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Research Article

NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ASSAY OF ALFUZOSIN HYDROCHLORIDE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, economic, rapid, precise reverse phase high pressure liquid chromatographic method has been developed and assay was validated for the determination of Alfuzosin hydrochloride in tablet dosage form. The method was carried out on a inertsil ODS-3V (150 x4.6 mm,5µ) column with a mobile phase consisting acetonitrile, water, tetrahydrofuran and perchloric acid in the ratio of 250:740:10:1 and filtered through 0.45µ cellulose filters. The flow rate was set at 1.0 ml/min with u.v detection at 245 nm. Retention time and injection volume set at 1.0 ml/min and 10µl respectively. The percentage recovery of Alfuzosin was found to be 98.8 and the developed method was validated in terms accuracy, precision, linearity, robustness and ruggedness, The proposed method is useful in terms of Alfuzosin hydrochloride in tablet dosage form.

Keywords: RP-HPLC, Alfuzosin hydrochloride, Method development, Method Validation.

INTRODUCTION

Alfuzosin hydrochloride is chemically designated as (R,S)-N-[3-[(4-amino-6,7-dimethoxy-2-quizolinyl) methylamino]propyl]tetra hydro-2-furancarboxamide hydrochloride[1].Alfuzosin belongs to a class of drugs called alpha-blockers. It is used to treat the signs and symptoms of benign enlargement of the prostate, by increasing the flow in urine which is reduced by benign prostatic hypertrophy[2,3]. Detailed survey of literature revealed several methods based on different techniques for its determination in human plasma and in pharmaceutical formulations[4]. Many trials has been done to develop and validate assay by using RP-HPLC[5] for the determination of Alfuzosin hydrochloride in tablet dosage form[6]. The present RP-HPLC[7] method was validated the following ICH guidelines[8].

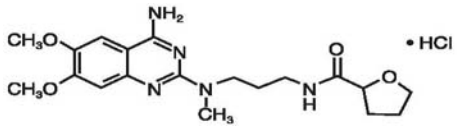

Fig. 1: Chemical structure of Alfuzosin hydrochloride

MATERIALS AND METHODS

Reagents and chemicals

Acetonitrile HPLC grade was procured from E.Merck Ltd, Mumbai. Methanol, perchloric acid, tetrahydrofuran AR grade were procured from S.D. fine chemicals, Hyderabad. Water HPLC grade was prepared using Millipore purification system. Alfuzosin hydrochloride reference standard procured from Dr.Reddy's laboratories, Hyderabad.

Instrumentation

The HPLC system consists of a water Empower 2487 having 2866 photodiode array detector system, which was connected with the help of Millineum 32 software for data integration and processing. Inertsil ODS-3V (150 X 4.6 mm) 5µ column was used for the analysis.

HPLC conditions

The contents of the mobile phase were acetonitrile, water, tetehydrofuran, perchloric acid in the ratio of 250: 740: 10: 1. These were filtered through 0.45µ membrane filter and degassed by sonication before use. The flow rate of mobile phase was optimized to 1.0 ml / min. The run time was set at 10 min and column temperature was maintained at ambient. The volume of injection was 10 µl, and the eluent was detected at 245 nm.

Method development

Preparation of standard solution: About 25 mg of Alfuzosin hydrochloride was accurately weighed and transferred to a 250 ml volumetric flask, 50 ml of acetonitrile added, dissolved and diluted to volume with water to get concentration of 100 mcg / ml.

Preparation of sample solution: Five tablets each contained 10 mg of Alfuzosin hydrochloride were dropped in to 500 ml volumetric flask, 100 ml of acetonitrile added, rotary shaking done till tablets were disintegrated. Then 300 ml water added and sonicated for 30 minutes. Sample diluted to volume with water and mixed to get concentration 100 mcg / ml. A portion of the solution was centrifuged at 3500 rpm for 15 minutes to get clear solution Then 10µl of standard and sample solutions were injected to HPLC.

Assay method: With the optimized chromatographic conditions a steady base line was recorded. Standard and sample solution of Alfuzosin hydrochloride was injected and chromatograms were recorded. Retention times of standard and sample solutions found to be 8.38 and 8.50 respectively. The concentration of the drug was calculated using the following formulation.

Sample area x standard dilution x potency x average weight / tablet standard area x sample dilution x 100

Method validation

System suitability

Standard solution prepared by using Alfuzosin hydrochloride working standard and injected five times in to the HPLC system. System suitability parameters were evaluated and found to be with in the limits. The RSD for peak areas from five replicate injections of Alfuzosin hydrochloride was found to be 0.71 % and tailing factor for Alfuzosin hydrochloride was found to be 1.07. Results were represented in table-1.

Table 1: System suitability of Alfuzosin hydrochloride

Injection number	Alfuzosin	Acceptance criteria
01	6281877	The % relative standard
02	6318990	deviation of peak areas
03	6265967	of Alfuzosin should not
04	6316030	be more than 2.0
05	6383062	
Mean	6313185	
% RSD	0.71	

Linearity

A graph was plotted to "concentration" verses "area" in linearity section. Correlation coefficient was found to be 0.999. Graph was represented in figure-1. Results were summarized in table-2.

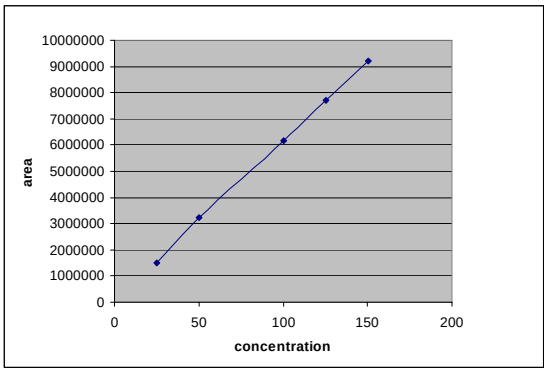

Fig. 2: Linearity of detector response

Table 2: Linearity of Alfuzosin Hydrochloride

Spike level	Areas
25%	1518384
50%	3237136
100%	6178894
125%	7690491
Coefficient of Correlation (r)	9216549
Slope (m)	0.999
Intercept (b)	61002.2
	78067.67

Specificity

Study of placebo interference from excipients was conducted. Placebo interference checked by taking placebo in 500 ml volumetric flask in triplicate, equivalent to about the weight of placebo. There was no interference at retention time of Alfuzosin peak.

Accuracy

The accuracy of method was determined by recovery experiments. These were performed in triplicate by spiking with equivalent amount of Alfuzosin raw material in to each volumetric flask for each spike level to get concentration of Alfuzosin equivalent to 50%,75%,100%,125% and 150% of the Alfuzosin as per proposed method. Average percentage recovery Alfuzosin was found to be with in the limits. Results were summarized in table 2

Ruggedness

System to system/column to column/analyst to analyst variability

Ruggedness of the system was determined by carrying out the experiment on different instruments like Schimadju HPLC and water alliance HPLC by different operators using different columns of similar type like Kromacil C18 and Inertsil –ODS C18 column. The average percentage drug recovery for two systems/columns/analysts was found to be 101.2 (system-1) and 99.4 (system-2) with a relative standard deviation of 0.7% and 0.8% respectively.

Bench top stability of standard and test preparation

Bench top stability of Alfuzosin in standard and test preparation was established over a period of about two days, which was analyzed with a freshly prepared standard each time. The difference in percentage assay of standard and test preparation from initial to two days was found to be with in the limits. Results were summarized in table 3.

Table 3: Ruggedness of Alfuzosin hydrochloride

Time in days	% Assay of standard preparation	Difference from initial day	% Assay of test preparation		Difference	
			Test-1	Test-2	Test-1	Test-2
Intial	99.7	NA	101.1	100.1	NA	NA
24 hrs	99.9	0.2	99.3	99.8	1.8	0.3
48 hrs	101.4	1.7	100.9	102.2	0.2	2.1

Robustness

Robustness was determined by slight changes in mobile phase composition and flow rate. Organic phase in mobile phase changed from 90 % to 110 % instead of 100 %, by giving five replicate injections of standard. Flow rate variation was checked by changing flow rate from 0.8 ml/min to 1.2 ml/min instead of 1.0 ml/min. Robustness of Alfuzosin standard was found to be with in limits. Results were presented in table 4.

Table 4a: Robustness of Alfuzosin hydrochloride effect of variation in composition of Organic Phase in Mobile Phase

System suitability parameters	Observed value			Acceptance criteria
	90% organic phase	100% organic phase	110 % organic phase	
Tailing factor of Alfuzosin peak in standard	0.98	1.07	1.1	NMT 2.0

Table 4b: Robustness of Alfuzosin hydrochloride effect of variation in Flow Rate

System suitability parameters	Observed value with Flow rate			Acceptance criteria
	0.8 ml/min	1.0 ml/min	1.2 ml/min	
Tailing factor of Alfuzosin peak in standard	1.13	1.07	1.09	NMT 2.0

RESULTS AND DISCUSSION

The HPLC procedure was optimized to develop stable assay method. The method was tested for system suitability by injecting five replicate injections of pure drug. It was tested with different mobile phase compositions and different pH ranges with different columns, at different flow rates with detection at 245 nm. The results of analysis shows that the amount of drug was in good agreement with the label claim of formulation. The proposed method is simple and does not involve laborious time consuming sample preparation.

CONCLUSION

The proposed RP-HPLC method for estimation of assay in tablet dosage form is accurate, precise, linear, simple and rapid. Hence the

present RP-HPLC method is suitable for quality control of raw materials, formulations and dissolution studies.

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