

Rapid Analysis of Limonin Citrus Extract Using the Hitachi LaChromUltra® Liquid Chromatography System

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Citrus fruits are known to have a multitude of health benefits, including high vitamin content, high fiber content, and other benefits that are currently being explored. Limonin is a compound that is present at low levels in lime seeds (*Citrus angustifolia*). This compound is generally used as a flavor enhancer, but recently has been shown to inhibit induced colon cancer in rats¹. Other possible cancer-fighting properties could be attributed to limonin, and this compound is worth studying for these reasons. The compound itself is part of a family of similar compounds: citrolimonin, limonine, and liminoic acid Δ -lactone, which could act in conjunction to halt carcinogenesis² in certain cancer cell lines. Usual analysis time for limonin is 20 minutes³, but this application has cut the run time to 6.5 minutes.

The present method describes the low-level analysis of limonin by ultra-fast HPLC, using the Hitachi LaChromUltra ultra high-speed liquid chromatography system. The method shows the standard curve, system suitability, and reproducibility of the compound. Overall, this method could be useful in determining the levels of this possibly life extending cancer fighting compound in therapeutic remedies.

Experimental Conditions

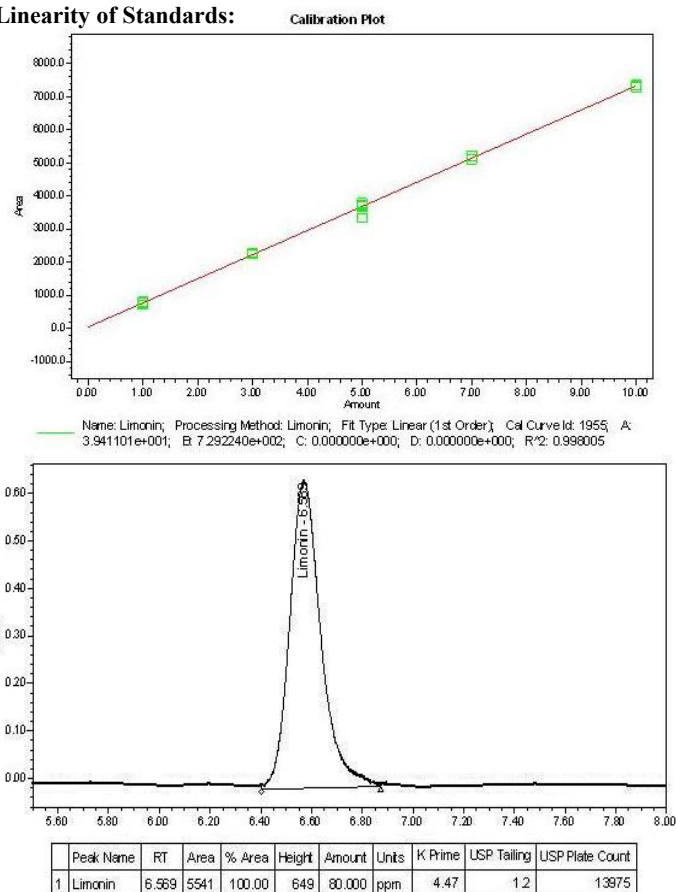
Module	Conditions and Other
Pump (L-2160U)	Isocratic; 60/40 water/acetonitrile Flow rate: 0.4 mL/min
Autosampler (L-2200U)	40, 50, 60, 80, and 85 μ L injection volume, of limonin diluted to 1 μ g/mL in acetonitrile, 10°C
Oven (L-2300U)	30°C
Detector (L-2455U)	Diode Array Detector Lambda Max: 217 nm
Column	Hitachi RP C-18 150mmX4.6mm 3 μ m column
Standards	Sigma-Aldrich® limonin from citrus seeds >99% purity P/N L9647-5MG

Results

80 ppm Standard %RSD, n=3

Peak Name: Limonin								
	Injection	Peak Name	RT	Area	% Area	Height	Amount	Units
	1	Limonin	6.569	5541	100.00	649	80.000	ppm
	2	Limonin	6.570	5373	100.00	648	80.000	ppm
	3	Limonin	6.567	5646	100.00	653	80.000	ppm
	Mean		6.569	5520.0		653.6	80.000	
	Std. Dev.		0.001	137.6		8.2	0.000	
	% RSD		0.02	2.5		1.3	0.00	

Linearity of Standards:



Discussion

Hitachi's LaChromUltra Liquid Chromatography System, equipped with a 3- μ m particle size Hitachi column, reduces the analysis time for limonin by up to 3 times. The LaChromUltra is designed to take full advantage of the increased efficiency associated with smaller particle sized columns, while giving the needed sensitivity for low-level detection. Reproducibility (<3%RSD), linearity ($r^2 > 0.998$), and system suitability ($N = 13975$, $k' = 4.47$, $T = 1.2$) are shown even at these low levels.

References:

- 1- Tanaka, T. Kohno, H. Tsukio, Y. Honjo, S. et al.; Citrus Liminoids Obacunone and Limonin Inhibit Azomethane-Induced Colon Carcinogenesis in Rats, *Biofactors* 13(1-4):213-218, 2000.
- 2 - Limonin Monograph: *U.S. National Library of Medicine*, National Institutes of Health Bethesda, MD, 2004.
- 3 - Widmer, W. Haun, C. *Chapter 5: Analysis of Limonin and Flavonoids of Citrus Extracts by Direct Injection*, American Chemical Society, 1998.

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