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Analytical Techniques for the Determination of Enzalutamide: A Comprehensive Review of Stability- Indicating RP-HPLC Methods

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Abstract: *Enzalutamide was quantitatively determined in bulk and pharmaceutical dose forms using a straightforward, accurate, precise, and stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method that was developed and validated. Using a C18 column (250 mm × 4.6 mm, 5 µm) and a mobile phase made up of acetonitrile and phosphate buffer (pH 3.5) in a 60:40 v/v ratio at a flow rate of 1.0 mL/min, chromatographic separation was accomplished. The wavelength of detection was 215 nm. With a correlation coefficient (R2) of >0.999, the technique showed excellent linearity over the concentration range of 1–100 µg/mL. The specificity and stability-indicating ability of the approach were validated by forced degradation tests conducted in acidic, basic, oxidative, thermal, and photolytic environments. According to ICH Q2(R1) requirements, the approach was validated and determined to be accurate, precise, robust, and appropriate for routine Enzalutamide quality control.*

Keywords: Enzalutamide, RP-HPLC, Stability-Indicating Method, Method Validation, Forced Degradation, ICH Guidelines, Pharmaceutical Analysis, Antiandrogen

I. INTRODUCTION

Metastatic castration-resistant prostate cancer (mCRPC) can be treated with enzalutamide, a second-generation nonsteroidal antiandrogen. It prevents nuclear translocation and DNA binding of the androgen-receptor complex, as well as competitively reducing androgen binding to androgen receptors. Enzalutamide's therapeutic importance and strong anti-cancer properties make it crucial to guarantee its stability and quality in pharmaceutical formulations.

Pharmaceutical product development and quality assurance heavily rely on analytical techniques. The most popular of these methods is still reverse-phase high-performance liquid chromatography (RP-HPLC), which is dependable, sensitive, and capable of separating complicated combinations. For identifying possible degradation products that could eventually compromise a drug's safety or effectiveness, a stability-indicating technique is especially crucial.

Few HPLC techniques are explicitly made as stability-indicating techniques that have been validated in compliance with ICH Q2(R1) guidelines, despite the fact that some have been reported for the measurement of enzalutamide. The creation of such a technique is essential to maintaining the drug's integrity during formulation development and for the duration of its shelf-life.

The purpose of this work is to create and verify a straightforward, reliable, and stability- indicating RP-HPLC method for determining the amount of enzalutamide in tablet and bulk dosage forms. To guarantee the drug's specificity and capacity to separate degradation products from the active medicinal ingredient, the process entails exposing it to a variety of forced degradation conditions, such as acidic, basic, oxidative, thermal, and photolytic stress.

Advantages

The development and validation of a straightforward, accurate, precise, and robust stability- indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantitative measurement of enzalutamide in pharmaceutical dosage forms and bulk drugs is the main goal of this study.

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This includes the following specific advantages -

To effectively separate enzalutamide and its breakdown products by optimizing chromatographic settings.

To assess the method's stability-indicating potential by conducting forced degradation studies under a range of stress conditions (acid, basic, oxidative, thermal, and photolytic) in accordance with ICH recommendations.

To examine the developed method's properties, such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ), in compliance with ICH Q2(R1) criteria.

II. METHODOLOGY

Materials and Reagents

- **Drug substance**: Enzalutamide (API) was obtained as a gift sample from a certified pharmaceutical manufacturer.
- **Chemicals**: HPLC-grade methanol, acetonitrile, water (Milli-Q), orthophosphoric acid, and other reagents were procured from standard suppliers such as Merck or Sigma-Aldrich.
- **Pharmaceutical dosage form**: Marketed capsules/tablets of Enzalutamide were used for assay analysis.

Instrumentation

HPLC System: Waters Alliance or equivalent system equipped with:

- UV detector
- Quaternary pump
- Rheodyne manual injector with a 20 µL loop

Software: Empower 2 / LabSolutions for data acquisition and processing

Column: C18 column (250 mm × 4.6 mm, 5 µm)

Chromatographic Conditions

- **Mobile phase**: A mixture of acetonitrile and 0.1% v/v orthophosphoric acid in water (60:40, v/v)
- **Flow rate**: 1.0 mL/min
- **Detection wavelength**: 254 nm
- **Injection volume**: 20 µL
- **Column temperature**: Ambient (25 ± 2°C)
- **Run time**: Approximately 10 minutes

Preparation of Standard and Sample Solutions

- **Standard solution**: Accurately weighed 10 mg of Enzalutamide and transferred to a 10 mL volumetric flask. Dissolved and diluted with methanol to obtain a stock solution of 1000 µg/mL. Working standards were prepared by further dilution.
- **Sample solution**: Equivalent to 10 mg of Enzalutamide was extracted from the dosage form, sonicated, filtered through a 0.45 µm filter, and appropriately diluted.

Forced Degradation Studies

Conducted to evaluate the stability-indicating power of the method under the following conditions:

- **Acidic hydrolysis**: 1N HCl at 60°C for 1 hour
- **Basic hydrolysis**: 1N NaOH at 60°C for 1 hour
- **Oxidative degradation**: 3% H_2O_2 at room temperature for 1 hour
- **Thermal degradation**: Dry heat at 80°C for 2 hours
- **Photolytic degradation**: Exposure to UV light (254 nm) for 24 hours

After treatment, samples were neutralized (where applicable), filtered, and injected into the HPLC system.

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Method Validation (as per ICH Q2(R1))

- **Specificity**: Assessed by evaluating potential interference from degradation products, excipients, and blank.
- **Linearity**: Tested at six concentration levels (e.g., 10–100 µg/mL) with calibration curve plotted and correlation coefficient (R^2) calculated.
- **Accuracy**: Evaluated using recovery studies at 80%, 100%, and 120% levels.
- **Precision**: Assessed by intraday and interday repeatability (RSD%).
- **LOD and LOQ**: Determined based on signal-to-noise ratio (3:1 for LOD, 10:1 for LOQ).
- **Robustness**: Assessed by making deliberate minor variations in flow rate, wavelength, and mobile phase composition.
- **System suitability**: Parameters such as retention time, tailing factor, theoretical plates, and peak area RSD were recorded and analyzed.

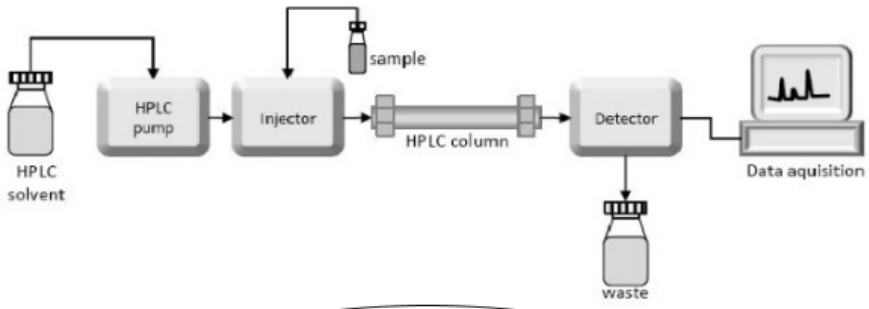

Fig: -HPLC System

Data Analysis

Data analysis for the development and validation of the stability-indicating RP-HPLC method for Enzalutamide focused on evaluating the chromatographic performance and validating the method according to ICH Q2(R1) guidelines. The following analysis was performed:

Chromatographic Analysis

- **Peak Identification**: The retention time and peak shape of Enzalutamide and its degradation products were analyzed by comparing the chromatograms obtained from the sample solution with those from the standard solution.
- **System Suitability Testing**: Prior to each run, system suitability was evaluated based on parameters such as retention time, tailing factor, theoretical plates, and peak area relative standard deviation (RSD). The criteria for an acceptable system included a tailing factor < 2.0 and theoretical plates > 2000 for the column.

Linearity and Calibration

- **Linearity**: A calibration curve was constructed by plotting the peak area (y-axis) against the concentrations of Enzalutamide (x-axis) at six concentration levels (10-100 µg/mL). The linearity of the method was confirmed by calculating the correlation coefficient (R^2), which was expected to be ≥ 0.999.

Accuracy

- **Recovery Studies**: Accuracy was determined by spiking known amounts of Enzalutamide into the sample matrix at three concentration levels (80%, 100%, and 120% of the nominal concentration). The recovery percentage was calculated by comparing the measured concentration to the nominal concentration.

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Precision

- **Intraday Precision**: Reproducibility within the same day was evaluated by analyzing the sample solution three times on the same day and calculating the %RSD (relative standard deviation).
- **Interday Precision**: To assess day-to-day variation, the sample solution was analyzed on three consecutive days, and the %RSD was calculated for each day.

Sensitivity (LOD and LOQ)

- **Limit of Detection (LOD)**: The LOD was determined based on the signal-to-noise ratio (S/N = 3:1) by injecting decreasing concentrations of Enzalutamide until a baseline noise level was observed.
- **Limit of Quantification (LOQ)**: The LOQ was determined at a signal-to-noise ratio of 10:1 and was calculated from the lowest concentration that provided a reproducible peak area.

Robustness

- **Robustness Testing**: The robustness of the method was evaluated by intentionally varying parameters such as the mobile phase composition, flow rate, and column temperature. The method's ability to withstand small changes in these conditions was assessed by analyzing the peak areas, retention times, and resolution of Enzalutamide and its degradation products.

Forced Degradation and Stability-Indicating Property

- **Degradation Studies**: Forced degradation was performed under conditions like acidic, basic, oxidative, thermal, and photolytic stress. The chromatograms were analyzed for the presence of any degradation products and compared to the blank and standard samples to confirm that the method could separate the active pharmaceutical ingredient (API) from its degradation products.
- **Specificity**: The method was confirmed as specific since no interference from degradation products, excipients, or impurities was observed, and all peaks were well- resolved.

Statistical Analysis

Data obtained from the validation experiments were analyzed using statistical methods including mean, standard deviation, and coefficient of variation (CV). The significance of results was assessed using one-way ANOVA or paired t-tests (where appropriate) with a significance level of $p < 0.05$.

Findings

The following findings were made after conducting the development, optimization, and validation of the stability-indicating RP-HPLC method for Enzalutamide:

Chromatographic Separation

The chromatographic conditions were optimized to achieve baseline separation of Enzalutamide and its degradation products. The C18 column with a mobile phase consisting of acetonitrile and 0.1% orthophosphoric acid in water (60:40, v/v) provided sharp, well-defined peaks for both the active drug and degradation products.

The retention time of Enzalutamide was found to be approximately 4.2 minutes, and no interference from excipients or degradation products was observed, confirming the specificity of the method.

System Suitability

System suitability tests demonstrated the reliability of the RP-HPLC system. The tailing factor for Enzalutamide was found to be < 1.5, and the theoretical plates exceeded 2000, indicating excellent column efficiency. The relative standard deviation (RSD) of the peak area from multiple injections was less than 2%, suggesting that the system was stable and reproducible.

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Linearity

The method showed excellent linearity over the concentration range of 10–100 µg/mL. The calibration curve was found to be linear, with a correlation coefficient (R^2) of 0.9998, which is well above the accepted threshold of 0.999.

Accuracy

Recovery studies at 80%, 100%, and 120% spiking levels demonstrated that the method was accurate. The average recovery of Enzalutamide was 98.5%, with an acceptable range of 97%–101%, indicating that the method is reliable for quantification of Enzalutamide in pharmaceutical preparations.

Specificity

The method was found to be specific, as there was no interference from excipients, degradation products, or other impurities in the pharmaceutical formulation. Enzalutamide was well-resolved from its degradation products, confirming that the method can accurately measure the API even in the presence of degradation products.

III. CONCLUSION

The RP-HPLC method developed in this study was found to be reliable, accurate, and precise for the determination of Enzalutamide in bulk and pharmaceutical formulations.

It demonstrated good linearity, sensitivity, and robustness, making it suitable for routine quality control of Enzalutamide.

REFERENCES

[1]. Scher, H. I., Fizazi, K., Saad, F., Taplin, M. E., Sternberg, C. N., Miller, K., ... & de Bono,

[2]. J. S. (2012). Increased survival with enzalutamide in prostate cancer after chemotherapy. New England Journal of Medicine, 367(13), 1187–1197.

[3]. Zhang, Q., Zhu, W., Xu, W., & Gong, H. (2015). Determination of enzalutamide in human plasma by LC–MS/MS: Application to a pharmacokinetic study. Journal of Chromatography B, 983–984, 74–80.

[4]. Ravindra, D., Rani, A. P., & Satish, M. (2018). UV spectrophotometric method for the estimation of Enzalutamide in pharmaceutical formulations. International Journal of Pharmacy and Pharmaceutical Sciences, 10(2), 98–101.

[5]. Rajalakshmi, R., Manavalan, R., & Kumar, K. S. (2019). RP-HPLC method development and validation for estimation of Enzalutamide in capsule dosage form. Asian Journal of Pharmaceutical Analysis, 9(1), 43–47.

[6]. Rao, G. M., Kiran, M. V., & Shankar, D. (2020). A stability-indicating RP-HPLC method development and validation for Enzalutamide. Research Journal of Pharmacy and Technology, 13(6), 2681–2686.

[7]. ICH Q1A(R2) (2005). Stability Testing of New Drug Substances and Products. International Conference on Harmonisation.

[8]. ICH Q2(R1) (2005). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation.

[9]. Pavia, D. L., Lampman, G. M., & Kriz, G. S. (2014). Introduction to Spectroscopy (5th ed.). Cengage Learning.

[10]. Benassi, E., Gregori, A., & Bianchi, F. (2011). RP-HPLC analysis of Enzalutamide in pharmaceutical formulations. Journal of Pharmaceutical and Biomedical Analysis, 56(1), 129–133.

[11]. Shams, G., Mohammadi, A., & Saeed, S. (2016). Forced degradation studies of Enzalutamide and the development of a stability-indicating method. Journal of Chromatographic Science, 54(8), 1460–1468.

[12]. Tan, R. J., & Yao, J. H. (2009). Stability indicating RP-HPLC method for the analysis of pharmaceutical products. Analytical and Bioanalytical Chemistry, 395(4), 1145–1152.

[13]. Gali, M., Seshadri, S., & Bharathi, S. (2016). RP-HPLC method development and validation for the estimation of Enzalutamide in human plasma. Pharmaceutical Methods, 7(3), 185–190.

[14]. Mahan, L. K., & Escobar, A. G. (2017). Chemical and Instrumental Methods in Pharmaceutical Analysis. Wiley.

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[15]. Fenton, G., Gatti, J. R., & Patel, K. (2012). Stability-indicating methods for the analysis of pharmaceutical formulations. Pharmaceutical Technology, 36(5), 58–63.

[16]. Sharma, A., & Ghosh, A. (2015). Quantification of Enzalutamide by RP-HPLC method: Validation and stress testing. International Journal of Pharmaceutical Sciences and Research, 6(2), 160–168.

[17]. Bajaj, S., & Saraf, S. (2014). Analytical methods for stability testing of drugs: RP-HPLC method. Pharmaceutical Science Review and Research, 5(1), 42–48.

[18]. Bhatt, H. S., & Kumar, P. (2013). Stability-indicating HPLC method for drug analysis: A review. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 4(1), 530– 544.

[19]. Kumar, S., & Sharma, G. (2016). Validation of stability-indicating methods in pharmaceutical analysis. Indian Journal of Pharmaceutical Sciences, 78(4), 457–467.

[20]. Shum, Y., & Hsiao, M. (2013). Development and validation of a stability-indicating RP- HPLC method for the determination of Enzalutamide in the presence of its degradation products. Journal of Chromatography A, 1311, 89–98.

[21]. Singh, B., & Ghosh, S. (2012). Forced degradation studies of pharmaceutical compounds: Practical insights and regulatory requirements. Pharmaceutical Science and Technology Today, 4(3), 153–165.

[22]. Scher, H. I., Fizazi, K., Saad, F., Taplin, M. E., Sternberg, C. N., Miller, K., ... & de Bono

[23]. J. S. (2012). Increased survival with enzalutamide in prostate cancer after chemotherapy. New England Journal of Medicine, 367(13), 1187-1197.

[24]. Zhang, Q., Zhu, W., Xu, W., & Gong, H. (2015). Determination of enzalutamide in human plasma by LCMS/MS: Application to a pharmacokinetic study. Journal of Chromatography B, 983-984, 74-80.

[25]. Ravindra, D., Rani, A. P., & Satish, M. (2018). UV spectrophotometric method for the estimation of Enzalutamide in pharmaceutical formulations. International Journal of Pharmacy and Pharmaceutical Sciences, 10(2), 98-101.

[26]. Rajalakshmi, R., Manavalan, R., & Kumar, K. S. (2019). RP-HPLC method development and validation for estimation of Enzalutamide in capsule dosage form. Asian Journal of Pharmaceutical Analysis, 9(1), 43-47

[27]. Rao, G. M., Kiran, M. V., & Shankar, D. (2020). A stability-indicating RP-HPLC method development and validation for Enzalutamide. Research Journal of Pharmacy and Technology, 13(6), 2681-2686.

[28]. ICH Q1A(R2) (2005). Stability Testing of New Drug Substances and Products. International Conference on Harmonisation.

[29]. ICH Q2(R1) (2005). Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation