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Development and validation of stability indicating assay method for the estimation of lafutidine and domperidone in capsule dosage form

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Abstract

A simple, sensitive, precise and specific high performance liquid chromatography method was developed and validated for the determination of Lafutidine and Domperidone in Lafutidine and Domperidone sustained release capsule. The separation was carried out by using a mobile phase consisting Acetonitrile: pH 6.5 phosphate buffer in ratio of 30:70. The column used was Xterra 250mm X 4.6mm, 5µ with flow rate 1 ml/min using UV detection at 276 nm. The retention time of Lafutidine and Domperidone were found to be 7.0 and 15.5 min respectively. Degradation study of Lafutidine and Domperidone in its capsule form was conducted under condition of hydrolysis, oxidation, thermal and photolysis, the results of analysis were validated statistically and by recovery studies (mean recovery = 99.94). The result of study showed that the proposed method is simple, rapid, precise and accurate, which is useful for the routine determination of Lafutidine and Domperidone in pharmaceutical dosage form.

Keywords: Lafutidine, Domperidone, Method development, Validation, Forced degradation.

Introduction

Lafutidine is 2-[(2-furylmethyl)sulfinyl]-N-((2Z)-4-{[4-(piperidin-1-ylmethyl)pyridin-2-yl]oxy}but-2-en-1-yl)acetamide (Fig. 1). It is a gastroprotective and antiulcer drug, which selectively block H2 receptors. Physical properties are white crystalline powder, soluble in methanol and freely soluble in glacial acetic acid, stable under ordinary condition.

Domperidone is 5-chloro-1-(1-[3-(2-oxo-2,3-dihydro-1Hbenzo[d]imidazol1yl)propyl piperidin-4-yl)-1H-benzo[d]imidazol2(3H)-one (Fig. 2). It is an antiemetic drug, which selectively block CTZ receptors. Physical properties are white crystalline powder, soluble in 0.1N HCl. This paper describes validated HPLC method for estimation of Lafutidine and Domperidone, a mobile phase consisting Acetonitrile: pH 6.5 phosphate buffer in ratio of 30:70. The column used was Xterra 250mm X 4.6mm, 5µ with flow rate 1 ml/min using UV detection at 276 nm.

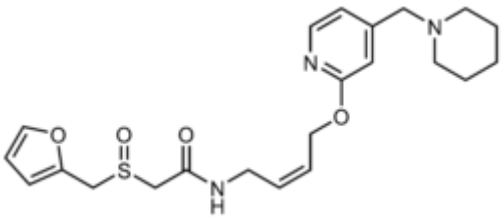

Fig. 1: Lafutidine

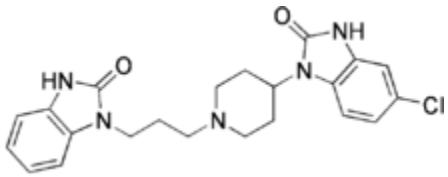

Fig. 2: Domperidone

Materials and Method

Equipments: HPLC equipped with pump, injector and PDA detector Waters 2695, 2996, HPLC equipped with pump, injector and UV detector, Waters 2695, 2487, HPLC equipped with pump, injector and UV detector, Agilent 1200 series, Balance-Sartorius, Mettler Toledo, Photo stability chamber-Newtronic, Oven- Techno Instrument.

Materials: Lafutidine standard (Alkem), Capsules were procured from FR&D department. Disodium hydrogen phosphate dehydrate (Merck) Acetonitrile (Merck) Orthophosphoric acid(Merck), MilliQ water, Hydrochloricacid(Merck), Sodium hydroxide(Merck), Column Xterra 250mmX4.6mm,5µ.

Preparation of 0.01 M Disodium hydrogen phosphate dihydrate buffer pH 6.5: Dissolve 4.45 gm Disodium hydrogen phosphate dihydrate in to 1000 ml water, mix and adjust pH 6.5 with orthophosphoric acid, Filter through 0.45µ nylon filter, mix and degas.

Preparation of mobile phase: Mix above Buffer and Acetonitrile in the ratio of 70:30, mixed and degassed.Use suitable High Performance Liquid Chromatography equipped with following:

Column: Xterra 250mm X 4.6mm, 5µ.
Flow rate: 1.0 ml/min
Wavelength: 276nm
Injection volume: 1µl
Column oven temperature: 30ºC
Sample compartment temp: 25ºC
Run time: 30 minutes
Diluent: Mobile phase

Preparation of standard Solution: Weigh accurately about 20 mg of Lafutidine working standard and 60mg

of Domperidone working standard transfer into a 100ml volumetric flask, add 70ml of methanol, sonicate to dissolve and make up the volume with methanol. Further dilute 5ml to 50ml with mobile phase.

Preparation of Sample solution: Weigh and transfer the content of 20 capsules and powder the content. Weigh accurately quantity of powder containing the equivalent of about 30 mg of Domperidone into a 100 ml volumetric flask. Add 70ml of methanol sonicate for 15 minutes with intermittent shaking and make up the volume with methanol. Filter through 0.45µ Nylon membrane filter or 0.45µ PVDF membrane filter. Further dilute 5ml to 25 ml mobile phase.

Procedure: Wash the column and equilibrate with mobile phase. Separately inject equal volumes (20µl) of the Standard preparation (Five replicate injections) and assay preparation (Duplicate injection) into the chromatograph. The System suitability parameters should be met. From the peak responses, calculate the content of Lafutidine and Domperidone in the sample.

The retention time of Lafutidine peak and Domperidone peak is about 7.0 minutes and 14.5 minutes respectively.

Evaluation of system suitability: From standard solution:

1. The % RSD for the peak areas of Lafutidine and Domperidone from five replicate injections should not be more than 2.0.
2. The tailing factor for Lafutidine and Domperidone should be not more than 2.0.

Validation Method

Linearity: The linearity of Lafutidine and Domperidone was performed using standard solution in the range of 14.74 mcg/mL to 27.37 mcg/mL of Lafutidine and 41.79mcg/mL to 77.60 mcg/mL of Domperidone(about 30% - 150% of test concentration). A graph was plotted with concentration (in mcg/ml) on x-axis and peak areas of Lafutidine and Domperidone on y-axis. Slope, y-intercept, correlation coefficient (r-value) and residual sum of squares (RSS) were determined Fig. 3. The results are tabulated in Table 1 and 2.

Table: 1: Concentration of Lafutidine in mcg/ml

Spike level in %	Concentration of Lafutidine in mcg/ml	Peak areas
70	14.74	170.74
80	16.84	196.24
90	18.95	219.67
100	21.05	244.18
110	23.16	269.34
120	25.26	294.71
130	27.37	320.09
Slope	11.79	
y-intercept	-3.11	
r-value	0.99993	
RSS	2.375757143	

Table 2: Concentration of Domperidone in mcg/ml

Spike level in %		Peak areas
70		1039.28
80	Concentration of Domperidone in mcg/ml	1195.35
90	41.79	1337.49
100	59.70	1487.39
110	65.67	1639.28
120	71.64	1793.47
130	77.60	1948.07
Slope	25.27	
y-intercept	-3.11	
r-value	0.99993	
RSS	2.375757143	

Accuracy: Placebo spiked with the known amount of 100% and 130% of test concentration as Lafutidine and Domperidone. The amount of Lafutidine and Domperidone was quantified as per the test method. The percentage recovery was calculated from the amount found and actual amount added. The results are tabulated in Table 3 and 4.

Table: 3: Recovery study of Domperidone

Level no/Spike level in %	Actual Amount of Domperidone added in mg	Actual Amount of Domperidone found in mg	%Recovery	%RSD
Level – 1 (70%)	20.43	20.06	98.19	0.78
	20.40	20.34	99.71	
	20.50	20.34	99.22	
Level – 2 (100%)	30.73	30.94	100.68	0.47
	30.77	31.14	101.20	
	30.79	30.87	100.26	
Level – 3 (130%)	39.58	39.85	100.68	0.49
	39.64	40.30	101.66	

	39.69	40.19	101.26	
	Over all Mean		100.32	
	Over all SD		1.109	
	Over all % RSD		1.11	

Table 3: Recovery study of Lafutidine

Level no/ Spike level in %	Actual Amount of Lafutidine added in mg	Actual Amount of lafu found in mg	%Recovery	%RSD
Level – 1 (70%)	69.552	68.184	98.03	0.36
	69.552	68.553	98.56	
	69.552	68.644	98.69	
Level – 2 (100%)	99.360	100.579	101.23	0.51
	99.360	101.279	101.93	
	99.360	100.271	100.92	
Level – 3 (130%)	129.169	128.195	99.25	0.67
	129.169	129.638	100.36	
	129.169	129.749	100.45	
Over all Mean			99.94	
Over all SD			1.351	
Over all % RSD			1.35	

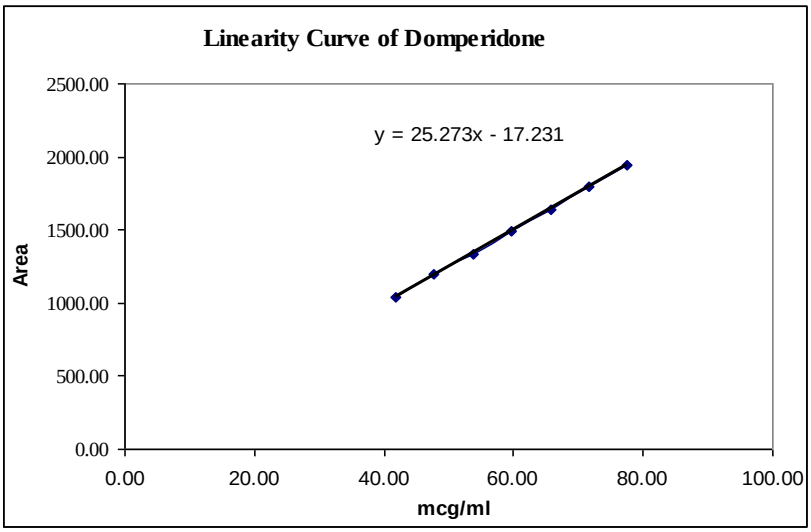

Fig. 3: Linearity of Domperidone

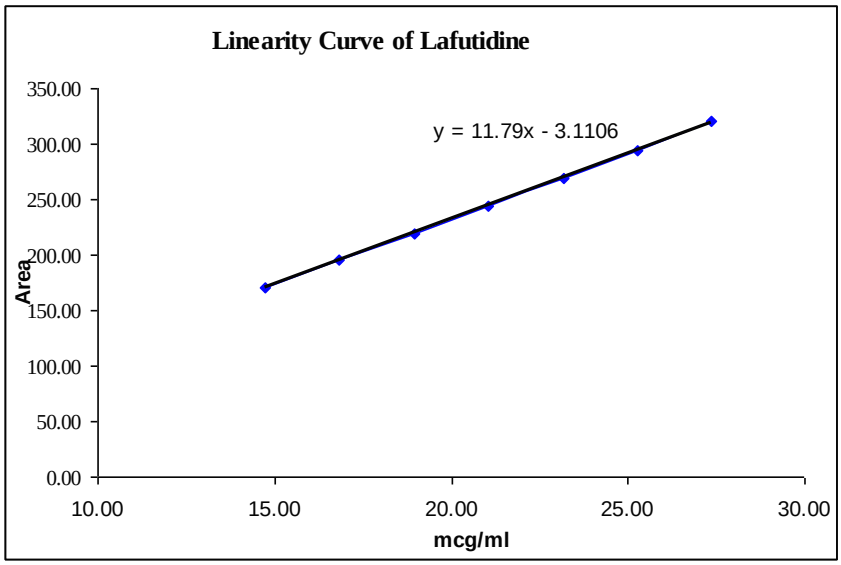

Fig. 3: Linearity of Lafutidine

Robustness: Robustness of the method was verified by deliberately varying the following instrumental conditions:

By changing the flow rate by ± 10%, By changing the temperature by ± 5°C, By changing the wavelength by ± 2nm, By changing the organic content by ± 2% (absolute), By changing the pH of buffer in mobile phase by ±0.1 units. The results are tabulated in Table 5 & 6.

Table 5: Robustness Data for Lafutidine

S. No.	I +(nm)	II -(nm)	III +(ml/min)	IV -(ml/min)	V +(pH)	VI -(pH)	VII +(ºC)	VIII -(ºC)	IX +(org)	X -(org)
1	95.83	96.60	95.11	95.94	98.67	95.26	97.44	97.60	100.40	99.90
2	97.14	99.90	94.89	95.96	98.62	95.06	97.86	98.27	100.26	100.40
3	97.22	97.93	95.09	95.73	99.63	95.03	97.53	97.78	101.58	101.47
Over all %RSD	0.54	1.04	1.14	0.72	1.03	1.09	0.40	0.52	1.89	1.83

Table 4: Robustness Data for Domperidone:

S. No.	I +(nm)	II -(nm)	III +(ml/min)	IV - (ml/min)	V +(pH)	VI -(pH)	VII +(ºC)	VIII -(ºC)	IX +(org)	X -(org)
1	100.38	100.46	99.02	99.55	100.41	97.42	101.61	101.61	102.47	101.76
2	102.08	101.71	98.72	99.82	100.35	97.40	101.99	102.21	102.00	102.11
3	100.59	99.75	98.94	99.70	101.34	96.87	101.93	101.94	103.68	103.30
Over all %RSD	0.53	0.56	1.00	0.62	0.37	1.83	0.56	0.61	1.05	0.90

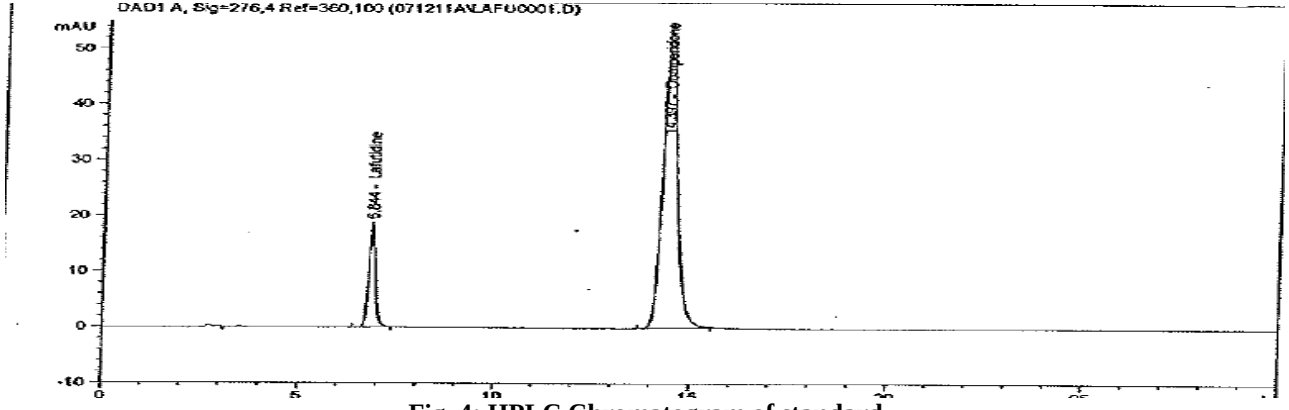

Fig. 4: HPLC Chromatogram of standard

Forced degradation: Forced degradation study was carried out by treating the sample under the following conditions. (Table 4 and Fig. 5-9)

a. Degradation by hydrochloric acid (Acid treated sample): Sample was treated with 5ml of 1N Hydrochloric acid and kept on water bath at 60°C for 20 minutes. Treated sample solution was analyzed as per the test method.
b. Degradation by sodium hydroxide (Base treated sample): Sample was treated with 5ml of 1N Sodium hydroxide and kept on water bath at 60°c for 15 minutes. Treated sample solution was analyzed as per the test method.
c. Degradation by hydrogen peroxide (Peroxide treated sample): Sample was treated with 5ml of 50% Hydrogen peroxide solution and kept on water bath at 60°c for 5 minutes. Treated sample solution was analyzed as per the test method.
d. Degradation by thermal (Heat treated sample): Sample was kept in oven at 105°C for about 30 hrs. Treated sample was analyzed as per the test method.
e. Degradation by UV –Visible light (UV-visible treated sample): Sample was exposed to UV light of about 200 watt hours/square meter and to visible light for about 1.2 million lux hours in photo stability chamber. Treated sample was analyzed as per the test method.

Table 5: Forced degradation (Lafutidine)

Condition	% Assay	% Degradation
Untreated Sample	97.14	-
Acid-Treated Sample	94.20	3.03
Base-Treated Sample	92.92	4.34
Peroxide-Treated Sample	94.32	2.90
Heat-Treated Sample	93.99	3.24
UV-visible Treated sample	96.24	0.93

Table 6: Forced degradation (Domperidone)

Condition	% Assay	% Degradation
Untreated Sample	100.84	-
Acid-Treated Sample	99.77	1.06
Base-Treated Sample	98.50	2.32
Peroxide-Treated Sample	98.46	2.36
Heat-Treated Sample	98.06	2.76
UV-visible Treated sample	99.66	1.17

Table 7: Summary of system suitability

Name of Experiment	Tailing factor	%RSD
System and Method precision, Filter paper	1.1	0.23
Recovery	1.1	0.18
Ruggedness	1.1	0.15
Forced degradation (UV)	1.1	0.33
Specificity	1.1	0.33

Table 8: Summary of system suitability

Name of Experiment	Tailing factor	%RSD
System and Method precision, filter paper	1.1	0.13
Recovery	1.1	0.24
Ruggedness	1.1	0.38
Forced degradation (UV)	1.2	0.27
Specificity	1.1	0.27

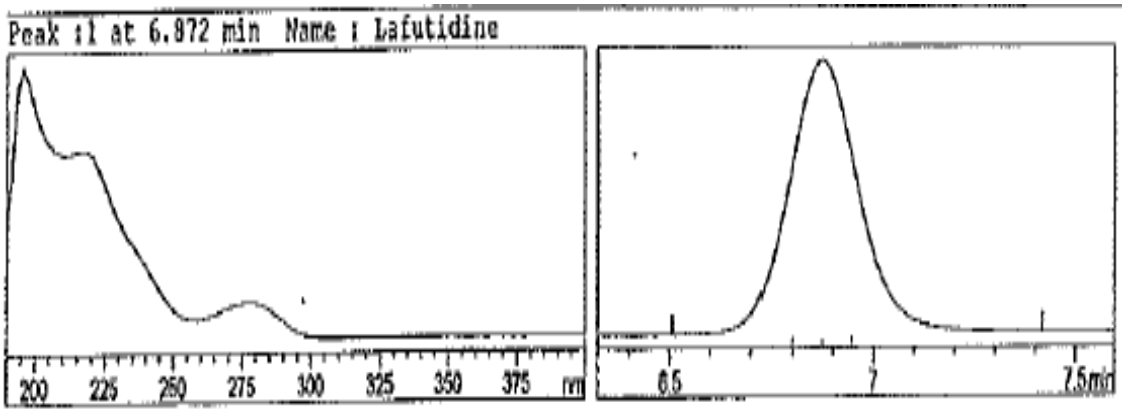

Fig. 5: Purity plot of control sample (Lafutidine)

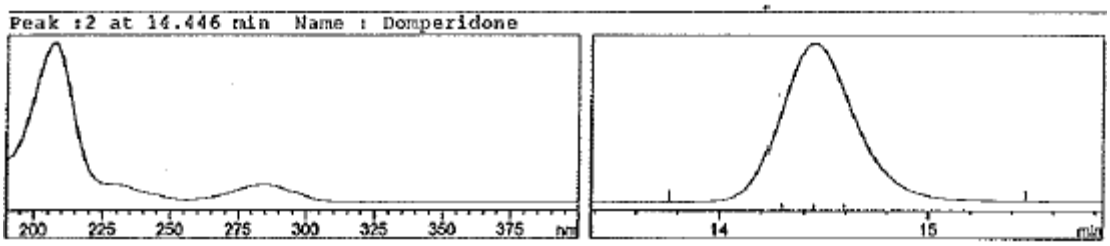

Fig. 6: Purity plot of control sample (Domperidone)

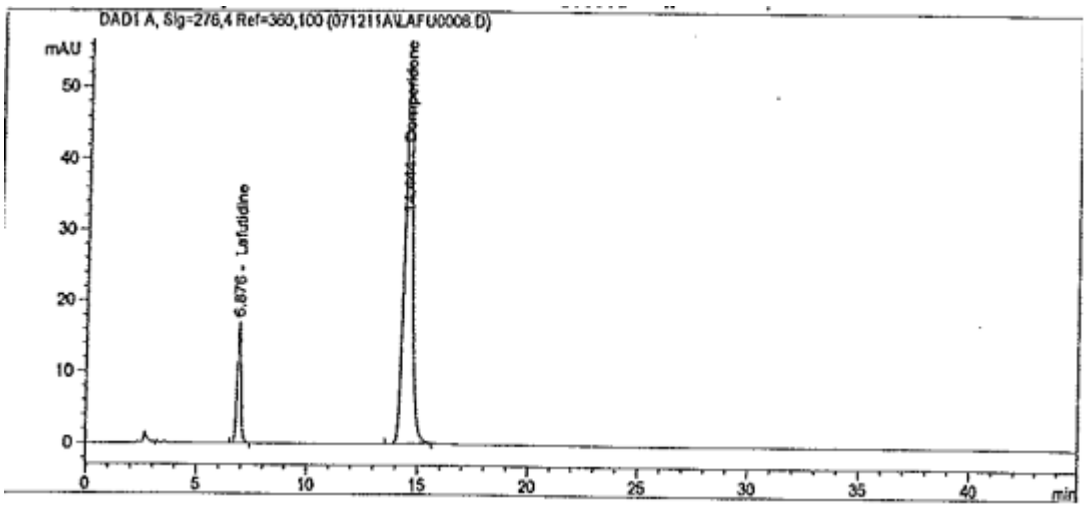

Fig. 7: HPLC Chromatogram of acid treated sample

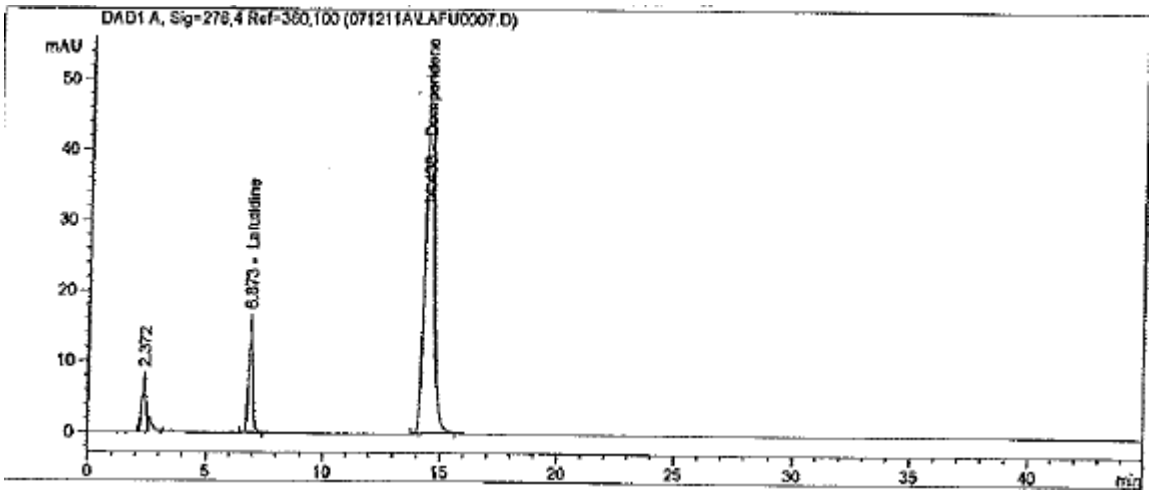

Fig. 8: HPLC Chromatogram of base treated sample

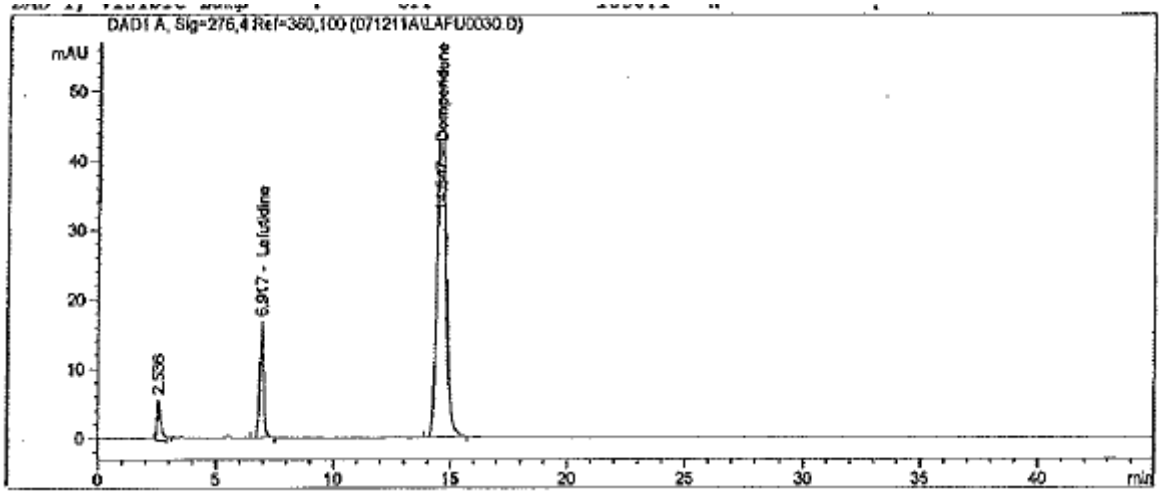

Fig. 9: HPLC Chromatogram of peroxide treated sample

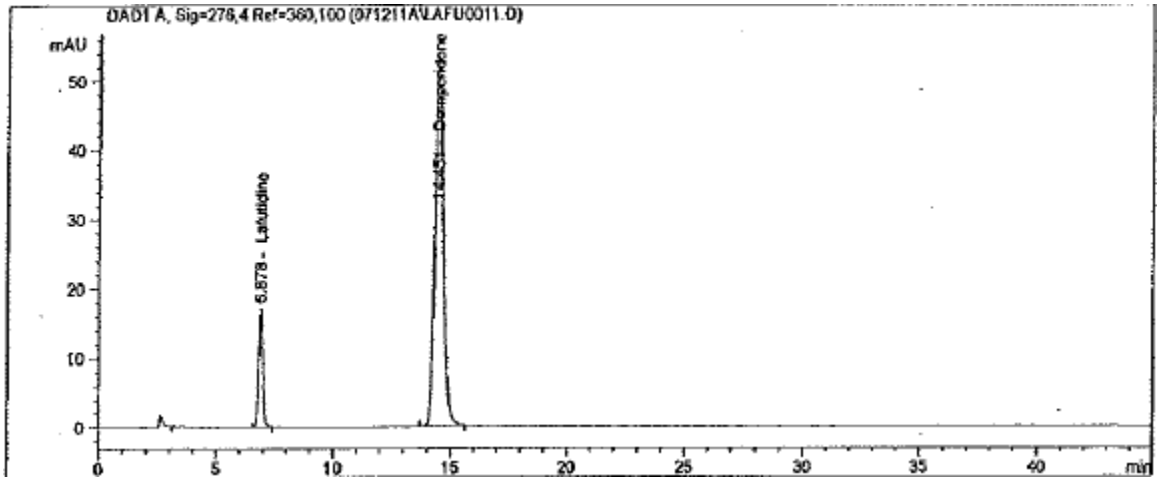

Fig. 10: HPLC Chromatogram of heat treated sample

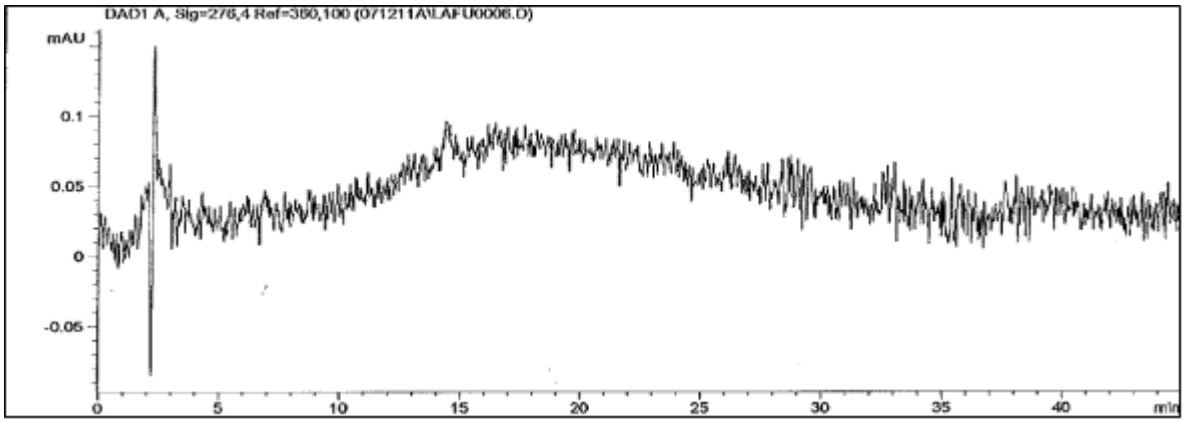

Fig. 11: HPLC Chromatogram of UV visible treated sample

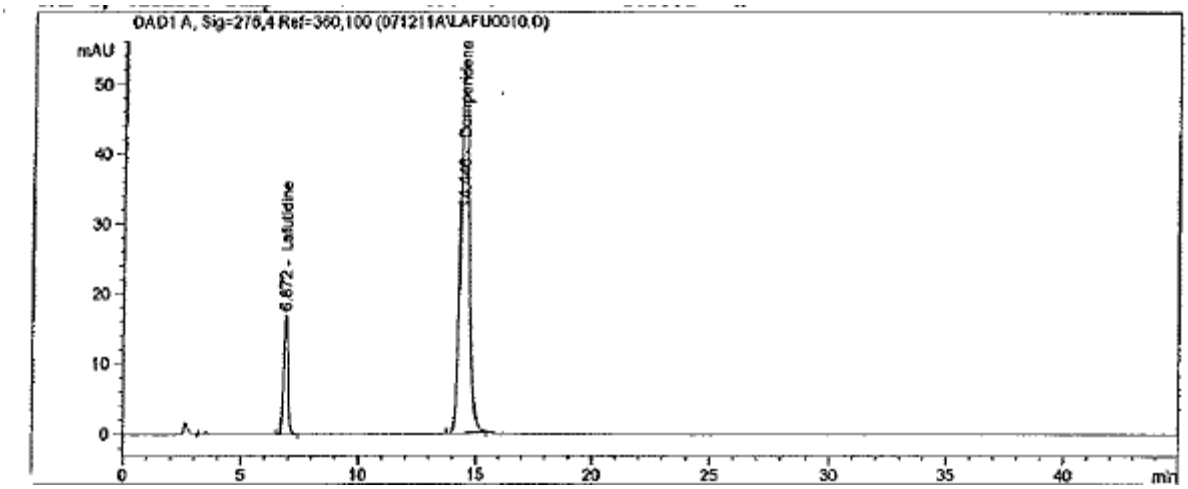

Fig. 12: HPLC Chromatogram of blank

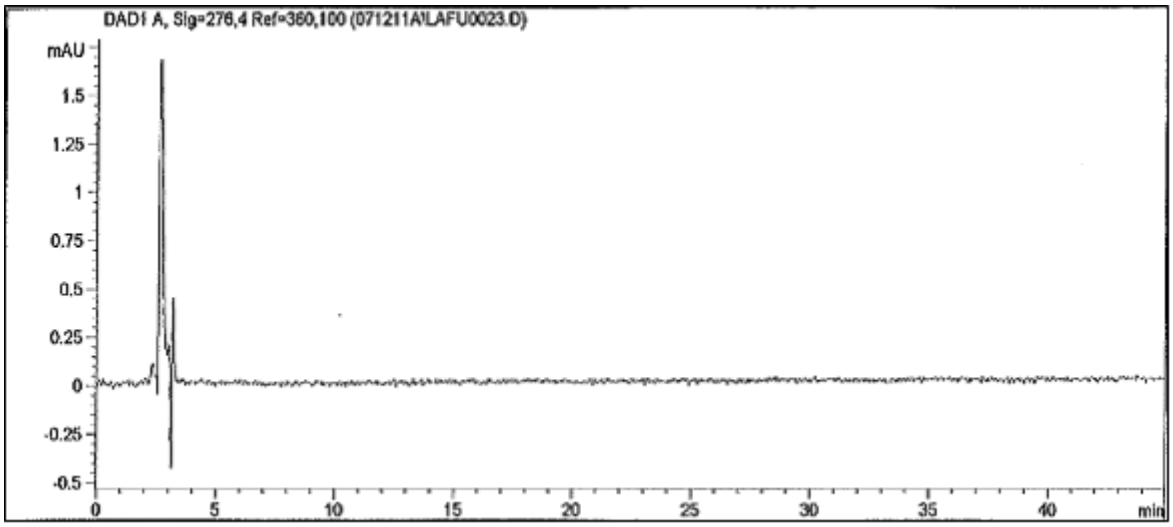

Fig. 13: HPLC Chromatogram of placebo

Result and Discussion

In order to develop an effective method for the analysis of the drugs in pharmaceutical formulations, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection of wavelength, ideal mobile phase and their proportions, optimum pH and concentration of standard solution were studied. The method developed with Column Xterra 250mm X 4.6mm, 5µ using flow rate: 1.0 ml/min, wavelength 276 nm at room temperature. The linearity of Lafutidine and domperidone was performed using standard solution in the range of 14.74 mcg/mL to 27.37 mcg/mL and 41.79 mcg/mL to 77.60 mcg/mL respectively (about 30% - 160% of test concentration).

Conclusion

The HPLC method for the assay of Lafutidine and Domperidone in Lafutidine (10mg IR) and Domperidone (10mg IR + 20mg SR) capsule was found to be simple, precise, accurate, rapid and validated. The mobile phase is simple to prepare and economical. The sample recoveries in formulation were in good agreement with their label claim. Hence it can be easily and conveniently adopted for routine analysis of Lafutidine and Domperidone in capsule.

Acknowledgement

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References

1. Indian Pharmacopoeia, (2007) Ghaziabad; Indian pharmacopoeia commission.
2. British Pharmacopoeia,(2009) London; British pharmacopoeia commission.
3. Singh S.S and Bakshi, M.,(2002), Development of validated stability indicating assay methods- critical review. J. Pharm. Biom. Anal., 28, 1011-1040.
4. Sarah K. Branch.,(2005), Guidelines from the International Conference On Harmonisation (ICH) review. J. Pharm. Biom. Anal. 38, 798-805.

5. ICH, (2003), Stability testing of new drugs substances and products, Q1A (R2). International conference on Harmonization, Geneva.
6. D.D. Hong, M. Shah,(2000) Development and validation of HPLC stability indicating assays, In: Drug stability: Principles and Practices, J.T. Carstensen, C.T. Rhodes, Marcel Dekker, New York.329-384.
7. Klick, S. et.al, (2005), Toward a generic approach for stress testing of drug substance and drug products. Pharm. Tech., 2, 48-66.
8. Singh S.S and Bakshi, M., (2004), ICH Guidance in practice: Establishment of inherent stability of Secnidazole and development of a validated stability indicating HPLC assay method. J. Pharm. Biom. Anal., 36, 769-775.
9. Acharya, M.M., (1999), Pharmaceuticals stability Testing and studies, an overview, The Eastern Pharmacist, May, 31-36.
10. Jadhav K., Dhamecha A and Patil M.B.,(2011), Application of UV spectrophotometric method for easy and rapid estimation of lafutidine in bulk and pharmaceutical formulation. Pharm Methods. 2(4):264–267.
11. Kulkarni A.S., Mane V. B.,(2012), Method development and validation for the simultaneous determination of Omperazole and Domperidone in solid dosage form by RP-HPLC . International J. of Pharm. and Pharm. Sci. ISSN- 0975-1491 Vol 4, Suppl 5, 534-540.
12. Chitlange S.S., Mulla A.I., and Wankhede S.B.,(2010). A validated RP-HPLC method for simultaneous estimation of Dexrabeprazole and Domperidone in pharmaceutical dosage form. Pelagia Research Library Der Pharmacia Sinica, 1 (1):42-47.
13. ICH Harmonised Tripartite Guideline.(1994) Code Q2A-Text on Validation of Analytical Procedure Step-3 Consensus Guideline. Geneva, Switzerland.
14. ICH Harmonised Tripartite Guideline.(1994)Code Q2B-Validation of Analytical Procedure Methodology Step-4 Consensus Guideline. Geneva, Switzerland.
15. Beckett AH, Stenlake JB.(2002) Practical Pharmaceutical Chemistry. 4th ed., New Delhi; CBS Publishers and Distributors, 157-66.
16. ICH Q2 (R1): (2005) validation of analytical procedures: text and methodology.
17. Snyder L.R., Kirkland J.J., (1997), Practical HPLC method development, Wiley inter science publication, Networks, 686-772.
18. USP NF,(2009) The official Compendia of Standards, 1, 734.
19. Willard H.L., Dean J.A., Settle F.A., Instrumental method analysis,7th edition, CBS publishers & distributers New Delhi, 626-629.
20. Sethi P.D.,(2001). HPLC, Quantitative analysis of pharmaceutical formulation, CBS Publisher and Distributor, New Delhi.
21. Mendham J., Denny R. C., Barnes J. D., Thomas M., Vogel's textbook of quantitative chemical analysis, Dorling Kindersley Pvt. Ltd. India.