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Method Development and Validation for the Simultaneous Estimation of Nebivolol hydrochloride and Amlodipine besylate in Tablet Dosage Forms by RP-HPLC

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ABSTRACT

A simple, specific and precise high performance liquid chromatographic method was developed and validated for Nebivolol HCl and Amlodipine besylate in a combined tablet dosage form. Nebivolol hydrochloride, a long acting, cardioselective β-blocker and Amlodipine besylate, a calcium channel blocker are considered in combination for the treatment of hypertension. The chromatographic separation was performed using Eclipse XDB plus C_{18} Column (4.6 × 150 mm, 5µm particle size). The mobile phase consisted of combination of 0.01 M Ammonium acetate buffer (pH 4.5) and Acetonitrile in the ratio of 50:50 v/v at a flow rate of 0.8 ml/min with the detection wavelength at 265 nm. Both the drugs showed good linearity in the concentration range of 10-50µg/ml and 5-25µg/ml respectively with a correlation coefficient of 0.999 for both drugs. The retention time for Nebivolol HCl and Amlodipine besylate were found to be 3.553 and 2.970 min respectively. The developed analytical method was validated for linearity, precision, accuracy, ruggedness, robustness, specificity and system suitability, which were within the acceptance limits according to ICH guidelines. The developed method can successfully be employed for routine quality control of Nebivolol HCl and Amlodipine besylate in combined tablet dosage forms.

Keywords: HPLC, Hypertension, method development, validation.

INTRODUCTION

Nebivolol HCl is an antihypertensive, which is a competitive and selective β1 receptor antagonist. [1] It is chemically known as α, α^1 – [imino bis (methylene)] bis [6 – fluoro-3, 4-dihydro-2H-1-benzopyran-2-methanol]. Nebivolol HCl is commonly used for the management the
pharmacological activity is attributable to the d – enantiomer [Fig. 1].

Amlodipine besylate is chemically 2 – [(2-aminoethoxy) methyl] – 4–(2–chlorophenyl) – 1, 4 – dihydro – 6 – methyl – 3, 5 – pyridine carboxylic acid 3 – ethyl 5 – methyl ester. It is a dihydropyridine calcium antagonist used in the treatment of hypertension [Fig. 2]. It is a long acting calcium channel blocker, which inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. [2]

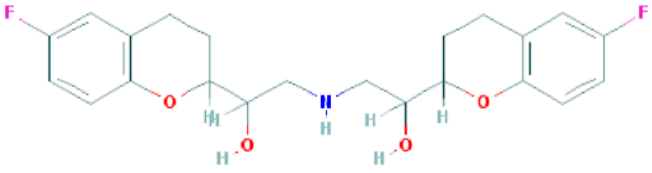

Fig. 1: Chemical structure of Nebivolol HCl

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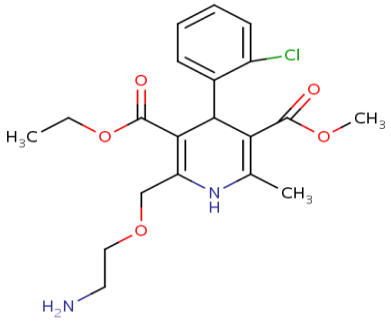

Fig. 2: Chemical structure of Amlodipine besylate

The literature survey revealed that several HPLC [1-8] and UV [9-14] methods have been reported for the simultaneous estimation of Nebivolol HCl and Amlodipine besylate in pharmaceutical dosage forms. However, HPLC is the most commonly used analytical method for the estimation of these drugs, either individually or in combination with other drugs.

The present work was intended to develop and validate a simple, sensitive and rapid analytical method with optimum chromatographic conditions for simultaneous quantification of Nebivolol HCl and Amlodipine besylate in pharmaceutical dosage forms. The developed method was validated as per ICH guidelines [15-17], and can be applied successfully in routine quality control of Nebivolol HCl and Amlodipine besylate in bulk and in combined dosage forms.

MATERIALS AND METHODS

Equipment

An Agilent model - 1220 Infinity LC – HPLC system having Agilent openLAB CDS (EZ Chrome) software "version A.04.05" equipped with a variable wavelength detector (VWD) and a manual injector was used. It was manufactured by Agilent Technologies, USA. An Eclipse XDB plus C_{18} Column (4.6 × 150 mm, 5µm particle size) was used for the analytical separation and quantification of the mixtures. An ELICO (LI 120) pH meter was used for adjusting the pH of the buffer.

Reagents and Chemicals

Pure samples of Nebivolol HCl and Amlodipine besylate were obtained from Micro Labs, Bangalore. The commercial tablet (NEBISTAR-SA) containing 5 mg of Nebivolol HCl and 2.5 mg of Amlodipine besylate was employed in the study.

HPLC grade acetonitrile and millipore water were purchased from Thermo Fisher Scientific India Pvt. Ltd., Mumbai. Analytical grade glacial acetic acid and ammonium acetate were purchased from Merck Speciailty, Mumbai and Finar chemicals Limited, Ahmedabad respectively.

Chromatographic Conditions

A freshly prepared 50:50 v/v mixture of 0.01 M Ammonium acetate buffer (pH 4.5) and acetonitrile was used as the mobile phase. Both ammonium acetate buffer and acetonitrile were filtered through a 0.45µ membrane filter and sonicated before use. The flow rate was adjusted to 0.8 ml/min, the injection volume was 20µL and the detection wavelength was 265 nm.

Preparation of Analytical Solutions

Preparation of Mobile Phase

A mixture of Acetonitrile and Ammonium acetate buffer was mixed in the ratio of 50:50 v/v. Then the resulting solution was filtered through 0.45µ nylon membrane filter, degassed and used as the mobile phase.

Preparation of buffer

0.77 g of ammonium acetate was accurately weighed and transferred to 1000 ml volumetric flask. It was dissolved by adding small quantities of distilled water and finally made up to 1000 ml with distilled water. The pH of the buffer was adjusted to 4.5 with glacial acetic acid, filtered through 0.45µ nylon membrane filter and finally degassed.

Nebivolol HCl standard solutions

Stock solution: 100 mg of Nebivolol HCl was accurately weighed and transferred to a 100 ml volumetric flask. 50 ml of mobile phase was added, sonicated for 15 minutes and cooled to room temperature. The solution was diluted and made up to 100 ml with mobile phase to get a 1000µg/ml solution.

Working standard solution: 10 ml of stock solution was diluted to 100 ml with the mobile phase to obtain a 100µg/ml solution.

Amlodipine besylate standard solutions

Stock solution: Amlodipine besylate equivalent to 50 mg of Amlodipine was accurately weighed and transferred to 100 ml volumetric flask. 50 ml of mobile phase was added, sonicated for 15 minutes and cooled to room temperature. The solution was diluted and made up to 100 ml with mobile phase to get a 500µg/ml solution.

Working standard solution: 10 ml of stock solution was diluted to 100 ml with the mobile phase to obtain a 50µg/ml solution.

Preparation of mixed standard stock solution

100 mg of Nebivolol HCl and 50 mg of Amlodipine besylate were weighed accurately into a 100 ml volumetric flask and dissolved in sufficient quantity of mobile phase and finally made up to 100 ml with the mobile phase.

Working standard solution: 10 ml of standard stock solution was taken into a 100 ml volumetric flask and diluted to 100 ml with mobile phase to get a concentration of 100µg/ml of Nebivolol HCl and 50µg/ml of Amlodipine besylate.

Calibration of the method

Standard stock solutions of Nebivolol HCl and Amlodipine besylate were diluted to get the working standard concentrations in the range of 10-50µg/ml and 5-25µg/ml respectively. Five replicates of each dilution were injected into the column and chromatograms were recorded. The corresponding calibration curve was constructed by plotting concentrations against the respective peak areas. The regression equation was obtained and the values of

slope-a, intercept-b, and correlation coefficient (R^2) were determined.

Preparation of sample solutions

20 tablets were weighed accurately and crushed to fine powder. Each tablet contained 5 mg of Nebivolol HCL and 2.5 mg of Amlodipine besylate. The quantity of powder containing the drugs equivalent to 25 mg of Nebivolol HCl and 12.5 mg of Amlodipine besylate was weighed and dissolved in sufficient quantity of mobile phase in a 25 ml volumetric flask and finally made up to volume with the mobile phase. The solution was filtered through 0.45µ nylon membrane filter. The amount of Nebivolol HCl and Amlodipine besylate present in tablet formulation was calculated by comparing the peak area of the standard with that of sample. The amount of drugs in tablet was calculated by using the formula given below:

$$\%\ \text{Assay} = \frac{\text{Sample Avg peak area}}{\text{Standard Avg peak area}} \times \frac{\text{Wt. of drug (mg)}}{\text{dilution of standard}} \times \frac{\text{dilution of tablet solution}}{\text{wt. of Sample}} \times \frac{\%\ \text{Purity}}{100} \times \frac{\text{Avg wt}}{\text{Lable Claim}} \times 100$$

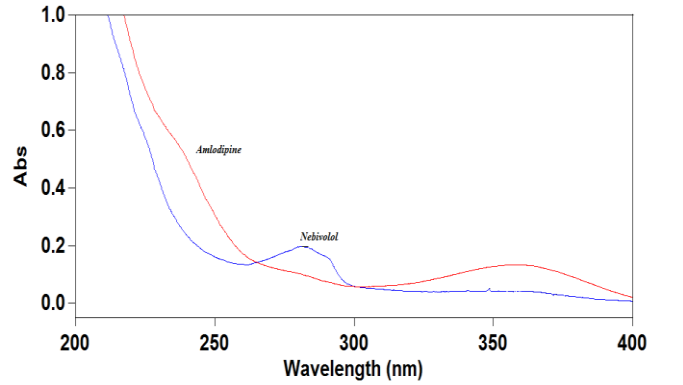

Fig 3: UV overlain absorption plot of Nebivolol HCl and Amlodipine besylate

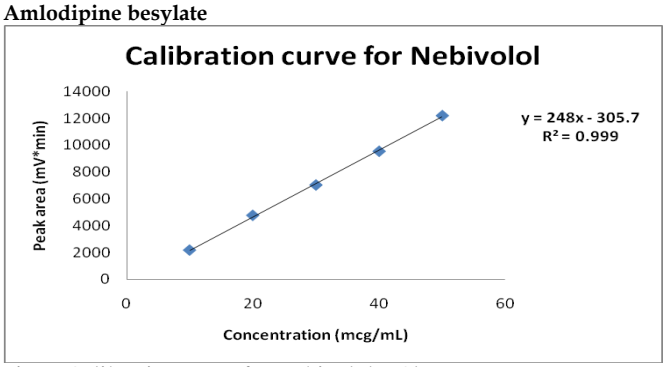

Fig. 4: Calibration curve for Nebivolol HCl

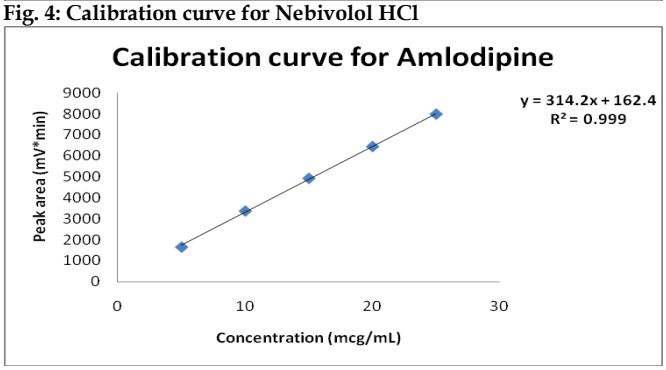

Fig. 5: Calibration curve for Amlodipine besylate

HPLC-Method Development and Validation

An analytical method for the estimation of Nebivolol HCl and Amlodipine besylate in tablets was developed and validated according to ICH guidelines. Analytical variable parameters such as linearity, precision, accuracy (percent recovery), specificity and system suitability were tested using the optimized chromatographic conditions and instruments.

Linearity

The calibration curves constructed by analyzing a series of concentrations of each drug ranging from 10-50µg/ml for Nebivolol HCl and 5-25µg/ml for Amlodipine besylate showed good linearity. The regression equations and correlation coefficients for Nebivolol HCl and Amlodipine besylate were found to be y = 248x-305.7; 0.999 and y = 314.2x+162.4; 0.999 respectively and the results were shown in Table 1 and Fig. 3, 4 & 5.

Accuracy (percent Recovery)

The accuracy study was performed on 80 %, 100 % and 120 % of the analytical method target concentrations of Nebivolol HCl and Amlodipine besylate. Standard and sample preparations were injected into HPLC system and three determinants for each concentration level were obtained. The percentage recoveries of Nebivolol HCL and Amlodipine besylate were calculated using standard at the same concentration at each concentration level as shown in Table 2.

Precision

System Precision: System precision was checked by injecting five replicate preparations of the standard drug solutions of Nebivolol HCl (20µg/ml) and Amlodipine besylate (10µg/ml). The corresponding peak areas were measured and % RSD calculated.

Method Precision: The method precision study was performed for five replicate sample preparations of marketed formulation containing Nebivolol HCl (20µg/ml) and Amlodipine besylate (10µg/ml). The corresponding peak areas were measured and % RSD calculated as exhibited in Table 3.

Robustness

Robustness of the developed analytical method was tested by evaluating the affect of small variations in analytical method parameters such as change in flow rate from 0.8 ml/min by ±0.1 ml/min, change in wavelength by ±5 nm and change in the mobile phase B ratio by ± 5 %. The chromatograms were recorded and the results are shown in Table 4.

Ruggedness

Ruggedness of the proposed method was determined by spiking five replicates of tablet formulation containing Nebivolol HCl (30µg/ml) and Amlodipine besylate (15µg/ml) prepared by another analyst into another HPLC system. The retention time and peak areas were obtained. The mean and % RSD were found to be within the acceptance criteria as shown in Table 5.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified (signal to noise ratio of 10). LOD and LOQ

were calculated as 3.3∂/S and 10∂/S, respectively as per ICH guidelines, where ∂ is the standard deviation of the response and S is the slope of the calibration plot. The results are presented in Table 1.

Table 1: Optimized chromatographic conditions of the proposed method

Parameter	Nebivolol HCl	Amlodipine besylate
Calibration range (µg/ml)	10-50	5-25
Retention time (min)	3.553	2.970
Slope (b)	258.79	343.43
Intercept (a)	-305.7	+162.4
LOD	0.20	0.07
LOQ	0.61	0.23
Correlation coefficient	0.999	0.999
Theoretical plates	3889	5108
Symmetry factor	0.98	1.09
Resolution	2.76	

Table 2: Recovery studies and Assay results of the proposed method

Drug	Amount of standard drug added to pre-analyzed formulation (µg/ml)	Amount recovered	Mean % recovery ± S.D (n=5)	% Assay
Nebivolol HCl	20	19.99	100.86±0.23	99.69
	30	30.11		
	40	40.91		
Amlodipine besylate	10	10.20	100.76±0.31	99.19
	20	20.08		
	30	29.98		

Table 3: System and Method Precision of the proposed method

Drug	Spiked level (µg/ml) (n=5)	System precision		Method precision	
		S.D	% R.S.D	S.D	% R.S.D
Nebivolol HCl	20	12.25	0.26	24.29	0.52
Amlodipine besylate	10	33.09	0.98	31.92	0.97

Table 4: Robustness of the proposed method

Parameter	Set values	RT (min)		Peak Area (mV*min)	
		Nebivolol HCl	Amlodipine besylate	Nebivolol HCl	Amlodipine besylate
Flow rate (mL/min)	0.7	3.545	2.949	4701.535	3315.725
	0.8	3.509	2.925	4752.220	3359.359
	0.9	3.512	2.950	4638.623	3346.260
Wavelength (nm)	260	3.540	2.959	4701.225	3360.520
	265	3.544	2.942	4758.730	3372.925
	270	3.510	2.935	4681.360	3325.026
Change in Mobile phase B	45 %	3.505	2.920	4618.757	3295.760
	50 %	3.503	2.955	4762.230	3361.325
	55 %	3.711	3.012	4629.352	3315.260

Table 5: Ruggedness of the proposed method

S. No	parameter	Nebivolol HCl	Amlodipine besylate
1	Analyst - 01	100.21% w/w	101.43% w/w
2	Analyst - 02	99.97% w/w	99.72% w/w
3	Acceptance criteria	90-110%w/w	90-110%w/w

Specificity

The specificity of the method was determined to check whether there is any interference due to presence of excipients, impurities or other components with the retention time of analytical peaks which may affect the specificity of the analytical method. The HPLC chromatograms were recorded for the drug-matrix (mixture of the drug and excipient) which showed almost no interfering peaks within retention time ranges.

System suitability

Five replicates of working mixed standard solution were injected and the parameters like theoretical plate number (N), symmetry factor (K) and resolution are calculated to check the system suitability. The results are presented in Table 1.

RESULTS AND DISCUSSION

The present study was aimed to develop a sensitive, precise and accurate method for simultaneous estimation of Nebivolol HCl and Amlodipine besylate in combined tablet dosage forms by RP-HPLC. An Eclipse XDB plus C_{18} Column (4.6 × 150 mm, 5µm particle size) was chosen as the stationary phase for the separation and determination of Nebivolol HCl and Amlodipine besylate. For the optimization of the mobile phase, various mixtures consisting of acetonitrile, methanol and 0.01 M Ammonium acetate buffer were examined at different ratios. The choice of the optimum composition is based on chromatographic response factor. A composition of 50:50 v/v of 0.01 M Ammonium acetate buffer and Acetonitrile provided an efficient separation of Nebivolol HCl and Amlodipine besylate with sufficient retention times. The injection volume was 20µL and the variable wavelength detector was set at 265 nm. The run time was 10 min. A flow rate of 0.8 ml/min was found to be optimum from the studied range of 0.5-2.0 ml/min, which gave optimum retention time, base line stability and noise. The retention times for Nebivolol HCl and Amlodipine besylate were found to be 3.553 min and 2.970 min respectively.

The chromatograms for the validation studies were recorded and shown in Fig. 6-9. The developed method was applied to the simultaneous estimation of Nebivolol HCl and Amlodipine besylate in commercial tablets. Satisfactory results were obtained for each compound showing good agreement with label claim, as shown in Table 2. The linear dynamic range was 10-50µg/ml and 5-25µg/ml for Nebivolol HCl and Amlodipine besylate respectively. The regression coefficients were found to be about 0.999 for both the drugs indicating good linearity of the method as exhibited in Table 1. The recovery study was performed on 80 %, 100 % and 120 % of the target concentrations of Nebivolol HCl and Amlodipine besylate sample preparations and their percentage recovery was found to be 100.86 % and 100.76 % respectively as a mean % recovery of all determinants at the three concentration levels as shown in Table 2. It indicates that the method is accurate and the commonly used excipients present in the tablet formulation did not interfere with the proposed method. The precision of the method was

determined from the peak areas of five homogeneous sample preparations. The system and method precisions obtained for Nebivolol HCl and Amlodipine besylate were found to be less than 2 % (% RSD) as shown in Table 3, indicating that the method is quite precise.

The LOD and LOQ for Nebivolol HCl and Amlodipine besylate were found to be 0.20µg/ml; 0.61µg/ml and 0.07µg/ml; 0.23µg/ml respectively. The optimum HPLC conditions like detection wavelength, ratio of mobile phase and flow rate set for the proposed method have been slightly modified as a means to evaluate the robustness of the method. The results indicated that the selected factors remained unaffected by small variations in these quantities, as shown in Table 4. The result for method ruggedness was found to be 100.21 % for Nebivolol HCl and 101.43 % for Amlodipine besylate as shown in Table 5.

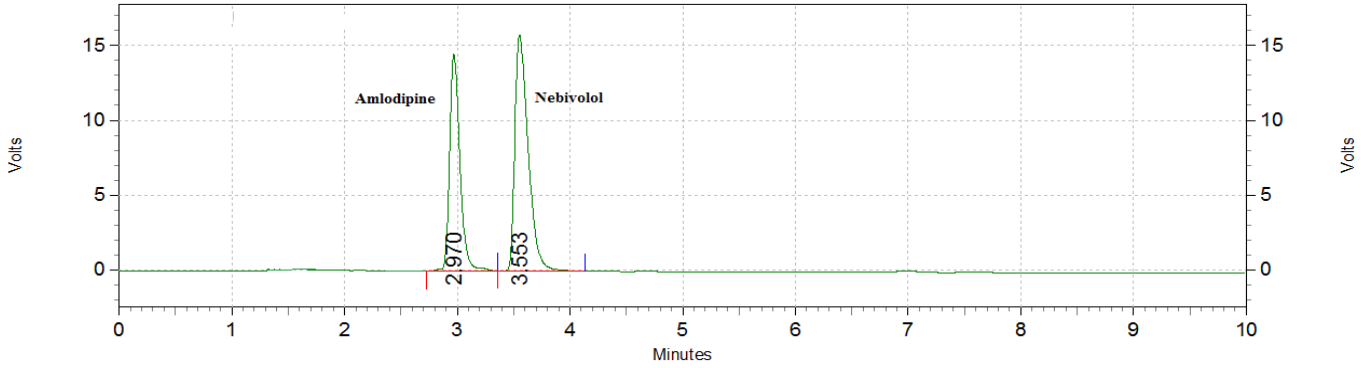

Fig. 6: Chromatogram for Standard solution

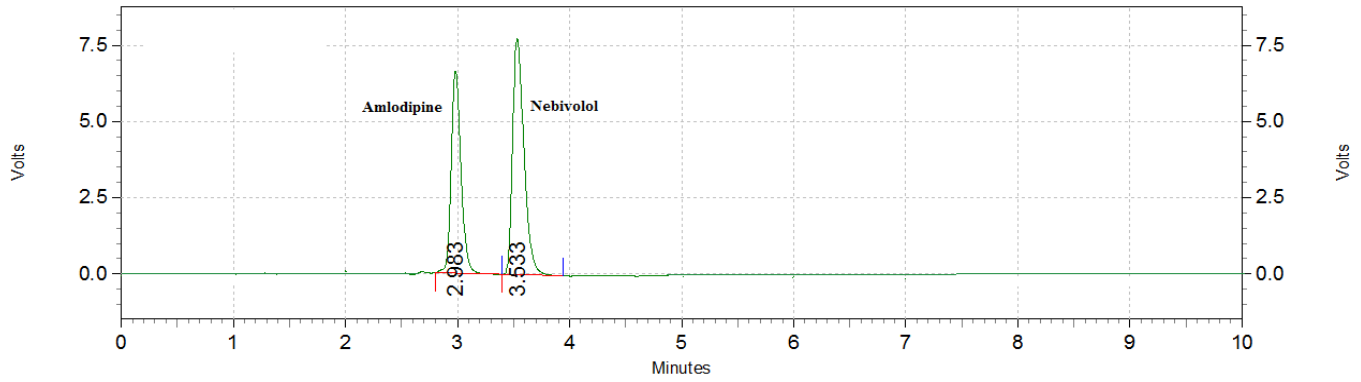

Fig. 7: Chromatogram for Test solution

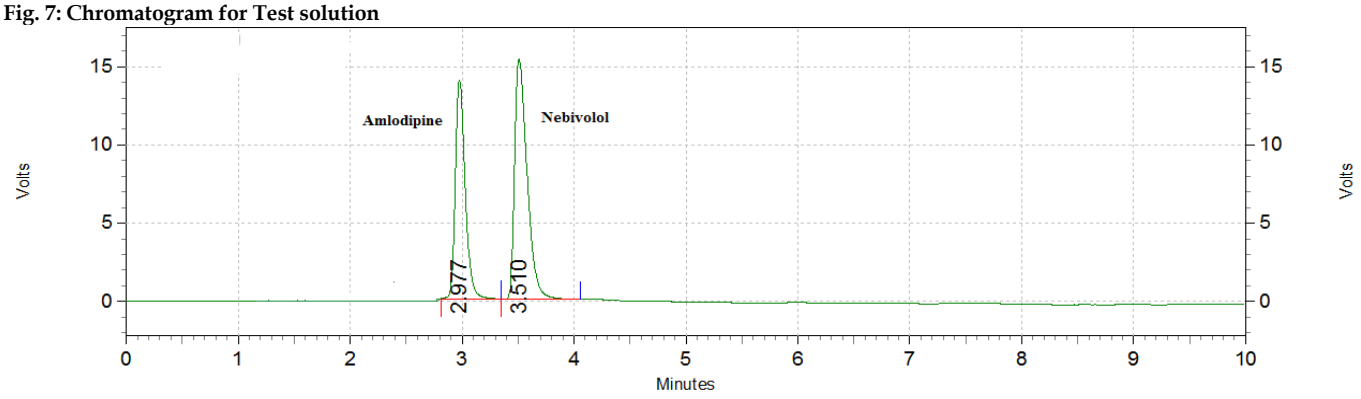

Fig. 8: Chromatogram for Specificity

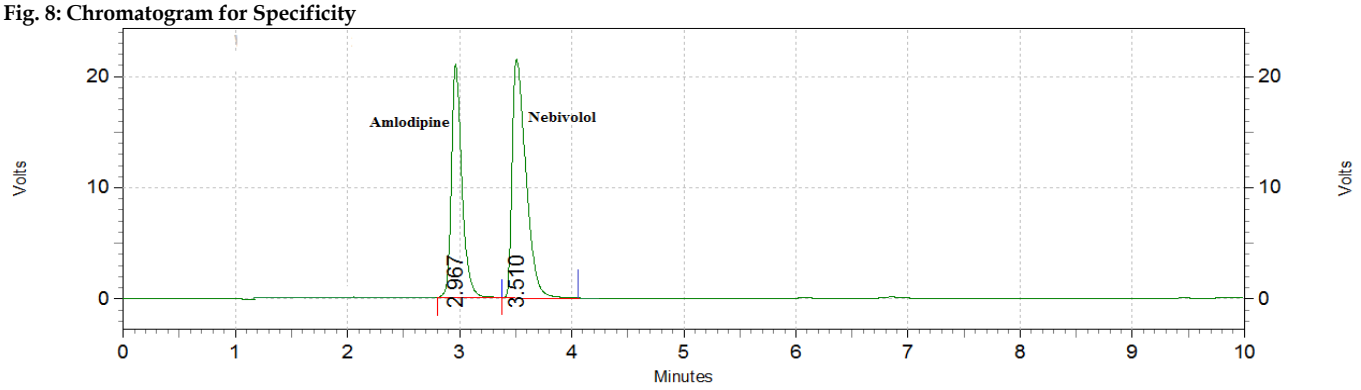

Fig. 9: Chromatogram for System suitability

The absence of additional peaks in the chromatograms showed non-interference of the common excipients used in the tablets indicating that the method is specific for the drugs under study. System suitability results such as theoretical plates, tailing factor and resolution were found to be 3889 & 5108 (theoretical plates), 0.98 & 1.09 (symmetry factor) and 2.76 (resolution) respectively for Nebivolol HCl and Amlodipine besylate.

The validation of the developed method for the required parameters as per ICH guidelines showed that all the parameters were within the acceptance criteria. The proposed method was applied for the simultaneous determination of Nebivolol hydrochloride and Amlodipine besylate in marketed tablet dosage form. The assay results confirmed with the label claim of the formulation. It can be concluded that the proposed HPLC method is sufficiently sensitive and reproducible for simultaneous estimation of Nebivolol HCl and Amlodipine besylate in tablet dosage forms.

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REFERENCES

1. Kolasani A, Kumar GVS, Puranik SB, Sridhar KA. Stability Indicating HPLC method for estimation of S-amlodipine besylate and Nebivolol hydrochloride in bulk drugs and marketed formulation. International Journal for Pharmaceutical Research Scholars 2012; 1: 217-223.
2. Sudhakar M, Venkateshwara rao J, Devika GS, Ramesh Petchi R. A Validated RP-HPLC method for simultaneous estimation of Nebivolol hydrochloride and S-Amlodipine besylate in tablet dosage forms. International Journal of Chemical and Pharmaceutical Sciences 2010; 1: 28-32.
3. Panchumarthy R, Garipati DR. Isocratic separation of four Beta blockers with Amlodipine by C_{18} RP-HPLC: Application to Amlodipine determination in pharmaceutical dosage forms. Int Res J Pharm. 2013; 4: 88-96.
4. Prabhakaran V, Sekar S, Baskar E, Raghuprasath VR. Method development and validation for the simultaneous estimation of Nebivolol hydrochloride and S (-) Amlodipine besilate in tablet dosage form by RP–HPLC. International Journal of Current Trends in Pharmaceutical Research 2013; 1: 19-29.
5. Ravisankar P, Devala Rao G, Krishna Chaitanya M, Devadasu CH, Srinivasa Babu P. Rapid Separation of five Anti-Hypertensive agents - Atenolol, Metoprolol, Hydrochlorothiazide, Amlodipine and Nebivolol: Application to estimation of Metoprolol Succinate in tablet dosage form. Journal of Chemical and Pharmaceutical Research 2013; 5: 215-228.
6. Jain M, Tiwari S, Mishra VK, Shukla S, Sheikh S. Simultaneous estimation of amlodipine besylate and nebivolol hydrochloride in combined dosage form by RP-HPLC. International Journal of Pharmacy & Life Sciences 2010; 1: 428-432.
7. Ajit P, Manish H, Vikas V, Godwin L, Sudesh B, Amjad A *et al*. Development and validation of a Mass Compatible RP-HPLC method for simultaneous determination of Amlodipine Besylate and Nebivolol Hydrochloride in tablet dosage form. Int J Ph Sci. 2013; 5: 1965-1970.
8. Sharma D, Jain A, Shrivastava A. Simultaneous estimation of amlodipine besylate and nebivolol hydrochloride in tablet dosage forms by reverse phase high performance liquid chromatographic using ultraviolet detection. Pharmaceutical Methods 2011; 2: 9-14.
9. Mishra P, Shah K, Gupta A. Spectrophotometric methods for simultaneous estimation of Nebivolol hydrochloride and Amlodipine besylate in tablets. International Journal of Pharmacy and Pharmaceutical Sciences 2009; 1: 55-61.
10. Patil PB, Chavan CB, Jagtap DA, Mohite SK, Magdum CS. Simultaneous Estimation of Nebivolol hydrochloride and Amlodipine besylate by UV Spectrophotometric Method. International Journal of ChemTech Research 2012; 4: 1241-1246.
11. Doshi N, Sheth A, Patel CN. Validated RP-HPLC method for simultaneous estimation of Olmesartan, medoxomil, Hydrochlothiazide and Amlodipine besylate in newly designed pharmaceutical dosage form. International Research Journal of Pharmacy 2012; 3: 178-182.
12. Patel A. Development and validation of derivative spectroscopic method for simultaneous determination of Nebivolol hydrochloride and s-Amlodipine besylate in combined dosage form. Int J Pharm Bio Sci. 2013; 4: 379 – 391.
13. Swethanagini V, Vasanth PM, Ramesh T, Ramesh Malothu. A Validated RP HPLC Method for Simultaneous Estimation of Nebivolol and Hydrochlorothiazide in Combined Dosage Forms. International Journal of Bio-Pharma Research 2012; 01: 20-24.
14. Patel DB, Mehta FA, Bhatt KK. Simultaneous estimation of Amlodipine Besylate and Indapamide in a pharmaceutical formulation by a High Performance Liquid Chromatographic (RP-HPLC) method. Sci. Pharm. 2012; 80: 581–590.
15. Code Q2A - Text on validation of analytical procedure, 1994, ICH Harmonized Tripartite Guideline.
16. Code Q2B - Analytical validation & Methodology, 1996, ICH Harmonized Tripartite Guideline.
17. Code $Q2R_1$ - Validation of analytical procedures text and methodology, 2005, ICH Harmonized Tripartite Guideline.

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