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# Simultaneous estimation of alfuzosin hydrochloride and dutasteride by validated RP -HPLC method

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## ABSTRACT

This research paper describes validated reverse phase liquid chromatographic method for the simultaneous estimation of alfuzosin hydrochloride and dutasteride in bulk drug and tablets. The HPLC separation was achieved on a Phenomenex $C_{18}$ column (250 mm id, 4.6 mm, 5 µm particle size) using methanol:0.02M ammonium acetate buffer (85:15, v/v), pH 9.5 adjusted with 0.1 % w/v triethylamine, as mobile phase at a flow rate of 1.0 ml/min. Quantification was achieved with photo diode array detection at 274 nm over the concentration range 1–50 µg/ml and 1-20 µg/ml with mean % recovery of 98.92% and 99.76% for alfuzosin hydrochloride and dutasteride, respectively by proposed RPLC method. Proposed method is simple, precise, accurate, sensitive and applicable for the routine simultaneous estimation of alfuzosin hydrochloride and dutasteride in bulk drug and in formulations.


## KEYWORDS

Alfuzosin hydrochloride;
Dutasteride;
RP-HPLC;
Benign prostatic hyperplasia.

## INTRODUCTION

Alfuzosin hydrochloride (ALF), (R,S)-N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl)methyl amino] propyl] tetrahydro-2 furancarboxamide hydrochloride, is selective antagonist of $\alpha_1$ adrenoreceptor exhibits selectivity for $\alpha_1$ receptors in the human prostate. ALF is used to reduce urinary obstruction and relieve the symptoms associated with symptomatic benign prostatic hyperplasia (BPH). Chemically dutasteride (DUTA) is (5α, 17β – N - {2,5 bis (trifluoromethyl)phenyl} – 3 – oxo - 4 azaandrost – 1 – ene – 17 -carboxamide. It is a specific inhibitor of steroid Type II 5α-reductase, an intracellular enzyme that converts the androgen testosterone into 5α-dihydrotestosterone (DHT) hormone responsible for prostate growth. Combination of both the drugs is indicated for the treatment of symptomatic BPH in men with an enlarged prostate[1-3]. Literature survey revealed different analytical methods like titrimetric[4-5], LC-MS[6-7], HPLC[8-13], HPTLC[14-15] spectrofluorometric and spectrophotometric[16-18], and voltammetric[19] methods for determination of ALF and DUTA in biological fluids and in their single dosage form. LC-MS-MS[20] method was also found for simultaneous estimation of ALF and DUTA in biological fluid. So it was thought of interest to develop simple, precise, accurate, specific and sensitive RP-HPLC method for simultaneous estimation of ALF and DUTA in bulk as well in their combined dosage form.

## EXPERIMENTAL

### Materials and methods

A Shimadzu (Columbia, MD) RP-HPLC instrument (LC-2010AT vp) equipped with an photodiode array detector, Phenomenex (Torrance, CA) $C_{18}$ column (250 mm × 4.6 mm id, 5 mm particle size) and Class-VP software were used. Sartorius CP224S analytical balance (Gottingen, Germany) and ultrasonic cleaner (Frontline FS 4, Mumbai, India) were used during the study. ALF and DUTA pure powder were kindly gifted by reputed pharmaceutical company. HPLC grade methanol and AR grade ammonium acetate and triethylamine were purchased from S.D fine chemical, Mumbai, India. The HPLC grade water was prepared by triple glass distillation and filtered through a nylon 0.45 μm – 0.47 mm membrane filter. Tablets were purchased from the local pharmacy.

### Chromatographic conditions

The chromatographic separation was performed on Phenomenex $C_{18}$ (250 mm × 4.6 mm id, 5 μm particle size) column at ambient temperature. The mobile phase consisting of methanol:0.02M ammonium acetate buffer (pH 9.5 adjusted with 0.1 % w/v triethylamine) (85:15, v/v) was filtered through a nylon 0.45 μm-47 mm membrane filter and degassed before use and was pumped at a flow rate of 1 ml/min. The elution was monitored at 274 nm and the injection volume was 20 μl.

### Preparation of standard solutions

A separate standard stock solution (1 mg/ml) of ALF and DUTA was prepared by transferring accurately weighed 25 mg of both and dissolving and diluting up to the mark with methanol in the 25 ml separate volumetric flask. Accurately measured 10 ml of standard stock solution of ALF and 5 ml of standard stock solution of DUTA were transferred to separate 100 ml volumetric flask and diluted up to mark with methanol to achieve final concentration 100 μg/ml for ALF and 50 μg/ml for DUTA.

### Preparation of sample solution

Thirty tablets of two different brands were weighed and powdered. A quantity of tablet powder equivalent to 20 mg of ALF and 1 mg of DUTA was transferred to a 100 ml volumetric flask containing 50 ml methanol and sonicated for 30 minutes. The solution was then filtered through a nylon 0.20 μm-47 mm membrane filter and volume was made up to 100 ml. the solution was then centrifuged and supernant liquid was collected having 200 μg/ml of ALF and 10 μg/ml of DUTA. An aliquot of 2 ml from the above solution was transferred to 10 ml volumetric flask and volume was made up to the mark with methanol to get 40 μg/ml of ALF and 2 μg/ml of DUTA. This solution was analyzed under the chromatographic conditions as described above.

### Method validation

The developed RP-HPLC method was validated for various parameters like linearity, precision, accuracy, specificity, robustness, system suitability, limit of detection (LOD) and (LOQ) as per ICH guideline[21].

Linearity of the method was evaluated at seven concentration levels by plotting the calibration curves over the concentration range from 1-50 μg/ml and 1-20 μg/ml, for ALF and DUTA, respectively. Accurately measured of working standard solutions of ALF (0.1, 0.5, 1, 2, 3, 4 and 5 ml) and DUTA (0.2, 0.4, 0.8, 1.6, 2.4, 3.2 and 4 ml) were transferred to a series of 10 ml volumetric flasks and diluted up to mark with methanol. 20 μl aliquots of each solution were injected and analyzed under the chromatographic conditions as described above. Calibration curves were constructed by plotting peak areas versus concentration of ALF and DUTA and the regression equations were calculated. Accuracy of the method was determined by adding known amounts of standard solutions of drugs (50, 100, and 150 % level) to previously analyzed sample solutions. The amount of drug was analyzed by applying these values to the regression equation of the calibration curves of both drugs and % recovery was calculated. Precision of the method was determined with respect to both repeatability and intermediate precision. The repeatability of the instruments was checked by repeatedly injecting ($n$ = 6) standard solution of both drugs. The intraday and interday precision of the proposed methods were determined by estimating the responses three times on same day and on three different days for three different concentrations of both drugs. To determine the robustness of the developed method,

experimental conditions like flow rate, ratio of mobile phase, wavelength, injection volume and column temperature were deliberately altered and the response of the ALF and DUTA was recorded and % RSD was calculated. Specificity of the method was confirmed from the resolution factor and peak purity data of ALF and DUTA. The limit of detection (LOD) of the both drugs was found by the trial and error method (visual) by injecting progressively low concentrations of standard solutions. The lowest concentration of the range at which the analyte can be quantified with acceptable accuracy and precision was selected as the limit of quantification (LOQ).

## RESULTS AND DISCUSSION

To optimize the HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for ALF and DUTA were obtained with a mobile phase consisting methanol:0.02M ammonium acetate buffer (pH 9.5 adjusted with 0.1 % w/v triethylamine) (85:15, v/v) at a flow rate of 1 ml/min to get better reproducibility and repeatability. Quantification was carried out at 274 nm based on the peak area. Complete resolution with clear baseline was obtained with retention time of 3.115 min for ALF and 5.260 for DUTA (Figure 1).

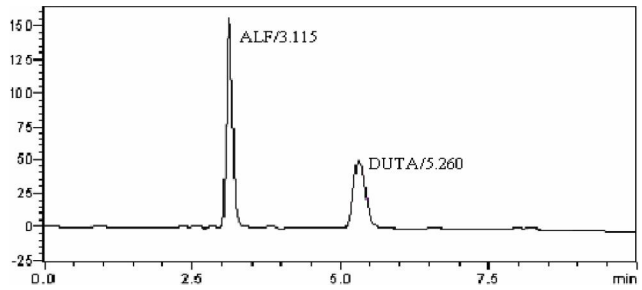


**Figure 1 : Chromatogram of standard solution of alfuzosin hydrochloride (40 µg/ml) and dutasteride (16 µg/ml)**

System suitability test parameters for ALF and DUTA for the proposed method are reported in TABLE 1.

Linear correlation was obtained between peak areas and concentrations in range of 0.5-16 µg/ml for ALF and 1-50 µg/ml for DUTA. Regression parameters are shown in TABLE 2 and high values of correlation coefficients reveals that the method is linear in specified range. Precision was measured in terms of % RSD. The % RSD values of method precision of ALF and DUTA obtained were 0.389 and 0.513%, respectively and for interday precision obtained were 0.739-1.114% and 0.891-1.580%, while for intraday precision obtained were 0.674-0.957% and 0.735-0.918% (Table 2), for ALF and DUTA, respectively indicates that the proposed method is repeatable and precise. LOD for ALF and DUTA was found to be 0.2 and 0.5 µg/ml and LOQ for TAM and FINA was found to be 0.5 and 1.0 µg/ml, respectively. These data shows that method is sensitive for determination ALF and DUTA (TABLE 2).

**TABLE 1 : System suitability test parameters of alfuzosin hydrochloride and dutasteride**

| Parameters | Mean[b] ± % RSD[a] | |
|---|---|---|
| | ALF | DUTA |
| Retention time, min | 3.120 ± 0.471 | 5.253 ± 0.396 |
| Tailing factor | 1.291 ± 0.775 | 1.172 ± 0.989 |
| Asymmetry factor | 1.305 ± 1.682 | 1.195 ± 1.007 |
| Theoretical plates | 3416.52 ± 1.117 | 5041.16 ± 0.879 |
| Capacity factor | 2.92 ± 1.286 | 3.51 ± 0.528 |
| Resolution | 8.625 ± 0.361 | |

**RSD[a] = Relative standard deviation, Mean[b] = Average of five determinations**

**TABLE 2 : Summary regression characteristics and validation parameters of alfuzosin hydrochloride and dutasteride**

| Parameters | ALF | DUTA |
|---|---|---|
| Linearity range (µg/ml) | 1-50 | 1-20 |
| Correlation coefficient ($r^2$) | 0.9987 | 0.9972 |
| Regression equation y = mx+c | | |
| Slope (m) | 44457.8 | 6377.56 |
| Intercept (c) | 54788.8 | 3912.3 |
| LOD (µg/ml) | 0.2 | 0.5 |
| LOQ (µg/ml) | 0.5 | 1 |
| Repeatability (% RSD[a], , $n^b$ =6) | 0.389 | 0.513 |
| Precision (%RSD[a]) | | |
| Intraday ($n^b$ = 3) | 0.258-0.481 | 0.663-0.952 |
| Interday ($n^b$ = 3) | 0.534-0.892 | 0.893-1.191 |

**RSD[a] = Relative standard deviation, n[b] = No. of replicate**

Mean % recoveries ± SD obtained were 98.92 ± 1.067 % and 99.76 ± 0.776 % for ALF and DUTA, respectively suggests accuracy of the proposed method (TABLE 3). Specificity of the method was confirmed from the resolution factor and peak purity data of the analyte. The resolution factor obtained for the ALF and

DUTA from the nearest resolving peak was > 2 in all samples and peak purity data obtained for ALF and DUTA were 0.9997 and 0.9999 suggests that no other excipient is co-eluted with the drug and the peak of the drug is pure in nature and hence the developed method is specific. Robustness of the method was estimated by changing the mobile phase composition (± 2%), flow rate (± 0.1 ml), wavelength (± 1 nm), pH (± 0.1 unit), injection volume (± 1µl) and column temperature (40°C ± 3°C ) and % RSD values calculated for all these changes were less than 2 indicate that method is robust.

The proposed validated method was successfully applied to determine ALF and DUTA in their combined tablet dosage form. The results obtained for ALF and DUTA were comparable with the corresponding labeled amounts (TABLE 4). The results of the assay indicate that proposed method is selective for the simultaneous estimation of ALF and DUTA without interferences from the excipients present in the tablets.

**TABLE 3 : Accuracy data of alfuzosin hydrochloride and dutasteride**

| Drug | Level | Amount Of Sample Taken (µg/ml) | Amount Of Standard Spiked (µg/ml) | Mean % Recovery ± SD[a], (n[c]=5) |
|---|---|---|---|---|
| ALF | I (50 %) | 20 | 10 | 99.40 ± 1.591 |
| | II (100 %) | 20 | 20 | 99.22 ± 1.193 |
| | III (150 %) | 20 | 30 | 98.15 ± 1.017 |
| DUTA | I (50 %) | 1 | 0.5 | 99.49 ± 0.668 |
| | II (100 %) | 1 | 1 | 100.67 ± 0.777 |
| | III (150 %) | 1 | 1.5 | 99.14 ± 0.861 |

**SD[a] = Standard deviation, n[b] = Number of replicate**

**TABLE 4 : Assay results of alfuzosin hydrochloride and dutasteride in tablets by RP-HPLC method**

| Formulation | Label claim (mg/tab) | | Amount found (mg/tab) | | % Assay ± SD[a] (n[b]=5) | |
|---|---|---|---|---|---|---|
| | ALF | DUTA | ALF | DUTA | ALF | DUTA |
| Tablet 1 | 20 | 1 | 20.034 | 0.999 | 100.17 ± 0.689 | 99.92 ± 1.083 |
| Tablet 2 | 20 | 1 | 20.148 | 1.017 | 100.74 ± 0.765 | 101.70 ± 0.986 |

**SD[a] = Standard deviation, n[b] = Number of replicate**

## CONCLUSION

A gradient RP-HPLC method has been developed and validated for simultaneous estimation of ALF and DUTA in tablets. The proposed RP-HPLC method is simple, precise, accurate, specific and sensitive and has ability to separate the drug from solvent and excipients usually found in the dosage form. The method is suitable for routine simultaneous analysis of ALF and DUTA in their combined dosage form and quality control of the raw material and formulations.

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