STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF LAFUTIDINE IN TABLET DOSAGE FORM BY RP-HPLC

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ABSTRACT

Objectives: To perform stability indicating analytical method development and validation of Lafutidine in tablet dosage form by reverse phase HPLC method. Materials and Methods: The method was developed employing isocratic RP-HPLC and validated for assay of Lafutidine in tablet dosage form. The method employs the use of a UV detector. Results: The developed assay method was found to be accurate, precise, reproducible and robust and was linear over a concentration range of 20-100µg/mL. Conclusion: The developed method was tried for its applicability in stability indicating assay. Various stress studies were carried out and the method was able to estimate Lafutidine, but was unable to estimate the degradation products of acid and alkaline stress conditions. The method was able to quantify degradation products in oxidative stress conditions.

Keywords: Lafutidine, RP-HPLC, Validation, Method development.

INTRODUCTION

Lafutidine is second generation histamine H2-receptor antagonist which inhibits gastric acid secretion. It is used for the treatment and prophylaxis of gastric and duodenal ulcers. It is also used for gastric mucosal lesion due to its gastroprotective action and in pre-anesthetic medication. Chemically Lafutidine[1] is 2-[[2-furylmethyl]sulfinyl]-N-[[2-(4- (piperidin-1-ylmethyl)pyridin-2-yl)oxy] but – 2 – en – 1 – y ] acetamide. Its empirical formula is C22H35N5O8S. Lafutidine is a white or almost white crystalline powder with a molecular mass of 431.55 g/mol. It is freely soluble in glacial acetic acid, soluble in methanol and very slightly soluble in water. Its absorption [2] does not depend on presence of food in stomach and binds to plasma proteins (approx. 88%) at therapeutic concentration. Its metabolism[3] occurs in liver by microsomal enzyme CYP3A4 (mainly) and CYP2D6 and its major metabolites are hydroxylated Lafutidine and Sulfonyl Lafutidine. It is not official in Indian pharma guidelines. Literature review revealed that there are few reported methods[4-9] which has certain shortcomings and is discussed in summary chart given in Table 1.

This study has been done with the objective to develop and validate stability indicating analytical method for estimation of Lafutidine in tablet dosage form by RP-HPLC as per ICH Q2(R1) guidelines. Structure of Lafutidine is given below. (Fig. 1)

Table 1: Summary of reported methods for Lafutidine

<table>
<thead>
<tr>
<th>Author</th>
<th>Title</th>
<th>Mobile phase and column</th>
<th>Run time</th>
<th>Retention time</th>
<th>Linearity range</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. Sumithra et al*</td>
<td>Analytical method development and validation of Lafutidine in tablet dosage form by RP-HPLC</td>
<td>Buffer (mixture of Potassium dihydrogen orthophosphate and dipotassium hydrogen orthophosphate pH 6.0) : Acetonitrile – 30: 70 Column Hypersil silica C18 (250x4.6mm, 5µ)</td>
<td>12 min.</td>
<td>7.755 min.</td>
<td>27-81µg/mL</td>
<td>NA</td>
</tr>
<tr>
<td>M. Jagadeeswaran et al5</td>
<td>Simultaneous determination of Lafutidine and Domperidone in capsule by High performance liquid chromatography</td>
<td>Buffer (20mM potassium dihydrogen orthophosphate) : Acetonitrile: Methanol-50:35:15 Column- Phenomenex C18 (250x4.6mm, 5µ)</td>
<td>10 min.</td>
<td>4.3 min.</td>
<td>80-120µg/mL</td>
<td>LOD 1.989µg</td>
</tr>
<tr>
<td>Parekh Ravishkumar R et al6</td>
<td>Development and validation of stability indicating RP-HPLC method for estimation of Lafutidine in bulk and pharmaceutical dosage form</td>
<td>Buffer (25mM sodium dihydrogen phosphate) : Acetonitrile – 35:65 Column- Grace C18, (250 x 4.6mm, 5µ) Mobile phase A- Buffer (20mM Diammonium hydrogen phosphate) : ACN – 80:20 v/v Mobile phase B- Buffer (20mM Diammonium hydrogen phosphate) : ACN – 30:70 v/v Column - Acquity UPLC BEH-shield RP 18 (100 x 3.0mm, 1.7µ)</td>
<td>10.0 min.</td>
<td>4.076</td>
<td>10-100 µg/mL</td>
<td>LOD 0.39µg</td>
</tr>
<tr>
<td>Aniket S Joshi et al7</td>
<td>Development and validation of stability indicating UPLC method for the determination of Lafutidine and its impurities in bulk and pharmaceutical dosage form</td>
<td>Buffer (25mM sodium dihydrogen phosphate) : Acetonitrile – 35:65 Column- Grace C18, (250 x 4.6mm, 5µ)</td>
<td>16 min.</td>
<td>5.04</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
MATERIALS AND METHOD

Lafutidine working standard (potency 99.6% w/w), Placebo and Lafutidine tablets were obtained from Alkem laboratories ltd. HPLC grade Acetonitrile, Disodium hydrogen phosphate dihydrate buffer, Ortho phosphoric acid, Hydrochloric acid, sodium hydroxide and Hydrogen peroxide were obtained from Merck, India ltd, Mumbai. HPLC instrument used was Agilent 1100 series with photodiode array detector and equipped with Chem station chromatographic software.

HPLC assay method development and validation: [10-12]

A UV spectrum of Lafutidine at a concentration of 50µg/mL was recorded by scanning in the UV range of 200-400 nm. Lafutidine was showing absorbance maxima at 276 nm. Different trial was tried using different column, mobile phase composition, buffer pH, flow rate and injection volume to obtain an optimized method. Validation [13] was carried out as per ICH Q2 (R1) guidelines.

Stress testing: [14-16]

Forced degradation studies were carried out on tablet dosage form and in different stress conditions. Acid hydrolysis was carried out by treating with 0.5 N HCl and alkaline hydrolysis was carried out by treating with 0.1 N NaOH at room temperature for 4 hours. Oxidative degradation was carried out by treating with Hydrogen peroxide (3%v/v) at room temperature for 4 hours. Thermal degradation was carried out by exposing the sample to 80°C for 4 hours.

RESULTS AND DISCUSSION

Various trials had been taken during method development and degradation study, the peak purity of the Lafutidine was main concern so as to develop a stability indicating assay method. Different trials were planned to develop a simple, sensitive, precise, accurate stability indicating assay method. The optimized chromatographic condition for assay of Lafutidine consists of the mobile phase composition ACN:disodium hydrogen phosphate dihydrate buffer (pH 6.0±0.05) in the ratio 30:70 using the X-Terra C$_18$ (250X4.6mm, 5µ) column with the flow rate of 1 mL/min. The injection volume was 20µL and column was maintained at temperature 30°C. The detection wavelength was 276 nm and the assay concentration was 50µg/mL with the run time of 12 min.

Chromatograph obtained with the optimized chromatographic condition is shown below. (Fig. 2)

![Chemical structure of Lafutidine](image1)

![Chromatogram of Lafutidine with optimized chromatographic condition](image2)
0.36 & 0.57 while for the change in detection wavelength (±2 nm) was found to be 0.84 & 1.23 for Lafutidine, proving that the method is robust with change in temperature, flow rate, pH and detection wavelength.

Lafutidine was subjected to different stress condition during forced degradation study. A summary of forced degradation study is tabulated in Table 2 and Chromatogram for oxidative degraded preparation is given in (Fig. 4)

**Table 2: Summary of forced Degradation study**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Mode of degradation</th>
<th>Condition</th>
<th>% degradation w.r.t control</th>
<th>Purity factor</th>
<th>Purity threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acid hydrolysis</td>
<td>0.5 N Hydrochloric acid at room temperature for 4 hours</td>
<td>7.05</td>
<td>999.935</td>
<td>999.854</td>
</tr>
<tr>
<td>2</td>
<td>Alkaline hydrolysis</td>
<td>0.1 N Sodium hydroxide at room temperature for 4 hours</td>
<td>17.15</td>
<td>999.948</td>
<td>999.867</td>
</tr>
<tr>
<td>3</td>
<td>Thermal degradation</td>
<td>80˚C for 4 hours</td>
<td>5.65</td>
<td>999.937</td>
<td>999.880</td>
</tr>
<tr>
<td>4</td>
<td>Oxidative degradation</td>
<td>3% v/v at room temperature for 4 hours</td>
<td>28.46</td>
<td>999.939</td>
<td>999.836</td>
</tr>
</tbody>
</table>

**CONCLUSION**

A simple, sensitive and specific isocratic RP-HPLC method was developed for assay of Lafutidine in tablet dosage form. The developed method consisted of mobile phase of disodium hydrogen phosphate dihydrate buffer (pH 6.0±0.05) and acetonitrile (70:30) with isocratic programming. X-Terra C18 (250mm×4.6mm, 5μ) column used as stationary phase with a flow rate of 1.0 mL/minute. The retention time of Lafutidine was found to be 6.969 min. Proposed method was found to be linear in the concentration range of 20.0 to 80.0 µg/mL with r² of 0.9998 for assay of Lafutidine. Precision study showed that the percentage relative standard deviation was within the range of acceptable limits, and the mean recovery was found to be 100.70 % for assay of Lafutidine. The developed method was validated as per ICH Q2A (R1) guideline and meets the acceptance criteria. The developed method is considered to be reliable and suitable for the routine quality control and is also able to quantify degradation product in oxidative stress condition.

**REFERENCES**

1. Lafutidine full prescribing information, Dosage & side effects [online]; [cited 2013 May 15]; Available from: www.mims.com/usa/drug/info/lafutidine


