

01/2008:1547 – *stationary phase*: spherical octadecylsilyl silica gel for chromatography R (4 µm) with a carbon loading of 7 per cent,

– *temperature*: 30 °C.

*Mobile phase*: mix 20 volumes of acetonitrile R and 80 volumes of a solution prepared by adding 5.0 mL of glacial acetic acid R to 1000.0 mL of water R.

*Flow rate*: 1.0 mL/min.

*Detection*: spectrophotometer at 282 nm.

*Injection*: 10 µL; inject test solution (a) and reference solutions (a), (b), (c) and (d).

*Run time*: 5 times the retention time of neohesperidin-dihydrochalcone which is about 10 min.

*Relative retention* with reference to neohesperidin-dihydrochalcone: impurity B = about 0.4; impurity D = about 0.7; impurity F = about 1.2; impurity G = about 3.7.

*System suitability*:

– *resolution*: minimum of 2.5 between the first peak (neohesperidin-dihydrochalcone) and the second peak (impurity F) in the chromatogram obtained with reference solution (d),

– chromatogram obtained with reference solution (a) is similar to the chromatogram provided with *neohesperidin-dihydrochalcone CRS*.

*Limits*:

– *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (2 per cent),

– *impurity D*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (c) (2 per cent),

– *any other impurity*: not more than 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent),

– *total of all impurities apart from impurity B*: not more than 2.5 times the area of the principal peak in the chromatogram obtained with reference solution (c) (2.5 per cent),

– *disregard limit*: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

**Heavy metals** (2.4.8): maximum 10 ppm.

2.0 g complies with limit test D. Prepare the standard using 2 mL of lead standard solution (10 ppm Pb) R.

**Water** (2.5.12): maximum 12.0 per cent, determined on 0.200 g.

**Sulfated ash** (2.4.14): maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (2.2.29) as described in the test for related substances.

*Injection*: 10 µL; inject test solution (b) and reference solutions (a) and (d).

*System suitability*:

– *resolution*: minimum of 2.5 between the first peak (neohesperidin-dihydrochalcone) and the second peak (impurity F) in the chromatogram obtained with reference solution (d),

– *repeatability*: reference solution (a).

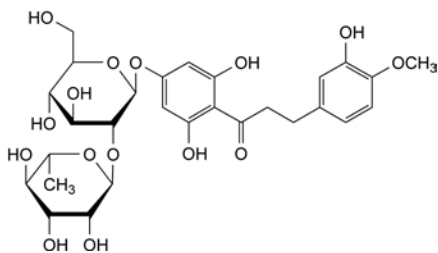
Calculate the percentage content of C<sub>28</sub>H<sub>36</sub>O<sub>15</sub> using the chromatogram obtained with reference solution (a) and the stated content of C<sub>28</sub>H<sub>36</sub>O<sub>15</sub> in *neohesperidin-dihydrochalcone CRS*, correcting for the water content of the substance to be examined.

STORAGE

Protected from light.

## NEOHESPERIDIN-DIHYDROCHALCONE

### Neohesperidin-dihydrochalconum



C<sub>28</sub>H<sub>36</sub>O<sub>15</sub>  
[20702-77-6]

M<sub>r</sub> 613

#### DEFINITION

1-[4-[[2-O-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]-oxy]-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)-propan-1-one.

*Content*: 96.0 per cent to 101.0 per cent (anhydrous substance).

#### CHARACTERS

*Appearance*: white or yellowish-white powder.

*Solubility*: practically insoluble in water, freely soluble in dimethyl sulfoxide, soluble in methanol, practically insoluble in methylene chloride.

#### IDENTIFICATION

A. Infrared absorption spectrophotometry (2.2.24).

*Comparison*: *neohesperidin-dihydrochalcone CRS*.

B. Examine the chromatograms obtained in the assay.

*Results*: the principal peak in the chromatogram obtained with test solution (b) is similar in retention time and size to the principal peak in the chromatogram obtained with reference solution (a).

#### TESTS

**Appearance of solution.** The solution is clear (2.2.1) and not more intensely coloured than reference solution Y<sub>4</sub> (2.2.2, Method II).

Dissolve 0.25 g in *methanol R* and dilute to 25 mL with the same solvent.

**Related substances.** Liquid chromatography (2.2.29).

*Test solution (a).* Dissolve 0.10 g of the substance to be examined in *dimethyl sulfoxide R* and dilute to 50.0 mL with the same solvent.

*Test solution (b).* Dilute 10.0 mL of test solution (a) to 20.0 mL with *dimethyl sulfoxide R*.

*Reference solution (a).* Dissolve 50.0 mg of *neohesperidin-dihydrochalcone CRS* in *dimethyl sulfoxide R* and dilute to 50.0 mL with the same solvent.

*Reference solution (b).* Dissolve 4.0 mg of *neohesperidin-dihydrochalcone impurity B CRS* in *dimethyl sulfoxide R* and dilute to 100.0 mL with the same solvent.

*Reference solution (c).* Dilute 1.0 mL of test solution (a) to 100.0 mL with *dimethyl sulfoxide R*.

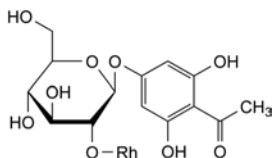
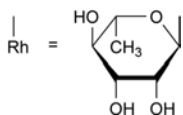
*Reference solution (d).* In order to prepare *in situ* impurity F and impurity G, suspend 0.10 g of the substance to be examined in 10.0 mL of a 100 g/L solution of *sulfuric acid R*. Heat the sample for 5 min on a water-bath. Dilute immediately 1.0 mL of the resulting solution to 50.0 mL with *dimethyl sulfoxide R*.

*Column*:

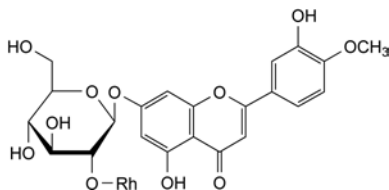
– *size*: *l* = 0.15 m, *Ø* = 3.9 mm,

## IMPURITIES

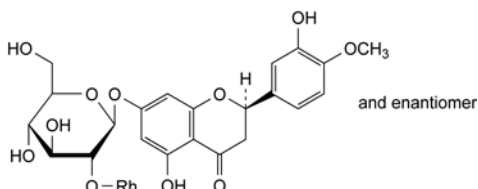
01/2008:0197



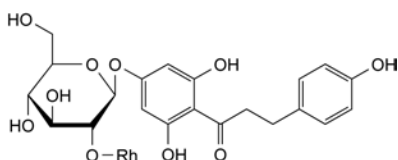
- A. 1-[4-[[2-O-(6-deoxy- $\alpha$ -L-mannopyranosyl)- $\beta$ -D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]ethanone (phloracetophenone neohesperidoside),



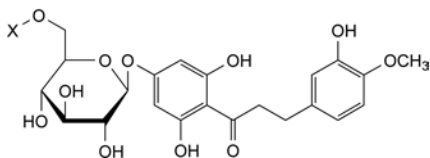
- B. 7-[[2-O-(6-deoxy- $\alpha$ -L-mannopyranosyl)- $\beta$ -D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one (neodiosmin),



- C. (2*RS*)-7-[[2-O-(6-deoxy- $\alpha$ -L-mannopyranosyl)- $\beta$ -D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4H-1-benzopyran-4-one (neohesperidin),

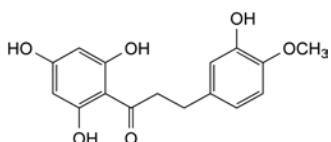


- D. 1-[4-[[2-O-(6-deoxy- $\alpha$ -L-mannopyranosyl)- $\beta$ -D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(4-hydroxyphenyl)propan-1-one (naringin-dihydrochalcone),



- E. X = Rh: 1-[4-[[2-O-(6-deoxy- $\alpha$ -L-mannopyranosyl)- $\beta$ -D-glucopyranosyl]oxy]-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (hesperidin-dihydrochalcone),

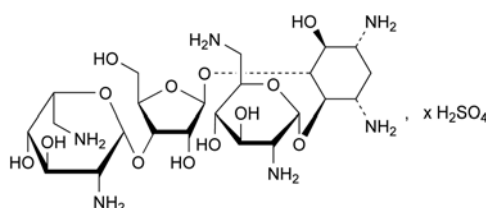
- F. X = H: 1-[4-( $\beta$ -D-glucopyranosyloxy)-2,6-dihydroxyphenyl]-3-(3-hydroxy-4-methoxyphenyl)propan-1-one (hesperetin-dihydrochalcone 7'-glucoside),



- G. 3-(3-hydroxy-4-methoxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one (hesperetin-dihydrochalcone).

## NEOMYCIN SULFATE

## Neomycini sulfas

 $C_{23}H_{46}N_6O_{13} \cdot xH_2SO_4$  $M_r$  615 (base)

## DEFINITION

Mixture of sulfates of substances produced by the growth of certain selected strains of *Streptomyces fradiae*, the main component being the sulfate of 2-deoxy-4-O-(2,6-diamino-2,6-dideoxy- $\alpha$ -D-glucopyranosyl)-5-O-[3-O-(2,6-diamino-2,6-dideoxy- $\beta$ -L-idopyranosyl)- $\beta$ -D-ribofuranosyl]-D-streptamine (neomycin B).

*Content*: minimum of 680 IU/mg (dried substance).

## CHARACTERS

*Appearance*: white or yellowish-white powder, hygroscopic.

*Solubility*: very soluble in water, very slightly soluble in alcohol, practically insoluble in acetone.

## IDENTIFICATION

- A. Examine the chromatograms obtained in the test for related substances.

*Results*:

- the retention time of the principal peak in the chromatogram obtained with the test solution is approximately the same as that of the principal peak in the chromatogram obtained with reference solution (e),
- it complies with the limits given for impurity C.

- B. It gives reaction (a) of sulfates (2.3.1).

## TESTS

**pH** (2.2.3): 5.0 to 7.5.

Dissolve 0.1 g in *carbon dioxide-free water R* and dilute to 10 mL with the same solvent.

**Specific optical rotation** (2.2.7): + 53.5 to + 59.0 (dried substance).

Dissolve 1.00 g in *water R* and dilute to 10.0 mL with the same solvent.

**Related substances.** Liquid chromatography (2.2.29).

*Test solution.* Dissolve 25.0 mg of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

*Reference solution (a).* Dissolve 25.0 mg of *framycetin sulfate CRS* in the mobile phase and dilute to 50.0 mL with the mobile phase.

*Reference solution (b).* Dilute 5.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

*Reference solution (c).* Dilute 1.0 mL of reference solution (a) to 100.0 mL with the mobile phase.

*Reference solution (d).* Dissolve the contents of a vial of *neamine CRS* (corresponding to 0.5 mg) in the mobile phase and dilute to 50.0 mL with the mobile phase.

*Reference solution (e).* Dissolve 10 mg of *neomycin sulfate CRS* in the mobile phase and dilute to 100.0 mL with the mobile phase.

*Column*:

- *size*:  $l = 0.25$  m,  $\varnothing = 4.6$  mm,
- *stationary phase*: base-deactivated octadecylsilyl silica gel for chromatography R (5  $\mu$ m),