Research Article

Development and Validation of RP-HPLC Method for the Determination of Nintedanib in Pharmaceutical Dosage Form

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Received: 04-03-2023; **Revised:** 22-05-2023; **Accepted:** 30-05-2023; **Published on:** 15-06-2023.

ABSTRACT

A simple, reliable, sensitive, precise, rapid, and reproducible RP-HPLC method was developed and validated for the determination of Nintedanib in the pharmaceutical dosage form. Separation was achieved under optimized chromatographic conditions on a Poroshell C18 isocratic column, (4.5 mm i.d. X 150 mm, 3.5 µm particle size maintained at ambient temperature). The mobile phase consisted of methanol, and acetonitrile in the ratio 50:50 v/v, isocratic elution at a flow rate of 1 mL/ min at ambient temperature, and using a PDA detector to monitor the elute at 379.5 nm. The retention time of Nintedanib was found to be 1.239 min and the calibration curve was a linear function of the drug in the concentration range of 2-10 ppm (r^2 = 0.9999). The limit of detection and the limit of quantitation was found to be 0.039911843 µg/mL and 0.120944979 µg/mL respectively. The recovery (accuracy) studies were performed and the percentage recovery was found to be 98.06 - 99.32 % w/w. The proposed method was validated as per ICH guidelines. Thus, the developed reversed-phase HPLC method was found to be feasible for the determination of Nintedanib in pharmaceutical formulations.

Keywords: RP - HPLC, Nintedanib, Validation, ICH guidelines.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2023.v80i02.008

DOI link: http://dx.doi.org/10.47583/ijpsrr.2023.v80i02.008

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). A survey of literature revealed that few chromatographic, spectrophotometric, and hyphenated methods are reported for the determination of Nintedanib individually and in combination. HPLC [1-6], HPTLC[7], UPLC [8-9], and LC-MS/MS [10], in biological fluids like human and rat plasma. However, a few HPLC methods have been reported hitherto for the determination of Nintedanib in bulk and pharmaceutical dosage forms. In this communication, we report a simple, accurate, precise RP-HPLC method for the estimation of Nintedanib in pharmaceutical dosage forms.

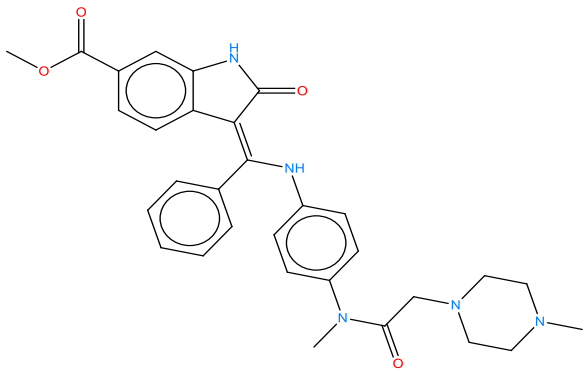

Figure 1: Chemical Structure of Nintedanib

MATERIALS AND METHODS

Instruments:

HPLC was performed on Waters (Alliance) 2695, Poroshell C_{18} column (4.5×150 mm,3.5 µm) (Based on 99.999 % ultra-high purity silica). The HPLC system was equipped with "Empower Pro" software. In addition, a Weighing balance (ESSAE VIBRA AJ (0.001g), ESSAE-Teraoka Ltd, digital pH meter (ELICO LI 120, ELICO India Ltd., a sonicator (spectra lab, model UCB 40),

UV-Visible Spectrophotometer (Systronics model-2203) were used in this 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.

Chromatographic conditions:

Table 1: Optimized Chromatographic Conditions

Parameter	Chromatographic conditions
Instrument	Waters (Alliance) 2695,
Column	Poroshell C_{18} column (4.5 mm i.d. X 150 mm, 3.5 µm particle size) (based on 99.999 % ultra-high purity silica)
Detector	Photo Diode Array Detector.
Mobile phase	Methanol: Acetonitrile (50:50% v/v)
Flow rate	1.0 mL/minute
Detection wavelength	UV at 379.5 nm
Run time	5 minutes
Coloum Temperature	29 °C
The volume of the injection loop	50 µL
Retention time (R_t)	1.239 minutes
Theoretical plates (th.pl) (efficiency)	4948
Theoretical plates per meter (t.plm)	1,36,960
Tailing factor (asymmetry)	1.12

Preparation of reagents and standards:

Preparation of Mobile phase:

The mobile phase consisted of a mixture of Methanol: Acetonitrile (50:50 % v/v) and was filtered through a 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of Stock and standard working solutions:

Accurately 100 mg of pure NINT was weighed and transferred into a 100 mL clean and dry volumetric flask, and the mobile phase was added, if necessary, sonicate to dissolve. The volume was brought up to the mark with the mobile phase. This is the primary stock solution of NINT with a concentration of 1000 ppm. The secondary stock solution is prepared by adding 1 mL of primary stock solution in a 100 mL volumetric flask and making up the volume with Methanol: Acetonitrile (diluent) having a concentration range of 10 ppm. Five standard working solutions were prepared for the calibration graph by adding defined volumes of the secondary stock solution and diluting with the mobile phase. The concentrations of NINT and 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm respectively. The linearity data of Nintedanib was shown in table 2.a and the calibration plot was shown in figure 2.

Table 2.a: Linearity data of Nintedanib by HPLC

Concentration (ppm)	Retention time, (R_t)	Peak area, mAU
0	-	0
2	1.229	296081
4	1.239	600692
6	1.239	901288
8	1.238	1201884
10	1.239	1501980

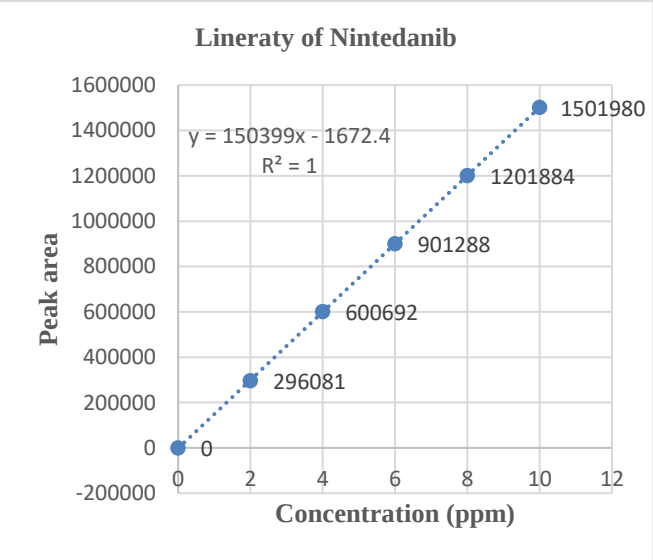

Figure 2: Calibration graph of Nintedanib by RP- HPLC

Table 2.b: Linear regression data of Nintedanib by RP-HPLC

Parameter	Method
Detection wavelength (ƛmax)	379.5 nm
Linearity range (ppm)	2-10
Regression equation (y=mx+c)	150339x -1672.4
Slope (b)	150339x
Intercept (a)	1672.4
Standard error of estimation (S_e)	1818.274
Correlation coefficient (r^2)	0.9999
Standard error of slope ($S_{b)}$	217.325
Standard error of intercept (S_a)	1315.970

Sample preparation:

Accurately weigh not less than twenty capsules, and the average was calculated. The NINT capsules were opened to get homogeneous powder. Weigh an amount of capsule powder 100 mg of NINT accurately and transfer it into a 100 mL volumetric flask. The 20 mL mobile phase was

added and placed in an ultrasonicator bath until dissolution was completed. The mobile phase was added to bring up the volume to the mark. From the above solution, pipette out 1.0 mL of the sample solution into a 100 mL volumetric flask and dilute with the mobile phase up to the mark mix well. This solution was filtered through a 0.45 µm nylon filter and degassed by sonication. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the sample solution was loaded in the injection port's 20 µL fixed sample loop.

Validation of the developed method:

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired product meeting its pre-determined specifications and quality characteristics. The method was validated as per ICH Q2 (R1) guidelines [11-14]

System suitability

Table 3: System suitability parameters

System suitability parameters	Nintedanib
Tailing factor (T)*	1.12
Number of Theoretical Plates	4948
Theoretical plates per meter (N)*	1,36,960
Retention time	1.239 min
SD for peak area and Retention time	0.00238
% RSD (For peak area) and Retention time	0.16658

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation have been completed. The system suitability parameters like theoretical plates, retention time, and tailing factor, were studied and found satisfactory.

Linearity

The linearity of the method is a measure of how well a calibration plot of response versus concentration approximates a straight line. The linearity of the method was confirmed over the concentration range of 2-10 ppm. A series of dilutions were prepared by using the working standard solutions. From the working standard solutions, 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were pipetted out into a 10 mL volumetric flask and diluted with Methanol: Acetonitrile and finally makeup to the volume with the diluent. The resulting solutions were labeled as 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. The calibration curves were constructed by plotting peak area versus concentration, and the linearity was calculated by the least square regression method. The calibration curve is shown in figure 2. The linearity data of NINT is presented in table 2.b.

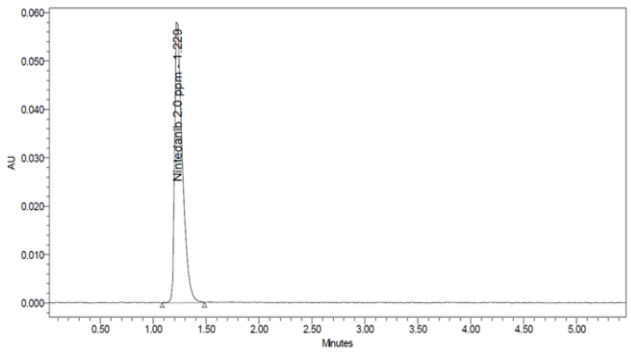

Figure 3.a: Standard chromatogram of (2 ppm)

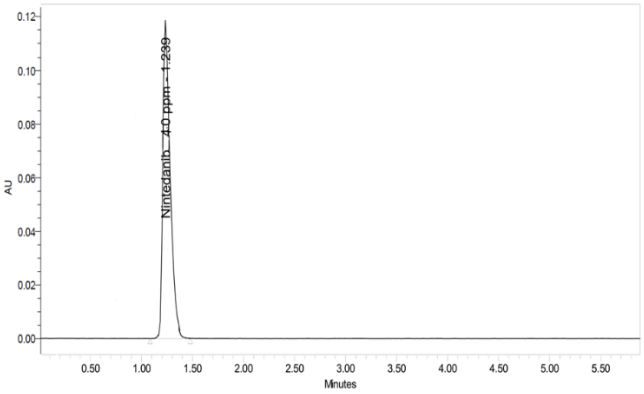

Figure 3.b: Standard chromatogram of Nintedanib (4 ppm)

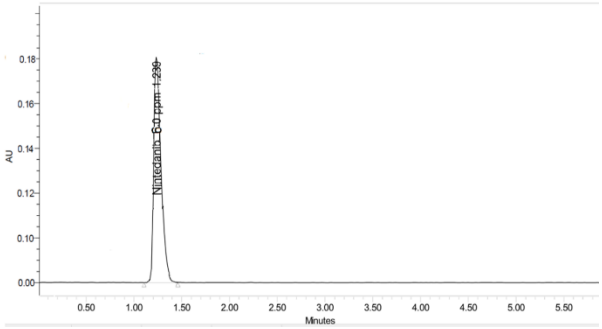

Figure 3.c: Standard chromatogram of NINT (6 ppm)

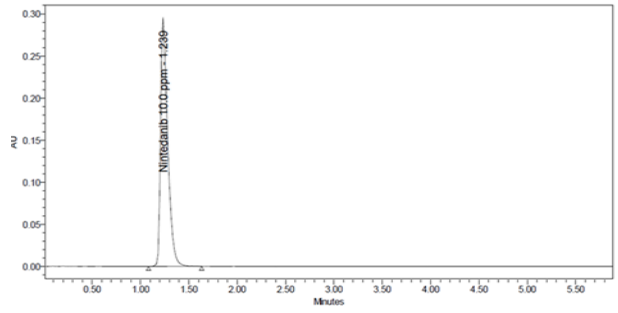

Figure 3.d: Standard chromatogram of NINT (8 ppm)

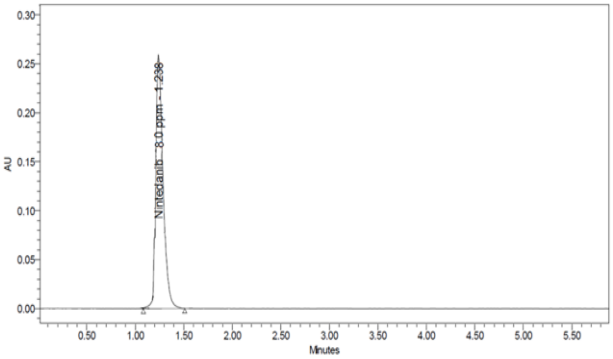

Figure 3.e: Standard chromatogram of NINT (10 ppm)

Table 4.a: Column performance and result table (2 ppm)

Area	Area percent	Height	Retention time	Asymmetry factor	Theoretical plates (USP)
296081	100 %	57954	1.229	1.174	4948

Table 4.b: Column performance and result table (4 ppm)

Area	Area percent	Height	Retention time	Asymmetry factor	Theoretical plates (USP)
600692	100 %	117856	1.239	1.150	4998

Table 4.c: Column performance and result table (6 ppm)

Area	Area percent	Height	Retention time	Asymmetry factor	Theoretical plates (USP)
901288	100 %	176774	1.239	1.113	4321

Table 4.d: Column performance and result table (8 ppm)

Area	Area percent	Height	Retention time	Asymmetry factor	Thel. plates (USP)
1201884	100 %	247822	1.238	1.111	5698

Table 4.e: Column performance and result table (10 ppm)

Area	Area percent	Height	Retention time	Asymmetry factor	Theoretical plates (USP)
1501980	100 %	294640	1.239	1.17478	6945

Precision

System precision

Six replicate recordings of peak area at 379.5 nm of 6 ppm concentration standard solution showed a % relative standard deviation (% RSD) less than 2, which indicates reproducibility and thereby the precision of the system.

Method precision (intraday and Interday)

Method precision was determined by performing the assay of a sample under tests of repeatability (Intra-day precision) shown in table 5.b. and intermediate precision (Inter-day precision) is shown in table 5.c. Performing for two consecutive days by two analysts, at different working concentrations. The percentage relative standard deviation (% RSD) was calculated, which is within the acceptable criteria of not more than 2.0.

Table 5.a: Results of system precision:

Injection No.	Area Response
1	901288
2	901290
3	901266
4	901300
5	901580
6	901280
Mean	901334
Standard deviation	121.0488
% Relative standard deviation	0.01342996

Table 5.b: Results of Intra-day precision

Sample	Injection no.	% Assay	Mean	SD	% RSD*
Nintedanib	1	99.8	99.75	0.104881	0.1051
	2	99.7			
	3	99.7			
	4	99.9			
	5	99.8			
	6	99.6			

*Average of six determination

Table 5.c: Results of Inter-day precision

Sample	Injection no.	% Assay	Mean	SD	%RSD
Nintedanib	1	99.8	99.78	0.098319	0.09853
	2	99.9			
	3	99.7			
	4	99.7			
	5	99.7			
	6	99.9			

*Average of six determinations.

Accuracy (Recovery studies):

The accuracy of the method was evaluated by the standard addition method. In this method, the volume of the test solution was taken as constant and standard Nintedanib was added in increasing amounts equivalent to 50 %, 100 %, and 150 % levels to each test solution. A known amount of standard NINT of 6 ppm concentration was added to the pre-analyzed samples in triplicate solutions. The percent recovery of the triplicate solutions was determined an average of the percent recovery was calculated. The results were presented in table 6.

Table 6: Results of the Accuracy study

S.No	Concentration % of spiked level	Mean % Recovery	% RSD
1	50 %	99.65	0.452
2	100 %	98.06	1.403
3	150 %	99.32	0.432

*Average of triplicate injections - Acceptance Criteria at each level should be 98.0-102.0 %

Study design:

Precision at different levels of the analytical method was determined in the concentration range of 50 %, 100 %, and 150 %. %RSD of the area of six replicates of linearity solution at 50 %, 100 %, and 150 % levels is less than 2.0. Hence precision at different levels of the method is established

Specificity

The specificity of the method was evaluated by assessing whether excipients and other additives that are usually present in pharmaceutical formulations of NINT do not interfere with the peaks of the analyte under optimum conditions. Specificity is shown in table 7.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and indicates its reliability during normal usage. For the determination of a method's robustness, parameters such as variation in detector wavelength are varied within a realistic range, and the quantitative influence variable is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. The absorbance was measured, and the assay was calculated six times. The results of robustness are presented in table 8.

Table 7: Results of Specificity study for Nintedanib

Name of the solution	Retention (R_t) minutes
Mobile Phase (blank)	No Interference at the Retention time of the analyte peak
Placebo	No Interference at a Retention time of the analyte peak
Nintedanib 10 ppm (Sample)	1.239 min

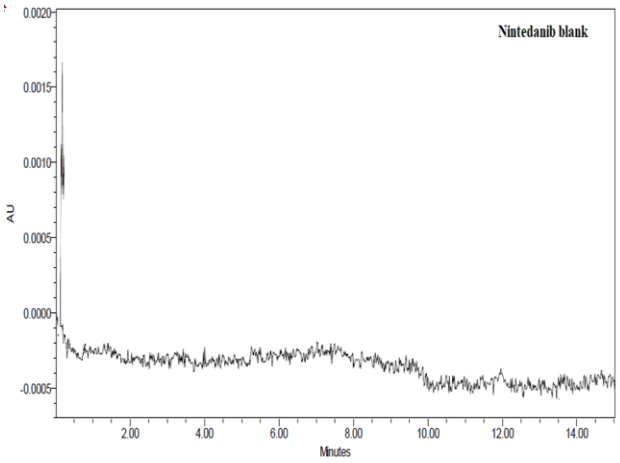

Figure 4: NINT Placebo chromatogram

LOD & LOQ:

The limit of Detection is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. The limit of quantification is the lowest concentration of the analyte in a sample that can be determined with acceptable precision and accuracy. The LOD&LOQ is shown in table 9.

Table 8: Robustness results of NINT

Parameters	Optimized	Used	Retention time (min)	Plate count $	Peak asymmetry #	Remarks
Flow rate (± 0.2 mL/min)	1.0 mL/min	0.8 mL/min	1.290 min	6980	1.12	*Robust
		1.0 mL/min	1.239 min	6945	1.174	*Robust
		1.2 mL/min	1.198 min	6650	1.16	*Robust
Detection wavelength (± 5 nm)	379.5	374.5 nm	1.235 min	6935	1.15	*Robust
		379.5 nm	1.239 min	6945	1.174	*Robust
		384.5 nm	1.250 min	6950	1.16	*Robust
Mobile phase composition MeOH: ACN (50:50 % v/v)	50:50 v/v	55:45 v/v	1.245 min	6940	1.180	*Robust
		50:50 v/v	1.239 min	6945	1.174	*Robust
		45:55 v/v	1.230 min	6950	1.165	*Robust

Acceptance criteria (Limits): #Peak asymmetry<1.5, $Plate count >2000, *Significant change in retention time

Table 9: LOD & LOQ results of NINT by HPLC

Limit of Detection (LOD)	0.039911843 µg/mL
Limit of Quantification (LOQ)	0.120944979 µg/mL

Assay of Nintedanib in capsules:

Twenty capsules of NINT marketed formulation was weighed and powdered. A quantity of capsule powder of Nintedanib 100 mg equivalent to 100 mg of Nintedanib was accurately weighed and transferred into a 100 mL volumetric flask containing a 20 mL mobile phase. The solution was sonicated for extracting the drug for about 15 minutes, filtered through a Whatman filter paper (0.45 µm) nylon filter, and added mobile phase to get the volume up to the mark. Transfer 1 mL of filtered sample solution to a 50 mL volumetric flask and made up to volume with the mobile phase to get 5 ppm solution. The absorbance of the resulting solution was measured at 379.5 nm, and the amount of NINT was computed from its calibration plot. The Nintedanib capsules were analyzed using the developed method. The assay results were compiled and found satisfactory, and the results of the analysis matched with a percent label claim of marketed NINT capsules. The mean percentage found and the % RSD values in the table showed that the proposed method can be adopted for the determination of Nintedanib in pharmaceutical capsules. The representative sample chromatogram of NINT is shown in figure 5.

Table 10: Assay results of NINT by HPLC

S.No	Formulation	Labelled claim	Amount found	* Mean % recovery ±SD	% RSD
1	**Nintib**	**100**	**98.97**	**98.97±0.002**	**0.00202**

*Average six determinations, SD: Standard deviation, RSD: Relative standard deviation

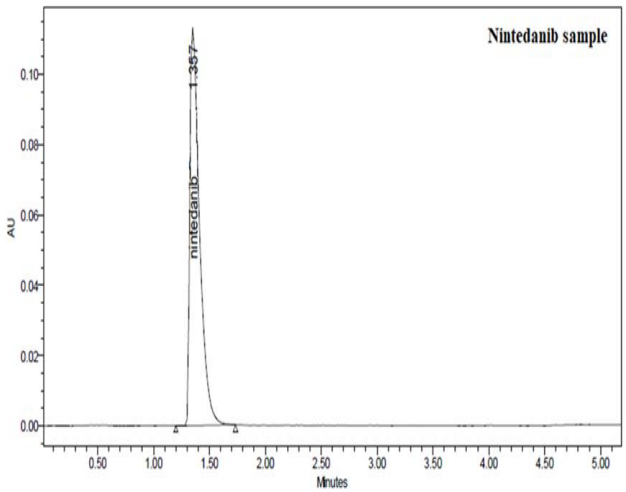

Figure 5: NINT sample chromatogram

RESULTS AND DISCUSSION

In HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried to separate title ingredients.

The objective of this study was to develop a rapid and sensitive RP-HPLC method for the analysis of NINT in bulk drug and pharmaceutical dosage forms by using the most commonly employed C_{18} column with PDA detection. Initially, various mobile phase compositions were tried to elute the drug. Mobile phase ratio and flow rate were selected based on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor), run time, and resolution. The system with Methanol: Acetonitrile (50:50 v/v) and 1mL/min flow rate were selected. The retention time for NINT was found to be 1.239 min The calibration was linear in the concentration range of 2 –10 ppm for

NINT. The specificity of the chromatographic method was tested by injecting sample concentration prepared from the marketed formulation. The response was compared with that obtained from the standard drug. The chromatogram confirms the presence of NINT at 1.239 min without any interference. Precision was evaluated using six replicates of the solution, which were prepared and analyzed. Method validation following ICH guidelines indicated that the developed method had high sensitivity with LOD of 0.039911843 µg/mL and LOQ of 0.120944979 µg/mL. The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters such as a change in flow rate to 1.0 mL and changing detection wavelength 379.5 nm and mobile phase composition change to 50:50 ratio of MeOH: ACN. These values indicated that the method was quite robust.

The proposed method was validated in accordance with ICH parameters and was applied for the analysis of the same in marketed formulations. The content of each component in the formulation was estimated by comparing the peak area of the test sample with that of the peak area of the standard, which is found to be 98.97 ± 0.002 % w/w. The development of an analytical method for the determination of drugs by Reverse Phase HPLC has received considerable attention in recent years because of their importance in the quality control of drugs and drug products. Hence, the developed RP-HPLC method can be adopted for the routine analysis of NINT in the pharmaceutical dosage form in quality control laboratories.

CONCLUSION

This paper describes the first RP-HPLC method developed and fully validated for the quantitative determination of Nintedanib in pharmaceutical dosage forms. Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. The method was completely validated shows satisfactory results for all the method validation parameters tested and the method was free from the interference of the other active ingredients and additives used in the formulation. In fact, results of the study indicate that the developed method was found to be Rapid, simple, reliable, accurate, linear, selective, sensitive, economical, reproducible, has a short run time, and only requires low-cost technology which makes this method an economical alternative for most clinical laboratories. Hence it can be concluded that this method may be employed for the routine quality control analysis of Nintedanib in active pharmaceutical ingredient API and pharmaceutical preparations.

ACKNOWLEDGMENT

The authors thank Hetero Labs Limited, Jeedimetla, Hyderabad for providing Nintedanib as a gift sample for this work. They also thank chairman Dr. L. Rathaiah, Vignan Pharmacy College, and, P. Srinivasa Babu, Principal of Vignan Pharmacy College for providing the necessary facilities to carry out this research work.

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Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.