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Development and Validation of Dual Wavelength Method for Simultaneous Estimation of Nebivolol Hydrochloride and Hydrochlorothiazide In Tablet Dosage Form

Megha D. Madhekar*[1], Narendra M. Gowekar[1], Kedareshwar G. Jadhav[1], Darshali S. Desai[1], Pooja P. Dhanawade[1], Savita N. Gowekar[2].

1. Department of Pharmaceutical Chemistry, Singhad institute of pharmaceutical sciences, Pune University, Lonavala(Pune), Maharashtra, INDIA.

2. BVU Poona Collage of Pharmacy, Erandwane, Pune, Maharashtra, India

ABSTRACT

The present work describe simple, sensitive, rapid, accurate, precise and economic dual wavelength spectrophotometric method for the simultaneous estimation of Nebivolol hydrochloride and Hydrochlorothiazide in combined tablet dosage form. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of component of interest". The utility of this method is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The method was based on determination of Nebivolol hydrochloride at 246 nm and 292 nm and Hydrochlorothiazide at 264 nm and 295 nm. The two drugs follow Beer's law over the concentration range of 5-30 µg/ml. The method was successfully applied to pharmaceutical dosage form. The results of analysis have been validated as per ICH guidelines.

Keywords: Nebivolol hydrochloride, Hydochlorothiazide, Dual wavelength spectrophotometric method, Validation.

*Corresponding Author Email: madhekar.megha@gmail.com

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INTRODUCTION

Hydrochlorothiazide (HCTZ) is a thiazide class diuretic drug (Figure 1). This reduces the volume of the blood, decreasing blood return to the heart. Hydrochlorothiazide is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones. The recommended dose of hydrochlorothiazide for treating high blood pressure is 25 mg to 50 mg per day.[1,2]

Nebivolol hydrochloride (NH) belongs to well-known class of third generation beta blocker (Figure 2). It has both selective beta blocking and vasodialating effects. The drug is used in the treatment of arterial hypertension.[3,4]

Literature survey reveals that a few analytical methods like HPTLC, HPLC and some spectroscopic methods[5-8] were reported for estimation of nebivolol HCl and hydrochlorothiazide, alone and in combination. As per our knowledge there is no any dual wavelength method was found to be reported in literature for the simultaneous estimation of nebivolol HCl and hydrochlorothiazide. Hence the aim of the present work was to develop an simple and accurate method for the simultaneous estimation of nebivolol HCl and hydrochlorothiazide in bulk and pharmaceutical dosage form.

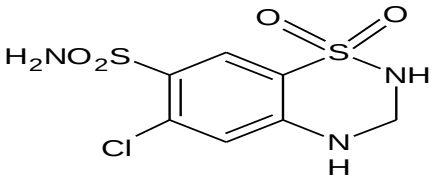

Figure 1: Chemical Structure of Hydrochlorothiazide

IUPAC name: 6-chloro-1,1-dioxo-3,4-dihydro- 2*H*-1,2,4- Benzothiadiazine-7-sulfonamide[9]

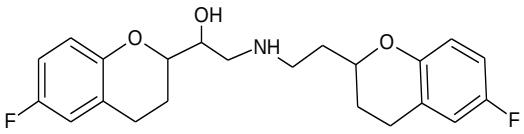

Figure 2: Chemical Structure of Nebivolol HCl

IUPAC name: α, α'[iminobis(methylene)]bis [6-fluro-3, 4-dihydro -2H-1-benzopyran –2-methanol] hydrochloride [9]

Dual Wavelength Method

The utility of dual wavelength method is to calculate the unknown concentration of a component of interest present in a mixture containing both the components of interest and an unwanted interfering component by the mechanism of the absorbance difference between two points on the mixture spectra. This is directly proportional to the concentration of the component of interest,

independent of the interfering components. The pre-requisite for dual wavelength method is the selection of two such wavelengths where the interfering component shows same absorbance whereas the component of interest shows significant difference in absorbance with concentration.[10]

MATERIALS AND METHODS:

Instrumentation:

A JASCO V-530 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. For weighing Shimadzu AUX220 (max 200g, d = 10 mg) balance was used.

Reagents and Chemicals:

Nebivolol HCl and hydrochlorothiazide [Bulk Drug] were obtained as gift samples. Methanol was used throughout the analysis.

Marketed Formulation:

The marketed formulation analyzed was Nebicard-H tablet manufactured by, Torrent pharmaceuticals Ltd.

Each tablet contains

5 mg Nebivolol HCl and

12.5 mg Hydrochlorothiazide.

Preparation of Standard Stock Solution:

An accurately weighed quantity of Nebivolol hydrochloride (10 mg) and Hydrochlorothiazide (10 mg) were transferred to a separate 10 ml volumetric flasks and dissolved and diluted up to the mark with methanol to obtain standard solutions having concentrations of Nebivolol HCl (1mg/ml) and HCTZ (1mg/ml). From the resultant solutions, 1 ml of each of the solutions were pipette out and diluted to 10 ml with methanol to get final concentration of 100 µg/ml.

DEVELOPMENT OF METHOD:

The solutions of NH and HCTZ were scanned in the wavelength range of 200-400nm. From the overlain spectra two wavelengths 246 and 292 nm were selected as λ_1 and λ_2 for estimation of the NH. HCTZ shows same absorbance at these wavelengths. Similarly, two wavelengths 264 and 295 nm were selected for estimation of HCTZ (Figure 3). For calibration curve, from the working standard solutions, appropriate dilutions in the range of 5 - 30 µg/ml for NH and HCTZ were prepared and analyzed. Mixed standards were prepared in the ratio of 1:2.5, as the formulation contains NH and HCTZ 5 mg and 12.5 mg respectively.

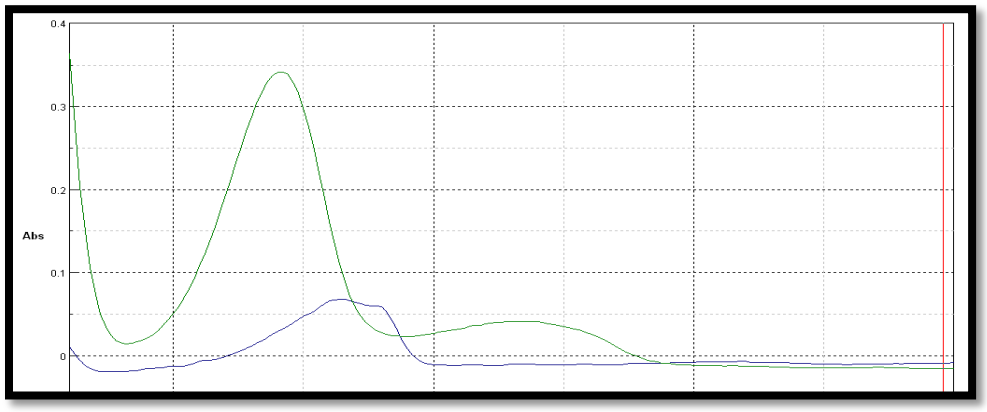

Figure 3: Overlain spectra of NH and HCTZ (Selection of wavelength)

Assay of tablet formulation by dual wavelength spectrophotometry:

Ten tablets were weighed and crushed to obtain a fine powder. An accurately weighed tablet powder equivalent to average weight of one tablet was transferred to 100 ml volumetric flask and dissolved in 50 ml of methanol with the aid of ultrasonication for 10 mins. The volume was made up to the mark using methanol as solvent. The resulting solution was filtered through whatman filter paper no.41. From the above prepared solution, further dilutions were prepared to get the final concentration. The absorbance was measured at the selected wavelengths and concentrations were determined. The analysis was done in triplicate.

METHOD VALIDATION [11]

Linearity and range:

Aliquots of standard stock solutions of NH and HCTZ were diluted with methanol to get final concentrations in range of 5-30 µg/ml. The difference in absorbance was plotted against the concentration to obtain calibration curve.(Figure 4 and Figure 5).

Precision:

Precision of the method was determined by performing interday variation and intraday variation studies. In interday variation, the absorbance of standard solutions of three concentrations of both the drugs NH and HCTZ were analysed in triplicate. Results are reported in Table 3.

Recovery studies:

To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels. Known amount of the two drugs was added to pre-analyzed tablet powder and percentage recoveries were calculated. Results are given in Table-4.

RESULTS AND DISCUSSION:

In this method, two specific wavelengths 246 and 292 nm were selected for the estimation of NH

where HCTZ shows same absorbance. Similarly 264 and 295 nm were selected for the estimation of HCTZ where NH shows same absorbance. The linearity ranges for both the drugs were found to be 5-30 µg/ml with regression coefficient of 0.999 and 0.998 for NH and HCTZ respectively (Table 1 and 2).

Table 1: Linearity data for NH

Concentration	Difference in Abs
5	0.0531
10	0.108
15	0.1618
20	0.2112
25	0.2646
30	0.3141

Table 2: Linearity data for HCTZ

Concentration	Difference in Abs
5	0.2807
10	0.5352
15	0.7993
20	1.0536
25	1.3551
30	1.6554

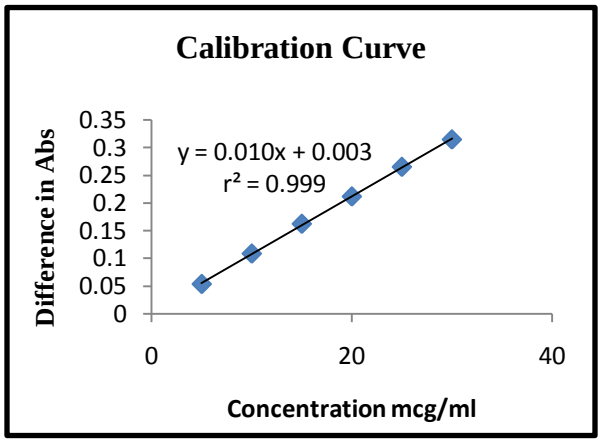

Figure 4: Calibration curve for NH

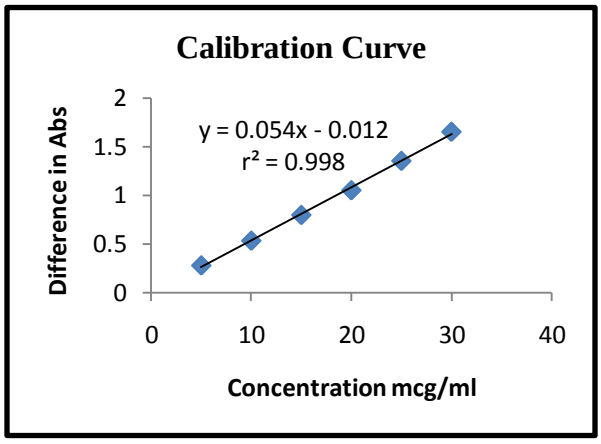

Figure 5: Calibration curve for HCTZ

Table 3: Precision data for NH and HCTZ

	Fortified amount (µg/ml)	Amount found (µg/ml)		% RSD	
		NH	HCTZ	NH	HCTZ
Intraday	5	5.28	4.92	1.58	0.65
(n = 3)	15	15.13	15.01	1.15	0.45
	25	25.09	25.25	0.99	0.14
Interday	5	5.2	4.99	1.34	0.70
(n = 3)	15	14.9	15.07	0.50	0.29
	25	24.81	25.02	0.80	0.10

Precision (%RSD) was found to be less than 2% and recovery study with SD less than 2%, indicates that the method is precise and accurate. The LOD and LOQ were found to be 0.339 and 1.028 µg/ml, respectively for NH and 0.122 and 0.372 µg/ml, respectively for HCTZ. The results obtained are in good agreement with corresponding labeled amount.

Table 4: % Recovery Data of NH and HCTZ

Concentration % of spiked level	**Amount of drug added(µg/ml)**		**Amount of pure drug found**		**% Recovery**	
	Pure drug	Formulation	NH	HCTZ	NH	HCTZ
80 % sample 1	6	10	6.009	6.19	100.15	103.27
80 % sample 2	6	10	5.87	6.10	97.83	101.7
80 % sample 3	6	10	5.95	6.06	99.16	101.14
					Mean- 99.4	Mean- 102.03
					%RSD-1.17	%RSD-1.08
100 % sample 1	10	10	10.08	10.36	100.8	103.60
100 % sample 2	10	10	10.19	10.21	101.9	102.15
100 % sample 3	10	10	9.88	10.19	98.8	101.94
					Mean- 100.5	Mean- 102.56
					%RSD-1.56	%RSD-0.88
120 % sample 1	14	10	13.73	14.37	98.13	102.64
120 % sample 2	14	10	13.97	14.13	99.78	100.95
120 % sample 3	14	10	14.11	14.20	100.78	101.4
					Mean- 99.56	Mean- 101.68
					%RSD-1.34	%RSD-0.89

CONCLUSION**:**

The most striking feature of the dual wavelength method is its simplicity and rapidity. The proposed dual wavelength method gives accurate and precise results for determination of Nebivolol hydrochloride and Hydrochlorothiazide in marketed formulation (tablet) without prior separation and is easily applied for routine analysis. The proposed method was successfully applied to determination of these drugs in commercial tablets.

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