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Method development and validation of Enzalutamide pure drug substance by using liquid chromatographic technique

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ABSTRACT

A Simple, Precise, Accurate and rapid liquid chromatography (RP-HPLC) method has been improved for the estimation of Enzalutamide drug substance by utilizing Zorbax SB phenyl (250mm× 4.6mm, 3µ) column. The Isocratic mode of elution was dev1+eloped. The mobile phase A was 5.0 grams of Ammonium Acetate in water and the pH 4.2 was adjusted with Trifluoracetic acid and the mobile phase B was Acetonitrile. The diluent was Acetonitrile. The mobile phase was set up in the proportion of (45:55 v/v) (Mobile phase A & B) by maintaining a flow rate of 1.0ml per minute at ambient temperature. The wavelength was fixed at 280nm in UV-Vis detector. The Retention time of Enzalutamide was 7.853min. The method was linear over the concentration range of 0.5 to 1.2mg/ml. The percentage mean recovery for Enzalutamide was observed to be 98% to 102% and the RSD was observed under 2%. The proposed technique was a new method of analysis for assessment of Enzalutamide drug substance by RP-HPLC method. And the method was observed to be appropriate for the standard examination of Enzalutamide in pure drug substance. The method was carried out based on International Conference on Harmonization (ICH) guidelines.

KEY WORDS: Enzalutamide, Ammonium Acetate, Acetonitrile, Validation, RP-HPLC.

1. INTRODUCTION

Enzalutamide (fig.1) is chemically 4-(3-(4-cyano-3-(trifluoromethyl) Phenyl)-5, 5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluro-N-methylbenzamide. The therapeutic indication of EZA is treatment of adult men for metastatic castration resistant prostate cancer (mCRPC) patients who undergone docetaxel therapy. EZA is non-steroidal androgen inhibitor, acts by binding to the ligand-binding domain of androgen receptor (AR) and diminished nuclear translocation, DNA binding and recruitment of AR co-activators also suppress prostate cancer cell growth by activating the TGF-ß pathway. It has higher affinity to androgen receptor than original anti-androgens. Phase III clinical trials have shown that Enzalutamide enhances the personal satisfaction of metastatic mutilation safe prostate cancer patients and shows advantages in general survival. Enzalutamide (Xtandi) is currently approved in the treatment of men with mCRPC pre-and post-chemotherapy at the recommended dose of 160mg once daily. The Enzalutamide and its reactive metabolites are highly protein bound (97-98% and 95% respectively)

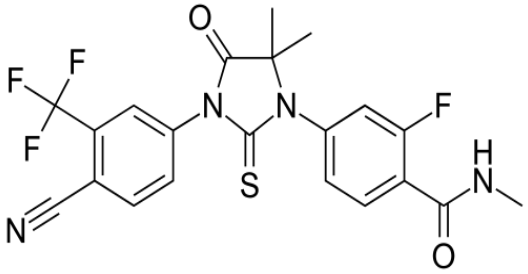

Figure.1. Chemical strucure of Enzalutamide

Literature survey reveals that there are few analytical methods were reported for the estimation of Enzalutamide drug substance. HPLC-UV, HPLC and LC-MS/MS analytical methods are reported for compounds either individually (or) mixed with other dosage form. There is a need of new analytical method development for the analysis of Enzalutamide pure drug substance in accordance with ICH guidelines.

The purpose of current research article was to develop selective, accurate and sensitive method development by using RP-HPLC techniques

2. MATERIALS AND METHOD

Instrumentation: Different types of equipment's viz digital weighing balance (Sartorius Me235S), HPLC system (Agilent 1200 series chrome Leon Dionex corporation Ver 6.80), Column Zorbax SB Phenyl (250×4.6mm, 3µm), Sonicator (Elma E-A126) pH meter (Pica^{+}) Vacuum pump (Wadegati) and other glassware's were used.

Chemicals and Solvents: The working standards of Enzalutamide was gifted from in K.R.R drugs: Hyderabad, India, All HPLC grades of Ammonium Acetate was gotten from Rankem (India). Trifluoracetic acid and purified water was procured from Merck (India) Pvt. Ltd. Acetonitrile was gotten from Fisher Scientific Pvt. Ltd.

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Chromatographic conditions: The isocratic mode of elution was utilized and the mobile phase consisting of Ammonium acetate buffer and Acetonitrile (45:55% V/V). For the filtration purpose 0.45 µm (HPLC grade) nylon filter paper was used. The column Zorbax SB phenyl (250×4.6mm, 3µm) was used for determination. The flow rate was 1.0ml/min and ambient temperature was maintained throughout the process. The sample was injected as volume of 10µl. prior to injection of solutions, column was fixed before 15min with mobile phase was running through the system. The UV detector was set at wavelength of 280nm.

Preparation of Ammonium Acetate buffer: About 5.0g of Ammonium Acetate was weighed accurately and transfer in to 1000ml of water, dissolved it and the pH was maintained to 4.2 with dilute trifluoro acetic acid and filter through 0.45µ nylon filter. The resulting solution was sonicated.

Preparation of Mobile Phase: Mix the above buffer solution of 450ml (45%) and Acetonitrile 550ml HPLC grade (50%) and sonicated for 5min. Filter through 0.45µ filter under vacuum filtration.

Preparation of Standard solution: The qualitative estimation was made for the standard drug and the impurities was found and removed from the drug substance. 50.0mg of Enzalutamide standard was taken in to 50ml of volumetric flask. Dissolve the substance by using diluent and sonicate it. And the volume was filled with diluent. From this solution pipetted out 5.0ml of solution in to 50ml volumetric flask and make up the volume with diluent and stir well. Here the diluent is acetonitrile.

Preparation of Sample solution: About 50.0mg of Enzalutamide sample in to a 50ml volumetric flask. Dissolve the substance by using diluent and sonicate it. And filled up to the mark by using diluent. Further transfer 5.0ml of solution in to 50ml volumetric flask and add diluent up to the mark. Here the diluent is acetonitrile.

Method Development: To set up and validate an accurate method for estimation of Enzalutamide drugs in tablet formulations, primary test was performed. Different chromatographic conditions were estimated for the estimation of Enzalutamide in pure drug dosage form. The pure drug substance of Enzalutamide was injected in to the HPLC system and run by using standard organic solvents. Water, Acetonitrile, Ammonium Acetate, were tested to find the good Conditions for the Separation of Enzalutamide in focus to develop good symmetrical peak. In placed of Water acetate buffer was used and it was observed that buffer, Acetonitrile gave satisfactory results. This mobile phase system was tried with different proportions. Finally, the optimal condition of the mobile phase was chosen as Ammonium Acetate butter (pH 4.2): ACN in the proportion of 45:55% v/v. This composition of the mobile phase was shown the drug very well. All measurements were carried out at ambient temperature of the column. The flow rate was optimizing by using various flow rate conditions were studied. The optimal flow rate was 1.0ml/min for the present work.

Validation of quantitative HPLC method: As per ICH guidelines the analysis was carried out. The parameters assessed were Specificity, Linearity, Precision, Accuracy, Limit of detection (LOD) and Limit of Quantification (LOQ).

Specificity: Specificity of a systematic technique is its capacity to measure precisely and particularly the concentration of analyte with no impedance from different types of diluents. Solvents of standard and sample solutions were injected in to liquid chromatography.

Linearity: For the validation of linearity, a minimum of 5 linearity levels of different concentration of Enzalutamide in the range of 50 to 150% of working level concentration of Enzalutamide in to the HPLC system. Plotting the graph of the peak area responses against the concentration and determine the correlation coefficient (r^2), Y- intercept and % RSD of response factor were shown in table.1.

Accuracy: The percentage of accuracy was 50%, 100%&150% each Level was injected three times. The data was represented in table.2.

Precision: Precision of the method was studied as system precision, Method precision and intermediate precision. The standard deviation and the %RSD were mentioned in table.3.

LOD and LOQ: The detection and quantification limits for the Enzalutamide was performed and calculated by using S/N (Signal to Noise) ratio method. The values were tabulated in table.4.

Robustness: The robustness study was carried out by change in the composition of mobile phase concentration and change in the pH was made to evaluate the impact on the method. The method was robust by change in mobile phase composition and pH change.

System suitability: System suitability test was conducted by using standard stock solution of Enzalutamide. And was injected five times in to HPLC system and the values were recorded.

3. RESULTS AND DISCUSSION

Enzalutamide can be effectively analyzed by the RP-HPLC method with Ammonium acetate buffer (pH:4.2): Acetonitrile (45:55v/v) at wavelength of 280nm. The R_t of drug was obtained to be 7.853min. The total time of analysis was less than 15min.

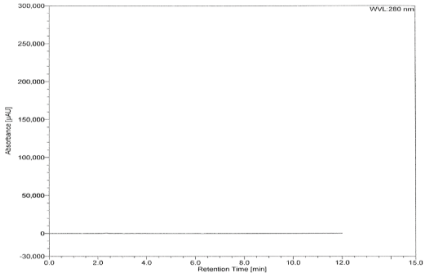

Figure.2. Chromatogram showing blank

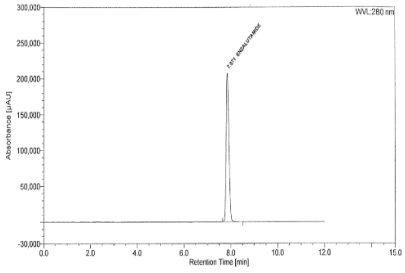

Figure.3. Chromatogram of Standard injection

Standard Chromatogram Values

S. No	Peak Name	R_t	Area	Height	USP Plate Count	USP Tailing
1	Enzalutamide	7.871	1693252	207590	21409	1.0

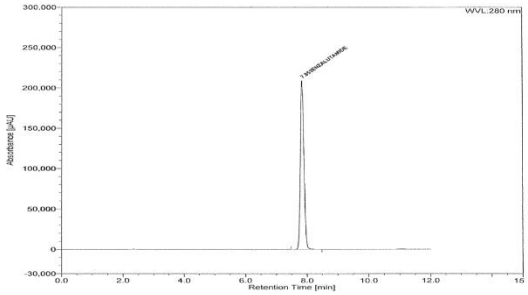

Figure.4. Chromatogram of Sample injection

Sample Chromatogram Values

S. No	Peak Name	R_t	Area	Height	USP Plate Count	USP Tailing
1	ENZ	7.853	1707474	209194	21570	1.1

From the specificity of the method, it was observed that there is no impedance of different substances in the R_t of the analytical peak. The theoretical plates were 21570. The tailing factor was 1.1 within the limit.

The linearity study was performed for the concentration range of 500-1500ppm of Enzalutamide and the correlation coefficient was obtained to be 1.0.

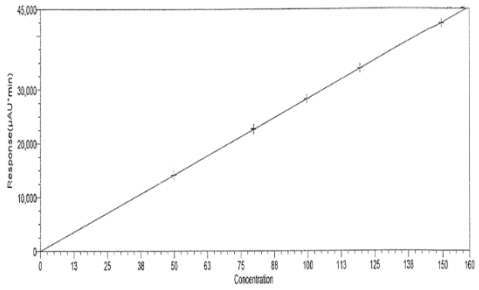

Figure.5. Calibration graph for Enzalutamide

Table.1. Linearity results for Enzalutamide from level -I to level-V

S. No	Linearity Level	Concentration	Area
1	I	50% (500ppm)	849824
2	II	80% (800ppm)	1362540
3	III	100% (1000ppm)	1697419
4	IV	120% (1200ppm)	2035321
5	V	150% (1500ppm)	2537797
Correlation Coefficient			1.000

The accuracy study was performed for % recovery of Enzalutamide at 50%, 100% and 150%. The percentage recovery for Enzalutamide is 100.3% the % recovery at each level and mean recovery should be 98-102%. The Accuracy results were tabulated in the table.2.

Table.2. Accuracy results for Enzalutamide

% Concentration (at specification level)	% Recovery	Mean recovery
50%	99.6 %	99.8%
	98.7 %	
	101.3 %	
100%	101.7%	100.6%
	98.6%	
	101.5 %	
150%	100.7%	100.4%
	98.9%	
	101.8%	

The precision study %RSD was resulted as under 1%. For Enzalutamide, the system precision indicates that the system has good reproducibility. In the method precision study %RSD was observed as 0.3%, which indicates that the method has good repeatability. In the intermediate system precision study, %RSD was observed as 0.6%. For Enzalutamide which indicates that the system has good reproducibility. The precision of results was shown in table.3.

Table.3. Precision studies for Enzalutamide

Injection	System Precision	Intermediate Precision	Method Precision
	Area (Enzalutamide)	Area (Enzalutamide)	Area (Enzalutamide)
Injection1	1693252	1716147	1707474
2	1692923	1723534	1707162
3	1692639	1730706	1711838
4	1692806	1720512	1704012
5	1690136	1715671	1703474
Average	1692351	1721314	1706792
SD	1258.4	0.587	0.337
% RSD	0.1%	0.6%	0.3%

The results of LOD was found to be 0.03 for Enzalutamide and LOQ Was found to be 0.05

Table.4. LOD and LOQ values of Enzalutamide

Drug name	LOD	LOQ
Enzalutamide	0.03	0.05

Robustness of the sample was prepared and run by changing the variations in mixture of mobile phase affected the method significantly. The pH concentrations were done at ambient temperature for Enzalutamide. The validated method was robust in less concentration of mobile phase composition.

Table.5. Robustness studies for change in organic compound in mobile phase

S. No	Change in organic composition in the mobile phase	Enzalutamide	
		USP plate count	USP Tailing
1	10% less	20473	1.0
2	Actual	21595	1.0
3	10% more	20939	1.1

Similarly, by changing the pH concentration from 4.2 to 4.4 as per the assay method. The method was robust only in low pH concentration.

Table.6. Robustness study for change in pH

S. No	Change in pH concentration	Enzalutamide	
		USP plate count	USP Tailing
1	4.0	21151	1.0
2	Actual	21595	1.0
3	4.4	20664	1.1

The system suitability parameter like theoretical plates, Tailing factor (T) were calculated and were observed to be more than 2000. And the proposed RP-HPLC technique was accurate and precise as presented in the table.7.

Table.7. System suitability Parameters

S. No	**Parameter**	**Enzalutamide**
1	Retention time	7.853
2	Theoretical plates	21570
3	Tailing Factor	1.1

4. SUMMARY AND CONCLUSION

The proposed technique was estimated as to be specific, Precise, Accurate, rapid and economical for estimation of Enzalutamide in pure drug substance. This method was validated as per ICH guidelines. The sample recoveries in all formulations were in good agreement with their respective label claims.

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