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Stability Indicating HPLC-UV Method for Simultaneous Estimation of Pantoprazole, Domperidone and Drotaverine

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Abstract: A stability indicating method for the simultaneous estimation of Pantoprazole (PPZ), Domperidone (DPD), and Drotaverine (DRT) using HPLC has been developed and validated. These drugs were separated through SD's Millenium C18 column (100x4.6 mm i.d., 5-µm particle size) with a mobile phase containing methanol, acetonitrile and 0.02M dipotassium hydrogen phosphate (pH 7.0), in the ratio of 20:33:47 (v/v/v) at a flow rate of 1mL/min for PPZ, 2.5mL/min for DPD and 1mL/min for DRT. Further, PPZ and DRT were detected at a wavelength of 290nm, and DPD at 240nm, based on the peak area. Parameters such as linearity, precision, accuracy, recovery and specificity were studied as per ICH guidelines. The retention time of PPZ, DPD and DRT were 2.5, 6.01 and 11.8 min. respectively. The limit of detection (LOD) was determined to be 0.01721µg/ml, 0.0115µg/ml, and 0.0212µg/ml for PPZ, DPD, and DRT, respectively. Lower limit of quantitation (LLOQ) was determined to be 0.0573µg/ml, 0.0385µg/ml, and 0.0706µg/ml for PPZ, DPD, and DRT, respectively. The linear ranges were found as 0.25-16 µg/ml for PPZ& DRT (n=7) and 0.125-8 µg/ml for DPD (n=7).The correlation coefficient for all components was found to be 1. In order to check the selectivity of the method for pharmaceutical preparations, forced degradation studies were also carried out.
Keywords : RP-HPLC, Pantoprazole, Domperidone, Drotaverine, Stability Studies, Degradation products.

Introduction

Multi-factorial origin is associated with Peptic ulcer disease; worldwide population is affected by it and it is a major source of morbidity and mortality [1]. Although cause of peptic ulcer is a very controversial subject but evidences suggest that stressful physical or mental situation, poor lifestyle including overindulging in rich and fatty foods, alcohol abuse, and consumption of tobacco [2], overuse of painkillers such as aspirin, ibuprofen, and naproxen are the major causes of peptic ulcer. Burning, aching, gnawing pain, back pain, bloating or nausea after eating and vomiting are the common symptoms of peptic ulcer [3]. In clinical practice, a combination of proton pump inhibitor, prokinetic agent (antiemetic) and antispasmodic drugs are prescribed for treatment of acid-peptic disorders including erosive gastro esophageal reflux disease (GERD) and non-erosive reflux disease (NERD) [4-7].

Pantoprazole (PPZ) is a proton pump inhibitor (PPI) and is the first line treatment for acid-peptic disorders. It, selectively and irreversibly, inhibits the proton pump (H^+/K^+-ATPase) that performs the final step in the acid secretary process [8, 9]. Domperidone (DPD) is a prokinetic drug which acts by selectively antagonizing the peripheral dopaminergic D2 receptors in the gastrointestinal wall, thereby enhancing gastrointestinal peristalsis and motility and increasing lower esophageal sphincter tone. The increased Gastro

intestinal motility facilitates the movement of acid contents further down in the intestine, preventing reflux esophagitis and thereby controlling nausea and vomiting [10]. Drotaverine (DRT), is an analogue of papaverine, and is used mainly as an antispasmodic and smooth-muscle relaxant in pain associated with gastrointestinal and biliary colic and postsurgical spasms. It acts by inhibition of phosphodiesterase enzyme, which leads to reduction in contraction of smooth muscles. It is used in gastric ulcer diseases and gastro-intestinal cancer [11, 12]. Proton pump inhibitors, prokinetic agents and antispasmodic drugs are commonly prescribed for the treatment of acid peptic disorders either individually or in combination with proton pump inhibitor and prokinetic agents along with antispasmodic drugs [13, 14]. However, no combinations are available in the market containing proton pump inhibitors, prokinetic agents and antispasmodic type drug in a single dose for the treatment of peptic ulcer. A formulation containing this type of combination will have an advantage of cost effectiveness and increased patient compliance by reducing the number of pills that a patient takes. So it worthwhileto develop a new combination including proton pump inhibitor (Pantoprazole), a prokinetic agent (Domperidone) and an antispasmodic drug (Drotaverine) in a single pill for treatment of acid-peptic disorders. For the development of such a type of new combination, a validated assay method is mandatory. Several HPLC methods [15-23] have been reported in the literature for the estimation of PPZ, DPD and DRT, individually and in combination with other drugs but, to the best of our knowledge, no analytical method is published for the simultaneous estimation of the PPZ, DPD and DRT. Therefore, as a pre requisite to develop a new formulation, an attempt was made to develop and validate a stability indicating assay method, as per International Conference on Harmonization(ICH)Guidelines[24], for the simultaneous estimation of PPZ, DPD and DRT using HPLC as a tool.

This work describes development, validation, and application of a new, simple, selective and reliable HPLC-UV method for the simultaneous estimation of PPZ, DPD and DRT, in bulk API powder or in tablet dosage forms. The method was validated for its specificity and stability indicating properties such as forced hydrolytic, oxidative, photolytic, dry heat degradation and stability in Simulated Gastric Fluid. A stability indicating method is required for estimation of stability of drug substance and drug products. It can detect changes with time in the chemical, physical or microbiological properties of drug substances or drug products. For the development of medicinal products of reliable quality and efficacy, recognized instability of constituents should be defined under ambient and biologically relevant conditions. Stability study in simulated gastric fluid (SGF) gives an important consideration about the stability of drugs in the GI tract. Stability in gastric juice is of prime importance for the drugs intended for oral administration. These fluids are the perfect media to determine the stability of drug candidates *in vitro*. In the present study, the proposed combination was tested *in vitro* using SGF.

Thus, a method has been developed such that it exhibits excellent chromatographic performance and is simple, precise, accurate, reproducible, economic, selective, sensitive, and specific. The method has been thoroughly validated and can be recommended for routine analysis and checking quality during stability studies of the cited drugs. These studies may help facilitate pharmaceutical development in areas such as formulation development, manufacturing, and packaging, in which the knowledge of chemical behavior can be used to improve a drug product [25].

Experimental

Pantoprazole sodium (100%) was procured as a gift sample from Jubilant Life sciences, and Domperidone (99.60%) and Drotaverine hydrochloride (99.54%) were procured similarly from Akums Drugs and Pharmaceutical Ltd. POX-40, DOMSTAL & DROTIN tablets were purchased from local market (Lucknow, India). Acetonitrile and methanol were of HPLC grade and were supplied by M/S SD Fine Chemicals (Mumbai). Dipotassium hydrogen phosphate and phosphoric acid were of analytical-reagent grade and supplied by SD Fine Chemical (Mumbai). HPLC grade water was obtained from "MILLIPORE Direct Q3" water filter. Analysis was performed with a Shimadzu chromatograph (LC solution software) equipped with a Rheodyne (7725 I) injector valve with 20-µL loop, an LC-20 AD Prominence solvent-delivery system, and a SPD-20A Prominence UV/VIS detector set at 290 nm (PPZ & DPD) and 240 nm (DRT). The equipment was controlled by a PC work station.

Preparation of Stock and Standard Solutions

Stock solutions, at concentrations of 1000 µg/ml each of PPZ, DPD and DRT were prepared separately in methanol. The stock solutions were protected from light and stored in a refrigerator to avoid degradation.

Aliquots of the stock solution were diluted with the mobile phase to yield standard solutions of 0.25, 0.5, 1, 2, 4, 8 and 16µg/ml for PPZ and DRT and concentrations of 0.125, 0.25, 0.5, 1, 2, 4, and 8µg/ml for DPD. Calibration curves were established in the ranges described above. Alternatively, the corresponding regression equation was derived.

Sample Preparation for Tablet Assay

Ten tablets ofPOX-40, DOMSTAL and DROTIN were crushed separately into a fine homogenous powder. Tablet powder equivalent to average weight of one tablet was weighed and taken separately in 100 ml volumetric flasks. Powder was dissolved in 40 ml of mobile phase and sonicated. Volume was made up to 100 ml with the mobile phase to obtain a concentration of PPZ, DPD and DRT as 400, 100 and 400 µg/ml. Solutions were centrifuged at 3000 rpm for 20 minutes. Supernatant was collected and further diluted with the mobile phase to obtain concentrations of 200, 100 and 200 µg/ml. Further, the aliquots of stock solutions were withdrawn and mixed and finally diluted with the mobile phase to prepare test solutions of different concentrations.

Forced Degradation and Stability Indicating Studies

The API of PPZ, DPD and DRT were subjected to various forced degradation conditions to effect partial degradation of the drug, preferably in 5-20% range. Forced degradation 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 40 (PPZ and DRT) and 20 (DPD) µg mL^{-1}, separately and in mixture. After degradation, these samples were diluted with mobile phase to achieve the nominal concentration of 8.0 (PPZ and DRT) and 4.0 (DPD) µg mL^{-1}.

Acid Hydrolysis

Solutions for acid degradation studies were prepared in methanol and 0.1 M hydrochloric acid (10:40, *v/v*) at room temperature (25 °C). It was observed that acid hydrolysis was a fast reaction and was almost completed within 10 min of the sample preparation. Therefore, the samples were analyzed after this period of time.

Alkaline Hydrolysis

Solutions for alkaline hydrolytic studies were prepared in methanol and 0.1 M sodium hydroxide (10:40, *v/v*) at room temperature (25 °C) and the resultant solutions were analyzed 10 min after preparation.

Neutral Hydrolysis

Solutions for neutral degradation studies were prepared in methanol and water (10:40, *v/v*) and the resultant solutions were heated on a water bath at 90 °C for 20 min. The mixture was then allowed to cool at room temperature and analyzed.

Oxidative Degradation

Solutions for use in oxidative degradation were prepared in methanol and 6% hydrogen peroxide (10:40, *v/v*) at room temperature (25 °C) and the resultant solutions were filtered using syringe filters and analyzed after 10 min.

Photo Degradation

Solutions for photo degradation studies were prepared in methanol and water (10:40, *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.

API in powder forms were exposed to dry heat (100 °C) in an oven for 8 h. The API was then removed from the oven and test sample was prepared in methanol.

Stability in Gastric Fluid

To determine the stability of the mixture in gastric fluid, the combination was subjected to simulated gastric fluid *in vitro*. For this, the mixture was spiked in 150 ml of simulated gastric fluid and then the resulting solution, at a concentration of 40 (PPZ and DRT) and 20 (DPD) µg mL^{-1}, was placed in an incubator at 37 °C. Sampling was done at time intervals of 15 minutes for 1.5 hours. The withdrawn samples were diluted with the

mobile phase to prepare test solutions of concentration 8.0 (PPZ and DRT) and 4.0 (DPD) µg mL^{-1}. These test solutions were analyzed by the developed stability indicating method.

Results

Selection and Optimization of the Chromatographic Conditions

Separation was achieved on a SD's Millenium C-18 reversed-phase column (100 cm × 4.6 mm i.d., 5-µm particle) equipped with guard column. The analytical system was washed daily with 60 mL of a 1:1 mixture of water and methanol.

For mobile phase optimization, initially all the drugs were dissolved in methanol. Then the solution of drugs at a concentration of 10 µg/ml was injected individually in order to determine their retention time in different mobile phases and it was found that the mobile phase containing methanol: 0.01M ammonium acetate buffer (60:40,v/v) of pH6 and pH7, did not given the proper separation of three drugs. Mobile phase containing methanol:0.01M ammonium acetate buffer(pH7), (60:40,v/v), the elution time of domperidone and drotaverine was not found satisfactory. Mobile phase (methanol: 0.01M ammonium acetate buffer of pH 6 in the ratio of 60:40, v/v,) peaks of domperidone and drotaverine were found to interfere with each other. Mobile phase containing methanol: ACN: 0.02M K_2HPO_4buffer (20:33:47, v/v/v) of pH6 and methanol: ACN: 0.02M K_2HPO_4 buffer (20:33:47, v/v/v) of pH5, resolution of peaks were not found satisfactory. However, mobile phase containing methanol: ACN: 0.02M K_2HPO_4 buffer (20:33:47, v/v/v) of pH7, pantoprazole and domperidone were satisfactorily resolved. Therefore, this optimized mobile phase was selected for further method development and validation. Before analysis, the mobile phase and the sample solutions were degassed by using of a sonicator (Ultrasonic Cleaner Toshan Industries PVT Ltd Hardwar India).The resulting mobile phase was filtered through a 0.45-µm membrane filter (Millipore, Ireland).

For the selection of wavelength, dilutions of all the three drugs in mobile phase were examined with a UV spectrophotometer and the UV absorption spectra were obtained. The appropriate wavelength, for simultaneous estimation of PPZ, DPD and DRT, was determined by overlaying the UV spectra (in methanol and mobile phase) of these three drugs shown in Figure 1. The study of spectra of the three drugs indicated three wavelengths of interest as 240, 290 and 319 nm. Further experiments revealed that at 319 nm, the absorption of pantoprazole was very low and domperidone did not show a peak. Therefore, the further work was carried on two wavelengths, i.e. 240 nm and 290 nm. The suitable wavelength for analysis was found to be 290 nm for PPZ and DPD, and 240 nm for DRT. Retention time for pantoprazole and domperidone were 2.7 and 6.04 (at 1ml/min flow rate) respectively. The third drug, Drotaverine, had a retention time of 9.7 min at flow rate of 2ml/min. Thus, some programming was needed. To ensure proper resolution and efficient analysis time, it was decided to program the flow rate and wavelength in time programmed as 1-7.5 min: 1 ml/min (λ 290nm); 7.70- 13.70 min: 2.5ml/min (λ 240nm); 14.00-15.00: 1ml/min (λ 290nm).

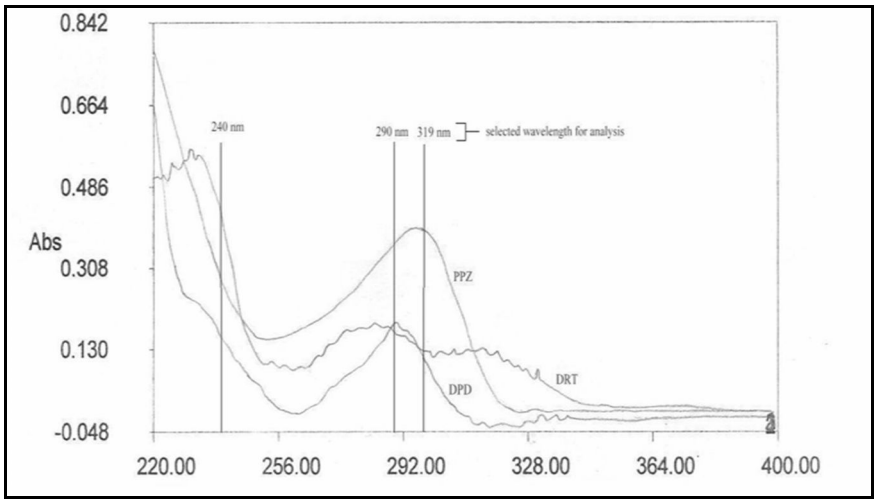

Figure 1: Overlay UV spectra of analytes.

Under these conditions, typical retention times for PPZ, DPD and DRT were 2.5, 6.01and 11.8 min respectively. The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in the standard solutions.

Method Validation

The quality control (QC) sample were prepared at three concentrations (0.5, 2 and 8µg/ml) for PPZ and DRT and (0.25, 1and 4 µg/ml) for DPD. The developed stability indicating HPLC method was validated according to ICH guidelines. The validation parameters included linearity, accuracy, precision, sensitivity, selectivity and specificity.

Linearity

Linearity was established over the concentration range of 0.25-16 µg/ml for PPZ and DRT (n=7) and 0.125-8 µg/ml for DPD (n=7). Peak areas (y) of PPZ, DPD and DRT were plotted against their respective concentrations (x) and linear regression analysis was performed on the resultant calibration curves. Correlation coefficients (r^2) were found to be one after pooling all the data of different days together for all three analytes and Correlation coefficients value for all the compounds prove that the method were linear in the specific range. The linear equations were: y = 54278x + 2473 for PPZ, y = 29992x + 816.8 for DPD and y = 18969x + 631.1 for DRT.

Accuracy/ Recovery

The accuracy of the method was confirmed by conducting a recovery study at three different concentrations by triplicate analysis (3 concentrations × 3 replicates = 9 determinations), in accordance with the ICH guidelines. Results from the accuracy study are reported in Table 1.

Table 1: Result of Recovery Studies

Drug	Quantity taken (µg/mL)	% Recovery	Avg. % Recovery	% CV
PPZ	0.50 2.00 8.00	97.05 100.84 99.35	99.08	0.26 1.16 1.18
DPD	0.25 1.00 4.00	97.14 101.93 99.98	99.68	0.36 1.29 1.23
DRT	0.50 2.00 8.00	98.52 100.84 98.82	99.39	0.39 1.09 0.91

Precision

Intra-day and inter-day precision were studied. Three injections of the QC sample were given on the same day and the percent relative standard deviations (%RSD) expressed as %CV were calculated to determine intra-day precision. These studies were also repeated on three different days to determine inter-day precision. The data obtained from experiments are tabulated in Table 2.

Table 2: Results of Precision

Drug	Actual Concentration (µg/mL)	Measured Concentration (µg/ml), Std. Dev., %CV	
		Intra-day Precision (n=3)	Inter-day Precision (n=9)
PPZ	0.50 2.00 8.00 0.25	0.4802, 0.0013, 0.29100.84 2.0168, 0.0242, 1.19 7.9475, 0.095, 1.19 0.2428, 0.0009, 0.39 99.98	0.4813, 0.0134, 2.79 2.0279, 0.0237, 1.17 7.9647, 0.0991, 1.24 0.2371, 0.0071, 2.41

DPD	1.00 4.00 0.50	1.0093, 0.0134, 1.33100.84 3.9993, 0.0497, 1.24 0.4799, 0.002, 0.42	1.0231, 0.0229, 2.24 4.0339, 0.0663, 1.63 0.4832, 0.0099, 2.06
DRT	2.00 8.00	2.0145, 0.0224, 1.11 7.9037, 0.0723, 0.91	2.0254, 0.0207, 1.02 7.9778, 0.0701, 0.87

Specificity and Selectivity

Under the proposed chromatographic conditions PPZ, DPD and DRT were completely separated from each other. Specificity was assessed by comparing the chromatograms obtained from tablet and blank solutions and from the standard drug solutions indicated in Figure 2. The chromatograms obtained from stressed and untreated samples are shown in Figure 3.

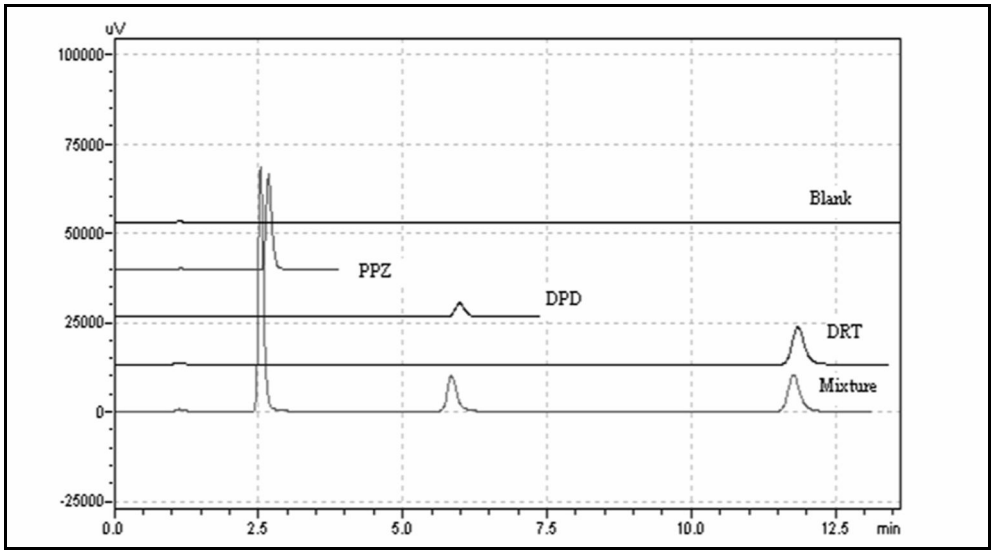

Figure 2. Chromatograms of analytes and blank sample.

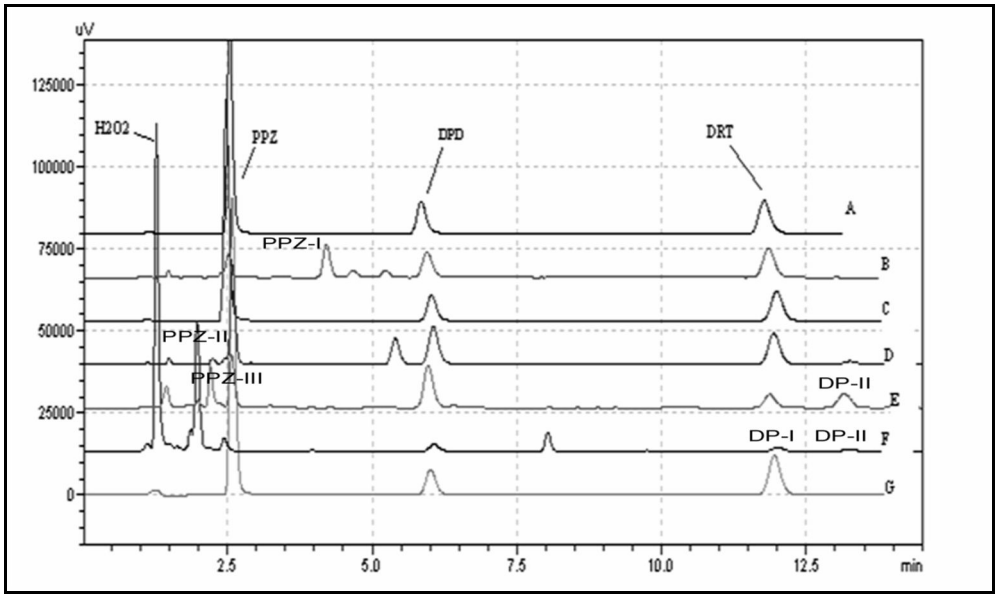

Figure 3. Representative chromatograms of analytes obtained under stress conditions.
A: untreated sample; B: acid hydrolysis; C: base hydrolysis; D: Neutral hydrolysis; E: Photo degradation; F: Oxidative degradation; G: Temperature stress. Degradant peaks of pantoprazole (PPZ-I =Acid, PPZ-II= oxidative and PPZ-III= photo degradants). Degradant peaks of drotaverine (DP-I, DP-II= oxidative and photo degradants).

Limit of Detection (LOD) and Lower Limit of Quantitation (LLOQ)

The limit of detection (LOD) and lower limit of quantitation (LLOQ) for the three drugs were determined according to ICH guidelines Q 2 (R1). LOD and LLOQ were calculated 'based on the standard deviation of the response and slope' and were defined as 3.3σ/S and 10 σ/S, respectively.

System Suitability Parameters

System suitability parameters were calculated for all the three quality control samples. Retention time, theoretical plate number, tailing factor, resolution and capacity factor were also calculated and the results were compared. The Compared results are presented in Table 3.

Table 3: System Suitability Parameters

Parameters	PPZ	DPD	DRT
Retention Time	2.7 ± 0.001 %CV=0.05	5.86±0.004 %CV=0.08	11.77±0.003 % CV= 0.02
Theoretical PlateNumber	3467.456±50.51 %CV = 1.45	5939.97 ± 55.97 %CV = 0.94	15069.12 ± 116.93 %CV = 0.77
Tailing Factor (10%)	1.33 ± 0.002 %CV = 0.19	1.27 ± 0.01 %CV = 1.09	1.23 ± 0.007 %CV = 0.56
Resolution	7.30 ± 0.006 %CV = 0.08	13.71 ± 0.08 %CV = 0.59	17.10 ± 0.06 %CV = 0.40
Capacity Factor (K')	1.29 ± 0.006 %CV = 0.48	4.23 ± 0.05 %CV = 1.27	9.49 ± 0.09 %CV = 1.03

Note: All values are expressed as mean ± s.d. and rsd (%cv).

Stability of Solutions

The stability of standard solution as well as sample solution in HPLC grade water was examined and no chromatographic changes were observed within 24hr at room temperature. Stock solutions were prepared in HPLC grade methanol and were found to be stable for at least two weeks when stored refrigerated at 4^0C.During these periods the retention times and peak areas of the drugs remained unchanged and no significant degradation was observed.

Assay of Tablet Formulation

Three dilutions of different concentration range were prepared from tablet stock solutions. These solutions were injected (20µL) for the quantitative analysis. The quantities of PPZ, DPD and DRT were calculated by extrapolating the peak area from the calibration plot, shown in Table 4. The mean retention time (±SD) of PPZ, DPD and DRT were 2.64(±0.04), 6.01(±0.08) and 11.81(±0.09) min, respectively.

Table 4: Results from Assay of Tablet Formulation

Drug	Label claim (mg/tablet; n=9)	Amount found (mg)	Drug content (%)
PPZ	40	40.382	100.95
DPD	10	10.192	101.92
DRT	40	40.763	101.93

Stability in Simulated Gastric Fluid

When drug is introduced in the body, it experiences an environment with pH varying from 1.5 (stomach) to 7 (duodenum) to 8.0 (colon). So, to ascertain whether the introduced combination can withstand such pH variation; the combination was incubated with SGF (pH1.2) and it was found that DPD and DRT were quite stable while PPZ was degraded and produced some chromophoric degradants. Further to ascertain that these degradants peaks are of PPZ only; PPZ was incubated with SGF individually. Samples were withdrawn at time intervals and compared. The observed chromatograms are shown in the Figure 4.

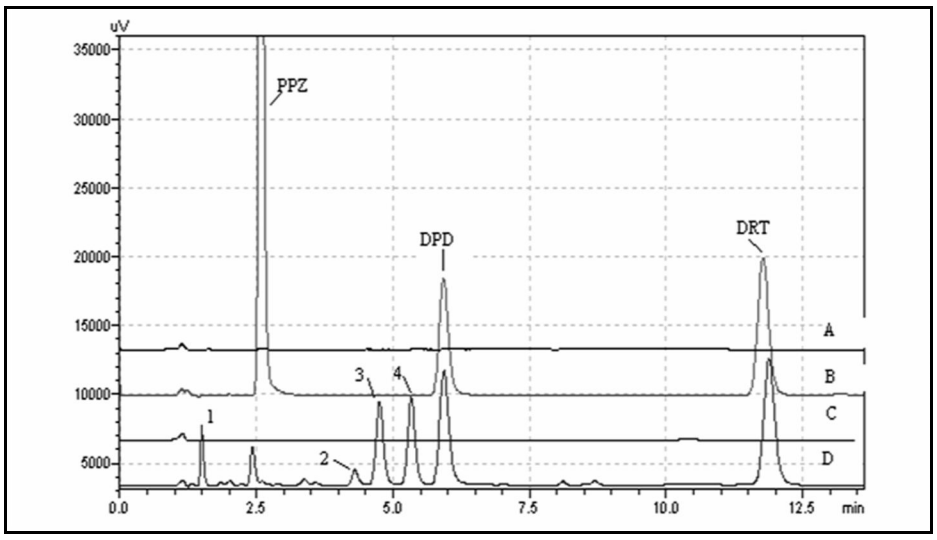

Figure 4: Overlay chromatograms of gastric stability experiments. A: Blank sample in mobile phase; B: Mixture formulation; C: Blank sample in SGF; D: Mixture in SGF.

Degradation Behavior of PPZ, DPD and DRT

According to International Conference on Harmonization (ICH) guidelines, forced degradation studies are mandatory during development of chromatographic procedures particularly when degraded products are unknown or not available.

Forced degradation studies were performed under stress conditions and resulting chromatograms are depicted in Figure 3. The percentage degradation of PPZ, DPD and DRT in various stress conditions are depicted in Figure 5. Pantoprazole has been found to be highly susceptible to low pH [26] and undergoes 95% degradation in SGF, forming degradation peaks 1, 2, 3 and 4 as shown in Figure 4. The possible PPZ degradation products, sulfenic acid or sulfonamide analogues (Figure 6) are being suggested based on the studies of Sivakumar et al. [10], Tutunji et al. [27]. Instability of pantoprazole in acidic environment was also evident from the results of acid degradation study of pantoprazole, showing 87% degradation with almost similar degradation peaks (Figure 3) as with SGF. Domperidone and drotaverine were more stable in acidic conditions, with 7% and 9% degradation respectively. In alkaline hydrolysis, all the three drugs showed a minimal degradation of 8%, 10% and 9%, respectively, without showing a peak. Neutral hydrolysis resulted in 55% and 30% degradation of pantoprazole and domperidone, respectively. However, drotaverine was quite stable and undergoes only a minimal degradation of 5%. Hence, it may be concluded that the stability of PPZ was pH dependent; with increased pH the rate of degradation decreased. In oxidative stress conditions; 90-95% degradation was observed in all the three drugs with degradation peaks at 1.9, 8.02 and 13.22 min for the mixture. To ascertain the origin of these peaks, individual drugs in similar conditions were analyzed. Degradation of drotaverine resulted in two peaks at 8.02 and 13.22 min. and their degradants; possibly drotaveraldine (DPI) and perparaldine (DPII) (Figure 6) have been identified based on the studies of Suganthi et al. [28]. The peak at 1.9 min was considered to be of pantoprazole. In case of pantoprazole, the rise in degradant peak area was in correspondence with the fall in parent peak, indicating that PPZ was decomposed to a chromophoric degradant. The oxidative degradants were possibly the sulphone or N-oxide analogues of PPZ as shown in Figure 6. The proposed degradant structures are based on the earlier report [29]. Photolytic degradation causes the degradation of PPZ, DPD and DRT to 77%, 38% and 60% respectively. PPZ was observed, with one major potential degradation product at 2.0 min and a cluster of minor degradation products are formed between 1.0- 2.5 min. (Figure 3).Dry heat degradation (thermal stress) results in a nominal degradation without generating any peak.

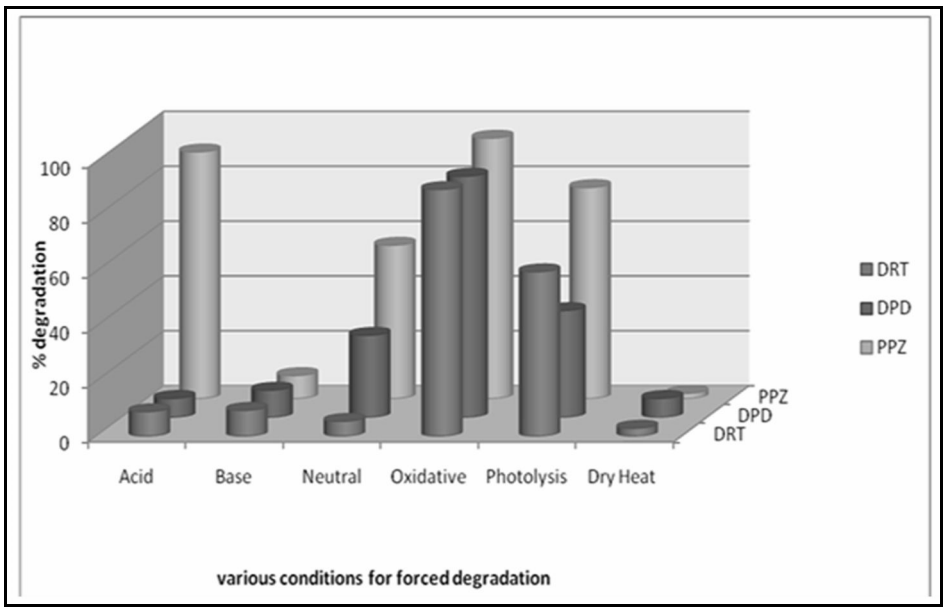

Figure 5: Degradation behavior of analytes under stressed conditions.

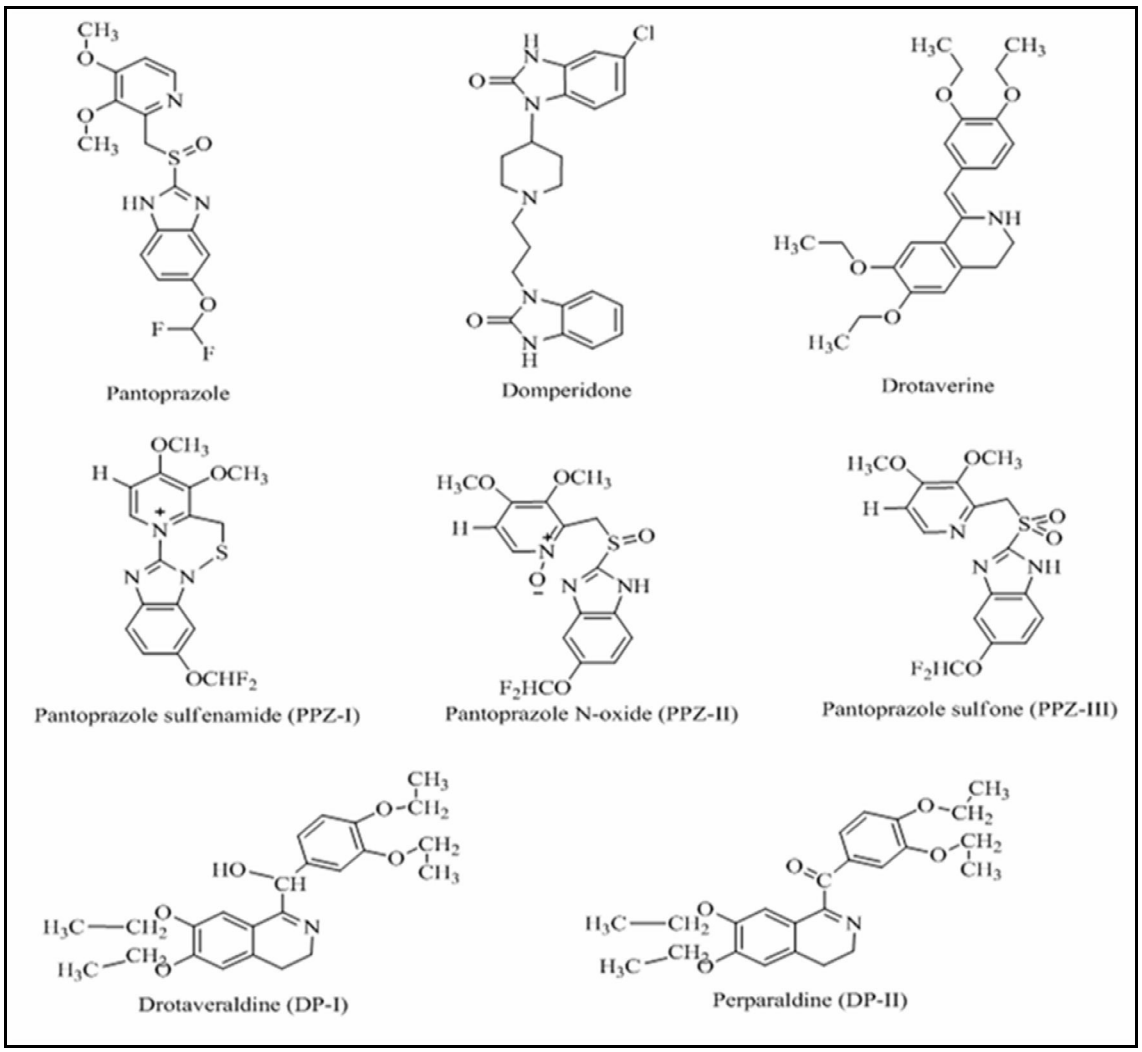

Figure 6: Chemical structures of analytes and possible degradation products.

Discussion

The aim of the study was to develop and validate an efficient stability-indicating RP-HPLC method for the simultaneous estimation of PPZ, DPD and DRT. Optimum chromatographic conditions were determined by varying column chemistry, solvent type, solvent strength (volume fraction of organic solvents in the mobile phase and pH of the buffer solution), detection wavelength and flow rate. After trying C18 columns of different particle size and lengths, the final choice of the column giving satisfactory resolution and runtime was the SD's Millenium C18 column (100 cm × 4.6 mm i.d., 5-µm) equipped with a guard column. A series of aqueous mobile phases, containing di-potassium hydrogen phosphate buffer solutions of different pH, in combination with methanol and acetonitrile, were also tested. The pH was not varied below 5 because pantoprazole is reported to be unstable at low pH [26]. The best result was obtained with di-potassium phosphate buffer of pH 7. The flow rate, determined by testing the effect of flow rate on peak area and resolution, was programmed as 1-7.5 min: 1 ml/min (λ 290nm); 7.70- 13.70 min: 2.5ml/min (λ 240nm); 14.00-15.00: 1ml/min (λ 290nm). The appropriate wavelength for simultaneous estimation of PPZ, DPD and DRT was determined by overlaying the UV spectra (in methanol and mobile phase). The proposed method was accurate (%RSD< 3%), precise and reproducible (%RSD< 3% for the intra-day and inter-day precision, thus confirming the method to be sufficiently precise).The specificity study revealed the absence of any undesired peak in the area of interest. Also, there was no extraneous peak present and eluted at the retention time of PPZ, DPD and DRT, when the tablet excipients and blank samples were analyzed. The linearity was observed by linear regression equation method and was established over the broad linear range of 0.25-16 µg/ml for PPZ & DRT (n=7) and 0.125-8 µg/ml for DPD (n=7).The difference between peak area of a particular drug as single and in combination was within the acceptable limit, showing that the drugs did not have any interaction in a mixture. The sample solutions were stable over the period of analysis (7 days); stability was assessed on 1st, 3rd and 7th day of analysis. The method offers the advantages of higher sensitivity **(**LOD was determined to be 0.01721µg/ml, 0.0115 µg/ml and 0.0212µg/ml for PPZ, DPD and DRT, respectively and LLOQ was determined to be 0.0573µg/ml, 0.0385µg/ml and 0.0706µg/ml for PPZ, DPD and DRT, respectively), decreased organic solvent consumption due to low retention time, and small sample volume (20µL). The results of analysis of marketed formulation of PPZ, DPD and DRT were 100.91, 101.92 and 101.95%, respectively and indicate that the method is selective for the routine analysis of PPZ, DPD and DRT in industry. Thus, the estimations of dosage forms were accurate and within the acceptance level. System suitability parameters indicate the adequacy of the proposed HPLC method for the routine analysis of PPZ, DPD and DRT in bulk and pharmaceutical dosage form. Capacity factor for PPZ, DPD and DRT was found to be 1.29, 4.23 and 9.49, respectively, indicating that the peaks are well resolved with respect to each other. Tailing factor, 1.23- 1.33, reflected good peak symmetry. High resolution values, 7.30, 13.71 and 17.10, among the peaks can be attributed to good separation. Higher number of theoretical plates, 3467.456, 5939.97 and 15069.12, indicated high column efficiency. RSD values less than 1.5%, expressed as %CV, indicated good injection repeatability.The proposed method could effectively separate the drugs from their degradation products, resulted from stress conditions. Moreover, the results of the forced degradation studies of each drug indicate a high degree of specificity of this method for PPZ, DPD and DRT. Stability in simulated gastric fluid (SGF) was also performed and results indicate that PPZ was susceptible to SGF and undergoes 95% degradation.The method was subsequently applied on the available marketed formulations of the drugs. The advantages offered by this method make it highly convenient for quantification of PPZ, DPD and DRT.

From the results, it can be concluded that an isocratic stability-indicating HPLC-UV method has been developed for the simultaneous estimation of PPZ, DPD and DRT .The proposed method is simple, accurate, precise, specific and has the ability to separate the drug from excipients found in the tablet dosage forms, so that it can be used as standard method for the simultaneous estimation of pantoprazole, domperidone and drotaverine in tablets/capsules using the HPLC system. Since the method was successfully applied for the estimation of selected drugs in bulk API powder or in tablet dosage forms as well; therefore this method can also be adopted for the study of pharmaceutical release patterns of the drugs while designing the new dosage forms. The results of analysis of the marketed dosage forms, by the proposed method are highly reproducible, reliable and are in good agreement with the label claims of the drugs. The method can be applied even to the analysis of stability samples obtained during accelerated stability experiments, as no interference was found under various stress conditions.

The results of the study indicate that the proposed combination was stable and the method could effectively estimate all the three drugs in a formulation. The proposed method may be applied to the analysis of samples obtained during extended accelerated stress degradation studies. Furthermore, several analytical data are required prior to the clinical trials of a drug combination. The method may be useful to estimate these drugs

in biological fluids with slight modification thereby assisting in their pharmacokinetic profile in the combination.

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References

1. Cho C.H., Koo M.W.L., Garg G.P., Ogle C.W., Stress-induced gastric ulceration: Its etiology and clinical implications, Scand J Gastroenterol., 1992,27,257-262.
2. Levenstein S., Kaplan G.A., Smith M.W., Psychological predictors of peptic ulcer incidence in the Alameda county study, J. Clin. Gastroenterol. , 1997, 24, 140-146.
3. Digestive Disorder Health Center. Understanding Ulcer, http:// www.webmed.com. (Accessed 13june 2013).
4. Salas M., Ward A., Caro J., Are proton pump inhibitors the first choice for acute treatment of gastric ulcer? A meta-analysis of randomized clinical trials, BMC Gastroenterol., 2002, 15, 2-17.
5. Blasko G., Pharmacology: A mechanism of action and clinical significance of a convenient antispasmodic agent: Drotaverine, J. Am. Med. India., 1998, 1, 63-9.
6. Zota K.R., Agrawal S., Zota K.C., Zota M.C., Zota H.M., A novel combined pharmaceutical composition containing diclofenac and methods of making and using the same, WO2013005226A, January 10, 2013.
7. American International Health Alliance **(**2002**)** Protocol for diagnosis and treatment of peptic ulcer in adults. WWW.aiha.com/en/resourcelibrary/products (accessed august 12, 2013).
8. Hardman J.G., Limbird L.E., Goodman and Gilman: The Pharmacological Basis of Therapeutics, Eleventh ed., McGraw-Hill: New York, 2006, 968.
9. Bharathi V., Hotha K.K., Jagadeesh B., Simultaneous estimation of four proton pump inhibitors-Lansoprazole, omeprazole, pantoprazole and rabeprazole: Development of a novel generic HPLC-UV method and its application to clinical pharmacokinetic study, Biomed. Chromatogr., 2009, 23,732-739.
10. Sivakumar T., Manavalan R., Valliappan K., Stability-indicating HPLC method for simultaneous determination of pantoprazole and domperidone from their combination drug product, Chromatographia, 2008, 67, 42.
11. Panigrahi D., Sharma R., Development and validation of an RP-HPLC method for simultaneous analysis of drotaverine and omeprazole in a tablet dosage form, Acta Chromatogr., 2008, 20, 439-450.
12. Rajmane V.S., Gandhi S.V., Patil U.P., Sengar M.H., Simultaneous determination of drotaverine hydrochloride and aceclofenac in tablet dosage form by spectrophotometry, Eurasian J. Anal. Chem., 2009, 4, 185.
13. Zhan L., Jing Y., Ziqiang Z., Luyong Z., Simultaneous determination of omeprazole and domperidone in dog plasma by LC-MS method, J. Chromatogr. Sci., 2009, 47, 881-884.
14. Zhiyong X., Xiaoyan C., Fengdan J., Dafang Z., Simultaneous determination of pantoprazole and its two metabolites in dog plasma by HPLC, J. Chromatogr. Sci., 2005, 43, 271-275.
15. Azhlwar S., Ravi T.K., Stability indicating HPLC method for simultaneous determination of drotaverine and aceclofenac, Int. J. Pharm. Pharm. Sci., 2011,3, 245-250.
16. Kumar R., Singh P., Singh H., Development and validation of RP-HPLC method for simultaneous estimation of naproxen and pantoprazole in pharmaceutical dosage form, Int. J. Pharm. Res. and Develop., 2011,2, 227-232.
17. Bharathi V., Hotha K.K., Jagadeesh B., Simultaneous estimation of four proton pump inhibitors-Lansoprazole, omeprazole, pantoprazole and rabeprazole: Development of a novel generic HPLC-UV method and its application to clinical pharmacokinetic study, Biomed. Chromatogr., 2009, 23,732-739.
18. Sivakumar T., Manavalan R., Valliappan K., Development and validation of a reversed- phase HPLC method for simultaneous determination of domperidone and pantoprazole in pharmaceutical dosage forms, Acta. Chromatogr., 2007, 18,130-142.
19. Dahivelkar P.P., Bari S.B., Bhoir S., Bhagwat A.M., High performance liquid chromatographic estimation of drotaverine hydrochloride and mefenamic acid in human plasma, Iranian J. Pharm. Res., 2009,8, 209-215.

20. Patel B., Patel M., Patel J., Suhagia B., Simultaneous determination of omeprazole and domperidone in capsules by RP-HPLC and densitometric HPTLC, J. Liq. Chromatogr. Rel. Tech., 2007, 30, 1749–1762.
21. Dahivelkar P.P., Mahajan V.K., Bari S.B., Shirkhedkar A.A., Fursule R.A., Surana S.J., Simultaneous derivative and multi-component spectrophotometric determination of drotaverine hydrochloride and mefenamic acid in tablets, Indian J. Pharm. Sci., 2007,69,812-814.
22. Ramakrishna N.V.S., Vishwottam K.N., Wishu S., Koteshwara M., High-performance liquid chromatography method for the quantification of pantoprazole in human plasma, J. Chromatogr. B, 2005,822, 326-329.
23. Kobylinska M., Kobylinska K., High performance liquid chromatographic analysis for the determination of domperidone in human plasma, J. Chromatogr. B, 2000,744, 207–212.
24. International conference on harmonization, ICH Guideline, Validation of analytical procedures technical requirements for registration of pharmaceuticals for human use: Text and methodology Q2 (R1), International Conference on Harmonization, Geneva, Switzerland, November 2005.
25. International conference on Harmonization, ICH Guideline, Validation of analytical procedures technical requirements for registration of pharmaceuticals for human use: Stability testing of new drug substances and products Q1A (R2), International Conference on Harmonization, USA, February 2003.
26. El-Sherif Z.A., Mohamed A.O., El-Bardicy M.G., El-Tarras M.F., Reversed-phase high performance liquid chromatographic method for the determination of lansoprazole, omeprazole and pantoprazole sodium sesquihydrate in presence of their acid-induced degradation products, Chem. Pharm. Bull., 2006,54,814-818.
27. Tutunji M.F., Qaisi A.M., El-Eswed B., Tutunji L.F., An *in vitro* investigation on acid catalyzed reactions of proton pump inhibitors in the absence of an electrophile, Int. J. Pharm., 2006, 323,110-116.
28. Azhlwar S., Ravi T.K., Stability indicating HPLC method for simultaneous determination of drotaverine and aceclofenac, Int. J. Pharm. Pharm. Sci., 2011,3,245-250.
29. Badwan A.A., Nabulsi L.N., Omari A.L., Daraghmeh M.M., Ashour M., Abdoh A.M., Jaber A.M., Pantoprazole sodium, in K. Florey (Ed), Analytical profiles of drug substances and excipients, Academic Press, Elsevier Science, New York, 2002, 213-259.

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