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Research Article

DEVELOPMENT AND VALIDATION OF RP HPLC METHOD FOR THE ESTIMATION OF ALFUZOSIN IN TABLET FORMULATION

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Abstract

A new simple, rapid, precise and accurate assay method was developed forestimation of Alfuzosin in pure form and tablet form. The analyte was separated on a C_8 (Primesil) column (5 µm, 4.6mm* 250 mm).The mobile phase was Acetonitrile:0.05 % Ortho Phosphoric Acid(40:60, v/v) after adjusting pH to 3.2 this pumped at 0.7 mL/min flow rate it passes all the parameters satisfactorily. The UV detector was operated at 242 nm for the determination of all the drugs. Linearity, accuracy and precision were found to be acceptable over the concentration ranges of 10-50 µg/ml for Alfuzosinwith a $R^2$of value0.997. The optimized methods proved to be specific, robust and accurate for the quality control of drugs in bulk drug and pharmaceutical formulations.

Keywords: Alfuzosin, Method Validation, HPLC, C8 Column

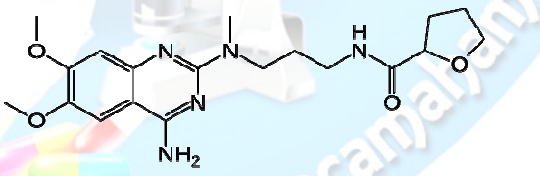
INTRODUCTION

Drugs play a vital role in the progress of human civilization by curing diseases. Analytical chemistry is divided into two branches qualitative and quantitative[1]. Today a majority of the drugs used are of synthetic origin. These are produced in bulk and used for their therapeutic effects in pharmaceutical formulations. Pharmaceutical product quality is of vital importance for patient safety. Pharmaceutical analysis is the branch of pharmacy that is responsible for developing sensitive, reliable and accurate methods for the estimation of drugs in pharmaceutical dosage forms and biological fluids.[2]

Alfuzosin (AFN) is a selective antagonist of postsynaptic alpha1-adrenoreceptors, which are located in the prostate, bladder base, bladder neck, prostatic capsule, and prostatic urethra.[3,4,5,6] AFN is chemically 3-[(4-amino-6,7-dimethoxy-quinazolin-2-yl)- methyl-amino]propyl] tetrahydrofuran- 2-carboxamide.[7] Several methods are available in the literature for the determination, most of these methods are for the determination of Alfuzosin separately in bulk and formulation. Analytical methods reported for quantitative determination of Alfuzosin individually in pharmaceutical formulations by HPLC[8,9,10,11], HPTLC[11], UV[12,13], colorimetry[14,15,16] and in biological fluids by HPLC[17,18].

According to the Literature review, there are only few HPLC method reported for the determination of Alfuzosin but they are very complex, time consuming. Therefore, an attempt has been made to develop simple, accurate, precise and rapid RP-HPLC methods for determination of ATN.

Figure No.1. Chemical structures of Alfuzosin



EXPERIMENTAL

Chemicals and reagents

Alfuzosinis obtained as generous gift sample from Swapnroop drug and pharmaceutical. Commercial Alfusin® tablets that were manufactured by Cipla.

Ltd., containing Alfusin (AFN) 10 mg, were collected from local market. Acetonitrile, methanol and water used were of HPLC grade (Merck, India). Ortho-phosphoric acid was AR grade (Merck, India). A 0.2 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Apparatus

The chromatographic system (Younglin (S.K) Geadient System) consisted of a prominence solvent delivery module, a manual injector with a 20 µL fixed loop and a UV-visible detector. The separation was performed on a C_8 (Primesil) column (5 µm, 4.6mm* 250 mm) at an ambient temperature. Chromatographic data were recorded and processed using Autochro -3000 software. An Fast clean ultrasonicate cleaner (India) was used for degassing the mobile phase.Shimadzu UV 1800 double beam UV visible spectrophotometer and Sansui DJ-150S-S electronic balance were used for Spectrophotometric and weighing purposes respectively.

Chromatography Conditions

Chromatographic separations of active substance was obtained by using C_8 (Primesil) column (5 µm, 4.6mm* 250 mm),.Mobile phase Acetonitrile:0.05 % Ortho Phosphoric Acid(40:60, v/v) was prepared, filtered through a 0.2 µm nylon filter and degassed for 5 min in an ultrasonicator. The mobile phase was pumped through the column at 0.7 mL/min flow rate. Analyses were carried out at ambient temperature with detection at 242 nm. The injection volume was 20 µL and each analysis.

Standard Solutions

Accurately weight and transfer 10mg Alfuzosin working standard into 10 mL volumetric flask as about diluent acetonitrile completely and make volume up to the mark with the same solvent to get 1000µg/mL standard (stock solution) and 15 min sonicate to dissolve it and the resulting stock solution 0.1mL was transferred to 10 mL volumetric flask and the volume was made up to the mark with mobile phase Acetonitrile: (0.05%OPA) Water, prepared in (4mLACN : 6mL WATER v/v) solvent.

Sample Solution

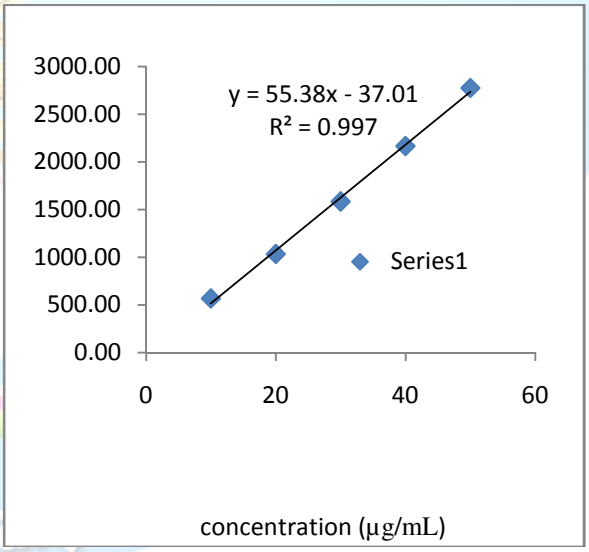
Weigh 20 Alfusin tablets and calculated the average weigh, accurately weigh and transfer the sample equivalent to 0.075 mg Alfuzosin into 10 ml volumetric flask. Add about 8ml of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm filter. Further pipette 0.3ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents.(30 µg/ml). The amounts of Alfuzosin per tablet were calculated by extrapolating the value of area from the calibration curve.

Validation of Proposed Method

Calibration curve (linearity)

Accurately measured aliquots of working standard solutions equivalent to 10-50 µg/mL AFN, transferred to series of 10 mL volumetric flasks and the contents of the flasks were diluted to volume with mobile phase. A 20 µL aliquot of each solution was injected in triplicate into the liquid chromatography. The conditions including the flow rate of mobile phase at 0.7 mL/min, detection at 242 nm. A calibration curve for each drug was obtained by plotting area under the peak versus concentration.

Figure No.2. Calibration curve of Alfuzosine.



Accuracy (% recovery)

Recovery studies were carried out by adding a known amount of pure drugs AFN to a pre ana-

lyzed sample solution. These studies were carried out by spiking 80%, 100% and 120% respective drug. The recovery studies showed that the results were within acceptable limits, above 99% and below 101%. The results are given in (Table 2).

Table No.1. Regression analysis of the calibration curves for Alfuzosin in the proposed HPLC Method.

Parameter	Alfuzosin
Linearity Range (µg/mL)	10-50
Detection Wavelength (nm)	242
Slope ± SD	55.38
Intercept ± SD	37.01
Correlation coefficient	0.997

SD- Standard deviation, Mean of three determinations

Table No.2. Summary of the validation parameters for the proposed HPLC method.

Parameter	Alfuzosin
LOD	0.4258µg /mL
LOQ	1.2896 µg /mL
Accuracy,%	99.78-100.39%
Repeatability (%RSD, n = 5)	0.44%
Precision (RSD, %)	Bellow 2
Interday, n = 3	99.20-101.65%
Intraday, n = 3	97.45-99.00%

LOD = Limit of detection.
LOQ = Limit of quantification
RSD = Relative standard deviation.

Table No.3. Assay results for the dosage form using the proposed HPLC method.

Formulation	Alfuzosin	%RSD
Alfusin	99.56±0.34	0.33

SD= Standard deviation, 5 determinations.

Method precision (repeatability)

The precision of the developed method was assessed in terms of repeatability, intraday and interday precision by analyzing six replicate standard samples. The % R.S.D. values of the results corresponding to the peak area and retention time were expressed for intra-day precision and on 3 days for inter-day precision.

Intermediate precision (reproducibility)

The intraday and interday precisions of the proposed method were determined by estimating the corresponding responses 5 times on the same day and on 5 different days for present method. The results are reported in terms of relative standard deviation (RSD).

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ of the drug were calculated using the equations according to International Conference on Harmonization (ICH) guidelines.

Robustness

The mobile phase composition was changed in (±1 mL/ min^{-1}) proportion and the flow rate of Acetonitrile in the mobile phase composition (±1 mL/ min^{-1}) and the change in detection wavelength (±1 mL/ min^{-1}) and the effect of the results were examined and it was performed using 10µg/mL solution of Alfuzosin in triplicate.

Specificity

Specificity is the ability of the analytical method to measure analyte response in presence of interferences including degradation products and related substances. Specificity was checked by determining AFN in laboratory prepared and in mixture containing different degradation products.

System suitability Test

In the system suitability test tertiary solution of 10 µg/mL of AFN (n=6) was prepared and injected. Then the system suitability parameters like retention time, theoretical plates, tailing factor and resolution were calculated from the chromatogram.

Table No. 4. System suitability test parameters for Alfuzosin for the proposed HPLC method.

System Suitability Parameters	Mean of Five Determination System Suitability Parameters
Retention Time (min)	4.833
Area	1079.65
Theoretical Plate Number	14122.3
Tailing Factor	1.2363
Resolution	0.0000

RESULTS AND DISCUSSION

The absorption spectra of AFN greatly overlap; so conventional determination of these compounds in mixture is not possible. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for AFN were obtained with a mobile phase consisting of Acetonitrile:0.05 % Ortho Phosphoric Acid(40:60, v/v), pH 3.2 adjusted using *o*-phosphoric acid. Quantification of the drugs was performed at 242 nm. Peak of the component with clear baseline separation was obtained.

Validation of the Proposed Method

Linearity

Linear correlation was obtained between peak areas and concentrations of AFN in range of 10–50µg/mL. The linearity of calibration curves was found to be acceptable over the concentration ranges of 10–50µg/mL for AFN with a R^2 0.997 value.(Table- 1, Fig- 2). The results show that good correlation existed between the peak area and concentration of the analysts.

Accuracy

The recovery experiments were performed by the standard addition method. The recoveries obtained were between99.78 to 100.39for AFN(Table 2). The high values indicate that the method was accurate.

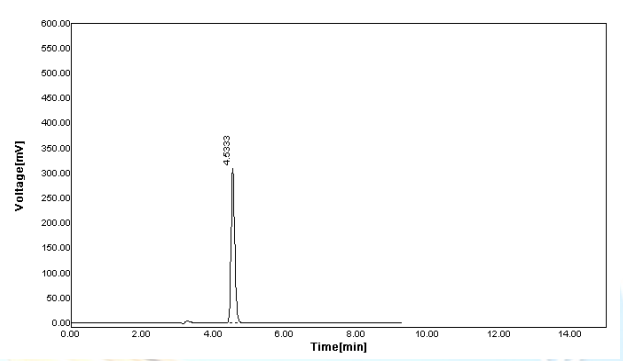
Method precision

Precision study was carried out using parameter like method repeatability study which showed that results were within acceptable limit i.e. % RSD below 2.0 indicating that the method is reproducible. The results are shown in (Table No.2).

Intermediate precision

The intraday RSD value for AFN was found to be 0.33-0.57%, the interday RSD values for AFN was 0.10–0.20% respectively. The % RSD (< 2%) values indicate that the method was sufficiently precise (Table 2).

Figure No.3. Standard chromatogram of marketed formulation.



LOD and LOQ

LOD values for AFN was found to be 0.4258µg/mL respectively. LOQ values for ATN was found to be 1.2896µg /mL (Table 2). These data showed that the method was sensitive enough for the determination of AFN.

Robustness

The method was found to be robust with no significant changes on test result upon change of analytical conditions like different flow rate, pH of mobile phase and detection wavelength. It was shown that the standard deviation bellow 1 and % RSD is less than 2 for all results and the method passes the robustness test.

System Suitability Test

A solution of 10µg/mL of AFN (n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated. The results (Table 4) obtained from system suitability tests are in agreement with the official requirements.

CONCLUSIONS

The proposed LC method presented in this paper

has advantages of simplicity, accuracy, precision and convenience for separation and quantitation of AFN in combination and can be used for the assay of their respective dosage form. Moreover, the proposed LC method is a stability indicating assay method that can determine AFN in presence of their degradation products. Thus, the proposed LC method can be used for the quality control of AFN in typical laboratories.

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