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Characterization of Degradation Products of Selpercatinib by Mass Spectrometry: Optimization of Stability-Indicating HPLC Method for Separation and Quantification of Process Related Impurities of Selpercatinib

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The study aimed to investigate a novel approach by utilizing liquid chromatography in coupled with mass spectrometry (LC-MS) to resolve, analyze and characterize significantly less quantity of stress degradation products (DPs) of selpercatinib along with its process related impurities. The analytes resolved on ZORBAX Eclipse (250 mm) stationary phase that was maintained employing 0.5 M sodium perchlorate at pH 5.4 methanol and acetonitrile in 50:20:30 (v/v) pumped at 0.8 mL/min isocratic flow and 241 nm wavelength. The method produces very high correlate linear curve in 30-240 µg/mL for selpercatinib and 0.03-0.24 µg/mL for its impurities with significantly low detection limit of 0.01 µg/mL for impurities. Selpercatinib pure compound was subjected to stress studies and chromatographic results confirm the formation of four DPs, which were characterized with the interpretation of their mass fragmentation pattern. The DPs were identified as 6-hydroxy-4-(6-(6-((6-methoxypyridin3-yl)methyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)pyridin-3-yl)pyrazolo[1,5-*a*]pyridine-3-carbonitrile (DP 1), 6′-(6-((6-methoxypyridin-3-yl)methyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)-1,2-dihydro-[3,3′-bipyridin]-5-ol (DP 2), (6-hydroxy-4-(6-(6-(pyridin-3-ylmethyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)pyridin-3-yl)pyrazolo[1,5-*a*]pyridine-3-carbonitrile (DP 3), 1-amino-6′-(6-((6-methoxypyridin-3-yl)methyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)-1,2-dihydro-[3,3′-bipyridin]-5-ol (DP 4), 6-(2-hydroxy-2-methylpropoxy)-4-(6-(3-methylpiperazin-1-yl)pyridin-3-yl)pyrazolo[1,5-*a*]pyridine-3-carbonitrile (DP 5) and 6-(2-hydroxy-2-methylpropoxy)-4-(6-(3-methylpiperazin-1-yl)pyridin-3-yl)pyrazolo[1,5-*a*]pyridine-3-carbonitrile (DP 6). Based on findings, it was concluded that this method was effectively applied for the regular quantification of impurities of selpercatinib in formulations. Additionally, it demonstrated applicability for the identification of both known and unknown impurities of selpercatinib.

Keywords: Selpercatinib, Impurity analysis, Degradation studies, Degradation products, Mass spectral characterization.

INTRODUCTION

The efficacy of a pharmaceutical product (drug) strictly associated with its microbial, chemical and physical stability. The occurrence of impurities and possible formation of degradation products (DPs) in a drug product can affect its distribution, absorption, metabolism and excretion and plays significant role on drug safety profile [1]. In early drug development stages, pharmaceutical companies routinely performing forced/stress degradations to evaluate the failure stability and toxicity due to DPs [2]. The drug degradation process evaluation against various conditions and structural characterization of DPs was considered as an integral part in pharmaceutical product development [3]. Furthermore, it was pivotal in the drug manufacturing process, determination of its shell life, formulation as well as packaging. Hence, stress studies accelerate generation of DPs by drug exposer to various physico-chemical conditions to assess both degradation pathways and stability [4].

The regulatory agencies like FDA (Food & Drug Administration), WHO (World Health Organization) and ICH (International Council for Harmonization), *etc.* recommend stress exposure of drug to acid, base, dry heat, oxidation (peroxide) and UV light conditions among others along with their protocols including pH, temperature and stress exposer time [5,6]. The high-performance liquid chromatography (HPLC) or ultra HPLC coupled with high resolution mass spectrometry (HRMS) and/or with suitable detectors represent most commonly employed analytical techniques for both structural and quantitative evaluation of DPs [7].

Selpercatinib (Fig. 1a), marketed under the brand name Retevmo, is a targeted therapy drug used in the treatment of certain types of cancers. It belongs to a class of medications known as tyrosine kinase inhibitors (TKIs) [8]. Selpercatinib is specifically designed to target abnormalities in genes like RET (rearranged during transfection), which can drive the growth of cancer cells. Selpercatinib has shown effectiveness in treating various cancers with RET gene alterations, including non-small cell lung cancer (NSCLC), medullary thyroid cancer (MTC) and certain other solid tumors [9]. Clinical trials have demonstrated promising results, leading to its approval by regulatory agencies for specific indications. Common side effects may include high blood pressure, fatigue, diarrhea and changes in liver function [10].

The review of literature was conducted to identify various analytical methods reported for quantification of selpercatinib. One HPLC method [11] reported for evaluation of selpercatinib in dosage forms whereas one HPLC-MS/MS [12] bioanalytical method reported for analyzing selpercatinib in combination with pralsetinib, brigatinib and lorlatinib. Existing literature indicates a lack of reported analytical methods for quantifying genotoxic impurities in selpercatinib and no one has characterized the stress degradation compounds of selpercatinib. Therefore, this article introduces an optimized HPLC method for quantifying genotoxic impurities of selpercatinib and LCMS/MS characterization of DPs. There is no method for quantifying process contaminants and characterizing stress degradation products in the literature. This work aims to develop a simple HPLC method for resolution and quantification of process associated impurities of selpercatinib. Further, the study also intended to identify DPs generated due to stress of selpercatinib using LCMS/MS analysis.

EXPERIMENTAL

Selpercatinib drug (98.55% purity), its impurity 1, 2 and 3 (Fig. 1) were gifted by Eli Lilly and Company (India) Private Limited, Hyderabad, India. The tablet formulation with 80 mg of selpercatinib having Retevmo® brand was brought from the local pharmacy. HPLC-grade methanol, acetonitrile and Milli-Q® water were acquired from Merck Chemicals, Mumbai, India. Reagent-grade chemicals, including hydrochloric acid, hydrogen peroxide, sodium acetate, acetic acid and sodium hydroxide obtained from Fisher Scientific, Mumbai, India.

This study utilizes Agilent 1100 instrument, USA with quaternary isocratic pump (G1311 A). Impurities of selpercatinib drug were introduced employing temperature variable autosampler (G1329A) with 0.1 to 1500 µL injection capacity. Programmable ultraviolet (UV) detector (G1314 A) was employed for detecting column eluents and chromatographic visualization was done with the coupling of Agilent ChemStation software. Waters LCMS system from Japan employing triple quadrupole mass detector and MassLynx software was employed for LCMS study.

Preparation of standard solution: Selpercatinib pure drug and impurities were individually prepared at 1 mg/mL (1000 µg/mL) concentration. This involved precisely weighed 50 mg of compound into a 50 mL flask that contain 25 mL of diluent. Analytes were then dissolved in diluent with the help of sonicator. Following this, undissolved analytes removed employing 0.2 µm filters and flask filled till mark to achieve a concentration of 1000 µg/mL for selpercatinib and impurities separately.

Preparation of test solution: Retevmo® capsules with 80 mg dosage were employed in preparing sample solution. Precisely weighed fine tablet powder equivalent to 25 mg selpercatinib drug was taken in 25 mL flask containing 15 mL diluent. Analytes in formulation were then dissolved in diluent with the assistance of sonicator. Following this, undissolved capsule particles were removed by employing 0.2 µm filters and flask filled till mark and then diluted to standard concentration. This solution was utilized for assessing method efficiency for evaluation of formulation assay.

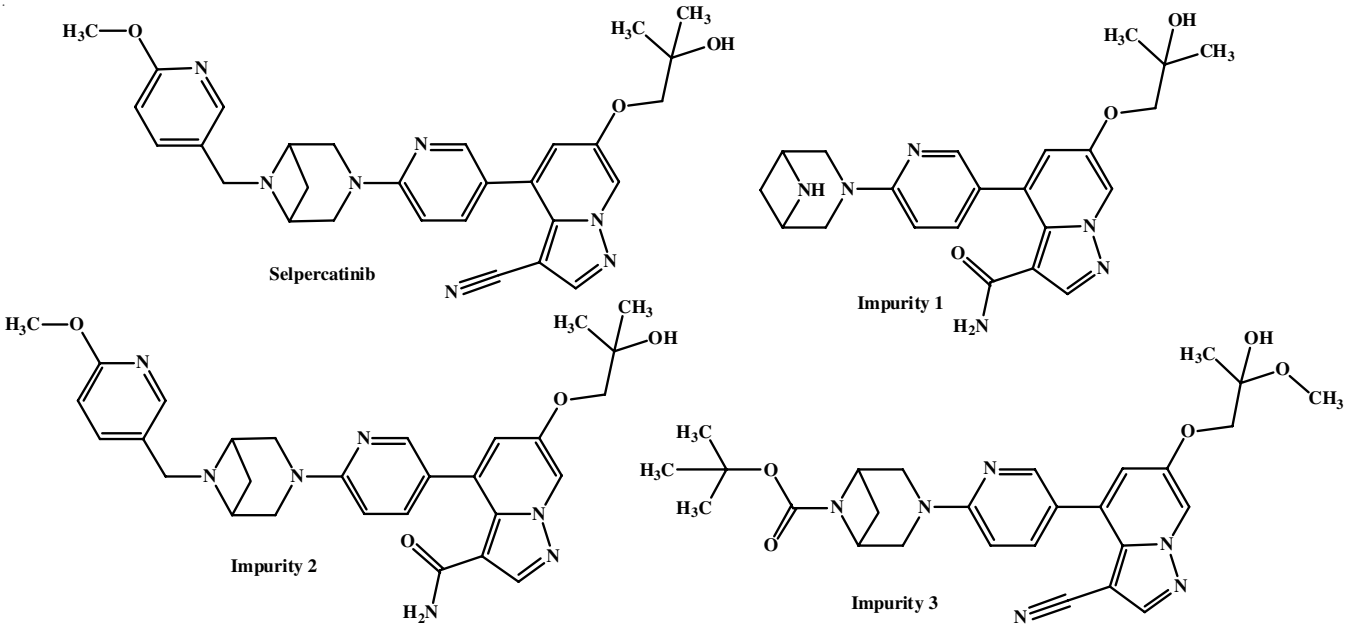

Fig. 1. Selpercatinib and its impurities

Method optimization: The optimal detector wavelength for detecting pure selpercatinib drug and its impurities was determined with UV-visible spectrophotometer. A 10 µg/mL of selpercatinib and its impurities was individually scanned and resulting absorption spectra confirmed appropriate wavelength. Stationary phases of various configurations from different manufacturers were tested for achieving finest resolution of analytes. The mobile phase was fine-tuned by adjusting the various mobile phases with varying pH levels and flow rate. The method which resolved analytes effectively was investigated for validation.

Method validation: The developed method underwent validation to ensure its suitability analyzing selpercatinib and its impurities. Parameters such as specificity, accuracy, precision, linearity, sensitivity, ruggedness and reproducibility were evaluated according ICH guidelines [13-15] and literature [16-23].

The method sensitivity was assessed by measuring the responses at signal-to-noise ratio (s/n) and s/n of 10:1 and 3:1, respectively was considered as quantification limit (LOQ) and detection limit (LOD). The predetermined concentration of impurities at nominal concentration was assessed in the proposed method. Linearity was assessed by analyzing calibration standard solutions at various concentration levels, ranging from the LOQ level to the maximum upper concentration. The calibration range was finalized by least squares linear regression that produces high correlation.

Method precision was assessed by evaluating selpercatinib and its impurities at LOQ level and middle concentration in linearity. It was assessed by performing intraday (with in day), interday precision (day change) and ruggedness (analyst change). The %RSD (relative standard deviation) of area response was determined and % RSD of not more than 2 was treated as acceptable.

The impact of minor variations in proposed conditions of resolution and analysis of selpercatinib and its impurities was examined in robustness. In this, both positive and negative variation in column temperature, wavelength, pH and composition of mobile phase was performed. The deviation in resultant chromatographic results was summarized and results were carefully assessed for robustness evaluation. Method accuracy was assessed by performing spiked recovery at four independent levels in calibration range (LOQ, 50%, 100% and 150% levels of specification limit). Triplicate analysis was performed in every level and % recovery was evaluated by correlation with calibration results.

Stability indicating ability of proposed method was verified through forced degradation studies. Five independent conditions, includes base, peroxide, acid, thermal and ultraviolet stress studies, were performed for standard selpercatinib. After subjecting the samples to stress conditions, they underwent neutralization, dilution to the desired concentration and analysis in optimized method. The acquired chromatograms and chromatographic responses were then utilized to evaluate stability indicating features of method.

The characterization of DPs involves careful interpretation of mass spectral results. The analysis incorporated a splitter directing 40% of column eluents and eluents recorded employing mass detector operating in electrospray ionization (ESI) positive ionization mode. Essential conditions, including capillary, fragmentor and skimmer voltages, were carefully configured and nitrogen gas was employed for drying and nebulization.

The suggested approach was utilized to identify and quantify process-related impurities of selpercatinib drug in samples. Both Retevmo® solution, spiked with a predetermined strength of impurities and unspiked solution were subjected to analysis using optimized method. The impurities percentage assay was evaluated by correlating area response of individual impurity to its equivalent linear curve.

RESULTS AND DISCUSSION

The absence of any analytical method in the literature for quantifying process related impurities of selpercatinib, present work sought to establish a straightforward HPLC method for evaluation of process-related impurities 1, 2 and 3 of selpercatinib. Different configurations of columns including amino, octadecylsilane (ODS), phenyl-hexyl and cyano columns were considered for best resolution of selpercatinib along with impurities in the study. The mobile phase composition with suitable pH range was optimized by varying different composition of mobile phase solvents including buffer strength and composition. The acetate and phosphate buffers with pH value of 3-6 were varied for resolution of selpercatinib impurities. The hydrophilic ionizable functional groups in the analytes such as –OH, –C=N, –NH, *etc.*, were effectively resolved with the mobile phase containing pH buffers and hence various buffers with different pH value was studied for effective resolution. Using phosphate buffer in mobile phase produces asymmetric peaks with poor resolution was observed. The inclusion of water in mobile phase fails to separate the analytes, hence, these were not studied in the optimization process. Efficient separation of selpercatinib and its impurities was accomplished employing a ZORBAX Eclipse (250 × 4.6 mm, 5.0 µm) column using 0.5 M sodium perchlorate solution at pH 5.4 methanol and acetonitrile in 50:20:30 (v/v) pumped isocratically at 0.8 mL/min. Column temperature, sample injection volume and detector wavelength were consistently held at 35 ºC, 20 µL and 241 nm, respectively throughout the analysis.

Under these conditions, the peaks representing selpercatinib and its impurities exhibited symmetric shapes, with a resolution between nearby peaks exceeding 2. Identification of individual analyte in standard solution involved by injecting individual standard solutions and correlating individual retention times (t_R) with the standard. The t_R observed at 7.34 min for selpercatinib, 12.26 min for impurity 1, 3.37 min for impurity 2 and 8.39 min for impurity 3. The method efficiency was confirmed to be falls within acceptable limits for tail factor, theoretical plates and resolution (Table-1). Chromatograms of the placebo and impurities spiked selpercatinib solution are presented in Fig. 2. The chromatographic results suggest that this method is specific.

The system suitability of selpercatinib and its impurities was confirmed, with acceptance criteria set at theoretical plates (N) of > 2000, resolution (R_S) of > 2 and asymmetric factor

TABLE-1
SUMMARY OF THE RESULTS OBSERVED IN METHOD VALIDATION

Parameter	Selpercatinib	Impurity 1	Impurity 2	Impurity 3
Retention time	7.34	12.26	3.37	8.39
Relative retention time	–	1.67	0.46	1.14
Relative response factor	–	0.057	0.045	0.065
R_S	11.45	9.95	–	4.26
A_S	0.93	0.98	0.96	1.03
N	5436	10650	3244	8977
Range (µg/mL)	30-240	0.03-0.24	0.03-0.24	0.03-0.24
Slope	8691.5	346699	276680	441013
Intercept	1921.6	- 168.2	211.67	1890.5
r^2	0.9991	0.999	0.9992	0.9992
Intra-day precision	0.31	0.35	0.36	0.18
Inter-day precision (day 1)	0.16	0.15	1.57	0.54
Inter-day precision (day 2)	0.31	0.61	0.43	0.16
Ruggedness	0.58	0.59	0.92	0.44
Accuracy at 50 % level[$]				
Prepared (µg/mL)	90	0.09	0.090	0.090
Recovered (µg/mL)	89.56	0.090	0.090	0.090
% Recovery	99.51	99.60	99.74	99.52
% RSD	0.94	0.81	0.91	1.13
Accuracy at 100 % level[$]				
Prepared (µg/mL)	120	0.120	0.120	0.120
Recovered (µg/mL)	119.41	0.120	0.120	0.119
% Recovery	99.50	99.94	99.64	99.54
% RSD	0.95	0.30	0.98	1.15
Accuracy at 150 % level[$]				
Prepared (µg/mL)	150	0.15	0.150	0.150
Recovered (µg/mL)	149.17	0.149	0.150	0.150
% Recovery	99.44	99.64	99.72	99.76
% RSD	1.21	0.96	0.83	1.00
Sensitivity				
LOD (µg/mL)	–	0.009	0.009	0.009
LOQ (µg/mL)	–	0.03	0.03	0.03

r^2 = Correlation coefficient; [$]Average results (n = 3); [$$]Average results (n = 3)

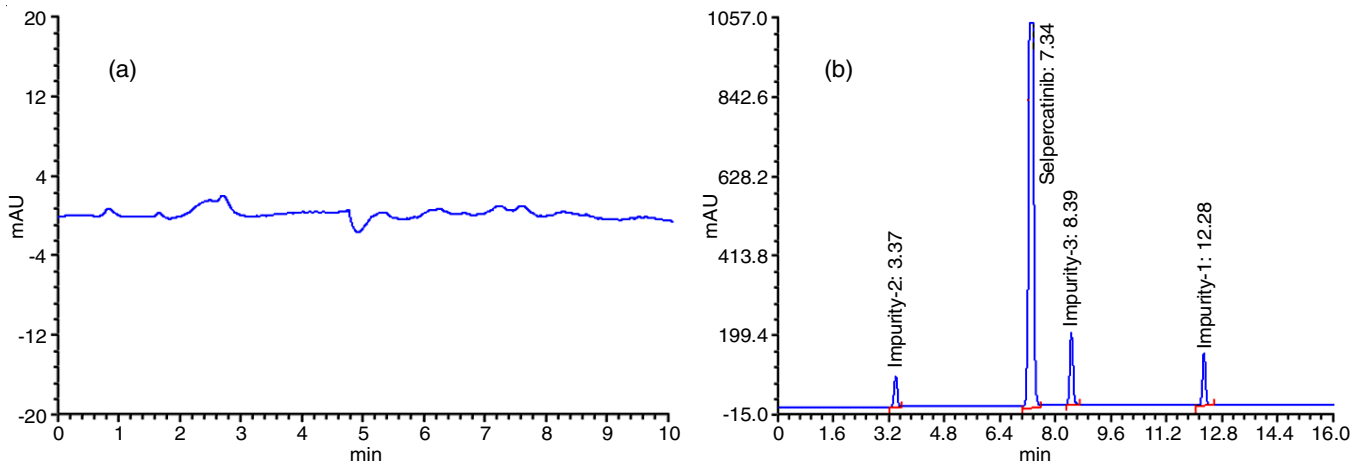

Fig. 2. Chromatograms observed in specificity study, (a) Chromatogram observed for analyzing the placebo solution in the developed method that doesn't show any chromatographic detections; (b) Standard chromatogram observed for analysing selpercatinib pure drug solution spiked with 0.1 % impurities that clearly show well resolved, retained symmentic peaks corresponds to analytes in the study

(A_S) of ≤ 2.0, adhering to guidelines. The observed N, R_S and A_S values for analyte peaks fell within permissible level (Table-1) indicating that method is appropriate for analyzing selpercatinib and its impurities.

The sensitivity of the method was assessed by evaluating LOD and LOQ of impurities using the s/n method. The results revealed LOD values of 0.009 µg/mL, 0.03 µg/mL for impurity 1, 2 and 3, respectively, indicating the high sensitivity of method at very low concentrations (Table-1). Linear dilutions of impurities, basing on detection limit, were prepared to determine the quantification limit, with the concentration of selpercatinib designed to contain 0.1% of each impurity. Calibration curves

were obtained within linear ranges of 30-240 µg/mL for selpercatinib and 0.03-0.24 µg/mL for impurities. The linear calibration curves demonstrated the applicability pf method for quantifying impurities at very low concentrations.

Method precision and reproducibility were assessed through intra-day, inter-day precision and ruggedness. Permissible % RSD of < 2 was achieved for selpercatinib and its impurities (Table-1), demonstrating the precision and ruggedness of the method for analyzing impurities of selpercatinib.

Accuracy of the method was determined by the recovery (R%) of pre-determined amounts of analyte in a placebo, which was assessed with formula $R\% = C_{found} \times 100/C_{taken}$. Three successive replicate injections of control samples, containing concentrations of 90 µg/mL, 120 µg/mL and 150 µg/mL for selpercatinib spiked with 0.1% of the investigated impurities, were conducted. Selpercatinib and the studied impurities exhibited an acceptable % recovery range of 98-102%. The % RSD at each level was achieved to be less than 2%, underscoring the method accuracy. A summary of validation results in this optimized method is tabulated in Table-1.

Method robustness was assessed by intentionally introducing slight modifications to the proposed method to evaluate its efficacy in resolving and quantifying selpercatinib and its impurities. In this investigation, variations were made in the mobile phase composition to 50:25:25 (v/v) and 50:15:35 (v/v) of sodium acetate and methanol, labeled as MP 1 and MP 2, respectively. The mobile phase pH was adjusted to 5.3 in pH 1 and 5.5 in pH 2. Wavelength changes included 246 nm in WL 1 and 236 nm in WL 2, while column temperature was modified to 30 ºC in CT 1 and 40 ºC in CT 2. The % area response change along with system suitability of selpercatinib and its impurities under these altered conditions are summarized in Table-2. No considerable changes were observed in any altered conditions and confirmed the robustness of the method.

Forced degradation studies were conducted to identify the potential degradation products, providing insights into degradation pathway and molecule stability. The ability of an analytical method to indicate stability is crucial for confirming product shelf life. Thus, method stability indicating capability was established and DPs formed were characterized through LCMS/MS. The percentage of degradation was evaluated as 9.84% in acidic conditions, 8.57% in basic conditions, 7.18% in peroxide, 6.99% in thermal and 5.17% in UV light degradation studies. The UV light and thermal degradation studies showed nominal significant degradation, in thermal degradation with no detectable degradation products. In acid degradation, a high % degradation of 9.84% was observed and the chromatogram revealed DP 1 and 4 at t_R of 2.26 min and 5.25 min, along with impurity 3. Base degradation visualizes two DPs at t_R of 3.80 min (DP 2) and 9.91 min (DP 5). Peroxide degradation showed the peaks corresponding to DP 3 and DP 6 retained at 4.09 min and 10.05 min, respectively along with impurity 1.

The results of the peak purity tests employing PDA detector verified that selpercatinib peak exhibited purity and homogeneity in studied stress tests. The mass balance of each stressed results fell within the range of 97.00% to 99.15%. The negligible alteration in the assay of selpercatinib in the presence of impurities, coupled with the peak purity results from stress samples, provides confirmation of the specificity and stability-indicating capability of method. Table-3 outlines the results and Fig. 3 displays chromatograms obtained during the forced degradation study.

LCMS/MS characterization of DPs: The LCMS/MS analysis was employed to characterize the DPs generated during

TABLE-2
ROBUSTNESS RESULTS

Changed condition	Parameter	Selpercatinib	Impurity 1	Impurity 2	Impurity 3
MP 1	% Change	0.14	0.68	0.86	0.23
	t_R	5546	10643	3244	8903
	N	0.92	0.99	0.95	1.02
MP 2	% Change	0.34	0.03	0.21	0.31
	t_R	5688	10765	3203	8956
	N	0.93	0.98	0.95	1.04
pH 1	% Change	0.25	0.53	0.48	0.23
	t_R	5453	10760	3209	9077
	N	0.91	0.95	0.94	1.04
pH 2	% Change	0.37	0.76	0.65	0.63
	t_R	5409	10776	3299	8992
	N	0.94	0.96	0.95	1.04
WL 1	% Change	0.17	0.15	0.81	0.41
	t_R	5456	10734	3205	8967
	N	0.91	0.94	0.95	1.01
WL 2	% Change	0.46	0.26	0.16	0.81
	t_R	5509	10702	3319	8944
	N	0.93	0.99	0.95	1.02
CT 1	% Change	0.65	0.17	0.01	0.13
	t_R	5403	10657	3245	8945
	N	0.91	0.99	0.93	1.02
CT 2	% Change	0.82	0.20	0.41	0.14
	t_R	5573	10763	3219	8904
	N	0.93	0.99	0.97	1.02

TABLE-3
FORCED DEGRADATION RESULTS OF SELPERCATINIB

Condition	% Degradation[$] of selpercatinib	% Assay[$] of selpercatinib	% Mass balance[$]	DPs identified
Acid	9.84	90.16	99.15	Two DPs (DP 1 and 4) were identified
Base	8.57	91.43	98.34	Two DPs (DP 2 and 5) were identified
Peroxide	7.18	92.82	97.98	Two DPs (DP 3 and 6) were identified
Thermal	6.99	93.01	96.88	No degradation product identified
UV light	5.17	94.83	97.00	One DP (DP 3) was identified

[$]Average of three replicate experiments; Mass balance = sum of drug assay & total impurities

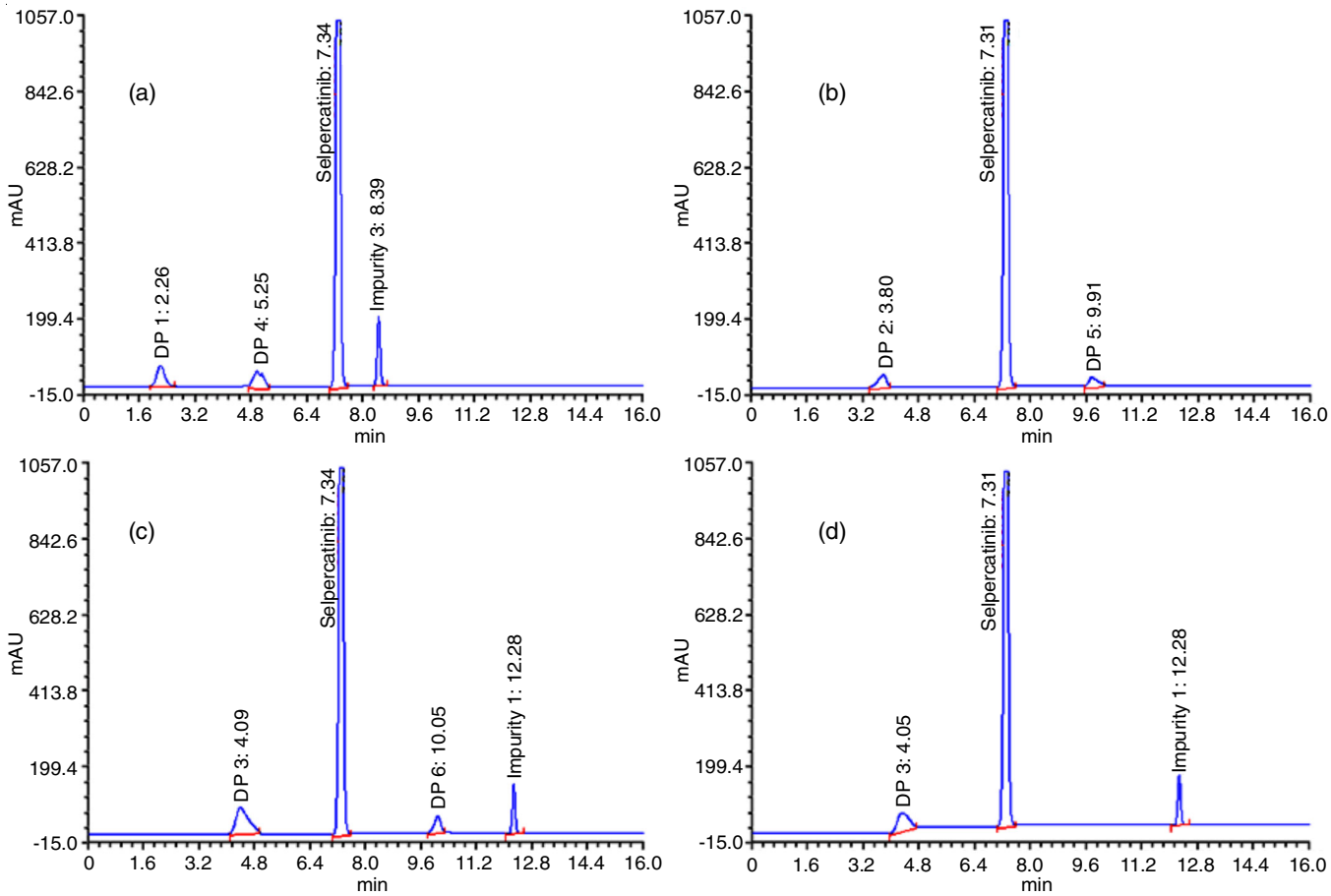

Fig. 3. Chromatograms observed in stress degradation study of selpercatinib, (a) Acid degradation chromatogram clearly showing well resolved peaks for DP 1 and DP 4, (b) Base degradation chromatogram showing peaks correspond to DP 2 and DP 5, (c) Peroxide degradation chromatogram showing peaks correspond to DP 3 and DP 6, (d) UVdegradation chromatogram showing peak correspond to DP 3

the forced degradation of selpercatinib. The forced degradation chromatogram revealed the presence of six distinct DPs, designated as DP 1 to 6, as confirmed by their respective retention times. All DPs, alongside the standard selpercatinib, displayed high intense molecular ions in positive ionization mode ($[M+H]^+$). Collision-induced dissociation spectra of selpercatinib and its DPs were recorded to acquire the structural information. The mass spectra of selpercatinib DPs are illustrated in Fig. 4.

The ESI/MS spectrum of DP 1, visualized at 2.2 min, reveals a prominent parent ion at *m/z* 454 (M+1). The spectra visualize significant fragment ion at *m/z* 161 (M+1) with the molecular formula $C_8H_6N_3O$. The sequential correlation of mass fragmentation date, the molecular structure of DP 1 was proposed with chemical name of 6-hydroxy-4-(6-(6-((6-methoxypyridin-3-yl)methyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)pyridin-3-yl)pyrazolo[1,5-*a*]pyridine-3-carbonitrile and its fragmentation mechanism is presented in Fig. 5.

DP 2, identified at a retention time (t_R) of 3.8 min in base degradation chromatograms, underwent characterization through LCMS/MS analysis. The fragmentation spectra of DP 2, depicted in Fig. 5b, revealed the fragments corresponding to the parent ion at *m/z* of 392 (M+1), confirming molecular weight of DP 2 as 391.46. The fragmentation spectra display a prominent fragment ion at *m/z* of 161 (M+1) with the molecular formula $C_8H_6N_3O$. DP 2 characterized as 6′-(6-((6-methoxypyridin-3-yl)methyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)-1,2-dihydro-[3,3′-bipyridin]-5-ol. Peak purity CID studies of DP 2

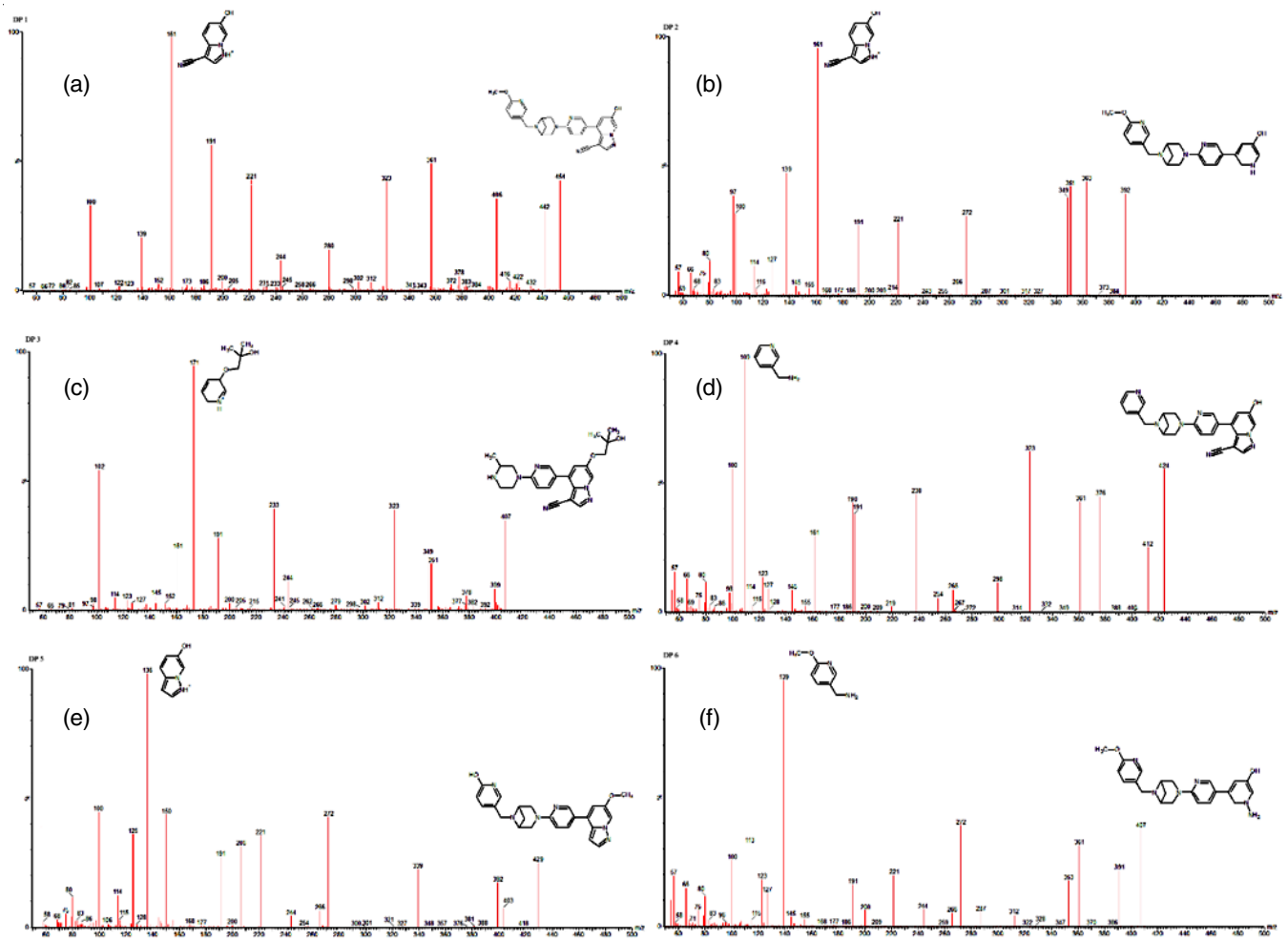

Fig. 4. Mass spectra of DPs formed during forced degradation study; mass fragmentation spectra identified at t_R of 2.2 min (a), 3.8 min (b), 4.0 min (c), 5.2 min (d), 9.9 min (e) and 10.1 (f) for DP 1, DP 2, DP 3, DP 4, DP 5 and DP 6, respectively

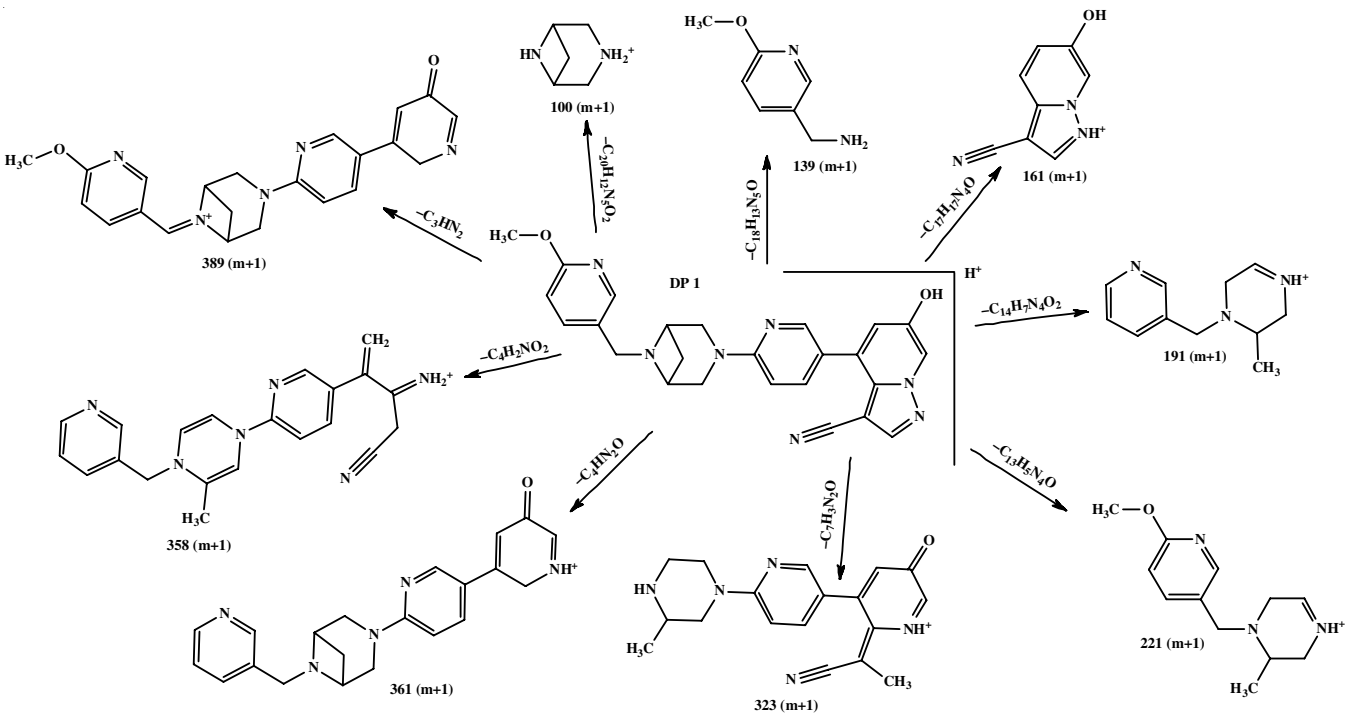

Fig. 5. Mass fragmentation pattern of DP 1

confirmed it as degradation products of DP 1 observed in the study. The fragmentation pattern proposed for DP 2 is shown in Fig. 6.

The peroxide stress chromatogram exhibits DP 3 at t_R of 4.0 min. The mass spectra clearly depict a parent ion at *m/z* 407 (M+1), suggesting a possible molecular formula of $C_{22}H_{26}N_6O_2$. DP 3 was generated by the elimination of C_7H_5NO from selpercatinib. An abundant fragment ion at *m/z* of 171 (M+1) indicate the molecular structure of DP 3, which is characterized as (6-hydroxy-4-(6-(6-(pyridin-3-ylmethyl)-3,6-diazabicyclo-[3.1.1]heptan-3-yl)pyridin-3-yl)pyrazolo[1,5-*a*]pyridine-3-carbonitrile. The proposed fragmentation pattern of DP 3 of selpercatinib, based on the mass fragmentation, is presented in Fig. 7.

The acid degradation chromatogram clearly resolve DP 4 at t_R of 5.2 min and product ion identified with molecular formula of $C_{24}H_{21}N_7O$ by losing C_5H_{10} of selpercatinib and the compound was confirmed as 1-amino-6′-(6-((6-methoxypyridin-3-yl)methyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)-1,2-dihydro-[3,3′-bipyridin]-5-ol. Peak purity CID studies for DP 4 conclusively establish as a degradation products of DP 1 observed in this study. The mass spectrum (Fig. 5d) illustrates a prominent fragment ion at *m/z* of 109 (M+1), resulting from the loss of $C_{18}H_{13}N_5O$ from DP 4. The fragmentation pattern of DP 4 identified in the study is presented in Fig. 8.

The base degradation chromatogram clearly visualizes a peak at 9.91 min and was designated as DP 5, which was not detected in other stress studied performed in the study. The mass fragmentation spectra of DP 5 reveal a prominent parent ion at *m/z* 429 (M+1) when observed under positive ionization mode. Furthermore, the spectrum exhibits fragment ions at *m/z* 136 (M+1), which result from the loss of $C_{17}H_{17}N_4O$. Based on the acquired data (Fig. 9), DP 5 has been identified as 6-(2-hydroxy-2-methylpropoxy)-4-(6-(3-methylpiperazin-1-yl)-pyridin-3-yl)pyrazolo[1,5-*a*]pyridine-3-carbonitrile, possessing a molecular formula of $C_{24}H_{24}N_6O_2$.

The proposed mass fragmentation pattern of DP 6 is illustrated in Fig. 10. The peroxide induced stress degradation chromatogram of selpercatinib visualizes a well resolved and retained peak corresponds to DP 6 at 10.05 min. The mass fragmentation spectra shows parent ion at *m/z* 407 (M+1) corresponds to molecular mass of DP 6. The spectrum visualizes the fragment ion at *m/z* of 139 formed by losing $C_{15}H_{16}N_4O$, 272 formed by losing $C_7H_7N_2O$ and *m/z* 361 (M+1) formed by losing CH_4NO. The collection of these product ions, in conjunction with the parent ion, serves to confirm that DP 6 as 6-(2-hydroxy-2-methylpropoxy)-4-(6-(3-methylpiperazin-1-yl)pyridin-3-yl)-pyrazolo[1,5-*a*]pyridine-3-carbonitrile, with a molecular formula of $C_{22}H_{26}N_6O_2$.

The results confirmed the identification of six distinct degradation products (DPs) during the forced degradation study of selpercatinib. These compounds were characterized through LCMS/MS analysis and structures of DPs formed are presented in Fig. 11.

The method employed for evaluation of process-related impurities in formulations, focusing on the Retevmo® brand formulation of selpercatinib in this study. Both impurity-spiked and unspiked formulation solutions of selpercatinib were analyzed. The chromatogram for the impurity spiked sample analysis clearly visualizes the impurities along with the standard selpercatinib. Conversely, the unspiked formulation analysis visualizes with no detection of impurities 1, 2 and 3. This

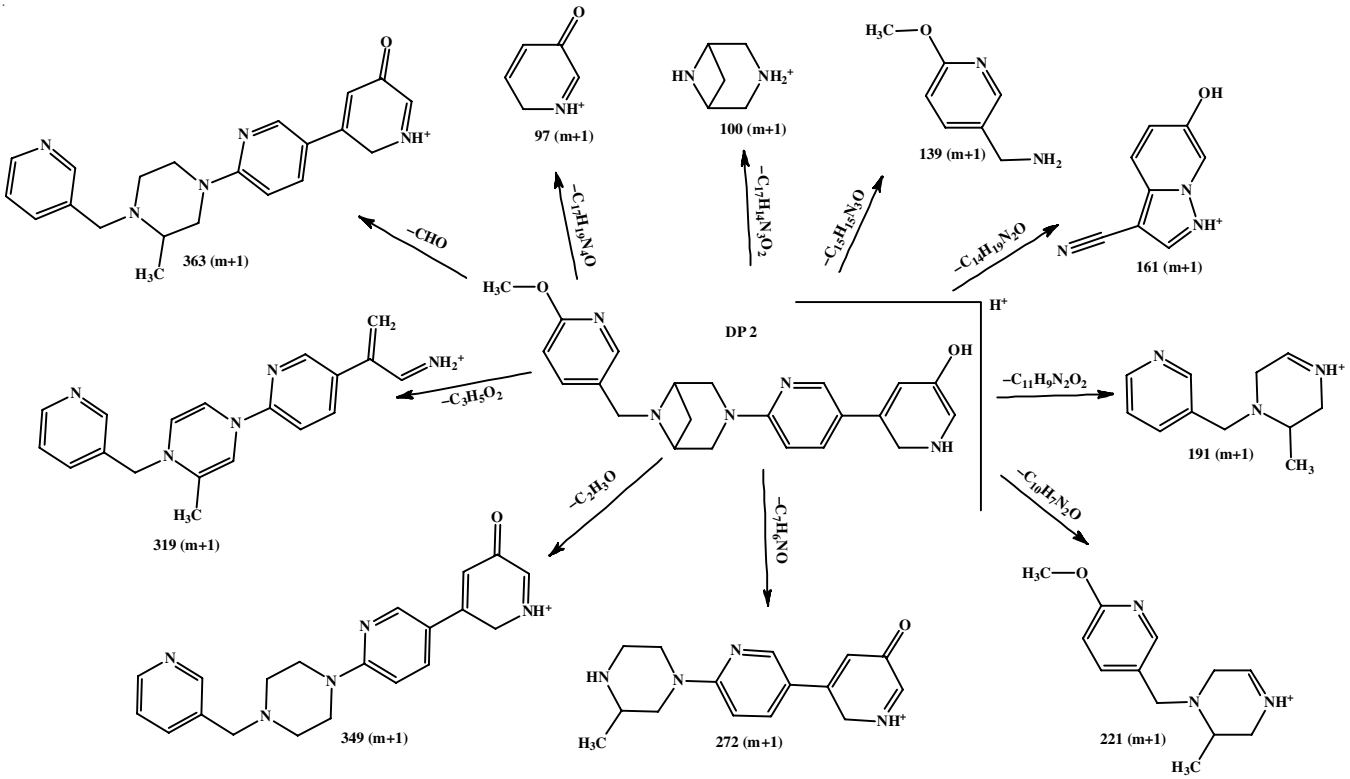

Fig. 6. Mass fragmentation pattern of DP 2

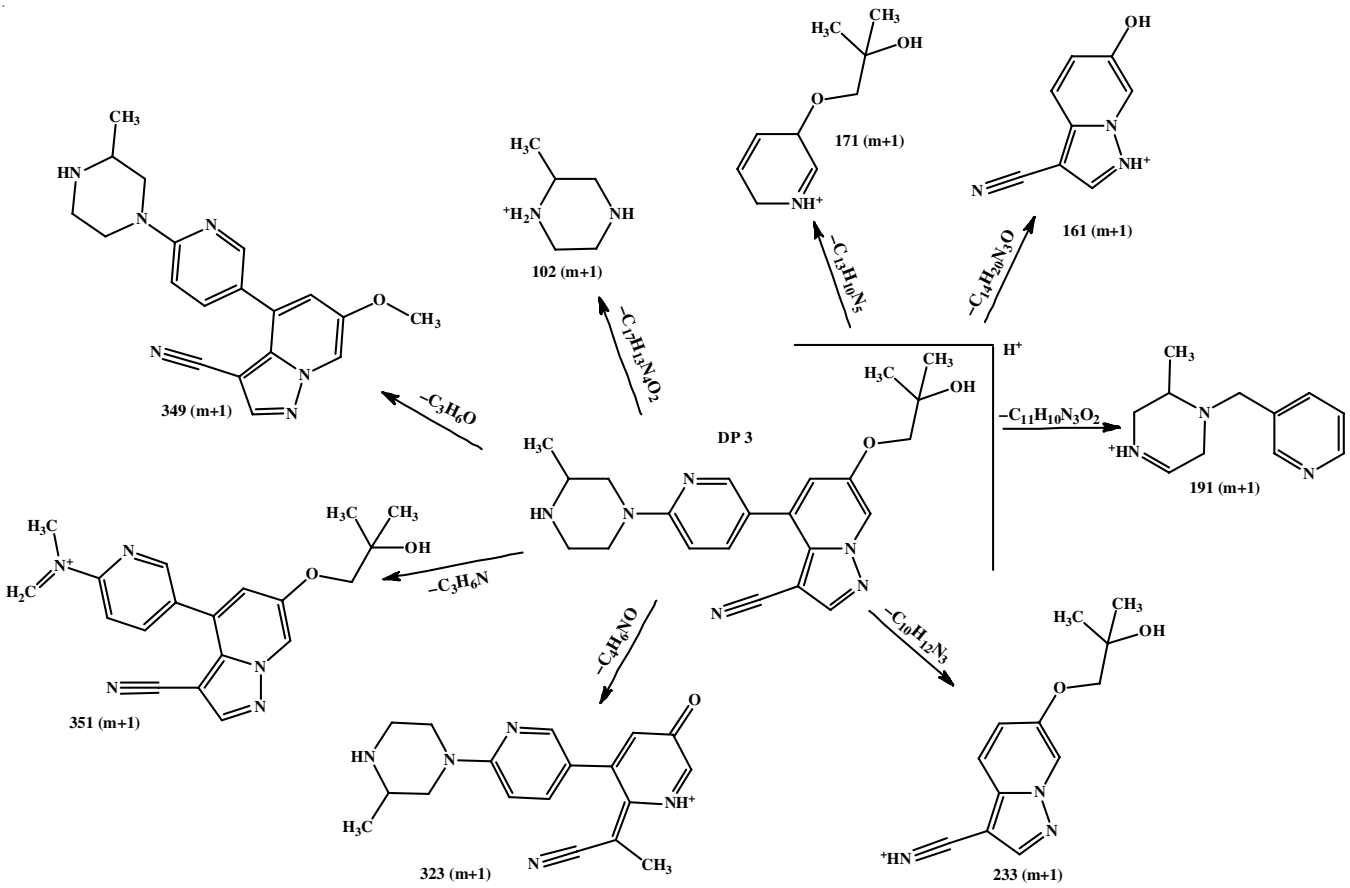

Fig. 7. Mass fragmentation pattern of DP 3

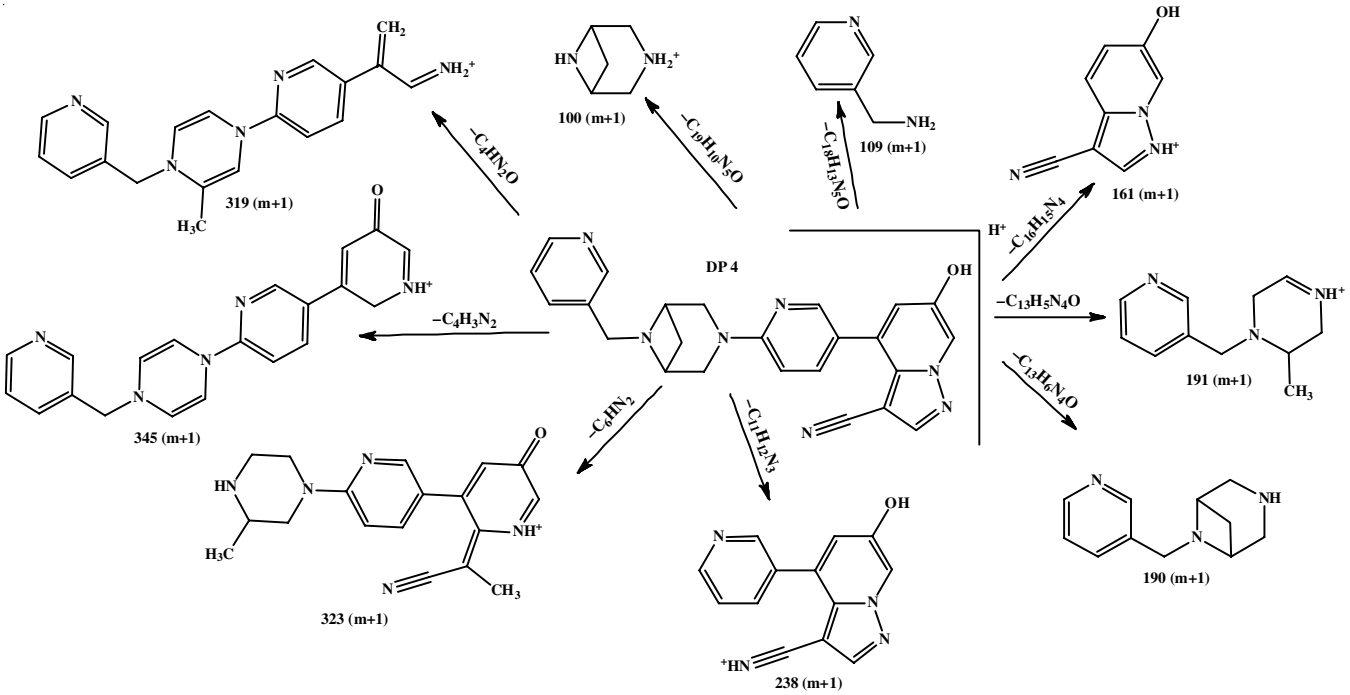

Fig. 8. Mass fragmentation pattern of DP 4

indicates that either these impurities were absent in formulation or they were present lesser than detection limit. Additionally, no visualization of formulation excipients or other unknown compounds in both the spiked and unspiked formulation was observed. Hence, method approved to be adequate for regular quality evaluation of process related impurities of selpercatinib. Fig. 12 shows the spiked and unspiked formulation chromatograms of selpercatinib.

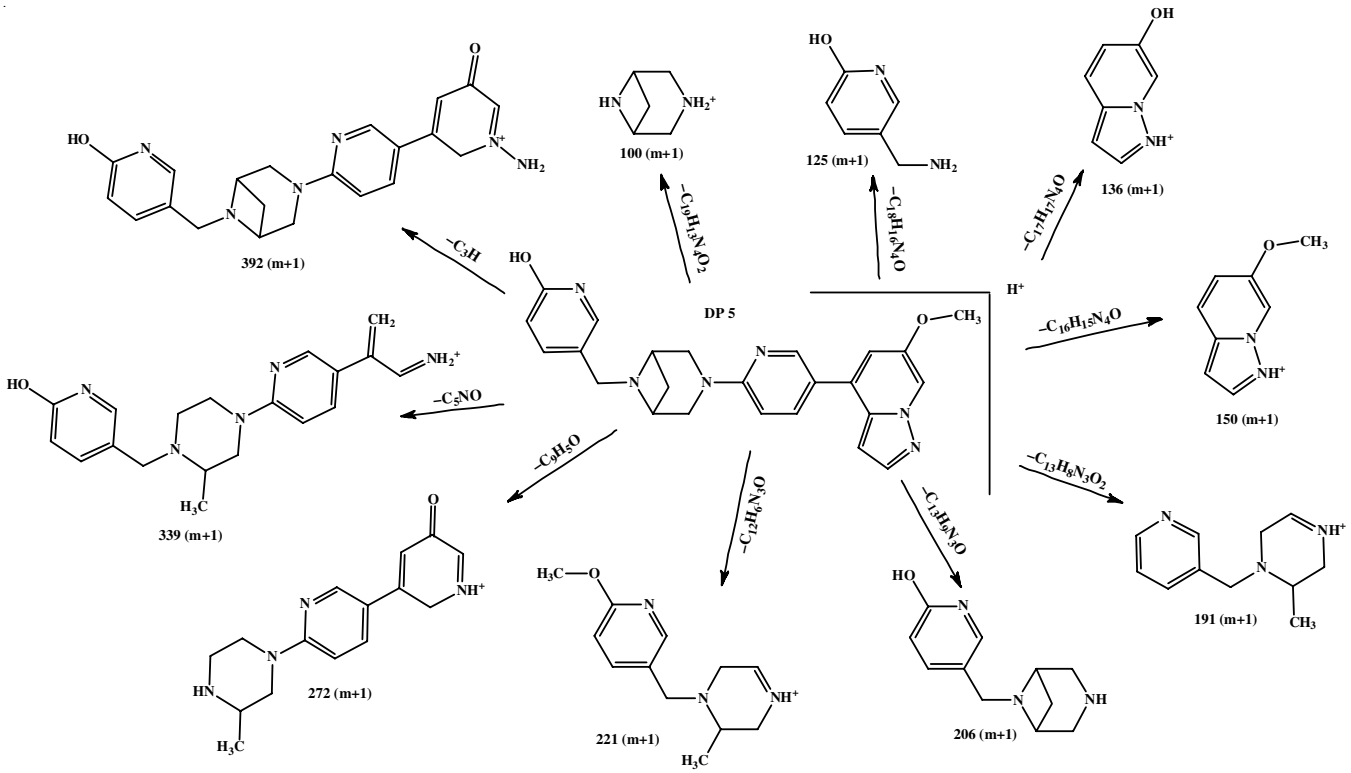

Fig. 9. Mass fragmentation pattern of DP 5

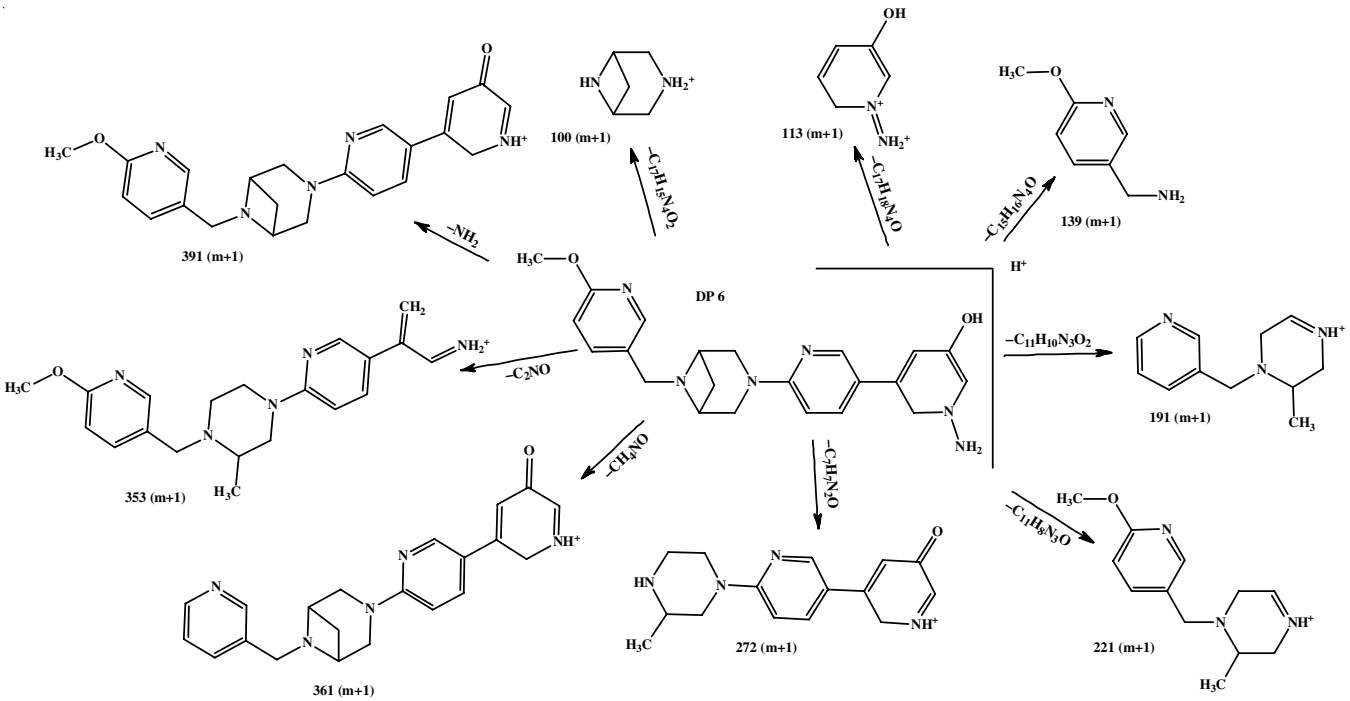

Fig. 10. Mass fragmentation pattern of DP 6

Conclusion

This work introduces and validates a stability indicating isocratic HPLC method for the determination of process related impurities in selpercatinib. The drug was subjected to several degradation circumstances, and chromatograms show different degradation products (DPs) for each stress level. The drug exhibited significant degradation under acidic and basic conditions, with notable degradation products, while the thermal stress resulted in the minimal degradation without the appearance of degradation compounds. The structures of DPs were elucidated using LCMS/MS, providing valuable insights into degradation pathway and stability of selpercatinib. This validated isocratic HPLC method is demonstrated to be specific, linear, precise and accurate. This method is beneficial for the identification

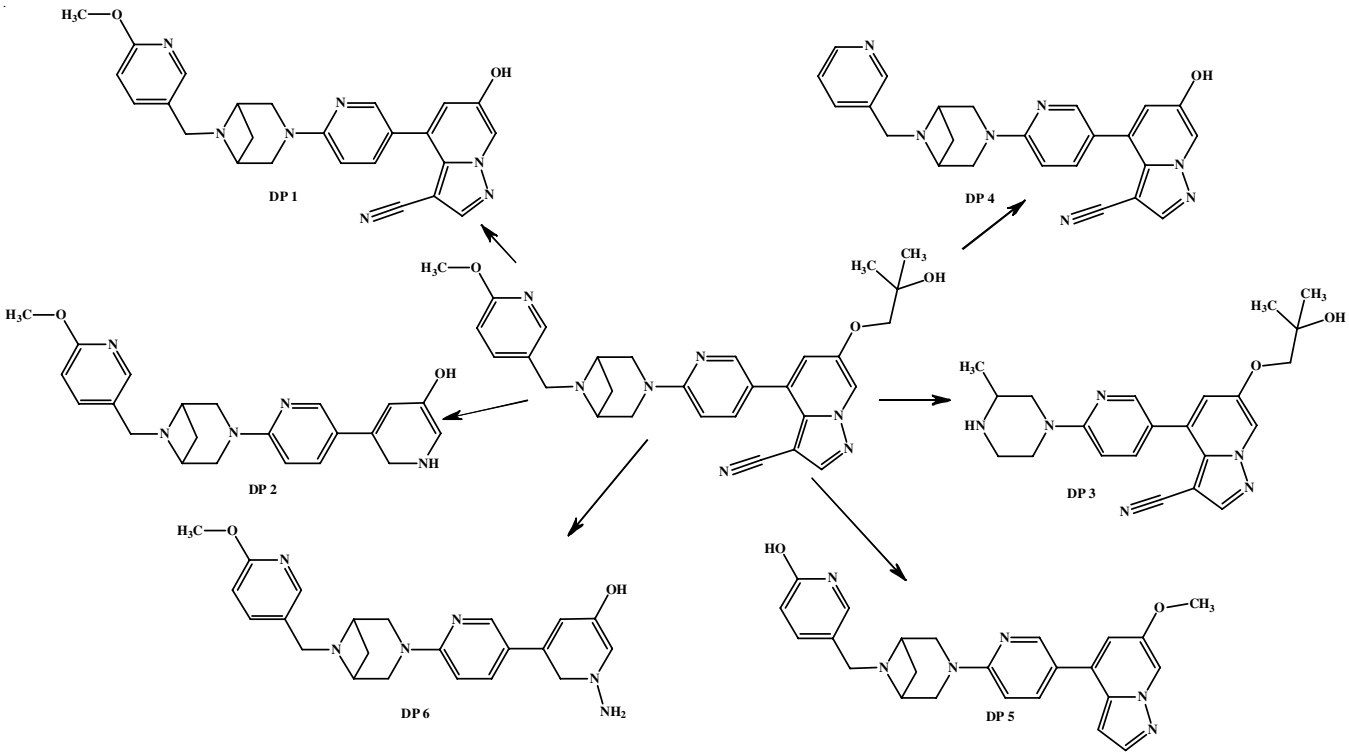

Fig. 11. Degradation products of selpercatinib drug

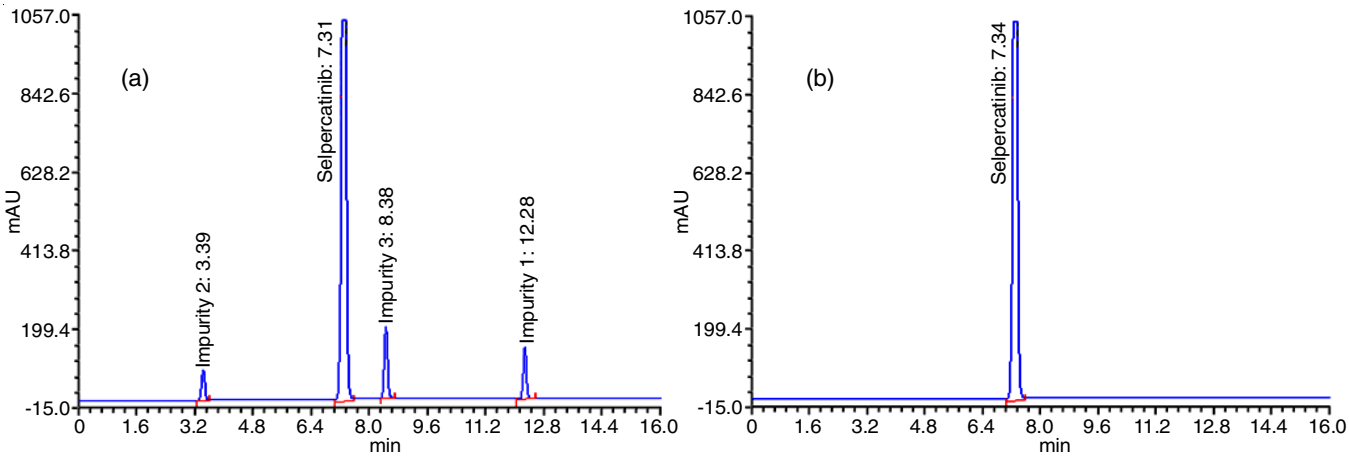

Fig. 12. Formulation chromatograms of selpercatinib in the proposed method, (a) 0.1% Impurities spiked Retevmo® solution; (b) No impurity spiked Retevmo® solution

and quantification of both process related impurities as well as DPs in selpercatinib drug.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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