Asian Journal of Chemistry Vol. 20, No. 7 (2008), 5192-5196

Simultaneous Spectrophotometric Estimation of Nebivolol Hydrochloride and Hydrochlorothiazide in Tablets

B. DHANDAPANI*, K. SURESH KUMAR, R. KARTHIKEYAN,
M. RAJA and A. SOMASUNDARAM
Department of Pharmaceutical Analysis, Nehru College of Pharmacy
Pampady, Thrissur-680 597, India
E-mail: dhandapanirx@gmail.com

Two simple, accurate and precise methods for simultaneous estimation of nebivolol hydrochloride (NEB-H) and hydrochlorothiazide (HCT) in combined dosage form have been described. First method employs formation and solving of Q-absorbance equation at 275 nm (isoabsorptive point) and at 282.5 nm (λ_{max} of NEB-H). The second method involves formation and solving of simultaneous equation using 282.5 and 271.5 nm (λ_{max} of HCT) using methanol as solvent. The result of analysis has been validated statistically and by recovery studies with standard deviation < 1.0 % was found. The proposed methods were successfully applied for estimation of nebivolol hydrochloride and hydrochlorothiazide in combined tablet formulation.

Key Words: Spectrophotometric estimation, Nebivolol hydrochloride, Hydrochlorothiazide.

INTRODUCTION

Nebivolol hydrochloride[1] (NEB-H) is a benzopyran antihypertensive drug (β_1-blocker) and chemically it is α,α'-[imino*bis*(methylene)]*bis*[6-fluoro-3,4-dihydro-2*H*-1-benzopyran-2-methanol hydrochloride. Hydrochlorothiazide[2] (HCT) is a 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide which is used as a diuretic. The combination of nebivolol hydrochloride and hydrochlorothiazide is newly introduced in market and used in the treatment of hypertension. Several methods such as HPLC[3-5], HPTLC[6], spectrophotometry[7] and non-aqueous potentiometric titration[8] are reported for the estimation of hydrochlorothiazide. Reports are available for estimation of NEB-H by HPLC[9-14]. Moreover, the literature survey revealed that so far, no method has been reported for estimation of NEB-H and HCT in combined dosage forms, hence an attempt is made to develop a simple, accurate and economical analytical method. This paper describes two simple UV spectrophotometric methods for simultaneous estimation of NEB-H and HCT in tablets using methanol as solvent.

EXPERIMENTAL

A Shimadzu UV/Vis double beam spectrophotometer (model: UV-1700) with auto corrected wavelength accuracy of ± 0.3 nm and 1 cm UV matched quartz cells were used. Gift samples of nebivolol hydrochloride (NEB-H) and hydrochlorothiazide (HCT) procured from Micro Labs, Bangalore. Tablets of brand Nebistor-H, Nebilong-H containing NEB-H (5 mg) and HCT (12.5 mg) were procured from a local Pharmacy shop.

Nebivolol hydrochloride (NEB-H) standard stock solution was prepared by weighing 25 mg of NEB-H, transferred to a 25 mL volumetric flask and volume was made up to 25 mL with to get a concentration of 1 mg/mL, from this solution, an aliquot of 2.5 mL was withdrew and it was diluted to 25 mL with methanol. Similarly, the standard solution of hydrochlorothiazide (HCT) were also made on the same guidelines.

For selection of analytical wavelength for the Q-absorbance method (Method-1), the stock solutions of NEB-H and HCT were separately diluted with methanol to get a concentration of 10 and 1 μg/mL, respectively and scanned in the wavelength range of 200-400 nm. From the overlay spectra of both drugs, wavelengths 275 nm (isoabsorptive point) and 282.5 nm (λ_{max} of NEB-H) were selected for the formation of Q-absorbance equation. For calibration curve, stock solutions of NEB-H and HCT were appropriately diluted to obtain concentration range of 10-50 and 1-5 μg/mL, respectively. The absorbance of NEB-H was measured at 282.5 and 275 nm and calibration curves were plotted. Similarly, the absorbance of HCT was measured at 282.5 and 275 nm, calibration curves were plotted. The absorptivities (A1 %, 1 cm) of each drug at both the wavelengths were also determined.

The absorbance and absorptivity values at the particular wavelengths were calculated and substituted in the following equation, to obtain the concentration.

$$C_{NEB\text{-}H} = (Q_M - Q_Y) \times A_1/(Q_X - Q_Y) \times a_{x1}$$

$$C_{HCT} = (Q_M - Q_X) \times A_1/(Q_Y - Q_X) \times a_{x1}$$

where, $C_{NEB\text{-}H}$, C_{HCT} are concentration of NEB-H, HCT respectively, A_1 is absorbance of sample at 275 nm, a_{x1} is the absorptivity of NEB-H at 275 nm, Q_x was obtained using the equation (absorptivity of NEB-H at 282.5 nm)/ absorptivity of NEB-H at 275 nm, Q_y was obtained by using (absorptivity of HCT at 282.5 nm)/(absorptivity of HCT at 275 nm) and Q_M from (absorbance of sample at 282.5 nm)/(absorbance of sample at 275 nm).

For the selection of analytical wavelength in simultaneous equation method (method 2), the spectrum of NEB-H and HCT of method 1 was used and wavelength 271.5 nm (λ_{max} of HCT) and 282.5 nm (λ_{max} of NEB-H) were selected for the formation of the simultaneous equations. For calibration curves, stock solutions of NEB-H and HCT in the concentration of range

of 10-50 and 1-5 μg/mL, respectively. The absorbance of NEB-H and HCT were measured at 282.5 and 271.5 nm, calibration curves were plotted. The absoptivities of both the drugs at both the wavelengths were determined.

The absorbance and the absorptivity values at the particular wavelength were calculated and substituted in the following equation, to obtain the concentration.

$$C_{NEB\text{-}H} = (A_1a_{x2} - A_2a_{x1})/(a_{x2}a_{y1} - a_{x1}a_{y2})$$

$$C_{HCT} = (A_2a_{y1} - A_1a_{y2})/(a_{x2}a_{y1} - a_{x1}a_{y2})$$

where, $C_{NEB\text{-}H}$, C_{HCT} are concentration of NEB-H and HCT, respectively, A_1 is the absorbance of sample at 282.5 nm, A_2 is the absorbance of sample at 271.5 nm, a_{x1} is the absorptivity of NEB-H at 282.5 nm and a_{x2} is the absorptivity of NEB-H at 271.5 nm, a_{y1} is the absorptivity of HCT at 282.5 nm and a_{y2} is the absorptivity of HCT at 271.5 nm.

20 Tablets of brand Nebicard-H (Torrent Pharma), Nebilong-H (Micro labs) label claim 5 mg of NEB-H and 12.5 mg of HCT were weighed, average weight determined and finely powdered. Appropriate quantity of powder from each tablet equivalent to 12.5 mg of HCT was accurately weighed and following standard addition method, 120 mg of NEB-H was accurately weighed and added to achieve 10:1 ratio (NEB-H and HCT) shaken vigorously for 15 min and filtered. Necessary dilutions of filtrate were made with methanol to get final concentration 10 μg/mL of NEB-H and 1 μg/mL of HCT. Absorbance of this solution was measured at 282.5, 275 and 271.5 nm and values were substituted in the respective formulae (methods 1 and 2) to obtain concentration and performing recovery studies by standard addition method in which preanalyzed samples were taken and standard drug was added at three different level and the results are shown in Table-1.

The overlay spectra of both the drugs showed that the peaks are well resolved, thus satisfying the criteria for obtaining maximum precision, based on absorbance ratio[15]. The criteria being the ratios $(A_2/A_1)/(a_{x2}/a_{x1})$ and $(a_{y2}/a_{y1})/(A_2/A_1)$ should lie outside the range 0.1-2.0 for precise determination of (Y) and (X), respectively where A_1/A_2 represents the absorbance of mixture at λ_1 and λ_2, a_{x1} and a_{x2} denote absorptivities of (X) at λ_1 and λ_2 and a_{y1} and a_{y2} denote absorptivities of (Y) at λ_1 and λ_2, respectively. In this context, the above criterion was found to be satisfied for NEB-H (X) and HCT (Y). Where λ_1 (275 nm) and λ_2 (282.5 nm) for Q-absorbance method, λ_1 (282.5 nm) and λ_1 (271.5 nm) for simultaneous equation method.

Two wavelengths that could serve as isoabsorptive points were 271.5 and 307 nm as determined by evaluation of overlay spectra. By comparing absorptivity of both the drugs at these wavelengths 275 nm was found suitable for the analysis. Since both the drugs gave same absorptivity at this wavelength. Hence 275 and 282.5 nm were selected for Q-absorbance equation.

TABLE-1
SUMMARY OF VALIDATION PARAMETERS

Parameters	Method 1		Method 2	
	NEB-H	HCT	NEB-H	HCT
Linearity range (μg/mL)	10-50	1-5	10-50	1-5
Correlation coefficient (r)	1.0000[a] 0.9999[b]	0.9992[a] 0.9994[b]	1.0000[a] 0.9992[c]	0.9992[a] 0.9999[c]
Intercept	0.00038[a] 0.00043[b]	0.00233[a] 0.00719[b]	0.00038[a] 0.00914[c]	0.00233[a] 0.00466[c]
Slope	0.01813[a] 0.01304[b]	0.0580[a] 0.1091[b]	0.0181[a] 0.0091[c]	0.0580[a] 0.1274[c]
Standard deviation (SD)	0.3392[a] 0.2440[b]	0.1086[a] 0.2041[b]	0.3392[a] 0.1711[c]	0.1086[a] 0.2448[c]
Standard error (SE)	0.1385[a] 0.0996[b]	0.0443[a] 0.8334[b]	0.1385[a] 0.0698[c]	0.0443[a] 0.0999[c]
Limit of detection (LOD) (μg/mL)	0.0617[a] 0.0617[b]	0.0094[a] 0.0061[b]	0.0617[a] 0.0617[c]	0.0094[a] 0.0063[c]
Limit of quantification (LOQ) (μg/mL)	0.1870[a] 0.1871[b]	0.0187[a] 0.0187[b]	0.1870[a] 0.1871[c]	0.0187[a] 0.0192[c]
Coefficient of variation CV)	0.7477[a] 0.7474[b]	0.7370[a] 0.7295[b]	0.7477[a] 0.7231[c]	0.7370[a] 0.7390[c]
Intra day (% RSD)*	0.1066[a] 0.1477[b]	0.3390[a] 0.1756[b]	0.1066[a] 0.3472[c]	0.3390[a] 0.1490[c]
Inter day (% RSD)*	0.1092[a] 0.1507[b]	0.6172[a] 0.4924[b]	0.1092[a] 0.7326[c]	0.6172[a] 0.4060[c]
Repeatability (% RSD)**	0.1426[a] 0.1365[b]	0.5520[a] 0.4402[b]	0.1426[a] 0.6553[c]	0.5520[a] 0.3630[c]
% Recovery (std. mixture)	100.15	98.72	102.83	99.44

Method 1 = Q-absorbance method; Method 2 = Simultaneous equation method, a = 282.5 nm, b = 275 nm, c = 271.5 nm.
*Mean of three measurements. **Mean of six measurements.

In simultaneous equation method two wavelengths *i.e.* λ_{max} of both the drugs were required, the spectra of HCT showed three distinct peaks one at around 226, 271.5 and 317 nm. The 271.5 nm was selected for analysis of HCT. The λ_{max} of NEB-H was 282.5 nm, which was used for the estimation.

RESULTS AND DISCUSSION

The proposed methods were successfully used to estimate the amount of nebivolol hydrochloride (NEB-H) and hydrochlorothiazide (HCT) present in two of the marketed tablet formulations. The assay values for both the tablets were comparable with corresponding labeled amounts and the validation parameters of proposed methods are summarized in Table-2. The result of analysis has been validated statistically and by recovery studies with standard

deviation < 1.0 % was found. On observing the validation parameters both the methods were found to be sensitive, precise, accurate, specific and reproducible and can be used for the routine simultaneous estimation of nebivolol hydrochloride and hydrochlorothiazide in formulations.

TABLE-2
ANALYSIS OF COMMERCIAL FORMULATIONS

Formulations	Method 1		Method 2	
	NEB-H	HCT	NEB-H	HCT
Nebicard-H (I)				
% Recovery	99.7100	99.4100	100.2000	98.9700
Standard deviation (SD)	0.2759	0.1693	0.2772	0.1996
Standard error (SE)	0.1593	0.0977	0.1061	0.1152
Coefficient of variation (CV)	0.1593	0.9137	0.9150	0.9597
Nebilong-H (II)				
% Recovery	100.0100	100.2500	100.7500	99.7500
Standard deviation (SD)	0.1825	0.1120	0.1825	0.1320
Standard error (SE)	0.1054	0.0646	0.1054	0.0767
Coefficient of variation (CV)	1.0000	0.9857	0.9973	1.0020

Method 1 = Q-absorbance method; Method 2 = simultaneous equation method.

REFERENCES

1. S. Budavari, The Merck index, Merck and Co., Inc., Whitehouse Station, NJ, edn. 12, p. 1103 (1994).
2. S. Budavari, The Merck index, Merck and Co., Inc., Whitehouse Station, NJ, edn. 12, p. 818 (1994).
3. W.J. Bachman and J.T. Stewort, *J. Chromatogr. Sci.*, **28**, 123 (1990).
4. F. Decross, W. Vanden Bossche and P.De Moerioose, *Chromatographia*, **20**, 477 (1985).
5. Z. Smahi, J.P. Huvenne and M. Traisnel, *Ann. Pharm. Fr.*, **50**, 167 (1992).
6. M. Lu, G. Wu, S. Chen and Z. Ruan, *Nanjing Yao Xueyuan Xuebo*, **1**, 1 (1983) (In Chinese).
7. O.D. Chandwani, P.P. Dahibhate, S.S. Kadam and S.R. Dhaneswar, *Indian Drugs*, **33**, 401 (1996).
8. Pharmacopoeia of India, The Controller of Publications, New Delhi, Vol. 1, pp. 370-371 (1996).
9. R. Woestenborghs, L. Embrechts and J. Heykants, in eds.: J.E. Reid and J.P. Leppard, HPLC Fluorescence Method for the Determination of New β1-adrenoceptor blocking agent nebivolol in Human Plasma, in Methodological Surveys in Biochemistry and Analysis, Plenum Press, Vol. 18, pp. 215-216 (1988).
10. K.R. Rajeswari, G.G. Sankar, A.L. Rao, D.B. Raju and J.V.L.N.S. Rao, *Asian J. Chem.*, **17**, 1259 (2005).
11. H.Y. Aboul-Enein and I. Ali, *Pharmazie*, **56**, 214 (2001).
12. H.Y. Aboul-Enein, *Biomed. Chromatogr.*, **17**, 113 (2003).
13. M. Thevis, G. Opfermann and W. Schanzer, *Biomed. Chromatogr.*, **15**, 393 (2001).
14. N.V.S. Ramakrishna, K.N. Vishwottam, M. Koteshwara, S. Manoj, M. Santosh and D.P. Varma, *J. Pharm. Biomed. Anal.*, **39**, 1006 (2005).
15. A.G. Davidson, A.H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th Edn, Part-II, CBS Publisher, New Delhi, India, p. 275 (1997).

(*Received*: 6 June 2007; *Accepted*: 15 April 2008) AJC-6520