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Simultaneous estimation of amlodipine besylate and nebivolol hydrochloride in combined dosage form by RP-HPLC

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Abstract

A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the simultaneous estimation of Amlodipine Besylate and Nebivolol hydrochloride in pharmaceutical dosage forms. The method was carried out on a Luna C-18, 5 µ column with a mobile phase consisting of 0.005M ammonium acetate solution, acetonitrile and triethylamine in the ratio 60:40:0.1 (v/v) and pH 3.0 was adjusted with orthophosphoric acid. Detection was carried out at 269nm at a flow rate of 1.5 ml/min. The retention time of Amlodipine and Nebivolol was 3.911 and 5.818 min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation. The proposed method can be used for the estimation of both the components in simultaneously in pharmaceutical tablet formulation. The method was found to be linear in the range of 10-30 µg/ml for both Amlodipine and Nebivolol. In the linearity study, regression equation and coefficient of correlation for Amlodipine and Nebivolol were found to be y = 59687x + 56654, r^2= 0.9923 and y = 32052x + 37541, r^2= 0.9992, respectively.

Keywords: Amlodipine, Nebivolol, RP-HPLC, Triethylamine, Acetonitrile, Ammonium acetate.

Introduction

Amlodipine Besylate (ADB), chemically, 2-[(2-aminoethoxy) methyl]- 4- (2-chlorophenyl) -1, 4-dihydro- 6-methyl-3, 5-pyridinedicarboxylic acid 3-ethyl,5-methyl ester, is an anti-hypertensive and an antianginal agent in the form of the besylate salt, Amlodipine besylate. Amlodipine is a calcium channel blocker (dihydropyridine) used as an anti-hypertensive and in the treatment of angina. Like other calcium channel blockers, amlodipine acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and hence reducing blood pressure; in angina it increases blood flow to the heart muscle.

Nebivolol hydrochloride (NVH) is chemically known as α, α- [iminobis(methylene)] bis[6-flouro-3,4-dihydro-2H-1-benzopyran-2-methanol]hydrochloride (Figure 1). t is a highly selective β1-blocker with nitric oxide-mediated vasodilatory actions and beneficial effects on vascular endothelial function. Nebivolol is used in the management of hypertension. It is given by mouth as the hydrochloride although doses are expressed in terms of base. The usual dose is 5 mg daily. An initial dose of 2.5 mg daily is employed in the elderly and in patients with renal impairment [1-2].

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Many methods have been described in the literature for the determination of Amlodipine Besylate and Nebivolol hydrochloride individually and in combination with other drugs either individually or in combination with other drugs. However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms[3-12]. The aim of this work was to develop an RP-HPLC method with ultraviolet detection for the simultaneous determination of Amlodipine Besylate and Nebivolol hydrochloride in pharmaceutical dosage forms. The present RP-HPLC method was validated following the ICH guidelines[13].

Material and methods

Reference standard of Amlodipine Besylate and Nebivolol hydrochloride were obtained as gift sample by Cadila Healthcare Ltd., Ahemdabaad. Commercial Preparation of ADB and NVH (Amlopres - NB, Cipla) was purchased from local market. HPLC grade acetonitrile was procured from Rankem (Mumbai, India). Ammonium acetate, triethylamine and othophosphoric acid AR grade were procured from Qualigens fine chemicals, Mumbai. Double distilled water, prepared in our laboratory was used throughout the experiment. Mobile phase was filtered using 0.45µ nylon filters made by mllipore (USA).

Apparatus and chromatographic conditions

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), UV detector (set at 269 nm) , Rheodyne 7725i injector with 20 µl loop volume. Software Class-VP 6.01 data station was applied for data collecting and processing (Shimadzu, Tokyo, Japan). A Luna C-18, (250X4.6 mm i.d., 5 µ) was used for the separation. The mobile phase was composed of mixture of 0.005M ammonium acetate solution, acetonitrile and triethylamine in the ratio 60:40:0.1 (v/v) and pH 3.0 was adjusted with orthophosphoric acid. It was filtered through a 0.45 µ membrane filter and degassed for 10 mins. Peak identity was confirmed by spectrum and retention time comparison and the HPLC system was operated at room temperature.

Preparation of standard solution

Standard solution of the pure drug was prepared by dissolving 5 mg of each of working standard of Amlodipine Besylate and Nebivolol hydrochloride in a 50 ml volumetric flask using 20 ml of mobile phase and sonicated until the reference solution completely dissolves. Then the volume was made up to the mark with the mobile phase. Further dilute 10 ml of this solution to 50 ml with mobile phase. The mixture was sonicated for 5 min.

Preparation of sample solution

Twenty tablets were accurately weighed and their average weight was calculated. The tablets were ground using pestle and mortar to a homogenized powder. A quantity of tablet powder equivalent to 5 mg of ADB and NVH was weighed and transferred into a 50 ml volumetric flask. 30 ml of mobile phase was added and sonicated for 30 minutes for extracting all the drugs from the excipients and to ensure complete solublization of drug and solution make up to 50ml with mobile phase. The excipeints were separated by filteration through a 0.45µm membrane filter. Discard initial few ml and after that 10 ml of filtered solution was diluted up to 50ml with mobile phase.

Before injection, both standard and sample solution was filtered through 0.45µm membrane filter. Inject separately 20 µl of the standard and sample solutions in 3 replicates and measure the response of major peak due to ADB and NVH.

Results and Conclusion

The objective of this study was to develop simultaneous estimation of two components under isocratic conditions. The mobile phase was used in different ratios. Finally a mixture of 0.005M ammonium acetate solution, acetonitrile and triethylamine in the ratio 60:40:0.1 (v/v), proved to be effective mixture than the other mixture used for the separation. The mentioned chromatographic conditions revealed to provide better resolution between ADB and NVH in a reasonable time of 3.911 and 5.889 min, respectively. The optimum wave length for detection was 269 nm, no indigenous interfering compounds eluted at the retention times of the drugs.

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Validation of the Method

The method was validated, in accordance with ICH guidelines, for linearity, accuracy, precision, specificity, limit of detection and limit of quantification.

Linearity

Linearity was assessed with the aid of serially diluted calibration solutions as mentioned above. The standards were injected separately. Calibration graphs were plotted on the basis of triplicate analysis of each calibration solutions. Linear calibration plot for the proposed method were obtained by analyzing five solutions in the concentration range of 10-30 µg/ml for both ADB and NVH The peak area of drugs was potted against the corresponding concentration to construct a linear regression equation and to calculate the value of correlation coefficient. Linear correlations were obtained over the range studied, with correlation coefficients ≥ 0. 999 for the drugs. In case of ADB regression equation was found to be y = 59687x + 56654, (r^2= 0.9923) for NVH it was y = 32052x + 37541, (r^2= 0.9992).

Precision

The precision of the method was done by triplicate analysis of tablet preparations. The precision was also studied in terms of intraday changes in peak area of drug solution on the same day and on three different days over a period of one week. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the results are given in Table-1.

Table-1. Precision study

Drug	Interday precision	Intraday precision
	RSD(%)	RSD (%)
Amlodipine Besylate	0.472	0.579
Nebivolol hydrochloride	0.262	0.281

Accuracy

Accuracy was performed by the method of standard addition at three different levels, by multiple level recovery studies. Three levels of solution were made which correspond to 80, 100 and 120% of the nominal analytical concentration. Each level was made in triplicate. These solutions were then analyzed for recovery studies and consistent values by replicated injections cum analysis. The recovery and relative standard deviation for each of the analytes are given in Table. 2. From the recovery study it is evident that the method is highly accurate and can give excellent results.

Table-2. Accuracy study

Drug	Level (%)	Recovery (%)	RSD (%)
Amlodipine Besylate	80	99.89	0.208
	100	100.58	0.142
	120	100.29	0.076
Nebivolol hydrochloride	80	99.27	0.318
	100	100.84	0.184
	120	99.45	0.049

Specificity

Specificity was tested against standard compounds and against potential interferences in the presence of placebo. The comparison of the chromatograms of the synthetic placebo mixture and the spiked drug solution revealed that there was no interference of placebo with the peaks of ADB and NVH in sample solution . Peak purity for ADB and NVH was tested by comparing spectra acquired at the start (S), apex (A), and end (E) of the peaks. Peak purity plots indicated that the peaks of drugs were pure and did not have any coelution peak. No interference from placebo was observed at the retention time of the drugs. Therefore, it was concluded that the method is specific.

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Limit of Detection and Quantification

Limit of Detection (LOD) and Limit of Quantification (LOQ) were estimated from the signal to noise ratio. LOD was found to be 7.41 ng/ml and 14.62 ng/ml for amlodipine and nevibolol respectively. Whereas LOQ was found to be 9.08 ng/ml and 28.5 ng/ml for amlodipine and nevibolol respectively.

System suitability

System suitability tests are an integral part of chromatographic method. They were used to verify that the reproducibility of the chromatographic system is adequate for the analysis. The application of the method was checked by analyzing the ADB and NVH in commercial tablets. The results are given in table. 4 which shows high recovery and low RSD (%) values.

Table. 4 System Suitability Parameters

Parameters	Amlodipine Besylate	Nebivolol hydrochloride
Tailing Factor	1.436	1.424
Resolution	-	6.147
Retention Time	3.911	5.818
No. of Theoretical Plates	4250.540	3738.672

Assay of Tablets

The validated method was applied for the assay of commercial tablets containing ADB (5mg) and NVH (5 mg). Each sample was analysed in triplicate after extracting the drug as mentioned in sample preparation under materials and method section and injections were carried out in triplicate. A typical chromatogram obtained from a sample solution is shown in Fig. 3. Results of analysis are shown in table-5.

Table-5. Result of analysis of Amlodipine Besylate and Nebivolol hydrochloride in tablets

Drug	n	Amount claimed (mg per tablet)	Amount found (mg per tablet)	Mean recovery	RSD (%)
Amlodipine Besylate	5	5.0	5.04	100.55	0.888
Nebivolol hydrochloride	5	5.0	5.01	100.2	1.02

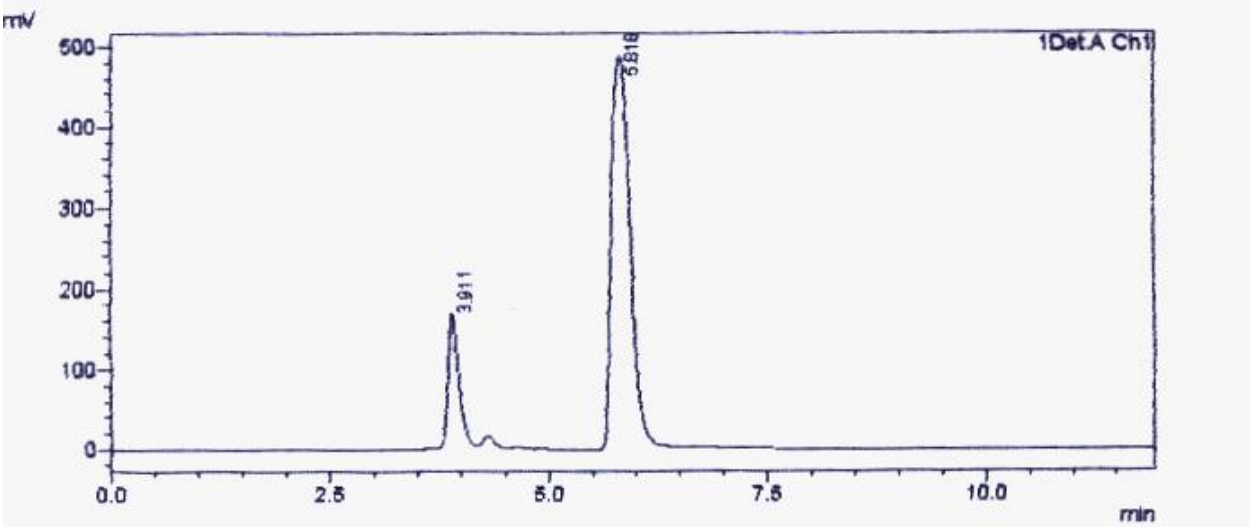

Fig. 1 Typical chromatogram obtained from Amlodipine Besylate (RT=3.911) and Nebivolol hydrochloride (RT=5.818)

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A RP-HPLC method has been developed for the simultaneous estimation of Amlodipine Besylate and Nebivolol hydrochloride in pharmaceutical dosage forms, using the UV detector. The proposed RP-HPLC method for simultaneous assay of Amlodipine Besylate and Nebivolol hydrochloride in combined tablets dosage forms is simple, precise, specific and highly accurate and less time consumption for analysis could be recorded. So, it can be employed for the routine analysis as method for simultaneous estimation of Amlodipine Besylate and Nebivolol hydrochloride. Hence this RP-HPLC method is suitable for quality control of raw materials and formulations, and also for dissolution studies. It can be used for bioequivalence studies in plasma.

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