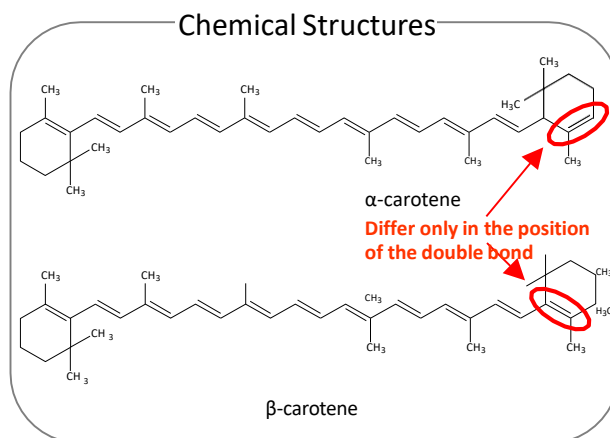


α -Carotene and β -carotene are a type of carotenoid and widely known as precursors to vitamin A in human body. They also have antioxidant activity.

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.

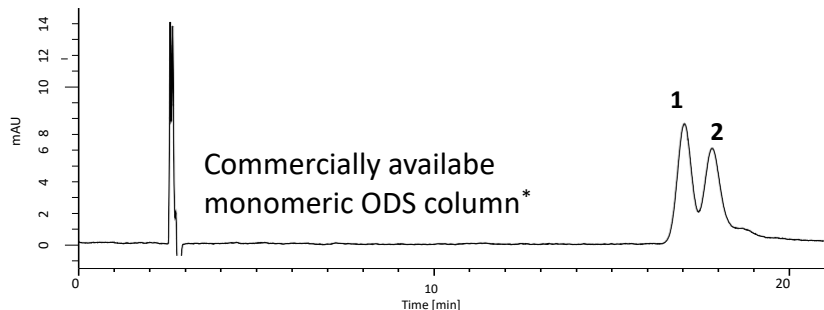
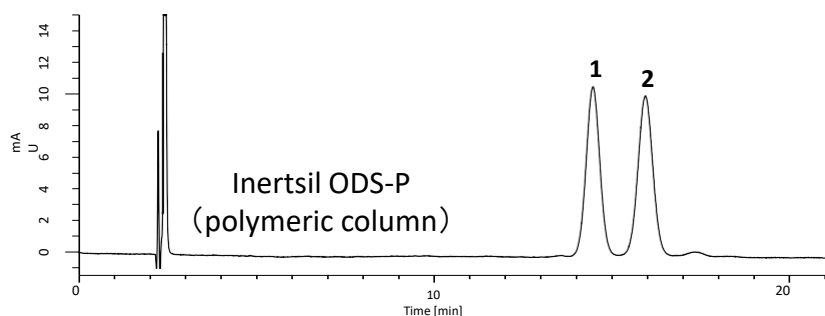
(K. Suzuki)



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.

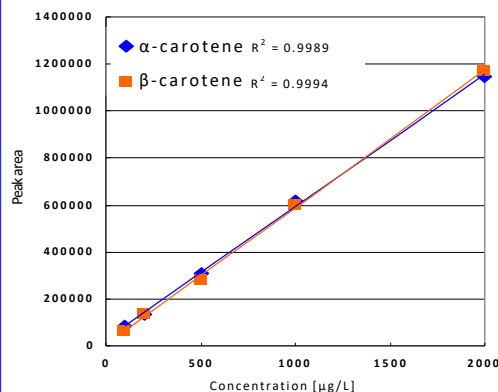
1. α -Carotene (2.0 mg/L)
2. β -Carotene (2.0 mg/L)



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

Conditions

- Column** : Inertsil ODS-P
(5 μ m, 250 x 4.6 mm I.D.)
Cat.No. 5020-02002
- Eluent** : A) CH₃OH
B) C₂H₅OH
A/B = 90/10, v/v
(gradient mixer)
- Flow rate** : 1.0 mL/min
- Col. Temp.** : 40 °C
- Detection** : PDA 455 nm
- Inj. Vol.** : 20 μ L



Calibration curves obtained with
Inertsil ODS-P column

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 °C 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

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

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

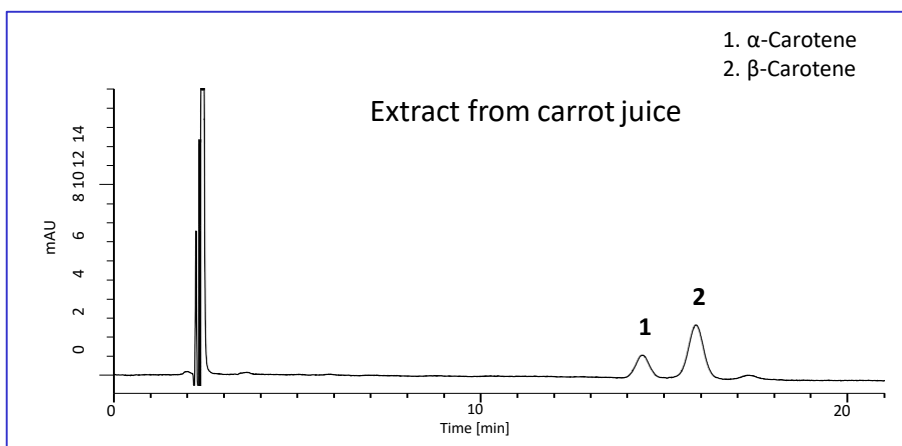
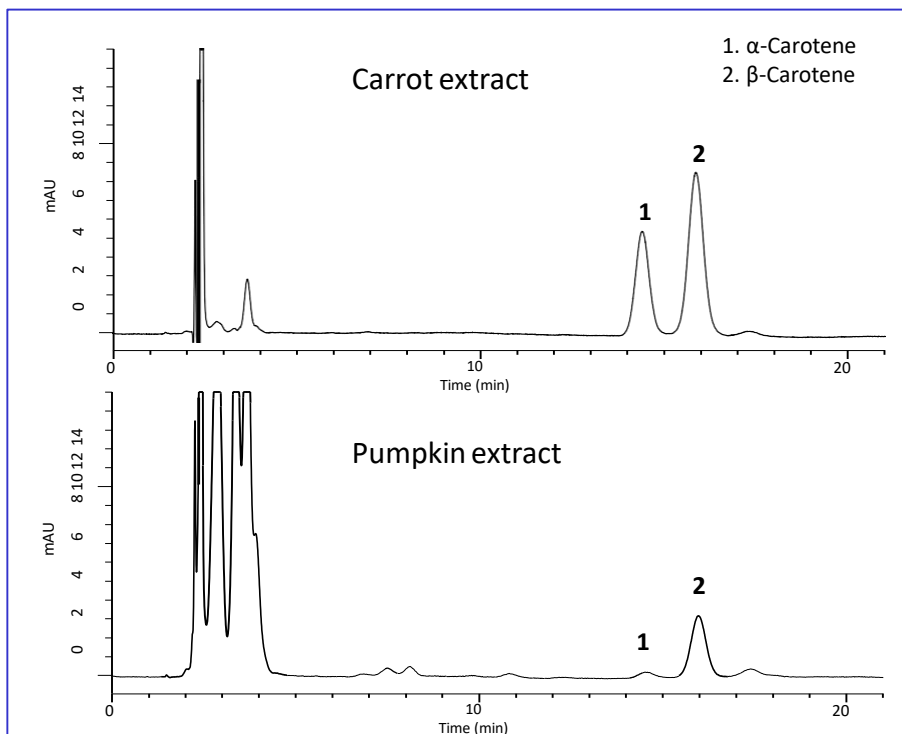
Liquid-liquid extraction

- Organic layer
- Aqueous layer
- Repeat procedure *A twice

Concentration

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

HPLC



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