Synthesis and Characterization of Novel Sulphoxide prodrug of Famotidine

Surendran Vijayaraj*[1], Bayupureddy Omshanthi[2], Sriram Anitha[2], Kokilam Perumal Sampath Kumar[3]

[1]Department of Pharmaceutical Sciences, NIMS University, Jaipur, Rajasthan 303121, India
[2]Department of Pharmaceutical Analysis, Sree Vidyanikethan College of Pharmacy, Tirupathi, Andhra Pradesh 517101, India
[3]Department of Pharmaceutical Sciences, Coimbatore Medical College, Coimbatore, Tamil Nadu 641014, India

ABSTRACT

Background of the work: Famotidine, a H_2 receptor antagonist is the drug of choice to treat ulcers in stomach (gastric and duodenal), erosive esophagitis (heartburn or acid indigestion) and gastroesophageal reflux disease (GERD). Drug molecules with limited aqueous solubility are becoming increasingly prevalent in the research and development portfolios of discovery focused pharmaceutical companies. Prodrugs are an established concept to overcome barriers like poor solubility to drug's usefulness. **Methods:** As aqueous solubility is an important parameter to enhance the bioavailability of drug, novel sulphoxide prodrug of famotidine was synthesized. The synthesized prodrug was characterized by IR, NMR, Mass and DSC. Physicochemical characterization was done by partition coefficient and aqueous solubility studies. Chemical hydrolysis study was done in simulated gastric and intestinal fluids. **Results and discussion:** Decrease in Log P value, -0.74 of sulphoxide prodrug compared to -0.60 of famotidine, indicates the increase in hydrophilic property of synthesized sulphoxide derivatives of famotidine. Aqueous solubility increment of 6.7 fold was found in sulphoxide prodrug compared to famotidine. Invitro chemical hydrolysis profiles revealed that the synthesized sulphoxide derivatives of famotidine are chemically stable in Simulated Gastric fluid pH 1.2 and Simulated Intestinal Fluid pH 7.4. Conclusion: Hence the synthesized novel sulphoxide prodrug shown better aqueous solubility than existing drug and can be effectively used in therapy.

Keywords: Famotidine, Sulphoxide prodrug, Characterization, aqueous solubility, Characterization, Chemical Hydrolysis, Log P.

INTRODUCTION

Famotidine (FM) chemically 3-([2-(diamino-methyleneamino) thiazol-4-yl] methyl-thio)-N' Sulfamoyl propimidamideis a histamine H_2 receptor blocker (Figure 1). FM is used to treat and prevent ulcers in stomach and intestine.[1] FM is a poorly water soluble drug and is poorly absorbed from lower gastro-intestinal tract. Dissolution is rate limiting step in the process of drug absorption.[2] There are many stated techniques for solubility enhancement of FM like complexation, solid dispersion, micronization.[3–5] prodrug is an efficient technique to enhance solubility of drugs. prodrugs are defined as a biologically inactive derivative of a parent drug molecule that usually requires a chemical or enzymatic transformation within the body to release the active drug, and possess improved delivery properties over the parent molecule.[6] There are no reported methods for preparation of prodrug in solubility enhancement of FM. The main purpose of present study is to increase aqueous solubility of Famotidine by pro-drug approach. A Huge sum of money has been invested in new drug research and it will take around 15 years to develop a new chemical entity whereas pro-drug strategy is aimed to enhance the effectiveness of existing drug hence it is economic and time saving approach.Therapy enhancement via successful delivery of a therapeutic agent is the principal goal of drug delivery research. Achieving therapeutic efficacy of any pharmaceutical dosage form mainly depends

Submission Date : 25-06-14
Revision Date : 18-08-14
Accepted Date : 03-09-14

DOI: 10.5530/ijper.48.4.6

Address for correspondence:
Mr. Vijayaraj Surendran
Department of Pharmaceutical Sciences, NIMS University, Jaipur, Rajasthan-303121, India
Phone: +91-9032774923
E-mail: vijaysurender85@gmail.com

www.ijper.org

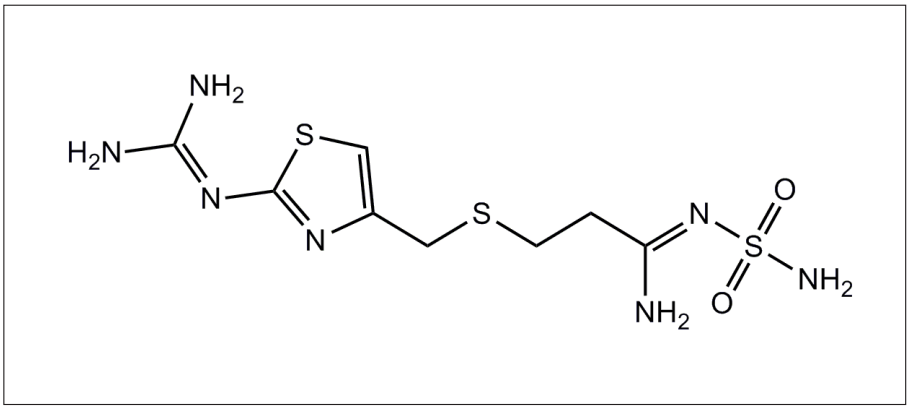

Figure 1: Structure of Famotidine

upon the availability of drug with desired concentration to the target site.[7] The bio availability of poorly water-soluble drug like FM is a well-known difficulty to be coped with during drug delivery. The current research aims to resolve aforementioned issue by Prodrug approach. Sulphoxide prodrug of FM was synthesized and the synthesized prodrug was investigated by FT-IR, $1H^1$ NMR, Mass and DSC studies. Aqueous solubility studies were performed to ensure the enhancement in solubility.

MATERIALS AND METHODS

Instruments and Chemicals

Melting points were determined on a Differential Scanning Calorimeter (DSC) apparatus. Aluminum sheets coated with silica gel 60 F254 of Merck were used for TLC. Photo microscopic images were taken using Olympus research microscope. Elemental analysis was performed using Carlo-Erba model 1108. The IR spectra were recorded in KBr discs on FT-IR Bruker IFS 55 spectrophotometer and wave numbers are reported in cm^{-1}. The $1H^1$NMR spectra were obtained on a Bruker DRX-300 spectrometer (75 MHz) in DMSO. Chemical shifts were recorded in ppm (d) relative to TMS as an internal standard. High resolution mass spectra were recorded on an Agilent 5975 MSD series Direct Inlet Probe system using electro spray ionization technique. All chemicals used were of analytical grade procured from SD fine, Himedia, and E. Merck while standard drug of Famotidine was purchased from Yarrow Chem Products, Mumbai.

Synthesis of Sulphoxide prodrug:[8–10]

One mole of Famotidine and 2 equivalents of urea hydrogen peroxide adduct (equimolar quantities of urea and hydrogen peroxide) were transformed into the beaker and temperature of 85°C was maintained in electric water-bath for 30 min (Figure 2). TLC studies were performed to ensure the completion of the reaction. The product obtained was filtered and collected. Recrystallization of the product yielded creamy white crystals which were filtered and collected. Physical characterization of synthesized prodrugs were done by TLC analysis using pre-coated aluminium plates with ethyl acetate/methanol/water (8:1.5:0.3 v/v/v) as mobile phase. Rf values of prodrugs and reactants were calculated and compared.

Spectral and Thermal Characterization

Pressed pellet technique was adapted for FT-IR analysis, drug admixed with KBr were made in to disc and was analyzed in spectral range of 4000 to 400 cm^{-1} and IR spectrum was recorded. $1H^1$, C^{13} NMR, Mass and DSC studies were carried out for the synthesized prodrug.

Partition Coefficient

The partition coefficient of product was determined in n-octanol/water system (10:10) by standard technique. Product (drug or prodrug) was accurately weighed (10 mg) and added to 10 ml each of n-octanol and aqueous phase. The mixture was shaken using mechanical shaker for 24 hrs until equilibrium was reached. Phases were separated by separating funnel and aqueous phase was analyzed for amount of product after appropriate dilution. Procedure was performed in triplicate.[11]

$$K_d = \frac{[\text{solute}]_o}{[\text{solute}]_{aq}} = \frac{C_o}{C_{aq}}$$

Where k_d is partition Coefficient

C_o = Concentration of solute distributed organic phase

C_{aq} = Concentration of solute distributed in aqueous phase

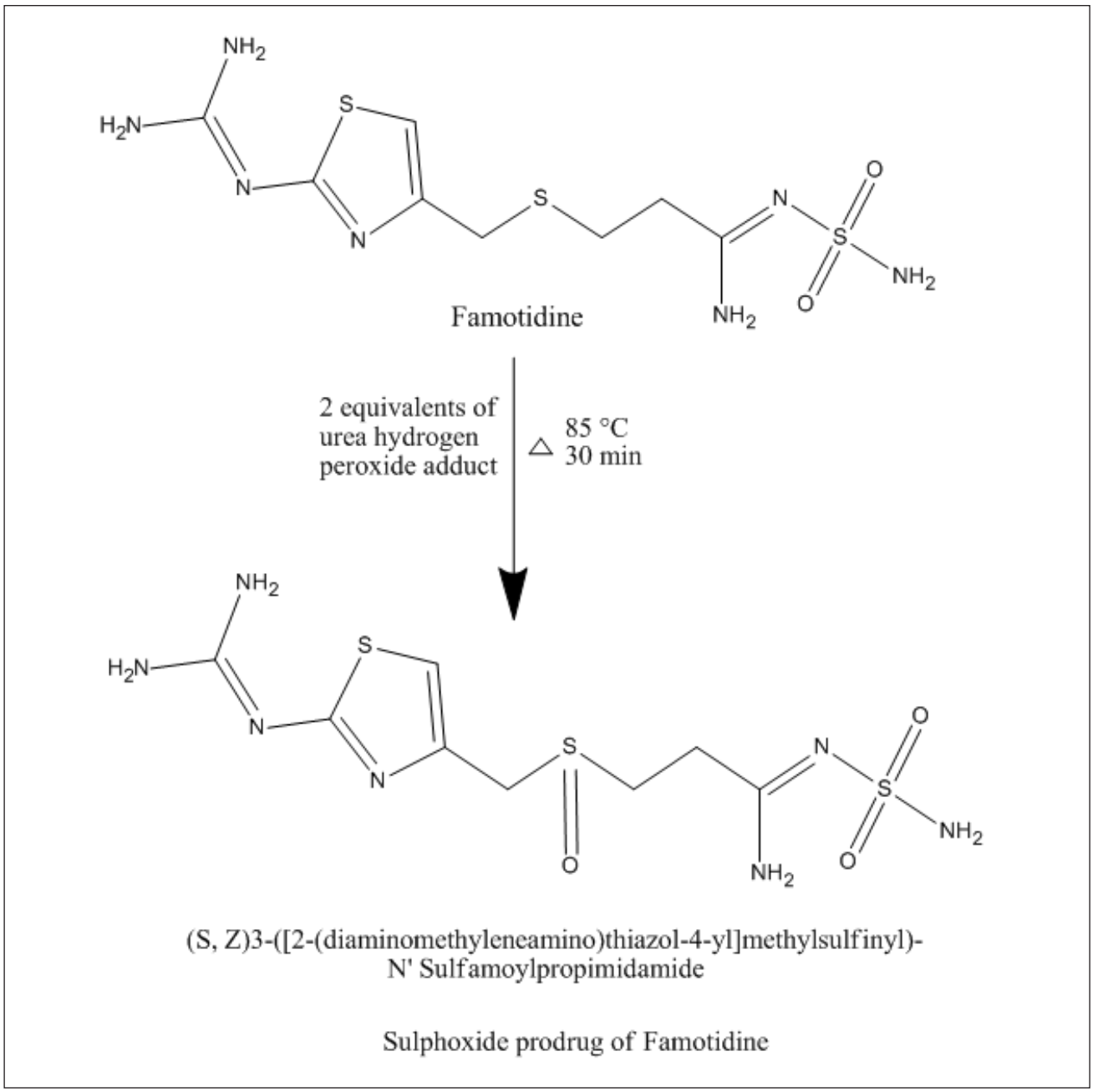

Figure 2: Chemical scheme for synthesis of sulphoxide prodrug of Famotidine

Aqueous Solubility

Equilibrium solubility was determined by a "shake-flask" method[13].The aqueous solubility of compound was determined by adding excess amount of drug beyond its saturation limit in sealed conical flask containing 10 ml of water. This conical flask is placed in a mechanical shaker for 48 hrs (This duration was previously tested to be sufficient to reach equilibrium). The solvent was filtered through Whatmann filter paper No.42 and the portion of the filtrate was suitably diluted with water. Solutions were analyzed by using UV spectrophotometer at 281 nm, which was the absorption maxima and drug concentrations were calculated.[12]

Chemical Hydrolysis Study:[13]

The rate of chemical hydrolysis of the prodrug was determined in Simulated Gastric Fluid (SGF, pH 1.2) and Simulated Intestinal Fluid (SIF, pH 7.4) at 37°C. Solution of 10 mg of synthesized prodrug was placed in dissolution basket containing 90 ml of SGF/ SIF individually. An aliquot of 15 ml of this solution was withdrawn repeatedly and kept in test tubes maintained at 37 ± 0.5°C. At a definite interval of time (0.5, 1, 2 up to 8 h), an aliquot was withdrawn to different test

tubes and was transferred to micro centrifuge tubes followed by addition of methanol to make up the volume. The tubes were placed in freezing mixture in order to arrest further hydrolysis, followed by vortexing at high speed for 5 min. After vortexing, the tubes were centrifuged at high speed 3000 rpm for 5 min. A 5 ml of clear supernatant obtained from each tube was measured on UV spectrophotometer for the amount of free drug released after the hydrolysis of prodrug in SGF and SIF at 281 nm

RESULTS AND DISCUSSION

Sulphoxide prodrug of FM was synthesized by using hydrogen peroxide-urea adduct and its percentage yield was found to be 85.48%. TLC studies have shown the Rf values of FM and its Sulphoxide prodrug to be 0.67 cm and 0.75 cm respectively, which ensures the formation of prodrug.

Photo microscopic images of FM and its Sulphoxide prodrug was observed at 45X. (Figure 3). Morphology of the synthesized Sulphoxide prodrug was different from that of sulphoxide prodrug. Elemental analysis of synthesized prodrug shows C: 27.19, H: 4.28, N: 27.74, O: 13.58, S: 27.22.

IR spectrum of sulphoxide prodrug of FM exhibits characteristic peak at 3401.8 NH stretching 1330.5 cm^{-1} asymmetric SO_2 stretching vibration, 1251.7 cm^{-1} aliphatic CN stretching , 667.2 cm^{-1} S-N stretching vibration. Shift in S-N stretch occurs in sulphoxide prodrug when compared to that of FM indicates the formation of a sulfoxide.(Figure 4, 5).

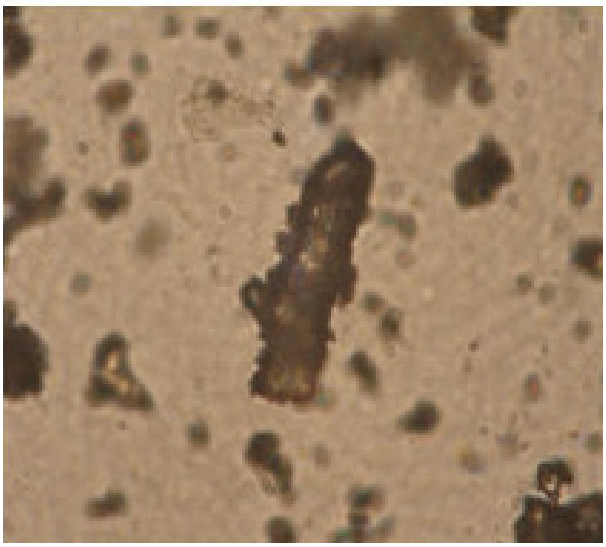

Figure 3: Photo microscopic image of famotidine at 45X magnification.

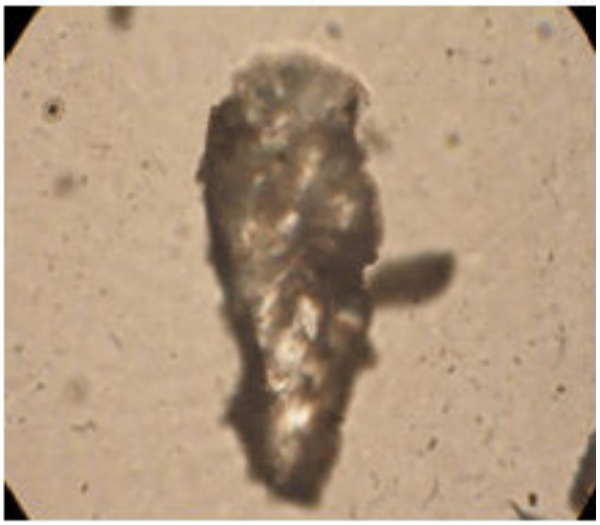

Figure 4: Photo microscopic image of sulphoxide prodrug of famotidine at 45X magnification.

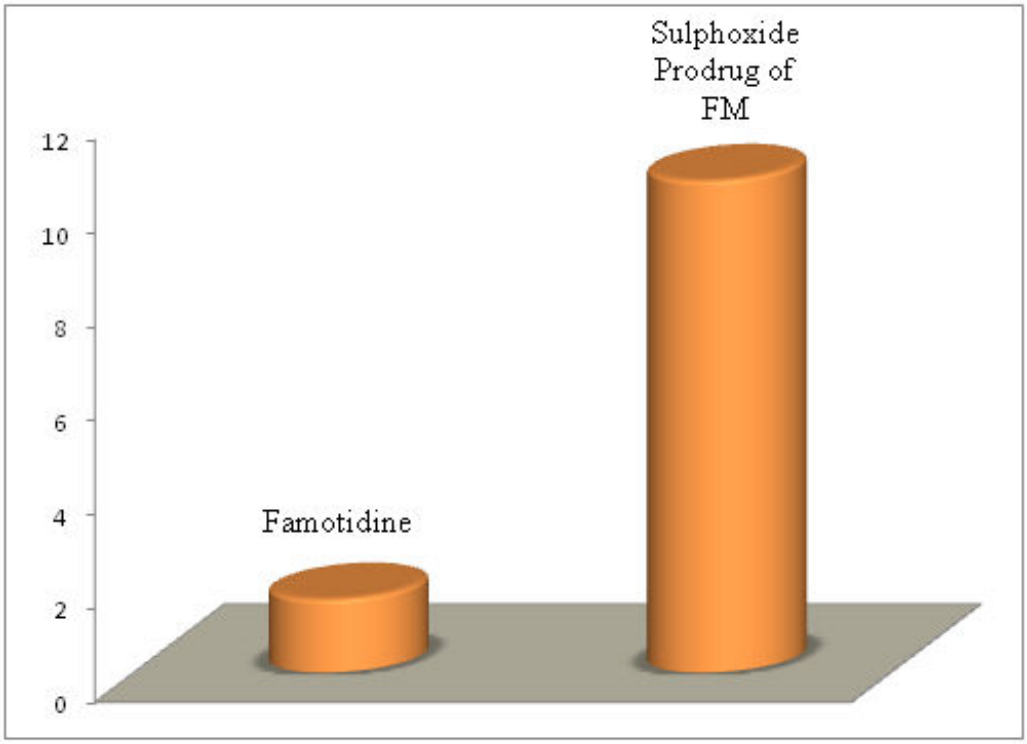

Figure 5: Aqueous solubility of famotidine and synthesized sulphoxide prodrug.

1H[1] NMR of sulphoxide prodrug of FM (DMSO) δ in ppm: 8.26 (s,1H, NH protons of sulfonamide), 7.35 (s,2H, NH protons of propionamide), 6.84 (s ,1H, Thiazole protons), 6.56 (s, 4H, NH_2 protonsof guanidine), 3.62 (s,2H, CH_2 methylene), 2.73 (t ,2H, -CH_2 protons), 2.52 (t,2H, -CH_2 protons). There is an increase in chemical shift value of methylene protons adjacent to sulphur atom in synthesized compared to FM. This down field effect may be due to deshielding nature of highly electronegative heteroatom attached to sulphur. Hence it indicates presence of oxygen atom near to sulphur group. (Figure 6, 7). C[13] NMR of Sulphoxide prodrug of FM (DMSO) δ in ppm: 33.42, 42.93, 51.76, 114.96, 156.71, 157.[8]2, 167.20 (Figure 8).

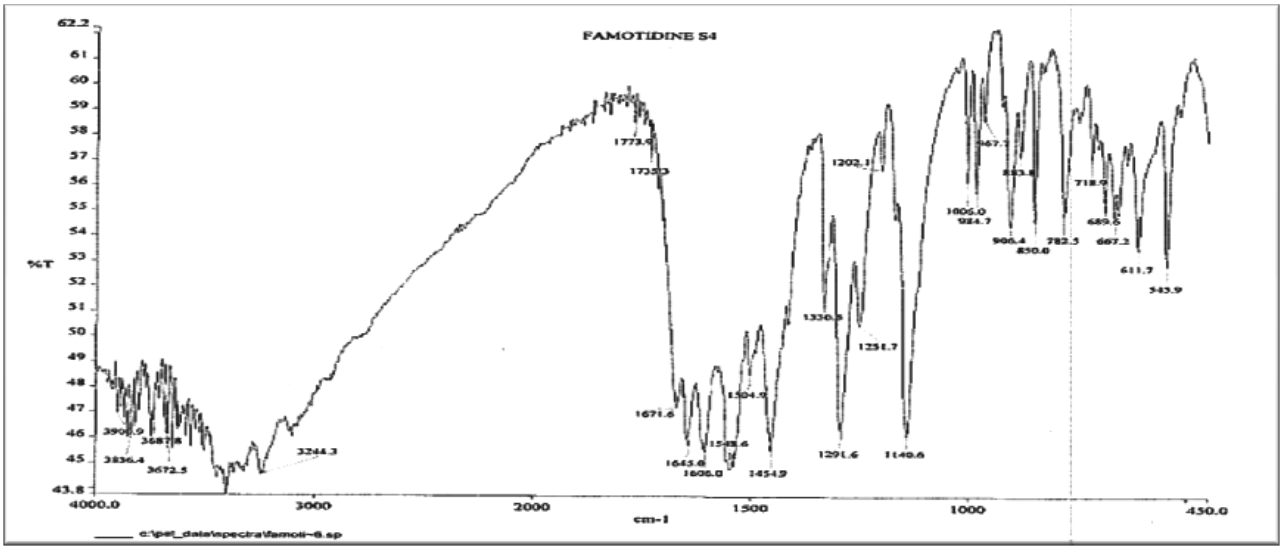

Figure 6: IR spectra of synthesized sulphoxide prodrug of famotidine.

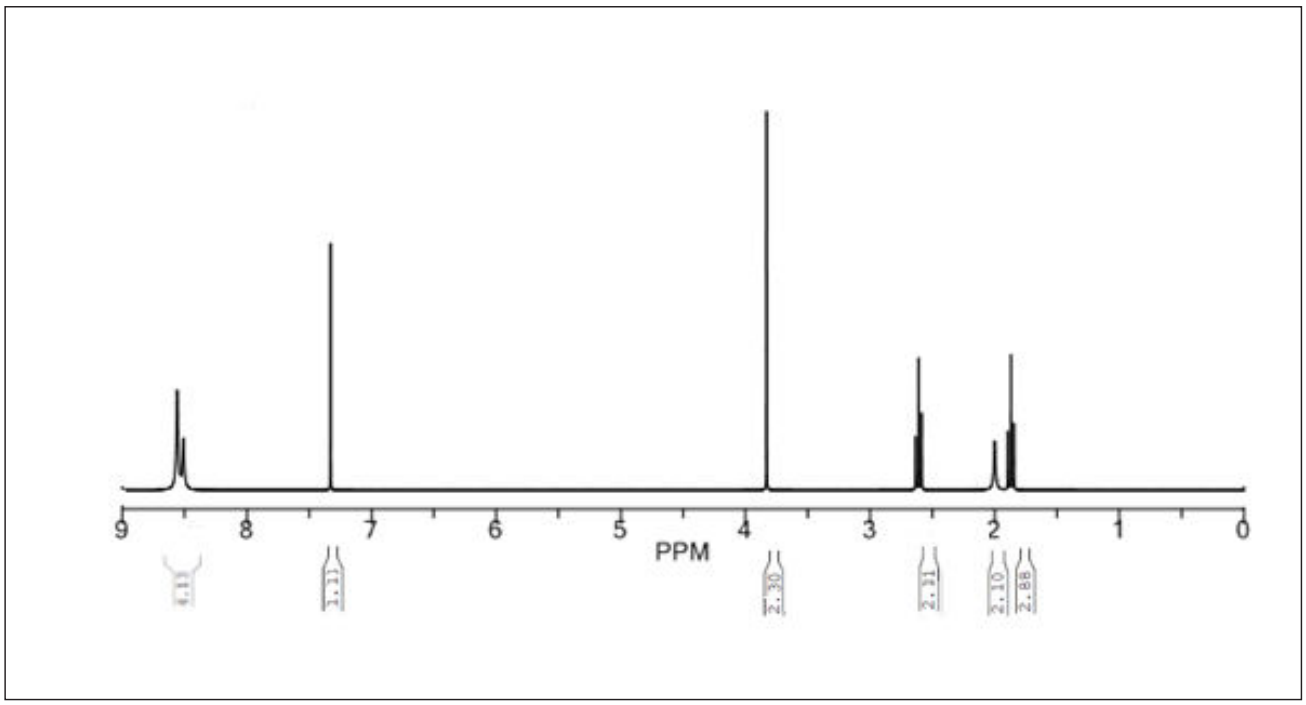

Figure 7: NMR Spectra of famotidine.

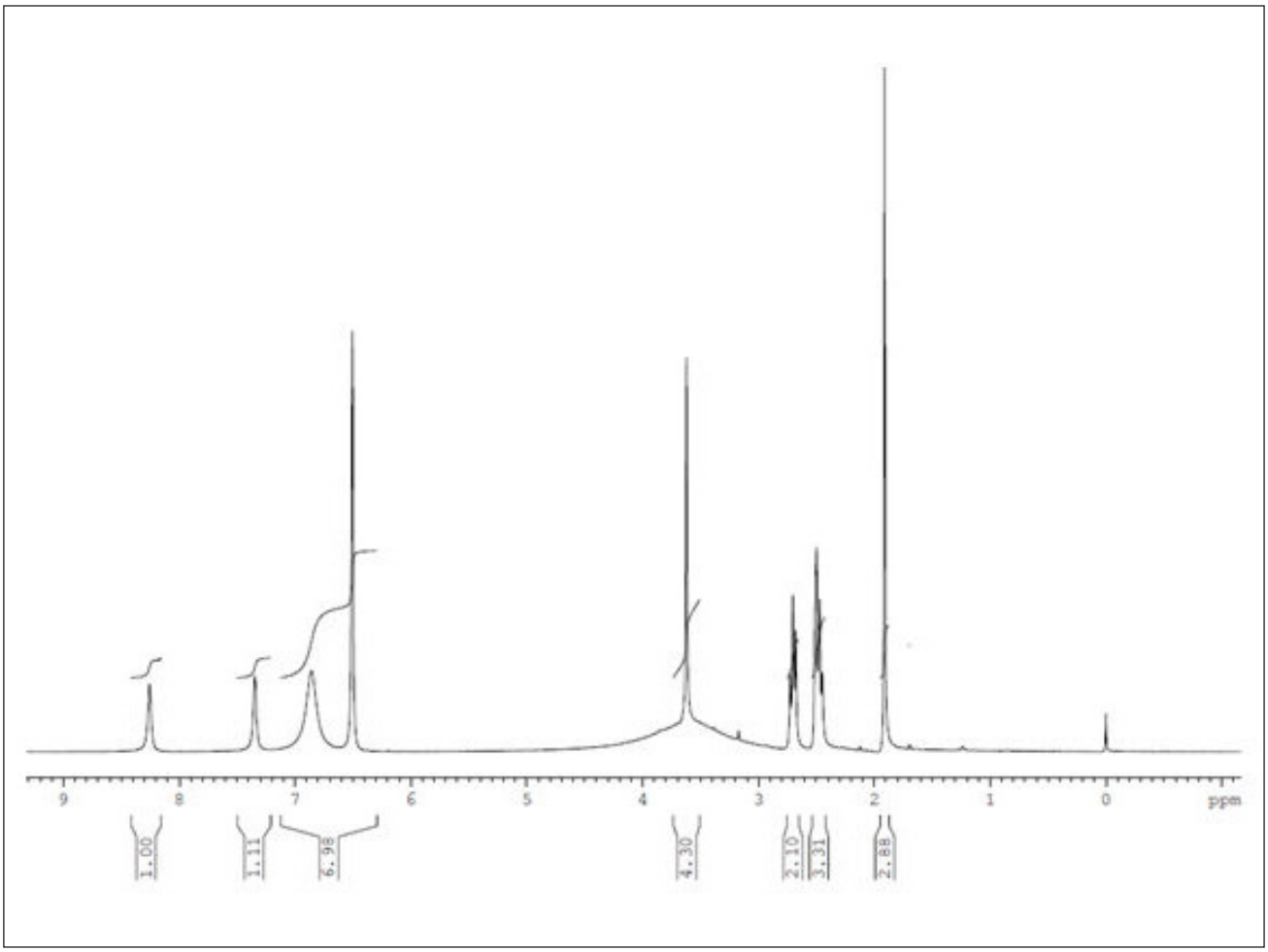

Figure 8: NMR spectra of synthesized sulphoxide prodrug of famotidine.

The mass spectrum of FM represents characteristic parent ion peak at m/z = 336 (M-1 peak, $C_8H_{15}N_7O_2S_3$) and base peak at m/z value = 80 (Figure 9). The mass spectrum of synthesized sulphoxide prodrug FM exhibits parent ion peak at m/z = 352 (M-1 peak, $C_8H_{15}N_7O_3S_3$), from parent peak, N-sulfomoylformidamide has been cleaved leaving 2-(4-(propylsulfinylmethyl-2-yl) guanidine of m/z = 247, $C_8H_{14}N_4OS_2$. Further 2-(4-(propylsulfinylmethyl-2-yl) guanidine has been fragmented into guanidine (m/z = 61, CH_5N_3, base peak) and N-ethylidienemethane sulfonamide (m/z = 121, $C_3H_7NO_2S$) (Figure 10).

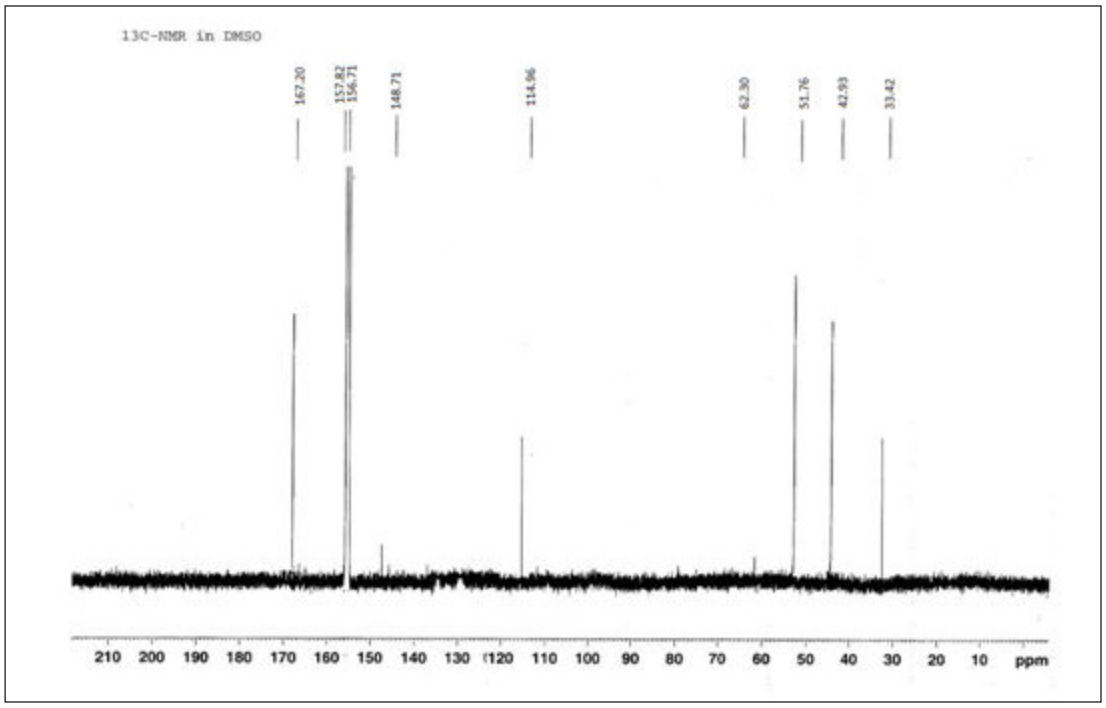

Figure 9: ^{13}C NMR of synthesized sulphoxide prodrug of Famotidine.

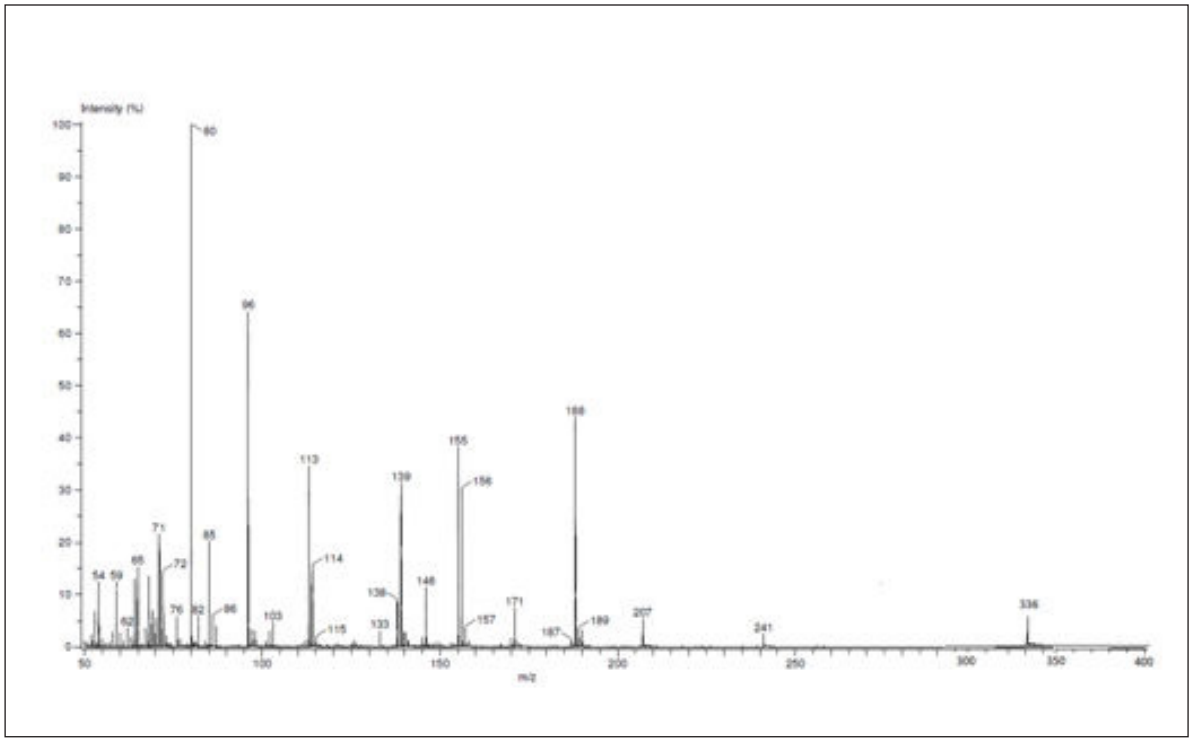

Figure 10: Mass spectra of Famotidine.

From the IR, NMR & Mass studies, the molecular structure for synthesized sulphoxide prodrug was predicted and the proposed structure was confirmed to be sulphoxide derivative of Famotidine & the molecular formula was found to be $C_8H_{15}N_7O_3S_3$.

DSC experiments were carried out to study the thermal behavior of the synthesized prodrug in relation to the individual drug. DSC study of FM shows endothermic peak at 166.4°C, while DSC study of sulphoxide prodrug shows sharp endothermic peaks at 146.34°C respectively. The sharp endothermic values of synthesized prodrug and the individual drug agreed with the measured melting range in the melting point determination. The thermal profile of synthesized prodrug was distinct, with a different melting transition from that of individual drug. This indicates the formation of novel prodrug (Figure 11, 12).

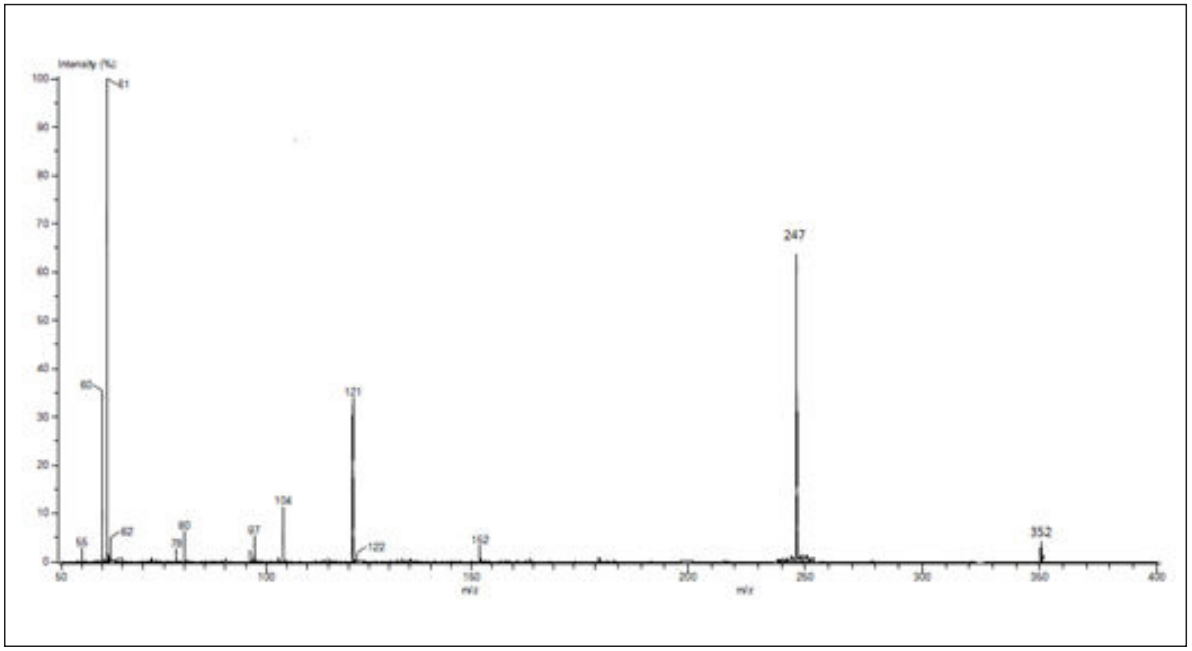

Figure 11: Mass spectra of synthesized sulphoxide prodrug of famotidine.

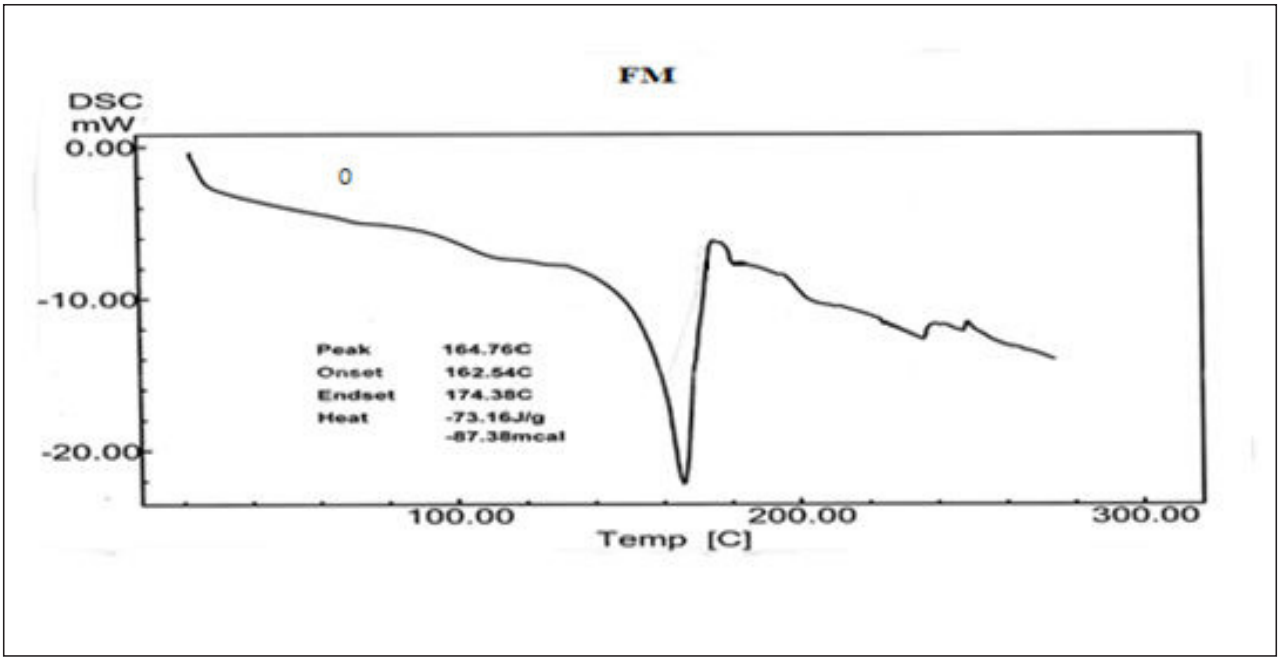

Figure 12: DSC thermogram of famotidine.

Log P value was calculated from Partition coefficient studies and was found to be -0.60 and -1.02 for drug and prodrug respectively. Decrease in log P values ensures increase in hydrophilic character of synthesized pro-drug.

Aqueous solubility of FM and sulphoxide prodrug was calculated in mg/ml and was found to be 1.565 and 10.512 for FM and prodrug of FM respectively (Figure 13).

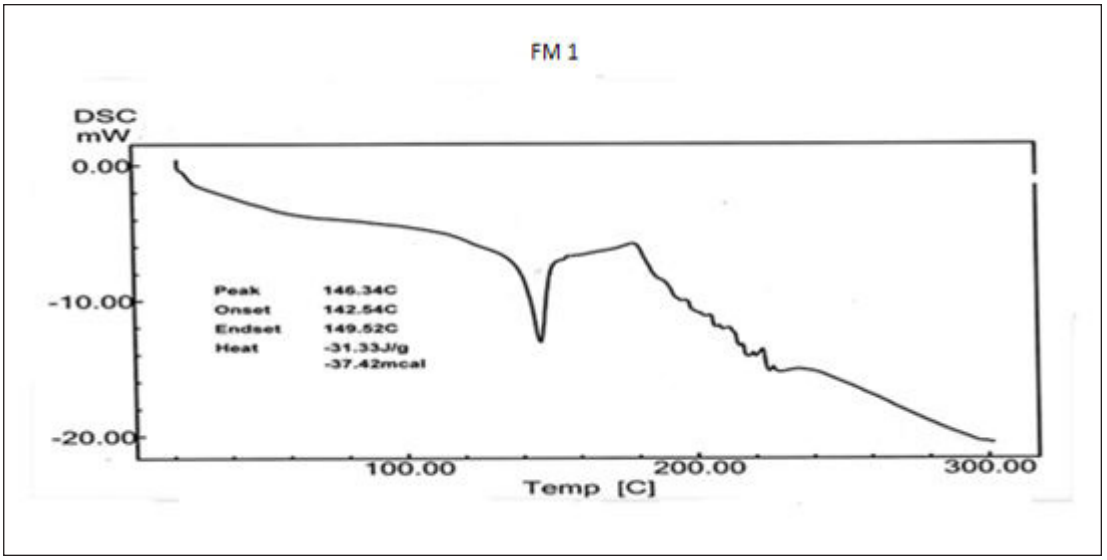

Figure 13: DSC thermogram of synthesized sulphoxide prodrug of famotidine

Chemical hydrolysis study of sulphoxide prodrug in buffer solutions(Simulated Gastric Fluid, pH 1.2 and Simulated Intestinal fluid, pH 7.4) at 37°C was performed. The Rate of hydrolysis, Hydrolysis constant and $t_{½}$ of synthesized Sulphoxide prodrug was found to be 94.77% and 90.71% at 165 min, $9.53 \times 10^{-3}$and 3.96×10^{-3}, 72 and 175 min in SGF and SIF respectively. Table 1.

Table 1: Kinetic Data for the Chemical Hydrolysis of Sulphoxide prodrug of FM

Sulphoxide prodrug	pH	Hydrolysis %					K_{obs} min^{-1}	$t_{1/2}$(min)
		1 min	15 min	45 min	105 min	165 min		
SGF	1.2	72.97	80.45	84.95	92.45	94.77	9.53×10^{-3}	72
SIF	7.4	82.16	83.13	85.79	88.54	90.71	$3.96 * 10^{-3}$	175

CONCLUSION

Novel Sulphoxide prodrug of Famotidine was synthesized by using hydrogen peroxide and urea adduct. The prepared prodrug exhibits good solubility, reasonable *in vitro* chemical stability in acidic and alkaline medium. Partition coefficient studies ensure the increase in hydrophilicity of the synthesized prodrug. These properties make the novel Sulphoxide prodrug of Famotidine effective in treating gastro intestinal problems with enhanced bio availability.

ACKNOWLEDGEMENT

The authors are thankful to Department of Science and Technology, India and IICT, Hyderabad for their financial assistance in spectral studies. They are Grateful to Dr. V. Murugesan for his aid in spectral interpretation. They are also thankful to the management of Sree Vidyanikethan College of Pharmacy, Tirupathi for providing necessary laboratory facilities to carry out the work.

CONFLICT INTEREST

Authors have no conflict of interest.

REFERENCES

1. Patel AR, Vavia PR. Preparation and Evaluation of Taste Masked Famotidine Formulation Using Drug/ β-Cyclodextrin/ Polymer Ternary Complexation Approach. AAPS Pharm Sci Tech. 2008; 9(2): 544–50.
2. Hassan MA, Suleiman MS, Najib NM. Improvement of the *in vitro* dissolution characteristics of Famotidine by inclusion in β-cyclodextrin. Int. J. Pharm. 1990; 58(1): 19–24.
3. Dobetti L. inventor; Eurand International SPA, assignee. Fast disintegrating tablets. US patent 6596311 B1*;* 2003 July 22.
4. Brown D. Orally disintegrating tablets-taste over speed. Drug Del Tech. 2003; 3(6): 58–61.
5. Seager H. Drug-delivery products and the Zydis fast-dissolving dosage form. J. Pharm. Pharmacol. 1998; 50(4): 375–82.
6. Bundgaard H. Design of prodrug. Amsterdam: Elsevier; 1985.
7. Shargel L, Susanna WC, Andrew BC. Applied Biopharmaceutics and Pharmacokinetics. 4thEd. India: Tata Mcgraw hill; 2007.
8. Lulinski P. Eco-friendly Oxidative Iodination of Various Arenes with a Urea-Hydrogen Peroxide Adduct (UHP) as the Oxidant. Synthesis. 2004; 3: 441–5.
9. Iran S, Abdolreza R, Samira KA. Novel tridentate Schiff base dioxo-molybdenum (VI) complex: Synthesis, crystal structure and catalytic performance in green Oxidation of sulfides by urea hydrogen peroxide. Polyhedron. 2009; 28(4): 733–8.
10. Rajender SV, Kannan PN. The Urea-Hydrogen Peroxide Complex: Solid-State Oxidative Protocols for Hydroxylated Aldehydes and Ketones (Dakin Reaction). Nitriles, Sulfides, and Nitrogen Heterocycles. Org. lett. 1999; 1(2): 189–91.
11. OECD. Guidelines for the Testing of Chemicals. Partition Coefficients; 1981.
12. Baka E, Comer JEA, Takacs-Novak K. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as a model compound. J. Pharm. and Biomed. Anal. 2008; 46(2): 335–41.
13. Mahdi MF, Alsaad HN. Design, Synthesis and Hydrolytic Behavior of Mutual prodrugs of NSAIDs with Gabapentin Using Glycol Spacers. Pharmaceuticals. 2012; 5(10): 1080–91.