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RESEARCH ARTICLE

Spectrophotometric determination of famotidine using benzoin as derivatizing agent

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Abstract

A simple and sensitive spectrophotometric procedure has been developed for the determination of famotidine, based on derivatization with benzoin. The reaction was monitored at 304.5 nm. The effect of pH, derivatizing reagent concentration, reaction time and temperature were examined and optimized. The reaction was repeatable with relative standard deviation (RSD) ± 0.14 % (n=5).The Beer's Law was obeyed within concentration range of 2.50-12.5 µg/ml. The reaction was selective and a number of pharmaceutical additives, amino acids and matrix of deprotonized serum and urine of healthy volunteer did not affect the determination with relative error within ± 2.36 %. Four pharmaceutical preparation of famotidine were analyzed and percentage recovery were within 94.0-97.50 % and RSD for analysis were within 0.08-0.41 %.The recovery of famotidine from deprotenized serum and urine was 98.4-98.6 % and 96.9-98.0 % within RSD 0.08-0.16 %..

1. Introduction:

Famotidine,(3-(((2-((aminoiminomethyl)amino)-4-thiazoyl)methyl)thio)-N-(aminosulfonyl) propanimidamide) (Fig.1) is a potent competitive and reversible inhibitor of histamine action at H2 receptors[(L. Zhang, et al; 1998). Famotidine is used in many countries for the treatment and maintance therapy of active duodenal ulcer and gustric ulcer. It is also indicated for the treatment of heartburn, acid indigestion and sour stomach due to consuming food and beverages which are known to causes these symptoms (L. Zhang, et al; 1998). Famotidine is readily, but incompletely absorbed from gastrointestinal tract with peak concentration in plasma occurring about 2 h after administration by mouth (D.M.Comoli-Richards and S.P.Clissold, 1986).A small portion of the drug is metabolized in liver, but mostly of it is execrated in urine. The famotidine is recommended to the patients 40 mg daily as theraptic doze (Z.Zarghi, et al; 2005)

Several analytical procedures have been developed for the determination of famotidine from pharmaceutical preparations and biological fluids. The analytical procedures are mainly based on spectrophotometry (A. Abu Zahi,et al; 1999,K. Kelani, et al; 2002, M. I. Walash, et al; 2009),spectroflurimetery (M. I. Walash,et al; 2009), potentiometry (M.M. Ayad et al; 2002) ,HPLC (Z.Zarghi et al; 2005,J. Novakovic, 1999, H. Haurer,1990, B. Carker,et al;1997, M. A. Campanero,et al; 2001, T. C. Dowling and R. F. Frye,1999), flow injection analysis(N.Helali,et al; 2005, M. Walash et al; 2005)Capillary zone electrophoresis (N. Helali,et al; 2008) and gas chromatography (S. A. Majedano and M. Y. Khuhawar; 2012)

Among these procedures HPLC is more frequently reported for the analysis of famotidine from the drugs and biological fluids. Some of these methods involve solid phase extraction for pre concentration and removal of interferences (L. Zhang et al; 1998, S. Wanwimolruk, et al; 1991) and others require solution extraction procedure before reverse phase HPLC(T. C. Dowling and R. F. Frye;1999)

Spectrophotometric methods are simple and involve less expensive equipment and adequate sensitivity and selectivity is achieved by using suitable derivatizing reagent. The spectrophotometric methods are based on the measurement of natural absorbance at 286 nm (N. Narendraet al; 2012) or after derivatization with benzoquinone S.A.K.Ahmed et al;1999, p-chloroanilic acid (H.A.Muhammad and Bull ; 2000)chloranil, 2,3-dichloro-5,6-dicyano-

1,4-benzoquinine dichloronitrophenol(B. V. Kamathet al; 1992) and ninhydrin (N.Rahman et al; 2003).The spectrophotometric procedures are also described based on the extraction of ion pair complex between famotidine and each of bromocesol green and bromothymol blue (Z.A.Z.Ali,Set,al 1999,S.M.Blaih et al;1994)

The molecular structure of famotidine is characterized by the presence of guanidine group and the compounds containing guanide groups are reported to react with benzoin to form 2-substituted amino-4-5-diphenyl-imidzole (M. Kai,et.al;1983a, M. Kai,et.al;1984b), and this initiated the study. The proposed method based on derivatization with benzoin is simple and sensitive for determination of famotidine in pharmaceutical preparations, spiked and real human plasma and urine.

2. Material and Methods:

2.1: Chemical and solutions

All the chemicals used were of reagent grade; freshly prepared deionized distilled water was used throughout the study. Famotidine (Sigma, St. Louis, MO, USA) was used. The stock solution (1 mg/mL) of drug was prepared by dissolving 100 mg of drug in methanol (30 ml) and further dilution to 100 ml was carried out with deionized water. Methanol (RDH chemical Co. Spring valley, USA) and benzoin (Fluka,Buchs, Switzerland) were used. Guaranteed reagent grade hydrochloric acid 37%,potassium chloride, acetic acid, sodium acetate, ammonium acetate, sodium tetraborate, boric acid, sodium bicarbonate, sodium carbonate, ammonium chloride, ammonia solution and sodium hydroxide were from E-Merck Darmstadt, Germany. Buffer solution (0.1 M) between pH 1 and 11 at 0.5 unit interval were prepared from the following; potassium chloride adjusted with hydrochloric acid (pH 1-2), acetic acid-sodium acetate (pH 3-6), ammonium acetate(pH 7), boric acid –sodium tetraborate (pH 7.5-8.5), sodium bicarbonate- sodium carbonate (pH 9),ammonium chloride-ammonia solution (pH 10), sodium chloride-sodium hydroxide (pH 11). Benzoin solution (4.0 mmol) was prepared by dissolving 85 mg benzoin in 100 ml of methanol.

2.2: Equipment

The pH measurements were made with an Orian 420 A pH meter (Orion Research inc. Boston, USA) with combined glass electrode and reference internal electrode. Spectrophotometric studies were carried out with double beam Perkin Elmer Lambda 35 (Perkin Elmer,Singhpour), UV and Visible spectrophotometer. The spectrophotometer was controlled by computer with lambda 35 software.The measurements were made with dual 1 cm quartz cuvettes.

2.3: Spectrophotometric Procedure

An aliquot of solution (0.5-1.0 ml) containing 12.5-62.5 µg of famotidine was transformed to 5 ml volumetric flask and was added 0.6 ml benzoin solution (4.0mmole in methanol w/v), 0.5 ml potassium hydroxide (2.0 mole), 0.5 ml sodium thiosulphate (0.2 mole)and 0.6 ml 2-mercapthanol (80 mmol) and the contents were heated at 70^{o} C for 10 min. The reaction matrix was allowed to cool at room temperature and 0.5 mlof borate buffer pH 8.5 was then added and volume was made to the mark with methanol. The absorption spectrum of the solution was measured against reagent blank within 400-270 nm.

2.4: Analysis of Famotidine in Pharmaceutical Preparation

At least five tablets of each Ulfam (Focus &Rulz PharmaceuticalsPvt. Ltd., Islamabad, Pakistan), Femme (Batala Pharmaceuticals, Gujranwala, Pakistan), Famotin (Efroze, Korangi Industrial Area, Karachi , Pakistan) and Facid (Matador Chemicals,Lahore, Pakistan) with labeled amount of 40 mg/tablet were weighed and crushed to fine powder. The powder corresponding to one tablet was weighed and dissolved in methanol water (1:1 v/v). The solution was filtered and final volume and adjusted to 100 ml. The solution (5 ml) was further diluted to 25 ml and the solution (1 ml) was taken and analyzed following spectrophotometric procedure. The quantitation was made from the linear calibration curve prepared from standard famotidine solution using regression equation based on $y = ax + b$.

2.5: Analysis of Famotidine in Pharmaceutical Preparation using Standard Addition:

Four pharmaceutical preparations Ulfam, Femme, Famotin and Facid containing famotidine were analyzedby spectrophotometer.5 tablets from each of the pharmaceutical preparations were weighed and ground to fine powder. The drug in the pharmaceutical preparations were also examined by the standard addition technique. Three different concentrations solutions from 25 µg/mlwere taken and analyzed by spectrophotometric procedure. The quantitation was made from external calibration curve and from an increase in the absorbance with added amount of famotidine.

2.6: Analysis of Famotidine from Spiked Serum Samples:

The blood sample (5 ml) was collected from healthy volunteers who has not taken any medicine at least onepreceding week.The blood sample was allowed at room temperature for one hour and centrifuged at 3000 g for

20 min. The supernatant layer was collected and was added 5 ml of methanol. The mixed contents were again centrifuged at 3000 g for 15 min. The clear supernatant layer was collected .The aliquot of solution (1 ml) of deprtonized serum was taken and added famotidine 8.5 µg/ml and 11.5 µg/ml and spectrophotometric procedure was followed. The quantitation was made from external calibration curve.

2.7:**Analysis of Famotidine from Spiked urine samples:**

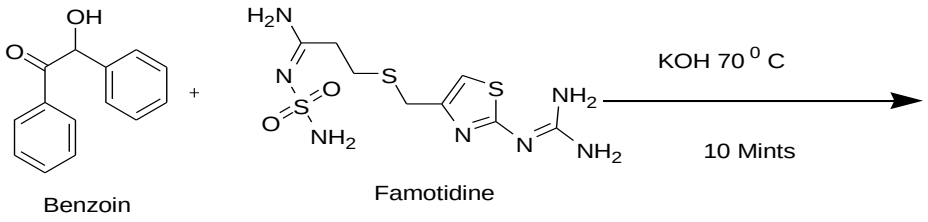
Urine (5 ml) from the morning urine samples collected from healthy volunteers who has not taken any medicine at least one preceding week was added methanol(5 ml).The content were mixed well and centrifuged at 3000 g for 20 min. The clearsupernatant layer was collected. Three aliquot of deprotinized urine samples were added 8.5 µg/ml and 11.5µg/ml of famotidine and spectrophotometric procedure was followed. The quantitation was made from external calibration curve.

The blood and urine samples from healthy volunteers (employee and students of Institute of Advance Research Studies in Chemical Sciences University of Sindh Jamshoro Pakistan) were collected. The blood samples were collected in EDTA tubes by vein puncture with a disposable syringe. Morning urine samples of healthy volunteers were collected in clean plastic bottles. The volunteers taking part in the study were informed about the aim and objectives of the study and verbal consent to participate in the project was obtained.

3: Result and Discussion:

Famotidine is commonly used drug and a number pharmaceutical companies sell their products containing famotidine with different names. Thus for quality assurance the analysis of pharmaceutical preparation for famotidine contents is paramount. Spectrophotometric methods are simpler and equipment is available in most of analytical laboratories. Famotidine give maximum absorbance at 285 nm with molar absorptivity of $7.2x10^2$ $Lmol^{-1}cm^{-1}$,butafter derivatization it absorbs maximally at 304.5 nm (Fig.4) with molar absorptivity of $4.900x10^4$ $Lmol^{-1}$ cm^{-1}.Thus for sensitive and selective determination, the derivatization reaction with benzoin was examined. The benzoin reacts with famotidine is alkaline medium in the presence of mercaptoethnol and sodium thiosulphate to form the imidazole derivative. The pH of the derivative was then adjusted to give maximum absorbance. The derivatization condition and spectrophotometric determination were therefore examined and optimized. The effect of the addition of the amounts of derivatizing reagent, potassium hydroxide, sodium thiosuphate, mercaptoethanol and warming time and temperature were examined, followed by the addition of buffer solution to obtain maximum absorbance. The addition of derivatizing reagent (4 mmole) was varied from 0.2 to 1.0 ml at interval of 0.1 ml and maximum absorbance was observed with 0.6 ml and above and was used (Fig.1). The addition of KOH (2M) was varied from 0.2-1.0 ml at an interval of 0.1 ml and maximum absorbance was observed with 0.5 ml and was selected. The volume of mercaptoethanol (8 mmole) was varied 0.2-1.0 ml with an interval of 0.1 ml and maximum absorbance was observed with 0.6 ml and was selected. Similarly the volume of sodium thiosulphate (0.2M) was from 0.2 - 1.0 ml at an interval of 0.1 ml and maximum absorbance was noted with 0.4 ml and was selected. The effect of warming temperature was varied from 50-90 ^{0}C at an interval of 10 ^{o}C and better absorbance was observed at 70 ^{0}C and was selected. Similarly warming time was varied from 5-20 min. at interval of 5 min. and maximum absorbance was obtained at warming time of 10 min. and was selected. After the formation of derivatives the addition of 0.5 ml of buffer solution was varied between pH 1-10 initially at unit interval, and then at 0.5 unit interval. Maximum absorbance was noted with the addition of borate buffer pH 8.5 (Fig.3) and was selected. The derivative once formed was highly stable and did not show any change in absorbance up to 16 h. using the optimized conditions. The derivatization and absorbance at 305.5 nm was repeatable (n=5) with relative standard deviation (RSD) 0.14 %.

Fig.1



Benzoin

Famotidine

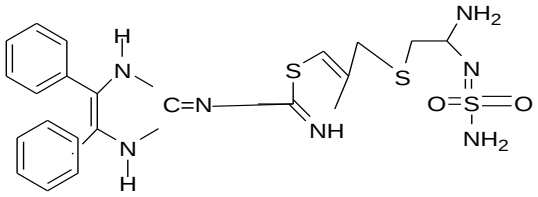


Fig.2 Effect of concentration of Reagent Benzoin

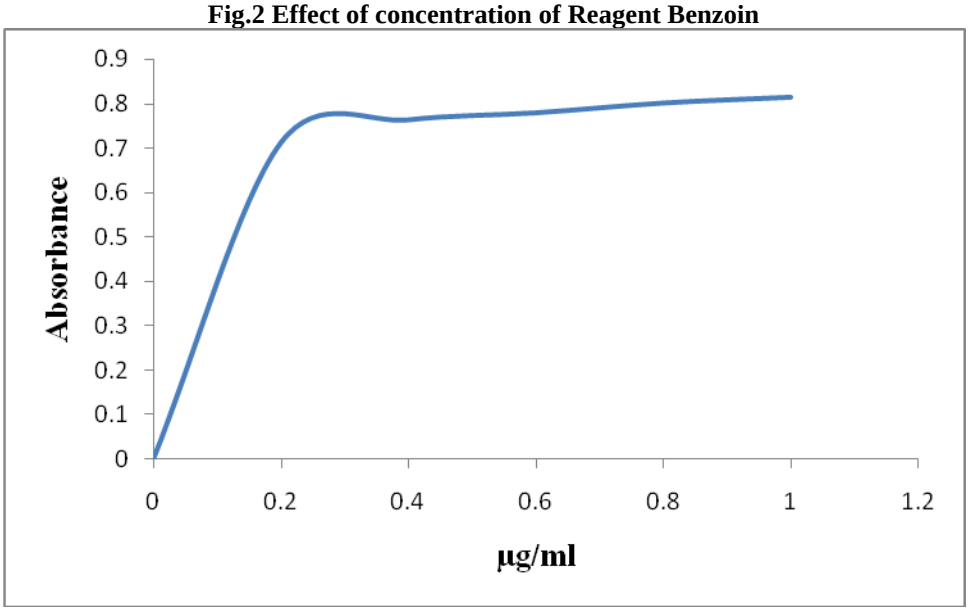


Fig.3. Effect of pH

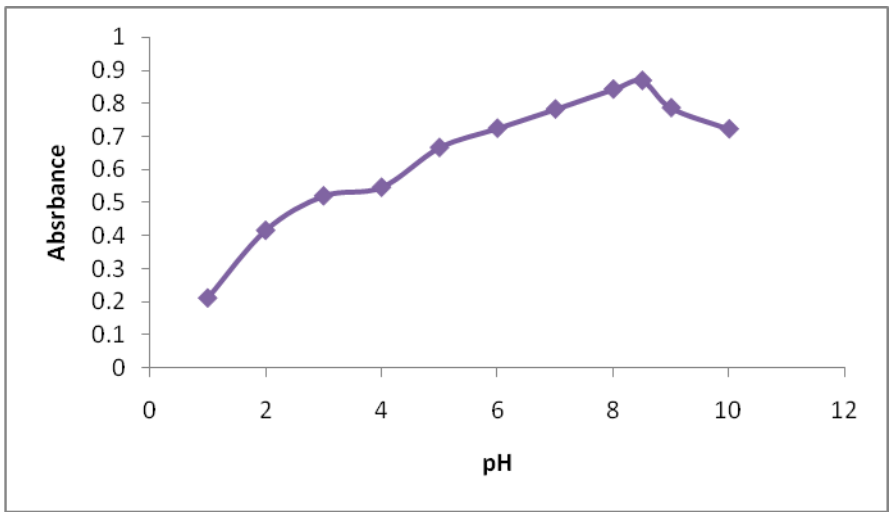


Fig: 8 Absorption Spectrum of the Derivative

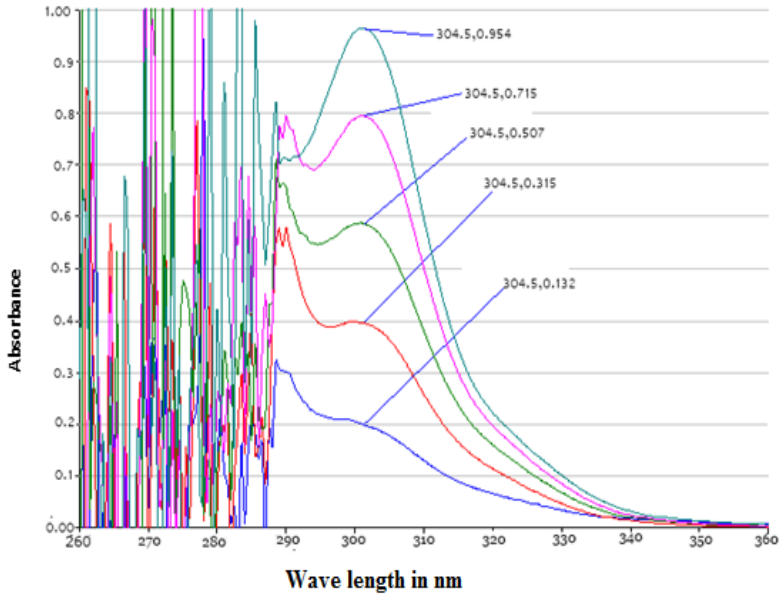


Table 1: Regression and Optical characteristics of derivative	
Parameters	Values
Wavelength of Derivative	304.50 nm
Beer's law range	2.50-12.50 µg/ml
Coefficient of correlation	0.995
Slope	0.074
Intercept	0.034
Molar absorpitivity	$4.900x10^4$ L mol^{-1} cm^{-1}

Table: 2 Drug Analysis for Famotidine from pharmaceutical Preparations.

S.No	Name of product	Compounds contain	Labelled amount mg/tablet	Amount found mg/tablet	CV n=4	%age Error
1	Ulfam	Famotidine	40 40	37.96 39.00	0.414 0.081	5.1 2.5
2	Fermim	Famotidine	40 40	38.50 38.80	0.414 0.271	3.75 3
3	Famotin	Famotidine	40	38.60	0.129	3.5
4	Facid	Famotidine	40	37.5	0.414	6.25

Table 3: Recovery of Famotidine from Spiked Serum and Urine:

Samples	Conc.µg/ml Added	Found conc. µg/ml (C.V) n=4	Percentage Recovery
Serum	8.50	8.17(0.11)	96.40
	11.50	11.31 (0.12)	98.60
Urine	8.50	8.16 (0.16)	96.20
	11.50	11.22(0.08)	97.60

Table: 4 Analysis of intra-day and inter day variation of Famotidine solution

S.No	Amount taken µg/ml	Intra-day		Inter-day	
		Absorbance	CV	Absorbance	CV
1	2.5	0.133	0.970	0.131	2.240
2	5	0.314	0.303	0.313	0.840
3	7.5	0.506	0.160	0.507	0.530

The effect of change in concentration of famotidine as derivative of the benzoin verses absorbance was plotted and a linear relationship was observed which obeyed, the Beer's law within the concentration 1.25-12.5 µg/ml with coefficient of determination (r^2) 0.995 and molar absorptivity of $4.9x10^4$ $L.mole^{-1}cm^{-1}$(Table.1)

The effect of drug additives, amines and amino acids for the possible interfering effects on the determination of the famotidine was examined. The additives were examined at the same concentration and at ten times the concentration of famotidine. The compounds examined were starch, glactose,lactose, talc, magnesium stearate, sucrose,gum acacia gelatin, calcium stearate, methylparabenpropylparaben,sodium chloride, sodium lauryl sulfate (SLS) hydroxyethyl cellulose, histamine, glycine and vitamin C and the compounds did not interfere with relative error within ±2.36 %.

The repeatability of the derivatization and determination intera (n=5) and inter day were examined by the same operation on the same and different days and RSD observed were within 0.16 - 0.97 % and 0.53-2.24%

respectively (Table.4). Test solutions of famotidine (n= 4) with in calibration range were analyzed and relative error was obtained within ± 2.4 %.

Five pharmaceutical preparations each Ulfam, Fermim, Famotinand Facid containing with labeled amount of 40 mg/ tablets were analyzed after extraction of the active ingrident in methanol-water. The amounts found were within 37.5-39.0 mg/tablet with RSD within 0.08-0.41%.The relative error from labeled value was observed within 2.5-6.25 % (Table.2). The pharmaceutical preparations were also analyzed by standard addition method and results agreed with direct calibration with the recovery within 99.2-99.70 %.

Deproteinized serum and urine samples of healthy volunteers were spiked with standard solution of famotidine and analysis was carried out for spectrophotometric procedure. The amount of famotidine recovered from the serum and urine were calculated within 96.4-98.6% and 96.20-97.60 % respectively with RSD within 0.08-0.16 % (Table.3).The results indicate that the matrix of deproteinizedserum and urine did not interfere the determination of the drug. The results have been achieved because of selectivity of reaction of derivatizing reagent benzoin with the analyte.

5:Conclusion:

Analytical procedure has been developed for the determination of famotidine from pharmaceutical preparation and spiked deprotinized serum and urine after derivatization with benzoin. The reaction was selective and a number of pharmaceutical additives, amines and amino acids did not interfere in the determination.

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