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Impurity Profiling Of Some Anti - Cancer Drugs With Special Reference To Repotrectinib

Shah Utsav Jagdishbhai[1]*, Dr. Bhaveshkumar H. Patel[2]
[1]*Department of Pharmaceutical Chemistry, K B Institute of Pharmaceutical Education and Research, utsavshah3944@gmail.com
[2]Kadi Sarva Vishwavidyalaya University, drbhavesh24@gmail.com

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 Repotrectinib in its pharmaceutical dosage form. Also, a forced degradation study of Repotrectinib was carried out including acid, alkali, peroxide, reduction, thermal and hydrolysis. The method was based on isocratic elution using a mobile phase consisting of Acetonitrile: Ammonium acetate pH - 2.4 / OPA (20:80 % v/v) at a flow rate of 1.0 ml/min, with Agilent Eclipse XDB (150 mm x 4.6 mm, 3.5 µm) column. Detection wavelength was 268 nm. In addition, Degradation Products were identified for Acid, Peroxide, Reduction and Hydrolysis forced degradation condition as DP – 1, DP - 2, DP - 3 and DP - 4 respectively and were verified by LC – MS/MS. Their Possible degradation pathway were also drawn. Linearity was observed for 10 - 60 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 Repotrectinib 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: *Repotrectinib, Stability - Indicating assay, Forced degradation, UPLC – MS/MS, Impurity, Anti- Cancer.*

INTRODUCTION:

"Cancer is when abnormal cells grow quickly and spread beyond their normal area, potentially invading nearby parts of the body and moving to other organs in a process called metastasis". [1, 2].
"Repotrectinib is indicated to treat ROS1 (reactive oxygen species 1) - positive Non - Small Cell Lung cancer. Repotrectinib is an inhibitor of proto-oncogene tyrosine-protein kinase ROS1 (ROS1) and of the tropomyosin receptor tyrosine kinases (TRKs)". [4, 5].
Impurity profiling, which involves finding and measuring these impurities, is now getting more attention from regulatory agencies. "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, only single Bio - analytical method for Mouse plasma in combination with other drug is only reported, but any method regarding Impurity Profiling of Anti – Cancer Drugs (i.e. Repotrectinib) is not available. [10-12].

MATERIAL AND METHODS:

RP - HPLC Method Development

Selection of Elution Mode

Reverse phase chromatography was chosen because it's mostly recommended for ionic and moderately to non-polar compounds. It is not only easy to use and convenient, but it also works very well in terms of efficiency, stability, and consistency. For separating our drug Repotrectinib, a C18 column with dimensions 150 mm x 4.6 mm and a particle size of 3.5 µm was selected. An isocratic method was used because it provides good reliability and maintains performance over a longer period of column use.

Wavelength Selection

The optimum wavelength for detection was selected at 268 nm.

Prepare a standard solution of Repotrectinib (40 µg/ml)

First, take 4 mg of Repotrectinib and dissolve it in 10 ml of methanol. Then, take 1 ml of this solution and add methanol to bring the total volume up to 10 ml. After this, the drug solution was scanned. The resulting spectra is shown in Figure 1.

Mobile Phase selection and optimization

Based on several trials, the mixture of Acetonitrile: Ammonium acetate pH - 2.4 / OPA (20:80 % v/v) at a flow rate of 1.0 ml/min performed better than other mobile phase mixtures in terms of peak shape, theoretical plate count, and asymmetry. The details of the optimized trial is shown in Figure 2.

The chromatographic conditions are listed in Table 1 and they met good system suitability parameters.

RESULT AND DISCUSSION [8, 9]:

A. METHOD VALIDATION SUMMARY:

1. Specificity:

The chromatograms for Repotrectinib show no interference from the blank and placebo samples. This means the developed method is specific. The chromatograms are shown in Figure 3 and Figure 4 respectively.

2. Linearity and Range:

Linearity was discovered by drawing a calibration curve of the area of peak concentration against its corresponding concentration (25%, 50%, 75%, 100%, 125%, and 150%). It was possible to deduce from this calibration curve that the graph represented a straight line within the range of 10 to 60 µg/ml of Repotrectinib. Y is calculated as 74631.70x + 11664.57 and (R^2 as 0.9992). 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 Repotrectinib at a concentration of 40 µg/ml was assessed by measuring the same solution six times.

The % RSD for Repotrectinib was 0.90.

Intermediate Precision:

A standard solution containing 40 µg/ml of Repotrectinib was analyzed six times on the same day using different instruments and by different analysts.

The % RSD for Repotrectinib was 0.92.

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: -

A 40 µg/ml drug solution was placed into three separate flasks. Then, 50%, 100%, and 150% of the standard solution were added to each flask, and each was diluted to a total volume of 10 ml. The area of the peak for each solution was measured at a wavelength of 268 nm. The amount of Repotrectinib in each solution was determined, and the percentage recovery for each level was calculated. The approach was successful because the recuperation values were within the scope. Table 5 shows accuracy results.

5. LOD and LOQ: -

The limit of detection and limit of quantification for Repotrectinib were determined to be 0.600 µg/ml and 2.000 µg/ml respectively.

6. Robustness: -

During the assessment of the chromatographic method, variations in flow rate, phase composition, and pH were tested. The RSD values were found to be within acceptable ranges. The robustness results are listed in Table 6.

7. Analysis of marketed formulation by developed method:

Take powder amount equal to 16.7 mg of Repotrectinib and place it into a 10 ml volumetric flask. Then, take 1 ml of this mixture and add mobile phase to bring the total volume up to 10 ml. Next, take 1 ml from the standard stock solution and transfer it to a 10 ml volumetric flask, then add mobile phase until the volume reaches the mark. The solution was filtered using Whatmann filter paper No. 41. Inject 20 µL of the solution for assay analysis.

Assay was found to be 100.6 % label claim.

B. FORCED DEGRADATION STUDIES: [13-16]

Preparation of Repotrectinib Sample Stock Solution:-

Accurately weigh 16.7 mg of Repotrectinib sample and transferred into a 10 ml volumetric flask add 7 ml of diluent and make up to the mark as final volume.

1. Acid Degradation

0.1 N Degradation

Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of 0.1N HCl. Heat it by refluxing at 50°C for 2 hours. Let it sit for 15 minutes. Then add 1 ml of 0.1N NaOH to neutralize the solution. Fill the flask to the mark with diluent and mix thoroughly.

1 N Degradation

Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of 1N HCl. Heat it by refluxing at 60°C for 2 hours. Let it sit for 15 minutes. Then add 1 ml of 1N NaOH to neutralize the solution. Fill the flask to the mark with diluent 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. Heat it by refluxing at 50°C for 2 hours. Leave it for 15 minutes. Then add 1 ml of 0.1N HCl to neutralize the solution. Fill the flask to the mark with diluent and mix thoroughly.

1 N Degradation

Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of 1N NaOH. Heat it by refluxing at 60°C for 2 hours. Leave it for 15 minutes. Then add 1 ml of 1N HCl to neutralize the solution. Fill the flask to the mark with diluent and mix thoroughly.

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. Let it sit for 15 minutes. Then dilute it to the mark with diluent 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. Let it sit for 15 minutes. Then dilute it to the mark with diluent and mix thoroughly. **DP - 2** was seen in the chromatogram during Peroxide degradation.

4. Thermal Degradation

105°C for 3 hrs Degradation

50 mg of sample was placed at 105°C for 3 hours, and then the sample was tested. 40 mg of the sample was put into a 100 ml volumetric flask and filled up to the mark. Then, 1 ml of this mixture was taken and diluted to 10 ml using the diluent.

105°C for 6 hrs Degradation

50 mg of sample was placed at 105°C for 6 hours, and then the sample was tested. 40 mg of the sample was put into a 100 ml volumetric flask and filled up to the mark. . Then, 1 ml of this mixture was taken and diluted to 10 ml using the diluent.

5. Reduction Degradation

3% Sodium bisulfate Degradation

Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of 3% sodium bisulfate solution. Heat it under reflux at 60 °C for 2 hrs. Let it sit for 15 minutes. Then add more diluent to bring the total volume up to 10 ml and mix well.

10% Sodium bisulfate Degradation

Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of 10% sodium bisulfate solution. Heat it under reflux at 60 °C for 2 hrs. Let it sit for 15 minutes. Then add more diluent to bring the total volume up to 10 ml and mix well. **DP - 3** was observed in the chromatogram during Reduction degradation.

6. Neutral Hydrolysis Degradation

HPLC Water Degradation

1. Take 1 ml of the sample into a 10 ml volumetric flask and add 1 ml of HPLC water. Reflux at 80 °C for 02 hrs. Let it stand for 15 minutes. Then add more diluent to fill it up to the mark and mix well.
2. Take 1 ml of the sample into a 10 ml volumetric flask and add 3 ml of HPLC water. Reflux at 90 °C for 05 hrs. Let it stand for 15 minutes. Then add more diluent to fill it up to the mark and mix well. **DP - 4** was observed in chromatogram in Hydrolysis degradation.

- In the Acid degradation DP-1 was identified; in Peroxide degradation DP-2; in Reduction degradation DP-3; and in Hydrolysis degradation DP-4. 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 Repotrectinib. 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 04 Degradation Products DP1, DP2, DP3 and DP4 were identified. Their MS Spectra, and Possible degradation Pathways are mentioned in Figure 7 to 15.

CONCLUSION:

A simple, specific, accurate and precise RP-HPLC method has been developed and validated as per ICH guideline for Estimation of Repotrectinib in its pharmaceutical dosage form. Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, Specificity were tested. Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, specific and robust. It can be successfully adopted for routine quality control analysis of Repotrectinib in its pharmaceutical dosage form without any interference. The degradation behavior of the drug was examined under acidic, alkali, peroxide, reduction, hydrolysis and thermal stress conditions. The drug remained stable under alkali and thermal conditions but broke down under acidic, peroxide, hydrolysis, and reduction conditions. The breakdown products were identified through [M+ H] + ion detection, and their structures were confirmed using UPLC-MS/MS along with correct mass measurements. The UPLC method was supported as per ICH guidelines and can be applied to the marketed formulations.

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Annexures:

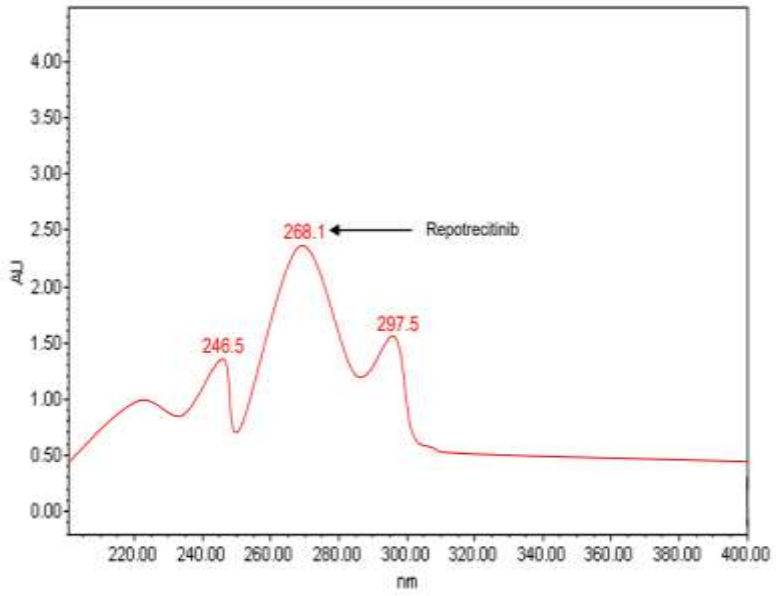


Figure 1: UV Spectra of Repotrectinib (40 µg/ml) in Methanol

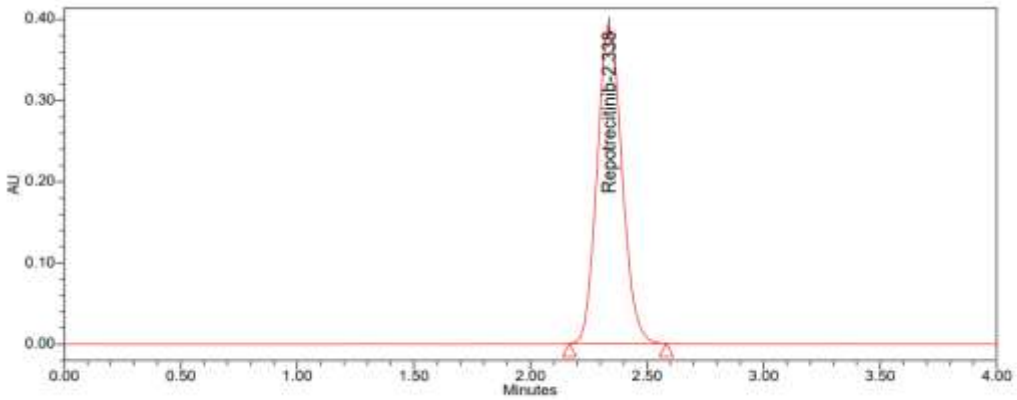


Figure 2: Optimized Trial / Acetonitrile: Ammonium Acetate (20:80 % v/v) pH, 2.4 / Ortho-Phosphoric Acid

Table 1: Chromatographic Conditions

Column	Agilent Eclipse XDB (150 mm x 4.6 mm, 3.5 µm)
Mode of Elution	Isocratic
Mobile phase	Acetonitrile : Ammonium acetate pH - 2.4 / OPA (20:80 % v/v)
Detection Wavelength	268 nm
Injection volume	10 µL
Flow rate	1.0 ml/min
Column Temperature	25°C
Run time	05 minutes
Retention Time	2.338
Theoretical Plates	12266
USP Tailing	1.12

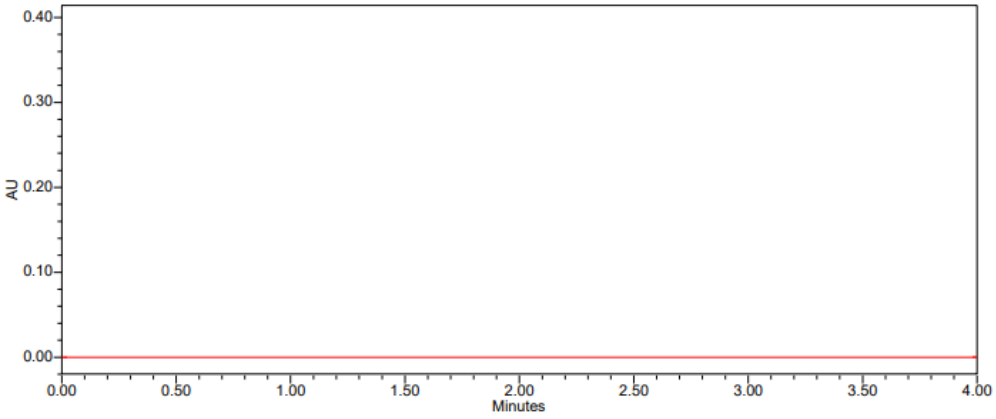


Figure 3: Chromatogram of Repotrectinib Blank

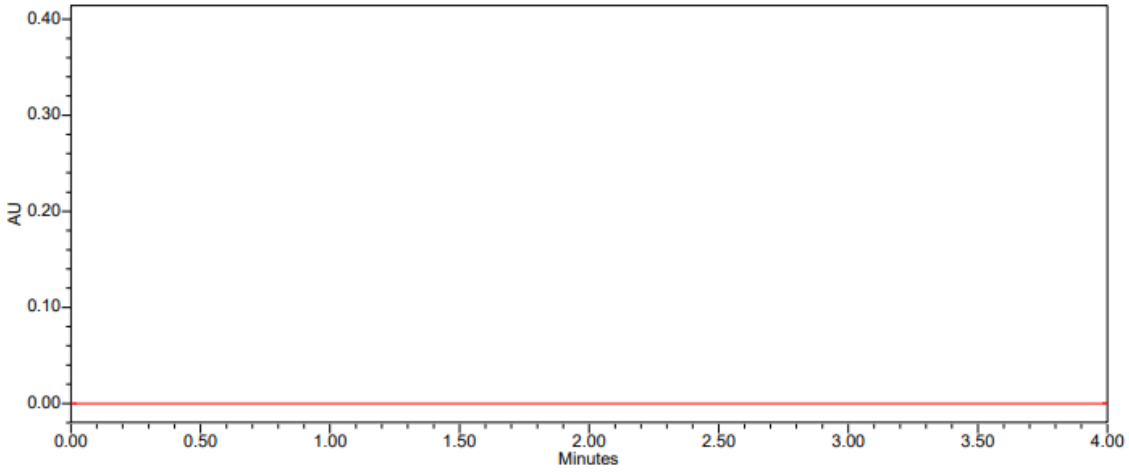


Figure 4: Chromatogram of Repotrectinib Placebo

Table 2: Linearity data for Repotrectinib

Sr. No.	Concentration (µg/ml)	Area
1	10.00	831393

2	20.00	1499261
3	30.00	2194419
4	40.00	2906102
5	50.00	3816581
6	60.00	4506553

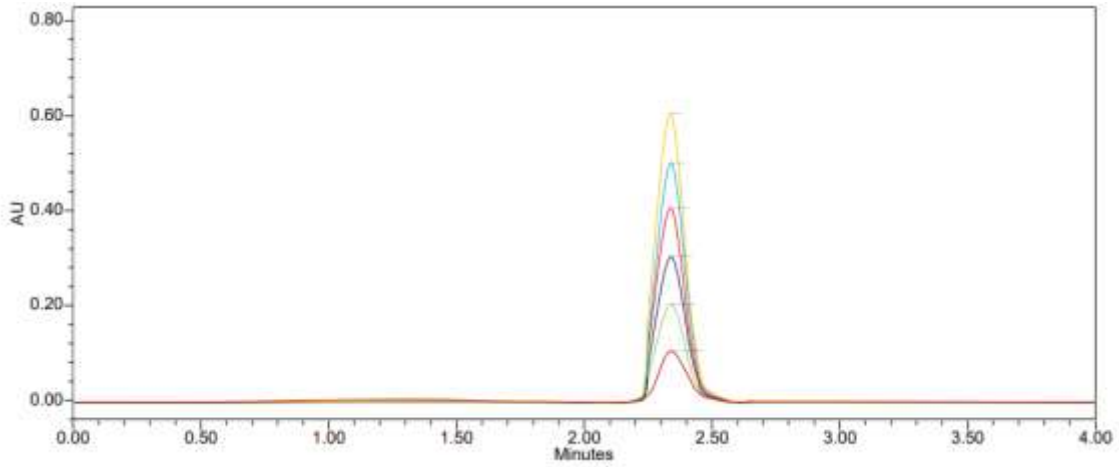


Figure 5: Overlay chromatogram of different concentrations of mixtures of Repotrectinib

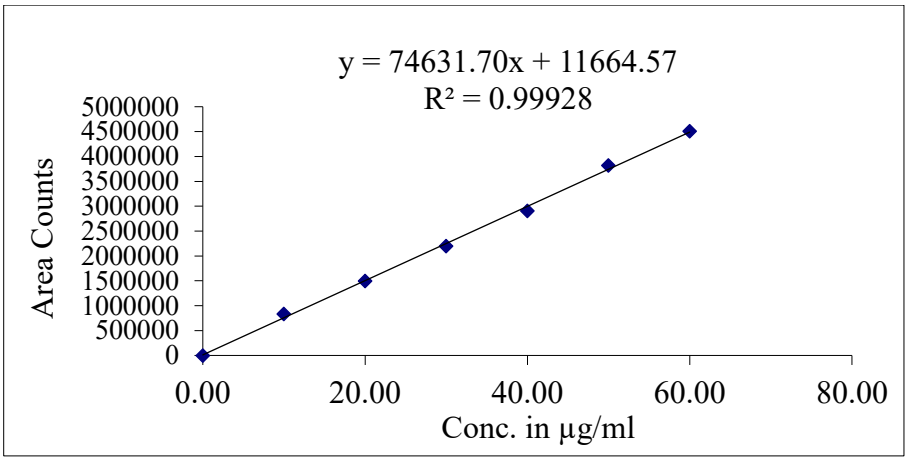


Figure 6: Calibration Curve of Repotrectinib (10 – 60 µg /ml)

Table 3: Repeatability / Method Precision

Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	40	2900847	99.9 ± 0.899	0.90
		2897995		
		2933999		
		2917151		
		2963482		
		2942710		

Table 4: Intermediate Precision

Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	40	2930654	100.4 ± 0.927	0.92
		2977351		
		2953262		
		2915471		
		2933031		
		2902356		

Table 5: Recovery data for Repotrectinib

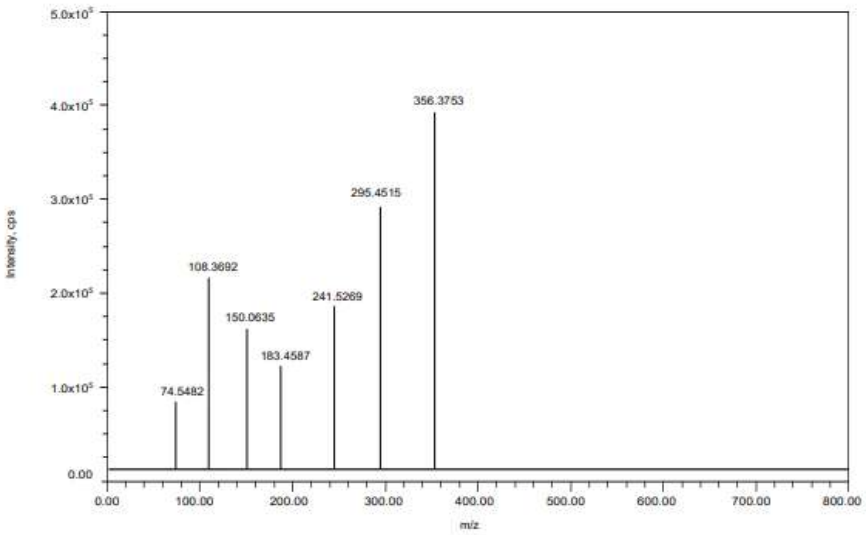
SR. NO.	Conc. Level (%)	Sample + Amount of API (mg)	Actual API Added (mg)	Amount recovered (mg)	% Recovery	% Mean Recovery ± S.D
1	50 %	2	2	1.97	98.5	99.9 ± 1.33
2		2	2	1.999	100.0	
3		2	2	2.023	101.2	
4	100 %	4	4	3.968	99.2	99.8 ± 0.55
5		4	4	3.997	99.9	
6		4	4	4.011	100.3	
7	150 %	6	6	6.079	101.3	101.2 ± 0.71
8		6	6	6.028	100.5	
9		6	6	6.112	101.9	

Table 6: Robustness data for Repotrectinib

SR. NO.	Area at Flow rate (- 0.1 ml/min)	Area at Flow rate (+ 0.1 ml/min)	Area at Mobile phase (-10%)	Area at Mobile phase (+10%)	Area at pH (-0.2)	Area at pH(+0.2)
1	3230105	2762279	3199870	2633129	3048759	2856947
2	3294649	2784826	3140360	2672307	3052342	2825146
3	3267997	2723007	3163200	2651989	3039567	2859748
% R.S.D	0.96	1.15	0.96	0.75	0.21	0.64

Table 7: Forced Degradation Data

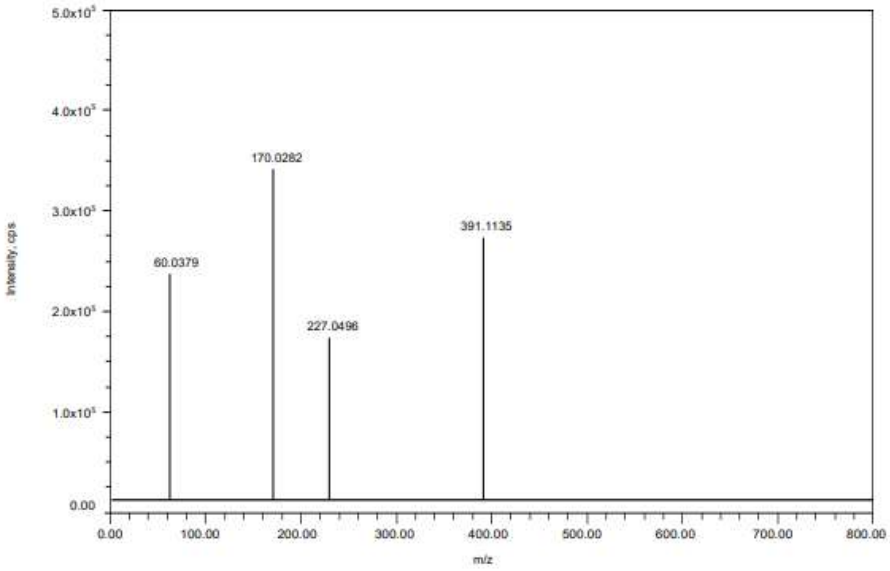
Sample Condition	% Label Claim	% Degradation	Purity Angle	Purity Threshold
Control	100	0	0.131	1.181
Acid – 1 N	87.4	12.6	0.137	1.185
Alkali - 1 N	97.3	2.7	0.134	1.186
Peroxide – 10 %	85.3	14.7	0.366	1.528
Reduction – 10%	88.8	11.2	0.138	1.186
Thermal - 6 hrs	99.2	0.8	0.136	1.181
Hydrolysis – 3 ml water	90.3	9.7	0.157	1.182



Multiple Reaction Monitoring-MRM of the Repotrectinib using Positive Polarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Repotrectinib	356.3753	295.4515

Figure 7: Repotrectinib MS Spectra



Multiple Reaction Monitoring-MRM of the Repotrectinib DP 1 using PositivePolarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Repotrectinib DP 1	391.1135	170.0282

Figure 8: DP – 1 MS Spectra

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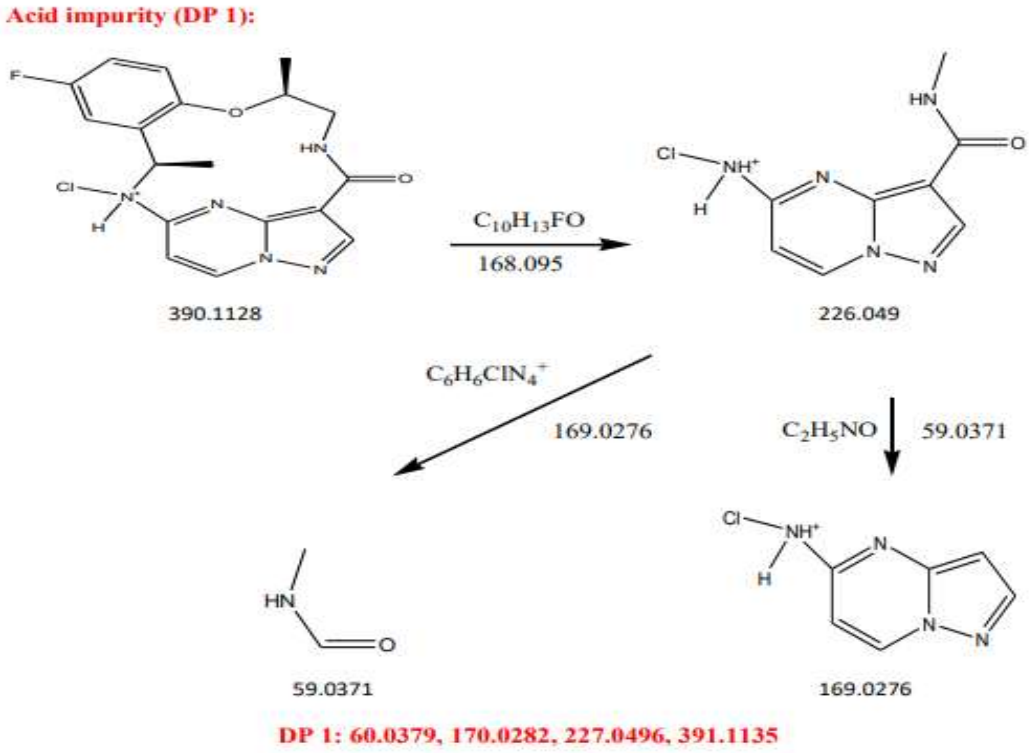
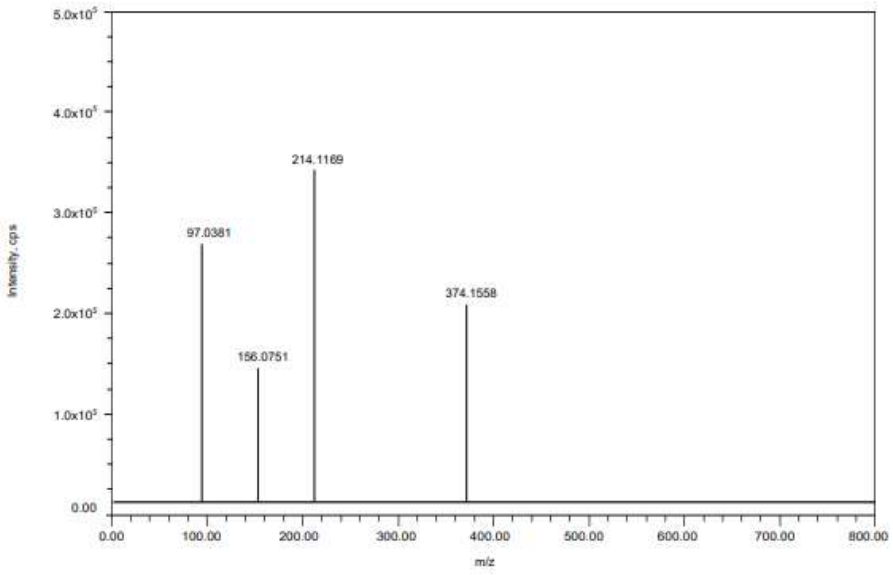


Figure 9: Fragmentation Mechanism of DP – 1 Possible Pathway



Multiple Reaction Monitoring-MRM of the Repotrectinib DP 2 using PositivePolarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Repotrectinib DP 2	374.1558	214.1169

Figure 10: DP – 2 MS Spectra

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://www.theaspd.com/ijes.php

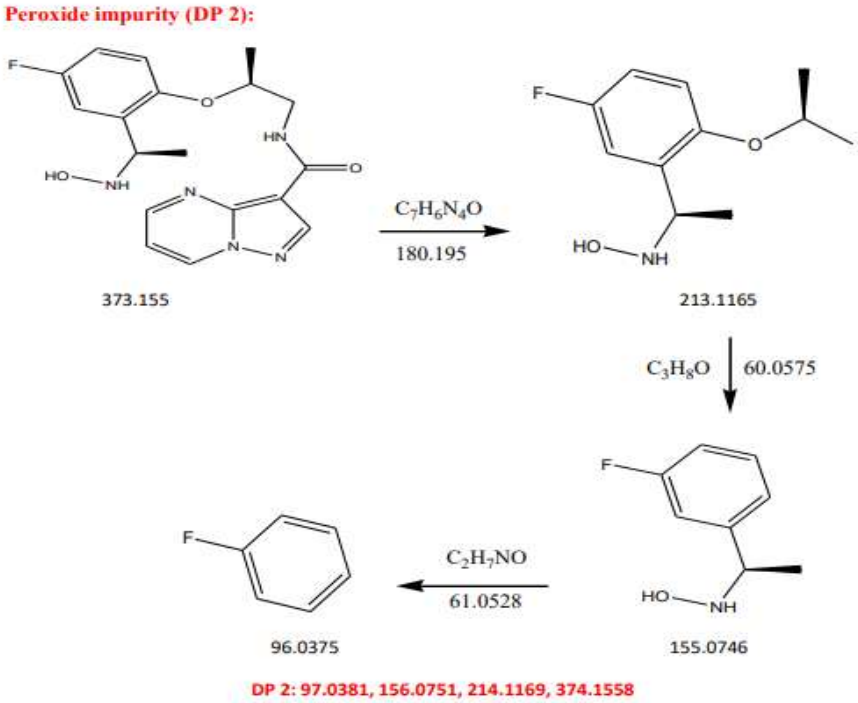
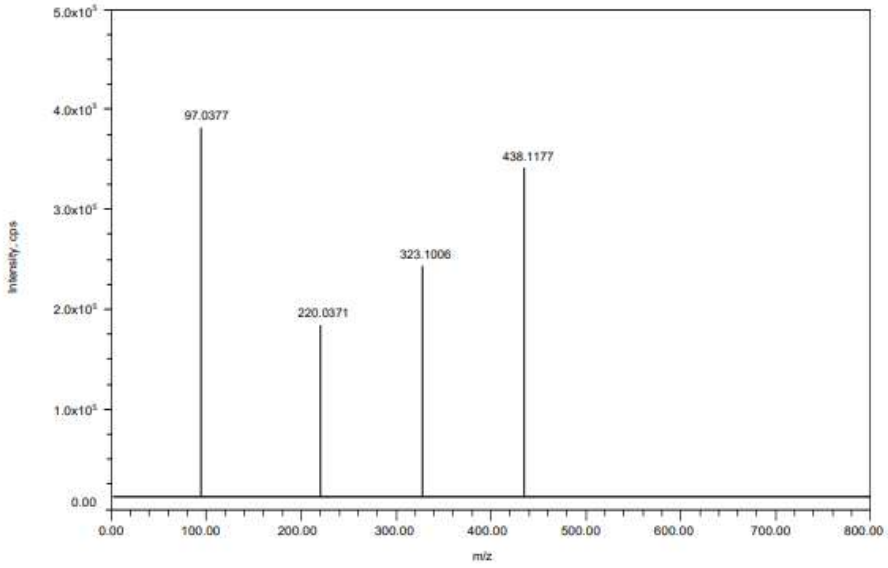


Figure 11: Fragmentation Mechanism of DP – 2 Possible Pathway



Multiple Reaction Monitoring-MRM of the Repotrectinib DP 3 using PositivePolarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Repotrectinib DP 3	438.1177	97.0377

Figure 12: DP – 3 MS Spectra

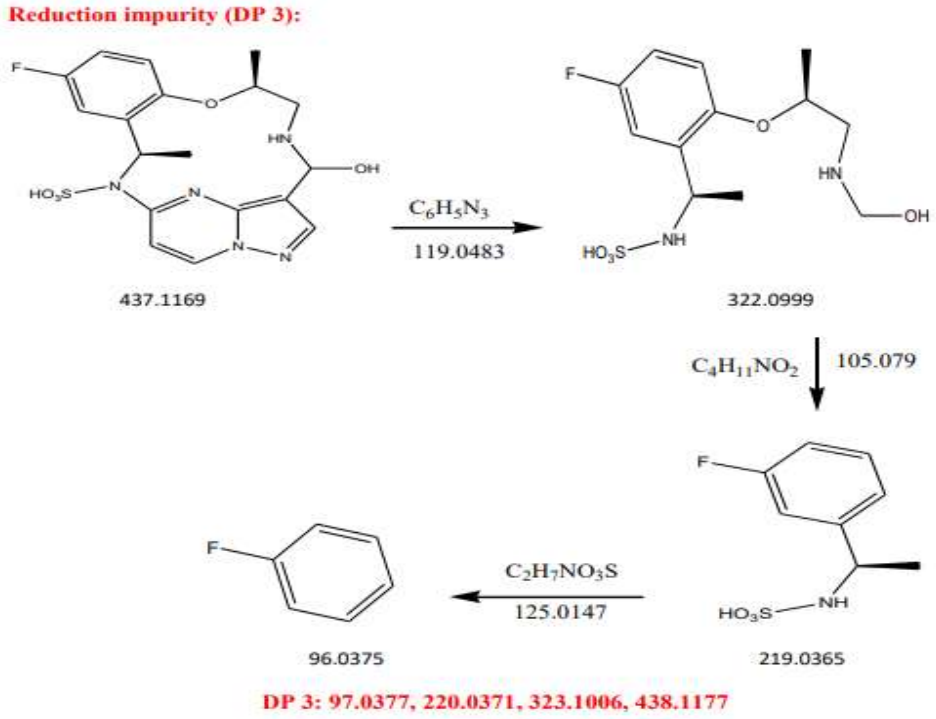
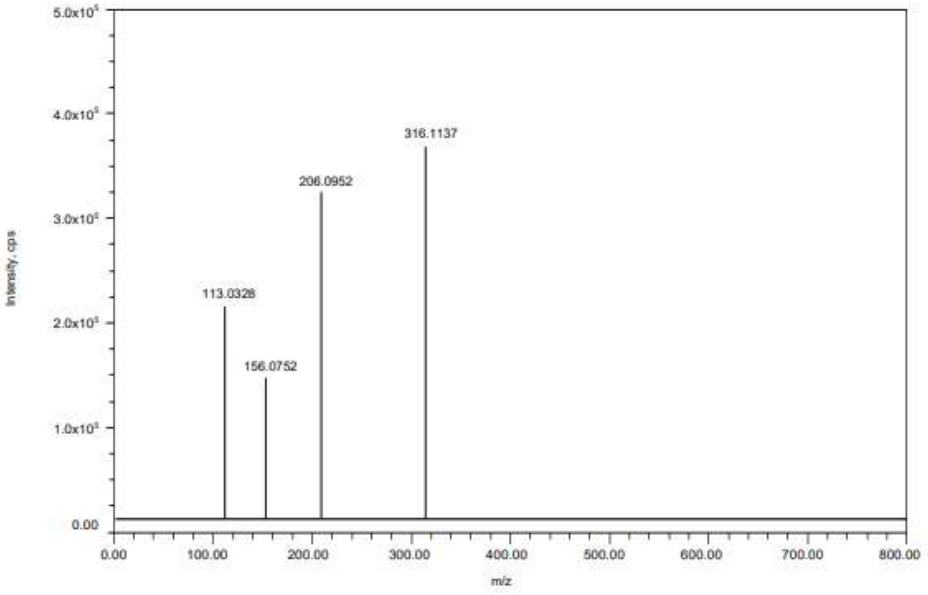


Figure 13: Fragmentation Mechanism of DP – 3 Possible Pathway



Multiple Reaction Monitoring-MRM of the Repotrectinib DP 4 using PositivePolarity

Analogue	Precursor Ion (m/z)	Daughter Ion with the Highest Intensity (m/z)
Repotrectinib DP 4	316.1137	206.0952

Figure 14: DP – 4 MS Spectra

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ISSN: 2229-7359
Vol. 11 No. 23s, 2025
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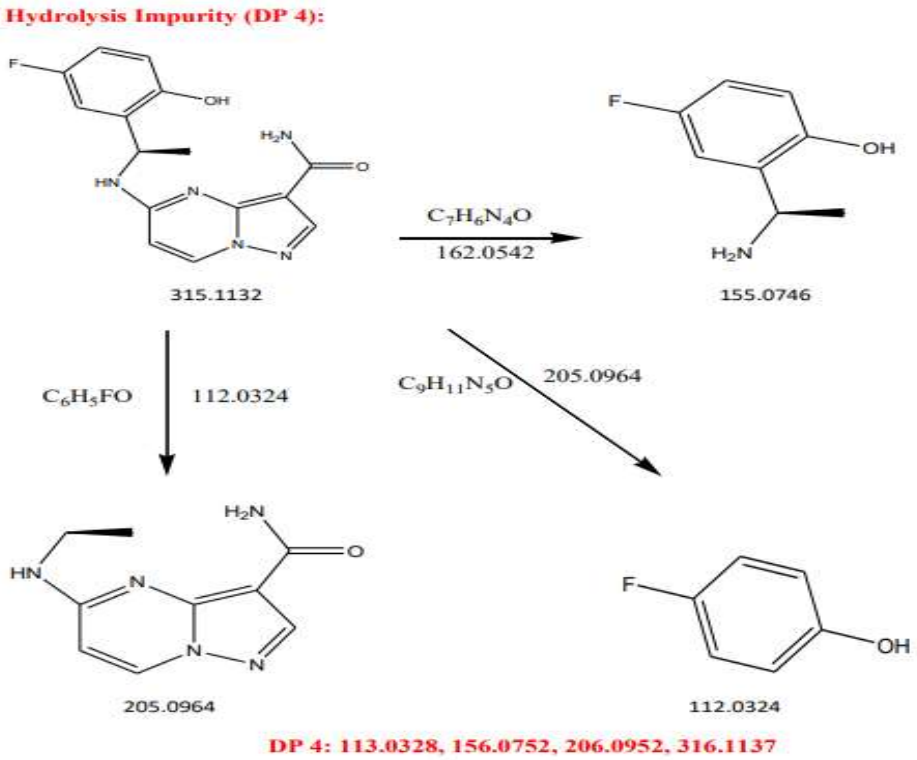

Figure 15: Fragmentation Mechanism of DP – 4 Possible Pathway