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Development and validation of spectrophotometric method for simultaneous determination of nebivolol and hydrochlorthiazide in tablets

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of nebivolol and hydrochlorothiazide in combined tablet dosage form. The method is based on the simultaneous equations for analysis of both the drugs using methanol as solvent. Nebivolol has absorbance maxima at 281 nm and hydrochlorothiazide has absorbance maxima at 270.5 nm in methanol. The linearity was obtained in the concentration range of 5-60 µg/ml for both nebivolol and hydrochlorothiazide. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The mean recovery was 99.86 ± 1.01 and 100.6 ± 1.34 for nebivolol and hydrochlorthiazide, respectively. The method was found to be simple, sensitive, accurate, precise, rapid and cost effective and can be applicable for the simultaneous determination of nebivolol and hydrochlorothiazide in pharmaceutical tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

Keywords: Nebivolol, Hydrochlorothiazide, Recovery, Simultaneous equations method, Tablet, Validation.

1. INTRODUCTION

Nebivolol (NEB) is chemically 1-(6-fluorochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl]amino}ethanol[1], is a cardio selective β-blocker, used in the treatment of hypertension and heart failure[2]. It is official in Indian Pharmacopoeia (IP)[3]. Literature survey reveals spectrophotometric[4] and HPLC[5] method for estimation of NEB in pharmaceutical dosage form. Literature survey also reveals HPTLC[6], spectrophotometric[7-10] and HPLC[11] methods for determination of NEB in combination. Hydrochlorothiazide (HCTZ) is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide[12], is a thiazide diuretic, used in hypertension[13]. It is official in IP[14], British Pharmacopoeia (BP)[15] and United States Pharmacopoeia (USP)[16]. Literature survey reveals HPLC[17] method for its estimation in biological fluid. Literature survey also reveals spectrophotometric[18-21] and HPLC methods[22] method for estimation of HCTZ in combination. The present manuscript describes simple, sensitive, accurate, precise, rapid and economical spectrophotometric method for simultaneous determination of NEB and HCTZ in pharmaceutical tablet dosage form.

2. MATERIALS AND METHODS

Apparatus

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and materials

NEB and HCTZ powder was kindly gifted by Zydus Cadila Pharmaceutical Ltd, Ahmedabad, Gujarat with 99.96 % purity. The commercial fixed dose combination product containing 5 mg NEB and 12.5 mg HCTZ was procured from the local market. Methanol AR grade was procured from S. D. Fine Chemicals Ltd, Mumbai.

Preparation of standard stock solutions

Accurately weighed portions of NEB (10 mg) and HTZ (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtain standard solution having concentrations of NEB (100 µg/ml) and HTZ (100 µg/ml).

Table 1: Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/mL)	Amount of standard spiked (%)	Mean% Recovery ± SD*
NEB	I	5	50	101.2 ± 1.56
	II	5	100	99.64 ± 0.57
	III	5	125	98.75 ± 0.89
HCTZ	I	12.5	50	102.1 ± 1.87
	II	12.5	100	99.36 ± 0.82
	III	12.5	125	100.2 ± 1.32

* Mean % Recovery ± SD of five observations

Preparation of sample solution

Twenty tablets were weighed and powdered. The tablet powder equivalent to 5 mg of NEB and 12.5 mg of HTZ was accurately weighed and transferred in to a 100 ml volumetric flask. Methanol (50 ml) was added to it and sonicated for 20 min and diluted up to the mark with methanol. The solution was filtered through Whatman filter paper No. 41. This solution is expected to contain 50 µg/ml NEB and 125 µg/ml HTZ. The above solution (1.0 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of NEB (5 µg/ml) and HTZ (12.5 µg/ml).

Determination of the analytical wavelengths

The standard solutions of NEB (10 µg/ml) and HCTZ (10 µg/ml) were scanned separately in the UV range of 200 - 400 nm. Data were recorded at an interval of 1 nm. From the spectra of both drugs, two analytical wavelengths i.e. 281 nm (λ_{max} of NEB) and 270.5 nm (λ_{max} of HCTZ) were selected and absorbances were measured at these selected wavelengths.

Method

The standard solution of NEB and HCTZ (10 µg/ml) were prepared separately in methanol and scanned in the wavelength range of 200 – 400 nm to determine the λ_{max} of both the drugs. The λ_{max} of NEB and HCTZ were found to be 281 nm and 270.5 nm, respectively. Six working standard solutions having concentration 5, 10, 20, 30, 40 and 60 µg/ml for both NEB and HCTZ were prepared in methanol using the standard stock solutions. The absorbance of resulting solutions was measured at 281 nm and 270.5 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of NEB and HCTZ in sample solution was determined by solving the respective simultaneous equations[23] generated by using absorptivity coefficients and absorbance values of NEB and HCTZ at these wavelengths.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[24].

Linearity (Calibration curve)

Calibration curves were plotted over a concentration range of 5-60µg/ml for both drugs. Accurately measured standard working solutions of NEB and HCTZ (0.5, 1, 2, 3, 4 and 6 ml) were transferred to a series of 10 ml of volumetric flasks separately and diluted to the mark with methanol, and absorbances were measured at 270.5 nm and 281.0 nm for both drugs. The calibration curves were constructed by plotting absorbances Vs concentrations.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of NEB and HCTZ (20µg/ml) without changing the parameters for the simultaneous equation method.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed methods were determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a

period of 1 week for 3 different concentrations of standard solutions of NEB and HCTZ (10, 20 and 40 µg/ml). The results were reported in terms of percent relative standard deviation (% RSD).

Table 2: Analysis of NEB and HCTZ by proposed method

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 3)	
	NEB	HCTZ	NEB	HCTZ	NEB	HCTZ
I	5	12.5	5.08	12.73	101.6 ± 1.35	101.8 ± 1.02
II	5	12.5	5.04	12.42	100.8 ± 0.63	99.36 ± 1.54

Accuracy (recovery study)

The accuracy of the methods was determined by calculating recoveries of NEB and HCTZ by the standard addition method. Known amounts of standard solutions of NEB and HCTZ were added at 75, 100 and 125 % levels to prequantified sample solutions of NEB and HCTZ (5 and 12.5 µg/ml, respectively). The amounts of NEB and HCTZ were estimated by applying the obtained values to the respective simultaneous equations.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drug wee derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

Table 3: Regression analysis data and summary of validation parameters

[a] LOD = Limit of detection [b] LOQ = Limit of quantitation

Parameters	Nebivolol		Hydrochlorthiazide	
Wavelength (nm)	270.5	281.0	270.5	281.0
Beer's Law Limit (µg/ml)	5 – 60	5 – 60	5 - 60	5 – 60
Molar Absorptivity (1 $mole^{-1}$ cm^{-1})	3100	5800	2200	8500
Sandell's sensitivity (µg/cm^2/0.001absorbance unit)	0.022	0.073	0.033	0.084
Regression equation (y = a + bc)				
Slope (b)	0.0071	0.0124	0.0713	0.0272
Intercept (a)	0.0061	0.0314	0.0167	0.0091
Correlation Coefficient (r^2)	0.9991	0.9995	0.9990	0.9995
LOD[a]	1.33	1.12	1.29	0.83
LOQ[b]	4.39	3.69	4.25	2.73
Repeatability (% RSD[c], n[d] = 6)	1.17	0.73	0.24	0.74
Precision (% RSD)				
Interday (n = 6)	0.18-1.90	0.15-1.75	0.12-0.95	0.17-1.25
Intraday (n = 6)	0.25-2.05	0.20-1.96	0.22-1.85	0.32-1.65
Standard Error of Mean (SEM)	0.5246	0.3273	0.1076	0.3318

[c] RSD = Relative standard deviation [d] n = number of determinations

ANALYSIS OF NEB AND HCTZ IN COMBINED DOSAGE FORMS

Pharmaceutical formulation of NEB and HCTZ were purchased from local pharmacy. The absorbance of final solution was measured at 281 and 270.5 nm for quantification of NEB and HTZ, respectively. The amounts of NEB and HCTZ present in sample solutions were determined by fitting the response into the simultaneous equation for NEB and HTZ.

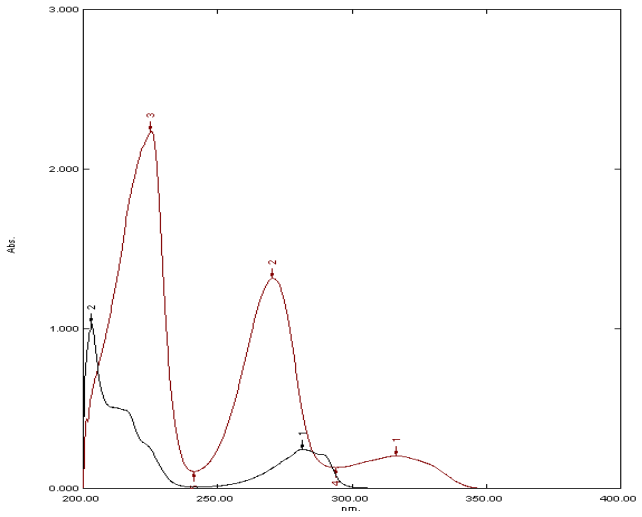

Fig 1: Overlain absorption spectra of Nebivolol (281 nm) and Hydrochlorthiazide (270.5 nm) in methanol

3. RESULTS AND DISCUSSION

The standard solutions of NEB and HTZ were scanned separately in the UV range, and zero-order spectra for NEB and HTZ (Figure 1) were recorded. Maximum absorbance was obtained at 281 nm and 270.5 nm for NEB and HTZ, respectively. These two wavelengths can be employed for the determination of NEB and HTZ without any interference from the other drug in their combined formulations.

The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). The method was successfully used to determine the amounts of NEB and HCTZ present in the tablet dosage forms. The results obtained were in good agreement with the corresponding labeled amount (Table 2). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in Table 3. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined dosage form.

4. CONCLUSION

Based on the results, obtained from the analysis of using described method, it can be concluded that the method has linear response in the range of 5 – 60 µg/ml for both NEB and HCTZ. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and is in good agreement with label claim of the drugs. The additive usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of NEB and HCTZ. The method was found to be simple, sensitive, rapid and economic and can be used for the routine analysis of NEB and HCTZ in combined dosage form.

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