WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 7, 119-130. Review Article ISSN 2277– 7105

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF RABEPRAZOLE SODIUM AND LAFUTIDINE INCOMBINED DOSAGE FORM

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Article Received on
12 March 2023,

Revised on 02 April 2023,
Accepted on 23 April 2023

DOI: 10.20959/wjpr20237-27892

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ABSTRACT

A simple, rapid, specific, accurate and precise RP-HPLC method was developed for the simultaneous estimation of Lafutidine and Rabeprazole Sodium in combined dosage form. A Hi Q sil C-8 Column (250 mm × 4.6 mm, 5 µm) in Isocratic mode with mobile phase containing Acetonitrile**:** Phosphate Buffer (70:30 v/v) pH 7.3 was used. The flow rate was 1.0ml/min and effluents were monitored at 230 nm. The retention time of Rabeprazole Sodium and Lafutidine was found to be 3.658 min and 5.408 min respectively. Validation parameters such as linearity, precision, accuracy, limit of detection(LOD), limit of quantification (LOQ), robustness were determined according to the International Conference on Harmonization (ICH) Q2R1 guidelines. The detector response was linear in the range for Lafutidine 10-60 µg/ml and for Rabeprazole Sodium 20-120 µg/ml. The proposed method was successfully applied for the simultaneous estimation of LAF and RAB in pharmaceutical dosage form.

KEYWORDS: Lafutidine (LAF), Rabeprazole Sodium (RAB), RP-HPLC method.

INTRODUCTION

Lafutidine is chemically 2-(furan-2-ylmethylsulfinyl)-N-[4-[4-(piperidin-1-ylmethyl) pyridin-2- yl]oxybut-2-enyl] acetamide[1] (Fig.1). Lafutidine (LAF) is the new generation H2-receptor antagonist. It blocks the production of acid by acid producing cells in the stomach and blocks histamine H2- receptors in the stomach and prevents histamine mediated gastric acid secretion.

Rabeprazole Sodium is chemically 2-[[[4-(3-Methoxypropoxy)-3-Methyl-2-Pyridinyl]-Methyl]Sulfinyl]-1H-Benzimidazole Sodium salt.[1,2] (Fig.2). Rabeprazole sodium (RAB) is a potent proton pump inhibitor that suppress gastric acid secretion by specific inhibition of the gastric H+/K+-ATPase enzyme system at the secretory surface of the gastric parietal cand is used in the treatment of gastro-oesophageal reflux disease (GERD) and duodenal ulcers.

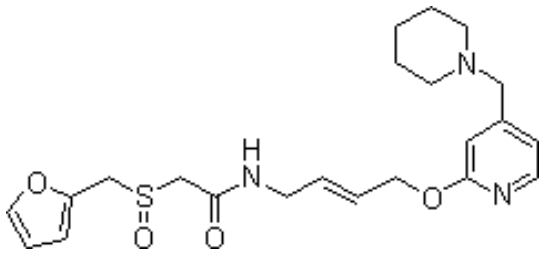

Fig. 1: Chemical Structure of Lafutidine.

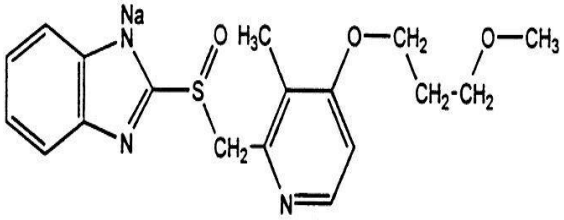

Fig. 2: Chemical Structure of Rabeprazole Sodium.

Literature survey revealed that a number of analytical methods have been reported for the estimation of Lafutidine (LAF) such as Spectrophotometry[3,4], HPLC[5], RP-HPLC[6], HPTLC[7], LC-ESI-MS[8], HPLC-MS[10] in single dosage form and RP-HPLC[18] in combination with other drugs. For estimation of Rabeprazole Sodium (RAB) spectrophotometry[11], HPLC[12,13] methods in single dosage form and RP-HPLC[17] in combination with other drugs have been reported. For simultaneous estimation of LAF and RAB in combined dosage form. Spectrophotometry[14,15], RP-HPLC[16] methods have been reported. A successful attempt has been made to estimate two drugs simultaneously by RP-HPLC method. The present work demonstrates simple, rapid, accurate, reproducible and economical method for the simultaneous determination of Lafutidine and Rabeprazole Sodium in tablet formulation by RP-HPLC.

Instruments

1 UV

A Jasco double beam UV–visible spectrophotometer, Model: V-630, with a fixed bandwidth (1.5nm) and 1-cm quartz cell was used for Spectral and absorbance measurements. In

addition, electronic balance, micropipette and sonicator were used in this study.

2. RP-HPLC

The HPLC system (Jasco HPLC) consisted of a pump with manual injection facility. The capacity of loop was 20 µl. The detector consisted of a UV-VIS spectrophotometer operated at a wavelength 230 nm. The software used was Borwin. The column used was Hi Q sil C-8(250 mm × 4.6 mm, 5 µm). Absorbance measurements were made on UV- detector. The pH meter used was of Hanna Instruments (Model: HI 98107, USA). All the weights were taken on an electronic Contech balance and sonication of mobile phase was done using sonicator Spectralab model UCB 40.

Development of RP-HPLC Method

Chromatographic methods offer an advantage in terms of sensitivity and selectivity. These methods can be used for routine analysis of dosage forms where two or more drugs are present together. RP-HPLC method was developed for simultaneous estimation of Lafutidine and Rabeprazole sodium.

Validation of Proposed RP-HPLC Method

1. Linearity

Linearity of the method was determined by constructing calibration curves. Standard solutions of LAF and RAB in increasing range concentrations 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 curve.

2. Precision

Precision of the method was determined by performing intraday variation and interday variation studies. For Intra day precision six replicates were injected on the same day and the percent relative standard deviation (%RSD) was calculated. For inter-day precision repeatability study was performed by injecting the six replicates of the same concentration on different days and the percent relative standard deviations (%RSD) was calculated.

3. Accuracy (Recovery Studies)

To check accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard LAF and RAB were added to pre- analyzed sample and content was determined.

4. Limit of Detection (LOD)

The LOD is estimated from the calibration curves used to determine method linearity. The LOD may be calculated as;

LOD = 3.3 x (SD / Slope)

Where, SD = the standard deviation of Y- interceptSlope = slope of the calibration curves.

5. Limit of Quantification (LOQ) The LOQ may be calculated as; LOQ = 10 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

6. Robustness

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 and pH on the area of chromatograms were studied. The method was found to be unaffected by small changes in method parameters change in flow rate and pH. **Change in.**

Conditions for Robustness

Condition	Change Level
Change in flow rate	± 0.2 ml/min
Change in pH	± 0.2

Analysis of marketed formulation

Twenty tablets containing LAF and RAB (10:20mg) were weighed and average weight was determined. Tablet powder equivalent to 10 mg of LAF and 20 mg of RAB was transferred to 100 ml volumetric flask, dissolved in mobile phase. Solution was ultrasonicated for 20 min. filtered through Whatman filter paper No. 42 µ. Filtrate was diluted with mobile phase to obtain final concentration. Chromatogram was recorded at 230 nm. Content of drugs in sample solution was calculated by comparing mean peak area of sample with that of the standard.

Introduction to Spectroscopy

Spectroscopy is the branch of science dealing with the study of interaction of electromagnetic radiation with matter.

Spectroscopy is further divided into

1. Atomic Spectroscopy

This deals with the interaction of electromagnetic radiations with atoms, which are most commonly present in their lowest energy state called the ground state.

It is further divided into:

i) Atomic emission

ii) Atomic absorption

2. Molecular Spectroscopy

This deals with the interaction of electromagnetic radiation with molecules.

1.2.1 Absorption Spectroscopy

All atoms and molecules are capable of absorbing energy in accordance with certain restrictions depending upon structures of substances. Energy may be furnished in the form of electromagnetic radiation. The kind and amount of radiation absorbed by the molecule depends on the nature and the number of molecules interacting with the radiation. The study of these dependencies is called absorption spectroscopy. The basis of UV- Visible spectrophotometry is the absorption of the UV- visible radiation, which causes the electronic transition within the molecules by the radiant energy of definite and narrow wavelength of monochromatic radiation.

Light absorption in the UV-visible radiation causes the transition of an electron from a ground state and relaxation of energy takes place very rapidly. The important consequences of rapid relaxation of the excited states are not appreciably disturbed by absorption of light energy from any source. Therefore, the fraction of light absorbed from an incident beam is independent of the intensity of these beams. This fact was expressed quantitatively which was integrated to obtain Beer's and Lambert's law.

Beer's and Lambert's law

$A = \log I_o/I_t = abc.$

where, A = Absorbance of the solution at a particular wavelength of the light beam.

I_o = Intensity of the incident light beam.

I_t = Intensity of beam after passing through solution.

a = Absorptivity of molecule at the wavelength of beam.b = Path length of cell in cm.

c = Concentration of solution in g/lit.

On passing electromagnetic radiation in the ultra-violet and visible regions through a compound with multiple bonds, a portion of the radiation is normally absorbed by the compound. The absorption of radiation is due to the subtraction of energy from the radiation beam when electrons in orbitals of lower energy are excited into orbitals of higher energy. Energy absorbed in the ultraviolet region by complex organic molecules causes transitions of valence electrons in the molecules

These transitions are

a) **n π^* transitions:** These type of transitions are shown by unsaturated molecules which contain atoms such as oxygen, nitrogen and sulphur. These transitions exhibit a weak band in their absorption spectrum. These are forbidden transitions.

b) **$\sigma \rightarrow \sigma^*$ transitions:** These transitions can occur in compounds in which all the electrons are involved in single bonds and there are no lone pair of electrons.

c) **n $\rightarrow \sigma^*$ transitions:** Saturated compounds with lone pair(non-bonding) of electrons undergo these transitions in addition to σ.

d) **$\pi \rightarrow \pi^*$ transitions:** A π π* transition corresponds to the promotion of an electron from a bonding π orbital to an antibonding π* orbital. This transition can in principle occur in any molecule having a π electron system.

High-Performance Liquid Chromatography [HPLC]

In the modern pharmaceutical industry, high-performance liquid chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. It is ideal for the analysis of many drugs in both dosage forms and biological fluids due to its simplicity, high specificity and good of sensitivity.

1.3.1 Types of HPLC

The principle characteristic defining the identity of each technique is the dominant type of molecular interactions employed. There are three basic types of molecular forces: ionic forces, polar forces, and dispersive forces. There are four main types of HPLC techniques are:

I. Normal phase HPLC

Retention by interaction of stationary phase`s polar surface with polar parts of sample molecules.

II. Reverse phase HPLC

Retention by interaction of stationary phase`s non polar hydrocarbon chain with non polar part of sample molecules.

III. Ion exchange chromatography

Retention by reversible ionic bonds to charged groups on stationary phase.

IV. Ion pair chromatography

Ionic sample molecules are ionically bound to an ion-pair reagent.

V. Affinity chromatography

Affinity chromatography separates proteins on the basis of a reversible interaction between a protein (or group of proteins) and a specific ligand coupled to a chromatographic matrix. The technique is ideal for a capture or intermediate step in a purification protocol and can be used whenever a suitable ligand is available for the protein(s) of interest. With high selectivity, hence high resolution, and high capacity for the protein(s) of interest, purification levels in the order of several thousand-fold with high recovery of active material are achievable. Target protein(s) is collected in a purified, concentrated form.

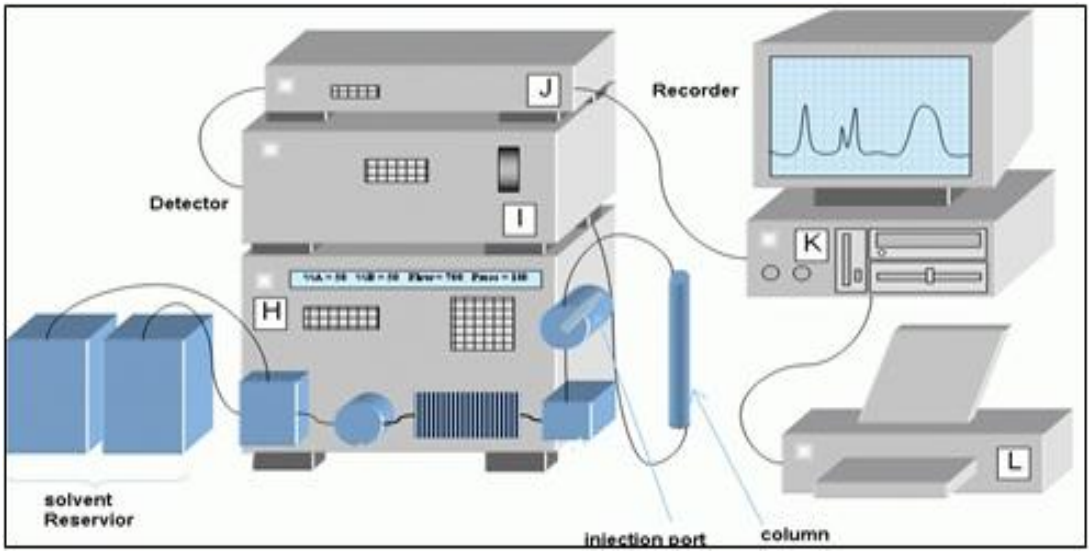

Figure 5: Diagram of HPLC instrument.

Also, HPLC can be classified by separation mechanism or by the type of stationary phase includes

1) Partition or liquid-liquid chromatography
2) Adsorption or liquid-solid chromatography

3) Ion - exchange or ion chromatography
4) Size - exclusion chromatography
5) Affinity chromatography
6) Chiral chromatography

1.3.2. Basic elements of HPLC system (Instrumentation)

Today's HPLC requires very special apparatus, which includes: -

1. Extremely precise gradient mixers (optional).
2. HPLC high pressure pumps with very constant flow.
3. Unique high accuracy, low dispersion, HPLC sample valves.
4. Very high efficiency HPLC columns with inert packing materials.
5. High sensitivity low dispersion HPLC detectors.
6. High-speed data acquisition systems.
7. Low dispersion connecting tubes for valve to column and column to detector Typical HPLC system consists of the following main components:

Solvent Reservoirs

Sufficient amount of HPLC solvents should be store for continuous operation of the system. It should be equipped with an online degassing system and special filters to isolate the solvent from the influence of the environment.

Pump

This provides the constant and continuous flow of the mobile phase through the system; most modern pumps allow controlled mixing of different solvents from different reservoirs.

Sample injector

This allows an introduction (injection) of the analyte mixture into the stream of the mobile phase before it enters the column. Most modern injectors are auto samplers, which allow programmed injections of different volumes of samples that are withdrawn from the vials in the auto sampler tray.

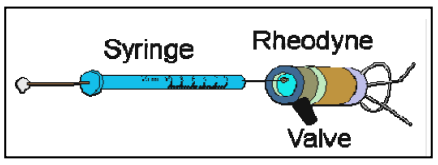

Figure 6: Diagram of Loop injector.

Column

This is the heart of HPLC system; it actually produces a separation of the analyte from mixture. A column is the place where the mobile phase is in contact with the stationary phase, forming an interface with enormous surface. Most of the chromatography development in recent years went toward the design of many different ways to enhance this interfacial contact.

Detector

This is a device for continuous measurement of specific physical and chemical properties of the column effluent. The most common detector used in pharmaceutical analysis is UV (ultraviolet), which allows monitoring and continuous measurement of the UV absorbance at a selected wavelength or over a span of wavelengths (diode array detection). Appearance of the analyte in the detector flow-cell causes the change of the absorbance. If the analyte absorbs greater than the background (mobile phase), a positive signal is obtained.

ASSAY OF RABEPRAZOLE TABLTES

For the analysis of the dosage form 20 tablets of RBZ (20mg) were weighed. Powder equivalent to 10mg of RBZ was taken in to a 100ml volumetric flask. The formulation first dissolved in 0.1 N NaOH (25 ml) and sonicated for about 10-15 min. finally made up the volume with 0.1N NaOH. The solution was filtered and final dilution of the sample (10µg/ml) was prepared and measured the absorbance against blank at 292nm. The amount of Rabeprazole was computed by using the equation referring to the calibration curve (Table-2).

Analysis of commercial dosage form lafutidine

Twenty tablets were weighed and powdered finely. A quantity of tablet powder equivalent to 10 mg of Lafutidine was accurately weighed and transferred to 10 ml volumetric flak and sonicated for 10 min. The solution was further filtered by whattmann filter paper. The solution was further diluted with 0.01 N HCL and 0.01 N NaOH to get the required concentration of 10µg/ml. The amount of drug International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 4, Suppl 4, 2012 Academic Sciences present in the sample solution was determined using the calibration curve of standard drug.

CONCLUSION

The proposed method for the assay of Lafutidine and Rabeprazole Sodium is very simple and rapid The method was validated for linearity, precision, accuracy and robustness. It could

be used for the rapid and reliable determination of Lafutidine and Rabeprazole Sodium in tablet formulation. The validated method was applied for the assay of commercial formulation containing Lafutidine and Rabeprazole Sodium.

ACKNOWLEDGEMENT

It is great pleasure for me to acknowledge my Parents, Project guide, Management of STE'S Smt. Kashibai Navale College of Pharmacy for providing necessary facilities. We express gratitude to the Emcure Pharmaceutical Pvt. Ltd. for sending drug gift samples none the less all my friends, who have contributed towards the conception of this research work.

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