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Validated stability indicating UPLC method for the simultaneous determination of famotidine and ibuprofen in combined dosage form

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ABSTRACT

A simple, precise and accurate stability indicating ultra-performace liquid chromatography (UPLC) method was developed and validated for the simulataneous quantitative determination of Famotidine and Ibuprofen in the presence of degradation products. The separation achieved on Acquity UPLC column with simple gradient method. The mobile phase A contains a mixture of pH 6.0 sodium acetate buffer and methanol in the ratio of 80:20 (v/v) and mobile phase B contains a mixture of pH 6.0 sodium acetate buffer and Mehtanol in the ratio of 30:70 (v/v). The peaks were monitored at 260 nm wavelength. This method was validated for accuracy, precision, linearity, and robustness. The method was also found to be stability indicating. Famotidine was found to degrade significantly in oxidative, acid and base stress conditions. The degradation products were well resolved from main peak of Famotidine and Ibuprofen, thus proved the stability indicating power of the method.

KEYWORDS

Ibuprofen;
Famotidine;
UPLC;
Method development;
Stability indicating.

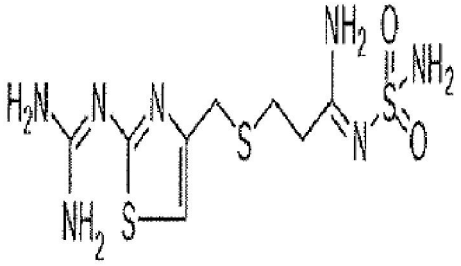
INTRODUCTION

Famotidine (FAM), 3-(((2-((aminoiminomethyl) amino)-4-thiazolyl)methyl)thio)-N'-(aminosulfonyl) propanimidamide, is a potent,competetive and reversible inhibitor of histamine action at the H2 receptor. It is used for the treatment of duodenal and gastric ulcers. The empirical formula of Famotidine is $C_8H_{15}N_7O_2S_3$ and its molecular weight is 337.43. Famotidine is available in 20 mg and 40 mg for oral administration[1].

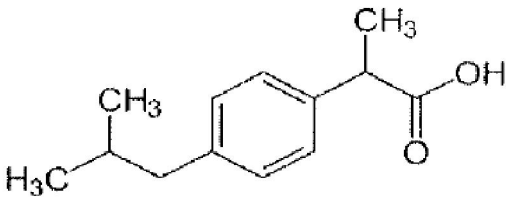
Ibuprofen (IBU) ((2*RS*)-2-[4-(2-Methylpropyl) phenyl]propanoic acid), is a nonsteroidal anti-inflammatory drug, which is available in 400 mg, 600 mg, and 800 mg tablets for oral administration. It is indicated for relief of the signs and symptoms of rheumatoid arthritis and osteoarthritis, for relief of mild to moderate pain and also indicated for the treatment of primary dysmenorrhea. The empirical formula of Ibuprofen is $C_{13}H_{18}O_2$ and its molecular weight is 206.29[1].

U.S.Food and Drug Administration has approved Duexis (Ibuprofen/Famotidine), a novel tablet formulation containing a fixed-dose combination of Ibuprofen (800 mg) and Famotidine (26.6 mg) [Figure 1] in 2011. In combination Ibuprofen treats the symptoms of arthritis, Famotidine helps reduce the risk of ulcers in the stomach or intestines that can be caused by long-term use of ibuprofen[2]. So far, few liquid chromatography procedures have been described for the individual determination of Ibuprofen and Famotidine[3-12]. These procedures were developed to estimate either Ibuprofen

or Famotidine individually from formulation or plasma. Whereas no single method has been reported to estimate simultaneously from formulation. The available individual methods does not solve the shorter development time for generic drug companies. Hence it is necessary to develop a simultaneous, rapid, accurate and validated method for the determination of FAM and IBU from combined dosage form.



(a) Chemical structer of famotidine



(b) Chemical structer of ibuprofen

Figure 1 : Chemical structures of famotidine and ibuprofen

Ultra performance liquid chromatography (UPLC) is a advanced technique in liquid chromatography, which enables significant reduction in separation time and solvent consumption. Literature indicates that UPLC system allows about nine fold decrease in analysis time as compared to the conventional HPLC system using 5µm particle size analytical columns and about three fold decrease in analysis time in comparison with 3µm particle size analytical columns without compromise on overall separation.

EXPERIMENTAL

Apparatus

Acquity UPLCTM system (Waters, Milford, USA) used consisting of a binary solvent manager, a sample manager and a UV detector. The output signal was monitored and processed using empower software, water bath equipped with MV controller (Julabo, Seelbach, Germany) was used for hydrolysis studies. Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability studies were performed in a dry air oven (MACK Pharmatech, Hyderabad, India).

Reagents and chemicals

Ibuprofen and Famotidine tablets were received from formulation development department of Dr. Reddy's laboratories limited, Hyderabad, India. Sodium acetate trihydrate, Triethylamine, glacial acetic acid, Methanol for HPLC were purchased from Merck, Darmstadt, Germany and water used was obtained by using Millipore MilliQ Plus water purification system.

UPLC conditions

The Separation was achieved on Acquity UPLC BEH Shield C-18,100 mm x 2.1 mm and 1.7 µm particle size, using gradient flow program. The buffer used for mobile phase and diluent was 0.05 M sodium acetate buffer and 2 ml of Triethyl amine in 1000 ml of MilliQ water and adjusted the pH to 6.0 with Glacial acetic acid. Mobile phase A was a mixture of pH 6.0 buffer and Methanol in the ratio of 80: 20(v/v); respectively and the mobile phase B contains a mixture of pH 6.0 Buffer and Methanol in the ratio of 30:70 (v/v), respectively. The flow rate of mobile phase was set as 0.35 mLmin-1. The UPLC gradient program was set as: time (min)/% solution B: 0.01/15,1.2/100,2.5/100,3.0/15 and 4.0/15. The column temperature was maintained at 30°C and the detector was monitored at a wavelength 260 nm. The injection volume was 1.0µL.

Standard solutions

A standard solution containing 3200 µg/ml of IBU and 106 µg/ml of FAM was prepared by dissolving IBU and FAM in diluent (60:40(v/v) pH 6.0 Sodium acetate buffer and Methanol).

Analysis of formulation

Twenty tablets each containing 800 mg of IBU and 26.6 mg of FAM were weighed to determine the average weight and powdered in a mortar with pestle. A quantity of powder equivalent to 26.6 mg of FAM and 800 mg of IBU were weighed and transferred into a 250 ml volumetric flask, added 175 ml of diluent and sonicated for 30 minutes with intermediate shaking and

then made up to volume with diluent. Filtered through 0.45µm nylon membrane filter by discarding first few mL filtrates and injected into the UPLC.

System suitability criteria

Relative standard deviation for peak areas of FAM and IBU for five injections of standard should not be more than 2.0% and tailing should not be more than 2.0 for both FAM and IBU.

Forced degradation studies

In order to establish whether the analytical method and the assay were stability-indicating, FAM and IBU tablet powder was stressed under various conditions to conduct forced degradation studies.

Oxidation

Solutions for use in oxidation studies were prepared in 10% H2O2 and exposed to 60°C for 30 minutes.

Acid degradation

Solutions for acid degradation studies were prepared in 2M hydrochloric acid and exposed to 60°C for 30 minutes.

Alkali degradation

Solutions for alkali degradation studies were prepared in methanol and 2M sodium hydroxide and exposed to 60°C for 30 minutes.

Temperature stress

Temperature Degradation study was performed by exposing the drug content at 105°C for 6 hours.

Photo stability

Sun light study was performed by exposing the drug content for sun light over a period of ten days.

RESULTS AND DISCUSSION

UPLC method development and optimization

Generic manufacturers do not incur the cost of drug discovery. Sometimes, reverse engineering is used to develop bioequivalent versions to existing drugs[13]. Before starting the development to separate the FAM and IBU a thorough literature search was done. Both IBU and FAM tablets are official in USP with different chromatographic conditions, column and diluent. In USP monograph IBU was analyzed by using pH 3.0 chloroacetic acid as buffer for mobile phase, FAM was analyzed by using pH 6.0 Sodium acetate trihydrate buffer along with Triethyl amine in the mobile phase. IBU is acidic drug with pka 4.6 and FAM is a weakly basic drug with pka 7.1. Based on the molecular properties of both IBU and FAM, pH 6.0 Sodium acetate trihydrate buffer with triethyl amine was choosed for mobile phase along with Methanol in the ratio of 80:20 (*v/v*). Initial method development trials performed to separate both FAM and IBU using isocratic flow method, but IBU was eluting at around 15 minutes and not a symmetrical peak. So gradient trials performed to reduce the run time and for better peak shape of IBU. Both FAM and IBU standards injected in different stationary phases like C18, C8 and BEH shield RP 18. In all the columns the tailing factor for FAM is less than 1.5, but tailing factor of IBU is more than 1.8 in C8 and fronting was observed in C18 column. The embedded polar group into the bonded phase ligand in BEH shield RP 18 column [Figure 2] helped to reduce the tailing factor of IBU less than 1.5. The method was optimized to separate major degradation products formed under different stress conditions. The main target of the chromatographic method is to separate closely eluting degradation products; mainly the degradants formed during degradation of FAM. The resolution between the peak at 1.059 RT and FAM was 1.9 in acid degradation, between FAM and peak at 1.244 RT was 1.7 in base degradation. FAM is sensitive to acid, base and peroxide degradation conditions and IBU was highly stable and not degrading in all the degradation conditions. Both FAM and IBU were eluting within 4 minutes of time with mobile phase A 80:20(*v/v*) and B 30:70 (*v/v*) of pH 6.0 buffer and methanol ratio. The ratio was finalized after confirming the good separation in between FAM and IBU along with degradation products [Figure 3].

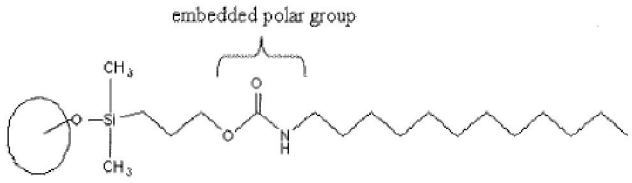
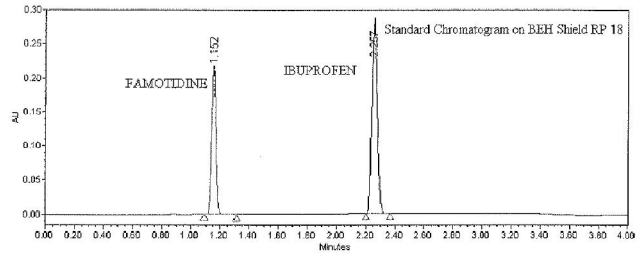
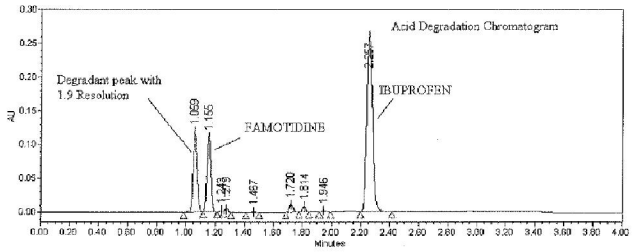


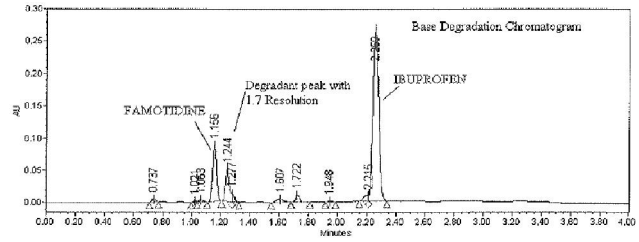
Figure 2 : Embedded polar group in acquity BEH shield RP 18 column



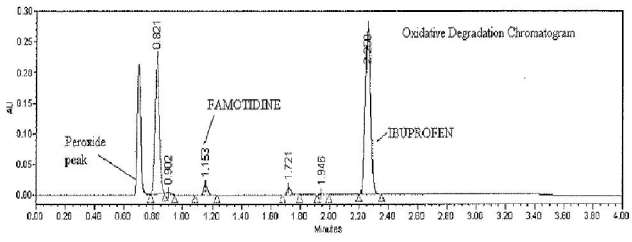
(a) Standard chromatogram on BEH shield RP 18



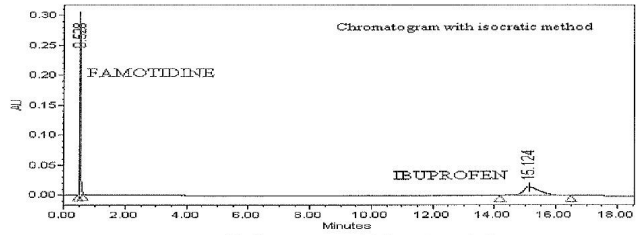
(b) Acid degradation chromatogram



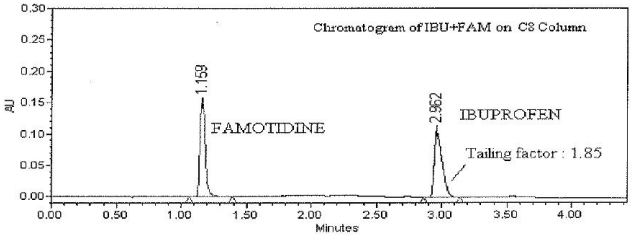
(c) Base degradation chromatogram



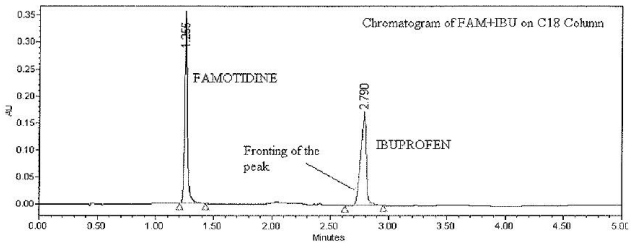
(d) Oxidative degradation chromatogram



(e) Chromatogram with Isocratic method



(f) Chromatogram in acquity C8 column



(g) Chromatogram in acquity C18 column

Figure 3 : (a) Standard chromatogram on BEH shield RP 18; (b) Acid degradation chromatogram ; (c) Base degradation chromatogram;(d) Oxidative degradation chromatogram;(e) Chromatogram with Isocratic method; (f) Chromatogram on Acquity C8 column;(g) Chromatogram on Acquity C18 column

Validation

Method validation was performed as per ICH guidance[14-16] for simultaneous determination of FAM and IBU in the formulations.

System suitability

To establish system suitability standard solution was injected five times and the chromatographic parameters like relative standard deviation for replicate injections of FAM and IBU, the tailing factor for both FAM and IBU peaks were evaluated. The relative standard deviation for five replicate injections of both FAM and IBU was found to be less than 2.0% and the tailing factor for both FAM and IBU peaks were less than 2.0. The results meet the system suitability criteria.

Precision

Precision of the assay was investigated with respect to both repeatibilty and reproducibility. Repeatability was performed by injecting six replicate samples with the concentration of 3200 µg/ml of IBU and 106 µg/ml of FAM respectively. The % RSD values for FAM and IBU were 0.6 and 0.8. Different analyst from the same laboratory evaluated the intermediate

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TABLE 1 : Precision, % assay of six samples

FAM		IBU	
99.2		100.5	
98.8		101.2	
100.6	Mean: 99.5	102.0	Mean: 101.0
98.6	% RSD: 0.7	100.0	% RSD: 0.6
100.1		101.0	
99.6		101.5	

precision of the method. This was performed by assaying the six samples of FAM and IBU tablets against qualified reference standard. The percentage assays of six samples were calculated. The results are summarized in TABLE 1.

Accuracy

The accuracy of an analytical method expresses the nearness between the reference value and found value.

TABLE 2 : Results of accuracy (% recovery)

	Spiked level (%, n=3)	Amount added (µg/mL)	Amount recovered (µg/mL)	% recovery
FAM	50	53	52.8	99.6
	100	106	105.2	99.2
	150	159	156	98.1
IBU	50	1600	1585	99.1
	100	3200	3195	99.8
	150	4800	4765	99.3

The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100% and 150% of target test concentration (106 µg mL^{-1} of FAM, 3200 µg mL^{-1} of IBU in tablets. The results obtained are shown in TABLE 2.

Linearity

The calibration curves plotted for FAM and IBU were linear over the concentration range of 53-159 µg/ml for FAM and 1600-4800 µg/ml for IBU. Peak areas were plotted against concentrations and calculated correlation coefficient from the resultant curve. The Correlation coefficient values of FAM and IBU are 0.999 & 0.998.

TABLE 3 : Peak purity results of FAM and IBU

Stress condition	Purity angle		Purity threshold		Purity flag	
	FAM	IBU	FAM	IBU	FAM	IBU
Acid stress	0.521	0.062	0.806	0.562	NO	NO
Base stress	0.235	0.359	0.715	0.921	NO	NO
Peroxide stress	0.533	0.210	0.649	0.295	NO	NO
Heat stress	0.062	0.404	0.258	0.554	NO	NO
Light stress	0.421	0.285	0.951	0.826	NO	NO

LOD and LOQ

The LOQ is the level above which quantitative results may be obtained with a specified degree of confidence; LOD is the lowest concentration level that can be determined to be statistically different from a blank. The LOQ that produced the requisite precision and accuracy was found to be 15 µg/ml for FAM and 3µg/ml for IBU, respectively. The LOD for both FAM and IBU were found to be 6 µg/ml and 1µg/ml, respectively.

Selectivity

The results of stress testing studies indicated a high degree of selectivity of this method for FAM and IBU. Photodiode array detection was used as an evidence to prove the selectivity of the method, and to evaluate the homogeneity of the drug peaks. Typical chromatograms obtained following the assay of sample and stressed samples are shown in Figure 2. There is no degradation observed for IBU, FAM is sensitive to Acid, Base and Peroxide. FAM degraded up to 40 % in both Acid and Base degradation conditions, all the degraded peaks were well separated with both FAM and IBU. Peak purity was evaluated for all degradation conditions and found both FAM and IBU peaks are homogenous. The results are summarized in TABLE 3.

Robustness

In all the deliberate varied chromatographic conditions (Flow rate, column temperature and composition of organic solvent), no significant difference observed in system suitability.

CONCLUSION

A simple, rapid, accurate and precise stability indicating UPLC analytical method has been developed and validated for the routine analysis of FAM and IBU in tablet dosage form. The results of stress testing un-

dertaken according to the international conference of harmonization (ICH) guidelines reveal that the method has the ability to separate these drugs from their degradation products. Method validation results proved the method is precise, selective, accurate, linear, robust and stability indicating.

ACKNOWLEDGEMENT

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REFERENCES

[1] http://www.Rxlist.com
[2] http://www.drugs.com/mtm/famotidine-and-ibuprofen.html
[3] United state pharmacopoeia, **34**, 3101.
[4] United state pharmacopoeia, **34**, 2795.
[5] Ling Zhong, K.C.Yeh; Journal of Pharmaceutical and Biomedical Analysis, **16**, 1051–1057 **(1998)**.
[6] A.Zarghi, H.Komeilizadeh, M.Amini, L.Kimiagar; Pharm,. Pharmacol.Commun., **4**, 77-80 **(1998)**.
[7] M.T.Mohite, S.N.Shet, S.Shaikh, V.R.Vaidya, R.S.Karodi; IJRAP, **1(2)**, 475-479 **(2010)**.
[8] A.Zarghi, A.Shafaati, S.M.Foroutan, A.Khoddamc; Journal of Pharmaceutical and Biomedical Analysis, **39**, 677–680 **(2005)**.
[9] M.G.Quaglia, E.Donati, S.Fanali, P.Catarcini; Societa chimica italiana, **58(9)**, 699-705, Sep **(2003)**.
[10] H.Farrar, L.Letzig; J.Chromatogr.B, **780**, 341-348 **(2002)**.
[11] M.R.Whelan, J.L.Ford; J.Pharm.Biomed.Anal, **30**, 1355-1359 **(2002)**.
[12] A.Reddy, M.Hashim; International Journal of Pharmaceutics, **335**, 63-69 **(2007)**.
[13] http://en.wikipedia.org/wiki/Generic_drug
[14] ICH Guide Q2A: Text on vsalidation of analytical procedures: Term and definition, International Conference on harmonization.
[15] ICH Guide Q2B: Validation of analytical procedures: Methodology, International Conference on harmonization.
[16] ICH Guide Q1B: Photo stability testing of new active substances and medicinal products.