



Analysis of Bitter Compounds, in Orange Juice using HPLC

Angelika
Gratzfeld-Huesgen

Food

Abstract

The following compounds are examples of flavoring agents used in food products:

- lupulon and humulon (hop bittering compounds)
- vanillin
- naringenin and hesperidin (bittering compounds)

Three major classes of compounds are used as flavoring agents: essential oils, bitter compounds, and pungency compounds. Although the resolution afforded by gas chromatography (GC) for the separation of flavor compounds remains unsurpassed, HPLC is the method of choice if the compound to be analyzed is low volatile or thermally unstable.

Sample preparation for bitter compounds in orange juice¹

The samples were prepared according to Carrez 1 and 2. This method uses potassium ferrocyanide and zinc sulfate for protein precipitation.

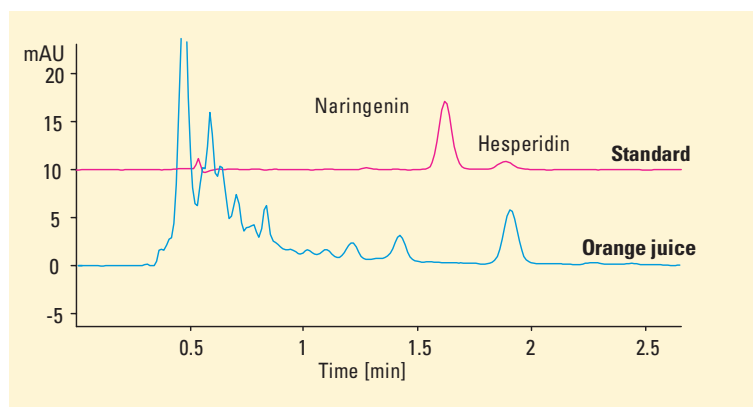


Figure 1
Analysis of bitter compounds in orange juices

Conditions

Column 125 ~ 4 mm Hypersil BDS, 5 µm

Mobile phase

A = water + 0.15ml/l H₂SO₄
(conc.), pH = 2.4

B = ACN

Gradient

start with 20% B; at 3 min 20% B
at 5 min 90% B; at 6 min 20% B

Flow rate 2 ml/min

Post time 1 min

Column compartment 40 °C

Injection vol 1 µl

Detector

UV-DAD detection wavelength 260/80 nm,
reference wavelength 380/80 nm

Sample preparation

The orange juice was prepared
according to Carrez 1 and 2



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Chromatographic conditions

The HPLC method presented here for the analysis of hesperidin and naringenin is based on reversed-phase chromatography. UV spectra were evaluated as an additional identification tool.

HPLC method performance

Limit of detection
1 ng (injected amount),
for DAD S/N = 2

Repeatability
of RT over 10 runs <0.2 %
of areas over 10 runs <1 %

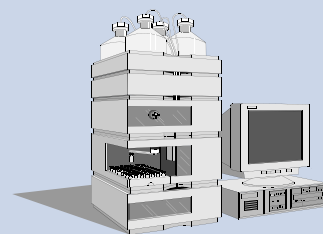
References

1.
Official Methods of Analysis; Horwitz, W., Ed.; 14th ed.; AOAC: Arlington, VA, **1984**; secs 12.018–12.021.

Equipment

Agilent 1100 Series

- vacuum degasser
- quaternary pump
- autosampler
- thermostatted column compartment
- diode array detector, Agilent ChemStation + software



Angelika Gratzfeld-Huesgen is application chemist at Agilent Technologies, Waldbronn, Germany.

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