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Application Note

► Determination of Naringine and Hesperidine in Fruit Juices

Category	Food
Matrix	Fruit juice
Method	HPLC
Keywords	Fruit juice, Flavonoids, Polyphenols, Naringine, Hesperidine
Analytes	Naringine, Hesperidine
ID	**VFD2**, December 2007

Summary

Naringine and Hesperidine in fruit juices have been analyzed be high-performance liquid chromatography at a detection wavelength of 285 nm.

Introduction

Citrus fruits contain a variety of Flavonoids, including flavorless Hesperidine (the principal Flavonoid in oranges and mandarines) and bitter Naringine (the principal Flavonoid in grapefruits). Hesperidine is found in high concentrations in the fruit peel of oranges and mandarines and in substantially lower concentrations in the juice of these fruits. Naringine is also found to be similarly distributed in oranges, although only in trace amounts [1-3]. If orange peels are pressed under high pressure, the Hesperidin portion increases, resulting in a decrease in the quality of the beverage. An increase in the portion of Hesperidin in orange juice (or of naringin in grapefruit juice) from peel extract ("pulp wash") can be thereby monitored. Even adulterations of orange juice with grapefruit juice can be detected through the concentrations of Naringine and Hesperidine. With a Smartline HPLC system from Knauer, a simple and fast analysis of Naringine and Hesperidine can be carried out on a Eurospher 100 C18 column. By using the Smartline UV Detector 2600, spectrum comparison for confidence in peak identification is possible throughout the entire run.

Experimental Sample Preparation

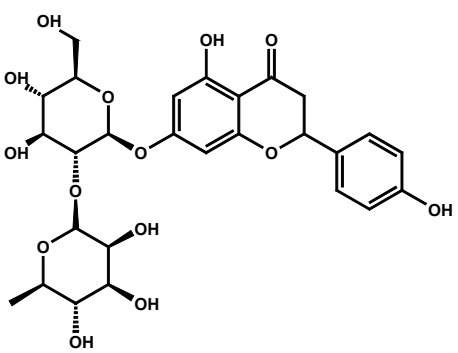
Protein precipitation was carried out on 50 ml of each fruit juice being examined with 1 ml each of Carrez I and II solutions. The samples were centrifuged and the supernatant was filtered through a 0.45 µm membrane. The injection volume for the standards and fruit juice samples was 5 µl. Since the stability of the sample solution is limited, sample measurement must take place within 24 h of sample preparation.

Experimental Preparation of Standard Solution

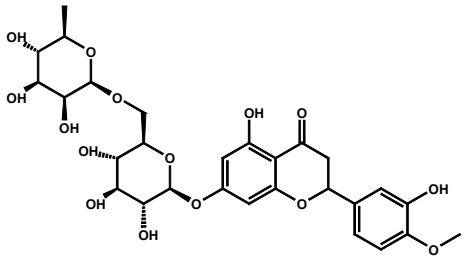
Calibration solutions for Naringine and Hesperidine were prepared in the concentration range 0.1 ppm to 600 ppm. In order to ensure the standards' solubility, the analytes were first dissolved in 5 ml dimethylformamide in a volumetric flask before being brought up to volume.

On the basis of the UV- spectra (Fig. 1) Naringine and Hesperidine were detected at 285 nm.

Chemical Structures



Naringine



Hesperidine

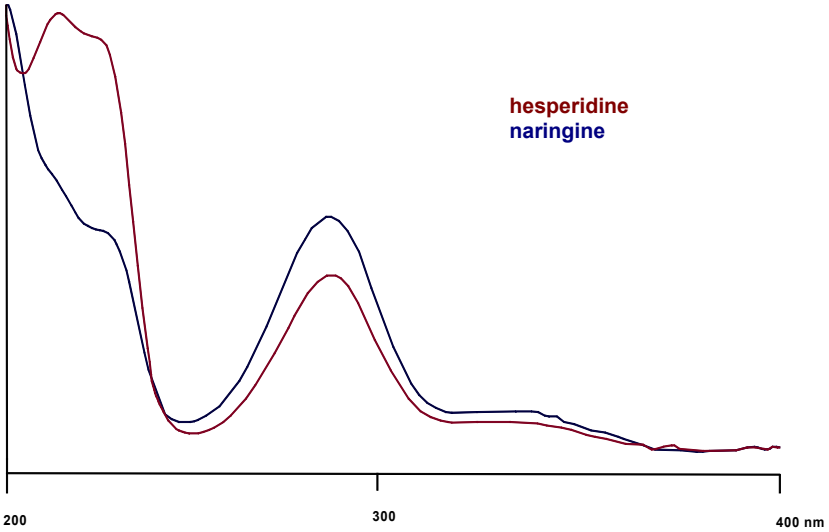


Fig. 1

UV spectra of Hesperidine (red) and Naringine (blue)

Method Parameters

Column	Eurospher 100-5 C18, 250 x 4 mm
Eluent	5 mM ammonium acetate / ACN 75 : 25 (v/v), adjusted to pH 4.45 with acetic acid
Flow rate	1 ml/min
Injection volume	5 µl
Column temperature	40 °C
System pressure	approx. 113 bar
Detection	UV at 285 nm

Results

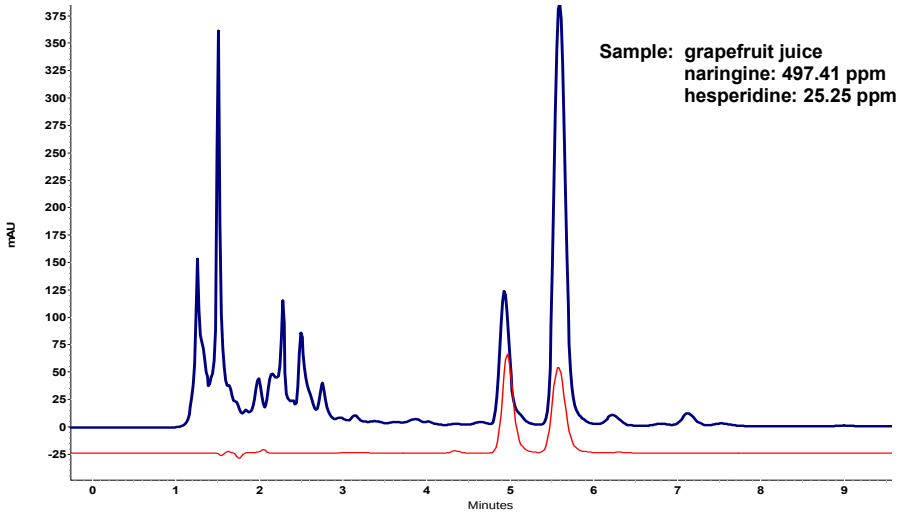


Fig. 2

Sample chromatogram of grapefruit juice (blue) overlaid with calibration standard (red)

Fruit juice	Naringine	Hesperidine
Sample 1: Multivitamin	2.82 ppm	31.66 ppm
Sample 2: Orange juice 1	0.85 ppm	11.44 ppm
Sample 3: Orange juice 2	28.03 ppm	79.25 ppm
Sample 4: Grapefruit juice	497.41 ppm	25.25 ppm

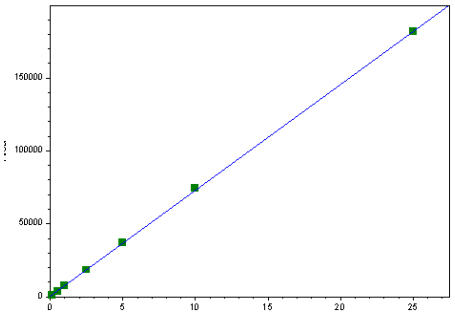


Fig. 3 (left)

Calibration curve of Hesperidine (r = 0.999883)

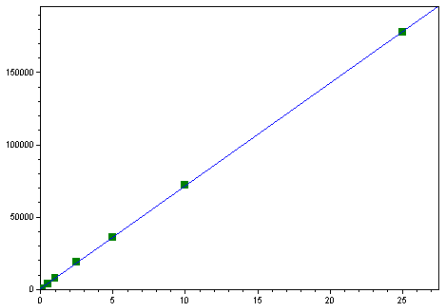


Fig. 4 (right)

Calibration curve of Naringine (r = 0.999950)

Method Performance

Limit of detection	0.1 ppm
Linearity (r^2)	Hesperidin: 0.999883 Naringine: 0.999950
Linearity range	0.1 – 600 ppm
Retention time precision*	
Peak area precision*	

*repeatability calculated over 5 replicate runs

Conclusion

For the analysis of Hesperidine and Naringine, one multivitamin juice, two orange juices and one grapefruit juice were investigated. A representative chromatogram is shown is figure 2. An additional verification of the calculated sample amount was always available by monitoring the spectra. The results indicate a marked decrease in quality when comparing orange juice 1 to orange juice 2. In order to measure the amount of Naringin in grapefruit juice, the calibration curve was extended up to 600 ppm. The results for the grapefruit juice and for orange juice 2 indicate that a clearly higher portion of the additional Flavonoids in the extract were achieved in the process of pressing the fruit.

References

[1] F.I. Kanaze, Ch. Gabrieli. E. Kokkalou, M. Georgarkis and I. Nniopas, J. Pharm. Biomed. Anal. , 33, 243 (2003)

[2] W.-J. Hsu, M. Berhow, G.H. Robertson, S. Hasegawa, Journal of Food Science 63 (1), 57–60 (1998)

[3] V. Carbone , P. Montoro , N. de Tommasi , C. Pizza, J Pharm Biomed Anal. 2004 Feb 4;34 (2):295-304

Physical Properties of recommended Column

Eurospher 100 C18 material can be universally used in different application areas, e.g. water soluble vitamins, steroids, catecholamines, sedatives.

Stationary phase	Eurospher 100-5 C18
USP code	L1
Pore size	100 Å
Pore volume	0,9 ml/g
Particle size	5 µm
Form	spherical
Surface area	350 m^2/g
% C	15
Endcapping	yes
Dimensions	250 x 4 mm
Order number	25DE181ESJ

Recommended Instrumentation

This application requires an isocratic HPLC equipped with degasser, autosampler, column oven, and multi-wavelength UV detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
Smartline Pump 1000, incl. 10 ml pump head	A50303
Smartline Manager 5000 with degasser	A5316
Autosampler 3900	A1508
Smartline Column Oven 4000	A5300
Smartline UV Detector 2600 PDA	A5200
10 mm Flow Cell	A4061
ChromGate Software	A1493
ChromGate PDA License for Detector 2600	A1459

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