

RESEARCH ARTICLE

Development and Validation of a Stability-Indicating Reversed-Phase High-Performance Liquid Chromatography Method for Elagolix Sodium using Quality by Design Approach

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ABSTRACT

This study developed a stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method for the estimation of elagolix sodium of the bulk drug by using a quality by design (QbD) approach. In a QbD approach, Box-Behnken screening is based on critical method parameters, i.e., initial % acetonitrile (ACN), column temperature, and flow rate. The interaction effect of this parameter [retention time, number of theoretical plates (NTP), and tailing factor] was evaluated in 3D response graphs. The plots revealed the final chromatographic conditions of the method. The present paper describes a new, simple, precise, accurate, and development for estimation of elagolix sodium by the RP-HPLC method. The described chromatographic method was standardized using a C18 column (Inertsil ODS-3 C18 column 250 × 4.6 mm, 5 μ) with gradient elution and mobile phase containing 0.05% trifluoroacetic acid:acetonitrile (55:45 v/v) at a flow rate of 1 mL/min. The eluents were detected by the photo-diode array (PDA) detector at 275 nm. The linearity study of elagolix sodium was found in the concentration range of 1 to 3 μg/mL, and the correlation coefficient (r^2) was found to be 0.9992%. The developed method was successfully applied to the bulk drug, as well as, successfully applied for forced degradation studies. Forced degradation studies, include acid hydrolysis, base hydrolysis, oxidation, thermal degradation, and photolytic degradation of elagolix sodium.

Keywords: Elagolix sodium, QbD, Stability indicating assay method, Validation.

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INTRODUCTION

The fundamental premise behind QbD is that quality is designed into the process at the beginning to establish a thorough understanding of the response of the system quality to system parameters, leading ultimately to the establishment of the design space for the method. The QbD approach is a recent trend in analytical method development, and it helps a lot if it gets properly implemented. QbD approach suggests looking into the quality of the analytical process during the development stage itself. It says that quality should be built into the process design rather than tested into the final results of the analytical process.^{1,9} Traditional chromatographic method development has always involved the time-consuming process of varying one system parameter at a time, examining its effect on the method and system operation.

This generally requires a large number of experimental runs, and in most situations, the developed method requires further

development. Elagolix sodium belongs to the GnRH class of drugs.² Medicine is used in the treatment of pain associated with endometriosis in women. Elagolix is a GnRH receptor antagonist that inhibits endogenous GnRH signaling by binding to GnRH receptors in the pituitary gland. Administration of elagolix results in dose-dependent suppression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), leading to decreased blood concentrations of the ovarian sex hormones, estradiol, and progesterone.³

Stability indicating assay methods are reported for the simultaneous determination of elagolix sodium in the presence of their degradants and their associated main impurities. The procedure gave acceptable results with fresh products but gave overestimation during the analysis of stability samples and aged products. Therefore, the focus in the present study was to develop a stability-indicating HPLC method for the combination, by degrading the drugs together under various

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stress conditions according to ICH. The drugs were separated from degradation products on an RP-HPLC column.¹¹

MATERIAL AND METHODS

Chemicals and Reagents

The working standard of elagolix sodium was obtained as gift samples from Dr. Reddy's Pharmaceuticals Private Limited, Hyderabad, India. HPLC grade methanol procured from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, trifluoroacetic acid (Merck), Water use Millipore-Merck, and HPLC grade acetonitrile procured from Finar.

Equipment

The HPLC system consisted of waters 2996 photodiode array detector. The chromatographic separations were performed using C18 Inertsil ODS (250 × 4.6 mm × 5 μ) column, eluted with the mobile phase at the flow rate of 1 mL/min. The mobile phase consisted of ACN and 0.05% trifluoroacetic acid (45:55 v/v). Measurements were made with an injection volume of 10 μL and ultraviolet (UV) detection at 275 nm. For the analysis of forced degradation samples, the photodiode array detector was used in scan mode with a scan range of 220 to 400 nm and the desired peak coverage of 100%. The output signal was integrated using. Peak homogeneity was expressed regarding peak purity and was obtained directly from the spectral analysis report obtained using the software as mentioned above.

Solution Preparation

100 mg of elagolix sodium was accurately weighed and transferred to a 100 mL volumetric flask. The sample was dissolved in a sufficient amount of methanol and the volume was made up to the mark (1,000 μg/mL) (solution A). 1 mL of this solution A was diluted to 10 mL with methanol (100 μg/mL) (solution B). Further, 1 mL of this solution B was diluted to 10 mL with diluent (mobile phase) (10 μg/mL) (solution C). Solution C was used to carry out the validation parameters.

Initial Method Development

Choice of Wavelength

10 mg of elagolix sodium working standard was weighed accurately and transferred to a 10 mL volumetric flask and the volume was made up with methanol to give a concentration of 1,000 μg/mL (solution A). Solution A was further diluted with methanol to get a concentration of 10 μg/mL. UV spectrum of a solution having a concentration of 10 μg/mL was recorded using methanol as blank. It showed absorbance at a wavelength of 275 nm, so it was selected λ_{max} for elagolix sodium.

Choice of Diluent

A suitable diluent for elagolix sodium was selected by checking its solubility in methanol, acetonitrile, and distilled water. Finally, methanol was chosen based on the solvent which gave optimum solubility.

Choice of Column

Different stationary phases, C8, and C18 columns along with the mobile phase constituted of different ratios of water,

acetonitrile, and methanol were tried to get the desired separation of peaks. After carrying out the basic trials, the C18 column shows optimum retention than the C8 column. Hence, the C18 column was selected for further method development.

Method Optimization by QbD Approach

The QbD approach for simultaneous RP-HPLC method development: The concepts described in ICH guidelines Q8 to Q10 are commonly referred to as QbD in a nutshell. QbD can be defined as a systematic approach that begins with a predefined objective, and it mainly focuses on the product, its process, and its control based on a logical and profound knowledge of the science involved and quality risk management.⁹ Different combinations of water and methanol were used for the optimization of the mobile phase.

The following steps can divide method development using QbD approach:

Analytical Target Profile

The primary aim was to develop a more robust method and validation of a developed method for the estimation of elagolix sodium. QbD approach was applied to get a method operable design region (MODR). Analytical target profile (ATP) is the target setting process in the approach of developing the analytical method. The method performance expectations, such as, specificity, linearity, range, precision, accuracy, detection limit, quantification limit, robustness, and system suitability assumed as predefined targets according to the ICH guidelines for the analytical method development.

Risk Assessment (RA)

Quality risk management (QRM) (ICH Q9) is a systematic process for the assessment, control, communications, and review of risks to the quality across the lifecycle. RA is an integral part of the analytical QbD process. Their use facilitates identification and ranking of parameters that could impact method performance and conformance to the ATP. Risk assessment approaches that begin with mapping tools, such as, flow-chart and Ishikawa fishbone diagram, as shown in Figure 1 to evaluate risk.

Design Space

The multinational combination and interaction of input variables and process parameters that have been demonstrated to assure the quality of the data produced by the method. The selection

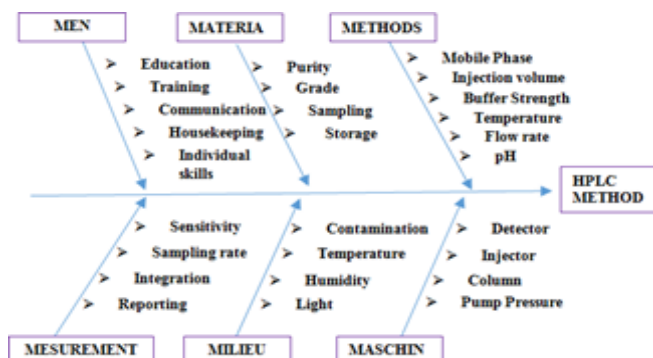


Figure 1: Ishikawa fishbone diagram

of critical process parameters (CPP) and critical quality attribute (CQA) is an integral part of design space formation.

Critical Quality Attribute (CQA)

CQA is a “physical, chemical, and biological property or characteristic that should be within an appropriate limit, range to ensure the desired product quality.” Tailing factor, NTP, and retention time achievement considered critical quality attributes for this method as shown in Table 1.

Critical Process Parameters (CPP)

Critical method parameters, i.e., flow rate, buffer pH, and organic modifier are process inputs that have a direct and significant influence on CQAs when they are varied within the regular operation range. The range of the CPP was selected from the data obtained in initial trials.

Control Strategy

From the aspect of the analytical method development (AMD) view, the control strategy may be defined as the controls on input factors to a method that ensures the method meets both traditional criteria and wider performance-related goals. The controls can include the quality of drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, and frequency of monitoring and control (ICH Q10).

Continuous Improvement throughout Product Life Cycle

Method performance can be improved throughout the method development. The analyst has opportunities to placed inventive approaches to improve quality. Process performance can be monitored to make sure consistency in quality. The QbD approach avails the continuous improvement throughout the method life cycle; this is the distinguishing point from the conventional method which is a much-frozen process.

RESULTS AND DISCUSSION

Software-aided Method Optimization

The Box-Behnken design is an independent quadratic design in that it does not contain an embedded factorial or fractional factorial design. In this design, the treatment combinations are

at the midpoints of edges of the process space and the center. These designs are rotatable (or near rotatable) and require three levels of each factor. The designs have limited capability for orthogonal blocking compared to the central composite designs.

Statistical analysis was used to identify the significant influential chromatographic factors and their interaction impact on the six responses, i.e., retention time, tailing factor, and NTP of elagolix sodium. The analysis of 3D response surface plots and predicted vs. actual plots were used to estimate as to which method parameter gave the most acceptable responses as shown in Figures 2 to 10. The response variables, i.e., retention time, tailing factor, and number of theoretical plates (NTP) was statistically evaluated as given in Table 1. The design summary is shown in Table 2.

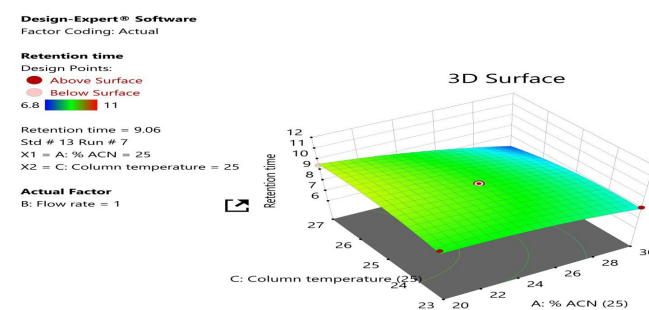


Figure 2: Effect of column temperature and initial organic content on the retention time of elagolix sodium

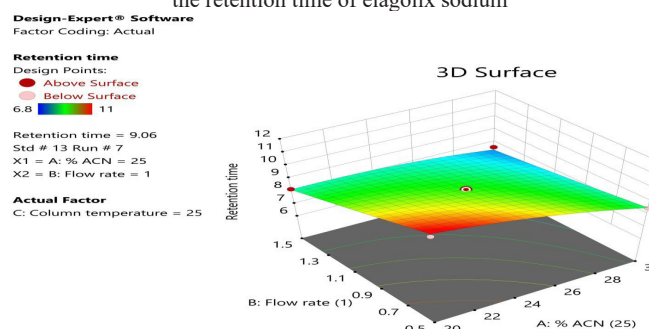


Figure 3: Effect of flow rate and initial organic content on retention time of elagolix sodium

Table 1: Factors screening by Box-Behnken design

Run	Initial % ACN	Flow rate	Column temperature	Retention time (Rt)	NTP	Symmetry factor (SF)
1	30	1	23	8.1	6,200	1.05
2	20	1	23	9.2	5,400	1.15
3	30	0.5	25	8.5	6,021	1.3
4	30	1.5	25	7.6	5,365	0.98
5	20	0.5	25	11	4,500	1.06
6	25	0.5	23	10	6,249	1.25
7	25	1	25	9.06	6,529	1.1
8	25	1.5	27	8	5,500	1
9	30	1	27	6.8	6,154	1.23
10	25	1	27	9.5	4,953	0.96
11	25	1.5	23	7.1	4,753	0.99
12	20	1.5	25	8.2	4,058	1.09
13	25	0.5	27	9.75	4,278	1.2

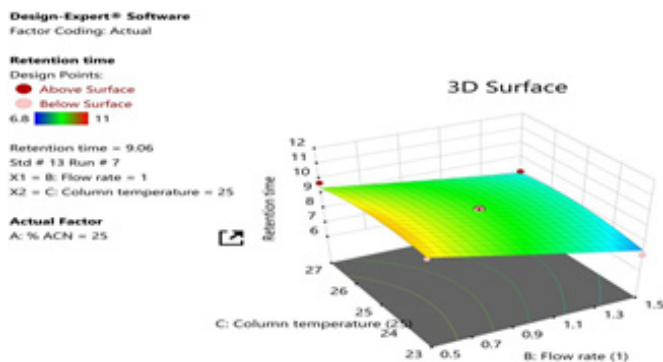


Figure 4: Effect of column temperature and flow rate on the retention time of elagolix sodium

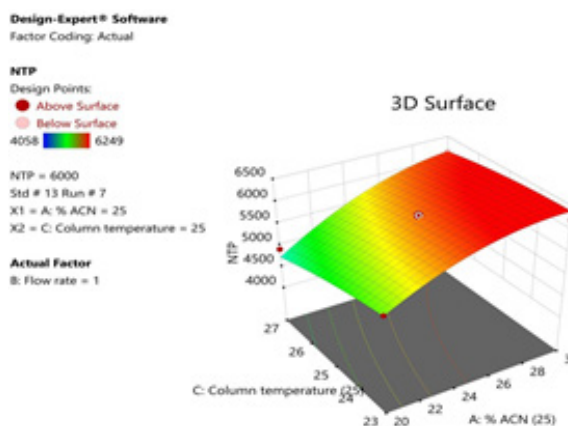


Figure 8: Effect of initial organic content and column temperature on NTP of elagolix sodium

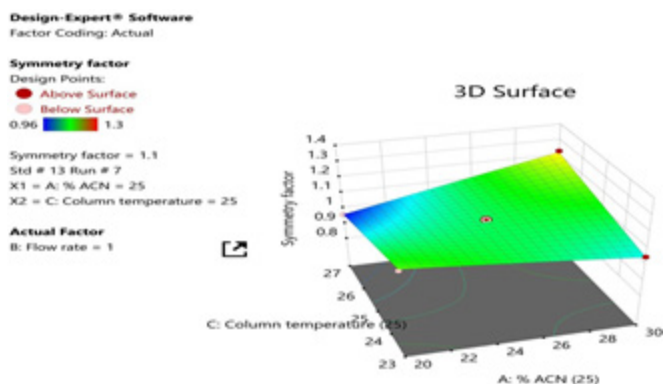


Figure 5: Effect of initial organic content and column temperature on symmetry factor of elagolix sodium

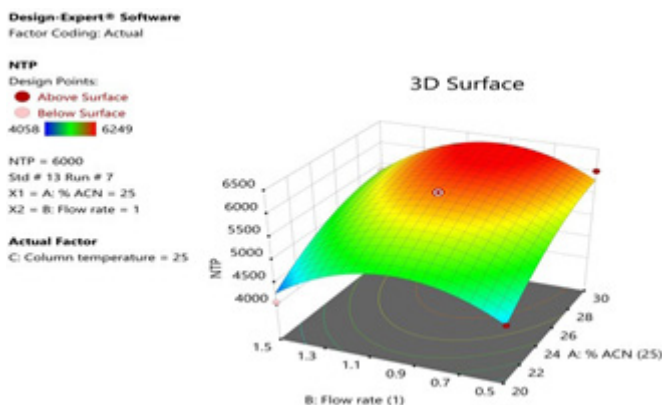


Figure 9: Effect of initial organic content and flow rate on NTP of elagolix sodium

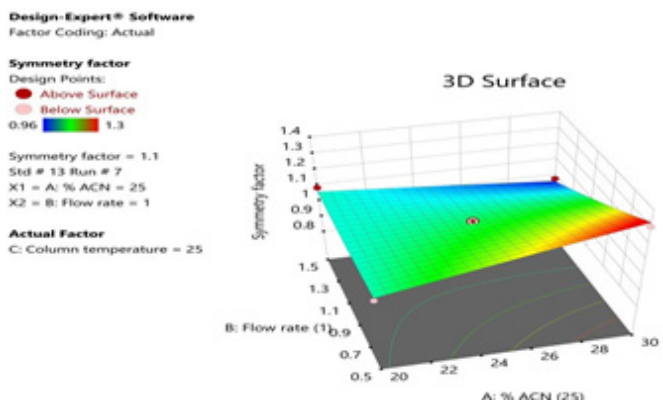


Figure 6: Effect of initial organic content and flow rate on the symmetry factor of elagolix sodium

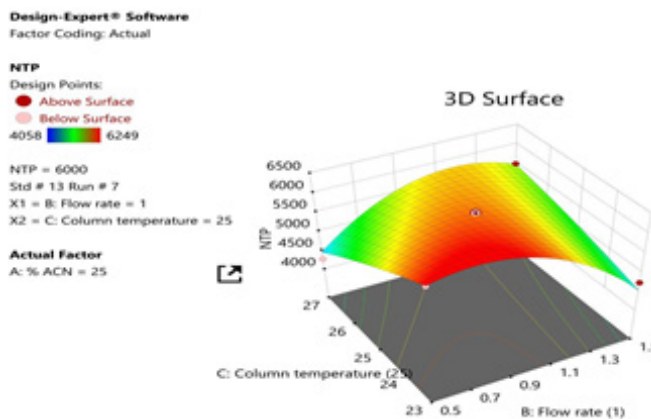


Figure 10: Effect of and flow rate column temperature on NTP of elagolix sodium

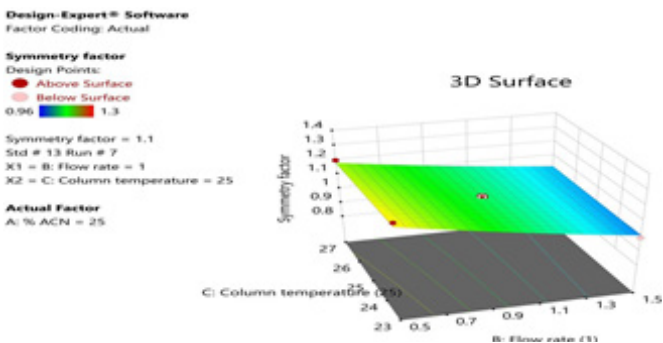


Figure 7: Effect of flow rate and column temperature on symmetry factor of elagolix sodium

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Validation

System Suitability

The elagolix sodium standard solution of 2 µg/mL was injected in six replicates. The mean system suitability parameters were obtained and are summarized in Table 3.

Linearity

Linearity was evaluated over the range of 50 to 150% of the working concentration 2 µg/mL in six replicates (1 to 3 µg/mL) for elagolix sodium. The calibration curve was plotted for the response (area) and concentration (amount) and the correlation coefficient (r^2) and y-intercept was calculated. The representative chromatograms, the calibration curve for linearity are shown in Figure 11.

Precision

Intraday precision (repeatability) of elagolix sodium was determined by taking six replicates of 50, 100, and 150% of working-level, i.e., 1, 2, and 3 µg/mL concentration at different time intervals and interday precision (intermediate precision) by six replicates of same three concentration on two consecutive days.

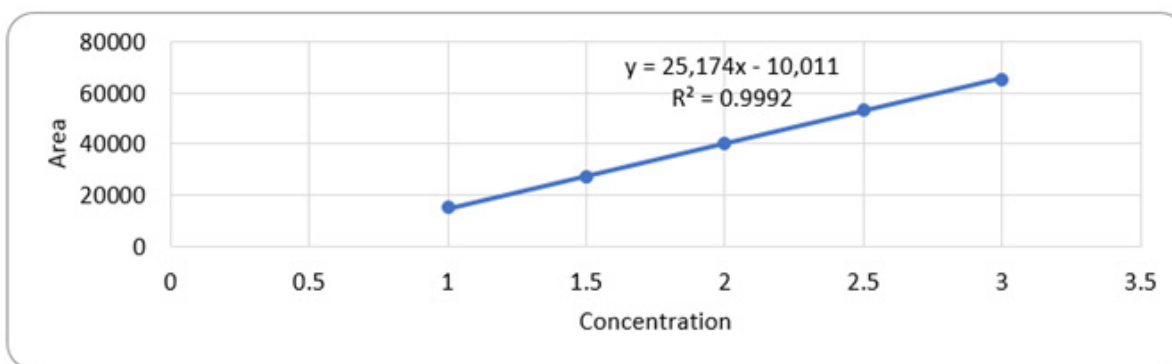


Figure 11: Calibration curve for elagolix sodium

Table 2: Design summary for optimization

Critical method parameters	Type	Low level	Medium level	High level	Final level
Column temperature	Numeric	23	25	27	25
Flow rate	Numeric	0.5	1	1.5	1
Initial % acetonitrile (ACN)	Numeric	20	25	30	45
Gradient range	Numeric	-	-	-	25–75%

Table 3: System suitability data for elagolix sodium

S. No.	System suitability parameters	Observations	Acceptance criteria
1.	Elagolix sodium standard solution	2 µg/mL	-
2.	Area % RSD	0.4%	NMT 2%
3.	Retention time	9.06	-
4.	NTP	6,529	NTP > 2,000
5.	Symmetry factor	1.1	0.8–2

Accuracy

The % recovery studies were carried out by spiking a known amount of elagolix sodium in the concentration of 50, 100, and 150% of the working level in triplicates. The chromatogram of each spiked was taken, and the total amount of drug was calculated and from which % recovery was calculated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were obtained by successively decreasing the concentration of elagolix sodium as long as a signal to noise ratio of not less than 3:1 and 10:1 is maintained, respectively. The representative chromatograms for LOD and LOQ were recorded.

Solution Stability

The stability of the drug solution was evaluated for three different concentrations (low, medium, and high), i.e., 1, 2, and 3 µg/mL. The analysis was performed at initial time zero and after those 6, 9, 24, 32, 48, and 72 hours.

Results of validation are summarized in Table 4.

Force Degradation

To prove the stability-indicating nature of the method, forced degradation studies were carried out by exposing the stock solution of the drug to the following conditions:

- Acid hydrolysis
- Base hydrolysis
- Oxidation degradation
- Thermal degradation
- Photolytic degradation

Acid hydrolysis: 25 mg of drug was accurately weighed and diluted to 25 mL with diluent. 12.5 mL of this solution was transferred to a round bottom flask and 2 mL of 1N HCl was added in a round bottom flask and this mixture was refluxed on a water bath for 6 hours at 60°C. After the reflux, the round bottom flask containing the stressed solution was cooled to room temperature, transferred to a 50 mL volumetric flask, neutralized with the corresponding base, and volume was made up with diluent. Finally, this solution was loaded into HPLC and the corresponding chromatogram was recorded as given in Figure 12.

Base hydrolysis: 25 mg of drug was accurately weighed and diluted to 25 mL with diluent. 12.5 mL of this solution was transferred to a round bottom flask and 2 mL of 5N NaOH was added in a round bottom flask and this mixture was refluxed on a water bath for 12 hours at 60°C. After the reflux, the round bottom flask containing the stressed solution was cooled to room temperature, transferred to a 50 mL volumetric flask, neutralized with the corresponding acid, and volume was made up with diluent. Finally, this solution was filtered through 0.45 µ filter paper and was loaded into HPLC and the corresponding chromatogram was recorded as given in Figure 13.

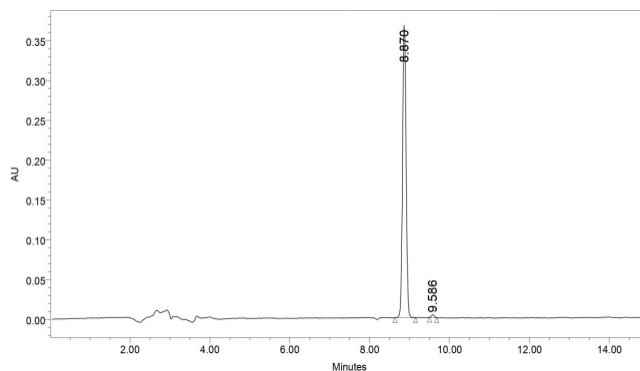


Figure 12: Chromatogram was recorded

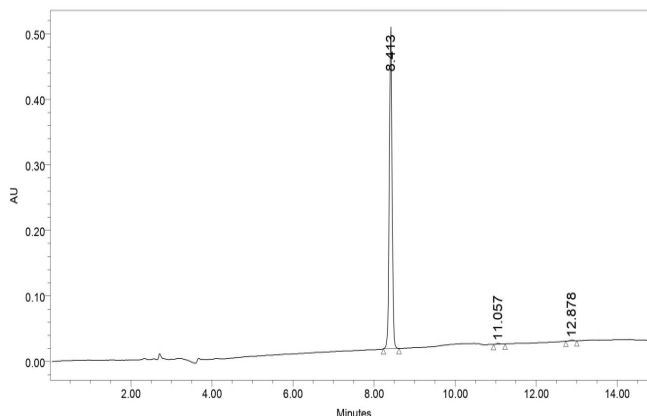


Figure 13: Corresponding chromatogram was recorded

Table 4: Statistical data of validation

S. No.	Validation parameters	Elagolix sodium monohydrate
1	System suitability	NTP-6529 % RSD-0.4% Asymmetry factor-1.1
2	Specificity	No interference was found at the Rt of the analyte
3	Linearity	$R^2 = 0.9992$ (linear) $y = 0.25174x - 10011$
4	Accuracy	100.75%
5	Interday precision (% RSD)	0.86%
6	Intraday precision (% RSD)	0.87%
7	LOD	0.2 µg/mL
7	LOQ	0.5 µg/mL
8	Solution stability (% RSD)	0.76%

Table 5: Result of forced degradation studies

S. No.	Degradation condition	Retention time of degradation products (min)	Elagolix sodium percent of degradation
1	Acid hydrolysis	9.586	0.8
2	Base hydrolysis	11.05, 12.87	0.25 + 0.37 = 0.62
3	Oxidative degradation	-	No degradation
4	Thermal degradation	-	No degradation
5	Photolytic degradation	-	No degradation
6	Total degradation	-	1.5%

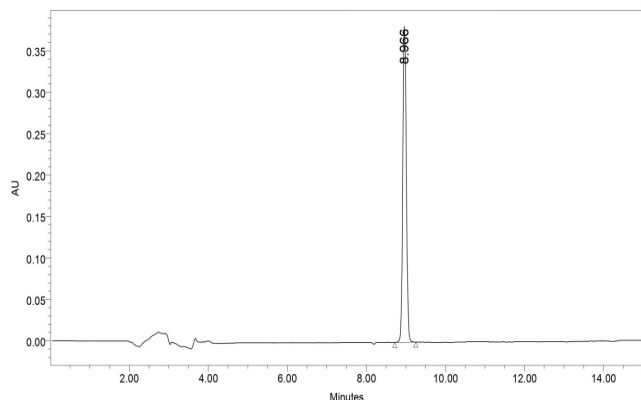


Figure 14: Corresponding chromatogram was recorded

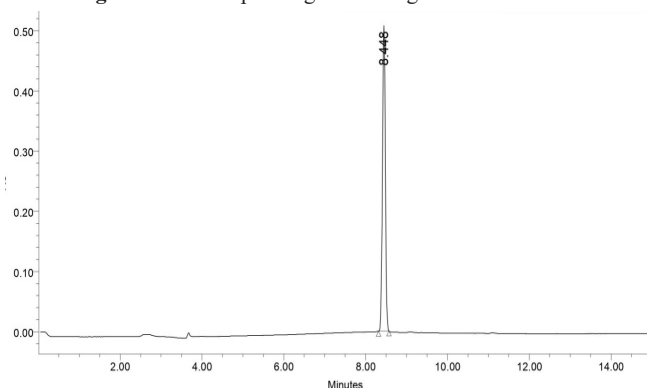


Figure 15: Corresponding chromatogram was recorded

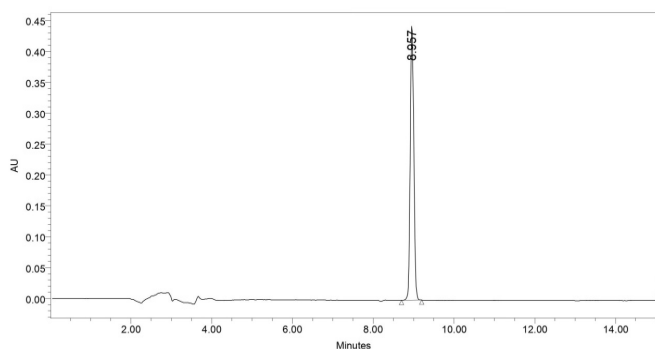


Figure 16: Corresponding chromatogram was recorded

Oxidative degradation: 25 mg of drug was accurately weighed and diluted to 25 mL with diluent. 12.5 mL of this solution was transferred to a round bottom flask, and 5 mL of 6% hydrogen peroxide was added in a round bottom flask and this mixture kept as such at room temperature for 30 minutes. After 30 minutes, the round bottom flask containing the stressed

solution was transferred to a 50 mL volumetric flask, and the volume was made up with diluent. Finally, this solution was filtered through 0.45 μ filter paper and was loaded into HPLC and the corresponding chromatogram was recorded. The corresponding chromatogram was recorded as given in Figure 14.

Thermal degradation: 25 mg of drug was accurately weighed and diluted to 25 mL with diluent. 12.5 mL of this solution was transferred to a round bottom flask and this was refluxed on a water bath for 12 hours at 60°C. After the reflux, the round bottom flask containing the stressed solution was cooled to room temperature, transferred to a 50 mL volumetric flask, and volume was made up with diluent. Finally, this solution was filtered through 0.45 μ filter paper and was loaded into HPLC and the corresponding chromatogram was recorded. The corresponding chromatogram was recorded as given in Figure 15.

Photolytic degradation: The photolytic degradation was carried out by exposing drug substances, i.e., elagolix sodium (10 mg) in the UV chamber for one week. After 1-week, the drug substance was dissolved in a 10 mL volumetric flask and then the volume was made up with diluent. 2.5 mL of this solution was further diluted to 10 mL with diluent. Finally, this solution was loaded into HPLC, and the corresponding chromatogram was recorded. The corresponding chromatogram was recorded as given in Figure 16.

Results of forced degradation studies are summarized in Table 5.

DISCUSSION

The forced degradation studies indicate that the drug is susceptible to acid hydrolysis and base hydrolysis. The degradation observed for acid hydrolysis was found to be 0.8% and the base hydrolysis was found to be 0.62%. The representative chromatograms of the forced degradation studies reveal that all the degradation products were fully resolved, this indicates the specificity of the method. Thus, the method can be employed for monitoring the stability of elagolix sodium in bulk drugs.

CONCLUSION

The developed and validated RP-HPLC method was found to be simple, precise, accurate, and was found to meet the validation parameters as per current guidelines. The developed method using the QbD approach was found to be more reproducible, decreases the number of trials, as well as, failure. The

developed method can be successfully applied for the routine analysis of drugs in bulk form.

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