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Development and Validation of RP-HPLC Method for the Determination of Anticancer Drug Brigatinib

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Abstract

Now-a-days, one of the most devastating and life-threatening disorders is cancer. Various types of cancer based on the organ affected are namely lung cancer, breast cancer, stomach cancer, colon cancer etc. A number of anticancer drugs are obtainable among which tyrosine kinase inhibitor is the superior one. The available tyrosine kinase inhibitors are namely Crizotinib, Ibrutinib, Osimertinib, Brigatinib. Among them, brigatinib is the recent INN drug which belongs to second generation of tyrosine kinase inhibitor and indicated for non-small cell lung cancer. Literature review shows that there are no developed methods available for determination of brigatinib except UPLC method. In the present study, authors aimed to develop and validate RP-HPLC method for determination of brigatinib. Regarding RP-HPLC method, C18 column (ID: 5 micron*100 Å) was employed as stationary phase while combination of methanol and distilled water (75%:25%) was used as mobile phase. The retention time of brigatinib was found 5.6 min from the chromatogram. The regression equation was y = 53344x - 239.6 with coefficient of determination (R^2) 0.999 and correlation coefficient (r) 0.9994; indicating excellent linearity of the calibration curve obtained by the newly developed method. Percent (%) RSD for robustness, ruggedness, precision was below 2. Limit of detection and limit of quantification were found to be 0.332 µg/mL and 1.00 µg/mL, respectively. The findings ensure the suitability of the developed method as an established one to assay brigatinib in any quality control laboratory.

Keywords: Brigatinib; Method validation; RP-HPLC; Quality control; ICH guidelines; Limit of quantification (LOQ)

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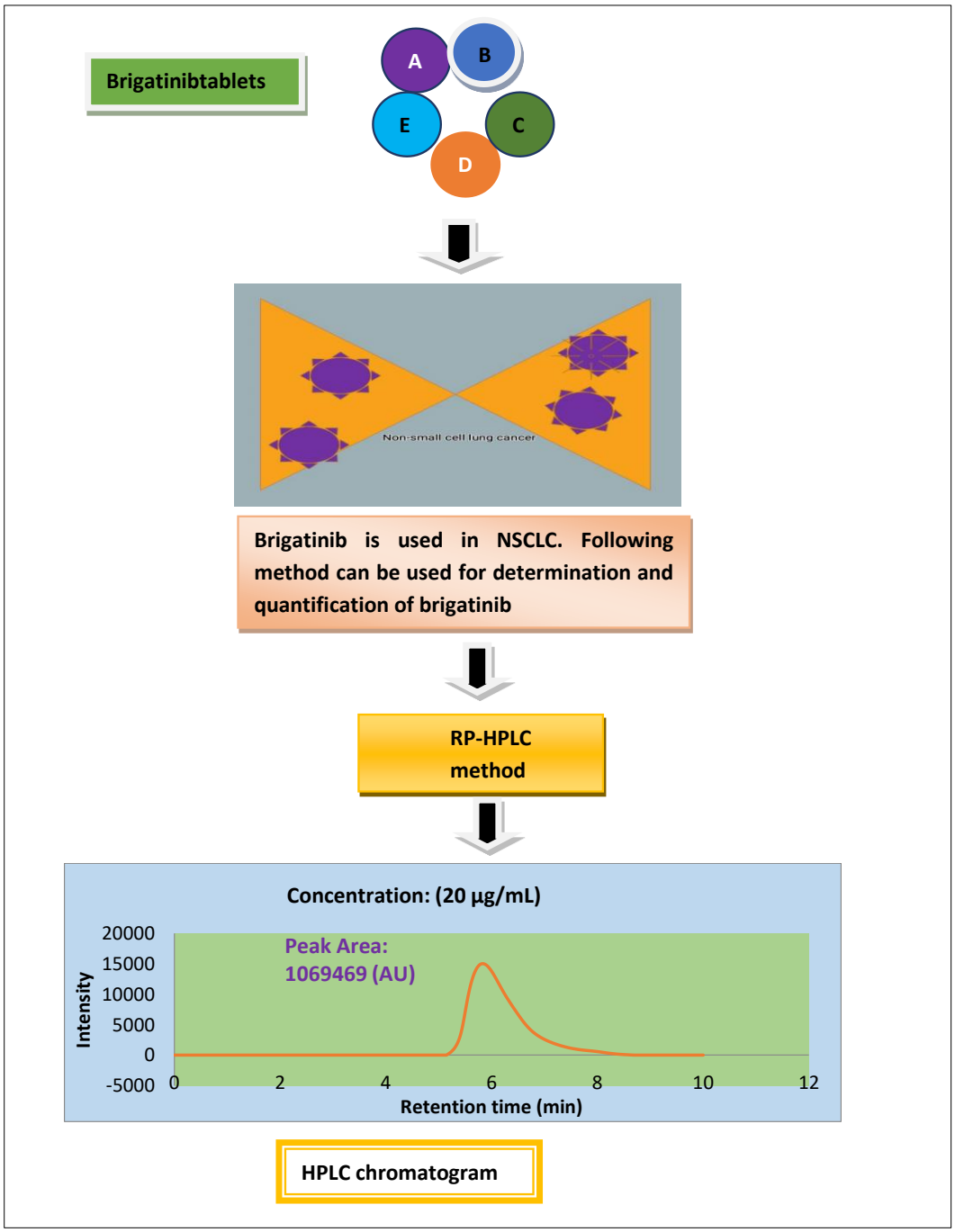
Graphical abstract



1. Introduction

Human life becomes disease amenable and injurious due to unhealthy lifestyle successively. Ultimate outcomes are various breakneck and dangerous diseases. Cancer is one of the life-threatening conditions which is the eventuality of uncontrolled growth and spread of abnormal cells in body [1]. Cancer can be of different types based on the origin namely lung cancer, colon cancer, liver cancer, stomach cancer, bladder cancer etc. Cancer prevention is mostly possible by maintaining proper and healthy lifestyle along with avoiding cancer triggers viz. dietary factors, environment pollutions, certain infections, less physical exercise, obesity etc. [2]. Besides surgery, radiation therapy, stem cell therapy etc. the most prevalent therapy is chemotherapy using anticancer agents. The familiar anticancer agents include alkylating agents, antibiotics, topoisomerase inhibitors, antimetabolites, tyrosine kinase inhibitors and mitotic inhibitors [3].

Anaplastic lymphoma kinase, a kind of tyrosine kinase inhibitor (TKI) is recently used in various cancer treatments. Rearrangement of the anaplastic lymphoma kinase (ALK) gene is responsible for 5% of non-small cell lung cancers (NSCLCs) [4]. Brigatinib is the novel second-generation ALK-TKI which has shown preclinical activity, with consistent clinical efficacy in crizotinib-intolerable patients [5, 6, 7, 8, 9]. The chemical name of brigatinib is 5-Chloro-2-N-{4-[4-(dimethylamino)piperidin-1-yl]-2-methoxyphenyl}-4-N-[2 (dimethylphosphoryl) phenyl] pyrimidine-2, 4-diamine and its structure is given below (Figure 1).

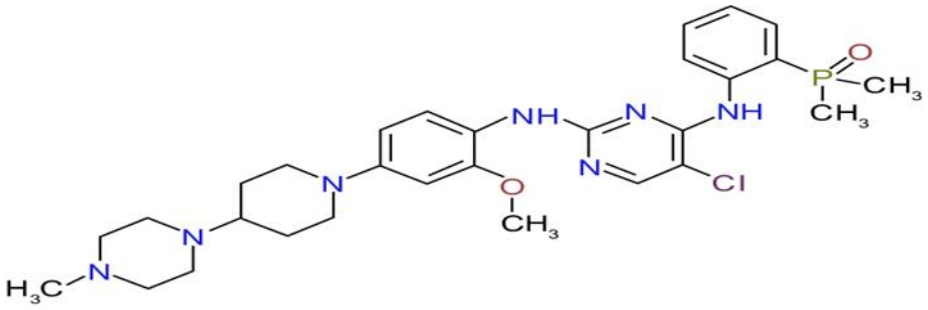


Figure 1 Structure of brigatinib (from Goggle)

Method development refers to developing a specific method for any purpose while method validation ensures that the method is completely developed and suitable for its intended use. However, literature review showed that no method has yet been developed and reported on RP-HPLC for brigatinib although this technique is widely used and authentic in pharmaceutical analysis for various drugs [10, 11, and 12]. Therefore, the authors took interest in developing methods based on RP-HPLC for the quantification of brigatinib. The developed method will be helpful for other researchers and manufacturers who are working and interested in this field.

2. Literature review

Comprehensive literature review using Goggle Scholar, PubMed, internet, and Sci-hub was executed. Only one article was cited in the literature for the determination of brigatinib, but it was on RP-UPLC (Reverse Phase-Ultra Performance Liquid Chromatography) method development and validation for brigatinib [13]. However, it is an expensive and less prevalent LC system in pharmaceutical companies around the globe especially in relatively less developed countries. RP-HPLC method [10, 11, 12, 14, 15, 16, 17, 18, 19, and 20] is comparatively more available, simple and widely used for various drugs but it was not developed and reported yet for the analysis of brigatinib. Therefore, authors of the present study aimed to develop and validate RP-HPLC method for the analysis of brigatinib. Literature review showed method development is the development of a suitable method whereas any developed method must comply with several parameters for being validated as per ICH guidelines [10, 11, 12, 20, 21, 22 23, 24, 25, 26, 27]. The parameters include specificity, linearity, accuracy, precision, ruggedness, robustness, etc.

Aims and Objectives

Brigatinib is a novel and recently launched INN drug indicated for the treatment of non-small cell lung cancer. Literature review displayed clearly that though RP-UPLC was reported but no developed and validated RP-HPLC method is available in the literature for the quantification of brigatinib. RP-UPLC unit itself is much costlier and less available in most of the pharmaceutical companies compared to that of RP-HPLC.Moreover, an expensive (0.1 % triethylamine: ACN; 20:80) solvent system was used in RP-UPLC.

Therefore, the aims and objectives of the present research work were to develop and validate RP-HPLC method for analysis of brigatinib, and using less expensive solvent mixture (distilled water: methanol; 25%: 75%).

3. Experimental

3.1. Reagents & Solvents

Distilled water, HPLC grade methanol (Merck), HPLC grade Ethanol (CARLO ERBA), HPLC grade Acetonitrile (Chemsavers), reagent grade HCl, HPLC grade DMSO (WOLDO) were the required reagents and those were purchased from local vendors except distilled water. It (distilled water) was prepared in the laboratory.

3.2. Reference standard and Samples

Brigatinib reference standard (100.8 %), brigatinib raw material, Brigatinib 180 mg tablet of a renowned pharmaceutical company was used in the present study.

3.3. Materials

The required materials and glassware were volumetric flasks (25 mL, and 100 mL), pipettes (5 mL, 10 mL, and 20 mL), a beaker (100 mL), Aluminum foils, Parafilm foils, Filter papers, Droppers, Test tubes, Glass slides, a Measuring cylinder (100 mL), and Sample tubes.

3.4. Apparatus

The necessary apparatus included in the present study were HPLC device (Shimadzu, SPD-20AV, Japan), Luna C18 column (ID: 5-micron x 100 Å), Electronic Balance (Shimadzu, Japan), Precision balance (Mettler Toledo, Switzerland), Laboratory Oven (Labtech, India), Filter apparatus, Sonication Degasser (Sonorex digital, Bandelin), pH meter (HANNA, Portugal).

4. RP-HPLC method Development

4.1. Solubility profile

To select the right solvent, the solubility profile of brigatinib was examined with some solvent. It was found that brigatinib is soluble in methanol, acetonitrile, and combination of methanol and distilled water at the ratio 75%:25% (methanol: water). Brigatinib is found to dissolve in ethanol, and DMSO too in mild hot condition.

4.2. Selection of mobile phase

If only methanol is used as mobile phase, the pressure of C18 column is raised, therefore combination of methanol and distilled water (75%:25%) after various trial was decided to use as mobile phase. Moreover, easy availability of methanol and water, and also less price of methanol over acetonitrile, and DMSO contributed to selection of the mobile phase.

4.3. Optimization of chromatographic conditions

4.3.1. Selection of column

C18 column (ID: 5-micron x 100 Å) was used as stationary phase, which gives a larger surface area to mobile phase has to travel across.

4.3.2. Selection of mobile Phase

Combination of methanol and distilled water at the ratio (75%:25%) gave optimum performance as mobile phase and hence it was used as the mobile phase in the present study.

4.3.3. Selection of flow rate

Flow rate was selected at 1 mL/min. In this case, pump A (which pumps methanol) was adjusted to 0.75 mL/min and pump B (which pumps distilled water) was adjusted to 0.25 mL/min.

4.3.4. Selection of injection volume

Injection volume was selected as 10 μL.

4.3.5. Selection of Run time

The peak of brigatinib was found within 5 min to 8.5 min (Figure 2), so run time was selected as 10 min.

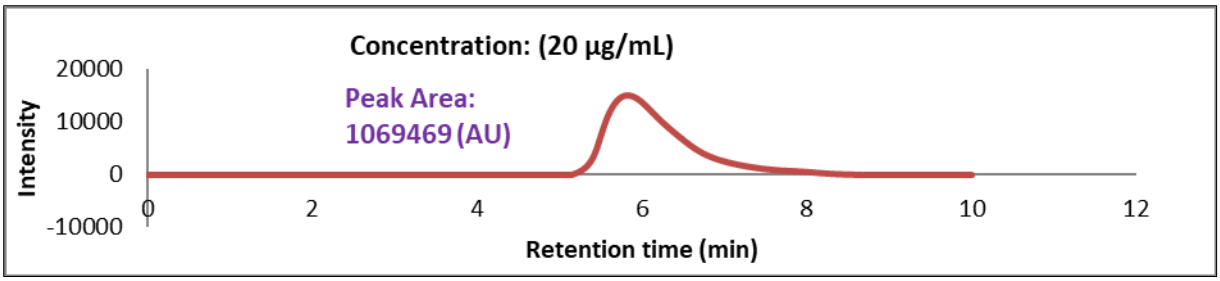


Figure 2 Chromatogram of brigatinib

4.3.6. Selection of Wavelength

Brigatinib showed two absorption bands in the UV region of its spectrum, which were 210 nm and 283 nm. Both of the wavelengths were used for the detection of brigatinib and hence to optimize the chromatogram:

Interference of the mobile phase around 5-6 min was observed when detection wavelength was set at 210 nm. But there was no interference of mobile phase at around 5-6 min when the detection wavelength 283 nm was used. That is why the 283 nm wavelength was selected as detection wavelength in the present chromatography experiments. Therefore, it was concluded that the retention time at 5.6 min (Fig. 2) was for the brigatinib under the optimized RP-HPLC conditions. And it was the first-time report for the retention time of brigatinib with the optimized chromatographic conditions shown in Table 1. It, thus, created the basis for the development of the RP-HPLC method under the present work.

Table 1 Optimized Conditions for RP-HPLC

Parameters	**Conditions in the Developed Method**
Column	C18 (ID: 5-micron x100 Å)
Mobile Phase	(75% Methanol:25% Distilled water)
Flow rate	1 mL/min
Injection volume	10 µL
Run time	10 min
Detection Wavelength	283 nm
Temperature	25° C
Type of elution	Isocratic
Detector	UV detector
Retention time	5.6 min

4.4. Determination of Calibration Curve for the RP-HPLC Method with the Standard Brigatinib:

First of all, 0.004 g standard brigatinib (powder) was taken in a 100 mL volumetric flask. Then, a small quantity of methanol was added into the flask, and the flask was shaken rigorously. Then methanol was added up to the mark to make the concentration of the solution 40 µg/mL. This was the stock solution of the standard brigatinib. A serial half-dilution was performed with the stock solution for the remaining work. Five (5) diluted standard solutions (20 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL) were thus prepared with the standard brigatinib stock solution (40 µg/mL) and RP-HPLC was done with the respective solution and their chromatogram were recorded.

Peak areas for the five standard solutions were plotted on Y-axis and the concentrations under those areas were plotted on X-axis to prepare a calibration curve, and the obtained curve is shown below (Figure 3):

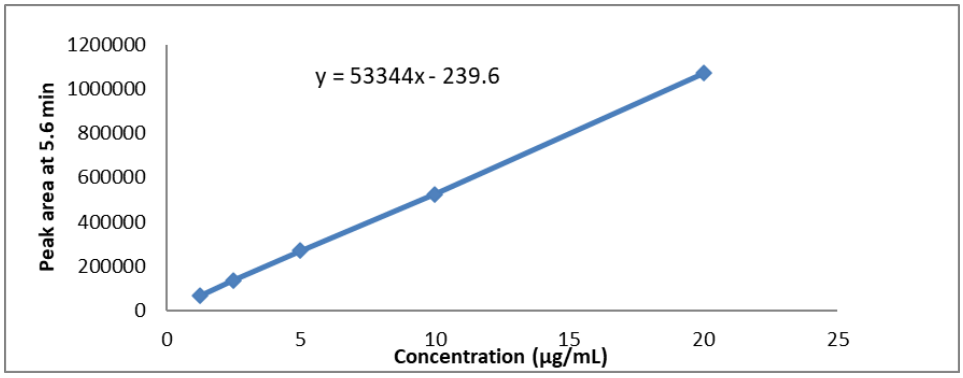


Figure 3 Calibration curve of RP-HPLC developed method for brigatinib

5. RP-HPLC method validation

The developed RP-HPLC method was subjected for validation as per the ICH guidelines and discussed below:

5.1. Linearity

Stock solution was diluted to make various solutions of different concentration such as 20 µg/mL, 10 µg/mL, 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL.

The concentrations of the five diluted standard solutions were plotted on X-axis and the peak areas corresponding to the respective concentration of the sample solutions at 210 nm were plotted (Fig. 3) on Y-axis to prepare a calibration curve, and to find the regression equation, coefficient of determination, and range.

5.2. Dynamic range

Dynamic range was calculated by observing the upper concentration and the lower concentration at which linearity, precision, accuracy was observed properly from the calibration curve (Fig. 3).

5.3. Specificity/ Selectivity

In a solution of brigatinib having concentration 10 µg/mL, small quantities of starch, lactose, magnesium stearate were added which are the possible interfering materials used in tablet dosage form. The specificity of RP-HPLC method was evaluated by calculating percentage recovery of the drug brigatinib.

5.4. Precision

5.4.1. Intraday precision / Repeatability

Repeatability of RP-HPLC method was analyzed by assaying six replicates of each of three concentration levels 10µg/mL, 5 µg/mL, and 2.5 µg/mL during the same day, under the same experimental conditions by the same analyst. Precision was expressed as % RSD.

5.4.2. Inter day precision / Intermediate Precision

Intermediate precision RP-HPLC method was evaluated by assaying three replicates of each of three concentration levels 20µg/mL, 10 µg/mL, and 5 µg/mL on three different days, under the same experimental conditions. Precision was expressed as % RSD.

5.5. Accuracy

The accuracy of RP-HPLC method was evaluated by recovery studies. In a solution of brigatinib having concentration 5 µg/mL, brigatinib was further added to make the concentration of solutions 10 µg/mL, 15 µg/mL, and 20 µg/mL. The accuracy was expressed as percentage recovery of the drug.

5.6. Ruggedness

Ruggedness of RP-HPLC method was evaluated by assaying six replicates for each of three concentration levels 20μg/mL, 10 μg/mL, and 5 μg/mL on a separate day by different analyst and HPLC device. Ruggedness was expressed as % RSD.

5.7. Robustness

Robustness of RP-HPLC method was determined by evaluating the influence of small but deliberate change in temperature (by providing small heat), pH (by addition of small amount dilute HCl), wavelength (by evaluating at 283, and 260 nm), and solvent (methanol, combination of methanol and distilled water). The robustness was expressed as % RSD.

5.8. Limit of Detection

Standard deviation and slope were calculated by analyzing the data of calibration curve. Limit of detection was calculated by using following formula:

LOD = (3.3* Standard deviation)/Slope

5.9. Limit of Quantification

Standard deviation and slope were calculated by analyzing the data of calibration curve. Limit of quantification was calculated by using following formula:

LOQ = (10*Standard deviation)/Slope

6. Result and Discussion

6.1. Linearity

By plotting concentrations of the five diluted standard solutions (as stated earlier in the section 4.4) on X-axis and peak areas of the respective standard solutions on Y-axis, the calibration curve (Fig. 3) with regression equation (y = 53344x - 239.6), coefficient of determination (R^2 = 0.999, Table 2), correlation coefficient (r = 0.9994) was found. Coefficient of determination value indicated excellent data fit and linearity of the calibration curve of the developed RP-HPLC method under the present work.

Table 2 Results of Regression Analysis for the RP-HPLC Method

Dynamic or Linear range	1.25-20 (μg/mL)
Slope	53344
Intercept	239.6
Coefficient of determination	0.999

6.2. Dynamic range

The dynamic range for RP-HPLC method was found 1.25 μg/mL to 20 μg/mL at which linearity, was observed properly (Fig. 3). In the mentioned range, precision and accuracy were evaluated following ICH guidelines (ICH – Q2B). The obtained results are presented in the (Table 4, 5, 6 and 7). It is evident from the (Table 4, 5, 6 and 7) that % RSD value did not exceed the ICH guideline value 2%. It means the developed method was properly validated and it can be applied for the quantification of brigatinib in the bulk with the appropriate precision and accuracy.

6.3. Specificity/ Selectivity

The percentage recovery of drug was 99.83 % – 101.3 % (Table 3) although small quantities of starch, lactose, magnesium stearate were present in the sample matrix as stated earlier under the section 5.3. The results showed that the analyte was almost completely interaction free from the excipients and thus the results indicated the specificity of the developed method for the detection and estimation of brigatinib from the bulk and the dosage form.

Table 3 Results of Selectivity/Specificity Test Using RP-HPLC

Concentration added (µg/mL)	Recovered Amount (µg/mL)	% Recovery
10	10.13	101.3
10	10.02	100.2
10	9.983	99.83
Standard Deviation	0.0765	0.7646
Mean	10.0443	100.4433
% RSD	0.7612	0.7612

6.4. Precision

6.4.1. Intraday precision / Repeatability

Percent (%) RSD for 6 replicates of brigatinib solution having concentration 10 µg/mL was 0.5 (Table 4), (%) RSD for 6 replicates of brigatinib solution having concentration 5 µg/mL was 0.46 (Table 5) and percent (%) RSD for 6 replicates of brigatinib solution having concentration 2.5 µg/mL was 1.74 (Table 6). All the evaluated values of % RSD were less than 2% indicating excellent intraday precision of the results obtained by the developed method.

Table 4 Results of Repeatability Test 1 Using RP-HPLC

Sample number	Concentration (µg/mL)	Peak area (AU)
1	10	525359
2	10	525240
3	10	519960
4	10	520987
5	10	524896
6	10	526340
Standard deviation		2638.765317
Mean		523797
% RSD		0.503776333

Table 5 Results of Repeatability Test 2 Using RP-HPLC

Sample number	Concentration ((µg/mL)	Peak area (AU)
1	5	269940
2	5	268734
3	5	270324
4	5	269873
5	5	266970
6	5	269780

Standard deviation	1245.858807
Mean	269270.1667
% RSD	0.462679851

Table 6 Results of Repeatability Test 3 Using RP-HPLC

Sample number	**Concentration ((µg/mL)**	**Peak area (AU)**
1	2.5	135294
2	2.5	135035
3	2.5	133940
4	2.5	129568
5	2.5	136032
6	2.5	135028
Standard deviation		2342.801379
Mean		134149.5
% RSD		1.746410817

6.4.2. Inter day precision / Intermediate Precision:

Percent (%) RSD for of solution having concentration 20 µg/mL tested on three different days was 0.108 (Table 7), % RSD for solution having concentration 10 µg/mL tested on three different days was 0.24 (Table 7) and % RSD for solution having concentration 5 µg/mL tested on three different days was 0.18 (Table 7). All the evaluated values of % RSD were less than 2 indicating good intermediate precision of the results obtained by the developed method.

Table 7 Results of Inter Day Repeatability Test Using RP-HPLC

Conc. ((µg/mL)	**Average peak area on Day 1**	**Average peak area on Day 2**	**Average peak area on Day 3**	**Standard Deviation**	**Mean**	**% RSD**
20	525359	526340	526348	568.704	526015.7	0.1081
10	269940	271245	270322	670.929	270502.3	0.2480
5	135294	135803	135567	254.724	135554.	0.1879

6.5. Accuracy

The accuracy of RP-HPLC method was confirmed by recovery studies. The percentage recovery was from 99.85 to 101.3 % (Table 8) which indicated accuracy of the results obtained by the new method. So, the developed method has high degree of accuracy for the determination of brigatinib.

Table 8 Results of Accuracy Test Using RP-HPLC

Previous concentration (µg/mL)	**Amount added (µg/mL)**	**Amount Found (µg/mL)**	**% Recovery**
5	5	9.985	99.85
5	10	15.02	101.3
5	15	19.98	99.9

6.6. Robustness

There is no significant change in retention time of brigatinib after deliberate changing of temperature, pH, and flowrate as well as injection volume. Low % RSD values indicated the robustness of the developed method (Table 9).

Table 9 Results of Robustness Test Using RP-HPLC

Parameters		**% RSD**
Temperature	Normal condition (25^{0}C)	0.76
	Temperature change (+ 5^{0}C)	0.82
Flow rate	1 ml/min	0.76
	1.2 ml/min	0.82
pH	Normal condition	0.76
	After little change	0.78
Wavelength	283 nm	0.76
	260 nm	1.04
Injection volume	10µL	0.76
	12 µL	0.94

6.7. Limit of Detection

Limit of detection was 0.33 µg/mL which was the lowest concentration of brigatinib that was detected by the method.

6.8. Limit of Quantification

The limit of quantification was 1.00 µg/mL which was the lowest concentration of brigatinib that could be quantified by the method.

6.9. Application of this method to assay brigatinib in Tablet

The method was used for determination of brigatinib in Brigacent brand (180 mg, Incepta Pharma, Bangladesh) tablet dosage form. The results showed (Table10) percentage recoveries were high (99.99-100.05) and % RSD values were low (0.13), which confirmed the method is suitable for routine determination of brigatinib in pharmaceutical preparations.

Table 10 Results of Tablet (180 g) assay using RP-HPLC

Concentration added (µg/mL)	**Recovered Amount (µg/mL)**	**% Recovery**
180 mg	179.99	99.99
180 mg	179.96	99.8
180 mg	180.09	100.05
Standard Deviation	0.068068593	0.1305
Mean	180.0133333	99.947
% RSD	0.037813084	0.1306

7. Conclusion

The present research work emerges with the development and validation of RP-HPLC method for the detection and quantification of brigatinib. Which was simpler and more cost effective than other previously developed similar methods, namely RP-UPLC method. ICH guidelines were followed to successfully establish the method for analysis of brigatinib in raw material and pharmaceutical dosage forms. The developed method is found simple, accurate, precise, rapid, sensitive, and specific for determination of brigatinib. The newly developed RP-HPLC method is highly recommended for routine use in quality control of brigatinib in any quality control and assurance laboratory.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that no competing interest exist.

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