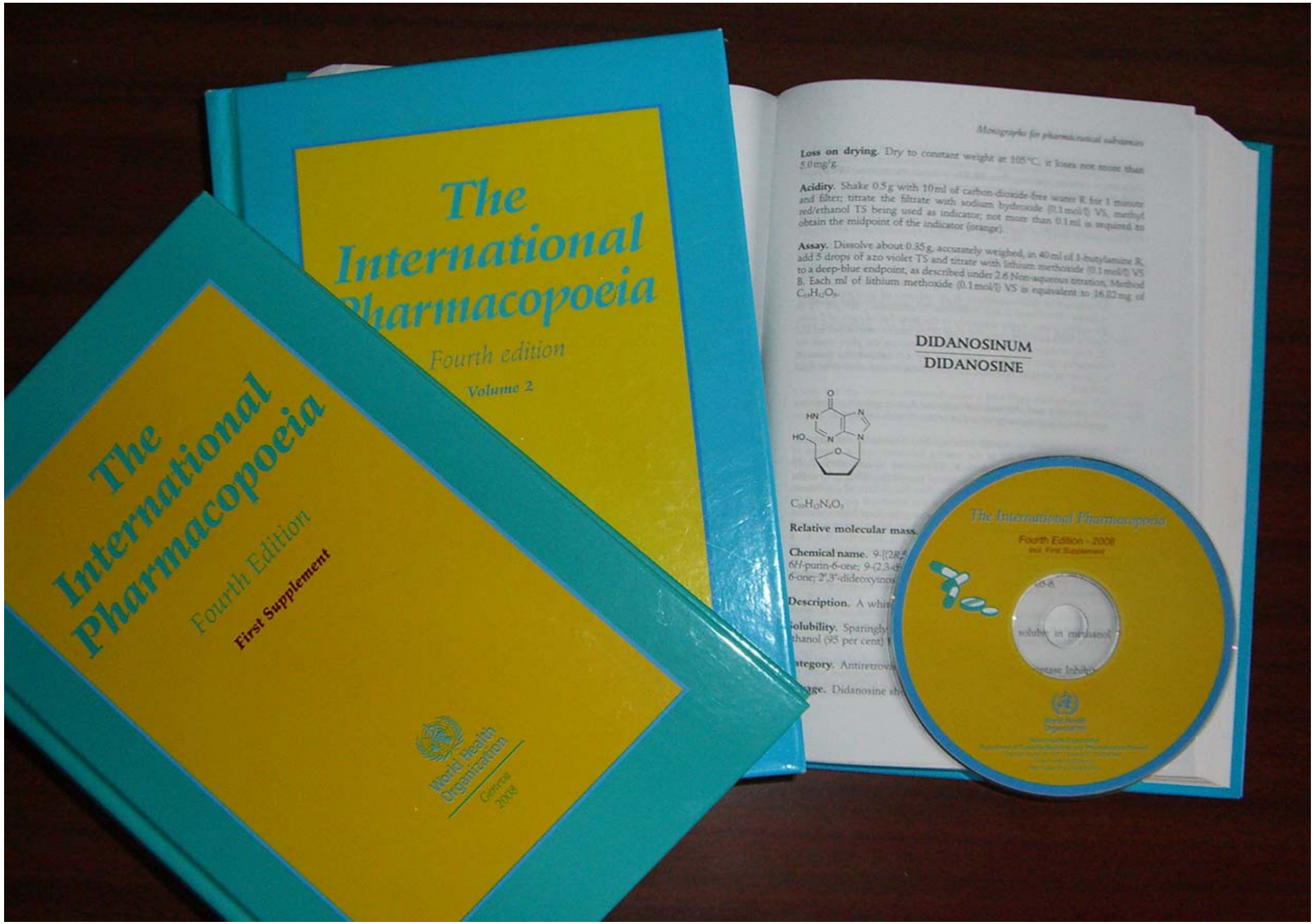
The International Pharmacopoeia
Fourth edition
Volume 2
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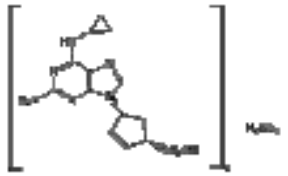
Fourth Edition
First Supplement
World Health Organization
General 2008
Monographs for pharmaceutical substances
Loss on drying. Dry to constant weight at 105 °C; it loses not more than 5.0 mg/g.
Acidity. Shake 0.5 g with 10 ml of carbon-dioxide-free water R for 1 minute and filter; titrate the filtrate with sodium hydroxide (0.1 mol/l) VS, methyl red/ethanol TS being used as indicator; not more than 0.1 ml is required to obtain the midpoint of the indicator (orange).
Assay. Dissolve about 0.35 g, accurately weighed, in 40 ml of 1-butylamine R, add 5 drops of azo violet TS and titrate with lithium methoxide (0.1 mol/l) VS to a deep-blue endpoint, as described under 2.6 Non-aqueous titration, Method B. Each ml of lithium methoxide (0.1 mol/l) VS is equivalent to 16.22 mg of $C_{10}H_{12}O_5$.
DIDANOSINUM
DIDANOSINE
$C_{10}H_{12}N_4O_3$
Relative molecular mass.
Chemical name.
Description.
Solubility.
Category.
Dosage.
The International Pharmacopoeia
Fourth Edition - 2008
incl. First Supplement

ABACAVIR SULFATE



Chemical name in accordance with IUPAC nomenclature rules

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

Relative molecular mass. 670.8

Chemical name. Abacavir sulfate is (1*S*,4*R*)-4-[2-Amino-6(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol hemisulfate; CAS Reg. No. 188062-50-2.

CAS number

Description. White to almost white powder.

Solubility. Freely 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 (1*R*, 4*S*)-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 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 cu
nm).

International chemical reference substance (ICRS)

intensity to that obtained with solution B.

A.2 Carry out the test as described under 1.14.1 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.

Cross-reference to a general method

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; $[\alpha]_D^{20°}$ between −52 ° and −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 1.14.4 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 20µl 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).

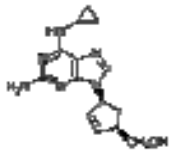
In the chromatogram obtained with solution (3), the impurity peaks are eluted at the following relative retention with reference to abacavir (retention time about 19 minutes): impurity C about 0.6; impurity D about 1.05; impurity E about 1.10; impurity B about 1.3; impurity F about 1.7. The test is not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

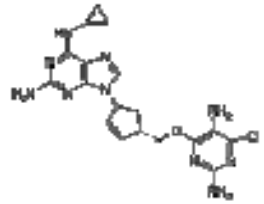
Assay. Dissolve about 0.300 g, accurately weighed, in 50 ml of water and titrate with sodium hydroxide (0.1 mol/l) VS, determining the end-point potentiometrically.

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

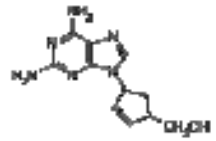
Impurities



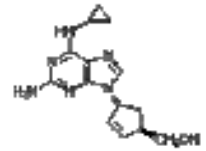
A. (1*R*, 4*S*)-abacavir sulfate enantiomer [see under Manufacture],



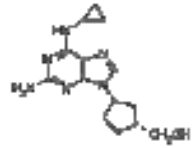
B. N^6-cyclopropyl-9-((1*R*,4*S*)-4-{[(2,5-diamino-6-chloro-4-pyrimidinyl)oxy]methyl}-2-cyclopenten-1-yl)-9*H*-purine-2,6-diamine,



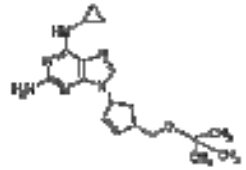
C. [(1*S*,4*R*)-4-(2,6-diamino-9*H*-purin-9-yl)-2-cyclopenten-1-yl]methanol,



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



E. {(1*R*,4*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-dimethylethyl)oxy]methyl}-2-cyclopenten-1-yl)-9*H*-purine-2,6-diamine.

ABACAVIRI COMPRESSI
ABACAVIR TABLETS

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

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

Labelling. The designation of the container of Abacavir tablets should state that the active ingredient is in the sulfate form and the quantity should be indicated in terms of the equivalent amount of abacavir.

Additional information. Strength in the current WHO Model list of essential medicines: 300 mg of abacavir. Strength in the current WHO Model list of essential medicines for children: 300 mg of abacavir.

Requirements

Cross-reference to the general monograph

Comply with the monograph for "Tablets".

Definition. Abacavir tablets contain Abacavir sulfate. They contain not less than 90.0% and not more than 110.0% of the amount of abacavir ($C_{14}H_{18}N_6O$) stated on the label.

Identity tests

• Either tests A, C and D, or tests B, C and D 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 Thin-layer chromatography, using silica R6 as the coating substance and a mixture of 8 volumes of dichloromethane R, 2 volumes of 2-propanol R as the mobile phase. Apply separately to the plate

5 µl of each of the following 2 solutions in methanol R. For solution (A) shake a quantity of the tablets containing the equivalent of 25 mg of abacavir with 5 ml, filter, and use the clear filtrate. For solution (B) use 6 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 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. See method A described under Assay. The retention time of the principal peak in the chromatogram obtained with solution (1) is similar to that in the chromatogram obtained with solution (2).

C. To a quantity of powdered tablets containing the equivalent of 15 mg abacavir add 100 ml of water R, shake and filter. Dilute 5 ml of the filtrate to 50 ml with the same solvent. The absorption spectrum (1.6) of the resulting solution, when observed between 220 nm and 320 nm, exhibits a maximum at about 291 nm.

quantity of the powdered tablets containing the equivalent of) mg of abacavir add 5 ml of water R and shake. The resulting yields reaction A described under 2.1 General identification tests as characteristic of sulfates.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using the chromatographic conditions as described under Assay method A.

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

For solution (1) transfer a quantity of the powdered tablets containing the equivalent of 10 mg of abacavir 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 solution (1), (2) and (3) and of 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 0.7; 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 due to abacavir and impurity D is at least 1.5.