DRAFT MONOGRAPH ON SOFOSBUVIR

(SOFOSBUVIRUM)

Draft Proposal for inclusion The International Pharmacopoeia

(November 2019)

DRAFT FOR COMMENTS

Please send any comments you may have on this draft working document to Dr Herbert Schmidt, Technical Officer, Medicines Quality Assurance, Technologies Standards and Norms (email: schmidt@who.int) by 15 February 2020.

Working documents are sent out electronically and they will also be placed on the WHO Medicines website (http://www.who.int/medicines/areas/quality_safety/quality_assurance/guidelines/en/) for comments under the “Current projects” link. If you wish to receive our draft guidelines, please send your e-mail address to jonessi@who.int and your name will be added to our electronic mailing list.

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Please send any request for permission to:

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### SCHEDULE FOR THE ADOPTION PROCESS OF DOCUMENT QAS/19.799: SOFOSBUVIR (SOFOSBUVIRUM)

<table>
<thead>
<tr>
<th>Description</th>
<th>Date</th>
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<tbody>
<tr>
<td>First draft received from collaborating laboratory.</td>
<td>April 2019</td>
</tr>
<tr>
<td>Discussion at the consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.</td>
<td>2-3 May 2019</td>
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<tr>
<td>Discussion at the Fifty-fourth meeting of the Expert Committee on Specifications for Pharmaceutical Preparations.</td>
<td>14-18 October 2019</td>
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<tr>
<td>Discussion at the consultation on Screening Technologies, Laboratory Tools and Pharmacopoeial Specifications for Medicines.</td>
<td>2020</td>
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<tr>
<td>Further follow-up action as required.</td>
<td></td>
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[Note from the Secretariat. The monograph on Sofosbuvir is proposed for inclusion in The International Pharmacopoeia.]
DRAFT PROPOSAL FOR INCLUSION IN
THE INTERNATIONAL PHARMACOPOEIA

SOFOSBUVIR
(SOFOSBUVIRUM)

Molecular formula. $\text{C}_{22}\text{H}_{29}\text{FN}_3\text{O}_9\text{P}$

Relative molecular mass. 529.5

Graphic formula.

Chemical name. Propan-2-yl $N$-$\{(S)$-$\{(2R,3R,4R,5R)$-$5$-$($2,4$-$dioxo$-$3,4$-$dihydropyrimidin$-$1(2H)$-$yl)$-$4$-$fluoro$-$3$-$hydroxy$-$4$-$methyloxolan$-$2$-$yl$]-methoxy)$\}$phenoxyphosphoryl$]-L$-alaninate; CAS Reg. No. 1190307-88-0.

Description. A white to off-white powder.

Solubility. Slightly soluble in water R, freely soluble in dehydrated ethanol R and acetone R, soluble in 2-propanol R and insoluble in heptane R.

Category. Antiviral (Hepatitis C viral polymerase nucleotide inhibitor)

Storage. Sofosbuvir should be kept in a well-closed container and stored at a temperature below 30 °C.

Additional information. Sofosbuvir may exhibit polymorphism.

Definition. Sofosbuvir contains not less than 97.5 % and not more than 102.0 % of $\text{C}_{22}\text{H}_{29}\text{FN}_3\text{O}_9\text{P}$ with reference to the anhydrous substance.
Identity tests

- Either test A alone or tests B and 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 sofosbuvir RS or with the reference spectrum of sofosbuvir.

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

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 sofosbuvir in the chromatogram obtained with solution (2).

C. Carry out the test as described under 1.14.1 Thin-layer chromatography, using silica gel R2\(^1\) as the coating substance and a mixture of 6 volumes of dichloromethane R, 1 volume of methanol R, 4 volumes of ethyl acetate R and 0.1 volume 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), use 1 mg of the test substance per mL. For solution (B), use 1 mg of sofosbuvir 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 sofosbuvir in the chromatogram obtained with solution (B).

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\(^1\) Silica gel on TLC alu foils from Fluka are suitable.
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 3; determine the heavy metals content according to method A; not more than 20 µg/g.

Water. Carry out the test as described under 2.8 Determination of water by the Karl Fischer method, Method A, using about 0.200 g of the substance; the water content is not more than 10 mg/g.

Related substances. Carry out the test as described under 1.14.4 High-performance liquid chromatography, using a column (150 mm x 4.6 mm) packed with end-capped, 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:

As mobile phase A, use a mixture of 21 volumes of 0.05 % phosphoric acid (~1440 g/L) TS, 77 volumes of water R and 2 volumes of acetonitrile R. As mobile phase B, use a mixture of 21 volumes of 0.05 % phosphoric acid (~1440 g/L) TS and 79 volumes of acetonitrile R.

Use the following gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (% v/v)</th>
<th>Mobile phase B (% v/v)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 2.5</td>
<td>100</td>
<td>0</td>
<td>Isocratic</td>
</tr>
<tr>
<td>2.5 - 27.4</td>
<td>100 to 0</td>
<td>0 to 100</td>
<td>Linear gradient</td>
</tr>
<tr>
<td>27.4 - 38</td>
<td>0</td>
<td>100</td>
<td>Isocratic</td>
</tr>
<tr>
<td>38 - 45</td>
<td>100</td>
<td>0</td>
<td>Re-equilibration</td>
</tr>
</tbody>
</table>

Operate with a flow rate of 1.5 mL per minute. As a detector, use an ultraviolet spectrophotometer set at a wavelength of 260 nm and, for impurities F and G, at 205 nm. Maintain the column temperature at 30 °C.

Prepare the following solutions using as diluent a mixture of 50 volumes of mobile phase A and 50 volumes of mobile phase B. For solution (1), dissolve 50.0 mg of the test substance and dilute to 100.0 mL. For solution (2), dilute 1.0 mL of solution (1) to 100.0 mL. For

² XSelect C18 was found suitable.
solution (3), dilute 5.0 mL of solution (2) to 100.0 mL. For solution (4), dilute 1.0 mg of sofosbuvir for peak identification RS (containing sofosbuvir and the impurities A, B, C, D and E) in 5.0 mL. For solution (5), dissolve 30.0 mg of pentafluorophenol R and dilute to 100.0 mL. Dilute 5.0 mL of this solution to 100.0 mL. Dilute 5.0 mL of this solution to 100.0 mL.

Inject 20 µL each of solution (3) and (4). The test is not valid unless in the chromatogram obtained with solution (4) the peak-to-valley ratio (Hp/Hv) is at least $3^3$, where Hp is the height above the extrapolated baseline of the peak due to impurity A and Hv is the height above the extrapolated baseline at the lowest point of the curve separating this peak from the peak due to sofosbuvir. Also, the test is not valid unless in the chromatogram obtained with solution (3) the signal-to-noise of the peak due to sofosbuvir is at least 10.

Inject alternately 20 µL each of solutions (1), (2) and (5).

Use the chromatograms obtained with solutions (4) and (5) and the relative retentions below to identify the impurity peaks.

The impurities, if present, are eluted at the following relative retentions with reference to sofosbuvir (retention time about 16 minutes): impurity A about 0.98; impurity B about 1.05; impurity C about 1.41; impurity D about 0.38; impurity E about 0.93, impurity F about 1.13; impurity G about 1.57.

In the chromatogram obtained with solution (1):

- the area of any peak corresponding to either impurity A or B is not greater than 0.15 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.15 %);
- the area of any peak corresponding to impurity C, when multiplied by a correction factor of 1.5, is not greater than 0.15 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.15 %);
- the area of any peak corresponding to impurity D, when multiplied by a correction factor of 0.5, is not greater than 0.15 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.15 %);

\[3^3\] A value for the Hp/Hv has to be determined based on the available sofosbuvir for peak identification RS.
• the area of any peak corresponding to impurity E is not greater than 0.3 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.3 %);

• the area of any peak corresponding to either impurities F or G, recorded at 205 nm, is not greater than the area of the peak due to pentafluorophenol in the chromatogram obtained with solution (5) and recorded at 205 nm (0.15 %);

• the area of any other impurity peak is not greater than 0.1 times the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (0.10 %).

• The sum of the corrected areas of any peak corresponding to impurities C and D and the areas of all other impurity peaks, recorded at 260 nm, is not greater than the area of the peak due to sofosbuvir in the chromatogram obtained with solution (2) (1.0 %).

Disregard any peak with an area less than the area of the peak due to sofosbuvir in the chromatogram obtained with solution (3) (0.05 %).

**Assay.** Carry out the test as described under 1.14.4 *High-performance liquid chromatography* using the conditions given under “Related substances” with the following modifications:

As the mobile phase use a mixture of 65 volumes of mobile phase A and 35 volumes of mobile phase B.

Prepare the following solutions using mobile phase as diluent. For solution (1), dissolve 50.0 mg of the test substance and dilute to 100.0 mL. For solution (2), dissolve 50.0 mg of sofosbuvir RS and dilute to 100.0 mL.

Inject alternately 20 µL each of solutions (1) and (2). Record the chromatograms for 20 minutes.

Measure the areas of the peaks corresponding to sofosbuvir obtained in the chromatograms of solutions (1) and (2) and calculate the percentage content of sofosbuvir (C_{22}H_{29}FN_{3}O_{9}P), using the declared content of C_{22}H_{29}FN_{3}O_{9}P in sofosbuvir RS.
### Impurities

**A.** Propan-2-yl $N$-[(R)-{[(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy}phenoxyphosphoryl]-L-alanine (Rp isomer) (process related impurity).

**B.** Propan-2-yl $N$-[(S)-{[(2R,3R,4R,5R)-4-chloro-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-hydroxy-4-methyloxolan-2-yl]methoxy}phenoxyphosphoryl]-L-alanine (chloro analogue) (process related impurity).

D. 1-[(2R,3R,4R,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl)-3-methyloxan-2-yl]pyrimidine-2,4(1H,3H)-dione (fluorouridine) (process related impurity and degradation product).

E. Ethyl $N$-[(S)-[(2R,3R,4R,5R)-5-[(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyloxan-2-yl]methoxy]phenoxyphosphoryl]-L-alaninate (ethyl analogue) (process related impurity).

F. Pentafluorophenol (process related impurity).


Reference substances invoked

Sofosbuvir RS

International Chemical Reference Substance (ICRS) to be established.
Sofosbuvir for peak identification RS (containing sofosbuvir and the impurities A, B, C, D and E)

International Chemical Reference Substance (ICRS) to be established.

Sofosbuvir Reference Spectrum

Reference spectrum of sofosbuvir to be established.