LT028

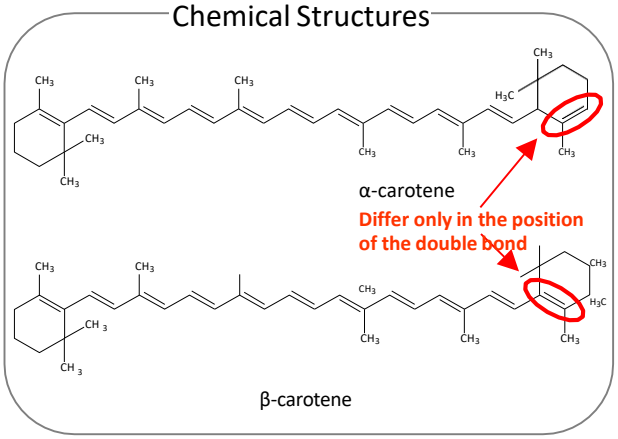
GL Sciences Inc.

Analysis of α-Carotene and β-Carotene in Food by HPLC

α-Carotene and β-carotene are a type of carotenoid and widely known as precursors to vitamin A in human body. They also have antioxidant activity.

The two carotenes are very similar in chemical structure. Since the difference is only in the position of a carbon-carbon double bond, it is difficult to separate them with widely-used monomeric ODS columns. To solve this problem, Inertsil ODS-P, which is a polymeric ODS column and superior in steric recognition, was used as a separation column in this note. (K. Suzuki)

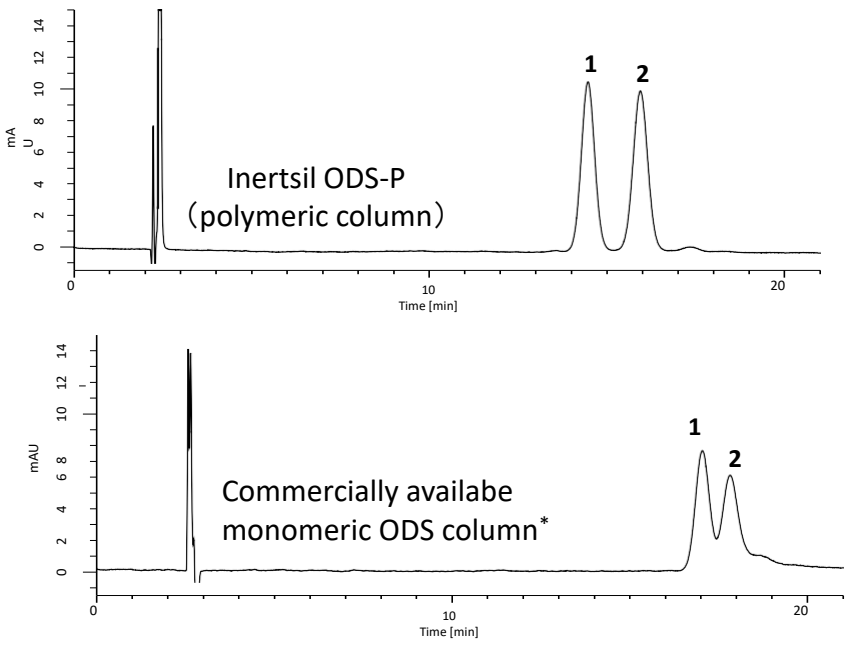
Chemical Structures



Chromatograms obtained from standard solution

When a commercially available monomeric column was used, α-carotene and β-carotene were not separated within 20 minutes. However, Inertsil ODS-P provided good separation of the two carotenes.

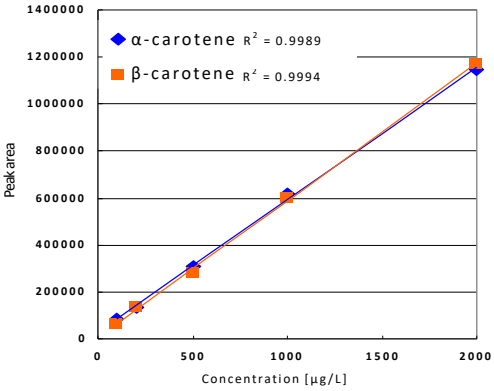
1. α-Carotene (2.0 mg/L)
2. β-Carotene (2.0 mg/L)



* The mobile phase composition was adjusted to A/B = 45/55.

Conditions

Column	: Inertsil ODS-P (5 µm, 250 x 4.6 mm I.D.) Cat.No. 5020-02002
Eluent	: A) CH_3OH B) C_2H_5OH A/B = 90/10, v/v (gradient mixer)
Flow rate	: 1.0 mL/min
Col. Temp.	: 40 ℃
Detection	: PDA 455 nm
Inj. Vol.	: 20 µL



Calibration curves obtained with Inertsil ODS-P column

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Analyses of food samples

Sample pretreatment 1

Vegetables
- 0.5 g
- 3 % Pyrogallol ethanol solution 10 mL
- 60 % KOH aqueous solution 1 mL

Saponification
- Heat at 70 ℃ for 30 min
- Place in an ice-water bath to cool down
- 1 % NaCl aqueous solution 22.5 mL
- Hexane-ethyl acetate = 9/1 (v/v) 15 mL
- Shaking for 5 min

*A

Liquid-liquid extraction
- Organic layer
- Aqueous layer
  - Repeat procedure *A twice

Concentration
- Evaporate under reduced pressure
- Dissolve in 2 mL of ethanol

HPLC

Sample pretreatment 2

Carrot juice
- 2 g
- 3 % Pyrogallol ethanol solution 20 mL
- Sodium sulfate anhydrous 10 g
- Shaking for 5 min

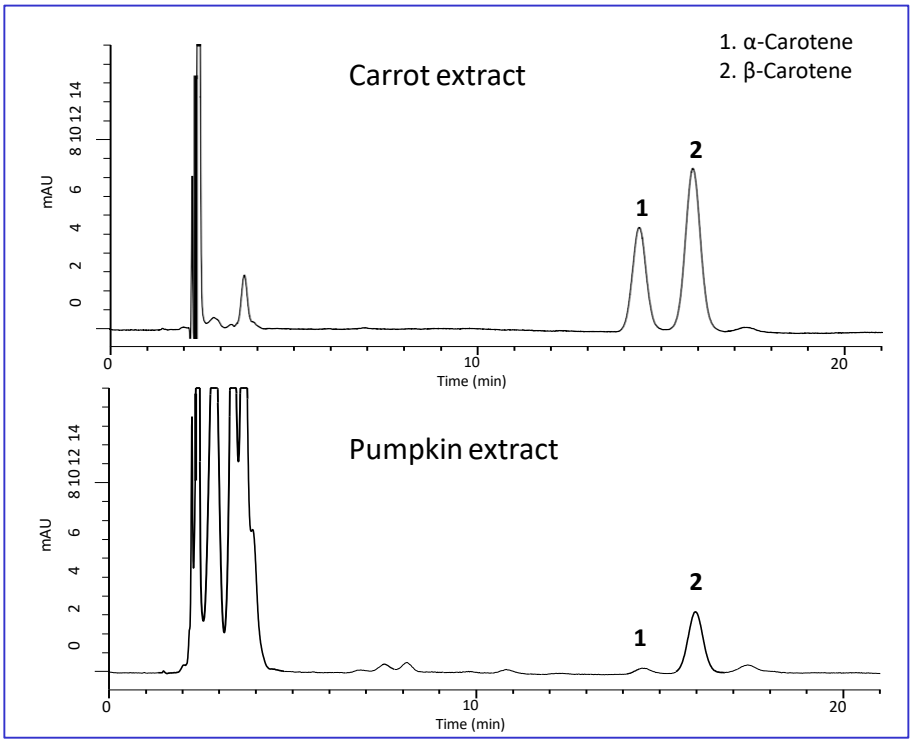
*B

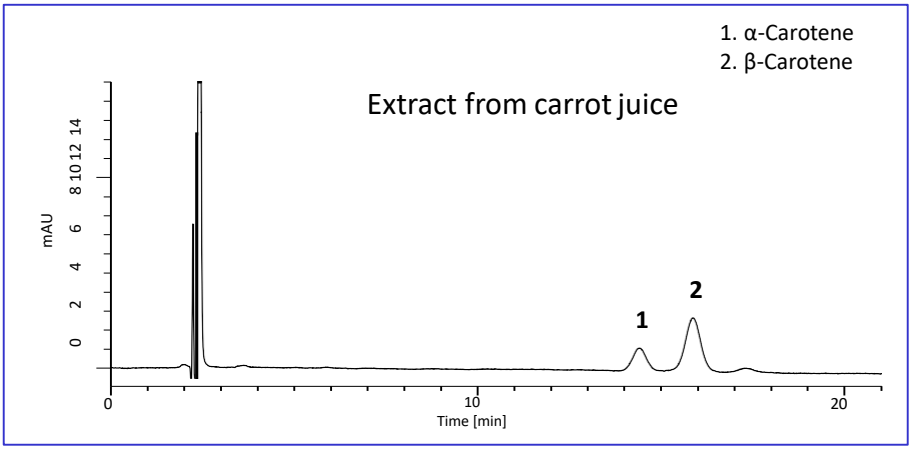
Centrifuge
- Supernatant
- Residue
  - Repeat procedure *B twice
- 1 % NaCl aqueous solution 23 mL
- 2-Propanol 6 mL
- Hexane-ethyl acetate = 9/1 (v/v) 15 mL
- Shaking for 5 min

Liquid-liquid extraction
- Organic layer
- Aqueous layer
  - Repeat procedure *A twice

Concentration
- Evaporate under reduced pressure
- Dissolve in 10 mL of ethanol

HPLC





GL Sciences, Inc. Japan
22-1 Nishishinjuku 6-Chome
Shinjuku-ku, Tokyo,
163-1130, Japan
Phone: +81-3-5323-6620
Fax: +81-3-5323-6621
Email: world@gls.co.jp
Web: www.glsciences.com

GL Sciences B.V.
De Sleutel 9
5652 AS Eindhoven
The Netherlands
Phone: +31 (0)40 254 95 31
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (ShangHai) Ltd.
Tower B, Room 2003,
Far East International Plaza,
NO,317 Xianxia Road,
Changning District.
Shanghai, China P.C. 200032
Phone: +86 (0)21-6278-2272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn

GL Sciences, Inc. USA
4733 Torrance Blvd. Suite 255
Torrance, CA 90503
Phone: 310-265-4424
Fax: 310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

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