DACLATASVIR DIHYDROCHLORIDE

(DACLATASVIRI DIHYDROCHLORIDUM)

Proposal for The International Pharmacopoeia

(January 2019)

DRAFT FOR COMMENT

Should you have any comments on this draft, please send these to Dr Herbert Schmidt, Medicines Quality Assurance Programme, Technologies Standards and Norms, Department of Essential Medicines and Health Products, World Health Organization, 1211 Geneva 27, Switzerland; email: schmidt@who.int by 31 March 2018.

In order to speed up the process for receiving draft monographs and for sending comments, please let us have your email address (to jonessi@who.int) and we will add it to our electronic mailing list. Please specify if you wish to receive monographs.

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SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/18.762:

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>First draft received from collaborating laboratory</td>
<td>March 2018</td>
</tr>
<tr>
<td>Discussion at the consultation on quality control laboratory tools and specifications for medicines</td>
<td>2–4 May 2018</td>
</tr>
<tr>
<td>Draft revision sent out for public consultation</td>
<td>June–July 2018</td>
</tr>
<tr>
<td>Preparation of Rev 1 considering the comments received during the public consultation</td>
<td>August 2018</td>
</tr>
<tr>
<td>Presentation to WHO Expert Committee on Specifications for Pharmaceutical Preparations</td>
<td>October 2018</td>
</tr>
<tr>
<td>Revision 1 sent out for public consultation</td>
<td>February–March 2019</td>
</tr>
<tr>
<td>Discussion at the consultation on screening technologies and pharmacopoeial specifications for medicines</td>
<td>2-3 May 2019</td>
</tr>
<tr>
<td>Further follow-up action as required</td>
<td></td>
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</tbody>
</table>

[Note from the Secretariat. The monograph on Daclatasvir dihydrochloride is proposed for inclusion in The International Pharmacopoeia.

The methods and specifications were drafted based on information provided by manufacturers and found in the scientific literature and on laboratory investigations.]
Daclatasvir dihydrochloride

(Daclatasviri dihydrochloridum)

Molecular formula. C_{40}H_{50}N_{8}O_{6}\cdot 2\text{HCl}

Relative molecular mass. 811.8

Graphic formula

![Graphic formula of Daclatasvir dihydrochloride]

Chemical name. Dimethyl N,N'-(1,1'-biphenyl)-4,4'-diylbis[1H-imidazole-4,2-diyl][(2S)-pyrrolidine-2,1-diyl][(2S)-3-methyl-1-oxobutane-1,2-diyl]]dicarbamate (IUPAC), Carbamic acid, N,N'-(1,1'-biphenyl)-4,4'-diylbis[1H-imidazole-5,2-diyl-(2S)-2,1-pyrrolidinediyli(1S)-1-(1-methylethyl)-2-oxo-2,1-ethanediyl]]bis-, C,C'-dimethyl ester (CAS); CAS Reg. No. 1009119-65-6

Description. A white to pale yellow powder.

Solubility. Freely soluble in water, soluble in methanol, and very slightly soluble in dimethylformamide.

Category. Antiviral (nonstructural protein 5A inhibitor).

Storage. Daclatasvir dihydrochloride should be kept in a tightly closed container.

Additional information. Daclatasvir dihydrochloride may exhibit polymorphism.
Requirements

Manufacture. The production method is validated to demonstrate that genotoxic halogenated biphenyl derivatives are adequately controlled in the final product.

Definition. Daclatasvir dihydrochloride contains not less than 97.0% and not more than 102.0% (“Assay”, method A) or not less than 98.5% and not more than 101.5% (“Assay”, method B) of C₄₀H₅₀N₈O₆.2HCl, calculated with reference to the anhydrous substance.

Identity tests

- Either tests A, D and E or tests D and E together with any one of tests B or C may be applied.

A. 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 daclatasvir dihydrochloride RS or with the reference spectrum of daclatasvir dihydrochloride.

If the spectra thus obtained are not concordant repeat the test using the residues obtained by separately dissolving the test substance and daclatasvir dihydrochloride RS in a small amount of methanol R and evaporating to dryness. The infrared absorption spectrum is concordant with the spectrum obtained from daclatasvir dihydrochloride RS.

B. Carry out text B.1 or, where a diode array detector is available, test B.2.

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

The absorption spectrum (1.6) of a 10 µg per mL solution of the test substance in methanol R, when observed between 230 nm and 400 nm, exhibits one maximum at 314 nm.
B.2  Carry out the test as described under 1.14.4 High-performance-liquid chromatography using the conditions given under “Assay”, method A. Record the UV spectrum of the principal peak in the chromatograms with a diode array detector in the range of 230 nm to 400 nm. The retention time and the UV spectrum of the principal peak in the chromatogram obtained with solution (1) correspond to the retention time and the UV spectrum of the peak due to daclatasvir in the chromatogram obtained with solution (2).

C.  Carry out the test as described under 1.14.1 Thin-layer chromatography using silica gel R4 or similar as the coating substance and a mixture of 77 volumes of ethyl acetate R, 15 volumes of methanol R and 8 volumes of water R as the mobile phase. Apply separately to the plate 2 μL of each of the following 2 solutions in methanol R containing (A) 10 mg of the test substance per mL and (B) 10 mg of daclatasvir dihydrochloride RS per mL. After removing the plate from the chromatographic chamber allow it to dry in air or in a current of cool air. Examine the chromatogram in ultraviolet light (365 nm). The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

Dip the plate in modified Dragendorff reagent TS. Dry it and examine the chromatogram in daylight. The principal spot obtained with solution (A) corresponds in position, appearance and intensity with that obtained with solution (B).

D.  Carry out test D.1 or, where HPLC and the indicated chiral column are available, test D.2.

D.1  Determine the specific optical rotation (1.4) using a 10 mg/mL solution of the test substance in methanol R and calculate with reference to the anhydrous substance:

\[ \alpha_{D}^{25} = -92.0 \text{ to } -102.0. \]

D.2  Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions and solutions given under “Impurity A (daclatasvir enantiomer).” The retention time of the principal peak obtained with solution (1) corresponds to the retention time of the peak due to daclatasvir in the chromatogram obtained with solution (3).
E. Dissolve 20 mg of the test substance in 20 mL methanol R; the solution yields reaction A described under 2.1 General identification tests as characteristic of chlorides.

**pH value.** pH of a 10 mg/mL solution, 2.5-3.5.

**Impurity A (daclatasvir enantiomer).** Carry out test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (15 cm x 4.6 mm) packed with particles of silica gel, the surface of which has been modified with chemically-bonded cellulose *tris* (3,5-dichlorophenyl carbamate) (3 µm).\(^1\) As mobile phase use a mixture of 30 volumes of 1.58 g per litre ammonium bicarbonate R in water and 70 volumes of acetonitrile R.

Operate at a flow rate of 1.0 mL per minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 320 nm. Maintain the column temperature at 40 °C.

Prepare the following solutions in mobile phase. For solution (1) dissolve 25.0 mg of the test substance in 50.0 mL. For solution (2) dilute 5.0 mL of solution (1) to 100.0 mL. Dilute 2.0 mL of this solution to 100.0 mL. For solution (3) use a solution containing 0.01 mg daclatasvir impurity A RS and 0.01 mg daclatasvir dihydrochloride RS per mL.

Inject 10 µL of solution (3).

The test is not valid unless the resolution factor between the peaks due to daclatasvir (retention time about 4.5 minutes) and impurity A (relative retention of about 1.6) is at least 3.0.

Inject alternately 10 µL of solution (1) and (2).

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to impurity A is not greater than 1.5 times the area of the peak due to daclatasvir in the chromatogram obtained with solution (2) (0.15%).

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\(^1\) A Lux i-Cellulose-5 column or a Chiralpak IC-3 column were found suitable.
Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography using the conditions given under “Assay”, Method A with the following modifications:

Use the following conditions for gradient elution:

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>80</td>
<td>20</td>
<td>Isocratic</td>
</tr>
<tr>
<td>1–25</td>
<td>80 to 55</td>
<td>20 to 45</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>25–30</td>
<td>55 to 30</td>
<td>45 to 70</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>30–35</td>
<td>30</td>
<td>70</td>
<td>Isocratic</td>
</tr>
<tr>
<td>35–37</td>
<td>30 to 80</td>
<td>70 to 20</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>37–45</td>
<td>80</td>
<td>20</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Prepare the following solutions using as diluent a mixture of 80 volumes of mobile phase A and 20 volumes of mobile phase B. For solution (1) dissolve 25.0 mg of the substance to be examined and dilute to 50.0 mL. For solution (2) dilute 1.0 mL of solution (1) to 100.0 mL. Dilute 5.0 mL of this solution to 50.0 mL. For solution (3) use a solution containing 0.5 mg of daclatasvir for peak identification RS (containing daclatasvir and the impurities B, E, G, H, I and J) per mL.

Inject alternately 10 µL of solutions (1), (2) and (3).

The impurities, if present, are eluted at the following relative retentions with reference to daclatasvir (retention time about 17 minutes): impurity J about 0.21; impurity I about 0.62; impurity H about 0.76; impurities B and C about 1.12; impurity F about 1.16; impurities D and E about 1.22; impurity K about 1.39; impurity L about 1.66 and impurity G about 1.82.

Use the relative retentions and the chromatograms supplied with daclatasvir for peak
identification RS and obtained with solution (3) to identify the peaks due to the impurities B, E, G, H, I and J in the chromatograms.

The test is not valid unless in the chromatogram obtained with solution (3) the peak-to-valley ratio (Hp/Hv) is at least 20, where Hp is the height above the extrapolated baseline of the peak due to the co-eluting impurities B and C and Hv is the height above the extrapolated baseline at the lowest point of the curve separating the peak due to daclatasvir from the peak due to the co-eluting impurities B and C.

In the chromatogram obtained with solution (1):

- the sum of the areas of any peak corresponding to impurities B and C (impurities B and C co-elute) is not greater than 1.5 times the area of the peak due to daclatasvir obtained with solution (2) (0.15%);
- the sum of the areas of any peak corresponding to impurities D and E (impurities D and E co-elute) is not greater than 1.5 times the area of the peak due to daclatasvir obtained with solution (2) (0.15%);
- the area of any peak corresponding to impurity J, when multiplied by a correction factor of 0.74, is not greater than 1.5 time the peak due to daclatasvir obtained with solution (2) (0.15%);
- the area of any peak corresponding to impurity G, when multiplied by a correction factor of 1.46, is not greater than 1.5 time the peak due to daclatasvir obtained with solution (2) (0.15%);
- the area of any peak corresponding to impurities I or H is not greater than 1.5 times the area of the peak due to daclatasvir obtained with solution (2) (0.15%);
- the area of any other impurity peak is not greater than the area of the peak due to daclatasvir obtained with solution (2) (0.10%);
- the sum of the areas of all impurity peaks is not greater than 10 times the area of the principal peak obtained with solution (2) (1.0%). Disregard any peak with an area less than 0.5 times the area of the peak due to daclatasvir obtained with solution (2) (0.05%).

**Water.** Determine as described under 2.8 Determination of water by the Karl Fischer method, Method A, using 0.500 g of the substance; the water content is not more than 10 mg/g.
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 metals content according to Method A; not more than 20 μg/g.

Sulfated ash (2.3). Not more than 1.0 mg/g.

Assay

- Either method A or method B may be applied.

A. Carry out test as described under 1.14.4 High-performance liquid chromatography using a stainless steel column (15 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 (3.5 μm).²

Use the following conditions for gradient elution:

- mobile phase A: 0.1 % (v/v) solution of trifluoroacetic acid R;
- mobile phase B: mixture of 50 volumes of methanol R and 50 volumes of acetonitrile R.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
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<td>Linear gradient</td>
</tr>
<tr>
<td>13–16</td>
<td>60 to 15</td>
<td>40 to 85</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>16–18</td>
<td>15</td>
<td>85</td>
<td>Isocratic</td>
</tr>
<tr>
<td>18–20</td>
<td>15 to 70</td>
<td>85 to 30</td>
<td>Return to initial composition</td>
</tr>
<tr>
<td>20–25</td>
<td>70</td>
<td>30</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate at a flow rate of 1.0 mL/minute. As a detector use an ultraviolet spectrophotometer set at a wavelength of 304 nm. For identity test B.2 use a diode array.

² A XBridge C18 column or a Zorbax SB C18 column were found suitable.
detector in the range of 230 nm to 400 nm. Maintain the column at a temperature of
40 °C.

Prepare the following solutions using as diluent a mixture of 70 volumes of mobile phase
A and 30 volumes of mobile phase B.

For solution (1) dissolve 50.0 mg of the substance to be examined and dilute to 100.0
mL. Dilute 10.0 mL of this solution to 50.0 mL. For solution (2) dissolve 50.0 mg of
daclatasvir dihydrochloride RS and dilute to 100.0 mL. Dilute 10.0 mL of this solution to
50.0 mL.

Inject alternately 20 µL each of solutions (1) and (2).

Measure the areas of the peak responses obtained in the chromatograms from solutions
(1) and (2) and calculate the percentage content of daclatasvir dihydrochloride
(C_{40}H_{50}N_{8}O_{6}·2HCl) using the declared content of C_{40}H_{50}N_{8}O_{6}·2HCl in daclatasvir
dihydrochloride RS.

B. Dissolve about 0.3 g, accurately weighed, in 5 mL water and add 20 mL of ethanol (~750
g/L) TS. Titrate with sodium hydroxide (0.1 mol/L) VS, determining the end-point
potentiometrically. Each mL of sodium hydroxide (0.1 mol/L) VS is equivalent to 40.59
mg of C_{40}H_{50}N_{8}O_{6}·2HCl.

**Impurities**

A. dimethyl N,N'-([1,1'-biphenyl]-4,4'-dibis{1H-imidazole-4,2-diyl-[(2R)-pyrrolidine-2,1-
diyl][[(2R)-3-methyl-oxobutane-1,2-diyl]]}dicarbamate (daclatasvir enantiomer)
(synthesis related impurity)
B. methyl N-[(2R)-1-{(2S)-2-[4-(4'-{2-[{(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-}
 methylbutanoyl]pyrrolid-2-yl}-1H-imidazol-4-yl}][1,1'-biphenyl]-4-yl}-1H-imidazol-2-
yl]pyrrolid-1-yl}]-3-methyl-1-oxobutan-2-yl]carbamate (RS/SS-daclatasvir
diastereoisomer) (synthesis related impurity)

C. methyl N-[(2S)-1-{(2R)-2-[4-(4'-{2-[{(2S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-}
methylbutanoyl]pyrrolid-2-yl}-1H-imidazol-4-yl}][1,1'-biphenyl]-4-yl}-1H-imidazol-2-
yl]pyrrolid-1-yl}]-3-methyl-1-oxobutan-2-yl]carbamate (SR/SS-daclatasvir
diastereoisomer) (synthesis related impurity)

D. dimethyl N,N'-(1,1'-biphenyl)-4,4'-diylbis{1H-imidazole-4,2-diyl-[(2R)-pyrrolidine-2,1-
diyl](2S)-3-methyl-1-oxobutane-1,2-diyl})dicarbamate (SR/SR-daclatasvir
diastereoisomer) (synthesis related impurity)
E. dimethyl $N,N'$-([1,1'-biphenyl]-4,4'-diylbis{1$H$-imidazole-4,2-diyl-[(2S)-pyrrolidine-2,1-diyl][(2R)-3-methyl-oxobutane-1,2-diyl]})dicarbamate (RS/RS-daclatasvir diastereoisomem) (synthesis related impurity)

F. methyl $N$-[(2S,2S)-1-[(2S)-2-[4-(4'-{(2S)-1-[(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl]}pyrrolidin-2-yl]-1$H$-imidazol-4-yl][1,1'-biphenyl]-4-yl]-1$H$-imidazol-2-yl]pyrrolidin-1-yl]-3-methyl-1-oxopentan-2-yl]carbamate (synthesis related impurity)

G. methyl $N$-[(2S)-1-[(2S)-2-[4-(4'-{(2S)-1-[(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoyl]}pyrrolidin-2-yl]-1$H$-imidazol-4-yl][1,1'-biphenyl]-4-yl]-1,3-oxazol-2-yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate (synthesis related impurity)

H. methyl $N$-[(2S)-1-[(2S)-2-[4-(4'-{(2S)-1-acetylpyrrolidin-2-yl]-1$H$-imidazol-4-yl][1,1'-biphenyl]-4-yl]-1$H$-imidazol-2-yl]pyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl]carbamate (synthesis related impurity)
I. methyl N-[(2S)-1-{1'(2S)-2-[4-(4'-{2-[(2S)-pyrrolidin-2-yl]-1H-imidazol-4-yl]1,1'\-biphenyl]-4-yl]-1H-imidazol-2-yl]pyrrolidin-1-yl}]-3-methylbutan-2-yl]carbamate (synthesis related impurity)

J. 4,4'-([1,1'-biphenyl]-4,4'-diyl)bis{2-[(2S)-pyrrolidin-2-yl]-1H-imidazole} (synthesis related impurity)

K. di-tert-butyl [1,1'-biphenyl]-4,4'-diylbis{1H-imidazole-4,2-diyl-{(2S)-pyrrolidine-1-carboxylate}} (synthesis related impurity)

L. 1,1'-([1,1'-biphenyl]-4,4'-diyl)diethanone (synthesis related impurity)

Reference substances to be established

Daclatasvir dihydrochloride ICRS

Daclatasvir impurity A ICRS

Daclatasvir for peak identification ICRS (containing daclatasvir and the impurities B, E, G, H, I and J)