International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.8, pp 288-300, 2016

Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of NSAIDS-Antiulcer Agent Combination

Hemlata, Savita Upadhyay, Shailendra K. Saraf*

Division of Pharmaceutical Chemistry, Faculty of Pharmacy, Babu Banarasi Das Northern India Institute of Technology, Lucknow-226028, U.P., India.

Abstract : A specific, accurate, precise and reproducible stability-indicating HPLC method has been developed and subsequently validated for the simultaneous determination of Paracetamol (PCM), Pantoprazole (PPZ) and Ibuprofen (IBU) in pharmaceutical dosage forms. The separation was performed on a Rankem Princeton Spher-100, C_{18} (150×4.6mm), 100A, 5µm column using methanol: disodium hydrogen phosphate buffer (adjusted to pH 7 using orthophosphoric acid) in the ratio of 60:40 (v/v) as the mobile phase. The flow rate was adjusted to 1ml/min for PCM, PPZ and 1.5ml/min for IBU. Quantitation was achieved with UV detection at 222 nm, based on peak area with linear calibration curves, at seven concentration levels ranging from 1-64µg/ml for PCM, PPZ and IBU in individual as well as in combined dosage form. It demonstrated good linearity with r^2 >0.998 for all the drugs. The method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and robustness. The proposed method was successfully applied for the analysis of pharmaceutical formulations containing PCM, PPZ and IBU, in the presence of degradation products formed under various stress conditions, and no interference from the excipients was observed.
Keywords: RP-HPLC, Paracetamol, Pantoprazole, Ibuprofen, Stability-indicating method.

Introduction

Rheumatoid arthritis is a long–lasting chronic autoimmune disorder characterized by joint swelling, joint tenderness, and destruction of synovial joints, leading to severe disability and premature mortality. This may result in a low red blood cell count, inflammation around the lungs and the heart. Fever and low energy may also occur. The cause of rheumatoid arthritis is not very clear but it is believed to involve a combination of genetic and environmental factors. Family history is an important risk factor because it is strongly associated with the inherited tissue type major histocompatibility complex (MHC) antigen HLA-DRB1 and the gene PTPN22 and PAD14. The clinical manifestation of Rheumatoid Arthritis (RA) can be preceding by the presence of autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA)[1, 2, 3]. The inflammation and thickening of the joint capsule is due to the body's immune system attacking the joints. It also affects the underlying bone and cartilage. RA primarily affects joints, but it also affects other organs in more than 15-25% of individuals. People with RA are more prone to atherosclerosis, risk of myocardial infraction, stroke, fibrosis of the lungs and renal amyloidosis can occur as a consequence of chronic inflammation [4]. The goal of the treatment is to reduce pain, decrease inflammation, and improve a person's overall functioning and this may be helped by exercise, balancing rest and the use of splints and braces, pain medications, steroids, and NSAIDs[5].

Paracetamol N-(4-hydroxy phenyl acetamide) is widely used as an analgesic and antipyretic drug. It is well tolerated and lacks many of the side effects of aspirin. So, it is commonly used for the relief of fever, headaches, minor aches and pains as well as for the management of more severe pains[6]. Paracetamol cannot suppress the inflammation of rheumatoid arthritis because its mechanism of action is related to the selective inhibition of COX-2.The 2-arylpropionic acid derivative, Ibuprofen [RS-2-(4-isobutyl-phenyl) propionic acid], is one of the most potent oral antipyretic, analgesic and non-steroidal anti-inflammatory drug [NSAID] used extensively in the treatment of acute and chronic pain, osteoarthritis, rheumatoid arthritis and related conditions. Its side effects are gastrointestinal haemorrhage and ulceration[7]. The production of prostaglandins, prostacyclins, and thromboxanes from arachidonic acid is inhibited by NSAIDs by covalently modifying the enzyme cyclooxygenase (COX) and irreversibly inhibiting the ability of arachidonic acid to bind to the active site on the enzyme. Chronic administration of NSAIDs has been linked to ulcer disease[8]. Ibuprofen is indicated in the treatment of osteoarthritis and rheumatoid arthritis. Pantoprazole is a substituted benzimidazole with antiulcer activities, which acts as a proton pump inhibitor that suppresses gastric acid secretion by an interaction with H^+/K^+- ATPase in gastric parietal cells[9].

The combination of paracetamol and ibuprofen works effectively in relieving pain and inflammation related to arthritis. However, ibuprofen has a tendency to cause ulcer, and thus pantoprazole may be added in the combination to reduce the risk of ulceration. 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. A validated HPLC assay is mandatory for the development of such a type of new combination. Literature survey revealed that a few analytical methods for the analysis of these drugs had been reported, individually or with other combinations[10-22].However, there is no method reported for the simultaneous estimation of these drugs in combination.

The present study targets at the development and subsequent validation of a stability- indicating HPLC assay for the determination of pantoprazole, ibuprofen and paracetamol in combined dosage form as per International Conference on Harmonization (ICH) Guidelines[23, 24]. For the development of medicinal products of reliable quality and efficiency, recognized instability of constituents should be defined under ambient and biologically relevant conditions. The stability of a drug substance in gastric and intestinal fluids provides evidence whether the drug loss from the gastrointestinal tract takes place by intestinal permeation or by a degradation process in the gastrointestinal fluids prior to membrane absorption. Stability study in SGF and SIF gives an important consideration about the stability of drugs in different environments of the stomach, small intestine and colon. These fluids are the perfect media to determine the stability of drug candidates *in vitro*.

The stability indicating method exhibited excellent chromatographic performance and was simple, precise, accurate, selective, sensitive and showed good resolution. To validate the stability-indicating power of the developed analytical method, PPZ, IBU and PCM were subjected to forced degradation studies including the effect of hydrolysis (acidic, alkaline and neutral), oxidation, photolysis and dry heat. The knowledge of chemical behaviour can be used to improve a drug product during the pharmaceutical development, manufacturing, and packaging which are facilitated by the stress degradation studies.

Experimental

Paracetamol and pantoprazole sodium were procured as a gift sample from Jubilant Life Sciences and ibuprofen was obtained from BBDNIIT, Lucknow. Disodium hydrogen orthophosphate dihydrate (AR grade), and orthophosphoric acid (AR grade) were obtained from S.D. Fine-Chem Limited (Mumbai) and methanol (HPLC grade) was obtained from RFCL Limited (New Delhi). HPLC grade water was obtained from "MILLIPORE Direct Q3" water filter. The tablet formulations, Calpol (Paracetamol 500mg), Brufen (Ibuprofen 400mg) and Pan (Pantoprazole 40mg) were purchased from the local market. Chromatographic separation was performed on a Shimadzu Liquid Chromatographic System equipped with 20AD UFLC pump, UV/VIS detector and Rheodyne (7725 I) injector valve with 20µl fixed loop. LC-Solution was applied for data collecting and processing (Shimadzu, Japan). The Rankem Princeton Spher-100, C_{18} (150×4.6mm), 100A, 5µm column, equilibrated with mobile phase, i.e. methanol: phosphate buffer (adjusted to pH 7 using orthophosphoric acid) in the ratio of 60:40 v/v, was used. The flow rate was maintained at 1mL/min for PCM, PPZ and 1.5ml/min for IBU, with detection at 222nm. Analysis was performed at ambient temperature.

Preparation of Stock and Standard Solutions

Stock solution, at concentrations of 1000µg/ml each of PCM, PPZ and IBU were prepared separately in the mobile phase. The stock solutions were protected from light and stored in a refrigerator to avoid degradation. Aliquots of the stock solution were appropriately diluted with the mobile phase to yield standard solutions of 1-64µg/ml of PCM, PPZ and IBU, individually.

Sample Preparation for Tablet Assay

Ten tablets of Calpol-500, Pan-40 and Brufen-400 were accurately weighed and finely powdered separately. A quantity of powder equivalent to 500mg of paracetamol, 40mg of pantoprazole and 400mg of ibuprofen was transferred separately into 100 ml volumetric flasks and dissolved in the mobile phase. Solutions were centrifuged at 3000 rpm for 20 minutes. The supernatant was collected and further diluted with the mobile phase to get final concentration of 3-12µg/ml of PCM, PPZ and IBU in individual as well as in combined dosage form.

Forced Degradation and Stability Indicating Studies

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 samples for stability studies were prepared by dissolving each API, or drug product, in methanol and later diluted with distilled water, aqueous hydrochloric acid, aqueous sodium hydroxide or aqueous hydrogen peroxide solution at a concentration of 12µg/ml of PCM, PPZ and IBU, separately and in mixture. After degradation, these samples were diluted with the mobile phase to achieve the nominal concentration.

Acid Hydrolysis

The solutions for acid degradation studies were prepared in methanol and 0.1M HCl (5:25, v/v) at room temperature (25ºC). The resultant solutions were analyzed after 10 minutes.

Alkaline Hydrolysis

The solutions for alkaline hydrolysis studies were prepared in methanol and 0.1 M sodium hydroxide (5:25, *v/v*) at room temperature (25 °C). The resultant solutions were analyzed 10 min after preparation.

Neutral Hydrolysis

The solutions for neutral degradation studies were prepared in methanol and HPLC grade water (5:25, v/v) at room temperature (25ºC). 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 then analyzed.

Oxidation Studies

The solutions for oxidation studies were prepared in methanol and 3%H_2O_2 (5:25, v/v) at room temperature (25ºC). The samples were kept for 1 hr. and then analyzed.

Photo Stability Studies

Drug powders were exposed to UV light at a short wavelength for 8 hrs. The solutions for photo stability studies were prepared in the mobile phase at room temperature (25ºC). The samples were kept for 1 hr. and then analyzed.

Temperature Stress Studies

Drug powders were exposed to dry heat in an oven at 50ºC for 1hr. The solutions for temperature stress studies were prepared in the mobile phase at room temperature (25ºC). The samples were kept for 1 hr. and then analyzed.

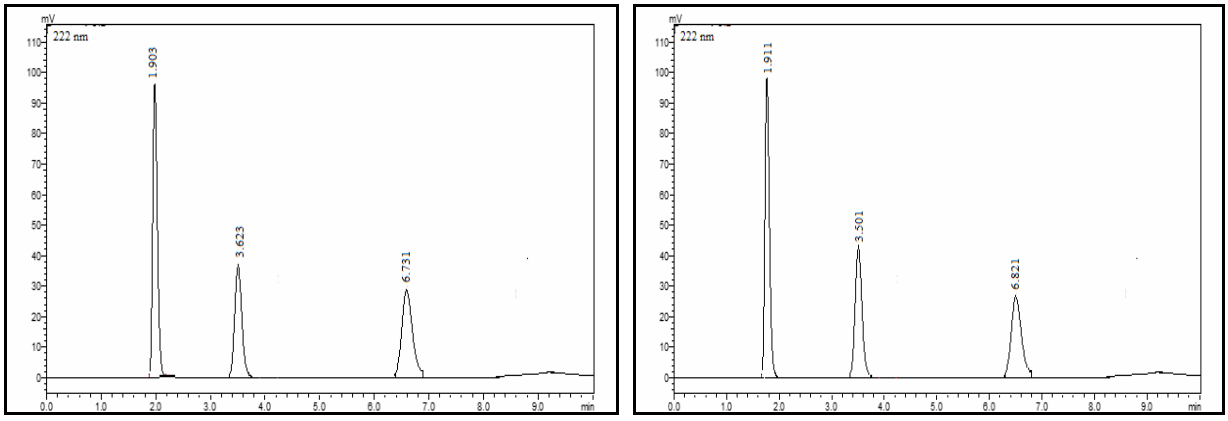
Stability Studies in Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) USP

In the present study, the proposed combination was tested *in vitro* using SGF and SIF. For this purpose, 30 mg of the mixture was spiked in 300 ml of SGF and SIF separately, and then the resulting stock test solution was placed in an incubator at 37ºC. Sampling was done at time intervals of 15 min for 1.5 hrs. The samples were withdrawn and diluted with the mobile phase to prepare the test solutions. These test solutions were analysed by the developed method. [25]

Results

Selection and Optimization of Chromatographic Conditions

The work was focussed on optimization of the conditions for simple, rapid, low cost and less time consuming analysis including the selection of proper column, mobile phase, wavelength, pH and flow rate to obtain satisfactory results. Preliminary studies involved trying different C18 Princeton reversed-phase columns (Figure 1b).Many mobile phases and their different proportions were tried at different pH and finally methanol: phosphate buffer (adjusted to pH 7 using orthophosphoric acid) in the ratio of 60:40 v/v was selected, which gave good resolution and acceptable system suitability parameters. With the optimized chromatographic conditions, a steady baseline was recorded. The retention time of PCM, PPZ and IBU were found to be 1.94, 3.64 and 6.75min, respectively. The flow rate was maintained at 1ml/min for PCM, PPZ and 1.5ml/min for IBU, with detection at 222nm.



(a) **(b)**

Figure1. (a) Chromatogram for the mixture of drugs at 222 nm; (b) Chromatogram for the mixture of drugs on Rankem Princeton Spher-100, C18, (150 x 4.6mm), 100A, 5µm

The level of each factor was studied for determining the optimum values and responses, which are summarized in Table 1. The central composite design can be applied to optimize the separation and to assist the development of better understanding of the interaction of several chromatographic factors on separation quality. The selection of factors for optimization was based on preliminary experiments, and prior knowledge from literature, as well as certain instrumental limitations.

Table 1. Method Development Conditions: Flow rate was programmed (1.0ml/min for 0.01-5.0 min), (1.5ml/min for 5.01-8.0 min).

Trial	Type of column	Mobile phase composition	Wavelength for PCM, PPZ, IBU	pH	Defect
1.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.025M Na_2HPO_4 Buffer: Acetonitrile (60:40)	247,293,222	7	More retention time, and less number of theoretical plates
2.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.025M Na_2HPO_4 Buffer: Acetonitrile (60:40)	247,293,222	6	More RT, Peak height very less
3.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	CH_3OH: Acetonitrile: 0.02M K_2HPO_4 Buffer(20:33:47)	247,293,222	7	More retention time, and less number of theoretical plates
4.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	CH_3OH: Acetonitrile: 0.02M K_2HPO_4 Buffer(20:33:47)	247,293,222	6	More RT
5.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.02M Na_2HPO_4 Buffer: CH_3OH (40:60)	247,293,222	7	Peak intensity not satisfactory
6.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.02M Na_2HPO_4 Buffer: CH_3OH (40:60)	247,293,222	6	Less peak intensity
7.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.025M Na_2HPO_4 Buffer: CH_3OH (40:60)	247,293,222	7	Well resolved with satisfactory peak intensity
8.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.025M Na_2HPO_4 Buffer: CH_3OH (40:60)	220,220,210	7	More RT in case of IBU
9.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.025M Na_2HPO_4 Buffer: CH_3OH (40:60)	256,256,220	7	Low peak intensity and increased RT in case of IBU
10.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.025M Na_2HPO_4 Buffer: CH_3OH (40:60)	260,260,256	7	Low peak intensity and increased RT in case of IBU

11.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.025M Na_2HPO_4 Buffer: CH_3OH (40:60)	270,270,270	7	Low peak intensity and increased RT in case of IBU
12.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.025M Na_2HPO_4 Buffer: CH_3OH (40:60)	293,293,293	7	IBU peak was not observed till 20 min.
13.	VARIAN (250×4.6 mm), C18, 5µm.Lot no. 27-31-60	0.025M Na_2HPO_4 Buffer: CH_3OH (40:60)	222	7	Resolution was not good
14.	RANKEM PRINCETON SPHER- 100, C18, (150 x 4.6mm), 100A5µm	0.025M Na_2HPO_4 Buffer: CH_3OH (40:60)	222	7	Less RT, more theoretical plates, less tailing, symmetrical peak shape, good resolution

Method Validation

The quality control (QC) samples were prepared at three concentration ranges of 3µg/ml, 12µg/ml and 48µg/ml for PCM, PPZ and IBU individually and in the mixture. The developed stability-indicating method was validated according to ICH guidelines. The validation parameters addressed were linearity, accuracy, precision, specificity, selectivity, limit of detection, limit of quantitation and robustness.

Linearity

The linearity of the method was determined at seven concentration levels ranging from 1-64 µg/ml for PCM (n=7), PPZ (n=7) and IBU (n=7), in individual as well as in the combined dosage form. The calibration curve was constructed by plotting response factor against concentration of the drugs. The slope and intercept value for calibration curve was y=71285x + 133977 (r^2=0.998) for PCM, y=108690x + 188113 (r^2=0.998) for PPZ and y= 43034x + 57336 (r^2=0.999) for IBU, respectively.

Accuracy/ Recovery

Accuracy of the method was observed by recovery result, accurately spiked with different concentrations (3concentration x 3 replicate's = 9 determinations) of the active ingredients, in accordance with the ICH guidelines. Results from the accuracy study are reported in Table 2.

Table2. Results of Recovery Studies

Drug	Amount Taken (µg/ml)	Amount Recovered (µg/ml)	% Recovery	%Covarience(CV)
PCM	3	2.89	96.43	2.82
	12	12.10	100.83	0.62
	48	48.03	100.06	0.44
PPZ	3	3.07	102.33	0.95
	12	11.89	99.12	1.25
	48	47.92	99.83	1.52
IBU	3	2.89	96.33	0.64
	12	12.67	105.58	0.26
	48	48.05	100.10	0.19

Precision

The precision was evaluated by inter-day and intra-day variation studies, at three concentration levels for each compound. For intra-day studies, injections of standard and sample solutions were made. The mean, S.D, and %CV of peak area were calculated. For inter-day variation studies, injections of standard and sample solutions were made for three consecutive days. The mean, S.D. and %CV of peak area were calculated. The data obtained from experiments are tabulated in Table 3.

Table 3. Results of Precision Studies

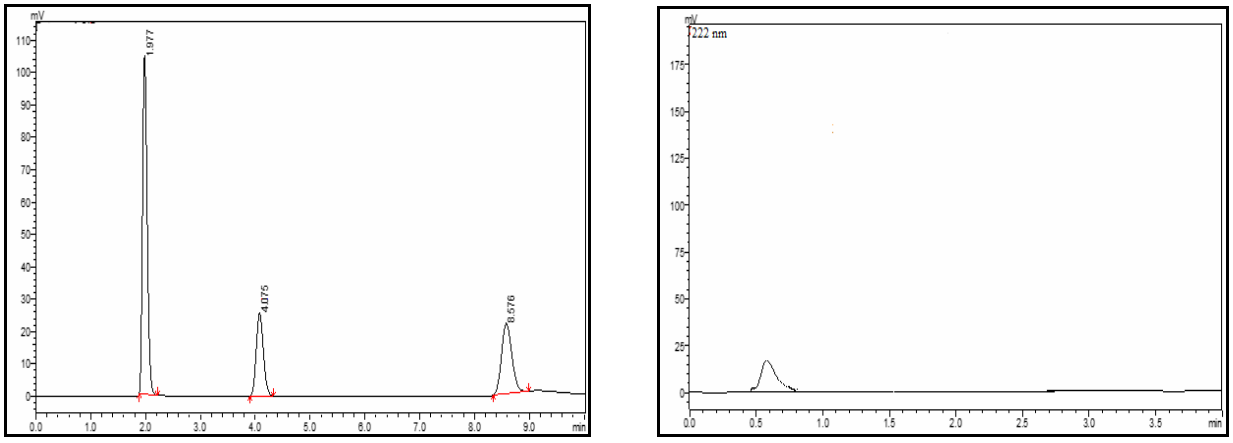
Drug	Actual conc. (µg/ml)	Intra-day Precision (n=3)			Inter-day Precision (n=9)					
		Mean	Std. Dev.	%CV	Area (mV)			Mean	Std. Dev.	%CV
					1st day	3rd day	7th day			
PCM	3	31346.7	1357	1.33	31521.23	31510.91	31508.23	31590.01	210	0.66
	12	92827.67	136	0.15	92567.4	92477.3	92785.4	92589.40	206	0.22
	48	375141.2	2150	0.57	381348.2	383456.2	383786.2	381357.20	5383	1.41
PPZ	3	60907.1	533	0.88	53325.83	53545.83	53893.83	53319.83	9680	1.16
	12	174457.9	403	0.23	171565.1	175488.1	178896.1	171356.10	3997	2.33
	48	667409.9	695	0.11	655666.2	658802.2	655896.2	655567.20	26229	2.01
IBU	3	19594.4	154	0.79	18125.44	18225.44	18345.44	18105.44	1649	2.11
	12	73901.8	69	0.09	68638.50	68785.26	68797.56	68855.56	5887	1.55
	48	308240.2	224	0.07	276745.5	275856.9	275853.9	276856.50	31093	1.23

Robustness

The robustness/ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters such as variations of pH in a mobile phase, variations in mobile phase composition, different columns (different lots and/or suppliers), temperature, flow rate etc. and provides an indication of its reliability and evaluates the system suitability parameters during normal usage.

Specificity and Selectivity

The interference between peaks was investigated by the analysis of blank samples from the other compounds, i.e. PCM, PPZ and IBU. Under the proposed chromatographic conditions PCM,PPZ, and IBU were completely separated from each other as depicted in Figure 2.The chromatogram obtained from stressed and untreated samples are shown in Figure 3.



(a) **(b)**

Figure2. (a) Chromatogram of the mixture of drugs; (b) Chromatogram of mobile phase (blank sample)

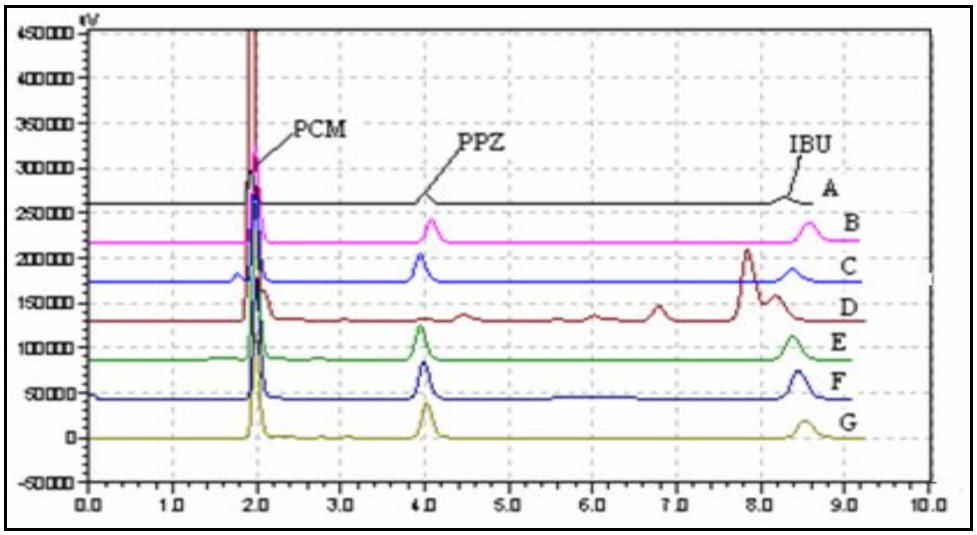


Figure 3. Representative chromatograms of analytes obtained under stress conditions. A.Untreated sample; B. Acid hydrolysis; C. Alkaline hydrolysis; D. Neutral hydrolysis; E. Oxidative degradation; F. Dry heat degradation; G. Photo degradation

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ for PCM, PPZ and IBU were determined according to ICH guideline Q_2B. LOD and LOQ were calculated on the basis of standard deviation of the response and slope using the formula:

LOD= Standard Deviation x 3.3/slope

LOQ= Standard Deviation x 10/slope

System Suitability Parameters

System suitability parameters ensure the working of the system in a proper way during the analysis. The values obtained demonstrated the suitability of the system for the analysis of these drugs, in individual as well as in a combined dosage form. The results are presented in Table 4.

Table4. System Suitability Parameters

Parameters	PCM	PPZ	IBU
Regression equation Y= mx+c; Slope; Intercept	y=71285x+133977; 71285; 133977	y=43034x+57336; 43034; 57336	y=108690x+188113; 108690; 188113
Correlation coefficient(r^2)	0.998	0.999	0.998
Retention time	1.93	6.78	3.62
Theoretical plate number	125.10	507.83	112.28
% Recovery (Accuracy)	100.83	105.58	99.12
% RSD (Precision)	0.147	0.09	0.23
Tailing factor	1.0	1.2	1.1
Capacity factor	1.36	7.38	3.50
LOD (µg/ml)	0.077	0.182	0.182
LOQ (µg/ml)	0.114	0.386	0.198
Resolution factor	2.45	6.14	4.93

Y= mx+c; m: slope, c: y axis intercept.

Stability of Sample Solution

In order to demonstrate the stability of samples, the solutions were analyzed over a period of at least two weeks when stored refrigerated at 4°C. Solutions containing PCM, PPZ and IBU (concentration range = 1

to 64µg/ml) were prepared in HPLC grade methanol and injected three times (n = 3), in individual as well as in a combined form.

Assay of Marketed Tablet Formulation

The percentage of individual drugs found in formulations was calculated and are presented in Table 5. The results of analysis showed that the estimated quantity of drugs were in good agreement with the label claim.

Table5. Results of Tablet Analysis

Drug	Label claim (mg/tablet; n=9)	Amount found (mg)	Drug content (%)
PCM	500	502.16	102.16
PPZ	40	40.34	100.95
IBU	400	400.12	101.20

Stability Study in Gastrointestinal Fluids on Drugs in Combination

In freshly prepared SGF, it was observed that there was 25 % degradation in paracetamol within 1.5 hr of incubation. Paracetamol was found to be stable with slight reduction in drug peak area and peak height. PPZ was found to be labile. Approximately, 78 % of the initial amount of IBU was degraded. IBU was unstable with a slight decrease in drug peak area and peak height. Degradation products were obtained at a retention time of 5.66 min and 9.83 min for PPZ and IBU, respectively. The identity of PCM, PPZ and IBU were confirmed by comparison with authentic standards, as shown in Figure 4. In SIF, PPZ and IBU were found to be labile. The identity of PCM, PPZ and IBU were confirmed by comparison with an authentic standard, as shown in Figure 5.

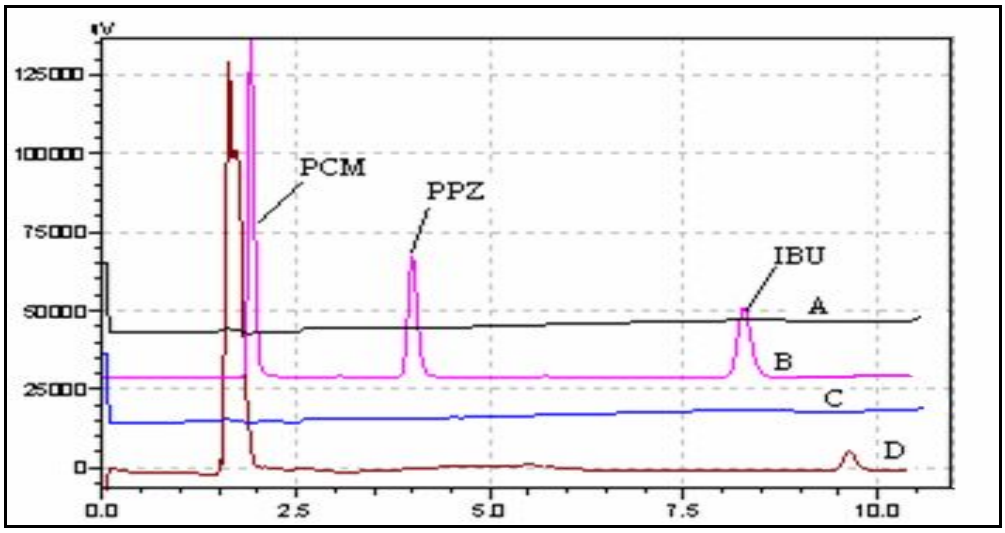


Figure 4. Overlay chromatogram of simulated gastric fluid studies. A: Blank sample (Mobile phase); B: Mixture formulation; C: Blank sample (SGF); D: Mixture in SGF

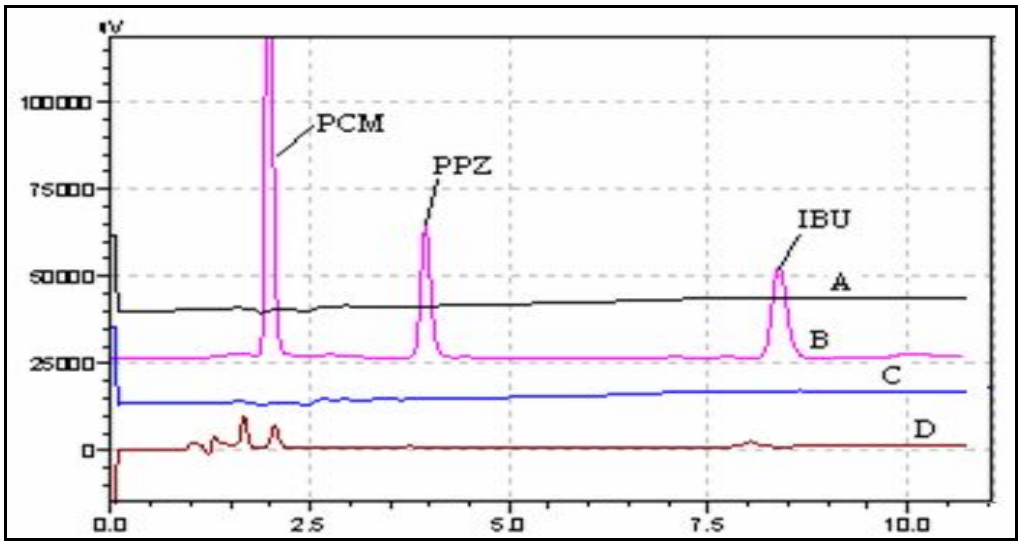


Figure5. Overlay chromatogram of simulated intestinal fluid studies. A: Blank sample (Mobile phase); B: Mixture formulation; C: Blank sample (SIF); D: Mixture in SIF

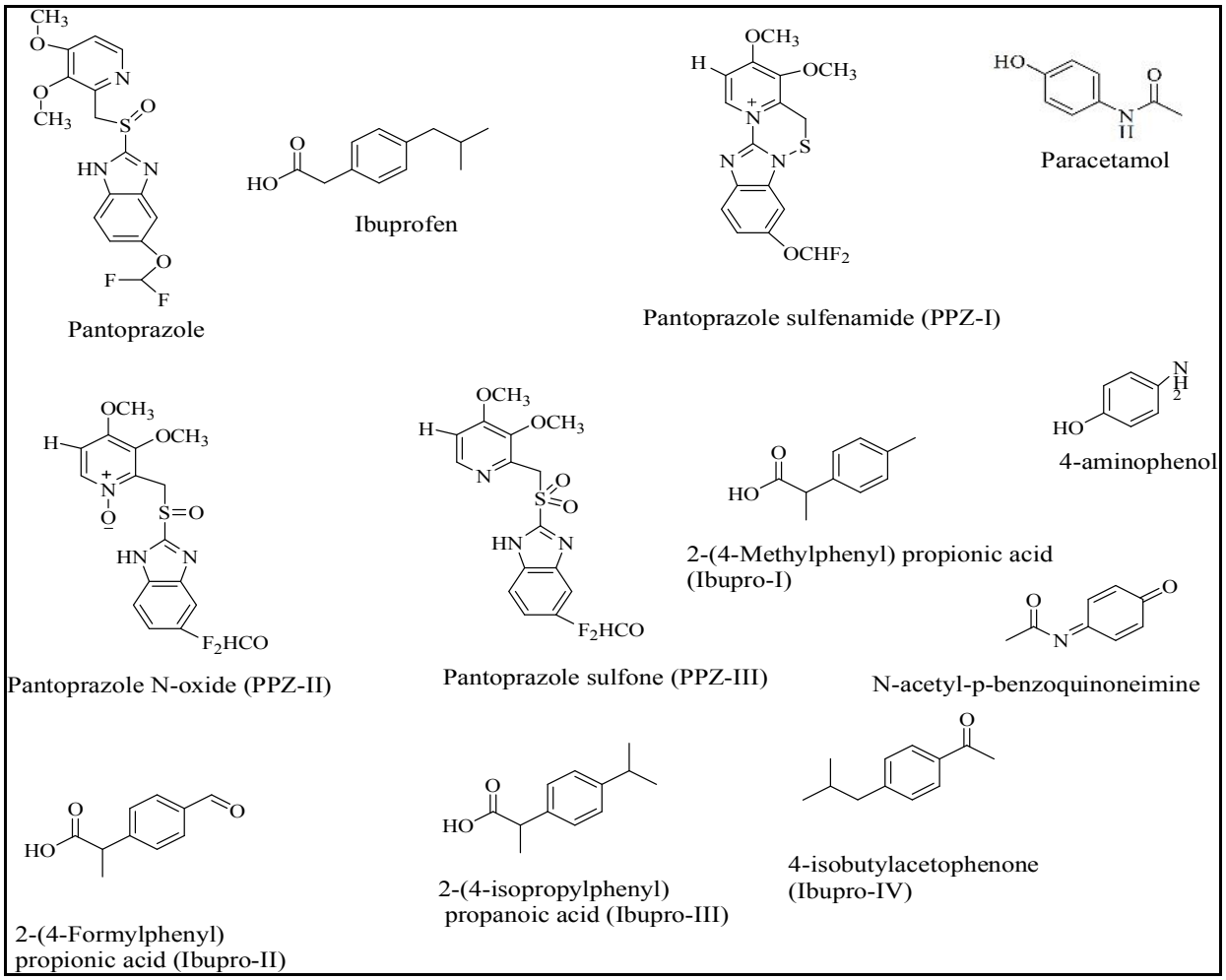


Figure 6. Chemical structures of analytes and possible degradation products

Degradation behaviour

Forced degradation studies of PCM, PPZ and IBU were carried out under various stress conditions and the extents of degradation of the analytes are depicted in Figure 3. PCM was found to be stable under acidic and alkali stress conditions, with slight decrease in drug peak area. PCM undergoes 20.91% decomposition under

acidic condition and 20.78% decomposition under alkaline condition. The possible structures of PCM acid degradant and alkali degradant, 4-amino phenol (Fig.5.12 a), are suggested based on the studies of Meda *et al*.[26] However, this drug was sufficiently stable under neutral condition, resulting in 20.64% degradation. Under oxidation stress, almost 10.48% of PCM was degraded, forming an oxidative degradant, i.e. N-acetyl-p-benzoquinoneimine (Figure 6). PCM was stable in both, solution and solid state, under UV light exposure as well as thermal stress conditions. A 25.79% decomposition of PCM was observed with minor degradation product, possibly 4-amino phenol (Figure 6) by thermal reaction[27]. In photo degradation studies, almost 10.10% of PCM was degraded. PPZ was highly susceptible to low pH and underwent 69.69% decomposition under acidic stress condition. The possible structure of PPZ acid degradants, sulfenic acid or sulfenamide analogues (Figure 6), are suggested based on the studies of Tutunji *et al*. and Qaisi *et al*.[28, 29]. Further, PPZ was sufficiently stable under basic conditions, resulting in 25.83% degradation. Hence, it was found that the stability of PPZ was pH dependent; the rate of degradation decreased with an increase in pH[30]. Under oxidation stress, almost 19.99% of PPZ was degraded. The oxidative degradants, possibly the sulphone of PPZ (Figure 6) or N-oxide analogues of PPZ (Figure 6), formed by the oxidation reaction of sulfinyl moiety or pyridine nitrogen, were lacking any therapeutic effect. Under thermal stress condition, PPZ was moderately stable showing 25.278% degradation. When PPZ was exposed to UV light at short wavelength, 20.21% degradation was observed. IBU was stable under acidic stress condition, resulting in 25.27% degradation. The structures of IBU acid degradants, 2-(4-formyl phenyl) propionic acid (Figure 6) or 2-(4-methyl phenyl) propionic acid (Figure 6) or 4-isobutylacetophenone (Figure 6), are suggested based on the studies of Velagaleti *et al*.[31] The drug was sufficiently stable under basic and neutral conditions, resulting in 20.53% and 25.59% degradation, respectively. Possible structures of IBU alkali degradant and neutral degradant, 2-(4-isopropyl phenyl) propionic acid (Figure 6), are suggested based on the studies of Dantu *et al*.[32] In contrast, IBU was relatively stable at all degradation conditions, resulting in 10.82%, 10.79% and 10.71% degradation under oxidation, thermal and photolytic stress conditions respectively. The possible structure was 4-isobutylacetophenone (Figure 6), based on the studies of Velagaleti *et al*.[31], for oxidation, thermal and photolytic degradants, respectively.

Discussion

The aim of this work was developing and validating a simple, specific, accurate, precise and stability-indicating HPLC method for the simultaneous estimation of PCM, PPZ and IBU in pharmaceutical dosage forms. Preliminary studies involved trying different C_{18} Princeton reversed-phase columns. Many mobile phases and their different proportions were tried at different pH and finally methanol: phosphate buffer (adjusted to pH 7 using orthophosphoric acid) in the ratio of 60:40 v/v was selected, which gave good resolution and acceptable system suitability parameters. With the optimized chromatographic conditions, a steady baseline was recorded. The retention time of PCM, PPZ and IBU were found to be 1.93, 3.64 and 6.74min, respectively. The flow rate was maintained at 1ml/min for PCM, 1ml/min for PPZ and 1.5ml/min for IBU with detection at 222nm. System suitability parameters indicated the adequacy of the proposed HPLC method for the routine analysis of PCM, PPZ and IBU in bulk and pharmaceutical dosage forms. The values of capacity factor for PCM, PPZ and IBU indicated that the peaks were well resolved with respect to each other. Tailing factor reflected good peak symmetry. The resolution values among the peaks could be attributed to good separation. Higher number of theoretical plates indicated high column efficiency. RSD values less than 1.0%, expressed as % CV, indicated good injection repeatability. 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 peaks in the area of interest. Also, there was no extraneous peak present and eluted at the retention time of PCM, PPZ and IBU, when the tablet excipients and blank samples were analyzed. The linearity results showed that an excellent correlation existed between response factor and concentration of drugs within the concentration range, showing that the drugs did not have any interaction in the 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 high sensitivity, less organic solvent consumption due to low retention time, and small sample volume (20µL). The results of analysis of marketed formulations of PCM, PPZ and IBU showed that the method was selective for the routine analysis of PCM, PPZ and IBU in the industry. Thus, the estimations of dosage forms were accurate and were within the acceptance level.

Conclusion

In this work, a simple, sensitive, accurate, linear, precise, reproducible, repeatable, specific and robust stability-indicating RP-HPLC method was established for the determination of PCM, PPZ and IBU, in the presence of their degradants. The behaviour of PCM, PPZ and IBU under different stress conditions was studied. The possible degradation in the gastrointestinal tract was checked using SGF and SIF studies. The proposed method may be applied to the analysis of samples obtained during extended accelerated stress degradation studies. Several analytical data are required prior to the clinical trials of a drug combination. The method may be slightly modified to estimate these drugs in biological fluids, thereby assisting in their pharmacokinetic profile in the combination.

Acknowledgments

The authors are thankful to All India Council for Technical Education (AICTE, MODROBS Scheme) for the grant provided for instrumentation facilities and Dr. R.C. Gupta, Ex. Scientist G & Head, Pharmacokinetic and Metabolism Division, CDRI for his guidance and valuable suggestions.

References

1. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, Birnbaum NS., *et al*. 2010 Rheumatoid arthritis classification criteria. Arthritis & Rheumatism.,2010,62,2569-2581.
2. Aho K, Heliovaara M, Maatela J, Tuomi T, Palusuo T. Rheumatoid factors antedating clinical rheumatoid arthritis. J Rheumatol., 1991,18,1282-1284.
3. Rantapaa-Dahlqvist S, De Jong BA, Berglin E, Hallmans G, Wadell G, Stenlund H, *et al*. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum.,2003,48, 2741-2749.
4. Majithia V, Geraci SA. Rheumatoid arthritis: diagnosis and management. Am. J. Med., 2007,120,936-939.
5. Efthimiou P, Kukar M. Complementary and alternative medicine use in rheumatoid arthritis: proposed mechanism of action and efficacy of commonly used modalities. Rheumatology International., 2010, 30, 571-586.
6. Graham G, Scott K. Mechanism of action of Paracetamol. Am. J. Ther., 2005, 12, 46-55.
7. Gowekar NM, Wadher SJ. Simultaneous estimation of formoterol fumarate dihydrate and fluticasone propionate dry powder inhalation formulation by RP-HPLC.; International Journal of PharmTech Research., 2016, 9, 164-170.
8. Roberts S, Mc Donald JM. Inhibitors of Gastric acid Secretion. In Burger's Medicinal Chemistry & Drug Discovery. Edited by Donald J. Abraham, 6th ed. Virginia, A John Wiley and Sons, Inc; 1998, 91-92.
9. Gorle AP, Shinde JS. Development and validation of stability indicating assay method of ofloxacin in bulk and pharmaceutical dosage form by RP-HPLC.; International Journal of PharmTech Research., 2016, 9, 289-298.
10. Mishra PK, Upadhyay S, Tripathi AC, Saraf SK. Stability Indicating HPLC-UV Method for Simultaneous Estimation of Pantoprazole ,Domperidone and Drotaverine.; International Journal of PharmTech Research.,2015,8, 912-923.
11. Ahirao PK, Pawar RP. Simultaneous quantitation of Famotidine and Ibuprofen in pharmaceutical dosage by using validated stability indicating LC method. Res. J. Pharmaceutical Sci., 2013, 2(4), 1-9.
12. Ghosh S, Venkatesh S, Ravikumar BVV. Development of stability indicating RP-HPLC method and validation for the estimation of vilazodone hydrochloride.; International Journal of PharmTech Research., 2015, 7, 204-211.
13. Schaich M., Kioefer B., Leigh, M., Dissolution test results showing biorelevant media made from SIF powder original are the same as media prepared using methylene chloride. Bio. Relevent. Com., 2013, 1(3), 1-14.
14. Sareen SMD, Sinaga SM, Muchlisyam. Development method for determination of ternary mixture of paracetamol, Ibuprofen and caffeine in tablet dosage form using zero- crossing derivative spectrophotometric.; International Journal of PharmTech Research., 2015, 7, 349-353.

15. Gnana RM, Geetha G, Sangaranarayanan A. Simultaneous stability indicating method development and validation for related compounds of Ibuprofen and Paracetamol tablets by RP-HPLC method. J. Chromat Separation Techniq.,2012, 3 (8), 1-5.
16. Rele RV. Validation method of oxolamine citrate from bulk drug and pharmaceutical formulation by High Performance Liquid Chromatography. International Journal of PharmTech Research., 2015, 7, 549-553.
17. Bajaj S, Singla D, Sakhuja N. Stability testing of pharmaceutical products. J. App. Pharm. Sci.,2012, 02 (03), 129-138.
18. Tsvetkova GB, Pencheva PI, Zlatkov BA, Plamen TP. Development and validation of RP-HPLC for simultaneous determination of Ibuprofen and Paracetamol in fixed dose combinations. Int. J. Pharm. Sci. Rev.Res.,2012, 16(1), 13-16.
19. Madhukar A, Kannappan N. RP-HPLC method for the simultaneous estimation of cilnidipine and metoprolol succinate in bulk and tablet dosage form in biorelevant media (FaSSIF).; International Journal of PharmTech Research., 2015, 7, 172-184.
20. Bharathi V, Hotha KK, 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.
21. Kaushita B, Lekhya Priya C, Bhaskara Rao KV, HPLC analysis and antioxidant activities of hydroethanolic leaf extract of Kaempferia galangal Linn.; International Journal of PharmTech Research., 2015, 7, 422-431.
22. Martindale, The complete drug reference, 33rd edition., Pharmaceutical Press: London, 2002, 541.
23. International Conference on Harmonization, ICH Guideline, Stability testing of new drug substances and products Q1A (R2), International Conference on Harmonization, Geneva, 2003.
24. Sherje AP, Londhe V. Stability indicating HPLC method for determination of paliperidone in bulk. International Journal of PharmTech Research., 2015, 8,157-163.
25. Krishna M, Nadre M, Sherikar AV, Reddy R. Stability indicating analytical method validation for determination of related substances by RP-HPLC for phenytoin sodium in phenytoin sodium capsule.; International Journal of PharmTech Research., 2015, 8, 78-87.
26. Meda H, Chitra KP, Bhimavarapu R, Kanikanti D. Forced Degradation Study of Paracetamol in Tablet Formulation Using RP-HPLC. Bulletin. Pharm. Res., 2011, 1(3), 13-17.
27. Sherif ZA, Mohamad AO, Bardiey MG, Tarras MF. Reversed-phase high performance liquid chromatographic method for the determination of Pantoprazole sodium sesquihydrate, Omeprazole and Lansoprazole in presence of their acid-induced degradation products. Chem. Pharm. Bull., 2006, 54, 814-818.
28. Tutunji MF., Qaisi AM, Eswed B, Tutunji LF. Stability-indicating HPLC method for the simultaneous determination of pantoprazole, rabeprazole, lansoprazole and domperidone from their combination dosage forms. Int. J. Pharm., 2006, 323, 110-116.
29. Qaisi AM, Tutunji MF, Tutunji LF. Acid decomposition of omeprazole in the absence of thiol: A differential pulse polarographic study at the static mercury drop electrode (SMDE). J. Pharm. Sci., 2006, 95, 384-391.
30. Bhadwan AA, Nabulsi LN, Omari MM, Daraghmeg NH, Ashour MK, Abdoh AM, Jaber AMY. Pantoprazole sodium: Analytical profile of drug substances and excipients. Elsevier Sci., 2002, 213-259.
31. Velagaleti R, Burns P, Anderson P, Farmer S.; Forced degradation of ibuprofen in bulk drug and tablets. Pharm. Tech., 2002, 28-42.
32. Rao DD., Shakil SS., Mukkanti K., Development and validation of an UPLC method for rapid determination of ibuprofen and diphenhydramine citrate in the presence of impurities in combined dosage form, J. Chromatogr. Sci., 2011, 49, 281-286.

*****