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A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ANTI-CANCER DRUG BRIGATINIB IN BULK AND MARKETED PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC CHROMATOGRAPHIC TECHNIQUE

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ABSTRACT

Objective: A rapid, sensitive, selective, and reproducible reversed-phase high-performance liquid chromatographic method has been developed and validated for the determination of Brigatinib (BRG), is an anaplastic lymphoma kinase inhibitor used to treat anaplastic lymphoma kinase positive metastatic non-small cell lung cancer. **Methods:** The chromatographic separation was carried out in an isocratic mode on a Phenomenex Luna C18, 100A, 5µm, 250mm x 4.6mm i.d. column with a mobile phase consisting of methanol and acetonitrile containing in the ratio of 80:20% v/v at a flow rate of 1.0 ml/min. The run time was maintained for 8.0 min and detection was monitored at 271 nm. **Results:** The calibration plot was linear over the concentration range of 6–16 µg mL−1 with limits of detection and quantification values of 0.5 and 0.15ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.85%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%. **Conclusion:** This method was found to be simple, selective, precise, accurate, and cost-effective. Hence, the method can be successfully applied to analyze the Brigatinib in bulk form and marketed pharmaceutical dosage forms.

KEYWORDS: Brigatinib, RP-HPLC, Method Development, Validation, Precision.

INTRODUCTION

Brigatinib, originally named AP26113, is a reversible dual inhibitor of anaplastic lymphoma kinase (ALK) and epidermal growth factor receptor (EGFR). It presents selectivity against the mutant forms of EGFR compared to the wild-type.[1] It also exhibits selectivity against 9 different Crizotinib-resistant mutants of the EML4-ALK fusion gene, which is a pivotal player in the transformation of susceptible lung parenchyma. Brigatinib is a Kinase Inhibitor. The mechanism of action of Brigatinib is as a Tyrosine Kinase Inhibitor, and Cytochrome P450 3A Inducer. Brigatinib is used to treat a certain type of non-small cell lung cancer (NSCLC) that has spread to other parts of the bodys. Brigatinib is in a class of medications called kinase inhibitors.[2] It works by blocking the action of an abnormal protein that signals cancer cells to multiply. Brigatinib, originally named AP26113, is a reversible dual inhibitor of anaplastic lymphoma kinase (ALK) and epidermal growth factor receptor (EGFR). It presents selectivity against the mutant forms of EGFR compared to the wild-type. It also exhibits selectivity against 9 different Crizotinib-resistant mutants of the EML4-ALK fusion gene, which is a pivotal player in the transformation of susceptible lung parenchyma.[3] Brigatinib was developed by Ariad Pharmaceuticals, a subsidiary of Takeda Pharmaceutical Company Limited, and FDA-approved on April 28, 2017. The IUPAC Name of Brigatinib is 5-chloro-4-N-(2-dimethyl phosphoryl phenyl)-2-N-[2-methoxy-4-[4-(4-methyl piperazin-1-yl) piperidin-1-yl] phenyl] pyrimidine-2, 4-

diamine. The Chemical Structure of Brigatinib is shown if follows.

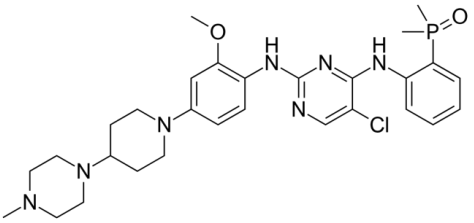

Fig-1: Chemical Structure of Brigatinib.

Method Development

Instruments Used

Table-1: List of Instrument used.

S. No.	Instruments/Equipments/Apparatus
1.	**HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	**ELICO SL-159** UV – Vis spectrophotometer
3.	Electronic Balance **(SHIMADZU ATY224)**
4.	Ultra Sonicator**(Wensar wuc-2L)**
5.	Thermal Oven
6.	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.
7.	P^H Analyser **(ELICO)**
8.	Vacuum filtration kit **(BOROSIL)**

Chemicals / Reagents Used

Table-2: List of Chemicals used.

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
4.	Dipotassium hydrogen orthophosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
5.	Potassium dihydrogen Orthophosphate	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
6.	Ortho Phosphoric acid	96%	A.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric Acid	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
8.	Sodium Hydroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
9.	Hydrogen Peroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai

Method Development and its Validation for Brigatinib by RP-HPLC

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It is scanned in the UV spectrum in the range of 200 to 400nm.

This has been performed to know the maxima of Brigatinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Brigatinib.[4] While scanning the Brigatinib solution we observed the maxima at 226 nm.

The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450. The scanned UV spectrum is shown below,

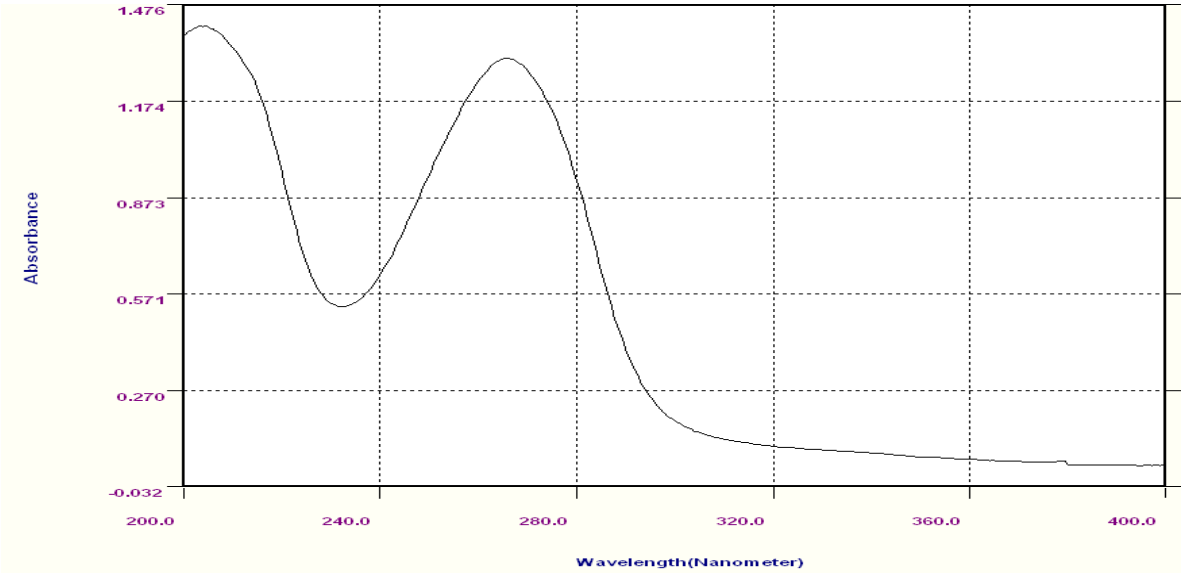

Fig-2: UV Spectrum for Brigatinib (271 nm)

Sample & Standard Preparation for the Analysis

25 mg of Brigatinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.

Further dilution was done by transferring 0.4ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.[5]

Optimization of Chromatographic Conditions

The chromatographic conditions were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.[6]

Table 3: Summary of Process Optimization.

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol : Water = 75:25	1.0ml/min	271nm	Very Low response	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol : Water = 50:50	1.0ml/min	271nm	Low response	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Acetonitrile: Water = 60:40	1.0ml/min	271nm	Tailing peaks	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol :Acetonitrile=50:50	1.0ml/ min	271nm	Broad Peak	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol :Acetonitrile =80:20	1.0ml/ min	271nm	Tailing peaks	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Acetonitrile: Methanol =65:35	1.0ml/ min	271nm	Good Peak	Method accepted

Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions obtained from experiments can be summarized as below.[7]

Table-4: Summary of Optimized Chromatographic Conditions.

Mobile phase	Methanol :Acetonitrile = 80:20% v/v
Column	Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.
Flow rate	1.0 ml/ min.
Wavelength	271nm
Sampling System	Automatic
Temp. of Auto sampler	Ambient
Volume of Injection	10µl
Run time	08 min.
Mode of Separation	Isocratic

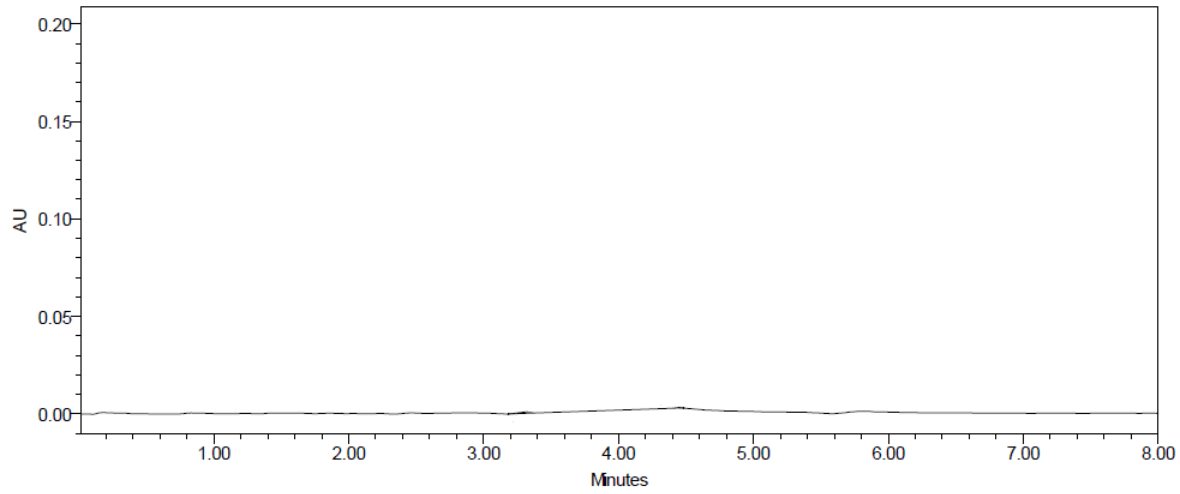

Fig-3: Chromatogram for Blank Solution.

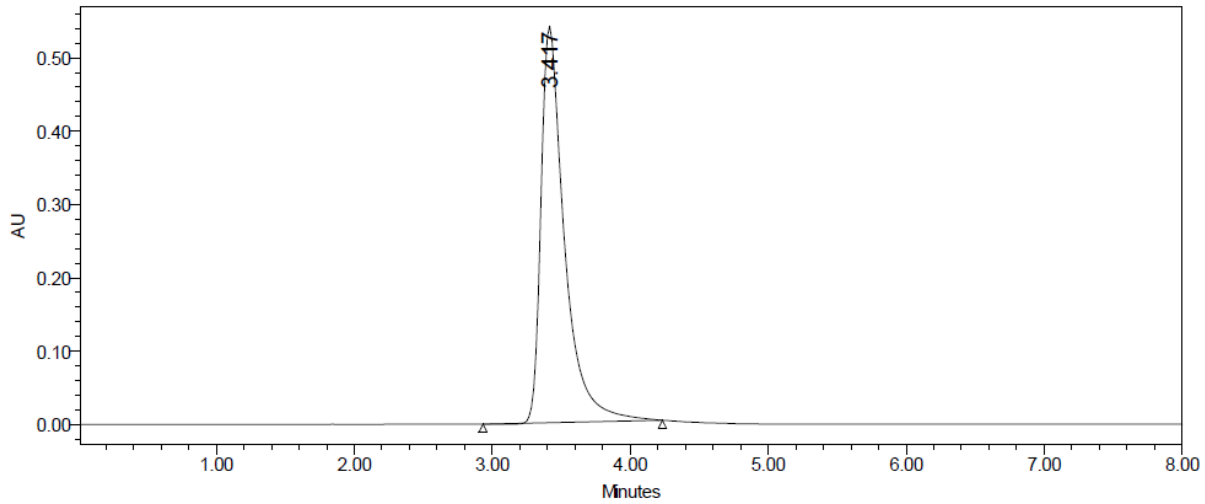

Fig-4: Chromatogram of Brigatinib in Optimized Condition.

Preparation of Mobile Phase

650mL (65%) of Acetonitrile and 350mL of Methanol (35%) were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.[8]

Sample & Standard Preparation for the Analysis

25 mg of Brigatinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.4ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.[9]

Method Validation

Linearity and Range

Standard solutions of Brigatinib in the concentration range of 0 µg/ml to 16 µg/ml were obtained by transferring (0.6, 0.8, 1.0, 1.2, 1.4, 1.6ml) of Brigatinib stock solution (1000ppm) to the series of 10 ml volumetric flasks. The volumetric flasks were made up to the mark with mobile phase. The solutions were filtered

through a 0.45 µm membrane filter and degassed under ultrasonic bath. The final resulted solutions were injected into HPLC the system. The run time/stop time maintained was 8 min and the various types of peak areas were measured.[10-13] The calibration data are shown in Table 5 and calibration curve data are shown in figure-5.

Table-5: Calibration Data for Brigatinib.

S. No.	Conc. (µg/ml)	Mean Peak Area
1	0	0
2	6	61233
3	8	84610
4	10	110247
5	12	130435
6	14	153354
7	16	172043

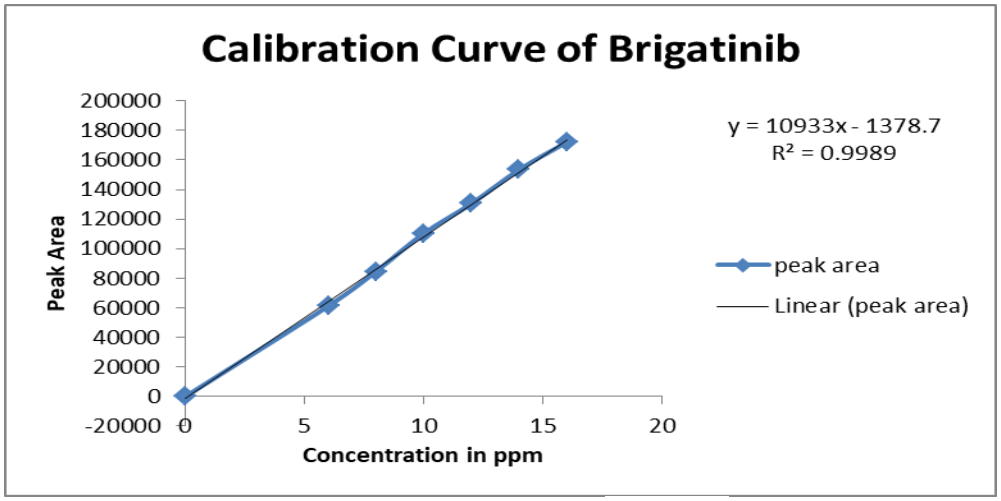

Fig-5: Calibration Curve for Brigatinib.

RESULT AND DISCUSSION

Linearity range was found to be 0-16 µg/ml for Brigatinib. The correlation coefficient was found to be 0.998 and the slope was found to be 10933 and intercept were found to be 1378 for Brigatinib.

Accuracy

Accuracy: Recovery Study of Brigatinib

The accuracy of the proposed developed method the % recovery studies were carried out by adding different quantities (80%, 100%, and 120%) of pure drug of Brigatinib was taken and added to the prepared pre-analyzed formulation of concentration 10µg/ml. From that % recovery values were measured.[14-16] The results were shown in Table-6.

Table 6: Data of Recovery Studies for Brigatinib.

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	8	8.105	93435	101.312	Mean= 100.0163% S.D. = 1.293505 % R.S.D.= 1.293294
S_2 : 80 %	8	7.898	91287	98.725	
S_3 : 80 %	8	8.001	92356	100.012	
S_4 : 100 %	10	10.195	115135	101.95	Mean= 101.4033% S.D. = 0.613379 % R.S.D.= 0.60489
S_5 : 100 %	10	10.152	114687	101.52	
S_6 : 100 %	10	10.074	113879	100.74	
S_7 : 120 %	12	12.171	135647	101.425	Mean= 100.6053% S.D. = 0.730041 % R.S.D. = 0.725649
S_8 : 120 %	12	12.044	134324	100.366	
S_9 : 120 %	12	12.003	133897	100.025	

Precision

Repeatability

Repeatability was assessed using six time repetition of working concentration.[17]
The results are shown in Table 7.

Table-7: **Data Showing Repeatability Analysis for Brigatinib.**

HPLC Injection Replicates of Brigatinib	Area Under the Curve
Replicate – 1	112546
Replicate – 2	113824
Replicate – 3	111351
Replicate – 4	111584
Replicate – 5	112419
Replicate – 6	112572
Average	**112382**
Standard Deviation	**876.7543**
% RSD	**0.7801**

RESULT AND DISCUSSION: The repeatability study which was conducted on the solution having the concentration of about 10 µg/ml for Brigatinib (n =6) showed a RSD of 0.7801% for Brigatinib. It was concluded that the analytical technique showed good repeatability.[18]

Intermediate Precision

Intra-Assay & Inter-Assay

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Brigatinib revealed that the proposed method is precise.[19]

Table 8: Results of Intra-Assay & Inter-Assay for Brigatinib.

Conc. of Brigatinib (API) (µg/ml)	Observed Conc. of Brigatinib (µg/ml) by the Proposed Method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	8.07	0.27	8.95	0.34
10	10.39	0.39	10.56	0.65
12	11.19	0.23	12.01	0.34

RESULT AND DISCUSSION: The intraday and interday studies results show that the mean % RSD was found to be within acceptance limit i.e. (≤2%). Hence it was concluded that there was no significant difference for the assay, which was tested within the day and between the days[20]. So, we concluded that the proposed method at selected wavelength was found to be precise.

Method Robustness

The influence of small changes in optimized chromatographic conditions such as changes in Flow Rate (± 0.1ml/min), Organic Phase (±5^{0}C), Wavelength of detection (±2nm) & Mobile Phase (±2%) studied to determine the robustness of the method are also in favour of (Table-9, RSD (%) < 2%) the proposed RP-HPLC method was used for the analysis of Brigatinib (API).[21-23]

Table-9: Result of Method Robustness Test.

Change in Parameter	% RSD
Flow (1.1 ml/min)	1.13
Flow (0.9 ml/min)	0.09
More Organic	0.78
Less Organic	0.72
Wavelength of Detection (273 nm)	0.77
Wavelength of detection (269 nm)	0.59

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantization (LOD and LOQ) can be determined by the following equations. These equations are based on the signal to noise ratio.[24] These two equations are useful for the determination of LOD and OQ.

L.O.D. = 3.3 (SD/S).

L.O.Q. = 10 (SD/S)

Where,

SD = Standard deviation Response

S = Slope of the Calibration curve

The slope S and standard deviation response values are obtained from the calibration curve of the analyte (Drug).

RESULT AND DISCUSSION: The LOD was found to be 0.05 µg/ml and LOQ was found to be 0.15 µg/ml for Brigatinib which represents that sensitivity of the method is high.

System Suitability Parameter

This includes the type of equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be examinated. The following system suitability test parameters were determined.[25] The obtained data are shown in Table 10.

Table-10: Data of System Suitability Parameter.

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	2.81
2	Asymmetry	T ≤ 2	Brigatinib =0.26
3	Theoretical plate	N > 2000	Brigatinib =2986

Assay of Brigatinib in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system.[26-28]

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded.

ASSAY

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of Test obtained with test preparation

AS = Peak Area of Standard obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Assay was performed as described in previous chapter. Results obtained are tabulated below.

Table-11: Assay of Brigatinib Tablets.

Brand Name of Tablets	Labelled Amount of Drug (mg)	Mean (± SD) Amount (mg) Found by the Proposed Method (n=6)	Mean (± SD) Assay (n = 6)
Alunbrig Tablets (Takeda Pharmaceutical Company Limited)	30	29.285 (± 0.486)	99.854 (± 0.567)

RESULTS AND DISCUSSION: The assay of Alunbrig Tablets containing Brigatinib was found to be 98.854%.

Forced Degradation Studies

The results of the stress studies indicated the specificity of the method that has been developed. Brigatinib was stable in photolytic and acidic stress conditions.[29-32] The result of forced degradation studies are given in the following table-12.

Table-12: Results of Forced Degradation Studies of Brigatinib API.

Stress Condition	Time	Assay of Active Substance	Assay of Degraded Products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	85.69	14.31	100.0
Basic Hydrolysis (0.1M NaOH)	24Hrs.	83.47	16.53	100.0
Wet heat	24Hrs.	79.86	20.14	100.0
UV (254nm)	24Hrs.	87.92	12.08	100.0
3 % Hydrogen peroxide	24Hrs.	80.81	19.19	100.0

SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for simultaneous analysis of Brigatinib different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Phenomenex Luna C_{18},

100A, 5µm, 250mm x 4.6mm i.d. column was preferred because using this column peak shape, resolution and absorbance were good. Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Brigatinib it is evident that most of the HPLC work can be accomplished in the wavelength range of 271 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 10 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay & stability which can help in the simultaneous analysis of Brigatinib in different formulations.

BIBLIOGRAPHY

1. https://go.drugbank.com/drugs/DB12267
2. https://pubchem.ncbi.nlm.nih.gov/compound/Brigatinib
3. https://en.wikipedia.org/wiki/Brigatinib
4. "Principles of Instrumental Analysis", 5th edition, Harcourt Publishes Int Company, Skoog, Holler and Nieman, Chapter 28, p.726-766.
5. "HPLC Columns" Theory, Technology and Practice. Uwe D. Neue, Wiley-VC.
6. Handbook of HPLC, Vol.78, by Elena Katz et al. Marcel Dekker Inc.
7. "Instrumental Methods of Chemical Analysis", 5th Edition, Galen W. Ewing, McGraw Hill Book Company, 1988.
8. "HPLC in Pharmaceutical Industry", Fong and Long, Marcel Dekker Series.
9. "Instrumental Method of Chemical Analysis" by Chatwal Anand, Himalaya Publishing House, p.no.615-623.
10. Dr. Kealey and P.J Haines, Analytical Chemistry, 1stedition, Bios Publisher, 2002; P1-7.
11. "Practical Pharmaceutical Chemistry", 4th edition, Part 2, by Beckett and Stenlake, CBS Publishers and Distributors, P.No.157-174.
12. Govt. of India, Ministry of Health and Family Welfare. Vol. 2. Delhi: Publication by Controller of Publication; 2007. Indian Pharmacopoeia, 484–554.
13. British Pharmacopoeia. (International Ed.) 1993; Vol. 1:429, 483. Published on the Recommendation of the Medicines Commissions Pursuant to Medicines Act, 1968; 1993.
14. United States Pharmacopoeia 29 NF 24, Published on the Recommendation of the Medicines Commissions Pursuant to Medicines, page no. 587.
15. Skoog, West, Holler, Crouch, "Fundamentals of analytical chemistry", eighth edition, 2009 (Indian edition), Cengage learning India Pvt ltd, New Delhi, Page no. 271-280.
16. A.V Kasture, K.R Mahadik, S.G Wadodkar, H.N. More, "A textbook of pharmaceutical analysis, Instrumental methods", Nirali Prakashan, vol.2, 9th edition, page no. 5-7: 28-30.
17. Settle FA, In: Handbook of Instrumental Techniques for Analytical Chemistry. 1st Ed, Singapore, Pearson Education Inc, 2004.
18. Willard HH and Dean AJ. Instrumental Methods of Analysis. CBS Publishers and distributors, 7th Ed, 1986; 513-515.
19. Connors AK. In: A Text Book of Pharmaceutical Analysis. A Wiley Interscience Publication, 3rd Ed, 2005; 373-400.
20. Ahuja S. In: High Pressure Liquid Chromatography of Comprehensive Analytical Chemistry. Elsevier Publications, 2006.
21. Principles and Methods. In: Amesham Biosciences of Reversed Phase Chromatography, 6-8.
22. Synder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development, 2nd Ed, John Wiley and Sons Inc. Canada, 1997.
23. Mohammad T et al., HPLC Method Development and Validation for Pharmaceutical Analysis- A Review. International Pharmaceutica Sciencia, 2012; 2(3): 14.
24. Snyder LR, Kirkland JJ and Glajch JL. In: Practical HPLC Method Development. 2nd ed, 2001.
25. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 22-23.
26. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc, 2006; 88-92.
27. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva, 1996; 11. (CPMP/ICH/281/95).
28. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4): 17-25.
29. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al. International Journal of Analytical and Bioanalytical Chemistry, accepted, 20 November 2015.
30. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC Chromacademy.
31. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.
32. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
33. Mossammat Rima Akter 1, Md. Saddam Hossain 2, K M Khairul Alam 1 and Md Rafiquzzaman 1, *, Development and Validation of RP-HPLC Method for the Determination of Anticancer Drug Brigatinib, GSC Biological and Pharmaceutical Sciences, 2023; 23(03): 030–041, https://doi.org/10.30574/gscbps.2023.23.3.0215.
34. J L Gulikers 1 2, A J van Veelen 1 2 3, E M J Sinkiewicz 1, Y M de Beer 1, M Slikkerveer 1, L M L Stolk 1, V C G Tjan-Heijnen 4, L E L Hendriks 5, S Croes 1 2, R M J M van Geel 1 2, Development and validation of an HPLC-MS/MS method to simultaneously quantify Brigatinib, Lorlatinib,

Pralsetinib and Selpercatinib in human K2-EDTA plasma, Biomed Chromatogr, 2023 Jun; 37(6): e5628. Doi: 10.1002/bmc.5628. Epub 2023 Apr 12.