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Research Article

# STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DOMPERIDONE AND LAFUTIDINE IN BULK AND THE PHARMACEUTICAL DOSAGE FORM

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**ABSTRACT**

A stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Domperidone and Lafutidine in bulk and pharmaceutical dosage form. Chromatography was carried on Thermo Hypersil BDS $C_{18}$ (250mm x 4.6i.d, 5µm) column with mobile phase comprising of dipotassium hydrogen phosphate (0.1M) buffer and methanol in the ratio 60:40 v/v. The flow rate was adjusted to 1.0ml/min with UV detection at 280nm. The retention times of Domperidone, Lafutidine were found to be 1.813 min, 8.949 min respectively. The different analytical parameters such as accuracy, linearity, precision, robustness, limit of detection(LOD), limit of quantification (LOQ) were determined according to the International Conference on Harmonization (ICH) Q2B guidelines. The detector response was linear in the range of 180-540µg/ml, 60-180µg/ml for Domperidone and Lafutidine, respectively. In the linearity study, the regression equation and coefficient of correlation for Domperidone and Lafutidine, were found to be (y =16040x+1400, $r^2$ = 1),(y = 22541x+3490, $r^2$ = 1) respectively. The proposed method is highly sensitive, precise and accurate & hence was successfully applied for the reliable quantification of active pharmaceuticals present in the commercial formulations. Degradation products produced as a result of stress studies did not interfere with the detection of Domperidone and Lafutidine and the assay can thus be stability-indicating.

**Keywords:** Domperidone, Lafutidine, RP-HPLC, Simultaneous estimation, Stability- indicating method.

## INTRODUCTION

**Domperidone (DOM)** is a dopamine antagonist with antiemetic properties. It stimulates gastro-intestinal motility and is used as an antiemetic for the short-term treatment of nausea and vomiting. DOM is known chemically as 5-chloro-1-[1-[3-(2, 3-dihydro-2-oxo-1H-benzimidazol-1-yl) propyl]-4-piperidinyl]-1,3-dihydro-2H-benzimidazol-2-one.

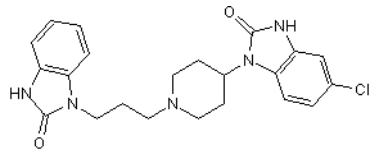


**Fig. 1: Domperidone (DOM) chemical structure**

**Lafutidine (LAF)** is a second generation histamine $H_2$- receptor antagonist used as anti-ulcerative agent. Antisecretory drugs are used in the treatment and prophylaxis of peptic ulcer disease, some are also employed in other disorders associated with gastric hyperacidity such as gastro-oesophageal reflux disease (GERD) and dyspepsia. They may be divided into Histamine $H_2$- receptor antagonists ($H_2$- antagonists), which act by blocking Histamine $H_2$- receptors on gastric parietal cells, thereby antagonizing the normal stimulatory effect of endogenous histamine on gastric acid production. It is indicated in hyperacidity, NSAID induced gastritis, gastric and duodenal ulcers and also used as pre-anaesthetic medication. Apart from $H_2$- receptor blockade activity, it has additional gastro protective action. Therefore not only inhibit acid secretion but also provide gastric mucosal protection. LAF is known chemically as 2-(furan-2-ylmethylsulphinyl)-N-[(Z)-4-[4-(piperidinyl-methyl)-pyridin-2-yl)oxybut-2-enyl]acetamide.

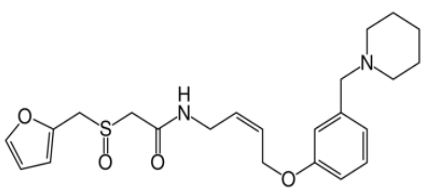


**Fig. 2: Lafutidine (LAF) chemical structure**

Several analytical procedures have been proposed for the quantitative estimation of Lafutidine separately and in combination with other drugs. HPLC and UV methods for estimation of Lafutidine alone in pharmaceutical preparation have been reported. Domperidone in combination with Lansaprazole, Pantaprazole are also available.

None of the reported analytical methods describe a simple, economical and stability-indicating RP-HPLC method for the simultaneous determination of DOM & LAF in presence of their degradation products. The objective of this work was to develop and validate an economic stability-indicating, rapid reversed-phase high performance liquid chromatographic method for the quality control of Domperidone and Lafutidine in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time. The method was validated and found to be accurate, precise and reproducible.

## MATERIAL AND METHODS

### Apparatus

Waters e2695 Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC.

Other Apparatus: Electronic Balance, Sonicator, 0.45µ membrane filter.

Pharmaceutical grade Domperidone and Lafutidine were kindly supplied as a gift sample by Dr. Reddys Laboratory, Hyderabad, Andhra Pradesh, India. Methanol was of HPLC grade and collected from E. Merck, Darmstadt, Germany. Dipotassium hydrogen Phosphate were analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO purification system.

### Commercial Formulation

Domperidone and Lafutidine tablets available in the market as Lafudac-D in composition of Lafutidine (10mg), Domperidone (30mg). The samples were properly checked for their manufacturing

license numbers, batch numbers, production, expiry dates and stored properly.

**Preparation and Selection of Mobile phase**

The preliminary isocratic studies on a reverse phase $C_{18}$ column with different mobile phase combination of Dipotassium hydrogen phosphate buffer and methanol were studied for simultaneous estimation of both drugs. The optimal composition of mobile phase determined to be Buffer: Methanol (60:40 v/v) and filtered through 0.45µ membrane filter.

**Preparation of standard solution**

18mg Domperidone and 6mg Lafutidine was dissolved in 50ml of Diluent (methanol) to get stock solution of Domperidone (360µg/ml) and Lafutidine (120µg/ml). This is taken as a 100% concentration. Solution containing mixture of Domperidone and Lafutidine of five different concentrations (50%, 75%, 100%, 125% and 150% of target concentration) were prepared in the same way.

**Preparation of Sample solution**

Sample solution containing both the drugs was prepared by dissolving tablet powder into Diluent (methanol). Ten tablets were weighed separately. Their average weights were determined. Powder of tablets equivalent to three tablets weight were weighed and taken in a 100ml volumetric flask, dissolved in diluents and shaken and sonicated for about 10 minutes, then filtered through 0.45µ membrane filter. The filtered solution was further diluted in the diluents to make the final concentration of working sample equivalent to 100% of target concentration.

**Chromatographic Conditions**

The mobile phase, a mixture of Dipotassium hydrogen phosphate buffer and methanol (60:40 v/v) pumped at a flow rate of 1.0ml/min through the column ($C_{18}$; 5µ, 4.6 x 250 mm, Thermo Hypersil BDS) at 45° C. The mobile phase was degassed prior to use under vacuum by filtration through a 0.45µ membrane filter. Both drugs showed good absorbance at 280 nm, which was selected as wavelength for further analysis.

**Forced Degradation studies**

The pharmaceuticals Lafudac-D capsules containing Lafutidine-10mg with Domperidone-30mg were subjected to various forced degradation conditions to effect the partial degradation of the drug preferably in the range 20-80%. 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 drug product in methanol and later diluted with either distilled water, hydrochloric acid, sodium hydroxide or hydrogen peroxide solution at a concentration of 360µg/ml Domperidone, 120µg/ml Lafutidine .

**Acidic degradation studies**

Powder of tablets equivalent to three tablets weight were weighed and taken in a 100ml volumetric flask. To it 10ml of 0.1N HCl was added and sonicated for 5 minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. The sample solution was neutralized using 10ml of 0.1N NaOH and diluted up to the mark with Diluent (methanol). Mixed well and filter through 0.45µm filter. The filtered solution was further diluted in the diluents to make the final concentration of working sample equivalent to 100% of target concentration and injected into HPLC system.

**Base degradation studies**

Powder of tablets equivalent to three tablets weight were weighed and taken in a 100ml volumetric flask. To it 10ml of 0.1N NaOH was added and sonicated for 5 minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. The sample solution was neutralized using 10ml of 0.1N HCl and diluted up to the mark with Diluent (methanol). Mixed well and filter through 0.45µm filter. The filtered solution was further diluted in the diluents to make the final concentration of working sample equivalent to 100% of target concentration and injected into HPLC system.

**Neutral degradation studies**

Powder of tablets equivalent to three tablets weight were weighed and taken in a 100ml volumetric flask. To it 10ml of water was added and sonicated for 5 minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. The sample solution was diluted up to the mark with diluent (methanol). Mixed well and filter through 0.45µm filter. The filtered solution was further diluted in the diluents to make the final concentration of working sample equivalent to 100% of target concentration and injected into HPLC system.

**Peroxide degradation**

Powder of tablets equivalent to three tablets weight were weighed and taken in a 100ml volumetric flask. To it 10ml of 3% Hydrogen peroxide ($H_2O_2$) was added and sonicated for 5 minutes. Refluxed under heat at 60 degrees in a heating mantle for 2 hours. The sample solution was diluted up to the mark with Diluent (methanol). Mixed well and filter through 0.45µm filter. The filtered solution was further diluted in the diluents to make the final concentration of working sample equivalent to 100% of target concentration and injected into HPLC system.

**Photo-stability studies**

Powder of tablets equivalent to three tablets weight were weighed and taken in a 100ml volumetric flask. To it 10ml of methanol was added and sonicated for 5 minutes. The resultant solution was exposed to natural sunlight during the daytime for 8 hrs. The sample solution was diluted up to the mark with Diluent (methanol). Mixed well and filter through 0.45µm filter. The filtered solution was further diluted in the diluents to make the final concentration of working sample equivalent to 100% of target concentration and injected into HPLC system.

**Thermo degradation**

Powder of tablets equivalent to three tablets weight were weighed and taken in a 100ml volumetric flask and kept in oven under heat at 105 degrees for about 8 hrs. Then the sample solution was diluted up to the mark with Diluent (methanol). Mixed well and filter through 0.45µm filter. The filtered solution was further diluted in the diluents to make the final concentration of working sample equivalent to 100% of target concentration and injected into HPLC system.

**Development and validation of HPLC method**

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of Domperidone and Lafutidine in tablet dosage form. The experiment was carried out according to the official specifications of USP-30, ICH-1996 and Global Quality Guidelines-2002. The method was validated for the parameters like system suitability, specificity, linearity, precision, accuracy, LOD, LOQ and robustness.

**System Suitability**

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Domperidone and Lafutidine. Various chromatographic parameters such as retention time, peak area, tailing factor, theoretical plates of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

**Specificity**

Specificity test determines the effect of excipients on the assay result. To determine the specificity of the method, standard sample of Domperidone and Lafutidine were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

**Linearity**

Linearity of the method was determined by constructing calibration curves.

Standard solutions of Domperidone and Lafutidine of different concentrations level (50%, 75%, 100%, 125% and 150%) were used for this purpose. Each measurement was carried out in 6 replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.

**Accuracy (Recovery Studies)**

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Domperidone and Lafutidine were added to pre- analyzed samples and were subjected to the proposed HPLC method.

**Precision**

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

**Robustness of Method**

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and ± 5ºC change in temperature.

**RESULTS AND DISCUSSION**

Results of system suitability are summarized in Table 1. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

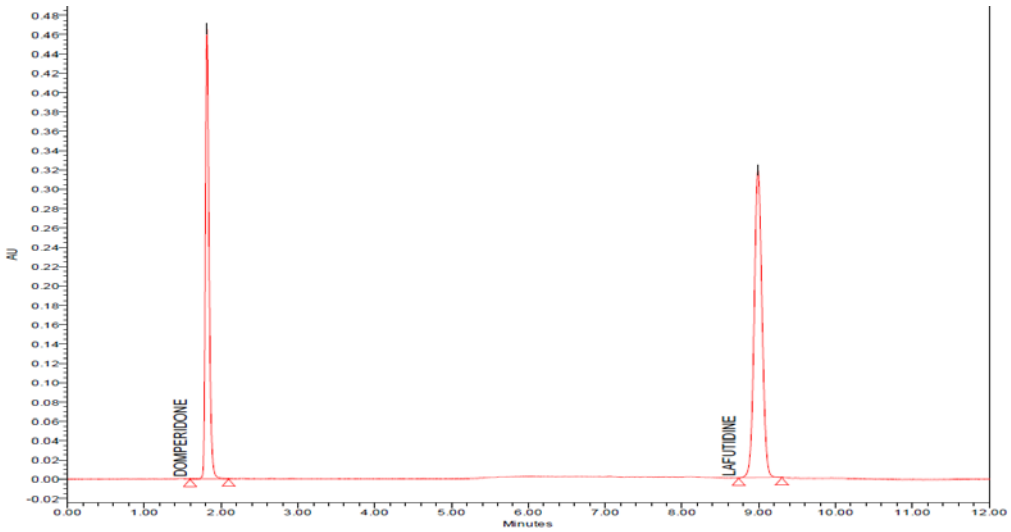


**Fig. 3: Typical chromatogram of Domperidone and Lafutidine in marketed formulation.**

| Name | Retention time | Area | USP Tailing | USP Plate Count |
|---|---|---|---|---|
| Domperidone | 1.815 | 1606674 | 1.325 | 7178 |
| Lafutidine | 8.990 | 2255970 | 1.054 | 37719 |

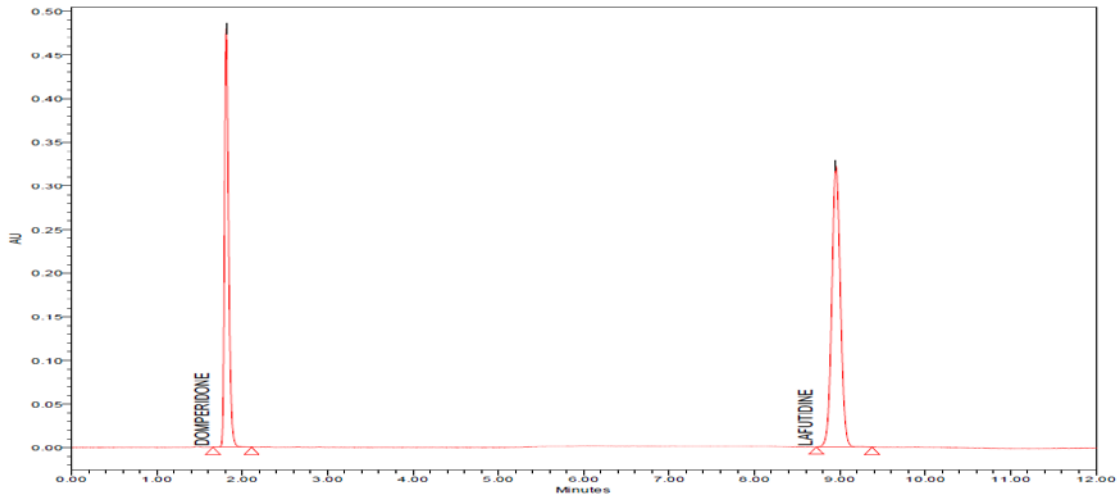


**Fig. 4: Typical Chromatogram of standard Domperidone and Lafutidine**

| Name | Retention time | Area | USP Tailing | USP Count | s/n |
|---|---|---|---|---|---|
| Domperidone | 1.812 | 1608403 | 1.356 | 7064 | 971.192 |
| Lafutidine | 8.953 | 2274219 | 1.053 | 37735 | 652.431 |

**Table 1: Result of system suitability tests of Domperidone and Lafutidine**

| Parameters | Domperidone | Lafutidine |
|---|---|---|
| Linearity range | 180-540 µg/ml | 60-180 µg/ml |
| Correlation coefficient | 1 | 1 |
| Slope | 16040x+1400 | 22541x+3490 |
| Retention time | 1.812 | 8.953 |
| USP plate count | 7064 | 37735 |
| Tailing factor | 1.356 | 1.053 |
| Limit of Detection (LOD) | 30.78ng/ml | 79.66ng/ml |
| Limit of quantification(LOQ) | 93.27ng/ml | 241.39ng/ml |

Chromatograms shown in figure 3 and figure 4 explain that retention time for standard sample and commercial product of Domperidone and Lafutidine are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective. A linear relationship between peak areas (average peak areas of six replicates) versus concentrations was observed for Domperidone and Lafutidine in the range of 50% to 150% of nominal concentration. Correlation coefficient was 1 for Domperidone and 1 for Lafutidine which prove that the method is linear. Calibration curve of Domperidone and Lafutidine are shown in Fig 3 and 4.

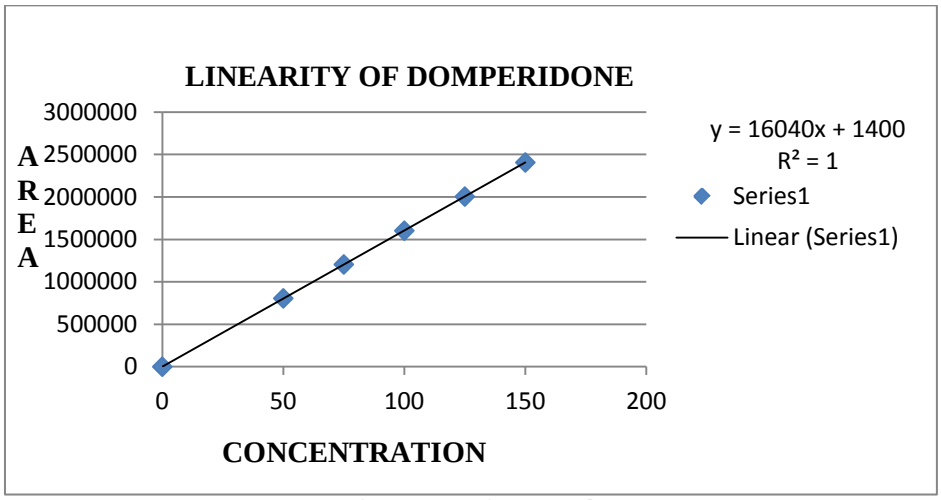


**Fig. 5: Linearity of Domperidone**

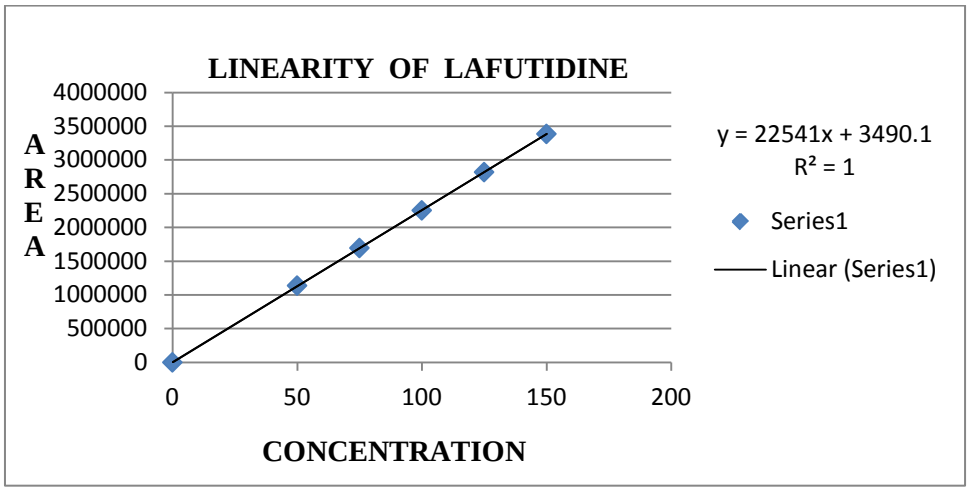


**Fig. 6: Linearity of Lafutidine**

**Table 2: Intraday and inter day precision result of Domperidone and Lafutidine**

| Drug | %RSD (Intra day) | % RSD (inter-day) |
|---|---|---|
| Domperidone | 0.169 | 0.138 |
| Lafutidine | 0.123 | 0.2037 |

**Table 3: Accuracy (% recovery) results of Domperidone and Lafutidine**

| Sample No | Domperidone | | | |
|---|---|---|---|---|
| | Spiked Amount(mg) | Recovered Amount (mg) | % Recovered | % Average Recovery |
| 1 | 9 mg | 9.09 mg | 101 | 100.33% |
| 2 | 18 mg | 18 mg | 100 | |
| 3 | 27 mg | 27 mg | 100 | |
| | | **Lafutidine** | | |
| 1 | 3 mg | 3 mg | 100 | 99.3 % |
| 2 | 6 mg | 5.94 mg | 99 | |
| 3 | 9 mg | 8.91 mg | 99 | |

Results of Intraday and inter day variability were summarized in table 2. Intraday variability was done from 9.00 am to 6.00 pm on the same day. % RSD of peak areas was calculated for various run. The method is highly precise as % RSD of peak area was less than 1% in all tests.

Results of accuracy study are presented in table 3. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 100.33% for Domperidone and 99.33% for Lafutidine. All the results indicate that the method is highly accurate. The results of robustness of the present method showed that small changes were made in the flow rate and temperature did not produce significant changes in analytical results which are not significant, we can say that the method is robust.

**Degradation behavior**

The results of stress studies indicated that the specificity of the method has been developed. Domperidone undergoes 8.8% degradation in acidic media, 8.18% degradation in basic media, 8.02% degradation in peroxide, 7.26% thermal degradation, with little degradation in sunlight and water. Thus, the compound is more acid-labile.

Lafutidine undergoes 11.65% degradation in acidic media, 16.32% degradation in basic media, 16.05% degradation in peroxide, with little degradation in water, sunlight and in dry heat. Thus, the compound undergoes maximum degradation in 3% $H_2O_2$ and 0.1N NaOH. Results of forced degradation studies were presented in table 5.

**Table 4: Results for robustness test of Domperidone and Lafutidine**

| Parameters count | Changes | RT | USP Tailing | USP Plate |
|---|---|---|---|---|
| | | **Domperidone** | | |
| Flow rate (ml/min) | 0.8 | 2.262 | 1.347 | 7562 |
| | 1.2 | 1.509 | 1.330 | 6853 |
| Temperature | $40^0$ C | 1.815 | 1.340 | 7058 |
| | $50^0$ C | 1.816 | 1.330 | 7049 |
| | | **Lafutidine** | | |
| Flow rate (ml/min) | 0.8 | 10.37 | 1.063 | 35980 |
| | 1.2 | 8.069 | 1.049 | 40323 |
| Temperature | $40^0$ C | 9.069 | 1.050 | 36339 |
| | $50^0$ C | 8.973 | 1.064 | 38084 |

**Table 5: Results of forced degradation studies**

| Stress condition | Time(hours) | % Assay of active substance | |
|---|---|---|---|
| | | DOM | LAF |
| Acid hydrolysis ( 0.1N HCl) | 2 | 91.19 | 88.35 |
| Base hydrolysis (0.1N NaOH) | 2 | 91.82 | 83.68 |
| Neutral hydrolysis (water) | 2 | 94.15 | 94.66 |
| Oxidation (3% $H_2O_2$) | 2 | 91.98 | 83.95 |
| Photolysis (direct sunlight) | 8 | 98.21 | 94.15 |
| Thermal degradation ( $105^0$ C) | 8 | 92.74 | 93.19 |

**CONCLUSION**

The new HPLC method developed and validated for simultaneous estimation of Domperidone and Lafutidine pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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