C. Examine the chromatograms obtained in the test for related substances in ultraviolet light at 254 nm. The principal spot obtained with test solution (b) is similar in position and size to the principal spot in the chromatogram obtained with reference solution (a).

D. Solution S (see Tests) is strongly acid (*2.2.4*).

TESTS

Solution S. Dissolve 2.50 g in *water R* and dilute to 100.0 ml with the same solvent.

Appearance of solution. Solution S is clear (*2.2.1*) and colourless (*2.2.2, Method II*).

Specific optical rotation (*2.2.7*). Dilute 10.0 ml of solution S to 25.0 ml with *water R*. The specific optical rotation is + 46 to + 49, calculated with reference to the dried substance.

Related substances. Examine by thin-layer chromatography (*2.2.27*), using a *TLC silica gel F_{254} plate R*.

Test solution (a). Dissolve 0.80 g of the substance to be examined in 6 ml of a mixture of equal volumes of *glacial acetic acid R* and *water R* and dilute to 10 ml with *ethanol R*.

Test solution (b). Dilute 1 ml of test solution (a) to 10 ml with *ethanol R*.

Reference solution (a). Dissolve 80 mg of *N-acetyltyrosine CRS* in a mixture of 3 volumes of *water R*, 3 volumes of *glacial acetic acid R* and 94 volumes of *ethanol R* and dilute to 10 ml with the same mixture of solvents.

Reference solution (b). Dilute 0.5 ml of test solution (b) to 10 ml with *ethanol R*.

Reference solution (c). Dissolve 40 mg of *tyrosine CRS* in 20 ml of a mixture of equal volumes of *water R* and *glacial acetic acid R* and dilute to 50 ml with *ethanol R*.

Apply separately to the plate 5 µl of each solution. Develop over a path of 10 cm using a mixture of 10 volumes of *water R*, 15 volumes of *glacial acetic acid R* and 75 volumes of *ethyl acetate R*. Allow the plate to dry in air. Examine in ultraviolet light at 254 nm. Any spot in the chromatogram obtained with test solution (a), apart from the principal spot, is not more intense than the spot in the chromatogram obtained with reference solution (b) (0.5 per cent). Spray with *ninhydrin solution R* and heat at 100 °C to 105 °C for 10 min. Examine in daylight. Any spot corresponding to tyrosine is not more intense that the spot in the chromatogram obtained with reference solution (c) (1 per cent).

Chlorides (*2.4.4*). Dilute 10 ml of solution S to 15 ml with *water R*. The solution complies with the limit test for chlorides (200 ppm).

Sulphates (*2.4.13*). Dissolve 1.0 g in *distilled water R* and dilute to 20 ml with the same solvent. The solution complies with the limit test for sulphates (200 ppm).

Ammonium. Prepare a cell consisting of two watch-glasses 60 mm in diameter placed edge to edge. To the inner wall of the upper watch-glass stick a piece of *red litmus paper R* 5 mm square and wetted with a few drops of *water R*. Finely powder the substance to be examined, place 50 mg in the lower watch-glass and dissolve in 0.5 ml of *water R*. To the solution add 0.30 g of *heavy magnesium oxide R*. Briefly triturate with a glass rod. Immediately close the cell by putting the two watch-glasses together. Heat at 40 °C for 15 min. The litmus paper is not more intensely blue coloured than a standard prepared at the same time and in the same manner using 0.1 ml of *ammonium standard solution (100 ppm NH_4) R*, 0.5 ml of *water R* and 0.30 g of *heavy magnesium oxide R* (200 ppm).

Iron (*2.4.9*). In a separating funnel, dissolve 0.5 g in 10 ml of *dilute hydrochloric acid R*. Shake with three quantities, each of 10 ml, of *methyl isobutyl ketone R1*, shaking for 3 min each time. To the combined organic layers add 10 ml of *water R* and shake for 3 min. The aqueous layer complies with the limit test for iron (20 ppm).

Heavy metals (*2.4.8*). Dissolve 2.0 g in *water R* and dilute to 20 ml with the same solvent. 12 ml of the solution complies with limit test A for heavy metals (10 ppm). Prepare the standard using *lead standard solution (1 ppm Pb) R*.

Loss on drying (*2.2.32*). Not more than 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

Pyrogens (*2.6.8*). If intended for use in the manufacture of parenteral dosage forms without a further appropriate procedure for the removal of pyrogens, it complies with the test for pyrogens. Inject per kilogram of the rabbit's mass 1.0 ml of a freshly prepared solution in *water for injections R* containing per millilitre 10.0 mg of the substance to be examined and 9.0 mg of pyrogen-free *sodium chloride R*.

ASSAY

Dissolve 0.180 g in 50 ml of *carbon dioxide-free water R*. Titrate with *0.1 M sodium hydroxide,* determining the end-point potentiometrically (*2.2.20*).

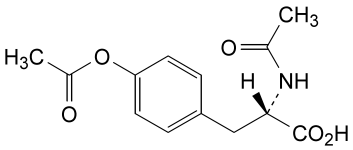
1 ml of *0.1 M sodium hydroxide* is equivalent to 22.32 mg of $C_{11}H_{13}NO_4$.

STORAGE

Store protected from light. If the substance is sterile, store in a sterile, airtight, tamper-proof container.

IMPURITIES

A. tyrosine,

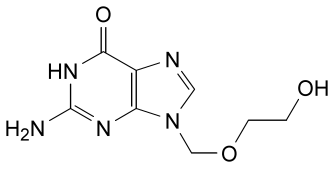


B. (2*S*)-2-(acetylamino)-3-[4-(acetoxy)phenyl]propanoic acid (diacetyltyrosine).

01/2008:0968

ACICLOVIR

Aciclovirum



$C_8H_{11}N_5O_3$ M_r 225.2

[59277-89-3]

DEFINITION

Aciclovir contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of 2-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-6*H*-purin-6-one, calculated with reference to the anhydrous substance.

CHARACTERS

A white or almost white, crystalline powder, slightly soluble in water, freely soluble in dimethyl sulphoxide, very slightly soluble in ethanol (96 per cent). It dissolves in dilute solutions of mineral acids and alkali hydroxides.

IDENTIFICATION

Examine by infrared absorption spectrophotometry (*2.2.24*), comparing with the spectrum obtained with *aciclovir CRS*.

TESTS

Appearance of solution. Dissolve 0.25 g in *0.1 M sodium hydroxide* and dilute to 25 ml with the same solvent. The solution is clear (*2.2.1*) and not more intensely coloured than reference solution Y_7 (*2.2.2, Method II*).

Related substances

A. Examine by thin-layer chromatography (*2.2.27*), using *silica gel* GF_{254} *R* as the coating substance.

Prepare the solutions immediately before use.

Test solution. Dissolve 0.1 g of the substance to be examined in *dimethyl sulphoxide R* and dilute to 10 ml with the same solvent.

Reference solution. Dissolve 5 mg of *aciclovir impurity A CRS* in *dimethyl sulphoxide R* and dilute to 10 ml with the same solvent. Dilute 1 ml of the solution to 10 ml with *dimethyl sulphoxide R*.

Apply to the plate 10 µl of each solution. Keep the spots compact by drying in a current of warm air. Allow the plate to cool and develop over a path of 10 cm with a mixture of 2 volumes of *concentrated ammonia R*, 20 volumes of *methanol R* and 80 volumes of *methylene chloride R*. Allow the plate to dry in air and examine in ultraviolet light at 254 nm. In the chromatogram obtained with the test solution, any spot with an R_F value greater than that of the principal spot is not more intense than the spot in the chromatogram obtained with the reference solution (0.5 per cent).

B. Examine by liquid chromatography (*2.2.29*).

Test solution. Dissolve 50.0 mg of the substance to be examined in 10 ml of a mixture of 20 volumes of *glacial acetic acid R* and 80 volumes of *water R* and dilute to 100.0 ml with the mobile phase.

Reference solution (a). Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

Reference solution (b). Dissolve 5 mg of *aciclovir CRS* and 5 mg of *aciclovir impurity A CRS* in a mixture of 20 volumes of *glacial acetic acid R* and 80 volumes of *water R* and dilute to 25.0 ml with the same mixture of solvents. Dilute 1.0 ml of the solution to 10.0 ml with the mobile phase.

Reference solution (c). Dissolve 7 mg of *guanine R* in *0.1 M sodium hydroxide* and dilute to 100.0 ml with the same solution. Dilute 1.0 ml to 20.0 ml with the mobile phase.

The chromatographic procedure may be carried out using:

– a stainless steel column 0.10 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (3 µm),

– as mobile phase at a flow rate of 2 ml/min a mixture prepared as follows: dissolve 6.0 g of *sodium dihydrogen phosphate R* and 1.0 g of *sodium decanesulphonate R* in 900 ml of *water R* and adjust to pH 3 ± 0.1 with *phosphoric acid R*; add 40 ml of *acetonitrile R* and dilute to 1 litre with *water R*,

– as detector a spectrophotometer set at 254 nm,

– a loop injector.

Inject 20 µl of each solution. Record the chromatograms for 7 times the retention time of aciclovir. The test is not valid unless in the chromatogram obtained with reference solution (b), the number of theoretical plates calculated for the peak due to impurity A is at least 1500 and its mass distribution ratio is at least 7 (V_0 can be calculated using *dimethyl sulphoxide R*). In the chromatogram obtained with the test solution: the area of any peak corresponding to guanine is not greater than that of the peak in the chromatogram obtained with reference solution (c) (0.7 per cent); the area of any peak apart from the principal peak and any peak corresponding to guanine is not greater than the area of the peak in the chromatogram obtained with reference solution (a) (0.5 per cent) and the sum of the areas of such peaks is not greater than twice the area of the peak in the chromatogram obtained with reference solution (a) (1 per cent). Disregard any peak with an area less than 0.05 times that of the principal peak in the chromatogram obtained with reference solution (a).

Water (*2.5.12*). Not more than 6.0 per cent, determined on 0.500 g.

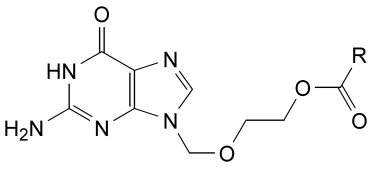
Sulphated ash (*2.4.14*). Not more than 0.1 per cent, determined on 1.0 g.

ASSAY

Dissolve 0.150 g in 60 ml of *anhydrous acetic acid R*. Titrate with *0.1 M perchloric acid*, determining the end-point potentiometrically (*2.2.20*). Carry out a blank titration.

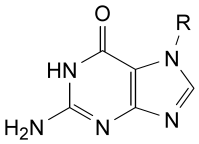
1 ml of *0.1 M perchloric acid* is equivalent to 22.52 mg of $C_8H_{11}N_5O_3$.

IMPURITIES



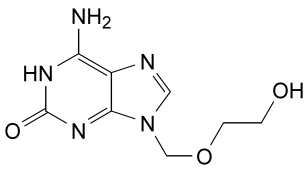
A. R = CH_3: 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate,

D. R = C_6H_5: 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl benzoate,

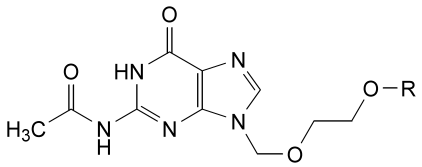


B. R = H: 2-amino-1,7-dihydro-6*H*-purin-6-one (guanine),

C. R = CH_2-O-CH_2-CH_2-OH: 2-amino-7-[(2-hydroxyethoxy)-methyl]-1,7-dihydro-6*H*-purin-6-one,



E. 6-amino-9-[(2-hydroxyethoxy)methyl]-1,9-dihydro-2*H*-purin-2-one,



F. R = H: *N*-[9-[(2-hydroxyethoxy)methyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl]acetamide,

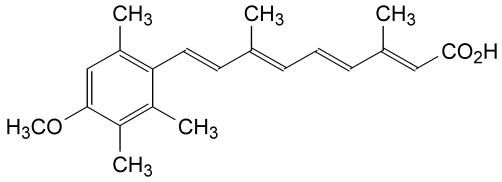
G. R = CO-CH_3: 2-[[2-(acetylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl acetate,

H. R = CO-C_6H_5: 2-[[2-(acetylamino)-6-oxo-1,6-dihydro-9*H*-purin-9-yl]methoxy]ethyl benzoate.

01/2008:1385
corrected 6.0

ACITRETIN

Acitretinum



$C_{21}H_{26}O_3$ M_r 326.4
[55079-83-9]

DEFINITION

(all-*E*)-9-(4-Methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid.

Content: 98.0 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: yellow or greenish-yellow, crystalline powder.

Solubility: practically insoluble in water, sparingly soluble in tetrahydrofuran, slightly soluble in acetone and in ethanol (96 per cent), very slightly soluble in cyclohexane.

It is sensitive to air, heat and light, especially in solution.

Carry out all operations as rapidly as possible and avoid exposure to actinic light; use freshly prepared solutions.

IDENTIFICATION

First identification: B.

Second identification: A, C.

A. Ultraviolet and visible absorption spectrophotometry (*2.2.25*).

Test solution. Dissolve 15.0 mg in 10 ml of *tetrahydrofuran R* and dilute immediately to 100.0 ml with the same solvent. Dilute 2.5 ml of this solution to 100.0 ml with *tetrahydrofuran R*.

Spectral range: 300-400 nm.

Absorption maximum: at 358 nm.

Specific absorbance at the absorption maximum: 1350 to 1475.

B. Infrared absorption spectrophotometry (*2.2.24*).

Preparation: discs.

Comparison: *acitretin CRS*.

C. Examine the chromatograms obtained in the assay.

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

TESTS

Related substances. Liquid chromatography (*2.2.29*). *Maintain the sampler at 4 °C.*

Test solution (a). Dissolve 25.0 mg of the substance to be examined in 5 ml of *tetrahydrofuran R* and dilute immediately to 100.0 ml with *anhydrous ethanol R*.

Test solution (b). Dilute 10.0 ml of test solution (a) to 25.0 ml with *anhydrous ethanol R*.

Reference solution (a). Dissolve 25.0 mg of *acitretin CRS* in 5 ml of *tetrahydrofuran R* and dilute immediately to 100.0 ml with *anhydrous ethanol R*. Dilute 10.0 ml of this solution to 25.0 ml with *anhydrous ethanol R*.

Reference solution (b). Dissolve 1.0 mg of *tretinoin CRS* in *anhydrous ethanol R* and dilute to 20.0 ml with the same solvent. Mix 5.0 ml of this solution with 2.5 ml of reference solution (a) and dilute to 100.0 ml with *anhydrous ethanol R*.

Reference solution (c). Dilute 2.5 ml of reference solution (a) to 50.0 ml with *anhydrous ethanol R*. Dilute 3.0 ml of this solution to 20.0 ml with *anhydrous ethanol R*.

Column:

– *size l* = 0.25 m, Ø = 4 mm;

– *stationary phase*: microparticulate *octadecylsilyl silica gel for chromatography R* (5 µm) with a specific surface area of 200 m^2/g, a pore size of 15 nm and a carbon loading of 20 per cent;

– *temperature*: 25 °C.

Mobile phase: a 0.3 per cent *V/V* solution of *glacial acetic acid R* in a mixture of 8 volumes of *water R* and 92 volumes of *anhydrous ethanol R*.

Flow rate: 0.6 ml/min.

Detection: spectrophotometer at 360 nm.

Injection: 10 µl of test solution (a) and reference solutions (b) and (c).

Run time: 2.5 times the retention time of acitretin.

Retention time: impurity A = about 4.8 min; tretinoin = about 5.2 min; acitretin = about 6.2 min; impurity B = about 10.2 min.

System suitability: reference solution (b):

– *resolution*: minimum 2.0 between the peaks due to acitretin and tretinoin; if necessary, adjust the concentration of *anhydrous ethanol R*.

Limits:

– *impurities A, B*: for each impurity, not more than the area of the peak due to acitretin in the chromatogram obtained with reference solution (c) (0.3 per cent);

– *total*: not more than the area of the peak due to acitretin in the chromatogram obtained with reference solution (b) (1.0 per cent);

– *disregard limit*: 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c).

Palladium: maximum 10.0 ppm.

Atomic absorption spectrometry (*2.2.23, Method I*).

Test solution. Introduce 2.0 g into a quartz beaker and add 3 ml of *magnesium nitrate solution R*. Heat in a muffle furnace to 350 °C at a rate of 40 °C/min to incinerate the content. Ignite at about 450 °C for 8 h and then at 550 ± 50 °C for a further hour. Dissolve the residue in a mixture of 0.75 ml of *hydrochloric acid R* and 0.25 ml of *nitric acid R*, warming gently. Cool, then transfer the solution into a volumetric flask containing *water R* and dilute to 50.0 ml with the same solvent.