

Analysis of Zeaxanthin by Single Quadrupole LC/MS

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Key Words

- Finnigan™ Surveyor™ HPLC
- Finnigan Surveyor MSQ™
- Age-related Macular Degeneration
- PDA Detection
- Zeaxanthin

Introduction

(3R,3'R)-zeaxanthin is one of several dietary carotenoids found in human ocular tissue. Research has shown that concentrations of zeaxanthin, as well as other carotenoids, are significantly lower than normal in the macula of persons diagnosed with neovascular age-related macular degeneration (AMD). One mechanism by which macular carotenoids may provide protection against AMD may involve its antioxidant function. Thus, these carotenoids may be ultimately beneficial in protecting the eye from light-induced oxidative damage.

Ongoing research in the area of AMD involves determining the metabolic pathways of dietary carotenoids in animal (bird) models. Isotopically labeled zeaxanthin can be created in culture grown in medium dissolved in deuterated water. The deuterated zeaxanthin can in turn be fed to birds to investigate dietary and non-dietary metabolic pathways of this carotenoid. In such studies a reliable method of identification and quantitation of zeaxanthin from tissue extracts is needed.

Goal

In this report, deuterated zeaxanthin, produced in bacterial culture, was extracted, purified, and subsequently characterized and quantified using a single quadrupole LC/MS system. This technique provides a simple and reliable way of identifying and quantifying zeaxanthin for metabolic studies.

Experimental Conditions

Deuterated zeaxanthin was produced by flavobacterium multivorum grown in media components such as glucose and yeast extract dissolved in deuterated water. The zeaxanthin was extracted and purified by preparative HPLC as described previously¹, dried, and dissolved in HPLC mobile phase containing methylene chloride and methanol. The resulting solution was centrifuged at high speed to remove particulates prior to LC/MS analysis.

¹Factorial analysis of tricarboxylic acid cycle intermediates for optimization of zeaxanthin production from *Flavobacterium multivorum*. P. Bhosale, A.J. Larson, and P.S. Bernstein. *J Appl Microbiol.* 2004;96(3):623-9.

Chromatographic Conditions

Instrument:	Finnigan Surveyor HPLC System with PDA detector
LC Column:	25 cm × 4.6 mm i.d. (Hypersil™)
Packing:	5 μm cyano (Microsorb)
Flow Rate:	1.0 mL/min
Mobile Phase:	hexane:methylene chloride:methanol:N,N'-di-isopropylethylamine (80:19.2:0.7:0.1 v v ⁻¹), normal phase
Gradient:	Isocratic
Injection Volume:	20–100 μL full loop
Column Temperature:	25°C (ambient)
Detection Wavelength:	450 nm

Mass Spectrometer Conditions

Instrument:	Finnigan Surveyor MSQ
Ionization:	Atmospheric pressure chemical ionization (APCI)
Polarity:	Positive
Probe Temperature:	550°C
Cone Voltage:	80.0 eV
Scan Range:	520–700 Da

Results

Zeaxanthin extracted from flavobacterium multivorum grown in heavy water was subjected to LC/APCI-MS analysis to determine the degree of deuteration. Figure 1 shows full-scan data for the zeaxanthin sample in its undeuterated (A), partially deuterated (B), and fully deuterated forms (C), obtained with the Finnigan Surveyor MSQ in positive APCI mode. The small amount of undeuterated zeaxanthin ion at *m/z* 569.5 (Figure 1, C) was also observed in the deuterated mixture. MS analysis revealed 55–70% replacement of hydrogen by deuterium atoms as indicated by the molecular mass cluster at around *m/z* 600.

APCI-MS in the positive ion mode is a very popular technique for detection of hydrophobic carotenoids because positive ion fragmentation leads to small fragmentation patterns, which give specific signals for each carotenoid. Growth conditions could not prevent atmospheric moisture from entering into the growth media. Thus, signals of undeuterated zeaxanthin were seen in some preparations of deuterated zeaxanthin, as Figure 1(C) shows.

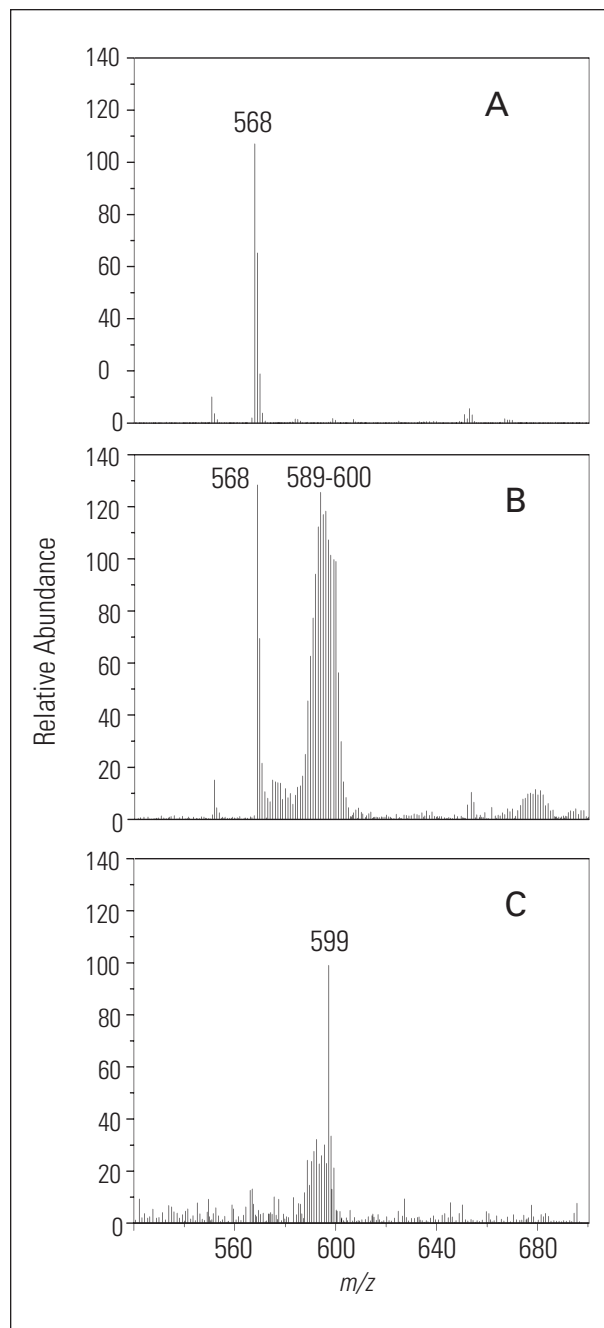


Figure 1: Full-scan zeaxanthin spectral data obtained from *Flavobacterium multivorum* grown in heavy water enriched medium: (A) undeuterated zeaxanthin, (B) partially undeuterated zeaxanthin with deuterated zeaxanthin, and (C) deuterated zeaxanthin.

The mass at m/z 599.0 was chosen as the quantitation ion. SIM analysis was completed for standard concentrations of 25 pg, 50 pg, 100 pg, 200 pg, 400 pg, 500 pg, 1 ng, and 2 ng. Intensity of the quantitation ion at each of these concentrations is shown in Figure 2.

The calibration data for these levels of deuterated zeaxanthin is shown in Figure 3. The SIM chromatogram for the 25 pg/ μ L standard gave a signal-to-noise ratio of 12 to 1. The correlation coefficient (R^2) was 0.9988 (Figure 3).

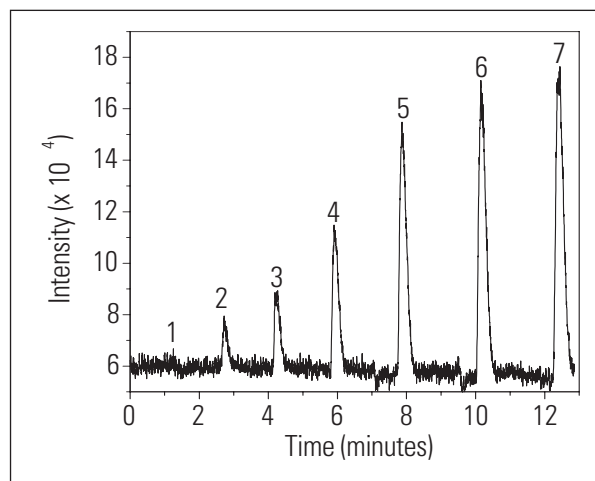


Figure 2: Intensity of m/z 599.0 for deuterated zeaxanthin at 25 pg (1), 50 pg (2), 100 pg (3), 200 pg (4), 400 pg (5), 1 ng (6), and 2 ng (7) as determined by loop injection analysis and single ion monitoring.

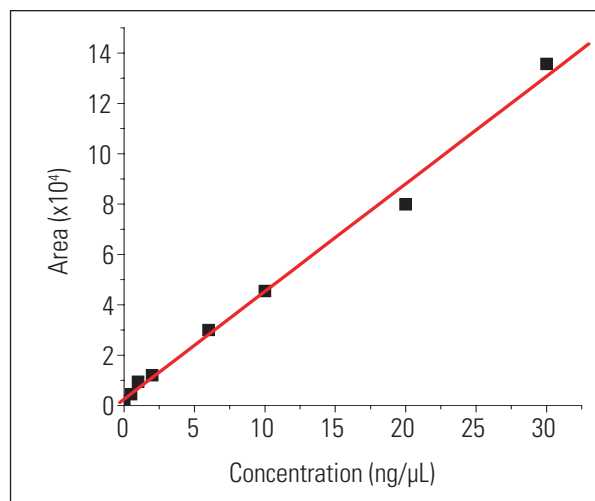


Figure 3: Zeaxanthin calibration data.

Discussion and Conclusion

A simple LC/MS method for separation and quantitation of zeaxanthin was demonstrated. APCI was used in the positive mode to identify this compound, since this technique leads to small fragmentation patterns typically seen with hydrophobic carotenoids. The data show excellent linearity and sensitivity for this compound that would allow for determination in the ppm range.

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