Development and Validation of Spectrophotometric method for Simultaneous estimation of Lafutidine and Domperidone in combined dosage form by area under curve method

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Abstract
A simple, accurate, rapid and precise spectrophotometric method has been developed for simultaneous estimation of Lafutidine and Domperidone in pharmaceutical dosage form. This method was based on UV spectrophotometric determination of two drugs, using Area under Curve method. It involves measurement of area under curve in the range of 268-278 nm (For Lafutidine) and 282.2-292.2 nm (For Domperidone) for the analysis in methanol. The linearity was observed in the concentration range of 2-12 µg/ml for Lafutidine and 3-18 µg/ml for Domperidone. The method showed good reproducibility and recovery with % RSD less than 2. Method was found to be rapid, specific, precise and accurate, can be successfully applied for the routine analysis of Lafutidine and Domperidone in bulk, and combined dosage form without any interference by the excipients. The method was validated according to ICH guidelines.

Key words:
Lafutidine, Domperidone, Area under Curve Method, Ultraviolet Spectrophotometry

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Introduction
Lafutidine (LAF) is 2-(furan-2-ylmethylsulfinyl)-N-[4-{4-(piperidin-1-ylmethyl) pyridin-2-yl] oxybut-2-enyl]acetamide[1]. It belongs to the class of H2-receptor antagonists. Used in the treatment of peptic ulcer and gastro-oesophageal reflux disease (GERD). The literature reports some of the analytical methods like LC–ESI–MS method for the quantitation of

The validation of method carried out as per ICH guidelines [25]. However, there are no reported methods for simultaneous estimation of both drugs in combination. This paper presents two simple, rapid, reproducible and economical method for the simultaneous estimation of both the drugs.

Experimental Condition

Instrument

A Shimadzu UV-VIS Spectrophotometer 1800 with 1.0 cm matched quartz cells was used.

Materials and Method

Standard gift samples of Lafutidine and Domperidone were procured from Ajanta Pharmaceuticals Ltd, Mumbai and Dwarkesh Pharmaceuticals Pvt. Ltd, Gujarat. Combined dosage formulation containing Lafutidine and Domperidone were purchased from local market (LAFUDAC-D).

Preparation of standard stock solution (100 µg/ml)

The stock solution (100 µg/ml) of LAF and DOM were prepared separately by dissolving accurately about 10 mg of drug in Methanol and the volume was made up to 100 ml with Methanol to prepare standard stock solution (100 µg/mL).

Preparation of Calibration curve of LAF and DOM

The standard stock solution (100 µg/ml) of LAF and DOM were further diluted to obtain the final concentration 2, 4, 6, 8, 10, 12 µg/ml and 3, 6, 9, 12, 15, 18 µg/ml respectively. Both the solutions were scanned in the spectrum mode from 200.0 nm to 400.0 nm. The maximum absorbance of LAF and DOM was observed at 273 nm and 287.2 nm, respectively.
From the overlain spectra of both drugs obtained after scanning of standard solution of LAF and DOM, area under the curve in the range of 268-278 nm and 282.2-292.2 nm was measured for the analysis respectively. The absorptivity values calculated. The calibration curve was plotted with concentration v/s area under the curve and regression equation was calculated.

Determination of Absorptivity values:
Absorptivity = Absorbance/ Concentration of that component in gm/l.

Concentration of LAF and DOM was calculated using following formula;
\[
C_{\text{LAF}} = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2} \quad (1)
\]
\[
C_{\text{DOM}} = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2} \quad (2)
\]
Where,
C_{\text{LAF}} = Concentrations of LAF,
C_{\text{DOM}} = Concentrations of DOM,
A_1 = Area at 268-278 nm,
A_2 = Area at 282.2-292.2 nm,
ax_1 = Absorptivity value of LAF at 268-278 nm,
ax_2 = Absorptivity value of LAF at 282.2-292.2 nm,
ay_1 = Absorptivity value of DOM at 268-278 nm,
ay_2 = Absorptivity value of DOM at 282.2-292.2 nm.

Procedure for the assay of LAF and DOM in Capsule
For the estimation of drugs in the commercial formulations, twenty capsules containing 10 mg of LAF and 30 mg of DOM were weighed and average weight was calculated. The capsules were crushed and powdered in glass mortar. For the analysis of drugs, quantity of powder equivalent to 5 mg of LAF and 15 mg of DOM was transferred to 50 ml volumetric flasks and dissolved in sufficient quantity of Methanol. It was sonicated for 30 mins and volume was made up to obtain a stock solution of 10 µg/ml LAF (maintaining 30 µg/ml of DOM). This solution was then filtered through Whatmann filter paper no. 1. The concentration of both LAF and DOM was determined by measuring absorbances of sample solutions in wavelength range of 268-278 nm (for LAF) and 282.2-292.2 nm (for DOM) using equation 1 and 2. Results of capsule analysis are shown in Table 1.
Validation: The method was validated according to ICH guidelines to study linearity, accuracy, precision, LOD and LOQ.

Linearity
The measurement of linearity was evaluated by analyzing different concentrations of the standard solution of LAF and DOM. For both the methods, the Beer law was obeyed in the concentration range 2-12 µg/ml and 3-18 µg/ml for LAF and DOM respectively (Table-3). The absorbance was plotted against the corresponding concentrations to obtain the calibration graphs.

Precision
The reproducibility of the proposed methods was determined by performing tablet assay at different time intervals on same day (Intra-day precision) and on three different days (Inter-day precision) (Table - 3).

Limit of Detection and Limit of Quantitation

The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines were used to calculate the LOD and LOQ (Table - 3). The detection limit (LOD) may be expressed as: LOD = 3.3 σ/S and the quantitation limit (LOQ) may be expressed as: LOQ =10 σ/S Where, σ = the standard deviation of the response S = the slope of the calibration curve.

Accuracy (% Recovery studies)
To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (50%, 100% and 150%). Percent recovery was calculated for LAF and DOM, by this method (Table-2). Here three times repetition done with proposed procedure.

Table No: 1 Result of analysis of capsule Formulation:

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Label claim</th>
<th>Amount Found</th>
<th>% label claim*</th>
<th>S.D.</th>
<th>R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAF</td>
<td>10 mg</td>
<td>9.880</td>
<td>98.80</td>
<td>0.058459</td>
<td>0.591664</td>
</tr>
<tr>
<td>DOM</td>
<td>30 mg</td>
<td>30.02</td>
<td>100.06</td>
<td>0.132552</td>
<td>0.441545</td>
</tr>
</tbody>
</table>

* Indicates mean of six determinations.

Table 2: Result of Accuracy

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level of Recovery</th>
<th>Amt. of drug taken µg/ml</th>
<th>Amt of std.drug taken(spiked amt) µg/ml</th>
<th>% of drug estimated*</th>
<th>R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAF</td>
<td>0%</td>
<td>2</td>
<td>0</td>
<td>98.89</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>2</td>
<td>1</td>
<td>99.56</td>
<td>0.0352</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>2</td>
<td>2</td>
<td>99.09</td>
<td>0.177</td>
</tr>
<tr>
<td></td>
<td>150%</td>
<td>2</td>
<td>3</td>
<td>100.90</td>
<td>0.099</td>
</tr>
<tr>
<td>DOM</td>
<td>0%</td>
<td>6</td>
<td>0</td>
<td>100.21</td>
<td>0.0905</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>6</td>
<td>3</td>
<td>99.77</td>
<td>0.0918</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>6</td>
<td>6</td>
<td>100.30</td>
<td>0.0498</td>
</tr>
<tr>
<td></td>
<td>150%</td>
<td>6</td>
<td>9</td>
<td>99.43</td>
<td>0.131</td>
</tr>
</tbody>
</table>

*Average of three determinations. LAF=Lafutidine, DOM=Domperidone
Table 3: Regression analysis and Validation Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LAF</th>
<th>DOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range</td>
<td>2-12 µg/ml</td>
<td>3-18 µg/ml</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9977</td>
<td>0.9957</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraday</td>
<td>0.75-1.71</td>
<td>0.57-1.89</td>
</tr>
<tr>
<td>Interday</td>
<td>0.80-1.69</td>
<td>0.57-1.29</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.6440</td>
<td>0.8029</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>1.9515</td>
<td>2.4331</td>
</tr>
<tr>
<td>Assay (% Recovery)</td>
<td>98.89-100.90</td>
<td>99.43-100.30</td>
</tr>
</tbody>
</table>

Result and Discussion

The present work provides an accurate, reproducible, sensitive method for the simultaneous analysis of LAF & DOM in bulk and capsule formulation. Linear relationships between drug concentrations were obtained over the range of at 2-12 µg/ml & 3-18 µg/ml for LAF and DOM respectively. Under experimental conditions described assay of capsule, linearity, accuracy studies and precision, LOD and LOQ were estimated. Correlation coefficient was found to be > 0.995. The results of commercial capsule formulation are presented in Table 1. The % assay was found to be 98.89- 100.90% for LAF and 99.43-100.30% for DOM, and S.D. and R.S.D. for six determinations of capsule sample, by this method, was found to be less than 2.0 indicating the precision of this method. No interference was observed from the pharmaceutical adjuvants /excipients.

Conclusion

The UV spectrophotometric method was developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method are within limits, indicating high degree of precision of the method. The results of the recovery studies performed indicate the method to be accurate. Hence, it can be concluded that the developed spectrophotometric method is accurate, precise and can be employed successfully for the estimation of LAF and DOM in bulk and formulation.

Acknowledgment

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