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STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PEXIDARTINIB IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, economical, precise and accurate RP-HPLC method for Pexidartinib in its Pharmaceutical Dosage Form has been developed. A reverse phase high performance liquid chromatographic method was developed for the Pexidartinib in its Pharmaceutical Dosage Form. The separation was achieved by C18 (250mm x 4..6 mm, 5µ) column and Buffer, pH 4.5: Acetonitrile (75:25) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 233 nm. Retention time of Pexidartinib was found to be 7.237 min. The method has been validated for linearity, accuracy and precision. Linearity observed for Pexidartinib 10-30 µg/ml. Developed method was found to be accurate, precise and rapid for estimation of Pexidartinib in its Dosage Form. The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation, Considerable Degradation was found in alkaline degradation. The proposed method was successfully applied for the estimation of Pexidartinib in Pharmaceutical dosage form.

KEYWORDS: Pexidartinib, Stability Indicating RP-HPLC Method, Validation.

1. INTRODUCTION

Pexidartinib is an oral small-molecule and selective tyrosine kinase inhibitor (TKI) that works by inhibiting the colony-stimulating factor (CSF1)/CSF1 receptor pathway. Pexidartinib was approved by the FDA on August 2, 2019 as the first systemic therapy for symptomatic tenosynovial giant cell tumor (TGCT). Pexidartinib targets the CSF1/CSF1R

pathway as a selective CSF1R inhibitor. It stimulates the autoinhibited state of the CSF1R by interacting with the juxtamembrane region of CSF1R, which is responsible for folding and inactivation of the kinase domain, and preventing the binding of CSF1 and ATP to the region.

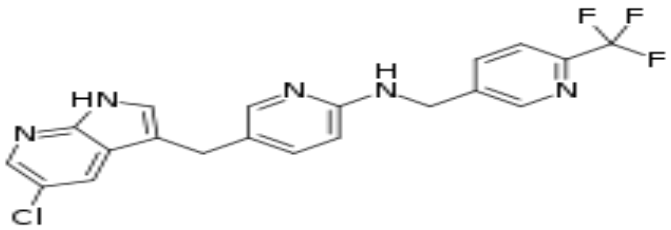

Figure 1: Chemical structure of Pexidartinib.

Literature review revealed that only one method was reported for the estimation of Pexidartinib in Bulk form. So, the aim of present work is to develop and validate stability indicating RP-HPLC Method for the estimation of Pexidartinib in Capsule dosage form as per ICH guidelines.

2. MATERIALS AND METHODS

Chemicals and Reagents

A sample of Pexidartinib was obtained as a gift from Sotac Healthcare, Ahmedabad India. Methanol and Acetonitrile of HPLC grade, KH_2PO_4 and NaOH of LR grade, HCl and H_2O_2 of LR grade and Water of Milli-Q grade were used. All the chemicals were procured from the Finar Chemicals Limited, India.

Apparatus and Instrumentation

Chromatographic separation achieved by the Shimadzu HPLC system (Model: LC-10 AT) with SPD 20A UV detector and LC solution software, Hypersil BDS C18 (250 × 4.6 mm, 5µ) column, Shimadzu analytical balance, Lab man pH meter and Ultrasonicator.

Preparation of standard solutions

Pexidartinib standard stock solution: (200µg/mL): A 20 mg of Pexidartinib was weighed and transferred into a 100 mL volumetric flask. Volume was made up to the mark with methanol.

Preparation of working solution Pexidartinib (20 µg/mL): Take 1 mL from the Pexidartinib stock solution and transferred into 10 mL volumetric flask and volume made up to the mark with mobile phase which was used in particular trials.

Phosphate Buffer preparation: Taken 6.8 gm Phosphate buffer and it was transferred into 1000 mL beaker and 200 mL of water added and shaken for few minutes then volume was made up with water, pH was adjusted by adding 1% O-Phosphoric acid or 0.1M KOH.

Diluent: Mobile phase.

Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study drug solutions of Pexidartinib (20 ppm) was prepared in Methanol. This drug solution was than scanned in UV region of 190-400 nm and maximum Absorbance was recorded. Solution was scanned between 190 - 400 nm. Wavelength What Gives maximum Absorbance was selected from the above Spectra shown in figure 2.

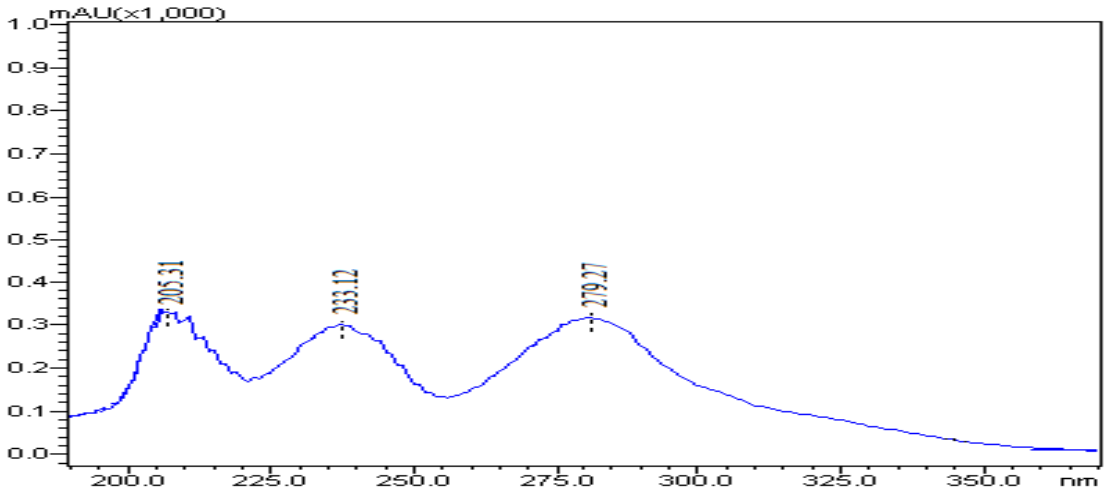

Figure 2: UV Spectra of Pexidartinib.

HPLC Method Development

Trial contains various mobile phase which are considered of Methanol and Water in different proportions and different volumes at different flow rate were tried. On the basis of various trial the mixture of Buffer, pH 4.5: Acetonitrile (75:25) at 1 mL/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and asymmetry shown in figure 3. It was observed that developed chromatographic condition provides better separation of Pexidartinib at 7.237 min.

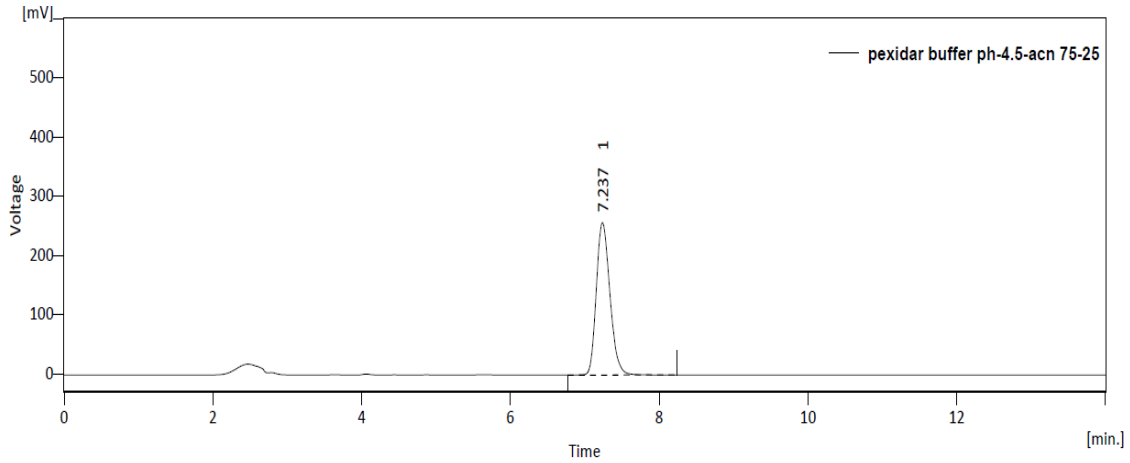

Figure 3: Optimized Chromatogram of Pexidartinib.

Optimization of HPLC Method

The developed HPLC method was optimized with a view to develop stability indicating method. The method was optimized on C_{18} (250× 4.6 mm, 5µ) Hypersil BDS with isocratic method of elution using the mobile phase Buffer, pH 4.5: Acetonitrile (75:25) at a flow rate of 1.0 mL/min and 20 µL injection volume. The detection was carried out at 233 nm.

Forced Degradation Studies

Stability-Indicating method are based on the characteristic structural, chemical or biological properties of each active ingredient of a drug product, is quantitative analytical methods and will differentiate separately active ingredient from its degradation products so that the active ingredient content can be exactly evaluated. Degradation studies were carried out under different stress conditions like Acidic, Alkaline, Oxidation, Photo and Thermal.

Acid degradation

Acid decomposition studies were performed by transferring 1 mL of stock solution into 10 mL of volumetric flask. Then 2 mL of 0.1 N HCl solution was added and mixed well and put for 2, 4 and 6 hrs at room temperature. After time period 2 mL of 0.1 N NaOH added to neutralize the solution and make up the volume with diluent to get 20 µg/mL for Pexidartinib. Results of acid hydrolysis is shown in figure 4.

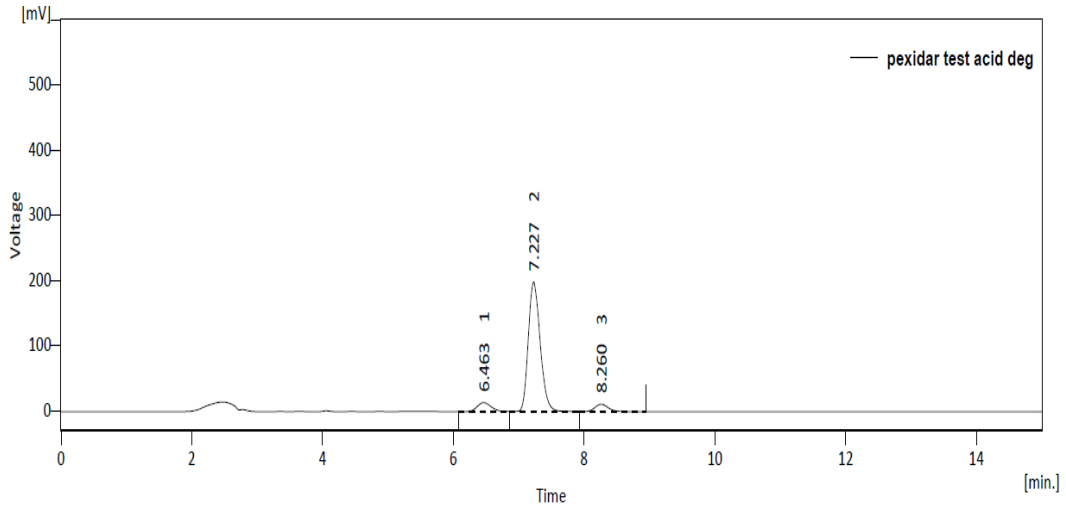

Figure 4: Chromatogram of Acid degradation.

Base degradation

Base decomposition studies were performed by transferring 1 mL of stock solution in to 10 ml of volumetric flask. 2 mL of 0.1 N NaOH solutions was added and mixed well and put for 2, 4 and 6 hrs at room temperature. After time period 2 mL of 0.1 N HCl added to neutralize the solution and make up the volume with diluent to get 20 µg/mL for Pexidartinib. Results of base hydrolysis is shown in figure 5.

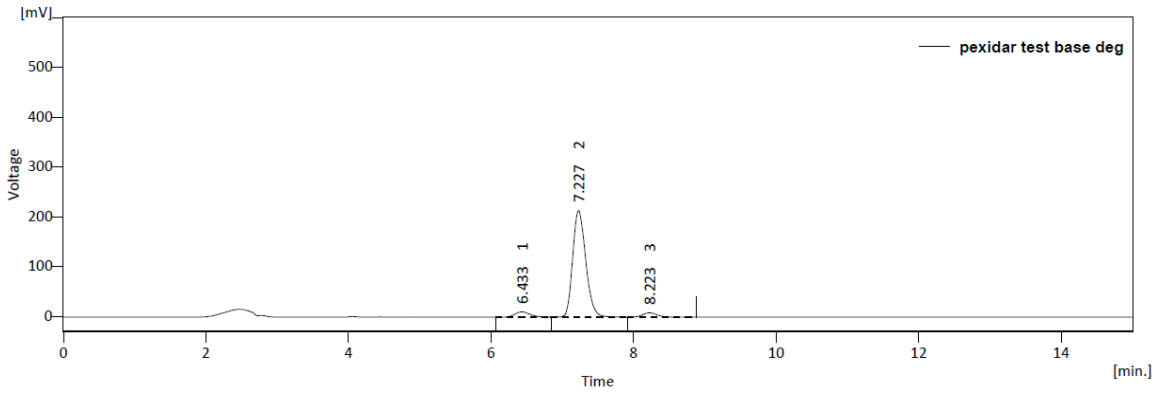

Figure 5: Chromatogram of Base degradation.

Oxidation degradation

Oxidation decomposition studies were performed by transferring 1 mL of stock solution into 10 mL of volumetric flask. 2 mL of 0.1 N HCl solutions was added and mixed well and put for 2, 4, 6 hrs at room temperature. After time period make up the volume with diluent to get 20 µg/mL for Pexidartinib. Results of Oxidation degradation is shown in figure 6

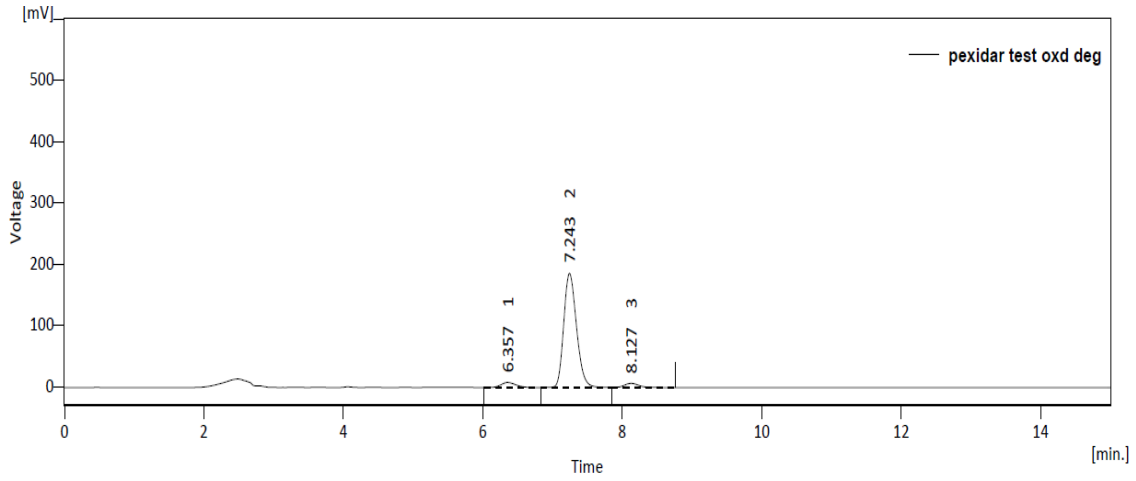

Figure 6: Chromatogram of Oxidation degradation.

Photo degradation

Photo decomposition studies were performed by transferring 1 mL of stock solution in to 10 ml of volumetric flask. Volumetric flask was kept in UV Chamber for 12, 18 and 24 hrs. After time period make up the volume with diluent to get 20 µg/ml for Pexidartinib. Results of Photo degradation is shown in figure 7.

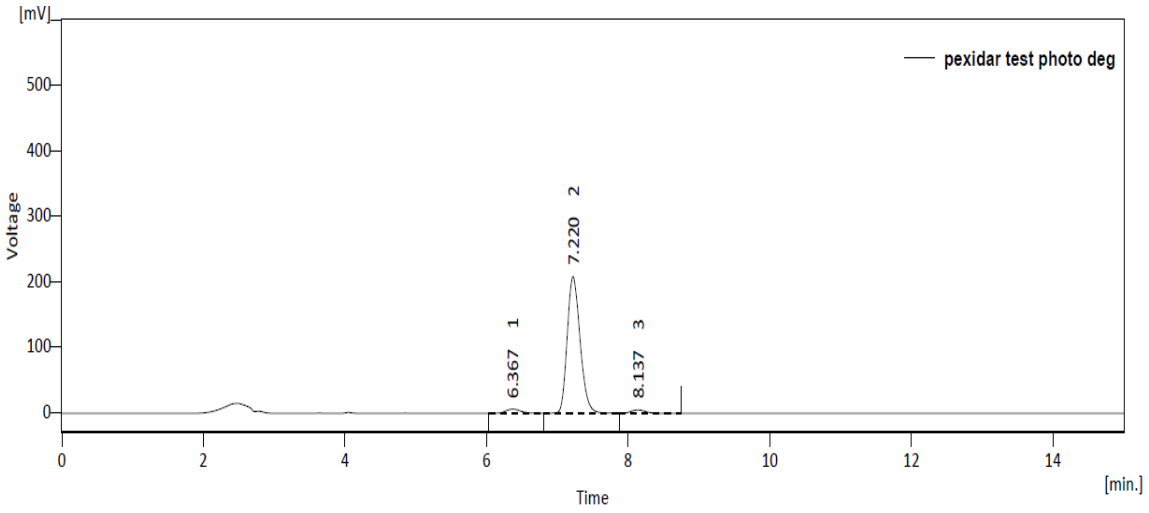

Figure 7: Chromatogram of Photo degradation.

Thermal degradation

20 mg of Pexidartinib was taken in 100 mL volumetric flask and put in oven for 2, 5 and 10 hrs at 80^0C temperature, than after volumetric flask was removed and cool at room temperature, volume was made up with mobile phase, 1 mL of this solution was transferred in 10 mL volumetric and volume was made up with diluent to get 20 µg/mL for Pexidartinib. Results of Thermal degradation is shown in figure 8.

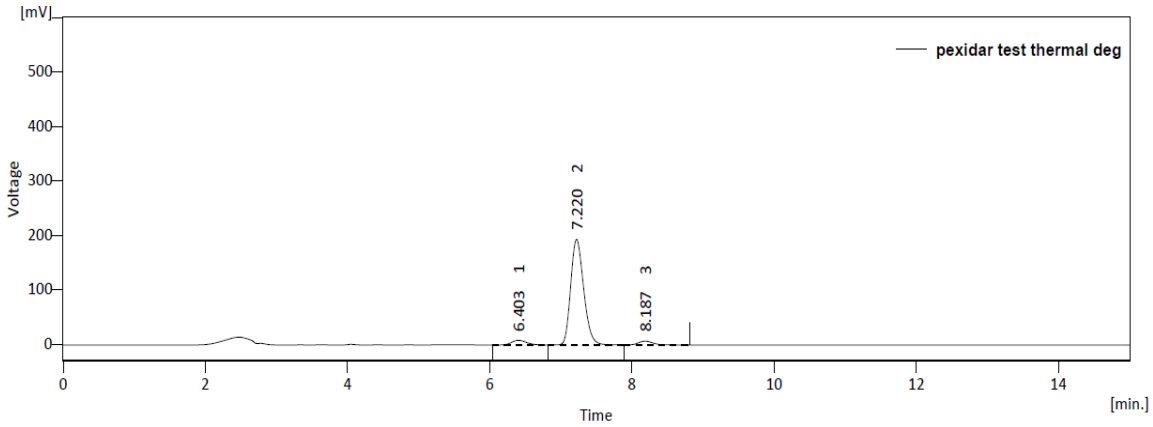

Figure 8: Chromatogram of Thermal degradation.

Table 1: % Degradation Calculation.

Pexidartinib		
Area of Standard		3174.13
Condition	**Area of Standard**	**% Degradation**
Acid	2564.58	19.20
Base	2658.31	16.25
Thermal	2500.45	21.22
Oxidation	2406.59	24.18
Photo	2634.05	17.02

Validation of Developed Method

Linearity

By analysis of standard solution in range of 10-30 µg/mL, the linearity for Pexidartinib was evaluated.

The graph of peak area obtained verses respective concentrations were plotted in term of slope, intercept and correlation co-efficient value.

For Pexidartinib calibration curve, Correlation co-efficient (r^2) was observed 0.9999.
The regression line equation for Pexidartinib is as following:

For Pexidartinib **y = 162.32x + 10.195**

Table 2: Data for Linearity.

Sr. No	**Concentration (µg/ml)**	**Area**
1	10	1640.960
2	15	2441.050
3	20	3241.512
4	25	4080.511
5	30	4879.326

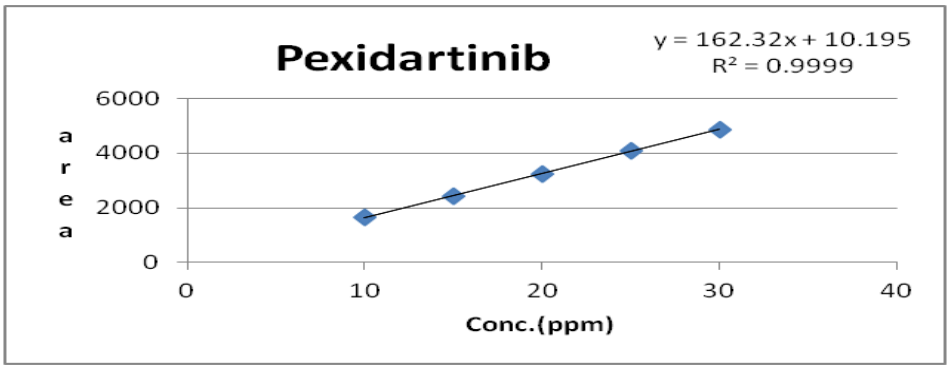

Figure 9: Calibration Curve of Pexidartinib (10-30 µg/mL).

Precision

A. Repeatability

Repeatability is measured by six times injecting the standard solution containing Pexidartinib (20µg/mL) and peak areas were determined and calculation was done to obtain %R.S.D.

The % RSD for Pexidartinib (20 µg/mL) was found to be 0.80

Table 3: Repeatability data.

Pexidartinib (50 µg/mL)				
Sr. No.	**Conc. (µg/ml)**	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
1.	20	3299.84	3312.00±26.44	0.80
		3274.13		
		3327.92		
		3343.36		
		3331.70		
		3295.02		

B. Intraday Precision

Standard solution having Pexidartinib concentration (10, 20, 30 µg/mL) was injected three times in same day and peak areas were determined and calculation was done to obtain %R.S.D.

Intraday precision for Pexidartinib was found in range of 0.41-1.95 % RSD.

Table 4: Intraday precision data.

Sr. No.	Conc. (µg/mL)	Mean ± S.D (n=6)	% R.S.D
1	10	1655.17±32.27	1.95
2	20	3226.47±37.72	1.17
3	30	4923.15±20.09	0.41

C. Interday Precision

Standard solution having Pexidartinib concentration (10, 20, 30 µg/mL) was injected three times in different days and peak areas were determined and calculation was done to obtain

%R.S.D.

Interday precision for Pexidartinib was found in range of 0.66-1.64% RSD

Table 5: Interday precision data.

Sr. No.	Conc. (µg/mL)	Mean ± S.D (n=6)	% R.S.D
1	10	1676.68±27.47	1.64
2	20	3188.82±20.98	0.66
3	30	4921.98±40.02	0.81

Accuracy

10 µg/mL drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10 mL. The area of each solution peak was measured at 233 nm. The amount of Pexidartinib was calculated at each level and % recoveries were computed.

Table 6: Recovery data.

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% RSD
1	80 %	10	8	8.09	101.13	1.35
2		10	8	7.98	99.79	
3		10	8	7.87	98.43	
4	100 %	10	10	10.06	100.59	0.69
5		10	10	9.94	99.39	
6		10	10	9.94	99.39	
7	120 %	10	12	12.09	100.73	0.94
8		10	12	11.97	99.79	
9		10	12	11.86	98.86	

LOD and LOQ

The LOD was estimated from the set of 3 calibration curves used to determination linearity. The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)

Where, SD= Standard deviation of Y-intercepts of 3 calibration curves.

Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine Linearity. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of Y-intercepts of 3 calibration curves.

Table 7: LOD and LOQ data.

LOD	LOQ
LOD = 3.3 x (SD / Slope) = 3.3 x (12.28/162.35) = 0.25 µg/ml	LOQ = 10 x (SD / Slope) = 10x (12.28/162.35) = 0.82 µg/ml

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.1 mL/min) 0.9 mL/min and 1.1 mL/min.
2. Ratio of Mobile phase was changed (±2) Buffer: Acetonitrile (77:23) and Buffer: Methanol (73:27)
3. Ratio of pH was changed (±0.2) pH 4.8 and pH 5.2

Table 8: Robustness data.

Sr. No.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase(-2)	Area at Mobile phase(+2)	Area at pH (- 0.2)	Area at pH (+0.2)
1	3340.87	3049.09	3362.26	3064.04	3236.51	3244.71
2	3301.81	3019.81	3372.45	3088.35	3234.40	3212.60
3	3393.39	3074.06	3305.05	3017.47	3162.58	3247.33
% R.S.D	1.37	0.89	1.31	0.60	1.09	1.18

Application of Method in marketed formulation

Take Capsule powder equivalent to 20 mg Pexidartinib and Transfer into 100 mL volumetric flask and add 60 mL of mobile phase and shake the solution for 15 minutes and make up the volume with mobile phase. (Stock solution 200 ppm), take 1mL from this solution and transfer into 10 mL volumetric flask and make the volume with mobile phase. (Working solution 20 ppm)

Table 9: Assay results.

Sr. No.	Label claim (mg)	Result (mg)	% Assay	Average % assay	SD	%RSD
1	200	198.57	99.29	99.55	0.24	0.24
2	200	199.49	99.74			
3	200	199.23	99.61			

3. RESULTS AND DISCUSSION

The analytical method for the estimation of Pexidartinib was developed and validation. Also, forced degradation study was carried out. Linearity of the Pexidartinib was found to be linear and Correlation co-efficient (r^2) was observed 0.9999. The % RSD values for Repeatability, Intraday and Interday precision was found less than 2% (i.e. 0.80%, 0.41–1.95% and 0.66-1.64% respectively). LOD and LOQ values was found 0.25 µg/mL and 0.82 µg/mL. Estimation of accuracy was carried out in terms of %Recovery, is found to be 98.43-101.13%.

Forced degradation studies was carried out as per the ICH recommendations and the developed stability indicating RP-HPLC method could separate drug from degradation products formed under various stressed conditions.

Table 10: Summary of Developed method.

Sr. no.	Parameter	Result
1	Linearity range	10.30 µg/mL
2	Regression equation	y = 162.32x + 10.195
3	Correlation coefficient	r^2= 0.9999
4	Precision (%RSD)	
	Repeatability	0.80
	Intraday	0.41-1.95
	Interday	0.66-1.64
5	Accuracy (% Recovery)	98.43-101.13
6	LOD	0.25 µg/mL
7	LOQ	0.82 µg/mL
8	%Assay	99.55

4. CONCLUSION

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, selective, robust and rapid and can be applied successfully in routine analysis for the estimation of Pexidartinib in its Pharmaceutical Dosage Form.

5. ACKNOWLEDGEMENTS

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