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VISIBLE SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ENZALUTAMIDE IN BULK AND FORMULATION DOSAGE

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ABSTRACT

Enzalutamide is an oral non-steroidal anti-androgen used for the treatment of prostate cancer. It is commercially available as XTANDI®. No visible spectrometric method has been reported for estimation of enzalutamide in formulation sample. In the present work, two visible spectrophotometric methods have been developed for quantitative determination of enzalutamide in bulk drug and formulation samples. These methods are validated for irinotecan with two chromogenic reagents namely 1,2 naphthoquinone-4-sulphonic acid (NQS),3-methyl benzothiazolinone hydrazine (MBTH) at λ_{max} of 630nm and 453nm respectively. The calibration curves were linear over a concentration range from 10-60 µg/ml for method 1 and 2.5-25 µg/ml for method 2. The relative standard deviations were less than 1% and average recovery was above 99.60%.These visible spectrophotometric methods at the respective absorption maxima enabled determination of the drug with no interference from the excipients.

KEYWORDS: *Ultraviolet-Visible Spectrophotometry, enzalutamide, 1,2 naphthoquinone-4-sulphonic acid (NQS),3-methyl benzothiazolinone hydrazine (MBTH).*

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INTRODUCTION

Enzalutamide[1] is chemically 4-{3-[4-cyano-3-(trifluoro methyl) phenyl]-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl}-2-fluoro-N-methyl benzamide(Figure 1). It has a molecular formula of $C_{21}H_{16}F_4N_4O_2S$ and a molecular weight of 464.44 g/mol. Enzalutamide is an oral non-steroidal anti-androgen[2] for prostate cancer[3]. Enzalutamide is an androgen receptor[4] inhibitor used for the treatment of patients with metastatic castration-resistant prostate cancer (CRPC). It was first launched in 1995 as a combination treatment (with surgical or medical castration) for advanced prostate cancer and subsequently launched as monotherapy for the treatment of earlier stages of the disease. Few chromatographic techniques like LC-MS/MS[5], LC- tandem MS[6] and HPLC[7] were reported for analysis of the drug. No single UV spectroscopy is reported for estimation of enzalutamide by the formation of complex.The present study describes simple, sensitive, accurate, rapid and economical spectrophotometric methods for the estimation of enzalutamide in bulk samples & tablet dosage forms, according to ICH guidelines [8].

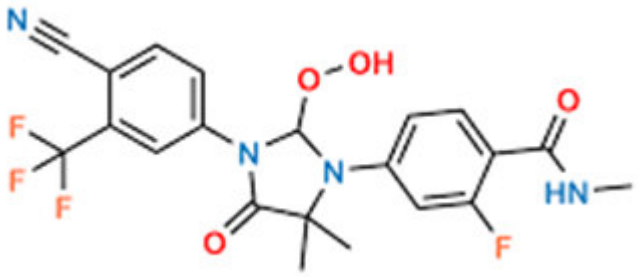

Figure 1
Structure of Enzalutamide

MATERIALS AND METHOD

Instrument
Pharmaspec-1700 Ultraviolet-Visible spectrophotometer (double beam) was used for all spectral measurements.Digisun model DI-707 pH meter was used for all the pH measurements.

Materials
Enzalutamide was obtained as gift sample from Mylan Laboratories. The reagents 1, 2 naphthoquinone-4-sulphonic acid (NQS),3-methyl benzothiazolinone hydrazine (MBTH),ferric chloride, sodium hydroxide and methanol purchased from Rankem, AR were used as they are without any further purification.

Preparation of standard drug solution
Method 1
A standard drug solution of enzalutamide was prepared by dissolving 100mg of drug in 100ml of methanol in a standard volumetric flask to obtain a stock solution of 1 mg/ml.

Method 2
A volume of 10ml of 1mg/ml solution was further diluted to 100 ml with methanol to get 100µg/ml working standard.

Preparation of sample solution
A quantity of the powder from tablets equivalent to 1 mg of drug dissolved in 10 ml methanol, filtered and analyzed by taking an aliquot and treated as per the procedure for standard.

Methodology for Bulk Drug Sample
Method 1
Aliquots of standard drug solution (1.0-6.0 ml) were transferred into series of 10 ml graduated test tubes, 2 ml of ferric chloride and 1 ml of MBTH were added to each test tube, mixed well and made up to 10 ml with methanol. The absorbance of resulting solution was measured at 630nm against reagent blank (Figure 6) prepared simultaneously and a linear graph was obtained.

Method 2
Aliquots of standard drug solution (0.25-2.5 ml) were transferred into series of 10 ml graduated test tubes, 0.5 ml of NQS and 2 ml of NaOH were added to each test tube, mixed well and made upto 10 ml with methanol.
The absorbance of resulting solution was measured at 453 nm against reagent blank (Figure 7) prepared simultaneously and a linear graph was obtained.

Methodology for Formulation drug sample
Method 1
A volume of 1 ml of the sample solution was transferred into 10 ml graduated test tube, 2 ml of ferric chloride and 1 ml of MBTH were added to the test tube, mixed well and volume made upto 10 ml with methanol. The absorbance of resulting solution was measured at 630 nm against reagent blank prepared simultaneously.

Method 2
A volume of 1 ml of the sample solution was transferred into 10 ml graduated test tube, 0.5 ml of NQS and 2 ml of NaOH were added to the test tube, mixed well and volume made up to 10 ml with methanol. The absorbance of resulting solution was measured at 453 nm against reagent blank prepared simultaneously.

Accuracy and Recovery Studies

Commercially available tablets of Enzalutamide (Table 1) were analyzed by the proposed methods and as additional check on the accuracy of the method, recovery experiments were also conducted by spiking known amounts of pure drug in pre-analyzed formulation. The recovery was calculated in each of the case using the regression line equation developed under the Linearity experiment[8]. Assay results of the proposed methods were compared with that of reference method and statistically evaluated using one-way ANOVA with post-test followed by Dunnett multiple comparison test. The means of the proposed methods are not significantly different from that of reference method ($P > 0.05$). The assay and accuracy results were presented in Table 2. The interference studies indicated the common additives and excipients present in formulations did not interfere with the proposed methods.

Table 1
Commercially Available Formulations of Enzalutamide

Generic Name	Proprietary Name	Dosage Form	Content
Enzalutamide	XTANDI® is provided as liquid-filled soft gelatin capsules for oral administration. Each capsule contains 40 mg of enzalutamide as a solution in caprylo caproyl polyoxyl glycerides. The inactive ingredients are caprylo caproyl polyoxyl glycerides, butylated hydroxyl anisole, butylated hydroxyl toluene, gelatin, sorbitol sorbitan solution, glycerin, purified water, titanium dioxide, and black iron oxide.	Capsules	40 mg

Table 2
Result of recovery studies (n=6)

Sample[a]	Labelled Amount (mg)	Amount obtained (mg)[b]			Percentage Recovery[b,c]	
		Proposed method		Reference method	Method1	Method2
		Method1	Method2	UV		
T_1	40	99.86±0.43	99.95±0.64	99.82±0.27	99.86±0.03	99.90±0.01
T_2	40	99.94±0.70	99.84±0.75	99.82±0.27	99.90±0.02	99.66±0.05

a - T1 and T2 are the tablets from different batches
b – Mean ± SD of 6 determinations.
c – 20 mg of pure drug was added and recovered.

For both the samples T1 and T2 One-way ANOVA with post-test followed by Dunnett multiple comparison tests were performed[10]. The results showed that $P > 0.05$ and the means of the proposed methods are not significantly different from that of reference method

Linearity

By using the method of least squares regression analysis[8] was performed to evaluate the slope (m), intercept (b) and correlation coefficient (r) was computed from various concentrations .The graph showed negligible intercept as described by the regression equation $y = mx + b$ where y is the absorbance and x is the concentration in µg/ml. Calibration curves for method 1 and method 2 were shown in Figures 2 and Figure3. The spectral analysis showed that λ_{max} of enzalutamide in method 1 is 630 nm and method 2 is 453 nm. The calibration curve was obtained for a series of concentration in the Beer's range of 10-60 µg/ml for method 1 and 2.5-25µg/ml for method 2.

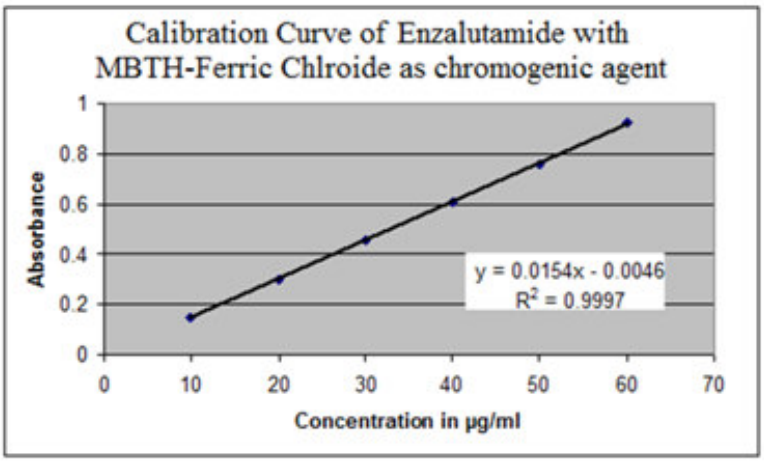

Figure 2
Calibration Curve of Enzalutamide with MBTH and $FeCl_3$

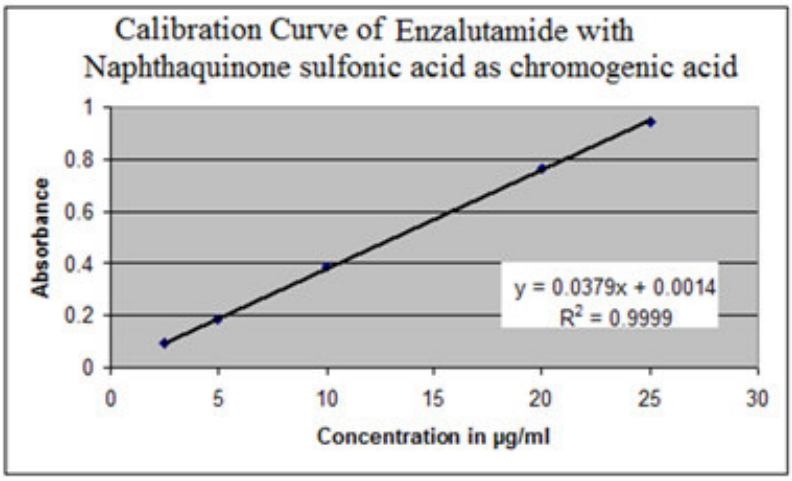

Figure 3
Calibration Curve of Enzalutamide with NQS

The optical characteristics such as molar absorptivity, Beer's law limits, absorption maxima and Sandell's sensitivity are presented in Table 3.

Table 3
Optical characteristics and precision of the methods

Parameter	Method 1	Method 2
λ_{max} (nm)	630	453
Beer's law range (µg/ml)	10-60	2.5-25
Molar extinction coefficient(a) ($L.mole^{-1} cm^{-1}$)	0.35×10^4	0.89×10^5
Sandell's sensitivity ($\mu g/cm^2/0.001$)	0.657	0.026
Regression equation (y = mx + c)		
Slope (m)	0.0015	0.0379
Intercept (c)	-0.0046	0.0014
Correlation coefficient (r)	0.9998	0.9999
Precision (%Relative Standard Deviation)	0.11	0.12

- Molar extinction coefficient(a)[8] = A/(b*c)
  A - Absorbance
  b - Pathlength
  c – Concentration
- Sandell's sensitivity calculated as per ICH guidelines[8]
- y=mx+c

Where, y is absorbance of of standard solution, x is concentration, m and c are slope and intercept of line respectively.

- Correlation coefficient (r)[8]

$$r = \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\sqrt{[\Sigma(x-\bar{x})^2(y-\bar{y})^2]}}$$

  y - y-coordinate
  $\bar{y}$ – mean of y values
  x - x coordinate
  $\bar{x}$ – mean of x values
- % Relative Standard Deviation[8] - S*100/ x

Precision

The reproducibility of method 1 and method 2 was evaluated by analysing the enzalutamide samples of concentration 60 µg/ml for method 1 and concentration of 25µg/ml for method 2 for inter-day and intra-day studies[8].Intraday precision was determined by analyzing three different concentrations of the drug for three times in a same day. Inter day precision was determined by analyzing the same concentrations of the solutions at three different days. The results tabulated in Table 4 and Table 5, the % RSD calculated for both methods proved satisfactory.

Table 4
Interday precession studies

Method	Mean	Standard deviation	% RSD
Method 1	0.924	0.001	0.111
Method 2	0.945	0.001	0.115

mean $(\bar{x})$ - $\frac{\text{Sum of six observations (X)}}{6\ (N)}$

Standard deviation $(S) - \sqrt{(\Sigma(x-\bar{x})^2 /N-1)}$ where x is absorbance

$\%\ RSD - S*100/\bar{x}$

Table 5
Intraday precession studies

Method	% RSD			Average %RSD
	Day 1	Day 2	Day 3	
Method 1	0.11	0.11	0.12	0.11
Method 2	0.12	0.12	0.12	0.12

Mean $(\bar{\bar{x}})$ - $\frac{\text{Sum of six observations (X)}}{6\ (N)}$

Standard deviation $(S) - \sqrt{(\Sigma(x-\bar{x})^2 /N-1)}$ where x is absorbance

$\%\ RSD - S*100/\bar{x}$

Average %RSD – $\frac{\%RSD(Day\ 1)+\%RSD(Day\ 2)+\%RSD(Day\ 3)}{3}$

RESULTS AND DISCUSSION

Zamir *et al* [9] reported simple area under curve (AUC) method for Enzalutamide by using UV spectroscopy. The sample solution in methanol was scanned for UV range 200nm to 400nm .The λ_{max} for enzalutamide is 236nm within Beer-Lambert's law in concentration range of 3 µg/mL to 15 µg/mL. In the present work two methods have been developed for the estimation of enzalutamide from tablet formulation, based on formation of colored complexes with MBTH - $FeCl_3$ and NQS respectively. The drug showed absorption at λ_{max} 630nm in method 1 over concentration range of 10-60µg/ml and 453nm in method 2 over concentration range of 2.5-25µg/ml (Table 3). Regression equations of calibration curves are y=0.0154x+0.0046(R^2=0.9997) for method I (Figure 2) and y=0.0379x+0.0014 (R^2=0.9999) for method II (Figure 3). Two samples were analysed and found that the percentage of drug content for method I is 99.86% and 99.94%, for method II is 99.95% and 99.84% respectively (Table 2). Interday and intraday precession studies were carried out and % RSD in found within limit (<1%) (Table 4 and Table 5).

Optimization of parameters for method 1 and method 2

Method 1

2ml of $FeCl_3$ (0.3% w/v) is added to the sample followed by 1ml of MTBH(0.2 % w/v) and stirred manually .The time taken for formation of colored complex is 5 minutes at temperature of 29^{0}C.The stability of coloured complex is >60 minutes.

Method 2

2ml of NaOH (2% w/v) is added to the sample followed by addition of 0.5ml NQS(0.5% w/v).The time taken for formation of complex is 15 minutes at a temperature of 29^{0}C.The stability of coloured complex is >40 minutes.

Chemistry of the colored species formed

Method 1

Enzalutamide has a secondary amino group in the molecular structure making it possible to undergo oxidative coupling[10] of the drug with MBTH in ferric chloride. Under the reaction conditions, MBTH loses two electrons and one proton on oxidation forming the electrophilic intermediate which has been postulated to be the active coupling species. The intermediate reacts with amine by electrophilic attack on the aromatic ring of the amine and the resulting intermediate is spontaneously oxidized with an oxidant to form the colored species via oxidative coupling mechanism (Figure 4).

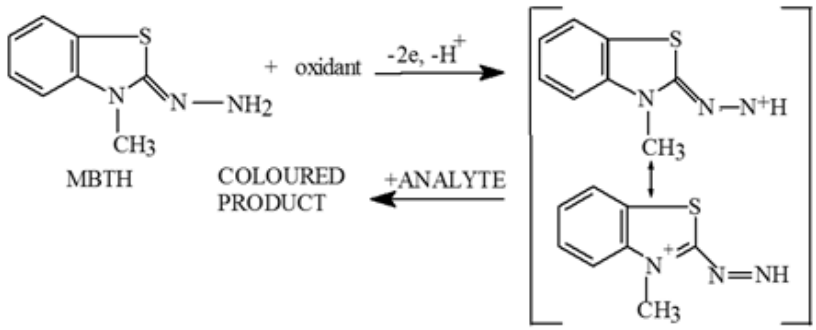

Figure 4
Mechanism for formation of colored complex with MBTH

Method 2
The method is based on the formation of a coloured derivative between Enzalutamide and 1,2-naphthoquinone-4- sulphonic acid sodium salt (NQS)[11].

The reaction between enzalutamide and NQS is a simple condensation reaction with the elimination of $NaHSO_3$ (Figure 5).

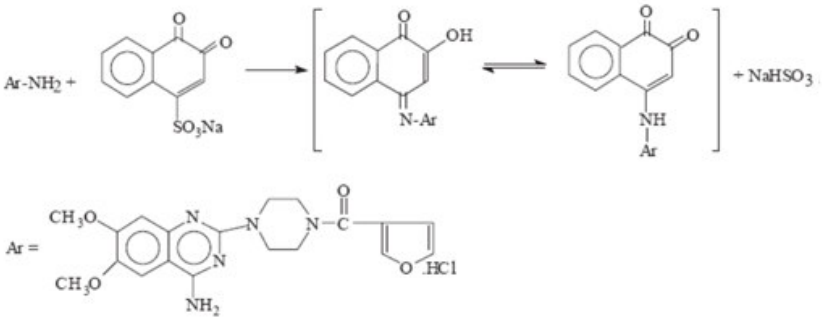

Figure 5
Mechanism for formation of colored complex with NQS

Absorption Maximum
Absorption spectra of Enzalutamide for method 1 and method 2 were shown in figures 6 and figure 7.

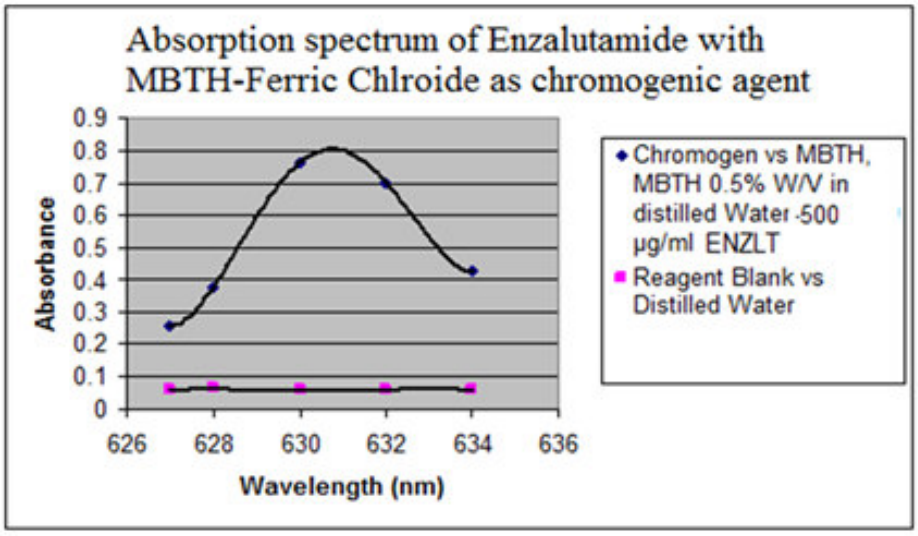

Figure 6
Absorption Spectrum of Enzalutamide by method 1

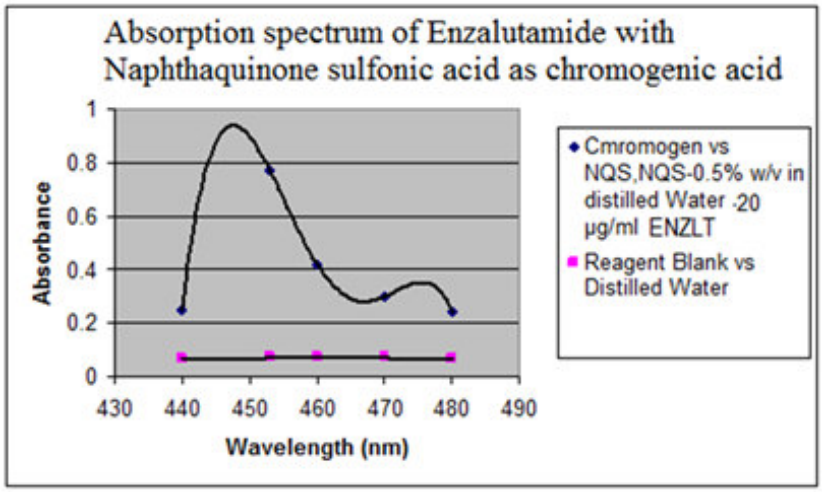

Figure 7
Absorption Spectrum of Enzalutamide by method 2

CONCLUSION

The proposed visible spectrophotometric methods enable quantitative determination of enzalutamide in bulk drug samples and tablets. Efficient visible spectrophotometric detection at the respective absorption maxima enabled determination with no interference from the excipients. The calibration curves were linear over a concentration range from 10-60 µg/ml for method 1 and 2.5-25 µg/ml for method 2. The relative standard deviation's (R.S.D.) were less than 1% and average recovery was above 99.60%. The proposed methods are fast, sensitive, precise, accurate, and efficient and can be used in for analysis in quality control laboratories.

CONFLICT OF INTEREST

Conflict of interest declared none.

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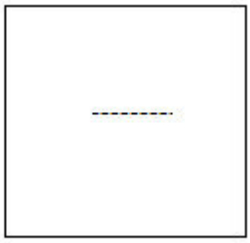

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