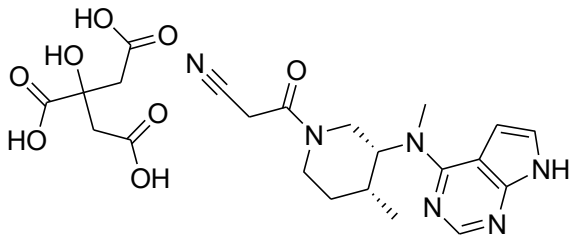
Tofacitinib Citrate



$C_{22}H_{28}N_6O_8$ Mol. Wt. 504.5

Tofacitinib Citrate is 3-{(3R,4R)-4-methyl-3-[methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino]piperidin-1-yl}-3-oxo propanenitrile 2-hydroxypropane-1,2,3-tricarboxylic acid.

Tofacitinib Citrate contains not less than 98.0 per cent and not more than 102.0 per cent of $C_{22}H_{28}N_6O_8$, calculated on the anhydrous basis.

Category. Rheumatoid Arthritis.

Description. A white to off-white powder.

Identification

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

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

Tests

Specific optical rotation (2.4.22). +26.0° to +32.0°, determined in a 0.25 per cent w/v solution in *water*.

Related substances. Determine by liquid chromatography (2.4.14).

Solvent mixture. 80 volumes of *water* and 20 volumes of *acetonitrile.*

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture, with the aid of ultrasound for 5 minutes and dilute to 50.0 ml with the solvent mixture.

Reference solution (a). A solution containing 0.0002 per cent w/v, each of, *DCT IPRS, diastereomer of tofacitinib citrate impurity IPRS* and 0.1 per cent w/v of *tofacitinib citrate IPRS* in the solvent mixture.

Reference solution (b). A 0.00015 per cent w/v solution of *tofacitinib citrate IPRS* in the solvent mixture.

Reference solution (c). Dilute 3.0 ml of reference solution (b) 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 (5 µm), (Such as ACE 5 C-18-PFP),
- column temperature: 35º,
- mobile phase: A. a buffer solution prepared by dissolving 2.72 g of *potassium dihydrogen orthophosphate* in 1000 ml of *water*,
  B. *acetonitrile*,
- a gradient programme using the conditions given below,
- flow rate: 1 ml per minute,
- spectrophotometer set at 210 nm,
- injection volume: 10 µl.

Time (in min.)	Mobile phase A (per cent v/v)	Mobile phase B (per cent v/v)
0	79	21
15	79	21
25	35	65
30	35	65
32	79	21
40	79	21

Name	Relative retention time	Correction factor
Citric acid	0.22	-
DCT[1]	0.34	0.67
Tofacitinib	1.0	-
Diastereomer of Tofacitinib Citrate[2]	1.18	-

[1]N-methyl-N[(3R,4R)-4-methylpiperidin-3yl]-7H-pyrrolo[2,3-d]pyrimidin-4-amine,
[2]3-[(3RS, 4SR)-4-Methyl-3- [methyl (7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino]piperidin-1-yl]-3- oxopropanenitrile.2-Hydroxy propane-1,2,3-tricarboxylic acid.

Inject reference solution (a), (b) and (c). The test is not valid unless the resolution between the peaks due to tofacitinib and diastereomer of tofacitinib citrate impurity is not less than 3.5 in the chromatogram obtained with reference solution (a), the column efficiency is not less than 9000 theoretical plates, the tailing factor is not more than 1.5, the relative standard deviation for replicate injections is not more than 5.0 per cent for tofacitinib peak in the chromatogram obtained with reference solution (b) and the signal to noise ratio is not less than 10 in the chromatogram obtained with reference solution (c).

Inject reference solution (b) and the test solution. In the chromatogram obtained with the test solution, the area of any peak corresponding to DCT and diastereomer of tofacitinib impurity, each of, is not more than 3.33 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent), the area of any other secondary peak is not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (0. 3 per cent) and the sum of areas of all the secondary peaks is not more than 6.7 times the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent). Ignore the peak due to citric acid and any peak with an area less than 0.33 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.05 per cent).

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

Water (2.3.43). Not more than 0.5 per cent, determined on 0.5 g.

Assay. Determine by liquid chromatography (2.4.14).

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

Test solution. Dissolve 50 mg of the substance under examination in the solvent mixture and dilute to 100.0 ml with the solvent mixture. Dilute 1.0 ml of the solution to 10.0 ml with the solvent mixture.

Reference solution. A 0.005 per cent w/v solution of *tofacitinib citrate IPRS* in the solvent mixture.

Chromatographic system

- a stainless steel column 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: a mixture of 75 volumes of a buffer solution prepared by dissolving 1.36 g of *potassium dihydrogen orthophosphate* and 1.75 g of *dipotassium hydrogen orthophosphate* in 1000 ml of *water* and 25 volumes of *acetonitrile*,
- flow rate: 1 ml per minute,
- spectrophotometer set at 215 nm,
- injection volume: 20 µl,

Inject the reference solution. The test is not valid unless the column efficiency is not less than 1500 theoretical plates, the tailing factor is not more than 2.0 and the relative standard deviation for replicate injections is not more than 1.0 per cent.

Inject the reference solution and the test solution.

Calculate the content of $C_{22}H_{28}N_6O_8$.

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

2.4.26 Solubility.

Tofacitinib Citrate. Very **s**lightly soluble in *water* and *methanol* and practically insoluble in *ethanol.*