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Application of ultra-high performance supercritical fluid chromatography for the determination of carotenoids in dietary supplements

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ABSTRACT

A quick and simple ultra-high performance supercritical fluid chromatography-photodiode array detector method was developed and validated for the simultaneous determination of 9 carotenoids in dietary supplements. The influences of stationary phase, co-solvent, pressure, temperature and flow rate on the separation of carotenoids were evaluated. The separation of the carotenoids was carried out using an Acquity UPC^2 HSS C18 SB column (150 mm × 3.0 mm, 1.8 μm) by gradient elution with carbon dioxide and a 1:2 (v:v) methanol/ethanol mixture. The column temperature was set to 35 °C and the backpressure was 15.2 MPa. Under these conditions, 9 carotenoids and the internal standard, β-apo-8′-carotenal, were successfully separated within 10 min. The correlation coefficients (R^2) of the calibration curves were all above 0.997, the limits of detection for the 9 carotenoids were in the range of 0.33–1.08 μg/mL, and the limits of quantification were in the range of 1.09–3.58 μg/mL. The mean recoveries were from 93.4% to 109.5% at different spiking levels, and the relative standard deviations were between 0.8% and 6.0%. This method was successfully applied to the determination of 9 carotenoids in commercial dietary supplements.

1. Introduction

Carotenoids are natural fat-soluble pigments present in plants, algae, and microorganisms. Humans cannot synthesize the carotenoids necessary to maintain normal health, and must acquire them through food and dietary supplements [1,2]. Epidemiological studies have found that increased carotenoid intake can reduce the incidence of several chronic conditions, such as cancer, heart disease, vascular disease, retinal diseases, and degenerative diseases [3]. Furthermore, α-carotene, β-carotene, and β-cryptoxanthin are important for humans as they function as vitamin A precursors [4]. The carotenoids lutein and zeaxanthin are found abundantly in human retina macula lutea, and a growing number of studies have indicated that they protect the retina by filtering out blue light, and play a significant role in reducing the risk of visual loss from age-related macular degeneration [5]. Lycopene is a representative carotenoid found in tomatoes, and has attracted increasing research interest over the last decade owing to the link between lycopene-rich foods or supplements and the prevention or treatment of prostate cancer [6]. Astaxanthin and fucoxanthin are two major marine carotenoids that have also received increasing attention in recent years. This is because their superior antioxidant activity allows them to perform as free radical scavengers and counteract oxidative stress processes, consequently reducing the risk of cardiovascular problems, acute inflammation, and UV-light damage [7,8]. Antioxidant properties are also observed with canthaxanthin, which has been used as a component of tanning pills and as food coloring [9,10].

Owing to the beneficial effects of these compounds, an increasing numbers of carotenoid dietary supplements have appeared on the market in recent years. Therefore, methods for the fast and accurate determination of carotenoids in dietary supplements are urgently required in order to ensure their quality and protect the interests of consumers.

HPLC coupled with UV–Vis absorbance detection or diode array detection (DAD) is widely used for the measurement of carotenoids in dietary supplements [11–14]. C18 and C30 columns are those most commonly used. Many studies have reported that the C30 column is more suitable for carotenoid determination and allows much better separation of carotenoid structural isomers, such as lutein and zeaxanthin, and α-carotene and β-carotene [15–17]. Ultra-high performance liquid chromatography (UHPLC)

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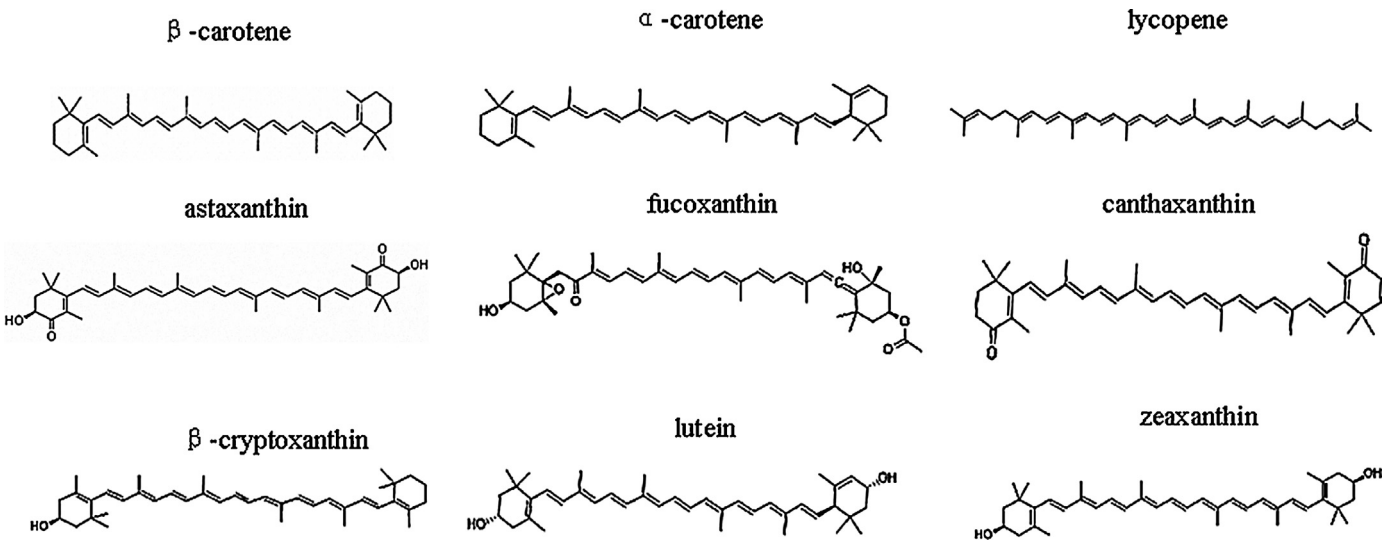

Fig. 1. Chemical structures of nine carotenoids in the present study.

with sub-2 μm particle-size columns is generally considered offering shorter analysis times, smaller peak widths and higher chromatographic resolution compared to conventional HPLC [18]. However, until now, there are no commercially available UHPLC C30 stationary phase columns. Bijttebier et al. compared the separation of complex carotenoid mixtures between the HPLC C30 column and different UHPLC columns. The study found that the overall performance of HPLC C30 column was better than all the tested UHPLC columns in the carotenoids separation. However, the main drawbacks of this method were the long analysis times [18]. Long analysis times not only mean large solvent volume usage which is not environmentally friendly, but also are not suitable for the determination of unstable compounds.

Supercritical fluid chromatography (SFC) is a suitable alternative method for carotenoids determination. CO_2 is the most used mobile phase in SFC. Above its critical pressure (P_C = 7.3 MPa) and temperature (T_C = 31 °C) the supercritical fluid has lower viscosity and higher diffusivity relative to conventional liquids, and higher density and dissolving capacity compared with conventional gases. These unique properties make SFC an effective analytical method for thermally unstable and involatile compounds. Relative to the corresponding HPLC separation, SFC allows higher flow rates and shorter run times. Furthermore, due to less organic solvent consumption, this technology is considered to be a green approach [19–25]. Owing to the low polarity of CO_2, SFC is efficient for the separation of hydrophobic compounds. In addition, the SFC mobile phase is also very flexible, and organic co-solvents, such as methanol, can be added to change its polarity. However, the addition of these co-solvents increase critical parameters to higher pressure and temperature figures which are depending on the nature and proportion of the co-solvents [26], such as when the mobile phase CO_2-MeOH 70:30 was used, critical parameters increased to 135 °C and 16.8 MPa [27]. Therefore, the majority of today's separations are actually not performed under a supercritical state but rather in subcritical conditions (with $P > P_C$ and $T < T_C$) [26,27]. The addition of polar cosolvents enhances the fluid solvent strength, enabling simultaneous determination of different polarity carotenoids [28,29].

Recent technological advancements that employed sub-2 μm particles in UHPLC have been transferred to ultra-high performance supercritical fluid chromatography (UHPSFC) [30]. The aim of this work was to develop a rapid and reliable method using ultra-high performance supercritical fluid chromatography coupled to photodiode array detector (PDA) and sub-2 μm particle columns for the determination of 9 selected carotenoids, including lycopene, α-carotene, β-carotene, β-cryptoxanthin, astaxanthin, fucoxanthin, canthaxanthin, lutein and zeaxanthin in dietary supplements.

2. Experimental

2.1. Chemicals and materials

β-Carotene, α-carotene, lutein, zeaxanthin, fucoxanthin (all ≥95.0%), β-cryptoxanthin, astaxanthin (≥97.0%), lycopene (≥90.0%), canthaxanthin (≥98.8%), and the internal standard (IS) trans-β-Apo-8′-carotenal (≥96.0%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The chemical structures of the 9 carotenoids are shown in Fig. 1. Methanol and ethanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). Dichloromethane (DCM), and dimethyl sulfoxide (DMSO) were purchased from Dikma Scientific (Lake Forest, CA, USA). Butylated hydroxytoluene (BHT) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All reagents were of HPLC-grade or better. Ultrapure water of purity ≥18.0 Ω-cm was prepared using a milli-Q system (Millipore, Millford, MA, USA).

2.2. Standard solution preparation

Stock solutions of each carotenoid and IS were prepared in DCM + 0.1% BHT. The concentrations of these solutions were determined using a Shimadzu – 2600 UV–Vis spectrophotometer. Before measuring absorbance, the stock solutions were diluted in suitable solvents (hexane for β-carotene, α-carotene, lycopene and astaxanthin; ethanol for fucoxanthin, β-cryptoxanthin, lutein and zeaxanthin; petroleum ether for canthaxanthin; chloroform for IS). The wavelengths of UV/visible absorbance detection and extinction coefficients used for the calculation of the exact concentrations of the standards are as follows: 471 nm, 3450 for lycopene; 445 nm, 2710 for α-carotene; 453 nm, 2592 for β-carotene; 470 nm, 2100 for astaxanthin; 466 nm, 2200 for canthaxanthin; 452 nm, 1601 for fucoxanthin; 452 nm, 2350 for β-cryptoxanthin; 445 nm, 2550 for lutein; 450 nm, 2540 for zeaxanthin and 461 nm, 2640 for IS. Absorbances of the solutions were between 0.200 and 0.800, and were recorded in triplicate. Individual working solutions of each carotenoid and IS prepared in DCM-ethanol 1:2 (v/v) were injected for purity determination in the UHPSFC system. Purity of

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