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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF BICALUTAMIDE IN TABLETS BY RP- HPLC AND QUALITATIVE ANALYSIS OF CEFAZOLIN SODIUM BY DIFFERENT ANALYTICAL TECHNIQUES

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ABSTRACT

An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Bicalutamide in pharmaceutical formulation. In this method Shimadzu technologies Phenomenex (250×4.6mm; particle size 5µm) column with mobile phase consisting of 0.5g of sodium dihydrogen phosphate buffer (pH adjusted to 2.9 with ortho phosphoric acid) and acetonitrile in ratio of 70:30 v/v was used. The detection wavelength is 271nm, flow rate 1.0 ml/min and the retention time was 9.302 min. The linearity was found in the range of 50µg/ml to 250µg/ml and shows a correlation coefficient of 0.998. Cefazolin sodium exhibits antibiotic activity. Analysis of cefazolin sodium was done by different analytical techniques like UV, HPLC. In UV the

absorbance of the solution was observed over a spectral range from 200nm to 400nm using 0.1 M $NaHCO_3$ solution as blank which shows maximum at 257nm. In this gradient method Shimadzu technologies Inertsil (150x4.6mm; particle size 5µm) column with mobile phase consisting of 6.8g potassium dihydrogen ortho phosphate buffer(P^H adjusted to 6.8± 0.05 with sodium hydroxide) and 3.4g of potassium dihydrogen ortho phosphate in water was taken and is mixed with acetonitrile in the ratio of 80:20v/v was used. The detection wavelength is 254nm, column oven temperature is 35°C, flow rate 1.5ml/min, retention time is 35.278 mins. Hence, an optimized, accurate, sensible, precise method was provided by which we can determine Bicalutamide in pure and pharmaceutical formulations in a cost efficient manner by RP-HPLC and analysis of cefazolin sodium was done by UV and HPLC.

Key words: Bicalutamide, HPLC, Validation, Cefazolin sodium, UV

INTRODUCTION

Bicalutamide (casodex) is a non – steroidal antiandrogen **[1]** which inhibits the action of androgen when taken orally for the treatment of D_2 metastatic carcinoma in combination with luteinizing hormone **[2]** and also used for treating skin, hair related condition when taken along with oral contraceptives and high testosterone levels which caused due to polycystic ovary syndrome and in combination with an estrogen is acclimated for feminizing hormone therapy **[3]**.

It is chemically N-[4-cyano-3(triflouromethyl) phenyl]-3-[(4-flourophenyl)sulphonyl)]-2-hydroxy-2-methyl propanamide **[4]** exists in fine white to off – white powder form which is practically insoluble in water and slightly soluble in chloroform and absolute ethanol , niggardly soluble in methanol, soluble in acetone and tetrahydrofuran with a melting point at 193°C **[5]** shows protein binding of 99.6%, elimination half – life of 7 – 10 days, metabolized in liver through hydroxylation and glucuronidation **[3]**. The present investigation mainly focus in developing an accurate, precise, easy, sensible, fast, efficient, analytical method, for determination of Bicalutamide and to reduce run time, cost for analysis by RP-HPLC method and validated approbated to ICH guidelines **[4]**. Cefazolin sodium is classified under the category of a semi synthetic cephalosporin analog in which broad-spectrum antibiotic activity is attained by inhibition of bacterial cell wall synthesis which is chemically [(5-methyl-1,3,4-thiadiazol-2-yl)sulfanyl]methyl-8-oxo-7[(2-(tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylase.

Cefazolin sodium has a molecular formula of $C_{14}H_{13}N_8NaO_4S_3$ which is a semi synthetic antimicrobial agent showing potent bactericidal action against gram-

positive bacteria and moderate action against gram-negative bacteria and microorganisms **[6]**.

MATERIALS AND METHODS

All the glassware's used were of Borosilicate glass and all the solvents used were of HPLC grade and prepared solutions were filtered through water filter 0.45 µm. This research work has performed during the 2019 – 2020 year at Chalapathi institute of pharmaceutical sciences, lam, Guntur, Andhra Pradesh.

Instruments:

U.V.Visible spectrophotometer (Labindia® 3092, UV.Win software, PDA detector), HPLC (Shimadzu, prominence-I, LC-2030C 3D plus, Liquid chromatography, column Phenomenex, (250x4.6mm, 5 µ particle size, software- Lab solutions), Analytical balance (Essae, Teraoka. Pvt. Ltd), P^H meter (Labindia®), Sonicator (Loba Life), Water filter (PISCO 0.45µm Millipore).

Chemicals and Reagents:

Bicalutamide, Cefazolin (sample provided by pharmaceutical industry), Methanol HPLC Grade (sdfcl, sd fine chem. limited. 1502, Marathon Icon, Lower Parel, Mumbai – 400 013). Acetonitrile, purified water, sodium dihydrogen ortho phosphate, phosphoric acid, sodium bicarbonate, potassium dihydrogen ortho phosphate (Thermo Fisher Scientific India Pvt. Ltd. B – Wing, Delphi, Hiranandani Business Park, Powai, Mumbai 400076).

Chromatographic Conditions:

1. Prepare a filtered and degassed mixture of buffer and Acetonitrile in the ratio of 70:30 v/v at pH 2.9 at a flow rate of 1.0 ml/min and Phenomenex column (250×4.6mm, 5µ) was used as stationary phase. The detection wavelength is 271nm.

2. 20mg of Cefazolin sodium was accurately weighed and dissolved in 0.1 M $NaHCO_3$ solution and the volume is made up to the mark with diluent (water). The absorbance of the solution was observed over a spectral range from 200nm to 400nm using 0.1 M NaHCO3 solutions as blank. 6.8g potassium dihydrogen Ortho phosphate was dissolved in 800ml water p^H was adjusted to 6.8 $\pm$0.05 with sodium hydroxide and diluted with water (mobile phase A). 3.4g of potassium dihydrogen ortho phosphate in 500ml water was taken and is mixed with 500ml acetonitrile, it was filtered through 0.45µ filter and degassed (mobile phase B). A gradient method of mobile phase A:B in the ratio of 80:20v/v, at a flow rate of 1.5ml/min and Inertsil column (150x4.6mm; particle size 5µm) was used as stationary phase. The detection wavelength is 254nm.

Method development:

Method development and optimization of chromatographic parameters for the

estimation of Bicalutamide tablets by RP-HPLC and qualitative analysis of Cefazolin by UV, HPLC are discussed below.

Selection of solvent:

1. Solubility studies for Bicalutamide revealed the solubility of drug in methanol. Bicalutamide was insoluble in water. It is soluble in acetone and tetrahydrofuran and slightly soluble in chloroform and absolute ethanol. Several trials were performed to know the suitable solvent system for dissolving the Bicalutamide and finally methanol is selected as correct solvent for conducting analytical procedure.

2. Solubility studies for cefazolin revealed the solubility of drug in water. Very slightly soluble in alcohol. It is soluble in saline TS and in dextrose solution. Several trials were performed to know the suitable solvent system for dissolving the Cefazolin sodium and finally water is selected as correct solvent for conducting analytical procedure.

Selection of detector wavelength:

1. An UV spectrum of 10µg/ml Bicalutamide in 10ml diluents (methanol) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum, wavelength of 271nm was selected. At this wavelength Bicalutamide standard showed good absorbance.

2. An UV spectrum of 20µg/ml cefazolin sodium in 10ml diluents (water) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum, wavelength of 257nm was selected. At this wavelength Cefazolin standard showed maximum absorbance.

Preparation of standard solution:

1.10mg of Bicalutamide was accurately weighed into 10ml volumetric flask and volume was made up to the mark with mobile phase. Solution was cooled to room temperature. 0.5ml of above solution transferred into 10ml volumetric flask and diluted to volume with methanol.

2. 20mg of Cefazolin sodium was accurately weighed into 10ml volumetric flask and the volume was made up to the mark with mobile phase. Solution was cooled to room temperature. 0.5ml of above solution transferred into 10ml volumetric flask and diluted to volume with water.

Preparation of calibration curve:

From the above prepared Bicalutamide stock solution, appropriate dilutions were prepared to get the final concentrations of 50, 100, 150, 200, 250, µg/ml and absorbance was taken at (λ) max 271nm. Average of such five sets of values was taken for standard calibration plot, and the calibration curve was plotted. Calibration curve was done by plotting Bicalutamide concentration on X-axis and their respective absorbance's on Y-axis.

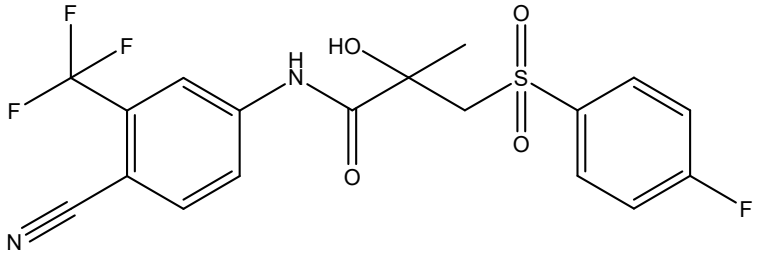

Figure 1: Chemical structure of Bicalutamide

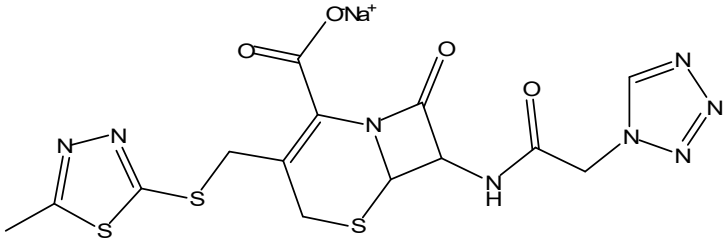

Figure 2: Chemical structure of Cefazolin Sodium

Method validation:

Validation is a process which was performed to provide a high rate of assurance. It is documented evidence that will consistently produce meeting, its predetermined specifications and quality attributes. The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. The method was validated for parameters such as system suitability, precision, accuracy, specificity, linearity, ruggedness, robustness, LOD, LOQ.

System suitability:

The system suitability studies were performed using done 60mg of standard drug. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable. The system suitability studies were done with accurately weighing equivalent to 10mg of Bicalutamide dosage form. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable.

Linearity:

The linearity study was performed for the concentration of 50µg/ml to 250µg/ml level. Each level was injected into chromatographic system. Correlation coefficient was calculated using peak areas. The linearity study was performed the correlation coefficient of Bicalutamide was found to be 0.998 respectively.

Specificity:

The system suitability for specificity was carried out to determine whether there is any interferences of any impurities in retention time of analytical peak. The study was performed by injecting blank. The specificity test was performed for Bicalutamide. It was found that there was no interference of impurities in retention

time of analytical peak. The method show excellent specificity with Bicalutamide eluting at retention of 9.513 minutes. No interference was observed with mobile phase.

Accuracy:

The accuracy study was performed for 50µg/ml, 100µg/ml and 150µg/ml for Bicalutamide. Each level of preparation was injected thrice into chromatographic system. % recovery was calculated using peak areas. The % recovery was found to be 101.6 to 99.70% respectively. (NLT 98% and NMT 102%).

Precision:

- ❖ Repeatability
- ❖ Intermediate Precision

Repeatability:

The precision study was performed for six injections of Bicalutamide. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of %RSD was calculated using peak areas that are obtained by injecting standard preparation.

Intra-day precision:

Intra-day precision was carried out on same day, same HPLC system, using same column at different times. Calculated average area and % RSD for 12 tests.

Acceptance criteria:

Relative standard deviation of % Assay results should not more than 2.0%.

Inter-day precision:

Inter-day precision was carried out on same HPLC system, using same column on another day. The average area was calculated and %RSD for 6 replicate injections of standard drug solutions.

Limit of Detection and Quantification:

Detection Limit:

The Detection Limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily qualitated as an exact value.

Calculation of S/N ratio:

From blank average Baseline signal obtained = 42.43 µ V

From LOD solution signal obtained = 0.00948 µ V

LOD = 3.3×σ /s = 3.3×0.00948/42.43 = 0.000737

Acceptance criteria:

For LOD solution the ratio of S/N shall be 3.

Quantitation Limit:

The lowest amount of analyte which can be determined quantitatively in a sample with suitable precision and accuracy is known to be the Quantitation limit.

Calculation of S/N ratio:

From Blank the average Baseline signal obtained = 42.43 µ V

From LOQ solution signal obtained = 0.00948 µ V

LOD = 10σ/s = 10×0.0098/42.43 = 0.02342

Acceptance criteria:

For LOQ solution the ratio of S/N shall be 10.

RESULTS AND DISCUSSION

A sensible, optimized method was developed for estimation of Bicalutamide in pure and pharmaceutical formulations and validation was done and qualitative analysis of Cefazolin sodium tablets by UV and HPLC was carried out and the results are presented in **Table 9** and **Figure 4-9**.

Table 1: Calibration data of Bicalutamide

S. No.	Concentration (µg/ml)	Absorbance
1	50	40010153
2	100	71974028
3	150	100161386
4	200	140570565
5	250	175034264

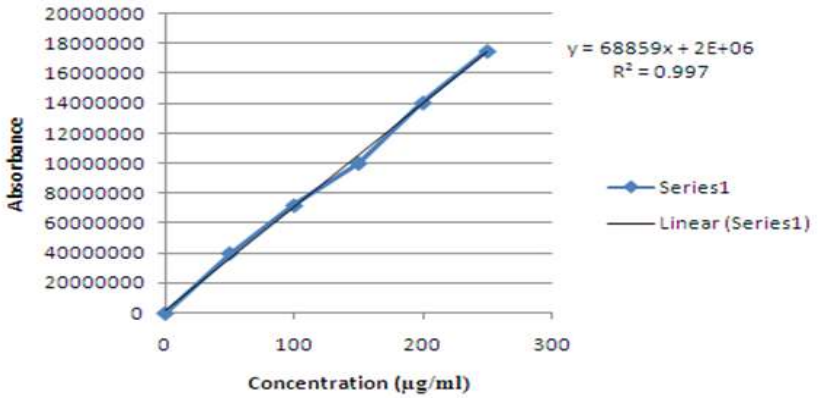

Figure 3: Calibration curve of Bicalutamide

Table 2: Showing results from System suitability

System suitability parameters	Results (average)
%RSD	0.16
Tailing factor	1.12
Plate count	5384
No. of theoretical plates	4890

Table 3: Linearity data of Bicalutamide

S.No.	Linearity Level	Concentration (µg/ml)	Peak area
1	I	50	40010153
2	II	100	71974028
3	III	150	100161386
4	IV	200	140570565
5	V	250	175034264
Correlation Coefficient			0.998

Table 4: Showing results from Specificity

Showing results for Specificity study – Standard preparation					
S. No.	Drug Name	Retention time	Peak area	Theoretical plates	Tailing factor
1	Bicalutamide	9.523	40010153	5267	1.10
Showing results for Specificity study – Sample preparation					
S. No.	Drug name	Retention time	Peak area	Theoretical plates	Tailing factor
1	Bicalutamide	9.513	40010026	5211	1.12

Table 5: Showing result from Accuracy study

Level of recovery	Amount of drug spiked (µg/ml)	Drug recovered	% Recovery	Mean	SD	%RSD
50	9.6	9.62	100.2	100.4	0.346	0.34
		9.62	100.2			
		9.68	100.8			
100	12	12.23	101.9	101.6	0.974	0.95
		12.08	100.6			
		12.31	102.5			
150	14.4	14.26	99.02	99.70	0.6451	0.64
		14.21	99.8			
		14.45	100.3			

Table 6: Showing results from Repeatability, intra-day, inter-day precision

Showing results from precision study – Repeatability				
S. No.	Peak Name			Peak area
1	Bicalutamide			71972014
2	Bicalutamide			71972290
3	Bicalutamide			71971988
4	Bicalutamide			71972209
5	Bicalutamide			71972017
6	Bicalutamide			71972065
Mean	-			71972097
SD	-			1648.33
%RSD	-			0.12
Showing results from precision study – Intraday				
Conc. µg/ml	Peak area			Statistical parameters
50	42728675			Mean:42725146
	42729089			S.d:123.5
	42717674			%RSD:0.34
100	71972209			Mean:71972018
	71972017			S.D:1407.15
	71972069			%RSD:0.06
150	100161405			Mean:100161530
	100161609			S.D:1227.72
	100161576			%RSD:0.06
Showing results from precision study – Interday				
Conc. µg/ml	Peak area			Statistical parameters
	Day-1	Day-2	Day-3	Mean:42725156
50	42719873	42729089	42726690	S.D:3133.3
				%RSD:0.09
100	71972209	719172017	71972069	Mean:71972019
				S.D:1357.0
				%RSD:0.09
150	100161405	100161609	100161576	Mean:100161530
				S.D:1242.28
				%RSD:0.07

Table 7: Showing results from cefazolin sodium standard preparation

S. No.	Name	Retention time	Area
1	Cefazolin Sodium	36.339	491624

Table 8: Showing results from cefazolin sodium sample preparation

S. No.	Name	Retention time	Area
1	Cefazolin Sodium	35.278	45336997

Table 9: Showing results from validation

Validation parameter	Acceptance Criteria	Results
System suitability	The RSD should be NMT 2% for each activity	%RSD is 0.16
Specificity	The interference of the diluents/placebo is considered insignificant, if the chromatogram of the placebo shows no peak, at the retention time of analyte peak	No peaks are eluted at the retention time of Bicalutamide.
System precision	The %RSD of 6 replicate injections should be NMT 2.0%	%RSD is 0.12
Method precision	The %RSD calculated on 6 determinations of assay value should be NMT 2%	%RSD is 0.10
Linearity	The correlation coefficient should be NLT 0.99	0.998
Accuracy	The average recovery is NLT 98% and NMT 102%. Hence, the method is considered to be accurate.	The sample concentrations were injected thrice and the results for accuracy were found to be within the limit.

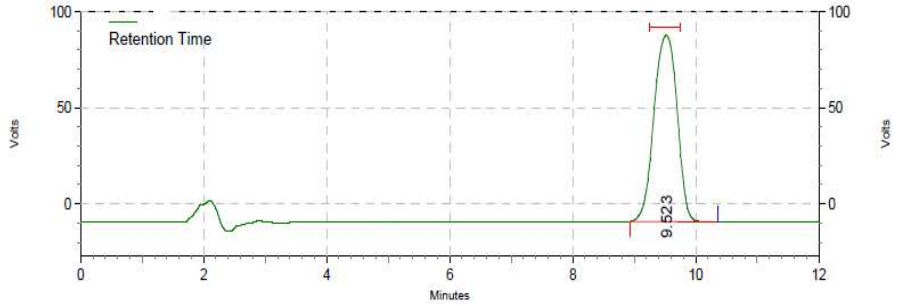

Figure 4: Chromatogram showing bicalutamide standard preparation

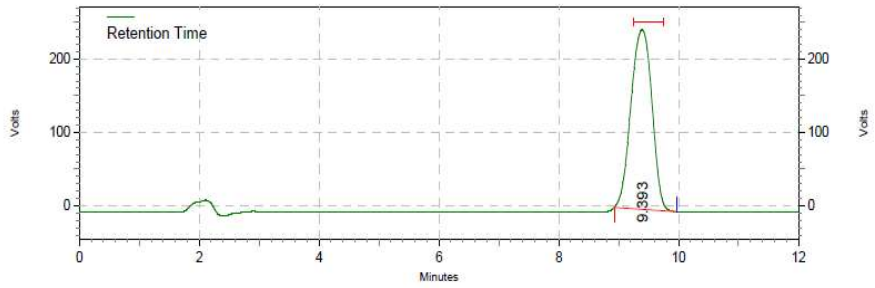

Figure 5: Chromatogram showing bicalutamide sample preparation

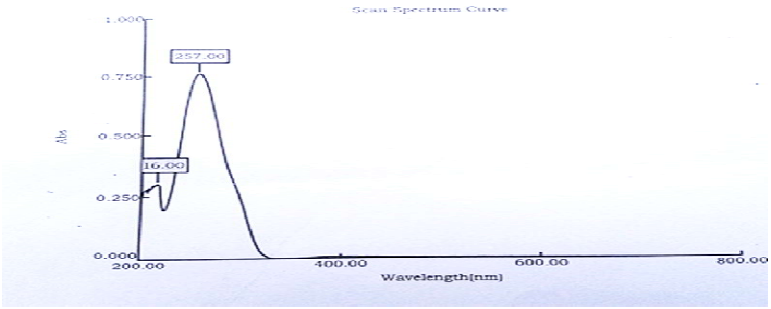

Figure 6: UV spectrum of cefazolin sodium

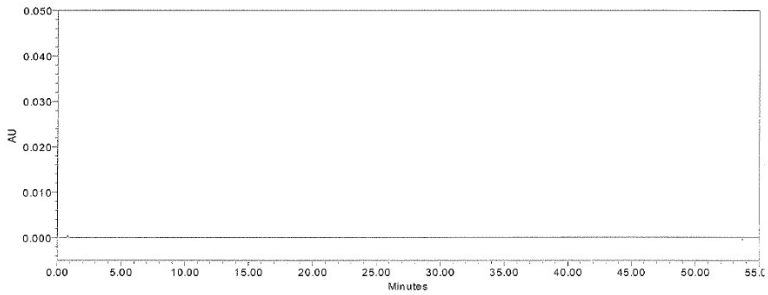

Figure7: Blank chromatogram of cefazolin sodium

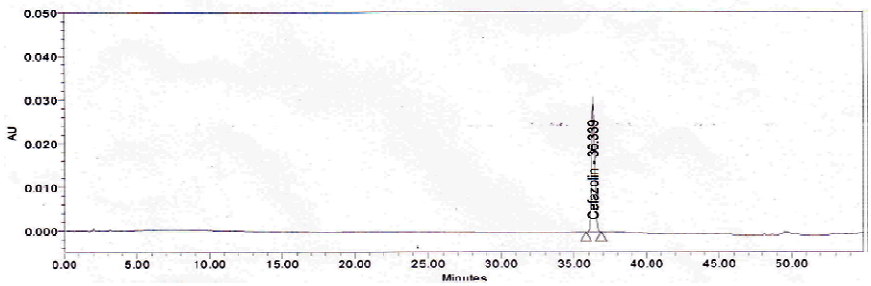

Figure 8: Chromatogram showing cefazolin sodium standard preparation

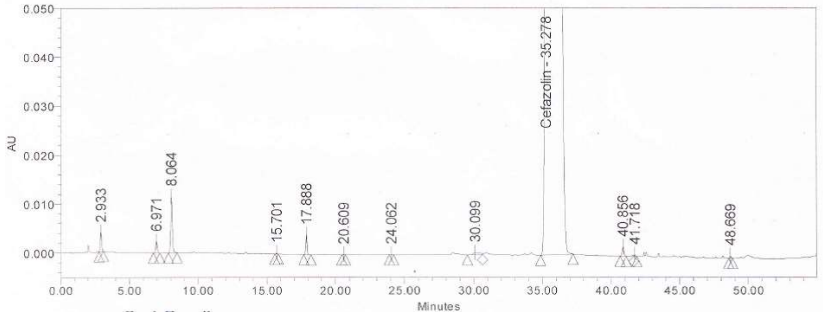

Figure 9: Chromatogram showing cefazolin sodium sample preparation

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tools of analysis. An optimized method was developed for determination of Bicalutamide in tablets by RP-HPLC. The mobile phase used consists of Buffer containing sodium dihydrogen phosphate and mobile phase ratio of sodium dihydrogen phosphate (p^H 2.9): acetonitrile. Phenomenex C_{18} (250×4.6mm, 5µ particle size) column was used as the stationary phase. The detection was carried out using UV detector at 271nm. The solutions are chromatographed at a constant flow rate of 1.0ml/min. The retention time for Bicalutamide was around 9.5min.The quantitative estimation was carried out on the capsule using RP HPLC. Quantitative results are processed by statistical validation. The values of %RSD are less than 2.0%, indicating the accuracy and precision of the method. The percentage recoveries were found to be 101.6% for Bicalutamide. Qualitative analysis of Cefazolin tablets was performed by using UV, HPLC techniques. From analytical and statistical data obtained the methods could

be successfully applied to identify Cefazolin Sodium by means of these analytical techniques (UV, HPLC). These methods can also be used for quantitative estimation of Cefazolin Sodium by UV, HPLC.

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