Separation of a complex carotenoid isomer mixture

Carotenoids tend to form geometric isomers, so they can occur as all-trans (all-E) or as cis (Z) isomers. They arise, for example, from the effects of light or heat on vegetables and fruits or their extracts. They also occur naturally or are formed in the human body. Due to the different shape of the Z isomers (kinked shape instead of linear and rigid), the isomers can differ drastically in their properties such as solubilisation or adsorption as well as transport in the human body. Lycopene, for example, occurs predominantly in foods as the all-E isomer, while in the human body it predominantly occurs as the Z isomer. This means that the lycopene is either immediately isomerised by the human body or the all-E form is poorly absorbed.

Therefore, appropriate analytical methods for detailed profiling of carotenoid isomers in nutritional studies are of great importance. This application note demonstrates the separation of up to 48 carotenoids by detection at different wavelengths (285, 347 and 450 nm) [1].

Standards of lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, lycopene, phytoene and phytofluene dissolved in ethanol were heated at 80 °C for 30 min to achieve stereomutation. A mixture of these extracts was used to optimise the separation.

Table 1: Chromatographic conditions.

Column:	YMC Carotenoid (3 µm) 150 x 4.6 mm ID
Part No.:	CT99S03-1546WT
Eluents:	A) Methanol containing 0.1 % ammonium acetate B) Methyl-tert-butyl ether (MTBE)
Gradient:	0–15 %B (0–45 min), 15–60%B (45–55 min), 60–70%B (55–60 min), 70%B (60–65 min), 70–0%B (65–70 min)
Flow rate:	1 mL/min
Temperature:	25 °C
Injection:	50 µL
Detection:	UV at 285, 347 and 450 nm
Sample:	Stereomutated standard solutions Plasma sample

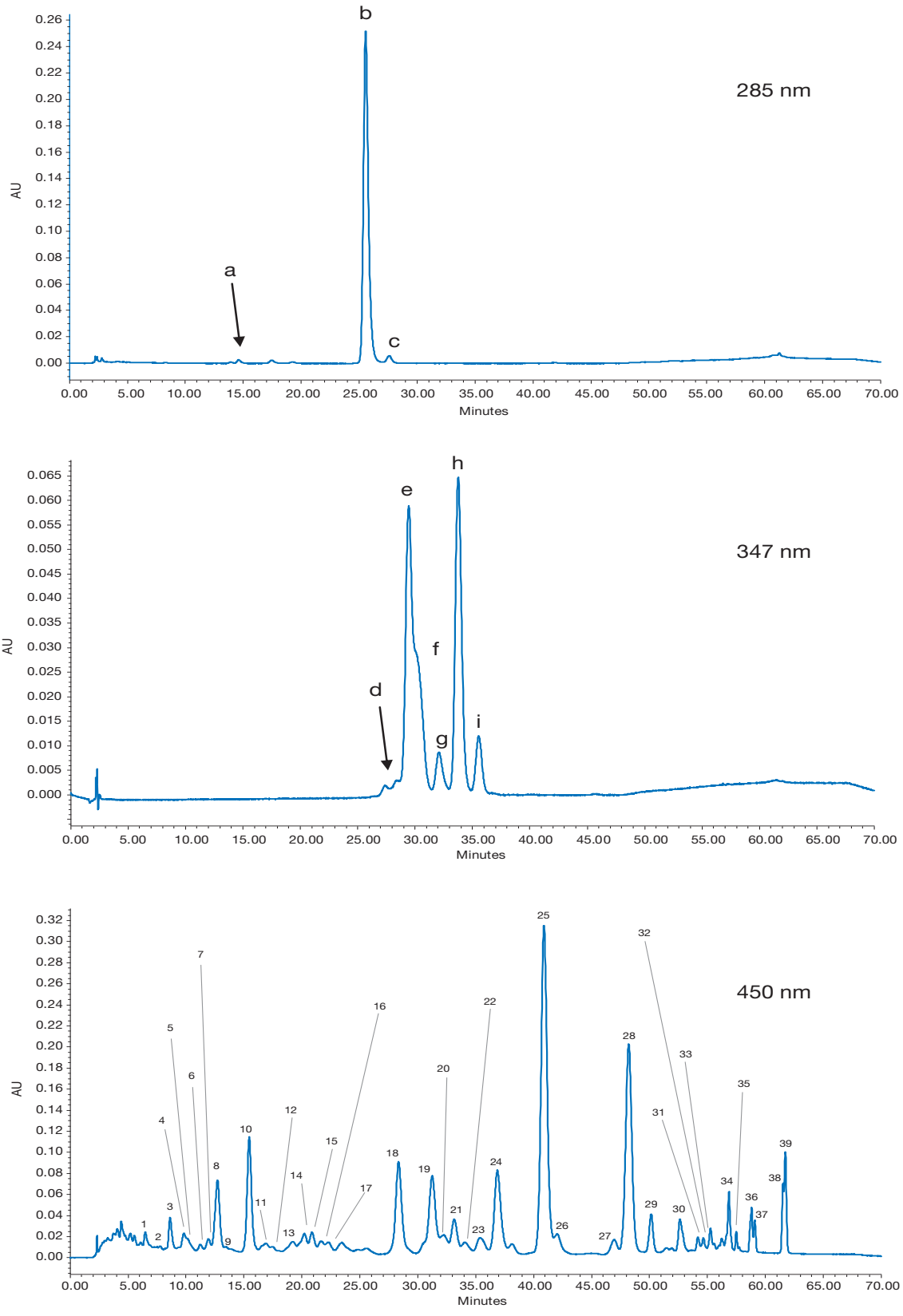

Figure 1: Chromatograms corresponding to the mixtures of isomers obtained by thermal stereomutation: phytoene (285 nm), phytofluene (347 nm), rest of carotenoids studied (450 nm) [1].

Table 2: Allocation of the peaks from Figure 1 [1].

Peak	Rt (min)	Isomer	Detection wavelength (nm)
a	14.5	Z-phytoene isomer 1	285
b	25.5	15-Z-phytoene	
c	27.6	All-E-phytoene	
d	28.5	Phytofluene isomer 1	347
e	29.5	Phytofluene isomer 2	
f	30.0	Phytofluene isomer 3	
g	32.1	Phytofluene isomer 4	
h	33.7	All-E-phytofluene	
i	35.4	Phytofluene isomer 6	
1	6.8	Di-Z-lutein	450
2	7.7	Di-Z-lutein	
3	8.6	Di-Z-lutein	
4	9.8	15Z-lutein	
5	10.0	Di-Z-zeaxanthin	
6	10.8	13Z- or 13‘Z-lutein	
7	11.6	13Z-zeaxanthin	
8	12.2	All-E-lutein	
9	12.3	15Z-zeaxanthin	
10	15.2	All-E-zeaxanthin	
11	16.3	9Z-lutein	
12	16.9	Di-Z-b-cryptoxanthin	
13	19.3	15Z-b-cryptoxanthin	
14	19.8	9‘Z-lutein	
15	20.3	13Z-b-cryptoxanthin	
16	20.9	13‘Z-b-cryptoxanthin	
17	22.0	9Z-zeaxanthin	
18	28.4	All-E-b-cryptoxanthin	
19	31.0	13Z-a-carotene	
20	32.3	9Z-b-cryptoxanthin	
21	32.8	13‘Z-a-carotene	
22	34.2	9‘Z-b-cryptoxanthin	
23	35.1	15Z-b-carotene	
24	36.6	13Z-b-carotene	
25	40.5	All-E-a-carotene	
26	41.8	9Z-a-carotene	
27	46.6	9‘Z-a-carotene	
28	47.9	All-E-b-carotene	
29	50.0	9Z-b-carotene	
30	52.0	Di-Z-lycopene isomer	
31	53.7	Di-Z-lycopene isomer	
32	54.9	Di-Z-lycopene isomer	
33	55.9	Di-Z-lycopene isomer	
34	56.6	15Z-lycopene	
35	57.3	Di-Z-lycopene isomer	
36	58.2	5Z,9Z- or 5Z,9’Z-lycopene	
37	58.6	9Z-lycopene	
38	61.5	All-E-lycopene	
39	61.8	5Z-lycopene	

With this method a human plasma sample 24 h after the consumption of carotenoid containing vegetables and fruits (carrot, celery, beets, parsley, lettuce, watercress, spinach and tangerine) was analysed. Several carotenoids were found (see Figure 2). Phytoene was not detected. Furthermore, three unidentified peaks (I – III) were detected, which do not match with the carotenoid isomers previously studied. These can be allocated to 3'-epilutein (I) and 3'-dehydrolutein (II,III) due to their absorption maxima. These substances have been detected in human serum and/or human retina in previous studies [2,3].

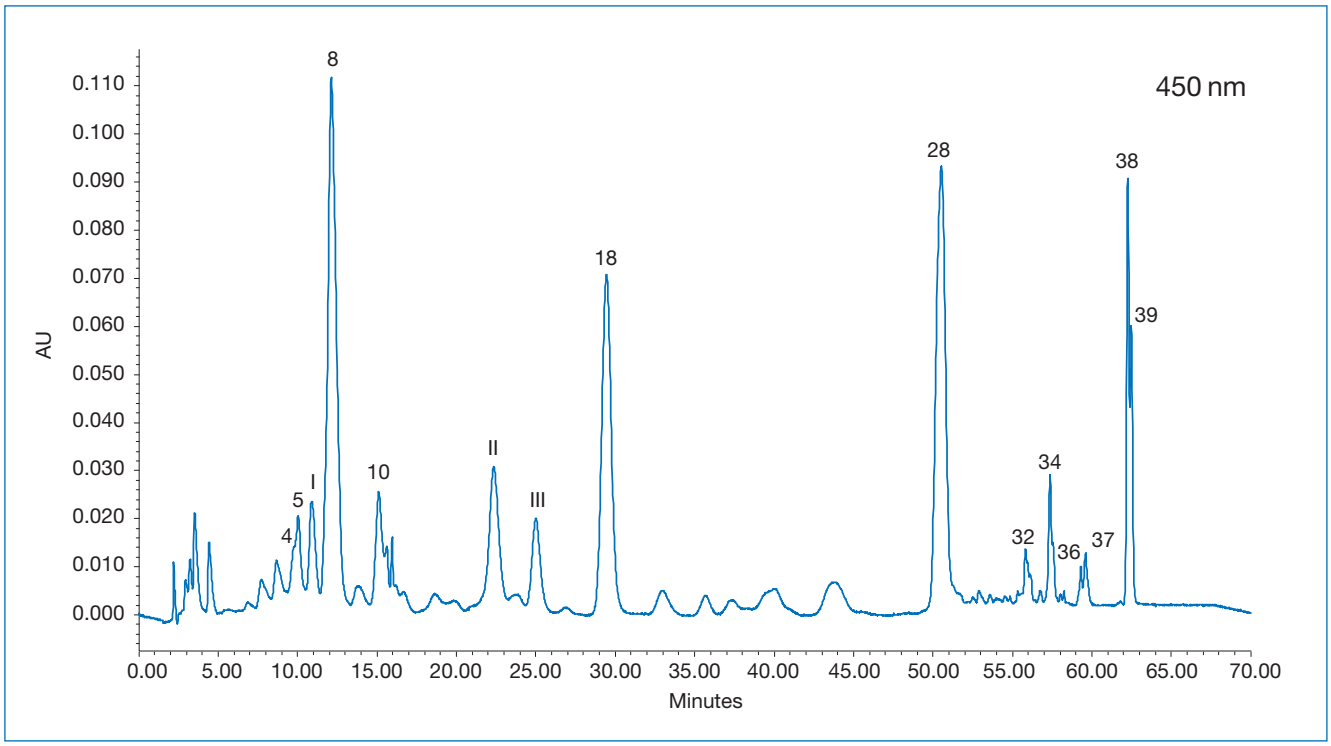

Figure 2: Analysis of a human plasma sample 24 h after the consumption of a mix of vegetables and fruits [1].

Literature

[1] A. J. Melendez-Martinez, C.M. Stinco, C. Liu, X.-D. Wang. A simple HPLC method for the comprehensive analysis of cis/trans (Z/E) geometrical isomers of carotenoids for nutritional studies, Food Chem. 138 (2013)1341-1350

[2] G. Britton, S. Liaaen-Jensen, H. Pfander, Carotenoids. Handbook (2004), Switzerland: Birkhäuser.

[3] W. Schalch, J.T. Landrum, R.A. Bone, Nutrition and health (2009), Vol. 5, pp. 301–334