ISSN No: 2287-6898
Research Article

IJBPR

A Validated RP HPLC Method for Simultaneous Estimation of Nebivolol and Hydrochlorothiazide in Combined Dosage Forms

Swethanagini V[1], Vasanth PM[1], Ramesh T[2], Ramesh Malothu*[2]

[1]Department of Pharmaceutical Analysis, UCEV-JNTUK, Vizianagaram, A.P, India

[2]Department of Biotechnology, UCEV-JNTUK, Vizianagaram, A.P, India

Received for publication: July 13th 2012; **Revised**: September 09th 2012; **Accepted**: October 24th 2012.

Corresponding Author: Dr. Ramesh Malothu, Head and Assistant Professor, Department of Pharmacy and Biotechnology, UCEV-JNTUK, Vizianagaram-535002, A.P, India

ABSTRACT

An accurate, Precise, Simple and Economical High Performance Liquid Chromatographic method for the estimation of Nebivolol and Hydrochlorothiazide was developed and validated. The determination was performed by the using of two phases one is stationary phase it's a Thermo BDS Hypersil C18 column having 250 x 4.6mm 5µ, and another one is mobile phase containing 0.1N phosphate buffer and Acetonitrile at the ratio 50:50%v/v. The flow rate was 1ml/min and effluents were monitored at 282nm. The retention time of Nebivolol and Hydrochlorothiazide was 3.5 and 5.4 min respectively. The developed method was validated for specificity, system suitability, precision, linearity, accuracy, Limit of Detection, Limit of Quantification, robustness, and ruggedness. Recovery of Nebivolol and Hydrochlorothiazide in formulations was found to be in the range of 100%, 100%, and 101% respectively. And the correlation coefficient was 0.999. Hence, it was concluded that the developed method is suitable for routine analysis of these combination due to its less analysis time.

Keywords: Hydrochlorothiazide, Nebivolol, RP HPLC, Method Development and Validation.

INTRODUCTION

Nebivolol Hydrochloride (NEB) is chemically designated as 1-(6-fluorochroman-2-yl)-{[2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} ethanol[12] (Figure.1). It is a racemate of two enantiomers with four chiral centers, Nebivolol is a highly selective β1-blocker with nitric oxide mediated vasodilatory actions and beneficial effects on vascular endothelial function. Nebivolol is used in the treatment of hypertension. It blocks the β adreno-receptor effect of adrenaline and nor-adrenaline[9,11]. Nebivolol reduces heart rate, rate of myocardial contractility, decrease systemic blood pressure and increase diastolic pause. Nebivolol is preferable in patients with bronchospasm, diabetes, peripheral vascular disease or Reynaud's Phenomenon[7]. Nebivolol mode of action is lowering blood pressure (BP) by reducing peripheral vascular resistance, and significantly increases stroke volume with preservation of cardiac output. The net hemodynamic effect of nebivolol is the result of a balance between the depressant effects of beta-blockade and an action that maintains the cardiac output [14]. NEB reaches peak value within 0.5-2 hrs. The oral bioavailability of the drug averages 12 % in fast metabolizers and 96 % in slow metabolizers2. NEB is not official in I.P., B.P. and U.S.P [6]. Hydrochlorothiazide (HCT) is chemically 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulfonamide 1, 1-dioxide (Figure.2). It is thiazide diuretic, used in the treatment of hypertension and edema associated with moderate congestive heart failure. It increases the rate of urine excretion 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. Hydrochlorothiazide is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones. On prolonged thiazide treatment, plasma volume and ECF returns to normal but their hypotensive effect continues due to reduced sensitivity of vascular beds to circulating catecholamine and angiotensin. It shows peak effect within 4- 6 hrs and eliminated in 10-12 hrs, most rapidly excreted in urine in unchanged form[2,3,5,9,13,]. The combination of NEB and HCZ are choice drugs for many low rennin hypertension. Literature survey reveals that there is number of analytical methods for estimation of NEB and HCZ including UV Spectroscopy[1,4], HPLC[3,9,14] and HPTLC[8,10,11]. The purpose of this investigation was to develop and validate a simple, rapid, sensitive, precise, accurate and specific reverse phase HPLC method.

Figure.1: Structure of Nebivolol

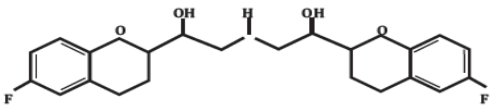
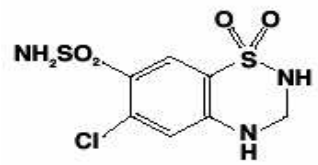


Figure 2. Structure of Hydrochlorothiazide



MATERIALS AND METHODS

Apparatus:

Waters e2695 Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software.

Materials:

Acetonitrile was HPLC grade and collected from E. Merck, Darmstadt, Germany. Potassium di hydrogen ortho phosphate was analytical reagent grade supplied by Fischer Scientific Chemicals and Potassium phosphate di basic was supplied by Rankem. HPLC grade Water was obtained from a Milli-QRO water purification system. Methanol was supplied by Rankem.

Commercial Formulation:

NEB and HCT in combined tablets are available in the market as NEBIOL-H. Those are Fixed-dose combinations of 5mg and 12.5mg. The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly.

Buffer Preparation:

Weighed 0.2625g of Potassium phosphate dibasic and 1.3625g of Potassium di hydrogen orthophosphate and dissolved in 500 ml of water and filtered through 0.45 µ filter and degassed.

Preparation of mobile phase:

Buffer solution and Acetonitrile were mixed in the ratio of 50:50% and degassed.

Preparation of Nebivolol standard solution:

Accurately weighed quantity of 10mg Nebivolol was transferred to a100ml volumetric flask, dissolved in 25ml of methanol and sonicated for 20 minutes and the solution was made up the final volume with methanol. From the above stock solution take 50ml was transferred to 100ml volumetric flask and make up the final volume with methanol. The solution was filtered with 0.45µ filter and sonicated for 15min.

Preparation of Hydrochlorothiazide standard solution:

Accurately weighed quantity of 25mg Hydrochlorothiazide was transferred to a 50ml volumetric flask, dissolved in 25ml of methanol and sonicated for 20mins and the solution was made up the final volume with methanol. From the above stock solution take 10ml was transferred to 100ml volumetric flask and make up the final volume with methanol. The solution was filtered with 0.45µ filter and sonicated for 15min.

Preparation of sample solution:

The tablet dosage form contains Hydrochlorothiazide and Nebivolol Twenty tablets were weighed accurately and crushed to fine powder and powder equivalent to 250mg of NEB was accurately weighed and transferred in to 50ml volumetric flask and mobile phase was added to dissolve the sample. To make up the volume with mobile phase & filtered with 0.45µ filter paper. From the above stock solution take 5ml was transferred to 100 ml volumetric flask and make up the volume with mobile phase. The solution was filtered with 0.45µ filter and sonicated for 15min.

Chromatographic Conditions:

The mobile phase pumped at a flow rate of 1 ml/min through the column (C18; 4.6 X 250 mm, 5µ, Thermo BDS column) at 30ºC, and injection volume is 10 µl, wavelength used was 282nm, and runtime was 7 min's.

Recommended procedure:

The HPLC system was stabilized for 30 minutes by following the chromatographic conditions as described. One blank followed by 5 Injections of standard solution was injected. The retention time and the average peak areas for each standard were recorded. Two Injections of working sample solution was injected and the results were recorded. The amount of drug present in sample solution was calculated. Chromatograms for the standards are shown in figure 3 and figure 4.

Figure.3: Chromatogram for standard

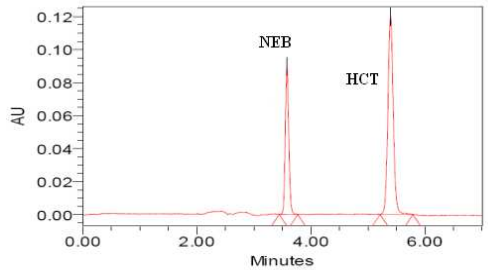
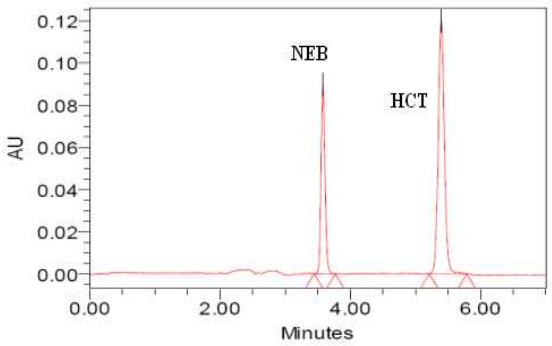


Figure.4: Chromatogram for sample



RESULTS

Validation of HPLC method:

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of NEB and HCT in tablet dosage form. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, LOD, LOQ, and robustness.

System Suitability:

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of NEB and HCT. The developed method was validated according to ICH Guidelines. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters. The result was shown in the table.1.

Table.1: Result of System Suitability Tests of Nebivolol and Hydrochlorothiazide.

PARAMETERS	NEB	HCT
Linearity range	50 to 150%	50 to 150%
Correlation coefficient	0.999	0.999
Retention time	3.5	5.4
Resolution Factor	000	12.6
USP plate count	10998	11256
Tailing factor	1.1	1.06

Selectivity:

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard samples of NEB and HCT were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another. Chromatograms shown in figure.4 and explain that retention time for standard and sample are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective.

Linearity:

Linearity of the method was determined by constructing calibration curves. Standard solutions of NEB and HCT of different concentrations level (50%, 75%, 100%, 125%, and 150%) were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients were 0.999 for the drugs which prove that the method is linear. The graphs were shown in the figure.5 and figure 6.The linearity data for NEB and HCT was shown in table.2.

Figure 5: Linearity of Nebivolol

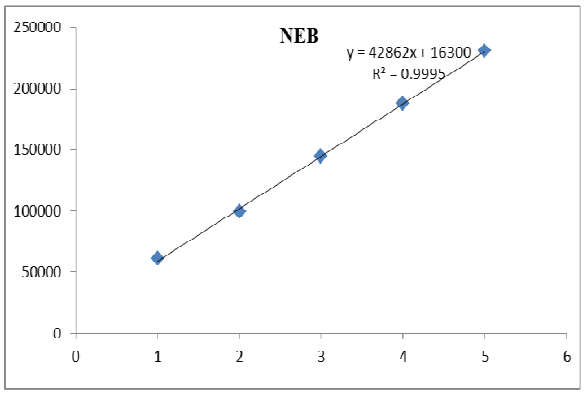


Figure 6: Linearity of Hydrochlorothiazide.

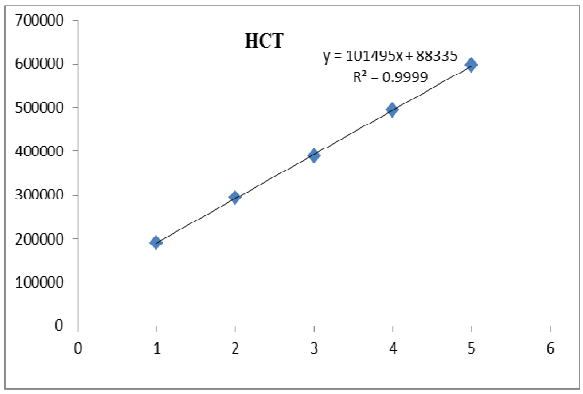


Table.2: Linearity Of Nebivolol And Hydrochlorothiazide

S.No.	Conc.	Area of NEB	Area of HCT
1	50	60998	190346
2	75	99709	292774
3	100	144678	389755
4	125	187769	494030
5	150	231278	597193

Recovery:

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 100% and 6 replicate injections at 50% and 150%. Known amounts of standard NEB and HCT were added to pre-analyzed samples and were subjected to the proposed HPLC method. The measured value was

obtained by recovery test. Spiked amount of both the drugs were compared against the recovery amount. % Recovery was 100%. The results are shown in table.3

Table.3: Accuracy of Nebivolol and Hydrochlorothiazide

S.No.	Spike Level	% Recovery of NEB	Mean % Recovery	% Recovery of HCT	Mean % Recovery
1	50%	101	100	99	99
2	50%	101		98	
3	50%	100		98	
4	50%	99		99	
5	50%	99		99	
6	50%	100		100	
1	100%	100	100	100	100
2	100%	100		100	
3	100%	100		100	
1	150%	101	101	100	100.6
2	150%	102		101	
3	150%	100		101	
4	150%	102		100	
5	150%	102		101	
6	150%	101		101	

Precision:

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. The values are shown in table.4.

Table.4: Precision of Nebivolol and Hydrochlorothiazide.

S.No.	Sample Area-NEB	Sample Area-HCT	% Assay NEB	% Assay HCT
1	144678	386741	99	99
2	144565	384652	98	99
3	146681	385214	98	99
4	144780	383573	98	98
5	147763	386871	99	101
6	144236	386061	99	100
Avg Assay			99	99
STD			0.33	0.97
% RSD			0.33	0.98

Robustness of Method:

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and ± 5ºc change in temperature. The values are shown in table.5.

Table.5: Results for Robustness Test of Nebivolol and Hydrochlorothiazide.

Parameters Count	Changes	RT	USP Tailing	USP Plate
Flow rate(ml/min)	Flow 1(low)	4.46, 6.78	1.05	9331
	Flow 2(high)	2.98, 4.53	1.12	7200
Temperature	Temp1(low)	3.58, 5.43	1.06	8149
	Temp1(high)	3.56, 5.6	1.04	8879

Ruggedness:

System to system variability: System to system variability on two HPLC systems was carried out to get the ruggedness of assay method. The result was shown in Table 6.

Table.6: System To System Variability for Nebivolol and Hydrochlorothiazide

S.No.	ASSAY% OF NEBIVOLOL (SYSTEM 1)	ASSAY% OF HYDROCHLORO THIAZIDE (SYSTEM 1)	ASSAY% OF NEBIVOLOL (SYSTEM 2)	ASSAY% OF HYDROCHLORO THIAZIDE (SYSTEM 2)
1	99.2	99	100.4	99
2	99.5	99	99.9	99
3	99.3	99	100.6	100.6
4	99.2	98	100.4	100.4
5	100.2	101	99.5	101
6	99.5	100	100.5	100
Average	99.5	99	100.2	99
% RSD	0.37	0.97	0.44	0.86

HPLC column to column variability:

Column to column variability on two HPLC systems was carried out to get the ruggedness of assay method. The result was shown in Table.7.

Table.7: Column To Column Variability for Nebivolol and Hydrochlorothiazide

S.No.	ASSAY % OF NEBIVOLOL (COLUMN 1)	ASSAY% OF HYDROCHLORO THIAZIDE (COLUMN 1)	ASSAY % OF NEBIVOLOL (COLUMN 2)	ASSAY% OF HYDROCHLORO THIAZIDE (COLUMN 2)
1	99.2	99	99.6	100.3
2	99.5	99	99.6	99.4
3	99.3	99	98.7	99.7
4	99.2	98	100.0	100.1
5	99.5	101	99.7	99.2
6	100.2	100	99.0	100.5
Average	99.5	99	99.4	99.9
% RSD	0.37	0.97	0.50	0.91

CONCLUSION

The new Reverse phase HPLC method developed and validated for simultaneous determination of Nebivolol and Hydrochlorothiazide pharmaceutical dosage forms and

assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid dosage forms. This method was doing in simple manner but founded rapidly accurate values, so method will be use full for quality control department, formulation and other departments

REFERENCES

1. Ankit B Chaudhary, Rakesh K Patel, Sunita A Chaudhary, Krupa V Gadhvi, Estimation Of Valsartan And Hydrochlorothiazide In Pharmaceutical Dosage Forms By Absorption Ratio Method, IJABPT Vol-1,Issue-2,2010

2. British Pharmacopoeia, Her Majesty's Stationery Office, London, Vol-II, 1993

3. Chandra Mohan Rao Kota, Useni Reddy Mallu, Hani, A Simple Gradient RP-HPLC Method For The Quantification Of Hydrochlorothiazide, Amlodipine Besylate And Nebivolol Hydrochloride In Pharmaceutical Dosage Forms, IJRRPAS, Vol-1, Issue-2, 2011.

4. Chaudhary AB, Patel RK, Estimation of Valsartan and Nebivolol in pharmaceutical dosage forms by absorption ratio method. IJRPS Vol-1,108-112,2010

5. Indian Pharmacopoeia, The Controller of Publication, New Delhi, Vol-I, 1996

6. Ketan Shah, Desai TR, Atanukumar Behera, Ranju S Pal, Kundlik M Girhepunje, Development of spectrophotNEBtric methods for estimation of nebivolol hydrochloride and hydrochlorothiazide simultaneously, in bulk and tablet dosage form, RJPBCS, Vol-1,Issue-2,2010

7. KV Shah, PR Tirgar, DB Sheth, TR Desai, Simultaneous Estimation Of Nebivolol Hydrochloride And Hydrochlorothiazide In Bulk And In A Tablet Dosage Form By Multicomponent And Simultaneous Estimation Method, Pharma Science Monitor, Vol-2, Issue-1, 2011

8. Mahesh Dangi, Darshana Chaudhari, Mayur Sinker, Veena Racha and Mrinalini.C. Damle, Stability Indicating HPTLC Method for Estimation of Nebivolol Hydrochloride and Amlodipine Besylate in Combination, Eurasian J. Anal. Chem. 5(2): 161-169, 2010

9. Manzoor Ahmed, Manohara YN, Ravi MC, Development and validation of RP-HPLC method for simultaneous estimation of nebivolol hydrochloride and hydrochlorothiazide in combined tablet dosage form

10. Reddy, TS Devi, Validation of a high-performance thin-layer chromatographic method, with densit NEBtric detection, for quantitative analysis of Nebivolol hydrochloride in tablet formulations. JPC-Modern TLC Vol. 20(2), 2007.

11. ST Kumbhar, GK Chougule, VS Tegeli, GB Gajeli, A Validated HPTLC Method for Simultaneous Quantification of Nebivolol and Hydrochlorothiazide in Bulk and Tablet Formulation.

12. The British Pharmacopoeia Commission. British Pharmacopoeia. Vol. I. London: the Stationery Office Books; 1993

13. United States Pharmacopoeia, 26th ed: Unites states Pharmacopoeial Convention, Rockville, MD, 2003

14. Y Gupta, A Shrivastava, D Duggal, A Patel, S Agrawal, A new RP-HPLC method for simultaneous estimation of nebivolol hydrochloride and hydrochlorthiazide in dosage forms, Journal of young pharmacists, Vol-1,Issue-3,2009

Source of support: Nil

Conflict of interest: None Declared