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HPLC Determination of Metformin, Famotidine and Ranitidine by Derivatization with Benzoin from Drugs and Biological Samples

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Abstract

A novel High Performance Liquid Chromatography (HPLC) method has been developed based on pre column derivatization with benzoin for determination of metformin, famotidine and ranitidine. The separation was achieved from C18 column when eluted isocratically, the solution of the drugs with methanol, water, acetonitrile and Tetra Hydrofuran (THF) (40:40:16:4 v/v) with a flow rate at 1 mL/min. UV detection was found to be 268 nm. Linear calibrations range was obtained with 2.5-12.5 μ g/ml, with limits of detection (LODs) 0.091-0.30 μ g/ml. The total run time for elution was 3.5 min. The derivitization, separation and quantitation was repeatable (n=4) in terms of retention time and peak height/peak area with relative standard deviation (RSD) within 0.84-1.55% and 0.68-1.17% respectively.

The method was applied for the analysis of metformin, famotidine and ranitidine from pharmaceutical preparations, human serum and human urine. The possible interfering effects of the sample matrix were checked by the analysis by standard addition method and matrix effect was not indicated.

Keywords: High Performance Liquid Chromatography (HPLC); Metformin; Famotidine; Ranitidine; Derivatization; Benzoin

Introduction

The guanidine compounds are characterized by the presence of guanidine functional group. These are compounds with a carbon surrounded by three amino functions. The hydrogen atom of the amino function may be substituted [1]. These are basic compounds and many of these are present in biological fluids and in tissues at low concentration in human and animals [2]. Metformin (N,N-dimethylguanidine) (Figure 1) is used as an oral antihyperglycemic drug in the management of non- insulin dependent diabetes mellitus [3,4]. However high concentration of metformin in plasma has been associated with an increase in the incidence of lactic acidosis, particularly in patient in acute renal failures [5,6]. It is slowly and incompletely absorbed from gastrointestinal tract and in excreted unchanged in urine [7].

Famotidine, (3-(((2-((aminoiminomethyl)amino)-4-thiazoyl)methyl)thio)-N-(aminosulfonyl)propanimidamide) (Figure 1) is a potent competitive and reversible inhibitor of histamine action at H2 receptors and is used for the treatment of active duodenal ulcer, gastric ulcer, heartburn, acid indigestion, sour stomach and Zollinger-Ellison syndrome [8,9]. The famotidine is commonly given to the patients [10].

Ranitidine HCl, N-(2-[(5-(dimethylaminomethyl)furan-2-yl) methylthio]ethyl)-N-methyl-2-nitroethene-1,1-diamine HCl (RAN) (Figure 1) is an inhibitor of gastric acid secretion that is used in the treatment of gastric and duodenal ulcers. It competitively inhibits the action of histamine on the H2 receptors of parietal cells [11]. There are many analytical methods that have been reported for the analyses of

these important drugs. Metformin has been determined by spectrophotometer and High Performance Liquid Chromatography (HPLC) [12-19]. Famotidine by spectrophotometry, spectrofluorimetry, voltammetric, potentiometric, flow injection analysis, HPLC and Capillary Zone Electrophoresis (CZE). Ranitidine by spectrophotometry, polarography Cyclic Voltammetry (CV), potentiometric sensors, the flow injection analysis. HPLC, CZE and Gas Chromatography (GC) [20-45]. The physicians prescribe famotidine or ranitidine together with the metformin for the treatment of duodenal ulcer to the diabetic patients. Kai et al. [45] reported that gunidino compounds react with benzoin to give single product 2substituted amino-4,5-diphemyl imidazole ring. Metformin and famotidine are pharmaceutical products containing gunadino functional group and were examined for pre column derivatization with benzoin followed by HPLC separation and UV detection. Ohta et al. [46] reported HPLC determination of metformin using benzoin as derivatizing agent. Alamgir et al. reported spectrophotometric and spectroflurometric procedures for the determination of famotidine in pharmaceutical preparation and biological fluids by derivatization with benzoin from drug and biological samples [21,22]. Ranitidine does not contain typical guandino functional group (A carbon atom surrounded by three amino functions) but contain a carbon attached to the two amino functions. The ranitidine react with benzoin to form derivative. The present work examines the reaction of ranitidine with benzoin for the simultaneous HPLC separation and determination of metformin, famotidine and ranitidine by pre column derivatization with benzoin from drugs and biological samples.





Figure 1: Structural diagrams of Metformin, Famotidine and Ranitidine.

Experimental

Chemicals and solutions

All the chemicals used were of G.R or analytical grade, freshly prepared deionized distilled water was used throughout the study. Metformin, famotidine and ranitidine (Sigma, Buchs, Switzerland), benzoin (Fluka, Buchs, Switzerland), and methanol (RDH Chemical Spring Valley, CA, USA) were used. The stock solution (1 mg/ml) was prepared by dissolving appropriate amount of drug in methanol and further dilution were carried out in deionized water. G.R grade Hydrochloric Acid (HCl 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 purchased from E-Merck Darmstadt, Germany. Buffer solution (0.1 M) between pH 1 and 10 at unit interval were prepared from the following; hydrochloric acidpotassium chloride (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).

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 study was carried out with double beam Perkin Elmer Lambda 35 (Perkin Elmer, Singapore), spectrophotometer with dual 1 cm silica cuvettes. The spectrophotometer was controlled by computer with Lambda 35 software. Mass spectrometry of ranitidine–benzoin derivative was recorded at HEJ Research Institute of Chemistry on Jeol IMS 600 mass spectrometer. Chromatographic studies were carried out on an Agilent HPLC system 1100 series (Agilent Technology USA) equipped with G1300A LC pump, G1315B diode array detector and 7725 Rhedyne injector. Data was obtained by the computer with Chemstation data acquisation software. Reverse phase LC was performed isocratically at room temperature using column Phenomenex C18, 5 μ m (150 × 4.6 mm id) throughout the study.

Preparation of ranitidine-benzoin derivative

Ranitidine (0.001 M) dissolved in methanol (10 ml) was added benzoin (0.001 M) dissolved in methanol (15 ml). A few drops of potassium hydroxide (2 M) were then added and content were reflexed for 30 min. Half of the solvent was distilled off and remaining solution was cooled at 4°C overnight. The precipitate obtained was filtered and recrystallized from methanol (Melting point 248 \pm 2°C). Mass spectrum shows M+ relative intensity 525 (1%).

Spectrophotometric procedure

In 5 ml volumetric flask was transferred solution containing 0.5-2.5 ml of 25 μ g/ml metformin, famotidine or ranitidine separately. Each was added 0.6 ml benzoin solution (8 mmole), 0.5 ml (2 M) potassium hydroxide, 0.5 ml of 2-merecaptoethanol (8 mmole) and 0.5 ml (0.2 mmol) sodium thiosulphate. The content were heated at water bath at 70-80°C for 10 min. and allowed to cool at room temperature (30°C). Then added sodium tetraborate buffer 1 mL (0.1 M) pH 8.5. The volume was made to 5 mL with methanol. The absorption spectra of the solutions were recorded against reagent blank within 450-200 nm.

HPLC procedure

An aqueous solution containing metformin, famotidine and ranitidine within the final concentration range from 2.5-12.0 μ g/ml each was placed in 5 mL volumetric flask and spectrophotometric procedure was followed. The solution 20 μ L was injected on column Phenomenex C18, 5 μ m (150 × 4.6 mm id) and eluted isocratically with methanol-water- acetonitrile and THF(40: 40: 16: 4 v/v) mobile phase with a flow rate of 1 mL min-1. UV detection was at 268 nm.

Analysis of metformin, famotidine and ranitidine in pharmaceutical preparations

Five tablets from each for Metformin tablets Glucophage (Merck, Pvt., Ltd. Quetta, Pakistan), Metphage ((Efroze Chemical Industries (Pvt.) Ltd Karachi, Pakistan). Neodipar (Sanofi Korangi Industrial Area Karachi-74900) collected from local market (Hyderabad, Sindh) were weighed and ground to fine powder. Similarly five tablets of each Ulfam (Focus & Rulz Pharmaceuticals Pvt. Ltd., Islamabad, Pakistan), Femim (Batala Pharmaceuticals, Gujranwala, Pakistan), Famotin (Efroze, Korangi Industrial Area, Karachi, Pakistan) and Facid (Matador Chemicals, Lahore, Pakistan), for famotidine. Zentac (Glax Smith Kiln Pakistan Limited Karachi), Reneph (Epharm Laboratories, Marriot Road, Karachi) for ranitidine were treated as above for metformin tablets. The powder of (0.10 g) of a tablet was weighed and dissolved in methanol and water (1:1 v/v). The solution was filtered through Whatman filter paper 42 and volume was adjusted to 100 ml in volumetric flask. The solution containing the active ingredient

within the calibration range of each drug was taken and HPLC procedure was followed.

The quantitation was made from the calibration curve based on linear regression equation "y=ax+b" prepared from standard solution of metformin, famotidine and ranitidine. The drugs in the pharmaceutical preparations were also examined by the standard addition technique. Two solutions (1.0 ml each) prepared from a pharmaceutical preparations were transferred to 5 ml volumetric flasks. A solution was added standard of active ingredient corresponding to 1.25 μ g/ml metformin, 3.75 μ g/ml famotidine and 5.0 μ g/ml ranitidine. Both the solutions were processed as HPLC procedure. The quantitation was made from an increase of response with added standard.

Analysis of metformin, famotidine and ranitidine from spiked deproteinized serum and urine

Blood sample (5 ml) and urine sample (5 ml) collected from healthy volunteers who had not taken any medicine during a proceeding week were incubated at 30° C for 1 h. and centrifuged at 3000 g for 15 min. The supernatant layer was collected and was added twice the volume (5 ml) of methanol. The contents were again centrifuged at 3000 g for 15 min. The supernatant layer was collected. Solution (1 mL) was transferred to a 5 mL volumetric flask, and was added metformin, famotidine and ranitidine corresponding to 4 and 7 μ g/ml at final concentration. The liquid chromatographic procedure was then followed. The quantification was carried out from linear regression equation "y=ax+b" derived from external calibration curve. A solution of deproteinized serum (1 ml) was transferred to 5 ml volumetric flask and was not added any drug and was processed as HPLC procedure and was treated as blank.

The blood samples from healthy volunteers (employee and students of Institute of Advance Research Studies in Chemical Sciences University of Sindh Jamshoro Pakistan) 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. The volunteers informed that they did not take medicine during at least one preceding week.

Results and Discussion

Metformin and famotidine are reported to react with the benzoin to form derivative [21,22,46]. The reaction of the benzoin toward ranitidine was examined. Pure benzoin-ranitidine derivative was prepared by refluxing together methanol solution of benzoin and ranitidine in alkaline medium. The precipitate isolated was used to record mass-spectrum of the derivative and indicates M+ Mass corresponding to spectrum at m/z 525 followed by a loss of -CH₃ with peak at 510. The base peak was observed at 207 (100%), beside these peaks, other peaks were appeared as 429, 355, 281, 73 and 44 which support the formation of derivative and its fragmentation pattern (Figure 2).



Attempt was made to elute and separate benzoin derivatives of metformin, famotidine and ranitidine from HPLC column Phenomenex C-18, 5 μ m (150 cm × 4.6 mm id) using simple isocratic modes. Different solvent systems with their different compositions were tried, and complete separation with symmetrical peaks were obtained when eluted with methanol- water- acetonitrile and tetrahydrofuran (40: 40: 16: 4 v/v) with a flow rate of 1 ml/min. UV detection was at 268 nm. The derivatization conditions of ranitidine with benzoin were examined in terms of concentration of derivatizing reagent potassium hydroxide, 2-merecaptoethanol, and sodium thiosulphate added for each determination. Heating time and temperature and final addition of buffer solution for pH adjustment were also optimized. The conditions which gave maximum response were considered as optimum. A similar condition as reported for famotidine was observed as reported [21,22].

Quantitation

The optimized HPLC conditions for elution and separation of metformin, famotidine and ranitidine were used to draw linear calibration curve for the drug by plotting average peak height/peak area (n=4) against concentration and linear response were obtained with 2.5-12.5 μ g/ml with coefficient of determination(r2) within 0.997-0.993 (Table 1).

The limit of detection and limit of quantitation were measured as signal to noise ratio (3:1) and (10:1) and were obtained with 0.091-0.30 μ g/ml and 0.30-1.01 μ g/ml respectively (Table 1). The separation and quantitation were repeatable in terms of retention time and peak height/peak area with relative standard deviation (RSD) within 2.8%.

The repeatability of the analytical procedure inter day (n=4) and intraday (n=4) was examined at the final concentration of 5.0 μ g/ml and 7.50 μ g/ml of each drug by the same operation for the analysis of drug mixture on different and same day and RSD were obtained within 4.0% (Table 1).

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S. No.	Drug	Calibration range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Coefficient of determination(r2)	Linear regression equation
1	Metformin	2.50-12.50	0.1	0.3	0.997	Y=0.8817x+0.0643
2	Famotidine	2.50-12.50	0.3	0.8	0.995	Y=0.767x+0.1048
3	Ranitidine	2.50-12.50	0.091	1.01	0.993	Y=1.1293x+0.0029

Table 1: Analytical parameters for the HPLC of drugs.

Sample analysis

The pharmaceutical preparations Metphage, Glucophage (500 mg tablet) and Glucophage (250 mg tablet) for metformin Ulfam, Fermim, Famotin, Facid acid for famotidine, Zentac and Reneph for ranitidine were analysed following the procedure for the analysis of pharmaceutical preparations. The results of analysis were agreed with

the labelled values by manufacturer with the relative deviation within 0.44-5.5% The RSD for replicate analysis (n=4) were obtained within 0.15-5.5%. The test mixture (n=4) within the calibration range were analysed for the simultaneous determination of the drugs and relative error was obtained within \pm 3.8% as shown in Table 2.

S. No.	Drug	Brand name	Amount labeled in mg/ tablet	Amount found in mg/ tablet	% age recovery	Relative Deviation%	RSD% n=4
1 Metformin		Metphage	850	846.3	99.56	0.44	0.15
	Metformin	Glucophage	500	495.9	99.18	0.82	0.16
	Glucophage	250	247.5	99.04	0.96	0.16	
		Ulfam	40	38.6	96.5	3.5	5.2
2 Fa	Famotidine	Femme	40	38.8	97	3	1.7
		Famotin	20	18.9	94.5	5.5	2.8
		Famocid	40	38.5	96.25	3.75	1.2
3	Ranitidine	Zentac	150	148.27	98.84	0.72	2.7
		Reneph	150	147.6	98.4	1.6	0.82

Table 2: Analysis of Metformin, Famotidine and Ranitidine in Pharmaceutical Preparations.

The pharmaceutical additives methylparabin, propylparabin, gum acacia, lactose, fructose, glucose, sodium chloride, lauryl sulphate, and ascorbic acid were added at twice the concentration drug and analysis was carried out flowing analytical procedures. These additives did not affect the determination with relative error within \pm 4.0% when compared with same concentration of standard drug without addition of the pharmaceutical additives.

The analysis of pharmaceutical preparations was further conformed by standard addition. The pharmaceutical were processed as analysis of pharmaceutical preparation and were appropriately diluted to bring the concentration within the calibration range for metformin, famotidine and ranitidine. Each of the pharmaceutical preparations were spiked with corresponding to drug at the concentration of 1.25 μ g/ml metformin, 3.75 μ g/ml famotidine and 5.0 μ g/ml ranitidine. The derivatization and chromatographic elution was carried out as analytical procedure. The quantitation of the results indicate an agreement with the observed value by linear calibration and recovery of drug from pharmaceutical were calculated within 97.53-99.70% as shown in Figure 3 and Table 3.





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	1	1					
S. No.	Drug	Sample	Amount of standard added μg/ml	Amount found in µg/ml	%age recovery	Relative error%	RSD% n=4
1	Metformin	Serum	2.5	2.48	99.2	0.8	0.62
			5	4.92	98.4	0.6	1.4
		urine	2.5	2.45	99.18	0.82	2.18
			5	4.96	99.2	1.8	2.1
2	Famotidine	Serum	2.5	2.47	96.5	3.5	3.55
			5	4.93	98.6	1.4	2.15
		urine	2.5	2.47	97	3	0.54
			5	4.94	98.8	1.2	1.6
3	Ranitidine	Serum	2.5	2.46	98.84	0.72	0.78
			5	4.92	98.4	1.6	0.65
		Urine	2.5	2.48	98.4	1.6	3.28
			5	4.95	99.1	0.9	1.25

Table 3: Analysis of metformin, famotidine and ranitidine from spiked deproteinized serum and urine.

The blood and urine samples of healthy volunteers who had not taken any medicine at least one preceding week were deproteinized with methanol. An aliquot (1 ml) of deproteinized blood and urine samples were added standard solution within calibration range and processed as analytical procedure. The amount found agreed with the spiked values with relative error within \pm 0.60-3.50 and RSD of the replicate analysis (n=3) was within 3.5% and recovery was calculated 96.50-99.20% (Figure 4 and Table 4,5).



Figure 4: HPLC separation of standards Metformin (1), Benzoin (reagent), Famotidine (2), Ranitidine (3) and Benzoin reagent (R).

S. No.	Con. taken µg/ml	Con. added µg/ml	Height in mV	Total Amount in µg/ml	Amount found in µg/ml	%age Found	RSD% n=4
1	Metformin 6.25	1.25	44.43	7.50	7.40	98.67	1.40
3	Famotidine 6.25	3.75	59.37	10.00	9.97	99.70	2.20
4	Ranitidine 6.25	5.0	65.12	11.25	10.98	97.53	1.28

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Amount taken	Inter-day		Intra-day		
Conc. µg/ml	Height in mV	RSD% n=4	Height in mV	RSD% n=4	
Metformin 2.5	68.54	3.2	67.9	0.98	
Famotidine 2.5	72.92	3.92	71.68	1.2	
Ranitidine 2.5	81.25	2.96	82.45	3.4	

Table 4: Standard addition method for metformin famotidine and ranitidine.

Table 5: Analysis of inter day and intera day variation of Metformin,Famotidine and Ranitidine solutions.

Conclusion

An analytical procedure has been developed for HPLC determination of metformin, famotidine and ranitidine after derivatization with benzoin. The limits of detection were observed within 0.091-0.30 μ g/ml. The method was applied for the analysis of pharmaceutical preparations and spiked deproteinized blood and urine samples with RSD within 0.44-5.50%. The observed values agreed with labelled and spiked amount with relative deviations within 0.60-3.50%.

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