Int. J. Chem. Sci.: 8(3), 2010, 1464-1472

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF ALFUZOSIN HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, rapid, accurate, precise and reproducible reverse phase high performance liquid chromatographic method has been developed for the estimation of alfuzosin hydrochloride (AFZ) in bulk and in pharmaceutical formulations. The quantification was carried out using Cyberlab capcell pak, ODS C_{18} (250 × 4.6 mm i.d., 5 µm particle size) column in an isocratic mode, with mobile phase comprising water : acetonitrile : methanol in the ratio of 75 : 15 : 10 (% v/v/v).The flow rate was at 0.8 mL/min and the detection was carried out at 246 nm. The retention time of the drug was found to be 2.59 min and the method produced linear response in the concentration range of 2-12 µg/mL (R ~ 0.9997). The recovery studies were also carried out and % RSD from reproducibility was found to be 0.334. The proposed method was statistically evaluated and can be applied for routine quality control analysis of alfuzosin hydrochloride in tablets.

Key words: RP-HPLC, Alfuzosin hydrochloride, Tablets.

INTRODUCTION

Alfuzosin hydrochloride[1] (Fig. 1) is chemically, (R, S)-N-[3-[(4-amino-6, 7-dimethoxy-2-quinazolinyl) methyl amino] propyl] tetrahydro-2-furancarboxamide hydrochloride. It is a selective antagonist of post-synaptic alpha$_1$-adrenoreceptors[2], which are located in the prostate, bladder base, bladder neck, prostatic capsule, and prostatic urethra. It is indicated for the treatment of the signs and symptoms of benign prostatic hyperplasia. It acts by selective blockade for alpha$_1$ -adrenergic receptors in the lower urinary tract, which causes smooth muscle in the bladder neck and prostate to relax, resulting in improved urine flow and a reduction in symptoms of benign prostatic hyperplasia (BPH).

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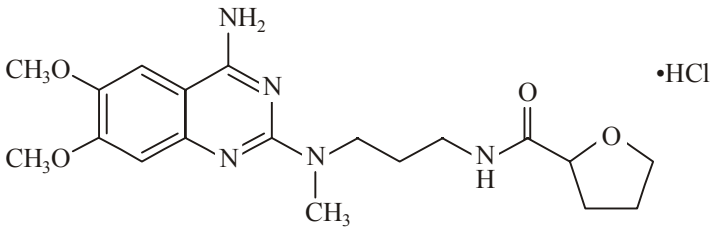

Fig. 1: Structure of alfuzosin hydrochloride

Literature survey reveals that few analytical methods have been reported for the determination of alfuzosin hydrochloride (AFZ) in pharmaceuticals and biological fluids. AFZ was determined in human plasma by LC-MS/MS[3], chiral HPLC[4,5], HPLC method using fluorescence detection[6,7], HPTLC method[8] and spectrophotometry[9-11].

The objective of this study is to develop a simple, fast, selective, accurate, precise and sensitive RP-HPLC-UV method for the determination of alfuzosin hydrochloride in bulk and in pharmaceutical dosage forms (tablets) suitable for routine quality control analysis.

EXPERIMENTAL

Chemicals and reagents

Alfuzosin hydrochloride working standard was received as gift sample from Dr. Reddy's Laboratories Ltd., Hyderabad, India. Alfusin-10 mg tablets, manufactured by Cipla Ltd and Alfoo 10 mg tablets, manufactured by Dr. Reddy's Laboratories Ltd., Hyderabad, India were procured from local pharmacy. HPLC grade water, methanol and acetonitrile were purchased from Merck, Mumbai, India.

Instruments and chromatographic conditions

The method development study was carried out isocratically on a high performance liquid chromatograph using Cyber Lab LC-100 separation module equipped with a Rheodyne injector 7725i, single pump, 20 µL fixed sample loop, 25 µL Hamilton syringe and detection was carried out using Ultraviolet detector. Cyberlab digital balance was used for weighing purpose.

Chromatographic separation was carried out at room temperature with Capcell Pak ODS C_{18} (250 × 4.6 mm with 5 µm particles) column. Mobile phase containing water : acetonitrile : methanol in the ratio of 75 : 15 : 10 (% v/v/v), was filtered through 0.45 µ membrane filter and degassed in a sonicator for 10 min before use. The flow rate of mobile

phase was maintained at 0.8 mL/min and detection was done using UV detector at 246 nm. The injection volume of both; standards and samples were 20 µL (10 µg/mL).

Procedure

Preparation of standard

A stock solution containing 1 mg/mL of alfuzosin hydrochloride was prepared by completely dissolving 100 mg of pure drug in 100 mL of distilled water. A working standard solution containing 100 µg/mL was prepared by diluting 5 mL of stock solution (1000 µg/mL) into 50 mL of distilled water. Linearity solutions ranging 2-12 µg/mL of alfuzosin hydrochloride were prepared from the above working standard solution (100 µg/mL) by diluting in 10 mL volumetric flask with mobile phase. Initially, the mobile phase was pumped for 30 min to saturate the column thereby to get the baseline corrected as shown in Fig. 2. Then solutions prepared as above were filtered through 0.45 µ membrane filter and then 20 µL of the filtrate was injected each time into the column at a flow rate of 0.8 mL/min. Evaluation of the drug was performed with UV-Visible detector at 246 nm after the drug solution of 10 µg/mL in distilled water was scanned in UV-Visible spectrophotometer SL-164 in the range of 200-350 nm against distilled water as blank and λ_{max} was found at 246 nm (Fig. 3). Peak area was recorded for all the peaks. The plot of peak area vs. the respective drug concentration gives the calibration curve. The retention time of alfuzosin hydrochloride standard was found to be 2.59 minutes (Fig. 4).

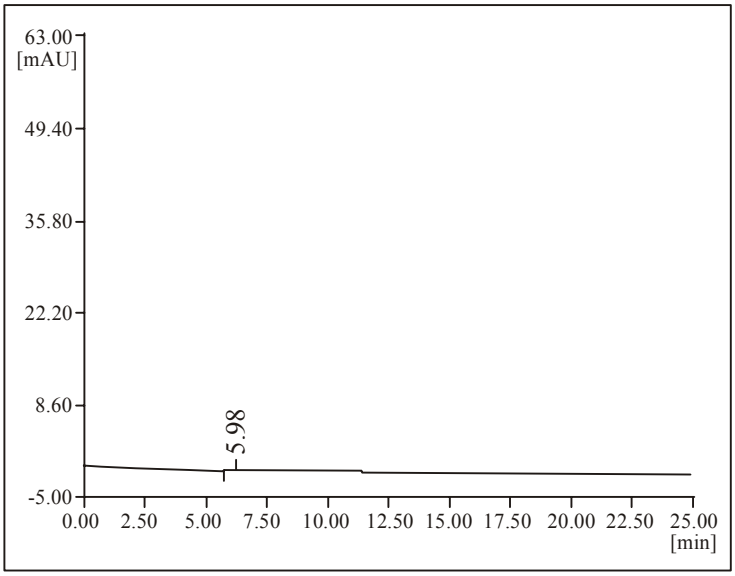

Fig. 2: Chromatogram of alfuzosin hydrochloride blank

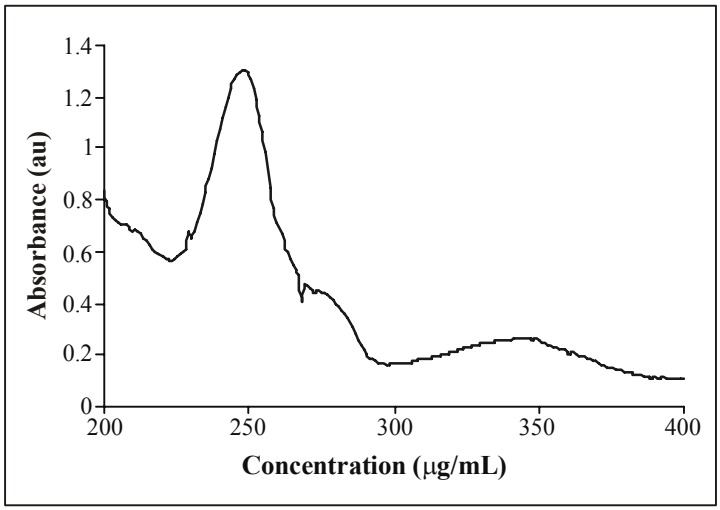

Fig. 3: Absorption spectrum of alfuzosin hydrochloride (10 µg/mL) in distilled water

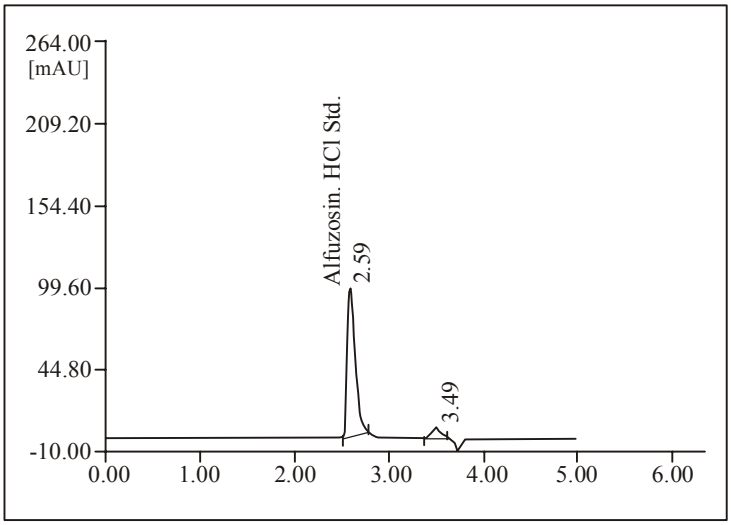

Fig. 4: Chromatogram of alfuzosin hydrochloride standard

Analysis of alfuzosin hydrochloride in tablet dosage forms

Twenty tablets each containing 10 mg alfuzosin hydrochloride were accurately weighed and powdered. A quantity of the powder equivalent to 50 mg was taken into a 50 mL volumetric flask and 30 mL distilled water was added. Then solution was sonicated for 10 min., dissolved and then made upto the volume with the distilled water and filtered through a 0.45 µ membrane filter. Then 10 mL of the above filtrate was transferred into a

100 mL volumetric flask and diluted to the mark with distilled water to obtain working standard solution of 100 µg/mL. This solution (10 mL) was further diluted to 100 mL with mobile phase to obtain a concentration of 10 µg/mL. Then 20 µL of the above solutions were injected each time into the column at a flow rate of 0.8 mL/min. The retention time of alfuzosin hydrochloride samples were found to be 2.57 and 2.59 minutes as shown in Fig. 5 and Fig. 6.

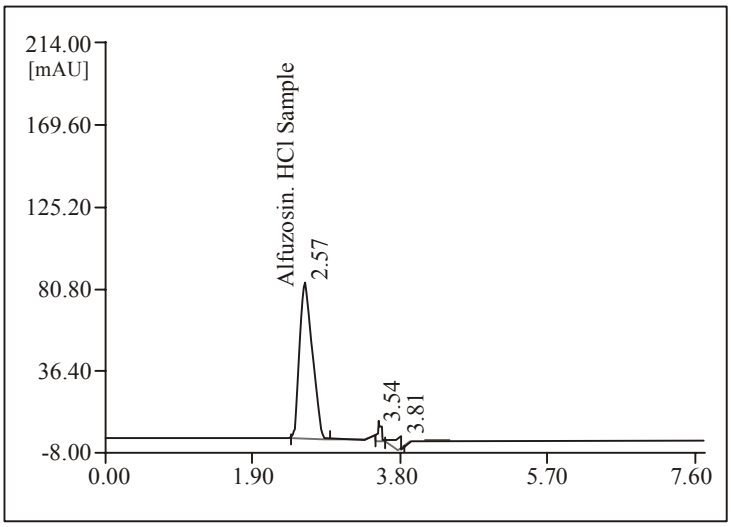

Fig. 5: Chromatogram of alfuzosin hydrochloride sample-1 (in tablet)

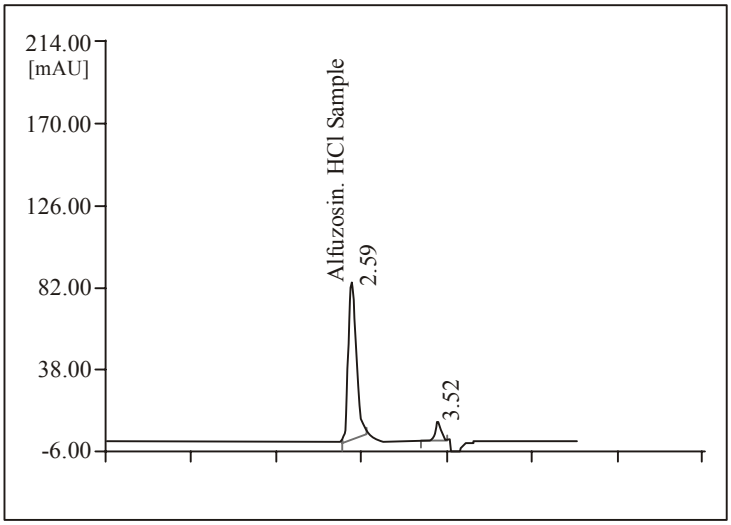

Fig. 6: Chromatogram of alfuzosin hydrochloride sample-2 (in tablet)

RESULTS AND DISCUSSION

The present study was carried out to develop a simple, fast, accurate and precise RP-HPLC method for the analysis of alfuzosin hydrochloride in bulk and in tablet dosage forms. For the determination of alfuzosin hydrochloride, different compositions of mobile phases were employed. Initially, a mobile phase consisting of acetonitrile and water in the ratio of 70 : 30 (% v/v) was tried where broad peak shape and more retention time were observed. Then the composition of mobile phase was changed to methanol and water in the ratio of 60 : 40 (% v/v); but in these conditions, more retention time and tailing were observed. Similarly, different trails were tried by adjusting compositions of water and methanol and water and acetonitile but finally the ratio was changed to 75 : 15 : 10 (% v/v/v) water : acetonitrile : methanol, where alfuzosin hydrochloride was eluted at around 2.59 min with symmetric peak shape and shorter retention time. The results of system suitability parameters are given in the Table 1.

Table 1: Results of system suitability parameters of alfuzosin hydrochloride in standard and in tablet formulations

Parameter	Standard	Sample-1	Sample-2
Retention time (min.)	2.59	2.57	2.59
Peak area response	57277.2	56892.4	56975.6
Theoretical plates (n)	4104.2	3560.8	3922.3
Tailing factor (t)	1.65	1.59	1.68

Linearity was determined from calibration graph plotted using peak area response versus concentration of the standard solutions and it was found to be obeyed in the concentration range of 2-12 μg/mL with a good linear relationship (r = 0.9997) (Fig. 7). The regression curve was constructed by linear regression fitting and its mathematical expression was y = 6046.3 x –3247.27 (where y is the peak area and x is the concentration of alfuzosin hydrochloride).

Precision of the developed method was studied by repeatedly injecting alfuzosin hydrochloride standard and sample solutions for six times (n = 6). The % RSD was found to be 0.334 and 0.527, respectively.

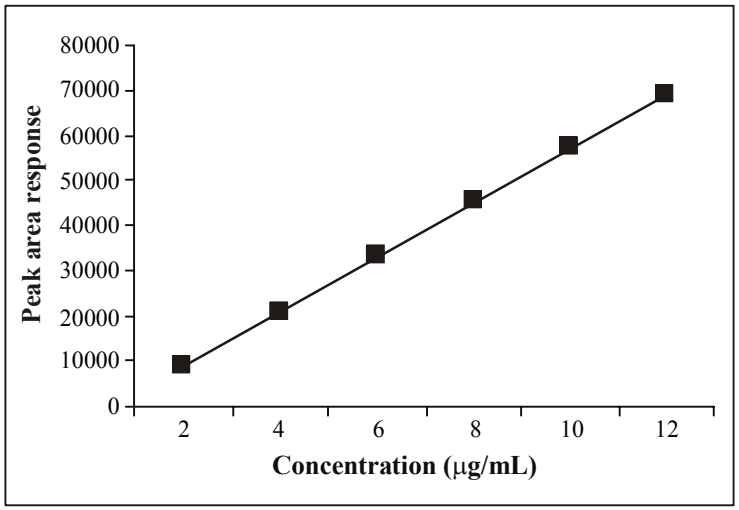

Fig. 7: Linearity graph of alfuzosin hydrochloride

The drug content (assay) in the tablets was quantified using the proposed RP-HPLC method. The mean amount of alfuzosin hydrochloride in two different brands of tablet dosage forms is given in Table 2. The tablets were found to contain 99.3 % and 99.4 % of the drug. It can be concluded that the proposed RP-HPLC method is sufficiently sensitive and reproducible for the analysis of alfuzosin hydrochloride in tablet dosage forms within a short analysis time.

Table 2: Results of assay in marketed formulation

Brand	Standard peak area	Sample peak area	Labelled amount (mg/tab)	Amount found (mg/tab)	% Assay
Alfusin	57277.2	56892.4	10	9.93	99.3
Alfoo	57277.2	56975.6	10	9.94	99.4
			Mean	99.35	
			% RSD	0.07	

The accuracy of the method was evaluated by performing recovery studies by analyzing three different concentration levels ranging from 50-150 % of the test concentrations. The percentage recovery was calculated and results are presented in Table 3.

Table 3: Results of accuracy (Recovery studies, n = 3)

Standard concentration	Amount added (µg/mL)	Amount recovered (µg/mL)	Average % recovery	% RSD
50 %	5	14.97	99.80	0.37
100 %	10	20.12	100.6	0.29
150 %	15	24.98	99.92	0.52

The developed method was validated according to the standard procedure and the summary of results is presented in Table 4.

Table 4: Summary of validation parameters

Parameters		Result
Linearity	Range (µg/mL)	2-12 µg/mL
	Correlation coefficient (r)	0.9997
	Slope	6046.3
	Intercept	-3247.27
	Regression equation	Y = 6046.3 x - 3247.27
System precision (n = 6)	% RSD	0.334
Method precision (n = 6)	% RSD	0.527
Accuracy	Mean % average recovery	99.92
Assay	Mean % assay	99.35
Specificity	Specific	No interference of other peak

ACKNOWLEDGEMENT

The authors are thankful to Dr. Reddy's Laboratories Ltd, Hyderabad, India for providing the gift sample of alfuzosin hydrochloride and also thankful to Nimra College of Pharmacy, Ibrahimpatnam, Vijayawada for providing the necessary facilities to carry out the research work.

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Accepted : 04.03.2010