LC–MS/MS Profiling of Stress-Induced Degradation Products of Avapritinib and Development of a Stability-Indicating HPLC Method for Its Quantification and Impurity Analysis

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The stability and purity of pharmaceutical compounds are critical for ensuring drug efficacy and patient safety. Avapritinib, a tyrosine kinase inhibitor used to treat gastrointestinal stromal tumors and other cancers, requires a robust analytical method for its quantification and stability assessment. This study presents a validated stability-indicating high-performance liquid chromatography (HPLC) method for the separation, identification, and quantification of avapritinib and its impurities under various stress conditions. The method utilizes an X-Bridge C18 (250 mm × 4.6 mm, 5 µm) column with a mobile phase composed of acetonitrile and a methanol–phosphate buffer mixture (pH 3.6, 0.01M) in a 70:30 v/v ratio, employing a linear gradient elution at 0.9 mL/min with UV detection at 251 nm. The method exhibited high selectivity and resolution (Rs > 2), excellent linearity (r^2 > 0.999) within 25–150 µg/mL for avapritinib and 0.25–1.5 µg/mL for its impurities, and precision with RSD% below 2%. Recovery studies confirmed accuracy (99.58% for avapritinib, 99.72–100.17% for impurities). Forced degradation studies identified significant degradation under acidic and photolytic conditions, leading to the formation of distinct degradation products (ADP 1, ADP 2, PDP 1, PDP 2, and PDP 3). Structural elucidation via mass spectrometry revealed degradation pathways, with ADP products forming under acidic conditions and PDP products under photolytic stress. The developed method provides a reliable tool for avapritinib quantification and stability assessment, offering critical insights into its degradation behavior for improved therapeutic safety and efficacy.

Keywords: avapritinib, degradation products, mass spectrometry, fragmentation

Introduction

Avapritinib (Figure S1) is a potent drug that belongs to the selective tyrosine kinase inhibitor class, which was primarily used to treat advanced systemic mastocytosis and gastrointestinal stromal tumors [1]. It works by targeting mutations in KIT and platelet-derived growth factor receptor genes that show resistance to conventional therapies. In precision-targeted therapy, it reduces tumor progression by inhibiting aberrant kinase signaling [2]. It is also prescribed for adult patients who are suffering from indolent systemic mastocytosis [3]. The regular side effects associated with the use of avapritinib include nausea, fatigue, edema, increased lacrimation, decreased appetite, vomiting, rash, dizziness, and constipation. The possible side effects include fatigue/asthenia, abdominal pain, cognitive impairment, edema, nausea, vomiting, decreased appetite, hair color changes, diarrhea, constipation, rash, dizziness, and increased secretion of tears.

The therapeutic significance such as efficacy and safety of avapritinib necessitates a thorough understanding of its stability and degradation behaviour under various stress conditions. The forced degradation studies, which are also known as stress testing, provide significant insights into the intrinsic stability of avapritinib and identify potential DPs. In these studies, pure avapritinib was exposed to different stress conditions to simulate potential DPs and evaluate the possible degradation pathways during manufacturing, storage, and administration [4,5]. The identification/characterization of DPs helps to optimize formulation strategies, select appropriate excipients and packaging material, and establish proper storage conditions to enhance the shelf life of drug products [6]. Additionally, some DPs may be

toxic or pharmacologically active and these DPs need to evaluate their toxicological risk to prevent adverse effects [7].

The HPLC coupled with mass spectrometry (LC–MS/MS) is a powerful analytical technique that was widely utilized for the identification and characterization of DPs [8]. LC–MS/MS provides structural determination of unknown compounds and helps to understand the fragmentation patterns of drugs. In addition, the optimization of a stability-indicating HPLC method is essential for routine quality control analysis to ensure accurate quantification of the drug, its impurities and DPs [9,10].

In literature, estimation in pharmaceutical formulation by using HPLC [11-15], UPLC-MS/MS determination in rat plasma [16], LC-MS/MS analysis in rat plasma [17], clinical studies of avapritinib [18], and SAR studies [19]. The literature reported several studies on the analytical profiling of avapritinib but no literature is available on the comprehensive characterization of its DPs. This research aimed to bridge this gap by employing LC–MS/MS for structural elucidation of stress-induced DPs of avapritinib. Additionally, this study intended to propose a robust stability-indicating HPLC method by following ICH guidelines to ensure the reliable quantification of avapritinib and its known impurities in pharmaceutical formulations. The key objectives of this study are to establish a stable analytical method capable of distinguishing avapritinib from its known impurities and degradation products with high sensitivity and selectivity. Additionally, the developed HPLC method will be validated according to ICH guidelines regarding specificity, linearity, precision, accuracy, robustness, and stability. The study will also subject avapritinib to various stress conditions to determine its degradation behavior, and major degradation products will be identified and elucidated using tandem mass spectrometry.

This study is significant from both regulatory and pharmaceutical perspectives. The comprehensive degradation profiling of avapritinib will aid in better understanding its stability. The developed stability-indicating HPLC method will serve as a reliable analytical tool for analysis of avapritinib and its known impurities in the study. Based on availability, the impurities 1, 2, and 3 of avapritinib (Figure S2) were used in this study.

Experimental part

Reagents. The pure form of avapritinib along with its impurities was obtained from Blueprint Medicines Corporation as a gift sample. Methanol, acetonitrile, water, LR and AR grade chemicals like NaOH, HCl, H_2O_2, and buffer salts, all HPLC grade chemicals are procured from Qualigens Fine Chemicals, Mumbai, Maharashtra, India.

Equipment. The Alliance (Japan) HPLC system equipped with a binary pump (2695), online degasser, injector with fixed volume loop (20 µL), and UV detector was utilized to perform LC analysis. LC–MS/MS analysis was performed using an Alliance LC system (Waters, Japan) coupled to a MicrOTOF-Q mass spectrometer made by Bruker Daltonics, Bremen, Germany. The LC system consisted of a binary pump with online degasser, autosampler, column oven, and UV detector. Data processing was done using MassLynx software. The mass analyser was operated in positive ESI mode with an m/z range of 10–1000. In addition, a GT Sonic sonicator (India) was used for the preparation of samples.

Preparation of standard solution

The 1000 µg/mL stock solution was prepared by dissolving an equivalent weight of 50 mg of pure form in a 50 mL flask having 25 mL of methanol, mixed thoroughly, and sonicated the solution for 3 mins for complete solubility of the drug. After the sonication solution was filtered through a 0.22µ µm nylon membrane filter paper and then made up to the mark, resulting in a solution of 1000 µg/mL, this solution is stored and used for further. In the same pattern, impurities are prepared, and a 1% solution of impurity was mixed with standard and used for analysis.

Stress studied samples

The stress degradation behavior of avapritinib was evaluated according to stress conditions set for ICH Q1A (R2). The stress studies conducted on the drug were exposure to hydrolysis, oxidation, dry heat, and photolysis conditions. Optimizations of stress conditions were done such that the results will have achieved 10-15% degradation of the drug substance. All the analysis utilized HPLC with a detector at 251 nm while characterizing the same products with the LC-MS/TOF. The hydrolytic degradation was done by heating 1 mL of 1N HCl (acid), 1N NaOH (base), and water (aqueous) with 1 mL of drug solution at 80°C for 12 hours separately. After exposure, acid-treated samples were neutralized with an equivalent strength of base, and vice versa. In oxidative degradation, 1 mL of the drug solution with a concentration of 1000 µg/mL was left for 24 hours with 1 mL of 10% H_2O_2 at room temperature. In thermal degradation, the solid drug was heated in an oven at 80°C for 7 days in a sealed glass ampoule while a control sample was kept at room temperature. The photostability of both the solid and solution state drug samples was studied. A solution of avapritinib at 1000 µg/mL, and the solid drug was subjected to 1.25 million lux hours of fluorescent light and 200 Wh/m² of UV light with control samples protected by aluminum foil. Photolytic conditions for the solution state showed the degradation of the drug, while for the solid state, the drug was stable. Samples were subsequently diluted to reach 100 µg/mL concentration of the avapritinib and 1% for the impurities with a mixture of solvent A and B in 70:30 v/v at pH 3.6 in preparation for the HPLC analysis.

Optimization of HPLC method for avapritinib and impurities

To optimize a simple and economical LC method, several attempts were made with solvents A and B in 70:30 v/v, solvent A is acetonitrile and solvent B is the combination of methanol, and 0.01M phosphate buffer (pH 3.6) in 50:20 v/v system as mobile phase solvents with X-bridge (250 × 4.6 mm; 5 µm particle size) and 251 nm detector wavelength. The optimization was first carried out on the individual samples of avapritinib and impurities and then on their mixture. The specificity of the method was checked by peak purity and resolution for impurities and avapritinib [20]. The linearity was established using solutions prepared from a stock solution at six concentration levels (25–150 µg/mL) for avapritinib and 0.25-1.5 µg/mL for the studied impurities. The triplicate of linearity solutions was analysed and the peak area versus concentration was processed using least squares linear regression analysis to determine the correlation coefficient to test accuracy. The sample mixture of avapritinib and its impurities spiked with three known concentrations, each of 75, 100, and 125 µg/mL for avapritinib and 0.75, 1, 1.25 µg/mL for impurities were analyzed in three replicates, and the % recovery results were calculated to evaluate method accuracy. The precision and reproducibility within a single day and in multiple days were proved by analysing avapritinib and its impurities at a 100 % recovery level concentration solution.

LC-MS/TOF Fragmentation Analysis of avapritinib

The breakdown pattern of avapritinib was analysed through LC-MS/TOF. The mass spectra were recorded within 10–1000 m/z range using positive ESI mode. The methanolic solution of avapritinib at 100 µg/mL having 1% known impurities and its stress degradation sample solutions were directly injected through a syringe pump into the MS/TOF system. The mass analyser parameters were adjusted to achieve the molecular ion peak and further optimization of mass fragment parameters brought characteristic fragment ions [21-22]. All the mass peaks recorded were given as fourth-decimal accuracy. The stressed sample mixture was further analysed for LC-MS/TOF using the same optimized conditions as that for avapritinib. An analytical gradient elution method was applied during analysis, and the fragmentation pattern of each degradant was obtained from the mass spectra and accurate m/z values.

Results and discussions

The chromatographic separation was accomplished by using an X-bridge C18 column (250 mm × 4.6 mm; 5 µm), equipped with the linear gradient elution system set at 0.9 mL/min, UV detection at 251 nm. The mobile phase used was a combination of solvent A and B in 70:30 v/v, solvent A is acetonitrile, and solvent B is the combination of methanol, and 0.01M phosphate buffer (pH 3.6) in 50:20 v/v, and the same concentration was used for the dilution process. The gradient program comprises of times/%B as 0-2 mins (10%), then 2.1 min - 5 mins (25%), 5.1 min-7 mins (35%), 7.1-8 min (25%) and 8.1-11 mins (10%). The specificity, selectivity, linearity, accuracy, and precision of the proposed method were validated. The standard chromatogram is given in Figure 1.

The standard and stress degradation solution in the optimized method shows high selectivity and stability-indicating capability because all degradant peaks appear to be adequately resolved. The resolution (Rs) of the peaks was greater than 2 with permissible symmetry and system suitability. The response of avapritinib was linear in 25 to 150 µg/mL range for avapritinib and 0.25 to 1.5 µg/mL for its impurities with correlation coefficient of higher than 0.999 (Table 1). The precision studies were carried out in terms of intra- and inter-day studies using 100 % level solution of avapritinib and its impurities. The achieved results are summarized in Table 1 with RSD% values of below 2% providing the high precision of the method. The method accuracy was assessed via recovery studies using the standard addition method, where formulation samples were spiked with known concentrations of avapritinib at three levels. The mean recovery was found to be 99.58% for avapritinib, 100.17% for impurity 1, 99.72% for impurity 2, and 99.74% for impurity 3 demonstrates the method's accuracy (Table 2).

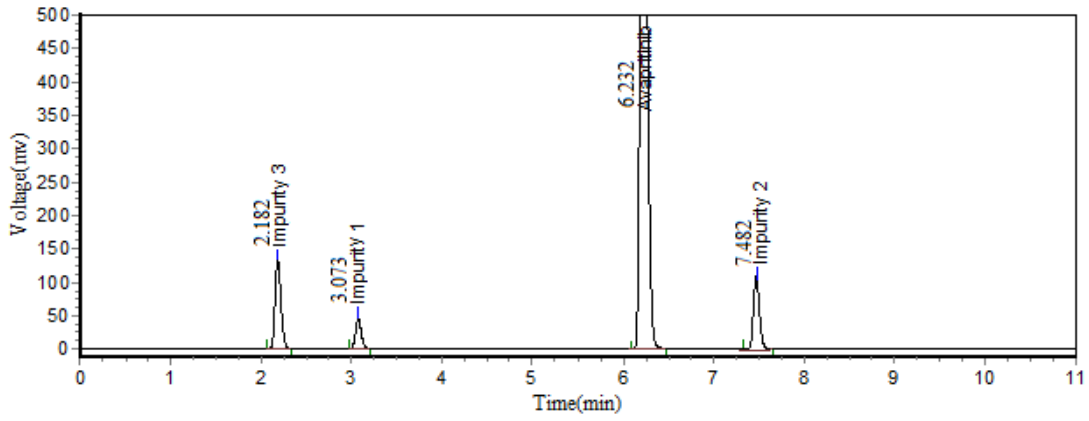

Figure 1. Standard chromatogram of avapritinib and its impurities.

Table 1. Precision study of avapritinib and its studied impurities.

Parameter	Avapritinib	Impurity 1	Impurity 2	Impurity 3
Linearity range (µg/mL)	25-150	0.25-1.5	0.25-1.5	0.25-1.5
Regression equation	y = 9861.7x + 654.17	y = 33702x + 742.95	y = 55879x + 269.41	y = 77448x + 1663.8
Correlation coefficient	0.9999	0.9994	0.9999	0.9984
Intraday precision (n=6)				
Mean Area value	977080.2	33911.4	55652.3	77452.4
SD	1696.32	110.47	157.64	185.08
%RSD	0.174	0.326	0.283	0.239
Interday precision (Day 1)				
Mean Area value (n=3)	974418.5	34046.9	55837.6	77606.7
SD	2596.45	122.83	95.10	312.76
%RSD	0.266	0.361	0.170	0.403
Interday precision (Day 2)				
Mean Area value (n=3)	976705.9	34034.3	55764.4	77401.8
SD	1317.52	195.16	209.98	164.45
%RSD	0.135	0.573	0.377	0.212
Ruggedness (Analyst 1)				
Mean Area value (n=3)	976019.0	34038.5	55859.1	77525.8
SD	2854.83	81.77	253.45	47.49
%RSD	0.292	0.240	0.454	0.061
Ruggedness (Analyst 2)				
Mean Area value (n=3)	976657.3	33920.0	55764.7	77464.1
SD	1976.30	87.58	113.50	191.52
%RSD	0.202	0.258	0.204	0.247

Table 2. Recovery results of avapritinib and its studied impurities.

Parameter	Injected concentration (µg/mL)	Estimated concentration (µg/mL) mean±SD	% Recovery	RSD (%)
Avapritinib	75	74.89±0.46	99.85	0.617
	100	99.43±0.44	99.43	0.443
	125	124.3±0.8	99.47	0.639
Impurity 1	0.750	0.750±0.004	100.26	0.563
	1.000	1.01±0.01	100.66	1.124
	1.250	1.24±0.01	99.58	0.855
Impurity 2	0.750	0.750±0.006	99.42	0.812
	1.000	1.000±0.004	99.82	0.363
	1.250	1.250±0.004	99.91	0.296
Impurity 3	0.750	0.750±0.003	99.42	0.456
	1.000	0.99±0.01	99.45	1.149
	1.250	1.25±0.01	100.36	0.589

Stress degradation study

The degradation through acidic and photo stress was apparent for avapritinib whereas its stability was achieved in oxidative, base, and thermal stress. The DPs produced under stress conditions were labelled as ADP 1, ADP 2, PDP 1, PDP2, and PDP 3 according to their elution order and formed stress conditions in the HPLC chromatogram as shown in Figure S3.

Mechanism of formation of DPs

Under strong acidic conditions, the piperazine ring of avapritinib can undergo ring-opening hydrolysis to form an open-chain amine, which is further broken

down into primary amines. As a result of this, primary amine derivatives formed as DPs and were identified at 1.283 min, and 2.538 min, which were named ADP 1 and DP 2. The stress exposure may weaken the N–CH_3 bond of the pyrazole ring and lead to the cleavage of the N–CH_3 bond. This cleavage generates a pyrazole radical (N•) and a methyl radical (•CH_3). The highly reactive methyl radical may further react with molecular oxygen, resulting in the formation of formaldehyde (HCHO) as a byproduct. This process yields an N-demethylation compound as DP which was retained at 5.865 min and was designated as PDP 2. Under UV stress conditions, avapritinib undergoes Piperazine Ring Dehydrogenation and forms Dehydrogenation compound as DP. In this stress study, the ROS attack the piperazine ring, leading to hydrogen abstraction (H• loss) from the nitrogen-bound carbon atoms (α-carbon positions) of the piperazine ring to form UV DP 2. This compound undergoes N-demethylation reaction as per UV DP 1 and forms UV DP 3. The detailed mechanism is presented in Figure S4.

Fragmentation pathway of ADP 1 and ADP 2

In acid degradation, two DPs are formed at 1.283 min and 2.538 min, they are named as ADP 1 and ADP 2 based on the elution of time. Avapritinib was broken down into two structural units, one with a molecular formula of C12H13FN4 named ADP 1, and the second one with a molecular formula of C10H10N6, which was named as ADP 2, because they are formed under acidic conditions, so that's named ADP 1 and ADP 2. Formation of ADP 1 is formed by the loss of $C_{10}H_{14}N_6$ form avapritinib. Formed ADP 1 has core functional groups like fluorophenyl and pyrimidine used for receptor binding. ADP 1 is again formed into two fragments based on the possible splits, one is formed by the cleavage at the C–C bond between the pyrimidine ring, and the second is the chiral carbon (adjacent group to the amine and fluorophenyl). This resulted in one fragment remaining the fluorophenyl moiety along with an extended conjugated system, forming a nitrilium ion, and second one is a pyrimidine ring along with another fluorophenyl-substituted ethyl group which is maintains stability and aromaticity. The transformation from $C_{11}H_9FN$ to $C_{10}H_8F$ occurs with the loss of one nitrogen (N) atom, one hydrogen atom (H), and a carbon atom (C), primarily due to the loss of the nitrile (-CN) group along with hydrogen, resulting in a fragment with 147.16 m/z. This resulted fragment again loss the acetylene group and forms another fragment with the molecular formula C_8H_6F (121.13 m/z). Already formed one of the fragment ions of 214.21 m/z is formed from one product ion by the loss of a single carbon, hydrogen, and a single fluorine atom, resulting in a fragment is $C_{11}H_8N_3$ with a 182.20 m/z value. The loss of two carbon atoms from the 182.20 m/z, forms one product ion with $C_9H_8N_3$ with 158.17 m/z, this formed product ion loss of intermediate C_3H_2 form one more and last product ion of 120.13 m/z with molecular formula of $C_9H_8N_3$. ADP 2 forms two basic product ions by the loss of the Cyanide radical and another one by the loss of the primary amine, resulting fragments are 188.20 m/z and 147.17 m/z. Product ion of 188.20 m/z forms another product ion by the loss of C_3H_3N resulted fragment is 135.14 m/z, this formed product again loss the C_3 atoms to form another product ion with a mass of 99.11 m/z with C_3H_7N formula. Product ion of 147.17 m/z form another product ion of 117.12 m/z by the loss of C_3H_7N group resulted in fragment name is N-methylidyne-2-(2H-pyrrol-4-yl)ethynaminium, this product ion again loss single Carbon, Hydrogen and Nitrogen group resulted product ion is C_6H_4N with 90.10 m/z which is named as 2H-pyrrol-4-ylethynylium. Available successive fragments of ADP 1, and ADP 2 were given in Figure S5 and mass spectra was given in Figure S7.

Fragmentation pathway of PDP 1, PDP 2, and PDP 3

In the photolytic degradation of avapritinib, three DPs are formed, they are named PDP 1, PDP 2, and PDP 3 based on their elution time. PDP 1 eluted to 3.557 min, PDP 2 eluted to 5.865 min, and PDP 3 was eluted to 6.765 mins. In photolytic degradation, the loss of a simple molecule of CH2 results in the formation of PDP 1, the loss of two hydrogen bonds leads to the formation of PDP2, and the loss of CH_6 leads to the formation of PDP 3. Molecular formula of PDP 1 is $C_{25}H_{25}FN_{10}$ with 484.53 m/z value, while comping to the fragmentation pathway of PDP 1 it forms three product ions by the loss of amine group (NH_2), another one is by loss of Benzyl cyanide (C_8H_7N), and third fragment by the loss of $C_{14}H_{13}N_8$ resulted fragments are with m/z of 468.50 ($C_{25}H_{23}FN_9$), m/z 348.38 ($C_{17}H_{18}N_9$), and m/z 191.22 ($C_{11}H_{12}FN_2$). First formed product ion was further formed another fragment by the loss of single Carbon atom, Hydrogen atom and single Nitrogen atom i.e. CHN, resulted fragment is with 441.48 m/z and with $C_{24}H_{22}FN_8$ molecular formula, resulted fragment is loss phenyldiazonium and resulted to form $C_{18}H_{17}FN_6$ with 336.36 m/z ({(E)-[4-{5-[1-(4-fluorophenyl)ethenyl]-1,2-dihydropyrimidin-2-yl}-3,4-dihydropyrazin-1(2H)-yl] methylidene}cyanamide). Second product ion of m/z 348.38 fragment also produce another product ion by the loss acrylonitrile group i.e. C_3H_3N and resulted fragment is $C_{14}H_{15}N_8$ (295.32 m/z), this formed product ion form another product fragment by the loss of $C_2H_2N_2$ resulted fragment formula is $C_{12}H_{13}N_6$ with 241.27 m/z. Methane group was lost from the 241.27 m/z resulting fragment is $C_{11}H_9N_6$ (221.22 m/z). Third fragment formed at the starting fragmentation pattern, i.e., C11H12FN2, forms another product ion by the loss of formamidine, resulting in a fragment of C10H8F (147.16 m/z). This fragment loses two carbon atoms to form a fragment of 123.14 m/z (C_8H_8F). PDP 2 forms two fragment ions by the loss of amino group (NH_2), and the C_8H_7FN resulted product ions

formulas are $C_{26}H_{21}FN_9$ (478.50 m/z), and $C_{18}H_{16}N_9$ (358.37 m/z). Formed product ion of 478.50 m/z is loss CHN group and form fragment ion of $C_{25}H_{20}FN_8$ with m/z 451.47, this product ion loss $C_{14}H_{10}N_6$ resulted formed fragment ion is $C_{11}H_{10}FN_2$ with m/z of 189.20 and it again loss CH_2N_2 resulted to form $C_{10}H_8F$ with m/z of 147.16, this formed product ion again loss C_2H_2 to form another fragment ion which is identified as (4-ethynylphenyl)fluoronium. Already formed product ion of m/z 358.37 is involved in fragmentation pathway and forms one product ion by the loss of acrylonitrile group, resulting in a formed product ion of C15H13N8 with a mass of 305.31 m/z. This formed product ion is $C_{15}H_{13}N_8$ with mass of 305.31 m/z, this formed product ion is again lost $C_3H_3N_2$ to form the last fragment ion of m/z 239.25. PDP 3 is formed under photolytic degradation with an eluted time of 6.765 min. PDP 3 mainly forms two product ions by the loss of ammonia and methanediazonium, resulting fragments are with the formula of $C_{25}H_{19}FN_9$, and $C_{24}H_{18}FN_8$. Molecular formula of $C_{25}H_{19}FN_9$ loses $C_{13}H_{11}N_7$, resulting in the product ion formula of $C_{12}H_8FN_2$. This formed fragment ion again loses $C_{12}H_8$ and forms the product ion with a mass of m/z 147.16, resulting from the loss of the Acetylene group and forming a fragment ion of C_8H_6F (121.13 m/z). The already formed fragment ion of m/z 437.45 forms another product ion by the loss of 1,3-Diethynyl-5-fluorobenzene ($C_{10}H_5F$). The resulting fragment ion has a molecular formula of $C_{14}H_{13}N_8$. This fragment ion loses a methyl group (CH_3) to form a product ion with a molecular formula of $C_{13}H_{10}N_8$ (m/z 278.27). Fragmentation pathway of PDP 1, PDP 2 and PDP 3 is given in Figure S6. Masses of all DPs are given in Figure S7.

Conclusion

In this study, a precise and reliable chromatographic method was successfully developed and validated for the analysis of avapritinib and its impurities. The separation was achieved using an X-bridge C18 column with a linear gradient elution system, demonstrating high specificity, selectivity, and stability-indicating capability. The developed method effectively resolved all degradant peaks with resolution (Rs) values greater than 2, ensures adequate separation and quantification of analytes. The stress degradation study reveals that avapritinib exhibited notable degradation under acidic and photolytic conditions, whereas it remained stable under oxidative, basic, and thermal stress conditions. The DPs identified in acid and photolytic stress conditions were thoroughly characterized based on their elution order and fragmentation pathways. The major acidic degradation products (ADP 1 and ADP 2) were formed due to piperazine ring hydrolysis and subsequent cleavage reactions, leading to the formation of stable intermediates. The photolytic degradation products (PDP 1, PDP 2, and PDP 3) resulted from the loss of methyl, hydrogen, and other functional groups, forming distinct degradation compounds. The proposed degradation pathways, supported by mass spectral analysis, provided comprehensive insights into the breakdown mechanisms of avapritinib under stress conditions. The fragmentation studies further confirmed the structural integrity and transformation of degradation products, with mass spectrometric evidence highlighting key product ions and their successive fragmentation patterns. The observed degradation behavior and formation of stable degradation products suggest that avapritinib's structural stability is influenced by specific stress conditions, emphasizes the necessity of controlled storage conditions to maintain drug integrity. This study contributes valuable information regarding the degradation behavior of avapritinib, which can aid in formulation development, stability studies, and regulatory submissions. Further research may explore additional stability parameters and the potential impact of DPs on pharmacological efficacy and safety.

References

1. Wu C. P., Lusvarghi S., Wang J. C., Hsiao S. H., Huang Y. H., Hung T. H., Ambudkar S. V. Avapritinib: A Selective Inhibitor of KIT and PDGFRα that Reverses ABCB1 and ABCG2-Mediated Multidrug Resistance in Cancer Cell Lines. *Mol Pharm.* 2019, 16 (7), 3040-3052. https://doi.org/10.1021/acs.molpharmaceut.9b00274

2. Mazzocca A., Napolitano A., Silletta M., Spalato Ceruso M., Santini D., Tonini G., Vincenzi B. New frontiers in the medical management of gastrointestinal stromal tumours. *Ther. Adv. Med. Oncol.* 2019, 11, 1758835919841946 https://doi.org/10.1177/1758835919841946

3. Li J., Zhang X., Deng Y., Wu X., *et al*., Efficacy and Safety of Avapritinib in Treating Unresectable or Metastatic Gastrointestinal Stromal Tumors: A Phase I/II, Open-Label, Multicenter Study. *Oncologist*. 2023, 28 (2), 187-e114 https://doi.org/10.1093/oncolo/oyac242

4. Amgoth K. M., Tummala S. R. LC-MS/MS approach for the quantification of five genotoxic nitrosoimpurities in varenicline. *J. Pharm. Res.* 2022, 26 (6), 1685-1693. https://doi.org/10.29228/jrp.259

5. Rajesh Varma Bhupatiraju., Srinivasa Kumar B., Pavani Peddi., Venkata Swamy Tangeti. An effective HPLC method for evaluation of process related impurities of Letermovir and LC-MS/MS characterization of forced degradation compounds. *J. Chem. Metrol.* 2023, 2 (2),181-198. https://doi.org/10.25135/jcm.98.2311.2975

6. Tummala S. R., Gorrepati N., Tatapudi H. K. Head Space GC-MS/MS Method for Quantification of Five Nitrosoamine-Genotoxic Impurities in Metformin HCl. *Curr. Pharm. Anal*. 2024, 20 (8), 944 –952. https://doi.org/10.2174/0115734129332940240919113159

7. Rajesh Varma Bhupatiraju., Bikshal Babu Kasimala., Lavanya Nagamalla., Fathima Sayed. Structural evaluation of degradation products of

Loteprednol using LC-MS/MS: Development of an HPLC method for analyzing process-related impurities of Loteprednol. *Anal. Sci. Technol.* 2024, 37 (2), 98-113. https://doi.org/10.5806/AST.2024.37.2.98

8. Tummala S. R., Amgoth K. P. Development of GC-MS/MS Method for Simultaneous Estimation of Four Nitrosoamine Genotoxic Impurities in Valsartan. *Turk. J. Pharm. Sci.* 2022, 19 (4), 455-461. https://doi.org/10.4274/tjps.galenos.2021.17702

9. Ravinder Bairam., Hemant Kumar Tatapudi., Vijay Srinivas Pothula., Likhitha Akaram., Sambasiva Rao Tummala., Naveena Gorrepati. Analytical quality by design approach in RP-HPLC method development for the quantification of mirabegron and solifenacin succinate in pharmaceutical formulation. *Lett. Appl. Nano. BioScience*. 2024, 14 (1), 1-12. https://doi.org/10.33263/LIANBS141.048

10. Rajesh Varma Bhupatiraju., Pavani Peddi., Subhashini Edla., Kandula Rekha., Bikshal Babu Kasimala. Green Analytical Approach for HPLC Method Development for Quantification of Sorafenib and Its Pharmacopeia Impurities: LC–MS/MS Characterization and Toxicity Prediction of Stress Degradation Products. *Sep. sci. plus.* 2024, 7 (9), e202400106. https://doi.org/10.1002/sscp.202400106

11. Suvasish Mishra., Koushik Sarker., Avijit Ghosh., Abhijit Saha., Subrata Sen. A Validated Stability Indicating RP-HPLC method for estimation of Avapritinib in bulk and tablet dosage form. *Int. J. App. Pharm.* 2022, 14 (2), 95-101. https://dx.doi.org/10.22159/ijap.2022v14i2.43432.

12. Sruthi Uppari., Gade Sammaiah. Stability Indicating RP-HPLC method development and validation for the quantitative estimation of avapritinib in API form and marketed pharmaceutical dosage form. *Int. j. pharm. res. Health. sci.* 2023, 11 (3): 3636-3643.

13. Thakur Gajender Singh., Jagadeesh Babu Vadlakonda., Rama Mohan Gupta V. Analytical method development and validation of avapritinib by RP-HPLC method. *WJPPS*. 2020, 9 (10), 1834-1840. https://dx.doi.org/10.20959/wjpps202010-17283

14. Rasagna P., Kalyani G. Development and validation of analytical RP-HPLC method for the quantitative determination of avapritinib in pure substances and marketed pharmaceutical tablet forms. *IJMRGE*. 2024, 05 (01), 100-107.

15. Sridhar Babu R., Syed Hafeez Ahmed., Shaik Mohd Khasim., Syeda Nusrath Fatima., Qayam Ali Khan., Lubna Hashmi., Hajira Samreen., Muneeza Fatima. RP-HPLC method development and validation for the estimation of avapritinib in bulk form and marketed pharmaceutical dosage form. *IJMRGE.* 2024, 05 (01), 161-167.

16. Xuegu Xu., Shunbin Luo., Qiang Yang., Y ingjie Wang., Wenlong Li., Guanyang Lin., Ren-ai Xu. Development and validation of the quantitative determination of avapritinib in rat plasma by a bioanalytical method of UPLC-MS/MS. *Arab. J. Chem.* 2021, 14 (6), 103152. https://doi.org/10.1016/j.arabjc.2021.103152

17. Gowri Kusuma Kumari., Kantipudi Rambabu. Bio-analytical Method Development and Validation for avapritinib in Rat Plasma by LC-MS/MS. *J. Pharm. Sci. & Res.* 2021, 13 (3), 134-137.

18. Baher Salman I., Roshdy Saraya E., Yasser Hassan F., Ahmed Hassan I., Hany Batakoushy A., Mohamed Abdel-Aal A. A., Ahmed Al-Harrasi., Adel Ehab Ibrahim. A green bioanalytical spectrofluorimetric approach for estimation of avapritinib anti-tumor drug; application to quality control and clinical studies. *Bioanal.* 2025, 17 (1), 31-40. https://doi.org/10.1080/17576180.2025.2451518

19. Teuber A., Schulz T., Fletcher B. S., Gontla R., Mühlenberg T., Zischinsky M.L., Niggenaber J., Weisner J., Kleinbölting S. B., Lategahn J., Sievers S., Müller M. P., Bauer S., Rauh D. Avapritinib-based SAR studies unveil a binding pocket in KIT and PDGFRA. *Nat. Commun.* 2024, 15, 63-71. https://doi.org/10.1038/s41467-023-44376-8

20. I.C.H. Validation of Analytical Procedures: Text and Methodology, Q2 (R1), International Conference on Harmonization IFPMA, Geneva, 2005.

21. Rajesh Varma Bhupatiraju., Srinivasa Kumar B., Venkata Swamy Tangeti., Kandula Rekha., Fathima Sayed. LC and LC-MS/MS studies for identification and characterization of related substances and degradation products of abrocitinib. *Toxicol. Int.* 2024, 31 (2), 321-334. https://doi.org/10.18311/ti/2024/v31i2/36370

22. Rajesh Varma B., Pavani P., Venkata Swamy T., Rekha K., Sreenivasa Rao B. Characterization of Pimavanserin stress degradation products by LCMS/MS and optimization of green analytical HPLC method for quantification of pimavanserin and its impurities. *Asian J. Chem.* 2024, 36, 2452-2460. https://doi.org/10.14233/ajchem.2024.32475