Injection: 10 µl.

Relative retention with reference to chlorogenic acid:
caftaric acid = about 0.8; caffeic acid = about 1.5;
cynarin = about 1.6; echinacoside = about 1.7; cichoric acid = about 2.3.

System suitability: reference solution:
- resolution: minimum 10 between the peaks due to caffeic acid and chlorogenic acid.

Locate the peaks due to caffeic acid and chlorogenic acid using the chromatogram obtained with the reference solution. Locate the peaks due to echinacoside and cynarin using the chromatogram in Figure 1821.–1.

Calculate the percentage content of echinacoside from the following expression:
\[ \frac{A_1 \times C_2 \times 100 \times 2.221}{A_2 \times C_1} \]

- \( A_1 \) = area of the peak due to echinacoside in the chromatogram obtained with the test solution;
- \( A_2 \) = area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution;
- \( C_1 \) = concentration of the test solution, in milligrams per millilitre;
- \( C_2 \) = concentration of chlorogenic acid in the reference solution, in milligrams per millilitre;
- 2.221 = peak correlation factor between chlorogenic acid and echinacoside.

STORAGE
Store uncomminuted.

DEFINITION
1,4-[2-O-(6-Deoxy-α-1-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 sulphoxide, 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.
Neohesperidin-dihydrochalcone

**TESTS**

**Appearance of solution.** The solution is clear (2.2.1) and not more intensely coloured than reference solution Y4 (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 sulphoxide 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 sulphoxide R.

Reference solution (a). Dissolve 500 mg of neohesperidin-dihydrochalcone CRS in dimethyl sulphoxide 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 sulphoxide 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 sulphoxide 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 sulphuric 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 sulphoxide R.

**Column:**
- **size:** t = 0.15 m, Ø = 3.9 mm,
- **stationary phase:** spherical octadecylsilica 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 twice 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 twice 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.

**Sulphated 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_{28}H_{36}O_{15} using the chromatogram obtained with reference solution (a) and the stated content of C_{28}H_{36}O_{15} in neohesperidin-dihydrochalcone CRS, correcting for the water content of the substance to be examined.

**STORAGE**

Protected from light.

**IMPURITIES**

A. 1-[4]-(2-O(4-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl)oxy]-2,6-dihydroxyphenyl]ethanone (phloroacetophenone neohesperidoside),

See the information section on general monographs (cover pages)
Neomycin sulphate

DEFINITION
Mixture of sulphates of substances produced by the growth of certain selected strains of *Streptomyces fradiae*, the main component being the sulphate 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]-\(\delta\)-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 sulphates (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 sulphate 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.