WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 1, 131-154. Review Article ISSN 2277– 7105

THE NOVEL ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR RELATED SUBSTANCES OF NINTEDANIB ESYLATE BY RP-HPLC METHOD

***Yogendra B. Parmar, Dharmesh Shah, Yashraj A. Majmudar, Ketul C. Kaka, Arpan S. Patel, Pankaj D. Kankad and Uday G. Sartanpara**

BDR Lifesciences. Pvt. Ltd. R.S No.578, Near Effluent Channel, Luna Village, Taluka-Padra, Vadodara District, Gujrat-391440, India.

Article Received on
23 October 2020,

Revised on 13 Nov. 2020,
Accepted on 03 Dec. 2020

DOI: 10.20959/wjpr20211-18950

***Corresponding Author**
Yogendra B. Parmar
BDR Lifesciences. Pvt. Ltd. R.S No.578, Near Effluent channel, Luna Village, Taluka-Padra, Vadodara District, Gujrat-391440, India.

ABSTRACT

An accurate, sensitive and rapid gradient reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and validated for related substances of Nintedanib Esylate. HPLC analysis was performed on YMC Triart, C18 (250 x 4.6) mm, 3µm. Column temperature maintained at 35°C conditions. Chromatographic separation was achieved with mobile phase gradient program at flow rate of 1.0mL/min. The injection volume was 10µl. The UV detection wavelength was 245nm. The method suitability was checked and validated according to the ICH guidelines Q2 (R1) for specificity, linearity, accuracy, precision, limit of quantification, limit of detection. Limit of detection of each impurity was found to be less than 0.031% w/w indicating that the developed method is highly sensitive. The calibration curve of each impurity was found to be linear within the concentration range of about 0.10µg/ml to 2.0µg /ml. The regression data for calibration curve shows good linear relationship. Correl coefficient (r^2) of each impurity was found to be greater than 0.998. The Recovery was found to be accurate for each impurity within the spike concentration range of about 0.10µg/ml to 1.5µg /ml. The Recovery of each impurity was found to be between 80% to 120%. The experiment results are given in detail in this research article.

KEYWARD: RP-HPLC, Method validation, Method Development, Nintedanib Esylate, Related substances.

INTRODUCTION

Nintedanib Esylate is chemically known as Ethanesulfonic acid; Methyl (3Z)-3-[({4-[N-methyl-2-(4-methylpiperazin-1-yl)acetamido]phenyl}amino)(phenyl)methylidene]-2-oxo-2,3-dihydro-1H-indole-6-carboxylate, Having molecular formula $C_{33}H_{39}N_5O_7S$ and molecular weight 649.76 g/mol.

Nintedanib, sold under the brand names **Ofev** and **Vargatef**, is an oral medication used for the treatment of idiopathic pulmonary fibrosis and along with other medications for some types of non-small-cell lung cancer.[1]

In March 2020, it was approved for use in the United States to treat chronic fibrosing (scarring) interstitial lung diseases (ILD) with a progressive phenotype (trait). It is the first treatment for this group of fibrosing lung diseases that worsen over time that was approved by the U.S. Food and Drug Administration (FDA).[2]

Common side effects include abdominal pain, vomiting, and diarrhea. It is a small molecule tyrosine-kinase inhibitor, targeting vascular endothelial growth factor receptor, fibroblast growth factor receptor and platelet derived growth factor receptor.

Ofev was developed by Boehringer Ingelheim. It received U.S. Food and Drug Administration (FDA) approval for use for Idiopathic Pulmonary Fibrosis (IPF) in 2014 - one of only two drugs available for treating IPF - and numerous studies since have demonstrated its effectiveness in slowing the progressive, terminal lung disease.[3]

Nintedanib is used for the treatment of idiopathic pulmonary fibrosis. It has been shown to slow down decrease in forced vital capacity.[4] And it also improves people's quality of life.[5] Nintedanib does not improve survival in people with IPF.[6] It interferes with processes like fibroblast proliferation, differentiation and laying down extracellular matrix.[7] The National Institute for Health and Care Excellence (NICE) recommends Nintedanib in cases of IPF where the FVC is 50-80% of predicted. NICE recommends discontinuation of therapy if a person's FVC decreases by 10% or more in a 12-month period, indicating disease progression despite treatment.[8]

It is also used in combination with docetaxel as a second-line treatment for adult patients with locally advanced, metastatic, or locally recurring non-small cell lung cancer of adenocarcinoma histology. It is unclear how this combination compares to other second

line agents as the comparisons have not been done as of 2014.[9] The chemical structure of Nintedanib Esylate is shown in Figure 1.

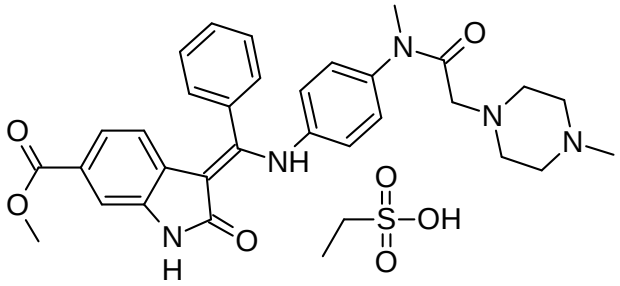


Figure 1: structure of Nintedanib Esylate.

Molecular formula: $C_{33}H_{39}N_5O_7S$

Molecular weight: 649.76 g/mol

IUPAC Name: Ethanesulfonic acid; Methyl (3Z)-3-[({4-[N-methyl-2-(4-methylpiperazin-1-yl)acetamido]phenyl}amino)(phenyl)methylidene]-2-oxo-2,3-dihydro-1H-indole-6-carboxylate, molecular formula is $C_{33}H_{39}N_5O_7S$ and molecular weight is 649.76 g/mol.

Nintedanib esylate does not have chiral centres. The double bond at C-3 of the indole moiety allows for E/Z isomerism, but the active substance is the Z-isomer.

There is no single pharmacopeial monograph available for this drug substance or drug product and no HPLC method is available in literature for quantification of Nintedanib Esylate related substances. In this research paper, development of HPLC method for the simultaneous detection and quantitative determination of six impurities in Nintedanib Esylate drug substance has been reported. Limit of detection (LOD), limit of quantification (LOQ) and linearity were established as per ICH guidelines. The limit of unknown impurity have been considered as 0.1% in accordance with ICH guideline based on maximum daily dose (0.3g/day).[10] The developed chromatographic method can resolve related substances with acceptable resolution to achieve good chromatography and the optimized methodology have been validated to accomplish ICH guidelines on validation.[11] The chemical structure of Nintedanib Esylate Related substances Impurity-1 to Impurity-6 are shown in Figure 2.

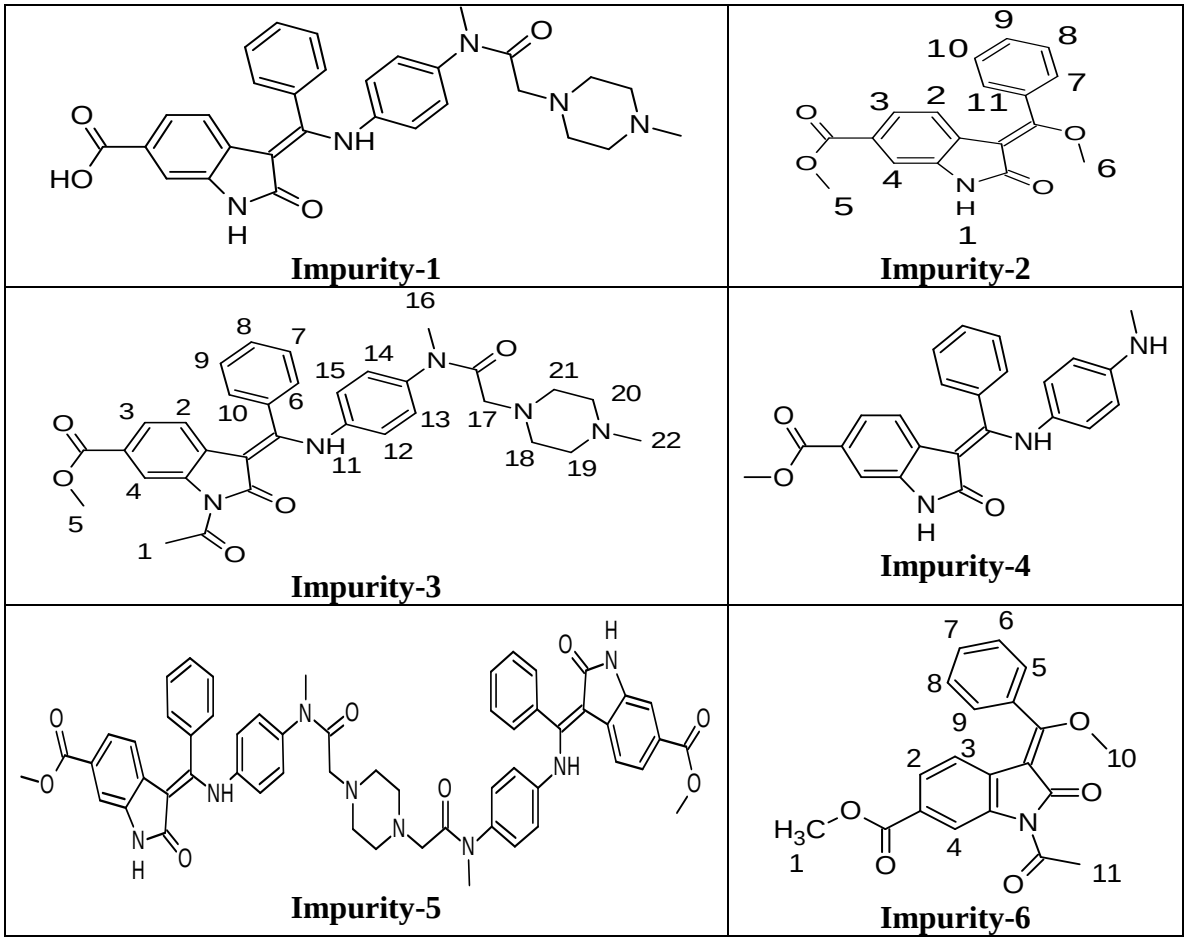


Figure 2: structure of Nintedanib Esylate Related substances Impurity-1 to Impurity-6.

Nintedanib Esylate Related substances Impurity-1 to Impurity-6 properties

Impurity-1

Molecular Formula: $C_{30}H_{31}N_5O_4$

Molecular Weight: 525.6 g/mole

Chemical Name: (*Z*)-3-((4-[methyl-2-(4-methylpiperazin-1-yl))acetamido]phenyl]amino] phenylmethylene]-2-oxoindoline-6-carboxylic acid

Impurity-2

Molecular Formula: $C_{18}H_{15}NO_4$

Molecular Weight: 309.31g/mol

Chemical Name: Methyl (3*Z*)-3-[methoxy (phenyl)methylidene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylate.

Impurity-3

Molecular Formula: $C_{33}H_{35}N_5O_5$

Molecular Weight: 581.60g/mol

Chemical Name: 4-[4-(Carbamoylamino)-3-chlorophenoxy]-7-methoxyquinoline-6-carboxamide

Impurity-4

Molecular Formula: $C_{24}H_{21}N_3O_3$

Molecular Weight: 399.45 g/mole

Chemical Name: Methyl-(E)-3-(([4-(methylamino)phenyl]amino]phenylmethylene]-2-oxoindoline-6-carboxylate

Impurity-5

Molecular Formula: C56H52N8O8

Molecular Weight: 965.06g/mol

Chemical Name: dimethyl 3,3'-(((((2,2'-(piperazine-1,4-diyl)bis(acetyl))bis (methylazanediyl)) bis(4,1-phenylene))bis(azanediyl))bis(phenylmethanylylidene))(3Z,3'Z)-bis(2-oxoindoline-6-carboxylate)

Impurity-6

Molecular Formula: $C_{20}H_{17}NO_5$

Molecular Weight: 351.35 g/mole

Chemical Name: Methyl (3*Z*)-1-acetyl-3-[methoxy (phenyl)methylidene]-2-oxo-2,3-dihydro-1*H*-indole-6-carboxylate

Methodology

Equipment

High performance liquid chromatography: Thermo scientific model Ultimate 3000

Balance: Radwag model MAY5.4Y

pH Meter: Mettler Toledo model five easy plus

Reagents required

Acetonitrile (HPLC grade)

Water (Milli-Q or HPLC grade).

Ammonium acetate (AR grade)

Ammonia solution (AR grade)

Chromatographic condition

Column	**:**	**YMC triart, C18 (250 X 4.6)mm, 3µm Part No.TA12S03-2546WT Make: YMC**		
Wavelength	:	UV detector at 245nm		
Flow rate	:	1.0mL/min.		
Column temperature	:	35°C		
Sample cooler temperature	:	25°C		
Injection volume	:	10µL		
Run time	:	80minute		
Gradient	:	**Time (min)**	**Mobile phase A (%v/v)**	**Mobile phase B (%v/v)**
		0	75	25
		5	75	25
		40	45	55
		50	45	55
		55	30	70
		70	30	70
		73	75	25
		80	75	25

Buffer preparation: Accurately weigh and transfer about 770mg of ammonium acetate in 1000mL water in 1000mL bottle sonicate to dissolve and filter the solution through 0.45µ nylon membrane filter. Adjust the pH 10.0 ± 0.05 using liquor ammonia (25%, AR grade) and Mix well.

Mobile phase-A preparation: Transfer 800mL of buffer solution and 200mL of acetonitrile in to 1000mL bottle. Mix well and sonicate to degas.

Mobile phase-B preparation: Transfer 200mL of buffer solution and 800mL of acetonitrile in to 1000mL bottle. Mix well and sonicate to degas.

Diluent preparation: Transfer 300mL of water solution and 700mL of acetonitrile in to 1000mL bottle. Mix well and sonicate to degas.

Blank preparation: Use diluent as a blank.

System suitability stock solution preparation: Accurately weigh and transfer about 25mg Impurity-3 and 25mg Impurity-2 working standard into a 50mL volumetric flask. Add 10mL of diluent to dissolve and dilute up to mark with diluent and mix well. Transfer 1mL of this

solution into a 50mL volumetric flask then add 20mL of diluent. Mix and dilute up to mark with diluent. (Concentration of Impurity-3 and Impurity-2 is 10ppm each).

System suitability solution preparation: Accurately weigh and transfer about 20mg of Nintedanib esylate working standard and take 3.0mL of system suitability stock solution into a 20mL volumetric flask then add 5mL of diluent. Mix and dilute up to mark with diluent. (Concentration of Nintedanib esylate, Impurity-3 and Impurity-2 are 1000ppm, 1.5ppm & 1.5ppm respectively).

Standard stock solution preparation: Accurately weigh and transfer about 25mg of Nintedanib esylate working standard into a 25mL volumetric flask. Add 10mL of diluent to dissolve. Dilute up to mark with diluent and mix well. (Concentration of Nintedanib esylate is 1000ppm).

Standard solution preparation: Take 1mL of above solution into a 100mL volumetric flask then add 20mL of diluent. Mix and dilute up to mark with diluent. Future dilute Take 5mL of above solution into a 50mL volumetric flask then Add 10mL of diluent. Mix and dilute up to mark with diluent. (Concentration of Nintedanib esylate is 1ppm).

Test solution preparation: Accurately weigh and transfer about 25mg of Nintedanib esylate test into a 25mL volumetric flask. Add 10mL of diluent sonicate to dissolve. Dilute up to the mark with diluent and mix well. (Concentration of Nintedanib esylate is 1000ppm).

Sequence Table

Sr. No.	Name of solution	Number of injection
01	Blank	01
02	System suitability solution	01
03	Standard solution	06
04	Test solution	01
05	Bracketing standard solution	01

System suitability criteria

1. % RSD of Nintedanib peak area of six replicate injections in standard solution should not be more than 5.0%.
2. Retention time of Nintedanib peak should be within the range of 23.0min to 25.0min and Impurity-3 peak should be within the range of 40.0min to 41.5min. Change the column, if retention time not achieve within the range.

3. Resolution between Nintedanib peak and Impurity-2 peak is not less than 6.0 in system suitability solution.
4. RRT should be calculate against the peak of Impurity-2 observed in system suitability solution

Retention time, relative retention factor and relative response factor given below table

Sr. No	Name of component	~RT (min)	RRT	RRF
1	Impurity-2	27.2	1.00	0.83
2	Nintedanib	23.0 to 25.0	0.85 to 0.92	1.00
3	Impurity-6	49.8	1.83	0.91
4	Impurity-3	40.0 to 41.5	1.47 to 1.53	1.57
5	Impurity-1	2.2	0.08	0.98
6	Impurity-4	42.1	1.55	1.69
7	Impurity-5	47.1	1.73	1.08

Calculation

Calculate the each impurity as per given below formula,

$$\text{Known and unknown impurity (\%)} = \frac{AT}{AS} \times \frac{WS}{25} \times \frac{1}{100} \times \frac{5}{50} \times \frac{25}{WT} \times \frac{1}{RRF} \times P$$

Where,

AT = Area of known or unknown impurities in test solution chromatogram.

AS =Average area of Nintedanib peak in standard solution.

WS =Weight of standard for standard solution in mg.

WT =Weight of test sample in mg

P =%Potency of standard

RRF = Relative response factor

Total impurity = Sum of all known and unknown impurities.

Analytical Method development

Column selection

Cromasil C18 (250 x 4.6)mm 5µm, Symmetry C18 (250x4.6) 5µ, Inertsil ODS 2V (250x4.6) 5µ, Hypersil BDS (250x4.6)mm 5µm, Zorbax SB C18 (250 x 4.6)mm 5µm, Zorbax RX C8 (250 x 4.6)mm 5µm etc. columns were used in experiments with different buffers and suitable of first acidic pKa value 3.7 but in which was observed not symmetrical peak as require. And finally tried and getting suitable and symmetrical chromatography observed

with Xterra MS C18 (250 x 4.6) mm 5µm, YMC triart C18 (250 x 4.6)mm 5µm columns with suitable buffer of second basic pKa value 10.86. Hence this column as YMC triart C18 (250 x 4.6) mm 5µm is more suitable for this method.

pH selection

pH selection on the basis of Nintedanib pKa value 3.7 and 10.86.

Wavelength selection

Wavelength extract from PDA Detector at 245nm suitable for Nintedanib and Impurity-1 to Impurity-6.

System suitability selection

Day to day analysis of Nintedanib RS by HPLC with same column of YMC trairt, The observation of retention time for Nintedanib peak and Impurity-3peak was found movement within the range about 23.0min to 25.0min and 40.0min to 41.5min respectively, So to provide and obtain accurate and precise results the resolution should be important parameter in the system suitability and it should be as the resolution is between Nintedanib peak and Impurity-2 peak is not less than 6.0, RRT must be calculate against the impurity-2 and it retention time of impurity -3 must be retain in the range of 40min to 41.5min range in system suitability solution.

Quantification with RRF purpose in calculation

Impurities of Nintedanib API cannot be physically use every time of routine analysis. So to avoid the preparation and solution stability of impurities.

Column care

Column must be wash with composition of (water: acetonitrile: trifluroaceticacid) (500:500:1) for at least 1.0hours to obtain the resolution require in SSTS. After that further wash with composition of water and acetonitrile (500:500) for 0.5hours and for long storage must be wash with pure (100%) acetonitrile.

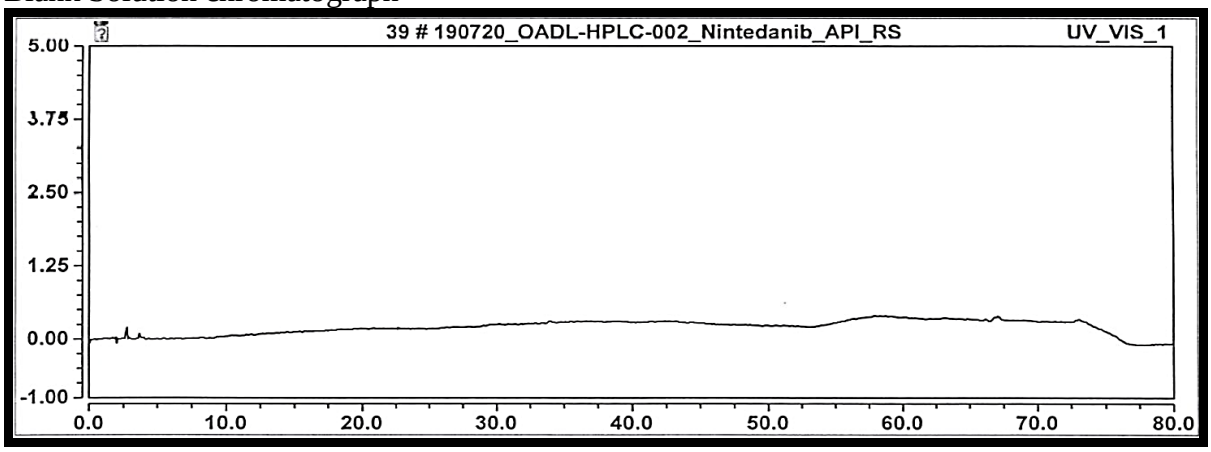
Design of Experiment at to be final level.

<table>
<tr><th>Objective</th><th>Method details</th><th>Observation</th><th>Conclusion/Optimized</th></tr>
<tr><td colspan="4">Design of Experiment -01</td></tr>
<tr>
<td>1. Related substances method develops for Nintedanib API.
2. Work on pKa value 3.7
3. Selection of detection wavelength.
4. Selection of mobile phase.
5. Selecting column.</td>
<td>Column: X-tera MS C18, (250 x 4.6)mm, 5µm,
Column temp: 25°C,
Auto Sample Temp.: 25°C,
Wavelength: 250nm, 210nm
PDA Detector : ON
Flow rate: 1.0mL/min,
Injection volume: 10µL,
Run time: 55min,
Pump: Gradient programme,
<table>
<tr><th>T me</th><th>A</th><th>B</th></tr>
<tr><td>0.0</td><td>95</td><td>5</td></tr>
<tr><td>5.0</td><td>95</td><td>5</td></tr>
<tr><td>30.0</td><td>30</td><td>70</td></tr>
<tr><td>4 .0</td><td>30</td><td>70</td></tr>
<tr><td>45.0</td><td>95</td><td>5</td></tr>
<tr><td>55.0</td><td>95</td><td>5</td></tr>
</table>
Buffer: 10mM NaH_2Po_4 in1000mL water adjust pH 3.01 with OPA.
Mobile Phase-A: Buffer (800): ACN (200),
Mobile Phase-B: Buffer (200): ACN (800),
Diluent: Water 500mL+ACN 500mL
Sample preparation: 1000ppm</td>
<td>1. Baseline was very poor.
2. All peaks are separate and heaving good peak shape and pure.
3. Impurity separate with main peak.
4. Blank interference observed at Impurity-6 peak.
5. Nintedanib peak observed with tailing.
6. UV Maxima for Nintedanib is 245nm.
7. May be column change with suitable pH value.</td>
<td>1. Wavelength for Nintedanib peak is selected 245nm.
2. Nintedanib peak shape should be improve as symmetrically for RS
3. Further Experiment required and to be achieved remaining objective should be check in next Experiment.</td>
</tr>
<tr><td colspan="4">Design of experiment -02</td></tr>
</table>

<table>
<tr>
<td>1. Related substances method develops for Nintedanib API.
2. Selected wavelength 245nm.
3. Work on pKa value 10.86
4. Selection of mobile phase.
5. Selection of column.</td>
<td>Methodology as per above Experiment – 01 Excluding below,
Column: YMC triart C18, (250 x 4.6)mm, 3µm,
Wavelength: 245nm,
Run time: 60min,
Pump: Gradient programme,
<table>
<tr><td>Time</td><td>A</td><td>B</td></tr>
<tr><td>0.0</td><td>75</td><td>25</td></tr>
<tr><td>5.0</td><td>75</td><td>25</td></tr>
<tr><td>40.0</td><td>45</td><td>55</td></tr>
<tr><td>50.0</td><td>45</td><td>55</td></tr>
<tr><td>53.0</td><td>75</td><td>25</td></tr>
<tr><td>60.0</td><td>75</td><td>25</td></tr>
</table>
Buffer: 10mM Ammonium Acetate in1000mL water adjust pH 9.00 with Ammonia.
Diluent: Buffer 500mL+ACN 500mL</td>
<td>1. Baseline observed without interference.
2. One unknown imp merge with API main peak in tailing.
3. All peaks are separate.
4. Nintedanib peak shape good & pure.
5. Impurity separate with main peak.
6. Buffer:ACN diluent not suitable due to degradation.</td>
<td>1. YMC Triate column to be use.
2. Diluent Water:ACN (1:1) to be use
3. Experiment with increase pH for resolution purpose.
4. Further Experiment required, to separate and resolve unknown impurity with API main peak.</td>
</tr>
</table>

<table>
<tr><th>Objective</th><th>Method details</th><th>Observation</th><th>Conclusion/Optimized</th></tr>
<tr><td colspan="4">Design of experiment -03</td></tr>
<tr>
<td>1. Separate and resolve one unknown impurity elute with API peak tailing.
2. Experiment with YMC Triate column, 10pH Buffer and water : ACN Diluent Used</td>
<td>Methodology as per above Experiment – 02 Excluding below,
Column temp: 35°C,
Run time: 80min,
Pump: Gradient programme,
<table>
<tr><td>Time</td><td>A</td><td>B</td></tr>
<tr><td>0.0</td><td>75</td><td>25</td></tr>
<tr><td>5.0</td><td>75</td><td>25</td></tr>
<tr><td>40.0</td><td>45</td><td>55</td></tr>
<tr><td>50.0</td><td>45</td><td>55</td></tr>
<tr><td>55.0</td><td>30</td><td>70</td></tr>
<tr><td>70.0</td><td>30</td><td>70</td></tr>
<tr><td>73.0</td><td>75</td><td>25</td></tr>
<tr><td>80.0</td><td>75</td><td>25</td></tr>
</table>
Buffer: 10mM Ammonium Acetate in1000mL water adjust pH 10.00 with Ammonia.
Diluent: Water 300mL+ACN 700mL
Sample preparation: 1000ppm</td>
<td>1. Baseline observed without interference.
2. All peaks are separate.
3. Nintedanib peak shape good & pure.
4. Impurity separate with main peak.
5. Impurity-3 and API peak retention time increase day to day.
6. Height and Theoretical plates decrease day to day.
7. Tailing factor increase day to day.
8. Test spike stable up to 24 hrs.
9. RRT calculate against Impurity-2</td>
<td>1. As per this methodology and observation that the method to be final.
2. This method include system suitability of API, Impurity-2 and Impurity-3 for resolution and RT. shown in figure 4.</td>
</tr>
</table>

Blank Solution chromatograph



System suitability Solution chromatograph

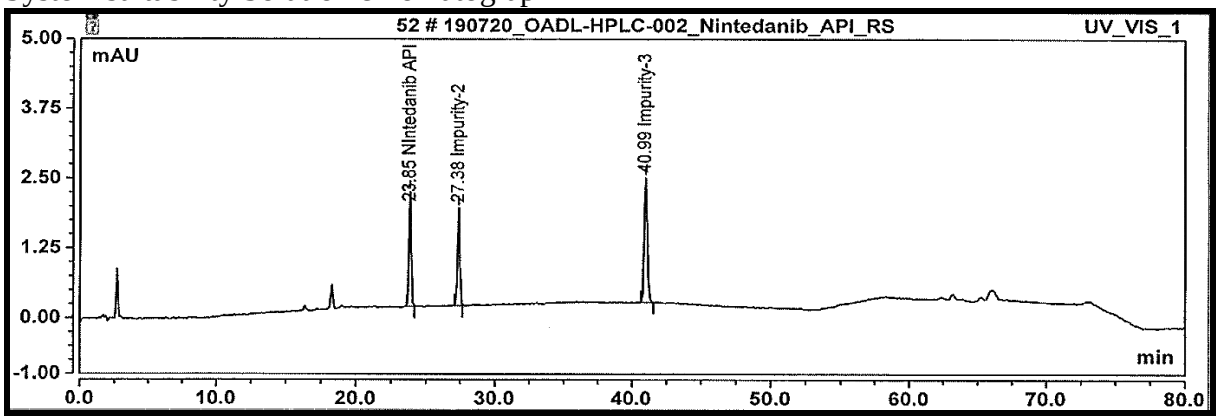


Figure 4: Chromatogram of Blank, System suitability solution.

Method validation

The proposed method was subjected to validation for various parameters like Specificity, precision, LOD, LOQ, linearity and accuracy in accordance with international conference on harmonization guidelines.

Specificity

Specificity is the ability to assessing unequivocally of analytic in the presence of components which may be expected to be present. For determination of specificity, blank, all individual related substances solutions were prepared and injected to confirm the individual retention times. The solutions of Nintedanib drug substance (Control Sample) and Nintedanib spiked with known related substances at specification level (Spiked Sample) were prepared and injected into HPLC. Peak purity was established by using Chromeleon Software. A typical representative HPLC chromatogram of Nintedanib drug substance spiked with all related substances is shown in Figure 5. The specificity results are tabulated in Table 1.

Preparation

System suitability stock solution preparation: Accurately weigh and transfer about 25mg Impurity-3 and 25mg Impurity-2 working standard into a 50mL volumetric flask. Add 10mL of diluent to dissolve and dilute up to mark with diluent and mix well. Transfer 1mL of this solution into a 50mL volumetric flask then add 20mL of diluent. Mix and dilute up to mark with diluent. (Concentration of Impurity-3 and Impurity-2 is 10ppm each).

System suitability solution preparation: Accurately weigh and transfer about 20mg of Nintedanib esylate working standard and take 3.0mL of system suitability stock solution into a 20mL volumetric flask then add 5mL of diluent. Mix and dilute up to mark with diluent. (Concentration of Nintedanib esylate, Impurity-3 and Impurity-2 are 1000ppm, 1.5ppm & 1.5ppm respectively).

Standard stock solution preparation: Accurately weigh and transfer about 25mg of Nintedanib esylate working standard into a 25mL volumetric flask. Add 10mL of diluent to dissolve. Dilute up to mark with diluent and mix well. (Concentration of Nintedanib esylate is 1000ppm).

Standard solution preparation: Take 1mL of above solution into a 100mL volumetric flask then add 20mL of diluent. Mix and dilute up to mark with diluent. Future dilute Take 5mL of above solution into a 50mL volumetric flask then Add 10mL of diluent. Mix and dilute up to mark with diluent. (Concentration of Nintedanib esylate is 1ppm)

Test and Test spike preparation describe in accuracy parameter. And all individual related substances preparation from stock solution of precision parameter.

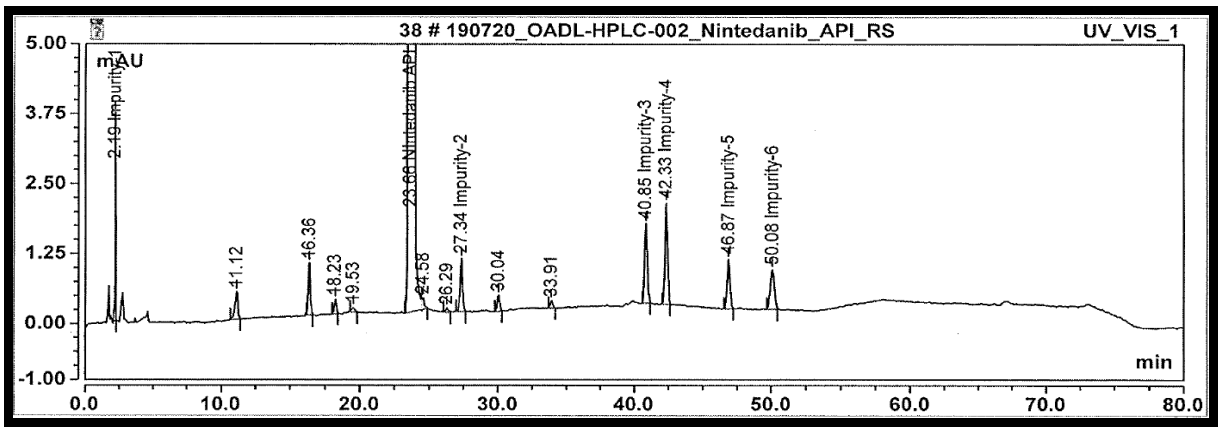


Figure 5: A typical representative HPLC chromatogram of Nintedanib drug substance spiked with related substances.

Table 1: Specificity experiment Results.

Name of content	~ RT (min)	RRT	Peak purity	
			Standard	Sample Spike
Impurity-1	2.19	0.08	967	974
Nintedanib	23.66	0.87	996	996
Impurity-2	27.34	1.00	998	998
Impurity-3	40.85	1.49	1000	1000
Impurity-4	42.33	1.55	999	999
Impurity-5	46.87	1.71	998	1000
Impurity-6	50.08	1.83	1000	1000

There is no any interference observed at the retention time Nintedanib, Impurity-1, Impurity-2, Impurity-3, Impurity-4, Impurity-5 and Impurity-6 which were well separated. Peak purity require minimum 950 as per above table peak was found pure. So method is specific.

System suitability

System suitability test was an integral part of the method development to verify that the system is adequate for analysis of Nintedanib to be performed. Six replicate injections of standard preparation were injected and relative standard deviation of peak area was determined. Then Resolution and retention time determined from system suitability solution. Day to day analysis of Nintedanib RS by HPLC with same column of YMC triart, The observation of retention time for Nintedanib peak and Impurity-3peak was found movement within the range about 23.0min to 25.0min and 40.0min to 41.5min respectively, So to provide and obtain accurate and precise results the resolution should be important parameter in the system suitability and it should be as the resolution is between Nintedanib peak and Impurity-2 peak is not less than 6.0, RRT must be calculate against the impurity-2 and it retention time of impurity -3 must be retain in the range of 40min to 41.5min range in system suitability solution. The system suitability results are shown in Table 2.

Preparation

As per specificity parameter.

Table 2: System suitability experiment Results.

Standard	Nintedanib Area
Injection -1	14.81
Injection -2	14.54
Injection -3	14.66
Injection -4	14.71
Injection -5	14.65

Injection -6	14.38
Mean	14.63
% RSD	1.02

System suitability observation

1. % RSD of Nintedanib peak area of six replicate injections in standard solution should not be more than 1.02%.
2. Retention time of Nintedanib peak is 23.66 and Impurity-3 peak is 40.85.
3. Resolution between Nintedanib peak and Impurity-2 peak is 11.60 in system suitability solution.

Precision

The repeatability of standard solution was assessed by preparing the solution to get the concentration about 1µg/ml of Nintedanib, impurity-1, impurity-2, impurity-3, impurity-4, impurity-5 and impurity-6. Preparation of solution is shown in table no 3.1. All the results were reported in terms of % RSD. The precision preparation and experiments results are given in Table 3.2.

Table 3.1: Precision preparation.

Impurity Name	Potency	Wt (mg)	Dilution	Taken ml	Dilution (stock-2)	Taken ml	Dilution	Conc. In ppm
Nintedanib	99.30	25.694	25	1	100	1	10	1.0206
Impurity-1	86.49	2.467	25	10				0.8535
Impurity-2	93.47	25.893	25	1				0.9681
Impurity-3	94.02	24.74	25	1				0.9304
Impurity-6	91.54	2.528	25	10				0.9258
Impurity-4 (Stock-3)	97.98	0.520	50			1		1.0190
Impurity-5 (Stock-4)	95.69	0.604	50 (First 25ml Methanol sonicate 10 min Than made up to Acetonitrile)			1		1.1559

Table 3.2: Precision experiment results.

Name	Area						
	Impurity-1	Nintedanib	Impurity-2	Impurity-3	Impurity-4	Impurity-5	Impurity-6
Injection -1	11.04	14.81	12.51	20.82	25.01	18.43	12.09
Injection -2	11.77	14.54	12.11	20.57	25.21	18.06	11.89
Injection -3	11.47	14.66	12.24	20.63	25.13	18.09	10.96
Injection -4	11.72	14.71	12.35	20.80	25.17	17.72	11.22
Injection -5	11.94	14.65	12.55	21.15	25.03	17.45	11.45
Injection -6	11.34	14.38	11.93	20.29	24.53	17.99	11.42
Mean	11.55	14.63	12.28	20.71	25.01	17.96	11.51
%RSD	2.85	1.02	1.94	1.39	1.00	1.87	3.64

LOD, LOQ

The limit of detection and limit of quantitation shall be determined based on Standard deviation of response and slop value obtained from the linearity study of related substance for impurity-1, impurity-2, impurity-3, impurity-4, impurity-5 and impurity-6 and Nintedanib. LOD and LOQ values are presented in Table 4.1 to Table 4.7

Linearity

The linearity of analytical method is its ability to elicit test results that are directly proportional to concentration of analyte in sample within given range. The linearity is expressed in terms of correlation co-efficient of linear regression analysis. Prepare the solution to gate concentration about 0.1-2.0µg/ml for impurity-1, impurity-2, impurity-3, impurity-4, impurity-5 and impurity-6 and Nintedanib. The graph was plotted for peak area vs. conc. for the drug. The statistical values are presented in Table 4.1 to Table 4.7 and Figure 6.

Table 4.1: Statistical evaluation of linearity and LOD/LOQ for Nintedanib.

ppm	Solution In %	ml of each stock-2, 3, 4	Dilution	Actual. ppm	Nintedanib Area
0.100	10%	0.10	10	0.1021	1.89
0.300	30%	0.30	10	0.3062	4.77
0.500	50%	0.50	10	0.5103	7.79
1.000	100%	1.00	10	1.0206	14.61
1.500	150%	1.50	10	1.5309	21.62
2.000	200%	2.00	10	2.0411	27.99
Slope					13.4936
STYEX					0.2400
LOD (ppm)					0.0587
LOQ (ppm)					0.1779
LOD (%)					0.006
LOQ (%)					0.018
Intercept					0.7173
Correlation Coefficient					0.9996

Table 4.2: Statistical evaluation of linearity and LOD/LOQ for Impurity-1.

ppm	Solution In %	ml of each stock-2, 3, 4	Dilution	Actual. ppm	Impurity-1 Area
0.100	10%	0.10	10	0.0853	1.22
0.300	30%	0.30	10	0.2560	3.68
0.500	50%	0.50	10	0.4267	6.06
1.000	100%	1.00	10	0.8535	11.74
1.500	150%	1.50	10	1.2802	17.41
2.000	200%	2.00	10	1.7070	22.55

Slope	13.1773
STYEX	0.2303
LOD (ppm)	0.0577
LOQ (ppm)	0.1748
LOD (%)	0.006
LOQ (%)	0.017
Intercept	0.3217
Correlation Coefficient (r^2)	0.9994
RRF	0.98

Table 4.3: Statistical evaluation of linearity and LOD/LOQ for Impurity-2.

ppm	Solution In %	ml of each stock-2, 3, 4	Dilution	Actual. ppm	Impurity-2 Area
0.100	10%	0.10	10	0.0968	1.26
0.300	30%	0.30	10	0.2904	3.82
0.500	50%	0.50	10	0.4840	6.23
1.000	100%	1.00	10	0.9681	11.61
1.500	150%	1.50	10	1.4521	16.99
2.000	200%	2.00	10	1.9362	21.97
Slope					11.2078
STYEX					0.2983
LOD (ppm)					0.0878
LOQ (ppm)					0.2662
LOD (%)					0.009
LOQ (%)					0.027
Intercept					0.5483
Correlation Coefficient (r^2)					0.9988
RRF					0.83

Table 4.4: Statistical evaluation of linearity and LOD/LOQ for Impurity-3.

ppm	Solution In %	ml of each stock-2, 3, 4	Dilution	Actual. ppm	Impurity-3 Area
0.100	10%	0.10	10	0.0930	2.65
0.300	30%	0.30	10	0.2791	6.69
0.500	50%	0.50	10	0.4652	11.21
1.000	100%	1.00	10	0.9304	20.51
1.500	150%	1.50	10	1.3956	30.72
2.000	200%	2.00	10	1.8608	40.20
Slope					21.2153
STYEX					0.2975
LOD (ppm)					0.0463
LOQ (ppm)					0.1402
LOD (%)					0.005
LOQ (%)					0.014
Intercept					0.8987
Correlation Coefficient (r^2)					0.9996
RRF					1.57

Table 4.5: Statistical evaluation of linearity and LOD/LOQ for Impurity-4.

ppm	Solution In %	ml of each stock-2, 3, 4	Dilution	Actual. ppm	Impurity-4 Area
0.100	10%	0.10	10	0.1019	2.96
0.300	30%	0.30	10	0.3057	8.39
0.500	50%	0.50	10	0.5095	12.99
1.000	100%	1.00	10	1.0190	24.92
1.500	150%	1.50	10	1.5285	36.89
2.000	200%	2.00	10	2.0380	46.86
Slope					22.7696
STYEX					0.7010
LOD (ppm)					0.1016
LOQ (ppm)					0.3079
LOD (%)					0.010
LOQ (%)					0.031
Intercept					1.2864
Correlation Coefficient (r^2)					0.9986
RRF					1.69

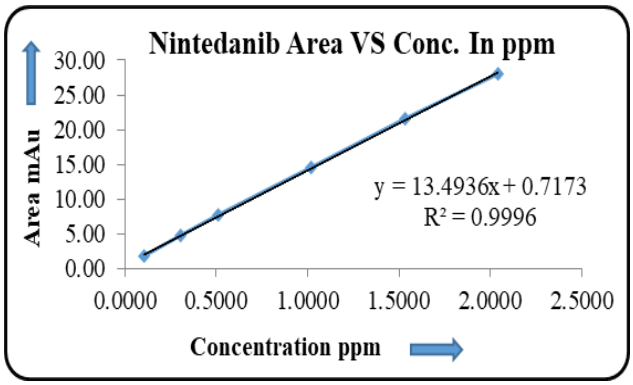
Table 4.6: Statistical evaluation of linearity and LOD/LOQ for Impurity-5.

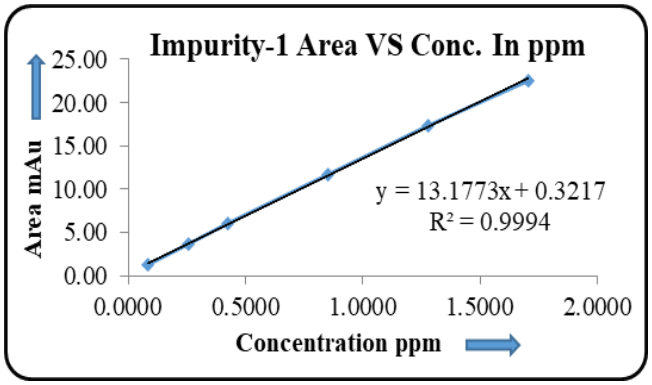
ppm	Solution In %	ml of each stock-2, 3, 4	Dilution	Actual. ppm	Impurity-5 Area
0.300	30%	0.30	10	0.3468	5.17
0.500	50%	0.50	10	0.5780	8.98
1.000	100%	1.00	10	1.1559	18.43
1.500	150%	1.50	10	1.7339	27.50
2.000	200%	2.00	10	2.3119	36.80
Slope					16.0685
STYEX					0.1160
LOD (ppm)					0.0238
LOQ (ppm)					0.0722
LOD (%)					0.002
LOQ (%)					0.007
Intercept					-0.3128
Correlation Coefficient (r^2)					1.0000
RRF					1.08

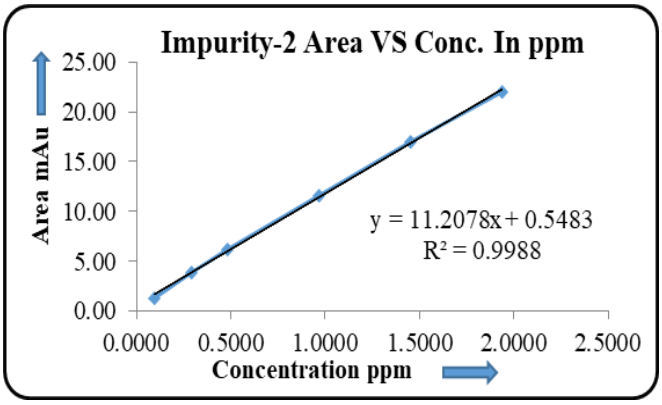
Table 4.7: Statistical evaluation of linearity and LOD/LOQ for Impurity-6.

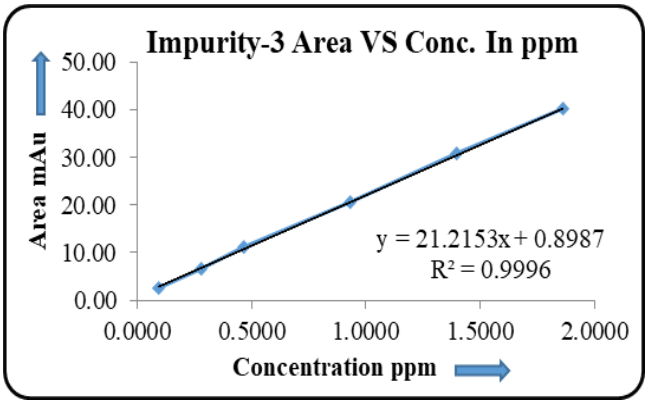
ppm	Solution In %	ml of each stock-2, 3, 4	Dilution	Actual. ppm	Impurity-6 Area
0.100	10%	0.10	10	0.0926	1.29
0.300	30%	0.30	10	0.2777	4.04
0.500	50%	0.50	10	0.4629	5.83
1.000	100%	1.00	10	0.9258	11.71
1.500	150%	1.50	10	1.3887	17.44
2.000	200%	2.00	10	1.8516	23.06
Slope					12.3046

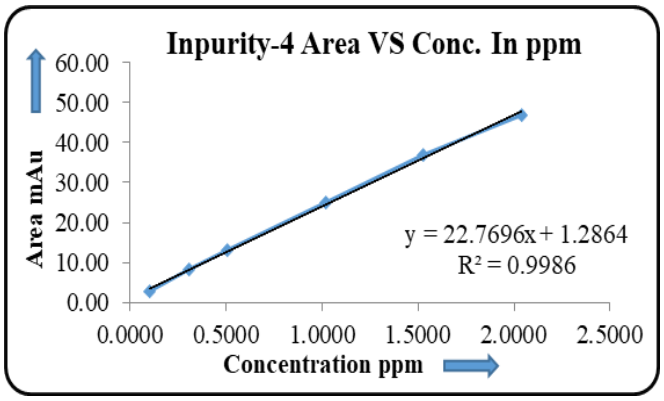
STYEX	0.1983
LOD (ppm)	0.0532
LOQ (ppm)	0.1612
LOD (%)	0.005
LOQ (%)	0.016
Intercept	0.3093
Correlation Coefficient (r^2)	0.9996
RRF	0.91

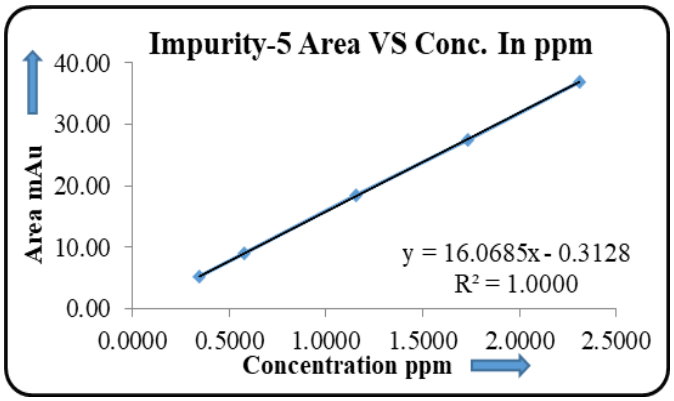












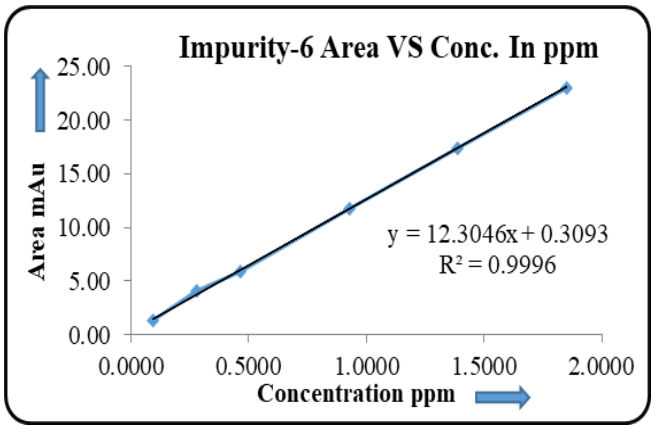


Figure 6: Linearity plot for Nintedanib and Related substances Impurity-1 to Impurity-6.

Accuracy

The accuracy of the method was determined by analyzing Nintedanib samples spiked with related substances at different levels (10, 30, 50, 100 and 150% of specification levels).

Standard solution

As per specificity parameter.

Accuracy stock solution preparation

Refer Stock-2, 3, 4 from precision parameter.

The percentage recovery values for all the impurities are calculated and tabulated in Table 5.1 to table 5.7

Table 5.1: Accuracy system suitability standard solution.

Injection No.	Area
1	14.81
2	14.54
3	14.66
4	14.71
5	14.65
6	14.38
Average	**14.63**
%RSD	**1.02**

Table 5.2: Accuracy for impurity-1.

Recovery Level Designation	Weight of Sample (mg)	ml of each stock- 2, 3, 4	Dilution (mL)	Actual Con. (%)	Actual Added Amount (%)	Area	% Result	Recovered %Result	Recovery
As such Test	25.871	NA	25	NA		0.97	0.0067	NA	
10%	25.255	0.250	25	0.0853	0.0085	2.32	0.0164	0.0097	113.72
30%	24.914	0.750	25	0.2560	0.0256	4.90	0.0350	0.0283	110.55
50%	24.681	1.250	25	0.4267	0.0427	6.89	0.0497	0.0430	100.77
100%	25.440	2.500	25	0.8535	0.0854	13.70	0.0959	0.0892	104.51
150%	25.326	3.750	25	1.2802	0.1280	20.01	0.1406	0.1339	104.59

Table 5.3: Accuracy for impurity-2

Recovery Level Designation	Weight of Sample (mg)	ml of each stock- 2, 3, 4	Dilution (mL)	Actual Con. (%)	Actual Added Amount (%)	Area	% Result	Recovered %Result	Recovery
As such Test	25.871	NA	25	NA		0.00	0.0000	NA	
10%	25.255	0.250	25	0.0968	0.0097	1.12	0.0093	0.0093	96.07

30%	24.914	0.750	25	0.2904	0.0290	3.31	0.0279	0.0279	96.07
50%	24.681	1.250	25	0.4840	0.0484	5.53	0.0471	0.0471	97.31
100%	25.440	2.500	25	0.9681	0.0968	10.59	0.0875	0.0875	90.38
150%	25.326	3.750	25	1.4521	0.1452	15.79	0.1310	0.1310	90.21

Table 5.4: Accuracy for impurity-3.

Recovery Level Designation	Weight of Sample (mg)	ml of each stock-2, 3, 4	Dilution (mL)	Actual Con. (%)	Actual Added Amount (%)	Area	% Result	Recovered %Result	Recovery
As such Test	25.871	NA	25	NA		0.00	0.0000	NA	
10%	25.255	0.250	25	0.0930	0.0093	1.81	0.0080	0.0080	86.02
30%	24.914	0.750	25	0.2791	0.0279	6.35	0.0283	0.0283	101.40
50%	24.681	1.250	25	0.4652	0.0465	9.98	0.0449	0.0449	96.52
100%	25.440	2.500	25	0.9304	0.0930	20.64	0.0902	0.0902	96.95
150%	25.326	3.750	25	1.3956	0.1396	30.47	0.1337	0.1337	95.80

Table 5.5: Accuracy for impurity-4.

Recovery Level Designation	Weight of Sample (mg)	ml of each stock-2, 3, 4	Dilution (mL)	Actual Con. (%)	Actual Added Amount (%)	Area	% Result	Recovered %Result	Recovery
As such Test	25.871	NA	25	NA		2.09	0.0083	NA	
10%	25.255	0.250	25	0.1019	0.0102	4.45	0.0182	0.0099	97.15
30%	24.914	0.750	25	0.3057	0.0306	8.54	0.0354	0.0271	88.65
50%	24.681	1.250	25	0.5095	0.0510	13.73	0.0574	0.0491	96.37
100%	25.440	2.500	25	1.0190	0.1019	26.61	0.1080	0.0997	97.84
150%	25.326	3.750	25	1.5285	0.1529	37.62	0.1533	0.1450	94.86

Table 5.6: Accuracy for impurity-5.

Recovery Level Designation	Weight of Sample (mg)	ml of each stock-2, 3, 4	Dilution (mL)	Actual Con. (%)	Actual Added Amount (%)	Area	% Result	Recovered %Result	Recovery
As such Test	25.871	NA	25	NA		12.1	0.0755	NA	
30%	24.914	0.750	25	0.3468	0.0347	17.47	0.1133	0.0378	109.00
50%	24.681	1.250	25	0.5780	0.0578	19.89	0.1302	0.0547	94.64
100%	25.440	2.500	25	1.1559	0.1156	30.94	0.1965	0.1210	104.68
150%	25.326	3.750	25	1.7339	0.1734	40.87	0.2607	0.1852	106.81

Table 5.7: Accuracy for impurity-6.

Recovery Level Designation	Weight of Sample (mg)	ml of each stock-2, 3, 4	Dilution (mL)	Actual Con. (%)	Actual Added Amount (%)	Area	% Result	Recovered %Result	Recovery
As such Test	25.871	NA	25	NA		0.00	0.0000	NA	
10%	25.255	0.250	25	0.0926	0.0093	1.15	0.0087	0.0087	93.95

30%	24.914	0.750	25	0.2777	0.0278	3.60	0.0277	0.0277	99.75
50%	24.681	1.250	25	0.4629	0.0463	5.15	0.0400	0.0400	86.41
100%	25.440	2.500	25	0.9258	0.0926	12.55	0.0946	0.0946	102.18
150%	25.326	3.750	25	1.3887	0.1389	18.80	0.1423	0.1423	102.47

Stability of solutions

Standard solution and sample solution spiked with impurities were prepared and analyzed initially and at different time intervals by keeping the solutions at room temperature (~ 25°C). Experimental results show that Standard solution is stable up to 36 hours at 25°C+2°C. Sample solution spiked with impurities are stable up to 24 hours at 25°C+2°C.

CONCLUSION

A reverse phase stability indicating HPLC method was developed and validated for the quantitative determination of related substances of Nintedanib esylate. The present research work will help the manufacturers and suppliers of Nintedanib esylate to quantify and qualify the quality in terms of purity based on experimental results. Thus, it can be used for routine analysis, quality control and for determining quality during the stability studies of pharmaceutical analysis.

ACKNOWLEDGEMENT

I am thankful to analytical development department of BDR Lifesciences Pvt. Ltd. for providing me research facility for this project.

REFERENCES

1. "Vargatef EPAR". European Medicines Agency (EMA), 20 January 2020. Retrieved, 9 March 2020.
2. "FDA Approves First Treatment for Group of Progressive Interstitial Lung Diseases". U.S. Food and Drug Administration (FDA) (Press release). 9 March 2020. Retrieved, 9 March 2020.
3. Ahluwalia N, Shea BS, Tager AM. "New therapeutic targets in idiopathic pulmonary fibrosis. Aiming to rein in runaway wound-healing responses". American Journal of Respiratory and Critical Care Medicine, 2014; 190(8): 867–78. doi:10.1164/rccm.201403-0509pp. PMC 4299574. PMID 25090037.
4. Mazzei ME, Richeldi L, Collard HR. "Nintedanib in the treatment of idiopathic pulmonary fibrosis". Therapeutic Advances in Respiratory Disease, 2015; 9(3): 121-9. doi:10.1177/1753465815579365. PMID 25862013.

5. Dimitroulis IA (September 2014). "Nintedanib: a novel therapeutic approach for idiopathic pulmonary fibrosis". Respiratory Care, 2014; 59(9): 1450–5. doi:10.4187/respcare.03023. PMID 24782550.
6. Brunton L, Knollman B, Hilal-Dandan R. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 13th Edition. McGraw Hill Professional, 2017. ISBN 9781259584749.
7. Wollin, Lutz; Wex, Eva; Pautsch, Alexander; Schnapp, Gisela; Hostettler, Katrin E.; Stowasser, Susanne; Kolb, Martin. "Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis". European Respiratory Journal, 2015. doi:10.1183/09031936.00174914. ISSN 0903-1936. PMID 25745043.
8. "Nintedanib for treating idiopathic pulmonary fibrosis" (PDF). National Institute for Health and Care Excellence (NICE), 27 January 2016. TA379. Retrieved 7 August. Lay summary, 2019.
9. Popat S, Mellemgaard A, Fahrbach K, Martin A, Rizzo M, Kaiser R, et al. "Nintedanib plus docetaxel as second-line therapy in patients with non-small-cell lung cancer: a network meta-analysis". Future Oncology, 2014; 11(3): 409–20. doi:10.2217/fon. 14.290. PMID 25478720.
10. The International Conference on Harmonization, Q3A (R2), Impurities in New Drug Substances: Text and Methodology, 2006.
11. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure: Text and Methodology, 2005.