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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

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ABSTRACT: This research paper describes validated reverse phase high-performance liquid chromatography (RP-HPLC) and high performance thin layer chromatography (HPTLC) methods for the estimation of alfuzosin hydrochloride in bulk and in pharmaceutical formulations. The RP-HPLC separation was achieved on phenomenex C_{18} column (250 mm, 4.6 mm i.d, 5 µm particle size) using water-methanol-acetonitrile (60+30+10, v\v\v) as the mobile phase at a flow rate of 1.0 mL/min at an ambient temperature. Quantification was achieved with ultra-violet (SPD-10A VP) detection at 245 nm over the concentration range 0.2–8 µg/mL with recovery in the range of 99 – 101.26 % for ALH by RP-HPLC method. The HPTLC separation was achieved on an aluminium-backed layer of silica gel $60F_{254}$ using toluene-methanol-triethylamine (3+1+0.2, v+v+v) as mobile phase. Quantification was achieved with HPTLC detection at 245 nm over the concentration range 50-400 ng/spot with recovery in the range of 98.67 – 101.28 % for ALH. Both the methods are simple, precise, and sensitive, and extended to routine analysis of ALH in bulk as well as pharmaceutical formulation.
Key words: Alfuzosin HCl, RP-HPLC, HPTLC, Benign prostatic hyperplasia (BPH).

INTRODUCTION

Alfuzosin HCl (ALH), chemically as (R,S)-N-[3-[(4-amino-6,7- dimethoxy- 2-quinazolinyl) methyl amino] propyl] tetrahydro-2 furancarboxamide hydrochloride with an empirical formula of $C_{19}H_{27}N_5O_4{\cdot}HCl$ and a molecular weight of 425.9 g/mol (Figure 1), is selective antagonist of α_1-adrenoreceptor and used in the treatment of Benign Prostatic Hyperplasia (BPH). BPH is the enlargement of the prostate, frequently occurring in men over the age of 50. Unfortunately, approximately 75 % of men above 50 years of age have a measurable enlargement of the prostate. ALH is a α_1-adrenoreceptor blocker, can cause smooth muscle in the bladder neck and prostate to relax, resulting in an improvement in urine flow and a reduction in symptoms of BPH (1-7). Literature survey revealed a selective, sensitive and rapid Liquid chromatography–tandem mass spectrometry method for the determination of Alfuzosin in human plasma (8). Direct high-performance liquid chromatographic method is also reported for the determination of enantiomers of ALH in plasma (9). High-performance liquid chromatographic determination with fluorimetric detection is available for estimation of ALH in biological fluids (10). Determination of ALH in pharmaceuticals, human serum and simulated gastric juice by voltammetry method is also found in literature (11).

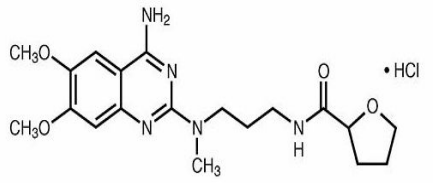

Figure 1. Structure of Alfuzosin HCl

ALH is determined by spectrophotometric and colorimetric methods in pharmaceutical preparations (12, 13, 14 and 15). ALH is estimated by HPLC method with column switching (16). A Stability indicating spectrophotometric and spectrofluorimetric, HPLC and HPTLC methods are also found for ALH in bulk as well as pharmaceutical formulations (17, 18). Any simple HPLC and HPTLC methods are not found in the

literature survey. So it was thought of interest to develop a simple, sensitive, accurate and precise method for analysis of ALH in bulk as well as pharmaceutical formulations.

EXPERIMENTAL

Apparatus

A shimadzu's HPLC (LC-10AT vp) equipped with UV-Visible detector (SPD-10A VP), manual injector of 20µl loop, Phenomenox (Torrance, CA) C_{18} (250 mm * 4.6 mm i.d., 5µm particle size) Column and winchrom software were used. For HPTLC, a Linomat V autosprayer, Scanner-III, flat bottom and twin trough developing chambers
and viewing cabinet with dual wavelength UV lamps (Camag, Muttenz, Switzerland) used. HPTLC plates used were of silica gel with fluorescent indicator 254 nm, layer thickness 0.2 mm, 10*10 cm, aluminium backing (E. Merck KGaA, Darmstadt, Germany). Sartorius CP224S analytical balance (Gottingen, Germany) and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the study.

Reagents and Materials

ALH bulk powder was kindly gifted by Cipla Pharmaceutical Limited, Mumbai (India), 99.95 % purity. HPLC grade methanol and acetonitrile were purchased from S. d. fine Chemical (Ahmedabad, India).). The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 µm –0.47 µm membrane filter (Gelman Laboratory, Mumbai, India). Tablets of ALH were purchased from the local pharmacy.

Chromatographic Conditions

(a) RP-HPLC method - A Phenomenox C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) was used at an ambient temperature. The mobile phase water-methanol-acetonitrile (60+30+10, v/v/v) was pumped at a flow rate of 1 mL/min. The mobile phase was filtered through nylon 0.45 µm-0.47 µm membrane filter and degassed before use. The elution was monitored at 245 nm and the injection volume was 20 µL.

(b) HPTLC method - Solutions of the ALH was applied to silica gel 60F254 HPTLC plates (10*10 cm) by means of a Linomat V automatic spotter equipped with a 100 µL syringe and operated with settings of band length, 6 mm; distance between bands,
8 mm; distance from the plate edge, 10 mm; and distance from the bottom of the plate,
10 mm. The plate was developed in a twin trough chamber previously saturated for 30 min with the mobile phase, toluene – methanol - triethylamine (3+1+0.2, v/v/v/) to 8 cm. The spots on the air-dried plate were scanned with a Scanner III at 245 nm using the deuterium source.

Preparation of ALH Standard Stock Solutions

(a) RP-HPLC method - Accurately weighed ALH (10 mg) was transferred to a 100 mL volumetric flask and dissolved and diluted to the mark with distilled water to obtain a standard solution of ALH (100 µg/mL). This solution (10 mL) was further diluted to 100 mL with distilled water to obtain a working standard solution with ALH (10 µg/mL) for the RP-HPLC method.

(b) HPTLC method - Accurately weighed ALH (10 mg) was transferred to a 100 mL volumetric flask and dissolved in and diluted to the mark with distilled water to obtain a standard solution of ALH (100 µg/mL). This solution was used as working standard solution.

Preparation of Sample Solutions

Twenty tablets of ALH were weighed and powdered. Tablet powder equivalent to 10 mg of ALH was transferred to 100 mL volumetric flask and 80 mL distilled water is added.. The solution was sonicated for 15 min, and the final volume was made with same to obtain solution of ALH (100 µg/mL). The mixture was then filtered through a nylon 0.20 mm-0.47 mm membrane filter. The above solution was suitably diluted with distilled water to obtain final solution of ALH (10 µg/mL) for RP-HPLC method and ALH (100 µg/mL) for HPTLC method.

Method Validation

Both the methods are validated for its linearity range, accuracy, precision, sensitivity and specificity. Method validation is carried out as per ICH Guidelines (19).

Linearity

(a) Calibration curve (linearity) of the RP-HPLC method - Calibration curve was constructed by plotting peak areas vs concentrations of ALH solutions, and the regression equations was calculated. The calibration curve was plotted over the concentration range 0.2–8 µg/mL. Accurately measured standard working solution of ALH (0.2, 0.8, 1, 2, 4 and 8 mL) were transferred to a series of 10 mL of volumetric flasks and diluted up to the mark with distilled water. Aliquots (20 µL) of each solution were injected under the operating chromatographic conditions described as above.

(b) Calibration curve (linearity) of the HPTLC method - Calibration curve was plotted over a concentration range of 50–400 ng/spot for ALH. Accurately prepared standard solution of ALH (0.5, 1, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mL) were applied to the plate. The calibration curve was constructed by plotting peak areas versus concentrations with the help of win-CATS software.

Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of ALH by the standard addition method. Known amounts of standard solutions of ALH (75, 100, and 125 %) for the RP-HPLC method and HPTLC method were added to prequantified sample solution of tablet powder. The amounts of ALH were estimated by applying these values to the regression equation of the calibration curve.

Method Precision (% Repeatability)

The precision of the instruments was checked by repeatedly injecting (n = 6) solutions of ALH (1µg/mL) for the RP-HPLC method and by repeated scanning of the same spot (n = 6) of ALH (200 ng/spot) without changing

the position of plate for the HPTLC method. Repeatability was reported in terms of percentage relative standard deviation (% RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentration of ALH (0.2, 2, and 8 µg/mL) for the RP-HPLC method and ALH (50, 200, and 400 ng/spot) for HPTLC method. The results were reported in terms of percentage relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were calculated using the following equations as per International Conference on Harmonization (ICH) guidelines (19).

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where σ = the standard deviation of the response and S = Slope of calibration curve.

Analysis ALH in Tablet Powder

Tablets of ALH of two brands were purchased from local pharmacy. The responses of the solutions of that tablet dosage forms were measured at 245 nm for quantification by using HPLC and HPTLC instruments described as above. The amount of ALH present in sample solutions was determined from regression equation of both the methods.

RESULTS AND DISCUSSION

(a) RP-HPLC Method

To optimize the RP-HPLC parameters, several mobile phases of different compositions were tried. A satisfactory separation and good peak symmetry for ALH was obtained with a mobile phase consisting of Water-Methanol-Acetonitrile (60+30+10, v\v\v). Quantification was achieved with UV detection at 245 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained. (Figure 2)

(b) HPTLC Method

Several mobile phases were tried to accomplish good distance of ALH. Using the mobile phase Toluene-Methanol-Triethylamine (3+1+0.2, v+v+v), better distance was attained at Rf value of 0.63 for ALH. A wavelength of 245 nm was used for the quantification of the drug. Resolution of the peaks with clear baseline separation was found. (Figure 3)

Validation of the Proposed Method

Linearity - Linear correlation was obtained between peak areas and absorbance vs concentration of ALH in range of 0.2–8 µg/mL and 50–400 ng/spot, respectively for RP-HPLC and HPTLC method. The linearity of the calibration curves was validated by the high value of correlation coefficients of regression. (Table 1)

Accuracy - The recovery experiments were carried out by the standard addition method. The recoveries obtained were 99.12-101.26 % by RP-HPLC and 98.67-101.28 % by HPTLC method for ALH. The high values indicate that both methods are accurate. (Table 2)

Method precision - The % RSD values for ALH were found to be 0.68 % using RP-HPLC and 0.75 % for HPTLC method. The low values % RSD indicates the proposed methods are repeatable. (Tables 2)

Intermediate precision - The low % RSD values of intraday and interday (0.304-0.685% and 0.841-1.002%) and (0.516-0.784% and 0.925-1.048%) variations for ALH, respectively by RP-HPLC and HPTLC methods, reveal that the proposed methods are precise. (Table 2)

LOD and LOQ - LOD for ALH was found to be 125.82 ng/mL and 20.55 ng/spot, respectively for RP-HPLC and HPTLC method. LOQ for ALH was found to be 192.36 ng/mL and 45.96 ng/spot, respectively for RP-HPLC and HPTLC method. These data show that both the methods are sensitive for the determination of ALH. (Table 2)

Analysis of ALH in tablet dosage form

The proposed validated methods were successfully applied to determine ALH in their tablet dosage forms. The results obtained for ALH were comparable with the corresponding labelled amounts. (Table 5)

CONCLUSIONS

The results of the analysis of tablet dosage forms by the proposed methods are highly reproducible and reliable and are in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of ALH by the proposed method. So both the methods can be used for the routine analysis of the ALH in their tablet dosage forms.

Table 1. Regression analysis of the calibration curves for ALH for the proposed RP-HPLC and HPTLC methods

Parameters	ALH	
	RP-HPLC	HPTLC
Concentration range	0.2-8 (µg/mL)	50-400 (ng/spot)
Slope	740.352	14.466
Standard deviation of the slope	2.618	0.0384
Intercept	143916	455.99
Standard deviation of the intercept	845.423	0.0083
Correlation coefficient	0.9955	0.9985
Standard deviation of the correlation coefficient	0.00053	0.00045

Table 2. Summary of validation parameters for ALH by proposed RP-HPLC and HPTLC methods

Parameters	ALH	
	RP-HPLC method	HPTLC
LOD[a]	125.82 ng/mL	20.55 ng/spot
LOQ[b]	192.36 ng/mL	45.96 ng/spot
Accuracy (%RSD[c])	99.12-101.26	98.67-101.28
Repeatability (%RSD[c], *n* = 6)	0.68	0.75
Precision (%RSD)		
Interday (*n* = 3)	0.841 – 1.002	0.925-1.048
Intraday (*n* = 3)	0.304 - 0.685	0.516-0.784

[a]LOD = Limit of detection.
[b]LOQ = Limit of quantification.
[c]%RSD = Percent relative standard deviation

Table 3. System suitability test parameters for ALH for the proposed RP-HPLC method.

Parameters	ALH ± % RSD[a]
Retention time (min)	2.86 ± 0.067
Tailing factor	0.95 ± 0.094
Asymmetry	1.08 ± 0.110
Theoretical plates	166220 ± 0.162

[a]%RSD = Percent relative standard deviation.

Table 4. System suitability test parameters for ALH for the proposed HPTLC method

Parameters	ALH ± % RSD[a]
Rf value	0.63 ± 0.01
Area average	3494.16 ± 0.83

[a]%RSD = Percent relative standard deviation.

Table 5. Assay results for ALH in pharmaceutical formulation using the proposed RP-HPLC and HPTLC methods.

Parameters	ALH ± SD[a] (n^b = 6)	
	RP-HPLC method	HPTLC
Brand A	99.53 ± 0.621	99.74 ± 0.638
Brand B	100.02 ± 0.484	101.14 ± 0.482

[a]S.D = Standard deviation.
[b]*n* = Number of determinations

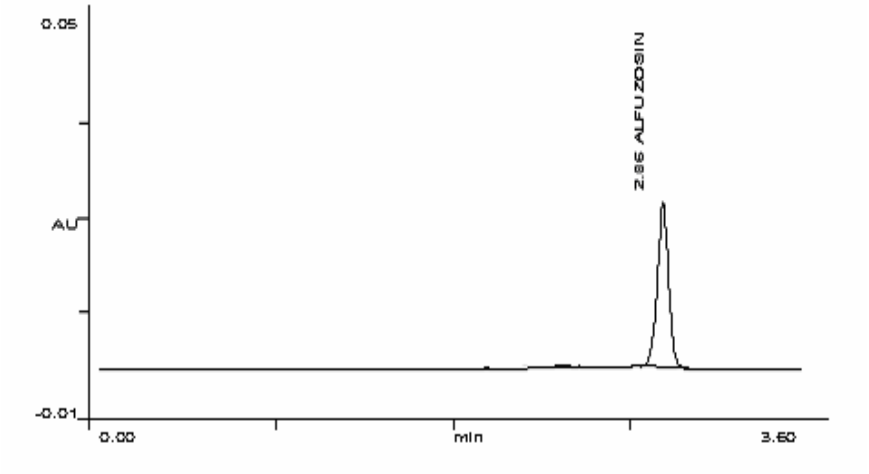

Figure 2. A typical HPLC chromatogram of ALH at 245 nm

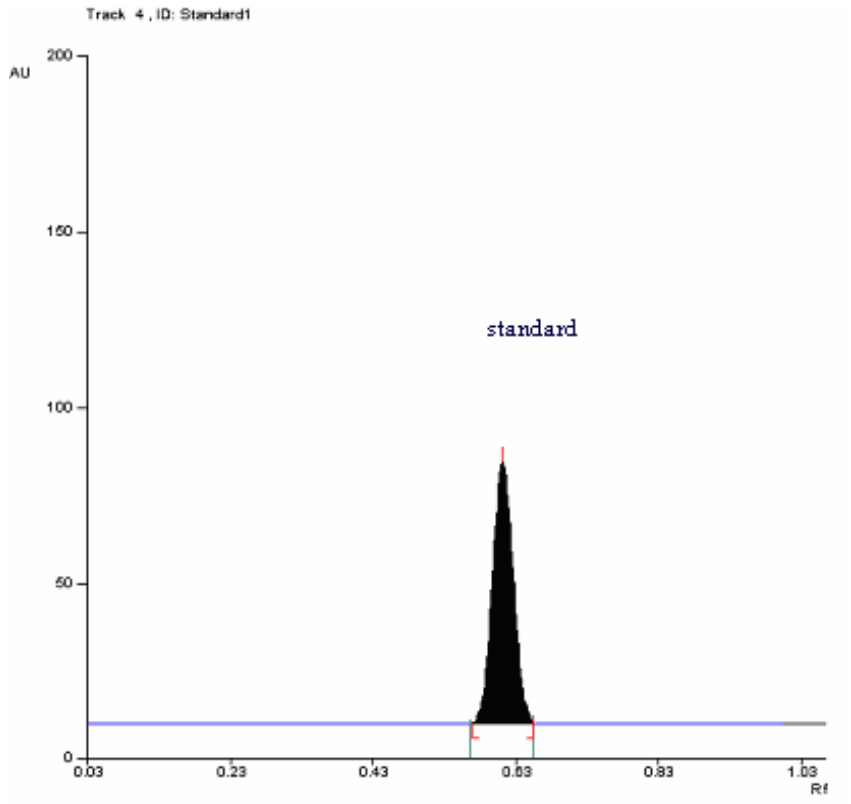

Figure 3. A typical HPTLC densitogram of ALH

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