Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 8 Issue 11 November 2024

Review Article

New Stability Indicating RP-UPLC Method for the Estimation of Nintedanib Capsules

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DOI: 10.31080/ASPS.2024.08.1125

Received: October 14, 2024

Published: October 26, 2024

Abstract

Nintedanib is used to treat idiopathic pulmonary fibrosis. It is also used to treat people with a chronic interstitial lung disease. A new RP-UPLC method has been developed for the estimation of Nintedanib in pharmaceutical formulations and the method was and validated. UPLC Acquity Waters – Empower software 2.0 versions equipped with photodiode array detector with Phenomenex C18 (50 x 2.1 mm, 1.6 µm) was used with a mobile phase consisting of Acetonitrile: dihydrogen phosphate buffer (pH adjusted to 3.0 with ortho phosphoric acid) (30:70) and flow rate of 0.2 mL/min (UV detection at 271nm) with a run time of 2.0 mins. Nintedanib has shown linearity 25–150 µg/mL with linear regression equation y = 28788.86 x + 24028.04 (r2 = 0.99958). The LOQ was found to be 1.0 µg/mL and the LOD was found to be 0.30 µg/mL. Nintedanib was subjected to forced degradation and the method was validated as per ICH guidelines.

Keywords: Nintedanib; RP-UPLC; Stability INDICATING; Validation; ICH GUIDELINES

Introduction

Nintedanib (Figure 1), chemically methyl (3*Z*)-3- [[4- [methyl- [2-(4-methyl piperazine-1-yl) acetyl] amino] aniline]-phenyl methylidene]-2-oxo-1H-indole-6-carboxylate is a multiple tyrosine kinase inhibitor for platelet-derived growth factor receptor, fibroblast growth factor receptor, and vascular endothelial growth factor receptor [1]. Nintedanib is an yellow crystalline solid that melts at 244 °C to 251 °C. It has poor solubility in water, and somewhat better solubility in dimethyl sulfoxide at 25 g/L. The molecular formula of Nintedanib is $C_{31}H_{33}N_5O_4$ and Molecular weight of 539.636 g/mol. Literature survey reveals that Nintedanib was estimated by HPLC [2-6] in pharmaceutical dosage forms and also in biological fluids.

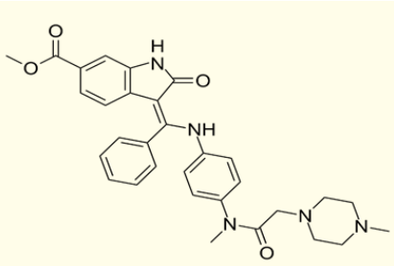

Figure 1: Chemical Structure of Nintedanib.

Materials and Methods

Instrumentation

Chromatographic separation was achieved by UPLC Acquity Waters – Empower software 2.0 versions equipped with photodiode array detector with Phenomenex C18 (50 x 2.1 mm, 1.6 µm) was used for the present study.

Preparation of phosphate buffer solution

1.36 grams of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in water in a 1L volumetric flask, pH was adjusted to 3.0 with OPA and finally filtered through 0.22 µ membrane filter paper.

Preparation of stock and working standard solutions

Accurately weigh and transfer 10mg of Nintedanib, working standard into a 10ml volumetric flask, add 7ml of diluent and sonication are used to thoroughly dissolve the sample and bring the volume to the desired level. From this stock solution 1 mL was taken in to a 10 mL volumetric flask, and diluent was added to make remaining volume This solution of 100 µg/mL of Nintedanib used as standard and future diluted as required.

Preparation of sample solution

Accurately weighed and transfer 18 mg of Nintedanib sample into a 10 mL clean dry volumetric flask add diluent and sonicate

it up to 30 mins to dissolve, and centrifuge for 30 min. to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45-micron Injection filter. Further pipette 1 mL of the above stock solutions into 10 ml volumetric flask and dilute up to the mark with diluents.

Method validation

Linearity

A series of 25-150 µg/mL Nintedanib solutions were prepared from the stock solution with mobile phase and 20 µL of each of these solutions were injected in to the UPLC system. The mean peak area of Nintedanib were calculated from the chromatograms and a calibration curve was drawn by taking the concentration of the Nintedanib solutions on the x-axis and the corresponding mean peak area values on the y-axis.

Precision, accuracy and robustness

Intraday and inter-day precision were studied using three different concentrations of Nintedanib on the same day and on three consecutive days respectively and the % RSD was calculated. The accuracy of the assay method was evaluated in triplicate at three concentration levels (50, 100 and 150%), and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Nintedanib in the drug product and the percentage recovery was calculated. The robustness of the method was assessed by exposing the drug solution to different analytical conditions purposely changing from the original optimized conditions. The effects so obtained were summarized to calculate the % RSD and has to be less than 2.0% specifying that the proposed method was robust.

Forced degradation studies

Forced degradation studies were performed to determine the ability of the drug to withstand its properties in the applied stress conditions. Nintedanib was exposed to different stress conditions.

Acidic degradation

Pipette 1 ml of the afore mentioned solution was added to a 10 ml vacuum flask, followed by 1 ml of 1N HCl. The vacuum flask was then maintained at 60°C for 1 hour before being neutralised with 1 N NaOH and diluted to 10ml with diluent. Filter the solution using 0.22-micron syringe filters and transfer to bottles.

Alkaline degradation

Pipette 1 ml of above solution into a 10ml volumetric flask and add 1ml of 1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 1 hour and then neutralized with 1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns' syringe filters and place in vials.

Thermal degradation

50 mg of Nintedanib standard was taken in petridish and kept in hot air oven at 105^0 C for 3 hours. Then the analyzed standard was taken and diluted with diluents and injected into UPLC and analysed.

Peroxide degradation

Pipette 1 ml above stock solution was added to a 10 ml vacuum flask, 1 ml of 3 percent w/v hydrogen peroxide was added to the flask and the volume was built up to the mark using diluent. The vacuum flask was then maintained at 60°C for 1hour. After that, the vacuum flask was left at room temperature for 15 minutes. Filter the solution using 0.22micron syringe filters and transfer to bottles.

Reduction degradation

Pipette 1ml of above-stock solution was added to a 10ml vacuum flask, 1ml of 10% Sodium bisulphite was added to a flask and the volume was built up to the required volume with diluent. The vacuum flask was then maintained at 60°C for 1 hour. After that, the vacuum flask was left at room temperature for 15 minutes. Filter the solution using 0.22 µ syringe filters and transfer to bottles.

Photolytic degradation

Nintedanib sample was placed in Photo stability chamber for 3 hours. Then the sample was taken and diluted with diluents and injected into UPLC and analysed.

Hydrolysis degradation

Pipette 1ml of above-stock solution was added to a 10ml vacuum flask, 1ml of HPLC grade water was added to a flask and the volume was built up to the required volume with diluent. The vacuum flask was then maintained at 60˚C for 1 hour. After that, the vacuum flask was left at room temperature for 15 minutes. Filter the solution using 0.22micron syringe filters and transfer to bottles.

Assay of Nintedanib capsules

Twenty capsules were procured and powder equivalent to 25 mg Nintedanib was extracted using the mobile phase in a 25 ml volumetric flask. The solution was sonicated for half an hour and filtered through 0.45 mm membrane filter and 20 µL of this solution was injected in to the UPLC system and the peak area was noted at its retention time from the resultant chromatogram.

Results and Discussion

A new RP-UPLC method has been developed for the estimation of Nintedanib in pharmaceutical formulations and the method was and validated. UPLC Acquity Waters-Empower software 2.0 versions equipped with photodiode array detector with Phenomenex C18 (50 x 2.1 mm, 1.6 µm) was used with a mobile phase consisting of Acetonitrile: dihydrogen phosphate buffer (pH adjusted to 3.0

with ortho phosphoric acid) (30:70) and flow rate of 0.2 mL/min (UV detection at 271nm) with a run time of 2.0 mins. The optimized chromatographic conditions were shown in Table 1 and the system suitability was shown in Table 2. The chromatograms of blank and the standard Nintedanib were shown in Figure 2A and Figure 2B respectively.

Table 1: Optimised chromatographic conditions.

Parameters	Observation
Injection volume	5 µl
Mobile Phase	Acetonitrile: KH_2PO_4 pH-3.0/OPA (30:70)
Column	Phenomenex C18 (50 x 2.1mm, 1.6µm)
Detection Wave Length	271 nm
Flow Rate	0.2 mL/min
Runtime	2 min
Temperature	Ambient (25° C)
Mode of separation	Isocratic mode

Table 2: System suitability data of Nintedanib.

Sample	Peak area	Retention	Theoretical plate	Tailing factor
Injection-1	2963560	0.968	7025	0.71
Injection-2	2912874	0.962	7036	0.72
Injection-3	29360.34	0.960	7028	0.70
Injection-4	2955341	0.966	7011	0.73
Injection-5	2942218	0.969	7010	0.69
Injection-6	2913021	0.971	7028	0.65

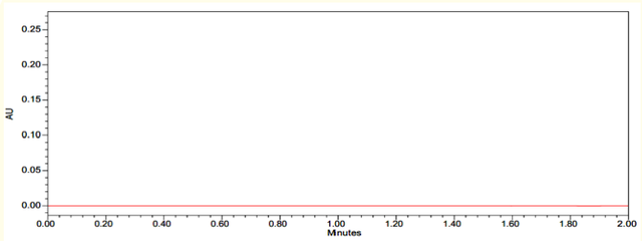

Figure 2a: Blank

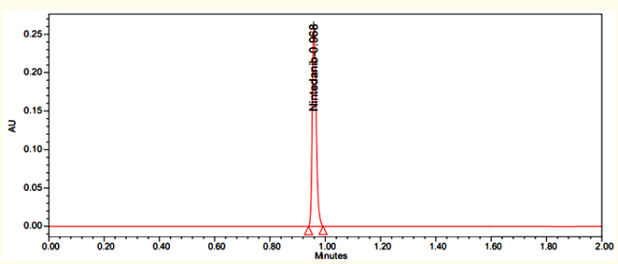

Figure 2b: Standard chromatogram of Nintedanib.

Linearity, precision, accuracy and robustness

Nintedanib has shown linearity 25–150 µg/mL with linear regression equation y = 28788.86 x + 24028.04 with correlation coefficient 0.99958 (Table 3). The LOD and LOQ of Nintedanib were found to be 0.30 µg/mL and 1.00µg/ml. The calibration curve was shown in Figure 3.

Table 3: Linearity study of Nintedanib.

Conc. (µg/mL)	Peak area	*Peak area ±SD
25	715220	715224 ± 4.58
	715229	
	715223	
50	1485769	1485763 ± 4.72
	1485762	
	1485760	
75	2201547	2201543 ± 3.60
	2201540	
	2201542	
100	2956341	2956333 ± 6.65
	2956332	
	2956328	
125	3658246	3658240 ± 5.50
	3658240	
	3658235	
150	4265207	4265209 ± 8.18
	4265202	
	4265218	

*Mean of three replicates

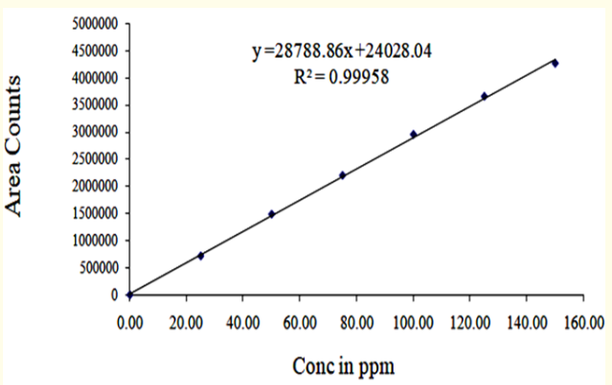

Figure 3: Calibration curve for Nintedanib at 271nm.

The method precision and intermediate precision studies were performed and the % RSD was found to be less than 2% (Table 4). The accuracy of the method was proved by the standard addition method and the results were shown in Table 5. The robustness of the assay method was established by introducing small changes in the chromatographic conditions which include detection wave-

Table 4: Precision study of Nintedanib.

S. No.	Conc. ((µg/mL)	Mean peak area		*Assay	
		Method precision	Intermediate precision	Method precision	Intermediate precision
1	18	2910417	2934307	99.1	99.7
2	18	2925326	2953204	99.6	100.4
3	18	2953268	2934619	100.5	99.7
4	18	2932514	2944124	99.8	100.1
5	18	2951501	2965761	100.5	100.8
6	18	2934712	2951240	99.9	100.3

Table 5: Accuracy study of Nintedanib.

Level	Standard Conc. (µg/mL)	Peak area	Amount added (mg)	Amount found (mg)	% Recovery	* % Recovery %RSD
50%	50	1455647	5.0	4.956	99.1	99.6 (1.170)
	50	1482287	5.0	5.047	100.9	
	50	1450264	5.0	4.938	98.8	
100%	100	2956320	10.0	10.065	100.7	100.2 (0.530)
	100	2945175	10.0	10.030	100.3	
	100	2925104	10.0	9.960	99.6	
150%	150	4360154	15.0	14.845	99.0	99.0 (0.510)
	150	4385624	15.0	14.931	99.5	
	150	4339712	15.0	14.780	98.5	

*Mean of three replicates.

length, percentage of acetonitrile in the mobile phase and flow rate and the results were shown in Table 6.

Assay of Nintedanib capsules

The purity of marketed capsule formulation was determined by utilizing optimized method and assay was found to be 100.7 % w/w.

Table 6: Robustness results of Nintedanib.

Parameter	Condition	Standard peak area	*Mean peak area	Tailing factor	Plate count	* Assay (% RSD)
Flow rate Change (mL/min)	0.18	2704269	2179450	0.80	6940	99.7 (0.51)
	0.20	2963560	2963558	0.71	7025	99.8 (0.32)
	0.22	3058524	3056525	0.69	7114	99.6 (0.35)
Organic Phase change	27:73	2654268	2638833	0.87	6812	100 (0.81)
	30:70	2912874	2912872	0.72	7036	100.1 (0.18)
	33:67	3258257	3240669	0.70	7213	99.8 (0.50)

*Mean of three replicates.

Forced degradation studies

Nintedanib was eluted at 0.968 min. Nintedanib has undergone acidic hydrolysis, thermal degradation, alkaline hydrolysis, peroxide degradation, hydrolysis, photolysis and the results were shown in Table 7 and the resultant chromatograms were shown in Figure 4.

Table 7: Stress degradation studies of Nintedanib.

Degradation studies	% Degradation*	Theoretical Plates	Tailing factor
Control	0	7086	0.73
Acidic degradation (1N HCl)	13.3	7061	0.78
Alkaline degradation (1N NaOH)	12.9	7025	0.70
Peroxide degradation (3% w/v hydrogen peroxide)	14.8	7057	0.83
Reduction (10% Sodium bisulphite)	2.4	7068	0.80
Hydrolysis (HPLC grade water for 12 Hours)	0.9	7040	0.69
Thermal degradation (105^{0}C for 3 hours)	3.6	7025	0.67
Photolytic degradation (1.2 million flux hours for 2-3 days)	4.5	7039	0.65

*Mean of three replicates.

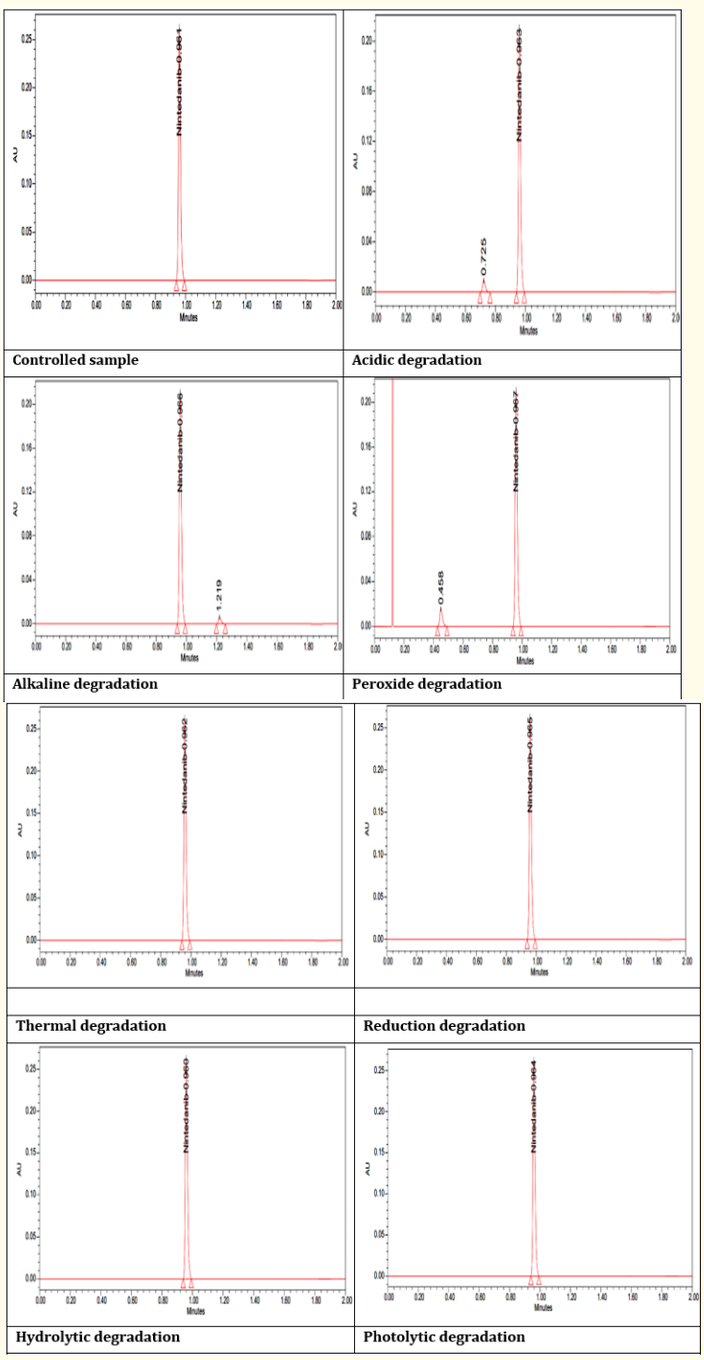

Figure 4: Typical chromatograms of Nintedanib during forced degradation studies.

Conclusion

The RP-UPLC techniques were validated as per ICH guidelines and found to be simple, economical and robust for the quantification of Nintedanib capsules.

Acknowledgement

The authors are grateful to Glenmark Pharmaceuticals (India) for supplying gift samples of Nintedanib. The authors have no conflict of interest.

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