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Research Article

QUANTITATIVE ANALYSIS OF ELACESTRANT IN PHARMACEUTICAL DOSAGE FORMS BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY WITH EMPHASIS ON STABILITY ASSESSMENT

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ABSTRACT

Objectives: Elacestrant, an estrogen receptor antagonist, inhibits the proliferation of estrogen-dependent cancer cells. A novel, sensitive, and user-friendly liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for the identification and quantification of elacestrant in pharmaceutical formulations.

Methods: The analytical method employed an Agilent Eclipse XDB-C18 column with dimensions of 150 × 4.6 mm and a particle size of 3.5 µm. The chromatographic separation was achieved using isocratic elution at a flow rate of 1.0 mL/min. For detection, a photodiode array (PDA) detector was utilized in conjunction with tandem mass spectrometry (MS/MS), enabling both qualitative and quantitative analysis with high sensitivity and specificity.

Results: The method demonstrated excellent linearity over a concentration range of 25–150 µg/mL (R^2=0.99979). The limit of detection and limit of quantitation were found to be 0.3 µg/mL and 1.0 µg/mL, respectively. Intra- (0.189) and inter-day (0.405) precision studies showed relative standard deviations (% RSDs) of <1%. Recovery rates ranged from 99.20% to 101.30%, and the percentage RSD values ranged from 0.36 to 0.645. The robustness of the new approach is demonstrated by the observed change in peak area, which was within limits, or <8.0%. Elacestrant has lower susceptibility toward thermal, photolytic (light/ultraviolet), and reduction degradation conditions, whereas its sensitivity toward peroxide, acid, alkali, and hydrolysis degradation conditions is evident from the high degradation levels. Mass spectrum give Molecular ion: m/z: 459.6463, base peak m/z: 174.630 and give another fragment ions m/z: 388.5172, m/z: 303.41652, m/z: 232.316.

Conclusion: The developed LC-MS/MS method is accurate, precise, robust, and linear for the determination of elacestrant in tablet formulations. With recovery rates within acceptable limits and degradation behavior consistent under stress conditions, the method is suitable for routine quality control and stability testing of pharmaceutical products containing elacestrant.

Keywords: Elacestrant, Liquid chromatography-tandem mass spectrometry, International Council for Harmonisation Q_2 (R1), Photodiode array, % Relative standard deviation.

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INTRODUCTION

Elacestrant (Fig. 1) is an oral anticancer drug used to treat breast cancer [1,2]. It is commercially available under the trade name Orserdu. The biological targets of endogenous estrogens, such as estradiol are the estrogen receptors (ER), which are antagonistic to elacestrant, an antiestrogen [1]. Musculoskeletal pain, nausea, elevated cholesterol, elevated liver enzymes, increased triglycerides, exhaustion, decreased hemoglobin, vomiting, increased alanine transaminase, increased aspartate transaminase, decreased sodium, increased creatinine, headache, constipation, diarrhea, hot flashes, abdomen pain, and upset stomach are among the most frequent adverse effects of elacestrant [3].

In January 2023, elacestrant received approval for medicinal use in the United States [1,2,4,5], and in September 2023, it received approval in the European Union [6,7].

Elacestrant is prescribed to treat postmenopausal women or adult men who have advanced or metastatic breast cancer that has progressed after at least one line of endocrine therapy and is ER-positive, human epidermal growth factor receptor 2-negative, and ESR1-mutated [2,3]. A biological target of endogenous estrogens such as estradiol, elacestrant is an antiestrogen, or antagonist of the ERs [5]. In particular, it inhibits the ER alpha (ERα) [5]. Elacestrant causes the ERα to degrade, making it a selective ER degrader as well [5,8].

Approximately 10% of elacestrant is bioavailable orally [5]. It is concentration-independent and has a plasma protein binding rate of above 99% [5]. The cytochrome P450 enzyme CYP3A4 metabolizes elacestrant in the liver, with CYP2A6 and CYP2C9 contributing less [5]. Elacestrant has a half-life of 30–50 h for elimination [5]. Urine contains 7.5% of it, whereas feces contain 82% [5].

No reports of liquid chromatography methods were found in the literature review. The findings of the suggested approach show that elacestrant in bulk and pharmaceutical formulations may be determined using the devised method. This standard approach is appropriate for use in industry quality control testing.

METHODS

Materials

Ultra-performance liquid chromatography (UPLC)-quality solvents and analytical-grade chemicals were supplied by Merck Chemicals, a Mumbai-based company. Hyderabad's SJS Pharmaceuticals Lab supplied the standards for any potential impurities in any of the selected drugs. MilliQ water and high-performance liquid chromatography (HPLC) analyses were used.

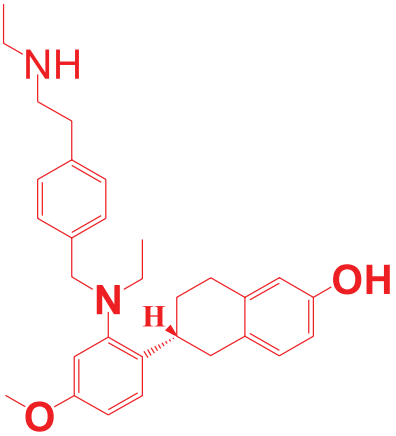

Fig. 1: Structure of elacestrant

Instruments
Digital balances (DENVER brand, SI234 model; Shimadzu AUX-220) and digital pH meters (Elico LI-120) were used to weigh and measure the samples' pH.

UPLC
An Agilent1290 Infinity II LC System (Pump: Quaternary; Software: Empower 2.0) with a photodiode array (PDA) detector was used to estimate elacestrant.

Mass spectrometer
An electrospray ionization (ESI) interface-equipped SCIEX QTRAP 5500 mass spectrometer was connected to the UPLC system. To evaluate the chromatogram's data, SCIEX software was utilized.

Chromatographic conditions
Method development and validation were conducted using the Agilent1290 Infinity II LC System (Pump: Quaternary; Software: Empower 2.0). A 150×4.6 mm, 3.5 µm Agilent Eclipse XDB column with an isocratic elution was used for the separation, which was kept at room temperature. Before use, the mobile phase acetonitrile (ACN)+ Ammonium formate pH-3.0/ortho-phthalaldehyde (OPA) (50:50) was filtered through a 0.22 m nylon membrane filter and degassed in an ultrasonication bath. The mobile phase flow rate was set at 1.0 mL/min. Ten microliters was the injection volume. The analysis was carried out using the step isocratic software. Using an ESI electron spray ionizer detector, tandem mass spectrometry (MS/MS) performed the detection. Data processing and collection were done using SCIEX software.

Preparation of ammonium formate buffer solution
1.45 µ membrane filter paper is used to filter 6.30 g of ammonium formate that has been dissolved in 1 L of HPLC-grade water with a pH of 3.0 that has been adjusted with OPA.

Preparation of mobile phase
Ammonium formate pH-3.0/OPA and ACN were mixed in a 50:50 ratio to create the mobile phase. To exclude any contaminants that could affect the final chromatogram, it was filtered through a 0.45 µ membrane filter.

Preparation of standard solution
Transfer 10 mg of the elacestrant working standard into a 10 mL clean, dry volumetric flask, weigh it precisely, add diluent, and sonicate to dissolve it completely. Then, use the same solvent to get the volume up to the desired level. (Stock solution). 1 mL of the aforementioned stock solutions should then be pipetted into a 10-mL volumetric flask and diluted with diluent to the appropriate level (100 ppm of elacestrant).

Sample solution preparation
After precisely weighing and transferring 24.9 mg of the elacestrant sample into a 10 mL clean, dry volumetric flask, add the diluent, sonicate it for 30 min to dissolve it, and centrifuge it for 30 min to dissolve it fully. Use the same solvent to get the volume up to the desired level. After that, it is filtered using a stock solution 0.45 µ injection filter. In addition, pipette 1 mL of the stock solutions mentioned above into a 10 mL volumetric flask, then dilute with diluent to the appropriate level (100 ppm of elacestrant).

Methodology for the evaluation (validation)
Specificity, linearity, range, accuracy, precision, sensitivity (limit of quantitation [LOQ] and limit of detection [LOD]), and robustness were among the analytical performance metrics that were validated in accordance with the Food and Drug Administration and International Council for Harmonisation (ICH) Q2B recommendations [9-10].

Linearity
By injecting six distinct test sample solutions in triplicate, a calibration curve for each impurity was created to assess the method's linearity. The standard stock solutions were used to create six distinct test sample solutions with varying concentrations (LOQ, 50%, 75%, 100%, 125%, and 150%). Using a calibration curve, correlation coefficients (R2) were computed for every analyte. To determine the relative response factor, the slope of the calibration curves was utilized.

For preparation of 50% solution
Weigh the elacestrant sample precisely, and then place 12.45 mg into a 10 mL clean, dry volumetric flask. Add the diluent, sonicate to dissolve it fully, and then use the same solvent to get the volume up to the desired level (A stock solution). In addition, pipette 1 mL of the stock solutions mentioned above into a 10 mL volumetric flask, then dilute with diluent to the appropriate level (50 ppm of elacestrant).

Preparation of 100% solution
Weigh the elacestrant sample precisely, and then place 24.9 mg into a 10 mL clean, dry volumetric flask. Add the diluent, sonicate to dissolve it fully, and then use the same solvent to get the volume up to the desired level (A stock solution). In addition, pipette 1 mL of the stock solutions mentioned above into a 10 mL volumetric flask, then dilute with diluent to the appropriate level (100 ppm of elacestrant).

Preparation of 150% solution
Weigh the elacestrant sample precisely, and then place 37.35 mg into a 10 mL clean, dry volumetric flask. Add the diluent, sonicate to dissolve it fully, and then use the same solvent to get the volume up to the desired level (A stock solution). In addition, pipette 1 mL of the stock solutions mentioned above into a 10 mL volumetric flask, then dilute with diluent to the appropriate level (150 ppm of elacestrant).

Robustness
To examine the system appropriateness characteristics, the chromatographic conditions were purposefully altered to assess the method's resilience. The impact of minor adjustments to the organic content (±5%) and mobile phase flow rate (±0.2 mL/min) was examined to assess the method's resilience.

Preparation of degradation parameters
Degradation parameters preparation shows in Table 1.

RESULTS AND DISCUSSION

Method development

Determination of working wavelength (λ_{max})
The drug was estimated using the isobestic wavelength. For interconvertible compounds, the wavelength at which the molar absorptivity is the same is known as the isobestic point. To precisely estimate the medication, this wavelength was utilized.

The PDA detector was used to scan the wavelength of maximum absorption of the drug solution in the mixture of ACN and ammonium formate pH-3.0/OPA (50:50) between 200 and 400 nm, using ACN and ammonium formate pH-3.0/OPA (50:50) as a blank. At 287 nm, the

absorption curve displays the isobestic point (Fig. 2). Thus, using the high-performance liquid chromatography chromatographic procedure, a detector wavelength of 287 nm was used.

A variety of parameters, including the column, mobile phase composition, buffers, pH, flow rate, wavelength, and others, were tuned in conjunction with the isocratic elution using mobile phases of varying compositions until the elacestrant peak was well separated and the system suitability conditions were satisfactory.

The isocratic elution with several mobile phase compositions was performed until the elacestrant peak was discrete and the system suitability requirements were met, in addition to modifying the column, mobile phase composition, buffers, pH, flow rate, wavelength, and other parameters.

Table 1: Degradation parameters preparation

S. No.	Degradation	Conditions
1	Acid degradation	0.1 mL of 1N HCl and heated at 70°C for 1 h
2	Alkali degradation	1 mL of 1N NaOH and heated at 70°C for 1 h
3	Thermal degradation	Exposed at 80°C for at least 72 h
4	Peroxide degradation	0.5 mL of 30% H_2O_2 at 70°C for 1 h
5	Reduction degradation	1 mL of 10% sodium bisulfate and heated at 70°C for 1 h
6	Photolytic degradation	Exposed to 1.2 Million lux hours of light
7	Hydrolysis degradation	Heated at 70°C for 30 min

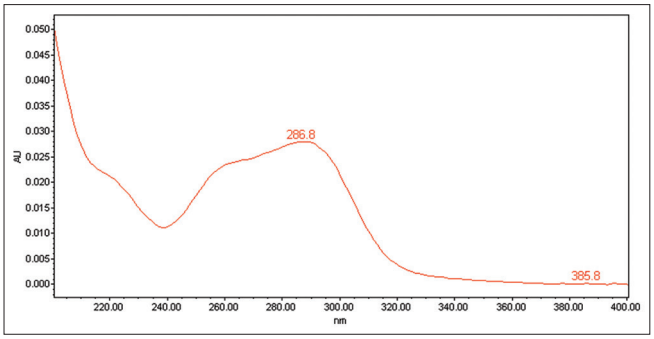

Fig. 2: Photodiode array- spectrum of elacestrant

The first phase trails (Fig. 3) in Table 2 were used in three different experimental trials using Symmetry C18 (150 mm×4.6, 3.5 µm) and organic solvents. With an isocratic separation mode (40:60; 45:65, 65:35; v/v), the tests used different mobile phase ratios (mobile phase ACN+Ammonium formate pH-3.0/OPA at a flow rate of 1.0 mL/min). The column's temperature was maintained at 25°C for 6–10 min during the run. Ten microliters of the modified sample were added to the UPLC equipment. In these trails, it is evident that the Baseline is insufficient and that the system suitability circumstances are outside the bounds.Trials in the second phase were carried out. Using the first phase trails in Table 2, two independent experimental trials were conducted using an Agilent Eclipse XDB (150×4.6 mm, 3.5 µ) and organic solvents. At a flow rate of 1.0 mL/min, the studies used several mobile phase ratios (mobile phase ACN+0.1% trifluoroacetic acid), each driven in an isocratic separation mode (80:20; 70:30, 65:35; v/v). For 6–10 min during the run, the column's temperature was maintained at 25°C. The UPLC device was filled with 10 µL of the modified sample. In these trails, it is evident that the baseline is insufficient and that the unknown peak and baseline drift are present (Fig. 4).

In conclusion, the Third Phase Trial (Table 2) was conducted using the same column and organic solvents, but with the mobile phase ratios changed to a 50:50 ratio. To maintain the 1.0 mL/min flow rate in certain circumstances, a PDA detector was used in combination with a reversed-phase (RP) column that was comparable to the Agilent Eclipse XDB (150×4.6 mm, 3.5 µ). ACN+Ammonium formate pH-3.0/OPA (50:50) were used as the mobile phase in an isocratic elution technique (Fig. 5).

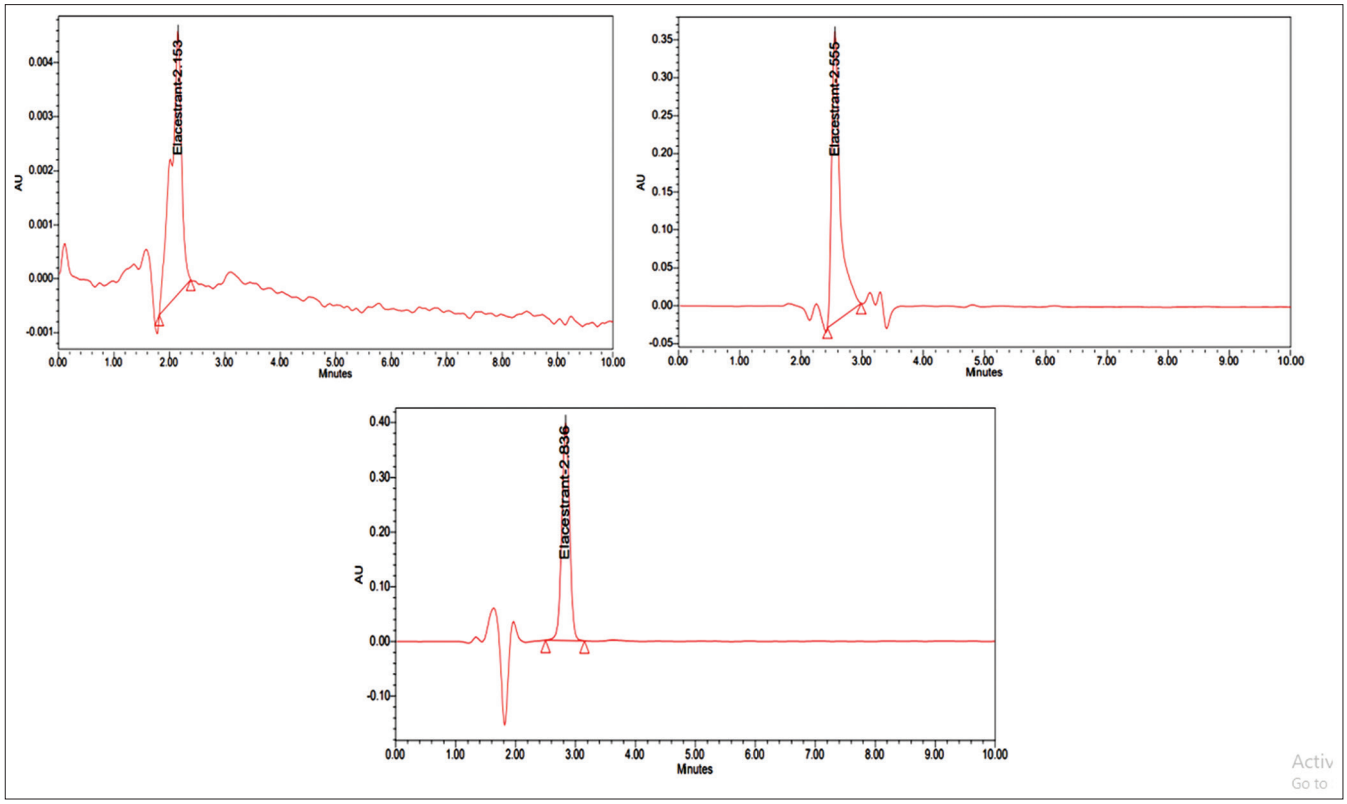

Fig. 3: Chromatograms obtained from symmetry C18 (150 mm × 4.6, 3.5 µm)

Table 2: Conducted trails and observation

S. No.	Column	Mobile phase and wavelength	Flow rate and run time	Observation
1	Symmetry C18 (150 mm×4.6, 3.5 µm)	ACN+0.1% TFA (80:20), 200–400 nm	1 mL/min, 10 min	System suitability conditions are not within the limit
2	Symmetry C18 (150 mm×4.6, 3.5 µm), 287 nm	ACN+0.1% TFA (70:30), 287 nm	1 mL/min, 10 min	Peak shape is not good
3	Symmetry C18 (150 mm×4.6, 3.5 µm)	ACN+0.1% TFA (65:35)	1 mL/min, 10 min	Baseline is not sufficient
4	Agilent eclipse XDB (150×4.6 mm, 3.5 µ)	Acetonitrile+Ammonium formate pH-3.0/OPA (40+60) and 287 nm	1 mL/min and 10 min	Unknown peak and baseline drift are observed
5	Agilent Eclipse XDB (150×4.6 mm, 3.5 µ)	Acetonitrile+Ammonium formate pH-3.0/OPA (45+55) and 287 nm	1 mL/min and 10 min	Response of the peak is very high
6	Agilent Eclipse XDB (150×4.6 mm, 3.5 µ)	Acetonitrile+Ammonium formate pH-3.0/OPA (50+50) and 287 nm	1 mL/min and 6 min	This method is suitable for validation

ACN: Acetonitrile, TFA: Trifluoroacetic acid

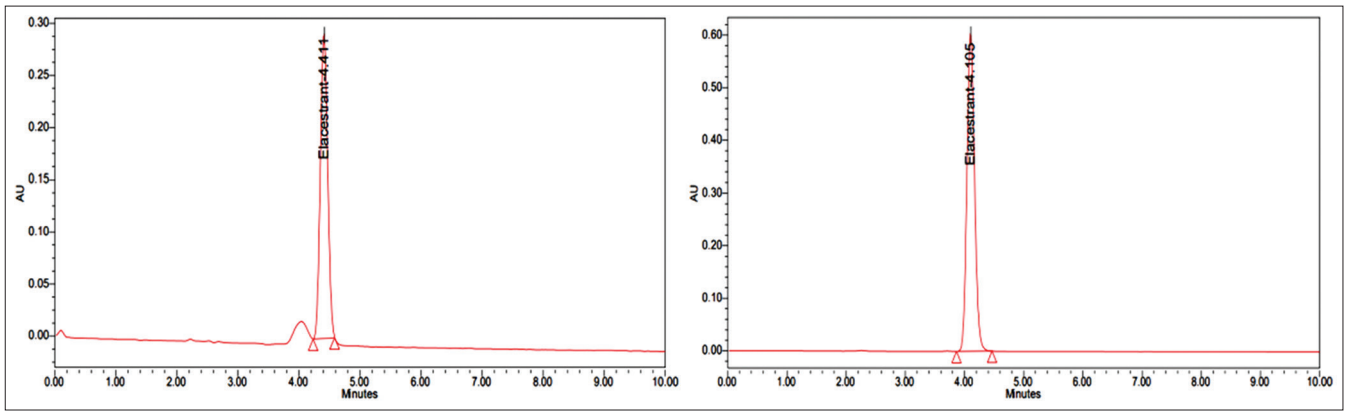

Fig. 4: Chromatogram obtained from Agilent Eclipse XDB (150 × 4.6 mm, 3.5 µ) column

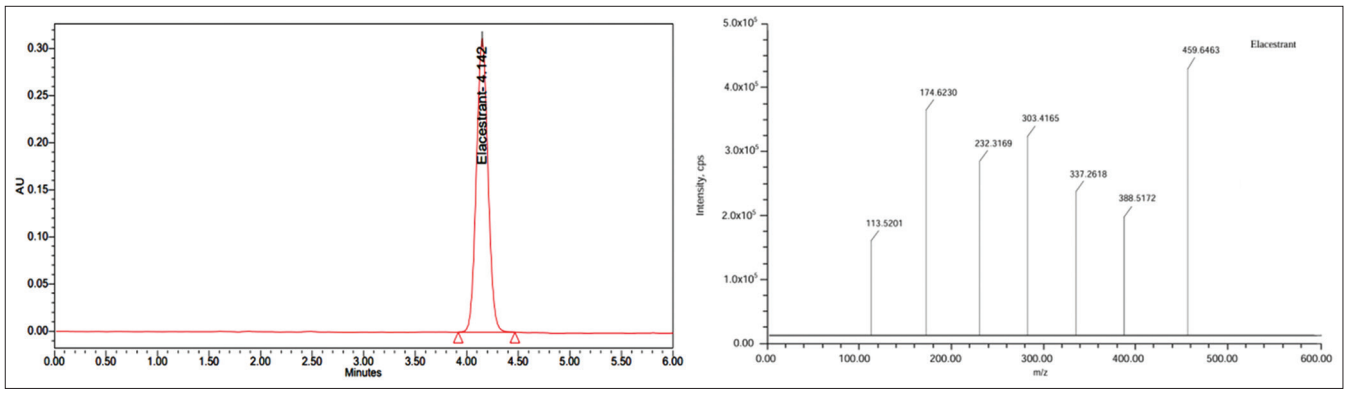

Fig. 5: Optimized chromatogram and optimized mass spectrum

In summary, the same column and organic solvents were used in the Third Phase Trial (Table 2), but the mobile phase ratios were adjusted to 50:50. In some cases, a PDA detector was utilized in conjunction with an RP column similar to the Agilent Eclipse XDB (150×4.6 mm, 3.5 µ) to maintain the 1.0 mL/min[1] flow rate. A 50:50 mobile phase consisting of ACN and ammonium formate pH-3.0/OPA was employed in an isocratic elution procedure (Fig. 5). A non-polar chemical called elacestrant breaks down ERs specifically. Despite being non-polar, the Agilent Eclipse XDB (150×4.6 mm, 3.5 µ) [11] column's polar silanols may interact with the elacestrant and reduce its retention duration. RP UPLC may use hydrogen bonds or dipole-dipole interactions to make this contact easier to elute. This path has a good response area, tailing factor, and resolution. At 4.142 min, the elacestrant peaked with a tailing factor of 0.92 and a peak area of 3323715. Table 3 shows the optimum conditions for this study.

In a mass spectrometer, elacestrant, which has a molecular ion at m/z 459.6463, fragments to produce distinctive fragment ions that reveal structural details. Molecular Ion [M+H]+, m/z 459.6463. The following fragment ions are produced by the molecular ion: 113.5201, 174.6230, 232.3169, 303.4165, 337.2618, and 388.5172. The base peak is 174.6230 out of the total fragment ions (Fig. 6).

Mass spectrometer conditions

Positive ion ESI interface mode was used to operate the mass spectrometer. To measure elacestrant, the multiple reactions monitoring mode has been used. The working parameters are listed in Table 4. Mass spectrum give molecular ion: m/z: 459.6463, base peak m/z: 174.630 and give another fragment ions m/z: 388.5172, m/z: 303.41652, m/z: 232.316.

Stability indicating studies

Carried out the forced degradation study with a purpose of understanding the specificity and stability-indicating nature of the proposed method. As a part of degradation studies, chromatograms were recorded under diverse conditions of stress. All degradant peaks are well separated from each other and also from elacestrant peak in each degradation condition. Hence, no interference was found from its degradation products. Based on the data, it can be concluded that the proposed method is a specific and stability-indicating method. Table 5 shows the stress-induced degradation results of the elacestrant. Elacestrant has lower susceptibility toward thermal (1.3), photolytic (2.6) (light/ultraviolet), and reduction degradation conditions, where as its sensitivity toward Peroxide (14.7%), Acid (12.5), Alkali (10.9) and Hydrolysis (10.6) degradation conditions is evident from the high degradation levels. These results signify the suitability of this method for routine quality control analysis as it is established to be selective. A purity angle less than the purity threshold is found by the empower program (Fig. 7a-h). The data indicates that the peak is homogeneous.

Method validation

The HPLC process was designed and improved in accordance with ICH (2005) requirements (Q2R1) [12]. Each verified parameter is covered in depth in the section below.

Table 3: Optimized chromatographic conditions

Parameters	Observation
Instrument used	Waters Alliance e-2695 HPLC
Injection volume	10 µL
Mobile phase	ACN+Ammonium formate pH-3.0/OPA (50:50)
Column	Agilent Eclipse XDB (150×4.6 nm, 3.5 µ)
Detection wavelength	287 nm
Flow rate	1 mL/min
Runtime	6 min
Temperature	Ambient (25°C)
Mode of separation	Isocratic mode

HPLC: High-performance liquid chromatography, ACN: Acetonitrile

Table 4: Optimized spectrometer conditions

S. No	Parameter	Observation
1	Collision energy	14 V
2	Ion spray voltage	5500 V
3	Source temperature	550°C
4	Drying gas temperature	120–250°C
5	Collision gas	Nitrogen
6	Drying gas flow stream	5 mL/min
7	Declustering potential	40 V
8	Entrance potential	10 V
9	Exit Potential	7 V
10	Dwell time	1 s

Table 5: Degradation studies

Degradation parameters	% Assay of degraded sample (A1)	% Degradation w.r.t. control sample (B1*)	PA	TH
Control	100	0	1.449	3.215
Acid	87.5	12.5	1.452	3.267
Alkali	89.1	10.9	1.433	3.232
Peroxide	85.3	14.7	1.485	3.286
Reduction	96.3	3.7	1.442	3.255
Thermal	98.7	1.3	1.471	3.249
Photolytic	97.4	2.6	1.436	3.292
Hydrolysis	89.4	10.6	1.419	3.264

PA: Purity angle, TH: Threshold. B1*=(100−A1)/100*100

System suitability and specificity

To ensure the suitability of the present method for the anticipated application, a system suitability test was conducted. System suitability (Fig. 8) parameters at optimized conditions for the present method were noticed within the acceptable criteria and are compiled in Table 6. The satisfactory tabulated values of system suitability parameters indicate the substantiated performance of the system. To verify the interference from blank, placebo (Fig. 9) and degradation products, specificity study was performed. At the retention time of elacestrant, neither diluents nor placebo has shown interference. Less than two tailing factor value and more than 2000 as the number of theoretical plates for the elacestrant peak in the UPLC chromatogram confirms the system suitability.

To verify the existence of any interference from either degradation at the elacestrant retention time, a specificity study was performed. At the retention time of 4.142 min for the elacestrant, the absence of peak interference from blank indicates the specificity of the present method.

Generation of molecular ion

The general fragmentation pattern of elacestrant, along with the corresponding theoretical mass-to-charge (m/z) ratios, shows good agreement with the experimental data obtained from mass spectrometry. This correlation supports the proposed fragmentation pathways. The only noticeable variation arises from differences in protonation, which can influence the ionization state and, consequently, the observed mass spectral peaks.

Mode of fragmentation with respective mass per electron ration (m/z) by mass spectroscopy

Elacestrant, with a molecular ion at m/z 459.6463, (Fig. 10) undergoes fragmentation in a mass spectrometer, yielding characteristic fragment ions that provide structural information. m/z 459.6463 (Molecular Ion [M+H]+): This represents the protonated molecular ion of elacestrant. It's the starting point for the fragmentation cascade. m/z 388.5172: This fragment likely arises from the loss of a neutral molecule or radical, possibly involving the cleavage of a bond within one of the side chains or functional groups of elacestrant. m/z 303.4165: This suggests further fragmentation of the m/z 388.5172 ion. The difference in mass indicates another neutral loss, again likely from a side chain or functional group. The specific structure of the m/z 303.4165 fragment is essential for understanding the fragmentation mechanism. m/z 232.3169: This fragment indicates continued breakdown of the molecule. The mass difference from m/z 303.4165 points to another specific neutral loss. This step could involve the loss of a more complex or substituted group. m/z 174.6230 (Base Peak): The base peak, being the most abundant ion in the spectrum, represents the most stable fragment formed. This suggests a highly favored fragmentation pathway leading to this particular ion. m/z 113.5201: This is the smallest observed fragment. It likely represents a smaller, highly stable fragment of the elacestrant molecule, possibly a portion of one of the side chains or a core structural element (Fig. 11).

Linearity

Elacestrant was injected at varying concentrations [13-15] (25–150 µg/mL) to assess linearity and observed linearity in the calibration curve that was created (Figs. 12 and 13). To create the analytical curve, the area under the curve was plotted versus the drug concentration. The regression equation, as shown in Table 7, was determined to be y=33013.26x+30501.71, with a correlation coefficient of 0.99979.

Precision

Precision is a measure of the variability of results [16] in repeated examinations of the Elacestrant sample after duplicate experimental setups. The investigations were conducted for both precisions, that is, method and intermediate precisions (MP and IP), using six replicates to validate the present approach. We performed the IP under several

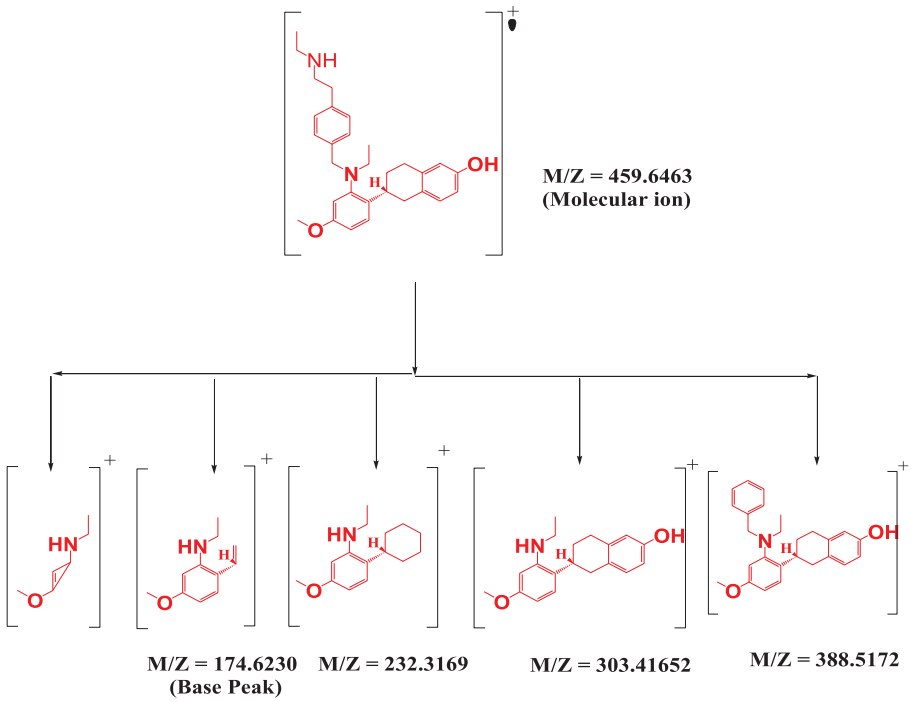

Fig. 6: Optimized mass spectrum fragmentation

Table 6: System suitability parameters for elacestrant

S. No	Parameter	Elacestrant
1	Retention time	4.142
2	Plate count	19349
3	Tailing factor	0.92
4	Purity angle	1.449
5	Purity threshold	3.215
6	Resolution	----
7	%RSD	0.21

%RSD: % Relative standard deviations

Table 7: Results of linearity for elacestrant

S. No.	Elacestrant	
	Conc. (µg/mL)	Peak area
1	25.00	858753
2	50.00	1671560
3	75.00	2562175
4	100.00	3336503
5	125.00	4187600
6	150.00	4928881
Regression equation	y=33013.26x+30501.71	
Slope	33013.26	
Intercept	30501.71	
R^2	0.99979	

Table 8: Results of precision (method and intermediate) study

S. No.	% Assay	
	M.P.	I.P.
1	99.75	100.94
2	99.53	100.67
3	100.10	100.12
4	99.77119	99.78
5	100.02	100.53
6	99.91	99.98
Mean	99.85	100.34
Standard deviation	0.188	0.406
%RSD	0.189	0.405

Values are given in mean±standard deviation; n=6.
M.P.: Method precision, I.P.: Intermediate precision at 100 µg/mL, %RSD: % Relative standard deviations

Table 9: Precision studies system suitability parameters

System suitability parameter	M.P.*	I.P.*
USP tailing factor	0.92	0.99
USP plate count	19349	20238
Retention time (min)	4.142	4.162
Purity Angle	1.449	1.344
Threshold	3.215	3.313
Peak area	3326760	3342968
SD of area	6214.76	13413.48
% RSD of area	0.187	0.401

M.P.: Method precision, I.P.: Intermediate precision, SD: Standard deviation, %RSD: % Relative standard deviations. Values are given in mean±standard deviation; n=6.
*from six standard injections

situations (instrument, column, and analyst). For MP and IP, the computed percentage relative standard deviation (RSD) values were 0.189 and 0.405, respectively (Fig. 14), and they fall within the ICH-recommended acceptable range (Table 8). It suggests a high degree of precision and a robust procedure. The percentage RSD results were 0.189 for the procedure MP and 0.405 for the IP. An assay with a precision of >99% indicates that the procedure is accurate (Table 9).

Accuracy

Accuracy, the most important parameter in the analytical process, is represented by adding a known amount of medicine to the sample and then calculating the recovery percentage. The accuracy of the analytical procedure is assessed by repeatability. Six replicate preparations were used to conduct the accuracy investigation with 50%, 100%, and 150% recovery levels before being injected into a chromatograph (Fig. 15) [17-18]. The results, which include the estimated percentage recovery from the chromatographic peak region at each recovery level, are displayed in Table 10. The percentage RSD values range from 0.3.6 to 0.645, and the recovery percentage was

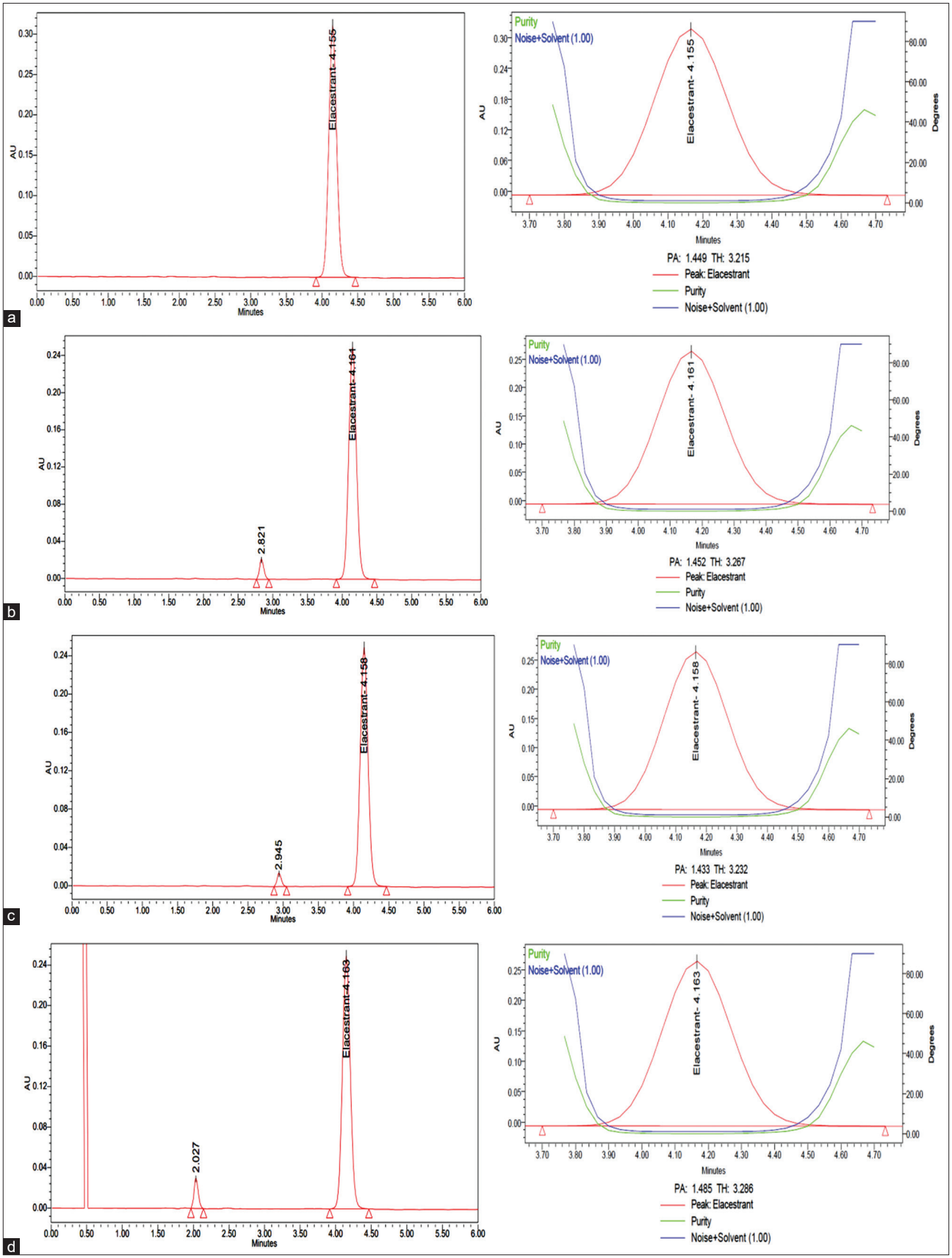

Fig. 7: (a) Chromatograms of control degradation and purity plot (b) Chromatogram of acid degradation and purity plot (c) Chromatogram of alkali degradation and purity plot (d) Chromatogram of peroxide degradation and purity plot (e) Chromatogram of reduction degradation and purity plot (f) Chromatogram of thermal degradation and purity plot (g) Chromatogram of photodegradation and purity plot (h) Chromatogram of hydrolysis degradation and purity plot

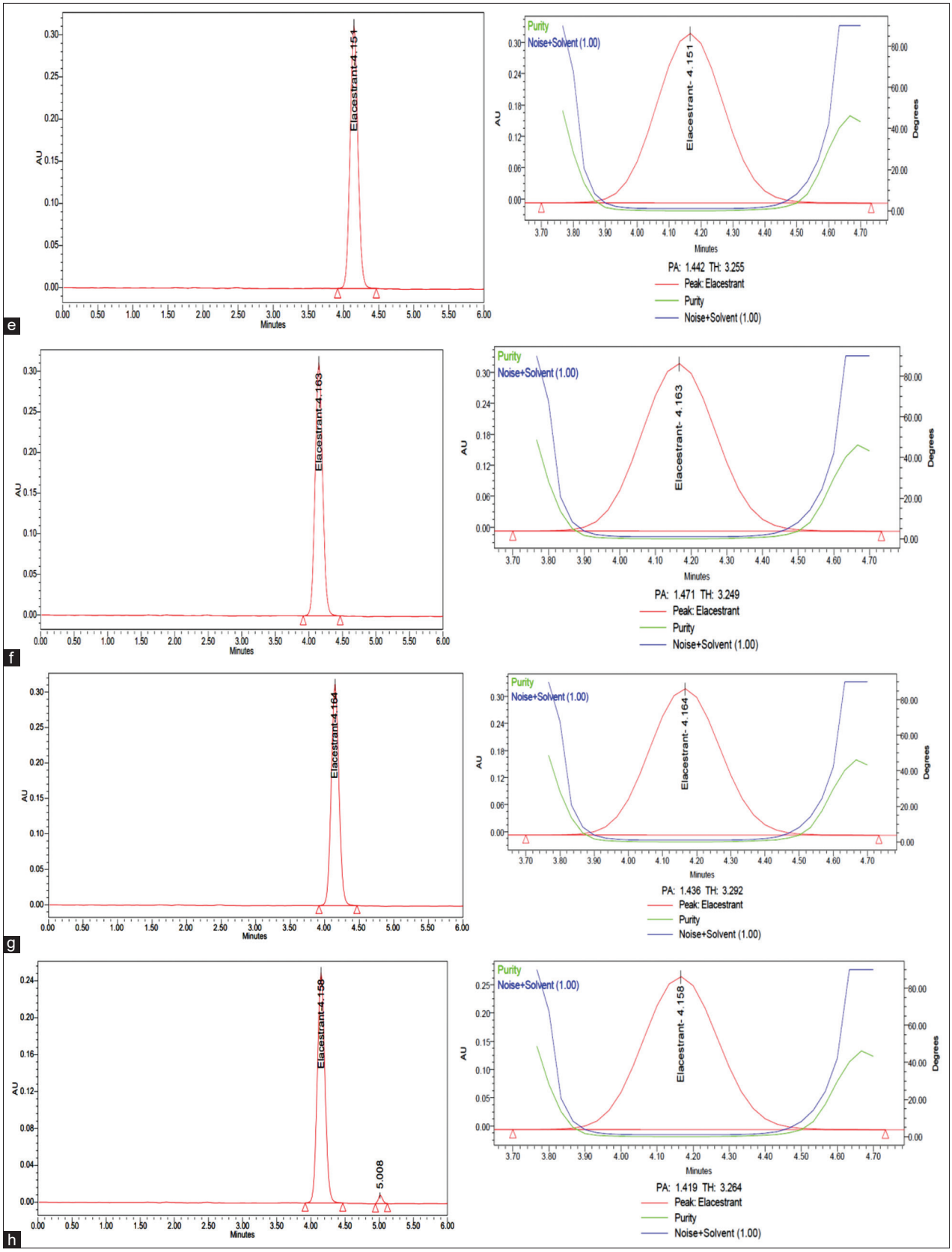

Fig. 7: ***(Continued)***

determined to be between 99.20 and 101.30. The analyte's recovery values fall within the allowed ranges since the recovery findings do as well. Thus, it shows that the approach that was suggested to estimate elacestrant was accurate.

Robustness

Using a standard solution of 10.0 µg/mL of elacestrant, the parameters (flow rate [Figs. 16 and 17] and organic phase) were carefully adjusted before their effects on the procedure were assessed [19-20].

Table 10: Accuracy results of elacestrant by HPLC method

%Concentration (at specification level)	Area	Average amount added (mg)	Amount found (mg)	% Recovery	Statistical values	
50	1671560	5	5.02	100.4	Mean	99.6666667
	1652558	5	4.97	99.4	SD	0.643
	1649567	5	4.96	99.2	%RSD	0.645
100	3369284	10	10.13	101.3	Mean	100.6
	3339286	10	10.04	100.4	SD	0.624
	3329308	10	10.01	100.1	%RSD	0.621
150	4995881	15	15.02	100.1	Mean	99.83
	4983792	15	14.98	99.9	SD	0.305
	4962896	15	14.92	99.5	%RSD	0.306

HPLC: High-performance liquid chromatography, SD: Standard deviation, %RSD: % Relative standard deviations. Values are given in mean±standard deviation; n=3

Table 11: Results of robustness/ruggedness experiment of elacestrant

Altered parameter	Actual condition	Altered condition	RT (Min)	Theor plates	Tail factor	Peak area	% Change in peak area
Control condition	NA	---	4.142	19349	0.92	3323715	--------
Flow rate	1.0 mL/min	(0.8 mL)	4.142	19458	0.97	3167452	4.7
		1.2 mL	4.018	19316	0.86	3406947	2.63
Organic phase change	50:50	(45:55)	4.402	19479	0.99	3057416	7.82
		(55:45)	3.921	19263	0.91	3561411	7.77

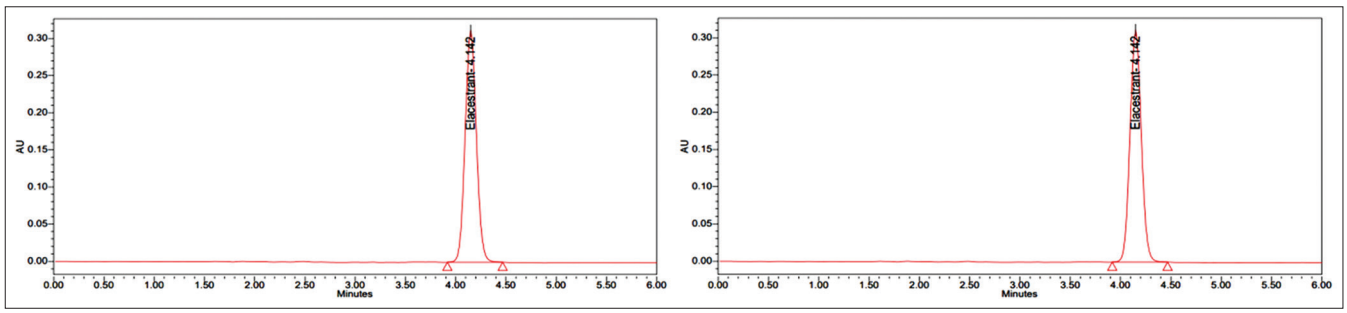

Fig. 8: Optimized chromatogram and standard chromatogram

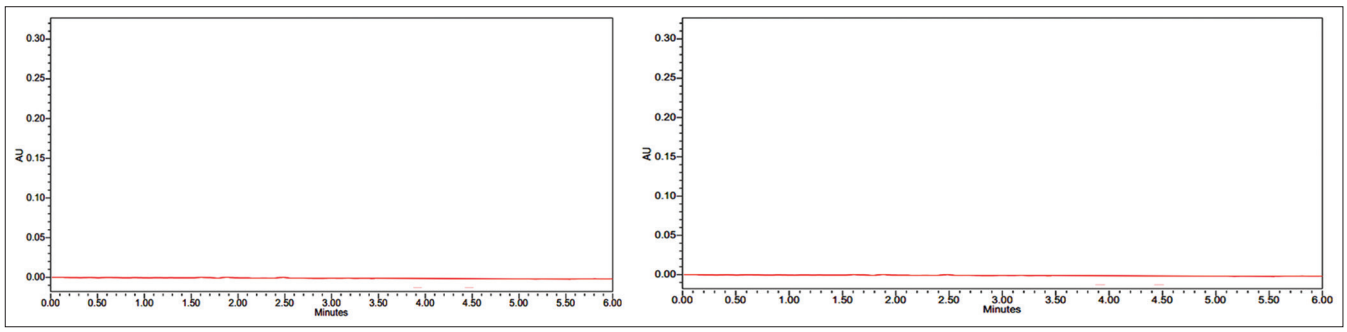

Fig. 9: Chromatogram of blank and placebo

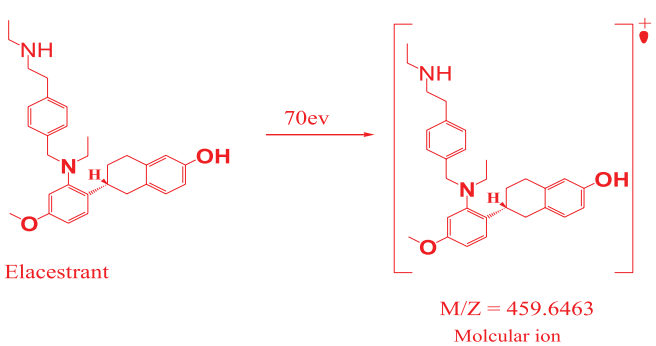

Fig. 10: Formation of molecular ion

The deliberate departure of experimental parameter values had no discernible effect on retention duration, theoretical plates, tailing factor, or peak area (Table 11). The content of the organic solvent varied by ±5% and flow rate changed ±0.8 mL. The robustness of the new approach is demonstrated by the observed change in peak area, which was within limits, or <8.0%.

LOD and LOQ

Elacestrant's lowest detectable and quantifiable concentrations, with acceptable S/N ratio criterion of 3 and 10 according to the present approach, were found to be 0.3 µg/mL and 1.0 µg/mL, respectively [21]. It is clear from decreased LOD and LOQ values (Fig. 18) that the present approach is applicable to a wide range of concentrations.

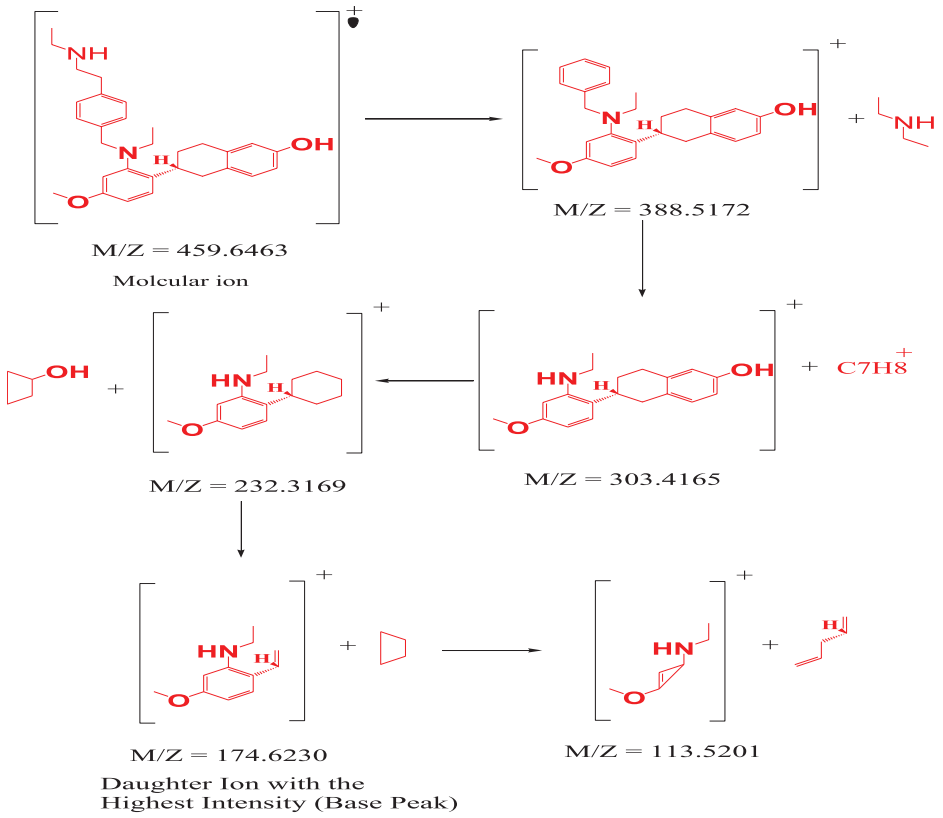

Fig. 11: Fragmentation of elacestrant

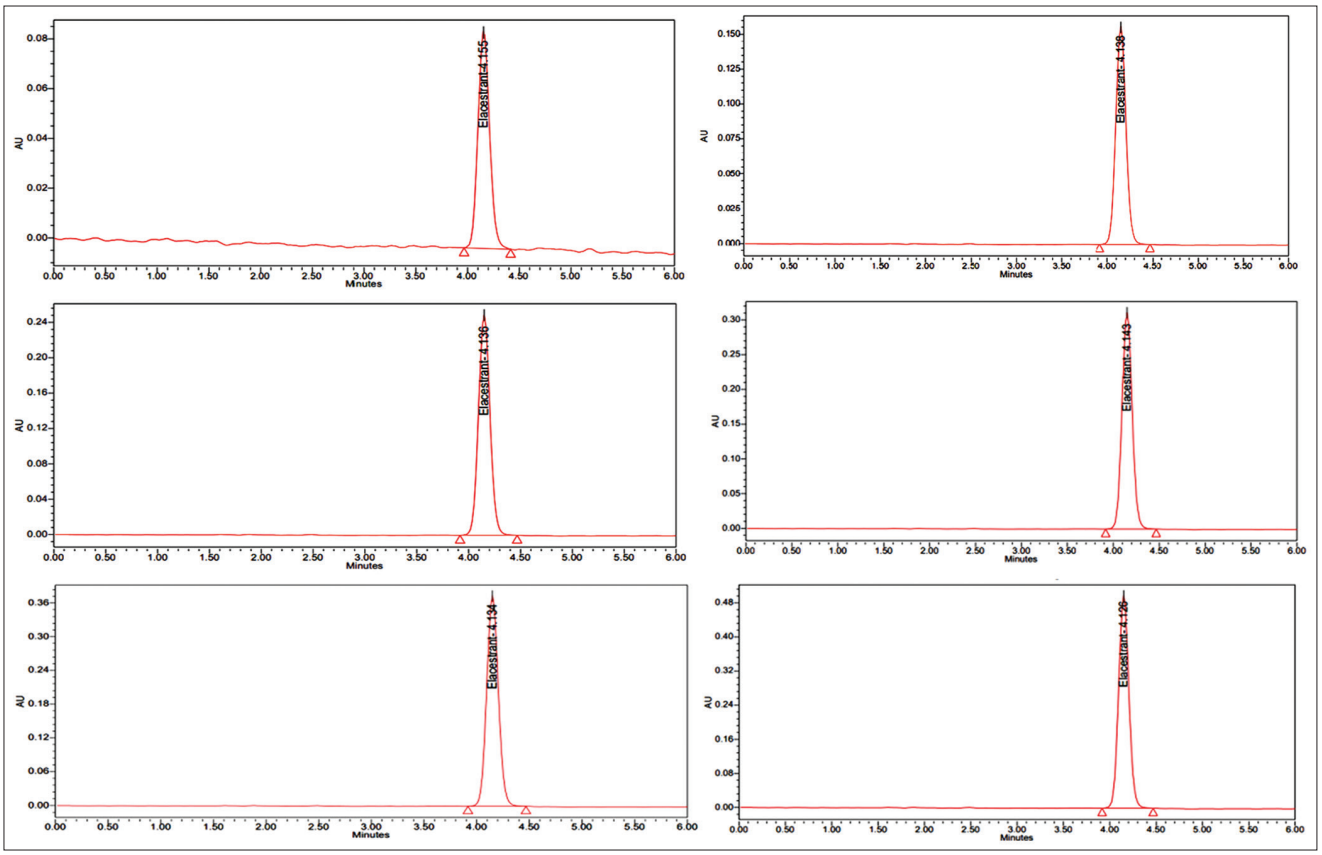

Fig. 12: Chromatogram of linearity- 25%, 50%, 75%, 100%, 125% and 150%

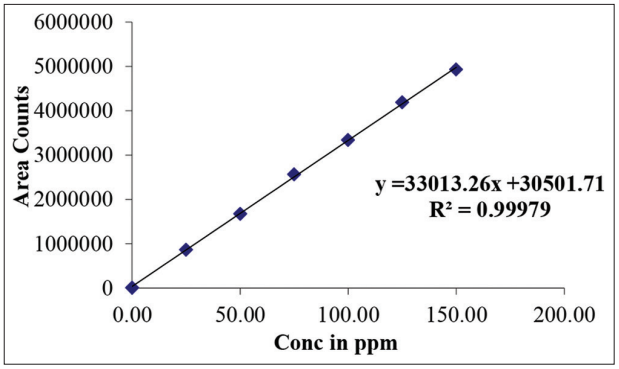

Fig. 13: Calibration curve for elacestrant

Pharmaceutical formulation analysis

Elacestrant's recovery values are good (Table 12), hence the aforesaid method was used to determine how much of it should be in the tablet formulation. These days, the UPLC approach can be employed in quality control labs in developing nations since, in addition to spectrophotometers [21-24], UPLC instruments are reasonably priced for these labs. To determine elacestrant in tablet formulations, the present approach is used in accordance with the present ICH recommendations (Fig. 19).

CONCLUSION

The developed liquid chromatography-MS/MS new method for the identification and quantification of elacestrant in pharmaceutical formulations has proven to be highly sensitive, accurate, and reliable.

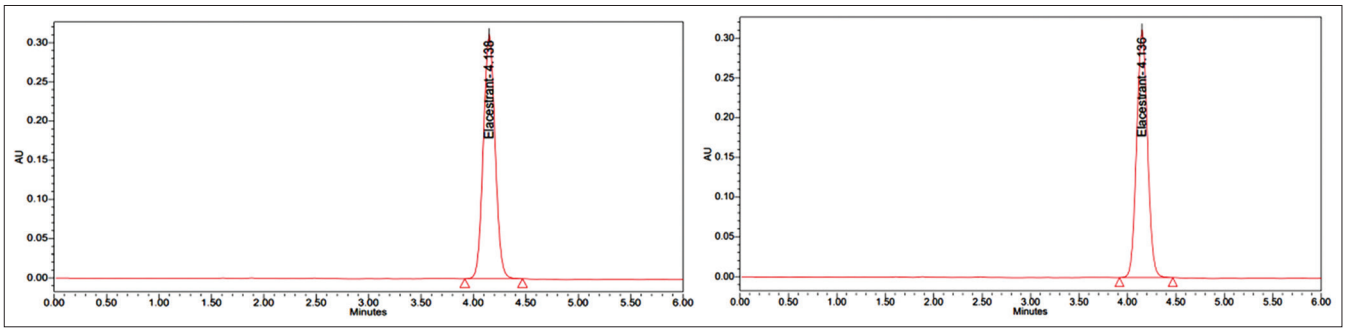

Fig. 14: Method precision and intermediate precision chromatograms

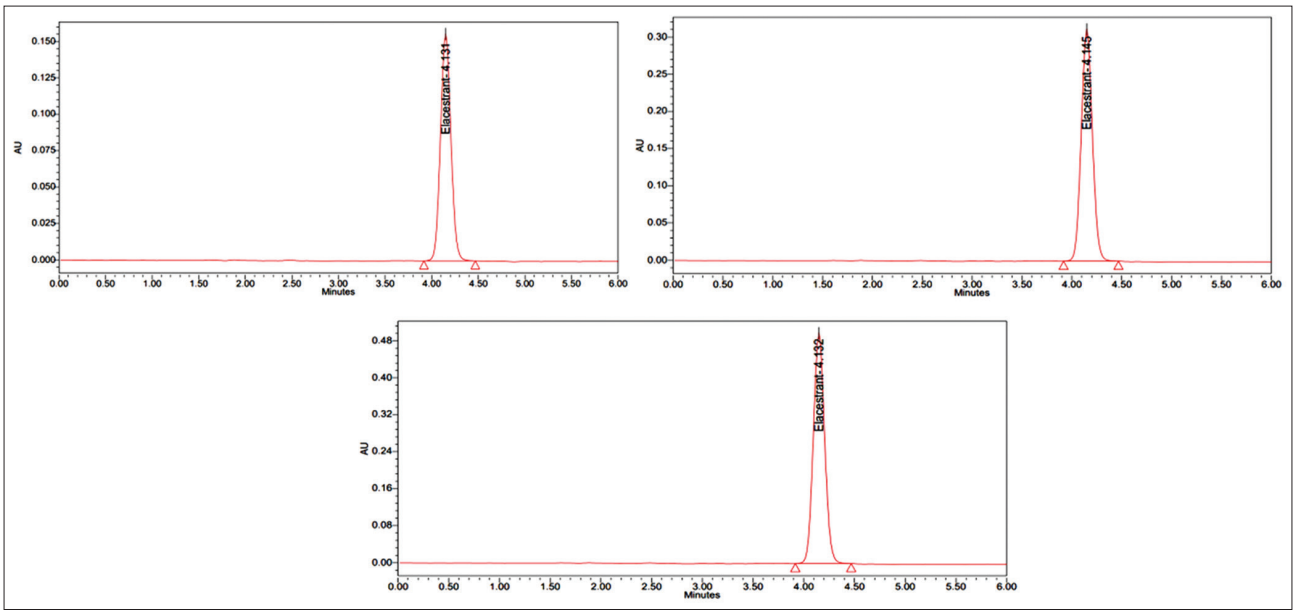

Fig. 15: Chromatogram of accuracy 50%, 100% and 150%

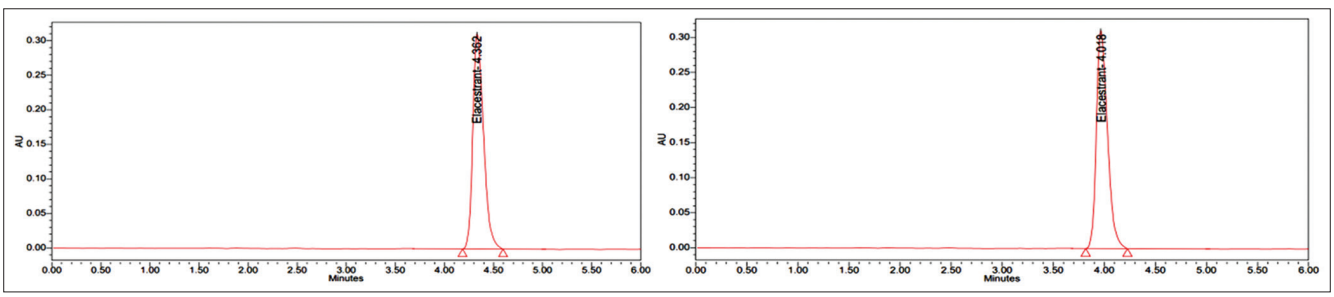

Fig. 16: Chromatogram for less flow rate and more flow rate

Table 12: Pharmaceutical formulation assay

S. No.	Brand name	Form	Dosage	Amount prepared	Amount found	% Assay
1	Orserdu	Tablet	345 mg	10 µg/mL	10.02 µg/mL	100.2

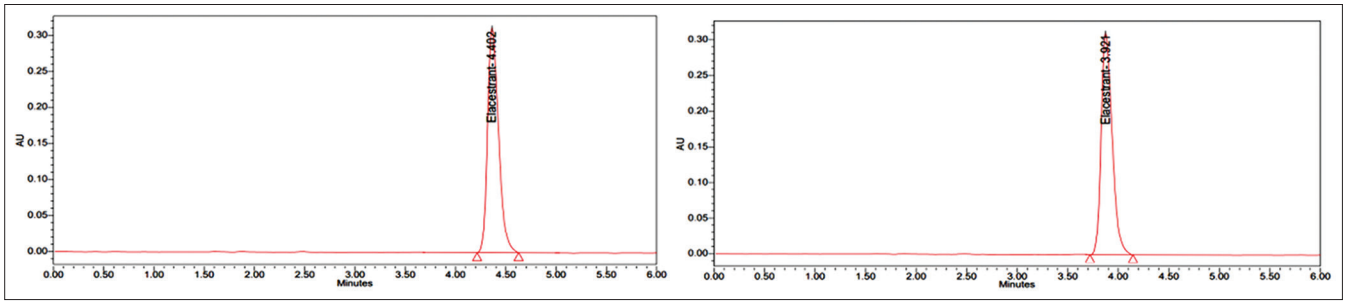

Fig. 17: Chromatograms for less and more organic phase

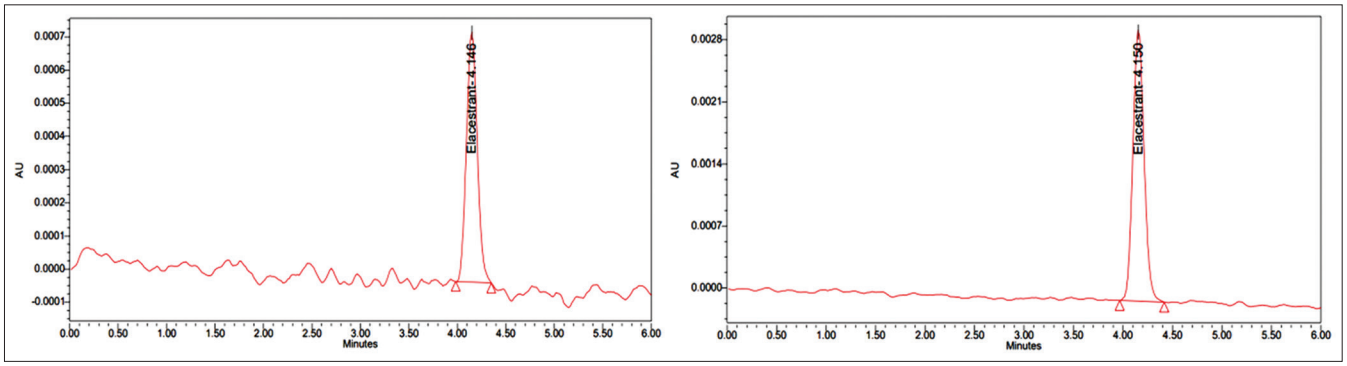

Fig. 18: Chromatogram for limit of detection and limit of quantitation

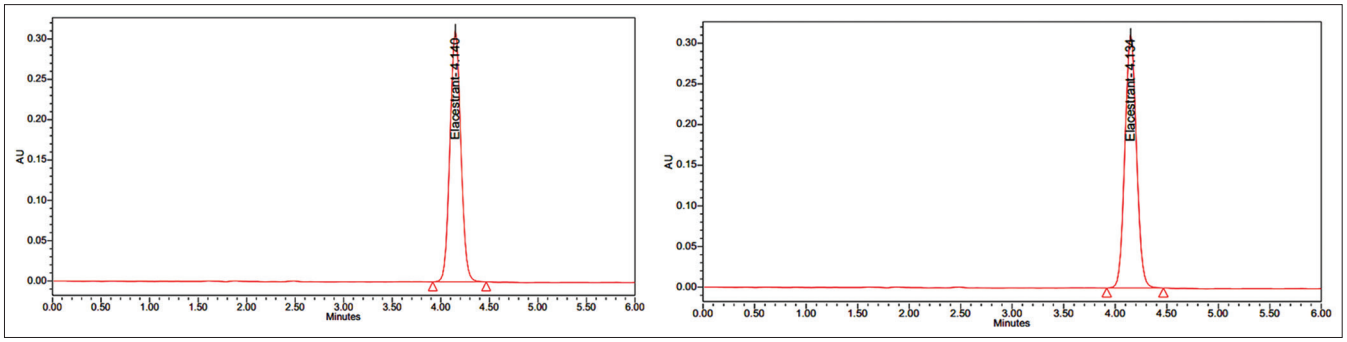

Fig. 19: Chromatograms of pharmaceutical formulation assay

The method exhibited excellent linearity over the concentration range of 25–150 µg/mL, with a correlation coefficient (R^2) of 0.99979. The LOD and LOQ were determined to be 0.3 µg/mL and 1.0 µg/mL, respectively, confirming the method's high sensitivity. Precision studies yielded intra-day and inter-day %RSD values of 0.189 and 0.405, respectively, well below the acceptable limit of 2%, indicating excellent reproducibility. Recovery rates ranged from 99.20% to 101.30%, with corresponding %RSD values between 0.36 and 0.645, confirming the method's accuracy and reliability. Robustness testing showed that variations in peak area remained within acceptable limits (<8.0%), demonstrating the method's stability under minor operational changes. Forced degradation studies further confirmed the method's stability-indicating capability. Elacestrant remained relatively stable under thermal, photolytic, and reductive stress conditions, while significant degradation was observed under oxidative (peroxide), acidic, alkaline, and hydrolytic conditions. Mass spectrometric analysis confirmed the identity of elacestrant with a molecular ion at m/z 459.6463, a base peak at m/z 174.630, and additional fragment ions at m/z 388.5172, 303.4165, and 232.316. Overall, the validated method is statistically robust and well-suited for routine quality control, stability studies, and regulatory compliance in pharmaceutical analysis of elacestrant.

STUDIES INVOLVING PLANTS

Not applicable.

DATA AVAILABILITY STATEMENT

Data included in article/supp. material/referenced in the article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Given by all the authors.

AUTHOR'S CONTRIBUTION

The study was proposed by Subhashini Kanthti, who also created the technique, carried out the trials, and gathered the data. The data were examined by Giri Prasad Gorumutchu. The project was overseen by Rudraraju Ramesh Raju. The original draft was written by Subhashini Kanthti, and all contributors helped with the final manuscript's review and editing.

CONFLICTS OF INTEREST

None.

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REFERENCES

1. Orserdu- elacestrant tablet, film coated. Daily Med. 2023 Feb 08. Archived from the original on 11 February 2023. Retrieved on 22 February 2025.
2. Orserdu EP. European Medicines Agency (EMA); 2023 Oct 09. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/orserdu [Last accessed on 2025 Apr 04].
3. FDA Approves Elacestrant for ER-Positive, HER2-Negative, ESR1-Mutated Advanced or Metastatic Breast Cancer. US Food and Drug Administration(FDA); 2023 Jan 27. Available from: https://web.archive.org/web/20230202074507/https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-elacestrant-er-positive-her2-negative-esr1-mutated-advanced-or-metastatic-breast-cancer [Last accessed on 2025 Apr 04].
4. Available from: https://www.accessdata.fda.gov/drugsatfda-docs/appletter/2023/217639orig1s000ltr.pdf
5. Stemline Therapeutics Inc. A Wholly Owned Subsidiary of Menarini Group, Receives Approval from U.S. FDA for Orserdu (Elacestrant) as the First and Only Treatment Specifically Indicated for Patients with ESR1 Mutations in ER+, HER2- Advanced or Metastatic Breast Cancer. Radius (Press Release). United States: Stemline Therapeutics Inc.; 2023 Jan 31.
6. Perkey C. Elacestrant (Orserdu). Oncology Times. 2023 Oct 20;45(20):7.
7. EC approves Menarini Group's Orserdu for Advanced or Metastatic Breast Cancer. Europe: PMlive; 2023 Sep 21.
8. Lloyd MR, Wander SA, Hamilton E, Razavi P, Bardia A. Next-generation selective estrogen receptor degraders and other novel endocrine therapies for management of metastatic hormone receptor-positive breast cancer: Current and emerging role. Ther Adv Med Oncol. 2022;14:1-25. doi: 10.1177/17588359221113694
9. Singh R, Dhaduk B, Dhalani J. A rapid quantification method for simultaneous determination of pendimethalin and metribuzin contents in suspoemulsion formulation. Results Chem. 2023;5:100779. doi: 10.1016/j.rechem.2023.100779
10. Dhaduk BB, Patel CB, Parsania PH. Reverse phase high-performance liquid chromatographic method development for simultaneous determination of biologically active and thermally stable 1, 1'-bis (3-methyl-4-hydroxyphenyl) cyclohexane and 1, 1'-bis (3, 5-dibromo-4-hydrophenyl) cyclohexane. Chem Der Sinica. 2015;6(4):62-71.
11. Agilent. Zorbax Column Selection Guide for HPLC Catalaoge. United States: Agilent; 2000.
12. International Conferences of Harmonization Validation of Analytical Procedures Test and Methodology. United States: Q2; 2005.
13. Kishore VN, Ramana GV, Nadh RV, Gorumutchu GP. Simultaneous quantification of tiagabine and its related substance by a stability-indicating RP-HPLC method. Rasayan J Chem. 2021;14(4):2236-45. doi: 10.31788/RJC.2021.1446412
14. Gorumutchu GP, Ratnakaram VN. Determination of mianserin using tropaeoline-OOO by ion pair formation. Int J Pharm. 2019;11(1):168-73. doi: 10.22159/ijap.2019v11i1.30125
15. Gorumutchu GP, Ratnakaram VN, Kishore VN. Ulipristal acetate determination using MBTH. Int J Res Pharm Sci. 2019;10(4):3369-75. doi: 10.26452/ijrps.v10i4.1646
16. Gorumutchu GP, Ratnakaram VN. Determination of mianserine using fe3+-phenanthroline by visible spectrophotometry. Res J Pharm Technol. 2019 January 31;12(1):209-12. doi: 10.5958/0974-360X.2019.00038.6
17. Prasad GG, Nadh RV, Kiran KK. Ion-associative complex formation for estimation of piperacillin. Int J Pharm Sci Res. 2019;10(1):117-24. doi: 10.26452/ijrps.v10i1.1787
18. Gorumutchu GP, Ratnakaram VN, Malladi S. Ion-pair formation for the determination of mianserin using fast sulphon black F. Asian J Pharmacol. 2018;12(4) Suppl: S822-8. doi: 10.22377/ijgp.v12i04.2261
19. Gorumutchu GP, Ratnakaram VN. Diazo coupling for the determination of selexipag by visible spectrophotometry. Int J Green Pharm. 2018;12(4):822-8. doi: 10.22377/ijgp.v12i04.2261
20. Gorumutchu GP, Ratnakaram VN. Extractive spectrophotometric determination of ulipristal acetate using naphthol blue black. Res J Pharm Technol. 2019;12(3):1347-52. doi: 10.5958/0974-360X.2019.00226.9
21. Kantheti S, Raju RR. A stability, accuracy, and robustness representing liquid chromatographic method for the quantification of zanubrutinib and its specified impurities. Int J Appl Pharm. 2023;15(5):210-20. doi: 10.22159/ijap.2023v15i5.48213
22. Kishore VN, Ramana GV. Simultaneous determination of tigecycline and its potential impurities by a stability-indicating RP-HPLC-UV detection technique. Int J Appl Pharm. 2022;14(1):75-82. doi: 10.22159/ijap.2022v14i1.41243
23. Gorumutchu GP, Ratnakaram VN. Extractive spectrophotometric determination of ulipristal acetate using naphthol blue black. Res J Pharm Technol. 2019;12(3):1347-52. doi: 10.5958/0974-360X.2019.00226.9
24. Sethi PD. HPLC Quantitative Analysis of Pharmaceutical Formulations. India: CBS Publications; 2001.
25. Bhadru B, Jakinaboina S, Rama Rao T. Analytical method development and validation of fenofibrate by uv spectroscopy. Int J Curr Res. 2024;16(11):30614-7. doi: 10.24941/ijcr.47963.11.2024
26. Yadav S, Kalra N, Arora P. Formulation and analytical evaluation of fast dissolving tablets of zidovudine. Int J Curr Pharm Revi Res. 2024;16(8):1-12.
27. Shah JH, Mehta T, Mukharya A, Chowdhury AR, Patel A, Mevada S, *et al*. Development of novel single HPLC method for simultaneous separation of multiple impurities in dexamethasone drug product. Int J Pharm Pharm Sci. 2024;16(11):24-31. doi: 10.22159/ijpps.2024v16i11.52010
28. Gorumutchu GP, Ratnakaram VN. Determination of mianserine using fe^{3+}-phenanthroline by visible spectrophotometry. Res J Pharm Technol. 2019 January 31;12(1):209-12. doi: 10.5958/0974-360X.2019.00038.6