Journal of Pharmaceutical Research International

33(46B): 154-162, 2021; Article no.JPRI.75665
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

High Pressure Liquid Chromatographic Method for the Determination of Mobocertinib in Pharmaceutical Dosage Form and Study of Its Degradation

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Authors' contributions

This work was carried out in collaboration between both authors. Author KR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GR managed the analyses of the study, managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i46B32927
Editor(s):
(1) Dr. P. Veera Muthumari, V.V. Vanniaperumal College for Women, India.
Reviewers:
(1) Safaa Fathy Saleh Ali, Jazan University & Fayuom University, Egypt.
(2) Muhua Feng, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, China.
Complete Peer review History: https://www.sdiarticle4.com/review-history/75665

Original Research Article

Received 10 August 2021
Accepted 19 October 2021
Published 19 October 2021

ABSTRACT

Aims: New validated method for the estimation of Mobocertinib using HPLC and study of its degradation.
Place and Duration of Study: Department of Chemistry, RVR & JC College of Engineering, Chowdavaram, Guntur, Andhra Pradesh, between February 2021 and August 2021.
Methodology: Using an X-bridge phenyl column (150 mm x 4.6 mm, 3.5 µ), acetonitrile, and 0.1 percent ortho phosphoric acid (OPA) (60:40 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 224 nm. Mobocertinib had a retention time of 2.271 minutes. The isocratic chromatography was performed at room temperature and took approximately five minutes to complete.
Results: Analysis was achieved within 5 min over an honest linearity within the concentration range from 6-90 µg/ml of Mobocertinib. Using a mathematical process, the suitability parameters of

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the system were investigated, and the results were found to be in acceptable limits. In a linear analysis, stages with regression coefficients of 0.999 were used. LOD and LOQ values were 0.075µg/ml and 0.248 g/ml for Mobocertinib. The drug was recovered at a rate of 98-102 percent, which means that the recovery is within reasonable limits.

Conclusion: The validation results were satisfactory, and the approach was found to be suitable for bulk and formulation analysis. The recommended procedure was found to be warranted according to ICH guidelines.

Keywords: Mobocertinib; development; validation; RP-HPLC; degradation studies.

1. INTRODUCTION

Mobocertinib, sold under the brand name Exkivity, is used for the treatment of non-small cell lung cancer [1,2]. The most common side effects include diarrhea [3,4], rash [5], nausea [6,7], stomatitis [8], vomiting [9], decreased appetite [10], paronychia [11,12], fatigue [13], dry skin, and musculoskeletal pain [14,15]. Mobocertinib is a small molecule tyrosine kinase inhibitor [16]. Its molecular target is epidermal growth factor receptor (EGFR) [17] bearing mutations in the exon 20 region [18]. It is a first-in-class oral treatment to target EGFR Exon20 insertion mutations.Mobocertinib is indicated for adults with locally advanced or metastatic non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) exon 20 insertion mutations, as detected by an FDA-approved test, whose disease has progressed on or after platinum-based chemotherapy [19]. Mobocertinib specifically inhibits EGFR (epidermal growth factor receptor, ErbB1 [20]) and HER 2 [21] (human epidermal growth factor receptor 2, ErbB2). Chemical structure of Mobocertinib is shown in Fig. 1.

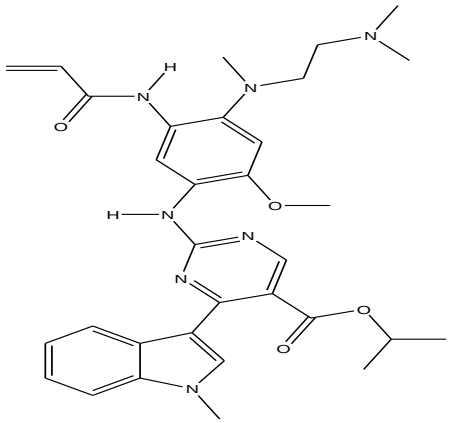


Fig. 1. Chemical structure of Mobocertinib

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

There is only one LC-MS/MS method reported in the literature, but this method was developed only for the analysis of mobocertinib in rat plasma. Till today there were no HPLC reports were developed for the quantification of Mobocertinib. Hence, the developed HPLC method was utilized for the estimation of the drug in bulk and pharmaceutical dosage form.

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

The reagents were purchased from Merck (India) Ltd., Mumbai, India: Acetonitrile, Ortho phosphoric acid (OPA) (purity-99.9 percent) and water (HPLC grade). Glenmark Pharmaceutical Private Ltd., Andheri (E), Mumbai, India provided an API (purity-99.9%) for Mobocertinib as a reference standard.

2.2 Equipment

Using an e-2695 chromatographic system and a PDA 2996 detector, we utilised a quaternary pump and a PDA. Empower software version 2.0 was used to analyse the chromatographic data.

2.3 Chromatographic Conditions

To conduct chromatography using isocratic conditions, an X-bridge phenyl (150 mm x 4.6 mm, 3.5 µ) column was utilised at temperature using a Chromatographic conditions separation was administered in isocratic mode at temperature employing an X-bridge phenyl (150 mm x 4.6 mm, 3.5 µ) column. Ortho phosphoric acid (0.1%) and acetonitrile (40:60 v/v) with a flow rate of 1 mL/min were used as a mobile phase in this experiment. Injection volume was 10 µl, and the eluent was found at 224 nm, as the maximum concentration of Mobocertinib was

found at this wavelength. So, it was decided to use the wave length of 224 nm.

2.4 Diluent

Mobile phase was used as diluents.

2.5 Preparation of Standard Solution

60 mg of Mobocertinib working standard was added to 100 ml of the flask and the solution was diluted to the required volume with the diluent. Dilute 5 ml of the prepared solution with diluents to a final volume of 50 ml.

2.6 Preparation of Sample Solution

Mix 138 mg of Mobocertinibsample (each capsule contains 40 mg of Mobocertinib) with 70 ml of diluents and sonicate to dissolve it. Then, add the remaining 30 ml of diluents to the mark. Use more diluents to dilute the sample solution, mixing thoroughly.

2.7 Forced Degradation

As far as release and stability studies are concerned, this proposed technique is an improvement on previous techniques, as it enables the use of both of these approaches. The following process steps are all part of the forced degradation study required by the ICH guidelines: acid, base, oxidation, reduction, and thermal degradation. In conclusion, it appears that the drugs under consideration were stable even though degraded peaks were observed, as they are dependent on the type of chromatography used.

2.7.1 Acid degradation

1 ml of the sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, to which 1 ml of 1N HCl was added and left to stand for 15 minutes. After 15 min add 1 ml of 1N NaOH and make up to the diluent mark. Filter the solution using syringe filter and injected into HPLC system.

2.7.2 Alkali degradation

1 ml of the sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, 1 ml of 1N NaOH was added, and the mixture was left to stand for 15 minutes. After 15 minutes, add 1 mL of 1N HCl to bring the solution up to the required concentration. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

2.7.3 Peroxide degradation

1 ml of sample stock solution was moved to a volumetric flask of 10 ml, add 1 ml of 30% hydrogen peroxide solution and make up to the mark with diluents. Filter the solution using syringe filter and injected into HPLC system.

2.7.4 Reduction degradation

Using a volumetric flask with a capacity of 10 ml, transfer 1 ml of sample stock solution and add 1 ml of 30% sodium bisulphate solution, then dilute to the required concentration with diluents. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

2.7.5 Thermal degradation

During the 6 hour baking period, the sample solution was kept at 105°C. The resulting solution was injected into a high-performance liquid chromatography system.

2.7.6 Hydrolysis degradation

1 ml of sample stock solution was transferred to a volumetric flask with a capacity of 10 ml, to which 1 ml of HPLC water was added, and the volume was brought up to the required level with diluents. Use a syringe filter to filter the solution, which will then be injected into the HPLC system.

3. RESULTS AND DISCUSSION

The purpose of this study was to develop a simple, accurate, and rapid RP-HPLC method for the estimation of Mobocertinib in bulk and pharmaceutical dosage form. To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally 0.1% ortho phosphoric acid buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredient. 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 X-bridge phenyl column of 150 x 4.6mm, 3.5 µ

with a PDA detector. The mobile phase flow rate has been done at 224 nm in order to obtain enough sensitivity. The retention time for Mobocertinib was 2.271 minutes. According to the ICH guidelines the proposed method was validated. Table 1 depicts the chromatographic parameters applied for the method.

3.1 Specificity

There was no blanketing of Mobocertinib until the molecules had been retained for the set period of time. The chromatogram in Fig. 2 shows an empty chromatogram.

3.2 System Suitability

Stabilization was performed for 60 minutes to encourage a constant base line. The system suitability was checked by dispensing six Mobocertinib-branded injections, which each contained 60 µg/ml of Mobocertinib, and assessing the results. A theoretical plate count of 6851 was derived for Mobocertinib, while the tailing factor was 1.12. These values were deemed acceptable. To gather all the data, the chromatography software will be utilised (Empower 2.0). Fig. 3 shows Satandard chromatogram and Table 2 gives System precision results.

3.3 Linearity

A standard solution containing 60 µg/ml of Mobocertinib was prepared to determine the linearity of the tactic (100 percent of the targeted level of the assay concentration). For this problem, it was necessary to perform sequential dilutions of the given solutions at concentrations ranging from 10 percent, 25 percent, 50 percent, 100 percent, 125 percent, 150 percent of the target concentrations. Because they were pumped, the peaks are used to map calibration curves on to the data points. It was found that the correlation coefficient between these analytes was 0.999. The results of the linearity tests and the Fig. 4, which displays the calibration plot of Mobocertinib, are shown in Table 3. The values of slope, intercept and correlation coefficient were acquired from the linearity calculation sheet.

Table 1. Optimized chromatographic conditions

Parameter	Proposed method
Stationary Phase	X-bridge phenyl (150 x 4.6 mm, 3.5 µ)
Mobile Phase	0.1% OPA : Acetonitrile (40:60)
Injection Volume	10 µl
Flow Rate	1.0 mL/min
Column Temperature	Ambient
Wave Length	224 nm
Run Time	5.0 min
Retention time of Mobocertinib	2.271 min

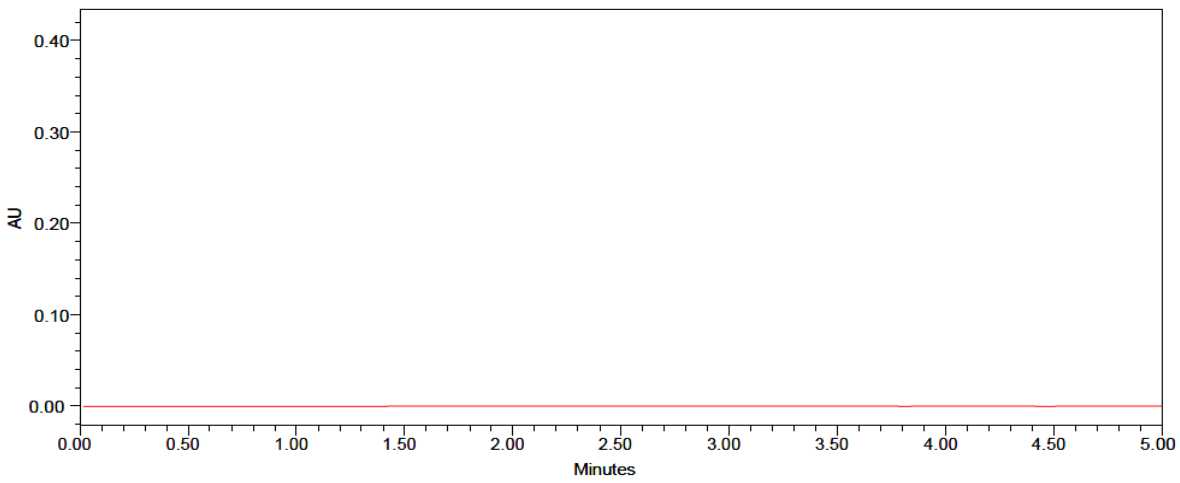


Fig. 2. Chromatogram of blank

Table 2. Results of system suitability

Parameter	Mobocertinib
Theoretical plate count	6851
Tailing factor	1.12
Resolution	-
Retention time	2.271 min

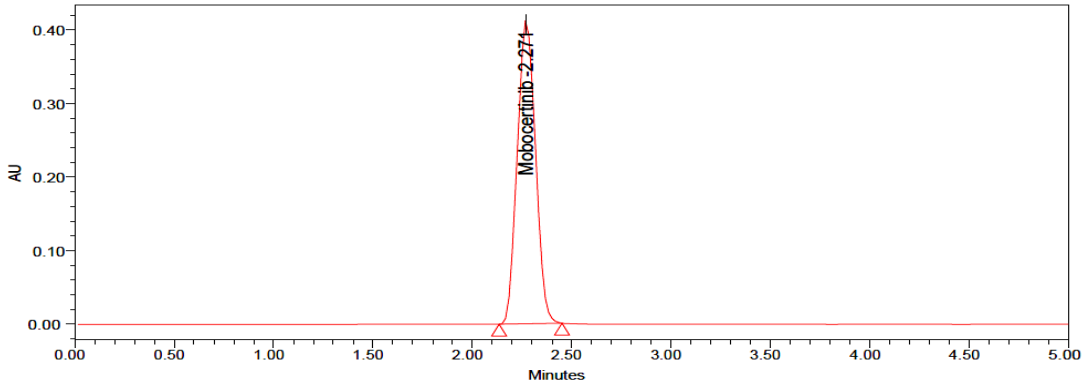


Fig. 3. Chromatogram of standard

Table 3. Results of linearity

S. No	Mobocertinib	
	Concentration (µg/mL)	Area
1	6.00	396971
2	15.00	931459
3	30.00	1947935
4	60.00	3819390
5	75.00	4763458
6	90.00	5746340
CC	0.99996	
Slope	63682.47	
Intercept	4170.18	

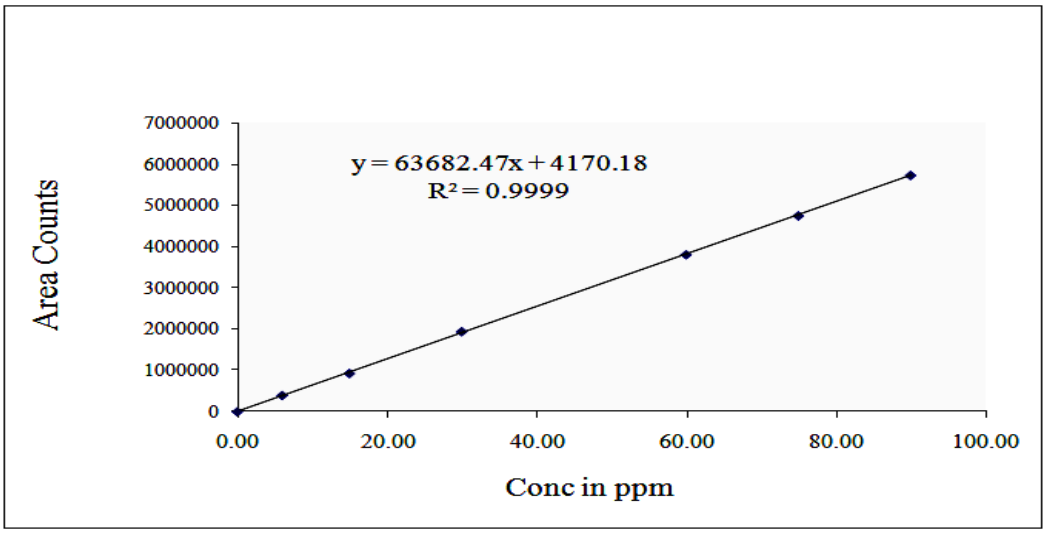


Fig. 4. Calibration plot of Mobocertinib

3.4 Limit of Detection and Quantification

The concentration level at which the analyte are reliably detected and quantified is the limit of detection and quantification. Mobocertinib had a LOD concentration of 0.075 µg/ml and a S/N value of 5. The concentration of Mobocertinib in the LOQ was 0.248 µg/ml, and the S/N value was 23. S/N is the ratio of signal to noise.

3.5 Precision

Six samples of an identical batch were prepared, and then the method precision of the process was examined. Due to solution injection, the maximum responses from these six separate samples won't calculate mean and percentage RSD values. This method was found to be precise, with an RSD of 2%, and the RSD percentage of the specimen or share assay values was nearly 100%. Table 4 gives the method precision results. Sampling chromatogram (Fig. 5).

Table 4. Results of method precision

S. No.	Area of Mobocertinib
1	3832418
2	3801582
3	3814570
4	3828269
5	3817321
6	3851651
Mean	3824301.83
Std. dev	17258.1513
% RSD	0.45

3.6 Accuracy

Effectiveness was established through recovery studies that were conducted in 3 separate concentrations (50 percent, 100 percent and 150 percent). 30, 60, and 90 µg/ml concentrations of API were made. According to the specified test method, the solution was injected into three solutions of increasing concentration, which allowed for the assay to be performed. In between 99.6 and 100.3 percent of Mobocertinib, the recovery values were observed. The recovery values for the share price were found to be two percent. Table 5 presents the accuracy results.

Table 5. Results of accuracy

Accuracy	Amount of Mobocertinib	% Recovery
50*****	30	99.7
100*	60	99.6
150*	90	100.3

** Results are mean recovery of three sample preparations*

3.7 Ruggedness

The HPLC method, observer, and column were investigated to see if the chromatographic patterns changed significantly when a different tactic was used. It is proof of the quality of the long-standing process that the RSD percentage was less than 2 percent. Ruggedness results are shown in Table 6.

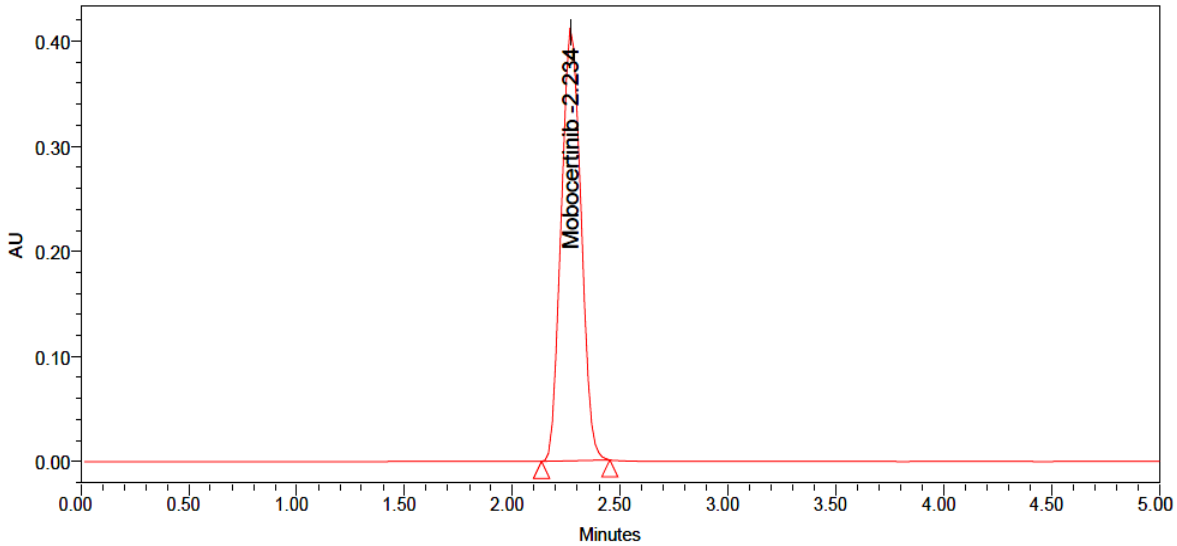


Fig. 5. Chromatogram of sample

Table 6. Results of intermediate precision

S. No.	Area of Mobocertinib	% RSD
1	3858214	0.79
2	3813206	
3	3815427	
4	3876539	
5	3804362	
6	3861488	

3.8 Robustness

According to RSD's tests, the robustness of the tactic brought in only 2% of RSD. The slightly varied parameters such as flow (±0.2 mL/min) and organic content in the mobile phase (±10 percent) were eliminated in favour of the optimised methods. Robustness results are shown in Table 7.

3.9 Stability

The ordinary and sample solutions were studied from initial to 24 hours, stored at RT, by examining the stability techniques. Injections were given at different time intervals, and the percentage of the assay made at the time of the first injection was about 2 percent less than that made 24 hours later. In storage conditions, there is no effect for Mobocertinib. Stability results are shown in Table 8.

Table 7. Results of robustness

Parameter	% RSD of Mobocertinib
Flow (0.8 mL/min)	1.32
Flow (1.2 mL/min)	0.15
Organic phase (54:46)	0.11
Organic phase (66:34)	0.12

3.10 Forced Degradation

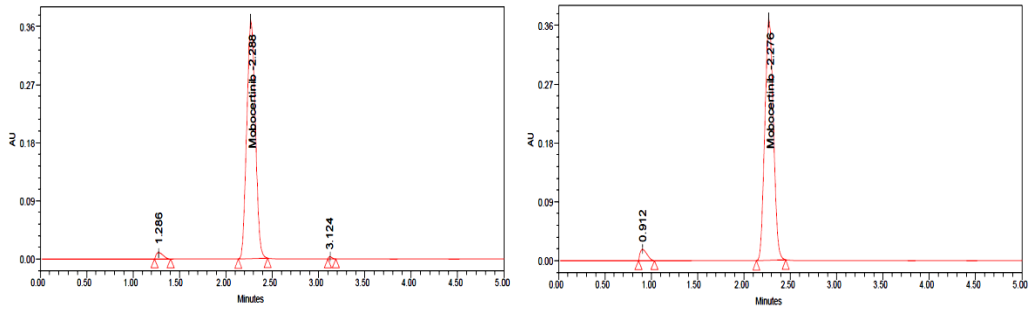
In acid condition 14.3% of Mobocertinib was degraded, in alkali condition 13.5% of the drug was degraded, in peroxide condition 16.1 of the drug was degraded, in reduction condition 13.4% of the drug was degraded, in thermal condition 4.1% of the drug was degraded and in hydrolysis condition 1.8% of the mobocertinib drug was degraded. Results of forced degradation are shown in Table 9 and the chromatograms were shown in Fig. 6.

Table 8. Stability results of Mobocertinib

Time intervals	Mobocertinib (% assay)	% Deviation
Initial	100.2	0.00
6 Hrs	99.3	-0.90
12 Hrs	99.1	-1.10
18 Hrs	98.5	-1.70
24 Hrs	97.3	-2.89

Table 9. Results of forced degradation

Stress Parameter	Mobocertinib	
	% degradation	% Assay
Acid degradation (1N HCl)	14.3	85.7
Alkali degradation (1N NaOH)	13.5	86.5
Peroxide degradation (30% Peroxide)	16.1	83.9
Reduction degradation (30% sodium bi sulphate)	13.4	86.6
Thermal (sample, 105°C, 6 Hrs)	4.1	95.9
Hydrolysis (1 ml HPLC water)	1.8	98.2



Acid degradation Alkali degradation

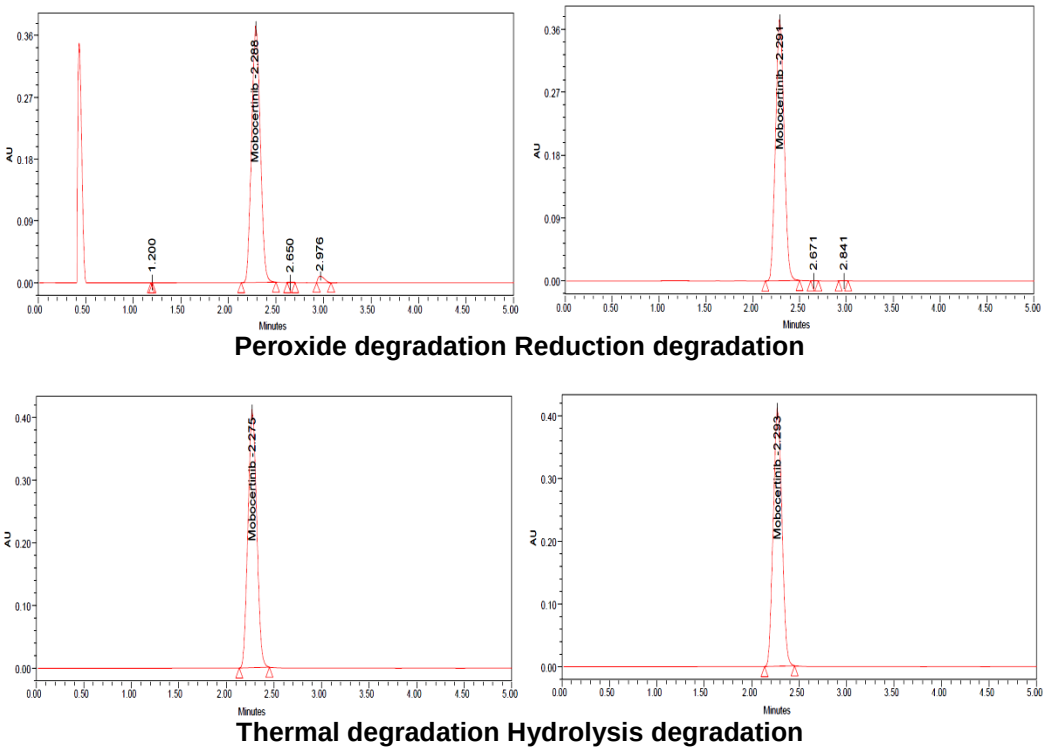


Fig. 6. Chromatogram of forced degradations

4. CONCLUSION

We present in this article simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Mobocertinib. All the products of degradation formed during the stress conditions and the active pharma ingredient are well separated and peaks were well resolved from each other and separate with an appropriate retention time indicating that the proposed method to be fast, simple, feasible and affordable in assay condition. The advantage of the method is the common chromatographic conditions are adopted for assay. Therefore the developed method showed it can be used for routine analysis of production standards and to verify the quality of drug standards during stability studies.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors express their appreciation to Professor Dr. Kantipudi Rambabu for offering helpful advice and guidance. I want to thank the research facility, Vijayawada, India, at full to Shree icon pharmaceutical laboratories, as they allowed this research to take place.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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