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Development and Validation of an RP- HPLC Method for Determination of PulmonaryKinase Inhibitors Drug Nintedanib in Bulk and Tablets

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ABSTRACT
Nintedanib is a small molecule that targets multiple receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (nRTKs). Nintedanib inhibits the following RTKs: platelet-derived growth factor receptor (PDGFR) α and β, fibroblast growth factor receptor (FGFR) 1-3, vascular endothelial growth factor receptor (VEGFR) 1-3, and Fms-like tyrosine kinase-3 (FLT3). The new sensitive and rapid RP-HPLC method was developed for the determination of Nintedanib in bulk and pharmaceutical dosage forms; it was validated according to ICH and FDA guidelines. The RP-HPLC analysis was performed on the Thermo 2080 systemequipped with a Scientific ARP-C18 (250 mm X 4.6 mm), 5µ column, with a mixture Acetonitrile: water (80:20 % v/v) as the mobile phase, at the flow rate of 1.0 mL/min. Detection was performed at the wavelength (λ) of 210 nm, and the retention time of Nintedanib was found to be 4.42 min. The total run time was 10 min. The calibration plot gave linear relationship over the concentration range of 20-100 µg/ml. The LOD and LOQ were 4 and 12.5ng/ml, respectively. The accuracy of the proposed method was determined by recovery studies and was found to be 99.93%. Repeatability testing for both standard and sample solutions showed that the method is precise within the acceptable limits. RSD% of the determination of precision was <2%. The results of robustness and solutions stability studies were within the acceptable limits as well. The proposed method showed excellent linearity, accuracy, precision, specificity, robustness, LOD, LOQ, and system suitability results within the acceptance criteria. In addition, the main features of the developed method are low run time and retention time around 4.42 min.

Key Words: RP-HPLC, Nintedanib, Repeatability, Pharmaceutical, Method development.

I. INTRODUCTION:

Various medicines are introduced into the world-market and also, that is growing every year. As a result, evaluation of quality and efficacy of these medicines are important Right from the beginning of discovery of each medicine[1]. Active pharmaceutical substances and the secondary pharmaceutical product(s) i.e. the dosage forms having each single or multi-component formulated product are needed to be investigated. The validation of an analytical method is generally used to demonstrate the method is suitable for the intended use[2]. The use of analytical methods during drug development and manufacturing gives information as potency, impurities, drug characteristic such as crystal form, drug release, and drug uniformity, degradation product etc. Nintedanib is a small molecule that targets multiple receptor tyrosine kinases (RTKs) and non-receptor tyrosine kinases (nRTKs). Nintedanib inhibits the following RTKs: platelet-derived growth factor receptor (PDGFR) α and β, fibroblast growth factor receptor (FGFR) 1-3, vascular endothelial growth factor receptor (VEGFR) 1-3, and Fms-like tyrosine kinase-3 (FLT3)[3]. The analytical way deals with quality standards which are assigned for products so to have desirable efficacy of the medicines. It is assumed that drug/medicine complying with those standards is having desired effect on use. The decision to release or reject a product is based on one or more type of control action[4].

Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision,

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detection, and quantitation limits. According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines[5].

In this research, a new sensitive and rapid RP-HPLC method was developed for the determination of Nintedanib in pharmaceutical dosage forms, and this method was validated according to ICH and FDA guidelines.

II. MATERIALS AND METHODS:

Instrumentation: Thermo 2080 system 4000 Quaternary pump, UV 6000 PDA detector with a Scientific ARP-C18 (250 mm X 4.6 mm), 5µ column.

Chemicals and Reagents: Pharmaceutical grade sample of Nintedanib was obtained from Thermosil fine chem, Pune. Ofev (Nintedanib) capsules 100 mg were purchased from the local market. Acetonitrile HPLC grade and other chemicals obtained from Thermosil Fine Chem, Pune.

Chromatographic Conditions: Acetonitrile: Water (80:20 % v/v) mixture is prepared and filtered through 0.45 µ membrane filter and then degassed. The analyteswere conducted on an analytical column ARP-C18 (250 mm X 4.6 mm), 5µ. With a detection wavelength of 210 nm. Theoperating temperature of the column was set at 25°C. Injection volume was 20µL, and the flow rate was maintainedat 1.3mL/min. The run time was 4.42 minutes.

Preparation of Standard Solution

A standard solution of Nintedanibwas prepared by dissolving an accurately weighed amount of Nintedanib100 mg in 100 ml of the mobile phase, and then 1 mL of the resulting solution was diluted to 100 mL by the same solvent to obtain a standard solution of Nintedanib.

Sample preparation of Nintedanib

Twenty tablets were weighed and finely powdered. The powder equivalent to 100 mg of Nintedanib was accurately weighed and transferred to volumetric flask of 100 ml capacity containing 50 ml of the Acetonitrile: water (80:20 % v/v) and sonicated for 30 min. This solution was carefully filtered through Whatman filter paper and the final volume was made with Acetonitrile: water (80:20 % v/v) to get the solution of 1000 mcg/ml. From this solution, 10ml was taken in 100 ml standard volumetric flask and diluted to 100 ml with Acetonitrile: water (80:20 % v/v) to give a solution of 100 mcg / ml.The prepared solution was filtered through 0.45 µm membrane filters.

Method Validation

The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy, precision, sensitivity (LOQ and LOD), and robustness.

Specificity: Specificity is one of the significantly features of HPLC, and it refers to the ability of the analytical method to discriminate between the analyte and the other components in the complex mixture. Specificity of the method was evaluated by injecting 10 µl solutions of standard, sample, blank, and placebo separately.

Linearity: To evaluate the linearity and range of the method, different standard solutions were prepared by diluting the standard stock solution with the mobile phase in deferent concentrations of Nintedanib: 20, 40, 60, 80 and 100 µg/ml. Linear regression analysis was used to evaluate the linearity of the calibration curve by using the least square linear regression method.

Sensitivity: Limit of detection (LOD)/limit of quantitation (LOQ) of Nintedanib were determined by analyzing different solutions of Nintedanib and measuring the signal-to-noise ratio. The limit of detection (LOD) is the concentration that gives a signal-to-noise ratio of approximately 3:1, while the limit of quantification (LOQ) is the concentration that gives a signal-to-noise ratio of approximately 10 :1 with %RSD of less than 10%.

Accuracy: The accuracy of the assay method was determined by recovery studies at three concentration levels (50%, 100%, and 150%), i.e., 30, 60, and 90 µg/ml, and three samples from each concentration were injected. The percentage recovery of added Nintedanib and RSD were calculated for each of the replicate samples.

Precision: TheIntermediate precision and Repeatability precision of the proposed methods were determined by several measurements of standard solution and sample solution, respectively. System precision was established by ten measurements of the standard solution at the 100% concentration levels on the same day. Method precision was established by six assay determinations of the sample solution at the 100% concentration levels on the same day. The RSD of obtained results was calculated to evaluate repeatability results.

Robustness: Robustness of the method was verified by applying minor and deliberate changes in the experimental parameters, for example: (i) Column

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temperature: ±5° C (ii) Flow rate: ±0.2 mL/min (iii) Wavelength: ±3 nm (iv) Mobile phase composition, organic composition ±5%Change was made to evaluate its effect on the method. Obtained data for each case was evaluated by calculating % RSD and percent of recovery.

III. RESULTS AND DISCUSSION

Method Development and Optimization

Several physical and chemical properties of Nintedanib were obtained from the literature. The analytical method was developed to select preliminary reversed phase HPLC chromatographic conditions, including detection wavelength, mobile phase, stationary phase, and sample preparation procedure. For this purpose, a series of trials were performed by varying the ratio of acetonitrile and water and optimizing the chromatographic conditions on the Thermo 2080 system 4000 Quaternary pump, UV 6000 PDA detector with a Scientific ARP-C18 (250 mm X 4.6 mm), 5µ column. The results of method optimization are summarized in Table 1. The mobile phase consisting of acetonitrile and Water in the ratio 80 : 20 v/v with a flow rate of 1.3 mL/min, injection volume 20 µl, run time 4.42 min, and column temperature 25°C at wavelength (λ) 210 was optimized as the best chromatographic conditions for the entire study where Nintedanib was eluted forming symmetrical peak shape, resolution and suitable analysis time with retention time about 4.42 min.

Method Validation

Specificity

The chromatograms of standard and sample are identical with nearly same retention time. No interference due to placebo at the retention time of analyte which shows that the method was specific. The chromatograms for specificity studies (standard, placebo and blank) are in Figure No. 1,2 and 3.

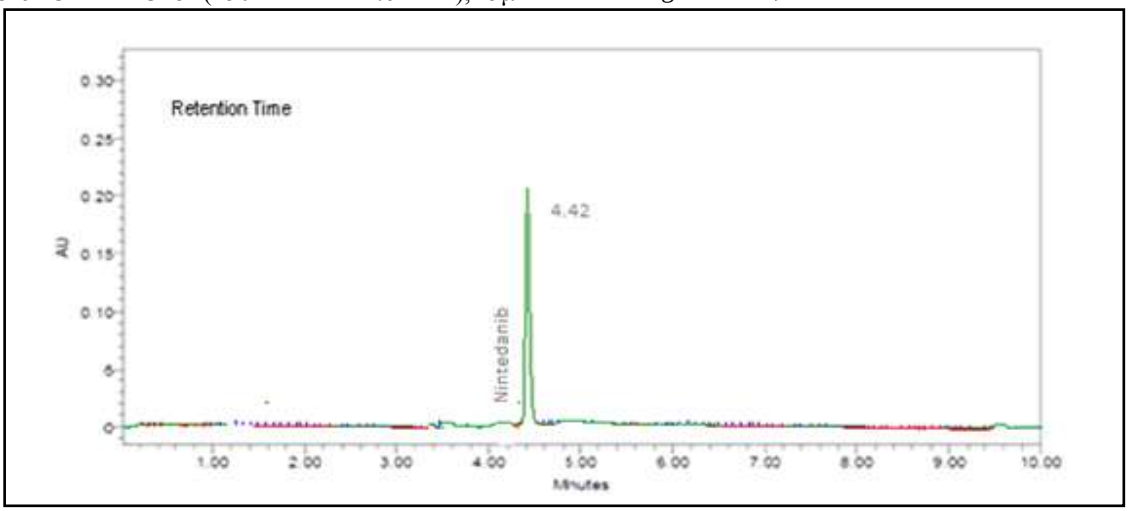

Figure No. 1: Standard chromatogram of Nintedanib

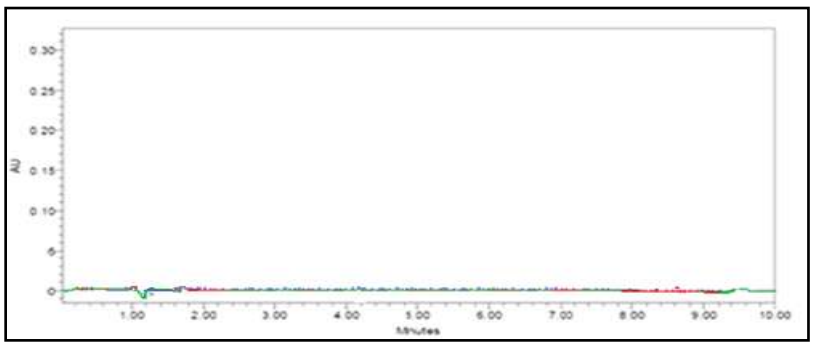

Figure No.2: Chromatogram for placebo interference

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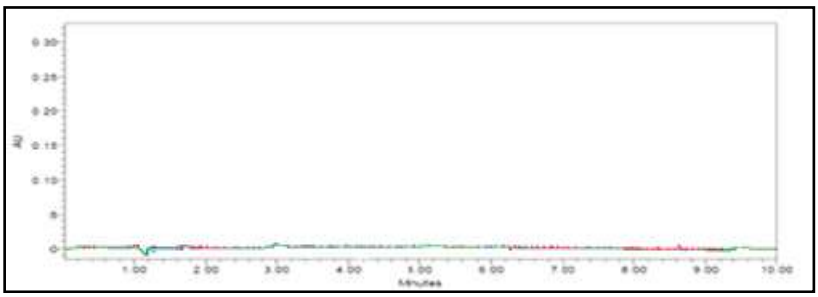

Figure No. 3: Chromatogram for blank interference

Chromatogram of blank and placebo is not showing any peak at the retention time of analyte peak. There is no interference due to blank and placebo at the retention time of analyte. Hence the method is specific.

Linearity

Appropriate volume from the stock solution was diluted to get the final concentration of 20,40,60,80 and 100µg/mL for Nintedanib. Calibration range was observed in the concentration range of 20 to 100 µg/ml for Nintedanib (r^2= 0.998). Chromatograms for the linearity are shown in Figure No.4. The data for linearity is tabulated in Table No.1.

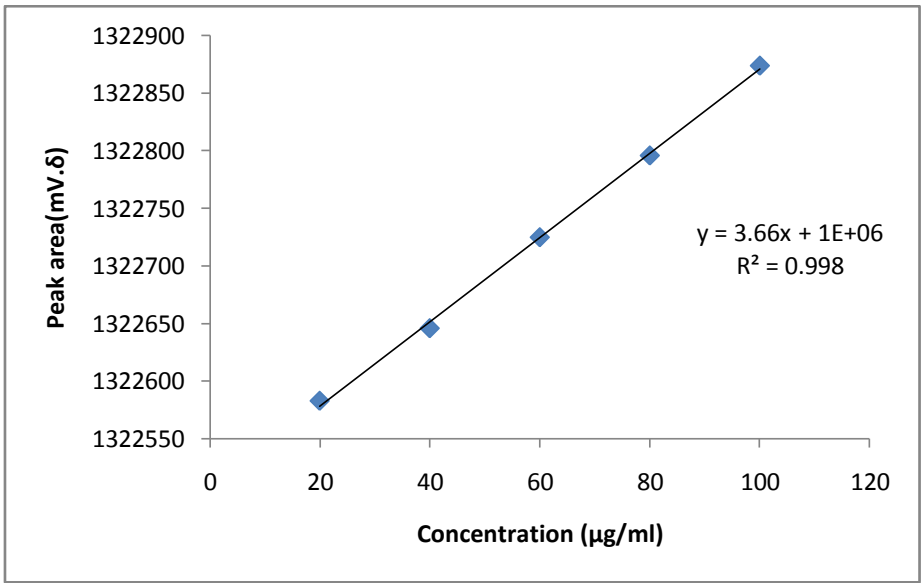

Figure No.4: Linearity curve for Nintedanib

\For the Nintedanib, Linearity was found with the concentration range of 20-100 ppm and line of equation found to be y = 3.66x + 1E+06 with R^2 value 0.9988, indicates the method is linear**.**

Table No. 1: Linearity results for Nintedanib by RP-HPLC

Sr. no	Linearity level	Concentration (µg/ml)	Peak area(mV.δ)
1	I.	20	1322583
2	I.	40	1322646
3	I.	60	1322725
4	I.	80	1322796
5	I.	100	1322874

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Correlation Coefficient: 0.9988

Accuracy (% Recovery)

The recovery experiment was performed by the standard addition method. The mean recoveries were found to be 99.3 ± 0.21. The recovery result indicates that the proposed method is accurate.

Table No.2: Accuracy results for Nintedanib by HPLC

Sample No.	Spike Level	% Recovery	Mean % Recovery
1	50%	99.8%	100.2%
		99.4%	
		99.0%	
2	100%	99.3%	99.3%
		99.6%	
		99.1%	
3	150%	99.0%	99.2%
		99.2%	
		99.5%	

Method Precision Repeatability

The RSD values of Nintedanib found to be 0.42 % at 210 nm Low values indicate that method is repeatable Table No.3.

Table No. 3: Repeatability test for HPLC method of Nintedanib

Injection No	Peak area(mV.δ)	% Recovery
1	1322833	99.4%
2	1322829	100%
3	1322832	99.0%
4	1322850	99.8%
5	132245	99.2%
Mean	1322837.8	99.48%
SD	**0.0080**	
%RSD	**0.42**	

Intermediate Precision: (Reproducibility)

The RSD values of Nintedanib are 0.42, which reveal that the method is precise.

Table No.4: Intermediate precision results for Nintedanib by HPLC

Parameter	% Assay
Mean	99.10
SD	0.00010
% RSD*	0.38

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Robustness

a) Effect of variation in flow rate

As the % RSD of retention time and asymmetry were within limits for variation in flow. Hence the allowable flow rate should be within 1.2 ml to 1.4 ml.Results are tabulated in Table no.5.

Table No. 5 : Robustness results for Nintedanib by HPLC (Change in flow rate)

Sr. No	Flow rate (ml/min)	Robustness results
		USP Tailing
1	1.2	1.53
2	1.3	1.48
3	1.4	1.48

b) Effect of variation in mobile phase concentration

As the % RSD of retention time and asymmetry were within limits for variation in mobile phase composition (± 2%). The results of robustness for effect of variation in flow rate are tabulated in Table No.6

Table No.6: Robustness results for Nintedanib by HPLC (Change in mobile phase composition)

Sr. No	Mobile phase composition	Robustness results
		USP Tailing
1	Mobile phase -2%	1.36
2	Mobile phase -2%	1.32

The method proved to be robust for the variations in the flow rate and mobile phase proportion assessed.

Sensitivity: Limit of detection (LOD)/limit of quantitation (LOQ) of Nintedanib were determined by analyzing different solutions of Nintedanib and measuring the signal-to-noise ratio. The limit of detection (signal-to-noise ratio ≥ 3) for Nintedanib was calculated to be 4 ng/ml. The value of the lower limit of quantification was found to be 12.5 ng/ml

IV. CONCLUSION:

In the present research, a fast, simple, accurate, precise, and linear RP- HPLC method has been developed and validated for Nintedanib, and hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for Nintedanib. In addition, the main features of the developed method are short run time and retention time was 4.42 min. The method was validated in accordance with ICH guidelines and also method is robust enough to reproduce accurate and precise results under different chromatographic conditions. The validation study shows that the developed method is accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient. RSD (%) and standard deviations which make it versatile and valuable for determination of Nintedanib in pharmaceutical dosage forms. From the comprehensive validation conducted, it was concluded that the method is stable and could be used throughout shelf life of the drug.

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