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DEVELOPMENT AND VALIDATION OF A STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF BICALUTAMIDE IN PHARMACEUTICAL FORMULATIONS

[1*]G.NAVEEN KUMAR REDDY, [2]V.V.S.RAJENDRA PRASAD, [2] PRASHANT KUMAR MAHARANA

[1]CMJ University, Shillong, Meghalaya.
[2] Sitha Institute of Pharmaceutical Sciences, JNTU, Hyderabad.
Corresponding author E mail: naving29@gmail.com

ABSTRACT

Simple, rapid, sensitive, accurate, robust & rugged stability indicating analytical method for determination of Bicalutamide in pharmaceutical formulations is developed and validated by using HPLC & applied the developed and validated method for determining the assay of bicalutamide in tablets. The method developed is more simple, robust and accurate than the existing methods. Chromatography was performed with mobile phase containing 0.5g of sodium dihydrogen orthophosphate dihydrate & acetonitrile adjusted to pH 3.0 with orthophosphoric acid, filtered and degassed, with a flow rate of 1.0 mL/min, Inertsil ODS-2,250 X 4.6 mm, 5µ column & UV detection at 270nm.The method was validated for linearity, accuracy, ruggedness, robustness, precision & bench top stability of sample & standard solution. Bicalutamide tablets were subjected to different stress conditions like acid, alkali, peroxide & UV studies and checked for its specificity, degradation & stability. The developed method was very rapid, specific, accurate, robust, rugged and stable.

KEY WORDS

Bicalutamide, Assay method, HPLC, Stability indicating method.

INTRODUCTION

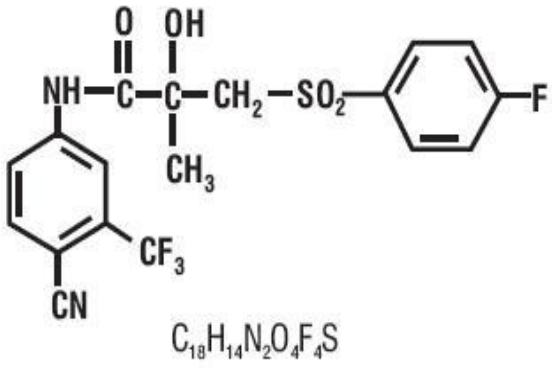
A stability-indicating method is "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and drug product. A stability-indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities."[1]

Bicalutamide designated chemically as N-[4-cyano-3-(trifluoromethyl) phenyl]-3-[(4-fluorobenzene) sulfonyl]-2- hydroxy-2-methylpropanamide with an empirical formula of $C_{18}H_{14}F_4N_2O4S$ and a molecular weight of 430.374 g/mol (**Fig.1**). Bicalutamide is soluble in acetone and tetrahydrofuran; slightly soluble in 100% ethanol or methanol; practically insoluble in water. It has a pKa of 12. [2-6].

Bicalutamide is an antineoplastic hormonal agent primarily used in the treatment of prostate cancer. Bicalutamide is a pure, non-steroidal anti-androgen with affinity for androgen receptors (but

not for progestogen, estrogen or glucocorticoid receptors). It competitively inhibits the action of androgens by binding to cytosol androgen receptors in the target tissue which stimulate the growth of normal and malignant prostatic tissue. Prostate cancer is mostly androgen-dependent and can be treated with surgical or chemical castration.[7]

Fig. No.1: Bicalutamide Hydrochloride



A few methods for the determination of Bicalutamide in pharmaceutical formulations by HPLC, HPTLC and UV appear in literature. This paper reports an improved, rapid, sensitive HPLC method with UV detection, useful for estimating the assay of Bicalutamide in pharmaceutical formulations. This method can be used for routine quality control analysis. The method was validated by parameters such as linearity, accuracy, precision, robustness, ruggedness, sample and standard solution stability and forced degradation studies[8-10].

EXPERIMENTAL

Reagents

HPLC grade Acetonitrile (HPLC Grade, Fischer), Sodium Dihydrogen Ortho Phospahte dihydrate (AR, Merck), Hydrochloric Acid (AR, Rankem), Sodium hydroxide (AR, Rankem), Hydrogen peroxide (AR, Rankem), Ortho phosphoric acid (AR, Rankem), Water (Milli Q water). Bicalutamide pure drug substance was kindly supplied by Hetero Labs, India. Ingredients used for placebo were lactose monohydrate, sodium starch glycolate type a potato, povidone, magnesium stearate, hypromellose 2910 (6mpa.s), hypromellose 2910 (6 mpa.s), polyethylene glycols and titanium dioxide.

Instrumentation

A liquid chromatograph (Shimadzu) system equipped PDA detector. The HPLC system was well equipped with LC Solutions software for data processing. Other instruments like Sartorius analytical balance, Metrohm pH meter and Biotechnics sonicator were used in sample and standard preparations and for forced degradation studies.

METHODOLOGY

Chromatographic conditions:

The analytical column used was Inertsil ODS-2,250 X 4.6 mm, 5µ.The mobile phase was 0.5g of sodium dihydrogen orthophosphate dihydrate & acetonitrile adjusted to pH 3.0 with orthophosphoric acid,filtered and degassed.It has a flow rate of 1.0mL/min, injection volume of 20µL with ambient column oven temperature and sample tray temperature with isocratic elution & UV detection at 270nm & a run time of 20 min.

Standard, sample, mobile phase and diluent preparation:

Fig. No.2: Blank-Diluent

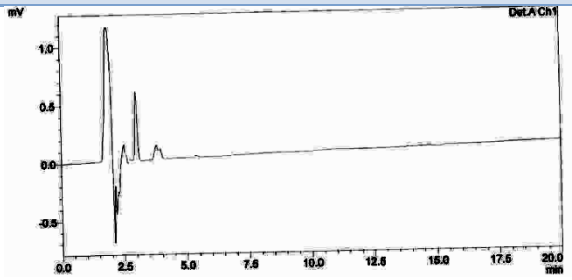
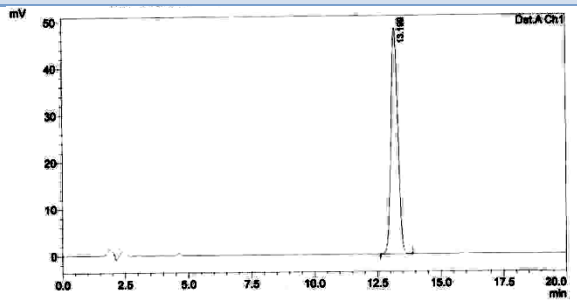


Fig.No.3: Standard



Diluent: Mobile phase is used as diluent.

Preparation of mobile phase: Weigh accurately 0.5g of sodium dihydrogen orthophosphate dihydrate into 1000mL beaker.Add 500 mL of water and dissolve then add 500mL of acetonitrile mix and adjust the pH to 3.0 with orthophosphoric acid,filter and degas.

Preparation of standard solution:

Weigh accurately 10.0mg of Bicaultamide standard and transfer in to 10mL volumetric flask, dissolve and dilute to volume with diluent. Further transfer 1.0mL of the solution in to 20mL volumetric solutionand dilute to volume with diluent (or prepare a solution containing 0.05mg/mL of bicaultamide in diluent).

Preparation of Test solution:

Weigh accurately about 65.0mg of Bicalutamide tablets powder equivalent to 25mg of Bicaultamide and transfer in to 25mL volumetric flask. Dissolve and dilute to volume with diluent and filter this through 0.45µ nylon membrane filter.Further transfer 1.0mL of above solution in to 20mL volumetric flask,dilute to volume with diluent.

RESULTS & DISCUSSION:

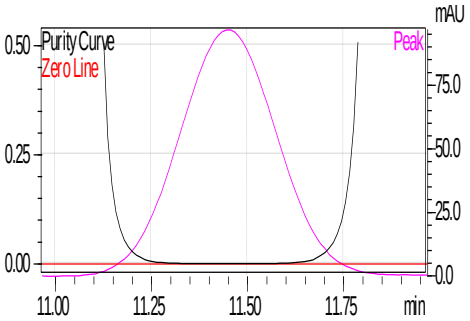
Specificity:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. [11]. Specificity was demonstrated by injecting a blank, placebo and standard solution. No interference was seen at the retention time of analyte. The specificity was also demonstrated by induced degradation of bicalutamide formulation samples to acid degradation, alkali degradation, peroxide degradation, U.V. degradation. Peak Purity index was checked and the results are tabulated in **Table No.:1.Figures 4-8** represents different stress conditions.

Table No.: 1

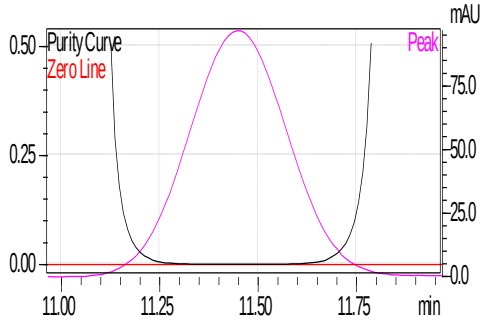
BICAULTAMIDE FORCED DEGRADATION		
Stress Condition	Peak purity index	Single point threshold
Acid Stress	0.999997	0.977552
Alkali Stress	1.000000	0.993260
Peroxide Stress	0.999975	0.993456
U.V. Stress	0.999997	0.993221
Unstressed sample	1.000000	0.993221
Acceptance Criteria	Peak purity index should be not less than0.995	

Fig. No.4: Acid Stressed Placebo Solution



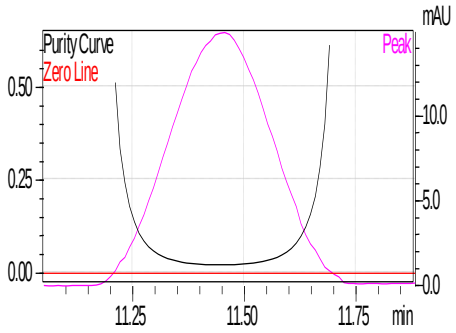
ID≠	: 1
Retention time	: 12.179
Compound Name	: Bicalutamide
Impurity	: Not detected
Peak purity index	: 0.999997
Single point threshold	: 0.977552

Fig.No.5: Alkali Stressed Placebo



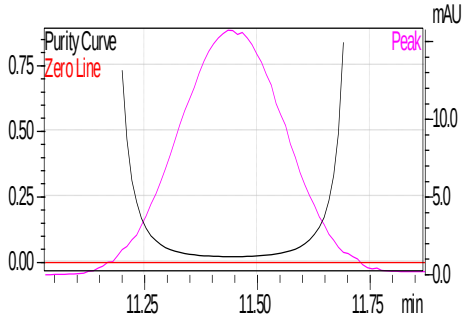
ID≠	:1
Retention time	: 12.185
Compound Name	: Bicalutamide
Impurity	: Not detected
Peak purity index	: 1.000000
Single point threshold	: 0.993260

Fig.No.6: Peroxide Stressed Placebo



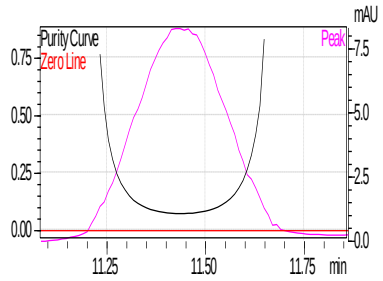
ID≠	: 1
Retention time	: 12.186
Compound Name	: Bicaultamide
Impurity	: Not detected
Peak purity index	: 0.999975
Single point threshold	: 0.993456

Fig.No.7: UV Stressed Placebo



ID≠	*: 1*
Retention time	*: 12.200*
Compound Name	*: Bicalutamide*
Impurity	*: Not detected*
Peak purity index	*: 0.999997*
Single point threshold	*: 0.993221*

Fig.No.8: Unstressed sample



ID≠	**: 1**
Retention time	**: 12.185**
Compound Name	**: Bicalutamide**
Impurity	**: Not detected**
Peak purity index	**: 1.000000**
Single point threshold	**: 0.993221**

System suitability Testing:

System suitability testing is used to verify that the reproducibility of the system is adequate for the analysis to be performed. System suitability is done by preparing and injecting the standard solution 6 times and calculating its RSD. Other parameters like tailing and theoretical plates should also be taken in to consideration. Results are tabulated in **Table No.:2**

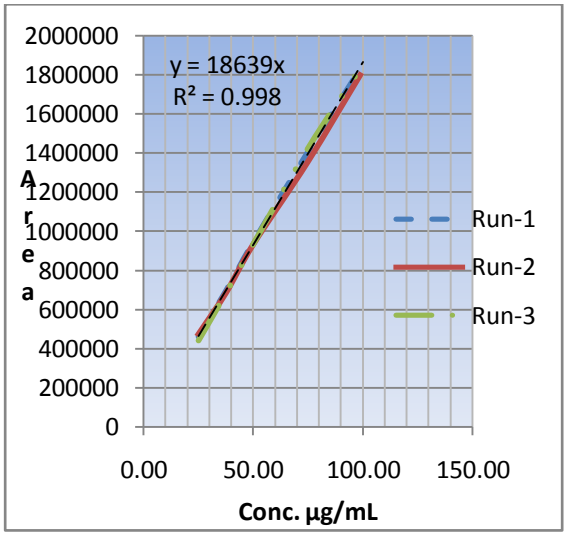
Table No.:2

BICAULTAMIDE SYSTEM SUITABILITY										
Injection No.:	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**STDEV**	**RSD**	***Limits***
Standard Area:	908913	909413	910417	911958	912126	912422	910875	1505	0.2	RSD NMT 2.0%
Theoretical Plates	10539	10560	10536	10522	10531	10540	10538	13	0.1	NLT 2000
USP tailing	1.03	1.03	1.03	1.03	1.03	1.03	1	0	0.0	NMT 2.0
RT	12.404	12.411	12.401	12.939	12.396	12.398	12	0	1.8	

Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample[11]. The linearity of the test method was performed by plotting a graph between concentration of the test solution on X-axis and response of the corresponding solutions on Y-axis from 50% to 150% of test concentration and calculated the correlation coefficient, it was found to be 0.999.The results are tabulated in **Table No.:4** and the graphs are represented as **Fig No.:9.**

Fig.No.9: Linearity



Limit of detection (LOD) and limit of quantification (LOQ):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy [11].

Calculated the LOD & LOQ, with the calculations obtained from evaluation of the calibration curve of the linearity. LOD and LOQ values are less than the minimum linearity concentration. The calculations and results are tabulated in **Table. No.:3**

Table No.:3

BICAULTAMIDE- LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)				
S.No.	Injection No.	Slope	Y-Intercept	R^2
1	Inj-1	18580.3	4147.3	0.999
2	Inj-2	17965.4	22449.2	0.999
3	Inj-3	18922.7	19907.1	0.998
Average		18489.4667	15501.2000	0.9987
STDEV		485.071	9914.578	0.001
LOD=3.3 x σ/S				
LOD	1.8	ppm		
LOQ=10 x σ/S				
σ = Standard deviation of y-intercepts of regression line				
S= slope of the linearity curve				
LOQ	5.4	ppm		

Bench top stability of standard & test preparation:

Performed the assay of Bicalutamide as per the test method in duplicate and kept the standard and test solutions on the bench top for 48 Hrs. Injected at initial, 24 Hrs and 48 Hrs. Calculated the difference between initial and bench top stability samples for % assay of Bicalutamide for test solutions and similarity factor for standard solutions were found to be within limits. The results are tabulated in

Table No.:5

Table No.:4

Bicaultamide Weighed(mg)	BICAULTAMIDE-LINEARITY			Conc. (µg/mL)	Conc. against std Conc.(50µg/mL)
	Diluted to(mL)	mL	mL		
25	100	2	20	25.00	50
25	100	3	20	37.50	75
25	100	4	20	50.00	100
25	100	6	20	75.00	125
25	100	8	20	100.00	150

BICAULTAMIDE-LINEARITY

Run	% Conc.	Conc. Of Bicaultamide (µg/mL)	Area of Bicaultamide	Slope	Y-intercept	R^2
1	50%	25.00	462058	18580.3	4147.318966	1.000
	75%	37.50	695425			
	100%	50.00	945000			
	125%	75.00	1407500			
	150%	100.00	1852595			
2	50%	25.00	475025	17965.4	22449.23276	1.000
	75%	37.50	685425			
	100%	50.00	934502			
	125%	75.00	1358792			
	150%	100.00	1823562			
3	50%	25.00	442535	1865423.0	-19907.08621	0.999
	75%	37.50	684525			
	100%	50.00	935465			
	125%	75.00	1425623			
	150%	100.00	1852595			
Average				633989.5811	2229.821839	0.999
Standard Deviation				1066452.67	21243.16	0.00
Acceptance criteria: Coefficient of correlation shall be NLT 0.999						

Table No.:5

BICAULTAMIDE BENCH TOP STABILITY OF STANDARD SOLUTION						
Time(Hrs)	Day	Std. Wt.	Response	Fresh Std Wt.	Response of fresh std.	Similarity Factor
Initial	Initial	10.12	939200			
24 Hrs	Day-1	10.12	938458	10.16	942345	1
48 Hrs	Day-2	10.12	935245	10.18	940125	0.99
BICAULTAMIDE BENCH TOP STABILITY OF TEST SOLUTION-1						

Time(Hrs)	Day	Weight (mg)	Response of sample	% Assay	Difference from Initial	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
Initial	Initial	65.45	932458	99.6	NA	
24 Hrs	Day-1	65.45	925489	98.9	0.7	
48 Hrs	Day-2	65.45	928345	99.6	0.0	
BICAULTAMIDE BENCH TOP STABILITY OF TEST SOLUTION-2						
Time(Hrs)	Day	Weight (mg)	Response of sample	% Assay	Difference from Initial	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
Initial	Initial	65.85	938900	100.3	NA	
24 Hrs	Day-1	65.85	940238	100.5	-0.2	
48 Hrs	Day-2	65.85	939455	100.8	-0.5	

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found[11]. Performed the accuracy of test method using bicalutamide placebo at 50%, 100%, 150% spike levels. The % assay at each spike level was found to be between 95.0-105.0% of the labeled amount. The results are tabulated in **Table No:6**

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility [11].

Method precision:

Determined the precision of the test method by preparing & injecting 6 test solutions of Bicalutamide formulations in to the chromatograph and recorded the results. The average % assay was found to be 100.4 with % RSD of 0.62. The results are tabulated in **Table No.:7**

Intermediate precision:

Performed the assay of Bicalutamide by following the same procedure as that of Method precision but on a different day and by a different analyst. The average % assay was found to be 99.4% with % RSD of 0.39.Overall RSD when compared with Method precision is 0.73. The results are tabulated in **Table No.:8&9**

Table No.:6

Standard Preparation	10	mg	1	Potency	99.8
	10		20		
Sample Preparation	Wt. of sample taken in mg		12.5	Label Claim	50
	200		200		
Standard Area		930456	Average Wt. in mg		65
BICAULTAMIDE-ACCURACY					
Spike level	Wt. of sample taken in mg		Sample area	% Recovery	Average

50%_01	32.45	473422	101.7	103.1
50%_02	31.56	472143	104.3	
50%_03	31.87	471842	103.2	
100%_01	65.65	944136	100.3	100.5
100%_02	65.02	940752	100.9	
100%_03	65.45	943384	100.5	
150%_01	98.50	1361496	96.4	97.0
150%_02	98.87	1372400	96.8	
150%_03	98.12	1377288	97.9	
Acceptance criteria:% Average recovery shall be between 95.0% -105.0%				

Table No.: 7

BICAULTAMIDE ANALYTICAL METHOD VALIDATION-ASSAY										
Method Parameter			Method Precision							
Std. wt. & Dilution	10.25	1	Tablet Wt.	Spl. wt. & Dilution	Wt. of sample taken	50	1	Label claim (mg)	50	
	10	20	65				20	Potency (%)	99.8	
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample Inj-1	Area of sample Inj-2	Average area of sample	Assay %	Average (%)	STDEV	% RSD
1	940384	1.54	65.56	895732	906209	900971	96.41	97.05	1.57970	1.6
2	945541	1.54	65.68	889977	891619	890798	95.15			
3	949321	1.54	65.64	889659	896773	893216	95.47			
4	951150	1.54	65.62	921865	922029	921947	98.57			
5	952464	1.54	65.34	908506	915701	912104	97.93			
6	954372	1.54	65.75	925844	925032	925438	98.75			
Average	947772	1.54	65.60							
STDEV	4883.26	0.00	% RSD of 6 replicate injections is not more than 2.0							
%RSD	0.52	0.00								

Table No.:8

BICAULTAMIDE ANALYTICAL METHOD VALIDATION-ASSAY										
Method Parameter			**Intermediate Precision**							
Std. wt. & Dilution	10.12	1	Tablet Wt.	Spl. wt. & Dilution	Wt. of sample taken	50	1	Label claim (mg)	50	
	10	20	65				20	Potency (%)	99.8	
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample Inj-1	Area of sample Inj-2	Average area of sample	Assay %	Average (%)	STDEV	% RSD
1	931053	1.28	65.54	925084	925128	925106	99.10			
2	929777	1.28	65.87	910174	920401	915288	97.56			
3	946095	1.29	65.32	921362	932548	926955	99.63			
4	932566	1.29	65.32	931254	909582	920418	98.93	98.92	0.85534	0.86
5	935677	1.32	65.32	931198	927870	929534	99.91			
6	940548	1.29	65.32	915530	915268	915399	98.39			
Average	935034	1.29	65.45							
STDEV	6563.81	0.01	% RSD of 6 replicate injections is not more than 2							
%RSD	0.70	1.14								

Table No.:9

BICAULTAMIDE ANALYTICAL METHOD VALIDATION-ASSAY							
Method Parameter		Method & Intermediate Precision combinedly					
Method Precision		Intermediate Precision					
S.No.	% Drug content	S. No.	% Drug content	Difference	Average of both Method & Intermediate precision	STDEV of both Method & Intermediate precision	%RSD of both Method & Intermediate precision
1	96.41	1	99.10	-2.7	98.0	1.558	1.59
2	95.15	2	97.56	-2.4			
3	95.47	3	99.63	-4.2			
4	98.57	4	98.93	-0.4			
5	97.93	5	99.91	-2.0			
6	98.75	6	98.39	0.4			
Limits: Overall RSD when compared with Method precision should be not more than 2%.							

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage [10]. Robustness was performed by injecting the Bicalutamide standard solution in to the UPLC by altering the Flow rate, Column oven temperature and also by changing the pH of the buffer & composition of the organic solvent from the normal chromatographic conditions. The results are tabulated in **Table No.:10**

Calculation:

%Assay:

$$\frac{At}{As} \times \frac{Ws}{10} \times \frac{1}{20} \times \frac{25}{Wt} \times \frac{20}{1} \times \frac{P}{100} \times \frac{100}{L} \times 100 =$$

Where

At=Area of test solution
As=Area of standard solution
Ws=Weight of standard taken
P=Potency of bicalutamide working Std. on as is basis
Avg. Wt. =Avg. Wt. of 20 tablets
LC=Label claim
Wt=Weight of tablets

CONCLUSION

The reported HPLC method was proved to be simple, rapid, specific & reproducible. The validation data indicates good specificity, precision, accuracy & reliability of the method. The developed method has many advantages like isocratic mode of elution, easy sample preparation, and can be used for routine quality control analysis of bicalutamide formulations.

Table No.:2

BICAULTAMIDE SYSTEM SUITABILITY										
Injection No.:	1	2	3	4	5	6	Mean	STDEV	RSD	*Limits*
Standard Area:	908913	909413	910417	911958	912126	912422	910875	1505	0.2	RSD NMT 2.0%
Theoretical Plates	10539	10560	10536	10522	10531	10540	10538	13	0.1	NLT 2000
USP tailing	1.03	1.03	1.03	1.03	1.03	1.03	1	0	0.0	NMT 2.0
RT	12.404	12.411	12.401	12.939	12.396	12.398	12	0	1.8	

Table No.:3

BICAULTAMIDE- LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)				
S.No.	Injection No.	Slope	Y-Intercept	R^2
1	Inj-1	18580.3	4147.3	0.999
2	Inj-2	17965.4	22449.2	0.999
3	Inj-3	18922.7	19907.1	0.998
Average		18489.4667	15501.2000	0.9987

STDEV		485.071	9914.578	0.001
LOD=3.3 x σ/S				
LOD	1.8	ppm		
LOQ=10 x σ/S				
σ = Standard deviation of y-intercepts of regression line				
S= slope of the linearity curve				
LOQ	5.4	ppm		

Table No.:4

Bicaultamide Weighed(mg)	BICAULTAMIDE-LINEARITY Diluted to(mL)	mL	mL	Conc. (µg/mL)	Conc.against std Conc.(50µg/mL)
25	100	2	20	25.00	50
25	100	3	20	37.50	75
25	100	4	20	50.00	100
25	100	6	20	75.00	125
25	100	8	20	100.00	150

BICAULTAMIDE-LINEARITY

Run	% Conc.	Conc. Of Bicaultamide (µg/mL)	Area of Bicaultamide	Slope	Y-intercept	R^2
	50%	25.00	462058			
	75%	37.50	695425			
	100%	50.00	945000	18580.3	4147.318966	1.000
	125%	75.00	1407500			
1	150%	100.00	1852595			
	50%	25.00	475025			
	75%	37.50	685425			
	100%	50.00	934502	17965.4	22449.23276	1.000
	125%	75.00	1358792			
2	150%	100.00	1823562			
	50%	25.00	442535			
	75%	37.50	684525			
	100%	50.00	935465	1865423.0	-19907.08621	0.999
	125%	75.00	1425623			
3	150%	100.00	1852595			
Average				633989.5811	2229.821839	0.999
Standard Deviation				1066452.67	21243.16	0.00
Acceptance criteria: Coefficient of correlation shall be NLT 0.999						

Table No.:5

BICAULTAMIDE BENCH TOP STABILITY OF STANDARD SOLUTION						
Time(Hrs)	Day	Std. Wt.	Response	Fresh Std Wt.	Response of fresh std.	Similarity Factor
Initial	Initial	10.12	939200			
24 Hrs	Day-1	10.12	938458	10.16	942345	1
48 Hrs	Day-2	10.12	935245	10.18	940125	0.99
BICAULTAMIDE BENCH TOP STABILITY OF TEST SOLUTION-1						

Time(Hrs)	Day	Weight (mg)	Response of sample	% Assay	Difference from Initial	
Initial	Initial	65.45	932458	99.6	NA	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
24 Hrs	Day-1	65.45	925489	98.9	0.7	
48 Hrs	Day-2	65.45	928345	99.6	0.0	
BICAULTAMIDE BENCH TOP STABILITY OF TEST SOLUTION-2						
Time(Hrs)	Day	Weight (mg)	Response of sample	% Assay	Difference from Initial	
Initial	Initial	65.85	938900	100.3	NA	Difference in Assay results of Initial,24 & 48 Hrs shall be NMT 2.0
24 Hrs	Day-1	65.85	940238	100.5	-0.2	
48 Hrs	Day-2	65.85	939455	100.8	-0.5	

Table No.:6

Standard Preparation	10	mg	1	Potency	99.8
	10		20		
Sample Preparation	Wt. of sample taken in mg		12.5	Label Claim	50
	200		200		
Standard Area		930456	Average Wt. in mg		65

BICAULTAMIDE-ACCURACY				
Spike level	Wt. of sample taken in mg	Sample area	% Recovery	Average
50%_01	32.45	473422	101.7	103.1
50%_02	31.56	472143	104.3	
50%_03	31.87	471842	103.2	
100%_01	65.65	944136	100.3	100.5
100%_02	65.02	940752	100.9	
100%_03	65.45	943384	100.5	
150%_01	98.50	1361496	96.4	97.0
150%_02	98.87	1372400	96.8	
150%_03	98.12	1377288	97.9	
Acceptance criteria:% Average recovery shall be between 95.0% -105.0%				

Table No.: 7

BICAULTAMIDE ANALYTICAL METHOD VALIDATION-ASSAY										
Method Parameter			Method Precision							
Std. wt. & Dilution	10.25	1	Tablet Wt.	Spl. wt. & Dilution	Wt. of sample taken	50	1	Label claim (mg)	50	
	10	20	65				20	Potency (%)	99.8	
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample Inj-1	Area of sample Inj-2	Average area of sample	Assay %	Average (%)	STDEV	% RSD
1	940384	1.54	65.56	895732	906209	900971	96.41	97.05	1.57970	1.6
2	945541	1.54	65.68	889977	891619	890798	95.15			
3	949321	1.54	65.64	889659	896773	893216	95.47			

4	951150	1.54	65.62	921865	922029	921947	98.57			
5	952464	1.54	65.34	908506	915701	912104	97.93			
6	954372	1.54	65.75	925844	925032	925438	98.75			
Average	947772	1.54	65.60							
STDEV	4883.26	0.00	% RSD of 6 replicate injections is not more than 2.0							
%RSD	0.52	0.00								

Table No.:8

BICAULTAMIDE ANALYTICAL METHOD VALIDATION-ASSAY										
Method Parameter			Intermediate Precision							
Std. wt. & Dilution	10.12	1	Tablet Wt.	Spl. wt. & Dilution	Wt. of sample taken	50	1	Label claim (mg)	50	
	10	20	65				20	Potency (%)	99.8	
Std. No.	Standards	USP Tailing	Weight of sample taken	Area of sample Inj-1	Area of sample Inj-2	Average area of sample	Assay %	Average (%)	STDEV	% RSD
1	931053	1.28	65.54	925084	925128	925106	99.10	98.92	0.85534	0.86
2	929777	1.28	65.87	910174	920401	915288	97.56			
3	946095	1.29	65.32	921362	932548	926955	99.63			
4	932566	1.29	65.32	931254	909582	920418	98.93			
5	935677	1.32	65.32	931198	927870	929534	99.91			
6	940548	1.29	65.32	915530	915268	915399	98.39			
Average	935034	1.29	65.45							
STDEV	6563.81	0.01	% RSD of 6 replicate injections is not more than 2							
%RSD	0.70	1.14								

Table No.:9

BICAULTAMIDE ANALYTICAL METHOD VALIDATION-ASSAY							
Method Parameter		Method & Intermediate Precision combinedly					
Method Precision		Intermediate Precision					
S.No.	% Drug content	S. No.	% Drug content	Difference	Average of both Method & Intermediate precision	STDEV of both Method & Intermediate precision	%RSD of both Method & Intermediate precision
1	96.41	1	99.10	-2.7	98.0	1.558	1.59
2	95.15	2	97.56	-2.4			
3	95.47	3	99.63	-4.2			
4	98.57	4	98.93	-0.4			
5	97.93	5	99.91	-2.0			
6	98.75	6	98.39	0.4			
Limits: Overall RSD when compared with Method precision should be not more than 2%.							

Table No.:10

BICAULTAMIDE ANALYTICAL METHOD VALIDATION-ASSAY					
Method Parameter			Robustness		
Change in Flow Rate (0.8mL/min)			Change in Flow Rate (1.2mL/min)		
Std. No.	Standards	USP Tailing	Std. No.	Standards	USP Tailing
1	1014245	1.31	1	873814	1.28
2	1014707	1.32	2	886672	1.29
3	996485	1.32	3	870171	1.28
4	1020140	1.3	4	888470	1.28
5	992453	1.31	5	905903	1.28
6	995621	1.32	6	897269	1.27
Average	1005609	1.31	Average	887050	1.28
STDEV	12038.21	0.01	STDEV	13580.24	0.01
%RSD	1.20	0.6	%RSD	1.53	0.5
Change in pH of Mobile Phase(2.8)			Change in pH of Mobile Phase(3.2)		
Std. No.	Standards	USP Tailing	Std. No.	Standards	USP Tailing
1	944221	1.23	1	943291	1.22
2	943291	1.24	2	942929	1.22
3	942990	1.24	3	943245	1.24
4	939203	1.24	4	945678	1.24
5	943867	1.24	5	940060	1.24
6	942040	1.24	6	945060	1.22
Average	942602	1.24	Average	943377	1.23
STDEV	1828.43	0.00	STDEV	1967.49	0.01
%RSD	0.19	0.3	%RSD	0.21	0.9
Change in Org Phase Comp (90%)			Change in Org Phase Comp (110%)		
Std. No.	Standards	USP Tailing	Std. No.	Standards	USP Tailing
1	930203	1.29	1	915958	1.31
2	932561	1.29	2	928299	1.31
3	933969	1.28	3	924180	1.32
4	935885	1.26	4	929582	1.31
5	938261	1.27	5	941644	1.32
6	941377	1.27	6	942354	1.32
Average	935376	1.28	Average	930336	1.32
STDEV	4031.33	0.01	STDEV	10214.15	0.01
%RSD	0.43	0.9	%RSD	1.10	0.42

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***Corresponding Author:**
G.NAVEEN KUMAR REDDY
G-4, GARUDADRI TOWERS, BALAJI NAGAR
KUKATPALLY, HYDERABAD-500 072,
ANDHRA PRADESH, INDIA
TEL.:+91 99634 04443
E-Mail :naving29@gmail.com