RP-LC Determination of Dissociation Constants and Quantitative Estimation of Antiulcer drugs, Famotidine, Nizatidine and Ranitidine in their Dosage Forms

Antiülser İlaçlardan Famotidin, Nizatidin ve Ranitidin'nin Ayrışma Sabitlerinin RP-LC Tayini ve Dozaj Formlarından Kantitatif olarak Belirlenmesi

Research Article

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ABSTRACT

Dissociation constant value (pK_a) is key parameter for predicting the extent of the ionization of a drug molecule at different pH. In this study, pK_a values of famotidine, ranitidine and nizatidine in different percentages of acetonitrile-water binary mixtures (10%, 15%, and 20%, v/v) were determined from the mobile phase pH dependence of retention factor with reverse phase liquid chromatographic method (RPLC). From calculated pK_a values, the aqueous pK_a values of studied compounds were estimated by mole fraction of acetonitrile. Moreover, the correlation established between retention factor and the pH of the water-acetonitrile mobile phase was used to determination of optimum separation condition. In order to validate the optimized condition, famotidine, ranitidine and nizatidine were studied in their dosage forms. A X-Terra C-18 reverse-phase column (250x4.6mm I.D., 5 μm particles) was preferred to carry out the developed method. Mobile phase of acetonitrile-methanol-water (10:6:84, phosphate buffer pH: 6.5) and diode array detection system at wavelengths 210 and 320 nm were used as separation conditions. Under studied conditions detection limits of 0.5678 μg/mL for famotidine, 0.3100 μg/mL for ranitidine and 1.3144 μg/mL for nizatidine were found. The parameters, linearity, precision, accuracy, limit of detection, and limit of quantitation were studied according to U.S. Pharmacopoeia.

Key Words
Antiulcer Drugs, Famotidine, Ranitidine, Nizatidine, pK_a, HPLC, Method validation.

ÖZET

Ayrışma sabiti değeri (pK_a), farklı pH değerinde, bir ilaç molekülünün iyonlaşma derecesini tahmin etmek için önemli bir parametredir. Bu çalışmada, Famotidin, Ranitidin ve Nizatidin'in farklı yüzdelerdeki asetonitril-su ikili karışımlarındaki (%10, %15, %20, v/v) pK_a değerleri, sıvı kromatografisi yöntemi (RPLC) alıkonma faktörü ve hareketli faz pH değişimi ile belirlenmiştir. Hesaplanan pK_a değerlerinden incelenen bileşiklerin su ortamı pK_a değerleri, asetonitrilin mol kesri kullanılarak tahmin edilmiştir. Ayrıca, alıkonma faktörü ve su-asetonitril hareketli fazının pH'sı arasında kurulan ilişki optimum ayırma koşulunun belirlenmesi için kullanılmıştır. Optimize edilmiş koşulu valide etmek için, Famotidin, Ranitidin ve Nizatidin kendi dozaj formlarından incelenmiştir. X-Terra C-18 ters-faz kolonu (250 x 4.6 mm ID, 5 um partikül), geliştirilen yöntem için tercih edilmiştir. Hareketli faz olarak asetonitril-metanol-su (10:6:84, fosfat tamponu pH: 6.5), 210 ve 320 nm dalga boylarında diyot dizi algılama sistemi ayrım koşulları olarak kullanılmıştır. Çalışılan koşullarda Famotidin'in tayin sınırı 0.5678 ug/mL, Ranitidin ve Nizatidin için sırasıyla 0.3100 ug/mL ve 1.3144 ug/mL bulunmuştur. Doğrusallık, kesinlik, doğruluk, duyarlılık sınırı ve kantitatif tayin sınırı parametreleri, ABD Farmakopesine göre incelenmiştir.

Anahtar Kelimeler
Antiülser ilaçlar, Famotidin, Ranitidin, Nizatidin, pK_a, HPLC, Methot validasyonu.

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INTRODUCTION

Famotidine (FAM), nizatidine (NIZ) and ranitidine (RAN) are H2-receptor antagonists which have been used for the treatment of gastro-oesophageal reflux disease and gastric and duodenal ulceration [1]. FAM is chemically named as 3-[({2-[(diaminomethylidene) amino]-1,3-thiazol-4-yl}methyl)sulfanyl]-N′-sulfamoylpropanimidamide and NIZ, N-(2-[(2-[(dimethylamino)methyl]thiazol-4-yl)methylthio] ethyl)-N-methyl-2-nitroethene-1,1-diamine and RAN, N-(2-[(5-[(dimethylamino)methyl]furan-2-yl)methylthio]ethyl)-N-methyl-2-nitroethene-1,1-diamine [1] (Figure 1).

pK_a values are useful physicochemical parameters describing the extent of ionization of functional groups with respect to pH. This parameter is important in pharmaceutical drug discovery and development [2]. pH-metric titrations and spectrophotometric analysis are routinely used for pK_a determination [3]. However, there are limitations to those procedures, such as poor solubility or a lack of a chromophore. In the recent years, liquid chromatography (LC) was introduced to determine acid dissociation constants of ionogenic substances [4]. The advantages of the LC methods are as follows: a very small quantity of the analyte required for assay and no requirement of the high purity of the sample. Small amount of sample is enough to study with RPLC and somewhat impurity in sample and low solubility in water are not problem to determine pK_a values in RPLC analysis [5]. In recent years, capillary electrophoresis has also been used for that purpose [6,7].

There have been several reports on the determination of these drugs in pharmaceutical preparations and biological fluids, including the use of liquid chromatography [8-13], capillary electrophoresis [14,15]. More complex or sophisticated liquid chromatography methods have also been reported for the individual analysis of H2 antagonists, including HPLC–MS [16-18], paired-ion HPLC-UV [19,20], post-column florescence derivatisation [21], HPLC-TLC [22] and supercritical chromatography [23]. Most of these methods require either solid-phase or liquid-phase extraction procedures which are time consuming.

In this study, pK_a values of FAM, NIZ and RAN were determined in 10%, 15% and 20% (v/v) acetonitrile (ACN)-water binary mixtures via the liquid chromatographic method. Literature results showed that the pK_a values of these drugs in water media were found in one study [24]. Also, rapid, environmentally acceptable, newly developed and validated reversed-phase liquid chromatographic method was used for the determination of famotidine (FAM), nizatidine (NIZ) and ranitidine (RAN) in pure form and pharmaceutical preparations.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals were of analytical reagent grade and all solvents were of HPLC grade. Reference samples of famotidine, nizatidine and ranitidine were obtained from DEVA Pharm. Ind, ACTAVIS Pharm. Ind. and DEVA Pharm. Ind., respectively. Their pharmaceutical dosage forms were purchased from Famoser® (40 mg famotidine) by BIOFARMA Phar. Ind., Axid® (150 mg nizatidine) by ABBOTT Pharm. Ind. In addition, Ranitab® (150 mg ranitidine) by DEVA Pharm. Ind. and Acetonitrile (HPLC grade) and methanol (HPLC grade) were obtained from Merck (Merck, Darmstadt, Germany).

Sodium hydroxide (Merck, Darmstadt, Germany) and o-phosphoric acid (Riedel-De Haen, Germany) and potassium hydrogen phthalate (standard buffer) were used for pH adjustment.

Apparatus

The LC analysis was carried out on a Shimadzu RP-LC system with a pump (LC-20 AD), a DAD detector system (SPD-M 20 A) and column oven (CTO 20 AC). X-Terra RP-18 column (250 mm × 4.60 mm ID, 5 μm) was used at 30°C. The measurement of mobile phase pH is of great importance for the purpose of pK_a determination. pH measurements of the mobile phase were carried out with a Mettler Toledo MA 235 pH/ion analyzer (Schwerzenbach, Switzerland) using combined glass electrode. pH values of the mobile phases were measured against a 0.05 mol kg^{-1} potassium hydrogen phthalate solution as primary standard reference, dissolved in the appropriate acetonitrile-water

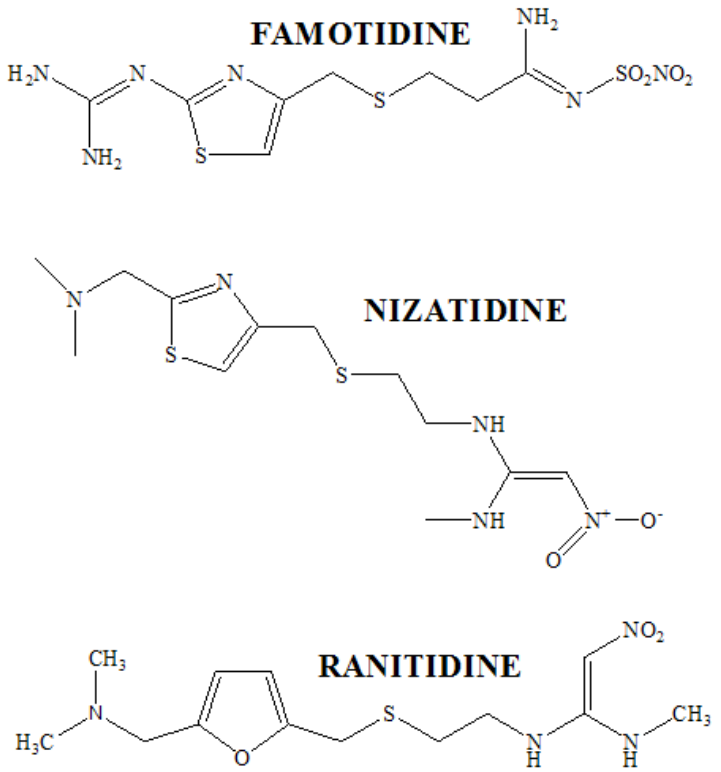

Figure 1. Chemical structures of studied compounds.

medium in accordance with IUPAC rules. Water used for the preparation of all aqueous solutions, with conductivity 18.2 mS.cm^{-1}, was obtained using a Zeneer Power I water system (Human Corp. Korea).

Procedures

In this study, the mobile phases assayed were ACN-water at 10, 15, and 20% (v/v) for determination of pK_a values of FAM, NIZ and RAN. The pH of the mobile phase containing 20 mM phosphoric acid was adjusted between 3.0 and 10.0 the addition of sodium hydroxide. The flow rate of the mobile phase was maintained at 1 mL.min^{-1} and injection volume was 20 µL.

The retention times (t_R) were determined from three separate injections for each mobile phase composition and pH values. Retention factors for each compound and mobile phase were calculated using the expression $k= (t_R-t_0)/t_0$. The dead time (t_0) was measured by injecting uracil solution (0.1% w/v in water), which was established for each mobile phase composition and the pH studied. The pK_a values of the studied compounds were determined from k/pH data pairs by means of the nonlinear regression program NLREG [25].

Preparation of standard solutions

Stock solutions (100 µg.mL^{-1}) of drugs were prepared in acetonitrile. Working solutions were prepared by dilution of stock solutions with the corresponding mobile phase to 10 µg.L^{-1}. All stock and working solutions were protected from light and stored in a refrigerator at about 4°C.

The concentration of FAM was varied in the range 0.5-12 µg.mL^{-1}, and the concentration of NIZ (internal standard-IS) was maintained at a constant level of 2.0 µg.mL^{-1}. The concentration of NIZ was varied in the range 0.5-12 µg.mL^{-1}, and the concentration of RAN (IS) was maintained at a constant level of 2.0 µg.mL^{-1}. The concentration of RAN was varied in the range 0.5-12 µg.mL^{-1}, and the concentration of NIZ (IS) was maintained at a constant level of 2.0 µg.mL^{-1}, respectively.

The calibration curves for LC analysis were constructed by plotting the ratio of the peak area of the drug to that of internal standard against the drug concentration.

Table 1. The pK_a values of studied compounds in ACN-water mixtures and water media.

Compounds	NLREG pK_a				
	10% (v/v) ACN	15% (v/v) ACN	20% (v/v) ACN	Water	This work
Famotidine	6.598±0.039	6.749±0.077	6.851±0.094	6.780	6.383
Nizatidine	6.566± 0.034	6.756±0.038	6.859±0.089	6.825	6.323
Ranitidine	8.117±0.019	8.297±0.011	8.339±0.040	8.657	7.946

Analysis of tablets

Ten tablets were weighed and finely powdered. The required amounts of these powders, equivalent to stock solution of 50 μg.mL^{-1} FAM, 30 μg.mL^{-1} NIZ and 30 μg.mL^{-1} RAN, were weighed and transferred into 100 mL volumetric flasks and diluted with ACN. The prepared solution was sonicated for 15 min to complete dissolution.

These solutions were filtered and the filtrate was collected in clean flasks. Appropriate solutions were prepared by taking suitable aliquots of clear filtrate and adding the appropriate IS solutions.

To verify the accuracy and reproducibility of the methods and to determine whether the excipients show any interference used in formulations, recovery experiments were carried out.

For this purpose, certain amounts of pure standard were added to the pre-analyzed injectable dosage forms of these drugs (and at constant levels of IS).

RESULTS AND DISCUSSION

Determination of pK_a values

In the present work, pK_a values of these drugs were determined in different solvents compositions. Table 1 shows the pK_a values of FAM, NIZ and RAN obtained by means of HPLC using the nonlinear regression program NLREG in ACN–water mixtures of 10, 15, and 20% (v/v), respectively. The influence of ACN content on pK_a values of FAM, NIZ and RAN was as expected: in all cases, there were increased in the pK_a values as ACN increased in the ACN–water binary mixtures.

In this study, the retention factors were determined for each mobile phase composition and pH studied. In Figure 2, data pairs of k/pH for studied compounds in 10%, 15% and 20% (v/v) ACN are shown and the correlation between the experimental capacity factors of the compounds studied over the whole experimental pH range was good.

Typical sigmoid shape curves were obtained in Figure 2, showing the dependence of the analyte retention factors upon the pH of the mobile phase. In general, the retention of the investigated analytes increased with increasing of the pH. Dissociation constant values of FAM, NIZ and RAN were determined in the water rich region of acetonitrile - water mixtures. pK_a of FAM, NIZ and RAN were calculated at three different organic solvent-water mixtures. To obtain the best aqueous pK_a values from pK_a data extrapolation method has been tried. pK_a values were plotted against to acetonitrile mole fraction (X of organic solvents). This method was applied using the following equation

$$pK_a = aX + b \qquad (1)$$

The intercepts of these linear equations obtained from this approach were the aqueous pK_a values of studied compounds (Table 2). There is actually a linear relationship between of the FAM, NIZ and RAN and mole fraction of acetonitrile in the binary mixtures. For chromatographic study, the resulting of linear equations for studied compounds are provided in Table 2.

Validation of the analytical methods

An X-Terra C18 column was used as the stationary phase for the simultaneous and individual determination of the selected compounds. The influence of pH of the mobile phase and column temperature were examined in order to optimize the chromatographic conditions for RP-LC determination of these drugs. The pH 6.5 was selected as optimum value with best

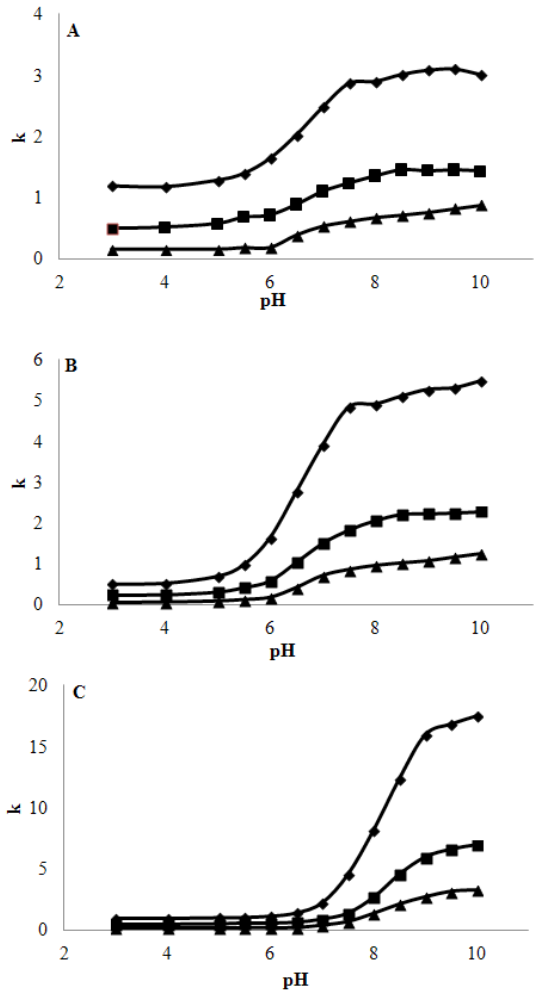

Figure 2. Plot of chromatographic retention factor, k, vs. the pH of mobile phase with 10% (v/v) ACN-water: ♦, 15% (v/v) ACN-water: ■, 20% (v/v) ACN-water: ▲. (A) Famotidine, (B) Nizatidine, (C) Ranitidine.

Table 2. The linear equations between experimental pK_a values and the mole fraction of acetonitrile.

Compounds	Equilibrium	R^2
Famotidine	pK_a = 6.1734x + 6.3831	R^2= 0.9835
Nizatidine	pK_a = 7.142x + 6.3226	R^2 = 0.9654
Ranitidine	pK_a = 5.3937x + 7.9456	R^2 = 0.8746

Table 3. Statistical evaluation of the calibration data of compounds by RP-HPLC.

	FAM	NIZ	RAN
Linearity range (μg.mL^{-1})	0.5-12	0.5-12	0.5-12
Slope	0.2550	0.7234	0.5683
Intercept	-0.0015	0.2002	0.1145
Correlation coefficient	0.9991	0.9994	0.9990
Limit of detection (μg.mL^{-1})	0.1874	0.4337	0.1023
Limit of quantification (μg.mL^{-1})	0.5678	1.3144	0.3100

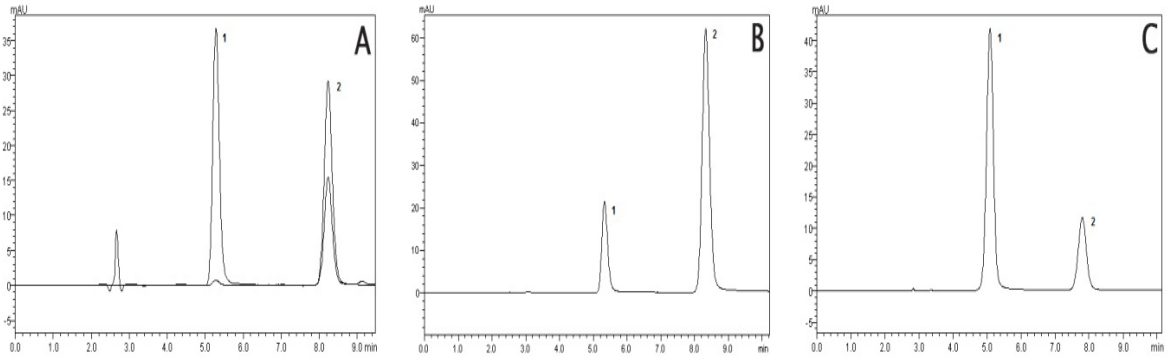

Figure 3. Chromatogram obtained from tablet dosage forms: A: FAM (4 μg.mL^{-1}) (1)–IS (2 μg.mL-1), B: NIZ (4 μg.mL^{-1}) (2)-IS (2 μg.mL^{-1}) (1) and C: RAN (4 μg.mL^{-1}) (1)-IS (2 μg.mL^{-1}) (2).

Table 4. Results of the analysis of pharmaceutical dosage forms.

	FAM	NIZ	RAN
Labeled claim (mg)	40.00	150.00	150.00
Amount found (mg)	40.46	151.42	150.58
R.S.D.%	0.83	1.02	0.31
Bias %	0.46	0.95	0.13
Added (mg)	20.00	75.00	75.00
Found (mg)	20.00	75.33	75.38
Average recovered (%)	100.00	100.44	100.52
R.S.D.% of recovery	0.12	0.37	0.18
Bias %	0.01	0.52	0.78

peak asymmetry and retention values. The pH of the mobile phase has always been adjusted with 20 mM ortho-phosphoric acid. The column temperature was set to 30^{0}C. Finally, the mobile phase acetonitrile-methanol-water (10-6-84 v/v/v) with 20 mM H_3PO_4 (at pH 6.5) at a flow rate of 1.0 mL.min^{-1} was found to be the most suitable carrier for RP-LC analysis.

Linearity was established by least squares linear regression analysis of the calibration curve [26-27]. The constructed calibration curves were linear over the concentration ranges of 0.5-12 μg.mL^{-1} for FAM, NIZ and RAN. Peak area ratios of FAM, NIZ and RAN and the IS were plotted versus their respective concentrations in the mobile phase. Correlation coefficients were higher than 0.999 for these drugs (Table 3). The low error values of the slope and the intercept show the precision of the proposed method. The LOD (limit of detection) and LOQ (limit of quantitation) were calculated using the standard deviation (s) of response and the slope (m) of the calibration curve as LOD=3.3 s/m; LOQ=10 s/m [26,27]. The LOQ that produced the requisite precision and accuracy was found to be 0.5678 μg.mL^{-1} for FAM, 1.3144 μg.mL^{-1} for NIZ and 0.3100 μg.mL^{-1} for RAN.

Analysis of commercial samples

Famoser® (containing 40 mg FAM), Axid® (containing 150 mg NIZ) and Ranitab® (containing 150 mg RAN) were analyzed. The results obtained from analysis of the tablet forms are summarized in Table 4. The amounts found were in conformity with the values claimed by the manufacturers. Figure 3 shows the chromatograms of these drugs obtained after injection of the solutions of their pharmaceutical dosage forms with the IS. As shown in Figure 3, the substances were eluted

as well-shaped, symmetrical, single peaks, well separated from the solvent front. No interfering peaks were obtained in the chromatogram due to tablet excipients. High percentages of recovery data were obtained.

Recovery studies were performed on tablet powder to obtain accuracy and precision of the proposed technique. The recovery of the procedure was measured by spiking already analyzed samples of blisters with the known concentrations of standard solutions of the drugs. The recovery analysis results for all techniques are shown in Table 4. It was concluded that the proposed methods are sufficiently accurate and precise to be applied to pharmaceutical dosage forms. High percentages of recovery data were obtained.

CONCLUSION

This work represents the first study dealing with the chromatographic pK_a determination of FAM, NIZ and RAN at three different acetonitrile percentages in the water rich region of acetonitrile-water binary mixtures. Aqueous pK_a values from pK_a data extrapolation method were investigated. The reliability of the LC methodology was examined statistically. A validated RP-HPLC method has been developed for the determination of FAM, NIZ and RAN in tablet dosage forms. The proposed method is simple, rapid, accurate, precise and specific. This method can be successfully employed for FAM, NIZ and RAN quantification of pharmaceutical preparations.

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