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New Analytical Method And Its Validation For The Estimation Of Dorzolamide Hydrochloride In Bulk And Marketed Formulation

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Abstract: Dorzolamide Hydrochloride in presence of acidic medium reacts with excess amount of potassium bromide-bromide and remaining Potassium bromide-bromate reacts with Safranin to produce pink colour. The final stock solution was made to produce 100µg/ml with distilled ethanol. The λ_{max} was found to be 540nm for assay. The linearity was found in concentration range of 10-50µg/ml. The correlation coefficient was found 0.9998. The regression equation was found as Y=0.0192x+0.0062. The method has been validated according to ICH Guidelines.

Key Words: Dorzolamide Hydrochloride, Safranin, Potassium bromide-bromate, linearity, correlation coefficient and regression equation.

Introduction:

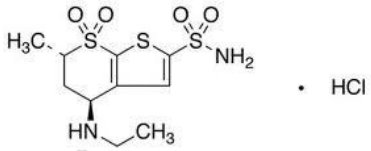
Chemical analysis may be reasonably stated as the application of a process or series of processes in order to quantify and identify a substance, the components of a solution or mixture, or determination of the structures of chemical compounds.[1] Spectrometric methods are a large group of analytical methods that are based on atomic and molecular spectroscopy.

Photometric techniques are amongst the most important instrumental techniques available to the pharmaceutical analysts. The basis of all these instrumental techniques is that they measure the interaction of electromagnetic radiation with matter in quantised energy levels.[2] Spectrometric methods are a large group of analytical methods that are based on atomic and molecular spectroscopy.[3]colorimetric assays generally consists of adding a reagent to the assay preparation or to the substance being tested, to produce a color that is compared with that of a standard preparation that has been prepared simultaneously and contains approximate quantity of the reference standard[4]. In general in analysis the first step is to determine the nature of the sample that is complete qualitative information and then further proceed for quantitative information by accuracy, LOD, LOQ etc[5]. Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use[6]. The parameters for method validation have been defined in different working groups of national

and international committees and are described in the literature[7]. Work carried out on Dorzolamide is pertaining Voltammetry, RP-HPLC, Densitometry etc.[8-14]

Dorzolamide Hydrochloride(trade name Trusopt) is a carbonic anhydrase inhibitor. It is an anti-glaucoma agent and topically applied in the form of eye drops. Dorzolamide Hydrochloride is used to lower increased intraocular pressure in open-angle glaucoma and ocular hypertension. The combination of Dorzolamide with Timolol is marketed as Cosopt .

Fig No: 1



Chemical structure: Fig No: 1

IUPAC Name: (4S, 6S)-2-ethylamino-4-methyl-5,5-dioxo-5λ^6,7-diethiabicyclo[4.3.0]nona-8,10 diene-8-sulfonamide.

Empirical formula: $C_{10}H_{16}N_2O_4S_3$, HCl
Physical state: White to off white, crystalline powder.
Molecular weight: 360.91
Solubility: Soluble in water and slightly soluble in ethanol and methanol.[15-16]

Materials And Methods:

A Shimadzu, UV Spec-1700, digital spectrophotometer and Jasco V-630 spectrophotometer with 1 cm matched quartz cells was used for all spectral and absorbance measurement. Pure drug was procured from Indoco Remedies Pvt Ltd, Verna-Goa as a gift sample. Steam distilled water was used for all dilution purposes throughout the work. Basic apparatus like calibrated volumetric flasks, pipette, beakers and graduated pipettes were used.

Experimental:

Dorzolamide Hydrochloride was determined spectrophotometrically in bulk and marketed formulation by using 2M hydrochloric acid and 550µg/ml of potassium bromide-bromate as a strong oxidizing agent.

Preparation of standard stock solution of Dorzolamide Hydrochloride:

Standard stock solution prepared by accurately weighing 100 mg of Dorzolamide Hydrochloride in 100 ml calibrated volumetric flask and made up the volume with distilled ethanol up to 100 ml.

Preparation of the working standard stock solution:

From the above standard stock solution A 10ml was pipetted using a 10ml volumetric pipette. This pipetted solution was transferred carefully to another 100ml volumetric flask and dissolved further with distilled ethanol up to the 100ml mark to obtain a 100µg/ml solution (Stock solution B). This solution was used for further work.

Preparation of 550µg/ml (1:0.1) [KBr: KBrO3] Potassium bromide –bromate reagent:

Accurately 1 gm. of potassium bromide and 0.1gm of potassium bromate was weighed and transferred to a 100ml volumetric flask. The volume was made up with distilled water dissolving the contents. Further 5ml of the above solution was pipetted out into a 100ml volumetric flask and the volume was made up to the mark.

Preparation of 2M HCl:

17ml of concentrated HCl was dissolved in100ml of distilled water.

Preparation of 0.025% of Safranin:

To 0.025gms of Safranin approximately 5ml of distilled ethanol was added in a100ml volumetric flask. The flask was shaken to dissolve the contents the solution was then made up with distilled water up to the mark.

Preliminary investigation:

Colorimetric Method For The Estimation Of Dorzolamide Hydrocholride

Optical Characteristics:

Determination of Absorption maximum:

To 1ml of drug solution (10µg/ml) from Stock solution (B) in a 10ml volumetric flask. To the flask add 2ml of 550µg/ml of Potassium bromide-bromate solution and 1.5ml of 2M Hydrochloric acid. Keep the flask aside for 20 minutes, add 0.5ml 0.025% Safranin and take the absorbance reading against the blank. The solutions were scanned in UV spectrophotometer between 800-400 nm. Absorption maxima obtained is given in Fig No: 2

Selection of analytical concentration range

For the spectrophotometric analysis, determination of the concentration range which obeys Beer-Lambert's law is necessary for accuracy and reproducibility.

Different concentrations of Dorzolamide Hydrochloride were prepared from the working standard stock solution (Stock solution B) ranging from 2µg/ml to 30µg/ml and their absorbance were read at 540nm.

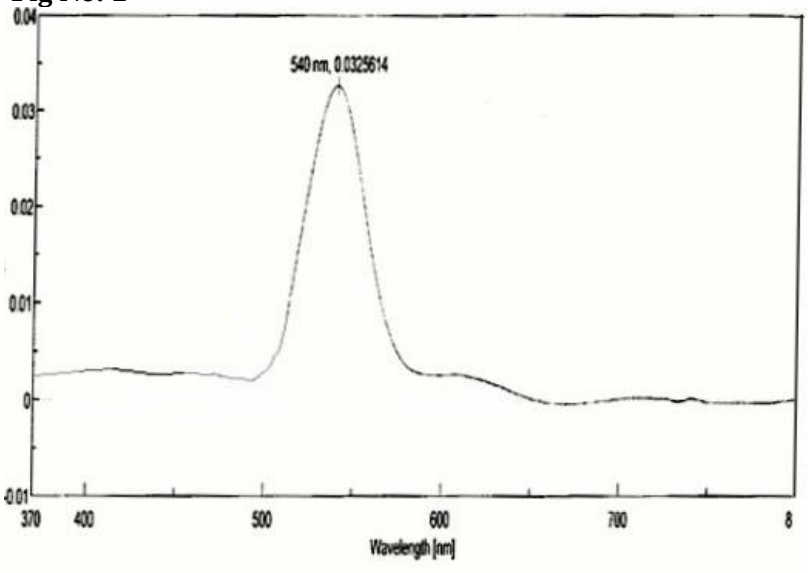
Preparation Of The Standard Curve

A series of concentration of 10-50µg/ml were prepared of drug solution from Stock Solution (B) in 10ml volumetric flasks by adding 1ml, 2ml, 3ml, 4ml and 5ml respectively. To each appropriately labeled flask we add 2ml of 550µg/ml of Potassium bromide-bromate solution and 1.5ml of 2M Hydrochloric acid. Keep the flask aside for 20 minutes and 0.5ml of 0.025% Safranin, take the absorbance reading at 540 nm. The graph and the absorbance for the above as given below in Table No: 1 and Fig No: 2.

Table No: 1 Standard curve for Dorzolamide Hydrochloride

Sr No	Volume of working standard of drug (ml)	Concentration in µg/ml	Absorbance at 540nm Mean ± S.D. (n=6)
1	1.0ml	10µg/ml	0.2025±0.001871
2	2.0ml	20µg/ml	0.3935±.0004231
3	3.0ml	30µg/ml	0.582±0.006481
4	4.0ml	40µg/ml	0.7815±0.001966
5	5.0ml	50µg/ml	0.960±0.005933

Fig No: 2

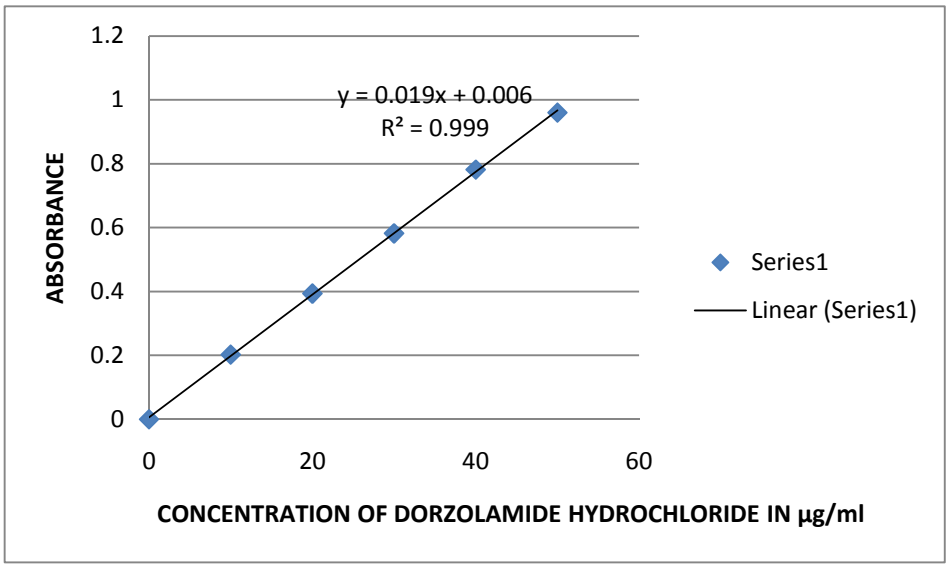


Validation Of The Method

Linearity

Calibration curve was plotted over a concentration range of 10 – 50 µg/ml for Dorzolamide Hydrochloride. Accurately measured standard working solutions of Dorzolamide Hydrochloride (1, 2, 3, 4, and 5ml) were transferred to one set of a series of 10 ml volumetric flasks. Solutions were made up to volume with distilled ethanol. A spectrum was recorded by placing drug solutions and diluent in sample and reference cells respectively. The absorbance was measured at 540nm (Peak maxima) and was plotted vs. concentration to give calibration curve, and regression equation and correlation coefficient was calculated and the calibration curve of amplitude of absorbance against concentration of the drug showed linearity shown in Table No: 1 and Fig No:3.

Fig No: 3 Standard curve for Dorzolamide hydrochloride



Sensitivity

The sensitivity of the proposed method for measurement of was Dorzolamide Hydrochloride estimated in terms of limit of detection [LOD] and limit of quantification [LOQ]. The LOD and LOQ were calculated by using the slope and SD of response (intercept). The mean slope value and SD of response were obtained after plotting six calibration curves. The LOD and LOQ obtained are reported in Table No: 2.

Precision

The precision of the method was established by system precision and method precision. System Precision was subjected to intraday and inter-day variation studies.

a) System Precision:

Intraday precision was determined by using three different levels of drug concentrations (10, 20, 30 µg/ml) prepared from stock solution-II and each level was analysed three times in a day. Same procedure was followed for three different days to study the Inter-day precision shown in Table No: 3.

b) Method Precision:

Method precision was determined by using sample solution of drug concentrations 10, 20, 30, 40, and 50µg/ml) and it was analyzed six times in a day by the same analyst shown in Table no:4 .

Table No: 2 Statistical Data

Parameter	Dorzolamide Hydrochloride at 540nm
Linear Range (µg/ml)	10-50
Regression Equation* (y)	y=bx+a: 0.0192x+0.0062
Slope (b)	0.0192
Intercept (a)	0.006316
Correlation coefficient (R^2)	0.9998
Standard deviation of slope	0.000121
Standard deviation of intercept	0.001038
Limit of Detection (µg/ml)	0.0207
Limit of quantition(µg/ml)	0.0629

Table No: 3 System Precision data for Dorzolamide Hydrochloride by colorimetric method

Sr. No	Concentration (µg/ml)	Inter-day Precision		Intra-day Precision	
		Mean ± S.D	RSD	Mean ± S.D	%RSD
1	**10**	0.199±0.0017	0.0087	0.202±0.0031	0.0156
2	**20**	0.393± 0.0011	0.0029	0.392±0.0028	0.0071
3	**30**	0.570 ± 0.0005	0.0009	0.575±0.0023	0.0040

Table No: 4 Repeatability data (Method Precision) for Dorzolamide Hydrochloride at 540 nm

Concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml
Absorbance	0.193	0.405	0.583	0.778	0.956
	0.194	0.402	0.582	0.782	0.952
	0.197	0.404	0.585	0.784	0.951
	0.195	0.407	0.582	0.785	0.955
	0.192	0.405	0.579	0.783	0.954
	0.194	0.402	0.578	0.782	0.952
Mean.	0.1941	0.4041	0.5815	0.7823	0.9533
Std. Dev.	0.0017	0.0019	0.0025	0.0024	0.0019
RSD	0.8871	0.4803	0.4450	0.3095	0.2062

n = 6 determination

Accuracy

Recovery studies by the standard addition method were performed to study the accuracy of the proposed method. Preanalysed samples of LH (4 µg/ml) were spiked with 80, 100 and 120 % extra Dorzolamide Hydrochloride standard and the mixture were analysed with the proposed method. Accuracy was assessed as the % Recovery at each concentration level. Data obtained from accuracy study are given in Table No: 5.

Ruggedness

To establish ruggedness of the proposed method, assays for two different concentrations of Dorzolamide Hydrochloride were performed by two different analysts. The results of assays are represented as % Recovery with SD and % RSD showing the ruggedness of the proposed method shown in Table No: 6.

Robustness

The absorbance readings of 10µg/ ml were measured at different laboratory using different spectrophotometer by another analyst and the %RSD values obtained to verify their robustness given in Table No: 7.

Specificity:

Refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour given in Table No: 8.

Table No: 5 Determination of Accuracy, Recovery studies

Amt. of sample Dorzolamide hydrochloride µg/ml	Amt. of Pure drug Dorzolamide hydrochloride %	Amt. of Pure drug Dorzolamide hydrochloride µg/ml	Amt. of drug recovered Dorzolamide hydrochloride µg/ml	Mean % Recovery ± SD
20	80%	16	15.91	99.43±0.6717
20	100%	20	20.11	100.56±0.6328
20	120%	24	23.84	99.35±0.8671

Table No:6 Ruggedness results for Dorzolamide Hydrochloride at 540 nm by colorimetric method

Sr. No.	Concentration (µg)	Analyst I		Analyst II	
		Amount found (µg)	(%) Recovery ± SD	Amount found (µg)	(%) Recovery ± SD
1	10	10.03	100.36± 0.8576	9.93	99.31±0.3880
2	20	19.93	99.5±0.4949	20.05	99.91±0.6328

Table No: 7 Robustness data for Dorzolamide hydrochloride at 540 nm

Conc. µg/ml	Instrument 1	%RSD	Instrument 2	%RSD
10	0.2023±0.0015	0.755	0.2052±0.0004	0.219

Table No:8 Specificity and Selectivity study

Study	Dorzolamide Hydrochloride
Specificity	Specific
Selectivity	Selective

Determination Of Dorzolamide Hydrochloride From Dosage Form:

Analysis of formulation:

An opthalmic marketed formulation, Dorzolamide HCl eye drop solution 2%(Indoco Remedies Pvt Ltd.) was obtained for all analytical study. Solution equivalent to 1000 µg/ml was pipetted from the marketed formulation and added to the a 1000ml volumetric flask .the volume was made up to 1000ml using distilled ethanol. The flask was shaken and volume was made up to the mark with distilled water to give a solution of 1000 µg/ml(Stock Solution A). From Stock solution B 2ml was pipetted out into a 10ml volumetric flask. Further appropriately we add 2ml of 550µg/ml of Potassium bromide-bromate solution and 1.5ml of 2M Hydrochloric acid. The flasks are kept aside for 20 minutes and the absorbance is read at 540 nm against the reagent blank.

0.5ml of the drug solution from Stock solution B was taken in a 10ml volumetric flask to this 1ml of 550µg/ml of Potassium bromide-bromate reagent and 1ml of 2MHCl was added and kept aside for 20 minutes. After this 0.1ml of 0.01% 0f Safranin solution was added and the volume was up with distilled ethanol. The absorbance was recorded The graph and the absorbance for the above are as given below in Table No: 9.

Stability of color:

Procedure: To 1ml of the drug solution pipetted from the working standard solution(Stock solution B) in a 10ml volumetric flask 2ml of 550µg/ml Potassium bromide-bromate and 1.5ml of 2M HCl were added, shaken and kept was kept aside for 20minutes .Thereafter 0.5ml of 0.025% 0f Safranin solution was added. The absorbance

was measured against the reagent blank at 540 nm. The reagent blank was prepared same as the drug solution except the drug solution. The absorbance was recorded after a period of every 5 minutes in a time span of 90 minutes. The results obtained have been tabulated in Table no:10 graph is given in Fig No:4.

Table no: 9 Assay Results of Marketed Formulation

Formulation	Actual concentration of Dorzolamide Hydrochloride(µg/ml)	Amount obtained of Dorzolamide Hydrochloride (µg/ml)	%Dorzolamide Hydrochloride
Opthalmic solution	20 µg/ml	19.41 µg/ml	97.05%

Table No:10 Stability of the coloured species of Dorzolamide Hydrochloride

Sr. No	Volume of drug solution in 10ml volumetric flasks(100µg/ml)	Time (Minutes)	Absorbance at 540nm
1	1ml	0	0.232
2	1ml	5	0.24
3	1ml	10	0.237
4	1ml	15	0.226
5	1ml	20	0.226
6	1ml	25	0.223
7	1ml	30	0.223
8	1ml	35	0.221
9	1ml	40	0.222
10	1ml	45	0.221
11	1ml	50	0.22
12	1ml	55	0.221
13	1ml	60	0.221
14	1ml	65	0.221
15	1ml	70	0.22
16	1ml	75	0.218
17	1ml	80	0.218
18	1ml	85	0.218
19	1ml	90	0.218

Fig No: 4 Graph of Stability of coloured species of Dorzolamide Hydrochloride

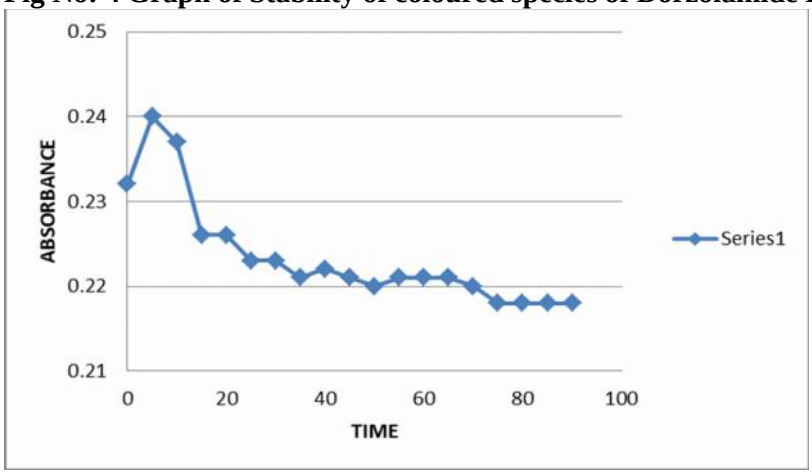
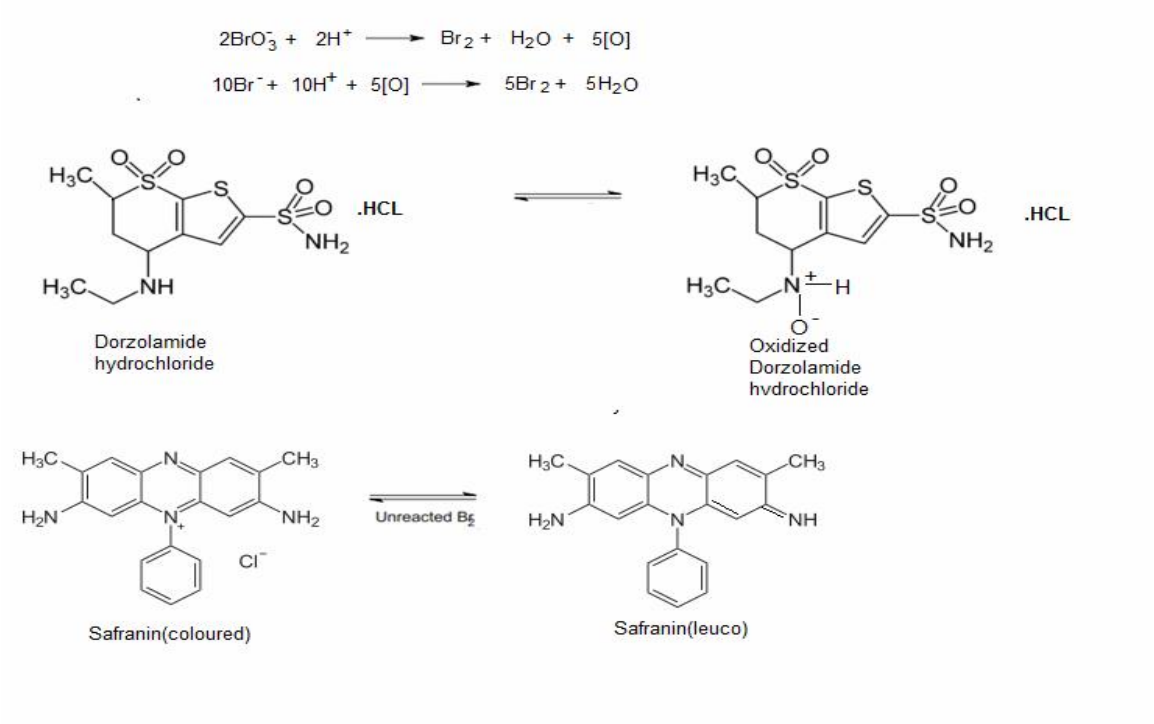


Fig No:5 Chemical reaction



Result And Discussion:

Potassium bromide bromate is strong oxidizing agent. Its react with Dorzolamide Hydrochloride in presence of acidic medium. When Potassium bromide bromate is added in excess amount it produced light yellow color complex of Dorzolamide Hydrochloride. Remaining Potassium bromide bromate now readily reacts with Safranin & oxidizes it. Unreacted Safranin molecules gives pink color. So, color of the final solution indirectly indicates the amount of drug present. The reaction is explained in Fig No: 5.

Conclusion:

For routine analytical purpose, it is always necessary to establish methods capable of analysing number of samples in a short time period with due accuracy and precision to obtain desired results. In view of the above fact, some simple analytical method was planned to develop with sensitivity, accuracy, precision and economical. In the present investigation, colorimetric method for the quantitative estimation of Dorzolamide Hydrochloride in bulk drug and pharmaceutical formulations has been developed.

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References:

1) Mendham J, Denny RC, Barnes JD, Thomas M, Sivasankar B. Vogel's text book of quantitative chemical analysis. 6th ed. New York: John Wiley & sons, Inc; 2009. p. 1.
2) Skoog DA, Holler FJ, Crouch SR. Instrumental analysis. India ed. 2007. p. 1, 32-3, 158-9.
3) Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. 4th ed. New Delhi: CBS Publishers and Distributors; 1997. p. 1.
4) Kasture AV, Wadodkar SG, Mahadik KR, More HN. Pharmaceutical Analysis. 11th ed. Nirali Prakashan; 2004. p. 183.
5) Willard, Meritt, Dean,Settle; Instrumental Methods of Analysis, seventh edition; Pg no:3,4.
6) Sharma PP. Validation in pharmaceutical industry. 1st ed. 2007. p. 363-82.

7) Nash RA, Alfred HW. Pharmaceutical process validation. 3rd ed. 2008. p. 507-23.
8) Erk N. Simultaneous determination of Dorzolamide hydrochloride and Timolol maleate in eye drops by two different spectroscopic methods. J Pharm Biomed Anal 2002; 28(2):391-7.
9) Lories IB. Application of TLC densitometry, first derivative UV spectrphotometry and ratio derivative spectrophotometry for determination of Dorzolamide Hydrochloride and Timolol Maleate. Journal of Pharmaceutical and Biomedical analysis 2002; 27(5): 737-46.
10) Erk N. Voltammetric and HPLC determination of Dorzolamide Hydrochloride in eye drops. Die Pharmazie-An International Journal of Pharmaceutical Sciences 2003;58(12):870-3.
11) Erk N. Simultaneous RP-HPLC determination of Dorzolamide Hydrochloride and Timolol Maleate in pharmaceutical preparations. Die Pharmazie-An International Journal of Pharmaceutical Sciences 2003; 5(7):491-3.
12) Novak TJ, Berwick L. Solid phase extraction by HPLC of Dorzolamide Hydrochloride. Journal of Liquid Chromatography and Related Technologies 1998; 21(2):143-5.
13) Sharath M, Channabasavaraj KP, Jose GB, Jagadish SM. Stability indicating RP-HPLC method for analysis of Dorzolmide Hydrochloride in the bulk drug and in its pharmaceutical doasage form. International Journal of Pharamaceutical Sciences 2011; 3(3):100-5.
14) Kanchan RU, Shikha MNR, Rane RB. Simultaneous RP-HPLC determination of Dorzolamide Hydrochloride and Timolol Maleate in pharmaceutical preparations. Trade Science Inc 2008; 7(8):126-7.
15) http://en.wikipedia.org/wiki/DORZOLAMIDE HYDROCLORIDE 15/11/2011; 16:42.
16) Drugs information online: drugs.com: http://www.drugs.com/sfx/dorzolmide 15/11/2011;16:50 .

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