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Development and Validation of RP-HPLC Method for Dorzolamide Hydrochloride in Bulk and Pharmaceutical Dosage form

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ABSTRACT

A New simple, precise, accurate and rapid RP-HPLC method was proposed for determination of Dorzolamide hydrochloride from pure and its dosage form. A Symmetry Hypersil C_{18} (250 × 4.6mm, 5µ) column in isocratic mode with mobile phase phosphate buffer (pH 6.2): Acetonitrile (60:40) at a flow rate of 1ml/min. The effluent was monitored at 254nm. The retention time was 3.337min for Dorzolamide hydrochloride. The linearity range was found to be 20 – 120 µg/ml. The developed method was validated for parameters like specificity, accuracy, ruggedness and robustness and ascertained values were found to be within limits. The method has significant advantages in terms of shorter analysis time, selectivity and accuracy then previously reported method and indicates that the method can be considered suitable for carrying out quality control &routine determination of Dorzolamide hydrochloride in bulk and pharmaceutical dosage form.

Keywords: Dorzolamide hydrochloride, RP-HPLC, isocratic, Validation.

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INTRODUCTION

Dorzolamide (4S-trans)-4-(ethyl amino)-5,6-dihydro-6-methyl-4H thieno[2,3-b]thiopyran-2-sulfonamide 7,7-dioxide monohydrochloride is optically active, carbonic anhydrase inhibitor [1], molecular weight 360.9 structure is shown as in figure 1.

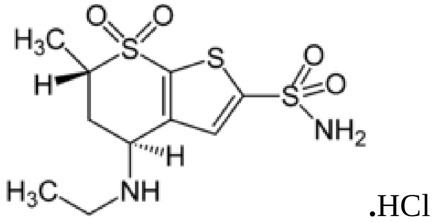


Figure 1: Structure of Dorzolamide Hydrochloride

It is an anti-glaucoma agent by decreasing the production of aqueous humor. It is optically applied in the form of eye drops to lower increased intra ocular pressure in an open angle glaucoma and ocular hypertension. There are many methods reported for determination of dorzolamide in individual and combined dosage form, viz, spectophotometric, LC-MS, RP-HPLC, capillary electrophoretic method. In the present investigation an attempt was made to develop and validate simple, precise, accurate isocratic RP-HPLC method with optimum run time for estimation of dorzolamide.

MATERIALS AND METHODS

List of chemical Used:

Dorzolamide hydrochloride working standard, formulation-DORZOX from cipla laboratories (Hyderabad, India), potassium dihydrogen phosphate (analytical grade), methanol (HPLC grade), acetonitrile (HPLC grade), othophosphoric acid (analytical grade) were purchased from Merck (Mumbai,India), water (HPLC grade) loba chemicals, dipotassium hydrogen phosphate (analytical reagent) S .D. fine chemicals.

Instrumentation:

Waters HPLC system (E2695 separation module and 2489 detector) -software EMPOWER, UV visible spectrophotometer-software NICOLET evolution, seperations and quantitation was done on a symmetry hypersil BDS 250x4.6 mm, 5 µm C 18 column.

Chromatographic conditions:

The column used for separation was a symmetry hypersil BDS 250x4.6mm, 5µm C 18 column. The mobile phase was prepared [5], by mixing phosphate buffer (pH 6.2): acetonitrile in a ratio of 60:40(v/v). The mobile phase was filtered using 0.45µm filter and degassed by ultra sonic vibrations prior to use. The flow rate was 1ml/min, column was maintained at ambient temperature, and detection wavelength was selected as 254nm.

Method development: [2, 4,8,13]

The method development was started with determination of absorbance maxima, at 254 nm as selected wavelength. The trails carried out to obtain an optimized method showing improved peak shape, plate count, and asymmetry. A detailed literature survey was conducted and the report states that the time of analysis for the determination of dorzolamide was time taking, hence the present work was carried out to develop a simple precise method development for the estimation of dorzoamide with less analysis time and the trails were conducted with mobile phase combination of phosphate buffer: acetonitrile in the ratio of 55:45 v/v using hypersil ODS column 150x4.6 mm 5µm in a flow rate 1.5 ml/min with a retention time 1.87 shown symmetric peak was good but retention time was too low and phosphate buffer :water in the ratio of 60:40v/v intersil ODS column 250x4.6mm in 5µm C18 column with a flow rate of 1 ml/min of retention time 6.420 with a theoretical plates 4077 shown peak symmetry was good and efficiency is low. Phosphate buffer pH 6.2: acetonitrile in ratio of 60:40v/v was an optimized method with a retention time 3.31 and flow rate of 1ml/min, and theoretical plates was found to be 5345 figure 2 and 3.

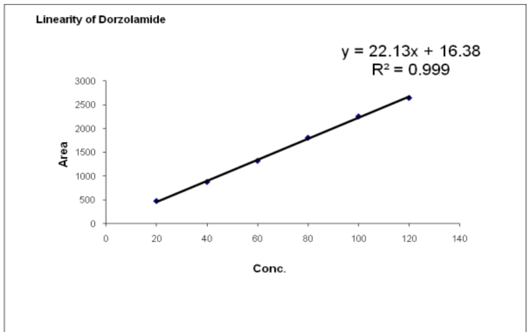


Figure 5: Showing linearity curve of Dorzolamide hydrochloride

Preparations of Mobile phase, Standard solution and sample solutions:

Preparation of mobile phase:

Preparation of buffer:

1.625 mg of potassium hydrogen phosphate was dissolved in 550 ml of water and 0.3gms of di potassium hydrogen phosphate in 450 ml water, both the solution were mixed and pH was adjusted to 6.2 using orthophosphoric acid. buffer was filtered through 0.45µ membrane filter.

Preparation of mobile phase:

600 ml of the buffer was mixed with 400 ml of acetonitrile; the resulting solution was sonicated for about 5 min in a sonicator.

Preparation of standard solution:

25 mg of drug was weighed and transferred to 25 ml volumetric flask containing 10 ml of HPLC grade milli Q water and ultra sonicated for 10 min and then volume was made up to 25 ml with HPLC grade milli Q water. The solution was filtered using whatmann filter paper NO. 41 from the filtrate appropriate dilutions were made in mobile phase.

Preparation of sample solution:

The 5 ml vial of opthalamic solution containing 2%(w/v) dorzolamide hydrochloride [9], was taken and 1 ml of opthalamic solution of dorzolamide hydrochloride was transferred to 200 ml standard flask and dissolved in HPLC grade milli Q water and diluted to volume upto the mark with same solvent, which was assayed and quantified.

Assay procedure:

20µl of standard and sample solutions were injected into an injector of RP-HPLC, from the peak area, standard amount of drug in sample were computed. Chromatograms of standard and sample were recorded.

Method validation[3, 10, 12]

The method validation was done as per the ICH guidelines, and accordingly the parameters evaluated were Specificity, precision, accuracy, linearity, ruggedness, robustness and system suitability studies. For all the parameters %RSD were calculated.

Specificity:

Specificity of an analytical method is its ability to measure accurately and specifically the concentration of analyte without interference from other API, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid chromatography. Retention times of sample and standard were compared.

Accuracy:

Accuracy refers to the closeness of a measured value to a standard or known value. The percentage recovery was studied for 80%, 100% and 120%, each level was injected three times dated are shown in table 1.

Linearity and Range:

The linearity of an analytical procedure is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within given range, was studied by analyzing six analyte concentrations of drug ranging from 20-120µg/ml shown in figure 5 and table 2.

Precision:

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from multiple sampling of same homogenous sample under prescribed conditions. This experiment was conducted to prove the repeatability of the assay results obtained by quantification methodology. System precision, method precision and intermediate precision was performed.

System precision:

20µl of standard solution was injected for six times and measured the peak area for all six injections in HPLC. The % RSD for the area of six replicate injections was calculated.

Method precision:

20µl of sample solution was injected for six times and the peak area of the resulting chromatogram was used for the calculation of standard deviation and relative standard deviation shown in figure 4.

Intermediate precision:

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by same samples under different conditions. The standard solutions was injected two times for two days, calculated the mean and %RSD table 3.

Robustness and system suitability test:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small variations in method parameters. For 20µl standard solution flow rate changed from 1ml/min to 1.1 and 0.9 ml/min, pH ±0.2, wave length ±2nm.

System suitability test [11] ensures the complete testing system suitable for intended application (instruments, agents, columns, analysts) system suitability parameters were found to be within acceptable limits. The suitability and stability of method was observed by injecting standard solution %RSD, efficiency and tailing factor was determined.

RESULTS AND DISCUSSION

The present investigation is aimed to develop analytical method and validate for dorzolamide hydrochloride in bulk and pharmaceutical dosage form. Several trails were carried out to develop an optimize method showing good peak shape, plate count and asymmetry. The developed method, was found to be simple, precise, and accurate and rapid by RP-HPLC method, by using Hypersil C18 (250×4.6mm, 5µ) column in isocratic mode with mobile phase consist of phosphate buffer pH 6.6: acetonitrile (60:40v/v), flow rate 1ml/min and effluent monitored at

254nm. The retention time of the compound was shown symmetric peak at 3.37. The percentage purity was calculated was found to be 99.86%v/v.

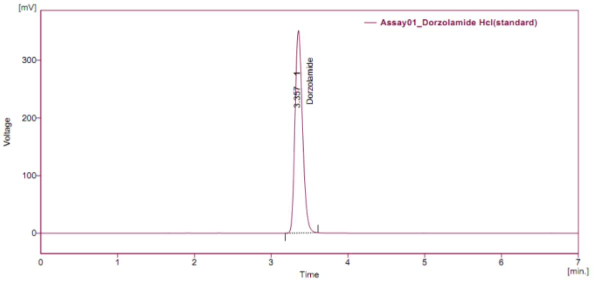


Figure 2: Chromatogram showing Dorzolamide hydrochloride standard solution.

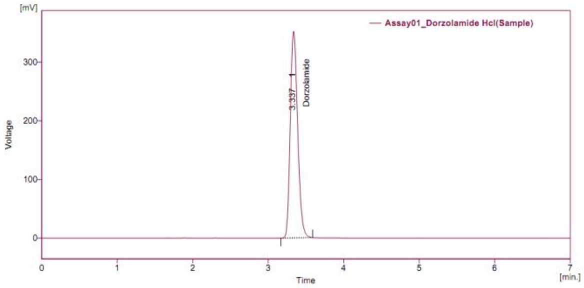
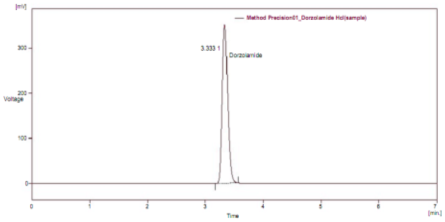


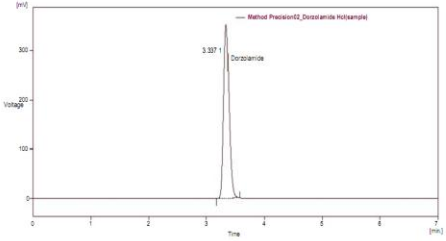
Figure 3: Chromatogram showing Dorzolamide hydrochloride sample solution

Chromatogram showing Dorzolamide hydrochloride sample solution -1



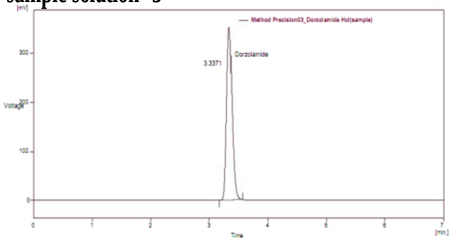
Drug	Retention time(min)	Area (mV.sec)	Asymmetry	Efficiency
DH	3.333	2342.445	1.36	5410

Chromatogram showing Dorzolamide hydrochloride sample solution -2



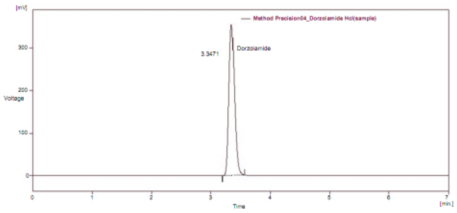
Drug	Retention time(min)	Area (mV.sec)	Asymmetry	Efficiency
DH	3.337	2349.550	1.36	5421

Chromatogram showing Dorzolamide hydrochloride sample solution -3



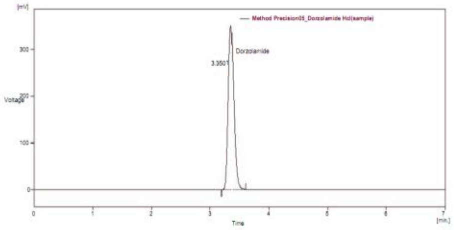
Drug	Retention time(min)	Area (mV.sec)	Asymmetry	Efficiency
DH	3.337	2349.803	1.36	5421

Chromatogram showing Dorzolamide hydrochloride sample solution -4



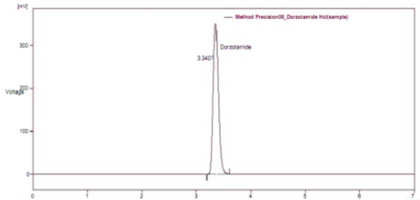
Drug	Retention time(min)	Area (mV.sec)	Asymmetry	Efficiency
DH	3.347	2358.859	1.27	5454

Chromatogram showing Dorzolamide hydrochloride sample solution -5



Drug	Retention time(min)	Area (mV.sec)	Asymmetry	Efficiency
DH	3.350	2367.816	1.36	5138

Chromatogram showing Dorzolamide hydrochloride sample solution -6



Drug	Retention time(min)	Area (mV.sec)	Asymmetry	Efficiency
DH	3.401	2327.836	1.36	5138

Figure 4: Chromatogram showing the Method Precision Studies

The developed method was validated as per ICH guidelines for the various parameters. The specificity of the proposed method proven that excipients and blank peak do not interfere in the retention time of the analyte. Hence the Dorzolamide hydrochloride showed good specificity at retention time 3.337.

The accuracy of the method was determined by measuring the drug percentage recovery by for the 80%, 100%, and 120%. The percentage recovery was found to be within the limit of 98.36-99.1%v/v(Table 1)

Table 1: showing accuracy data of Dorzolamide hydrochloride

Solution	Concentration(µg/ml)		%recovery	Mean % recovery	SD	RSD
	Formulation	Pure drug				
S$_1$ (80%)	100	81.2	96.93%	98.36	1.27	1.29
	100	79.8	98.84%			
	100	80.3	99.33%			
S$_2$ (100%)	100	100.5	99.59%	98.45	1.46	1.48
	100	100.3	96.80%			
	100	101.2	98.97%			
S$_3$ (120%)	100	120.6	99.48%	99.1	1.57	1.59
	100	121.2	95.80%			
	100	11.99	98.97%			

The precision of the method was ascertained from determinations of peak areas of six replicate injection of standard drug (system precision) and fixed amount of sample drug(method precision).

The % Relative Standard Deviation for system precision was found to be 0.52 and the % Relative Standard Deviation for method precision was found to be 0.409. The ruggedness was evaluated by injecting standard solutions of dorzolamide hydrochloride on different day by using different columns and by different analyst and %RSD was found to be 0.63 and 0.509.

The linearity was determined as linearity regression of the claimed analyte concentration of the range 20-120µg/ml. the calibration curve obtained by plotting peak area versus concentration was linear and the correlation coefficient was found to be 0.999.

Table 2: Linearity of Dorzolamide HCl concentration ranging from 20-120 µg/ml.

S.No	Solution	Conc.(µg/ml)	Area
1	L1	20	475.632
2	L2	40	878.252
3	L3	60	1323.707
4	L4	80	1809.351
5	L5	100	2258.266
6	L6	120	2649.204
Correlation coefficient (r^2)			0.999

The robustness was carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature, and flow rate and theoretical plates and tailing factor were observed and was found to be above 5000(theoretical plates) and not more than 2 (tailing factor). The system suitability parameters like theoretical plates(N), tailing factor(T) were calculated and were found to be more than 2000 and not more than 2 and ascertained that proposed RP-HPLC method was accurate and precise.

Table 3: Ruggedness or intermediate Precision of Dorzolamide hydrochloride working standard (Different columns of same dimensions on different days and Different analysts on different system)

S.No	Parameters	Retention time	Area
1	Morning	3.363	2386.603
2	Evening	3.313	2359.407
3	Second day	3.367	2361.782
AVG		3.357	2369.264
SD		0.03	15.06
%RSD		0.898	0.63
1	Analyst1 Instrument 1	3.313	2359.407
2	Analyst2 Instrument 2	3.323	2342.458
AVG		3.318	2350.933
SD		0.07	11.98
%RSD		0.213	0.509

Table 4: Showing Robustness results for Dorzolamide hydrochloride

Parameter	Variation	Theoretical plates	Tailing factor
Flow rate	+0.1	5744	1.33
	-0.1	5932	1.35
pH	+0.5	5642	1.33
	-0.5	5242	1.36
Wavelength	+2	5210	1.4
	-2	5927	1.3

Table 5: Showing results for System suitability parameters of Dorzolamide hydrochloride

S.No	Retention time	Area	Theoretical plates	Tailing factor
1	3.367	2361.782	5519	1.30
2	3.363	2367.138	5869	1.32
3	3.363	2386.603	5869	1.417
4	3.370	2397.778	5892	1.417
5	3.357	2419.247	5486	1.32
Avg.	3.364	2386.51	5727	**1.35**
SD	0.004	23.374		
%RSD	0.14	0.979		

CONCLUSION

The proposed method was found to be simple, precise, and accurate and rapid for determination of Dorzolamide hydrochloride from API and pharmaceutical dosage form. Chromatographic separation was achieved by using symmetry Hypersil C_{18} (250 × 4.6mm, 5μ) column in isocratic mode with mobile phase consist of phosphate buffer (pH 6.2): Acetonitrile (60:40 v/v). The flow rate was 1ml/min and effluent was monitored at 254nm. The peak was eluted at 3.337min for Dorzolamide hydrochloride. The proposed method was validated for parameters like specificity, accuracy, ruggedness and robustness and ascertained values were found to be within limits. The method has significant advantages, in terms of shorter analysis time, selectivity, and accuracy than previously reported. The extraction method gave consistent and reproducible recovery for analyte from formulated preparation, with no interferences. The validation study indicates that method can be considered suitable for carrying out quality control and routine determination of Dorzolamide hydrochloride in bulk and pharmaceutical dosage form.

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