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Simultaneous Quantification of Famotidine and Ibuprofen in Pharmaceutical Dosage by Using Validated Stability Indicating LC Method

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Abstract

A reverse phase liquid chromatographic analytical method was developed for the simultaneous determination of Famotidine and Ibuprofen content in tablet dosage form. The chromatographic separation was achieved by using YMC Cyano column with mobile phase containing a gradient mixture of 0.05M ammonium acetate in water as mobile phase A and acetonitrile as mobile phase B at a flow rate of 1.0 ml/min. Both of the analyte were quantified with a UV detector at 270 nm. Famotidine and ibuprofen tablet were subjected to the stress conditions of oxidation, acid hydrolysis, base hydrolysis and thermolysis and photolysis. The tablet was found to be stable under thermal and photolytic conditions but degradation was observed in base hydrolysis, acid hydrolysis and oxidation. The stability-indicating capability of this method was demonstrated by adequate separation of the degradation peaks from those of the actives in the stress degraded samples. The method was validated for linearity, specificity, system suitability, precision and accuracy in accordance with ICH guidelines. The proposed method was applied to quantify famotidine and ibuprofen in the combined pharmaceutical tablet and stability studies of the same.

Keywords: Famotidine, Ibuprofen, HPLC, Stability indicating method, stress testing, pharmaceutical preparations.

Introduction

Famotidine is chemically 3-([2-(diaminomethyleneamino) thiazol- 4-yl] methylthio)-N-sulfamoylpropanimidamide (figure-1a)[1]. It is commonly used in the treatment of peptic ulcer disease and gastro esophageal reflux disease. Usually Famotidine is histamine H_2-receptor antagonist which blocks the action of histamine on stomach cells and reduces acid production. Famotidine is useful in promoting the healing of stomach and duodenal ulcers and reducing ulcer pain. Famotidine has been effective in preventing recurrence of ulcers when given in low doses for prolonged periods of time. Famotidine is used for treating heartburn and in healing ulceration and inflammation of the esophagus resulting from acid[2-6].

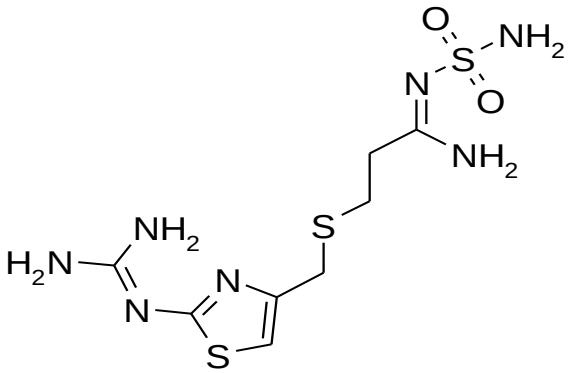


Figure-1a
Chemical structure of Famotidine

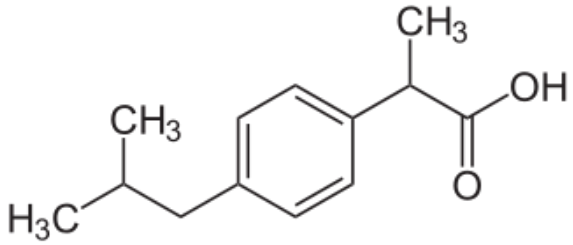


Figure-1b
Chemical structure of Ibuprofen

Ibuprofen is chemically (RS)-2-9(4-(2-methylpropyl)phenyl) propionic acid (figure-1b) is an non-steroidal anti-inflammatory drug that is commonly used for the relief of symptoms of arthritis, fever, primary dysmenorrheal (menstrual pains) and as an analgesic (medication given to reduce pain without resulting in loss of consciousness). Ibuprofen also showed antiplatelet effect (protects from blood clots)[7-9].

Simultaneous determination of Famotidine and related impurities in pharmaceuticals was reported. Chromatographic separation was accomplished within 10 minutes on a porous graphitic carbon column using acetonitrile and water containing 0.5% pentane sulphonic acid in the ratio of 50:50, v/v as the mobile phase. Separation was achieved with a flow rate of 1 ml/min. and at a detection wavelength of 265 nm. The calibration curves were linear over a concentration range 1.5 to 100 µg/ml[11]. A rapid and specific HPLC method was reported for quantitative analysis of Famotidine in pharmaceutical dosage forms[12]. A stability indicating HPLC analytical method

was reported Famotidine in the presence of its degradation products. The method was used to investigate the kinetics of degradation of the drug in an acidic solution[13]. HPLC method using a monolithic column was reported for quantification of Famotidine in plasma. The assay enables the measurement of Famotidine for therapeutic drug monitoring with a minimum detectable limit 5.0 ng/ml[14]. HPLC method with column switching and an IS for the quantitative determination of Famotidine in human plasma was reported. Famotidine and the IS were isolated from plasma samples by cation exchange solid phase extraction with SCX cartridges[15].

Kinetic spectrophotometric method for the determination of Ibuprofen in pharmaceutical formulations was reported. Ibuprofen was determined in an acidic ethanolic medium by monitoring the rate of appearance of 1-nitroso-2-naphthol, resulting from the displacement by Ibuprofen of Co(III) from the tris(1-nitroso-2-naptholato)cobalt(III) complex[16]. A rapid CE method for the determination of Ibuprofen and pseudoephedrine hydrochloride in combine tablets was reported. CE was performed in 0.025 M phosphate buffer solution (pH 8.1). The two compounds were completely separated within 3 minute and quantified with a UV detector at 210 nm[17]. Determination of Ibuprofen in ointments by RP-HPLC was reported[18]. A literature survey reveals that several methods were reported for the estimation of Famotidine and Ibuprofen a lonely, but none of the reported method describes the analysis of both drugs simultaneously.

We developed and validated a short runtime stability indicating HPLC method for the simultaneous determination of Famotidine and Ibuprofen in pharmaceutical combine dosage form.

Material and Methods

Famotidine and Ibuprofen drug standards were provided by Hetero drugs, India. Their combine formulated tablet "Duexis" were purchased from market. HPLC grade acetonitrile, sodium hydroxide (NaOH), hydrochloric acid (37% purity) and ammonium acetate were purchased from Rankem laboratories, India. Hydrogen Peroxide (H_2O_2) was purchased from Acros Organics, USA. Water used was obtained after reverse osmosis (Milli Q unit) of triple distilled.

Chromatographic conditions: Analysis was carried out using Agilent–1100 HPLC which consisted of binary pump with two solvent lines, an online solvent degassing unit, an auto sampler, a sample cooling unit with temperature controller, a column oven and PDA detector. Chromatographic separation was achieved using YMC Cyano, (150 × 4.6 mm, 5 µm) YMC, INC. USA column, at 30 °C oven temperature. The mobile phase consisted of 0.05 M ammonium acetate in water as mobile phase A and 100% acetonitrile as mobile phase B. The compounds were separated using a linear gradient starting from A/B = 95/05 upto 2 minute and changing to A/B = 80/20 to 10 minute. The condition was maintained upto 15 minute and the concentration was returned to A/B =95/05 after 3 minute and hold for 2 minute. The flow rate was 1.0 ml/min. Famotidine and Ibuprofen was quantified at a wavelength of 270 nm. Twenty micro liters of sample was injected in the HPLC system. Data acquisition and peak integration were achieved using Agilent chemstation software.

Standard stock solution: About 27 mg Famotidine (99.13%) and 800 mg Ibuprofen (99.01%) was accurately weighed, transferred into a 100 ml volumetric flask and dissolved in diluent. Diluent used was the mixture of water and acetonitrile in ratio of 50:50, v/v. A standard solution was prepared from this stock solution by transferring 10 ml of solution to a 100 ml volumetric flask and diluted with diluent. This solution contains 27 µg/ml and 800 µg/ml of Famotidine and Ibuprofen respectively.

Preparation of sample: Ten tablets were weighed and finely powdered. A quantity of powder equivalent to 27 mg of Famotidine and 800 mg of Ibuprofen was transferred in a 100 ml volumetric flask. To this flask, 50 ml of diluent was added and the solution was sonicated for 25 minute with intermittent shaking. Make up the volume with diluent and centrifuged at 10,000 rpm for 10 minute. From the centrifuged solution, 5 ml of clear solution was transferred to a 50 ml volumetric flask and diluted with diluent.

Procedure for forced degradation study: Specificity is the capability of analytical method to measure the concentration of analyte without any interference of impurities, degradation products, excipients or related substances. Stress degradation of tablet was carried out to evaluate the specificity of method for potential degradation products of both the compounds. Tablet powder was subjected to acidic (0.1 M HCl), basic hydrolysis (0.1M NaOH), oxidation (30% hydrogen peroxide), thermolysis and photolysis.

Acidic degradation: Weighed and transfer powder equivalent to 27 mg of Famotidine and 800 mg of Ibuprofen to 100 ml volumetric flask. 10 ml 0.1N HCl solution was added to it and heated the mixture at 60^0C for 3 hrs in water bath. Allowed the solution to attend ambient temperature and neutralized with 0.1N NaOH solution to pH 7. The volume is made up with diluent. Further dilute the solution to achieve the assay level concentrations of both analytes.

Alkali degradation: Weighed and transfer powder equivalent to 27 mg of Famotidine and 800 mg of Ibuprofen to 100 ml volumetric flask. 30 ml diluent and 10 ml 0.1N NaOH solution was added and heated the mixture at 60^0C for 3 hrs in water bath. Neutralized the solution with 0.1N HCl solution to pH 7 and the volume is made up with diluent. Further dilute the solution to achieve the assay level concentrations of both analytes.

Oxidative degradation: Weighed and transfer powder equivalent to 27 mg of Famotidine and 800 mg of Ibuprofen in 100 ml volumetric flask. 30 ml diluent and 5 ml 30% hydrogen peroxide was added to it and heated the mixture at 60^0C for 1 hr in water bath. Allow the solution to attend ambient temperature and the volume was made up with diluent. Further dilute the solution to achieve the assay level concentrations of both analytes.

Thermal degradation: Powder was kept in Petri dish at 90 ^{0}C for 24 hrs. The solution was prepared to achieve the assay level concentrations of both analytes.

UV-Short (254 nm) degradation: Powder of drug product exposed to UV short light for 24 hrs. The solution was prepared in diluent to achieve assay level concentrations of both analytes.

UV-Long (366 nm) degradation: Powder of drug product exposed to UV long light for 24 hrs. The solution was prepared in diluent to achieve assay level concentrations of both analytes. Peak purity check was performed for Famotidine and Ibuprofen peak after each stress condition.

Method validation: The proposed HPLC method was validated for specificity, system suitability, linearity, sensitivity, precision and accuracy for each compound according to ICH guidelines[19].

System suitability: System suitability was evaluated from six replicate injections of standard solution at 100% assay concentration of Famotidine and Ibuprofen. The capacity factor, tailing factor, retention time and theoretical plates were calculated from the standard solution chromatogram.

Precision: The system precision was determined by six replicate injections of standard solution at working level concentrations of Famotidine and Ibuprofen. The mean, SD and percentage RSD of peak area were calculated. Method precision was determined by calculating percentage RSD for six measurements of tablet solution. Intermediate precision was evaluated by calculating the percentage RSD for six measurements of tablet solution by using different analyst.

Linearity: Linearity was verified by analyzing seven concentrations of Famotidine and Ibuprofen mix standard solutions making triplicate injections for each concentration. Primary mixed standard stock solution of Famotidine and Ibuprofen was prepared (0.27 mg/ml and 8.0 mg/ml respectively) by dissolving the standards in the diluent. Calibration standards were prepared over a range 0.4 to 40 µg/ml for Famotidine and 0.7 to 1200 µg/ml for Ibuprofen.

Accuracy/recovery: Accuracy of the method was demonstrated by preparing placebo samples spiked with 80, 100 and 120% of nominal concentrations of Famotidine and Ibuprofen present in the tablet. Each concentration level was prepared in triplicate. Percentage recoveries were used to express accuracy.

Limit of detection and Limit of quantification: For determining the LOD and LOQ, a specific calibration curve was constructed using the analytes response and concentrations in the range of LOD and LOQ. LOD and LOQ were calculated by using following equations:

LOD = Cd x Syx /b

LOQ = Cq x Syx /b

Where, Cd/Cq is coefficient for LOD/LOQ; Syx is residual variance due to regression; b is slope.

Precision at LOQ was checked by analyzing six standard solutions prepared at LOQ level and calculating the percentage RSD of peak area.

Robustness: The robustness is the ability of method to remain unaffected by small changes in parameters. To determine robustness of the method, experimental conditions were purposely altered and assay, peak tailing, theoretical plates and peak area percentage RSD were evaluated. The flow rate of mobile phase was 1.0 ml/min. To study the effect of flow rate it was changed to 0.1 units from 1.0 to 0.9 ml/min. and 1.1 ml/min. The effect of column temperature was studied at 28^{o}C and 32^{o}C instead of 30^{o}C, while other mobile phase components were kept constant. The effect of mobile phase composition was studied by changing initial composition of mobile phase to 0.05 M ammonium acetate and acetonitrile in ratio 97:0, v/v and 93:07, v/v. At all conditions sample was assayed in triplicate. The effect of detection wavelength was studied at 266 nm and 274 nm.

Results and Discussion

Optimization of the chromatographic conditions: Famotidine and Ibuprofen are completely different in their structural polarity having pKa values about 7.1 and 4.5 respectively. Therefore it is very critical to develop common LC method for the assay of both analytes. To develop stability indicating assay method it is necessary to separate the both analyte peaks from degradants peaks. To achieve this, different column stationary phases like C_{18}, CN,

C_8 tried with different mobile phases containing buffers like phosphate, ammonium acetate and trifluoroacetic acid with different pH (3-9) and organic modifier (acetonitrile) were used.

Our objective of chromatographic method development was to achieve optimal resolution between both analyte peaks, tailing factor should be less than 2, retention time in between of 3 to 10 minute. From development trials it was clear that Cyano stationary phase have good selectivity than other stationary phases. The chromatographic separation of both analytes from its degradants was achieved using YMC Cyano, (150 × 4.6 mm, 5 µm) YMC INC. USA column, when employed isocratic mobile phase but retention time for Ibuprofen was too long. Therefore attempt then made with gradient method using the same column. In all above mentioned trials, YMC Cyano

column shows better performance as compared to other C_{18} or C_8 columns. It was determined that mixture of aqueous solution of 0.05 M ammonium acetate and acetonitrile using gradient at the flow rate of 1.0 ml/min and column temperature at 30°C. Both analytes peak shape with less tailing, resolved from degradants and the chromatographic analysis time was found to be less than 20 minute. In optimized conditions its degradants were well separated. Typical retention time of famotidine and Ibuprofen peaks were about 7.0 and 9.0 minute respectively.

Results of forced degradation studies: The stress conditions were designed to result in approximately 5 to 20% degradation of the active pharmaceutical agent[20]. Overstressing the samples may generate irrelevant degradants which would never be seen in stability studies and under stressing may fail to generate degradants that may be generated in stability studies[21]. All degradation samples were analyzed.

The drug product showed extensive degradation in acid hydrolysis, alkali hydrolysis and oxidative condition. Table-2 indicates the extent of degradation, peak purity and assay of both analytes under various stress conditions. Chromatographic peak purity data was obtained from the spectral analysis report. The peak purity value was found to be greater than 990 indicates a homogeneous peak and thus establishing the specificity of assay method. Figure 2a to 2e showed the chromatograms of tablet solution, diluent, placebo solution, Famotidine specificity solution and Ibuprofen specificity solution respectively. Figure 2f to 2h showed alkali hydrolysis degradation of tablet, oxidative degradation of tablet and acid hydrolysis degradation of tablet respectively. Figure 3a to 3c showed peak purity spectra of Famotidine in presence of it degradants in alkali hydrolysis, oxidative and acid hydrolysis respectively. Figure 3d to 3f showed peak purity spectra of Ibuprofen in presence of it degradants in alkali hydrolysis, oxidative and acid hydrolysis respectively. Assay of Famotidine and Ibuprofen was unaffected by the presence of other degradants which confirms the stability indicating power of the method.

Results of method validation: The system suitability parameters measured from six replicate injections of Famotidine and Ibuprofen mix standard solution were retention time, capacity factor, theoretical plates, tailing factor and the resolution between both analytes. Results are tabulated in table-1.

Percentage RSD in the study of repeatability of the assay (method precision) for Famotidine and Ibuprofen was 0.8 and 1.1 respectively. Percentage RSD in the study of ruggedness (intermediate precision) for Famotidine and Ibuprofen was 0.7 and 1.3 respectively. These results suggest that the developed method is precise for both the analytes. Results are tabulated in table-3.

The results obtained for the accuracy study indicated that the average recovery at each concentration for both analytes was 100 ± 2% and the percentage RSD at each concentration level was less than 2.0. Thus establishing the accuracy of the method. Results are tabulated in table-4.

Calibration curves were linear over the range of 0.4 to 40 µg/ml for Famotidine and 0.7 to 1200 µg/ml for Ibuprofen with a correlation coefficient of 0.9992 and 0.9997 respectively. The results indicate a linear relationship between peak areas and concentrations. Observed LOQ values for Famotidine and Ibuprofen were 0.4 µg/ml and 0.7 µg/ml respectively. Observed LOD values for Famotidine and Ibuprofen were 0.13 µg/ml and 0.22 µg/ml respectively. Results are tabulated in table-3. In robustness study the all deliberated changes % assay of both analytes was 100 ± 2% and the percentage RSD at each condition was less than 2. Results are tabulated in table-5.

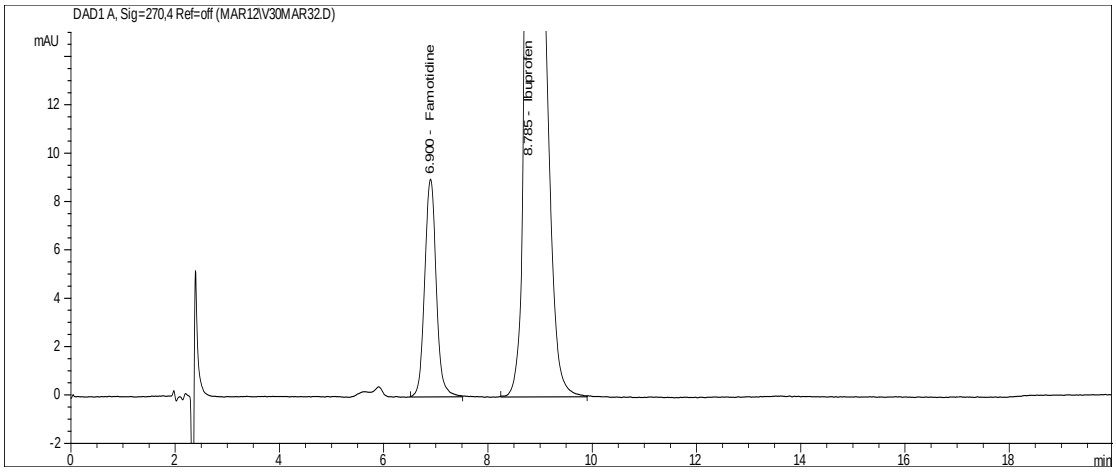
Table-1
System suitability data

Analyte	RT (Min.)	Capacity factor	Resolution	Theoretical Plate Count	USP Tailing
Famotidine	6.9	3.51	-	7863	1.2
Ibuprofen	8.8	4.47	5.3	9591	1.1

Table-2
Results of analysis of forced degradation study

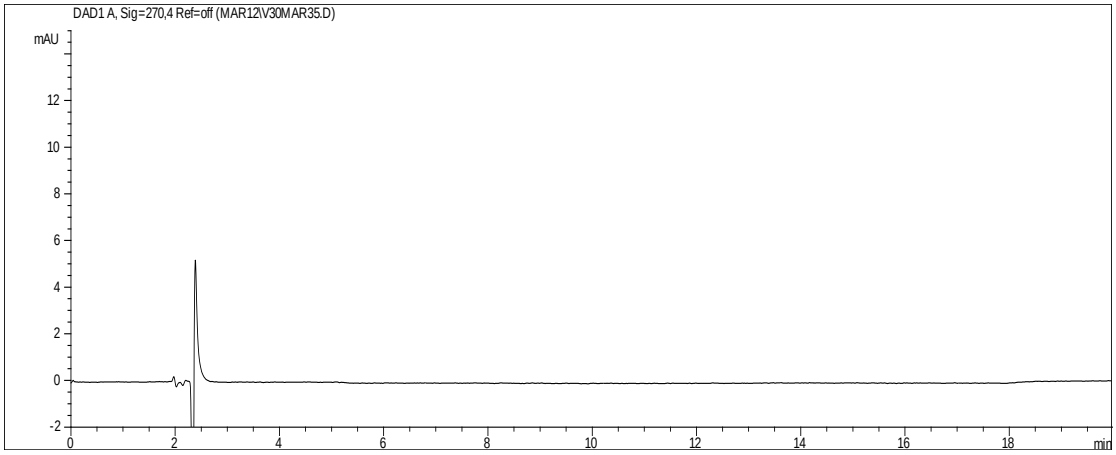
Stress condition	Peak purity*		Assay (%)	
	Fam.	Ibu.	Fam.	Ibu.
Acidic (60 °C/3 hrs)	999.368	999.868	88.56	98.69
Alkali (60 °C/3 hrs)	999.523	999.731	92.63	99.11
Oxidative (60 °C/1hrs)	999.725	999.699	85.12	98.86
Thermal (90 °C/24 hrs)	999.829	999.629	99.23	99.05
UV-short (24 hrs)	999.925	999.801	99.01	99.42
UV-long (24 hrs)	999.919	999.933	99.16	99.78

*peak purity values in the range of 990-1000 indicate a homogeneous peak



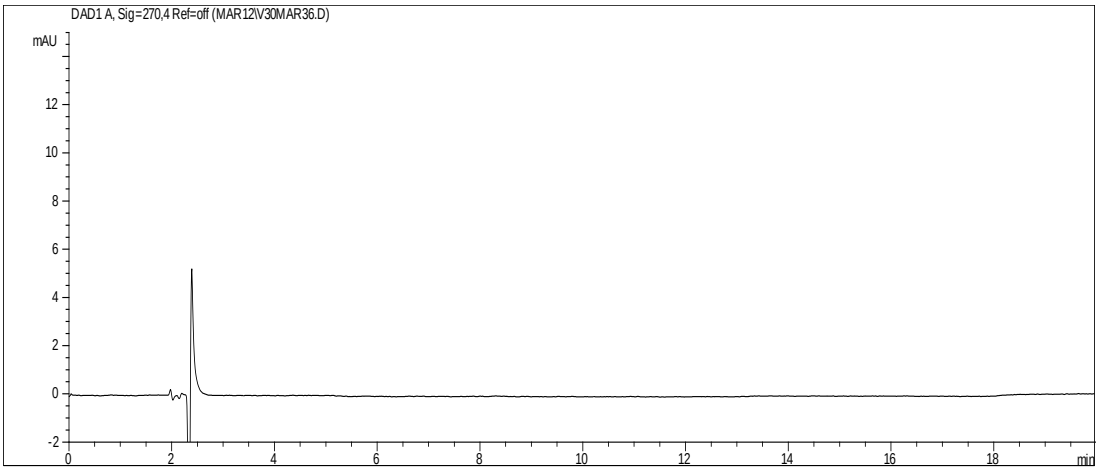
Time (min)

Figure-2a
Chromatogram of tablet solution



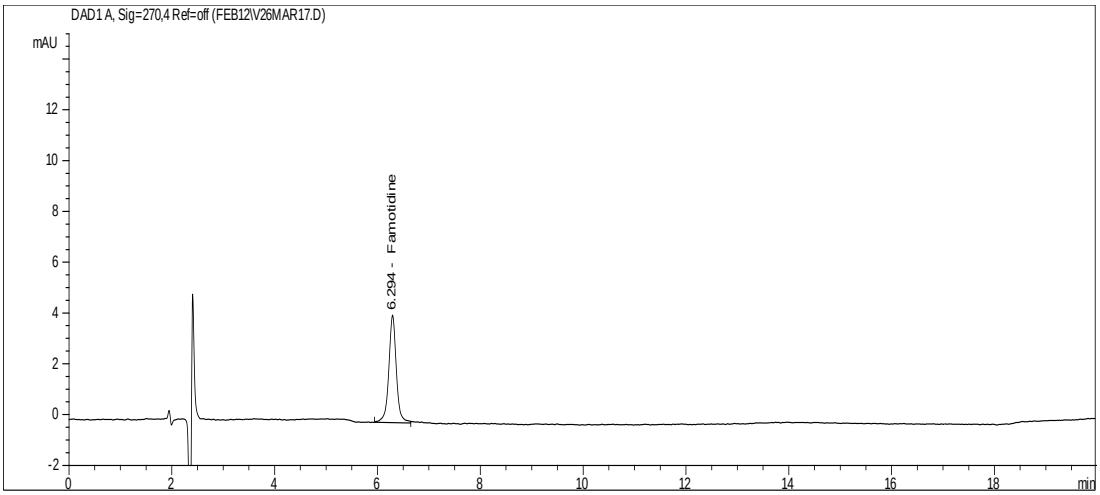
Time (min)

Figure-2b
Chromatogram of diluent



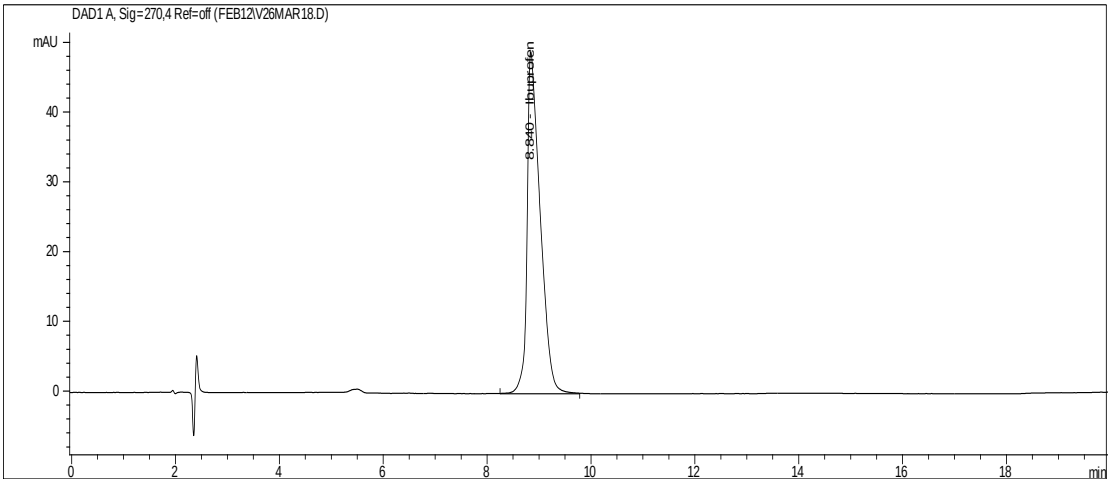
Time (min)

Figure-2c
Chromatogram of placebo



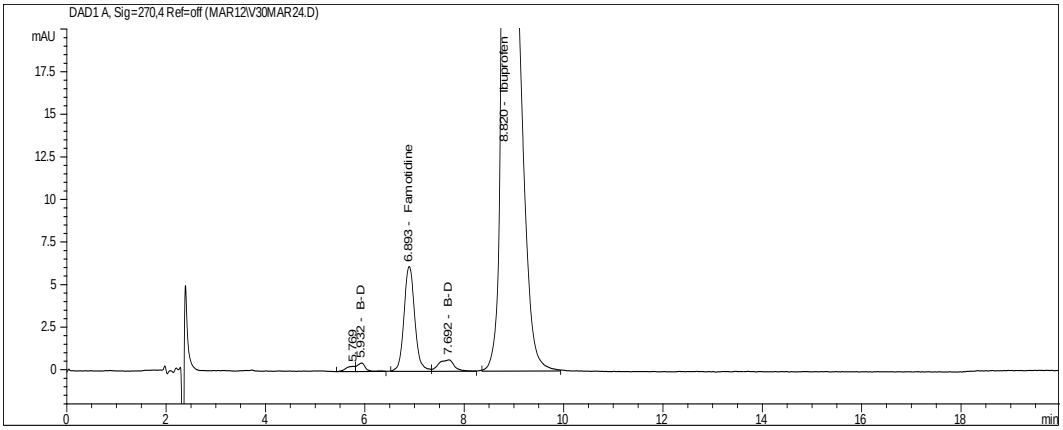
Time (min)

Figure-2d
Chromatogram of Famotidine solution



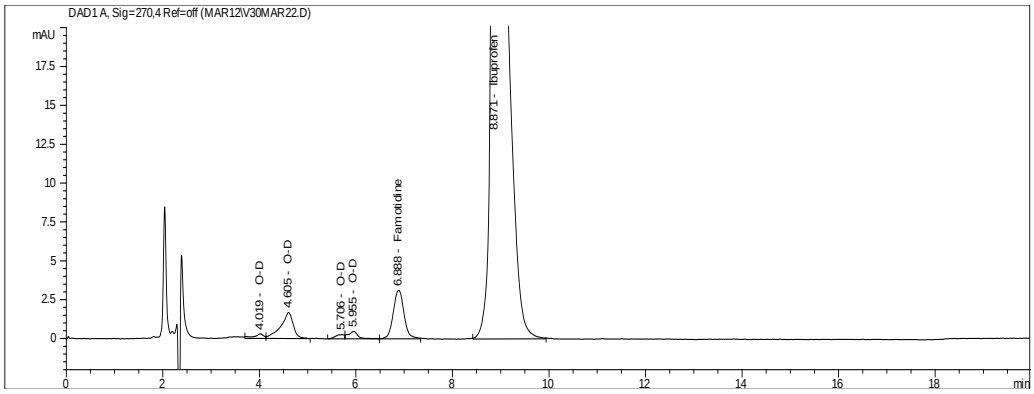
Time (min)

Figure-2e
Chromatogram of Ibuprofen solution



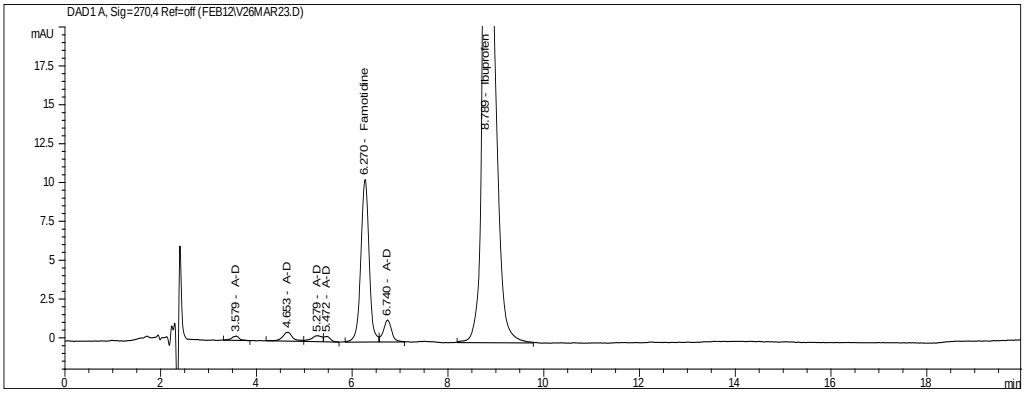
Time (min)

Figure-2f
Chromatogram of alkali hydrolysis degradation of the tablet



Time (min)

Figure-2g
Chromatogram of oxidation degradation of tablet



Time (min)

Figure-2h
Chromatogram of acid hydrolysis degradation of tablet

Table-3
Validation summary

Parameters	Famotidine	Ibuprofen
Method Precision (% RSD)	0.8	1.1
Intermediate Precision(% RSD)	0.7	1.3
LOQ (µg/ml)	0.4	0.7
LOD (µg/ml)	0.13	0.22
Linearity range (µg/ml)	0.4 to 40	0.7 to 1200
Regression equation	y = 7.0212x + 3.1592	y = 5.2896x + 2.9358
r2	0.9992	0.9997

Table-4
Results of accuracy/recovery

% Level	Amount spiked (mg)	Amount found (mg)	Recovery (%)	RSD (%)
Famotidine				
80	21.45	21.32	99.19	1.1
100	27.56	26.99	99.65	0.7
120	32.96	32.56	99.16	0.9
Ibuprofen				
80	643.12	632.11	98.39	1.2
100	804.23	801.59	99.13	0.9
120	965.23	960.12	98.99	0.8

Table-5
Results of analysis of robustness study

Parameter	Variation	Rs	Assay (%)	
			Fam.	Ibu.
Flow rate (± 10%)	0.9 ml/min.	5.3	99.12	99.50
	1.1 ml/min.	5.1	99.10	99.78
Organic modifier (± 2%)	3 ml	4.4	98.97	98.97
	7 ml	5.4	99.30	99.62
Column Oven temperature (± 2 °C)	28 °C	5.3	99.89	98.99
	32 °C	5.1	99.20	99.62
Wavelength ((± 4 nm)	266 nm	5.0	99.78	99.81
	274 nm	5.0	99.32	99.73

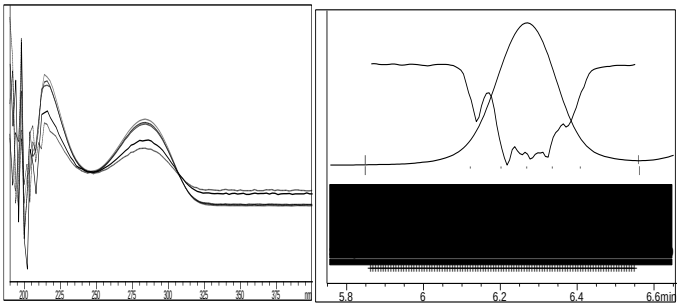


Figure-3a
Peak purity spectrum of Famotidine in alkali hydrolysis

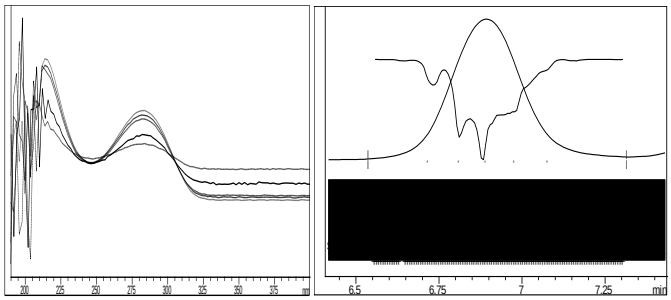


Figure-3b
Peak purity spectrum of Famotidine in oxidation

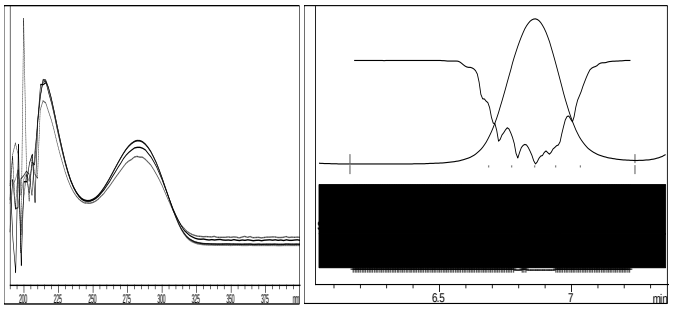


Figure-3c
Peak purity spectrum of Famotidine in acid hydrolysis

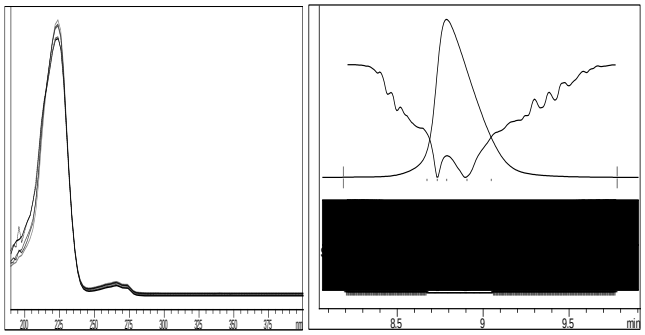


Figure-3d
Peak purity spectrum of Ibuprofen in alkali hydrolysis

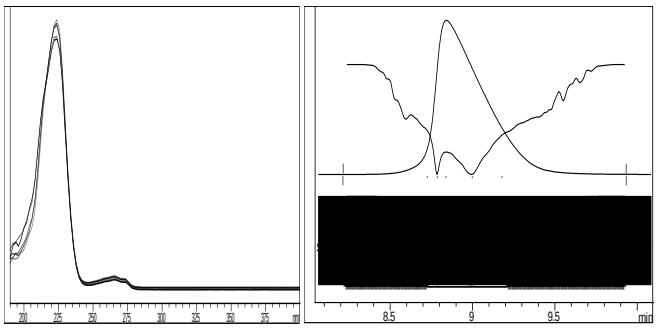


Figure-3e
Peak purity spectrum of Ibuprofen in oxidation

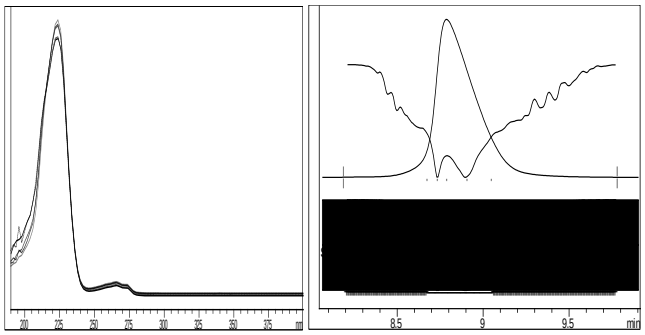


Figure-3f
Peak purity spectrum of Ibuprofen in acid hydrolysis

Conclusion

In this study, we described a stability indicating HPLC method for simultaneous determination of Famotidine and Ibuprofen which has been successfully validated. The accuracy and precision are good with a good resolution between both the analytes. The method is sensitive enough to detect degradation peaks which are not co eluting with those of the active analytes as shown in the chromatograms. Therefore, this method is suitable for simultaneous determination of Famotidine and Ibuprofen in combination tablet. This method has been successfully applied to study the drug excipients compatibility and stability study of the tablets.

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