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Full Length Original Research Paper

Simultaneous estimation of Rabeprazole Sodium and Lafutidine in Bulk and Pharmaceutical dosage form by RP-UPLC Method

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Abstract:

Objective: A reversed phase stability-indicating Ultra-Performance Liquid Chromatographic (UPLC) assay method was developed and validated for quantitative determination of Rabeprazole Sodium & Lafutidine in bulk drug and marketed dosage form. Method: a Phenomanex, C18 column, 150 × 2.5 mm, in Isocratic mode with mobile phase containing Acetonitrile: buffer (0.01 M Potassium di-hydrogen orthophosphate) pH 6.8 (60:40% v/v) was used. The flow rate was 1.2ml/min and detection was made at 215 nm. The retention time of Rabeprazole Sodium and Lafutidine was found to be 3.1 min and 5.8 min respectively. The developed method was validated using various analytical parameters *viz.*, accuracy, linearity, precision, specificity, system suitability, robustness according to International Conference on Harmonization (ICH) Q2R1 guidelines. Result: The detector response was linear in the range of 40-120 µg/ml, 80-240 µg/ml for Lafutidine and Rabeprazole Sodium respectively and the readings of all the validation parameters are within the acceptance criteria. Conclusion: The Proposed UPLC method is reliable for the simultaneous estimation of Rabeprazole Sodium & Lafutidine in bulk and other solid dosage forms.

Keywords: Rabeprazole Sodium, Lafutidine, RP-UPLC, ICH guidelines.

Introduction

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 H_2-receptor antagonist. It blocks the production of acid by inhibiting acid producing cells in the stomach and blocks histamine H_2-receptors in the stomach and prevents histamine mediated gastric acid secretion. It is advised in hyperacidity, NSAID induced gastritis, gastric and duodenal ulcers and also used as preanesthetic medication. Apart from H_2-receptor blockade activity, it has additional gastro protective action. Therefore it is used not only to inhibit acid secretion but also to provide gastric mucosal protection. [1, 2]

Rabeprazole Sodium is chemically 2-(((4-(3-Methoxypropoxy)-3- Methyl-2-Pyridinyl)-Methyl) Sulfinyl)-1H-Benzimidazole Sodium salt. Rabeprazole sodium (RAB) is a Potent Proton Pump inhibitor that suppresses 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. [3, 4]

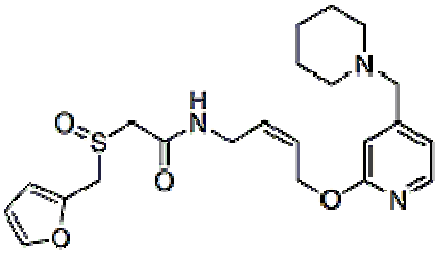

Figure 1: Chemical Structure of Lafutidine

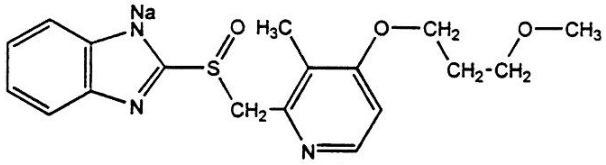

Figure 2: Chemical Structure of Rabeprazole Sodium

UPLC is a rising chromatographic separation technique whose packing materials have smaller particle size lesser than 2.5µm which improves the speed, resolution and sensitivity of analysis. When many scientists experienced separation barriers with conventional HPLC, UPLC extended and expanded the utility of chromatography. The main advantage is reduction of analysis time which also reduces solvent consumption. The analysis time, solvent consumption and analysis cost are very important factor in many analytical laboratories. The time consumption during optimization of new methods can also be greatly reduced. This results in many analysis in a day and quick results which is of very importance to the industries and research laboratories. [5, 6, 7]

Literature survey [8-15] 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 by 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 by RP-UPLC method.

Materials and Methods

Instrument

UPLC Model: Mexera Shimadzu UPLC system

Sample injector: S-5200

Pump: P-3000

Fixed Capacity Loop: 10 µl

Detector: PDA detector

Column: Phenomenex, C18 column, 150 mm.

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 Macleods & Ipca Laboratories Ltd, Mumbai, respectively. Acetonitrile and Water were of HPLC grade and procured from E. Merck, Darmstadt, Germany. Potassium di-hydrogen orthophosphate and analytical reagent grade supplied by Fischer Scientific Chemicals.

Marketed formulation

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

Preparation and Selection of Mobile phase [4-10]

The preliminary isocratic studies on a reverse phase phenomenex C18 column with different mobile phase combination of Acetonitrile and Potassium di-hydrogen orthophosphate buffer were studied for simultaneous estimation of both drugs. The optimal composition of mobile phase determined to be Acetonitrile : 0.01M Potassium di-hydrogen orthophosphate pH 6.8 (60:40% v/v) and filtered through 0.22µ membrane filter.

Preparation of standard stock solution [11-12]

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 phase Acetonitrile:0.01M Potassium di-hydrogen orthophosphate pH 6.8

(50:50 v/v))and diluted to the mark with same solvent (concentration of stock solutions is 1000µg/ml of LAF and 1000µg/ml of RAB respectively). Appropriate volume of aliquots from standard lafutidine and rabeprazole sodium stock solutions were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with mobile phase to give solution(s) containing 40, 60, 80,100,120µg/ml LAF and 80,120,160,200,240 µg/ml RAB.

Preparation of Sample solution [11-12]

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.

Optimized Chromatographic Conditions

The mobile phase, Acetonitrile : 0.01M Potassium di-hydrogen orthophosphate pH 6.8 (60:40% v/v) pumped at a flow rate of 1.2 ml/min through the column Phenomanex, C18 column, 150 mm. The mobile phase was degassed prior to use under vacuum by filtration through a 0.22µ membrane filter. Both drugs showed good absorbance at 215 nm (isobestic point), which was selected as wavelength for further analysis.

Development and Validation of RP-UPLC Method [11-18]

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 UPLC method.

Precision

Precision of the method was determined by performing intraday variation, interday variation and method repeatability studies. Three replicates of three different concentrations were injected on the same day and the percent relative standard

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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 concentration and the percent relative standard deviations (%RSD) were calculated.

Robustness

To evaluate the robustness of the developed RP-UPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate and temperature on the Area of Chromatograms were studied. The method was found to be unaffected by small changes ± 0.2 change in flow rate and temperature.

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 same mobile phase at 215 nm at a flow rate of 1.2 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.

RESULT 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

Sr. No	Rabeprazole			Lafutidine		
	Rt (min)	Theoretical Plates	Tailing Factor	Rt (min)	Theoretical Plates	Tailing Factor
1	3.290	7251	0.92	5.940	4791	1.11
2	3.258	7138	0.91	5.875	4805	1.12
3	3.298	7214	0.92	6.031	4768	1.12
4	3.300	7129	0.92	6.078	4763	1.12
5	3.290	7198	0.91	5.940	4764	1.11
Avg.	3.2872	7186	0.916	5.973	4778	1.116
%RSD	0.516	0.719	0.598	1.354	0.39	0.491

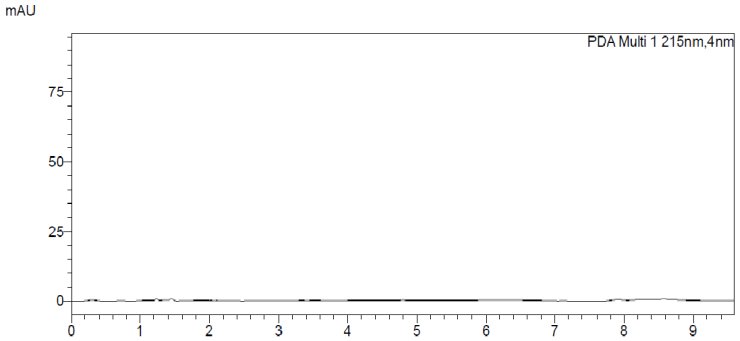

Figure 3: Specificity chromatogram of Diluent

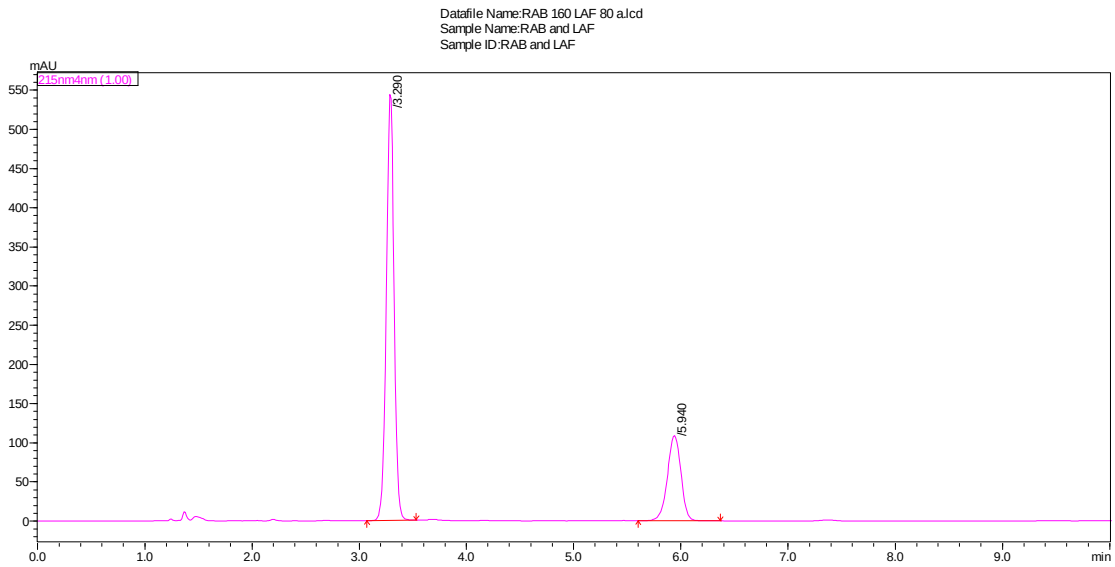

Figure 4: Specificity chromatogram of Standard RAB & LAF

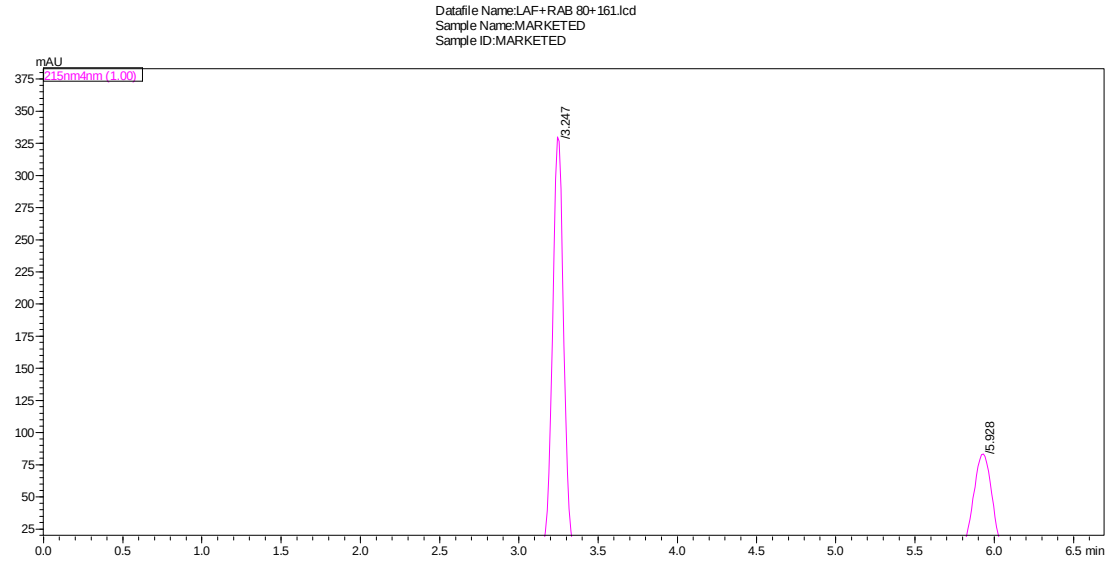

Figure 5: Specificity chromatogram of Marketed Product (Lafumac Plus)

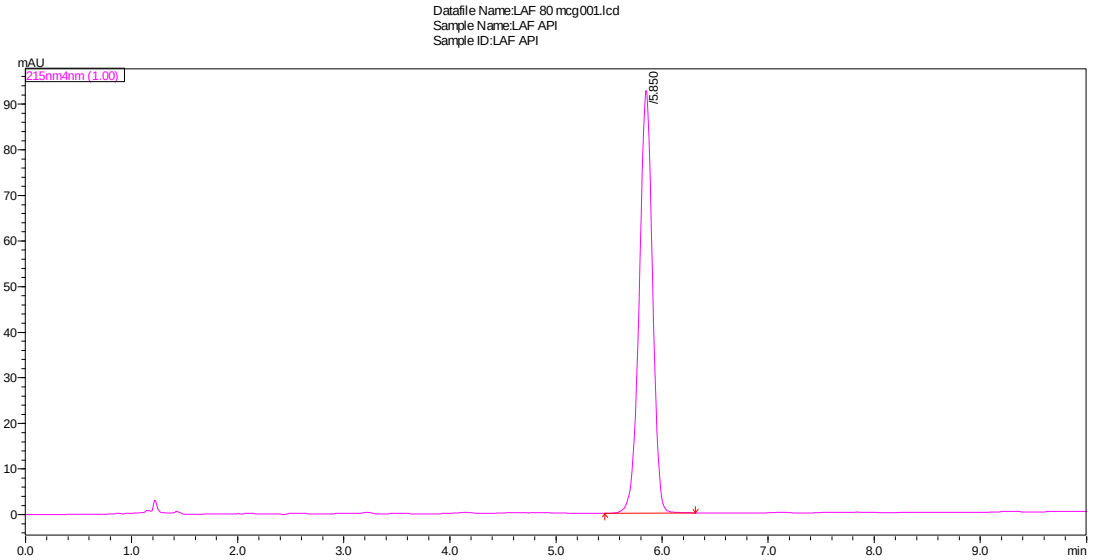

Figure 6: Specificity Chromatogram of standard LAF (80µg/ml)

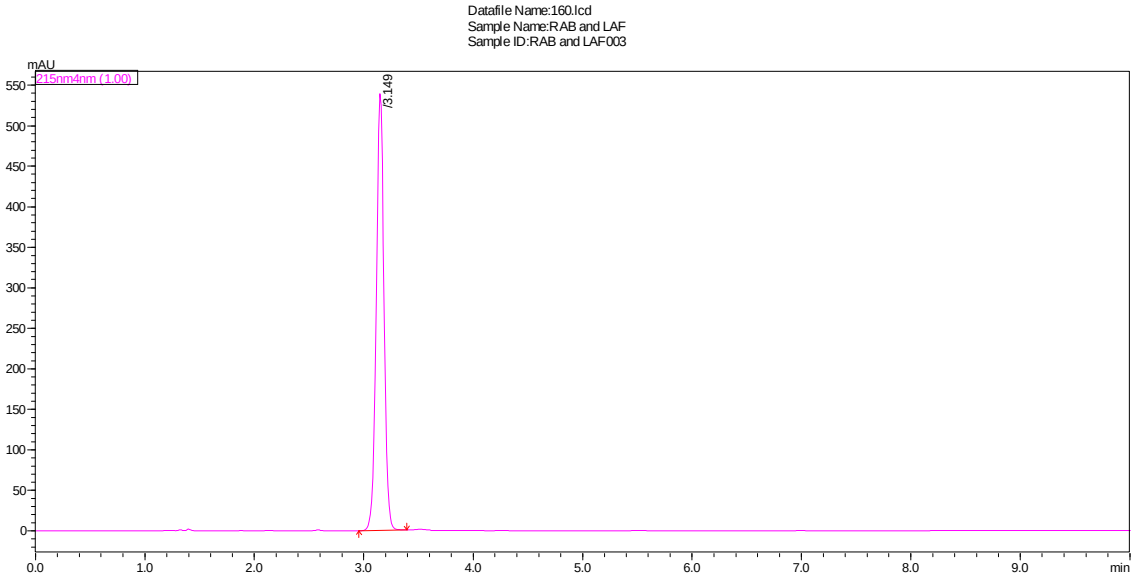

Figure 7: Specificity Chromatogram of standard RAB (160µg/ml)

Sr. No.	Conc.(µg/ml)		Area	
	LAF	RAB	LAF	RAB
1	40	80	432378	1298594
2	50	100	568004	1704224
3	60	120	687766	1957219
4	80	160	971213	2725789
5	100	200	1230301	3417221

Table 2: Result of Linearity for LAF and RAB

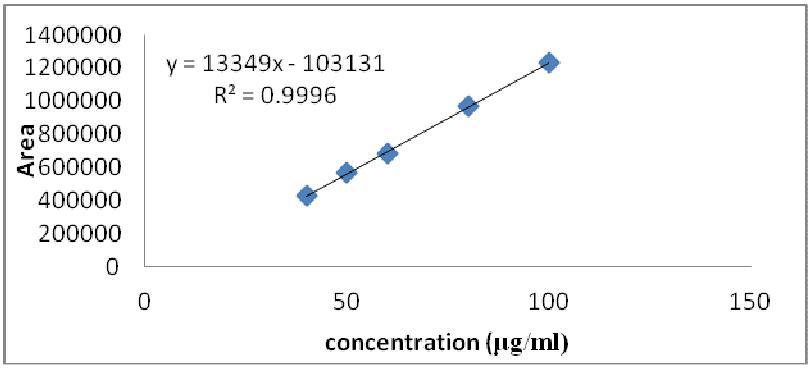

Figure 8: Calibration curve of Lafutidine

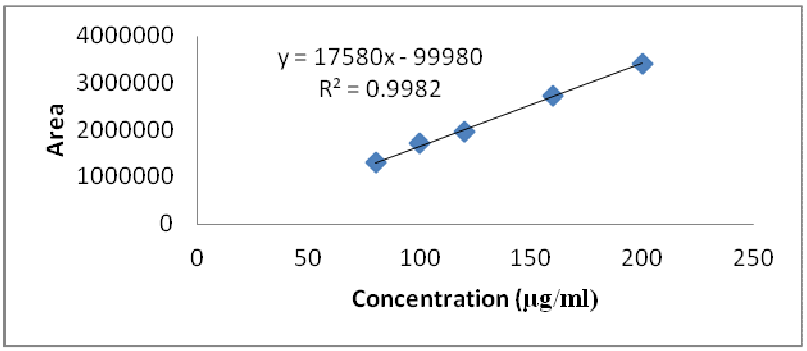

Figure 9: Calibration curve of Rabeprazole Sodium

Amt of drug in sample (mg)	Amt of drug added (mg)	Amt measured (mg)	% recovery	Average of % recovery	%RSD
40	20	59.74088	99.568	99.267	1.759
40	20	60.50514	100.842		
40	20	58.4376	97.389		
40	40	79.79341	99.742	98.998	1.539
40	40	80.00662	100.008		
40	40	77.79645	97.246		
40	60	100.3778	100.378	100.194	1.085
40	60	101.1779	101.118		
40	60	99.02689	99.027		

Table 3: Data derived from Accuracy experiment- Lafutidine

Amt. of drug in sample (mg)	Amt. of drug added (mg)	Amt. measured (mg)	% recovery	Average of % recovery	%RSD
80	40	118.738	98.948	100.038	1.338
80	40	119.561	99.634		
80	40	121.838	101.532		
80	80	161.597	100.998	99.911	1.032
80	80	159.664	99.790		
80	80	158.313	98.946		
80	120	199.189	99.595	99.803	0.700
80	120	198.461	99.231		
80	120	201.167	100.584		

Table 4: Data derived from Accuracy experiment- Rabeprazole Sodium

Drug	Condition	Area		Theoretical plates	
		Mean	%RSD	Mean	%RSD
Rabeprazole	Flow rate 1.0ml/min	1303423	1.090	7186	0.854
	Flow rate 1.4ml/min	1302670	1.485	7195	0.746
	Temp. 38ºC	1294157	1.046	7201	0.781
	Temp. 42ºC	1293301	1.372	7178	0.981
Lafutidine	Flow rate 1.0ml/min	433293	1.160	4778	1.012
	Flow rate 1.4ml/min	430511	1.159	4772	0.991
	Temp. 38ºC	435542	1.488	4782	0.978
	Temp. 42ºC	436843	1.495	4766	0.845

Table 5: Data derived from Robustness study

Drug	Concentration	Area	Mean Area	% RSD
Lafutidine	40µg/ml	443567	440279	0.681
		439576		
		437694		
	80µg/ml	942671	947590.3	0.474
		951483		
		948617		
	120µg/ml	1298345	1291871	1.163
		1302576		
		1274691		
Rabeprazole	80µg/ml	1311654	1305441	0.469
		1305275		
		1299394		
	160µg/ml	2743844	2704834	1.263
		2680272		
		2690386		
	240µg/ml	3962107	3979830	0.469
		3978082		
		3999300		

Table 6: Determination of Intraday Precision

Drug	Conc	Area	Mean Area	% RSD
Lafutidine	40µg/ml	437539	441017.7	1.371
		448028		
		437576		
	80µg/ml	954226	953037.3	0.128
		951787		
		953099		
	120µg/ml	1381352	1395381	1.289
		1389134		
		1415656		
Rabeprazole	80µg/ml	1294576	1288457	1.647
		1305942		
		1264853		
	160µg/ml	2648364	2680136	1.103
		2685214		
		2706831		
	240µg/ml	3875164	3809063	1.504
		3773594		
		3778432		

Table 7: Determination of Interday Precision

Brand Name	Drug	Label Claim	Amount Found	% Label claim	S.D	R.S.D
Lafumac Pluse	LAF	10 mg	10.196	101.96	0.309994624	0.30
	RAB	20 mg	20.38	101.90	0.48354593	0.47

Table 8: Analysis of Marketed Product

Parameters		LAF	RAB
Linearity Range		40-100 µg/ml	80-200 µg/ml
Correlation Co-efficient		0.999	0.998
% Recovery		99.0%	99.9%
Precision (% RSD)	Interday	1.1	1.5
	Intraday	0.8	0.5
Robustness (% RSD)			
Flow rate	1.0 ml/min	1.160	1.090
	1.4 ml/min	1.159	1.485
Temperature	38ºC	1.488	1.046
	42ºC	1.495	1.372
Specificity	Specific		

Table 9: Summary of Validation parameters

Conclusion

The proposed UPLC method is found to be specific, accurate, precise and rapid for determination of LAF and RAB in combination. The proposed method was also applied for the estimation of these drugs in commercial dosage form and was successfully estimated. This method is reliable and can be useful for the rapid estimation in industries during inprocess quality control testing.

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