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Article

Spectrophotometric Determination of Labetalol in Pure and Dosage Forms

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ABSTRACT

In this paper, a sensitive, accurate, and rapid spectrophotometric method has been reported for the determination of labetalol (LBT) in pure and pharmaceutical formulations. The proposed method is based on the formation of charge-transfer complex between the drug and 2, 3-dichloro-5, 6-dicyano-p-benzoquinone. The absorbance of the formed charge-transfer complex was measured at 460 nm. The developed method was evaluated in terms of standard deviation (SD), relative SD, correlation coefficient, limit of detection, and limit of quantitation. Molar absorptivity and Sandell's sensitivity were calculated at the optimum experimental conditions. The applicability of the proposed method was ascertained by recovery studies which indicated that the present methods can be successfully applied for the determination of LBT in pure and pharmaceutical formulations.

Key words: Spectrophotometric method, Labetalol, 2, 3-Dichloro-5, 6-dicyano-p-benzoquinone, Charge-transfer complex, Molar absorptivity.

1. INTRODUCTION

Labetalol (LBT), 5-[1-hydroxy-2-(1-methyl-3-phenylpropylamino) ethyl]salicylamide, (Figure 1) is a non-cardiovascular β-blocker. It possesses some intrinsic sympathomimetic activity. It has α_1-blocking properties which decrease peripheral vascular resistance. It is used to induce hypotension during surgery. It is the first adrenergic antagonist capable of blocking both α and β receptors.

Spectrophotometric methods are simple, low cost, and reliable for the determination of drugs when compared to other methods [1]. Several analytical methods such as liquid chromatography–mass spectrometry [2,3], capillary electrophoresis [4,5], polarography [6], nuclear magnetic resonance spectroscopy [7], and spectrofluorimetry [8-10] have been reported for the determination of the drug in its pure and commercial dosage forms. In addition, some spectrophotometric methods have also been developed for the quantitation of the LBT [11,12]. The survey of literature has shown that no spectrophotometric method was reported so far, for the determination of LBT with 2, 3-dichloro-5, 6-dicyano-p-benzoquinone (DDQ).

In the present investigation, a new spectrophotometric method has been developed that is simple, accurate, and reproducible for the determination LBT based on the formation of charge-transfer complex reaction with DDQ to give colored solution and can be used for the determination of LBT in bulk and its formulations.

2. EXPERIMENTAL

2.1. Instrumentation

A Shimadzu ultraviolet (UV)-visible spectrophotometer (UV-160A) with a matched pair of 10 mm quartz cells was utilized for all measurements. Mettler Toledo Analytical Balance (accuracy 0.1 mg) was used for weighing all the samples.

2.2. Materials and Reagents

LBT was procured from Sigma-Aldrich. Formulations were purchased from local market. All the chemicals used were of analytical reagent grade. Double-distilled water is used throughout the experiment. A stock solution of LBT was prepared by dissolving accurately weighed 100 mg of pure drug in 100 ml of water and sonicated to get required concentration of 1 mg/ml. Further, it was diluted with double-distilled water as required for the present investigation.

2.3. Method Development

Standard drug solution was transferred into a series of clean and dry volumetric flasks. To each flask, 3.2 ml of 0.2% DDQ solution was added and brought up to the volume with acetonitrile to get the required concentrations. A wine red color was developed, and absorbance was measured at 410 nm against the reagent blank.

2.4. Procedure

Accurately weighed amount of LBT was transferred into clean and dry volumetric flask and diluted to get the required concentration and analyzed by above-mentioned procedure.

20 tablets of each formulation were weighed and grinded to make a fine powder. A quantity of 100 mg was taken into volumetric flask and analyzed as described above.

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3. RESULTS AND DISCUSSION

3.1. Absorption Spectrum

Various volumes of LBT were taken in volumetric flasks. 2 ml of 0.2% DDQ solution in acetonitrile was added to each flask and diluted up to the mark with corresponding solvent. The maximum absorbance of the resultant solution was measured at the wavelength of 460 nm against the reagent blank (Figure 2).

3.2. Effect of the DDQ Reagent Concentration

It was found that 2.0 ml of 0.2% DDQ produced maximum intensity of the chromogen that was unaffected by further addition of reagent. Hence, 2.0 ml of the reagent solution was used for further study.

3.3. Effect of the Solvent

Polar and non-polar solvents such as chloroform, methanol, 1, 2-dichloroethane, acetone, and acetonitrile were tested to select the suitable solvent for the analysis of drug. Of all the solvents, acetonitrile was found to be the best solvent that exhibited a maximum optical density when compared to other solvents.

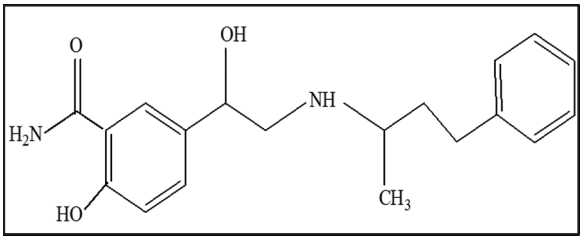

Figure 1: Structure of labetalol.

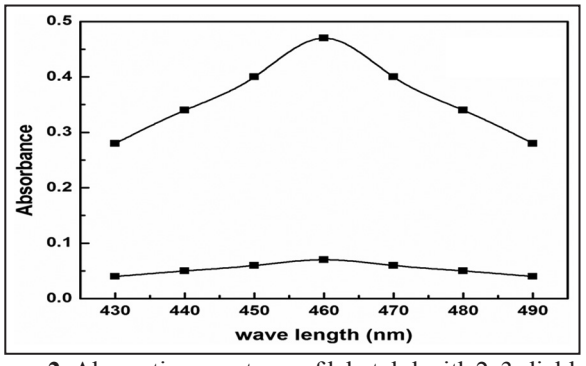

Figure 2: Absorption spectrum of labetalol with 2, 3-dichloro-5, 6-dicyano-p-benzoquinone.

3.4. Analytical Method Validation

Linearity of the LBT drug solution was studied and calibration plots were constructed with the obtained results (Figure 3). From the calibration plots, a linear correlation was found between the absorbance and concentration. The obtained results such as Beer's law limit, Sandell's sensitivity, and molar absorptivity are reported in Table 1.

During the evaluation of robustness, some parameters such as concentration of drug and reagent, wavelength range, and shaking time were interchanged. The capacity remains unaffected by small deliberate and shaking time. Method ruggedness was expressed as % reflex sympathetic dystrophy of the same procedure applied by two analysts and in two different instruments on different days. The results showed no statistical differences between different analysts and instruments, suggesting that the developed methods were robust and rugged.

The limit of detection and limit of quantitation was calculated using the following formulae.

$$LOD = \frac{3.3s}{S}$$

Where,

$$LOQ = \frac{10s}{S}$$

s = Standard deviation

S = Slope of the calibration curve.

The obtained accuracy results proved that the recovery values in the drug and in pharmaceutical formulations are within the acceptance criteria and details were presented in Tables 2 and 3.

3.5. Applications

Blood and urine samples were collected from healthy volunteers. The samples (10.0 ml) were centrifuged at 3000 rpm/min for 10 min. The solutions were filtered and preserved in the absence of light at 40°C. To this, various concentrations of LBT samples were added and analyzed by the developed method. The results are given in Table 4.

Table 1: Spectral characteristics of the drug with reagent

λmax (nm)	Beer's law limit (µg/ml)	Molar absorbance (L/mol/cm)	Sandell's sensitivity	Correlation coefficient (r^2)	Slope (m)	Intercept (c)	% RSD	Color	LOD	LOQ
460	5-26	2.304×10^4	0.0021	0.9995	0.226	0.0099	0.2128	Pinkish red	0.1327	0.4419

RSD: Reflex sympathetic dystrophy, LOD: Limit of detection, LOQ: Limit of quantitation

Table 2: Evaluation of accuracy and precision results of the proposed method in bulk form

Taken mg/ml	Intraday				Interday			
	*Found mg/ml	Recovery %	±SD	% RSD	*Found mg/ml	Recover %	±SD	% RSD
2	1.99	99.33	0.006	0.29	1.98	99.17	0.012	0.58
4	3.98	99.42	0.015	0.38	3.96	99.08	0.006	0.15
6	5.97	99.44	0.006	0.10	5.96	99.33	0.010	0.17

*Average of six determinations. SD: Standard deviation, RSD: Reflex sympathetic dystrophy

Table 3: Evaluation of accuracy and precision results of the proposed method in pharmaceutical dosage form

Pharmaceutical formulation	Taken mg/ml	Intraday				Interday			
		*Found mg/ml	Recovery %	±SD	% RSD	*Found mg/ml	Recovery %	±SD	% RSD
Gravidol	4	3.94	98.50	0.020	0.51	3.97	99.17	0.021	0.52
Labebet	6	5.98	99.61	0.006	0.10	5.96	99.39	0.012	0.19
Trandate	8	7.91	98.83	0.031	0.39	7.98	99.71	0.006	0.07

*Average of six determinations. SD: Standard deviation, RSD: Reflex sympathetic dystrophy

Table 4: Method accuracy from recovery studies

Sample	Added mg/ml	*Found mg/ml	Recovery %	±SD	% RSD
Blood samples	0.2	0.197	98.67	0.001	0.29
	0.4	0.397	99.25	0.002	0.44
	0.6	0.596	99.39	0.002	0.35
	0.8	0.791	98.88	0.003	0.33
Urine samples	0.4	0.397	99.17	0.001	0.29
	0.6	0.597	99.44	0.001	0.10
	0.8	0.796	99.50	0.002	0.25
	1.0	0.987	98.67	0.006	0.59

*Average of six determinations. SD: Standard deviation, RSD: Reflex sympathetic dystrophy

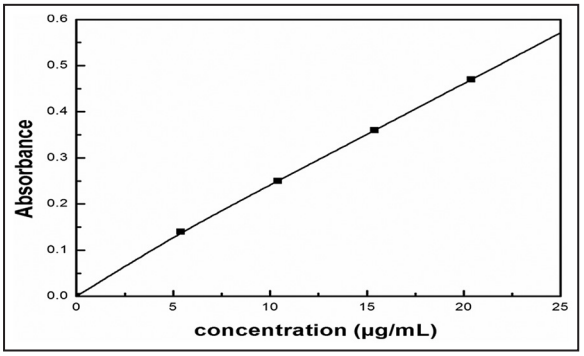

Figure 3: Calibration plot of labetalol.

4. CONCLUSION

In the current developed method, the drug LBT was estimated in bulk, in pharmaceutical formulations and in biological fluid samples. The linearity of the calibration standards of the drug by spectrophotometric method was good from the result of correlation coefficient. This indicates that the proposed method was accurate, reliable, and reproducible for the estimation of the drug LBT in bulk, in the pharmaceutical formulations and in biological fluid samples.

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***Bibliographical Sketch**

Prof. P. Venkateswarlu has more than 30 years of teaching and research experience in the Department of Chemistry, Sri Venkateswara University, Tirupati. He guided 25 Ph.D. students. He has more than 130 publications in internationally reputed journals which are indexed in Science Citation Index. He is the reviewer for many Elsevier journals.