Use of Charge Transfer Complex Formation Reaction in Spectrophotometric Determination of Timolol

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Abstract— A simple, sensitive and accurate spectrophotometric method has been developed for the determination of timolol in bulk and pharmaceutical formulations. The current technique is based on charge transfer reaction between the drug and reagent para-chloranilic acid (PCA). When the standard solution of the drug is allowed to react with PCA, a reddish-pink color is acquired and the maximum absorbance is measured at 543nm. The Beer's law is obeyed in concentration range of 6.7–24.8µg/ml in the current investigation. The suggested method is effectively applied for the analysis of timolol in bulk and pharmaceutical formulations and in biological fluid samples with excellent recovery and reproducibility.

Keywords- Timolol, para-chloranilic acid (PCA), spectrophotometric method, Beer's law, pharmaceutical formulations, biological fluid samples.

I. INTRODUCTION

Timolol, chemically ((S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol) is a first generation beta blocker essentially used in the treatment of high blood pressure, heart attacks, angina (heart ache) and in the prevention of migraine headache1. The drug is also utilized in the healing of open angle and secondary glaucoma. Blocking the stimulating actions of sympathetic nervous system allows the heart to relax and beat more leisurely is done by the drug timolol. Specifically, the quantity of blood that heart pumps is decreased. It is officially recognized in United States Pharmacopoeia[2] and British Pharmacopoeia[3]. In the literature, it was determined by Voltametry[4], HPLC[5] and HPTLC[6]. Dorzolamide and timolol maleate were analyzed simultaneously by spectrophotometry[7] and TLC/ratio derivative spectrophotometry[8]. Nagori[9] developed HPLC methods with narrow linearity range for dorzolamide and timolol.

Because of the complexity and expensive equipment involved in the existing methods, there is a need to develop simple, less expensive, more selective method for the determination of timolol. Hence in the present investigation, an attempt was made to develop a new spectrophotometric method for determination of timolol in bulk, pharmaceutical formulations and in biological fluid samples.

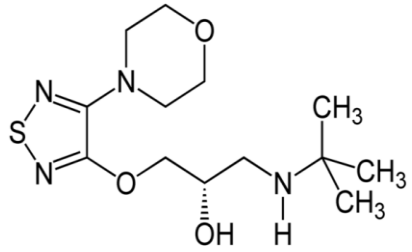

Fig.1. Structure of timolol

II. EXPERIMENTAL

A. Apparatus

The spectral measurements were carried using Schimadzu UV-visible double beam spectrophotometer (model 2450) with 1 cm matched quartz cells.

B. Materials and reagents

Acetonitrile, chloroform, methanol, 1, 4-dioxane and PCA were procured from Merck. Timolol maleate acquired from Sun Pharmaceutical Industries, Bangalore, India. Commercial dosage in the form of drops Iotim plus and Timolet plus were purchased from local market. All the chemicals used were of analytical grade. Double distilled water was used for all the experimental studies.

C. Preparation of standard solutions

The standard stock solution of timolol was prepared by adding precisely weighed 25mg of timolol into a 50ml standard flask and dissolved in acetonitrile. From this, 10ml of standard stock solution was diluted to 50ml with acetonitrile to get 100µg/ml of timolol. Further dilutions were prepared from stock solution.

D. Procedure

Fresh aliquots of the drug were taken into a series of standard flasks containing various concentrations of the drug (12–20µg/ml). To these standard flasks, 0.1% of PCA was added. Instantaneously subsequent to the addition of reagent as a result of formation of charge transfer complex, a pink colour was obtained and showed a maximum absorbance at 543nm

against the blank and the drug amount was computed from the calibration curve.

III. RESULTS AND DISCUSSION

A. Absorption spectrum

The reaction of timolol as n-electron donor with PCA as π-acceptor results in the formation of reddish pink product which exhibits maximum absorption at 543 nm (Fig. 2) due to the formation of the corresponding PCA radical anion. Thus, the absorption band at 543 nm was utilized for further experiments.

B. Effect of concentration of reagent

To determine the optimum concentration of reagent for maximum intensity of colour development, different volumes of reagent were allowed to react with a fixed concentration of drug. It was found that with 1.6ml volume of 1% PCA solution, the colour intensity was extremely elevated and stable even upon the addition of few drops of reagent. Hence, 1.6ml volume of 1% PCA was utilized for the entire investigations.

C. Effect of reaction time

The optimum reaction time was calculated based on the intensity of colour developed upon the addition of reagent to the standard drug solution. The colour was developed immediately after the addition of reagent and it was stable for 90 minutes and all the required readings were taken in the précised period.

D. Effect of solvent

For the dissolution of the reagent some solvents like acetonitrile, methanol 1, 4 dioxane were checked for suitability. Among those, 1, 4 dioxane produced maximum absorbance; hence it was selected as suitable solvent for dissolution of the PCA reagent.

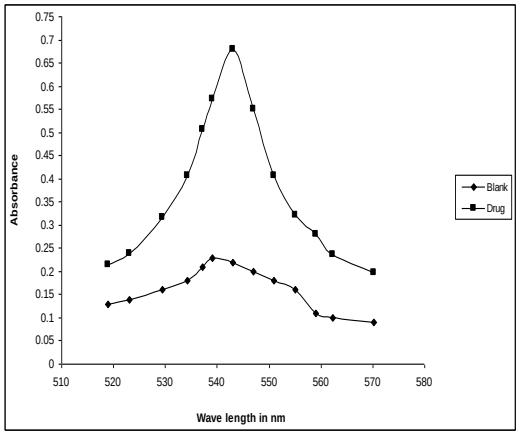

Fig.2. Absorption spectrum of timolol with PCA

IV. Method validation

The proposed method was validated according to ICH guidelines for the successful quantitative determination of timolol. The characters of validation such as linearity, accuracy, precision, specificity, limit of detection, limit of quantification, robustness and ruggedness were studied.

A. Linearity

Linearity of the method was studied calibration plots were obtained from the results. From the calibration plots a linear correlation was found between the absorbance and the concentration ranges (Fig. 3). The optical characters like Beer's law limit, Sandell's sensitivity, molar absorptivity were given in table1.

B. Robustness and Ruggedness

Few analytical parameters like pH range, concentrations of both drug and reagents and shaking time were interchanged and it was noticed that the results were unaffected which confirms the robustness of the proposed method.

Percentage of relative standard deviation is a measure of ruggedness. The experiments were repeated by two different analysts using two different instruments in two different days. From the results it was observed that there was no statistical difference between the above said two analysts and instruments, hence the developed analytical method was confirmed to be robust.

C. Accuracy

Accuracy was ascertained from the recovery studies by standard addition method. The recoveries of the timolol by the proposed method were observed very near to 100 percent which proves that the method was accurate.

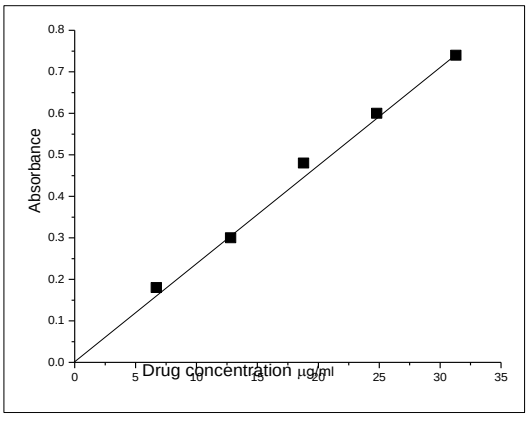

Fig. 3. Calibration plot of timolol with PCA

D. Precision

Precision is to measure the ability to create reproducible results. The precision of the present method was evaluated by intra-day and inter-day experiments by analyzing the same concentrations of the solutions on three different days. The RSD% was less than one proved that there was no considerable difference, which proves that the developed method was precise (table 2).

E. Detection and quantification limit

Sensitivity of the method can be expressed in terms of LOD and LOQ of the developed method. LOD is 3s/S and LOQ is 10s/S where s is the standard deviation of the replicate determination values and S is the slope of the calibration graph.

F. Recovery

For recovery studies of selected drug timolol, samples of different concentrations were analysed by the proposed method and the recovery percentages were found to be accurate which indicates the accuracy and selectivity of the proposed method.

V. Applicability of the proposed method

Assay of pharmaceuticals, serum and urine samples

The standard solution of timolol in its dosage form, Timolet plus (20mg/5ml) was prepared accurately by taking 25ml of drug solution in 100 ml volumetric flask and diluted upto the mark. The resulting solution shows a concentration of 1 mg/ml. From this stock solution 1 ml was pipetted out to a 10 ml volumetric flask and diluted up to the mark with distilled water and utilized for further analysis (table 3).

Blood and urine samples were collected from donor's and were centrifuged at 3000 rpm for nearly 10 minutes. The resulted solutions were filtered and stored in the absence of light at a temperature of 4^{o}C. From these solutions, different concentrations of the drug were determined by the proposed analytical method (table 4).

VI. CONCLUSION

In the present work, a reliable, sensitive, accurate and selective spectrophotometric method was developed for the determination of timolol in both bulk and in pharmaceutical formulations and compared the results with reference method[10] and found that the results are in good agreement with those of reference method. The method was linear which is evident from the values of correlation coefficient. The proposed analytical technique was also unaffected by interferences due to the excipients and other impurities present in the pharmaceutical formulations. Thus the proposed method was more accurate for the estimation of the timolol in its pharmaceutical forms and in biological fluids than the existing methods and can be applied successfully for regular quality control.

Table 1. Optical characteristics of proposed method

Parameter	Value
λmax (nm)	543
Beer's law limit (µg/ml)	6.7-24.8
Molar absorbance (L.mol-1 cm-1)	0.6
Sandells sensitivity (µg.cm-2/0.001 A.U)	0.001667
Correlation coefficient (r2)	0.99702
Slope (m)	0.023132
Intercept (c)	0.03106
%RSD	0.166667
Colour	Reddish pink
LOD	0.129692
LOQ	0.431873

Table 2. Evaluation of intra-day and inter-day accuracy

Taken mg/ml	Inter day *Found mg/ml	Recovery%	±SD	% RSD
3	2.98	99.48	0.015	0.501
6	5.95	99.22	0.020	0.349
2	**1.98**	**99.00**	**0.01**	**0.505**
4	**3.96**	**99.08**	**0.02**	**0.385**
	Intra day			
3	**2.97**	**99.01**	**0.020**	**0.67**
6	**5.94**	**98.98**	**0.026**	**0.44**
2	**1.98**	**98.83**	**0.015**	**0.78**
4	**3.97**	**99.17**	**0.015**	**0.39**

***Average of five determinations**

Table 3. Determination in pharmaceutical dosage

Formulation	Taken mg/ml	*Found mg/ml	Recovery %	±SD	%RSD
Iotim plus Timolet plus	4	3.95	98.75	0.030	0.76
	6	5.96	99.28	0.032	0.54
	8	7.97	99.62	0.020	0.25
	10	9.95	99.50	0.043	0.43

***Average of five determinations**

Table 4. Method accuracy from recovery assay

Sample	Added mg/ml	*Found mg/ml	Recovery %	±SD	RSD%
Serum samples	2	1.980	99.000	0.005	0.505
	4	3.967	99.167	0.006	0.634
	6	5.943	99.056	0.005	0.541
	8	7.947	99.332	0.006	0.567
Urine samples	2	1.973	98.667	0.011	1.055
	4	3.970	99.250	0.007	0.666
	6	5.960	99.333	0.003	0.336
	8	7.947	99.334	0.003	0.291

***Average of five determinations**

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