A VALIDATED HPTLC METHOD FOR DETERMINATION OF NINTEDANIB IN BULK DRUG

Debanchal Dutta[1], Soumyajit Das[2], Manik Ghosh[1]*

[1]Birla Institute of Technology, Mesra, Ranchi, Jharkhand (835215), INDIA

[2]Bristol Myers Squibb, E. City Phase – I, Bengaluru, Karnataka (560100), INDIA

*Corresponding Author's E-mail: manik@bitmesra.ac.in

Tel.: + 916512276247; Fax: + 916512275401

Abstract

A simple, rapid, precise and accurate HPTLC method was developed and validated for the estimation of Nintedanib, a novel tyrosine kinase inhibitor used in idiopathic pulmonary fibrosis, in bulk drug. Chromatography was carried out using silica gel 60 F_{254} TLC plate and mobile phase Chloroform: Methanol in the ratio 7:3 v/v. The densitometric determination was done at 386 nm. Regression analysis data for the calibration plot were indicative of a good linear relationship between response and concentration over the range of 800-3200 ng/band. The variance (r) was found to be 0.999. The LOD & LOQ were found to be 83.357 ng/band & 252.599 ng/band respectively. The method was validated according to ICH Q2R1 guideline. The method was precise and accurate with %RSD 0.5323 (intraday) and 0.6939 (interday) respectively and percentage recoveries in the range 99.65 % – 101.43 %.

Keywords: Nintedanib, HPTLC, idiopathic pulmonary fibrosis

INTRODUCTION

- Nintedanib (NTB), an inhibitor of tyrosine kinase, marketed under the brand names “Ofev” & “Vargatef” used to treat Idiopathic pulmonary fibrosis (IPF).
- Chemically known as methyl(3z)-3-[[4-[methyl-[2-(4-methylpiperazin-1-yl)acetyl]amino]anilino]phenylmethylidene]-2-oxo-1H-indole-6-carboxylate.
- It acts by selectively binding to the intracellular ATP binding pocket of fibroblast growth factor receptor (FGFRs), vascular endothelial growth factor receptor (VEGFRs) & platelet-derived growth factor receptor (PDGFRs) and thereby inhibiting them.
- Generally, IPF is incurable, the available drugs just reduce the advancement of the disease and slow down the drop-in lung functioning by obstructing the fibrotic process signaling pathways.

Rationale

- Literature review suggest that various methods involving UPLC, LC-MS, UV are already reported for the estimation of NTB in bulk drug, formulation, rat plasma & human plasma.
- Lin et al. (2016) worked on simultaneous determination of NTB and its metabolite in rat plasma by UPLC-MS/MS.
- Darwish et al. (2016) performed a rapid validated LC-MS for NTB quantification in human plasma.
- But, till date a validated HPTLC method for the estimation of NTB in bulk drug is not reported.
- The current manuscript is an attempt to report a validated HPTLC method as per ICH Q2(R1) guidelines for estimation of NTB. This study is designed to develop a simple, rapid, precise & accurate HPTLC method for determination of NTB in bulk drug and to validate such as per ICH guidelines.

Materials and Methods

Materials

Pure NTB API was received as gift sample from MSN Laboratories Private Ltd, India. HPLC grade solvents were procured from Spectrochem Pvt. Ltd. Mumbai, India.

Instrumentation

A Hamilton microliter syringe (Linomat syringe 659.0014, Hamilton-Bonaduz Schweiz, Camag, Switzerland), pre-coated silica gel aluminum Plate 60 F254, (20 cm × 10 cm, 100 m thickness; E. Merck, Darmstadt, Germany), Linomat 5 sample applicator (Camag, Switzerland), twin trough chamber (20 cm × 10 cm; Camag, Switzerland), TLC Visualizer 2 (Camag, Switzerland) for photo documentation, and a TLC scanner 4 (Camag, Switzerland) operated by the visionCATS software (version 2.5, Camag, Switzerland) were used while performing the study. Electronic analytical balance (AUW 220, Sartorius Corp., Germany) was used for accurately weighing of drug.

Preparation of standard solution

Standard NTB was accurately weighed (100 mg) and was transferred to a 100 ml volumetric flask. NTB was dissolved in methanol and the volume was made up to the mark to obtain a stock solution of 1000 µg/ml solution. 20 ml was pipetted out from the prepared stock solution and was further diluted with methanol to obtain working concentration of 200 ng/µl.

Mobile Phase Optimization

The mobile phase was optimized to Chloroform: Methanol in the ratio of 7:3 after several trials.

Chromatographic Procedure

Different working concentrations of NTB were prepared from the standard solution. Bands with bandwidth of 8 mm were applied on a pre-coated silica gel 60 GF254 aluminum plates with sample applicator Linomat 5. Twin trough glass chamber was used for linear ascending development. The mobile phase comprised of chloroform: methanol (7:3 v/v) and 20 min was set as the chamber saturation time at room temperature. The development was allowed till 80 mm. After the development, plates were dried with the help of an air dryer and scanned using densitometer with Camag TLC scanner 4 using visionCATS software. All measurements were taken at 386 nm in the reflectance–absorbance mode, with 6.00 mm × 0.45 mm (micro) slit dimension, 20 mm/s scanning speed, and 100 m/step data resolution. Reported areas of respective bands were used for calculation.

Method validation

Developed HPTLC method was validated for linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in accordance with ICH Q2(R1) guideline.

Linearity: Concentrations from 800-3200 ng/band having same volume (8µl) of NTB were applied in triplicate on HPTLC plate. A graph of obtained peak areas versus the corresponding concentration were plotted & evaluated using linear regression analysis.

Sensitivity: LOD & LOQ of the method was calculated by the standard deviation of the obtained peak areas. A known concentration was taken and spotted six times on HPTLC plate. Slope of the calibration curve was obtained after performing linearity. Calculation was done using the formula stated by ICH in its guideline:

$$\text{Limit of Detection (LOD)} = 3.3 \times \sigma/S$$
$$\text{Limit of Quantitation (LOQ)} = 10 \times \sigma/S$$

Where, ‘σ’ is the standard deviation of the *y*-intercept of the regression line, and ‘S’ is the slope of the calibration curve.

Precision: Precision was determined by performing intraday and interday precision studies. Intraday precision was evaluated by taking differently weighed working concentration of NTB (1600ng/band) and spotting in triplicates on the same day and Interday precision was performed in the same way using the above-mentioned concentration in three different days. Then %RSD was calculated from the obtained peak areas.

Accuracy: The accuracy of the method was performed using standard addition method. Standard NTB was spiked into the standard solution at 80 %, 100 % & 120 % levels consisting 1280 ng/band, 1600 ng/band & 1920 ng/band respectively. Then based on the measured peak areas % recovery was calculated which was found to be within 100 ± 2 %.

Robustness: Small and deliberate variations in method parameters like change in the mobile phase ratio and saturation time were made and their effect on response was observed. Finally, % RSD was calculated

Results and Discussion

Wavelength selection: The sensitivity of the method relies on the selection of correct wavelength. The developed plate was scanned in densitometer under reflectance mode in UV-Visible region from 200-700nm using Camag TLC scanner 4. A clear resolute peak was obtained at 386 nm and was chosen as the working wavelength.

Method Optimization: Optimization of chromatographic conditions were done to obtain a method appropriate for NTB determination in bulk drug. At preliminary stage, various combinations of mobile phase and in different ratios such as Chloroform: Ethyl acetate (7:3, 8:2, 9:1) and Chloroform: Methanol (9:1, 8:2, 7:3) were tested with 20 min saturation time. Finally, mobile phase Chloroform : Methanol (7:3) was chosen for performing the experiment since the R_f (0.58 ± 0.02) and the resolution of the band were satisfactory compared to the above tested combinations.

Method validation

Linearity: "Linearity of an analytical procedure is its ability (within a range) to provide a response directly proportional to the concentration of the analyte". NTB showed good correlation coefficient (r^2 =0.999) in the concentration range of 800-3200 ng/band with equation y=0.7256x+1954.4 (Fig 1).

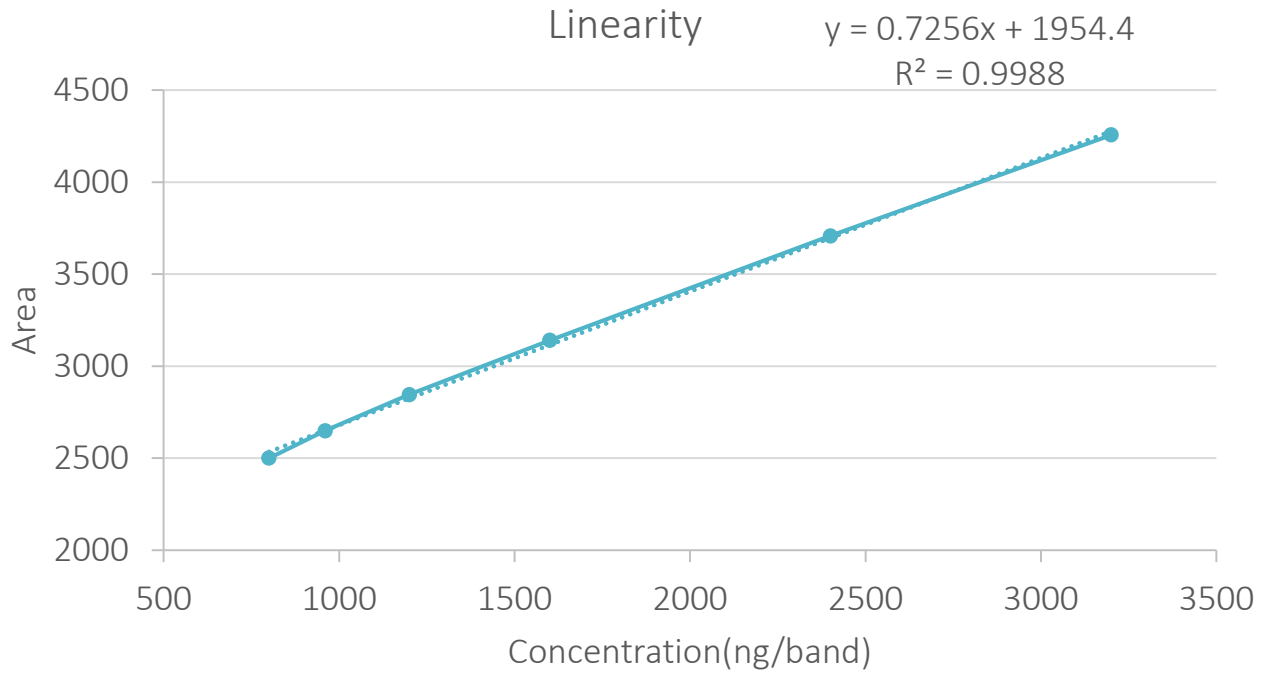

Fig 1. Linearity of NTB from 800-3200 ng/band; y=0.7256x+1954.4; r^2= 0.999

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD & LOQ for NTB were obtained as 83.357 ng/band and 252.599 ng/band respectively thereby indicating the developed method's sensitivity.

Precision: Intraday & interday precision was performed for precision studies. In intraday precision, the experiment was performed three times in a day by spotting working concentration in triplicates. Interday precision was performed by repeating the experiment in the same above-mentioned manner in different days. %RSD of the observed peak areas were calculated for both intraday (Table 1) & interday precision (Table 2) and was obtained as 0.303–0.682 & 0.343–1.114 respectively.

Table 1. Intraday precision of NTB (n=3).

Concentration (ng/band)	Area			Average Area	SD	%RSD
1600	3151	3132	3140	3141.00	09.539	0.303
1600	3179	3155	3136	3156.67	21.548	0.682
1600	3149	3177	3162	3162.67	14.011	0.443

Table 2. Interday precision of NTB (n=3); Concentration 1600 ng/band.

Day	Area			Average Area	SD	%RSD
Day 1	3141	3156	3162	3153.00	10.817	0.343
Day2	3138	3166	3121	3141.67	22.723	0.723
Day 3	3168	3114	3180	3154.00	35.157	1.114

Accuracy: It was performed by spiking standard NTB at 80%, 100% & 120% concentration levels into the standard solution. % recoveries were calculated and was obtained as 99.65 % to 101.43 %. It was within the acceptable range of 100 ± 2% (Table 3).

Table 3. Accuracy of NTB.

Sample	% of nominal	Standard (ng/band)		% Recovery
		Spike	Found	
1	80	1280	1292.127	100.95
2	100	1600	1622.876	101.43
3	120	1920	1913.201	99.65

Robustness: It was performed by making small and deliberate changes in the mobile phase ratio & saturation time and their corresponding response were recorded. % RSD was calculated and was found within the acceptable range of less than 2%.

Table 4. Robustness with change in mobile phase ratio and saturation time

Change in mobile phase ratio (Chloroform : Methanol :: 7:3 ± 0.2)			
Solvent Ratio	R_f	Area ± SD	%RSD
6.8 : 3.2	0.58 ± 0.02	3142.83 ± 17.81	0.566
7.2 : 2.8	0.58 ± 0.02	3152.50 ± 28.27	0.896
Change in saturation time (20 min ± 2 min)			
Time (min)	R_f	Area ± SD	%RSD
18	0.58 ± 0.01	3147.00 ± 28.69	0.911
22	0.58 ± 0.03	3144.67 ± 29.57	0.940

Conclusion

The present study establishes a simple, precise, accurate and robust validated HPTLC method, suitable for estimation of NTB in bulk drug.

Acknowledgment

Authors are thankful to MSN Laboratories Pvt. Ltd., India for supplying the gift sample and Central Instrumentation Facility, BIT Mesra, Ranchi for HPTLC facilities.

Bibliography

A.Y. Kamble, M. Mahadik, L. Khatal, S.R. Dhaneshwar, Validated HPLC and HPTLC Method for Simultaneous Quantitation of Amlodipine Besylate and Olmesartan Medoxomil in Bulk Drug and Formulation, Analytical letters January 2010, 43(2):251-258·

D. Lin, L.M. Qiao , Y.N. Zhang , Y. Liu , X.S. Liu, Simultaneous determination of nintedanib and its metabolite by UPLC–MS/MS in rat plasma and its application to a pharmacokinetic study, Journal of Pharmaceutical and Biomedical Analysis 117(2016) 173-177.

D. Lin, L.M. Qiao, Y.N. Zhang, Y. Liu, X.S. Liu, Simultaneous determination of nintedanib and its metabolite by UPLC–MS/MS in rat plasma and its application to a pharmacokinetic study, Journal of Pharmaceutical and Biomedical Analysis 117(2016) 173-177.

G. Kleinschmidt, in: J. Ermer, J.H.M. Miller (Eds.), Method Validation in Pharmaceutical Analysis. A Guide to Best Practice, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2005.

H.W. Darwish, M.W. Attwa1, A.A. Kadi1, Rapid validated liquid chromatographic method coupled with Tandem mass spectrometry for quantification of nintedanib in human plasma, Tropical Journal of Pharmaceutical Research November 2016, 15 (11) 2467-2473.

International Conference on Harmonization, ICH Q2 (R1): Validation of Analytical Procedures: Text and Methodology, ICH Secretariat, Geneva, 2005.

K.F. Fodor, B. Renger, Z. Vegh, The frustrated reviewer- recurrent failures in manuscript describing validation of quantitative TLC/HPTLC procedures for analysis of pharmaceuticals, Journal of Planar Chromatography 2010, 23(3).

P. Shah , J. Patel, K. Patel, T. Gandhi, Development and validation of an HPTLC method for the simultaneous estimation of Clonazepam and Paroxetine hydrochloride using a DOE approach, Journal of Taibah university of science 11 (2017) 121-132.

S.V. Gandhi, N.D. Dhavale, V.Y. Jadhav, S.S. Sabnis, Spectrophotometric and reversed-phase high-performance liquid chromatographic methods for simultaneous determination of escitalopram and clonazepam in combined tablet dosage form, J. AOAC Int. 91 (2008) 33–38.

T.B. Solanki, P.A. Shah, K.G. Patel, Central composite design for validation of HPTLC method for simultaneous estimation of olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide in tablets, Indian J. Pharm. Sci. 76 (2014) 179–187.

Venkatachalam, V.S. Chatterjee, Stability-indicating high performance thin layer chromatography determination of Paroxetine hydrochloride in bulk drug and pharmaceutical formulations, Anal. Chim. Acta 598 (2007) 312–317.

Thank You