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Development and validation of a RP-HPLC Method for Simultaneous Estimation of Atenolol and Nitrendipine in Tablet Dosage Form

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ABSTRACT

A rapid high performance liquid chromatographic method has been developed and validated for the estimation of Atenolol and Nitrendipine simultaneously in combined dosage form. A Phenomenox C-18 column having dimensions of 4.6×250 mm and particle size of 5 µm in isocratic mode, with mobile phase containing a mixture of methanol: acetonitrile: water (40:40:20 v/v) (pH adjusted to pH 3.0 using orthophosphoric acid) was used. The mobile phase was pumped at a flow rate of 1.5 ml/min and the eluents were monitored at 235 nm. The selected chromatographic conditions were found to effectively separate Atenolol (R_t: 2.61 min) and Nitrendipine (R_t: 6.11 min) having a resolution of 11.097. The method was validated in terms of linearity, accuracy, precision, and specificity, limit of detection and limit of quantitation. Linearity for Atenolol and Nitrendipine were found in the range of 30-70 µg/ml and 6-14 µg/ml, respectively. The percentage recoveries for Atenolol and Nitrendipine ranged from 99.05-100.51% and 99.14-101.60%, respectively. The limit of detection and the limit of quantitation for Atenolol were found to be 1.96 µg/ml and 5.95 µg/ml respectively and for Nitrendipine were found to be 0.34 µg/ml and 1.03 µg/ml, respectively. The method was found to be robust and can be successfully used to determine the drug content of marketed formulations.

Key words: Atenolol, Nitrendipine, RP-HPLC, acetonitrile, methanol, water, validation.

INTRODUCTION

Atenolol is a β1-selective (cardioselective) β-adrenergic receptor blocking agent without membrane stabilizing or intrinsic sympathomimetic (partial agonist) activities. This preferential effect is not absolute however, and, at higher doses, Atenolol inhibits β2-adrenoreceptors, chiefly located in the bronchial and vascular musculature [1]. Atenolol is also used to treat myocardial infarction (heart attack) and arrhythmias (rhythm disorders), angina (chest pains), and disorders arising from decreased circulation and vascular constriction, including migraine. Atenolol may be used alone or concomitantly with other antihypertensive agents including thiazide-type diuretics, hydralazine, prazosin, and α-methyldopa [2].

Nitrendipine is a 1, 4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridine dicarboxylic acid ethyl, methyl ester is a calcium channel blocker with vasodilatory properties, present in several commercial preparation administrated in the treatment of hypertension[3]. Literature survey revealed that several methods have been reported estimation of Atenolol and Nitrendipine individually or in combination with other drugs in pharmaceutical dosage forms and/or in biological fluids [4-20]. However, no HPLC method has been reported so far for the estimation of these two drugs simultaneously in combined dosage forms. Hence, in the present study, a new reversed-phase high performance liquid chromatography method was developed and validated for the simultaneous estimation of Atenolol and Nitrendipine in tablets. The method was validated for accuracy, precision, specificity, detection limit, quantitation limit and robustness as per 'ICH' guidelines [21] . Present work describes rapid, accurate, reproducible, and economical methods for simultaneous estimation of these drugs in tablet formulation.

MATERIALS AND METHODS

Reference standard of Atenolol and Nitrendipine were obtained as gift sample by Concept Pharmaceuticals Pvt. Ltd. Aurangabaad. Tablet formulation containing labeled amount of Atenolol (50mg) and Nitrendipine (10 mg). (Cardif beta-10, Concept Pharmaceuticals Pvt. Ltd. Aurangabaad) was procured from the local pharmacy. HPLC grade acetonitrile and methanol was procured from Rankem (Mumbai, India). Ortho phosphoric acid was procured from Qualigens fine chemicals, (Mumbai, India). Double distilled water, prepared in our laboratory was used throughout the experiment. Mobile phase was filtered using 0.45µ nylon filters made by millipore (USA).

The liquid chromatographic system consisted of the following components are Shimadzu HPLC LC-2010 AHK unit and Agilent 1100 system with variable wavelength programmable UV/Vis detector and Rheodyne injector with a 20 µl fixed loop. Chromatographic analysis was performed on a Phenomenox Luna C-18 with 250×4.6 mm internal diameter and 5 µm particle size. The mobile phase was composed of mixture of acetonitrile, methanol and water in the ratio of 40:40:20 v/v v (pH adjusted to 3.0 with orthophosphoric acid). It was filtered through a 0.45 µ membrane filter and degassed for 10 minutes. The flow rate of the mobile phase was maintained at 1.5 ml/min. Detection was carried out at 235 nm at 25ºC.

Preparation of standard solution

Standard solution was prepared by dissolving 50 mg of Atenolol and 10 mg of Nitrendipine working standard in a 100 ml volumetric flask using 50 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 5 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 50 mg of Atenolol and 10 mg of Nitrendipine was weighed and transferred into a 100 ml volumetric flask. 50 ml of mobile phase was added and sonicated for 30 minutes and solution was made up to 100ml with mobile phase. The excipeints were separated by filteration through a 0.45µm membrane filter. Discard initial few ml and after that 5 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 Atenolol and Nitrendipine.

RESULTS AND DISCUSSION

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 Atenolol and Nitrendipine in sample solution. No interference from placebo was observed at the retention time of the drugs. Therefore, it was concluded that the method is specific.

Linearity

Appropriate aliquots of standard stock solutions of Atenolol and Nitrendipine were diluted mobile phase to obtain final concentrations in the range of 30-70 µg/ml of Atenolol and 6-14 µg/ml of Nitrendipine. The solutions were injected in triplicates for each concentration using a 20 µl fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average content of the drug versus respective concentrations and regression equations were computed for Atenolol and Nitrendipine. The plots of average content Vs respective concentration of Atenolol and Nitrendipine were found to be linear in the range of 30-70 µg/ml and 6-14 µg/ml with coefficient of correlation (r^2) 0.9989 and 0.9992 for Atenolol and Nitrendipine respectively.

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 1.964 µg/ml and 0.342 µg/ml for Atenolol and Nitrendipine respectively. Whereas LOQ was found to be 5.952 µg/ml and 1.036 µg/ml for Atenolol and Nitrendipine respectively.

Precision

Repeatability of the method was validated by performing six replicate assays of the homogeneous sample. Results were calculated in terms of %RSD of the content of Atenolol and Nitrendipine. Method was also validated for intermediate precision by comparing the performance of the method on different day by different chemist. Six replicate assays of homogeneous sample were performed using the same procedure and chromatographic conditions, % RSD of the contents of Atenolol and Nitrendipine were calculated. This results and repeatability (performed on previous day, by different chemist) results were compared and given in table-1.

Table no.1 Precision study results

Drug	Repeatability	Intermediate precision	
		Day -1	Day -2
Atenolol	0.710	0.350	0.539
Nitrendipine	0.822	0.661	0.675

Accuracy

Accuracy was performed by the method of standard addition at three different levels, by multiple level recovery studies. Preanalyzed sample solution was spiked with Atenolol and Nitrendipine in the same proportion as that present in the tablet formulation. Spiking was done at three

different concentrations 80%, 100%, and 120% of the label claim. Accuracy of the method was studied by calculating the recovery of the spiked samples. Recovery and relative standard deviation for Atenolol and Nitrendipine is given in table-2.

Table no.2. Accuracy study results

Drug	Level (%)	Recovery (%)	RSD (%)
Atenolol	80	99.865	0.692
	100	99.525	0.573
	120	100.014	0.665
Nitrendipine	80	100.460	0.990
	100	100.714	0.764
	120	99.750	0.809

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 atenolol and nitrendipine in commercial tablets. The results are given in table. 3.

Table no.3 System Suitability Parameters

Parameters	Atenolol	Nitrendipine
Tailing Factor	1.211	1.158
Resolution	-	10.988
Retention Time	2.621	5.162
No. of Theoretical Plates	5489.672	4069.358

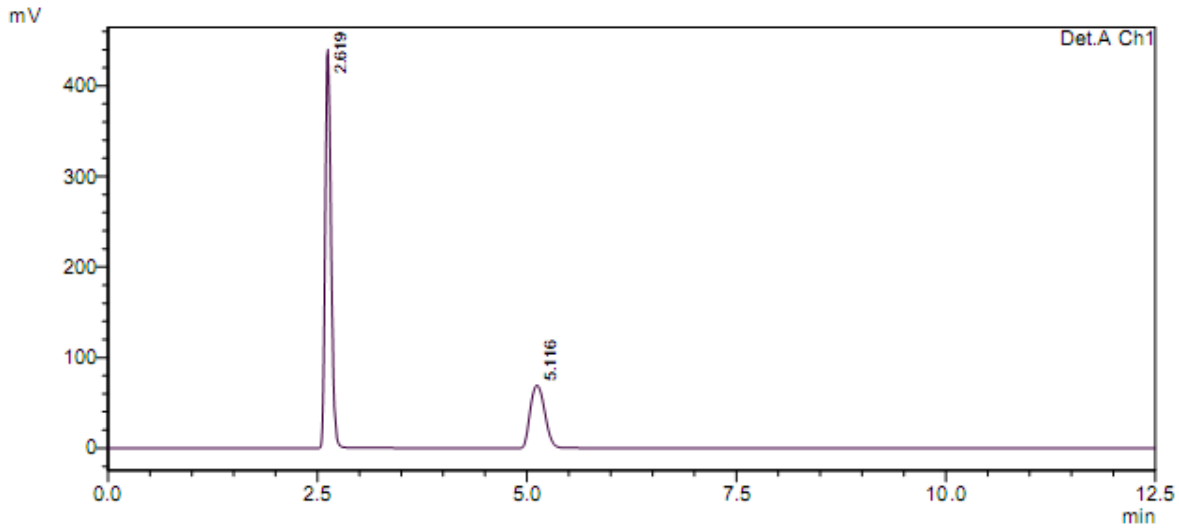

Fig.1.Typical chromatogram of Atenolol and Nitrendipine

Assay of Tablets

The validated method was applied for the assay of commercial tablets containing atenolol (50mg) and nitrendipine (10 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.no.1. Results of analysis are shown in table-4.

Table no.4 Result of analysis of Atenolol and Nitrendipine in tablets (n=3)

Drug	Amount claimed (mg per tablet)	Amount found (mg per tablet)	Mean recovery	RSD (%)
Atenolol	50.00	50.06	100.13	0.563
Nitrendipine	10.00	10.03	100.30	0.607

CONCLUSION

The developed RP-HPLC method for simultaneous assay of Atenolol and Nitrendipine 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 for simultaneous estimation. 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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