Sofosbuvir and Daclatasvir Tablets

Sofosbuvir and Daclatasvir Dihydrochloride Tablets

Sofosbuvir and Daclatasvir Tablets contain daclatasvir dihydrochloride equivalent to not less than 95.0 per cent and not more than 105.0 per cent of the stated amounts of daclatasvir, $C_{40}H_{50}N_8O_6$ and sofosbuvir, $C_{22}H_{29}FN_3O_9P$.

Usual strength. Sofosbuvir, 400 mg and Daclatasvir, 60 mg.

Identification

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

Tests

Dissolution (2.5.2).

Apparatus No. 2 (Paddle),
Medium. 1000 ml of *phosphate buffer pH 6.8* with 0.75 per cent w/v *polyoxyethylene glycol dodecyl ether (Brij 35)* solution prepared by dissolving 6.8 g of *potassium dihydrogen phosphate* and 0.9 g *sodium hydroxide* in 1000 ml of *water*, adjusted to pH 6.8 with *0.2 M sodium hydroxide.* Add 7.5 g of *polyoxyethylene glycol dodecyl ether* and sonicate to dissolve and mix,
Speed and time. 75 rpm and 15 minutes.

Withdraw a suitable volume of the medium and filter.

Determine by liquid chromatography (2.4.14).

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

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

Reference solution (a). A 0.132 per cent w/v solution of *daclatasvir dihydrochloride IPRS* in *methanol*.

Reference solution (b). Dissolve 40 mg of *sofosbuvir IPRS* in 70 ml of the solvent mixture, add 5.0 ml of reference solution (a) 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 µm) (Such as Inertsil ODS 3V),
- sample temperature: 10°,
- mobile phase: A. 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 2.5 with *dilute orthophosphoric acid,*

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 262 nm for sofosbuvir and 318 nm for daclatasvir,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
10	45	55
15	20	80
17	75	25
22	75	25

The elution order is the daclatasvir followed by sofosbuvir peak.

Inject reference solution (b) at 318 nm and 262 nm. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent for daclatasvir and sofosbuvir peak, respectively.

Inject reference solution (b) and the test solution at 318 nm (for daclatasvir) and at 262 nm (for sofosbuvir).

Calculate content of $C_{40}H_{50}N_8O_6$ and $C_{22}H_{29}FN_3O_9P$ in the medium.

Q. Not less than 80 per cent of the stated amount of $C_{40}H_{50}N_8O_6$ and $C_{22}H_{29}FN_3O_9P$.

Related substances.

For Daclatasvir –

Determine by liquid chromatography (2.4.14).

Test solution. Disperse a quantity of powdered tablets containing 60 mg of Daclatasvir in 70 ml of *methanol*, with the aid of ultrasound for 30 minutes with intermittent shaking and dilute to 100.0 ml with *methanol*. Centrifuge the solution at 5000 rpm for10 minutes. Use the clear supernatant.

Reference solution. A 0.033 per cent w/v solution of *daclatasvir dihydrochloride IPRS* in *methanol.* Dilute 1.0 ml of the solution to 100.0 ml with *methanol.*

Chromatographic system

- a stainless steel column 25 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (Such as ACE 5 C18-HL),
- sample temperature: 10°,
- mobile phase: A. a 0.136 per cent w/v of *potassium dihydrogen phosphate* in *water*, add 2.0 ml of *triethylamine* and mix, adjusted to pH 2.5 with *orthophosphoric acid,*
  B. a mixture of 10 volumes of *water* and 90 volumes of *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.2 ml per minute,
- spectrophotometer set at 300 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	95	5
8	95	5
15	85	15
25	85	15
35	75	25
45	70	30
55	60	40
65	50	50
85	45	55
90	48	52
95	20	80
105	20	80
110	95	5
120	95	5

Name	Relative retention time
Coupled amine hydrochloride impurity*	0.49
Mono impurity*	0.85
Acetyl impurity*	0.87
Daclatasvir (Retention time is about 41minutes)	1.0
SSSR-Diastermer*	1.08
RSSR-Diasteromer*	1.14
Oxazolidine impurity*	1.48

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

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any secondary peak is not more than 0.4 times the area of the principal peak in the chromatogram obtained with the reference solution (0.2 per cent). Ignore the peak due to sofosbuvir and any peak with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

For Sofosbuvir –

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

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

Test solution. Disperse a quantity of powdered tablets containing 0.4 g of Sofosbuvir in the 75 ml of the solvent mixture, with the aid of ultrasound for 30 minutes with intermittent shaking and dilute to 100.0 ml with the solvent mixture. Centrifuge the solution at 5000 rpm for about 10 minutes. Use the clear supernatant.

Reference solution. A 0.002 per cent w/v solution of *sofosbuvir IPRS* in the solvent mixture.

- sample temperature: 25°,
- spectrophotometer set at 262 nm.

Name	Relative retention time	Correction factor
Fluoro uridine impurity	0.14	0.49
Ethyl analog impurity*	0.91	---
Sofosbuvir Rp isomer*	0.97	---
Sofosbuvir	1.00	---
Chloro analog impurity*	1.06	---
Penta fluoro phenyl impurity*	1.09	---
Disubstitued impurity*	1.53	---
Phosphoramidate intermediate impurity*	1.73	---

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

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0.

Inject the reference solution and the test solution. In the chromatogram obtained with the test solution the area of any peak corresponding to fluoro uridine impurity is not more than 0.4 times the area of the principal peak in the chromatogram obtained with the reference solution (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 the reference solution (0.2 per cent). Ignore any peak due to daclatasvir and with an area less than 0.1 times the area of the principal peak in the chromatogram obtained with the reference solution (0.05 per cent).

The sum of all the impurities (daclatasvir and sofosvuvir impurities) is not more than 1.0 per cent.

Uniformity of content. Complies with the test stated under Tablets.

Determine by liquid chromatography (2.4.14).

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

Test solution. Disperse 1 intact tablet in 5 ml of *water*, with the aid of ultrasound for 10 minutes. Add 30 ml of *methanol,* sonicate for 60 minutes with intermittent shaking and dilute to 50.0 ml with *methanol*. Dilute 5.0 ml of the solution to 100.0 ml with the solvent mixture.

Reference solution. A 0.066 per cent w/v solution of *daclatasvir dihydrochloride IPRS* in *methanol*. Dilute 1.0 ml of the solution to 10.0 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 Inertsil ODS 3V),
- column temperature: 35°,
- sample temperature: 10°,
- mobile phase: A. 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 2.5 with *dilute orthophosphoric acid,*

  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1.3 ml per minute,
- spectrophotometer set at 318 nm.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	75	25
2.5	45	55
4	75	25

6	75	25

Inject the reference solution. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{40}H_{50}N_8O_6$ in the tablet.

Other tests. Comply with the tests stated under Tablets.

Assay. Determine by liquid chromatography (2.4.14).

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

Test solution (a). Weigh and powder 20 tablets. Disperse a quantity of powder containing 0.3 g of Daclatasvir in 20 ml *water,* with the aid of ultrasound for 10 minutes. Add 180 ml of *methanol,* sonicate for 60 minutes with intermittent shaking and dilute to 250.0 ml with *methanol.* Centrifuge at 5000 rpm for 10 minutes. Dilute 5.0 ml of clear supernatant to 100.0 ml with the solvent mixture.

Test solution (b). Dilute 10.0 ml of test solution (a) to 25.0 ml with the solvent mixture.

Reference solution. A solution containing 0.066 per cent w/v of *daclatasvir dihydrochloride IPRS* and 0.16 per cent w/v of *sofosbuvir IPRS* in *methanol*. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Use chromatographic system as described under Dissolution.

Inject the reference solution at 318 nm and 262 nm. The test is not valid unless the tailing factor is not more than 2.0 and the relative standard deviation for replicate injection is not more than 2.0 per cent for daclatasvir and sofosbuvir peak, respectively.

Inject the reference solution and test solution (a) at 318 nm (for daclatasvir).

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

Calculate the content of $C_{40}H_{50}N_8O_6$ and $C_{22}H_{29}FN_3O_9P$ in the tablets.

Each mg of daclatasvir hydrochloride, $C_{40}H_{52}Cl_2N_8O_6$ is equivalent to 0.91 mg of daclatasvir, $C_{40}H_{50}N_8O_6$.

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

Labeling. The label states the strength in terms of the equivalent amount of daclatasvir and sofosbuvir.