ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development of spectrophotometric methods for estimation of nebivolol hydrochloride and hydrochlorothiazide simultaneously, in bulk and tablet dosage form

Ketan Shah [1], Desai TR[1], Atanukumar Behera[2], Ranju S Pal[3], Kundlik M Girhepunje[3]*

[1]R. K. College Of Pharmacy, Kasturbadham, Rajkot, Gujarat, India-360 003.
[2]Shree Leuva Patel Trust Pharmacy Mahila College, Amreli, Gujarat, India- 365601
[3]Sagar Institute of Research and Technology, Bhopal, Madhya Pradesh, India.

ABSTRACT

The goal of the current investigation is to develop new spectrophotometric methods viz. AUC method and first derivative spectroscopy method, for simultaneous determination of Nebivolol hydrochloride (NEB) and Hydrochlorothiazide (HCT) in bulk and in combined tablet dosage form, which were used for the validation of linearity, accuracy and precision. One of these methods involved for solving of simultaneous equations based on measurement of AUC at two wavelengths range 312-322 nm and 285-295 nm. Another method was first derivative spectroscopy, which was adapted to eliminate spectral interference. This method employs formation and solving of simultaneous equation using 292 nm (λ_1) and 279 nm (λ_2) as two analytical wavelengths. Both the drugs obey linearity with absorbance in the concentration ranges employed for these methods. The methods have been validated statistically and by recovery studies. These methods were found to be simple, sensitive, rapid, accurate, reproducible and economical.

Keywords: Nebivolol hydrochloride, Hydrochlorothiazide, Area under curve, Derivative spectroscopy

*Corresponding author
Mob. No. 09328029067
Email: kundlik@gmail.com

ISSN: 0975-8585

INTRODUCTION

Nebivolol hydrochloride (NEB), chemically α, α'- [Iminobis (methylene)] bis [6-fluoro-3, 4-dihydro-2H-1-benzopyran-2-methanol] [1] a beta-adrenergic blocker[2-3] & hydrochlorothiazide (HCT), chemically 6-Chloro-3, 4-dihydro-7-sulfamoyl-2H-1, 2,4-benzothiadiazine 1,1-dioxide[4] a thiazide diuretic[5] are known for a synergistic therapeutic effect in essential hypertension [6]. Combination of these two drugs usually prescribed in the initial management of essential hypertension [6]. Fixed combination of NEB (5 mg) and HCT (12.5 mg) are being marketed.

For HCT, I.P. [7] and B.P. [8] describes the spectrophotometric determination and U.S.P.[9] by HPLC method. NEB is not official in I.P., B.P. and U.S.P.

Literature survey reveals spectrofluorimetric method[10] for the estimation of NEB and few HPLC[11-15] methods for HCT. There was no simultaneous estimation for NEB and HCT are reported. The paper presents two simple, accurate, reproducible and economical methods for the estimation of NEB and HCT in bulk and in multicomponent formulation.[16]

A Shimadzu UV/Visible spectrophotometer model 1601 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm with automatic wavelength correction with a pair of 10mm quartz cells. A Shimadzu electronic analytical balance (AX-200) was used for weighing the sample. An ultrasonic cleaner (Art No.400014CL) was used for sonicating the tablet powder. Nebivolol Hydrochloride, Hydrochlorothiazide and Methanol -AR grade were used in the study.

Standard Stock solutions (100 µg/ml) of NEB and HCT were prepared by dissolving separately 10 mg of drug in methanol. The scanning of NEB and HCT were carried out in the range of 200-400 nm against methanol as blank for obtaining the overlain spectra. For simultaneous estimation of NEB and HCT, mixed standard of 20 µg /ml solution of NEB and 50 µg/ml solution of HCT were prepared by diluting appropriate volumes of the standard stock solutions. This mixed standard was used for method-A and method-B. The optical characteristics and regression values for the calibration curve are presented in Table 1.

MATERIALS AND METHODS

Nebivolol hydrochloride was a kind gift from Torrent Pharmaceuticals, Ahmedabad. and Hydrochlorothiazide was a kind gift from Li-taka Pharmaceuticals, Pune. All other chemicals were of analytical grade.

Preparation of samples

Method-A:

1) Standard Stock solutions (100 µg/ml) of NEB and HCT were prepared by dissolving separatively 10 mg of drug in methanol. From the standard drug solution appropriate dilutions were made to obtain two sets of solutions for each drug. The sets were prepared with concentration of 1,2,5,10,20,30,40,50,60,70,80 µg /ml for NEB and 1,2,5,10,20,30,50,60,70,80 µg /ml for HCT. Both the drugs were scanned in the range of 400-200nm.

2) For simultaneous estimation of NEB and HCT, 20 µg /ml solution of NEB and 50 µg/ml solution of HCT were prepared by diluting appropriate volumes of the standard stock solutions. The scanning of the solutions of NEB and HCT were carried out in the range of 200 to 400 nm for obtaining the overlain spectra. . AUC of standard solution were recorded at selected wavelength ranges 285-295 nm and 312-322 nm. Mixed standard for the pure drug was prepared from the stock solutions.

3) Mixed standards containing 20 µg/ml for NEB and 50 µg/ml for HCT were prepared and their AUC were recorded at selected wavelength ranges 312-322 nm and 285-295 nm.

Concentration of NEB and HCT in the powder mixture is found by using equation (i) and (ii).

$$C_{NEB} = \frac{X_{H\ 312\text{-}322} * AUC^{M}_{285\text{-}295} - X_{H\ 285\text{-}295} * AUC^{M}_{312\text{-}322}}{X_{H\ 312\text{-}322} * X_{N\ 285\text{-}295} - X_{H\ 285\text{-}295} * X_{N\ 312\text{-}322}} \quad \text{(i)}$$

$$C_{HCT} = \frac{X_{N\ 285\text{-}295} * AUC^{M}_{312\text{-}322} - X_{N\ 312\text{-}322} * AUC^{M}_{285\text{-}295}}{X_{H\ 312\text{-}322} * X_{N\ 285\text{-}295} - X_{H\ 285\text{-}295} * X_{N\ 312\text{-}322}} \quad \text{(ii)}$$

Where,

(1) C_{NEB} = Concentration of Nebivolol Hydrochloride

(2) C_{HCT} = Concentration of Hydrochlorothiazide

(3) $AUC^{M}_{312\text{-}322}$ = Area under curve of mixture at wavelength range 312-322 nm

(4) $AUC^{M}_{285\text{-}295}$ = Area under curve of mixture at wavelength range 285-295 nm

(5) $X_{H\ 312\text{-}322} = \frac{\text{AUC of HCT at wavelength range 312-322 nm}}{\text{Concentration of HCT in gm / liter}}$

(6) $X_{H\ 285\text{-}295} = \frac{\text{AUC of HCT at wavelength range 285-295 nm}}{\text{Concentration of HCT in gm / liter}}$

(7) $X_{N\ 312\text{-}322} = \frac{\text{AUC of NEB at wavelength range 312-322 nm}}{\text{Concentration of NEB in gm / liter}}$

(8) $X_{N\ 285\text{-}295} = \frac{\text{AUC of NEB at wavelength range 285-295 nm}}{\text{Concentration of NEB in gm / liter}}$

By putting the values in equation (i) and (ii)

$$C_{NEB} = \frac{105.113 * AUC^{M}_{285\text{-}295} - 81.189 * AUC^{M}_{312\text{-}322}}{105.113 * 139.67 - 81.189 * 0.0} \quad \text{(iii)}$$

$$C_{HCT} = \frac{139.67 * AUC^{M}_{312\text{-}322} - 0.0 * AUC^{M}_{285\text{-}295}}{105.113 * 139.67 - 81.189 * 0.0} \quad \text{(iv)}$$

4) Twenty tablets (brand name Nebicard-H and manufactured by Torrent Pharmaceuticals Ltd., Ahmedabad) were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 10 mg was transferred to a 100ml volumetric flask and dissolved in about 25 ml of methanol. After the immediate dissolution, the volume was made up to the mark with methanol. The solution was kept for sonication for about 20 minutes .The solution was filtered through Whatmann filter paper No.41 and was diluted to prepare the concentration of 20 µg/ml NEB and 50 µg/ml HCT.

The AUC were recorded at selected wavelength ranges 285-295 nm and 312-322 nm and the amount of drug present in the sample solution were obtained by using equation in the same manner as that was used with pure mixed standards.[17]

Method –B:

1) The standard solutions of 20 µg /ml of both the drugs were scanned in the range of 400-200 nm, the absorbance spectra, thus obtained were derivatised to remove the interference of absorbing species. From the examination of overlain first derivative spectra of NEB and HCT, 292 nm (λ_1) and 279 nm (λ_2) were selected as working wavelength for first derivative spectroscopy. From the standard drug solution appropriate dilutions were made to obtain two sets of solutions for each drug. The sets were prepared with concentration of 5, 10,

ISSN: 0975-8585

20, 30, 40, 50 µg /ml for NEB and 5,10,20,30,40,50,60 µg /ml for HCT. Absorbances were recorded in the first derivative mode of UV-Visible spectrophotometer.

2) For simultaneous estimation of NEB and HCT using first derivative spectrum, 20 µg /ml solution of NEB and 50 µg/ml solution of HCT were prepared by diluting appropriate volumes of the standard stock solutions. The scanning of the solutions of NEB and HCT were carried out in the range of 400 to 200 nm. Absorbances and absorptivities of standard solution were recorded at selected wavelengths λ_1 and λ_2.

3) Mixed standard for the pure drug was prepared from the stock solutions. Mixed standards containing 20 µg/ml for NEB and 50 µg/ml for HCT were prepared and their absorbances were recorded at selected wavelengths λ_1 and λ_2.
Concentration of NEB and HCT in the powder mixture is found by using equation (i) and (ii).

$$A_1 = (-1.20611)C_{NEB} + (-0.20471)\, C_{HCT} \qquad \text{(i)}$$

$$A_2 = (0.4041666)\, C_{NEB} + (-5.19757)\, C_{HCT} \qquad \text{(ii)}$$

The concentration of C_{NEB} and C_{HCT} can be obtained by solving equation (i) and (ii).

$$C_{NEB} = \frac{A_1 \times (-5.19757) - A_2 \times (-0.20471)}{(-1.20611) \times (-5.19757) - (0.4041666) \times (0.20471)} \qquad \text{iii) and}$$

$$C_{HCT} = \frac{A_2 \times (-1.20611) - A_1 \times (0.4041666)}{(-1.20611) \times (-5.19757) - (0.4041666) \times (0.20471)} \qquad \text{(iv)}$$

Where (1) (-1.20611) and (0.4041666) are absorptivities of NEB at λ_1 and λ_2 respectively.
(2) (-0.20471) and (-5.19757) are absorptivities of HCT at λ_1 and λ_2 respectively.
(3) A_1 and A_2 are absorbances of mixtures at λ_1 and λ_2 respectively.
(4) C_{NEB} & C_{HCT} are concentrations in gm / liter.

Preparation and analysis of tablet sample solution:

Twenty tablets (brand name Nebicard-H and manufactured by Torrent Pharmaceuticals Ltd., Ahmedabad) were weighed and crushed to a fine powder. An accurately weighed powder sample equivalent to 10 mg was transferred to a 100 ml volumetric flask and dissolved in about 25 ml of methanol. After the immediate dissolution, the volume was made up to the mark with methanol. The solution was kept for sonication for about 20 minutes .The solution was filtered through whatmann filter paper No.41 and was suitably diluted to get a final concentration of 20 µg/ml NEB and 50 µg/ml HCT. [18]

The absorbances were recorded at selected wavelengths 292 nm (λ_1) and 279 nm (λ_2) of the first derivative mode and the concentrations of the two drugs in the sample solution were obtained by using equation (iii) and (iv). The analysis procedure was repeated six times with the same batch of tablets.

RESULTS AND DISCUSSION

The wavelength range selected for Nebivolol Hydrochloride was between 285- 295 nm at which Nebivolol Hydrochloride contributes to a larger AUC as compared to Hydrochlorothiazide. For Hydrochlorothiazide the wavelength range selected was between 312-322 nm under which only Hydrochlorothiazide contributes to AUC, because in this region Nebivolol HCl showed no spectrum. AUC of NEB and HCT were obtained at both the wavelength range 285-295 nm and 312-322 nm against methanol as blank for solving equation. NEB and HCT showed linearity with AUC in the range 0-80 µg /ml at their respected selected wavelength range. Co-efficients of correlation were found to be 0.9999 for NEB and 0.9999 for HCT.

In simultaneous estimation of NEB and HCT, AUC of standard solution were recorded at selected wavelength ranges 285-295 nm and 312-322 nm Which shown in fig.1.

NEB and HCT showed linearity with absorbance in the range 0-50 µg /ml and 0-60 µg /ml at λ_1 and λ_2 respectively shown in fig. 2. For NEB Co-efficient of correlation were found to be 0.9987 and 0.9944 at λ_1 and λ_2 respectively, and for HCT Co-efficient of correlation were found to be 0.9936 and 0.9996 at λ_1 and λ_2 respectively.

The results of analysis and statistical validation for the marketed tablet formulation are reported in Table-2. The results of recovery studies conducted by the addition of different amounts of pure drugs at 80%, 100% and 120% levels to a tablet solution were found to be satisfactory which shown in the Table-3. The results of recovery studies indicated that the method was accurate and reproducible.

The results of the analysis of pure drug and statistical validation data are given in Table-1 for both the methods.

The results of the tablet analysis, recovery studies and statistical validation data are given in Table-2 and 3 for both the methods.

The proposed method for simultaneous estimation of NEB and HCT by simultaneous equation method in combined sample solutions was found to be simple, accurate and reproducible. Once the equations are established, analysis requires only the measuring of the absorbances of the sample solution at the two wavelengths selected, followed by few simple calculations. Both the methods can be employed for routine analysis of the drugs in quality control, R & D laboratories in pharmaceutical industries.

ACKNOWLEDGEMENTS

The authors thank M/S Torrent Pharmaceuticals, Ahmedabad and M/S Li-taka Pharmaceuticals, Pune for supplying gift samples of Nebivolol Hydrochloride and Hydrochlorothiazide respectively to carry out this work.

TABLE 1. STATISTICAL VALIDATION OF PURE DRUGS

Name of Component	Amount Present (µg/ml)	Method	Mean*	% Co-efficient of Variation
NEB	20	Method-A	100.68±1.113	1.105±0.454
	20	Method-B	99.27±1.325	1.335±0.5410
HCT	20	Method-A	99.82±0.728	0.729±0.297
	20	Method-B	99.84±0.968	0.9703±0.395

NEB is Nebivolol Hydrochloride and HCT is Hydrochlorothiazide,
Method-A is AUC method, method-B is first derivative method.* Here Mean is the average of (n=6) results.

TABLE 2. STATISTICAL VALIDATION OF TABLET

Name of Component	Amount Present (µg/ml)	Method	Mean*	% Co-efficient of Variation
NEB	20	Method-A	101.06±0.620	0.613±0.253
	20	Method-B	99.04±0.193	0.195±0.079
HCT	20	Method-A	99.280±0.733	0.739±0.199
	20	Method-B	98.760±0.460	0.466±0.187

NEB is Nebivolol Hydrochloride and HCT is Hydrochlorothiazide,
Method-A is AUC method, method-B is first derivative method.* Here Mean is the average of (n=6) results.

ISSN: 0975-8585

TABLE 3. RECOVERY STUDIES

Level of % Recovery	Name of Drug	Amount Present (mg/tab)	Amount of Standard added (mg/tab)	% Recovery* ± SD		% Co-efficient of Variation ± SE	
				A	B	A	B
80	NEB	5	4	100.170 ±0.136	100.800 ±1.992	0.1363 ±0.078	1.976 ±0.350
	HCT	12.5	10	99.090 ±0.504	101.13 ±0.952	0.508 ±0.029	0.942 ±0.050
100	NEB	5	5	101.27 ±0.345	99.98 ±1.709	0.341 ±0.099	1.709 ±0.086
	HCT	12.5	12.5	99.390 ±0.223	99.820 ±1.611	0.225 ±0.029	1.614 ±0.030
120	NEB	5	6	99.710 ±0.560	99.810 ±1.867	0.561 ±0.023	1.870 ±0.078
	HCT	12.5	15	100.06 ±1.169	100.19 ±1.387	1.168 ±0.074	1.384 ±0.023

* Here % recovery is average of three results at each level.
A is AUC method and B is first derivative method.

Fig.1: Overlain Spectra of NEB and HCT
Overlain spectra showing area under curve of Nebivolol hydrochloride (NEB) and Hydrochlorothiazide (HCT) in methanol.

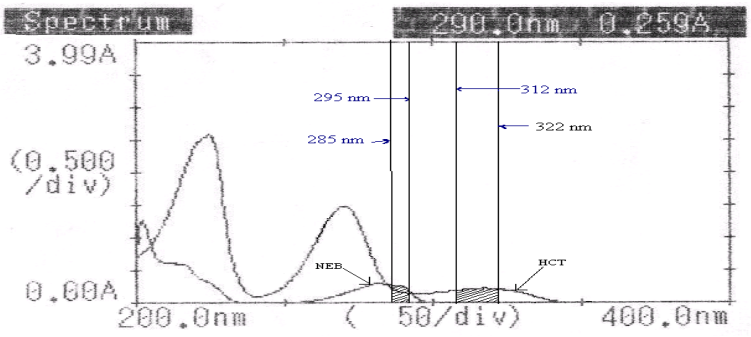
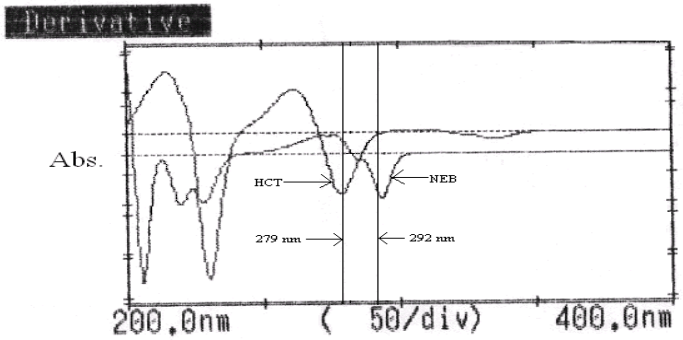


Fig.2: Overlain spectra of NEB and HCT
UV absorption first derivative spectra of Nebivolol hydrochloride (NEB) and Hydrochlorothiazide (HCT) in methanol.



ISSN: 0975-8585

REFERENCES

[1] Budavari S. The Merck Index, Merck & Co. Inc. Whitehouse Station. New Jersey, USA. 2001; 13th ed.:52.
[2] Sweetman SC. Martindale: The Complete Drug Reference. Pharmaceutical Press, London, UK 2005;34th ed.:64.
[3] Bennett PN, Brown MJ. Clinical Pharmacology, Churchill Livingstone, London 2003; 9th ed.: 474.
[4] Florey K. Analytical Profiles of Drug Substances. Academic Press, London 2005; 10: 405.
[5] Joel GH, Lee EL. Goodman and Gillman's The Pharmacological Basis of Therapeutics. Mc Graw- Hill, Medical Publishing Division, USA 2001; 10th ed.: 260-773.
[6] Jain SK, Jain D, Tiwari M, Chaturvedi SC. Indian J Pharm Sci 2002; 64: 267.
[7] Indian Pharmacopoeia, Published by the Controller of Publication, New Delhi 1996; 1: 370.
[8]. British Pharmacopoeia, Published by Her Majesty's Stationery Office, London 1993; 2: 942.
[9] United States Pharmacopoeia, United States Pharmacopoeial Convention. Inc., Rockville, MD 2003; 26th ed: 909.
[10] Rajeswari KR, Sankar GG, Rao AL. Indian Pharmaceutical Congress, Hyderabad, Scientific Abstracts GP 69; 298.
[11] Carlucci G, Palumbo G, Mazzeo P, Quaglia MG. J Pharm Biomed Anal 2000; 23: 185.
[12] Carlucci G, Carlo V D, Mazzeo P. Anal Lett 2000; 33: 2491-2500.
[13] Kanumula GV, Bhanu Raman. Indian Drugs 2000; 37: 38.
[14] Moussa BA, EL Kousy NM. Pharm Week Bl Sci 1985; 7: 79.
[15] Erram SV, Tipnis HP. Indian Drugs 1994; 31: 16.
[16] Beckett AH, Stanlake JB. Practical Pharmaceutical Chemistry, Part Two, CBS Publishers and Distributors, New Delhi 4th ed.: 281-307.
[17] Erk N. J Pharm Biomed Anal 2002; 27: 901-902.
[18] Shirkee PP, Tamhanc VA, Kulkarni MA, Patel MK, Tirodkar VB, Sethi PP. Indian Drugs 1994; 31: 494.