LC–MS/MS characterization of stress degradation products of Ripretinib along with development and validation of a stability-indicating HPLC method for quantification of Ripretinib

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Abstract

The green analytical HPLC methods are highly effective for quantifying drugs because these methods reduce the usage of harmful chemicals and generate less waste leading to minimal environmental influence. Various compositions of green solvents including ethanol and water with buffers were utilized as mobile phase. The conditions that produce acceptable results were studied for valuation in terms of linearity, accuracy, precision and sensitivity. This method was applied for the sepration and characterization of degradation products (DPs) of ripretinib using LCMS/MS. The method comprises of Ascentis Express C18 (2.1 mm × 100 mm, 2.7 micron) column along with ethanol and 0.01% formic acid in water, in 65:45 (v/v) at pH 4.3 at 0.7 mL/min flow and 261 nm wavelength. Acceptable results were obtained for Ripretinib. A stress test revealed six different DPs in acid, base and peroxide breakdown tests, labelled as DP 1 to 6 based on order of elution.

The applicability of MS^n studies and mass fragmentation confirms DP 1 as 7-amino-3-(5-amino-2-bromo-4-fluorophenyl)-1-ethyl-1,6-naphthyridin-2(1H)-one 1-oxide, DP 2 as 1-[5-(7-amino-1-ethyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-bromo-2-fluorophenyl]urea and DP 3 as 7-amino-3-(5-amino-2-bromo-4-fluorophenyl)-1-ethyl-1,6-naphthyridin-2(1H)-one, DP 4 as 3-(5-amino-2-bromo-4-fluorophenyl)-1-ethyl-7-(methylamino)-1,6-naphthyridin-2(1H)-one, DP 5 as {4-bromo-5-[1-ethyl-7-(methylamino)-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl]-2-fluorophenyl}carbamic acid and DP 6 as 3-(2-bromo-4-fluoro-5-(3-phenylureido)phenyl)-1-ethyl-7-(methylamino)-2-oxo-1,2-dihydro-1,6-naphthyridine 1-oxide. The GAPI (Green Analytical Procedure Index) and AGREE (Analytical GREEnness) tools were utilized to assesses the greenness of method. This study found that the method works well for measuring Ripretinib and characterized its DPs.

Keywords: Ripretinib, LCMS, Degradation products, Green analytical method.

Introduction

Ripretinib is a tyrosine kinase inhibitor for the treatment of advanced gastrointestinal stromal tumors (GIST) in patients whose disease has progressed following prior therapies; it inhibits several alternative, kinase-insertion mutation-positive forms of the KIT and PDGFRA kinases involved in the pathogenesis of GIST[8]. Ripretinib is a drug approved by the FDA on June 2020, designed to block the signaling pathways supporting tumor growth. It is administered orally and is often used when there is cross resistance to other treatments like imatinib[15].

Ripretinib is administered orally, at 150 mg daily, with or without food. Omitted doses should be overlooked if the dose is scheduled in fewer than 8 hours from the time the dose was omitted[3]. Frequent adverse events are alopecia, fatigue, nausea, myalgia, hand-foot syndrome, hypertension, anorexia and diarrhoea[1]. Greater potential side effects would include increased risks of cutaneous squamous cell carcinoma and melanoma, hypertension, becoming severe cases and cardiac conditions, to include LV diminished ejection fraction[9].

Ripretinib is chemically designated as 1-(4-bromo-5-(1-ethyl-7-(methylamino)-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-2-fluorophenyl)-3-phenylurea. The empirical formula is $C_{24}H_{21}BrFN_5O_2$ and the molecular weight is 510.3582 g/mol and its structure is represented in figure 1.

There is insufficient information on a thorough analysis of degradation products and the proposed structures for the ripretinib. Some studies have conducted estimation of ripretinib using fluorescence detection by HPLC and propylene carbonate as an ecofriendly solvent[2,5], LC-MS/MS pharmacokinetic profile in rat plasma[12], pharmacokinetic effect in Beagle dogs by UPLC-MS/MS[4], potential mutagenic impurities by UPLC-MS/MS[16].

Green analytical methods follow green chemistry principles that aim to reduce the use of harmful chemicals, to produce less waste and to save resources. These methods can also lower costs and an improve efficiency, making them a good

choice for accurately and reliably measuring ripretinib in pharmaceutical products. This research focuses on developing an eco-friendly method using HPLC to measure ripretinib and to study how it breaks down using LC-MS/MS.

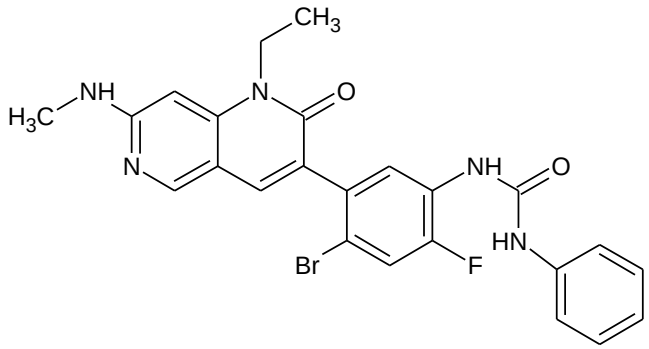


Figure 1: Molecular structure of Ripretinib

Material and Methods

Chemicals and reagents: The ripretinib is standard drug with purity greater than 99.20%, formulation QINLOCK 50 Mg tablet was generously provided by Dr. Reddy's Laboratory, located in Hyderabad, India. The chemicals employed in this study were all of analytical grade and were sourced from Merck Chemicals, Mumbai. Additionally, high-purity solvents required for HPLC analysis including ethanol, Formic acid and water were obtained from Merck Chemicals, Mumbai.

Instrumentation: The analytical experiments used were Agilent 2695 series HPLC-UV system (USA) with binary pump and degasser. This system also features an autosampler and a compartment that controls the temperature of the column, ensuring consistent conditions throughout the analysis. The data collected from these experiments were integrated through Empower 3 software. The detailed characterization of DPs was performed on an Agilent 1290 series LC-MS/MS system (USA) paired with a Q-TOF mass detector. The ionization of analytes including the DPs was performed using Electrospray Ionization (ESI) in positive mode. The raw data obtained were exported and analyzed through Mass Hunter Workstation software. The pH of the samples was carefully measured using a pH meter from Mettler Toledo (Switzerland). The samples were fully dissolved before analysis with the assistance of an ultrasonic bath (Oscar Ultrasonic Pvt. Ltd., India).

Standard solution preparation: In the process of preparing stock solution, exactly 10 mg of standard substance was carefully weighed and then transferred into 10 mL flask. 5 mL of ethanol was added to this flask and then sonicated to achieve complete solubility of standard in ethanol separately. After ensuring complete solubility, it was filtered through a nylon membrane filter with a pore size of 0.22 µm to remove any impurities. Once filtered, the solution was diluted to a 10 mL mark with additional ethanol to reach 1000 µg/mL concentration. The prepared stock solution was then stored for future use as a standard solution.

Forced degradation study: The stress study was conducted following ICH Q1 A (R2) guidelines[7] as reported in literature[13]. In acid degradation, 100 mg of the drug was dissolved in a 100 mL flask with 50 mL diluent, then mixed with 5 mL of 2N HCl and heated at 80°C for 8 hours. After three days, it was neutralized with 2N NaOH and diluted to volume. A portion was filtered and adjusted to 100% concentration for analysis. For base degradation, 100 mg of drug was combined with 5 mL of 3N NaOH and 45 mL diluent, heated at 80°C for 8 hours, neutralized with 3N HCl and diluted for testing. In oxidative degradation, 100 mg of drug was dissolved with diluent and 5 mL of 30% H_2O_2, then stored in the dark for three days. The filtered solution was diluted to 100% concentration. The pure drug was also exposed to heat at 80°C for 2 days and to UV light at room temperature for 3 days before being diluted and analyzed.

Analytical method validation: Method validation was performed according to Q2 (R1) guidelines prescribed by ICH[6] and the literature available[10,14]. System suitability was evaluated by repeatedly injecting known strength of ripretinib solution. The resultant chromatographic results including resolution, tailing factor, theoretical plate count and % RSD of the area were summarized to ensure the method's performance. Linearity was determined by analysing six different concentrations of ripretinib and the resultant area response was plotted against its concentration. The linear relationship with correlation coefficient of greater than 0.999 was treated as acceptable. To assess method accuracy, ripretinib was spiked at levels ranging from 50% to 150% of target concentration. These spiked analysis results were correlated with calibration results to confirm method reliability. Precision and ruggedness were examined by analyzing 100% level solution in calibration range under various conditions including same day, day change and analyst change.

The resultant area response was used to evaluate % RSD and the results confirm the method's repeatability and reproducibility. Robustness was validated by introducing minor intentional changes to method parameters such as detector wavelength, composition and flow of mobile phase. The impact on % area response and system performance in every change were observed to assess method robustness. Sensitivity was evaluated by determining the LOD and LOQ for ripretinib using the slope and standard error from the calibration curve.

Characterization of degradation products: Stressed solutions were analyzed on an HPLC-MS/MS system under proposed optimized conditions. The method allowed the detection of peaks associated with ripretinib and DPs. The mass range covered during the analysis was from 10 to 500 m/z. In LC-MS analysis, 25% of column eluents were

allowed to pass through the MS system by fixing a splitter between the column and the mass spectrometer detector.

Evaluation of method greenness: The proposed quantitative method was green-evaluated through GAPI and AGREE on metric tools[11]. The green evaluation specifically helps to estimate different parameters for ensuring the safety of the drug by reducing the health and environmental impact. This estimation includes aspects such as sample collection, the method used, solvents and reagents used in the method, energy expenditure, steps of disposal of wastes and other pertinent considerations. Among them, GAPI is highly effective in examining environmental sustainability factors. In this evaluation, different color combinations were used like green, red and yellow.

The whole figure is divided into six different parts, where each part signifies different factors such as the origin of the sample, the process type that it undergoes, sample preparation, the chemicals and reagents used, the instruments and most importantly, the type of quantitative analysis, which is signified by the letter "O". The AGREE method was used to determine the extent of environmental sustainability. The method by metric is based on the twelve principles of analytical green chemistry. This current study aims to develop AGREE, GAPI and environmental sustainability methods for the achievement of safe and effective qualitative and quantitative analysis of ripretinib.

Results and Discussion

The optimization started with a Waters C18 column. Many organic modifiers were tried to get the desired asymmetric peak shape, resolution and minimum tailing. However, the first set of conditions did not provide an acceptable asymmetric peak shape. Given the above, changes were introduced into the mobile phase and the pH was altered to attempt to yield better results but did not produce permissible results. Hence, a Ascentis Express C18 column with dimensions of 100 × 2.1 mm and particle size 2.7 µm was employed. A mobile phase of ethanol and 0.01% formic acid in water in the ratio of 65:45 (v/v) was utilized which provided adequate resolution with an asymmetric peak shape of low tailing. Therefore, these conditions were optimized in view of the development of the method for the analysis of ripretinib. Under such optimized conditions, the wavelength maximum was set at 261 nm, based on the absorption wavelength of ripretinib.

Degradation Study of Ripretinib: Ripretinib was investigated for the degradation behavior under different stress conditions using HPLC. The stress conditions performed and the results noticed were tabulated in table 1.

Table 1 displays the data obtained from the stress study indicating that under the heat and light conditions no degradation compounds were noticed. However, the drug degraded when subjected to acidic, basic and oxidative environments (Figure 2). In these sensitivity tests, five new peaks were found in the chromatograms at retention times of 1.93 min, 2.21 min, 3.51 min, 4.56 min, 6.21 min and 7.95 min which were considered to be DPs. Based on the time they elute, these degradation products were named DP 1, DP 2, DP 3, DP 4, DP 5 and DP 6 respectively and were analyzed using the LC-MS/MS technique.

LC–MS/MS studies of DPs: The exact mass of ripretinib and its DPs was determined by analyzing their detailed high-resolution mass fragmentation patterns. The accurate mass predictions for ripretinib and its degradation structures were obtained by calculating the Ring and Double Bond (RDB) values and following the nitrogen rule to ensure accuracy. The elemental compositions of the protonated degradation products and their resulting ions are listed in table 2. Figures 3 and 4 illustrate the fragmentation pathways for each of the degradation products, DP 1 through DP 6, showing how each one breaks down.

DP 1: Protonated DP 1 is formed under peroxide degradation study which is given in figure 3. Total molecular weight of DP 1 is 393.2103 g/mol with $C_{16}H_{14}BrFN_4O$ of molecular formula. DP 1 is eluted at 1.93 min in the peroxide degradation study. From DP 1, total molecular formula loss of CH_3O resulted in formation of $C_{15}H_{17}BrFN_4O$ product with 368.22 g/mol as the molecular weight of the formed product. From this product, another product was formed due to the loss of C_2H_4O to form $C_{13}H_{13}BrFN_4$ with a molecular weight of 324.17 g/mol. By-product ion of $C_{13}H_{13}BrFN_4$ forms another by product ion that is $C_{12}H_{12}BrFN_3$ with 297.14 of molecular weight. Product ion of $C_{12}H_{12}BrFN_3$ also forms C_8H_8BrFN by the loss of $C_4H_4N_2$. Last product ion is formed due to the loss of C_2H_2, with molecular weight of 191.02. Figure 3 illulistrated the degradation pathway of DP 1 and mass spectrum is given in figure 4. Finally formed DP 1 molecular name is 7-amino-3-(5-amino-2-bromo-4-fluorophenyl)-1-ethyl-1,6-naphthyridin-2(1H)-one 1-oxide.

DP 2: DP 2 is generated specifically under acid degradation conditions, with a molecular formula of $C_{17}H_{15}BrFN_5O_2$ and a molar mass of 420.23 g/mol. During this test, DP 2 elutes at 2.21 min. The loss of a CH_3O group from DP 2 yields a fragmented ion with the formula $C_{14}H_{16}BrFN_5O$ and a mass of 369.21 m/z, known as (1Z)-N-[amino(hydroxy)methyl]-4-bromo-3-[(E)-2-(4,6-diaminopyridin-3-yl)ethenyl]-6-fluorocyclohexa-2,4-dien-1-iminium. This fragmented ion further loses a CH_2N_2O group, forming another ion with the molecular formula $C_{13}H_{14}BrFN_3$ and mass of 311.17 m/z, named 2-amino-5-[(E)-2-(6-bromo-4-fluorocyclohexa-1,5-dien-1-yl)ethenyl]pyridin-4(3H)-iminium.

Next, the compound C_6H_2BrF is eliminated, resulting in a product with formula $C_7H_{12}N_3$ and a mass of 138.18 m/z, identified as 6-amino-3-ethenyl-2,5-dihydropyridin-4(3H)-iminium. This ion further forms a product with formula $C_5H_9N_2$ and a mass of 97.13 m/z. The mass spectrum for DP 2 is shown in figure 5, with the full degradation pathway illustrated in figure 3. Based on this pattern, DP 2 is

identified as 1-[5-(7-amino-1-ethyl-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-bromo-2-fluorophenyl]urea.

DP 3: DP 3 is formed exclusively in acid degradation study with $C_{16}H_{14}BrFN_4O$ as molecular formula and 377.21 is the molar mass of the formed compound. In this stress\ condition, peak was eluted at 3.51 min. Loss of the CHO group from DP 3 forms the fragmented product $C_{15}H_{13}BrFN_4$ with a mass of 348.19 m/z known as 2-amino-5-[(E)-2-(5-amino-2-bromo-4-fluorophenyl)ethenyl]-N-ethylidynepyridin-4-aminium.

Table 1
Forced degradation conditions for Ripretinib

Condition	Stressor	Conditions	Duration	% degradation	DPs
Acid	HCl (2N)	80°C	8 days	16.3	DP 1, 2 & DP 3
Base	NaOH (3 N)	80°C	8 hours	12.4	DP 4
Oxidative	H_2O_2 (30%)	Room temperature	3 days	13.5	DP 5 & 6
Thermal	---	80°C	2 days	1.23	---
UV	200 W h/m^2	Room temperature	3 days	1.05	---

Table 2
Elemental composition study results of Ripretinib and its DPs

Compound	MF	m/z Calculated	m/z observed	Error in ppm	Fragmentation (m+1)
Ripretinib	$C_{24}H_{21}BrFN_5O_2$	510.3670	510.3682	2.351	439.0128 391.0564 375.0215 311.1302 104.0494
DP 1	$C_{16}H_{14}BrFN_4O_2$	393.2103	393.2101	0.508	369.2236 325.1703 298.1455 218.0576 192.0201
DP 2	$C_{17}H_{15}BrFN_5O_2$	420.2357	420.2356	0.238	370.2115 312.1721 218.0144 139.1895 98.1377
DP 3	$C_{16}H_{14}BrFN_4O$	377.2109	377.2107	0.530	349.1921 325.1708 298.1455 218.0575 192.0201
DP 4	$C_{17}H_{16}BrFN_4O$	391.2375	391.2376	0.255	365.1915 347.2011 177.1945 125.1630
DP 5	$C_{18}H_{16}BrFN_4O_3$	435.2470	435.2467	0.689	409.2011 351.1650 323.1549 259.1093 206.0468
DP 6	$C_{24}H_{21}BrFN_5O_3$	526.3576	526.3575	0.190	452.2688 393.2017 363.1755 312.1721 204.0308 137.1737

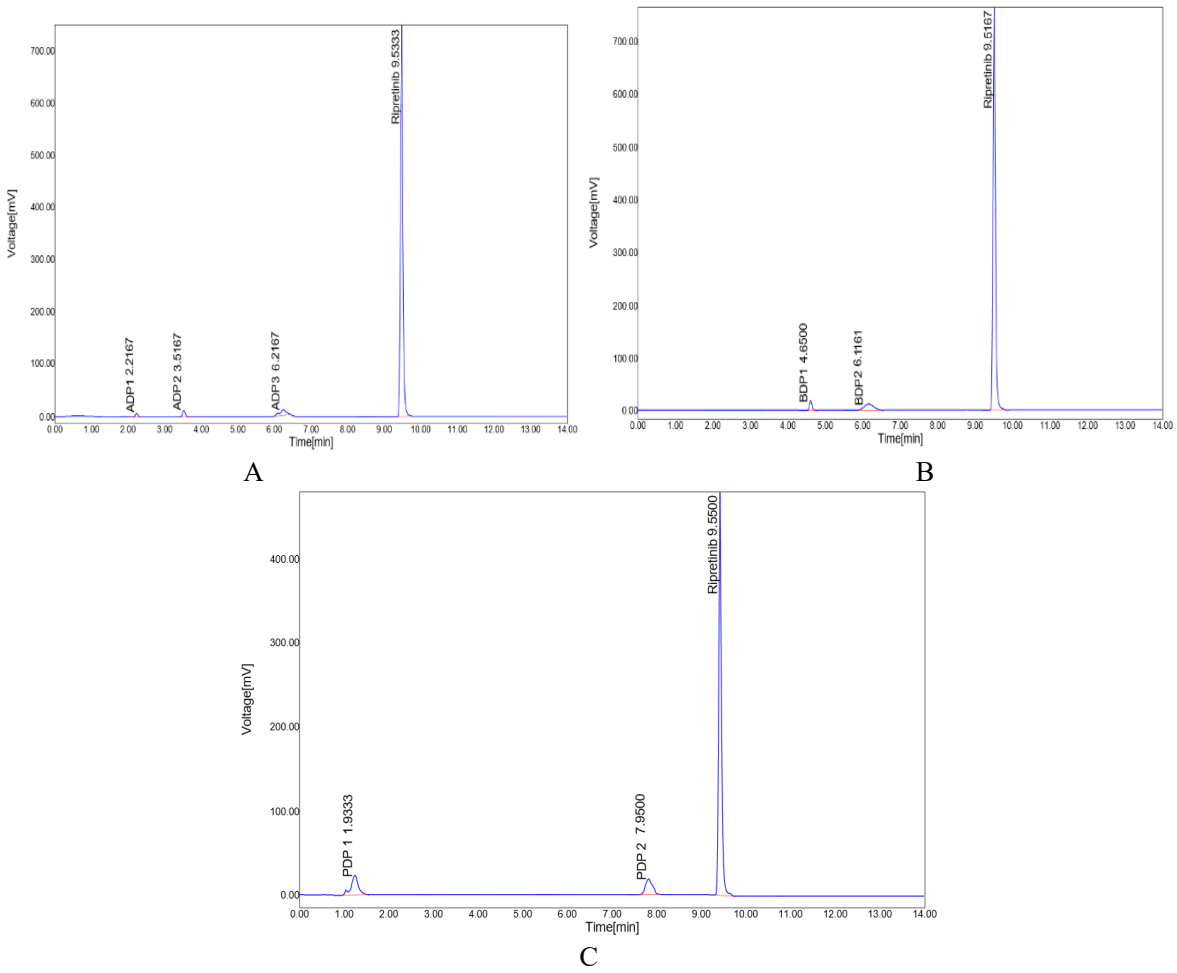


Figure 2: Acid (A), Base (B) and Oxidative (C) degradation chromatogram that resolve degradation products in the proposed method

This fragmented ion again loses C_2 group and formed another product with molecular formula of $C_{13}H_{13}BrFN_4$ with 324.17 m/z of molar mass. This formed product name is 2-amino-5-[(E)-2-(5-amino-2-bromo-4-fluorophenyl) ethenyl]pyridin-4(3H)-iminium. From the formed compound, CHN was eliminated to form another product with a 297.14 m/z, with $C_{12}H_{12}BrFN_3$ molecular formula. This formed compound name is ((2Z,3E)-4-(5-amino-2-bromo-4-fluorophenyl)-2-[(methylideneamino)methylidene]but-3-en-1-iminium.

From this link, $C_4H_4N_2$ is separated to form another fragmented ion C_8H_8BrFN with 217.05 m/z. This formed compound name is *4-bromo-5-ethenyl-2-fluoroanilinium*. From this, again loss of C_2H_2 forms another fragment with 191.02 m/z with molecular formula of C_6H_6BrFN. Officially formed compound name is 4-bromo-2-fluoroanilinium. The proposed whole degradation pathway of DP 3 is given in figure 3. Mass spectrum of DP 3 is presented in figure 6. By all this pattern, proposed DP 3 name is N-(2-chlorodibenzo[b,f][1,4]oxazepin-11-yl)-N-methylethane-1, 2-diamine.

DP 4: DP 4 is formed exclusively in base degradation study with $C_{17}H_{16}BrFN_4O$ as molecular formula and 391.23 g/mol is the molar mass of the formed compound. Peak was eluted at 4.65 min in the base stress condition. Loss of the C_2H_3 group from DP 4 forms the fragmented product of $C_{15}H_{13}BrFN_4O$ with a mass of 364.19 m/z is known as 3-(5-amino-2-bromo-4-fluorophenyl)-2-hydroxy-7-(methylamin o)-1,6-naphthyridin-1-ium. This formed fragmented ion, again loses H group and formed another product with molecular formula of $C_{15}H_{14}BrN_4O$ with 346.20 m/z of molar mass. This formed product name is 3-(6-bromo-3-iminocyclohexa-1,5-dien-1-yl)-2-hydroxy-7-(methylamino) -1,6-naphthyridin-1-ium.

From the formed compound, C_6H_4BrFN was eliminated to form another product with a 176.19 m/z, with $C_9H_{10}N_3O$ as molecular formula. This formed compound name is 2-hydroxy-7-(methylamino)-1,6-naphthyridin-1-ium. From this link, C_3O is separated to form another fragmented ion $C_6H_{10}N_3$ with 124.16 m/z. This formed compound name is 4-amino-5-methylpyridin-2(1H)-iminium.

The proposed whole degradation pathway of DP 4 is given in figure 7. Mass spectrum of DP 4 is presented in figure 8. By all this pattern, proposed DP 4 name is 3-(5-amino-2-bromo-4-fluorophenyl)-1-ethyl-7-(methylamino)-1,6-naphthyridin-2(1H)-one.

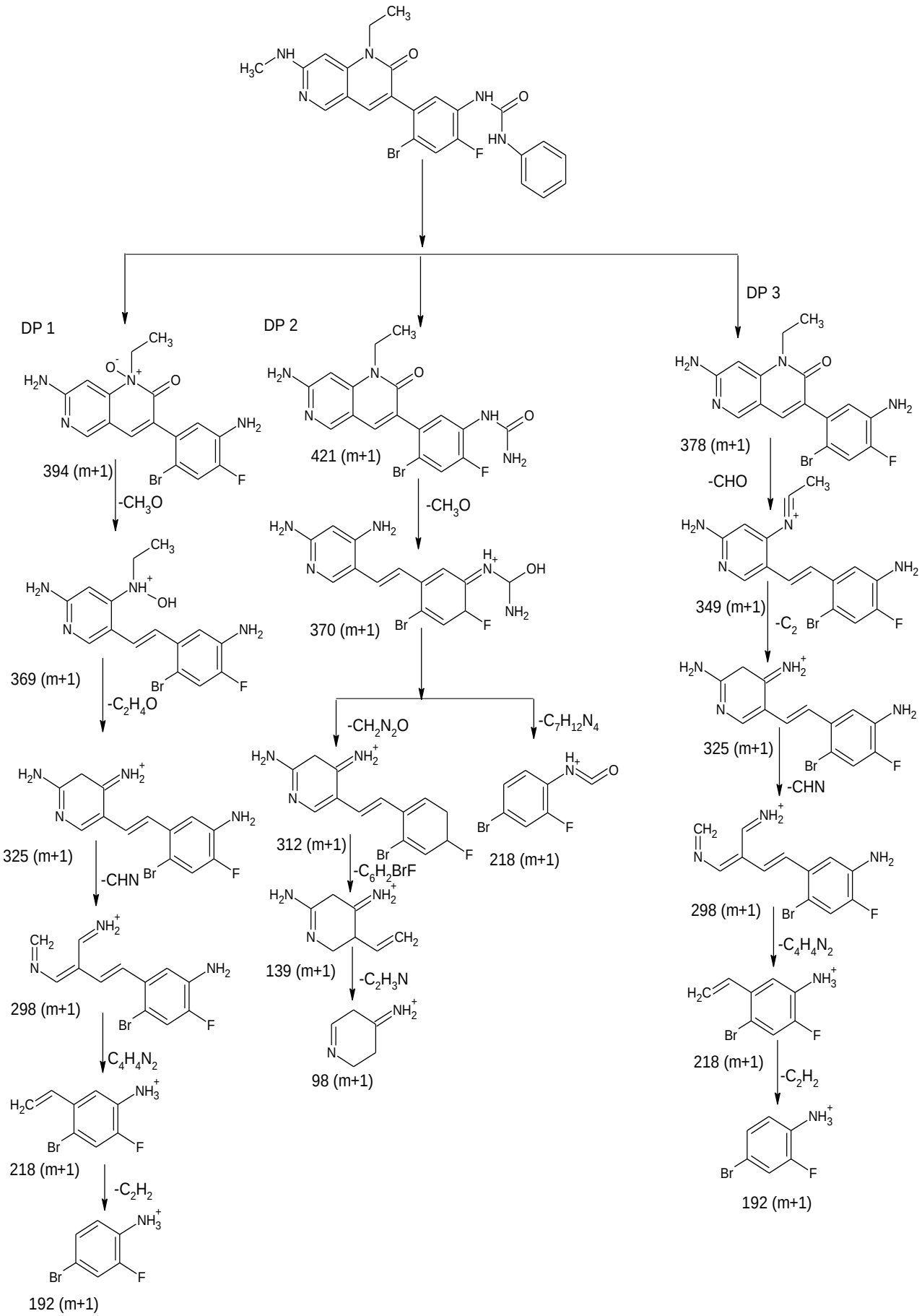


Figure 3: Degradation pathway for DP 1, 2 and 3

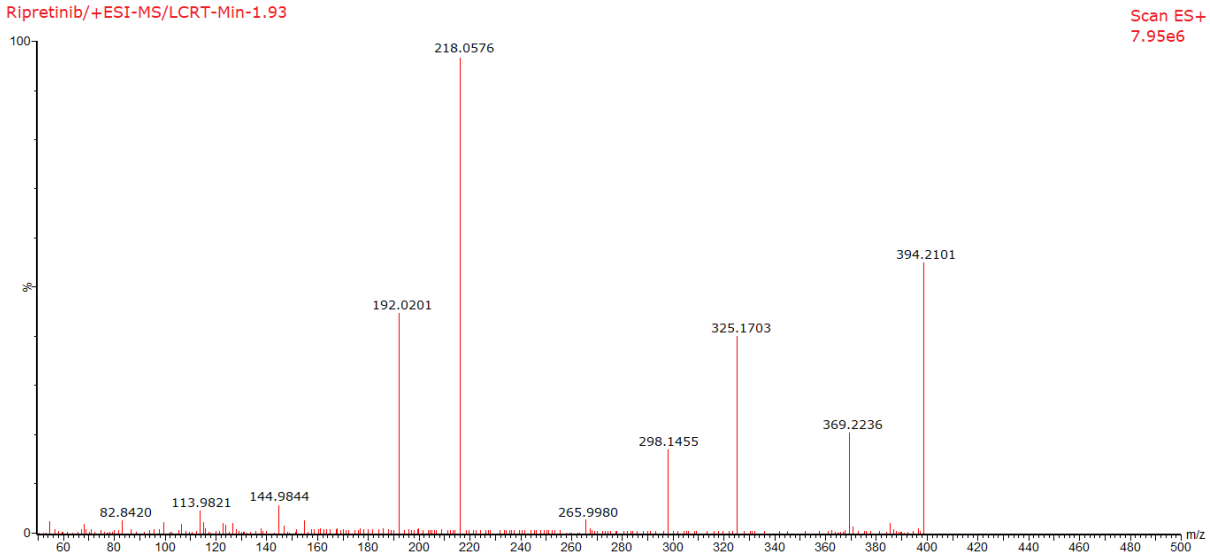


Figure 4: Mass spectrum of DP 1

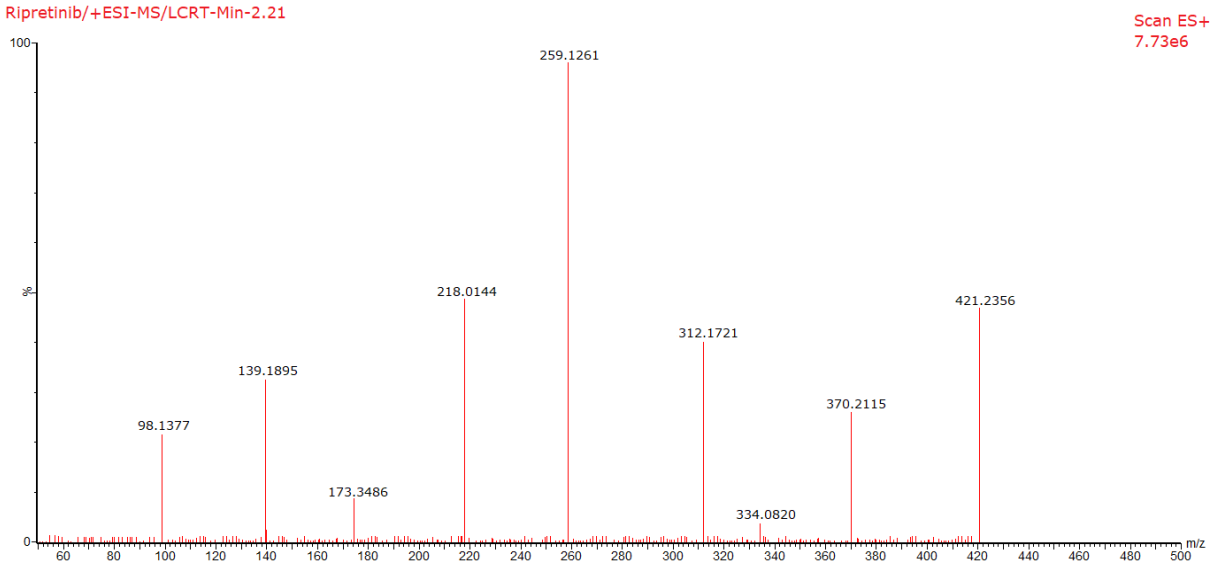


Figure 5: Mass spectrum of DP 2

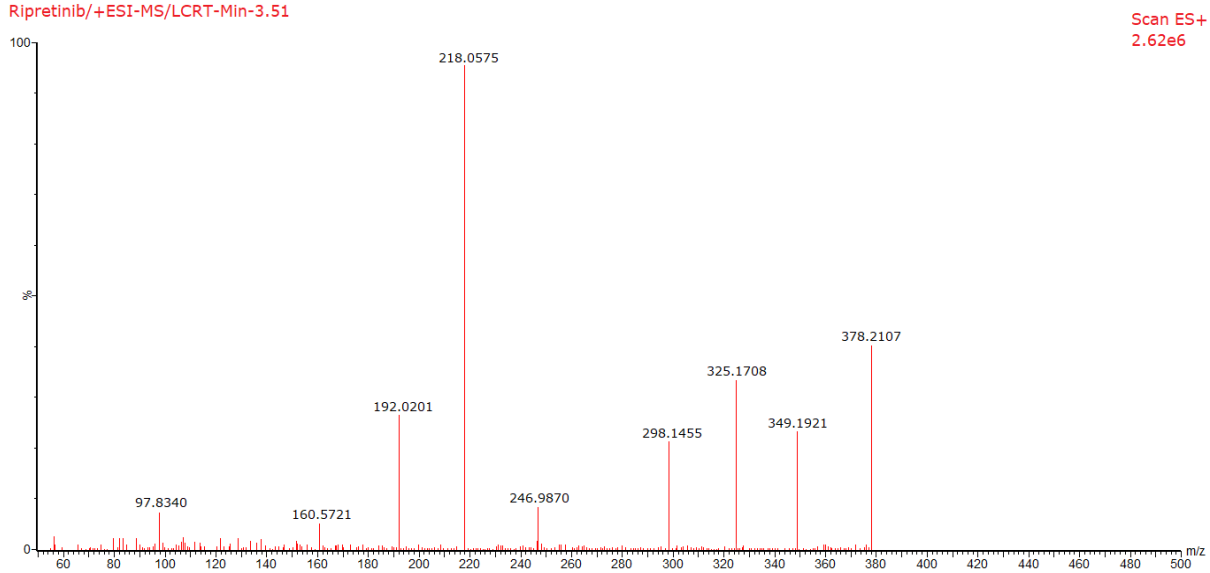


Figure 6: Mass spectrum of DP 3

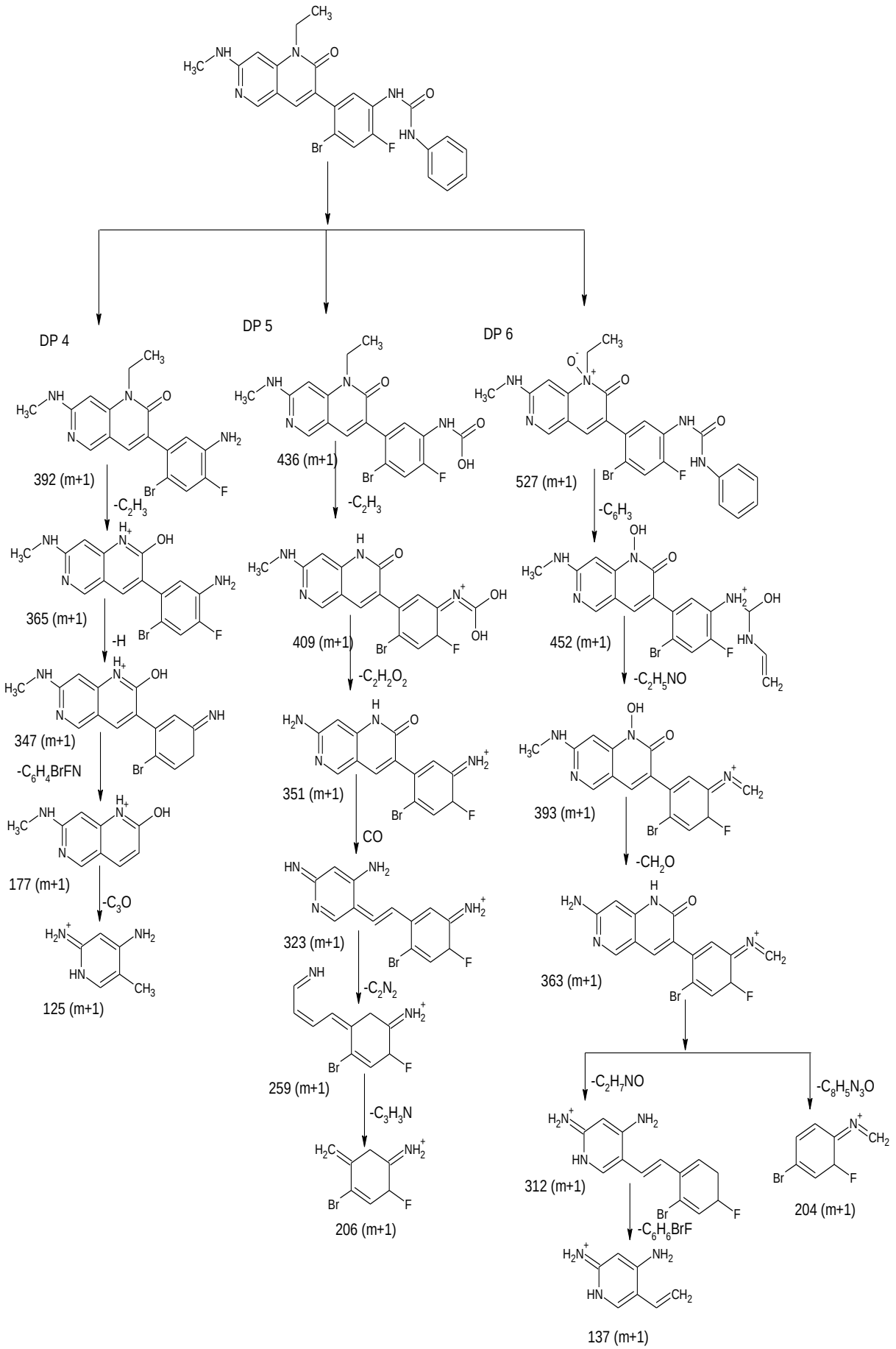


Figure 7: Degradation pathway for DP 4, 5 and 6

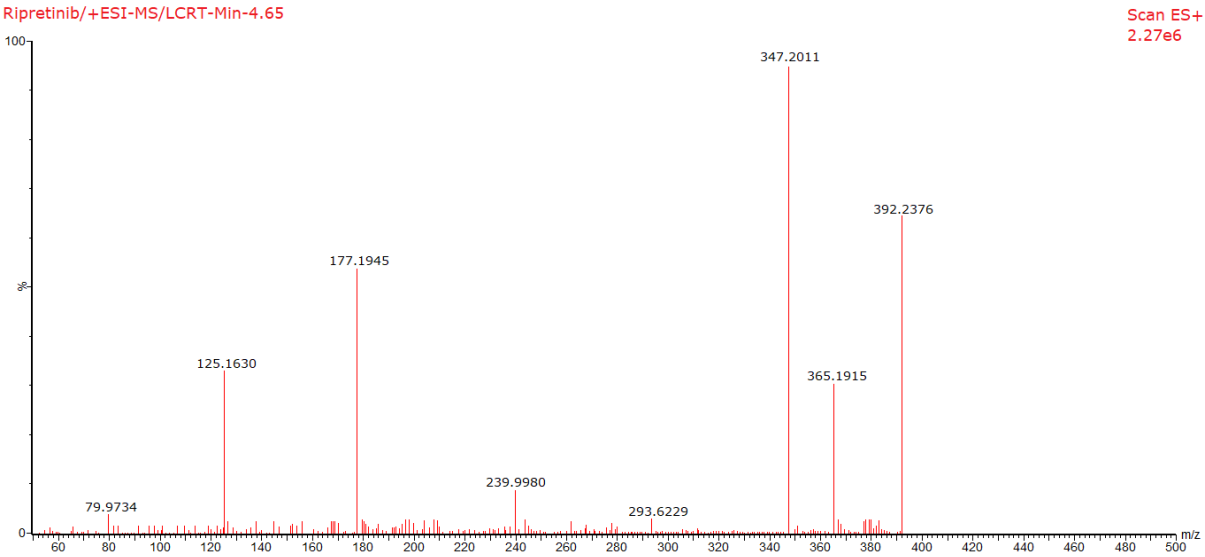


Figure 8: Mass spectrum of DP 4

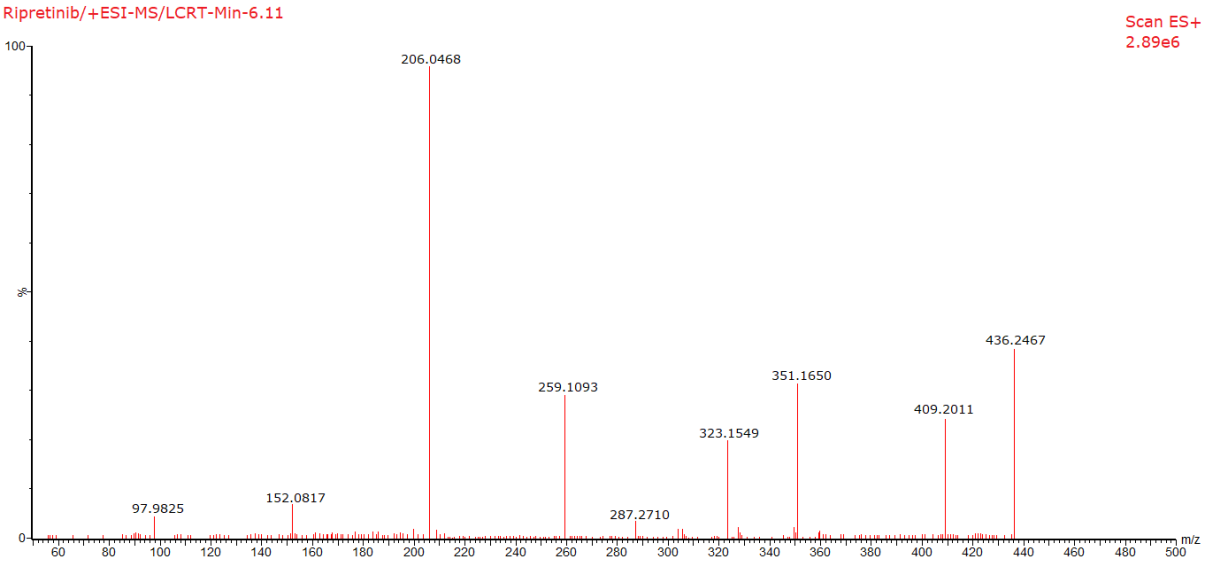


Figure 9: Mass spectrum of DP 5

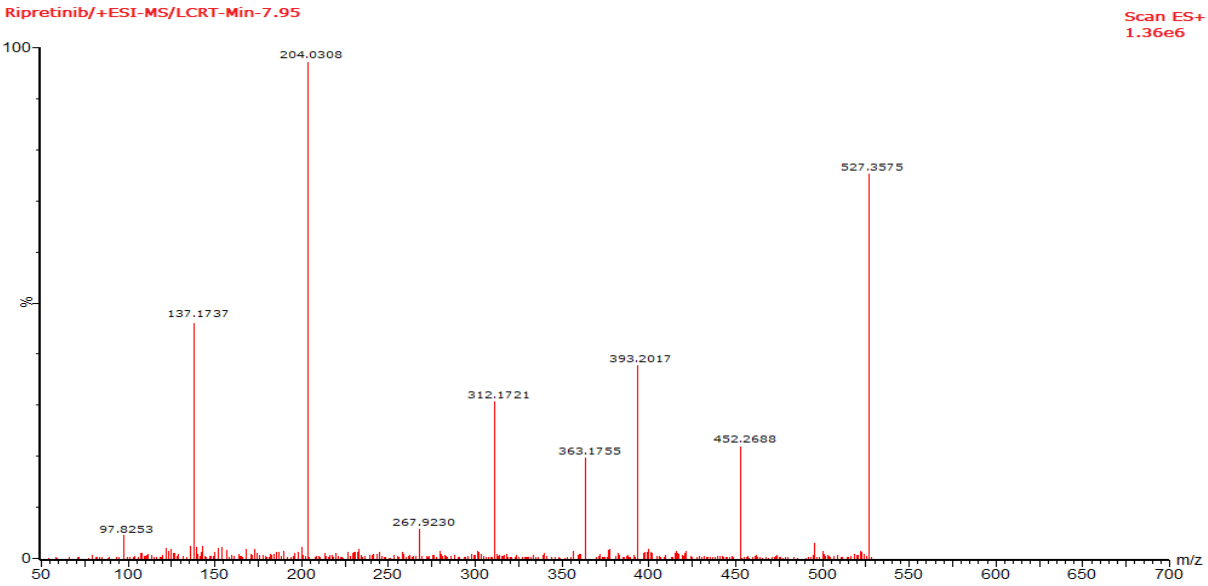


Figure 10: Mass spectrum of DP 6

DP 5: DP 5 is formed exclusively in base and acid degradation study with $C_{18}H_{16}BrFN_4O_3$ molecular formula and 435.24 g/mol as the molar mass of the formed compound. In this stress condition, peak was eluted at 6.11 min in the base and acid stress condition. Loss of the C_2H_3 group from DP 5 forms the fragmented product ion of $C_{16}H_{15}BrFN_4O_3$ with a mass of 408.20 m/z known as 4-bromo-N-(dihydroxymethylidene)-6-fluoro-3-[7-(methylamino)-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl]cyclohexa-2, 4-dien-1-iminium. From this formed fragmented ion, $C_2H_2O_2$ group is eliminated to form another product with molecular formula of $C_{14}H_{11}BrFN_4O$ with 350.16 m/z of molar mass. This formed product name is 3-(7-amino-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl)-4-bromo-6-fluorocyclohexa-2,4-dien-1-iminium. From the formed compound, CO was eliminated to form another product with a 322.15 m/z, with $C_{13}H_{11}BrFN_4$ molecular formula.

This formed compound name is 3-[2-(4-amino-6-iminopyridin-3(6H)-ylidene)ethenyl]-4-bromo-6-fluorocyclohexa-2, 4-dien-1-iminium. From this link, C_2N_2 is separated to form another fragmented ion $C_{10}H_{11}BrFN_2$ with 258.10 m/z. This formed compound name is (5Z)-4-bromo-2-fluoro-5-[(2Z)-4-iminobut-2-en-1-ylidene]cyclohex-3-en-1-iminium. From this, again loss of C_3H_3N forms another fragment ion with 205.04 m/z with molecular formula of C_7H_8BrFN and name is 4-bromo-2-fluoro-5-methylidenecyclohex-3-en-1-iminium. The proposed whole degradation pathway of DP 5 is given in figure 7. Mass spectrum of DP 5 is presented in figure 9. By all this pattern proposed DP 5 name is {4-bromo-5-[1-ethyl-7-(methylamino)-2-oxo-1,2-dihydro-1,6-naphthyridin-3-yl]-2-fluorophenyl}carbamic acid.

DP 6: Protonated DP 6 is formed under peroxide degradation study which is given in figure 7. Total molecular weight of DP 6 is 526.35 g/mol with $C_{24}H_{21}BrFN_5O_3$ of molecular formula. DP 6 is eluted at 7.95 min in the peroxide degradation study. From DP 6, total molecular formula loss of C_6H_3 resulted in formation of $C_{18}H_{18}BrFN_5O_3$ product with 451.16 g/mol as the molecular weight of the formed product. From this product, another product is formed due to the loss of C_2H_5NO to form $C_{16}H_{13}BrFN_4O_2$ with a molecular weight of 392.20 g/mol. By-product ion of $C_{16}H_{13}BrFN_4O_2$ forms another by product that is $C_{15}H_{11}BrFN_4O$ with 362.17 of molecular weight. Product ion of $C_{15}H_{11}BrFN_4O$ also forms two product ions, one of the formed compounds is $C_{13}H_{14}BrFN_3$ by the loss of C_2H_7NO and another formed product is C_7H_6BrFN with 203.03 of m/z formed by the loss of $C_8H_5N_3O$.

From compound of $C_{13}H_{14}BrFN_3$, another product ion is formed due to the loss of C_6H_6BrF, with molecular weight of 136.17 m/z. The degradation pathway of DP 6 is elucidated in figure 7 and mass spectrum is given in figure 10. Finally formed DP 6 molecular name is 3-(2-bromo-4-fluoro-5-(3-phenylureido)phenyl)-1-ethyl-7-(methylamino)-2-oxo-1,2-dihydro-1,6-naphthyridine 1-oxide.

Method validation

Linearity: In the linearity study, we used the linear method to determine the relation of the concentration of ripretinib to their area measurement responses. The linear relationship was seen in from 5 to 30 µg/mL of ripretinib. Regression equation observed for ripretinib is $y = 21850x - 22817$ ($R^2 = 0.9997$). These results justify that the method adopted is suitable for both qualitative and quantitative determination of ripretinib in the concentration ranges studied.

Precision and ruggedness: The values of the precision and ruggedness assay percentages and the corresponding % RSD of ripretinib are precise and reproducible (Table 3). The measured assay value for ripretinib is 99.75 %. As far as the precision is concerned the % RSD of 0.494 for intraday measurements for ripretinib are 0.437, 0.297 in interday precision day 1 and 2 for day 2 for ripretinib and 0.58% RSD for ruggedness studies. This suggests that daily estimates are quite dependable. Based on the results, the values were all approaching very close to 100% confirming that the analytes were accurately estimated in the proposed method.

Robustness: The robustness results shown in table 4 highlight that the analytical method used for testing ripretinib was dependable under different conditions. The mobile phase was adjusted to a mixture of ethanol and 0.01% formic acid in water in the ratio of 60:40 (v/v), with an assay percentage of 98.88%. Changing the mobile phase composition to a 70:30 ratio (v/v) improved the assay percentage to 99.87%. Similarly, the method produced reliable results when the flow rates of the mobile phase and the detector wavelength were altered. These findings indicate that the method consistently performs well, providing reliable resolution, peak shape, plate count and assay percentage across different conditions.

Table 3
Precision and ruggedness results of Ripretinib

S.N.	Analyte	Ripretinib
1	% Assay	99.75
2	% RSD in Intraday precision	0.494
3	% RSD in Interday precision Day 1	0.437
	Day 2	0.297
4	% RSD in Ruggedness	0.580

Table 4
Robustness results of Ripretinib

Condition changed	Tailing factor	% Change	% Assay
Ethanol and 0.01% formic acid in water in the ratio of 60:40 (v/v)	0.99	1.016	98.88
Ethanol and 0.01% formic acid in water in the ratio of 70:30 (v/v)	0.98	0.130	99.87
0.75 mL/min flow rate	1.01	0.547	99.45
0.65 mL/min flow rate	0.99	0.010	99.99
266 nm wavelength	1.02	0.768	99.23
256 nm wavelength	0.99	0.439	99.56

Table 5
Accuracy results of Ripretinib

Target Amount in µg/mL	Spiked amount in µg/mL	Final amount in µg/mL	Area values	% recovery	%RSD
10	5	15	295870.6	99.23	0.346
10	5	15	297775.3	99.87	
10	5	15	296165.2	99.33	
10	10	20	418067.1	100.19	0.525
10	10	20	414946.3	99.44	
10	10	20	413861.4	99.18	
10	15	25	526646.5	100.54	0.491
10	15	25	523765.5	99.99	
10	15	25	521513.1	99.56	

Table 6
Solution stability of Ripretinib

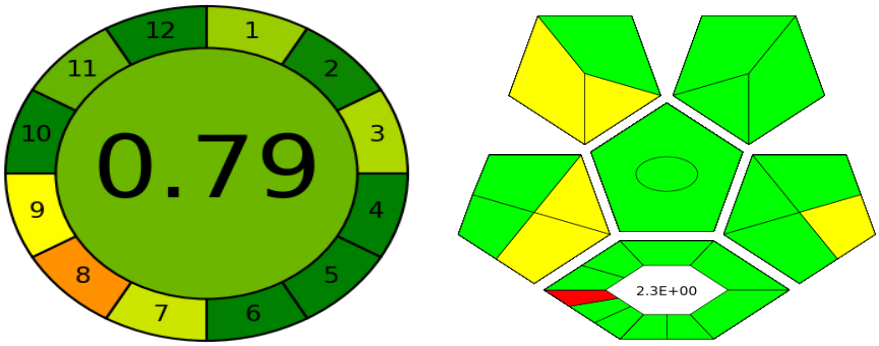
Time Interval	Standard	Sample
0 hour (Initial)	99.91	98.66
24 hours (after)	99.89	98.74
48 hours (after)	99.71	98.61
% difference	0.18	0.13

Accuracy: Accuracy was tested at three concentration levels: 50%, 100% and 150%. For 50%, 15 µg/mL of ripretinib was used; for 100%, 20 µg/mL and for 150%, 25 µg/mL were used. The recovery rates for Ripretinib were 99.48% at 50%, 99.60% at 100% and 100.03% at 150%, all within acceptable limits. The results of accuracy are given in table 5.

Solution stability: To assess solution stability, a 100% standard solution of ripretinib was stored at room temperature and tested at various intervals. The stability was evaluated by comparing the % difference between injected samples and standards. The %differences were 0.13% for the sample and 0.18% for the standard indicating minimal degradation and excellent stability of ripretinib solutions over 48 hours. Stability results are presented in table 6.

Assessment of Green Analytical Chemistry: This study was designed with a strong emphasis on environmentally friendly practices in developing a simple analytical procedure to analyse ripretinib and its degradation products. Unlike traditional methods that rely on harmful solvents like acetonitrile and methanol, this approach utilizes safer and green solvents such as ethanol and water. These solvents were carefully selected for preparing both the mobile phase and sample solutions to reduce the environmental impact of the process. Furthermore, a 100 mm column was chosen for the analysis that allows shorter analysis run time. This column not only decreases the amount of solvent utilized but also reduces energy consumption that makes the method more sustainable and efficient.

The AGREE and GAPI software tools were utilized to evaluate the environmental impact of the proposed analytical method. The analytical method demonstrated a high AGREE score of 0.79 (Figure 11A) which exhibits strong alignment with green chemistry principles. The method excels in areas such as minimal sample, solvent usage, non-hazardous chemicals, energy efficiency and comprehensive environmental assessment, which collectively contribute to its sustainable profile. The GAPI tool further provided a detailed visual overview, using pictograms and pentagrams to illustrate the method's environmental impact.



(A) AGREE **(B) GAPI**

Figure 11: Green assesment pictograms noticed in AGREE and GAPI tools for the proposed analytical procedure

The analysis showed mostly green and yellow pictograms indicating no significant environmental concerns (Figure 11B). The GAPI rating of 2.3E+00 confirmed the method's low environmental impact. The yellow pictograms were associated with sample handling and preparation, suggesting that there are areas where further improvements could be made to reduce environmental risks.

This study finding can be one of the choices for the analysis of Ripretinib with environment sustainability.

Conclusion

This study successfully developed and validated a green analytical HPLC method for evaluating DPs of ripretinib. This method utilizes eco-friendly solvents such as ethanol and water, minimizes the use of harmful chemicals and reduces waste, thereby diminishing its environmental impact. Comprehensive optimization of the mobile phase, along with rigorous validation for parameters like linearity, accuracy, precision and sensitivity, ensured the method's reliability. The identification of six distinct degradation products through LCMS/MS further underscores the method's effectiveness in structural characterization.

The evaluation of greenness using GAPI and AGREE tools confirms that the developed technique significantly reduces hazardous solvent usage while maintaining excellent chromatographic performance. In conclusion, this green analytical approach is a significant advancement for the quantification of Ripretinib and its DPs, contributing to safer and more sustainable pharmaceutical practices.

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