Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

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

HIREN D. ANTALA[1*]

[1]Department of Pharmaceutical Quality Assurance, Noble Pharmacy College, Junagadh, Gujarat, India. Email: hirenantala21@gmail.com

Received: 06 May 2013, Revised and Accepted: 12 Aug 2013

ABSTRACT

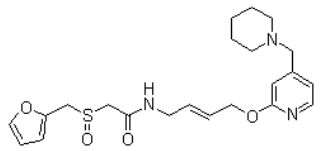
Objective:A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Lafutidineand RabeprazoleSodium in combined dosage form.Method:A Thermo Hypersil, C18 column, 250 mm × 4.6 mm, 5µm in Isocratic mode with mobile phase containing Acetonitrile:0.02M Pottasiumdihydrogenortho phosphate pH 7.2 (50:50 v/v) was used.The flow rate was 1.5ml/min and effluents were monitored at 215 nm. The retention time of Rabeprazole Sodium and Lafutidinewas found to be 2.99 min and 8.13 min respectively.Result:The different analytical parameters such as accuracy, linearity, precision, robustness, limit of detection(LOD), limit of quantification (LOQ) were determined according to the International Conference on Harmonization (ICH) Q2R1 guidelines. The detector response was linear in the range of 40-120 µg/ml, 80-240 µg/ml for Lafutidineand RabeprazoleSodium respectively.Conclusion: The Proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Pharmaceutical dosage form.

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

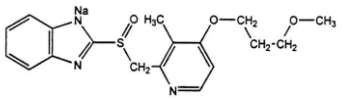
INTRODUCTION[1]

Lafutidine is chemically 2-(furan-2-ylmethylsulfinyl)-N-[4-[4-(piperidin-1-ylmethyl) pyridin-2-yl]oxybut-2-enyl]acetamide. Lafutidine is not official in any pharmacopoeias. Lafutidine is the new generation H2-receptor antagonist. It blocks the production of acid by acid producing cells in the stomach and blocks histamine H_2-receptors in the stomach and prevents histamine mediated gastric acid secretion. It is indicated in hyperacidity, NSAID induced gastritis, gastric and duodenal ulcers and also used as preanesthetic medication. Apart from H2-receptor blockade activity, it has additional gastro protective action. Therefore not only inhibit acid secretion but also provide gastric mucosal protection.

Rabeprazole Sodium is chemically 2-[[[4-(3-Methoxypropoxy)-3-Methyl-2-Pyridinyl]-Methyl]Sulfinyl]-1H-Benzimidazole Sodium salt. Rabeprazole sodium (RBP) 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 Gastroesophageal reflux disease (GERD) and duodenal ulcers. It has a faster onset of action and lower potential for drug interaction compared to Omeprazole.



Chemical Structure of Lafutidine



Chemical Structure of Rabeprazole Sodium

Literature survey[15-21] revealed that a number of analytical methods have been reported for the estimation of Lafutidine(LAF) and Rabeprazole Sodium(RAB) in individual and combination with other drugs are spectrophotometry, HPLC, RP-HPLC, HPTLC,but not even single method was reported for the simultaneous estimation of LAF and RAB in their combined dosage form.

MATERIALS AND METHODS

Instrument

HPLC Model**:** Analytical Technologies Limited

Sample injector**:** S-5200

Pump**:** P-3000

Fixed Capacity Loop**:** 20 µl

Detector**:** UV-3000

Column**:** Thermo Hypersil, C18 column, 250 mm × 4.6 mm, 5µm

Other instruments**:**

Double beam UV-visible spectrophotometer (SHIMADZU, Model 1800)

Ultrasonicator**:** PEI, Ultra sonic bath

pH meter**:**Chemiline, CL-180, Labline technology pvt ltd.

Electronic analytical balance **:**(AUX-220), Uni Bloc-SHIMADZU

Volumetric flask: 10, 25, 50, 100 ml (RASAYAN-Borosilicate glass)

Pipettes**:** 1, 2, 5, 10 ml,

All instruments and glass wares were calibrated.

Reagents and chemicals

Pure drug samples of LAF and RAB were provided as a gift sample from Alkem Laboratories Ltd, MumbaiandCadila Pharmaceuticals Ltd, Ahmedabad respectively. Acetonitrile and Waterwere of HPLC grade and collected from E. Merck, Darmstadt, Germany. Pottasium dihydrogenortho phosphate and Sodium hydroxide were analytical reagent grade supplied by Fischer Scientific Chemicals. Water.

Marketed formulation

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

Preparation and Selection of Mobile phase

The preliminary isocratic studies on a reverse phase C18 column with different mobile phase combination of Acetonitrile and Pottasiumdihydrogenortho phosphate buffer were studied for simultaneous estimation of both drugs. The optimal composition of mobile phase determined to be Acetonitrile**:** 0.02M Pottasiumdihydrogenortho phosphate pH 7.2 (50:50 v/v) and filtered through 0.45µ membrane filter.

Preparation of standard stock solution

Accurately weighed quantity of LAF (100 mg) and RAB (100 mg) was transferred in to two separate 100 ml volumetric flasks,

dissolved in diluents(mobile phaseAcetonitrile**:**0.02M Pottasiumdihydrogenortho phosphate pH 7.2 (50:50 v/v))and diluted to the mark with same solvent. (Stock solutions: 1000µg/ml of LAFand 1000µg/ml of RAB).Appropriate volume of aliquots from standard Lafutidine and Rabeprazole Sodium stock solutions were transferred to same volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with mobile phase give a solution containing 40, 60, 80,100,120µg/ml LAF and 80,120,160,200,240 µg/ml RAB.

Preparation of Sample solution

Twenty Capsules were weighed and content crushed to obtain a fine powder. An accurately weighed powder equivalent to about 100 mg of Lafutidine and 200 mg of Rabeprazole Sodium was transferred to 100 ml volumetric flask and the volume was made up to the mark using mobile phase. The solution was sonicated for 20 minutes. The solution was filtered through whatman Filter Paper No.42. First few ml of filtrate were discarded. 8.0 ml of the solution from above filtrate was diluted to 100 ml with mobile phase to make the final concentration of working sample equivalent to 100% of target concentration.

Chromatographic Conditions

The mobile phase, phaseAcetonitrile**:**0.02M Pottasiumdihydrogen ortho phosphate pH 7.2 (50:50 v/v) pumped at a flow rate of 1.5 ml/min through the column Thermo Hypersil, C18 column, 250 mm × 4.6 mm, 5µm. The mobile phase was degassed prior to use under vacuum by filtration through a 0.45µ membrane filter. Both drugs showed good absorbance at 215 nm, which was selected as wavelength for further analysis.

Development and Validation of Rp-Hplc Method [11,12]

System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of Lafutidine and Rabeprazole Sodium. Various chromatographic parameters such as retention time, peak area, tailing factor, theoretical plates of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Specificity

Specificity test determines the effect of excipients on the assay result. To determine the specificity of the method, standard sample of Lafutidine and Rabeprazole Sodium were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

Linearity

Linearity of the method was determined by constructing calibration curves.

Standard solutions of Lafutidine and Rabeprazole Sodium of different concentrations level (50%, 75%, 100%, 125% and 150%) 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 curves and correlation coefficients.

Accuracy (Recovery Studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard Lafutidine and Rabeprazole Sodium were added to pre- analyzed samples and were subjected to the proposed HPLC method.

Precision

Precision of the method was determined by performing intraday variation, interdayvariation and method repeatability studies.Three replicates of three different concentrations, were injected on the same day and the percent relative standard deviations (%RSD) were calculated to determine intra-day precision. These studies were also repeated on three consecutive days to determine inter-day precision.Repeatability study was performed by injecting the six replicates of the same concentrationand the percent relative standard deviations (%RSD) were calculated.

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 ± 0.2 change in flow rate andpH.

Limit of Detection (LOD)

The LOD is estimated from the set of 6 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- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Limit of Quantification (LOQ)

The LOQ is estimated from the set of 6 calibration curves used to determine methodlinearity. 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.

Analysis of marketed formulation

Twenty Capsules were weighed and content crushed to obtain a fine powder. An accurately weighed powder equivalent to about 100 mg of Lafutidine and 200 mg of Rabeprazole Sodium was transferred to 100 ml volumetric flask and the volume was made up to the mark using mobile phase. The solution was sonicated for 20 minutes. The solution was filtered through whatman Filter Paper No.42. First few ml of filtrate were discarded. 8.0 ml of the solution from above filtrate was diluted to 100 ml with mobile phase. The prepared sample solution was chromatographed for 10 minutes run time using mobile phase at 215 nm and a flow rate of 1.5 ml/min. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated by fitting peak area responses into the equation of the straight line representing the calibration curves for Lafutidine and Rabeprazole sodium.

RESULTS AND DISCUSSION

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

Table 1: Result of System suitability for LAF and RAB

S. No.	Standard Response (mAU*S)	
	Lafutidine	Rabeprazole Sodium
1	82911.632	86189.498
2	82935.192	86632.982
3	83325.415	86557.138
4	82963.872	86720.762
5	83291.154	86804.424
6	83155.868	86138.766

Average	**83097.166**	**86507.261**
SD	**185.283**	**278.906**
%RSD	**0.22**	**0.32**
Retention Time	**8.12**	**2.98**
Theoretical plates	**12097**	**6153**
Tailing Factor	**0.874**	**1.078**
Resolution	**23.05**	

Table 2: Result of Solvent suitability

Time	**Standard Response (mAU*S)**	
	LAF	**RAB**
0 hrs	81575.283	86441.938
6 hrs	81800.450	86793.506
12 hrs	81294.776	86777.727
18 hrs	81667.812	86835.935
24 hrs	81684.639	86525.503
Average	81604.592	86674.9218
SD	190.7941418	178.300169
% RSD	0.23	0.21

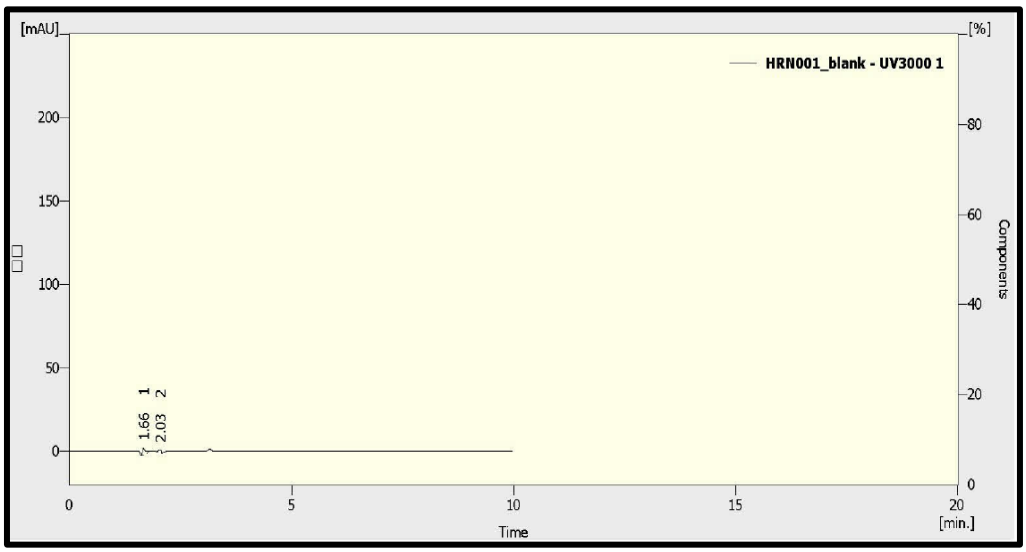


Fig. 1: Chromatogram of Diluent for Specificity

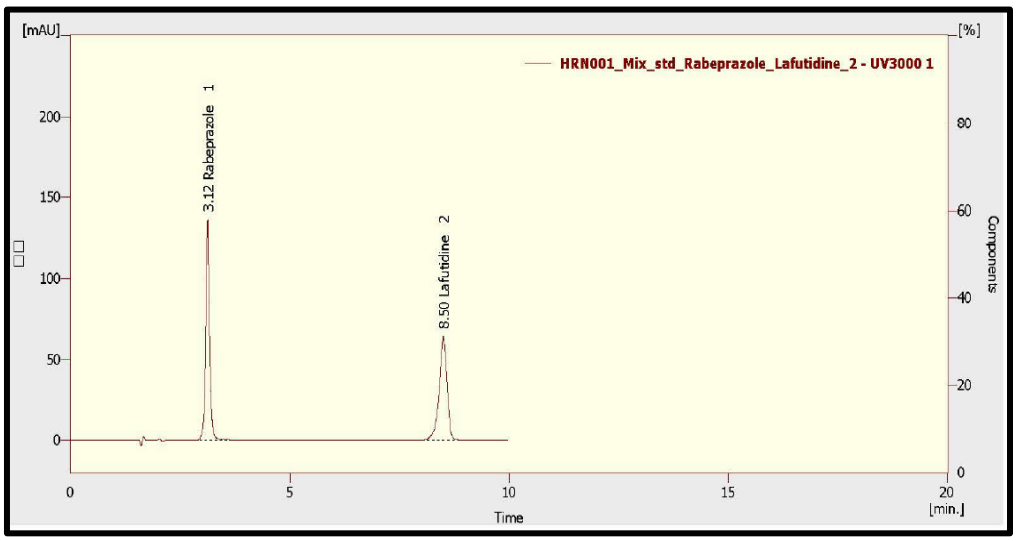


Fig. 2: Chromatogram of standard LAF(80µg/ml) and RAB (160 µg/ml)for Specificity

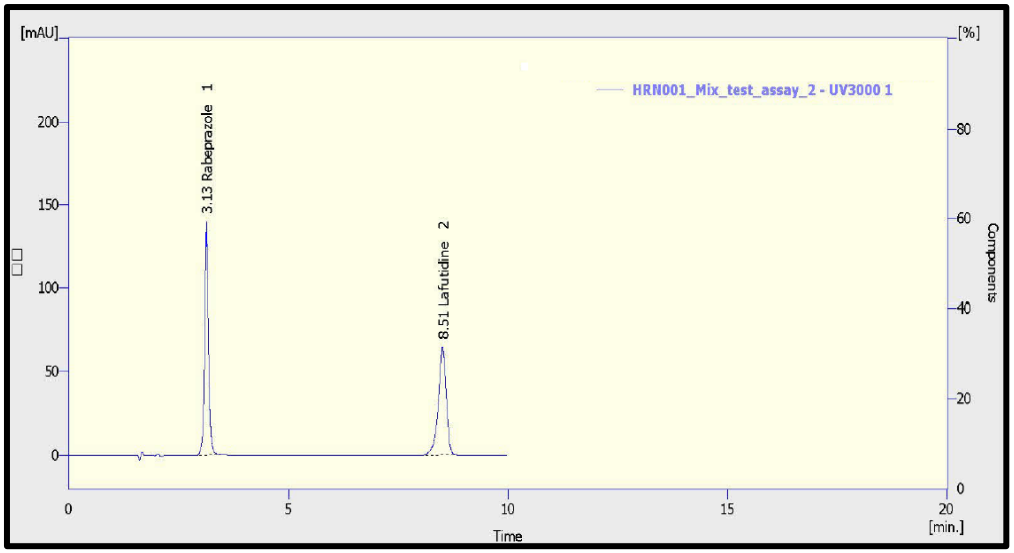


Fig. 3: Chromatogram of Marketed formulation (*LAFUMAC PLUS*)LAF(80µg/ml) and RAB (160 µg/ml)for Specificity

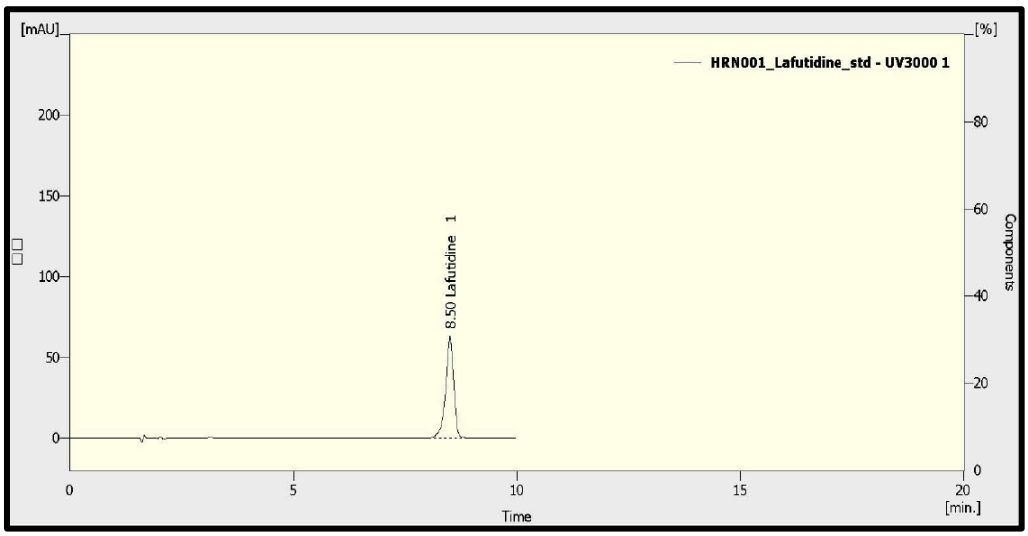


Fig. 4: Chromatogram of standard LAF(80µg/ml)for Specificity

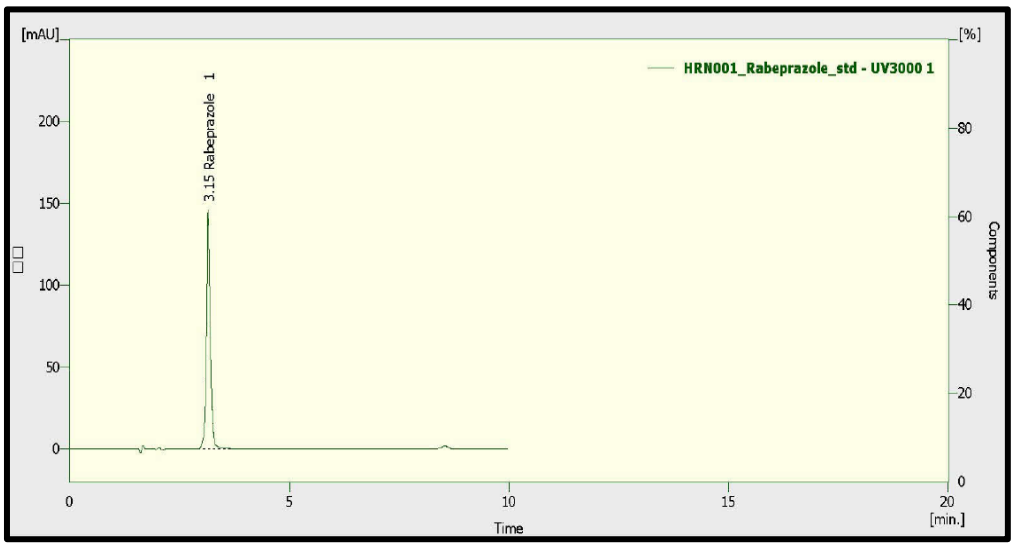


Fig. 5: Chromatogram of standard RAB(160µg/ml)for Specificity

Table 3: Result of Linearity for LAF and RAB

Lafutidine		Rabeprazole Sodium	
Concentration (µg/ml)	**Mean Peak Area* (mAU*S)**	**Concentration (µg/ml)**	**Mean Peak Area* (mAU*S)**
40	39679.483 ±787.143	80	43851.262 ±522.660
60	60948.4 ±1008.715	120	64273.594 ±629.936
80	82231.1 ±1435.660	160	87048.8 ±47.423
100	99759.9 ±382.012	200	108265.3 ±67.556
120	121430.7 ±609.465	240	126982.4 ±67.113

***n=6**

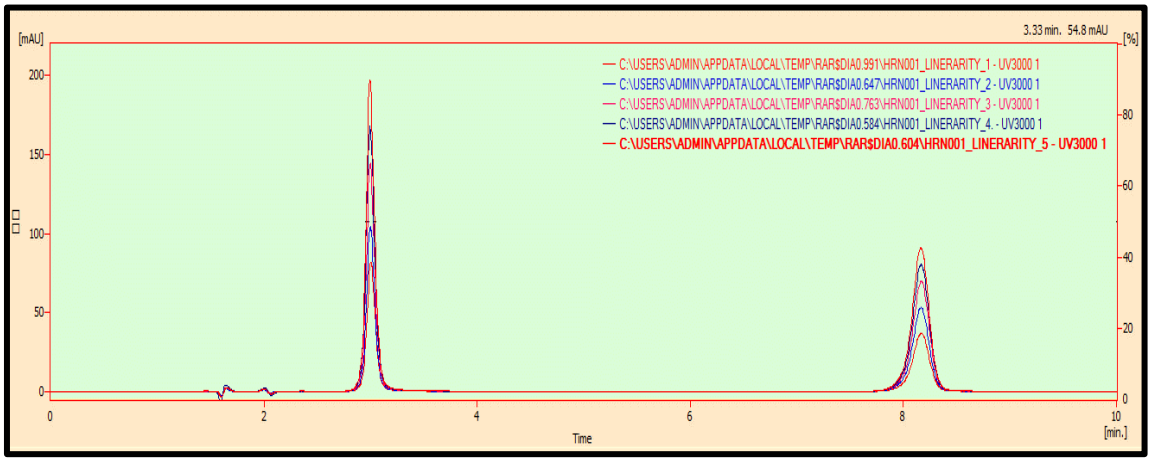


Fig. 6: Chromatogram of Linearity for LAF (40-120µg/ml) and RAB (80-240µg/ml)

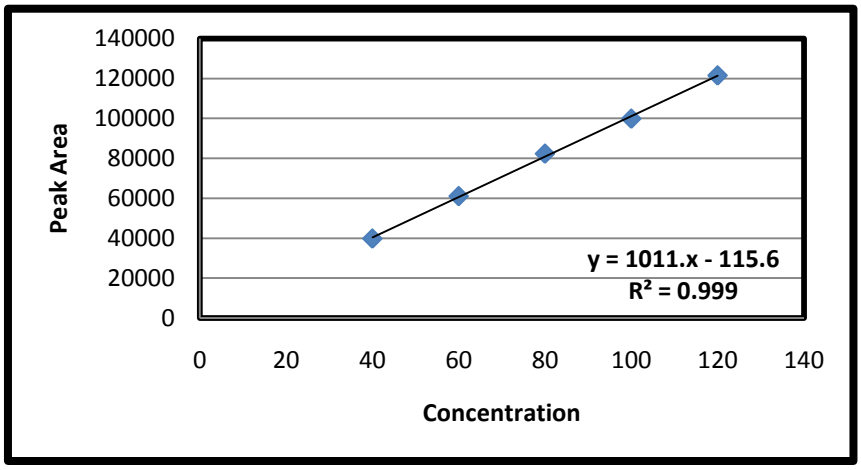


Fig. 7: Calibration curve of LAF

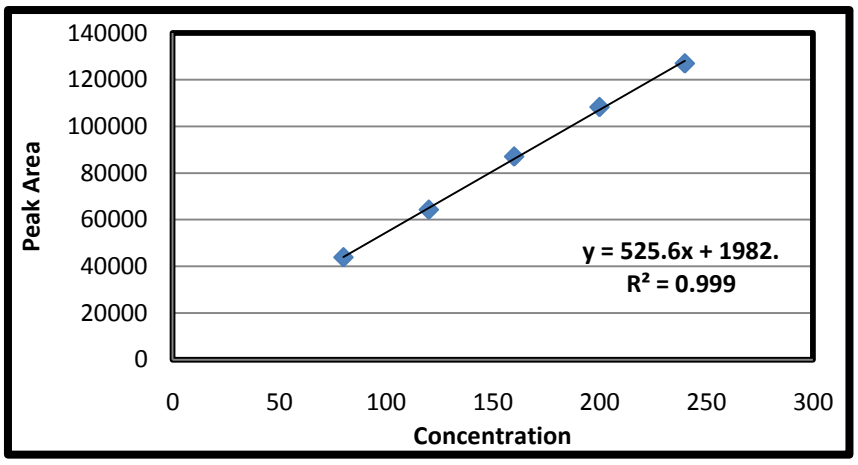


Fig. 8: Calibration curve of RAB

Table 4: Results of Repeatability for LAF and RAB

Lafutidin		Rabeprazole Sodium	
Concentration (µg/ml)	**Peak Area (mAU*S)**	**Concentration (µg/ml)**	**Peak Area (mAU*S)**
80	83732.9	160	88050.34
80	83538.84	160	88546.27
80	83359.02	160	88658.05
80	83656.06	160	88825.36
80	83422.77	160	88861.64
80	83688.58	160	88298.49
Mean	83566.36	**Mean**	88540.024
SD	151.711	**SD**	315.263
%RSD	0.18	**%RSD**	0.36

Table 5: Results of Intraday precision for estimation of LAF and RAB

LAF Concentration (µg/ml)	Peak Area (mAU*S)* ±S.D.	%RSD	RAB Concentration (µg/ml)	Peak Area (mAU*S)* ±S.D.	%RSD
40	40322.4 ±158.2846	0.4	80	44822.68 ± 305.3427	0.68
80	80821.02 ± 718.9966	0.89	160	84430.75 ± 740.3407	0.88
120	115111.6 ±348.03684	0.3	240	123717.2 ± 955.2996	0.77

***n=3**

Table 6: Results of Interday precision for estimation of LAF and RAB

LAF Concentration (µg/ml)	Peak Area (mAU*S)* ±S.D.	%RSD	RAB Concentration (µg/ml)	Peak Area (mAU*S)* ±S.D.	%RSD
40	39793.867 ±571.38	1.43	80	44286.057 ±622.349	1.4
80	81717.123 ±1182.227	1.45	160	85817.679 ±1330.04	1.55
120	114050.3413 ±1267.13	1.11	240	122723.674 ±1815.6	1.47

***n=3**

Table 7: Results of Accuracy (%Recovery) for LAF Concentration of Preanalyzed sample of Lafutidine: 39.82 µg/ml

Level of recovery	Amt of Std LAF spiked (µg/ml)	Total amt of LAF (µg/ml)	Amt of LAF found (µg/ml)	Amount of LAF recovered (µg/ml)	% Recovery	Mean % recovery ± SD
	20	60	60.57	20.75	103.7	102.98
50%	20	60	60.40	20.58	102.9	±
	20	60	60.29	20.47	102.35	0.6788
100%	40	80	79.47	39.65	99.13	100.81
	40	80	80.85	41.03	102.57	±
	40	80	79.85	40.03	100.75	1.72
150%	60	100	97.56	57.74	96.23	97.42
	60	100	98.79	58.97	98.28	±
	60	100	98.47	58.65	97.75	1.064

Table 8: Results of Accuracy (%Recovery) for RAB Concentration of Preanalyzed sample of Rabeprazole Sodium: 80.84 µg/ml

Level of recovery	Amt of Std RAB spiked (µg/ml)	Total Amt of RAB (µg/ml)	Amt of RAB found (µg/ml)	Amount of RAB recovered (µg/ml)	% Recovery	Mean % recovery ± SD
50%	40	120	120.97	40.13	100.33	98.98
	40	120	119.90	39.06	97.65	±
	40	120	120.42	39.58	98.95	1.34
100%	80	160	160.18	79.34	99.18	98.21
	80	160	158.39	77.55	96.94	±
	80	160	159.64	78.8	98.5	1.15
150%	120	200	198.81	117.97	98.31	99.69
	120	200	202.21	121.37	101.14	±
	120	200	200.37	119.53	99.61	1.42

Table 9: Results of LOD for Lafutidine and Rabeprazole Sodium

Parameters	Lafutidine	Rabeprazole Sodium
Mean Slope (n=6)	1011.05	525.6
SD (n=6)	635.9232459	478.0856269
LOD (µg/ml)	2.08	3.0

Table 10: Results of LOQ for Lafutidine and Rabeprazole Sodium

Parameters	Lafutidine	Rabeprazole Sodium
Mean Slope (n=6)	1011.05	525.6
SD (n=6)	635.9232459	478.0856269
LOD (µg/ml)	6.3	9.09

Table 11: Result of Robustness byChange in Flow Rate

S. No.	Flow rate 1.7 ml/min (+0.2ml/min)		Flow rate 1.3 ml/min (-0.2ml/min)	
	LAF Area (mAU*S)	RAB Area (mAU*S)	LAF Area (mAU*S)	RAB Area (mAU*S)
1	77295.45	82812.66	97235.39	102474.1
2	76396.4	81912.7	96245.5	101474.2
3	78194.5	83712.5	98239.5	102474.6
4	78597.73	82548.62	97258.63	102264.36
5	76583.25	83358.25	97525.54	103925.3
6	76325.82	81995.25	96840.25	101828.5
Mean	77232.191	82723.330	97224.11767	102406.8
SD	973.2334	722.122	667.5953024	841.360345
% RSD	1.26	0.87	0.69	0.82

Table 12: Result of Robustness byChange in pH

S. No.	pH 7.4 (+0.2)		pH 7.0 (-0.2)	
	LAF Area (mAU*S)	RAB Area (mAU*S)	LAF Area (mAU*S)	RAB Area (mAU*S)
1	81460.549	86906.462	83008.84	89239.538
2	81621.312	85231.241	81940.257	87710.668
3	81575.283	86441.938	82263.401	88190.382
4	81150.32	86605.32	82568.52	87725.63
5	81825.68	86523.36	82458.86	88024.29
6	81152.55	85826.35	83052.2	89125.63
Mean	81464.28233	86255.7785	82548.67967	88336.023
SD	269.5741345	614.1513529	430.1868266	681.4478446
% RSD	0.33	0.71	0.52	0.77

Analysis of marketed formulation

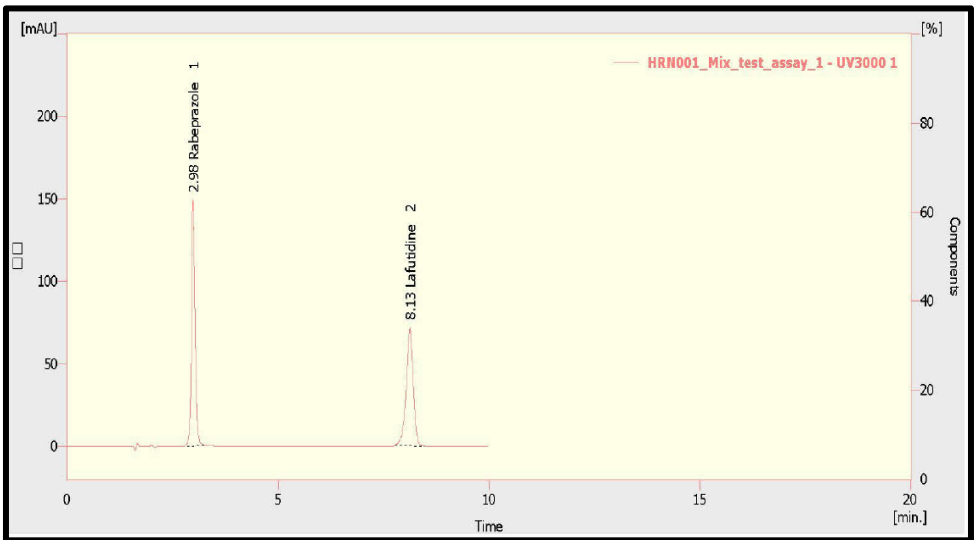


Fig. 9: Chromatogram of marketed formulation (*LAFUMAC PLUS*)

Table 13: Analysis of marketed formulation

Brand name	Drugs	Label Claim (mg)	Amount Found (mg)	% Label Claim*	S.D.	%RSD
LAFUMAC	LAF	10	10.196	101.96%	0.309994624	0.30
PLUS	RAB	20	20.38	101.90%	0.48354593	0.47

***n=**

Table 14: Summary of Validation parameters

S. No	Parameters	Lafutidine	Rabeprazole Sodium
1	Linearity Range	40-120µg/ml	80-240µg/ml
2	Correlation coefficient (R^2)	0.999	0.999
3	Precision (%RSD)	0.18	0.36
	1.Repeatability(n=6)	0.53	0.78
	2. Intraday precision (n=3)	1.33	1.47
	3. Interday precision (n=3)		
4	Accuracy (% Recovery) (n=3)	102.98	98.98
	Level 1 (80%)	100.81	98.21
	Level 2 (100%)	97.42	99.69
	Level 3 (120%)		
5	LOD (µg/ml)	2.08	3.0
6	LOQ (µg/ml)	6.3	9.09
7	Specificity	Specific	
8	Robustness	Complies < 2.0 %RSD	
9	Solvent suitability	Complies < 2.0 %RSD	
10	Assay	101.96%	101.90%

CONCLUSION

The proposed method gives specific, accurate, precise, and rapid results for determination of LAF and RAB in combined formulation. The proposed method was also successfully applied for the estimation of these drugs in commercial dosage form.

ACKNOWLEDGEMENT

It is great pleasure for me to acknowledge my parents and all of my friends, who have contributed towards the conception, origin and nurturing and successful completion of this research work.

REFERENCES

1. Castell D, Murray J, Tutuian R, Arnold R, "Review article:the pathophysiology of gastro-oesophageal refluxdisease-esophageal manifestations", Aliment PharmacolTher, 20thEdn., 2004, pp 14-25
2. Shethi PD, HPLC- Quantitative analysis of pharmaceutical formulations; 1stEdn; CBS publishers & Distributors, New Delhi, 2001, pp 3-141.
3. Ahuja S, and Michael W, Hand book of pharmaceutical analysis by HPLC; 1stEdn, Elsevier Academic Press, 2005, pp 44-54.
4. Kazakevich Y, and Lobrutto R, HPLC for Pharmaceutical Scientists; 1stEdn, John Wiley & Sons Inc, New Jersey, 2007, pp 987-1051.
5. Snyder LR, Kirkland JL, and Glajch JL, Practical HPLC Method Development; 2ndEdn, Wiley, New York, 1997, pp 1-19.
6. Braithwatte A, Smith FJ, Chromatographic Methods; 5thEdn, Kluwer Academic Publisher, London, 1996, pp 27-29.
7. SGE Analytical Science, "How do I Develop an HPLC Method?", Technical Article,2001, http://www.sge.com/uploads/db/Rl/dbr/y8NOG4YTZB2MDHivmfg /Ta-0010-h.pdf
8. Valko K, Snyder LR, and GlajchJ,"Retention in reversed-phase liquid chromatography as a function of mobile phase composition." J. Chromatogr., A, 1993, 656(2), 501–20.
9. Snyder LR and Schunk TC, "Retention mechanism and the role of the mobile phase in normal-phase separation on amino-bonded-phase columns." Analytical Chemistry, 1982, 54(11),pp. 1764–72.
10. Huber JFK, Vander Linden R, Ecker E, Oreans M, "Column switching in high pressure liquid chromatography." J. Chromatogr., A, 1973, 83(2), pp.267-71.
11. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1). 2005.
12. United States Pharmacopoeia 30, Validation of Compendial Methods, Rockville MD USA, United States Pharmacopeial Convention Inc; 2007, pp 1225.
13. Robert AN, Alfred HW. Pharmaceutical Process Validation An international, 3rd ed, Marcel Dekker, New York.2005; pp.515-22.
14. The Merck Index, An encyclopedia of chemicals, drugs and biologicals, 14th ed., published by Merck Research Laboratories, pp.5344-5347
15. Sumithra M, Shanmuga P, Srinivasulu K,"Analytical Method Development and Validation of Lafutidine in Tablet dosage form by RP-HPLC", International Journal of ChemTech Research ,3(3), July-Sept 2011, pp. 1403-1407
16. SanthoshaB, Ravindranath A, Sundari C, " Stability indicating RP-HPLC method for the simultaneous estimation of Domperidone and Lafutidine in bulk and the pharmaceutical dosage form", International Journal of Pharmacy and Pharmaceutical Sciences, 4(4), 2012, pp.589-594
17. Reddy M, Bodepudi C, Shanmugasundaram P, "Method Development and Validation of Rabeprazole in Bulk and Tablet dosage form by RP-HPLC Method" ,International Journal of ChemTech Research 3(3) , July-Sept 2011, pp.1580-1588
18. Heda A, Gadade D, Kathiriya J and Puranik P, "HPLC Method Development and Validation for Simultaneous Analysis of Diclofenac Sodium and Rabeprazole Sodium", E-Journal of Chemistry, 2010, pp. 386-390
19. Padmalatha M, Snehalatha T, Ramya S, Kanakadurga M, "A simple and validated RP-HPLC method for the simultaneous estimation of Rabeprazole and Levosulpiride in bulk and pharmaceutical dosage forms", International Research Journal of Pharmaceutical and Applied Sciences, Int. Res J Pharm. App Sci., 2012, 2(2), pp.99-106
20. Akkamma H, Sai Kumar S, Sreedhar C, Rao S, Kanagala S, Manogna K, "Development and Validation of New Analytical Method for Simultaneous Estimation of Domperidone and Rabeprazole in Pharmaceutical Dosage Forms", Research Journal of Pharmaceutical, Biological and Chemical Sciences: july-sep-2012,3(3),pp. 705-71.