Construction of Metabolic Profiling Method for Carotenoid Oxidation Products using Supercritical Fluid Chromatography Coupled with Tandem Mass Spectrometry

Atsuki Matsubara[1]*, Takato Uchikata[1], Masakazu Shinohara[2], Masaru Yoshida[2], Eiichiro Fukusaki[1], Takeshi Bamba[1]

1. Department of Biotechnology, Graduate School of Engineering, Osaka University, 2. The Integrated Center for Mass Spectrometry, Graduate School of Medicine, Kobe University

OSAKA UNIVERSITY

Outline

Purpose

- We tried to construct an analytical system for carotenoids and their oxidation products which were biomarker candidates using SFC/MS/MS

Result

- Structure of minor carotenoids could be characterized with high sensitivity using product ion scan
- Screening of carotenoids and their epoxides could be achieved in 20 min using SFC
- Some carotenoid epoxides were successfully detected in human serum

SFC for metabolite analysis

Features of SF chromatography (SFC)

- High speed
- High resolution

→ High-throughput analysis

- Possible to change the polarity of the mobile phase considerably by the addition of a modifier.

→ Wide separation modes

- Useful for analysis of hydrophobic compounds.

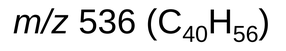
SFC is expected to be a suitable analytical tool for metabolites

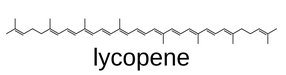
TO apply SF technologies to biometrics analysis…

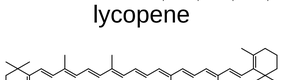
We are constructing analysis system for various metabolites using SFC coupling to mass spectrometry

Carotenoids

m/z 536 ($C_{40}H_{56}$)

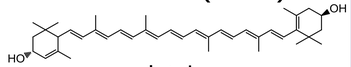






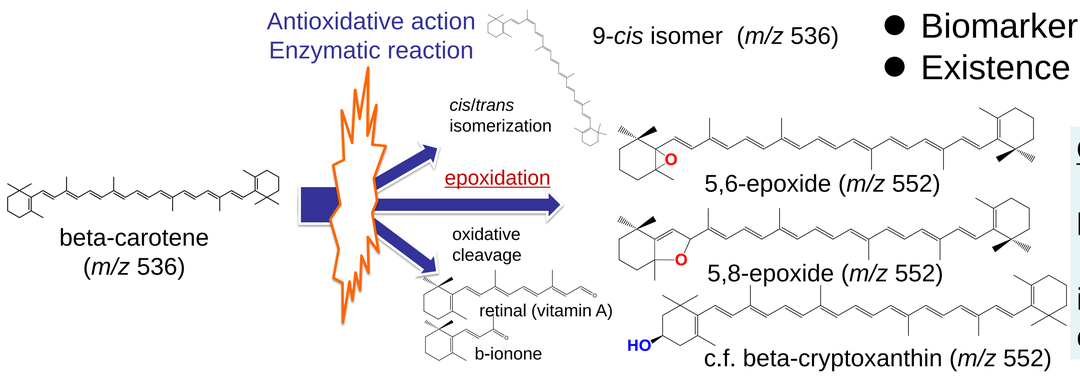
m/z 552 (+O)

m/z 568 (+2O)



- C40 isoprenoid
- Provitamin A
- Fat-soluble anti-oxidants
- Easily modified by environmental factors

Carotenoid oxidation products



- Biomarker candidates
- Existence of many isomers

Objectives

・Application of SFC for better separation

・Acquisition of structure information of minor compounds by MS/MS

Methods

SFC conditions

Analytical SFC Method Station, Waters

Mobile phase : Carbon Dioxide

Modifier : MeOH
with 0.1% (w/v) $HCOONH_4$
10-30% (20min)

Oven temperature : 35°C

Back pressure : 10 MPa

Column: Hibar Purosphere RP18e
(4.6 x 250 mm, 5 μm)

MS conditions (SRM)

Xevo TQ, Waters

Ionization method :
Electrospray ionization (ESI)

Polality : Positive

Capillary Voltage: 2.5 kV

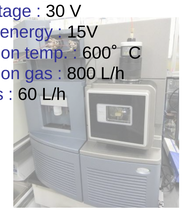
Cone voltage : 30 V

Collision energy : 15V

Desolvation temp. : 600° C

Desolvation gas : 800 L/h

Cone gas : 60 L/h



MS conditions (Product ion scan)

QTRAP5500, AB SCIEX

Ionization method :
Electrospray ionization (ESI)

Polality : Positive

Curtain gas : 40 psi

Collision gas : 5

Ion spray voltage : 5500 V

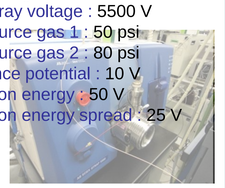
Ion source gas 1 : 50 psi

Ion source gas 2 : 80 psi

Entrance potential : 10 V

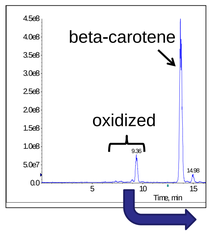
Collision energy : 50 V

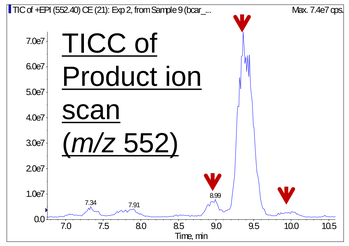
Collision energy spread : 25 V



Results

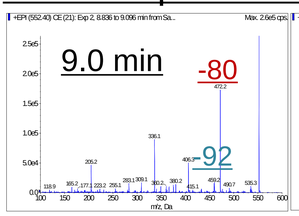
Analysis of oxidized beta-carotene standard

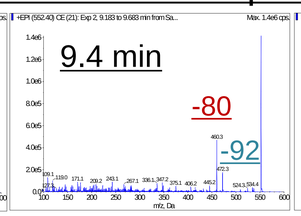


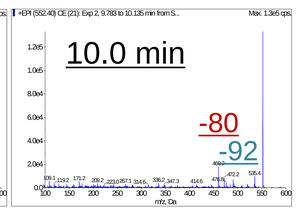


Method for oxidation: reaction with *m*-chloroperoxybenzoic acid for 2 hours at room temp.

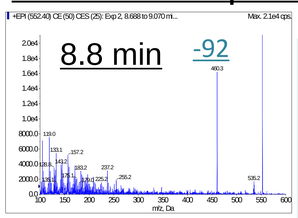
MS/MS spectra of the arrowed peaks







c.f. MS/MS spectrum of beta-cryptoxanthin



Structure of minor carotenoids could be characterized using product ion scan

NL92

→ common NL among carotenoids

NL80

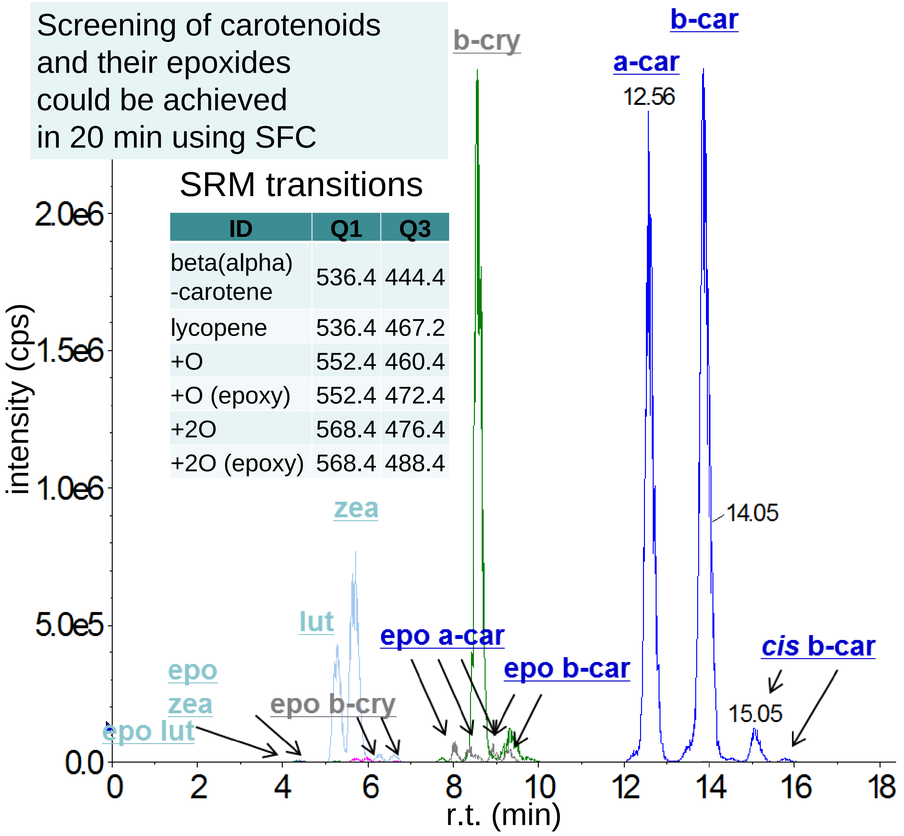
→ specific NL for carotenoid epoxides

Chromatogram obtained from standard oxidation product mixture

Screening of carotenoids and their epoxides could be achieved in 20 min using SFC

SRM transitions

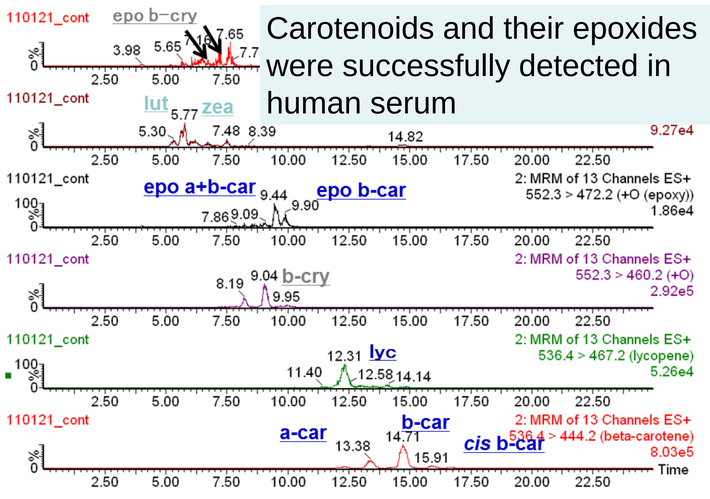
ID	Q1	Q3
beta(alpha) -carotene	536.4	444.4
lycopene	536.4	467.2
+O	552.4	460.4
+O (epoxy)	552.4	472.4
+2O	568.4	476.4
+2O (epoxy)	568.4	488.4



Limit of detection for each carotenoid

Carotenoid	Detection limit (fmol)
beta-carotene	0.17
alpha-carotene	0.17
beta-cryptoxanthin	0.16
lutein	0.16
zeaxanthin	1.6

Analysis of human serum



Carotenoids and their epoxides were successfully detected in human serum