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Identification and Characterization of Degradation Products by Using MS-MS Studies for Developed and Validated Stability Indicating HPTLC Method for Estimation of Nintedanib Esylate in Pharmaceutical Dosage Form

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Abstract: Nintedanib esylate was widely reported as tyrosine kinase inhibitor. As there is no HPTLC method was reported for nintedanib drug the objective of this work is to develop simple, precise, fast, accurate and validated HPTLC method for estimation of this drug. Also, to carry out forced degradation study of nintedanib esylate and identification of isolated stressed sample of nintedanib esylate by MS-MS studies. HPTLC is the widely recognized for its reliability and for its sensitivity. A validated method was developed using camag HPTLC. Stability studies were carried out as per standard guidelines. The degradant peaks were then subjected for its characterisation and identification by using MS-MS studies. The method is found to be simple precise and accurate. The Rf value is found to be 0.53. Linearity shows 0.9979. The stability studies shows that the sample is degraded under acidic, basic and oxidative stress condition. MS-MS studies revels that there is formation of 10 degradation products which was characterized and identified. Proposed HPTLC method developed and validated which is successfully applicable for pharmaceutical dosage form containing this drug. The characterization and identification of degradation products may be use further for impurity profiling of nintedanib esylate

Keywords: Nintedanib Esylate, HPTLC, Isolation of Degradation Product, MS-MS Identification.

INTRODUCTION

The nintedanib esylate is chemically methyl (3Z) -3- [[4-{ [methyl – [2 – (4 – methyl piprazin -1- - yl] amino } aniline (phenyl) methylidene) – 2 Oxo – 2,3 – dihydro - -1 – H – indole -6 – carboxylate, (Z) – N – methyl – 2 (4 – methyl piperzin – 1-yl) – N – (4 (((2 – oxoindolin – 3 – ylidone (phenyl) methyl) amino) phenyl) acetamide (fig no .1) Idiopathic pulmonary fibrosis is commonly present in the six or seventh decade of life and arises more frequently in man than women. It is characterized by fibrosis of the lung parenchyma and destruction of lung function. It is due to the repetitive alveolar epithelial cell injury and deregulation repair, in which there is wild proliferation of lung fibroblasts and distinction of fibroblast into myofibroblast, which extremely deposit extracellular medium proteins in the interstitial space.[1-5]

The nintedanib is the tyrosine –kinase inhibitor. & used for preventing idiopathic pulmonary fibrosis & non-small lung cancer. It can mostly target the 3 receptors (i) Targeting vascular endothelial growth

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factor receptor (ii) Fibroblast growth factor receptor (iii) Platelet derived growth factor receptor[3]. It is an indolinane derivative & similarly as anti- angiogenic drug. It can block the kinas activity by inhabiting the intercellular ATP-binding pockets of specific tyrosine kinase and binding type was travelled from fibroblast growth factor recptor-1 and vascular endothelial growth factor receptor. It is initially established by Boehringer Ingelheim and sold under the brand names OFEV and VARGATEF. It is available in 100 and 150mg strength of dosage forms. It is approved by FDA (2014) and European Commission for the management of idiopathic pulmonary fibrosis (2015).[6-11]

The literature survey reveals that, there is no HPTLC method was reported. So, the aim of the present study is to develop stability indicating HPTLC method for estimation of nintedanib esylate in pharmaceutical dosage form and to characterize the degradation products by using MS-MS studies. The structure of drug shown in fig no.1.

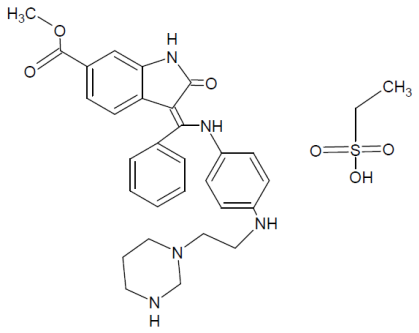


Fig.1: Structure of Nintedanib esylate

MATERIALS AND METHOD

Chemical and regents- Nintedanib esylate is received as a gift sample from in- house R&D laboratory of suven life Sciences. The analytical grade dichlomethane, methanol, ammonia, HCl, NaOH, H_2O_2 was used.(2)

Instrumentation- For the validation of the nintedanib esylate CAMAG LINOMAT 5 automatic applicator is used. The capacity a syringe is about 100 µL (glass syringe). For the saturation of mobile phase the twin chamber is used (CAMAG 10 × 10 cm) and win CATS 1.4.0 software (Camag) was used for the present study. [12]

Chromatographic conditions– The 0.4µL sample solution and standard solution is applied on TLC plate with help of micro syringe (band size: 6 mm) and six samples are applied on TLC plate and the plates were run in different solvent systems. In an attempt to achieve the desired Rf value range (0.2-0.8) with a compact band, several trials were made by using different solvent systems containing non-polar solvents and relatively polar like dichloromethane : methanol, dichloromethane: toluene: formic acid, acetone: toluene different concentration levels were tried in order to determine the best conditions for the effective separation of nintedanib esylate among the different mobile phase combination tested Dichloromethane :methanol: ammonia (4 : 6 : 0.1 v/v) give compact bands which showed symmetrical peak on chromatogram and desired Rf value. The Rf value with the standard deviation was 0.53 ± 0.3 for nintedanib esylate.

Preparation of sample solution- Accurately weighed quantity of 10.0 mg nintedanib esylate was transferred to 10.0 ml volumetric flask, added 5 ml of methanol and ultrasonicated for 10 minutes, volume was then make up to the mark with methanol. Diluted 1.0 ml of above solution to 10.0 ml with methanol (Conc. obtained 100 µg /ml) [13]

Method validation

The all parameter is validated as per ICH guideline.

Linearity- The altered concentration of Nintedanib esylate (100 -600ng per band) is applied on TLC plate by using microsyringe with the help of automatic applicator. The concentration vs peak is being record.[14-19]

Precision-The inter and intraday precision in carried out using same concentration **(**0.4µL).The interday precision is carried out in same day but different interval of time and intraday is done on successive days.

Accuracy – The accuracy study is carried out in 3 levels that are 80%, 100% and 120%. An accurately weighed the powder ~ to 10mg nintedanib esylate and transferred in to the 9 different 10 ml volumetric flask and add 8 mg, 10 mg and 12 mg of powder into the flask. Sonicate and filter using whatman filter paper (120mm size).

Limit of Detection and Limit of Quantitation- LOD is the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOQ is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. It is calculated by using formula

$$LOD = 3.3 \times S.D \div Avg \qquad LOQ = 10 \times S.D \div Avg$$

Robustness– It is designed by altering the concentration of mobile phase (±0.1ml), saturation time (5, 10,15min) and total volume of mobile phase (20.2).

Force degradation–The degradation study is carried out by introducing the sample into the various stress conditions like acidic (0.1 N HCl), oxidative (3% H2O2), alkaline (0.1N NaOH). For neutral degradation the sample is kept on water bath at 80°c for 2 hrs. For thermal and photo degradation the sample is Kept at 100°c for 1 hrs and 24 receptively.

Isolation and Identification of Degradation Products by HPTLC-MS/MS

The degradation product is isolated and categorize by applying the degradation sample on TLC plate. (9µl-band). Then the plate is placed in the optimized chromatographic conditions and air dried. After drying observed under UV cabinet. The degradation product is identified and marked. The identify degradation product is scrapped out and saturated overnight with methanol. After one day the sample is filtered using what man filter paper and the sample is inserted into the further MS/MS study.

RESULT AND DISCUSSION

Optimization of mobile phase – The optimization of the mobile phase is a critical step in HPTLC. The Dichloromethane: Methanol: Ammonia (4:6:0.1 v/v/v) show good result at 0.53±0.3 (Fig no.2) and its 3D spectra is shown in fig no. 3

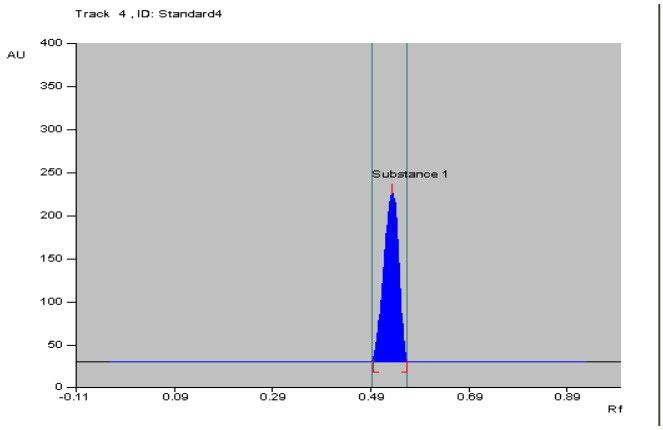


Fig. 2: Typical densitiogram of Nintedanib esylate

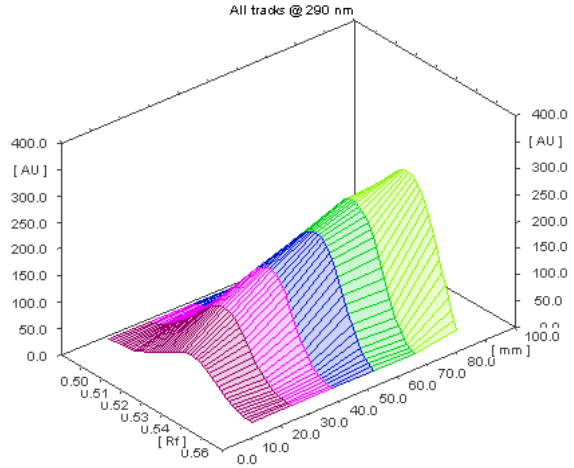


Fig. 3: 3D graph of linearity of Nintedanib esylate at 290.0 nm

Linearity and calibration curve – The linearity curves was found to be linear and regression coefficient was found to be 0.9979. The equation of line is y = 175.057x+11.419.The results were shown in Fig no 4 and Table no 1.

Table 1: Standard calibration data for Nintedanib esylate

Sr. No.	Concentration ng/band	Mean peak area 290 nm
1.	100	13337.55
2.	200	2426.48
3.	300	3579.4
4.	400	4904.2
5.	500	5653.4
6.	600	7129.9

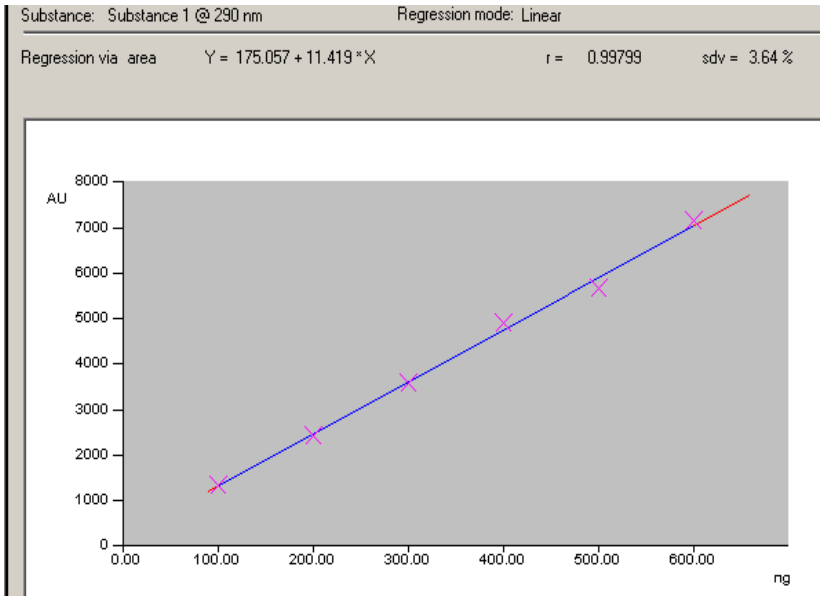


Fig. 4: Calibration curve of Nintedanib esylate at 290.0 nm

METHOD VALIDATION

The method was validated as per Q2 (Analytical validation) ICH guideline. The results for precision, recovery, robustness and LOD/LOQ were mentioned in Table No 2 to Table No 6.

Table 2: Statistical evaluation of precision of developed method

Drug	Intra-day Precision			Inter-day Precision		
	% Label claim*	S.D. (±)	R.S.D.	% Label claim*	S.D. (±)	R.S.D.
Nint.	120.4	0.69	0.69	120.5	0.31	0.31

Table 3: Results of recovery studies

Sr. No.	Level of recovery	Wt. of capsule powder taken (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery
1.	80 %	22.11	8.0	7.8	98.5
		22.16	8.1	7.6	96.5
		22.13	8.2	7.9	99.5
2.	100 %	22.15	10	9.9	99
		22.12	10.2	9.8	98
		22.10	10.3	9.7	97.2
3.	120 %	22.11	12	11.9	99
		22.18	12.2	11.7	97.7
		22.16	12.1	11.8	98.9

Table 4: Statistical validation for recovery study

Level of recovery	% Recovery*	S.D. (±)	R.S.D.
80%	99.3	0.39	0.39
100%	98.6	0.43	0.43
120%	98.5	0.38	0.38

Table 5: Results of LOD and LOQ

Parameters	Nintedanib esylate
LOD (µg/band)	0.9643µg/ml
LOQ (µg/band)	2.94µg/ml

Table 6: Result of robustness study

Factor	Level	Peak area	Rf
Mobile phase composition (± 0.1 ml)			
3.8:6.1:0.1	– 0.1	5689.22	0.51
4:6:0.1	0	5743.63	0.53
4.1:6.1:0.1	+ 1	5868.23	0.56
	R.S.D.	**0.82725**	**0.012**
Duration for chamber saturation (± 5 min)			
10 min	- 5	5679.11	0.51
15min	0	5743.63	0.53
20 min	+ 5	5877.33	0.58
	R.S.D.	**0.904439**	**0.018**
Development to scanning			
5 min	-	5602.00	0.51
10 min	-	5643.63	0.52
15 min	-	5888.43	0.57
	R.S.D	**1.461558**	**0.017**

Force degradation study – The degradation study of Nintedanib esylate is done. It shows the maximum degradation in acidic condition and the minimum in photolytic condition. There results were shown in Table No 7 and Figure No 5 to Figure No 9.

Table 7: Result of degradation studies

Sr. No.	Stress Condition	Temperature and Time	% assay of active substance	Rf of degraded product
1.	Acid (0.1 N HCl)	80^0 C for 3hrs	80.8%	0.05,0.36,0.37, 0.57,0.69
2.	Alkali(0.1N NaOH)	80^0 C for 30 min	81.18%	0.05,0.09,0.57
3.	Oxide (3% H_2O_2)	80^0 C for 1hrs	80.5%	0.69
4.	Neutral	80^0 C for 3 hrs.	93.8%	0.88
5.	Thermal	60^0 C for 1 hrs.	98.4%	0.09,0.89
6.	UV	80^0 C for 24 hrs.	92.9%	0.86

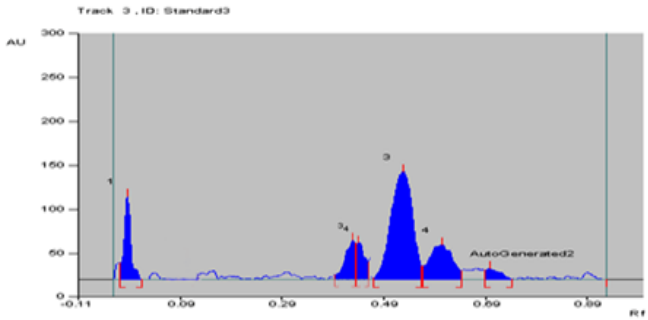


Fig. 5: Densitogram of acid (0.1 M HCl) treated sample

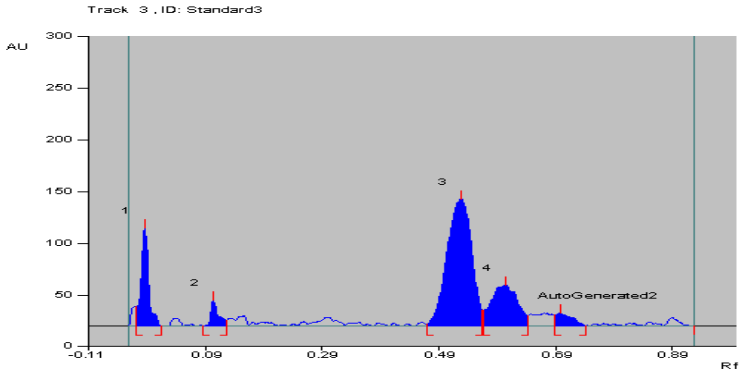


Fig. 6: Densitogram of alkali (0.1M NaOH) treated sample

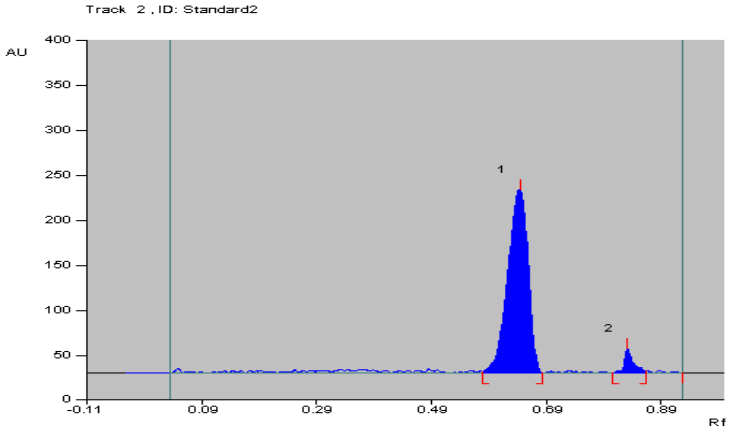


Fig. 7: Densitogram of oxide (3% H_2O_2) treated sample

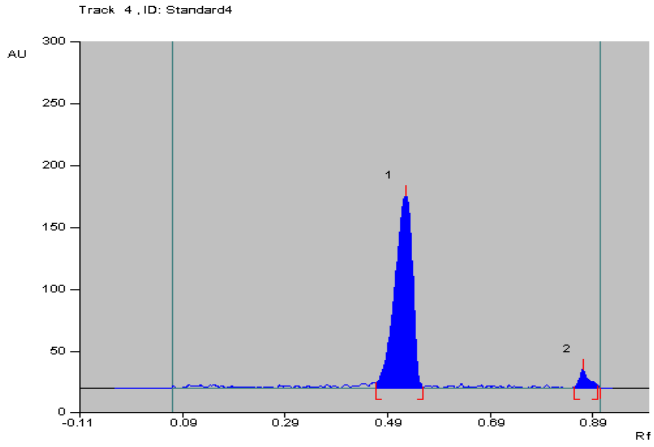


Fig. 8: Densitogram of the sample exposed to neutral hydrolysis

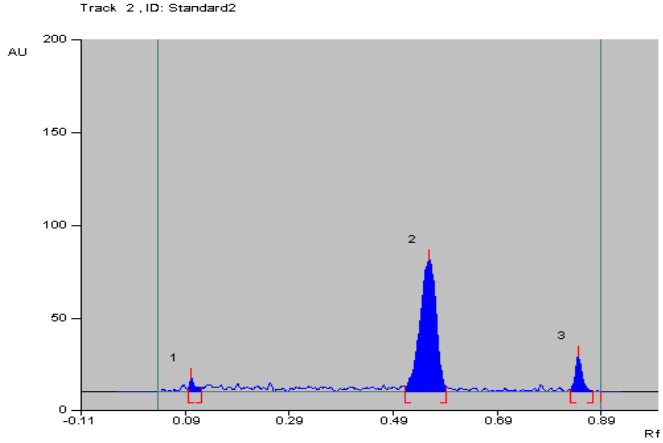


Fig. 9: Densitogram of sample exposed to heat

Isolation and identification of degrade product by HPTLC-MS/MS (Tandem mass spectroscopy) method.

The degradation product was isolated by using HPTLC method and the structures of degrandants product are determined by using LC-MS study. The fragmentation pattern of the drug was established by carrying out MS-MS studies in positive electro spray ionization (ESI) mode in the mass range of 50–1500 daltons (Da). The drug (concentration of 5µg/ml) was directly infused using a syringe pump into MS/MS in methanol: water (50:50v/v). To optimized the mass parameters which clearly inform about the molecular ion peak of the drug. These were further modified to get complete fragmentation of the drug. High purity nitrogen was used as the nebulizer as well as the auxiliary gas. Fragmentation of various precursor ions formed in MS/MS studies was achieved at different collision energies

The five degrdant is observed in the acidic stress condition, four in alkaline stress condition and one in oxidative stress condition. In acidic stress condition ester hydrolysis may takes place at 6 carboxylate position of the drug (molecular formula C18H33N5O4: weight-525.25) followed by loss of CH3OH to yield DP-1 (C30H31N5O4, weight: 555.06) after further fragmentation pathway was observed. I.e.DP-2, DP-3 DP-4, DP-5 which is shown in figure no 10 to Figure No 14.

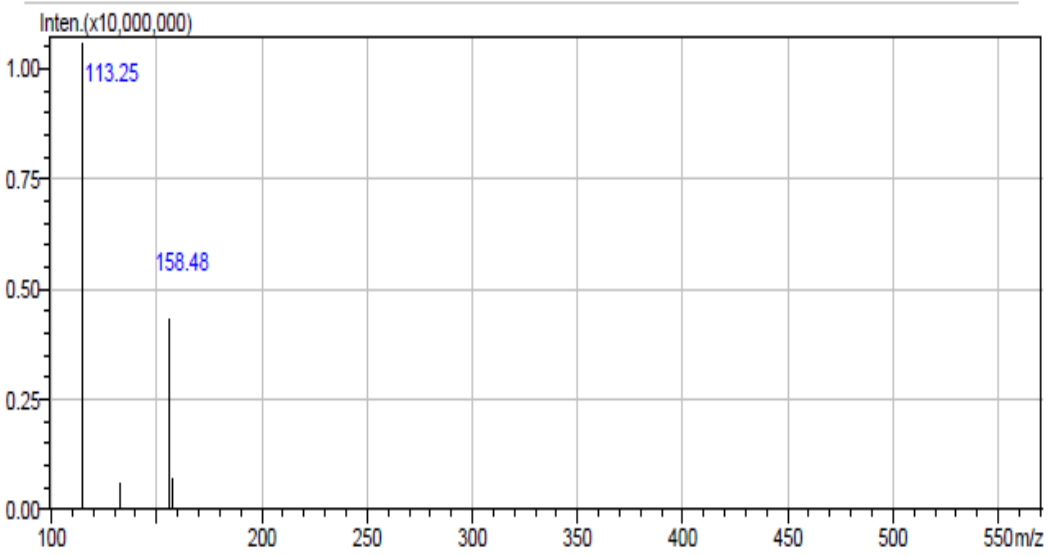


Fig. 10: MS/MS spectrum of the DP-2(m/z 158.48)

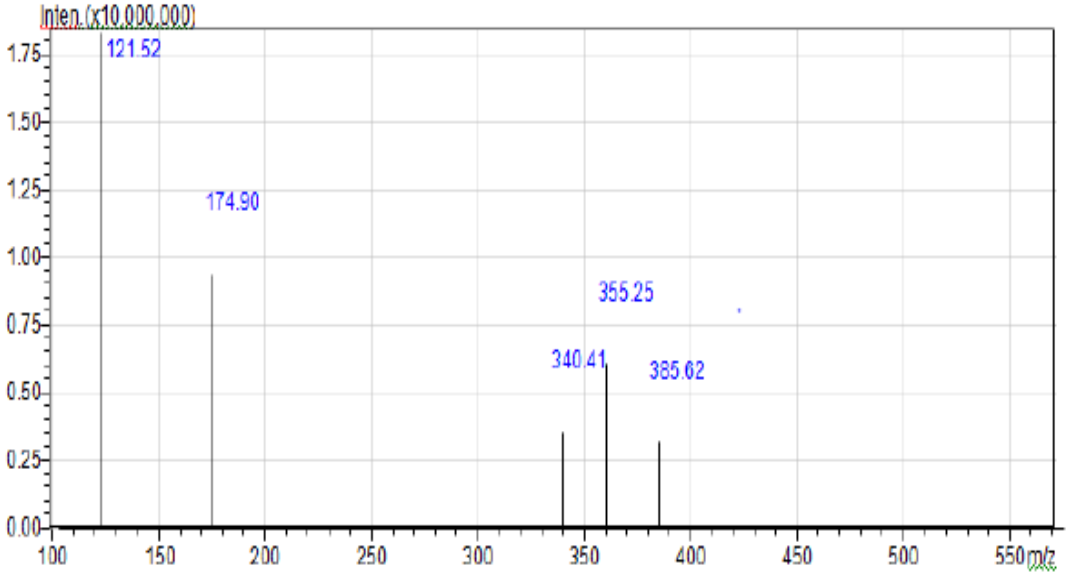


Fig. 11: MS/MS spectrum of the DP-3(m/z 385.62)

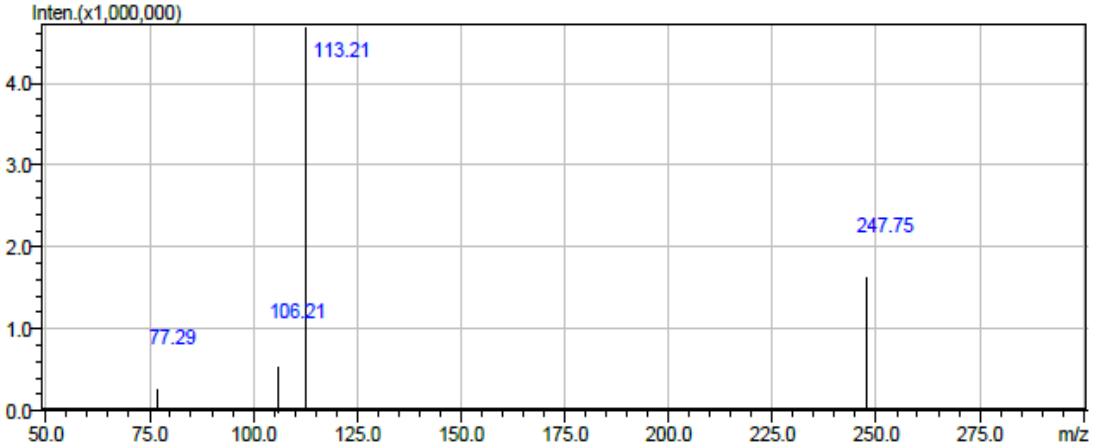


Fig.12: MS/MS spectrum of DP-4(m/z 247.75)

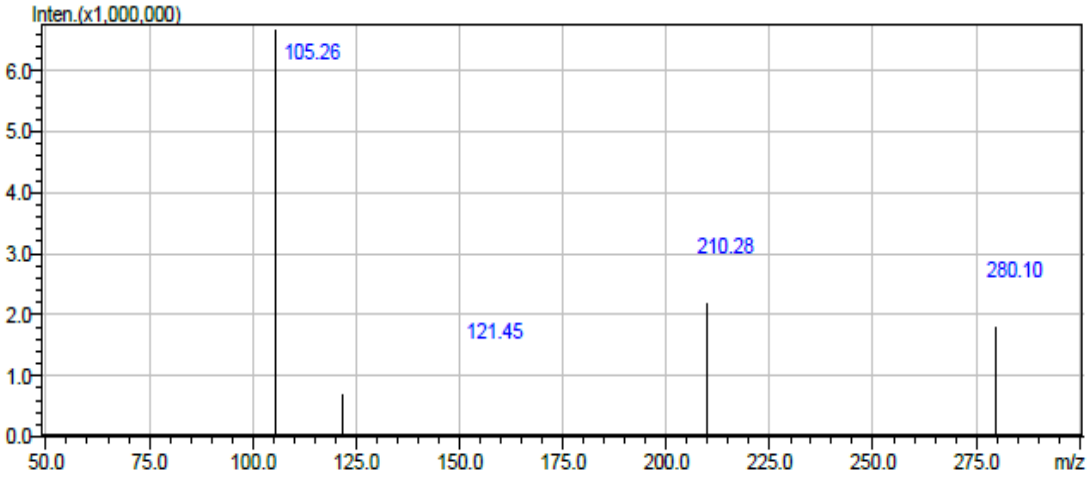


Fig. 13 MS/MS spectrum of DP-5 (m/z 280.10)

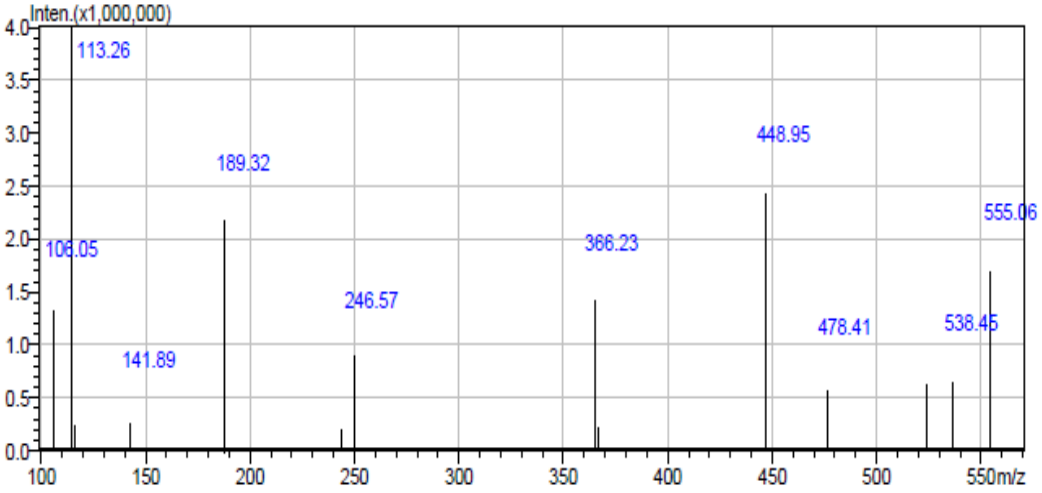


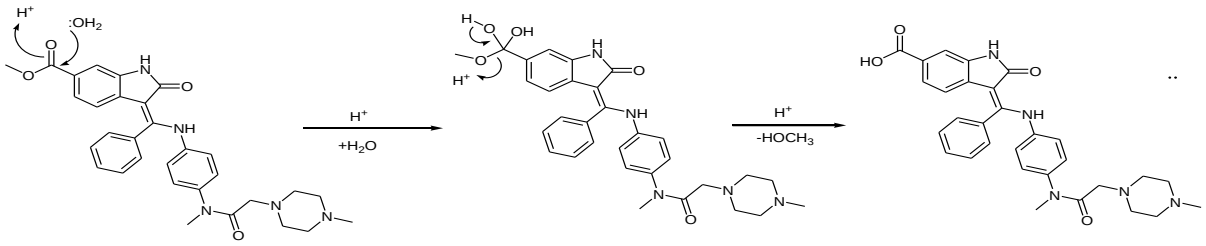
Fig. 14: MS/MS spectrum of DP-6(m/z 555.248)

The alkaline stress condition follows the same degradation pathway of the acidic stress condition, but one degredant was not detected in alkaline condition having (m/z 247.168) fig.no 13.

In oxidative stress condition DP-6 was formed by attack of the hydrogen peroxide at 3-NH position following peroxide mechanism of free radical and formation of oxime, which is then reduced ton yield DP-6. (Molecular formula: C31H33N5O2, Weight: 555.248) fig.no.15.

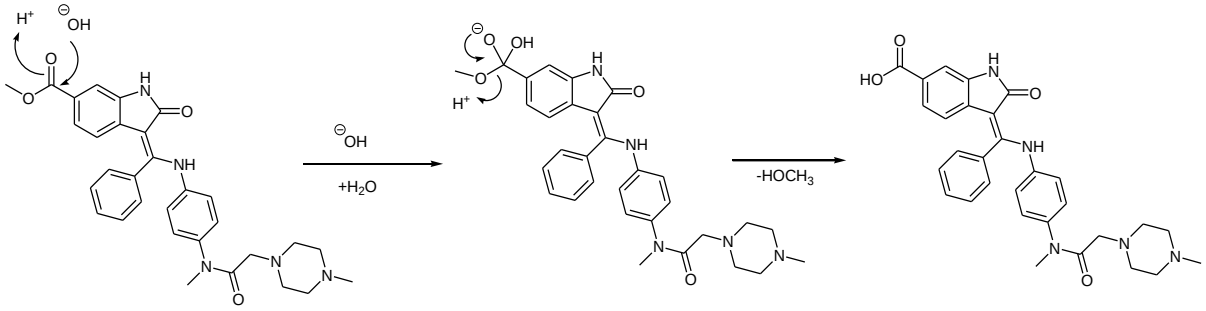
Fragmentation Pattern of the Nintedanib Esylate

The possible degradation pathways were proposed in Fig no 15 to Fig 18.



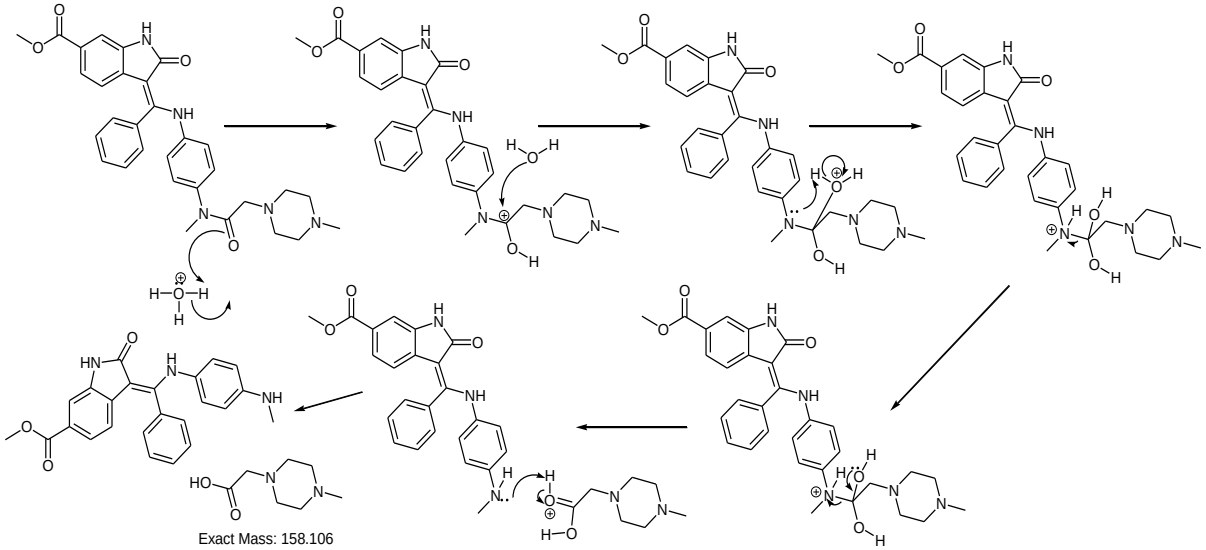
Exact Mass: 525.238

Fig. 15



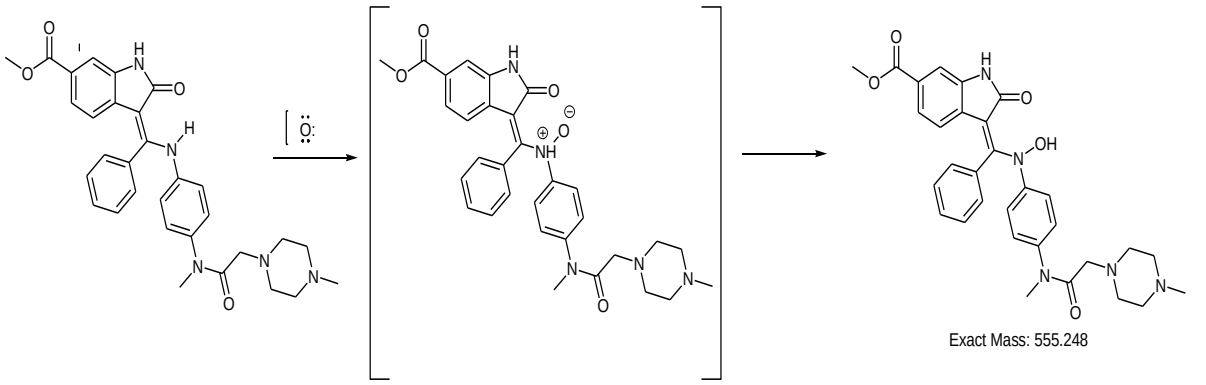
Exact Mass: 525.238

Fig. 16



Exact Mass: 158.106

Fig. 17



Exact Mass: 555.248

Fig. 18

CONCLUSION

The stability indicating HPTLC methods was established and validated for estimation of nintedanib esylate bulk and in its capsule formulation. The developed **HPTLC** method helps to separate drug as well as all the degradation products of Nintedanib esylate which proved its stability indicating nature. The use of **HPTLC** method helps to separation and isolation of drug as well as degradation products and the MS/MS help to identify the structure of each degradation product as a result we can understand degradation pathway of drug molecule. The ms-ms studies show ten degradation products in acid, alkaline, oxidative condition.

It is a most versatile technique and is known for uniformity, purity profile, assay values and precision and accuracy of results. It can handle several samples of even divergent nature and composition. It is accepted as a time-saving and most economical machine practically with minimum trouble shootings.

The Proposed HPTLC method was found to be a more sensitive, precise, economic and less time consuming and cost effective.

Conflict of Interest: None to declare

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REFERENCES

[1] Wollin L, Kolb M. “Mode of action of Nintedanib in the treatment of idiopathic pulmonary fibrosis”. ERJ Express march 5, 2015 p.g no1-2.

[2] Purnachand D , Madhusudhanreddy B “Development and Validation of a Simple and Sensitive Stability indicating RP-HPLC assay method for determination of nintedanib and stress degradation studies” Jcpr 2015 p.g no 774-775

[3] Bhole R, Wankhede S, Stability Indicating HPTLC Method for Simultaneous Estimation of Empagliflozin and Linagliptin in Pharmaceutical Formulation. Anal Chem Lett 2017; p.g no 76-85.

[4] Bhole R & Tamboli F (2018) Development and Validation of Stability Indicating HPTLC-MS Method for Estimation of Empagliflozin in Pharmaceutical Dosage Form, Analytical Chemistry Letters, 2018,:2, p.g no 244-256

[5] ICH Q2A; Guidelines on validation procedure; definition and terminology, federal Register 1995;pg no 60:11260

[6] ICH Q2B Guidelines on validation of analytical procedure; methodology, federal register 1196; pg. no 60:27464

[7] ICH, (Q2R1), Harmonized Tripartite Guideline, Validation of analytical procedures: Text and Methodology, IFPMA. Geneva 2005; pp. 1- 13.

[8] ICH (Q1AR2). Harmonized tripartite guideline, stability testing of new drug substances and products, IFPMA. In: Proceedings of the International Conference on Harmonization. Geneva; 2003.

[9] Bhole R, Wankhede S,Stability Indicating HPTLC Method for Simultaneous Estimation of Empagliflozin and Linagliptin in Pharmaceutical Formulation. Anal Chem Lett 2017; 7:1: p.g no 76-85.

[10] GJ Roth; G Linz ; P Sieger; Wall; Hilberg; T Bock.US Patent 7119093,2006.

[11] GJ Roth; G Linz ; P Sieger; Wall; Hilberg; T Bock.US Patent 7989474,2011.

[12] P.L. Mc Cormack, Drugs, 2015, 75,129-139.

[13] Ellis P, Kaiser R, 2010, 16, 2881-2889.

[14] Villiers D, Lestremau F, A 2006, 1127,60-96

[15] Skoog DA, Holler FJ, Crouch SR. Fundamental of Instrument Analysis. 8thed.India;Thomson BOOKS/Cole;2007.p 1-5

[16] Madhukar A, Prince A, Vijay K. (2011) Simple and sensitive analytical method development and validation of metformin Hcl by RP-HPLC, Int J Pharm Pharm Sci. 3(3): p.g no 117-120.

[17] Thorat S, padmane S, Tajne M, Ittadwar A; " Development and validation of simple, Rapid and Sensitive UV, HPLC and HPTLC Methods for estimation of pirfenidone in tablet dosage form" J. Chil.chem .soc 61, (2016) p.g no 2978-2980.

[18] Xiao, Min, LI, Jun-wei; Sun, Wei; Luo, Shun-bin; Hu, Guo-xin "HPLC Determination of pirfenidone in rat plasma and its pharmacokinetics"**:** Chinese Journal of Pharmaceutical Analysis, Volume 32, Number 2, 2012, p. 202- 205(4)

[19] Chunduri R, Dannana G; Development and Validation of LC-MS/MS Method for Quantification of Teneligliptin in Human plasma and its application to a Pharmacokinetic study; World Journal of Pharmacy and Pharmaceutical Sciences, 2016, 5(5): p.g no 838-850.