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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF BICALUTAMIDE BY UV, FIRST ORDER, AND SECOND ORDER DERIVATIVE SPECTROPHOTOMETERY

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ABSRACT

The present study was an attempt to develop a simple, sensitive, precise, accurate, and low economical method and validated the method for determination of Bicalutamide by UV, First and Second order derivative spectrophotometry. The solvent was used Ethanol and maximum absorbance(λ_{max}) was found to be 272.20nm, 257nm, 228.20nm for UV, first and second order derivative spectrophotometry. Beers law obeyed at the concentration range of 3-11µg/ml, 8-12µg/ml, and 10-18µg/ml for UV, First and Second order derivative spectrophotometry respectively. The correlation coefficient for all the three methods were found to 0.999, and % recovery was found to be 100.05, 99.76 & 99.87% for UV, First and Second order derivative spectrophotometry respectively. The proposed method has been validated as per ICH guidelines for Linearity, Accuracy, Precision, Specificity, LOD, and LOQ. The method was also applied for the degradations studies. The LOD values were found to be 0.04µg/ml, 0.12µg/ml, 0.14µg/ml and LOQ values were found to be 0.13µg/ml, 0.41µg/ml, 0.45µg/ml for UV, First and Second order derivative spectrophotometer respectively. The developedmethod was validated successfully for the estimation of Bicalutamide in bulk and dosage form.

KEYWORDS: UV-Visible spectroscopy, Bicalutamide, Stability studies, UV, First and second order derivative.

INTRODUCTON

Bicalutamide is chemically, (2RS)-4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'(trifluoromethyl) propionanilide is an orally active, nonsteroidal antiandrogen.[1]

Adrenocarcinoma of prostate is most common solid neoplasm diagnosed in men.[2,3,4&5] Primary treatment of prostate cancer includes androgen ablation therapy or surgical castration.[6,7&8]

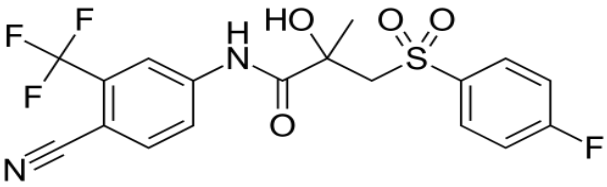

Fig 1: Chemical structure of Bicalutamide.

A few UV Spectroscopic and RP-HPLC methods havebeen reported in the literature for the estimation of Bicalutamide.[1-15] An attempt has been made to develop asimple and reliable UV Spectroscopic method for theestimation of Bicalutamide in tablet dosage form. It becomes essential to develop a simple, sensitive, accurate, précised and reproducible method for the estimation of drug samples. Our main concern is development and validation of UV Spectrophotometric method as per ICH guidelines.

MATERIALS AND METHODS

Bicalutamide was obtained (50 mg tablets) from Hetero labs private limited, Hyderabad, A.P, India was collected from local market. All the chemicals used were of A.R. grade procured.

For all the Spectrophotometric methods, Shimadzu model 1800 UV-VIS spectrophotometer with spectral bandwidth of 1nm, wavelength accuracy of ±0.3nm and a pair of 1 cm matched quartz cells of 10 mm optical path length, detector used was silicon photodiode.

STANDARD SOLUTIONS

Standard solution

Accurately weigh 10mg of Bicalutamide and transfer into 10ml volumetric flask and add some quantity of ethanol and sonicated for 5 min for solubility of Bicalutamide make up to mark with ethanol and concentration was attained 1000µg/ml. The final concentration of the Bicalutamide was obtained 7µg/ml. The absorbance was measured at 271 nm.

Sample solution

Accurately weighed 20 tablets and the average weight of the tablets were determined. The tablets were crushed into a fine powder by using motor and pestle, then accurately weighed and transferred 57.81mg of powder which is equivalent to 10mg of Bicalutamide and

transferred into 10ml volumetric flask and add ethanol, sonicated for 5min. The volume was made up to the mark with ethanol and concentration was obtained 1000 µg/ml. The final concentration was obtained 7µg/ml and measured absorbance at 271 nm.

METHOD VALIDATION

The validation of the method was performed according to ICH guidelines. The different validation parameters were studied Specificity, linearity, Precision, accuracy, limit of detection, limit of quantification and robustness.[19]

System suitability parameters

The method was optimized at different system suitability parameters like wavelength, absorbance, temperature and concentration. The wavelength were fixed at 271,257and 225 for zero, first and second order spectra respectively. The absorbance's were fixed at 0.445, 0.025 and 0.013 for zero, first and second order spectra respectively. The optimized concentrations were 7, 10 and 14µg/ml for zero, first and second order spectra respectively. The spectra's were showed in figure no 2-6.

Table No: 1. Optimized parameters of Zero, First & Second order spectra.

Zero Order Spectra			
S.NO	**Type**	**Wavelength(nm)**	**Absorbance**
1	Standard	271	0.445
2	Test	271	0.401
3	Solvent	Methanol	
First order Spectra			
1	Standard	257	0.025
2	Solvent	Methanol	
Second Order Spectra			
1	Standard	225	0.013
2	Solvent	Methanol	

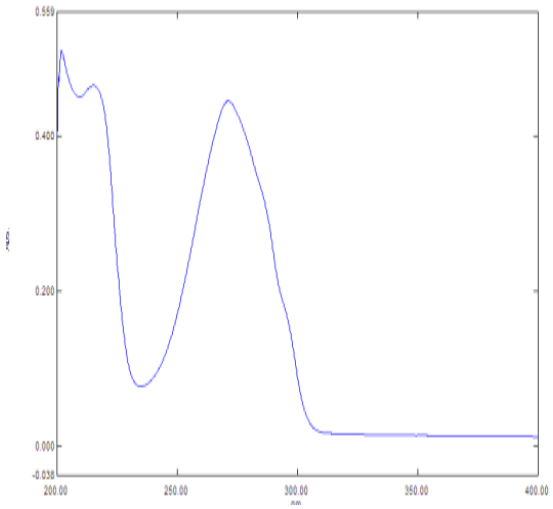

Figure:2 Zero order Standard spectrum

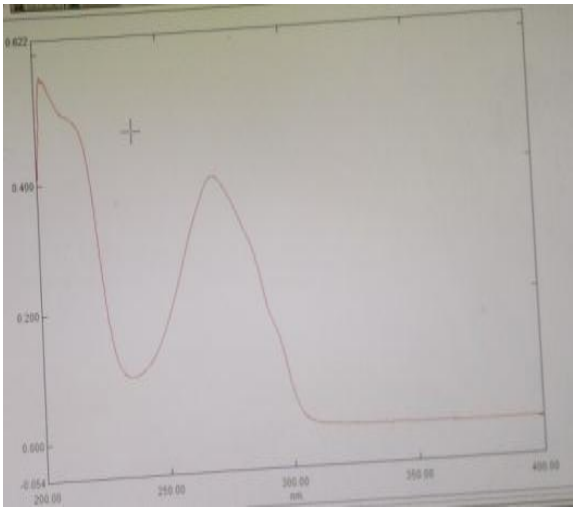

Figure:3Zero order sample spectrum

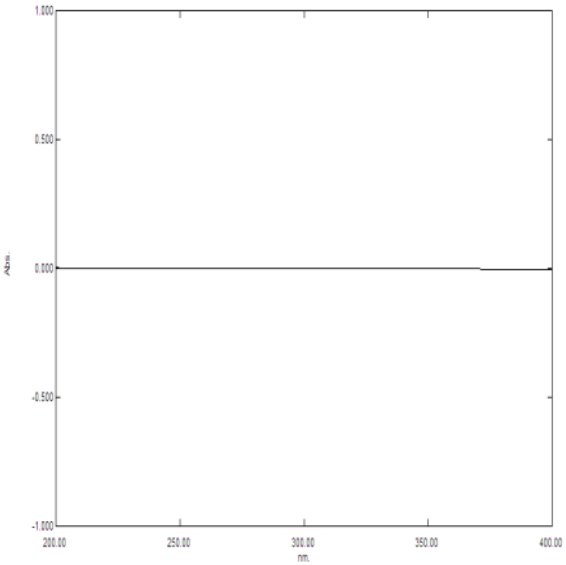

Figure: 4 Zero order blank spectrum

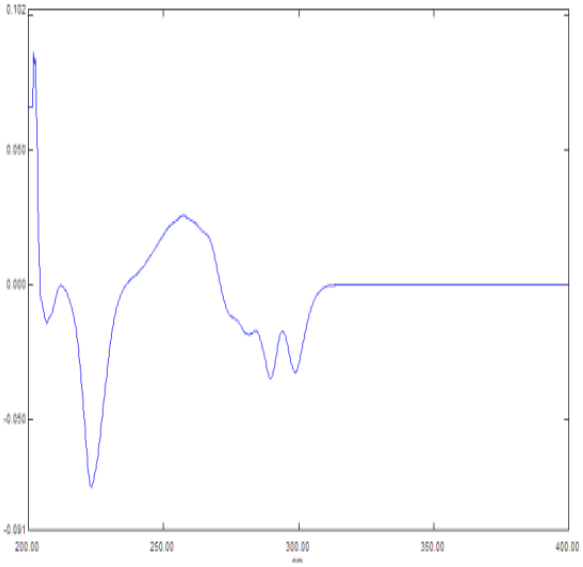

Figure: 5 First order spectra Bicalutamide

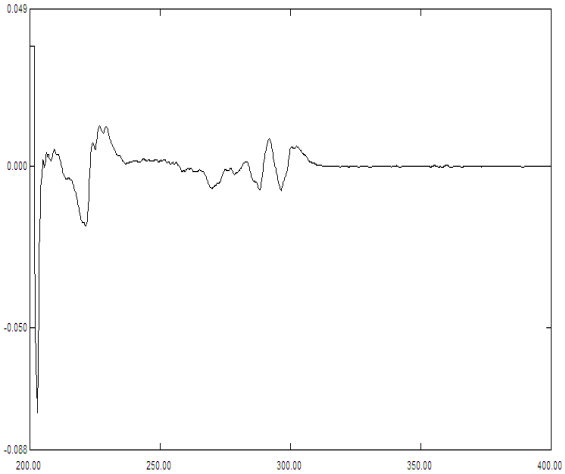

Figure: 6 Second order spectra of Bicalutamide.

Specificity

The specificity of the method was calculated by measuring the absorbance for solvent, standard and sample solutions prepared as per optimized method and measured any interference of solvent, sample. There was no interference observed method showed specificity.

Linearity

The linearity of the method was studied by calibration curve (absorbance Vs concentration). The pure solution was checked at concentration range from 3-11µg/ml for zero order, 8-12µg/ml for first order10-18 for second order spectra respectively. The r^2 value was found to be 0.999 for all spectrophotometric methods. The linearity curves showed in figures 7, 8& 9.

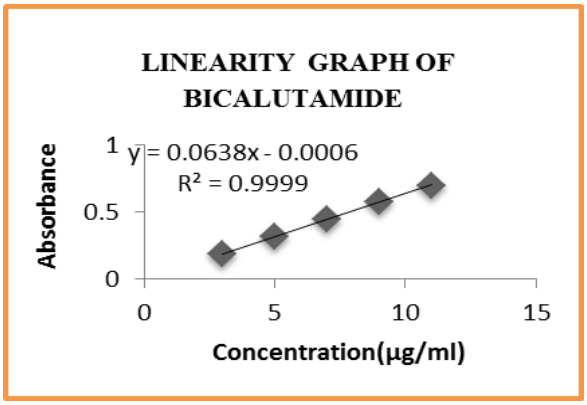

Figure: 7 Linearity graph of Zero order spectra

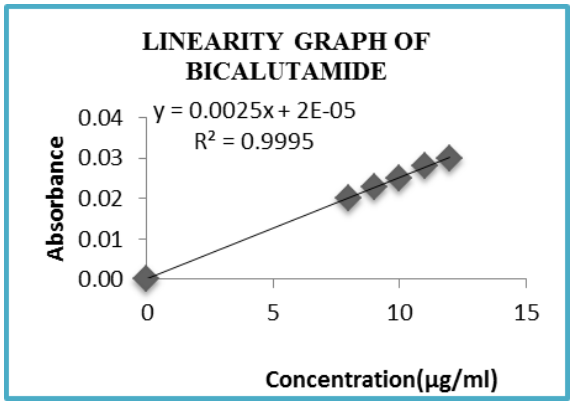

Figure: 8 Linearity graph of first order spectra.

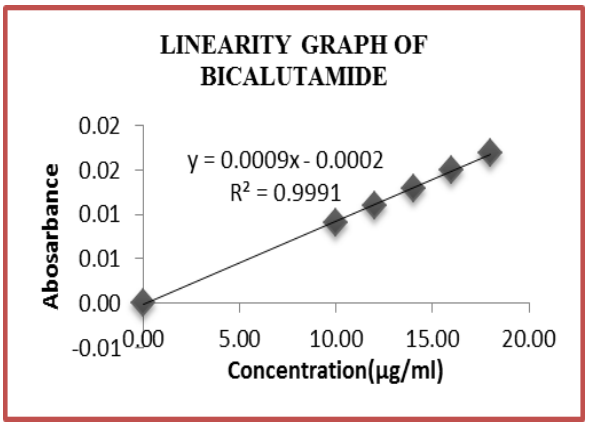

Figure: 9 Linearity graph of second order spectra.

Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the method was determined by intraday precision and

intermediate precision. The repeatability of the method was studied by six replicate measurements of standard and sample solutions. The % RSD values of zero, first, second derivative spectra were obtained less than 2% as directed by ICH guidelines. The results were showed in table no 2 & 3.

Table No 2: Results of Intraday Precision for Bicalutamide.

Method	9.30 AM			1.30 PM			5.30 PM		
	Abs	% Assay	%RSD	Abs	% Assay	%RSD	Abs	% Assay	%RSD
Zero order	0.440	98.81	0.19	0.439	98.66	0.24	0.437	98.21	0.44
First order	0.025	100.01	0.42	0.024	99.61	0.16	0.024	99.61	0.66
Second order	0.013	100.01	0.32	0.012	99.24	0.58	0.012	99.24	0.69

Table No: 3 Results of Interday Precision for Bicalutamide.

Method	Day 1			Day 2			Day 3		
	Abs	% Assay	%RSD	Abs	% Assay	%RSD	Abs	% Assay	%RSD
Zero order	0.441	99.11	0.19	0.439	98.66	0.28	0.437	98.21	0.46
First order	0.025	100.01	0.47	0.024	99.61	0.17	0.024	99.61	0.49
Second order	0.0130	100.01	0.34	0.0128	98.27	0.66	0.0129	99.24	0.66

Accuracy

The accuracy of the method was studied by standard recovery process. The solution was analysed at three concentration levels of 50%, 100% and 150%. The mean recovery study was performed under optimized concentrations of zero, first and second derivative spectrophotometer. The results were obtained in between 98-102% of all spectrophotometric methods. Results was showed in table no 4.

Table No 4: Results of Accuracy for Bicalutamide.

S.NO	Accuracy level	Weight of sample	Abs	Amount added	Amount found	%Recovery
Zero order derivative spectra						
1	50%	46.24	0.351	5.57	5.53	99.34
2	100%	57.81	0.443	6.96	6.97	100.19
3	150%	69.372	0.534	8.35	8.40	100.62
First order derivative spectra						
1	50%	28.905	0.012	2.49	2.47	99.26
2	100%	57.81	0.024	9.99	10.0	100.31
3	150%	86.715	0.037	22.49	22.46	99.90
Second order derivative spectra						
1	50%	28.905	0.006	3.49	3.47	99.52
2	100%	57.81	0.013	13.99	14.00	100.12
3	150%	86.715	0.019	31.49	31.48	99.98

LOD and LOQ

The limit of detection (LOD)and quantification limits were studied by signal to noise ratio 3:1 and 10:1 ratios. The detection limit and quantitation limit were obtained 0.04 & 0.13 µg/ml for zero, 0.12 & 041µg/ml for first order and, 0.14 & 0.45 µg/ml for second order derivative spectra respectively.

Table No:5 Results of LOD and LOQ for Bicalutamide.

Parameter	Result		
	Zero order	First order	Second order
Slope	0.0638	0.0025	0.0009
STDEV	0.0008	0.0001	0.0004
LOD	0.04µg/ml	0.12µg/ml	0.14µg/ml
LOQ	0.13µg/ml	0.41µg/ml	0.45µg/ml

Robustness

The robustness of the method was performed by deliberate change in parameters like wavelength ±2 nm and temperature ± 5℃ with optimized standard solution concentrations of zero order derivative spectra. The method was not affected with changed parameters of wavelength and temperature. The results was showed in table no 6.

Table No: 6 Results of Robustness for Bicalutamide.

S. No	Parameter	Condition	Absorbance	% Assay
1	Low	269.00	0.438	98.44
2	Actual Wavelength	271.00	0.445	100.01
3	High	273.00	0.449	100.91
4	Low (20°C)	271nm	0.437	98.21
5	Actual Temperature (25°C)	271nm	0.440	98.89
6	High(30°C)	271nm	0.442	99.34

Force degradation study of Bicalutamide

The forced degradation studies were performed on Bicalutamide, there was no interference of excipients with drug. It proves capability of the method for the analysis of drug with different stress conditions. Differet stress indicated studies wereconducted like acid(0.1N HCl, refluxed for 4H at room temp), Base(0.1NNaOH refluxed for 6H at room tempera ture), H2O2(3%H2O2Storedatroomtemperaturefor5H), hydrolytic at 80oC and UV-light (nearUV≥200for3days).

Table No: 10 Results of zero order degradation studies of Bicalutamide.

S. No	Condition	Absorbance	% Assay	% Degradation
1	Acid(0.1HCl for 4hrs at room temperature)	0.409	91.92	8.08
2	Base(0.1N NaOH for 6hrs at room temperature)	0.415	93.27	6.73
3	H_2O_2 (3% at room temp for 5 hrs)	0.425	95.52	4.48
4	UV-light (200 nm≥ For 3 days)	0.400	89.90	10.10

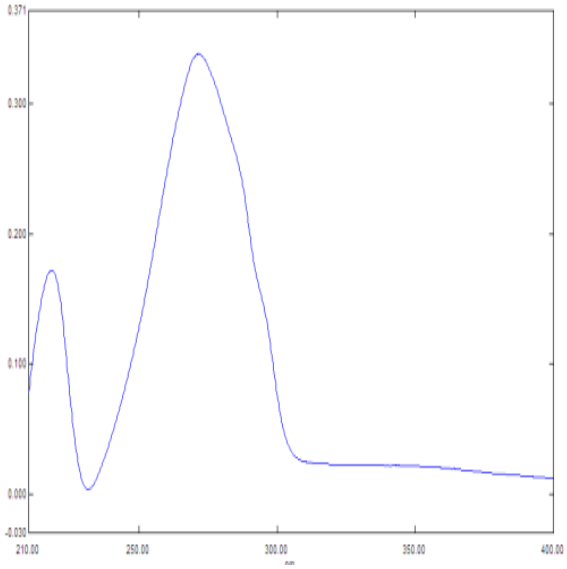

Figure No: 10 Spectrum for acid degradation

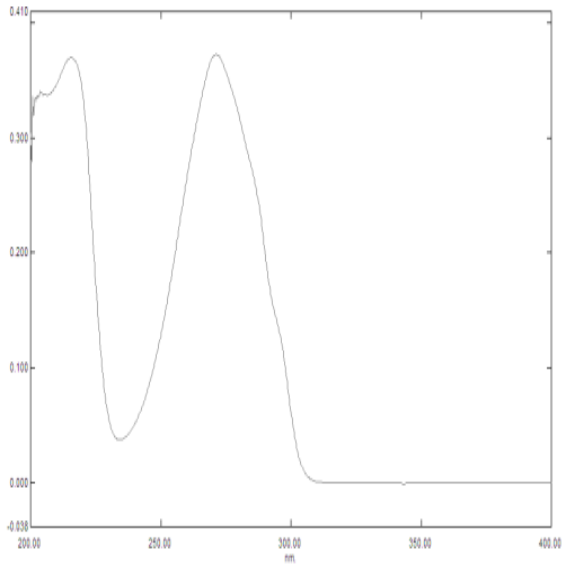

Figure No: 11 Spectrum for base degradation

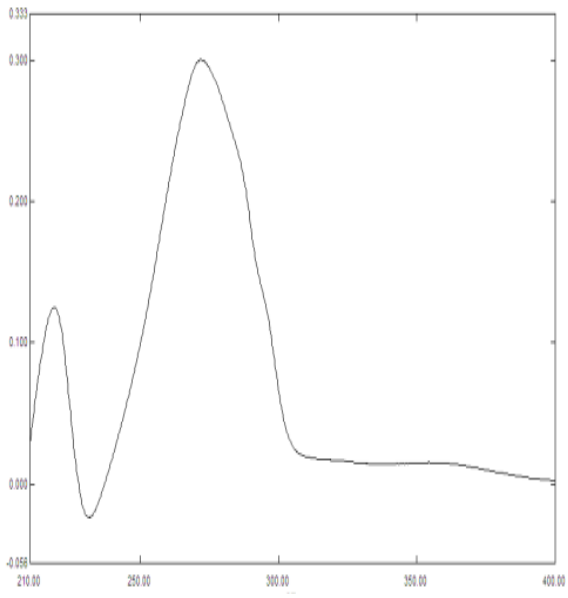

Figure No: 12 Spectrum for Peroxide degradation

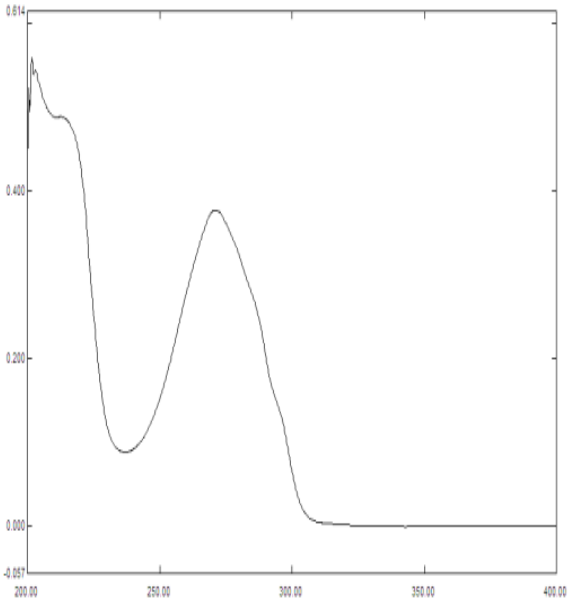

Figure No: 10 Spectrum for Photo degradation

CONCLUSION

The most striking features of the method was its simplicity, lessconsuming solvent in sample preparations such as extraction of solvents, heating, degassing which are needed for UV, First order, Second order. It can be concluded that the proposed methods was fully validated and found to be simple, sensitive, accurate, precise, reproducible, and robust and relatively inexpensive. So the developed method can be easily applied for the routine Quality Control analysis of Bicalutamide in pharmaceutical preparations. The other active ingredients and excipients usually present in the pharmaceutical dosage forms did not interfere in the estimation.

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