Original Article

Development And Quantification Of Isothiocyanate Impurity In Enzalutamide Drug Substance By Using Rp-Hplc Technique

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Abstract

In the routine pharmaceutical industry manufacturing process sometimes few unknown impurities will trigger during its quality assessment practice. Those unknown impurities might be impact on downstream synthesis and which leads to the quality of active pharmaceutical ingredients (API's). A new, simple, rapid, selective, precise and accurate reverse phase high performance liquid chromatography method has been developed for quantification of isothiocyanate impurity in Enzalutamide drug substance. The separation was achieved by using stationary phase Phenomenex Kinetex (100 x 2.1 mm, 3.5µm) column and the mobile phase consists of two channels A and B. channel-A: 0.1% formic acid buffer and channel-B: acetonitrile: water (85:15 v/v) in the proportion of gradient elution. The flow rate is 0.5 mL/min. The column temperature was maintained at 40°C and sample cooler temperature was maintained at 5°C, injection volume 5 µL and wavelength 295nm. The retention time of isothiocyanate impurity was noted to be 13.7 min respectively. The limit of detection (LOD) and limit of quantitation (LOQ) for isothiocyanate impurity 0.7492 µg/mL and 2.7372 µg/mL respectively. The Linearity of isothiocyanate impurity was carried out at different concentrations ranging from 2.281-13.686 µg/mL and correlation coefficient was found to be 0.9998. Precision of the method was performed by injecting standard injections, the %RSD value of isothiocyanate impurity was found to be 1.36%. Accuracy was confirmed by recovery studies the %recovery of shown in the range of LOQ and 150% and the results obtained were found to be within the limits. Hence the method was found to be accurate. The method was validated as per ICH guidelines.

KEY WORDS: Isothiocyanate, LOD and LOQ, Enzalutamide drug substance, ICH guidelines, Validation.

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 structures of Enzalutamide and Isothiocyanate impurity was shown in **Figure 1 and Figure 2.**

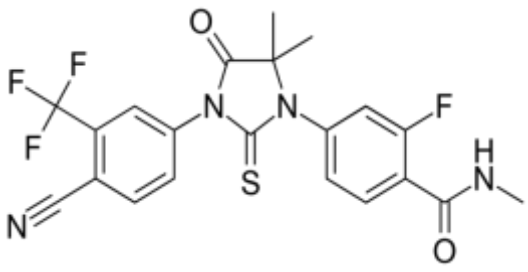

Figure 1. Chemical structure of Enzalutamide

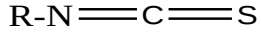

Figure 2. Chemical structure of isothiocyanate

In the synthesis process of Enzalutamide drug substance, 4-Isothiocyanoyl-2-(trifluoromethyl) benzonitrile is a reagent used was used as a key intermediate to synthesize thioimidazolinone compounds and is a potential anti-prostate cancer drug.

In the available literature, few analytical methods had been reported for the quantification of isothiocyanates[14-18]. However, no method was reported for the quantification of isothiocyanate in Enzalutamide drug substance finally, a sensitive HPLC method was reported for the determination of quantification isothiocyanate impurity in Enzalutamide drug substance and the method was validated for specificity, linearity, accuracy and precision experiments[19].

MATERIALS AND METHODS

Chemicals and Reagents

Formic acid (AR grade), Acetonitrile, Methanol, Ethanol (HPLC grade) and water, reagents and chemicals were procured from merck chemicals. Mumbai, India.

Instrumentation

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 study.

Preparation of mobile phase-A

Transferred 1 mL of the formic acid solution into 1000 mL milli-Q water and mixed well. Filtered the solution with 0.22 µm nylon membrane filter and sonicate to degas.

Preparation of mobile phase-B

Prepared a mixture of 850 mL of Acetonitrile and 150 mL of water in the ratio of 85:15 (%v/v). Filtered the solution with 0.22 µm membrane filter and sonicate to degas.

Preparation of diluent

Acetonitrile used as a diluent.

Preparation of blank

Diluent used as a blank

Preparation of standard stock solution

Weighed accurately 1.215 mg of isothiocyanate standard into a 50 mL volumetric flask, added 25 mL diluent, sonicate for 2 minutes to dissolve, diluted to volume with diluent and mixed well. Further diluted 1.0 mL of this solution into a 100 mL volumetric flask, made up to volume with diluent and mixed well.

Preparation of standard solution

Transferred 5 mL of the standard stock solution into 25 mL volumetric flask, diluted to volume with diluent and mixed well.(Concentration of the isothiocyanate standard solution contains 9 µg/mL of with respect to 50.0 mg/mL Enzalutamide test concentration).

Preparation of sample solution

Weighed accurately 250 mg of the test sample into a 5 mL volumetric flask, added 2.5 mL diluent, sonicated to dissolve, diluted to volume with diluent and mixed well.

Preparation of spiked sample solution

Weighed accurately 250 mg of the test sample into a 5 mL volumetric flask, added 2.5 mL diluent, sonicated to dissolve then added 1.0 mL of isothiocyanate standard stock solution, diluted to volume with diluent and mixed well.

Method Development

The objective of the general method is to quantification of isothiocyanate impurity at low level with selectivity in Enzalutamide drug substance. The sample diluent, column oven temperature, sample cooler temperature, gradient program and injection volume and HPLC parameters were investigated and optimised using isothiocyanate standard solution or isothiocyanate standard spiked to sample solutions.

Selection of wavelength

UV-spectroscopic analysis of Enzalutamide drug substance showed maximum UV absorbance (λmax) at 210 nm and isothiocyanate impurity showed maximum absorption 295 nm respectively.

Selection of mobile phase

The method development was started with by using stationary phase Phenomenex Kinetex (100 x 2.1 mm, 3.5µm) column and the mobile phase consists of two channels A and B. channel-A: 0.1% formic acid buffer and channel-B: acetonitrile in the proportion of gradient elution. The HPLC gradient program was time (min)/B% v/v: 0/50, 10/50, 11/100, 20/100, 21/50, 25/50. The flow rate is 1.0 mL/min. The column temperature was maintained at 40°C and sample cooler temperature was maintained at 5°C, injection volume 5µL and wavelength 295nm. There was no proper resolution of isothiocyanate impurity from blank and Enzalutamide sample peaks and peak interferences are present.

For the next trial flow rate was changed from 1.0 mL/min to 0.5 mL/min remaining chromatographic parameters are constant. There was no proper resolution of isothiocyanate impurity peak from Enzalutamide sample peaks.

For the next attempt mobile phase-B was changed from 100% acetonitrile to acetonitrile and water in the ratio of 85:15 v/v. remaining chromatographic parameters are constant. The proper resolution of isothiocyanate impurity peak separated from Enzalutamide sample peaks achieved.

Selection of diluent for sample and standard preparation

The sample diluent is important for a HPLC method as it affects the sensitivity and accuracy. Several sample diluents were evaluated including methanol, acetonitrile, ethanol and water alone and in combination. The use of water alone as the sample diluent led to irreproducible results, water solubility of drug (7.85 µg/mL). A combination of water with the organic diluents 1:1 ratio were studied led to irreproducible results. The reproducibility of isothiocyanate impurity spiked to Enzalutamide was evaluated and the results demonstrated that the acetonitrile sample diluent showed the best sensitivity and selectivity.

Selection of injection volume for sensitivity

One of the key objectives of the method development was to achieve adequate sensitivity for low level isothiocyanate analysis. The isothiocyanate method sensitivity was further optimized by the evaluating the effect of injection volume on the noise level and S/N value of a 9 ppm isothiocyanate standard solution. Several HPLC injection microliter (µL) including 5µL, 10µL and 20µL were studied. The optimal injection volume and adequate signal was observed using the 5µL injection volume injection parameter.

Optimized chromatographic conditions

Chromatographic analysis was performed on Waters 2695 HPLC system. The chromatograms are recorded and analysed Empower^3 software. The separation was achieved by using stationary phase Phenomenex Kinetex (100 x 2.1 mm, 3.5µm) column and the mobile phase consists of two channels A and B. channel-A: 0.1% formic acid buffer and channel-B: acetonitrile: water (85:15 v/v) in the proportion of gradient elution. The HPLC gradient program was time (min)/B% v/v: 0/50, 10/50, 11/100, 20/100, 21/50, 25/50. The flow rate is 0.5 mL/min. The column temperature was maintained at 40°C and sample cooler temperature was maintained at 5°C, injection volume 5µL and wavelength 295nm.

RESULTS AND DISCUSSION

Specificity

The method specificity was validated for potential interference from blank, standard, sample and spiked sample solution. There are no detectable peaks in the chromatograms of blank, standard, sample and spiked sample. The isothiocyanate peak in the chromatogram of 9 ppm isothiocyanate spiked sample solution is sufficiently resolved from all other peaks before and after Isothiocyanate peak.

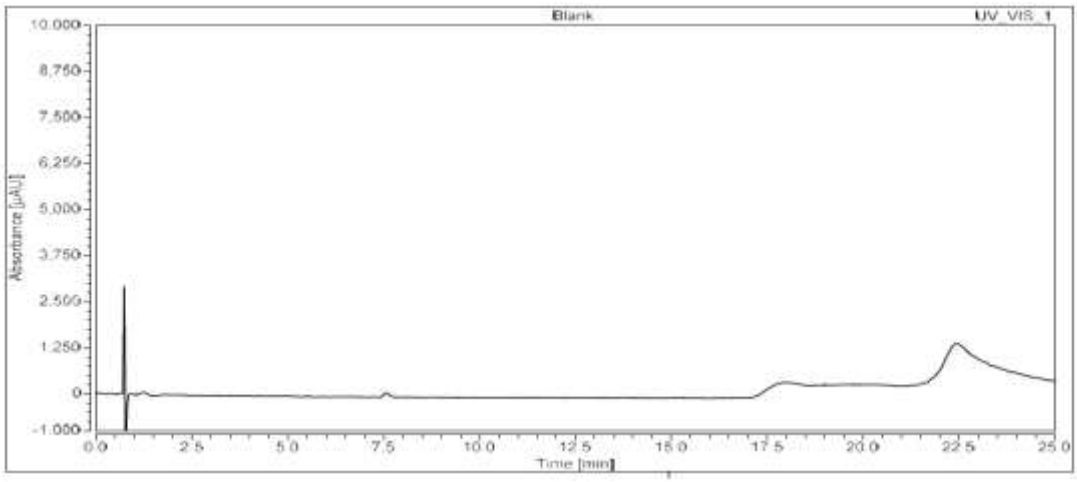

Figure 3. Typical chromatogram of blank

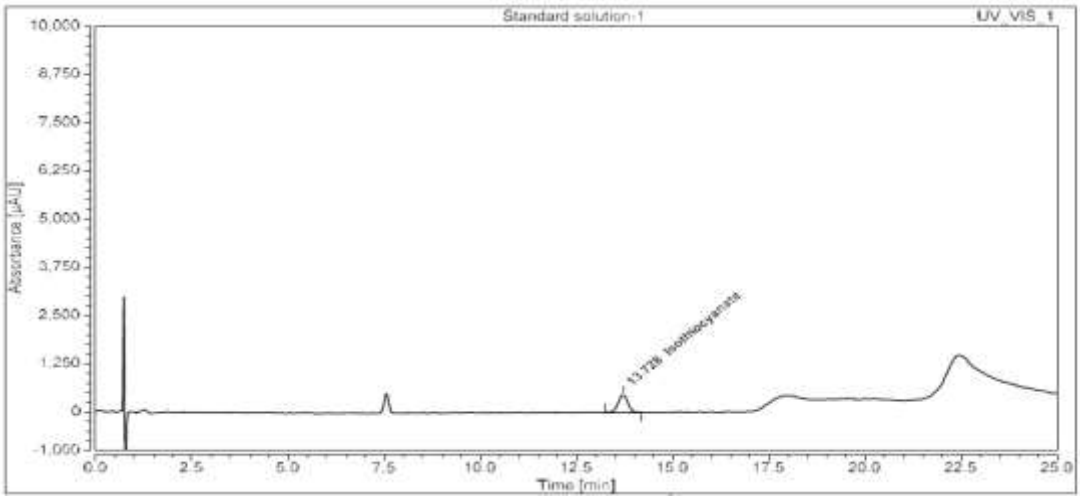

Figure 4. Typical chromatogram of standard

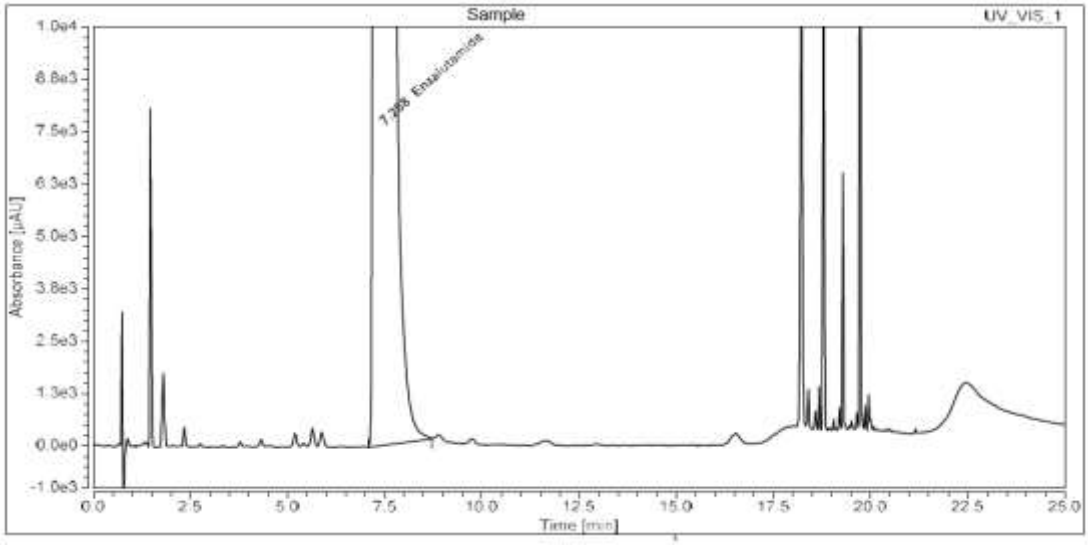

Figure 5. Typical chromatogram of sample

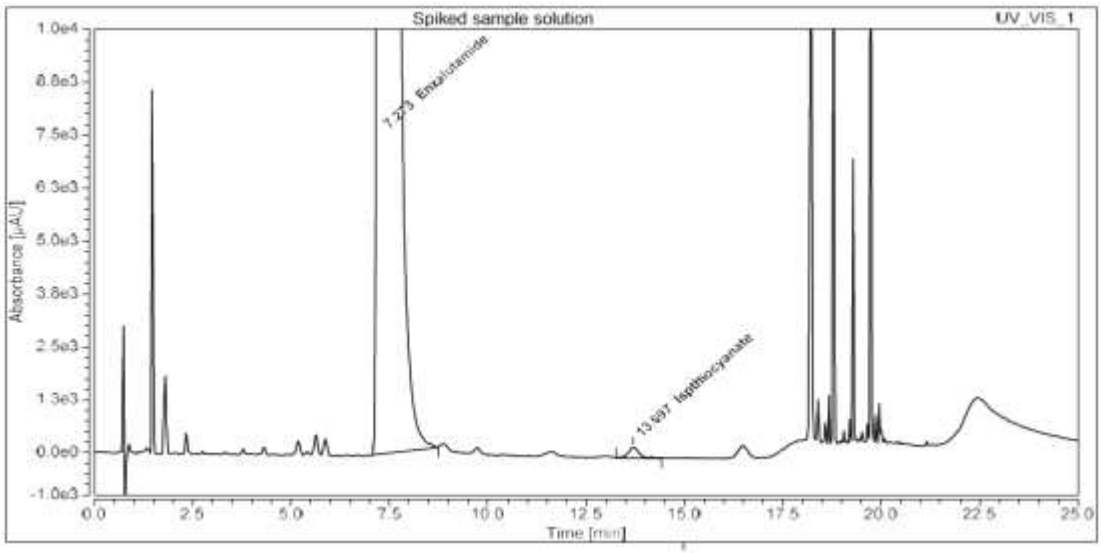

Figure 6. Typical chromatogram of spiked sample

System precision

System precision was demonstrated by preparing standard solution as per method and chromatographed the same into HPLC system in six replicated injections of standard solution. The peak areas of analyte were recorded for these standard injections. The system precision was evaluated by computing the % Relative standard deviation for the peak area of these standard injections.

Detection limit (LOD), Quantitation limit (LOQ)

A solution containing 2.7372 ppm of isothiocyanate standard was injected six times. The RSD of areas and S/N ratios for each standard were calculated. A solution containing 0.7492 ppm of isothiocyanate standard was injected three times. The worst found signal to noise ratio for each peak was greater than 3 in each injection. All the peaks were detected in all the three injections.

Therefore, the quantitation limit (QL) and the detection limit (DL) was thus set at 2.7372 ppm and 0.7492 ppm, respectively.

Method precision

Precision (repeatability) was evaluated from the recovery data. Recovery data was determined by injecting six sample solutions spiked isothiocyanate 9 ppm at specification level. The samples were prepared as per the analytical method.

Linearity and Range

The linearity of isothiocyanate was evaluated from 2.281 ppm to 13.686 ppm (six levels with duplicate preparations at each level). The peak areas were plotted against the corresponding concentrations and the linear regression was performed.

Accuracy

Accuracy was determined by analyzing the triplicate preparation of isothiocyanate standard at low 2.2 ppm, 4.5 ppm, 9 ppm and 13.5 ppm levels in the presence of Enzalutamide drug substance as per the analytical method. The accuracy as % recovery was calculated from the experimental concentrations of isothiocyanate standards by the theoretical concentrations. The recovery of ranged from 99.8% to 105.6% were obtained for the three concentrations levels.

Solution stability

The stability of standard, sample and spiked sample solutions were prepared in duplicate and stored at ambient laboratory conditions (25°C), refrigeration (2-8°C), respectively. Therefore, the standard solution, sample solution and spiked sample solution were stable for 24 hrs at refrigerated temperature conditions. The standard solution, sample solution and spiked sample were unstable at room temperature conditions.

Table 1. Validation data of Enzalutamide for the quantification of isothiocyanate

Parameter	**isothiocyanate**
LOD (ppm)	0.7492
LOQ (ppm)	2.7372

Precision at LOQ level (RSD, %)	4.20
System Precision at sixth level (RSD, %)	1.36
Method Precision at sixth level (RSD, %)	0.64
Linearity range (µg/mL)	LOQ-150
Correlation coefficient	0.9880
Slope	886.0525
Intercept	86.5333
% of Y-intercept	1.05
Accuracy at LOQ (mean recovery, %)	105.6
Accuracy at 50 (mean recovery, %)	101.4
Accuracy at 100 (mean recovery, %)	100.8
Accuracy at 150 (mean recovery, %)	99.8

CONCLUSION

The simple method was developed for the quantification of isothiocyanate impurity in Enzalutamide drug substance at trace level concentration have been developed and validated as per ICH guidelines. The results showed that the developed method is simple, fast, sensitive, suitable and specific for the effectiveness of the method was ensuring by the specificity, precision, LOD, LOQ, linearity, accuracy and solution stability. Hence, the method well suit for their intended purposes and can be successfully applied for the release testing of Enzalutamide in bulk and pharmaceutical dosage forms.

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CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

REFERENCES

1. Sethi PD. High Performance Liquid Chromatography: Quantitative Analysis of Pharmaceutical Formulations (1st Ed). CBS Publishers and Distributors. 2001;116-117.
2. Synder LR, Kirkland JJ, Glajch LJ. Practical HPLC Method Development (2nd Ed). John Wiley and sons. 1997; 2-10.
3. Sethi PD. Quantitative Analysis of Drugs in Pharmaceutical Formulations (3rd Ed). CBS Publishers and Distributors. 2011; 51.
4. Willard HH, Merrit LL, Dean JA, et al. In Instrumental Methods of Analysis (6th Ed). C.B.S. Publishers. 1989;1-12.
5. Conners KA. In Textbook of Pharmaceutical Analysis. A Wiley Publication. 1999;16-22.
6. Skoog DA, West DM, Holler FJ, et al. In Fundamental of Analytical Chemistry (8th Ed). Cengage Learning. 1992;2-3.
7. Bradley L. In Remington, Instrumental Method of Analysis (21st Ed). Lippincott Williams and Wikkins. 2005;633.
8. Lachman L, Lieberman HA, Kanig JL. The Theory and Practice of Industrial Pharmacy (3rd Ed). Varghese Publishing. 374-411.
9. Pod F, Brian C, Jones E. Pharmaceutical Capsules (2nd Ed). Pharmaceutical press. 2004;61-93.
10. Raymond C, Paul J, Sian C. Handbook of Pharmaceutical Excipients (5th Ed). Published by Pharmaceutical press. 2006;103-04.
11. CHMP assessment report. Introduction to drug and its use, chemical properties and mechanism of action. European Medicines Agency. 2013.
12. Astellas. NICE Final Appraisal Determination (FDA) recommends use of prostate cancer treatment XTANDI prior to chemotherapy. 2015.
13. Astellas. Enzalutamide phase 2 terrain trial demonstrated statistically significant increase in PFS as compared to Bicalutamide in metastatic prostate cancer. 2015.
14. Agrawal SS, Yallatikar T, Gurjar P. Reversed-phase high-performance liquid chromatographic method development and validation for allyl isothiocyanate estimation in phytosomes of Brassica nigra extract. Journal of Advanced Pharmaceutical Technology & Research. 2019;10:126-31.
15. Pilipczuk T, Kusznierewicz B, Chmiel T, Przychodzen W, Bartoszek-Pączkowska A. Simultaneous determination of individual isothiocyanates in plant samples by HPLC-DAD-MS following SPE and derivatization with N-acetyl-l-cysteine. Food Chemistry. 2017; 214: 587-596. DOI: 10.1016/j.foodchem.2016.07.125
16. Agerbirk N, De Nicola, G R, Olsen, C E, Müller C, and Iori R. (2015). Derivatization of isothiocyanates and their reactive adducts for chromatographic analysis. Phytochemistry, 2015; 118:109–115. http://dx.doi.org/10.1016/j.phytochem.2015. 06.004.
17. Aires A, Mota VR, Saavedra MJ, Monteiro AA, Simoes M, Rosa EAS, and Bennett RN. Initial in vitro evaluations of the antibacterial activities of glucosinolate enzymatic hydrolysis products against plant pathogenic bacteria. Journal of Applied Microbiology. 2009; 106(6): 2096–2105. http://dx.doi.org/10.1111/j.1365-2672.2009.04181.x.

18. Aissani N, Tedeschi P, Maietti A, Brandolini V, Garau VL, and Caboni P. (2013). Nematicidal activity of allylisothiocyanate from horseradish (Armoracia rusticana) roots against Meloidogyne incognita. Journal of Agricultural and Food Chemistry. 2013; 61(20): 4723–4727. http://dx.doi.org/10.1021/jf4008949.
19. International Conference on Harmonization of technical requirements for registration of pharmaceutical for human use, ICH harmonized tripartite guideline, Validation of analytical procedures: Text and methodology, Q2 (R1), step 4 (2005).