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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF AMLODIPINE AND ATENOLOL IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

Objective: The objective of this study was to develop a simple, efficient, specific, precise and accurate Reverse phase High Performance liquid chromatographic method for the simultaneous estimation of Amlodipine and Atenolol in bulk drugs and tablet dosage form.

Methods: The chromatographic separation was achieved using reverse phase C18 column; BDS C18 column (250 mm x 4.6 mm x 5µm). The mobile phase used was a mixture of Phosphate buffer (1gm of Sodium dihydrogen phosphate and 2gm of Di Potassium hydrogen phosphate in 1000ml ml of water); pH 5(adjusted with Ortho phosphoric acid) Methanol and Acetonitrile in the ratio of 40: 30: 30(v/v) at isocratic mode and eluents were monitored at 213 nm using UV-Visible spectrophotometer as the detector.

Results: With the optimized method, the retention times of Amlodipine and Atenolol were found to be 2.540 and 5.950 respectively with theoretical plate count and asymmetry as per the ICH limits. The method has shown a good linearity in the concentration range of 6-14µg/ml for Amlodipine and 60-140µg/ml for Atenolol with a regression coefficient of 0.9983 for both drugs. The % assay of Atenolol and Amlodipine were 99.22% and 99.47%. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were 0.40µg/ml and 1.22µg/ml for Amlodipine and 5.26µg/ml and 15.94µg/ml for Atenolol respectively. The proposed method is accurate with (with percentage mean recoveries 99.97% for Amlodipine and 99.20% for Atenolol), precise, robust, stable and specific.

Conclusion: The proposed method was validated in accordance with ICH guidelines and hence can be successfully applied to the simultaneous estimation of Amlodipine and Atenolol in tablet formulations.

Keywords: RP-HPLC, Amlodipine, Atenolol, LOD, LOQ.

INTRODUCTION

Amlodipine is 3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine- 3, 5-dicarboxylate (Figure 1). Amlodipine is long acting calcium channel blocker used as anti-hypertensive and in treatment of angina. Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels; in angina it decreases blood flow to the heart muscle. It is official in USP-2010, BP-2012, and IP-2007 [1, 2, and 3]

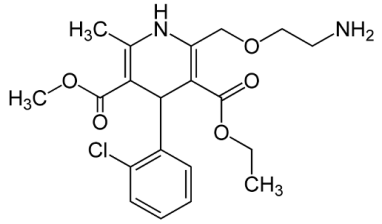

Fig. 1: It shows structure of Amlodipine

Atenolol is chemically2-(4-{2-hydroxy-3-[(propan-2yl)amino] propoxy} phenyl) acetamide(Figure 2), is a β-blocker seem to be equally effective as an antihypertensive, antianginal and antiarrhymthmic drug widely used as Cardiovascular drug in combination with Amlodipine. Atenolol competes with sympathomimetic neurotransmitters such as catecholamines for binding at beta (1)-adrenergic receptors in the heart and vascular smooth muscle, inhibiting sympathetic stimulation. It is official in BP-2012 [4].

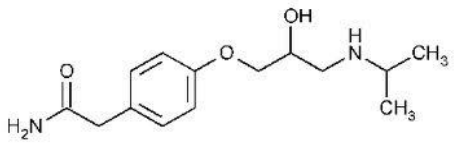

Fig. 2: It shows structure of Atenolol

Hypertension is amongst the major health disorder as it does not have any symptoms and in most cases it is not detected. Hypertension is a significant risk factor for cardiovascular disease, which includes heart attacks, strokes, congestive heart failure and even kidney failure. In such case combination of Beta blockers and Calcium channel blockers are given.

For the simultaneous estimation of the drugs present in multicomponent dosage forms, HPLC method is considered to be most suitable since this is a powerful and rugged method. Many methods have been reported in the literature for the estimation of Amlodipine and Atenolol individually and in combination [6-10]. However, there is no simple method with shorter run times has been reported for the simultaneous estimation of Amlodipine with Atenolol. The present investigation was aimed at developing a fully validated RP-HPLC method for the simultaneous estimation of Amlodipine and Atenolol in bulk and pharmaceutical combined dosage forms that is more economical, simple, precise and accurate than the previous methods.

MATERIALS AND METHODS

Instrumentation

A Shimadzu (Japan) HPLC system consisting of two LC-20AT pumps, SIL-20A auto sampler and BDS C_{18} (250mmx4.6mm, 5µ) column were used. Ultra violet detection was achieved with a SPD-20A UV-VIS detector (Shimadzu, Japan). The drug analysis data were acquired using Spinchrome software running under Windows XP on a Pentium PC.

Reagents and Chemicals

Atenolol and Amlodipine pure drug samples were provided by Aurobindo Pharma Hyderabad. Acetonitrile and Methanol were of HPLC grade and were purchased from Rankem India. Fixed dose combination capsules (Brand name: Amlokind AT) containing 5mg of Amlodipine and 50mg of Atenolol were procured from local pharmacy, Hyderabad, India.

Chromatographic Conditions

The mobile phase, a mixture of buffer, Methanol and Acetonitrile (40:30:30v/v) pumped at a flow rate of 1.0ml/min through the column (BDS C18;5µ,4.6x250mm)at ambient temperature.

(Buffer preparation: 1gm of Sodium dihydrogen phosphate and 2gm of Di Potassium hydrogen phosphate is dissolved in 1000ml of water. pH- 5.0 is adjusted with ortho phosphoric acid). The mobile phase was filtered through 0.45µm filters to remove all fine particles and gases and degassed for 10min by sonication. Samples of 20µl were injected into the HPLC system and the effluents were analyzed at 225nm, with runtime of 10min.

Preparation of standard solution

Weighed accurately 50mg of Atenolol and 5mg of Amlodipine in 100ml volumetric flasks and made up the volume with Methanol. From the above solution 5ml is pippetted and diluted to 50ml with methanol.

Preparation of sample solution

20 tablets were accurately weighed and the average weight was calculated. The tablets were grinded to fine powder with the help of mortar and pestle. Then, the amount of powder equivalent to average weight of a tablet was transferred to a volumetric flask, dissolved in methanol and shaken for about 10 min then filtered through filter paper. The filtered solution was further diluted in the methanol to make the final concentration. Then 20 µl of standard and sample solutions were injected into column and chromatogram was recorded.

Development and Validation of HPLC method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of Atenolol and Amlodipine in tablet dosage forms. The experiment was carried out according to the official specifications of USP– 30, ICH- 1996, Global Quality Guidelines- 2002. The method was validated for the parameters like system suitability, selectivity, linearity, accuracy, precision, and robustness.

System suitability

A standard solution was prepared using Atenolol and Amlodipine working standard as per the test method and was injected six times into the HPLC system. The parameters namely USP plate count, peak asymmetry factor and resolution for the standard solutions were calculated.

Selectivity

Selectivity was determined in the presence of common excipients used in the tablet formulation. Sample containing 100% Atenolol and Amlodipine was injected first. Then the samples mixed with three different placebo formulations were injected to find out the selectivity of the method.

Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of Atenolol and Amlodipine at different concentrations level (60%, 80%, 100%, 120%, and 140%) were used for this purpose. Before injection of the solutions, the column was equilibrated for at least 30min with the mobile phase. Each measurement was carried out in six replicates to verify the reproducibility of the detector response at each concentration level. The peak areas of the chromatograms were plotted against the concentrations of Atenolol and Amlodipine to obtain the calibration curves. The five concentrations of the standard were subjected to regression analysis to calculate calibration equation and correlation coefficients.

Accuracy

Accuracy is the closeness in agreement between the accepted true value or a reference value and the actual result obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed.

Precision

Method Precision was determined by injecting six replicates of drug sample solution. The retention times and peak areas of six replicates are recorded. The precision is expressed as the % RSD of Peak areas and it should not be more than 2%.

Robustness

The robustness of the method was assessed by altering the some experimental conditions such as, by changing the flow rate from 0.9 to1.1 ml/min, amount of diluents (10% to 15%) the temperature of the column (28°C to 32°C) and pH of the mobile phase.

Limit of detection and limit of quantitation

Limit of detection and limit of quantitation represent the concentration of analyte that would yield signal to noise ratio of 3 for LOD and 10 for LOQ respectively. To determine LOQ and LOD serial dilutions of mixed standard solution of Amlodipine and Atenolol was made from standard solution. The samples were injected in LC system and measured signal from the samples was compared with those of blank samples.

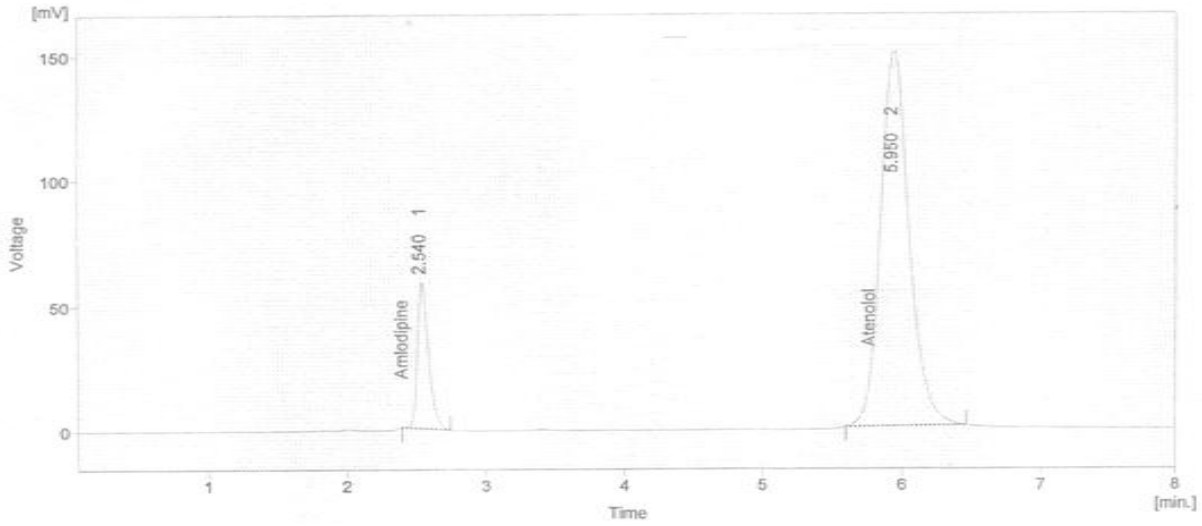

Fig. 3: It shows chromatogram of Amlodipine and Atenolol in Standard preparation

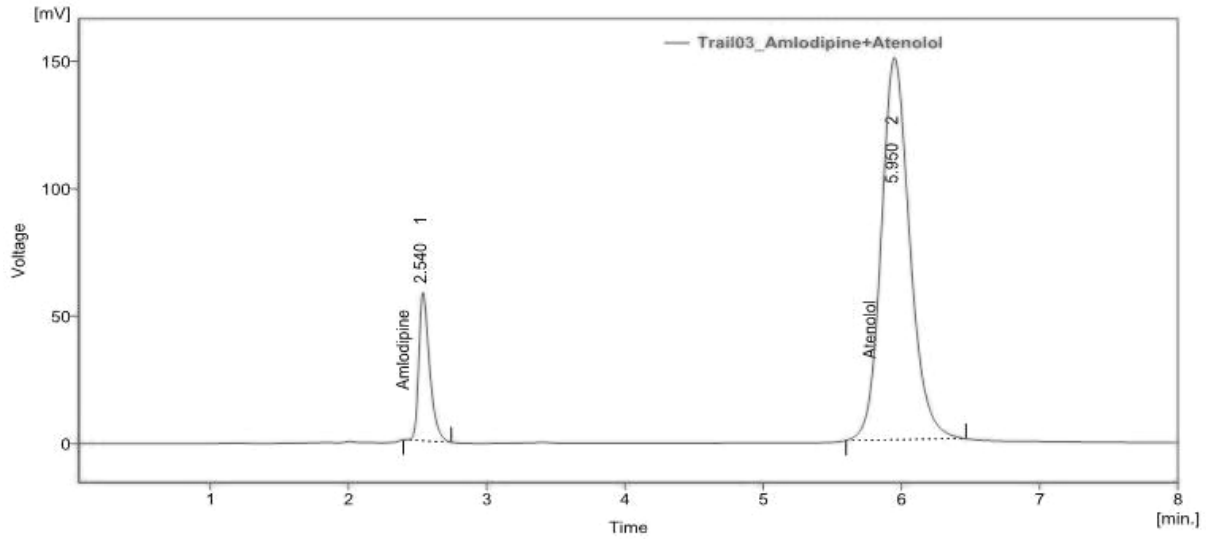

Fig. 4: It shows chromatogram of Amlodipine and Atenolol in sample preparation

RESULTS AND DISCUSSION

Method Development

System suitability

The system suitability tests were carried out to evaluate the resolution and reproducibility of the system for the analysis. Table1 summarized the test results of system suitability study.

System suitability studies were carried out on the method and %RSD values of retention times, peak areas, asymmetry, and theoretical plate count were found to be less than 2% for both Amlodipine and Atenolol standards.

Linearity

The linearity of the developed method was determined in triplicate at different concentrations ranging from 6-14 µg/ml for Amlodipine and 60-140 µg/ml for Atenolol.

The regression analysis equation for Amlodipine and Atenolol was y = 25.825x+44.389 and y = 19.843x+137.21, Regression coefficient (R^2) was 0.9983 for both drugs, showing good linearity. The results confirmed the linearity of the standard curves over the range studied and the excellent reproducibility of the assay method.

Table 1: It shows result of system suitability tests of Amlodipine &Atenolol

Parameters	Observation*	%RSD
Amlodipine		
Peak area	315.941	0.84451
Retention time(min)	2.538	0.0610
Theoretical plates	5137.333	0.1312
Tailing factor	1.787	1.56
Atenolol		
Peak area	2155.614	0.648
Retention time(min)	5.956	0.316
Theoretical plates	5137.333	29.669
Tailing factor	1.266	1.508
*mean of 6 replicate values		

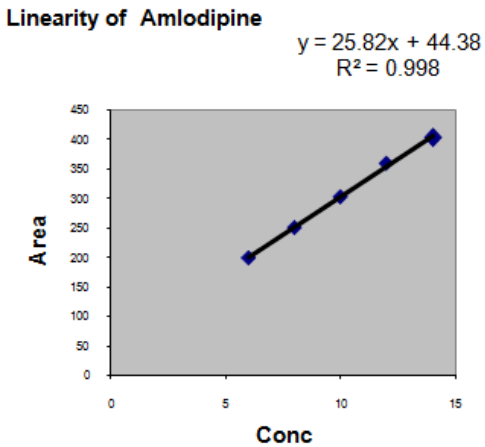

Fig 4: It shows linearity plot of Amlodipine

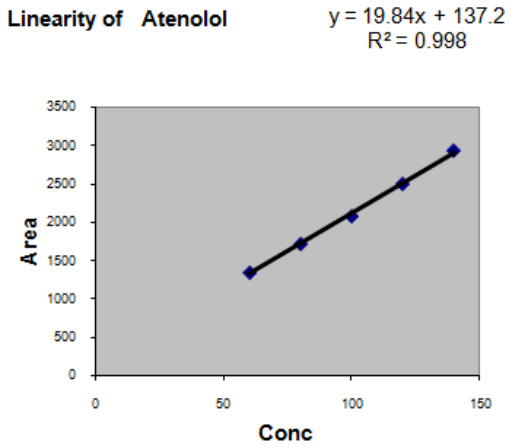

Fig 5: It shows linearity plot of Atenolol

Accuracy

Table 2: It shows recovery of Amlodipine

Amlodipine	Standard mcg	% Recoveries	% Mean recovery
	8	100.41	
	10	99.56	99.97
	12	99.96	

Table 3: It shows recovery of Atenolol

Atenolol	**Standard mcg**	**%Recoveries**	**%Mean recovery**
	80	99.90	
	100	99.27	99.20
	120	98.42	

The percentage mean recoveries are 99.97% for Amlodipine and 99.20% for Atenolol respectively.

Precision

Method Precision: Method precision was determined by injecting sample solutions of concentration Atenolol (50 µg/mL) and Amlodipine (5 µg/mL) for six times are prepared separately.

The chromatograms were recorded and the results were summarized in Table 2. % RSD of retention time and peak areas obtained for Atenolol were 0.44 and 0.30 respectively and for Amlodipine were 0.84 and 0.18 respectively.

Method Precision was observed as the %RSD values for the retention times and peak areas of Amlodipine and Atenolol in both standard and sample preparations were found to be less than 2%.

Table 4: It shows method precision results for Amlodipine and Atenolol

Parameters	**Observation**	**%RSD**
Amlodipine		
Retention time	2.560	0.84
Area	319.264	0.18
Atenolol		
Retention time	6.015	0.44
Area	2203.867	0.30

Robustness

As part of the robustness, deliberate changes in the flow rate and detection wavelength were made to evaluate the impact on the method and retention times were significantly changed.

Limit of detection and Limit of quantitation

The LOD for this method was found to be 0.40µg/mL for Amlodipine and 5.26µg/mL for Atenolol respectively. The LOQ for this method was found to be 1.22µg/mL for Amlodipine and 15.94µg/mL for Atenolol respectively.

Table 5: Robustness test of Amlodipine and Atenolol

Parameters	**Changes**	**%Recovery of amlodipine**	**% recovery of atenolol**	**%target**
Flow rate(ml/min)	0.9	99.8	99.3	100%
	1.1	99.7	99.4	100%
Column temperature(°c)	28	99.7	99.5	100%
	30	99.6	99.5	100%

Table 6: It shows LOD and LOQ for Amlodipine & Atenolol

Amlodipine	**mcg**	**Area**
LOD	0.40	10.45
LOQ	1.22	31.65
Atenolol		
LOD	5.26	104.46
LOQ	15.94	316.54

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were 0.40µg/ml and 1.22µg/ml for Amlodipine and 5.26µg/ml and 15.94µg/ml for Atenolol respectively.

Ruggedness was carried out by changing analyst and environment conditions, all the parameters are within the limits. The specificity of the method was established by determining the interferences of peaks of diluent or excipients. These results indicate that the method is sensitive enough to carry out the routine analysis of Amlodipine and Atenolol combination dosage forms.

CONCLUSION

The proposed high-performance liquid chromatographic method has been evaluated over the accuracy, precision and linearity and proved to be more convenient and effective for the quality control and identity of Atenolol and Amlodipine in pharmaceutical dosage forms.

The measured signals were shown to be precise, accurate and linear ad suitable for intended purpose. Moreover, the lower solvent consumption along with the short analytical run time of 6 minutes leads to an environmentally friendly chromatographic procedure that allows the analysis of a large number of samples in a short period of time. Therefore, this HPLC method can be used as a routine sample analysis.

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