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Impurity Profiling of Some Anti - Cancer Drugs With Special Reference to Lazertinib

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Abstract:
A simple, specific, accurate and precise stability-indicating reversed-phase liquid chromatographic method had been developed and validated as per ICH guideline for Estimation of Lazertinib in its pharmaceutical dosage form (Tablet). Also, a forced degradation study of Lazertinib was performed for conditions like acid, alkali, peroxide, reduction, thermal and hydrolysis. The method was based on isocratic elution using a mobile phase mixture as Acetonitrile: 0.1% Formic Acid, pH - 2.25 / OPA (50:50 % v/v) at a flow rate of 1.0 ml/min, with Waters X-Terra RP-18 (150 mm x 4.6 mm, 3.5µm) column. Detection wavelength was 241 nm. In addition, Degradation Products were identified for Acid, Alkali, Peroxide, Reduction and Hydrolysis forced degradation condition as DP – 1, DP - 2, DP - 3, DP – 4 and DP - 5 respectively and were verified by LC – MS/MS. Their Possible degradation pathway were also drawn. Linearity was observed for 20 - 120 µg / ml. For accuracy recovery data the approach was successful because the recuperation values were within the scope. For Precision and Robustness the RSD percentage were determined to be within reasonable limits. It can be successfully adopted for routine quality control analysis of Lazertinib in its pharmaceutical dosage form without any interference. The forced degradation products were identified [M+ H] + ion, and the proposed structures were supported by UPLC–MS/MS experiments combined with correct mass evaluations. The UPLC method was supported as per ICH guidelines and can be applied to the marketed formulations.
Keywords: *Lazertinib, Stability - Indicating assay, Forced degradation, UPLC – MS/MS, Impurity, Anti- Cancer.*

INTRODUCTION:

Cancer occurs when cells grow out of control and spread to other areas of the body, possibly moving to different organs through a process known as metastasis. [1, 2].
Lazertinib is indicated for the first-line treatment of adult patients with locally advanced or metastatic non-small cell lung cancer (NSCLC). Lazertinib is an oral, third-generation, epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI). [4, 5].
Impurity profiling, which means finding and measuring these impurities, is now receiving more focus from regulatory agencies. There is strong requirement to have unique specifications/standards with regard to impurities. According to the International Council for Harmonisation (ICH), the maximum daily dose and identification threshold to be considered is as follows: for doses ≤2g/day, the threshold is 0.10% or 1mg per day intake (whichever is lower), and for doses ≥2g/day, the threshold is 0.05%. [3].

A Literature survey revealed that, Not any stability Indicating RP – HPLC method is reported for the analysis of Lazertinib as individual drug and one analytical method for LC–MS/MS determination of BLU-945(Kinase Inhibitor category) in Rat Plasma is only reported, but any method regarding Impurity Profiling of Anti – Cancer Drug (i.e. Lazertinib) is not available. [10-12].

MATERIAL AND METHODS:

RP - HPLC Method Development

Selection of Elution Mode

Reverse phase chromatography was picked because it's commonly suggested for ionic and moderately to non-polar substances. It's not just easy to use and convenient, but it also performs precisely well in terms of efficiency, stability, and consistency. For separating our drug Lazertinib, a C18 column with measurements as 150 mm x 4.6 mm and a particle size of 3.5 µm was finalized. An isocratic method was used because it offers good reliability and keeps the performance consistent even after long-term use of the column.

Wavelength Selection

The detection wavelength at 241 nm was considered as optimum.

Preparation of Standard solution of Lazertinib (80 µg/ml)

Take 8 mg of the weighed amount of Lazertinib and dilute it to a total volume of 10 ml using Methanol. Then, from this solution, take 1 ml and dilute it to a final volume of 10 ml with Methanol. After this, the drug solution was scanned. The resulting Spectra is shown in Figure 1.

Selection of Mobile phase and its optimization

After conduction of several trials by testing different mobile phases such as Acetonitrile, Trifluoroacetic acid, and Formic acid using various ratios to determine the best results, a mixture of acetonitrile and 0.1% formic acid in a 50:50 (%v/v) ratio at a pH of 2.25, with a flow rate of 1.0 ml/min, was found to provide better performance compared to other mobile phase mixtures. This combination gave improved peak shape, higher theoretical plate count, and lower asymmetry.

The details of the optimized trial is mentioned in Figure 2. The chromatographic conditions are listed in Table 1, and they achieved suitable system suitability parameters.

RESULT AND DISCUSSION [8, 9]:

A. METHOD VALIDATION SUMMARY:

1. Specificity:

The chromatograms of Lazertinib show no interference from the blank and placebo samples. Therefore, the developed method is considered specific. The chromatograms are displayed in Figure 3 and Figure 4 respectively.

2. Linearity and Range:

The linearity of Lazertinib was checked by analyzing standard solutions in the concentration range of 20 to 120 µg/ml. Different volumes of 0.25, 0.50, 0.75, 1, 1.25 and 1.50 ml were taken from the stock solution of Lazertinib, which has a concentration of 80 µg/ml. These volumes were transferred into 10 ml volumetric flasks, and the flasks were filled to the mark with mobile phase to prepare solutions with concentrations of 20, 40, 60, 80, 100 and 120 µg/ml for Lazertinib.

In terms of slope, intercept and correlation co-efficient value, the graph of peak area obtained versus respective concentration was plotted. Y is calculated as 30226.74x + 7055.46 and (R^2 as 0.9998). From the linearity calculation sheet, the slope, intercept, and correlation coefficient values were found. The results were shown in Table 2 and Overlay Chromatogram as well as Calibration Curve were mentioned in Figure 5 and Figure 6 respectively.

3. Precision:

Repeatability / Method Precision:

The repeatability of peak area measurement for Lazertinib at a concentration of 80 µg/ml was evaluated by measuring the same solution six times. The % Relative Standard Deviation (% RSD) for Lazertinib was calculated to be 0.45.

Intermediate Precision:

A standard solution with 80 µg/ml of Lazertinib was tested six times on the same day using different instruments and different analysts. The % Relative Standard Deviation (% RSD) for Lazertinib was determined to be 0.75.

The method is considered precise, as the Relative Standard Deviation (RSD) values are below 2%. Table 3 and 4 shows the data for method precision and intermediate precision results.

4. Accuracy: -

80 µg/ml drug solution was taken in three different flask. Spiked 50%, 100%, 150% of standard solution in it and diluted up to 10 ml. The peak areas of each solution were measured at a wavelength of 241 nm. The amount of Lazertinib in each solution was determined, and % recoveries were calculated for each level. The method worked well since the recovery values stayed within the expected range. Table 5 displays the accuracy outcomes.

5. LOD and LOQ: -

The lowest amount of Lazertinib that can be detected was found to be 0.48 µg/ml and the lowest amount that can be accurately measured was 1.6 µg/ml.

6. Robustness: -
During the assessment of the chromatographic method, variations in flow rate, Mobile phase composition, and pH were tested. The RSD values were found to be within acceptable ranges. The robustness findings are shown in Table 6.

7. Analysis of marketed formulation by developed method:
Take Tablet powder equivalent to 80 mg of Lazertinib, was transferred to a 100 ml volumetric flask. Now, 01 ml was taken from that and made upto 10 ml with Mobile phase. Take 1 mL from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase. The solution was filtered through Whatmann filter paper No. 41. Inject 20 µL of the solution for assay analysis.
Assay was found to be 99.3 % label claim.

B. FORCED DEGRADATION STUDIES: [13-16]
Preparation of Lazertinib Sample Stock Solution
Take accurately weigh 13 mg of the Lazertinib sample and put it into a 10 ml volumetric flask. Then add the diluent until the total volume reaches the mark.

1. Acid Degradation
0.1 N Degradation
Take 1 ml of the sample and put it into a 10 ml volumetric flask. Then add 1 ml of 0.1N HCl. Heat the mixture under reflux at 50°C for 2 hours. Wait for 15 minutes. After that, add 1 ml of 0.1N NaOH to neutralize the solution. Then add more diluent to bring the total volume up to 10 ml and mix thoroughly.

1 N Degradation
Take 1 ml of the sample and put it into a 10 ml volumetric flask. Then add 1 ml of 1N HCl. Heat the mixture under reflux at 60°C for 2 hours. Wait for 15 minutes. After that, add 1 ml of 1N NaOH to neutralize the solution. Then add more diluent to bring the total volume up to 10 ml and mix thoroughly. **DP - 1** was observed in chromatogram in Acid degradation.

2. Alkali Degradation:
0.1 N Degradation
Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of 0.1N NaOH into it. Reflux the same at 50°C for 2 hours. Now, stand the solution as it is for 15 minutes. Then add 1 ml of 0.1N HCl to neutralize the solution. Fill the flask to the top with diluent and shake it well to mix thoroughly.
1 N Degradation
Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of 1N NaOH into it. Reflux the same at 60°C for 2 hours. Now, stand the solution as it is for 15 minutes. Then add 1 ml of 1N HCl to neutralize the solution. Fill the flask to the top with diluent and shake it well to mix thoroughly. **DP - 2** was seen in chromatogram in Alkali degradation.

3. Peroxide Degradation
3 % Peroxide Degradation
Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of 3% hydrogen peroxide in flask. Let it sit for 15 minutes. After that, add diluent to bring the total volume up to 10 ml and mix thoroughly.
10 % Peroxide Degradation
Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of 10% hydrogen peroxide in flask. Let it sit for 15 minutes. After that, add diluent to bring the total volume up to 10 ml and mix thoroughly. **DP - 3** appeared in the chromatogram during the Peroxide degradation process.

4. Thermal Degradation
105°C for 3 hrs Degradation
A sample of 50 mg was heated at 105°C for 3 hours, and then the sample was tested. 8 mg of the sample was placed in a 10 ml volumetric flask and filled up to the mark. Next, 1 ml of this solution was taken and mixed with diluent to make a total volume of 10 ml.

105°C for 6 hrs Degradation

A sample of 50 mg was heated at 105°C for 6 hours, and then the sample was tested. 8 mg of the sample was placed in a 10 ml volumetric flask and filled up to the mark. Next, 1 ml of this solution was taken and mixed with diluent to make a total volume of 10 ml.

5. Reduction Degradation

3% Sodium bisulfate Degradation

Take 1 ml of the sample and put it into a 10 ml volumetric flask. Add 1 ml of 3% sodium bisulfate solution. Heat the mixture under reflux at 60 °C for 2 hrs. Allow it to stand for 15 minutes. Then add more diluent until the total volume reaches 10 ml and mix thoroughly.

10% Sodium bisulfate Degradation

Take 1 ml of the sample and put it into a 10 ml volumetric flask. Add 1 ml of 10% sodium bisulfate solution. Heat the mixture under reflux at 60 °C for 2 hrs. Allow it to stand for 15 minutes. Then add more diluent until the total volume reaches 10 ml and mix thoroughly. **DP - 4** was found in the chromatogram during Reduction degradation.

6. Neutral Hydrolysis Degradation

HPLC Water Degradation

1. Take 1 ml of the sample and put it into a 10 ml volumetric flask. Add 1 ml of HPLC water into it. Heat it gently at 80 °C for 02 hrs. Wait for 15 minutes. Then add more diluent until the flask is reached to the mark. Shake it well to mix thoroughly.
2. Take 1 ml of the sample and put it into a 10 ml volumetric flask. Add 3 ml of HPLC water into it. Heat it gently at 90 °C for 05 hrs. Wait for 15 minutes. Then add more diluent until the flask is reached to the mark. Shake it well to mix thoroughly. **DP - 5** was observed in the chromatogram during Hydrolysis degradation.

❖ In the Acid degradation DP-1 was identified; in Alkali degradation DP-2; in Peroxide degradation DP-3; in Reduction degradation DP-4; and in Hydrolysis degradation DP-5. The overall degradation conditions along with the percentage of degradation are listed in Table 7.

C. Mass Spectroscopy and Degradation Products (DPs) with Possible Degradation Pathway:

LC/MS Conditions

Instrument:

- Waters, alliance e - 2695 model HPLC provided with column oven, Auto sampler and degasser was operated for analysis.
- The HPLC system was coupled to SCIEX QTRAP 5500 mass spectrometer equipped with electrospray ionization interface.
- SCIEX software was used for the interpretation of the data of the chromatogram.

Mass spectrometer conditions:-

The mass spectrometer was operated in positive ion electrospray ionization mode. Multiple reactions monitoring was used to measure Lazertinib. The working parameters were set as follows:

▪ Collision energy: 15 V, Ion spray voltage: 5500 V
▪ Source temperature: 550 °C, Drying gas temperature: 120-250 °C
▪ Collision gas: nitrogen, Drying gas flow stream: 5 L/min
▪ Delustering potential: 40 V, Entrance potential: 10V
▪ Exit Potential: 7 V, Dwell time: 1 sec

Total 05 Degradation Products DP1, DP2, DP3, DP4 and DP5 were identified. Their MS Spectra, and Possible degradation Pathways are mentioned in Figure 7 to 17.

CONCLUSION:

A simple, specific, accurate and precise RP-HPLC method has been developed and validated as per ICH guideline for Estimation of Lazertinib in its pharmaceutical dosage form (Tablet). Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, Specificity were tested. All these tests showed that the method is linear, accurate, precise, specific, and robust.

This method can be used regularly for quality control analysis of Lazertinib in its tablet form without any unwanted interference.

The drug's breakdown was studied under different stressful conditions under acidic, alkali, peroxide, reduction, hydrolysis and thermal stress conditions.

The drug was found to be stable in thermal conditions and unstable in acidic, alkali, peroxide, hydrolysis and reduction conditions. The degradation products were identified [M+ H] + ion, and the proposed structures were supported by UPLC–MS/MS experiments combined with correct mass evaluations. The UPLC method was supported as per ICH guidelines and can be applied to the marketed formulations.

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Annexures:

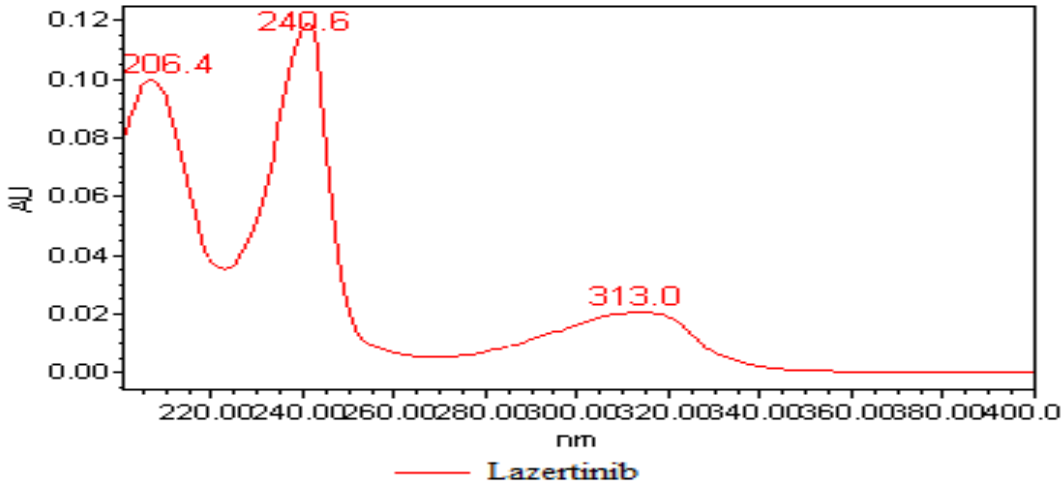


Figure 1: UV Spectra of Lazertinib (80 µg/ml) in Methanol

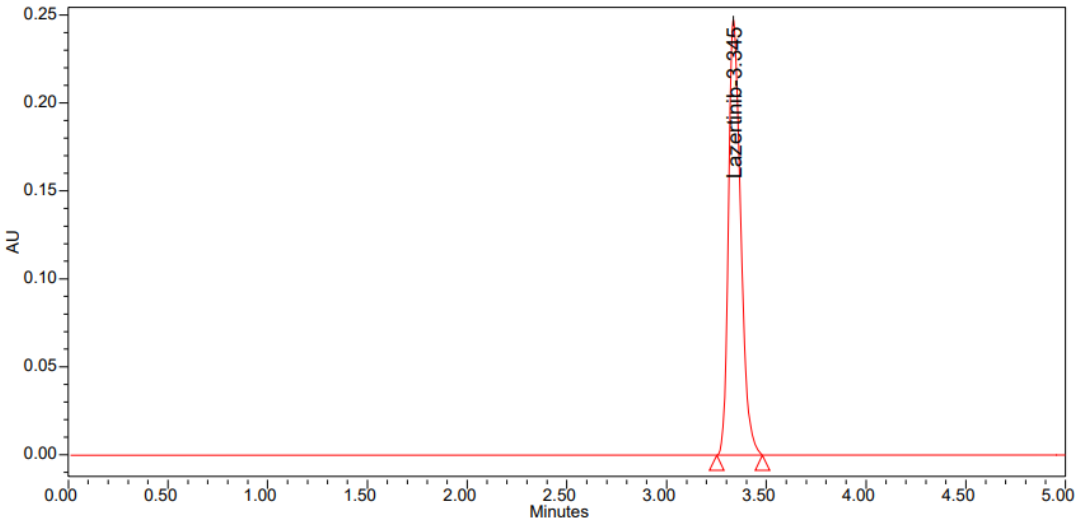


Figure 2: Optimized Trial / Acetonitrile: 0.1% Formic acid (50:50 % v/v) pH, 2.25 / Ortho-Phosphoric Acid

Table 1: Chromatographic Conditions

Column	Waters X-Terra RP-18 (150 mm x 4.6 mm, 3.5µm)
Mode of Elution	Isocratic
Mobile phase	Acetonitrile: 0.1% Formic acid , pH-2.25 / OPA (50:50 % v/v)
Detection Wavelength	241 nm
Injection volume	10 µL
Flow rate	1.0 ml/min
Column Temperature	25°C
Run time	05 minutes
Retention Time	3.345
Theoretical Plates	17865
USP Tailing	0.91

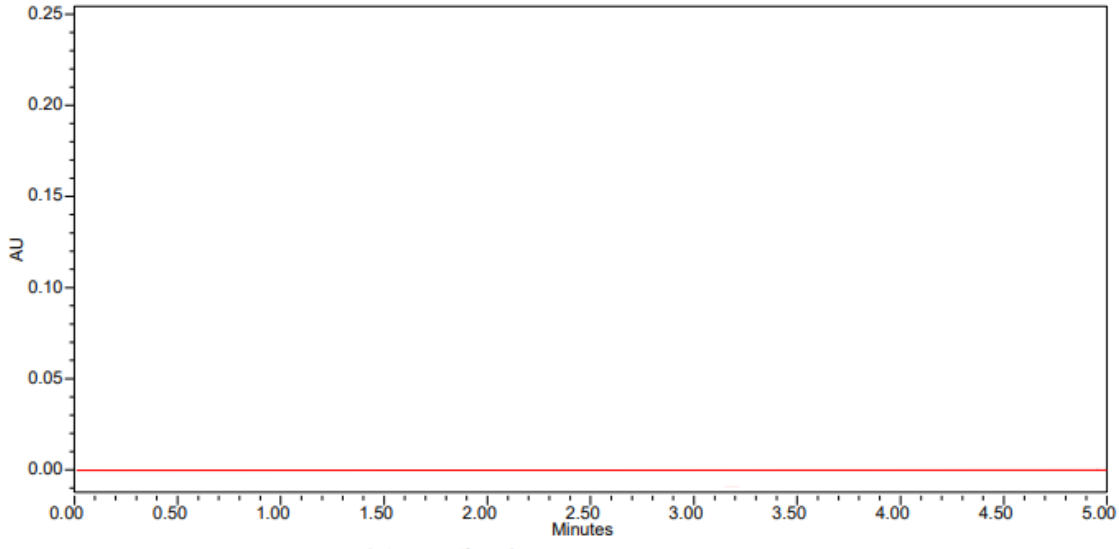


Figure 3: Chromatogram of Lazertinib Blank

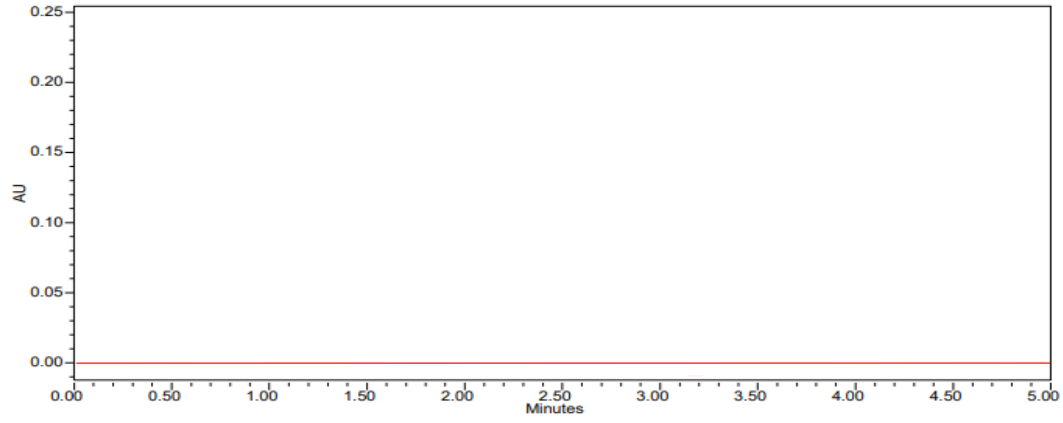


Figure 4: Chromatogram of Lazertinib Placebo

Table 2: Linearity data for Lazertinib

Sr. No.	Concentration (µg/ml)	Area
1	20.00	609247
2	40.00	1203427
3	60.00	1857694
4	80.00	2435410
5	100.00	3003041
6	120.00	3635802

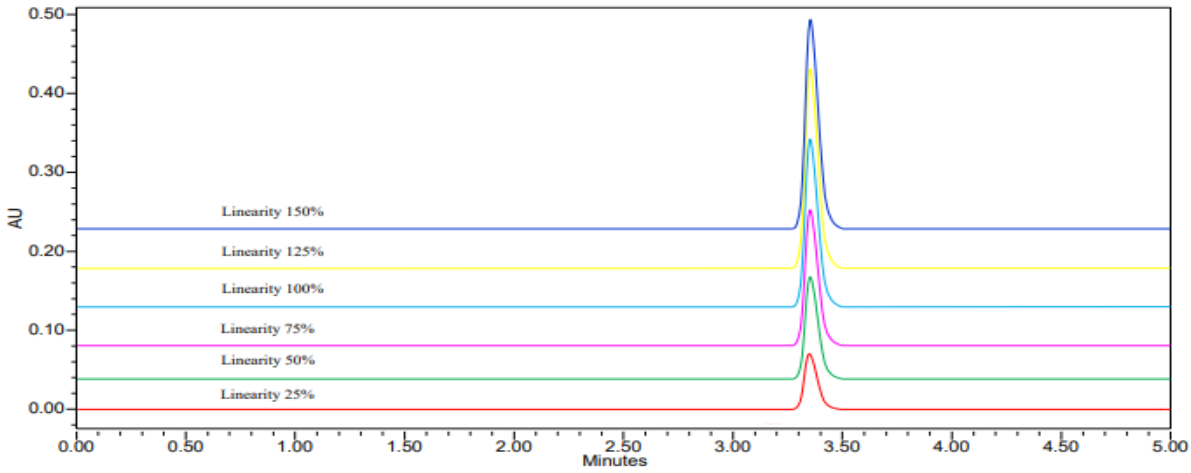


Figure 5: Overlay chromatogram of different concentrations of mixtures of Lazertinib

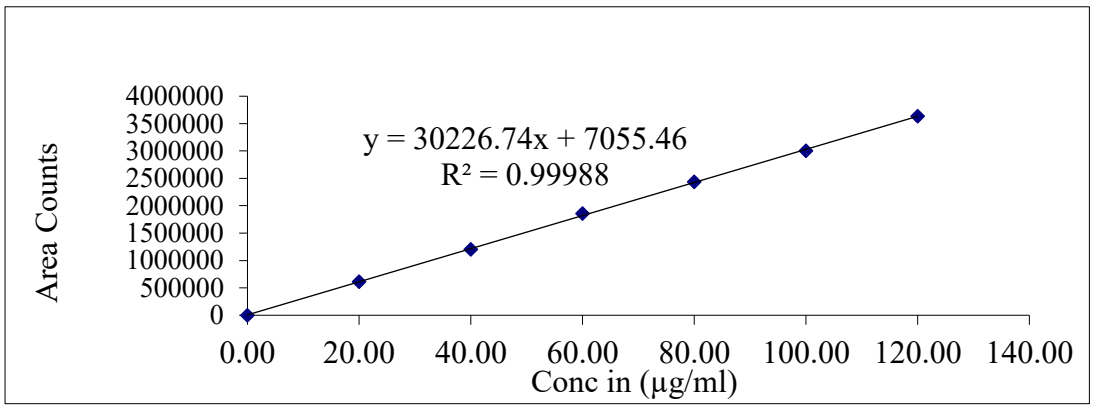


Figure 6: Calibration Curve of Lazertinib (20 – 120 µg /ml)

Table 3: Repeatability / Method Precision

Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	80	2430847	99.9 ± 0.445	0.45
		2417995		
		2423999		
		2447151		
		2433482		
		2442710		

Table 4: Intermediate Precision

Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	80	2440654	99.8 ± 0.747	0.75
		2417351		
		2423262		
		2415471		
		2433031		
		2462356		

Table 5: Recovery data for Lazertinib

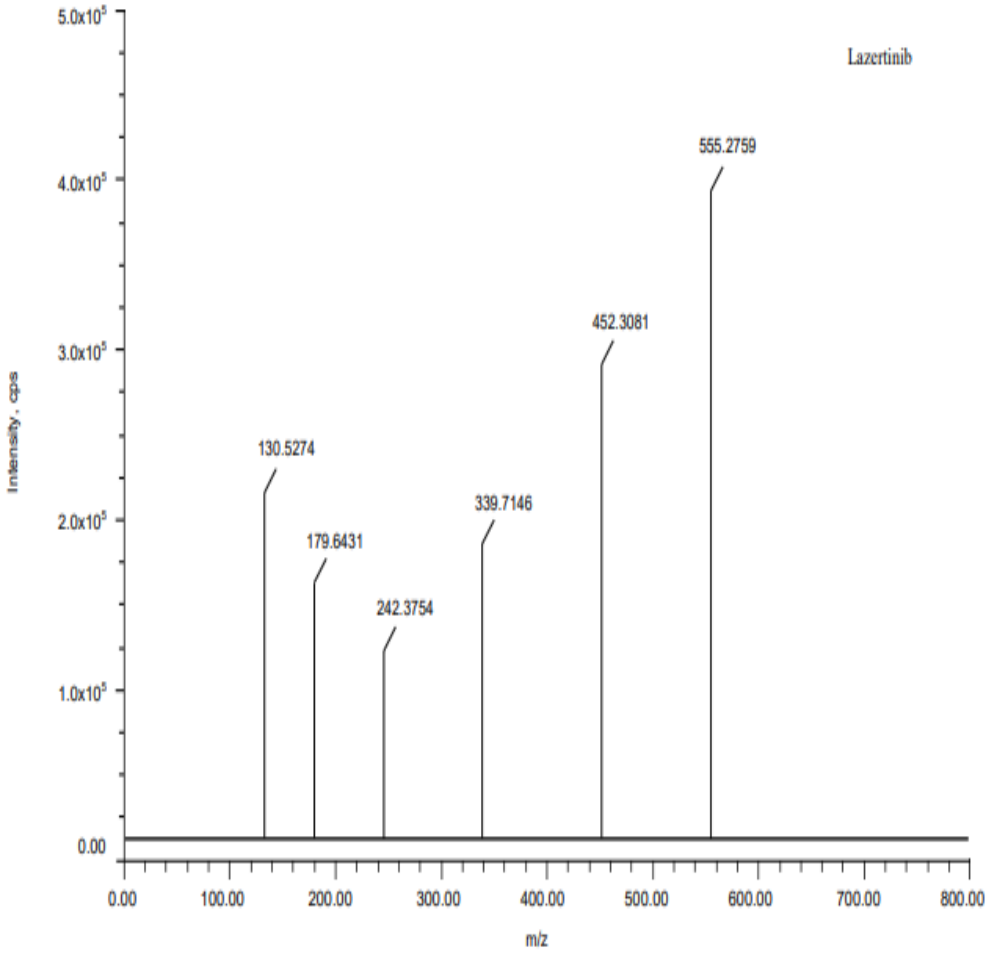
SR. NO.	Conc. Level (%)	Sample + Amount of API (mg)	Actual API Added (mg)	Amount recovered (mg)	% Recovery	% Mean Recovery ± S.D
1	50 %	4	4	3.984	99.6	99.9 ± 0.52
2		4	4	4.018	100.5	
3		4	4	3.98	99.5	
4	100 %	8	8	7.950	99.4	99.7 ± 0.37
5		8	8	7.977	99.7	
6		8	8	8.009	100.1	
7	150 %	12	12	11.935	99.5	99.6 ± 0.41
8		12	12	12.009	100.1	
9		12	12	11.917	99.3	

Table 6: Robustness data for Lazertinib

SR. NO.	Area at Flow rate (- 0.1 ml/min)	Area at Flow rate (+ 0.1 ml/min)	Area at Mobile phase (-5)	Area at Mobile phase (+5)	Area at pH (-0.25)	Area at pH(+0.25)
1	2332279	2690105	2285129	2799870	2518365	2332657
2	2374826	2684649	2272307	2780360	2506205	2354126
3	2363007	2667997	2281989	2763200	2487541	2358451
% R.S.D	0.93	0.41	0.27	0.65	0.61	0.61

Table 7: Forced Degradation Data

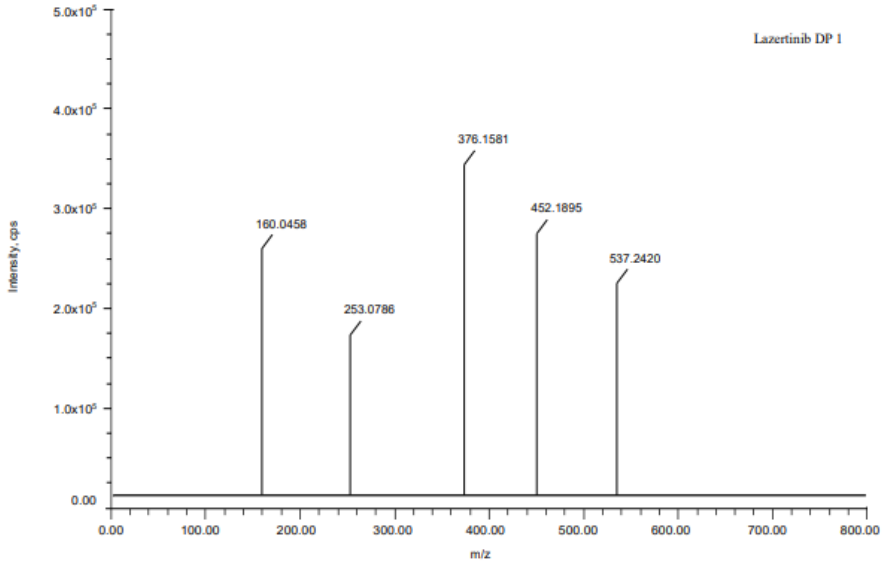
Sample Condition	% Label Claim	% Degradation	Purity Angle	Purity Threshold
Control	100	0	1.324	4.778
Acid – 1 N	88.9	11.1	1.387	4.763
Alkali - 1 N	86.5	13.5	1.361	4.719
Peroxide – 10 %	84.2	15.8	1.396	4.750
Reduction – 10%	87.4	12.6	1.303	4.728
Thermal - 6 hrs	97.4	2.6	1.313	4.733
Hydrolysis – 3 ml water	86.2	13.8	1.358	4.741



Multiple Reaction Monitoring-MRM of the Lazertinib using PositivePolarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Lazertinib	555.2759	452.3081

Figure 7: Lazertinib MS Spectra



Multiple Reaction Monitoring-MRM of the Lazertinib DP 1 using Positive Polarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Lazertinib DP 1	537.2420	376.1581

Figure 8: DP – 1 MS Spectra

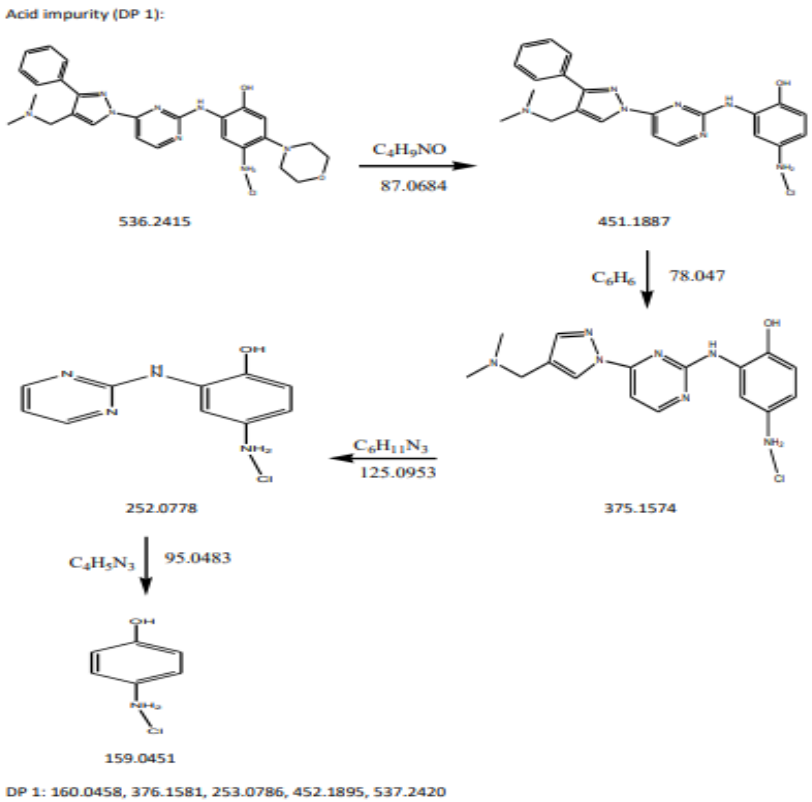
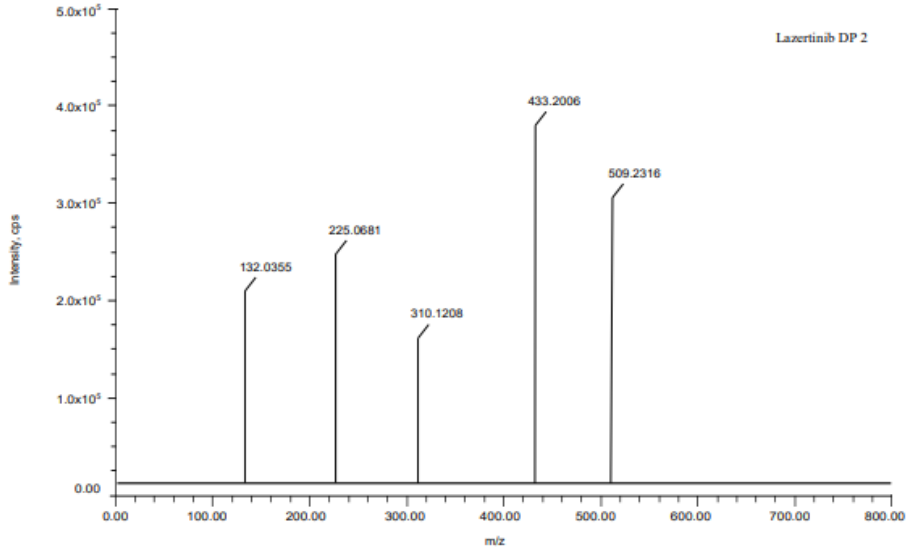


Figure 9: Fragmentation Mechanism of DP – 1 Possible Pathway



Multiple Reaction Monitoring-MRM of the Lazertinib DP 2 using Positive Polarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Lazertinib DP 2	509.2316	433.2006

Figure 10: DP – 2 MS Spectra

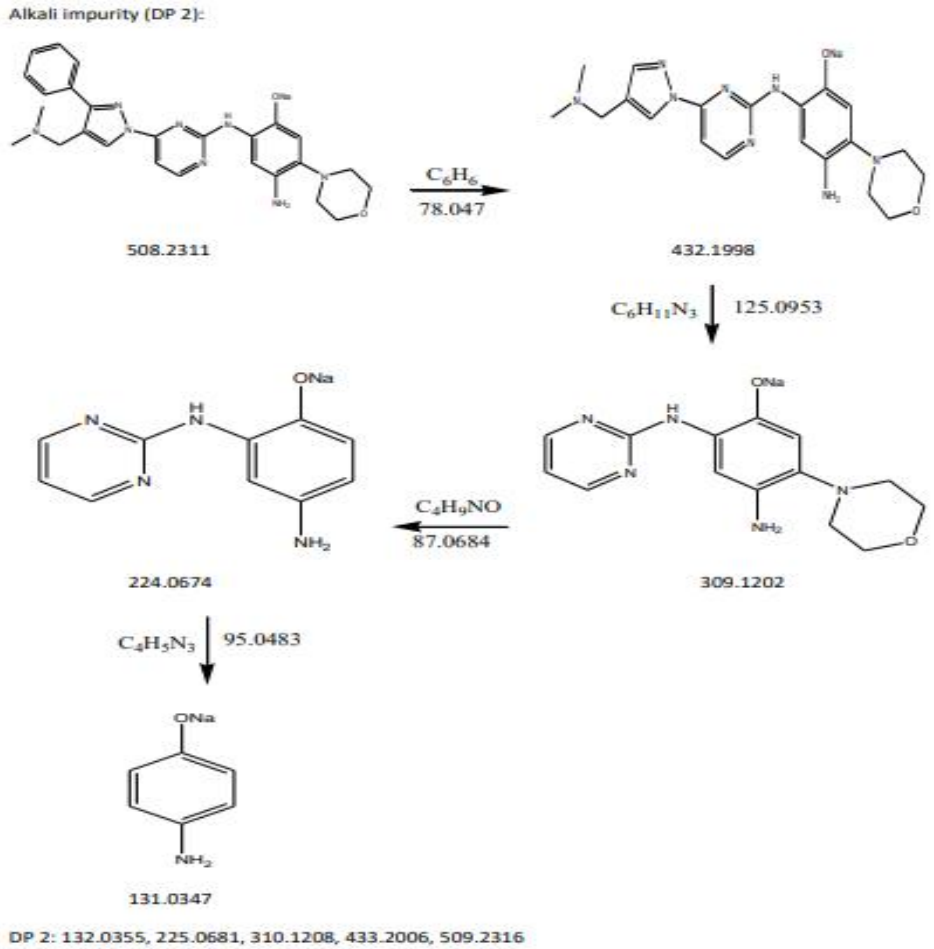
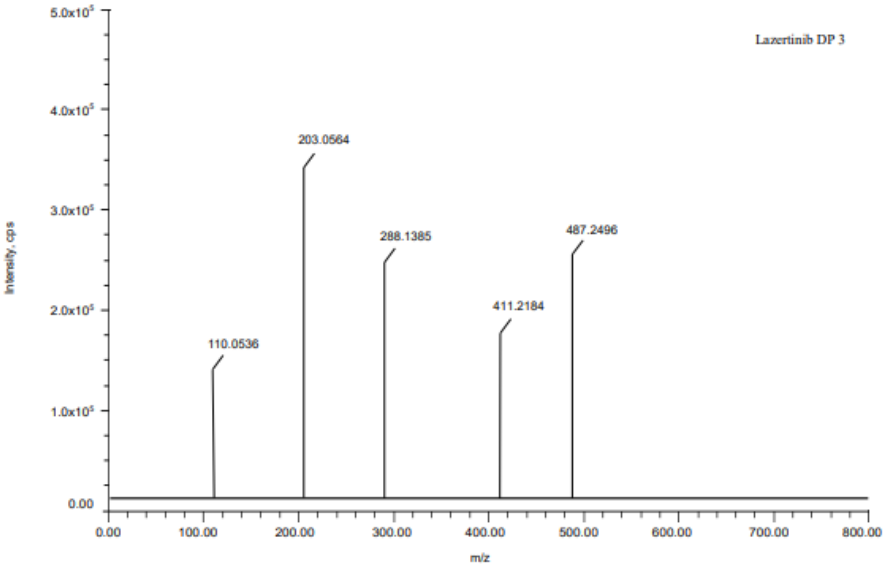


Figure 11: Fragmentation Mechanism of DP – 2 Possible Pathway



Multiple Reaction Monitoring-MRM of the Lazertinib DP 3 using Positive Polarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Lazertinib DP 3	487.2496	203.0564

Figure 12: DP – 3 MS Spectra

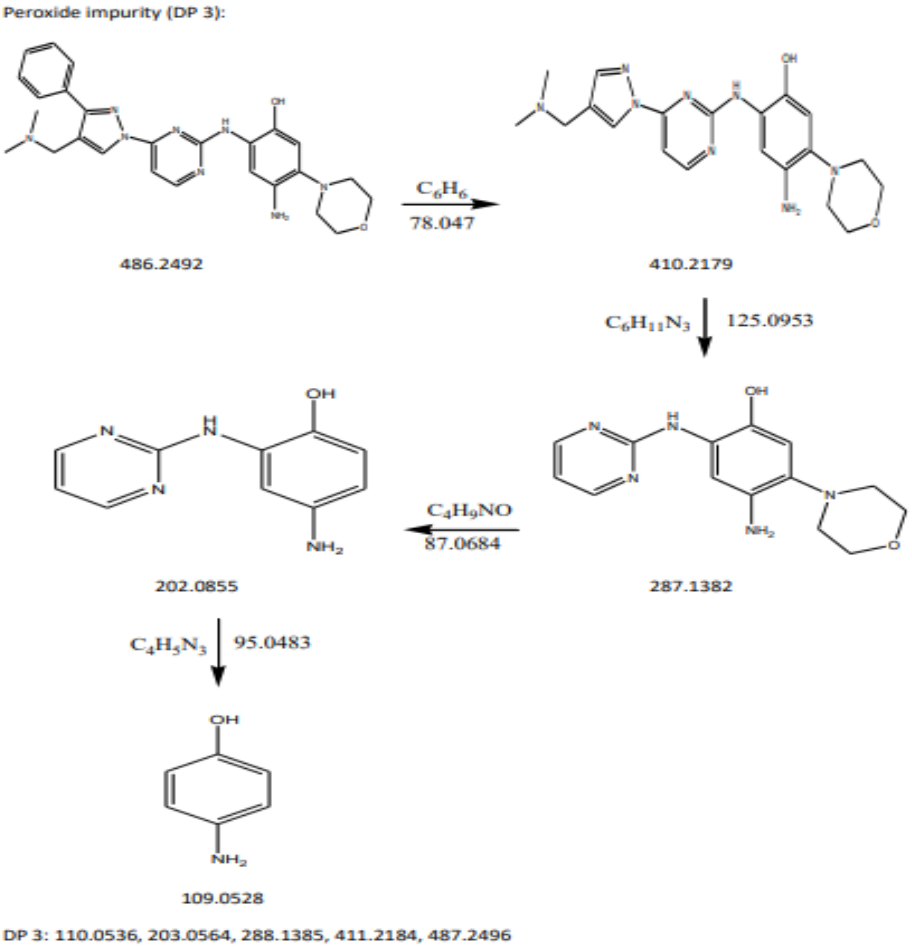
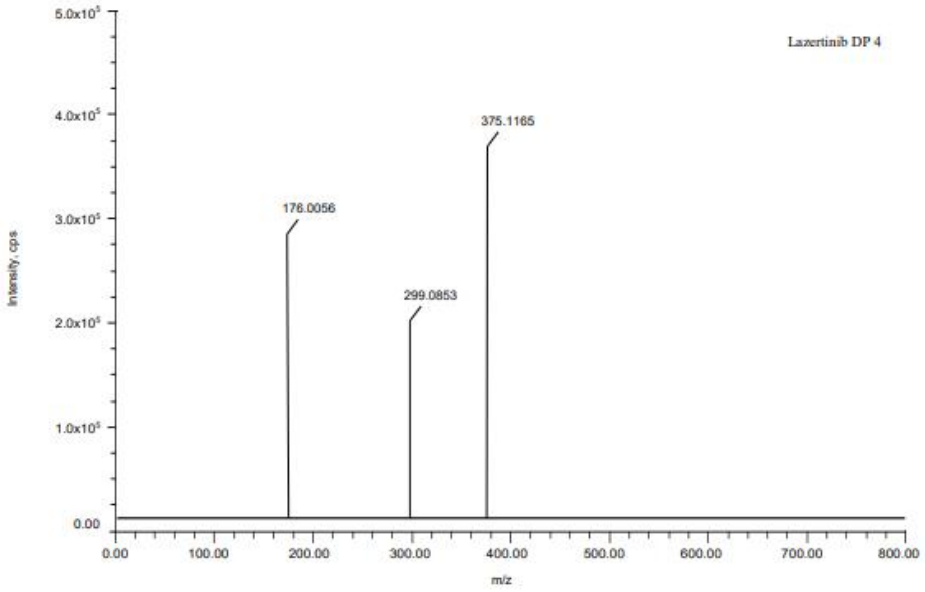


Figure 13: Fragmentation Mechanism of DP – 3 Possible Pathway



Multiple Reaction Monitoring-MRM of the Lazertinib DP 4 using Positive Polarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Lazertinib DP 4	375.1165	176.0056

Figure 14: DP – 4 MS Spectra

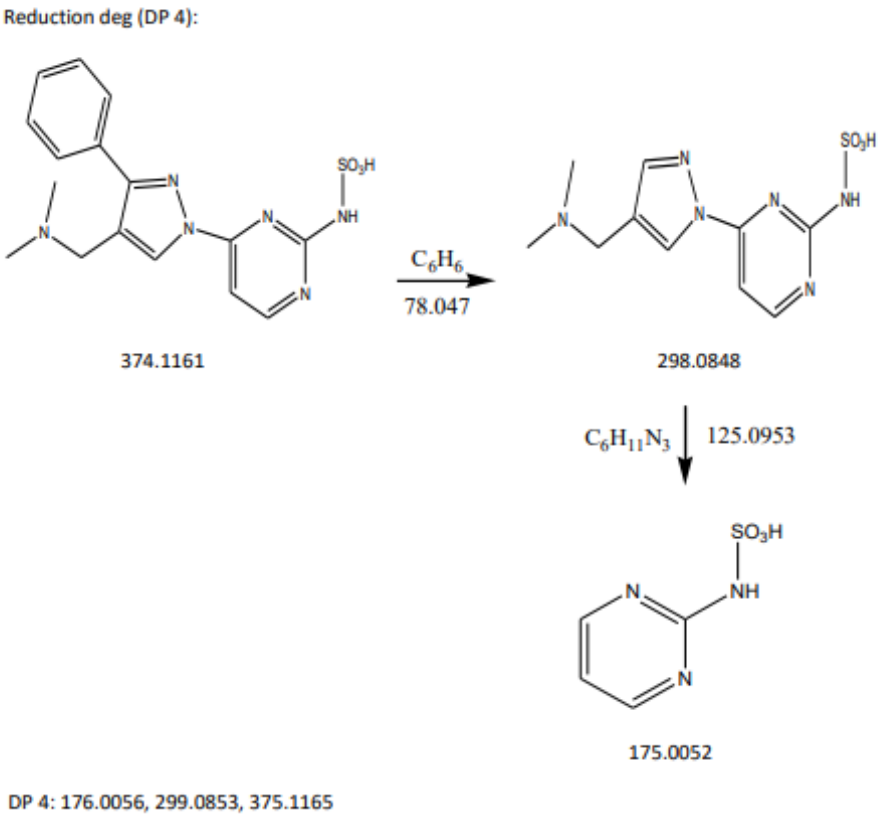
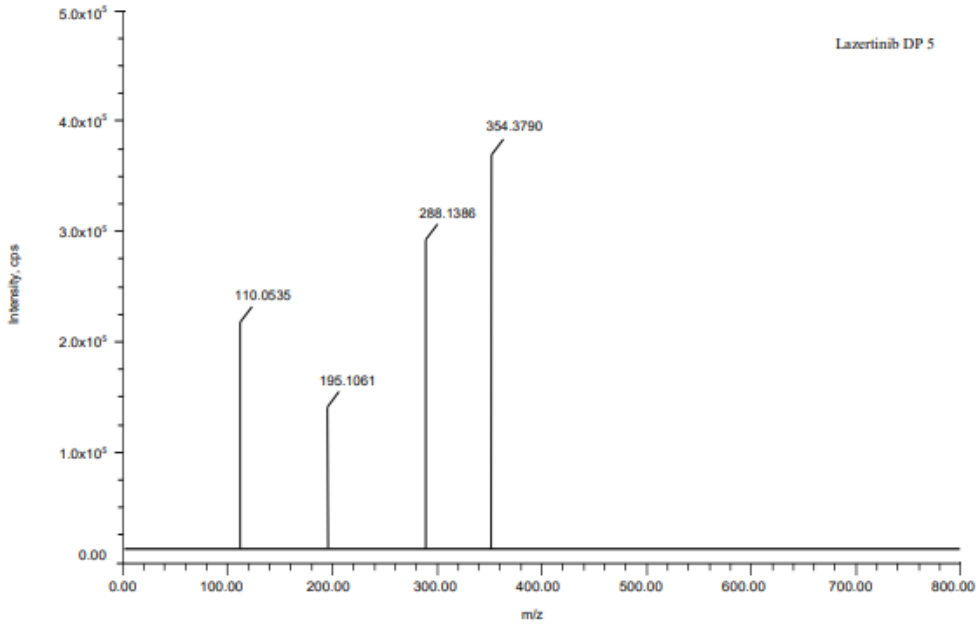


Figure 15: Fragmentation Mechanism of DP – 4 Possible Pathway



Multiple Reaction Monitoring-MRM of the Lazertinib DP 5 using Positive Polarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Lazertinib DP 5	354.3790	288.1386

Figure 16: DP – 5 MS Spectra

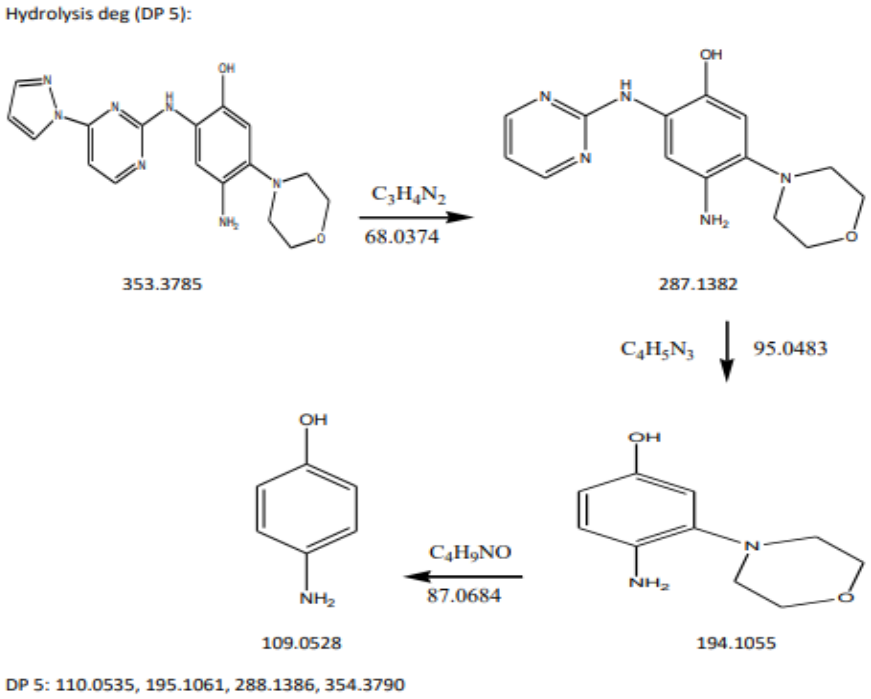


Figure 17: Fragmentation Mechanism of DP – 5 Possible Pathway