

Research Article

Development and Validation of Liquid Chromatographic Method for Estimation of Ibuprofen and Famotidine in Combined Dosage Form

**Dimal A. Shah, Dixita J. Suthar, Sunil L. Baldania,
Usman K. Chhalotiya, and Kashyap K. Bhatt**

*Indukaka Ipcowala College of Pharmacy, Phase IV, Beyond GIDC, New Vallabh Vidyanagar, P.B. No. 53,
P.O. Vithal Udyognagar, Gujrat-388 121, India*

Correspondence should be addressed to Dimal A. Shah, dimalgroup@yahoo.com

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An isocratic, reversed phase-liquid-chromatographic assay method was developed for the quantitative determination of ibuprofen and famotidine in combined-dosage form. A Brownlee C_{18} , $5\ \mu\text{m}$ column with mobile phase containing water : methanol : acetonitrile (30 : 60 : 10, v/v/v) was used. The flow rate was 1.0 mL/min, and effluents were monitored at 264 nm. The retention times of ibuprofen and famotidine were 4.9 min and 6.8 min, respectively. The linearity for ibuprofen and famotidine was in the range of 2–20 $\mu\text{g/mL}$ and 0.1–10 $\mu\text{g/mL}$, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. The method was successfully applied to the estimation of ibuprofen and famotidine in combined dosage form.

1. Introduction

Ibuprofen (IBU) is chemically (RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid [1, 2]. The empirical formula of IBU is $C_{13}H_{18}O_2$, with a molecular weight of 206.2 g/mole (Figure 1). It is a nonsteroidal anti-inflammatory drug, and it inhibits prostaglandin biosynthesis by blocking the enzyme cyclooxygenase, which converts arachidonic acid to prostaglandin. It is used as analgesic, antipyretic, and anti-inflammatory drug [3]. Famotidine (FAM) is chemically 3-[(2-[(diaminomethylidene)amino]-1,3-thiazol-4-yl)methyl] sulfanyl)-N' sulfamoyl propanimidamide [1, 2]. It has an empirical formula $C_8H_{15}N_7O_2S_3$ and a molecular weight of 337 g/mole (Figure 2). It is a histamine-2 receptor blocker. Histamine stimulates cells lining of stomach to produce acid. Famotidine blocks the action of histamine on stomach cells, thus reducing production of acid by the stomach [3]. The combination dosage form of ibuprofen and famotidine is

available in the market, and it is indicated in the treatment of arthritis.

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) [4–7], 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.

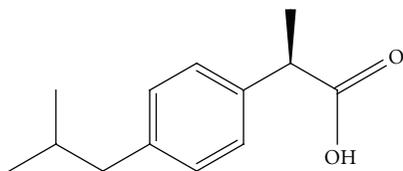


FIGURE 1: Structure of ibuprofen.

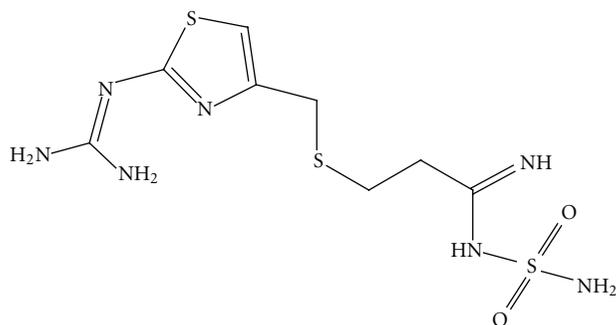


FIGURE 2: Structure of famotidine.

2. Experimental

2.1. Apparatus. The liquid chromatographic system consists of PerkinElmer series 200 LC (Shelton, USA) equipped with a series 200 UV detector, series 200 quaternary gradient pump, and manual injector rheodyne valve with 20 μL fixed loop. The analytes were monitored at 264 nm. Chromatographic analysis was performed on Brownlee C_{18} column having 250 mm \times 4.6 mm i.d. and 5 μm particle size. All the drugs and chemicals were weighed on Shimadzu electronic balance (AX 200, Shimadzu Corp., Japan).

2.2. Reagents and Materials. Analytically pure FAM and IBU were obtained as gift samples from Blue Cross Laboratory limited, Mumbai, India, and Mercury Laboratories Limited, Vadodara, India, respectively. HPLC grade acetonitrile, methanol, and water were obtained from E. Merck Ltd., Mumbai, India. Tablet formulation (DUEXIS, Horizon Pharma, USA) containing labeled amount of 800 mg of ibuprofen and 26.6 mg of famotidine was used for the study.

2.3. Chromatographic Conditions. The Brownlee C_{18} column equilibrated with mobile phase water : methanol : acetonitrile (30 : 60 : 10, v/v/v) was used. The flow rate was maintained at 1 mL/min, eluent was monitored with UV detector at 264 nm, and the injection volume was 20 μL . Total run time was kept 10 min.

2.4. Preparation of Standard Stock Solutions. IBU and FAM were weighed (25 mg each) and transferred to two separate 25 mL volumetric flasks and dissolved in few mL of mobile phase. Volumes were made up to the mark with mobile phase to yield a solution containing 1000 $\mu\text{g}/\text{mL}$ of IBU and FAM, respectively. Aliquots from the stock solutions of IBU

and FAM were appropriately diluted with mobile phase to obtain working standard of 100 $\mu\text{g}/\text{mL}$ of IBU and FAM, respectively.

3. Method Validation

The method was validated for accuracy, precision, linearity, detection limit, quantitation limit, and robustness.

3.1. Linearity. Appropriate aliquots of IBU and FAM working standard solutions were taken in different 10 mL volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 2, 5, 10, 15, 20 $\mu\text{g}/\text{mL}$ of IBU and 0.1, 0.5, 1, 4, 10 $\mu\text{g}/\text{mL}$ of FAM, respectively. The solutions were injected using a 20 μL fixed loop system, and chromatograms were recorded. Calibration curves were constructed by plotting average peak area versus concentrations, and regression equations were computed for both the drugs.

3.2. Precision. The repeatability studies were carried out by estimating response of IBU (10 $\mu\text{g}/\text{mL}$) and FAM (1 $\mu\text{g}/\text{mL}$) six times, and results are reported in terms of relative standard deviation. The intraday and interday precision studies (intermediate precision) were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations of IBU (2, 10, 20 $\mu\text{g}/\text{mL}$) and FAM (0.1, 1, 10 $\mu\text{g}/\text{mL}$), and the results are reported in terms of relative standard deviation.

3.3. Accuracy. The accuracy of the method was determined by calculating recoveries of IBU and FAM by method of standard additions. Known amount of IBU (0, 4, 8, 12 $\mu\text{g}/\text{mL}$) and FAM (0, 0.1, 1, 4 $\mu\text{g}/\text{mL}$) were added to a prequantified sample solution, and the amount of IBU and FAM was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

3.4. Detection Limit and Quantitation Limit. The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines:

$$\begin{aligned} \text{LOD} &= 3.3 \times \frac{\sigma}{S}, \\ \text{LOQ} &= 10 \times \frac{\sigma}{S}, \end{aligned} \quad (1)$$

where σ is the standard deviation of y -intercepts of regression lines and S is the slope of the calibration curve.

3.5. Robustness. Robustness of the method was studied by deliberately changing the experimental conditions like flow rate and percentage of organic phase.

3.6. Solution Stability. Stability of sample solutions was studied at $25 \pm 2^\circ\text{C}$ for 24 h.

3.7. System Suitability. A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of IBU and FAM to be performed. System suitability test of the chromatography system was performed before each validation run. Five replicate injections of a system suitability standard and one injection of a check standard were made. Area, retention time (RT), tailing factor, asymmetry factor, and theoretical plates for the five suitability injections were determined.

3.8. Analysis of Marketed Formulation. Twenty tablets were weighed accurately and finely powdered. Tablet powder equivalent to 800 mg IBU and 26.6 mg of FAM was taken in 100 mL volumetric flask. Methanol (50 mL) was added to the above flask, and the flask was sonicated for 15 minutes. The solution was filtered using whatman filter paper no. 41, and volume was made up to the mark with the mobile phase.

Appropriate volume of the aliquot was transferred to a 10 mL volumetric flask, and the volume was made up to the mark with the mobile phase to obtain a solution containing $12 \mu\text{g/mL}$ of IBU and $0.4 \mu\text{g/mL}$ of FAM. The solution was sonicated for 10 min. It was injected as per the above chromatographic conditions and peak areas were recorded. The quantifications were carried out by keeping these values to the straight line equation of calibration curve.

4. Results and Discussion

4.1. Optimization of Mobile Phase. The objective of the method development was to resolve chromatographic peaks for active drug ingredients with less asymmetric factor.

The mobile phase water : methanol : acetonitrile (30:60:10, v/v/v) was found to be satisfactory which gave two symmetric and well-resolved peaks for IBU and FAM. The retention time for IBU and FAM were 4.9 min and 6.8 min, respectively (Figure 3). The resolution between IBU and FAM was found to be 4, which indicates good separation of both the compounds. The asymmetric factors for IBU and FAM were 1.3 and 0.8, respectively. The mobile phase flow rate was maintained at 1 mL/min. Overlaid UV spectra of both the drugs showed that IBU and FAM absorbed appreciably at 264 nm; so detection was carried out at 264 nm.

4.2. Method Validation. The calibration curve for IBU was found to be linear in the range of 2–20 $\mu\text{g/mL}$ with a correlation coefficient of 0.9988. The calibration curve for FAM was found to be linear in the range of 0.1–10 $\mu\text{g/mL}$ with a correlation coefficient of 0.9972. The regression analysis of calibration curves is reported in Table 1. Instrument precision was determined by performing injection repeatability test, and the RSD values for IBU and FAM were found to be 0.58% and 0.47%, respectively. The intraday and intraday precision studies were carried out, and the results

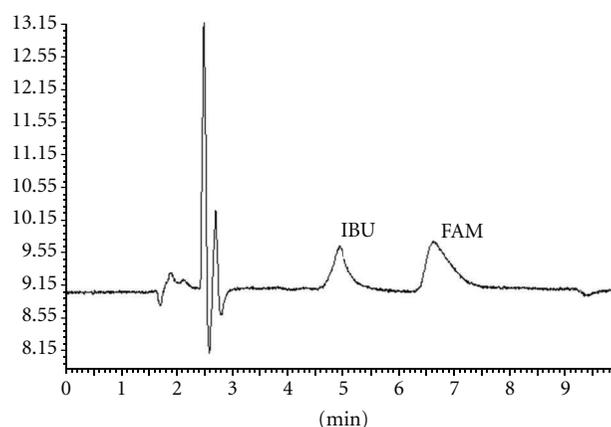


FIGURE 3: Liquid chromatogram of IBU and FAM.

TABLE 1: Regression analysis of calibration curve.

Parameter	IBU	FAM
Linearity ($\mu\text{g/mL}$)	2–20	0.1–10
Correlation coefficient (r)	0.998	0.997
Slope of regression	1973.26	25880.80
Standard deviation of slope	14.97	29.47
Intercept of regression	535.06	7544.28
Standard deviation of intercept	2.96	0.30

are reported in Table 2. The low RSD values indicate that the method is precise.

The accuracy of the method was determined by calculating recoveries of IBU and FAM by method of standard addition. The recoveries were found to be 97.60–100.42% and 99.85–101.47% for IBU and FAM, respectively (Table 2). The high values indicate that the method is accurate.

The detection limits for IBU and FAM were found to be $0.65 \mu\text{g/mL}$ and $0.033 \mu\text{g/mL}$, respectively, while quantitation limits were found to be $2 \mu\text{g/mL}$ and $0.1 \mu\text{g/mL}$, respectively. The above data shows that a nanogram quantity of both the drugs can be accurately and precisely determined. Robustness study was performed by deliberately changing the experimental conditions like flow rate from 1 mL/min to 0.8 mL/min and 1.2 mL/min. The composition of mobile phase was changed, varying the proportion of methanol by 5%. In both conditions, the recovery of both the drugs was determined and the RSD was found to be less than 2%.

System suitability test was carried out, and the results are summarized in Table 3.

Stability of standard and sample solution of IBU and FAM were evaluated at room temperature for 24 hr. Both the drugs were found to be stable with a recovery of more than 97%.

4.3. Analysis of Marketed Formulations. The proposed method was successfully applied to the determination of IBU and FAM in their combined dosage form. The % recovery was found to be 100.01 ± 0.72 and 98.56 ± 0.37 ,

TABLE 2: Summary of validation parameters.

Parameters	IBU	FAM
Retention time (min)	4.9	6.8
Detection limit ($\mu\text{g/mL}$)	0.65	0.033
Quantitation limit ($\mu\text{g/mL}$)	2	0.1
Accuracy (%)	97.60–100.42%	99.85–101.47%
Precision (RSD ^a , %)		
Intraday precision ($n = 3$)	0.75–0.93%	0.72–0.94%
Interday precision ($n = 3$)	1.02 = 1.53%	0.92–1.51%
Instrument precision (RSD ^a)	0.58%	0.47%

^aRSD is relative standard deviation, and “ n ” is number of determinations.

TABLE 3: System suitability parameter for the proposed method.

Parameter	IBU	FAM
Retention time (min)	4.9	6.8
Theoretical plates	4560	6400
Tailing factor	1.3	0.8
Base width (sec)	29.49	42.22

TABLE 4: Analysis of marketed formulation.

Formulations	Labelled Amount (mg)		% Recovery ^b	
	IBU	FAM	IBU	FAM
A	800	26.6	100.01 \pm 0.72	98.56 \pm 0.37

^b mean value \pm standard deviation of three determinations; tablet formulation A is DUEXIS (Horizon Pharma, USA) containing labeled amount of 800 mg of ibuprofen and 26.6 mg of famotidine.

respectively, for IBU and FAM (Table 4), which were comparable with the corresponding labeled amounts.

5. Conclusion

The proposed study describes stability indicating LC method for the estimation of IBU and FAM combination in mixture. The method was validated and found to be simple, sensitive, accurate, and precise. The method was successfully used for determination of drugs in their pharmaceutical formulation.

Acknowledgments

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