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Research Article

A New Stability Indicating UV Method Development And Validation For The Quantification Of Enzalutamide In Bulk And Pharmaceutical Combined Dosage Form

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ABSTRACT

Enzalutamide's bulk and mixed dosage forms may now be quantitatively estimated using a new, sensitive, and straightforward UV approach. Enzalutamide is an androgen-receptor signalling pathway inhibitor as well as a competitive inhibitor of dihydrotestosterone, the active metabolite of testosterone. At 240 nm, the drug's maximum absorption was discovered. Since methanol was identified to dissolve enzalutamide, it was utilized as a solvent to perform the work. The method's ultimate linearity range was determined to be 3-11 µg/ml, with an R2 value of 0.9996. A %RSD of less than 2, which is well within, denotes a strong and precise approach. The 100–101% recovery rate was discovered, demonstrating the accuracy of the procedure. The approach was validated in accordance with the ICH criteria, and good findings were obtained. The values for LOD and LOQ were found to be 0.1288 and 0.390, respectively. Studies on the drug's stability reveal that it is unstable in a variety of stress situations, including oxidation, base, acid and water.

INTRODUCTION

Spectroscopy is a branch of science that studies the interaction between EMR and matter. The basic law that relates absorbance and concentration is the Beer-Lambert law.

Beer-Lambert Law:

Beer-Lambert's law is an essential relationship in spectroscopy that describes the connection between the concentration of a solution and the amount of light absorbed by that solution. The law states that the absorbance (A) of a sample is directly proportional to the concentration (c) of the absorbing species in the sample and the path length of the light through the sample. (1-9) Enzalutamide is a competitive inhibitor of dihydrotestosterone, the active metabolite of testosterone, and an inhibitor of the androgen-receptor signalling pathway. (10)

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Table 1: Drug profile of Enzalutamide

Criteria	Features
IUPAC NAME	4-(3-(4-cyano-3-(trifluoromethyl)phenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl)-2-fluoro-N-methylbenzamide
Molecular Formula	$C_{21}H_{16}F_4N_4O_2S$
Molecular Weight	464.44 g/mol
Appearance	White powder
λ_{max}	210nm
Solubility	Soluble in Methanol, poorly soluble in Water
Melting Point	197-200°C

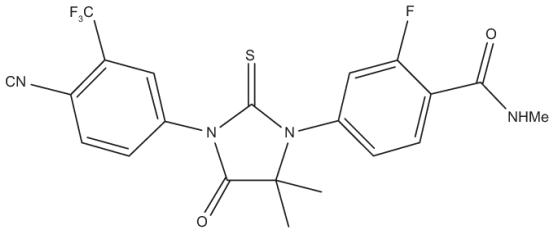

Figure 1: Structure of Enzalutamide

A search through the literature revealed that the drug has been examined with a variety of analytical approaches, notably HPLC, RP-UPLC, UPLC-MS-MS, and LC-tandem mass spectrometry. The development of a basic, precise, reliable, and repetitive Visible Spectrophotometric method for the quantification of Enzalutamide in bulk and in combined dosage form is described in the current work. According to ICH Guidelines, the developed approach was validated. (11-25)

MATERIAL AND METHODS:

Chemicals and reagents:

Instrumentation:

The proposed work was carried out on a UV Spectrophotometer, which is a double beam double detector configuration with a 1 cm quartz cuvette cell. All weighing was done on PGB-200 model weighing balance.

Selection of Solvents:

On the basis of solubility study Methanol was selected as the solvent for dissolving Enzalutamide.

METHOD DEVELOPMENT:

Preparation of Standard Stock Solutions of Enzalutamide:

Stock Solution A (1mg/ml):

Accurately weighed 10mg of Enzalutamide into a 10ml volumetric flask, dissolved in 5ml methanol and made up the volume to 10ml with distilled water.

Stock Solution B (100µg/ml):

1ml of stock solution A was taken in 10ml volumetric flask and added 4ml of methanol and made up the volume with water.

Dilutions:

Further serial dilutions were done by taking 0.3, 0.5, 0.7, 0.9, 1.1ml of stock B and made up the volume with Methanol and water up to 10ml to give concentrations 3,5,7,9,11µg/ml.

Selection of Analytical Wavelength:

An appropriate aliquot portion of 0.7ml from stock solution B was transferred to 10 mL volumetric flasks; the volume was made up to the mark using Methanol and water (7µg/ml working standard). Drug solution was scanned against a Methanol blank between 200 nm to 400 nm range. The drug showed λmax at 240nm.

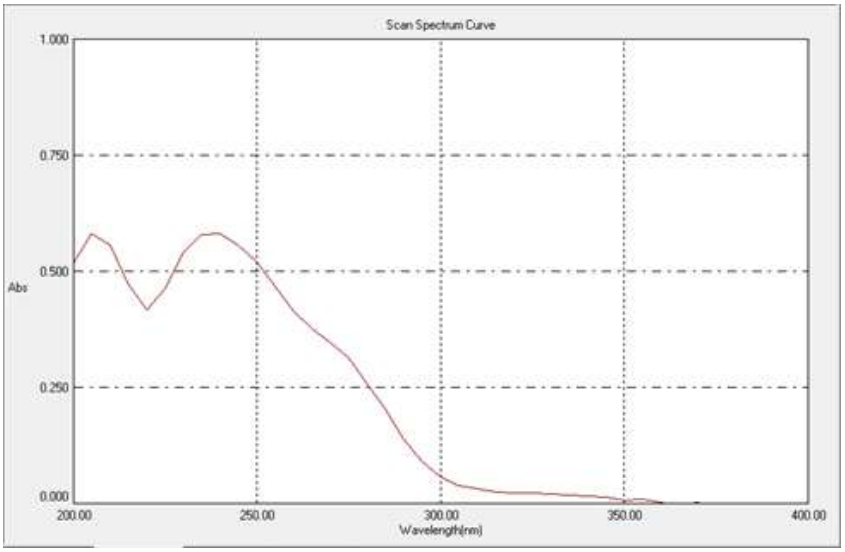

Figure 2: UV spectrum of Enzalutamide (240nm)

METHOD VALIDATION:

The suggested approach has undergone rigorous validation in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness, ruggedness and assay.

Determination of Linearity:

A working standard solution of the drug was divided into five separate 10 mL volumetric flasks using an appropriately measured proportionate fraction. Methanol and water were used to make up the volume to the required level in order to produce concentrations (3–11µg/mL). The absorbance of these solutions was measured at 240nm. The calibration curve was displayed in a concentration versus absorbance graph.

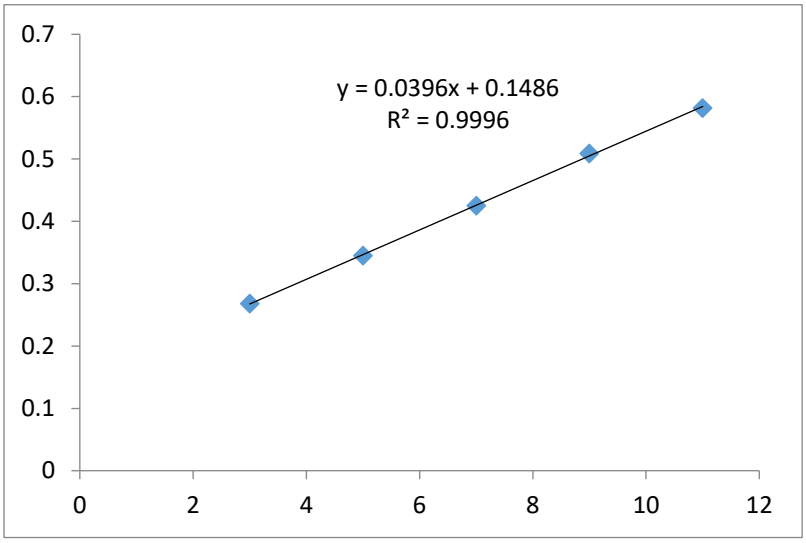

Figure 3: Calibration Curve of Enzalutamide

Accuracy:

On the basis of a recovery, research carried out using the conventional addition approach, the proposed method's accuracy was determined. The tablet powder was re-analysed using the recommended method after being mixed with a known quantity of standard medication solutions to make final concentrations of 50%,100%, and

150%. The absorbance recorded and the % recoveries were calculated.

Precision:

Precision was determined as intra-day and inter-day variations. The results of the intra-day and inter-day precision research were determined by calculating absorbance at the same drug concentrations (7 ppm) three times on the same day and three different days over the course of a week at a wavelength of 240nm. The results were reported.

Ruggedness:

The suggested method's durability was assessed through the analysis of portions from uniform slots by two different analysts under similar operational and environmental circumstances. The results were reported accordingly.

Robustness:

Robustness was obtained by performing the analysis at two different wavelengths (±5 nm). The results were reported.

LOD & LOQ:

LOD & LOQ gives information about the sensitivity of the method. The values reported indicate that the method is highly sensitive.

DEGRADATION STUDIES:

Stock preparation:

Prepared 7µg/ml stock solution by taking 0.7ml ml of stock B solution and dissolving in methanol and water (5:5) in 10ml volumetric flask.

Acid degradation:

Added 1ml of 1N HCl to 1 ml of working standard and made up the volume to 10 ml with solvent and kept the prepared solution aside for 24 hrs.

Alkaline degradation:

To 1 ml of working standard solution, 1 ml of 1N NaOH was added and made up the volume to 10 ml with solvent and kept aside for 24hrs.

Oxidative degradation:

To 1 ml of the working standard solution added 1 ml of H2O2 and made up the volume to 10 ml with solvent.

Degradation by hydrolysis: To 1 ml of the working standard solution added 1 ml of water and made up to the mark with solvent.

Assay:

Table no 2 Assay Results

Formulation	Label claim mg/tab	Amount found Mean ±S.D	Assay	%RSD
Tablet	40mg	99.89 ± 0.0064	99.89	0.0064

RESULTS AND DISCUSSION:

Method Validation:

Linearity:

The calibration curve has a regression coefficient of 0.9996 and displayed linearity in the 3–11 µg/ml range.

Table 3: Linearity of Enzalutamide

Sr. No.	Enzalutamide	
	CONC	ABS
1	3	0.268
2	5	0.345
3	7	0.425
4	9	0.509
5	11	0.582
Regression equation	y = 0.0396x + 0.1486	
R^2	0.9996	

Accuracy:

The % RSD of the present work was found to be within and less than 2. The % recovery was between 99-100%.

Table 4: Accuracy data of the UV method

%Sample Spiked	Sample (Tablet)	Standard (7 ppm)	Mean	SD	%RSD	%Recovery
50%	1	0.35	0.674	0.001	0.148368	99.6337
	1	0.35				
	1	0.35				
50%	1	0.7	0.814	0.001	0.12285	101.0989
	1	0.7				
	1	0.7				
50%	1	1.35	0.95	0.001	0.105263	100.6105
	1	1.35				
	1	1.35				

Precision:

The % RSD of Enzalutamide was found to be 0.232 for intraday precision and. 0.232 for interday precision, respectively.

Table 5: Intraday Precision Results

	10AM Mean± SD	%RSD	1PM Mean ±SD	%RSD	4PM Mean ±SD	%RSD
LQC (3PPM)	0.26717± 0.00075	0.28176	0.26667± 0.00103	0.3873	0.267 0.000632	0.236875
MQC (7PPM)	0.±4225 0.001049	0.248239	0.±4225 0.001378	0.32625	0.423167 0.000983	0.232342
HQC (11PPM)	0.5075± 0.000548	0.107926	0.508167 0.000753	0.148135	0.508± 0.000632	0.124499

Table 6: Interday precision Values

	10AM Mean± SD	%RSD	1PM Mean ±SD	%RSD	4PM Mean ±SD	%RSD
LQC (3PPM)	0.26757± 0.000837	0.31277	0267± 0.000632	0.236875	0.2665± 0.001049	0.393549
MQC (7PPM)	0.422667± 0.001862	0.440512	0.42233± 0.001211	0.286755	0.423167± 0.000983	0.232342
HQC (11PPM)	0.508167± 0.000983	0.193478	0.5075± 0.001049	0.206662	0.507833± 0.001169	0.230203

Robustness:

A small variation of the wavelength (±5nm) was applied to the presented method and it was found that the %RSD was within the limits and the values were 0.136061and 0.135954.

Table 6: Robustness Values

Conc (µg/ml)	240		
		235nm	245nm
7	Mean	0.42433	0.424667
7	SD	0.000577	0.000577
7	%RSD	0.136061	0.135954

Ruggedness:

Two different analysts performed the ruggedness studies under same conditions and it was found that the % RSD was within the limits

Table 7: Ruggedness Result

Conc(µg/ml)		240nm	
		Analyst 1	Analyst 2
7	Mean	0.425	0.4253333
7	SD	0.001	0.001155
7	%RSD	0.235294	0.271481

LOD & LOQ:

The study for LOD & LOQ was carried out and the obtained results are described in the table below.

Table 8: LOD & LOQ Values

Drug	LOD	LOQ
Enzalutamide	0.128	0.390

Stability Studies:

A 7µg/ml solution of Enzalutamide was obtained and its stability was tested (24hours) in 0.1N HCl, 0.1N NaOH, Hydrogen Peroxide, and water. The results are shown in below table.

Table 9: Stabilities Studies of Enzalutamide

Solution	% Degradation	
	Day 1	Day 2
HCl	47%	58%
NaOH	67%	70%
H_2O_2	3%	5%
Water (H_2O)	2%	5%

DISCUSSION:

Using UV spectroscopy, we created a method in this work for estimating Enzalutamide in both bulk and pharmaceutical combined dosage forms. With the use of various techniques like HPLC, RP-UPLC, and others, there have been numerous methods. We used UV spectroscopy to carry out the method's development and validation.

CONCLUSION:

Based on the aforementioned findings, it can be said that the suggested method is straightforward, sensitive, exact, repeatable, and affordable to determine enzalutamide in bulk and combined dosage form. The drug's R2 value is 0.9996, and an %RSD of less than 2 suggests the method is robust and precise. The lowest LOD value can be used to determine the method's sensitivity. % Degradation indicates high level of degradation that means the drug is highly unstable in different stress conditions.

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