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Research

Newer rp-hplc method development and validation for the simultaneous estimation of lafutidine and rabeprazole in dosage form

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Lafutidine and Rabeprazole, in its pure form as well as in tablet dosage form. Chromatography was carried out on an Altima C18 (4.6mm x 150mm, 5µm) column using a mixture of ACN, Methanol and Phosphate buffer pH4.6 (10:25:65 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 265nm. The retention time of the Lafutidine and Rabeprazole was 2.088, 6.068 ±0.02min respectively. The method produces linear responses in the concentration range of 10-50mg/ml of Lafutidine and 20-100mg/ml of Rabeprazole. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Lafutidine and Rabeprazole, RP-HPLC, Validation, Accuracy.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

HPLC

HPLC is also called as high-pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

MATERIALS AND METHODS

Lafutidine-Suralabs, Rabeprazole -Suralabs, Water and Methanol for UPLC-LICHROSOLV (MERCK), Acetonitrile for UPLC- Merck, Acetic Acid-Merck.

HPLC method development

Trails

Preparation of standard solution

Accurately weigh and transfer 10 mg of Lafutidine and Rabeprazole working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.3 ml of Lafutidine and 0.6ml of Rabeprazole from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Water, Methanol: Phosphate buffer and ACN: Water with varying proportions. Finally, the mobile phase was optimized to TEA buffer (pH 4.0), Methanol in proportion 65:35 v/v respectively.

Optimization of Column: The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Gemini C18 (4.6×250mm) 5µ was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : 40ºC
Column : Phenomenex Gemini C18 (4.6×250mm) 5µ
Mobile phase : Methanol: TEA Buffer (65:35 v/v)
Flow rate : 1ml/min
Wavelength : 230nm
Injection volume : 10µl
Run time : 6minutes

Validation

Preparation of buffer and mobile phase

Preparation of Triethylamine buffer (pH-4.0): Take 6.0ml of Triethylamine in to 750ml of HPLC water in a 1000ml volumetric flask and mix well. Make up the volume up to mark with water and adjust the pH to 4.0 by using Orthophosphoric acid, filter and sonicate.

Preparation of mobile phase: Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Buffer: Methanol: ACN (65:25:10v/v/v)
Column : Altima C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 265 nm
Column temp : 38ºC
Injection Volume : 10 µl
Run time : 14 minutes

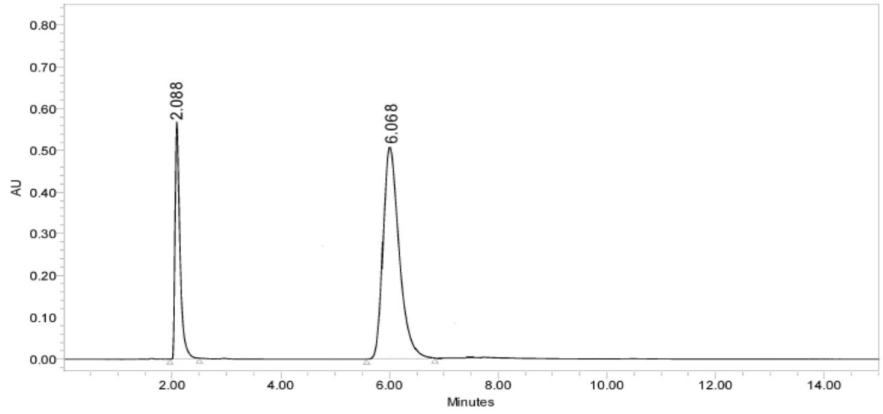

Fig 1: Optimized Chromatogram

Table 1: Peak Results for Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Lafutidine	2.088	3425413	567933		1.0	5565.5
2	Rabeprazole	6.068	1629854	517733	2.5	1.1	5355.2

From the above chromatogram it was observed that the Lafutidine and Rabeprazole peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

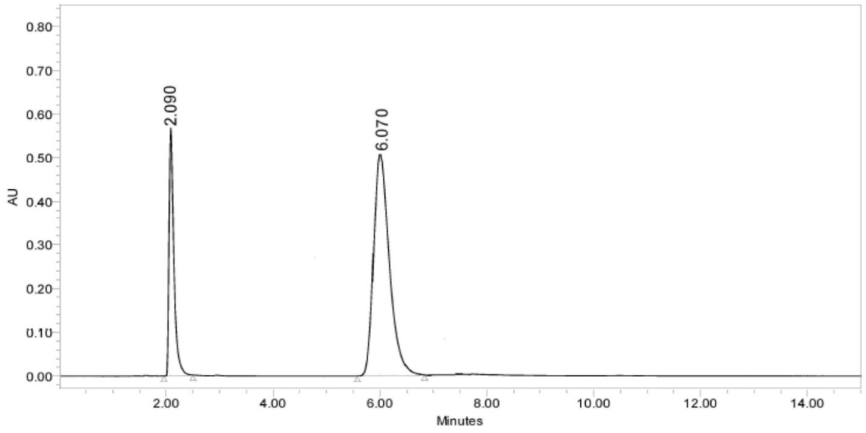

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No.	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Lafutidine	2.090	3468547	567933		1.0	5565.5
2	Rabeprazole	6.070	16289441	517733	2.5	1.1	5355.2

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

system suitability

Table 3: Results of system suitability for Lafutidine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lafutidine	2.080	3569412	567917	5568.0	1.0
2	Lafutidine	2.080	3465125	517719	6359.2	1.1
3	Lafutidine	2.080	3598154	567933	5565.5	1.0
4	Lafutidine	2.081	3586491	517733	5355.2	1.1
5	Lafutidine	2.081	3582694	567917	6348.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of method precession for Rabeprazole

S. No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rabeprazole	2.080	3582264	567917	5568.0	1.0	2.5
2	Rabeprazole	2.080	3586491	517719	5359.2	1.1	2.5
3	Rabeprazole	2.080	3598154	567933	5565.5	1.0	2.5
4	Rabeprazole	2.081	3564125	517733	5355.2	1.1	2.5
5	Rabeprazole	2.081	3569412	562173	5568.0	1.0	2.5
Mean			3580089				
Std. Dev			13609.81				

% RSD	0.380153

- *%RSD for sample should be NMT 2.*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 5: Peak results for assay standard

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Lafutidine	2.087	3425681	567917		1.0	5568.0	1
2	Rabeprazole	6.067	16235984	517719	2.5	1.1	5359.2	1
3	Lafutidine	2.088	3425413	567933		1.0	5565.5	2
4	Rabeprazole	6.068	16298543	517733	2.5	1.1	5355.2	2
5	Lafutidine	2.088	3465423	567933		1.0	5545.5	3
6	Rabeprazole	6.068	16265213	517733	2.5	1.1	5352.1	3

Assay (Sample)

Table 6: Peak results for Assay sample

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Lafutidine	2.089	3469821	567917		1.0	6568.0	1
2	Rabeprazole	6.069	16259845	517719	2.5	1.1	5359.2	1
3	Lafutidine	2.090	3468547	567933		1.0	5565.5	2
4	Rabeprazole	6.070	16287531	517733	2.5	1.1	5355.2	2
5	Lafutidine	2.090	3468143	567813		1.0	5391.1	3
6	Rabeprazole	6.070	16282431	517623	2.5	1.1	5564.0	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

==16276602 / 16266580 × 10/60 × 60/0.0136 × 99.6/100 × 0.4102/300 × 100
= 100.1%

The % purity of Lafutidine and Rabeprazole in pharmaceutical dosage form was found to be 100.1%.

Linearity
Chromatographic data for linearity study
Lafutidine

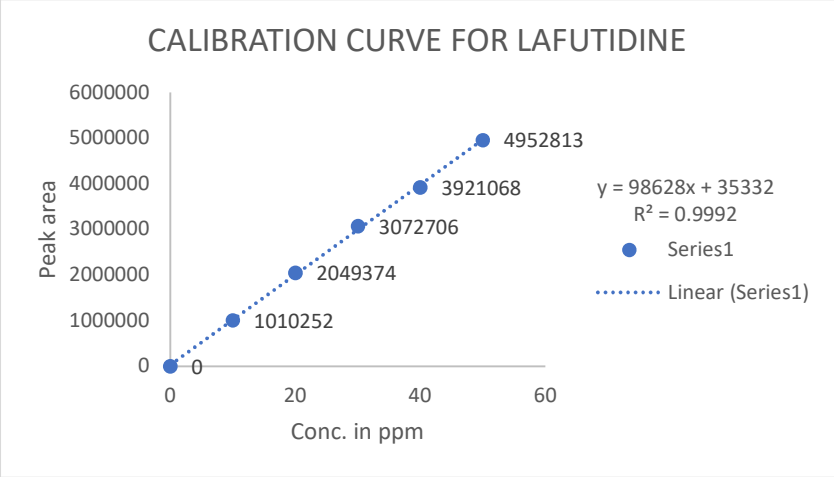

Fig 3: Calibration Graph for Lafutidine

Rabeprazole

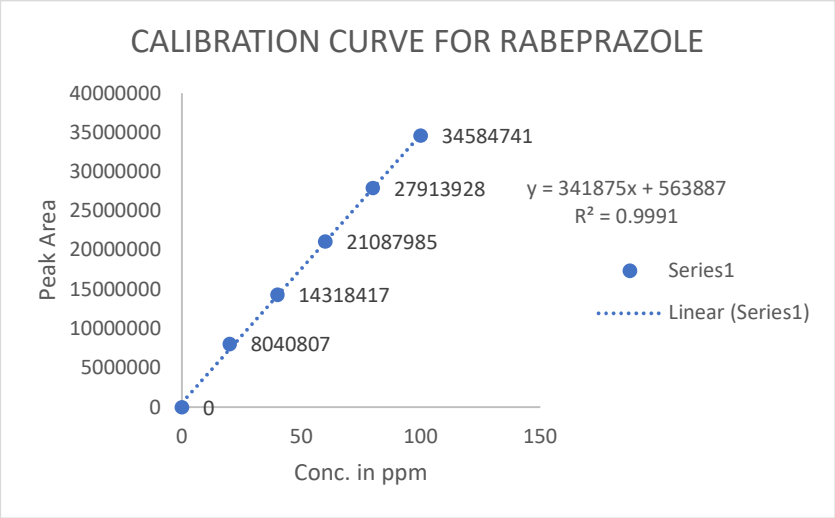

Fig 4: Calibration Graph for Rabeprazole

Precision
Repeatability

Table 7: Results of repeatability for Lafutidine

S.No.	Name	Rt	Area	Height	USP Plate Count	USP Tailing
1	Lafutidine	2.084	3569412	567917	5568.0	1.0
2	Lafutidine	2.083	3465125	517719	5359.2	1.1
3	Lafutidine	2.082	3598154	567933	5565.5	1.0
4	Lafutidine	2.081	3586491	517733	5355.2	1.1
5	Lafutidine	2.080	3582694	567917	5568.0	1.0
Mean			3560375			
Std. Dev			54225.61			
% RSD			1.523031			

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 8: Results of method precision for Rabeprazole

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rabeprazole	2.080	3582264	567917	5568.0	1.0	2.5
2	Rabeprazole	2.081	3586491	517719	5359.2	1.1	2.5
3	Rabeprazole	2.082	3598154	567933	5565.5	1.0	2.5
4	Rabeprazole	2.083	3564125	517733	5355.2	1.1	2.5
5	Rabeprazole	2.084	3569412	562173	5568.0	1.0	2.5
Mean			3580089				
Std. Dev			13609.81				
% RSD			0.380153				

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision
Day 1

Table 9: Results of Intermediate precision for Lafutidine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lafutidine	2.081	3481579	567917	5568.0	1.0

2	Lafutidine	2.082	3458121	517719	5359.2	1.1
3	Lafutidine	2.083	3426581	567933	5565.5	1.0
4	Lafutidine	2.084	3465712	517733	5355.2	1.1
5	Lafutidine	2.085	3451476	567917	5568.0	1.0
6	Lafutidine	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

- *%RSD of five different sample solutions should not more than 2.*

Table 10: Results of Intermediate precision for Rabeprazole

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rabeprazole	6.061	15481579	567917	5568.0	1.0	2.5
2	Rabeprazole	6.062	15369852	517719	5359.2	1.1	2.5
3	Rabeprazole	6.063	15248454	567933	5565.5	1.0	2.5
4	Rabeprazole	6.064	15874692	517733	5355.2	1.1	2.5
5	Rabeprazole	6.064	15236547	567933	5568.0	1.0	2.5
6	Rabeprazole	6.064	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 11: Results of Intermediate precision Day 2 for Lafutidine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Lafutidine	2.081	3481579	567917	5568.0	1.0
2	Lafutidine	2.082	3458121	517719	5359.2	1.1
3	Lafutidine	2.083	3426581	567933	5565.5	1.0
4	Lafutidine	2.084	3465712	517733	5355.2	1.1
5	Lafutidine	2.085	3451476	567917	5568.0	1.0
6	Lafutidine	2.085	3452106	567514	5359.2	1.1
Mean			3455929			
Std. Dev			18188.92			
% RSD			0.5			

- *%RSD of five different sample solutions should not more than 2.*

Table 12: Results of Intermediate precision for Rabeprazole

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Rabeprazole	6.061	15481579	567917	5568.0	1.0	2.5
2	Rabeprazole	6.062	15369852	517719	5359.2	1.1	2.5
3	Rabeprazole	6.063	15248454	567933	5565.5	1.0	2.5
4	Rabeprazole	6.064	15874692	517733	5355.2	1.1	2.5
5	Rabeprazole	6.064	15236547	567933	5568.0	1.0	2.5
6	Rabeprazole	6.064	15217547	567133	5359.2	1.1	2.5
Mean			15404779				
Std. Dev			251289.4				
% RSD			1.6				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Accuracy

Table 13: The accuracy results for Lafutidine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1543793	15	15.2	101.9	100.9%
100%	3035883	30	30.4	101.4	
150%	4451005	45	44.7	99.4	

Table 14: The accuracy results for Rabeprazole

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1084420	30	30.07	100.2	99.6%
100%	2096069	60	59.6	99.4	
150%	3112684	90	89.3	99.3	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 15: Results for Robustness

Lafutidine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	3425413	2.088	5568.2	1.0
Flow rate of 0.9 mL/min	3425282	3.111	5922.2	1.2
Flow rate of 1.1 mL/min	3517879	1.880	5868.8	1.2
Less aqueous phase	3175485	3.101	5836.2	1.2
More aqueous phase	3365431	1.881	5282.6	1.1

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Rabeprazole

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate of 1.0 mL/min	2029854	6.068	5359.2	1.1
Flow rate of 0.9 mL/min	1738319	7.101	5999.1	1.2
Flow rate of 1.1 mL/min	1638304	5.007	5989.2	1.1
Less aqueous phase	1973724	7.108	5387.2	1.1
More aqueous phase	2102838	5.008	5938.1	1.1

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Lafutidine and Rabeprazole in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps. Lafutidine And Rabeprazole was freely soluble in ethanol, methanol and sparingly soluble in water. ACN, Methanol and Phosphate buffer pH4.6 (10:25:65 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Lafutidine and Rabeprazole in bulk drug and in pharmaceutical dosage forms.

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