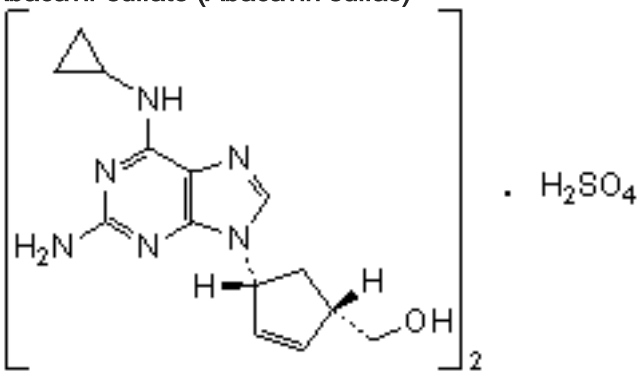
Abacavir sulfate (Abacaviri sulfas)



$(C_{14}H_{18}N_6O)_2, H_2SO_4$

Relative molecular mass. 670.7.

Chemical name. Abacavir sulfate is bis({(1S,4R)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]cyclopent-2-en-1-yl}methanol) sulfate; CAS Reg. No. 188062-50-2.

Description. White to almost white powder.

Solubility. Soluble in water.

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

Storage. Abacavir sulfate should be kept in a well-closed container.

Requirements

Definition. Abacavir sulfate contains not less than 99.0% and not more than 101.0% of $(C_{14}H_{18}N_6O)_2,H_2SO_4$ calculated with reference to the anhydrous substance.

Manufacture. The production method is validated to demonstrate that the substance, if tested, would comply with a limit of not more than 0.5% for (1R, 4S)-abacavir enantiomer using a suitable chiral chromatographic method.

Identity tests

• Either tests A, B, D and E or tests C, D and E may be applied.

A. Carry out test A.1 or, where UV detection is not available, test A.2.

A.1 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using silica gel R6 as the coating substance and a mixture of 8 volumes of dichloromethane R and 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 5 mg of the test substance per mL and (B) 5 mg of abacavir sulfate RS per mL. After removing the plate from the chromatographic chamber allow it to dry exhaustively in air or in a current of cool air. Examine the chromatogram in ultraviolet light (254 nm).

The principal spot obtained with solution (A) corresponds in position, appearance and intensity to that obtained with solution (B).

A.2 Carry out the test as described under *1.14.1 Chromatography*, Thin-layer chromatography, using the conditions described above under test A.1 but using silica gel R5 as the coating substance. Spray with vanillin/sulfuric acid TS1. Heat the plate for a few minutes at 120 °C. Examine the chromatogram in daylight.

The principal spot obtained with solution A corresponds in position, appearance and intensity to that obtained with solution B.

B. The absorption spectrum (1.6) of a 15 µg per mL solution, when observed between 210 and 300 nm, exhibits a maximum at about 291nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 399 and 441 nm.

C. Carry out the examination as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from abacavir sulfate RS or with the *reference spectrum* of abacavir sulfate.

D. Determine the specific optical rotation (1.4) using a 10 mg/mL solution and calculate with reference to the anhydrous

substance; $[\alpha]_D^{20\,°C}$ = -53° to -57°.

E. A 10 mg/mL solution yields reaction A described under 2.1 General identification tests, as characteristic of sulfates.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under 2.2.3 Limit test for heavy metals, Procedure 1; determine the heavy metal content according to Method A; not more than 20 µg/g.

Sulfated ash (2.3). Not more than 2.0 mg/g.

Water. Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A. Use 1.0 g of the test substance. The water content is not more than 5 mg/g.

Related substances

Carry out the test as described under *1.14.1 Chromatography*, High-performance liquid chromatography, using a stainless steel column (15 cm x 4.6 mm), packed with octadecylsilyl silica gel for chromatography (5 µm).

The mobile phases for gradient elution consist of a mixture of Mobile phase A and Mobile phase B, using the following conditions:

Mobile phase A: 0.05% solution of trifluoroacetic acid R.

Mobile phase B: 85 volumes of methanol R and 15 volumes of water.

Time (min)	Mobile phase A (%v/v)	Mobile phase B (%v/v)	Comments
0 - 20	95 to 70	5 to 30	Linear gradient
20 - 35	70 to 10	30 to 90	Linear gradient
35 - 40	10 to 95	90 to 5	Return to initial composition
40 - 45	95	5	Re-equilibration

Operate with a flow rate of 0.8 mL per minute and the column oven temperature at 30 °C. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Prepare the following solutions in the dissolution solvent prepared by diluting 1 mL of phosphoric acid (~1440g/l) TS in 1 litre of water.

For solution (1) dissolve 10 mg of the test substance in the dissolution solvent and dilute to 50.0 mL with the same solvent. For solution (2) dilute 5.0 mL of solution (1) to 50.0 mL with the dissolution solvent. Then dilute 5.0 mL of this solution to 50.0 mL with the same solvent. For solution (3) dissolve 5 mg of abacavir sulfate for system suitability RS (containing abacavir sulfate and impurities B to F) in the dissolution solvent and dilute to 25 mL with the same solvent.

Inject separately 20 µl each of solutions (1), (2) and (3) and of the dissolution solvent in the chromatographic system. Examine the blank chromatogram for any extraneous peaks and disregard the corresponding peaks observed in the chromatogram obtained with solution (1).

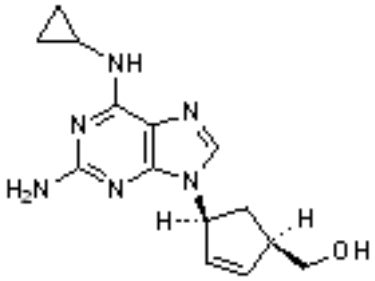
In the chromatogram obtained with solution (3) the impurity peaks are eluted at the following relative retention with reference to abacavir (retention time about 19 minutes): impurity C about 0.6; impurity D about 1.05; impurity E about 1.10; impurity B about 1.3; impurity F about 1.7. The test is not valid unless the resolution between the peaks corresponding to abacavir and impurity D is at least 1.5.

In the chromatogram obtained with solution (1) the area of any individual peak corresponding to impurity C, D, E, B or F is not greater than 0.3 times the area of the principal peak obtained with solution (2) (0.3%). The area of any other impurity peak is not greater than 0.1 times the area of the principal peak obtained with solution (2) (0.1%). The sum of the areas of all peaks, other than the principal peak, is not greater than the area of the principal peak obtained with solution (2) (1%). Disregard any peak with an area less than 0.05 times the area of the principal peak obtained with solution (2) (0.05%).

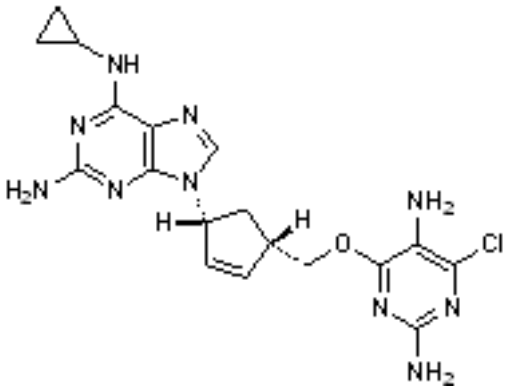
Assay. Dissolve about 0.300 g, accurately weighed, in 50 mL of water and titrate with sodium hydroxide (0.1 mol/l) VS, determining the end-point potentiometrically.

Each mL of sodium hydroxide (0.1 mol/l) is equivalent to 33.54 mg of $(C_{14}H_{18}N_6O)_2,H_2SO_4$.

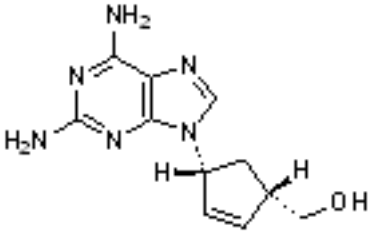
Impurities



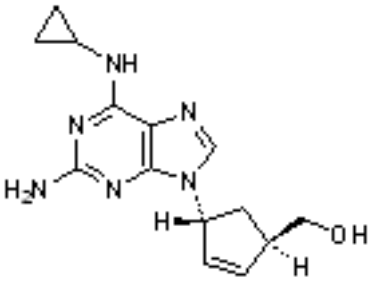
A. {(1*R*,4*S*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-en-1-yl}methanol (abacavir enantiomer) (see under Manufacture),



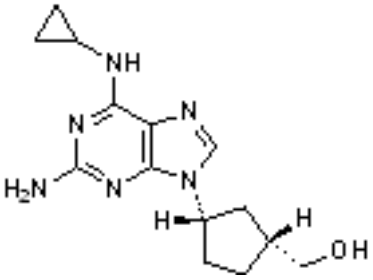
B. N^6-cyclopropyl-9-[(1*R*,4*S*)-4-{[(2,5-diamino-6-chloropyrimidin-4-yl)oxy]methyl}cyclopent-2-en-1-yl]-9*H*-purine-2,6-diamine,



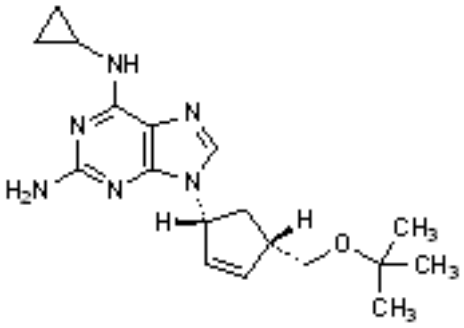
C. [(1*S*,4*R*)-4-(2,6-diamino-9*H*-purin-9-yl)cyclopent-2-en-1-yl]methanol,



D. {(1*R*,4*R*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopent-2-en-1-yl}methanol,



E. {(1*R*,3*S*)-3-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]cyclopentyl}methanol,



F. N^6-cyclopropyl-9-{(1*R*,4*S*)-4-[(1,1-dimethylethoxy)methyl]cyclopent-2-en-1-yl}-9*H*-purine-2,6-diamine.