INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF IBUPROFEN AND FAMOTIDINE IN BULK AS WELL IN PHARMACEUTICAL DOSAGES FORM BY USING PDA DETECTOR

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Article Received on: 21/02/14 Revised on: 03/03/14 Approved for publication: 11/04/14

DOI: 10.7897/2230-8407.050464

ABSTRACT
This study was designed to develop and validate a simple, sensitive, precise, and specific reverse phase high-performance liquid chromatographic (RP-HPLC) method for the determination of Ibuprofen and Famotidine in bulk and its tablet dosage forms. The RP-HPLC separation was carried out by reverse phase chromatography on XTerra column C_{18} (4.6 X 150 mm, 3.5 µm Make: ACE) or equivalent with a mobile phase composed Sodium Dihydrogen Ortho Phosphate and the pH has been adjusted to 2.5 by Orthophosporic Acid and Acetonitrile in the ratio of 30:70 v/v in isocratic mode at a flow rate of 1.2 ml/min. The run time was maintained for 8 minutes. The detection was monitored at 236 nm. The Accuracy was calculated and the % Recovery was found 98.4 %-101.8 % for the drug Ibuprofen and 98.4 %-100.5 % for the drug Famotidine respectively and reproducibility was found to be satisfactory. The calibration curve for Ibuprofen was linear from 100 to 200 ppm for the drug Ibuprofen and 3.32- 6.65 ppm for the drug Famotidine respectively. The inter-day and intra-day precision was found to be within limits. The proposed method has adequate sensitivity, reproducibility, and specificity for the determination of Prasugrel in bulk and its tablet dosage forms. The limit of detection and limit of quantification for Ibuprofen were found to be 0.18 µg/ml and 0.63 µg/ml respectively. The limit of detection and limit of quantification for Famotidine were found to be 0.046 µg/ml and 0.15 µg/ml respectively. The present work was undertaken with the aim to develop and validate a rapid and consistent RP- HPLC in which the peaks will be appear with a short period of time as per ICH guideline. The proposed method is simple, fast, accurate, and precise for the quantification of Ibuprofen & Famotidine in the dosage form, bulk drugs as well as for routine analysis in quality control.
Keywords: High Performance Liquid Chromatography; Sodium Dihydrogen Ortho Phosphate; Acetonitrile; Ibuprofen; Accuracy; LOD; LOQ; ICH guideline.

INTRODUCTION

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[1-2]. 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[4-6]. To the best of our knowledge, few liquid chromatography procedures were described for the individual determination of Ibuprofen (Figure 1) and Famotidine (Figure 2), 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 rapid, accurate, and validated RP-HPLC method for the simultaneous determination of Ibuprofen and Famotidine from combined dosage form for generic drug development. A literature survey regarding quantitative analysis of these drugs revealed that attempts have been made to develop analytical methods for the estimation of ibuprofen alone and in combination with other drugs by liquid chromatographic (LC)[7], HPTLC[8–10], supercritical fluid chromatography[11], and Spectrophotometric methods[12]. Famotidine is official in British Pharmacopoeia and United States Pharmacopoeia. A literature survey revealed that liquid chromatographic (LC)[13], HPTLC[14] and Spectrophotometric methods[15] have been reported for the estimation of famotidine. There is no method reported for the estimation of IBU and FAM in combined dosage form. The present study involves development and validation of liquid chromatographic method for the estimation of IBU and FAM in combined dosage form.

MATERIALS AND METHOD

Chemicals and Reagents Used

The following chemicals were procured for the process: Water [HPLC Grade], Methanol [HPLC Grade], Acetonitrile [HPLC Grade], Ibuprofen and Famotidine [Working standards], Orthophosphoric Acid and Sodium Dihydrogen Ortho Phosphate all the chemicals were procured from

Standard Solutions and the tablets were collected from the Local market.

Apparatus and Chromatographic Conditions

The proposed method was performed on High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector. Chromatographic separation was achieved at ambient temperature on column Symmetry C_{18} (4.6 x 150 mm, 3.5 µm). The flow rate and run time was set to 1.2 ml/min and 8 minutes respectively. Analytical balance Afcoset ER-200A and pH meter Adwa – AD 1020 were used. The wavelength selected was 236 nm. The injection volume was 20 µl.

Preparation of Phosphate buffer

The buffer solution was prepared by dissolving accurately weighed 2.5 milligrams of Sodium Dihydrogen Ortho Phosphate and transferred into a clean and dry 1000 ml volumetric flask, dissolved and diluted with 1000 ml water [HPLC Grade]. The final pH of the buffer was adjusted to 2.5 by using Orthophosphoric Acid.

Preparation of mobile phase and diluent

The Mobile Phase was prepared by mixing 300 ml (30 %) of the above buffer and 700 ml of Acetonitrile [HPLC Grade] (70 %) and degassed in an ultrasonic water bath for 10 minutes. Then the resultant solution was filtered through 0.45 µ filter under vacuum filtration. The Mobile phase was used as Diluent.

Preparation of the Ibuprofen and Famotidine Standard and Sample Solution

Preparation of Stock solution

The stock solution was prepared by weighing accurately 10 mg Ibuprofen and Famotidine and transferred into a clean and dry 100 ml volumetric flask. About 70 ml of diluent was added and sonicated. The volume was made up to the mark with the same diluent. From the above prepared Stock solution pipette out 0.49 ml and 1.5 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added up to the mark to get final concentration.

Preparation of Sample Solution

The sample solution was prepared by weighing equivalently 10 mg of Ibuprofen and Famotidine and transferred into a 100 ml clean and dry volumetric flask and about 70 ml of diluent was added and sonicated to dissolve it completely and the volume made up to the mark with the same solvent. From above prepared stock solution pipette out 1.5 ml of solution and transferred into a clean and dry 10 ml volumetric flask, the diluent was added up to the mark 10 ml to get final concentration. The standard and sample solutions were injected five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas.

System Suitability

The Tailing factor for the peaks due to Ibuprofen and Famotidine in Standard solution should not be more than 1.5. The Theoretical plates for the Ibuprofen and Famotidine peaks in Standard solution should not be less than 2000. The system suitability of the method was checked by injecting five different preparations of the Ibuprofen and Famotidine standard. The parameters of system suitability were checked.

Assay calculation for Ibuprofen and Famotidine

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.Wt.}{Label\ Claim} \times 100$$

Where, AT = average area counts of sample preparation, AS = average area counts of standard preparation, WS = Weight of working standard taken in mg, WT = Weight of test taken in mg, DS = Dilution of standard solution, DT = Dilution of sample solution, P = Percentage purity of working standard

System Suitability Results for Ibuprofen

- The Tailing factor obtained from the standard injection was 1.3.
- The Theoretical Plates obtained from the standard injection was 3894.8.

Assay Result for Ibuprofen

$$\frac{29797433}{2972766} \times \frac{10}{100} \times \frac{1}{1} \times \frac{5}{10} \times \frac{100}{31.04} \times \frac{1}{1} \times \frac{10}{5} \times \frac{99.9}{100} \times \frac{155.2}{50} \times 100 = 100.1\%$$

System Suitability Results for Famotidine

- The Tailing factor obtained from the standard injection was 1.3.
- The Theoretical Plates obtained from the standard injection was 2775.1.

Assay Results for Famotidine

$$\frac{116733}{117283} \times \frac{10}{100} \times \frac{1}{1} \times \frac{0.5}{10} \times \frac{100}{310.4} \times \frac{1}{1} \times \frac{10}{0.5} \times \frac{99.8}{100} \times \frac{155.2}{5} \times 100 = 99.4\%$$

Table 1: Precision result for the drug Ibuprofen

Injection	Area
Injection-1	1591698
Injection-2	1599546
Injection-3	1596156
Injection-4	1594522
Injection-5	1589429
Average	1594270
Standard Deviation	3921.3
%RSD	0.25

Table 2: Precision result for the drug Famotidine

Injection	Area
Injection-1	216449
Injection-2	210815
Injection-3	210584
Injection-4	213409
Injection-5	210592
Average	212370
Standard Deviation	2573. 3
%RSD	1.21

Table 3: Ruggedness result for the drug Ibuprofen

Injection	Area
Injection-1	1679469
Injection-2	1681508
Injection-3	1643419
Injection-4	1624651
Injection-5	1649866
Average	1655783
Standard Deviation	24391.8
%RSD	1.47

Table 4: Ruggedness result for the drug Famotidine

Injection	Area
Injection-1	220614
Injection-2	229991
Injection-3	224299
Injection-4	225129
Injection-5	224163
Average	224839
Standard Deviation	3362.5
%RSD	1.50

Table 5: Accuracy result for the drug Ibuprofen

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50 %	1695030	5.0	5.08	101.5 %	100.6 %
100 %	3284722	10.0	9.84	98.4 %	
150 %	5100544	15.0	15.2	101.8 %	

Table 6: Accuracy result for the drug Famotidine

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50 %	213703	5.0	4.9	98.4 %	99.6 %
100 %	433623	10.0	9.9	99.9 %	
150 %	654208	15.0	15.0	100.5 %	

Table 7: Linearity result for the drug Ibuprofen

S. No.	Linearity Level	Concentration	Area
1	I	100 ppm	1056290
2	II	125 ppm	1320357
3	III	150 ppm	1588900
4	IV	175 ppm	1849003
5	V	200 ppm	2128917
Correlation Coefficient			0.999

Table 8: Linearity result for the drug Famotidine

S. No.	Linearity Level	Concentration	Area
1	I	3.32 ppm	140332
2	II	4.15 ppm	182258
3	III	4.98 ppm	210426
4	IV	5.81 ppm	254916
5	V	6.65 ppm	287232
Correlation Coefficient			0.998

Table 9: Result for effect of variation in flow rate for the drug Ibuprofen

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1.	1.1	2164	1.6
2.	1.2	2189	1.5
3.	1.3	2036	1.72

Table 10: Result for effect of variation in flow rate for the drug Famotidine

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1.	1.1	2068	1.7
2.	1.2	2158	1.3
3.	1.3	2083	1.72

Table 11: Result for effect of variation in mobile phase composition for the Drug Ibuprofen (Organic Phase)

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2087	1.9
2	Actual	2189	1.5
3	10% more	2038	1.9

Table 12: Result for effect of variation in mobile phase composition for the Drug Famotidine (Organic Phase)

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2078	1.9
2	Actual	2158	1.3
3	10% more	2096	1.8

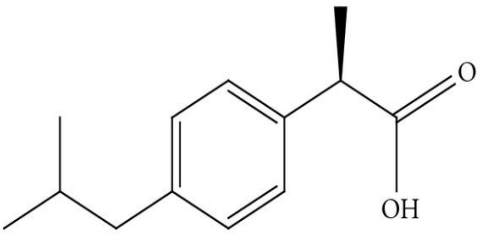

Figure 1: Chemical Structure of Ibuprofen

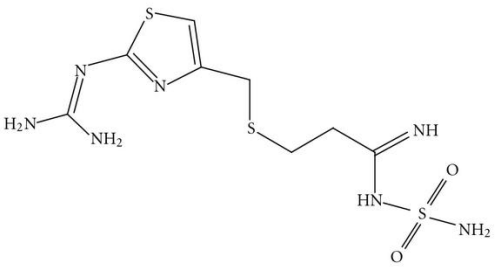

Figure 2: Chemical Structure of Famotidine

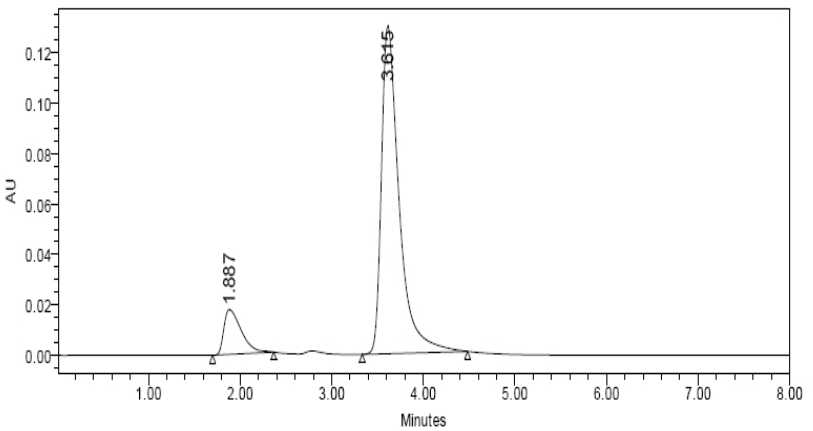

Figure 3: Optimization chromatogram for Ibuprofen and Famotidine

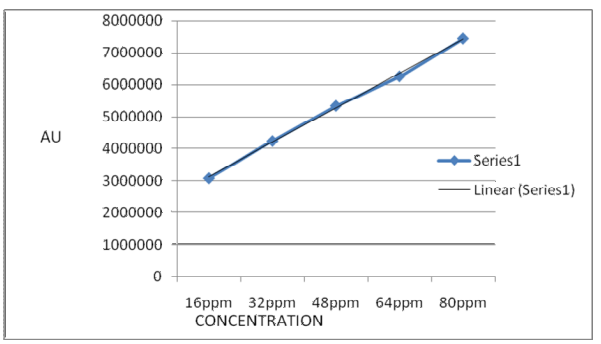

Figure 4: Calibration curve for the drug Ibuprofen

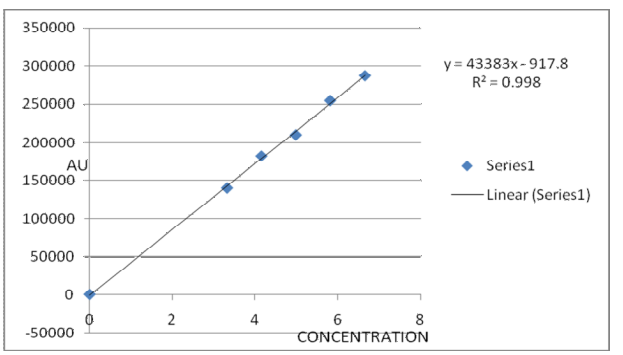

Figure 5: Calibration curve for the drug Famotidine

RESULTS AND DISCUSSION

The present work was undertaken with the aim to develop and validate a rapid and consistent RP-HPLC method development in which the peaks will be appear with short period of time as per ICH Guidelines. The proposed method was simple, fast, accurate and precise method for the Quantification of drug in the Pharmaceutical dosage form, bulk drug as well as for routine analysis in Quality control. Overall the proposed method was found to be suitable and accurate for the Quantitative determination of the drug in tablet dosage form. The method was simple, precise, accurate and sensitive and applicable for the simultaneous determination of Ibuprofen and Famotidine in bulk drug and in combined dosage forms. The High performance liquid chromatography (RP-HPLC) methods was developed and validated for simultaneous estimation of Ibuprofen and Famotidine in bulk drug and in combined dosage forms. The RP-HPLC separation was achieved on a Symmetry C_{18} (4.6 X 150 mm, 3.5 µm) in an Isocratic Mode. The mobile phase was composed of Phosphate Buffer (30 %) whose pH was adjusted to 2.5 by using Orthophosphoric Acid and Acetonitrile [HPLC Grade] (70 %). The flow rate was monitored at 1.2 ml per min. The wavelength was selected for the detection was 236 nm. The run time was 8 minutes.

The retention time found for the drugs Ibuprofen and Famotidine were 1.887 minutes and 3.615 minutes respectively. It was represented in Figure 3.

Method Validation

Method was validated according to ICH guidelines for validation of analytical procedures[16-17].

Precision

The Precision data for the drugs Ibuprofen and Famotidine were represented in Table 1 and 2. The % RSD for sample should be NMT 2. The % RSD for the standard solution was found to be 0.25 and 1.21 for the drugs Ibuprofen and Famotidine respectively, which is within the limits hence the method was precise.

Intermediate Precision

When the drugs Ibuprofen and Famotidine were analyzed by the proposed method in the intra and inter-day (Ruggedness) variation, a low coefficient of variation was observed it was represented in Table 3 and 4, which shows that the developed RP-HPLC method was highly precise. The % RSD was found to be 1.47 and 1.50 for the drugs Ibuprofen and Famotidine respectively, which is within the limits.

Accuracy

The standard solution with Accuracy -50 %, Accuracy -100 % and Accuracy -150 % were injected into chromatographic system and calculated the amount found and amount added for Ibuprofen and Famotidine and further calculated the individual recovery and mean recovery values (Table 5 and 6). The % recovery was found to be 98.4 %- 101.8 % for the drug Ibuprofen. The % recovery was found to be 98.4 % - 100.5 % for the drug Famotidine.

Linearity

In order to test the linearity of the method, five dilutions of the working standard solutions for the drugs Ibuprofen and Famotidine were prepared. The linearity was established in the range of 100 to 200 ppm for the drug Ibuprofen and 3.32 to 6.65 ppm for the drug Famotidine. The data were represented in Table 7 and 8. Each of the dilution was injected into the column and the Linearity Curve was represented in Figure 4 and 5. The Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which was in the acceptance limit.

Limit of Detection and Limit of Quantification

The Limit of detection and limit of quantification of the method were calculated basing on standard deviation of the response and the slope (s) of the calibration curve at approximate levels of the limit of detection and limit of quantification. The LOD for the drugs Ibuprofen and Famotidine were found to be 0.18 µg/ml and 0.63 µg/ml respectively. The LOQ for the drugs Ibuprofen and Famotidine were found to be 0.046 µg/ml and 0.15 µg/ml respectively. The Signal to noise ratio should be 3 for LOD. The results obtained were within the limit. The Signal to noise ratio should be 10 for LOQ solution. The results obtained were within the limit.

Robustness

The Robustness of the method was found out by testing the effect of small deliberate changes in the chromatographic conditions in the chromatographic conditions and the corresponding peak areas. The factors selected for this purpose were flow rate and percentage composition variation in Phosphate Buffer and Acetonitrile in the mobile phase. The method was found to be robust enough that the peak area was not apparently affected by small variation in the chromatographic conditions. The system suitability parameters were within the limits and shown in Table 9, 10, 11 and 12.

CONCLUSION

The complexity of problems encountered in pharmaceutical analysis with the importance of achieving the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in estimation of drugs. It was concluded that the proposed new RP-HPLC method developed for the quantitative determination of Ibuprofen and Famotidine in bulk as well as in its formulations was simple, selective, sensitive, accurate, precise and rapid. The method was proved to be superior to most of the reported methods. The mobile phases were simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence the method can be easily adopted as an alternative method to report routine determination of Ibuprofen and Famotidine depending upon the availability of chemicals and nature of other ingredients present in the sample. The method also finds use in clinical, biological and pharmacokinetic studies for the drug Ibuprofen and Famotidine. The method was validated as per ICH guidelines, and validation acceptance criteria were met in all cases. The proposed method can be use in future for the clinical, biological and pharmacokinetic studies of Ibuprofen and Famotidine.

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Cite this article as:
Sumathi Gracy, S. Ashutosh Kumar, Manidipa Debnath, J. V. L. N. Seshagiri Rao, D. Gowri Sankar, T. Anuradha Mounika. RP-HPLC analytical method development and validation for simultaneous estimation of ibuprofen and famotidine in bulk as well in pharmaceutical dosages form by using PDA detector. Int. Res. J. Pharm. 2014; 5(4):300-305 http://dx.doi.org/10.7897/2230-8407.050464

Source of support: Nil, Conflict of interest: None Declared