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VALIDATION OF DEVELOPED METHOD BY RP-HPLC FOR SIMULTANEOUS ESTIMATION OF FAMOTIDINE AND IBUPROFEN IN HUMAN PLASMA AND STUDYING THE STABILITY OF THE DRUGS IN PLASMA

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ABSTRACT

The RP-HPLC separation was carried out by reverse phase chromatography on a Symmetry C_{18} (4.6 x 150 mm, 3.5 µm, make: XTerra) with a mobile phase composed of sodium dihydrogen ortho phosphate [pH 2.5] and acetonitrile in the ratio of 30:70 v/v in an isocratic mode at a flow rate of 1.2 mL/min. The run time was maintained for 8.0 min. The detection was monitored at 236 nm. The accuracy was calculated in human plasma and the % recovery was found 99.80 - 99.85 for famotidine and 99.56 -99.85.5 for ibuprofen and reproducibility was found to be satisfactory. The calibration curve for famotidine in human plasma was linear over 3.32 to 6.65 µg/mL and 100- 200 µg/mL for ibuprofen in human plasma respectively. The inter-day and intra-day precision in human plasma was found within limits. The proposed method has adequate sensitivity, reproducibility, and specificity for the determination of famotidine and ibuprofen in plasma. The LLOQ obtained by the proposed method in human plasma were 1.24 and 5.0 µg/mL for famotidine and ibuprofen respectively. The proposed method is simple, fast, accurate, and precise for the quantification of famotidine and ibuprofen in plasma as per the ICH guidelines.

Keywords: HPLC, Famotidine, Ibuprofen, Accuracy, LLOQ.

INTRODUCTION

Famotidine, 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 g/mol. Famotidine is available in 20 mg and 40 mg for oral administration (1-4).

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Ibuprofen, ((2*RS*)-2-[4-(2-Methylpropyl) phenyl] propanoic acid) is a non-steroidal anti-inflammatory drug, which is available in 400, 600, 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 g/mol.

To the best of our knowledge, few liquid chromatography procedures were described for the individual determination of famotidine (Figure I) and ibuprofen (Figure II), these procedures were developed to estimate either famotidine or ibuprofen 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 method in plasma and validate liquid chromatographic method for the simultaneous estimation of famotidine and ibuprofen in combined dosage form for generic drug.

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 (11-13), HPTLC (14-16), supercritical fluid chromatography (17), and spectrophotometric methods (18-19). Famotidine is official in British Pharmacopoeia (20) and United States Pharmacopoeia (21).

There is no method reported for the simultaneous estimation of ibuprofen and famotidine in combined dosage form. The present study involves development and validation of liquid chromatographic method for the estimation of ibuprofen and famotidine in combined dosage form in plasma.

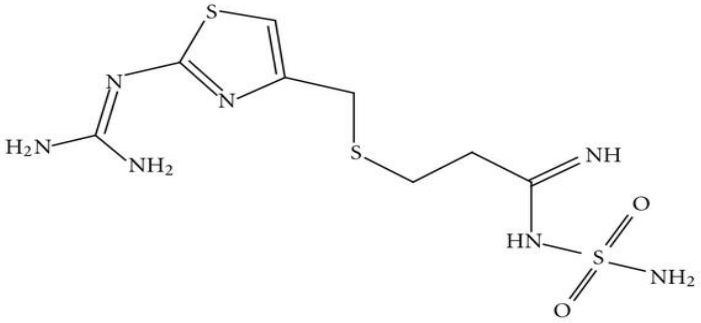

Figure 1. Chemical Structure of Famotidine

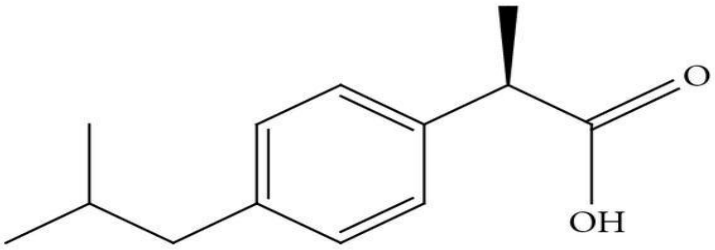

Figure 2. Chemical Structure of Ibuprofen

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MATERIALS AND METHOD (22)

Chemicals and reagents used: The reference samples of ibuprofen and famotidine were supplied by M/s Pharma Train, Hyderabad**.** HPLC grade water (prepared by using 0.45 Millipore Milli-Q) was procured from Standard Reagents, Hyderabad. HPLC grade methanol and acetonitrile were purchased from Merck, Mumbai. The chemicals used for preparation of buffer include sodium dihydrogen ortho phosphate (Finar Chemicals, Ahmedabad), and orthophosphoric acid (Standard Reagents, Hyderabad). The processed plasma was collected from M/s. Pharma Train, Hyderabad, and Andhra Pradesh.

Apparatus and chromatographic conditions: The equipment used was High Performance Liquid Chromatography Equipped with Auto Sampler and DAD or UV Detector. The column Symmetry C_{18} (4.6 x 150 mm, 3.5 µm, make: XTerra) or equivalent was selected. The flow rate was monitored at 1.2 mL per min. The detection was carried out at 236 nm. The injection volume selected 20 µL, the temperature of the column oven was maintained at 25 °C, the detector used was Photo diode array and the run time was 8.0 min.

0.45 µ membrane filters (Advanced Micro Devices Pvt. Ltd., Chandigarh, India) were used for filtration of various solvents and solutions intended for injection into the column. The ultra violet spectra of the drugs used for the investigation were taken on a Lab India UV 3000 spectrophotometer for finding out their λ_{max} values. Solubility of the compounds was enhanced by sonication on an ultra sonicator (Power Sonic 510, (Hwashin Technology).

All the weighing in the experiments were done with an Afcoset electronic balance. The Hermle microlitre centrifuge Z100 (model no 292 P01) was used for the centrifugation process and Remi equipment (model no- CM101DX) Cyclomixer was used.

Glassware: All the volumetric glassware used in the study was of Grade A quality Borosil.

Preparation of phosphate buffer (23): The buffer solution was prepared by dissolving 2.5 g of sodium dihydrogen ortho phosphate in 900 mL of HPLC grade water in a 1000 mL clean and dry flask. The mixture was stirred well until complete dissolution of the salt. Further 100 mL of water was added the pH was adjusted to 2.5 with 1 % ortho phosphoric acid.

Preparation of mobile phase: The mobile phase consisted of phosphate buffer (pH 2.5) and acetonitrile [HPLC grade] (30:70 v/v) was filtered through a 0.45 µ membrane filter, sonicated and degassed. This was used as diluent for further studies also.

Preparation of standard solution of famotidine and ibuprofen: 10 mg famotidine was weighed accurately and transferred into a 100 mL clean and dry volumetric flask. Initially, the drug was mixed with 70 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up to the mark with the same solvent. Similarly, about 10 mg ibuprofen was weighed accurately and transferred into a 10 mL clean and dry volumetric flask. Initially, the drug was mixed with 7 mL of diluent. The solution was sonicated for 15 min for complete dissolution of the drug. The final volume was made up to the mark with the same solvent.

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From the above prepared stock solutions 0.5 mL of famotidine and 1.5mL ibuprofen were pipetted out into a 10 mL clean and dry volumetric flask and it was diluted up to the mark with diluent. This mixed stock solution contains 5.0 µg/mL of famotidine and 150.0 µg/mL of ibuprofen.

Spiking of famotidine and ibuprofen into plasma and their extraction from plasma (By protein precipitation method): From the above prepared mixed stock solution (5.0 µg/mL of famotidine and 150.0 µg/mL of ibuprofen), 0.5 mL was pipetted out and spiked into 0.5 mL of plasma in a polypropylene tube (Torson's). Then the tube was cyclo mixed for 5 min. Then 1.0 mL of acetonitrile was added to the tube and centrifuged for 20 min at 3000 rpm. Further the supernatant liquids were collected in another eppendorf tube and 20 µL supernatant was injected into the analytical column.

VALIDATION OF DEVELOPED METHOD (24-31)

1. **Selectivity*:*** An aqueous mixture of famotidine and ibuprofen (5.0 µg/mL of famotidine and 150 µg/mL of ibuprofen) was prepared and injected into the column and the retention times were checked and any interference at the retention times were checked by comparing the response in the blank. No interference was observed at the retention times for famotidine and ibuprofen extracted from plasma. The method was found to be precise and specific. A typical chromatogram of famotidine and ibuprofen in plasma is shown in figure 3.

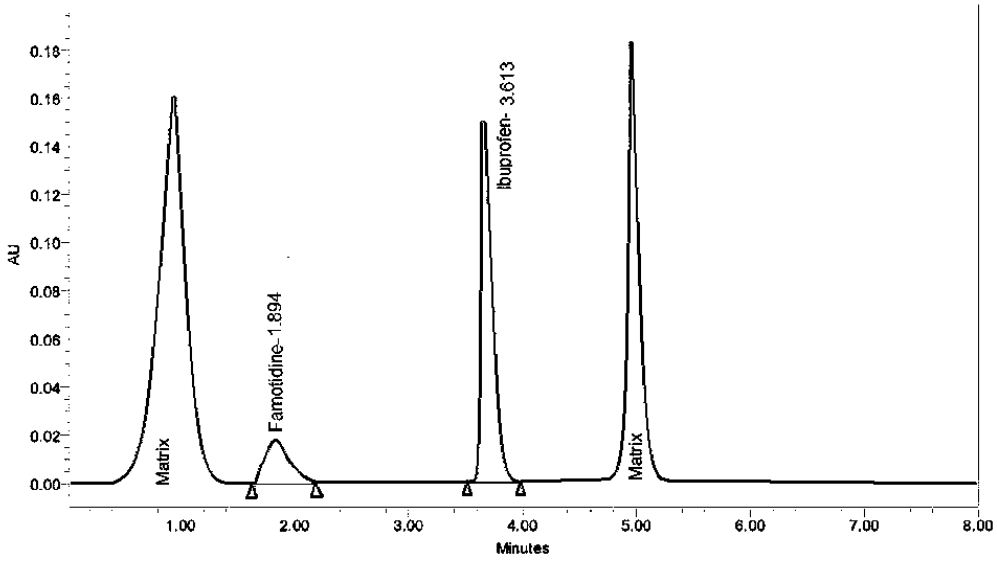

Figure 3. A typical chromatogram of famotidine and ibuprofen standard drugs in plasma

2. **Sensitivity*:*** To determine the sensitivity in terms of LLOQ, 'Lower Limit of Quantification' where the response of LLOQ must be at least five times greater than the response of interference in blank matrix at the retention time of the analyte (s). The LLOQ obtained by the proposed method were 1.24 and 5.0 µg/mL for famotidine and ibuprofen respectively.

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3. Precision: To check the intra and inter-day variations of the method, solutions containing 5.0 µg/mL of famotidine and 150 µg/mL of ibuprofen were subjected to the proposed HPLC method of analysis and results obtained were noted. The precision of the proposed method i.e. the intra and inter-day variations in the peak areas of the drugs solutions in plasma were calculated in terms of percent RSD and are represented in Table 1, 2, 3 and 4 and Figure 4 and 5. A statistical evaluation revealed that the relative std. dev. of the drugs at linearity level for 6 injections was less than 2.0.

Table 1. Intra-day precision of proposed method for Famotidine in plasma

Injection	Retention Time	Area
Injection-1	1.910	21644
Injection-2	1.909	21081
Injection-3	1.909	21058
Injection-4	1.910	21340
Injection-5	1.908	21059
Injection-6	1.909	21085
Average	1.909	21211
Standard deviation	0.001	238.1
% RSD	0.04	1.12

Table 2. Intra-day precision of proposed method for Ibuprofen in plasma

Injection	Retention Time	Area
Injection-1	3.632	159169
Injection-2	3.631	159954
Injection-3	3.630	159615
Injection-4	3.630	159452
Injection-5	3.626	158942
Injection-6	3.633	159617
Average	3.630	159458
Standard deviation	0.002	359.4
%RSD	0.1	0.22

Table 3. Inter-day precision of proposed method for Famotidine (on 3 consecutive days n = 6) in plasma

Days	Retention Time	Area
Day -1*	1.888	22061
Day -2*	1.889	22699
Day -3*	1.899	22429
Average	1.892	22396
Standard Deviation	0.01	320
%RSD	0.32	1.4

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Table 4. Inter-day precision of proposed method for Ibuprofen (on 3 consecutive days n = 6) in plasma

Days	Retention Time	Area
Day -1*	3.608	167946
Day -2*	3.615	168150
Day -3*	3.611	164341
Average	3.611	166812
Standard Deviation	0.003	2143
%RSD	0.1	1.3

***Average of Six injections**

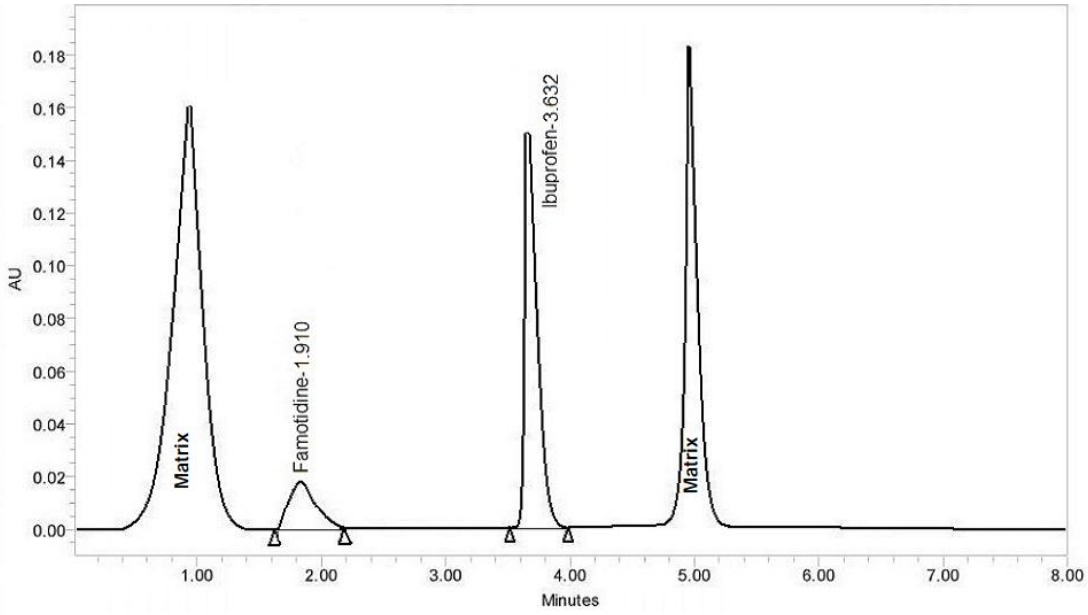

Figure 4. Typical chromatogram of Famotidine and Ibuprofen in plasma for intra-day precision

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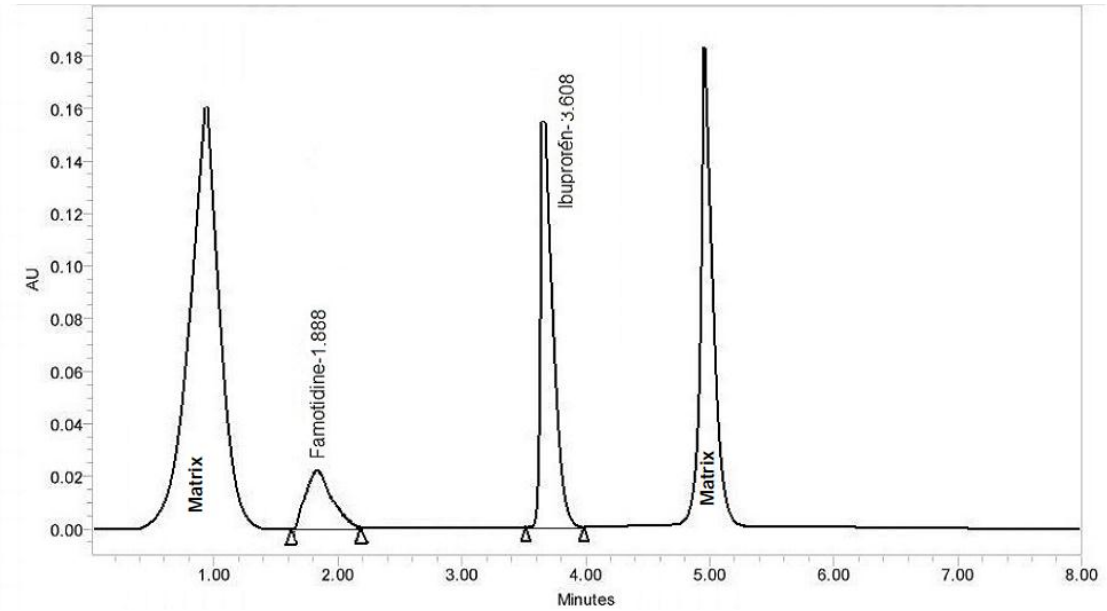

Figure 5. Typical chromatogram of Famotidine and Ibuprofen in plasma for inter-day precision

4. **Accuracy*:*** To determine the accuracy of the proposed method, recovery studies were carried out by analyzing (8.0, 10.0, 12.0 mg of famotidine and ibuprofen) of pure drugs. The solutions were suitably diluted at 5.0 µg/mL concentration of famotidine and 150 µg/mL concentration of ibuprofen. Then each dilution was injected thrice (n=3). The percent recoveries of the drugs were calculated. The results are shown in table 5 and 6.

Table 5. Accuracy data of the proposed method for Famotidine in plasma

Conc. level	% recovery	Avg. % recovery	Amount recovered (mg)	SD	% RSD
8.0	99.88	99.80	7.99	0.012	0.14
	99.67		7.97		
	99.85		7.99		
10.0	99.98	99.85	10	0.015	0.15
	99.82		9.98		
	99.74		9.97		
12.0	99.88	99.80	11.99	0.015	0.13
	99.67		11.96		
	99.85		11.98		

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Table 6. Accuracy data of the proposed method for Ibuprofen in plasma

Conc. level	% recovery	Avg. % recovery	Amount recovered (mg)	SD	% RSD
8.0	99.80	99.56	7.98	0.029	0.36
	99.13		7.93		
	99.74		7.98		
10.0	99.80	99.80	9.98	0.00	0.00
	99.79		9.98		
	99.81		9.98		
12.0	99.80	99.80	11.98	0.00	0.00
	99.79		11.98		
	99.81		11.98		

5. Linearity: In order to find out the linearity range of the proposed HPLC method in plasma, curves were constructed by plotting peak areas obtained for the analyte against their concentrations. A good linear relationship (r^2=0.996) was observed between the concentrations of famotidine and ibuprofen and their corresponding peak areas. The relevant regression equations were y = 44044x – 4392 for famotidine (r^2 = 0.996) and y = 10696x – 15647for ibuprofen (r^2 = 0.999) (where y is the peak area and x is the concentration of famotidine and ibuprofen (µg/mL)). The linearity ranges for famotidine and ibuprofen and their corresponding graphs are shown in Figure 6 and 7.

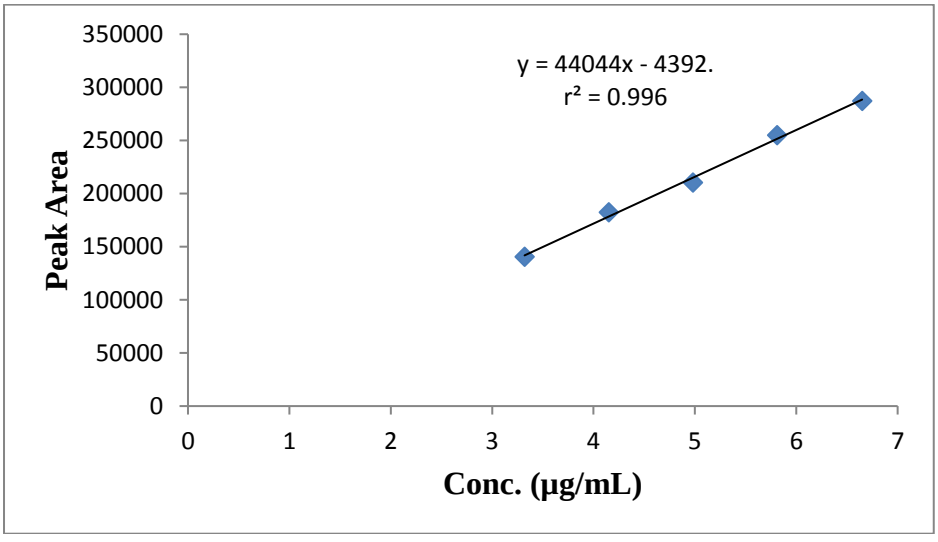

Figure 6. Calibration curve for Famotidine in plasma

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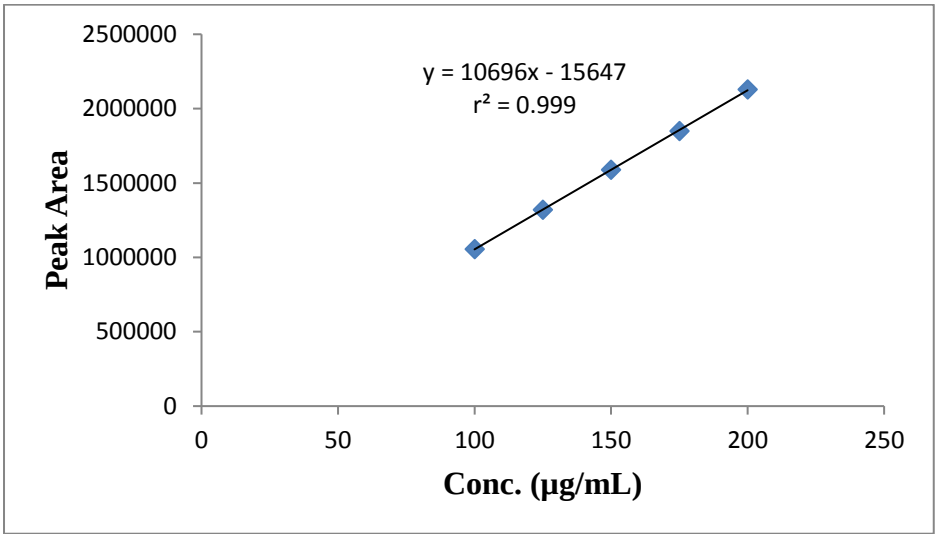

Figure 7. Calibration curve for Ibuprofen in plasma

6. Stability (31): All stability determinations used a set of samples prepared from a freshly made stock solution of the analyte in the appropriate analyte-free, interference-free biological matrix. The stock solutions of the analyte for stability evaluation were prepared in an appropriate solvent at known concentrations. To test the stability of the drug extract, it was subjected to

a) Freeze and thaw stability at -20 ºC ± 2 ºC,
b) Short term stability for period of 24 hours stored at room temperature,
c) Long term stability for period of 15 days stored at 4 ºC.

Before all these studies, the freshly prepared samples were spiked into the plasma and extracted and collected in vial and injected into HPLC system. All the stability samples compared against the standard stock solution assessed for stability. The results are presented in table 7 and 8, and Figure 7, 8, 9 and 10 (the figures in the table are in peak area units).

Table 7. The stability data for Famotidine in plasma

Sr. No.	Standard Sample	Freeze and Thaw Stability Sample	Short term Stability Sample	Long term Stability Sample
1.	21040	20750	20349	19853
2.	21044	20755	20336	19842
3.	21052	20748	20359	19864
Mean	21045	20751	20348	19853
SD	6	4	12	11
% RSD	0.03	0.02	0.06	0.06
Assay	---------	98.6	96.69	94.33

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Table 8. The stability data for Ibuprofen in plasma

Sr. No.	Standard Sample	Freeze and Thaw Stability Sample	Short term Stability Sample	Long term Stability Sample
1	158988	156796	152660	151913
2	158892	156999	152482	151924
3	158796	156793	152785	151909
Mean	158892	156863	152642	151915
SD	96	118	152	8
% RSD	0.06	0.08	0.10	0.01
Assay		98.72	96.12	95.61

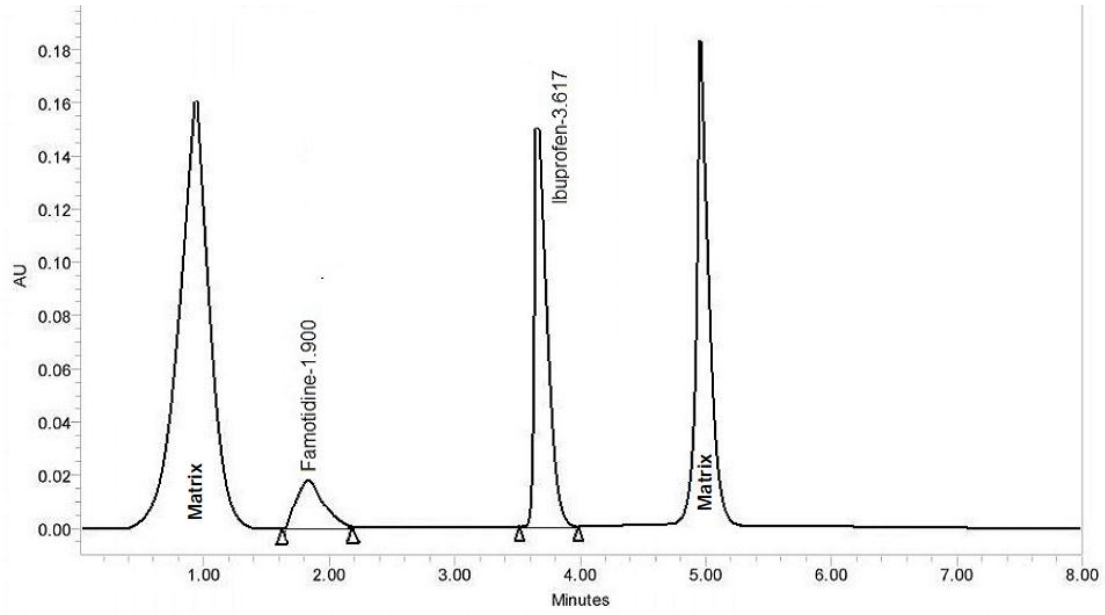

Figure 7. Typical chromatogram for standard samples of Famotidine and Ibuprofen in plasma for stability studies

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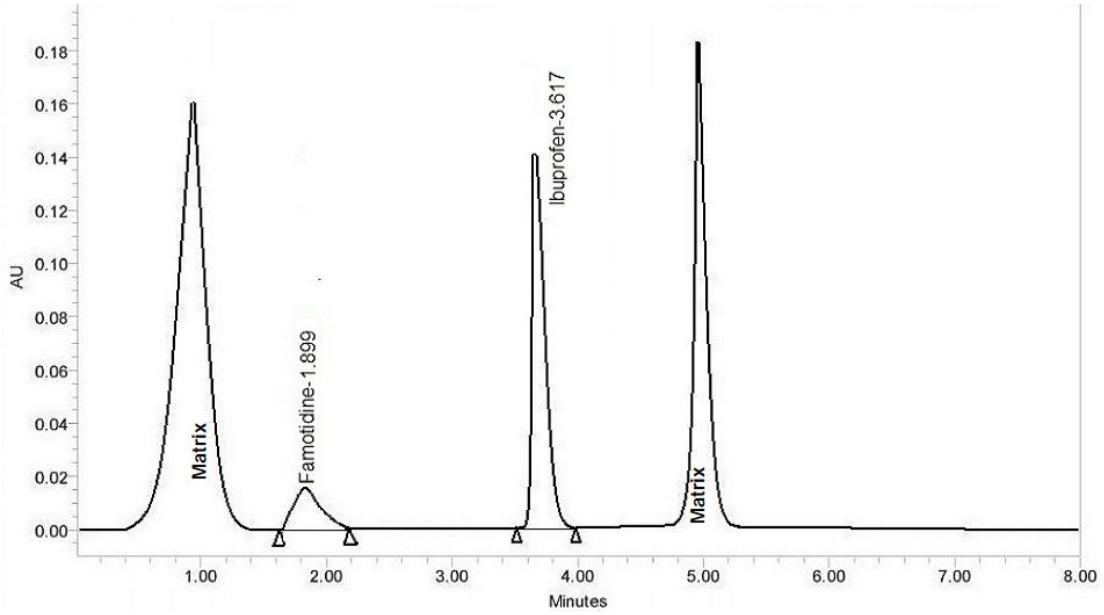

Figure 8. Typical chromatogram for freeze thaw samples of Famotidine and Ibuprofen in plasma for stability studies

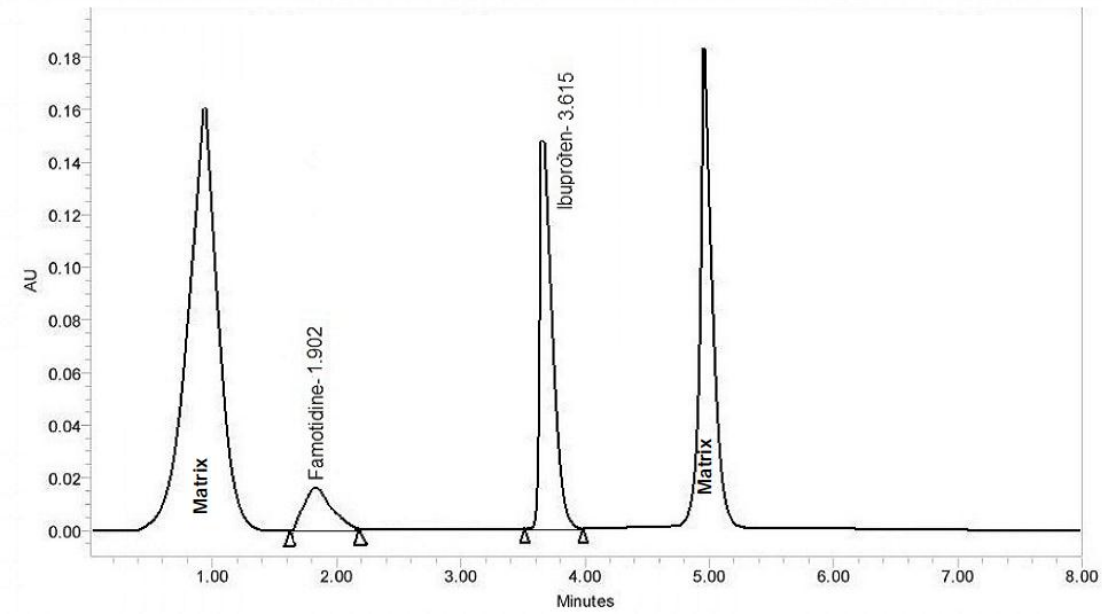

Figure 9. Typical chromatogram for short term stability samples of Famotidine and Ibuprofen in plasma for stability studies

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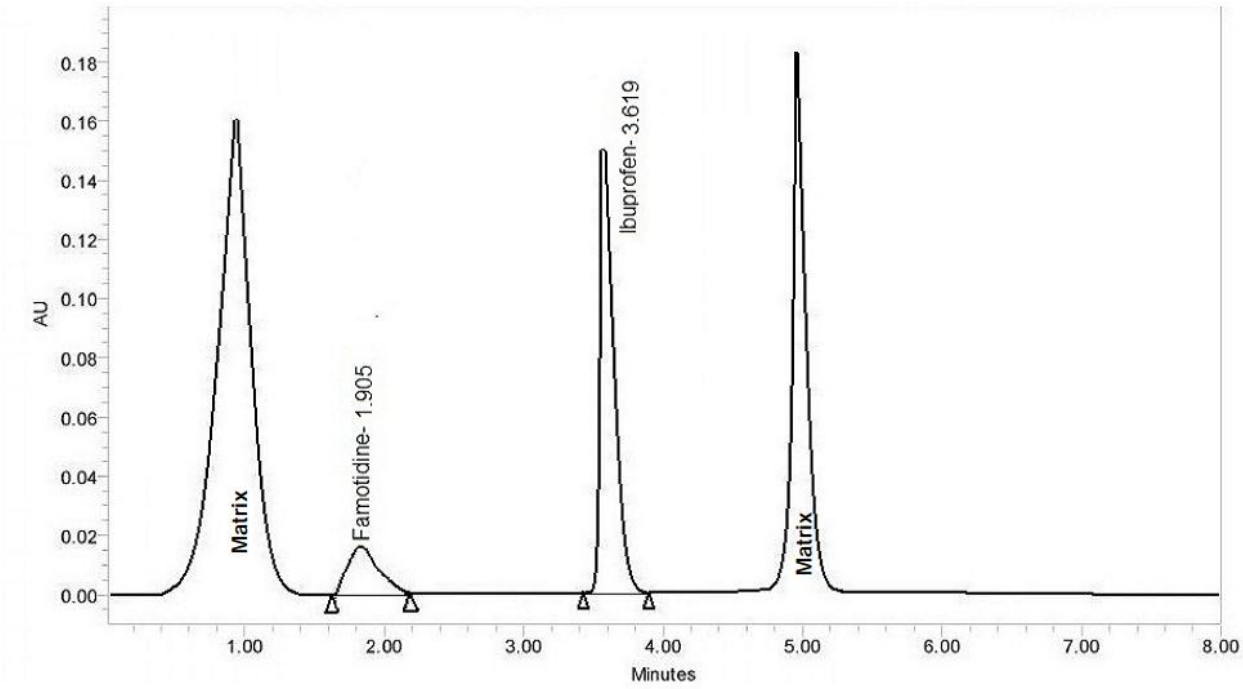

Figure 10. Typical chromatogram for long term stability samples of Famotidine and Ibuprofen in plasma for stability studies

RESULT AND DISCUSSION

The use of phosphate buffer (pH 2.5) and acetonitrile (HPLC Grade) in the ratio of 30:70 (v/v) resulted in peak with good shapes and resolution. A flow rate of 1.2 mL/min was found to be optimum in the 0.4-1.5 mL/min range resulting in short retention time, baseline stability and minimum noise.

The LLOQ obtained for famotidine and ibuprofen by the proposed method in plasma were1.24 and 5.0 µg/mL respectively. The retention times obtained for famotidine and ibuprofen in plasma were1.894and 3.613 min respectively. Quantitative linearity of drugs in plasma was obeyed in the concentration ranges of 3.32-6.65 µg/mL for famotidine and 100-200 µg/mL for ibuprofen respectively. The relevant regression equations were $y = 44044x - 4392$ for famotidine ($r^2 = 0.996$) and $y = 10696x - 15647$ for ibuprofen ($r^2 = 0.999$) (where y is the peak area and x is the concentration of famotidine and ibuprofen (µg/mL)**.** The intra-day and inter-day drugs variations in plasma by the proposed method in plasma showed an RSD less than 2 %, indicating that the method is precise. The corresponding mean recoveries of the drugs in plasma were 99.56-99.80 %. This reveals that the method is quite accurate. The RSD obtained for the drugs spiked in plasma for stability studies were less than 2 %.

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CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous determination of famotidine and ibuprofen. The method was validated as per ICH guidelines and all the parameters met within the acceptance criteria. Applicability of this method for simultaneous estimation of famotidine and ibuprofen in plasma was confirmed. Hence, this method is specific can be easily and conveniently adopted for routine quality control analysis of the above drugs.

ACKNOWLEDGEMENTS

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