DEVELOPMENT AND VALIDATION FOR ESTIMATION OF IBUPROFEN AND FAMOTIDINE IN SYNTHETIC MIXTURE BY RP-HPLC METHOD


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ABSTRACT
In the present work, simple, sensitive, accurate and precise RP-HPLC method have been developed for the quantitative estimation of Famotidine and Ibuprofen individually as well as in synthetic mixture. RP-HPLC method in which determination of Famotidine and Ibuprofen was carried on a reverse phase C18 column using a mobile phase consisting of Methanol: Water (90: 10 % v/v) pH adjusted at 3.5 by Orthophosphoric acid. The mobile phase was pumped at a rate of 1.2 ml/min and the detection was carried out at 266 nm. The peaks obtained were sharp with retention time of 5 min for Famotidine and 7.4 min for Ibuprofen. The peaks were well resolved with a resolution factor of 5.78. The linearity was found to be in the range of 4-20 μg/ml and 120-600 μg/ml with (r² = 0.9998, and r² = 0.9997). The peaks obtained were sharp having clear baseline separation with a retention time of 5.0 ± 0.013 and 7.4 ± 0.012 min for Famotidine and Ibuprofen respectively. LOD for both the drugs were 0.097 μg/ml and 4.32 μg/ml respectively and LOQ for both the drugs were found to be 0.32 g/ml and 14.26 g/ml respectively.

Keywords: Famotidine, Ibuprofen, RP-HPLC method, Simultaneous Estimation, ICH.

INTRODUCTION
Famotidine is competitive histamine H₂ receptor antagonist. Its main effect is inhibition of gastric secretion [1, 2]. Chemically it is 3-[(2-[((diaminomethylidene)amino]-1,3-thiazol-4-yl)methyl) sulfanyl]-N'-sulfamoyl propanimidamide [3]. Famotidine is official in I.P. [4], B.P. [5], U.S.P. [6], J.P. [7] and E.P [8], which includes Potentiometric titration, HPLC method for estimation of Famotidine (FAMO).

Ibuprofen is prototypical non-steroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic properties [1, 2]. Chemically it is 2-[4-(2-methylpropyl) phenyl] propionic acid [9]. Ibuprofen is official in I.P. [4], B.P. [5], U.S.P. [6], J.P. [7] and E.P [8], which includes Acid Base Titration, GC, and HPLC method for estimation of Ibuprofen (IBU).
Famotidine and Ibuprofen are available in a combined tablet dosage named DUEXIS (Horizon Pharma). It was approved by US-FDA \cite{10}. The combination of these two drugs is not official in any pharmacopoeia. Literature review \cite{11-28} shows that numbers of analytical methods are available for estimation of both the drugs either alone or in combination with other drugs. Based on our current and ongoing referencing work, till date, we have not come across any official and reported analytical methods for simultaneous estimation of both the drugs in synthetic mixture. Therefore, the objective is to develop a RP-HPLC method for simultaneous estimation of Famotidine and Ibuprofen in their synthetic mixture and to validate the developed method according to ICH guidelines \cite{29}.

**MATERIALS AND METHODS**

**Instruments:**

a) Thermo Electron Corporation, HPLC system with auto sampler.

i. Liquid chromatography: Thermo LC.


iii. Column: Lichrosphere C\textsubscript{18} column (250 X 4.6 mm, 5 µm).


v. Software: SN 4000, chromquest.

b) CL 54+ Toshcon industries Pvt. Ltd, Digital pH meter.
c) Shimadzu UV-1800, UV-Visible double beam Spectrophotometer.
d) Shimadzu AUX-220, Electronic analytical balance.
e) Sonica Ultrasonic Cleaner, Sonicator.

Reagents and Chemicals:
a) Standard Famotidine (FAMO) and Ibuprofen (IBU) were kindly gifted by Vaibhav Analytical Laboratories, Ahmedabad, India.
b) Methanol of HPLC grade (Rankem, RFCL chemicals Pvt. Ltd.)
c) Methanol (A.R. Grade - Chemco Chemicals Ltd.)
d) Water of HPLC grade (Rankem, RFCL chemicals Pvt. Ltd.)
e) Orthophosphoric acid (OPA) (Analytical reagent grade).

Methodology (RP-HPLC Method)

Preparation of combined stock solution of Famotidine and Ibuprofen:
Accurately weighed FAMO (25 mg) and IBU (750 mg) were transferred to a 25 ml volumetric flask and dissolved and diluted up to the mark with methanol to produce 1000 µg/ml for FAMO and 30000 µg/ml for IBU. The solution was labeled as Stock Solution 1 (SS1). Further dilution was made from SS1 to produce 100 µg/ml for FAMO and 3000 µg/ml for IBU. The final solution was labeled as Standard Solution 2 (SS2). The SS2 was filtered through 0.45 µm Nylon 66 (N66) 47 mm membrane filter paper and first few drops of filtrate were discarded.

Selection of wavelength for Detection:
By appropriate dilution of each standard stock solution of FAMO and IBU with methanol, and from that concentration FAMO 12 µg/ml and IBU 360 µg/ml were prepared and each solution was scanned using double beam UV visible spectrophotometer and spectra were overlaid. From overlain spectra of FAMO and IBU, 266 nm was selected as analytical wavelength for multicomponent analysis using HPLC method. (Fig. 1)

Selection and preparation of Mobile phase:
The standard drug of FAMO and IBU were injected into the HPLC system and run in solvent system. Mobile phases like methanol and water was tried in order to find the best conditions for separation of FAMO and IBU. It was found that methanol water gives
A satisfactory result and the optimal composition of the mobile phase was determined to be methanol: water (90:10 v/v) and pH adjusted to 3.5 with Orthophosphoric acid (OPA).

Chromatographic conditions:
- **Column**: Licrosphere C$_{18}$ (250 X 4.6mm, 5 µm)
- **Mobile Phase**: Methanol: Water (90:10 V/V)
- **Wavelength**: 266nm
- **Flow Rate**: 1.2 ml/min
- **Injection Volume**: 20µl
- **Column Temperature**: ambient

Preparation of the calibration curve:
From the SS2 further dilution was made by mobile phase to produce diluted solution of FAMO in range of 4-20 µg/ml and for IBU in range of 120-600 µg/ml. Calibration curve of FAMO was obtained by plotting the mean peak area against concentration (µg/ml) for FAMO. Linear relation was obtained between mean peak area and concentration of the drug in the range of 4-20 µg/ml (4, 8, 12, 16 and 20 µg/ml) (Table 1 and Fig. 6) and for IBU the range of 120-600 µg/ml (120, 240, 360, 480 and 600 µg/ml) (Table 2 and Fig. 7).

Method validation:
- **Linearity and Range** (n = 5):
The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity. The linearity response was determined by analyzing different sample solution in the range of 4-20 µg/ml (4, 8, 12, 16 and 20 µg/ml) for FAMO and 120-600 µg/ml (120, 240, 360, 480 and 600 µg/ml) for IBU (Fig. 5).
- **Accuracy** (n = 3):
Accuracy studies were carried out to determine the suitability and reliability of the proposed method. Accuracy studies were carried out adding a known quantity of standard drug to the pre-analyzed sample solutions and were analyzed by proposed method.
Accuracy was determined by calculating the % Recovery of FAMO and IBU by the standard addition method in which, known amounts of standards powders of FAMO and IBU at 50%, 100% and 150% levels were added to the pre-analyzed samples.

**Precision:**

A) **Repeatability** (n=6):

Repeatability study was carried out by analyzing the six different prepared same concentration sample of FAMO (12 µg/ml) and IBU (360 µg/ml).

B) **Intraday** (n=3):

In intraday precision from above solution make three replicates of sample containing FAMO (4, 12 and 20 µg/ml) and for IBU (120, 360 and 600 µg/ml) and was analyzed 3 times at different time intervals in the same day at their selected wavelength.

C) **Interday** (n=3):

In interday precision from above solution make three replicates of sample containing FAMO (4, 12 and 20 µg/ml) and for IBU (120, 360 and 600 µg/ml) and was analyzed at same time on different days at their selected wavelength.

**Specificity:**

Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. The specificity of the RP-HPLC method was determined by comparison with chromatogram of standard and sample of drugs, blank and placebo.

**LOD and LOQ:**

The LOD and LOQ were estimated from the set of 5 calibration curves.

The LOD may be calculated as, $LOD = 3.3 \times (SD/Slope)$ and

The LOQ may be calculated as, $LOQ = 10 \times (SD/Slope)$

Where,

$SD = \text{Standard deviation of the Y- intercepts of the 5 calibration curves.}$

Slope = Mean slope of the 5 calibration curves.
ESTIMATION OF FAMOTIDINE AND IBUPROFEN IN SYNTHETIC MIXTURE BY PROPOSED METHOD (n=5)

3.7.1 Preparation of synthetic mixture:
Accurately weight 25 mg of FAM and 750 mg of IBU and transfer it in to a mortar then add 236.02 mg of MCC (LR Grade), 3% Crosscarmellose sodium (LR Grade), 1.6% PVP K30 (LR Grade), 1.5% Mg. stearate (LR Grade) and finally 1.5% of Talc (LR Grade). Mix it in mortar for 15 min.

Above mixture was transferred to 25 ml volumetric flask and dissolved in sufficient quantity of methanol. The contents were ultrasonicated for 10 min and final volume made up with methanol. The solution was than filtered through Whatman filter paper (No. 41). From above solution 1.0 ml aliquots of the solution was taken and transferred to 10 ml volumetric flask. Volume was made up to the mark with the methanol to produce solution containing FAMO (100 µg/ml) and IBU (3000 µg/ml). From this solution aliquots of 0.4 ml of the solution was taken and transferred to 10 ml volumetric flask. Volume was made up to the mark with the methanol to produce solution containing FAMO (12 µg/ml) and IBU (360 µg/ml). This solution was used for the estimation of FAMO and IBU (Table 5 and Table 6).

RESULTS AND DISCUSSION
A simple, accurate and precise RP-HPLC method was successfully developed using Methanol: Water (90: 10 % v/v) as mobile phase with pH adjusted at 3.5 by Orthophosphoric acid. The peaks obtained were sharp with retention time of 5 min for Famotidine and 7.4 min for Ibuprofen. The peaks were well resolved with a resolution factor of 5.78. The method was precisely applied to the synthetic mixture and the results obtained were accurate and reproducible. The data for all the validation parameters are summarized in Table 4. The % assay results of FAMO and IBU indicate that the developed method was successfully utilized for the estimation of FAMO and IBU in their synthetic mixture.
Figure 1
Selection of Wave length

Figure 2
chromatogram of Famotidine (12µg/ml)
Figure 3
chromatogram of Ibuprofen (360 µg/ml)

Figure 4
chromatogram of Ibuprofen (360 µg/ml) and Famotidine (12 µg/ml)

Figure 5
Overlaid chromatogram for Famotidine and Ibuprofen
### TABLE 1: PEAK AREA OF FAMOTIDINE AT VARIOUS CONCENTRATIONS (N=5)

<table>
<thead>
<tr>
<th>FAMO (µg/ml)</th>
<th>Area mean ± S.D.</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>159021±776.46</td>
<td>0.49</td>
</tr>
<tr>
<td>8</td>
<td>290868.6±1134.59</td>
<td>0.39</td>
</tr>
<tr>
<td>12</td>
<td>422960.2±677.24</td>
<td>0.16</td>
</tr>
<tr>
<td>16</td>
<td>558095.2±859.35</td>
<td>0.15</td>
</tr>
<tr>
<td>20</td>
<td>681805.4±808.68</td>
<td>0.12</td>
</tr>
</tbody>
</table>

![Figure 6](image)

Calibration curve for Famotidine

### TABLE 2: PEAK AREA OF IBUPROFEN AT VARIOUS CONCENTRATIONS (N=5)

<table>
<thead>
<tr>
<th>IBU (µg/ml)</th>
<th>Area mean ± S.D.</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>129389.2±353.57</td>
<td>0.27</td>
</tr>
<tr>
<td>240</td>
<td>236627.4±348.91</td>
<td>0.15</td>
</tr>
<tr>
<td>360</td>
<td>346521.6±385.92</td>
<td>0.11</td>
</tr>
<tr>
<td>480</td>
<td>458493±465.81</td>
<td>0.10</td>
</tr>
<tr>
<td>600</td>
<td>557027.6±822.01</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Figure 7
Calibration curve for Ibuprofen

**TABLE 3: SUMMARY OF REGRESSION ANALYSIS OF FAMOTIDINE AND IBUPROFEN**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Famotidine</td>
<td>Y=32820x+28711</td>
<td>0.9998</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>Y=896.79x+22569</td>
<td>0.9997</td>
</tr>
</tbody>
</table>

**TABLE 4: SUMMARY OF VALIDATION AND SYSTEM SUITABILITY**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>FAMO</th>
<th>IBU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>5.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.12</td>
<td>1.03</td>
</tr>
<tr>
<td>Resolution factor</td>
<td>5.78</td>
<td></td>
</tr>
<tr>
<td>Theoretical plates</td>
<td>7173</td>
<td>6681</td>
</tr>
<tr>
<td>Linearity</td>
<td>4.20 μg/ml</td>
<td>120-600 μg/ml</td>
</tr>
<tr>
<td>Accuracy (n=3)</td>
<td>99.12-100.72%</td>
<td>99.56-100.70%</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability (%cv, n=6)</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Intraday (n=3)</td>
<td>0.13-0.36</td>
<td>0.14-0.42</td>
</tr>
<tr>
<td>Interday (n=3)</td>
<td>0.14-0.44</td>
<td>0.15-0.45</td>
</tr>
<tr>
<td>LOD</td>
<td>0.097</td>
<td>4.32</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.32</td>
<td>14.26</td>
</tr>
</tbody>
</table>
TABLE 5: ASSAY RESULT OF FAMOTIDINE AND IBUPROFEN (N = 5)

<table>
<thead>
<tr>
<th>DRUG</th>
<th>ACTUAL CONC. (mg)</th>
<th>CONC. FOUND (mg)</th>
<th>% CV</th>
<th>% PURITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAMO</td>
<td>25</td>
<td>24.99</td>
<td>0.61</td>
<td>99.76%</td>
</tr>
<tr>
<td>IBU</td>
<td>750</td>
<td>745</td>
<td>0.74</td>
<td>99.70%</td>
</tr>
</tbody>
</table>

CONCLUSION

The developed RP-HPLC method was found to be simple, rapid, accurate, sensitive and specific methods for the estimation of FAMO and IBU. The % assay results of 99.76% for FAMO and 99.70% for IBU indicate that the developed method was successfully utilized for the estimation of FAMO and IBU in their synthetic mixture in routine analysis.

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REFERENCES


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