World Health Organization

DARUNAVIR TABLETS

(DARUNAVIRI COMPRESSI)

Draft proposal for inclusion for *The International Pharmacopoeia*

(13 August 2024)

DRAFT FOR COMMENTS

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For any technical questions, you may contact **Dr Herbert Schmidt**, Technical Officer, Norms and Standards for Pharmaceuticals, Technical Standards and Specifications (schmidth@who.int), with a copy to Ms Bezawit Kibret (kibretb@who.int)
Comments should be submitted through the online platform by **20 October 2024**. Please note that only comments received by this deadline will be considered for the preparation of this document.
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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/20.830:

DARUNAVIR TABLETS

(DARUNAVIRI COMPRESSI)

Description	**Date**
Monograph drafted based on information received from manufacturers and on laboratory investigations.	February 2020
Discussion at the consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.	27-29 April 2020
Discussion at the Consultation on Quality Control and Pharmacopoeial Specifications for medicines.	April 2023
Discussion at the Consultation on Quality Control and Pharmacopoeial Specifications for Medicines.	May 2024
Draft monograph sent out for public consultation.	August – October 2024
Presentation at the 58th Meeting of the Expert Committee on Specifications for Pharmaceutical Preparations	October 2024
Further follow-up action as required.	

DARUNAVIR TABLETS (DARUNAVIRI COMPRESSI)

Category. Antiretroviral. Protease inhibitor.

Storage. Darunavir tablets should be kept in tightly closed containers at a temperature not exceeding 30 °C.

Additional information. Strength in the current WHO Model List of Essential Medicines (EML): 75 mg, 400 mg, 600 mg and 800 mg of darunavir. Strength in the current WHO EML for children: 75 mg of darunavir.

Labelling. The designation of the container of Darunavir tablets should state that the active ingredient is Darunavir. Where Darunavir is in the ethanol solvate form, the label so indicates. The quantity should be indicated in terms of darunavir or the equivalent amount of darunavir.

Requirements

Comply with the monograph on *Tablets*.

Definition. Darunavir tablets contain Darunavir. They contain not less than 90.0% and not more than 110.0% of the amount of Darunavir ($C_{27}H_{37}N_3O_7S$) stated on the label.

Identity tests

- Either tests A or tests B and C or tests C and D may be applied.

A. To a quantity of the powdered tablets, nominally containing 25 mg of darunavir, add 5 mL of dehydrated ethanol R. Shake the suspension and filter. Add 0.2 mL of the filtrate to 300 mg potassium bromide. Dry the treated potassium bromide in an oven at 100 °C for 10 minutes. Add again 0.2 mL of the filtrate and dry at 100 °C for further 60 minutes. Prepare a disc and carry out the test as described under *1.7 Spectrophotometry in the infrared region*. The infrared absorption

spectrum is concordant with the spectrum obtained from darunavir RS similarly treated or with the reference spectrum of darunavir.

[Note from the Secretariat. Anhydrous darunavir will be used to record the reference spectrum.]

B. Carry out the test as described under *1.14.4 High-performance liquid chromatography* using the conditions given under "Assay". The retention time of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time of the peak due to darunavir in the chromatogram obtained with solution (2).

C. Use solution (1) as described under "Assay". Dilute 10.0 mL of this solution to 50.0 mL using a mixture of 50 volumes of water R and 50 volumes of acetonitrile R as the diluent (10 µg/mL). Record an absorption spectrum of the solution in the range from 200 nm to 400 nm as described under *1.6 Spectrophotometry in the visible and ultraviolet regions*. The spectrum exhibits a maximum at 266 nm.

Alternatively, in combination with identity test B, where a diode array detector is available, record the UV spectra of the principal peaks in the chromatograms with a diode array detector in the range of 200 nm to 400 nm. The retention time and UV spectrum of the principal peak in the chromatogram obtained with solution (1) corresponds to the retention time and the UV spectrum of the peak due to darunavir in the chromatogram obtained with solution (2).

D. Carry out the test as described under *1.14.1 Thin-layer chromatography*, using silica gel R2 as the coating substance and a mixture of 48 volumes of dichloromethane R, 25 volumes of methanol R, 22 volumes of ethyl acetate R and 5 volumes of ammonia (~260 g/L) TS as the mobile phase.

Apply separately to the plate 10 µL of each of the following solutions in methanol R. For solution (A), add 5 mL of methanol R to a quantity of the powdered tablets, nominally containing 25 mg of darunavir, shake and filter. For solution (B), use a

solution containing 5 mg of darunavir RS per mL. After removing the plate from the chromatographic chamber, allow it to dry in a current of air. Examine the chromatogram in ultraviolet light (254 nm). The principal spot obtained with solution (A) corresponds in position, appearance and intensity to the spot due to darunavir in the chromatogram obtained with solution (B).

Dissolution. Carry out the test as described under *5.5 Dissolution test for oral dosage forms* using as the dissolution medium 900 mL of a solution of 2% sodium laurilsulfate R in sodium dihydrogen phosphate buffer pH 3.0 and rotating the paddle at 75 revolutions per minute.

Prepare the dihydrogen phosphate buffer pH 3.0 by dissolving 6.90 g sodium dihydrogen phosphate R in about 800 mL of water R, adjusting the pH to 3.0 with Phosphoric acid (105 g/L) TS and diluting to 1000 mL with water R.

At 45 minutes, withdraw a sample of 10 mL of the medium through an in-line filter. Dilute to a concentration in the range of 0.04 to 0.07 mg/mL with dissolution medium.

Determine the content as described under *1.14.4 High-performance liquid chromatography* using the conditions given under "Assay".

For the reference solution, weigh and transfer 50.0 mg of darunavir RS into a 100.0 mL volumetric flask and add 80 mL of dissolution medium. Sonicate for 10 minutes and dilute to volume. Dilute 10.0 mL of this solution to 100.0 mL with dissolution medium.

Measure the areas of the peaks corresponding to darunavir obtained in the chromatograms of the sample and reference solution. For each of the tablets tested, calculate the total amount of darunavir ($C_{27}H_{37}N_3O_7S$) in the medium using the declared content of darunavir ($C_{27}H_{37}N_3O_7S$) in darunavir RS.

Evaluate the results as described under *5.5 Dissolution test for solid oral dosage forms, Acceptance criteria*. The amount of darunavir released is

- not less than 80% (Q) for tablets containing 75 mg, 400 mg and 600 mg of Darunavir, or
- not less than 75% (Q) for tablets containing 800 mg of Darunavir.

Related substances. 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 particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3.5 µm).[1]

Use the following conditions for gradient elution:

Mobile phase A: a mixture of 90 volumes of 0.01 M potassium dihydrogen phosphate (~1.361 g/L) TS and 10 volumes of acetonitrile R.

Mobile phase B: a mixture of 30 volumes of 0.01 M potassium dihydrogen phosphate (~1.361 g/L) TS and 70 volumes of acetonitrile R.

Time (minutes)	**Mobile phase A (% *V*/*V*)**	**Mobile phase B (% *V*/*V*)**	**Comments**
0-2	100	0	Isocratic
2–55	100 to 0	0 to 100	Linear gradient
55–55.1	0 to 100	100 to 0	Return to initial composition
55.1–60	100	0	Re-equilibration

Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 264 nm. Maintain the column temperature at 35 °C.

Prepare the following solutions using a mixture of 50 volumes of water R and 50 volumes of acetonitrile R as a diluent. For solution (1), weigh and powder 20 tablets.

[1]A Zorbax-SB-C18 column has been found suitable.

Transfer a quantity of the powdered tablets, nominally containing 250.0 mg of darunavir, into a 500.0 mL volumetric flask and add 300 mL. Sonicate and shake the flask for 10 minutes. Dilute to volume and filter. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. For solution (3), dilute 5.0 mL of solution (3) to 50.0 mL. For solution (4), prepare a solution containing darunavir for peak identification RS (containing darunavir and the impurities A, C, E, F and D) as described in the leaflet of the reference substance.

Inject 75 µL each of solutions (1), (2), (3) and (4).

Use the chromatogram obtained with solution (4) and the chromatogram supplied with darunavir for peak identification RS to identify the peaks due to the impurities A, C, E, F and D.

The impurities are eluted, if present, at the following relative retention with reference to darunavir (retention time about 36 minutes): impurity M about 0.67; impurity A about 0.84; impurity P about 0.98; impurity O about 1.03; impurity C about 1.11; impurity E about 1.13; impurity D about 1.15; impurity F about 1.16; impurity T about 1.39; impurity G about 1.40; impurity H about 1.43.

The test is not valid unless, in the chromatogram obtained with solution (4), the resolution factor between the peaks due to impurity D and due to impurity F is at least 1.0. Also, the test is not valid unless, in the chromatogram obtained with solution (3), the peak due to darunavir is obtained with a signal-to-noise ratio of at least 20.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity E is not greater than 0.4 times the area of the peak due to darunavir in the chromatogram obtained with solution (2) (0.40 %);
- the area of any peak corresponding to impurity C is not greater than 0.3 times the area of the peak due to darunavir in the chromatogram obtained with solution (2) (0.30 %);

- the area of any peak corresponding to impurity A, when multiplied by a correction factor of 1.27, is not greater than 0.25 times the area of the peak due to darunavir in the chromatogram obtained with solution (2) (0.25 %);
- the area of any peak corresponding to impurity F, when multiplied by a correction factor of 1.64, is not greater than 0.25 times the area of the peak due to darunavir in the chromatogram obtained with solution (2) (0.25 %);
- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 1.35, is not greater than 0.2 times the area of the peak due to darunavir in the chromatogram obtained with solution (2) (0.2 %);
- the area of any other impurity peak is not greater than 0.2 times the area of the peak due to darunavir in the chromatogram obtained with solution (2) (0.20 %).
- The sum of the areas of all impurity peaks, including the corrected areas of any peaks corresponding to impurities A, D and F, is not greater than 2 times the area of the peak due to darunavir in the chromatogram obtained with solution (2) (2.0 %). Disregard any peak with an area, or in the case of impurities A, D and F a corrected area, of less than the area of the peak due to darunavir in the chromatogram obtained with solution (3) (0.10%).

Assay. 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 particles of silica gel, the surface of which has been modified with chemically-bonded octadecylsilyl groups (3.5 µm).[2]

As the mobile phase use a mixture of 30 volumes of mobile phase A and 70 volumes of mobile phase B.

Operate with a flow rate of 1.0 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 264 nm. Maintain the column at a temperature of 35 °C.

[2]A Zorbax-SB-C18 column has been found suitable.

Prepare the following solutions using a mixture of 50 volumes of water R and 50 volumes of acetonitrile R as a diluent. For solution (1), weigh and powder 20 tablets. Transfer a quantity of the powdered tablets, nominally containing 250.0 mg of darunavir, into a 500.0 mL volumetric flask and add 300 mL. Sonicate and shake the flask for 10 minutes and dilute to volume. Dilute 10.0 mL of this solution to 100.0 mL and filter. For solution (2), dilute 50.0 mg of darunavir RS in 100.0 mL. Dilute 10.0 mL of this solution to 100.0 mL

Inject 10 µL each of solution (1) and (2) and record the chromatograms for 22 minutes. The retention time of darunavir is about 6 minutes.

Measure the areas of the peaks corresponding to darunavir obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of darunavir ($C_{27}H_{37}N_3O_7S$) in the tablets using the declared content of darunavir ($C_{27}H_{37}N_3O_7S$) in darunavir RS.

Impurities

- The impurities limited by the requirements of this monograph include those listed in the monograph on Darunavir.

Reference substances to be established.

Darunavir for peak identification RS (containing darunavir and the impurities A, C, E, F and D)

- ICRS to be established.

Darunavir RS

- ICRS to be established.

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