International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol.13 | Issue 2 | Apr - June -2024

www.ijpir.com

DOI : https://doi.org/10.61096/ijpir.v14.iss2.2024.114-120

ISSN: 2231-3656

Print: 2231-3648

Research

Development and Validation of UV Spectrophotometric Method for Estimation of Alfuzosin Hydrochloride in Bulk and its Tablets

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Published on: 24 Apr 2024

Published by:
DrSriram Publications

Abstract

This technique is commonly used for the quantitative estimation of the drug from their formulation as well as for studying their metabolites and their estimation in the biological fluids. Alfuzosin is a selective α_1-adrenoreceptor blocking agent. A simple and sensitive UV spectrophotometric method was developed and validated for the determination of alfuzosin hydrochloride in bulk, pharmaceutical formulations. The estimation carried out by using methanol as solvent. The absorbance was measured at 350 nm. The method was linear in the range of 10-30 µg/ml with correlation coefficient value 0.994. The recovery was found to be 97.21-99.86%. The relative standard deviation was found to be less than 2. The method was validated with respect to accuracy, precision, assay, ruggedness, and robustness, limit of detection and limit of quantitation. Due to the flexibility, accuracy and high precision, the developed method can be employed in routine analysis of bulk and dosage forms.

Keywords: Alfuzosin hydrochloride, adrenergic antagonist, spectrophotometry, hypertension.

INTRODUCTION

Alfuzosin hydrochloride is chemically (R,S)-N-[3-{(4-amino-6,7-dimethoxy-2-quinazolinyl) methylamino}propyl] tetrahydro-2-furan carboxamide hydrochloride is antagonist of α_1-adrenergic receptors used in the treatment of prostatic hyperplasia [1,2].

Alfuzosin hydrochloride with an empirical formula of $C_{19}H_{27}N_5O_4 \cdot HCl$ (Figure 1) and molecular weight is 425.9 g/mol. Alfuzosin is alpha-adrenergic blocker and relaxes the muscles in the prostate and bladder neck, making it easier to urinate. It is used to improve urination in men with benign prostatic hyperplasia and has been tried in the treatment of hypertension [3].

Alfuzosin hydrochloride has oral bioavailability of 60% under fasting conditions. It is a white to off-white crystalline powder which melts at approximately 240 ^{0}C with molecular weight 425.91 g/mol is freely soluble in water, sparingly soluble in alcohol and practically insoluble in dichloromethane[4].

Present work deals with UV-spectrophotometric method for estimation of alfuzosin hydrochloride from bulk and tablets.

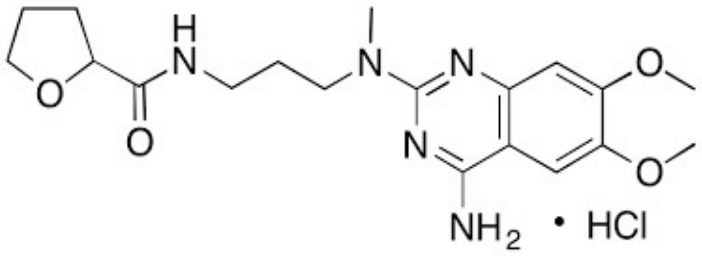

Fig 1: Structure of alfuzosin hydrochloride

Most of the pharmaceutical industries perform quantitative chemical analysis to ensure that the raw material used and the final product thus obtained meets certain specification and to determine how much of each component are present in the final product. Standard analytical procedure for newer drugs or formulation may not be available in Pharmacopoeias; hence it is essential to develop newer analytical methods which are accurate, precise, specific, linear, simple and rapid [5,6].

By considering the impediments of the reported methods such as lack of broad linearity range, economical, present work was aimed to develop and validate a simple, sensitive, precise and accurate UV detection method estimation on alfuzosin in bulk and in pharmaceutical dosage forms according to ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used were of analytical grade. All the solution were freshly prepared with double distilled water. Pure raw material of alfuzosin hydrochloride was obtained as a gift sample.

Pharmaceutical formulation

The formulation (tablets) used were purchased from local Apollo Pharmacy, Hyderabad, Telangana, India (Alfuzosin 10 mg).

Equipments

UV-Visible spectrophotometer with UV Win software. Weighing balances and matching quartz cells with a 1 cm cell path length were utilized along with the mentioned equipment, which had automatic wavelength accuracy of 0.1 nm. Electronic digital balance was used for quantification.

Solvent selection

Number of trails were done to find out the ideal solvent for dissolving the drug alfuzosin hydrochloride. The solvents such as double distilled water, methanol, acetonitrile and ethanol were tried based on the solubility of the drug.

Selection of detection wavelength [7,8]

Appropriate volume 1 ml of standard stock solution of alfuzosin hydrochloride was transferred into a 10 ml volumetric flask, diluted to a mark with methanol to give concentration of 10 µg/ml. The resulting solution was scanned in the UV range (200-400 nm).

Preparation of stock solution [9-11]

A precisely weighed, 10 mg of alfuzosin hydrochloride was transferred to 10 ml clean and dry volumetric flask. Then few ml of methanol was added and dissolved the drug by vigorous shaking. The volume was then made up to the mark with methanol to obtain the stock solution of 1000 µg/ml.

Preparation of working standard solution

From stock solution 1 ml was pipetted out further diluted to 10 ml with methanol to get the solution having the concentration of 100µg/ml.

Preparation of calibration curve

From the above working standard solution, pipetted out 1 ml, 1.5 ml, 2 ml, 2.5 ml, and 3 ml diluted to 10 ml using methanol to produce 10, 15, 20, 25, and 30 µg/ml solutions respectively. The absorbance of the solutions at the λ_{max} of 350 nm using methanol as blank was measured. The calibration curve was plotted by taking concentration on X-axis and absorbance on Y-axis. The curve shows linearity in the concentration range of 2 to 10 µg/ml. The correlation co-efficient (r^2) was found to be 0.994.

Procedure for assay of tablets [12]

20 Tablets of alfuzosin hydrochloride marketed formulations were weighed and powdered. A quantity of tablet powder equivalent to 50mg of alfuzosin hydrochloride was transferred to 100 ml volumetric flask and volume was made up to the mark with methanol. The absorbance of the resulting solution was measured at 350 nm and the amount of alfuzosin hydrochloride was computed from its calibration plot.

Analytical method validation [13-15]

These current validation characteristics describe the validation parameters stated by the International Conference on Harmonization [ICH] guidelines.

Linearity

The linearity of an analytical procedure is its ability to obtain test results, which are directly proportional to the concentration of analyte in the sample. Linearity can be assessed by performing single measurements at several analyte concentrations. A linearity correlation coefficient above 0.994 is acceptable for most methods, especially for major components in assay methods. The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under prescribed conditions. Precision was determined by intra-day and inter-day study. The repeatability of the method was evaluated by carrying out the assay 3 times on the same day and intermediate precision was evaluated by carrying out the assay on 3 consecutive days for the sample solution. The percent relative standard deviation (% RSD) was calculated.

Accuracy (Recovery studies)

The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted true value. Accuracy studies were performed at three different levels (80%, 100% and 120%) by standard addition method and the samples were analyzed in triplicate by the proposed method. Known amount of standard alfuzosin hydrochloride at 80%, 100% and 120% of pre-determined sample was added to a pre-quantified tablet sample.

Ruggedness

Method ruggedness is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

Robustness

The concept of robustness of an analytical procedure has been defined by the ICH as “a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters”. The most important aspect of robustness is to develop methods that allow for expected variations in the separation parameters. For the determination of a method’s robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method’s robustness range. The absorbance was measured and assay was calculated for six times.

LOD and LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOD = 3.3 × σ/S

LOQ = 10 × σ/S

Where, σ = Standard deviation of the response, and

S = Slope of the calibration curve.

RESULTS

New spectrophotometric method has been developed for the determination of alfuzosin hydrochloride in bulk and tablet formulation. The absorption spectra were recorded in the wavelength region of 200-400 nm by UV spectrophotometer.

Determination of absorbance maxima

From the above working standard solution, 1 ml was pipetted out into a 10 ml volumetric flask and the volume was made up to the mark with methanol to prepare a concentration of 10 µg/ml. The sample was then scanned in UV/Vis-spectrophotometer in the range 200-400 nm using methanol as blank and the wavelength corresponding to maximum absorbance was found to be 350 nm.

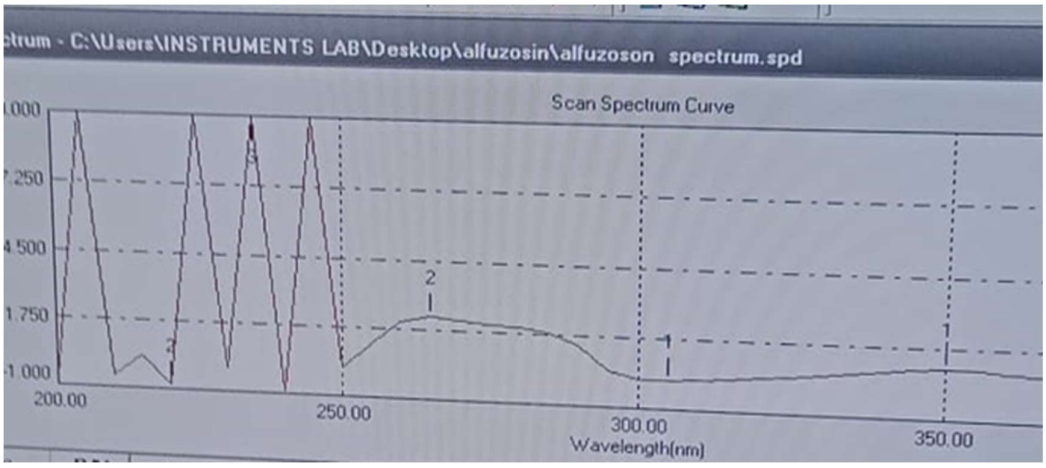

Fig 2: Absorbance maxima of alfuzosin hydrochloride

Table 1: Linearity of alfuzosin

S. No.	Conc. (µg/ml)	Absorbance
1	0	0
2	2	0.154
3	4	0.321
4	6	0.441
5	8	0.614
6	10	0.768

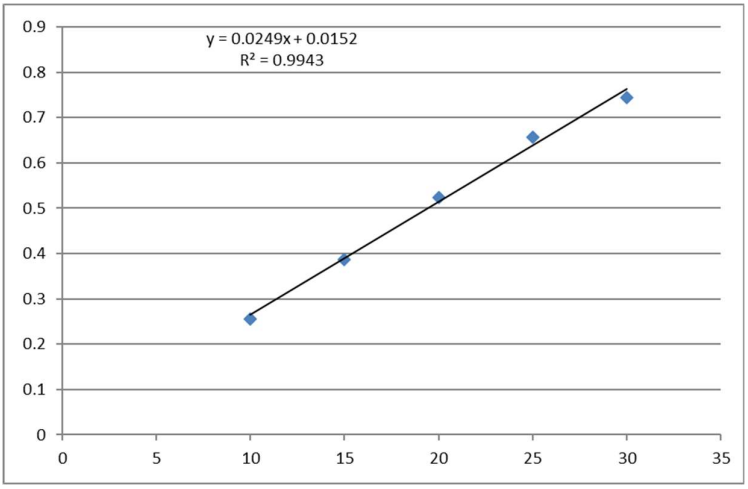

Fig 3: Linearity graph of alfuzosin

Table 2: Precision results

S. No.	Conc. (µg/ml)	Absorbance (intraday)	Absorbance (interday)
1	30	0.773	0.781
2	30	0.771	0.783
3	30	0.772	0.782
4	30	0.771	0.780
5	30	0.772	0.789
6	30	0.774	0.783
Mean		**0.7735**	**0.7945**
Std. Dev.		**0.000707**	**0.0021**
% RSD		**0.09140**	**0.266**

Table 3: Results for accuracy

Level of adding	Amount added (µg/ml)	Amount recovered (µg/ml)	Average
80	4.8	4.67	97.21
100	6	5.89	98.16
120	7.2	7.01	99.86

Table 4: Results for robustness study

S. No.	Wavelength	Absorbance
1.	348 nm	0.774
2.	350 nm	0.761
3.	352 nm	0.772

Table 5: Results for ruggedness

S. No.	Analyst	% RSD
1	Analyst-1	0.719
2	Analyst-2	0.701

Table 6: Results for assay

Brand name	Drug	Labelled amount	Mean	SD	% Assay	% RSD
Alfuzosin	Alfuzosin hydrochloride	10 mg	100.115	1.40	99.12	0.0139

Table 7: Results of LOD and LOQ

Drug	LOD	LOQ
Alfuzosin hydrochloride	0.305 µg/ml	0.927 µg/ml

DISCUSSION

It was observed that the drug showed maximum absorbance at 350 nm which was selected as the wavelength for detection. The absorption maxima curve was shown in Figure 2. The proposed method obeyed Beer's law in the concentration range of 10-30 µg/ml with good correlation coefficient of $r^2 = 0.994$. Calibration data was represented in Table 1. Beer's law range was confirmed by the linearity of the calibration curve of alfuzosin hydrochloride was show Figure 3.

Precision of the method was reported in terms of relative standard deviation and it should be evaluated by using a minimum of 3 determinations over which shows % RSD less than 2 indicates that the method was precise and the results are presented in Table 2. Recovery studies were carried out for the developed method by addition of known amount of standard drug solution of alfuzosin hydrochloride to pre-analyzed tablet sample solution at three different concentration levels. The resulting solutions were analyzed by the proposed methods. The recovery (Table 3) was in the range of 97.21-99.86%. The results were reported to be within the limits. In fact, there was no difference in mean assay results of the method obtained from two instruments of different manufacturers.

For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range and the quantitative influences of the variables were determined. The absorbance was measured and assay was calculated for s results of robustness were represented in Table 4. The results are within the specified limits which states that this method is robust. The results of alfuzosin hydrochloride ruggedness were reported in Table 5. The developed method was applied to the analysis of tablet formulations found to be within the proposed limits and the mean %assay value was found to be 99.12%.

The assay results are given in Table 6. The limit of detection and limit of quantitation for estimation of alfuzosin hydrochloride were 0.3059 µg/ml, 0.927 µg/ml respectively, and illustrated in Table 7. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method.

CONCLUSION

For the determination of alfuzosin from API and pharmaceutical dosage form a simple, precise, reliable, accurate, economical and rapid method was developed. The developed method that was validated to parameters such as specificity, linearity, accuracy, precision, robustness, LOD, LOQ and system suitability according to ICH guidelines showed values within limits. The validation study suggests that this method can be considered appropriate in performing quality control and routine procedural determinations of alfuzosin in bulk and pharmaceutical dosage form. Statistical analysis of the data showed that the method was precise, accurate, reproducible and selective for the analysis of alfuzosin hydrochloride. The method was successfully employed for the determination of alfuzosin hydrochloride in commercially available tablet dosage form.

Acknowledgement
The authors are highly thankful to CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad, Telangana, India for providing all the facilities to carry out the work.

Conflict of Interest
Declared none

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