

Ph. Eur. Monograph 2359: Dorzolamide Hydrochloride Related Substances on Luna® 5 µm C18(2)

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Overview

Dorzolamide hydrochloride is used for the treatment of glaucoma and ocular hypertension. It is administered in solution as eye drops.

In 2017 Dorzolamide hydrochloride was prescribed more than one million times in the US.

In this application note we are showing the effective separation of Dorzolamide hydrochloride from its impurities and related substances according to Ph. Eur. Monograph 2359. The fully porous Luna 5 µm C18(2) columns met the system suitability requirement for the separation between Dorzolamide and [2-[[[(4S,6S)-6-methyl-7,7-dioxo-2-sulfamoyl-4,5,6,7-tetrahydro-7λ⁶-thieno[2,3-*b*]thiopyran-4-yl]amino]ethyl]boronic acid (impurity C, $R_s \geq 2.0$). The relative retention of impurity C related to the retention of Dorzolamide was 0.883.

All reference solutions were prepared as indicated in Ph. Eur. monograph 2359 for Dorzolamide hydrochloride. The following certified reference standards (CRS) were purchased from the European Directorate for the Quality of Medicines & HealthCare (EDQM) – Council of Europe; Postal address: 7 Allée Kastner CS 30026 F - 67081 Strasbourg (France):

- Y0000726 Dorzolamide hydrochloride CRS
- Y0000750 Dorzolamide for system suitability CRS

LC-UV Conditions

Column: Luna 5 µm C18(2)

Dimension: 250 x 4.6 mm

Part No.: [00G-4252-E0](#)

Mobile Phase: A = Acetonitrile/3.7 g/L KH₂PO₄ (65:935 v/v)

B = Acetonitrile

Gradient: Time (min)	%B
0	0
15	0
30	50
37	0
44	0

Flow Rate: 1.5 mL/min

Detector: UV @ 254 nm

Injection: 10 µL

System: Agilent® 1260 Infinity II

Table 1: Preparation of test and reference solutions

Solution	Composition
Test solution	Dissolve 30 mg of the substance to be examined (for this study: Dorzolamide hydrochloride CRS) in the mobile phase and dilute to 50.0 mL with the mobile phase.
Reference solution (a)	Dilute 1.0 mL of the Test solution to 100.0 mL with the mobile phase A. Dilute 1.0 mL of this solution to 10.0 mL with the mobile phase A.
Reference solution (b)	Dissolve 2 mg of dorzolamide for system suitability CRS (containing impurity C) in 2 mL of mobile phase A.



Chromatographic Results

Figure 1: Reference solution (a) on Luna® 5 µm C18(2)

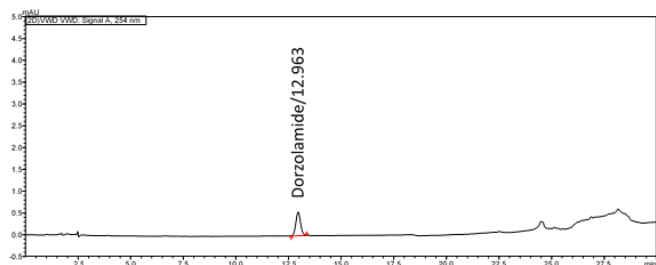


Table 2: Results reference solution (a) on Luna 5 µm C18(2)

Inj. No.	t _R Dorzolamide HCl (min)	Peak Area
1	12.963	8346
2	12.967	8292
3	12.962	8362
4	12.957	8313
5	12.965	8208
6	12.950	8326
Average	12.961	8308
%RSD	0.048	0.659

Figure 2: Reference solution (b) on Luna 5 µm C18(2)

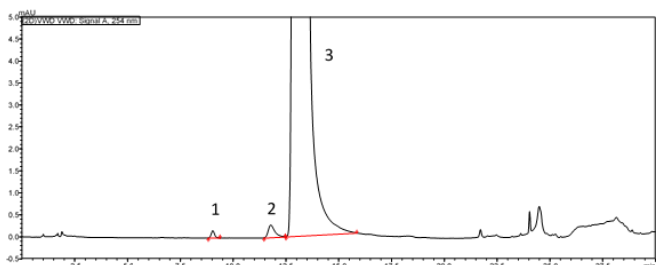


Table 3: Results reference solution (b) on Luna 5 µm C18(2)

Inj. No.	t _R Unknown (min)	t _R Imp. C (min)	t _R Dorzolamide (min)	Resolution (Dorzolamide /Impurity C)
	Peak 1	Peak 2	Peak 3	Peak 3/2
1	9.038	11.784	13.349	2.811
2	9.032	11.782	13.345	2.812
3	9.028	11.774	13.344	2.844
4	9.023	11.771	13.338	2.833
5	9.031	11.775	13.347	2.845
6	9.010	11.755	13.333	2.858
Avg.	9.027	11.774	13.343	2.834
% RSD	0.107	0.088	0.045	0.672

Figure 3: Test solution on Luna 5 µm C18(2)

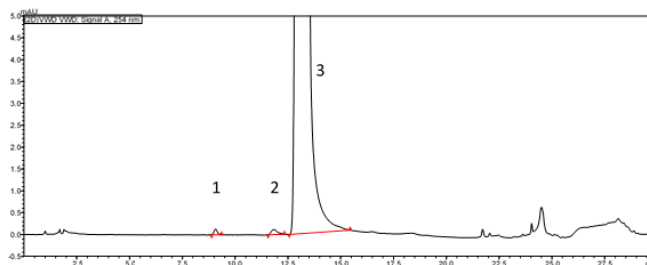


Table 4: Results test solution on Luna 5 µm C18(2)

Injection No.	t _R Dorzolamide (min)	Peak Area	Resolution (Dorzolamide/ Impurity C)
	Peak 3	Peak 3	Peak 3/2
1	13.257	8624765	2.857
2	13.247	8633281	2.853
3	13.242	8629076	2.933
4	13.240	8605320	2.936
5	13.232	8597617	2.938
6	13.230	8613157	2.919
Average	13.241	8617203	2.906
% RSD	0.075	0.164	1.379

Order Information

Luna 5 µm Analytical Columns (mm)

Phases 250 x 4.6

C18(2) [00G-4252-E0](#)



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