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Carotenoids and apocarotenoids determination in intact human blood samples by online supercritical fluid extraction-supercritical fluid chromatography-tandem mass spectrometry

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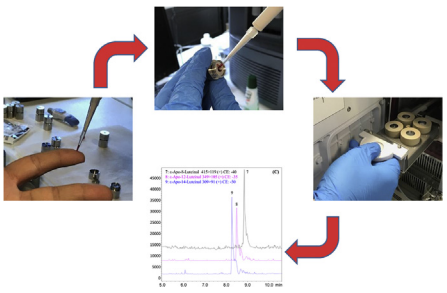
HIGHLIGHTS

- A SFE-SFC-QqQ/MS method was developed to the bioactives determination in human blood.
- Some apocarotenoids were detected in intact human blood for the first time.
- Selected carotenoid were directly rapidly screened in intact human blood.
- A remarkable small quantity of sample was required for the analyses.

GRAPHICAL ABSTRACT



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ABSTRACT

A direct on-line method based on the coupling of supercritical fluid extraction and supercritical fluid chromatography with triple quadrupole mass spectrometry detection (SFE-SFC-QqQ/MS) for selected carotenoids determination and apocarotenoids detection in intact human blood was developed for the first time. Carotenoids and apocarotenoids were identified by using the available standard together with full scan, selected ion monitoring (SIM), and multiple reaction monitoring (MRM) experiments. Moreover, β-Cryptoxanthin, Zeaxanthin, β-Carotene and Capsanthin were directly quantified by the developed methodology, using a multiple reaction monitoring (MRM) approach; the determined average content of β-carotene was 123.8 nmol L^{-1} (range 18.7–485.1 nmol L^{-1}), of β-cryptoxanthin was 385.3 nmol L^{-1} (range 72.5–1920.3 nmol L^{-1}), of zeaxanthin was 396.9 nmol L^{-1} (range < LoD – 1795.8 nmol L^{-1}) and of capsanthin was 38.9 nmol L^{-1} (range < LoD – 188.4 nmol L^{-1}). Analyses were carried out on 10 μL aliquots of intact blood samples without any preliminary treatment; the online extraction and chromatographic separation time was just over 20 min. The method was validated in terms of linearity, precision, limits of detection and quantification, and accuracy. Interestingly, β-apo-12′-carotenal, apo-10′-zeaxanthinal, apo-12′-zeaxanthinal, apo-14′-zeaxanthinal, ε-apo-8-luteinal, ε-apo-12-luteinal and

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ε-apo-14-luteinal were detected in human blood, together with two zeaxanthin fatty acid esters, for the first time.

1. Introduction

Carotenoids are naturally occurring pigments synthesized by plant and some microorganisms, usually consisting of a C_{40} backbone, with beneficial health properties [1]. Chemically they can be divided into carotenes (hydrocarbons) and xanthophylls, bearing oxygen containing functional groups. Quite often in nature, hydroxycarotenoids are esterified with fatty acids because the esterification provides greater stability to the molecule; both monoesters or diesters can be formed depending upon the xanthophyll moiety. Although around 40 different carotenoids are commonly present in the human diet, only about 20 have been reported in human blood and tissues; β-carotene, α-carotene, lycopene, lutein, zeaxanthin and β-cryptoxanthin are the most common carotenoids found in human blood and tissues [2–6]. Although carotenoid esters have been detected in human skin [7] and in human colostrum [8], only two reports are available in the literature on the detection of carotenoid esters in human serum or plasma [9,10].

Carotenoids oxidative and enzymatic cleavage products called apocarotenoids are very important bioactive molecules in plants [11,12] and, some of them, have also been reported in humans where they may exert unique biological activities as, for example, transcriptional regulation [13,14]. Multiple carotenoid cleavage dioxygenase enzymes (CCDs) have been identified in plants, whereas, cytoplasmatic β-carotene-15-15′-oxygenase 1 (BCO1) and mitochondrial β-carotene-9′-10′-oxygenase 2 (BCO2) have been characterized as mammalian carotenoid cleavage enzymes, respectively responsible for cleavage at the carotenoid central double bond and for carotenoids eccentric cleavages [11–14].

Relatively few studies are available in the literature on the apocarotenoids detection in human blood samples, and those available studies were focused on the apo-lycopenals and β-apocarotenals occurrence in either serum, plasma or red blood cell [15–18]. Natural β-apo-carotenoids have been suggested to function as naturally occurring antagonists of retinoic acid receptors with possible implications as modulators in the cardiovascular diseases and cancer preventions [18]. Available analytical reports for detecting carotenoids and apocarotenoids in blood samples are based on liquid extraction and liquid chromatography approaches, with relatively long analytical times and substantial organic solvent waste [2–6,9,10,14–26]; to the best of authors knowledge, only two reports are available in the literature on the application of a supercritical fluid chromatography coupled with mass spectrometry methodology for the carotenoids detection in human plasma [27,28], and no reports are available in the literature on the direct online extraction and determination of carotenoids and apocarotenoids by a supercritical fluid extraction-supercritical fluid chromatography-mass spectrometry (SFE-SFC-MS) methodology. The use of atmospheric pressure chemical ionization (APCI) for the carotenoids analysis has rapidly grown; in fact, it efficiently ionize not only xanthophylls and carotenes but also carotenoids esters [26]. Therefore, the aim of this research was focused on the development of an on-line method based on the coupling of supercritical fluid extraction and supercritical fluid chromatography with triple quadrupole mass spectrometry detection (SFE-SFC-APCI/QqQ/MS) for the carotenoids and apocarotenoids detection in intact human blood samples, for the first time; moreover, a further aim was the direct quantification of selected carotenoids, belonging to different carotenoids chemical classes, by the developed methodology also for the first time. The detection of compounds present in very low amounts like the carotenoids and apocarotenoids in human blood samples, that could be used as biomarkers in large clinical or epidemiological studies, needs the development of very sensitive methodologies [29]; therefore, the development of an analytical platform like the SFE-SFC-QqQ/MS one, could be considered as a rapid and sensitive single evaluation tool for a reliable quali-quantitative profiling of carotenoids and apocarotenoids in human blood samples and also in other biological fluids.

2. Materials and methods

2.1. Chemicals

All the reagents and solvents used were of analytical or HPLC grade and were purchased from Merck KGaA (Darmstadt, Germany). Carotenoids standards, namely, β-apo-8′-carotenal, β-carotene, β-cryptoxantin, capsanthin, zeaxanthin and lutein were purchased from Extrasynthese (Genay, France). Human blood was used for the method validation. Stock solutions of the carotenoids compounds were prepared by using a solution of MeOH 90% and $CHCl_3$ 10% at a concentration level of 10,000 mg L^{-1} and stored in dark vials at −20 °C. Calibration standard solutions were prepared in human blood (matrix matched calibration); these solution were used to determine the recoveries by considering only the carotenoids standard amounts used in the different spikings.

Standard mixtures of carotenoids at the 5000 and 100 mg L^{-1} levels, in MeOH, were used for 5000 μg L^{-1} and 100 μg L^{-1} spiking levels. The two solutions were used to measure recovery.

2.2. Apocarotenoid standards preparation

Apocarotenals were generated by oxidative cleavage of the parent carotenoid by potassium permanganate according to the conditions reported by Rodriguez & Rodriguez-Amaya for β-carotene [30], and also described by Giuffrida et al., for zeaxanthin and capsorubin [31] and here applied to lutein standard as well; therefore, a series of β-apo-carotenals, apo-zeaxanthinals, and ε-apo-luteinals were generated by oxidation of β-carotene, zeaxanthin, and lutein respectively. In the conditions used the potassium permanganate generated oxidative cleavage of the double bonds of the studied carotenoids and gave apocarotenoids without further oxidation to carboxylic acid functions. Briefly, in a round bottom amber vial, a solution of the parent carotenoid (1.0 mol equiv.) in dichloromethane containing several drops of water was treated with $KMnO_4$ (2.6 mol equiv.) at room temperature with stirring for 12 h. After the reaction was complete, the formed apocarotenoids mixture was filtered through celite to remove MnO_2 and the filtrate was washed several times with water (until the color of excess $KMnO_4$ was removed), dried over anhydrous Na_2SO_4 and concentrated in controlled vacuum. The residue was dissolved in 2 mL of methanol/MTBE (1:1) prior to SFC-MS analysis, as described in Section *2.5*.

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