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Research Article

RP-HPLC Method Development and Validation for the Determination of Alfuzosin HCl in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

The present study was undertaken to develop a simple, rapid, accurate and sensitive reverse phase HPLC method for determination of Alfuzosin HCl in bulk powder and pharmaceutical dosage form. The chromatographic separation was accomplished on Hypersill ODS (250×4.6mm, 5µm) column using a mobile phase methanol: water is 90:10 (v/v) at a column temperature of 25°C. The eluents were monitored at 244 nm and total run time was 5 min with a flow rate of 1 ml/min. The drug was well resolved on the stationary phase and the retention time was found as 1.3±0.89 min with injection volume of 20µl.The method was found to be linear at concentration ranges of 10-50µg/ml with correlation coefficient of 0.999. The method was validated for linearity, precision, robustness and accuracy as per ICH guidelines. The results of all the validation parameters were well within their acceptance values (%RSD <2.0 specified by the USP, ICH and FDA), which prove applicability of the proposed method for routine analyses and quality-control assay of Alfuzosin HCl in pharmaceutical preparations.

INTRODUCTION

Alfuzosin HCl is widely used an alpha-adrenergic blocker drug to treat Benign Prostatic Hyperplasia (BPH) approved by the FDA in June 2003. It is a selective antagonist of post-synaptic alpha-1 adrenoceptors, which are located in the lower urinary tract. Blockade of these adrenoceptors causes relaxation of the muscles in the prostate and bladder neck, making it easier to urinate and a reduction in symptoms of BPH [1]. It is freely soluble in water and readily absorbed after oral administration. The bioavailability of alfuzosin is 49% and extensively metabolized by liver. It is excreted by fecal (69%) and renal (24%) routes. Chemically, it is designated as *(R, S)-N*-[3-[(4-amino-6,7-dimethoxy-2-quizolinyl) methyl amino] propyl] tetra hydro-2-furancarboxamide hydrochloride with the empirical formula of $C_{19}H_{27}N_5O_4$.HCl (Figure 1) and its molecular mass is 389.449g/mol. Alfuzosin is a basic compound with a pK_a value of 8.13 and is stable under normal conditions of temperature and light [2].

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Alfuzosin HCl is a white to off-white crystalline powder, and is sparingly soluble in alcohol, and practically insoluble in dichloromethane.

A detailed literature survey revealed that there is no official method available for the estimation of Alfuzosin in oral formulations. Moreover, few analytical methods have been reported for the determination of Alfuzosin in pharmaceuticals and biological fluids. Alfuzosin was determined in biological fluids by HPLC [3-5], LC-ESI-MS/MS [6], LC-MS [7] and voltammetric methods [8-9]. Alfuzosin was also determined in pharmaceutical formulations including RP-HPLC [10-11], HPLC and HPTLC [12-14], UPLC [15], spectrophotometry [16], colorimetry [17] and voltammetry [8].

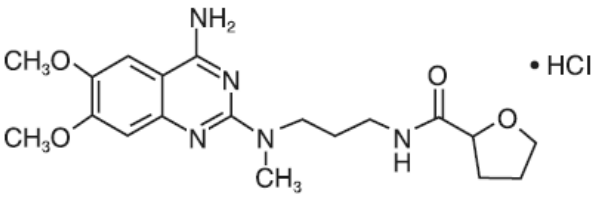

Figure 1: Chemical structure of Alfuzosin HCl

Furthermore, in this study the aim has been designed to develop a new more accurate, simple, precise, reproducible method for the

quantitative HPLC analysis of Alfuzosin HCl in bulk and tablet dosage form and was validated as per ICH guidelines.

MATERIALS AND METHODS

Materials

The reference sample of Alfuzosin HCl was obtained as generous sample from Eskayef Bangladesh Ltd. The branded formulation commercially available Alfasin tablet was procured from the local market, which contain 10mg of Alfuzosin HCl. HPLC grade acetonitrile and methanol was procured from the Active Fine Chemicals Ltd., Bangladesh. Water for Injection (WFI) was obtained as a gift from Globe Pharmaceutical Ltd. All chemicals and reagents used were of analytical grade in the present study.

Instrumentation

The analysis of drugs was carried out on HPLC system on a C18 column. An array detector and an injector with 20µl sample loop. A 20µl syringe was used for injecting the samples. A double-beam Shimadzu UV-1800 visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using an ultrasonic bath sonicator. An Axis balance was used for weighing the materials.

HPLC Condition

The contents of the mobile phase were methanol and water in the ratio of 90:10. These were filtered through 0.45µ membrane filter and degassed by sonication before use. The flow rate of mobile phase was optimized to 1.0 ml/min. The run time was set at 5 min and column temperature was maintained at 25°C. The volume of injection was 20µl, and the eluent was detected at 244 nm.

Preparation of Standard Solution

About 25 mg of Alfuzosin Hydrochloride was accurately weighed and transferred into a 50 ml volumetric flask. Then the reagent methanol was added to make up the volume up to the mark and the mixture was sonicated. 2.0 ml of this solution was taken into another clean and dry 25 ml volumetric flask and then diluted up to the mark using methanol.

Preparation of Sample Solution

Ten tablets were weight accurately and powdered. A quantity of tablet powder equivalent to 25mg of Alfuzosin HCl was accurately weighed and transferred to a clean and dry 50ml volumetric flask. After this the methanol was added to make up the volume up to the mark and then the mixture was sonicated for 5 min. The solution was filtered through Whatman No. 42 filter paper. After filtration 2.0 ml of this solution was taken into another clean and dry 25 ml volumetric flask and diluted up to the mark with methanol.

Preparation of Mobile Phase

For the preparation of mobile phase, HPLC grade methanol and water was mixed, filtered and degassed in such a way that the final volume consists in the ratio of 90:10 v/v.

Preparation of Rinsing Solvent

A mixture of methanol and water as 50:50 v/v was prepared and mixed.

Preparation of Calibration Curve

Standard solution was serially diluted with methanol respectively to obtain varying concentrations of stock solutions as (10.0, 20.0, 30.0, 40.0, and 50.0µg/ml) for Alfuzosin HCl to prepare calibration curve.

Method Validation

The proposed method was validated for linearity, limit of detection, limit of quantification, precision, and accuracy as per International Conference on Harmonization (ICH) guidelines [18-19].

Linearity

The linearity of an analytical procedure is its ability of produce results that are directly proportional to the concentrations of an analyte in the samples. The determination was repeated three times at each concentration level. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Limit of Detection (LOD)

Limit of Detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. LOD was determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected. LOD was calculated using the following equations [20].

$$LOD = 3.3 \times S_o / b$$

Where S_o and b are the standard deviation of the response and the slope of the calibration curve.

Limit of Quantification (LOQ)

Limit of quantification (LOQ) is defined as the lowest concentration that can be quantified reliably with a specified level of accuracy and precision. LOQ was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte could be quantified with acceptable accuracy and precise. LOQ was calculated using the following equations [20].

$LOQ = 10 \times S_o/b$

Where S_o and b are the standard deviation of the response and the slope of the calibration curve.

Precision

The precision of the HPLC method was verified by studying repeatability and intraday and inter day variation. Repeatability studies were performed by analysis of concentration of 20µg/ml six times on the same day. Intraday precision of test method is demonstrated by three injections of the same batch (same concentration) of samples at initial, 24 and 48 hrs. Inter-day precision of test method is demonstrated by three injections of the same batch (same concentration) of samples on three successive days.

Accuracy

The accuracy of the analysis was evaluated by determination of recovery at three different concentrations, equivalent to 80%, 100%, and 120% of the amount in the pre-analysed dosage form as ICH guidelines and average recoveries were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered. The flow rate of the mobile was 1 ml/min. To study the effect of flow rate on the resolution, it was changed by 0.8 ml/min. The effect of the column temperature on resolution was studied at 30°C instead of 25°C.

Potency test

The validated method has been applied for the determination of potency of the tested tablets.The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

RESULTS AND DISCUSSION

The spectra of diluted solutions of the Alfuzosin HCl were recorded on UV spectrophotometer and the maximum absorbance was found at 244nm. To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like water, methanol and acetonitrile with or without different buffers in different combinations were tested as mobile phases on a C18 stationary phase. A mixture of methanol and water in ratio of 90:10 v/v was proved to be the most suitable of all the combinations since the chromatographic peaks were better defined and resolved and almost free from tailing. Flow rate of the mobile phase were changed from 0.5-2.0 ml/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found that 1.0ml/min flow rate was ideal for successful elution of the analyte. No interference was observed in blank and placebo solution of Alfuzosin HCl in the trail injections with a runtime of 10 min. The above optimized chromatographic conditions were followed for estimation of Alfuzosin HCl in bulk and tablet dosage form. The conditions used for chromatography were optimized on the basis of experimentation. The method was validated in accordance with ICH guidelines for linearity, precision, robustness and accuracy. The mobile phase methanol: water (90:10 v/v) enables good resolution and separation using HiQSil C18HS column. The retention time (Rt) values, was 1.3±0.89 min and detection wavelength was 244 nm (Figure 2).

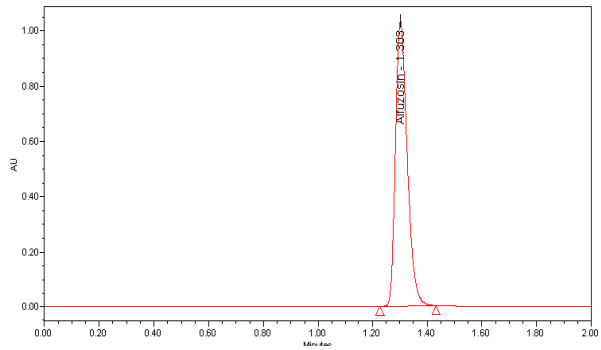

Figure 2: Chromatogram of Alfuzosin HCl standard

A superior linear relationship was obtained when the area was plotted against the concentrations in the range of 10-50 µg/ml (Table1, Figure3). The correlation coefficient of the calibration curve was 0.9998 indicating good linearity with representative linear equation y = 39123x – 5033.3. The limit of detection was found 0.33µg/ml while the limit of quantification was 1.00µg/ml (Table 1).

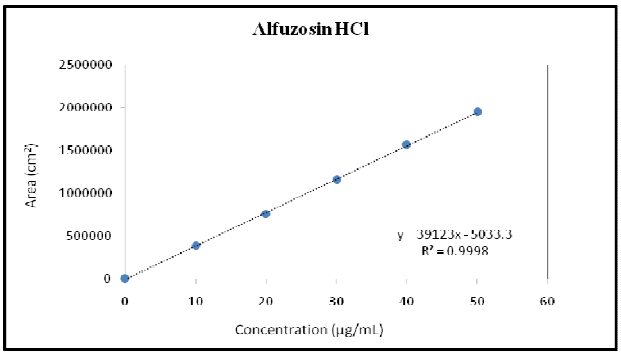

Figure 3: Calibration curve of Alfuzosin HCl

Table 1: Linearity and range test of the developed RP-HPLC method for the determination of Alfuzosin HCl in the pharmaceutical formulation

Conc. of standard (µg/ml)	Peak area (cm^2)	Statistical analysis	Pass/ Remark
10	392107	Regression correlation coefficient (R^2)= 0.9998 Regression equation y = 39123x - 5033.3	Passed
20	784214		
30	1176321		
40	1568428		
50	1960535		
Limit of detection (LOD)			0.33 µg/ml
Limit of quantification (LOQ)			1.00µg/ml

Table 2: Results of repeatability studies

Injection No.	Peak area	Theoretical value (mg)	Average found (mg)	SD	% RSD
1	377358	9.69	9.55	0.17	1.84
2	360012	9.25			
3	371551	9.55			
4	379017	974			
5	368968	9.48			
6	374298	9.62			

Table 3: Results of intermediate precision (intra-day and inter-day precision)

Parameters	Intra-day precision	Inter-day precision
Mean	9.58	9.56
Standard deviation	0.14	0.17
SEM	0.06	0.07
RSD (%)	1.50	1.43

SEM: Standard error of mean; RSD: Relative standard deviation

Precision is validated by studying the repeatability studies indicate the precision under the same operating condition over a short interval of time and intermediate precision in terms of intra-day variation and inter-day variation. The results of repeatability studies, intra-day and inter-day precision were listed in Table 2 and 3 respectively. Robustness study data (Table4) reveal that the method remained unaffected by small, deliberate changes in the flow rate and temperature. The RSD values of the data obtained are below 2% indicating that method is reliable for normal usage. The results of recovery study indicate that any small change in the drug concentration in the solution could be accurately determined by the proposed methods (Table 5).

Table 4: Results of robustness studies

Injection No.	Area	Theoretical Value (mg)	Average found (mg)	SD	% RSD
1	377500	9.70	9.56	0.17	1.81
2	371551	9.55			
3	378120	9.72			
4	361888	9.30			
5	366168	9.41			
6	376674	9.68			

The validated newly method was successfully applied for the analysis of Alfuzosin HCl in pharmaceutical dosage forms (tablets). The potency of marketed formulation was determined by this validated method and the results are presented in Table 6.Percentage estimation of drug content from tablet dosage form by this method was 98.57% with standard deviation (SD) <2. This value indicates the suitability of this method for routine analysis of Alfuzosin HCl in tablet dosage form.

CONCLUSION

The outcomes of the current study advocated that the proposed HPLC method is simple, accurate, robust, less time consuming, cheap and reproducible for estimation of Alfuzosin HCl in bulk and tablet dosage form without interference from the tablet excipients. This chromatographic validated method showed high sensitivity, acceptable linearity and accuracy according ICH guidelines.

Table 5: Results of recovery data study of Alfuzosin HCl

Injection No.	Level of recovery	Amount Added	Amount Found	% Recovery	Mean %Recovery	SD*	RSD
1	80%	8.12	8.10	99.75			
2	80%	8.12	8.00	98.52	99.05	0.05	0.63
3	80%	8.12	8.03	98.89			
4	100%	10.15	10.12	99.70			
5	100%	10.15	10.16	100.1	99.76	0.03	0.30
6	100%	10.15	10.10	99.51			
7	120%	12.18	12.15	99.75			
8	120%	12.18	12.17	99.92	99.94	0.02	0.21
9	120%	12.18	12.20	100.16			

*Mean of three determinations.

Table 6: Potency determination of marketed formulation of Alfuzosin HCl

Dosage form	SN	Sample code	Label claim (mg)	Amount found (mg)	Potency (%)	Mean±SD
Tablet	1	ALF-1	10	9.85	98.50	98.57±0.5
	2	ALF-2	10	9.90	99.00	
	3	ALF-3	10	9.79	97.90	
	4	ALF-4	10	9.89	98.90	

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REFERENCES

[1] Goodman and Gilman's. 2001. The Pharmacological Basics of Therapeutics. 10th ed., McGraw Hill, London.

[2] Guinebault P, Broquaire M, Colafranceschi C, Thénot JP. High-performance liquid-chromatographic determination of alfuzosin in biological fluids with fluorimetric detection and large-volume injection. J Chromatogr A 1986; 353: 361-9.

[3] Niu CQ, Ren LM. Chiral separation and preparation of three new antagonists of alpha1-adrenoceptors by chiral mobile phase HPLC. Yao XueXueBao 2002; 37: 450-3.

[4] Carlucci G, Di-Giuseppe E, Mazzeo P. Determination of alfuzosin in human plasma by high-performance liquid chromatography with column-switching. J LiqChromatogr 1994; 17: 3989-97.

[5] Krstulovic AM, Vende JL. Improved performance of the second generation alpha1-AGP columns: applications to the routine assay of plasma levels of alfuzosin hydrochloride. Chirality 1989; 1: 243-5.

[6] Mistri HN, Jangid AG, Pudage A, Rathod DM, Shrivastav PS. Highly sensitive and rapid LC–ESIMS/ MS method for the simultaneous quantification of uro selective α1-blocker, alfuzosin and an antimuscarinic agent, solifenacin in human plasma. J Chromatogr B 2008; 876: 236-44.

[7] Wiesner JL, Sutherland FCW, Van Essen GH, Hundt HK, Swart KJ, Hundt AF. Selective, sensitive and rapid liquid chromatography-tandem mass spectrometry method for the determination of alfuzosin in human plasma. J Chromatogr B 2003; 788: 361-8.

[8] Uslu B. Voltammetric Analysis of Alfuzosin HCl in Pharmaceuticals, Human Serum and Simulated Gastric Juice. Electroanalysis1990; 14: 866-70.

[9] Rashedi H, Nourouzi P, Ganjali M. Determination of alfuzosin by hybrid of ionic liquid-graphenenano-composite film using coulometric FFT linear sweep voltammetry. Int J Electro Sci 2013; 8: 2479-90.

[10] Madhusudhan, P., Radhakrishna, M.R. and Devanna, N. Method development and validation of Alfuzosin HCl and Dutasteride in pharmaceutical dosage form by RP-HPLC. Int J Novel Tends PharmaSci 2015; 5: 94-101.
[11] Patil VP, Devdhe SJ, Kale SH, Nagmoti VJ, Kurhade SD, Girbane YR, Gaikwad MT. Development and validation of new RP-HPLC method for the estimation of Alfuzosin Hydrochloride in Bulk and Tablet Dosage Form. Am J Ana Che 2013; 4: 34-43.
[12] Dhoka MV, Harale RB, Bhankele SC, Damle MC, Vidwans OS, Kshirsagar SJ. Development, validation and comparative statistical evaluation of HPLC and HPTLC method for determination of Alfuzosin in Human plasma. J Che Pharm Res 2012; 4: 3207-14.
[13] Fayed AS, Shehata MA, Hassan NY, Elweshahy SA. Validated HPLC and HPTLC stability-indicating methods for determination of alfuzosin hydrochloride in bulk powder and pharmaceutical formulations. J Sep Sci 2006; 29: 2716-24.
[14] Patel DB, Patel NJ. Development and validation of reverse phase high performance liquid chromatography and high performance thin layer chromatography methods for estimation of alfuzosin hydrochloride in bulk and in pharmaceutical formulations. Int J Chem Tech Res 2009; 1: 985-90.
[15] Brinda1 MN, Reddy VK, Goud ES. Development and validation of an ultra-performance liquid chromatographic method for uniform of dosage of alfuzosin hydrochloride tablets. IntJ PharmBiolSci 2014; 4: 54-60.
[16] Saiyed N, Patel D, Desai S. Development and validation of first order derivative spectrophotometric method for estimation of Alfuzosin Hydrochloride and Solifenacin Succinate in combined dosage form. Int J Pharm PharmSci2015; 2: 175-83.
[17] Ishaq BM, Prakash KV, Kumar CH, Rani, GU, Ramakrishna P. Colorimetric determination of alfuzosin HCl in pharmaceutical formulations. J Pharm Res 2011; 4: 226-8.
[18] ICH. 2003. Harmonised Tripartitle Guideline, Stability Testing of New Drug Substances and Products. Q1A (R1), USA, pp. 1-18.
[19] ICH. 2005. Harmonised Tripartite Guideline, Validation of analytical procedures: text and methodology, International Conference on Harmonization. Q2 (R1), Geneva.
[20] Banik S, Karmakar P, Miah MAH. Development and Validation of a UV-Spectrophotometric Method for Determination of Vildagliptin and Linagliptin in Bulk and Pharmaceutical Dosage Forms. Bangladesh Pharm J 2015; 18: 163-8.