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A VALIDATED GRADIENT RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ATENOLOL AND AMLODIPINE IN COMBINED DOSAGE FORM

CHAUDHARY M* AND PRIYA

School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun-248001, Uttarakhand, India

***Corresponding Author: Meenu Chaudhary: E Mail: 1979mchaudhary@gmail.com**

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ABSTRACT

The presented work offers a validated gradient method based on RP-HPLC enabling fast quantification of Amlodipine and Atenolol simultaneously in tablet formulations. The whole analysis is founded upon RP-HPLC separation using ODS-A C18 (250 mm x 4.6 mm, particle size-5µm) column maintained at 35°C. The mobile phase comprises a buffer, acetonitrile, and methanol. 0.9ml/min flow rate was set together with 20µl of injection volume and the entire analysis has been carried out at 228 nm. The chromatogram showed that there were well-separated Atenolol and Amlodipine peaks. Over the concentration range of 25 to 75µg/ml for Atenolol and 2.5 to 7µg/ml for Amlodipine a linear relationship of the drug concentration with peak areas was observed. With the recovery between 99.88% to 101.66% for Atenolol and 99.34% to 102.54% for Amlodipine, the current approach is proven to be accurate. The approach demonstrated high precision as for both the drugs percent Relative standard deviation was below 2%. Atenolol and Amlodipine in tablet formulation can therefore be analyzed using this method, which is more convenient as well as reliable than the other methods.

Keywords: Gradient, RP-HPLC, Simultaneous, Quantification

INTRODUCTION

One of the most common and serious health problems emerging nowadays is hypertension **[1]**. High blood pressure is associated with many significant cardiovascular disorders, including kidney failure, CHF (congestive heart failure), strokes, as well as heart attacks. Among the treatments, beta-blockers are the efficacious as well as safest drugs to fight against high blood pressure and Atenolol comes from this beta-blocker pharmacological class **[2]**. Atenolol is an antagonist or blocker of beta-1 adrenergic receptors, named so since it has the ability to specifically bind to and block this receptor **[3]**. It functions as it competes against catecholamine to bind with the heart's as well as smooth muscle's adrenergic receptors (β1) which so inhibits sympathetic activation **[4]**. The main purpose of adrenergic stimulation includes increasing the rate of heartbeat. Atenolol helps in lowering the cardiac output, and heart rate together with systolic and diastolic B.P by inhibiting stimulation **[5]**.

Chemically, Atenolol is characterized as 4-(2-hydroxy-3-[(1-methylethyl) amino] propoxy] benzeneacetamide **[6] (Figure 1)**. For oral administration the various dosage forms available for Atenolol are - capsules, tablets, and also syrup. This medicine is usually offered as a tablet in three distinct strengths i.e.25, 50, and 100 mg **[7]**.

Amlodipine is an antihypertensive medication that belongs to the calcium channel blocker class of drugs. It acts by inhibiting Calcium from entering the cell membranes of smooth muscle and myocardial cells, resulting in decreased contractile function and dilating coronary as well as systemic arteries, subsequently lowering blood pressure **[8]**. Since Amlodipine has a long half-life, therefore dosed only once a day and this also promotes compliance by patients **[9]**. Chemically Amlodipine is characterized as 3-~{O}-ethyl 5-~{O}-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate **[10] (Figure 2)**. An oral dose of Amlodipine comes in 2.5, 5, and 10 mg strengths. Generally, the advised initial dose is 5mg per day whereas 10 mg per day is the highest dose **[11]**.

In comparison to monotherapy, an antihypertensive drug combination can produce numerous advantages such as regulating the blood pressure better, provide a simple dosing schedule, causes a reduction in side effects **[12]**, and because beta-blockers together with calcium channel

blockers represent effective hypertensive medications, their action takes a synergistic approach to control high blood pressure. According to Li-Ping *et.al.,* Amlodipine and Atenolol work synergistically to decrease as well as stabilize the blood pressure and when the two drugs are present in dose ratio 10:1 the effect is considered to be most potent **[13]**. Because HPLC is a fast as well as robust method, it is thought to be most appropriate for the simultaneous quantification of compounds contained in the multicomponent dosage form. The purpose of this work has been to thoroughly validate an HPLC gradient method which is much cost-effective, and efficient as per the ICH guidelines for determining Amlodipine and Atenolol simultaneously in the pharmaceutical tablet dosage form.

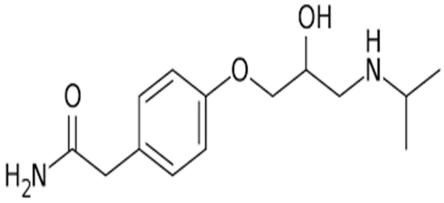

Figure 1: Structure of Atenolol

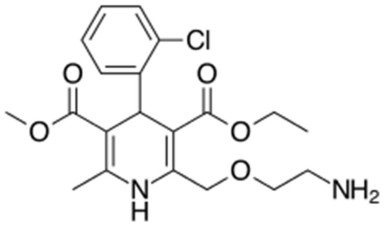

Figure 2: Structure of Amlodipine

MATERIAL AND METHODS

Instrumentation and reagents: The HPLC system – Shimadzu was employed for the chromatographic separation that was integrated with UV detector along with autosampler. An LC software program was used for gathering and processing the analysis data. Pure samples of Amlodipine and Atenolol were obtained from Arene life science ltd. And Kopran research lab. HPLC grade methanol and acetonitrile were used (from merk life science) and other reagents were of AR grade including potassium dihydrogen phosphate. Throughout the work, milli Q water has been used for preparations and in the study, tablets containing 5 mg Amlodipine and 50 mg Atenolol was employed.

Preparation of mixed Standard solution: Atenolol stock solution (A) having 1000µg/ml concentration and Amlodipine stock solution (B) having 250µg/ml concentration were prepared by dissolving respective working standard with diluent A and at temperature, not surpassing 10˚C sonicated for few minutes to dissolve. For preparation of mixed standard solution from stock solution A – 5ml and from stock solution B -2ml were pipette out and transferred into volumetric flask of 100ml

including diluent A volume was made up to the mark.

Test solution: five tablets were transferred after weighing into a volumetric flask of 250 ml and about 150ml of diluents A was added. At temperature, not surpassing 10˚C it was sonicated to dissolve and using diluent A volume was made up to 250ml. For 5 minutes at 1000 RPM, the solution got centrifuged. Thereafter, into a volumetric flask of 100ml, 5 ml of supernatant was taken and with diluent B volume was made up.

Optimized chromatographic conditions: The stationary phase enabling analytes chromatographic separation was a ODS-A C18 (250 mm x 4.6 mm, 5m) column maintained at temperatures of 35˚C. With the U.V detector, the analysis was reported at 228 nm. For injecting samples, 20µl of injection volume was selected. Flowing at 0.9 ml/min, a gradient mobile phase comprising of Buffer, Acetonitrile, and Methanol was utilized.

METHOD VALIDATION

The process confirming that analytical procedures utilized to perform a certain test are appropriate in order to be used for the purpose for which they were developed is called method validation. ICH guidelines were used to validate the developed method for all parameters.

The system suitability test is done to demonstrate the proper functioning of the system prior to HPLC analysis. Prior to beginning analysis, the acceptance criteria of system suitability testing must be met. 5 replicate injections of a mixed standard solution having 100% concentration of Atenolol and Amlodipine were injected into the HPLC system for assessing the system suitability. In order to determine the method precision, six test solutions were prepared and injected into the HPLC system on same day. Whereas in similar manner six test solutions were prepared and injected into different HPLC systems on a different day by a different analyst. Based on the assay results of these six test preparations, %RSD was calculated to evaluate the precision of the method. In order to assess the method's linearity working test solutions of Atenolol and Amlodipine at various concentrations covering 50-150% were prepared. The responses to these solutions were recorded after they were introduced into the system. The linearity curve was generated by plotting the peak areas of the chromatogram against the concentrations of Atenolol and Amlodipine. To verify the method's accuracy, recovery studies were conducted using a recovery sample in which a placebo was spiked using predetermined quantities of

Atenolol and Amlodipine at a concentration of 50–150 percent of the working standard in triplicates. The method's specificity is utilized to inspect for possible excipients interference with the analytical peak's retention time, potentially altering the analytical method's specificity. In the presence of prevalent excipients that were involved in the formulation of tablets, specificity was determined

RESULTS AND DISCUSSION

The system suitability checks are criteria that ensure proper system performance. The peak area percent RSD, USP tailing factor, as well as theoretical plates, were all estimated as instrument performance characteristics. The percent RSD for Atenolol and Amlodipine peak areas was below 2%, with values of 0.104 and 0.208 respectively. Mean USP tailing factor for Atenolol was 1.485 and for Amlodipine was 1.471. With theoretical plates above 2000 for both. The acceptance requirements were met by all of the parameters assessed. The findings revealed that for the analysis the chromatographic system was suitable. For method precision and intermediate precision, Atenolol & Amlodipine have % content between 90 to 110 %, and the RSD percentage of results was within a 2% limit that made it evident that the method developed is precise. The **Table 1** represents the method and intermediate precision. In the concentration range of 25 to 75µg/ml and 2.5 to 7 µg/ml for Atenolol and Amlodipine respectively a linear relationship of the drug concentration with peak areas was observed. **Table 2** provides data for linearity. The method is said to be linear as the data obtained is within the specification limits. Amounts of Amlodipine and Atenolol that were recovered (accuracy) ranged from 99.88% to 101.66% for Atenolol and 99.34% to 102.54% for Amlodipine. **Table 3** reports the accuracy (recovery) result. The presented method is found to be extremely accurate as a result of the high percentage of the recovery. Since extra peaks were not found indicating that there were no interference due to the presence of excipients contained in tablets showing that for the drugs being studied, the method is unique or specific **(Figure 3)**.

Table 1: Method and Intermediate precision results of Atenolol and Amlodipine

Drug	Method precision			Intermediate precision		
	%assay (n=6)	S.D	% RSD	%assay (n=6)	S.D	% RSD
Atenolol	98.25	0.71	0.72	98.72	0.76	0.77
Amlodipine	98.46	0.67	0.68	98.94	0.63	0.64

Table 2: Linearity data for Atenolol and Amlodipine

Parameter	Atenolol	Amlodipine
Linearity range	25 to 75µg/ml	2.5 to 7 µg/ml
Slope	50155	57002
Intercept	30395	8535
Correlation coefficient	0.999	0.999

Table 3: Accuracy (recovery) result of Atenolol and Amlodipine

Spike level %	Atenolol			Amlodipine		
	Recovery Mean % (n=3)	S.D	% RSD	Recovery Mean % (n=3)	S.D	% RSD
50	100.88	0.39	0.38	102.21	0.33	0.3
100	100.5	0.56	0.56	100.04	0.61	0.61
150	101.31	0.32	0.31	99.78	0.41	0.41

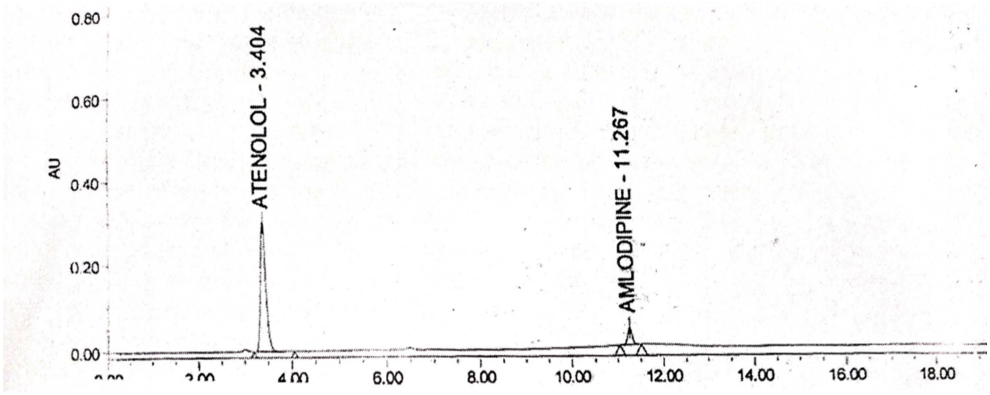

Fig. 3: chromatogram for specificity

CONCLUSION

It was discovered, based on the findings, that a unique, efficient, as well as highly reliable RP-HPLC method estimating quantitatively Amlodipine and Atenolol simultaneously in pharmaceutical tablet dosage form employing gradient program, confirmed being linear, specific, accurate, precise and also fit the purpose for which it was intended. This study completed provides documented evidence of the analytical method being effective in analyzing these drugs quantitatively therefore can be readily implemented as a routine estimation method

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