Stability-indicating HPLC method for the simultaneous determination of pantoprazole, rabeprazole, lansoprazole and domperidone from their combination dosage forms

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Abstract

A specific, accurate, precise and reproducible stability-indicating HPLC method has been developed and subsequently validated for the simultaneous determination of pantoprazole, rabeprazole, lansoprazole and domperidone in commercial tablets. The proposed HPLC method utilizes Phenomenex Gemini C18 column (150 mm x 4.6 mm i.d., 5 µm) and mobile phase consisting of methanol-acetonitrile-20 mM dipotassium hydrogen phosphate and phosphoric acid buffer pH 7.0 (20:33.11:46.89, v/v/v) at a flow rate of 1.10 mL/min. Quantitation was achieved with UV detection at 280 nm based on peak area with linear calibration curves at concentration ranges 1.0-10µg/ml for pantoprazole & rabeprazole, 0.75-7.5µg/mL for lansoprazole and 0.5-5.0µg/mL for domperidone ($R^2 > 0.999$ for all drugs). The method was validated in terms of accuracy, precision, linearity, limits of detection, limits of quantitation and robustness. This method has been successively applied to pharmaceutical formulation and no interference from the tablet excipients was found. Pantoprazole, rabeprazole, lansoprazole, domperidone and their combination drug product were exposed to acid, base and neutral hydrolysis, oxidation, dry heat and photolytic stress conditions and the stressed samples were analyzed by the proposed method. As the proposed method could effectively separate the drug from its degradation products, it can be employed as stability-indicating method for the determination of instability of these drugs in bulk and commercial pharmaceutical formulations.

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INTRODUCTION

Safety and efficacy of pharmaceuticals are two fundamental issues of importance in drug therapy.

Instability of pharmaceuticals can cause a change in physical, chemical, pharmacological and toxicological properties of the active pharmaceutical ingredients (API), thereby affecting its safety and efficacy. Hence, the pharmacists should take cognizance of various factors such as drug stability, possible degradation products, mechanisms and routes of degradation and potential interactions with excipients utilized in the formulation to ensure the delivery of their therapeutic values to patients. In order to assess the stability of a drug product, one needs an appropriate analytical methodology, so called the stability indicating methods which allow accurate and precise quantitation of the drug, its degradation products and interaction products, if any. In recent times, the development of stability-indicating assays has increased enormously [1–3], using the approach of stress testing as outlined in the International Conference on Harmonization (ICH) guideline Q1AR2 [4] and even this approach is being extended to drug combinations [5–7]. This ICH guideline requires that stress testing on API and drug products should be carried out to establish their inherent stability characteristics which should include the effect of temperature, humidity, light, oxidizing agents as well as susceptibility across a wide range of pH. However, there are no detailed regulatory guidelines that direct how stress testing is to be done and hence stress testing has evolved into an "artful science" that is highly dependent on the experience of the pharmaceutical industries or the individuals directing the studies [8]. The knowledge gained from stress testing can be useful for (1) the development of stable formulation and appropriate packaging design, (2) controlling of manufacturing and processing parameters, (3) identification and isolation of toxic degradants during API synthesis, (4) recommendation of appropriate storage conditions and shelf-life determination and (5) designing and interpreting environmental studies, as the degradation of the drug in the environment will often

be similar to degradation observed during stress-testing studies. It is also recommended that analysis of stability samples should be done through the use of a validated stability-indicating testing method.

Pantoprazole (PP), Rabeprazole (RP) and Lansoprazole (LP) (Fig. 1) belong to a class of antisecretory compounds, the substituted benzimidazoles that suppress gastric acid secretion by specific inhibition of the H^+/K^+ ATPase enzyme system at the secretory surface of the gastric parietal cell [9]. They are used for the treatment of acid-peptic diseases such as duodenal, gastric and oesophegeal ulceration [10]. Domperidone (DP) (Fig. 1) is a potent dopamine antagonist used for the treatment of nausea and vomiting. Nowadays, the mixtures of these active components are present in pharmaceutical formulations as capsules and tablet forms. Thus, the pharmacology of Pantoprazole, Rabeprazole, Lansoprazole and domperidone corroborates their use in combined dosage form to treat various gastro intestinal disorders in particular for hyperacidity frequently associated with gastro intestinal dysmotility. Combination drug products of Pantoprazole, Rabeprazole and Lansoprazole with domperidone are hence widely marketed and successfully used in the treatment of gastro esophageal reflux disease and non ulcer dyspepsia. Several HPLC methods have been cited in the literature for the estimation of PP[11-13], RP[14,15], LP [16-18] and DP [19-22] there seems to be no reports concerning methods for the simultaneous determination of all the four analytes (PP, RP, LP and DP) using HPLC in the commercial pharmaceutical preparations has been published. Hence, recently we have developed an optimized reversed-phase HPLC method for the routine quality control analysis of PP, RP, LP and DP simultaneously from tablets and capsule dosage forms. The method gave acceptable results for fresh quality control samples, but gave overestimation during analysis of stability samples and aged products, as it lacks assay

specificity in presence of their degradation products. Further, no stability-indicating method has been reported in literature for simultaneous determination of PP, RP and LP with DP in presence of their degradants.
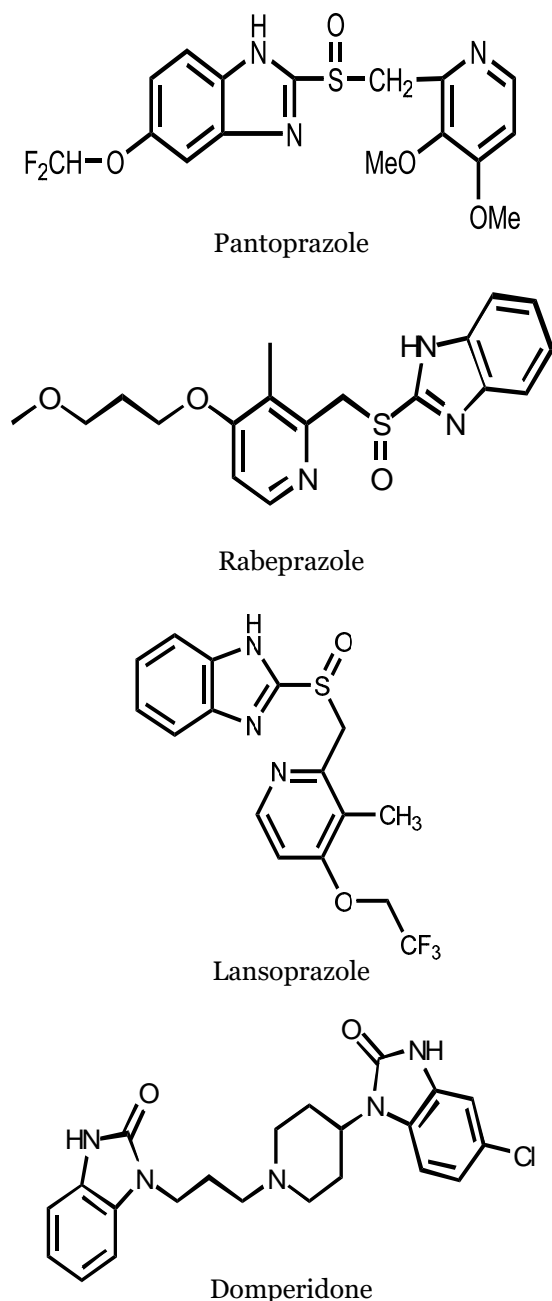

Pantoprazole

Rabeprazole

Lansoprazole

Domperidone

Fig 1: The chemical structures of analytes

Therefore, the present study targets the development and subsequent validation of a stability-indicating HPLC method for the simultaneous determination of PP, RP, LP and DP in presence of their degradants. To establish the stability indicating nature of the

method, forced degradation of each API and drug product was performed under stress conditions and stressed samples were analyzed by the proposed method. The proposed LC method was able to separate all drugs from degradants generated during forced degradation studies.

EXPERIMENTAL

Chemicals and Reagents

Working standards of domperidone, pantoprazole, rabeprazole lansoprazole and diclofenac sodium (IS) were donated by M/S. Pharma analytical Lab., Puducherry, India. The pharmaceuticals Pantocid-D capsules (PP-20 mg with DP-10 mg), Rabby-DM tablets (RP-20mg with DP-10 mg) and Lancer-DM (LP-15mg with DP-10 mg) were purchased from Sun pharmaceuticals (J&K, India) Elixir Life Care (P) LTD., (Chennai, India) and East West Pharma, (Haridwar India) respectively. Acetonitrile and methanol were of HPLC grade and dipotassium hydrogen phosphate and phosphoric acid were of analytical-reagent grade supplied by M/S SD fine Chemicals (Mumbai, India). Sodium hydroxide, hydrochloric acid and hydrogen peroxide were of analytical-reagent grade from Qualigens Fine Chemicals (Mumbai, India). HPLC grade water was obtained following distillation in glass and passage through a Milli-Q Academic system (Millipore, Bangalore, India) and was used to prepare all solutions.

HPLC instrumentation and conditions

Chromatographic measurements were made on a Shimadzu (Tokyo, Japan) model which consisted of an LC10AD and LC10 ADvp solvent delivery module, SPD 10A UV-visible detector, a Rheodyne injector (model 7125, USA) valve fitted with a 20 μL loop, and UV detector (SPD-10A). The system was controlled through a system controller (SCL-10A) and a personal computer using Shimadzu chromatographic software (LC Solution, Release 1.11SP1). Chromatographic separations were carried out on a

Phenomenex Gemini C18 analytical column (150 mm x 4.6 mm i.d., 5 μm) connected with a Phenomenex C18 guard cadridge (4 mm x 3 mm i.d., 5 μm) using a mobile phase consisting of methanol – acetonitrile – 20 mM dipotassium hydrogen phosphate and phosphoric acid buffer pH 7.0 (20:33.11:46.89 v/v/v) at a flow rate of1.10 mL/ min. In order to increase the sensitivity for the less concentrated compound (i.e., DP) and to decrease the background from mobile phase a wavelength of 280 nm was selected for detection. The injection volume of the sample was 20 μL. The HPLC system was used in an air-conditioned laboratory atmosphere (20 ± 2 °C).

Preparation of stock and standard solutions

Stock solutions at concentrations of 1000μg /mL each of PP, RP, LP and DP were prepared separately in methanol. The stock solutions were protected from light and stored at 4 °C to avoid degradation. Aliquots of the stock solutions of PP, RP, LP and DP were diluted with mobile phase to yield standard solutions of 1, 2, 5, 7 and 10 μg /ml for PP& RP, 0.75, 2.25, 3.75, 5.25, 7.5μg/ml for LP and concentrations of 0.5, 1.0, 2.5, 3.5 and 5.0 μg/ ml for DP Calibration curves reporting peak areas of PP, RP, LP and DP versus drug concentrations were established in the ranges described above.

Sample preparation for tablet assay

Twenty tablets were weighed and finely powdered. In the case of capsule dosage, the contents of the capsule were mixed thoroughly. An amount of pharmaceutical products powder equivalent to 10 mg of DP with 20 mg of PP, 10 mg of DP with 20mg of RP, and 10 mg of DP with 15mg of LP were accurately weighed and transferred in a 50ml volumetric flask and to this, 25 mL of the mobile phase was added. This mixture was subjected to sonication for 10 min for complete extraction of drugs and the solution was made up to the mark with mobile phase to obtain a concentration of PP, RP, LP and DP as 5.0, 5.0, 3.75 and 2.5 μg/ml respectively. The solution was centrifuged at 4000 rpm for 10 min; the clear supernatant was collected and filtered through a 0.2μm membrane filter (Gelman Science, India) and 20 μl of this solution was injected for HPLC analysis.

FORCED DEGRADATION STUDIES OF API AND TABLETS

The pharmaceuticals Pantocid-D capsules containing (PP-20 mg with DP-10 mg), Rabby-DM tablets containing (RP-20mg with DP-10 mg) and Lancer-D containing (LP-15mg with DP-10 mg) were subjected to various forced degradation conditions to effect partial degradation of the drug preferably in 20−80% range[23]. The forced degradation studies were performed not only for the drug product, but also for API of PP, RP, LP and DP to determine whether any observed degradation occurred because of drug properties or was due to drug–excipient interactions. Moreover, the studies provide information about the conditions in which the drug is unstable so that measures can be taken during formulation to avoid potential instabilities. The stability samples were prepared by dissolving each API or drug product in methanol and later diluted with either distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide or aqueous hydrogen peroxide solution at a concentration of 100 (PP and RP), 75 (LP) and 50 (DP) μg/ml separately. After degradation, these samples were diluted with mobile phase to achieve the nominal concentration of 5.0 (PP and RP), 3.75 (LP) and 2.5(DP) μg/ml, which was based on their label strength in tablets.

Acid hydrolysis

Solutions for acid degradation studies were prepared in methanol and 0.1 M hydrochloric acid (20:80, v/v) at room temperature (22 °C). It was observed that both acid and base hydrolysis was a fast reaction for both drugs and almost completed within 10 min of the sample preparation, therefore the samples were analyzed after this period of time.

Base hydrolysis

Solutions for base degradation studies were prepared in methanol and 0.1 M sodium hydroxide (20:80, v/v) at room temperature (22 °C) and the resultant solutions analyzed 10 min after preparation.

Neutral hydrolysis

Solutions for neutral degradation studies were prepared in methanol and water (20:80, v/v) and the resultant solutions heated on a water bath at 90 °C for 20 min. The mixture was then allowed to cool at room temperature, filtered using syringe filters and analyzed.

Oxidation studies

Solutions for use in oxidation studies were prepared in methanol and 6% hydrogen peroxide (20:80, v/v) at room temperature (22 °C) and the resultant solutions were filtered using syringe filters and analyzed after 10 min.

Photostability studies

Solutions for Photostability studies were prepared in methanol and water (20:80, v/v) and the resultant solution was exposed to natural sunlight during the day time for 8 h. The degraded sample was then filtered using syringe filters and analyzed.

Temperature stress studies

Tablets and API in powder forms were exposed to dry heat (100 °C) in an oven for 8 h. The API and tablet powders were then removed from the oven and an aliquot of tablet powder equivalent to the weight of one tablet were prepared for analysis as previously described.

RESULTS AND DISCUSSION

HPLC method development

Our earlier HPLC method was optimized with respect to mobile phase composition, buffer concentration and flow rate to achieve an optimal chromatographic condition for the separation and simultaneous quantitation of PP, RP, LP and DP from Pantocid-D capsules containing (PP-20 mg with DP-10 mg), Rabby-DM tablets containing (RP-20mg with DP-10 mg) and Lancer-D containing (LP-15mg with DP-10 mg). During optimization, the pH of the aqueous phase was not varied and maintained at 7.0, as this could influence the stability of Proton- pump inhibitors [24]. This optimized method employs phenomenex Gemini C18 column (150 mm · 4.6 mm i.d., 5 µm) and mobile phase consisting of methanol–acetonitrile– 20 mM dipotassium hydrogen phosphate and phosphoric acid buffer pH 7.0 (20:33.11:46.89, v/v/v) for the separation of PP, RP, LP and DP without affecting the stability of these analytes. However, this method does not give data on specificity for the estimation of the four analytes in the presence of their degradants. Therefore, as an attempt to develop stability-indicating assay, the same optimal chromatographic conditions have been tried to separate these analytes from their degradation products generated during forced degradation studies. The only modification of the optimized method in the present work was that no internal standard was employed to avoid confusion with the probable degradants of internal standard that arise from stress studies. Using this customized optimized method, it was possible to separate PP, RP, LP, DP and their degradation products without any interference and thus, the assay can be considered stability-indicating.

Validation of the method

The developed stability-indicating method was validated according to ICH [25, 26] guidelines. The validation parameters addressed were linearity, limit of detection and quantitation, accuracy, precision, specificity and robustness.

Linearity

Linearity was established over the concentration range of 1.0-10µg/ml, 1.0-10µg/ml, 0.75-7.5µg/ml, and 0.5-5.0µg/ml for PP (n = 6), RP (n=6), LP (n=6) and DP (n = 6), respectively. Peak areas (y) of PP, RP, LP and DP were plotted versus their respective concentrations (x) and linear regression analysis performed on the resultant calibration curves. Correlation coefficients (R^2) were found to be more

than 0.999 for all the analytes. Typically, the mean of the regression equations were: $y = 48752x + 16.66$, $y = 37331x + 45.44$, $y = 45345x - 452.1$, $y = 38557x + 501.4$ for PP, RP, LP and DP, respectively.

Limit of Detection and Quantitation

The limit of detection (LOD) and quantitation (LOQ) for PP, RP, LP and DP were determined according to ICH guideline Q2B [26]. LOD was defined as $3.3\,\sigma/S$ and LOQ was $10\,\sigma/S$ based on 'standard deviation of the response and slope' of the calibration curve specially constructed in a low region of 0.05 to 1.0% of the target analyte concentration [27]. The standard deviation of y-intercepts of the regression lines was used as σ (the standard deviation of the response) and S is the slope of the calibration curve. The LOD and LOQ were estimated as 1.16 and 3.50 ng/ml for PP, 1.54 and 4.68 ng/ml for RP, 1.76 and 5.36 ng/ml for LP, 2.8 and 8.42 ng/ml for DP respectively.

Accuracy/Recovery

Accuracy of the method was determined by performing the recovery experiment at 80, 100 and 120% levels of the labeled amount of the analytes in the commercial formulation. Three replicate samples of each concentration level were prepared by spiking the standard drugs with the placebo or tablet excipients and the %recovery at each level (n = 3), and mean %recovery (n = 9) were determined (Table 1). The recoveries for PP, RP, LP and DP were found to be 99.86, 99.90, 100.02 and 100 %, respectively, which were within acceptable ranges of 100 ± 2%

Precision

Six injections, of three different concentrations, were given on the same day and the percent relative standard deviations (%RSD) were calculated to determine intra-day precision. These studies were also repeated on six consecutive days to determine inter-day precision. The data obtained from precision experiments are given in Table 2. The %RSD values for the intra-day precision study were ≤ 2 and for the inter-day study ≤ 3, confirming that the method was sufficiently precise [28].

Specificity

The results of forced degradation studies of each drug in the presence of their degradation products indicated a high degree of specificity of this method for PP, RP, LP and DP. The degradation product of each of the parent compounds was found to be similar for the Pantocid-D capsules, Rabby-DM tablets and Lancer-D capsules with that of API powders assessed. Typical chromatograms obtained following the assay of untreated and stressed samples of API and formulations are shown in Fig. 2.

Robustness test

Robustness of the proposed method was assessed with respect to small alterations in the acetonitrile concentration (33.11 ± 0.5%), the pH value (7.0 ± 0.2) and the buffer concentration (20 ± 2.0 mM). The degree of reproducibility obtained as a result of small deliberate variations in the method parameters has proven that the method is robust and the data are summarized in Table 3.

Table 1: Results of accuracy experiment using proposed method

Spiked levels	PP (*n* = 3)			RP (*n* = 3)			LP (*n* = 3)			DP (*n* = 3)		
	Taken (mg)	Recovered (mg)	Recovery (%)	Taken (mg)	Recovered (mg)	Recovery (%)	Taken (mg)	Recovered (mg)	Recovery (%)	Taken (mg)	Recovered (mg)	Recovery (%)
80%	16.13	16.13	99.97	16.12	16.1	99.85	12.02	12.02	99.94	8.03	8.03	100
100%	20.10	20.04	99.71	20.06	20.02	99.83	15.09	15.08	99.95	10.13	10.12	99.89
120%	24.01	24.02	99.92	24.06	24.07	100.04	18.02	18.05	100.18	12.02	12.04	100.13
Mean %recovery (*n* = 9)			99.86			99.90			100.02			100.00
% RSD			0.138			0.116			0.135			0.12

Table 2: Results of Precision experiments using proposed method

Actual concentration (µg mL⁻¹)	Measured concentration (µg mL⁻¹)			
	Intra-day precision		Inter-day precision	
	Mean, SD	%RSD	µg mL⁻¹, SD,	%RSD
Pantoprazole (*n* = 6)				
1.0	0.99 , 0.01	1.38	0.98 ± 0.01	1.82
5.0	5.03 , 0.04	0.86	4.94 ± 0.06	1.25
10.0	9.95 , 0.06	0.63	9.74± 0.19	2.03
Rabeprazole (*n* = 6)				
1.0	0.99 , 0.01	1.97	0.97 ± 0.02	2.27
5.0	5.05 , 0.08	1.70	4.91 ± 0.11	2.37
10.0	10.01, 0.04	0.49	10.10 ± 0.11	1.18
Lansoprazole (*n* = 6)				
0.75	0.74 , 0.00	1.09	0.73 ± 0.01	1.86
3.75	3.74 , 0.03	0.99	3.73 ± 0.02	0.56
7.5	7.50, 0.01	0.21	7.48 ± 0.02	0.26
Domperidone (*n* = 6)				
0.5	0.50 , 0.00	1.78	0.49 ± 0.01	2.58
2.5	2.52 , 0.01	0.56	2.48 ± 0.00	0.32
5.0	5.09 , 0.05	1.15	4.98 ± 0.06	1.23

Table 3: Results of Robustness test of the proposed method

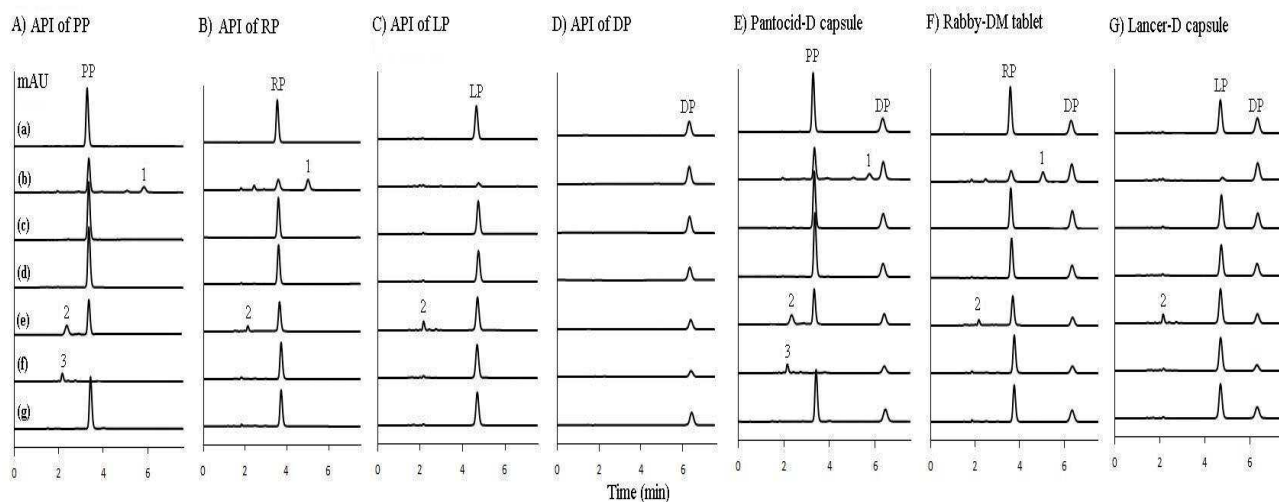| Parameter	Modification	Pantoprazole (% Recovery)	Rabeprazole (% Recovery)	Lansoprazole (% Recovery)	Domperidone (% Recovery)
	32.61	99.2	99.8	99.79	99.89
MeCN conc. (%)	33.11	99.6	100.64	99.86	98.91
	33.61	99.1	99.4	99.6	98.03
	6.8	99.5	99.8	99.79	99.8
pH value	7.0	99.1	100.64	99.86	99.8
	7.2	99.4	99.4	99.6	100.4
	18	99.2	99.8	99.79	99.89
Buffer conc. (mM)	20	99.6	100.64	99.86	98.91
	22	99.1	99.4	99.6	98.03
Mean		99.31	99.94	99.75	99.29
%RSD		0.21	0.54	0.11	0.86

Fig 2: Representative chromatograms of API (PP, RP, LP, DP) and Pantocid-D capsule, Rabby-DM tablet and Lancer-D capsule obtained under stress conditions (a)untreated sample; (b) acid hydrolysis (0.1M HCL, 22 °C, 10min); (c) base hydrolysis (0.1M NaOH, 22°C, 10 min); (d) neutral hydrolysis (water, 90°C, 20 min); (e) oxidative degradation (6% H2O2,22°C,10 min); (f) photolytic degradation (sunlight 8 h); and (g) dry heat degradation (100°C, 8 h), showing 1-acid, 2- oxidative and 3- photolytic degradation peaks
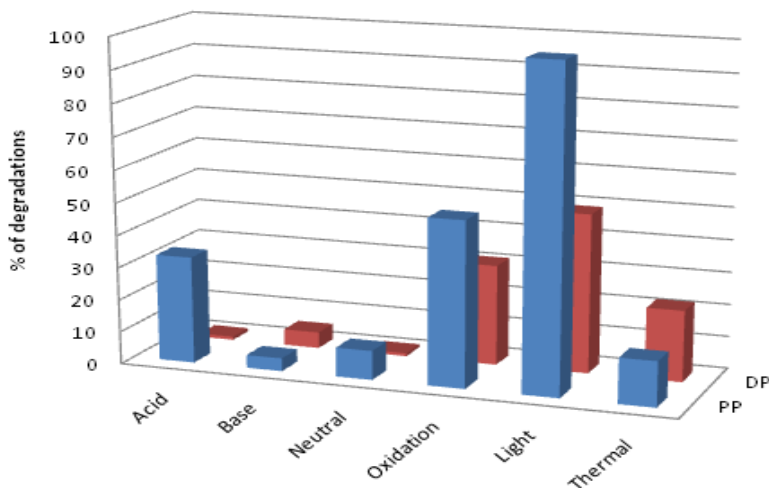
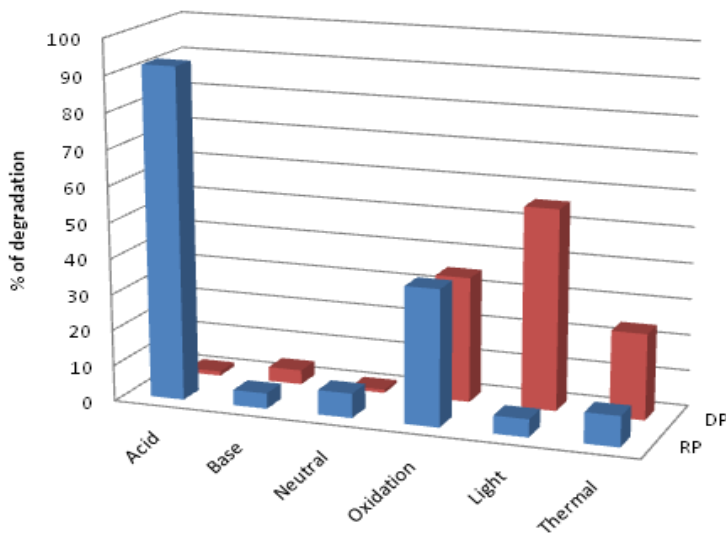**Fig 3:** Percentage degradation of PP and DP obtained from Pantocid-D capsules under various stress conditions

Fig 4: Percentage degradation of RP and DP obtained from Rabby-DM tablets under various stress conditions
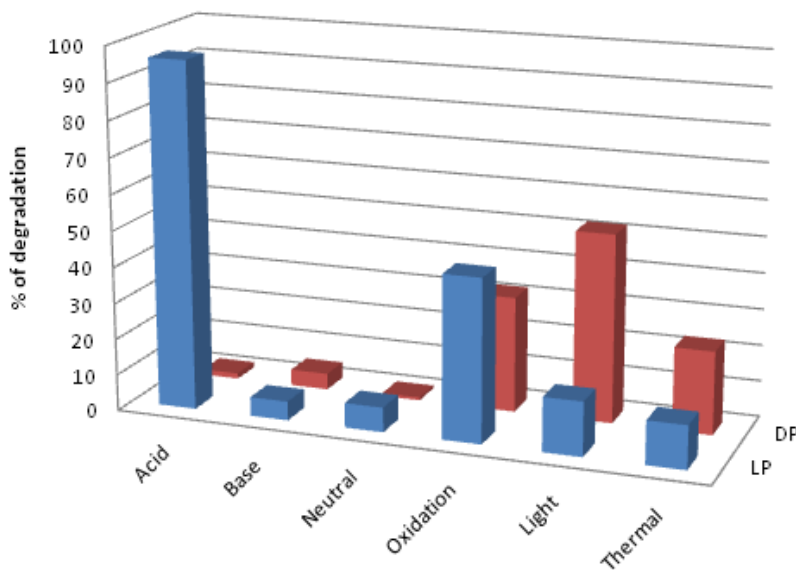
Fig 5: Percentage degradation of LP and DP obtained from Lancer-D capsules under various stress conditions

Degradation behavior

Forced degradation studies of API (PP, RP, LP, DP) and formulations (Pantocid-D capsules, Rabby-DM tablets and Lancer-D capsules) were carried out under various stress conditions and resultant chromatograms are depicted in Fig. 2 and the extent of degradation of the two analytes in Pantocid-D capsules, Rabby-DM tablets and Lancer-D capsules are shown in Fig. 3, Fig. 4 and Fig. 5 respectively. The degradant product formed from each drug has been identified by comparing the respective chromatograms of each API with formulations obtained after forced degradation studies.

Proton pump inhibitors are highly susceptible to low pH [29] and PP is no exception and undergoes 33 and 32% decomposition under acidic stress condition for both pure API and capsules forms, respectively, forming a major acid degradant peak at tR = 5.6 min (Fig. 2A-(b) and E-(b)) were suggested based on the studies of Tutunji et al. [30] and Qaisiet al. [31]. On the other hand, this drug was sufficiently stable under basic and neutral degradation conditions, resulting only 4 and 9% degradation in tablets, respectively. Hence, it was found that the stability of PP was pH dependent; the rate of degradation decreased with increased pH. These results are in accordance with the previously published reports [24, 32, 33]. In contrast, DP was relatively stable at all hydrolytic stress conditions, resulting 1, 0.94 and 5% degradation in tablets under acidic, neutral and basic stress conditions.

In oxidation stress condition, almost 51% of PP was degraded in tablets, forming a major oxidative degradation product at tR = 2.25 min (Fig. 2E-(e)). In this case, the rise in degradant peak area was in correspondence with the fall in parent peak, indicating that PP was decomposed to a chromophoric degradant. This oxidative degradants possibly the sulphone or N-oxide analogues of PP formed by the oxidation reaction of sulfinyl moiety or pyridine nitrogen, was lacking any therapeutic effect

[34]. DP degradation of about 31% was found under oxidative stress condition, with no degradation peaks observed in the chromatogram (Fig. 2E-(e)).

When tablets in solution state were exposed to direct sunlight, almost complete degradation (>99%) of PP was observed, with one major potential degradation product at tR = 2.09 min and formation of a cluster of minor degradation products between tR ranges of 2.20–2.80 min (Fig. 2E-(f)). But, no degradation peaks could be identified for DP, although 54% of DP was decomposed, which might be due to lack of chromophore in the degradation product formed. This test showed that PP in aqueous methanolic solutions are very sensitive followed by DP to sunlight exposure. Under dry heat stress condition, PP and DP in tablets were moderately stable showing 14 and 22% degradation [35].

Rabeprazole is known to be an acid labile drug and undergoes 92.33 and 91% decomposition under acidic stress condition for both pure API and tablets forms, respectively, forming a major acid degradant peak at tR = 4.92 min (Fig. 2B-(b) and F-(b)). On the other hand, this drug was sufficiently stable under basic and neutral degradation conditions, resulting only 4.33 and 6.86 % degradation in tablets, respectively. Hence, it was found that the stability of RP was pH dependent; the rate of degradation decreased with increased pH [36]. In contrast, DP was relatively stable at all hydrolytic stress conditions, resulting 1.2, 0.94 and 4% degradation in tablets under acidic, neutral and basic stress conditions.

In oxidation stress condition, almost 38% of RP was degraded in tablets, forming a major oxidative degradation product at tR = 2.06 min (Fig. 2F-(e)). In this case, the rise in degradant peak area was in correspondence with the fall in parent peak [36]. DP degradation of about 35% was found under oxidative stress condition, with no degradation peaks observed in the chromatogram (Fig. 2F-(e)).When tablets in solution state were exposed to direct

sunlight, RP was relatively stable showing 5% degradation[37], although 56% of DP was decomposed, with no degradation peaks in the chromatogram (Fig. 2F-(f)). Under dry heat stress condition, RP and DP in tablets were moderately stable showing 8.56 and 24% degradation.

Analysis of Lansoprazole drug substance stressed by acid, base, neutral, hydrogen peroxide, light and heat revealed that the compound is stable under alkaline conditions, heat, and light; however, it is sensitive to acid and oxidation [38]. LP undergoes almost 97 and 96% decomposition under acidic stress condition for both pure API and capsules forms, respectively, with no major potential degradation product and formation of a cluster of minor degradation products between tR ranges of 1.7–2.1 min (Fig. 2C-(b)). This LP acid degradants possibly the, sulfide, was suggested based on the studies of Jeffrey Selenka et al [39]. On the other hand, this drug was sufficiently stable under basic and neutral degradation conditions, resulting only 5.25 and 6.82% degradation in tablets, respectively. Hence, it was found that the stability of LP was pH dependent; the rate of degradation increased with decreased pH. These results are in accordance with the previously published report [40].In contrast; DP was relatively stable at all hydrolytic stress conditions, resulting 1.5, 0.8 and 4.5% degradation in tablets under acidic, neutral and basic stress conditions.

In oxidation stress condition, almost 45% of LP was degraded in tablets, forming a major oxidative degradation product at tR = 2.11 min (Fig. 2G-(e)). In this case, the rise in degradant peak area was in correspondence with the fall in parent peak. This oxidative degradants possibly the sulphone, the proposed degradant was based on the earlier report Jeffrey Selenka et al [38]. DP degradation of about 35% was found under oxidative stress condition, with no degradation peaks observed in the chromatogram (Fig. 2G-(e)). When tablets in solution state were exposed to direct sunlight, 15% degradation of LP

and 52% of DP was observed, with the formation of a cluster of minor degradation products between tR ranges of 1.9–2.11 min (Fig. 2G-(f)). But, no degradation peaks could be identified for DP, although 52% of DP was decomposed. Under dry heat stress condition, LP and DP in capsules were moderately stable showing 12 and 23% degradation. The degradation products of PP, RP, LP, and DP were found to be similar for all the formulations (Pantocid-D capsules, Rabby-DM tablets and Lancer-D capsules) and API powders assessed. In contrast, the decomposition of DP in API samples was not correlated with the tablet samples, however, less degradation was found in tablet samples. This protective effect may be ascribed to the excipients used in the tablet formulation.(Fig. 2).The stability of stock solutions (stored at 4 °C for 1 week) was determined by quantitation of each drug in solution in comparison to the response obtained for freshly prepared standard solutions. No significant changes (<2%) were observed for the chromatographic responses for the stock solutions analyzed, relative to freshly prepared standards.

Assay of commercial product

The validated method was applied to the determination of PP, RP and LP with DP in commercially available Pantocid-D capsules Figure 2E-(a), Rabby-DM tablets Figure 2F-(a), Lancer-D capsules Figure 2G-(a), illustrates a typical HPLC chromatogram obtained following the assay of Pantocid-D capsules, Rabby-DM tablets and Lancer-D capsules. The result of the assays (n = 6) undertaken yielded 99.84% (%RSD = 0.30%) and 99.95% (%RSD = 0.44%) of label claim for PP and DP, 99.84% (%RSD = 0.40%) and 99.88% (%RSD = 0.44%) of label claim for RP and DP, 99.94% (%RSD = 0.13%) and 99.91% (%RSD = 0.45%) of label claim for LP and DP, respectively.

CONCLUSION

An isocratic stability-indicating HPLC- UV method has been developed for the estimation of PP, RP and LP with DP in the presence of degradation products. The proposed method is simple, accurate, precise, specific, and has the ability to separate the drugs from degradation products and excipients found in the pharmaceutical dosage forms. The method is suitable for use in routine analysis of both drugs in bulk API powder or in pharmaceutical dosage forms. The method can be applied even to the analysis of stability samples obtained during accelerated stability experiments, as no interference was found with the degradants formed under various stress conditions.

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