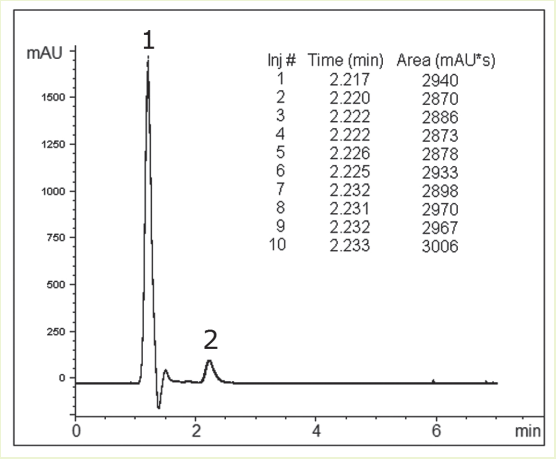
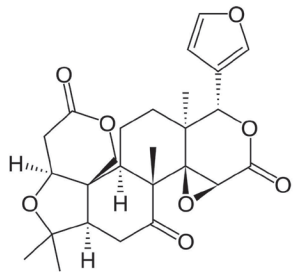
Limonin in Orange Juice

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 Bidentate C18™**, 4µm, 100Å

Catalog No.: 40018-75P

Dimensions: 4.6 x 75 mm

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

Injection vol.: 1 microL

Flow rate: 0.5mL/min

Detection: UV 220 nm (PerkinElmer HPLC)

Sample: 500 ppm of 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 of 10 injections shown in the figure.

Peaks: 1. Orange juice matrix
2. Limonin

t_0: 0.9 min

Discussion

When limonin determination is done by HPLC, the main drawback of the method is poor reproducibility of the results. The use of the Cogent Bidentate C18 column and a simple isocratic method helped to achieve repeatability of the results. %RSD for peak areas was 1.64 for 10 consecutive injections. Peak 1 (present in the chromatogram of filtered juice and spiked filtered juice) represents the matrix of this challenging sample. It is well resolved from the limonin peak, visible in the spiked juice sample. The developed method is fast, reproducible, and can be used to monitor limonin levels in the production of juice. The presented chromatogram shows 10 overlaid injections.

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