Spectrophotometric Determination of Metoprolol with P- Chloranilic Acid by Charge Transfer Reaction

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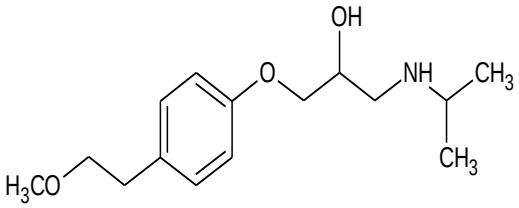
Abstract— In the present investigation, a simple and sensitive spectrophotometric method was developed for the assay of metoprolol in bulk and dosage forms. The developed method was purely based on charge transfer complex reaction between the drug, a n-electron donor and p-chloranilic acid (PCA), a π- acceptor in non-aqueous medium. PCA forms charge transfer complex with metoprolol with the formation of reddish pink chromogen. The maximum absorption band was recorded at 538nm. Beer's law was obeyed in the concentration range of 6.5-24.6µg/ml. The proposed method has been successfully applied for the analysis of metoprolol in bulk, pharmaceutical formulations and biological fluid samples with high accuracy and precision.

Keywords- Metoprolol, para chloranilic acid (PCA), spectrophotometric method, Beer's law, charge transfer complex, pharmaceutical formulations.

I. INTRODUCTION *(HEADING 1)*

Metoprolol, (*RS*)-1-(isopropylamino)-3-[4-(2-methoxy-ethyl)phenoxy] propan-2-ol[1] is a beta blocker, used in the treatment of disorders like hypertension, angina pectoris, arrhythmia, Congestive Heart Failure (CHF) and myocardial infraction[2]. This drug is officially listed in Martindale and the Extra Pharmacopoeia[3]. In British Pharmacopoeia, the drug is determined by potentiometric titration method[4]. It was determined by several analytical methods such as thin layer chromatography[5], infrared spectroscopy[6], high performance liquid chromatography[7], electrophoresis[8], electrochemical method[9], Gas Chromatography- Mass Spectrometry (GC-MS)[10-11], LC-MS[12] and LC-MS-MS[13] and spectrophotometric method[14]. These methods, however, suffer from disadvantages such as lack of sensitivity, accuracy and selectivity.

Hence in the present investigation, a new spectrophotometric method has been developed which is simple, cost effective, selective, accurate and rapid for the determination of metoprolol in bulk, in pharmaceutical formulations and in biological fluid samples.



Structure of Metoprolol

Fig.1. Structure of metoprolol

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

Chloroform, acetonitrile, methanol, 1, 4-dioxane and PCA were procured from Merck. metoprolol was procured from Astra Zeneca, India. The commercial dosage of the drug in the form of tablet metolar (50 mg) and betaloc (50 mg) 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

Accurately weighed 100 mg of metoprolol was taken in100 ml standard flask and dissolved in methanol to get the resulting solution of concentration 1mg/ml. From this stock solution, 10 ml was pipetted out into a 100 ml standard flask and made up to the mark with methanol to get 100 µg/ml concentration. PCA stock Solution was prepared by mixing 250 mg of PCA in 100 ml of 1, 4-dioxane (11.96x 10-3 M). The required working solutions of reagent were prepared by diluting the stock solution.

D. Procedure

Fresh sample of metoprolol solution with concentrations ranging from 0.6 – 1.4 ml (6–14 µg/ml) was transferred to clean and dry 10 ml volumetric flasks. To each flask, 1ml of PCA solution of concentration 4.6 x 10^{-3} mol / dm^{-3} was added, the maximum absorbance was measured at 538 nm against the blank and amount of the drug in the solution was computed from calibration curve.

III. RESULTS AND DISCUSSION

A. Absorption spectrum

The reaction of metoprolol as n-electron donor with PCA as π-acceptor results in the formation of reddish pink product which exhibits maximum absorption at 538 nm (Fig. 2) due to the formation of the corresponding PCA radical anion. Thus, the absorption band at 538 nm was utilized for further experiments.

B. Effect of concentration of reagent

The effect of concentration of the reagent on the colour intensity developed was obtained by adding different volumes of PCA to a fixed concentration (12 µg / ml) of the drug and it was found that 10ml of 4.6 x 10^{-3} mol/dm^{-3} PCA was produced maximum colour intensity and it was unaffected by the further addition of reagent.

C. Effect of reaction time

The optimum reaction time was measured for the formation of colour by the addition PCA to metoprolol solution at laboratory temperature. Upon the addition of reagent, the colour developed instantaneously. The colour remained stable for 2 hours.

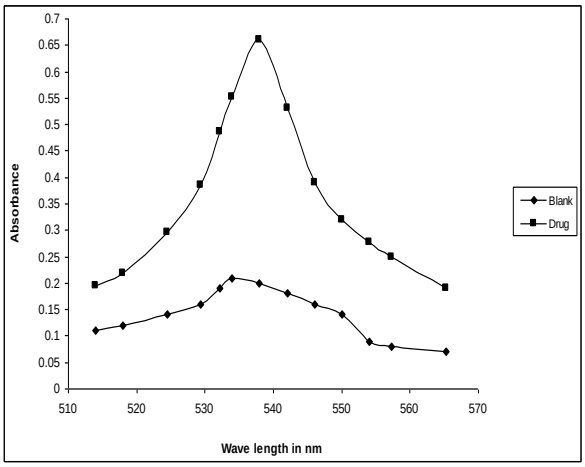


Fig.2. Absorption spectrum of metoprolol with PCA

D. Effect of solvent

Metoprolol was dissolved in several solvents and the absorbance in each solvent was recorded at a fixed concentration of drug and reagent. Among all the solvents, methanol was selected as a suitable solvent. In the case of reagent, the colour intensity was observed very high when it was dissolved in 1, 4-dioxane. So, methanol and 1, 4-dioxane was chosen as the best solvents for the drug and reagent throughout the experiment.

IV. Method validation

For the quantitative analysis of metoprolol, the method was validated according to ICH guidelines in terms of linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantification (LOQ) robustness and ruggedness. Standard calibration curve was constructed by plotting absorbance versus concentration (Fig. 3). The linearity of calibration graph was proved by high values of correlation coefficient. The molar absorbtivity, LOD and LOQ of the coloured complexes and relative standard deviation for the proposed method was also calculated and presented in table 1.

A. Robustness and Ruggedness

For the evaluation of robustness, some parameters like pH range, concentrations of the drug and reagents and shaking time were interchanged. Even after that, the results were unaffected. Ruggedness of the method was expressed as the percentage of relative standard deviation for the proposed method developed by two analysts in two different instruments in two different days. The results proved that there was no statistical difference between the above said two analysts and instruments which conclude that the developed method was robust and rugged.

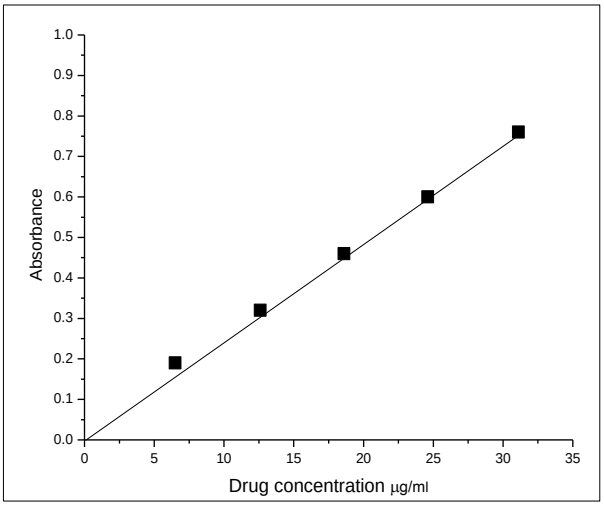


Fig.3. Calibration plot of metoprolol

TABLE 1. OPTICAL CHARACTERISTICS OF PROPOSED METHOD

Parameter	Value
λmax (nm)	538
Beer's law limit (µg/ml)	6.5-24.6
Molar absorbance (L.mol-1 cm-1)	0.58
Sandells sensitivity (µg.cm-2/0.001 A.U)	0.002
Correlation coefficient (r2)	0.998
Slope (m)	0.023
Intercept (c)	0.024
%RSD	0.172
Colour	Reddish-pink
LOD	0.132
LOQ	0.439

B. Accuracy

To study the accuracy of the proposed analytical method, recovery studies were carried out in bulk drug and in biological fluid samples. The results proved that the developed method is highly accurate.

C. Precision

Precision is to measure the ability to create reproducible results. The intra-day and inter-day precision was determined by analyzing the same concentration of the solutions in three different days. RSD% of the precision was less than one which proves that there was no considerable difference for the assay which was tested in inter-day and intra-day (table 2).

D. Limit of detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ indicate sensitivity of the proposed method. LOD is 3s/S and LOQ is 10s/S. S is the slope of the calibration graph and s is the standard deviation of the replicate determination values.

TABLE 2. EVALUATION OF ACCURACY

Taken mg/ml	Inter day				Intra day			
	*Found mg/ml	Recovery%	±SD	% RSD	*Found mg/ml	Recovery%	±SD	% RSD
2	1.98	99.06	0.004	0.210	1.976	98.83	0.005	0.29
4	3.98	99.55	0.007	0.181	3.984	99.61	0.006	0.16
2	1.97	98.17	0.01	0.778	1.982	99.12	0.012	0.63
4	3.96	99.08	0.02	0.525	3.961	99.02	0.030	0.78

***Average of five determinations**

TABLE 3. DETERMINATION OF IN PHARMACEUTICAL DOSAGE

Formulation	Taken mg/ml	*Found mg/ml	Recovery %	±SD	%RSD
Metolar	5	4.95	99.01	0.040	0.710
	15	14.98	99.88	0.010	0.0706
Betaloc	10	9.96	99.63	0.015	0.153
	20	19.98	99.91	0.008	0.0400

***Average of five determinations**

TABLE 4. RECOVERY ASSAY

Sample	Added mg/ml	*Found mg/ml	Recovery %	±SD	RSD%
Serum samples	2	1.972	98.667	0.021	1.055
	4	3.974	99.333	0.015	0.384
	6	5.950	99.168	0.030	0.504
	8	7.947	99.333	0.042	0.524
Urine samples	2	1.980	99.000	0.010	0.505
	4	3.973	99.334	0.006	0.145
	6	5.970	99.500	0.011	0.168
	8	7.940	99.250	0.026	0.333

***Average of five determinations**

V Applicability of the proposed method

A. Assay of pharmaceuticals

The contents of 4 tablets were weighed and finely powdered. A portion of powder equivalent to 50mg of the drug was taken into 50ml standard flask and dissolved in small portion of methanol and made up to the mark with the same solvent. The contents in the flask were filtered with whatmann No. 41 filter paper and washed well with methanol for the complete recovery of the drug. The resulting concentration of the solution was found to be 1 mg/ml. Required aliquots were taken from the above stock solution for further experiments of recovery. The results obtained were satisfactory with high accuracy (table 3).

B. Assay in serum and urine samples

Serum and urine samples were collected from healthy donors and centrifuged at 3000 rpm per min. for nearly 10 min. The resulted solutions were filtered and preserved in the absence of light at a temperature of 4^{o}C. These samples were spiked with the drug and analyzed by the proposed method. From the

results obtained (table 4), it can be concluded that the proposed method could be successfully applied to recover metoprolol from biological samples.

VI. Conclusion

In the present method, metoprolol was determined in bulk, tablet form and in biological fluid samples and compared the results with those of reference methods[15,16]. The linearity of the developed method was good which is evidenced from correlation coefficient values. The proposed method was free from interference due to the excipients and other impurities present in the tablet forms. The high recovery values obtained indicate that the proposed method is more selective, sensitive, reliable and accurate than the existing methods for the estimation metoprolol in bulk, tablets and biological fluid samples.

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