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Characterization of Prasugrel Degradation Profile by Several Oxidative Reagents using HPLC and LC-MS/MS Technique

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Prasugrel, a derivative of thienopyridine functions as a prodrug that exerts irreversible inhibition on platelet ADP receptors by specifically targeting the P2Y12 receptor. In pharmaceuticals, the stability of the product plays a crucial role in establishing the shelf life of product. The second common degradation pathway next to hydrolysis is the oxidation. In practice, the oxidative stress studies were carried out using hydrogen peroxide (H_2O_2), free radicals, oxygen purging, transition metals, singlet oxygen, Fenton reagent, *etc.* This study compares the oxidative degradation behaviour of prasugrel hydrochloride using different oxidative stressors and was analyzed by HPLC using a method that could separate degradation products individually and from the drug. The degradation products formed under different stress conditions were characterized using LC-TOF-MS/MS. Analysis of accelerated stability samples of prasugrel formulation revealed that the degradation products formed under azobisisobutyronitrile (AIBN) and H_2O_2 stress conditions were simulated with the stability data. This study on prasugrel showed that the degradation products formed with H_2O_2 were also observed under hydrolytic conditions. In the pharmaceutical industry, H_2O_2 is the only oxidative stressor (< 95%) used to study the oxidation degradation of the sample. The present work has demonstrated that AIBN serves as a valuable oxidative stressor for accurately assessing the oxidative stability of drug.

Keywords: Degradation products, Prasugrel hydrochloride, Oxidative stressors, Azobisisobutyronitrile.

INTRODUCTION

Prasugrel HCl (PSL), a derivative of thienopyridine, is a platelet activation and irreversible acting by aggregation inhibitor on the P2Y12 receptor on the platelet [1]. It is a prodrug that inhibits ADP (adenosine diphosphate receptor) receptor [2]. Pharmaceutical drugs should be stable and maintain quality under various conditions *e.g.*, during production, transportation, warehouse storage, hospital and community pharmacies, retail outlets and the home [3]. Drug substances have different molecular structures and are vulnerable to various chemical degradation pathways, including hydrolysis, oxidation and photodegradation and complex interactions with excipients and other pharmaceutical compounds [4,5]. Oxidation is a widely recognized chemical degradation mechanism affecting liquid and solid pharmaceutical formulations [6]. Oxidation states as the electron loss from a molecule (rise in the oxidation number). It can also be stated as a rise in oxygen levels or a reduction in hydrogen atom concentration [7].

Various sources for oxidative degradation of the drug are the presence of head space oxygen, presence of highly reactive impurities such as peroxides, formic acid and aldehydes present in the excipients [8] (like lactose, microcrystalline cellulose), different oxidizing agents used during the manufacturing process and permeability of packaging material to atmospheric oxygen. The different mechanisms of drug oxidation are auto-oxidation, nucleophilic/electrophilic addition and electron transfer process [6]. The type of oxidative mechanism depends on the chemical structure of drug, oxidizing agents used during the manufacturing process and the presence of reactive oxygen species as impurities [9].

A number of oxidizing species can be formed by oxygen, including the mild reducing agent superoxide, the oxidizing agent hydrogen peroxide (H_2O_2) and the singlet oxygen molecule. Besides this, metals also catalyze the oxidative degradation of drugs [10]. Boccardi [11] conducted a comparative analysis of degradation profiles resulting from several oxidative stressors like H_2O_2, transition metals and free radicals for tetra-

zepam, dextromethorphan hydrobromide, phenylbutazone and triuperazine dihydrochloride [11]. Among the oxidative stressors, the oxidation mediated by free radicals closely resembled the actual oxidative degradation observed after prolonged storage of the pharmaceutical products [12]. The prevention of oxidative degradation can be achieved by controlling the impurity concentrations such as heavy metals, peroxides, scavengers or antioxidants (vitamin C and vitamin E) or chelating agents by using appropriate containers and packaging material containing an antioxidant [7].

It has been specified in the ICH (International Conference on Harmonization) guidelines Q1A (R2) that stress studies should be done on the drug substance (DS) and should include testing under various conditions like hydrolysis, oxidation, temperature, humidity and photolysis [13]. The guideline specifies the conditions for performing stress studies under humidity, temperature and photolysis have been specified. But despite oxidation being the second most common degradation pathway in pharmaceuticals, any regulatory guidelines do not address the specific parameters related to oxidative stress studies. In practical applications, the oxidative stress studies commonly involve the use of H_2O_2, free radicals, oxygen purging, singlet oxygen, transition metals, AIBN (azobisisobutyronitrile) and Fenton reagent, *etc.* Few literature reports have compared the oxidative degradation profile using different oxidative stressors [14]. The present study endeavored to perform the accelerated stability studies on drug, carry out stress studies on the drugs using different oxidative stressors and develop an HPLC method that resolves all the degradation products (DPs) formed. Comparing the degradation profile formed under various stress conditions with the accelerated stability data and mass fragmentation on the drug and degradation products. Characterization of the degradation products along with degradation mechanism under each stress condition was performing using LC-MS/TOF technique. Various aspects of oxidative stress include H_2O_2, radical initiator, pressurized oxygen, transition metals and bubbled oxygen studied at various concentrations, temperatures and the study duration. The 95% of oxidizing agents used in the industries include H_2O_2, followed by the radical initiator.

The drug prasugrel HCl is reported to undergo the oxidative degradation and hydrolysis. A literature survey revealed that few analytical methods like LC-MS, UPLC, HPTLC and HPLC has been reported [15-24] and no identification and characterization of oxidative degradation products using AIBN (azobisisobutyronitrile) as oxidative stressor is not reported yet.

EXPERIMENTAL

Pure prasugrel HCl as a gratis sample received from Ind-Swift Pharmaceuticals Ltd. (Dera Bassi, India). The oxidative stressor azobisisobutyronitrile (AIBN) was purchased from Avra Synthesis Pvt. Ltd., Hyderabad, India. The analytical grade buffer salts and other chemicals were procured from Merck Pvt. Ltd. (Mumbai, India). The ultra-pure water was obtained from ELGA water purification unit (Bucks, England).

Characterization: Humidity chamber (KBF720, WTC Binder, Tuttlingen, Germany) was used for conducting the accelerated stability studies at 40 ± 2 ºC/75% RH ± 5% RH. The Waters 2998 HPLC (Waters, USA) equipped with photodiode array (PDA) detector system and Empower software version 6.13 was used to analyze the degradation sample of prasugrel HCl. Inertsil C-18 column (250 mm × 4.6 mm, particle size 5 µm) (Bellefonte, USA) was used the separation of DPs from prasugrel HCl. The ionization source and analyzer were used in mass studies was carried out using LC-ESI-TOF-MS/MS, consisted with HPLC-1100 series (Agilent Technologies, Germany) comprising of an auto-injector (G1313A), online degasser (G1379A), column oven (G1316A), binary pump (G131A). The MicroTOF-Q spectrometer was used in MS system (Bruker Daltonik, Germany). Hystar software (version 3.1) was employed from the same source. ESI was employed in both positive and negative ionization modes. A calibration solution consisting of 5 mM sodium formate (Sigma-Aldrich, India) solution was utilized. In positive ESI mode, the masses were calibrated by employing internal reference ions with *m/z* values of 158.9640, 226.9515, 294.9389, 362.9263, 430.9137, 566.8866 and 634.8760. The pH analyzer (MA 235, Mettler Toledo, Switzerland) was employed for the purpose of calibrating and verifying the pH of buffers. Additional equipment utilized in the study includes ultra-sonicator (3210, Branson Ultrasonics Corporation, USA), analytical balance (AG 135, Mettler Toledo, Switzerland) and auto pipettes (Eppendorf, Germany).

Methodology of stress studies: The drug prasugrel HCl is insoluble in water, so the mixture of acetonitrile (ACN) and H_2O in the ratio of 50:50 v/v was used as a diluent for preparing stock solutions [4]. For hydrolytic conditions, the initial stock (2 mg/mL stock) solution was prepared in 50:50 v/v of ACN:H_2O kept in the reflux, maintained at 40 ºC and an equivalent volume of the stock solution was diluted with the stressor (1 mg/mL). The sampling was done for every 1 h and analyzed on the HPLC for 3 days. For every stress condition four samples were generated, including a blank solution stored under room temperature (RT), zero-time samples containing the prasugrel HCl at room temperature and blank and the prasugrel HCl solution subjected to stress conditions. For the current study, six oxidative stressors were used [25].

For H_2O_2, equal quantities of 10% H_2O_2 solution and stock solution of prasugrel HCl (1 mg/mL) in ACN:H_2O (50:50 v/v) were mixed and stored at room temperature. A blank solution was prepared containing ACN:H_2O and H_2O_2. While for AIBN, equal quantities of 20% AIBN and prasugrel HCl stock solution (1 mg/mL) were mixed and kept at 40 ºC in the stability chamber. Two blanks were prepared, one containing an ACN solution of prasugrel HCl and the other an initiator solution. Samples were collected at regular intervals for 3 days. For oxygen purging, prasugrel HCl was taken in an ampoule and oxygen was purged, sealed and stored at room temperature. The samples were then analyzed after one month. In case of transition metal ion spiking, the stock solutions were prepared for prasugrel HCl and spiked with $FeCl_3$ and $CuSO_4$ to get a final concentration of 500 ppm, respectively. The blank solutions were prepared to contain ACN:H_2O with metal ion solution. All the solutions were kept at 40 ºC in the stability chamber, Sampling was done every 24 h for 3 days. After sampling, added EDTA to the stress samples

TABLE-1
OPTIMIZED HPLC AND LC-MS/MS METHOD THAT SEPARATES ALL THE DEGRADATION PRODUCTS

Column, column temperature and detection wavelength	Mobile phase A	Mobile phase B	Gradient program at flow rate of 1 mL/min (Tmin A:B)
Inertsil C-18 (250 × 4.6) mm 5 µm, 30 °C, 230 nm	10 mM Ammonium formate (pH 2.8)	Acetonitrile (ACN)	T0-5 70:30; T30 35:75; T40 10:90; T45 10:90; T46 70:30; T50 70:30

at 2:1 ratio to chelate with the transition metals. Before injecting into the HPLC, the samples were centrifuged to remove the precipitate.

In Tween test, a Tween-80 in water (10% w/v) solution of was prepared. Prasugrel HCl was then dissolved into the solution to get a final concentration of 1 mg/mL and 6.48 mg of anhydrous $FeCl_3$ was added directly into the vial to give 10 mM Fe^{3+}. Both the stressed solution and blank were stored at room temperature. Samples were taken at 1, 2 and 3 days time points, diluted in the diluent and injected. All the oxidatively stressed samples were analyzed by HPLC in a gradient mode using a mobile phase composed of ACN and 10 mM ammonium formate buffer (pH-2.8).

Stability indicating assay method (SIAM): Few reports are available regarding stress studies on prasugrel HCl [18,19, 21]. Several modifications were made, like changes in the concentrations of the mobile phase buffers, changing the buffer pH, changes in gradient, flow rate and molarity of the buffer component. The prasugrel HCl, method development was initiated on an Inertsil C-18 column, the organic modifier used was acetonitrile and the aqueous phase was ammonium formate buffer (10 mM, pH adjusted to 2.8 using formic acid). The wavelength of detection was optimized at 230 nm. The optimized gradient program chromatogram is shown in Table-1.

MS/TOF, MS^n and H/D exchange studies on prasugrel HCl: The MS/MS method was developed in LC-ESI-TOF-MS/MS in two different methods. One was for molecular ion peak and the other was for fragmentation pattern. Samples were analyzed at a concentration of 5 ppm. Also, MS^n studies were carried out to determine each fragment's origin to elucidate the fragmentation pathway. The H/D exchange studies were also carried out to verify the structures of various fragments with the knowledge of exchangeable hydrogens. Deuterated methanol was used for H/D exchange studies. For the H/D exchange studies above solution was injected into MS/TOF. The optimized MS/TOF method is shown in Table-2.

TABLE-2
DEVELOPED AND OPTIMIZED MS/TOF PARAMETERS

Methods	Conditions	Prasugrel HCl	
		$[M+H]^+$	Fragment
Transfer	Funnel 1 RF (Vpp)	200	180
	Funnel 2 RF (Vpp)	220	200
	ISCID energy (eV)	5.0	5.0
Quadrupole	Ion energy	4.0	3.0
	Low mass (*m/z*)	300	150
Collision cell	Collision energy (eV/z)	7.0	18
	Transfer time (µs)	70	48
	Collision RF (Vpp)	450	300
	Pre pulse storage (µs)	2.0	10
Detector	Source (V)	-1200	-1200

LC-MS/TOF data on the degradation products (DPs): The LC-MS method was developed with volatile buffer (10 mM ammonium formate buffer adjusted pH to 3.0 with formic acid). The two mass methods developed earlier were used to characterize degradation products. Using the above data, the structural elucidation was carried out systematically.

RESULTS AND DISCUSSION

Prasugrel mass fragmentation behaviour: The mass spectrum of prasugrel HCl (Fig. 1) shows the separation of prasugrel HCl and its degradation products (DPs). In total, five fragments were formed from prasugrel HCl (Fig. 2). The molecular ion peak of *m/z* 374, when taken for MS2 studies, got fragmented into four major ions of *m/z* 332, 314, 206 and 177. The ions of *m/z* 332 was produced due to the removal of the $COCH_3$ group from prasugrel HCl (*m/z* 374). Further in MS3 studies, the fragmentation of *m/z* 332 into three ions of *m/z* 314, 206 and 154. The formation of *m/z* 314 ion due to the neutral loss of H_2O. In the MS4 step, a fragmentation of *m/z* 206 ion into *m/z* 177 ion, on the removal of CH_3NH_2, which in turn fragmented to an *m/z* 149 ion on the removal of RCOR group. The data obtained from H/D exchange studies were also used to confirm the presence of labile hydrogen(s) of the proposed fragments. The MS^n and MS/TOF data are listed in Tables 3 and 4, respectively and the optimization of stress conditions is listed in Table-5.

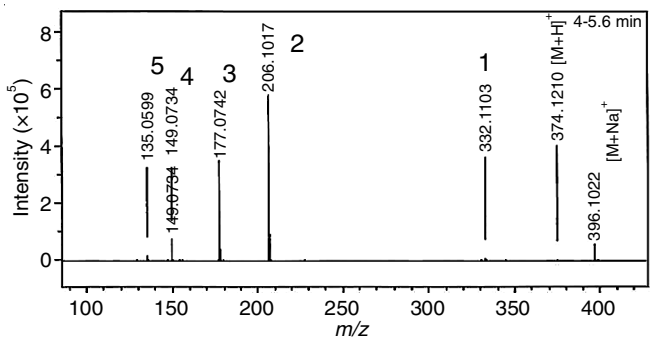

Fig. 1. MS/TOF spectra of Prasugrel HCl

TABLE-3
MS^n FRAGMENTATION PATTERN OF PRASUGREL HCl DRUG

MS^n	Precursor ion	Product ions
MS^n	374	332, 314, 206, 177
MS^n	332	314, 206, 154
MS^n	206	177, 135

Degradation behaviour: Total 6 DPs were formed from prasugrel HCl, as shown in Figs. 3 and 4. The DPs formed under each forced degradation condition differed qualitatively and quantitatively. In H_2O_2, prasugrel HCl underwent extensive

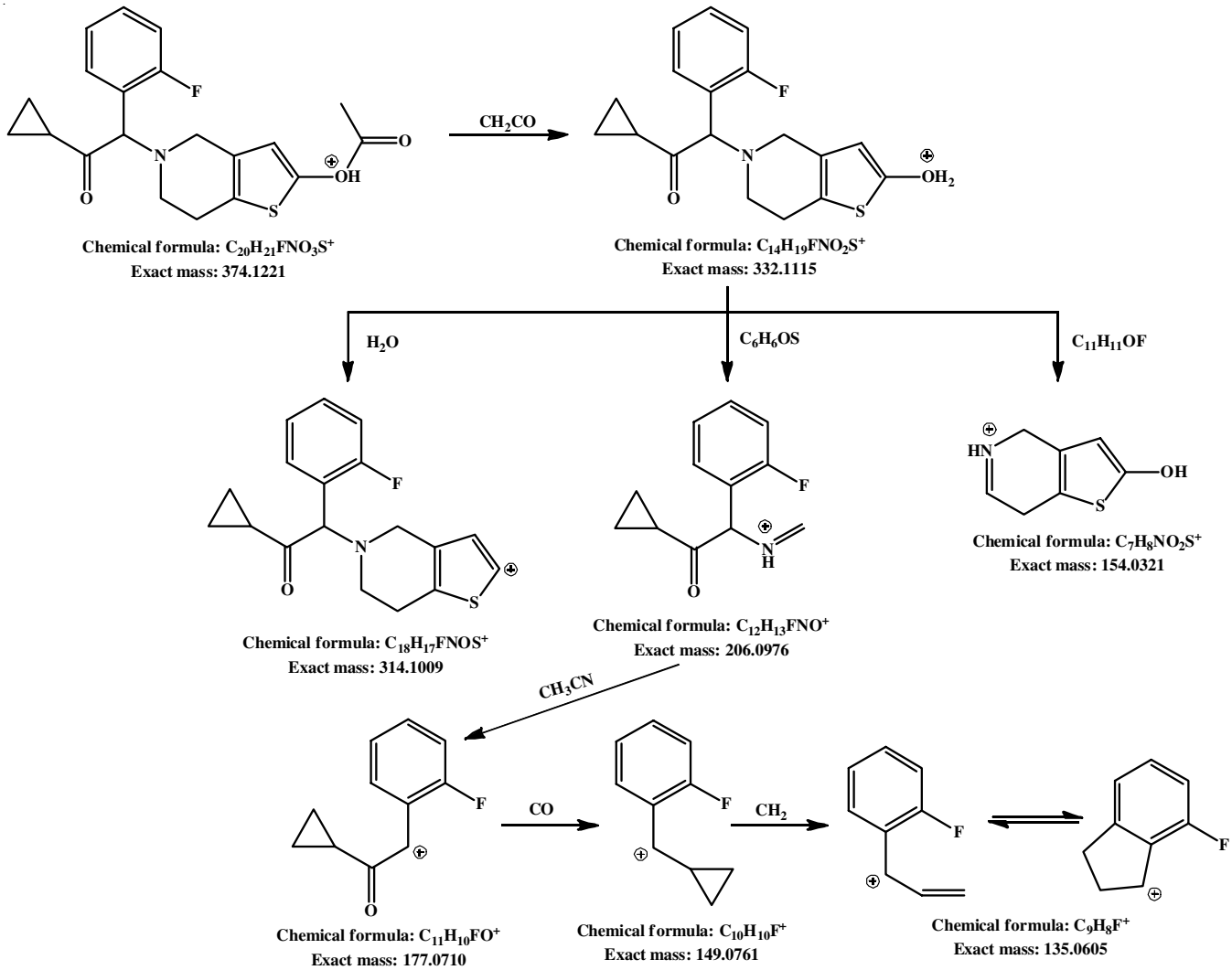

Fig. 2. Mass fragmentation pattern of Prasugrel HCl along with exact masses

TABLE-4
MS/QTOF AND H/D EXCHANGE DATA OF PRASUGREL HCl DRUG

Peak No.	MS/TOF data (amu)	Best possible molecular formula	Exat mass of the most probable structure (amu)	Error in mmu	RDB	Possible parent ion	Difference from parent (amu)	Possible molecular formulae of the loss	H/D exchange data (amu)	No of labile hydrogens
$[M+H]^+$	374.1210	$C_{20}H_{21}NO_3FS^+$	374.1220	-1.068	10.5	$[M+H]^+$	–	–	375.1266	1
1	332.1103	$C_{18}H_{19}NO_2FS^+$	332.1115	-1.204	9.5	$[M+H]^+$	42.0107	CH_2CO	333.1153	1
2	206.1017	$C_{12}H_{13}NOFS^+$	206.0975	4.132	6.5	1	126.0086	C_6H_6OS	208.1069	2
3	177.0742	$C_{11}H_{10}OF^+$	177.0710	3.181	6.5	2	29.0275	CH_3N	177.0713	–
4	149.0734	$C_{10}H_{10}F^+$	149.0761	-2.705	5.5	3	28.0008	CO	149.0672	–
5	135.0599	$C_9H_8F^+$	135.0605	-0.554	5.5	4	14.0135	CH_2	135.0543	–

TABLE-5
OPTIMIZED EXPERIMENTAL CONDITIONS FOR STRESS STUDIES ON PRASUGREL HCl DRUG

Oxidative stressor	Temp.	Solvent used	Drug conc.	Stressor Conc.	Duration of exposure
H_2O_2	40 °C	ACN:H_2O	1 mg/mL	10%	1 h
AIBN	40 °C	ACN:H_2O	1 mg/mL	20%	2 days
Transition metals	40 °C	ACN:H_2O	1 mg/mL	500 ppm of $FeCl_3$ and $CuSO_4$	3 days
Tween-80	RT	ACN:H_2O	1 mg/mL	10% Tween-80 and 10mM $FeCl_3$	3 days
Oxygen purging	RT	–	1 mg/mL	–	30 days
Hydrolytic condition	40 °C	ACN:H_2O	1 mg/mL	–	3 days
Accelerated stress condition	40 °C/75%RH	ACN:H_2O	1 mg/mL	–	6 min

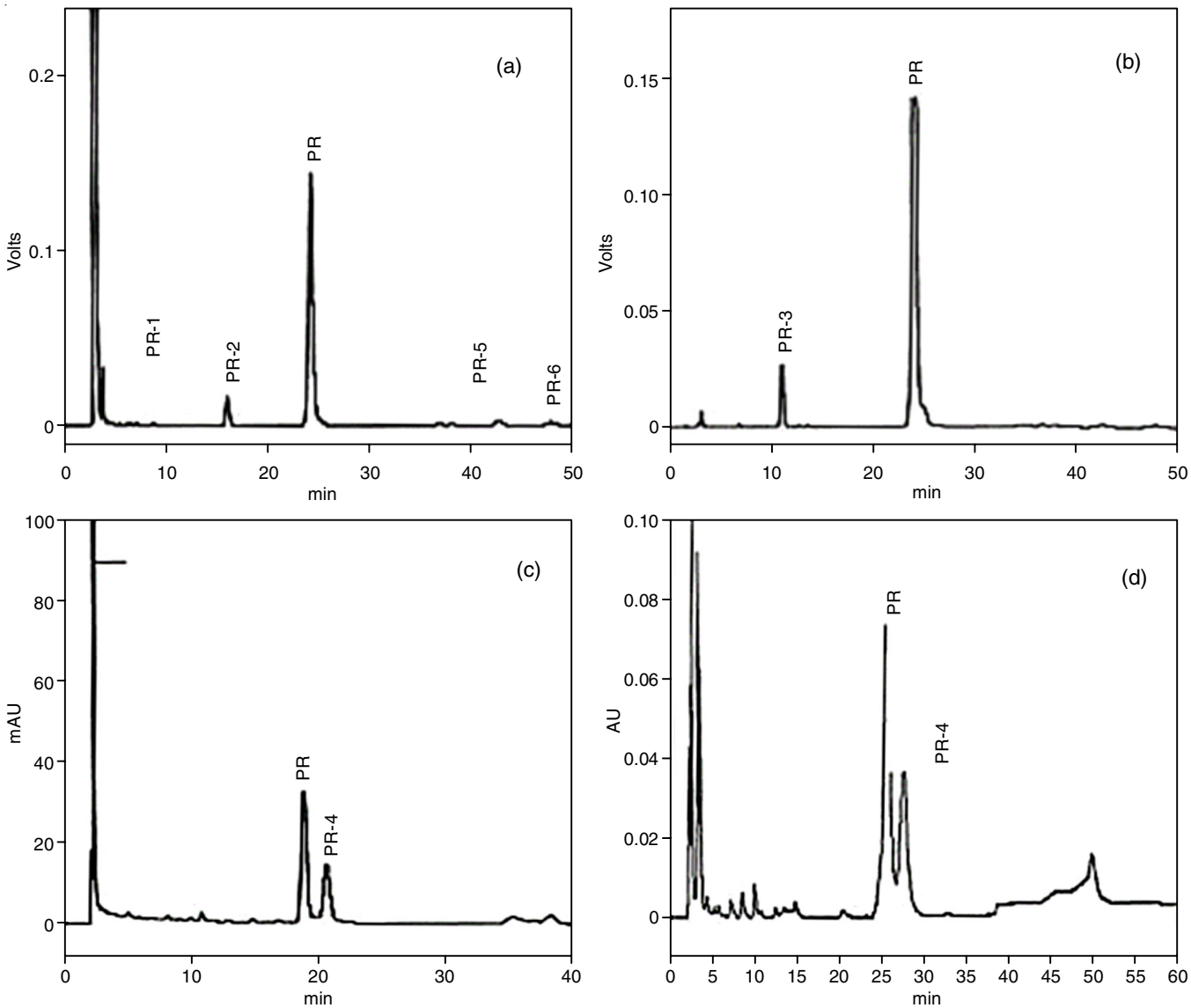

Fig. 3. HPLC chromatograms of Prasugrel HCl in individual stress conditions (a) H_2O_2; (b) AIBN; (c) $FeCl_3$; (d) $CuSO_4$

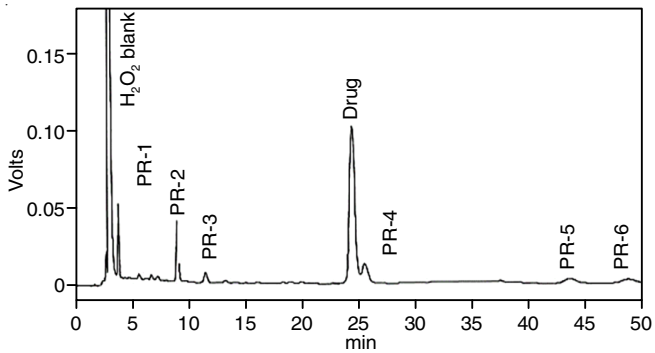

Fig. 4. HPLC Chromatogram showing separation of all the degradation products of Prasugrel HCl

degradation within 1 h at room temperature, resulting in more than 20% of prasugrel HCl degradation. Total 4 DPs were formed in 10% H_2O_2. In case of AIBN, a different DPs was observed and the extent of degradation was less than H_2O_2. Prasugrel HCl was found to be stable in case of oxygen purging and Tween-80. With $FeCl_3$ and $CuSO_4$, again a different DPs was observed and the area of the DPs was more in $CuSO_4$ when compared with $FeCl_3$.

LC-MS/TOF studies on DPs in oxidative conditions: To characterize DPs, prasugrel HCl degraded samples were analyzed using LC-MS/TOF studies. The observed mass values, exact mass values, plausible molecular formulae, RDB and error in mmu of each DPs are also listed in Table-6.

TABLE-6
MS/TOF DATA OF DEGRADATION PRODUCTS OF PRASUGREL HCl DRUG IN OXIDATIVE CONDITION

DP's	Observed mass [M+H] (amu)	Molecular formulae (exact mass, RDB, error in mmu)
PR-1	398.1096	$C_{18}H_{21}FNO_6S^+$ (398.1068, 8.5, 2.788)
PR-2	206.0980	$C_{12}H_{13}NOF^+$ (206.0975, 6.5, 2.932)
PR-3	372.1013	$C_{20}H_{19}N_3FS$ (372.1064, 11.5, -5.118)
PR-4	302.1157	$C_{17}H_{17}NO_3F$ (302.1186, 9.5, -2.998)
PR-5	332.1104	$C_{18}H_{19}NO_2FS^+$ (332.1115, 9.5, -1.104)
PR-6	332.1104	$C_{18}H_{19}NO_2FS^+$ (332.1115, 9.5, -1.104)

Characterization of DPs: The DPs were characterized using LC-MS/TOF technique.

PR-l (*m/z* 398): The DP1 observed mass is 398.1096 Da and the exact mass was 398.1068 Da ($C_{18}H_{21}FNO_6S^+$) is calculated using elemental composition calculator (ECC). From the difference between the exact and observed mass, an error of 2.788 mmu was observed. The two major fragments were showed as *m/z* 380 and 318 ions formed from the DP1 (398.1096 *m/z*), the difference of 18 Da and 80 Da suggests the neutral loss of H_2O and SO_3. A detailed mass fragmentation pattern of PR-l is shown in Fig. 5.

PR-2 (*m/z* 206): The observed mass of DP was 206.0980 Da and the exact mass was 206.0975 Da ($C_{12}H_{13}NOF^+$) using ECC. An error of 0.432 mmu was determined based on

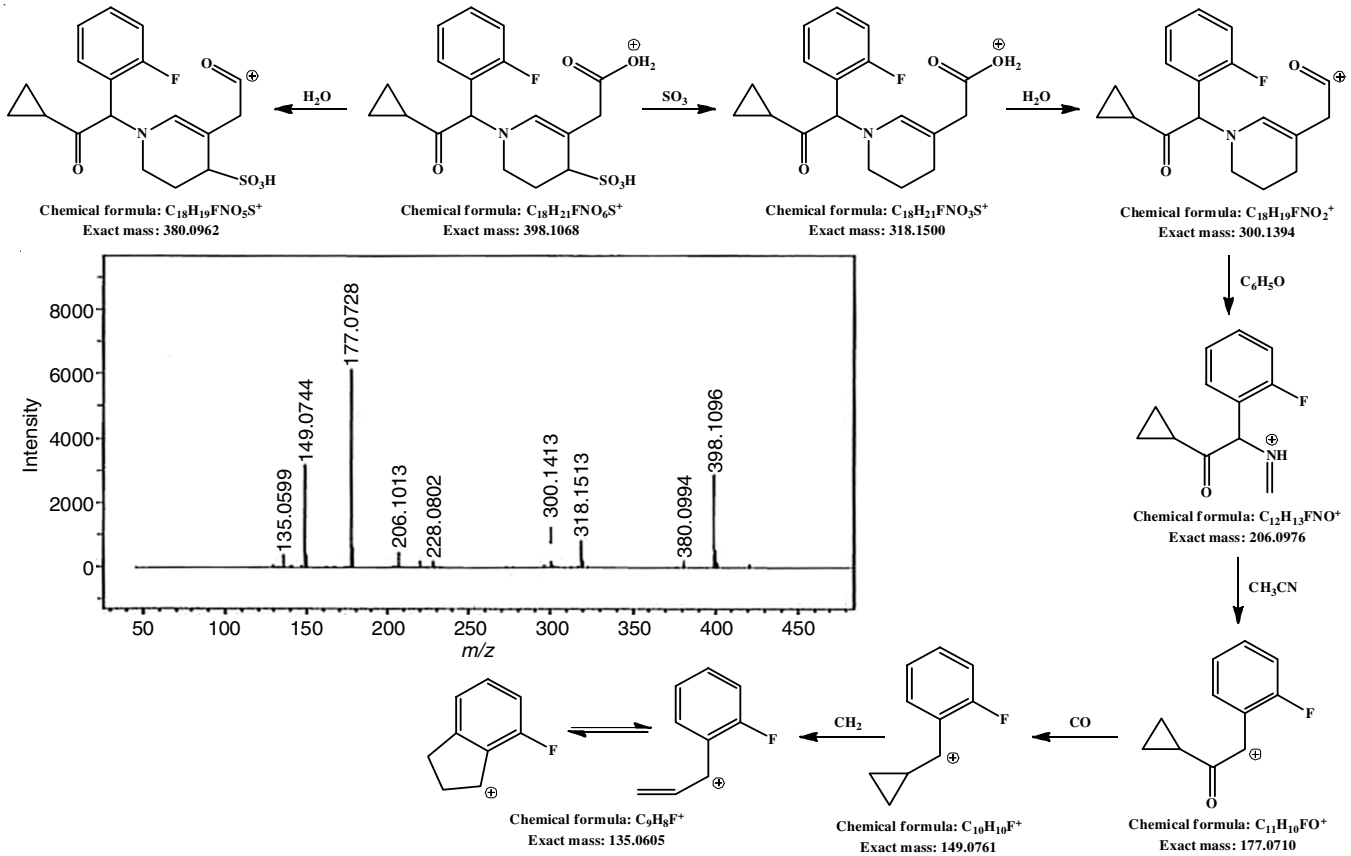

Fig. 5. Line spectra and fragmentation pattern of PR-1

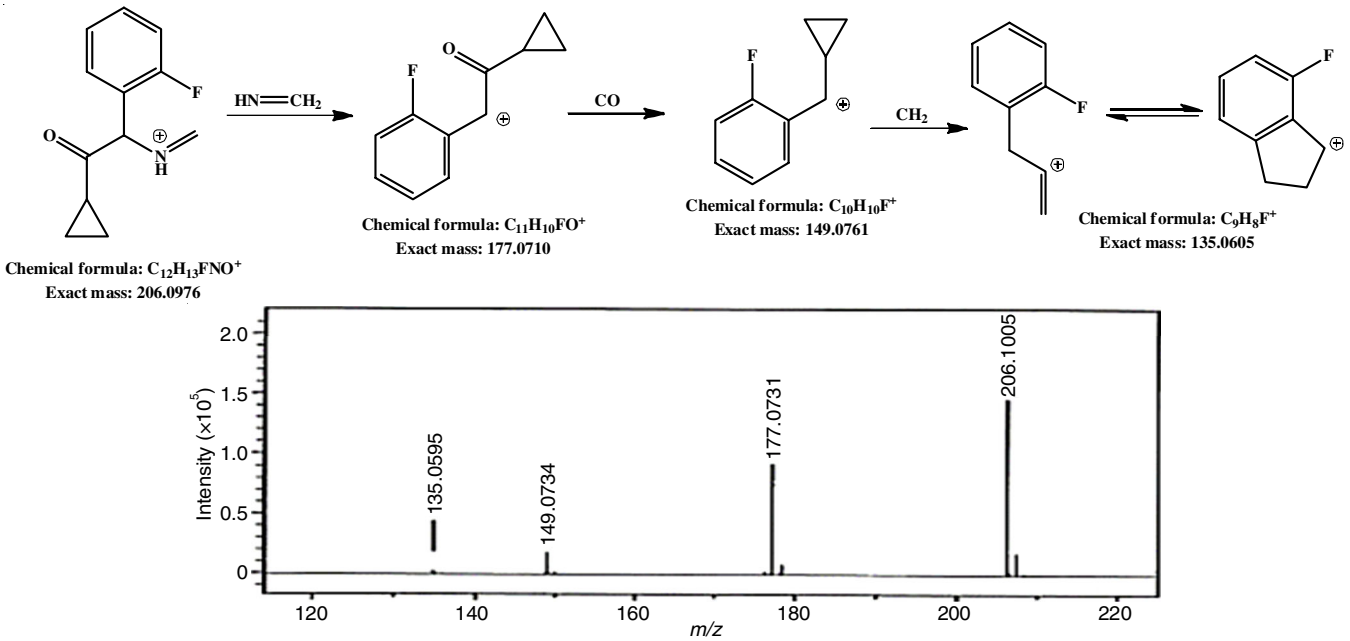

Fig. 6. Line spectra and fragmentation pattern of PR-2

difference between the exact and observed mass. The fragmentation pattern of PR-2 into 1 major *m/z* 177 ion is explained in Fig. 6.

PR-3 (*m/z* 372): The observed mass of DP was 372.1013 Da and the exact mass was 372.1064 Da ($C_{20}H_{19}N0_3FS^+$) was calculated using ECC. The associated error was -5.118 mmu from the observed mass and the exact mass. It showed 1 major fragmentation of *m/z* 330 ion. The mass fragmentation pattern is shown in Fig. 7.

PR-4 (*m/z* 348): The observed mass of DP was 302.1157 Da. It formed adducts 324.098 (Na^+ adduct) and 340.0725 (K^+ adduct). Further, 302.1157 ions fragmented to give ions of *m/z* 286.128, 177.072 and 149.072. The degradation pathway is shown in Fig. 8.

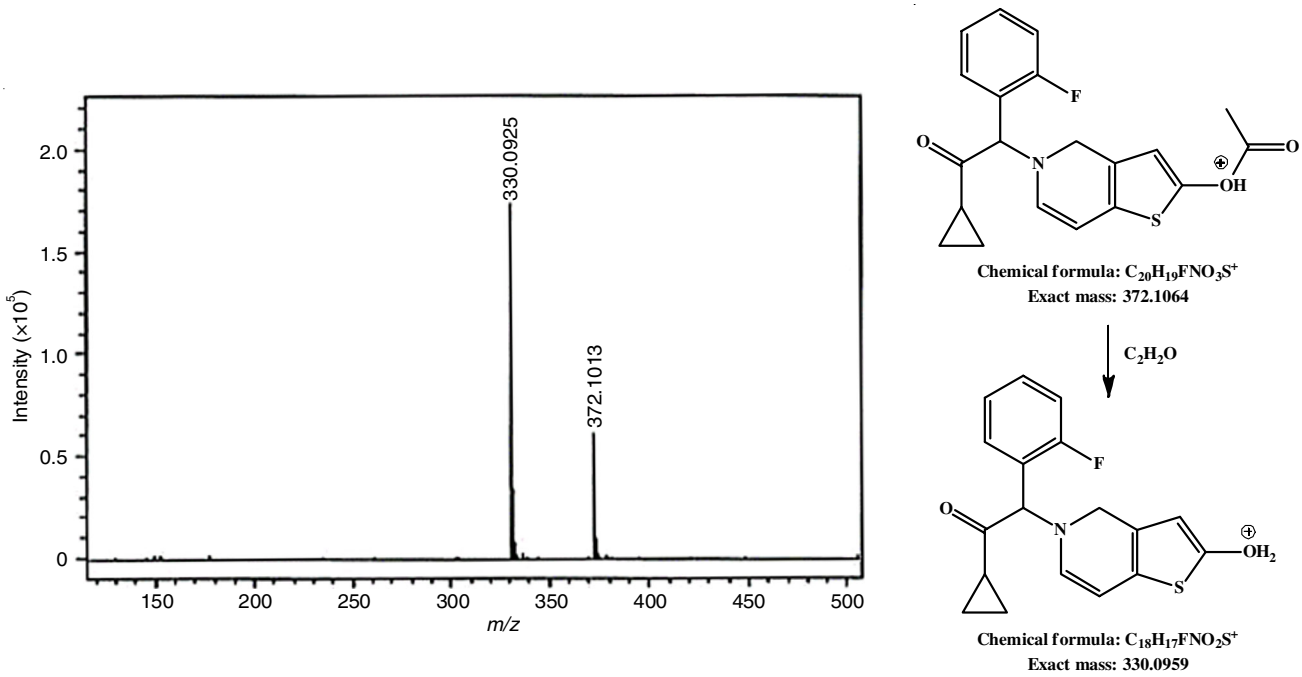

Fig. 7. Line spectra and fragmentation pattern of PR-3

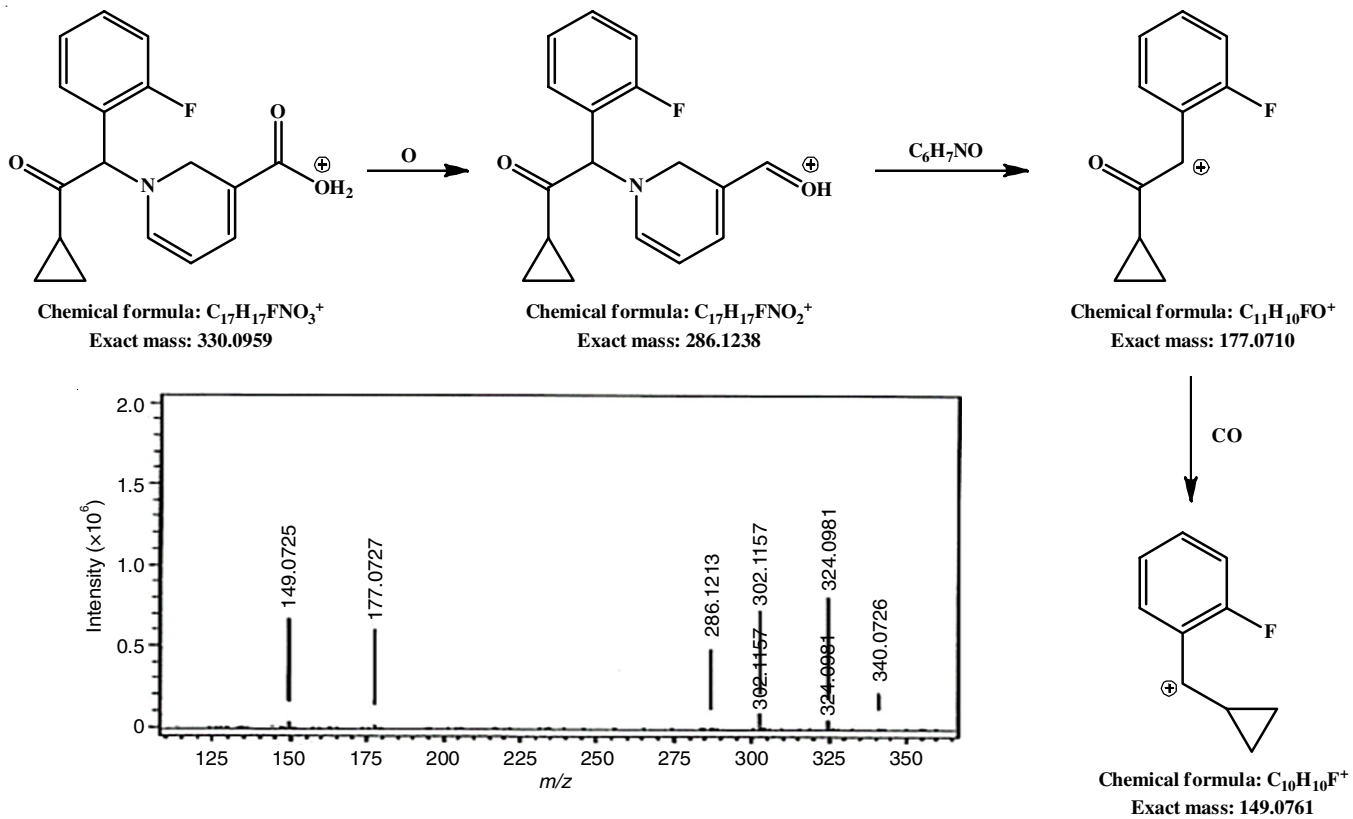

Fig. 8. Line spectra and fragmentation pattern of PR-4

PR-5 and PR-6 (*m/z* 332): The observed mass of these two DPs was same *i.e.* 332.1104 Da. The exact mass was found to be 332.1155 Da ($C_{18}H_{19}NO_2FS^+$) with the help of an ECC. Its fragmentation pattern is shown in Fig. 9.

Stability analysis for drug product (formulation): To confirm the degradation nature of prasugrel HCl in the tablets, the formulation was kept in the accelerated stability chamber, maintained at 40 ºC/75% RH. The tablets were analyzed after 1 month. The prasugrel HCl was stable as no DPs were formed in one month sample. The formulation was then analyzed after 6 months. 9 DPs were formed of which four were previously observed in the stress samples under AIBN and H_2O_2 stress conditions. The 5 DPs leftover were characterized using LC-MS/TOF. In LC-MS chromatogram, all the peaks were visible compared to the UV chromatogram. Hence, the LC-MS/TOF chromatogram of prasugrel HCl tablets is shown in Fig. 10. The DPs PR-7, 14 and 15 were observed in H_2O_2 stress condition while PR-10 under AIBN. The line spectra of these four DPs are presented in Fig. 11. The remaining five DPs were not observed in any of the stress conditions. Hence efforts were made to characterize the newly formed DPs.

LC/MS-TOF studies on DPs: The characterization of DPs and stability samples of prasugrel HCl tablets were analyzed using LC/MS-TOF studies. The observed mass values,

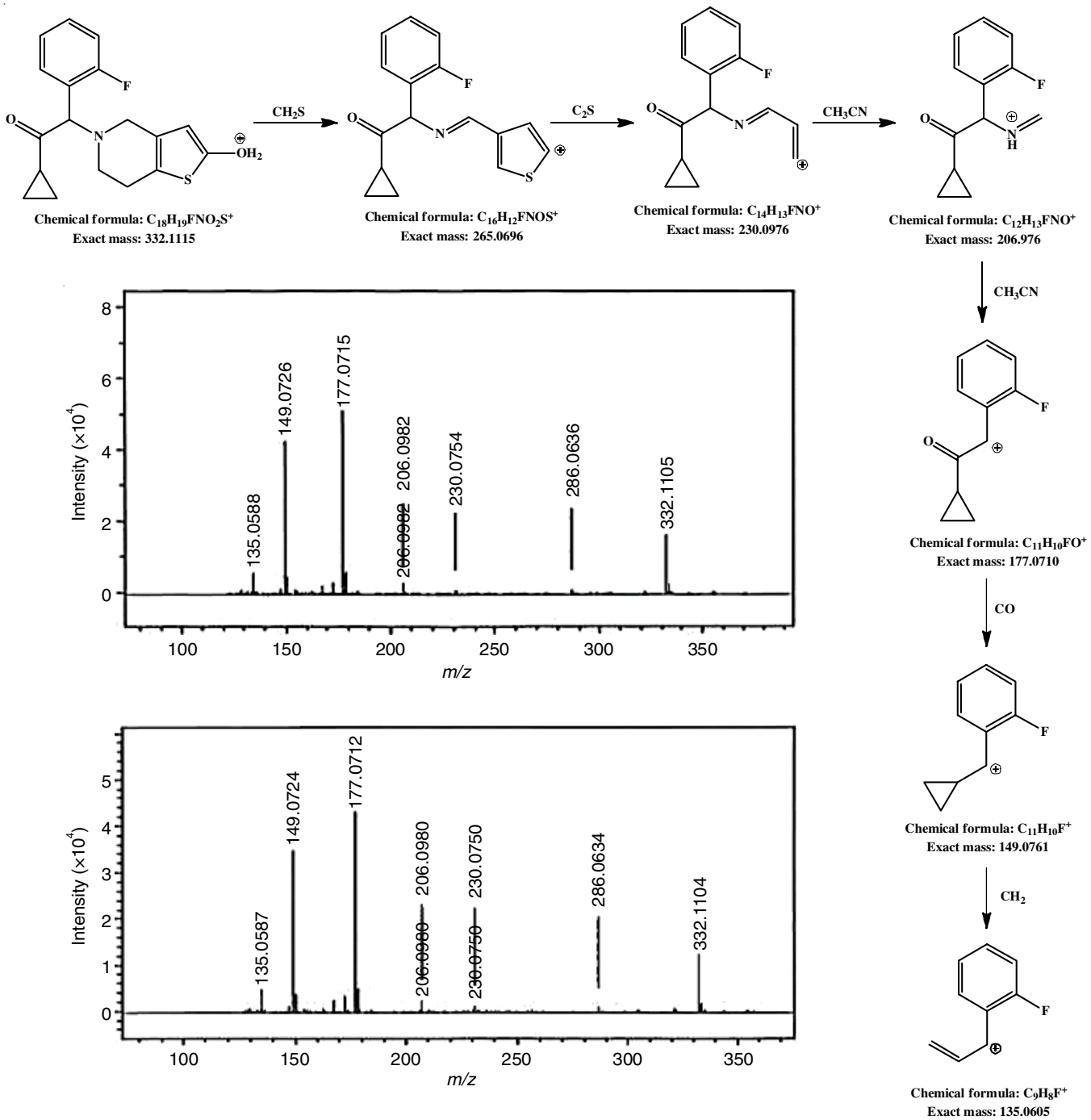

Fig. 9. Line spectra and fragmentation pattern of PR-5 and PR-6

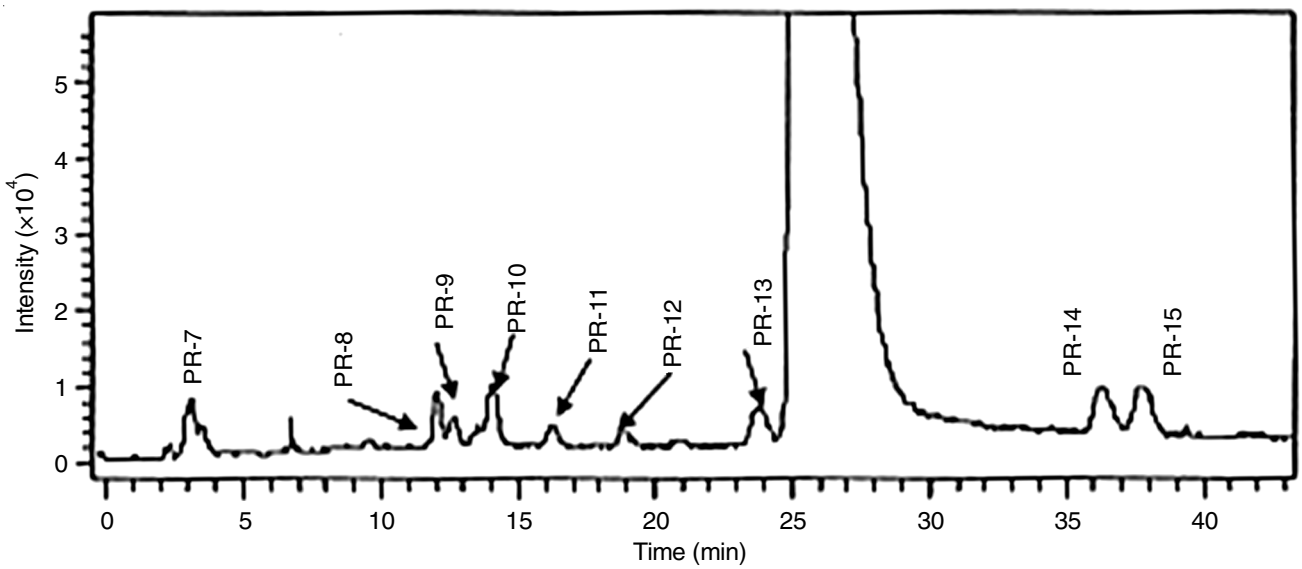

Fig. 10. Chromatogram showing the degradation products formed in the stability sample of Prasugrel HCl

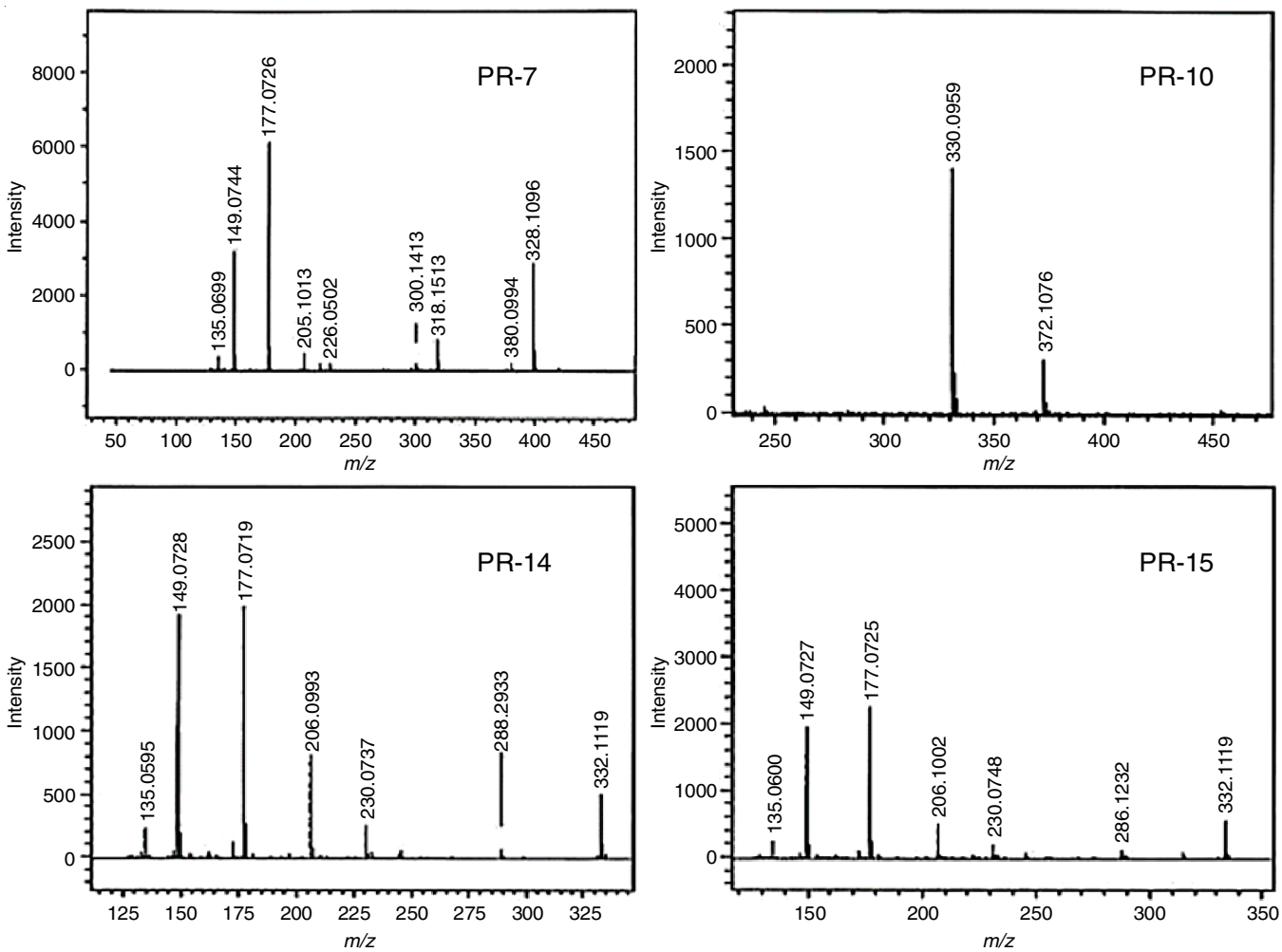

Fig. 11. Line spectra of PR-7, PR-10, PR-14 and PR-15

exact mass, best possible molecular formulae, RDB and error in mmu of each DP are listed in Table-7.

Characterization of DPs of prasugrel HCl: A total of 5 DPs PR-8 (*m/z* 357), PR-9 (*m/z* 286), PR-11 (*m/z* 230), PR-12 (*m/z* 230), PR-13 (*m/z* 348) were characterized using LC/MS-TOF; the degradation pathway is discussed below.

PR-8 (*m/z* 357): The observed mass of DP was 357.1287 Da and the exact mass was 357.1193 Da ($C_{20}H_{20}NO_2FS$) with

TABLE-7
MS/TOF DATA OF DPs IN PRASUGREL HCl DRUG
IN ACCELERATED STABILITY CONDITION

DP's	Observed mass [M+H] (amu)	Molecular formulae (exact mass, RDB, error in mmu)
PR-7	398.1096	$C_{18}H_{21}FNO_6S^+$ (398.1068, 8.5, 2.788)
PR-8	357.1287	$C_{20}H_{20}NO_2FS^+$ (357.1193, 11.0, 9.371)
PR-9	286.1248	$C_{17}H_{17}NO_2F^+$ (286.1237, 9.5, 1.017)
PR-10	372.1076	$C_{20}H_{19}NO_3FS$ (372.1064, 11.5, 1.382)
PR-11	230.0727	$C_{14}H_{13}NOF$ (230.0975, 8.5, -24.868)
PR-12	230.0787	$C_{14}H_{13}NOF$ 230.0975, 8.5, -18.868)
PR-13	348.1083	$C_{18}H_{19}NO_3FS$ (348.1064, 9.5, 1.8822)
PR-14	332.1155	$C_{18}H_{19}NO_2FS^+$ (332.1115, 9.5, 3.996)
PR-15	332.1155	$C_{18}H_{19}FNO_2FS^+$ (332.1115, 9.5, 3.996)

the help of an ECC. The error was found to be 9.371 mmu. A mass fragmentation pattern of PR-8 is given in Fig. 12.

PR-9 (m/z 286): The observed mass of DP was 286.1248 Da and the exact mass was 286.1237 Da ($C_{17}H_{17}NO_2F$) with the help of ECC. It is associated with an error of 1.017 mmu. Its fragmentation pattern is shown in Fig. 13.

PR-11 (*m/z* 230): The observed mass of DP was 230.0727 Da and with the use of an ECC, the exact mass was found to be 230.0975 Da ($C_{14}H_{13}NOF$). An error of -24.808 mmu was calculated by the difference between the exact and observed mass. Its fragmentation pattern is shown in Fig. 14.

PR-12 (*m/z* 230): The DP observed mass was 230.0787 Da and with the help of an ECC, the exact mass was found to

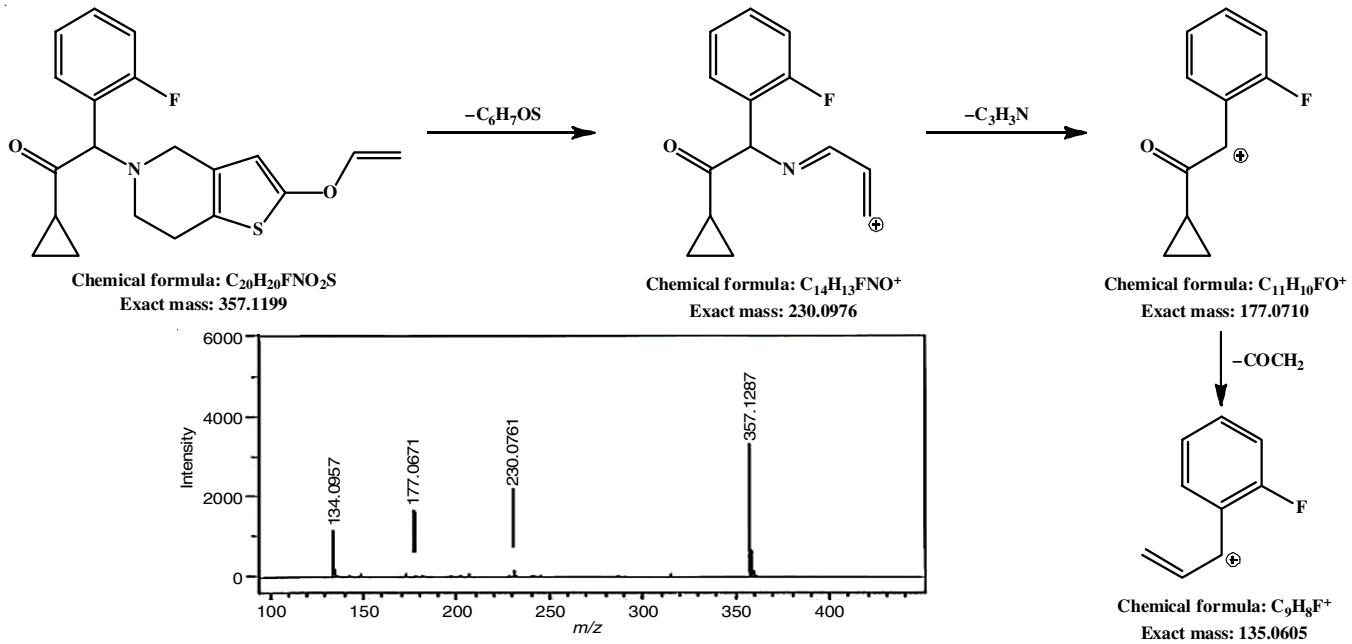

Fig. 12. Line spectra and fragmentation pattern of PR-8

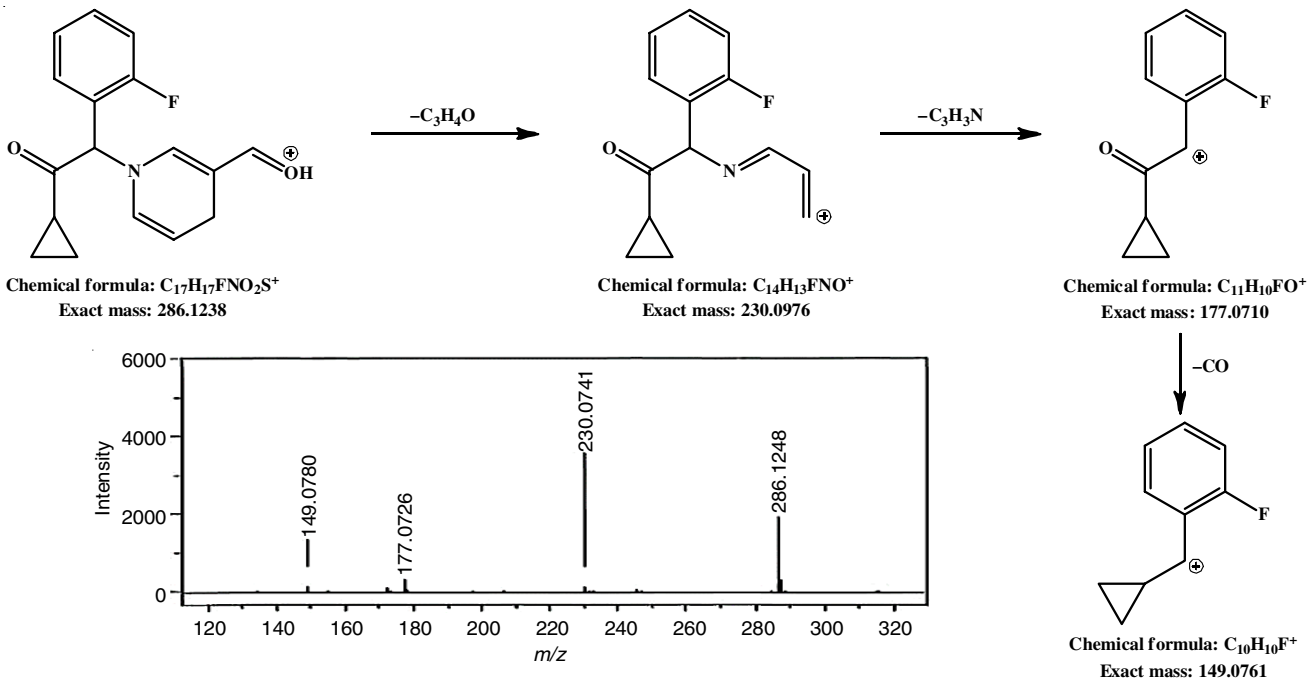

Fig. 13. Line spectra and fragmentation pattern of PR-9

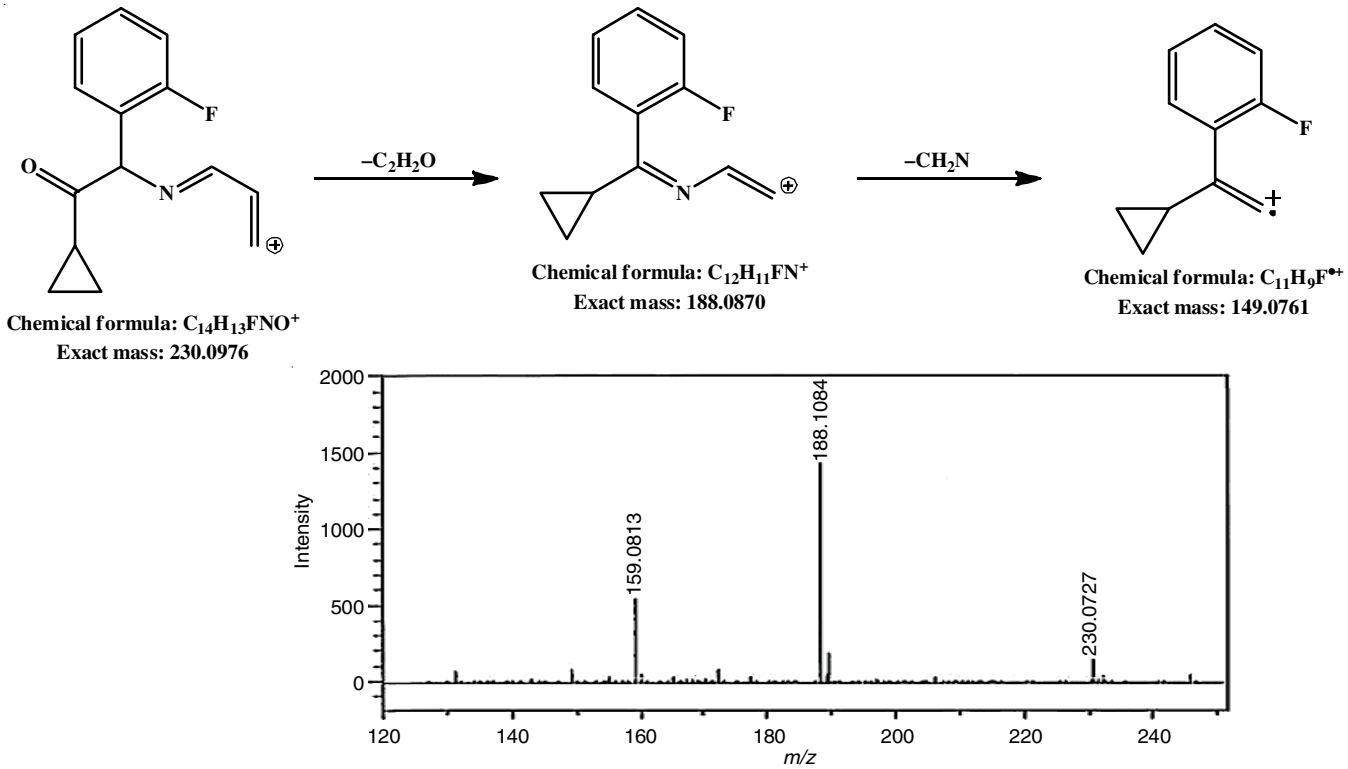

Fig. 14. Line spectra and fragmentation pattern of PR-11

be 230.0975 Da ($C_{14}H_{13}NOF$). An error of -18.868 mmu was observed by calculating the difference between the exact and observed mass. Its fragmentation pattern is shown in Fig. 15.

PR-13 (*m/z* 348): The DP observed mass was 348.1083 Da and with the help of an ECC, the exact mass was found to be 348.1064 Da ($C_{14}H_{13}NOF$). An error of 1.8822 mmu was

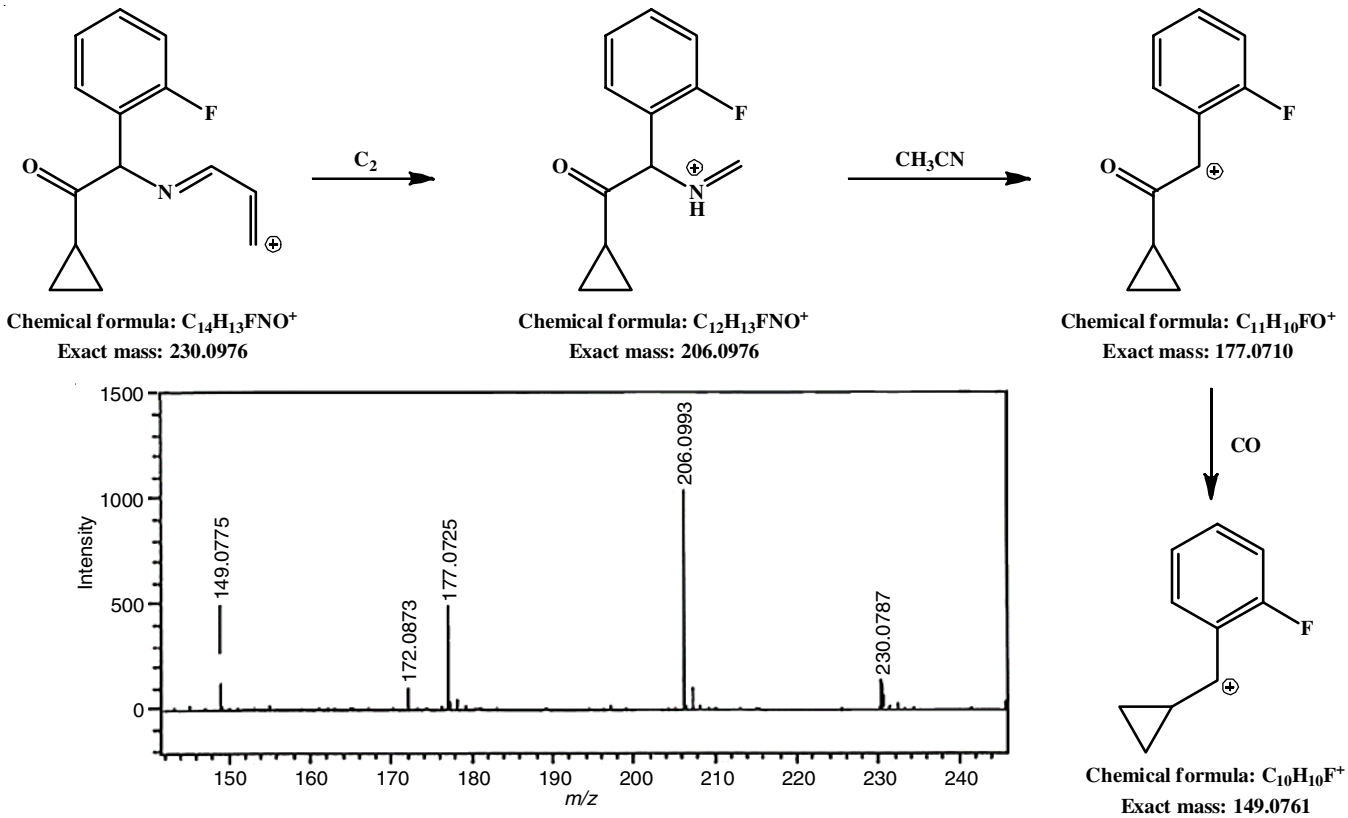

Fig. 15. Line spectra and fragmentation pattern of PR-12

established by calculating the difference between the exact and observed mass. The fragmentation pattern is shown in Fig. 16.

Comparison of DPs formed from oxidative conditions with the stability samples: The degradation products PR-7, PR-14 and PR-15 formed from the stability samples were also observed in H_2O_2 stress conditions, while PR-10 formed from the stability samples were also observed under AIBN. The exact masses and the comparison of the degradation products formed under various conditions like H_2O_2, AIBN, transition metals, hydrolytic condition and accelerated conditions (40 ºC, 75% RH, 6 months) are listed in Table-8.

Conclusion

The degradation behaviour of prasugrel HCl was different in different oxidative stress conditions were studied. With H_2O_2, a total of four degradation products were observed. In case of AIBN and transition metals ($FeCl_3$, $CuSO_4$) a single degradation product was found and characterized using LC-MS/MS technique. In hydrolytic conditions, a total six degradation products were formed and the formulated tablets kept in accelerated storage conditions (40 ºC, 75% RH, 6 M) obtained total nine degradation products, out of which four were from previously observed in the stress samples under AIBN and H_2O_2 stress

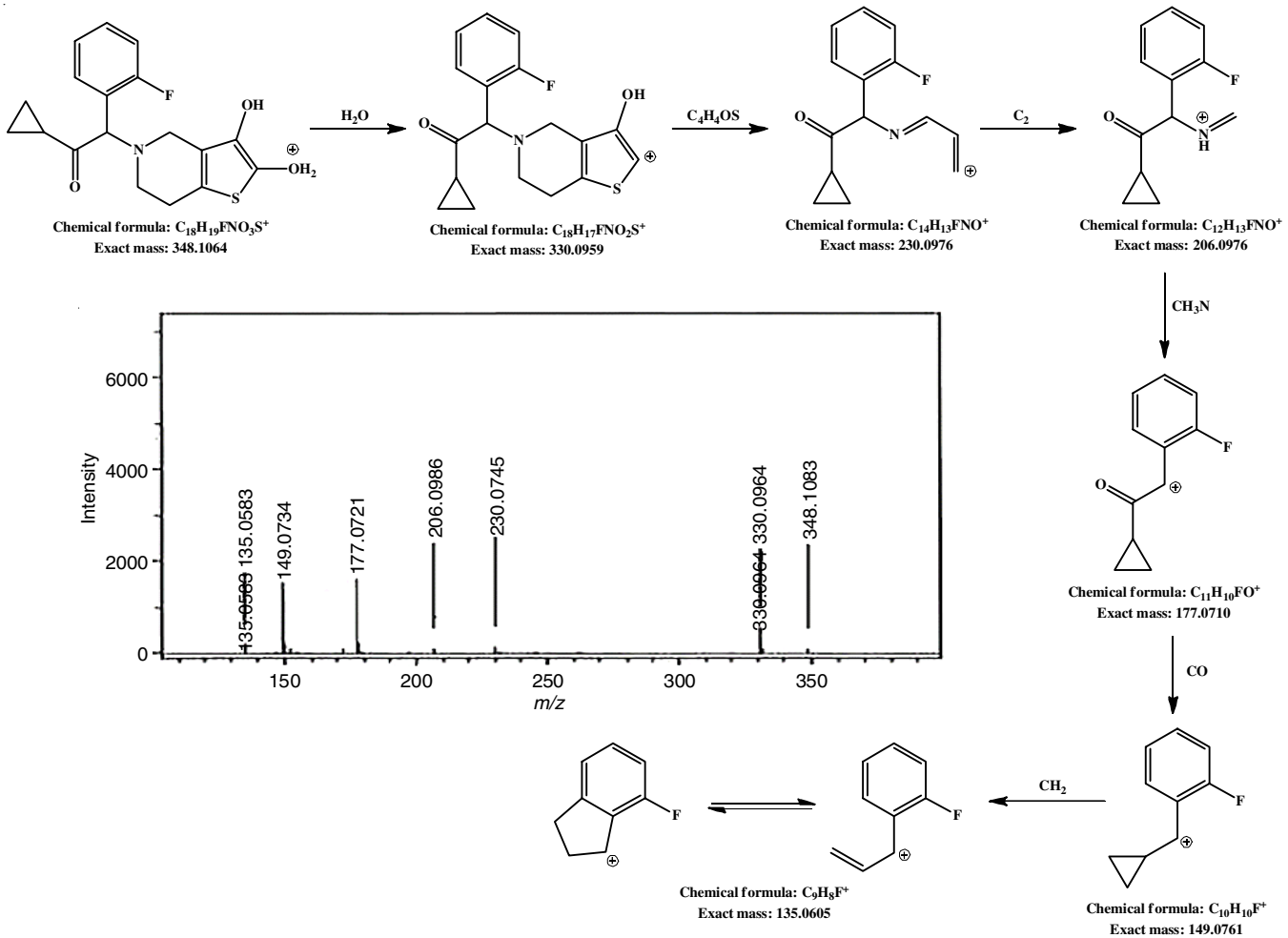

Fig. 16. Line spectra and fragmentation pattern of PR-13

TABLE-8
OBSERVED DEGRADATION PRODUCTS MASS IN DIFFERENT CONDITIONS [M+H] (amu)

H_2O_2 condition	AIBN condition	$FeCl_3$ and $CuSO_4$ condition	Hydrolytic condition	40 °C/75% RH 6 M Accelerated condition
PR-1, 398.1096	PR-3, 372.1076	PR-4, 302.1157	PR-1, 398.1096	PR-7, 398.1096 (≈ PR-1)
PR-2, 206.0976			PR-2, 206.0980	PR-8, 357.1287
PR-5, 332.1155			PR-3, 372.1013	PR-9, 286.1248
PR-6, 332.1155			PR-4, 302.1157	PR-10, 372.1076 (≈ PR-3)
			PR-5, 332.1104	PR-11, 230.0727
			PR-6, 332.1104	PR-12, 230.0787
				PR-13, 348.1083
				PR-14, 332.1155 (≈ PR-5)
				PR-15, 332.1155 (≈ PR-6)

conditions. The remaining five degradation products were also characterized. The study revealed that the degradation products formed with H_2O_2 are also observed under hydrolytic condition. Hence, H_2O_2 does not represent the actual oxidative stability of the drug. A single degradation product was observed in case of AIBN; the degradation profile induced by AIBN and H_2O_2 was simulated with the accelerated stability data (40 ºC, 75% RH for 6 months). Thus, the present study signifies that AIBN is also a useful oxidative stressor that generates the degradation profile, which represents the oxidative environment of the drug. The pharmaceutical and chemical industries use only H_2O_2 (more than 95%) as an oxidative stressor. This work signifies the importance of using AIBN as a useful oxidative stressor and provides a more accurate oxidative degradant than H_2O_2.

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CONFLICT OF INTEREST

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

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