IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2015 Vol.:2, Issue:4

Development and Validation of First Order Derivative Spectrophotometric Method for Estimation of Alfuzosin Hydrochloride and Finasteride in Combined Dosage Form

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ISSN 2349-7203
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Submission: 11 March 2015
Accepted: 21 March 2015
Published: 25 March 2015

www.ijppr.humanjournals.com

Keywords: Spectrophotometric method, First derivative, alfuzosin hydrochloride, finasteride

ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric method for the simultaneous determination of alfuzosin hydrochloride and finasteride in combined tablet dosage form. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra were obtained in methanol and the determinations were made at 258 nm for alfuzosin hydrochloride and 223 nm for finasteride. The linearity was obtained in the concentration range of 2-12 µg/ml for both alfuzosin hydrochloride and finasteride. The mean % recovery was 100.79 and 99.89 % for alfuzosin hydrochloride and finasteride respectively. The method was found to be simple, sensitive, accurate, precise and was applicable for the simultaneous determination of alfuzosin hydrochloride and finasteride in tablet dosage form.

1. INTRODUCTION

Alfuzosin hydrochloride (ALFU) is chemically (R,S)-N-[3- [(4-amino-6,7-dimethoxy-2 quinazolinyl) methylamino] propyl] tetrahydro-2-furancarboxamide hydrochloride, its molecular weight is 425.91g/mol with an empirical formula $C_{19}H_{27}N_5O_4.HCl$[1]. 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) [2]. It is official in USP and BP. USP [3] and BP [4] describes Potentiometry titration method for its estimation. Literature survey reveals UV spectrophotometric method [5], RP-HPLC method [6], HPLC & HPTLC [7], stability indicating HPLC & HPTLC method [8], stability-indicating spectrophotometric and spectrofluorimetric methods[9], colorimetric determination[10] for the estimation of alfuzosin hydrochloride in single and combination of other drugs.

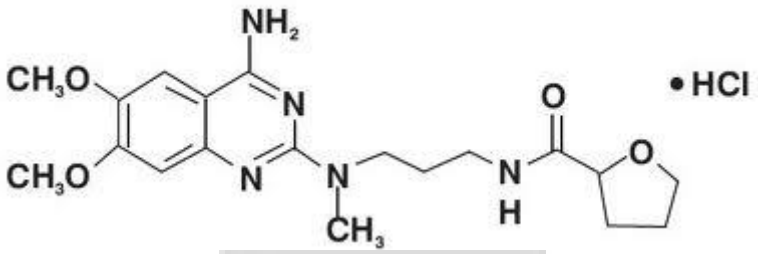

Figure 1: Chemical Structure of ALFU

Finasteride (FINA) is chemically N- (1,1-dimethylethyl) – 3-oxo-4-aza-5-androst-1-ene- 17-carboxamide, its molecular weight is 372.6 g/mol with empirical formula $C_{23}H_{36}N_2O_2$[11]. 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[12]. It is official in IP, BP and USP. IP [13], BP [14] and USP [15], which describes liquid chromatography method for estimation. Literature survey reveals UV spectrophotometric method [16], RP-HPLC [17], RP-HPLC-PDA method [18], stability indicating LC method [19] for the estimation of finasteride in single and combination with other drugs.

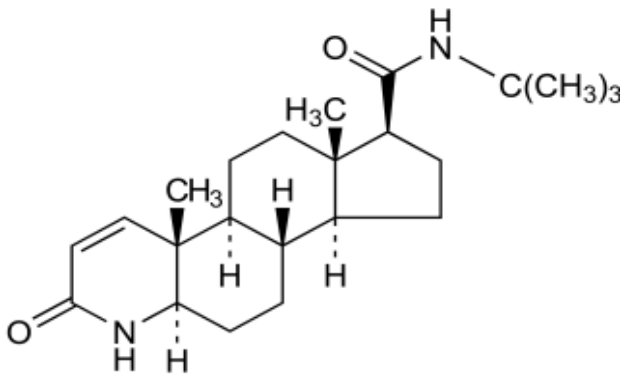

Figure 2: Chemical structure of FINA

Alfuzosin hydrochloride (ALFU) is an alpha adrenergic blocker. It works by relaxing the muscle in the prostate and bladder neck, making it easier to urinate. Finasteride (FINA) is class of drug that an 5-alpha reductase inhibitors, which block the action of the 5-alpha reductase enzyme that converts testosterone to 5- α dihydrotestosterone (DHT). 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. The present manuscript describes simple, sensitive, accurate, precise, rapid and economic first order derivative spectrophotometric method for simultaneous determination of alfuzosin hydrochloride and finasteride in pharmaceutical tablet dosage form.

2. MATERIALS AND METHODS

2.1 Apparatus: UV-Visible Double Beam Spectrophotometer, (Shimadzu-1800, Japan) with computer software UV Probe 2.33, with spectral slit width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched cells used to measure absorbance of all the solutions. Digital Balance Shimadzu Model ATX 224, Japan, Bath sonicator, Toshcol Instruments was used in the study.

Reagents and Materials: ALFU and FINA API powder was kindly gifted by Sun pharmaceuticals Ltd, Vadodara, Gujarat, India. Methanol (AR grade) was procured from Aatur Instru-Chem.

2.2 Preparation of standard stock solutions

Accurately weighed portion of ALFU & FINA 10 mg were transferred to a separate 100 ml volumetric flask and dissolved and diluted to mark with methanol to obtain standard solution having concentration of ALFU (100 µg/ml) and FINA (100 µg/ml).

2.3 Selection of scanning range and sampling wavelength

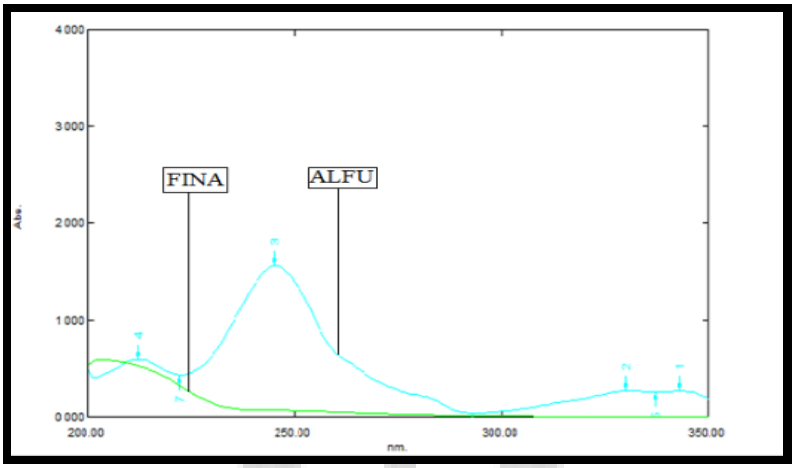

Figure 3: Overlain zero order derivative spectra of ALFU and FINA

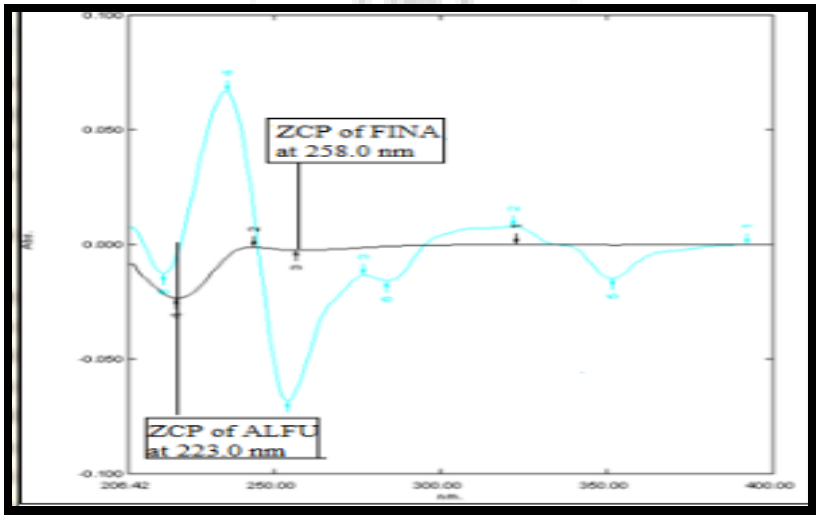

Figure 4: Overlain first order derivative spectra of ALFU & FINA

The standard solutions of ALFU (10 µg/ml) and FINA (10 µg/ml) were scanned separately in the UV range of 200-400 nm. The zero-order spectra thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 2 nm. The two spectra were overlain and it appeared that ALFU showed zero crossing at 223 nm, while FINA showed zero crossing at

258 nm. At the zero crossing point (ZCP) of FINA (258 nm) ALFU showed a first-derivative absorbance, whereas at the ZCP of ALFU (223 nm), FINA showed a first-derivative absorbance. Hence 258 and 223 nm was selected as analytical wavelengths for determination of ALFU and FINA respectively. These two wavelengths can be employed for the determination of ALFU and FINA without any interference from the other excipient in their combined dosage formulations.

2.4 Analysis of ALFU and FINA in combined tablet dosage form

Twenty Tablets were weighed and powdered. The powder equivalent to 5 mg of ALFU and 5 mg of FINA was transferred to a 50 ml volumetric flask. Methanol (50 ml) was added to it and sonicated for 10 min. The solution was filtered through Whatman filter paper No. 45 and the volume was adjusted up to the mark with methanol. This solution is expected to contain 100 µg/ml of ALFU and 100 µg/ml of FINA. This solution (0.4 ml) was taken into a 10 ml volumetric flask and the volume was adjusted up to mark with methanol to get a final concentration of ALFU (4 µg/ml) and FINA (4 µg/ml). The responses of the sample solution were measured at 258 nm and 223 nm for quantitation of ALFU and FINA respectively. The amounts of the ALFU and FINA present in the sample solution were calculated by fitting the responses into the regression equation for ALFU and FINA in the proposed method.

Table 1: Assay results for the combined dosage form

Formulation (Tablet)	Label claim (mg/tablet)		Amount found (mg/tablet)		% label claim ± S.D (n=3)	
	ALFU	FINA	ALFU	FINA	ALFU	FINA
	5	5	4.98	4.90	99.60 ± 0.005774	98.00 ± 0.005774

S. D. is Standard deviation and n is number of determinations

3. Validation of proposed method

3.1 Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-12 µg/ml for each ALFU and FINA. Accurately measured standard solutions of ALFU and FINA (0.2, 0.4, 0.6, 0.8, 1.0 and

1.2 ml) were transferred to a series of 10 ml of volumetric flasks, separately from 100 µg/ml and diluted to the mark with methanol, and first-derivative absorbances (D1) were measured at 258 nm for ALFU and 223 nm for FINA. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

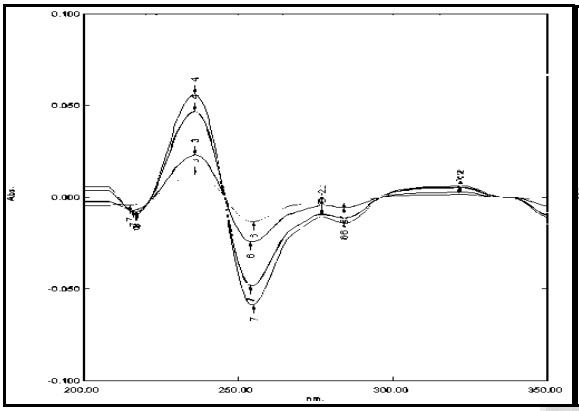

Figure 5: Overlain spectra of ALFU

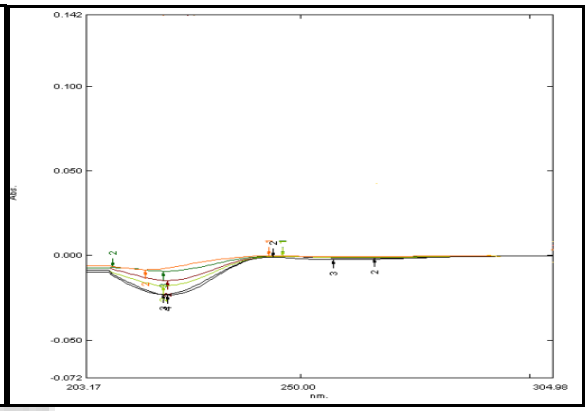

Figure 6: Overlain spectra of FINA

3.2 Precision

3.2.1 Repeatability

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solution (*n*= 6) for ALFU and FINA (6 µg/ml) without changing the parameter of the first-derivative spectrophotometry method.

3.2.2 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 and FINA (6, 8 and 10 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

3.3 Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of ALFU and FINA by the spiking method. Known amounts of standard solutions of ALFU and FINA were added at 80, 100 and 120 % level to prequantified sample solutions of ALFU and FINA (4:4 µg/ml). The

amounts of ALFU and FINA were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for six times.

Table 2: Accuracy of proposed method (n=3)

Drug	Level	Amount taken (µg/ml)	Amount added (µg/ml)	Total amount of drug (µg/ml)	% recovery (n = 3)	% RSD
Alfuzosin hydrochloride	80%	4	3.8	7.8	100.83	1.43
	100%	4	4.0	8.0	100.79	1.36
	120%	4	4.2	8.2	100.75	1.30
Finasteride	80%	4	3.8	7.8	99.79	0.36
	100%	4	4.0	8.0	100.78	1.87
	120%	4	4.2	8.2	99.12	1.53

3.4 Specificity

The specificity of an analytical method is ability to measure accurately an analyte in presence of interferences like synthetic precursor, excipients, degradants, or matrix component. Comparison of UV spectrum of standard mixture and formulation shows specificity of method. The derivative spectrophotometric method is able to access the analyte in presence of excipients, and, hence, it can be considered specific.

3.5 Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived from the calibration curves by using the following equations as per International Conference on Harmonization (ICH) guidelines.

Limit of Detection and Limit of Quantitation were calculated using following formula

$$LOD = 3.3(SD) / S$$

$$LOQ = 10 (SD) / S,$$

Where SD=standard deviation of response (absorbance) and S= slope of the calibration.

4. RESULTS AND DISCUSSION

The standard solutions of ALFU and FINA were scanned separately in the UV range, and zero-order spectra (Figure 3) thus obtained was then processed to obtain first-derivative spectra. Data were recorded at an interval of 2 nm. The two derivative spectra showed maximum absorbance at 258 nm (ZCP of FINA) for ALFU and 223 nm (ZCP of ALFU) for FINA. First-derivative absorbances (D1) were recorded 258 nm for ALFU and 223 nm for FINA (Figure 4). First derivative spectra give good quantitative determination of both the drugs at their respective without any interference from the other excipient in their combined dosage formulations. Second and third-ordered derivative spectra of the drugs were not tested because the first-order spectra give satisfactory ZCPs and good quantitative determination of both the drugs without any interference.

Table 3: Optical and Regression Analysis Data and Validation Parameter of first derivative results of ALFU and FINA

PARAMETERS		First derivative UV spectrophotometry	
		ALFU at 258 nm	FINA at 223 nm
Concentration range (µg/ml)		2-12 µg/ml	2-12 µg/ml
Molar absorptivity (L mol-1cm-1)		0.8764×10^{6}	0.4218×10^{6}
Sandell's Sensitivity (g/cm2/0.001 absorbance unit)		0.4583	0.4736
Slope		0.0048	0.0019
Intercept		0.0032	0.0019
Correlation coefficient		0.9964	0.9968
Accuracy (recovery, n = 3) ± S.D	80%	100.83 ± 0.000577	99.79 ± 0.000173
	100%	100.79 ± 0.000577	100.78 ± 0.000321
	120%	100.75 ± 0.000577	99.12 ± 0.000289
LOD (µg/ml)		0.39	0.19
LOQ (µg/ml)		1.2	0.60
Precision			
Repeatability (% RSD, n=6)		1.50	1.09
Intraday (n = 3)		0.97-1.48 %	0.47-1.94 %
Interday (n = 3)		1.08-1.20	0.28-1.15%

The % assay ± S.D. were found to be for ALFU 99.60 ± 0.005774 & for FINA 98.00 ± 0.005774, respectively (Table 1). No interference was observed from the pharmaceutical excipients. The method was successfully applied to pharmaceutical formulation, with no interference from excipients as indicated by the results of recovery study (Table 2). The repeatability, intraday precision and interday precision were expressed in terms of relative standard deviation (RSD). For intraday and interday precision % RSD for ALFU and FINA was found to be satisfactory (Table 3). Results of all validation parameters are shown in (Table 3). Hence, the proposed method was evaluated statistically and was validated in terms of linearity, accuracy and precision. The present work provides an accurate and sensitive method for the analysis of ALFU and FINA in tablet formulation.

5. CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 2-12 µg/ml for both ALFU and FINA with co-efficient of correlation, (r2) = 0.9964 and (r2) = 0.9968 for ALFU and FINA respectively. The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The additives usually present in the pharmaceutical formulation of the assayed sample did not interfere with determination of ALFU and FINA. The method can be used for the routine analysis of the ALFU and FINA in combined dosage form without any interference of excipients.

6. ACKNOWLEDGMENT

The authors are thankful to Sun Pharmaceutical Ltd, Vadodara, Gujarat, India for providing gift sample of alfuzosin hydrochloride and finasteride for research. The authors are highly thankful to Pioneer Pharmacy Degree College, Vadodara, Gujarat, India for providing all the facilities to carry out the work.

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