Analytica Chimica Acta xxx (2018) 1–9

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A rapid LC-MS method for qualitative and quantitative profiling of plant apocarotenoids

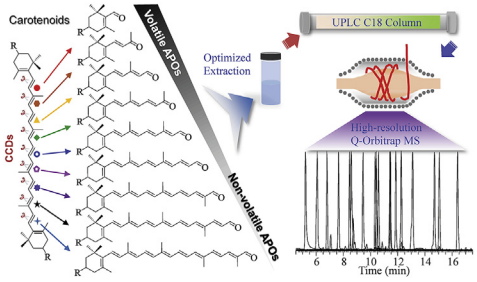
Jianing Mi, Kun-Peng Jia, Jian You Wang, Salim Al-Babili*

King Abdullah University of Science and Technology (KAUST), Biological and Environmental Sciences and Engineering Division, The Bioactives Lab, Thuwal 23955-6900, Saudi Arabia

HIGHLIGHTS

- We developed a LC-MS method to analyze volatile and non-volatile apocarotenoids.
- We optimized LC-MS conditions to detect simultaneously C_{10}- to C_{30}-apocarotenoids.
- We applied the new validated method to profile 20 apocarotenoids in plant samples.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 May 2018
Received in revised form
28 June 2018
Accepted 1 July 2018
Available online xxx

Keywords:
Apocarotenoids
UHPLC-Q-obitrap-MS
Qualitative and quantitative analysis
Oryza sativa
Spinacia oleracea
Vitamin A

ABSTRACT

Carotenoid cleavage products (apocarotenoids; APOs) exert important biological functions in light perception and as vitamin A source, signaling molecules, hormone precursors, pigments and volatiles. However, an analytical method that allows simultaneous profiling of these diverse compounds is still missing. We developed an efficient method to analyze APOs present in plant tissues, which is based on ultra-high performance liquid chromatographic separation and high-resolution hybrid quadrupole-Orbitrap (Q-Orbitrap) mass spectrometry (MS). Our approach allowed unambiguous identification and quantification of volatile and non-volatile APOs in a single run. Modified sample preparation and optimized ultra-high performance liquid chromatography (UHPLC)-MS parameters permitted the measurement of APOs in *Oryza sativa* seedlings and *Spinacia oleracea* leaves, unraveling 20 endogenous APOs with chain lengths ranging from C_{10} to C_{30}, confirmed by high-resolution MS, MS/MS data and using synthetic standards. Our experimentation demonstrates that the usage of methanol with 0.1% butylated hydroxytoluene facilitates the extraction of both short-chain and long-chain APOs from plant materials. In addition, our validated analytical method allows the quantitative analysis of APOs with a wide content range from 2.5 pg/mg to 10 ng/mg dried weight. The adoption of the analytical protocol, as described in this study, realizes the measurement of volatile APOs by using a LC-MS method, hence, allowing informative and reliable profiling of APOs, which is important for determining the content of these compounds in food and crucial for understanding their function and metabolism in plants.

Abbreviations: APOs, apocarotenoids; Q-Orbitrap, quadrupole-Orbitrap; MS, mass spectrometry; UHPLC, ultra-high performance liquid chromatography; ABA, abscisic acid; SL, strigolactone; CCDs, carotenoid cleavage dioxygenases; ROSs, reactive oxygen species; NCEDs, 9-*cis*-epoxy carotenoid cleavage dioxygenases; SLE, solid-liquid extraction; LLE, liquid-liquid extraction; SPE, solid-phase extraction; SFE, supercritical fluid extraction; HS-SPME, headspace solid-phase microextraction; GC-MS, gas chromatography-mass spectrometry; SFC-MS, supercritical fluid chromatography-mass spectrometry; MeOH, methanol; ACN, acetonitrile; IPA, 2-propanol; FA, formic acid; BHT, butylated hydroxytoluene; IS, internal standard; LOD, limits of detection; LOQ, limits of quantification; RSDs, relative standard deviations; RT, retention time.

* Corresponding author.
E-mail address: salim.babili@kaust.edu.sa (S. Al-Babili).

https://doi.org/10.1016/j.aca.2018.07.002
0003-2670/

1. Introduction

Carotenoids are isoprenoid compounds generally consisting of a C_{40}-backbone equipped with an extended conjugated double bond system, and are synthesized by all photosynthetic organisms and many heterotrophic microorganisms [1–3]. In plants, carotenoids are essential constituents of photosystems, indispensable for light-harvesting and photoprotection [3–5]. Carotenoids also exert a ubiquitous function as precursors of a diverse class of compounds that are generally called apocarotenoids (APOs) and which include vitamin A and its derivatives, the phytohormones abscisic acid (ABA) and strigolactone (SL) [6,7], the fungal pheromone trisporic acid [8,9], pigments, such as the citrus citraurin [10,11], and several biologically important volatiles, including β-ionone and the signaling molecule β-cyclocitral [12,13]. APOs are produced from carotenoids via oxidative cleavage of specific double bonds, which is catalyzed by carotenoid cleavage dioxygenases (CCDs) [14,15]. In addition, these compounds can be formed by less-specific enzymes (e.g., lipoxygenases and peroxidases) [16] or by non-enzymatic oxidation processes triggered by reactive oxygen species (ROSs) processes [17]. In plants, APOs are present in different types of plastids, such as leucoplasts and chromoplasts, and in different tissues, including flowers and roots [18]. Due to the diversity of carotenoid precursors (more than 750 to date), multiple cleavage sites, and modifications after cleavage, structures of APOs vary in functional groups and length of the polyene-chain [18].

Evidence is emerging for the importance of APOs for different aspects of plant's life. As visual and volatile signals, APOs attract animals for flower pollination and seed dispersal [19,20]. Moreover, some APOs, such as β-cyclocitral, are plastid-retrograde signaling molecules mediating the transcriptional response of plants to oxidative stress [17]. It was also shown that β-cyclocitral and/or β-ionone treatment increases the photooxidative stress tolerance of Arabidopsis [12]. The volatile β-ionone also exhibits antifungal and antimicrobial activity [21]. Primary carotenoid cleavage products can be further modified into phytohormones, such as ABA and SL, which play an important role in plant's response to drought and other abiotic stress conditions, plant's pathogen defense, and establishing plant's shoot and root architecture [22–24]. Finally, there is also an increasing evidence for the role of yet unidentified APOs in regulation of carotenoid biosynthesis, plastid development and the formation of lateral roots [25,26]. In addition to retinal, long chain plant APOs are important constituents of human nutrition, as they might be a source for vitamin A [27]. In addition, β-carotene-derived APOs, such as β-apo-14′-carotenal and β-apo-13-carotenone, have been reported as retinoid receptor antagonists, which may modulate retinoic acid-dependent biological processes and impact human health [28]. Hence, determining the APOs profile in plant material is required to better understand their biological functions in plants and is also relevant from a nutritional point of view.

Carotenoid cleavage dioxygenases are ubiquitous enzymes that differ in their substrate- and stereo-specificity, as well as in targeted double bond. In plants, CCDs are divided into 9-*cis*-epoxy carotenoid dioxygenases (NCEDs) that form the ABA precursor xanthoxin from 9-*cis*-violaxanthin or 9′-*cis*-neoxanthin and CCDs, an in-homogenous group of enzymes that cleave different carotenoids and/or APOs at different sites [29,30]. The CCD family of *Arabidopsis thaliana* includes 5 NCEDs and the four CCDs: CCD1, CCD4, CCD7 and CCD8. CCD7 and CCD8 catalyze the stereospecific conversion of 9-*cis*-β-carotene, formed by the carotene isomerase DWARF27, into the strigolactone precursor carlactone [31]. As deduced from *in vitro* studies, CCD7 cleaves 9-*cis*-β-carotene at the C9′-C10′ double bond, yielding β-ionone and 9-*cis*-β-apo-10′-carotenal [32–35]. In the next step, CCD8 catalyzes the formation of carlactone, a non-canonical, tri-oxygenated CCD product carrying a lactone ring, via a less understood combination of reactions, including repeated dioxygenation and intramolecular rearrangement [15,31]. Interestingly, *in vitro* studies showed that CCD8, when incubated with all-*trans*-β-apo-10′-carotenal, also catalyzes usual cleavage reaction leading to β-apo-13-carotenone [36]. Thus, the determination of APOs *in planta* is crucial to evaluate for identifying and understanding the substrate specificity of CCDs.

Due to their divergent physical properties, the extraction of APOs from biological material is a challenging step in analyzing these compounds [37], particularly if simultaneous isolation of volatile and non-volatile APOs is intended. The most frequently referenced methods for APOs extraction and pre-concentration from biological material comprised solid-liquid extraction (SLE) and liquid-liquid extraction (LLE) [38–40], solid-phase extraction (SPE) [41,42], supercritical fluid extraction (SFE) [43,44], and headspace solid-phase microextraction (HS-SPME) [45]. For LC-MS analysis, SLE, LLE and SPE are generally used as sample preparation methods, while HS-SPME is specific for gas chromatography-mass spectrometry (GC-MS). On-line SFE is recently used in the supercritical fluid chromatography (SFC)-MS analysis of APOs [43]. In referenced extraction methods, different solvents have been established to extract volatile or non-volatile APOs, such as ethanol/tert-butylmethylether/tetrahydrofuran (9/5/1, v/v/v) for the isolation of various β-apocarotenals, 5,6-epoxy-β-carotenal, retinol, and retinal [46]; diethylether for the extraction of long-chain β-apocarotenals, 5,6-epoxy-, and 5,8-epoxy-β-apocarotenals [47]; acetone for extracting volatile APOs, such as β-ionone [48]. However, these extraction methods mainly focus on either non-volatile or volatile APOs. Similarly, analysis of volatile and non-volatile APOs requires different analytical methods, i.e. GC-MS, SFC-MS, and UHPLC-MS. For example, β-apo-8′, β-apo-10′, β-apo-12′, and β-apo-14′, β-apo-15-carotenal and β-apo-13-carotenone were detected by using HPLC-MS [49,50] or SFC-MS [39,43,44], while volatile APOs (e.g., β-ionone and α-ionone) have been determined by GC-MS [50,51]. Thus, a method that allows simultaneous, sensitive and reliable quantification of both volatile and non-volatile APOs is needed.

Here, we developed a method for the analysis of volatile and non-volatile APOs with chain lengths ranging from C_{10} to C_{30}, by using UHPLC-Q-Orbitrap-MS. For this purpose, we first optimized the extraction conditions, to collect both types of APOs from plant material. Next, we improved the UHPLC resolution of individual compounds, to simultaneously analyze volatile and non-volatile APOs from a single plant extract. Finally, we used this method to quantitatively profile APOs in rice and spinach samples.

2. Materials and methods

2.1. Chemicals

LC-MS grade methanol (MeOH), acetonitrile (ACN), 2-propanol (IPA), formic acid (FA), and butylated hydroxytoluene (BHT, purity ≥ 99%) were purchased from Sigma-Aldrich (Taufkirchen, Germany). LC-MS grade water; HPLC grade n-hexane, dichloromethane, ethyl acetate, and acetone were purchased from VWR International, LLC. (Pennsylvania, USA). APO standards including 3-OH-β-cyclocitral, β-cyclocitral, 3-OH-β-ionone, β-ionone, 3-OH-β-apo-11-carotenal, β-apo-11-carotenal, 3-OH-β-apo-13-carotenone,

Please cite this article in press as: J. Mi, et al., A rapid LC-MS method for qualitative and quantitative profiling of plant apocarotenoids, Analytica Chimica Acta (2018), https://doi.org/10.1016/j.aca.2018.07.002