HITACHI
Inspire the Next

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

Hitachi High Technologies America, Inc.

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 rats[1]. 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 carcinogenesis[2] in certain cancer cell lines. Usual analysis time for limonin is 20 minutes[3], 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<br>Flow rate: 0.4 mL/min |
| Autosampler (L-2200U) | 40, 50, 60, 80, and 85 µL injection volume, of limonin diluted to 1µg/mL in acetonitrile, 10°C |
| Oven (L-2300U) | 30°C |
| Detector (L-2455U) | Diode Array Detector<br>Lambda Max: 217 nm |
| Column | Hitachi RP C-18 150mmX4.6mm 3 µm column |
| Standards | Sigma-Aldrich® limonin from citrus seeds<br>>99% purity P/N L9647-5MG |

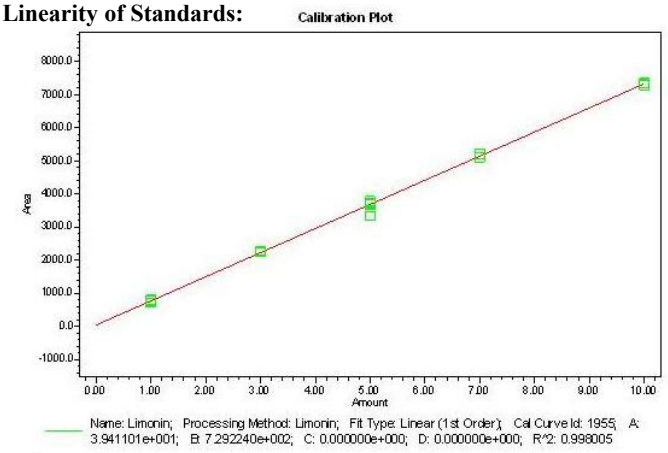
## Results

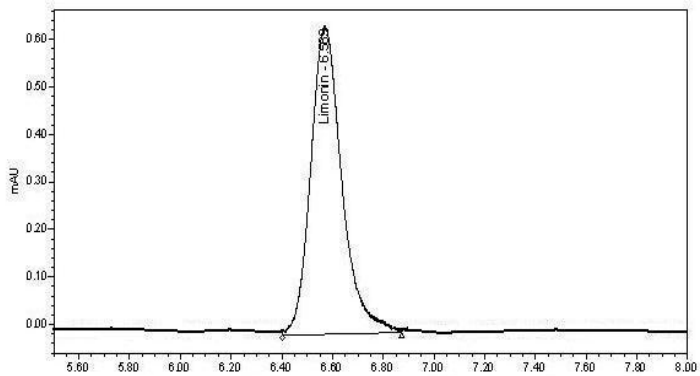
**80 ppm Standard %RSD, n=3**

**Peak Name: Limonin**

| | Injection | Peak Name | RT | Area | % Area | Height | Amount | Units |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | Limonin | 6.569 | 5541 | 100.00 | 649 | 80.000 | ppm |
| 2 | 2 | Limonin | 6.570 | 5373 | 100.00 | 648 | 80.000 | ppm |
| 3 | 3 | Limonin | 6.567 | 5646 | 100.00 | 663 | 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:**




| | Peak Name | RT | Area | % Area | Height | Amount | Units | K Prime | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Limonin | 6.569 | 5541 | 100.00 | 649 | 80.000 | ppm | 4.47 | 1.2 | 13975 |

## Discussion

Hitachi's LaChromUltra Liquid Chromatography System, equipped with a 3-µm 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 Carcinogenisis 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.

**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