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Original Article

MS/MS and HPLC Characterization of Forced Degradation Products of Clopidogrel and Pantoprazole Sodium

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ABSTRACT

Objective: To develop a simple, precise and accurate reverse phase high performance liquid chromatographic method for the evaluation of Clopidogrel (CLP) and Pantoprazole Sodium (PAN)succeeding degradation studies as per the ICH guidelines.
Methodology: The degradants and active drugs were separated by thin layer chromatography and exposed to structural elucidation by mass spectrometry with electron spray ionization (ESI) technique. The degradation products were detected in alkali and acid hydrolysis for Clopidogrel; whereas Pantoprazole Sodium exhibited degradation on exposure to light.
Results & Discussion: The base peak of CLP with m/z of 501.6 was formed in positive ESI mode. The daughter ion of CLP was observed at m/z of 418.3. Two degradants with m/z of 524.1 and m/z of 507.8 were formed by acid hydrolysis to give a carboxylic acid derivative and another by dehydrogenation respectively. Degradation product with m/z of 553.3 was di-sodium adduct of CLP and was formed in alkaline hydrolytic condition. The base peak of photo degradant of PAN with m/z of 419.1 was formed in positive ESI mode showed same m/z value of the base peak of PAN.
Conclusion: The obtained results indicate that structural modification occurred in PAN molecule due to photolytic degradation. This article sheds light on the mechanisms for the formation of degradation products for CLP and PAN. A linear gradient mode of elution was used for separation of CLP, PAN and their degradants on a C18column using a PDA detector. The developed and validated method was used for the analysis of marketed tablets of CLP and PAN in combination.

Keywords: RP-HPLC, Mass Spectrometry, Clopidogrel, Pantoprazole sodium, Structural elucidation and Forced degradation products.

1. INTRODUCTION

International Conference on Harmonization (ICH) guidelines directs the establishment of stability indicating assays. Thus forced degradation studies have become an integral part of the drug development. The guidelines instructs to conduct forced degradation studies under a variety of conditions, such as acid/base, light, heat, humidity, oxidation, and

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separation of active drug from degradation products[1]. These forced degradation experiments are crucial in the drug development process to facilitate: drug formulation design, stability indicating method development, selection of packaging and storage conditions, better understanding of the culpability of the drug molecule, and also solving stability related issues[2].

Appropriately, the present study sheds light to establish inherent stability of Clopidogrel (CLP) and Pantoprazole Sodium (PAN) through forced degradation studies under several ICH recommended test conditions, with the structural recognition of their degradation products and to develop a stability-indicating method. CLP chemically known as methyl (2S)-2-(2-chlorophenyl)-2-{4H,5H,6H,7H-thieno[3,2-c]pyridin-5-yl}acetate;sulfuric acid,$C_{16}H_{18}ClNO_6S_2$, Fig 1A).

Clopidogrel bisulfate is an irreversibly alters the platelet receptor for adenosine diphosphate (ADP), thereby blocking the binding of ADP to its receptor, inhibiting ADP-mediated activation of the glycoprotein complex GPIIb/IIIa, and inhibiting fibrinogen binding to platelets and platelet adhesion and aggregation.CLP is an antiplatelet agent used to inhibit blood clots in a variety of conditions such as peripheral vascular disease, coronary artery disease, and cerebrovascular disease.PAN is known as sodium 6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methanesulfinyl]-1H-1,3-benzodiazol-1-ide, $C_{16}H_{14}F_2N_3NaO_4S$, Fig. 1B). PAN is a proton pump inhibitor drug used for short-term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease.

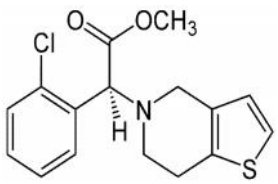

Fig 1.A: Structure of Clopidogrel

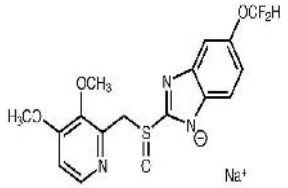

Fig 1.B: Structure of Pantoprazole Sodium

Literature survey reveals that CLP has been analyzed in pure and pharmaceutical formulations by spectroscopic technique [3, 4,] for Stress Degradation Studies and Stability- Indicating assay method by HPLC[5, 6,] determination of CLP in tablet dosage form[7] and impurity profiling by RPHPLC[8], determination of CLP by LC-MS/MS analysis[9, 10, 11,] assay by thin layer chromatography(TLC)[12, 13, 14] and simultaneous determination of CLP and PAN by spectrophotometric method[15],RP-HPLC in tablet dosage form[16, 17], by Vierordt's method in tablet dosage form[18], by LC–MS–MS in human plasma[19]. PAN is an official drug and is included in European pharmacopoeia 6.0 (EP) wherein the assay procedure for PAN includes non-aqueous titration[20]. Literature survey reveals several bioanalytical methods reported for the analysis of PAN. These include Spectrophotometric determination of PAN in pharmaceutical dosageform[21 - 25], Stress Degradation Studies and Stability-Indicating assay method by HPLC[26 – 29]and by HPTLC[30], determination of PAN in tablet dosage form[31, 32], HPLC determination of PAN inhuman plasma[33] and urine[34], human aqueous humor and serum using liquid chromatography electro spray ionization tandem mass spectrometry(LC/ESI-MS–MS)[35, 36], LC–MS[37] or LC–ESI–MS– MS[38] for determination of PAN and related substances in bulk drugs and formulations[39]. Other methods included the use of capillaryelectrophoresis[40] and HPLC for chiral separation[41], and stability indicating assay method by HPTLC[42].

Simultaneous determination of CLP and PAN by spectrophotometric method in tablet and capsule[15],by RP-HPLC method for tablets [16, 17, 18,] by LC–MS–MS in human plasma [19] was the reported method for quantization of both the drugs in combination. The implementation of HPTLC procedures is commonly found in product development and analytical laboratories [43, 44, 45]. No stress degradation studies and stability indicating assay method for simultaneous estimation of both the drugs were found to be reported. Thus, there was a need to develop a suitable validated analytical method for the simultaneous determination of CLP and PAN. This paper describes a simple and sensitive RP-HPLC method for the simultaneous analysis of CLP and PAN with the MS-MS characterization of forced degradation products. The proposed methods were validated as per the guidelines of the ICH [46].

2. METHODOLOGY

2.1 Chemicals and Reagents: CLP and PAN were obtained as a gift sample from Wintac Limited, Bangalore. India. Marketed tablets of CLP and PAN(PLAVIX, Sanofi India Ltd., India, containing 75 mg of CLP and PAN-40, Alkem Pvt. Ltd. containing 40 mg of PAN)were obtained from a pharmacy. Millipore water was obtained fromMillipore Sigma (Model: Milli-Q® IQ 7000), and was filtered using 0.45 µm nylon filter. Acetonitrile (HPLC grade), methanol (HPLCgrade), buffers and all other chemicals of analytical grade were purchased from Merck Chemical Laboratories, Bangalore, India.

2.2 Instrumentation: The HPLC chromatograms were acquired on UFLC(SHIMADZU) equipped with LC solution software with UV-VIS-PDA detector at the sensitivity of 0.01. Separation was attained using phenomenex C8 column (250 × 4.6 mm, 5 µm). Themobile phase was a mixture of potassium dihydrogen orthophosphate buffer (pH-3.0) and acetonitrile (40:60 v/v) at flow rate 1.2 mL/min. The contents of mobile phase were filtered before use through membrane filter (0.45 µ). The optimized chromatographic conditions are shown in Table 1.

Table 1: Optimized Chromatographic conditions

Chromatographic Conditions:	
Column	C8 (250 x 4.6 mm. 5 µ) phenomenex
Flow rate	1.0 mL/min
Run time	10 min

Wavelength	243 nm and 220 nm for Pantoprazole sodium and Clopidogrel respectively
Injection Volume	20µL
Detector	PDA Detector
Elution	Isocratic
Mobile Phase	potassium dihydrogen orthophosphate buffer (pH-3.0) and acetonitrile (45:55 v/v)
Column-oven temperature	25 ± 5ºC

The identification and characterization of degradants weredone using MS-MS spectrometry. Mass spectra were acquired using Varian500 MS, IT Mass Spectrophotometer. ESI parameters used were: Capillary voltage – 100 eV, source temperature – 150 ºC, sample was introduced by direct insertion probe input gas pressure 90psig, scan range – m/z 50 to 1250, approx run time0.5 seconds, nebulizing gas used was nitrogen in positive ESI and air in negative ESI.Heated Electrospray Ionization (HESI-II) was used as the ionization source. Mass spectra were acquired usingCostar software. All the samples were prepared inmethanol.

2.3 Standard Solutions Preparation: Stock solutions of CLP (100 µg/ mL) and PAN (150 µg/ mL) was prepared, methanol was used as a solvent to make upto the mark. All the solutions were prepared freshly from the stock. Mobile phase comprised of ammonium acetate buffer and methanol in the ratio of 40:60. All the solutions were filtered using Millipore filter before injection.

2.4 Test Solution Preparation (Assay of tablets): Tablets (twenty) of CLP and PAN were weighed and converted to fine powder by crushing. 1.0mg equivalent weight of tablet powder was taken in a 10mL volumetric flask. The solvent (methanol) was added and sonicated for extraction of drug completely. Further the solution was filtered into another volumetric flask and the volume was made upto the mark with methanol. The test solution was mixed with mobile phase to provide 10µg/ mL and 15µg/mL of working solutions for CLP and PAN respectively.

2.5 Method Validation: The developed HPLC methodhas been validated for accuracy, precision, specificity/selectivity, linearity, limits of detection and quantitation and robustness. ICH guidelines were used for method validation. System suitability tests were performed to confirm reproducibility and suitability of the system.

2.5.1 Precision: System precision was evaluated by analyzing the standard solutions of CLP and PAN
(20 µg mL-1) six times on the same day. The acceptance criterion was ± 2 % for the % RSD of the peak area and retention time (RT). Six independent assays of a test sample of CLP and PAN were carried out to assess the precision of the method by carrying out on the same day. The% RSD of all the six obtained assays was evaluated. The intermediate precision was evaluated on different days.

2.5.2 Limits of detection (LOD) and Limit of quantification (LOQ): The LOD and LOQ was estimated. The obtained results were found to be well within the acceptance criteria as per ICH guidelines.

2.5.3 Linearity: Stock solution (100 µg mL^{-1}) of mixture of both drugs (CLP and PAN) was prepared using methanol. Linearity for CLP and PAN were evaluated at six concentration levels by serial dilutions of the stock solution. Three times the solutions were injected into the system. Concentration versus peak area data were evaluated with linear regression. Theslope and y-intercept of the calibration curve was recorded.

2.5.4 Specificity / Selectivity: The proposed methods were evaluated by determining the concentrations of CLP and PAN in the presence of degradants in order to verify the non-interference of degradation products with the quantitation of drugs.

2.5.5 Accuracy: Accuracy was estimated by the determination of recovery of the method at three different concentrations, corresponding to 50%,100%, and 150% of the test solution concentration for CLP and PAN by adding known amount of standard to the test solutions. The percentage recoveries were estimated after three injections of every solution.

2.5.6 Robustness: The effect of a minor deliberate change in the conditions was studied by carrying out robustness studies. Small change in the linear gradient method and the flow rate was done to test the robustness. Resolution between the drug and the degradant peak was estimated. Flow rate was decreased from 1.0mL/min to 0.8mL/ min to study the effect of flow rate. Chromatograms were estimated by keeping other method conditions unchanged. The resolution was estimated in all the chromatograms.

2.5.7 Solution Stability: Stability of the test preparation was estimated by keeping the solution in a tightly closed volumetric flask at room temperature. Tests were carried out at 12, 24 and 72 h and the chromatograms obtained were estimated and compared with standard solution which was freshly prepared.

2.5.8 System suitability: The parameters pertaining to the system like retention time, peak area, peak purity, tailing factors, resolution and number of theoretical plates were studied. Standard solutions of CLP and PAN and their solutions subjected to forced degradation were estimated five times.

2.6 Forced degradation studies:

CLP and PAN were exposed to forced degradation independently and in combination as per the ICH guidelines. 1mg/mL solutions of CLP and PAN were prepared initially to use in the studies. Mobile phase was used to dilute the samples to obtain 10 µg/mL concentrations before injecting into HPLC.

Different conditions for forced studies are as follows:

2.6.1. Acid, Alkaline and Neutral degradation studies: The acid, alkaline and neutral degradation studies were carried out at 80°C for 8 h using 1M of Hcl and NaOH for acid and alkaline decomposition respectively.

2.6.2. Oxidation: Oxidative degradation studies of CLP and PAN was carried out using 2 % v/v hydrogen peroxide. 100 µg/ mL solutions of CLP and PAN were prepared for this study. Thus prepared solutions were kept for 48 h at room temperature.

2.6.3. Thermal degradation studies: The powdered samples of CLP and PAN were kept in a vial in an oven at 60 °C for one month.

2.6.4. Photo stability: CLP and PAN having concentration of 1mg/ mL in methanol were kept in sunlight for 15 days to check the stability under photo conditions. Standard was also prepared and was kept under dark.

2.6.5. Separation of Degradants: Methyl acetate, methanol and hexane were used in different ratio to separate the degradants from the mixture using thin layer chromatography (TLC). Mass spectrometry was used to characterize the isolated degradants.

3. RESULTS AND DISCUSSION

3.1 HPLC METHOD DEVELOPMENT AND OPTIMIZATION:

The drugs subjected to forced degradation studies were used to develop method with isocratic elution mode to identify the degradation pathways. 100mM ammonium acetate buffer (pH 3): methanol (30:70, v/v) was used for the separation of CLP from its degradants. 150mM potassium buffer (pH 3.5): methanol (60:40, v/v). Peaks were found to be merged when the above mobile phase was used for simultaneous estimation of CLP and PAN along with their degradants.

Therefore, linear gradient mode of elution was applied with a flow rate of 1.0mL/min and maximum wavelength of 275nm. A good resolution was obtained between the standard drugs and degradants.

The chromatograms obtained for drugs and their degradation products are shown in Fig. 2(A&B).

Fig 2: HPLC Chromatogram of (A) Standard Clopidogrel and Pantoprazole Hydrochloride (B) Degradants of Clopidogrel and Pantoprazole Hydrochloride

3.2 METHOD VALIDATION:

3.2.1 Precision: The % RSD for the system precision was found to be 0.667 % and 0.928 % for RT and peak area of CLP, and 0.616 % and 0.921 % for RT and peak area of PAN on the same day (intra-day) respectively. The % RSD for method precision was found to be 0.991 % (intra-day) and 0.801 % (inter-day) for peak area of CLP, and 0.661 % (intraday) and 0.817 % (inter day) for peak area of PAN respectively. The % RSD for intra-day and inter-day assays values obtained was found to be 1.12 %for CLP and 0.91 % for PAN. The values obtained for system and method precision are shown in Table 2.

Table 2: System and Method Precision Data (n = 6)

System Precision					Method Precision			
	CLP		PAN		CLP		PAN	
	RT (min)	Peak Area	RT (min)	Peak Area	Concentration (µg mL-1)			
					Intra day	Inter day	Intra day	Inter day
Mean	26.268	20678	4.153	645433	19.989	20.057	20.502	20.592
SD	0.028	2581.537	0.021	2107.263	0.104	0.105	0.067	0.158
% RSD	0.106	1.248	0.516	0.326	0.520	0.522	0.325	0.770

3.2.2 LOQ and LOD: The LOD for CLP and PAN was found to be 0.02 µg mL^{-1} and 0.01 µg mL^{-1}respectively. The LOQ for CLP and PAN was found to be 0.2 µg mL^{-1} and 0.1 µg mL^{-1}respectively.

3.2.3 Linearity: The calibration curve was found to be linear for CLP and PAN. Highly promising results were shown with good correlation between analyte and peak area. The results obtained are given in Table 3. The data obtained for peak area of CLP and PAN with their concentration was treated with linear regression estimation. The correlation coefficient obtained was found to be well within the acceptance criteria.

Table 3: Linearity Data

Analyte		CLP	PAN
Slope ± SD (n=3)		32847.28 ± 158.37	18342.53 ± 34.37
Intercept ± SD		7879.603 ± 841.85	4917.918 ± 466.59
R^2 ± SD		0.99897 ± 0.00032	0.99923 ± 0.00015
Confidence interval of slope at 95%	Lower	33240.68	5788.33
	Upper	32453.88	9970.88
Confidence interval of intercept at 95%	Lower	18257.40	779.65
	Upper	18427.67	9056.19

3.2.4 Selectivity / Specificity: The peak purity index was estimated for CLP and PAN to evaluate the selectivity of the developed method in the degradants. The photodiode array (PDA) detector was used to check the purity of the drug peak. The LC solutions software was used to check if the drug peaks are well resolved for the degradants peaks. The results obtained were well within the acceptance criteria

indicating that there was no interference between the analyte peak and the degradants. The results for specificity and system suitability are given in Table 4.

Table 4: Specificity and System suitability of PAN and CLP in different forced degradation studies

Stress degradation Conditions	RT (min)	Peak area	% Assay	Peak Purity	Resolution	Asymmetry	Capacity Factor	Theoretical Plates
CLP								
Standard	27.438	17406	100	995	18.12	0.97	1.62	3249
Acid	27.102	15162	87.33	999	3.96	1.39	2.85	2890
Alkaline	27.164	13821	79.40	994	4.64	1.10	2.92	4371
Neutral	27.082	16897	99.87	994	18.07	0.95	1.65	3985
Oxidation	27.077	17085	99.72	998	17.99	0.99	1.65	3256
Photo stability	27.129	16985	99.90	995	18.54	1.05	1.66	3996
Temp (60°C)	27.304	16542	99.30	993	18.15	1.12	1.70	3105
PAN								
Standard	4.585	58867	100	998	2.015	1.19	0.53	4490
Acid	4.509	55559	99.53	996	2.401	1.18	0.71	3360
Alkaline	4.457	56641	99.93	1000	2.432	1.06	0.72	3888
Neutral	4.498	57853	100	997	2.389	0.93	0.70	3171
Oxidation	4.514	57992	100	997	2.2	0.95	0.61	3636
Photo stability	4.533	33161	88.46	999	1.33	1.14	0.62	2640
Temp (60oC)	4.425	56248	100	998	2.202	1.07	0.61	3967

3.2.5 Accuracy:

Accuracy of the method was proven by the recovery tests carried out. Table 5 indicates the amount of drugs added to the aliquots of test solution and diluted. The results obtained are given in table 5. The study was done three times on the same day of analysis, for three consecutive days (n=9). The percentage recovery was found to be 100.52% and 99.91% for CLP and PAN respectively.

Table 5: Accuracy (N = 9)

Level %	Concentration of drug added (µg mL-1)	Total concentration of drug (µg mL-1)	Mean Recovery %	% RSD
CLP				
50	10	30	100.75	1.37
100	20	40	100.76	0.44
150	30	50	99.72	0.52
Mean	**100.41 %**	**0.875 %**		
PAN				
50	10	26	100.46	0.56
100	20	36	99.61	0.21
150	30	46	99.49	0.44
Mean	**99.85 %**	**0.68 %**		

3.2.6 Robustness: The forced degradation samples were subjected for assessment of robustness of the method. The resolution between the drug and the degradation products was also measured. The obtained results are tabulated in Table 6. The resolution did not change significantly with the small change in the flow rate of mobile phase. The %RSD was found to be less than 1%. The change in the buffer composition showed no significant change in the retention time (RT) of PAN, but a significant change was seen in the RT of CLP. Whereas, resolution between CLP and its degradant was not changed significantly. Thus, indicating that the developed method was robust for the estimation of CLP, PAN and their degradants simultaneously.

Table 6: Robustness of the Method

Change in flow rate for the Mobile Phase: 10 mM ammonium acetate, pH 4.5 : methanol (0-2 min, 50:50; 2-20 min, 20:80; 20-30 min, 50:50; 30-40 min, 50:50)								
RT							**% Assay**	
Flow rate (mL min-1)	**Photo CLP**	**STD CLP**	**PAN Deg 1**	**PAN Deg 2**	**PAN Deg 3**	**STD PAN**	**CLP**	**PAN**
1	4.980	5.193	18.126	21.524	26.987	34.667	99.85	98.99
1.2	3.60	4.457	14.833	16.805	20.162	27.163	100.21	99.84
Change in linear gradient time of the mobile phase; 10 mM ammonium acetate (pH 4.5) : methanol at a flow rate of 1.2 mL min^{-1}								
RT							**% Assay**	
Time min	**Photo CLP**	**STD CLP**	**PAN Deg 1**	**PAN Deg 2**	**PAN Deg 3**	**STD PAN**	**CLP**	**PAN**
0 – 1.5 1.5 – 35 35 – 45 45 – 50	3.872	4.591	17.284	19.926	25.347	37.043	100.26	100.05
0 – 2 2 – 30 30 – 40 40 – 50	3.522	4.455	13.204	14.912	18.597	26.559	101.80	99.19
0 – 2 2 – 20 20 – 30 30 – 40	3.500	4.461	14.883	16.857	20.162	27.168	99.60	99.62
0 – 3 3 – 20 20 – 26 26 –32 32 – 45	3.419	4.272	14.027	16.942	19.013	24.525	98.50	100.84
0 – 2 2 – 15 15 – 25 25 – 30 30 – 40	3.174	4.091	12.742	15.034	16.745	21.547	101.90	99.54

CLP – Clopidogrel, STD – Standard, PAN – Pantoprazole, Deg – Degradant

3.2.7 Solution Stability: The test samples and the standard drugs for assay were subjected to 72h stability studies and found to be stable. The results obtained indicates that the values and statistically significant having %RSD less than 2.

3.3 Assay of marketed tablets: The developed method was applied to assay the marketed tablets; the results obtained indicated that the %RSD was found to be 0.97% and 0.87% for PAN and CLP respectively.

3.4 Forced degradation studies: Totally PAN showed two degradants; in acidic condition 13.32% degradation was observed. In alkaline condition PAN showed 42.31% degradation. PAN did not show degradation in photo, oxidative, thermal and hydrolytic conditions. CLP when subjected to photolytic condition showed 12% degradation with one degradant. CLP didn't show any degradants in oxidation, acidic, alkaline and thermal conditions.

3.5 Elucidation of degradation products:

Photo degradant of CLP and alkali degradant of PAN were separated by adopting thin layer chromatography.

Separation of degradants of PAN was done using methanol: hexane: methyl acetate in the ratio 10:30:60 v/v/v. The degradants of CLP was done using methanol: hexane: methyl acetate in the ratio of 50:30:20 v/v/v. Mass spectrometry was used to identify all the degradants.

3.6 LC-PDA studies: The chromatograms obtained from HPLC, PDA spectra of CLP and its acid and alkaline degradants are given in **Fig. 3.** The PDA absorption spectra indicate that the acid degradation product **I** of CLPand alkaline degradation product **I** of CLP have identical spectra for UV absorbance. Also acid degradation product **II** and alkaline degradation product **III** of CLP display similar spectra. Whereas, a different spectra was observed with alkaline degradation product **I** and **III**, which revealed that both **I** and parent drug have different chromophore than product **III**. This indicates that during the conversion of parent drug CLP to its degradation product I and III, drug chromophore was lost or altered. The retention time of degradation product **II** of CLP is different than CLP standard. Whereas the spectrum of UV absorbance of degradation product **II** of CLP was matching with the spectrum of CLP standard. The obtained results in MS-MS studies shows that alkaline degradation product **II** of Pan is an adduct of PAN.

The HPLC chromatogram and PDA-UV spectra of PAN and its photo degradant are shown in Fig. 4. The PDA absorption spectra of PAN photo degradation product (RT-4.881 min) were found to be different than the standard PAN (RT-5.804 min). The standard PAN showed two wavelengths of maxima at 225.2 nm and 279.7 nm, whereas the photo degradation product of PAN showed absorption maxima at 254.5 nm and 279.9 nm. This indicates that PAN undergoes photo degradation.

Structural reorganization of PAN is seen very prominently from the results obtained by MS-MS fragmentation.

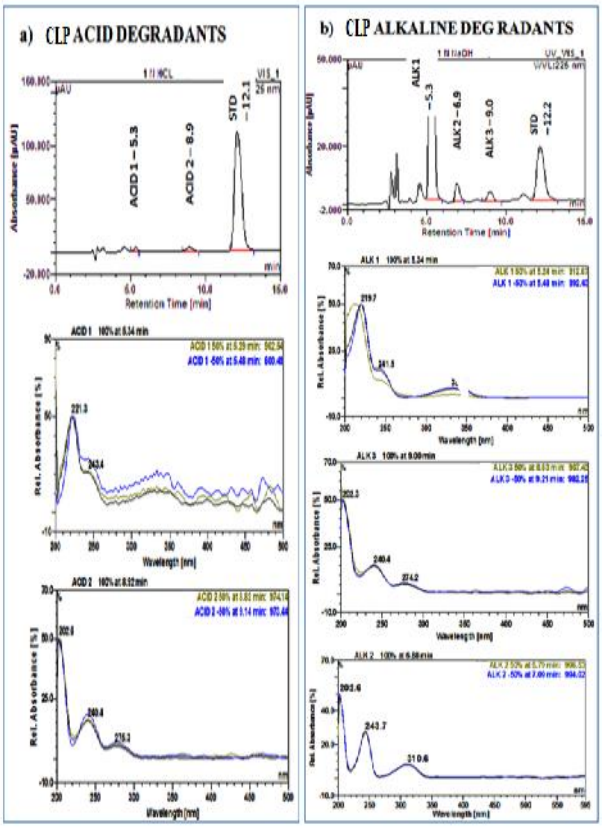

Fig 3: HPLC Chromatogram and PDA Absorption Spectra of: A) Acid Degradants of CLP & B) Alkaline Degradants of CLP

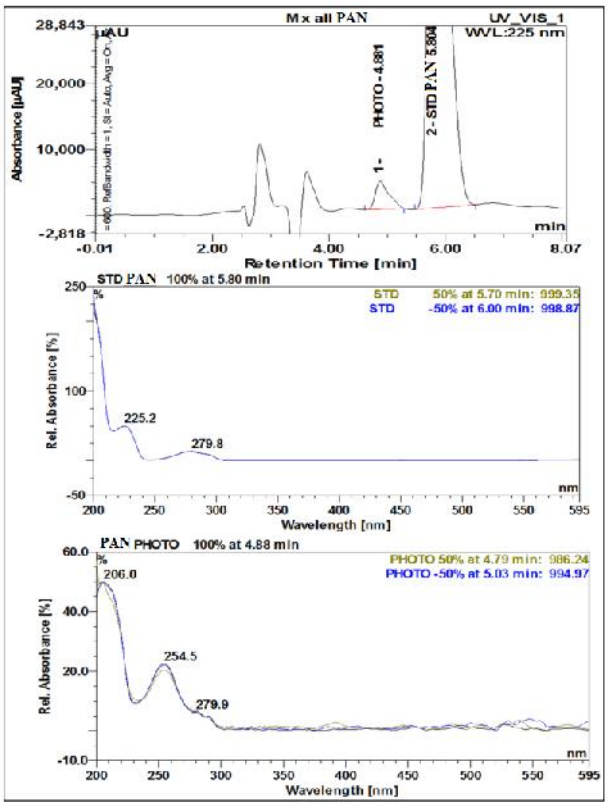

Fig 4: HPLC Chromatogram and PDA Absorption Spectra Of: A) Standard PAN and B) Photo Degradant of PAN

3.7 MS/MS fragmentation of CLP: The MS-MS spectrums and fragmentation arrangement of CLP is displayed in Fig. 5. The base peak of CLP was found with m/z value of 529.1. The daughter ion of CLP was seen at m/z of 461.2. The MS-MSspectra's, fragmentation patterns and hydrolysis mechanism of acid hydrolysis degradants of CLPare shown in Fig. 6 and Fig. 7 respectively. Two degradants with m/z of 546.2 and m/z of 527.5 were formed by acid hydrolysis to produce a derivative (I) and by dehydrogenation to give degradant (III) respectively. Degradation product I having m/z value of487.1 was formed by addition of 8 amu $[M+8]^+$that indicated the complete hydrolysis of the compound.

Degradation product III having m/z of 429.1 was composed with difference of 3 amu $[M-3H]^+$ expressing elimination of hydrogen molecule from CLP. The MS-MS spectrum and fragmentation pattern of alkaline hydrolysis of CLP is observed in Fig. 7. Degradation product II with m/z of 503.7 was formed by addition of 32amu $[M+32]^+$ indicating di-methyl compound of CLP.

Degradation product II of CLP was observed in alkaline hydrolytic condition only. The degradation product I and III of CLP was observed in both acidic and alkaline hydrolysis. The degradation products for CLP were interpreted and identified by us as (I) - methyl (+)-(S)-α-(2-chlorophenyl)-6-hydrothieno[3,2-c]pyridine-5(4H)- acetate (MW. 416.4) , (II) methyl, 6,7-dihydrothieno[3,2-c]pyridine-5(4H)- sulfate (MW. 413.9) and (III) methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5-sulfate (MW. 425.7). The major fragments of standard CLP is shown in Table 7 and its degradation products are given in Table 8.

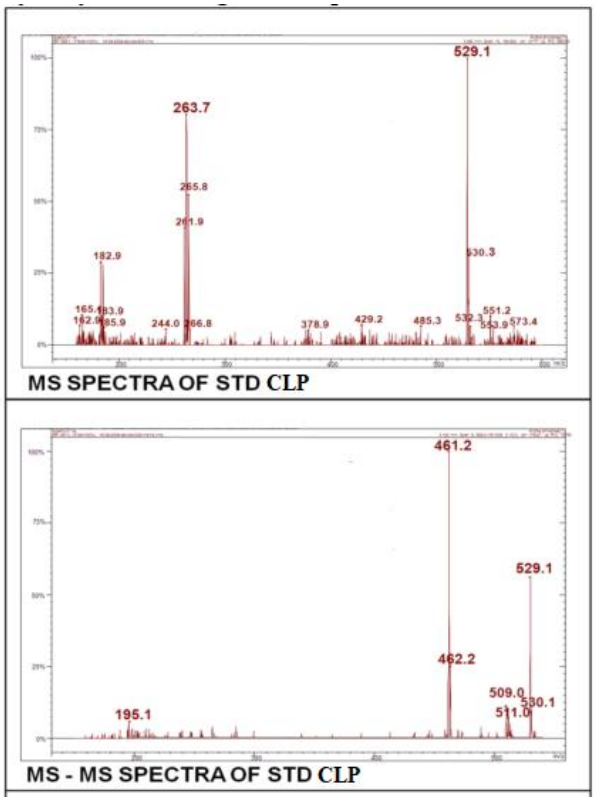

Fig 5: Mass Spectral Details of Standard CLP

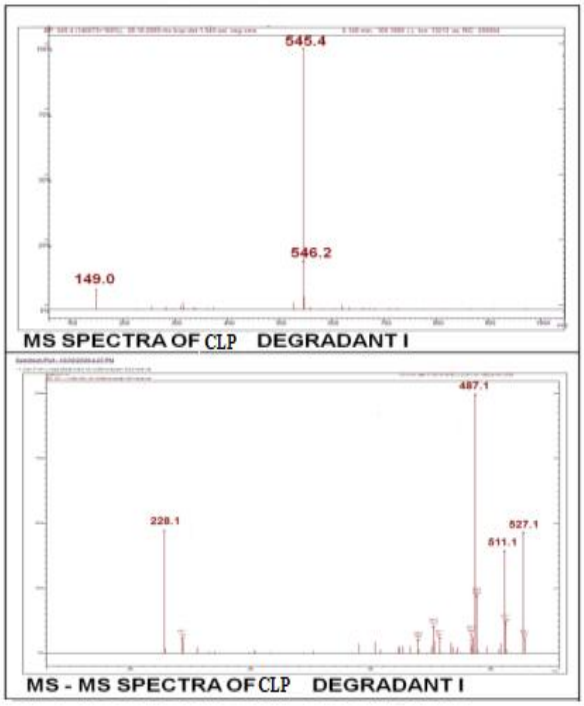

Fig 6: Mass Spectral Details of CLP Degradant I

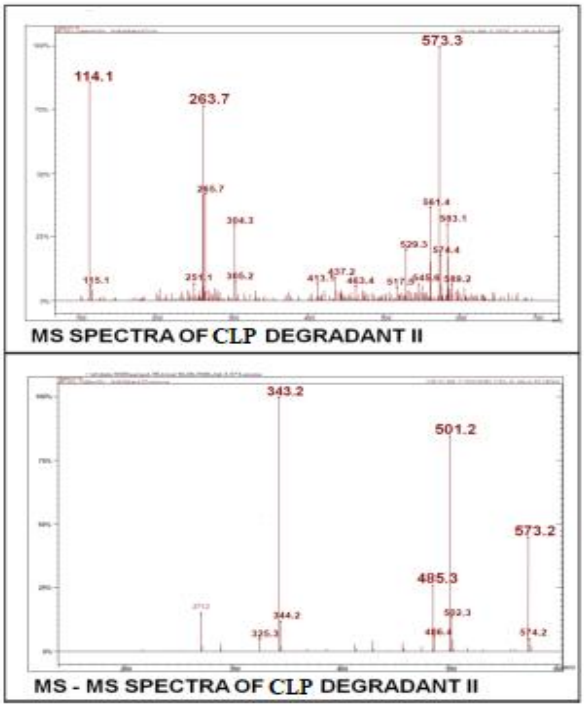

Fig 7: Mass Spectral Details of CLP Degradant II

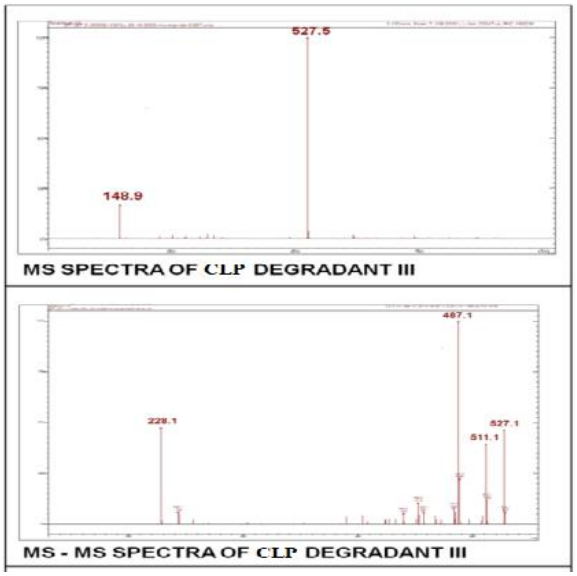

Fig 8: Mass Spectral Details of CLP Degradant III

Table 7: MS-MS Data of Standard CLP and PAN

Sl. No.	Experimental mass	Theoretical mass	Error in mmu	Difference from parent ion
CLP				
1	529.10	528.22	0.880	
2	509.00	509.53	-0.53	20.1
3	461.20	461.46	-0.26	67.9
4	195.10	19.108	-0.008	334.0
PAN				
1	409.10	408.17	0.93	
2	271.00	271.36	-0.36	138.10
3	228.10	228.29	-0.19	181.00
4	200.10	200.234	-0.134	209.00

Table 8: MS - MS Data of Degradation Products of CLP and PAN with their Major Fragments

Degradation products	Experimental mass	Theoretical Mass	Error in mmu	Major fragments		Error in mmu
				Experimental mass	Theoretical mass	
CLP						
Degradant I	545.40	545.54	-0.14	511.0	511.54	-0.54
				487.10	487.48	-0.38
				452.3	451.52	0.78
				228.10	228.12	-0.02
Degradant II	527.50	526.21	1.29	511.0	511.54	-0.54
				487.10	487.48	-0.38
				452.3	451.52	-0.38
				228.10	228.12	-0.02
Degradant III	573.30	572.49	0.81	501.20	500.23	0.97
				485.30	485.49	-0.19
				343.20	344.16	-0.96
				271.20	270.15	1.05
PAN						
Photo Degradant	409.10	408.19	0.91	271.00	271.36	- 0.36
				228.10	228.29	-0.19
				200.10	200.234	-0.13

3.8 MS/MS fragmentation of PAN: The MS-MS spectrum and fragmentation pattern of PAN is shown in Fig. 9. PAN base peak exhibited an m/z of 409 in the ESI mode. PAN showed high degradation in photostability investigation forming a degradant showing the same m/z value of409.1 as that of PAN base peak. The MS-MS spectrum and fragmentation pattern of PAN under photo degradation is shown in Fig. 10. This indicates that structural modification of PAN molecule occurred in photolytic degradation condition. The photodegradation product of PAN was identified as 5-(Difluoromethoxy)-3-[[(4, 4-dimethoxy-2-pyridyl) methyl] sulfinyl]-benzimidazole. The data for possible molecular formulae and major fragments of standard PAN and its photo degradation product is given in Table 6 and Table 7 respectively.

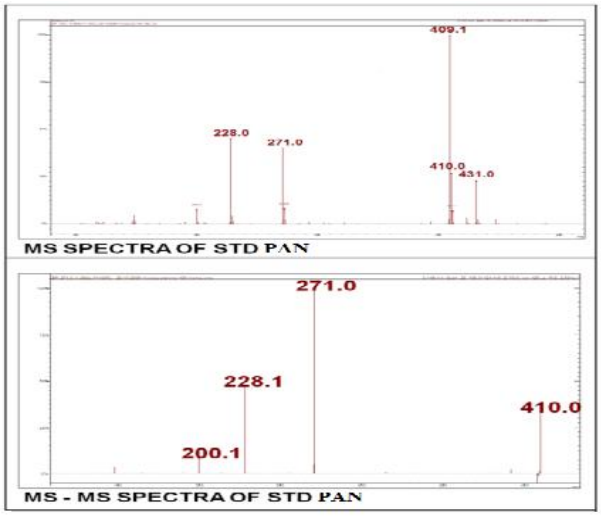

Fig 9: Mass Spectra of Standard PAN

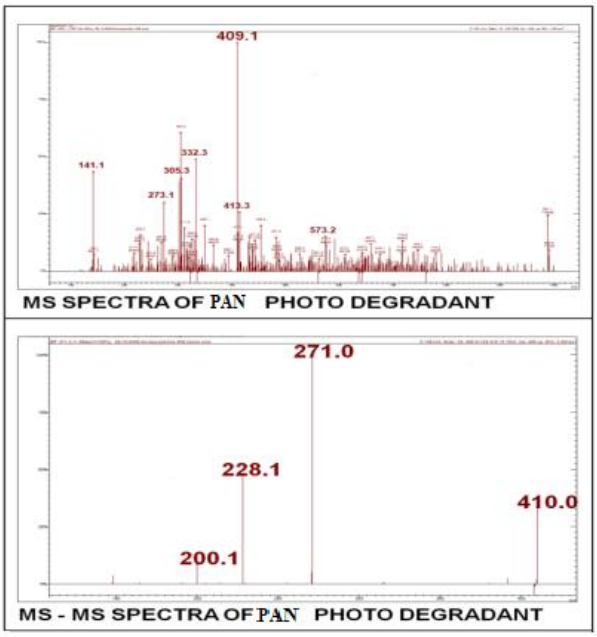

Fig 10: Mass Spectra of PAN Photo Degradant

4. CONCLUSIONS

The study conducted as per ICH guidelines on Pantoprazole (PAN) and Clopidogrel (CLP) assisted in the identification

of the degradation route of the drugs with the structural elucidation of degraded products.

The validated RP-HPLC method developed for the concurrent estimation of CLP and PAN under forced conditions was found to be simple, precise accurate and robust.

Thus the above conducted study was found to be unique and would be beneficial in analyzing stability samples in pharmaceutical industries and also for routine analysis of formulations.

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