SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF NEBIVOLOL HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE IN COMBINED TABLET DOSAGE FORM BY AREA UNDER CURVE METHOD

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ABSTRACT

The objective of the current study was to develop a simple, accurate, precise and rapid UV spectrophotometric method with subsequent validation using ICH suggested approach for the determination of antihypertensive pharmaceutical dosage form containing binary mixture of nebivolol hydrochloride (NEB) and hydrochlorothiazide (HCZ) using methanol as the solvent. The proposed area under curve method involves the measurement of area at selcted analytical wavelength ranges and performing the analysis using "Cramer's Rule" and "Matrix Method". Two analytical wavelength ranges selected were 295-285 nm and 312-322 nm for the estimation of NEB and HCZ. The linearity of the proposed method was investigated in the range of 5-70 µg/mL (r = 0.9998) for NEB and 5-70 µg/mL (r = 0.9998) for HCZ, respectively. The percentage mean recovery was found to be 98.90% for NEB and 99.26% for HCZ. Also the method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD value indicating high grade of precision of the method.

Key words: UV Spectrophotometric estimation, Nebivolol hydrochloride, Hydrochlorothiazide, Validation.

INTRODUCTION

Nebivolol hydrochloride (NEB) is chemically described as (±)-[2R*[R*[R*(S*)]]]-α, α' - [Iminobis (methylene)] bis [6-fluoro- 3, 4- dihydro- 2H-1-benzopyran- 2-methanol] hydrochloride[1,2]. It is a racemate of two enantiomers, the RSSS enantiomers possesses a

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favourable heamodynamic profile. It is a cardioselective third generation β_1 receptor blocking agent. It blocks the β adreno-receptor effect of adrenaline and nor-adrenaline, reducing heart rate, force of myocardial infarction, decreases systemic blood pressure and increases diastolic pressure. In addition to adrenergic blocking property, it possesses additional vasodilating activity mediated by L-argenine nitric oxide pathway, increasing the bioavalability of nitric oxide (NO), which produces vasodilation by enhancing cyclic guanosine monophosphate and also inhibits platelets aggregation and smooth muscle cell proliferation. Hydrochlorothiazide (HCZ) is chemically described as 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide[1,2]. It belongs to the class of thiazide diuretics, which is widely used in the treatment of hypertension and oedema associated with mild to moderate congestive heart failure. It increases the rate of urine excretion by the kidneys through decreased tubular reabsorption of sodium and chloride ions and by increasing osmotic transport of water to renal tubules, which in turn lowers the cardiac output and blood pressure[3,4].

Literature survey reveals that few methods have been reported for the determination of HCZ or NEB individually in biological fluids or in combination with other drugs in pharmaceutical dosage forms[5-11]. But no method has been developed for simultaneous estimation of NEB and HCZ in combined dosage form. The present manuscript describes a sensitive, simple, precise and accurate UV spectrophotometric method for simultaneous estimation of NEB and HCZ in combined dosage form with subsequent validation as per ICH guidelines[12].

EXPERIMENTAL

Chemicals and reagents

The working standards of NEB and HCZ were generous gifts obtained from Alembic Pharmaceuticals Ltd. (Baroda, India). Methanol (AR grade) was obtained from E. Merck Ltd. The combination formulation of NEB and HCZ (Label claim: Nebivolol 5 mg, as nebivolol hydrochloride and hydrochlorothiazide 12.5 mg), Nebicard-H tablets (Torrent Pharmaceuticals Ltd.) were purchased from the local market.

Instrument used

A Shimadzu UV/Visible spectrophotometer (Model 1700) with 1 cm matched quartz cells was used for spectrophotometric analysis. The spectra were recorded using specific program of the instrument (UV Probe 2.1), having specifications as- spectral band width 2 nm, wavelength accuracy $\pm$ 0.5 nm and wavelength readability 0.1 nm increment.

Preparation of standard stock solution and selection of analytical wavelength

The standard stock solutions 100 µg/mL, each of NEB and HCZ were prepared separately by dissolving accurately weighed working standards in small proportions of methanol and later diluted to desired volume with the same.

Appropriate dilutions of the above stock solutions were done to obtain a working solution of 30 µg/mL, each of NEB and HCZ. Both the solutions were seperately scanned in the wavelength region of 400-200 nm in the "Spectrum mode". On examination of the spectra, 295-285 nm was selected as working wavelenth range for NEB and 322-312 nm was selected as working wavelength range for HCZ, as, at the above selected wavelength range, the area under curve (AUC) remains constant, ideally obeying "Cramer's Rule" and Matrix Method" (Fig. 1 and 2).

Preparation of calibration curves

Appropriate dilutions were prepared form the standard stock solutions of NEB and HCZ, respectively to obtain a working concentration range of 5-70 µg/mL for both the drugs. Area under curve of the above solutions of NEB and HCZ were measured at their respective selected analytical wavelength ranges and their calibration curves were prepared by plotting area under curve (AUC) against concentration (Fig. 3 and 4).
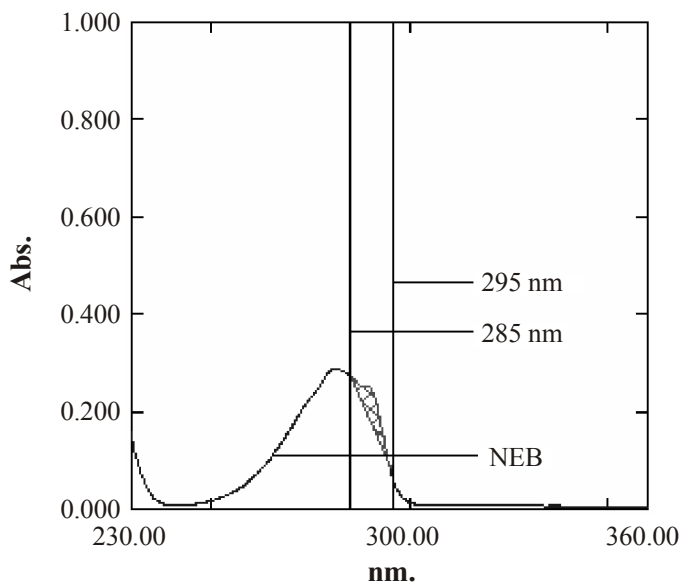

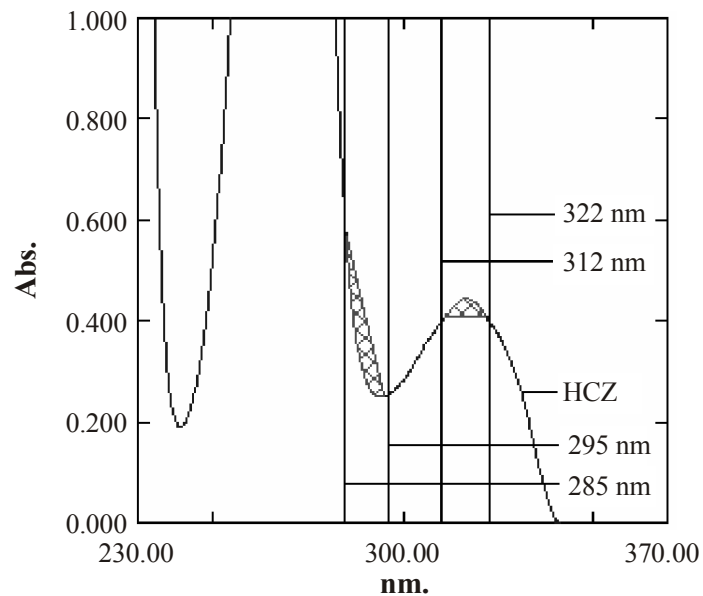**Fig. 1: Spectra showing area under curve of NEB at 295 – 285 nm and 322 – 312 nm, respectively**

**Fig. 2: Spectra showing area under curve of HCZ at 295 – 285 nm and
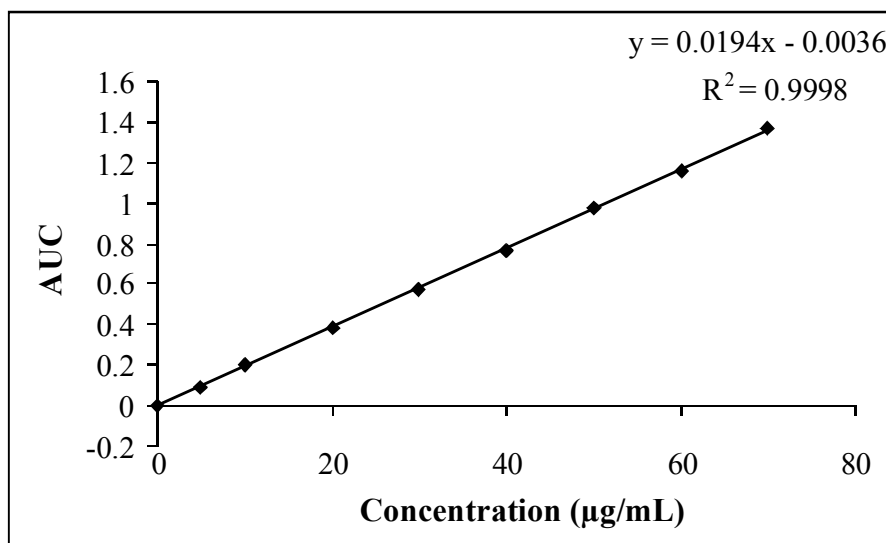322 – 312 nm, respectively**

**Fig. 3: Calibration curve of NEB at 295 - 285 nm in methanol by
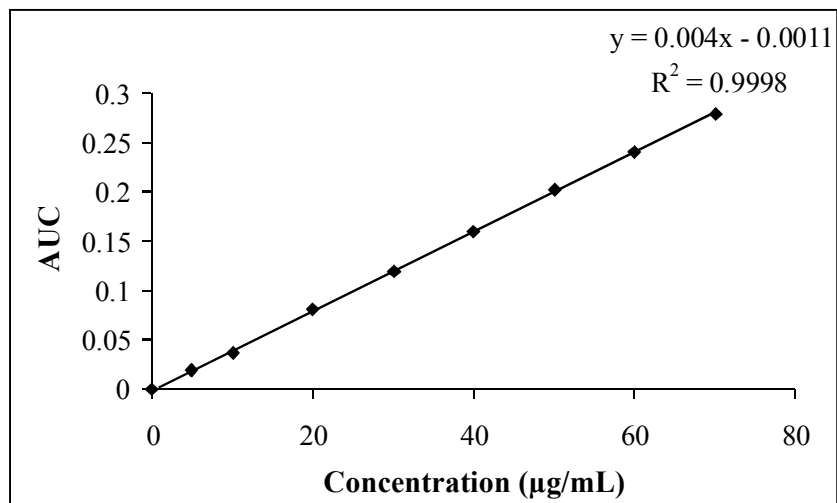area under curve method**

Fig. 4: Calibration curve of HCZ at 322 - 312 nm in methanol by area under curve method

Analysis of tablet dosage form

Twenty tablets were weighed, their mean weight was determined and finally they were crushed to obtain a fine powder. An amount of powdered mass equivalent to one tablet content was transferred into a 100 mL volumetric flask and dissolved in sufficient quantity of methanol. The contents were ultrasonicated for 20 minutes and the final volume was made up to the mark with methanol. The prepared solution was then filtered through Whatmann filter paper No. 41. Appropriate aliquot was pippetted out from the standard stock solution and was further diluted to obtain a mixture containing 20 µg/mL of NEB and 50 µg/mL of HCZ. The spectra of mixed sample solutions were recorded and analyzed by determining the AUC at selected analytical wavelength ranges applying the "Cramer's Rule" and "Matrix Method" (Fig. 5). It is defined as "The total area under curve of a mixture at a particular wavelength range is equal to the sum of area under curve of the individual components at same wavelength range".

$$C^M = \frac{X^N_{\lambda1-\lambda2} \, AUC_{\lambda3-\lambda4} - X^N_{\lambda3-\lambda4} \, AUC_{\lambda1-\lambda2}}{X^N_{\lambda1-\lambda2} \, X^M_{\lambda3-\lambda4} - X^N_{\lambda3-\lambda4} \, X^M_{\lambda1-\lambda2}} \qquad \ldots(1)$$

$$C^N = \frac{X^M_{\lambda1-\lambda2} \, AUC_{\lambda3-\lambda4} - X^M_{\lambda3-\lambda4} \, AUC_{\lambda1-\lambda2}}{X^N_{\lambda1-\lambda2} \, X^M_{\lambda3-\lambda4} - X^N_{\lambda3-\lambda4} \, X^M_{\lambda1-\lambda2}} \qquad \ldots(2)$$

Where,

$$X_{\lambda1-\lambda2} = \frac{AUC_{\lambda1-\lambda2}}{Conc.\,in\,g/lit.} \quad and \quad X_{\lambda1-\lambda2} = \frac{AUC_{\lambda3-\lambda4}}{Conc.\,in\,g/lit.}$$
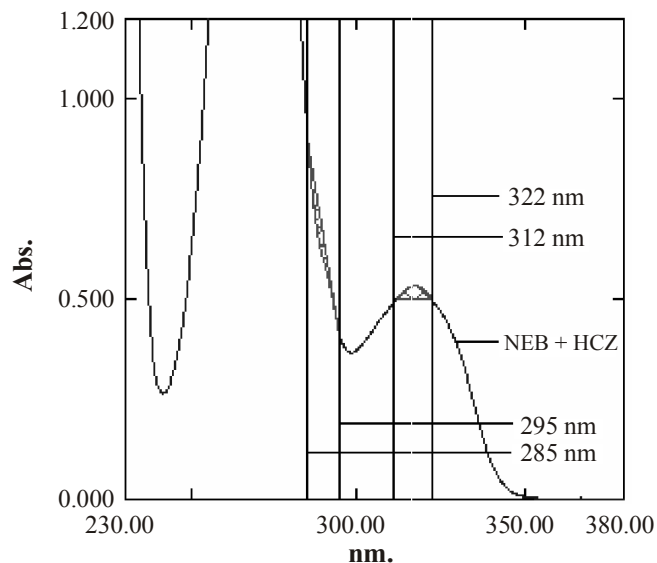

Fig. 5: Spectra showing area under curve of mixture at 295 – 285 nm and 322 – 312 nm, respectively

Method validation

The developed analytical method was subjected to validation with respect to various parameters such as linearity, limit of quantification (LOQ), limit of detection (LOD), accuracy, precision, recovery studies, specificity and reproducibility as per the ICH guidelines[12].

RESULTS AND DICUSSION

The present manuscript dealts with simultaneous estimation of NEB and HCZ in combined tablet dosage form by area under curve method using methanol as solvent. The developed method is based upon estimation of both the drugs by determining the area under curve at selected analytical wavelength ranges (295-285 nm and 322-312 nm) and solving the Cramer's equation.

The linearity of the proposed method was established by least square regression analysis of the calibration curve. The constructed calibration curves were linear over the

concentration range of 5-70 µg/mL for NEB (r = 0.9998) and HCZ (r = 0.9998), respectively (Table 1).

Table 1: Statistical analysis of the calibration curves of NEB and HCZ, respectively

Parameters	NEB	HCZ
Range (µg/mL)	5 - 70	5 – 70
Slope*	0.0194	0.004
Intercept*	-0.0036	-0.0011
Correlation coefficient (r)*	0.9998	0.9998
LOQ (µg/mL)*	5.0	2.0
LOD (µg/mL)*	1.0	0.5
Where, *n = 6		

Recovery studies were also performed to determine the accuracy and precision of the proposed method. Recovery experiments were performed at three levels, 80%, 100% and 120% of the labeled amount of both the drugs (5 mg NEB and 12.5 mg HCZ) in tablet formulation. Three replicate samples of each concentration levels were prepared and the percentage recovery at each level (n = 3)[12], and mean % recovery (n = 9)[12] were determined and summarized in Table 2. The mean (%) recovery was found to be 98.90% and 99.26% for NEB and HCZ, respectively.

Table 2: Recovery of NEB and HCZ in spiked standard drug solution

Level of (%) recovery	Amount present (mg)		Amount added (mg)		Amount found (mg)		Recovery* (%)	
	NEB	HCZ	NEB	HCZ	NEB	HCZ	NEB	HCZ
(80%)	5.0	12.5	4.0	10.0	8.85	22.26	98.37	98.94
(100%)	5.0	12.5	5.0	12.5	9.88	24.81	98.76	99.23
(120%)	5.0	12.5	6.0	15.0	10.95	27.39	99.55	99.61
	Mean (%) recovery**				RSD (%)**			
NEB	98.90				0.8226			
HCZ	99.26				0.5410			
Where, *n = 3 and **n = 9								

Intra-day precision was estimated by assaying the quality control sample of the tablet formulation containing 20 µg/mL of NEB and 50 µg/mL of HCZ, six times and the results were averaged for statistical evaluation. The statistical validation data for intra day precision is summarized in Table 3.

Table 3: Statistical validation data for determination of intra-day precision (n = 6)

Interpolated concentration (mean ± SD) *	RSD (%)*	SE (%)*
Nebivolol hydrochloride (µg/mL)		
20 19.95 ± 0.1716	0.8601	0.0700
Hydrochlorothiazide (µg/mL)		
50 49.82 ± 0.1869	0.3751	0.0763
Where, *n = 6		

Inter-day precision was evaluated by analyzing a set of quality control samples of the tablet formulation containing 20 µg/mL of NEB and 50 µg/mL of HCZ; six levels analyzed on three consecutive days. The statistical validation data (results averaged for statistical evaluation) for intra-day precision is summarized in Table 4.

Table 4: Statistical validation data for determination of inter-day precision (n = 3)

Interpolated concentration (mean ± SD) *	RSD (%)*	SE (%)*
Nebivolol hydrochloride (µg/mL)		
20 19.88 ± 0.1805	0.9079	0.1042
Hydrochlorothiazide (µg/mL)		
50 49.79 ± 0.2069	0.4155	0.1194
Where, *n = 3		

Both intra-day and inter-day variation were found to be showing less % RSD value indicating high grade of precision of the method.

The validation results obtained confirm the suitability of the proposed UV

spectrophotometric method for simple, accurate and precise analysis of NEB and HCZ in pharmaceutical preparations. The proposed method does not need prior separation of NEB and HCZ before analysis. In addition, the proposed method is suitable for application without interference of excepients and can be applied directly to the commercial preparations without previous treatment.

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