A Study of Stability Indicating Development and Validation of a Method for Simultaneous Estimation of Brigatinib and Alectinib Using Reverse Phase Ultra Performance Liquid Chromatography in Active Pharmaceutical Ingredient form

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ABSTRACT

Aims: New validated method for the simultaneous estimation of Brigatinib and Alectinib using UPLC and study of its degradation

Place and Duration of Study: Department of Chemistry, RVR & JC College of Engineering, Chowdavaram, Guntur, Andhra Pradesh, between March 2021 and April 2021.

Methodology: Using Luna C_{18} 100 x 2.6 mm, 1.6 µm column, acetonitrile, and 0.1 percent Tri ethyl amine (TEA) (80:20 v/v) as a mobile phase, the proposed method successfully achieved effective chromatographic separation with a flow rate of 1 mL/min and a wave length of 260 nm. The Braginib and Alectinib peaks were resolved within 5 minutes of elution time, with the Brigatinib peak eluting at 3.208 minutes and the Alectinib peak eluting at 1.757 minutes.

Results: The proposed method displays excellent linearity in the concentration ranges of 1.0 µg/ml to 15 µg/ml for Brigatinib and 5.0 µg/ml to 75 µg/ml for Alectinib. The RSD of robustness levels has a maximum of just 2 percent.

Conclusion: The accuracy, specificity, and sensitivity of the method were all found to be in line with ICH guidelines, when the procedure was developed and tested.

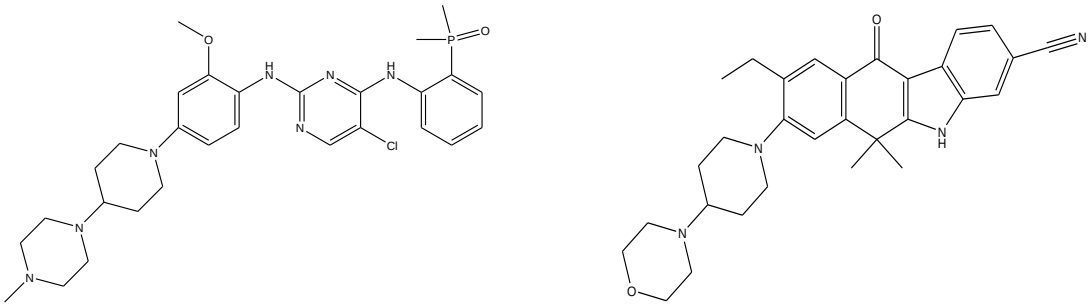
Keywords: ICH Guide lines, RP-UPLC, Brigatinib, Alectinib, Validation.

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Brigatinib, sold under the brand name Alunbrig among others, is a small-molecule targeted cancer therapy being developed by ARIAD Pharmaceuticals, Inc [1]. Brigatinib acts as both an anaplastic lymphoma kinase (ALK) [2, 3] and epidermal growth factor receptor (EGFR) [4, 5] inhibitor. Brigatinib could overcome resistance to osimertinib conferred by the EGFR C797S mutation if it is combined with an anti-EGFR antibody such as cetuximab or panitumumab [6]. Brigatinib is an inhibitor of ALK and mutated EGFR [7]. ALK was first identified as a chromosomal rearrangement in anaplastic large cell lymphoma (ALCL) [8, 9]. Genetic studies indicate that abnormal expression of ALK is a key driver of certain types of non-small cell lung cancer (NSCLC) [10, 11] and neuroblastomas [12, 13], as well as ALCL. Since ALK is generally not expressed in normal adult tissues, it represents a highly promising molecular target for cancer therapy. Epidermal growth factor receptor (EGFR) is another validated target in NSCLC. Additionally, the T790M “gatekeeper” mutation is linked in approximately 50 percent of patients who grow resistant to first-generation EGFR inhibitors. While second-generation EGFR inhibitors are in development, clinical efficacy has been limited due to toxicity thought to be associated with inhibiting the

native (endogenous or unmutated) EGFR. A therapy designed to target EGFR, the T790M mutation but avoiding inhibition of native EGFR is another promising molecular target for cancer therapy [14].

Alectinib (INN, marketed as Alecensa) is an oral drug that blocks the activity of anaplastic lymphoma kinase (ALK) [15] and is used to treat non-small-cell lung cancer (NSCLC). Alectinib has a low potential for interactions. While it is metabolised by the liver enzyme CYP3A4 [16, 17], and blockers of this enzyme accordingly increase its concentrations in the body, they also *decrease* concentrations of the active metabolite M4, resulting in only a small overall effect. Conversely, CYP3A4 inducers decrease alectinib concentrations and increase M4 concentrations. Interactions via other CYP enzymes and transporter proteins cannot be excluded but are unlikely to be of clinical significance. There are no contraindications under the US approval. The European approval only has the default remark about hypersensitivity [18] being a contraindication. Apart from unspecific gastrointestinal effects [19] such as constipation (in 34% of patients) and nausea (22%), common adverse effects in studies included oedema [20] (swelling; 34%), myalgia [21] (muscle pain; 31%), anaemia [22] (low red blood cell count), sight disorders, light sensitivity and rashes (all below 20%). Serious side effects occurred in 19% of patients; fatal ones in 2.8%. Chemical structures of Brigatinib and Alectinib were shown in figure 1.



A **B**

Fig. 1: Chemical structures of (A) Brigatinib and (B) Alectinib

To date, there have been no HPLC methods for Brigatinib and Alectinib estimation. Thus, the goal of the study is to predict Brigatinib and Alectinib, which is a pharmaceutical component, using RP-UPLC.

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

Merck (India) Ltd. provided acetonitrile, triethyl amine, and water in Worli, Mumbai, India. Glenmark Pharmaceuticals in Mumbai provided the APIs that served as reference standards for both Brigatinib and Alectinib.

2.2 Equipment

UPLC makes: The chromatographic device used was the Waters acquity, which included a quaternary pump, a PDA (photo diode array) detector, and the chromatographic programme Empower-2.0.

2.3 Chromatographic Conditions

UPLC system instrumentation was used to develop and validate the technique (Waters Acquity UPLC). Empower 2.0 software was used to process the data. Luna C_{18} column (100

x 2.6mm, 1.6 µm) was selected for use in the experiment. The compound was purified by isocratic elution using a mobile phase of 0.1% triethylamine buffer solution and acetonitrile in a 20:80 ratio. The pump was adjusted to pump 1.0 ml/min. UV detection was conducted at a wavelength of 260nm. The injection volume was 10 microliters, and the diluent was the same as the mobile process.

2.4 Preparation of standard solution

To get 10 mg of Brigatinib and 50 mg of Alectinib working requirements, put the contents of a 100ml volumetric flask in a sonicator for 15 minutes to break up the solids. Dilute volume with 70ml of diluents. Dilute 5 mL to 50 mL by using diluents.

3. RESULTS AND DISCUSSION

The purpose of this study was to develop a simple, accurate, and rapid RP-UPLC method for simultaneous Brigatinib and Alectinib estimation. To optimize the chromatographic conditions, different ratios of buffers (phosphate buffer, 0.1% Ortho phosphoric acid, 0.1% formic acid and 0.1% tri ethyl amine) and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. By using Acetonitrile and 0.1% formic acid (70:30) as mobile phase, the trial gave USP tailing of 1.02, 1.05 and USP resolution of 1.94 and USP plate count of 1763, 2442. The trial chromatogram was shown in figure 2.

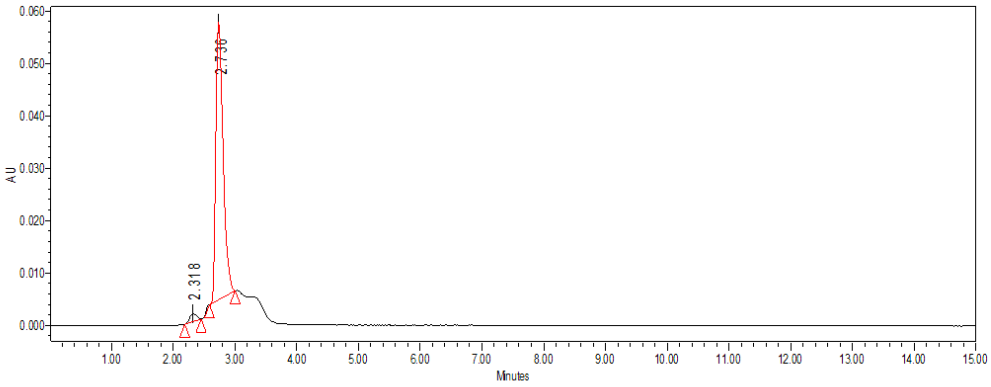


Fig. 2. Chromatogram of trial-1

Finally 0.1% tri ethyl amine buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method various stationary phases such as C_8, C_{18} and amino, phenyl columns were tested. From these trials the peak shapes were relatively good with Luna C_{18} column of 100 x 2.6 mm, 1.6 µ with a PDA detector. A buffer and acetonitrile mixture is part of the mobile process (20:80), the flow rate is 1.0ml/min and the column temperature is room temperature. Recovery data and peak sharpness are calculated based on finalization of diluent and standard solution concentrations, as well as injection volumes that are greater than the quantification maximum (LOQ). An isocratic concentration was used to achieve better resolution. Finally by using Luna C_{18} (100 x 2.6mm, 1.6 µm) column, 0.1% Tri ethyl amine : ACN 20:80 as mobile phase we got the optimized chromatogram by satisfying all the suitability conditions.

3.1 Method validation

The optimized RP-HPLC method was validated as per the ICH guidelines [23-25] with respect to system suitability, linearity and range, precision, accuracy,

and robustness. As seen in Table 1, the optimized conditions for the defined and validated UPLC process are listed.

Table 1. Optimized chromatographic conditions

S. No.	Parameter	Method Conditions
1	Column	Luna C_{18} 100 x 2.6mm, 1.6 µm
2	Flow rate	1 ml/min
3	Wave length	260nm
4	Injection Volume	10µl
5	Run time	5 min
6	Mobile phase	0.1% Tri ethyl amine : ACN 20:80

3.1.1 Specificity

Figure 3 is completely blank. No chromatographic interference was observed for placebo and blank samples at the retention times of Brigatinib and Alectinib.

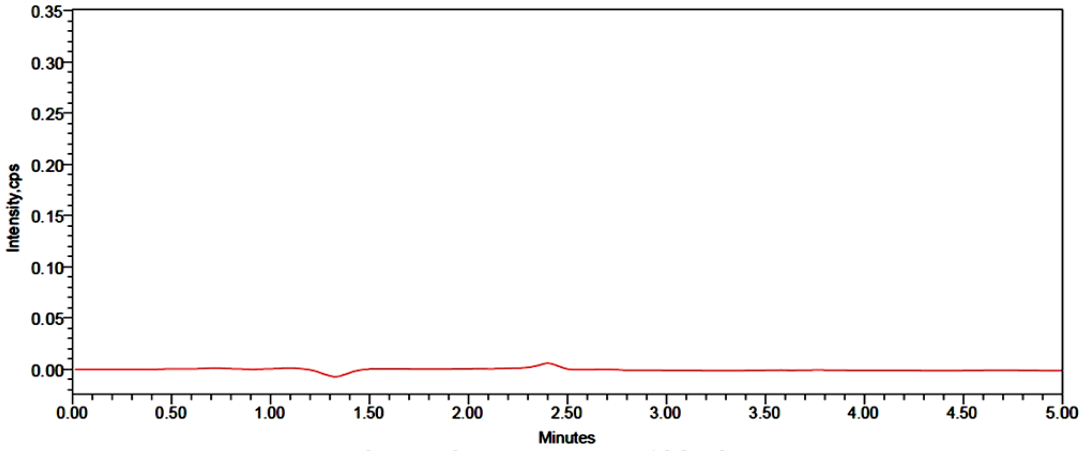


Fig. 3. Chromatogram of blank

3.1.2 System suitability

To run the UPLC, the standard solution was added to the system, and it was found that the system suitability parameters were in an acceptable range. The RSD percentage was determined using the average RSD (relative standard deviation) peak areas. The percentage of identical injections from the RSD fell within the recommended range. Table 2 and figure 4 show the obtained results.

Table 2: Results of system suitability

S. No	System suitability parameter	Acceptance criteria	Drug Name	
			Brigatinib	Alectinib
1	% RSD	Not more than 2.0	1.41	0.61
2	USP Tailing	Not more than 2.0	1.02	1.01
3	USP Plate count	Not less than 3000	3674	5692

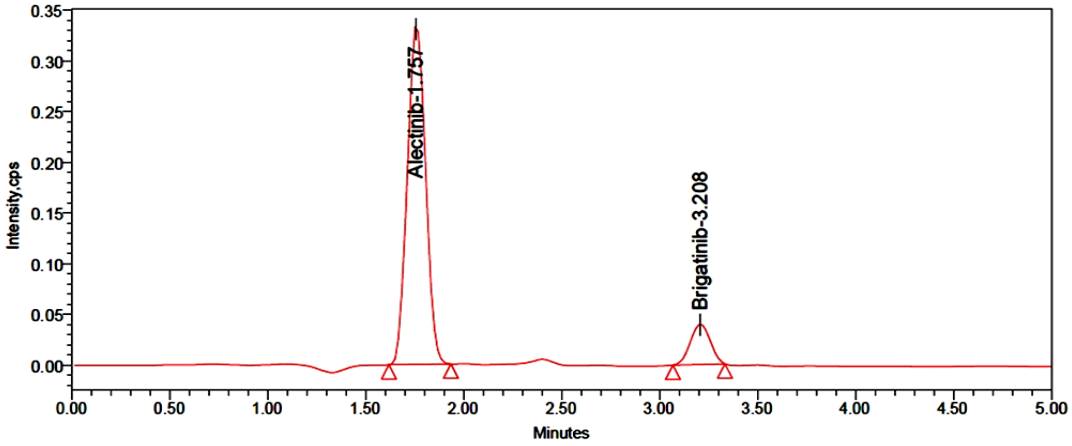


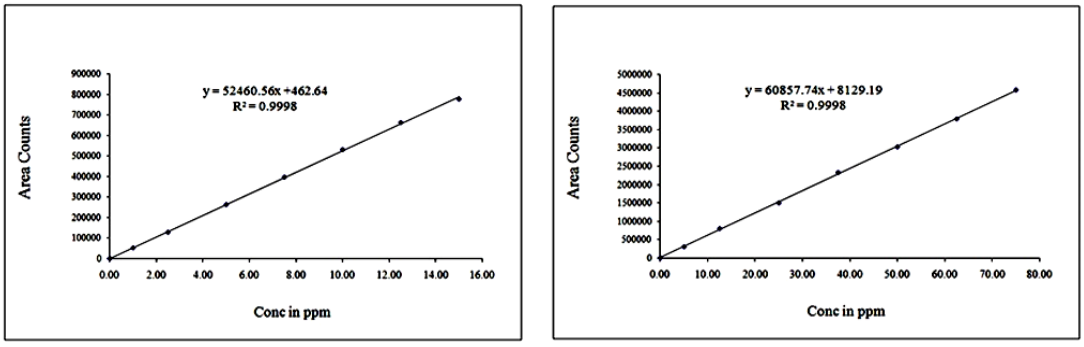
Fig. 4. Chromatogram of standard

3.1.3 Linearity

For Brigatinib, linearity concentrations of 1.0 µg/ml to 15 µg/ml were prepared, while for Alectinib, ranged from 5.0 µg/ml to 75 µg/ml. The regression equations for Brigatinib (CC-0.9998) and Alectinib (CC-0.9998) were Y=52460.56x+462.64 and Y=60857.74x+8129.19, respectively. Table 3 showed the results, and Figure 5 depicted the linearity map.

Table 3: Results of linearity

S. No.	Brigatinib		Alectinib	
	Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
Linearity-1	1.00	51812	5.00	303157
Linearity-2	2.50	127837	12.50	798487
Linearity-3	5.00	262790	25.00	1501250
Linearity-4	7.50	396985	37.50	2331664
Linearity-5	10.00	531081	50.00	3030781
Linearity-6	12.50	662775	62.50	3793848
Linearity-7	15.00	777061	75.00	4585293
Slope	52460.56		60857.74	
Intercept	462.64		8129.19	
CC	0.9998		0.9998	



a b

Fig. 5 Calibration plots of (a) Brigatinib and (b) Alectinib

3.1.4 Limit of detection and quantification

LOD and LOQ were calculated with the calibration curve method. A known RP-UPLC procedure was used to calculate the compound's LOD and LOQ by injecting standard solutions in increasing concentrations. In order to determine LOD and LOQ, the slope approach was employed, with LOQ being calculated as 10x/S and LOD as 3.3x/S, where S is the calibration curve slope and is the response standard deviation. Brigatinib's LOD and LOQ concentrations were 0.013µg/ml and 0.043 µg/ml and Alectinib's were 0.063 µg/ml and 0.208 µg/ml respectively.

3.1.5 Precision

To pinpoint the accuracy of the procedure, the entire analytical process was put to the test by evaluating standard solution preparation and the end results. At least six different determinations were employed to establish repeatability, and the relative standard deviation was established using this information. Based on the data found in Table 4 the following points are made, sample chromatogram was shown in figure 6.

Table 4: Results of method precision

Analyte	Std Conc.	%RSD
Brigatinib	10	1.27
Alectinib	50	0.62

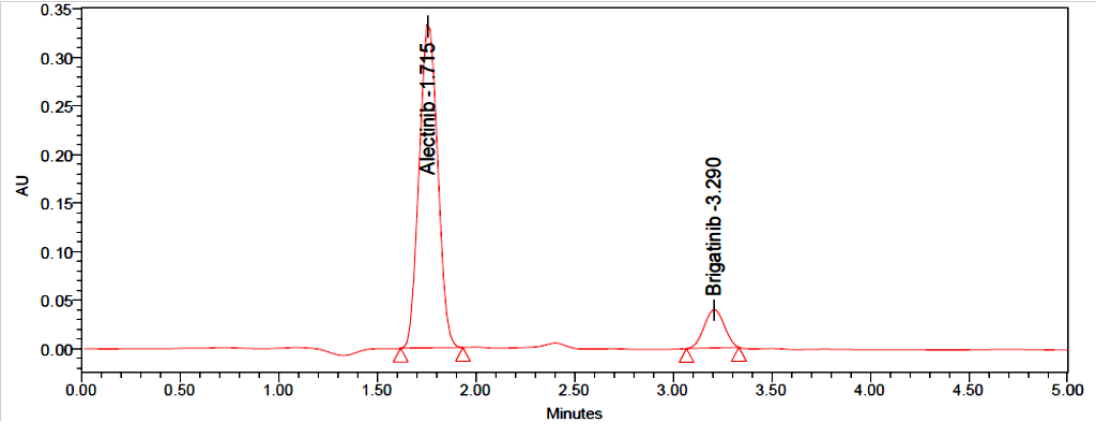


Fig. 6. Chromatogram of sample

3.1.6 Accuracy

The method's accuracy was confirmed through the recovery experiments on three different levels (50 percent, 100 percent and 150 percent). Preparations containing Brigatinib concentrations of 5, 10, and 15 micrograms per millilitre and Alectinib concentrations of 25, 50, and 75 micrograms per millilitre were created. The 98 to 102 percent recovery percentages were found. The accuracy findings for Brigatinib and Alectinib were presented in table 5.

Table 5: Results of accuracy

Accuracy	% Recovery	
	Brigatinib	Alectinib
50*	100.0	99.1
100*	99.4	99.4
150*	99.0	100.5

* Results are mean recovery of three sample preparations

3.1.7 Ruggedness

Six duplicates of a standard solution were sampled on a separate day, using a different analyst and device. Means and % RSD values were obtained for locations of maximum peaks. Findings found in Table 6 are shown in the chart below.

Table 6: Results of intermediate precision

Analyte	Std. Conc.	%RSD
Brigatinib	10	0.88
Alectinib	50	1.41

3.1.8 Robustness

Despite a small flow rate variance (0.2ml) and organic solvent (10 percent) in its chromatographic condition, no significant difference in RSD is made in robustness. Findings are shown in Table 7.

Table 7: Results of robustness

S.No	Parameter name	% RSD for purity	
		Brigatinib	Alectinib
1	Flow (0.8ml/min)	1.32	0.99
2	Flow (1.2ml/min)	0.84	0.63
3	Organic solvent (+10%) (88:12)	0.57	0.82
4	Organic solvent (-10%) (72:28)	0.71	1.35

3.1.9 Forced degradation

This proposed method is effective for both release and stability studies, and as such, can be seen as a better technique for stability. Acid, base, oxidation, reduction, and thermal degradation are all part of the forced degradation study required by the ICH requirements. Dependent on the type of chromatography used, it is apparent that the drugs under consideration were stable during the stress testing even though degraded peaks were observed (Table 9). Acid degradation and Peroxide degradation chromatograms were shown in figure 7 and 8.

3.1.9.1 Acid degradation

The acid degradation method involves introducing 1ml of 1N HCl to a 50ml volumetric flask, heating the flask for 30 minutes at 60°C, then marking the flask with diluent before adding 1ml of 1N NaOH. The final product is obtained after filtering the solution using a 0.45 nylon syringe filter.

3.1.9.2 Alkali degradation

The alkali degradation process begins with the measurement of 50ml of standard solution, followed by the addition of 1ml of 1N NaOH, which is then heated at 60°C for 30 minutes. This is followed by the addition of 1ml of 1N HCl, and the process is ended by diluting the mixture. The final product is obtained after filtering the solution using a 0.45 nylon syringe filter.

3.1.9.3 Peroxide degradation

The following procedure was used to decompose the materials: The solutions, 5 mL of normal solution and 1 mL of 30% H2O2, are placed in volumetric flasks, then warmed for 30 minutes at 60°C and allowed to cool before combining with diluent. The solution can be filtered using a 0.45 nylon syringe filter.

3.9.4 Reduction degradation

The degrading protocol was as follows: In a 50 mL volumetric flask, 5 mL of normal solution is put in, followed by 1 mL of 30% sodium bicarbonate solution. The entire contents are then

heated to 60°C for 15 minutes, and then cooled down to 40°C. To filter the solution, use a 0.45-micron nylon syringe filter.

3.1.9.5 Thermal degradation

The test product was put in an oven heated to 105°C for six hours and then refluxed for 30 min at 60°C. The solution was injected into the UPLC system as a result.

3.1.9.6 Hydrolysis degradation

Standard solution of 5 ml is placed in to a 50 ml volumetric flask, and 2 ml of UPLC water is added. The flask is then heated to 60°C for 15 minutes before chilling with diluent. To filter the solution, use a 0.45-micron nylon syringe filter.

3.1.9.6 Photo degradation

A technique was performed where the standard solution was exposed to the sun for 12 hours, and then 60°C refluxed for 30 minutes. The UPLC technique requires normal water injection.

Table 9: Results of forced degradation

Degradation Condition	% Degradation of Brigatinib	% Degradation of Alectinib
Acid Degradation	14.2	11.8
Alkali Degradation	13.5	12.5
Peroxide Degradation	13.2	15.4
Reduction Degradation	14.9	13.8
Thermal Degradation	1.5	1.9
Photolytic Degradation	0.7	1.1
Hydrolysis Degradation	0.9	1.5

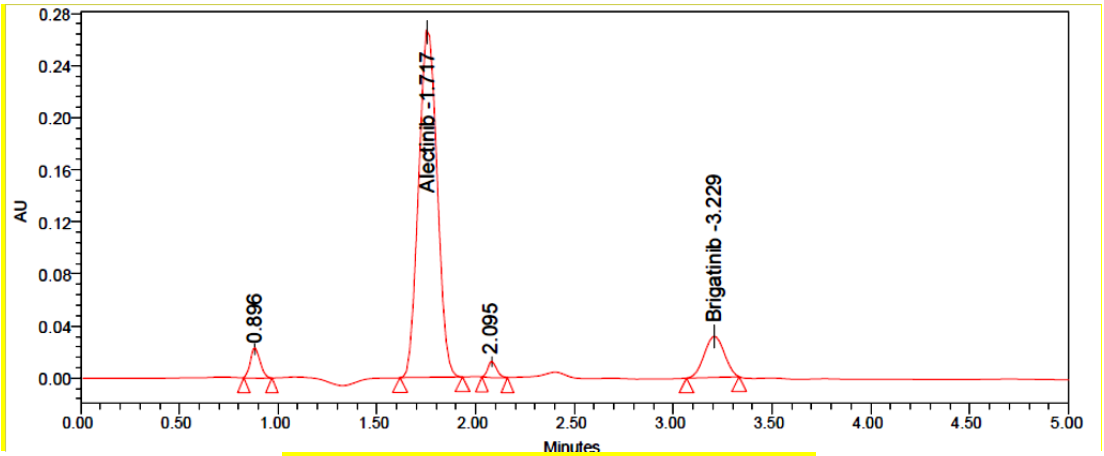


Fig. 7. Chromatogram of Acid degradation

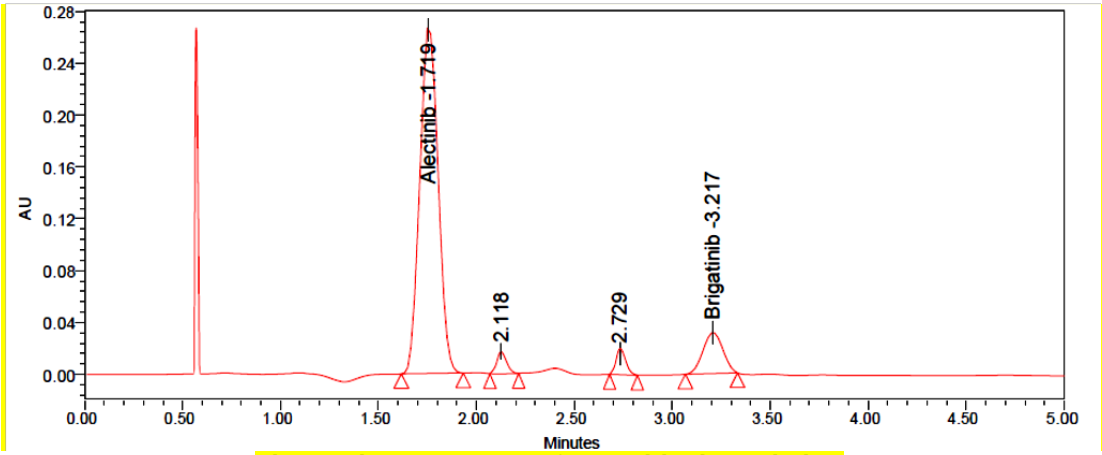


Fig. 8. Chromatogram of Peroxide degradation

4. CONCLUSION

In this article we present a simple, selective, validated and well defined stability that shows isocratic RP-UPLC methodology for the quantitative determination of Capecitabine and Docetaxel. All degradation products produced during stress conditions and the peaks were well separated and well resolved with an adequate retention time, indicating that the proposed method is quick, simple, feasible and affordable. Therefore the evolved chromatographic method can be effectively applied for regular investigation in drug research.

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COMPETING INTERESTS

No financial conflicts of interest.

AUTHORS' CONTRIBUTIONS

Dr Kantipudi Rambabu designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Gunturu Raviteja managed the analyses of the study, managed the literature searches. All authors read and approved the final manuscript.

CONSENT

This manuscript not published at any other journals.

ETHICAL APPROVAL

We are not performing any clinical trials in this study.

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