Degradation Studies of Alfuzosin Hydrochloride Using UV Spectroscopy

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***Abstract*-The degradation studies of Alfuzosin HCl were conducted to evaluate its stability under various stress conditions using UV spectroscopy. The drug was subjected to acidic, alkaline, oxidative, thermal, and photolytic conditions to simulate different environmental factors that could impact its stability. UV spectroscopy was utilized to observe alterations in the absorption spectrum of the drug, facilitating the identification of degradation products and the evaluation of degradation extent. The findings indicated that the linearity range for Alfuzosin hydrochloride was between 100-500 ng/ml at a wavelength of 245 nm. The correlation coefficient for Alfuzosin hydrochloride was determined to be 0.999. The detection limit was established at 1 ng/ml, while the quantification limit was set at 100 ng/ml. The percentage relative standard deviation (RSD) for interday and intraday precision was calculated to be 0.2441 and 0.2083, respectively. The analysis indicated that the concentration of degradation products of Alfuzosin hydrochloride was 77.1% following acid hydrolysis, 85.1% after oxidation, and 94% due to photolytic degradation. Consequently, the method developed for the estimation of Alfuzosin hydrochloride in pharmaceutical dosage forms and in bulk is characterized as straightforward, precise, reproducible, and cost-effective.**

Keywords: Alfuzosin HCl, Prostate cancer, Hydrolytic degradation, Oxidative degradation, Photolytic degradation

INTRODUCTION

Alfuzosin hydrochloride is a selective alpha-adrenergic antagonist employed in the management of benign prostatic hyperplasia (BPH) among older males. BPH results in the enlargement of the prostate gland, which can obstruct urine flow from the bladder, leading to urinary retention. This medication is highly soluble in water, facilitating its absorption following administration. The presence of food significantly improves its oral bioavailability. It is a white or off white crystalline powder. It is used in the symptomatic treatment of urinary obstruction caused by benign prostatic hyperplasia and has been tried in the treatment of hypertension.

DEGRADATION STUDIES

Degradation studies refer to experiments or research aimed at understanding how the quality or functionality of a substance (like a chemical, pharmaceutical product, material, or biological sample) deteriorates over time under various

environmental or stress conditions. The purpose of degradation studies is to evaluate the stability, lifespan, and performance of a product in real-world conditions and help predict its behavior over time. Degradation studies can be categorized into several distinct types. They are: Forced degradation, Hydrolytic degradation, Oxidative degradation, Thermal degradation, Photolytic degradation.

MATERIALS AND METHODOLOGY

Drug

• The reference standard of Alfuzosin Hydrochloride was purchased from Sami Labs, Bangalore

• The commercial product of Alfuzosin Hydrochloride Tablet was purchased from the market

Reagents Used

0.1M Sodium Hydroxide, 0.1M Hydrochloric acid, 5% Hydrogen Peroxide, Methanol, Distilled water

PREPARATION OF REAGENTS

Preparation of 0.1M Sodium hydroxide

4g of Sodium hydroxide pellets were weighed and dissolved in small amount of distilled water then made up the volume to 1000ml

Preparation of 0.1 M Hydrochloric acid

8.33ml of concentrated Hydrochloric acid was measured and diluted with distilled water to 1000ml

Preparation of 5% Hydrogen Peroxide

50ml of Hydrogen peroxide was diluted with distilled water and the volume made up to 1000ml

SOLUBILITY

Table No 1: SOLUBILITY

SOLVENT	ALFUZOSIN HYDROCHLORIDE
Water	Soluble
Methanol	Soluble
Ethanol	Soluble
Chloroform	Soluble
Acetone	Insoluble
Acetonitrile	Sparingly soluble

EXPERIMENTAL METHODOLOGY

Selection and Optimization of Solvent

Solvent methanol was optimized as it met all the requirements in terms of the stated time, peak quality and non-interference as required.

Table No 2: SELECTION AND OPTIMIZATION OF SOLVENT

SOLVENT	ALFUZOSIN HYDROCHLORIDE
Water	Soluble
Methanol	Soluble
Ethanol	Soluble
Chloroform	Soluble
Acetone	Insoluble
Acetonitrile	Sparingly soluble

Selection of wavelength

The wavelength at which maximum absorption takes place in UV analysis was at a range of 200 to 400nm and the λ max was at 245nm

Preparation of Standard Stock Solution

The standard stock solution of Alfuzosin Hydrochloride was prepared by dissolving 10mg of drug in methanol and the final volume was made up to 100ml with the same solvent in a volumetric flask to get a solution containing 1000ng/ml alfuzosin hydrochloride. (As)

Preparation of working standard

In a 10ml standard flask pipette out 1ml of alfuzosin hydrochloride and dilute it up to the mark with methanol to a concentration of 100 ng/ml the solution was scanned between 200 -400nm and 245nm was found to be the maximum wavelength of absorption. This wavelength was selected for the development of UV method for estimating alfuzosin hydrochloride.

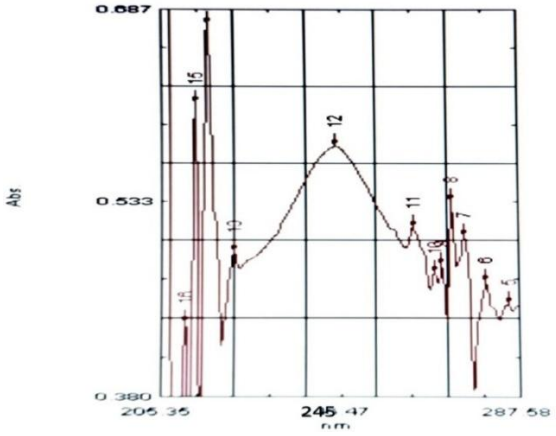

Fig No 1: UV Spectra of Alfuzosin Hydrochloride

METHOD VALIDATION

Validation is the process of creating written proof that ensures that a specific activity will consistently achieve the anticipated outcome, or a product that meets its predetermined requirements and high quality.

The approach has been proven to work in a variety of situations like Linearity, Accuracy, Precision, Limit of Detection and Limit of Quantification.

LINEARITY

Aliquots of working stock solution (Aw) was prepared with developed solvent to get a concentration range of 100-500ng/ml. The absorbance of resulting solution was measured at 245nm. A calibration curve using concentration vs. absorbance was plotted to study the Beer-Lamberts law and regression equation.

Table No 4: LINEARITY

Eq. of Line: y=0.001x+0.320		$R^2 = 0.999$
SL. No.	Concentration (ng/ml)	Mean Absorbance at 245nm
1	100	0.641
2	200	0.704
3	300	0.728
4	400	0.740
5	500	0.743
Mean		0.7112
SD		0.0421
RSD		0.0591
Slope		0.00137

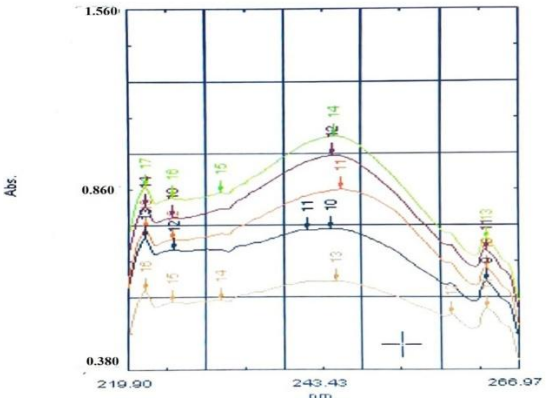

Fig No 2: Linearity spectra

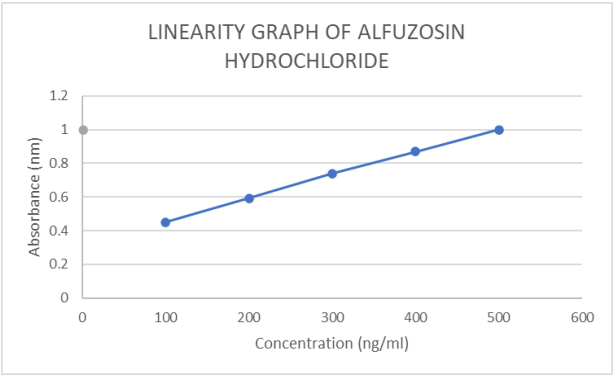

Fig no 3: Linearity graph

PRECISION

The interday and intraday was determined by the assay of sample solution on the same day or different days at different time in six replicates.

(1) Intraday Precision

In the intraday precision study was determined for 300ng/ml was analyzed 6 times for consecutive days (morning, afternoon, evening).

Table No 4: INTRADAY PRECISION

SL. NO.	Drug concentration	Absorbance	Standard Absorbance	Time
1.	Sample (300ng/ml)	0.738	0.740	30min
		0.741		
		0.740		
		0.737		
		0.738		
		0.740		
Average		0.739		
SD		0.00154		
RSD		0.20838		

(2) Interday Precision

The interday precision was determined for a solution of concentration 300ng/ml was analyzes for 3 times on different days.

Table No 5: INTERDAY PRECISION

SL.NO.	Concentration (ng/ml)	Day 1	Day 2	Day 3
1	300	0.740	0.738	0.740
2		0.743	0.741	0.743
3		0.743	0.740	0.737
4		0.742	0.738	0.741
5		0.741	0.738	0.742
6		0.742	0.738	0.738
Average		0.741	0.738	0.740
SD		0.001483	0.001612	0.002323
RSD		0.200134	0.218428	0.313918
Average RSD	0.24416			

ACCURACY

The accuracy study was performed using the standard addition method at 80%, 100% and 120% of standard Alfuzosin Hydrochloride solution as per ICH guidelines. The recover studies were performed three times at each level.

Table No 6: ACCURACY

SL. No	Concentration on(ng/ml)	Amount of drug(ng/ml)	Absorbance	Mean	SD	RSD
1.	80%	240	0.711	0.712	0.002236	0.314044
			0.712			
			0.715			
2.	100%	300	0.740	0.740	0	
			0.740			
			0.740			
3.	120%	360	0.763	0.764	0.001732	0.226701
			0.766			
			0.765			

LIMIT OF DETECTION (LOD)

The detection limit was determined by the analysis of sample with known concentration of analyte and by establishing the minimum level of which the analyte can be determined.

$DL = 3.3\alpha/S$

Where, α = the standard deviation of response, S= slope of the calibration curve

The limit of detection was found to be 1ng/ml

LIMIT OF QUANTIFICATION (LOQ)

The quantification limit was determined by the analysis of sample with known concentration of the analyte can be quantified acceptable accuracy and precision

$$QL=10\alpha/S$$

Where, α= the standard deviation of response, S=slope of the calibration curve

The limit of Quantification was found to be 100ng/ml

ANALYSIS OF MARKETED FORMULATIONS

The validated method was applied to the determination of Alfuzosin in tablets. Twenty tablets were assayed and the result are shown Table No: 8 indicating that the amount of drug in tablet sample was in good agreement with the label claim of the formulation s indicated by % recovery.

Table No 7: ASSAY OF MARKETED FORMULATION

SL. No		Concentration(ng/ml)	Actual Absorbance at 245nm	Acid Hydrolysis Absorbance at 245nm
1.	Blank	300	0.372	0.370
2.	Standard		0.742	0.675
3.	Sample		0.691	0.533

STABILITY STUDIES

The ICH guideline characterized stability testing of drug substances and product requires the stress testing to be carried out to enlighten the inherent stability characteristics of the active substance and also to produce a rapid identification of differences that might result from changes in the manufacturing processes. Vulnerability to oxidation, hydrolytic, photolytic and thermal stability are the required tests.

FORCED DEGRADATION STUDIES

Forced degradation studies serve to identify potential reactions that could lead to the degradation of a processed product. This process entails the degradation of drug products and substances under conditions that are more extreme than those used in accelerated stability testing, thereby producing degradation products that can be analyzed to evaluate the stability of the molecule.

HYDROLYTIC DEGRADATION USING 0.1M HCl

Standard Preparation

Aliquot of 1 ml of Alfuzosin hydrochloride (10mg dissolved in 10ml i.e.1 mg/ml) was transferred to a small round bottom flask. The prepared solutions were subjected to reflux for 2 h in a boiling water bath. The samples were cooled to room temperature (25^0 C), neutralized with an amount of acid equivalent to that of the previously added. From the resulting neutral solution, 2 ml was taken in cuvette and absorbance was recorded.

Sample Preparation

100mg equivalent of Alfuzosin hydrochloride tablet were crushed weighed and transferred to volumetric flask, dissolved 0.1M Hydrochloric acid to achieve a concentration of 1 mg/ml. The solution was kept at room temperature. Then the next day (1^{st} day), an aliquot solution was diluted with methanol to get final concentration of 300ng/ml. The solution was scanned in the UV region and the maximum absorbance was recorded. The same procedure was repeated for 3rd day and 5th day time interval. The obtained spectrum is compared with standard spectrum.

Blank Preparation

100ml of 0.1M HCl solution was taken in a 100ml volumetric flask. The solution was kept at room temperature the next day; an aliquot solution was diluted with methanol to get final concentration. This procedure is repeated for 3rd and 5th day. The procedure was repeated thrice. After the stipulated time. The absorption of the resulting solution showed maximum against reagent blank treated in the same way. Three such determination were made and the assay value was estimated.

Table No 8: HYDROLYTIC DEGRADATION OF ALFUZOSIN HYDROCHLORIDE USING 0.1M HCl

SL. No	Drug	Amount		%label claimed	%RSD
1	ALFUZOSIN HYDROCHLORIDE	Labelled	Found	87	0.983
		10	8.7		

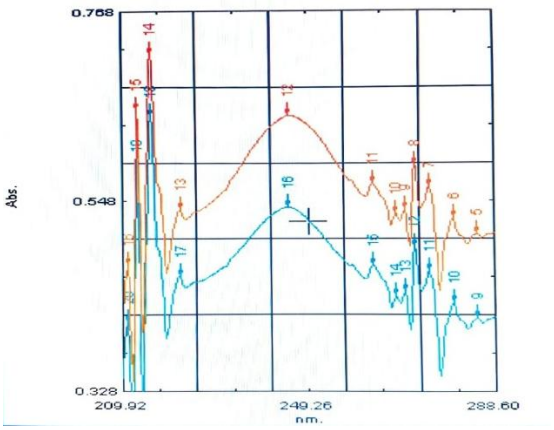

Fig No 4: Overlay spectra of hydrolytic degradation of Alfuzosin hydrochloride using 0.1M HCl

OXIDATION DEGRADATION USING 5% H_2O_2

Standard Preparation

100mg of alfuzosin hydrochloride was weighed and transferred to volumetric flask, dissolved to 5% H_2O_2 achieves a concentration of 1mg/ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with methanol to get final concentration of 300ng/ml. The solution was scanned in the UV region and the maximum absorbance was recorded. The same procedure was repeated for 60 mins and 90 mins time interval.

Sample Preparation

100mg equivalent of Alfuzosin hydrochloride tablets were crushed weighed and transferred to volumetric flask, dissolved 5% H_2O_2 to achieve a concentration of 1mg/ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with methanol to get final concentration of 300ng/ml. The solution was scanned in the UV region and the maximum absorbance was recorded. The same procedure was repeated for 60mins and 90mins time interval.

Blank Preparation

100ml of 5% H_2O_2 solution was taken in a 100ml volumetric flask. The solution was kept at room temperature. After 30mins aliquot solution was diluted with methanol to get final concentration. This is used as a blank. The procedure was repeated thrice after the stipulated time. The absorption of the resulting solution showed maximum against reagent blank treated in the same wat three such determinations were made and the assay value was estimated.

Table No 9: OXIDATIVE DEGRADATION OF ALFUZOSIN HYDROCHLORIDEUSING 5% H_2O_2

SL. No		Concentration(ng/ml)	Actual Absorbance at 245nm	Oxidative Degradation Absorbance at 245nm
1.	Blank		0.510	0.570
2.	Standard		0.782	0.640
3.	Sample	300	0.631	0.537

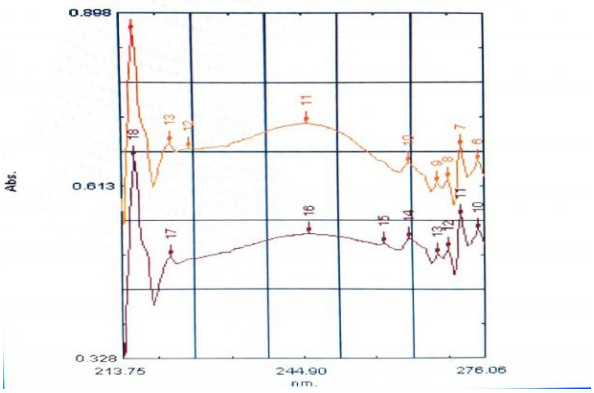

Fig No 5: Overlay spectra of oxidative degradation using 5% H_2O_2

IRRADIATION WITH ULTRAVIOLET LIGHT

A sample powder of Alfuzosin Hydrochloride (10 mg) was exposed to UV light (254 nm) for 48 h. The material was dissolved in 10 ml methanol. The solution was filtered with syringe filtration disk claimed concentration of 1 mg/ml. It was suitably diluted and a volume of 2 ml was taken in cuvette and absorbance was recorded. As well, an aqueous solution of Alfuzosin Hydrochloride (1mg/ml) was exposed to UV light (254 nm) for 48 h, and after diluting, a volume of 2 ml was taken in cuvette and absorbance was recorded.

Table No10: IRRADIATION WITH UV LIGHT

SL. No		Concentration(ng/ml)	Actual Absorbance at 245nm	Oxidative degradation absorbance at 245nm
1.	Blank		0.409	0.407
2.	Standard		0.742	0.538
3.	Sample	300	0.552	0.522

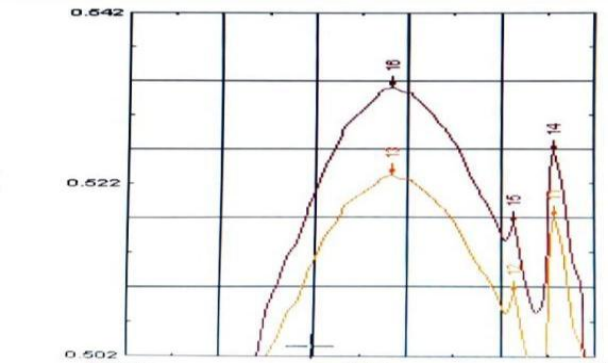

Fig No 6: Overlay spectra of irradiation of Alfuzosin hydrochloride with UV light

RESULTS AND DISCUSSION

The linearity range of Alfuzosin Hydrochloride was 100-500 ng/ml at a wavelength of 245 nm. The coefficient of correlation for Alfuzosin Hydrochloride was found to be 0.999. The percentage of Alfuzosin hydrochloride in the formulation was found to be 87%.

The validation parameters of Alfuzosin hydrochloride by UV spectroscopic method were summarized by as follows:

Table No 11: RESULTS OF VALIDATED METHOD DEVELOPED FOR ALFUZOSIN HYDROCHLORIDE

PARAMETERS	ANALYTIC DATA
Linearity range	100-500ng/ml
λmax	245nm
Slope	0.001
Intercept	0.320
Correlated coefficient Cr	0.999
LOD(ng/ml)	1ng/ml
LOQ(ng/ml)	100ng/ml
Intraday precision(%RSD)	0.20838
Interday precision(%RSD)	0.24416
Accuracy (%recovery)	87

From the forced degradation, it was observed that in case of photolytic degradation stability Alfuzosin Hydrochloride was most stable under the employed stress conditions. Maximum degradation was seen hydrolytic degradation using hydrogen peroxide. Nonetheless, the method was able to isolate completely the degradation products from the intact Alfuzosin Hydrochloride. This confirmed stability indicating property of the proposed method.

Tabel No 12: DEGRADATION STUDIES

SL No.	Stability Studies of Standard Alfuzosin Hydrochloride	Concentration Used (ng/ml)	Concentration Left After Degradation(ng/ml)	% Recovery
1.	Acid Hydrolysis	300	231.40	77.1
2.	Oxidation Degradation	300	255.3	85.1
3.	Photolytic Degradation	300	283.69	94

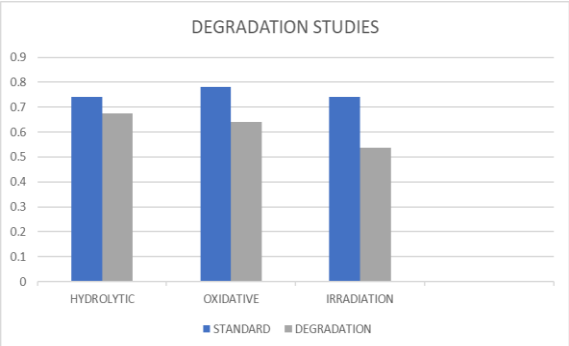

Fig No 7: Degradation studies

CONCLUSION

It could be concluded that the developed method for estimation of Alfuzosin Hydrochloride in pharmaceutical dosage form and in bulk is simple definite, reproducible, and economical. The values of accuracy, precision, LOD and LOQ were within the limits.

The present study involves the stress induced degradation studies such as acid hydrolytic degradation, oxidative degradation and photolytic degradation. Degraded samples were quantified by UV method and the results of bulk and samples are compared with that of standard.

Alfuzosin Hydrochloride is very sensitive so it is unstable in hydrolytic and oxidative but is stable in photolytic. Statistical analysis for the results clearly demonstrates that the method is suitable for the determination of Alfuzosin Hydrochloride in bulk and tablet without any interference from the degradation products, and it is endorsed for routine use in quality control industry laboratories.

FUTURE SCOPE

- To design and validate a method that signifies stability.
- To determine the degradation pathways of drug substance and drug products.
- Studying how degradation products might influence
- Improving the understanding of the drug's stability and safety
- Help to differentiate between hydrolytic, oxidative, thermal degradation of alfuzosin HCl and provide insights into the chemical structure of the degradation products.

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