



DIDANOSINE CAPSULES

Draft proposal for *The International Pharmacopoeia* (September 2010)

REVISED DRAFT FOR COMMENT

This document was provided by a quality control expert and was discussed at the recent WHO consultation on specifications for medicines and quality control laboratory issues. Previous comments received have been incorporated into this revised draft. Should you have any comments, please send these to Dr S. Kopp, Manager, Medicines Quality Assurance Programme, Quality Assurance and Safety: Medicines, World Health Organization, 1211 Geneva 27, Switzerland; fax: (+41 22) 791 4730 or e-mails: kopps@who.int with a copy to Ms C. Mendy mendyc@who.int by 29 October 2010.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/10.356
International Pharmacopoeia monograph on Didanosine capsules

	Date
Preparation of first draft by laboratory	February 2010
Discussion of the draft proposal in the consultation on specifications for medicines and quality control laboratory issues	10-12 May 2010
Mailing of revised draft monograph for comments	July 2010
Collation of comments received	August 2010
Revised draft discussed during video-/teleconference on specifications for medicines	25 August 2010
Revised draft mailed out for comments	September 2010
Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations	18-22 October 2010
Any further action as required	...

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DIDANOSINE CAPSULES

Category. Antiretroviral (Nucleoside Reverse Transcriptase Inhibitor).

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

Additional information. Strengths in the current WHO Model list of essential medicines: 125 mg, 200 mg, 250 mg, 400 mg. Strengths in the current WHO Model list of essential medicines for children: 125 mg, 200 mg, 250 mg, 400 mg.

The capsules usually contain enteric-coated beadlets.

Requirements

Comply with the monograph for "Capsules".

Definition. Didanosine capsules contain Didanosine. They contain not less than 90.0% and not more than 110.0% of the amount of Didanosine ($C_{10}H_{12}N_4O_3$) stated on the label.

Identity tests

• Either test A alone or tests B and C may be applied.

A. To a quantity of the contents of the capsules containing 50 mg of Didanosine add 10 ml of methanol R, shake to dissolve, and filter. Evaporate the filtrate to dryness. Carry out the examination with the residue as described under 1.7 Spectrophotometry in the infrared region. The infrared absorption spectrum is concordant with the spectrum obtained from didanosine RS or with the *reference spectrum* of didanosine.

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

B.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 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 the following 2 solutions in methanol R. For solution (A) shake a quantity of the contents of the capsules containing 25 mg of Didanosine with 5 ml, filter and use the clear filtrate. For solution (B) use 5 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 to that obtained with solution B.

B.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.

C. To a quantity of the contents of the capsules containing 20 mg of Didanosine add 100 ml of methanol R, shake and filter. Dilute 5 ml of the filtrate to 100 ml with the same solvent. The absorption spectrum (1.6) of the resulting solution, when observed between 210 nm and 300 nm, exhibits one maximum at about 250 nm.

Dissolution

[Note from Secretariat: the revised general monograph for Capsules states for those capsules in which the contents, rather than the shell, resist the action of gastric fluid, a suitable dissolution test should be carried out to demonstrate the appropriate release of the active substance. It is therefore intended to add such a dissolution method rather than a disintegration test as previously indicated.]

Related substances

Prepare fresh solutions and perform the tests without delay.

Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm), packed with octadecylsilyl base-deactivated silica gel for chromatography R (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 M solution of ammonium acetate R adjusted to pH 8.0 using ammonia (~100 g/l) TS.

Mobile phase B: methanol R.

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Comments
0 - 18	92	8	Isocratic
18 - 25	92 to 70	8 to 30	Linear gradient
25 - 45	70	30	Isocratic
45 - 50	70 to 92	30 to 8	Return to initial composition
50 - 60	92	8	Re-equilibration

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

For solution (1) transfer a quantity of the contents of the capsules containing 25 mg of Didanosine into a 50-ml volumetric flask. Add about 20 ml of the dissolution solvent, sonicate for about 15 minutes and make up to volume using the dissolution solvent. Filter a portion of this solution through a 0.45- μ m filter, discarding the first few ml of the filtrate. 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 5.0 mg of hypoxanthine R in the dissolution solvent and dilute to 100.0 ml with the same solvent. Dilute 1.0 ml of this solution to 20.0 ml with the same solvent. For solution (4) dilute 5.0 ml of solution (1) to 50.0 ml with the dissolution solvent. 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 20 μ l 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 at least 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 20 μ l each of solutions (1), (3), (4) and of the mobile phase in the chromatographic system.

In the chromatogram obtained with solution (2), the following peaks are eluted at the following relative retention times with reference to didanosine (retention time about 13-15 minutes): 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.

In the chromatogram obtained with solution (1) the area of any peak corresponding to impurity A (hypoxanthine) is not greater than the area of the principal peak in the chromatogram obtained with solution (3) (1.0%). 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 in the

chromatogram 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 in the chromatogram obtained with solution (4) (0.1%). The sum of the areas of all peaks, other than the principal peak and that, if any, corresponding to impurity A, is not greater than the area of the principal peak in the chromatogram obtained with solution (4) (0.5%). Disregard any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with solution (4) (0.05%).

Assay

- Either method A or method B may be applied.

A. Carry out the test under 1.14.4 High-performance liquid chromatography, using a stainless steel column (25 cm x 4.6 mm) packed with base deactivated particles of silica gel the surface of which has been modified with chemically bonded octadecylsilyl groups (5 µm)¹.

As the mobile phase use a solution prepared as follows: 90 volumes of a 0.05 M solution of ammonium acetate R adjusted to pH 8.0 using ammonia (~100 g/l) TS and 10 volumes of methanol R.

Prepare the following solutions using the mobile phase as diluent. For solution (1) weigh and mix the contents of 20 capsules and transfer a quantity containing about 25 mg of Didanosine, accurately weighed, into a 50-ml volumetric flask. Add about 40 ml of mobile phase, sonicate for about 5 minutes, allow to cool to room temperature, and make up to volume using the same solvent. Filter a portion of this solution through a 0.45-µm filter, discarding the first few ml of the filtrate. Dilute 1.0 ml of that solution to 100.0 ml with the mobile phase. For solution (2) use 5 mg of didanosine RS per ml of mobile phase.

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

Inject separately 20 µl each of solutions (1) and (2) and record the chromatograms for 1.5 times the retention time of didanosine.

Measure the areas of the peak responses obtained in the chromatograms from solution (1) and (2), and calculate the content of Didanosine (C₁₀H₁₂N₄O₃) in the capsules.

B. Weigh and mix the contents of 20 capsules and transfer a quantity containing about 25 mg of Didanosine, accurately weighed, to a 50-ml volumetric flask. Add about 25 ml of methanol R, sonicate for about 5 minutes, allow to cool to room temperature, and make up to volume using the same solvent. Filter a portion of this solution through a 0.45-µm filter, discarding the first few ml of the filtrate. Dilute 1.0 ml of the filtrate to 50.0 ml

¹ Hypersil BDS has been found suitable.

with the same solvent. Measure the absorbance (1.6) of this solution in a 1-cm layer at the maximum at about 250 nm against a solvent cell containing methanol R.

Calculate the content of Didanosine ($C_{10}H_{12}N_4O_3$) in the capsules using an absorptivity value of 45 ($A_{1cm}^{1\%} = 450$).

Impurities. The impurities limited by the requirements of this monograph include those listed in the monograph for Didanosine.

Revised draft for comment