4.1 Introduction

Alfuzosin hydrochloride is an alpha-adrenoreceptor blocker. It is used in the symptomatic treatment of urinary obstruction caused by benign prostatic hyperplasia (Fig 4.2) 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 \( \text{C}_{19}\text{H}_{27}\text{N}_{5}\text{O}_{4}\cdot\text{HCl} \). Its molecular weight is 425.9 g mol\(^{-1}\). 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. The chemical structure of alfuzosin hydrochloride is shown in Figure 4.1.

![Chemical structure of alfuzosin hydrochloride](image)

**Fig 4.1: Chemical structure of alfuzosin hydrochloride**
Alfuzosin is a quinazoline-derivative alpha-adrenergic blocking agent used to treat hypertension and benign prostatic hyperplasia. Accordingly, alfuzosin is a selective inhibitor of the alpha (1) subtype of alpha adrenergic receptors. In the human prostate, alfuzosin antagonizes phenylephrine (alpha (1) agonist)-induced contractions in vitro, and binds with high affinity to the alpha 1a adrenoceptor, which is thought to be the predominant functional type in the prostate. Studies in normal human subjects have shown that alfuzosin competitively antagonized the pressor effects of phenylephrine (an alpha (1) agonist) and the systolic pressor effect of norepinephrine. The antihypertensive effect of alfuzosin results from a decrease in systemic vascular resistance and the parent compound alfuzosin is primarily responsible for the
antihypertensive activity. Alfuzosin should not be combined with ketoconazole (Nizoral, Extina, Xolegel, Kuric), itraconazole (Sporanox), or ritonavir (Norvir), because they increase alfuzosin blood levels by preventing the breakdown of alfuzosin by the liver. Combining alfuzosin with blood pressure reducing medications may increase the risk of hypotension (low blood pressure). PDE-5 inhibitors, used primarily for erectile dysfunction (for example, vardenafil [Levitra, Staxyn], tadalafil [Cialis, Adcirca], and sildenafil [Viagra, Revatio]) add to the blood pressure lowering effects of alfuzosin and may result in orthostatic or postural hypotension [2-12].

Good number of analytical methods like Titration method [13], Voltammetry [14], Thermal analysis [15], Polymers method [16], HPLC-Fluorimetric detector [17-19], HPTLC [20-22], Spectrophotometric [23-32], HPLC methods [33-47] and UPLC method [48] are available for the determination of alfuzosin in biological samples, bulk material or pharmaceutical formulations, have been reported in literature.

**Fig 4.3: Different brands of alfuzosin hydrochloride tablets**


Vamsikrishna M et.al [28-30] proposed optimized and validated spectrophotometric methods for the determination of alfuzosin in pharmaceutical formulations. Ashour Safwan et.al [31] proposed a method for the spectrophotometric determination of alfuzosin hydrochloride in pharmaceutical preparations with some sulphonephthalein dyes. Adusle Prajakta V and team [32] proposed UV spectrophotometric methods for the estimation of alfuzosin in bulk and pharmaceutical dosage forms. Dipti B Patel et.al [33] developed new HPLC and thin layer chromatography methods for the determination of alfuzosin hydrochloride in bulk and formulations. A simple, rapid and reproducible high performance reversed phase liquid chromatographic method has been developed for the estimation of alfuzosin hydrochloride in bulk drug samples and pharmaceutical dosage forms using RP C-18 column by S. Appala raju [34]. The mobile phase consists of buffer (pH 3.8) and acetonitrile in the ratio 650:350 (v/v) respectively, and was pumped at \( 1.0 \text{ mL min}^{-1} \) at 30°C.

K.S. Bharath Kumar et.al [35] proposed simple HPLC method for the determination of alfuzosin hydrochloride in formulations. The separation was achieved on Inertsil ODS-3V 150X4.6 mm, 5µm column with a mobile phase consisting acetonitrile, water, tetrahydrofuran and perchloric acid in the ratio 250:740:10:1. The flow rate was \( 1.0 \text{ mL min}^{-1} \) with UV detection.
at 245 nm. A reverse phase high performance liquid chromatographic method has been
developed for the estimation of alfuzosin hydrochloride in pharmaceutical formulation using RP-
C18 column by Vandana P. Patil et.al [36]. The mobile phase (Tetrahydrofuran, acetonitrile and
buffer (pH 3.5) in the ratio 1:20:80) was pumped at a flow rate of 1.5 mL min$^{-1}$ and the eluents
were monitored at 254 nm. Y. Sreenivasa Reddy [37] developed, a reversed phase high
performance liquid chromatography (HPLC) dissolution method with ultraviolet detection at 245
nm for the determination of alfuzosin hydrochloride. Chromatographic separation was achieved
by using an analytical column Symmetry C18, 150 X 4.6 mm with a particle size of 5µm. The
system was operated at a column temperature 25°C using a mobile phase consisting of KH$_2$PO$_4$–
H$_3$PO$_4$ buffer (pH 3.0), acetonitrile and triethylamine (75:23:2) with a flow rate 1.5 mL min$^{-1}$.
Shivprasad et.al [38] proposed a simple, rapid, specific and sensitive reverse phase HPLC
method for the simultaneous estimation of alfuzosin hydrochloride (ALF) and dutasteride
(DUTA) in bulk powder and in pharmaceutical dosage form. The RP-HPLC separation was
performed on HiQ Sil C18 HS column (4.6 mm I.D X 250 mm) using the mobile phase
methanol: water 90:10 (v/v) at a flow rate of 1 mL min$^{-1}$ at an ambient temperature. The
quantitation by HPLC was achieved with UV detection at 244nm.

Mani Ganesh et.al [39] proposed an isocratic reversed phase high-performance liquid
cromatographic (HPLC) method with ultraviolet detection at 245 nm for the determination of
alfuzosin hydrochloride in dosage formulation. Good chromatographic separation was achieved
by using a stainless steel analytical column, Inertsil ODS-3V (5µm, 150x4.6 mm). The system
was operated at ambient temperature (25±2°C) using a mobile phase consisting of acetonitrile:
water: tetrahydrofuran: perchloric acid (250:740:10:1) at a flow rate of 1 mL min$^{-1}$. Ch Amrutha
varshini et.al [40] proposed a simple, rapid and accurate RP-HPLC method for the simultaneous

Jarosław Szulfera [48] proposed comparison of core–shell and totally porous ultra-high performance liquid chromatographic stationary phases based on their selectivity towards alfuzosin compounds.

The aim of the present study is to develop a rapid, simple, precise and accurate ultra-performance liquid chromatographic (UPLC) method for assaying of alfuzosin in pharmaceutical formulations. The developed method is validated as per the regulatory requirement which can be used for routine quality control applications. The method is validated according to International Conference on Harmonization (ICH) guidelines in terms of specificity, precision, accuracy,
linearity, range, ruggedness and robustness including with stability of mobile phase, standard and sample solutions.

4.2 Experimental

4.2.1 Reference substances, chemicals, reagents and samples

The entire experiment was performed using “class A” volumetric glassware. Pharmaceutical grade alfuzosin active pharmaceutical ingredient (API) and tablets were procured from Bio - Leo labs, Hyderabad. The chemicals like tetrahydrofuran, acetonitrile and perchloric acid were purchased from Merck, Mumbai. Millipore water was generated from TK water system. The analytical column used was Inertsil ODS-3.0 X 50 mm column with a particle size of 2 µm.

4.2.2 Instrumentation

Alfuzosin assay analysis was performed by using waters uplc (Milford, MA, USA) PDA system consisting of a quaternary solvent manager, a sample manager, column-heating compartment and photodiode array detector. This system was controlled and the output signal was monitored by waters empower software. Inertsil ODS-3, 50 X 4.6 mm with 3µm column was employed as stationary phase for chromatographic separation. Sartorius semi micro balance was used for all weighing’s and Thermo Orion pH meter was used for buffer pH adjustment. Sonication was carried out with Bandelin sonicator and rotary shaker was used for shaking of samples during preparation.

4.2.2.1 Standard preparation

Weighed accurately 25.0 mg of alfuzosin hydrochloride working standard and transferred into a 250 mL volumetric flask. 50 mL of acetonitrile and 100 mL of water were added and
sonicated to dissolve. The contents were diluted to the volume with water and mixed thoroughly.

### 4.2.2.2 Sample preparation

One tablet of alfoo (Dr. Reddys Laboratory, Hyderabad) was taken in a 100 mL dry volumetric flask. 20 mL of acetonitrile were added, sonicated with intermediate shaking until the tablets disintegrate and kept on a cyclomixer for 5 minutes. 50 mL of water were added and sonicated for 30 minutes with intermediate shaking. The solution was finally made up to the volume with water and mixed. A portion of the sample solution was centrifuged at 3500 RPM for 15 minutes and filtered through 0.45µm filter.

### 4.2.2.3 Blank preparation

A mixture of acetonitrile and water in the ratio 1:4 (v/v) was used as the blank.

### 4.2.2.4 Placebo preparation

Weighed accurately microcrystalline cellulose USP-NF (704 mg) and hypromellose 3 cps USP-NF (36 mg) (Dr. Reddys Laboratory, Hyderabad) and taken in a 100 mL dry volumetric flask. 20 mL of acetonitrile were added, sonicated with intermediate shaking until the tablets disintegrated and kept on a cyclomixer for 5 minutes. 50 mL of water were added and sonicated for 30 minutes with intermediate shaking. The solution was finally made up to the volume with water and mixed. A portion of this solution was centrifuged at 3500 RPM for 15 minutes and filtered through 0.45µm filter.

### 4.2.2.5 Mobile phase

A mixture of tetrahydrofuran, acetonitrile, water and perchloric acid in the ratio 10:220:770:1 (v/v) was used as the mobile phase.
4.2.3 Chromatographic conditions

The chromatographic column used was Inertsil ODS-3 with dimensions of 50 X 4.6 mm with 3 µm particle size. The isocratic method was employed with the mobile phase. The column temperature was maintained at 25°C and the detection was monitored at a wavelength of 245 nm. Injection volume was maintained as 5 µL and the mobile phase flow was set at 1.0 mL min⁻¹.

4.2.4 Evaluation of system suitability

From the chromatogram obtained (Fig:4.6) for the standard preparation, the tailing factor was found as 1.5 which is less than permitted limit (2.0) and the relative standard deviation of replicate injections was calculated as 0.1%. These values indicate the suitability of the proposed system for the determination of alfuzosin.

4.3 Results and discussion

4.3.1 Method development and optimization

Method development was initiated by the review of literature survey and studies on alfuzosin hydrochloride physical and chemical characteristics. The solubility of alfuzosin was tested in different solvents and identified that mixture of water and acetonitrile solution was suitable for extraction of alfuzosin from its tablets. Based on spectral profile and absorption characteristics of alfuzosin, UV detector at 245 nm wavelength was selected for its detection. Preliminary experiments were carried out under various chromatographic conditions as follows.

4.3.1.1 Optimization of chromatographic parameters

First trial
In this trial, the peak response observed for alfuzosin and theoretical plates was found very less as the peak was broad in shape. The elution time of omeprazole was found to be about 5 minutes which need to be reduced.

Second trial

To overcome the limitations of the above trail method, the experiment was repeated by changing the column dimensions and mobile phase composition.
In this trial, the peak response was found satisfactory for alfuzosin with good peak shape. The alfuzosin peak was eluted at about 3 minutes.

**Third trial**

To reduce the run time the flow rate was changed from 0.5 to 1.0 mL minute\(^{-1}\) and injection volume was also changed from 8 µL to 5 µL.

<table>
<thead>
<tr>
<th>Column</th>
<th>Inertsil ODS-3, 50 X 3.0 mm, 2 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Mixture of tetrahydrofuran, acetonitrile, water and perchloric acid in the ratio of 10:220:770:1 (v/v)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL minute(^{-1})</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 µL</td>
</tr>
<tr>
<td>Run time</td>
<td>2 minutes</td>
</tr>
<tr>
<td>Detection</td>
<td>Ultra violet detection at 245 nm.</td>
</tr>
</tbody>
</table>

In this trial, it was noticed that alfuzosin peak was eluted in 1.3 minutes with good peak shape in the chromatogram (Fig: 4.7).

From the results of all the above trials, it was finally concluded that the best optimal conditions for the quantitative separation and elution of alfuzosin are Inertsil ODS-3 with 50 mm length, 3.0 mm internal diameter and 2 µm particle size as stationary phase, degassed mixture of tetrahydrofuran, acetonitrile, water and perchloric acid in the ratio 10:220:770:1 (v/v) as mobile
phase with 1.0 mL minute\(^{-1}\) flow rate, 5 \(\mu\)L injection volume and detection at 245 nm with an UV detector.

### 4.3.2 Method Validation

The proposed test method was validated to include requirements of International conference on Harmonization (ICH) guidelines in terms of specificity, linearity, precision, accuracy, range, robustness and ruggedness. The stability of mobile phase, standard, sample solutions and system suitability were also examined.

#### 4.3.2.1 Specificity

The specificity parameter of the method was evaluated by injecting the blank, placebo, standard preparation and sample preparation into the chromatographic system and the retention times were measured. The recorded chromatograms are shown in Figures 4.4, 4.5, 4.6 and 4.7. No peak was observed at retention time of alfuzosin hydrochloride for blank and placebo. Specificity results of alfuzosin hydrochloride are given in Table 4.1.

![Chromatogram of the blank solution](image-url)

**Fig 4.4: Chromatogram of the blank solution**
Fig 4.5: Chromatogram of the placebo solution

Fig 4.6: Chromatogram of the standard solution
**Fig 4.7: Chromatogram of the sample solution**

**Table 4.1: Specificity results of alfuzosin**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Retention time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blank</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Placebo</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>1.303</td>
</tr>
<tr>
<td>4</td>
<td>Sample</td>
<td>1.317</td>
</tr>
</tbody>
</table>

**4.3.2.2 Precision**

To evaluate system precision, six sample preparations of alfuzosin solution were injected into an ultra-performance liquid chromatographic system under optimal conditions and the chromatograms were recorded. The relative standard deviation was calculated for alfuzosin and obtained as 0.37% (Table 4.2), which was found to be satisfactory against the prescribed limits.
Table 4.2: *Precision study of alfuzosin analysis*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>% Alfuzosin Hydrochloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision-1</td>
<td>98.62</td>
</tr>
<tr>
<td>Precision-2</td>
<td>98.40</td>
</tr>
<tr>
<td>Precision-3</td>
<td>98.23</td>
</tr>
<tr>
<td>Precision-4</td>
<td>98.02</td>
</tr>
<tr>
<td>Precision-5</td>
<td>97.62</td>
</tr>
<tr>
<td>Precision-6</td>
<td>97.90</td>
</tr>
<tr>
<td>Average</td>
<td>98.13</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.37</td>
</tr>
</tbody>
</table>

4.3.2.3 **Accuracy**

Different known aliquots of alfuzosin standard solution containing different known amounts, each one in triplicate, except lower and maximum concentrations were injected into the chromatographic column and the chromatograms were recorded under established experimental conditions. From the peak area values the mean recoveries of alfuzosin were evaluated and presented in Table 4.3.
Accuracy study found that the mean recovery of alfuzosin was between 99.84% and 102.05% at different concentration levels (10 – 300 µg mL\(^{-1}\)). The small variation in the recovery percentages of alfuzosin obtained at different concentration levels (Table 4.3) indicate that the obtained results are accurate.

### 4.3.2.4 Linearity

Different aliquots of standard alfuzosin hydrochloride solutions containing variable amounts of the analyte were injected into the chromatographic column and the chromatograms were recorded. The peak areas of the resultant chromatograms were recorded and tabulated in Table 4.4. The calibration plot drawn between the peak areas and concentration of the analyte (Fig: 4.8) showed that the method is suitable for the determination of alfuzosin in the range 10 - 300 µg mL\(^{-1}\).
### Table 4.4: Statistical data of alfuzosin linearity study

<table>
<thead>
<tr>
<th>Concentration (µg mL(^{-1}))</th>
<th>Area response</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>380326</td>
</tr>
<tr>
<td>50</td>
<td>1857895</td>
</tr>
<tr>
<td>100</td>
<td>3737830</td>
</tr>
<tr>
<td>150</td>
<td>5518170</td>
</tr>
<tr>
<td>200</td>
<td>7472364</td>
</tr>
<tr>
<td>300</td>
<td>10883041</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>0.9998</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>36479.072</td>
</tr>
<tr>
<td>Intercept</td>
<td>-1.140</td>
</tr>
<tr>
<td>DL (µg mL(^{-1}))</td>
<td>0.010</td>
</tr>
<tr>
<td>DQ (µg mL(^{-1}))</td>
<td>0.035</td>
</tr>
</tbody>
</table>

**Fig 4.8: The linearity graph of alfuzosin**
4.3.2.5 Robustness

The robustness study of the proposed method was carried out with respect to organic solvent, flow rate and column oven temperature. The chromatographic conditions were maintained same as per test method in each case. The results are shown in Table 4.5. From the obtained results, it was observed that there was no much variation in retention time, theoretical plates and asymmetry of alfuzosin peak, obtained at different deliberately varied conditions from the test method. Hence the method is robust for all the varied conditions.

Table 4.5: Robustness results of alfuzosin

<table>
<thead>
<tr>
<th>Robustness Condition</th>
<th>Tailing factor</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Condition</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Organic solvent (Acetonitrile) -10%</td>
<td>1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Organic solvent (Acetonitrile) +10%</td>
<td>1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Flow changed to 1.2 mL minute-1</td>
<td>1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Flow changed to 0.8 mL minute-1</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Column Temperature changed to 30°C</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Column Temperature changed to 25°C</td>
<td>1.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Conclusion

The literature survey revealed that almost all the reported chromatographic methods for the validation of alfuzosin are based on RP-HPLC principle. The present proposed method is the
first novel UPLC method. The method was validated according to the ICH guidelines which revealed that the method is selective, precise and accurate. The proposed UPLC method has the ability to separate alfuzosin from excipients found in the alfuzosin tablets and therefore can be applied to the analysis of samples at quality control. The method is rapid, direct, and specific and can be used for the routine analysis of alfuzosin drug in the tablets. The method may also be extended to evaluate the active drug substance.

The results of the proposed method were compared with those of one of the reported methods [34] to show the quality of the proposed method for the determination of alfuzosin (Table 4.6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HPLC Method [34]</th>
<th>Present UPLC Method</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>RP C-18 column (150)</td>
<td>Inertsil ODS-3, 50x 3.0</td>
<td>Shorter column gives less</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Mobile phase consists of buffer (pH 3.8) and acetonitrile in the ratio 650:350 (v/v)</td>
<td>Mixture of tetrahydrofuran, acetonitrile, water and perchloric acid in the ratio of 10:220:770:1 (v/v)</td>
<td>In reported method high pH buffer was used as mobile phase when compared with present method, High pH mobile phase damages silica based columns.</td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL minute(^{-1}) with isocratic mode</td>
<td>1.0 mL minute(^{-1}) with isocratic mode</td>
<td>Isocratic mode is simple, reliable &amp; gives constant baseline response and low flow rate increases the life time of column stationary phase.</td>
</tr>
<tr>
<td>Data Acquisition time</td>
<td>10 minutes per injection</td>
<td>2.0 minutes per injection</td>
<td>Less run time reduces solvent consumption and saves analysis time.</td>
</tr>
<tr>
<td>Linearity range</td>
<td>Linearity of the method covered from 0.02 µg mL(^{-1}) to 20 µg mL(^{-1}) of alfuzosin</td>
<td>Linearity of the method covered from 10 µg mL(^{-1}) to 300 µg mL(^{-1}) of alfuzosin</td>
<td>Applications will increase with increased range.</td>
</tr>
</tbody>
</table>

References:


44. Alain Rouchouse, Martine Manoha, Alain Durand, Jean Paul Thenot, *Direct high performance liquid chromatographic determination of the enantiomers of alfuzosin in plasma on a second-generation α1-acid glycoprotein chiral stationary phase*, *Journal of Chromatography A*, (1990), 506, 601-610.


