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UTILITY OF ANALYTICAL REAGENT FOR SPECTROPHOTOMETRIC DETERMINATION OF NEBIVOLOL HYDROCHLORIDE

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ABSTRACT

A simple, sensitive and rapid spectrophotometric method was developed for the determination of nebivolol hydrochloride (NBV) in pure and its formulations. The developed method was based on the formation of charge-transfer complex between the drug, an n-electron donor and π-acceptors, reagent 2, 3-dichloro-5, 6-dicyano-p-benzoquinone. The formed charge-transfer complexe absorbance was measured at nm and used for the estimation of NBV in its pure and commercial dosage forms. The developed method was obeyed the beer's law in the concentrate range 4-28 µg/ml, percentage of recovery was found 99.02 to 99.52% and method was validated as per ICH guidelines.

INTRODUCTION:

Nebivolol Hydrochloride (NBV) is chemically known as 1-(6-fluorochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} ethanol hydrochloride and is structurally given in **Fig.1** having with formula $C_{22}H_{26}F_2NO_4Cl$ and molecular weight 441.90 g/mol. It is cardioselective $beta_1$ receptor blocker with nitric oxide potentiating vasodilatory effect used in the treatment of hypertension and also for left ventricular failure[1]. It is highly cardioselective under certain circumstances[1]. Nebivolol is approximately 3.5 times more β1-adrenoceptor-selective than bisoprolol and other β1-adrenergic blockers in human myocardium and thus might be the most β1-adrenoceptor-selective antagonist available for clinical practice at the moment[2]. It is officially published in IP[3]. While reviewing literature, UV spectrophotometry[4], capillary electrophoresis[5], high performance thin-liquid chromatography (HPTLC) [6] and high-performance liquid chromatography (HPLC) [7, 8] were reported with UV detection for determination of nebivolol in pharmaceutical forms. Two methods were

reported on the separation of enantiomers and the determination of nebivolol metabolites[9]. To the best of our knowledge, no spectrophotometric methods were reported for estimation of colour complex formation of nebivolol hydrochloride with reagent of DDQ. A novel spectrophotometric method has been developed for the determination of nebivolol hydrochloride with reagents which is time saving, simple and reproducible in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

Materials and reagents: Nebivolol hydrochloride was procured from reputed pharmaceutical company as a free sample and its formulations i.e. Mucolite, Ambrodil and Ambrolite were purchased in local market, Tiurpati. All the chemicals used were of analytical reagent grade.

Instrumentation: All measurements were carried out using a Shimadzu UV-Visible spectrophotometer (UV-160A) with a matched pair of 10 mm quartz cells. Mettler Toledo analytical balance (accuracy 0.1 mg) was used for weighing all the samples.

Preparation of standard solution: Standrad solution was prepared by dissolving 100mg of NBV in 100ml volumetric flask in DMSO. The concentration of resulting solution was 1 mg/ml and further diluted to obtain required concentrations for the present investigation.

Preparation of reagent: 0.20% (w/v) of reagent solution was prepared by dissolving 0.2g of DDQ compound in 100 ml of acetonitrile in standard volumetric flask, sonicated and used as such.

Method Development: Freshly prepared aliquots of NBV in the range of 4-28µg/ml were transferred to a series of clean and dry volumetric flasks and added 2.2 ml of 0.2% DDQ solution to each flask followed by mixing of the contents to obtain wine red chromogen. The maximum absorbance of the formed charge transfer complex was measured at 450 nm against the reagent blank.

Procedure for analysis of pure drug

Accurately weighed an amount of pure drug BRH was transferred into clean and dry volumetric fl ask, subsequently diluted with water to get the required concentration and analyzed by above mentioned the procedure.

Procedure for Assay of Pharmaceuticals: Twenty tablets were weighed and grinded into fine powder. A quantity of grinded powder eqavalent to 100 mg was taken into volumetric flask dissolving with DMSO and analysed solution by using as stated above methods.

RESULTS:

The molecular interaction between electron donors and acceptors is generally associated with the formation of intensely colored charge transfer complexes, which absorb in the visible region [9]. The photometric methods based on molecular interactions are simple and appropriate since they result in the rapid formation of the complexes. NBV is n-electron donor and will form charge-transfer complexes with selected reagents which act as π-acceptors.

Absorption Spectrum: Fresh aliquots of 0.2% DDQ solution was added into various volumes of NBV solution and measured the maximum absorbance of wine red chromogen at 450 nm against the reagent blank.

Effect of reagent concentration: To obtain optimum concentration of reagent, standard solution was allowed to react with 0.2% DDQ solution in the range of 0.2-2.8 ml and found that high intensity chromogen was formed at 2.2 ml.

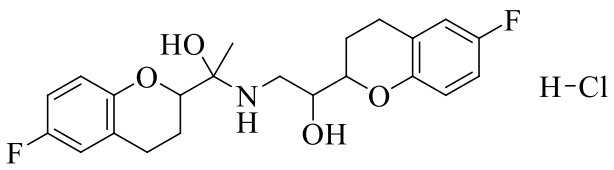

Fig. 1 Structure of nebivolol hydrochloride

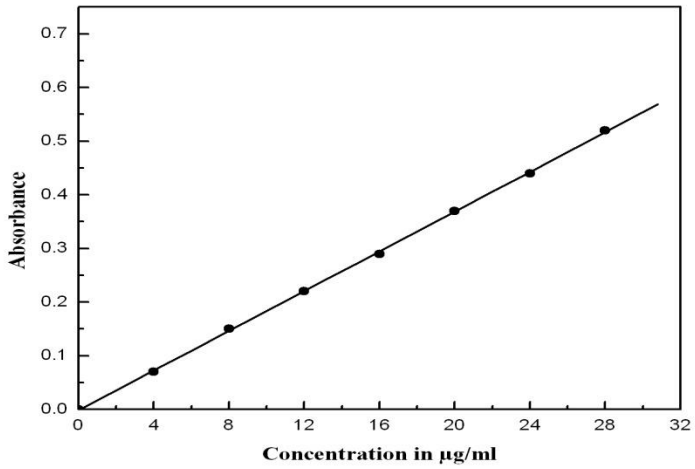

Fig.2 Calibration plot of nebivolol hydrochloride

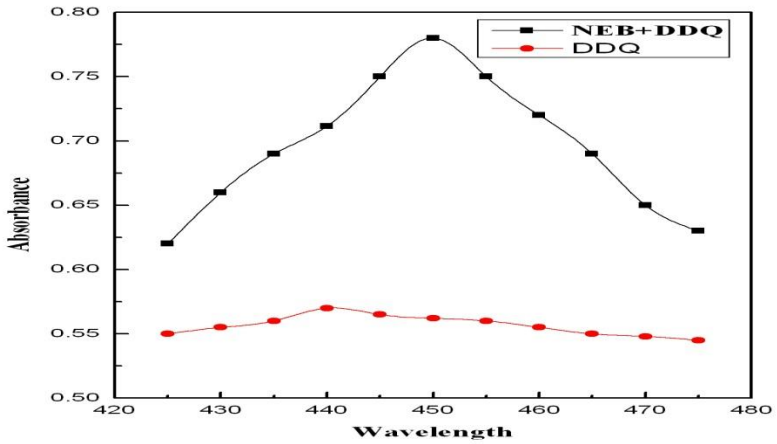

Fig.3 Absorption spectrum of nebivolol hydrochloride with DDQ

Table 1: Spectral characteristics of the drug with reagent

Parameter	DDQ method
λmax (nm)	450
Beer's law limit (µg/ml)	4 - 28
Molar absorbance (L.mol-1 cm-1)	6.0982
Sandell's sensitivity (µg.cm-2/0.001 A.U)	0.0014
Correlation coefficient (r2)	0.9954
Slope (m)	0.0175
Intercept (c)	0.0282
%RSD	0.1775
Colour	Wine Red
LOD	0.2314
LOQ	0.7012

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

Method	Taken mg/ml	Intra day				Inter day			
		*Found mg/ml	Reco-very %	± SD	% RSD	*Found mg/ml	Recov-ery%	± SD	% RSD
DDQ method	4	3.93	98.21	0.0098	0.2503	3.94	98.58	0.0207	0.5238
	6	5.93	98.81	0.0147	0.2483	5.96	99.25	0.0356	0.5984
	8	7.94	99.25	0.0141	0.1781	7.92	98.98	0.0387	0.4886

***Average of six determinations**

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

Method	Pharma-ceutical formulation	Taken mg/ml	Intra day				Inter day			
			*Found mg/ml	Reco-very %	± SD	% RSD	*Found mg/ml	Recov-ery%	± SD	% RSD
DDQ method	Nebicard	4	3.93	98.21	0.0343	0.8732	3.93	98.13	0.0321	0.8177
	Nebilong	6	5.92	98.67	0.0089	0.1511	5.95	99.08	0.0226	0.3799
	Nebistar	8	7.91	98.83	0.0081	0.1033	7.87	98.33	0.0388	0.4934

***Average of six determinations**

Table 4: Determination of recovery of nebivolol hydrochloride in pharmaceutical formulation

Name of the drug	Pharmaceutical formulation	Labeled amount (mg/ml)	*Found (mg/ml)	Recovery %	± SD	% RSD
Nebivolol hydrochlo-ride	Nebicard	5	4.95	99.02	0.0169	0.3597
	Nebilong	5	4.96	99.18	0.0206	0.4386
	Nebistar	5	4.98	99.52	0.0116	0.2393

***Average of six determinations**

Table 5: Determination of nebivolol hydrochloride in presence of excipients

Excipients	Amount taken mg/ml	*Found mg/ml	Recovery %	±SD	RSD%
Glucose	2	1.96	98.17	0.0186	0.9483
Sucrose	3	2.95	98.22	0.0216	0.7331
Lactose	4	3.94	98.50	0.0141	0.3589
Dextrose	2	1.97	98.67	0.0121	0.6137
Talc	3	2.96	98.61	0.0232	0.7831
Starch	4	3.95	98.83	0.0314	0.7946

***Average of six determinations**

Effect of the concentration of the drug: In the standard solution of range of 4-28µg/ml, a known standard volume of reagent was added for colour development and measured the maximum absorbance at 450 nm and all the results obeyed the Beer's law.

Method validation: The present developed method for determination of NBV was validated according to the International conference on harmonization (ICH) guidelines[17,18] with respect to the parameters like linearity, accuracy, precision, recovery and specificity, Limit of detection (LOD), Limit of quantitation (LOQ) and robustness. Standard calibration curve was constructed by plotting absorbance versus concentration [Fig.2] and regression equation was derived from the calibration plot. The linearity of calibration graphs was proved by the high values of the correlation coefficient and the small values of the y – intercept of the regression equation. Recovery studies were conducted by using standard addition method and obtained results reported in table 4 to proved accuracy of the method. To confirm the repetability, intra day and inter day analysis were performed and obtained results were reported in terms of % RSD in respective table 2 and table 3 and found no significanat variation. To assess the specificity and selectivity of developed method, the effect excipients like starch, lactose, glucose, sugar, talc etc. were studied. The results indicated in table 5 that there was no effect of interference from the excipients on the developed methods. The Sandell's sensitivity, LOD, LOQ of the resulting colored complexes were also calculated and reported in respective table 1.

DISCUSSION

Nebivolol hydrochloride is a cardioselective beta$_1$ receptor blocker and also used in treatment of hypertension.

Nebivolol is commercially available in the form of Nebest, Nebicard, Nebistar, Nebilong etc. In a laboratory experiment conducted on biopsied heart tissue, nebivolol proved to be the most β1-selective of the β-blockers tested, being approximately 3.5 times more β1-selective than bisoprolol. However, the drug's receptor selectivity in humans is rather more complex and depends on the drug dose and the genetic profile of the patient taking the medication. The nebivolol hydrochloride was analysed by using current developed method in both bulk and tablet form. The linearity of the calibration standards of the drug by spectrophotometric method was good from the result of correlation coefficient. The overall recovery of the drug by the proposed method was good. Hence, the proposed analytical method is free from interface due to the excipients and other impurities present in the tablet forms. LOD, LOQ, molar absorptivity and Sandal's sensitivity values indicated that the proposed analytical method, i.e. spectrophotometric method was more accurate, precious and cost effective for determination of drug in bulk and pharmaceutical formulations.

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