# Analysis of capsaicin by using HPLC and LC/MS/MS

Capsaicin is a pungent component contained in hot pepper, etc. Proper amount of capsaicin encourages salivation or perspiration, but if it is taken excessively, it may damage the mucosal membrane of stomach. This time, capsaicin and dihydrocapsaicin contained in two kinds of hot pepper were measured. Diode array detector and fluorescence detector of Primaide System were used for analysis and it was confirmed that fluorescence detector could analyze with higher sensitivity, which is presented as below. In addition, qualitative analysis was implemented using LC/MS/MS.

(R.Takahashi)

Analysis example of capsaicin standard sample

UV (280 nm)

FL (Ex 280 nm Em 320 nm)

**FL detector enables
the analysis in high sensitivity!**
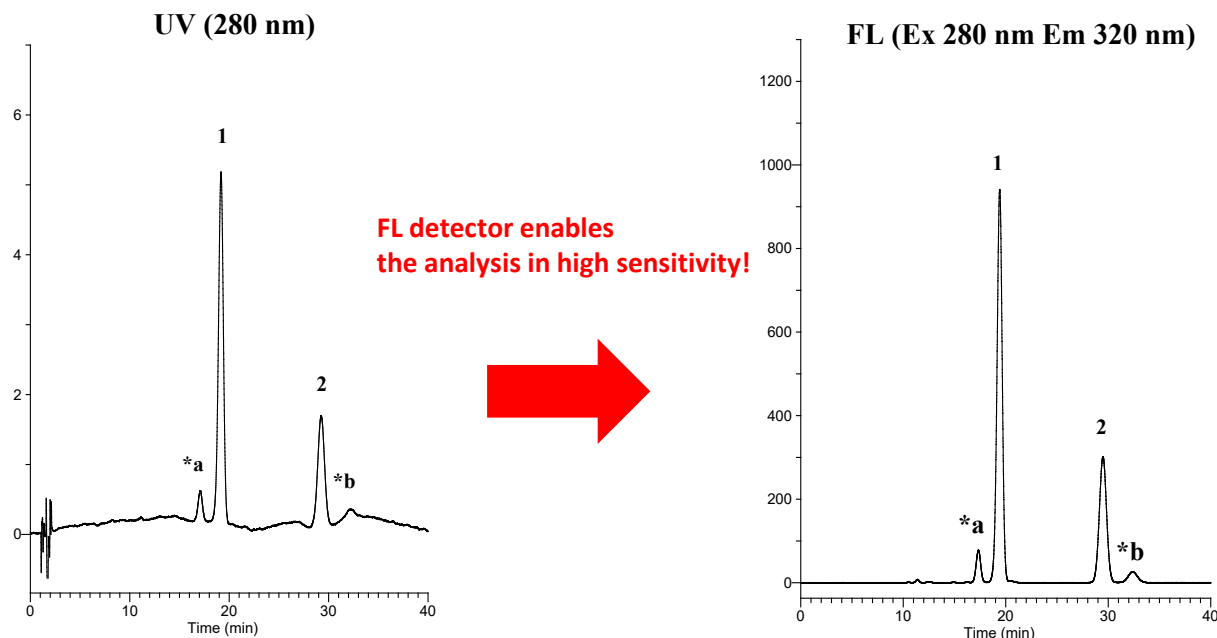
HPLC Conditions

Column	: InertSustain C18 (5 µm, 150 × 4.6 mm I.D.)
Eluent	: A) CH$_3$CN B) 0.1 % H$_3$PO$_4$ in H$_2$O
Flow rate	: 1.0 mL/min
Column temperature	: 40 $^\circ$C : UV 280 nm (DAD)
Detector	FL Ex 280 nm Em 320 nm
Injection volume	: 20 µL
Gradient	

Time (min)	A (vol%)	B (vol%)
0.0	40	60
40.0	40	60
45.0	100	0
55.0	100	0
56.0	40	60
65.0	40	60

Sample:

1. Capsaicin   18 mg/L
2. Dihydrocapsaicin   8 mg/L
*a. Nordihydrocapsaicin
*b. Homocapsaicin
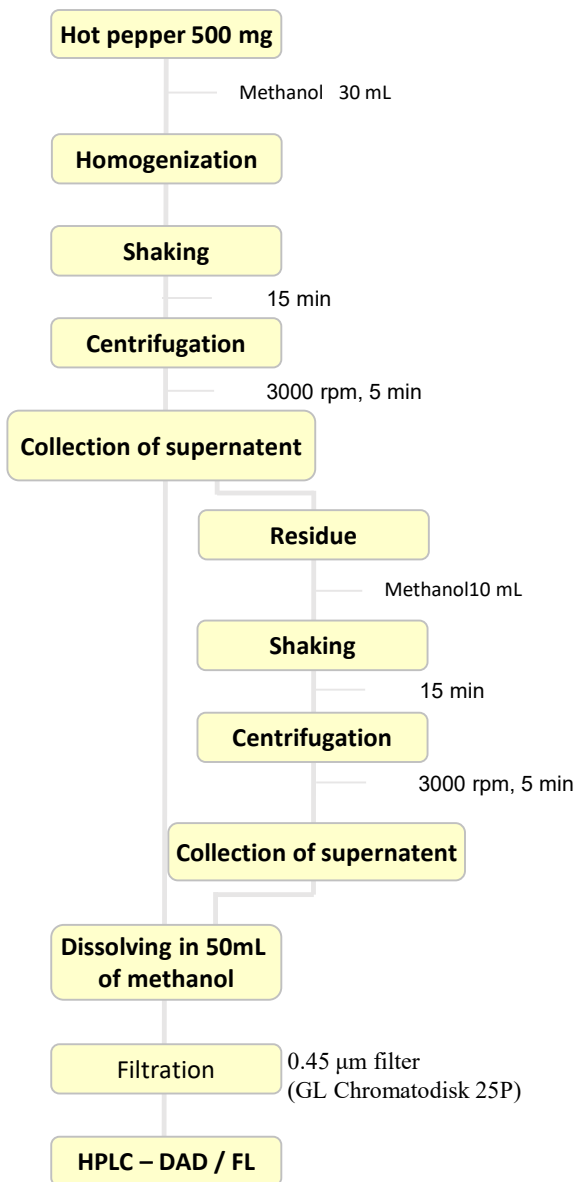
*a and *b were estimated by the result of measurement using LC/MS/MS and also the references.
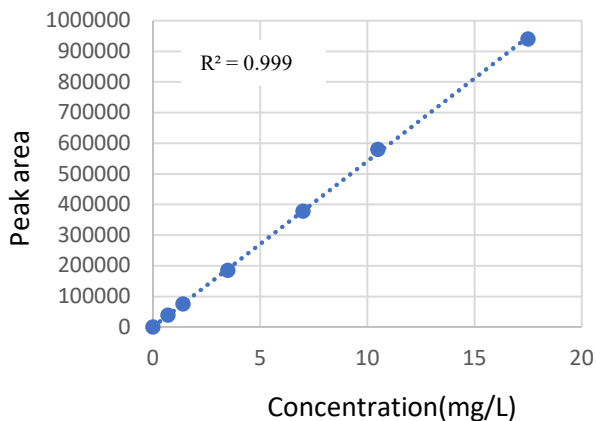
Structural formula

Capsaicin

Nordihydrocapsaicin

Dihydrocapsaicin
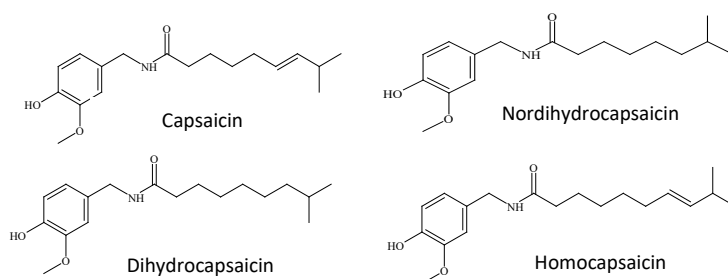
Homocapsaicin

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

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Example of sample preparation

Hot pepper 500 mg

— Methanol   30 mL

Homogenization

Shaking

— 15 min

Centrifugation

— 3000 rpm, 5 min

Collection of supernatent

Residue

— Methanol 10 mL

Shaking

— 15 min

Centrifugation

— 3000 rpm, 5 min

Collection of supernatent

Dissolving in 50mL of methanol

Filtration — 0.45 μm filter (GL Chromatodisk 25P)

HPLC – DAD / FL

Calibration curve

$R^2 = 0.999$

Capsaicin calibration curve
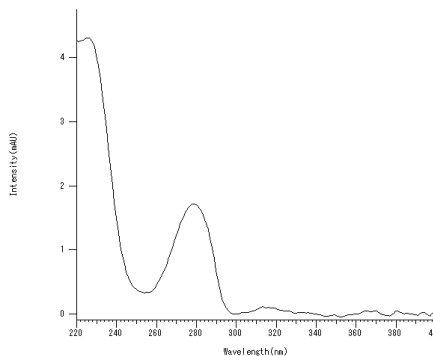(FL Ex 280 nm Em 320 nm)

$R^2 = 0.999$
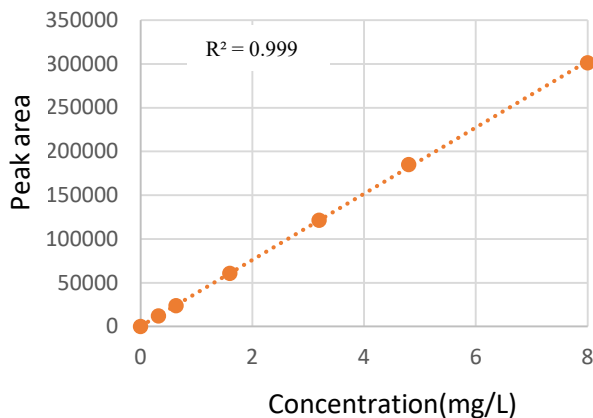
Dihydrocapsaicin calibration curve
(FL Ex 280 nm Em 320 nm)

UV spectrum of standard capsaicin

Capsaicin
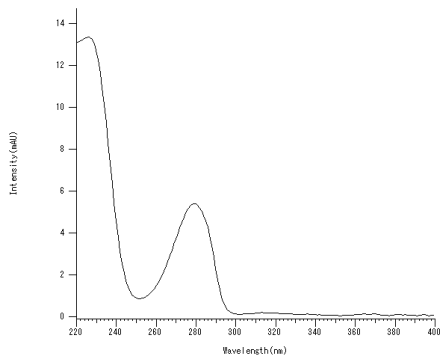
Dihydrocapsaicin

## Example of Hot Pepper Analysis

20 µL of sample solution after pretreatment was analyzed by HPLC. Same as the standard sample, by using fluorescence detector, the actual sample also can be analyzed with high sensitivity.
For the analysis of the variety containing small amount of capsaicin, the analysis by fluorescence detector is especially effective.

Sample:
1. Capsaicin
2. Dihydrocapsaicin
*a. Nordihydrocapsaicin
*b. Homocapsaicin

*a and *b were estimated by the result of measurement using LC/MS/MS and also the references.

Red Pepper Analysis

FL (Ex 280 nm Em 320 nm)
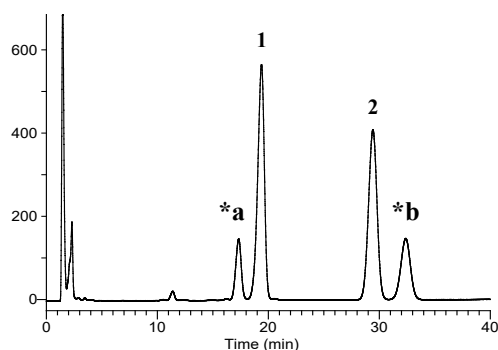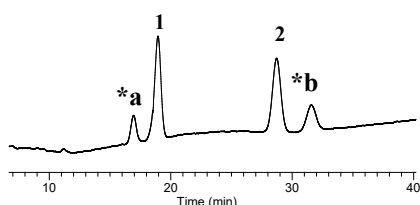
UV (280nm)

Green Pepper Analysis

FL (Ex 280 nm Em 320 nm)

UV (280nm)

2. *a. *b. Not detected
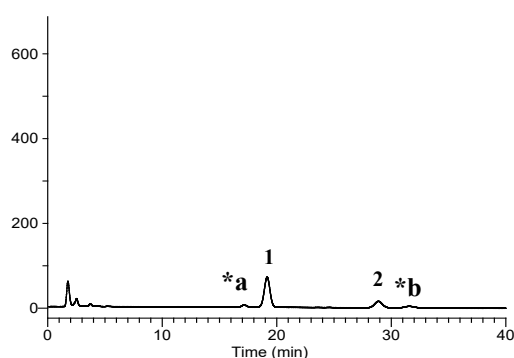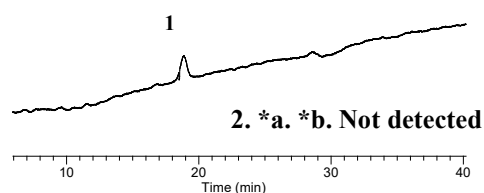
Products used

● Column
  InertSustain C18 (5 µm, 150 × 4.6 mm I.D.)
 Cat.No. 5020-07345

● Vial related products
  • Screw vial, 1.5mL,100 ea.
    Cat.No.1030-51022
  • Screw cap with septum
    , red / Silicon, white, thickness 1.0mm
    Cat.No.1030-51222

● Syringe filter
  • GL Chromatodisk 25P 0.45 µm
    Cat.No. 5040-28542
  • Plastic disposal syringe
    20 mL lure lock type, 100 ea.
    Cat. No. 1030-55120

How to select columns
This time for the analysis by HPLC, InertSustain C18 is used as a first choice column, but for the next LC/MS/MS high-speed analysis, InertSustain AQ-C18 having strong retention of hydrophilic compound is used, which may reduce the effect of ion suppression.

Red Pepper Analysis by Using LC/MS/MS

Using a common structure m/z 137 among capsaicin kinds as a product ion, precursor ion scan was implemented.
As a result of analysis, multiple compounds were detected.
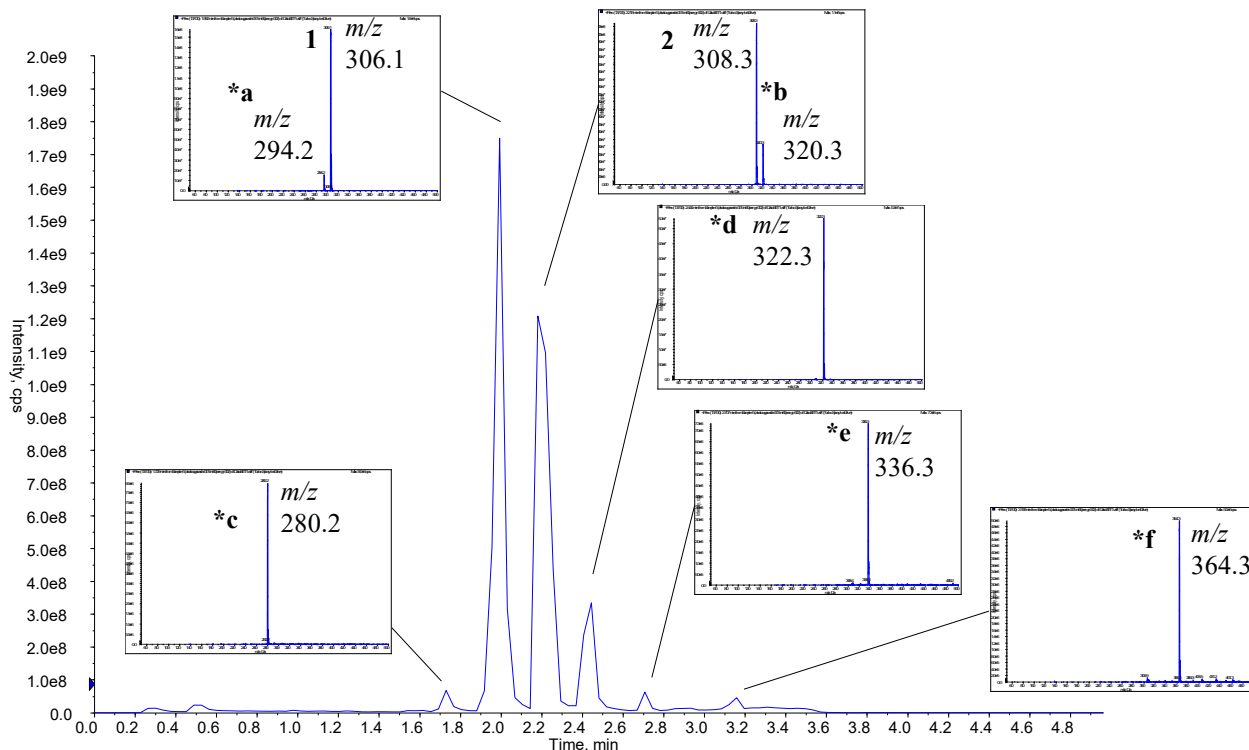
Conditions

Instrument	:	ExionLC HPLC system
		Q TRAP 6500+
Column	:	InertSustain AQ-C18 (1.9 μm, 50 x 2.1 mm I.D.)
Column Cat. No.	:	5020-89938
Eluent	:	A) CH_3CN
		B) 2 mM CH_3COONH_4 in H_2O

Time (min)	A (vol%)	B (vol%)
0.0	60	40
3.0	95	5
3.1	60	40
5.0	60	40

Flow rate	:	0.5 mL/min
Column Temp.	:	40 °C
Detector	:	LC/MS/MS (QTRAP 6500+ : ESI, Positive, Precursor ion scan m/z 50- 500 of 137)

CUR	CAD	IS	TEM	GS1	GS2	DP	EP	CE	CXP
20	10	3500	500	50	50	20	10	42	23

Injection Vol.	:	2 μL

Sample:

	M.W.	Precursorion m/z
1. Capsaicin	305.4	306.1
2. Dihydrocapsaicin	307.4	308.3
*a. Nordihydrocapsaicin	293.4	294.2
*b. Homocapsaicin	319.4	320.3
*c. Vanillyloctamide	279.4	280.2
*d. Homodihydrocapsaicin	321.5	322.3
*e. *f. unknown		
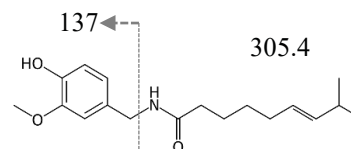
*a - *f were estimated by the result of measurement using LC/MS/MS and also the references.

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