



Isolation, identification and structural characterization of forced degradation products of mobocertinib using LC-MS/MS and NMR

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ABSTRACT

The main objective of this study is to develop and validate an LC-MS/MS stability-indicating method for the isolation, identification, and characterization of forced degradation products of mobocertinib under various stress conditions, utilizing LC-MS/MS and NMR techniques. Four degradation products (DP1, DP2, DP3 and DP4) were isolated. Degradation under acidic and oxidative conditions provides DP1 and DP4, respectively. Both DP2 and DP3 were obtained under basic conditions. Chromatographic development and separation were achieved using an ODS C18 column (Chemsil) with a constant flow rate of 1.0 ml/min. The mobile phase consisted of 40 % water containing 0.1 % formic acid in methanol, and detection was performed at a wavelength of 225 nm. The application of LC-MS/MS technology was utilized to separate the above degradation products. The LC-MS/MS method has validated which includes specificity, linearity, LOD and LOQ, precision, accuracy and robustness. The chemical structure of the degradation products were characterized by ¹H NMR and mass spectroscopy. The above listed degradation impurities are novel and not listed in the literature. In conclusion, this study provides vital information about mobocertinib's chemical stability and helpful in further drug development process.

Introduction

Mobocertinib chemically isopropyl 2-((5-acrylamido-4-((2-(dimethylamino)ethyl)(methyl) amino)-2-methoxyphenyl)amino)-4-(1-methyl-1H-indol-3-yl)pyrimidine-5-carboxylate (Fig. 1). It is authorized as orally accessible inhibitor of human EGFR exon 20 insertion mutations [1]. Mobocertinib and its active metabolites exerts its activity by irreversibly binding with EGFR and blocks exon 20 insertion mutation [2]. The inhibition of EGFR signaling causes cell death in tumor cells [3]. In the year 2021, FDA approved the mobocertinib for adults in the treatment of locally advanced or metastatic non-small cell lung cancer. The recommended dose is 160 mg orally once daily. Additionally, mobocertinib reported as an inhibitor of other EGFR family members, including ERBB2, HER2 and HER4. EGFR, HER-2&4 are tyrosine kinases which are frequently altered in a variety of tumor cells [4,5]. They play important roles in tumor cell proliferation and vascularization [6,7].

Any pharmaceutical product's safety and its efficacy is depends on chemical stability of the active pharmaceutical ingredient. The FDA and ICH framed the guidelines to understand the stability of the pharmaceutical molecules by subjecting them into forced degradation studies

[8–11]. Forced degradation studies are important criteria in the pharmaceutical product development. Forced degradation studies are carried out by subjecting the active pharmaceutical ingredient or drug product under various accelerated conditions such as acidic, basic, oxidation, light, thermal, and humidity [12,13]. The results of the forced degradation studies will provide an insight during selection of suitable formulation, storage condition, detection and quantification of drug-related impurities, and its shelf life. In addition, forced degradation studies provide valuable data to regulatory bodies enabling them to make decisions about drug approval and post-marketing surveillance.

The physical appearance of mobocertinib is light yellow color powder, freely soluble in DMSO and methanol and insoluble in water. The molecular weight is 585.7 g/mol. In the marketed preparations, mobocertinib is available as capsule on the name of Exkivity® and contains mobocertinib succinate. Analytical reports on method validation for mobocertinib are limited since it is a newly approved drug. Li and colleagues reported an LC-MS/MS technique for quantifying mobocertinib in rat plasma [14]. A new HPLC approach and forced degradation experiments were presented by Raviteja et al. for the measurement of mobocertinib in pharmaceutical dose [15]. However, no degradation products were isolated and reported. The LC-MS/MS approach has

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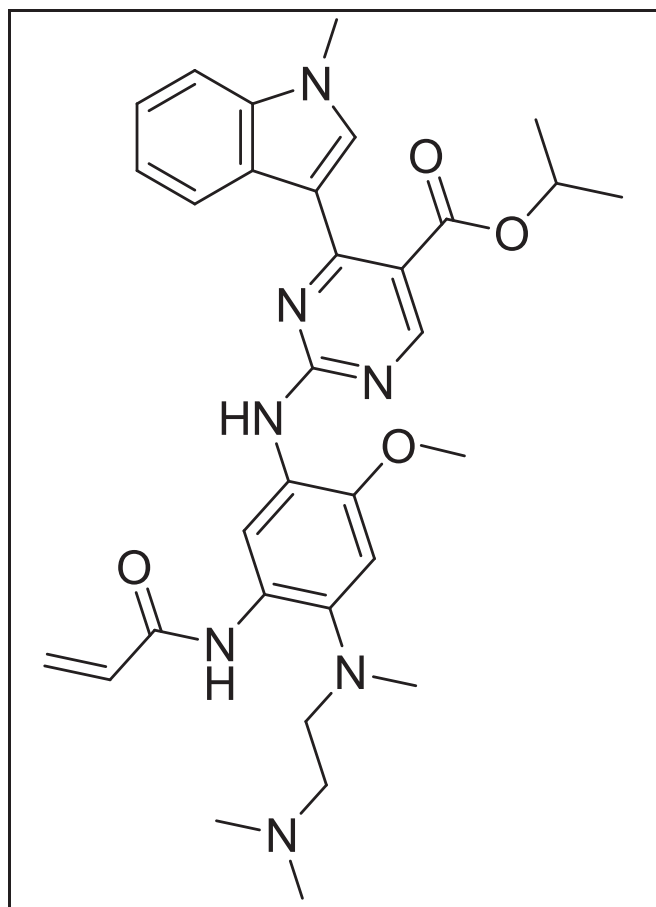


Fig. 1. Chemical Structure of Mobocertinib Drug.

proven to be efficacious in the identification, characterization, and separation of degradation products resulting from recently licensed anticancer medicines [16–18]. In this research paper we developed a swift and highly sensitive LC-MS/MS approach to identify and characterize the degradation products of mobocertinib.

Experimental

Chemicals and reagents

A gift sample of mobocertinib was acquired from Sun Shine Chemical, China. Analytical reagents were sourced from Qualigens Fine Chemicals Pvt. Ltd. Mumbai, India, while acetonitrile and methanol were purchased from J.T. Baker Chemicals Pvt. Ltd. Mumbai, India. Additionally, NaOH and HCOOH were obtained from S.D. Fine-Chem Ltd. Mumbai, India, HCl from Merck Specialties Pvt. Ltd. Mumbai, India and ultra-pure water from Aquarch, Ahmedabad, India.

Instrumentation

For development and validation LC-MS/MS method for mobocertinib, LC-MS/MS system was implemented in positive ion mode. MS/MS are manufactured by Applied Bio systems Scix API 2000, USA and LC is manufactured by Agilent Infinity 1100 (Waldbronn, Germany). The mass spectrometer is equipped with ESI source along with triple quadrupole system. Sonicator from Frontline 1870, the pH meter is

Table 1

Linearity Response for Mobocertinib.

Linearity Level (%)	Concentration of Mobocertinib (µg/ml)	Area
50	0.2	3429.521
75	0.3	5161.627
100	0.4	7041.352
125	0.5	8974.846
150	0.6	11092.509

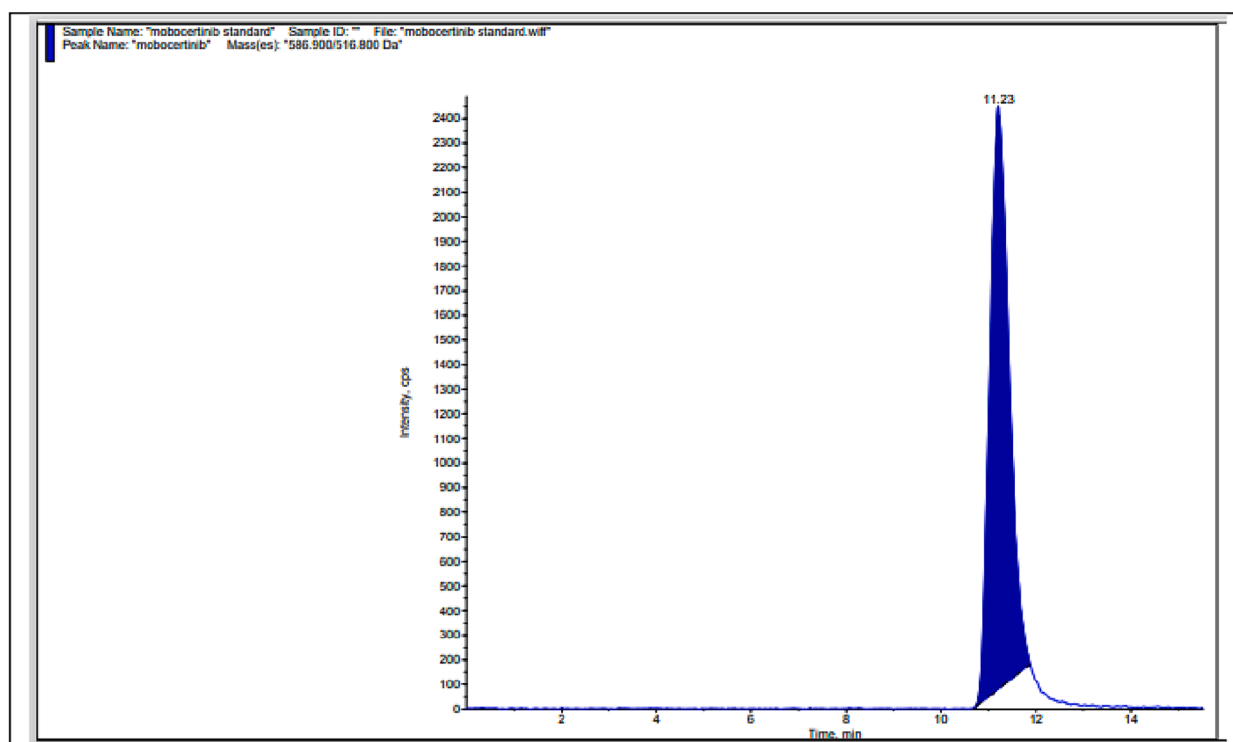


Fig. 2. Mobocertinib LC-MS/MS Chromatogram of Standard Solution.

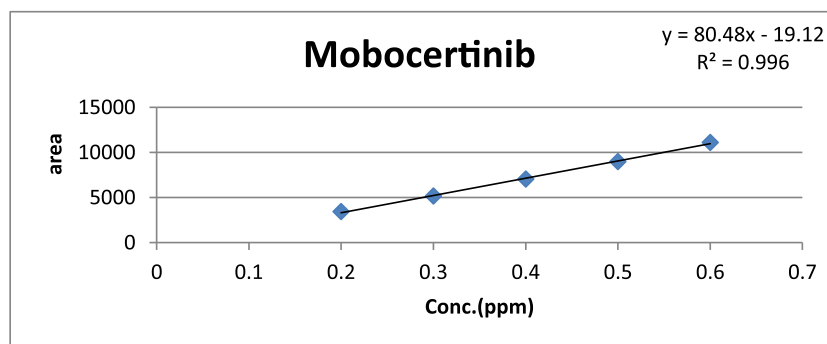


Fig. 3. Linearity Curve of Mobocertinib Drug.

Table 2

Result of Repeatability Data for Mobocertinib.

Conc. of Mobocertinib (µg/ml)	Mean Area ± S.D	% R.S.D
0.4	7061.87 ± 107.84	1.527

Table 3

Result of Precision Data for Mobocertinib.

Conc. of Mobocertinib (µg/ml)	Intraday Precision Mean Area ± S.D, % R. S.D	Interday Precision Mean Area ± S.D, % R. S.D
0.2	3522.96 ± 34.31, 0.974	3460.62 ± 52.43, 1.515
0.4	7044.36 ± 105.17, 1.493	7082.09 ± 86.90, 1.227
0.6	11056.85 ± 177.34, 1.604	10927.45 ± 157.45, 1.441

Table 4

Result of Accuracy Data for Mobocertinib.

Recovery Level	Sample amount (µg/ml)	Amount added (µg/ml)	% Recovery	%Mean Recovery ± S.D	% R. S.D
80 %	0.20	0.16	101.392	100.111 ± 1.172	1.172
	0.20	0.16	99.088	1.174	
	0.20	0.16	99.853		
100 %	0.20	0.20	99.447	100.449 ± 1.011	1.011
	0.20	0.20	101.477	1.015	
	0.20	0.20	100.423		
120 %	0.20	0.24	100.774	100.589 ± 1.574	1.574
	0.20	0.24	101.052	0.578	
	0.20	0.24	99.942		

Table 5

Result of Analysis of Marketed Formulation.

Capsule	Label Claim	Assay (% of label claim*) Mean ± S. D.	% R.S.D
Exkivity	40 mg	99.909 ± 1.751	1.752

manufactured by Analab Scientific Private Ltd, the hot air oven and photo stability chamber are from Kesar Control System (Ahmedabad, India). The entire study's sample weight was measured using a highly accurate calibrated Shimadzu ATX-224 microbalance (Kyoto, Japan). Isolation was achieved using preparatory Shimadzu HPLC equipment, which has an LC-20AR pump and a rheodyne injector linked to the detector (SPD-20AT). ^1H NMR was recorded using Bruker AVANCE 400 MHz spectrometer. All NMR spectra were recorded in DMSO and the chemical shifts values were expressed in ppm.

Table 6

Results of retention time and m/z values of DPs and its fragment ions of Mobocertinib.

Degradation Condition	Degradation Products (DPs)	Retention Time (min)	Molecular ion m/z value (Da)	Selected fragment ion m/z value (Da)
Acid	DP1	6.87	544.400	414.500
Base	DP2	5.58	618.700	517.600
	DP3	6.88	544.600	414.500
Oxidation	DP4	12.86	618.900	485.400

Preparation of standard stock and working standard solution

Mobile phase preparation: A mixture of water containing 0.1 % formic acid in methanol was prepared in the ratio of 40:60 and degassed by sonication.

Diluent preparation: Prepare a mixture of HPLC-grade water and methanol in a 40:60 (v/v) ratio.

Preparation of standard stock solution of mobocertinib: To prepare a concentration of 40 µg/ml of mobocertinib, transfer and dissolve 40 mg of mobocertinib into a 1000 ml volumetric flask with methanol.

Preparation of working standard solution of mobocertinib: Add diluent to a 100 ml volumetric flask containing 1.0 ml of mobocertinib standard stock solution to achieve a final concentration of 0.4 µg/ml.

Preparation of sample solution of mobocertinib: Weigh at least ten capsules and determine the net content of each capsule. Transfer 40 mg of mobocertinib capsule content into 1000 ml flask and add 600 ml of methanol and sonicate the solution for ten minutes. Finally, transfer 1.0 ml of the stock mixture into a 100 ml flask and prepare the sample by diluting it with diluent. Use a 0.45µ PTFE syringe filter to filter the sample.

Preparation of acid degradation solution of mobocertinib: Transfer 1.0 ml of the standard stock solution into a 100 ml round bottom flask, add 1.0 ml of 0.1 M HCl, and reflux at 60 °C for 4 h. After 4 h, cool the solution to room temperature, neutralize it, and achieve the final concentration 0.4 µg/ml by adding diluent.

Preparation of base degradation solution of mobocertinib: Transfer 1.0 ml of the standard stock solution into a 100 ml round bottom flask, add 1.0 ml of 0.1 M NaOH, and reflux at 60 °C for 4 h. After 8 h, cool the solution to room temperature, neutralize it, and dilute the reaction mixture with diluent to attain a final concentration 0.4 µg/ml.

Preparation of neutral degradation solution of mobocertinib: Transfer 1.0 ml of standard stock solution into a 100 ml round bottom flask, add 1.0 ml of water, and reflux at 60 °C for 72 h. After 72 h, cool the reaction mixture and make up the volume with diluent to obtain 0.4 µg/ml of mobocertinib.

Preparation of oxidative degradation solution of mobocertinib: Transfer 1.0 ml of standard stock solution into a 100 ml round bottom flask, add 1.0 ml of a 3.0 % hydrogen peroxide solution, and reflux for 4 h at 60 °C.

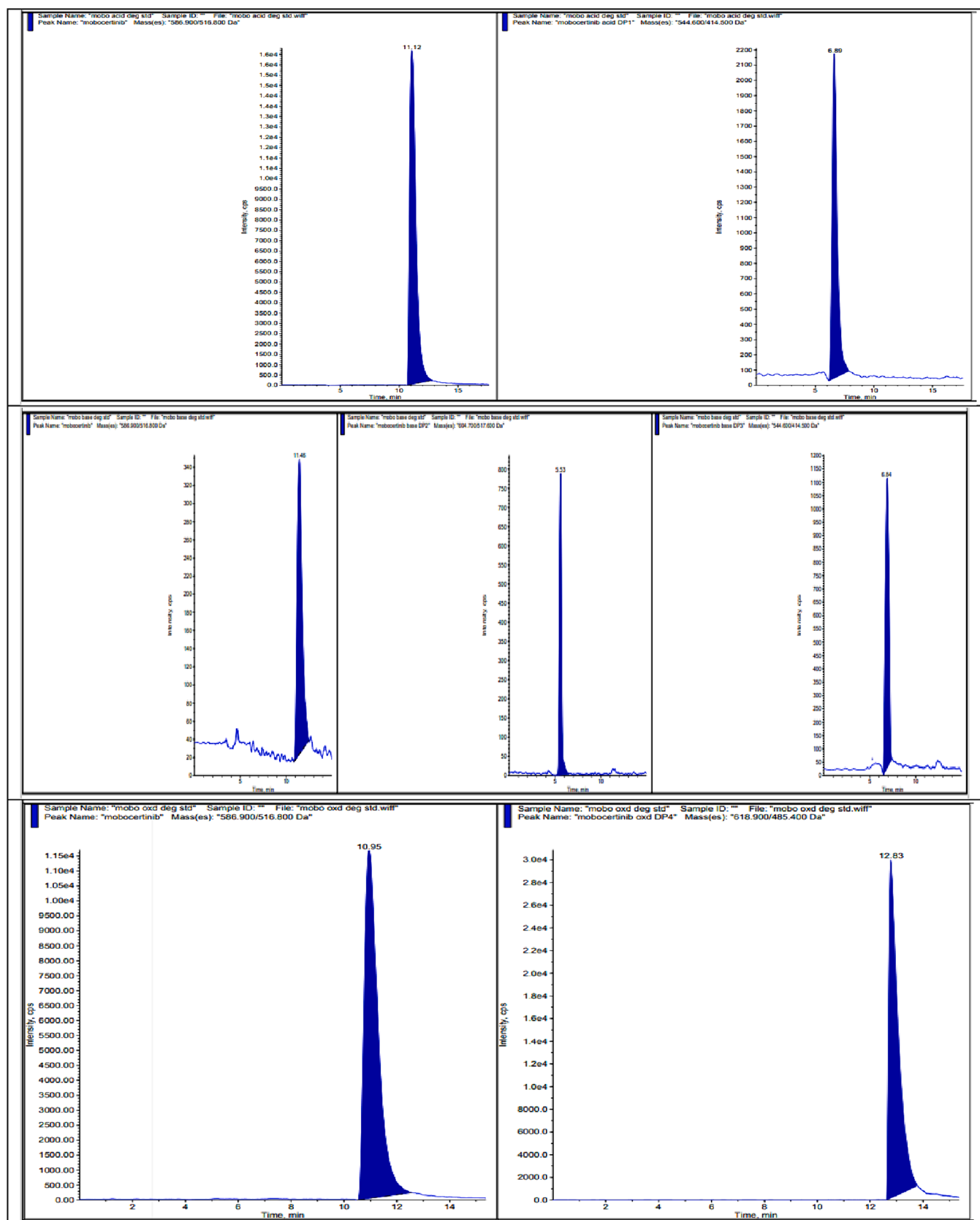


Fig. 4. Mobocertinib LC-MS/MS Chromatogram of Degradation Solution.

Table 7
Chromatographic Condition for HPLC.

Final Mobile Phase	0.1 % Formic acid in Water: Methanol (40:60)
Column	Chemsil ODS-C ₁₈ (150 mm X 4.6 mm, 5 μ m)
Column Temperature	35 °C
Flow Rate	1.0 ml/min
Wavelength	225 nm
Injection Volume	20 μ L

After 4 h, cool the reaction mixture to room temperature and dilute it with diluent to achieve a concentration of 0.4 μ g/ml of mobocertinib.

Preparation of thermal degradation solution of mobocertinib: 100.0 mg of mobocertinib standard in a petridish was allowed to expose in a hot air oven at 100 °C for 5 days. After 5 days, weigh and transfer about 40.0 mg of the powder into a 1000 ml volumetric flask and dissolve with methanol. Transfer 1.0 ml of the solution into a 100 ml volumetric flask and dilute it with diluent to obtain a concentration of 0.4 μ g/ml of mobocertinib thermal degradation solution.

Preparation of photo degradation solution of mobocertinib: Transfer 100.0 mg of mobocertinib standard in a petridish and allowed it in a photo stability chamber for 5 days. The rest of the procedure is similar to thermal degradation.

Forced degradation studies in the marketed dosage form (Exkivity capsule): The forced degradation studies in the marketed dosage form also studied by adopting similar condition as described for mobocertinib standard.

Results and discussion

LC-MS/MS chromatographic condition optimization for analysis of mobocertinib

The primary goals of the present work are: (1) Develop and validate a stability-indicating by using LC-MS/MS analytical technique and (2) Isolate, identify, and describe the forced degradation products of mobocertinib using LC-MS/MS and ¹H NMR. The stability indicating LC-MS/MS method was developed and validated using the 150 mm $\times$ 4.6 mm, 5 μ m Chemsil ODS C18 column. The temperature of the column was

maintained at 35 °C. 0.1 % formic acid in water and methanol at the ratio of 40:60 was utilized as mobile phase. A flow rate of 1.0 ml/min was maintained with an injection volume of 20 μ L and nitrogen was used as curtain gas. Positive ionization mode was used to record the mass spectra. The temperature of around 400 °C, curtain gas pressure of 20 psi, the ion spray voltage of 5500 V, ion source pressure of 50 psi for GS1 and 60 V for GS2 were the parameters for ion source optimization. Prior scanning, following parameters were checked: declustering, focusing, and entrance voltages of a multiple reaction monitoring scan are set to 45, 450, and 10 V, respectively. The mass spectrum of mobocertinib yielded 586.900 Da and 516.800 Da as molecular ion peak and major fragment peak, respectively. The standard chromatogram displays a peak at 11.21 min for the retention period of mobocertinib and the developed method is validated as per guideline of ICH (Fig. 2) [14].

Validation of LC-MS/MS chromatographic method

Specificity

Specificity is performed by injecting 20 μ L solutions of standard, sample and blank solution of mobocertinib individually. It was observed that the blank solution has no interference with the standard and sample solution of mobocertinib drug. Hence, all analyte peaks were pure and hence proved the specificity of the method.

Linearity and range

By least square method, linear regression analysis was established for a range of various concentration of mobocertinib. The calibration curve shown below gives good linearity in the range of 0.2–0.6 μ g/ml with a correlation coefficient (r^2) of 0.996. Results are given in Table 1 and Fig. 3.

LOD and LOQ

Using the established formulas, it was discovered that the LOD and LOQ for mobocertinib were 0.022 μ g/ml and 0.067 μ g/ml, respectively. The low values of LOD and LOQ show that the established method was sensitive, accurate, and precise since it could detect and quantify at extremely low concentrations.

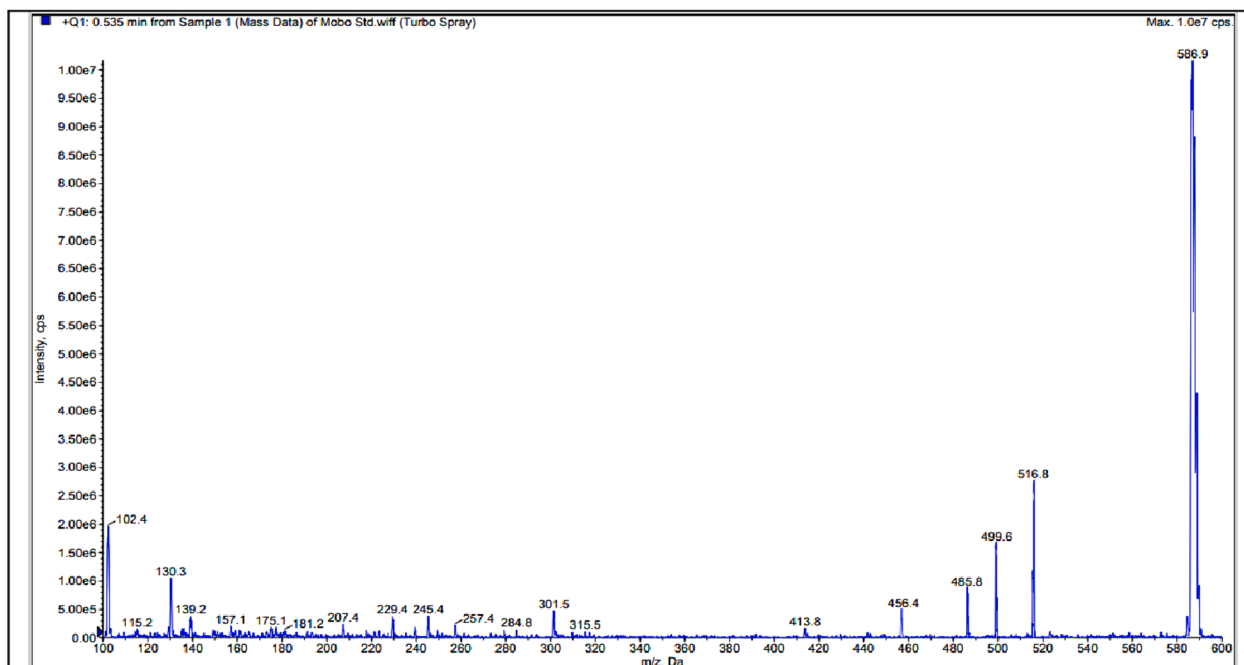


Fig. 5a. Mass Spectra of Mobocertinib Standard Solution.

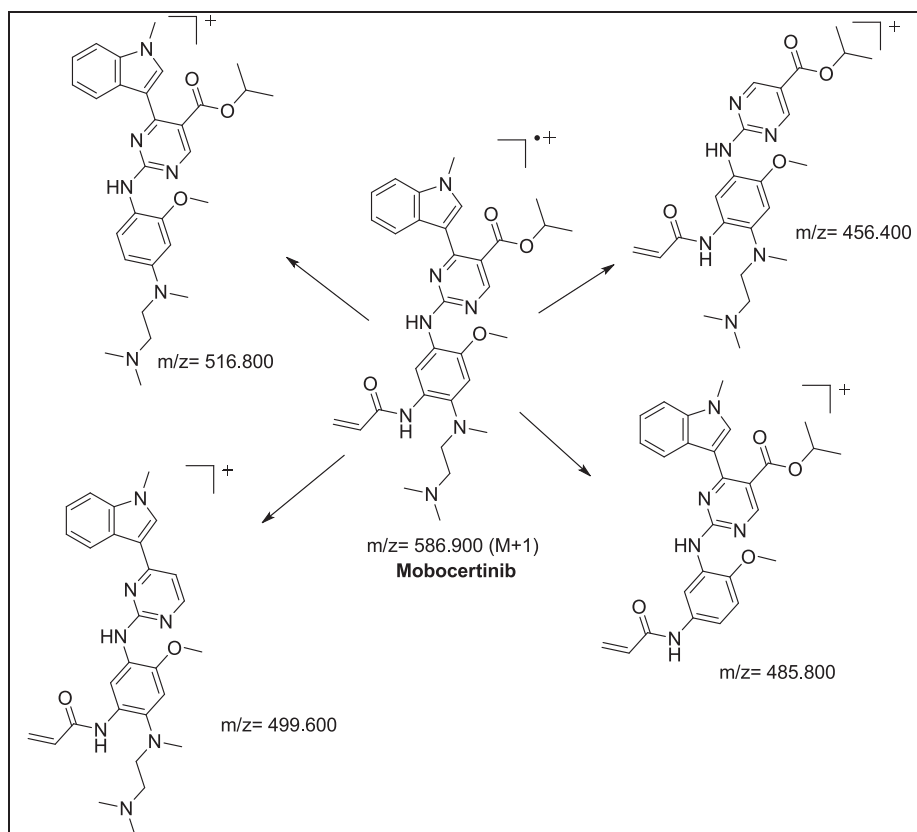


Fig. 5b. Proposed Fragmentation Pathway of Mobocertinib Standard Solution.

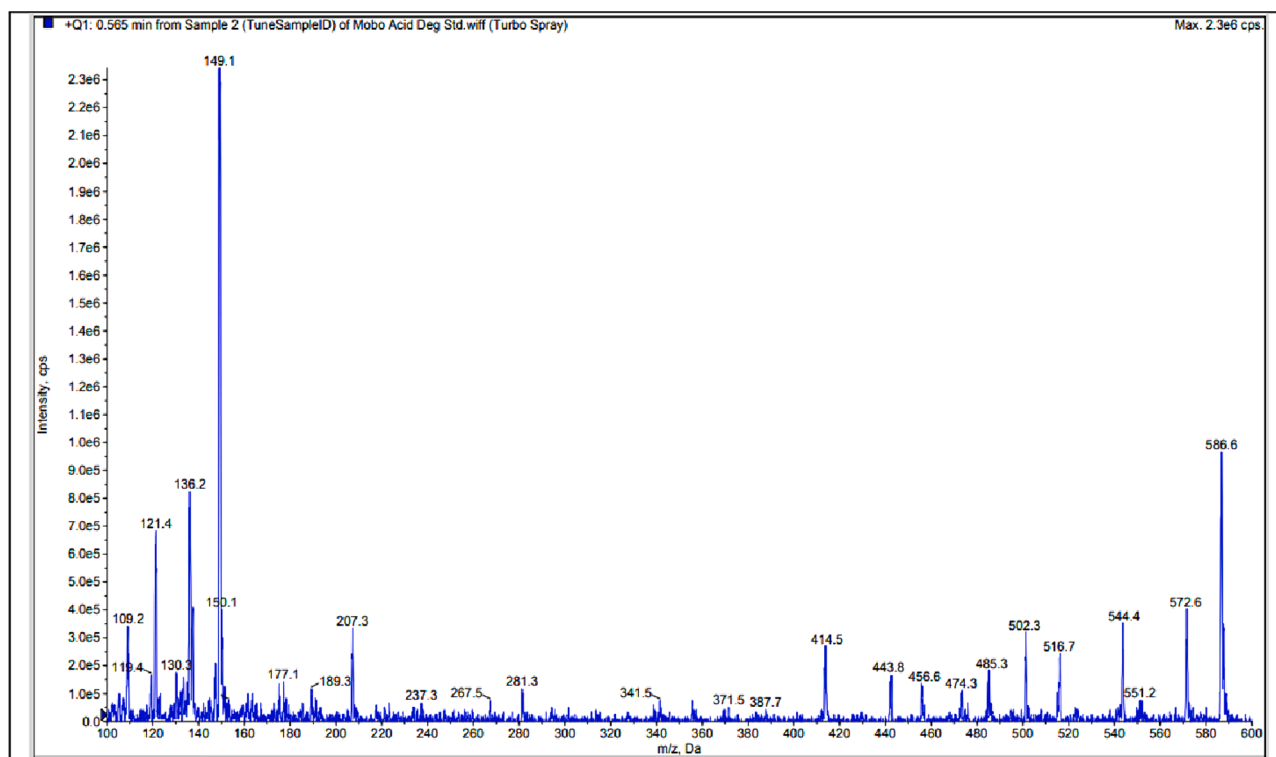


Fig. 6a. Mass Spectra of Acid Degradant Product (DP1) of Mobocertinib.

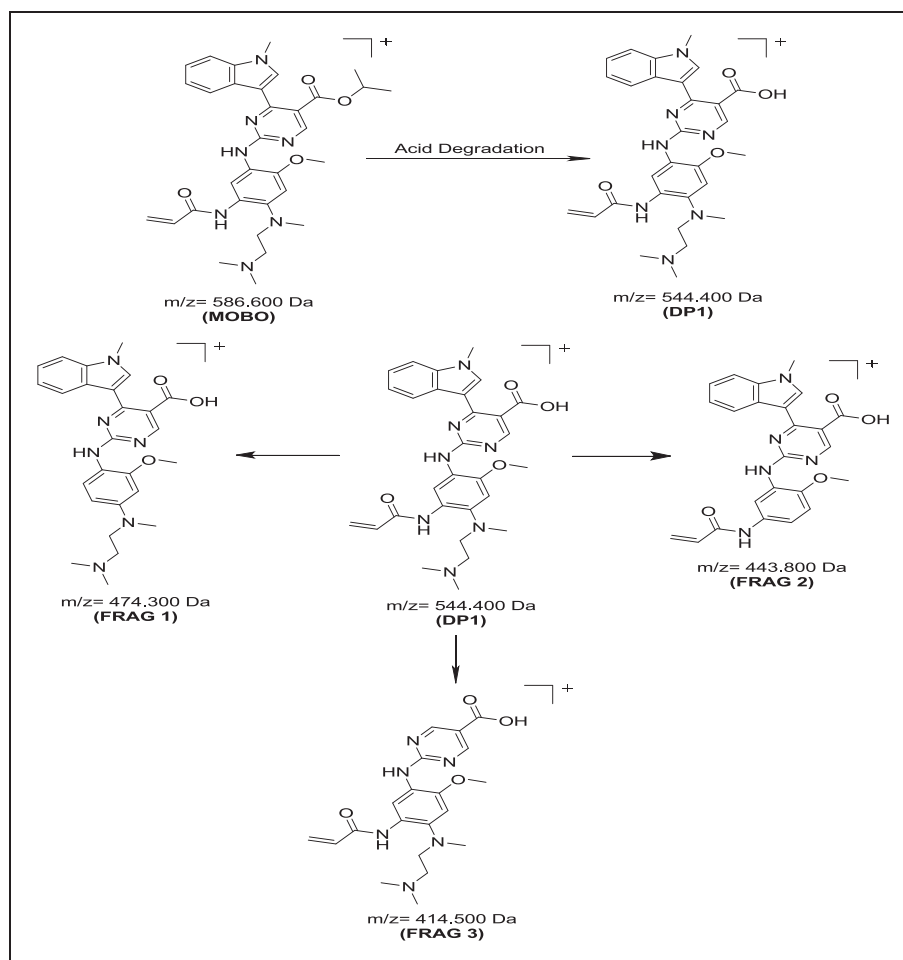


Fig. 6b. Proposed Fragmentation Pathway of Acid Degradant Product (DP1) of Mobocertinib.

Precision

Repeatability: From working standard solution of mobocertinib, six injections of mobocertinib were performed and the % RSD was calculated which should be less than 2.0 %. The results are shown in Table 2.

Intraday Precision: On the same day, three analyses of working standard solution containing (0.2, 0.4, and 0.6 $\mu\text{g/ml}$) of mobocertinib were performed. % RSD was computed for each analysis (Table 3).

Interday Precision: Three separate analyses of a working standard solution containing (0.2, 0.4, and 0.6 $\mu\text{g/ml}$) of mobocertinib were performed, and the % RSD was calculated and results were tabulated in Table 3.

Recovery

Recovery trials were conducted by adding standard drug to the sample at three distinct concentration levels (80 %, 100 %, and 120 %) while accounting for the bulk drug samples percentage purity. Each concentration of the sample was injected for three times to check the reproducibility and the same was determined from the percentage of RSD values to conclude the accuracy. Table 4 represents the percentage recovery values for mobocertinib at three different concentrations of 80 %, 100 %, and 120 % were found to be in the range of 98.89–101.28 %.

Robustness

To evaluate the robustness of the devised approach, small deliberate modifications in the chromatographic conditions, such as a change in mobile phase ratio (2.0 %), a change in column temperature (± 2 units) and a change in flow rate (± 0.2 units) were evaluated and % RSD is calculated which is less than 2.0 %.

Assay of marketed formulation of mobocertinib

A validated LC-MS/MS method was applied to find the amount of mobocertinib in Exkivity capsules. It is represented by the mean percentage recoveries as shown in Table 5.

Degradation behavior studies of mobocertinib on LC-MS/MS system

Mobocertinib was evaluated under various stress conditions including acidic, basic, neutral, oxidative, ultraviolet (UV), and temperature conditions. Mobocertinib was susceptible to degradation in the presence of acid, base, and oxidative stress. In the acidic condition, just one degradation product, denoted DP1, was formed. Two degradation products (DP2 and DP3) and one degradation product (DP4) were obtained from the base treatment and oxidative conditions, respectively. The retention period and m/z values of the mobocertinib's degradation products (DPs) are shown in Table 6 and Fig. 4.

Degradation behavior studies of mobocertinib on HPLC system

The developed and validated LC-MS/MS method is transferred to HPLC method using UV detector (225 nm) for the estimation of mobocertinib in acid, base, and oxidative conditions. The degradation solutions were prepared for HPLC chromatogram as similar to LC-MS/MS. 20 μL of final concentration 100 $\mu\text{g/ml}$ of various degradation solutions is injected to HPLC column to obtain HPLC chromatogram and its parameters are given in Table 7.

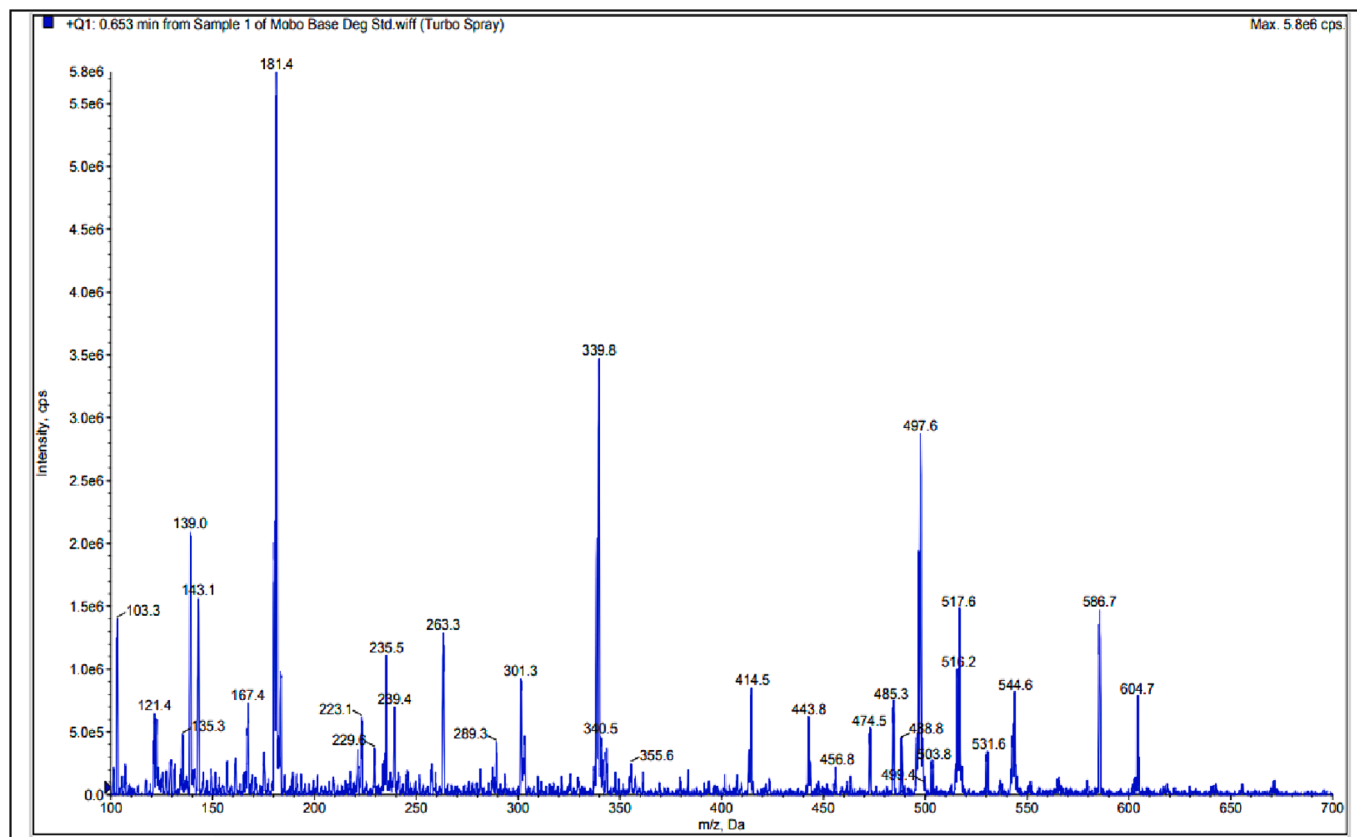


Fig. 7a. Mass Spectra of Base Degradant Product (DP2) of Mobocertinib.

Preparation, isolation and purification of forced degradant products of mobocertinib

Two sets of forced degradation solutions were prepared by adopting standard procedure as discussed earlier. One set for trial and the other for isolation purpose. Add 9.0 ml of methanol and 1.0 ml of 0.1 M HCl to 200 mg of mobocertinib and reflux for 4 h at 60°C, and cool it to room temperature. Neutralize the solution with 0.1 M NaOH. This solution is intended for the isolation of the acid degradation product (DP1) of mobocertinib. As previously discussed, two sets of solutions were prepared and used accordingly. Similarly, 250 mg of mobocertinib drug is transferred to 100 ml round bottom flask containing 9.0 ml of methanol and 1.0 ml of 0.1 M NaOH and refluxed at 60 °C for 8 h. After 8 h, cooled down the solution at room temperature and neutralized it with 0.1 M HCl. This solution is utilized for the isolation of the base degradation product (DP2 and DP3) of mobocertinib. For oxidative degradation, transfer about 200 mg of mobocertinib into a 100 ml round bottom flask containing 9.0 ml of methanol and 1.0 ml of 3.0 % H₂O₂ and reflux at 60 °C for 4 h. After 4 h, cool the mixture to room temperature. Isolation and purification of this solution provides DP4. Under the specified conditions, mobocertinib degradation products (DPs) were isolated by performing preparative HPLC. Chromanik C18 column measuring 150 mm × 20 mm with a particle size of 10 μm was used. Maintain the column temperature at around 35 °C. The degradant solutions were collected individually from the outlet according the retention period. The resultant degradation solutions were purified and processed through rotavapour and characterized by proton NMR to elucidate its structure.

Proposed pathway of mass fragmentation of mobocertinib drug

To understand the origin of each fragment multi-stage (MSⁿ) mass fragmentation analysis was employed. Four protonated fragments were

found at 516.80 Da (FRAG 1), 499.60 Da (FRAG 2), 485.80 Da (FRAG 3), and 456.40 Da (FRAG 4). Fig. 5a & Fig. 5b shows the mobocertinib mass spectra and the anticipated fragmentation mechanism, respectively.

Proposed pathway of mass fragmentation of DP1

Hydrolysis of the ester group was observed under acidic conditions and lead to acid degradation product (DP1), whose *m/z* ratio is approximately 544.400 Da (DP1). The possible fragments for DP1 are observed at *m/z* 474.30 Da (FRAG 1), 443.80 (FRAG 2), and 414.50 (FRAG 3). Fig. 6a and Fig. 6b display the mass spectra and the suggested fragmentation pathway of mobocertinib's acid degradant product (DP1), respectively.

Proposed pathway of mass fragmentation of DP2

Under basic condition, terminal alkene group of the amide is converted to alcohol as observed in Michael addition reaction. The DP2's *m/z* ratio is appeared at 604.700 Da (DP2). In addition, four protonated fragmentation ions are observed at 517.600 Da (FRAG 1), 516.200 Da (FRAG 2), 503.800 Da (FRAG 3), and 474.500 Da (FRAG 4). Fig. 7a. and Fig. 7b display the mass spectra and suggested fragmentation pathway of mobocertinib's basic degradant product (DP2), respectively.

Proposed pathway of mass fragmentation of DP3

The ester part of the isopropyl was hydrolyzed under basic conditions, as observed in acidic conditions. The structure of DP1 and DP3 are same. DP3 displayed similar fragmentation pattern as discussed in DP1. Fig. 8a and Fig. 8b display the mass spectra and the suggested fragmentation pathway of Mobocertinib's base degradant product (DP3).

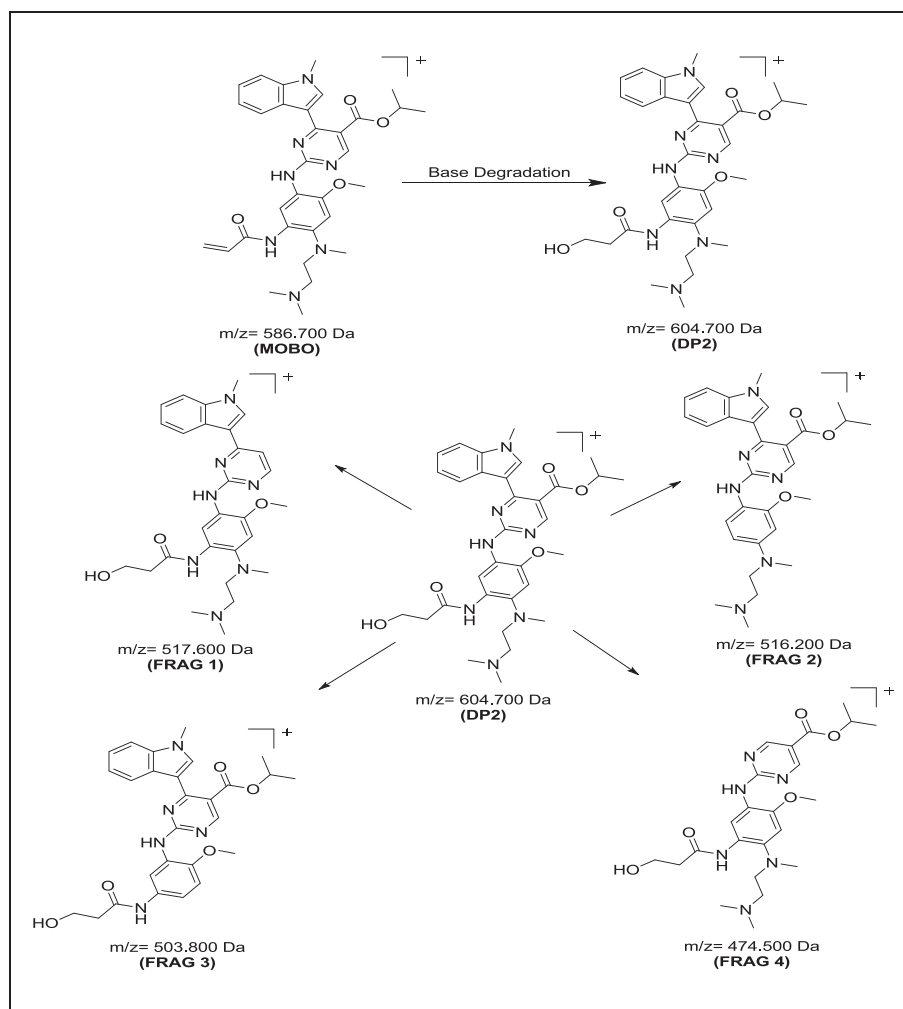


Fig. 7b. Proposed Fragmentation Pathway of Base Degradant Product (DP2) of Mobocertinib.

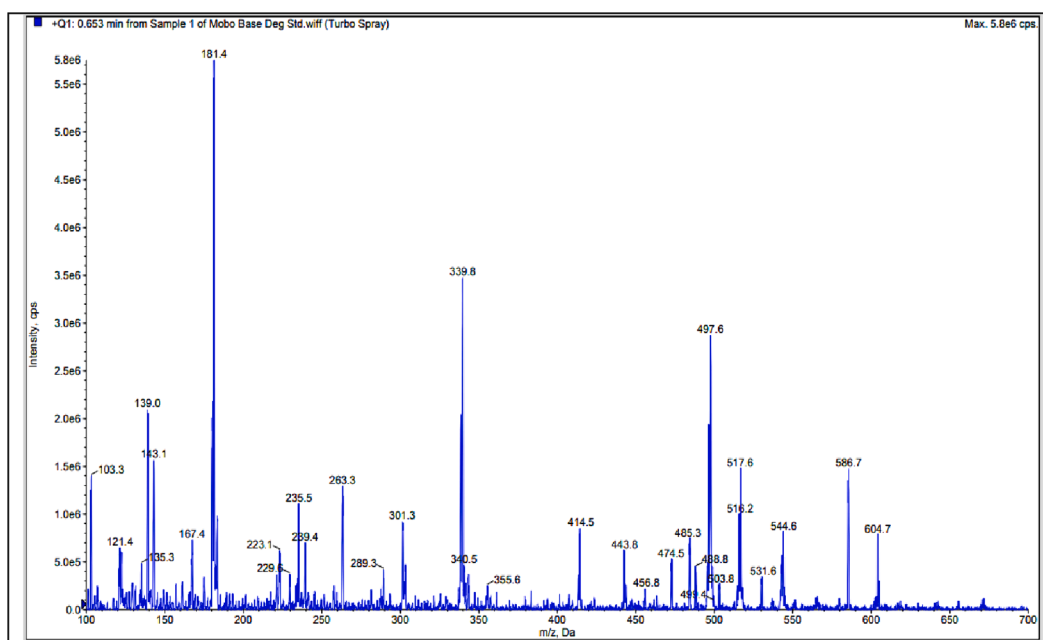


Fig. 8a. Mass Spectra of Base Degradant Product (DP3) of Mobocertinib.

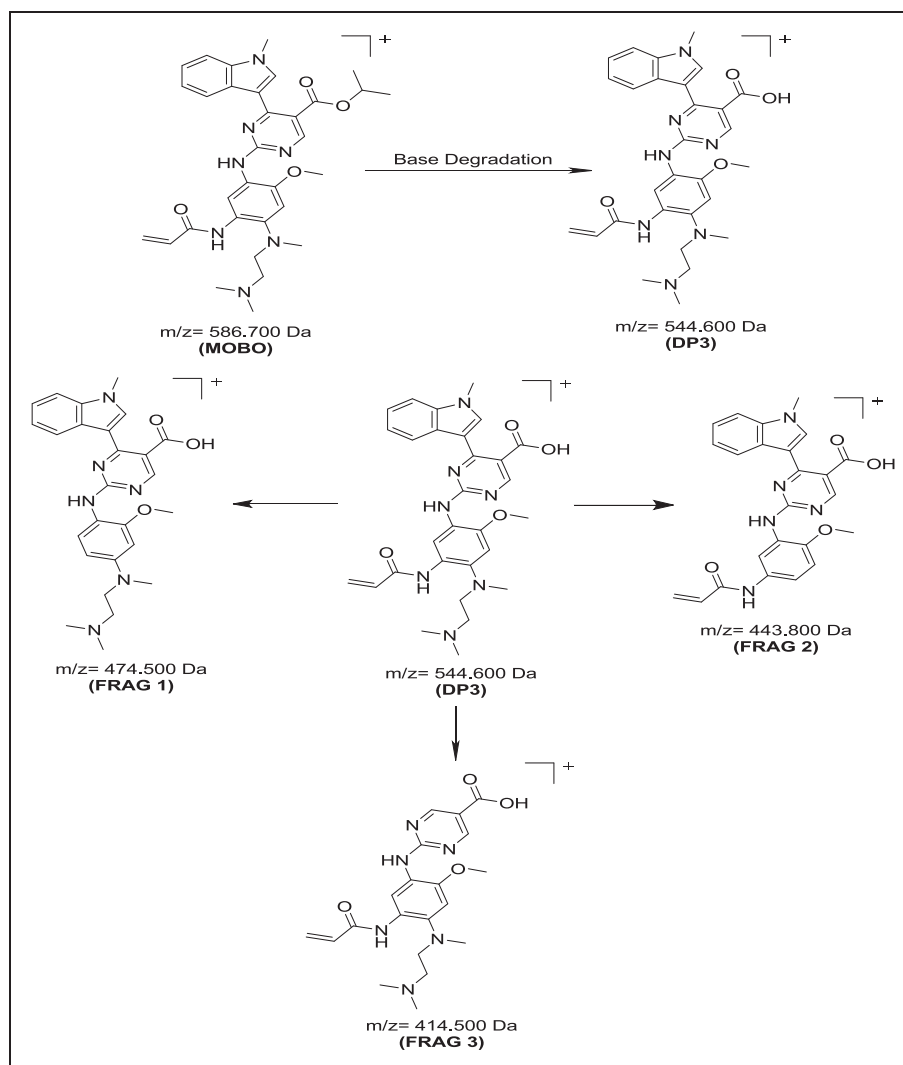


Fig. 8b. Proposed Fragmentation Pathway of Base Degradant Product (DP3) of Mobocertinib.

Proposed pathway of mass fragmentation of DP4

The hydrogen peroxide oxidizes the two tertiary nitrogen atom and form N-oxides and it is confirmed in the mass spectrum. In the mass spectra, it displayed a prominent peak at 618.90 Da (DP4). From the MS spectra, DP4 is further fragmented into four more clear peaks that can be viewed at 548.100 Da (FRAG 1), 531.700 Da (FRAG 2), 488.500 Da (FRAG 3), and 485.400 Da (FRAG 4). [Fig. 9a](#) and [Fig. 9b](#) depict the mass spectra and the estimated fragmentation pattern for DP4, respectively.

Characterization of DP1

DP1: MS m/z 544.400 [M + H]; IR (KBr) 3478 (NH stretching), 3200 (OH, broad peak), 1714 (C = O; Carboxylic acid), 1674 (C = O; Amide), 1616 (C = C) cm^{-1} ; ^1H NMR: 2.20 (6H, singlet, $-\text{CH}_3$), 2.29–2.32 (2H, triplet, $-\text{CH}_2$), 2.72 (3H, singlet, $-\text{CH}_3$), 2.86–2.89 (2H, triplet, $-\text{CH}_2$), 3.81 (3H, singlet, $-\text{CH}_3$), 3.87 (3H, singlet, $-\text{CH}_3$), 5.76–5.79 (1H, doublet of doublet, $\text{CH}_2 = \text{CH}$), 6.25–6.29 (1H, doublet of doublet, $\text{CH}_2 = \text{CH}$), 6.39–6.46 (1H, doublet of doublet, $\text{CH}_2 = \text{CH}$), 8.83 (1H, singlet, $-\text{NH}$), 10.17 (1H, singlet, $-\text{NH}$), 11.21 (1H, singlet, $-\text{OH}$), 7.01–8.67 (8H, multiplet, $-\text{ArH}$). [Fig. 10](#) illustrates the proton NMR spectrum of DP1.

Characterization of DP2

DP2: MS m/z 604.700 [M + H]; IR (KBr) 3618 (OH stretching, broad), 3254 (NH stretching), 1754 (C = O; ester), 1680 (C = O; Amide), 1637 (C = C) cm^{-1} ; ^1H NMR: 1.11–1.12 (6H, doublet, $-\text{CH}_3$), 2.12 (6H, singlet, $-\text{CH}_3$), 2.20–2.22 (2H, triplet, $-\text{CH}_2$), 2.30–2.32 (2H, triplet, $-\text{CH}_2$), 2.69 (3H, singlet, $-\text{CH}_3$), 2.84–2.86 (2H, triplet, $-\text{CH}_2$), 3.86 (3H, singlet, $-\text{CH}_3$), 3.90 (3H, singlet, $-\text{CH}_3$), 4.07–4.11 (2H, multiplet, $-\text{CH}_2$), 4.98–5.02 (1H, multiplet, $-\text{CH}$), 8.78 (1H, singlet, $-\text{NH}$), 10.30 (1H, singlet, $-\text{NH}$), 11.31 (1H, singlet, $-\text{OH}$), 7.09–8.52 (8H, multiplet, $-\text{ArH}$). [Fig. 11](#) illustrates the proton NMR spectrum of DP2.

Characterization of DP3

DP3: MS m/z 544.400 [M + H]; IR (KBr) 3476 (NH stretching), 3245 (OH, broad), 1715 (C = O; Carboxylic acid), 1686 (C = O; Amide), 1619 (C = C) cm^{-1} ; ^1H NMR: 2.20 (6H, singlet, $-\text{CH}_3$), 2.30–2.32 (2H, triplet, $-\text{CH}_2$), 2.72 (3H, singlet, $-\text{CH}_3$), 2.86–2.89 (2H, triplet, $-\text{CH}_2$), 3.83 (3H, singlet, $-\text{CH}_3$), 3.87 (3H, singlet, $-\text{CH}_3$), 5.76–5.79 (1H, doublet of doublet, $\text{CH}_2 = \text{CH}$), 6.25–6.29 (1H, doublet of doublet, $\text{CH}_2 = \text{CH}$), 6.39–6.46 (1H, doublet of doublet, $\text{CH}_2 = \text{CH}$), 8.83 (1H, singlet, $-\text{NH}$), 10.18 (1H, singlet, $-\text{NH}$), 11.21 (1H, singlet, $-\text{OH}$), 7.01–8.67 (8H, multiplet, $-\text{ArH}$). [Fig. 12](#) illustrates the proton NMR spectrum of DP3.

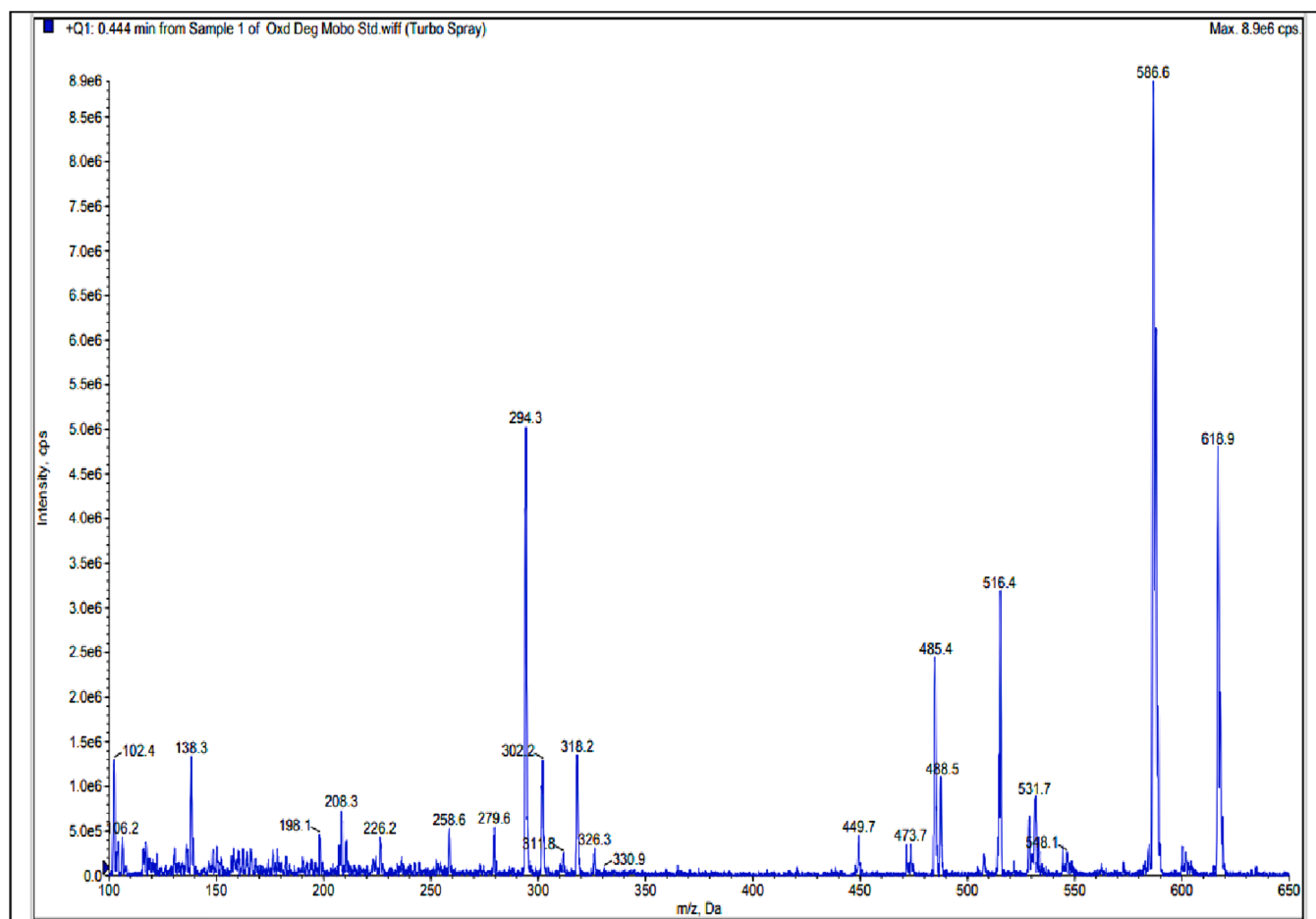


Fig. 9a. Mass Spectra of Oxidative Degradant Product (DP4) of Mobocertinib.

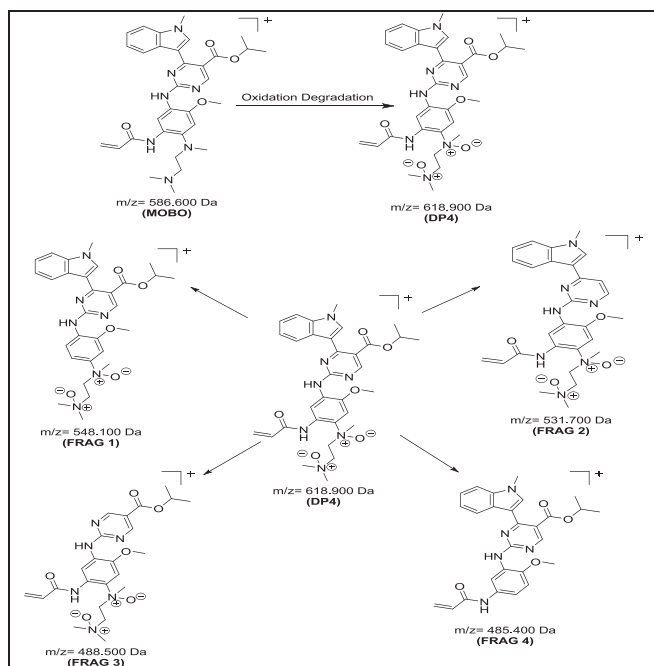


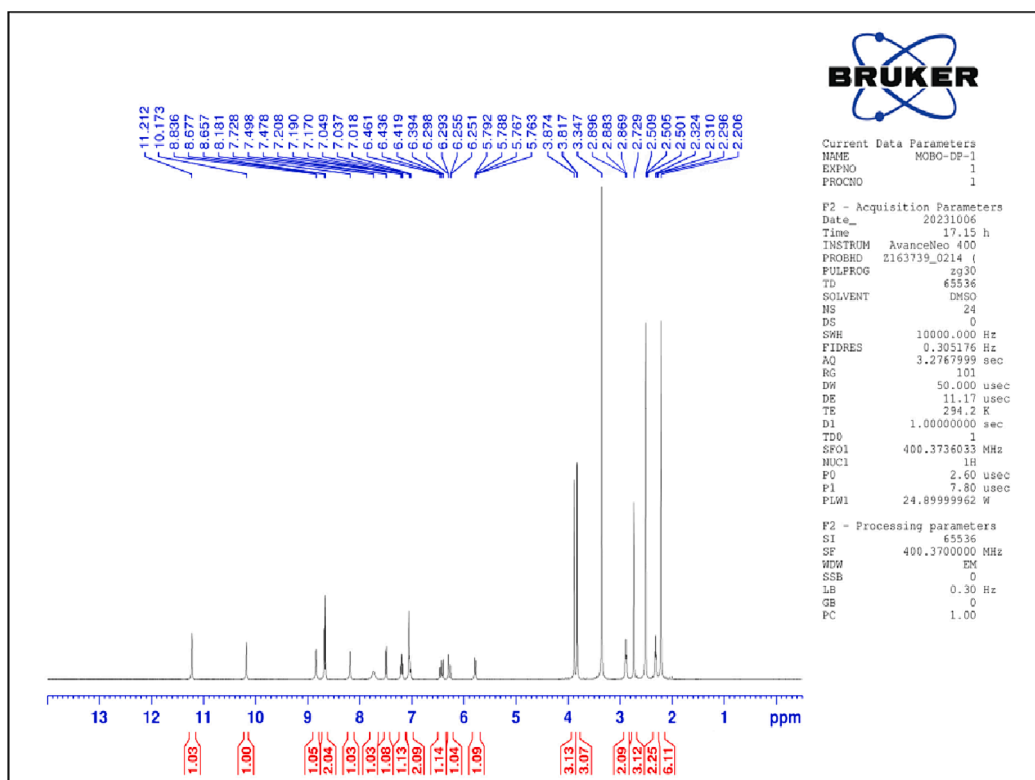
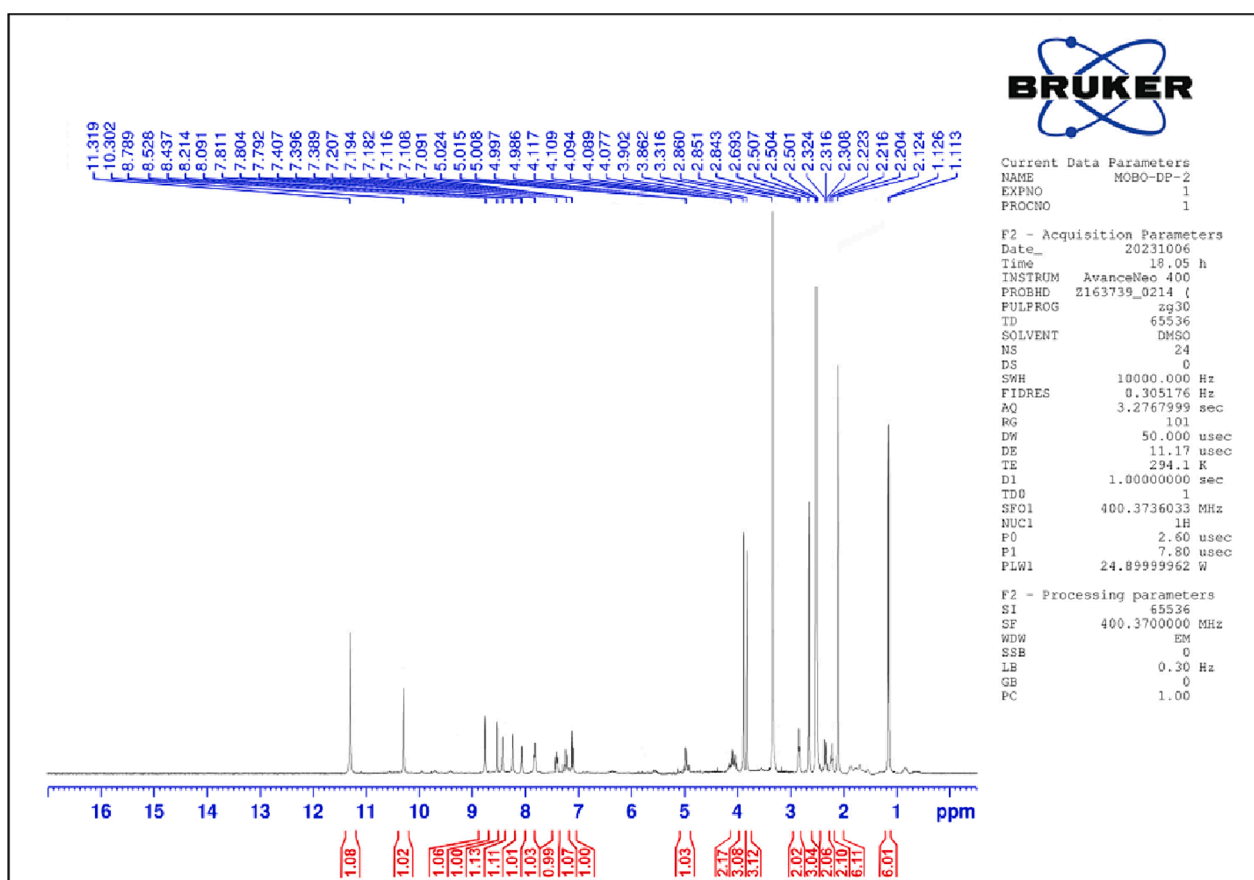
Fig. 9b. Proposed Fragmentation Pathway of Oxidative Degradant Product (DP4) of Mobocertinib.

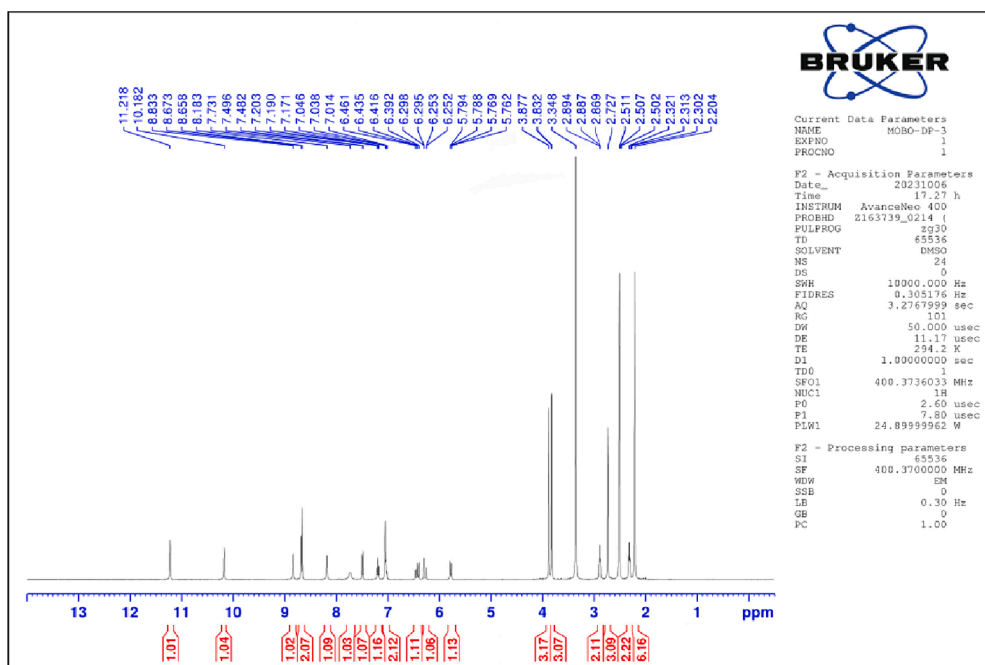
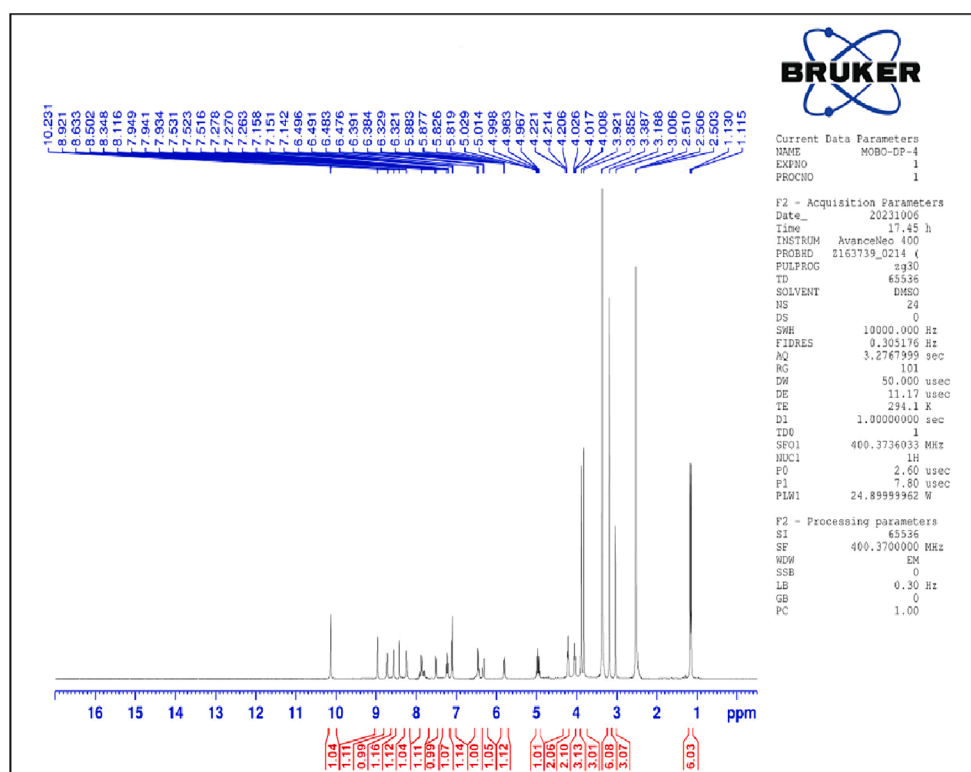
Characterization of DP4

DP4: MS m/z 618.900 $[M + H]^+$; IR (KBr) 3414 (NH stretching), 1741 ($C = O$; ester), 1683 ($C = O$; Amide), 1626 ($C = C$) cm^{-1} ; 1H NMR: 1.11–1.13 (6H, doublet, $-CH_3$), 3.00 (3H, singlet, $-CH_3$), 3.18 (6H, singlet, $-CH_3$), 3.85 (3H, singlet, $-CH_3$), 3.92 (3H, singlet, $-CH_3$), 4.00–4.02 (2H, triplet, $-CH_2$), 4.20–4.22 (2H, triplet, $-CH_2$), 4.96–5.02 (1H, multiplet, $-CH$), 5.81–5.88 (1H, doublet of doublet, $CH_2 = CH$), 6.32–6.39 (1H, doublet of doublet, $CH_2 = CH$), 6.47–6.49 (1H, doublet of doublet $-CH$), 8.92 (1H, singlet, $-NH$), 10.23 (1H, singlet, $-NH$), 7.14–8.63 (8H, multiplet, $-ArH$). Fig. 13 illustrates the proton NMR spectrum of DP4.

Conclusion

As per ICH guidelines, the forced degradation studies of mobocertinib was carried out under various conditions such as acidic, basic, oxidative, thermal and photolytic conditions. Two degradation products were isolated from basic conditions (DP2 and DP3) and one each from acidic (DP1) and oxidative conditions (DP4), respectively. Mobocertinib was found to be stable in thermal and photolytic conditions. The LC-MS/MS technology was utilized to study the degradation products. Additionally, LC-MS/MS is validated for specificity, linearity, precision, accuracy, robustness, LOD and LOQ. Later, the LC-MS/MS methodology was transferred to HPLC system and validated by with the set of parameters. Preparative HPLC is used to isolate the degradation products (DP1 to DP4). The isolated compounds were characterized by Infrared spectroscopy, proton NMR and mass spectroscopy. Chemically, the structure of DP1 and DP3 are same. All the isolated degradation

Fig. 10. ^1H NMR Spectra of Acid Degradant Product (DP1) of Mobocertinib.Fig. 11. ^1H NMR Spectra of Base Degradant Product (DP2) of Mobocertinib.

Fig. 12. ^1H NMR Spectra of Base Degradant Product (DP3) of Mobocertinib.Fig. 13. ^1H NMR Spectra of Oxidative Degradant Product (DP4) of Mobocertinib.

compounds were isolated for the first time and not reported in literature. In conclusion, the LC-MS/MS method is developed, validated and it may be helpful in evaluating the quality and stability of mobocertinib medications in the pharmaceutical formulations.

CRediT authorship contribution statement

Shweta Mishra: Validation, Methodology, Conceptualization. **Ashlesha Chauhan:** Supervision. **R. Ramajayam:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rechem.2024.101465>.

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