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(RESEARCH ARTICLE)

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Development and validation of RP-HPLC method for the simultaneous estimation of rabeprazole sodium and lafutidine in combined dosage form

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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

1. 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 cell and is used in the treatment of gastro-oesophageal reflux disease (GERD) and duodenal ulcers.

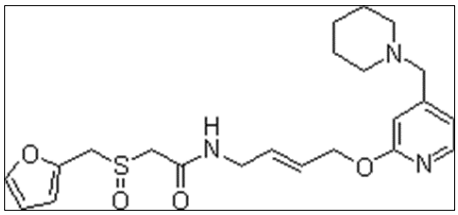

Figure 1 Chemical Structure of Lafutidine

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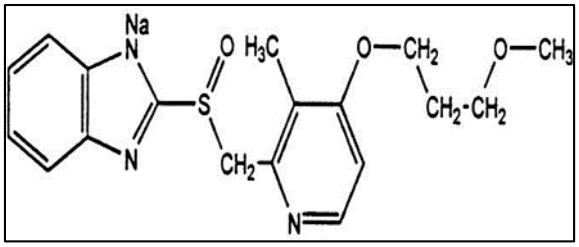

Figure 2 Chemical Structure of Rabeprazole Sodium

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.

2. Material and methods

2.1. Instrument

HPLC Model: 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. The software used was Borwin. The column used was Hi Q sil C- 8(250 mm × 4.6 mm, 5 µm).

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.

2.2. Reagents and chemicals

Pure drug samples of LAF and RAB were provided as a gift sample from Emcure Pharmaceuticals Ltd, Pune, India. Acetonitrile of HPLC grade was obtained from Thomas Baker. Analytical grade Potassium dihydrogen ortho phosphate and Sodium hydroxide were used.

2.3. Marketed formulation

The commercial formulation LAFUMACPLUS (Macleods Pharmaceuticals Ltd.,Mumbai) was purchased from local pharmacy. Each Capsule contains 10mg Lafutidine and 20mg Rabeprazole Sodium.

2.4. Preparation and Selection of Mobile phase

Acetonitrile and Potassium dihydrogen ortho phosphate buffer were selected as mobile phase on C-8 column after several trials of mobile phase combination. The optimum composition of mobile phase determined to be Acetonitrile: 0.01M Pottasium dihydrogen ortho phosphate (70:30 v/v) pH 7.3 and filtered through 0.45µ membrane filter.

2.5. Chromatographic Conditions

The mobile phase Acetonitrile: (0.01M) Potassium dihydrogen ortho phosphate (70:30 v/v) pH pumped at a flow rate of 1.0 ml/min through the column Hi Q sil C- 8(250 mm × 4.6 mm, 5 µm). The mobile phase was degassed prior to use by vacuum filtration through a 0.45µ membrane filter. Both drugs showed good absorbance at 230 nm, which was selected as wavelength for further analysis.

2.6. Preparation of standard stock solution

Accurately weighed quantity of LAF (100 mg) and RAB (100 mg) was transferred in to separate 100 ml volumetric flasks, dissolved in mobile phase and diluted to the mark with same to give stock solutions of concentration 1000µg/ml. Aliquots were withdrawn from standard Lafutidine and Rabeprazole Sodium stock solutions and transferred to 10 ml volumetric flasks. The volume was adjusted to the mark with mobile phase give working standard. of concentration

100µg/ml. For calibration curve, stock solutions of LAF and RAB were appropriately diluted to obtain working standard solutions in the increasing concentration range.

2.7. 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. (Table no1) The typical Chromatogram of LAF and RAB in tablet dosage form are shown in Fig 3.

Table 1 Analysis of marketed formulation

BRANDNAME: **LAFUMAC** *PLUS*	**Drugs**	**LabelClaim (mg)**	**Amount Found (mg)**	**% LabelClaim***	**S.D.**	**%RSD**
	LAF	10	9.9089	99.07	0.012	0.0129
	RAB	20	20.2273	101.47	0.216	0.213

*n=6 Mean of six determinations

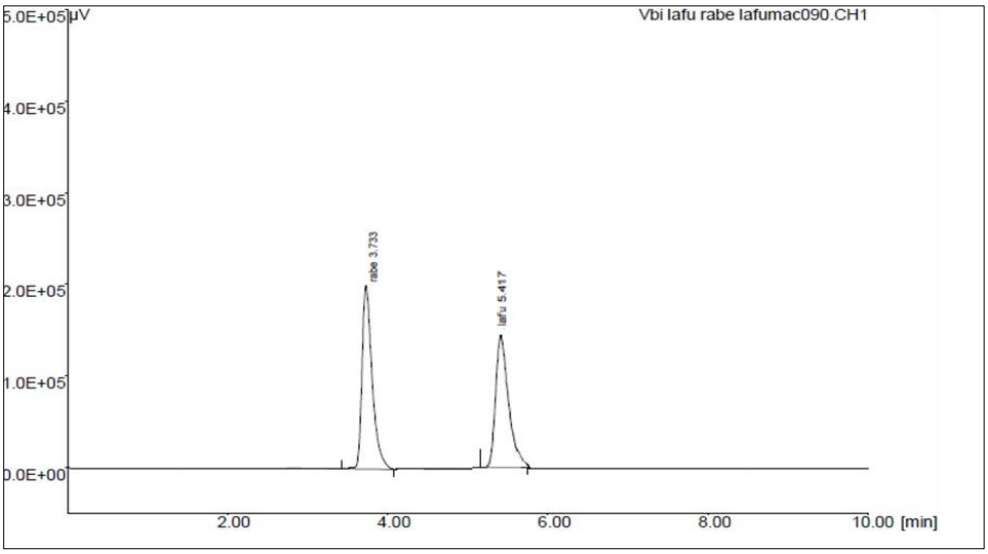

Figure 3 Analysis of marketed formulation

2.8. Development and validation of RP-HPLC method[20, 21]

2.8.1. 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. Linearity was found to be 10-60 µg/ml for LAF and 20-120 µg/ml for RAB.

2.8.2. 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.

2.8.3. 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.

2.8.4. Limit of Detection (LOD)

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

$$LOD = 3.3 \times (SD / Slope)$$

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

2.8.5. Limit of Quantification (LOQ)

The LOQ may be calculated as; $LOQ = 10 \times (SD / Slope)$

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

Slope = the mean slope of the 6 calibration curves.

2.8.6. 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.

3. Results and discussion

The proposed method was validated as per ICH guideline Q2R1. Results obtained for variousvalidation parameters are as follow:

Table 2 System suitability parameters for LAF and RAB

Sr. No.	Parameters	Rabeprazole Sodium	Lafutidine
1.	Retention time (min)	3.658	5.408
2.	No. of theoretical plates	3958.693	6164.964
3.	Tailing factor	1.73	1.49
4.	LOD (µg/ mL)	3.77964	3.81438
5.	LOQ(µg/ mL)	11.4535	11.5587
6.	Resolution	1.750	

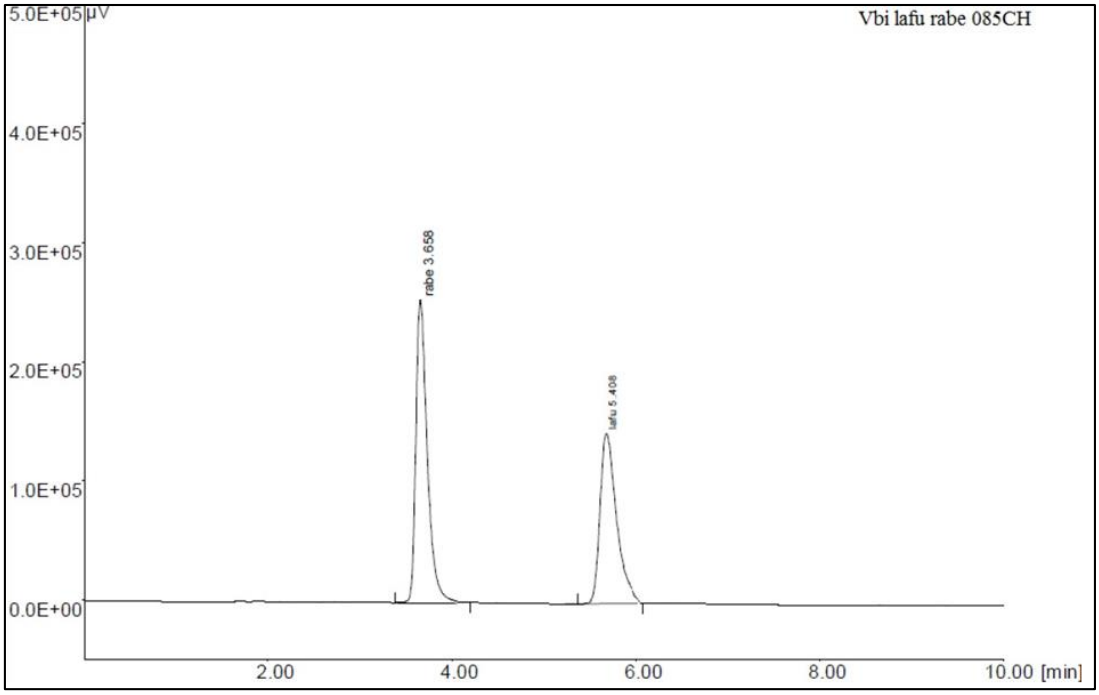

Figure 4 Chromatogram of standard LAF(100µg/ml) and RAB (200 µg/ml)

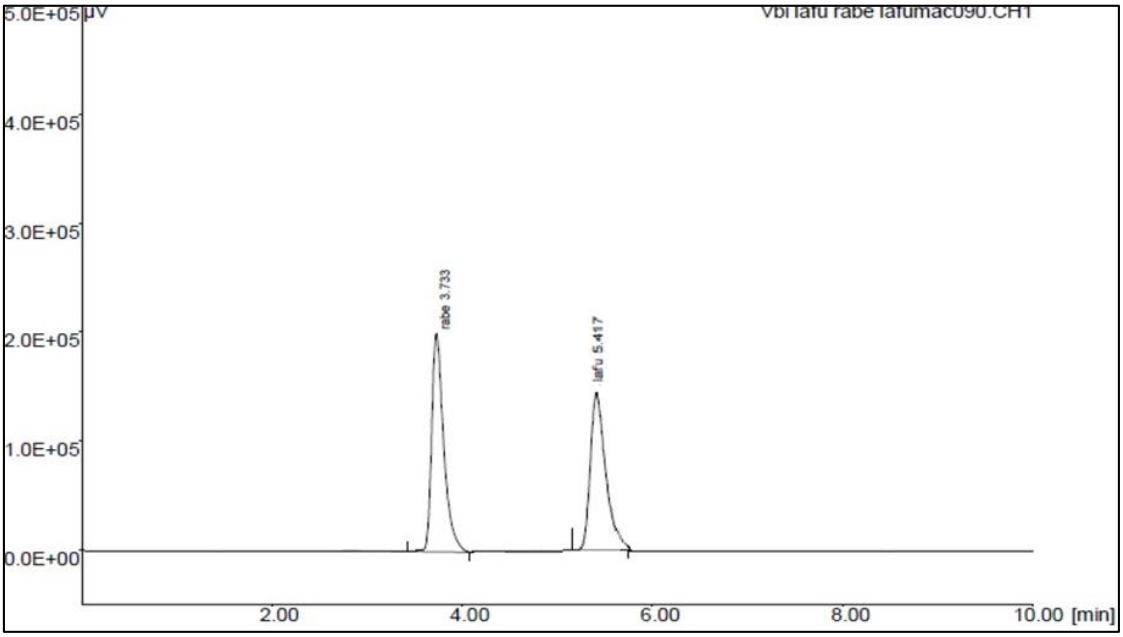

Figure 5 Chromatogram of Marketed formulation LAF(10µg/ml) and RAB (20 µg/ml)

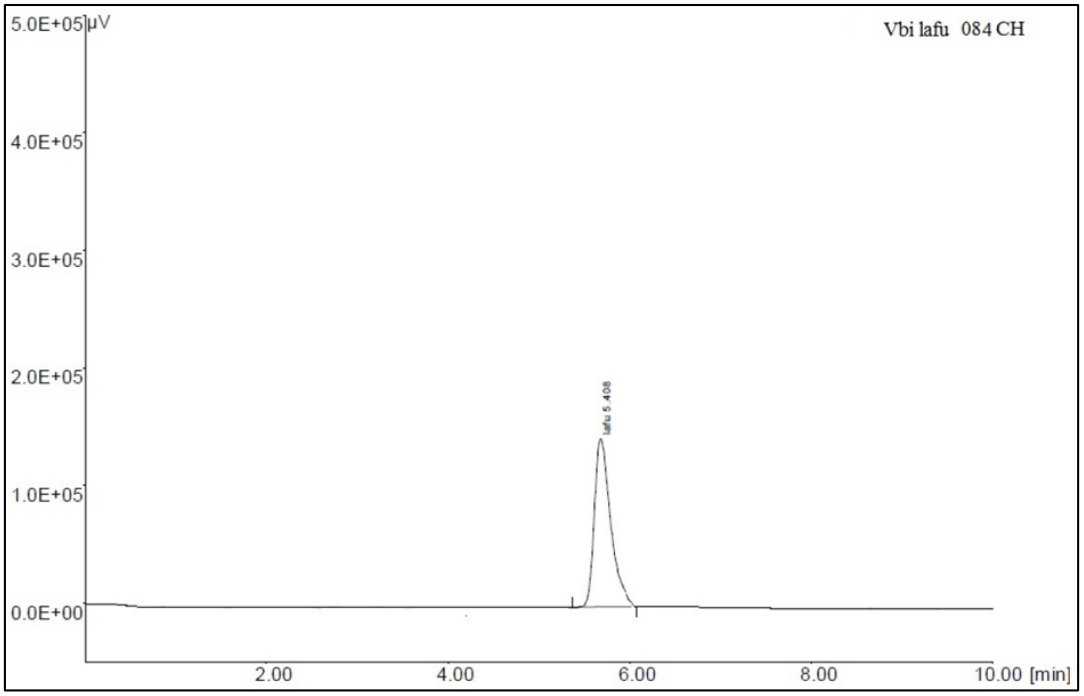

Figure 6 Chromatogram of standard LAF(100µg/ml)

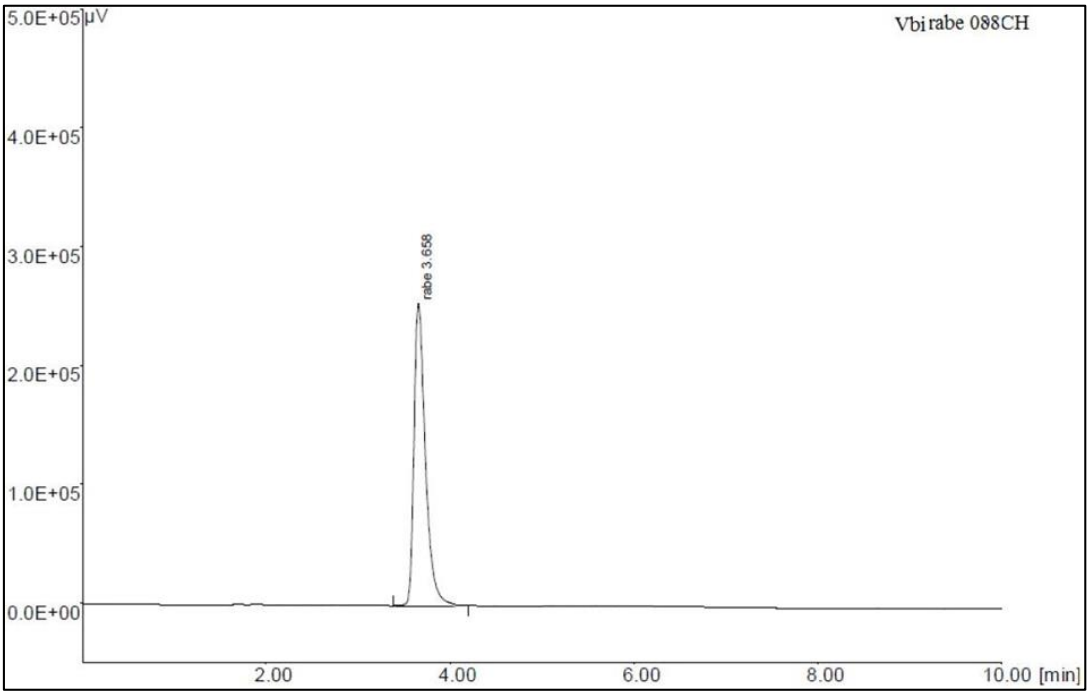

Figure 7 Chromatogram of standard RAB(200µg/ml)

Table 3 Linearity data of LAF and RAB

Sr. No.	Concentration (µg/ml)		Mean peak area (µv)	
	LAFU	RABE	LAFU	RABE
1	10	20	609143	1270353
2	20	40	1128824	2493983
3	30	60	1678298	3746508
4	40	80	2206141	5047704

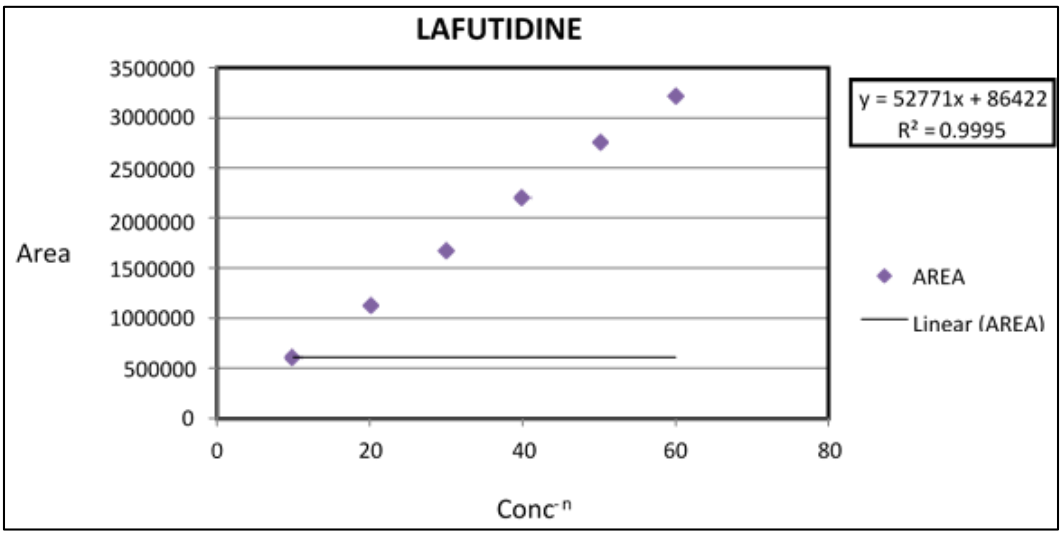

Figure 8 Calibration curve of LAF

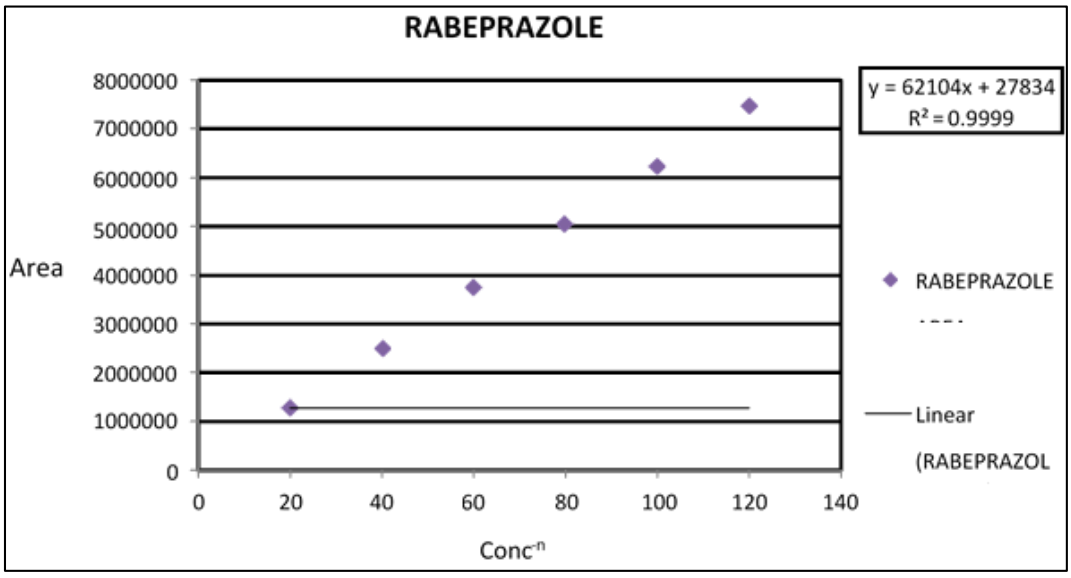

Figure 9 Calibration curve of RAB

Table 3 Linear regression data for calibration curves of LAFU and RAB

Parameters	**Lafutidine**	**Rabeprazole sodium**
Linearity range (µg/ml)	10-60	20-120
r2	0.999	0.999
Slope	52771	62104
Intercept	86422	28834

Table 4 Results of intra-day precision studies

Sr. No.	**Amount Claimed(µg/ml)**		**Amount Found(µg/ml)**		**% Amount Found**	
	LAFU	**RAB**	**LAFU**	**RAB**	**LAFU**	**RAB**
1	10	20	9.9088	20.3132	99.0888	101.556
2	10	20	9.9063	20.2951	99.0636	101.4757
3	10	20	9.9069	20.3289	99.0699	101.6447
4	10	20	9.9092	20.2614	99.0926	101.3074
5	10	20	9.9069	20.2273	99.0699	101.1369
6	10	20	9.9063	20.3416	99.0635	101.7082

Table 5 Results of inter-day precision studies

Sr. No.	**Amount Claimed(µg/ml)**		**Amount Found(µg/ml)**		**% Amount Found**	
	LAFU	**RAB**	**LAFU**	**RAB**	**LAFU**	**RAB**
1	10	20	9.9126	20.4742	99.1267	102.3711
2	10	20	9.9063	20.4400	99.0636	102.2003

Table 6 Results of Precision study for LAF and RAB

Parameter	**Drug**	**Amount of drug taken (mg/tablet)**	**Amount of drug found (mg)**	**% Mean amt. estimated***	**S.D.**	**% RSD**
INTRA-DAY	LAFU	10	9.9145	99.0621	0.02014	0.02033
	RABE	20	20.2614	101.46	0.2293	0.22601
INTER-DAY	LAFU	10	9.9069	99.0748	0.01278	0.0129
	RABE	20	20.3289	101.473	0.21627	0.2131

Table 7 Results of % Recovery studies for LAFU and RABE

Level of Recovery	Amt. Taken(µg/ml)		Amt. Added(µg/ml)		% Recovery		% MeanRecovery	
	LAFU	**RABE**	**LAFU**	**RABE**	**LAFU**	**RABE**	**LAFU**	**RABE**
80%	10	20	8	16	102.42	101.13	102.24	100.40
	10	20	8	16	102.03	100.13		
	10	20	8	16	102.29	99.13		
100%	10	20	10	20	99.91	100.05	101.28	100.05
	10	20	10	20	102.72	99.96		
	10	20	10	20	101.23	100.14		
120%	10	20	12	24	100.44	102.73	100.45	102.68
	10	20	12	24	100.45	102.66		
	10	20	12	24	100.46	102.66		

		80%	100%	120%
S.D.	LAFU	0.2018	1.4060	0.01048
	RAB	0.9998	0.883	0.0422
% RSD	LAFU	0.1974	1.3881	0.01043
	RAB	0.9985	0.882	0.0411

Table 8 Results of LOD and LOQ for Lafutidine and Rabeprazole Sodium

Parameter	LAFUTIDINE	RABEPRAZOLE SODIUM
LOD (µg/ml)	3.8143	3.7796
LOQ (µg/ml)	11.5587	11.4534

Table 9 Results of robustness studies

Factor	Level	Retention time of LAFU (min)	Retention time of RAB (min)
A: Flow Rate (ml/min)			
0.9	-1	3.80	5.59
1.0	0	3.65	5.40
1.1	+1	3.71	5.12
B: Percentage of Acetonitrile in the mobile phase (v/v)			
69	-1	3.60	5.38
70	0	3.65	5.40
71	+1	3.80	5.45

Table 10` Summary of Validation parameters

Sr.no.	Parameters	Lafutidine	Rabeprazolesodoium
1	Linearity Range	10-60	20-120
2	Correlation coefficient(R^2)	0.999	0.999
3	Precision (%RSD)		
	1. Intraday precision(n=6)	0.0129	0.2131
	3. Interday precision(n=6)	0.0442	0.4419
4	Accuracy (% Recovery)(n=3)		
	Level 1 (80%)	102.24	100.40
	Level 2 (100%)	101.28	100.05
	Level 3 (120%)	100.45	102.68
5	LOD (µg/ml)	3.8143	3.7796

4. 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.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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