Asian Journal of Chemistry Vol. 20, No. 2 (2008), 1648-1650

NOTE

Simultaneous Estimation of Nebivolol Hydrochloride and Hydrochlorothiazide in Tablets

B. DHANDAPANI*, K. SURESH KUMAR, J. DHARUMAN and K. GEETHA
Department of Pharmaceutical Analysis, K.M.C.H. College of Pharmacy
Kovai Estate, Kalapatti Road, Coimbatore-641 035, India
E-mail: dhandapanirx@gmail.com

A simple reverse phase liquid chromatographic method has been developed and validated for simultaneous determination of nebivolol hydrochloride and hydrochlorothiazide in combination. The separation was carried out using a mobile phase consisting of 0.4 % (v/v) triethylamine buffer of pH 3.0 and acetonitrile in the ratio of 70:30 (v/v). The column used was phenomenex C-18 with flow rate of 1.4 mL/min using PDA detection at 282 nm. The described method was linear over a concentration range of 5-25 and 12.5-62.5 µg/mL for the assay of nebivolol hydrochloride and hydrochlorothiazide. Atorvastatin (10 µg/mL) was used as internal standard. Results of analysis were validated statistically and by recovery studies.

Key Words: Nebivolol hydrochloride, Hydrochlorothiazide, Atorvastatin.

Nebivolol hydrochloride[1] (NEB-H) is a benzopyran antihypertensive drug (β_1 blocker) and chemically it is a α,α'-[iminobis(methylene)]bis[6-flouro-3,4,-dihydro-2H-1-benzopyran-2-methanol hydrochloride. Reports are available for estimation of NEB-H by HPLC[2-5] and other methods. Hydrochlorothiazide[1] (HCT) is a 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide which is used as a diuretics. Hydrochlorothiazide is official in IP, BP, USP and EP. Several methods[6-9] such as HPLC, HPTLC, spectrophotometry and non-aqueous potentiometric titration are reported. The combination of nebivolol hydrochloride (NEB-H) and hydrochlorothiazide is newly introduced in market and used in the treatment of hypertension. This paper describes validated RP-HPLC for simultaneous estimation of NEB-H and HCT in combination using 0.4 % triethylamine buffer of pH 3.0 and acetonitrile in the ratio of 70: 30 % (v/v). The column used was chromosil C-18 with flow rate of 1.4 mL/min using PDA detection at 282 nm.

Standard bulk drug sample of nebivolol hydrochloride, hydrochlorothiazide and atorvastatin were provided by Micro Lab. Ltd., Bangalore.

Tablets of combined dosage form were procured from the local market. All other reagents used were of HPLC grade. HPLC (Shimadzu LC-20AT) method was developed using phenomenex C_{18} ODS C_{18} column (250 × 4.6 mm i.d, 0.5 µ). Mobile phase selected for this method contained 70 parts of 0.4 % (v/v) triethylamine buffer (0.4 mL/100 mL) and 30 parts of acetonitrile adjusted to pH 3 with 0.1 % orthophosphoric acid that was filtered through 0.45 µ membrane filter. Flow rate employed was 1.0 mL/min. Detection of eluent was carried out at 282 nm using PDA detector. Method was developed using atorvastatin (ATR) as internal standard. Standard stock solutions of pure drugs were made separately in mobile phase containing 5-25 µg/mL of nebivolol hydrochloride, 12.5-62.5 µg/mL of hydrochlorothiazide and 10 µg/mL of atorvastatin and filtered through a 0.45 µ membrane filter. Each solution was injected and a chromatogram was recorded. Mean retention time for nebivolol hydrochloride 2.3 min, for hydrochlorothiazide 3.1 min and 5.3 min for atorvastatin.

Analysis of formulation: 20 Tablets of the formulation were weighed and the average weight per tablet was calculated. 20 Tablets were crushed and ground to a fine powder. Powder equivalent to 12.5 mg of hydrochlorothiazide was weighed and transferred to a 100 mL volumetric flask. The tablet powder was dissolved in the mobile phase and filtered through a membrane filter (0.45 µ). The sample solution was suitably diluted and used for the analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20 µL fixed-sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated six times and the peak areas were recorded. The peak area ratios of each of the drugs to the internal standard were calculated and the amount of each drug present per tablet was estimated from the respective calibration curves. The result of analysis reported in Table-1. The stability of the sample in mobile phase was analyzed after 24 h, it was found no change in analytical parameters[10].

TABLE-1
ANALYSIS OF FORMULATION

Sample	Amount (mg/tablet)		Label claim (%)	RSD* (%)
	Labeled	Estimated		
Nebivolol	5	4.98	99.6	0.2168
Hydrochlorothiazide	12.5	12.52	100.16	0.1987

*Each value is a mean of six observations.

Recovery studies: To study the accuracy, reproducibility and precision of the above methods, were carried out by addition of standard drug solution to pre-analyzed sample at different levels. Results of recovery studies and other parameters were found to be satisfactory and are reported in Table-2.

TABLE-2

Validation parameters	NEB-H	HCT
Linearity range (μg/mL)	5-25	12.5-62.5
R	0.9989	0.9992
LOD (ng/mL)	5	10
LOQ (ng/mL)	25	50
Intra day (% RSD)*	0.4575	0.6373
Inter day (% RSD)*	0.6727	0.6453
Repeatability (% RSD)*	0.3447	0.4820
Accuracy (%)	99-101	100-101
Peak purity index	1.0000	1.0000
Resolution factor (R_s)	5.418	-
Asymmetry factor (A_s)	0.95	
No. of theoretical plates (N)	6952	6671
Capacity factor (k')	0.330	-
High equivalent to theoretical plates (HETP)	21.575	22.482
Tailing factor	1.327	1.423
Selectivity factor (α)	3.639	

* Each value is a mean of six observations.

The developed RP-HPLC method for simultaneous estimation of two drugs from combined dosage form utilising C_{18} column and 0.4 % triethylamine and acetonitrile as mobile phase. Detection of eluent was carried out using PDA detector at 282 nm. The method was developed using atorvastatin as internal standard. The run time per sample is less than 6 min. The excipients in the formulation did not interfere in the accurate estimation of nebivolol hydrochloride and hydrochlorothiazide. Since none of the methods is reported for simultaneous estimation of nebivolol hydrochloride and hydrochlorothiazide from combined dosage form, this developed method can be used for routine analysis of two components in formulation.

REFERENCES

1. S. Budavari, The Merck Index, Merck and Co., Inc., Whitehouse Station, NJ, edn. 12, p. 1103, 818 (1994).
2. H.Y. Aboul-Enein, *Pharmazie*, **56**, 626 (2001).
3. K.R. Rajeswari, G.G. Sankar, A.L. Rao, D.B. Raju and J.V.L.N. Seshagiri Rao, *Asian. J. Chem.*, **17**, 1259 (2005).
4. N.V.S. Ramakrishna, K.N. Vishwottam, M. Koteshwara, S. Manoj, M. Santosh and D.P. Varma, *J. Pharm. Biomed. Anal.*, **39**, 1006 (2005).
5. M. Thevis, G. Opfermann and W. Schanzer, *Biomed. Chromatogr.*, **14**, 393 (2001).
6. A.P. Argekar and J.G. Sawant, *Anal. Lett.*, **33**, 869 (2000).
7. B.N. Suhagia, R.R. Shah and D.M. Patel, *Indian J. Pharma. Sci.*, **67**, 37 (2005).
8. S. Erram and H.P. Tipnis, *Indian J. Pharm. Sci.*, **54**, 245 (1992).
9. S.S. Zarapkar and S.H. Rane, *Indian Drugs*, **37**, 589 (2000).
10. J.D. Carstensen, Drug Stability, Marcel Dekker, New York, edn. 2 (1990).

(*Received*: 2 March 2007; *Accepted*: 15 October 2007) AJC-6022