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Separation and quantification of 15 carotenoids by reversed phase high performance liquid chromatography coupled to diode array detection with isosbestic wavelength approach

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ABSTRACT

The manuscript presents the development of a new reverse phase high performance liquid chromatography (RP-HPLC) photo diode array detection method allowing the separation and quantification of 15 carotenoids (adonirubin, adonixanthin, astaxanthin, astaxanthin dimethyl disuccinate, asteroidenone, beta-apo-8′-carotenal, beta-apo-8′-carotenoic acid ethyl ester, beta-carotene, canthaxanthin, capsanthin, citranaxanthin, echinenone, lutein, lycopene, and zeaxanthin), 10 of which are feed additives authorised within the European Union. The developed method allows for the reliable determination of the total carotenoid content in one run using the corresponding *E*-isomer as calibration standard while taking into account the *E*/*Z*-isomers composition. This is a key criterion for the application of the method, since for most of the analytes included in this study analytical standards are only available for the *E*-isomers. This goal was achieved by applying the isosbestic concept, in order to identify specific wavelengths, at which the absorption coefficients are identical for all stereoisomers concerned. The second target referred to the optimisation of the LC conditions. By means of an experimental design, an optimised RP-HPLC method was developed allowing for a sufficient chromatographic separation of all carotenoids. The selected method uses a Suplex pKb-100 HPLC column and applying a gradient with a mixture of acetonitrile, tert-butyl-methyl ether and water as mobile phases. The limits of detection and limits of quantification ranged from 0.06 mg L^{-1} to 0.14 mg L^{-1} and from 0.20 mg L^{-1} to 0.48 mg L^{-1}, respectively.

1. Introduction

Carotenoids are a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls) consisting of eight isoprene building blocks. Because of the numerous conjugated double bonds and the cyclic end groups, carotenoids present a variety of stereoisomers with different chemical and physical properties. The most important forms commonly found among carotenoids are stereoisomers abbreviated as *E*- or *Z*-isomers. Stereoisomers of this type are interconvertible in solution and exert a marked influence on the physical properties. *E*- and *Z*-isomers do not only differ in their melting points, solubility and stability, but also in respect to absorption affinity, colour and colour intensity [1]. Also the ultraviolet/visible (UV/Vis) spectra of the *E*/*Z*-isomers show significant differences, for instance the appearance of a new absorbance band in the spectra of the Z-isomers compared to the corresponding *E*-isomers [2].

Apart from the nutritional importance in human and animal health as metabolic precursors of vitamin A and antioxidants, carotenoids are used for the direct colouring of foodstuff as well as for pigmentation of animal products via their addition to complete feedingstuffs. In this study we included 15 carotenoids (Fig. 1), namely adonirubin, adonixanthin, asteroidenone, echinenone, lycopene and the feed additives astaxanthin, astaxanthin dimethyl disuccinate, beta-apo-8′-carotenal, beta-apo-8′-carotenoic acid ethyl ester, beta-carotene, canthaxanthin, capsanthin, citranaxanthin, lutein, and zeaxanthin. The feed additives are authorised within the European Union under Regulation (EC) No 1831/2003 classified in the category "sensory additives" and functional group "colourants: substances which, when fed to animals, add colours to food of animal origin" [3]. For instance, astaxanthin and canthaxanthin are added to salmon and trout feed

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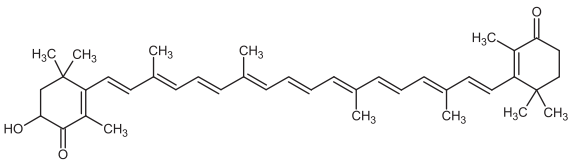
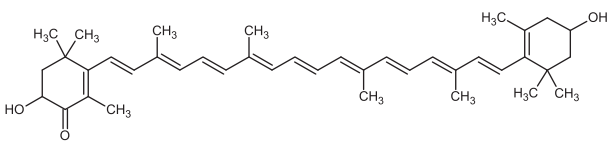
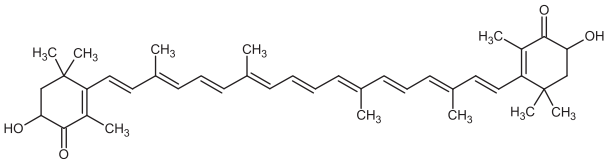


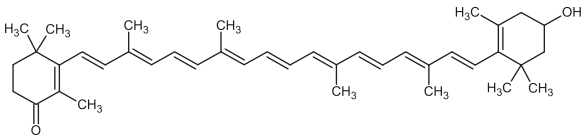
Adonirubin (ADR) $C_{40}H_{52}O_3$



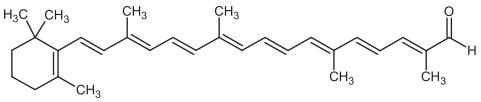
Adonixanthin (ADXN) $C_{40}H_{54}O_3$



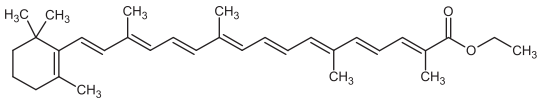
Astaxanthin (AXN) $C_{40}H_{52}O_4$



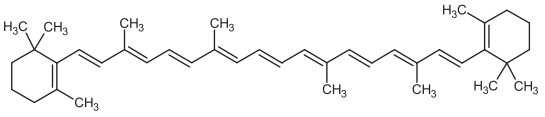
Asteroidenone (AST) $C_{40}H_{54}O_2$



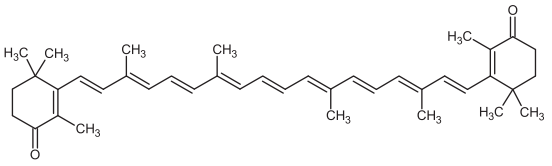
Beta-apo-8'-carotenal (BACAR) $C_{30}H_{40}O$



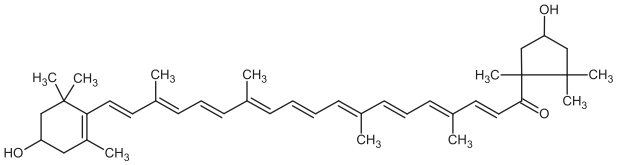
Beta-apo-8'-carotenoic acid ethyl ester (BACARE) $C_{32}H_{44}O_2$



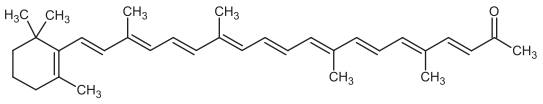
Beta-carotene (BCAR) $C_{40}H_{56}$



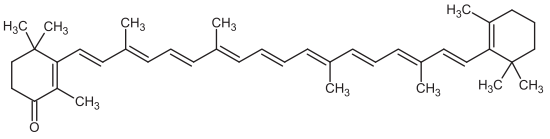
Canthaxanthin (CXN) $C_{40}H_{52}O_2$



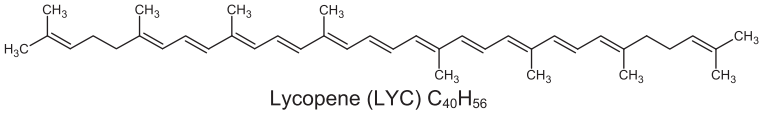
Capsanthin (CSN) $C_{40}H_{56}O_3$



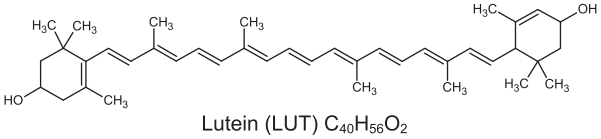
Citranaxanthin (CIXN) $C_{33}H_{44}O$



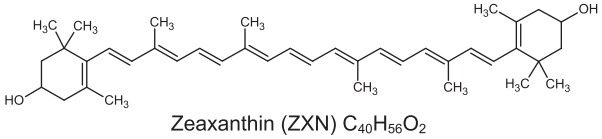
Echinenone (ECH) $C_{40}H_{54}O$



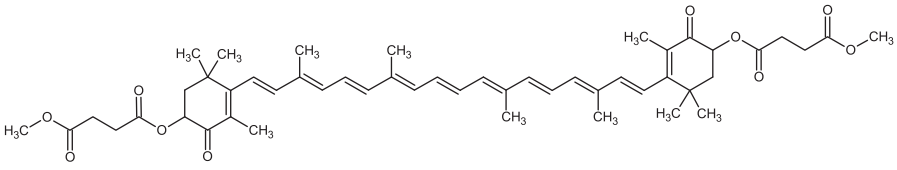
Lycopene (LYC) $C_{40}H_{56}$



Lutein (LUT) $C_{40}H_{56}O_2$



Zeaxanthin (ZXN) $C_{40}H_{56}O_2$



Astaxanthin dimethyl disuccinate (AXN-DMDS) $C_{50}H_{64}O_{10}$

Fig. 1. Chemical structures of the target all-*E* carotenoids.