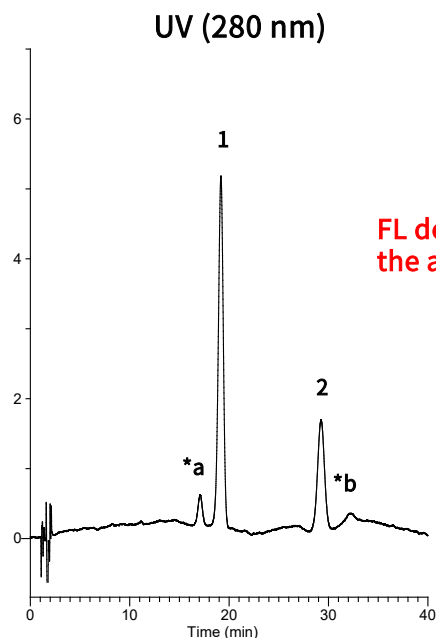
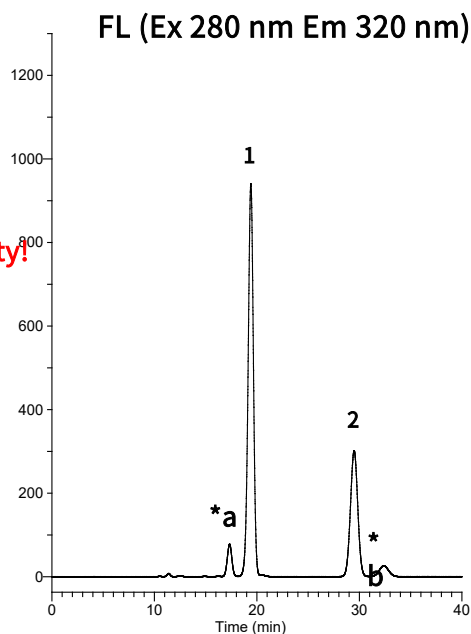


Capsaicin is a pungent component contained in hot pepper, etc. Proper amount of capsaicin encourages salivation or perspiration, but if it is taken excessively, it may damage the mucosal membrane of stomach. This time, capsaicin and dihydrocapsaicin contained in two kinds of hot pepper were measured. 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.

Analysis example of capsaicin standard sample



FL detector enables
the analysis in high sensitivity!



HPLC Conditions

Column : InertSustain C18
(5 μ m, 150 \times 4.6 mm I.D.)
Eluent : A) CH₃CN
B) 0.1 % H₃PO₄ in H₂O
Flow rate : 1.0 mL/min
Column temperature : 40 $^{\circ}$ C
Detector : UV 280 nm (DAD)
FL Ex 280 nm Em 320 nm

Injection volume : 20 μ L
Gradient

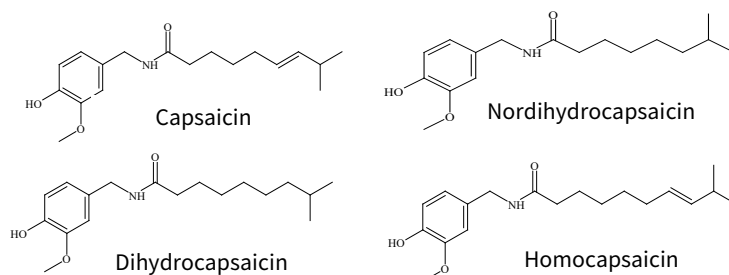
Time (min)	A (vol%)	B (vol%)
0.0	40	60
40.0	40	60
45.0	100	0
55.0	100	0
56.0	40	60
65.0	40	60

Sample:

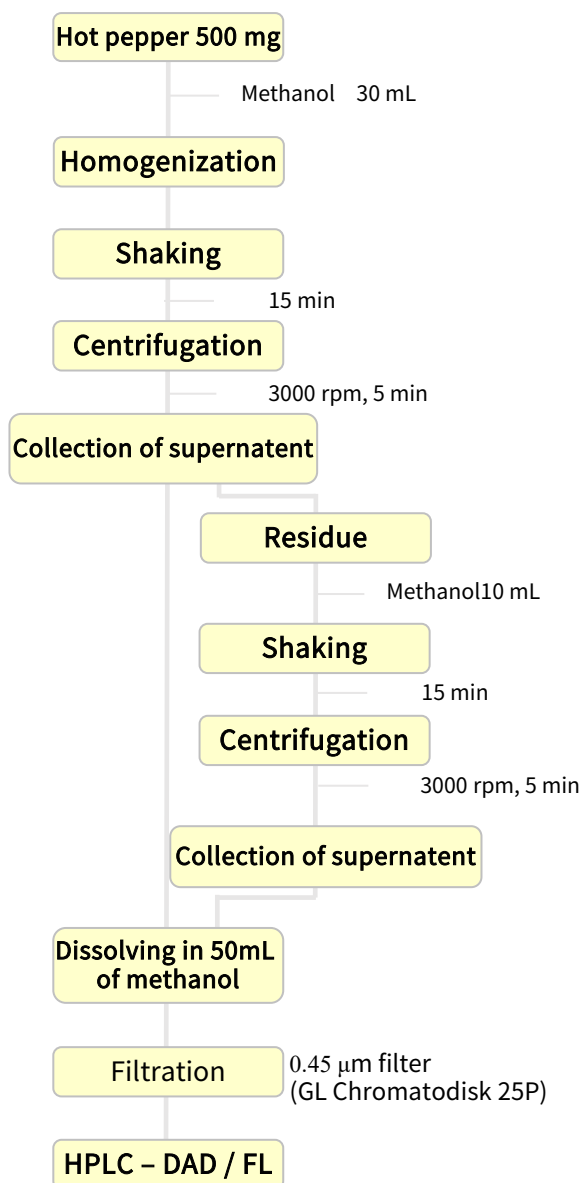
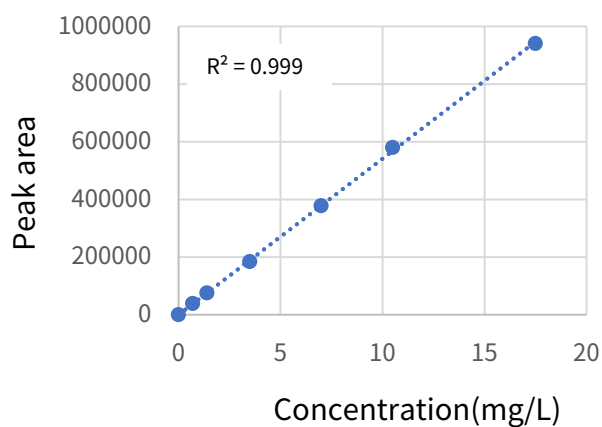
1. Capsaicin 18 mg/L
2. Dihydrocapsaicin 8 mg/L
*a. Nordihydrocapsaicin
*b. Homocapsaicin

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

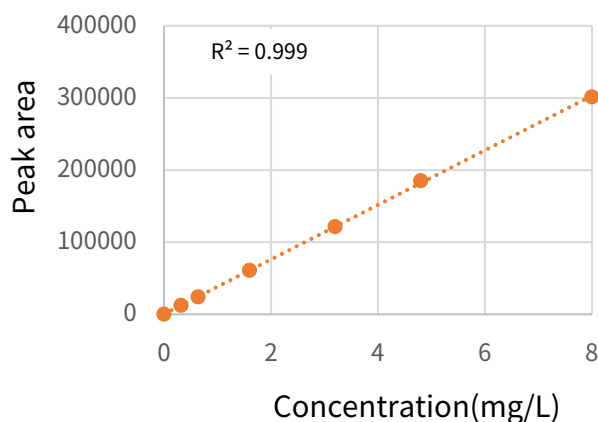
Structural formula



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

Example of sample preparationCalibration curve

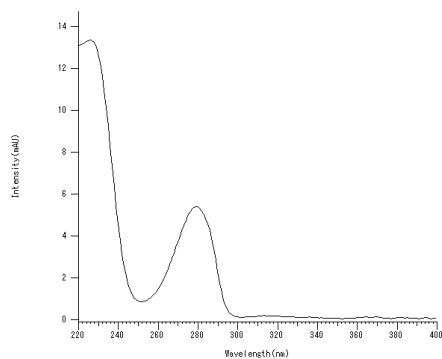
Capsaicin calibration curve
(FL Ex 280 nm Em 320 nm)



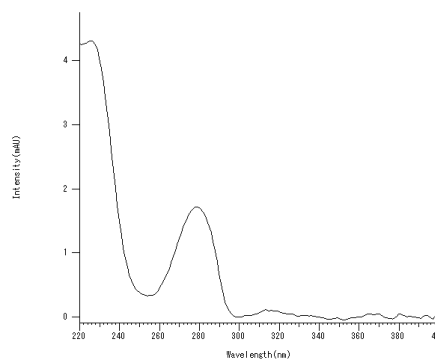
Dihydrocapsaicin calibration curve
(FL Ex 280 nm Em 320 nm)

UV spectrum of standard capsaicin

Capsaicin



Dihydrocapsaicin



Example of Hot Pepper Analysis

20 μ L 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.

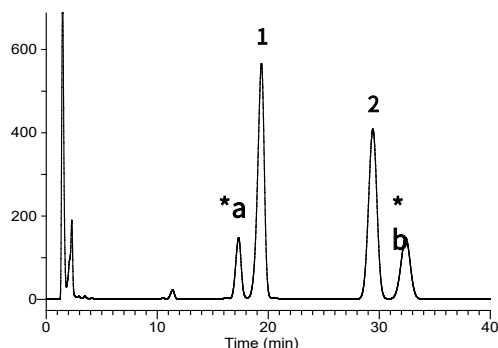
Sample:

capsaicin
dihydrocapsaicin
Nordihydrocapsaicin
Homocapsaicin

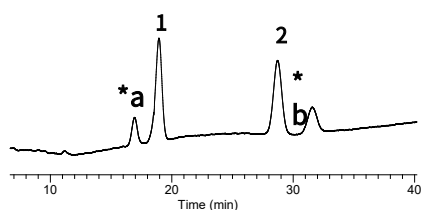
Peak 1 and 2 were estimated by the result of measurement using LC/MS/MS and also the references.

Red Pepper Analysis

FL (Ex 280 nm Em 320 nm)

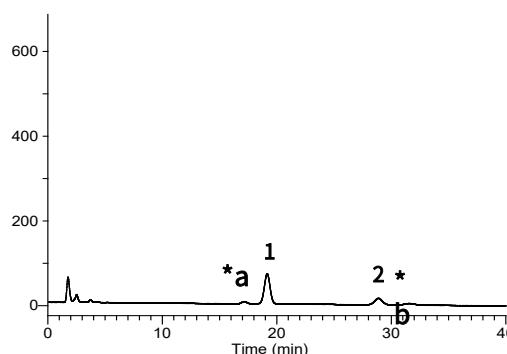


UV (280nm)

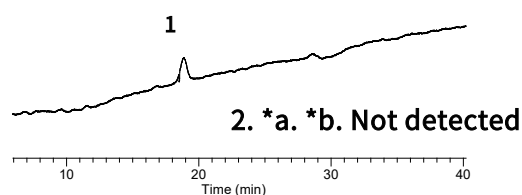


Green Pepper Analysis

FL (Ex 280 nm Em 320 nm)



UV (280nm)



Products used

●Column

InertSustain C18 (5 μ m, 150 \times 4.6 mm I.D.)
Cat.No. 5020-07345

●Syringe filter

- GL Chromatodisk 25P 0.45 μ m
Cat.No. 5040-28542
- Plastic disposal syringe
20 mL lure lock type, 100 ea.
Cat. No. 1030-55120

●Vial related products

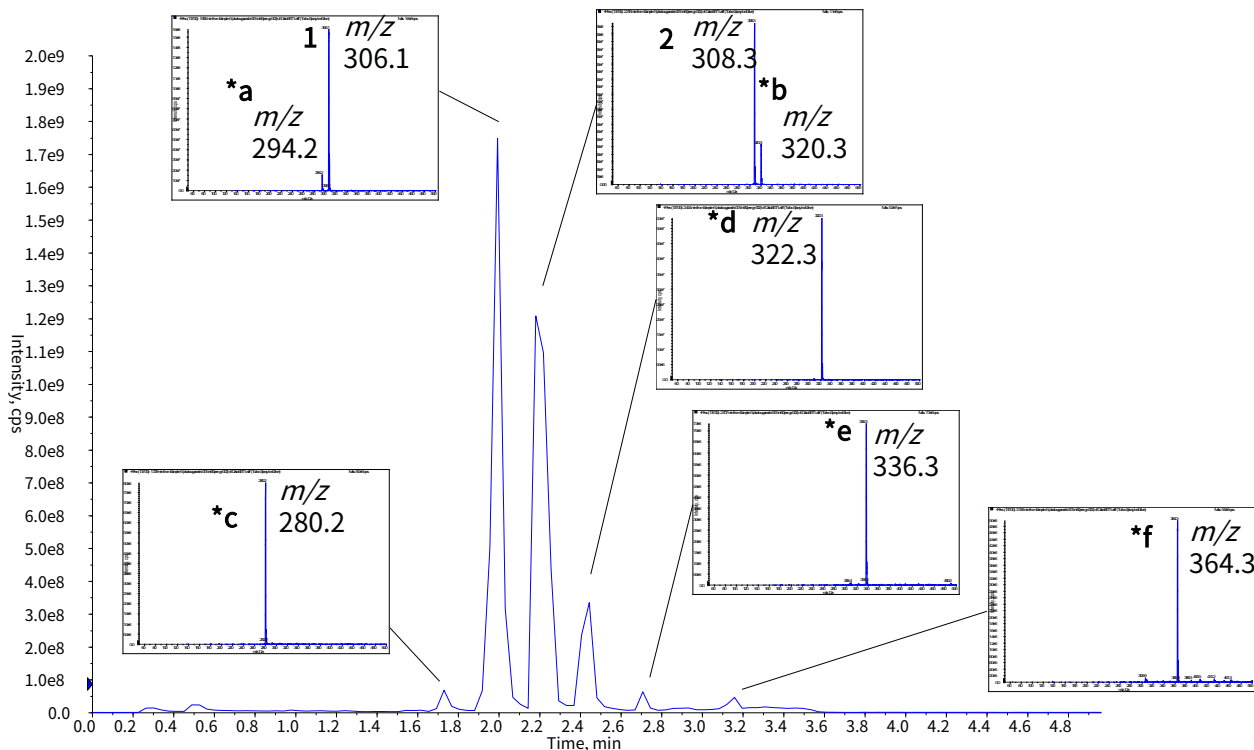
- Screw vial, 1.5mL, 100 ea.
Cat.No.1030-51022
- Screw cap with septum
, red / Silicon, white, thickness 1.0mm
Cat.No.1030-51222

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 μ m, 50 x 2.1 mm I.D.)

Column Cat. No.

: 5020-89938

Eluent

: A) CH_3CN
B) 2 mM $\text{CH}_3\text{COONH}_4$ 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 $^{\circ}\text{C}$

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

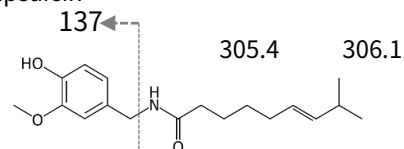
Injection Vol.

: 2 μL

Sample:

1. Capsaicin

M.W. Precursorion
 m/z



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.

GL Sciences disclaims any and all responsibility for any injury or damage which may be caused by this data directly or indirectly. We reserve the right to amend this information or data at any time and without any prior announcement.

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@glsc.co.jp
Web: www.glsciences.com

GL Sciences Inc. USA

4733 Torrance Blvd. Suite 255
Torrance, CA 90503
USA

Phone: +1-310-265-4424
Fax: +1-310-265-4425
Email: info@glsciencesinc.com
Web: www.glsciencesinc.com

GL Sciences B.V.

Dillenburgstraat 7C
5652AM, Eindhoven
The Netherlands

Phone: +31-40-254-9531
Email: info@glsciences.eu
Web: www.glsciences.eu

GL Sciences (Shanghai) Limited

Tower B, Room 2003
Far East International Plaza
No.317 Xianxia Road, Changning District
Shanghai, China 200051

Phone: +86-21-62782272
Email: contact@glsciences.com.cn
Web: www.glsciences.com.cn



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