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Spectrophotometric Method For Estimation Of Levobunolol In Bulk And Tablet Dosage Form

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Abstract: A simple and sensitive spectrophotometric method has been developed for the determination of Levobunolol in bulk and tablet dosage forms. The method was based on the charge transfer reactions of Levobunolol with 2, 3-dichloro-5, 6-dicyano-1, 4-benzquinone. The absorbance of the highly intensive coloured solution was measured at 470 nm against reagent blank treated similarly. Beer's law is obeyed in the concentration range of 50-250 µg/ml. Statistical analysis proves that the proposed method is reproducible and selective for the routine analysis of pharmaceutical formulations of Levobunolol.
Key words: Levobunolol(LV), Spctrophotometry, DDQ (2, 3-dichloro 5,6-dicyano-1,4-benzoquinone) .

INTRODUCTION

2, 3-dichloro-5, 6-dicyano-1, 4-benzquinone (DDQ):

2,3-Dichloro 5,6- dicyano- p-benzoquinone(DDQ) is an oxidizing[1], dehydrating agent[2] in synthetic organic chemistry as well as it is known for its interaction with drugs having donor sites in their structures, and form ion-pair charge transfer complexes which offers a basis for quantification of the drugs[3-6].

Levobunolol(LV):

Levobunolol hydrochloride chemically, 1(2*H*)-Naphthalenone,5-[3-[(1,1-dimethylethyl)amino]-2-hydroxy propoxy]-3,4-dihydro-,hydrochloride It is freely soluble in methanol[7] .The structure of levobunolol is given in fig.1.

***Uses*:** Levobunolol is used in the treatment of glaucoma.

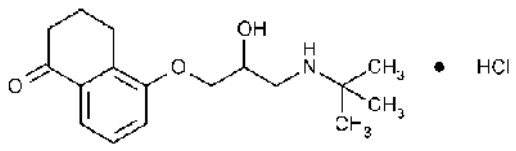

Fig.1: Levobunolol

***Doses*:** Betagan Eye-DPS 5 mg/ml is available in different trade names in Pharmaceutical market. The most commonly reported side effects with levobunolol are Ocular stinging, burning, decreased BP, decreased heard rate, transient ataxia, dizziness, lethargy, Headache, decreased comeal sensitivity and pruritus. Its quantification is reported by Salem H quoting earlier and different methods of determination[8]. Nersin made a precipitation based technique of levobunolol cation with tungsto-phosphate anion[9].

Only a few methods viz, HPLC, Spectrofluorimetry, electrophoresis, UV-visible spectrophotometry appeared in the literature for the determination of Levobunolol in bulk and pharmaceutical formulations. There is a need for simple spectrophotometric method for the analysis of Levobunolol in pharmaceutical formulations. UV-Visible spectrophotometry is the technique of choice in research laboratories, hospitals and pharmaceutical industries due to its low cost and inherent simplicity. The objectives of the work are to develop new spectrophotometric method for its estimation in bulk and tablet dosage form with good accuracy, simplicity, precission and economy. Hence the present work deals with the spectrophotometric estimation of Levobunolol using 2,3-dichloro 5,6-dicyano-1,4-benzoquinone .

MATERIALS AND METHODS

Instrumentation:

After due calibration of the instrument, spectral and absorbance measurements are made using ELICO UV-160 A double beam Spectrophotometer manufactured by M/S ELICO private Limited, Hyderabad, India. All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Reagents were prepared a fresh for every method.

Preparation of Standard solution of drug

Pure Levobunolol (50 mg) is dissolved in 50 ml methanol to obtain a stock solution of 1 mg/ml. The final concentration of Levobunolol is brought to 100 µg/ml with methanol.

Preparation of Reagent

DDQ (0.1% w/v): DDQ (2,3-dichloro 5,6-dicyano-1,4-benzoquinone) (Loba Chem., India) solution is prepared by dissolving 100 mg in 100 ml of methanol.

Procedure

The wavelength of maximum absorbance of the Levobunolol drug treated with DDQ solution is ascertained by the following procedure.

1ml of Levobunolol solution (100µg/ml) is transferred into a standard flask. To this solution 1.0 ml of DDQ reagent is added to form light orange colour solution. The final volume is brought to 10 ml with chloroform. The resultant solution is mixed well and allowed to stand for 5 min for complete the reaction. The absorbance of the orange colour solution is measured at the wavelength range of 400 to 600 nm, against the reagent blank (prepared in same manner omitting drug solution). The figure of absorbtion spectrum is given in fig.2.

Table.1: Optical characteristics

Parameters	Method - Value
λmax (nm)	470
Beer's law limit (µg/ml)	50-250
Correlation coefficient (r2)	0.999
Regression Equation (y=mx+c)	y= 0.01x + 0.003
Intercept (c)	0.003
Slope (m)	0.01

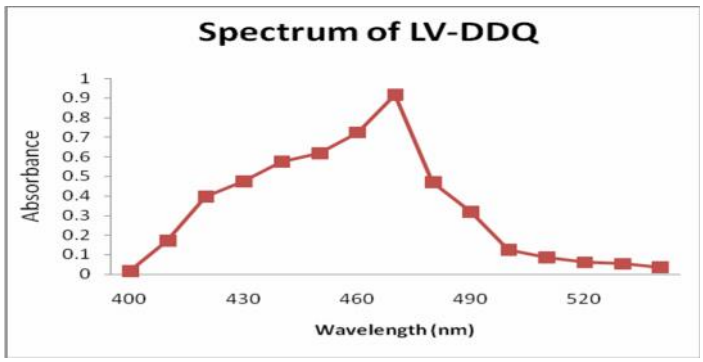

Fig: 2: Spectrum of Levobunolol-DDQ

From fig .2, it is clear that the Levobunolol drug treated with DDQ solution has maximum absorbance at 470 nm[10-13]. Hence, all further studies are made at 470 nm. The rapid formation of the complex leads to the widespread utility in the development of visible spectrophotometric methods for analysis of many pharmaceutical compounds[14-20].

***(c) Assay Procedure*:**

To study the effect of drug concentration on the absorbance of the charge transfer complex under optimal conditions now arrived is studied by the following method to know the suitability of the method for the assay of Levobunolol.

Various aliquots of the standard Levobunolol solution ranging from 0.5-2.5 ml are transferred into a series of standard flasks. To each flask, 1.0 ml of DDQ solution is added to produce light orange colour. The final volume is brought to 10 ml with chloroform. The reaction mixture in each flask is shaken well and allowed to stand for 5 min to complete the reaction. The absorbance of the orange colour solution is measured at 470 nm, against the reagent blank (prepared in similar manner omitting drug solution).

calibration graph is obtained by plotting absorbance values against the concentration of Levobunolol solution. The calibration curve is found to be linear over a concentration range of 50 to 250 μg/ml of Levobunolol. The linearity of the curve obtained indicates that it obeys Beer's law. The amount of Levobunolol present in the sample is read from the calibration graph. The results are presented in fig.3.

Analysis of tablets:

The method is then applied to the determination of the drug from the marketed tablet formulations. Tablets are weighed and contents are powdered and well mixed. The powder equivalent to 50 mg of Levobunolol is dissolved in chloroform, the volume is made upto 50 ml with chloroform, filtered, residue is washed with chloroform. Further dilution is made as described in the preparation of standard solution of Levobunolol. Further analysis is carried out as per procedure described above and results are summarized in the Table.2. The amount of drug present in the sample is estimated from calibration graph.

Average ± Standard deviation of six determinations, the t & F-values refer to comparision of the proposed method with reference method.

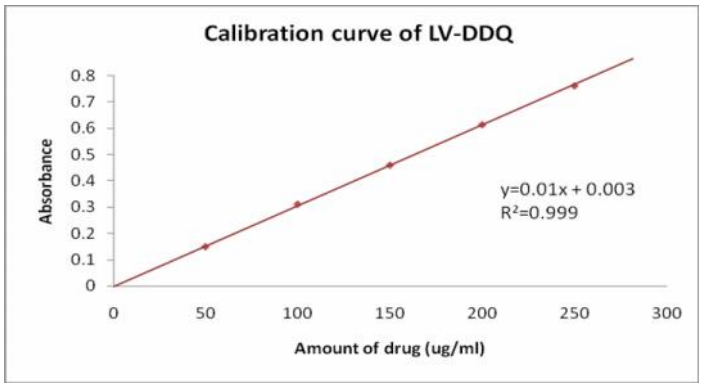

Fig.3: Calibration curve of Levobunolol

Table. 2: Assay of Levobunolol by DDQ method in pharmaceutical formulations:

Sample	**Labelled amount (mg)**	***Amount found by Reference method ± S.D***	***Amount found by proposed method ± S.D***	**% of Label claim**	**RSD%***	**t^*_{cal}**	**F***
Tablet1	10	9.972±0.011	9.966±0.027	99.66	0.272	0.541	0.173
Tablet2	100	99.972±0.011	99.958±0.029	99.58	0.280	1.163	0.164
Tablet3	5	4.974±0.010	4.953±0.033	99.26	0.680	0.820	0.093

RESULTS AND DISCUSSION

The developed method based on the reaction of Levobunolol as n-electron donor with acceptor, 2, 3-dichloro-5, 6-dicyano-1, 4-benzquinone. The absorption spectral analysis shows that the maximum absorbance of Levobunolol was found to be 470 nm. It was observed that the absorbance started decreasing above 1.0 ml of DDQ solution .Hence 1.0 ml of DDQ solution was used for further studies. The calibration curve was obtained for a series of concentration in the range of 50-250µg/ml . Statistical analysis of the results did not detect any significant difference in the performance of the proposed method to the reference method with respect to accuracy and precision as revealed by the Student's t-value [21] .The recovery technique was performed to study the accuracy and reproducibility of the proposed method. The results are shown in Table 2. The optical characteristics such as absorption maxima, Beer´s law limits are presented in Table 1. The regression analysis using method of least squares was made for the slope (m), intercept (c) and correlation (r) obtained from different concentrations and results are summarized.The percent relative standard deviation, standard deviation and student's 't' test values calculated from the six measurements of Levobunolol are presented in Table 2. Relative standard deviation values and standard deviation were low that indicates the reproducibility of the proposed method. In the student's 't' test, no significant differences were found between the calculated and theoretical values of both the proposed methods at 95% confidence level. The proposed method is found to be simple, precise, accurate and time saving, reproducible and can be conveniently adopted for routine analysis of estimation of Levobunolol in bulk drugs samples and pharmaceutical formulations.

CONCLUSION

The proposed method is found to be simple, precise, accurate, time saving and economic can be conveniently adopted for routine analysis of estimation of Levobunolol in bulk drug samples and pharmaceutical formulations as seen from the agreement of the amount of Levobunolol in the present method and the labeled amount of the pharmaceutical preparation.

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