Original Article

RP-UPLC method development and validation for the simultaneous estimation of ibuprofen and famotidine in pharmaceutical dosage form

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Abstract

Aim and Backrgound: A stability-indicating LC method was developed for the simultaneous determination of Ibuprofen and Famotidine in pharmaceutical dosage forms. **Materials and Methods:** The chromatographic separation was achieved on Acquity UPLC BEH C-18,50 mm x 2.1 mm and 1.7 µm column with gradient elution. The mobile phase A contains a mixture of 50 mM sodium acetate buffer (pH 5.5): methanol (85:15, v/v), and the mobile phase B contains a mixture of 50 mM sodium acetate buffer (pH 5.5): methanol (25:75, v/v). The flow rate was 0.3 mL min^{-1}, and the detection wavelength was 260 nm. **Results:** The limit of detection for Ibuprofen and Famotidine was 1.6 and 1.2 µg mL^{-1}, respectively. The limit of quantification (LOQ) for Ibuprofen and Famotidine was 5.1 and 4.3 µg mL^{-1}, respectively. **Conclusion:** This method was validated for accuracy, precision, and linearity. The method was also found to be stability indicating.

Key words: Famotidine, ibuprofen, ssimultaneous, sstability-indicating, UPLC

INTRODUCTION

Famotidine (FM), 3-(((2-((aminoiminomethyl)amino)-4-thiazolyl)methyl)thio)-N′-(aminosulfonyl) propanimidamide is a potent, competitive, and reversible inhibitor of histamine action at the H_2 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 (IB) ((2*RS*)-2-[4-(2-Methylpropyl)phenyl]propanoic acid) is a non-steroidal 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 for Ibuprofen is $C_{13}H_{18}O_2$ and its molecular weight is 206.29.[2]

To the best of our knowledge, few liquid chromatography procedures were described for the individual determination of Ibuprofen [Figure 1a] and Famotidine [Figure 1b].[2-17] These procedures were developed to estimate either Ibuprofen or Famotidine individually and from formulation or plasma, whereas no single method has been reported for their simultaneous estimation from the formulation. Hence, it is necessary to develop a stability indicating, rapid, accurate, and validated LC method for the simultaneous determination of Ibuprofen and Famotidine from combined dosage form for generic drug development.

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about

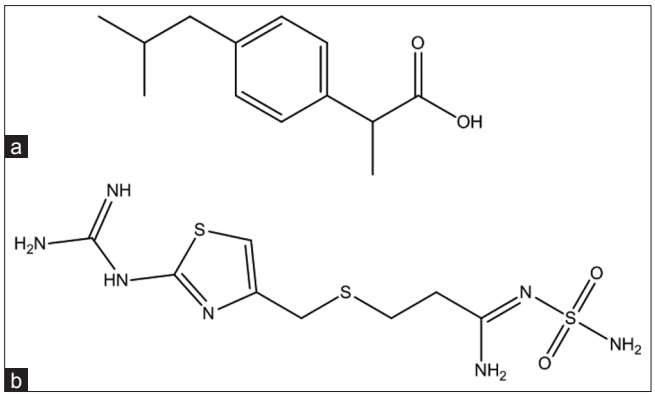

Figure 1: Chemical structure and chemical name of (a) Ibuprofen (b) Famotidine.

9-fold decreases in analysis time as compared to the conventional HPLC system using 5 µm particle size analytical columns, and about 3-fold decrease in analysis time in comparison with 3 µm 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 (Duexis) tablets (800 mg of Ibuprofen and 26 mg of Famotidine) were purchased from the pharmacy. 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

Chromatographic conditions

The chromatographic column used Acquity UPLC BEH C-18,50 mm x 2.1 mm and 1.7 µm particle size. The separation was achieved on a gradient method. 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 water and adjusted the pH to 5.5 with glacial acetic acid. Mobile phase A was a mixture of pH 5.5 buffer and methanol in the ratio of 85: 15(v/v), respectively, and the mobile phase B contains a mixture of pH 5.5 buffer and methanol in the ratio of 75:25 (v/v), respectively. The flow rate of mobile phase was set as 0.3 Ml min^{-1}. The UPLC gradient program was set as: Time (min)/% solution B: 0.01/10,1.6/100, 2.8/100, 3.0/10, and 3.5/10. The column temperature was maintained at 25°C, and the detector was monitored at a wavelength 260 nm. The injection volume was 1.5 µL.

Preparation of stock solutions

A standard solution containing 1600 µg/ml of IBU and 50 µg/ml of FAM were prepared by dissolving IB and FM in diluent (50:50 (v/v) pH 5.5 sodium acetate buffer and methanol).

Preparation of sample solution

Twenty tablets, each containing 800 mg of IB and 26 mg of FM, were weighed individually to determine the average weight and powdered separately in a mortar. A quantity of powder equivalent to 52 mg of FM and 1600 mg of IB were weighed and transferred into a 500 ml volumetric flask, added 300 ml of diluent and sonicated for 45 minutes with intermediate shaking and then made up to volume with diluent.

RESULTS AND DISCUSSION

Method development and optimization of stability indicating assay method

The method was optimized to separate major degradation products formed under varies stress conditions. I (pKa = 4.4) is acetic compound, whereas FM (pKa = 7.1) is basic compound. The main target of the chromatographic method is to get the separation for closely eluting degradation products. The degradation samples were run using different stationary phases like C18, C8, Cyano, and mobile phases containing buffers like phosphate, sulfate, and acetate with different pH (2-7) and using organic modifiers like acetonitrile and methanol in the mobile phase. But, the separation was satisfactory in the adopted chromatographic conditions only [Table 1 and Figure 2] the optimized conditions are, the mobile phase A was a mixture of pH 5.5 buffer and methanol in the ratio of 85:15(v/v), respectively, and the mobile phase B contains a mixture of pH 5.5 buffer and methanol in the ratio of 75:25 (v/v), respectively.

Specificity – forced degradation studies

Forced degradation studies were performed on IB

and FM to prove the stability-indicating property of the method. The stress conditions employed for degradation study of FM and IB include light exposure, heat (100°C), acid hydrolysis (1 N HCl), base hydrolysis (1 N NaOH), water hydrolysis, and oxidation (3% H_2O_2). For light studies, the monitoring period was 10 days, whereas for heat, acid, base, and water hydrolysis, it was 24 h. Oxidation was carried out for 2 h. Peak purity of the principal peak in the chromatogram of stressed samples of IB and FM tablets was checked using photo diode array detector.

Degradation was not observed in IB and FM stressed samples that were subjected to light and water hydrolysis. However, the degradation was observed under heat, oxidative conditions, base hydrolysis, and acid hydrolysis. The peak purity test results derived from PDA (Photo Diode Array detector) confirmed that the IB and FM peaks were pure and homogeneous in all the analyzed stress [Table 2]. This indicates that the method is specific and stability indicating.

Table 1: Results from system suitability test

Compound	USP resolution	USP tailing factor	No. of theoretical plates, N (USP tangent method)
Famotidine	-	1.2	3528
Ibuprofen	3.8	1.1	5869

Table 2: Summary of results from forced degradation experiments

Stress condition	Time	Assay of Famotidine (%)	Assay of Ibuprofen (%)	% Degradation
Acidic hydrolysis (1 N HCl)	24 h	88.5	99.2	11.3
Basic hydrolysis (1 N NaOH)	24 h	93.6	99.6	5.8
Oxidation (3% H_2O_2)	2 h	90.6	99.2	8.3
Aqueous hydrolysis	24 h	99.6	99.3	0.2
Thermal treatment (60 °C)	10 days	95.5	99.6	4.9
Light (photolytic degradation)	10 days	99.6	100.1	0.3

Validation

Method validation was performed as per ICH guidance for simultaneous determination of IB and FM in the formulations. The following validation characteristics were addressed, linearity, detection limit, quantification limit, precision, accuracy, robustness, ruggedness, and specificity.[18-20]

Precision

The precision of an analytical method gives information on the random error. It expresses of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. The percentage RSD values for the precision study was 0.5%, 0.6% (inter-day precision) and 0.5%, 0.6% (intra-day precision) for IB and FM, respectively. This is confirming good precision of the method. The results are summarized in Table 3.

Accuracy

The accuracy of an analytical method expresses the nearness between the reference value and found value. The accuracy of the method was evaluated in triplicate at three concentration levels, i.e. 50%, 100%, and 150% of target test concentration (52 μg mL^{-1} of FM, 1600 μg mL−1) of IBU in tablets. The results obtained are shown in Table 4.

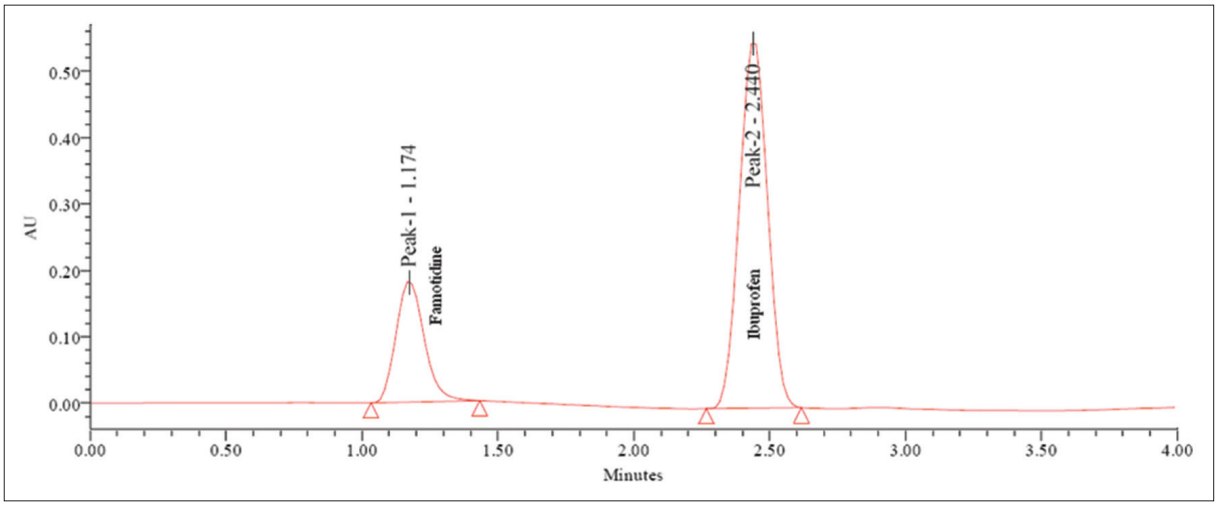

Figure 2: A typical HPLC chromatogram of Famotidine and Ibuprofen from tables.

Table 3: Results from determination of intermediate precision

Variation		RSD (%) for assay of Famotidine	RSD (%) for assay of Ibuprofen	Resolution (R_s) between Famotidine and Ibuprofen
Different system	Waters Aquity UPLC-1	0.5	0.6	3.8
	Waters Aquity UPLC-2	0.4	0.7	3.9
Different column	Batch-1	0.5	0.6	3.8
	Batch-2	0.3	0.5	3.8
Different analyst	Analyst 1	0.5	0.6	3.8
	Analyst 2	0.5	0.9	3.7

Table 4: Results from study of accuracy for drug product

Drug name	Recovery level	Recovery (%), *n* = 3	RSD (%)
Famotidine	50%	99.5	0.36
	100%	100.1	0.12
	150%	99.9	0..65
Ibuprofen	50%	100.1	0.47
	100%	99.9	0.60
	150%	99.6	0.55

Table 5: Results from study of robustness

Condition	Variation	Resolution (R_s) between Famotidine and Ibuprofen
Temperature (± 5 ºC of optimum temperature)	20 ºC	3.5
	30 ºC	3.9
Flow rate (± 0.16% of optimum flow rate)	0.27 mL min^{-1}	3.7
	0.33 mL min^{-1}	4.0
pH (± 0.2 unit of set pH)	5.3	3.6
	5.7	3.8
Organic variation (± 10%)	90%	3.6
	110%	3.9

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentration. The limit of detection of IB and FM was 1.72 and 0.54 µg mL^{-1}, respectively. The limit of quantification of IB and FM was 5.73 and 1.64 µg mL^{-1}, respectively.

Linearity

The calibration curves plotted for FM and IB were linear over the concentration range of 50-160 µg/ml for FM, 1600-4800 µg/ml for IB. Peak areas were plotted against concentrations, and linearity regression analysis performed for the resultant curve. The correlation coefficient values of FM and IB are 1.000 and 0.999.

Robustness

The robustness of a method is its capacity to remain unaffected by small changes in conditions. To determine the robustness of the method, the experimental conditions were deliberately altered and system suitability parameters like relative standard deviation for replicate injections of IB and FM peaks and the USP resolution factor between IB and FM peaks were evaluated. The mobile phase flow rate was 0.3 mL min^{-1}. This was changed by 0.03 units to 0.27 and 0.33 mL min^{-1}, and the effect was studied. Similarly, the effect of column temperature was studied at 20 and 30 °C instead of 25 °C. The effect of mobile phase organic composition was studied by ± 10%. The effect of mobile phase pH was studied by ± 0.2 units.

In all the deliberate varied chromatographic conditions (flow rate, column temperature, and composition of organic solvent), no significant difference observed in system suitability [Table 5].

CONCLUSION

A novel UPLC method proves to be simple, linear, precise, accurate, robust, rugged, and specific. The total runtime was 4 min, within which two drugs and their degradation products were separated. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is stability-indicating and can be used for simultaneous quantitative determination of the drugs IB and FM in presence of degradation products in stability by the industry. The adopted UPLC method can also be useful for the assay estimation of IB tablets, FM tablets individually also.

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