



METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ATENOLOL IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, rapid, precise and accurate reverse phase high performance liquid chromatography has been developed and validated for the estimation of Atenolol in tablet formulations. Separation was carried on Waters e 2695 Isocratic HPLC system separation module with EMPOWER 2 software, PDA detector waters 2998 and INERSIL ODS C18 analytical column, (4.6 x 250* 5µm), was operated in isocratic mode using water and acetonitrile (50:50v/v) as mobile phase and at a flow rate of 1.2 ml/min with detection wavelength of 228 nm, by an injection volume of 20 µl and entire separation was carried out at an ambient temperature for the HPLC system. The linearity was found in the range of 50-150 µg/ml and showed a correlation coefficient of 0.9981. The retention time of Atenolol was found to be 1.2. This study concluded that the proposed method was found to be accurate, reproducible, and consistent. The proposed method can be effectively used for the routine analysis of the drug in marketed formulations.

KEY WORDS: Atenolol, RP-HPLC, Method Development, Validation.

INTRODUCTION

Atenolol (ATN) is chemically¹, 2-[4-[(2 RS)-2-hydroxy-3-(1-methylethyl) amino] propoxy phenyl] acetamide (Fig 1), it is a beta adrenergic blocker for the treatment of hypertension and acts by lowering of the systolic and diastolic blood pressure by 15 to 20% in a single drug treatment. The important analytical functional groups present in Atenolol are hydroxyl, aminopropoxy, acetamide, and the literature survey suggested carrying out the separation in Reverse mode³. There are no specified and selective method for the estimation of Atenolol and a very few methods are available for the simultaneous estimation of ATN which include Simultaneous UV spectrophotometric methods for estimation of atenolol and amlodipine besylate in combined dosage form⁴, Analytical Method development and Validation of Losartan Potassium and Atenolol in combined dosage form by RP-HPLC⁵. From the validated data as per ICH guidelines the proposed method is simple, rapid, precise accurate and economic for Atenolol and this method can be effectively used for the routine analysis of these drugs in marketed formulations⁶.

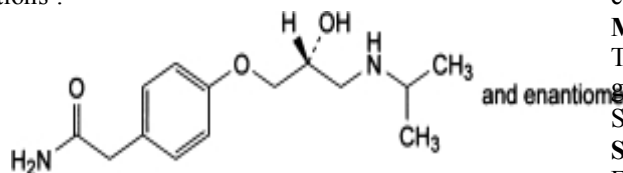


Figure 1 Structure of Atenolol

MATERIALS AND METHODS

Atenolol is given by Lyka laboratories, Mumbai. Atenolol , brand names of Atenolol Atormin(PCI pharmaceuticals limited), Benol-50(Indica laboratories PVT LTD), Tenormin(CIPLA,RANBAXY,ZYDUS) all are containing 50 mg of Atenolol were obtained from local pharmacy. Water used was of HPLC grade, Methanol was of analytical grade. The instruments used were waters electronic

balance, Elico pH meter, Soltec ultra sonicator, Waters e 2695 Isocratic HPLC system separation module EMPOWER 2 software, PDA detector waters 2998 and Analytical column was INERSIL ODS C18, (4.6 x 250* 5µm).

METHOD DEVELOPMENT

Preparation of Standard and Sample solutions

Procedure for Calibration curve of Atenolol

Accurately weighed quantity of 100 mg of Atenolol was dissolved in 100 ml volumetric flask with the mobile phase water and acetonitrile (50:50v/v). From this stock solution, concentrations of 50, 75, 100, 125 and 150 µg/ml solutions were prepared and constructed the calibration curve at a detection wavelength of 228 nm, which was used for estimation of marketed samples (Fig 3).

Assay Procedure for Sample Solution preparation:

Accurately weighed quantity of equivalent powder of 50 mg of Atenolol from 20 tablets of different tablets was dissolved in mobile phase water and acetonitrile (50:50v/v) in 100 ml volumetric flask and further diluted to fall in working range concentration for the estimation by using the calibration curves.

METHOD VALIDATION

The proposed method was validated according to ICH guidelines in terms of parameters like Accuracy, Precision, Specificity, Linearity, LOD, LOQ etc.

System Suitability Parameters

For system suitability, five replicates of standard solutions of Atenolol injected studied the suitability parameters like Plate number (N), Resolution (R) and Relative retention time (α), and Peak symmetry of samples (As) were studied with the help of standard chromatograms (Table 1).

Linearity and Range

The linearity of calibration curves (analyte to peak area ratio Vs concentration) in pure solution was checked over the concentration ranges of 50-150 µg/ml for Atenolol. The linearity was evaluated by linear regression analysis, using least squares method The calibration curves were linear in the

studied range and equations of the regression analysis obtained for Atenolol $Y = 31752 X + 60498$. Correlation coefficient values for Atenolol found to be 0.9981 (Table 2).

Accuracy

To study reliability, suitability and accuracy of the method, recovery studies were carried out, by adding a known quantity of the standard to the pre analyzed sample. The recovery was carried out at 50%, 100%, 150% level and the contents were determined from respective chromatogram. From the results obtained we conclude that the method was accurate (Table 3).

Precision

The Precision of test method was done by performing assay on five replicate determination of sample preparation at test concentration level (as per method of analysis) and calculated relative standard deviation of assay results. Five injections from standard solutions were injected and the peak areas were obtained and %RSD was calculated (Table 4).

Limit of Detection

Limit of detection is the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The minimum concentration at which the analyte can be detected was determined from the linearity curve by applying the formula (Table 1).

$$LOD = 3.3 SD/slope.$$

Limit of Quantitation

Limit of quantitation is the lowest concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantitation can be obtained from linearity curve by applying the following formula (Table 1).

$$LOQ = 10 SD/slope.$$

Table 1: System suitability Parameters

System Suitability Parameters	Atenolol
Linearity range	50-150 µg/ml
Tailing Factor	1.53
Number of theoretical Plates	2012
Retention time	1.200
LOD	0.0000915µg
LOQ	0.000305µg

Table 2: Linearity Data for Atenolol

Concentration of Atenolol (µg/ml)	Peak Area of Atenolol
50	363369
75	2512977
100	3225604
125	4114236
150	4716789

Table 3: Recovery studies for Atenolol

s.no	Inj.Sample	Spike level	Amount Present (µg/ml)	Amount Recovered	% Recovered
1	Atenolol	50 %	55.8442	57.1612	102.36
2		100 %	98.9487	101.3599	102.44
3		150 %	148.423	148.3834	99.97

Table 4: Precision of Atenolol

s.no:	Area of Atenolol
1	3291693
2	3291308
3	3330193
4	3294733
5	3298244
6	3286053
Mean	3298704
S.D	15946
%RSD	0.5

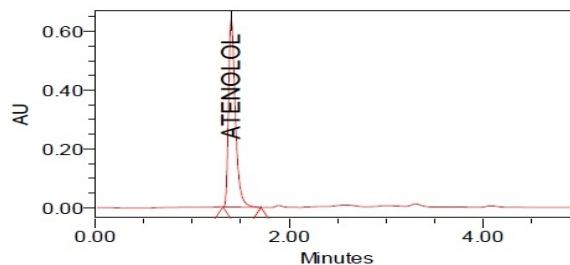


Figure 3a Standard chromatogram of Atenolol

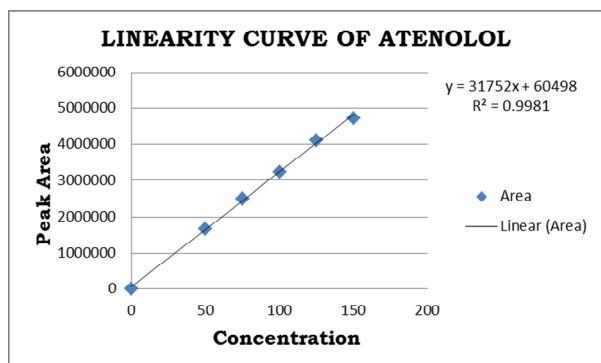


Figure 2 Linearity plot for Atenolol

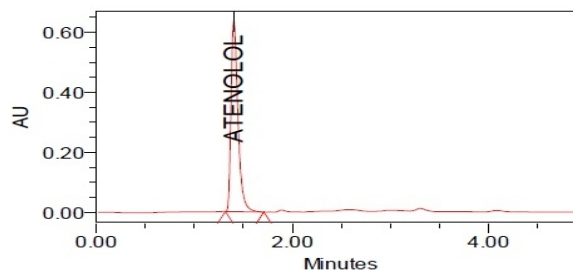


Figure 3b Sample chromatogram of Atenolol

RESULTS AND DISCUSSION

The Separation was carried on Waters e 2695 Isocratic HPLC system separation module with EMPOWER 2 software, PDA detector waters 2998 and INERSIL ODS C18 analytical column, (4.6 x 250* 5µm), was operated in isocratic mode using water and acetonitrile (50:50v/v) as mobile phase and at a flow rate of 1.2 ml/min with detection wavelength of 228 nm, by an injection volume of 20 µl and entire separation was carried out at an ambient temperature for the HPLC system.

Under the described experimental conditions, sharp peaks that belong to Atenolol were obtained at retention time of 1.2 min. System suitability studies were carried out in which the resolution between the peaks, tailing factor and number of theoretical plates were found and are presented (Table 1). The Linearity (Table 2) was obtained in the concentration range 50 to 150 µg/ml for Atenolol, with correlation coefficient of 0.9981. The Accuracy of the method was determined by performing recovery studies at 50%, 100%,

150% were found to within the limits (Table 3). The precision of the method was also found to be good (Table 4). The Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method (Table 1).

CONCLUSION

This study concluded that the proposed method was found to be accurate, reproducible, and consistent and could be effectively used for the routine analysis of these drugs in marketed formulations.

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