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Original Article

Development and Validation of Stability Indicating RP-HPLC Method for Quantitative Estimation of Enzalutamide in Enzalutamide Capsules Dosage Form

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ABSTRACT

Objectives: A precise, accurate and selective stability-indicating reverse phase high performance liquid chromatographic assay method has been developed for the quantitative estimation of Enzalutamide in Enzalutamide capsules dosage form. **Materials and Methods:** The separation was achieved by using a stationary phase Waters X-Bridge Shield RP18 (150 x 4.6 mm, 3.5µ) and the mobile phase consisted of perchloric acid buffer and acetonitrile in the proportion of (20:80 volume/volume). The run velocity was 1.2 mL/min. Enzalutamide was identified using UV detector at the wavelength of 210 nm. Column oven temperature 25°C and sample cooler temperature 25°C and infused quantity 20 µL, run time was 15 min. **Results:** As there is no meddling flanked by blank and placebo at the retention time of Enzalutamide. Degradation study results were shown significant degradation was observed in acid and oxidation (peroxide) stress condition. Hence it can be concluded that Enzalutamide is sensitive to acid and oxidation. To obtain system precision, a study was conducted with six replicate injections. %RSD was estimated from the peak areas of Enzalutamide establish to be 0.55% correspondingly. The relative standard deviation for method exactitude was establish to be 0.55%. The suggested HPLC technique was linear over the range of 100.6-301.8 µg/mL, the correlation coefficient was establish to be 0.9999. The accuracy studies were shown as % recovery for Enzalutamide 50% to150% level. The limit of % recovered revealed is in the assortment of 98 and 102% and the consequences obtained were establish to be within the limits. Hence the technique was establish to be accurate. The solution steadiness of the standard and samples are stable upto 48 hr on a bench top and refrigerator (2-8°C). The method is robust for changes like flow rate and column oven temperature. Performed the filter validation for sample solution 0.45 µm PVDF and 0.45 µm Nylon filterers are suitable for filtration. The method has validated as per ICH guidelines and all the validation parameters are satisfy the ICH Q2 specification acceptance limits. **Conclusion:** The developed method was validated for an assortment of parameters as per ICH guidelines like accuracy, precision, linearity, specificity, system suitability, solution stability and robustness. The consequences obtained were within the acceptance criteria. So, it can be concluded that the urbanized technique is simple, precise, cost-effective, eco-friendly, and safe and can be successfully employed for the routine analysis of Enzalutamide in bulk and pharmaceutical dosage forms.

Keywords: Enzalutamide, Liquid chromatography, Forced degradation, Validation.

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INTRODUCTION

Enzalutamide a androgen receptor antagonist suitable for the treatment of adult men with metastatic castration resistant prostate cancer. It is 4-{3-[4-cyano-3- (trifluoromethyl) phenyl]-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methylbenzamide. Enzalutamide is indicated for the treatment of adult men with metastatic castration-resistant prostate cancer who have received docetaxel therapy, compared with other anti-androgen, it shows reduced expression of androgen receptor dependent genes, decreased growth of prostate cancer cells, induction of cancer cell death and tumor regression. Molecular formula and molecular weight of Enzalutamide are $C_{21}H_{16}F_4N_4O_2S$ and 464.44 g/mol, respectively.[1-3] Enzalutamide is freely soluble in acetonitrile and absolute ethanol and practically insoluble in water.[4-13] The chemical structure of Enzalutamide was shown in Figure 1.

The fiction review discloses that there are no HPLC methods were statemented in major pharmacopoeias like USP, EP, JP and BP. Only a few methods were reported to date for the estimation of

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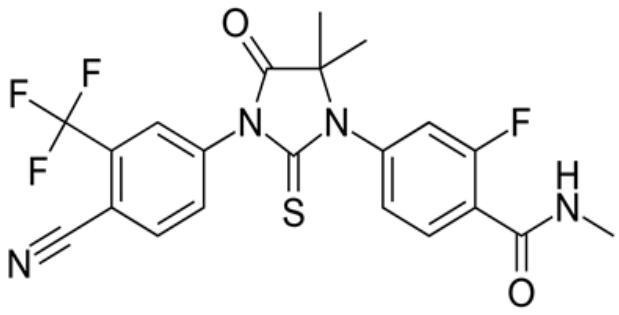

Figure 1: Chemical structure of Enzalutamide.

Enzalutamide by using UV Spectroscopy[14] and high performance liquid chromatography.[15-19] Most of the analytical methods for estimation of Enzalutamide in biological fluid were carried out by LC-MS/MS[20-22] methods were reported for the estimation of Enzalutamide in pharmaceutical dosage forms.

Hence we tried to develop stability indicating the HPLC technique for Enzalutamide in Enzalutamide in capsules dosage form. The present work describes a simple, stability indicating HPLC method for the determination of Enzalutamide in Enzalutamide in capsules dosage form according to ICH guidelines.[23,24]

MATERIALS AND METHODS

Chemicals and Reagents

Analytical-grade Perchloric acid, Acetonitrile, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide and water, reagents and chemicals were procured from Merck Chemicals. Mumbai, India.

Instruments and Equipment

Waters HPLC model: e2695 with DAD, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model) and Analytical Balance (Metller Toledo Model) were used in the present assay.

Method of Analysis

Preparation of perchloric acid buffer solution

Transferred 1.0 mL of Perchloric acid (70%) into a 1000 mL of irrigate and mix well. Filter through 0.45 µm membrane filter and sonicate to degas.

Preparation of mobile phase

Prepared a mixture of 200 mL of perchloric acid buffer and 800 mL of acetonitrile in the ratio of 20:80 (%volume/volume). Filter the solution with 0.45 µm membrane sift and sonicate to degas.

Preparation of diluent

Organized a mixture of 500 mL of irrigate and 500 mL of acetonitrile in the proportion of 50:50 (%volume/volume). Filter the solution with 0.45 µm membrane sift and sonicate to degas.

Preparation of standard solution

Weighed accurately 25.23 mg of Enzalutamide working standard into a 25 mL volumetric flask, added 15 mL acetonitrile, sonicate for 2 min to dissolve, diluted to quantity with diluent and mixed well.

Further diluted 5.0 mL of the above solution into a 50 mL volumetric flask, made up to volume with diluent and mixed well. (Concentration of the standard contains about 100µg/mL of Enzalutamide).

Preparation of test solution

Weighed accurately Enzalutamide 5 whole capsules and transferred into a 100 mL volumetric flask, added 75 mL diluent, sonicated for 30 min with intermediate shaking to dissolve, diluted to volume with diluent and mixed well. Centrifuge the solution at 5000 rpm for 5 min.

Further diluted 5.0 mL of this supernatant solution into a 100 mL volumetric flask, made up to volume with diluent and mixed well. (Concentration of the test solution contains about 100 µg/mL of Enzalutamide).

Preparation of placebo solution

Weighed accurately and transferred placebo powder (equivalent to 200 mg of Enzalutamide) into 100 mL volumetric flask, added 75 mL of diluent and sonicated for 30 min, with intermediate shaking, cool to room temperature and diluted to volume with diluent and mixed well. Centrifuge the solution at 5000 rpm for 5 min.

Further diluted 5.0 mL of this supernatant solution into a 100 mL volumetric flask, made up to volume with diluent and mixed well.

Instrumentation

Chromatographic analysis was performed on Waters X-Bridge Shield RP-18 (150 x 4.6 mm, 3.5µ) mobile phase consisting of perchloric acid buffer and acetonitrile in the ratio of (20:80 volume/volume). The flow rate was 1.2 mL/min, the column oven temperature was 25°C and the sampler cooler temperature was 25°C, the injection volume was 20 µL, and detection was performed at 210 nm using a photodiode array detector (PDA).

RESULTS

Method expansion and Optimization of Chromatographic Conditions

UV-spectroscopic analysis of Enzalutamide drug substance showed utmost UV absorbance (λ_{max}) at 210 nm correspondingly.

To develop a appropriate and robust HPLC technique for the fortitude of Enzalutamide in Enzalutamide in capsules dosage form, dissimilar mobile phases were employed to achieve a good peak shape. The technique expansion was started with Inertsil

ODS-3V (150x4.6mm, 3.5µm) with the following dissimilar mobile phase compositions similar to 0.1% orthophosphoric acid buffer and acetonitrile in the proportion of 85:15 volume/volume. It was examined that when Enzalutamide was infused, higher retention time and peak tailing were not satisfactory. The column stationary phase was not appropriate for the component. For the after that experiment alter the column as of Inertsil ODS-3V to Hypersil BDS. The compound Enzalutamide was eluted at void volume and the peak shape was not good. For the next trial change the column from Hypersil BDS to Waters X-Bridge Shield RP-18 (150 x 4.6mm, 3.5µ). The standard Enzalutamide was injected, peak fronting were not satisfactory.

For the next trial the mobile phase consisted of perchloric acid buffer and acetonitrile in the ratio of 20:80 volume/volume respectively, flow rate 1.2 mL/min, column temperature 25°C and sampler cooler maintained 25°C. UV detection was performed at 210 nm. The compound Enzalutamide was eluted at 6.20 min and the zenith shape was establish to be good. The chromatogram of Enzalutamide standard using the anticipated technique is shown in Figure 2 system suitability results of the method are presented in Table 1.

Method validation

The urbanized RP-HPLC technique was extensively authenticated for assay of Enzalutamide in Enzalutamide capsules formulation using the following parameters.

Specificity and System suitability

Blank and Placebo interference

A swot to establish the meddling of blank and placebo were conducted. Diluent and placebo were infused keen on the chromatograph in the distinct above chromatographic circumstances and the blank and placebo chromatograms were recorded. Chromatogram of blank solution Figure 3 showed no zenith at the retention time of Enzalutamide zenith. This indicates that the diluent solution used in sample preparation does not interfere with the inference of Enzalutamide in Enzalutamide capsules dosage form. Similarly chromatogram of the placebo solution Figure 4 showed no peaks at the retention time of Enzalutamide zenith. This indicates that the placebo used in sample preparation does not interfere with the inference of Enzalutamide in Enzalutamide capsules formulation. Similarly chromatogram of the standard and sample solution Figures 5 and 6.

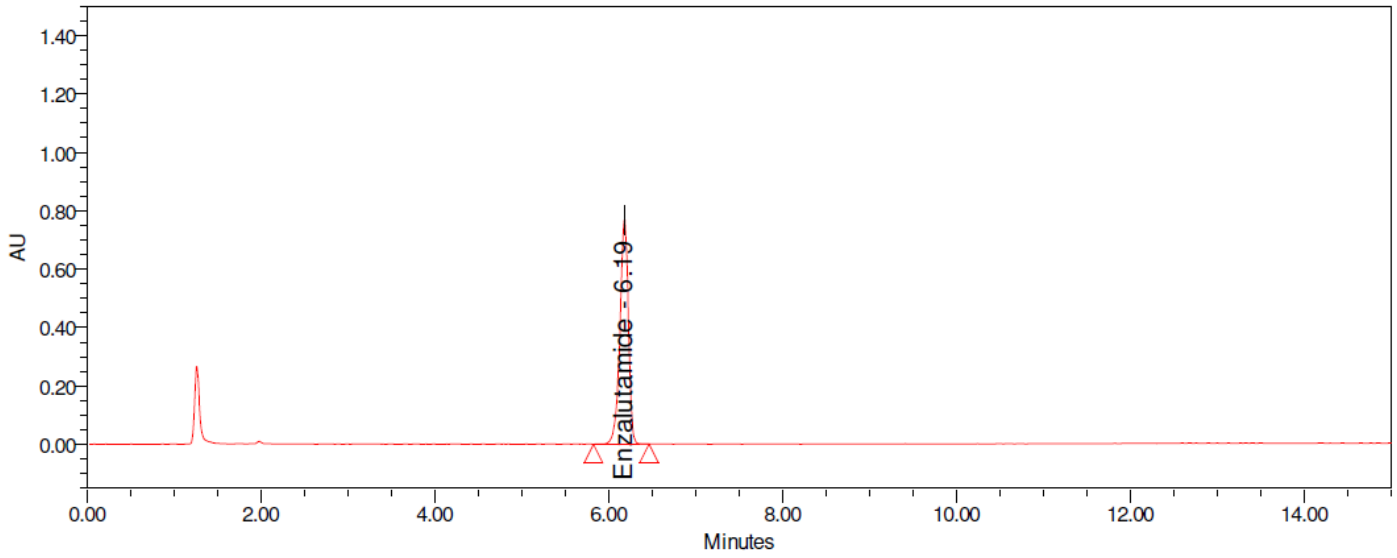

Figure 2: Typical chromatogram of Enzalutamide standard.

Table 1: Specificity results.

Sl. No	Name	Retention Time (min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution	6.19	No	No
4	Sample solution	6.19	No	No

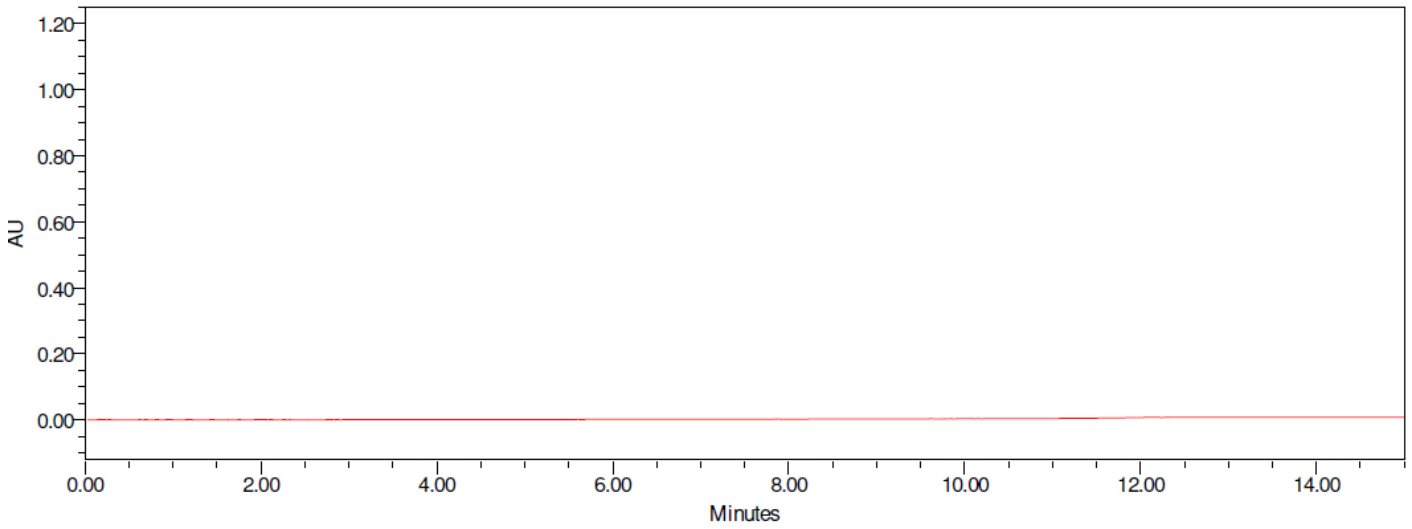

Figure 3: Typical chromatogram blank.

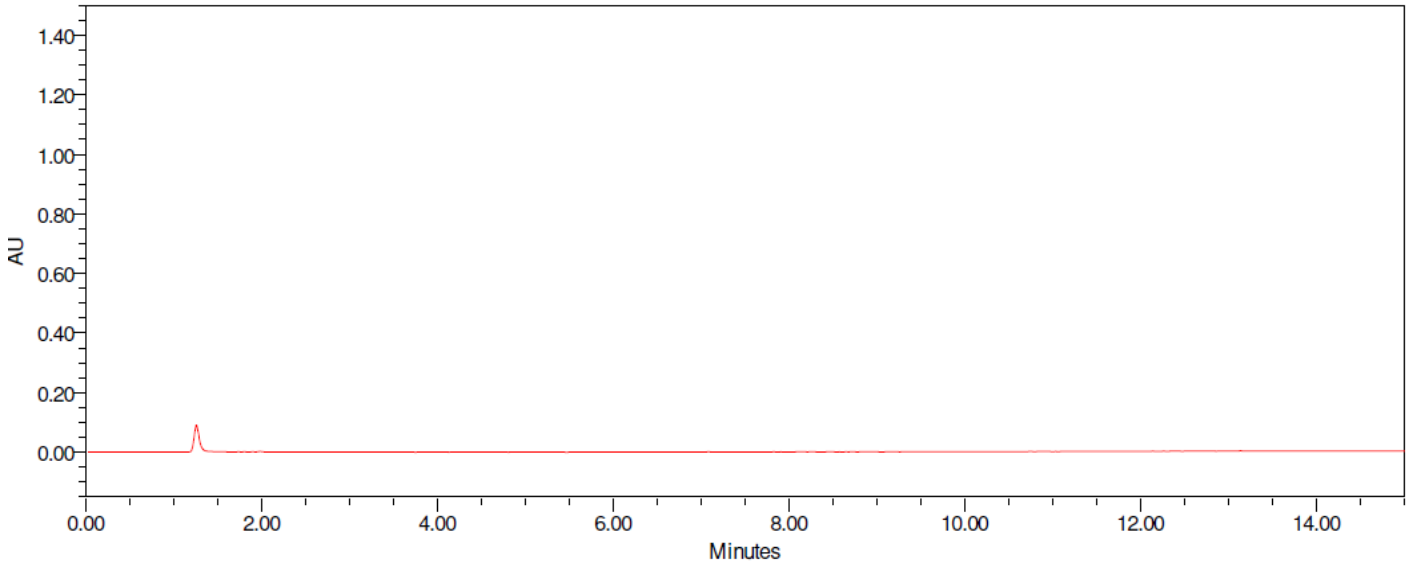

Figure 4: Typical chromatogram placebo.

Force Degradation studies

A swot was carry out to demonstrate the successful parting of degradants/impurities as of Enzalutamide. Separate portions of sample and placebo solutions were exposed to the following stress conditions to induce degradation. Stressed and unstressed samples were injected into the HPLC system with a PDA detector. The degradation study results were presented in Table 2.

Significant degradation was pragmatic in the acid and peroxide stress conditions. Hence it can be finished that Enzalutamide is responsive to acid and oxidation.

System precision

The criterion solution was arranged as per the test technique, infused keen on the HPLC system six times, and calculated the % RSD for the vicinity responses. The statistics were revealed in Table 3.

The relative standard deviation of six replicates criterion solution consequences were establish to be within the specification limit i.e. 0.55%.

Method precision

The exactitude of the test technique was estimated by doing an assay for six samples of Enzalutamide capsules (40 mg) as per the test technique. The content in mg and % label claim for Enzalutamide for each of the test preparation was calculated. The middling content of the six arrangements and % RSD for the six observations were determined. The statistics were revealed in Table 4.

Overall and individual % of Assay are complying as per test technique specification. The relative standard deviation of six assay preparations is 0.55%.

Table 2: Forced degradation results.

Stress condition	Degradation condition	% Assay	% Degradation
As such	Control sample	100.8	NA
Acid	0.1N HCl/60°C/1 Hr	92.1	8.7
Alkali	0.05N NaOH/60°C/1 Hr	97.4	3.4
Oxidative	3% H_2O_2/BT/1 Hr	88.1	12.7
Photolytic	1.2 million Lux hours	99.1	1.7
Photolytic	200 watt hours/m^2 for 7 days	99.6	1.2
Humidity	90%RH Exposed for 2 days	100.2	0.6
Thermal	105°C/1 day	98.2	2.6

Table 3: System precision results.

Sl. No.	No.of injections	Peak area
1	Inj-1	8393937
2	Inj-2	8378601
3	Inj-3	8300775
4	Inj-4	8373873
5	Inj-5	8354810
6	Inj-6	8279808
Average		8346967
STDEV		46122.3604
% RSD		0.55

Table 4: Method precision results.

Sl. No	No. of Preparations	% Assay
1	Preparation 1	100.1
2	Preparation 2	99.8
3	Preparation 3	100.7
4	Preparation 4	101.1
5	Preparation 5	99.9
6	Preparation 6	100.9
Average		100.4
SD		0.5529
%RSD		0.55

Linearity of detector response

The linearity of an analytical technique is its ability to obtain test consequences which has a definite mathematical relation to the attentiveness of the analyte. The linearity of response for Enzalutamide was concluded in the assortment of 50% to 150% (100.6-301.8 µg/mL for Enzalutamide). The calibration arc of the analytical technique was assessed by plotting attentiveness versus acme region and represented graphically. The correlation coefficient [r^2] was establish to be 0.9999. Therefore the HPLC technique was establish to be a linear criterion arc that was estimated and given in Figure 7 to demonstrate the linearity of the anticipated technique. From the data obtained which is given in Table 5 and the technique was establish to be linear within the anticipated assortment.

Accuracy

The accuracy of the test technique was established by preparing revival samples of Enzalutamide at 50% to 150% of the target attentiveness echelon. The revival samples were organized in triplicate preparations on Enzalutamide API spiked to placebo, and analyzed as per the proposed method for each concentration level except 50% and 150 %. The above samples were chromatographed and the percentage revival of each sample was estimated for the amount added. Evaluated the exactitude of the recovery at every level by computing the relative standard deviation of six preparations for 50% and 150% level recovery samples consequences. The statistics achieved which given in Table 6 and the technique was establish to be accurate.

Solution stability of analytical solutions

Solution constancy standards and sample solutions were established at an assortment of circumstances for instance bench top at room temperature and in refrigerator 2-8°C. The constancy of standard and sample solutions was establish by assessment of initially prepared criterion and sample solutions with freshly prepared criterion solutions. The statistics were revealed in Tables 7-11.

Standard and sample solutions are steady for 48 hr when stored at room temperature and 2-8°C.

Robustness studies

To authenticate the technique robustness the chromatographic performance at distorted circumstances was evaluated compared to the nominal conditions of the technique. The criterion solution was infused at each of the following distorted circumstances.

The technique is robust for modify like flow rate, column oven temperature, and the organic phase of the mobile phase.

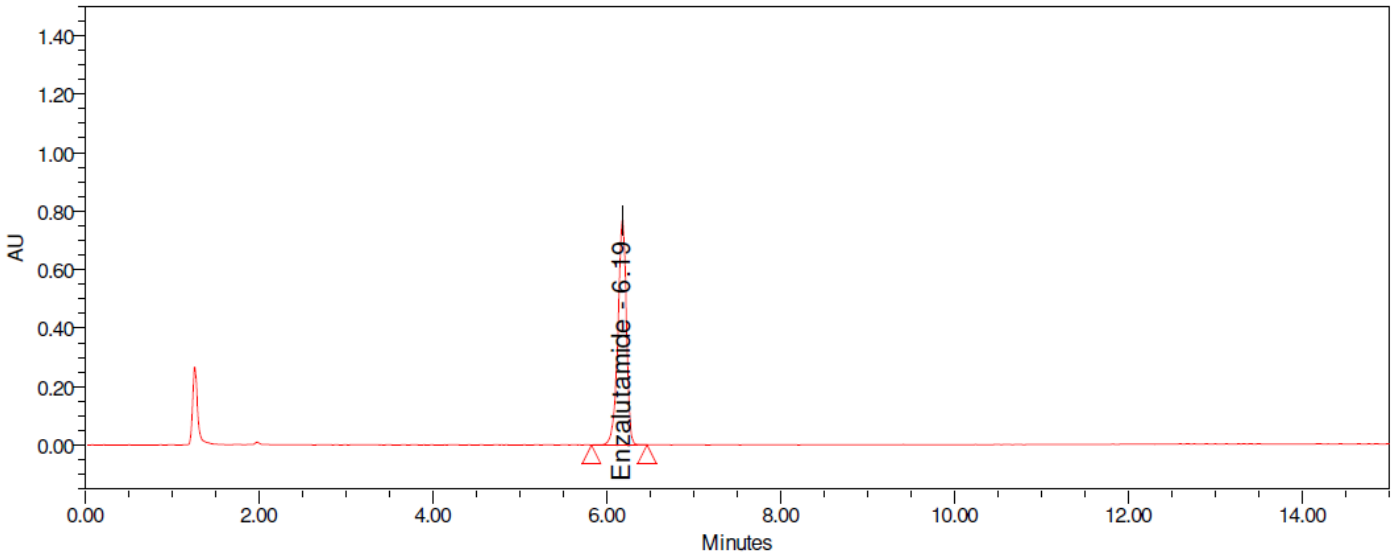

Figure 5: Typical chromatogram standard.

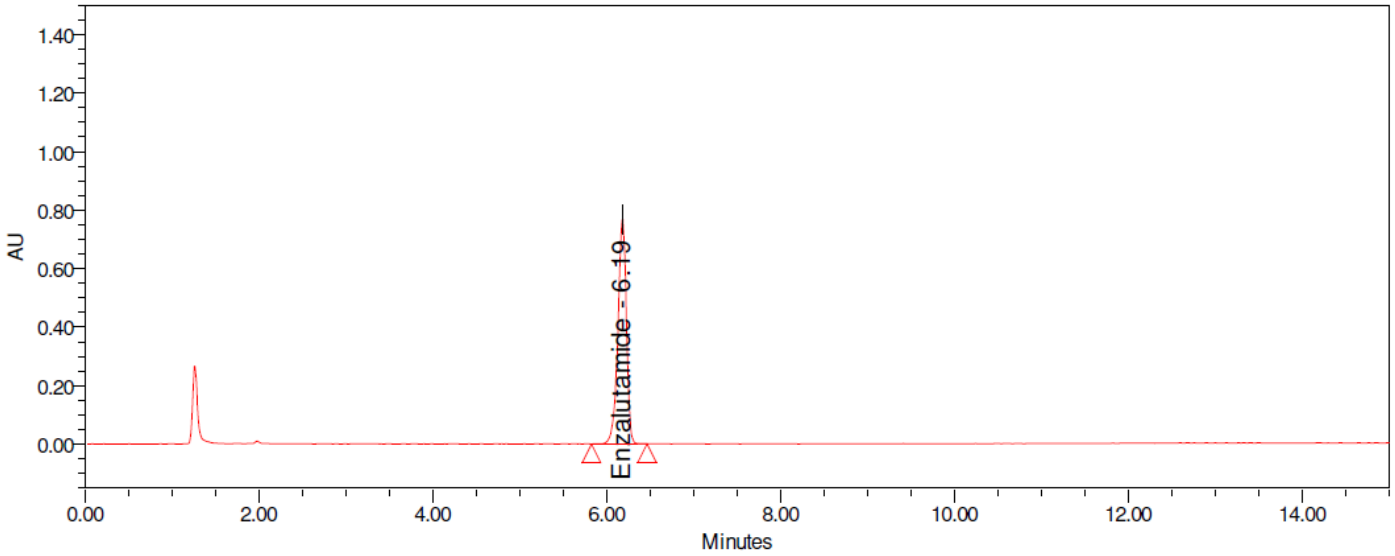

Figure 6: Typical chromatogram sample.

Filter validation

Performed the filter validation for sample solution, one portion of the solution was centrifuged and the other portion of the solution was filtered through 0.45 µm PVDF and 0.45 µm Nylon filters.

Filter validation parameter was established. Based on the above results and observations 0.45 µm PVDF and 0.45 µm Nylon filterers are suitable for filtration.

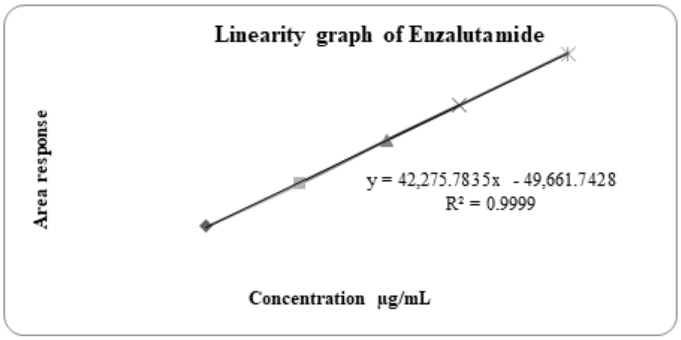

Figure 7: Calibration curve for Enzalutamide.

DISCUSSION

RP-HPLC technique for inference of Enzalutamide in Enzalutamide capsules dosage form was urbanized and authenticated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC technique was urbanized for the inference of Enzalutamide in Enzalutamide capsules dosage form. The optimized technique consists of a mobile phase consisting of perchloric acid buffer and acetonitrile in the proportion of (20:80 volume/volume) with Waters X-Bridge Shield RP-18 (150 x 4.6mm, 3.5µ) column. The retention time

Table 5: Linearity studies for Enzalutamide.

Sl. No	Linearity Level	Concentration (ppm)	Area response
1	Linearity at 50%	100.6	4236485
2	Linearity at 75%	152.912	6354632
3	Linearity at 100%	201.2	8472843
4	Linearity at 120%	241.44	10167478
5	Linearity at 150%	301.8	12709456
Correlation coefficient (r^2)			0.9999
Intercept			-49661.7428
Slope			42275.7835
% Y-intercept			-0.59

Table 6: Recovery studies for Enzalutamide.

% Level	µg added	µg found	% Recovery	Mean % Recovery
50% level-1	50.43	50.14	99.42	100.6
50% level-2	50.15	50.95	101.60	
50% level-3	50.41	50.81	100.79	
100% level-1	100.11	100.22	100.11	100.1
100% level-2	100.89	100.96	100.07	
100% level-3	100.16	100.26	100.10	
150% level-1	150.18	150.44	100.17	100.1
150% level-2	150.47	150.57	100.07	
150% level-3	150.54	150.45	99.94	

Table 7: Results for solution stability of standard.

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24hr	1.03	1.04
48hr	1.13	1.10

Table 8: Results for solution stability of sample at room temperature.

Time Interval	%Assay	%Assay difference
Initial	100.4	NA
24hr	100.0	0.4
48hr	99.4	1.0

Table 9: Results for solution stability of sample in Refrigerator.

Time Interval	%Assay	%Assay difference
Initial	100.4	NA
24hr	100.2	0.2
48hr	99.2	1.2

of Enzalutamide was establish to be 6.2 min. The urbanized technique was authenticated as per ICH Q2A (R1) instruction.

As there is no meddling flanked by blank and placebo at the retention time of Enzalutamide. Degradation swot consequences were revealed significant degradation was scrutinized in acid and oxidation stress circumstances. Hence it can be concluded that Enzalutamide is sensitive to acid and oxidation. To obtain system exactitude, a study was conducted with six replicate injections. %RSD was estimated from the acme areas of Enzalutamide found to be 0.55% correspondingly. The relative standard deviation for technique exactitude was establish to be 0.55%.

The anticipated HPLC technique was linear over the assortment of 100.6-301.8 µg/mL, the correlation coefficient was establish to be 0.9999.

The accuracy studies were exposed as % recovery for Enzalutamide 50% to 150% level. The limit of % recovered shown is in the range of 98% and 102% and the consequences obtained were establish to be within the limits. Hence the technique was found to be accurate.

The solution steadiness of the standard and samples are steady upto 48 hr on a bench top and refrigerator (2-8°C). The technique is robust for changes like flow rate and column oven temperature. Performed the filter validation for sample solution 0.45 µm PVDF and 0.45 µm Nylon filterers are suitable for filtration.

Table 10: Robustness studies Results.

Parameter		Theoretical plates	Tailing factor	%RSD of peak area
Flow variation ± 10%	1.4 mL	14984	0.98	0.11
	1.0 mL	18532	0.99	0.15
Temperature variation ± 5°C	30°C	19782	0.99	0.1
	20°C	18751	0.98	0.13

Table 11: Results for Filter validation.

Sl. No.	Filter details	Area Response	% Assay	The difference when compared to centrifuged
1	Centrifuged Sample	8446754	100.7	NA
2	0.45 µm PVDF Filtered Sample	8439947	100.1	0.6
3	0.45 µm Nylon Filtered Sample	8441124	100.1	0.6

CONCLUSION

The urbanized technique was authenticated for various parameters as stated by ICH instructions like accuracy, precision, linearity, specificity, system suitability, solution stability and robustness. The consequences obtained were within the acceptance criteria. So, it can be concluded that the urbanized technique is simple, precise, cost-effective, eco-friendly, and safe and can be successfully employed for the routine analysis of Enzalutamide in bulk and pharmaceutical dosage forms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

USP: United States Pharmacopeia; **EP:** European Pharmacopoeia; **JP:** Japanese Pharmacopoeia; **BP:** British Pharmacopoeia; **API:** Active pharmaceutical ingredients; **HPLC:** High-Performance Liquid Chromatography; **HPTLC:** High-Performance Thin-Layer Liquid Chromatography; **RT:** Retention Time; **ICH:** International Council on Harmonization; **SD:** Standard Deviation; **RSD:** Relative Standard Deviation.

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