

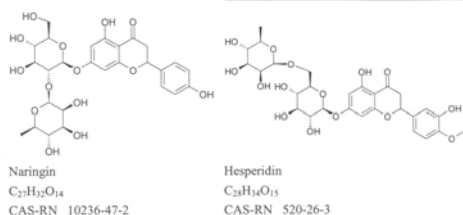
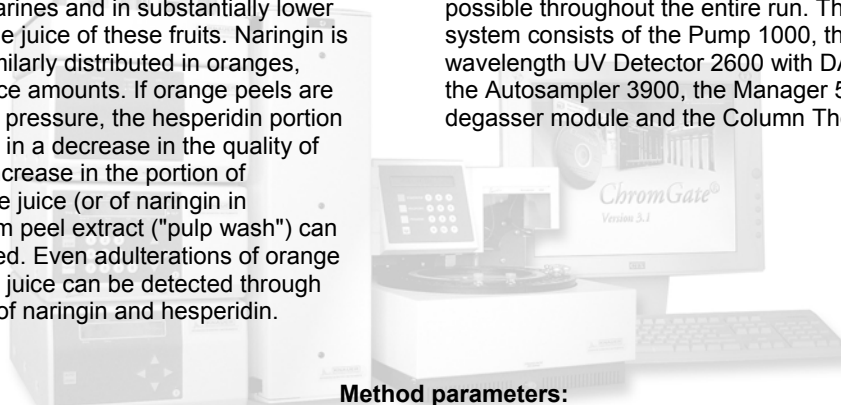
## Determination of Naringin and Hesperidin in Fruit Juices

S.Marten, J.Harms, Wissenschaftliche Gerätebau Dr. Ing. H.Knauer GmbH

### Introduction:

Citrus fruits contain a variety of flavonoids, including flavorless hesperidin (the principal flavonoid in oranges and mandarines) and bitter naringin (the principal flavonoid in grapefruits). Hesperidin is found in high concentrations in the fruit peel of oranges and mandarines and in substantially lower concentrations in the juice of these fruits. Naringin is also found to be similarly distributed in oranges, although only in trace amounts. 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 naringin and hesperidin.

With a Smartline HPLC system from Knauer, a simple and fast analysis of naringin and hesperidin 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. The Smartline system consists of the Pump 1000, the multi-wavelength UV Detector 2600 with DAD technology, the Autosampler 3900, the Manager 5000 with degasser module and the Column Thermostat 4000.

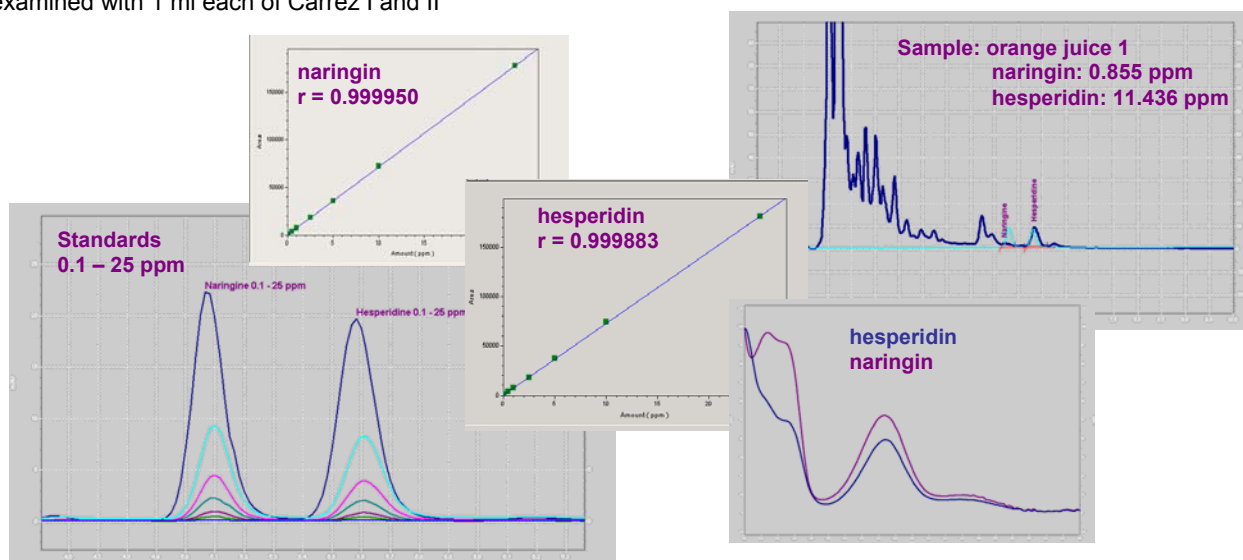


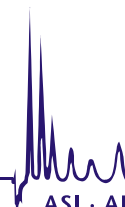
### Method parameters:

Column: Eurospher 100-5 C18, 250 x 4 mm (B6Y535)  
 Eluent: isocratic 5 mM ammonium acetate / ACN 75 : 25 (v/v), adjusted to pH 4.45 with acetic acid  
 Flow rate: 1 ml/min  
 Temperature: 40 °C  
 Injection volume: 5 µl  
 Detection: UV (Smartline 2600) at 285 nm

Calibration solutions for naringin and hesperidin were prepared in the concentration range 0.1 ppm to 100 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. 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.





## Results:

Calibration curves with regression coefficients (r) of 0.99988 or better were obtained for both naringin and hesperidin standards. The limit of detection for both compounds was established to be at 0.1 ppm. An additional verification of the calculated sample amount was always available by monitoring the spectra. For the analysis of hesperidin and naringin, one multivitamin juice, two orange juices and one grapefruit juice were investigated.

Fruit juice	Naringin	Hesperidin
Multivitamin	2.82 ppm	31.66 ppm
Orange juice 1	0.85 ppm	11.44 ppm
Orange juice 2	28.03 ppm	79.25 ppm
Grapefruit juice	497.41 ppm	25.25 ppm

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.