IJBPAS, April, 2023, 12(4): 1488-1497

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NINTEDANIB IN PHARMACEUTICAL DOSAGE FORM

PUJITHA K, DURGA GAYATHRI E, JAHNAVI M, SAHITHI M, TARUN SAI NAGENDRA G, SRINIVASA BABU P AND RAVI SANKAR P*

Department of Pharmaceutical Analysis, Vignan Pharmacy College, Vadlamudi, Guntur, A.P, India

***Corresponding Author: Dr. Ravi Sankar P: E Mail: banuman35@gmail.com**

Received 8th May 2022; Revised 16th June 2022; Accepted 27th Aug. 2022; Available online 1st April 2023

https://doi.org/10.31032/IJBPAS/2023/12.4.7014

ABSTRACT

A precise, simple, cost-effective, accurate Ultraviolet spectrophotometric method has been developed for the determination of Nintedanib in the Pharmaceutical dosage form. Nintedanib shows the highest λmax at 379.5 nm. The Nintedanib follows linearity in the concentration range of 0.2-1.0 µg/mL with a superior correlation coefficient value of 0.9999. The precision of the method was studied in intra-day and inter-day studies. The % RSD value is < 2 which indicates that the method is precise. The % recovery was found to be in the range lies between 99.75-99.85 %. The percentage assay of Nintedanib obtained was 99.93 %. The Proposed spectrophotometric method was validated as per the ICH Q2 (R_1) guidelines. The developed UV method is accurate, precise, and reproducible. Hence this rapid method can be feasible for the quality control analysis of Nintedanib in the pharmaceutical dosage form.

Keywords: Nintedanib, Validation, Ultraviolet spectroscopy, Method development

INTRODUCTION

The chemical name for Nintedanib is 1H-Indole-6-carboxylic acid,2,3--dihydro-3-[[[4-[methyl[(4-methyl-1-piperazinyl) acetyl] amino] phenyl] amino] phenyl methylene]-2-oxo-, methyl ester. It has the molecular formula $C_{31}H_{33}N_5O_4$ and a molecular weight of 539.6248g/mol. 1H-Indole-6-carboxylic acid,2,3--dihydro-3-[[[4-[methyl[(4-methyl-1-piperazinyl) acetyl] amino] phenyl] amino] phenyl methylene]-2-oxo-, methyl ester. Nintedanib indolinone-derived inhibitor of Multiple Receptor Tyrosine kinases (RTKs) and Non- Receptor Tyrosine kinases (nRTKs) selectively binds to and inhibits vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), and colony-stimulating factor 1 receptor (CSF1R) tyrosine kinases, which may result in the induction of endothelial cell apoptosis, the reduction in tumor vasculature, the inhibition of tumor cell proliferation and migration, and antifibrotic activity in pulmonary fibrosis. Nintedanib works by decreasing the blood supply to the cancer tumor to slow tumor growth. Nintedanib is used to treat Potential antiangiogenic, antifibrotic, and antineoplastic activities. Idiopathic Pulmonary Fibrosis (IPF). Literature Survey shows that the Nintedanib has been determined by HPLC **[1-6]**, HPTLC **[7]**, UPLC **[8-9]**, and LC-MS/MS **[10]**, in biological fluids like human and rat plasma. However, no UV spectrophotometric method has been reported for the estimation of Nintedanib in bulk and pharmaceutical dosage forms hitherto. Hence the major objective of the present research is to develop and validate a simple, precise, sensitive UV spectrophotometric method for Nintedanib in Capsule dosage form as per International Conference on Harmonization (ICH) Q2 (R2) guidelines **Figure 1**, shows the chemical structure of Nintedanib.

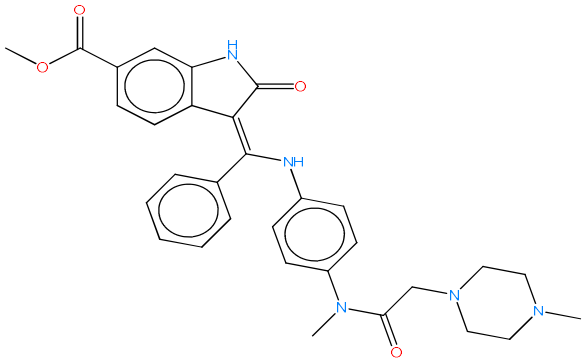

Figure 1: Chemical Structure of Nintedanib

MATERIALS AND METHODS

Instrument

A double beam ELICO SL 210UV spectrophotometer containing two matched quartz cells with a one cm light path was taken for measuring of absorbance of Nintedanib (0.1 mg sensitivity) balance was used for weighing. Ultra Sonicator bath Model no - 91250, PCI Ltd., Mumbai was used in this present study.

Chemicals and reagents:

Nintedanib was procured from Hetero Drugs Ltd., Hyderabad, and Telangana, India. The Nintedanib tablets containing 200 mg labeled claim of Nintedanib tablets were used for this study. Acetonitrile and CH_3OH were procured from E. Merck specialties, private Ltd., Mumbai, India.

Selection of solvent:

Plentiful trials were executed to find out the suitable solvent system for dissolving the Nintedanib. The solvents such as acetonitrile, methanol, and distilled water were tried based on the solubility of the drug. Nintedanib is soluble in solvents such as methanol- Acetonitrile and N-methyl pyrrolidine. Thus, methanol and acetonitrile were selected.

Selection of detection wavelength:

To determine the optimum λmax of Nintedanib, 10 µg/ml of the Nintedanib solution was prepared and scanned in the Ultra Violet wavelength range of 200 - 400 nm. It was observed that the drug showed maximum absorbance at 379.5 nm which was chosen as the detection wavelength for the estimation of Nintedanib.

Standard preparation solution:

A stock solution of Nintedanib at 1000 µg/ml is prepared in Methanol: Acetonitrile (50:50) by sonication. Dilution in Methanol: Acetonitrile (50:50) is made up at 0.2 µg/ml-1.0 µg/ml concentrations.

Preparation of Calibration curve:

From the above prepared Nintedanib stock solution, appropriate dilutions were prepared to get the ultimate concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 µg/ ml, and absorbance was taken at λ_{max} 379.5 nm. The average of such five sets of values was taken for the standard calibration plot, and the calibration curve was plotted. The calibration curve was done by plotting Nintedanib concentration on the x-axis and their respective absorbances on the y-axis.

Table 1: Calibration data of Nintedanib

S. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	0.2	0.1670
3	0.4	0.3216
4	0.6	0.4832
5	0.8	0.6433
6	1.0	0.8028

Table 2: Linear regression data

Parameter	Results
Detection wavelength (λ_{max})	379.5 nm
Beer's law limits (µg/ml)	0.2-1.0
Molar absorptivity (L. mole^{-1}cm^{-1})	8028
Sandell's sensitivity (µg /cm^2/0.001 absorbance unit)	0.001245
Regression equation (y= mx+ c)	0.7998x+0.0029
Slope (m) & Intercept (c)	0.7998 & 0.0029
Standard error of slope (S_m)	0.004562748
Standard error of intercept (S_c)	0.002762881
Standard error of estimate (S_e)	0.003817469
Correlation coefficient (r^2)	0.9999

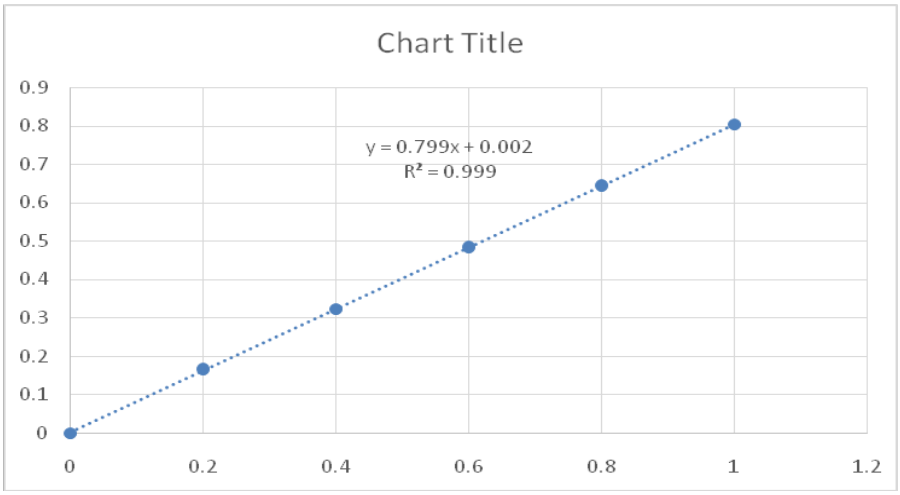

Figure 2: Calibration curve of Nintedanib

Table 3: Summary output Regression ANOVA Data of Nintedanib

	A	B	C	D	E	F	G	H	I
1	SUMMARY OUTPUT								
2									
3	*Regression Statistics*								
4	Multiple R	0.99993519							
5	R Square	0.999870384							
6	Adjusted R Square	0.99983798							
7	Standard Error	0.003817469							
8	Observations	6							
9									
10	ANOVA								
11		*df*	*SS*	*MS*	*F*	*Significance F*			
12	Regression	1	0.449671957	0.449671957	30856.3633	6.3004E-09			
13	Residual	4	5.82923E-05	1.45731E-05					
14	Total	5	0.449730249						
15									
16		*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
17	Intercept	0.003240952	0.002762881	1.173033519	0.305868163	-0.004430036	0.010911941	-0.004430036	0.010911941
18	X Variable 1	0.801491429	0.004562748	175.6597942	6.3004E-09	0.788823208	0.814159649	0.788823208	0.814159649

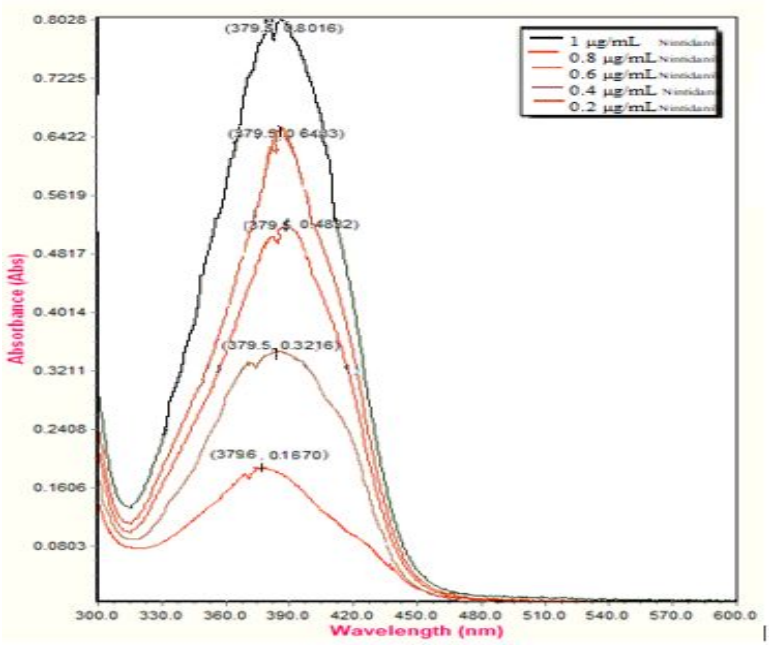

Figure 3: Ultraviolet Overlain Spectra of Nintedanib

Method development and validation

A lot of solvents were tested for solubility for Nintedanib, solvents such as Distilled water, Methanol, Acetonitrile, and N-methyl pyrrolidine at 10 µg/ml concentrations. Nevertheless, Nintedanib was soluble and stable in the MeOH-ACN mixture for a minimum of 24 hours at room temperature. Hence MeOH-ACN mixture was used for the detection of wavelength and preparation of standard and working concentration. To ensure the planned method for the pharmaceutical formulation, an assay of Nintedanib 200 mg capsules was utilized at working concentration. An assay for the working concentration of the sample at 379.5 nm was analyzed. UV spectrophotometric method is validated according to ICH Q2 (R1) **[11-15]**, guidelines for validation of analytical procedures. The method was validated for parameters such as specificity, linearity, range, accuracy, precision, LOD, LOQ, robustness, and system suitability testing.

Precision:

System precision: In system precision 0.6 µg/ml concentrations of six reproducible recordings of absorbance at 379.5nm were measured on the same day and corresponding responses were studied. The mean, SD, and % RSD were calculated.

Method precision

Method precision was estimated by conducting the assay of a sample under the test of repeatability (intraday precision) and intermediate precision was performed on

three successive days three times. Eventually, the mean, SD, and % Relative standard deviation were determined.

Accuracy (recovery studies):

Recovery studies of Nintedanib were accomplished by applying the standard addition method. To a known amount of the pre-analyzed drug sample 50 %, 100 %, and 150 % of standard drug substance were added and suitably diluted. The absorbances of the resultant solutions were measured at 379.5 nm. The amount recovered was determined by fitting the absorbance values in the calibration graph. At each level, 3 analyses were performed. % Mean recovery was calculated as shown in **Table 7**. The accepted limits of recovery are 97.66% - 99.83%. In fact, from the amount of Nintedanib was found and % recovery was estimated.

Ruggedness

Ruggedness is done by performing the proposed method on different instruments. In addition, this method is carried out by two different analysts and performing the method on different instruments to check the reproducibility **(Table 8)**.

Solution Stability: The Solutions of Nintedanib (Concentration 1.0 µg/ml) were tested for their stability at ambient temperatures. The absorbance values for 8hrs, 16 hrs, 24 hrs, 32 hrs, and 48 hrs were reproducible and absorbance variation was found to be less than 2% in both conditions **(Table 9)**.

Analysis of marketed formulation

The validated method was applied to the estimation of Nintedanib. 20 capsules were assayed and the results are represented in table 10 which indicates that the amount of drug in the tablet sample was in good agreement with the label claim of the formulation as indicated by the percentage recovery of 99.87% **(Table 10)**.

Table 4: Results of system precision

S. No	Absorbance
1	0.8028
2	0.8037
3	0.8017
4	0.8026
5	0.8029
Mean	0.80274
Standard deviation	0.000716
% Relative Standard deviation	0.089224

Table 5: Results of Method precision (Intraday precision)

S. No.	Concentration (µg/mL)	Inter-day Precision		
		Day1	Day2	Day3
1.	0.6	0.4832	0.4829	0.4830
2.	0.6	0.4832	0.4832	0.4831
3.	0.6	0.4833	0.4831	0.4832
4.	0.6	0.4831	0.4830	0.4832
5.	0.6	0.4830	0.4831	0.4829
6.	0.6	0.4829	0.4830	0.4830
Statistical validation data of Inter-day precision				
Parameter	SD	0.00014	0.00010	0.00012
	Mean	0.4831	0.4830	0.4830
	%RSD	0.0304	0.0217	0.0250

Table 6: Results of method precision (Inter day precision)

Concentration(µg/mL)	Absorbance			
	0hr	2hr	4hr	6hr
0.6	0.4832	0.4832	0.4832	0.4831
0.6	0.4835	0.4834	0.4831	0.4829
0.6	0.4830	0.4832	0.4829	0.4822
0.6	0.4831	0.4831	0.4830	0.4832
0.6	0.4832	0.4832	0.4831	0.4829
0.6	0.4832	0.4831	0.4830	0.4830
Statistical validation data of Intraday precision				
SD	0.00016	0.00010	0.00010	0.00035
Mean	0.4832	0.4832	0.4830	0.4828
% RSD	0.0346	0.0227	0.0217	0.0734

Table 7: Accuracy of results

Recovery level	Amount of standard drug added (µg/mL)	Amount of test added (µg/mL)	Total amount recovered (µg/mL)	% Recovery
50 %	0.3	0.3	0.595	99.16
100 %	0.6	0.3	0.879	97.66
150 %	0.9	0.3	1.198	99.83
Mean Recovery: 97.66-99.83%				

Table 8: Results of ruggedness

S.NO.	Absorbance for 0.6 µg/mL			
	Analyst-1	Analyst-2	Instrument-1	Instrument-2
1.	0.4832	0.4832	0.4831	0.4832
2.	0.4831	0.4829	0.4833	0.4830
3.	0.4832	0.4831	0.4832	0.4833
4.	0.4829	0.4838	0.4834	0.4834
5.	0.4835	0.4828	0.4830	0.4835
6.	0.4830	0.4832	0.4832	0.4832
Statistical validation data of Ruggedness				
SD	0.000207	0.00035	0.000141	0.000175
Mean	0.48315	0.483167	0.4832	0.483267
% RSD	0.042919	0.072488	0.029268	0.036237

Table 9: Solution Stability studies of Nintedanib

Time(hrs)	Absorbance 1.0µg/ml standard in ambient conditions
0	0.8028
8	0.8029
16	0.8028
24	0.8028
32	0.8030
48	0.8028

Table 10: Result of assay of pharmaceutical formulation Nintedanib

S. No.	Formulation	Amount present (mg)	Amount obtained* (mg)	% Purity (% w/w)
1	Nintib (Capsule)	200	199.87	99.93

RESULTS AND DISCUSSION

The ultraviolet spectra of Nintedanibwere scanned in the region between 200-400 nm. The overlay spectra of Nintedanib at different concentrations were absorbed maximum at 379.5nm, which was selected as the detection wavelength. The response of the Nintedanib was found to be linear in the concentration range of 0.2-1.0 µg/ml with a good correlation coefficient of r^2=0.999 **Figure 2** shows the Nintedanib linearity calibration curve and **Table 1** shows the calibration data of Nintedanib **Table 2** lists the linear regression data of the proposed UV method. **Figure 3** displays the overlay spectra of Nintedanib. The summary output (ANOVA) results of Nintedanib are summarized in **Table 3**. The system precision, and intermediate precision results, i.e., inter-day and intra-day precision of Nintedanib are tabulated in **Tables 4 - 6** respectively. The % RSD was less than 2 in all precision results cases which indicates that the method was precise. In this recovery, study accuracy was carried out by using a standard addition method at three different concentration levels (80%, 100%, and 120%). The mean percentage recovery at each level should be 97.66% - 99.83%. All the results are well within the acceptance criteria and results indicate that the method is accurate. Results are excellent and displayed in **Table 7**. Ruggedness was performed to check the reproducibility which showed the % RSD less than 2 which indicates that the method was rugged **(Table 8)**. The developed method was eventually applied for the quantification of Nintedanib in capsules. The mean % assay values were found to be 99.93 %. The amount of the drug in the capsule sample was in good agreement with the label claim of the formulation. The assay results are shown in **Table 10**.

CONCLUSION

The UV method was developed for the determination of Nintedanib. In this study, the precision and accuracy were < 2 % RSD. This method provides reproducible results

with high precision, and accuracy and was capable of analyzing Nintedanib in low concentrations. However, this UV method is simple, quick, and sensitive. The results proved that this method is successfully ideal for routine quality control testing of Nintedanib samples.

Acknowledgment

The authors are thankful to Hetero Labs for providing the sample of Nintedanib. We are highly grateful to Dr. L. Rathaiah, honorable chairman, of the Vignan group of institutions, Vadlamudi, Guntur for providing the necessary laboratory services to carry out the present investigation.

REFERENCES

[1] Dasari Purnachand, Arava Veera Reddy, Bhoomi Reddy Ramadevi, Ch. V. S. L. Kameswarrao, Gogireddy Surendra Reddy, Bethi Madhusudhanreddy, Development and validation of simple and sensitive stability-indicating RP-HPLC assay method for determination of Nintedanib and stress degradation studies, Journal of Chemical and Pharmaceutical Research,2015,7(8):774-782.

[2] Rohit Kumar, Vijaya Kumar Munipalli, Raman Mohan Singh, Sayali Warde, Validated RP-HPLC Method for Determination and Quantification of Nintedanib in Pharmaceutical Formulation, Journal of Advancement in pharmacology, 2020;1(1):38-47.

[3] Kohei Togami, Kenta Fukuda, Kotaro Yamaguchi, Sumio Chono, Hitoshi Tada, Facile and sensitive HPLC-UV method for determination of Nintedanib in rat plasma, International Journal of Pharmacy and Pharmaceutical Sciences,2018;10(6):133-137.

[4] Rohan S. Patil, Moreshwar P. Mahajan, Development and Validation of RP-HPLC Method for the Estimation of Nintedanib Esylate in Bulk and Its Formulation, A Journal of Pharmaceutical Science,2015;7(8):774-782.

[5] Santosh. A. Waghmare, M. Sumitra, QbD Based Development and Validation of RP-HPLC Method for Nintedanib Esylate: Application to Bioanalytical and Stability Study in Plasma, Analytical Chemistry Letters, 11 (3) 2021, 392 – 408.

[6] P. Ravi Sankar, A. Bhavani Sailu, M. M. Eswarudu, M. Nithya Satya, P. Sreeja, P. Roja, Sk. Rijwana, Analytical Methods for Determination of Different Members of FDA approved Tyrosine Kinase Inhibitors Like Dasatinib, Lapatinib, Imatinib, Sorafenib, Nintedanib, Sunitinib, and Pazopanib: A Review, Journal of Pharmaceutical Sciences & Research, Vol. 13(6), 2021, 313-318.

[7] Debanchal Dutta, Soumyajit Das, Manik Ghosh, Validated HPTLC Method for the Determination of Nintedanib in Bulk Drug, MDPI,2019;9(1):22.

[8] Vivek Dhiman, Ankit Balhara, Saranjit Singh, Shristy Tiwari, Samanthula Gananadhamu, M. V. N. Kumar Talluri, Characterization of stress degradation products of Nintedanib by UPLC, UHPLCQ-TOF/MS/MS and NMR: Evidence of a degradation product with a structure alert for mutagenicity, Journal of Pharmaceutical and Biomedical Analysis, 2021;199:114037.

[9] Snehal K Shukla, Hossam Kadry, Jayshil A Bhatt, Rasha Elbatanony, Fakhrul Ahsan, Vivek Gupta, Statistical optimization and validation of a novel ultra-performance liquid chromatography method for estimation of Nintedanib in rat and human plasma, Bioanalysis, 2020;12(3): 159–174.

[10] Hany W. Darwish, Mohamed W. Attwa, Adnan A. Kadi, Rapid validated liquid chromatographic method coupled with Tandem mass spectrometry for quantification of Nintedanib in human plasma, Journal of Pharmaceutical Research,2016; 15 (11): 2467- 2473.

[11] Ravisankar P, Naga Navya Ch, Pravallika D, Navya Sri D, A review on step-by-step analytical method validation, IOSR Journal of Pharmacy.2015;5:7-19.

[12] Ravi Sankar P, Sai Snehalatha K, Tabassum Firdose Shaik, Srinivasa Babu P, Applications of HPLC in Pharmaceutical Analysis. Int.J.Pharm.Sci.Rev.Res.2019; 59(1): 117-124.

[13] Panchumarthy Ravisankar, Anusha S, Supriya K, Ajith Kumar U, Fundamental chromatographic parameters, Int. J. Pharm. Sci. Rev.Res.2019; 55(2):46-50.

[14] Ravisankar P, Gowthami S, Devala Rao G, A review on analytical method development, Indian journal of research in pharmacy and Biotechnology, 2014;2:1183-1195.

[15] ICH Q2 (R1), Validation of analytical procedures, Text, and methodology, International Conference on Harmonization, Geneva, 2005;1-17.