HITACHI
Inspire the Next

Rapid Analysis of Capsaicinoids in Capsicum Oleoresin Using the Hitachi LaChromUltra® Liquid Chromatography System

Hitachi High Technologies America, Inc.

Capsaicinoids are a group of compounds produced by the seeds of members of the pepper plant family (Capsicum species). They have an irritating effect on mammalian mucous membranes, causing the burning sensation experienced when eating peppers. Medicinally, capsicum oleoresin, the extract of capsaicinoids from peppers, is used in topical creams and ointments to alleviate discomfort caused by arthritis, psoriasis, and other chronic pain conditions due to the release of endorphins that these creams cause[1].

Pharmaceutical companies that produce these topical medications test for the capsaicinoid content in their formulations (typically between 0.025-0.075%) using liquid chromatography. A major challenge in the measurement of the amount of capsaicinoids is the long run time (typically 65 minutes) of the analysis and the poor resolution between the individual capsaicinoids[2]. This application note illustrates how the Hitachi LaChromUltra® ultra high-pressure liquid chromatography system, in conjunction with a smaller particle-size stationary phase column, can overcome these limitations and bring the run time down to 13 minutes. Capsaicin (CAP) and dihydrocapsaicin (DHC) are the major capsaicinoids, representing over 80% of the total capsaicinoids.

Experimental Conditions

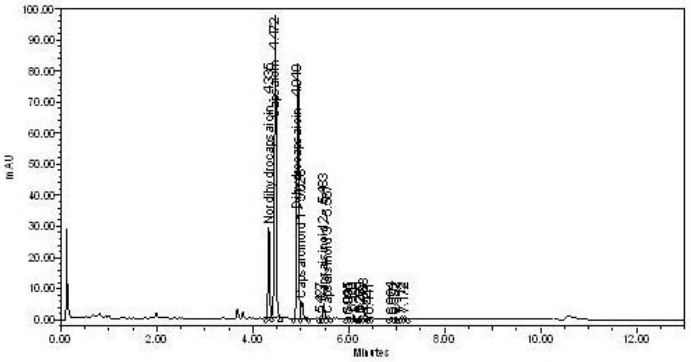
Module	Conditions and Other
Pump (L-2160U)	Mobile Phase A: 0.5% H_3PO_4 and B: ACN (See Gradient Profile Below), Run Time: 13 min.
Autosampler (L-2200U)	CAP Linearity Standards = 1.03, 0.75, 0.50, and 0.25 mg/mL, diluted 85/15 MeOH/water DHC Linearity Standards =1.07, 0.75, 0.50, and 0.25 mg/mL, diluted 85/15 MeOH/water Sample = Capsicum Oleoresin (ARL Lot #3742H), diluted as above, 10°C
Oven (L-2300)	40°C
Detector (L-2455U)	Photo Diode Array Detector Lambda Max: 280 nm
Column	Agilent® ZORBAX® Eclipse Plus C-18 1.8 µm 2.1x50mm

Time	%A	%B	FlowRate (mL/min)
0	90	10	1.0
5	50	50	1.0
7	10	90	1.0
10	10	90	1.0
10.1	90	10	1.0
13	90	10	1.0

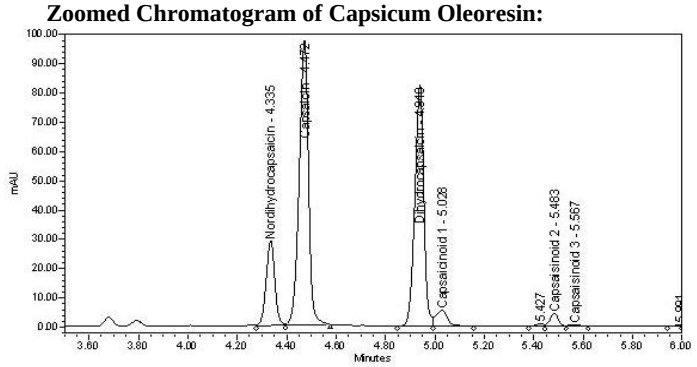
References:
1- Dray, A. "Mechanism of action of capsaicin-like molecules on sensory neurons". *Life Sci*. 51 (23): 1759–65, 1992.
2 – *Capsaicin Creams and Ointments Monograph: USP 32/NF 27*, United States Pharmacopoeia/National Formulary, Rockville, MD, 2009.

Results

Full Scale Chromatogram of Capsicum Oleoresin:



Zoomed Chromatogram of Capsicum Oleoresin:



Reproducibility of CAP, DHC, and Nordihydrocapsaicin NDHC (n=3):

Sample	%RSD CAP	%RSD DHC	%RSD NDHC
Capsicum Oleoresin	0.7	0.3	0.4

Discussion

Hitachi's LaChromUltra Liquid Chromatography System, equipped with a 1.8-µm particle size column, reduces the analysis time for capsaicinoids by up to 5 times over traditional methods. The LaChromUltra is designed to take advantage of the increased efficiency of smaller particle-sized columns, while giving the needed resolution for closely eluting compounds. Reproducibility (<0.7%RSD), linearity (r^2 > 0.996 for CAP and 0.997 for DHC), and system suitability (N > 40000, k' > 7.6, T > 1.0, and Rs > 1.5) are shown to be acceptable for all major capsaicinoids in the capsicum oleoresin. Capsaicinoids were confirmed by spectral analysis with the PDA.

Hitachi High Technologies America, Inc.
Life Sciences Division
5100 Franklin Drive
Pleasanton, CA 94588
Toll Free: (800) 548-9001
E-mail: sales-LS@hitachi-hta.com
Web site: www.hitachi-hta.com/ultra