Method of Analysis for Tofacitinib Citrate

Tofacitinib Citrate is (3R,4R)-4-methyl-3-(methyl-1H-pyrrolo[2,3-d] pyrimidin-4-amino)-β-oxo-1-piperidinepropionitrile,2-Hydroxy-1,2,3-propanetricarboxylic acid (1:1),

Calculated on dried basis, content of C16H20N6O.C6H8O7 should be ranging from 98.0% to 102.0%.

【Properties】

This product should be white to off-white powder. This product is soluble in dimethyl sulfoxide, slightly soluble in water, slightly soluble in methanol, slightly soluble in ethanol or acetone, and almost insoluble in acetonitrile.

【Identification】

In the chromatogram recorded under the content determination, the retention time of the main peak of the test solution should be consistent with the retention time of the main peak of the reference solution.

【Check】

Moisture Take this product, according to the moisture measurement method (the moisture should not be 0.5%)

【Related substances】

Take this product, accurately weighed, dissolved in a solvent (0.05% trifluoroacetic acid solution - acetonitrile (95:5)) to make a solution containing about 0.5mg per 1ml, as a test solution. Another amount of citric acid was added, and a solvent (0.05% trifluoroacetic acid solution-acetonitrile (95:5)) was dissolved to prepare a solution containing about 0.2 mg per 1 ml as a citrate reference solution. According to the chromatographic conditions under the content determination, accurately measure 10 μl of each of the test solution and the citrate reference solution, respectively, and inject them into the liquid chromatograph, and record the chromatogram for the impurity peak (white solvent) in the test solution. Except for

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peak and tannic acid peaks, the peak area of individual impurities shall not be greater than 0.2% according to the area normalization method, and the sum of the peak areas of each impurity shall not be greater than 1.0%.

【Residual solvent】

Take the appropriate amount of this product, accurately weighed, add dimethyl sulfoxide to make a solution containing about 0.1g per 1ml, as the test solution (currently used); another precision weigh methanol, ethanol, Appropriate amount of n-butanol, diluted with dimethyl sulfoxide to prepare a solution containing about 0.3 mg of methanol, 0.062 mg of ethanol and 0.5 mg of n-butanol per 1 ml, as a reference solution; determined by residual solvent assay. The fourth edition of the 2005 edition is determined by a capillary column with 7% cyanopropyl 7% phenyl-86% dimethylpolysiloxane (or similar polarity) as the stationary phase. The temperature is programmed and the column temperature is used. It was kept at 50 ° C for 5 min, heated at 30 °C / min to 200 °C for 5 min; the inlet temperature was 200 °C, and the detector temperature was 250 ° C. Precisely measure 1μl of the test solution and the reference solution, respectively, and inject them into the gas chromatograph, record the chromatogram, calculate the peak area according to the external standard method, the methanol should not exceed 0.3%, the ethanol should not exceed 0.5%, n-butanol should not Over 0.5%.

【Residue on ignition】

Take about 1.0g of this product and check it according to law (and the residualresidue must not exceed 0.1%)

【Heavy metal】

Take the residue left under the ignition residue and check it according to law (heavy metals should not exceed 20ppm).

【Enantiomer】

Solvent: [n-hexane: isopropanol: methanol = 1:1:1]:diethylamine=100:1
Take this product, add solvent to dissolve and dilute to make a solution containing about 0.5mg per 1ml, as the test solution; take appropriate amount of the mixed reference substance, dissolve and dilute with solvent to make 1mg solution per 1ml, as a mixture Spinel reference solution. Tested by high performance liquid chromatography (General Rule 0512). A chiral column (Chiralcel OD-H 250 x 4.6 mm 5 μm) was used as a mobile phase with n-hexane-ethanol (85:15) as the mobile phase; the detection wavelength was 220 nm; the column temperature was 25 °C, and the flow rate was 1.0 ml/min. 20 μl of the mixed reference solution was injected into the liquid chromatograph, and the enantiomers of tofacitinib and tofacitinib were

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sequentially peaked. The retention time of the enantiomers of tofatinib and tofacitinib is about 30 min and 35 min, the resolution between the two peaks is required to be ≥ 2.0, and the number of theoretical plates is greater than 2000. In addition, 20 μl of the test solution was accurately weighed and injected into a liquid chromatograph to record a chromatogram. Calculated by area normalization method, the enantiomer should not exceed 0.2%.

【 Citrate content 】

Take this product, accurately weighed, dissolved in water to make a solution containing about 1mg per 1ml, as a test solution. The sodium citrate (molecular weight of dihydrate: 294.10, molecular weight of anhydrous citric acid: 192.12) was accurately weighed and dissolved in water to prepare a solution containing about 0.4 mg per 1 ml as a reference solution. Using octadecylsilane bonded silica as a filler (250mm*4.6mm, 5μm); using 18.2mmol/L phosphate buffer, 0.1% isopropanol solution (take 2.4g potassium dihydrogen phosphate, dissolve and dilute with water)
To 1000ml, add 10ml of isopropyl alcohol, adjust the pH value to 2.4 with phosphoric acid to mobile phase A, use methanol as mobile phase B, flow rate is 1.0ml/min, column temperature is room temperature, linear gradient elution according to the following table, The detection wavelength was 210 nm. The number of theoretical plates is not less than 4,000.

Time (Min)	Mobile Phase A (%)	Mobile Phase B (%)
0	100	0
10	100	0
25	25	75
26	100	0
35	100	0

Precisely measure 20μl of the test solution into the liquid chromatograph and record the chromatogram; take the reference solution and measure it by the same method. Calculate the peak area as an anhydrate according to the external standard method, and the C6H8O7 should be 36.2%~40.0%.

【Content determination】

Measured by high performance liquid chromatography. Chromatographic conditions and system suitability test using octadecylsilane bonded silica as a filler (Kromasil C18, 4.6 × 100mm, 2.5μm); with 0.01mol / L potassium dihydrogen phosphate solution (take potassium dihydrogen phosphate 1.36g, Add 1000 ml of water to dissolve, adjust the pH to 7.0 with 1 mol/L potassium hydroxide as mobile phase A, use mobile phase B with acetonitrile, flow rate is 1.0 ml/min, column temperature is 30 ° C, linear gradient elution according to the following table The detection wavelength is 280 nm. The number of theoretical plates is not less than 4,000.

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Time (Min)	Mobile Phase A (%)	Mobile Phase B (%)
0	90	10
15	70	30
25	35	65
25.1	90	10
35	90	10

Determination method Take this product, accurately weighed, dissolved in a solvent (0.05% trifluoroacetic acid solution - acetonitrile (95:5)) to make a solution containing about 0.1 mg per 1 ml, as a test solution. Precisely measure 10 μl into the liquid chromatograph and record the chromatogram; take another reference and measure it by the same method. According to the external standard method, the peak area is calculated.

[Storage] Sealed and protected from heat and light.

[Validity Period] 24 months.

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