

1 Dorzolamide Hydrochloride and Timolol 2 Maleate Ophthalmic Solution

3 ドルゾラミド塩酸塩・チモロールマレイン酸塩点眼液
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5 Dorzolamide Hydrochloride and Timolol Maleate
6 Ophthalmic Solution contains not less than 93.0% and
7 not more than 107.0% of the labelled amount of dor-
8 zolamide (C₁₀H₁₆N₂O₄S₃: 324.44), and not less than
9 93.0% and not more than 110.0% of the labelled
10 amount of timolol (C₁₃H₂₄N₄O₃S: 316.42).

11 **Method of Preparation** Prepare as directed under Oph-
12 thalmic Liquids and Solutions, with Dorzolamide Hydro-
13 chloride and Timolol Maleate.

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

17 **Identification (1)** The retention times of the peak in the
18 chromatograms corresponding to dorzolamide obtained
19 from the sample solution and dorzolamide from the stand-
20 ard solution observed in the Assay (1) are the same.

21 **(2)** The retention times of the peak in the chromato-
22 grams corresponding to timolol obtained from the sample
23 solution and timolol from the standard solution observed in
24 the Assay (2) are the same.

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

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

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

31 **Purity (1)** Related substance 1—Use the sample solu-
32 tion obtained in the Assay (1) as the sample solution. Pipet
33 1 mL of the sample solution, add a mixture of diluted phos-
34 phoric acid (1 in 500) and acetonitrile (19:1) to make ex-
35 actly 100 mL, and use this solution as the standard solution.
36 Perform the test with exactly 20 μ L each of the sample so-
37 lution and standard solution as directed under Liquid Chro-
38 matography <2.01> according to the following conditions,
39 and determine each peak area by the automatic integration
40 method: the peak area of the related substance OB having
41 the relative retention time of about 1.2 to dorzolamide from
42 the sample solution is not larger than 2.4 times the peak area
43 of dorzolamide from the standard solution, and the peak
44 area of the related substance OA having the relative reten-
45 tion time of about 0.8 to dorzolamide from the sample solu-
46 tion is not larger than 1/5 times the peak area of dorzolamide
47 from the standard solution. The area of the peak other than

48 dorzolamide and the peaks mentioned above from the sam-
49 ple solution is not larger than 1/5 times the peak area of dor-
50 zolamide from the standard solution. Furthermore, the total
51 area of the peaks other than dorzolamide from the sample
52 solution is not larger than 2.5 times the peak area of dor-
53 zolamide from the standard solution.

54 *Operating conditions—*

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

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

60 *System suitability—*

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

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

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

73 **(2)** Related substance 2—Use the sample solution ob-
74 tained in the Assay (2) as the sample solution. Pipet 2 mL
75 of the sample solution, add the mobile phase to make ex-
76 actly 200 mL, and use this solution as the standard solution.
77 Perform the test with exactly 20 μ L each of the sample so-
78 lution and standard solution as directed under Liquid Chro-
79 matography <2.01> according to the following conditions,
80 and determine each peak area by the automatic integration
81 method: the area of the peak other than timolol and the peak
82 having the relative retention time of about 0.49 to timolol
83 obtained from the sample solution is not larger than 2/5
84 times the peak area of timolol from the standard solution.
85 Furthermore, the total area of the peaks other than timolol
86 and the peak having the relative retention time of about 0.49
87 to timolol, from the sample solution is not larger than 1/2
88 times the peak area of timolol from the standard solution.

89 *Operating conditions—*

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

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

95 *System suitability—*

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

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

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

103 **Foreign insoluble matter** <6.11> It meets the require-
104 ment.

105 **Insoluble particulate matter** <6.08> It meets the require-
106 ment.

107 **Sterility** <4.06> Perform the test according to the Mem-
108 brane filtration method: it meets the requirement.

109 **Assay (1)** Dorzolamide hydrochloride—Pipet a volume
110 of Dorzolamide Hydrochloride and Timolol Maleate Oph-
111 thalmic Solution, equivalent to about 2.5 mg of dorzolamide
112 ($\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_4\text{S}_3$), add a mixture of diluted phosphoric acid
113 (1 in 500) and acetonitrile (19:1) to make exactly 25 mL,
114 and use this solution as the sample solution. Separately,
115 weigh accurately about 22 mg of Dorzolamide Hydrochloride
116 RS (separately determine the water <2.48> in the same
117 manner as Dorzolamide Hydrochloride), dissolve in a mix-
118 ture of diluted phosphoric acid (1 in 500) and acetonitrile
119 (19:1) to make exactly 200 mL, and use this solution as the
120 standard solution. Perform the test with exactly 20 μL each
121 of the sample solution and standard solution as directed un-
122 der Liquid Chromatography <2.01> according to the follow-
123 ing conditions, and determine the peak areas, A_T and A_S , of
124 dorzolamide in each solution.

125 Amount (mg) of dorzolamide ($\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_4\text{S}_3$) in 1 mL of
126 Dorzolamide Hydrochloride and Timolol Maleate Ophthal-
127 mic Solution

$$128 = M_S / M_T \times A_T / A_S \times 1 / 8 \times d \times 0.899$$

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

131 M_T : Amount (g) of Dorzolamide Hydrochloride and Tim-
132 olol Maleate Ophthalmic Solution taken

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

135 *Operating conditions*—

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

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

142 Column temperature: A constant temperature of about
143 25°C.

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

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

148 Flowing of mobile phase: Control the gradient by mixing
149 the mobile phases A and B as directed in the following table.
150

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

151

152 Flow rate: 1.2 mL per minute.

153 *System suitability*—

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

158 System repeatability: When the test is repeated 6 times
159 with 20 μL of the standard solution, the relative standard
160 deviation of the peak area of dorzolamide is not more than
161 2.0%.

162 (2) Timolol maleate—Pipet a volume of Dorzolamide
163 Hydrochloride and Timolol Maleate Ophthalmic Solution,
164 equivalent to about 6.5 mg of timolol ($\text{C}_{13}\text{H}_{24}\text{N}_4\text{O}_5\text{S}$), add
165 the mobile phase to make exactly 25 mL, and use this
166 solution as the sample solution. Separately, weigh
167 accurately about 34 mg of Timolol Maleate RS, previously
168 dried at 100°C under reduced pressure for 3 hours, dissolve
169 in the mobile phase to make exactly 100 mL, and use this
170 solution as the standard solution. Perform the test with
171 exactly 20 μL each of the sample solution and standard
172 solution as directed under Liquid Chromatography <2.01>
173 according to the following conditions, and determine the
174 peak areas, A_T and A_S , of timolol in each solution.

175 Amount (mg) of timolol ($\text{C}_{13}\text{H}_{24}\text{N}_4\text{O}_5\text{S}$) in 1 mL of Dorzola-
176 mid Hydrochloride and Timolol Maleate Ophthalmic So-
177 lution

$$178 = M_S / M_T \times A_T / A_S \times 1 / 4 \times d \times 0.732$$

179 M_S : Amount (mg) of Timolol Maleate RS taken

180 M_T : Amount (g) of Dorzolamide Hydrochloride and Tim-
181 olol Maleate Ophthalmic Solution taken

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

184 *Operating conditions*—

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

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

191 Column temperature: A constant temperature of about
192 40°C.

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

197 Flow rate: 1.0 mL per minute.

198 *System suitability*—

199 System performance: Dissolve 44 mg of Timolol Maleate
200 RS in 4 mL of sodium hydroxide solution (1 in 250), warm
201 at 70°C for 15 hours, and add the mobile phase to make 25
202 mL. To 5 mL of this solution, add 28 mg of Dorzolamide
203 Hydrochloride RS to dissolve, add the mobile phase to
204 make 25 mL, and use this solution as the solution for system
205 suitability test. When the procedure is run with 20 µL of the
206 solution for system suitability test according to the above
207 operating conditions, the number of theoretical plates and
208 the symmetry factor of the peak of timolol are not less than
209 3000 and not more than 2.0, respectively. The resolution
210 between the co-eluting peak of dorzolamide and maleate,
211 having the relative retention time of about 0.49 to timolol,
212 and the peak, having the relative retention time of about
213 0.58 to timolol, is not less than 1.5, and the resolution
214 between the peaks having the relative retention times of
215 about 0.58 and about 0.70 to timolol is not less than 1.5.

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

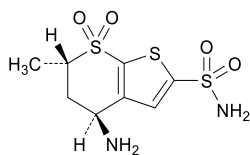
220 **Containers and storage** Containers—Tight containers.

221 Storage—Light-resistant.

222 **Others**

223 Related substance OA:

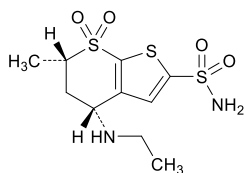
224 (4*S*,6*S*)-4-Amino-6-methyl-5,6-dihydro-4*H*-thieno[2,3-*b*]
225 thiopyran-2-sulfonamide 7,7-dioxide



226

227 Related substance OB:

228 (4*RS*,6*SR*)-4-Ethylamino-6-methyl-5,6-dihydro-4*H*-
229 thieno[2,3-*b*]thiopyran-2-sulfonamide 7,7-dioxide



230

and enantiomer

231 **Add the following to 9.01 Reference**

232 **Standards (1):**

233 **Timolol Maleate RS**

234