LT191

GL Sciences Inc.

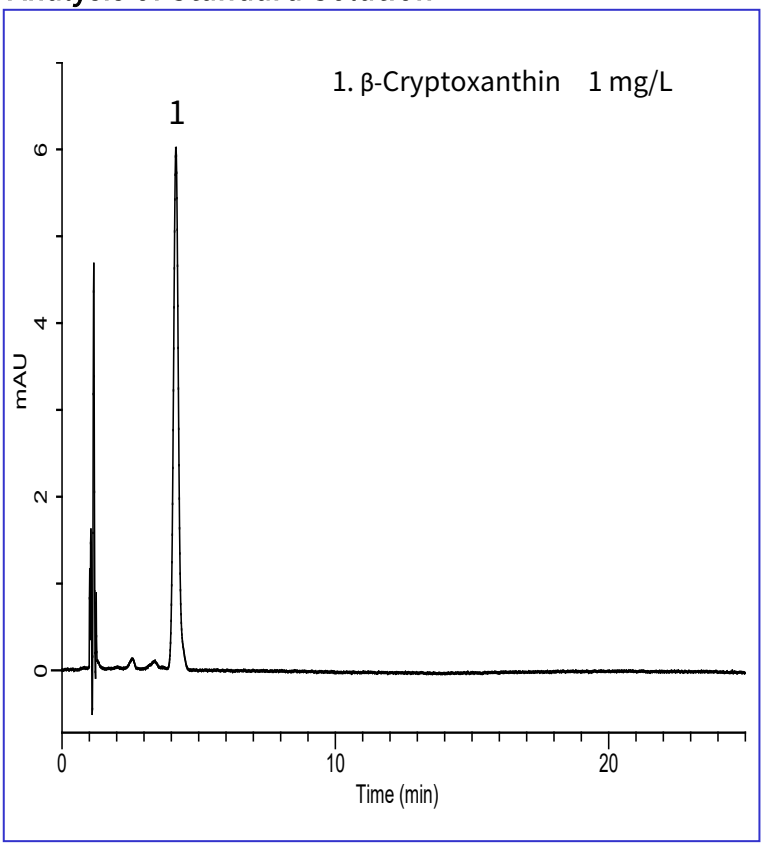
Analysis of β-cryptoxanthin in Citrus Unshiu

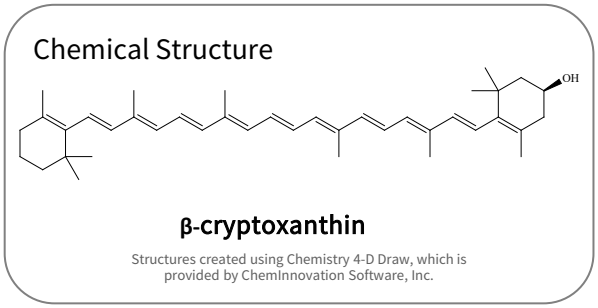
β-Cryptoxanthin is a carotenoid pigment found in the edible part of the fruit Citrus unshiu. In addition to its function as provitamin A, which is mainly metabolized to the essential nutrient vitamin A, it is also known to have a excellent antioxidant properties derived from the highly conjugated double bonds in the molecular framework. β-Cryptoxanthin is considered to be effective in preventing lifestyle-related diseases and in reducing the incidence of osteoporosis. Many foods, supplements, soft drinks, etc. are commercially available that take advantage of these beneficial effects. In this application note, separation and analysis was made with reference to the food sanitation inspection guidelines, and the JAS amendment. (S.Miura)

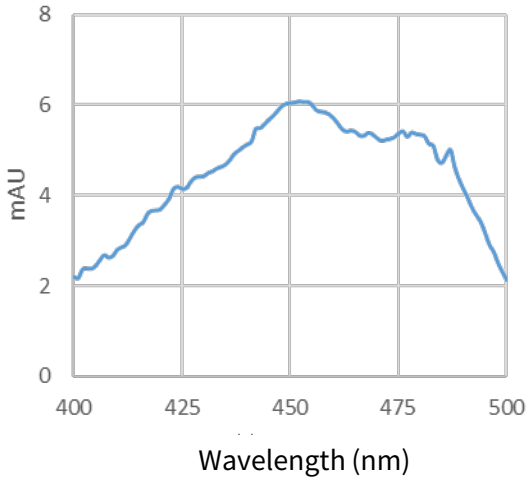
Chromatogram with Conditions Described in Japan Agricultural Standards(JAS) Amendment

The Japanese Agricultural Standards (JAS) amendment of 2019 describes HPLC conditions using an Inertsil ODS-3 column. In this application note we will introduce an example of analysis using Inert Sustain C18 column that has comparable performance but with greater inactivity. Using this column, it is possible to perform analysis with a separation pattern similar to ODS-3 column.

Analysis of Standard Solution







UV/Vis absorption spectrum

Conditions

Column	:	InertSustain C18 (5 µm, 150 x 4.6 mmI.D.)
Column Cat. No.	:	5020-07345
Eluent	:	0.12 mM Ascorbyl palmitate in (CH_3OH / $CHCl_3$ = 24/1, v/v)
Flow rate	:	1.5 mL/min
Col. Temp.	:	40 °C
Detection	:	VIS 455 nm
Injection Vol.	:	20 µL
Sample	:	Standard

Example of Analysis of β-cryptoxanthin

After removing the epicarp part of the Citrus unshiu fruit, it was crushed using a homogenizer, and extraction was performed in accordance with the 2019 Japanese Agricultural Standards (JAS) amendment. After saponification, the residue was dissolved and filtered to obtain a sample solution.

Pretreatment

Sample

- Weigh approximately 2.0 g

Extraction

- Add 15 mL of pyrogallol solution (30 g / L)
- Sodium sulfate 10 g
- Shake extract for 5 min
- Centrifuge for 5 min
- Collect the supernatant (volumetric flask) (②)

① (Shake extract for 5 min, Centrifuge for 5 min, Collect the supernatant (volumetric flask) (②))

- Add 15 mL of pyrogallol solution to the residue repeat ①, and collect the supernatant (③).
- Combine ② and ③ and add pyrogallol solution and make up the volume to 50mL.

Saponification

- Take 10mL of constant volume solution in a centrifuge tube
- 1mL of 60% Potassium Hydroxide Solution
- Heat (70 °C) for 30 minutes
- Cool (to room temperature)

Recover unsaponifiable agent

- 20mL of 1% sodium chloride solution
  5mL of 2-propanol
  Add 12mL of ethyl acetate/hexane (9:1) mixture
- Shake for 5 min
- Centrifuge for 5 min
- Collect the supernatant (eggplant flask) (②)
- Repeat ① for the residue and collect supernatant (③)
- Repeat operations ③ (④)
- Combine ②③④ and distill under reduced pressure.

Constant volume

- Make up the volume to 5 mL with ethanol

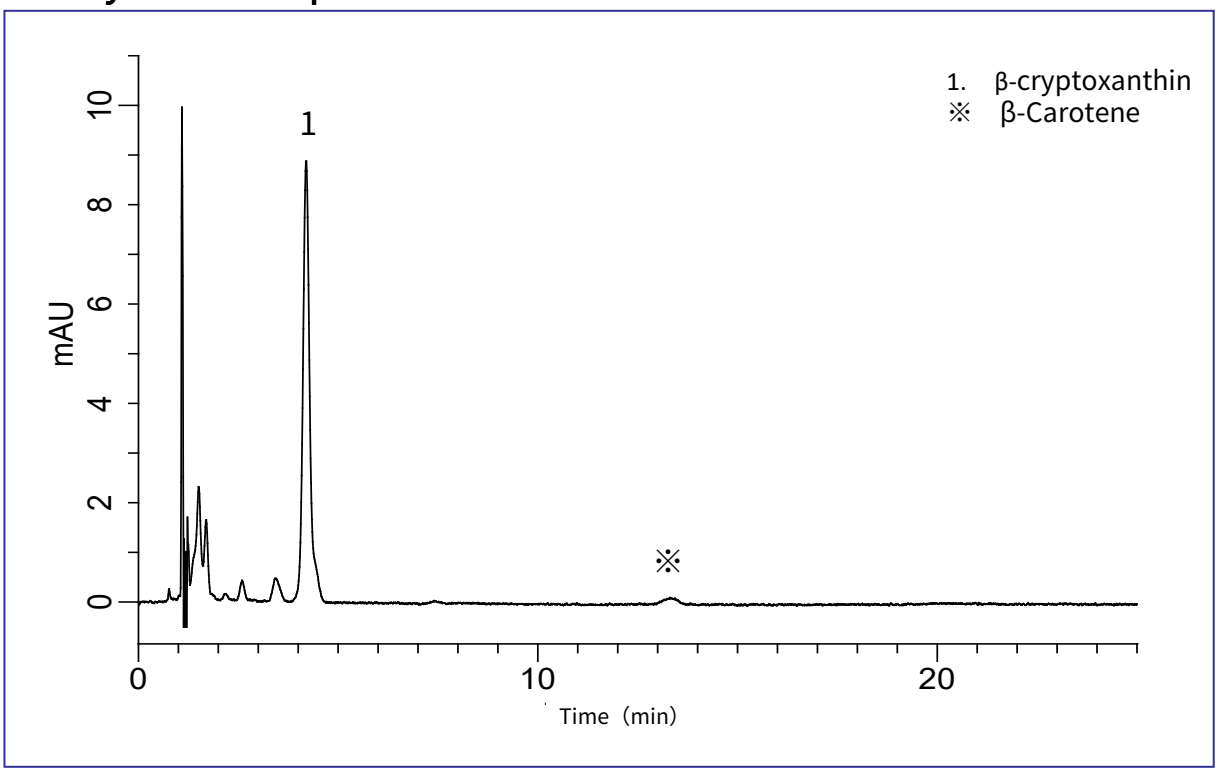
Filtration

- Pass the recovered liquid through a GL ChromatoDisc 13P (0.45 µm).

Sample solution

HPLC

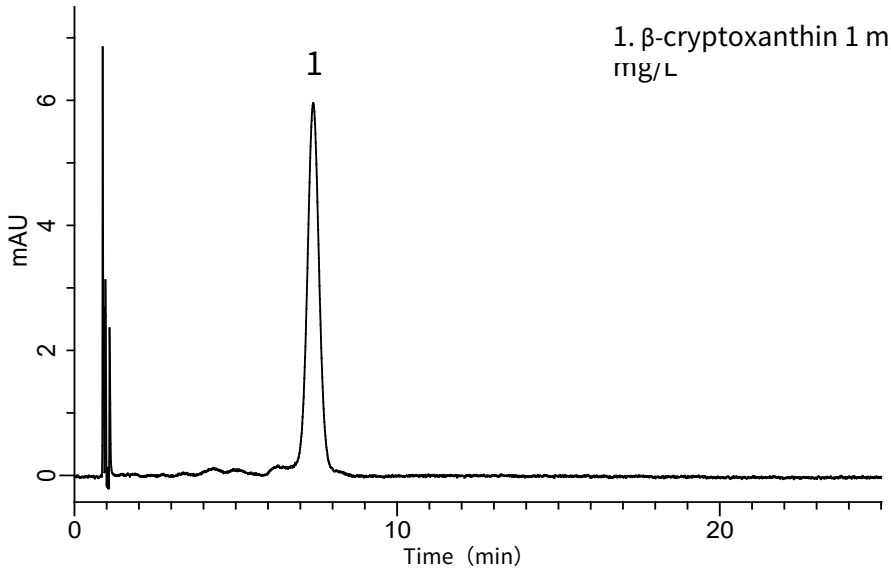
Analysis of Sample Solutions



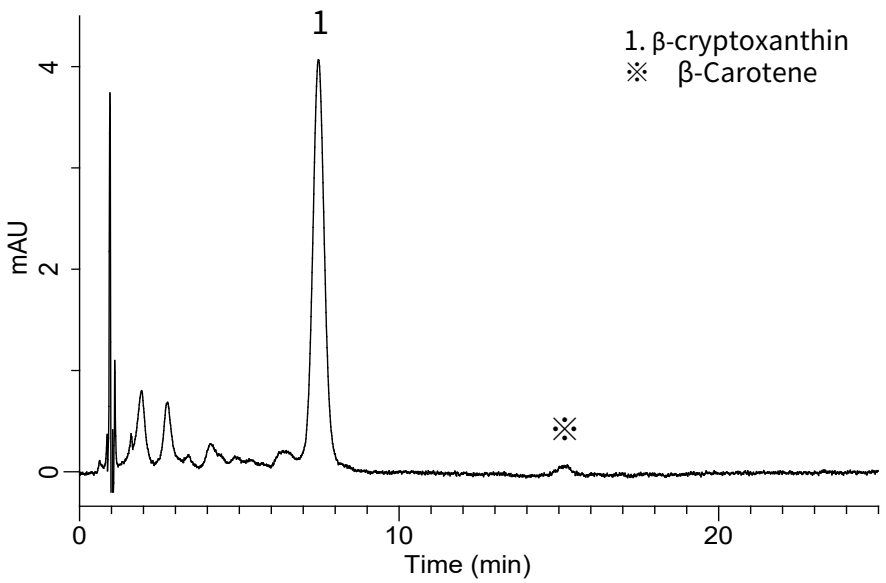
Chromatogram with Conditions Described in Food Hygiene Inspection Guideline (carotene analysis)

Since β-cryptoxanthin is a type of carotene, it is possible to perform analysis under the same eluent conditions used for carotene. In this application note (with reference to the food hygiene inspection guidelines) a relatively hydrophobic solvent was used as the eluent. In addition, the measurement was performed using an ODS column that makes it easy to recognize the difference in structures.

Standard

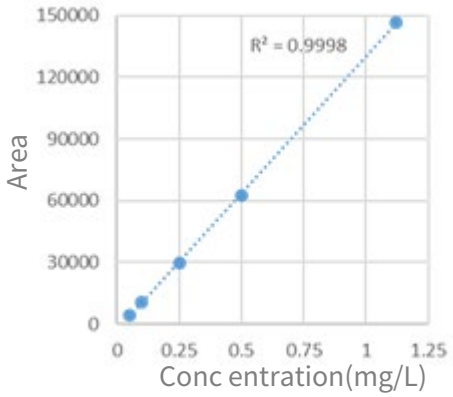


Sample



Conditions

Column	: Inertsil ODS-P (5 µm, 150 x 4.6 mmI.D.)
Column Cat. No.	: 5020-02001
Eluent	: A) CH_3CN B) CH_3OH C) THF A/B/C = 55/40/5, v/v/v
Flow Rate	: 1.5 mL/min
Col. Temp.	: 20 °C
Detection	: VIS 455 nm (5430 Diode Array Detecto
Injection Vol.	: 20 µL
Sample	: Standard



Calibration curve

Products used

- Analysis column
  - InertSustain C18 5 µm 4.6 x 150mmI.D.
    Cat.No. 5020-07345
  - Inertsil ODS-P 5 µm, 4.6 x 150 mmI.D.
    Cat.No. 5020-02001

- Vial related products
  - Polypropylene vial, 1.5mL, 100/pk
    Cat.No.1030-51019
  - Screw Cap with Septa PTFE Red/Silicon White, thickness 1.0mm
    Cat.No.1030-51222

- Syringe Filter
  - GL ChromatoDisc 13P
    Cat.No. 5040-28541
  - Plastic Disposal Syringe, 10mL, Luer-lock type
    Cat. No.1030-55110

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