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SHORT COMMUNICATION

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A simplified method for the HPLC resolution of α-carotene and β-carotene (*trans* and *cis*) isomers

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Abstract A new HPLC/DAD (Diode Array Detector) method is proposed for the identification of some carotene isomers. The operating conditions adopted permit the resolution of α-carotene, all-*trans*-β-carotene, 9-*cis*-β-carotene, 13-*cis*-β-carotene and 15-*cis*-β-carotene. Moreover, the chromatographic conditions reported are simplified in respect of those reported up to now. The method is applied to the determination of carotenoids in a dried *Dunaliella salina* extract, but it could be also applied to other organic matrices such as eggs.

Keywords HPLC · Carotene isomers

Introduction

Several authors [1, 2, 3, 4, 5,6] have reported the different biological activities of carotenoids in humans and some [4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,18] have proposed HPLC methods for the resolution of carotene isomers, especially of α-carotene, all-*trans*-β-carotene and *cis* isomers (9-*cis*, 13-*cis*, 15-*cis*) (Fig. 1). Among the methods reported, the chromatographic conditions suggested by Stahl et al. [6] give the best resolution.

In this paper, an alternative method is proposed that uses an RP-Amide (C16) column and methanol as the mobile phase. It has been used to analyse NB-Dunaliella Algae Powder (an extract of *Dunaliella salina* from Chile) to allow a direct comparison with Stahl's work. This analytical method can be also used to determine all-*trans*-β-carotenes in eggs and egg-based products; in this case lutein and zeaxanthin results were co-eluted.

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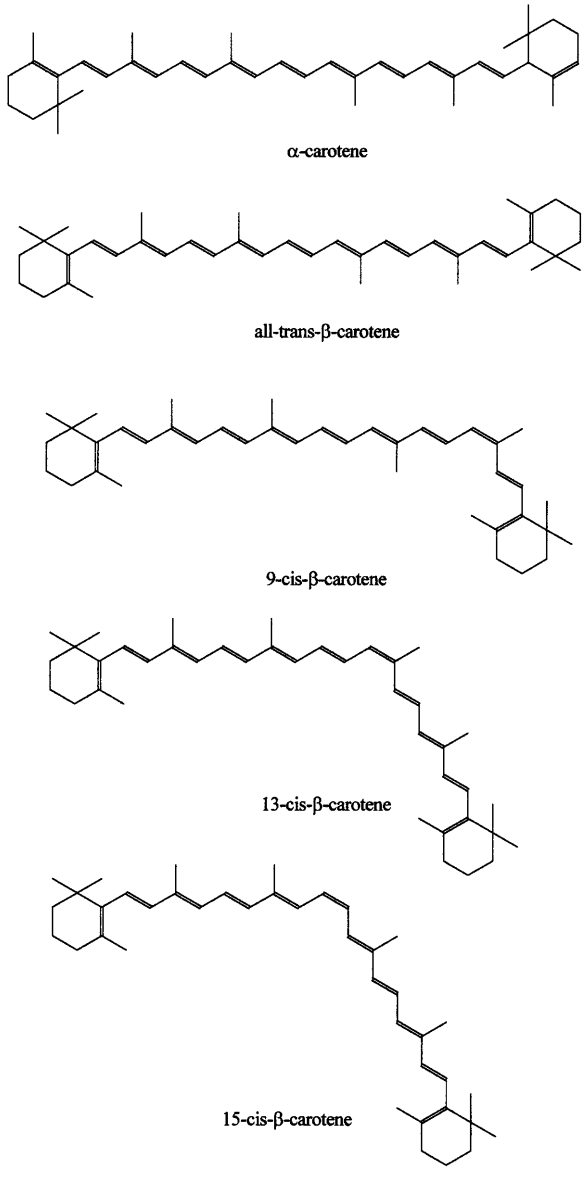


Fig. 1 Structures of α-carotene and β-carotene (*trans* and *cis*) isomers

Experimental

Samples

A dried extract of *Dunaliella salina* from Chile (NB-Dunaliella Algae Powder from Norbiotech LTDA) was analysed in order to verify the efficiency as far as the resolution of the isomers of β-carotene.

Reagents

n-Hexane (code 152496G, BDH, Poole, England), acetone (code 100014.2500, Merck, Darmstadt, Germany), methanol (code 15250, BDH, Poole, England) and toluene (code 8389, Merck, Darmstadt, Germany), acetonitrile (code 152516Q, BDH, Poole, England) and chloroform (code 100776B, BDH, Poole, England) were used for the extraction procedure. HPLC grade methanol was used as the mobile phase.

Reference materials

A mixture of α- and β-carotene (1:2) isomers (min 95% from Sigma-Aldrich, code C-4646, Steinheim, Germany), all-*trans*-β-carotene (min 95% from Sigma-Aldrich, code 85,555-3, Steinheim, Germany), xanthophyll (lutein) (min 70% from Sigma-Aldrich, code X-6250, Steinheim, Germany), zeaxanthin (kindly supplied by Roche, Milan, Italy).

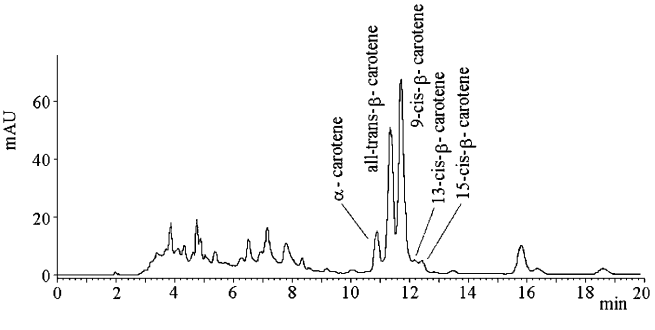


Fig. 2 HPLC/DAD chromatogram of an extract of a *Dunaliella salina* dried powder sample

Apparatus and operating conditions

An RP-Amide (C16) column (4.6 mm×250 mm, 5 µm, Supelco, Bellefonte, PA – code 505064) was used with methanol as mobile phase at a flow rate of 1 mL min^{-1}. Analyses were performed with an HPLC Shimadzu system (Shimadzu Italia s.r.l., Milan, Italy) consisting of: a) System Controller SCL-10Avp; b) two pumps LC-10ADvp; c) Rheodyne injection valve (7725i, 20 µL loop); d) diode array detector SPD-M10Avp performing detection at 453 nm; e) acquisition software Class VP-5-Shimadzu.

Reference materials were used to identify α- and all-*trans*-β-carotene; a direct comparison with HPLC traces obtained by the Stahl method [6] allowed the identification of *cis*-isomers of β-carotene.

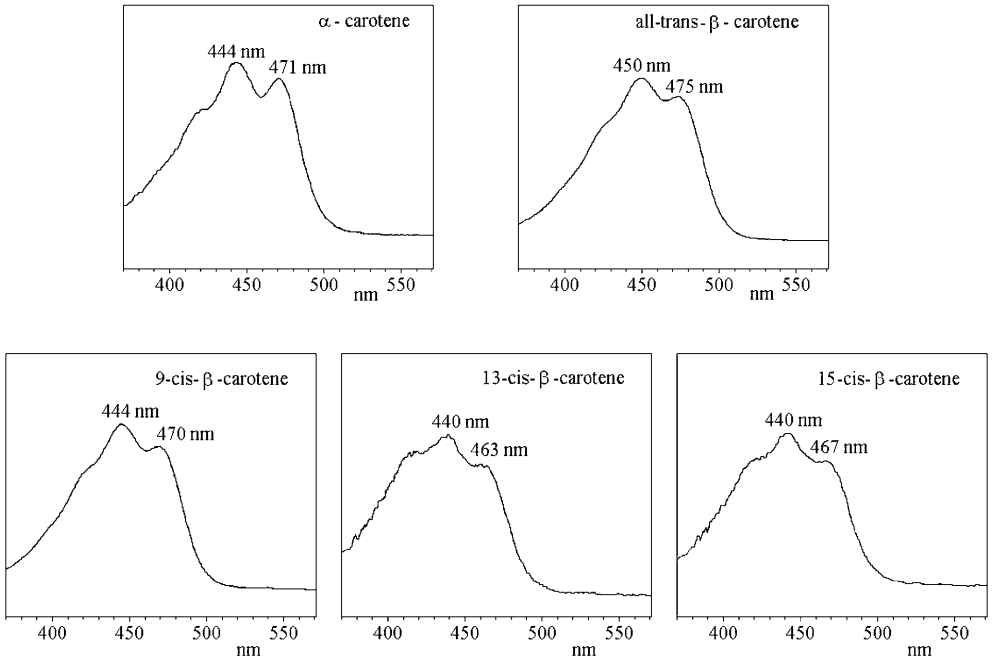
Extraction procedure

A sample of NB-Dunaliella Algae Powder (1–2 g) were extracted with 30 mL of n-hexane/acetone/methanol/toluene (29:24:21:26 w/w/w/w). The mixture obtained was shaken vigorously for 5 min. After separation of the organic phase the remaining residual phase was extracted again with 30 mL of the aforementioned solution. The recovered solution was dried with sodium sulfate, then evaporated to dryness under vacuum with a rotory evaporator (Büchi 461 Water Bath, Switzerland) at 35 °C and reconstituted with 40 mL of methanol/acetonitrile/chloroform (44:45:11 w/w/w).

Stahl chromatographic conditions [6]

A Suplex PKB 100 column (4.6 mm×250 mm, 5 µm, Supelco, Bellefonte, PA) with methanol/acetonitrile/2-propanol (54:44:2 w/w/w) as the mobile phase, at a flow rate of 1 mL min^{-1} and detection at 460 nm by diode array detector (Model 168, Beckman, Munich, Germany) were utilised. The method was applied to an extract of *Dunaliella salina* called Betatene (Henkel, La Grange, IL).

Fig. 3 DAD spectra of α-carotene and β-carotene (*trans* and *cis*) isomers with λ_{max} displayed



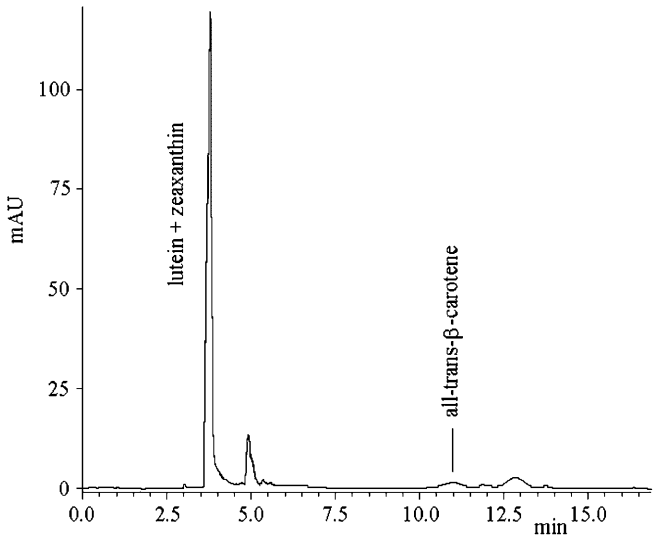


Fig. 4 HPLC/DAD chromatogram of a sample of egg yolk obtained under the same conditions used for *Dunaliella salina* (trace shown in Fig. 2)

Table 1 Comparison of the carotenoids content in an extract of a *Dunaliella salina* dried powder sample determined using the Stahl method and our method

Carotenoid[a]	Stahl method [6]		Our method	
	(%)	(CV%)	(%)	(CV%)
α-Carotene	8.3	10.4	9.0	8.5
all-*trans*-β-Carotene	36.4	6.5	36.7	4.6
9-*cis*-β-Carotene	52.1	4.1	50.7	3.5
cis-β-Carotene (13-*cis*, 15-*cis*)	3.2	13.2	3.6	10.1

[a]The results are expressed as rel. % of the carotenoids considered. Each % value results from the average of 5 consecutive injections

Results

Fig. 2 shows an HPLC/DAD chromatogram of an extract from the alga *Dunaliella salina* with identification of α-carotene, all-*trans*-β-carotene, 9-*cis*-β-carotene, 13-*cis*-β-carotene and 15-*cis*-β-carotene. Fig. 3 reports the spectra of the above mentioned isomers with the corresponding λ_{max}.

Fig. 4 shows the chromatographic resolution obtained for a sample of egg yolk using the same operating conditions reported for *Dunaliella salina*; these conditions allowed the identification of lutein and zeaxanthin (co-eluted) and all-*trans*-β-carotene (traces).

A calibration graph was determined by performing triplicate 20 μL injections of the all-*trans*-β-carotene reference material solutions. A straight calibration curve was obtained by plotting the area counts against the varying concentrations (mg L^{-1}) of all-*trans*-β-carotene following the equation:

$$\text{Concentration}\left(\text{mgL}^{-1}\right) = 0.3371 \times \text{Area counts} + 0.0322$$

The correlation coefficient of the curve is 0.9995. The instrumental detection limit is 2.0 μg L^{-1} of all-*trans*-β-carotene (corresponding to 8 μg 100 g^{-1} of organic matrix).

The method was compared with the one described by Stahl [6]: results of comparative analyses on the same sample of Dunaliella salina (*Dunaliella salina* from Chile, NB-Dunaliella Algae Powder, Norbiotech LTDA) are reported in Table 1.

Discussion

A routine procedure, which is more simple and less time consuming than existing ones, is developed for the HPLC resolution of α-, all-*trans*, 9-*cis*,13-*cis*, and 15-*cis*-β-carotene. This method is characterised by the use of an RP-Amide (C16) column and the use of only one solvent as the mobile phase.

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