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DEVELOPMENT AND VALIDATION OF A ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR UNIFORM OF DOSAGE OF ALFUZOSIN HYDROCHLORIDE TABETS

M.N Brinda[1*], V. Krishna Reddy[1], E.Sasikiran Goud[2]

[1*, 2]Sri Krishnadevaraya University, Department of chemistry, Anantapur, A.P, India

*Corresponding Author Email: sasikiranediga@gmai.com

ABSTRACT

Aim of the present work was to develop simple, shorter and effective UPLC method with UV detection (245nm) and subsequent validation for the content uniformity determination of alfuzosin hydrochloride in tablets. The method uses isocratic the mobile phase mixture of tetrahydrofuran, acetonitrile, water and perchloric acid in the ratio of 10:220:770:1 (v/v) on Inertsil ODS-3, 3.0 x 50 mm, 2µ column. The RSD for five injections was observed to 0.1 percentage and linearity range of (LOQ) 10 – 300 percentage of label claim established with 0.9998 correlation. The observed result shows that the method was rapid, precise, accurate and simple. The method was validated as per ICH guidelines.

KEY WORDS

Alfuzosin hydrochloride, Method development, UPLC, Validation.

INTRODUCTION

Alfuzosin hydrochloride is an alpha$_1$-adrenoreceptor blocker. It is used in the symptomatic treatment of urinary obstruction caused by benign prostatic hyperplasia and has been tried in the treatment of hypertension. Alfuzosin hydrochloride is chemically designated as N-{3-[(4-Amino-6, 7-dimethoxyquinazolin-2-yl(methyl)amino]propyl}tetrahydro-2-furamide hydrochloride[1]. The empirical formula of alfuzosin hydrochloride is $C_{19}H_{27}N_5O_4$•HCl. The molecular weight of alfuzosin hydrochloride is 425.9. Alfuzosin hydrochloride is a white to off-white crystalline powder that melts at approximately 240°C. It is freely soluble in water, sparingly soluble in alcohol, and practically insoluble in dichloromethane.

Few analytical methods have been reported for the determination of alfuzosin in pharmaceuticals and biological fluids. Alfuzosin was determined in human plasma by HPLC-tandem mass spectrometry [2], chiral mobile phase HPLC for chiral separation of three new enantiomers of alfuzosin, doxazosin and terazosin [3] and HPLC methods using fluorescence detection [4-8]. Voltammetric methods [9]. Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs and, therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds [10-13].The present work describes a simple, isocratic method for the determination of alfuzosin hydrochloride in tablets as for ICH guidelines.[14-17].

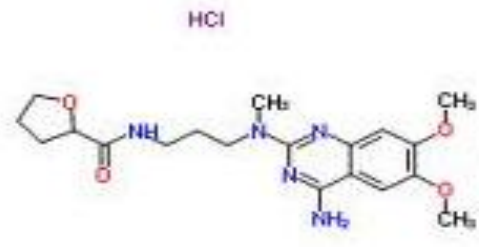

Figure: 1 Structure of alfuzosin hydrochloride

MATERIALS AND METHODS

Chemicals

Qualified standards and samples of alfuzosin hydrochloride were obtained from local laboratories and were used without any further purification. The chemicals like tetrahydrofuran, Acetonitrile and perchloric acid were purchased from Merck, Mumbai. Millipore water generated from TK water system. The analytical column used was Inertsil ODS-, 3.0 x 50 mm, 2µ

Instruments

An Acquity UPLC system manufactured by Waters which consist of Photo Diode Array (PDA) detector, Quaternary solvent manager, sample manager, column heating compartment was used for assay determination of alfuzosin hydrochloride. UPLC instrument was controlled by Waters Empower chromatographic software. A Inertsil ODS-, 3.0 x 50 mm column with particle size of 2µm was used as stationary phase for chromatographic separation and determination of alfuzosin hydrochloride.

Standard preparation

Weighed accurately 25.0 mg of alfuzosin hydrochloride working standard and transfer into a 250 ml volumetric flask, add 50 ml of acetonitrile and 100 ml of water sonicate to dissolve. Dilute to volume with water and mix well.

Sample preparation

Transferred one tablet into a 100 ml dry volumetric flask. Add 20 ml of Acetonitrile, sonicate with intermediate shaking until the tablet disintegrate and keep on a cyclomixer for 5 minutes. Add 50 ml of water and keep on a cyclomixer for 5 minutes and then sonicate for 30 minutes with intermediate shaking. And then make up the volume with water and mix. Centrifuge a portion of the sample at 3500 rpm for 15 minutes. Filter the sample through 0.45µ filter.

Chromatographic conditions

The chromatographic column used Inertsil ODS-3 with dimensions of 3.0 x 50 mm with 2 µm particle size. The isocratic method was employed with the mobile phase mixture of tetrahydrofuran, acetonitrile, water and perchloric acid in the ratio of 10:220:770:1 (v/v). The column temperature was maintained at 25.0^{o}C and detection was monitored at a wavelength of 245 nm. Injection volume was 5µl and the mobile phase flow was set at 1.0mL/min. The acetonitrie and water in the ratio of 1:4 v/v used as a diluent for preparation of solutions.

METHOD VALIDATION

The developed method for determination of alfuzosin hydrochloride was validated for system suitability along with method selectivity, specificity, linearity, range, precision, accuracy, range, ruggedness, robustness according to the ICH guidelines.

Method validation parameters

The system suitability was conducted using standard preparation and evaluated by injecting five replicate injections. Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting Diluent, placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte. Performed the linearity with alfuzosin hydrochloride in the range of 10 to 300% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also performed

precision at higher level by injecting six times into the chromatographic system.

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted using alfuzosin hydrochloride and evaluated by making six replicate injections. The Accuracy of the method by recoveries of alfuzosin hydrochloride sample solutions at different concentration levels ranging from 10 to 300%.The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions:

Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The wavelength of 245 nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify alfuzosin hydrochloride. Preliminary development trials were performed with various ODS columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Inertsil ODS-, 3.0 x 50 mm, 2µ, column there a significant improvement in the peak shapes with 1.5 tailing factor.

System suitability:

The RSD from five replicate injections of standard preparation was 0.1 %. Tailing factor for alfuzosin hydrochloride peak was 1.5.

Selectivity:

Performed the specificity parameter of the method by injecting diluent, standard preparation sample preparation and placebo preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved no peak observed at retention time of alfuzosin hydrochloride. Specificity results of alfuzosin hydrochloride given in the below **Table-2**.The selectivity chromatograms shown in the **Figures 2-6**

Table 2: Selectivity results of alfuzosin hydrochloride

S.No.	Sample	Retention time
1	Blank	-
2	Placebo	-
3	Standard	1.303
4	Sample	1.372

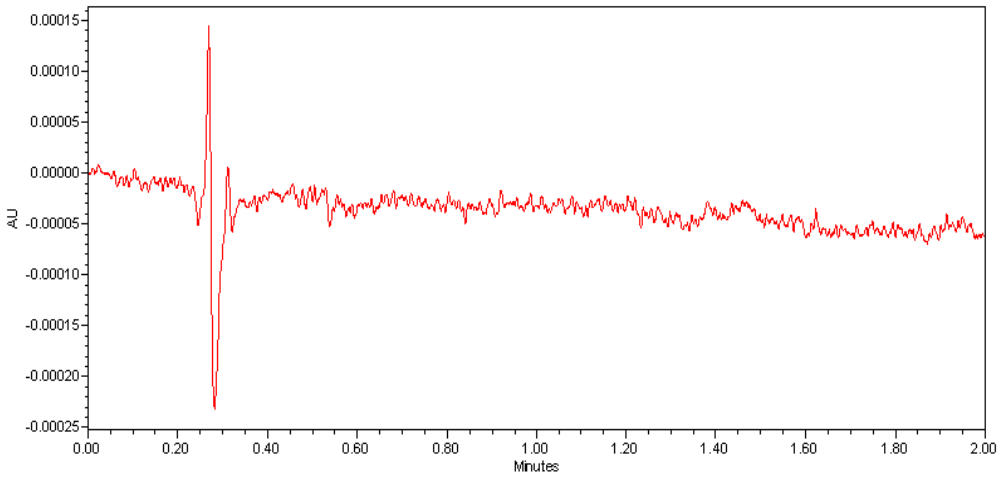

Fig: 2 Chromatogram of blank

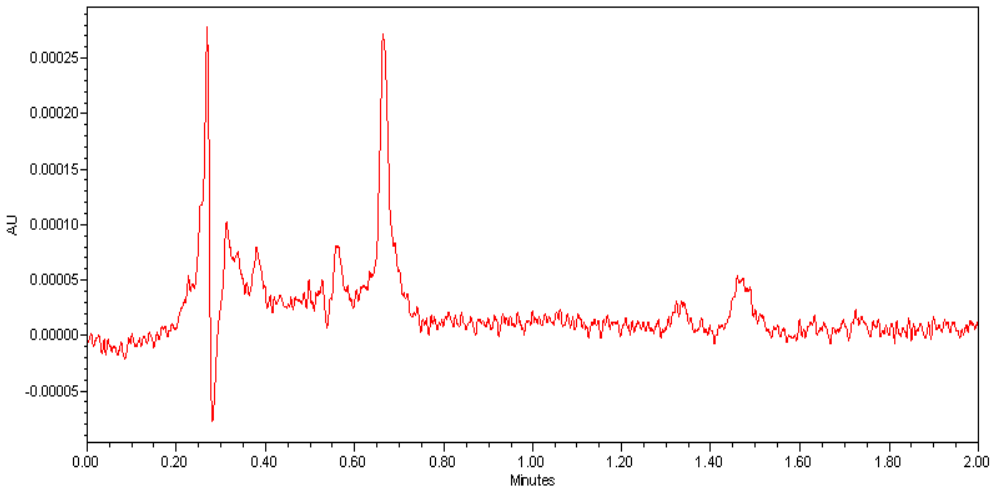

Fig: 3 Chromatogram of placebo

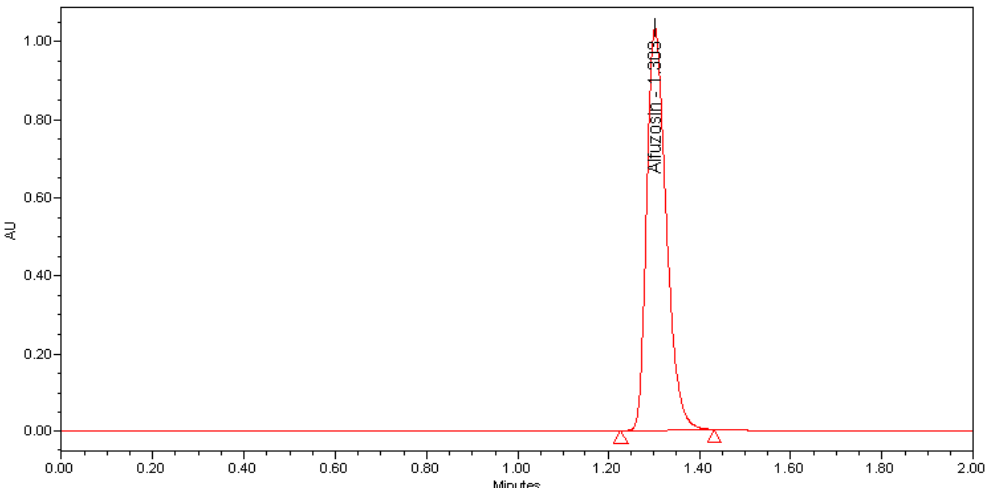

Fig: 4 Chromatogram of alfuzosin hydrochloride standard

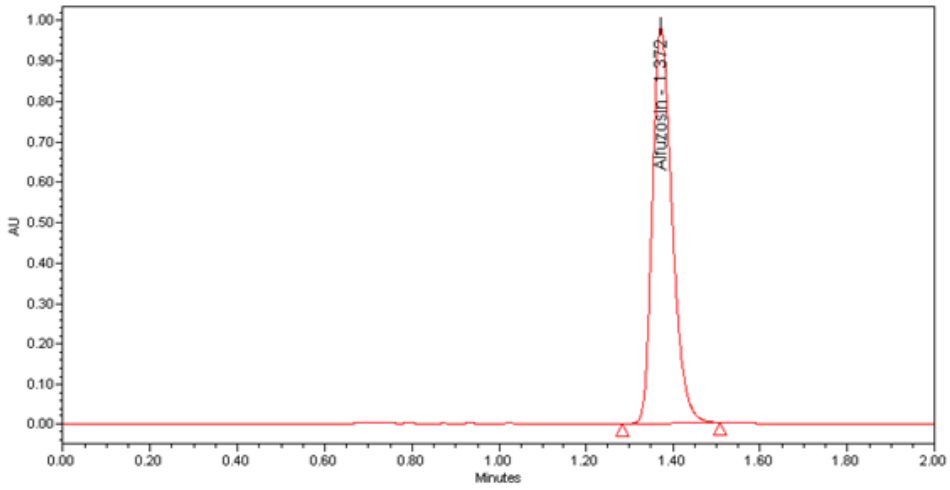

Fig: 5 Chromatogram of alfuzosin hydrochloride sample

Linearity:

To demonstrate the linearity with alfuzosin hydrochloride standard in the range of 10 to 300% of specification limit**.** Correlation coefficient of alfuzosin hydrochloride was 0.9998.The linearity results shown in the below **Table -3**.

Table 3: Linearity results of alfuzosin hydrochloride

S.No.	Concentration in ppm	Area response
1.	10	380326
2.	50	1857895
3.	100	3737830
4.	150	5518170
5.	200	7472364
6.	300	10883041

Accuracy:

Accuracy study found that the mean % of recovery was more than 97.0% and less than 103.0% at each level 10 to 300% of concentration levels, hence method is accurate. The accuracy results are given **Table-4**.

Table4: Accuracy results

S.No.:	Level in %	% Mean Recovery
1.	10	99.84
2.	50	100.46
3.	100	100.19
4.	150	102.05
5.	200	101.67
6.	300	100.99

Precision:

The precision of test method was validated by assaying six samples prepared on alfuzosin hydrochloride and calculate relative standard deviation of Assay results. The precision results are given **Table-5**.

Table: 5 Precision results

S.No.	% Alfuzosin Hydrochloride
Precision-1	98.7
Precision-2	101.0
Precision-3	107.0
Precision-4	98.5
Precision-5	98.5
Precision-6	101.1
Precision-7	106.9
Precision-8	101.3
Precision-9	98.8
Precision-10	101.2
Average	101.3
% RSD	3.16

Robustness

The method robustness was studied by injecting the system suitability solution at change in the percentage of organic modifier (Acetonitrile), flow rate, and column temperature. The results were obtained as shown in the below **Table-6**.

Table: 6 Robustness results

Condition	Tailing factor	% RSD
Limits	**NLT 2.0**	**NMT 2.0**
Normal Condition	1.5	0.1
Flow rate 0.8mL/min	1.6	0.5
Flow rate 1.2mL/min	1.6	0.3
Column Temperature 20°C	1.6	0.5
Column Temperature 30°C	1.6	0.3
Organic phase +10.0%	1.6	0.4
Organic phase -10.0%	1.7	0.7

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CONCLUSIONS

A simple isocratic UPLC method has been developed and validated for the determination of uniform of dosage form of alfuzosin hydrochloride tablets. The developed method has been found to selective, sensitive, precise, robust and stability indicating. The method can be directly adopted in quality control laboratories for routine analysis with respect to determination and quantification of alfuzosin hydrochloride and also for the analysis of stability samples.

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*Corresponding Author:
M.N BRINDA[*]
Sri Krishnadevaraya University,
Department of chemistry,
Anantapur, A.P, India