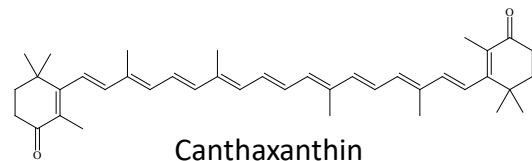
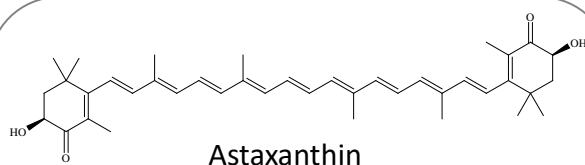


Astaxanthin and cantaxanthin are carotenoids that are used as color enhancers in aquatic animals. The residue limits for cantaxanthin, are set for livestock and fishery foods, and the test methods are described in "Testing methods for pesticide residues in foods, feed additives, or substances that are components of veterinary drugs" (Notification No. 0124001 of the Safety Division, PFSB dated January 24, 2005).

In this report, a High Performance Liquid Chromatography (HPLC) was used with a photodiode array detector (PDA), which is versatile for many diverse applications.

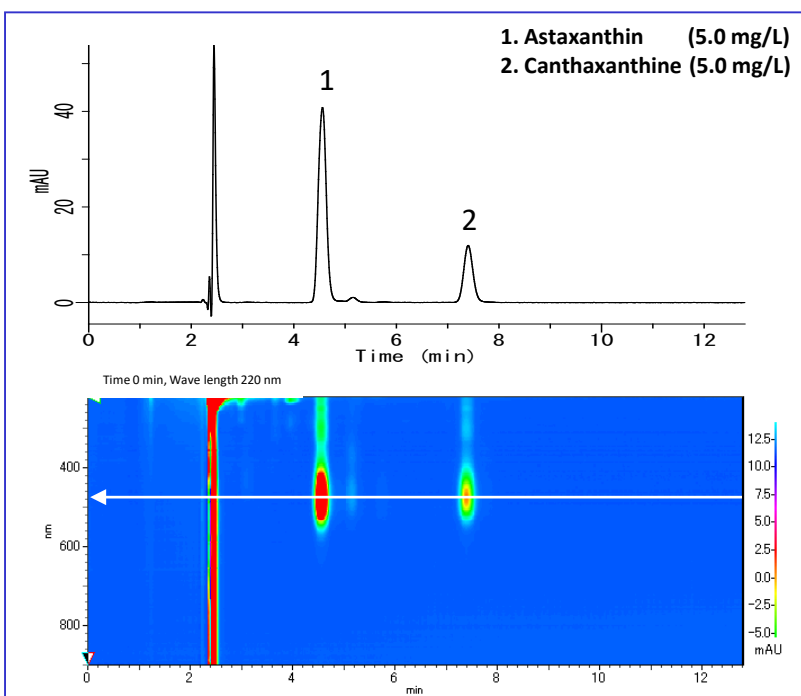
(K. Suzuki)

### Structural Formula



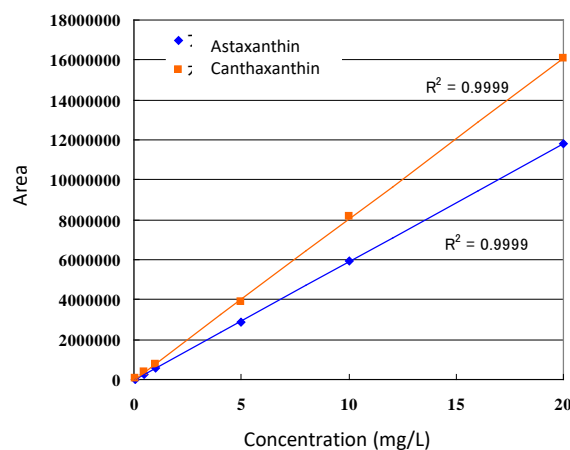
Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

## Example: Measurement of standards



### HPLC conditions

<b>Column</b>	: Inertsil ODS-SP (5 $\mu$ m, 150 x 4.6 mm I.D.)
<b>Eluent</b>	: A) CH <sub>3</sub> CH <sub>3</sub> B) 0.05 % TFA solution A/B = 97/3, v/v (gradient mixer)
<b>Flow rate</b>	: 0.8 mL/min
<b>Column temperature</b>	: 40 °C
<b>Detected</b>	: PDA 480 nm
<b>Injection volume</b>	: 20 $\mu$ L



Calibration curve

## Measurement example

### Sample pretreatment example

Pretreatment was performed according to the test method for cantaxanthin described above.

#### Sample

- Homogenization by fine cutting
- 5g

#### Extraction (1)

- Acetonitrile 30 mL
- Acetonitrile saturated *n*-hexane 20 mL
- Anhydrous sodium sulfate 10 g
- Homogenize
- Centrifugal separation 3000 rpm 10 min

#### Preparative isolation

- Residue + hexane layer

#### Extraction (2)

- Acetonitrile layer
- Acetonitrile 20 mL
- Shake
- Centrifugal separation 3000 rpm 10 min
- Acetonitrile layer

#### Collection

- 10 mL of *n*-propanol

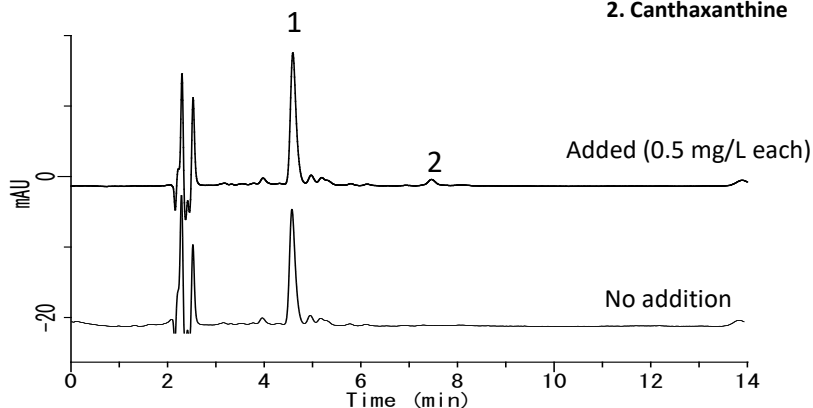
#### Concentration

- Concentration under reduced pressure at 40 °C or below 5 mL
- A constant volume of 10 mL with methanol.

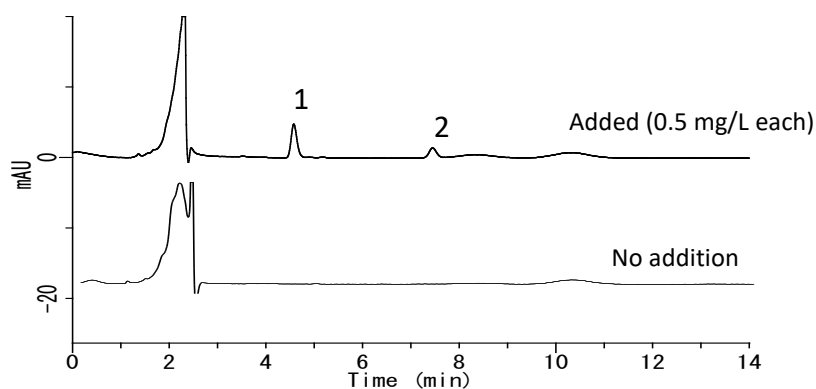
#### HPLC

### Wakame seaweed

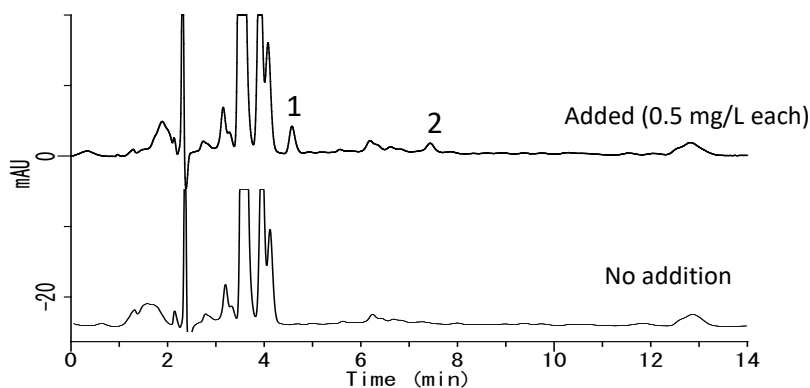
1. Astaxanthin
2. Canthaxanthine



### Shrimp extract



### Dried shiitake mushroom extract



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