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STABILITY INDICATING HPTLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF NINTEDANIB ESYLATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, rapid, precise and accurate High-Performance Thin Layer Chromatography (HPTLC) method was developed and validated for the estimation of Nintedanib esylate, a novel tyrosine kinase inhibitor used in idiopathic pulmonary fibrosis, in bulk drug. HPTLC method was developed for estimation of Nintedanib esylate by using pre-coated silica gel aluminium plate $60F_{254}$ (20x20) cm with 250 µm thickness Methanol: Chloroform: Ammonia (6:4:0.1 v/v/v) as a mobile phase. The forced degradation study of Nintedanib esylate includes oxidation, alkali hydrolysis, acid hydrolysis, thermal conditions, and photolytic conditions. According to the degradation study, Nintedanib esylate is stable under hydrolysis, oxidative, thermal, and photolytic conditions but degrades significantly under acidic and alkaline environments. HPTLC method showed linearity from 100-500 ng/band and accuracy (% recovery) was found to be 100.21- 100.57 % with correlation coefficient 0.9984. The precision was found to be less than 2 % RSD. The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 2.71 ng/band and 8.23 ng/band respectively. The method has been validated as per ICH guidelines. The developed HPTLC method can be applied for the routine analysis in pharmaceutical industry.

Keywords: Nintedanib esylate, HPTLC, Degradation study, Assay, Method development

INTRODUCTION

Nintedanib esylate competitively inhibits both nonreceptor tyrosine kinases (nRTKs) and receptor tyrosine kinases (RTKs). NRTK targets of Nintedanib esylate include

Lck, Lyn, and Src. RTK targets of Nintedanib esylate include platelet-derived growth factor receptor (PDGFR) α and β; fibroblast growth factor receptor (FGFR) 1, 2, and 3; vascular endothelial growth factor receptor (VEGFR) 1, 2, and 3; and FLT3. Its use in IPF is predicated on its inhibition of PDGFR, FGFR, and VEGFR, which increase fibroblast proliferation, migration, and transformation **[1]**.

Nintedanib esylate is chemically known as Ethane sulfonic acid; Methyl (3Z) -3-[({4-[Nmethyl-2-(4-methylpiperazin-1-yl) acetamidol phenyl} amino) (phenyl) methylidene]- 2-oxo-2,3- dihydro-1H-indole-6-carboxylate, It's 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 **[2-3]**.

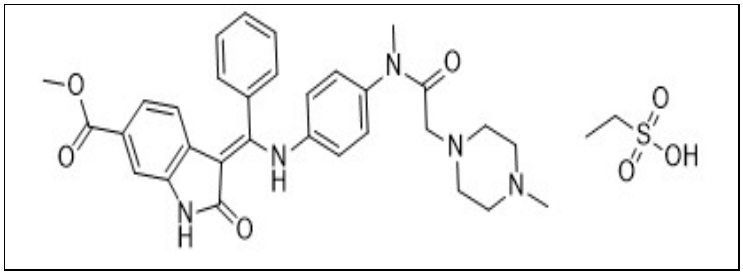

Figure 1: Structure of Nintedanib esylate

On literature survey it was found various method involving UPLC, LC-MS, UV, RP-HPLC and HPTLC methods are already reported for the estimation of Nintedanib esylate in bulk drug, formulation, rat plasma and human plasma **[4-17]**.

According to literature survey the method development approach specially focused on pharmaceutical development in stability indicating HPTLC method for Nintedanib esylate have not been widely discussed.

Therefore, there is an unmet need to develop a systemic analytical method development approach for pharmaceutical development using stability indicating method.

MATERIALS AND METHODS

Materials

Nintedanib esylate was received as gift sample from sun pharma, Vadodara, India.100 mg of Nintedanib esylate containing marketed formulation name as Nintib was procured from local market. Analytical grade chemicals and reagents were used for analysis.

Instrumentation

Pre-coated silica gel aluminium plate $60F_{254}$ (20x20 cm) with 250 µm thickness (E.Merck), Desaga-250 ùl dosing syringe (Hamilton), Desaga 200 ùl Applicator syringe (Hamilton), Desaga Applicator,

AS30win, Desaga Twin Through chamber 200x100 with stainless lid, Desaga TLC Scanner, Proquant Software, UV cabinet with dual wavelength UV lamp (254nm and 366nm)were used while performing the study.

Preparation of standard solution

Accurately weighed 10mg of quantity of Nintedanib esylate reference standard was transferred into 10 ml volumetric flask and dissolve in methanol and sonicated for about 2min with intermittent shaking and diluted up-to mark with methanol to give a stock solution having strength 1000µg/mL. From above stock solution transferred 1mL into 10mL and mark up to with methanol to give a working solution having strength 100µg/mL.

Mobile phase optimization

The mobile phase was optimized to Methanol: Chloroform: Ammonia in the ratio of 6:4:0.1after several trials.

Method validation

As per ICH guidelines Q2 (R1), the method validation parameters studied were solution stability, specificity, linearity, accuracy, precision, limit of detection and limit of quantification.

Linearity:

Calibration curve was plotted over a concentration range from 100-500 ng/band. The plate was developed in a developing chamber previously saturated with the mobile phase for 30 minutes. Each reading was the average of three determinations.

Accuracy:

Accuracy is the closeness of the test results obtained by the method to the true value. The accuracy of the method was determined by calculating recoveries of Nintedanib esylate by the standard addition method. Accuracy is performed at three levels 80, 100, 120 %. Calculated amount of Nintedanib esylate were added to a pre quantified tests solution of Nintedanib esylate. Each level was prepared in triplicate manner and each preparation was injected in duplicate.

Precision:

(a) Intra -day precision:

Intra-day precision was determined by analyzing of standard solution of Nintedanib esylate at three different concentration [200 ng/band,300 ng/band,400 ng/band] three time on same day (n =3) and % RSD for Nintedanib esylate was calculated.

(b) Inter -day precision:

Inter-day precision was determined by analyzing of standard solution of Nintedanib esylate at three different concentrations [200 ng/band,300 ng/band, 400 ng/band] over a period of 3 days (n = 3). The peak areas obtained were used to calculate mean and % RSD values.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ of the drug were calculated using equation according to ICH guidelines. LOD= 3.3 σ/s and LOQ=10σ/s were found.

Robustness:

The robustness was check by small changes chromatography condition like mobile phase (10 ± 0.5ml), saturation time (30 ± 5 min), solvent migration (80 ± 10 mm)

Analysis of marketed formulation:

From sample stock solution (100 µl), 10 µl was applied on the TLC plate and same procedure was followed as described in linearity.

Forced degradation studies:

Force degradation studies were performed in according to ICH guidelines and studies were carried out with API. Force degradation condition like acidic (0.1 N HCl reflux at 60˚ C for 8 hrs), basic (0.1 N NaOH reflux at 60˚ C for 8 hrs), peroxide (3% v/v H_2O_2 at room temperature for 6 hrs), thermal (at 100˚C for 24 hrs), photolytic (254 nm for 48 hrs) were applied to the drug sample.

RESULTS AND DISCUSSION:

Linearity:

The linearity of the method was investigation the concentration range of 100- 500 ng/band. And overlay chromatogram of Nintedanib esylate was shown in the **Figure 2**. The linearity and regression analysis data were shown in **Table 2**. The overlay chromatography and calibration curve shown in **Figure 3**.

Accuracy:

The accuracy study was carried out by the standard addition method. The percentage recovery was found in the range of 100.24-100.57 % **(Table 1)**. For Nintedanib esylate respectively, this indicates accuracy of the method.

Precision:

Precision is validated by studying the repeatability, inter-day and intra-day precision. The % RSD was found to be less than 2 for Nintedanib esylate was showed good precise of developed method and data was shown in **Table 2**.

Limit of detection and limit of quantification

The LOD and LOQ of the present method to be 2.71 and 8.23ng/band respectively.

Robustness

The deliberate change in performed mobile phase, solvent migration, saturation time and the results in terms of % RSD is less than 2, indicated good and satisfactory robustness of proposed method.

RESULT OF FORCED DEGRADATION STUDY

Optimized degradation conditions for Nintedanib esylate which are shown in **Table 3**. From degradation study it is found that Nintedanib Esylate is stable in case of oxidative, hydrolysis, thermal and photolytic condition while significant degradation found in case of acid and alkali hydrolysis.

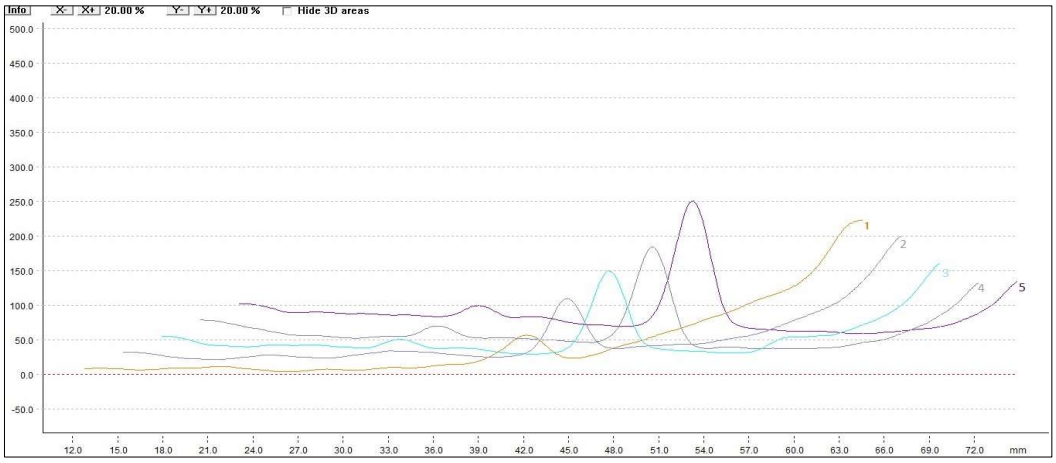

Figure 2: Overlay chromatogram of Nintedanib esylate standard (100–500ng/band)

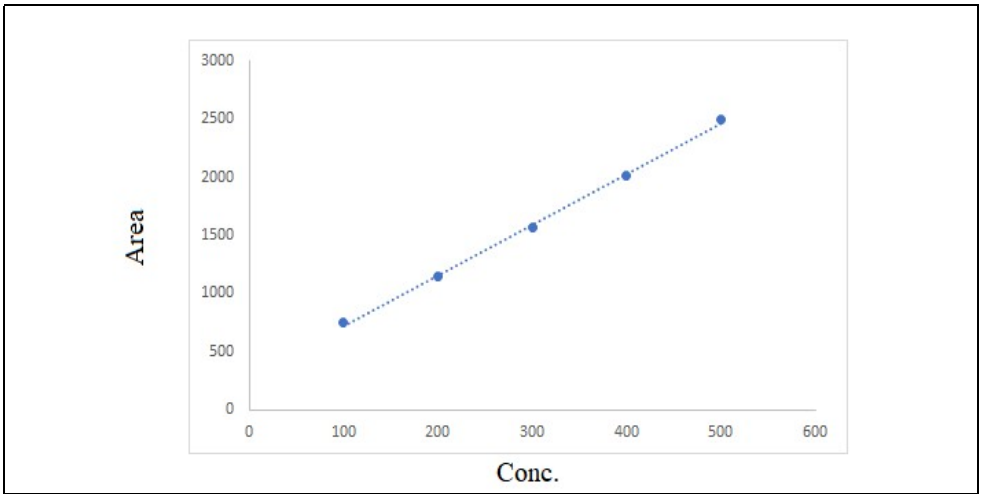

Figure 3: Calibration curve of Nintedanib esylate

Table 1: Result of recovery study (n=3)

Level	Amount of test (ng/band)	Amount of Std Added (ng/band)	Amount found mean* ± SD	%Recovery* ± SD	%RSD
80%	200	160	360.10 ± 0.15	100.57 ± 0.28	0.27
100%	200	200	400.08 ± 0.41	100.34 ± 0.68	0.21
120%	200	240	440.09 ± 0.52	100.24 ± 0.17	0.16

***Average of 3 determinations**

Table 2: Summary of validation parameters

Parameter		Nintedanib esylate
Linearity range (ng/band)		100-500ng/band
Correlation coefficient (r^2)		0.998
Precision	Repeatability	0.12%
	Intraday precision	0.11-0.36%
	Interday precision	0.09-0.16%
Limit of detection		2.71ng/band
Limit of quantification		8.23ng/band
Robustness		Robust

Table 3: Result of force degradation study

Sr. No.	Type of degradation	Peak Area	No. of Degraded Peak	% Degradation
1	Standard	1163.45	_	_
2	Acid Hydrolysis	978.59	1	15.88
3	Alkali Hydrolysis	988.46	1	15.29
4	Oxidative	1037.68	1	10.81
5	Thermal	1052.58	_	9.53
6	Photolytic	1067.53	_	8.25

CONCLUSION

HPTLC method was developed and validated as per the ICH guidelines. The developed method has been found suitable for routine estimation of Nintedanib esylate in marketed formulation. The developed method has been found to be simple, accurate, precise, sensitive and economic for The Estimation of Nintedanib esylate in capsule. Nintedanib esylate was degraded significantly under alkaline and oxidative conditions moderately in acidic and comparatively stable in thermal and photolytic conditions. It gives symmetric peak shape and good resolution for Nintedanib esylate. Hence, the method can be successfully used for the estimation of Nintedanib esylate in pharmaceutical dosage form in regular quality control testing and stability studies.

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