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Research Article

Estimation of ibuprofen and famotidine by (RP-HPLC)

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Abstract

A force degradation profile by RP-HPLC method was developed for Ibuprofen (IBU) & Famotidine (FMT) in combine tablet dosage form using Cosmosil RP-C18 (4.6 x 250mm, 5µm) in an gradient mode with mobile phase comprising of Methanol: Water (pH 2.5 using OPA) The flow rate was 0.7 mL/ min and effluent was monitored at 240 nm. The stress conditions selected on the basis of literature review and drug profile. The analysis of the marketed formulation shows the % RSD of 0.061 and 0.35 for IBU & FMT which fully agrees with system suitability. All the system suitability parameters were fully obeyed during generation of force degradation profile.

Keywords: IBU, FMT, RP-HPLC, Force, Stress, Degradation.

1. Introduction

Forced degradation studies are also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used. But these guidelines are very general in conduct of forced degradation and do not provide details about the practical approach towards stress testing. Although forced degradation studies area regulatory requirement and scientific necessity during drug development, it is not considered as a requirement for formal stability program [17- 18]. The review of literature[7-16] suggested that no stability indicating assay for the above combination is reported. The present work was undertaken with an objective to develop the force degradation profile for the above combination on RP-HPLC so as to support the development of stability testing program. Ibuprofen [Fig. 1] Chemically is (RS)-2-4-2- methylpropyl phenyl propanoic acid. It is white crystalline powder used as analgesic having solubility in methanol, ethanol and in water 21 mg/lt. While famotidine [Fig. 2] chemically is 3-2-(diaminomethylidene)amino-1,3-thiazol-4-ylmethylsulfanyl-N'-sulfamoylpropanimid amide. It is white to pale yellow crystalline. Used as anti-ulcer having solubility in methanol and freely soluble in glacial acetic acid, slightly soluble in water.[5, 6.-19, 20]

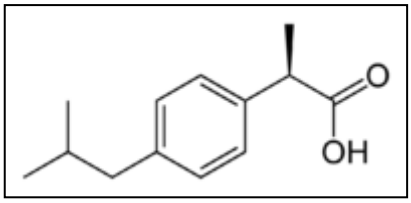

Fig. 1 Chemical Structure of Ibuprofen

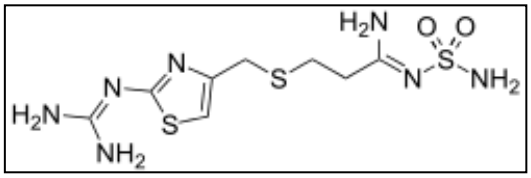

Fig. 2 Chemical Structure of Famotidine

2. Experimental

Reagents & Chemicals

Standard samples of IBU & FMT were received as gift samples from the Leben Laboratories Akola (Maharashtra) and Taj Pharmaceuitical Mumbai

(Maharashtra). The marketed formulation Duexis (Horizon Pharma) was purchased from the local market containing IBU 800 mg and FMT 26.6 mg and all the chemicals used were are of analytical grade.

Instruments

HPLC System of Younglin Quaternary pump with UV- VIS detector (190-990 nm) Software – Autochro. Analytical balance of citizen model CY 104 (microanalytical balance) was used for weighing purpose also the ultrasonicator serve well instruments model RC-SYSTEM MU-1700 used for sonication purpose.

Preparation of Standard Solutions

Standard Stock Solution (A) Accurately weighed quantity of IBU (30.0 mg) was transferred to 50.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of 600.0 µg/mL. The resultant solution was then sonicated for 10.0 min in ultrasonicator.

Standard Stock Solution (B) Accurately weighed quantity of FMT (10.0 mg) was transferred to 50.0 mL volumetric flask and dissolved in methanol. The volume was made up to mark with methanol to get final concentration of 200.0 µg/mL. The resultant solution was then sonicated for 10.0 min in ultrasonicator.

Working Standard Solution (C) 0.5 mL of solution (A) and 0.05 mL of solution (B) was transferred to 10.0 mL volumetric flask and then the volume was made up to the mark with mobile phase to get final concentration of (30.0 µg/mL of IBU & 1.0 µg/mL of FMT) respectively. The resultant solution was then sonicated for 10.0 min in ultrasonicator.

Optimization of Mobile Phase and Chromatographic Conditions

Procedure The chromatographic conditions were set as per the optimized parameters. The mobile phase was allowed to equilibrate with stationary phase as was indicated by a steady baseline. Solution (C) was injected in the Rheodyne injector (20.0 µL) and the respective chromatograms were recorded. Various mobile phases were tried by permutations and combinations and also by varying column, flow rate, column temperature and type of buffers with varying pH and solvents. The various mobile phases tried are as follows.

- **Trial 1** Methanol: Water (70: 30) pH 7.
- **Trial 2** Methanol: Water (75: 25) pH 7.
- **Trial 3** Methanol: Water (80:20) pH 7.
- **Trial 4** Methanol: Water (85: 15) pH 2.5.

Above mentioned mobile phases were tried. The mobile phase containing Methanol: water (85: 15) at pH 2.5, injection volume- 20.0 µL flow rate of 0.7 mL/min was selected, due to its high resolving power, sensitivity and suitability, for the determination of IBU and FMT. The chromatogram is shown in **Figure 3**. Hence the following optimized chromatographic parameters were selected to carry out further experimentation.

- **Column**: Comosil RP-C18 (4.6 x 250mm, 5µm).
- **Flow Rate** : 1 mL/min.
- **Wavelength** : 226.0 nm.
- **Injection Volume** : 20.0 µL.
- **Column Temperature** : Ambient.
- **Run Time** : 20.0 min.
- **Mobile Phase** : Methanol: Water (60:40).
- **pH** : 3 (Using OPA).

System Suitability Studies

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be carried out. It is performed to ensure that the system is operating properly and read to deliver results with acceptable accuracy and precision. The tests were performed by collecting data from five replicate injections of standard solutions.

Procedure The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Five replicate injections of mixed working standard solution (C) were injected in to the system, the chromatograms were recorded for both the drugs and the results are shown in **Table 1 & 2.**

Analysis of Marketed Formulation

Prepared as per the methodology adopted for laboratory mixtures

Preparation of Sample Solutions

Ten Tablets were weighed accurately and ground to fine powder. An accurately weighed quantity of Tablet powder equivalent to (800.0 mg of IBU & 26.6 mg of FMT) were transferred to 50.0 mL of volumetric flask and dissolved in sufficient amount of methanol. Then the volume was made up to the mark with methanol. The resultant solution was then filtered through whatman filter paper (no. 41). The filtered solution was then sonicated in ultrasonicator for 10.0 min. aliquot portions of 0.0075 mL was then transferred to the three separate 10.0 mL volumetric flask and then the volume was mad up to the mark with mobile phase to get final concentration of (120.0 µg/mL of IBU and 4.0 µg/mL of FMT) respectively.

Procedure Equal volume (20.0 µL) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The amount of drug in a Tablet was calculated using following formula

$$\text{mg/Tablet} = \frac{AT1 \times WS1 \times Ds \times P1}{AS1 \times WT \times Dt} \times \text{Avg. wt}$$

Where,

AT1 = Average area of IBU/FMT peaks in Test chromatogram

AS1 = Average area of IBU/FMT peaks in Standard chromatogram

Ds = Dilution factor for standard

Dt = Dilution factor for test

P1 = Potency of working standards of IBU/FMT of % w/w basis

Avg. wt = Average weight of 10 Tablets

Further calculate the amount of IBU/FMT present in % of Label claim using following formula

$$\% \text{ Label Claim} = \frac{\text{Assay (mg/Tablet)} \times 100}{\text{Label claim of IBU/FMT}}$$

The results are shown in **Table 3**, while chromatogram is shown in **Figure 4.**

Force Degradation Studies

In order to establish the force degradation profile and to determine whether the analytical method for assay was stability indicating, the Tablet formulation of IBU and FMT were subjected to various stress conditions to conduct forced degradation studies. Stress studies were carried out under the condition of acid/alkali hydrolysis, oxidation, neutral degradation in accordance with ICH Q1A (R2) guideline. Selection of stress conditions was primarily depends on the literature review and drug profile

The % degradation was evaluated by the following formula:

$$\% \text{ Degradation} = \frac{\text{Area of unstressed} - \text{Area of Stressed}}{\text{Area of unstressed}} \times 100$$

Approaches for Force Degradation

Acid Degradation

An accurately weighed quantity of tablet powder equivalent to (800.0 mg of IBU & 26.6 mg of FMT) was transferred to a round bottom flask to which 50.0 mL of 1.0N HCL has been added as degradation medium. It is then subjected to reflux on water bath at 70.0^0C for 1.0 hr. the resultant solution was then filtered through whatman filter paper (no. 41). Then 0.0057 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (90.0 µg/mL of IBU and 3.0 µg/mL of FMT) respectively.

Alkali Degradation

An accurately weighed quantity of Tablet powder equivalent to (800.0 mg of IBU & 26.6 mg of FMT) was transferred to a round bottom flask to which 50.0 mL of 1.0N NaOH has been added as degradation medium. It is then subjected to reflux on water bath at 70.0^0C for 1.0 hr. the resultant solution was then filtered through whatman filter paper (no. 41). Then 0.0057 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (90.0 µg/mL of IBU and 3.0 µg/mL of FMT) respectively.

Oxidative Degradation

An accurately weighed quantity of Tablet powder equivalent to (800.0 mg of IBU & 26.6 mg of FMT) was transferred to a round bottom flask to which 50.0 mL of 3.0% H_2O_2 has been added as degradation medium. It is then subjected to reflux on water bath at 70.0^0C for 1.0 hr. the resultant solution was then filtered through whatman filter paper (no. 41). Then 0.0057 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (90.0 µg/mL of IBU and 3.0 µg/mL of FMT) respectively.

Neutral Degradation

An accurately weighed quantity of Tablet powder equivalent to (800.0 mg of IBU & 26.6 mg of FMT) was transferred to a round bottom flask to which 50.0 mL of water has been added as degradation medium. It is then subjected to reflux on water bath at 70.0^0C for 1.0 hr. the resultant solution was then filtered through whatman filter paper (no. 41). Then 0.0057 mL of filtered solution was then transferred to 10.0 mL volumetric flask and volume was made up to the mark with mobile phase to get final concentration of (90.0 µg/mL of IBU and 3.0 µg/mL of FMT) respectively.

Procedure

Equal volume (20.0 µL) of each stress sample was injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area, retention time of the major peaks were measured. The respective chromatograms are shown in **Figure 5 to 9.**

3. Results and discussion

Optimization of Mobile Phase and Chromatographic Conditions

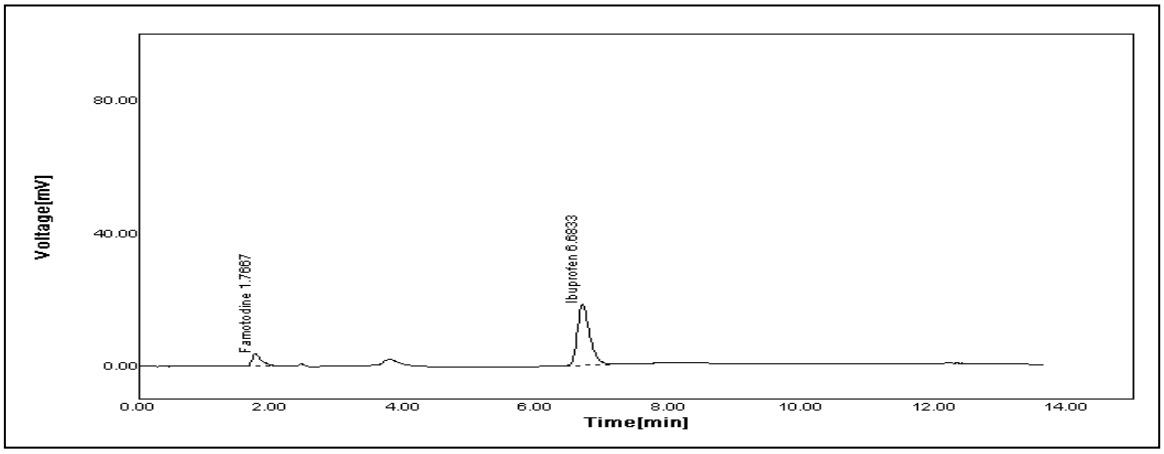

Fig. 3 Optimized Chromatogram of IBU & FMT

Observation

Good resolution with minimized tailing also proper peak shape and system suitability was observed within the limits. Hence the above chromatographic parameters are finalized.

System Suitability Studies

Table 1 Result of System Suitability Studies for (IBU)

System Suitability Test (IBU)					
Sr. No	**Area Reproducibility**	**Retention Time**	**Tailing Factor**	**Resolution**	**Theoretical Plates**
1	3086.20	6.616	1.348	19.00	6851
2	3083.11	6.612	1.344	19.05	6852
3	3088.31	6.641	1.352	19.01	6822
4	3087.25	6.661	1.325	18.99	6872
5	3086.16	6.651	1.331	19.00	6378
Mean	3086.206	6.6362	1.3364	19.01	6755.2
%RSD	0.019	0.679	1.026	0.573	1.812
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2**	**> 2000**

Observation

All the parameters of system suitability are observed within the limits for IBU.

Table 2 Results of System Suitability Studies for (FMT)

System Suitability Test (FMT)					
Sr. No	**Area Reproducibility**	**Retention Time**	**Tailing Factor**	**Resolution**	**Theoretical plates**
1	82.4619	1.816	1.205	0	3605.9
2	83.4516	1.912	1.221	0	3604.1
3	82.4618	1.901	1.252	0	3606.1
4	83.4612	1.951	1.241	0	3605
5	82.4722	1.916	1.224	0	3609
Mean	82.4617	1.9192	1.2348	0	3606.02
%RSD	1.32	0.318	1.117	0	0.0594267
Limit	**NMT 2%**	**NMT 1%**	**< 2**	**> 2**	**> 2000**

Observation

All the parameters of system suitability are observed within the limits for FMT.

Analysis of Marketed Formulation

Table 3 Results of Marketed Formulation Analysis

Sr. No.	**IBU**		**FMT**	
	Assay (mg)	**Assay (%)**	**Assay (mg)**	**Assay (%)**
1	120.85	99.83	4.47	99.91
2	119.24	99.83	4.49	99.92
3	119.02	99.80	4.46	99.91
Mean	119.70	99.82	4.47	99.913333
SD	0.1138	0.024119	0.3510	0.04520
% RSD	0.061	0.648763	0.35	0.417598

The proposed method was applied to the determination of IBU & FMT in marketed formulation the **mean % amount** found was **99.82 (IBU) & 99.91 (FMT)** with **% RSD** values is **NMT 2.0%** indicates the developed method was successfully applied for analysis of marketed formulation. All the results found are in good agreement with the label content of marketed formulation.

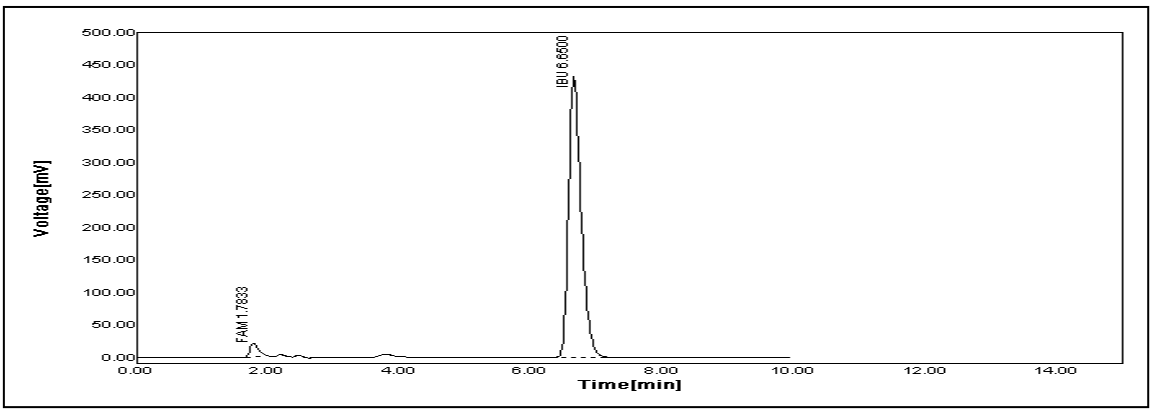

Fig. 4 Chromatogram of Marketed Formulation

Force Degradation Studies

Acid Degradation

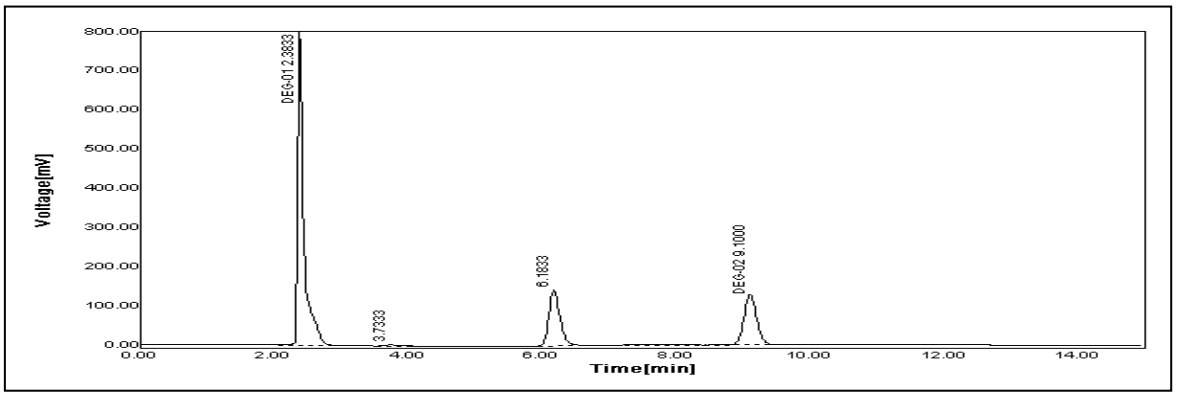

Fig. 5 Chromatogram of Acid Degradation

Acid degradation studies revels that both the drugs are capable of undergoing a strong degradation in acidic medium (1.0 N HCL) since both the drugs are basic in nature. A strong degradation was observed because of stronger ionization of both drugs under acidic medium owing to higher pKa of both the drugs.

Table 4 Overview of Acid Degradation

Name	Retention time	Area	%Area
Deg-1	2.3833	5247.64	58.81
FMT	3.7334	44.3834	0.50
IBU	6.1833	1612.2797	18.07
Deg-2	9.1000	2018.8269	22.62

Base Degradation

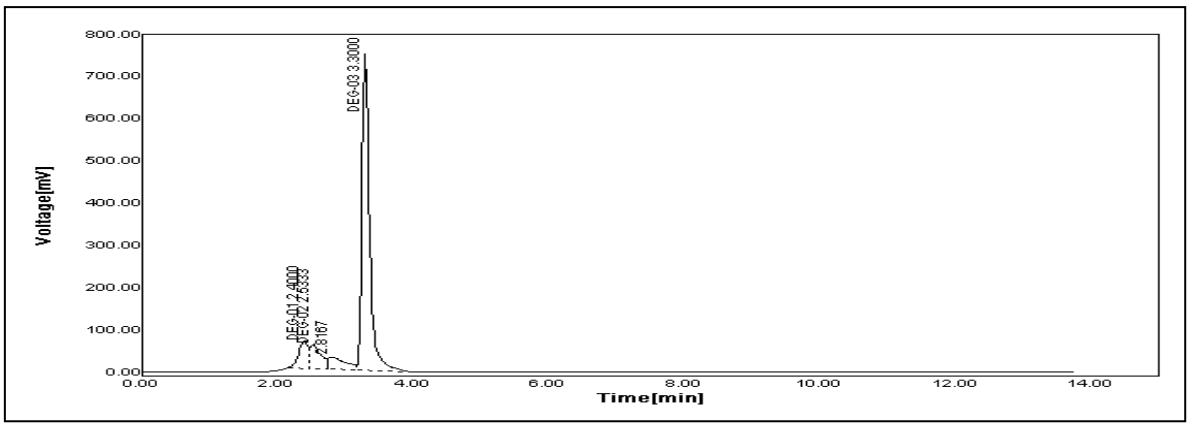

Fig. 6 Chromatogram of Base Degradation

A base degradation studies revels that both the drugs are also capable of undergoing the strong degradation under basic medium (1.0 N NaOH). Since a stronger degradation peaks were reported. both the drugs are strong basic in nature owing to higher pKa values.

Table 5 Overview of Base Degradation

Name time	Retention	Area	%Area
Deg-1	2.4000	633.6837	8.26
Deg-2	2.5333	399.2056	7.81
Deg-3	3.3000	5927.6650	77.24

Oxidative Degradation

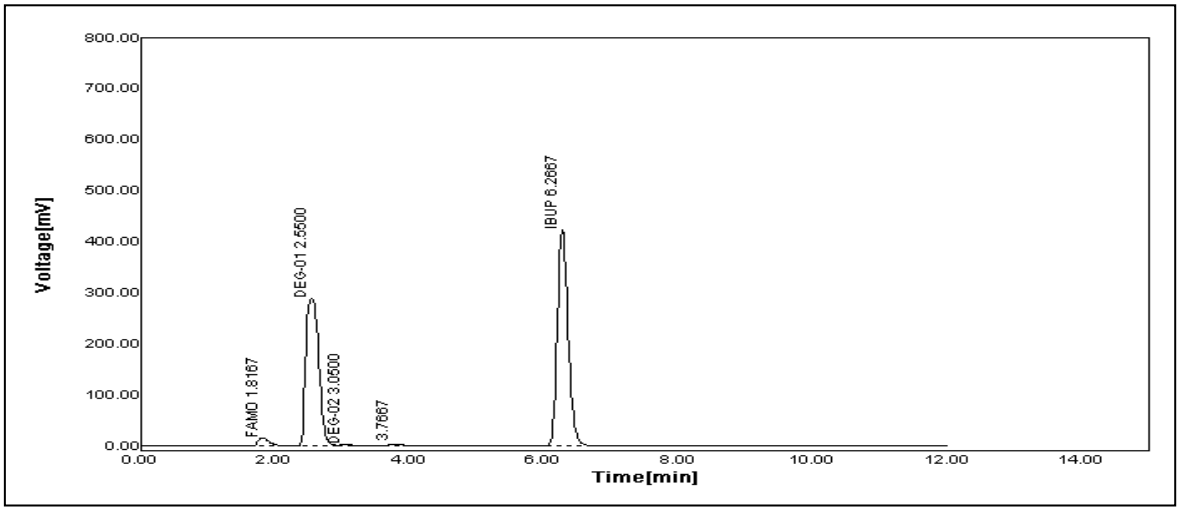

Fig. 7 Chromatogram of Oxidative Degradation

Famotidine undergoes strong oxidative degradation (3.0% H_20_2) since the famotidine is light sensitive in nature.

Table 6 Overview of Oxidative Degradation

Name time	Retention	Area	%Area
FMT	1.8167	190.3470	2.21
Deg-1	2.5500	3758.0488	43.62
Deg-2	3.0500	51.4413	0.60
IBU	6.2667	4539.7798	52.70

Neutral Degradation

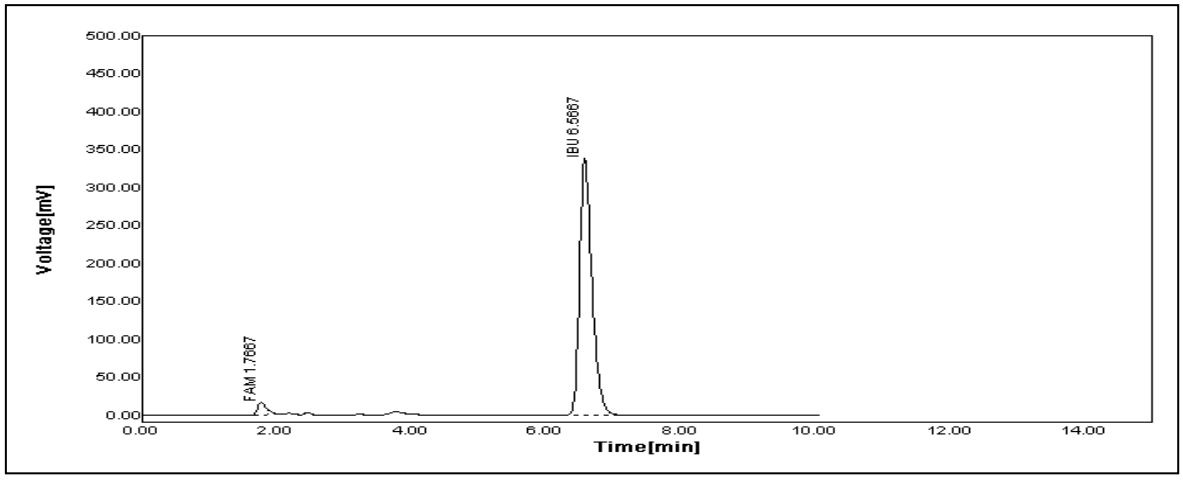

Fig. 8 Chromatogram of Neutral Degradation

A neutral degradation studies revels that the famotidine is capable of undergoing the neutral degradation (Water) owing to the water solubility of famotidine.

Table 7 Overview of Neutral Degradation

Neutral Degradation			
Name	**Retention Time**	**Area**	**% Area**
Famotidine	1.96	375.31	5.79
Deg-I	2.68	767.45	11.83
Ibuprofen	6.35	5343.51	82.38

References

[1]. Lough W.J., I.W. Wainer, "HPLC fundamental principles and practices," (1991), Blackie Academic and professional, 52-67.

[2]. International Conferences on Harmonization Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 2005, pp. 101-109.

[3]. ICH-Q1A (R2): Stability Testing of New Drug Substances and Products (Second Revision), FDA, Vol. 68, 2003,pp. 225, 657-678.

[4]. ICH-Q1B: Photostability Testing of New Drug Sub-stances and Products, FDA, Vol. 62, No. 95, 1997, pp.27115-27122.

[5]. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare. New Delhi; Published by the Controller of Publications; (2010), Vol.2, pp. 129.

[6]. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare. New Delhi; Published by the Controller of Publications; (2010), Vol.2, pp. 310.

[7]. Monika Bakshi, Saranjit Singh, Development of validated stability-indicating assay method critical review, Journal of Pharmaceutical and Biomedical Analysis 2002; 28(1): 1011–1040.

[8]. Gnana Raja M., Geetha G., Sangaranarayanan A. Simultaneous, Stability Indicating Method Development and Validation for related Compounds of Ibuprofen and Paracetamol Tablets by RP-HPLC Method. J Chromat Separation Techniq 2012; 2(3): 3:8.

[9]. V. Rajani Sekhar, Y. Padmanabha Reddy, P. Ramalingam, D. Harihara Theja, "RP-HPLC and UV-derivative spectrophotometry technique for the simultaneous estimation of ibuprofen and famotidine in pharmaceutical dosage form. Der Pharmacia Sinica 2013, 4(2):160-170.

[10]. Tummala Vijaya Bhaskara Reddy, Nallagari Sowjanya Reddy, Sanivarpu Ravi Prakash Reddy, Chintala Rambabu, Golkonda Ramu. Optimized and validated stability indicating RP-UPLC method for the determination of famotidine in pharmaceutical formulations", journal of pharmacy research2013; 6 (1) :865-869.

[11]. L. Peikova, M. Georgieva, B. Tsvekova., RP-HPLC method for simultaneous determination of ibuprofen and famotidine in pharmaceutical dosage form. Pharmacia 2014 ;6(1):35-42.

[12]. Nawel Helali, Lofti Monser., Stability indicating method for famotidine in pharmaceuticals using porous graphitic carbon column. J. Sep. Sci. 2008; 3(1): 276 – 282.

[13]. Ahirrao V. K. Pawar R. P. Simultaneous quantification of famotidine and ibuprofen in pharmaceutical dosage by using validated stability indicating LC method. Research Journal of Pharmaceutical sciences 2013;2(4): 1-9.

[14]. Karthik B. et al., Analytical Method De-velopment and Validation of Ibuprofen and Fa-motidine in Tablet Dosage Form by RP-HPLC Method. Journal of Pharmacy Research 2012; 5 (9): 4633-4635.

[15]. Krishnaveni G. et al. Simultaneous determination of famotidine and ibuprofen in combined pharmaceutical dosage form by RP-HPLC method. International Journal of Pharma and Bio Sciences 2013; 25 (3): 655-662.

[16]. Dimal A. et al., Development and validation of liquid chromatographic method for estimation of ibuprofen and famotidine in combined dosage form. ISRN Analytical Chemistry 2012; http://dx.doi.org/10.5402/2012/674392.

[17]. Jitendra Kumar et al. Recent Approaches for Impurity Profiling of Pharmaceuticals. International Journal of Advances in Pharmaceutics 2013; 2(3): 25-34.

[18]. Abhijeet Welankiwar et al. Photostability Testing of Pharmaceutical Products. Int Res J Pharm 2013; 4(9): 11-15.

[19]. http://Ibuprofen- Wikipedia, the free encyclopedia.

[20]. http://Famotidine- Wikipedia, the free encyclopedia