STABILITY INDICATING HPTLC METHOD DEVELOPMENT AND VALIDATION FOR CERITINIB IN BULK AND FORMULATION

Santosh V. Gandhi[a]*, Shivani R. Sawarkar[a] and Mrinalini C. Damle[a]

(Received 11 July 2022) (Accepted 19 October 2023)

ABSTRACT

Ceritinib is an anti-cancer of drug used in treatment of non-small cell lung cancer. A high-performance thin layer chromatography (HPTLC) method has been developed for ceritinib, which is stability indicating and simple, precise, and discriminating. The stationary phase used was aluminum-backed silica gel60 F_{254} plates with chloroform: methanol: glacial acetic acid as the mobile phase in the ratio of 8.5:1.5:0.5 (V/V/V). The retention factor was found to be 0.34 ± 0.02. The densitometric scanning was performed at 277 nm. The linear range for analysis was 100–600 ng band^{-1}, which gave good linear relationship with regression coefficient of 0.998. Method accuracy was proved by the recovery studies. The detection limit and quantification limit were 7.38 and 10.03 ng band^{-1}, respectively. Stress degradation studies like hydrolysis under different pH conditions, photolytic, thermal, and oxidative degradation were carried out as per ICH Q1A (R2) and Q1B guidelines. The method established was found to be robust, and thus it can be used as a stability-indicating method.

Keywords: Forced degradation, ceritinib, HPTLC, stability-indicating

INTRODUCTION

Ceritinib is a tyrosine kinase inhibitor that selectively, and potently inhibits anaplastic lymphoma kinase (ALK)[1]. Ceritinib acts to inhibit this enzyme and stop cell proliferation, ultimately halting cancer progression (Fig. 1).

As per the literature, there is a LC-MS/MS method for determination and quantification of potential genotoxic impurities in the ceritinib active pharmaceutical ingredient[2]. Bioanalytical HPLC and UPLC-MS/MS method for determination in rat plasma has also been reported[3,4]. Development and validation of stability indicating RP-HPLC method for determination of ceritinib[5,6] and forced degradation of ceritinib under stress conditions and structural interpretation of novel degradants using HR-MS/MS and NMR have been reported[7]. To our understanding, no report as per literature associated to stability indicating HPTLC is available. This current work describes a simple and easy stability indicating method for determination of ceritinib as per ICH guidelines.

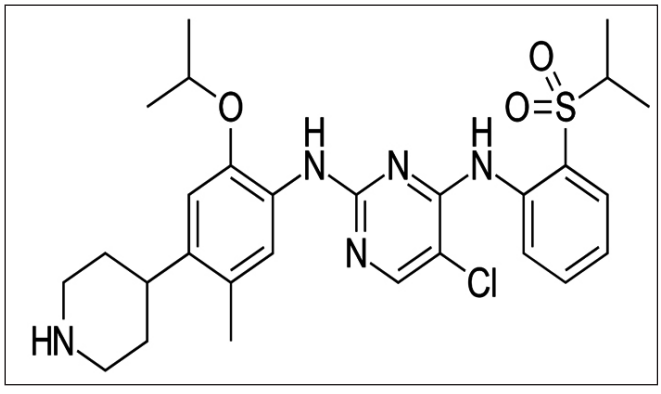

Fig. 1: Structure of ceritinib

MATERIALS AND METHODS

Materials

NATCO Pharmaceuticals, Hyderabad kindly provided ceritinib as a gift sample. AR grade chemicals like methanol, chloroform, sodium hydroxide, glacial acetic acid, hydrochloric acid and hydrogen peroxide were purchased from Loba Chemical Pvt. Ltd., Mumbai.

Instrumentation

HPTLC system (CAMAG) equipped with Linomat 5 sample applicator, TLC Scanner (III), Software

[a] Department of Pharmaceutical Chemistry, AISSMS College of Pharmacy, Kennedy Road, Near RTO, Pune - 411 001, Maharashtra, India
*For Correspondence: E-mail: santoshvgandhi@rediffmail.com

https://doi.org/10.53879/id.60.11.13584

[WINCATS (version 1.4.3)], twin trough glass chamber, application syringe [Hamilton (100 µL)], and aluminum TLC plate precoated with silica gel 60 F_{254} (Merck) were used in study. Other instruments used were electronic balance [Shimadzu (Model AY- 120)], UV- Visible spectrophotometer [JASCO (Model- V730)], hot air oven [BIOMEDICA], sonicator [PRAMA (Model SM15 US)] and stability chamber for photo degradation (Newtronic).

Standard stock solution

Solution of concentration of 1000 µg mL^{-1} was made by weighing accurately 10 mg of ceritinib and dissolving in 10 mL of methanol. It was then appropriately diluted with methanol to get 50 µg mL^{-1} as a working standard solution.

Detection of wavelength

Stock solution of ceritinib 10 µg mL^{-1} was prepared using methanol and UV spectrum was recorded. It showed maximum absorbance at 277 nm. Spectrum is shown in Fig. 2.

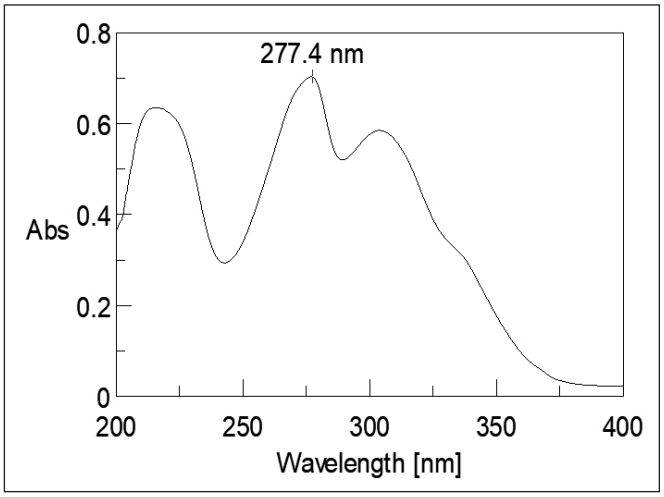

Fig. 2: UV spectrum of ceritinib in methanol (10 µg mL^{-1})

Chromatographic conditions

Chromatographic studies on the drug ceritinib were performed on aluminum plates previously covered with silica gel 60 F_{254} (10 cm × 10 cm size with 250 µm layer thickness). Samples were applied on the plate as a 6 mm wide band using a 100 µL syringe with a Linomat sample applicator. Mobile phase composed of chloroform:methanol:glacial acetic acid (8.5:1.5:0.5 V/V/V). Linear ascending development was carried out for 20 minutes under saturated conditions, with a migration distance of 80 mm. Densitometric scanning was performed at 277 nm assisted by the software, with a slit size of 4 x 0.45 mm.

Forced degradation studies

The degradation conditions were applied as per ICH guidelines Q1A (R2)[8,9]. The stress conditions such as strength of reagent and the time of exposure used were as follows (Table I):

Acid hydrolysis

To 5 mL of ceritinib stock solution (100 µg mL^{-1}), 1 mL of 2 N HCl was added and kept aside for 2 h. Then, volume was made upto 10 mL with methanol and 10 µL of the resultant solution was applied on TLC plate and developed (Fig. 3).

Base hydrolysis

To 5 mL of ceritinib stock solution (100 µg mL^{-1}), 1 mL of 2 N NaOH was added and kept aside for 2 h. Then

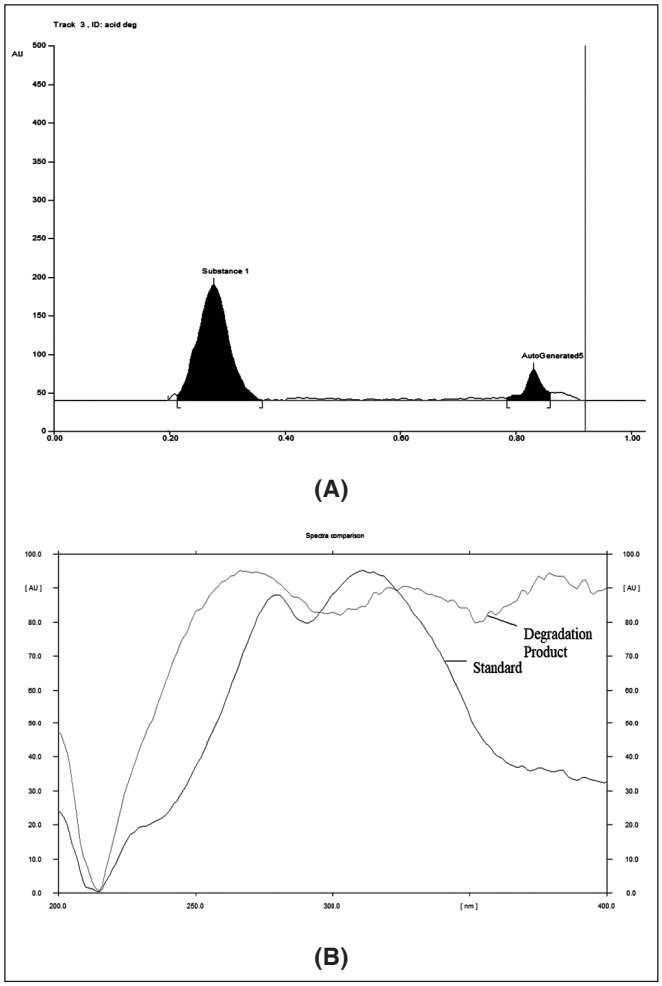

Fig. 3: A) 2D densitogram and B) spectral scan of peaks of acid hydrolysis

volume was made upto 10 mL with methanol and 10 µL of the resultant solution was applied on TLC plate and developed (Fig. 4).

Oxidative degradation using 30% hydrogen peroxide

To 5 mL of ceritinib stock solution (100 µg mL^{-1}), 1 mL of 30 % H_2O_2 was added and kept aside for 2 h. Then volume was made up to 10 mL with methanol and 10 µL of the resultant solution was applied on TLC plate and developed (Fig. 5).

Thermal degradation

20 mg of ceritinib was weighed and transferred to a clean, dried petri plate. The petri plate was placed in an oven at 105°C for 2 h. After 2 h, the sample was removed. 10 mg of the ceritinib was dissolved in methanol and further diluted to obtain 50 µg mL^{-1} solution. 10 µL of the resultant solution was applied on TLC plate and developed (Fig. 6).

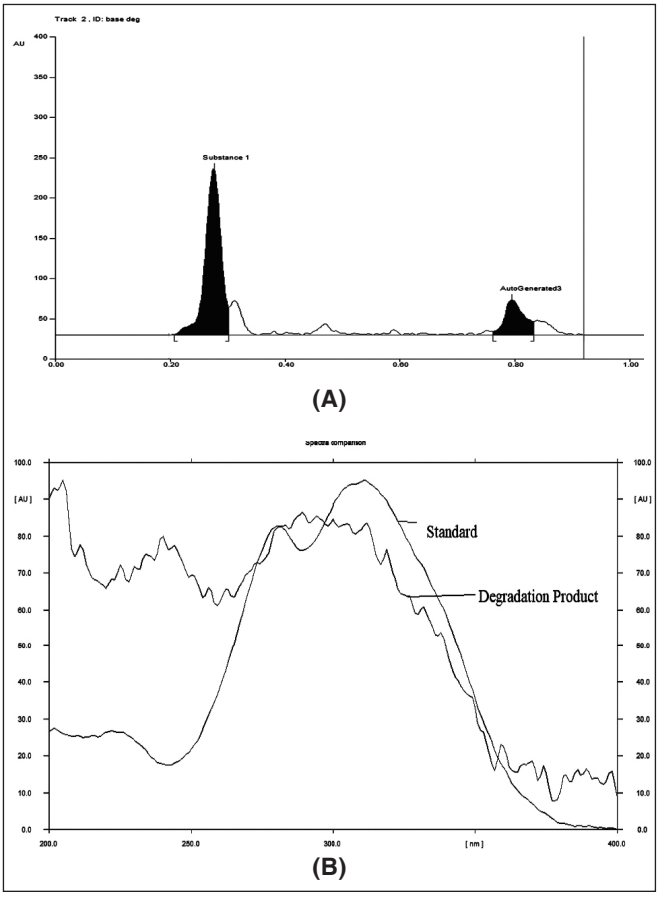

Fig. 4: A) 2D densitogram and B) spectral scan of peaks of base hydrolysis

PHOTOLYTIC DEGRADATION

UV light degradation

20 mg of ceritinib was transferred to dried petri plate and exposed to 200-watt h m^{-2} UV light. After exposure,

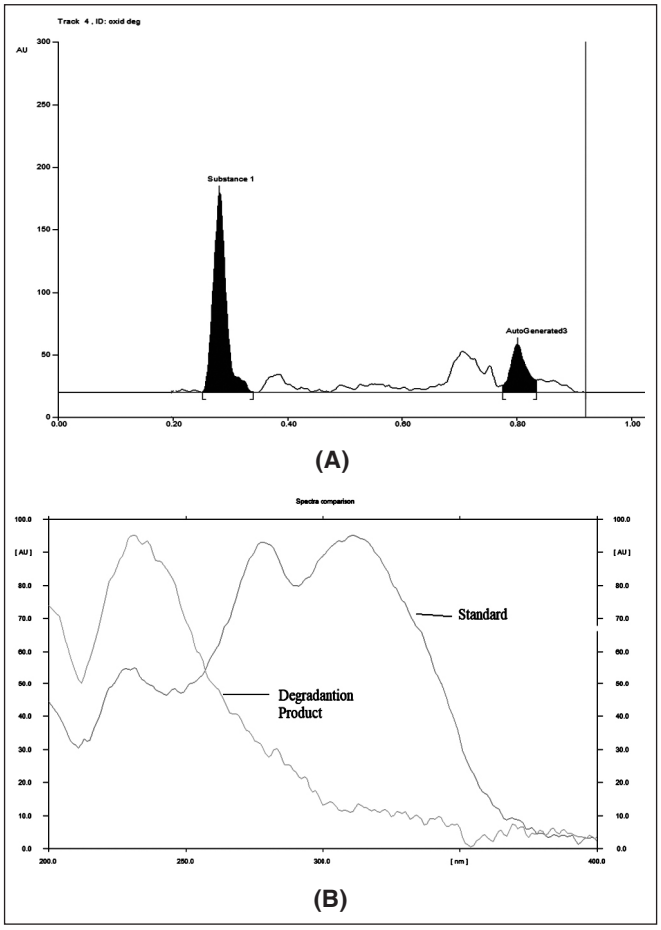

Fig. 5: A) 2D densitogram and B) spectral scan of peaks of oxidative degradation

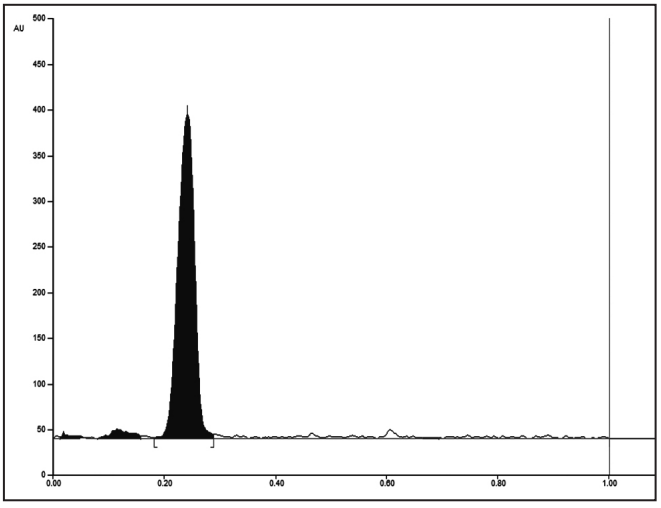

Fig. 6: 2D Densitogram of thermal degradation

drug (10 mg) was dissolved in 10 mL of methanol and further diluted to get 50 µg mL^{-1} solution. 10 µL of the resultant solution was applied on TLC plate and developed (Fig. 7).

Fluorescent light degradation

20 mg of ceritinib was transferred into dried petri plate and exposed to 1.2 million lux h of fluorescent light in photostability chamber. After exposure, 10 mg of drug was dissolved in 10 mL of methanol and further diluted to get 50 µg mL^{-1} solution. 10 µL of the resultant solution was applied on TLC plate and developed (Fig. 8).

METHOD VALIDATION

Method was validated as per ICH Q2(R1) guidelines[10].

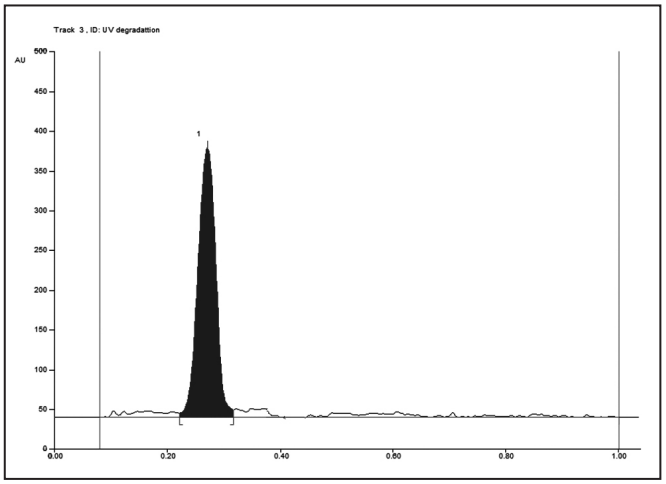

Fig. 7: 2D Densitogram after UV degradation

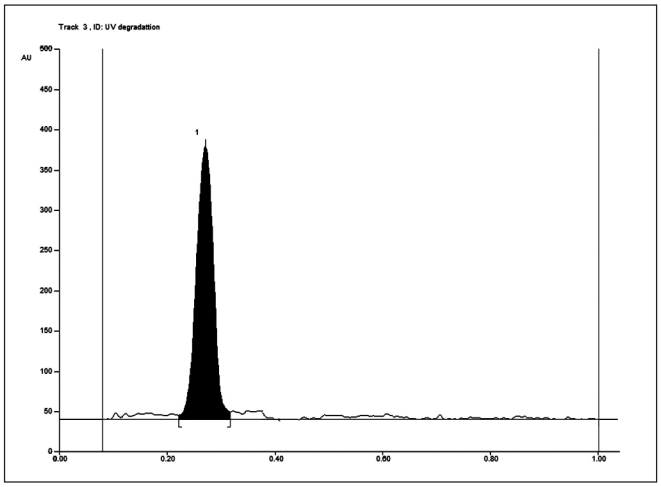

Fig. 8: 2D Densitogram after fluorescent degradation

Table I: Summary of forced degradation studies

Parameter	Stress condition	% Drug content	% degradation
Acid degradation	2 N HCl for 2 h	74.41	25.59
Alkaline degradation	2 N NaOH for 2 h	58.53	41.47
Oxidative degradation	30% H_2O_2 for 2 h	36.73	63.27
Thermal degradation	105^{0}C for 2 h	94.15	5.85
UV degradation	200 watt h m^{-2}	90.08	9.92
Fluorescence degradation	1.2 millon lux h	72.07	27.93

Table II: Results of linearity studies

Conc. (ng $band^{-1}$)	Area						Average	SD	%RSD
	1	2	3	4	5	6			
100	2394	2336	2356	2361	2357	2299	2351	28.79	1.225
200	4305	4338	4397	4307	4309	4387	4341	38.14	0.879
300	6302	6311	6384	6363	6333	6409	6350	38.56	0.607
400	8312	8312	8375	8345	8348	8599	8384	98.00	1.169
500	10437	10537	10657	10757	10888	10998	10712	193.37	1.805
600	12358	12452	12367	12387	12395	11987	12324	153.82	1.248

Linearity

Standard stock solution of ceritinib was diluted appropriately with methanol to have 50 µg mL^{-1} solution. Volume 2 µL, 4 µL, 6 µL, 8 µL, 10 µL and 12 µL were spotted on the TLC plate to get spotted amounts in the 100-600 ng $band^{-1}$ range. The plate was developed, and graph was plotted of area vs concentration in ng $band^{-1}$. Linearity was performed six times. The results were found linear with correlation coefficient (R^2) 0.998 and the regression equation y=20.29x+308.71. The linearity data is given in Table II and calibration curve and 3D densitogram shown in Fig. 9 and Fig. 10, respectively.

Precision

The method precision was checked for intra-day and inter-day precision at three different concentrations (200, 300, 400 ng $band^{-1}$). In intraday studies, three concentrations were analyzed on the same day. Interday

Table III (a): Results of intraday precision studies

Concentration in ng $band^{-1}$	Area	Amount recovered in ng $band^{-1}$	Recovery (%)	Avg ± SD	RSD (%)
200	4396	201.44	100.72	101.02± 0.22	0.223
	4417	202.48	101.24		
	4411	202.18	101.09		
300	6265	293.56	97.85	98.46± 1.19	1.212
	6238	292.23	97.41		
	6403	300.36	100.12		
400	8218	389.81	97.45	98.03±0.55	0.561
	8317	394.25	98.56		
	8317	394.69	98.67		

Table III (b): Results of interday precision studies

Concentration in ng $band^{-1}$	Area	Amount recovered in ng $band^{-1}$	Recovery (%)	Avg ± SD	RSD (%)
200	4379	200.61	100.30	99.74 ± 0.93	0.932
	4386	200.95	100.48		
	4303	196.86	98.43		
300	6352	297.85	99.28	99.76 ±0.48	0.481
	6371	298.78	99.59		
	6421	301.25	100.42		
400	8292	393.46	98.36	100.41±1.51	1.502
	8582	407.75	101.94		
	8501	403.76	100.94		

Table IV: Results of assay studies

Concentration (ng $band^{-1}$)	Concentration recovered (ng $band^{-1}$)	Percent recovered (%)	Mean ± RSD
200	198.93	99.47	100.81± 1.532
200	198.49	99.24	
200	198.34	99.17	
200	204.89	102.45	
200	203.56	101.78	
200	205.48	102.74	

Table V: Results of accuracy studies

Sr. No.	Level	Concentration in formulation (ng band^{-1})	Concentration of API added (ng band^{-1})	Area	% Recovery	Mean ± RSD
1	50%	200	100	6271	98.0	98.7±0.554
2		200	100	6337	99.0	
3		200	100	6345	99.2	
4	100%	200	200	8289	98.3	98.2±0.505
5		200	200	8221	97.5	
6		200	200	8315	98.6	
7	150%	200	300	10500	100.5	100.8±0.276
8		200	300	10566	101.1	
9		200	300	10544	100.9	

Table VI: Robustness data

Sr. No.	Parameter	% RSD
1	Wavelength variation ± 2 nm	1.23
2	Saturation time ± 2 min	1.17
3	Mobile phase composition ± 0.2 mL	1.02

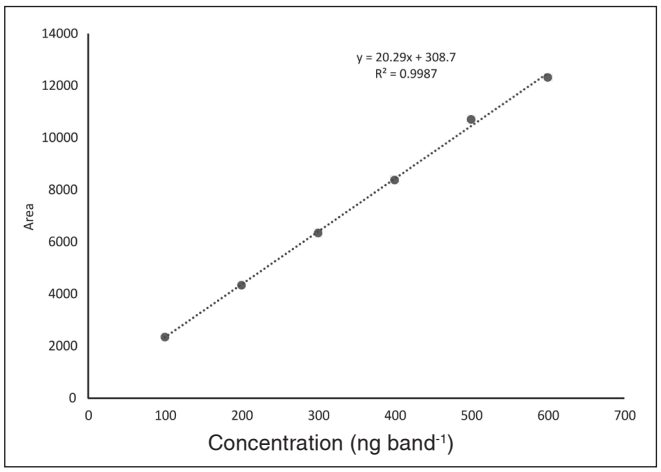

Fig. 9: Calibration curve of ceritinib

precision was checked on different days using the same method. The resulting peak areas were used to calculate the % RSD. For intraday and interday precision, results are given in Table III (a) and III (b). The % RSD was calculated, and values were found to be less than 2 %.

Detection limit and quantitation limit

The detection and quantitation limits of method were calculated from calibration curves. The equations were used to calculate with standard deviation of y-intercept

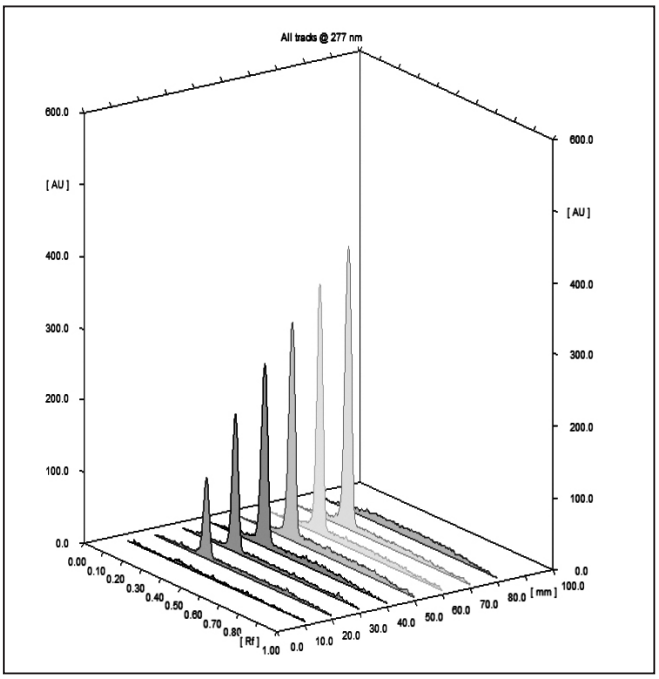

Fig. 10: Linearity of ceritinib

considered in calculation. The detection and quantification limits were 7.38 and 10.03 ng band^{-1}, respectively.

Assay

Assay was performed by spike blend method due to unavailability of the marketed preparation. 150 mg of ceritinib was blended with same amounts of lactose monohydrate and cellulose microcrystalline to have 450 mg of spike blend. The contents were properly mixed. The powder corresponding to 10 mg of ceritinib was weighed and dissolved in 10 mL of methanol, as described in preparation of stock solution (1000 µg mL^{-1}). The vial was sonicated for 10 minutes to allow complete dissolution

of ceritinib. Further dilution with methanol was carried out to get 100 µg mL^{-1} solution, which was again diluted with methanol to yield a concentration of 50 µg mL^{-1} and applied. The procedure was repeated six times. The assay results obtained are shown in Table IV.

Accuracy

Method accuracy was determined by standard addition method. To the synthetic mixtures of the product components known amounts of the API to be analyzed was added. The blend prepared for the assay was spiked with pure drug substance at 50 %, 100 % and 150% levels. This was evaluated utilizing 3 concentration levels covering the predefined range i.e. 3 concentrations/3 replicates. Obtained results are summarized in Table V.

Robustness

Robustness of the developed method was assessed by small but purposeful changes in mobile phase volume, saturation time and detection wavelength. Mobile phase volume was changed by ± 0.2 mL, saturation time was varied by ±2 min i.e., 18 min and 22 min. Detection wavelength was varied by ± 1 nm. One by one factor was varied at 200 ng $band^{-1}$ concentration to study effect on peak area of drug. As the % RSD values were less than 2, the method was found to be robust. Data is summarized in Table VI.

CONCLUSION

The proposed method quantitatively evaluated in terms of validation parameters as per ICH Q2 (R1) guideline. Consideration of all factors, indicate that the proposed HPTLC method is cost-effective, accurate and precise.

REFERENCES

1. https://en.wikipedia.org/wiki/Ceritinib accessed on 10 June 2021
2. Antolčić M., Runje M. and Galić N.: A simple and sensitive LC-MS/MS method for determination and quantification of potential genotoxic impurities in the ceritinib active pharmaceutical ingredient, **Anal. Methods,** 2020, 12(25), 3290-3295.
3. Du P., Guan Y., An Z., Li P. and Liu L.: A selective and robust UPLC-MS/MS method for the simultaneous quantitative determination of anlotinib, ceritinib and ibrutinib in rat plasma and its application to a pharmacokinetic study, **Analyst**, 2019, 144(18), 5462-5471.
4. Sakuntala M.S., Carey M.W. and Rao A.L.: Development and validation of HPLC method for determination of ceritinib in rabbit plasma using PDA detector, **Int. J. Pharm. Sci. Res.**, 2018, 9(7), 2897-2904.
5. Kumar C.N, Prathyusha V. and Kannappan N.: A novel validated stability indicating RP-HPLC method development for the estimation of ceritinib in it's bulk and finished dosage form as per ICH guidelines, **Der Pharm. Lett.**, 2014, 6(5), 339-351.
6. Adhao V.S., Sharma J. and Thakre M.: Development and validation of stability indicating RP-HPLC method for determination of ceritinib, **Indonesian. J. Pharm.**, 2018, 28(4), 241-248.
7. Vajjha S., Bommukuri V. and Rumalla C.S., Kaliyaperumal M. and Korupolu R.: Forced degradation of ceritinib under stress conditions: structural interpretation of novel degradants using HR-MS/MS and NMR, **Mater. Today: Proc.**, 2020, 33, 2067 - 2076.
8. Guidelines ICH, Stability testing of new drug substances and product. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, Q1A (R2). Geneva, Switzerland, 2003.
9. Guidelines ICH, Photostability testing of new drug substances and product. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, Q1A (R2). 1996.
10. Guidelines ICH, Validation of analytical procedures: text and methodology Q2 (R1). International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland, 2005.