Sofosbuvir Tablets

Sofosbuvir Tablets contain not less than 95.0 per cent and not more than 105.0 per cent of the stated amount of sofosbuvir, $C_{22}H_{29}FN_3O_9P$.

Usual strength. 400 mg.

Identification

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

Tests

Dissolution (2.5.2).
Apparatus No. 2 (Paddle),
Medium. 900 ml of a buffer solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* and 0.9 g of *sodium hydroxide* in 950 ml of *water,* adjusted to pH 6.8 and dilute to 1000 ml with *water*,
Speed and time. 75 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter, Measure the absorbance of the filtrate, suitably diluted, if necessary with the dissolution medium at about 262 nm (2.4.7). Calculate the content of sofosbuvir, $C_{22}H_{29}FN_3O_9P$ in the medium from the absorbance obtained from a solution of known concentration of *sofosbuvir IPRS* in the dissolution medium.

Q. Not less than 80 per cent of the stated amount of $C_{22}H_{29}FN_3O_9P$.

Related substances. Determine by liquid chromatography (2.4.14).

Buffer solution. Dissolve 0.77 g of *ammonium acetate* in 1000 ml *water,* adjusted to pH 4.0 with *dilute acetic acid.*

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

Test solution. Transfer 10 intact tablets to a 1000-ml volumetric flask, add about 600 ml of the buffer solution continuously stir flask vigorously using magnetic stirrer, till tablets are completely disintegrated, add 300 ml of *methanol* and stir vigorously for 60 minutes, dilute to volume with *methanol,* mix and filter. Centrifuge at 5000 rpm for 10 minutes. Dilute 3.0 ml of the clear supernatant to 25.0 ml with the solvent mixture.

Reference solution (a). A 0.00024 per cent w/v solution of *sofosbuvir IPRS* in the solvent mixture.

Reference solution (b). Dissolve 0.5 mg of *sofosbuvir Rp isomer IPRS* in 3.0 ml of *methanol* and dilute to 10.0 ml with the solvent mixture.

Reference solution (c). Dissolve 48 mg of *sofosbuvir IPRS* in 70 ml of the solvent mixture, add 1.0 ml of reference solution (b) and dilute to 100.0 ml with 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 X Select 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: 10µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	98	2
2.5	98	2
27.4	21	79
28	98	2
35	98	2

Name	Relative retention time	Correction factor
Fluoro uridine phosphate impurity[3]	0.21	0.93
Fluoro uridine impurity[1]	0.39	0.5
Uridine alanine phosphate impurity[2]	0.45	1.61
Uridine phenyl phosphate impurity[4]	0.61	1.11
Uridine isopropyl alanine phosphate impurity[5]	0.65	0.9
Phenol impurity	0.72	2.17
Ethyl analog*	0.93	—
Sofosbuvir Rp isomer*	0.98	—
Sofosbuvir (Retention time is about 16.5 minutes)	1.0	—
Chloro analog impurity*	1.05	—
Penta fluoro phenyl impurity*	1.13	—
Phosphoramidate sofosbuvir impurity*	1.41	—
Phosphoramidate intermediate impurity*	1.57	—

*Process impurity include for identification only and not included in the calculation of total degradation products.
[1]2'-deoxy-2'-fluoro-2'-methyluridine,
[2](2S)-2{[{[(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-ethyltetrahydrofuran-2-yl]methoxy}(hydroxyl) phosphoryl]amino}propanoicacid,
[3]2'-deoxy-2'-fluoro-2'-methyluridine5'-(dihydrogen phosphate),
[4]2'-deoxy-2-fluoro -5-O-[hydroxyl(phenoxy)phosphoryl]-2'-methyluridine,
[5]propan-2-yl(2S)-2-{[{[(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3hydroxy-4-methyltetrahydrofuran-2-yl]methoxy(hydroxyl).

Inject reference solution (a) 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 10.0 per cent in the chromatogram obtained with reference solution (a).

Inject reference solution (a) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to fluoro uridine impurity, uridine alanine phosphate impurity, phenol impurity, fluoro uridine phosphate impurity, uridine phenyl phosphate impurity and uridine isopropyl alanine phosphate impurity, each of, is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent), the area of any other secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent) and the sum of the areas of all the secondary peaks is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (1.0 per cent). Ignore any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14), as described under Related substances with the following modifications.

Reference solution. A 0.0048 per cent w/v solution of *sofosbuvir IPRS* in the solvent mixture.

Inject the reference solution. 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 2.0 per cent.

Inject the reference solution and the test solution.

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

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