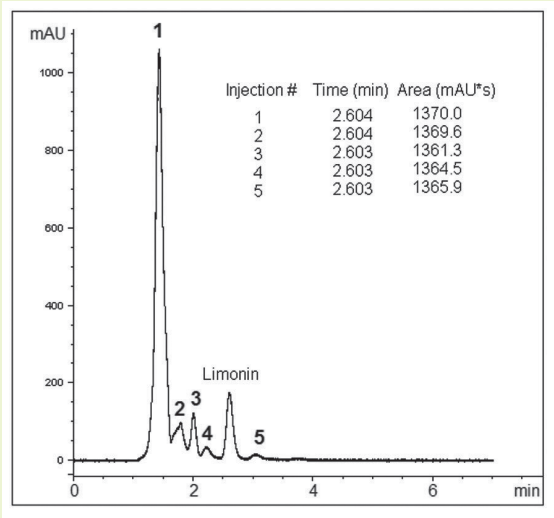
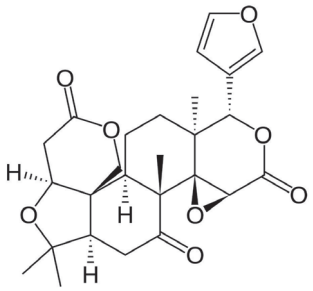
Limonin in Orange Juice

Separation from matrix peaks with reproducible results





Note: Limonin is a bitter compound which may negatively affect juice quality. The compound is found in the seeds and membrane tissue of the fruit. It is very important for groves to determine the level of limonin in juice so the correct recovery settings for the juice production can be set. The level of limonin can change dramatically from season to season. It also depends on the fruit size. The analysis of limonin is crucial in production of high quality non-bitter fruit juices.

Method Conditions

Column: **Cogent UDC Cholesterol™**, 4µm, 100Å

Catalog No.: 69069-7.5P

Dimensions: 4.6 x 75 mm

Solvents: 40% A: DI H_2O / 0.1% formic acid (v/v)
60% B: Acetonitrile / 0.1% formic acid (v/v)

Injection vol.: 1µL

Flow rate: 0.5mL/min

Detection: UV 220 nm (PerkinElmer HPLC)

Temperature: 25˚C

Sample: 500 ppm limonin standard in 20% DI H_2O / 0.1% formic acid / 40% acetonitrile / 40% methanol was prepared. Orange juice was filtered and injected as is (data not shown). Orange juice was spiked with 250 ppm limonin, filtered, and injected – chromatogram overlay shown in the figure.

Peaks: 1–5. Orange juice matrix Limonin

t_0: 0.9 min

Discussion

The Cogent UDC-Cholesterol column was used in the analysis of limonin in orange juice. The juice sample was spiked at the level of 250 ppm of limonin, filtered (MicroSolv Tech Corp. 0.45µm nylon), and injected. Several peaks from to the juice matrix were separated (peaks 1–5 in the figure), but still the limonin peak was well separated and easy to integrate. %RSD for peak areas was 0.271 and for retention times was 0.021 (5 consecutive injections, shown in figure). The developed method is fast and doesn't suffer from a lack of reproducibility of the data. It can be used to monitor limonin levels in the production of juice.

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