Simultaneous Estimation of Nebivolol hydrochloride and Amlodipine besylate by UV Spectrophotometric Method

Priyanka B.Patil*, Chetan B.Chavan, Dev A.Jagtap,

S.K. Mohite, C. S. Magdum

Rajarambapu College Of Pharmacy,Kasegaon ,Tal. Walawa, Dist.Sangali,MH,India.

*Corres. Author: patil.priyanka188@gmail.com

Abstract: A new, simple, rapid and novel spectrophotometric method has been developed for simultaneous estimation of Nebivolol hydrochloride and Amlodipine besylate. For this, simultaneous equation method is used. The method involved measurement of absorbance at two wavelengths, 280 nm and 239 nm, max of Nebivolol hydrochloride and Amlodipine besylate respectively. Beer's law obeyed in concentration range of 10-90 µg/ mL and 10- 45 µg/ mL for Nebivolol hydrochloride and Amlodipine besylate respectively. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific by no heating and no organic solvent extraction. This paper describes the development and validation of UV spectroscopic method for Simultaneous estimation of Nebivolol hydrochloride and Amlodipine besylate in combined solid dosage form.
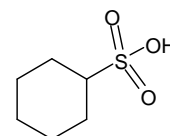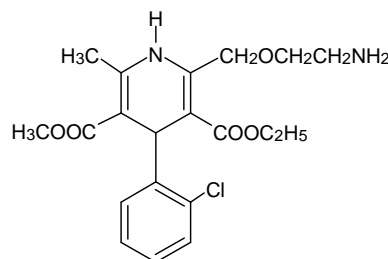**Key words:** Nebivolol hydrochloride, Amlodipine besylate, max, Simultaneous equation method.

INTRODUCTION:

Amlodipine besylate is a calcium channel blocker, chemically it is [3-ethyl-5-methyl (4RS)-2-[(2-aminoethoxy) methyl] -4- (2-chlorophenyl)-methyl-1-dihydropyridine-3, 5- dicarboxylate benzene sulfonate [1]. Amlodipine besylate is a dihydropyridine calcium channel blocker. Amlodipine besylate is a calcium antagonist that inhibits the transmembrane influx of calcium ions into vascular smooth muscles and cardiac muscles, which in turn affects their contractile process and results in reduced blood pressure [2].
Amlodipine Besylate is a peripheral arterial vasodilator that acts directly on the vascular smooth muscle to cause a reduction in peripheral vascular resistance and in blood pressure. Amlodipine Besylate is official in the Indian Pharmacopoeia, British Pharmacopoeia, and European Pharmacopoeia. .

Fig Amlodipine Besylate

Nebivolol Hydrochloride , ' [Iminobis (methylene) bis [6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] (Merck Index, 1996) is a $_1$-Blocker (Anti-Hypertensive), reduces peripheral vascular resistance, and significantly increases stroke volume, with preservation of cardiac output. [3]
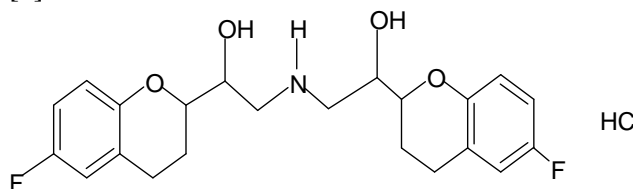
Fig. Nebivolol Hydrochloride

Many methods have been described in the literature for the determination of Nebivolol Hydrochloride and Amlodipine besylate individually and in combination with other drugs[5-16]. The proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific by no heating and no organic solvent extraction.

MATERIAL AND METHODS

Apparatus
Spectral runs were made on a Shimadzu UV-Visible spectrophotometer, model- 1800 was employed with spectral bandwidth of 0.5 nm and wavelength accuracy of ± 0.3 nm with automatic wavelength corrections with a pair of 10 mm quartz cells for all analytical work.

Reagents and chemicals
Nebivolol Hydrochloride and Amlodipine besylate were obtained from Dr. Reddy's Lab, Hyderabad, India as a gift sample and were used as working standards.
Methanol AR and double distilled water were used throughout the analysis. All the solutions were protected for light and were analyzed on the day of preparations.

Commercial formulation
A commercial pharmaceutical preparation of combination of and Nebivolol Hydrochloride and Amlodipine besylate that is AMLOPRESS-NB was purchased from the local market.

Selection of common solvent:
Methanol of analytical reagent grade was selected for stock solution as common solvent and further dilutions with double distilled water for developing spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

Preparation of Standard Solution:
10 mg each of pure AMB and NBH were weighed accurately and separately dissolved in methanol in a 10 ml volumetric flask and further diluted with the double distilled water, to get a 100 µg/ml solution.

Determining the Sampling Wavelength for Simultaneous Analysis:
By appropriate dilution of two standard drug solutions with methanol, solutions containing 10 µg/ml of NBH and 10 µg/ml of AMD were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both the drugs. NBH and AMD showed absorbance maxima at 280 nm (1) and 239 nm (2) respectively.

Selection of Method and Wavelength:
For estimation of NBH, simultaneous equation method employing 280 nm (1) as analytical wavelength was used. For estimation of AMD, 239 nm (2) was selected as the analytical wavelength.

Calibration curves
Seven standard dilutions of each drug were prepared separately having concentrations of 10-90 µg/mL. The absorbances of these standard solutions were measured at 280 nm and 239 nm and calibration curve was plotted. The absorptivity coefficients of the two drugs were determined using calibration curve.
NBH and AMD showed linearity with absorbance in the range of 10- 90 µg/ml and 10- 45 µg/ml at their respective maxima. Coefficients of correlation were found to be 0.9980 for NBH and 0.9970 for AMD. For simultaneous estimation of NBH and AMD, a series of standard solution were prepared by diluting appropriate volume of standard stock solutions.

Calibration curve for Amlodipine Besylate
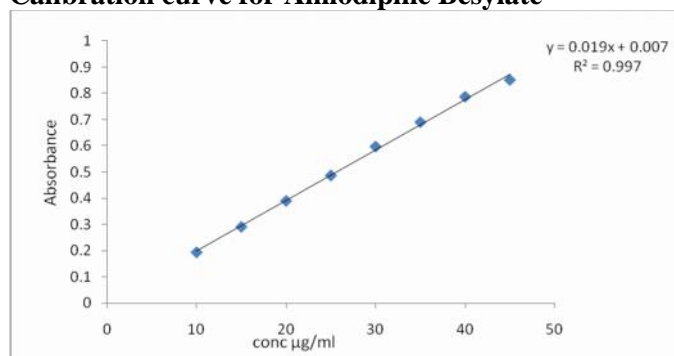
Calibration curve for Nebivolol Hydrochloride
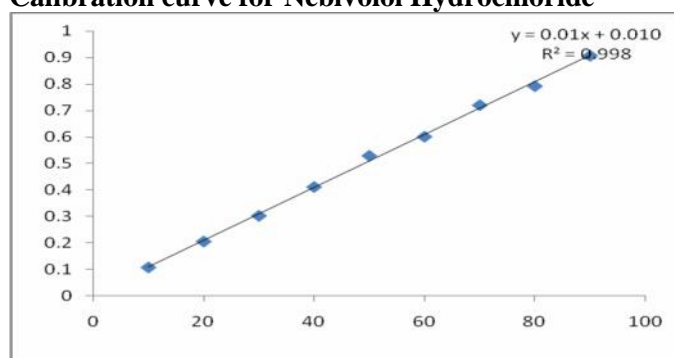
Preparation of standard solution

Sample solution containing both the drugs was prepared by dissolving 10 mg of each drug in 10mL volumetric flask using methanol to give stock solutions of 1000 μg/mL. From this stock solution, working standard solution of 10 μg/mL concentration was prepared by appropriate dilution with distilled water.The absorbance of this sample solution was measured at 280 nm and 239 nm and their concentrations were determined using proposed analytical methods.

Analysis of Marketed Formulation:

Marketed tablet formulation containing NBH 5mg and AMD 5 mg was analyzed using this method.

From the 20 tablets , an amount equivalent to 5 mg of NBH and 5 mg of AMD was weighed and dissolved in 40 mL of methanol and sonicated for 10 minutes. Then the solution was filtered through whatman filter paper no. 41 and then final volume of the solution was made up to 50 ml with methanol to get a stock solution containing 100μg/ml of NBH and AMD. Appropriate aliquots of NBH and AMD within the Beer's law limit were taken. The absorbance of resulting solutions was measured at 280 nm and 239 nm. The concentration of NBH and AMD present in the sample solution was calculated. The result of analysis of the tablet formulation is presented in table no. 3.

Table no.1 Slope, Intercept, Correlation coefficient of AMD

Slope	0.019
Intercept	0.007
Correlation coefficient	0.9970

Table 2: Slope, Intercept, Correlation coefficient of NBH

Slope	0.01
Intercept	0.010
Correlation coefficient	0.9980

Table 3: Results of analysis of tablet samples

Analyte	Label claim (mg/ tab)	% Label claim estimated* (Mean ± S.D.)
NBH	5	100.91 ± 0.3444
AMD	5	100.43 ± 0.5021

*Average of 6 determination, S.D. = Standard Deviation

Method validation

The method validation parameters like linearity, precision, accuracy, repeatability, limit of detection and limit of quantitation were checked as per ICH guidelines.

Linearity and range

The linearity for NBH and AMD were determined at seven concentration levels, ranging from 10-90μ/mL and 10- 45μ/mL using working standards. (Table 5)

2.12. Precision and accuracy

The precision of the method was evaluated by inter day and intra day variation studies. In intra day studies, working solutions of standard and sample were analyzed thrice in a day and percentage relative standard deviation (% RSD) was calculated. In the inter day variation studies, working solution of standard and sample were analyzed on three consecutive days and percentage relative standard deviation (% RSD) was calculated. The data is shown in table 5. The accuracy of the method was determined by recovery studies. The recovery studies were performed by the standard addition method at 80%, 100% and 120% level and the percentage recoveries were calculated and are shown in Table 5.

Limit of detection and limit of quantitation

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and result shown in Table 5.

LOD = 3.3 (/ S)

Where, S = slope of calibration curve, = standard deviation of the response. The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and result shown in Table 5.

LOQ = 10 (/ S)

Where, S = slope of calibration curve, = standard deviation of the response.

Recovery Studies:

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte. Recovery studies by spiking different concentrations of pure drug in the preanalyzed tablet samples within the analytical concentration range of the proposed method. The added quantities of the individual drugs were estimated by above method. The results of recovery studies were found to be satisfactory and the results are presented in table no. 4.

Table 4 Recovery study of NBH and AMD

Amt. of sample		Amt. of drug added		Percentage recovery	
NBH μg/ml	AMD μg/ml	NBH μg/ml	AMD μg/ml	NBH	AMD
5.0	5.0	4.0	4.0	100.27	99.99
5.0	5.0	5.0	5.0	99.91	100.23
5.0	5.0	6.0	6.0	99.89	100.01

**Table 5: Optical and Regression characteristics and validation parameters of method
For analysis of NBH and AMD**

Parameters	NBH	AMD
Linearity range (µg/ml)	10-90	10-45
Regression equation($y^* = mx + c$)	Y=0.01x+0.010	Y=0.019x-0.007
Slope	0.01	0.019
Intercept	0.010	0.007
Correlation Coefficient (r^2)	0.9980	0.9970
LOD (µg/ml)	0.35	0.22
LOQ (µg/ml)	1.07	0.68
Precision (RSD)		
Intra-day (n=3)	0.53	0.19
Inter-day (n=3)	0.55	0.35

Where, n=Number of determination, LOD=Limit of detection, LOQ= Limit of quantitation

RESULTS AND DISCUSSION:

The proposed method for simultaneous estimation of NBH and AMD in combined sample solutions was found to be simple, accurate and reproducible. Beer's law was obeyed in the concentration range of 10-90 µg/ml and 10- 45 µg/ml for Nebivolol Hydrochloride and Amlodipine besylate respectively. Coefficient of variation was found to be 0.9980 and 0.9970 for NBH and AMD, respectively. Once the equations are determined, analysis requires only the measuring of the absorbance of the sample solution at two wavelengths selected, followed by a few simple calculations.

CONCLUSION:

The most striking feature of this method is its simplicity and rapidity, non- requiring- consuming sample preparations such as extraction of solvents, heating, degassing, costly organic solvents which are needed for HPLC procedure. It is a new and novel method and can be employed for routine analysis in quality control analysis. The described method gives accurate and precise results for determination of Nebivolol Hydrochloride and Amlodipine besylate mixture in tablet.

REFERENCES:

1. Indian Pharmacopoeia, Vol.II, 2007, 96-98.
2. Tripathi K.D, Essentials of Medical Pharmacology, 6th ed, Jaypee Brothers, Medical publishers, New Delhi, 522-553.
3. Budavari S. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals.13th ed. Whitehouse Station (NJ): Merck Research Lab. Division of Merck 2001: 854.
4. Aboul-Enein H Y, Raluca-Ioana S, Jacobus F V. Analysis of several angiotensin-converting enzyme inhibitors using potentiometric, enantioselective membrane electrodes. Anal. Lett. 1999; 32: 623–632.
5. Bahrami G, and Mirzaeei SH, simple and rapid HPLC method for determination of Amlodipine in human serum with fluorescence detection and its use in pharmacokinetic studies. J Pharm Biomed Anal. 2004; 36: 163-168.
6. Chaudhari B and Patel NM. Development and Validation of HPLC method for simultaneous estimation of Atorvastatin Calcium and Amlodipine Besylate. J Pharm Research. 2006; 5:141-144.
7. Agrekar AP and Powar SG. Reverse phase High Performance Liquid Chromatographic determination of Ramipril and Amlodipine in tablets. J Pharm Biomed Anal. 2000; 21: 1137-1142.
8. Chaudhari BG, Patel NM and Sham PB. Stability Indicating RP-HPLC for simultaneous determination of Atorvastatin Calcium and Amlodipine Besylate from their combination

drug products. Chem Pharm Bull (Tokyo). 2007; 55: 241-246.

9. Pandya KK, et al. Detection and Determination of Total Amlodipine by high performance thin layer chromatography. J Chromatogr Biomed Appl., 1995; 667: 315- 320.

10. Meyyanthan SN and Suresh B. HPTLC method for the simultaneous determination of Amlodipine and Benazepril in their formulations. J Chrom. Sci., 2005; 43: 73- 75.

11. Dhandapani B, Thirumoorthy N. and Prakash D.J(2009) Development and Validation for the Simultaneous Quantification of Nebivolol Hydrochloride and Hydrochlorothiazide by UV Spectroscopy, RP-HPLC HPTLC in Tablets, ISSN:0973-4945; CODEN ECJHAOE- Journal of Chemistry. 2010; 7(2): 341-348.

12. SHAH K.V, Thumber B. L., Desai T. R. (2009) Simultaneous Estimation of Nebivolol Hydrochloride and Hydrochlorothiazide in Bulk and Tablet Dosage Form by Q-Analysis Method. International Journal of Pharmaceutical Science & Research. 2009; 1(2): 80-85.

13. Garg G.,Saraf S. and Saraf S. Development and validation of simultaneous estimation of enalapril maleate and amlodipine besylate in combined dosage forms. Trends Applied Sci. Res., 2008; 3: 278-284.

14. Kamble A.Y, Mahadik M.V., Khata L D. Dhaneshwar S.R. (2010) Validated HPLC and HPTLC Method for Simultaneous Quantitation of Amlodipine Besylate and Olmesartan Medoxomil in bulk drug and formulation, Analytical letters. 2010; 43(2): 251 – 258.

15. Vora D. N. and Kadav A. A. Development and Validation of a Simultaneous HPLC Method for Estimation of Bisoprolol Fumarate and Amlodipine Besylate from Tablets, Indian J Pharm Sci., 2008; 70(4): 542–546.

16. Ilango K, Kumar PB, Prasad VRV, Simple and rapid high performance thin layer chromato - graphy estimation of amlodipine from pharmaceutical dosage forms, Indian J. Pharm. Sci., 1997; 59(6):336-37.

*****