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Forced Degradation and Structural Elucidation of MET/RET Kinase Inhibitors: A Comprehensive Review on Tepotinib, Selpercatinib, and Capmatinib

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Abstract
Targeted kinase inhibitors for treating MET (mesenchymal-epithelial transition factor), RET REarranged during Transfection), and MET-induced non-small cell lung cancer (NSCLC) include drugs like tepotinib, selpercatinib and capmatinib. To ensure the quality and safety of these drugs, stability studies are to be conducted. This review includes recent forced degradation studies using complementary in silico and experimental studies. The MET inhibitor tepotinib, which is used for NSCLC, produced five DPs between the values of m/z 511-527 under acidic, basic, oxidative, and photolytic stress conditions using UHPLC-QTOF-MS/MS. Zeneth an in-silico software, predicted eight possible degradation products (DPs) of tepotinib under the simulated stress conditions, but only five degradation products were actually formed in lab experiments i.e DP-1, 2, 3, 4, 5. Derek & Sarah which are the toxicity prediction tools, scanned the predicted degradation products DP-3, DP-5 as nephrotoxic and DP-3 as mutagenic respectively. RET kinase inhibitor selpercatinib, which is used for NSCLC and thyroid cancer, under acidic forced degradation conditions (stress testing), formed a single impurity called sel-1 ($C_{28}H_{29}N_7O_3$), which was isolated using semi-preparative Liquid Chromatography with high purity and structurally elucidated by HRMS and NMR, revealing cytotoxicity against HepaRG and MKN-1 cells versus the parent drug. The MET inhibitor Capmatinib, which is used for NSCLC, under forced degradation conditions produced three major degradation products—DP1- DP3—where Degradation Product-1 is formed by acid & base hydrolysis, DP2 and DP3 are formed by photolysis, and DP2 was isolated and confirmed by using the ^{1}H NMR with the stability-indicating UHPLC-Q-TOF-MS method developed.

Keywords: MET inhibitors, RET inhibitors, forced degradation, stability studies, degradation products, in silico prediction

Introduction
Tepotinib, an orally administered drug under the brand Tepmetko, is a selective and potent mesenchymal-epithelial transition kinase inhibitor approved by the Food and Drug Administration in February 2021 for metastatic non-small cell lung cancer [1]. In March 2020, the Japanese Ministry of Health, Labour, and Welfare approved tepotinib to treat advanced or recurrent non-small cell lung cancer [2]. It is a newly approved drug which need degradation studies to be conducted under stress conditions like varying pH, temperature, oxidation, and light exposure, which may affect the stability of the drug substance and drug product over time. This helps identify degradation products as per International Council for Harmonisation Q1A(R2) guidelines [3]. Innovation in stability profiling uses in silico tools via Zeneth software, a computational approach to predict potential degradation products of a drug under different virtual stress conditions and virtual degradation product pathway mapping [4]. Tepotinib literature is limited to two liquid chromatography-mass spectrometry methods. One study included an in silico and experimental analysis of the metabolic aspect using liquid chromatography-mass spectrometry analysis conducted by Abdelhameed *et al*. Another study developed a liquid chromatography-tandem mass spectrometry method by Atwa *et al*. for quantification of tepotinib in human liver microsomes [5, 6]. Mahajan *et al*. addressed the gap by conducting stability profiling by integrating in silico predictions (Zeneth for degradation pathways/degradation products) with experimental ultra-high-performance liquid chromatography/liquid chromatography-quadrupole time-of-flight-

tandem mass spectrometry validation under International Council for Harmonisation stress conditions like acidic, basic, oxidative, thermal, photolytic in tepotinib. Toxicity was assessed using Derek and Sarah software. Tepotinib's therapeutic reliability is ensured by International Council for Harmonisation-aligned stability profiling [7]. Rearranged during transfection proto-oncogene is present on gene 10 of humans, which encodes a transmembrane receptor tyrosine kinase that helps in cell signaling. Rearranged during transfection variants include KIF5B, TRIM33, CCDC6, and NCOA4, which are fusion mutations, and M918T, which is a point mutation [8]. Rearranged during transfection is responsible for various cancers like non-small cell lung cancer and thyroid cancers. Rearranged during transfection mutations affect only 2% of non-small cell lung cancer cases; clinical trials are important due to the large global patient population [9]. Among non-smoking lung adenocarcinoma patients, young patients are prone to rearranged during transfection mutations [10]. Among 20% of thyroid cancer patients, rearranged during transfection fusion mutations have been identified [11]. In 2020, selpercatinib (LOXO-292) was approved by the United States Food and Drug Administration for market release [12], an oral selective rearranged during transfection inhibitor that blocks rearranged during transfection signaling and downstream molecules, resulting in cell proliferation of mutating tumor cells. Selpercatinib targets particular genetic defects specified to rearranged during transfection-driven cancers, enabling personalized treatment [13]. It is commonly used in the treatment of advanced, spreading lung cancer (non-small cell lung cancer) in adults as they mainly target both the primary and secondary rearranged during transfection mutations that delay or prevent cancer from becoming resistant toward the drug, improving long-term therapy [14]. Rearranged during transfection fusion-positive thyroid cancer and advanced or metastatic rearranged during transfection-mutant medullary thyroid cancer are also treated using selpercatinib [15]. During the chemical synthesis of selpercatinib, various intermediates are produced according to a published patent [16]. During the study of selpercatinib's related substances, researchers may discover new compounds with anti-tumor activity, leading to novel drug candidates discovery. Advanced analytical methods are needed to ensure the purity and potency of selpercatinib. For ensuring the purity of selpercatinib, preparative liquid chromatography plays a key role in isolating pure selpercatinib, its structurally similar products, and lowest concentrations. To isolate an impurity from vancomycin, Wang *et al*. used a new two-dimensional preparative liquid chromatography technique. Nonlinear preparative liquid chromatography was developed by Jin *et al*. and investigated the principle for purifying palmitoleic acid by using the octadecyl bonded stationary phase for purification of unsaturated fatty acids; for structural elucidation, high-resolution mass spectrometry and nuclear magnetic resonance are used. For development of stability-indicating assay methods and for forced degradation studies, these techniques are valuable [17]. Liquid chromatography-high-resolution mass spectrometry and nuclear magnetic resonance techniques are employed by Chaganti *et al*. for identification and characterization of degradation impurities of the ubrogepant drug; under forced degradation conditions, eight degradation impurities were formed through and they are structurally characterized [18]. In anti-human immunodeficiency virus drug cabotegravir, under forced degradation conditions, Kovac *et al*. identified four degradation impurities, which are isolated by using preparative liquid chromatography and structurally identified using nuclear magnetic resonance [19]. Saida *et al*. studied the antiretroviral drug dolutegravir under forced degradation conditions and obtained impurities degradation product-1 and degradation product-2, respectively, which are separated by preparative liquid chromatography; for accurate mass determination of degradation product-1 and degradation product-2, high-resolution mass spectrometry is used; for structural confirmation, nuclear magnetic resonance spectroscopic analysis is used [20]. Qin Wang and colleagues conducted a study on selpercatinib's acid-degradation impurity using high-resolution mass spectrometry and nuclear magnetic resonance to identify the impurity from the acid-stressed selpercatinib. The cytotoxicity studies were conducted on the impurity to determine its potential anti-tumor activity. These studies provided the reference data for the production, storage, and use of raw materials and formulations and kept the drug's raw ingredients and finished products safe, stable, and effective [21]. United States Food and Drug Administration approved capmatinib in May 2020 for the treatment of non-small cell lung cancer. In adult patients with metastatic non-small cell lung cancer, capmatinib is given orally that targets c-mesenchymal-epithelial transition factor receptor tyrosine kinase, which helps in organ regeneration and tissue repair. Capmatinib shows anti-tumor activity in human lung cancer; it inhibits mesenchymal-epithelial transition factor phosphorylation by human growth factor or mesenchymal-epithelial transition factor amplification, mesenchymal-epithelial transition factor-mediated downstream signaling protein phosphorylation, and mesenchymal-epithelial transition factor-dependent cancer cell proliferation [22, 23]. The forced degradation studies determine the stability of pharmaceuticals at different environmental factors that affect the stability of the drug and dosage form [24, 25]. To perform forced degradation studies under various stress conditions, International Council for Harmonisation guidelines Q1A and Q1B provide the guidance to develop a stability-indicating assay methods [26, 27]. The amide bond present is responsible for chemical degradation in capmatinib. During degradation studies, various stress conditions may cause the degradation of active ingredients during long-term storage, and degradation products are separated and analyzed using suitable analytical methods [28-32]. Zhou *et al*. conducted a pharmacokinetic study by developing a liquid chromatography-mass spectrometry/tandem mass spectrometry method for the quantitation of capmatinib in rat plasma [33]. Bhangare *et al*.'s seminal study addressed a critical gap in capmatinib by conducting degradation studies and elucidated degradation products under International Council for Harmonisation guidelines; ultra-high-performance liquid chromatography-quadrupole time-of-flight-tandem mass spectrometry and proton nuclear magnetic resonance are used for identification and characterization of the three major degradants. For quantification of amide degradation products, as per International Council for Harmonisation Q2(R1), a validated reversed-phase high-performance liquid chromatography stability-indicating method is used for characterization of first degradation product in capmatinib [34].

Materials and Methods

The reviewed studies employed various experimental protocols, instruments, and software for forced degradation and structural elucidation of tepotinib, selpercatinib, and capmatinib. For tepotinib, Mahajan *et al*. purchased the drug from Clearsynth Labs, Hyderabad, India. High-performance liquid chromatography-grade acetonitrile, formic acid (85% extra pure), and 30% weight/volume hydrogen peroxide, analytical-grade sodium hydroxide, sodium thiosulphate, and hydrochloric acid (37%) were purchased respectively from Sigma-Aldrich Chemicals Pvt. Ltd., Finar Chemicals, and Sisco Research Laboratories Pvt. Ltd. The Milli-Q water was obtained from the Milli-Q system (Evoqua Water Technologies LLC). Instruments used included a Sartorius CPA225D analytical balance calibrated for weighing all samples, an IKA RCT basic hot plate for conducting forced degradation studies purchased from Sigma-Aldrich Chemicals Pvt. Ltd., and photostability studies conducted in a Newtronic Lifecare Equipment's photostability chamber, Mumbai, as per International Council for Harmonisation guidelines [34]. Waters Acquity ultra-high-performance liquid chromatography Class H was used for separation of the degradation products of tepotinib under high-pressure gradients, BioQuaternary manager for accurate solvents mixing. For sample injection, Biosample manager-flow through needle injected 2 microliter sample at controlled temperature, and a photodiode array detector was used. The data integration and analysis were carried out using Empower 3 Software, which provided robust peak resolution at 260 nanometers. Agilent Infinity 1290 series ultra-high-performance liquid chromatography coupled with 6450 ultra-high definition accurate-mass quadrupole time-of-flight mass spectrometer with electrospray ionization was used for characterization of degradation products and for data acquisition, for chemical formulas and rings/double bonds values, Mass Hunter software and Frank Antolasic's calculator were used, respectively. This advanced tools selected by Rupali Mahajan *et al*. helped in identification of five major degradation products of tepotinib and also strengthened the collaboration between experimental results and in silico tools. Degradation studies were carried out by sampling a 500 microgram/milliliter tepotinib using a 1:1 volume/volume mixture of acetonitrile and water and subjected toward forced degradation conditions like acid, base, oxidative, neutral hydrolytic, and photolytic conditions as per International Council for Harmonisation guidelines. After stressing tepotinib samples, alkalinity was adjusted back to neutral pH, which prevents damage to the ultra-high-performance liquid chromatography column; filtration was done through a 0.22 micrometer nylon membrane to remove insoluble particles from the tepotinib samples, ensuring artifact-free preparations that prevent the clogging of the ultra-high-performance liquid chromatography column. This filtered sample was then subjected to separation in ultra-high-performance liquid chromatography using 0.1% formic acid in water mixed progressively with acetonitrile, which efficiently eluted the drug and its impurities with photodiode array detection, resulting in baseline-resolved peaks of five key degradation products, allowing precise quantification and identification. Integration of ultra-high-performance liquid chromatography to liquid chromatography-quadrupole time-of-flight mass spectrometry in electrospray ionization mode, using Mass Hunter software and an elemental calculator to match low-parts per million errors and rings/double bonds, verifying degradation product structures like N-dealkylated ones. In silico Zeneth predicted degradation paths, while Derek/Sarah Nexus tools identified toxicities in degradation products. For separating forced degradation products of tepotinib, 0.1% formic acid as mobile phase A and high-performance liquid chromatography-grade acetonitrile as mobile phase B were used; the flow rate was set to 0.2 milliliter/minute, the injection volume was set as 2 microliter. The total run time was set to 10 minutes. A photodiode array detector at a single wavelength at 260 nanometers was set for the analysis of samples. Liquid chromatography-quadrupole time-of-flight mass spectrometry was directly coupled with ultra-high-performance liquid chromatography using electrospray ionization parameters: 3500 volts capillary, 175 volts fragmentor, 65 volts skimmer voltages, 325 degrees Celsius gas temperature, 8 liters/minute flow, 35 pounds per square inch gauge nebulizer with nitrogen for drying, 2 microliter injections over 50-1000 mass-to-charge ratio range. Degradation product structures were elucidated by elemental calculator, minimizing parts per million mass errors and using rings/double bonds values for formula confirmation. Zeneth (8.1.1) was used to predict forced degradation pathways of tepotinib, toxicity and mutagenicity; Lhasa Limited's Derek Nexus 6.2.0, Sarah Nexus 3.2.0 for mutagenic potential, integrating results with experimental data were used. Mahajan *et al*. optimized ultra-high-performance liquid chromatography separation of tepotinib degradation products through trials by screening the buffers and columns; a mix of 0.1% formic acid in water and acetonitrile using the CSH C18 column at a slow flow of 0.2 milliliter per minute and normal room temperature were taken; this gave clear peaks where resolution was over, tailing under 1.5, and over 2000 theoretical plates. Peak purity was confirmed; five degradation products were formed under acid, base, peroxide stress, but tepotinib remained photostable [7].

For selpercatinib, the drug was purchased from Shenli-Kanghua, Jinan, China. High-performance liquid chromatography solvents-acetonitrile, methanol were purchased from Merck KGaA, Darmstadt, Germany; analytical reagents hydrochloric acid, phosphoric acid, sodium hydroxide, dimethyl sulfoxide, and triethylamine were purchased from China National Pharmaceutical Shanghai, China; 3-(4, 5-dimethylthiazole-2)-2, 5-diphenyltetrazolium bromide from Biyuntian Company, China; cell media-Dulbecco's Modified Eagle Medium, Roswell Park Memorial Institute-1640, fetal bovine serum were purchased from Gibco USA; dimethyl sulfoxide-d6 was purchased from Sigma-Aldrich, USA. Instrumentation used included Agilent reversed-phase high-performance liquid chromatography (1200) and semi-preparative liquid chromatography (1260 Infinity II), Varian Cary100 ultraviolet/visible spectrophotometer for absorbance; this study employed the AB SCIEX ZenoTOF 7600 high-resolution mass spectrometry system for the mass determination of degradation impurities, the Bruker AVANCE III HD-500 nuclear magnetic resonance spectrometer for detailed structural elucidation using proton and carbon-13 spectra, and the Merck Milli-Q water purification system to generate high-purity water for chromatographic and sample preparations. The acid degradation of selpercatinib generated a crude product, which was further processed to isolate the primary impurity. This degradant, designated sel-1, was obtained as a white amorphous powder following targeted chromatographic separation and solvent removal. Selpercatinib and its

impurity sel-1 were analyzed using high-performance liquid chromatography using Agilent 5 HC-C18 (2) column with diameter of 4.6 millimeter, 250 millimeter length, and 5 micrometer average particle size at 35 degrees Celsius with a flow rate of 1.0 milliliter/minute, ultraviolet detection at 235 nanometers, and 10 microliter injections using gradient elution from mobile phase A (90:10 acetonitrile:2 millimolar potassium dihydrogen phosphate buffer, pH 2.4 with 0.4% triethylamine) and phase B (acetonitrile): 0-2 minutes at 5% B, 2-15 minutes to 15% B, 15-30 minutes to 35% B, 30-35 minutes to 45% B, 35-36 minutes back to 5% B, and 36-45 minutes hold at 5% B. For structural elucidation, sel-1 was subjected to high-resolution mass spectrometry in a 100 nanogram/milliliter 50% aqueous methanol solution using heated electrospray ionization with parameters including 3500 volts spray voltage, capillary temperature at 320 degrees Celsius, probe heater 350 degrees Celsius, 45 liters/minute sheath gas, 15 liters/minute auxiliary gas, 100 microampere max spray current, and S-Lens radio frequency level of 50; while both selpercatinib and sel-1, 10 milligram each, were dissolved in dimethyl sulfoxide-d6, which underwent proton nuclear magnetic resonance with a frequency of 600 megahertz and carbon-13 nuclear magnetic resonance with 150 megahertz spectroscopy with a broadband observe probe. Cytotoxicity was assesed using 3-(4, 5-dimethylthiazole-2)-2, 5-diphenyltetrazolium bromide assay over five human cancer cell lines—MKN-1, A549, HepaRG, MDA-MB-231, and PANC-1—by seeding log-phase cells at 1 x 10^5/milliliter in the 96-well plates, treating with various concentrations of selpercatinib and sel-1 with eight replicate wells for 48 hours. After 48 hours, the supernatant was removed, wells washed twice with phosphate-buffered saline. Added 100 microliter 1 milligram/milliliter 3-(4, 5-dimethylthiazole-2)-2, 5-diphenyltetrazolium bromide and incubated for 4 hours. Discarded the supernatant, added 150 microliter dimethyl sulfoxide, and incubated in dark at 37 degrees Celsius for 30 minutes to solubilize the formazan crystals, measuring optical density at 490 nanometers; inhibition rates calculated as [(1 - experimental optical density/control optical density) x 100%], and deriving half maximal inhibitory concentration from dose-response curve [21].

For capmatinib, the drug was purchased from Chemscene (Jinay Pharmaceuticals, Maharashtra, India); analytical-grade ammonium formate was purchased from Sigma Aldrich, Bengaluru, India; high-performance liquid chromatography-grade acetonitrile, hydrochloric acid, formic acid, sodium hydroxide, and 3% hydrogen peroxide were purchased from Thermo Fisher Scientific India Pvt. Ltd.; purified water was prepared using Milli-Q system (Millipore, USA). Agilent 1260 Infinity II high-performance liquid chromatography system equipped with an Eclipse C18 column, quaternary pump, diode array detector, and autosampler, running Open Lab CDS EZ chrome software, were used for degradation study of capmatinib. Eutech pH 510 meter was used for pH measurements with standard buffers of pH 1.68, 4.0, 7.0, and 10.0. Agilent 1290 Infinity ultra-high-performance liquid chromatography coupled to a 6545 quadrupole time-of-flight mass spectrometer with electrospray ionization source, along with autosampler, quaternary pump, vacuum degasser, and temperature-controlled compartment, was used for liquid chromatography-mass spectrometry characterization of degradation products. Photolytic degradation was conducted using Allen Bradley neutronic photostability chamber per International Council for Harmonisation Q1B option 2, ultraviolet/white fluorescent lamps, while Heratherm hot-air oven was used for thermal and hydrolytic stresses. Structural elucidation of capmatinib and its degradation product 2 was done using proton nuclear magnetic resonance on a Bruker 500 megahertz instrument for determining the peak, using dimethyl sulfoxide-d6 for capmatinib and chloroform-d for degradation product 2, used for solubility, respectively; tetramethylsilane as a reference standard was taken for recording proton spectra, and delta parts per million scale as a representation of chemical shifts was used. Development and optimization of stability-indicating high performance liquid chromatography assay for capmatinib initially involved screening of various buffers like ammonium acetate, ammonium formate, formic acid for better peak display and for checking the compatibility of the buffers used, and the best selected was ammonium formate; selecting 10 millimolar ammonium formate as the aqueous mobile phase (A). Chromatographic separation used an Agilent Eclipse Plus C18 column with diameter of 250 millimeter, length of 4.6 millimeter, and particle size of 5 micrometer with acetonitrile as phase (B) in gradient elution of 10% B at 0-2 minutes, 30% B at 7-10 minutes, 40% B at 19-22 minutes, 10% B at 25-28 minutes. Flow rate was set as 1 milliliter/minute, injection volume was 10 microliter, and detection at 228 nanometers. System suitability was verified with six replicates of 100 parts per million capmatinib, and the system suitability parameters including the tailing factor, retention time, theoretical plates were checked for robust impurity resolution. The high-performance liquid chromatography assay for capmatinib was validated as per International Council for Harmonisation guidelines: linearity determined by calibration curves from the replicate injections, precision by repeated analyses at short and long terms at various levels, accuracy by recovery assessments, sensitivity by noise-based thresholds from the diluted solutions, and specificity determined by interference checks with formulation components against the controls [34].

Results

Liquid chromatography-quadrupole time-of-flight-tandem mass spectrometry elucidated five degradation products of tepotinib: degradation product-1 formed by hydrolysis of cyanide group into an amide; degradation product-2 was an oxidized form of degradation product-1 with a nitrogen-oxygen bond and key fragments from oxygen loss; degradation product-3 was a double-oxidized form with two such bonds and a unique fragment showing nitrogen oxidation in the ring; degradation product-4 acid hydrolysis to carboxylic acid, tepotinib-like fragments; degradation product-5 formed from peroxide stress by adding one oxygen atom (N-oxide) to the pyrimidine ring. Nephrotoxicity and mutagenicity for degradation product-3 and degradation product-5 emphasized these studies to mitigate risks from tepotinib degradation products, while advocating proper storage. This method showed robustness for quality control and ongoing research of pharmaceutical.

Selpercatinib exhibited a single high-performance liquid chromatography peak at 21.373 minutes before acid degradation. Followed by acid degradation, high-performance liquid chromatography analysis showed selpercatinib at 21.315 minutes along with multiple peaks of impurities, most abundant are unidentified impurity, sel-1, at 12.725 minutes; some impurities were not isolated due to low concentrations and were minor. Sel-1, with exact mass 511.2398, was purified using semi-preparative liquid

chromatography, sel-1 retention peak at 8.068 minutes and selpercatinib at 9.872 minutes, yielding a white amorphous powder with 99.1% purity in high-performance liquid chromatography at retention time of 12.622 minutes and ultraviolet maximum at 235 nanometers. High-resolution mass spectrometry indicated a single -CH2 loss from selpercatinib, while proton and carbon-13 nuclear magnetic resonance confirmed its structure as 6-(2-hydroxy-2-methylpropoxy)-4-(6-(6-((6-oxo-1, 6-dihydropyridin-3-yl)methyl)-3, diazabicyclo[3.1.1]heptan-3-yl)pyridin-3-yl)pyrazolo[1, 5-a]pyridine-3-carbonitrile, with residual solvent peaks for methanol and triethylamine hydrochloride. *In vitro* cytotoxicity assays across five tumor cell lines showed sel-1's half maximal inhibitory concentration values (microgram/milliliter) for MKN-1 was 30.96 (stronger than selpercatinib's 40.53), for A549 half maximal inhibitory concentration value was 41.87 (weaker than 8.56), for HepaRG half maximal inhibitory concentration value was 21.50 (stronger than 29.21), for MDA-MB-231 half maximal inhibitory concentration value was 53.11 (weaker than 8.61), and for PANC-1 half maximal inhibitory concentration value was 36.95 (weaker than 9.96).

Capmatinib under forced degradation testing as per International Council for Harmonisation, including acidic, basic, neutral, oxidative, thermal, and photolytic conditions, starting at 1000 parts per million and diluted the samples to 100 parts per million for analysis, showed stability under neutral, oxidative, and thermal stresses conditions; no impurities were detected, but exhibited acidic and basic hydrolysis produced a same impurity due to amide-to-carboxylic acid conversion, and photolytic exposure in solution phase showed two major impurities; in solid phase, it was stable. The degradation study of capmatinib was done using ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry and proton nuclear magnetic resonance spectroscopy. The liquid chromatography-mass spectrometry/tandem mass spectrometry fragmentation behavior of the parent drug was studied for structural characterization before identifying the degradation products. The quasi-molecular ion peak was identified at mass-to-charge ratio 413 [M+H]+, along with major fragments at 382 mass-to-charge ratio, 354 mass-to-charge ratio, and 327 mass-to-charge ratio due to gradual loss of methylamine, carbon monoxide, and hydrogen cyanide. These fragmentation patterns led to characterization of the degradation pathways. Three degradation products (degradation product 1-degradation product 3) were identified under hydrolytic and photolytic stress conditions. Hydrolytic degradation produced degradation product 1 due to amide-to-carboxylic acid conversion, while photolytic stress produced degradation product 2 and degradation product 3 due to oxidation and hydroxylation at the methylene bridge between quinoline and imidazole moieties. Degradation product 2 was isolated and characterized by using proton nuclear magnetic resonance, confirming the conversion of methylene oxidation to a carbonyl group. The degradation study of capmatinib, including the stability testing under neutral, oxidative, and thermal conditions, revealed robustness under neutral hydrolysis, oxidative, and thermal stress, but degradation under acidic hydrolysis, basic hydrolysis, and photolysis. The degradants included degradation product 1 formed due to methyl amide hydrolysis to carboxylic acid and degradation product 2, degradation product 3 formed due to methylene group oxidation/hydroxylation between quinoline-imidazole rings. Degradation product 2 was isolated using proton nuclear magnetic resonance. A validated liquid chromatography-mass spectrometry/tandem mass spectrometry stability-indicating method separated capmatinib and its degradants for analysis. This study characterized three degradants, established hydrolytic, photolytic pathways, and explained the degradation mechanisms.

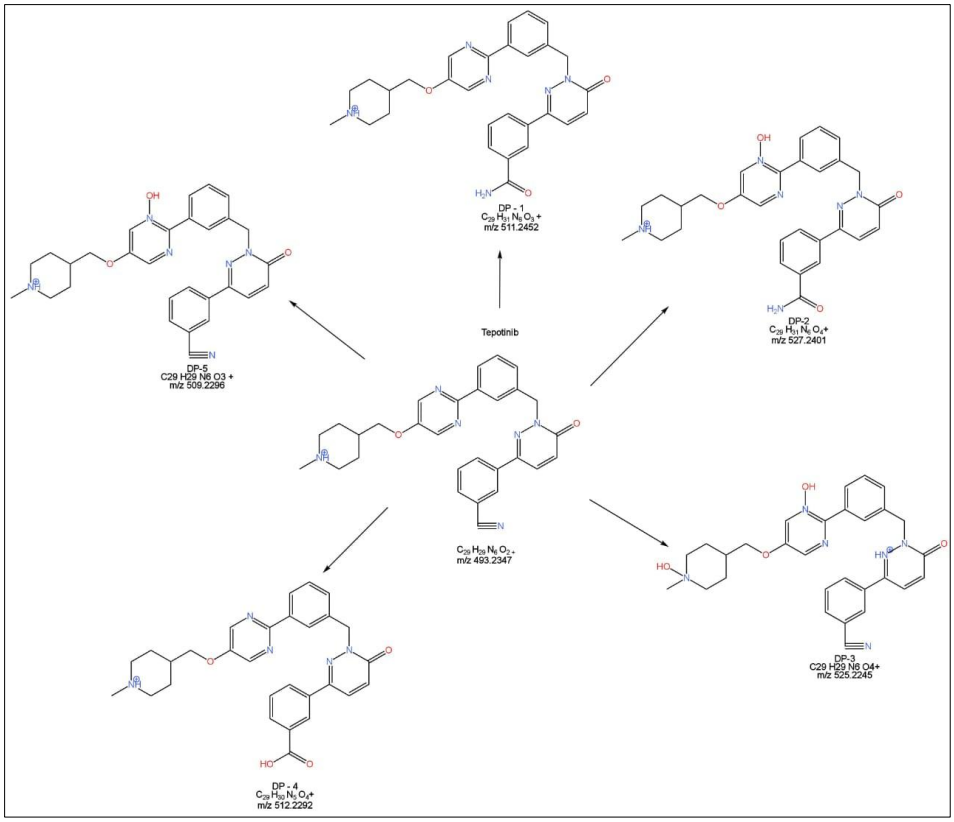

Fig 1: Degradation products of Tepotinib

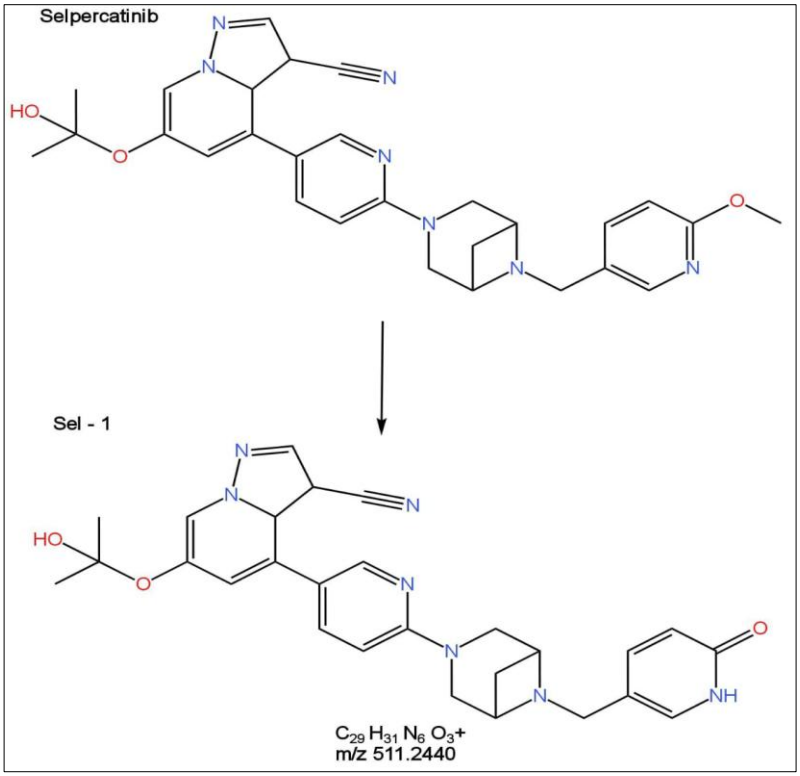

Fig 2: Degradation products of Selpercatinib

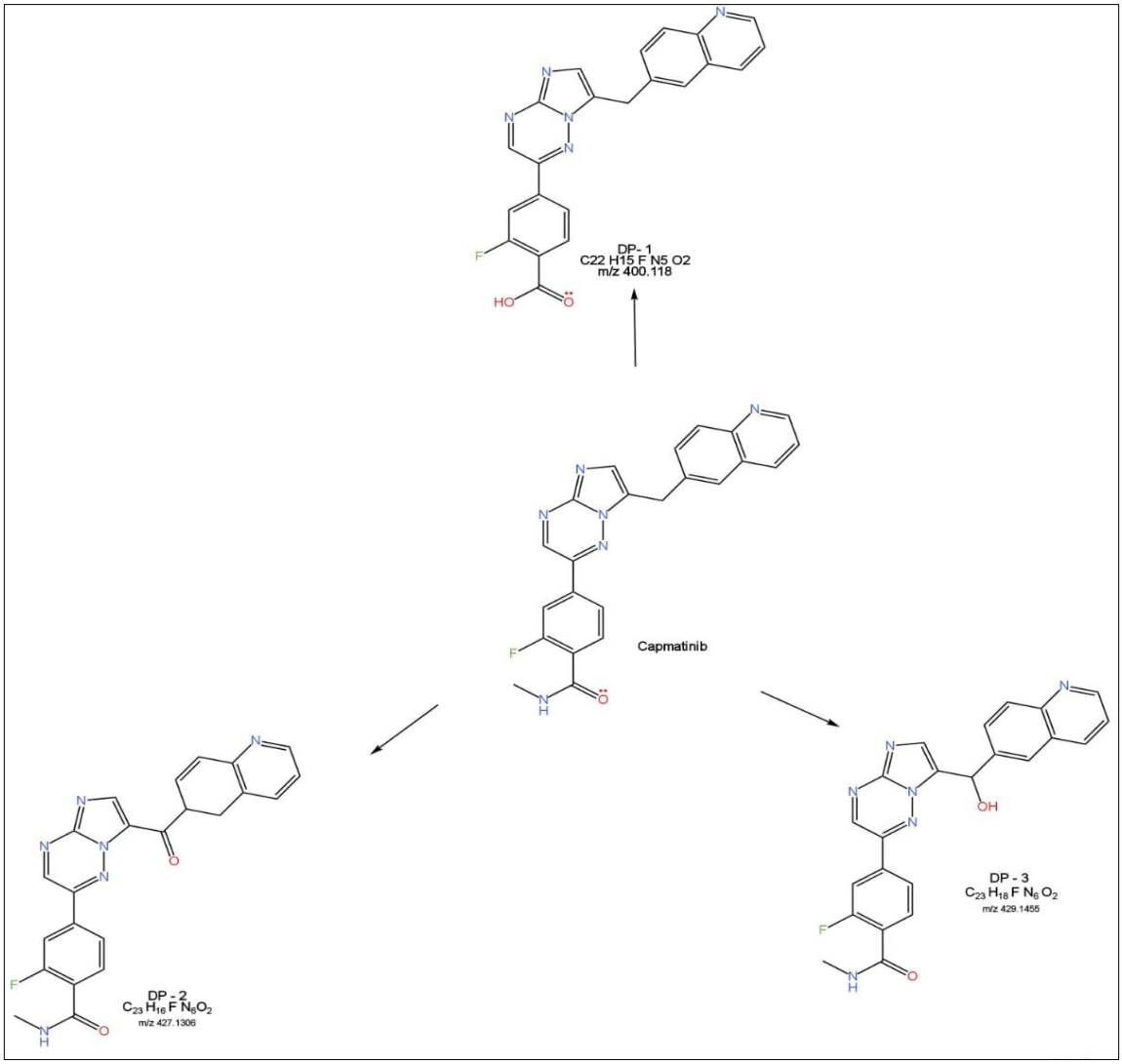

Fig 3: Degradation products of Capmatinib

Table 1: Analytical Method Validation for Capmatinib (9 Font size, Times New Roman, Normal, Bold)

Validation Parameter	Experimental conditions	Results	Interpretation
Linearity	Eight concentrations (20-160 µg/mL), triplicate injections	Correlation coefficient (R^2 = 0.999)	Excellent linearity across range
Intra-day Precision	Spiked levels: 80, 100, 120 µg/mL (n = 3, same day)	Avg. areas: 3747.22, 4519.90, 5365.39 mAU;%RSD = 0.567, 0.241, 0.629	Highly precise within-day analysis
Inter-day Precision	Spiked levels: 80, 100, 120 µg/mL (two consecutive days)	Avg. areas: 3724.44, 4498.12, 5278.89 mAU;%RSD = 0.258, 0.096, 0.108	Excellent reproducibility between days
Accuracy (Recovery)	80, 100, 120 µg/mL (triplicate)	Recovery = 100.78-104.06%;%RSD < 0.21%	Accurate and within acceptable limits
LOD (Limit of Detection)	Based on S/N = 3:1	0.25 ppm (S/N = 3.39)	Highly sensitive
LOQ (Limit of Quantitation)	Based on S/N = 10:1	0.5 ppm (S/N = 10.06)	Reliable for low-level quantification
Specificity	Drug tested with excipients (Mg stearate, MCC, crospovidone, mannitol, SLS)	No interference at CMT retention time	Method specific and selective

Table 2: Forced Degradation Conditions for Tepotinib, Selpercatinib, Capmatinib

Drug Name	Stress Condition	Stressor & Concentration	Sample Prep / Details	Temperature & Duration	Post-Processing
Tepotinib	Acid Hydrolysis	1 N HCl (1:1 v/v)	Stock solution mixed 1:1 with acid	70°C for 4 days	Neutralize with 1 N NaOH (200 µL/500 µL), add diluent, filter (0.22 µm nylon)
	Base Hydrolysis	0.1 N NaOH (1:1 v/v)	Stock solution mixed 1:1 with base	Room temp for 17 h	Neutralize with 0.1 N HCl (200 µL/500 µL), add diluent, filter (0.22 µm nylon)
	Oxidative	6% w/v H_2O_2 (1:1 v/v)	Stock solution mixed 1:1, kept in dark	Room temp for 6 h	Quench with sodium thiosulphate, add diluent, filter (0.22 µm nylon)
	Neutral Hydrolysis	Water (1:1 v/v)	Stock solution mixed 1:1 with water	70°C for 4 days	Add diluent, filter (0.22 µm nylon)
	Photolytic	UV (365 nm) & Visible (ICH Q1B)	Solid/solution not specified	4 days (≥200 Wh/m² UV; 1.2 million lux·h)	Add diluent, filter (0.22 µm nylon)
Selpercatinib	Acid Hydrolysis (for DP isolation)	4 M HCl (150 mL added to 300 mL drug solution)	600 mg in 300 mL solvent (2 mM KH_2PO_4 + 0.4% TEA, pH 2.5, in ACN:H_2O 10:90)	80°C for 40 h	Neutralize with 4 M NaOH, freeze-dry, redissolve in H_2O, centrifuge (12, 000 rpm, 10 min)
Capmatinib	Neutral Hydrolysis	Water	-	-	-
	Oxidative	3% H_2O_2	-	Not specified	-
	Thermal	Dry heat	Solid state	70°C	-
	Acidic/Basic Hydrolysis	Likely HCl/NaOH	-	-	-
	Photolytic (Solution)	UV/Visible light	Solution phase	-	-
	Photolytic (Solid)	UV/Visible light	Solid state	-	-

Table 3: Forced Degradation Product Analysis of Tepotinib, Selpercatinib, Capmatinib

Drug Name	Degradation Product (DP)	Stress Condition(s)	Observed m/z ($[M+H]^+$)	Molecular Formula	Key Structural Change / Difference from Parent	Key Analytical & Toxicity Insights
Tepotinib	DP-1	Acidic, basic, and trace peroxide hydrolysis	511.2440	$C_{29}H_{31}N_6O_3^+$	Not explicitly stated. Δ +18 Da (+H_2O) from parent suggests hydrolysis or addition.	UHPLC-QTOF-MS/MS; In-silico toxicity (Derek/Sarah): Skin sensitization = Equivocal; Nephrotoxicity = Negative; Mutagenicity = Negative.
	DP-2	Oxidative stress	527.2390	$C_{29}H_{31}N_6O_4^+$	Not explicitly stated. Δ +34 Da (+O_2) from parent suggests di-oxidation.	UHPLC-QTOF-MS/MS; Toxicity:Non-sensitizer; Nephrotoxicity=Negative; Mutagenicity = Negative.
	DP-3	Oxidative stress	525.2232	$C_{29}H_{29}N_6O_4^+$	Not explicitly stated. Δ +32 Da (+O_2) from parent with unsaturation change suggests oxidation/dehydrogenation.	UHPLC-QTOF-MS/MS; Toxicity: Skin sensitization= Equivocal; Nephrotoxicity=Equivocal; Mutagenicity = Inactive (Derek) / Positive (Sarah).
	DP-4	Acidic hydrolysis	512.2283	$C_{29}H_{30}N_5O_4^+$	Not explicitly stated. Δ +19 Da (+O_2, -N) from parent suggests	UHPLC-QTOF-MS/MS; Toxicity: Skin sensitization = Equivocal;

					oxidation with nitrogen loss.	Nephrotoxicity = Negative; Mutagenicity
	DP-5	Oxidative stress	509.2289	$C_{29}H_{29}N_6O_3^+$	Not explicitly stated. Δ +16 Da (+O) from parent suggests oxidation.	UHPLC-QTOF-MS/MS; Toxicity: Skin sensitization = Equivocal; Nephrotoxicity = Equivocal; Mutagenicity = Equivocal (Sarah).
Selpercatinib	Sel-1 (primary)	Acid-induced (major impurity)	511.2440	$C_{29}H_{31}N_6O_3^+$	Loss of one -CH_2 group from the parent molecule.	Structure confirmed by $^1H/^{13}C$ NMR. NMR spectra showed residual solvent peaks (methanol, triethylamine HCl).
	Other impurities	Acid-induced (minor)	-	-	-	-.
Capmatinib	DP1	Acidic / Basic Hydrolysis	400.118	$C_{22}H_{15}FN_5O_2$	Hydrolysis of methyl amide group → conversion to carboxylic acid.	Characterized by UHPLC-QTOF-MS/MS.
	DP2	Photolytic	427.1306	$C_{23}H_{16}FN_6O_2$	Oxidation of methylene bridge to form a carbonyl (C=O).	Characterized by UHPLC-QTOF-MS/MS and confirmed by ^1H NMR.
	DP3	Photolytic	429.1455	$C_{23}H_{18}FN_6O_2$	Hydroxylation at the methylene bridge (addition of OH).	Characterized by UHPLC-QTOF-MS/MS.

Discussion

This review covers forced degradation studies of tepotinib, selpercatinib, and capmatinib which are targeted kinase inhibitors that acts against non-small cell lung cancer and thyroid malignancies, this study also highlights stability studies and analytical techniques as per International Council for Harmonisation guidelines. Tepotinib showed changes to hydrolytic and oxidative stresses, producing five degradation products, indicates the toxicity i.e nephrotoxic and mutagenic, degradation product-3 and degradation product-5, it's necessary to optimize storage and formulation condition to avoid risks; a robust ultra-high-performance liquid chromatography method conforms its sensitivity. Selpercatinib produced sel-1 impurity, isolated using semi-preparative liquid chromatography, identified by high-resolution mass spectrometry/nuclear magnetic resonance, and showing cytotoxicity in HepaRG/MKN-1 cells, conforming Sel-1 as therapeutic drug. Capmatinib showed stablility under neutral, oxidative, and thermal stresses but degraded under hydrolytic conditions and photolytic conditions, with degradation product 2 confirmed by proton nuclear magnetic resonance and impurities conformed by liquid chromatography-mass spectrometry/tandem mass spectrometry, identifying pathways for quantification. Collectively, these studies for oncology drugs, encouraging clinical trials and personalized therapies. For Tepotinib study is done by in silico-experimental using Zeneth with ultra-high-performance liquid chromatography-quadrupole time-of-flight-tandem mass spectrometry/nuclear magnetic resonance. sel-1 can be repurposed; DPs of capmatinib under photolytic conditions need ICH Q1B photostability standards. Overall, forced degradation studies help in establishing the safe, effective MET/RET cancer drugs.

Conclusion

The forced degradation studies of tepotinib, selpercatinib, and capmatinib showed degradation products under various stress conditions, also determining their toxicity. Tepotinib showed five degradation products under hydrolytic and oxidative stresses conditionds, with nephrotoxic and mutagenic risks. Selpercatinib acid- impurity sel-1 provides promising cytotoxicity effect over cancer cell lines, which can be formulated as drug candidate. Capmatinib is stabile under neutral, oxidative, and thermal conditions but unstable under hydrolysis and photolysis stress conditions, forming three degradation products. These studies shows advanced analytical twchniques like ultra-high-performance liquid chromatography-quadrupole time-of-flight-tandem mass spectrometry, nuclear magnetic resonance, and in silico tools, storage conditions, and quality control for these kinase inhibitors in non-small cell lung cancer and thyroid cancer treatments are helpful in studying the drug stability. Future research should mainly focus on clinical studies and enhanced formulation of personalized oncology drugs.

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