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Qualitative and quantitative assessment of process related impurities in Brigatinib raw material and formulations using HPLC

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Abstract: The presence of process related impurities in any drug or the drug product was associated with its safety, stability and efficacy. The overall literature survey proved that there is no method published on the assessment of process related impurities in brigatinib. In this study, a simple, reliable and stable HPLC qualitative method was reported for quantification of process related impurities with easy and quick extraction procedure. The impurities along with standard brigatinib was resolved on Lichrospher® C18 (250 mm × 4.6 mm; 5 μm particle size) column in room temperature using methanol, acetonitrile, pH 4.5 phosphate buffer in 55:25:20 (v/v) at 1.0 mL/min as mobile phase and UV detection at 261 nm. The method produces well resolved peaks at retention time of 4.60 min, 12.28 min, 3.37 min, 7.34 min and 8.39 min respectively for brigatinib, impurity A, B, C and D. The method produces a very sensitive detection limit of 0.0065 μg/mL, 0.0068 μg/mL, 0.0053 μg/mL and 0.0058 μg/mL for impurity A, B, C and D respectively with calibration curve linear in the concentration range of 22.5-135 μg/mL for brigatinib and 0.0225-0.135 μg/mL for impurities. The method produces all the validation parameters under the acceptable level and doesn't produces any considerable changes in peak area response while minor changes in the developed method conditions. The method can effectively resolve the unknown stress degradation products along with known impurities with less % degradation. The method can efficiently resolve and quantify the impurities in formulation and hence can suitable for the routine quality analysis of brigatinib in raw material and formulation.

Key words: brigatinib, process related impurities, HPLC method development, method validation, formulation assay

1. Introduction

The pharmaceutical products were manufactured mostly by utilizing a synthetic approach or by adjusting a naturally occurring product by utilizing varied range of reactive reagents.[1] In this process, it was natural that nominal level of side products or the unreacted reagents were remains in the furnished

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product as impurity. The presence of these impurities in any furnished product may exhibit un-wanted toxicity that includes carcinogenicity and genotoxicity.[2] The regulatory agencies like European Medicines Agency, food and drug administration issued guidelines related to the presence of these impurities in any pharmaceutical product. Hence pharmaceutical companies were focusing to minimize these impurities in the pharmaceutical product to minimize the negative impact of impurities.[3]

Brigatinib (*Fig.* 1) belongs to anaplastic lymphoma kinase inhibitor and an epidermal growth factor receptor class drug prescribed for the treatment of anaplastic lymphoma kinase positive metastatic non-small cell lung cancer that was spread to other parts of body.[5] It exhibits its action by activating downstream signaling proteins and inhibits anaplastic lymphoma kinase phosphorylation. The common side effects such as lung disease, diabetes, high blood pressure, slow heartbeats, pancreas vision and breathing problems as well as liver and kidney disease.[6] Its chemical name is *5-chloro-2-N-{4-[4-(dimethylamino)piperidin-1-yl]-2-methoxyphenyl}-4-n-[2-(dimethylphosphoryl)phenyl]-pyrimidine-2,4-diamine* with molecular formula of $C_{29}H_{39}ClN_7O_2P$ and molar mass of 584.10 g/mol.

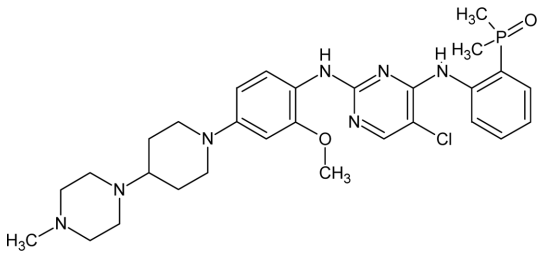


Fig. 1. Chemical structure of brigatinib.

The most commonly used route for the synthesis of brigatinib[7,8] was presented in *Fig.* 2. In the process of synthesis, there is a possibility of formation of process related impurities. Process related impurities are the un-wanted compounds that are originated from starting material, intermediates used during the synthesis process or the byproducts generated during the process of synthesis and that remains as un-wanted compound in the final compounds or its product. Based on the route of synthesis, there is a possibility of formation of four process related impurities namely impurity A, impurity B, impurity C and impurity D.

The impurity A was originated as byproduct (1.3 %) during the process of synthesis of brigatinib. The impurity A was formed by oxidation of *2-(Dimethyl-phosphinyl)aniline* which was used as intermediate reactant (**18**). Approximately 4.5 % of impurity B was remains in the final product as impurity and that was formed by pyrolysis of *5-Fluoro-2-nitroanisole* which was utilized as starting material (**4**) in presence of *dimethylformamide*. Very less quantity (approximately 0.16 %) of impurity C was formed by reduction of raw material **4** in presence of potassium carbonate.

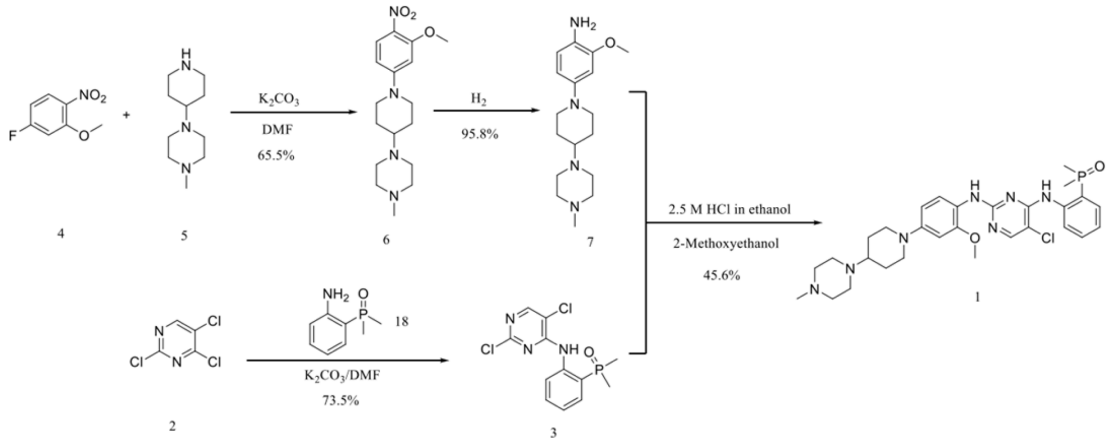
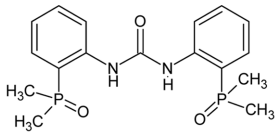
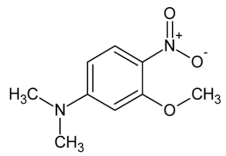


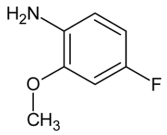
Fig. 2. Schematic representation for synthesis of brigatinib.[7,8]



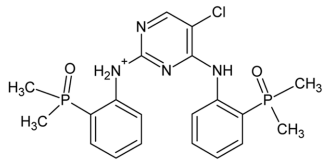
Impurity A [*1,3-bis(2-(dimethylphosphoryl) phenyl) urea*]
MF: $C_{17}H_{22}N_2O_3P_2$; **MM:** 364.32 g/mol



Impurity B [*(2-amino-5-(dimethylamino)-phenyl)dimethylphosphine oxide*]
MF: $C_9H_{12}N_2O_3$; **MM:** 196.20 g/mol



Impurity C [*4-fluoro-2-methoxyaniline*]
MF: C_7H_8FNO; **MM:** 141.14 g/mol



Impurity D [*4-Bromo-2-(1,1,1-trifluoro-2-methylpropan-2-yl)pyridine*]
MF: $C_{20}H_{23}ClN_4O_2P_2$; **MM:** 449.83 g/mol

Fig. 3. Molecular structure of impurities in the study.

In the process of synthesis of brigatinib, an approximately 1.2 % of impurity D was formed by acid-catalyzed decomposition of compound **3**.[7,8] *Fig*. 3 presents the process related impurities originated during the synthesis process.

The manufactures are continuously exploring the novel reactants or the raw materials for the synthesis of final product with very less impurities or the impurities under the permissible levels. There is a need of suitable analytical method for identification, quantification of these impurities for minimizing and controlling these impurities. HPLC is the versatile technique for identification, quantification of impurities in any pharmaceutical drug or drug product. The literature survey was conducted to identify suitable analytical method for quantification of brigatinib. In literature, few analytical methods published for determination of brigatinib in biological samples and reported the pharmacokinetic profile using LCMS/MS[9-12] and UPLC/MS.[13] Few bio-analytical methods reported for quantification of brigatinib in biological samples in combination with other drugs.[14-17] The review of literature suggested that there is no method available for the quantification of process related impurities in brigatinib pure drug and formulations. Hence, this study proposed to develop a simple and stable HPLC method for resolution, identification and quantification of process related impurities in brigatinib.

2. Experimental

2.1. Equipment's

The HPLC study was conducted on Agilent 1100 (USA) equipment that was equipped with quaternary solvent delivery pump (model G1311 A). The prepared standard and samples were injected into the system using temperature adjustable auto sampler (model G 1329A) with 0.1-1500 μL injection capacity. The injected samples were resolved with suitable C18 column and the resolved samples were detected through programmable ultraviolet (UV) detector (model G 1314 A). The detector response was integrated with Agilent chem-station version 2.0 software.

2.2. Chemicals and reagents

The brigatinib analytical pure drug having 98.54 % purity, its process related impurities A, B, C, and D along with its tablet formulation having brand Briganix® (90 mg) were obtained from Beacon Pharmaceuticals private limited, Kolkata, West Bengal. The solvent used in the study like methanol, acetonitrile were of HPLC grade and Milli-Q® water were purchased from Merck chemicals, Mumbai. The analytical reagent grade chemicals such as acetic acid, sodium acetate, hydrochloric acid (HCl), sodium hydroxide (NaOH),

and hydrogen peroxide were purchased from Fisher scientific, Mumbai.

2.3. Preparation of solutions

2.3.1. Brigatinib and impurity solutions

The brigatinib standard drug at a quantity of 25 mg was weighed accurately in a sterilized volumetric flask half filled with diluent (methanol). The flask was kept in an ultrasonic bath sonicator for 2 min to dissolve brigatinib completely in diluent. The brigatinib dissolved solution was filtered through 0.2 µm nylon membrane filter in to a clean and dry 25 mL volumetric flask and the final volume made up to 25 mL using the same diluent. The brigatinib standard solution at 1 mg/mL (1000 µg/mL) was obtained and the same procedure was used for preparing impurity A, B, C and D solution in the same concentration level separately. Then the solutions were separately diluted to known fixed concentration and selected concentration of standard and impurity solutions were mixed during the analysis.

2.3.2. Formulation solution

The Briganix® brand tablets of brigatinib with dosage of 90 mg were used for preparing the formulation solution in the study. One complete strip of tablets was grinded with a sterile mortar and pestle to a uniform fine powder. The tablet powder equivalent to 25 mg brigatinib pure drug was weighed accurately in to a sterile volumetric flask having 15 mL diluent. The brigatinib in the formulation powder was dissolved completely in the diluent with the help of an ultrasonic bath sonicator. The un-dissolved formulation excipients were removed by filtration through 0.2 µm nylon membrane filter and the volume was made up to the mark. The formulation solution at 1000 µg/mL concentration was obtained and was brought to precision level concentration in linearity before the analysis.

2.4. Method development

The method development aimed such that the method was simple and convenient that can resolve the four impurities of brigatinib along with standard. The preliminary conformation of the detector wavelength that can detect impurities of brigatinib was confirmed with UV-visible spectrophotometer. The pure brigatinib and its impurities solution at 10 µg/mL solution was scanned individually in 400-200 nm and the overlaid spectra suggest the wavelength suitable for simultaneous detection of brigatinib and its impurities. The stationary phase selection was conducted by analysing the precision level concentration of brigatinib spiked with 0.1 % impurities solution on different columns. During the column selection, different columns with different configuration were studied for best resolution of analytes. The composition, pH and flow of mobile phase were optimized by change in different solvent composition with different pH and flow rates. In each method development trail condition, precision level concentration of brigatinib spiked with 0.1 % impurities solution was analysed. The chromatographic response, peak shape and system suitability were summarized and the conditions that produce best results were considered as suitable for the analysis of brigatinib and its impurities.

2.5. Method validation

The method optimized for separation and analysis of process related impurities of brigatinib was validated in terms of range, linearity, precision, ruggedness, accuracy as per the procedure published in literature and guidelines issued by ICH.[18]

2.5.1. System suitability

The method system suitability was established by analysing precision level concentration solution in the proposed method. The chromatograms and chromatographic responses observed in this study were observed carefully for evaluating system suitability of method.

For evaluating the system suitability, the parameters such as retention time, theoretical plates (>2000), asymmetric (tail) factor (<2) and resolution (>2) was considered as acceptable as per guidelines.

2.5.2. Sensitivity

Prior to the evaluation of the range of analysis, the

LOD (limit of detection) and LOQ (limit of quantification) of process related impurities of brigatinib was determined in the developed method. The minimum concentration of impurities that can detect in the developed method was considered as LOD whereas the lowest quantifiable concentration was considered as LOQ and was evaluated by adopting signal (s) to noise (n) ratio method. The minimal concentration of impurities of brigatinib was analysed in the developed method and the chromatographic response (signal) along with baseline (noise) response was summarized. The signal to noise ratio of 3 and 10 was considered as LOD and LOQ respectively.

2.5.3. Linearity and range

The analytical range of brigatinib and its impurities was evaluated by considering the sensitivity results of impurities in the developed method. The standard solution of brigatinib was prepared such that the solutions contain 0.1 % of each impurity. The chromatographic response of each analyte was tabulated and calibration curve was plotted individually by considering obtained peak area response on y-axis and its prepared concentration on x-axis. The best fitted calibration range for each analyte was considered as suitable range of analysis in the developed method.

2.5.4. Precision

The middle concentration in the linearity level spiked with 0.1 % of each impurity was used for evaluating the repeatability and reproducibility of the developed method. The solution was prepared and injected six times in the same day (intraday), six times in three days (interday) and six times by three different analysts in the same day (ruggedness). The peak area response of each analyte in each study was tabulated and the % RSD was calculated for each analyte in each study. The % RSD of less than 2 was acceptable in each study as per the guidelines.

2.5.5. Robustness

The influence of minor variations in the developed method conditions for the separation and quantification of brigatinib and its impurities was evaluated in robustness. The ±5 mL variation in composition of mobile phase, ±5 nm variation in wavelength of detector and ±0.1 factor variation in mobile phase pH were made intentionally and the 100 % concentration of brigatinib containing 0.1 % of each impurity was injected in each changed method condition. The chromatographic response and the system suitability of the obtained chromatograms in each condition were summarized. The % change in the peak area response of each analyte was calculated by comparing it corresponding regression equation and a % change of less than 2 was considered as acceptable.

2.5.6. Recovery

In recovery/accuracy study, 50 %, 100 % and 150 % spiked levels of known standard concentration (100 %) in the calibration range was performed and the % recovery was calculated in each spiked level by comparing with calibration results. The % recovery of 98-102 was considered as acceptable.

2.5.7. Force degradation studies

The method applicability for the separation and analysis of stress degradation compounds generated during the stress exposer of brigatinib was confirmed by performing forced degradation studies. In this, the standard brigatinib at a quantity of 50 mg was separately mixed with 50 mL of 0.1 N HCl, 0.1 N NaOH and 3 % hydrogen peroxide solution for acid, base and peroxide degradation study respectively. The stressed samples were incubated for 24 hours to induce degradation in brigatinib drug. Then the solution was neutralized, diluted to 100 % concentration level and then analysed in the developed method. The standard brigatinib was taken in a petri dish and exposed to 60 ºC for 24 h in an air oven for thermal degradation and exposed to UV light at 254 nm for 24 h for photolytic degradation study. Then the stressed sample was diluted to 100 % concentration level and the dilute solution was analysed in the developed method. The chromatograms observed for each stress sample analysis was observed for evaluation of method efficiency for the separation and analysis of stress degradation compounds. The % degradation of

brigatinib was calculated by comparing the peak area response of stressed sample with the un-stressed sample of the same concentration level.

2.5.8. Sample analysis

The formulation solution of brigatinib with brand Briganix® - 90 mg was analysed in the developed method. The formulation solution spiked with known and concentration of the impurities was also analysed in the developed method. The % assay was calculated by comparing the formulation results with that of the calibration curve results.

3. Results and Discussion

The available analytical methods for analysis brigatinib in literature proved that there is no method reported for quantification of process related impurities of brigatinib in pure drug and its formulations. To fulfil the gap identified in literature, this study planned to develop a simple and stable HPLC method for estimation of four process related impurities of brigatinib viz., impurity A, B, C and D in formulations and pure drug. The method optimization process and results achieved in method optimization study were summarized in *Table* 1.

Table 1. Method development conditions tried during optimization process

S No	Mobile Phase composition	Result	Conclusion
1	MP: acetonitrile and acetate buffer pH 5.4 in 60: 40 (v/v); SP: Water spherisorb (250 mm) C18 column; WL: 261 nm; FR: 1.0 mL/min	No clear peak identified for brigatinib and impurities. Unidentified and unresolved detection was identified	Method Rejected
2	MP: methanol and acetate buffer pH 5.4 in 60: 40 (v/v); SP: Thermo C18 (250 mm) column; WL: 261 nm; FR: 1.0 mL/min	Unresolved peaks were identified in the chromatogram with baseline disturbances throughout run time. Results suggest that the column doesn't resolve the analytes	Method Rejected
3	MP: acetonitrile and acetate buffer pH 4.8 in 70:30 (v/v); SP: Phenomenex Luna (250 mm) C18 column; WL: 261 nm; FR: 1.0 mL/min	Peaks corresponds to impurity A and C were not resolved whereas the peaks correspond to impurity B and brigatinib was resolved. The peak symmetry of the detected peaks was noticed to be not acceptable with less peak area response.	Method Rejected
4	MP: methanol and 0.1 % aqueous orthophosphoric acid in 50:50 (V/V); SP: Phenomenex Luna (250 mm) C18 column; WL: 261 nm; FR: 1.0 mL/min	No individual peak was noticed in this condition suggest that the presence of orthophosphoric acid in the mobile phase doesn't resolve the analytes and hence was not used for further study	Method Rejected
5	MP: methanol and phosphate buffer pH 4.5 in 60:40 (v/v); SP: Lichrospher® C18 column; WL: 261 nm; FR: 1.0 mL/min	Peaks for brigatinib and its impurities was noticed but the resolution of impurity B and brigatinib was observed to be very poor whereas merge peak was noticed for impurity C and D. Impurity A peak was noticed to be well resolved and retained. This suggests the used buffer and column will able to resolve the analytes.	Method Rejected
6	MP: methanol, acetonitrile, phosphate buffer pH 4.5 in 70:10:20 (v/v); SP: Lichrospher® C18 column; WL: 261 nm; FR: 1.0 mL/min	Peaks for brigatinib and its impurities were noticed with acceptable resolution and less peak intensity. Results suggest that the presence of acetonitrile give positive influence on the separation of analytes.	Method Rejected
7	MP: methanol, acetonitrile, phosphate buffer pH 4.5 in 55:25:20 (v/v); SP: Lichrospher® C18 column; WL: 261 nm; FR: 1.0 mL/min	Method produces acceptable resolution and system suitability	Method Accepted

MP = Mobile phase composition; SP = Stationary phase; WL = Detector wavelength; FR = Flow rate of mobile phase

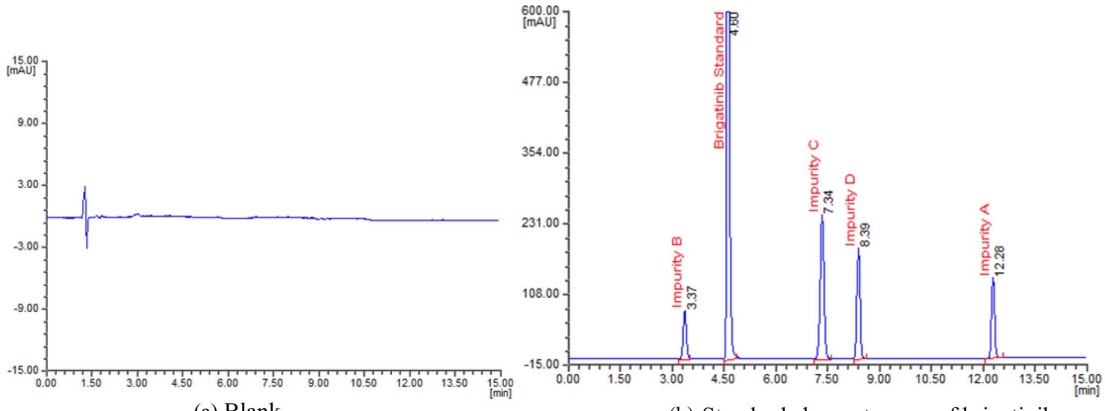
The optimization of method was concluded achieving suitable analytical conditions for the separation and analysis of process related impurities of brigatinib. The 55:25:20 (v/v) composition of methanol, acetonitrile, phosphate buffer pH 4.5 at 1.0 mL/min as mobile phase, Lichrospher® C18 (250 mm × 4.6 mm; 5 µm particle size) column in room temperature as stationary phase and UV detection at 261 nm was finalized as optimized conditions that effectively resolves the process related impurities of brigatinib. The chromatogram observed for blank solution (without analytes) analysis in the optimized method conditions (*Fig.* 4(a)) doesn't show any chromatographic response throughout the run time. The chromatogram identified for standard brigatinib spiked with 0.1 % impurities (*Fig.* 4(b)) show well resolved symmetric peaks represents brigatinib and its impurities in the study. In the chromatogram of blank and standard, there is no identification of unwanted detections throughout the run time proved the specificity of the method.

The chromatographic results achieved for brigatinib and its impurities in the study were tabulated for evaluating the system suitability of the developed method. The system suitability parameters such as tail factor, number of theoretical plates and resolution of brigatinib and its impurities were observed carefully. As tabulated in *Table* 2, the method produces acceptable system suitability results that suggest that the method was suitable for the separation and analysis of brigatinib.

The method sensitivity was evaluated in terms of detection and quantification limit. The detection limit was observed as 0.0065 µg/mL, 0.0068 µg/mL, 0.0053 µg/mL and 0.0058 µg/mL whereas the quantification limit observed as 0.0215 µg/mL, 0.0225 µg/mL, 0.0175 µg/mL and 0.019 µg/mL respectively for impurity A, B, C and D. The high quantification limit of 0.0215 µg/mL was considered as minimum concentration that detect impurities in the developed method and the same concentration was chosen as lowest concentration in the calibration curve. Based on this concentration, the standard and impurity concentration prepared such that the brigatinib solution contain 0.1 % of impurity.

The accurate fit calibration curve was obtained in the concentration level of 22.5-135 µg/mL for brigatinib and 0.0225-0.135 µg/mL for impurities in the study. The regression equation was observed as y = 8504.5x – 14482 (R^2 = 0.9999), y = 440538x + 2865.6 (R^2 = 0.9994), y = 599231x + 4319.2 (R^2 = 0.9991), y = 879165x + 7735.6 (R^2 = 0.9994) and y = 694530x + 6499.1 (R^2 = 0.9990) for brigatinib, impurity A, B, C and D respectively. The calibration curve was found to be linear with very high correlation coefficient for impurities in the study as well as standard brigatinib.

The 90 µg/mL concentration of standard brigatinib



(a) Blank

(b) Standard chromatogram of brigatinib spiked with 0.1 % impurities

Fig. 4. System suitability chromatogram in the developed method: (a) Blank, (b) Standard chromatogram of brigatinib spiked with 0.1 % impurities.

Table 2. Summary of the method validation results in the developed method

S No	Studied parameter	Results observed for				
		Brigatinib	Impurity A	Impurity B	Impurity C	Impurity D
1	Concentration in µg/mL	90	0.09	0.09	0.09	0.09
2	Retention time (min)	4.60	12.28	3.37	7.34	8.39
3	Peak Area	748948.2	43015.2	57419.5	86693.1	70102.4
4	Theoretical plates	6252	12145	4713	8794	9543
5	Tail factor	1.04	0.94	0.98	0.97	0.95
6	Resolution	5.80	14.57	--	9.32	4.28
7	Linearity range	22.5-135 µg/mL	0.0225-0.135 µg/mL	0.0225-0.135 µg/mL	0.0225-0.135 µg/mL	0.0225-0.135 µg/mL
8				Ruggedness study*		
8a	% change in MP 1	0.96	0.64	0.26	0.23	0.14
8b	% change in MP 2	0.99	0.14	0.06	0.49	0.65
8c	% change in pH 1	0.52	0.33	0.45	0.21	0.12
8d	% change in pH 2	0.85	0.82	0.04	0.50	0.51
8e	% change in WL 1	0.79	0.80	0.58	0.22	0.11
8f	% change in WL 2	0.78	0.92	0.15	0.58	0.89
9				% Recovery in* *		
9a	50 % recovery	98.43±0.36	98.71±0.33	98.78±0.86	98.57±0.41	98.63±0.29
9b	100 % recovery	99.01±0.69	99.59±0.91	98.83±0.76	99.12±0.65	100.26±0.41
9c	150 % recovery	99.95±0.60	99.48±0.70	100.17±0.73	100.55±0.41	100.91±0.54

*average of three replicate experiments; ** average ± standard deviation for three replicate experiments

solution spiked with 0.1 % each impurity was used for evaluation of repeatability and reproducibility of the developed method. The % RSD of peak area response in each study for each analyte was calculated and the % RSD of 0.21, 0.49, 0.24, 0.30 and 0.17 in intraday precision, 0.33, 0.63, 0.33, 0.41 and 0.27 in interday precision, 0.46, 0.53, 0.24, 0.44 and 0.40 ruggedness study respectively for brigatinib, impurity A, B, C and D.

The method robustness was evaluated by both positive and negative change in the method conditions such as the detector wavelength, mobile phase composition and pH was changed. The composition of MP (mobile phase) changed as methanol, acetonitrile, buffer in 50:30:20 (v/v) in MP 1 and 60:20:20 (v/v) in MP 2 changed study. The mobile phase pH changed as 4.4 (pH 1) and 4.7 (pH 2) whereas detector wavelength (WL) changed as 256 nm (WL 1) and 266 nm (WL 2). The % change in each changed condition was calculated and an acceptable % change of less than 2 (*Table* 2) was observed for brigatinib and its impurities. This confirms that the method was robust as there is no considerable change in the separation and detection of brigatinib and impurities when small change in the developed method conditions.

The spiked recovery at 50 %, 100 % and 150 % spiked levels by considering 90 µg/mL of brigatinib spiked with 0.1 % of each impurity was chosen for evaluating the method accuracy. The % recovery was calculated for each analyte in each recovery injection and the % RSD in each spiked level was calculated for brigatinib and its impurities. As shown in *Table* 2, an acceptable recovery in the range of 98-102% in each analysis and an acceptable % RSD of less than 2 was observed in each spiked level for brigatinib and its impurities. This proved that the method was accurate.

The method effectiveness for resolving stress degradation products generated during stress degradation study of brigatinib. The brigatinib pure drug was exposed to stress studies like acid, base, peroxide, thermal, UV light and the stress exposed sample was analysed in the developed method. The resultant chromatograms and its chromatographic response was carefully noticed for evaluating the method effectiveness. Among all stress conditions studied, acid degradation shows very high % degradation of 9.25 % and the chromatogram shows three degradation products (DPs) at a retention time of 2.5 min, 5.3 min

and 10.7 min. The chromatogram shows impurity B and D at its standard retention time. The % degradation of 8.51 % was noticed in base degradation and the chromatogram shows well resolved DPs at 1.24 min, 2.40 min and 9.40 min. In this condition, known impurity B was noticed at a retention time of 3.39 min. Three degradation products along with known impurity B was noticed in the chromatogram observed in peroxide degradation study with a % degradation of 5.65 min. No known impurity was noticed in the chromatogram of thermal degradation study. In this, three DPs were well resolved and retained at 2.18

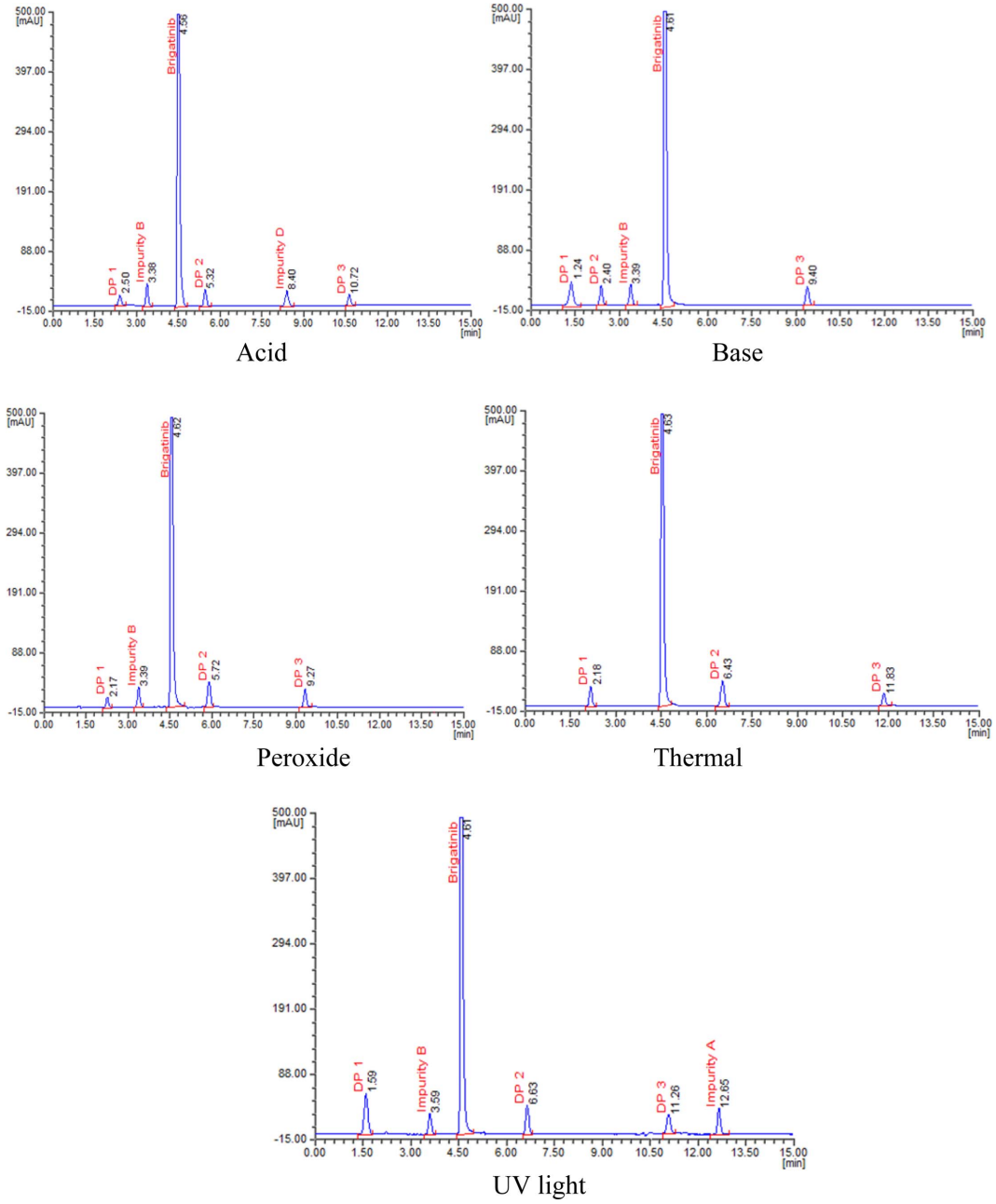
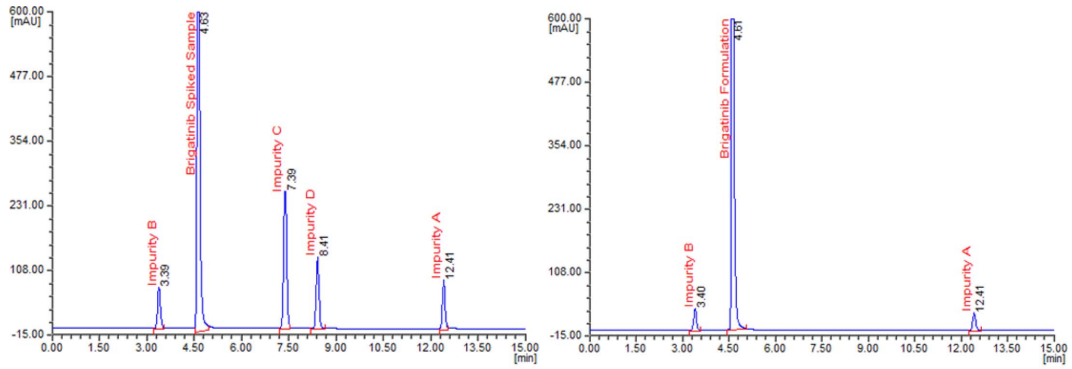


Fig. 5. Chromatogram identified in thermal degradation study.



Spiked sample chromatogram Un- spiked sample chromatogram

Fig. 6. Formulation chromatogram of brigatinib in the developed method.

min, 6.43 min and 11.83 min with a % degradation of 7.13%. In UV light degradation study, the % degradation of 8.09% was noticed with three DPs retained at 1.59 min, 6.63 min and 11.26 min. The known impurities A and B were also detected in the chromatogram. In all the degradation condition, there is no change in retention time of the brigatinib along with its known impurities in the study. Hence the method can separate and quantify the process related impurities in brigatinib. The chromatograms in this study were presented in *Fig*. 5.

The method optimized in the study was applied for resolution and quantification of process related impurities of brigatinib in samples. The formulation sample spiked with known concentration of impurities and un-spiked formulation solution was analysed in the developed method. The chromatogram identified for formulation solution spiked with impurities clearly show impurity A, B, C and D. The un-spiked formulation solution show peaks corresponds to A and B. This chromatogram doesn't shows peak corresponds to impurity C and D suggest that these impurities were under the detection limit in the sample. The % impurity content was calculated by substituting the peak area response of impurities identified in the sample with its corresponding calibration curve. The % assay was calculated to be 0.05 and 0.02 % respectively for impurity A and B. the quantity of impurities in the formulation was noticed to be under the permissible levels. The spiked and un-spiked formulation chromatogram doesn't show any unwanted detections or the peak corresponds to excipients throughout the run time. Hence it was concluded that the method was adequately suitable for resolution, identification and quantification of process related impurities of brigatinib.

4. Conclusions

This study reports a simple and sensitive HPLC method for resolution, identification and quantification of process related impurity A, B, C and D of brigatinib in synthetic mixture and formulations. The method produces very sensitive detection limit of 0.0065 µg/mL, 0.0068 µg/mL, 0.0053 µg/mL and 0.0058 µg/mL respectively for impurity A, B, C and D with a sensitive calibration curve range of 0.0225-0.135 µg/mL for impurities. The method can effectively resolve the un-known stress degradation products along with known impurities in the study and can adequately sufficient for quantification of process related impurities in sample. Hence it can be concluded that the method developed in the study was simple, sensitive and stable that can effectively resolve, identify and quantify the process related impurities in pure drug and formulations.

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