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VALIDATED UV SPECTROPHOTOMETRIC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF ALFUZOSIN HYDROCHLORIDE AND DUTASTERIDE IN BULK AND TABLET DOSAGE FORM

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Abstract

Analytical method development being a vital part of preformulation-formulation research and development obviates the need to develop reliable, effective, ecofriendly and cost effective methodologies for routine analysis of active pharmaceutical ingredients. UV spectroscopy is one of the earliest, yet of wide applications in drug analysis in different stages of formulations and quality control; despite the availabilities of sophisticated chromatographic and other hyphenated techniques. Current research attempts to develop simple, sensitive, accurate, precise and economical UV spectrophotometric methods for the simultaneous estimation of alfuzosin (AFZ) and dutasteride (DUT) in bulk and tablet formulations by simultaneous equation method. From the overlain spectra, 247.5 nm and 241 nm were selected as sampling wavelengths for AFZ and DUT respectively. Assay results showed 10.163 mg of AFZ and 0.504 mg of DUT were found in the tablet dosage form. The method was validated as per ICH guidelines. Linearity was obtained in the concentration range of 0.5−5 µg/mL for AFZ and 5−50 µg/mL for DUT. The %RSD for intraday and interday variations of AFZ was found to be 0.422±0.001 and 0.319±0.002 respectively. An intraday and interday variation of DUT was found to be 0.517±0.001 and 0.563±0.001 respectively. In both cases values were within the acceptance limit of < 2%. The mean percentage recovery values for AFZ and DUT were 99.79 % and 100.02% respectively, within the acceptance limit of 98% to 102%. From the high recovery values (> 98 %) it can be inferred that the method is free from the interference of excipients used in the formulation. Based on the results obtained the proposed method can be regarded as simple, accurate, precise, and reliable which can be employed for routine quality control of AFZ and DUT in bulk and combined tablet dosage forms.

Keywords: Analytical method development, Alfuzosin, Dutasteride, Validated, UV spectrophotometric.

Introduction

Analytical method development is an integral part of preformulation and formulation development research. With the development of both small and large scale pharmaceutical industries worldwide,

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there arises the urgent need to develop a properly validated, stability indicating, specific analytical method for the routine analysis of drugs. Rapid, simple, sensitive, cost effective analytical method development is of imperative necessity since the design of the drug delivery system is related to it. Moreover drug analysis is also necessary in various steps of formulation design and dissolution studies [1-5]. Sophisticated chromatographic methods with HPLC, HPTLC which are being employed for analysis are relatively expensive; many methods necessitate analyte extraction from respective sample matrices thus necessitating complicated sample preparation steps, use of internal standards for analysis increases the time required and error in recovery [6-23]. UV-vis spectrophotometric method is one of the earliest, yet easy, sensitive, relatively cost effective method applied for drug estimations in both small and large scale pharmaceutical R&Ds [1-5,24,25]. Multi-component formulations have gained a lot of importance now a days due to the greater patient compliance and acceptability, increased potency, multiple action, fewer side effects and faster relief. However analytical complexities of these multi-drug component dosage forms put forward considerable challenges to the analytical chemist during the analytical procedure [12,15,17]. The prime requisite is to develop new methods to analyze the drugs simultaneously and without interference. The UV methodology becomes much more beneficial and acceptable if it can simultaneously estimate more than one drug at a time.

Alfuzosin(AFZ), chemically known as N-{3-[(4-Amino-6,7-dimethoxyquinazolin-2-yl (methyl) amino] propyl} tetrahydro-2- furamide hydrochloride is a non-subtype specific alpha(1)-adrenergic blocking agent that exhibits selectivity for alpha(1)-adrenergic receptors in the lower urinary tract (**Fig.1**). Inhibition of these adrenoreceptors leads to the relaxation of smooth muscle in the bladder neck and prostate, resulting in the improvement in urine flow and a reduction in symptoms in benign prostate hyperplasia. Alfuzosin also inhibits the vasoconstrictor effect of circulating and locally released catecholamines (epinephrine and norepinephrine), resulting in peripheral vasodilation [24-26]. Dutasteride (DUT), chemically known as 17β-N-{2,5-bis (trifuoromethyl) -phenyl- carbamoyl} -4-aza-5-androst-1-en-3-one, is a dual 5-alpha-reductase inhibitor, blocking both type I and type II 5-alpha-reductase isoenzymes (**Fig.2**). DUT inhibits the conversion of testosterone to 5α-dihydro - testosterone (DHT). DHT is the androgen, which is primarily responsible for the initial development and subsequent enlargement of the prostate gland [27,28]. Both AFZ and DUT are used in the treatment of Benign Prostatic Hyperplasia (BPH) and lower urinary tract symptoms (LUTS) and the combined formulation is a good treatment option for BPH [29,30]. Literature reports some of the UV spectrophotometric, HPLC and HPTLC methods for the determinations of AFZ or DUT from dosage formulations or from biological matrices [6,8,10,12,13,19]. But the current research aims to develop and validate a UV-spectrophotometric method for the simultaneous estimation of AFZ and DUT in a combined dosage form by simultaneous equation method. There are no such parallel reportings of any UV method for the simultaneous estimation of this drug combination to the best of our knowledge.

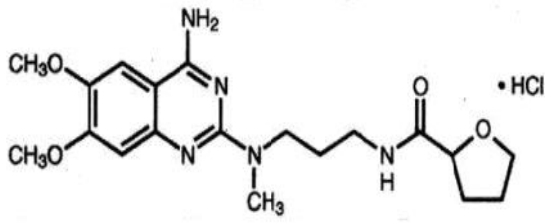

Fig. No. 01: Structure of Alfuzosin hydrochloride

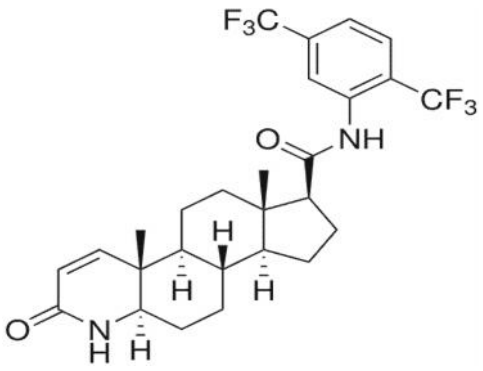

Fig. No. 02: Structure of Dutasteride

Materials and Methods

Instruments used

Electronic analytical balance (Shimadzu, Japan); Single beam UV-Vis spectrophotometer (Thermo Scientific Aquamate plus, India); Ultrasonic bath sonicator (Cyber labs, India) were used in the study.

Reagents and chemicals

Analytical pure drugs of AFZ and DUT were obtained as kind gift samples from Hetero Drugs, Hyderabad, India. The combined tablet formulation (**Alfusin-D**) with a labelled claim of AFZ 10 mg and DUT 0.5 mg respectively, were obtained from local drug store. Methanol of analytical grade was purchased from Merck, Mumbai.

Preparation of standard stock solution

Accurately weighed 10 mg of AFZ and 0.5 mg of DUT were transferred to a 10 mL volumetric flask separately, dissolved in methanol and finally made up to the volume to get the concentration of 1000 µg/mL of AFZ and 500µg/mL of DUT.

Selection of wavelength

The standard stock solutions of AFZ and DUT were further diluted with methanol to get the concentration of 10 µg/mL of each and the solutions were scanned between the UV range of 200–400 nm against methanol as blank. λ_{max} of AFZ was recorded to be at 247.5 nm while DUT showed λ_{max} of 241 nm.

Calibration curve

A series of dilutions were prepared from the standard stock solutions of AFZ and DUT to obtain the concentration of 0.5-5 µg/mL of AFZ and 5-50 µg/mL of DUT. Absorbances of the above solutions were measured at 247.5 nm and 241 nm and a calibration curve of absorbance against concentration was plotted and the regression coefficient (R^2) was also determined.

Determination of absorptivity coefficients

The absorptivity coefficients of both drugs (AFZ and DUT) were determined at selected wavelengths by using the formula: **$A=A\ (^{1\%}_{1cm})/b\ c$**.

The absorptivity values are then substituted in the following equations (1) and (2):

$A1 = ax1Cx + ay1Cy$…….(1)

$A2 = ax2Cx + ay2Cy$……..(2)

Where,

A1 and A2 are absorbances of sample at 247.5 nm and 241 nm respectively.

a_{X1} and a_{X2} are absorptivities of AFZ at 247.5 nm and 241 nm respectively.

a_{Y1} and a_{Y2} are absorptivities of DUT at 247.5 nm and 241 nm respectively.

Cx and Cy are concentrations of AFZ and DUT respectively.

Preparation of sample solution

Average weight of twenty tablets containing 10 mg of AFZ and 0.5 mg of DUT (labeled claim) was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, a standard addition method was used. An accurately weighed 9.5 mg of pure DUT was added to finely powdered sample to bring the concentration of DUT in linearity range. With this addition, the ratio of AFZ to DUT was brought to 1:1. Quantity of powder equivalent to 10 mg of AFZ and 10 mg of DUT powdered sample was transferred to a 50 mL volumetric flask, dissolved in sufficient quantity of methanol, sonicated and volume was adjusted up to mark with the same to obtain a stock solution of 1000 µg/mL of AFZ and DUT. This solution was then filtered through Whatmann filter paper.

Analysis of tablet dosage form

Aliquot portion of the above sample stock solution was diluted with methanol and the absorbance was measured at appropriate wavelengths and the concentrations of the two drugs were determined using equations (3) and (4). Analysis was done in triplicate.

$$Cx = \frac{A2ay1 - A1ay2}{Ax2\ ay1 - ax1\ ay2} \ldots\ldots (3)$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2\ ay1 - ax1\ ay2} \ldots\ldots (4)$$

Method validation

The proposed method was validated as per ICH guidelines in terms of linearity, precision, accuracy [31].

Linearity

A Series of solutions were prepared using AFZ and DUT standard stock solution at concentration levels from 0.5-5 µg/mL and 5-50 µg/mL respectively. The absorbances of the solutions were measured at 247.5 and 241 nm against methanol as blank. The calibration curves were constructed by plotting concentrations on x-axis and absorbance on y-axis. R^2 value not less than 0.99 was regarded as acceptance criterion.

Accuracy

The accuracy of the developed method was determined by recovery studies. Recovery studies were carried out at three different levels. The preanalyzed samples were spiked with 50, 100 and 150% of mixed standard solution. The mixtures were analyzed and the recoveries were determined. The study was carried out in triplicate. The mean % recovery of the AFZ and DUT at each level should be not less than 98.0% and not more than 102.0% was considered as the acceptance criterion.

Precision

Precision was studied to find out intra and inter-day variations in the test method of AFZ and DUT. Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using at three different days, and percentage relative standard deviation (%RSD) was calculated. The %RSD should not be more than 2.0%.

Table No. 01: Table for calibration curve of AFZ and DUT

Conc. of AFZ (µg/mL)	Absorbance (nm)	Conc. of DUT(µg/mL)	Absorbance (nm)
0.5	0.228	5	0.248
1	0.302	10	0.312
2	0.423	20	0.439
3	0.567	30	0.569
4	0.698	40	0.712
5	0.824	50	0.852
$R^2 = 0.99$		$R^2 = 0.99$	

Table No. 02: Linearity of Alfuzosin hydrochloride and Dutasteride

Parameters	AFZ	DUT
95% confidence intervals		
Slope	0.1276-0.1400	0.0125–0.0137
Y-intercept	-0.1473–0.1779	0.1645–0.1938
X-intercept	-1.388 to -1.057	-13.59
Goodness of Fit		
R^2	0.999	0.999
P value	< 0.0001	< 0.0001
Equation	Y = 0.1338X + 0.1626	Y=0.01318X+0.1792
Best fit values		
Slope	0.1338 ± 0.001960	0.0131±0.00018
Y-intercept	0.1626 ± 0.004821	0.1792±0.0046
X-intercept	-1.215	-13.59

Table No. 03: Absorptivity values of Alfuzosin HCl and Dutasteride

Absorptivity values of Afluzocin HCL		Absorptivity values of Dutasteride	
247.5(nm) –ax_1	8240	247.5(nm) –ay_1	4770
241(nm)- ax_2	7420	241(nm)- ay_2	5690

Table No. 04: Assay results of Alfuzosin HCl and Dutasteride

Drug	Labeled amount(mg)	Amount present (mg)	% Assay
AFZ	10	10.163	101.63
DUT	0.5	0.504	100.87

Table No. 05: **Accuracy studies of Alfuzosin HCl**

Sample No.	Spike Level (%)	Amount (µg / mL) added	Amount (µg / mL) Found	% Recovery	Statistical analysis	
	50	0.5	0.496	99.33	Mean	99.55
1	50	0.5	0.493	98.67	SD	1.011
	50	0.5	0.503	100.66	%RSD	1.016
	100	1.0	1.013	101.32	Mean	100.33
2	100	1.0	1.003	100.33	SD	0.993
	100	1.0	0.993	99.33	%RSD	0.990
	150	1.5	1.486	99.11	Mean	99.48
3	150	1.5	1.493	99.55	SD	0.337
	150	1.5	1.496	99.77	%RSD	0.338

Table No. 06: Accuracy studies of Dutasteride

Sample No.	Spike Level (%)	Amount (µg / mL) added	Amount (µg / mL) Found	% Recovery	Statistical analysis	
	50	5	4.96	99.35	Mean	99.57
1	50	5	4.93	98.71	SD	0.979
	50	5	5.03	100.64	%RSD	0.983
	100	10	9.96	99.67	Mean	99.78
2	100	10	9.93	99.35	SD	0.489
	100	10	10.03	100.32	%RSD	0.490
	150	15	15.16	101.06	Mean	100.71
3	150	15	15.19	101.28	SD	0.808
	150	15	14.96	99.78	%RSD	0.803

Table No. 07: Results of precision studies of two drugs

S.No.	Alfuzosin HCl			Dutasteride		
	Concentration	Intra day (%RSD)	Inter day (%RSD)	Concentration	Intra day (%RSD)	Inter day (%RSD)
1	2	0.591	0.852	20	0.475	0.574
2	3	0.455	0.269	30	0.269	0.835
3	4	0.219	0.431	40	0.214	0.280

Table No. 08: Summary of the validation parameters of the proposed method

S.No.	Parameters	Alfuzosin HCl	Dutasteride
1	Linearity (µg/mL)	0.5-5	5-50
2	Correlation Coefficient	0.9995	0.9994
3	Precision(%RSD) (i)IntradayPrecision	0.422	0.319
	(ii)Interday Precision	0.517	0.563
4	Accuracy (%recovery)	99.79	100.02
5	Tablet Assay (%)	101.63	100.87

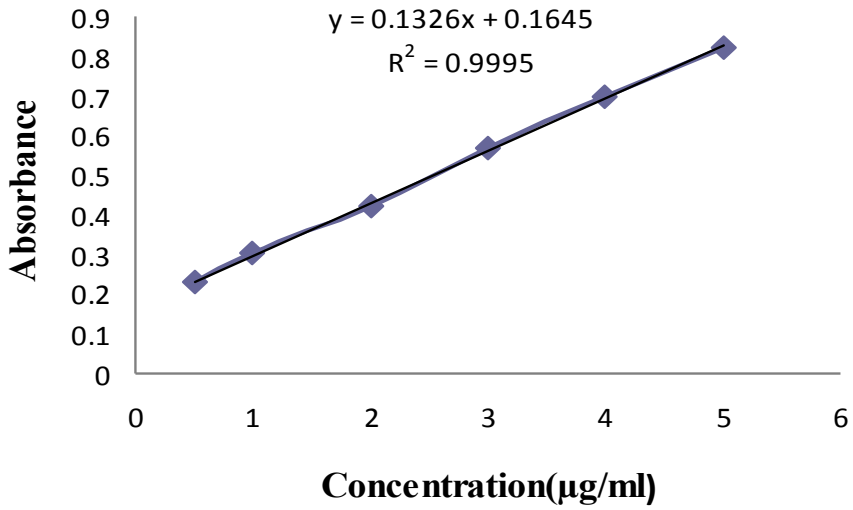

Fig. No. 03: Calibration curve of Alfuzosin hydrochloride

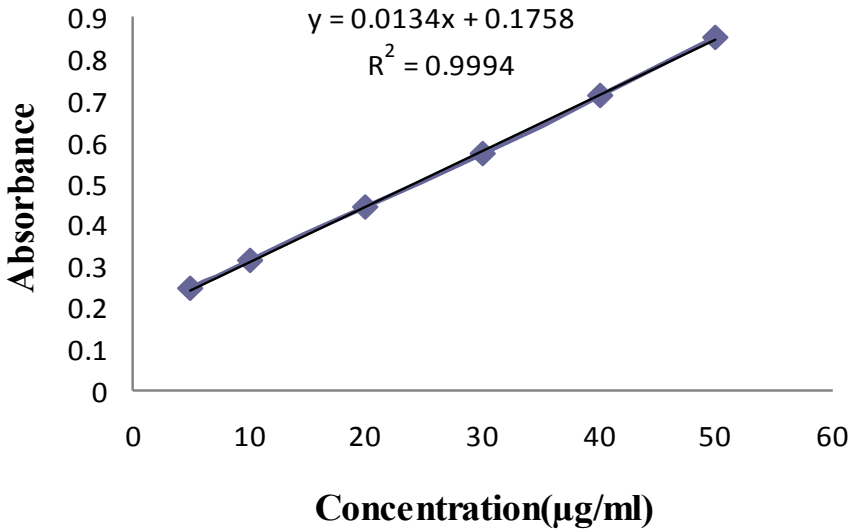

Fig. No. 04: Calibration curve of Dutasteride

Results

The standard calibration curves and linearity range of AFZ and DUT are presented in Table 1-2 and Fig.3-4. The absorptivity values and assay results of AFZ and DUT are presented in Table 3-4. In method validation, the results of accuracy studies of AFZ and DUT are depicted in Table 5-6 and precision of inter and intraday variations in Table 7. The summary of all validation parameters are presented in Table 8.

Discussion

The current research aims to develop a UV spectrophotometric method for the simultaneous estimation of AFZ and DUT in a commercially available tablet dosage. After considering the solubility of both the drugs, methanol was selected as the common solvent. From the overlain spectra, 247.5 nm and 241 nm were selected as sampling wavelengths for AFZ and DUT. The method was found to be very simple, reliable and requires knowledge of molar absorptivities of the components which should be determined very accurately. Next the only requirement is the measurement of absorbances at 247.5 nm and 241 nm. The calculations being very simple, can be done manually. The above said two wavelengths were selected to frame the simultaneous equation [1-5].

From the assay results (Table 3), the amounts of AFZ and DUT in the tablet dosage form were found to be 10.163 mg for AFZ and 0.504 mg for DUT which are satisfactory. The method was

validated as per ICH guidelines. Linearity was obtained in the concentration range of 0.5-5 µg/mL for AFZ and 5-50µg/mL for DUT (Table 1-2). The %RSD for intraday and interday variations of AFZ was found to be 0.422±0.001 and 0.319±0.002 respectively. An intraday and interday variation of DUT was found to be 0.517±0.001 and 0.563±0.001 respectively (Table 7). In both cases the results are within the acceptance limit of < 2% which indicates that the UV method developed has good precision. While validating the accuracy of the method (Table 5-6), it was found that the mean percentage recovery values were 99.79 % for AFZ and 100.02% for DUT, which are within the acceptance limit of 98% to 102% indicating the significant accuracy of the method. From the high recovery values (> 98 %) it can be inferred that the method is free from the interference of excipients used in the formulation. Based on the results obtained the proposed method can be regarded as simple, accurate, precise, and reliable which can be employed for routine quality control of AFZ and DUT in bulk and combined tablet dosage forms.

Conclusion

Properly validated simple, cost effective, time saving analytical techniques with the aid of UV are of immense benefit for the pharmaceutical R&Ds for routine drug estimations. The methodology becomes more acceptable if more than one drug can be simultaneously estimated by the same method since combined dosage formulations are becoming much popular from the point of therapeutic benefits and consumer acceptance. The current properly validated UV methodology is found to be successful for the simultaneous estimation of AFZ and DUT in combined tablet dosage form and expected to be beneficial for the routine estimations of the same.

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