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Spectrophotometric Determination of Phentolamine by Charge Transfer Complex Method.

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ABSTRACT

A new simple and specific spectrophotometric method for the determination of phentolamine has been developed. The method is based on charge transfer complex reaction between the drug, a n-electron donor and 2,3-dichloro-5,6-dicyano-1,4-benzquinone (DDQ), a π -acceptor in non-aqueous medium. DDQ forms charge transfer complex with phentolamine with the formation of red colour complex which is used to determine the drug spectrophotometrically. The maximum absorbance was obtained at 410 nm with apparent molar absorptivity of 1.1254 L mol^{-1} cm^{-1}. Beer's law was obeyed in the concentration range of 30-120 µg/ml with Sandel's sensitivity, slope and intercept 0.0033 µg/cm^{-2}, 0.0040 and -0.0014 respectively. Results of the analysis were validated statistically and by recovery studies. The average percentage recovery was found to be more than 99. The developed method was found to be free from interference of additives and impurities during estimation.

Key words: Phentolamine, DDQ, spectrophotometric method, Beer's law.

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INTRODUCTION

Phentolamine is chemically 3-[N (4,5-dihydro 1H-imidazol-2yl methyl) – 4-methylanilino] phenol. It is a reversible [1] non selective alpha –adrenergic antagonist. It is used in the treatment of hypertension and hypertensive emergencies. It is also used in the treatment of pheochromocytoma prior to the administration of beta blockers to avoid unopposed alpha stimulation [2,3]. It has diagnostic and therapeutic roles in complex regional pain syndrome [4]. Literature survey reveals that several analytical methods were reported for the determination of phentolamine in bulk and in formulations which include high-performance liquid chromatography [6,7], chemiluminescence [8], liquid chromatography-mass spectrometry [9] and thin layer chromatography [10].

But the above mentioned methods are very complex and expensive equipment is involved. Hence, there is a need to develop simple, less expensive and more selective method for the determination of phentolamine. Hence in the present investigation, an attempt was made to develop a new spectrophotometric method for determination of phentolamine in bulk, in pharmaceutical formulations and in biological fluid samples.

EXPERIMENTAL

Instrumentation

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

Chemicals and reagents

Acetonitrile, chloroform, methanol, 1, 4-dioxane and DDQ were procured from Merck. Phentolamine was acquired from Sun Pharmaceutical Industries, Bangalore, India. Commercial dosage forms were purchased from local market. All the chemicals used were of analytical grade. Double distilled water was used for all the experimental studies.

Preparation of standard stock solution

Phentolamine tablet powder equivalent to 100 mg of drug was transferred in to 100 ml volumetric flask containing 50 ml of methanol and kept for ultrasonication for 5 min, then it was diluted up to the mark with methanol and the solution was filtered through whatman filter paper 41, to get concentraction of 1mg/ml. From this solution 10 ml was pipetted out in to 100 ml volumetric flask and the volume was made up to the mark with methanol. The final concentration of phentolamine was brought to 100 µg/ml with methanol and used for the analysis. Working standard solutions were prepared by appropriate dilution of standard stock solution with methanol.

Procedure

The fresh aliquots of standard drug solution of phentolamine ranging from 0.2-1.0 ml (2-10 µg/ml) were transferred into a series of 10 ml volumetric flasks. To each flask, 1.0 ml of DDQ solution was added and kept on water bath for 20 min for complete colour development and cooled, then transferred the colored solution in to 100 ml separating funnel. The mixture was extracted twice with 10 ml chloroform by shaking for 2 min and then allowed to stand for clear separation of the two phases. The absorbance of the separated chloroform layer i.e reddish colored complex was measured at 410 nm against the reagent blank. Calibration graph was obtained by plotting absorbance values against the concentration of phentolamine solution. The calibration curve is found to be linear over a concentration range of 30-120 µg/ml of phentolamine.

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RESULTS AND DISCUSSION

Absorption spectrum

The reaction of phentolamine as n-electron donor with DDQ as π- acceptor results in the formation of red colour product which exhibits maximum absorption at 410 nm (Fig. 1) due to the formation of charge-transfer complex. Thus, the absorption band at 410 nm was utilized for further experiments. The optical characteristics such as Beer's law limit, Sandel's sensitivity, molar extinction coefficient and relative standard deviation were calculated for the method and results were summarized in Table 1.

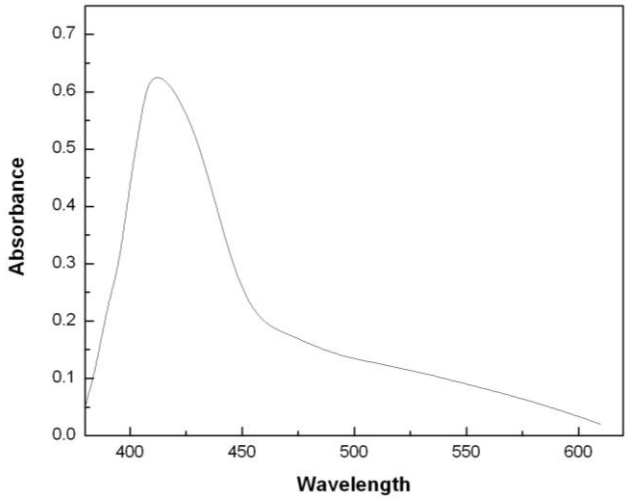

Figure 1: Absorption Spectrum of Phentolamine drug treated with DDQ solution

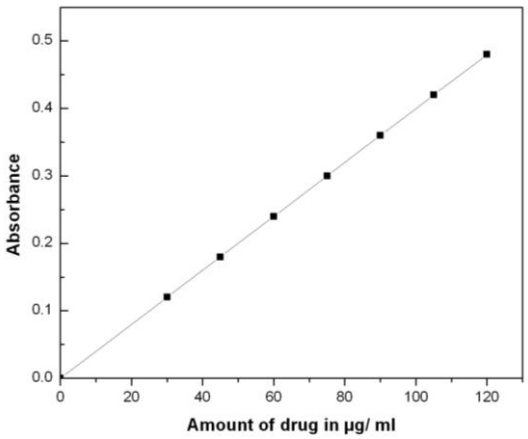

Figure 2: Calibration of curve of phentolamine

Validation of proposed method

Linearity

The standard calibration curve was plotted over the concentration range of 30-120 µg/ml for phentolamine, that gives the linear equation y = 0.0040 x -0.0014 (correlation coefficient r = 0.9992). A good correlation was seen between the absorbance and respective concentration of phentolamine.

Accuracy

The accuracy of the proposed analytical method was assessed by inter day and intraday recovery studies and the results were recorded in Table 2. These results revealed that any small change in the drug

concentration in the solution could be accurately determined by the proposed analytical method. The accuracy of the developed method was also proved by less standard deviation values and high recovery percentage.

Detection limit

The limit of detection (LOD) and limit of quantification (LOQ) were determined as follows. 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.

Effect of interference

The effect of the additives such as glucose, sucrose, lactose, dextrose, talc, starch which frequently added with the phentolamine in its dosage forms was studied. The results were recorded in Table 4. From the results, it was concluded that there is no significant effect of interferences on the recovery percentage of phentolamine by the proposed method.

Assay pharmaceutical formulations and in serum and urine samples

The standard solution of phentolamine in its dosage form was prepared and utilized for determination of drug in its pharmaceutical formulations as described above. The results were presented in Table 3. Blood and urine samples were obtained from healthy volunteers and serum was separated from blood by centrifugation at 5,000 rpm for 10 min. The resulted solutions were filtered and preserved in the absence of light at a temperature of 4^0C. These samples were spiked with the drug and analyzed by the proposed method and the results were given in Table 5.

Table 1: Optical Characteristics of the Proposed Methods

Parameters	value
$\lambda \max(nm)$	410
Beer's law limit (μg/ml)	30-120
Molar absorptivity (L.mol^{-1} cm^{-1})	1.1254
Sandal's sensitivity (μg.cm^{-2}/0.001 A.U)	0.00333
Slope(b)	0.0040
Intercept(a)	-0.0014
Correlation coefficient(r^2)	0.9988
% RSD	0.333
LOD	0.7446
LOQ	2.47978

*Y=a+bX, where Y is the absorbance and X concentration in $\mu g / ml$

Table 2: Evaluation of Interday and Intraday Accuracy

Added	Inter day				Intra day			
μg/ml	Found	Recovery %	±SD	%RSD	Found	Recovery %	±SD	%RSD
12	11.99	99.93	0.005	0.046	11.98	99.8	0.0057	0.045
13	12.99	99.94	0.005	0.042	12.97	99.8	0.0054	0.462
14	13.98	99.91	0.015	0.0036	13.95	99.7	0.0053	0.035
15	14.98	99.90	0.006	0.035	14.97	99.6	0.0067	0.0321

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Table 3: Assay of Phentolamine in Tablet Formulations

Labeled amount mg/ml	Amount found mg/ml	%Recovery	±SD	% RSD
250	249.53	99.33	0.0577	0.2854
250	249.53	99.33	0.0288	0.2906
250	249.43	99.64	0.0450	0.3016

*A verage of five determination based on label claim

Table 4: Determination of Phentolamine in Presence Of Excipients

Excipients	Amount taken mg/ml	*Found mg/ml	Recovery %	±SD	RSD %
Glucose	4	3.97	99.25	0.0200	0.503
Sucrose	6	5.97	99.50	0.2645	0.443
Lactose	8	7.98	99.79	0.0057	0.723
Dextrose	10	9.93	99.33	0.0288	0.290
Talc	12	11.97	99.77	0.0208	0.173
Starch	14	13.97	99.80	0.3511	0.148

* Average of five determinations

Table 5: Method Accuracy from Recovery Assay

Sample	Added mg/ml	*Found mg/ml	Recovery %	±SD	RSD%
Serum samples	0.8	0.78	98.54	0.0076	0.9688
	1	0.98	98.66	0.0057	0.5851
	1.2	1.19	99.55	0.0045	0.3774
	1.4	1.39	99.61	0.0045	0.3233
Urine samples	0.4	0.39	98.58	0.0040	0.9248
	0.6	0.59	99.55	0.0020	0.3484
	0.8	0.79	99.58	0.0020	0.2612
	2.0	1.98	99.41	0.0076	0.3841

*Average of five determinations

CONCLUSION

A simple, rapid and sensitive spectrophotometric method for the simultaneous determination of phentolamine in pharmaceutical formulations, human serum and urine sample has been developed and validated in this study. 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 can be applied successfully for regular quality control.

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