Optimization and Validation of Quantitative Spectrophotometric Methods for the Determination of Alfuzosin in Pharmaceutical Formulations

M. VAMSI KRISHNA* and D. GOWRI SANKAR
Pharmaceutical Analysis and Quality Assurance Division, University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003.
marothu_vamsi@rediffmail.com

Received 3 January 2007; Accepted 8 February 2007

Abstract: Three accurate, simple and precise spectrophotometric methods for the determination of alfuzosin hydrochloride in bulk drugs and tablets are developed. The first method is based on the reaction of alfuzosin with ninhydrin reagent in N, N'-dimethylformamide medium (DMF) producing a colored product which absorbs maximally at 575 nm. Beer’s law is obeyed in the concentration range 12.5-62.5 µg/mL of alfuzosin. The second method is based on the reaction of drug with ascorbic acid in DMF medium resulting in the formation of a colored product, which absorbs maximally at 530 nm. Beer’s law is obeyed in the concentration 10-50 µg/mL of alfuzosin. The third method is based on the reaction of alfuzosin with p-benzoquinone (PBQ) to form a colored product with λmax at 400 nm. The products of the reaction were stable for 2h at room temperature. The optimum experimental parameters for the reactions have been studied. The validity of the described procedures was assessed. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The proposed methods could be used for the determination of alfuzosin in pharmaceutical formulations. The procedures were rapid, simple and suitable for quality control application.

Keywords: Alfuzosin, Assay, Spectrophotometry, Ninhydrin, Ascorbic acid, p-Benzooquinone
Introduction

Alfuzosin Hydrochloride (AFZ) is a alpha 1-receptor blocker and is chemically known as N-[3-[(4-amino-6,7-dimethoxy-quinazolin-2-yl)-methyl-amino]propyl] oxolane-2-carboxamide hydrochloride. Figure 1 shows the structure of alfuzosin. It is used for the treatment of lower urinary tract symptoms associated with benign prostatic hyperplasia. Literature survey reveals that, few chromatographic methods have been reported for the estimation of AFZ. To the best of our knowledge, there is no work in the literature reported about the spectrophotometric method for the analysis of AFZ in either biological fluids or pharmaceutical formulations. Hence the author has made an attempt to develop three simple and sensitive spectrophotometric methods for the estimation of AFZ in bulk drugs and in pharmaceutical formulations. The methods are based on the reaction of primary amino group of alfuzosin with ninhydrin, ascorbic acid and p-benzoquinone (PBQ).

![Figure 1. Structure of alfuzosin](image)

Experimental

Apparatus

All spectral and absorbance measurements were made on a systronic model 106 digital spectrophotometer with 10mm matched quartz cells.

Materials and reagents

All chemicals used were of analytical reagent grade. AFZ was obtained from Dr.Reddy’s labs Hyderabad. The 2% ninhydrin solution was prepared in N, N'-dimethylformamide (DMF). Ascorbic acid solution (1%) was prepared by dissolving 1000 mg in 10 mL of distilled water, in a 100 mL volumetric flask and completing the volume with DMF. The 0.5% PBQ solution was prepared in methanol. 0.1 M phosphate (Na H_2PO_4) buffer solution was prepared and pH adjusted to 7.5 with NaOH.

Standard solution

Stock solution (1000µg/mL) was freshly prepared by dissolving 100mg of AFZ in 100mL of distilled water and then this was further diluted with distilled water so as to obtain working standard solutions of 250 µg/mL (Ninhydrin Method), 200 µg/mL (Ascorbic acid Method) and 300 µg/mL (PBQ Method).

General procedures

Ninhydrin method

In to 10 mL volumetric flasks, different aliquots of working standard solution (0.5- 2.5mL) were transferred to provide final concentration range 12.5 – 62.5 µg/mL. To each flask, 2 mL of 2% ninhydrin solution was added and diluted to volume with DMF. The solutions were heated on a boiling water bath for 10 minutes. The solutions were cooled to room temperature and made up to mark with DMF. The absorbance of each solution was
measured at 575 nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration of the alfuzosin. The content of the unknown was computed either from calibration curve or regression equation.

**Ascorbic acid method**

In 10 mL volumetric flasks, different aliquots of working standard solution (0.5-2.5 mL) were transferred to provide final concentration range 10.0 – 50.0 µg/mL. To each flask, 1.5 mL of 1% ascorbic acid solution was added and diluted to volume with DMF. The solutions were heated on a boiling water bath for 15 minutes. The solutions were cooled to room temperature and made up to mark with DMF. The absorbance of each solution was measured at 530 nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration of the alfuzosin. The content of the unknown was computed either from calibration curve or regression equation.

**PBQ method**

In 10 mL volumetric flasks, different aliquots of working standard solution (0.5-2.5 mL) were transferred to provide final concentration range 15.0 – 75.0 µg/mL. To each flask, 1.5 mL of 0.1 M phosphate buffer solution and 1.5 mL of PBQ reagent were successively added. The volume was made up to mark with distilled water and the solutions were heated on a boiling water bath for 10 minutes. The solutions were cooled to room temperature and made up to mark with distilled water. The absorbance of each solution was measured at 400 nm against the reagent blank. The calibration curve was constructed by plotting the absorbance versus final concentration of the alfuzosin. The content of the unknown was computed either from calibration curve or regression equation.

**Results and Discussion**

**Ninhydrin method**

Ninhydrin is a well-established reagent for the determination of certain amines, amino acids and thiophenes\(^7\). It has been extensively used in the determination of the compounds of pharmaceutical importance and in the kinetic studies\(^8-10\). The reaction is usually carried out by heating for a short time in an organic solvent (2-propanol, butanol, DMF) or in a mixture of water and organic solvent. The reaction product is measured between 550 and 580 nm depending on the reaction conditions\(^11\).

It has been suggested\(^12\) that ninhydrin was converted to \(\text{o-carboxyphenylglyoxal}\) in alkaline medium which would reduce ninhydrin to 2-hydroxyindan-1, 3-dione. In the present study, it combines with –NH\(_2\) group of alfuzosin to form amino derivative, which further undergoes condensation with ninhydrin to give diketoxyindylindene-diketoxyhydrindamine (Ruhemenn’s purple) with \(\lambda_{\text{max}}\) at 575 nm(Scheme 1 and Figure 2). The reaction between alfuzosin and ninhydrin in DMF resulted in the formation of diketoxyindylindene-diketoxyhydrindamine. Alfuzosin was capable of reaction with ninhydrin only at higher temperatures. Maximum color was obtained by heating on a boiling water bath for 10 minutes prolonged heating decreased the color intensity, and so the reaction time should be controlled. The developed color was stable for 2h. The effect of ninhydrin concentration on reaction rate was investigated using 0.5-2.5mL of 2% ninhydrin. It was found that increasing the volume of 2.0% ninhydrin solution would increase the absorbance of the reaction product up to 2.0 mL after which further increase in the volume of ninhydrin resulted in no change in the absorbance of reaction product. Thus 2.0 mL of 2% ninhydrin was adopted as the most suitable volume for maximum absorbance.
Ascorbic acid method

Ascorbic acid has been used\textsuperscript{13} as a sensitive reagent for the specific determination of amines in $N, N'$-dimethylformamide (DMF) medium. In this laboratory, we have used ascorbic acid as a regent for the determination of a pharmaceutical possessing amino group and proposed a reaction mechanism after an appropriate study\textsuperscript{14}. Alfuzosin, as a primary amine, reacts with ascorbic acid in DMF medium to produce a coloured product, which absorbed maximally at 530 nm (Figure 3). Under the specified experimental conditions, ascorbic acid undergoes oxidation resulting in the formation of dehydroascorbic acid\textsuperscript{15}. The carbonyl group of dehydroascorbate reacts with $\text{–NH}_2$ group of alfuzosin to form a purple colored condensation product. The reaction is proposed to proceed as shown in scheme 2.

\begin{center}
\textbf{Scheme 1. Suggested reaction pathway between alfuzosin and ninhydrin}
\end{center}

**Figure 2.** Absorption spectrum of reaction product between alfuzosin (Final concentration 50µg/mL) and ninhydrin.

**Figure 3.** Absorption spectrum of reaction product between alfuzosin (Final concentration 50µg/mL) and ascorbic acid.
Scheme 2. The suggested reaction pathway between alfuzosin and ascorbic acid
The reaction of alfuzosin and ascorbic acid in DMF medium involves two variables i.e. heating time and concentration of ascorbic acid. To study the effect of heating time, 500 μg of alfuzosin was mixed with 1.5 mL of 1% ascorbic acid in a boiling tube and heated on a boiling water bath at 95 ± 5°C. The absorbance was measured at 530 nm as a function of time. It was observed that the absorbance remained constant between 12 and 20 minutes of heating. Thus, 15 minutes of heating time was selected as an optimum value. The influence of volume of 1% ascorbic acid was critically examined. It was found that increasing the volume of 1% ascorbic acid solution would increase the absorbance of the reaction product up to 1.5 mL and above this volume the absorbance remained unaffected. Therefore, 1.5 mL of 1% ascorbic acid was used throughout the experiment.

\[ \text{Alfuzosin} + \text{PBQ reagent} \rightarrow \text{Reaction product} \]

**Scheme 3.** The suggested reaction pathway between alfuzosin and PBQ
p-Benzoinone (PBQ) method

PBQ reagent is used for the determination of an amino acid or amino group. Scheme 3 shows the possible reaction pathway predicted from literature\textsuperscript{16-18} and from results of the present work, where the free primary amine moiety of alfuzosin condenses with carbonyl group of PBQ to form the condensation product. Under the reaction conditions used, which include heating to 95 °C, it was observed that the product of the reaction of PBQ and alfuzosin shows $\lambda_{\text{max}}$ at 400 nm (Figure 4); for most pharmaceutical compounds\textsuperscript{16-18} the products of the reaction absorb in the range 390-670 nm, but most of them at about 490-500 nm. On changing the pH to 0.5, 6.0, 7.0 or 0.8, a shift of the maximum absorbance to shorter wavelengths was observed. Zaia \textit{et al}\textsuperscript{19} observed a similar shift to shorter wavelengths upon reaction of PBQ with proteins and amino acids.

![Figure 4. Absorption spectrum of reaction product between alfuzosin (Final concentration 50$\mu$g/mL) and PBQ reagent](image)

The absorptiometric properties of the colored species as well as the influence of different parameters on the color development are extensively studied to determine optimal conditions of the assay procedure. The reaction was studied as a function of the volume of the reagent, selectivity of the solvent, reaction time and stability. The optimum conditions were incorporated in to the general procedure.

\textbf{Optical and regression characteristics}

The optical characteristics such as Beer’s law limits, Sandell’s sensitivity, molar absorptivity, percent relative standard deviation (calculated from eight replicate samples
containing 3/4\textsuperscript{th} of the amount of the upper beer’s law limits) were calculated for all the methods and the results are summarized in Table 1. Regression characteristics like standard deviation of slope ($S_b$), standard deviation of intercept ($S_a$), standard error of estimation ($S_e$), % range of error (0.05 and 0.01 confidence limits) and detection limit were calculated for all the methods and are shown in Table 1.

Commercial formulation of alfuzosin was successfully analyzed by the proposed and reference methods. The values obtained by the proposed and reference methods are presented in Table 2. The reliability of the proposed method was checked by standard addition method. The results (Table 3) show that the mean recoveries were found in the range 100.34-101.14 with RSD $\leq$1.26% for ninhydrin method, 100.2-100.72 with RSD $\leq$1.40 for ascorbic acid method and 100.02-100.65 with RSD $\leq$1.26 for PBQ method.

### Table 1. Optical and Regression Characteristics, Precision and Accuracy of the Proposed Methods for alfuzosin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ninhydrin Method</th>
<th>Ascorbic acid Method</th>
<th>PBQ Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$, nm</td>
<td>575</td>
<td>530</td>
<td>400</td>
</tr>
<tr>
<td>Beer’s law limits, µg mL\textsuperscript{-1}</td>
<td>12.5 – 62.5</td>
<td>10.0– 50.0</td>
<td>15 - 75</td>
</tr>
<tr>
<td>Detection limits, µg mL\textsuperscript{-1}</td>
<td>0.682</td>
<td>0.216</td>
<td>0.313</td>
</tr>
<tr>
<td>Molar absorptivity, L mole\textsuperscript{-1} cm\textsuperscript{-1}</td>
<td>5.49 x 10\textsuperscript{3}</td>
<td>7.03 x 10\textsuperscript{3}</td>
<td>4.86 x 10\textsuperscript{3}</td>
</tr>
<tr>
<td>Sandell’s sensitivity</td>
<td>0.077</td>
<td>0.060</td>
<td>0.087</td>
</tr>
<tr>
<td>Regression equation ($Y = a + bC$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope ($b$)</td>
<td>1.29 x 10\textsuperscript{-2}</td>
<td>1.65 x 10\textsuperscript{-2}</td>
<td>1.46 x 10\textsuperscript{-3}</td>
</tr>
<tr>
<td>Standard deviation of slope ($S_b$)</td>
<td>0.70 x 10\textsuperscript{-4}</td>
<td>0.4 x 10\textsuperscript{-4}</td>
<td>0.2 x 10\textsuperscript{-4}</td>
</tr>
<tr>
<td>Intercept ($a$)</td>
<td>3.60 x 10\textsuperscript{-3}</td>
<td>3.0 x 10\textsuperscript{-3}</td>
<td>3.0 x 10\textsuperscript{-3}</td>
</tr>
<tr>
<td>Standard deviation of intercept ($S_a$)</td>
<td>2.94 x 10\textsuperscript{-3}</td>
<td>1.20 x 10\textsuperscript{-3}</td>
<td>1.20 x 10\textsuperscript{-3}</td>
</tr>
<tr>
<td>Standard error of estimation ($S_e$)</td>
<td>2.80 x 10\textsuperscript{-3}</td>
<td>1.14 x 10\textsuperscript{-3}</td>
<td>1.14 x 10\textsuperscript{-3}</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Relative standard deviation, %\textsuperscript{a}</td>
<td>0.224</td>
<td>0.125</td>
<td>0.110</td>
</tr>
<tr>
<td>% Range of error(Confidence limits)\textsuperscript{a}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05 level</td>
<td>0.187</td>
<td>0.105</td>
<td>0.092</td>
</tr>
<tr>
<td>0.01 level</td>
<td>0.277</td>
<td>0.156</td>
<td>0.136</td>
</tr>
<tr>
<td>% Error in bulk samples\textsuperscript{b}</td>
<td>-0.328</td>
<td>0.169</td>
<td>0.145</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Average of eight determinations, \textsuperscript{b}Average of three determinations

In $Y = a + bC$, $Y$ is absorbance and $C$ is concentration.

### Table 2. Results of analysis of tablet formulations containing alfuzosin

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labeled Amount mg</th>
<th>% Recovery*</th>
<th>% Recovery*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ninhydrin Method</td>
<td>Ascorbic acid Method</td>
</tr>
<tr>
<td>Tablets-1</td>
<td>10</td>
<td>100.12</td>
<td>100.09</td>
</tr>
<tr>
<td>Tablets-2</td>
<td>10</td>
<td>100.75</td>
<td>100.32</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Recovery amount was the average of five determinations

\textsuperscript{**}UV method developed in our laboratory
Table 3. Determination of Alfuzosin in pharmaceutical formulation by standard addition method

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Ninhydrin Method</th>
<th>Ascorbic acid Method</th>
<th>PBQ Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount, µg/mL</td>
<td>Recovery±R SD, %</td>
<td>Amount, µg/mL</td>
</tr>
<tr>
<td></td>
<td>Taken+ added</td>
<td>Found ±SD</td>
<td>Taken+ added</td>
</tr>
<tr>
<td>Tablets-I</td>
<td>15+20 35.12±0.36</td>
<td>100.34±1.02</td>
<td>20+20 40.08±0.13</td>
</tr>
<tr>
<td></td>
<td>20+30 50.25±0.53</td>
<td>100.5±1.05</td>
<td>25+25 50.24±0.21</td>
</tr>
<tr>
<td>Tablets-II</td>
<td>15+20 35.40±0.29</td>
<td>101.14±0.81</td>
<td>20+20 40.17±0.48</td>
</tr>
<tr>
<td></td>
<td>20+30 50.51±0.64</td>
<td>101.02±1.26</td>
<td>25+25 50.36±0.71</td>
</tr>
</tbody>
</table>
Conclusions

The data given above reveal that the proposed methods are simple, accurate and sensitive with good precision and accuracy. The proposed methods can be used as alternative methods to the reported ones for the routine determination of alfuzosin. This encourages their successful use in routine analysis of alfuzosin in quality control laboratories.

References

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