A Validated Stability Indicating RP-HPLC Method Development for Anticancer Drug Enzalutamide in Bulk and Pharmaceuticals

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ABSTRACT

A reproducible stability-indicating Reverse Phase-HPLC technique for the quantification of Enzalutamide in in pharmaceuticals was developed and validated. Chromatography was done on Inertsil ODS-C_{18} 5μm (250 mm × 4.6 mm) analytical column with acetonitrile: methanol: water in 40:30:30% v/v proportion as a mobile phase and 1 ml/min as a flow rate. Enzalutamide was detected at 237 nm UV-wavelength maximum. In the present work mobile phase used as diluents, developed technique was validated over 20-150μg/ml linear concentration range for Enzalutamide. This method established with linearity coefficient value of 0.99 and the percentage recovery was found to be 99.3%. This method was proven with LOD and LOQ findings of 0.53μg/ml and 1.61μg/ml respectively. The drug was degraded in acid and alkaline conditions and the percentage degradation values were 3.10 % and 4.54 % respectively. There was no degradation of drug when exposed to neutral, UV, thermal, sun-light and oxidative conditions. Drug was undergoing degradation when exposed to acid and alkaline conditions. The developed technique was useful in the routine quantitation of Enzalutamide.

Keywords: Enzalutamide, Prostate cancer, RP-HPLC, Stability studies.

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INTRODUCTION

Enzalutamide used in the treatment of prostate cancer, which inhibits the androgen receptor competitively and affects in different stages of signalling paths of cancer. It inhibits the binding of androgen to androgen-receptor, nuclear translocation and consequent interface with DNA. This will results in decreasing the propagation of prostate cancer-cells, which finally leads

to apoptosis and decrease in the tumour-volume. It was chemically designated as 4 -(3 -(4 –cyano-3-(trifluoromethyl) phenyl) -5, 5- dimethyl -4-oxo-2-thioxoimidazolidin-1-yl) -2-fluoro-*N*-methylbenzamide with molecular formula and weight of $C_{21}H_{16}F_4N_4O_2S$ and 464.44 g/mol. [1-4] It is a selective antagonist of androgen receptor (AR) silently and biologically targets the androgens like DHT (dihydrotestosterone) and testosterone. As the first generation Non-selective androgen antagonists (NSAA) bicalutamide, it does not produce AR-translocation to the cell nucleus. In addition to this it inhibits the binding of AR to DNA (deoxyribonucleic acid) and AR-tocoactivator proteins. [5-6] So far, it has been mentioned as an AR-signaling preventer in addition to antagonism. This drug is designated as a "second-generation" NSAA, because it has significantly increased efficiency as an anti-androgen comparative to so called "first-generation" NSAAs like bicalutamide and flutamide. It has only 2 to 3 times lesser attraction for the AR comparative to DHT and the endogenous ligand of AR in prostate gland. [3]

When LNCaP-cells (a cell-line of prostate cancer) bring about to express higher levels of AR (as in the advanced prostate cancer) were cured with this drug, the androgen-dependent gene expression-PSA and TMPRSS was decreased in comparative to bicalutamide where the gene expression was increased. In VCaP-cells which over express the AR, the drug induced apoptosis whereas the bicalutamide drug won't. [7-8] Furthermore, it acts as W741C-mutant AR antagonist in comparative to bicalutamide, which acts as agonist when bind to the W741C-mutant. Literature survey of drug discloses that very few analytical procedures on LC-MS/MS, UV and RP-HPLC were reported for the analysis of pharmaceuticals. [9-12] Hence the present research work is piercing to develop a sensitive, precise, economical, accurate and stability indicating technique for the quantification of Enzalutamide in API and dosage formulations.

MATERIALS AND METHODS
Liquid chromatographic system
Liquid chromatographic system (Shimadzu, Japan) consisted of a binary LC -20A CE pump, solvent degasifying system (DGU-20A), autosampler (SIL-HTC) and temperature controller (CTO-10 AS) for maintaining column temperature was used for the chromatographic elution of drug. Separation was achieved by optimized chromatographic conditions on Inertsil-ODS-C_{18} (250 × 4.6 mm) 5μm. All the chromatograms were processed and integrated using Empower-2 software.
Chemicals and Reagents
Enzalutamide pure drug obtained from Hetero drugs, Hyderabad, India. ACN and methanol of HPLC grade were procured form SD-Fine Chemicals, Mumbai, India. Other chemicals of analytical grade were bought from Qualigens chemicals, Mumbai, India.

Enzalutamide marketed formulations (Xtandi capsules) were obtained from local market for the sample analysis.
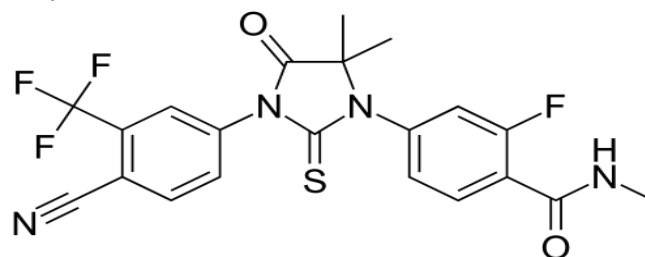

Fig. 1: Chemical structure of Enzalutamide

Mobile Phase preparation
It was processed by mixing HPLC-grade acetonitrile, methanol and water in 40:30:30% v/v proportion. The resultant mobile phase subjected for sonication for 10 to 15 min for degasification and filtered through 0.45 micron filter paper. In the present research work mobile phase were used as a diluent for sample and standard preparations.
Protocol for Standard Solution
Accurately weighed amount of 100 mg of Enzalutamide working standard was transferred to a 100 ml volumetric flask and drug was dissolved in few ml of mobile phase completely, then the final volume was make up to 100 ml using mobile phase (diluent). Further dilutions were made with diluent to get 60μg/ml.
Protocol for Sample Solution
10 capsules were weighed separately and the mean of 10 capsules were calculated and recorded. An accurately weighed amount of Enzalutamide equivalent to 100 mg was transferred and dissolved in 70 ml of diluent by sonicating the solution for 30 minutes in a 100 ml flask. Final volume was made up to the mark with diluent. The resulting solution was filtered and serial dilutions were processed to get 60μg/ml. The resultant solution filtered with the help of a 0.45μ membrane filter.
Liquid Chromatography conditions
Inertsil-ODS-C_{18} (250 × 4.6 mm) 5μm fixed phase was used for the separation at 25°C as column oven temperature. The mobile phase was infused through the fixed phase at 1 ml/min flow rate. In the LC system the infusion volume was 10μL. The photodiode array detection system was set to λmax of 237 nm for the detection and chromatographic run time was 5 minutes.
Validation
The developed and optimized technique was validated in compliance with ICH validation parameters. [13-20]
Precision
Method precision was assessed by infusing the six solutions of standard into the HPLC system and % RSD (relative standard deviation) was calculated.
Specificity
The method specificity was evaluated by comparing the drug solution with placebo solution by infusing

into chromatographic system and the resulting chromatograms observed for the interference of drug response with placebo peak response. [15]

Linearity

Linearity of the analytical method was analyzed by processing series of replicates ranging from 20-150µg/ml and infusing them into HPLC system.

Accuracy

Method accuracy was evaluated in the form of % recovery. The drug solution along with sample was prepared in three variable concentrations of 50.0, 100.0 and 150.0 percentage. Then the percentage recovery was estimated. [13-14]

Ruggedness

Ruggedness was assessed by infusing the 6 standard solutions into chromatographic system for different days. The %RSD was calculated.

Robustness

Robustness of the method was assessed by fluctuating the optimized analytical conditions like mobile phase composition by ± 5%, flow-rate by ± 0.1 ml/min and temperature of the oven by ± 5°C. [17-18]

Solution stability

Stability of solution was assessed by analyzing the standard drug solution after storage for 24 h at laboratory conditions.

Ruggedness

Ruggedness was evaluated by infusing the 6 solutions of standard into HPLC for different days. The %RSD was calculated. [20]

Forced degradation studies

Forced degradation studies were processed with acid, oxidative, alkaline, photolytic, thermal, and ultra violet (UV) degradations on sample. The drug sample was processed by exposing to these stress environments and the peak purity was determined from the resulting chromatograms, which indicates that the technique was effectively separated the degrade components from the standard. [19]

Acid degradation

It was processed by utilizing 0.1 M Hydrochloric acid solution. 10 mg of Enzalutamide pure drug was transferred in to a 10 ml volumetric flask. Drug was made solubilize in 5 ml of 0.1 M Hydrochloric acid and solution was exposed to 80°C temperature in a hot water bath. Samples were processed at different timings such as 0.0 min, 30.0 min, 1.0 h, 2.0 h, and 4.0 h. At variable time intervals, samples were collected and 5.0 ml of methanol was added vortexed for 5 min. From the resulting solution serial dilutions were processed get 60µg/ml with diluent. Finally the resulting solution was filtered through 0.22 micrometer filter paper and infused in to LC-system.

Alkali degradation

It was processed by utilizing 0.1 M sodium hydroxide solution. 10 mg of Enzalutamide pure drug was transferred in to a 10.0 ml volumetric flask. Drug was made solubilize in 5.0 ml of 0.01 M sodium hydroxide, and solution was exposed to 80°C temperature in a hot water bath. Samples were processed at different timings such as 0.0 min, 30.0 min, 1.0 h, 2.0 h, and 4.0 h. At variable time intervals, samples were collected and 5.0 ml of methanol was added vortexed for 5min. From the resulting solution serial dilutions were processed get 60µg/ml with diluent. Finally the resulting solution was filtered through 0.22 micrometer filter paper and infused in to LC-system.

Oxidative degradation

For oxidation, hydrogen peroxide (3%) selected as reagent. 10mg of the standard drug was transferred in to a 10.0 ml volumetric flask. Drug was made solubilize in 5.0 ml of H_2O_2. Resulting solution was kept a side for room temperature. Samples were processed at different timings as mentioned in acid degradation study and prepare 60µg/ml by serial dilution and infused into LC-system.

Neutral degradation

It was processed by utilizing distilled water. 10mg of the standard drug was transferred in to a 10.0 ml volumetric flask. Drug was made solubilize in 5.0 ml of distilled water, and solution was exposed to 80°C temperature in a hot water bath. Then the flask was heated on a water-bath to attain 80°C. Samples were processed at different timings as mentioned in acid degradation study and prepare 60µg/ml by serial dilution and infused into LC-system.

Thermal degradation

It was processed by placing the standard drug at 40°C in an incubator. Samples were collected at definite time intervals. The weighed amount of sample was added to 5 ml of HPLC-grade methanol and vortexed for 5 min. Final volume was made to get 1000µg/ml. From that serial dilutions were made to get 60µg/ml with diluent. It was vortexed and filtered with a 0.22µm filter, and 20µL of sample was infused into LC-system.

UV-degradation

100 mg of pure drug was weighed and transferred to a clean petri-dish. Then the petri-dish was positioned under a UV chamber by maintaining 30 cm distance. The petri-dish cover was removed for degradation. After 3 h, the UV-lamp was switched to off and 10 mg drug sample was taken and 1000µg/ml was processed with diluent, from which 60µg/ml concentration was processed by serial dilution. It was vortexed and filtered through a 0.22µm filter. Twenty microlitres of the sample was infused into LC-system.

Photolytic degradation

For photolysis condition, 100 mg of the pure drug was weighed and transferred to a clean petri-dish and closed. Then the petri-dish was exposed to direct sunlight. At different timings, 10mg of drug sample was taken out. From it, a stock solution of 1000µg/ml was processed with diluent, and 60µg/ml concentration was processed by serial dilution. It was vortexed and filtered through a 0.22µm filter. Twenty micro liters of the sample was infused into LC-system. [13, 19]
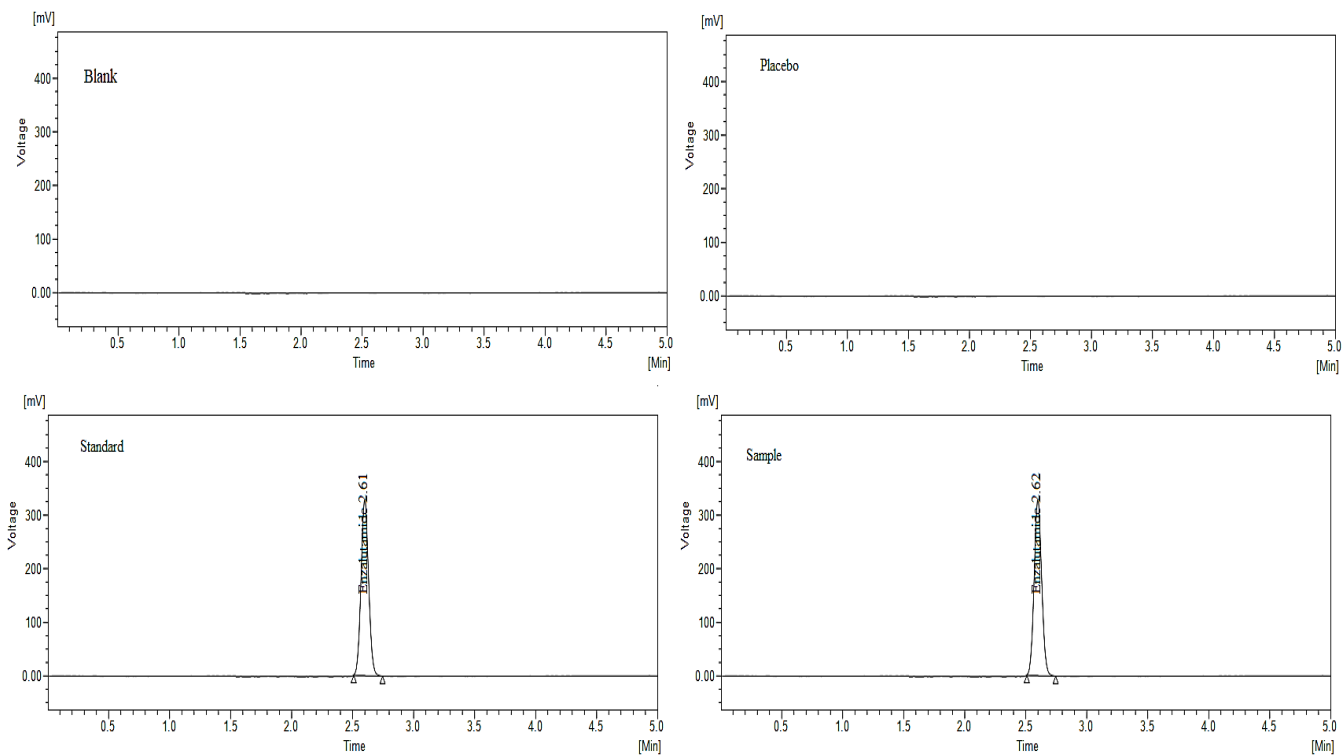
Fig. 2: Chromatograms of Enzalutamide Blank, Placebo, Standard and Sample
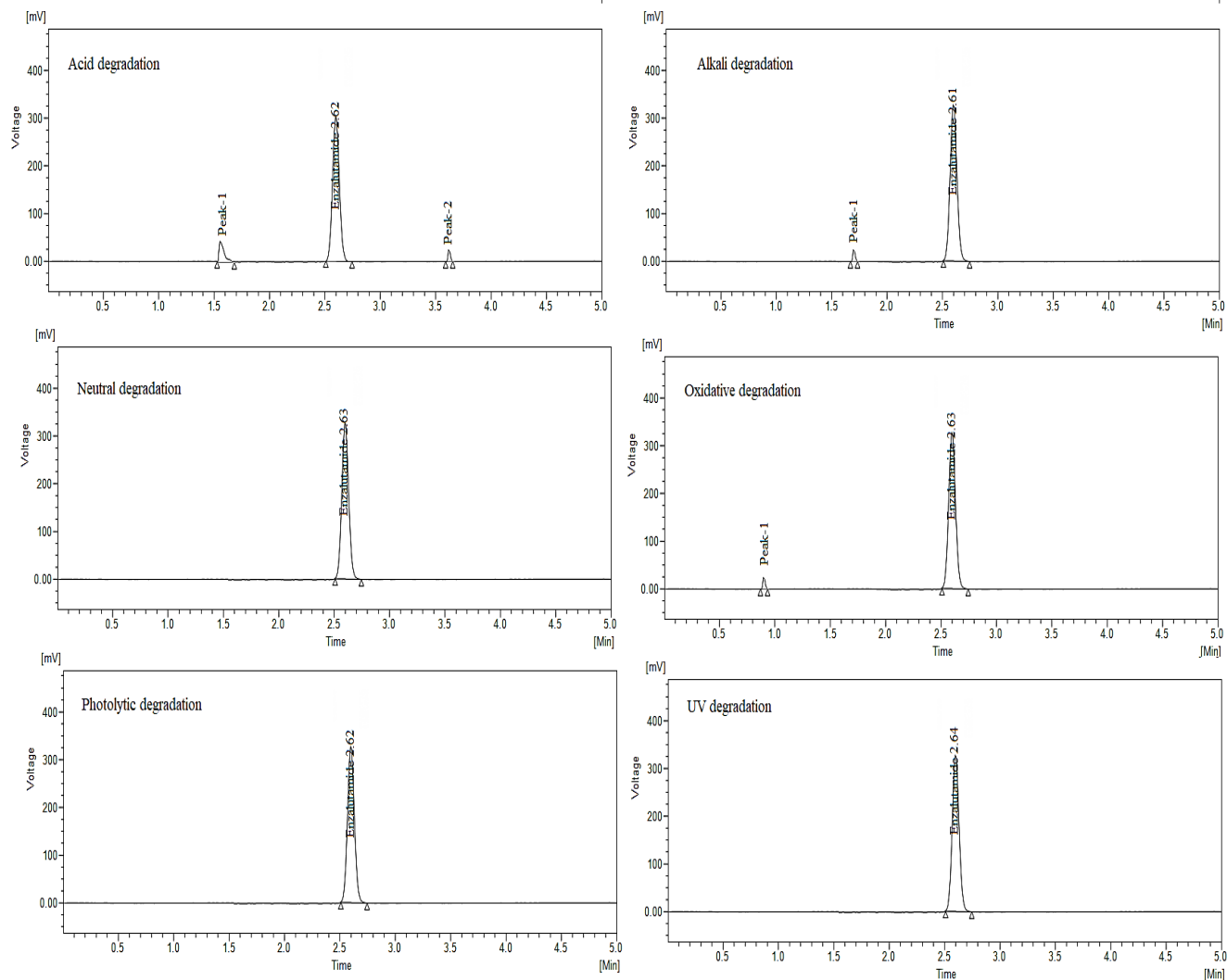

Fig. 3: Chromatograms of enzalutamide forced degradation studies

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Table 1: Validation results for Enzalutamide

Parameter	Result (mean ± SD)	
Precision (%RSD, n)	0.96 (100.08 ± 0.96)	
Specificity	No interference	
Accuracy (% Recovery, n mean±SD)	99.58% ± 0.28 - 99.89% ± 0.19	
Linearity	20-150µg/ml	
Correlation coefficient(r^2)	0.9998	
LOD concentration	0.18µg/ml	
LOQ concentration	0.58µg/ml	
Ruggedness(%RSD, n)	Day 1	Day 2
	0.92	0.82
Robustness (%RSD, n)	Decrease in flow rate 0.8	Increased flow rate 0.18
(Organic phase)	Decrease in mobile phase 0.4	Increased mobile phase (Organic) 0.65
	Decreased column temperature 0.5	Increased column temperature 1.02
Solution stability	Day 1 (0 h)	Day 2 (After 24 h)
(%RSD, n)	0.85	1.25
(% Assay, n)	100.12 ± 0.97	99.85 ± 1.05
USP-tailing	1.36	
USP theoretical plates	3789	

n–number of samples, i.e., six samples for estimation; SD- Standard deviation

Table 2: Forced degradation results for Enzalutamide

Stress condition	% Assay	% area of degradation peak	% Degradation
0.1M HCl for 4 h at 80°C	96.90	2.65	3.10
0.1 M NaOH for 4 h at 80°C	95.46	-	4.54
3% H_2O_2 for 4 h	96.20	-	3.80
Water for 4 h at 80°C	99.50	-	0.50
UV light for 3 h	98.12	-	1.88
(Thermal) 40°C for 6 h	98.34	-	1.66

RESULTS

Method development

Method was optimized after trials with different types of columns, mobile phase composition and flow rate. The optimized method was processed with Inertsil-ODS-C$_{18}$ (250 mm × 4.6 mm) 5µm analytical column with acetonitrile: methanol: water in the proportion of 40:30:30% v/v as mobile phase at 1 ml/min flow rate. Drug was detected at 237 nm UV-wavelength maximum. The chromatograms for the optimized method were shown in the Fig. 2.

Validation

The optimized technique was validated in compliance with ICH validation parameters and the results were shown in Table 1.

Forced degradation studies

Forced degradation studies [13] were processed for the enzalutamide drug by exposing the drug solution to different stress environments such as acidic (0.1 N hydrochloric acid for 4 h at 80°C), basic (0.1 N sodium hydroxide for 4 h at 80°C), peroxide (3% hydrogen peroxide for 4 h at 60°C), neutral (refluxing the drug in water for 4 h at 80°C), photolytic (UV-light for 3 h) and thermal (40°C for 6 h) conditions. All the findings of forced degradation findings were presented in Table 2 and respective chromatograms were shown in Fig. 3.

DISCUSSION

In the present specific RPHPLC-technique, linearity was assessed over the concentration level of 20-150µg/ml, the research work was successfully validated, and the validation constraints were within

the acceptable limits (Table 1 and Fig. 2). In this liquid chromatographic technique, the LOD and LOQ of drug were 0.18µg/ml and 0.58µg/ml, respectively. In present technique, Enzalutamide was exposed to its stress studies under different environments as per the ICH-guidelines. The neutral/hydrolytic degradation study of Enzalutamide reveals that no degradants were observed for 4.0 h in neutral environment. The findings of acid hydrolysis shown that degradation component peaks at 1.59 min and 3.63 min along with drug peak. From the chromatogram it was observed that 3.10% of drug was degraded in 0.1M hydrochloric acid at 80°C. Enzalutamide was degraded in 0.01 M sodium hydroxide at 80.0°C for 4.0 h, and the degradation peak was eluted at 1.72 min in the chromatogram. From the chromatogram the drug percentage degradation was 4.54%. Enzalutamide did not degrade after it was kept under direct sunlight for 21 days. Drug under UV-chamber for 48.0 h it was not underwent degradation, observed from the resulting chromatogram. The chromatogram of oxidative degradation of drug was showed that no degradants were formed after 4 h of exposure. The chromatogram of thermal degradation showed that enzalutamide was not degraded for 15 days at 40°C.

In this research article Stability-indicating Reverse Phase-HPLC technique for Enzalutamide was developed and validated. All the validation parameters: selectivity, accuracy, precision, recovery, robustness, and ruggedness were within the acceptance limit. The drug sample was resolved on Inertsil-ODS-C$_{18}$ (250 mm × 4.6 mm) 5µmanalytical column with acetonitrile: methanol: water in the proportion of 40:30:30% v/v as mobile phase at 1 ml/min flow rate. Enzalutamide was detected at 237 nm UV-wavelength maximum.The drug was degraded in acid and alkaline conditions and the percentage degradation values were 3.10% and 4.54% respectively. There was no degradation of drug when exposed to neutral, UV, thermal, sun-light and oxidative conditions. The drug was successfully estimated by this technique and it was

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useful for laboratories for the routine analysis of Enzalutamide in bulk and pharmaceuticals.

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