Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

Stress Degradation studies and Development of High Resolution New Validated Stability Indicating Analytical Method for Determination of Various Proton Pump Inhibitors in its Bulk and Multicomponent Pharmaceutical Dosage forms in the Presence of Degradation products as per ICH Guidelines

CH. Naveen Kumar[a,b]*, N. Kannappan[b], Mahendra Kumar CB[c]

[a]*Bright Labs, Kothapet,Dilshuknagar,Hyderabad,Telangana,INDIA.*
[b]*Department of Pharmacy, Faculty of Engineering and Technology, Annamalainagar, Annamalai University, Chidambaram, Tamilnadu, INDIA.*
[c]*Department of Pharmacy, St. Mary's College of Pharmacy, Secunderabad, Telangana, INDIA.*

Received on: 11-02-2015; Revised and Accepted on: 04-03-2015

ABSTRACT

A *Novel high resolution , sensitive,accurate ,robust & Rugged stability indicating analytical method was developed for simultaneous determination of four active pharmaceutical ingredients for the simultaneous Determination of four active ingredients including pantaprazole (PAN), Rabeprazole (RAB), Lansaprazole (LAN) and Domperidone(DOM) in its bulk and pharmaceutical dosage forms by RP-HPLC-DAD and the analytical separation was carried out by reverse phase chromatography on X Bridge (3x100mm;3.7 µm) C18 column with the gradient program. Sol-A is composed of Buffer pH 7.5 adjusted with Ortho phosphoric acid (10 ml of Triethylamine and 20 mM Potassium Dihydrogen Ortho Phosphate in to 1000ml of HPLC water) and Sol -B Mixture of Methanol and Acetonitrile in a ratio of 85:15 v/v and the M.P- A is a Mixture of Sol-A : Sol-B in the ratio of 90:10 v/v M.P-B consists of Sol-A : Sol-B in the ratio of 20:80 v/v . M.P-A (0-3min: 70-70, 3-7min:70-40, 7-20min:40-40, 20-21min:40-40,21-25min:70-70) with gradient programme the flow rate for the mobile phase elution is 0.5 ml per minute and the column oven temperature is maintained at 25^{0}c, run time was 25 minutes. The quantification was achieved with PDA detector and the effluents were monitored at 280 nm for four drugs and their combination drug products were subjected to various stress conditions. the calibration curves for all four drugs was found to be linear and the correlation coefficient for all four drugs is not less than (r^2=0.999).The LOD Concentration for PAN, RAB, LAN & DOM was found to be 0.782 µg/mL,0.897 µg/mL,0.142 µg/mL & 0.185µg/mL respectively. Then the LOQ Concentration for 2.524 µg/mL, 2.894 µg/mL, 0.459 µg/mL and 0.599µg/mLwere found respectively. There was no interference observed with excepients and degradation products in the determination of API and FP thus providing the stability indicating superiority of the method.*

Keywords: *High resolution, Forced degradation studies,RP-HPLC-PDA Detector, Pantoprazole(PAN), Rabeprazole(RAB), Lansoprazole(LAN), Domperidone(DOM); Stability-indicating Analytical method.*

INTRODUCTION

Pantoprazole(PAN) is proton-pump inhibitor that inhibits gastric acid by blocking the H+/K+-adenosine triphosphatase enzyme system of the gastric parietal cell [1-5]**.** Its application is in the short-term treatment of erosion and ulceration of the esophagus [6]**.** Pantoprazole is 5- (Difluoromethoxy) – [[(3,4-dimethoxy-2-Pyridiynyl) Methyl] sulphinyl] - 1H– benzimidazole. Its sodium form that is used in pharmaceuticals is known as Pantoprazole sodium has the structure given in **(Fig. 1).**

Rabeprazole sodium (RAB) is chemically known as 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulfinyl]-1H-benzimidazole sodium salt **(Fig. 2).** It is a proton pump inhibitor and used for the treatment of peptic ulcer or GERD [7-11].

Lansoprazole (LAN) is chemically 2-({3-methyl-4-(2, 2, 2-trifluoroethoxy)-2-pyridyl) methyl} sulfinyl benzimidazole **(Fig. 3),** is used as a gastric proton pump inhibitor. It has an empirical formula of C16H14F3N3O2S and a molecular weight of 369.36. Literature survey revealed HPTLC, spectrophotometric and spectrofluorometric methods for determination of lansoprazole in bulk, dosage forms, biological fluids and acid-induced degradation studies [12-15]**.**

Domperidone is chemically known as 5-chloro-1-[1-[3-(2, 3-dihydro-2-oxo-1Hbenzimidazol-1-yl) propyl] piperidin-4-yl]-2, 3-dihydro-1H-benzimidazol-2-one **(Fig. 4).** It is a gastro-kinetic and anti-emetic. It is a peripheral dopamine-2 receptor antagonistIt is official in B.P. The present work describes the development of a high resolution validated stability indicating RP-HPLC method which can quantify these components simultaneously from a combined dosage forms with high resolution and with proper stability indicating studies. A few chromatographic methods in dosage forms and biological fluids have been reported for the simultaneous determination of PAN, RAB, LAN, and DOM in multicomponent dosage forms [16-20] but there is no proper evidential stability indicating features in many papers so the attempt was made to optimize and developing stability indicating method with high resolution power. The present RP-HPLC method was validated following the ICH guidelines [21-23] the developed method can be successfully applied to the quality control of various proton pump inhibitors and for other analytical purposes.

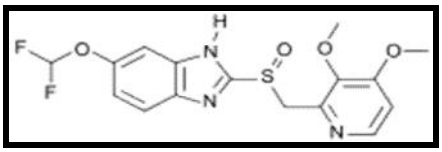

Fig. 1: Chemical structure of Pantoprazole(PAN)

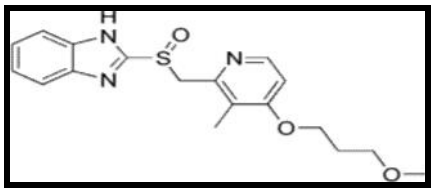

Fig. 2: Chemical structure of Rabeprazole(RAB)

***Corresponding author:**
CH. Naveen Kumar
Bright Labs, Kothapet,
Dilshuknagar, Hyderabad, Telangana, INDIA.
**E-Mail: naveen2626@gmail.com*

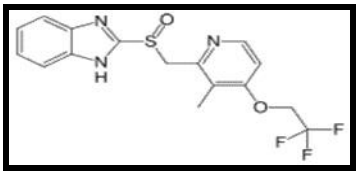

Fig. 3: Chemical structure of Lansoprazole(LAN)

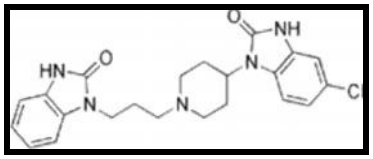

Fig. 4: Chemical structure of Domperidone(DOM)

EXPERIMENTAL

Materials & Methods:

Pharmaceutical grade working standards Pantoprazole (PAN), rabeprazole (RAB), lansoprazole (LAN), domperidone (DOM) were obtained from Dr.Reddys Labs, Hyderabad as a gratis samples. The HPLC-grade methanol was purchased from Merck. All other chemicals like acetonitrile, KH_2PO_4, 0. 22µ membrane filter, 0.45µ filter paper and solvent used were of analytical grade (Merk). High purity water was prepared by using a Milli-Q RO system (Millipore). All the chemical and reagents were purchased from Merck chemicals.

Instrumentation:

The analysis was performed using waters-2695(Model alliance) High Performance liquid chromatography waters auto sampler–PDA detector 996 by using, Empower-software version-2, analytical balance (MettlerToledo) UV/Visible-Detector (Standard cell) and data handling system (Autochrome-3000), pH meter (lab India), Sonicator. The column used is Waters X Bridge 3x100mm; 3.7 µm C18 withthe flow rate 0.5ml/min (Gradient elution).

Preparation of solutions:

Sol-A: is composed of Buffer pH 7.5 adjusted with Ortho phosphoric acid (10 ml of Triethylamine and 20 mM Potassium Dihydrogen Ortho Phosphate in to 1000ml of HPLC water)

Sol-B: Mixture of Methanol and Acetonitrile in a ratio of 85:15 v/v

Mobile phase (MP) Preparation:

M.P- A is a Mixture of Sol-A : Sol-B in the ratio of 90:10 v/v.

M.P-B consists of Sol-A: Sol-B in the ratio of 20:80 v/v with gradient program.

Preparation of blank solution:

Combination of Potassium dihydrogen orthophosphate buffer (pH-4.5) and Acetonitrile was mixed in the ratio of 30:70. This prepared solution was used as mobile phase. This solution was also used for specificity blank solution

Preparation of Placebo Solution:

The placebo Solution was prepared by dissolving the Specified amount Excipients in diluent (in house made).

Preparation of STD stock solution:

Standard solution of PAN, RAB, LAN and DOM-were prepared by dissolving 10 mg of each drug into 10 mL volumetric flask separately. Then dilution was made by adding 10 mL of the Diluent solution to 10 mL standard flask and making up the volume with the Diluent. The final concentration of each drug was found to be 1000µg/ml.

Preparation of STD solution:

From the Prepared individual Standard Stock Solution of PAN, RAB, LAN and DOM take 0.3 ml of PAN, RAB, LAN and DOM into a 10ml of standard flak to this add 10ml of diluent. Finally make up the solution upto the mark with diluent. The Final concentration of the individual was 30 µg/ml respectively.

Preparation of Test solution:

The test solution was prepared by taking an equivalent amount of PAN, RAB, LAN and DOM into a 10ml of volumetric flask make up with diluent,from that take 1ml into 10 ml of standard flask make up the solution with diluent. Final concentration of PAN, RAB, LAN and DOMwas 30 µg/ml respectively.

Optimization of HPLC Method:

The HPLC method was optimized and developed for simultaneous method for PAN, RAB, LAN and DOM. The mixed standard solution was injected in HPLC by the following chromatographic conditions.

The chromatographic separation was achived on X Bridge 3x100mm;3.7 µm C18, Gradient mode and the Mobile phase consists of Triethylamine and Potassium Dihydrogen Ortho Phosphate pH – 7.4) : Methanol and Acetonitrile in a ratio of 85:15 v/v and the flow rate of mobile phase was 0.5ml/min , run time was 25 min and the column temperature was maintained at Room temp(20-25^0 c),volume of injection loop was 20µl.detection was monitored at 280 nm. **(Table 1).**

Method validation:

The method validation was done according to the ICH guidelines. The following validation characteristic parameters are accuracy, precision, linearity, and specificity, LOD, LOQ, ruggedness and robustness.

1. Linearity and range: Linearity of the method was studied by injecting the mixed standard solutions with the concentration ranges from of 10-50µg/mL for PAN, RAB, LAN and DOM drug levels of increasing concentrations were prepared and injected six times into the HPLC system keeping the constant injection volume. The peak areas were plotted against the concentrations to obtain the linearity graphs.

2. Precision: The precision of the optimized method was evaluated by carrying out six independent assays of test sample. %RSD of six assay values was calculated. Intermediate precision was carried out by the samples by using another instrument and with different analyst.

3. Limit of Detection and Quantification: The LOD and LOQ procedures were performed on samples contain very lower concentrations of analytes under the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analytes that can be detected and quantified, with acceptable accuracy and precision.

4. Robustness: Robustness was studied by evaluating the effect of small variations in the chromatographic conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition. These chromatographic variations are evaluated for resolution between PAN, RAB, LAN and DOM

5. System suitability:

The system suitability parameters with respect of tailing factor, theoretical plates, repeatability and resolution between PAN, RAB, LAN and DOM peaks were defined.

6. Specificity:

The specificity of the analytical method is the ability of the method to estimate the analyte response in the presence of additional components such as impurities, degradation products and matrix [19]. The peak purity of PAN, RAB, LAN and DOM were assessed by comparing the Retention time of standard PAN, RAB, LAN and DOM good correlation was obtained between the Retention time of standard and sample of PAN, RAB, LAN and DOM. The specificity method was also evaluated to ensure that there were no interference products resulting from forced degradation studies.

7. Forced degradation study:

Forced degradation or Stress testing of a drug substance will help to identify the degradation products, which can help to establish the intrinsic stability of the molecule .All stress decomposition studies were performed at an initial drug concentration 200µg/mL of PAN, RAB, LAN and DOM.

The Stability indicating study of PAN, RAB, LAN and DOM were undergoes acid, alkali and oxidation degradation, photolysis and heat condition. Placebo Interference: The placebo (in the

present of excipients in tablet) sample were prepared as per the test method and analyzed in the HPLC. It expressed there is no additional peaks at the retention time of PAN, RAB, LAN and DOM in the chromatograph it indicates that there is no placebo interference.

Acid Degradation: Sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume upto the mark with mobile phase and analyzed using HPLC.

Alkali Degradation: Sample was treated with 3ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3ml of 1N hydrochloric acid, made the volume up to the mark with mobile phase and analyzed using HPLC.

Oxidative Degradation: PAN, RAB, LAN and DOM solutions of 200 and 20µg/ml were mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume up to the mark with mobile phase and analyzed using HPLC.

Photolytic Degradation: The PAN, RAB, LAN and DOM samples were kept under UV light for different time intervals (15mins – 7days) and made the volume upto the mark with mobile phase and analyzed using HPLC. Thermal Degradation: Samples were heated at 800 C for 15mins -60mins and 2200 C for 2-5mins and analyzed.

8. Accuracy: Accuracy was carried out by applying the method to drug sample (PAN, RAB, LAN and DOM combination of tablets) to which known amounts of PAN, RAB, LAN and DOM. Standard powder corresponding to 80,100 and 120% of label claim was added, mixed and the powder was extracted and determined by the system in optimized mobile phase. The experiment was performed in triplicate and percentage recovery, % RSD was calculated.

9. Analysis of marketed formulation: The marketed formulation was assayed by above description. The peak areas were monitored at 280nm and determination of sample concentrations were using by multilevel calibration developed on the same HPLC system under the same conditions using linear regression analyzed for PAN, RAB, LAN and DOM in the same way as described above.

RESULTS AND DISCUSSIONS

The simultaneous HPLC method was optimized and developed for PAN, RAB, LAN and DOM. The mixed standard solution was injected in HPLC by the following chromatographic conditions.The chromatographic separation was achived on X Bridge 3x100mm;3.7 µm C18, Gradient mode and the Mobile phase consists of Triethylamine and Potassium Dihydrogen Ortho Phosphate pH – 7.4) : Methanol and Acetonitrile in a ratio of 85:15 v/v and the flow rate of mobile phase was 0.5ml/min , run time was 25 min and the column temperature was maintained at Room temp(20-25^0 c),volume of injection loop was 20µl.detection was monitored at 280 nm

Method Development and Optimization:

The HPLC procedure was optimized with a view to develop a suitable LC method for the analysis PAN, RAB, LAN and DOM in fixed dose for bulk and combined dosage form. It was found that mobile phase consists of Triethylamine and Potassium Dihydrogen Ortho Phosphate(pH – 7.4) : Methanol and Acetonitrile in a ratio of 85:15 v/v has given good resolution, theoretical plates, and for PAN, RAB, LAN and DOM at the flow rate of 0.5 ml/min **(Table. 1; Fig. 5 & 6).**

Table No. 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (column)	X Bridge C18(3x100mm;3.7 µm)
Mobile Phase	Triethylamine and Potassium Dihydrogen Ortho Phosphate (pH – 7.4) : Methanol and Acetonitrile in a ratio of 85:15 v/v
pH	4.5
Flow rate (ml/min)	0.5ml/min
Run time (minutes)	25 mins
Column temperature (°C)	Room temp(20-25^0 c)
Volume of injection loop («l)	20µl
Detection wavelength (nm)	280 nm
Drugs RT (min)	6.738, 8.034, 12.00 & 17.786

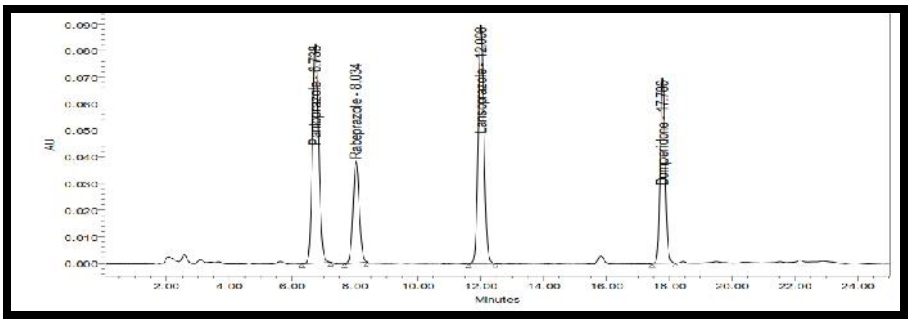

Fig. 5: Chromatogram of standard API MIXTURE (PAN, RAB, LAN and DOM)

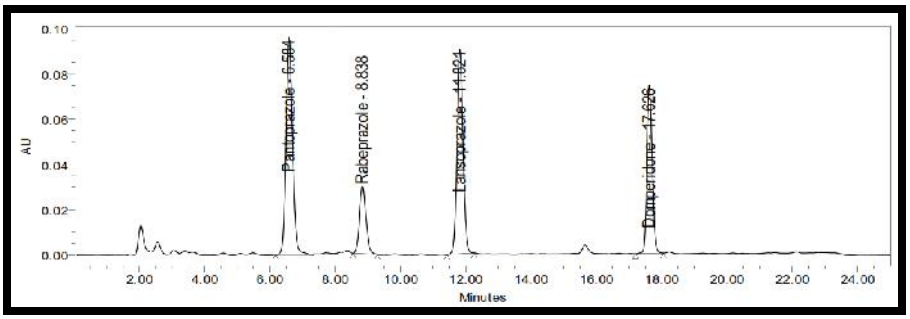

Fig. 6: Chromatogram of Sample DRUG PRODUCT MIXTURE (PAN, RAB, LAN and DOM)

Validation of Developed Method:

The method validation was done according to the ICH guidelines. The following validation characteristic parameters areaccuracy, precision, linearity, and specificity, LOD, LOQ and robustness.

1. Linearity: The linearity five levels of concentrations with correlation regression curves are obtained the conc. range of 10-50µg/mL for PAN, RAB, LAN and DOM. The reports of drug were found the linear in prepared conc. Where X was the conc of the drug in µg/ml & Y was area of the peak in the absorbance unit. The chromatograms were obtained during the linearity were shown in the (**Fig. 7-11 & Table 2 & 3.)**

Table No. 2: Linearity study of PAN and RAB

Linearity level	PAN		RAB	
	Conc. (µg/ml)	Mean Area	Conc. (µg/ml)	Mean Area
1	10	660991	10	280198
2	20	1002565	20	427899
3	30	1362223	30	559645
4	40	1709380	40	674064
5	50	2038876	50	822583
Correlation co-efficient		0.999		0.999
Slope		34626		13309
Intercept		31603		15359

Table No. 3: Linearity study of LAN and DOM

Linearity level	LAN		DOM	
	Conc. (µg/ml)	Mean Area	Conc. (µg/ml)	Mean Area
1	10	595717	10	416136
2	20	945502	20	644565
3	30	1226813	30	852720
4	40	1559380	40	1042333
5	50	1862327	50	1272924
Correlation co-efficient		0.999		0.999
Slope		31431		21113
Intercept		29421		21233

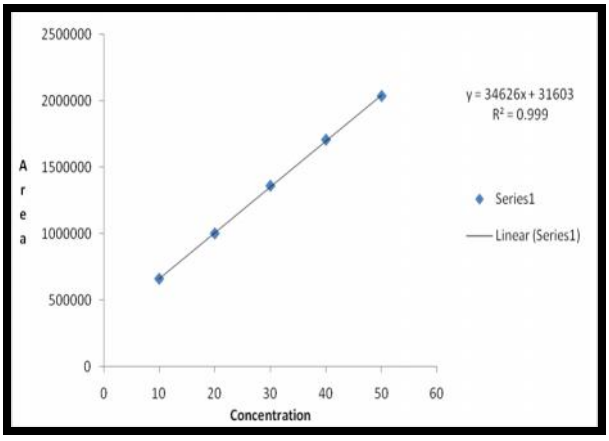

Fig. 7: Linearity curve for PAN

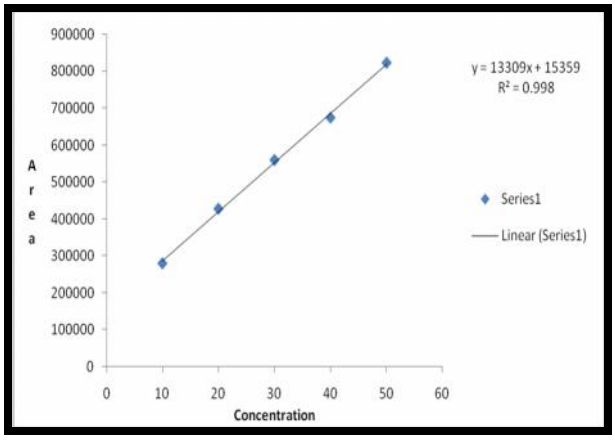

Fig. 8: Linearity curve for RAB

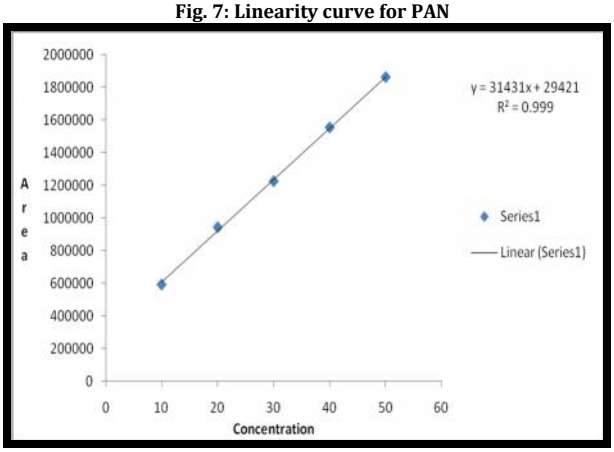

Fig. 9: Linearity curve for LAN

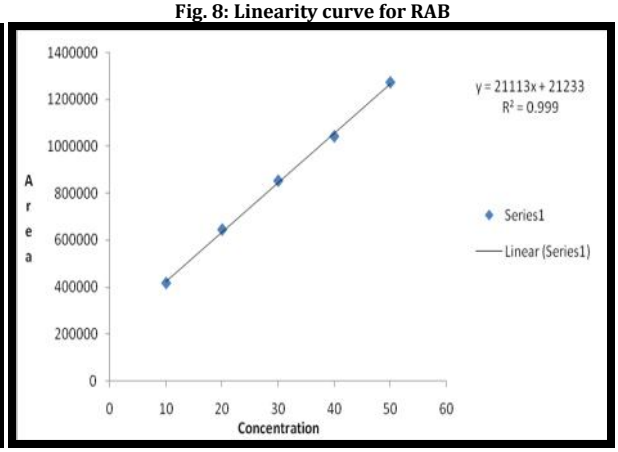

Fig. 10: Linearity curve for DOM

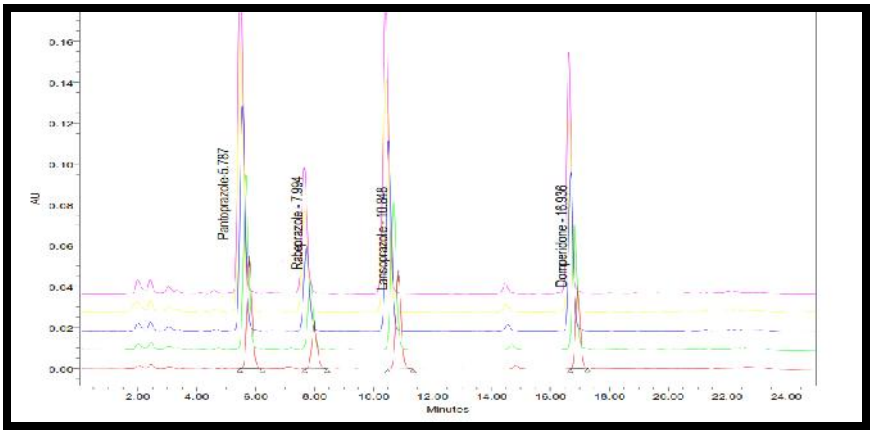

Fig. 11: Overlay linearity Chromatogram for linearity all Levels (1-5)

2. Precision: Precision of this analysis, as the intraday precision was evaluated by performing six individual test samples prepared & calculated the % RSD. Interday precision of this method was analyzed by the performing same the procedure with the various days by the person with the same developed environment. Resulting data of precision was given in the **Table 4 & 5 (Fig. 12)**. The % RSD values of the intra-day precision & interday precision study was < 2.0% for PAN, RAB, LAN and DOM. This is confirmed that method was precise.

Table No. 4: Precision study of PAN & RAB

Replicate	Area of PAN		Area of RAB	
	Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
1	1337044	1320156	568120	567458
2	1350710	1318264	569159	564231
3	1345738	1316584	564297	560254
4	1336237	1312564	564228	562148
5	1334899	1312569	564731	562387
6	1356021	1309547	558197	563214
Mean	1343441.5	1314947	564788.6	563282
St. dev.	8742.7	4032.6	3852.2	2433.104
% RSD	0.7	0.3	0.7	0.4

Table No. 5: Precision study of LAN & DOM

Replicate	Area of LAN		Area of DOM	
	Intra-day precision	Inter-day precision	Intra-day precision	Inter-day precision
1	1226085	1206548	843034	834682
2	1228107	1204976	842196	829654
3	1224297	1204876	843807	830264
4	1224183	1206348	843851	825467
5	1225141	1206489	841613	830268
6	1225637	1204897	845120	836149
Mean	1225575.1	1205689	843270.0	831080.7
St. dev.	1444.6	849.5604	1265.2	3834.934
% RSD	0.1	0.07	0.2	0.4

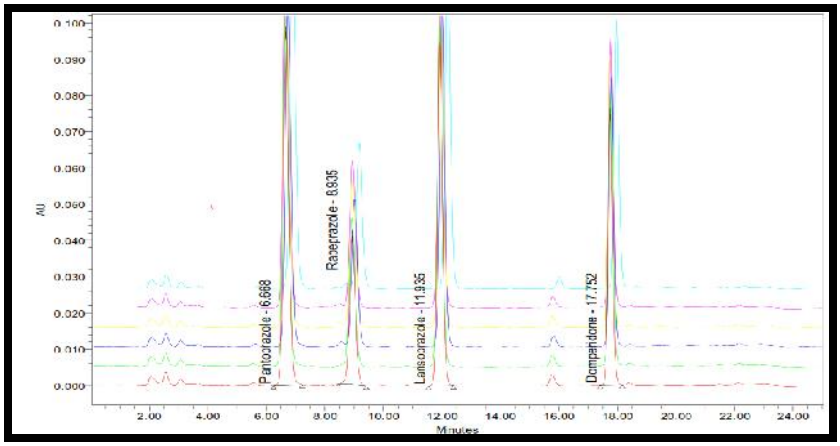

Fig. 12: Overlay precision Chromatogram for PAN, RAB, LAN and DOM

3. LOD and LOQ: Limit of detection (LOD) & the limit of quantifications (LOQ) are evaluated by the serial dilutions of PAN, RAB, LAN and DOM stock solutions in the ordered to be obtaining the signal to the noise ratio 3:1 for the LOD & 10:1 for the LOQ. Then the LOD Concentration for of PAN, RAB, LAN and DOM was found to be 0.782 µg/mL, 0.897 µg/mL, 0.142 µg/mL & 0.185µg/mL respectively. The LOQ Concentration for 2.524 µg/mL, 2.894 µg/mL, 0.459 µg/mL and 0.599µg/mL respectively. The chromatogram of the LOD and LOQ were shown in the **(Fig. 13 & 14)**.

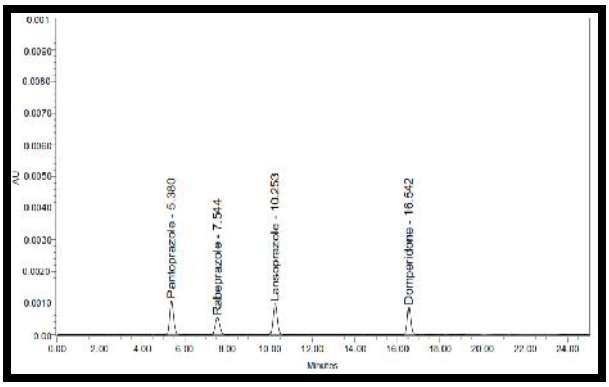

Fig. 13: Chromatogram of LOD study of PAN, RAB, LAN and DOM

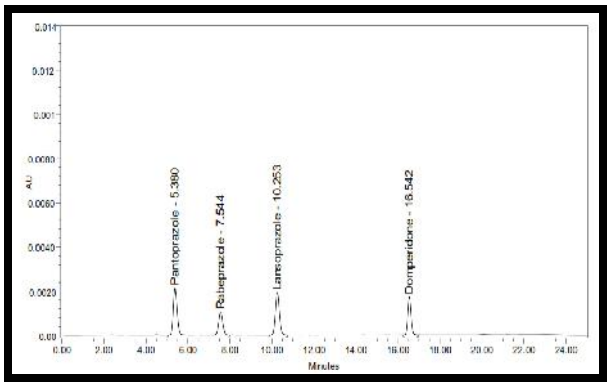

Fig. 14: Chromatogram of LOQ study of PAN, RAB, LAN and DOM

4. Specificity: The specificity is a method for drug establishing by the verifying for the interferences with drug quantification from degradation products are formed during forced degradation study and peak purity for PAN, RAB, LAN and DOM were found better under the various conditions. There were no other interferences of any other peaks and degradation products with the drug peaks.

5. System suitability: The system suitability parameters with respect of tailing factor, theoretical plates, repeatability and resolution between PAN, RAB, LAN and DOM peaks were defined five replicate injections of the standard solution were injected and asymmetry, resolution, % RSD of peak area and theoretical plate were determined. For system suitability, asymmetry parameters should not more than 2.0, resolution should be more than 3.0 theoretical plate should not less than 3000 & % RSD for peak area should not be more than 2.0%, were full fill during all validation parameters all parameter are within the range of ICH prescribed Limits **(Table.6).**

Table No. 6: System suitability parameters for PAN, RAB, LAN and DOM

System suitability parameters	PAN	RAB	LAN	DOM
Retention time (min)	6.738	8.034	12.00	17.786
Repeatability of retention time; %R.S.D (n=5)	0.01	0.05	0.05	0.05
Repeatability of peak area; %R.S.D= (S.D./Mean)×100	0.7	0.4	0.4	0.4
Resolution (Rs)	-	2.62	8.34	18.0
Tailing factor (asymmetric factor)	1.13	1.12	1.16	1.12
USP plate count	5406	9634	20687	56762
LOD (µg/mL)	0.782	0.897	0.142	0.185
LOQ (µg/mL)	2.524	2.894	0.459	0.599

6. Robustness: The robustness is studied by the evaluating effects of small but the deliberate differences in method condition. The results of robustness for developed methods were started in the **Table 7.** The results are shown during all the different conditions of the test solution wasn't affective & in the accordance with an actual one. The suitability also found better; hence this method was conformed as robust. The chromatograms were Obtained during the robustness were shown in the **Fig. 15-18.**

Table No. 7: Evaluation data of Robustness study of PAN, RAB, LAN and DOM

Parameters		Adjusted to	Mean Area [a]	Mean RT	SD	% RSD
PAN	Flow Rate As per method 0.5ml/min	0.4 ml/min	1541332	7.224	6117	0.4
		0.6ml/min	1030698	4.703	1220	0.1
	Temp as per method 25^0 c	Less temp 20^0 c	1541332	7.224	6116	0.4
		More Temp30^{0}c	1995853	7.263	9718	0.5
RAB	Flow Rate As per method 0.5ml/min	0.4 ml/min	966166	9.812	5482	0.6
		0.6ml/min	640352	6.613	2470	0.4
	Temp as per method 25^0 c	Less temp 20^0 c	965234	9.846	5482	0.6
		More Temp30^{0}c	959954	9.812	8257	0.9
LAN	Flow Rate As per method 0.5ml/min	0.4 ml/min	155790	12.904	7215	0.5
		0.6ml/min	1027610	9.160	1369	0.1
	Temp as per method 25^0 c	Less temp 20^0 c	1555970	12.324	7215	0.5
		More Temp30^{0}c	1586080	12.90	6526	0.4
DOM	Flow Rate As per method 0.5ml/min	0.4 ml/min	1071367	18.82	4080	0.88
		0.6ml/min	7111044	15.521	870	0.1
	Temp as per method 25^0 c	Less temp 20^0 c	1075684	18.82	4025	0.4
		More Temp30^{0}c	882990	18.82	846	0.1

[a] = 5 *Replicates;* [a] each of the value was indicates for mean of 3 injections

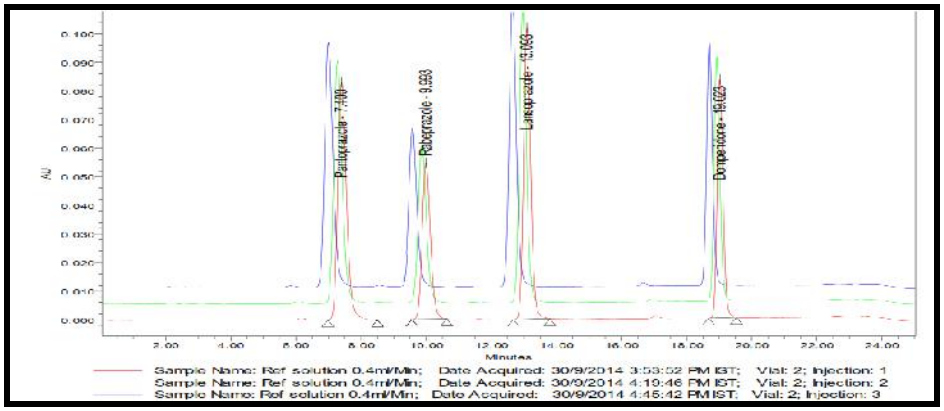

Fig. 15: Chromatogram of PAN, RAB, LAN and DOM (0.4 ml/min flow rate)

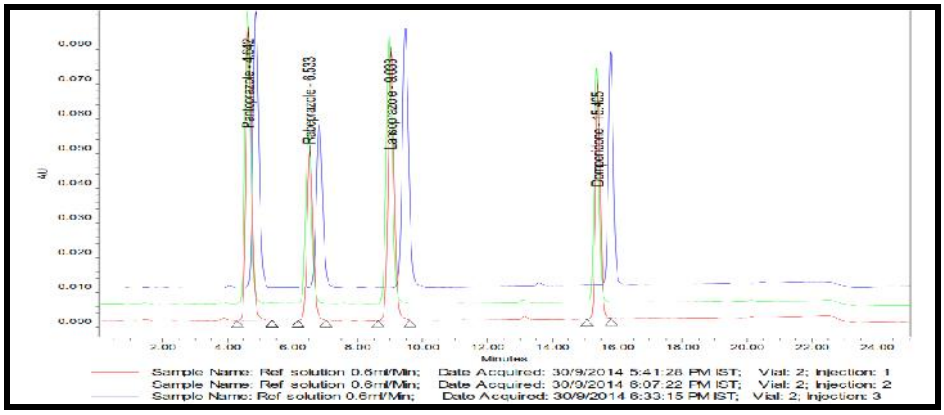

Fig. 16: Chromatogram of Chromatogram of PAN, RAB, LAN and DOM (0.6 ml/min flow rate)

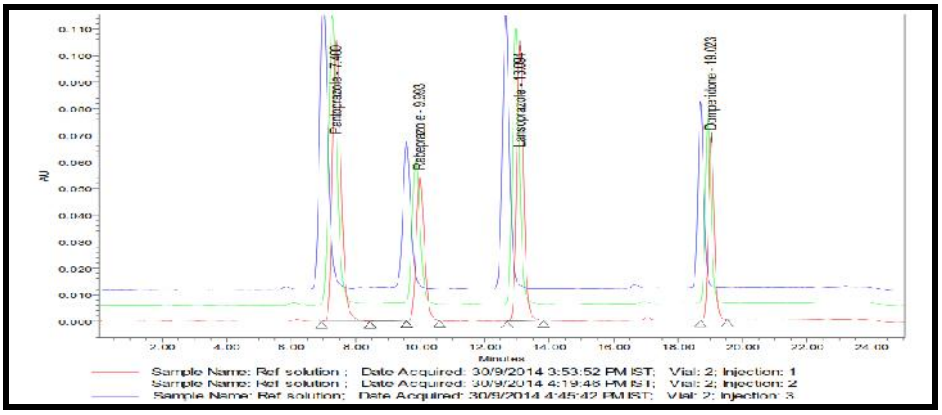

Fig.17: Chromatogram of Chromatogram of PAN, RAB, LAN and DOM (More temp 30^0 C)

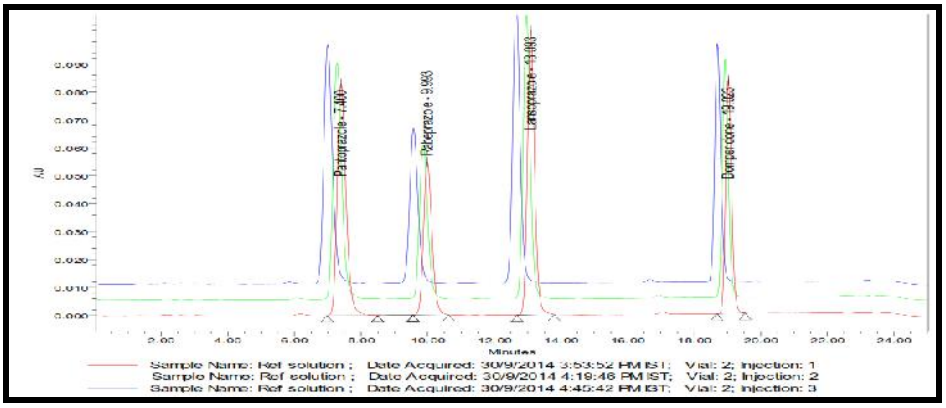

Fig. 18: Chromatogram of Chromatogram of PAN, RAB, LAN and DOM (less temp 20^0 C)

2.7. Solution stability study: Sample Stability was evaluated by shorting at the ambient temp & analysis was done in initial time, after 3hrs, 6 hrs, 12 hrs and 24 hrs. The analysis of the reports from all aged solutions was compared with those of from the freshly prepared solution (initial solution). **(Table 8-11)** shows results are obtained the stability of solution study at various intervals for a test preparations and it was conformed that the test solutions were stable upto the 24hrs at the ambient temp, because difference in the measured & the original values were < 2.0 %.

Table No. 8: Evaluation of solution stability for PAN

Replicate	Initial Area	Area After 3 hrs	Area After 6 hrs	Area After 12 hrs	Area After 24 hrs
1	1337044	1325698	1326987	1316594	1320156
2	1350710	1325478	1326548	1315879	1318264
3	1345738	1326587	1326547	1315697	1316584
4	1336237	1321698	1326598	1315475	1312564
5	1334899	1325698	1326874	1325691	1312569
6	1356021	1325489	1325694	1325497	1309547
Mean	1343441.5	1325108	1326541	1319139	1314947
St. dev.	8742.7	1720.105	453.9928	5014.574	4032.6
% RSD	0.7	0.1	0.03	0.3	0.3

Table No. 9: Evaluation data of solution stability for RAB

Replicate	Initial Area	Area After 3 hrs	Area After 6 hrs	Area After 12 hrs	Area After 24 hrs
1	568120	567453	567325	567592	567458
2	569159	564658	564368	569594	564231
3	564297	560214	560124	564452	560254
4	564228	562645	562658	564657	562148
5	564731	562321	562364	564354	562387
6	558197	562596	563368	558267	563214
Mean	564788.6	563314.5	563367.8	564819.3	563282
St. dev.	3852.2	2469.688	2395.558	3841.771	2433.104
% RSD	0.7	0.4	0.4	0.6	0.4

Table No. 10: Evaluation of solution stability for LAN

Replicate	Initial Area	Area After 3 hrs	Area After 6 hrs	Area After 12 hrs	Area After 24 hrs
1	1226085	1206548	1226085	1206548	1226085
2	1228107	1204976	1228107	1204976	1228107
3	1224297	1204876	1224297	1204876	1224297
4	1224183	1206348	1224183	1206348	1224183
5	1225141	1206489	1225141	1206489	1225141
6	1225637	1204897	1225637	1204897	1225637
Mean	1225575.1	1205689	1225575.1	1205689	1225575.1
St. dev.	1444.6	849.5604	1444.6	849.5604	1444.6
% RSD	0.1	0.07	0.1	0.07	0.1

Table No. 11: Evaluation data of solution stability for DOM

Replicate	Initial Area	Area After 3 hrs	Area After 6 hrs	Area After 12 hrs	Area After 24 hrs
1	843034	834682	843034	834682	834682
2	842196	829654	842196	829654	829654
3	843807	830264	843807	830264	830264
4	843851	825467	843851	825467	825467
5	841613	830268	841613	830268	830268
6	845120	836149	845120	836149	836149
Mean	843270.0	831080.7	843270.0	831080.7	831080.7
St. dev.	1265.2	3834.934	1265.2	3834.934	3834.934
% RSD	0.2	0.4	0.2	0.4	0.4

8. Recovery studies: The recovery of PAN, RAB, LAN and DOM was determined by the 3 various conc. levels. % recovery was found to be 99.01-100.01% for PAN, 99.77-99.91% for RAB, 99.92-100.13% for LAN and 98.43-99.92%for DOM **(Table 12).** The results are indicating that this method was accurate. Chromatograms obtained during the study of accuracy were shown in **Fig. 19-21.**

Table No. 12: Accuracy study of PAN, RAB, LAN and DOM

Analyst	Recovery levels	Actual Conc. (µg/mL)	Added Conc. (µg/mL)	Theoretical Conc. (µg/mL)	Found Conc. (µg/mL)	% Recovery	% RSD	% Error [a]
PAN	80 %	30	24	54	53.95	99.90	0.4	-0.09
	100 %	30	30	60	59.46	99.10	0.2	-0.9
	120 %	30	36	66	66.01	100.01	0.1	0.01
RAB	80 %	30	24	54	53.89	99.79	0.2	-0.20
	100 %	30	30	60	59.95	99.91	0.4	0.0
	120 %	30	36	66	65.88	99.81	0.1	-0.18
LAN	80 %	30	24	54	54.02	100.01	0.1	0.03
	100 %	30	30	60	65.95	99.92	03	0.0
	120 %	30	36	66	66.09	100.13	0.1	0.0

DOM	**80 %**	30	24	54	53.96	99.92	0.1	-0.07
	100 %	30	30	60	59.85	99.75	0.2	-0.25
	120 %	30	36	66	64.97	98.43	0.2	-1.56

[a] [found conc. – theoretical conc./theoretical conc.] x 100; Each value was indicates the mean of 3 injections.

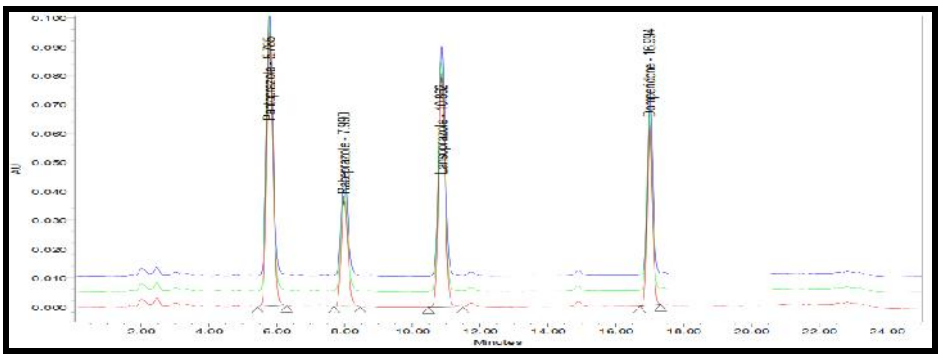

Fig. 19: Accuracy chromatogram for level-1 (80%)

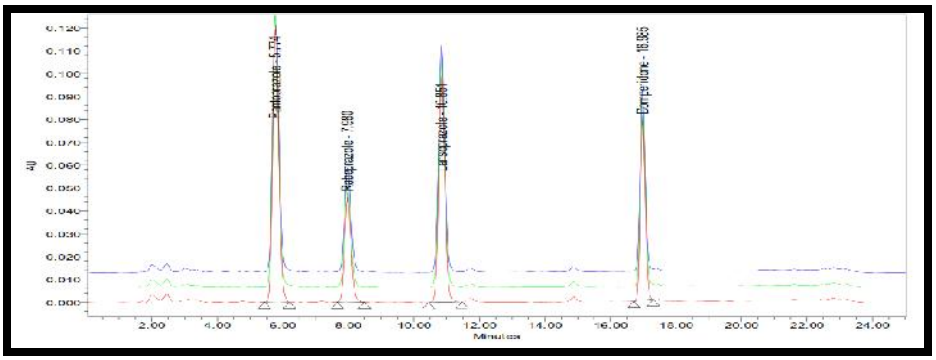

Fig. 20: Accuracy chromatogram for level-2 (100%)

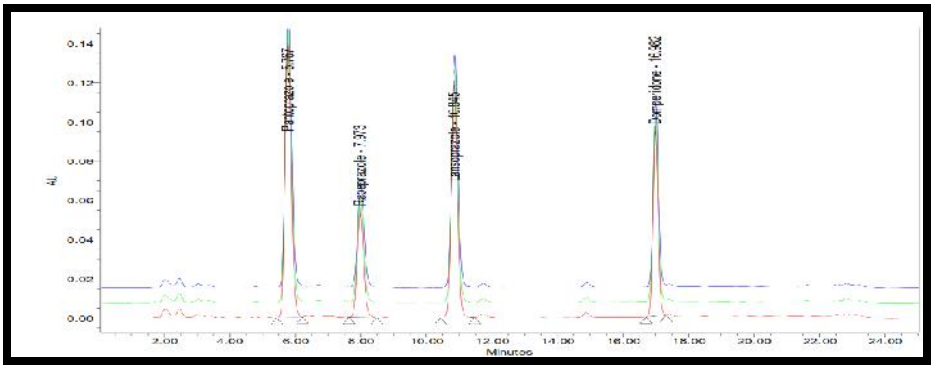

Fig. 21: Accuracy chromatogram for level-3 (120%)

9. Ruggedness: The ruggedness was studied by evaluating by different analysts but in the same chromatographic conditions. The results of ruggedness of developed method are started in the **Table 13 & 14.** The results are shown during by different analysts but in the same chromatographic condition of the test solution wasn't affected & in the accordance with the actual. The suitability parameters are also been found good; hence this method was concluded as rugged.

Table No. 13: Evaluation data of Ruggedness study of PAN & RAB

ID Precisions	No. of Injections	PAN		RAB	
		Peak Area	RT	Peak Area	RT
ID Precision - 1	1	1337044	6.653	568120	8.925
	2	1350710	6.665	569159	8.935
	3	1345738	6.668	564297	8.944
ID Precision - 2	1	1336237	6.678	564228	8.953
	2	1334899	6.739	564731	9.010
	3	1356021	6.891	558197	9.173
MEAN		1343441.5	6.71566	564788.6	8.99
STDEV		8742.7	0.0910	3852.2	0.0944
% RSD		0.7	1.3	0.7	1.05087

Table No. 14: Evaluation data of Ruggedness study of LAN & DOM

ID Precisions	No. of Injections	LAN		DOM	
		Peak Area	RT	Peak Area	RT
ID Precision - 1	1	1226085	11.931	843034	17.752
	2	1228107	11.935	842196	17.754
	3	1224297	11.955	843807	17.766
ID Precision - 2	1	1224183	11.960	843851	17.771
	2	1225141	12.008	841613	17.786
	3	1225637	12.201	845120	17.963
MEAN		1225575.1	11.998	843270.0	17.79867
STDEV		1444.6	0.1030	1265.2	0.081451
% RSD		0.1	0.8585	0.2	0.457624

10. Analysis of a commercial formulation:

Experimentally the results for the amount of PAN, RAB, LAN and DOM in tablets, expressed as a percentage of label claims were in good agreement with the label claims there by suggesting that there is no interaction from the excipients which are commonly present in formulation of tablets.

11. Degradation study:

In a order to to establish the inherent stability and stability indicating assay method and to determine whether the analytical methods were stable PAN, RAB, LAN and DOM dosage forms are stressed on the different conditions to applied degradation studies. The guidelines are expressed in ICH Q2A, Q3B, Q2B & FDA 21 CFR section of 211 all the required for development & for the validation of stability study.

The degradation of a sample was prepared by the transfer the individual tablet powder was equivalent to the weight of each tablet was transfer into 100 ml flask & it was treated under the acidic, alkaline, thermal, oxidizing and photolytic conditions. When degradation was complete the solution were left to equilibrate to the room temp & dil. with mobile phase to furnish the solutions of a concentration equivalent to a 30 µg/mL of PAN, RAB, LAN and DOM. The specific degradative conditions are described below.

Acid degradation study: The Acid degradation was done by sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs at 60ºC. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume up to the mark with mobile phase and analyzed using HPLC. The degrading drug content was found up to 7.68% in the acidic condition **(Fig. 22-24) & (Table 15, 16).**

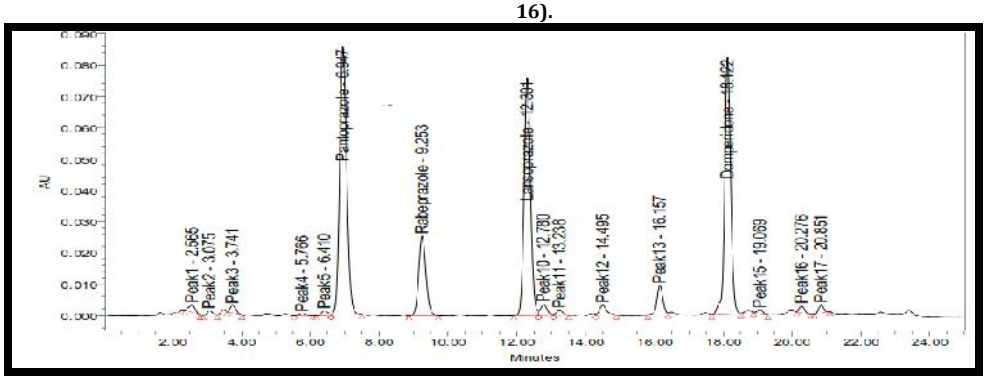

Fig. 22: Chromatogram of acidic forced degradation of PAN, RAB, LAN and DOM

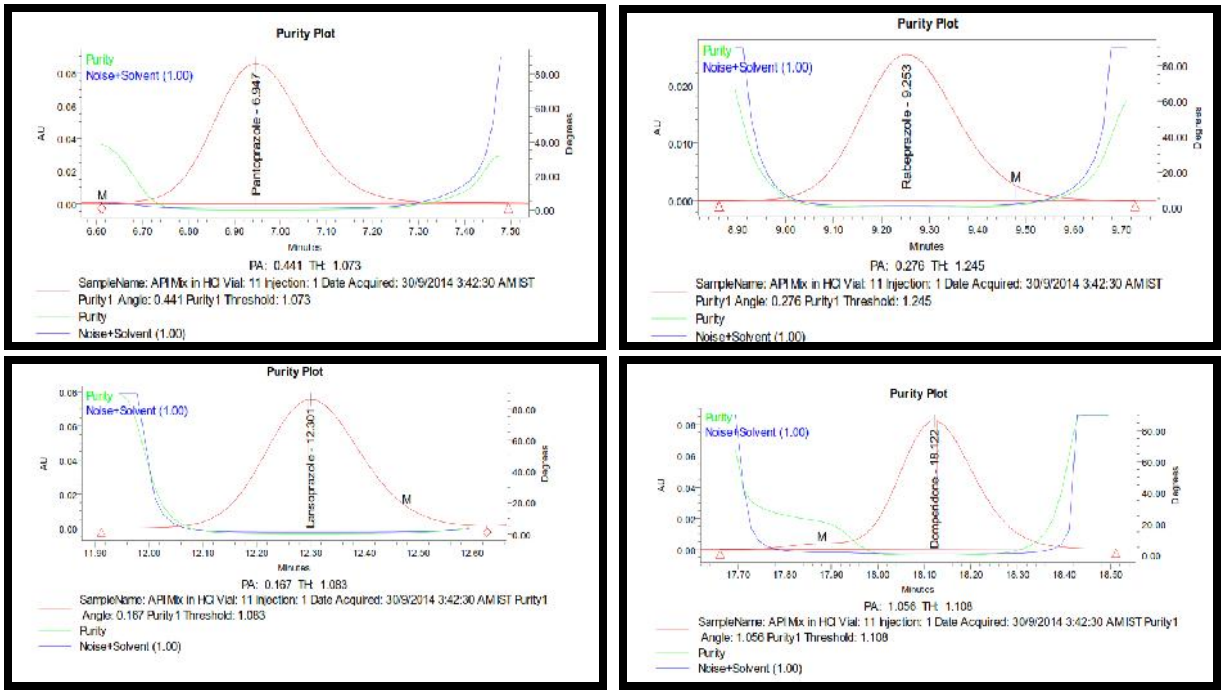

Fig. 23: Purity Plots for PAN, RAB, LAN and DOM in acidic forced degradation

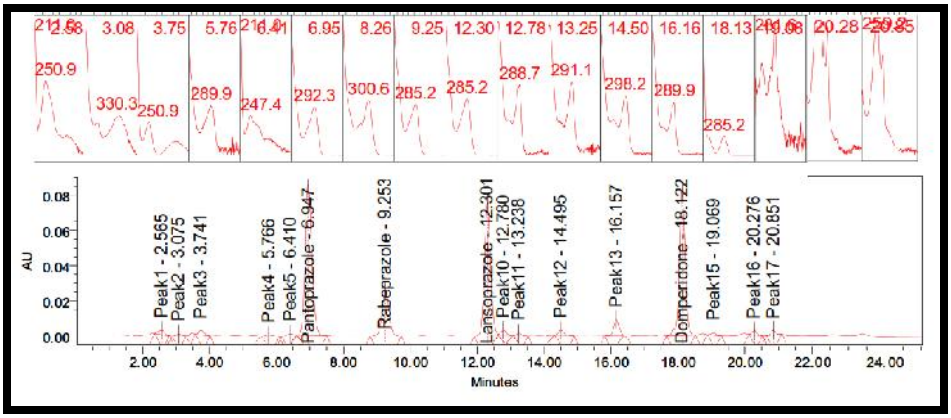

Fig. 24: Spectrum index for PAN, RAB, LAN and DOM in acidic forced degradation

Alkaline degradation: The Alkaline degradation was done by sample was treated with 3ml of 1N sodium hydroxide and kept the sample for 10hr. After 10hr solution was neutralized to add 3ml of 1N hydrochloric acid, made the volume up to the mark with irrelevant media and analyzed using HPLC. In alkali degradation study, it was found to be 7.78% of the degraded drug **(Fig. 25-27 & Table 15 & 16).**

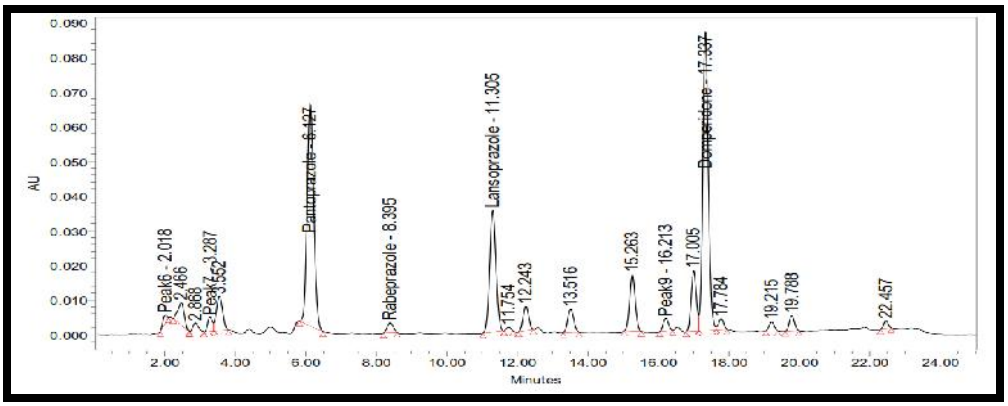

Fig. 25: Chromatogram of alkali forced degradation of PAN, RAB, LAN and DOM

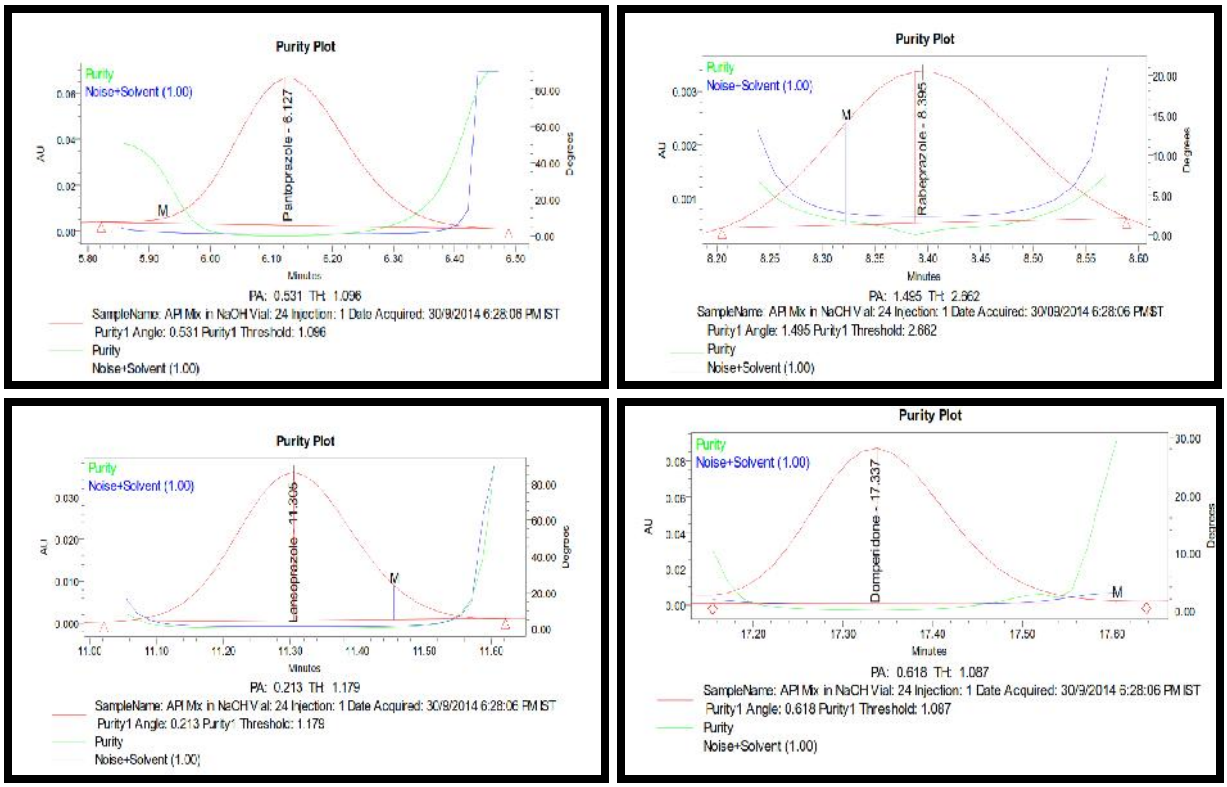

Fig. 26: Purity Plots for PAN, RAB, LAN and DOM in alkali forced degradation

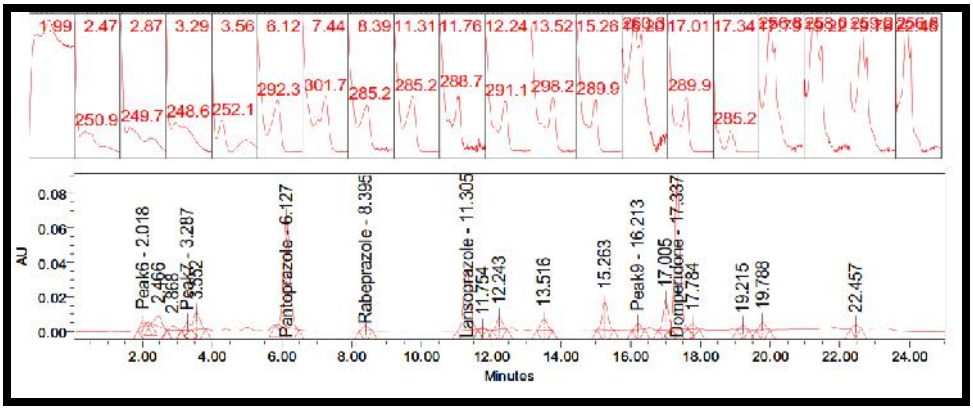

Fig. 27: Spectrum index for PAN, RAB, LAN and DOM in Base (Alkali) forced degradation

Oxidative degradation: The oxidative degradation was done by sample was mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with mobile phase and analyzed using HPLC. In oxidative degradation, it was found to be 11.07% of the degraded drug **(Fig. 28-30** & **Table 15 & 16).**

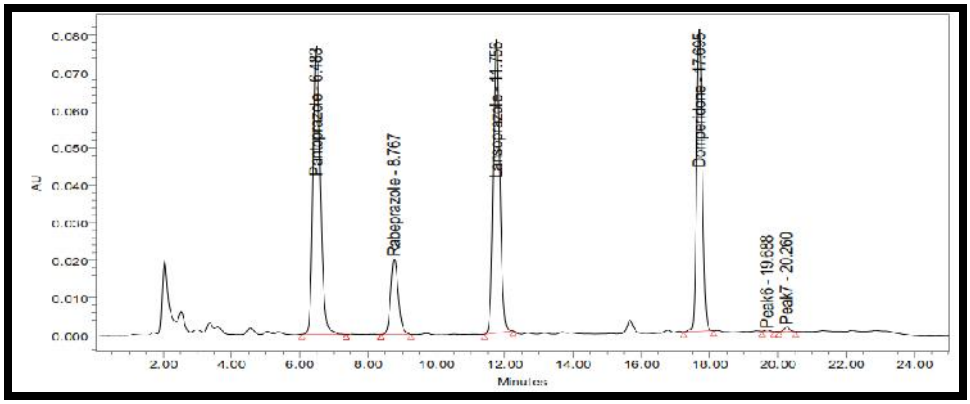

Fig. 28: Chromatogram of oxidative forced degradation of PAN, RAB, LAN and DOM

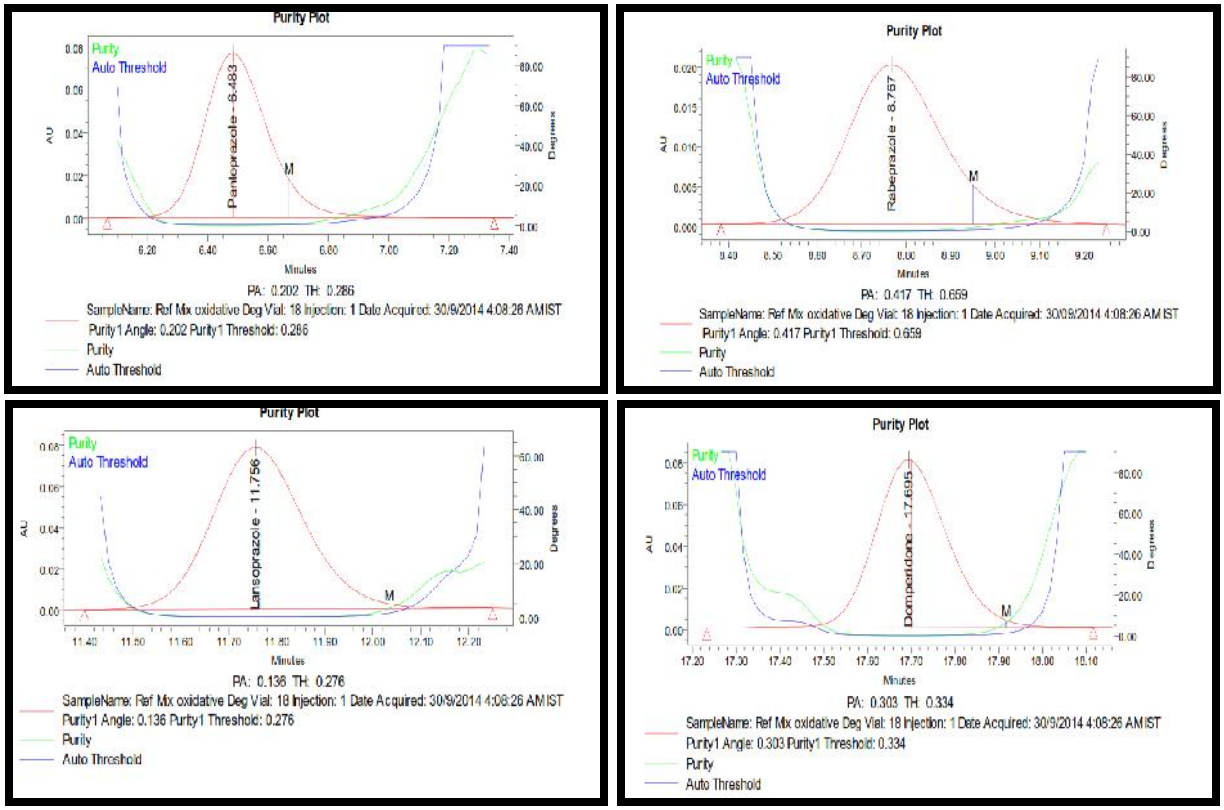

Fig. 29: Purity Plots for PAN, RAB, LAN and DOM in oxidative forced degradation

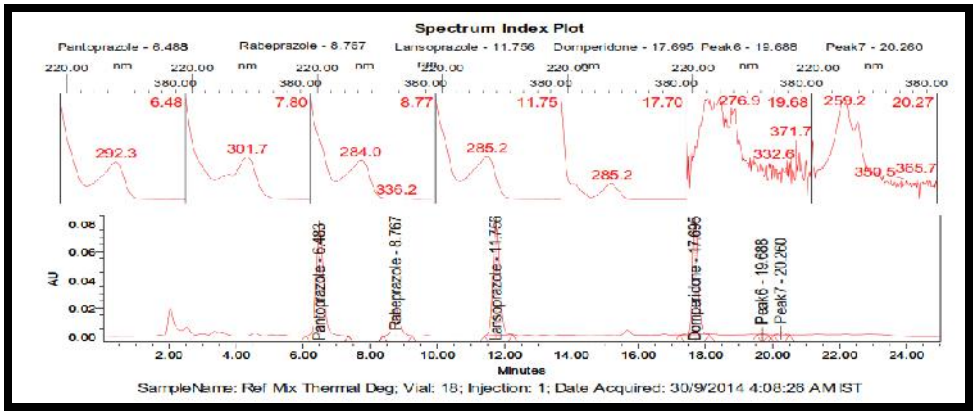

Figure 30: Spectrum index for PAN, RAB, LAN and DOM in oxidative forced degradation

Photolytic degradation: The photolytic degradation was done by exposing of drug content under the UV light for 15mins to 7days. There is 5.63% of the drug degradation observed in the above specific photolytic degradation condition **(Fig. 31-33 & Table 10 & 11).**

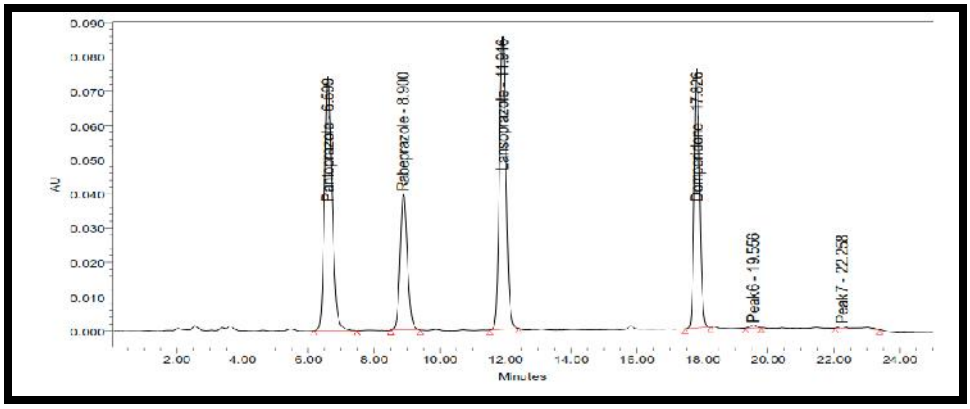

Figure 31: Chromatogram of UV-light degradation of PAN, RAB, LAN and DOM

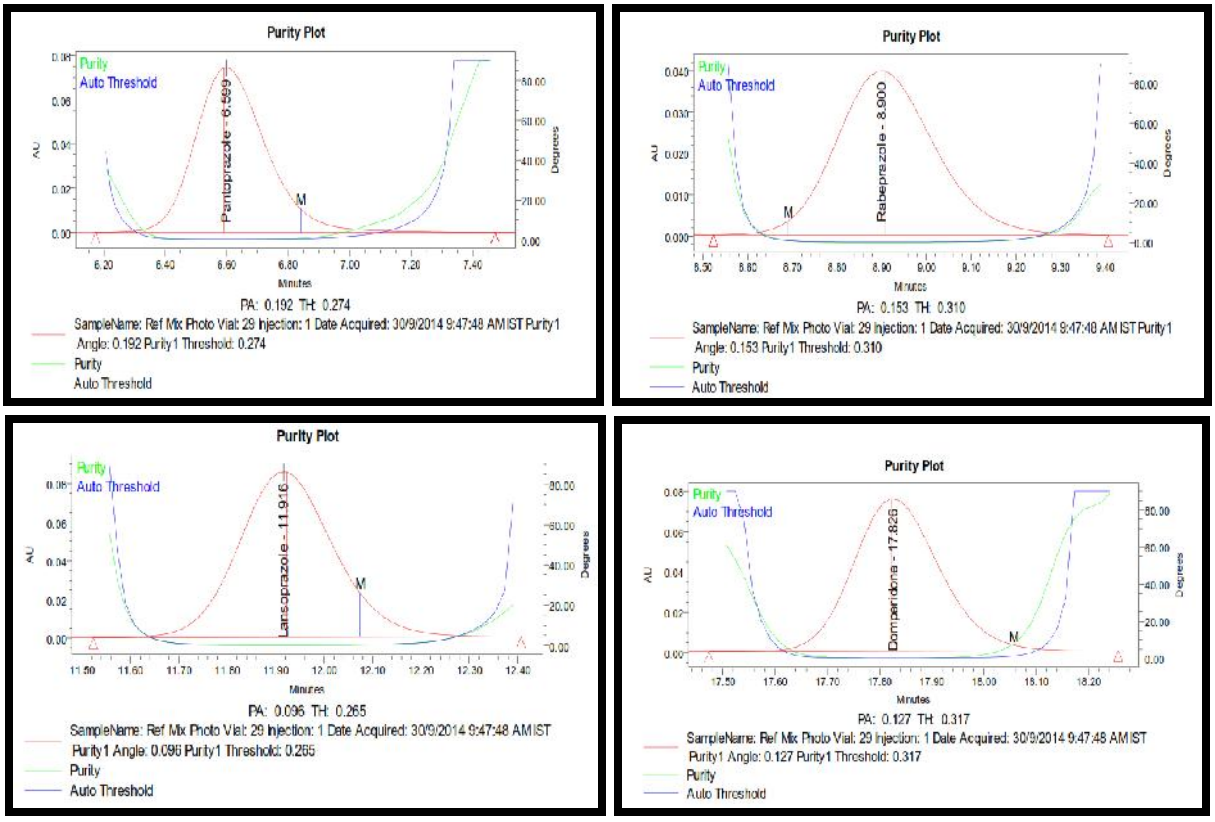

Figure 32: Purity Plots for PAN, RAB, LAN and DOM in UV-light degradation

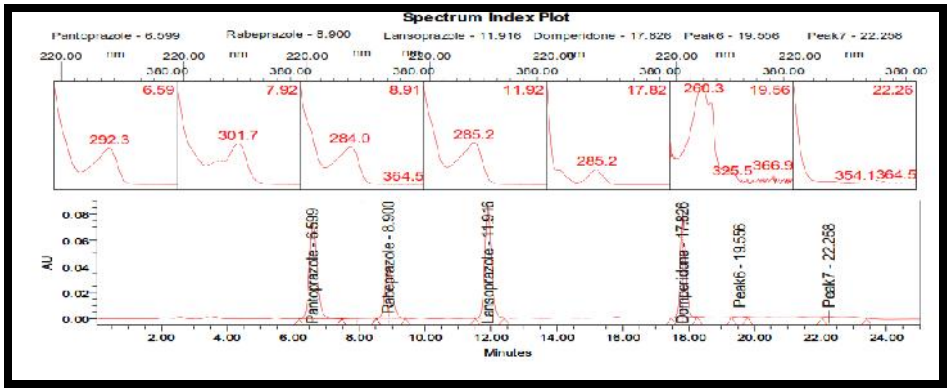

Fig. 33: Spectrum index for PAN, RAB, LAN and DOM in Photolytic forced degradation

Thermal degradation: The Thermal degradation is to be performing by the exposing the solid drug at the 80°C for 15mins to 60mins and at 220°C for 2-5mins. Resultant chromatogram of thermal degradation study **(Fig. 34-36 & Table 15, 16)** was indicates that the drug was found to be slightly stable under thermal condition. It was only 11.08% of the drug content were degraded.

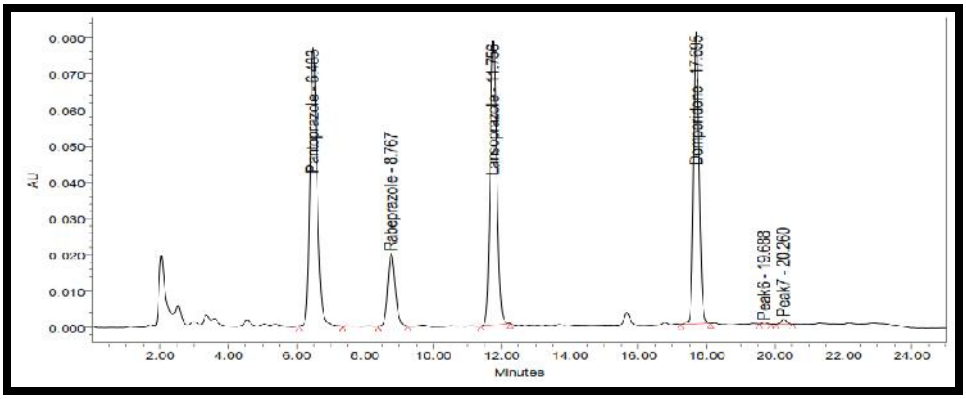

Fig. 34: Chromatogram of thermal degradation of PAN, RAB, LAN and DOM

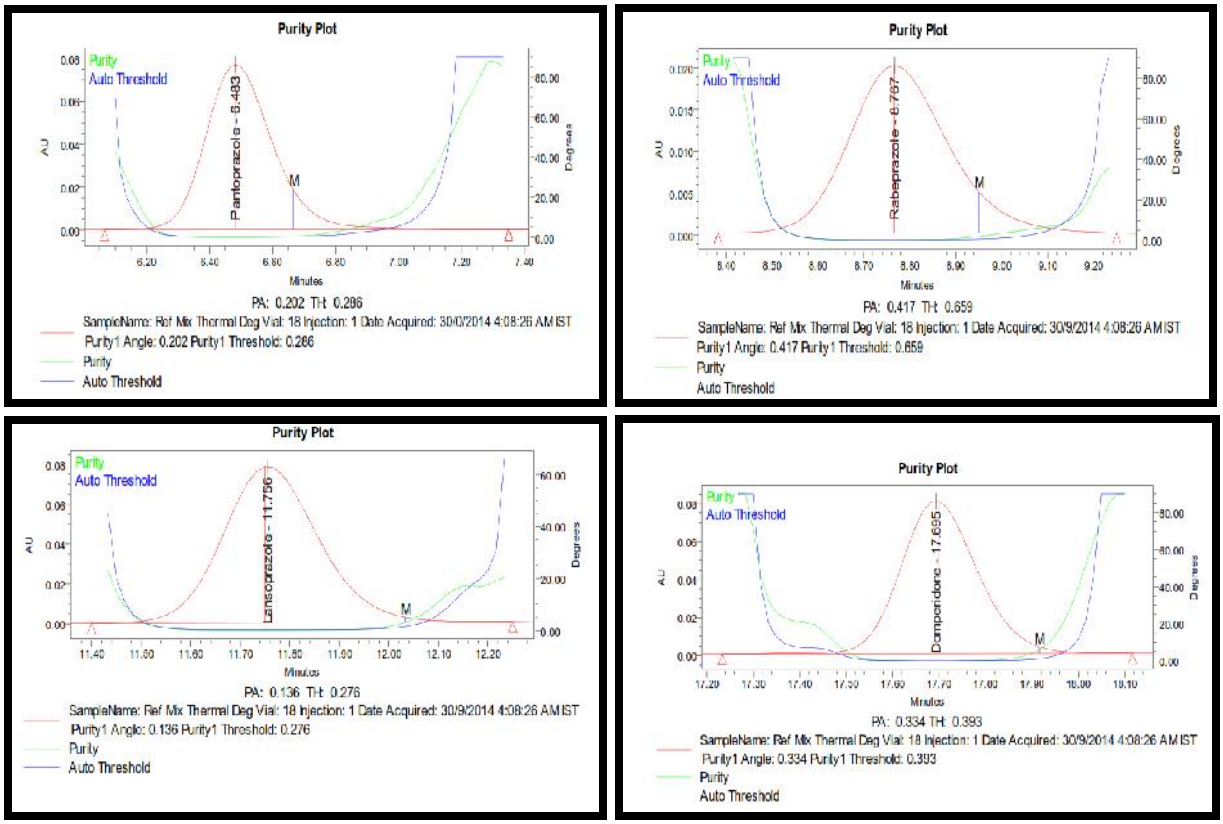

Fig. 35: Purity Plot for PAN, RAB, LAN and DOM in thermal degradation

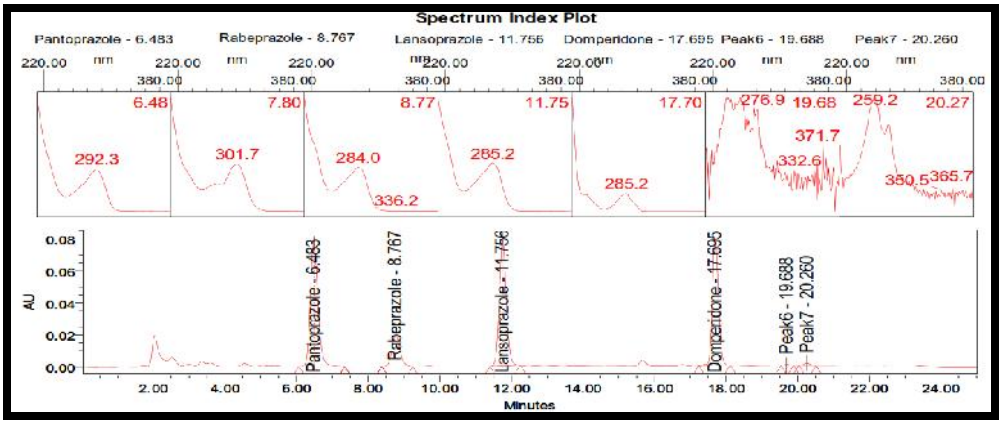

Fig. 36: Spectrum index for PAN, RAB, LAN and DOM in Thermal forced degradation

Table No. 15: Peak purity results of PAN, RAB, LAN and DOM

Stress Condition	*Purity Angle*				*Purity Threshold*			
	PANT	RABE	LANS	DOMP	PANT	RABE	LANS	DOMP
Acid Degradation	0.441	0.276	0.167	1.056	1.073	1.245	1.083	1.108
Alkali Degradation	0.531	1.495	0.213	0.619	1.096	2.662	1.179	1.087
Oxidative Degradation	0.202	0.417	0.136	0.303	0.286	0.659	0.276	0.334
Photolytic Degradation	0.192	0.153	0.096	0.127	0.274	0.310	0.265	0.317
Thermal Degradation	0.202	0.417	0.136	0.334	0.286	0.659	0.276	0.393

Table No.16: Percentage of degradation of PAN, RAB, LAN and DOM

Drug Name		*Acid*	*Alkali*	*Oxidative*	*Photolytic*	*Thermal*
PANTOPRAZOLE	*Std Area*			1343441		
	Sample Area	1219484	1219484	1219484	1219484	1219484
	% of Degradation	9.22%	8.48%	7.24%	3.41%	7.24%
RABEPRAZOLE	*Std Area*			564788		
	Sample Area	520888	520888	520888	520888	520888
	% of Degradation	7.77%	5.50%	10.83%	8.08%	10.84%
LANSOPRAZOLE	*Std Area*			1225575		
	Sample Area	1107974	1107974	1107974	1107974	1107974
	% of Degradation	9.59%	6.73%	13.29%	3.26%	13.29%
DOMPERIDONE	*Std Area*			843270		
	Sample Area	808342	808342	808342	808342	808342
	% of Degradation	4.14%	10.42%	12.95%	7.70%	12.95%
% Average of Degradation		7.68 %	7.78%	11.07%	5.63%	11.08%

CONCLUSION

A new high resolution RP-HPLC stability indicating method Described in this manuscript provides results which can resolve all the four proton pump inhibitor drugs in the presence of degradation products. Hence this method is convenient and reproducible can be used routinely in quality control of various proton pump inhibitor bulk drugs and also in multicomponent pharmaceutical dosage forms.

REFERENCES:

1. Dentinger PJ, Swenson CF, Anaizi NH. Stability of pantoprazole in an extemporaneously compounded oral liquid. Am J Health-Syst Pharm. 2002; 59:953–956.
2. M. Kobylinska, and k. Kobylinska J. of Chrom. B, Analytical sciences and applications 7, 1207 (2000).
3. Sivakumar Thanikachalam, Manavalan Rajappan, Valliappan Kannappan. Stability-Indicating HPLC Method for Simultaneous Determination of Pantoprazole and Domperidone from their Combination Drug Product,chromatographia 2008, 67, 41–47.
4. Zeinab Abdelaziz Et-Sherif, Afaf Osman Mohamad, El-Tarras RP-HPLC method for the determination of lansoprazole, omeprazole and Pantoprazole sodium sesquihydrate in presence of their acid-induced degradation products. 2006: 54(6) 814-818.
5. Ramakrishna NV, Vishwottam KN, Wishu S and Koteshwara M. High-performance liquid chromatography method for the quantification of pantoprazole in human plasma. J Chromatogr B. 2005; 822: 1-2.
6. Rahman N, Kashif M. Pharmazie. Initial-rate method for the determination of pantoprazole in pharmaceutical formulations using 1-fluoro 2,4- dinitrobenzene. 2005; 60: 197-200.
7. Roche VF. Roche, V.F. The chemically elegant proton pump inhibitors. Am J Pharm Educ. 2006; 70:1–11.
8. Reguri Buchireddy, Khagga Mukkanti, Polisetty srinivasulu, koduri Srinivas SV, A validated, Stability indicating, LC Method for rabeprazole sodium, 2008; 68: 275-280.
9. Crowther JB. Validation of pharmaceutical test methods. In: Ahuja S, Scypinski S (Handbook of modern pharmaceutical analysis. Academic press,New York, 2001;p 415–443.
10. Rindi, Fiocca, Miller, Europ. J. Gastroenterology and Hepatology, 17, 559 (2005).
11. El-Gindy A, El-Yazby F and Maher M. Spectrophotometric and chromatographic determination of rabeprazole in presence of its degradation products. J Pharm Biomed Anal.2003; 31: 229-242.
12. Masatomo M, Tada H, Suzuki T. Simultaneous determination of lansoprazole enantomers and their metabolites in plasma by liquid chromatography with solid-phase extraction. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 804:389-95.
13. Gerloff J, Mignot A, Barth H, Heintze K. Pharmacokinetics and absolute bioavailability of lansoprazole. Eur J Clin Pharmacol 1996; 50:293-7.
14. Susheel J.V., M. Lekha and T.K.Ravi. High Performance Thin layer Chromatographic Estimation of Lansoprazole and

Domperidone in Tablets. Indian J. Pharm. Sci, 2007 69(5): 684-686.
15. Dugger HA, Carlson JD, William Henderson, Erdmann GR, Mahmood Alam S, Ruwayda Dham and Quamruzaman. Bioequivalence evaluation of lansoprazole 30-mg capsules (Lanfast and Lanzor) in healthy volunteers. European Journal of Pharmaceutics and Biopharmaceutics.2001; 51: 153-157.
16. Dollery C. Therapeutic Drugs. Edinburgh, Scotland, Churchill Livingston, Kobylinska M and Kobylinska K. High-performance liquid chromatographic analysis for the determination of domperidone in human plasma. J Chromatogr B.2000; 744:207-212.
17. Zavitsanos AP, MacDonald C, Bassoo E and Gopaul D. Determination of domperidone in human serum and human breast milk by high-performance liquid chromatography-electrospray mass spectrometry. J Chromatogr B. 1999; 730: 9-24.
18. Hagino K, Yamamoto M and Kotaki H, Iga. Quantitative determination of domperidone in rat plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr B.1998; 720: 1-2.
19. M. Mondal, TK. Pal Jayakumar, Chattaraj, Banerjee, J. Indian Medical Association, 102, 26 (2004).
20. ICH, Q2A Text on validation of analytical procedures, international conference on Harmonization, Oct. 1996.
21. Chaibva FA, Walker RB. Development and validation of a stability-indicating analytical method for the quantitation of oxytocin in pharmaceutical dosage forms. J Pharm Biomed Anal.2007; 43:79-185.
22. International conference on harmonization (ICH), Q1AR2: stability testing of new drug substances, products IFPMA, 2003, Geneva.
23. Singh S, Bakshi M. Guidance on conduct of stress tests to determine inherent stability of drugs. Pharm Technol.2000; 24:1–14.

How to cite this article:

CH. Naveen Kumar et al.,: Stress Degradation studies and Development of High Resolution New Validated Stability Indicating Analytical Method for Determination of Various Proton Pump Inhibitors in its Bulk and Multicomponent Pharmaceutical Dosage forms in the Presence of Degradation products as per ICH Guidelines, J. Pharm. Res., 2015; 4(3): 135-150.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil