Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution

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Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution contains not less than 93.0% and not more than 107.0% of the labelled amount of dorzolamide ($C_{10}H_{16}N_2O_4S_3$: 324.44), and not less than 93.0% and not more than 110.0% of the labelled amount of timolol ($C_{13}H_{24}N_4O_3S$: 316.42).

Method of Preparation Prepare as directed under Ophthalmic Liquids and Solutions, with Dorzolamide Hydrochloride and Timolol Maleate.

Description Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution is a clear, colorless, and slightly viscous liquid.

Identification (1) The retention times of the peak in the chromatograms corresponding to dorzolamide obtained from the sample solution and dorzolamide from the standard solution observed in the Assay (1) are the same.

(2) The retention times of the peak in the chromatograms corresponding to timolol obtained from the sample solution and timolol from the standard solution observed in the Assay (2) are the same.

Osmotic pressure ratio Being specified separately when the drug is granted approval based on the Law.

Viscosity Being specified separately when the drug is granted approval based on the Law.

pH Being specified separately when the drug is granted approval based on the Law.

Purity (1) Related substance 1—Use the sample solution obtained in the Assay (1) as the sample solution. Pipet 1 mL of the sample solution, add a mixture of diluted phosphoric acid (1 in 500) and acetonitrile (19:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine each peak area by the automatic integration method: the peak area of the related substance OB having the relative retention time of about 1.2 to dorzolamide from the sample solution is not larger than 2.4 times the peak area of dorzolamide from the standard solution, and the peak area of the related substance OA having the relative retention time of about 0.8 to dorzolamide from the sample solution is not larger than 1/5 times the peak area of dorzolamide from the standard solution. The area of the peak other than dorzolamide and the peaks mentioned above from the sample solution is not larger than 1/5 times the peak area of dorzolamide from the standard solution. Furthermore, the total area of the peaks other than dorzolamide from the sample solution is not larger than 2.5 times the peak area of dorzolamide from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1).

Time span of measurement: For 18 minutes after injection of the sample solution.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay (1).

Test for required detectability: Pipet 2 mL of the standard solution, add a mixture of diluted phosphoric acid (1 in 500 mL) and acetonitrile (19:1) to make exactly 20 mL. Confirm that the peak area of dorzolamide obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL the standard solution.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dorzolamide is not more than 5.0%.

(2) Related substance 2—Use the sample solution obtained in the Assay (2) as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography *<2.01>* according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than timolol and the peak having the relative retention time of about 0.49 to timolol obtained from the sample solution is not larger than 2/5 times the peak area of timolol from the standard solution. Furthermore, the total area of the peaks other than timolol and the peak having the relative retention time of about 0.49 to timolol, from the sample solution is not larger than 1/2 times the peak area of timolol from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (2).

Time span of measurement: For 10 minutes after injection of the sample solution.

System suitability—

System performance and system repeatability: Proceed as directed in the system suitability in the Assay (2).

Test for required detectability: Pipet 10 mL of the standard solution, add the mobile phase to make exactly 100

mL. Confirm that the peak area of timolol obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL the standard solution.

Foreign insoluble matter <*6.11*> It meets the requirement.

Insoluble particulate matter <*6.08*> It meets the requirement.

Sterility <*4.06*> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (1) Dorzolamide hydrochloride—Pipet a volume of Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution, equivalent to about 2.5 mg of dorzolamide ($C_{10}H_{16}N_2O_4S_3$), add a mixture of diluted phosphoric acid (1 in 500) and acetonitrile (19:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Dorzolamide Hydrochloride RS (separately determine the water <*2.48*> in the same manner as Dorzolamide Hydrochloride), dissolve in a mixture of diluted phosphoric acid (1 in 500) and acetonitrile (19:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of dorzolamide in each solution.

Amount (mg) of dorzolamide ($C_{10}H_{16}N_2O_4S_3$) in 1 mL of Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution

$$= M_S / M_T \times A_T / A_S \times 1/8 \times d \times 0.899$$

M_S: Amount (mg) of Dorzolamide Hydrochloride RS taken, calculated on the anhydrous basis

M_T: Amount (g) of Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution taken

d: Density (g/mL) of Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 253 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25℃.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 500) and acetonitrile (19:1).

Mobile phase B: A mixture of acetonitrile and diluted phosphoric acid (1 in 500) (19:1).

Flowing of mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 — 15.0	100	0
15.0 — 15.1	100 → 0	0 → 100
15.1 — 20.0	0	100

Flow rate: 1.2 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution, the number of theoretical plates and the symmetry factor of the peak of dorzolamide are not less than 5000 and not more than 3.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution, the relative standard deviation of the peak area of dorzolamide is not more than 2.0%.

(2) Timolol maleate—Pipet a volume of Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution, equivalent to about 6.5 mg of timolol ($C_{13}H_{24}N_4O_3S$), add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 34 mg of Timolol Maleate RS, previously dried at 100℃ under reduced pressure for 3 hours, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <*2.01*> according to the following conditions, and determine the peak areas, A_T and A_S, of timolol in each solution.

Amount (mg) of timolol ($C_{13}H_{24}N_4O_3S$) in 1 mL of Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution

$$= M_S / M_T \times A_T / A_S \times 1/4 \times d \times 0.732$$

M_S: Amount (mg) of Timolol Maleate RS taken

M_T: Amount (g) of Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution taken

d: Density (g/mL) of Dorzolamide Hydrochloride and Timolol Maleate Ophthalmic Solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 295 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40℃.

Mobile phase: Dissolve 22.0 g of sodium dihydrogen phosphate monohydrate in water to make 2000 mL, and adjust to pH 2.8 with phosphoric acid. To 600 mL of this solution add 400 mL of methanol.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: Dissolve 44 mg of Timolol Maleate RS in 4 mL of sodium hydroxide solution (1 in 250), warm at 70℃ for 15 hours, and add the mobile phase to make 25 mL. To 5 mL of this solution, add 28 mg of Dorzolamide Hydrochloride RS to dissolve, add the mobile phase to make 25 mL, and use this solution as the solution for system suitability test. When the procedure is run with 20 μL of the solution for system suitability test according to the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of timolol are not less than 3000 and not more than 2.0, respectively. The resolution between the co-eluting peak of dorzolamide and maleate, having the relative retention time of about 0.49 to timolol, and the peak, having the relative retention time of about 0.58 to timolol , is not less than 1.5, and the resolution between the peaks having the relative retention times of about 0.58 and about 0.70 to timolol is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the solution for system suitability test, the relative standard deviation of the peak area of timolol is not more than 2.0%.

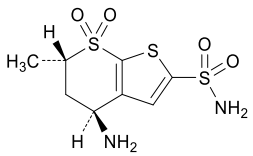
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Others

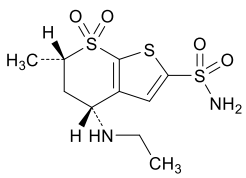
Related substance OA:

(4*S*,6*S*)-4-Amino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide



Related substance OB:

(4*RS*,6*SR*)-4-Ethylamino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide



and enantiomer

Add the following to 9.01 Reference Standards (1):

Timolol Maleate RS