ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Development and Estimation of Dorzolamide Hydrochloride by Different Spectroscopic Methods

S.Nagalakshmi*, N. Damodharan, J.Thanka and S. Seethalakshmi
[1]Department of Pharmaceutics, Sri Ramachandra University, Porur, Chennai, India,
[2]Department of Pharmaceutics, SRM college of Pharmacy, kattunkulathur, Chennai, India.
[3]Department of Pathology, Sri Ramachandra Medical college and research Institute, Sri Ramachandra University, Porur, Chennai, India.
[4]Department of Pharmacology, ESI medical college & PGIMSR, K.K.Nagar, Chennai, India.
***Corresponding author:E-Mail:nagalakshmimpharm@gmail.com**

ABSTRACT

Objective: U.V methods have been developed and validated for the qualitative estimation of Dorzalamide Hcl in bulk and in the formulation.

Method: Solvent used in the estimation was phosphate buffer pH 7.4. It was evident from the various concentrations Dorzalamide Hcl obeyed Beer's law in the concentration range of 3-24 µg / ml and the absorbance maxima was found to be at 253 nm. Other spectroscopic determinations such as FTIR, DSC were done in order to study the interactions between the drug and other polymers.

Conclusion: The developed method was successful and it has been applied for the estimation of Dorzalamide Hcl in bulk and in pharmaceutical dosage forms.

KEY WORDS: Qualitative, Phosphate buffer, concentration, absorbance, estimation, pharmaceutical dosage form.

1. INTRODUCTION

Development in almost all the field of science depends on the effective concentration rendered by systematic approach. In order to expand the frontiers of knowledge in all areas of science research is the only tool used for development. Analytical method development should be adopted in order to make it cost effective and to satisfy the intended purpose. They are required to analyze drug substance and drug product compound in all stages of its formulation development. Dorzalamide HCl is selected in order to assess the potency in pure and in the formulation which is not official in pharmacopeia. Extensive literature survey reveals that only few analytical methods have been reported for the estimation of Dorzalamide Hcl (Sharath, 2011).

Dorzalamide Hcl is an antiglaucoma agent and topically applied in the form of eye drops. Chemically is an (4s, 6s)- 4- (ethyl amino)- 6- methyl- 5, 6 dihydro- 4 h thieno (2, 3b) thiopyran -2- siphonamide 7, 7- di oxide hydrochloride). Dorzalamide Hcl is a carbonic anhydrase inhibitor and used to lower increased intraocular pressure in open angle glaucoma and ocular hypertension (Apurva, 2013; Gajan, 2011).

Hence there is a need to develop new method for its estimation in bulk and in pharmaceutical dosage form.

2. MATERIALS AND METHODS

Dorzalamide Hcl was obtained from Cipla ltd, Potassium di hydrogen orthophosphate, Sodium hydroxide all were laboratory reagent obtained from loba chemicals. U.V spectrophotometer of Perkin Elmer Lambda 25 was used. FTIR spectrophotometer of - JASCO 4100 type A was used.

Estimation of Dorzalamide HCl by U.V. spectrophotometer method: Based on the measurement of absorbance at 253 nm in solvent phosphate buffer pH 7.4 estimation was carried out (Nevin, 2002; Manjunatha, 2011).

Preparation of phosphate buffer pH 7.4: The Buffer was prepared by mixing 50 ml of 0.2 M monobasic potassium di hydrogen ortho phosphate with 39.1 ml of 0.2 m sodium hydroxide and diluting it with distilled water to produce 200 ml (Deshpande, 2004).

Standard calibration curve of Dorzalamide Hcl: Accurately weighed 10 mg of Dorzalamide Hcl was dissolved in 100 ml of phosphate buffer pH 7.4 to get a stock solution aliquots of (2, 4, 6, 8, 10 & 12µg/ml) were withdrawn and further diluted to 100 ml with buffer to obtain a concentration range of 3-24 µgm/ml. The absorbance of the resulting solution was measured at 253 nm on a U.V. spectrophotometer using phosphate buffer pH 7.4 as blank. The standard curve obtained by plotting a graph of absorbance Vs concentration (µg/ml). From the standard curve the amount of Dorzalamide Hcl in the bulk drug was estimated (Skoog, 2004; Chatwal, 2004).

Fourier transforms Infra-Red spectroscopy (FT-IR): In order to identify the functional groups present in the drug, polymer and formulation. To check whether interaction occurs between the drug polymer and formulations. FTIR studies were carried out in the spectrum scanning range from 400-4000$cm^{-1}$and the resolution is 1cm^{-1} which is the required range for this study (Clothup, 1950).

Differential Scanning Calorimeter (DSC): To detect any possible change in the physical state of drug, polymer and formulations DSC studies were carried out. The samples of (2-5 mg) were heated at a constant rate of 10ºC/min in an aluminum pan under a nitrogen (N_2) atmosphere. A similar empty pan was used as the reference.

3. RESULTS

U.V spectrophotometer: Calibration graph for the U.V spectrophotometric determination of Dorzolamide Hcl is given in the table 1 and fig 1& fig 2. This gives the absorbance value of Dorzolamide Hcl for various concentrations. Graph of Concentration Vs absorbance in which a linear correlation that exist indicates both are directly proportional with increase in concentration increases the absorbance value shows the linearity graph. Fig 2 shows the standard calibration curve with the slope 0.0285x and regression coefficient value 0.9994.and the Standard solution containing 3-24 µgm/ ml of drug in phosphate buffer pH 7.4 which indicated that it obeys beers lamberts law.

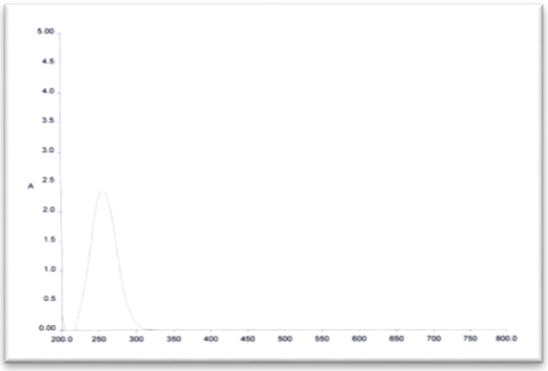

Figure.1. Calibration graph for standard solution of Dorzolamide Hcl in phosphate buffer pH 7.4. at 253 nm

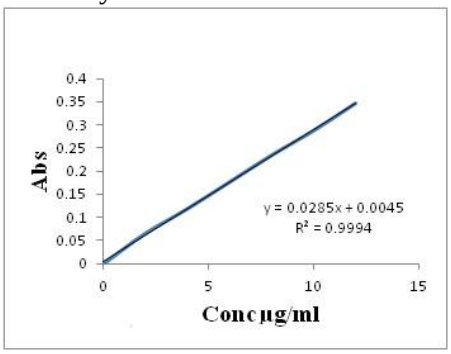

Figure.2. Linearity plot of Dorzolamide Hcl in phosphate buffer pH 7.4 at 253 nm

Table.1. Concentration Vs absorbance

Concentration µg/ ml	Absorbance at 253 nm± S.D *
0	0
2	0.0661 ± 0. 01
4	0.1190 ± 0. 04
6	0.1766 ± 0. 02
8	0.2346 ± 0. 03
10	0.2876 ± 0. 01
12	0.3466 ± 0. 04

***Average of three readings (n=3)**

DSC Interpretation: DSC data allow identification and characterization of a drug substance through the melting temperature and heat of fusion in case of crystalline substances. Polymorphic forms can also be identified by virtue of their different melting temperature. The drug dorzolamide showed endothermic reaction peak at 276.9º C whereas cholesterol showed the peak of melting point at 148.8 ºC which exhibited the exothermic reaction. The surfactant span 60 showed exothermic reaction peak at 59.3º c. whereas the formulation showed the exothermic reaction peak at 60.4 ºC which indicates the drug, cholesterol and span 60 incorporated indicating weak interaction between the Dorzolamide with cholesterol and span 60. From this data it was found that there were no significant interactions in this study.

FTIR Interpretation: The presence of functional groups was confirmed by using FT IR (Instrument- JASCO 4100 type A). The readings were obtained between 400 cm-1 to 4000 cm-1 using KBr pellet technique. FT IR was carried out for Dorzolamide HCl, Cholesterol, span 60 and mixture of (Drug+ cholesterol+ surfactant). The results of the analysis showed various stretching, bending and rocking vibration based on the groups present. The results were shown in tables 2-5 and fig 3-6.

Dorzolamide HCl:

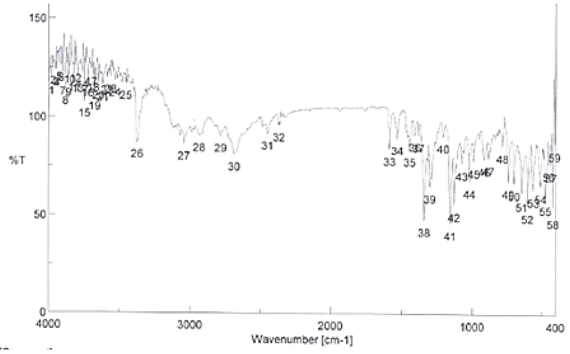

Figure.3. FTIR spectrum of the dorzolamide Hcl

Table.2. Interpretation of dorzolamide Hcl

Wave number (cm-1)	Functional groups
3794.26	NH stretching vibration
2786.63	C = H stretching vibration
1589.06	C= N stretching vibration
1535.06	N-H Bending vibrations
1344.14	C- H Bending vibrations
1158.04	SO_2 stretching vibration
783.9	N- H Rocking vibrations
644.1	C- H Rocking vibrations

Cholesterol:

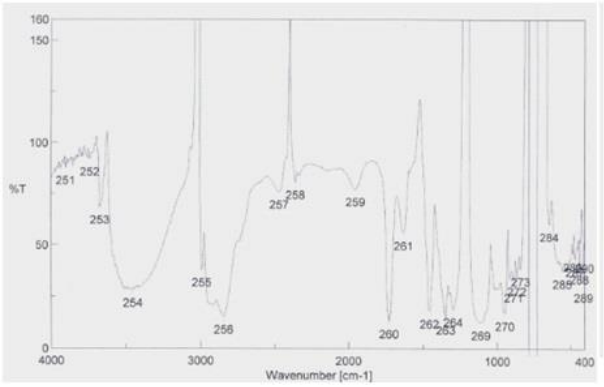

Figure.4. FTIR spectrum of the cholesterol.

Table.3. Interpretation of cholesterol

Wave number (cm-1)	Functional groups
3697.8	OH stretching vibration
2866.6	C = H stretching vibration
1466.6	C- H Bending vibration
799.3	C- C Bending vibrations

Span 60:

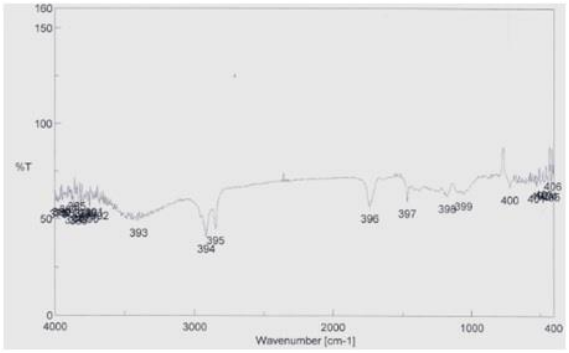

Figure.5. FTIR spectrum of the span 60

Table.4. Interpretation of Span 60

Wave number (cm-1)	Functional groups
3674.69	OH stretching vibrations
2917.7	C= C stretching vibrations
1737.55	C=O stretching vibrations
1468.5	C-H Bending vibrations
1179.2	C-C stretching vibrations
721.2	C-H Rocking vibrations

(Drug, surfactant, Cholesterol)

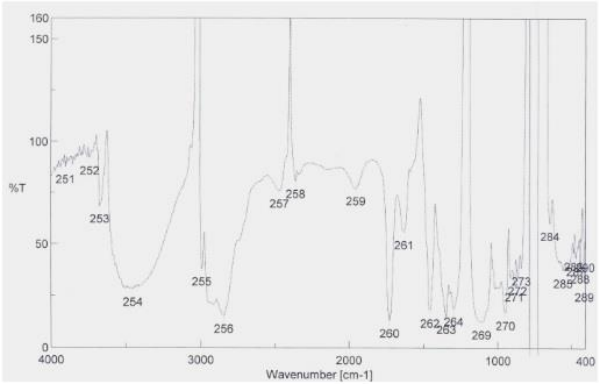

Figure.6.FTIR spectrum of the Dorzolamide HCl, span 60 and cholesterol

Table.5. Interpretation of drug, cholesterol and Span 60

Wave number (cm-1)	Functional groups
3670.8	N- H stretching vibrations
3450.9	O-H stretching vibrations
2846.4	C=C stretching vibrations
1955.47	C=O stretching vibrations
1729.8	C= N stretching vibrations
1633.4	N-H stretching vibrations
1458.8	C-H stretching vibrations
1350.8	O-H stretching vibrations
1112.7	C- O stretching vibrations
948.8	C-C stretching vibrations
865.8	C-H Rocking vibrations
840.8	N-H Rocking vibrations

DISCUSSION

From the above results obtained for various studies like Calibration curve, FTIR studies and DSC interpretation it was found that for the concentration of Standard solution containing 3-24 µgm/ ml of drug in phosphate buffer pH 7.4 which indicated that it obeys beers lamberts law. DSC studies indicated there were no significant interactions in this study from the peaks obtained. It was found that FTIR studies showed Dorzolamide HCl, Cholesterol, span 60 and mixture of (Drug+ cholesterol+ surfactant) there were no incompatibilities or interactions present also indicating various stretching, bending and rocking vibrations of functional groups.

4. CONCLUSION

It can be concluded that U.V spectrophotometeric method was used to estimate the amount of dorzolamide Hcl present in pure drug and in the pharmaceutical preparation. So the developed method could be suitable for the quantitative estimation of dorzalamide Hcl in pharmaceutical preparation.

ACKNOWLEDGEMENT

Authors are thankful to Cipla Pvt Ltd, Mumbai for providing pure sample of Dorzalamide Hcl and also to the management of Sri Ramachandra University for providing financial assistance through the Young faculty grant fellowship 'GATE project' to carry out the research work.

REFERENCES

Apurva LD, Ashish S, Sneha V, Rajashree G, Formulation, development and evaluation of long acting ophthalmic insitu gelling system of Dorzalamide Hcl, IJDDR, 5(40), 2013, 156-63.

Chatwal GR, Sharma A, Instrumental Methods of Chemical Analysis, Edn. 5, Himalaya Publishing House, Delhi, 1(1), 2004, 1-5.

Clothup NB, Spectra-Structure Correlations in the Infra-Red Region, J. Opt. Soc. Am, 40(6), 1950, 397- 400.

Deshpande SV, Funne SM, Mahaparale SP and Onkar PR, Development and Validation of UV Spectrophotometric Methods for Estimation of Timolol Maleate and Dorzolamide Hydrochloride in Bulk and Eye Drop Formulation. IJPCS, 3(4), 2004, 838-844.

Gajan D, Priya J, Dinesh K J, Gaurav A, Development and optimization of Dorzalamide Hcl and timolol maleate in-situ gel for glaucoma treatment. Asian J Pharma,1(4), 2011, 93-97.

Manjunatha KM, Kulkarni GT, Mruthyunjaya JH, Spectrophotometric determination of combined dosage form of matrix ocular inserts containing Dorzolamide Hcl and timolol maleate, Inventi Rapid: Pharm Ana & Qual Assur, 1(7), 2011, 21-25.

Nevin E, Simultaneous determination of Dorzolamide Hcl, timolol maleate in eye drops by two different spectroscopic methods, J Pharm biomed Anal, 28(2), 2002, 391-397.

Sharath HM, Babu G, Channabasavaraj KP, Modiya JS, Development and validation of spectrophotometric methods for estimation of Dorzolamide in bulk and pharmaceutical dosage form. IJPSR, 2 (4), 2011, 948-53.

Skoog DA, In Principle of Instrumental Analysis, Edn.5, Eastern Press, Bangalore, 2004.