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Development and Validation of a Stability-indicating Quality by Design-based HPLC Method for Simultaneous Quantification of Elacestrant and Genotoxic Nitroso Impurities

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Abstract

Nitroso impurities are a class of chemical contaminants that contain the nitroso group (–NO) and typically act as genotoxic agents. Hence, it is necessary to develop a sensitive and reliable analytical method for their accurate quantification to ensure drug safety and regulatory compliance. A Quality by Design (QbD) approach was systematically applied to develop and optimise a method for the separation and simultaneous quantification of elacestrant and its nitroso impurities. In method development, various column types and mobile phase compositions were screened based on the physicochemical properties of analytes, followed by utilising central composite design and response surface methodology. The critical method parameters, such as buffer composition and pH, were identified as key factors that affect the resolution and retention. Several experimental runs were analysed through 3D surface plots and contour graphs to fine-tune the conditions for the effective resolution of elacestrant and its impurities. The finalised method uses a Shim-pack C18 column at 35°C with a mobile phase of 10 mM ammonium acetate buffer (pH 4.3) and acetonitrile in a 65:35 (v/v) at 1.0 mL/min and detection at 238 nm. The method shows excellent linearity ($R^2 > 0.999$) across the tested concentration ranges for both elacestrant and its impurities. Precision studies show low %RSD values confirm the method's reproducibility. The high recovery rates (98–102%) at multiple recovery levels demonstrate the method's accuracy. The method shows high sensitivity with low detection and quantification limits, suggesting the method's suitability for trace-level quantification of impurities. Forced degradation experiments confirm the stability-indicating capability of the method with no interference or co-elution of analytes.

Major Findings: The developed QbD-based stability-indicating RP-HPLC method was found to be sensitive, precise, accurate, robust, and suitable for routine quality control, stability assessment, and regulatory monitoring of elacestrant and its genotoxic nitrosamine impurities in pharmaceutical formulations.

Keywords: Elacestrant, Method Development, Nitroso Impurities, QbD Approach, Stress Degradation Studies

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1. Introduction

The pharmaceutical companies significantly focus on the establishment of sensitive and robust analytical procedures to ensure the safety, quality, and efficacy of drug products[1]. Among various analytical methods, stability-indicating methods gained significant attention because these methods not only have the capability to quantify active

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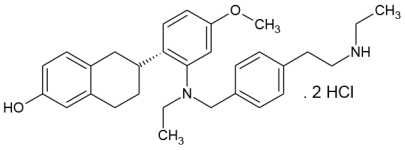
pharmaceutical ingredients (APIs) but are also applicable to quantify potential impurities and degradation products (DPs). The regulatory agencies mandate stringent quality control measures that necessitate the implementation of advanced analytical approaches. In this contest, HPLC has become one of the universally embraced methods used in impurity profiling and stability analysis of drugs and their products[2].

Elacestrant is an orally bioavailable selective estrogen receptor degrader approved for the treatment of postmenopausal women and men with hormone receptor-positive, HER2-negative advanced or metastatic breast cancer who have previously received endocrine therapy[3]. It inhibits the estrogen-driven tumour growth by binding to estrogen receptors and promoting their degradation. It offers a promising alternative treatment for patients who have developed resistance to conventional endocrine therapies like aromatase inhibitors and selective estrogen receptor modulators[4]. The common side effects associated with elacestrant include nausea, vomiting, fatigue, musculoskeletal pain, decreased appetite, and diarrhoea[5].

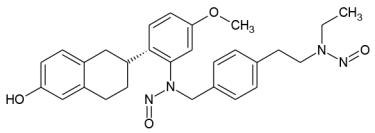
The nitroso impurities are a class of genotoxic contaminants that originate from reactions that involve nitrites, secondary or tertiary amines, and acidic conditions[6]. The nitroso compounds, especially the N-nitrosamines, are well-documented carcinogens, and to minimise patient risk, these impurities are regulated by stringent limits by the FDA and ICH. Even trace levels of nitroso impurities in pharmaceutical formulations can compromise drug safety, and this necessitates the development of highly sensitive analytical methods for their detection and quantification[7]. Their presence can also reduce drug stability and affect potency and therapeutic efficacy[8]. Hence, it is very crucial to propose robust quality control measures, such as stability-indicating HPLC methods for quantification of nitroso impurities, and to ensure regulatory compliance.

The QbD is a scientific method of drug product development focused on understanding the process and control to achieve the desired product quality. The QbD implementation in analytical method development assures the development of rugged, reproducible, and regulatory acceptable methodologies[9]. The major components of the QbD strategy are risk assessment, design of experiments (DoE), and method optimisation to determine the critical method parameters (CMPs) and critical quality attributes (CQAs) that affect analytical performance. Applying a QbD-based strategy in HPLC method development improves method robustness, reliability, and regulatory compliance[10].

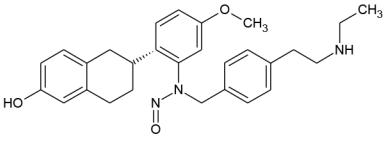
In literature, Conlan MG *et al.*,[11] reported pharmacokinetic and pharmacodynamic studies of



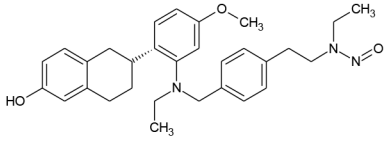
Elacestrant
Molecular Formula: $C_{30}H_{38}N_2O_2$. 2(HCl)
Molecular Weight: 458.6.2(36.5) g/mol



NNDND Impurity
Molecular Formula: $C_{28}H_{32}N_4O_4$
Molecular Weight: 488.6 g/mol
IUPAC Name: *(R)-N-Ethyl-N-(4-(((2-(6-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)-5-methoxyphenyl) (nitroso)amino) methyl) phenethyl) nitrous amide*



NNND Impurity
Molecular Formula: $C_{28}H_{33}N_3O_3$
Molecular Weight: 459.6 g/mol
IUPAC name: *(R)-N-(4-(2-(ethylamino) ethyl) benzyl)-N-(2-(6-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)-5-methoxyphenyl) nitrous amide*



NN Impurity
Molecular Formula: $C_{30}H_{37}N_3O_3$
Molecular Weight: 487.6 g/mol
IUPAC Name: *(R)-N-ethyl-N-(4-((ethyl(2-(6-hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)-5-methoxyphenyl) amino) methyl) phenethyl) nitrous amide*

Figure 1. Details of elacestrant and its nitroso impurities in the study.

elacestrant. Subhashini *et al.*,[12] reported UPLC based analytical method for quantification of elacestrant in formulations.

Kumar *et al.*,[13] reported a UPLC method for the quantification of process-related impurities of elacestrant. Hu *et al.*,[14] characterise the reported HPLC–MS/MS *in vitro* metabolites of elacestrant in Human Liver Microsomes. These reported methods are suitable for the quantification of elacestrant in formulations, biological samples, and process-related impurity analysis. Despite the growing regulatory concern about genotoxic nitroso impurities in pharmaceutical products, no validated analytical method has been reported for the simultaneous quantification of process-related genotoxic impurities in elacestrant. Furthermore, there is a lack of stability-indicating and QbD-based HPLC methods capable of detecting and quantifying these impurities at trace levels in compliance with current regulatory requirements. Hence, this study aims to develop and validate a sensitive and robust analytical method for the detection and quantification of nitroso impurities such as N, N-Dinitroso N-Desethyl (NNDND) impurity, N-Nitroso N-Desethyl (NNND) impurityand N-Nitroso (NN) impurity in elacestrant using HPLC. As no method has been reported for the quantification of selected nitroso impurities of elacestrant, this proposed study will be the first and best option for impurity profiling of elacestrant. The structure and systemic details of elacestrant and its nitroso impurities were presented in Figure 1.

2. Materials and Methods

2.1 Chemicals and Materials

The API of elacestrant, along with its three nitroso impurities and formulation (Orserdu-86 mg), was generously provided as a gift sample by Enanti Labs, Visakhapatnam, Andhra Pradesh, India. The HPLC-grade solvents purchased from Merck, Mumbai, were utilised to ensure accuracy and reliability of chromatographic experiments, whereas analytical reagent-grade chemicals utilised in this study were purchased from Fisher Scientific, Mumbai.

2.2 Instrumentation

The analytical method for quantification of elacestrant and its nitroso impurities was conducted on a Shimadzu (USA) HPLC system with binary LC 10AT vp pumps, a manual Injector, a Shim-pack (250 mm) GIST (Shimadzu) C18 column, and an SPD 10Avp UV-Visible detector. The entire chromatographic setup was operated through the SPD-10A VP system controller, whereas data collection and processing were conducted through the LC Solutions software platform.

2.3 Chromatographic Conditions

The separation of elacestrant and its nitroso impurities was achieved using a Shim-pack GIST C18 column (250 mm × 4.6 mm, 3.5 µm) under isocratic elution mode. The 10 mM ammonium acetate buffer (pH 4.3) and acetonitrile in a 65:45 (v/v) ratio at 1.0 mL/min provides optimal resolution and peak symmetry. The resolved analytes were recorded through a UV detector at 238 nm. The column temperature was set at 35°C to enhance chromatographic performance. The manual injector was used to introduce samples into the chromatographic system with an injection volume of 20 µL. The entire chromatographic analysis was performed under ambient conditions to ensure accurate and reproducible results.

2.4 Preparation of Standard and Calibration Solutions

An elacestrant solution of 10 mg was initially prepared by accurately weighing the substance and transferring it to a 10 mL volumetric flask. The drug was dissolved in a diluent made from a combination of equal parts methanol and acetonitrile, and the volume was adjusted to 10 mL to give a concentration of 1 mg/mL (1000 µg/mL). This stock solution was then diluted to make working standards between 5 and 30 µg/mL. Similarly, impurity solutions with concentrations between 0.050 and 3.0 µg/mL were prepared separately using the same diluent. Solutions were kept at 4°C for further analysis.

2.5 Method Development

The chromatographic parameters were systematically optimised to establish an effective HPLC method for quantification of elacestrant and its nitroso impurities[15,16]. Multiple stationary phases that include Phenomenex C18, X-Bridge Phenyl C18, and Shim-pack GIST C18 columns of 150 mm and 250 mm configurations were tested to determine the most suitable column that produces ideal retention characteristics and selectivity for elacestrant and its impurities. The mobile phase composition was

refined by optimising different aqueous buffer systems, such as acetate and phosphate buffers across various pH levels in combination with methanol and acetonitrile as organic modifiers that were tested. The chromatographic separation was carried out under isocratic elution mode, and the key method parameters, like flow rate and column temperature, were also carefully adjusted to enhance sensitivity and separation efficiency. Finally, the QbD approach was utilised to finalise the optimised conditions that have the ability to deliver reproducible and reliable results.

2.6 QbD Approach

The finalisation of analytical conditions that produce accurate, reliable, and regulatory compliance results for elacestrant and its nitroso impurities was performed by adopting the QbD approach. The essential Quality Target Product Profile (QTPP) attributes, such as retention time, peak symmetry, theoretical plates, and the resolution between elacestrant and its impurities, were identified to achieve a well-defined analytical method. These QTPPs were carefully optimised to enhance method efficiency and performance. The CQAs, such as mobile phase composition and its pH, were systematically controlled to maintain precision and reproducibility in the results. A central composite experimental design using Design Expert® software (Version 11.0, Stat-Ease Inc.) was utilised to examine the influence of these CQAs on method performance. The final method selection was based on its ability to provide superior separation efficiency with consistent and reproducible results. Furthermore, a thorough risk assessment was conducted by following ICH Q8 and Q9 guidelines to assess the robustness and ruggedness of method[17,18]. This assessment ensures that the procedure was consistent under slight differences in operating conditions and when used by various analysts. The optimised procedure exhibits excellent reliability, which makes it ideal for the quantification of elacestrant and its impurities in quality control analysis.

2.7 Method Validation

The optimised method was thoroughly validated in accordance with the ICH Q2 (R1) guidelines to ensure its accuracy, precision, and reliability for routine pharmaceutical analysis[19]. The key analytical parameters were evaluated to confirm method effectiveness. The method linearity was evaluated by analysing six different concentrations of elacestrant (5-30 µg/mL) and its impurities (0.050-3.0 µg/mL). A strong correlation coefficient (R^2 value) and a direct and proportional relationship between the concentration and peak response confirm the method's linearity. Method precision was determined by repeatability, analysing the mid-range concentration of elacestrant and its impurities at two-hour intervals (n = 6) within the same day (intraday precision), whereas the same concentration was evaluated over two consecutive days for inter-day precision. The relative standard deviation (%RSD) of peak area response was calculated, and a value of less than 2% indicates that the method was precise and reproducible. Method accuracy was validated through recovery studies in which known amounts of elacestrant and its impurities were spiked at three concentration levels (50%, 100% and 150%) within the established linearity range. The recovery percentage between 98% and 102% demonstrates that the method accurately measures the actual content of elacestrant and its impurities without interference. The method sensitivity was determined by calculating the limit of detection (LOD) and limit of quantification (LOQ) using the standard deviation of the y-intercept (σ) and the slope (SD) of the calibration curve. The formulas such as LOD = 3.3 × σ/SD and LOQ = 10 × σ/SD were applied to establish sensitivity levels of elacestrant and its impurities in the proposed method. The method's robustness was assessed by introducing minor variations in the finalised conditions, and then the results were utilised to determine the influence of these adjustments on method performance. Additionally, system suitability of resultant chromatograms was verified by evaluating retention time, theoretical plates, and peak asymmetry to ensure that the method remains consistent and reproducible for the routine analysis of elacestrant and its impurities in pharmaceutical formulations.

2.8 Forced Degradation Studies

Stress degradation experiments were conducted to evaluate the chemical stability of elacestrant under different stressful conditions. The experiments were designed to establish the susceptibility of the compound to degradation and to prove the selectivity and reproducibility of the analytical method developed[20]. Various stress conditions, such as acidic, alkaline, oxidative, reductive, thermal, photolytic, and hydrolytic degradation, were used to evaluate the possible degradation of elacestrant. In acid degradation, 50.8 mg

of elacestrant was weighed accurately and transferred to a 10 mL volumetric flask. It was then reacted with 1 mL of aqueous HCl (1N) and left to settle for 15 min to induce degradation. This reaction was then neutralised before diluting with the desired diluent. Alkaline and oxidative degradation involve almost the same procedure by replacing HCl with aqueous NaOH (1N) and peroxide (10%) solution. Similarly, reductive degradation was induced by adopting the same procedure by replacing HCl with 1 mL of an aqueous solution of sodium bisulfite (10%). 50 mg of elacestrant was subjected to 105°C for 6 hours to induce thermal degradation, and after heating, 20 mg of the exposed sample was dissolved in 7 mL of diluent, sonicated for 5 minutes, and then further diluted to the standard concentration before analysis. Photolytic degradation was assessed by subjecting 70 mg of elacestrant to a photostability chamber for 6 hours to mimic long-term light exposure, whereas the hydrolytic degradation was performed by incubating elacestrant with 1 mL of HPLC-grade water for 15 min, followed by dilution, sonication, and filtration. All samples exposed to stress were tested in the proposed HPLC method to identify the formation of DPs and determine the stability and specificity of the analytical method.

3. Results and Discussion

In this study, a systematic approach was employed to develop a reliable and efficient chromatographic method for the simultaneous quantification of elacestrant and its nitroso impurities. The method development was initiated with an extensive review of literature, along with a detailed evaluation of the physicochemical characteristics of elacestrant and its related impurities.

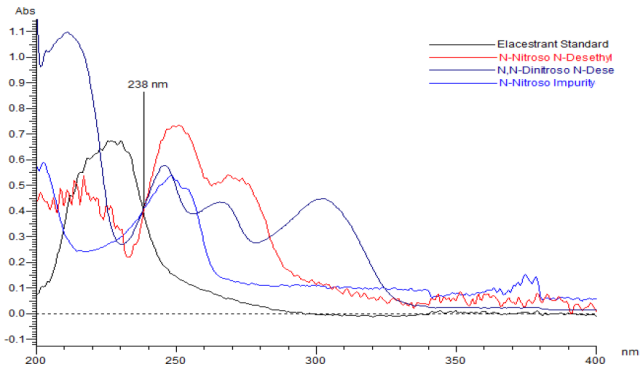


Figure 2. Overlay UV spectrum of elacestrant and its nitroso impurities.

The key chromatographic variables, such as stationary phase selection (column type), mobile phase, and detection wavelength, were carefully fine-tuned to achieve optimal resolution, sharp and symmetrical peak shapes, and high sensitivity. Initially, the UV absorption behaviour of elacestrant and its related nitroso impurities was investigated using a UV-visible spectrophotometer to identify a suitable wavelength for simultaneous detection. The overlay UV spectrum demonstrates that elacestrant and its nitroso impurities exhibit significant absorbance at 238 nm (Figure 2), and this was confirmed as the effective wavelength for detection.

Method development was initially carried out using a Phenomenex C18 column by employing methanol, acetonitrile, and water in a volumetric ratio of 50:30:20. The resultant chromatogram shows inadequate separation between elacestrant and its nitroso impurities, along with a disturbed baseline (Figure 3A). The usage of the X-Bridge Phenyl C18 column doesn't produce adequate separation of analytes. In this column, the peak corresponds to elacestrant eluting at a long retention time, whereas the overlapped impurity peak was noticed (Figure 3B). The utilisation of a Shim-pack column with a phosphate buffer as a mobile phase component displays peaks corresponding to analytes in the study. The resolution, symmetry, and response of identified peaks were noticed to be inadequate (Figure 3C), suggesting that the buffer utilised in the study doesn't have the capability to resolve the analytes. The introduction of ammonium acetate buffer in the mobile phase produces well-resolved peaks (Figure 3D), suggesting that the buffer was suitable for the resolution of analytes.

The QbD approach was applied to further improve the analytical method. This strategy helps in developing a reliable and strong method for the accurate analysis of elacestrant along with its nitroso impurities. The initial method development trials prove that the resolution between closely eluting impurities such as NNDND and NNND was a key target for method performance. This requirement was set as QTPPs and ensures the method produces high resolution between these two compounds throughout the analysis. Meanwhile, the buffer composition and its pH were identified as CQAs, and these two critical factors were evaluated using a statistical DoE approach as tabulated in Table 1. The composition of the buffer was considered as factor A and was studied within the range between 60.00% (coded as -1) and 70.00% (coded as +1), whereas the buffer pH was

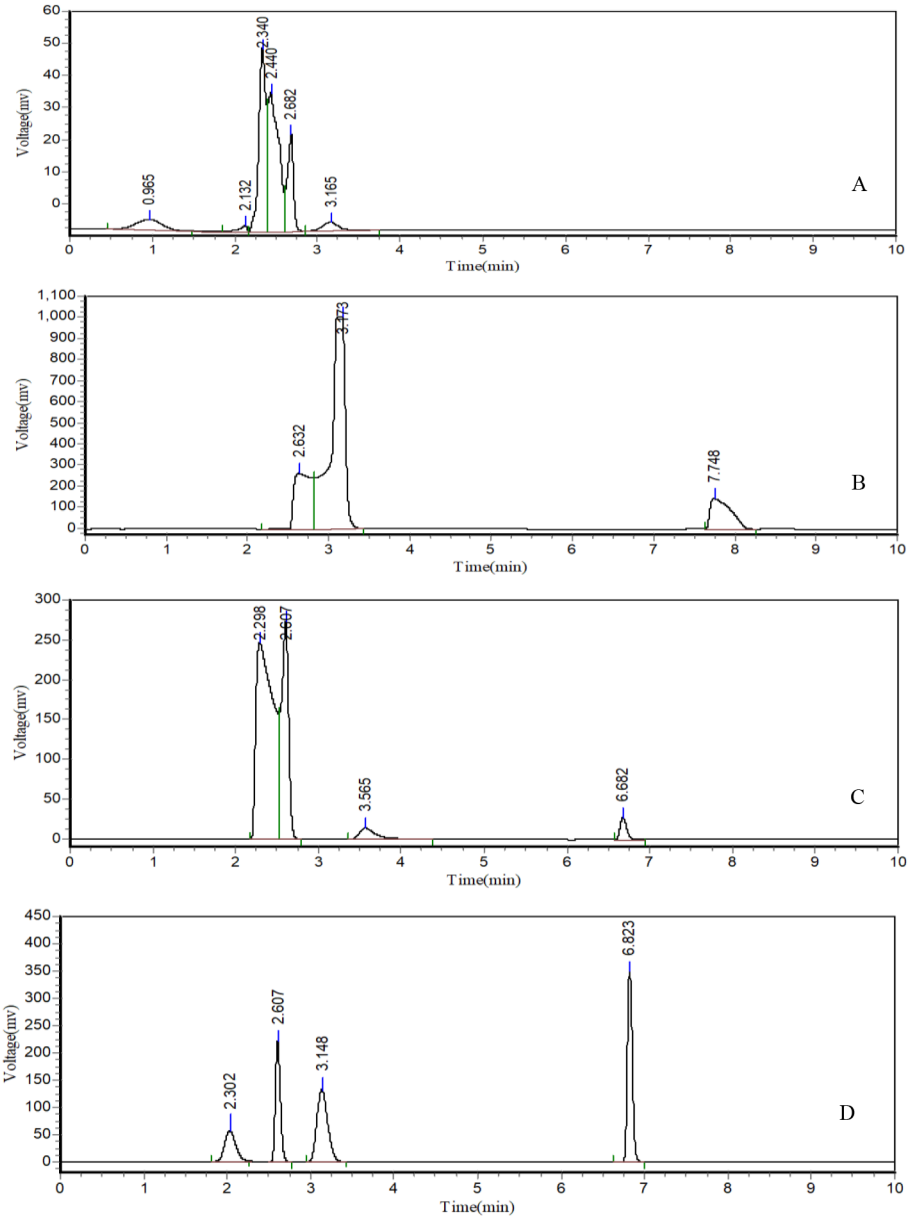


Figure 3 **(A-D).** Method optimisation chromatograms observed for analysing elacestrant and its nitroso impurities.

Table 1. Factors selected during optimisation of analytical method parameters for the analysis of elacestrant and its nitroso impurities

Factor	Name	Units	Type	Sub Type	Minimum	Maximum	Coded Low	Coded High	Mean	Standard Deviation
A	Buffer	count	Numeric	Continuous	60.00	70.00	-1 ↔ 60.00	+1 ↔ 70.00	65.00	4.710
B	pH	count	Numeric	Continuous	3.30	5.30	-1 ↔ 3.30	+1 ↔ 5.30	4.30	0.9428

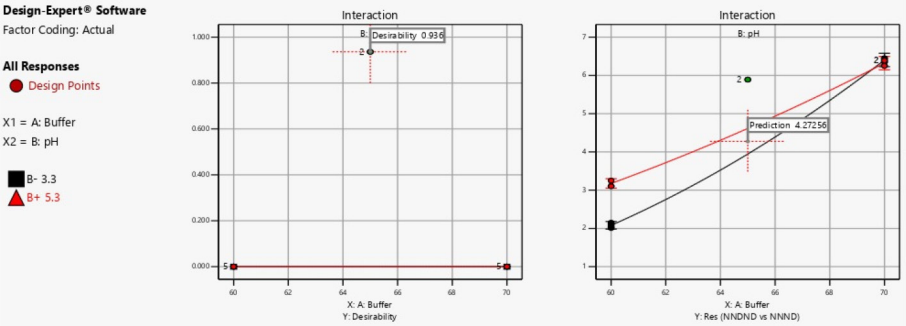


Figure 4. DoE interaction plots were noticed in the optimisation of buffer concentration and volume for the analysis of elacestrant and its nitroso impurities.

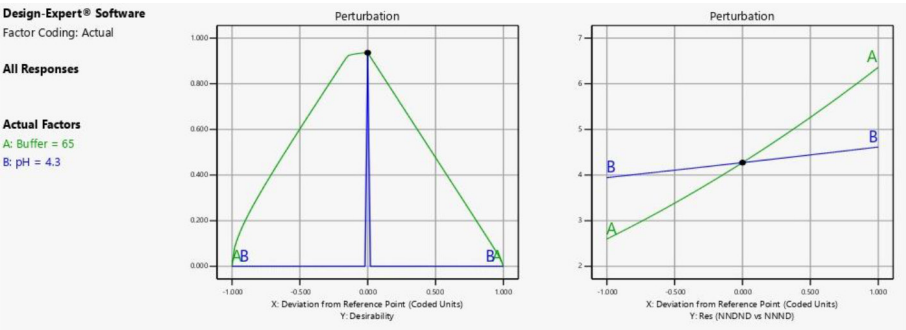


Figure 5. DoE perturbation plots were noticed in the optimisation of buffer concentration and volume for the analysis of elacestrant and its nitroso impurities.

considered as factor B and was evaluated within the range of pH 3.30 (coded as -1) to pH 5.30 (coded as +1).

The interaction plots (Figure 4) and perturbation plots (Figure 5) from Design-Expert® software show the influence of buffer concentration and pH on the resolution of NNDND and NNND impurities as well as the overall method desirability. At lower pH (3.3), both resolution and desirability remain low, even with changes in buffer levels. However, at higher pH (5.3), the resolution improves significantly as the buffer concentration increases and reaches the best separation at a buffer level of 70. Similarly, the desirability score was noticed to be very high (around 0.936) at a buffer pH of 5.3 with a buffer volume of 65, indicating that this is the most favourable condition for the method. Similarly, perturbation plots show that the desirability increases sharply as buffer concentration approaches the centre point (65) and then decreases, indicating that the method performs best at this specific buffer level. The buffer pH has minimal effect on desirability. As the buffer concentration increases, the resolution between NNDND and NNND improves steadily, whereas pH shows a slight positive effect.

The contour plots (Figure 6) were plotted to display buffer concentration influence and pH on both desirability and resolution between NNDND and NNND impurities. In the desirability plot (left), the highest desirability value of 0.96002 is observed near the centre, indicating an optimal region around buffer 65 and pH 4.3. The resolution plot (right) shows a gradual increase in resolution with an increase in buffer concentration, whereas the change in pH exhibits a small response. The predicted resolution value at the centre point is 4.27256 (rounded as 4.3), confirming that this combination of buffer and pH provides a good balance for achieving high resolution and method performance.

The influence of buffer strength and pH on the response is illustrated in the 3D surface plot (Figure 7, left). It is evident that as both buffer and pH increase, the response value also increases and reaches a peak at higher levels of both factors. This implies a synergistic effect where higher buffer and pH values together contribute significantly to an increase in the response value. The "Predicted *vs.* Actual" plot (Figure 7, right) shows a strong agreement between the predicted and actual values

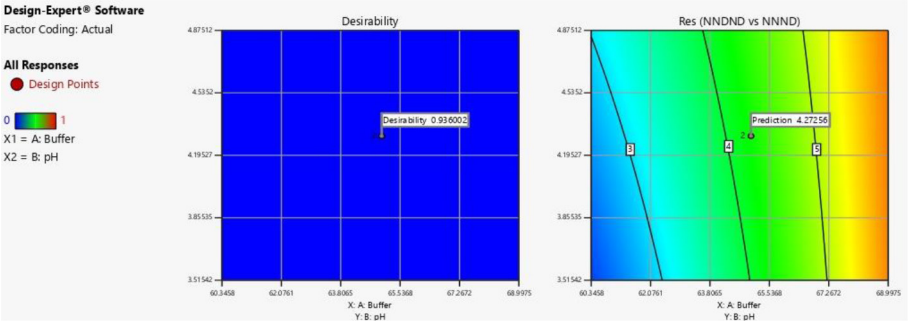


Figure 6. DoE contour plots were noticed in the optimisation of buffer concentration and volume for the analysis of elacestrant and its nitroso impurities.

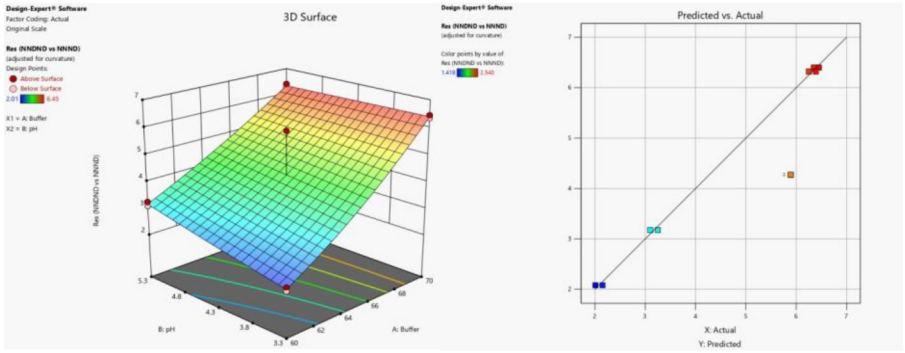


Figure 7. DoE 3D surface and predicted *vs.* actual plots noticed in the optimisation of buffer concentration and volume for the analysis of elacestrant impurities.

of the response, indicating that the model fits the data well. Most of the data points lie close to the diagonal line representing perfect prediction, suggesting the method's accuracy.

The result observed through the QbDDoE approach facilitates the successful optimisation of chromatographic conditions for the analysis of elacestrant and its nitroso impurities. The finalised method utilises a Shim-pack C18 column at 35°C using 10 mM ammonium acetate buffer (pH 4.3) and acetonitrile in a 65:35 (v/v) ratio at 1.0 mL/min and 238 nm wavelength. These optimised conditions ensure the precise and consistent quantification of elacestrant and its nitroso impurities with high method robustness and minimal variability. The optimised chromatogram observed in this study is displayed in Figure 8. The developed method exhibits excellent chromatographic performance for the separation of elacestrant and its nitroso impurities. The method elutes analytes at retention times of 2.065, 2.673, 3.873, and 4.482 min, respectively, for NNDND impurity, NNND impurity, NN impurity, and elacestrant, indicating a well-resolved elution profile. The method demonstrates good column efficiency and was reflected by observing theoretical plate counts of 5987 for NNDND, 8745 for NNND, 11857 for NN, and 14930 for the elacestrant, whereas the tailing factors for all peaks were within the acceptable range, suggesting symmetrical peak shapes with minimal distortion. Further, the resolution between successive peaks was satisfactory, confirming the method's ability to clearly distinguish and quantify elacestrant and its nitroso impurities.

The developed analytical method demonstrates a high degree of sensitivity, as evidenced by the determined LOD and LOQ for the studied impurities of elacestrant impurities. The LOD was determined to be 0.015 µg/mL, whereas the LOQ was calculated as 0.05 µg/mL. These values indicate that this method can reliably detect impurities at trace amounts, and these results highlight the method's suitability for applications requiring the analysis of extremely low concentrations. The linearity of impurities was constructed from the LOQ level and ensured that the linear concentration of the standard would be 100% higher than the concentration of impurities. The method shows excellent linearity over the concentration

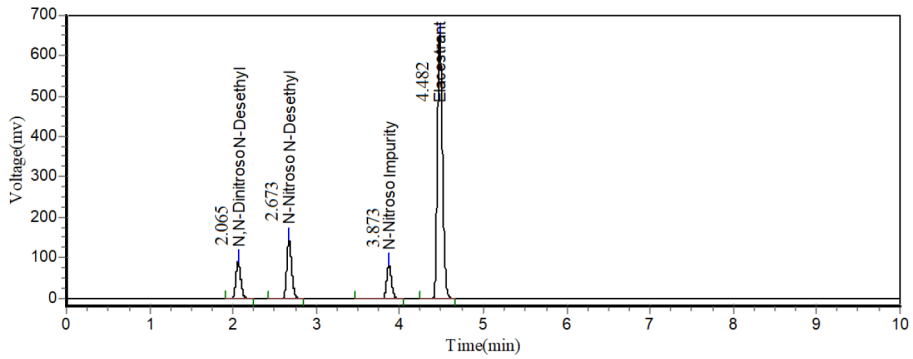


Figure 8. The chromatogram obtained under the optimised conditions clearly demonstrates the effective separation of elacestrant and its nitroso impurities.

range of 5 to 30 µg/mL for elacestrant, while its nitroso impurities exhibit linearity within 0.05 (LOQ) to 0.3 µg/mL. The linear regression equations were observed to be y = 267353x + 163824 (R^2 = 0.999) for elacestrant, y = 305684x + 673.32 (R^2 = 0.9998) for NNDND impurity, y = 455354x + 473.83 (R^2 = 0.9996) for NNND impurity, and y = 282500x + 346.37 (R^2 = 0.9995) for NN impurity. The correlation coefficients (R^2) values were found to be very close to unity, confirming a strong linear relationship between concentration and response. The precision of the method was evaluated through both intraday and interday studies. The % RSD values for elacestrant and its related impurities were all achieved within the generally accepted criteria of less than 2%. This result confirms that the method yields reproducible results over multiple runs and across different days. Method accuracy was assessed by recovery studies at three different spiking levels (50%, 100%, and 150%). The % recovery for elacestrant ranged from 99.24% to 100.29%, whereas nitroso impurities were in the range of 99.26% to 100.36% for the NNDND impurity, 98.28% to 100.66% for the NNND impurity, and 99.11% to 100.94% for the NN impurity. In all cases, the recovery values fell within the acceptable range of 98% to 102%, and the % RSD values remained below 2%, indicating that the method is both reliable and accurate for quantification. Method ruggedness was assessed by employing different analysts on separate days, and the %RSD results remain well within the permissible limit, demonstrating that the method remains consistent under variable conditions. The robustness of the method was determined by introducing deliberate minor changes in the experimental conditions, such as mobile phase composition, its pH, and detection wavelength. The results demonstrate that the developed method is reliable and consistent, even when subjected to slight variations in analytical parameters. Minor changes in method conditions display minimal fluctuations in peak areas, with percentage changes ranging from 0.12% to 0.53% for elacestrant and 0.01% to 1.43% for its impurities. These entire variations fell within the acceptable limit of less than 2%, confirming that the method is not significantly affected by small changes in experimental conditions. The summary of these validation results was tabulated in Table 2.

The forced degradation study was performed to assess the chemical stability of elacestrant when exposed to various stress conditions in the proposed method. The chromatogram of the control sample that was not subjected to any stress displays a single sharp peak corresponding to elacestrant, confirming its purity and indicating no degradation. Under acidic conditions (Figure 9A), the drug underwent 5.75% degradation, and the chromatogram reveals two additional peaks at 1.898 min and 3.182 min, suggesting the formation of DPs. Similarly, basic conditions (Figure 9B) induce 4.23% degradation with two new peaks at 2.532 min and 3.148 min indicates the chemical breakdown of elacestrant under alkaline stress. In the oxidative degradation study using hydrogen peroxide, 2.36% degradation was observed, with an additional peak appearing at 4.173 min, suggesting the partial degradation of elacestrant due to oxidation. In contrast, under other stress conditions studied, it displays minimal degradation with less than 2% degradation, and no new peaks were detected indicate that elacestrant remains stable under those conditions. Overall, the method effectively separates the elacestrant from its DPs, and nitroso impurities confirm that it is a reliable stability-indicating method suitable for routine analysis and long-term stability studies.

Table 2. Method validation summary results were noticed in the method proposed for the analysis of elacestrant and its nitroso impurities

Validation Parameter	Result			
	Elacestrant	NNDND	NNND	NN
Linearity[!]	5 to 30	0.05-0.3		
Slope	267353	305684	455354	282500
Intercept	163824	673.32	473.83	346.37
R^2	0.9990	0.9998	0.9996	0.9995
Intraday Precision $	0.305	0.282	0.358	0.847
Interday (day 1) $	0.268	0.159	0.253	0.283
Interday (day 2) $	0.453	0.316	0.322	0.246
Ruggedness (analyst 1) $	0.196	0.671	0.278	0.360
Ruggedness (analyst 2)C$	0.519	0.322	1.279	0.176
Robustness $	0.565	0.276	0.839	0.417
50% level recovery &	99.60	99.81	99.00	99.21
100% level recovery &	100.06	99.57	99.63	100.01
150% level recovery &	99.60	100.20	100.21	100.73
Mobile phase composition (+ change) $$	0.41	0.42	0.08	1.43
Mobile phase composition (- change)[$$]	0.53	0.75	0.41	1.00
Mobile phase pH (+ change) $$	0.12	0.85	0.64	0.26
Mobile phase pH (- change) $$	0.35	0.22	0.12	0.82
Wavelength (+ change) $$	0.25	0.01	0.22	0.07
Wavelength (- change) $$	0.53	0.64	0.45	0.01
LOD[!]	---	0.015		
LOQ[!]	---	0.05		

Results expressed in the units of **µg/mL** ([!]), % RSD units ([$]), percentage ([&]) and % change ([$$]); n = 3

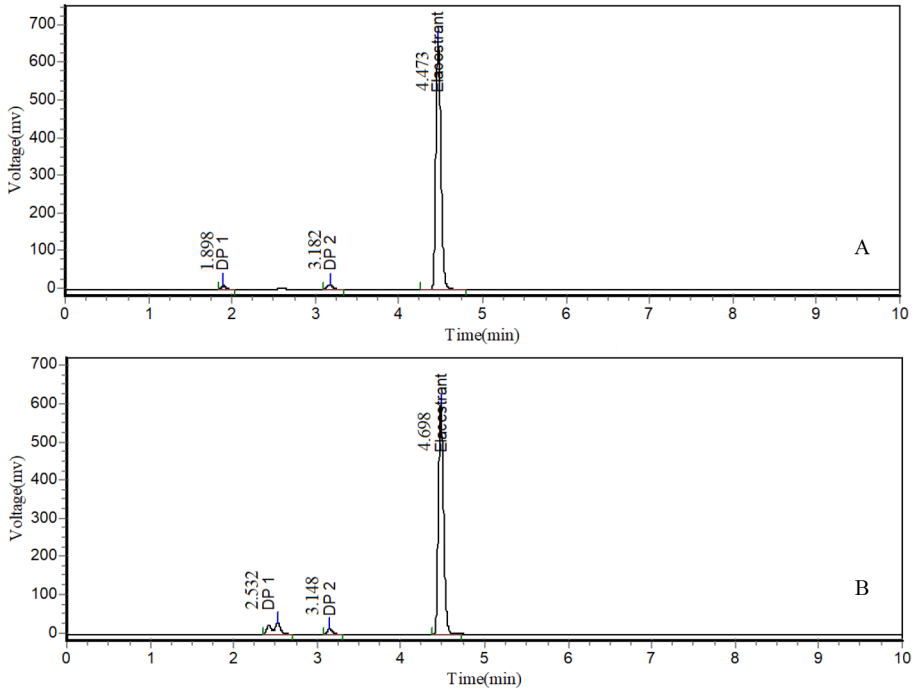


Figure 9. **(A)** Acid and **(B)** base stress degradation chromatograms of elacestrant in the proposed method.

The novelty of this study was proved by correlating the findings achieved in this study with available literature. In literature, no method offers the resolution and quantification of elacestrant and its nitroso impurities. Hence, this newly established method demonstrates adequate sensitivity, selectivity, and reliability that make it ideal for routine quality control and regulatory purposes. Importantly, the study was designed through a QbD approach that allows systematic optimisation of critical method parameters and ensures enhanced chromatographic resolution, better peak shapes, and accurate quantification of elacestrant and its nitroso impurities.

4. Conclusion

This study successfully proposed a robust, accurate, and highly reliable HPLC method for the simultaneous quantification of elacestrant and its nitroso impurities. During the method optimisation process, thorough and systematic strategies were employed with a special focus on key chromatographic parameters such as the type of column and the composition and pH of the mobile phase. The method was fine-tuned by adopting a QbD approach to achieve excellent resolution, sharp peak shapes, and enhanced sensitivity with high robustness under varied analytical conditions. The final method comprises a Shim-pack C18 column at 35°C with a mobile phase of 10 mM ammonium acetate buffer (pH 4.3) and acetonitrile in a 65:35 (v/v) at 1.0 mL/min. This setup provides efficient separation of elacestrant and its nitroso impurities with minimal peak tailing and consistent retention times. The developed method demonstrated high sensitivity with LOD and LOQ values of 0.015 µg/mL and 0.05 µg/mL, respectively proves its suitability for trace-level impurity analysis. Excellent linearity was achieved for elacestrant and its impurities with correlation coefficients greater than 0.999. The validation results prove that the method was precise, accurate, rugged, and robust, as evidenced by low %RSD values and recovery values within the acceptable range of 98–102%. Deliberate variations in chromatographic conditions produced minimal changes in analytical performance proves the reliability of the method during routine application. Forced degradation studies suggest that elacestrant undergoes significant degradation under acidic, basic, and oxidative stress conditions. Importantly, the developed method effectively separated elacestrant from its degradation products and nitroso impurities without interference and thereby proves its stability-indicating capability. Overall, this method proves to be highly suitable for routine quality control, impurity profiling, and regulatory applications of elacestrant and its nitroso impurities in pharmaceutical analysis.

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Author Contribution

Conceptualisation and methodology: K.S and M.M.; Software, validation, and formal analysis: K.S and M.M.; Investigation: M.M.A. and S.A.B.; Resources: R.S.; Data curation and writing—original draft preparation: K.S and M.M.; Writing—review and editing: K.S.; Visualisation: R.S.; Supervision: K.S., R.S., S.A.B., M.M.A. All authors have read and agreed to the published version of the manuscript.

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