Development and Validation of a Stability-Indicating RP-HPLC Method for the Determination of Alfuzosin Hydrochloride in Bulk and Tablet Dosage Form

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ABSTRACT

A specific and accurate HPLC method is developed for the determination of Alfuzosin inbulk drugs and in solid tablet dosage form. Best symmetric peak shape obtained with Symmetry C18, 150x4.6 mm, 5 μm column in an isocratic mode, with retention time 15 min.The mobile phase used was buffer, acetonitrile and tetrahydrofuran in the ratio of 810:180:10 v/v/v with flow rate 1.5 ml/min and wavelength monitored at 245 nm. As per ICH guidelines method has validated. Method has found linear in the range of 25-75 μg/ml. Method was found specific with respective of diluents, excipients and degradants.

Keywords: Alfuzosin Hydrochloride (ALH); Limit of Detection (LOD); Limit of Quantitation (LOQ)
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INTRODUCTION

Alfuzosin hydrochloride (ALH) is chemically known as N-[3-[(4-amino-6, 7-dimethoxy-quinazolin-2-yl)-methyl amino] propyl] tetrahydrofuran-2-carboxamide hydrochloride with an empirical formula of $C_{19}H_{27}N_5O_4 \cdot HCl$ (Figure 1) and molecular weight is 425.9 g/mol. ALH is alpha- adrenergic blockers [1] and relaxes the muscles in the prostate and bladder neck, making it easier to urinate. It is used to improve urination in men with benign prostatic hyperplasia [2-10].
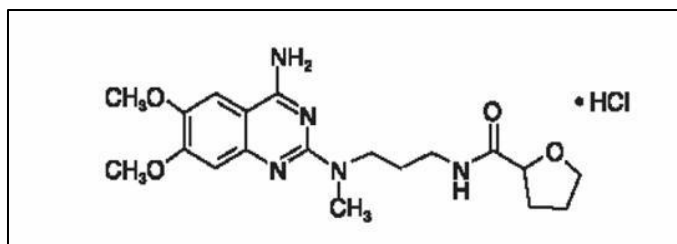

Figure 1: Alfuzosin hydrochloride

EXPERIMENTAL SECTION

Chemicals and reagents
Alfuzosin HCl was obtained as a gift sample from M/s Dr.Reddy's Laboratories Ltd, Hyderabad. Acetonitrile, Methanol (Finar) and water (Milli Q) used were of HPLC grade (Merck). Sodium perchlorate Potassium dihydrogen Orthophosphate and Ortho- Phosphoric Acid were obtained from Merck, Mumbai. Commercially available tablets (Votrient®- GSK RxIndia) were procured from local market.

Chromatography instrument

Quantitative HPLC was performed on liquid Chromatography, Alliance 2695, PDA detector module equipped with automatic injector with injection volume 25 µl, and 2487 UV-Visible Detector. A Symmetry C18, 150x4.6 mm, 5 µm was used. The column was maintained at 40ºC and eluted under isocratic conditions over 15.0 min at a flow rate of 1.5 ml/min.

Preparation of buffer

Weighed and transferred 6 g of sodium perchlorate into a beaker containing 1000 ml of water and sonicated to dissolve. Adjusted the pH of the solution to 3.5±0.05 with dilute perchloric acid and filtered the solution through 0.45µm membrane filter.

Preparation of mobile phase

Prepare a degassed mixture of buffer, acetonitrile and tetrahydrofuran in the ratio of 85:14:10 v/v/v.

Preparation of standard solution

Accurately weighed and transferred about 50 mg of Alfuzosin Hydrochloride working standard into a 100 ml volumetric flask and diluted to make up the volume with mobile phase and mix. 5.0 ml of the above solution was transferred into a 50 ml volumetric flask. Diluted to volume with mobile phase and mixed well.

Preparation of sample solution

Ten Alfoo tablets are weighed and then powdered. Accurately weighed and transferred tablets powder equivalent to about 20 mg of Alfuzosin Hydrochloride into a 200 ml volumetric flask. Later 120 ml of mobile phase was added and sonicated for not less than 10 minutes (maintain the sonicator bath temperature between 20-25°C) with occasional shaking. Dilute to volume and mix. Transfer 5.0 ml of the clear supernatant solution into a 10 ml volumetric flask, dilute to volume with mobile phase and mix.

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RESULTS AND DISCUSSION

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HPLC method development and optimization

Optimization of mobile phase and method development: Optimization of mobile phase was performed based on trial and error method. A series of trials were conducted in order to get proper optimized HPLC conditions. In the first instance several mobile phase compositions were tried such as buffer: methanol, buffer: acetonitrile in different ratios. Finally the mobile phase comprising of buffer and acetonitrile with Tetrahydrofuran in the ratio of 81:18:1 v/v was found to have sharp peak (Figure 2).

The optimized chromatographic conditions for the determination of Alfuzosin are represented in Table 1. After completion of method development and optimization method was validated as per ICH guideline [1]

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Table 1: Optimized chromatographic conditions of Alfuzosin

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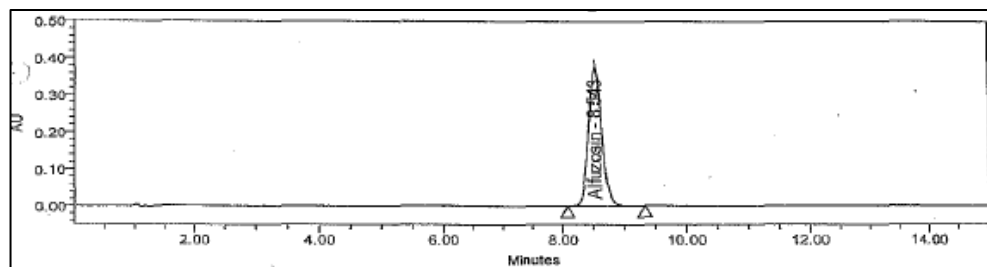|	
Column	Symmetry C18, 150×4.6mm, 5µm
Pump mode	Isocratic
Wavelength	245 nm
Mobile phase	Buffer :Acetonitrile 85:14:1 (v/v)
Injection Volume	25µl
Column Temp	40ºC
Flow rate	1.5 ml/min
Retention Time	8.5 minutes
Run time	20 minutes

Figure 2: Chromatogram showing the Alfuzosin at Retention time 8.5min

Validation

Linearity and range

Aliquots of standard Alfuzosin hydrochloride stock solution was taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Alfuzosin was in the range of 25-75 μg/mL. Each of these drug solutions (10 μL) was injected into the column, and the peak areas and retention times were recorded. Evaluation was performed with UV detector at 245 nm and the Calibration graph was obtained by plotting peak area versus concentration in μg/mL of Alfuzosin (Figure 3).

The plot of peak areas of each sample against respective concentration of Alfuzosin was found to be linear in the range of 25-75 μg/mL with correlation coefficient of 0.999 (Figures 4 and 5).
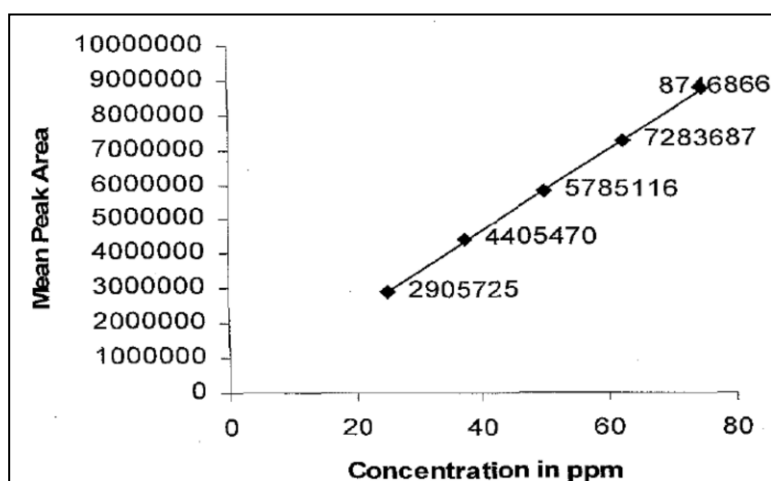

Figure 3: Calibration curve for Alfuzosin

Accuracy

Accuracy of the method was determined on the basis of recovery studies performed by standard addition method at different level of labeled claim (50%, 100% and 150%) of standard. Percentage of recovery for each case was calculated and was found to be 99.8 to 101.2. This was found to be well within the acceptance criteria of 98-102%.

Precision

The system precision of the proposed method was determined by injecting standard solution for five times and measured the area in HPLC. The method precision of the proposed method was determined by injecting six sample solutions into HPLC prepared individually. The % RSD for the areas of system precision and method precision were calculated and results are found to be 0.06 and 0.128 which were within the limit of 2.0%

Robustness

Robustness was studied by change in flow rate, Mobile phase variation, pH of the buffer. The percent relative standard deviation (% RSD) was calculated and it was found to within the range of 0.06 – 0.74 which is well within the range of %RSD 2.

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Specificity

Prepared blank solutions was inject into the chromatographic system. Blank chromatograms show no peak at the retention time of Alfuzosin due to impurities.
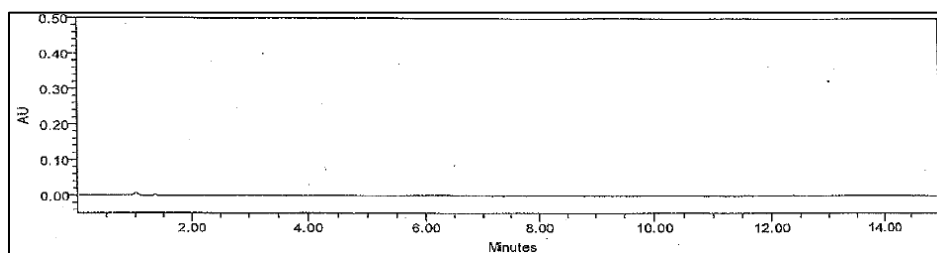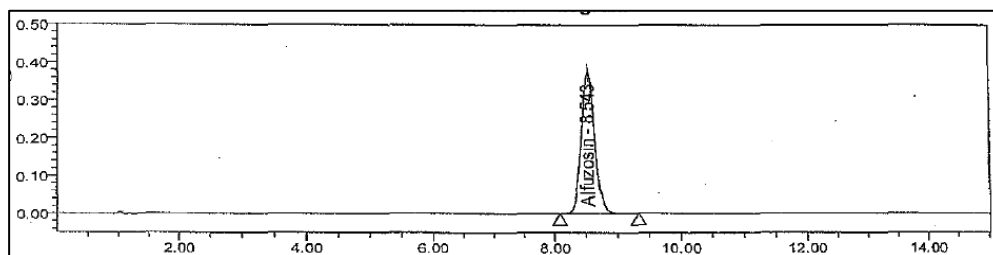

Figure 4: Blank chromatogram

Figure 5: Chromatogram of standard solution

Forced degradation studies

In order to establish stability indicating nature of the method, forced degradation studies was done i.e. Acid, Base and peroxide degradations have been carried (Figures 6 and 7).

From the studies found that there is no possible degradation occurs during acid, base and peroxide degradations. Also there is no co-eluting peaks with the Alfuzosin. Peak purity of Alfuzosin has passed. The Purityangle was found to be lessthan the purity threshold. Results of the forced degradation studies have been provided in Table 2.

Degradation Condition	Peak purity
Acid degradation	Passed
Base degradation	Passed
Peroxide degradation	Passed

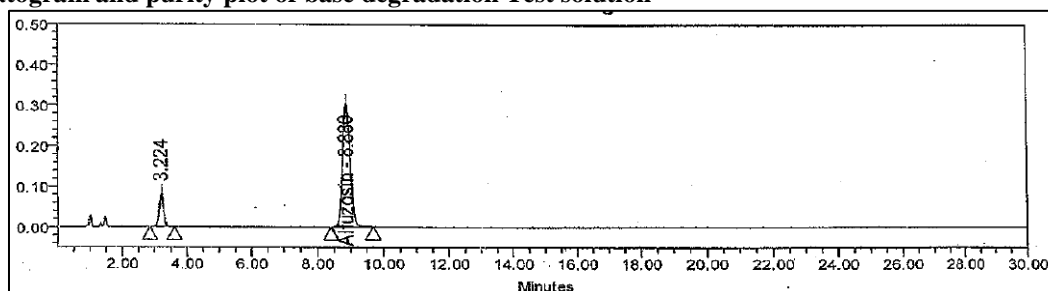**Table 2: Forced degradation studies results**

Chromatogram and purity plot of base degradation Test solution

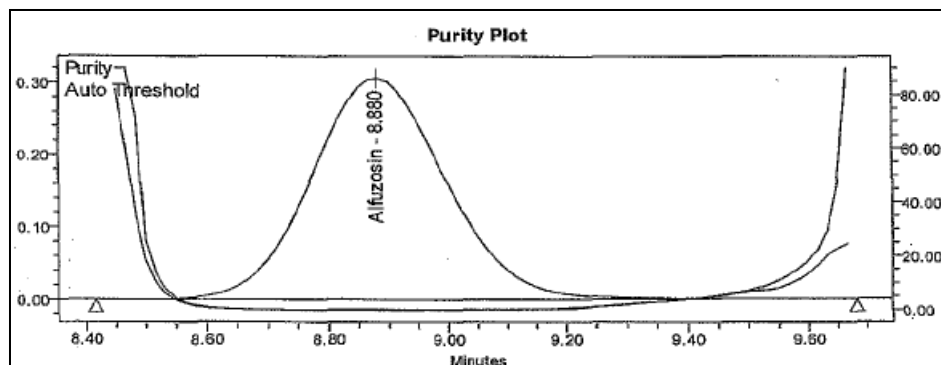**Figure 6: Chromatogram of Base degradation test sample**

Figure 7: Purity plot of Base degradation test sample

CONCLUSION

A simple Specific stability indicating liquid chromatographic method is developed for the quantification of Alfuzosin hydrochloride in Pharmaceutical dosage forms. This method is validated and it is found to be Specific, precise, accurate, Robust and linear for estimation of Alfuzosin hydrochloride. The method is stability-indicating and can be used for routine analysis of production sample and to check the stability samples of Alfuzosin hydrochloride in Pharmaceutical dosage forms.

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