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Analytical method development and validation of stability indicating HPLC method for estimation of pexidartinib in bulk and pharmaceutical dosage form

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Abstract
Stability indicating RP-HPLC method for estimation of Pexidartinib in bulk and pharmaceutical dosage form has been developed. The separation was achieved by Shimadzu LC-10AT Hypersil BDS C18 (25cm x 0.46cm) column and Phosphate Buffer (pH 3.5): Acetonitrile (70:30) as mobile phase at a flow rate of 1.0 mL/min. Detection was carried out at 225 nm. Retention time of Pexidartinib was found to be 6.517 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 bulk and pharmaceutical 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 the drug in commercial dosage form.

Keywords: Analytical, HPLC, estimation, pharmaceutical

1. Introduction
Tenosynovial Giant Cell Tumor (TGCT) is a group of rare tumors that form in the joints. TGCT is not typically cancerous, but it can grow and damage surrounding structures. These tumors grow in three areas of the joint: synovium: the thin layer of tissue that lines the inner joint surfaces bursae: fluid-filled sacs that cushion tendons and muscles around the joint to prevent friction tendon sheath: a layer of tissue around tendons. TGCTs are divided into types based on where they are and how quickly they grow. Localized giant cell tumors grow slowly. They start in smaller joints like the hand. These tumors are called giant cell tumors of the tendon sheath (GCTTS). Diffuse giant cell tumors grow quickly and affect large joints like the knee, hip ankle, shoulder or elbow. These tumors are called pigmented villonodular synovitis (PVNS). Both localized and diffuse TGCTs are found inside the joint. Diffuse giant cell tumors can also be found outside the joint. In rare cases, they can spread to sites like the lymph nodes or lungs.

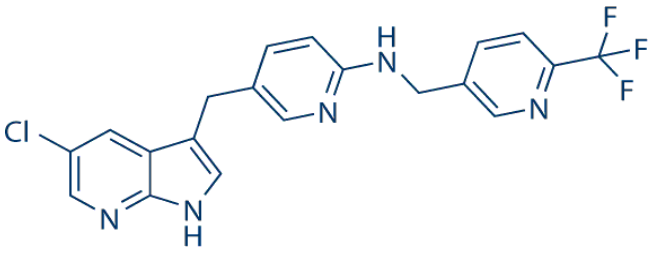

Fig 1: Structure of Pexidartinib Hydrochloride

Pexidartinib hydrochloride is chemically 5-[(5-chloro-1H-pyrrolo [2, 3-b]pyridin-3-yl)methyl]-N-[[6- (trifluoromethyl) pyridin-3-yl]methyl]pyridin-2-amine. It is a Kinase Inhibitor belonging to the class of Antineoplastic Agents. Pexidartinib is a small molecule tyrosine kinase inhibitor that targets colony stimulating factor 1 receptor (CSF1R), KIT proto-oncogene receptor tyrosine kinase (KIT), and FMS-like tyrosine kinase 3 (FLT3) harboring an internal tandem duplication (ITD) mutation. Overexpression of the CSF1R ligand promotes cell proliferation and accumulation in the synovium. *In vitro*, pexidartinib inhibited proliferation of cell lines dependent on CSF1R and ligand-induced autophosphorylation of CSF1R. Pexidartinib also inhibited the proliferation of a CSF1R dependent cell line *in vivo*. [1, 2]
From literature review and patent search we identified that some methods are reported for the analysis of drug. The dosage form containing pexidartinib hydrochloride is available in market. But no method has been reported for stability indicating estimation of Pexidartinib in bulk and pharmaceutical dosage form by RP-HPLC method.

Materials and Methods
Chemicals and Reagents
Pexidartinib Hydrochloride was obtained from Sreeni Labs. Other reagents used are Methanol (HPLC Grade), Potassium Dihydrogen (AR Grade), Water (HPLC Grade) and Acetonitrile (HPLC Grade).

HPLC and Chromatographic Condition
HPLC Model – LC-10AT equipped with UV Detector was employed in this method. Spinchrom software was used for peak integration along with data processing. The column used for separation of analytes is Hypersil BDS Column C_{18} (25cm x 0.46 cm). Isocratic mode of elution was employed. Ultrasonic Water Bath, pH meter, Analytical Balance AUX-200 are other equipments utilized. Phosphate Buffer (pH 3.5): Acetonitrile (50:50) as mobile phase, at a flow rate of 1 ml/min was used. Sample was analyzed at 225 nm at injection volume of 20 µl.

Preparation of Standard Solution
Pexidartinib Standard Stock Solution (200 µg/ml)
A 20mg of Pexidartinib was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with mobile phase.

Preparation of Standard Working Solution of Pexidartinib (20 µg/ml)

Take 1ml from Pexidartinib stock solution and transferred to a 10 ml Volumetric flask and volume made up to the mark by Mobile phase which was used in trials. [3-9]

Wavelength Selection

An ideal wavelength is the one that gives good response for the drugs that are to be detected. Solution of Pexidartinib was scanned between 200-400 nm. Wavelength 225 nm was selected from the overlay spectra of solution (Figure 2).

Various mobile phases were tried which consist of methanol, water, buffers in different proportions with various pH and different volumes at different flow rate. Phosphate Buffer (pH 3.5): Acetonitrile (50:50) as mobile phase, at a flow rate of 1 ml/min was finalized.

System Suitability Test

System Suitability Test were carried out on freshly prepared standard stock solution of Pexidartinib under optimized chromatographic condition and parameters were studied to evaluate the suitability of the system (Table 1)

Assay of Marketed Formulation

Accurately weighed amount of capsule powder equivalent to 20 mg of Pexidartinib was transferred carefully in a clean and dry 100 ml volumetric flask. Add 60 ml Mobile phase (methanol) and shake for 10 min and make up the volume with Mobile phase. The solution was filtered through Whatman filter paper mo.42. Take 1 ml from this solution and transferred to 10 ml volumetric flask and made up volume up to the mark with Mobile phase.

Validation of RP-HPLC Method

1. Specificity

To perform the Specificity, Solution of Pexidartinib was injected and interference was checked with the chromatogram of blank.

2. Linearity

The linearity for Pexidartinib was assessed by analysis of standard solution in range of 10-30 µg/ml. The linearity of the method was evaluated by linear regression analysis (Table 2, Figure 3, 4)

3. Precision

The precision (repeatability, intraday precision and interday precision) of the proposed method was evaluated by carrying out six independent assays of the sample. RSD (%) of six assay values obtained was calculated. (Table 4, 5)

4. Accuracy

Good recovery data of Pexidartinib was obtained by spiking 80 %, 100 % and 120 % of standard solution in it and diluted up to 10 ml. The area of solution peak was measured at 225 nm. The amount of Pexidartinib was calculated at each level and % recoveries was computed (Table 6)

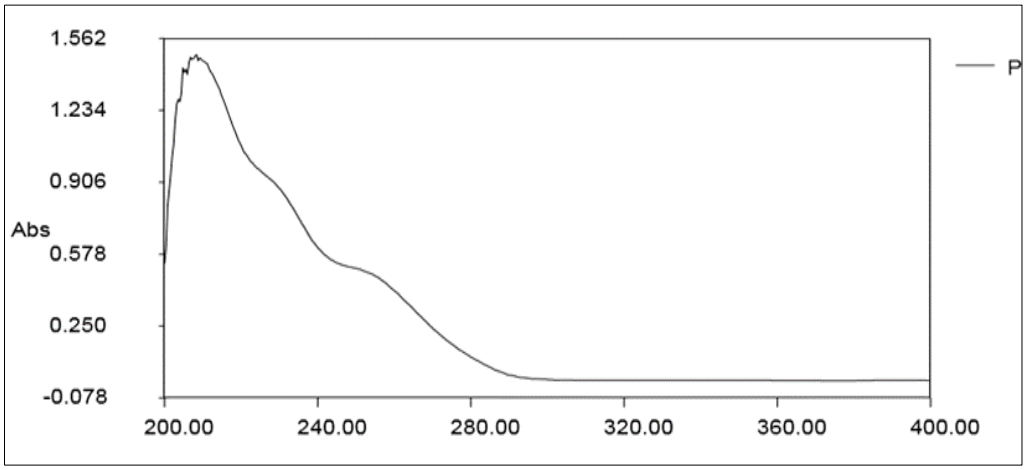

Fig 2: UV Spectra of Pexidartinib (225 nm selected)

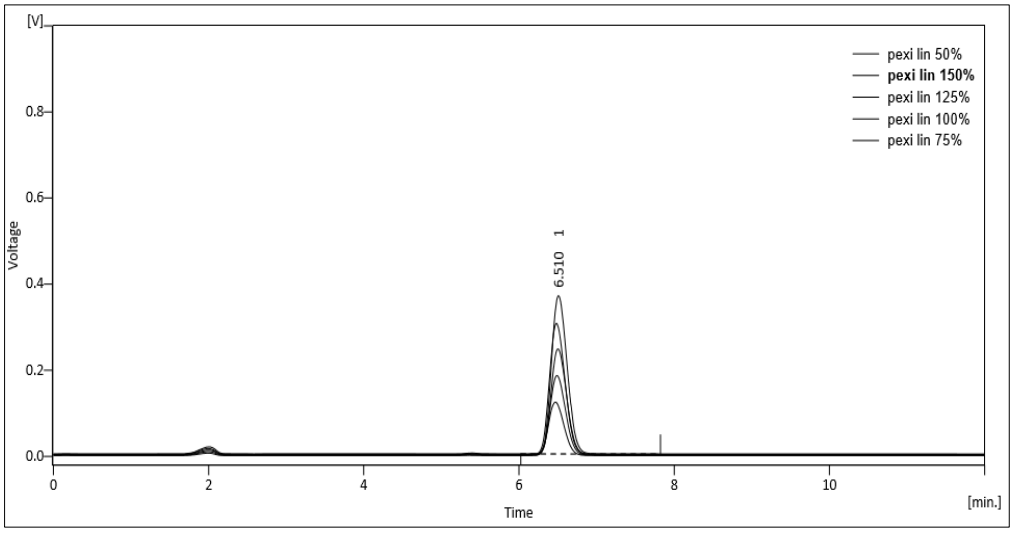

Fig 3: Linearity Overlay

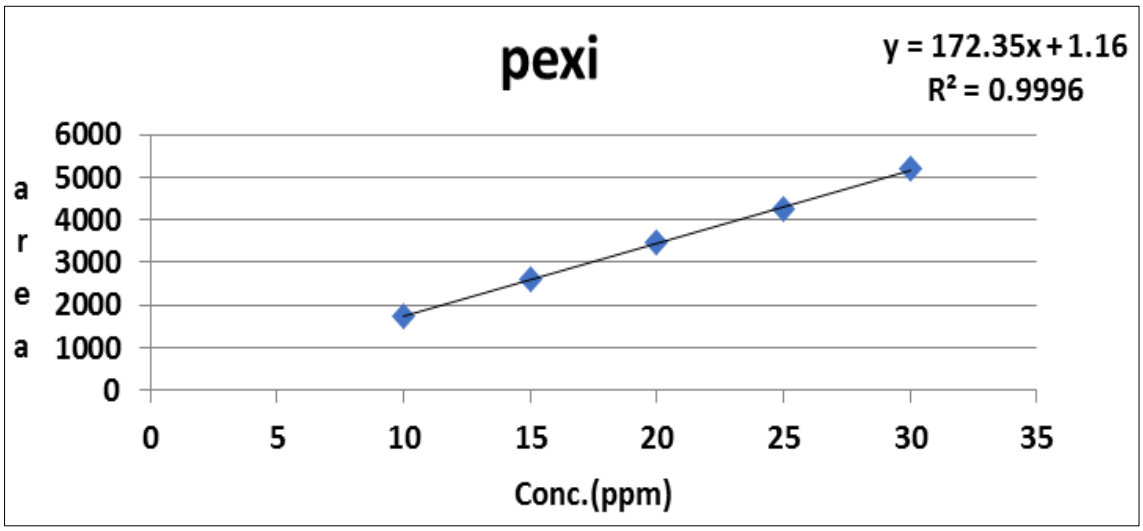

Fig 4: Calibration Curve of Pexidartinib (10-30 µg/ml)

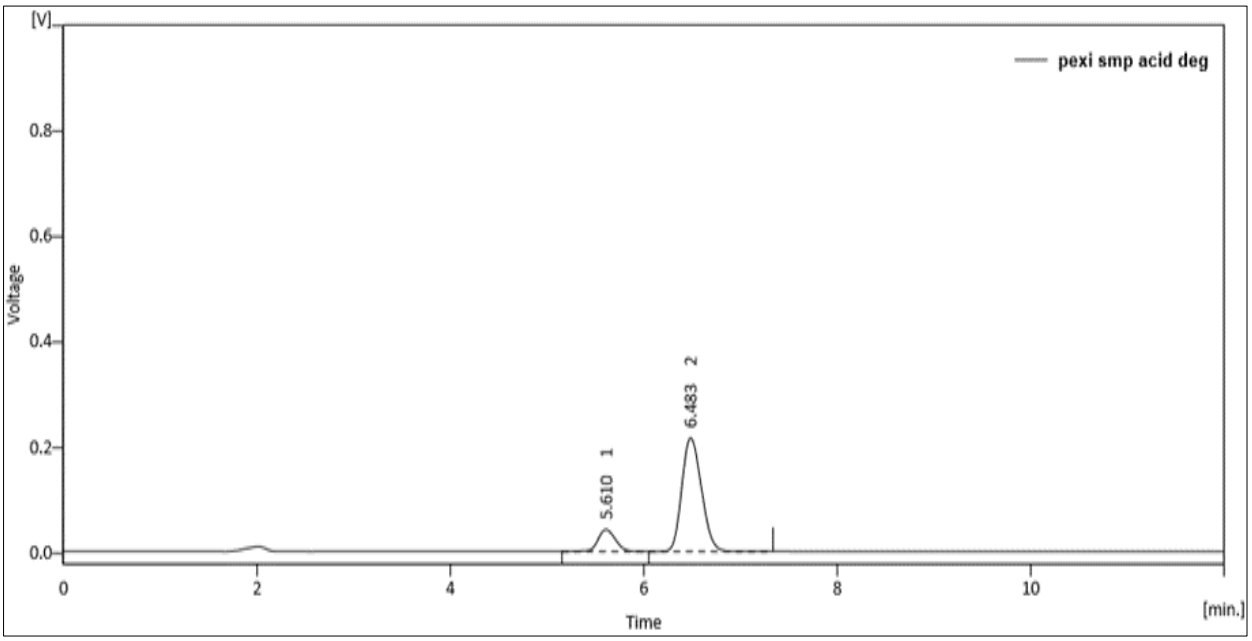

Fig 5: Acid Degradation Sample

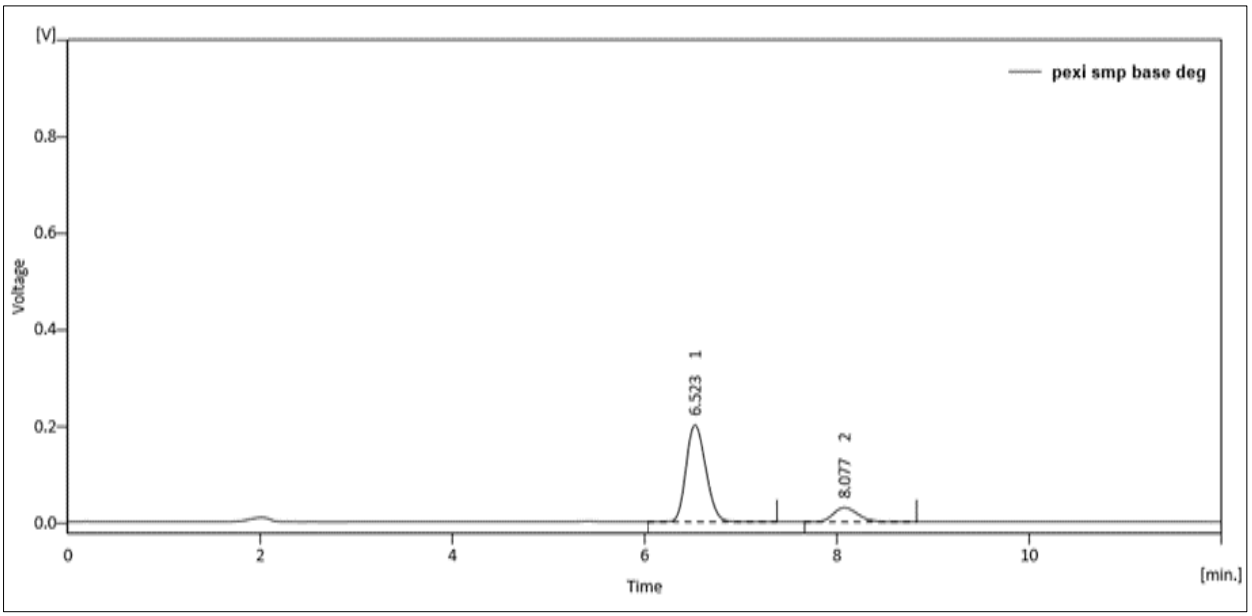

Fig 6: Base Degradation Sample

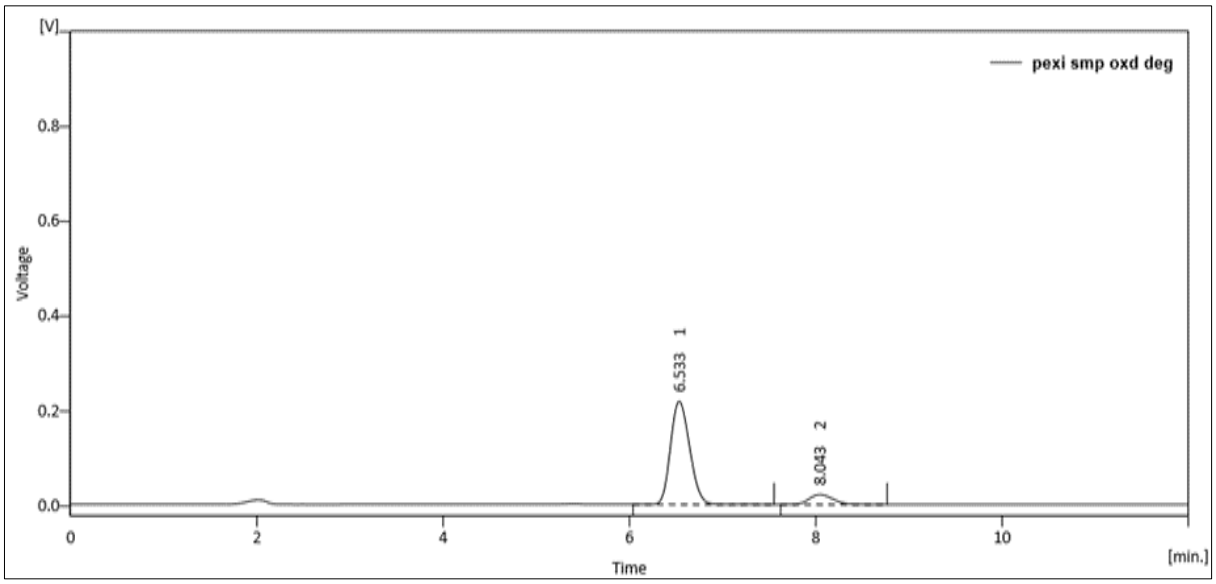

Fig 7: Oxidation Degradation Sample

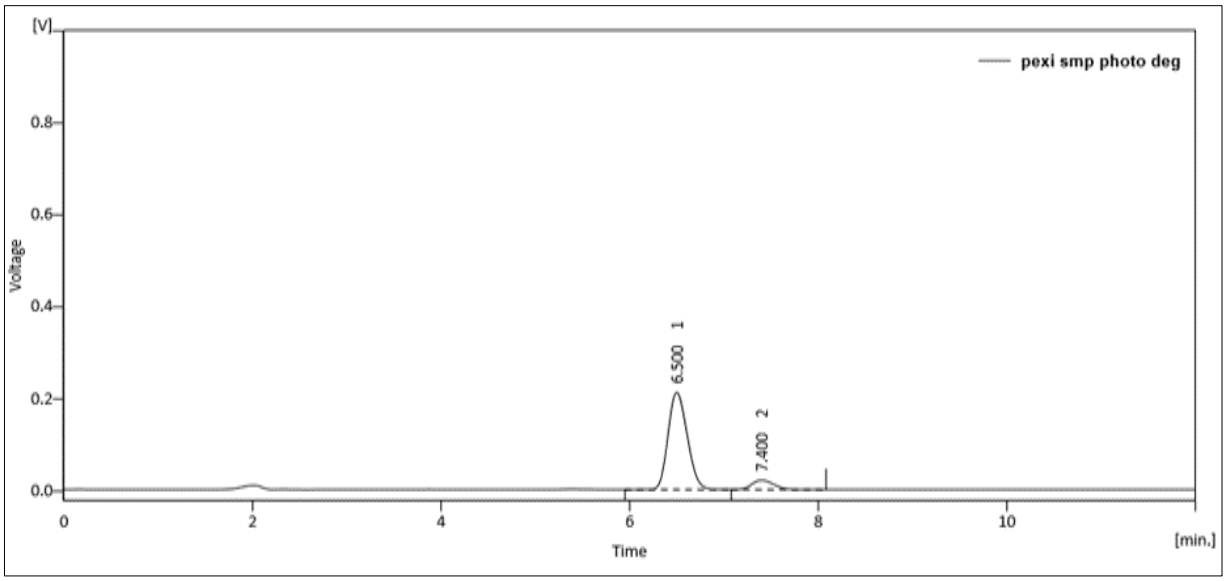

Fig 8: Photo Degradation Sample

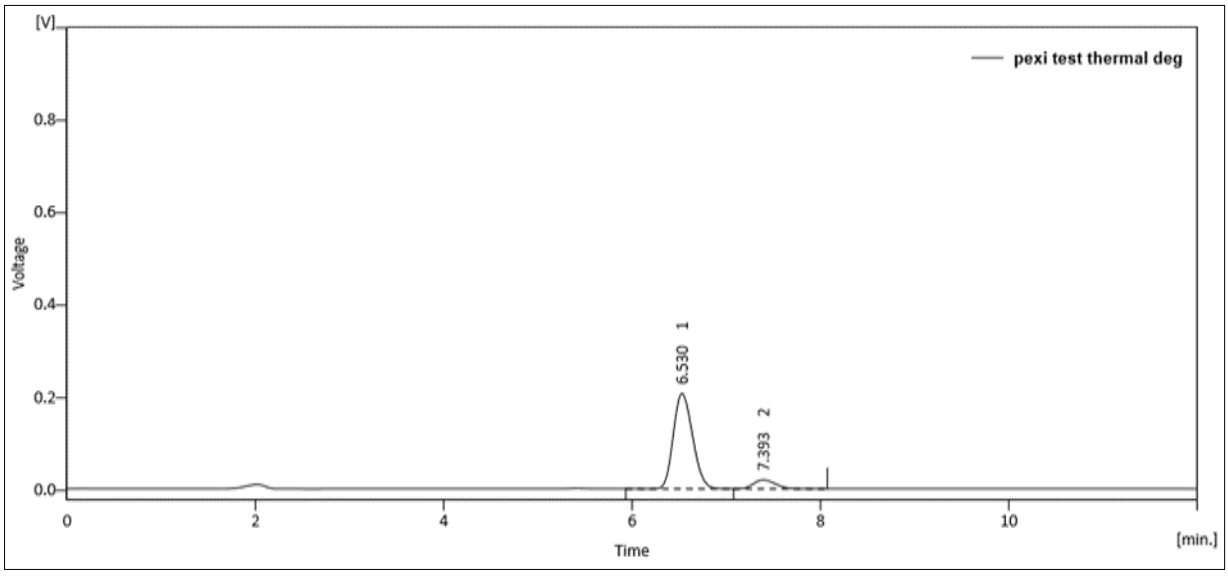

Fig 9: Thermal Degradation Sample

1. LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. (Table 8)
Then LOD and LOQ were calculated as follows:
LOD = 3.3* SD/ Slope of calibration curve
LOQ = 10* SD/ Slope of calibration curve
Where, SD = Standard deviation of intercepts

2. 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 (±0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (±0.2) 3.7 and 3.3.
3. Ratio of Mobile phase was changed (±2) Buffer (3.5): Acetonitrile (Table 7)

Forced Degradation Studies

1. Acid degradation

Blank: Take 2ml 1M HCL and 2ml 1M NaoH and transfer it into a 100ml volumetric flask and make up the volume with 10ml mobile phase.
Standard: Take 1ml of Pexidartinib stock solution and 2ml 1M HCL. Kept the solution for 4 hours and then neutralize with2ml 1M NaoH to stop degradation further. Now make up the volume with mobile phase.
Formulation degradation: Take 1ml from sample stock solution and add 2ml 1M HCL in it. Kept the solution for 4 hours and then neutralize with 2ml 1M NaoH to stop degradation further. Now make up the volume with mobile phase. (Table 9, Figure 5)

2. Base degradation

Blank: Take 2ml 0.1N NaoH and 2ml 0.1N HCL and transfer it into a 100ml volumetric flask and make up the volume with 10ml mobile phase.
Standard: Take 1ml of Pexidartinib stock solution and 2ml 0.1N NaoH. Kept the solution for 3 hours and then neutralize with 2ml 0.1N HCL to stop degradation further. Now make up the volume with mobile phase.
Formulation degradation: Take 1ml from sample stock solution and add 2ml 0.1NNaoH in it. Kept the solution for 3 hours and then neutralize with 2ml 0.1N NaoH to stop degradation further. Now make up the volume with mobile phase. (Table 9, Figure 6)

1. Oxidation Degradation

Blank: Take 2ml 3% Hydrogen Peroxide and transfer it into a 100ml volumetric flask and make up the volume with 10ml mobile phase.
Standard: Take 1ml of Pexidartinib stock solution and 2ml 3% Hydrogen Peroxide. Kept the solution 10 hours. Now make up the volume with mobile phase.
Formulation degradation: Take 1ml from sample stock solution and add 2ml 3% Hydrogen Peroxide in it. Kept the solution for 10. Now make up the volume with mobile phase. (Table 9, Figure 7)

2. Photo degradation

Blank: Transfer the stock solution to 10ml volumetric flask and make up the volume with mobile phase.
Standard degradation: The volumetric flask was kept in UV chamber for 24 hours. After that take 1ml of Pexidartinib standard stock solution and transfer to 10ml volumetric flask and make up to volume with mobile phase. (20µg)
Formulation degradation: The volumetric flask was kept in UV chamber for 24 hours. After that take 1ml of Pexidartinib sample stock solution and transfer to 10ml volumetric flask and make up to volume with mobile phase. (20µg) (Table 9, Figure 7)

3. Thermal Degradation

Blank: Transfer the stock solution to 10ml volumetric flask and make up the volume with mobile phase.
Standard degradation: The volumetric flask was kept at 105° for 16 hours. After that take 1ml of Pexidartinib standard stock solution and transfer to 10ml volumetric flask and make up to volume with mobile phase. (20 µg)
Formulation degradation: The volumetric flask was kept at 105° for hours. After that take 1ml of Pexidartinib sample stock solution and transfer to 10ml volumetric flask and make up to volume with mobile phase. (20 µg) (Table 9, Figure) [10]

Results and Discussions

The method developed for estimation of Pexidartinib was found to be simple, rapid, precise and accurate in its pharmaceutical dosage form. The maximum absorption wavelength of the sample drug solution was found at 230 nm. After number of trials were carried out, Buffer pH 3.5: Acetonitrile (50:50) was finalized as mobile phase as it was showing good peak shapes with significant amount of resolution. The retention time of Pexidartinib was found to be 6.517 min.

Table 1: System suitability parameter

Parameters	Pexidartinib
Retention Time	6.517
Theoretical Plates	4579
Asymmetry	1.308
Resolution	-

Table 2: Linearity data for Pexidartinib

Sr. No.	Concentration (µg/ml)	Area
1	10	1723.139
2	15	2596.842
3	20	3456.913
4	25	4267.315
5	30	5196.673

Table 3: Repeatability data for Pexidartinib

Sr. No.	Conc.(µg/ml)	Area	Mean ± S.D. (n=6)	% RSD
1	20	3419.74	3426.033 ± 55.553	1.621
		3444.734		
		3424.167		
		3354.329		
		3393.267		
		3519.963		

Table 4: Intraday Precision data for estimation of Pexidartinib

Sr. No.	Conc.(µg/ml)	Area Mean ± S.D. (n=6)	% RSD
1	10	1722.428 ± 28.974	1.682
2	20	3429.16 ± 53.970	1.573
3	30	5155.212 ± 67.801	1.315

Table 5: Interday Precision data for estimation of Pexidartinib

Sr. No.	Conc.(µg/ml)	Area Mean ± S.D. (n=6)	% RSD
1	10	1698.412 ± 30.221	1.779
2	20	3402.491 ± 36.0598	1.059
3	30	5119.05 ± 42.1735	0.823

Table 6: Recovery data for Pexidartinib

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D.
1	80%	10	8	8.09	101.07	100.14 ± 1.06
2		10	8	7.92	98.99	
3		10	8	8.03	100.36	
4	100%	10	10	10.07	100.74	99.59 ± 1.03
5		10	10	9.93	99.27	
6		10	10	9.88	98.76	
7	120%	10	30	12.15	101.29	99.94 ±1.33
8		10	30	11.84	98.64	
9		10	30	11.99	99.88	

Table 7: Robustness data for Pexidartinib

Sr. No.	Area at flow rate (+0.2 ml/min)	Area at flow rate (-0.2 ml/min)	Area at pH (+0.2)	Area at pH (-0.2)	Area at Mobile phase (+2)	Area at Mobile phase (-2)
1	3319.116	3569.953	3263.28	3620.517	3299.289	3612.157
2	3267.456	3596.866	3257.257	3624.539	3277.9430	3638.166
3	3307.171	3606.898	3245.581	3573.329	3237.344	3594.337
% RSD	0.820076	0.531974	0.276426	0.78966	0.961851	0.609746

Linearity and Range

In term of slope, intercept and correlation co-efficient value, the graph of peak area obtained verses respective concentration was plotted. Correlation co-efficient for calibration curve Pexidartinib was found to be 0.9996 (Table 2, Figure 3, 4)

The regression line equation for Pexidartinib is as following: y = 172.35x + 1.16

Precision

1. Repeatability

The data for repeatability of peak area measurement for Pexidartinib (20 µg/ml), based on six measurements of solution of Pexidartinib (20 µg/ml). The RSD value should not be more than 2%. The % RSD for Pexidartinib was found to be 1.621. (Table 3)

2. Intraday Precision

Standard solution containing (10, 20, 30 µg/ml) of Pexidartinib was analyzed three times on the same day and % RSD was calculated. (Table 4)

3. Interday Precision

Standard solution containing (10, 20, 30 µg/ml) of Pexidartinib was analyzed three times on the different day and % RSD was calculated. (Table 5)

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 225 nm. The amount of Pexidartinib was calculated at each level and % recoveries were computed. (Table 6)

LOD and LOQ

LOD and LOQ values from the regression equation of the Pexidartinib was found to be 0.5666 µg/ml and 1.717 µg/ml (Table 8).

Robustness

All the parameters like flow rate of mobile phase, pH of mobile phase and ratio of mobile phase were changed one by one by one and the % RSD was calculated and all the parameters were found to be within limits, less than 2% (Table 7). Summary of validation Parameters are mentioned in Table 8.

Forced Degradation Studies

Relatively higher amount of degradation was observed in alkaline condition. Whereas found to be stable at acidic, thermal, photolytic and oxidation condition (Table 9).

Assay

The % purity of Pexidartinib in marketed formulation was found to be 99.508 and the % RSD was found to be within limits, less than 2% (Table 10).

Table 8: Summary of Validation Parameters

Parameters		Pexidartinib
Linearity Range		10-30 µg/ml
Correlation coefficient		R^2 = 0.9996
Regression line equation		y = 172.35x + 1.16
Accuracy		% recovery=100.04%
LOD		0.5666 µg/ml
LOQ		1.717 µg/ml
Repeatability		% RSD= 1.621
Interday Precision		1.779, 1.059, 0.823
Intraday Precision		1.682, 1.573, 1.315
Robustness	pH (±0.2)	0.276, 0.789
	Flow rate (±0.2 ml)	0.820, 0.531
	Mobile phase ratio (±2 ml)	0.961, 0.609
Assay (% of label claim) Mean		99.508 ± 1.098

Table 9: Forced Degradation Studies

Degradation Method	% Degradation (Standard)	% Degradation (Sample)
Acid	13.84	12.93
Base	17.58	18.32
Oxidation	9.38	11.25
Photo	16.24	15.94
Thermal	14.63	14.41

Table 10: Assay of Marketed Dosage Form

Capsule	Turalio
Label claim	Pexidartinib 200 mg
Assay (% of label claim*) Mean ± S.D.	99.508 ± 1.098

Conclusion

A simple, accurate and precise stability indicating RP-HPLC method was developed and validated as per ICH guideline for estimation of Pexidartinib in dosage form. This method can be utilize for routine laboratory analysis and assay of Pexidartinib in dosage form.

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