Validated LC Method for the Estimation of Dorzolamide HCl (Carbonic Anhydrase Inhibitor) in Ophthalmic Solutions

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Abstract: A reverse phase HPLC method is described for the determination of Dorzolamide in eye drops. Chromatography was carried on an Inertsil ODS 3V column using Acetonitrile : (0.02M) 1,Octane Sulphonic acid buffer (pH 3.5) (36:64 v/v) on isocratic mode at a flow rate of 1 mL/min with UV detection at 254 nm. The detector response was linear in the concentration range 4-720 µg/mL. The limit of detection and limit of quantification are found to be 0.7041 and 2.3483 µg/mL respectively. The method was validated as per the ICH guidelines. The proposed method is rapid, accurate and precise and can be applied for the routine analysis of dorzolamide in ophthalmic solutions.

Keywords: Dorzolamide (DRZ), RP-HPLC, Eye drops, ICH.

Introduction

Dorzolamide¹⁻³ (Figure 1) is a carbonic anhydrase (CA) inhibitor. Dorzolamide is a sulfonamide and a highly specific carbonic anhydrase II (CA-II) inhibitor, which is the main CA iso-enzyme involved in aqueous humor secretion. Inhibition of CA-II in the ciliary processes of the eye decreases aqueous humor secretion, presumably by slowing the formation of bicarbonate ions with subsequent reduction in sodium and fluid transport. It is indicated for the reduction of elevated IOP in patients with open-angle glaucoma or ocular hypertension who are insufficiently responsive to beta-blockers. It reduces IOP by approximately 17-23% in patients with elevated IOP. Dorzolamide⁴ (DRZ), chemically [(4S, 6S) -4- (Ethylamino) -6-methyl- 5, 6- dihydro-4H thieno[2,3b] thiopyran- 2-sulphonamide 7, 7-dioxide hydrochloride] has a molecular formula C₁₀H₁₆N₂O₄S₃ and molecular weight 324.44. Martens-Lobenhoffer⁵ et al studied the pharmacokinetics of DRZ where as Almeida⁶ et al reported the effects of DRZ on rabbit central corneal thickness.

Literature survey reveals that liquid chromatographic⁷⁻⁸ methods, voltametric⁹, spectrophotometric¹⁰ and simultaneous determination of DRZ along with timolol was reported by TLC-densitometry¹¹, Ratio Derivative Spectrophotometry¹² and capillary electrophoresis¹³ in ophthalmic preparations. The aim of the present work is to develop a LC method for the determination of Dorzolamide in ophthalmic solutions and to validate as per the ICH guidelines.

Experimental

Dorzolamide was obtained from Cipla Ltd. (India) as gift sample. DORTAS (2%, 3 ml), DORZOX (2%, 5 ml) and OCUDOR eye drops (2%, 5 ml) brands are available in the local market. HPLC grade Acetonitrile (Merck) and 1- Octane Sulphonic acid (Spectrochem) and Ortho phosphoric acid were used for the entire work.

Instrument

Quantitative HPLC was performed on a binary gradient HPLC with Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695 separations module and a Waters 2695 PDA detector. Data acquisition was performed by the Empower pro-software operated on a Pentium® IV microprocessor. An Inertsil ODS 3V column (250 x 4.0 mm, 5 μ m) was used.

Chromatographic conditions

The mobile phase composed of Acetonitrile : (0.02M) 1-Octane Sulphonic acid buffer (pH adjusted to 3.5 with o-phosphoric acid) was used in the ratio of 36:64 v/v with flow rate of 1.0 ml/min. The mobile phase was degassed and filtered through 0.2 μ m membrane filter before pumping into HPLC system. Analysis was carried out at 254 nm.

Preparation of standard stock and sample solutions

Accurately weighed 100 mg of Dorzolamide pure drug was taken and dissolved in acetonitrile in a 100 mL volumetric flask and sonicated. The stock solution was further diluted with the mobile phase to get a series of solutions containing 4.0-720.0 μ g/mL of DRZ.

Validation

Linearity

10 μ l of each of the above prepared solutions (4.0-720.0 μ g/mL) were injected in to the HPLC system and the peak area (Table 1) was recorded from the respective chromatograms. A representative chromatogram is shown in Figure 2.

Figure 1. Chemical Structure of Dorzolamide (DRZ).

A calibration graph was drawn by plotting the concentration of Dorzolamide ($\mu\text{g/mL}$) on x-axis and the corresponding peak area on the y-axis (Figure 3).

Assay of commercial formulations

DORTAS (2%, 3 ml), DORZOX (2%, 5 ml) and OCUDOR eye drops (2%, 5 ml) brands were purchased from the local market and required sample solutions were made using the mobile phase and 10 μL of these formulation solutions were injected into the HPLC system and the peak areas were recorded. The results are given in Table 2.

Figure 2. A typical chromatogram of Dorzolamide by RP- HPLC.

Table 1. Calibration data of Dorzolamide.

Concentration $\mu\text{g/ml}$	Average Peak area (n=3)	% RSD
4	93857	0.141
8	175677	0.876
20	459296	0.324
40	891999	0.193
80	1792688	0.482
160	3559260	0.391
200	4412501	1.021
240	5333088	0.813
320	7093708	0.952
400	8776995	0.923
480	10432011	0.183
560	12136319	0.632
600	13184571	0.815
720	15813771	0.867

Precision

The precision of an analytical procedure expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The repeatability (intra-day precision) refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment. Intermediate precision (inter-day precision) involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts.

Figure 3. Calibration curve of Dorzolamide by RP- HPLC.

Table 2. Analysis of commercial formulations (Eye Drops).

Commercial Formulation	Labeled amount	Amount found	
	(%)	(%)	% Recovery
Brand I	2	1.9866	99.33 ± 0.25
Brand II	2	1.9870	99.35± 0.16
Brand III	2	1.9916	99.58 ± 0.19

**Average of six determinations.*

Table 3. Intra and inter - day precision for Dorzolamide (n = 3).

Conc.(µg/ml)	Intra-day precision		Inter-day precision	
	Mean Peak Area	RSD (%)	Mean Peak Area	RSD (%)
200	4401577 ± 501779.78	0.114	4439270 ± 40530.54	0.913
400	8784338 ± 59118.59	0.673	8837998 ± 72648.34	0.822
600	13169381± 48068.24	0.365	12988022 ± 132607.70	1.021

The intra-day repeatability was investigated using three separate sample solutions each at three different levels (200, 400 and 600 $\mu\text{g mL}^{-1}$) prepared as reported above, from the freshly reconstructed formulations. Each solution was injected in triplicate and the peak areas obtained were used to calculate means and RSD% values (Table 3).

The inter-day reproducibility was checked on three different days, by preparing and analyzing in triplicate four separate sample solutions from the reconstructed formulations at the same concentration level of intra-day repeatability; the means and RSD% values were calculated from peak areas.

Accuracy

The accuracy of an analytical method is the closeness of the test results to the true value. To assess accuracy, freshly prepared placebo of the DRZ pharmaceutical formulations were spiked with various amounts of pure DRZ at 80, 100 and 120%. Each solution was injected in triplicate and the peak areas were used to calculate means and RSD% values (Table 4) and compared with those obtained with standard DRZ solutions.

Table 4. Accuracy - Recovery data for Dorzolamide ($n = 3$).

Amount (%) of drug added to analyte	Theoretical content ($\mu\text{g mL}^{-1}$)	Mean Conc. found ($\mu\text{g mL}^{-1}$) $\pm$ SD	% Recovery	% RSD
80	360	360.13 $\pm$ 0.645	100.04	0.1792
100	400	395.43 $\pm$ 1.367	98.86	0.3459
120	440	438.83 $\pm$ 1.079	99.73	0.2459

Results and Discussion

The analysis of the chromatogram of Dorzolamide revealed the following efficiencies of the column: for Dorzolamide $N = 8676$ (where N represents theoretical plate number) and asymmetry 1.05. The limit of detection (LOD) and limit of quantification (LOQ) for Dorzolamide were found to be 0.7041 and 2.348 $\mu\text{g/mL}$ respectively. The retention time of Dorzolamide was found to be 6.027 min. The linearity was followed in the concentration range of 4.0-720.0 $\mu\text{g/mL}$ with regression equation $y = 21855x + 27183$ and correlation coefficient 0.999. The method is precise [0.822-1.021] (Inter-day) and 0.144-0.673 (Intra-day)] and accurate (0.1792-0.3459) as the RSD values were less than 2 %. The percentage of purity of Dorzolamide in commercial formulations is found to be 99.33-99.58.

Conclusion

The proposed liquid chromatographic method is simple, rapid, reproducible and economical for the analysis of Dorzolamide in pharmaceutical formulations and can be used successfully applied for the quantitative analysis of Dorzolamide in pharmaceutical formulations.

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References

1. Grover S, Apushkin MA, Fishman GA, *Am. J. Ophthalmol.*, 2006, **141**(5), 850.
2. Balfour J A, Wilde M I, *Drugs Aging*, 1997, **10**(5), 384.
3. Ponticello G S, Sugrue M F, Plazonnet B, Durand-Cavagna G, *Pharm Biotechnol.*, 1998, **11**, 555.
4. The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, Maryadele J.O' Neil. Eds, 14th Ed., Published by Merck Research Laboratories, Division of Merck and Co. Inc., Whitehouse Station, NJ, 2006, Monograph No. 3426, 579.
5. Martens-Lobenhoffer J, Banditt P, *Clin Pharmacokinet.* 2002, **41**(3), 197.
6. Almeida G C Jr, Faria e Souza S J, *Braz J Med Biol Res.*, 2006, **39**(2), 277.
7. Sharath H. M. , Channabasavaraj K. P, Jose G. Babu, Jagadish S. Modiya, *International Journal of Pharmacy And Pharmaceutical Sciences*, 2011, **3**(3), 100.
8. Erk N, *Pharmazie*, 2003, **58**(7), 491.
9. N Erk, *Die Pharmazie*, 2003, **58**(12), 870.
10. H. M. Sharath*, G babu Jose, K. P. Channabasavaraj, J. S. Modiya, *International Journal of Pharmaceutical Sciences and Research*, 2011, **2**(4), 948.
11. Nevin Erk. *J Pharm Biomed Anal*, 2002, **28**(2), 391.
12. Lories IB. *J Pharm Biomed Anal*, 2002, **27**(5), 737.
13. Ismail, Palabiyik; Mehmet, Caglayan; Feyyaz, Onur, *Chromatographia*, 2011, **73**(5), 541.
14. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (Nov. 6,) Validation of Analytical Procedures: Methodology, ICH Steering Committee, Geneva, Switzerland. 1996.