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# SIMPLE COST-EFFECTIVE STABILITY INDICATING METHOD DEVELOPMENT FOR THE ESTIMATION OF LAFUTIDINE HYDROCHLORIDE IN MARKETED FORMULATION BY USING RP-HPLC

**Ankit Tiwari, Mr. Govind Nagar, Dr. Alok pal Jain**
RKDF College of Pharmacy, SRK University, Bhopal (M.P.)

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**Abstract:**
*Lafutidine hydrochloride is an antiulcer drug used in the treatment of gastric and duodenal ulcers. An efficient, simple, and cost-effective stability indicating HPLC method was developed for the estimation of lafutidine hydrochloride in the marketed formulation. The method involves the use of a C18 reversed-phase column (250 mm x 4.6 mm, 5 µm) with a mobile phase consisting of acetonitrile and methanol (50:50, v/v). The flow rate was set at 1.0 mL/min. The method was validated for linearity, precision, accuracy, and robustness. The linearity was established in the concentration range of 5-25µg/mL. The method was found to be precise with % RSD values of less than 2 for intra-day and inter-day precision. The accuracy was within the range of near to 100% for lafutidine hydrochloride. The robustness of the method was satisfactory with %RSD values of 0.3-0.5. The developed method was found to be simple, precise, accurate, and cost-effective for the estimation of lafutidine hydrochloride in the marketed formulation.*

***Key words:*** *Lafutidine hydrochloride, Cost effective, Method development, Validation*

**Corresponding author:**
**Ankit Tiwari,**
*RKDF College of Pharmacy, SRK University, Bhopal (M.P.)*
*Mail id: dralokpaljain@gmail.com*

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## INTRODUCTION:

Analytical method development and validation plays an important role in the discovery, development and manufacture of pharmaceuticals. These methods used to ensure the identity, purity, potency and performance of drug products. There are many factors to consider when developing methods. The initially collect the information about the analyte's physiochemical properties (pKa, logP, solubility) and determining which mode of detection would be suitable for analysis. The majority of the analytical development effort goes into validating a stability indicating HPLC method. The goal of the HPLC method is to try and separate quantify the main active drug, any reaction impurities, all available synthetic intermediates and degradants[1-3].

Analysis can be divided in to two classes, i.e. Qualitative analysis and Quantitative analysis. Qualitative analysis gives an indication of the identity of the chemical species in the sample. Quantitative analysis estimates, how much quantity is present in a mixture[4]. Modern analytical chemistry is functioning by instrumental analysis. Separation of components in a mixture is based on their interaction between a stationary and a mobile phase. These interaction differences are achieved based on the properties such as polarity, electric charge (for ionic compounds), pH, functional groups and size of the molecule[5-6].

Different types of quantitative analytical techniques are there, for eg: HPLC, Gas chromatography, TLC, Ion chromatography and Column chromatography, UV/Visible spectroscopy, FT-IR, LC-MS, GC-MS, MASS and NMR.

High-performance liquid chromatography is a separation technique based on a solid stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption, or ion exchange processes, depending upon the type of stationary phase used[7-8]. Most of the drugs in multicomponent dosage forms can be analyzed by HPLC method for the reason that of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tiresome extraction and isolation procedures. HPLC method development is not very difficult when literature reference for the same or similar compounds to be analyzed can be found.

There are various fields of Pharmacy one of them is pharmaceutical analysis which plays a very significant role in quality control of pharmaceuticals through a rigid check of raw materials, in process samples till finished products. Quality is important in every product or service, but it is vital in medicine, as it involves life, so there should not be any compromises to quality of drug products. Secondly to maintain the quality of drugs is a regulatory and mandatory requirement and lastly it is crucial for business in modern and competitive market also. Quality of drugs can only be maintained by analyzing it using analytical method with high degree of accuracy and precision and should satisfy all other validation parameters. The objectives of the research are the development and validation of fast, reproducible, efficient, economic and environmentally viable chromatographic methods for the analyses of drug in pharmaceutical dosage form. The objective of present work is, to develop simple cost-effective RP-HPLC method for drugs in pharmaceutical dosage form. To validate the developed methods according to ICHQ2R1 & ICHQ2R2 guidelines to ensure their precision, accuracy, repeatability, reproducibility and other analytical method validation parameters.

## MATERIAL AND METHODS:

### Selection of Mobile Phase

Initially to estimate Lafutidine hydrochloride in fix dosage form number of mobile phase in different ratio were tried. Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50 v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

**Preparation of Stock Solution** Accurately weighed 10 mg of Lafutidine hydrochloride was transferred into 50 ml volumetric flasks separately and dissolved in 10 ml of methanol and sonicate for 10 min., then volume was made up to 50 ml with methanol and vortex it to get complete dissolution and then filtered by whatmann filter paper (no.41). Concentration of Lafutidine hydrochloride in methanol was 200 µg/ml. (stock- A)

**Preparation of Sub Stock Solution** 5 ml of solution was taken from stock-A of Lafutidine hydrochloride and transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent to give

concentration of 100 µg/ml (Stock-B).

**Preparation of Different Solution**
0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with methanol. This gives the solutions of, 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of Lafutidine hydrochloride.

**Linearity and Calibration Graph**
To establish the linearity of analytical method, a series of dilution ranging from 5-25 µg/ml was prepared. All the solution were filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 247nm and it was repeat for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

**Validation of Developed Method**
**Linearity**

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated[7]. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

**Specificity**

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components[8].

**Accuracy**

Recovery studies were performed to validate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

**Precision**

**The precision are established in three differences:**
**Repeatability**

**Intermediate precision**

a) Day to Day

b) Analyst to Analyst

**Reproducibility**

**Repeatability**
The repeatability was performed for five replicates at five concentrations in linearity range 5, 10, 15, 20 and 25µg/ml for Lafutidine hydrochloride indicates the precision under the same operating condition over short interval time[9].

**Robustness**

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, methanol: Acetonitrile (50:50% v/v), to (50:50% v/v).

**Detection Limit and Quantitation Limit**
The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve[10].

**Analysis of drugs in tablets formulation**

Tablet powder amount equal to 10mg of Lafutidine hydrochloride was taken in 100ml volumetric flask. This was than dissolve in 25 ml of methanol by sonication for about 10 minutes. The volume is made up to the mark by methanol and filtered by whatmann filter paper (no.41) and the filtrate was used to prepare samples of different concentration.

## RESULTS AND DISCUSSION:

Method Development and Validation for Estimation of Lafutidine hydrochloride in synthetic mixture using RP-HPLC. The RP-HPLC method was developed for estimation of Lafutidine hydrochloride in combined formulation by isocratically using acetonitrile: methanol in the ratio of 50:50 v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 247nm. Then developed method was validated by using various parameters. The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10µg/ml of Lafutidine hydrochloride were injected and chromatogram was recorded.

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded.

Specificity of the method was determined and the peaks of plasma, diluent, mobile phase and excipient of physical mixture did not interfere with standard peaks of Lafutidine hydrochloride. The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less then 2 indicate the accuracy of method.

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are less then 2 indicate the precision of method. The robustness of developed method was checked by changing in the deliberate variation in solvent.

Detection limit and quantitation limit of described method were observed as 0.85µg/ml and quantitation limit 2.14µg/ml respectively based on the SD of response and slope, which meet the requirement of new method. The results of the analysis of synthetic mixture were reported. The assay value of drugs was close to 100, SD and % RSD are less then 2 indicate the no interference of excipient in the estimation of drug. filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

**Table 1: Results of system suitability parameters**

| Parameters | Lafutidine hydrochloride |
|---|---|
| **No. of Theoretical Plates** | 3133±71.36 |
| **Tailing Factor** | 1.233±0.078 |
| **Retention time** | 3.028±0.088 |

**Table 2: Results of Linearity of Lafutidine hydrochloride**

| PARAMETER | Lafutidine hydrochloride |
|---|---|
| **Concentration (µg/ml)** | 5-25 |
| **Correlation Coefficient ($r^2$)*** | 0.999 |
| **Slope (m)*** | 22.32 |
| **Intercept (c)*** | -0.695 |

*value of five replicate

**Table 3: Results of recovery study**

| % Level | % MEAN±SD* |
|---|---|
| **80%** | 100.236±0.544 |
| **100%** | 98.857±1.575 |
| **120%** | 99.716±0.149 |

***** Value of three replicate and three concentrations.

**Table 4: Results of precision**

| Parameter | % MEAN±SD* |
|---|---|
| **Repeatability** | 99.188±0.506 |
| **Intermediate precision** | |
| **Day to day precision** | 99.074±0.667 |

***** Value of five replicate and five concentrations

**Table 5: Results of Robustness**

| Parameter | % MEAN±SD* |
|---|---|
| **Robustness** | 99.074±0.667 |

***** Value of five replicate and five concentrations

**Table 6: Assay of synthetic mixture**

| | **Lafutidine hydrochloride *** |
|---|---|
| Label Claim (mg) | **10mg** |
| % Found (mg) | 9.92 |
| % Assay | 99.20 |
| % RSD | 0.312 |

*Average of three determination

## CONCLUSION:

The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form. The Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

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