STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LANSOPRAZOLE AND DOMPERIDONE IN BULK AND ITS PHARMACUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT
A simple, selective, linear, precise, and accurate stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Lansoprazole and Domperidone from bulk and formulations. Chromatographic separation was achieved isocratically on a Waters C8 column (150×4.6 mm, 3.9 μ particle size) using a mobile phase, (OPA: Methanol) pH adjusted to 2.2 with orthophosphoric acid in the ratio of 80:20 v/v. The flow rate was 1ml/min and effluent was detected at 235nm. The retention time of Lansoprazole and Domperidone were 1.4 and 3.1 min respectively. Linearity was observed in the concentration range of 50-150μg/ml for Lansoprazole and Domperidone. Percent recoveries obtained for both the drugs were 100.00%. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, LOD and LOQ. The method developed was successfully applied for the analysis of simultaneous estimation of Lansoprazole and Domperidone capsules. Lansoprazole and Domperidone were exposed to acid, base, peroxide, thermal and photolytic stress conditions and the stressed samples were analyzed by proposed method.

KEYWORDS: Lansoprazole, Domperidone, Methanol, Phosphate buffer, orthophosphoric acid.
INTRODUCTION

Lansoprazole is chemically 2 - [[3-methyl-4-(2, 2, 2 - trifluoroethoxy) pyridin-2-yl] methyl sulfinyl]-1H-benzoimidazole and it is a proton pump inhibitor. Its active metabolite is absorbed with cytosine group in H+/K+ ATP ASE there by inhibiting the ability of parietal cells to produce gastric acid. It is used in the treatment of gastro-oesophageal reflux disease (GERD), peptic ulcer and Zollinger-Ellison syndrome. The structure of lansoprazole is shown in Figure 1.

Domperidone is chemically 5- chloro-1- [1- [3- (2-oxo-2, 3-dihydro-1H-benzimidazol-1-yl) propyl]- piperidin-4-yl]- 1, 3- dihydro- 2H benzimidazol- 2- one and it is used as gastrointestinal emptying (delayed) adjunct, a peristaltic stimulant, and also as an antiemetic and dopaminergic blocking agent. The structure of domperidone is shown in Figure 2.

MATERIALS AND METHODS

Chemicals: A commercial Capsule formulation Lancas-D from Casca Remedies Pvt Ltd, (Ambala Cantt, Haryana, India) containing 30mg of LAN and 10mg of DOM was obtained as a gift sample from Casca Remedies Pvt Ltd, (Ambala Cantt, Haryana, India) and used within their shelf life period. HPLC grade methanol was obtained from Merck Limited. Analytical grade Dipotassium Phosphate buffer was obtained from SD Fine (Mumbai, India). HPLC grade water was obtained by distilling deionizer water produced by a Milli-Q Millipore water system (Milford, MA, USA). All other chemicals used were of pharmaceutical or analytical grade.
Chromatographic conditions
The HPLC system (LC Waters, Milford, MA, USA) consisted of quaternary gradient system (600 Controller), in-line degasser (Waters, model AF), photodiode array detector (Water, 2998 model) and auto sampler (Waters). Data was processed using Empower2 software (Waters, Milford, MA, USA). Isocratic elution of the mobile phase OPA: Methanol pH adjusted to 2.2 with orthophosphoric acid in the ratio of 80:20 v/v. with the flow rate of 1 ml/min. Separation was performed on a particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material.

Integration of the detector output was performed using the Waters Empower software to determine the peak area. The contents of the mobile phase were filtered through a 0.45μm membrane filter and degassed by sonication before use. Mobile phase was used as diluents. The flow rate of the mobile phase was optimized to 1 ml/min which yields a column back pressure of 110–112 kg/cm² The run time was set at 6 min and a column temperature was maintained at 30°C. The volume of injection was 5μl, prior to injection of the analyte, the column was equilibrated for 30 with the mobile phase. The eluent was detected at 235 nm. The developed method was validated in terms of specificity, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness as per ICH guidelines. [17]

Preparation of standard stock solutions of lansoprazole and domperidone
Accurately weigh and transfer 60mg Lansoprazole and 20mg Domperidone working standard into a 100ml clean dry volumetric flask and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 5ml of Lansoprazole and Domperidone of above stock solution into a 25ml volumetric flask and dilute upto the mark with HPLC grade water. Fill it in two vials label it as STD1 and STD2.

Preparation of test Solution (Analysis of lansoprazole and domperidone in capsules)
Sample solution containing both the drugs was prepared by dissolving capsule pellets into Diluent. Accurately weighed capsule pellets were taken in a 100ml volumetric flask, dissolved in diluent and shaken and sonicated for about 30 minutes then filtered through 0.45μ membrane filter. The filtered solution was further diluted in the diluent to make the final concentration of working sample equivalent to 100% of target concentration.
RESULTS AND DISCUSSION
The present research work was designed at developing a rapid, sensitive, precise and accurate HPLC method for the simultaneous estimation of lansoprazole and domperidone in pharmaceutical dosage forms. In order to affect analysis of the component peaks under isocratic conditions, mixtures of water and methanol in different combinations with different pH were tested as mobile phase on a Waters C8 stationary phase. A binary mixture of OPA: methanol pH adjusted to 2.2 with orthophosphoric acid in the ratio of 80:20 v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and free from tailing. A flow rate of 1.0 ml/min of the mobile phase was found to be suitable.

METHOD DEVELOPMENT
After various trials, the following chromatographic conditions were finally optimized for the simultaneous estimation of lansoprazole and domperidone in a capsule dosage form. Mobile phase constitutes of OPA: Methanol pH adjusted to 2.2 with the ratio of 80:20 v/v. Detection wave length 235nm flow rate1.0 ml/min, after a steady baseline the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas were found and finally 120μg/ml of the standard solution of the individual samples of lansoprazole and domperidone and mixed standard solutions were injected and the chromatograms were recorded.

Figure 3: Typical chromatogram of lansoprazole and domperidone with detection at 235 nm.

The separation of Lansoprazole and Domperidone with retention times of 1.4 and 3.1min respectively. The typical chromatograms of the standard solutions were recorded for the repeatability and the respective chromatogram was given in Figure 3.
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<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Description/Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stationary Phase</td>
<td>Water’s C8 (150X4.6X3.9)</td>
</tr>
<tr>
<td>2</td>
<td>Mobile Phase</td>
<td>OPA (pH 2.2) and methanol in the ratio of 80:20 v/v</td>
</tr>
<tr>
<td>3</td>
<td>Flow rate</td>
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<tr>
<td>4</td>
<td>Detection Wavelength</td>
<td>235</td>
</tr>
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<td>5</td>
<td>Detector</td>
<td>Photo diode array</td>
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<tr>
<td>6</td>
<td>Injection</td>
<td>Auto sampler -Waters</td>
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<td>7</td>
<td>Rt’s</td>
<td>Lansoprazole – 1.4 Min Domperidone – 3.1 Min</td>
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<tr>
<td>8</td>
<td>Injection volume</td>
<td>5μl</td>
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<tr>
<td>9</td>
<td>Column Temperature</td>
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Method validation

After development of method, validation of the stability indicating method for simultaneous estimation of lansoprazole and domperidone was performed in accordance with ICH guidelines (International Conference on Harmonization (ICH) 2000) which include System suitability, Accuracy, Precision, Specificity, Linearity, LOD and LOQ, and Robustness.

Linearity

Calibration graphs were constructed by plotting peak area vs. concentration of lansoprazole and domperidone and the regression equations were calculated. The calibration graphs were plotted over 5 different linear concentrations in the range of 50-150 μg/ml for all the drugs. Aliquots (20 ml) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n = 6)]. The method was found linear over the concentration range of 50-150 μg/ml for lansoprazole and domperidone. Linearity curves of lansoprazole and domperidone were shown in figure 4 & 5 respectively.

![Fig 4: standard calibration curve for lansoprazole.](image-url)
Fig 5: standard calibration curve for domperidone.

Accuracy
The accuracy of the method was established by recovery studies i.e., external standard addition method. The known amount of standard was added at three different levels to pre-analyzed sample. Each determination was performed in triplicate. The mean recoveries obtained were 100.00% for lansoprazole and domperidone. The results of accuracy were tabulated in table 2.

Precision
The intraday and inter-day precision of the proposed method was determined by analyzing mixed standard solution of lansoprazole and domperidone at concentration 100 μg/ml 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation. The % RSD values for lansoprazole and domperidone were found to be 0.11% and 0.10% respectively.

Limit of detection (LOD) and limit of quantitation (LOQ)
The limit of detection (LOD) and limit of quantitation (LOQ) of lansoprazole and domperidone were determined by calculating the signal-to-noise (S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines. LOD values for lansoprazole and domperidone were found to be 0.07 ng/ml and 0.14 ng/ml respectively. LOQ values for lansoprazole and domperidone were found to be 0.33 ng/ml and 0.66 ng/ml respectively.

Robustness
The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical conditions like flow rate (0.1 ml min⁻¹), and pH of the
mobile phase (± 0.2). Stability of standard and test solution (prepared from the dosage form) was established by storage at 25 °C and 15 °C for 48 h. During the storage period, the test solutions were reanalyzed at intervals of 6, 12, 24, 36 and 48 h and assay was determined against appropriate fresh standard preparations.
TABLE 2: Assay of capsule dosage form

<table>
<thead>
<tr>
<th>SAMPLE NO</th>
<th>SAMPLE WEIGHT</th>
<th>LANSOPRAZOLE</th>
<th>DOMPERIDONE</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% ASSAY-1</td>
<td>% ASSAY-2</td>
</tr>
<tr>
<td>1</td>
<td>572.00</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>572.00</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>572.00</td>
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<td>100</td>
</tr>
<tr>
<td>6</td>
<td>572.00</td>
<td>99</td>
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</tr>
</tbody>
</table>

AVERAGE ASSAY: 99% 100 %

STD: 0.11 0.10

% RSD: 0.11 0.10

Forced degradation study

The pharmaceuticals Lancas-D capsules containing Lansoprazole-30mg with Domperidone 10mg 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.

Degradation behaviour

The results of stress studies indicated that the specificity of the method has been developed. Domperidone undergoes 5% degradation in acidic media, 7% degradation in basic media, 2% degradation in peroxide, 5% thermal degradation, with 8% degradation in sunlight. Thus, the compound is more acid-labile. Lansoprazole undergoes 5% degradation in acidic media, 9% degradation in basic media, 9% degradation in peroxide, 3% thermal degradation, with 10% degradation in sunlight. Results of forced degradation studies were presented in table 3.

TABLE 3: Degradation data.

<table>
<thead>
<tr>
<th>Nature of the Sample</th>
<th>Sample Weight</th>
<th>Lansoprazole</th>
<th>Domperidone</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sample Area - 1</td>
<td>% Assay - 1</td>
<td>% of Degradation</td>
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<tr>
<td>Acid</td>
<td>572.00</td>
<td>2443891</td>
<td>94</td>
</tr>
<tr>
<td>Base</td>
<td>572.00</td>
<td>2326604</td>
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</tr>
<tr>
<td>Peroxide</td>
<td>572.00</td>
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<td>90</td>
</tr>
<tr>
<td>Heat</td>
<td>572.00</td>
<td>3497161</td>
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<tr>
<td>Sunlight</td>
<td>572.00</td>
<td>2317279</td>
<td>89</td>
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CONCLUSION
The present results provide clear evidence that the proposed method can be successfully used for simultaneous determination of drug content in marketed formulations. The new HPLC stability indicating method developed and validated for simultaneous determination of Lansoprazole and Domperidone 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 developed methods were validated for various parameters as per ICH guidelines like Accuracy, precision, linearity, specificity, ruggedness, and robustness. The results obtained were within the acceptance criteria for all the parameters. The assay results conformed to the label claim of the formulation. The results for both the compounds were in good agreement with label claims. The method can be applied even to the analysis of stability samples obtained during accelerated stability experiments, as no interference was found with the degradants formed under various stress conditions. 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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