Impurity Profiling of Some Anti-Cancer Drugs/Capivasertib

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

KEYWORDS
Capivasertib, Stability - Indicating assay, Forced degradation, UPLC – MS/MS, Impurity, Anti – Cancer.

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 Capivasertib in its pharmaceutical dosage form. Also, a forced degradation study of Capivasertib 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 : 0.1% Triethyl Amine (50:50 % v/v) pH 2.5 / Formic acid at a flow rate of 1.2 ml /min with Kromasil C18 (150 mm x 4.6 mm, 5µm) column. Detection wavelength was 257 nm. In addition, Degradation Products were identified for Acid, Alkali, Peroxide and Thermal forced degradation condition as DP – 1, DP - 2, DP - 3 and DP - 4 respectively and were verified by LC – MS. Their Possible degradation pathways were also drawn. Linearity was observed for 50 - 300 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 Capivasertib 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.

Introduction:

➢ Cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as **Metastasis**.

➢ Capivasertib is used to treat hormone receptor-positive, HER2-negative, locally advanced or metastatic breast cancer. Capivasertib is an inhibitor of all 3 isoforms of serine/threonine kinase AKT (AKT1, AKT2, and AKT3) and inhibits phosphorylation of downstream AKT substrates.

➢ In the pharmaceutical world, an impurity is considered as any other organic material, besides the drug substance, or ingredients, arise out of synthesis or unwanted chemicals that remains with API's.

➢ Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now gaining critical attention from regulatory authorities.

➢ According to ICH, the maximum daily dose and Identification threshold to be considered is as follows; ≤2g/day 0.10% or 1mg per day intake (whichever is lower) and if maximum daily dose is ≥2g/day than 0.05%.

MATERIAL AND METHODS:

❑ Development of RP - HPLC Method :

❖ Selection of Elution Mode

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also better performing in terms of efficiency, stability and reproducibility. Here, C18 (150 mm x 4.6 mm, 3.5 µm) column was selected for separation of Capivasertib. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability.

➢ **Selection of wavelength**

The optimum wavelength for detection was set at 257 nm.

❖ **Preparation of Standard solution of Capivasertib (200 µg/ml)**

Take 20 mg of weighed quantity of Capivasertib and dilute it upto 10 ml with Methanol.
Further from above solution take 1 ml and make the final volume upto 10 ml with the use of Methanol.
After that this drug solution was scanned. The observed Spectra is mentioned in Figure 1.

❖ **Mobile Phase selection and Optimization**

On the basis of various trials the mixture of Acetonitrile : 0.1% Triethylamine (50:50 % v/v pH 2.5), at 1.2 ml/min flow rate, had proved to be better than the other mixtures of mobile phases in terms of peak shape, theoretical plate and asymmetry. The Optimized Trial details are mentioned in Figure 2. The Chromatographic Conditions are documented in Table 1, they were found with good system suitability parameters.

RESULT AND DISCUSSION:

A. METHOD VALIDATION SUMMARY:

1. Specificity: The Chromatograms of Capivasertib shows no interference for Blank and Placebo. So the Developed method is Specific. The Chromatograms were shown in Figure 3 and 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 50 to 300 µg/ml of Capivasertib. Y is calculated as **11424.04x + 25080.96** and (R^2 as 0.9997). 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 data for repeatability of peak area measurement for Capivasertib (200 µg/ml) based on Six measurements of same solution. The % RSD for Capivasertib was found to be 0.56.

Intermediate Precision: Standard solution containing (200 µg/ml) of Capivasertib was analyzed Six times on the same day on different instrument by different analyst. The % RSD for Capivasertib was found to be 0.40.
Method is precise, with Relative standard deviation (RSD) percentage values of less than 2%. Table 3 and 4 demonstrates data for method precision and Intermediate Precision results.

4. Accuracy: -

200 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 area of each solution peak was measured at 257 nm. The amount of Capivasertib was calculated at each level and % recoveries were computed. The approach was successful because the recuperation values were within the scope. Table 5 shows accuracy results.

5. LOD and LOQ: -

LOD and LOQ details for Capivasertib were calculated as 1.200 µg/ml and 2.000 µg/ml respectively.

6. Robustness:-

In the evaluation of chromatographic technique, the fluctuations in flow rate and the movement composition of phases and pH Changes were done. The RSD percentage was determined to be within reasonable limits. In Table 6, Robustness data are documented.

7. Analysis of marketed formulation by developed method:
Take Tablet powder equivalent to 200 mg of Capivasertib, was transferred to a 100 ml volumetric flask. Take 01 ml 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 above Solution 20 µL for Assay Analysis. Assay was found to be 100.1 % label claim.

B. FORCED DEGRADATION STUDIES:
❖ **Preparation of Capivasertib Sample Stock Solution :-**
Accurately weigh 44 mg of Capivasertib sample and transferred into a 10 ml volumetric flask and make up to the mark as final volume with diluent.

➢ **1. Acid Degradation**
0.1 N Degradation
Take 1 ml of sample into a 10 ml volumetric flask and add 1ml of 0.1N HCl. Reflux at 50 °C for 02 hrs. Leave it for 15 min. After 15 min add 1 ml of 0.1N NaOH to neutralize the solution and diluted to volume with diluent and mixed.

1 N Degradation
Take 1 ml of sample into a 10 ml volumetric flask and add 1ml of 1N HCl. Reflux at 60 °C for 02 hrs. Leave it for 15 min. After 15 min add 1 ml of 1N NaOH to neutralize the solution and diluted to volume with diluent and mixed.
DP - 1 was observed in chromatogram in Acid Degradation.

➢ **2. Alkali Degradation:**
0.1 N Degradation
Take 1 ml of sample into a 10 ml volumetric flask and add 1 ml of 0.1N NaOH at 50 °C for 02 hrs. Leave it for 15 min. After 15 min add 1ml of 0.1N HCl to neutralize the solution and diluted to volume with diluent and mixed.

1 N Degradation
Take 1 ml of sample into a 10 ml volumetric flask and add 1ml of 1N NaOH. Reflux at 60 °C for 02 hrs. Leave it for 15 min. After 15 min add 1 ml of 1N HCl to neutralize the solution and diluted to volume with diluent and mixed.
DP - 2 was observed in chromatogram in Alkali Degradation.

➢ **3. Peroxide Degradation**
3 % Peroxide Degradation
Take 1 ml of sample into a 10 ml volumetric flask and add 1 ml of 3% Hydrogen Peroxide. Leave it for 15 min. After it is diluted to volume with diluent and mixed.

10 % Peroxide Degradation
Take 1 ml of sample into a 10 ml volumetric flask and add 1 ml of 10% Hydrogen Peroxide. Leave it for 15 min. After it is diluted to volume with diluent and mixed.
DP - 3 was observed in chromatogram in Peroxide Degradation.

➢ **4. Thermal Degradation**
105 °C for 3 hrs Degradation
50 mg of sample was exposed at 105 °C for 3 hrs and the exposed sample was analyzed. 20 mg of sample was transferred into 100 ml volumetric flask and make up to the mark. Further dilute 1 ml to 10 ml with diluent.
DP - 4 was observed in chromatogram in Thermal Degradation.

➢ **5. Reduction Degradation**

3% Sodium bisulfate Degradation

Take 1 ml of sample into a 10 ml volumetric flask and add 1 ml of 3% Sodium bisulfate solution. Reflux at 60 °C for 02 hrs. Leave it for 15 min. After 15 min diluted to volume with diluent and mixed.

10% Sodium bisulfate Degradation

Take 1 ml of sample into a 10 ml volumetric flask and add 1 ml of 10% Sodium bisulfate solution. Reflux at 60 °C for 02 hrs. Leave it for 15 min. After 15 min diluted to volume with diluent and mixed.

➢ **6. Neutral Hydrolysis Degradation**

Take 1 ml of sample into a 10 ml volumetric flask and add 1 ml of HPLC water. Reflux at 80 °C for 02 hrs. Leave it for 15 min. After 15 min diluted to volume with diluent and mixed.
Take 1 ml of sample into a 10 ml volumetric flask and add 3 ml of HPLC water. Reflux at 90 °C for 05 hrs. Leave it for 15 min. After 15 min diluted to volume with diluent and mixed.

❖ In Acid degradation DP - 1, In Alkali degradation DP – 2, In Peroxide degradation DP - 3 and In Thermal degradation DP – 4 were identified. Overall Degradation Conditions with Percentage of Degradation were mentioned 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 managed in positive ion electrospray ionization interface mode. Multiple reactions monitoring mode has been applied to quantify the Capivasertib. Working parameters have been set as follows:

➢ Collision energy: 15 V , Ion spray voltage: 5500 V
➢ Source temperature: 550 ^{o}C , Drying gas temperature: 120-250 °C
➢ Collision gas: nitrogen, Drying gas flow stream: 5 L/min
➢ Declustering 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 Capivasertib in its pharmaceutical dosage form (Tablet).
❖ 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 Capivasertib in its pharmaceutical dosage form (Tablet) without any interference.
❖ The degradation actions of the drugs were examined under acidic, alkali, peroxide, reduction, hydrolysis and thermal stress conditions. The drug was found to be stable in hydrolysis and reduction and unstable in acidic, alkali, peroxide, and thermal 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.

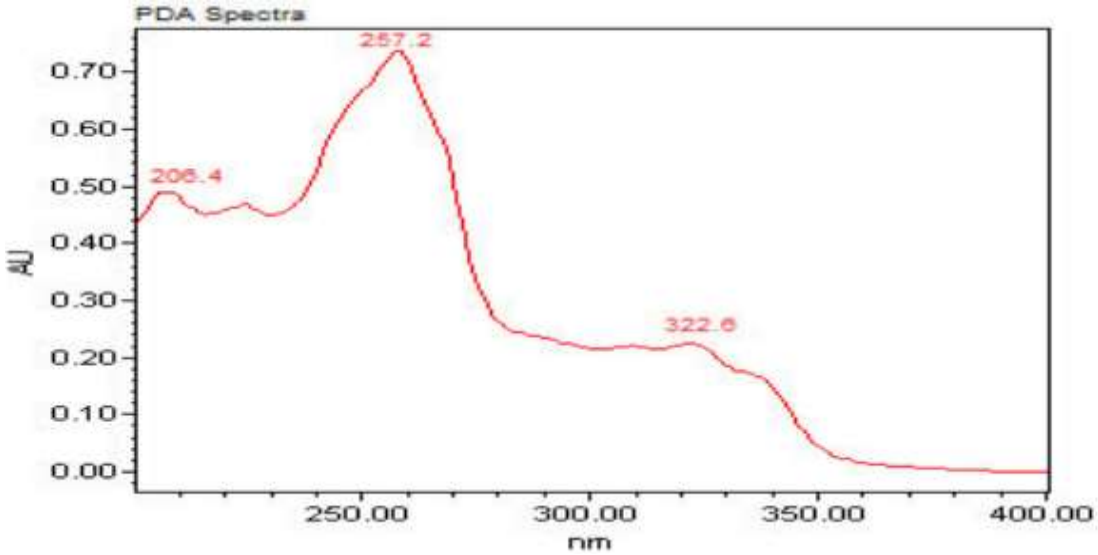


Figure 1: UV Spectra of Capivasertib (200 µg/ml) in Methanol

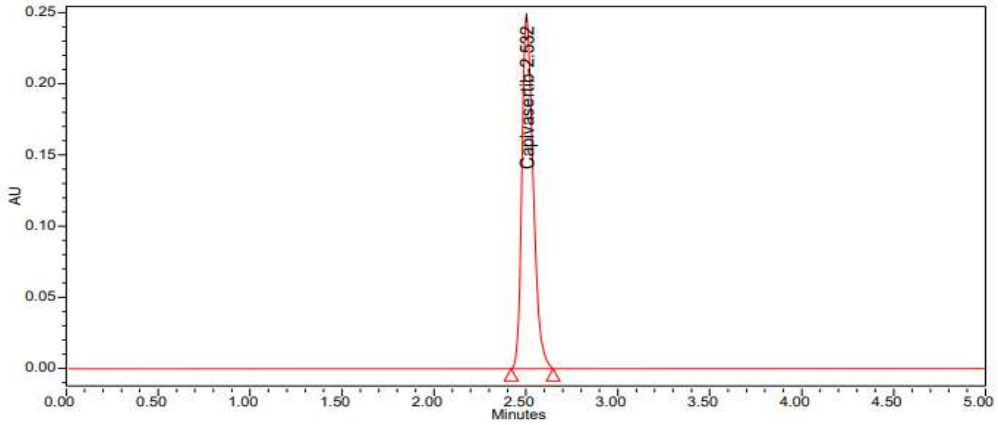


Figure 2: Optimized Trial / Acetonitrile: 0.1% Triethyl Amine (50:50 % v/v) pH, 2.5 /Formic acid

Table 1: Chromatographic Conditions

Column	**Kromasil C18 (150 mm x 4.6 mm, 5 µm)**
Mode of Elution	Isocratic
Mobile phase	Acetonitrile : 0.1% Triethyl Amine (50:50 % v/v) pH, 2.5 / Formic acid
Detection Wavelength	257 nm
Injection volume	10 µL
Flow rate	1.2 ml/min
Column Temperature	25°C
Run time	06 minutes
Retention Time	2.532

Theoretical Plates	6012
USP Tailing	0.91

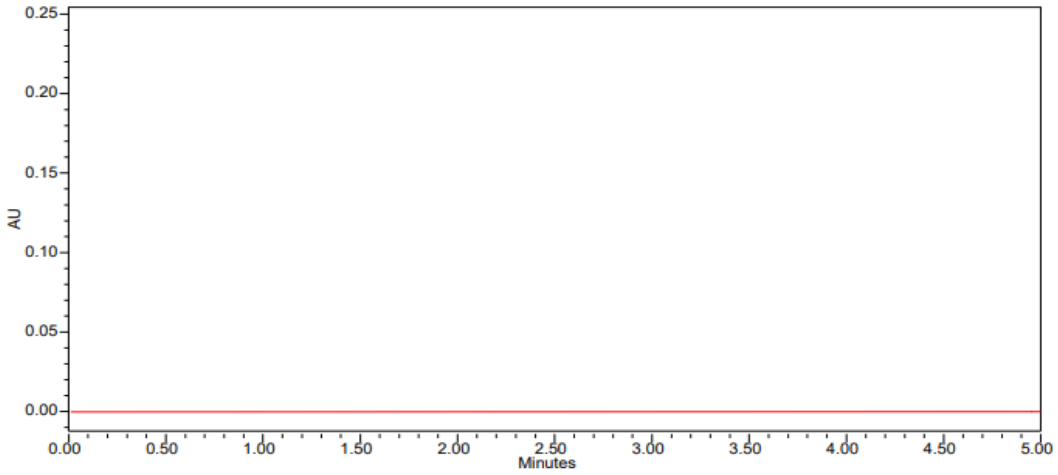


Figure 3: Chromatogram of Capivasertib Blank

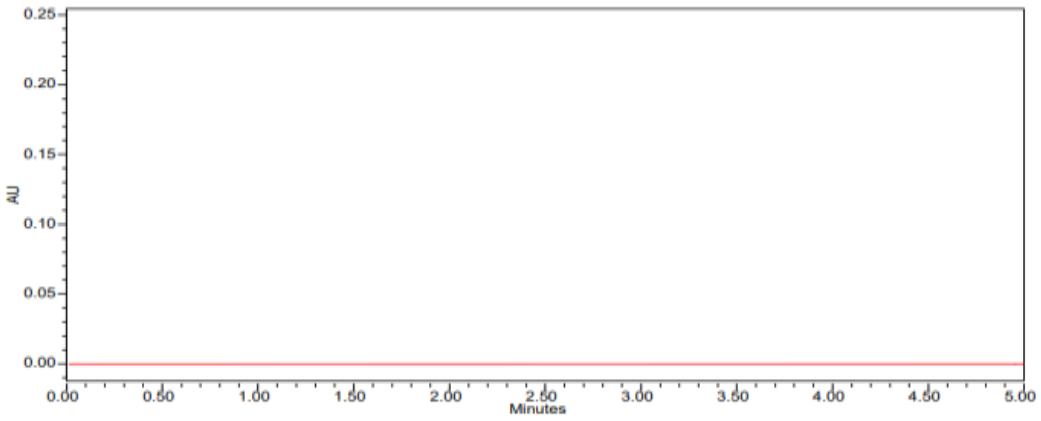


Figure 4: Chromatogram of Capivasertib Placebo

Table 2: Linearity data for Capivasertib

Sr. No	Concentration (µg/ml)	Area
1	50.00	589134
2	100.00	1178227
3	150.00	1767340
4	200.00	2356454
5	250.00	2845258
6	300.00	3434392

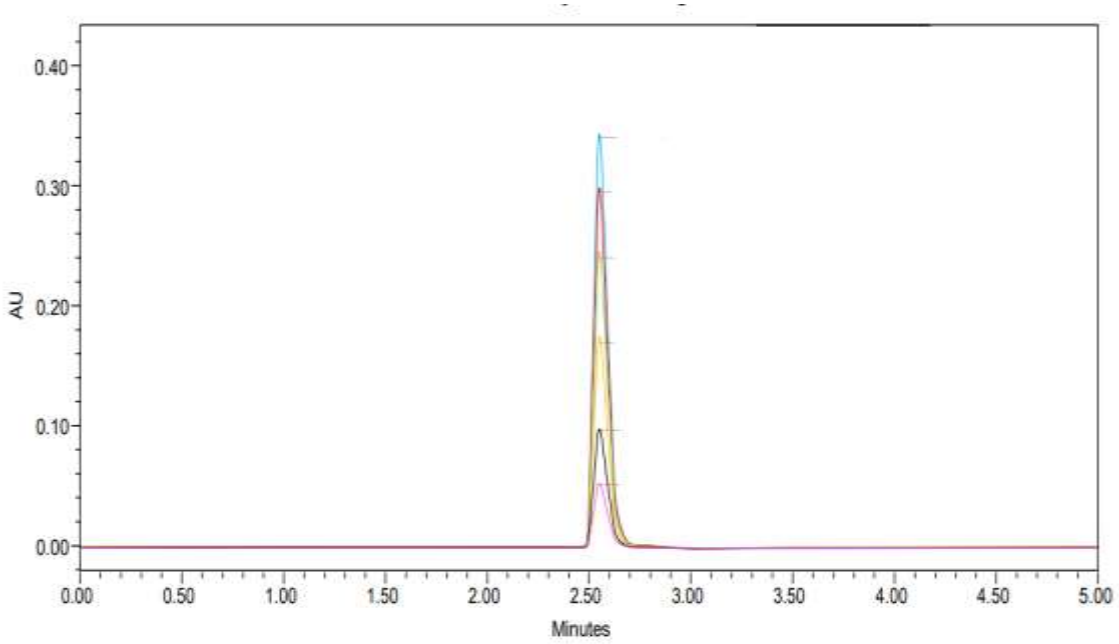


Figure 5: Overlay chromatogram of different concentrations of mixtures of Capivasertib

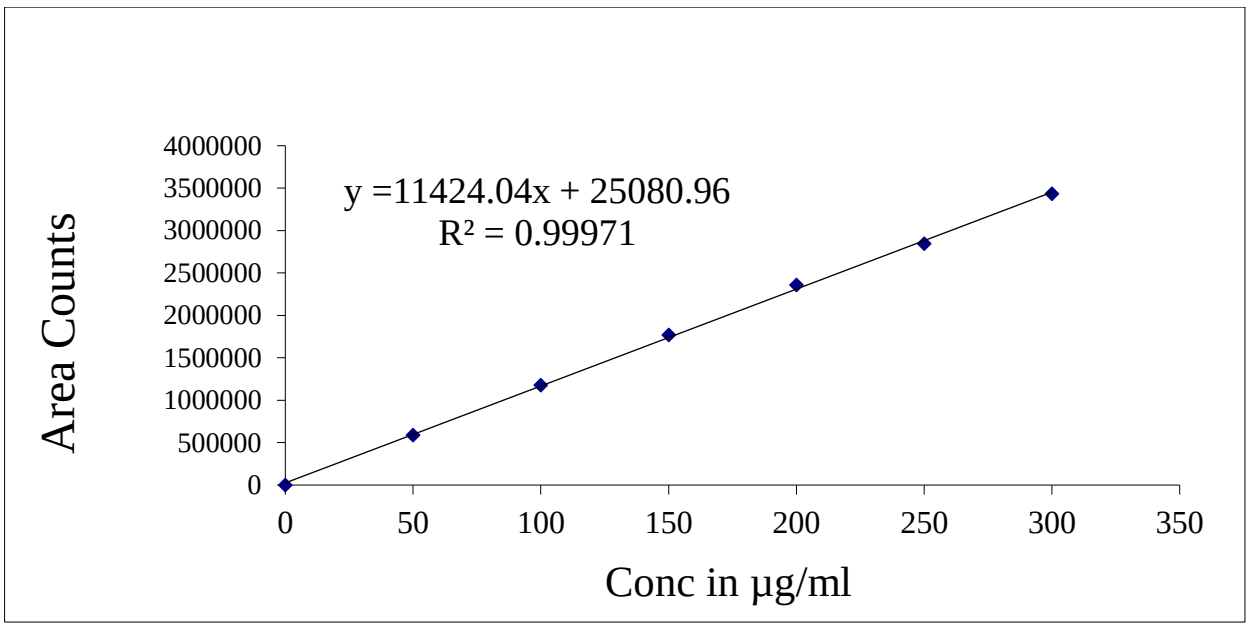


Figure 6: Calibration Curve of Capivasertib (50 – 300 µg /ml)

Table 3: Repeatability/ Method Precision

Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	200	2359764	99.9 ± 0.557	0.56
		2326437		
		2337466		
		2347229		
		2350401		
		2361015		

Table 4: Intermediate Precision

Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	200	2357642	100.0 ± 0.403	0.40
		2332674		
		2347145		
		2350219		
		2342113		
		2359001		

Table 5: Recovery data for Capivasertib

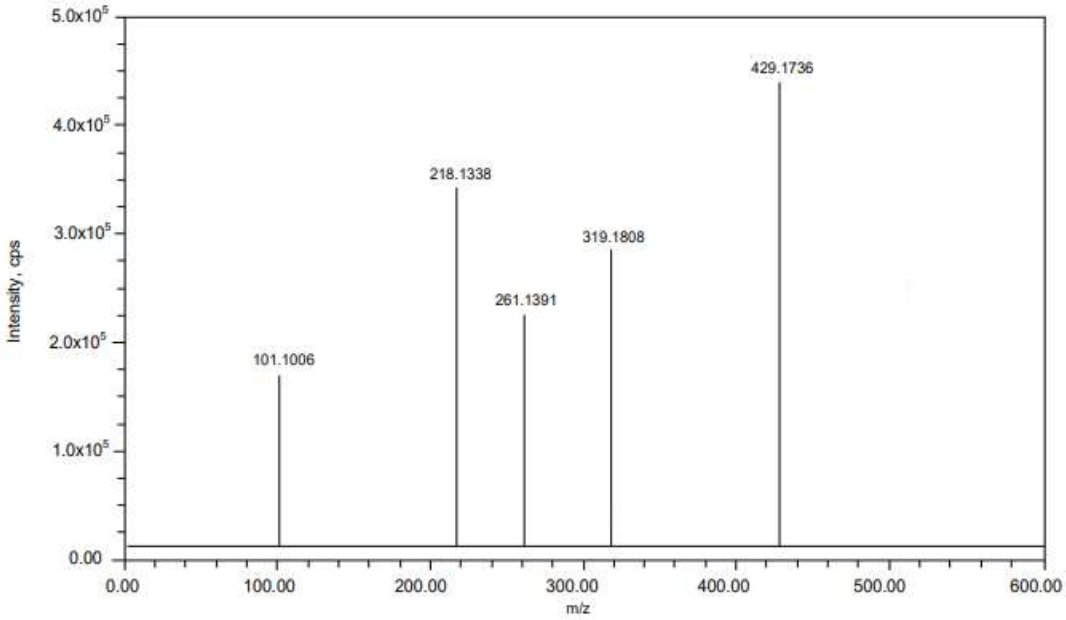
SR. NO.	Conc. Level (%)	Sample + Amount of API (mg)	Actual API Added (mg)	Amount recovered (mg)	% Recovery	% Mean Recovery ± S.D
1	50 %	10	10	10.07	100.7	100.6 ± 0.10
2		10	10	10.06	100.6	
3		10	10	10.05	100.5	
4	100 %	20	20	20.14	100.7	100.2 ± 0.53
5		20	20	19.93	99.7	
6		20	20	20.03	100.2	
7	150 %	30	30	30.22	100.7	100.3 ± 0.52
8		30	30	29.91	99.7	
9		30	30	30.1	100.3	

Table 6: Robustness data for Capivasertib

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 (+0.2)	Area at pH (-0.2)
1	2048799	2543316	1937404	2751643	2235694	2458569
2	2069711	2560849	1951473	2729516	2251203	2455846
3	2071093	2587878	1940212	2741559	2243275	2461284
% R.S.D	0.61	0.91	0.38	0.40	0.30	0.10

Table 7: Forced Degradation Data

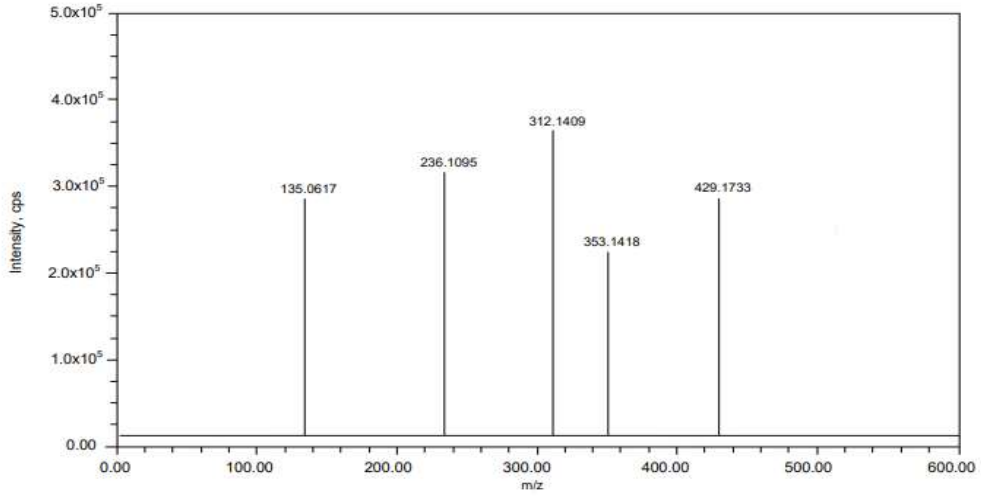
Sample Condition	% Label Claim	% Degradation	Purity Angle	Purity Threshold
Control	**100**	**0.0**	**0.512**	**8.971**
Acid – 1 N	87.5	12.5	0.567	8.942
Alkali - 1 N	86.2	13.8	0.533	8.983
Peroxide – 10 %	85.0	15.0	0.548	8.955
Reduction – 10%	95.4	4.6	0.591	8.912
Thermal - 3 hrs	89.8	10.2	0.542	8.957
Hydrolysis – 3 ml water	96.6	3.4	0.555	8.967



Multiple Reaction Monitoring-MRM of the Capivasertib using PositivePolarity

Analogue	Precursor Ion(m/z)	Daughter Ion with the Highest Intensity (m/z)
Capivasertib	429.1736	218.1338

Figure 7: Capivasertib MS Spectra



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

Analogue	Precursor Ion(m/z)	Daughter Ion with the Highest Intensity (m/z)
Capivasertib DP-1	429.1733	312.1409

Figure 8: DP – 1 MS Spectra

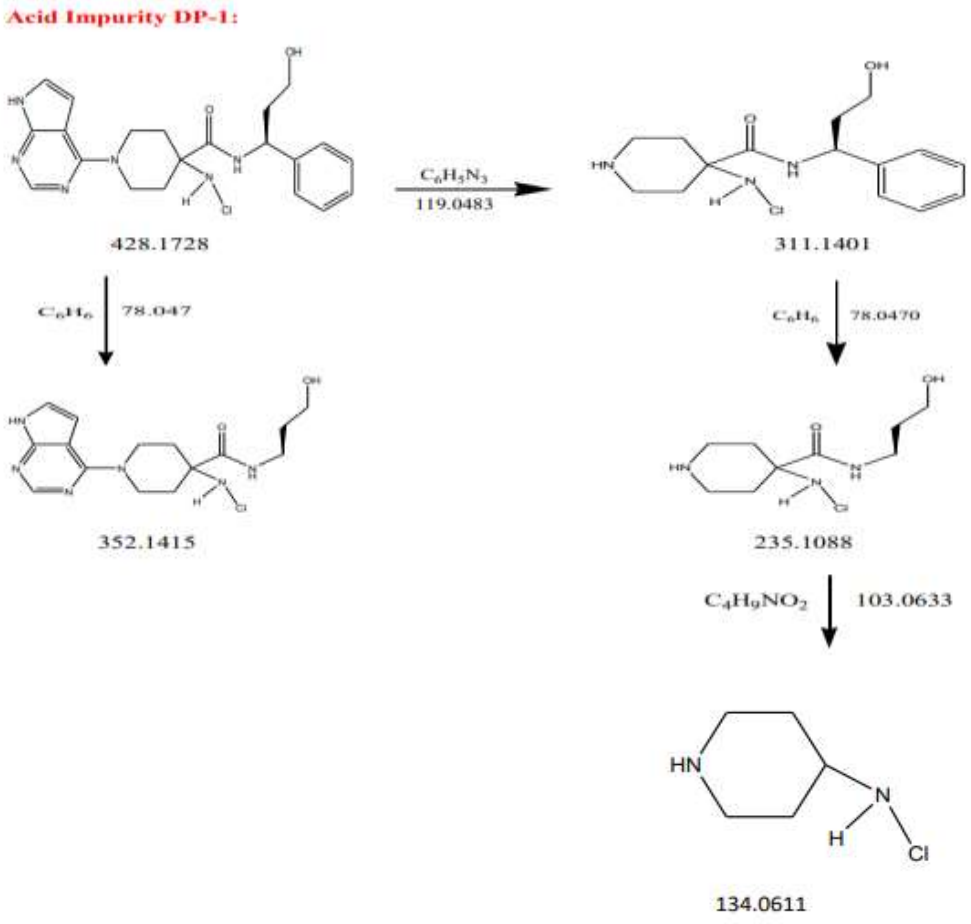
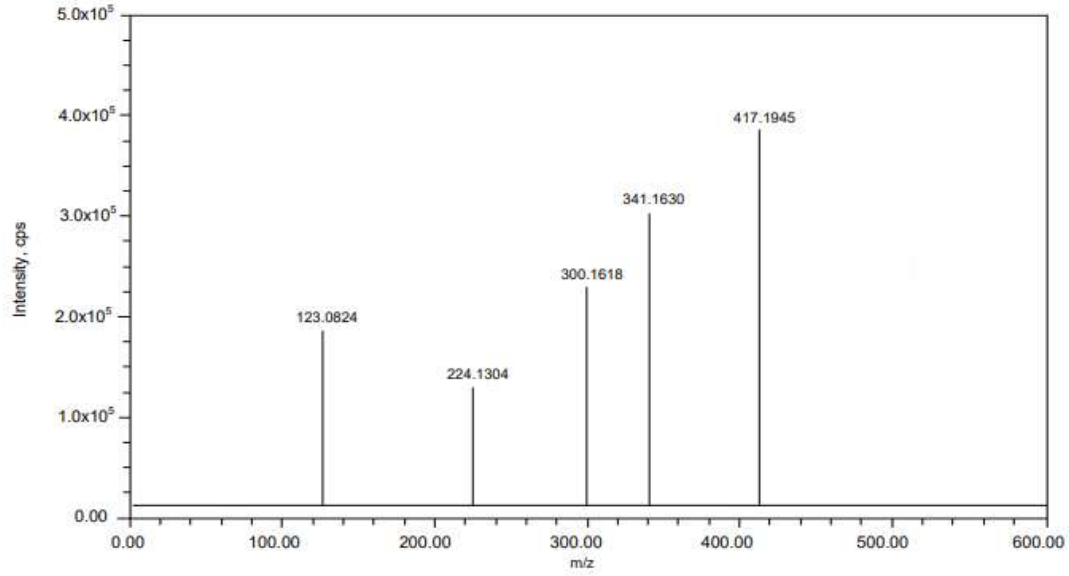


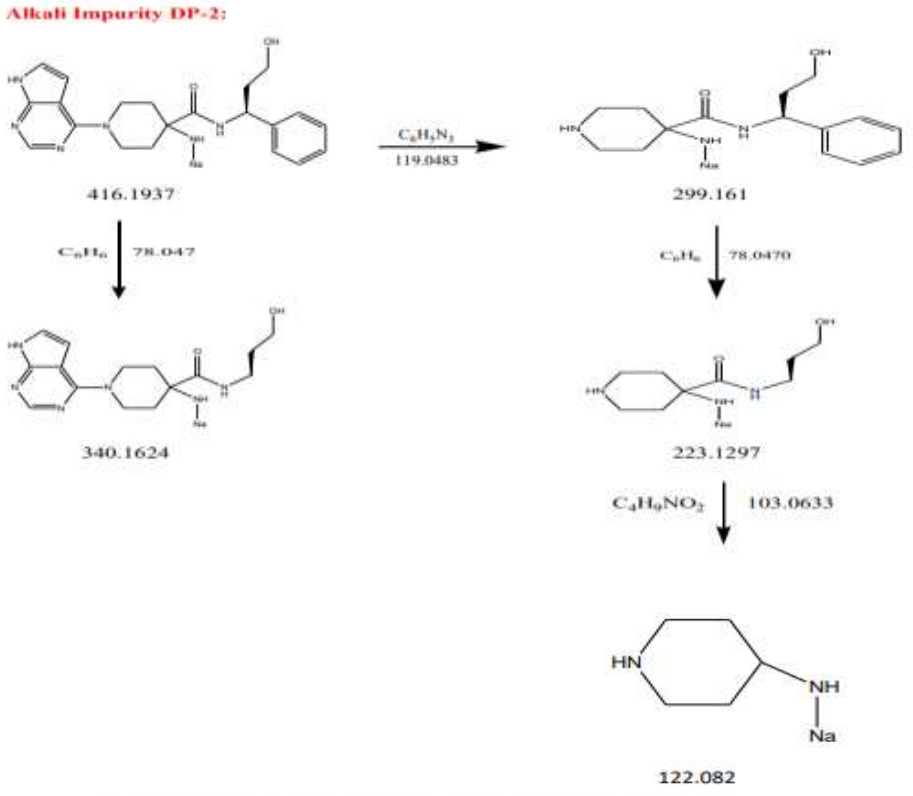
Figure 9: Fragmentation Mechanism of DP – 1 Possible Pathway



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

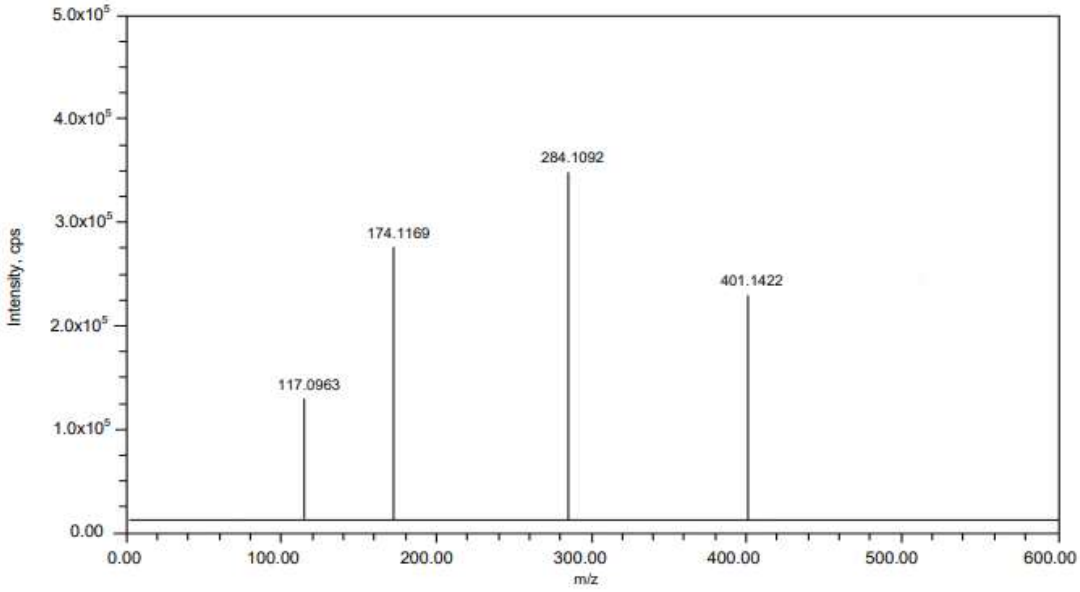
Analogue	Precursor Ion(m/z)	Daughter Ion with the Highest Intensity (m/z)
Capivasertib DP-2	417.1945	341.1630

Figure 10: DP – 2 MS Spectra



DP-2: 123.0824, 224.1304, 300.1618, 341.1630, 417.1945

Figure 11: Fragmentation Mechanism of DP – 2 Possible Pathway



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

Analogue	Precursor Ion(m/z)	Daughter Ion with the Highest Intensity (m/z)
Capivasertib DP-3	401.1422	284.1092

Figure 12: DP – 3 MS Spectra

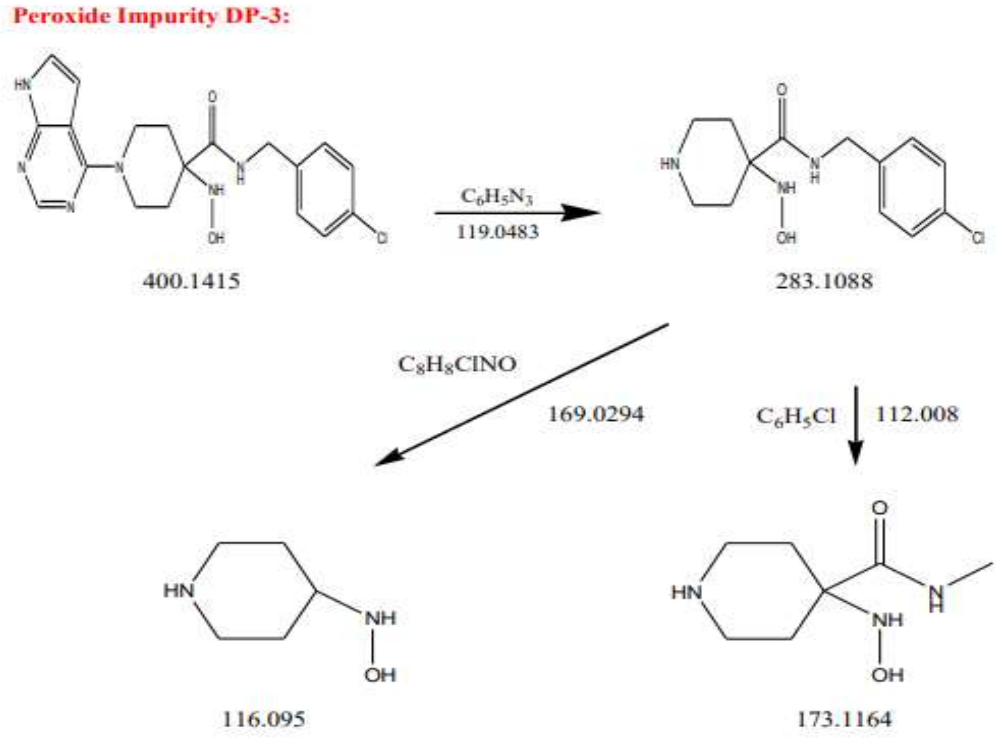
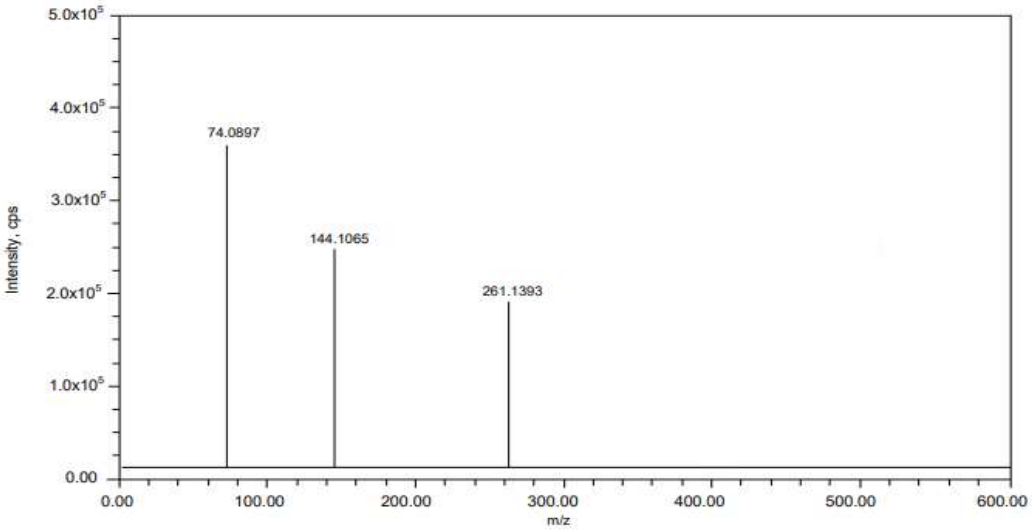


Figure 13: Fragmentation Mechanism of DP – 3 Possible Pathway



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

Analogue	Precursor Ion(m/z)	Daughter Ion with the Highest Intensity (m/z)
Capivasertib DP-4	261.1393	74.0897

Figure 14: DP – 4 MS Spectra

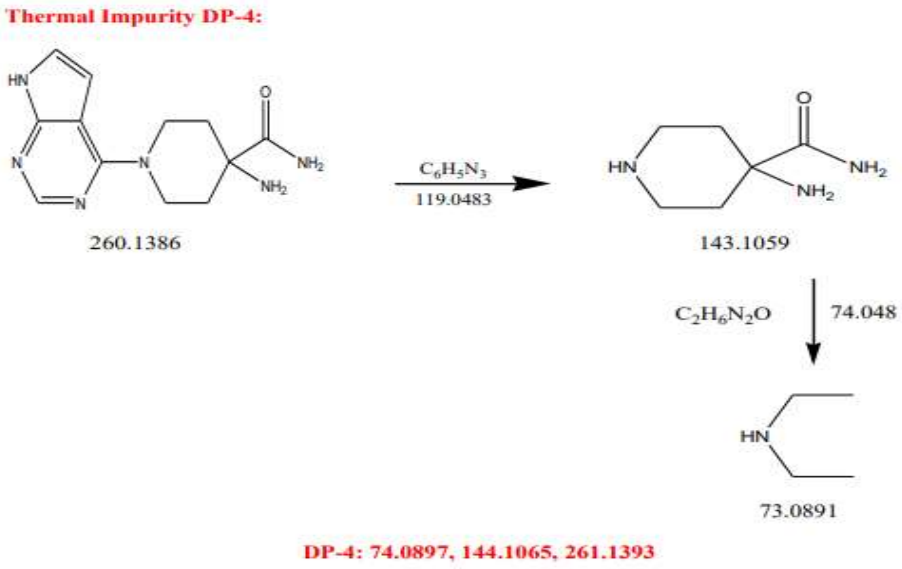


Figure 15: Fragmentation Mechanism of DP – 4 Possible Pathway

References

1. Cancer, "World Health Organization", Accessed Feb. 16, 2021. https://www.who.int/news-room/fact-sheets/detail/cancer
2. Tripathi KD., "Essentials of Medical Pharmacology", 7th Edition, Jaypee Brothers Medical Publishers (P) Ltd, New Delhi 110 002, India, 2013, pp 857-878.
3. ICH Guidelines Topic Q3A (R2): Impurities in new Drug Substances; October 2006 https://database.ich.org/sites/default/files/Q3A%28R2%29%20Guideline.pdf
4. Drug profile for Capivasertib: https://go.drugbank.com/drugs/DB12218
5. AstraZeneca "Truqap'', 17 Nov 2023: https://www.astrazeneca.com/
6. Willard HH, Merritt LL, Dean JA, Settle FA, Instrumental Methods of Analysis, 7thEdition, CBS Publishers & Distributors, New Delhi – 110002, India, 1988, pp 160 -178
7. Chatwal GR, Anand SK, Instrumental Methods of Chemical Analysis, 5th Edition, Himalaya Publishing House, New Delhi – 110003, India, 2007, pp 2.624-2.654
8. ICH guidelines, Q2 (R1): Validation of Analytical Procedures; Text and Methodology, Nov 2005, pp 1-13.
9. ICH guidelines, Q2 (R2): Validation of Analytical Procedures; Text and Methodology, Draft Version 24 Mar 2022, pp 1-15.
10. Gaur B., Sahu S. "Development and Validation of a Novel Stability-Indicating Reverse Phase High-Performance Liquid Chromatography Method for the Quantification of Capivasertib in Bulk Drug and Pharmaceutical Dosage Form", *International journal of pharmaceutical quality assurance and pharmaceutical analysis*, **2024**, Vol 15, Issue 2, 639 - 644.
11. Zhang Z, Nong L, Chen ML, Liu MH, Cheng WW. "A validated LC-MS/MS method for the quantification of Capivasertib in dog plasma: Application to its pharmacokinetics study." *Biomedical Chromatogr*aphy, **2020**, Oct, 34 (10), 195-202.
12. Qiu F., Norwood D., "Identification of Pharmaceutical Impurities", *Journal of Liquid Chromatography & Related Technologies*, **2007;** 30(5-7), 877–935
13. Blessy M. Patel R., "Development of forced degradation and stability indicating studies of drugs - A review", *Journal of Pharmaceutical Analysis, **2014**;* 4(3); 159-165.
14. Patel R., Patel P., "Stability Indicating HPLC Method Development – A Review", *INTERNATIONAL RESEARCH JOURNAL OF PHARMACY, **2011**;* 2(5); 79-87.
15. YULIANITA R., SOPYAN I., "FORCED DEGRADATION STUDY OF STATINS: A REVIEW", *International of Applied Pharmaceutics*, **2018;** 10(6), 38–42.
16. Singh S., Bakshi M., "Guidance on conduct of Stress tests to determine Inherent stability of drugs", *Pharmaceutical Technology On-Line*, **2000;** 1-14