Sofosbuvir and Velpatasvir Tablets

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Sofosbuvir and Velpatasvir Tablets

Sofosbuvir and Velpatasvir Tablets contain not less than 90.0 per cent and not more than 110.0 per cent of the stated amounts of sofosbuvir, $C_{22}H_{29}FN_3O_9P$ and velpatasvir, $C_{49}H_{54}N_8O_8$.

Usual strength. Sofosbuvir, 400 mg and Velpatasvir, 100 mg.

Identification

In the Assay, the principal peaks in the chromatogram obtained with the test solution correspond to the principal peaks in the chromatogram obtained with reference solution (c).

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 900 ml of acetate buffer prepared by dissolving 6.8 g of *sodium acetate trihydrate* and 5.0 g *cetyltrimethylammonium bromide* in 1000 ml of *water*, adjusted to pH 5.0 with *2M acetic acid*,
Speed and time. 75 rpm and 30 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

Test solution. Use the filtrate, dilute if necessary, with the dissolution medium.

Reference solution. A solution containing 0.089 per cent w/v of *sofosbuvir IPRS* and 0.0225 per cent w/v of *velpatasvir IPRS* in *methanol.* Dilute 5.0 ml of the solution to 10.0 ml with the dissolution medium.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Bakerbond Q2100),
- sample temperature: 8°,
- mobile phase: a mixture of 55 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml *water*. Add 2.0 ml of *triethylamine* and mix, adjusted to pH 2.3 with *dilute orthophosphoric acid*, and 45 volumes of *acetonitrile*,
- flow rate: 0.8 ml per minute,
- spectrophotometer set at 262 nm for sofosbuvir and 298 nm for velpatasvir,
- injection volume: 5 µl.

Inject the reference solution at 262 nm. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for sofosbuvir peak.

Inject the reference solution at 298 nm. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for velpatasvir peak.

Inject the reference solution and the test solution at 262 nm (for sofosbuvir) and at 298 nm (for velpatasvir).

Calculate content of $C_{22}H_{29}FN_3O_9P$ and $C_{49}H_{54}N_8O_8$ in the medium.

Q. Not less than 75 per cent of the stated amount of $C_{22}H_{29}FN_3O_9P$ and $C_{49}H_{54}N_8O_8$.

Related substances.

For Sofosbuvir –

Determine by liquid chromatography (2.4.14).

Solvent mixture. 30 volumes of *water* and 70 volumes of *methanol.*

Test solution. Disperse a quantity of the powdered tablets containing 400 mg of sofosbuvir in 70 ml of *methanol*, with the aid of ultrasound for 30 minutes with intermittent shaking and dilute to 100.0 ml with *water*. Centrifuge the solution at 5000 rpm for 10 minutes. Use the clear supernatant.

Reference solution (a). Dissolve 10.0 mg of s*ofosbuvir IPRS* in 3.5 ml of *methanol* with the aid of ultrasound for 5 minutes and dilute to 5.0 ml with *water*.

Reference solution (b). Dissolve 10.0 mg of *velpatasvir IPRS* in 3.5 ml of *acetonitrile* with the aid of ultrasound for 5 minutes and dilute to 5.0 ml with *water*.

Reference solution (c). Dilute 1.0 ml of reference solution (a) and 1.0 ml of reference solution (b) to 20.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution (d). Dilute 1.0 ml of reference solution (c) to 10.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (3.5 µm) (Such as XSelect HSS C -18),
- column temperature: 50^0,
- sample temperature: 8°,
- mobile phase: A. a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, add 2 ml of *triethylamine* and adjust to pH 2.8 with *orthophosphoric acid*,
  B. a mixture of 90 volumes of *acetonitrile*, 10 volumes of *water* and add 0.1 volume of *orthophosphoric acid*,
- a gradient programme using the conditions given below,
- 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)	Flow rate (ml per min.)
0	100	0	1.2
8	97	3	1.2
15	92	8	1.2
25	82	18	1.2
35	78	22	1.2
45	75	25	1.2
70	73	27	1.2
84	68	32	1.2
100	68	32	1.2
112	60	40	1.0
120	60	40	1.0
130	15	85	1.2
140	15	85	1.2
145	100	0	1.2
155	100	0	1.2

Name	Relative retention time	Correction factor
Fluoro uridine impurity[1]	0.15	0.49
Ethyl analog impurity[2*]	0.76	---
Rp-isomer[3*]	0.92	---
Penta fluoro phenol impurity[4*]	0.94	---
Amine free base impurity of velpatasvir[5]	0.99	1.16
Sofosbuvir (Retention time: about 78 minutes)	1.00	---
Chloro analog impurity[6*]	1.15	---
Disubstiuted impurity or phosphoramidate impurity[7*]	1.68	---
Phosphoramidate intermediate impurity [8*]	1.71	---

[*]Process impurity included for identification only and not included in the calculation of total degradation products.

[1]2'-Deoxy-2'-fluoro-2'-methyluridine.
[2](S)-ethyl 2-(((S)-(((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.
[3]Isopropyl ((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)-L-alaninate.
[4]2,3,4,5,6-Pentafluoro phenol,
[5]{(2S)-1[(2S,5S)-2-(9-{2-[(2S,4S)-4-(methoxymethyl)pyrrolidine-2yl]-1H-imidazol-5-yl}-1,11-dihydroisochromeno[4',3':6,7]naphthol[1,2-d] imidazole-2-yl)-5-methylprrolidin-1-yl]-3-methyl-1-oxobutan-2-yl}carbamate.
[6]Isopropyl ((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)-L-alaninate.
[7]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-methyl tetrahyrofuran-3-yl] oxy}(phenoxy)phosphoryl] amino} propanoate.
[8]propan-2-yl(2S)-2-{[(S)-pentafluorophenoxy)(phenoxy)phosphoryl]amino}propanoate.

Inject reference solution (c). The test is not valid unless the tailing factor for sofosbuvir peak is not more than 2.0.

Inject reference solution (c), (d) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to fluorouridine impurity is not more than twice the area of the sofosbuvir peak in the chromatogram obtained with reference solution (c) (0.5 per cent), the area of any other secondary peak is not more than twice the area of sofosbuvir peak in the chromatogram obtained with reference solution (c) (0.5 per cent). Ignore any peak with an area less than twice the area of sofosbuvir peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

Note- 1. Identify unknown impurities of sofosbuvir and velpatasvir, using velpatasvir Placebo (Placebo with sofosbuvir and without Velpatasvir) and sofosbuvir Placebo (Placebo with velpatasvir and without sofosbuvir).

2. Identify impurities of sofosbuvir, using velpatasvir Placebo at 260 nm and ignore these impurities in the test chromatogram at 295 nm.

For Velpatasvir –

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

- spectrophotometer set at 295 nm.

Name	Relative retention time	Correction factor
Diimidazole pharma impurity or Diimidazole impurity[1*]	0.78	---
CDMT impurity [2*]	0.86	---
Intermediate-I Dimer impurity[3*]	0.95	---
S-Moc Phenyl glycine isomer or Diastereomer impurity[4]	0.99	---
Velpatasvir (Retention time: about 112 minutes)	1.00	---
Lactone impurity[5]	1.07	0.98
Glycine dimer impurity[6*]	1.09	---

[*]Process impurity included for identification only and not included in the calculation of total degradation products.

[1]methyl {(1R)-2-[(2S,4S]-2-(5-[2-[(2S,5S)-1-[(2S)-2[(methoxycarbonyl)amino]-3-methylbutanoyl]-5-methylpyrrolidin-2-yl]-1,4,5,11-tetrahydro isochromeno[4',3':6',7'] naphthol[1,2-d]imidazole-9-yl}-1H-imidazol-2-yl)-4-(methoxymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl]carbamate.
[2]methyl {(2S)-1-[(2S,5S)-2-(9-[2-[(2S,4S)-1-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-(methoxymethyl)pyrrolidin-2-yl]-1H-imidazol-5yl]-1,11-dihydroisochromeno{4',3':6'7'} naphthol[1,2-d]imidazole-2-yl)-5-methylpyrrolidin-1-yl]-3-methyl-1-oxobutan-2-yl} carbamate.
[3]methyl {(1R)-2-[(2S,4S]-2-(5-[2-[(2S,4S)-1-[(2R)-2[(methoxycarbonyl)amino]-2-phenylacetyl}-4-(methoxymethyl)pyrrolidin-2-yl]-1,11-dihydro isochromeno[4',3':6',7'] naphthol-1,2-d]imidazole-9-yl}-1H-imidazol-2-yl)-4-(methoxymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl]carbamate.
[4]methyl {1S)-2-[(2S,4S)-2-(5-{2-[(2S,5S)-1-{(2S)-2-[(methoxycarbonyl)amino]-3-methylbutanoly}-5methylpyrrolidin-2-yl}-1,11-dihydroiso chromeno[4',3':6',7'}naphtho[1,2-d]imidazol-9-yl}-1H-imidazol-2-yl)-4-(methoxymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl}carbamate.
[5]methyl {(1R)-2-[(2S,4S]-2-(5-[2-[(2S,5S)-1-[(2S)-2[(methoxycarbonyl)amino]-3-methylbutanoyl]-5-methylpyrrolidin-2-yl]-11-oxo-1,11-dihydroisochromeno[4',3':6',7'] naphthol[1,2-d]imidazole-9-yl}-1H-imidazol-2-yl)-4-(methoxymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl]carbamate.
[6]methyl {(1R)-2-({(1R)-2-[(2S,4S)-2-(5-{5-2-[(2S,5S)-1-{(2S)-2[(methoxycarbonyl) amino]-3-methylbutanoyl}-5-methylpyrrolidin-2yl}-1,11-dihydroisochromeno[4',3':6',7'] naphthol[1,2-d]imidazole-9-yl}-1H-imidazol-2-yl)-4-(methoxymethyl)pyrrolidin-1-yl]-2-oxo-1-phenylethyl}amino)-2-oxo-1-phenylethyl]carbamate.

Inject reference solution (c). The test is not valid unless the tailing factor for velpatasvir is not more than 2.0.

Inject reference solution (c), (d) and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to the amine free base and lactone impurities, each of, is not more than twice the area of the velpatasvir peak in the chromatogram obtained with reference solution (c) (1.0 per cent), the area of any other secondary peak is not more than the area of velpatasvir peak in the chromatogram obtained with reference solution (c) (0.5 per cent). Ignore any peak with an area less than the area of velpatasvir peak in the chromatogram obtained with reference solution (d) (0.05 per cent).

The sum of all the impurities (velpatasvir and sofosvuvir specified and unspecified impurities) is not more than 2.0 per cent.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

Solvent mixture. Equal volumes of *methanol* and *water*.

Test solution. Disperse 10 intact tablets in 20 ml of *water*, with the aid of ultrasound for 10 minutes, add 400 ml of *methanol*, and stir with magnetic stirrer for 30 minutes. Further sonicate for 30 minutes with intermittent shaking, dilute to 500.0 ml with *methanol*. Centrifuge at 5000 rpm for 10 minutes. Dilute 3.0 ml of clear supernatant to 200.0 ml with the solvent mixture.

Reference solution (a). Dissolve 40 mg of *sofosbuvir IPRS* in 40 ml of *methanol* with the aid of ultrasound for 5 minutes and dilute to 50.0 ml with *methanol*.

Reference solution (b). Dissolve 40 mg of *velpatasvir IPRS* in 160 ml of *methanol* with the aid of ultrasound for 5 minutes and dilute to 200.0 ml with *methanol*.

Reference solution (c). Dilute 3.0 ml of reference solution (a) and 3.0 ml of reference solution (b) to 20.0 ml with the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm) (Such as Ace 5 C 18),
- column temperature: 35^0,
- sample temperature: 8°,
- mobile phase: A. a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen phosphate* in 1000 ml of *water*, add 2 ml of *triethylamine* and adjust to pH 2.8 with *orthophosphoric acid*,
  B. a mixture of 90 volumes of *acetonitrile*, 10 volumes of *water* and add 0.1 volume of *orthophosphoric acid*,
- a gradient programme using the conditions given below,
- flow rate: 1.1 ml per minute,
- spectrophotometer set at 262 nm for sofosbuvir and 298 nm for velpatasvir,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	80	20
8	68	32
18	48	52
25	48	52
28	15	85
40	15	85
43	80	20
50	80	20

Inject reference solution (c) at 262 nm. The test is not valid unless the resolution between the peaks due to sofosbuvir and velpatasvir is not less than 5.0, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for sofosbuvir peak.

Inject reference solution (c) at 298 nm. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 2.0 per cent for velpatasvir peak.

Inject reference solution (c) and the test solution at 262 nm and 298 nm respectively.

Calculate the content of sofosbuvir, $C_{22}H_{29}FN_3O_9P$ at 262 nm and velpatasvir, $C_{49}H_{54}N_8O_8$ at 298 nm in the tablets.

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