SLOVENSKI STANDARD
SIST EN 12148:1998

01-junij-1998

Sadni in zelenjavni sokovi - Določevanje hesperdina in naringina v sokovih citrusov - Metoda tekočinske kromatografije

Fruit and vegetable juices - Determination of hesperidin and naringin in citrus juices - Method using high performance liquid chromatography

Frucht- und Gemüsesäfte - Bestimmung von Hesperidin und Naringin in Zitrussäften - Hochleistungs-flüssigkeitschromatographisches Verfahren

Jus de fruits et de légumes - Détermination de la teneur en hespéridine et en naringine dans les jus d'agrumes - Méthode utilisant la chromatographie liquide a haute performance (HPLC)

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English version

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Frucht- und Gemüsesäfte - Bestimmung von Hesperidin und Naringin in Citrussäften - Hochleistungs-flüssigkeitschromatographisches Verfahren

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Contents

Foreword

This European Standard has been prepared by Technical Committee CEN/TC 174 "Fruit and vegetable juices - Method of analysis" the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 1997, and conflicting national standards shall be withdrawn at the latest by March 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard : Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This draft European Standard specifies a method for the determination of the hesperidin and naringin contents, using high performance liquid chromatography (HPLC), in fruit and vegetable juices and related products.

2 Normative references

This draft European Standard incorporates by dated or undated references, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN ISO 3696:1995 Water for analytical laboratory use - Specification and test methods

ISO 5725:1986 Precision of test methods - Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests

3 Symbols and abbreviations

3.1 Symbols

For the purposes of this standard the following symbols apply :

c	Substance concentration;
ρ	Content of hesperidin or naringin respectively in samples (mass concentration in milligrams per litre of juice).

3.2 Abbreviations

For the purposes of this standard the following abbreviations apply :

Hesperidin : hesperitin-7-rutinoside.
Naringin : naringenin-7-neohesperidoside.

HPLC	High performance liquid chromatography
UV	Ultraviolet
AUFS	Absorption units full scale

4 Principle

Hesperidin and naringin are extracted into a dimethylformamide solution. After heating at 90 °C a membrane filtered aliquot portion of the sample is separated by reversed phase HPLC using UV-detection and calculation is by the external standard method.

5 Reagents

5.1 General

Use only reagents of recognized analytical grade and only water in accordance with at least grade 1 of EN ISO 3696:1995.

5.2 Acetic acid

5.3 Acetic acid, c (CH_3COOH) = 0,01 mol/l

5.4 Acetonitrile, HPLC-grade

5.5 Di-ammonium oxalate monohydrate, $(NH_4)_2C_2O_4\ H_2O$

5.6 Dimethylformamide

5.7 Hesperidin (3.2)

5.8 Naringin (3.2)

5.9 Standard solution of naringin and hesperidin

For preparing the stock solution, 120 mg of naringin (5.8) and hesperidin (5.7) are dissolved in 20 ml of dimethylformamide (5.6) in a 100 ml volumetric flask. After the solids have dissolved, the solution is made up to volume with acetic acid solution (5.3). To prepare the hesperidin/naringin working standard solution (120 mg/l of each compound), the stock solution (1 200 mg/l) is diluted 1 in 10 with a mixture of acetic acid solution (5.3) and dimethylformamide (5.6) (8 to 2 volumes respectively). The working standard solution is stable for 30 days when stored at 4 °C. From the stock solution (1 200 mg/l) prepare further standards by dilution of the stock solution with a mixture of acetic acid solution (5.3) and dimethylformamide (5.6) (8 to 2 volumes) to give solutions containing 60 mg/l, 30 mg/l and 15 mg/l of both hesperidin and naringin.

5.10 Ammonium oxalate solution c ($(NH_4)_2\ C_2O_4\ .\ H_2O$) = 0,025 mol/l

The ammonium oxalate solution is prepared by dissolving 3,55 g of di-ammonium-oxalate monohydrate (5.5) in 1 000 ml of water.

5.11 Mobile phase for the HPLC-analysis

The mobile phase for the determination of hesperidin and naringin is prepared by mixing 200 ml of acetonitrile (5.4), 800 ml of water and 0,5 ml of acetic acid (5.2). The acetonitrile and water shall be measured separately and the composition of mobile phase shall be prepared exactly.

6 Apparatus

Usual laboratory apparatus and, in particular, the following :

6.1 Volumetric flask, 50 ml

6.2 Volumetric pipette, 10 ml

6.3 Disposable filter, non-sterile, hydrophilic (of pore size 0,45 µm) for the filtration of sample solutions

6.4 HPLC-column :

- HPLC-column C18, 250 mm x 4 mm, 5 µm
- HPLC-column, C18, 250 mm x 4,6 mm, 5 µm

6.5 HPLC-equipment :

pump, column (6.4) and UV detector.

6.6 Water bath, capable of reaching and maintaining 90 °C.

7 Procedure

7.1 Preparation of the test sample

Normally products shall not be pre-treated and their analysis by this method shall be on a volumetric basis, results being expressed per litre of the sample. The analysis of concentrated products may also be carried out on a volumetric basis, after dilution to a known relative density. In this case, the relative density shall be indicated. Based on a weighed sample and taking the dilution factor for analysis into account, the results may also be expressed per kilogram of product. In products with high viscosity and/or very high content of cells (for example pulp), determination on the basis of a weighed test sample is the usual procedure. Mix cloudy samples well before dilution.

7.2 Test procedure

7.2.1 Sample preparation

Keep the sample at ambient temperature. Mix samples well before opening and take the test sample (7.1) immediately. Pipette 10 ml of the test sample or diluted concentrated products (7.1) into the 50 ml-volumetric flask (6.1). Then add 10 ml of ammonium oxalate solution (0,025 mol/l) (5.10) and 10 ml of dimethylformamide (5.6). Mix the diluted samples thoroughly and dilute to volume (50 ml) with water. Heat the samples in a temperature controlled water bath (6.6) at 90 °C for 10 min. After cooling to room temperature, filter an aliquot portion of the solution through a membrane filter (6.3) prior to analysis by HPLC.

7.2.2 HPLC-measurement

After equilibration of the HPLC-system, analyse the samples (HPLC-equipment (6.5), column (6.4), mobile phase (5.11)).

When using a fixed wavelength detector, measurement is at a wavelength of 280 nm or in the case of variable wavelength detector 287 nm is used. Detection is at sensitivities between 0,05 to 0,2 AUFS.

Flow rate : Approximately 1 ml/min
Injection volume : 20 µl

The standard and sample solutions are injected in successive runs.

As the stability of the sample solutions is limited, the test shall be completed within 24 h.

8 Calculation

The calculation is made according to the external standard method by integration of the peak areas or by measuring the peak heights taking into account the retention times and the linearity of the calibration curve. During calculation, take into account any dilution factor and the relationship of the value to mass or volume. If a concentrated product has been diluted to single strength, report the relative density of the single strength sample.

Calculate the hesperidin and naringin content, ρ_H and ρ_N of each sample using the following formula :

$$\rho_H = \frac{A_{HP}}{RF_H} \times XF \qquad (1)$$

$$\rho_N = \frac{A_{NP}}{RF_N} \times XF \qquad (2)$$

where :

A_{HP} is the peak area for the hesperidin content of the sample ;