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Research Article

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Simultaneous Estimation of Nebivolol Hydrochloride and Hydrochlorothiazide in Tablets

Kamal Shah[1]*

[1]*Institute of Pharmaceutical Research, GLA University, Mathura, UP, 281406, India.*

***Email:** kamal0603@gmail.com*

ABSTRACT

A method was established for simultaneous valuation of Nebivolol hydrochloride (NBH) and Hydrochlorothiazide (HYT) from the tablet dosage form by partial simultaneous equation method, the method was found to be sensitive and accurate. The maxima of both the drugs were noted in 0.1 N hydrochloric acid. The NBH has lambda maxima at 283 nm and HYT at 270 and 320 nm. As NBH shows no absorbances at 320nm. So effective wavelengths designated were 283 nm and 270 nm for Method I, 283 nm, and 320 nm for Method II. The results of analysis for the method I exhibited greater values of standard deviation (SD), standard error of the mean (SEM), coefficient of variation (COV) and percentage range of error (within 95% confidence limit) (PROE), and thus showed less precision of the method. The results of method II analysis of marketed formulations expressively indicated fewer values. To test the accuracy and reproducibility, recovery experiments were performed. The fewer SD values depicted the novelty of the method. The method can be said to be reproducible, accurate, and precise. Thus, it was established that the method-II developed was an accurate one and can efficiently be simple, accurate, sensitive, and precise. Hence, the above two equations can be efficiently applied for the simultaneous determination of NBH and HYT in marketed formulations.

Key words: *Nebivolol hydrochloride, Hydrochlorothiazide, Simultaneous estimation, UV*

INTRODUCTION

The chemical name of Nebivolol hydrochloride (NBH) is 1-(6-fluoro-chroman-2-yl)–[2-(6-fluoro-chroman-2-yl) 2-hydroxyethylamino]-ethanol hydrochloride. An antihypertensive agent results in vasodilation due to the release of nitric oxide. It is said to be a beta1 selective adrenergic antagonist [1]. Hydrochlorothiazide is also used against high blood pressure coming underclass of thiazide diuretics. Chemically the hydrochlorothiazide is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide. Apart from its use against hypertension, it is also used in congestive heart failure [2-6], A review of works discovered an HPLC-fluorescence technique for nebivolol in human plasma and High-performance liquid chromatography enantiomeric separation of NBH on normal and reversed amylose centered chiral phases [7, 8]. UV or colorimetric estimation [9-12] can estimate NBH as a single entity. The nebivolol is already estimated by simultaneous equation method using amlodipine/valsartan with the parent drug [13-19]. Here in this manuscript, we have developed a novel procedure for the determination of NBH and HYT in combination, which may be further applied, to the dosage form available. The established procedure was validated as per the guidelines of ICH Q2 (R1).

MATERIALS AND METHODS

A spectrophotometer (UV/Visible) to make GBC Cintra 10 was utilized during the development of methods; the instrument has 10 mm matched quartz cells. The AR-grade reagents and solvents were used throughout the experiment. The marketed formulations were purchased from the local market containing NBH and HYT. The brand procured are Nodon-H and Nebicard-H. The pure API of NBH and HYT was received as a gift sample from Torrent Pharmaceuticals Ltd., Ahmedabad, and Ipca laboratories. Drugs were used as such without further purification.

Preparation of standard solutions

The pure API of NBH was 100 mg accurately weighed. It was solubilized in methyl alcohol by taking a volumetric flask of 100 mL. HYT was solubilized separately in methyl alcohol and taken in a volumetric flask of 100 mL. The final concentrations of NBH and HYT were made to (1000 µg/mL). This gives us a stock solution.

Selection of working wavelength

From stock solution of Nebivolol hydrochloride and Hydrochlorothiazide, working standard solutions of Nebivolol hydrochloride (A) (100 µg/mL) and Hydrochlorothiazide (B) (100 µg/mL) were made by suitable dilutions of the stock solutions with 0.1N HCl. Aliquots of 20 µg/mL were prepared from the working standard solution (A) and (B), by suitably diluting with 0.1N HCl. Then both were scanned separately in the range of 200-400 nm. An overlain spectrum of this scan was then recorded **(Figure 1)**. Then the mixture of both is scanned and recorded at 10 µg/mL **(Figure 2)**.

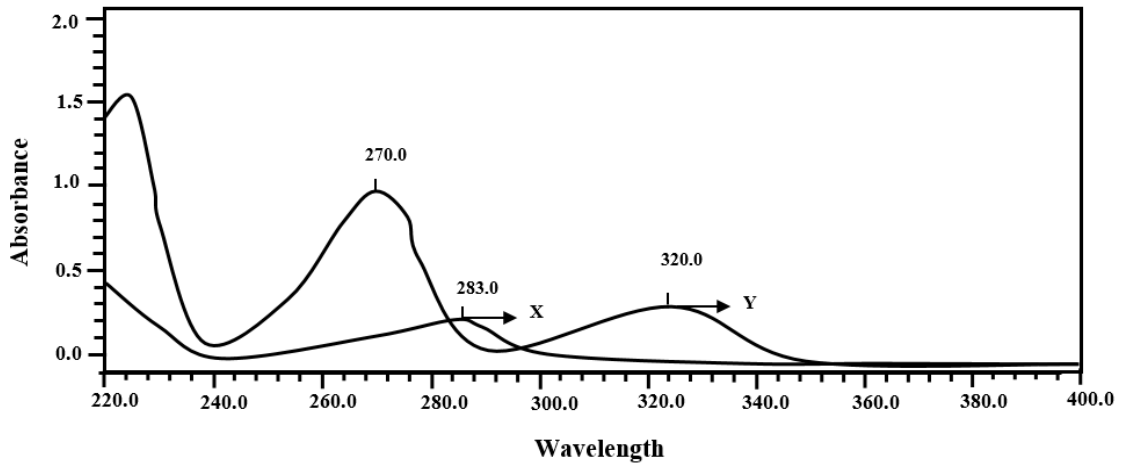

Figure 1. Overlain Spectra of Nebivolol Hydrochloride (X) (20 µg/mL) and Hydrochlorothiazide (Y) (20 µg/mL)

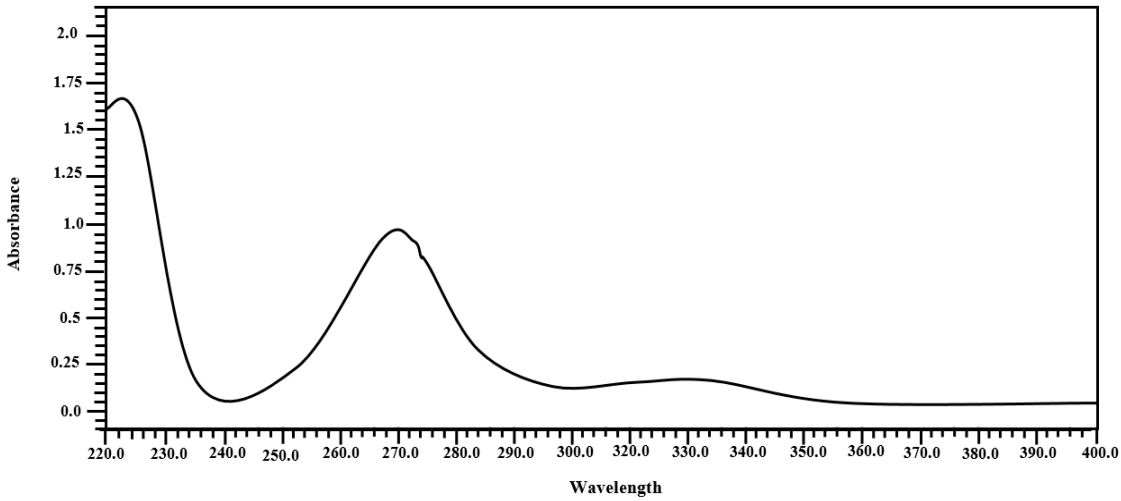

Figure 2. UV Scan of Mixture (10 µg/mL) of Nebivolol Hydrochloride and Hydrochlorothiazide

Overlain spectra exhibited the lambda maximum of NBH (20 µg/mL) at 283 nm and that of HYT (20 µg/mL) at 270 nm and 320 nm. Practical wavelengths designated were 270 nm and 283 nm for method-I (based on the simultaneous equation method). Various dilutions of Nebivolol hydrochloride and Hydrochlorothiazide from working standard solutions were prepared and absorbances were measured at selected wavelengths. The linearity curve for concentration versus absorbance was plotted.

Determination of absorption coefficient

The absorption coefficient of Nebivolol hydrochloride (A) in 0.1N HCl

The working standard of NBH i.e. 100 µg/mL was taken to prepare aliquots (0.5, 1.5, 2.5…7.5 mL) and were transferred to stoppered volumetric flasks of a capacity of 10 mL. The volumes were made with 0.1N HCl. The absorbances were measured for Nebivolol hydrochloride at 270 nm and 283 nm. It was found that Nebivolol hydrochloride followed Beer Lambert's law in the concentration range 5-75 µg/mL. The absorption coefficients were calculated for these concentrations.

Absorption coefficient of Hydrochlorothiazide (B) in 0.1N HCl

Similarly, the working standard of HYT (100 µg/mL) was taken to prepare aliquots (0.4, 0.8, and 1.2 …3.2 mL) and were transferred to stoppered volumetric flasks of a capacity of 10 mL. The volumes were made with 0.1N HCl. The absorbances were measured at 270 nm and 283 nm. It was found that Hydrochlorothiazide followed Beer Lambert's law in the concentration range 4-32 µg/mL. The absorption coefficients were calculated for these concentrations.

Absorption coefficient of Hydrochlorothiazide in 0.1N HCl at 320 nm

The same aliquots HYT was used to measure the absorbances at 320 nm. It was found that Hydrochlorothiazide followed Beer Lambert's law in the concentration range 4-32 µg/mL. The absorption coefficient was calculated for this concentration.

Development of simultaneous equation method

Method-I : (Lambda maximum designated were 270 nm and 283 nm)

A set of two simultaneous equations was developed using the absorption coefficents

$$A_1 = ax_1Cx + ay_1Cy \quad \text{at 270 nm} \tag{1}$$

$$A_2 = ax_2Cx + ay_2Cy \quad \text{at 283 nm} \tag{2}$$

Where Cx the concentration of Nebivolol hydrochloride and Cy the concentration of Hydrochlorothiazide.

A_1 = Absorbances at 270 nm
A_2 = Absorbances at 283 nm
ax_1 = absorption coefficients of Nebivolol hydrochloride at 270 nm
ax_2 = absorption coefficients of Nebivolol hydrochloride at 283 nm
ay_1 = absorption coefficients of Hydrochlorothiazide at 270 nm
ay_2 = absorption coefficients of Hydrochlorothiazide at 283 nm

Replacing the standards of ax_1, ax_2, ay_1 and ay_2, equations can be rewritten as

$$A_1 = 0.0069Cx + 0.0732Cy \quad \text{at 270 nm} \tag{3}$$

$$A_2 = 0.0110Cx + 0.0280Cy \quad \text{at 283 nm} \tag{4}$$

Method-II: A set of two simultaneous equations was developed using the absorption coefficients

$$A_3 = ax_3\,Cx + ay_3\,CY \quad \text{at 320 nm} \tag{5}$$

$$A_2 = ax_2\,Cx + ay_2\,CY \quad \text{at 283 nm} \tag{6}$$

Where Cx = Concentrations of Nebivolol hydrochloride
Cy = Concentrations of Hydrochlorothiazide
A_3 = Absorbances at 320 nm
A_2 = absorbances at 283 nm
ax_3 = absorption coefficients of Nebivolol hydrochloride at 320 nm
ax_2 = absorption coefficients of Nebivolol hydrochloride at 283 nm
but ax_3 = Zero,
ay_3 = absorption coefficients of Hydrochlorothiazide at 320 nm
ay_2 = absorption coefficients of Hydrochlorothiazide 283 nm

Substituting the values of ax_2, ay_3 and ay_2 equations (5) and (6) can be rewritten as

$$A_3 = 0.0134\ CY \quad (7)$$

$$A_2 = 0.0110\ Cx + 0.0280\ CY \quad (8)$$

RESULTS AND DISCUSSION

Estimation from tablets

Two of the marketed formulations, Nodon H (Cadila Pharma Pvt. Ltd.) and Nebicard H (Torrent Pharma Pvt. Ltd.) were taken in tablet dosage forms. The average weight of twenty tablets was calculated **(Table 1)**. Twenty tablets were taken separately and powdered, the powdered drug of quantity equivalent to 125 mg of Hydrochlorothiazide and 50 mg of Nebivolol hydrochloride was accurately weighed and taken to a volumetric flask of the capacity of 100 mL. These were then extracted with methyl alcohol (4 × 20 mL) and filtered, finally volume was made up. Further dilutions were made with 0.1N HCl to make the working standard solution conform to 125 µg/mL of Hydrochlorothiazide and 50 µg/mL of Nebivolol hydrochloride. This working standard solution was used to make aliquots of defined concentrations. This was made in a volumetric flask of 10 mL capacity in six replicates so that it comes within Beer's Lambert law limit. The absorbances were taken at 270 and 283 nm.

Table 1. Average Weight of Tablets

S.No.	Brand	Average weight (g) (Uncoated tablet)
1	Nodon-H	0.2307
2	Nebicard-H	0.2018

The results of analysis (Method I) of marketed formulations meaningfully displayed higher values of SD, SEM, COV, and PROE (within 95% confidence limits). These showed that the Method-I developed is not as accurate, sensitive, and precise as it should have been. Therefore, the two different maxima (i.e. 283 nm and 320 nm) were selected as the two workable wavelengths for Method-II. This method was based on a partial simultaneous equation. In Method II, tablets are extracted similarly as in the case of Method I. The working standard solutions (of method-I) corresponding to 50 µg/mL of Nebivolol hydrochloride and 125 µg/mL of Hydrochlorothiazide were taken for analysis. The working standard solution was used to prepare the aliquots in six replicates in a 10 mL volumetric flask. The volume was noted and absorbance was recorded at 283 nm and 320 nm. Substituting the absorbance value in equations (7) and (8), Drug content in tablets (the amount found), SD, COV, SEM, and PROE (within 95% confidence limits) was evaluated.
These data are incorporated in **Table 2**.

Table 2. Statistical Analysis of Marketed product

Formulation (Tablet)	Method	Composition of Tablet	Market claim* (mg/tab)	Calculated Amount* (mg/tab)	SD*	% RSD*	SE*

Nodon H	Method-I	Nebivolol hydrochloride Hyhrochlorothiazide	5 mg 12.5 mg	4.0145±0.070 12.8340±0.057	0.0889 0.0714	2.2151 0.5563	0.0362 0.0291
	Method-II	Nebivolol hydrochloride Hyhrochlorothiazide	5 mg 12.5 mg	4.9804±0.035 12.4551±0.026	0.0450 0.0327	0.9035 0.2625	0.0183 0.0133
Nebicard H	Method-I	Nebivolol hydrochloride Hyhrochlorothiazide	5 mg 12.5 mg	4.0289±0.062 12.8385±0.027	0.0785 0.0349	1.9484 0.2718	0.0320 0.0142
	Method-II	Nebivolol hydrochloride Hyhrochlorothiazide	5 mg 12.5 mg	4.9605±0.217 12.4725±0.017	0.0135 0.0219	0.2721 0.1755	0.1110 0.0089

Recovery studies were performed by the standard addition method. Pure drugs were added to the previously analyzed pharmaceutical preparation. In each formulation 10 mg of Hydrochlorothiazide and 5 mg of Nebivolol hydrochloride were added. The mixture was analyzed by the proposed method in multiple six readings. The recovery study data are incorporated in **Table 3**.

Table 3. Drug Recovery Data

Tablet Brand	Method	Percentage Recovery ± SD*	
		Nebivolol Hydrochloride	Hydrochlorothiazide
Nodon H	II	99.73 ± 0.1273	99.76 ± 0.2613
Nebicard H	II	99.48 ± 0.0687	99.75 ± 0.2701

*Average of six determinations.

CONCLUSION

In the present case, two equations as given below were developed.

At 270 nm $A_1 = 0.0069Cx + 0.0732 Cy$

At 283 nm $A_2 = 0.0110Cx + 0.0280Cy$

Using these equations, the concentrations of NBH and HYT were assessed in the marketed product. The results of the analysis displayed higher values of SD, SEM, COV, and PROE (within 95% confidence limit) and thus show low precision in this protocol.

Looking into the above facts another two equations were developed at 283 nm and 320 nm.

At 320 nm $A_3 = 0.0134 Cy$

At 283 nm $A_2 = 0.0110Cx + 0.0280Cy$

Using these equations once again, the drug content in commercial formulations was determined. These results were further evaluated statistically.

The results of the analysis of marketed formulations significantly displayed low values of SD, SEM, COV, and PROE (within 95% confidence limits) and thus exhibited the precision of the method. To test the accuracy and reproducibility, recovery experiments were performed. The percentage recovery was close to 100% for this method. The low SD values designated repetition, reproduction, and accuracy of the method. Thus, it can be determined that the method II established was simple, accurate, sensitive, and precise. Hence, the above two equations can be used effectively in the concurrent estimation of NBH and HYT in commercial products.

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FINANCIAL SUPPORT : None

ETHICS STATEMENT : The work is done by the author itself. In the experiment no animal or human data is used.

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