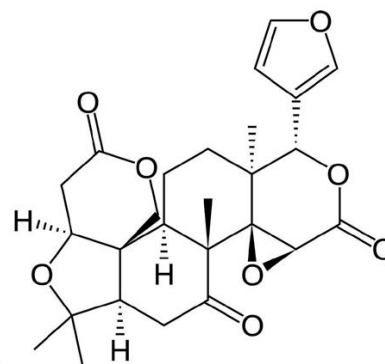# Limonin Repeatability in HPLC Analyses – AppNote

Separating Limonin from Matrix Peaks

When Limonin determination is done by HPLC, the main drawback of some Methods is poor Reproducibility of the results. Using the Method in this AppNote, a simple Isocratic Method, helped to achieve acceptable Repeatability of the results.

The %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 which is well resolved from the Limonin Peak, visible in the spiked juice sample. *The Chromatogram below shows 10 overlaid injections.*

Inj #	Time (min)	Area (mAU*s)
1	2.217	2940
2	2.220	2870
3	2.222	2886
4	2.222	2873
5	2.226	2878
6	2.225	2933
7	2.232	2898
8	2.231	2970
9	2.232	2967
10	2.233	3006

Peaks:

1. Orange juice matrix

2. Limonin

Method Conditions

Column: Cogent Bidentate C18™, 4µm, 100Å

Catalog No.: 40018-75P

Dimensions: 4.6 x 75mm

Mobile Phase:

   40% A: DI Water / 0.1% Formic Acid

   60% B: Acetonitrile / 0.1% Formic Acid

Injection vol.: 1 µL

Flow rate: 0.5mL / minute

Detection: UV @ 220nm

Sample Preparation: 500ppm of Limonin Standard in 20:40:40 DI Water with 0.1% Formic Acid / Acetonitrile / Methanol was prepared. Orange Juice was filtered and injected as is *(data not shown)*. Orange juice was spiked with 250ppm Limonin, filtered, and injected.

t₀: 0.9 minutes

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.

Attachment

No 280 Limonin Repeatability in HPLC Analyses pdf 0.2 Mb Download File