APTI ijper

Simultaneous Estimation of Nebivolol Hydrochloride and Valsartan and Nebivolol Hydrochloride and Hydrochlorothiazide in Pharmaceutical Formulations by UV Spectrophotometric Methods

S. N. Meyyanathan, Arunadevi S. Birajdar* and Bhojraj Suresh

Department of Pharmaceutical Analysis,
J.S.S. College of Pharmacy,Post Box No: 20, Rocklands, Ooty –643001, Tamilnadu.
Author for Correspondence: aruna_birajdar@rediffmail.com

Abstract

Two simple, precise and reproducible UV spectrophotometric methods, simultaneous equation method and Q-value analysis method, have been developed and validated for the simultaneous estimation of nebivolol hydrochloride and valsartan used as cardiovascular drugs available in capsule dosage form and nebivolol hydrochloride with hydrochlorothiazide used as antihistaminic H_1 blocker available in tablet dosage form. The methods are based on the measurement of absorbance of nebivolol hydrochloride and hydrochlorothiazide at 270.4 nm, 280.2nm and 270 nm and measurement of absorbance of nebivolol Hcl and valsartan at 246.6 nm, 280.2 nm and 275 nm respectively. These methods obeyed Beer's law in the concentration range of 0.5 - 2.5 µg /ml for nebivolol Hcl, 1.0 – 20 µg /ml for valsartan and 1.0–3.0 µg /ml for hydrochlorothiazide. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed methods. These methods were successfully applied to the determination of these drugs in pharmaceutical dosage forms.

Keywords : *Nebivolol hydrochloride; Valsartan; Hydrochlorothiazide; Simultaneous determination; Q-Analysis method; UV Spectrophotometry.*

INTRODUCTION

Nebivolol hydrochloride (NEB) is (±) [2R* R* R* (S *)] œ, œ [imino bis (methylene)] bis- [6- fluoro - 3,4 - dihydro - 2H - 1 - benzopyran - 2 - methanol] hydrochloride is an antihypertensive drug, It is a racemate of two enantiomers with four chiral centres. The SRRR-enantiomer (d-nebivolol) is a potent and cardio selective 1-adrenergic blocker. The RSSS-enantiomer (nebivolol) has a favourable hemodynamic profile[1-3]. Valsartan (VAL) (N-valeryl-N[[2-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl] valine, is an orally active, potent and specific competitive angiotensin II antagonist acting at the ATI receptor, which mediates all known effects of angiotensin II on the cardiovascular system. Valsartan is widely used in the treatment of hypertension[1,4]. Hydrochlorothiazide (HCT) is 6-chloro-3, 4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide one of the popular thiazide diuretics. HCT is often used in the treatment of hypertension, congestive heart failure, symptomatic edema and the prevention of kidney stones [1,5].

Indian Journal of Pharmaceutical Education and Research
Received on 20/8/2008 ; Modified on 18/8/2009
Accepted on 20/12/2009

Literature survey reveals that a few methods have been reported for estimation of Valsartan, Nebivolol hydrochloride individually[6,7] as well as for the combination of Valsartan and Hydrochlorothiazide with other drugs were reported[8,9]. The present paper describes two methods of two different combinations, NEB with VAS and NEB with HCT. To the best of our knowledge, no study has been described for the simultaneous determination of both combinations in pharmaceutical formulations by UV spectrophotometric methods. Therefore it is desirable to develop a simple and reproducible analytical methods.

MATERIALS AND METHODS

Materials

Pharmaceutical grade NEB from Cadila pharmaceutical Ltd. Ankleshwer, VAS from Hetero Labs. Hyderabad and HCT from Franco-Indian Pharma. Mumbai, India was used. All analytical grade chemicals and solvents were supplied by Merck, India.

Equipment

The Shimadzu UV-Visible Spectrophotometer-160A with data processing system was used. The sample solution was recorded in 1cm quartz cells against solvent blank over the range 200-400 nm. The optimal conditions

for recording the spectra to achieve good reproducibility included scan speed at 60nm s^{-1}, slit width at 2 nm.

PROCEDURE

Standard solutions and calibration curves

Stock solutions for spectrophotometric measurements were prepared by dissolving NEB, VAS and HCT in methanol to obtain concentration of 1 mg /ml for each compound. For calibration, series of above solutions were prepared containing NEB 0.5, 1.0, 1.5, 2.0, 2.5 µg /ml, VAS 1.0, 4.0, 8.0, 12.0, 16.0, 20.0 µg /ml and HCT 1.0, 1.5, 2.0, 2.5, 3.0 µg /ml by diluting the stock standard solution with methanol in standard volumetric flasks (10ml). The dilutions were scanned in the wavelength range of 200-400 nm. Two wavelengths selected for each formulation for the use of simultaneous equation were 280.2 & 246.6 nm for NEB with VAS combination and 280.2 & 270.4 nm for NEB with HCT combination as shown in Figure 1. and Figure 2. respectively. (A1%, 1cm) Є was calculated for each standard drug by measuring absorbance of 1% solution at 1cm path length. Similarly, mixed standard solutions were used for UV-spectrometric analysis by Simultaneous equation and Q-analysis Methods.

Simultaneous equation method

Twenty capsules were weighed, powdered and weighed accurately equivalent to 5 mg of NEB and 80 mg VAS. The twenty tablets were weighed, powdered and weighed equivalent to 5 mg NEB and 12.5 mg HCT were transferred to a 100 ml volumetric flask separately, dissolved in 50ml of methanol by ultrasonication for 20 min. The solutions were diluted with the same solvent, filtered through Whatmann No. 41 filter paper. The filtrate was diluted with methanol to get final dilution 1.0 µg/ml and 16.0 µg/ml of NEB and VAS or 1.0 µg/ml and 2.5 µg/ml NEB and HCT respectively.Absorbance of these solutions were measured at selected wavelengths as A_1 and A_2 and concentrations of the two drugs in each sample were calculated by using following equations. The method employed solving of simultaneous equations using Cramer's rule and matrices.

The simultaneous equations NEB and VAS analysis were

$A_1 = Є_{1v} \times C_1 + Є_{1n} \times C_2$ ..... (1)

$A_2 = Є_{2v} \times C_1 + Є_{2n} \times C_2$ ..... (2)

The simultaneous equations NEB and HCT analysis were

$A_1 = Є_{1v} \times C_1 + Є_{1H} \times C_2$ ..... (1)

$A_2 = Є_{2v} \times C_1 + Є_{2H} \times C_2$ ..... (2).

$Є_{1n}$ and $Є_{2\ n}$ absorptivity values of NEB at 280.2 nm wavelength

$Є_{1v}$and $Є_{2\ v}$ absorptivity values of VAS at 246.6 nm wavelength

$Є_{1H}$and $Є_{2\ H}$ absorptivity values of HCT at 270.4 nm wavelength

C_1 and C_2 concentrations of VAS and NEB respectively in sample solution.

Q value analysis method

From the overlain spectrum shown in Figure 1 for VAS and NEB two wavelengths were selected one at 275 nm as isoabsorptive point for both the drugs and at 246.6 nm. Similarly from the overlain spectrum shown in Figure 2 for HCT and NEB two wavelengths were selected one at 270 nm as isoabsorptive point for both the drugs and at 280.2 nm. The absorbance of the standard and sample solutions were prepared and measured in the same manner as in the previous method. The absorptivity values for both standard drugs at the selected wavelength obtained for simultaneous equation method were employed for determination of Q values and another wavelength used was isoabsorptivite point for each combination. The concentrations of drugs in sample solution were determined by using the following formula.

For estimation of VAS

$$C_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{A}{a_1} \qquad Q_0 = \frac{\text{Absorptivity of sample at 246.6 nm}}{\text{Absorptivity of sample at 275 nm}}$$

For estimation of NEB

$$C_2 = \frac{Q_0 - Q_1}{Q_2 - Q_1} \times \frac{A}{a_2} \qquad Q_1 = \frac{\text{Absorptivity of VAS at 246.6 nm}}{\text{Absorptivity of VAS at 275 nm}}$$

$$Q_2 = \frac{\text{Absorptivity of NEB at 246.6 nm}}{\text{Absorptivity of NEB at 275 nm}}$$

A = Absorbance of sample at isoabsorptive point

a_1 and a_2 = Absorptivities values of VAS and NEB respectively at iso-absorptivite point

For estimation of HCT

$$C_1 = \frac{Q_0 - Q_2}{Q_1 - Q_2} \times \frac{A}{a_1} \qquad Q_0 = \frac{\text{Absorptivity of sample at 280.2 nm}}{\text{Absorptivity of sample at 270 nm}}$$

For estimation of NEB

$$C_2 = \frac{Q_0 - Q_1}{Q_2 - Q_1} \times \frac{A}{a_2} \qquad Q_1 = \frac{\text{Absorptivity of HCT at 280.2 nm}}{\text{Absorptivity of HCT at 270 nm}}$$

$$Q_2 = \frac{\text{Absorptivity of NEB at 280.2 nm}}{\text{Absorptivity of NEB at 270 nm}}$$

A = Absorbance of sample at isoabsorptive point.

a_1 and a_2 = Absorptivities values of HCT and NEB respectively at iso-absorptivite point.

RESULTS AND DISCUSSION

The NEB, VAS and HCT were sparingly soluble in water so methanol was used as solvent for all standard and sample solution preparation. UV absorption spectrum exhibited maximum absorbance for NEB, VAS and HCT at 280.2, 246.6 and at 270.4 respectively. Standard 1% solutions were prepared and measured absorbance at both the wavelengths of each respective content of formulations for calculating ϵ_{1v}, ϵ_{2v} and ϵ_{1n} , ϵ_{2n} and $\epsilon_{1H,}$ ϵ_{2H} values. The absorbance of sample solutions was noted at the respective wavelengths for both formulations.

Similarly by calculating Q-values for each drug and then put these values in formula and determine content of each drug in formulation. Statistical parameters obtained were reported in Table 1. Recovery studies carried out for both the methods by spiking standard drug in the powdered formulations 80%, 100%, 120% amount of each dosage content as per ICH guidelines. The results of the recovery analysis are reported in Table 2.

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 12 h at room temperature. The retention time and peak area of NEB and VAS remained almost similar (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 12 h , which was sufficient time to complete the whole analytical process.

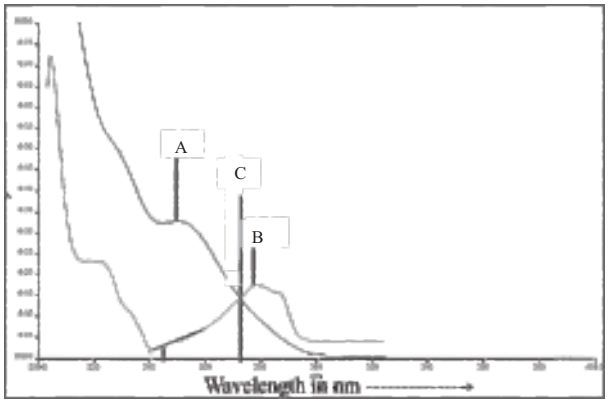
CONCLUSION

The proposed two spectrophotometeric methods assured required precision and accuracy. Both the methods were found to be simple, accurate, economical, reproducible and rapid. Hence, it can be employed for routine analysis.

ACKNOWLEDGEMENT

The authors are thankful to Cadila pharmaceutical Ltd. Ankleshwer, Hetero Labs.Hydrabad and Franco-Indian Pharma, Mumbai for generous gift samples of Pharmaceutical grade Nebivolol Hcl, Valsartan and Hydrochlorothizide respectively.The authors also thank Mr. Supe (Drug Inspector), Miss Krishnveni N., Mr. S. Rajan Mr. S. Murlidharan (CADRAT) for their co-operation. The authors are grateful to "His Holiness Jagadguru Sri Sri Shivarathree Deshikendra Mahaswamigalavaru" of Sri Suttur Mutt, Mysore and AICTE (QIP) cell for providing facilities to carry out this work.

Fig. 1: Overlain Spectra for Valsartan and Nebivolol hydrochloride

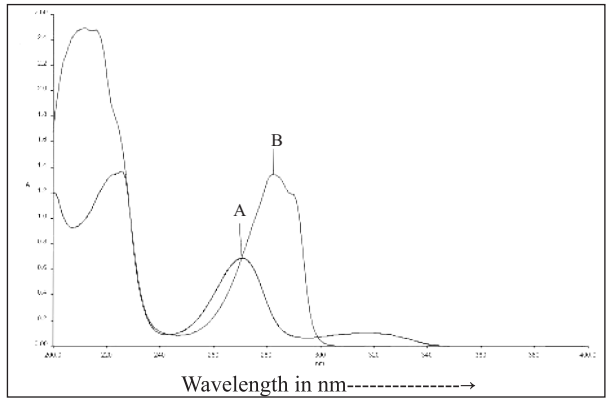


A: - Absorbance of Valsartan

B: - Absorbance of Nebivolol hydrochloride

C: - Absorbance at iosabsorptive point

Fig. 2: Overlain Spectra for Hydrochlorothiazideand Nebivolol hydrochloride



A: - Absorbance of Hydrochlorothiazide

B:- Absorbance of Nebivolol hydrochloride

Table No.1- Statistical parameters

Parameters	UV-Simulation equation method			
	NEB	VAS	NEB	HCT
Precision (%RSD)	1.48	1.50	1.24	1.10 %
coefficient of variation (R.S.D.)	1.49	2.63	1.25	2.12
Standard error	0.40	0.71	0.34	0.57
Correlation co-efficient®	0.998	0.999	0.998	0.999
Linearity Range (µg/ml)	0.5 - 2.5	1.0 - 20	0.5 - 2.5	1.0 - 3.0
Accuracy (%)	101.2	99	99.8	100.2

NEB - Nebivolol hydrochloride, VAS - Valsartan, HCT - Hydrochlorothiazide

Table No.2- Analysis of Tablet formulations

Method	Formulation	Label claim mg /dose		Amount found mg /dose		%Recovery ± SD*
Simultaneous	T_1	5	80	5.10	80.2	100.21±1.48
Equation Method	T_2	5	12.5	4.99	12.5	98.26±2.50
Q-Value	T_1	5	80	5.12	80.5	101.20±1.24
analysis Method	T_2	5	12.5	5.01	11.9	99.85±2.10

T_1 = Capsule formulation containing NEB 5 mg and VAS 80 mg per dosage.
T_2 = Tablet formulation containing NEB 5 mg and HCT 12.5 mg per dosage.
** = Average of 6 determinations*

REFERENCES

1. The Merck Index, Thirteenth edition, Merck Res. Lab. Division of Merck and Co. Inc, Whitehouse station, NJ 2001;1152,1767.
2. Aboul-Enein HY, Ali I. HPLC enantiomeric resolution of nebivolol on normal and reversed amylose based chiral phases. Pharmazie 2001; 56(3): 214-6.
3. Bundkirchen A, Brixius K, Bölck B, Nguyen Q , Schwinger RH. Beta 1-adrenoceptor selectivity of nebivolol and bisoprolol. A comparison of [3H] CGP 12.177 and [125I] iodocyanopindolol binding studies. Eur. J. Pharmacol. 2003; 460(1): 19-26.
4. Nozomu Koseki , Hiroto Kawashita, Hisanori Hara, Miyuki Niina, Makoto Tanaka, Ryosei Kawai, Yusuke Nagae, Naoki Masuda. Development and validation of a method for quantitative determination of valsartan in human plasma by liquid chromatography-tandem mass spectrometry . J. Pharm. Biomed. Anal. 2007; 43: 1769–74.
5. Hao Li, Yingwu Wang, Yao Jiang, Yunbiao Tang, Jiang Wang, Limei Zhao and Jingkai Gu. A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of valsartan and hydrochlorothiazide in human plasma. J Chromatogr B. 2007; 852 (1-2): 436-42.
6. Sevgi Tatar, Serap Sag?lık. Comparison of UV- and secondderivative-spectrophotometric and LC methods for the determination of valsartan in pharmaceutical formulation, J Pharm Biomed Anal 2002; 30: 371–75.
7. Ramakrishna NV, Vishwottam KN, Koteshwara M, Manoj S, Santosh M, Varma DP. Rapid quantification of nebivolol in human plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. J Pharm Biomed Anal 2005; 39(5):1006-13.
8. Eda S. atana , S¸adi Altınay , Nilgu¨n Gu¨nden Go¨g˘er , Sibel A. O_ zkan b, Zu¨hre S¸entu¨rk .Simultaneous determination of valsartan and hydrochlorothiazide in tablets by first-derivative ultraviolet spectrophotometry and LC. J Pharm Biomed Anal 2001; 25: 1009-13.
9. Patel LJ, Suhagia BN, Shah PB , Shah RR. Simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet dosage form by RP-HPLC method. Indian J Pharm Sci 2006; 68 (5): 635-38.