

QUANTITATION OF ALFUZOSIN HYDROCHLORIDE IN PHARMACEUTICAL FORMULATIONS BY RP-HPLC

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ABSTRACT

An isocratic reversed phase high-performance liquid chromatographic (HPLC) method with ultraviolet detection at 245 nm has been developed for the determination of alfuzosin hydrochloride in dosage formulation. Good chromatographic separation alfuzosin was achieved by using a stainless steel analytical column Inertsil ODS-3V (5 μ m, 15 cm x 0.46 cm). The system was operated at ambient temperature (25 \pm 2 $^{\circ}$ C) using a mobile phase consisting of acetonitrile : water : tetrahydrofuran : perchloric acid (250:740:10:1) at a flow rate of 1 ml/min. The calibration curve for alfuzosin hydrochloride was linear over the tested concentration range of 50%, 75%, 100%, 125% and 150% with reference to the label claim and a correlation coefficient of 0.999. The intra- and inter-run precision and accuracy results were 98.07 to 100.34 with the %RSD of 0.71% and tailings factor 1.07. The proposed method was validated for its selectivity, linearity, accuracy, and precision. The method was found to be suitable for the quality control of alfuzosin hydrochloride in bulk drug as well as in formulation.

Keywords: Alfuzosin, UV detection, RP-HPLC, dosage formulation, method validation.

INTRODUCTION

Alfuzosin hydrochloride or (RS)-N-[3-[(4-amino-6, 7-dimethoxy-2-quinazolonyl) methyl amino] propyl] tetrahydro-2-furanocarboxamide hydrochloride, is a white or off white crystalline powder melts at 240 $^{\circ}$ C. It is quinazolonyl derivative used to treat benign enlargement of the prostate (British Pharmacopoeia 2003, Martindale, The Extra Pharmacopoeia 2005; The Merck Index 2001). In literature some liquid chromatography method (Guine Bault *et al.*, 1986; Rouchouse *et al.*, 1990) and a visible spectrophotometric method (Vamsi Krishna and Gowri Sankar, 2007) for the analysis of Alfuzosin were reported for the determination of alfuzosin and its related substances in biological fluids like plasma, blood, urine and in formulation were reported but not a single method has been reported for its determination in bulk and solid dosage forms by RP-HPLC method. The main purpose of this study was to develop a simple and reliable method to quantitate Alfuzosin hydrochloride in a relatively short time with high linearity. Therefore, this study focused on the development of simple and rapid isocratic RP-HPLC method which can be employed for the routine analysis of Alfuzosin hydrochloride in bulk drug and formulations. The established method was validated with respect to specificity, linearity, precision, accuracy, and ruggedness.

EXPERIMENTAL

Materials and reagents

All chemicals and reagents were of HPLC grade quality. Alfuzosin hydrochloride was supplied from Dr. Reddy's laboratory Limited, Hyderabad, India. A stock solution of 1mg/ml alfuzosin hydrochloride was prepared in acetonitrile and water (1:4). More dilute solutions were made daily with same solvents just before use.

Pharmaceutical dosage form

Tablet label claim of 10 mg alfuzosin hydrochloride was procured from local market.

Chemicals

HPLC grade acetonitrile and phosphoric acid were purchased from Ranbaxy India Ltd., India. Other chemicals used were HPLC grade from Qualigens, Bombay, India. Tablet sample were purchase from market.

Instrumentation and chromatographic conditions

An Agilent HPLC system consisted of Perkin Elmer UV detector, an Inertsil ODS 3V-C18 (5 μ m, 15 cm x 0.46 cm) column was used for separation and the chromatograph were recorded using Empower software. The separation was carried out under isocratic elution with mobile phase was tetrahydrofuran, acetonitrile, water

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Table 1: Linearity, precision and accuracy (recovery) characteristics of alfuzosin

Linearity	Precision of the method		Accuracy of the test Method (Recovery studies)	
Concentration	Inter day*	Intra day** (3days)	Spike level	% Recovered†
25%	0.71%	0.80%	50%	98.5
50%			75%	98.3
100%			100%	98.4
125%			125%	99.0
150%			150%	99.2
Correlation coefficient(r)		0.9998		
Intercept		0.0003		
Slope		1.011		

* %RSD of 10 determinations, †RSD of six determinations at each level.

Table 2: Ruggedness and bench top stability data for alfuzosin

Bench top stability			Deviation from Initial Assay	Ruggedness			
Initial Assay	24hours	48hours		Analyst*		System*	
				1	2	1	2
99.7%	99.9%	101.4	0.2 to1.7	101.2%	99.4%	101.2%	99.4
%RSD (limit NMT 2.0%)				0.71%	0.80%	0.71%	0.8%

*Mean of six determinations.

and perchloric acid (10:220:770:1). The flow rate was 1.0 ml min⁻¹, the analyte was monitored at 245 nm, and the injection volume was 10µl.

Preparation of standard and sample solutions

Standard preparation

A standard stock solution of 25mg of alfuzosin hydrochloride in acetonitrile and water was prepared in volumetric flask. Working solutions of following concentrations 25%, 50%, 100%, 125% and 150% of the labeled amount alfuzosin 10.0mg tablets were prepared by diluting the stock solutions with the same solvent.

Procedure for tablets

Five tablets were weighed transferred into 500ml volumetric flask then 100ml of acetonitrile, water (1:4) was added and kept on a rotary shaker until tablets were disintegrated followed by 250ml of same solvent and sonicated for 30minutes with frequent shaking and the final volume was adjusted with the same solvent. From that a portion was centrifuged at 3500rpm for 20minuts, which was used for the estimation. The desired concentration for the drug was obtained by accurate dilution and the analysis was followed up as in the general analytical procedure.

RESULTS AND DISCUSSION

Development and optimization of isocratic HPLC conditions

A UV scan of alfuzosin hydrochloride showed a maximal absorbance at or near 245 nm. Initial method development

was conducted on a Inertsil, C18 (5µm, 15 cm x 0.46 cm) column was used for separation at ambient temperature. This column provides efficient and reproducible separations of nonpolar compounds. Consequently, it was selected for method development and validation of assay. Preliminary method development of suitable isocratic conditions to resolve alfuzosin hydrochloride on the C18 column was conducted with Acetonitrile: water: tetrahydrofuran at various ratios as the mobile phase for initial method development. A mobile phase of acetonitrile-water-Tetrahydrofuran and Perchloric acid with following ratio 250:740:10:1 was found to provide a reproducible, baseline resolved peak (figs. 1-2). These conditions allowed for separation of alfuzosin hydrochloride from tablet formulation.

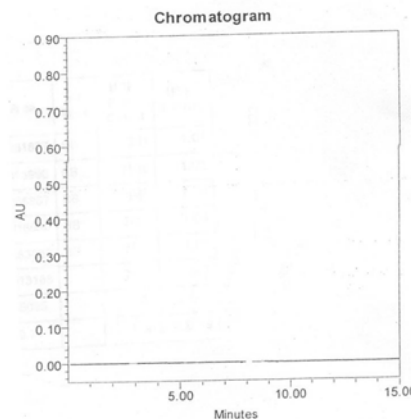


Fig. 1: Blank Chromatogram

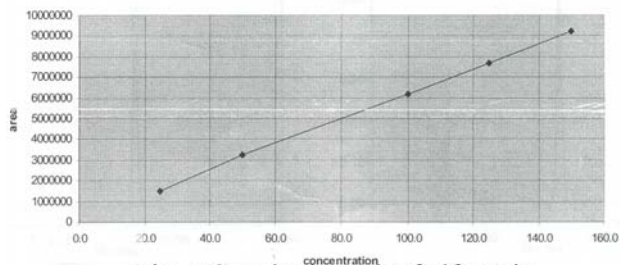


Fig. 2: Standard graph of alfuzosin.

The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. The specificity of the method was established through the placebo study on common additives used for tablets which shows no peak with placebo. For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [ICH 1996] and [USP 2003] have recommended the accomplishment of accuracy tests, precision, specificity, linearity, and robustness of the method. The calibration curve of alfuzosin was 25%, 50%, 100%, 125% and 150% were linear in the range as mentioned above of the labeled amount alfuzosin 10.0mg tablets. The representative linear equation was $y = 1.011x + 0.0003$ ($n = 6$; $r = 0.9999$) where y represents injected concentration and x is the recovered concentration (fig. 3).

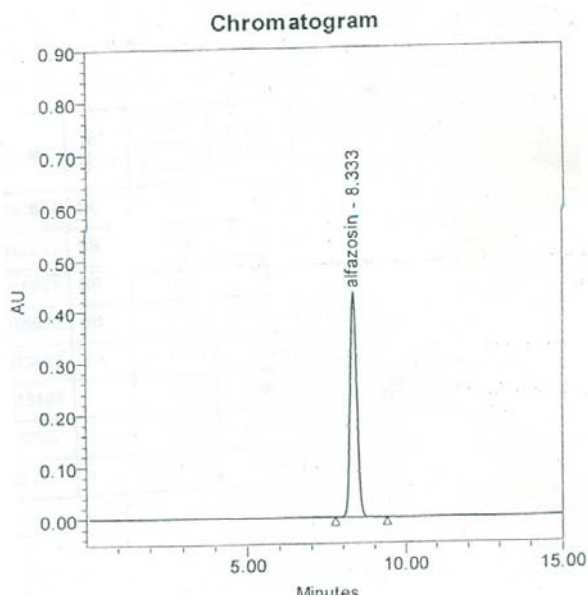


Fig. 3: Chromatogram of alfuzosin.

System suitability

The HPLC system was equilibrated with the initial mobile phase composition, followed by 5 injections of the same standard. These 5 consecutive injections were used to evaluate the system suitability on each day of method validation. The system suitability parameters including percentage RSD (Relative Standard Deviation) of peak

areas (0.71%) < 2 and tailing factor (1.07) < 2 . All parameters were satisfactory with good specificity for the stability assessment of alfuzosin hydrochloride. Theoretical plates of the column were > 3000 .

Intraday and inter-day accuracy and precision

The precision of the method was investigated with respect to repeatability. For intra-day precision, standard solution of fixed concentration was injected at various time interval and %RSD was noted for that injections (0.71% limit %RSD $< 2.0\%$). And the day-to-day precision was studied by injecting the same concentration of standard solution at various days and the %RSD was calculate (0.80 limit %RSD $< 2.0\%$) as shown in table 1. The recovery of alfuzosin hydrochloride in dosage formulation was performed at five concentration levels (50, 75, 100, 125 and 150%), accuracy of the method in dosage formulation ranged from 98.3% to 99.2% as shown in table 1.

Limit of quantitation and detection

The detection limit (LOD), taken as the lowest absolute concentration of analyte in a sample, which can be detected but not necessary quantified under the stated experimental condition, was, 5%. The limit of quantitation (LOQ), taken as the lowest concentration of analyte in a sample, which can be determined with acceptable precision and accuracy under the stated conditions, was 25% to the labeled amount alfuzosin 10mg tablets.

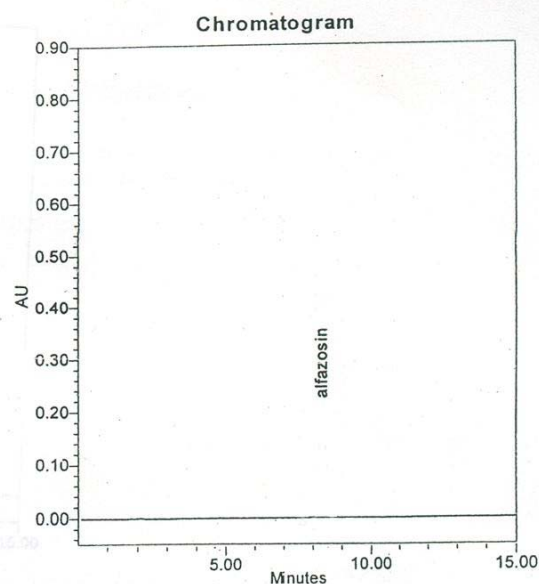


Fig. 3: Placebo study.

Specificity

The specificity of the chromatographic method was determined to ensure separation of alfuzosin hydrochloride by placebo (fig. 4) study and by blank run of mobile phase (fig. 1) which were free from interference of

solvent and commonly used tablet excipients. This is evidenced by the lack of interfering peaks in the chromatograms of placebo and blank run.

Ruggedness

The ruggedness was established by determining alfuzosin hydrochloride in dosage formulation using the different chromatographic system and the same column by two analysts. The assay result indicated that the method was capable with high precision (table 2).

Bench top stability

Stability of standard solution and sample solutions were determined by assay after 24 and 48 hours at room temperature against fresh standard solutions. It shows that the drug is stable and does not show much variation in the time span up to 48 hours (table 2).

CONCLUSION

A simple and reliable HPLC method for measuring alfuzosin hydrochloride in bulk pharmaceutical dosage formulation has been developed. A fully validated RP-HPLC procedure for the assay of alfuzosin hydrochloride drug in bulk and tablet formulation is described for the first time. Hence, it can be recommended for the routine quality control of this drug. The simplicity of the HPLC procedure, the short run time and the low volume of injection make this method suitable for quick and routine analysis. The intraday run and inter day run variability and accuracy results were within the acceptable limit.

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