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Diazotization and coupling reaction of alfuzosin with β-naphthol using sensetive spectrophotometric method

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Abstract: *A simple, specific and rapid spectrophotometric method was developed for the determination of alfuzosin in pharmaceutical formulations. The method is based on the diazotization and coupling reaction of alfuzosin with β-naphthol. The amino group in alfuzosin is diazotised with sodium nitrite and hydrochloric acid at 0°C temperature. After diazotisation, the diazonium salt is coupled with β-naphthol. The orange coloured chromogen formed in the method is stable for more than 24 hours. The orange coloured chromogen is used to determine alfuzosin the spectrophotometrically. The diazotized drug treated with β-napthol solution has maximum absorbance at 510 nm and concentration range of 30-180µg/ml. The molar absorptivity, sandall's sensitivity, slope and intercept were 1.4697 L.mole^{-1}cm^{-1}, 0.0021 µg.cm^{-2}, 0.0039 and 0.0057 for alfuzosin respectively. The developed method was found to be simple, specific, robust, accurate and precise for the determination of alfuzosin.*

Key words: *Alfuzosin, sodium nitrite, hydrochloric acid, β-naphthol and UVSpectrophotometric method.*

I. Introduction

Alfuzosin is chemically N-[3-[(4-amino-6, 7-di-methoxy-quinazolin-2-yl)-methyl-amino] propyl] tetra hydrofuran-2-carboxamide. Alfuzosin belongs to a class of drugs called alpha-blockers. It is used for increasing the flow of urine that is reduced by benign prostatic hyperplasia (BPH) [1]. It is a basic compound with a pKa value of 8.13 and is stable under normal conditions of temparature and light [2]. It works by relaxing the muscles in the prostate and bladder neck, making it easier to urinate. It is recently developed antagonist of a_{1}-post- synaptic adrenergic receptor, showing some myorelaxant effect. Alfuzosin is used in adult men to treat slow urination due to benign prostatic hyperplasis. Most men experience an improvement in urination in 2 to 3 weeks.

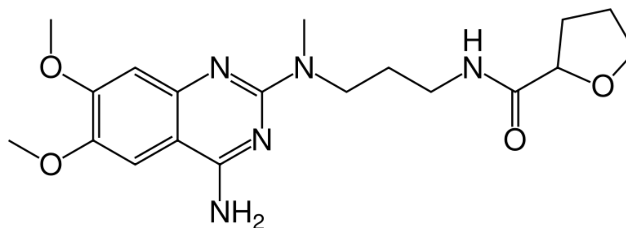

Figure 1.Structure of alfuzosin

The literature survey reveals that, different methods are available for the determination of alfuzosin including spectrophotometry [3-8], chromatography [9-15], voltametric [16], potentiometric [17], HPLC [18], HPTLC, Non aqueous titration, liquid chromatography-tandem mass spectrophotometry and colorimetric methods were reported for the estimation of alfuzosin hydrochloride from biological fluied and pharmaceutical formulations [19-22].

The above mentioned methods are very complex and expensive equipment is involved. The present investigation has made to develop simple, cost effective, selective, accurate and rapid for the determination of alfuzosin.

II. Experimental

2.1 Instrumentation

A shimadzu UV-visible double beam spectrophotometer (model 2450) with 1 cm matched quartz cells was used for all the spectral measurements.

2.2 Chemicals and reagents

0.1N sodium nitrite, 1% β-naphthal, 1%urea solution, 0.5N sodium carbonate, methanol,0.1N hydrochloric acid, β-naphthol were produced from merk. Alfuzosin was produced from sun pharmaceuticals

industries, Bangalore. All the chemicals used were of analytical grade. Double distilled water was used for all the experimental studies.

2.3 Preparation of the sample solution

50 mg of the drug is weighed accurately and transferred into a 50 ml beaker and mixed well with 30 ml of methanol. The solution is filtered and transferred into a 50 ml volumetric flask and the volume is made up to 50 ml with methanol. The concentration of the drug solutions is now 1mg/ml. This stock solution is further diluted to obtain the working concentration of 100 μg/ml.

2.4 Assay Procedure

In to a series of 10 ml volumetric flasks, various aliquots of the standard alfuzosin solution ranging from 0.2-1.0 ml are transferred. To each flask, 2.0 ml of 0.1N hydrochloric acid solution and 1.0 ml of cold 0.1N sodium nitrite solution are added. The resultant solution in each flask is well shaken and allowed to stand for five minutes at 0-5°C temperature for diazotization to complete. A 1.0 ml of 1% urea solution is added to each flask and the solution is shaken frequently to allow nitrogen gas to escape. Then, 1.0 ml of 0.5N sodium carbonate solution and 1.0 ml of 1% β-naphthol solution are added and the volume in each flask is made up to 10 ml with methanol. An orange colour is formed. The maximum absorbance of the orange coloured solution is measured at 510 nm against the reagent blank. Calibration graph is obtained by plotting absorbance values against the concentration of alfuzosin solution. The calibration curve is found to be linear over a concentration range of 30 to 180 μg/ml of alfuzosin. The amount of alfuzosin present in the sample is estimated from the calibration graph. The results are presented in figure 3.

2.5 Spectrum of diazotized alfuzosin treated with β-naphthol

The absorbance maximum of the diazotised drug treated with β-naphthol solution is ascertained by the following procedure.

1.0 ml of alfuzosin solution (100μg/ml) is transferred into a 10 ml volumetric flask. To this, 2.0 ml of 0.1N hydrochloric acid and 1.0 ml of cold 0.1 N sodium nitrite solutions are added. The resultant solution is well mixed, and then allowed to stand for five minutes at 0-5°C temperature for diazotization. To this solution, 1 ml of 1% urea solution is added and shaken frequently for nitrogen gas to escape. Then 1.0 ml of 0.5 N sodium carbonates and 1 ml of 1% β-napthol solution are added and the volume is made to 10 ml with methanol. The absorbance of the orange coloured form is measured in the wavelength range of 400 to 700 nm, against the reagent blank. The spectrum is given in figure. 2

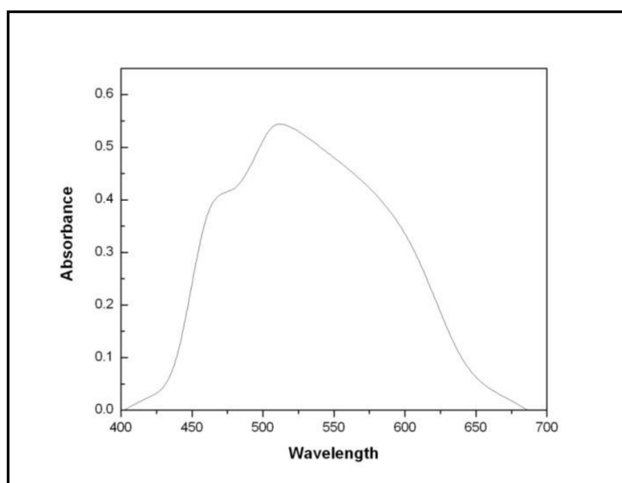

Figure 2: Spectrum of diazotized alfuzosin treated with β-naphthol

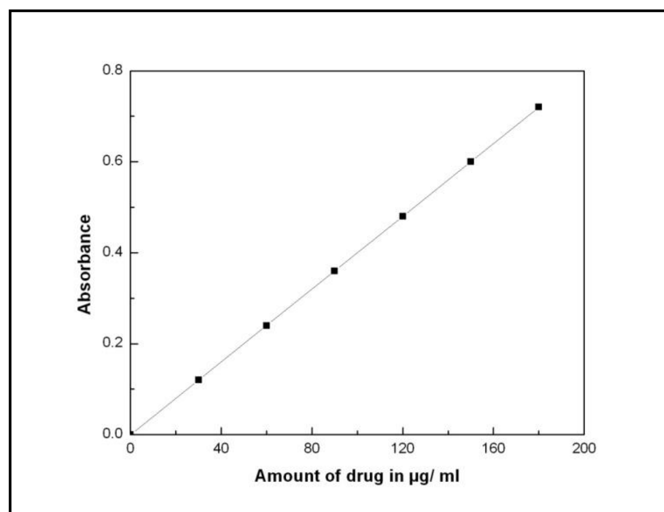

Figure 3: Calibration curve of alfuzosin

III. Method Validation

3.1 Linearity

For the quantitative analysis of the drug alfuzosin, the method was validated according to ICH guidelines and the characters of validation addressed are linearity, accuracy, precision, specificity, LOD, LOQ and robustness. The experimental conditions of the drug alfuzosin by spectrophotometric method, standard calibration curves with β-naphthol were constructed by plotting absorbance versus concentration. The statistical parameters were given in the regression equation calculated from calibration plots along with standard deviation. The linearity of calibration graphs are proved by high values of correlation coefficient and small values of y- intercept of the regression equation. The molar absorbtivities of the coloured complexes and relative standard deviation for the proposed spectrophotometric method were also calculated and shown in table 1.

3.2 Robustness and Ruggedness

In the study of robustness, some parameters like concentrations of the drug and reagents and shaking time were interchanged. Even after that, the results were unaffected by small deliberate and shaking time. The method of ruggedness was expressed as the percentage of relative standard deviation for the proposed method developed by two analysts in two different instruments in two different days. The results proved that there is no statistical difference between the above said two analysts and instruments which conclude the developed analytical method were robust and rugged.

3.3 Accuracy and precision

Recovery studies were carried out in bulk drug and in biological fluids, viz. Serum and urine samples were given in table 5. All the results are good within the acceptable boundary. The percentage recovery was calculated as

Percentage Recovery = [(a-b)/c] x 100.

Where 'a' is the total amount of the drug estimated.

'b' is the amount of the drug found on pre-analyzed basis (standard drug solution).

'c' is the amount of the pure drug added to the formulation.

The intra-day and inter-day precision was determined by analyzing the same concentration of the solutions on three different days. The precision calculated as inter-day and intra-day RSD% is less than 1 and proved that there is no considerable difference for the assay which is tested in inter-day and intra-day from pharmaceutical ingredients and biological samples. The results are presented in table 2.

3.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were determined by using the formula based on the standard deviation of the response and slope.

LOD and LOQ were calculated by using the equations.

LOD= 3x s/S and LoQ = 10xs/S.

Where's' is the standard deviation of the intercept.

S is the slope.

3.5 Effect of interferences

To study the selectivity of the proposed analytical method, the effect of the excipients viz. glucose, sucrose, lactose, dextrose, talc and Starch which frequently come with the drug alfuzosin in its dosage forms was studied. The results showed that there is no interference from the degradation which indicates a high selectivity of the proposed method in determining alfuzosin in its dosage form. These results are recorded in table 4.

3.6 Assay in pharmaceutical formulations and in serum and urine samples

Blood and urine samples were collected from donors, and centrifuged at 3000 rpm for nearly 10 min. The resulted solutions were filtered and preserved in the absence of light at a temperature of 4°C. From these solutions, various concentrations of the drug alfuzosin were analyzed with the help of proposed analytical method and these results were recorded in table 5. These results are indicating that, the proposed method can be successfully applied to recover alfuzosin in biological samples, viz. urine and serum.

IV. Results And Discussion

Alfuzosin undergoes diazotization when treated with sodium nitrate and hydrochloric acid. The excess nitrous acid during the diazotisation is removed by the addition of urea solution. The solution was shaken frequently to allow the nitrogen gas to escape. The diazonium cation reacts with the coupling reagent, β-naphthol by electrophilic substitution at the o-position of the coupling agent to produce an orange azo product. This wine red product shows maximum absorbance at 510 nm. The colour of the product is stable for more than 24 hours. The calibration curve (concentration vs absorbance) is linear over the range of 30-180 μg/ml of alfuzosin.

The optical characteristics of the proposed method such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in table 1. The molar absorptivity and Sandell's sensitivity values show that method is sensitive. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and results are summarized in the table 1. The value of correlation coefficient was 0.999, which indicated the good linearity of calibration lines. The percent relative standard deviation calculated from the five measurements of alfuzosin shown in table 3. The % RSD is less than 2, which indicates that the method has good reproducibility. The standard deviation values are low indicates high accuracy and reproducibility of the method. The't' calculated values are compared well with the theoretical value of 2.78 there by indicating that the precision of the method is good. There no effect of additives and excipients such starch, calcium lactose and glucose in the concentrations those present in general pharmaceutical preparations.

The proposed method is found to be simple, sensitive, selective, accurate, precise, and economical, and can be used in the determination of alfuzosin in bulk drug and its pharmaceutical dosage forms tablets in a routine manner.

TABLE 1: Optical Characteristics of Proposed Method

Parameters	value
λ max(nm)	510
Beer's law limit(μg/ml)	30-180
Molar absorptivity (L.mole^{-1} cm^{-1})	1.4697
Sandell's sensitivity (μg.cm^{-2}/0.001 A.U)	0.002174
Slope(b)	0.0039
Intercept(a)	0.0057
Correlation coefficient(r^2)	0.9985
%RSD	0.2173
LOD	0.7645
LOQ	2.5459

TABLE 2: Evaluation Of Inter Day and Intra Day Accuracy

Taken μg/ml	Inter day				Intra day			
	Found	Recovery %	±SD	%RSD	Found	Recovery %	±SD	%RSD
1	0.985	98.5	0.0072	0.732	0.998	99.8	0.0072	0.722
2	1.978	98.9	0.157	0.077	1.988	99.4	0.0015	0.077
3	2.997	99.9	0.0091	0.300	2.989	99.6	0.0090	0.3015
4	3.985	99.6	0.0092	0.022	3.987	99.6	0.0095	0.022

TABLE 3: Assay of Alfuzosin in Pharmaceutical Formulations

Tablets	Labelled amount mg/ml	Amount found mg/ml	%Recovery	±SD	% RSD
Tablet-1	5	4.86	97.33	0.057	0.186
Tablet-2	10	9.93	99.3	0.028	0.290
Tablet-3	15	14.94	99.64	0.045	0.301

TABLE 4: Determination of Alfuzosin in Presence of Excipients

Excipients	Amount taken mg/ml	*Found mg/ml	Recovery %	±SD	RSD %
Glucose	5	4.98	99.73	0.0152	0.3063
Sucrose	10	9.98	99.86	0.152	0.1529
Lactose	15	14.96	99.77	0.0115	0.0771
Dextrose	20	19.95	99.26	0.5571	0.8349
Talc	30	29.96	99.87	0.0152	0.0509
Starch	20	99.65	99.25	0.5543	0.8211

*** Average of five determinations**

TABLE 5: Method Accuracy from Recovery Assay

Sample	Added mg/ml	*Found mg/ml	Recovery %	±SD	RSD%
Serum samples	0.5	0.49	99.46	0.0020	0.4185
	0.6	0.59	99.55	0.0020	0.3484
	0.7	0.69	99.75	0.0011	0.1577
	0.8	0.79	99.25	0.0055	0.7012
Urine samples	0.5	0.49	99.66	0.0011	0.2317
	0.7	0.69	99.71	0.0010	0.1432
	0.9	0.89	99.66	0.0020	0.2229
	1.1	1.09	99.75	0.0020	0.1897

***Average of five determinations**

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