**ABSTRACT**

A simple, rapid, precise and accurate reverse phase high performance liquid chromatography has been developed and validated for simultaneous estimation of Atenolol and Hydrochlorothiazide in tablet formulations. HPLC Waters e2695 system equipped with Empower 2 Software with PDA Detector and Xterra ODS C18 column (4.6x 150*5μm particle size) was operated in isocratic mode using water and methanol (50:50v/v) as mobile phase and pumped at rate of 0.8ml/min and eluents were monitored using UV-Visible detector at 230nm. The linearity was found in the range of 50-150 μg/ml and shows a correlation coefficient of 0.999. The retention time of Atenolol and Hydrochlorothiazide was noted to be 1.885 and 2.442 mins, respectively. 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.

**Key Words:** Atenolol, Hydrochlorothiazide, RP-HPLC, Method Development.
INTRODUCTION

UV spectroscopic methods were developed for the simultaneous estimation of atenolol and indapamide, Atenolol and Aspirin, atenolol and amlodipine Besylate, Atenolol and Lercanidipine hydrochloride in bulk and combined tablet. Hydrotrophy method has been used for estimation of atenolol tablets using metformin hydrochloride as hydrotropic solubilizing agent and (LC-MS) method has been developed and validated for quantification of atenolol in human plasma. Atenolol and Losartan potassium has been developed by using RP-HPLC on RP C18 Column. UV-spectrophotometric methods have been developed for simultaneous determination of Nebivolol and Hydrochlorothiazide, in combined pharmaceutical dosage form. HPTLC method was used for determination of Enalapril Maleate and Hydrochlorothiazide in tablet dosage forms. HPLC, high-throughput bioanalytical method was used for Simultaneous estimation of Candesartan Cilexetil and Hydrochlorothiazide in tablet dosage form. Capillary electrophoresis method was used for the simultaneous determination of hydrochlorothiazide and Enalapril. Stability-indicating liquid chromatography method was developed for the quantitative simultaneous estimation of irbesartan (IRB) and hydrochlorothiazide (HCTZ) in combined pharmaceutical dosage form. So far, Atenolol and Hydrochlorothiazide combination using RP-HPLC method was not yet reported in literature.

So, the Objective of the present study was to develop a simple, rapid, specific and sensitive RP-HPLC method for simultaneous determination of Atenolol and Hydrochlorothiazide in tablet dosage form for a routine analysis and to validate the method according to ICH guidelines.

MATERIALS AND METHODS

Atenolol and Hydrochlorothiazide were generously given by Lyka laboratories, Mumbai having 99.66% and 99.33% purity. Atenolol and Hydrochlorothiazide combination tablet (brand name-ATEN-H) containing 50mg of atenolol and 25mg of Hydrochlorothiazide was obtained from local pharmacy. Water used was of HPLC grade, Methanol is of analytical grade. The instruments used are 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 is XTERRA C18, (4.6 x 150* 5µm).

Method Development Trials

50:50 ratios of water and Methanol was sonicated and used as mobile phase and different trails were performed to optimize the method. Trail-1 to 6 were done using INERTSIL ODS C 18 50*4.6*5, Trail-7 and 8 done using XTERA RP 18 ODS 150*4.6*5 and selected as optimised column for this study.

Trail-1: Retention time is very less, two peaks are not eluted, and so further trail is carried out.
Trial-2: Retention time is very less, two peaks are not eluted, and also spikes appeared so further trail is carried out.

Trial-3: Retention time is again decreased. So, further trail is carried out by increasing the injection volume.

Trial-4: Here unknown peaks are appeared so further trail is carried out.

Trial-5: Two peaks are eluted but the retention time is less so further trail is carried out.

Trial-6: Retention time is very less and tailing is also observed

Trial-7: Retention time is good but tailing is observed. So, further trail is carried out by changing the column.

Trial-8: Longer column is used two peaks were identified, the peaks of Atenolol and hydrochlorothiazide were good and retention time of both peaks was 1.885 and 2.442, there is a good resolution of 3.32. Among the 8 trials, the 8th trial was selected as the peaks are good with less retention time.

Preparation of Solutions

Mobile phase is prepared by mixing 500 ml of methanol and 500 ml of water (50:50). Standard was prepared by accurately weighing and transferring 28.1 and 50 mg of atenolol and hydrochlorothiazide working Standard into a 100 ml clean dry volumetric flask. 50 ml of diluent was added and sonicated for 10 minutes, and make up the volume to 100 ml. Sample preparation was done by powdering 3 tablets. Transferred the 150 mg weight of sample into 50 ml of clean, dry, volumetric flask and to this, added 20 ml of diluent and sonicated for about 20 minutes, further make up the volume with diluent. Pipetted out 5 ml of above solution into 50 ml of volumetric flask and made up to 50 ml and filtered through 0.45 micron filter.

Method Validation

After the method development the method was validated 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 and Hydrochlorothiazide were injected and 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.

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 and Hydrochlorothiazide respectively. The linearity was evaluated by linear regression analysis, using least squares method.

ACCURACY

Accuracy expresses the closeness of agreement between the value, which was accepted either as conventional true value or and accepted reference value (International standard e.g. pharmaceutical standard) and the value found (mean value) obtained by applying the test procedure a number of times. 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 analysed sample and recovery study were done. The recovery was carried out at 50%, 100%, 150% level and the contents were determined from respective chromatogram.

**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.

**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 is determined from the linearity curve by applying the formula.

\[ \text{LOD} = 3.3 \frac{\text{SD}}{\text{slope}} \]

**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.

\[ \text{LOQ} = 10 \frac{\text{SD}}{\text{slope}} \]

DISCUSSION AND CONCLUSION

In the present work, conditions were optimized for development and validation of a simple and accurate HPLC method for simultaneous estimation of Atenolol and Hydrochlorothiazide in combined pharmaceutical drug formulation. RP-HPLC method was developed by performing 8 trials. Finally, the chromatographic conditions optimised are XTERRA C18 column at 230nm using 50:50 ratio of water and methanol with flow rate of 0.8ml and injection volume of 20µl at run time for 5 minutes. The system suitability was evaluated by injecting five replicates of standard solutions of atenolol and Hydrochlorothiazide. The linearity of calibration curves of atenolol and Hydrochlorothiazide were checked over concentration ranges of 50-150µg/ml and linearity curves were plotted as shown in Figure.2, 3. Percentage recovery studies were carried out using spike level of 50%, 100% and 150% levels and the mean recovery was found to be 101,100 and 101% for atenolol and 102,101 and 100% for Hydrochlorothiazide and tabulated in Table.1. The precision was carried out by using 6 replicates of standard solution. The peak areas were obtained and the percentage RSD was found to be 0.3 for both atenolol and Hydrochlorothiazide which is shown in Table.2. The LOD values for atenolol and Hydrochlorothiazide were 0.00006µg and 0.00003µg and LOQ values were found to be 0.0002µg and 0.0001µg for atenolol and Hydrochlorothiazide respectively. 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.

REFERENCES


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<th>Amount Recovered (µg)</th>
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Table 1. Recovery studies for Atenolol and Hydrochlorothiazide
Table 2. Recovery studies for Atenolol and Hydrochlorthiazide

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Fig.1. Chromatogram of Standard- I (Atenolol & Hydrochlorthiazide)
Fig. 2. Linearity plot for Atenolol

Fig. 3. Linearity plot for Hydrochlorothiazide