ASSAY

Prepare the solutions immediately before use.

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

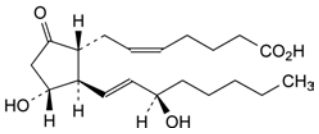
Injection: test solution (b) and reference solution (d).

Calculate the percentage content of $C_{20}H_{32}O_5$.

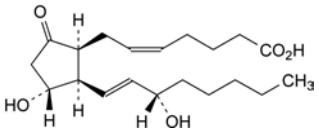
STORAGE

At a temperature not exceeding - 15 °C.

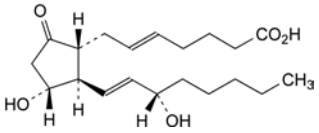
IMPURITIES



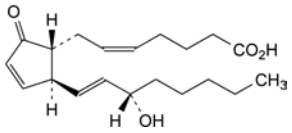
A. (*Z*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*R*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (15-epiPGE$_2$; (15*R*)-PGE$_2$),



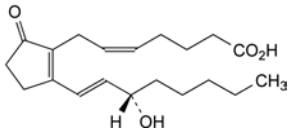
B. (*Z*)-7-[(1*S*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (8-epiPGE$_2$; (8S)-PGE$_2$),



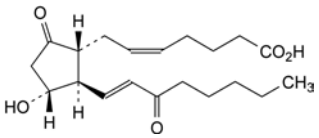
C. (*E*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (5-*trans*-PGE$_2$; (5*E*)-PGE$_2$),



D. (*Z*)-7-[(1*R*,2*S*)-2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopent-3-enyl]hept-5-enoic acid (PGA$_2$),



E. (*Z*)-7-[2-[(*E*)-(3*S*)-3-hydroxyoct-1-enyl]-5-oxocyclopent-1-enyl]hept-5-enoic acid (PGB$_2$),



F. (*Z*)-7-[(1*R*,2*R*,3*R*)-3-hydroxy-2-[(*E*)-3-oxo-oct-1-enyl]-5-oxocyclopentyl]hept-5-enoic acid (15-oxo-PGE$_2$; 15-keto-PGE$_2$).

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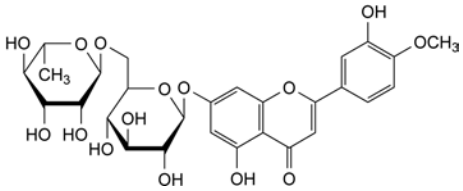
DIOSMIN

Diosminum



$C_{28}H_{32}O_{15}$ M_r 609

[520-27-4]

DEFINITION

7-[[6-*O*-(6-Deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one.

Substance obtained through iodine-assisted oxidation of (2*S*)-7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (hesperidin) of natural origin.

Content: 90.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance: greyish-yellow or light yellow hygroscopic powder.

Solubility: practically insoluble in water, soluble in dimethyl sulfoxide, practically insoluble in alcohol. It dissolves in dilute solutions of alkali hydroxides.

IDENTIFICATION

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *diosmin CRS*.

B. Examine the chromatograms obtained in the assay.

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

TESTS

Iodine: maximum 0.1 per cent.

Determine the total content of iodine by potentiometry, using an iodide-selective electrode (*2.2.36*), after oxygen combustion (*2.5.10*).

Test solution. Wrap 0.100 g of the substance to be examined in a piece of filter paper and place it in a sample carrier. Introduce into the flask 50 mL of a 0.2 g/L solution of *hydrazine R*. Flush the flask with oxygen for 10 min. Ignite the filter paper. Stir the contents of the flask immediately after the end of the combustion to dissolve completely the combustion products. Continue stirring for 1 h.

Reference solution. Dilute 2.0 mL of a 16.6 g/L solution of *potassium iodide R* to 100.0 mL with *water R*. Dilute 10.0 mL of the solution to 100.0 mL with *water R*.

Introduce into a beaker 30 mL of a 200 g/L solution of *potassium nitrate R* in *0.1 M nitric acid*. Immerse the electrodes and stir for 10 min. The potential of the solution (nT_1) must remain stable. Add 1 mL of the test solution and measure the potential (nT_2).

Introduce into a beaker 30 mL of a 200 g/L solution of *potassium nitrate R* in *0.1 M nitric acid*. Immerse the electrodes and stir for 10 min. The potential of the solution must remain stable (nR_1). Add 80 µL of the reference solution and measure the potential (nR_2).

The absolute value $|nT_2 - nT_1|$ is not higher than the absolute value $|nR_2 - nR_1|$.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 25.0 mg of the substance to be examined in *dimethyl sulfoxide R* and dilute to 25.0 mL with the same solvent.

Reference solution (a). Dissolve 25.0 mg of *diosmin CRS* in *dimethyl sulfoxide R* and dilute to 25.0 mL with the same solvent.

Reference solution (b). Dilute 5.0 mL of reference solution (a) to 100.0 mL with *dimethyl sulfoxide R*.

Reference solution (c). Dissolve 5.0 mg of *diosmin for system suitability CRS* in *dimethyl sulfoxide R* and dilute to 5.0 mL with the same solvent.

Column:

- *size*: *l* = 0.10 m, Ø = 4.6 mm,
- *stationary phase*: *octadecylsilyl silica gel for chromatography R* (3 µm),
- *temperature*: 40 °C.

Mobile phase: *acetonitrile R, glacial acetic acid R, methanol R, water R* (2:6:28:66 *V/V/V/V*).

Flow rate: 1.5 mL/min.

Detection: spectrophotometer at 275 nm.

Injection: 10 µL loop injector; inject the test solution and reference solutions (b) and (c).

Run time: 6 times the retention time of diosmin.

Relative retention with reference to diosmin (retention time = about 4.6 min): impurity A = about 0.5, impurity B = about 0.6, impurity C = about 0.8, impurity D = about 2.2, impurity E = about 2.6, impurity F = about 4.5.

System suitability: reference solution (c):

- *resolution*: minimum of 2.5 between the peaks due to impurities B and C.

Limits:

- *correction factors*: for the calculation of contents, multiply the peak areas of the following impurities by the corresponding correction factor: impurity A = 0.38; impurity F = 0.61,
- *impurity A*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),
- *impurity B*: not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (5 per cent),
- *impurity C*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),
- *impurity E*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),
- *impurity F*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (b) (3 per cent),
- *any other impurity*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),
- *total of other impurities and impurity A*: not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1 per cent),
- *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (10 per cent),
- *disregard limit*: 0.02 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent).

Heavy metals (*2.4.8*): maximum 20 ppm.

2.0 g complies with limit test C. Prepare the standard using 4.0 mL of *lead standard solution (10 ppm Pb) R*.

Water (*2.5.12*): maximum 6.0 per cent, determined on 0.300 g.

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

ASSAY

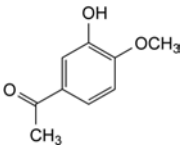
Liquid chromatography (*2.2.29*), as described in the test for related substances.

Injection: test solution and reference solution (a).

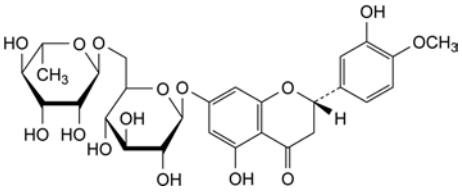
STORAGE

In an airtight container.

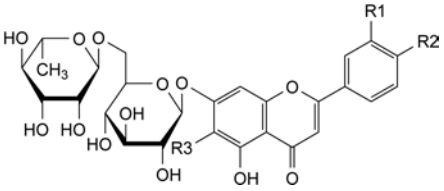
IMPURITIES



A. 1-(3-hydroxy-4-methoxyphenyl)ethanone (acetoisovanillone),



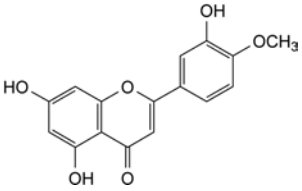
B. (2*S*)-7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one (hesperidin),



C. R1 = R3 = H, R2 = OH: 7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (isorhoifolin),

D. R1 = OH, R2 = OCH_3, R3 = I: 7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-6-iodo-4*H*-1-benzopyran-4-one (6-iododiosmin),

E. R1 = R3 = H, R2 = OCH_3: 7-[[6-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranosyl]oxy]-5-hydroxy-2-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one (linarin),

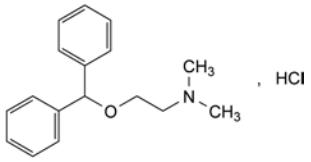


F. 5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one (diosmetin).

01/2008:0023
corrected 6.0

DIPHENHYDRAMINE HYDROCHLORIDE

Diphenhydramini hydrochloridum



$C_{17}H_{22}ClNO$ M_r 291.8
[147-24-0]

DEFINITION

2-(Diphenylmethoxy)-*N*,*N*-dimethylethanamine hydrochloride.

Content: 99.0 per cent to 101.0 per cent (dried substance).

CHARACTERS

Appearance: white or almost white, crystalline powder.

Solubility: very soluble in water, freely soluble in alcohol.

IDENTIFICATION

First identification: C, D.

Second identification: A, B, D.

A. Melting point (*2.2.14*): 168 °C to 172 °C.

B. Dissolve 50 mg in *alcohol R* and dilute to 100.0 mL with the same solvent. Examined between 230 nm and 350 nm, the solution shows 3 absorption maxima (*2.2.25*), at 253 nm, 258 nm and 264 nm. The ratio of the absorbance measured at the maximum at 258 nm to that measured at the maximum at 253 nm is 1.1 to 1.3. The ratio of the absorbance measured at the maximum at 258 nm to that measured at the maximum at 264 nm is 1.2 to 1.4.

C. Infrared absorption spectrophotometry (*2.2.24*).
Preparation: discs.
Comparison: *diphenhydramine hydrochloride CRS*.

D. It gives the reactions of chlorides (*2.3.1*).

TESTS

Solution S. Dissolve 1.0 g in *carbon dioxide-free water R* and dilute to 20 mL with the same solvent.

Appearance of solution. Solution S and a fivefold dilution of solution S are clear (*2.2.1*). Solution S is not more intensely coloured than reference solution BY_6 (*2.2.2, Method II*).

Acidity or alkalinity. To 10 mL of solution S add 0.15 mL of *methyl red solution R* and 0.25 mL of *0.01 M hydrochloric acid*. The solution is pink. Not more than 0.5 mL of *0.01 M sodium hydroxide* is required to change the colour of the indicator to yellow.

Related substances. Liquid chromatography (*2.2.29*).

Test solution. Dissolve 70 mg of the substance to be examined in the mobile phase and dilute to 20.0 mL with the mobile phase. Dilute 2.0 mL of the solution to 10.0 mL with the mobile phase.

Reference solution (a). Dilute 1.0 mL of the test solution to 10.0 mL with the mobile phase. Dilute 1.0 mL of this solution to 20.0 mL with the mobile phase.

Reference solution (b). Dissolve 5 mg of *diphenhydramine impurity A CRS* and 5 mg of *diphenylmethanol R* in the mobile phase and dilute to 10.0 mL with the mobile phase. To 2.0 mL of this solution add 1.5 mL of the test solution and dilute to 10.0 mL with the mobile phase.

Column:

– *size*: *l* = 0.25 m, Ø = 4.6 mm,
– *stationary phase*: *base-deactivated octylsilyl silica gel for chromatography R* (5 µm).

Mobile phase: mix 35 volumes of *acetonitrile R* and 65 volumes of a 5.4 g/L solution of *potassium dihydrogen phosphate R* adjusted to pH 3.0 using *phosphoric acid R*.

Flow rate: 1.2 mL/min.

Detection: spectrophotometer at 220 nm.

Injection: 10 µL.

Run time: 7 times the retention time of diphenhydramine.

Relative retention with reference to diphenhydramine (retention time = about 6 min): impurity A = about 0.9; impurity B = about 1.5; impurity C = about 1.8; impurity D = about 2.6; impurity E = about 5.1.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to diphenhydramine and to impurity A.

Limits:

– *correction factor*: for the calculation of content, multiply the peak area of impurity D by 0.7,
– *impurity A*: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent),
– *any other impurity*: not more than 0.6 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent),
– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent),
– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.250 g in 50 mL of *alcohol R* and add 5.0 mL of *0.01 M hydrochloric acid*. Carry out a potentiometric titration (*2.2.20*), using *0.1 M sodium hydroxide*. Read the volume added between the 2 points of inflexion.

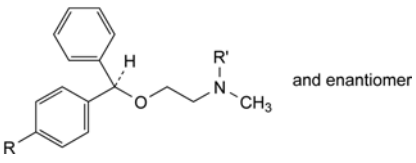
1 mL of *0.1 M sodium hydroxide* is equivalent to 29.18 mg of $C_{17}H_{22}ClNO$.

STORAGE

Protected from light.

IMPURITIES

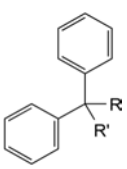
Specified impurities: A, B, C, D, E.



A. R = R′ = H: 2-(diphenylmethoxy)-*N*-methylethanamine,

B. R = R′ = CH_3: 2-[(*RS*)-(4-methylphenyl)phenylmethoxy]-*N*,*N*-dimethylethanamine,

C. R = Br, R′ = CH_3: 2-[(*RS*)-(4-bromophenyl)phenylmethoxy]-*N*,*N*-dimethylethanamine,



D. R = OH, R′ = H: diphenylmethanol (benzhydrol),

E. R + R′ = O: diphenylmethanone (benzophenone).