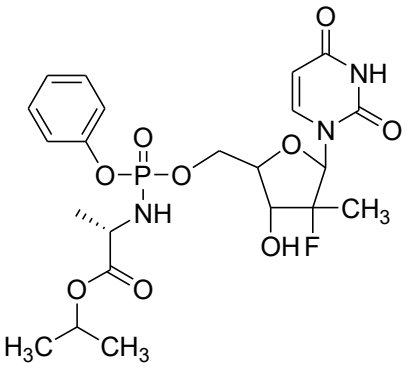
Sofosbuvir



$C_{22}H_{29}FN_3O_9P$ Mol. Wt. 529.5

Sofosbuvir is propan-2-yl (2S)-2-[[[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxy-phenoxyphosphoryl]amino]propanoate.

Sofosbuvir contains not less than 97.5 per cent and not more than 102.0 per cent of $C_{22}H_{29}FN_3O_9P$, calculated on the anhydrous basis.

Category. Antiretroviral.

Description. A white to off-white powder.

Identification

A. Determine by infrared absorption spectrophotometry (2.4.6). Compare the spectrum with that obtained with *sofosbuvir IPRS* or with the reference spectrum of sofosbuvir.

B. In the Assay, the principal peak in the chromatogram obtained with the test solution corresponds to the peak in the chromatogram obtained with reference solution (a).

Tests

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. A *0.01M ammonium acetate buffer* prepared by dissolving 0.77 g of *ammonium acetate* in 1000 ml of *water,* adjusted to pH 4.0 with *dilute acetic acid.*

Solvent mixture. 60 volumes of buffer solution and 40 volumes of *methanol.*

Test solution. Dissolve 48 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture.

Reference solution (a). A 0.048 per cent w/v solution of *sofosbuvir IPRS* in the solvent mixture.

Reference solution (b). Dilute 1.0 ml of reference solution (a) to 100.0 ml with the solvent mixture.

Reference solution (c). A solution containing 0.048 per cent w/v of *sofosbuvir IPRS* and 0.000048 per cent w/v of *sofosbuvir Rp isomer IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as Xselect HSS T3),
- sample temperature: 5°
- mobile phase: A. a 0.05 per cent v/v of *orthophosphoric acid* in *water,*
  B. *acetonitrile,*

– a gradient programme using the conditions given below,
– flow rate: 1.5 ml per minute,
– spectrophotometer set at 260 nm,
– injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
27.4	21	79
28	98	2
34	98	2

Name	Relative retention time	Correction factor
Fluoro uridine [1]	0.38	0.5
Ethyl analog[2]	0.93	-
Sofosbuvir Rp isomer[3]	0.98	-
Sofosbuvir	1.00	-
Chloro analog[4]	1.05	-
Pentafluoro phenol[5*]	1.13	-
Phosphoramidate sofosbuvir[6]	1.41	1.5
Phosphoramidate intermediate[7]	1.57	-

* This impurity is to be disregarded as controlled under Content of Pentafluoro phenol and Phosphoramidate intermediate test.
[1]2'-deoxy-2'-fluoro-2'-methyluridine,
[2](S)-2[(S)-[[(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihy2H-pyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyltetrahydro-2-furanyl]methoxy](phenoxy) phosphorylamino]propanoic acid-1-ethyl ester,
[3]propan-2-yl(2S)-2-{[(R)-{[(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl]methoxy}(phenoxy)phosphoryl]amino}propanoate,
[4]propan-2-yl(2S)-2-{[(S)-{[(2R,3R,4R,5R)-4-chloro-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-hydroxy-4-methyltetrahydrofuran-2-yl]methoxy}(phenoxy)phosphoryl]amino}propanoate,
[5]2,3,4,5,6-pentofluorophenol,
[6]propan-2-yl(2S)-2-{[{[(2R,3R,4R,5R)-2-[3S,5S)-5,8-dimethyl-3-oxido-6-oxo-3-phenoxy-2,7-dioxa-4-aza-3λ^5-phosphanon-1-yl]-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-4-methyltetrahydrofuran-3-yl]oxy}(phenoxy)phosphoryl]amino}propanoate,
[7]N-[(S)-(2,3,4,5,6-pentafluorophenoxy)phenoxyphosphinyl]-L-Alanine-1-methylethyl ester.

Inject reference solution (b) and (c). The test is not valid unless the resolution between the peaks due to sofosbuvir Rp isomer and sofosbuvir is not less than 1.5 in the chromatogram obtained with reference solution (c), the column efficiency is not less than 100000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent in the chromatogram obtained with reference solution (b).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak corresponding to fluoro uridine, Rp isomer, chloro analog and phosphoramidate sofosbuvir, each of, is not more than 0.15 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.15 per cent), the area of any peak corresponding to ethyl analog is not more than 0.3 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.3 per cent), the area of any other secondary peak is not more than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.1 per cent) and the sum of the areas of all the secondary peaks is not more than the area of the principal peak in the chromatogram obtained with the reference solution (b) (1.0 per cent). Ignore the peaks due to pentafluoro phenol and phosphoramidate intermediate and any peak with an area less than 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

Content of Pentafluoro phenol and Phosphoramidate intermediate. Determine by liquid chromatography (2.4.14).

Solvent mixture. 50 volumes of *acetonitrile* and 50 volumes of *water.*

Test solution. Dissolve 20 mg of the substance under examination in the solvent mixture and dilute to 10.0 ml with the solvent mixture.

Reference solution. A solution containing 0.0003 per cent w/v, each of, *pentafluoro phenol IPRS* and *phosphoramidate intermediate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 10 cm x 2.1 mm, packed with octadecylsilane bonded to porous silica (1.7 µm) (Such as Acquity UPLC BEH C18),
- column temperature: 45°,
- sample temperature: 5°,
- mobile phase: A. a 0.05 per cent v/v of *orthophosphoric acid* in *water,*
  B. *acetonitrile,*
- a gradient programme using the conditions given below,
- flow rate: 0.4 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 2µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	65	35
8	30	70
10	30	70
10.1	65	35
12	65	35

The relative retention time with reference to phosphoramidate intermediate for pentafluoro phenol is about 0.38.

Inject the reference solution. The test is not valid unless the column efficiency is not less than 3000 theoretical plates, the tailing factor is not more than 1.8 for pentafluoro phenol and the relative standard deviation for replicate injections is not more than 5.0 per cent for both the peaks.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to pentafluoro phenol and phosphoramidate intermediate, each of, is not more than the area of the principal peak in the chromatogram obtained with the reference solution (0.15 per cent).

Heavy metals (2.3.13). 1.0 g complies with the limit test for heavy metals, Method B (20 ppm).

Sulphated ash (2.3.18). Not more than 0.1 per cent.

Water (2.3.43). Not more than 1.0 per cent, determined on 1.0 g.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances.

Inject reference solution (a). The test is not valid unless the column efficiency is not less than 100000 theoretical plates, the tailing factor is not more than 1.5 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject reference solution (a) and the test solution.

Calculate the content of $C_{22}H_{29}FN_3O_9P$.

Storage. Store protected from moisture, at a temperature not exceeding 30°.

Solubility: Very slightly soluble in *water.*