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WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

DIDANOSINE
Final text for inclusion in *The International Pharmacopoeia*

DIDANOSINUM

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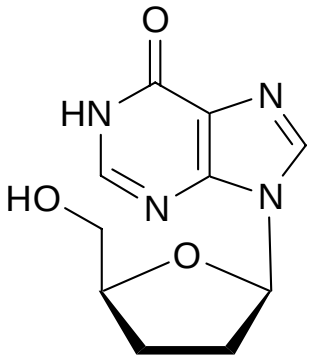


$C_{10}H_{12}N_4O_3$

Relative Molecular Mass. 236.2

Relative molecular mass.

Chemical name. 9-[(2*R*,5*S*)-5-(hydroxymethyl)tetrahydrofuran-2-yl]-1,9-dihydro-6*H*-purin-6-one; 9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one; 2',3'-dideoxyinosine (DDI); CAS Reg. No. 69655-05-6.

Description. A white to almost white powder.

Solubility. Sparingly soluble in water; slightly soluble in methanol R and ethanol (95 per cent) R

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

Storage. Didanosine should be kept in a tightly closed container.

Requirements

Didanosine contains not less than **98.5%** and not more than **101.0%** of $C_{10}H_{12}N_4O_3$, calculated with reference to the dried substance.

Identity test

- ***Either tests A and B, or test C 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 ‘‘Thin-layer chromatography’’ (Vol. 1, p. 83), using silica gel R6 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of didanosine 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 with that obtained with solution B.

A.2. Carry out the test as described under ‘‘Thin-layer chromatography’’ (Vol. 1, p. 83), using silica gel R5 as the coating substance and a mixture of 67 volumes of dichloromethane R, 20 volumes of acetonitrile R, 10 volumes of methanol R and 3 volumes of ammonia (~260 g/l) TS as the mobile phase. Apply separately to the plate 5 µl of each of 2 solutions in methanol containing (A) 1 mg of the test substance per ml and (B) 1 mg of didanosine 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. 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 with that obtained with solution B.

B. The absorption spectrum of a 10 µg/ml solution in methanol R, when observed between 210 nm and 300 nm, exhibits one maximum at about 250 nm; the specific absorbance ($A^{1\%}_{1cm}$) is between 435 to 485..

C. Carry out the examination as described under “Spectrophotometry in the infrared region” (Vol. 1, p. 40). The infrared absorption spectrum is concordant with the spectrum obtained from didanosine RS or with the *reference spectrum* of didanosine.

If the spectra are not concordant, use didanosine RS. Dissolve the sample in a small amount of methanol R, evaporate to dryness and carry out the IR spectrum with the residue as

mentioned above. Treat didanosine RS in the same way. The infrared absorption spectrum is concordant with the spectrum obtained from didanosine RS.

Specific optical rotation. Use a 10 mg/ml solution and calculate with reference to the dried substance;$[\alpha]_D^{20°C}$ –24° to –28°.

Heavy metals. Use 1.0 g for the preparation of the test solution as described under "Limit test for heavy metals", Procedure 3 (Vol. 1, p. 118); determine the heavy metals content according to Method A (Vol. 1, p. 119); not more than 20 µg/g.

Sulfated ash. Not more than 1.0 mg/g.

Loss on drying. Dry for 4 hours at 105°C; it loses not more than 5.0 mg/g.

Related substances

Note: Prepare fresh solutions and perform the tests without delay

Carry out the test as described under "High-performance liquid chromatography" (Vol. 5, p. 257), using a stainless steel column (25cm x 4.6mm), packed with octadecylsilyl base-deactivated silica gel for chromatography R (5µm)[1].

Maintain the column temperature at 20 – 25°C.

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

Mobile phase A: A 0.05 M solution of ammonium acetate R adjusted to pH 8.0 using ammonia (~260 g/l) TS.

Mobile phase B: Methanol R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)
0	92	8
18	92	8
25	70	30
45	70	30
50	92	8
60	92	8

Prepare the following solutions in a mixture of 92 volumes of mobile phase A and 8 volumes of mobile phase B (dissolution solvent).

[1] Hypersil BDS is suitable.

For solution (1) dissolve 5.0 mg of hypoxanthine R in the dissolution solvent and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml to 20.0 ml with the same solvent. For solution (2) dissolve 5 mg of didanosine for system suitability RS (containing impurities A to F) in the dissolution solvent and dilute to 10 ml with the same solvent. For solution (3) dissolve 25 mg of the test substance in the dissolution solvent and dilute to 50.0 ml with the same solvent. For solution (4) dilute 5.0 ml of solution (3) to 50.0 ml with the dissolution solvent. Then dilute 5.0 ml of this solution to 50.0 ml with the same solvent.

Operate with a flow rate of 1.0 ml per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of about 254 nm.

Use the chromatogram supplied with didanosine for system suitability RS and the chromatogram obtained with solution (2) to identify the peaks due to impurities A to F.

Inject 20µl of solution (2). The test is not valid unless the resolution factor between the peaks due to impurity (C) (2'-deoxyinosine) and impurity D (3'-deoxyinosine) is greater than 2.5, if necessary reduce the amount of methanol in the mobile phase and adjust the proportion of aqueous phase pH 8.0 accordingly.

Inject separately 20µl of solution (4) in replicate injections in the chromatographic system. The relative standard deviation for peak areas of didanosine in replicate injections of solution (4) is not more than 5.0%.

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

In the chromatogram obtained with solution (3), the following peaks are eluted at the following retention times ratio with reference to didanosine (retention time = about 13-15 min): impurity A = about 0.3; impurity B = about 0.4; impurity C = about 0.44; impurity D = about 0.48; impurity E = about 0.5; impurity F = about 0.8; impurity I = about 1.4; impurity G = about 1.6; impurity H = about 2.0.

Measure the areas of the peak responses obtained in the chromatograms from solutions (1), (3) and (4), and calculate the content of related substances as a percentage.

In the chromatogram obtained with solution (3) the area of any peak corresponding to impurity A (hypoxanthine) is not greater than the area of the principal peak obtained with solution (1) (0.5%). The area of any individual peak corresponding to impurities B, C, D, E, F or G is not greater than 0.2 times the area of the principal peak obtained with solution (4) (0.2%). The area of any other impurity peak is not greater than 0.1 times the area of the principal peak obtained with solution (4) (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 (4) (1.0%). Disregard any peak with an area less than 0.05 times the area of the principal peak obtained with solution (4) (0.05%).

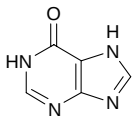
Assay

Dissolve about 0.200 g, accurately weighed, in 50 ml of glacial acetic acid R1 and titrate with perchloric acid (0.1 mol/l) VS as described under “Non-aqueous titration”; Method A (Vol. 1, p.131) determining the end point potentiometrically.

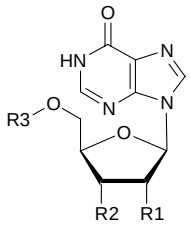
Each ml of perchloric acid (0.1 mol/l) VS is equivalent to 23.62 mg of $C_{10}H_{12}N_4O_3$.

Impurities

The following list of known and potential impurities that have been shown to be controlled by the tests in this monograph is given for information.



A. 1,7-dihydro-6*H*-purin-6-one (hypoxanthine)

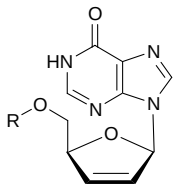


B. R1 = R2 = OH, R3 = H
9-β-D-ribofuranosyl-1,9-dihydro-6*H*-purin-6-one (inosine)

C. R1 = R3 = H, R2 = OH
9-(2-deoxy- β-D-*erythro*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2'-deoxyinosine)

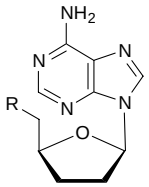
D. R1 = OH, R2 = R3 = H
9-(3-deoxy-β-D-*erythro*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (3'-deoxyinosine)

E. R1 + R2 = O, R3 = H
9-(2,3-anhydro-β-D-ribofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2',3'-anhydroinosine)



F. R = H

9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl]-1,9-dihydro-6*H*-purin-6-one; (2',3'-didehydro-2',3'-dideoxyinosine)

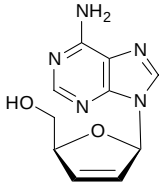


G. R = OH

9-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine (2',3'-dideoxyadenosine)

H. R = H

9-(2,3,5-trideoxy-β-D-*glycero*-pentofuranosyl)-9*H*-purin-6-amine (2',3',5'-trideoxyadenosine)



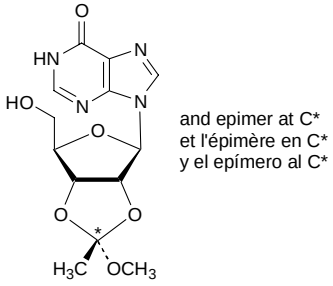
I. 9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-9*H*-purin-6-amine (2',3'-dideoxy-2',3'-didehydroadenosine)

J. structure as shown for impurities B to E where R1= R2 = H, R3 = CO-CH_3

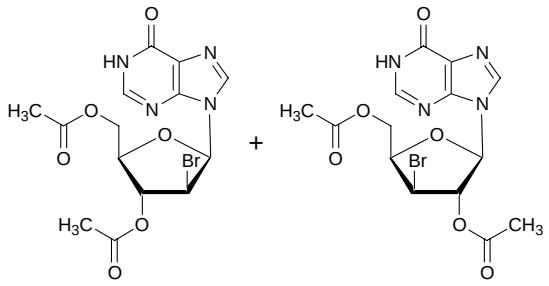
9-(5-*O*-acetyl-2,3-dideoxy-β-D-*glycero*-pentofuranosyl)-1,9-dihydro-6*H*-purin-6-one (didanosine acetate)

K. structure as shown for impurity F where R = CO-CH_3

9-(5-*O*-acetyl-2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-1,9-dihydro-6*H*-purin-6-one (2',3'-didehydrodidanosine acetate)



L.9-[2,3-*O*-[(1*RS*)-1-methoxyethylene]-β-D-ribofuranosyl]-1,9-dihydro-6*H*-purin-6-one (2',3'-*O*-(1-methoxyethylidene)inosine; "dioxalane")



M. mixture of 9-(3,5-di-*O*-acetyl-2-bromo-2-deoxy-β-D-arabinofuranosyl)-1,9-dihydro-6*H*-purin-6-one and 9-(2,5-di-*O*-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)-1,9-dihydro-6*H*-purin-6-one ("bromoesters")

Reagents

Hypoxanthine R. 1,7-dihydro-6*H*-purin-6-one; $C_5H_4N_4O$.

A commercially available reagent of suitable grade.

Description. A white, crystalline powder.

Solubility. Very slightly soluble in water, sparingly soluble in boiling water, soluble in dilute acids and in dilute alkali hydroxide solutions.

Melting point. Decomposes without melting at about 150°C.

Thin-Layer Chromatography. Examine as prescribed in the monograph on Mercaptopurine (Vol. 4, p.77-79); the chromatogram shows only one principal spot.

Silica gel for chromatography, octadecylsilyl, base-deactivated

A very finely divided silica gel, pretreated before the bonding of octadecylsilyl groups to minimise the interaction with basic components.

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