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Research Article

Stability-Indicating RP-HPLC Method Development and Validation for the Determination of Lerodalcibep in Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT

A reverse-phase high-performance liquid chromatography (RP-HPLC) method was investigated for the determination of lerodalcibep in bulk drug and pharmaceutical dosage form. Chromatographic separation was reported using a Kromasil C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of 0.1% trifluoroacetic acid and acetonitrile (60:40, v/v). The flow rate was 1.0 mL/min, detection was performed at 270 nm, and the column temperature was 30°C. The method was evaluated for system suitability, linearity, precision, accuracy, sensitivity, robustness, assay and forced-degradation behavior. The manuscript reports system precision of 0.3% RSD, intermediate precision of 0.5% RSD, mean accuracy of 99.44%, and mean assay of 99.68%. The reported limits of detection and quantification were 0.08 and 0.24, respectively; units and calculation details require confirmation. Calibration details and several method-specific experimental particulars must be verified against the original records before definitive conclusions on validation and stability-indicating capability are made.

INTRODUCTION

Analytical methods are essential for the identification, quantification and quality assessment of pharmaceutical substances in bulk materials and dosage forms. Chromatographic techniques, particularly high-performance liquid chromatography (HPLC), are widely used because they can separate analytes from components of

complex mixtures and support quantitative analysis.

Reverse-phase HPLC uses a relatively non-polar stationary phase and a comparatively polar mobile phase. Method performance depends on factors including stationary-phase chemistry, mobile-phase composition, flow rate, temperature, detection wavelength and sample preparation.

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Appropriate method development and validation are therefore required to establish that a procedure is suitable for its intended analytical purpose.

Lerodalcibep is described in the source manuscript as a PCSK9 blocker used with diet and exercise to lower LDL cholesterol in people with elevated cholesterol. The source also describes it as a synthetic fusion protein. These pharmacological and structural statements should be supported by suitable primary or authoritative references before submission.

The objective of this work was to develop and evaluate an RP-HPLC method for the determination of lerodalcibep in bulk drug and pharmaceutical dosage form, including assessment of relevant validation characteristics and forced-degradation behavior. The precise research gap and novelty statement should be supported by a review of previously published analytical methods for lerodalcibep.

MATERIALS AND METHOD

Chemicals and reagents

The chemicals and reagents used in the present study included acetonitrile (HPLC grade), water (HPLC grade), methanol (HPLC grade), potassium dihydrogen orthophosphate (AR grade), orthophosphoric acid (AR grade), and a Millipore membrane filter (0.2 μm).

Instrumentation and chromatographic conditions

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Lerodalcibep solutions. Sonicator (Ultrasonic

sonicator), PH meter (Thermo scientific), Micro balance (Sartorius), Vacuum filter pump, Kromasil C18 column (250 $\times$ 4.6 mm, 5 μm); mobile phase comprising 0.1% trifluoroacetic acid and acetonitrile (60:40, v/v); flow rate 1.0 mL/min; detection wavelength 270 nm; and column temperature 30°C.

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions:

Accurately weighed 25 mg of Lerodalcibep transferred 25ml and volumetric flasks, 3/4 Th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1000 $\mu\text{g/ml}$ of Lerodalcibep)

Preparation of Standard working solutions

(100% solution): 1ml of Lerodalcibep from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100 $\mu\text{g/ml}$ of Lerodalcibep)

Preparation of Sample stock solutions:

Accurately transfer 1.0 mL of the syringe solution (equivalent to 250 mg of Lerodalcibep) into a 100 mL volumetric flask. Add about 70 mL of diluent and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters. (2500 $\mu\text{g/ml}$ of Lerodalcibep)

Preparation of Sample working solutions

(100% solution): 0.4ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100 $\mu\text{g/ml}$ of Lerodalcibep)

Preparation of buffer:0.1%OPA Buffer but then specifies perchloric acid, while the optimized mobile phase is described as trifluoroacetic acid.



Method validation

System suitability and system precision

The 100 µg/mL standard solution was injected six times to assess system suitability. Report individual retention times and peak areas, mean, standard deviation, %RSD, theoretical plate count, tailing factor and any resolution values where relevant. The reported peak-area results were 667224, 664519, 666707, 665701, 664450 and 661750; mean 665059; standard deviation 1971.2; and %RSD 0.3. A separate chromatographic table reports retention times, peak areas, plate counts and tailing factors; reconcile these values with the final system-suitability.

Table 1: System suitability and system precision

Injection	Retention time (min)	Peak area	USP plate count	Tailing factor
1	2.465	656484	2788	1.47
2	2.468	657909	2856	1.43
3	2.468	653308	2770	1.42
4	2.470	652703	2890	1.48
5	2.471	655078	2762	1.49
6	2.481	654165	2890	1.46

plate counts above 2000 and tailing factors below 2. Include resolution where applicable and clearly identify which injection series supports each reported statistic.

Linearity

A range of 25–150% is reported. Add the nominal and actual concentrations, peak area at each level, replicate count, regression equation, slope, intercept, correlation coefficient and residual assessment, using the original calibration data. The summary reports $R^2 = 0.999$, slope = 6733.8, intercept = 961.27 and equation $y = 6733.8x + 961.27$; verify these against the calibration plot and raw data.

Accuracy

Accuracy was assessed at 50%, 100% and 150%. The reported individual recoveries were 99.40%, 99.44% and 99.50% at 50%; 99.53%, 99.17% and 99.75% at 100%; and 99.56%, 99.53% and 99.08% at 150%. The manuscript reports an overall mean recovery of 99.44%. Confirm the calculation and provide level-wise means and %RSD.

Table 2: Accuracy

Level	Amount spiked (µg/mL)	Amount recovered (µg/mL)	Recovery (%)
50%	50	49.70	99.40
50%	50	49.72	99.44
50%	50	49.75	99.50
100%	100	99.53	99.53
100%	100	99.17	99.17
100%	100	99.75	99.75
150%	150	149.33	99.56
150%	150	149.29	99.53
150%	150	148.62	99.08

Precision

System precision: peak areas 667224, 664519, 666707, 665701, 664450 and 661750; reported mean 665059, SD 1971.2 and %RSD 0.3.

Intermediate precision: peak areas 654576, 650719, 659325, 659160, 657208 and 655128; reported mean 656019, SD 3261.1 and %RSD 0.5. Describe analyst, day, instrument or other changed conditions as applicable.

Sensitivity: LOD and LOQ

The reported LOD and LOQ are 0.08 and 0.24. Confirm and state the units, concentration basis, calculation procedure, equations and supporting data. If calculated from calibration slope and response standard deviation, report those inputs. State whether LOQ was experimentally verified for acceptable precision and accuracy.

Robustness



The robustness testing with changes in flow rate, mobile-phase composition and column temperature, with duplicate injections and %RSD values within the stated limit. However, conditions differ between the method narrative and table. The table lists flow at 0.9 and 1.1 mL/min, mobile-phase compositions 55:45 and 45:55, and temperatures 27°C and 33°C; the narrative instead mentions 25°C and 35°C. Verify the actual settings, the meaning of the $\pm$ notation, and all results before finalizing.

Tale 3: Robustness

Parameter	Reported nominal condition	Reported variation	Reported %RSD
Flow rate	1.0 mL/min	0.9 mL/min	0.1
Flow rate	1.0 mL/min	1.1 mL/min	0.2
Mobile phase	60:40	55:45	0.4
Mobile phase	60:40	45:55	0.3
Column temperature	30°C	27°C	0.9
Column temperature	30°C	33°C	0.4

Degradation studies

Confirm experimental details, including reagent concentration, exposure time, temperature, neutralization/dilution steps and whether the reported percentage represents assay remaining or degradation. The oxidation, acid and alkali procedures specify 30 minutes at “600 c”; this has been interpreted nowhere in this report and must be confirmed from the laboratory record.

Table 4: Degradation studies

Stress condition	Drug reported as undegraded (%)	Drug reported as degraded (%)
Acid	95.45	4.55
Alkali	95.92	4.08
Oxidation	96.28	3.72
Thermal	97.08	2.92

UV	98.18	1.82
Water/neutral	99.17	0.83

Reported procedures include peroxide treatment with 20% hydrogen peroxide; acid and alkali treatment with 2N hydrochloric acid and 2N sodium hydroxide, respectively; dry heat at 105°C for 6 hours; UV exposure described as 7 days or 200 Wh/m²; and neutral hydrolysis in water under reflux for 6 hours at 60°C. Confirm and standardize these conditions. Include chromatograms, peak-purity evidence where available, mass balance and discussion of degradation-product separation before asserting stability-indicating performance.

ASSAY

The reported individual assay results were 99.86%, 99.75%, 99.78%, 99.62%, 99.74% and 99.33%; mean 99.68%, SD 0.188 and %RSD 0.2. Describe the calculation formula, standard/sample preparation and number of determinations.

Table 5: ASSAY

Sample	Assay (%)
1	99.86
2	99.75
3	99.78
4	99.62
5	99.74
6	99.33
Mean	99.68
SD	0.188
%RSD	0.2

Reported procedures include peroxide treatment with 20% hydrogen peroxide; acid and alkali treatment with 2N hydrochloric acid and 2N sodium hydroxide, respectively; dry heat at 105°C for 6 hours; UV exposure described as 7 days or 200 Wh/m²; and neutral hydrolysis in water under reflux for 6 hours at 60°C. Confirm and standardize these conditions. Include chromatograms, peak-purity evidence where



available, mass balance and discussion of degradation-product separation before asserting stability-indicating performance.

RESULTS AND DISCUSSIONS

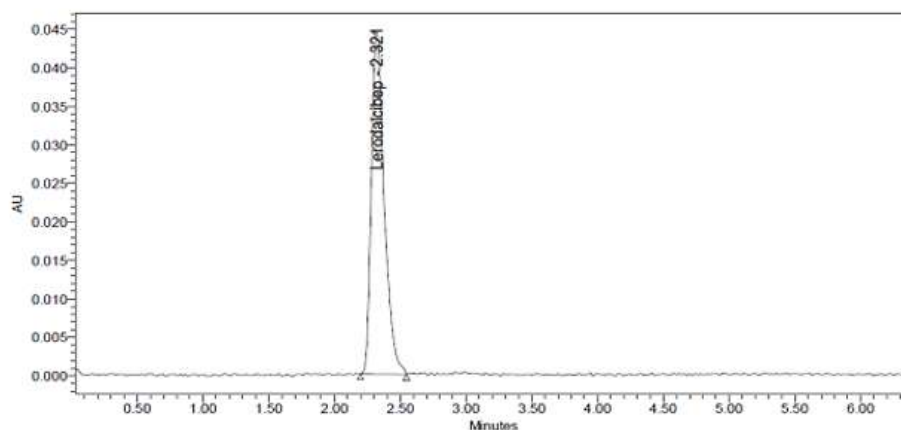


Fig 1: OPTIMIZED CHROMATOGRAM

Specificity:

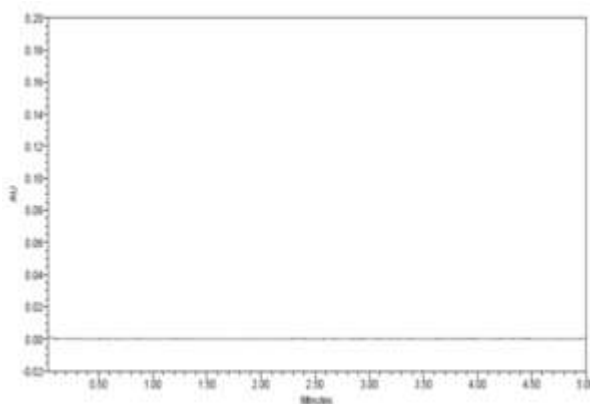
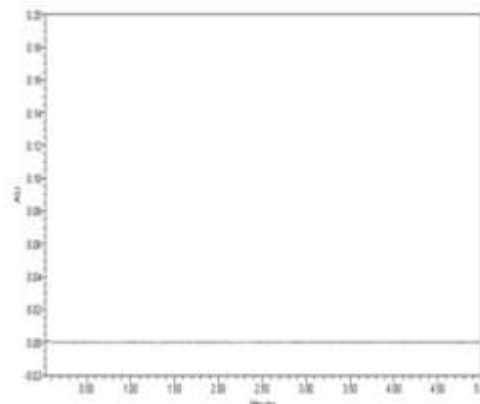
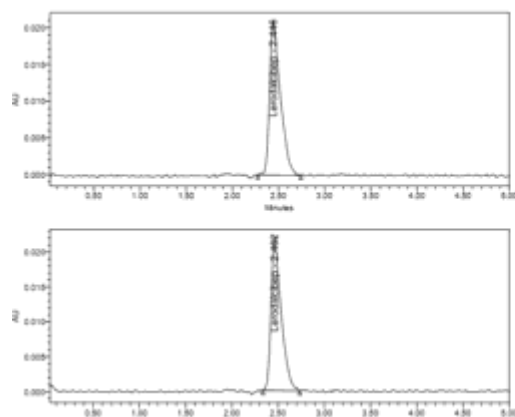


Fig 2: blank Chromatogram

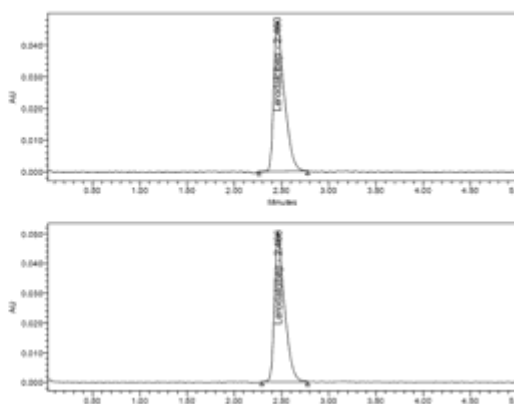


Placebo Chromatogram

Linearity:



LNT 25%



LNT 50%



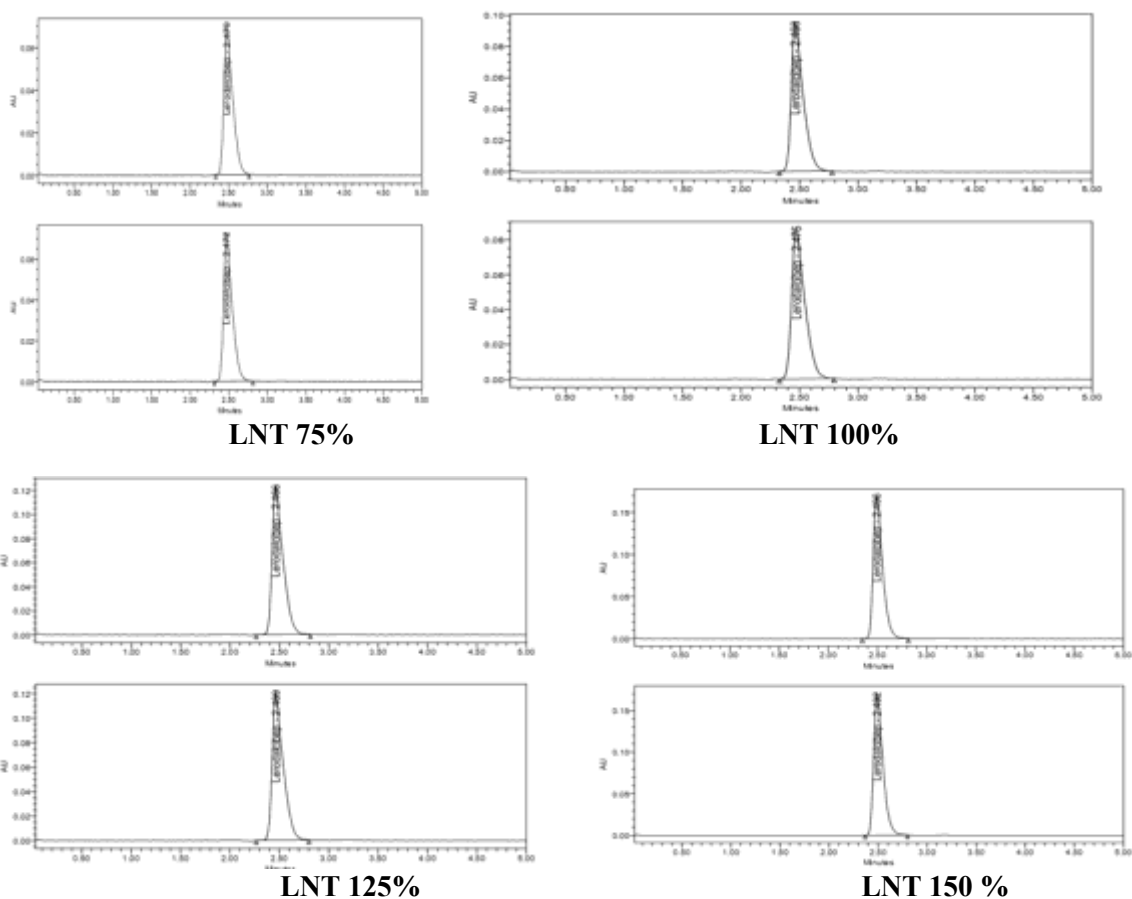
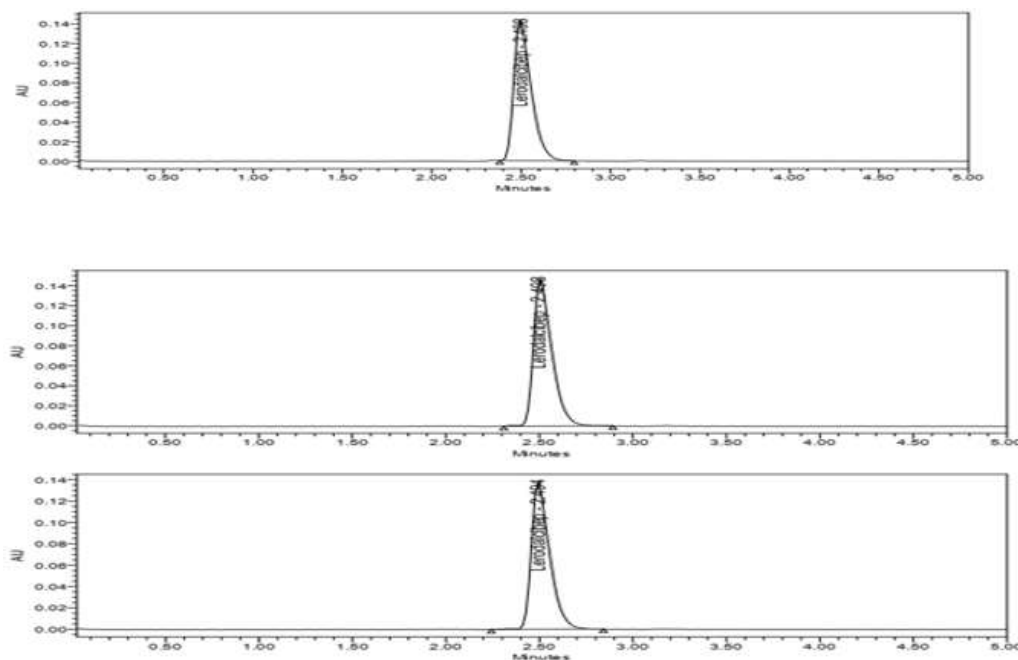
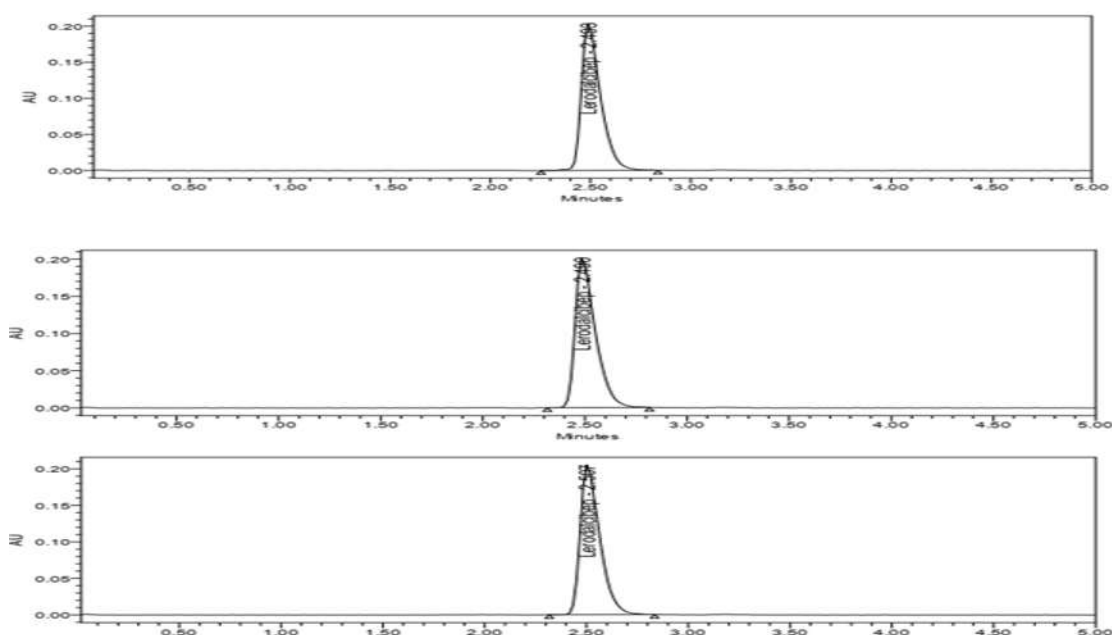


Fig 3: Linearity of 25%,50%,75%,100%,125%,150% Chromatogram

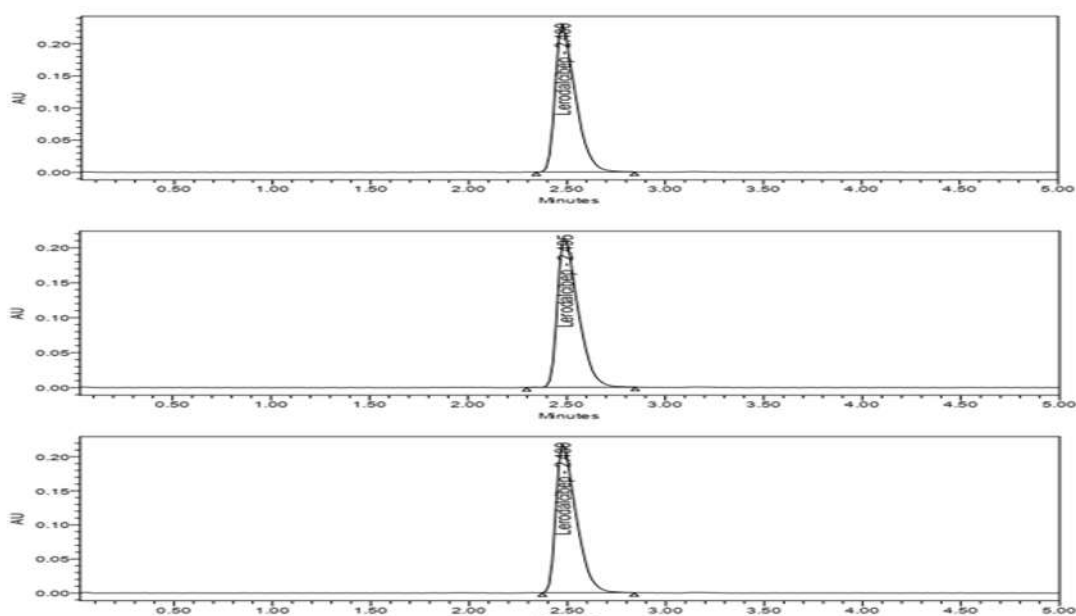
Accuracy:



Accuracy 50% Chromatogram



Accuracy 100% Chromatogram



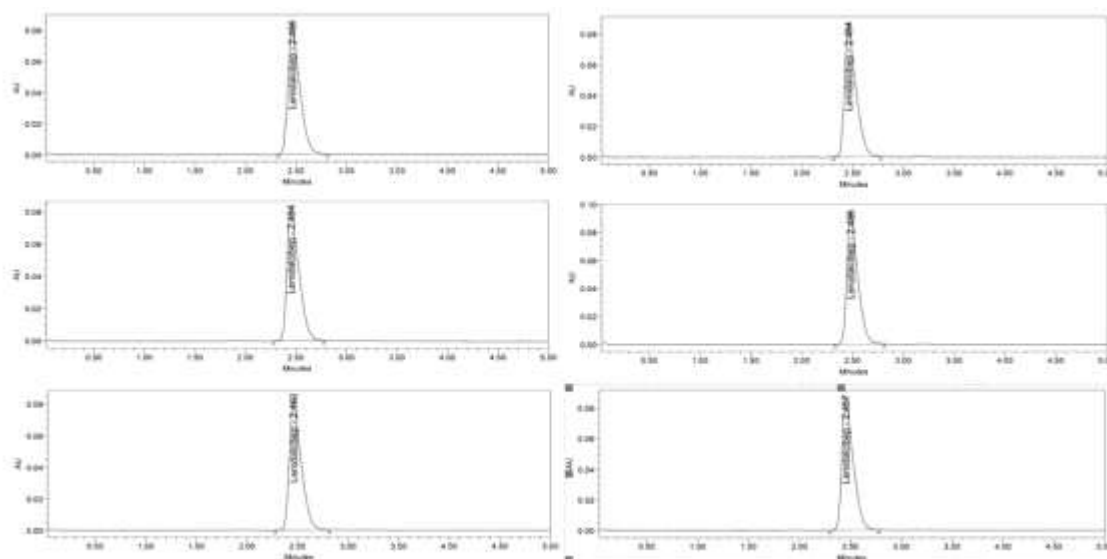
Accuracy 150% Chromatogram

Fig 4: Accuracy of 50%,100%,150% Chromatogram

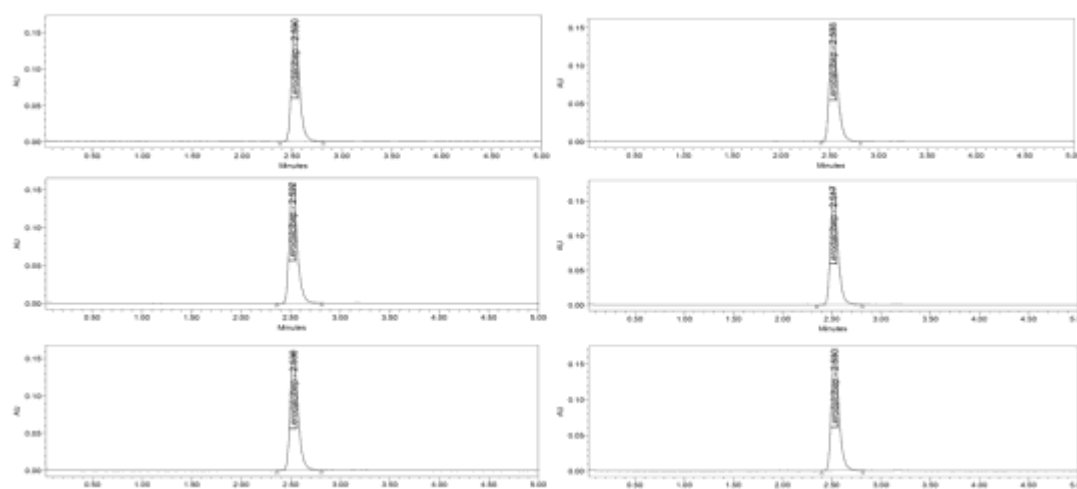
Precision:

Repeatability:





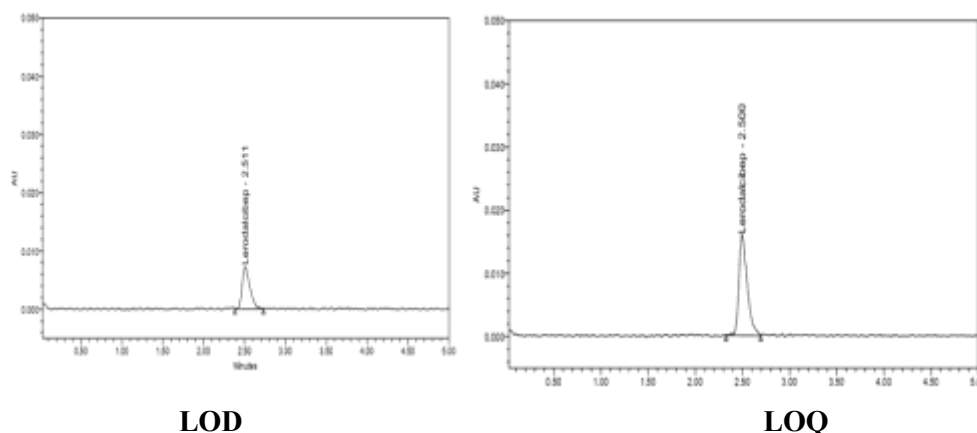
Repeatability Chromatogram



Intermediate precision Chromatogram

Fig 5: Repeatability Chromatogram, Intermediate precision Chromatogram

Sensitivity:



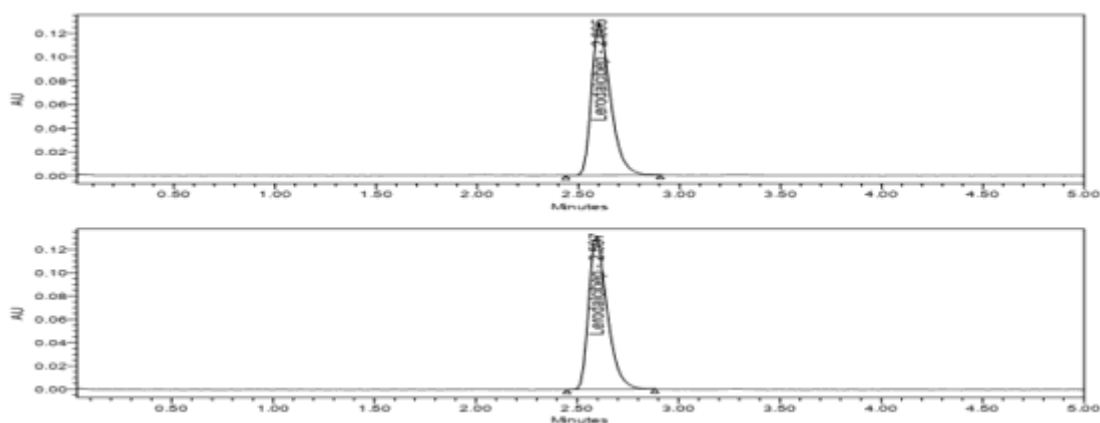
LOD

LOQ

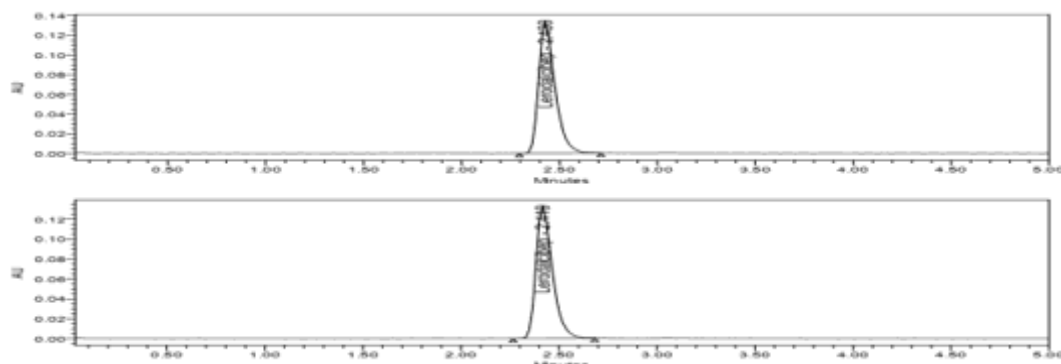
Fig 6: LOD, LOQ Chromatogram



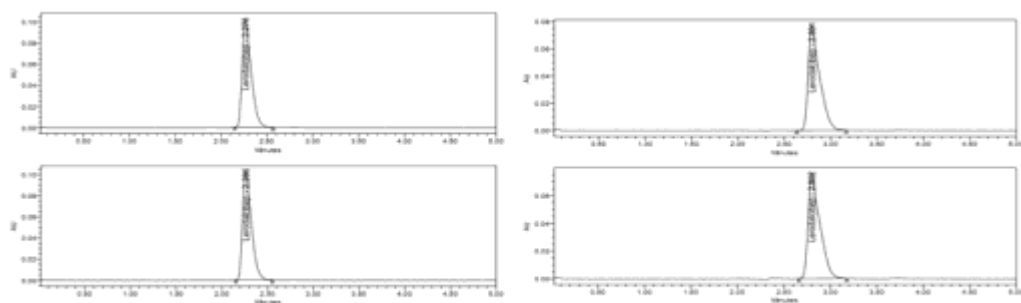
Robustness:



Flow minus Chromatogram of Lerodalcibep

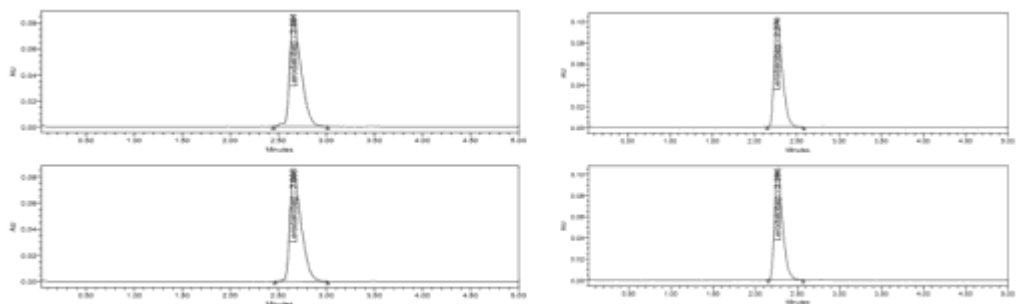


Flow plus Chromatogram of Lerodalcibep



Mobile phase minus

Mobile phase plus



Temperature minus

Temperature plus

Fig 7: Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C)



ASSAY:

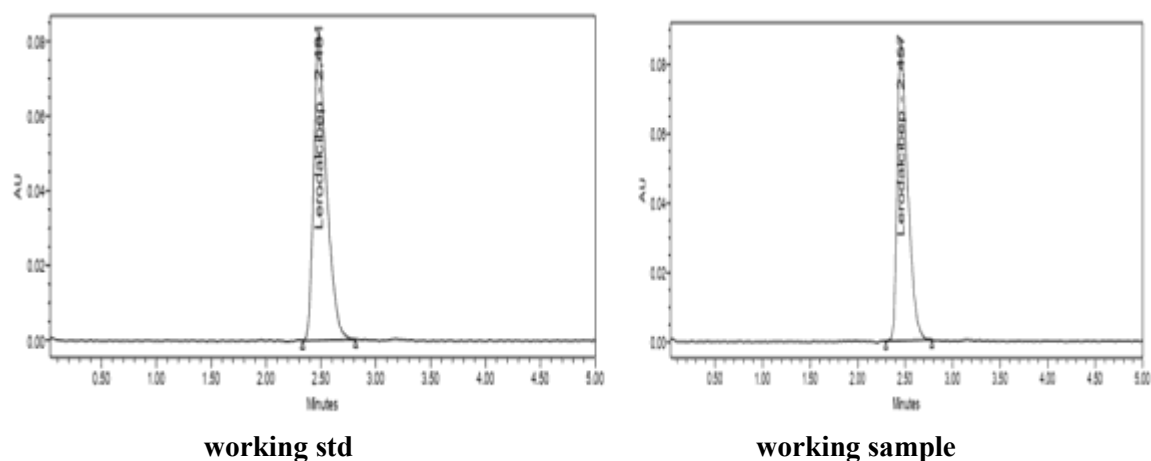


Fig 8: Assay Chromatogram of standard, Assay Chromatogram of sample

Degradation studies:

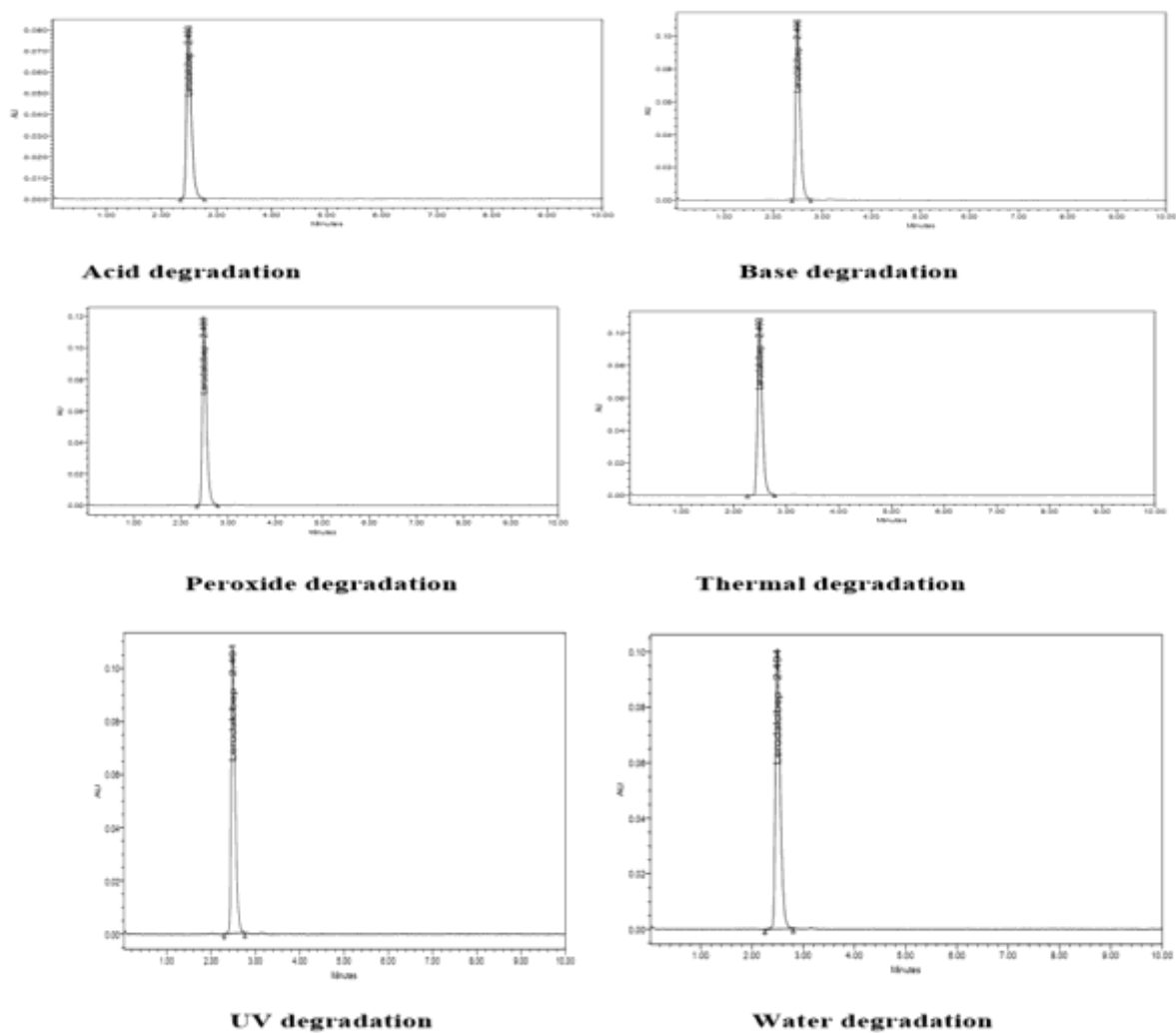


Fig 9: Acid, Base, Peroxide, Thermal, UV, Water degradation chromatogram

The reported chromatographic conditions were a Kromasil C18 column (250 × 4.6 mm, 5 µm), mobile phase of 0.1% trifluoroacetic acid: acetonitrile (60:40, v/v), flow rate 1.0 mL/min, detection at 270 nm and column temperature 30°C. Present representative chromatograms for blank, placebo, standard, linearity levels, precision, accuracy, robustness, assay and each stress condition with legible axes, peak labels and figure numbers.

System-precision and intermediate-precision results are reported as 0.3% and 0.5% RSD,

respectively. Mean recovery is reported as 99.44%, and mean assay as 99.68%. Linearity is summarized as $R^2 = 0.999$, although the full calibration dataset should be supplied. The stress table reports degradation from 0.83% to 4.55%. These summaries alone do not establish selectivity or stability-indicating capability; the chromatograms and peak-purity/separation evidence should be discussed and supplied.

SUMMARY OF REPORTED VALIDATION RESULTS

Parameters		Ierodalcibep	Limit
Linearity Range (µg/ml)		25-150 µg/ml	R< 1
Regression coefficient		0.999	
Slope(m)		6733.8	
Intercept(c)		961.27	
Regression equation (Y=mx + c)		y = 6733.8x + 961.27	
Assay (% mean assay)		99.68%.	90-110%
Specificity		Specific	No interference of any peak
System precision %RSD		0.3	NMT 2.0%
Method precision %RSD		0.2	NMT 2.0%
Accuracy %recovery		99.44%	98-102%
LOD		0.08	NMT 3
LOQ		0.24	NMT 10
Robustness	FM	0.1	%RSD NMT 2.0
	FP	0.2	
	MM	0.4	
	MP	0.3	
	TM	0.9	
	TP	0.4	

CONCLUSION

Chromatographic conditions used are stationary phase Kromasil c18 250 x 4.6 mm, 5µ. Mobile phase 0.1% Trifluoroacetic acid buffer: Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1ml/min, detection wave length was 270nm, column temperature was set to Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out

between 25% to 150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.2 for repeatability and 0.5 for intermediate precision. LOD and LOQ are 0.08µg/ml and 0.24µg/ml respectively. By using above method assay of marketed formulation was carried out 99.68% was present.

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