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Development and Validation of an RP-HPLC-UV Method for the Analysis of Drugs Used for Benign Prostatic Hyperplasia in Pharmaceutical Preparations

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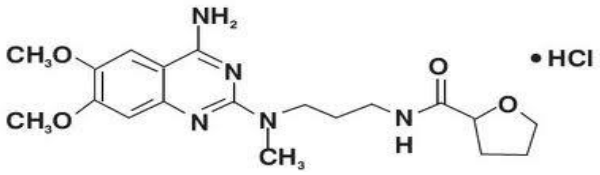
Abstract: The present work describes a simple, rapid, and reproducible reverse phase high performance liquid chromatography (RP-HPLC) with UV detection method for the simultaneous estimation of alfuzocin hydrochloride and finasteride in tablets which are used in benign prostatic hyperplasia therapy. The separation and quantification was achieved by Shim pack XR ODS (250 mm x 4.6 mm, 5 µm id) in isocratic mode, with mobile phase consisting of acetonitrile: water with 1 % Triethyl amine (TEA) (pH 7.0) (75:25 v/v) at a flow rate of 1 ml/min. Spectrophotometric detection was performed at a wavelength of 223 nm. The retention time of alfuzocin hydrochloride and finasteride was found to be 2.90 and 4.46 min, respectively. The developed method was validated according to ICH guidelines Q2 (R1). The method was validated for linearity, accuracy, and precision. Linearity for alfuzocin hydrochloride and finasteride were in the range 4-12 µg/ml for both drugs. The relative standard deviation values for intermediate precision studies were <1%. Statistical analysis of the data showed that the method was precise, accurate, reproducible and selective for the analysis of alfuzocin hydrochloride and finasteride. The method was successfully employed for the determination of alfuzocin hydrochloride and finasteride in commercially available tablet dosage form.

Keywords: *Alfuzocin hydrochloride, finasteride, RP-HPLC, validation, tablet*

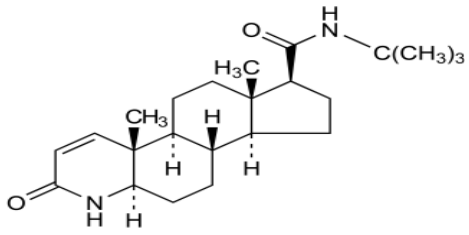
1 Introduction

Alfuzosin hydrochloride (ALFU) is chemically (R,S)-N-[3-[(4-amino-6,7-dimethoxy-2 quinazolinyl) methylamino] propyl] tetrahydro-2-furancarboxamide hydrochloride [Figure 1] (O'NEIL, M. J.2006). Alfuzosin hydrochloride (ALFU) is a selective alpha-1 adrenergic blocker commonly used the reduction of urinary obstruction and relief of associated manifestations in patient with symptomatic benign prostatic hyperplasia (BPH) (Harvey and Champe, 2009). It is official in United States Pharmacopoeia (USP) (UNITED STATES PHARMACOPOEIA, 2011) and British Pharmacopoeia (BP) (BRITISH PHARMACOPOEIA, 2010).

The pharmacopoeia describes potentiometry titration method for its estimation. Literature survey reveals UV spectrophotometric method (Kumar et al., 2008), RP-HPLC method (Kumar et al., 2010), HPLC & HPTLC (Patel and patel, 2009), stability indicating HPLC & HPTLC method (Salah et al., 2006), stability-indicating spectrophotometric and spectrofluorimetric methods (Fayed et al., 2007), colorimetric determination (Mohammed et al., 2011) for the estimation of alfuzosin hydrochloride in single and combination of other drugs.



(A)



(B)

Figure 1: Chemical structure of ALFU (A) and FINA (B)

Finasteride (FINA) is chemically N- (1,1-dimethylethyl) –

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3-oxo-4-aza-5-androst-1-ene- 17- carboxamide(O'NEIL, M. J.2006)[Figure 2]. Finasteride (FINA) is an specific inhibitor of steroid 5α-reductase; blocks conversion of testosterone by type 2 5α-reductase to 5α dihydrotestosterone (DHT). It is used for the treatment of the symptomatic benign prostatic hyperplasia and male pattern hair loss (androgenetic alopecia) in men (Bennett and Brown, 2008) It is official in Indian Pharmacopoeia (IP) , BP and USP. IP (Indian Pharmacopoeia, 2010), BP (British Pharmacopoeia, 2010) and USP (united state Pharmacopoeia, 2011), which describes liquid chromatography method for determination. Literature survey reveals UV spectrophotometric method (Thimmaraju et al., 2011), RP-HPLC (Basavaiah and Somashekar, 2007), RP-HPLC-PDA method (Sindhura et al., 2012), stability indicating LC method (Srinivas et a.l, 2011)for the estimation of finasteride in single and combination with other drugs.

Both the drugs in combination those are more effective than either single drug. This drug combination is mainly used for treatment of benign prostatic hyperplasia. This drug combination is not official in any pharmacopoeia, so no official method is available for the estimation of these two drugs in combined dosage forms. Literature survey of ALF and FINA revealed several methods for detecting these drugs individually but there is no method for their simultaneous estimation using RP-HPLC. These drugs were given as regimen for the treatment of benign prostatic hyperplasia. The developed method was validated as per ICH guidelines ([International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH, 1995) and its updated international convention. The linearity of response, precision, ruggedness and robustness of the described method has been checked.

2 Materials and Methods

2.1 Chemical and Reagents

ALFU and FINA Active Pharmaceutical Ingredient powder were kindly gifted by Sun pharmaceuticals Ltd, Vadodara, Gujarat, India. Acetonitrile, water, methanol (HPLC grade), TEA, Orthophosphoric acid were purchased from Aatur Instru-Chem., vadodara, Gujarat, India. All the other reagents used were of analytical grade.

2.2 HPLC instrumentations and conditions

The analysis was carried out on a HPLC system (Shimadzu-LC 20AD, japan) equipped with UV detector, pressure controlled by prominence pump and operated by LC SolutionSoftware. Shim pack XR C18 column (250 mm × 4.6 mm i.d., particle size 5 µm) was used for separation. Mobile phase used for separation was mixture containing acetonitrile: water (1% TEA pH 7with OPA) (75 : 25v/v). The flow rate was kept at 1.0 mL/min, column temperature was ambient (25°C), eluents were detected by UV detector at 223 nm, and the injection volume was 20 µL.

2.3 Preparation of Mobile Phase

Mobile phase was prepared by mixing 75 ml acetonitrile and 25 ml water containing 1% triethylamine adjusted pH 7.0 with ortho phosphoric acid. The mobile phase was sonicated for 10 min and then it was filtered through 0.45 µm membrane filter paper.

2.4 Preparation of Standard Stock Solution

A stock solution of ALFU and FINA (100 µg/ml) was prepared, by taking 10 mg of each drug, accurately weighed, in separate 100 ml volumetric flasks and dissolving in methanol and diluted to 100 ml with mobile phase up to the mark.

2.5 Preparation of sample solution

20 tablets were weighed, powdered & weight equivalent to 5 mg ALFU & 5 mg FINA was taken and transferred into a volumetric flask and made upto 50 ml with methanol, sonicated for 5 min, The solution was allowed to stand for 15 min with intermittent sonication to ensure complete solubility of the drug, take 2.0 ml solution and made upto 10 ml with mobile phase to get concentration 20 µg/ml within the linearity range of respective drugs and then filtered through 0.45 µ whatmann filter.

2.6 Method Validation

2.6.1 Linearity

The calibration curves were constructed by plotting peak areas Vs. concentration of ALFU and FINA, and the regression equations were calculated. The calibration curves were plotted over the concentration range 4-12 µg/ml for ALFU and FINA. Accurately measured standard working solutions of ALFU and FINA (0.4, 0.6, 0.8, 1.0 and 1.2 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µl) of each solution were injected under the operating chromatographic conditions.

2.7 Precision

2.7.1 Repeatability

The precision of the instrument was checked by repeated injecting (n=6) standard solutions of ALFU and FINA (8 µg/ml for ALFU and FINA) under same conditions and measurement of peak area, retention time and tailing factor on the same day and calculate SD and %RSD.

2.7.2 Intermediate precision

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of ALFU (6, 8, and 10 µg/ml) and FINA (6, 8, and 10 µg/ml). The result was reported in terms of relative standard deviation (%RSD).

2.8 Accuracy (recovery study)

Accuracy was determined by calculating recovery of ALFU and FINA by the spiking method. From working sample solution of test, 0.6 ml of solution were taken and increasing aliquots of combined working standard solution (1.6, 2.0 and 2.4 ml from 100 µg/ml of ALFU and 1.6, 2.0, 2.4 ml from 100 µg/ml of FINA) were added and peak area was measured.

2.9 Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Commonly used formulation excipients were spiked into a pre-weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

2.10 Robustness

The robustness is the ability of method to remain unaffected by small changes in parameters. The robustness of the method was determined by purposely altering experimental conditions and peak area, peak tailing, theoretical plates and resolution were calculated. To study the effect of flow rate, it was changed to 0.2 units from 1.0 ml/min to 0.8 ml/min and 1.2 ml/min. The effect of ratio of mobile phase was changed to 2 units from 75:25 to 77:23 and 73:27.

2.11 Limit of detection and limit of quantitation

LOD and LOQ of ALFU and FINA were determined by calibration curve method. Solutions of ALFU and FINA were prepared in the range of 4-12 µg/mL and injected in triplicate.

2.12 System suitability parameters

System suitability parameters were checked for proposed RP-HPLC method. 10 µg/ml of ALFU and 10 µg/ml of FINA were as prepared in mobile phase [acetonitrile: water + 1% triethylamine pH 7.0 adjusted with OPA (75:25)] was prepared as per dosage form ratio. Then chromatogram was recorded under finally optimized chromatographic conditions, and following parameters were checked for system suitability of proposed method likr Retention times (Rt) , theoretical plates (N) , resolution (RS), tailing factor (AS), capacity factor (k').

3 Results

3.1 Optimization of Chromatographic Conditions

To develop suitable RP-HPLC method for simultaneous estimation of ALFU and FINA, different chromatographic conditions were applied and optimized chromatographic conditions were developed [Figure 2].

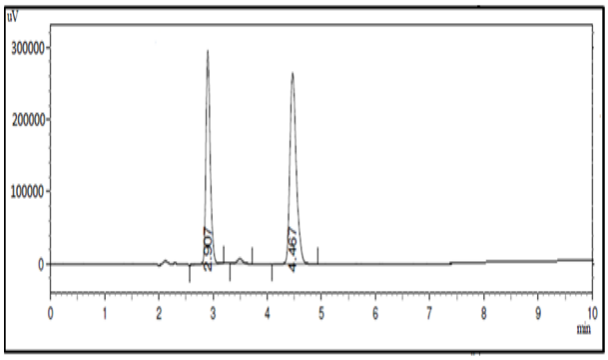

Figure 2. Optimized chromatogram of ALFU and FINA

Optimized chromatographic conditions are as follows:

Instrument: HPLC Shimadzu separation module LC-20AD Prominence liquid chromatograph,

Mobile phase: acetonitrile: water (1% TEA pH 7with OPA) (75 : 25v/v).

Column: Shim pack XR C18 (250 mm × 4.6 mm i.d., particle size 5 µm),

Injection volume: 20 µL,

Flow rate: 1.0 mL/min,

Detection wavelength: 223 nm,

Run time: 10 min,

Temperature: Ambient (25°C).

3.2 Validation

3.2.1 Linearity

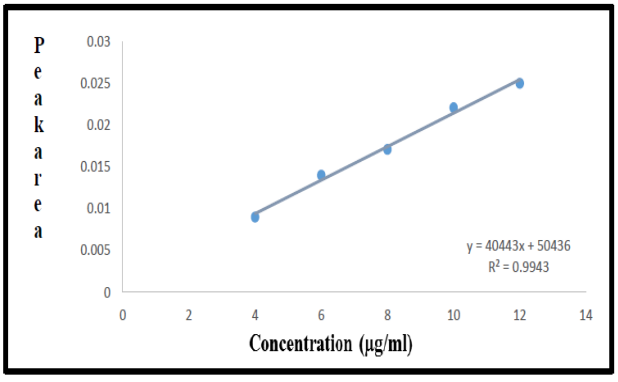

Figure 3. Calibration curve of ALFU

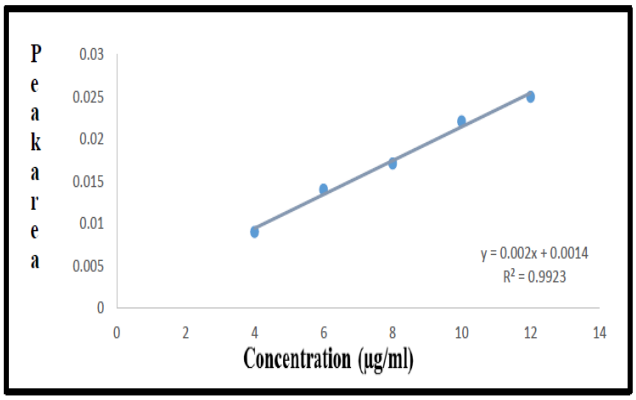

Figure 4. Calibration curve of FINA

The chromatographic method showed good linearity for ALFU and FINA in the range of 10-60 µg/ml for both which as shown in [Figure 3 and 4]. Correlation of coefficient value was found to be 0.9943 and 0.9934 for ALFU and FINA respectively.

3.3 Precision

3.3.1 Repeatability

The RSD values for ALFU and FINA were found to be 0.04% and 0.99 % respectively (Table 1). Relative standard deviation was less than 2%, which indicates that the proposed method is repeatable.

3.3.2 Intermediate precision

The low RSD values of intraday (0.01-0.22 % and 0.30-0.39 %) and interday (0.67-0.79 % and 0.07-1.05 %) for ALFU and FINA respectively, reveals that the proposed method is precise (Table 1).

Table 1: Repeatability and intermediate precision data of ALFU and FINA (n=6)

Parameter	ALFU	FINA
Repeatability	0.0424	0.9985
Interday	0.67- 0.79 %	0.07-1.05 %
Intraday	0.01-0.22 %	0.30-0.39

3.4 Accuracy

Table 2: Result of recovery study (n=6)

Drugs	Level	Amount present (µg/ml)	Amount added (µg/ml)	Total amount of drug (µg/ml)	% Recovery (n=3)	% RSD
ALFU	80%	6	4.8	10.8	99.96	0.07
	100%		6.0	12.0	99.78	0.37
	120%		7.2	13.2	100.38	0.33
FINA	80%	6	4.8	10.8	99.87	0.55
	100%		6.0	12.0	100.14	0.12
	120%		7.2	13.2	100.17	0.08

Accuracy was performed at 80%, 100% and 120% levels by spiking method. Each concentration was analyzed three times and average recoveries were measured (Table 2). Result obtained reveals that % recovery of ALFU and FINA was within acceptance criteria given in ICH i.e. 98-102%.

3.5 Specificity

Specificity data shown in (Table 3).

Table 3: Specificity Data

Drugs	Average standard area	Average sample area	%RSD
ALFU	412480	412489	0.46
FINA	128292	128269	0.87

3.6 Robustness

The robustness of the method was determined by making slight changes in the chromatographic conditions. The robustness of the proposed HPLC method was assessed for peak resolution and peak resolution and symmetric factor. The parameters investigated are: (1) Flow rate (±0.2 ml/min) (2) Mobile phase composition (±2). The robustness of the method shows that there were no marked changes in the chromatographic parameters, which demonstrates that the method developed is robust (Table 4).

Table 4: Robustness study of ALFU and FINA

Sr. No	Parameters	Variations	Assay ± SD		Assay ± SD	
			ALFU	FINA	ALFU	FINA
1	Flow rate (1± 0.2 ml/min)	0.8 ml/min	99.26±0.02	99.15±0.05	0.03	0.05
		1.0 ml/min	99.16±0.31	100.05 ±0.21	0.32	0.21
		1.2 ml/min	99.53±0.02	99.71±0.05	0.02	0.06
2	Mobile Phase (75:25 ± 2 v/v)	73:27(v/v)	99.65±0.05	99.71±0.05	0.05	0.06
		75:25(v/v)	99.16 ±0.31	100.05±0.21	0.32	0.21
		77:23(v/v)	99.50±0.08	99.63±0.07	0.08	0.07

3.7 Limit of detection and limit of quantitation

Table 5: LOD and LOQ data of ALFU and FINA

Drug	Range(µg/ml)	Linear regression equation	R^2	LOD(µg/ml)	LOQ(µg/ml)
ALFU	4-12	y = 40443x + 50436	0.9943	0.46	1.40
FINA	4-12	y = 0.002x + 0.0014	0.9923	0.86	1.61

LOD and LOQ of ALFU and FINA were determined by calibration curve method. Solutions of ALFU and FINA were prepared in the range of 4-12 µg/mL and injected in triplicate (Table 5).

3.8 System suitability testing

The resolution, number of theoretical plates, and peak asymmetry were calculated for the standard solutions. The stock solution containing 10 µg/mL was injected and repeated five times and the chromatograms were recorded. The resolution, number of theoretical plates, and peak asymmetry were calculated to determine whether the result complies with the recommended limit.(Table 6)

Table 6: System suitability parameters

Parameters	Data obtained	
	ALFU	FINA
Retention time (min)	2.90	4.46
Theoretical plates per column	7036	7360
Symmetry factor/ tailing factor	1.23	1.35
Capacity factor	4.06	1.1
Resolution	5.09	

3.9 Analysis of ALFU and FINA in marketed formulation (tablet)

Commercial pharmaceutical preparation of tablet, which were claimed to contain 5 mg Alfuzosin hydrochloride and 5 mg finasteride**.** The percentage recovery of ALFU and FINA in tablet was found to be in Table 7. % Assay of alfuzosin hydrochloride and finasteride was found in an acceptance limit so this method could be used for analysis of this combination.

Table 7: Assay of combined dosage form

	Amount taken (µg/ml)		Amount found (µg/ml)		% Label claim (Assay ± SD) (n=3)	
	ALFU	FINA	ALFU	FINA	ALFU	FINA
Formulation (Tablet)	20	20	19.83	20.11	99.16± 0.31	100.05 ± 0.21

4 Discussion

ALFU and FINA were given linear response from 4-12 µg/ml in RP-HPLC for both. Correlation of coefficient value was found to be 0.9943 for ALFU and 0.9934 for FINA. So the range of ALFU and FINA was found to be 10-60 µg/ml for both respectively. %RSD was less than 2, which indicates that the proposed method is repeatable and precise. Result obtained from accuracy study reveals that % recovery of ALFU and FINA was within acceptance criteria given in ICH i.e. 98-102%. The robustness study suggested that all the parameters have no significant influence on the determination. Results indicate that the selected factors remained unaffected by small variation of these parameters and %RSD was less than 2, which demonstrates that the proposed method was robust.

5 Conclusion

The proposed RP-HPLC method with UV detection was used for the simultaneous estimation of ALFU and FINA was found to be sensitive, accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials, in vitro dissolution study of combinational dosage formulations containing ALFU and FINA.

Conflict of Interests

The authors do not have a direct financial relation with the commercial identity mentioned in paper that might lead to a conflict of interests for any of the authors.

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