

# Analysis of Zeaxanthin by Single Quadrupole LC–MS

Gail Harkey, Thermo Electron Corporation, Schaumburg, Illinois, USA,  
Prakash Bhosale, University of Utah School of Medicine, Salt Lake City, Utah, USA.

## 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.

## 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 previously, 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.

## 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.

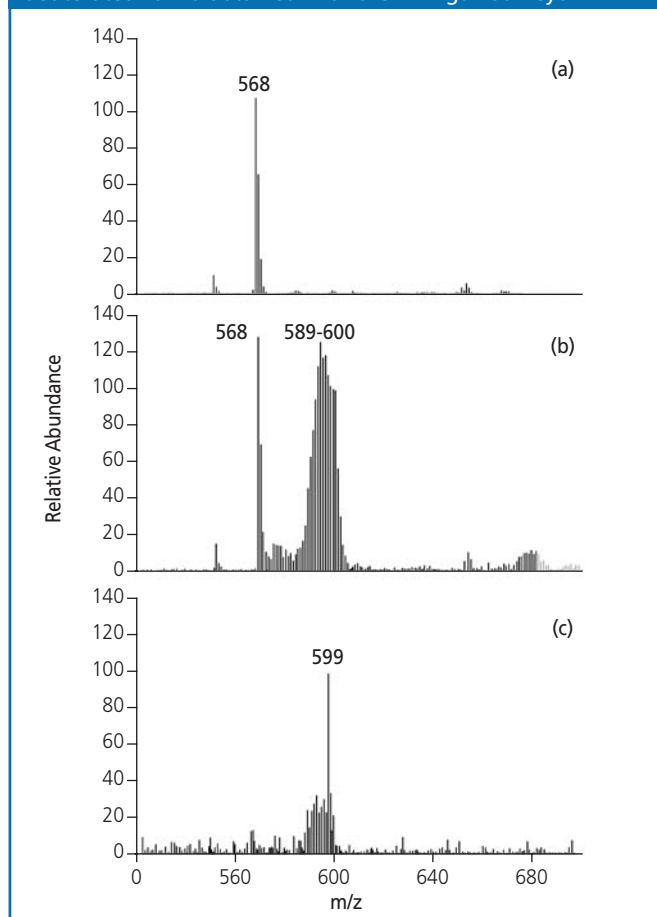
The mass at  $m/z$  599.0 was chosen as the quantification ion. SIM analysis was completed for standard concentrations of 25 pg, 50 pg, 100 pg, 200 pg, 400 pg, 500 pg, 1 ng and 2 ng. The SIM chromatogram for the 25 pg/ $\mu$ L standard gave a signal-to-noise ratio of 12 to 1. The correlation coefficient ( $R^2$ ) was 0.9988.

## Discussion and Conclusion

A simple LC–MS method for separation and quantification 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.

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

**Figure 1:** Full-scan data for the zeaxanthin sample in (a) its undeuterated, (b) partially deuterated and (c) fully deuterated forms obtained with the Finnigan Surveyor.



**Thermo**  
ELECTRON CORPORATION

**Thermo Electron Corporation**  
355 River Oaks Parkway  
San Jose, California 95134 USA  
tel. +1 561 688 8700, fax +1 561 688 8731  
e-mail: analyze@thermo.com  
website: www.thermo.com/chromatography  
Reader Service 232