



DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF IBUPROFEN AND FAMOTIDINE IN RAW MATERIAL AND TABLET DOSAGE FORM

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ABSTRACT

A simple, rapid, selective and sensitive (RP-HPLC) method was developed and validated for the simultaneous estimation of Ibuprofen and Famotidine in raw material and tablet dosage form. The chromatographic separation was performed on a C8 grace column (150 mm×4.6 mm, 5 µm) with a mobile phase comprising of mixture of 0.05mM potassium dihydrogen phosphate buffer and acetonitrile (85:15) pH 6.5 adjusted with 45% Potassium hydroxide, at a flow rate of 1 ml /min with detection at 265nm. As per international conference on harmonization (ICH) guidelines the method was validated for linearity, accuracy, precision, limit of quantitation and limit of detection. Linearity of Ibuprofen was found to be in the range of 300 – 900 mcg/ml and Famotidine was found to be in the range of 10- 30 mcg/ml. The correlation coefficients were 0.9998 for Ibuprofen and 0.9991 for Famotidine. The accuracy of the developed method was found to be 98.75 – 100.17 for Ibuprofen and 99.34 – 99.80 for Famotidine. The experiment shows the developed method is free from interference of excipients. It can be conveniently adopted for the routine quality control analysis of the tablet dosage form.

KEYWORDS: IBuprofen (IB), FAMotidine (FA) RP-HPLC.

INTRODUCTION

Ibuprofen is chemically (RS) – 2 – (4-(2-methyl propyl) Phenyl) propanoic acid.^[1] It is a nonsteroidal anti-inflammatory drug (NSAID) used for relief of symptoms of arthritis, fever and analgesic (pain reliever) especially where there is an inflammatory component and dysmenorrhea.

Famotidine is chemically 3 – [[2-(Diaminomethylen) amino] thiazol – 4 – yl] methylsulphonyl N – sulphonyl propanimidamide.^[1] It is a histamine H₂ – receptor antagonist that inhibits stomach acid production, and it is commonly used in the treatment of peptic ulcer diseases and gastroesophageal reflux disease. Combination of IB and FA is anti inflammatory and reduce the risk of ulcer.

Literature survey reveals that UV(1-9), HPLC(10-13), GC(14) methods are reported for the determination of IB and FA individually and combination with some other drugs. There are no reported RP-HPLC methods for simultaneous estimation of both drugs in combined dosage form.

Hence an attempt has been made to develop simple sensitive, rapid, accurate and precise RP-HPLC method

for the simultaneous estimation of IB and FA in bulk and in tablet dosage form.

MATERIALS AND METHOD

Instruments

The chromatography was performed on a Shimadzu (UFLC) (LC- 20AD) equipped with PDA detector and LC- solution software, Grace column C₈ (150 mm x 4.6 mm, 5 µm) was used as stationary phase. A Sartorius CP224S analytical balance (Goettingen, Germany), an ultrasonic cleaner (Frontline FS 4, Mumbai) pH meter instrument (Lab India) were used in the study.

Reagents and Materials

HPLC grade methanol and acetonitrile were procured from E-Merck (India) Ltd., Mumbai, Potassium dihydrogen phosphate was procured from Qualigens fine chemicals, Mumbai. Milli Pore and Milli Q Water was used to prepare all solutions.

Preparation of Mixed Standard Stock and Working Standard Solutions

An accurately weighed IB (300 mg) and FA (10 mg) were transferred to 100 mL volumetric flask, dissolved in 50 mL methanol and diluted up to mark with methanol. The standard stock solution had concentrations of IB

(300 mcg mL⁻¹) and FA (10mcg mL⁻¹) Accurately measured 2.5 mL of standard stock solution was transfer to 25 mL volumetric flask and diluted up to the mark with methanol. The working standard solution had concentrations of IB (300 mcg mL⁻¹ and FA (10 mcg mL⁻¹).

Preparation of Calibration Curves

Aliquots (1.0, 1.5, 2.0, 2.5, and 3.0 mL) of mixed working standard solutions of IB and FA each were transferred in a series of 10 mL volumetric flasks, and the volume was made up to the mark with methanol. An aliquot (10 mL) of each solution was injected under the operating chromatographic condition as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the concentration, and the regression equations were calculated. Each response was average of three determinations.

Preparation of Sample Solution

For determination of the content of IB and FA in tablets; the powder from twenty tablets were powdered and weighed. The powder equivalent to 300 mg IB and 10 mg of FA was transferred to a volumetric flask and dissolved in 60 mL of methanol. The solution was sonicated for 30 min. The extracts were filtered through nylon filter paper and the residue was washed thoroughly with methanol. To ensure completion the solution was filtered again through 0.45 µm filter paper, the extracts and washing were pooled and transferred to a 100 mL volumetric flask and volume was made up to 100 mL with methanol. Accurately measured 1.0 mL of standard sample stock solution was transferred to 10 mL volumetric flask, diluted up to the mark with methanol to get final working concentration of IB (300 mcg mL⁻¹) and FA (10 mcg mL⁻¹) An aliquot (10 mL) of sample solution was injected under the operating chromatographic conditions as described above and responses were recorded. The analysis procedure was repeated three times with tablet formulation.

Method Validation

The method validation parameters such as linearity, precision, accuracy, limit of detection, limit of quantification, specificity and solution stability etc. were ascertained according to the International Conference on Harmonization (ICH) guidelines.

Linearity

The calibration curves were plotted over a concentration range of (300-900 mcg mL⁻¹) for IB and (10-30 mcg mL⁻¹) for FA. Aliquots (1.0, 1.5, 2.0, 2.5 and 3.0 mL) of mixed working standard solutions of IB and FA each were transferred into a series of 10 mL volumetric flasks, and the volume was made up to the mark with methanol. An aliquot (10 mL) of each solution was injected under the operating chromatographic conditions as described above and responses were recorded. Calibration curves were constructed by plotting the peak areas versus the

concentration, and the regression equations were calculated.

Method Precision (Repeatability)

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of (300-900 mcg mL⁻¹) for IB and (10-30 mcg mL⁻¹) for FA without changing the parameters of the proposed method. The results were reported in terms of relative standard deviation (%RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for 3 different concentrations of standard solutions of IB and FA (300-900 and 10-30 mcg mL⁻¹). The results were reported in terms of relative standard deviation (% RSD)

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating the recoveries of IB and FA by the standard addition method. Known amounts of standard solutions of IB and FA were added at 80, 100, 120 % level to prequantified sample solution of IB and FA (300 mcg mL⁻¹ of IB and 10 mcg mL⁻¹ of FA). The amounts of IB and FA were estimated by applying obtained values to the respective regression line equations.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drugs were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by ICH guidelines.

$$\text{LOD} = 3.3 \times \sigma / S$$

$$\text{LOQ} = 10 \times \sigma / S$$

Specificity

Specificity of the method was established by observing the interferences of common excipients used for pharmaceutical dosage form with the principal peaks.

Solution Stability

The stability of the solution under study was established by analyzing the solution after 24 h. The cumulative % RSD of the results was calculated.

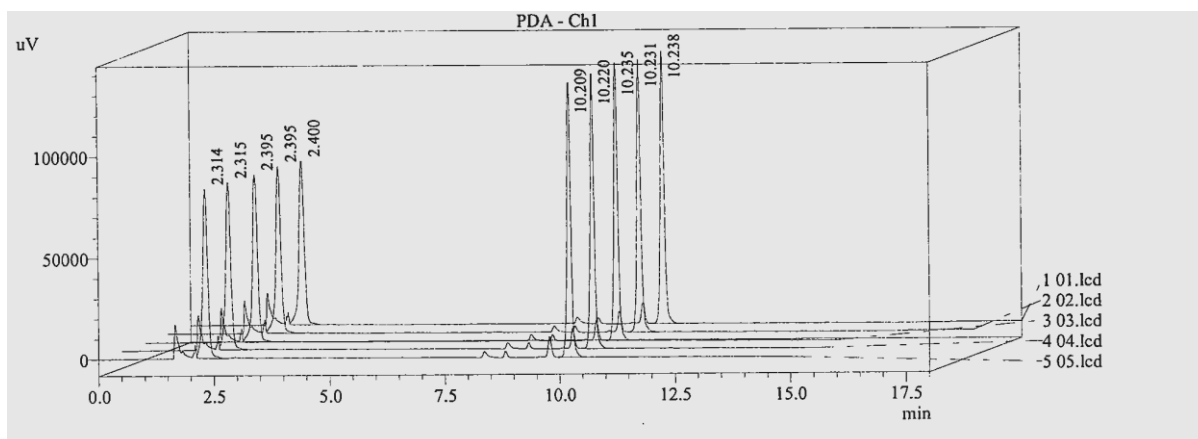


Figure 1: Chromatogram of Ibuprofen and Famotidine ($300\text{--}900\text{ mcg mL}^{-1}$ and $10\text{--}30\text{ mcg mL}^{-1}$) with corresponding retention time.

Table I: System suitability parameters.

Parameters	IB	FA
Retention time (min)	10.238	2.400
Tailing factor*	1.082	0.795
Asymmetry factor*	0.956	0.582
Theoretical plates*	56053	1950
Resolution	0.000	40.210

Table II : Linearity parameters for the simultaneous estimation of IB, FA

Parameters	IB	FA
Linearity range (mcg / mL)	300 – 900 (mcg / mL)	10 – 15 (mcg / mL)
r^2	0.9998	0.9991
Slope	1266.33	21268.74
Intercept	36861.60	177864.60

Table III : Recovery studies.

Drug Level	Amount of sample taken (mcg mL^{-1})	Amount of standard spiked (%)	% Recovery $\pm$ S.D. (n = 6)
IB I	0.5	80	98.75 $\pm$ 0.63
II	0.5	100	99.28 $\pm$ 0.36
III	0.5	120	100.17 $\pm$ 0.78
FA I	15	80	99.80 $\pm$ 0.78
II	15	100	99.34 $\pm$ 0.54
III	15	120	99.47 $\pm$ 0.63

Table IV: Estimation of IB and FA in Tablets.

Tablets	Label Claim (mg) IB FA	Amount found (mg) IB FA	% Label claim $\pm$ S.D. (n = 3) IB FA
I	800 26.6	801.04 26.52	100.13 $\pm$ 0.65 99.69 $\pm$ 0.73
II	800 26.6	806.90 26.83	100.86 $\pm$ 1.23 100.86 $\pm$ 1.45

RESULTS AND DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for IB and FA were obtained with a mobile phase comprising of phosphate buffer : acetonitrile (85:15 V/V) at a flow rate of 1.0 mL min^{-1} to get better reproducibility and repeatability. Quantification was achieved with PDA detection at 265 nm based on peak area. Complete resolution of the peaks with clear baseline was obtained (Fig.1). The retention

time for IB and FA were found to be 10.2 and 2.4 min, respectively (Fig.1). The results of system suitability parameters are given in (Table I).

Linear correlation was obtained between peak area versus concentrations of IB and FA in the concentration ranges of $300\text{--}900$ and $10\text{--}30\text{ (mcg mL}^{-1}\text{)}$. The correlation coefficient were 9998 for IB and .9991 for FA was obtained (Table II). The % RSD values for IB and FA were found to be $<2\%$ which indicates that the proposed

method is repeatable, reveal that the proposed method is precise. The accuracy of developed method was found to be 98.75-100.17 for IB and 99.30-99.80 for FA are shown in the (Table III) which indicates accuracy of the proposed method. LOD values for IB and FA were found to be 0.0872 mcg mL⁻¹ and 0.0366 mcg mL⁻¹ respectively and LOQ values for IB and FA were found to be 0.0264 mcg mL⁻¹ and 0.0999 mcg mL⁻¹ respectively. These data show that the proposed method is sensitive for the determination of IB and FA. It was observed that there is no interference of the excipients with the principal peak. Hence, the method is specific for the estimation IB and FA. The results of stability indicated no significant change in the assay results of the same solution (% RSD is less than 2) the confirming the stability of the drug in the solvent used for the analysis.

The amount of IB and FA present in the sample solutions were determined by fitting the responses into the regression equations of the calibration curve for IB and FA, respectively and the results obtained were comparable with the corresponding labelled claim (Table IV).

CONCLUSION

The proposed RP-HPLC method can be used for routine quality control analysis of IB and FA in raw material and pharmaceutical dosage forms.

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