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Simultaneous Determination of Nebivolol and Hydrochlorthiazide in Tablets by Derivative Spectrophotometry

Satish A. Patel*, Hemant M. Patel[1]

1. Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Kherva – 382711, Mehsana, Gujarat, India

2. Babariya Institute of Pharmacy, Vadodara-Mumbai National Highway, Varnama-391240, Vadodara, Gujarat, India

ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric method for the simultaneous determination of nebivolol and hydrochlorothiazide in combined tablet dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra were obtained in methanol and the determinations were made at 270.5 nm (ZCP of hydrochlorothiazide) for nebivolol and 282.5 nm (ZCP of nebivolol) for hydrochlorothiazide. The linearity was obtained in the concentration range of 5-100 µg/ml for nebivolol and 2-14 µg/ml for hydrochlorothiazide. The mean recovery was 100.04 ± 0.93 and 99.87 ± 1.16 for nebivolol and hydrochlorothiazide, respectively. The method was found to be simple, sensitive, accurate and precise and was 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.

Key words: Nebivolol, hydrochlorothiazide, recovery, first order derivative spectrophotometric method, tablet, validation.

*Corresponding Author Email: satishpatel_77@yahoo.com

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INTRODUCTION

Nebivolol (NEB) (Figure 1) 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) (Figure 2) 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]. IP, BP and USP describe non-aqueous titration, potentiometric titration and HPLC method, respectively for its estimation. 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.

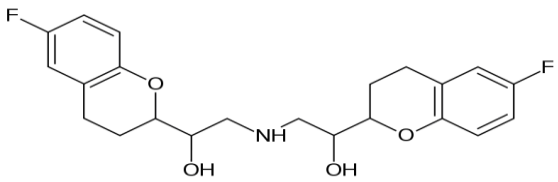

Figure 1: Chemical structure of NEB

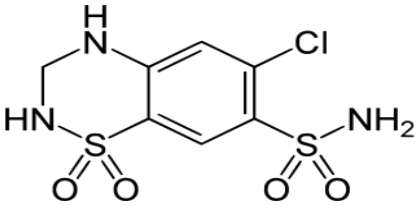

Figure 2: Chemical structure of HCTZ

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

Nebivolol and Hydrochlorthiazide bulk powder was kindly gifted by reputed Pharmaceutical Company, Gujarat (India), 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, India.

Preparation of standard stock solutions

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

Methodology

The spectrum of the standard solution was taken in the range of 200 nm to 400 nm. This spectrum of the drugs was converted to first derivative forms and ZCP'S (Zero Crossing Points) were determined and it were found to be 241.0 nm, 242.5nm, 282.5 nm for nebivolol and 241.5 nm, 270.5 nm, 293.5 nm for hydrochlorthiazide. The absorbance of the nebivolol solution was measured at 270.5 nm (ZCP of hydrochlorthiazide) and for the hydrochlorthiazide solution absorbance was measured at 282.5 nm (ZCP of nebivolol). The linearity was found in the range of 5-100 µg/ml and 2-14 µg/ml for nebivolol and hydrochlorthiazide respectively. The calibration curves for derivative spectroscopy were constructed by plotting drug absorbance at ZCP Vs concentration and regression equations were computed.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[23].

Linearity (Calibration curve)

Calibration curves were plotted over a concentration range of 5-100 µg/ml and 2-14 µg/ml for NEB and HCTZ respectively. Accurately measured standard working solutions of NEB (0.5, 1, 2, 4, 6, 8, and 10 ml) and HCTZ (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 ml) were transferred to a series of 10ml of volumetric flasks 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 (20 µg/ml and 10 µg/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 and 8, 10, 12µg/ml respectively). The results were reported in terms of relative standard deviation (RSD).

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 pre-quantified sample solutions of NEB and HCTZ (5 and 12.5µg/ml respectively). The amounts of NEB and HCTZ were estimated by applying the obtained values to the regression equation.

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 X σ/S

LOQ = 10 X σ/S

Where, σ = the standard deviation of the response

S = slope of the calibration curve.

Analysis of NEB and HCTZ in combined tablet dosage form

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

RESULTS AND DISCUSSION

The standard solutions of NEB and HCTZ were scanned separately in the UV range, and zero-order spectra (Figure 3) thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 1 nm. The two derivative spectra showed maximum absorbance at 270.5 nm (ZCP of HCTZ) for NEB and 282.5 nm (ZCP of NEB) for HCTZ. First-derivative absorbances (D1) were recorded 270.5 nm for NEB and 282.5 nm for HCTZ (Figure 4 and 5). First derivative spectra give good quantitative determination of both the drugs at their respective without any interference from the other drug in their combined dosage formulations. Second and third-ordered derivative spectra of the drugs were not tested because the first-order spectra give

satisfactory ZCPs and good quantitative determination of both the drugs without any interference.

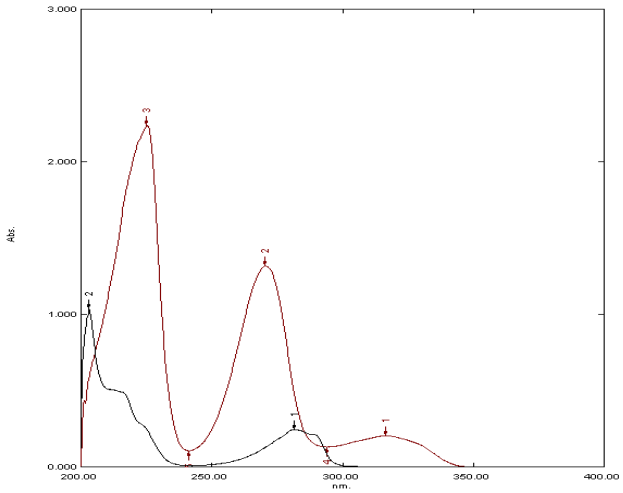

Figure 3: Overlain zero-order absorption spectra of NEB and HCTZ in methanol

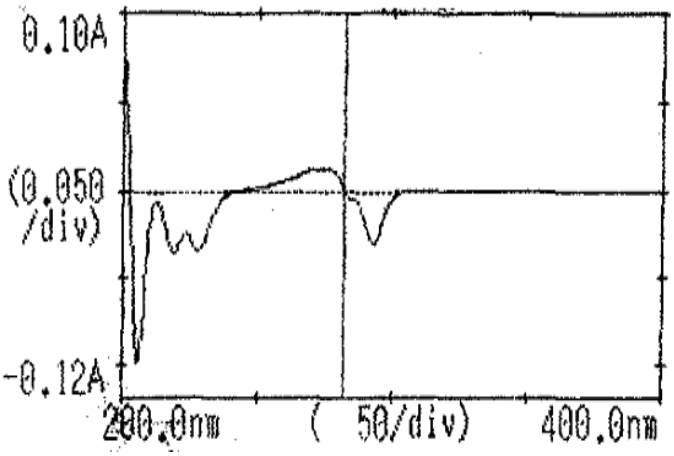

Figure 4: First order derivative spectra of NEB in methanol

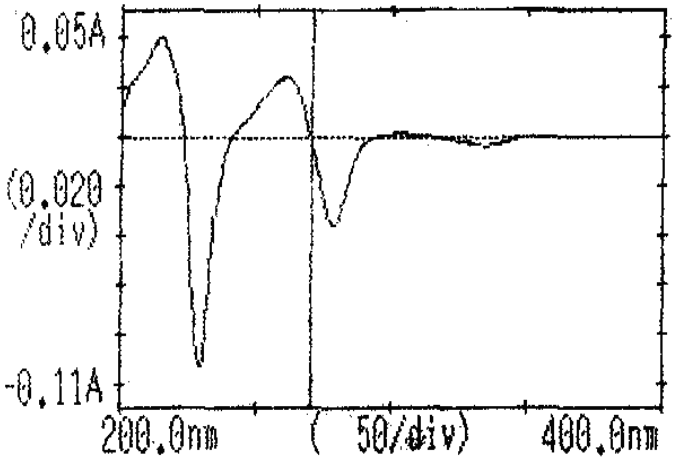

Figure 5: First order derivative spectra of HCTZ in methanol

Linear correlation was obtained between absorbance and concentrations of NEB and HCTZ in the concentration ranges of 5-100 µg/ml and 2-14 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table 1). The RSD values for NEB and HCTZ were found to be 1.61 and 0.19 %, respectively (Table 1). The low values of relative standard deviation (less than 2 %) indicate that the proposed method is repeatable. The low RSD values of interday (1.20-2.30 % and 0.15-1.75 %) and intraday (0.95-2.15 and 0.25-1.96 %) for NEB and HCTZ, respectively, reveal that the proposed method is precise (Table 1). LOD values for NEB and HCTZ were found to be 1.25 and 0.25 µg/ml, respectively and LOQ values for NEB and HCTZ were found to be 3.75 and 0.85 µg/ml, respectively (Table 1). These data show that proposed method is sensitive for the determination of NEB and HCTZ.

Table 1: Regression Analysis Data and Summary of Validation Parameters

Parameters	**First-derivative UV spectrophotometry**	
	NEB at 270.5 nm	**HCTZ at 282.5 nm**
Concentration range	5 – 100 µg/ml	2 – 14 µg/ml
Regression line equation	Y=0.0005x + 0.0023	Y=0.0042x + 0.0004
Slope	0.0005	0.0042
Intercept	0.0023	0.0004
Correlation coefficient	0.9995	0.9990
LOD[a]	1.25 µg/ml	0.25 µg/ml
LOQ[b]	3.75 µg/ml	0.85 µg/ml
Accuracy (% recovery, n = 6)	100.04 ± 0.93	99.87 ± 1.16
Repeatability (% RSD[c], n = 6)	1.61	0.19
Precision (% RSD)		
Interday (n = 6)	1.20 – 2.30%	0.15 – 1.75
Intraday (n = 6)	0.95 – 2.15%	0.25 – 1.96

[a]RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification

Table 2: Recovery Data for the Proposed Method

Drug	**Sample amount taken (µg/ml)**	**Standard amount added (%)**	**Mean % Recovery ± SD***
NEB	5	75 %	100.57 ± 1.02
	5	100 %	100.0 ± 0.85
	5	125 %	99.56 ± 0.97
HCTZ	12.5	75 %	99.65 ± 1.46
	12.5	100 %	99.60 ± 0.89
	12.5	125 %	100.37 ± 1.12

S. D. is Standard deviation and n is number of determinations

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.04 ± 0.93 and 99.87 ± 1.16 % for NEB and HCTZ, respectively (Table 2). The results of recovery studies indicate that the proposed method is accurate. The proposed validated

method was successfully applied to determine NEB and HCTZ in their combined dosage form. The results obtained for NEB and HCTZ were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of NEB and HCTZ in pharmaceutical dosage forms.

Table 3: Assay Results for the Combined Tablet Dosage Form

Tablet	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 6)	
	NEB	HCTZ	NEB	HCTZ	NEB	HCTZ
I	5	12.5	5.03	12.46	99.73 ± 0.53	101.5 ± 1.09
II	5	12.5	4.96	12.65	99.20 ± 0.37	101.2 ± 1.78

S. D. = Standard deviation, n = Number of determinations.

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 – 100 and 2 - 14µg/ml for Nebivolol and Hydrochlorthiazide, respectively with co-efficient of correlation, (r^2) = 0.9995 and (r^2) = 0.9990 for Nebivolol and Hydrochlorthiazide, respectively. 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 Nebivolol and Hydrochlorthiazide. The method can be used for the routine analysis of Nebivolol and Hydrochlorthiazide in combined dosage form.

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