ANALYSIS OF NINE CAPSAICINOIDS IN CAPSICUM PRODUCTS BY UPLC

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INTRODUCTION

- Capsaicinoids are the naturally occurring, pungency-producing components found in capsicum products such as red pepper, chili pepper, and oleoresins. They also exhibit a variety of biological properties that may affect human health.
- The common capsaicinoids are capsaicin (CAP), dihydrocapsaicin (DHC), nordihydrocapsaicin (NDC), nonivamide (NON), homocapsaicin (HC) and homodihydrocapsaicin (HDC) (Figure 1).
- The current standards for the analysis of capsaicinoids in capsicums and their extractives are the AOAC 995.03 and the ASTA Method 21.3. In these methods, NON co-elutes with CAP.
- **The scope** of this work is to develop a suitable UHPLC method that can separate NON and CAP as well as other capsaicinoids with a reasonable run time for the routine analysis of capsaicinoids in pepper and pepper-containing foods.

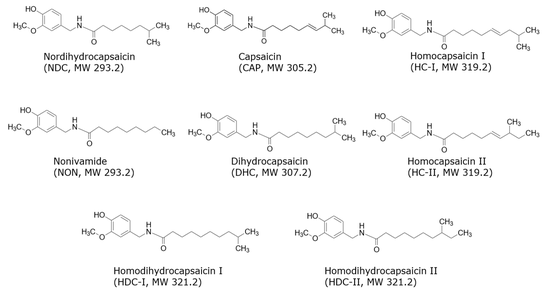


Figure 1. *Chemical structures and molecular weight (MW) of capsaicinoids.*

EXPERIMENTAL

LC conditions

LC System:	ACQUITY™ H-Class PLUS System (QSM) with ACQUITY Fluorescence Detector
MS system:	QDa™ Mass Detector (Performance)
Software:	Empower™ 3 CDS
Run time:	27.0 min
Column:	CORTECS™ T3 Column (1.6 µm, 2.1 × 150 mm)
Temp:	45 °C
Mobile phases:	A: water-acetonitrile mix (water/ACN 80/20 v/v, with 0.1% Formic acid).
	B: acetonitrile (with 0.1% formic acid)
Injection volume:	1.0 µL
Gradient program:	(see table)

Time (mi)	Flow Rate (mL/miin)	MP A (%)	MP B (%)	Curve
0.0	0.40	85.0	15.0	6
8.0	0.40	85.0	15.0	6
15.0	0.40	75.0	25.0	6
20.0	0.40	50.0	50.0	6
20.5	0.40	0.0	100.0	6
23.5	0.40	0.0	100.0	6
23.6	0.40	85.0	15.0	6
27.0	0.40	85.0	15.0	6

QDa settings:

Polarity:	ESI+	Probe Temp.:	600 °C
Capillary Voltage:	1.5 kV	Cone Voltage:	10 V
SIR channels:	m/z 294 (NDC and NON); m/z 306 (CAP); m/z 308 (DHC); m/z 320 (HC); m/z 322 (HDC)		
Scan mode:	m/z 60—650		
Sampling rate:	10 points/sec.		

Sample Preparation

Samples were prepared according to the ASTA Method 21.3 with minor modifications. Specifically, weigh accurately about 20 g wet samples, such as sauces, or 5 g dry samples, such as powders or flakes, into a 200 mL volumetric flask. Pipet 150 mL reagent alcohol into the flask and add a magnetic stirrer to aid boiling. Reflux vigorously with fast stirring for 5 hours. Allow to cool. Rinse the condenser with reagent alcohol into the flask. Dilute to volume with reagent alcohol. Filter 3-4 mL through a 0.2 µm nylon syringe filter into glass vials.

Quantification

The contents were determined using NON as the external standard. The resulting concentrations were converted to the individual capsaicinoid concentrations using their molecular weights.

RESULTS

1) Method Robustness

Three variables, the flow rate, the MP composition, and the column temperature, were investigated for their effects on the Rs (NON and CAP) using a partial factorial-factor design. Table 1 shows the range of the variables, the safe operating space, and the relevant instrument performance specifications. Figure 2 shows the 2D contour plots of the Rs against these variables. Table 2 shows the effects of column batch-to-batch variation on the separation of NON and CAP.

Table 1*. Variables and their ranges in the method robustness evaluation using a partial fractional-factor design. The safe operating region and the corresponding instrument performance limits are also shown.*

Variables	Units	Range	Safe operating space*	Instrument specs
Flow rate	ml/min	0.4 ± 0.05	0.4 ± 0.04	Flow rate error < 1.0%
% water in mobile phase	%	68 ± 3	68 ± 2	Composition error < 0.5%
Column Temp.	°C	45.0 ± 2.0	45.0 ± 2.0	Temp. error < 0.5

*: The operating region where Rs (for NON and CAP) of 1.5 or above can be obtained.

Figure 2. *2D contour plots of Rs against flow rate and initial mobile phase composition at various column temperatures. Boxes show the acceptable regions in Table 2. The shaded area represents Rs of 1.5 or less, and the unshaded area represents Rs >1.5.*

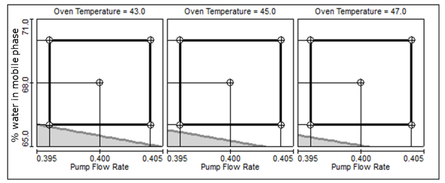


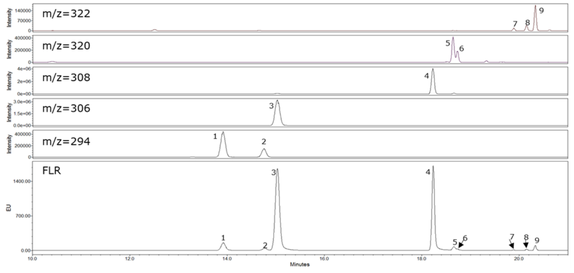
Table 2. *Effects of column batch-to-batch variation on the Separation of NON and CAP.*

Column batch #	RT (min)[a]		Plate count[a]		Rs[a]
	NON	CAP	NON	CAP	
0122	15.421 (0.05%)	15.682 (0.05%)	131000 (0.02%)	140000 (0.35%)	1.558 (0.09%)
0123	15.237 (0.01%)	15.501 (0.01%)	132000 (0.45%)	141000 (0.22%)	1.596 (0.07%)
0119	15.046 (0.06%)	15.301 (0.05%)	134000 (0.28%)	141000 (0.45%)	1.564 (0.03%)

Note: a: Mean and RSD (in parenthesis) are listed, n ≥3.

2) Peak ID

Figure 3. *Overlay of chromatograms of capsaicinoids in a natural capsaicin extract determined by fluorescence and MS detectors. Peaks: 1, NDC; 2, NON; 3, CAP; 4, DHC; 5, HC-I; 6, HC-II; 7, HDC isomer; 8, HDC-I; 9, HDC-II.*



3) Method Performance

Calibration

Table 3. *Calibration results and LOQ values for NON and CAP in UHPLC-FLR analysis.*

Compound	Retention time* (min)	Conc. range (mg/L)	Fitting mode	R²†	LOQ‡ (mg/L)
NON	14.63 ± 0.02	0 - 150	Linear through zero	0.9999	0.01
CAP	14.87 ± 0.03	0 - 150	Linear through zero	0.9999	0.01

*: Mean ± SD, n = 10. †: 7 concentration levels. ‡: Estimated at s/n=10.

Accuracy and precision

Table 4. *Results obtained from the spiking experiment and the analysis of a natural capsaicin for method accuracy.*

Spiking experiment results

Sample Matrix	Compound	Original level (mg/g)	Spiked level (mg/g)	Determined level after spiking (mg/g)	Total recovery (%)
Red pepper seasoning	CAP	1.823	0.863	2.656‡	98.9%
Hot sauce	CAP	0.028	0.018	0.046‡	99.6%

Reference material measurement results

Reference material	Compound	CoA value (mg/g)	Conc. determined (mg/g)	Accuracy (%)
Capsaicin (natural)	CAP	603	585 (0.88%)‡	97.0%
	NON	N/A	19 (0.86%)‡	N/A
	CAP+NON	603	604	100.2%

†: mean value of two measurements. The relative differences from mean are <2%.
‡: The mean and RSD (in parentheses) are shown, n=6.

4) Analysis of capsicum samples

Table 5. *Analysis of capsaicinoids in commercial food products*

Sample #	Matrix	Total SHU[a]	NDC (µg/g)	NON (µg/g)	CAP (µg/g)	DHC (µg/g)	HC[b] (µg/g)	HDC[c] (µg/g)	Total (µg/g)
1	Red Pepper Seasoning	51506	173.7	50.0	1884.6	1204.8	86.1	77.0	3476
2	Red Pepper	60747	152.9	56.1	2354.9	1320.6	125.0	85.5	4095
3	Dried Chili Sauce	2637	16.7	3.0	81.7	71.7	4.5	6.6	184
4	Chipotle Pepper	12847	63.9	14.7	381.0	376.4	32.8	45.5	914
5	Green Chili Pepper	24172	161.0	26.3	833.7	568.3	75.4	63.8	1729
6	Paprika Flakes	503	2.2	0.6	16.2	13.6	0.8	2.4	36
7	Chili Flakes	1840	8.8	1.8	56.5	52.3	4.2	6.3	130
8	Hot Sauce A	756	2.9	0.9	28.9	16.1	1.4	1.8	52
9	Hot Sauce B	225	0.5	0.2	8.5	5.2	0.4	0.5	15

Note: a: The sum of SHU from NDC, NON, CAP, and DHC; b: The sum of HC isomers (HC-I and HC-II); c: The sum of HDC isomers (3 peaks).

CONCLUSION

- **A UHPLC-FLR method that simultaneously separate and quantify nine capsaicinoids in foods within a 27-minute run time has been successfully developed.**
- **For the first time, the critical pair of capsaicin and nonivamide were adequately separated (Rs ≥1.5) within a 27 min total run time.**
- **This UHPLC-FLR analytical method can be conveniently implemented in the routine analysis labs where the existing standard methods are being used.**
- **This method could be applied to a wide range of analyses, such as the authentication of high commercial value capsicum products and the capsaicinoid related biological and medical activities studies.**

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