Determination of Chemical Stability of Various Famotidine Dosage Forms by UV –Visible Spectrophotometric Method and Data Analysis by R-GUI Stability Software

Sohail Hassan[1], Erum Zaheer[1,*], Iyad Naeem Muhammad[2], Amir Hassan[1], Mohsin Ali[3] and Masooda Qadri[3]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan

[2]Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan

[3]Department of Chemistry, Faculty of Science, University of Karachi, Karachi, Pakistan

Abstract: H_2 receptor antagonists are still the first line of therapy in treating gastro esophageal reflux diseases as well as other ulcers of the upper gastrointestinal tract. Accelerated stability studies of different brands of Famotidine tablets (20mg) and suspension(10mg/5ml),both liquid and dry, were carried out at 40 ^{o}C ± 2 ^{o}C (Temperature) and 75% R.H. ± 5% R.H. The assay of tablets was conducted by both HPLC and UV/Visible Spectrophotometric methods whereas for suspensions only UV/Visible Spectrophotometric method was used. The tests were conducted at 0, 1, 3 and 6 months as per guidelines of ICH for accelerated studies. The results of physical tests indicated that the dissolution of tablet decreases in all cases with time whereas disintegration of all brands was found within 15 minutes throughout the course of study while the hardness demonstrated to be decline with time. Kinetic treatment to determine rate constants and shelf lives indicated that dry suspension was more stable than liquids while the tablets showed stability for three years which was parallel to their claimed expiry. Among tablets, brand A was the most stable and among suspensions, brand C showed the longest stability. The stability studies were also carried out by using a software R-Gui (version 2.13) and results were compared with manually calculated results.

Keywords: Famotidine, Stability, Software generated shelf life, Formulations, ICH stability guidelines.

INTRODUCTION

Enteric diseases caused by the irregularities in the production or secretion of gastric acid or due to its retrograde or anterograde movements to the adjacent anatomical structures, though mild in their initial stages, can exert destructive effects on the epithelial lining of these structures which lack natural defense system against the acid. Over the years, various medicinal agents have been discovered and innovated to relieve the symptoms of these agonizing conditions. Though, the journey of these discoveries has travelled long from simpler acid neutralizing agents, through the mucosal protecting substances up to the modern production of proton pump inhibitors, the inhibitors of acid release by the stomach, in response to food and other stimuli have always been the mainstay of treatment and prevention of these pathological states.

In this regard, H_2 receptor antagonists (H$_2$RA), a class of histamine blockers hold a prominent position since distant past. Famotidine, is such an antagonist [1] which inhibits gastric acid release by blocking H_2 receptors present on gastric parietal cells. It was approved by FDA in mid 80s and was licensed by Merck & Co.

Unlike Cimetidine, the first H$_2$RA, Famotidine does not alter hepatic C-P450 drug metabolizing enzyme system and does not demonstrate interaction with other drugs [2].

On the other hand, in preference to Ranitidine, it has no adverse effects on platelets production, Futhermore, it has been reported that Famotidine possesses nine times and thirty two times greater strength in inhibiting gastric acid release as compared to Ranitidine and Cimetidine respectively [3]. These favorable safety profiles along with profound pharmacodynamic effects on gastric acid secretion and a convenient 12-hourly dosing schedule has made Famotidine exceedingly popular among H$_2$RAs.

Prescription Famotidine is used for short term treatment of gastro esophageal reflux disease, gastric and acute duodenal ulcers and is also recommended for maintainance therapy of the same. Pathological hypersecretory conditions of Zollinger-Ellison syndrome and multiple Endocrine Adenomas are also managed by Famotidine [4, 5]. By giving Famotidine there are reports of improvement in Schizophrenic symptoms in patients [6-8]. It is also used in patients on NSAIDs therapy to prevent the occurrence of peptic ulcers. Famotidine has also been reported to be used in combination with H$_1$-antagonist to prevent and treat acute allergic reaction of Urticaria [9]. Beside this, it is an effective alternate to proton pump inhibitors.

*Address correspondence to this author at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan; E-mail: erumzaheer86@gmail.com

Various methods for determining the stability of Famotidine have been proposed and practiced.

In 1992, kamath *et al.* [10] developed a spectrophotometric method for determination of famotidine in tablets or bulk powder.

In 2006, Tzanavaras *et al.* have employed Flow injection analysis to optimize and validate the dissolution test for Famotidine tablets [11]. The stability of Famotidine in an extemporaneously prepared oral liquid was studied, by Quercia *et al.* in 1993, for 30days at $4^{o}C$ and $24^{o}C$ (room temperature) [12]. The concentration of Famotidine remained above 90% of original concentration for 20 days at $4^{o}C$ and for15 days at $24^{o}C$. After 30 days, Famotidine concentration was reduced by 15% and 24% at $24^{o}C$ (room temperature) respectively. In1989, Suleiman *et al.* studied Kinetics of acid catalyzed hydrolysis of Famotidine in 0.01-0.10 M HCl solutions of 2 mg/ml using stability indicating HPLC assay with UV/Visible detection. The specific hydrogen ion catalysed rate constant was found to be 3.427/ M/ h at $37^{o}C$ and activation energy was determined to be 63.7 Kj/ mol [13]. Thermal degradation of Famotidine in aqueous solution was determined by stability indicating HPLC method, in 1992, by Junnarkar and Stavchansky [14]. Sulfacetamide and Famotidine were eluted in 13 min with retention times of 5.7 and 10 respectively. Linear relationship of 60µg/ ml was found between peak height ratio (Famotidine/Sulfacetamide) and Famotidine concentration. In concentration rangeof4-57µg/ ml coefficient of variation of assay method ranged from 0.2 to 5.1%. Junnarkar and Stavchansky, in 1995, studied Isothermal and nonisothermal decomposition of Famotidine in aqueous solution was over pH range of 1.71 to 10.0 for establishing expiration date of preparations [15]. In 2002, Degim and Agabeyoglu have compared isothermal and nonisothermal stability of nizatidine and Famotidine by using linear and logarithmic temperature programs rate constants and activation energies were calculated. Nonisothermal stability tests results were compared with results of isothermal stability tests and were found similar [16]. Degradations of Famotidine under basic conditions were investigated, in 25% ammonia solution and 2M NaOH, by Singh *et al.*, in 2002. Several degradation products were obtained and structures established by proton and carbon NMR, Mass spectroscopy [17]. UV/Visible stability demonstrating method for evaluation of Famotidine, Cimetidine and Ranitidine Hydrochloride in existence of their derivative was adopted by Kelani *et al.*, in 2002 [18].

MATERIALS AND METHODS

Required samples of tablets and suspensions in the form of commercially available formulations of Famotidine were collected from market of Karachi where as Famotidine standard, for reference, was gifted by Saffron Pharmaceuticals Pvt. Ltd, Faisalabad, Pakistan. All reagents were of HPLC grade obtained from Merck, Darmstadt, Germany. The water for HPLC was prepared by double glass distillation.

Assay of Tablets by UV/Visible Spectrophotometry

The contents of Famotidine tablets were determined by a developed and validated UV/Visible Spectrophotometric method [19].

Standard Preparation

Standard Famotidine solution (500 µg/ml) was prepared by dissolving 50mg pure drug in100ml of 0.1N HCl,10 ml of that solution was transferred to a 100ml volumetric flask and made up the volume upto 100 ml with0.1NHCl to obtain 50µg/ml. The absorbance was measured at 265nm using 0.1NHCl as blank.

Sample Preparation

For sample preparation, 20 tablets of Famotidine were grounded into fine powder. An accurately weighted quantity of powder equivalent to 50mg of Famotidine was transferred to a 100ml standard flask and mixed with 60ml of 0.1N HCl by shaking lasting for 20 minutes. The obtained mixture was filtered by using a Whatman no. 42 filter paper. 10 ml of the filterate was taken out using a pipette and transferred to a 100ml volumetric flask. Required volume of working concentration of 50µg/ml was obtained by using 0.1N HCl. The absorbance was measured at 265nm using 0.1NHCl as blank.

Assay by High Performance Liquid Chromatography (HPLC) Method

The content of Famotidine in tablets were determined by using official method of U.S.P 2010 [20].

Dissolutiontest

The dissolution test for Famotidine tablets was performed according to the official method of U.S.P 2010. Acceptable limit is not less than 75% [20].

Disintegration Test

One dosage unit (tablet) was placed in each of six tubes; a disc was added to each tube. Water was used

as an immersion fluid. The apparatus was operated at 37 ˚C ± 2 ˚C, with 30 Strokes / minutes for 30 minutes and then examined the state of the tablets. If 1 or 2 of the tablets failed to disintegrate completely, repeated the test on a further 12 tablets. If 16 out of 18 disintegrated completely then the tablets complied the test [20, 21].

Hardness Test

The hardness of Famotidine tablets of different brands stored under specified conditions of temperature and pressure were measured using Fugiwara hardnesstester and the values recorded in Kp (Kilopounds) units.

Assay of Famotidine Suspensions

For assay of Famotidine suspensions, a developed and validated UV/Visible Sectrophotometric method was used [22].

Preparation of Standard

Standard solution was prepared by dissolving accurately weighted 25 mg of Famotidine. Working standard, was taken in 50 ml volumetric flask and made upto mark with 0.1N HCl.

The solution was then vortexed and sonicated. In 50ml volumetric flask 2ml of the solution was diluted upto mark with 0.1NHCl, then vortexed and sonicated.The absorbance was measured at maximum wavelength 265nm using 0.1NHCl as blank.

Preparation of Sample

Suspension equivalent to 25mg of Famotidine was taken in a 50ml beaker and stirred with 30 ml 0.1N HCl until the suspension was completely dissolved. Resultant compound was transferred to a 50ml flask and made upto the mark with 0.1 n HCl. The solution was then centrifuged in a centrifuge tube for 15 minutes at 2500 RPM. The clear solution was poured in extraction flask added 40ml diethylether and extracted. After discarding the organic layer extraction was repeated thrice with 40ml ether. Aqueous layer was collected, heated on water bath at 60 °C for 5minutes,cooled and then diluted to 50ml with 0.1NHCl. The absorbance was measured at maximum wavelength of 265nm using 0.1N HCl as blank.

Table 1:   The Remaining% Content of the Active Ingredient in Famotidine Tablets (20mg) by UV/Visible Spectrophotometry

| Different brands of famotidine (20mg)	Months			
	0	**1**	**3**	**6**
BrandA	99.89	99.55	99.20	98.53
BrandB	99.22	98.84	98.51	97.52
BrandC	99.85	99.50	98.97	98.22
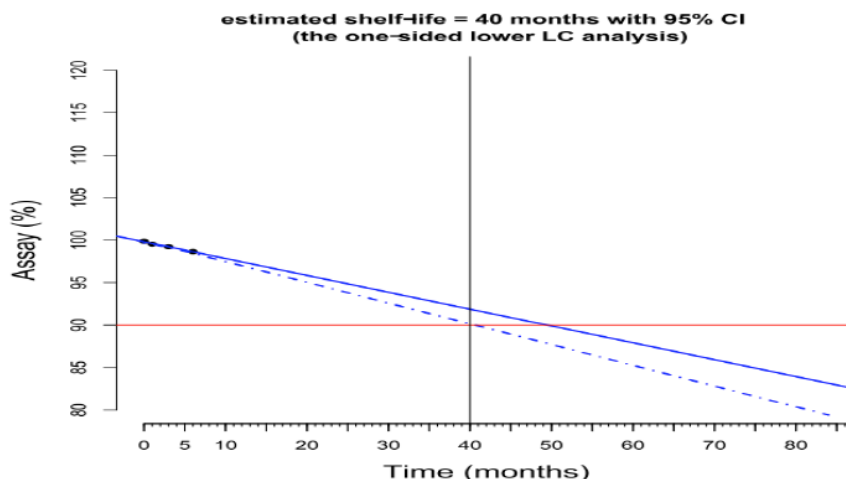
Figure 1: Estimation of shelf life of Famotidine tablet (20 mg) Brand A by UV assay.

RESULTS

In the present study the Famotidine stability was evaluated in tablet and suspension dosage form by UV-visible Spectrophotometery and verifying and comparing it with R-Gui stab software version 2.13 [23]. The results are mentioned in Table **1** while the software originated data is mentioned in Figures **1** and **2**. Tables
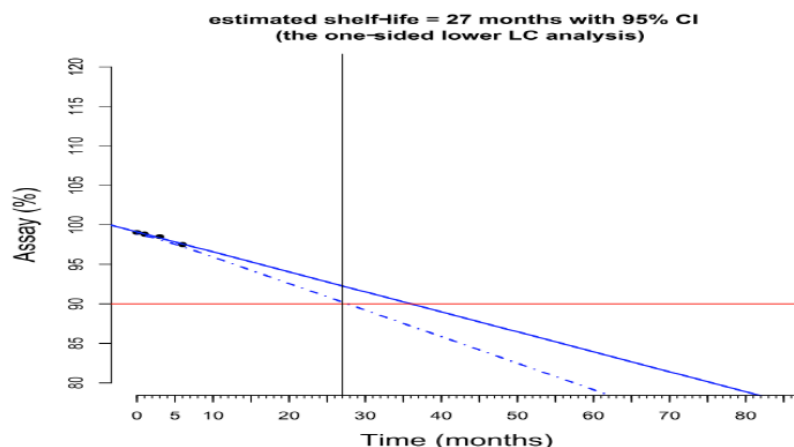
Figure 2: Estimation of shelf life of Famotidine tablet (20 mg) Brand B by UV assay.

Table 2: Software Originated Results for Apparent First – Order Rate Constant (kobs) for the Degradation of Famotidine Tablets by UV/Visible Spectrophotometry

Famotidine (20mg)	k_{obs} day^{-1}	Correlation coefficient	t_{90} years (months)
Brand A	8.750×10^{-5}	0.9875	3.34 (40months)
Brand B	1.296×10^{-4}	0.9751	2.25 (27months)
Brand C	9.459×10^{-5}	0.9875	3.08 (37months)

Table 3: The Remaining % Content of the Active Ingredient in Famotidine Suspensions (10mg/5ml)

| Different brands of famotidine suspensions	MONTHS			
	0	1	3	6
Brand A Dry Suspension	99.40	98.90	98.20	97.03
Brand A Liquid Suspension	99.80	99.12	97.86	95.71
Brand B Liquid Suspension	99.20	98.67	97.75	96.06
Brand C Liquid Suspension	99.80	99.14	98.27	96.95

Table 4: Software Originated Results for Zero– Order Rate Constant (k) for the Degradation of Famotidine Suspensions

| Famotidine suspensions (10mg/5ml)	K mg/5ml day^{-1}	Correlation coefficient	t_{90} years
Brand A (dry suspension)	1.506×10^{-3}	0.9979	1.83 (22months)
Brand A (liquid suspension)	2.559×10^{-3}	0.9992	1.08 (13months)
Brand B	2.067×10^{-3}	0.9981	1.34 (16months)
BrandC	1.848×10^{-3}	0.9952	1.5 (18months)
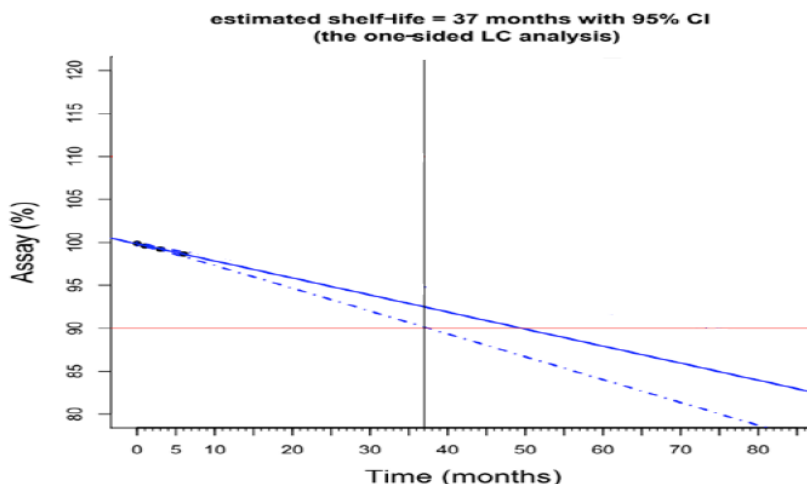
Figure 3: Estimation of shelf life of Famotidine tablet (20 mg) Brand C by UV assay.

2, **3** and **4** and Figures **4**, **5**, and **6** shows the dissolutions of Famotidine in tablets and suspensions.
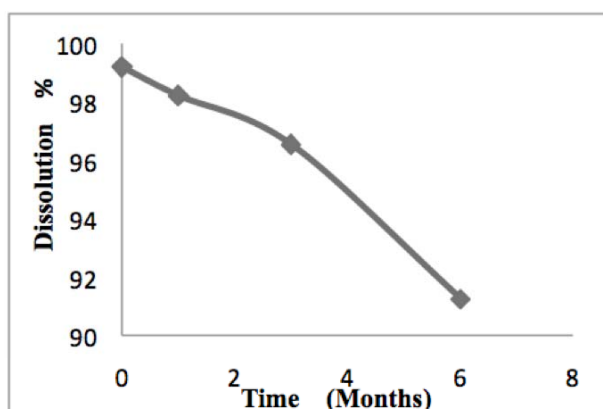
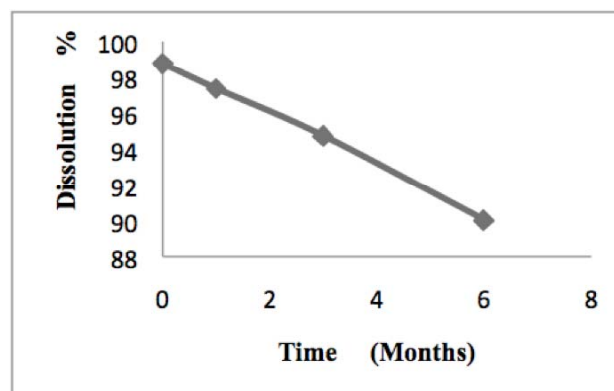**Figure 4:** Graph of Dissolution – Time of Famotidine (20mg) Brand A.
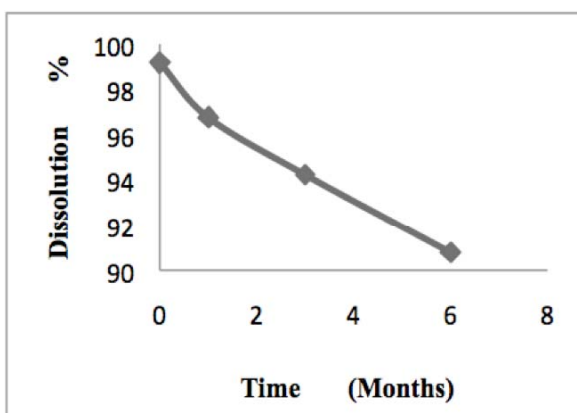
Figure 5: Graph of Dissolution – Time of Famotidine (20mg) Brand B.

DISCUSSION

The drug stability in any dosage form is always a matter of prime importance in wider feilds of pharmaceutical sciences. In the academia and industrial pharmacy, the drug stability studies in dosage forms are conducted as routine evaluation. The stability studies are also carried out to estimate the mechanism and phenomena of the active pharmaceutical ingredients' (APIs) degradation in crude and in dosage forms. Establishment of the shelf life or the expiration date is of pivotal importance in this regard. Moreover, the factors and the elements that influence the drug stability in drug product need to be studied as per the guidelines proposed by International Conference on Harmonization (ICH Q1E) [23].

Figure 6: Graph of Dissolution – Time of Famotidine (20mg) Brand C.

The stability of Famotidine in tablets and in liquid dosage forms has been reported in the literature [12, 24-26]. The drug products containing Famotidine available in Karachi market acclaim an expiry of three years in general. The present study is focused on evaluating the shelf life of Famotidine tablets and suspensions using R-package "stab" software having a single-factor analysis, for single-batch based on ICH Q1E specifications [23]. Zero order analysis was made
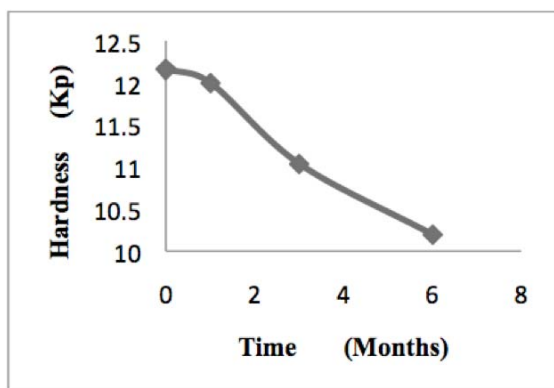
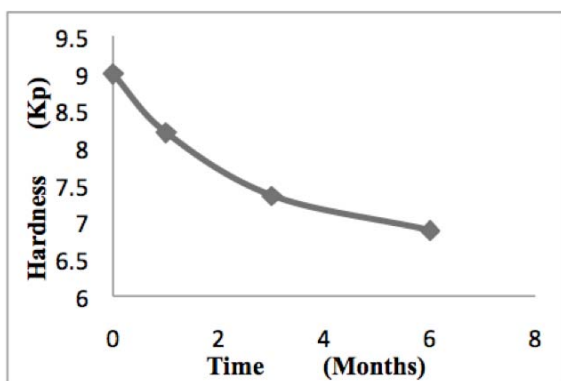**Figure 7:** Graph of hardness – time of Brand A Famotidine (20mg).

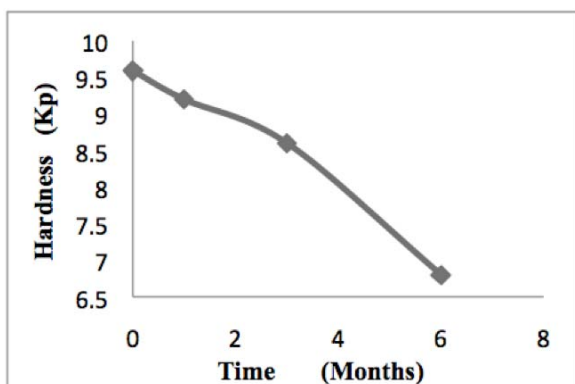**Figure 8:** Graph of hardness – time of Brand B Famotidine (20mg).

Figure 9: Graph of Hardness – Time of Brand C Famotidine (20mg).

at one sided Lower control analysis at 95% confidence interval. Brand A have shown a shelf life of 3.34 years, Brand B 2.25 years, Brand C 3.08 years. Brand A was found most stable whereas Brand B was the least stable. In case of suspensions, shelflives obtained were Brand A (Dry suspension) 1.83 years, Liquid Suspensions Brand A, B and C showed the shelf lives of 1.08 years , 1.34 years and 1.5years respectively. Among liquid suspensions Brand C was most stable Famotidine is available as an oral suspension (powder for reconstitution) all over the world in the concentration

of 40 mg/ 5ml and the patient is advised to discard unused suspension after 30 days (Figures **10-13**). In Pakistan liquid suspensions of Famotidine for oral use are prepared by different local pharmaceutical companies in the concentration of 10 mg/5ml and the expiry is stated to be two years, which has raised the question on its authenticity. Furthermore, research on stability has demonstrated that dry suspension is more stable than liquids which has prompted the pharmaceutical firms to shift their products to dry suspension but with the same expiry period of two years. Several reports related to this work have been published in the literature [27-31] that addressed the issue of drug stability conducted by software generated shelf lives.
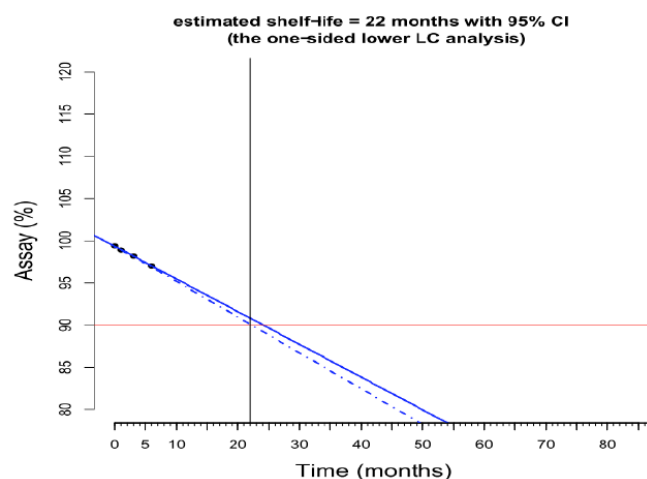

Figure 10: Estimation of shelf life of Famotidine suspension (10 mg/ 5 ml) Brand A (Dry suspension).
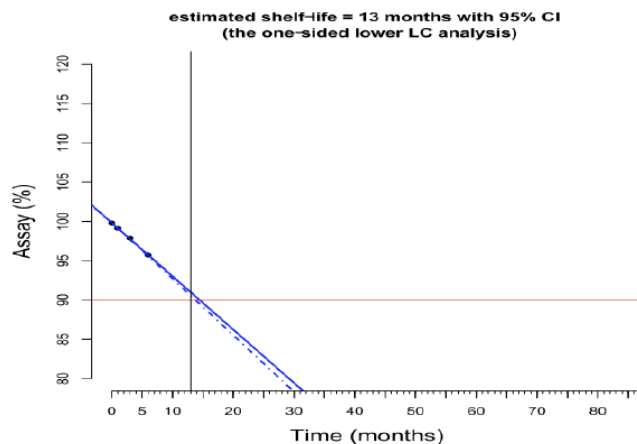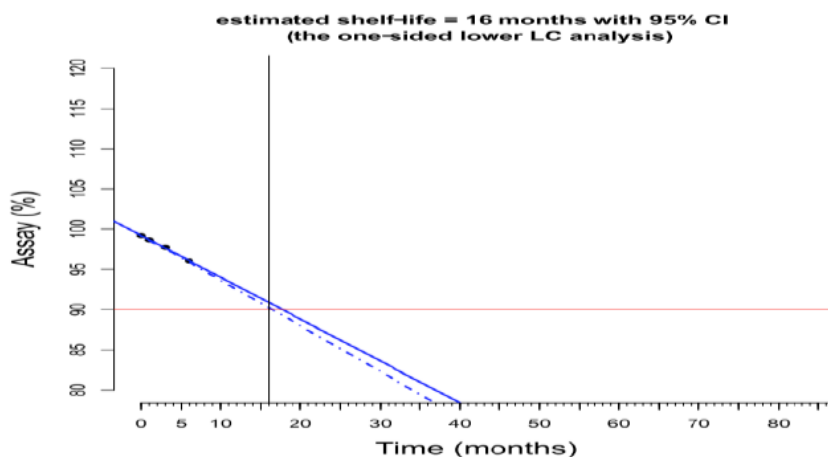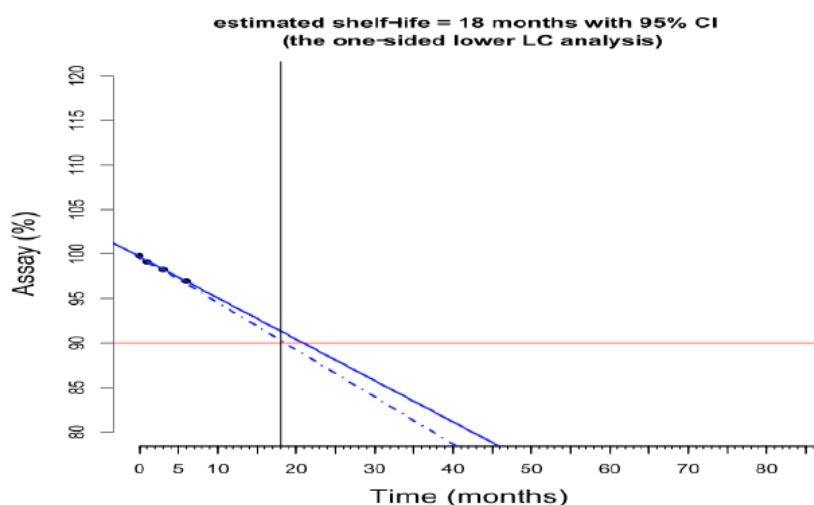

Figure 11: Estimation of shelflife of famotidine suspension (10 mg/ 5 ml) Brand A (liquid suspension).

CONCLUSION

Most of the tablets were found stable for three years which is their claimed expiry, whereas dry powder form

Figure 12: Estimation of shelf life of Famotidine suspension (10 mg/ 5 ml) Brand A (Liquid suspension).

Figure 13: Estimation of shelf life of Famotidine suspension (10 mg/ 5 ml) Brand A (Liquid suspension).

was found stable for two years as is claimed but the liquid suspensions failed to show stability parallel to their claimed expiry. The study suggests that rigorous monitoring of the drug stability in different pharmaceutical dosage forms is a dire need of the present time.

REFERENCES

[1]   Modlin IM, Sachs G. Inhibition of the Histamine-2 Receptor. In: Modlin IM, Sachs G, editors. Acid Related Diseases: Biology and Treatment. 2ed ed. Philadelphia: Lippincott William and Wilkins 2004; p. 138-40.

[2]   Humphries TJ, Merritt GJ. Review article: drug interactions with agents used to treat acid-related diseases. Alimentary pharmacology & Therapeutics 1999; 13(Suppl 3): 18-26. PubMed PMID: 10491725. Epub 1999/09/24. eng.

[3]   Cheli R, Perasso A, Giacosa A. Gastric secretion: a physiological and pharmacological approach. Verona: Cortina International; Raven Press, New York; 1986.

[4]   McEvoy GK, (ed.). AHFS Drug information. 95th ed. Bethesda, MD: American Society of Health-System Pharmacists 1995.

[5]   Gennaro AR. Remington's Pharmaceutical Sciences. 20th ed. Pennsylvania: Mack Publishing Co; 2000.

[6]   Kaminsky R, Moriarty TM, Bodine, J, Wolf DE, Davidson M. Effects of Famotidine on deficit symptoms of Schizophrenia. Lancet 1990; 335: 1351-52. http://dx.doi.org/10.1016/0140-6736(90)91237-5

[7]   Rosse RB, Kendrick K, Tsui LC, *et al.* Famotidine adjunctive pharmacotherapy of schizophrenia: a case report. Clin Neuropharmacol 1995; 18: 369-74. http://dx.doi.org/10.1097/00002826-199508000-00009

[8]   Martinez MC. Famotidine in the management of schizophrenia. Ann Pharmacother 1999; 33: 742-47. http://dx.doi.org/10.1345/aph.18262

[9]   Bet 2. Combination therapy with h2 and h1 antihistamines in acute, non-compromising allergic reactions. Emergency Medicine Journal 2008; 25(3): 165-6.

[10] Kamath BV, Shivram K, Vangani S. Spectrophotometry Determination of Famotidine by Charge Transfer Complexation. Anal Let 1992; 25(12): 2239-247.

[11] Tzanavaras PD, Verdoukas A, Balloma T. Optimization and validation of a dissolution test for famotidine tablets using flow injection analysis. Journal of Pharmaceutical and Biomed Analysis 2006; 41(2): 437-41. http://dx.doi.org/10.1016/j.jpba.2005.12.011

[12] Quercia RA, Jay GT, Fan C, Chow MS. Stability of Famotidine in an extemporaneously prepared oral liquid. American Journal of Health-System Pharmacy 1993; 50(4): 691-93.

[13] Suleiman MS, Najib NM, Hassan MA, Abdel-Hamid ME. Kinetics of the acid-catalysed hydrolysis. Int. journal of Pharmaceutics 1989; 54: 65-69.
http://dx.doi.org/10.1016/0378-5173(89)90166-X

[14] Junnarkar GH, Stavchansky S. Thermal degradation of famotidine in aqueous solution stability indicating assay. Analytical Lett 1992; 25: 1907-13.
http://dx.doi.org/10.1080/00032719208018261

[15] Junnarkar GH, Stavchansky S. Isothermal and non isothermal decomposition of famotidine in aqueous solution. Pharmaceutical Research 1995; 12: 599-604.
http://dx.doi.org/10.1023/A:1016222501079

[16] Degim Z, Agabeyoglu I. Nonisothermal stability tests of famotidine andnizatidine. II Farmaco 2002; 57: 729-35.
http://dx.doi.org/10.1016/S0014-827X(02)01266-1

[17] Singh S, Kumar S, Sharda N, Chakraborti AK. New findings on degradation of famotidine under basic conditions: Identification of a hitherto unknown degradation product and the condition for obtaining the propionamide intermediate in pure form. Journal of Pharmaceutical Sciences 2002; 91: 253-57.
http://dx.doi.org/10.1002/jps.1176

[18] Kelani KM, Aziz AM, Hegazy MA, Abdel-Fattah LS. UV/Visible Spectrophotometric stability indicating methods for quantitative determination of cimetidine, famotidine and ranitidine HCl in prescence of their oxidative derivatives. Analytical Lett 2002; 35: 1055-73.
http://dx.doi.org/10.1081/AL-120004555

[19] Zenita O, Basavaiah K, Ramesh PJ, Vinay KB. Simple and sensitive UV Spectrophotometric methods for determination of Famotidine in tablet formulations Farmacia 2011; 59: 647-56.

[20] United States Pharmacopeia and National Formulary USP33–NF28. Assay of Famotidine tablets. Rockville,MD: The United Pharmacopeial Convention, Inc.; 2010.

[21] International Pharmacopoeia. Disintegration test for tablets (5.3). 4th ed (4th supplement). Geneva: World Health Organization; 2014.

[22] Zaheer E, Hassan S, Ibraheem M, *et al*. Development and Validation of a UV Spectrophotometric method for determination of Famotidine in suspension. FUUAST Journal of Biology 2014; 4: 7-12.

[23] Lee HY, Wu PC, Lee YJ. Stab: an R package for drug stability data analysis. Comput Methods Programs Biomed 2010; 100: 140-48.
http://dx.doi.org/10.1016/j.cmpb.2010.03.002

[24] Mady FM, Abou-Taleb AE, Khaled KA, *et al*.Evaluation of carboxymethyl-beta-cyclodextrin with acid function: Improvement of chemical stability, oral bioavailability and bitter taste of famotidine. International Journal of Pharmaceutics 2010; 397: 1-8.
http://dx.doi.org/10.1016/j.ijpharm.2010.06.018

[25] Walash MI, El-Brashy A, El-Enany N, Kamel ME. Spectrofluorimetric Determination of Famotidine in Pharmaceutical Preparations and Biological Fluids. Application to Stability Studies. Journal of Fluorescence 2009; 19: 333-344.
http://dx.doi.org/10.1007/s10895-008-0421-3

[26] Wu YQ, Fassihi R. Stability of metronidazole, tetracycline HCl and famotidine alone and in combination. International Journal of Pharmaceutics 2005; 290: 1-13.
http://dx.doi.org/10.1016/j.ijpharm.2004.10.015

[27] Javed I, Ranjha NM, Massud A, Hussain L. Accelerated Stability Studies of Flurbiprofen Film Coated Tablets of Five Different National Brands in Pakistan. Journal of Drug Delivery and Therapeutics 2013; 3(2).

[28] Kiermeier A, Verbyla AP, Jarrett RG. Estimating a Single Shelf·Life for Multiple Batches. Australian & New Zealand Journal of Statistics 2012; 54: 343-358.
http://dx.doi.org/10.1111/j.1467-842X.2012.00685.x

[29] Liu W, Hsu J, Bretz F, Hayter A, Han Y. Shelf-life and its estimation in drug stability studies. Journal of Applied Statistics 2014; 41: 1989-2000.
http://dx.doi.org/10.1080/02664763.2014.898135

[30] Penjuri SCB, Damineni S, Ravouru N. Formulation and evaluation of verapamil hydrochloride transmucosal drug delivery system. Thai J Pharm Sci 2013; 37: 25-38.

[31] Muhammad IN, Shoaib MH, Yousuf RI, *et al*. Formulation development and optimization of Cefuroxime axetil tablets by Direct compression method and its Stability studies. Lat Am J Pharm 2012; 31: 271-278.