

Validation of an HPLC method for determining canthaxanthin and β -apo-8'-carotenal in food

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Abstract

This study validates an analytical method for quantifying canthaxanthin and β -apo-8'-carotenal, two carotenoid food additives that were recently approved in Japan; however, an official method for quantifying the contents of these compounds in foods is lacking. These compounds, which serve as food coloring agents with established Acceptable Daily Intakes (ADIs) of 0.025 and 0.05 mg/kg body weight/day, respectively, require reliable analytical methods for regulatory compliance and safety monitoring. We modified a method from the literature and evaluated its performance according to the Guidelines for Validation of Analytical Methods for Food Additives in Foods. The proposed method demonstrated good sensitivity with a lower quantification limit of 0.1 μ g/mL (0.001 g/kg food content) and good linearity within the tested concentration range. Recovery tests on four food products yielded satisfactory recovery rates, repeatability, and intra-laboratory precision, meeting all guideline criteria. The proposed technique provides a reliable analytical method for determining canthaxanthin and β -apo-8'-carotenal content in tested food products. These results support the potential applicability of this technique as an official quantification method for regulatory monitoring and food safety assurance.

Keywords : canthaxanthin, β -apo-8'-carotenal, HPLC, method validation

I Introduction

Carotenoids, which are fat-soluble compounds, are widely found in nature and have been used for various purposes, including food, livestock feed, and pharmaceuticals, given the diverse physiological functions and color properties of this class of chemicals¹⁾. Among these compounds, canthaxanthin and β -apo-8'-carotenal (Fig. 1) have unique properties and usefulness. Seafood such as salmon and shrimp, edible mushrooms, green algae, and even the feathers of flamingos contain canthaxanthin^{2, 3)}, which is known to give these materials a red-orange color⁴⁾. Globally, this pigment is used in feed additives to enhance the color of chicken egg yolks and meat. β -apo-8'-carotenal has an orange hue⁴⁾ and is an intermediate metabolite in the pathway leading to vitamin A synthesis⁵⁾. Moreover, β -apo-8'-carotenal occurs naturally in trace amounts in green and yellow vegetables and fruits⁶⁾.

According to the Japan Food Safety Commission, the safe daily amounts (Acceptable Daily Intakes; ADIs) for canthaxanthin and β -apo-8'-carotenal are 0.025 mg/kg body weight/day and 0.05 mg/kg body weight/day, respectively^{7, 8)}. Based on that safety review, canthaxanthin and β -apo-8'-carotenal were approved as food additives in Japan in 2015 and 2014, respectively. In Japan, these substances are permitted for use in food coloring based on specific usage criteria⁹⁾. Canthaxanthin is restricted to kamaboko with the maximum allowable amount of 0.035 g/kg, and is prohibited in hanpen, satsuma-age, tuna ham, fish sausage, and other similar products. Furthermore, β -apo-8'-carotenal is not allowed in kelp, meat, fresh fish, fresh shellfish, tea, seaweed, beans, or vegetables. Generally, for food additives with established usage standards, the so-called "Analytical Method for Food Additives in Foods"¹⁰⁾ is specified as a validated test method to determine conformance to the usage standards; for instance, this method