxanthin; 2, pre-β-cryptoxanthin; 3, β-cryptoxanthin; 4, lycopene; 4', 4'', cis-isomers of lycopene; 5, α-carotene; 6, β-carotene; 6', cis-isomer of β-carotene (13- and/or 15-cis-β-carotene); a, retinol; b, retinyl acetate; c, tocopherol; d, tocopherol acetate. The dotted lines indicate the positions of retinyl palmitate (e) and phytoene (f).

isomer in the down-slope of the peak), γ-carotene and β carotene are identified. In channel 2, monitored at 340-370-286-450 nm, we detect phytofluene and phytoene, without interfering with the β-carotene. The presence of phytoene and phytofluene has been demonstrated by several authors in vegetable and fruit samples (16,20, 21).

Figure 7 represents a serum extract (extracted with hexane and injected into THF) from a healthy subject. In channel 1, monitored at 450 nm, we determine the presence of lutein + zeaxanthin, pre-β-cryptoxanthin, β-cryptoxanthin, lycopene, α-carotene, β-carotene and a cis-isomer of β-carotene (probably 13 or 15-cis--β-carotene). Channel 2 was monitored at 313 nm (for retinol and retinyl acetate), 294 nm (for tocopherol and tocopherol acetate) and 325 nm (for retinyl palmitate).

In all the sera analyzed to date, the peak corresponding to lycopene shows two peaks in the down-slope, which we consider must correspond to isomers of lycopene, the presence of which in serum has been reported by other authors (10,17).

When the serum corresponding to figure 7 was concentrated three-fold, the presence of phytoene, phytofluene and retinyl palmitate was detected, although in very small amounts. The presence of phytoene and phytofluene in serum has been mentioned previously by other groups (10,22).

With this study, we have increased the number of carotenoids previously separated by us (13), and have further improved their resolution and detection limits. It has become possible to separate the cis-isomers of β-carotene, detect the presence of two isomers in lycopene, as well as to separate simultaneously phytoene and phytofluene. The analysis of fourteen carotenoids,

retinal, tocopherol, retinyl palmitate, retinyl and tocopherol acetate takes 12 minutes, and its applicability has been assayed in vegetable samples as well as in serum.

Given the broad range of carotenoids that can be determined, this method should provide interesting areas for further research on the changes produced during the processing of foods, as well as another means to study the influence of seasonal changes, ripeness, storing conditions and horticultural varieties. On the other hand, with respect to the analysis in serum, it provides the possibility to broaden the epidemiologic studies that relate these compounds to different diseases.

ACKNOWLEDGMENTS

We thank the F. Hoffmann-La Roche Co. (Basel, Switzerland) for their generous gift of carotenoid reference samples and Martha Messman for the preparation of the manuscript.

This investigation was partially funded by grant C-014/88 from the Fundación Banco Exterior (Spain) and by grant 89/0110 from the Fondo de Investigaciones Sanitarias (FISS), Spain.

REFERENCES

1. Sweeney, J.P. and March, A.C., Separation of carotene stereoisomers in vegetables, J. AOAC, 53, 937, 1970.

2. Tsukida, K., Saiki, K., Takii, T. and Koyama, Y., Separation and determination of cis/trans-β-carotenes by high-performance liquid chromatography, J. Chromatogr., 245, 359, 1982.

3. Bushway, R.J., Separation of carotenoids in fruits and vegetables by high performance liquid chromatography, J. Liq. Chromatogr., 8, 1527, 1985.

4. Bieri J.G., Brown, E.D. and Smith, J.C., Jr., Determination of individual carotenoids in human plasma by high performance liquid chromatography, J. Liq. Chromatogr., 8, 473, 1985.

5. Schwartz, S.J. and Patroni-Killam, M., Detection of cis-transcarotene isomers by two-dimensional thin layer and high performance liquid chromatography, J. Agric. Food Chem., 33, 1160, 1985.

6. Quackenbush, F.W., Reverse phase HPLC separation of cis-and trans-carotenoids and its application to β-carotenes in food materials, J. Liq. Chromatogr., 10, 643, 1987.

7. Chandler, L.A. and Schwartz, S.J., HPLC separation of cis-trans carotene isomers in fresh and processed fruits and vegetables, J. Food Sci., 52, 669, 1987.

8. Chandler, L.A. and Schwartz, S.J., Isomerization and losses of trans β-carotene in sweet potatoes as affected by processing treatments, J. Agric. Food Chem., 36, 129, 1988.

9. MacCrehan, W.A. and Schonberger, E., Determination of retinol, α-tocopherol, and β-carotene in serum by liquid chromatography with absorbance and electrochemical detection, Clin. Chem., 33, 1585, 1987.

10. Sowell, A.L., Huff, D.L., Gunter, E.W. and Driskell, W.J., Identification of cis-carotenoids in human sera analyzed by reversed-phase high-performance liquid chromatography with diode array detention, J. Chromatogr., 431, 424, 1988.

11. Koyama, Y., Hosomi, M., Miyata, A., Hashimoto, H., Reames, S.A., Nagayama, K., Kato-Jippo, T. and Shimamura, T., Supplementary and revised assignment of the peaks of the 7,9-, 9,9'-, 13,13'-, 9,13'-di-cis and 9,9',13-tri-cis isomers of β-carotene in high performance liquid chromatography using a column of calcium hydroxide, J. Chromatogr., 439, 417, 1988.

12. Khachik, F., Beecher, G.R. and Lusby, W.R., Separation, identification and quantification of the major carotenoids in extracts of apricots, peaches, cantaloupe and pink grapefruit by liquid chromatography, J. Agric. Food Chem., 37, 1465, 1989.

13. Olmedilla, B., Granado, F., Rojas-Hidalgo, E. and Blanco, I., A rapid separation of ten carotenoids, three retinoids, alpha-tocopherol and d-α-tocopherol acetate by HPLC and its application to serum and vegetable samples, J. Liq. Chromatogr., 13, 1455, 1990.

14. Pesek, C.A., Warthesen, J.J. and Tsaoukis, P.S., A kinetic model for equilibration of isomeric β-carotenes, J. Agric. Food Chem., 38, 41, 1990.

15. Rushin, W.G., Catignani, G.L. and Schwartz, S.J., Determination of β-carotene and its cis isomers in serum, Clin. Chem., 36, 1986, 1990.

16. Khachik, F., Beecher, G.R. and Whittaker, N.F., Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography, J. Agric. Food Chem., 34, 603, 1986.

17. Thurnham, D.I., Smith, E. and Singh Flora, P., Concurrent liquid-chromatographic assay of retinol, α-tocopherol, β-carotene, α-carotene, lycopene and β-cryptoxanthin in plasma with tocopherol acetate as internal standard, Clin. Chem., 34, 377, 1988.

18. De Ritter, E. and Purcell, A.E., Carotenoids as colorants and vitamin A precursors, Bauernfeind, J.C., ed., Academic Press, London, 1981, p. 883.

19. Edwards, C.G. and Lee, C.Y., Determination of β-carotene stereoisomers by spectrophotometric analysis, J. Food Sci., 51, 528, 1986.

20. Jen, J.J., Carotenoids of yellow and lutescent tomatoes, J. Agric. Food Chem., 22, 908, 1974.

21. Czinkotai, B., Daood, H. and Biacs, P., Analysis of carotenoids from paprika by HPLC, Chromatogram 10, 4, 1989.

22. Bendich, A. and Olson, J.A., Biological actions of carotenoids, FASEB J., 3, 1927, 1989.