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Spectrophotometric determination of ranitidine hydrochloride and famotidine using 1,2- naphthoquinone-4-sulphonate.

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ABSTRACT

Accurate, simple and sensitive spectrophotometric method was developed for the determination of ranitidine hydrochloride and famotidine in bulk and pharmaceutical dosage forms. The method was based on the reaction of these drugs with 1,2-naphthoquinone-4-sulphonate in alkaline medium, producing colored products measured at λ_{max} 488 nm and 468 nm for ranitidine HCl and famotidine, respectively. Beer's law was obeyed in the concentration range from (24-120 μg ml^{-1}) for ranitidine HCl and (6-20 μg ml^{-1}) for famotidine with molar absorpitivity 1.498 x 10^3 $Lmol^{-1}cm^{-1}$ and 1.441 x 10^4 $Lmol^{-1}cm^{-1}$ respectively .The proposed method was successfully applied for the determination of ranitidine HCl and famotidine in their dosage forms with good accuracy and precisions.

INTRODUCTION

Ranitidine hydrochloride is chemically known as *N*-[2-[[[5-[(Dimethylamino) methyl]-2-furanyl] methyl] thio] ethyl]-*N'*-methyl-2-nitro-1, 1-ethenediamine, hydrochloride. While famotidine is known as *N'*-(aminosulfonyl)-3-[[[2-[(diaminomethylene) amino]-4-thiazolyl] methyl]thio]- Propanimidamide.

Both drugs are officially listed in B.P.2011 and U.S.P.XXXII (2010). They are histamine H_2-receptor antagonist which competitively inhibit the action of histamine on the H_2-receptors of parietal cells and thereby reduce the gastric acid secretion under daytime and nocturnal basal conditions. They are widely used in the management of gastrointestinal disorders, such as aspiration syndrome, dyspepsia, gastro-esophageal reflux disease, peptic ulcer and Zollinger-Ellison syndrome. (Goodman and Gilman, 1996).

Various analytical methods have been reported for the assay of ranitidine HCl in its pure form as well as in pharmaceutical formulations. These methods include spectrophotometric, (Basavaiah and Somashekar, 2007; Ashwini *et al.*, 2010; Narayana *et al.*, 2010), potentiometric methods, (Eman *et al.*, 2011; Mohamed 2013) and HPLC methods. (Alaa, 2008; Bijay *et al.*, 2011; Nitish *et al.*, 2011).

A survey of the literature revealed that famotidine has been estimated in its pharmaceutical preparations by UV-spectrophotometry, (Darwish *et al.*, 2007; Dipali *et al.*, 2011; Lilia, 2012), HPLC, (Arayne *et al.*, 2010; Reddy *et al.*, 2012) and electrochemical methods (Ayad *et al.*, 2002; Tiwari *et al.*, 2008).

This study reports simple, sensitive, economical and accurate spectrophotometric method for the analysis of ranitidine hydrochloride and famotidine in their pure and pharmaceutical formulations. The results of the analysis were validated by statistical analysis and recovery studies. Common additives used as excipients in pharmaceutical formulations do not interfere in the determination of the studied drugs.

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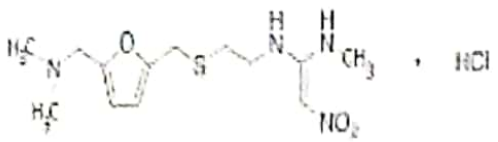
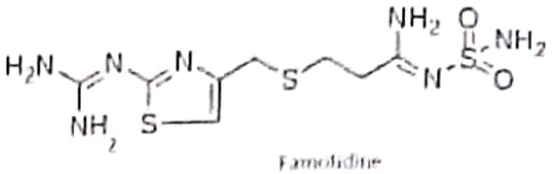


Ranitidine HCl



Famotidine

MATERIALS and METHODS

Apparatus

Labomed® Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1 cm quartz cells connected to windows compatible computer using UV Win 5 Software v5.0.5.

Thermostatically controlled (Wisebath) ® water bath.

Materials and reagents

All solvents and reagents were of analytical grade and distilled water was used throughout the work.

Materials

Ranitidine hydrochloride (Sigma Pharmaceutical Industries, Quesna City, Egypt)

Famotidine (Amoun pharmaceutical Co., El-obour city, Cairo, Egypt).

Pharmaceutical preparations

The following pharmaceutical preparations were analyzed:

Aciloc® tablets (Sigma Pharmaceutical Industries, Quesna City, Egypt) labeled to contain 300 mg ranitidine HCl per tablet.

Zantac® ampoules (Glaxo smithkline, Egypt) labeled to contain 50 mg ranitidine HCl per ampoule.

Antodine® tablets (Amoun pharmaceutical Co., El-obour city, Cairo, Egypt) labeled to contain 20 mg famotidine per tablet.

Reagents

1,2- Naphthoquinone-4-sulphonate (Fisher Scientific UK limited, UK).

Sodium hydroxide (Sd fine-chem limited, industrial estate, Mumbai, India).

Disodium hydrogen phosphate, sodium bicarbonate, methanol (EL-Nasr Pharm.Chem.Co., Egypt). **Preparation of materials and reagents**

Working solutions

Standard solution of ranitidine hydrochloride (1.2 mg/ml) was prepared by dissolving 120 mg of the pure drug in 100ml distilled water in a volumetric flask. Standard solution of famotidine (0.2 mg/ml) was prepared by dissolving 20 mg of the pure drug in the least amount of methanol, then completing to 100 ml with distilled water.

1,2-Naphthoquinone-4-sulphonate (NQS; Folin's reagent):

Fresh solution of 0.5% *w/v* NQS in distilled water was prepared daily.

Sodium hydroxide 0.05M.

General Procedures

Authentic drugs

Accurately measured aliquots of standard solutions containing (0.24-1.2 mg/ml) and (0.06-0.2 mg/ml) of ranitidine HCl and famotidine, respectively were transferred into a series of 10-ml volumetric flasks. To each flask, 1.5 ml NaOH and 2ml NQS were added for ranitidine HCl and 1ml NaOH and 1.5 ml NQS were added for famotidine and mixed well. The reaction solutions were allowed to proceed at room temperature for 20 and 15 minutes for ranitidine HCl and famotidine, respectively. The reaction mixtures were completed to the volume with distilled water. The absorbance of each solution was measured at λ_{max} 488 nm for ranitidine HCl and 468 nm for famotidine against the blank.

Pharmaceutical preparations

Aciloc tablets

Twenty tablets of Aciloc were weighed and finally powdered. An accurately weighed amount of the powder equivalent to the concentration of ranitidine HCl in the proposed method was extracted with 30 ml

distilled water three times, the filtrate was collected and transferred to 100 ml volumetric flask and completed to the mark with distilled water. Aliquots from this solution equivalent to those in authentic sample were used for the application of the proposed method applying standard addition techniques.

Antodine tablets

Twenty tablets of Antodine were weighed and finely powdered. An accurately weighed amount of the powder equivalent to the concentration of famotidine in the proposed method was extracted with 20 ml methanol three times, the filtrate was collected and transferred to 100 ml volumetric flask and completed to the mark with distilled water. Aliquots from this solution equivalent to those in authentic sample were used for the application of the proposed method applying standard addition techniques.

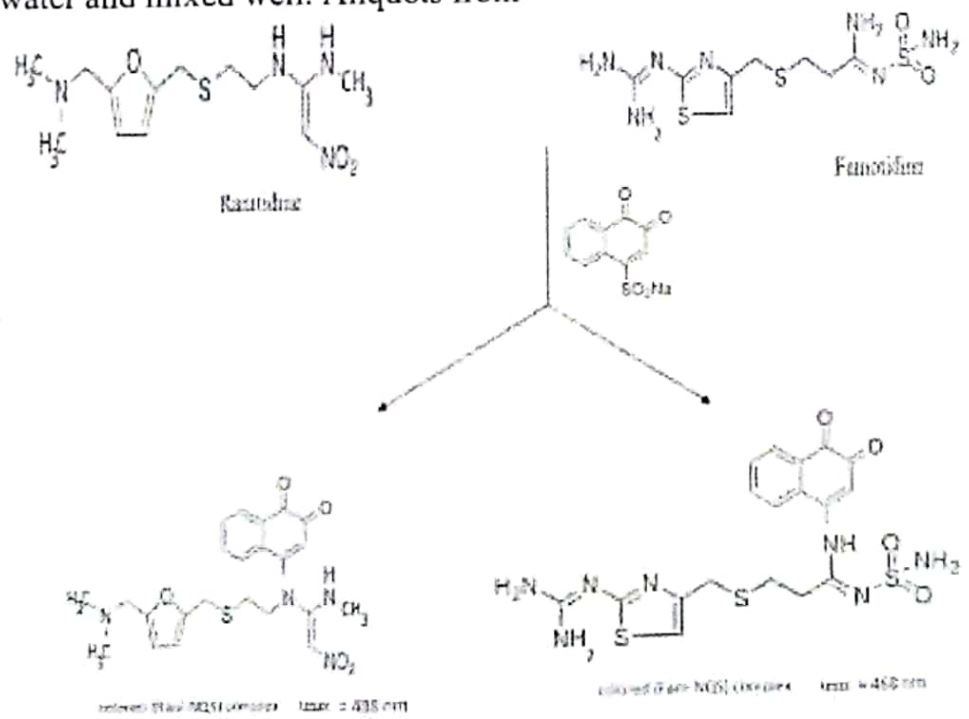
Ampoules

Ten ampoules of Zantac were carefully transferred to 250 ml volumetric flask; the volume was adjusted to 250 ml using distilled water and mixed well. Aliquots from this solution equivalent to ranitidine HCl in authentic sample were used for the application of the proposed method applying standard addition techniques as shown in Table (4).

RESULTS and DISCUSSION

1,2- Naphthoquinone-4-sulphonate (NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical drugs containing either primary or secondary amines. (Darwish *et al.*, 2009; Gurupadayya *et al.*, 2011; Rajeswari *et al.*, 2011; Ulu, 2011; Darwish *et al.*, 2012; Elbashir and Elwagee, 2012; Ashour and Bayram, 2013).

The present study shows the possibility of the reaction of NQS with ranitidine HCl and famotidine in alkaline media through nucleophilic substitution reactions. These drugs contain amino groups which can react with NQS, producing orange-red colored product in case of ranitidine HCl and reddish-brown colored adduct in case of famotidine, shown in reaction schemes. The suggested mechanism is explained below.



Reaction scheme between NQS and ranitidine HCl and famotidine.

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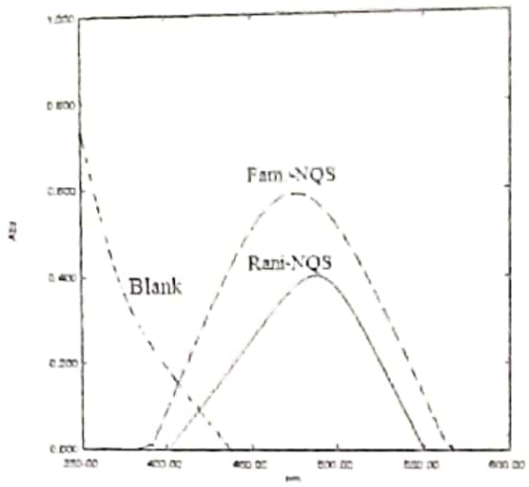

Figure 1. Absorption spectra for the reaction products of 0.5% *w/v* NQS and 96 µg ml^{-1} ranitidine HCl at λ_{max}488 nm and 14µg ml^{-1} famotidine at λ_{max} 468 nm.

Study of the experimental parameters

The different experimental parameters affecting the development of the reaction products were carefully studied and optimized. Such factors were changed individually while others were kept constant. These factors include reagent concentration, pH, temperature and reaction time.

i- Effect of the reagent concentration

The concentration of the reagent was investigated. Two ml and 1.5 ml of 0.5% *w/v* NQS solution for ranitidine HCl and famotidine were optimum reagent concentrations (Figure 2).

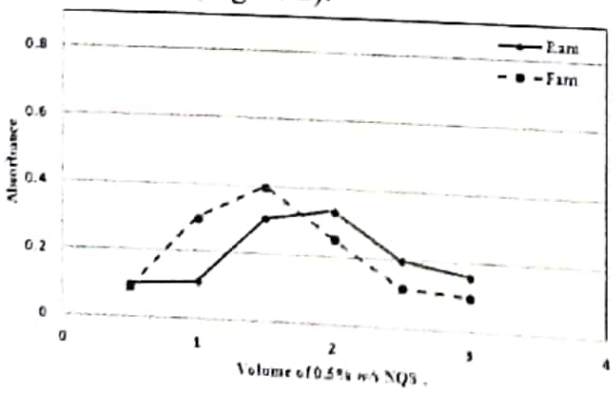

Figure 2. Effect of volume of 0.5% *w/v* NQS on the reaction with 40 µg ml^{-1} ranitidine HCl and 10 µg ml^{-1} famotidine.

ii- Effect of pH

To generate the nucleophile from the two cited drugs and activate the nucleophilic substitution reaction, alkaline medium was necessary. Different inorganic bases were tested: sodium hydroxide, potassium hydroxide, disodium hydrogen phosphate and sodium bicarbonate. The best results were obtained on using 1.5 ml and 1 ml of 0.05 M NaOH for ranitidine HCl and famotidine, respectively (Figure 3).

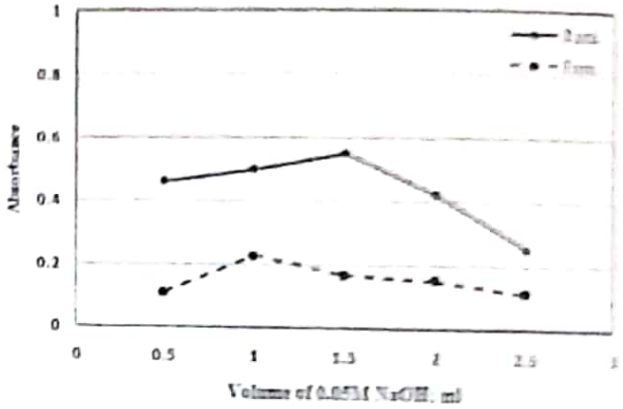

Figure 3. Effect of volume of 0.05M NaOH on the reaction products of 0.5% NQS with 40 µg ml^{-1} ranitidine HCl and 10 µg ml^{-1} famotidine.

iii- Effects of temperature and reaction time

The effect of temperature on the reaction was studied by carrying out the reaction at room temperature (25±2°C) and at varying elevated temperature (30 - 60°C). The results revealed that there was no significant difference between the absorbances that have been obtained at room temperature and those at elevated temperature. Finally, it was found that the reaction proceeded at room temperature for 20 and 15 minutes for ranitidine HCl and famotidine, respectively (Figures 4 and 5).

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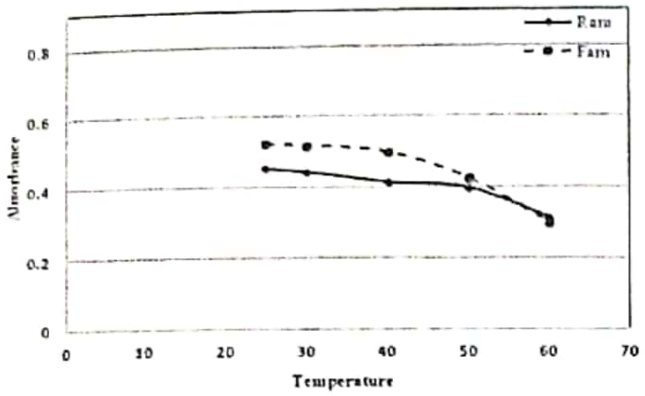

Figure 4. Effect of the temperature (°C) on the reaction of 0.5% NQS with 40 µg ml^{-1}ranitidine HCl and 10 µg ml^{-1} famotidine.

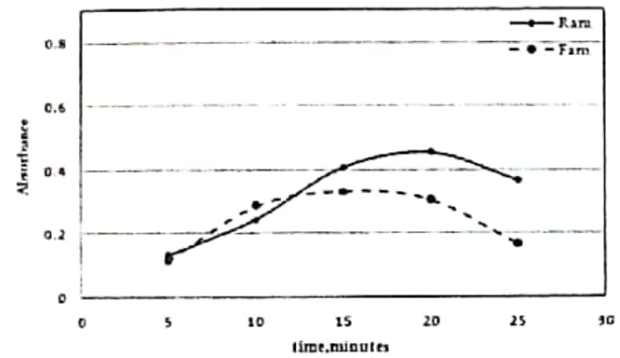

Figure 5. Effect of the time (minutes) on the reaction of 0.5% NQS with 40µg ml^{-1} ranitidine HCl and 10 µg ml^{-1} famotidine.

Stoichiometry of the reaction

The molar ratio of the reagent and both drugs in the reaction mixture was studied according to Job's method of continuous variation. The molar ratio was found to be 1:1 (drug: reagent) for both ranitidine HCl and famotidine (Figure 6) (Rose, 1964).

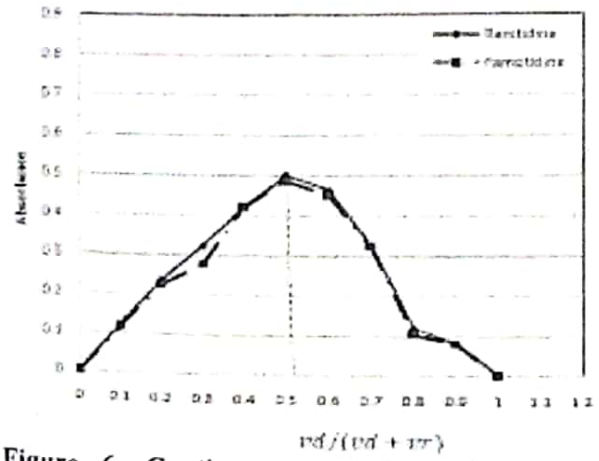

Figure 6. Continuous variation plots for the reaction between 2x10^{-3}M of NQS and 2x10^{-3}M of ranitidine HCl; 2x10^{-2}M Famotidine and 2x10^{-2}M NQS.

Validation of the proposed method

The validity of the proposed method was tested according to ICH recommendations. (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2005).

Linearity and Quantification

A linear relationship was obtained for the absorbance of NQS with the two cited drugs in the concentration ranges of (24-120 µg ml^{-1}) and (6-20 µg ml^{-1}) for ranitidine HCl and famotidine, respectively.

Under the optimized conditions, the optical and statistical parameters for the proposed methods are summarized in table (1). The molar absorpitivity, Sandell's sensitivity, correlation coefficients, slopes and intercepts were listed.

The good linearity of the calibration graph and the negligible scatter of the experimental points were clearly evident from the value of the correlation coefficient and variance.

The proposed method was successfully applied for the determination of pure drugs. Performance of the proposed method was assessed by comparing the calculated *t* and F values with the reference methods (Basavaiah and Somashekar, 2007; Walash *et al.*, 2005).

The results showed that the *t* and F values were less than the tabulated ones; indicating that there was no significant difference between the proposed and reference methods (Table 2).

Dosage forms containing ranitidine HCl and famotidne were analyzed by the proposed method applying the standard addition technique. The obtained results compared with the reference method and statistical analysis of the results showed that there is no interference from the common additive and excipients, indicating a high selectivity for determining the studied drugs in their dosage forms (Table 3).

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Table (1). Analytical parameters and spectral data for spectrophotometric determination of ranitidine HCl and famotidine through the proposed method.

Parameter		Ranitidine HCl	Famotidine
λmax, nm		488 nm	468 nm
Volume of 0.05M NaOH (ml)		1.5 ml	1 ml
Conc. of NQS		0.5% *w/v*	0.5% *w/v*
Volume of NQS (ml)		2 ml	1.5 ml
Temperature (°C)		Room temperature (25±2°C)	Room temperature (25±2°C)
Reaction time (min.)		20 min.	15 min.
Beer's law limits (μg ml^{-1})		24-120	6-20
Regression equation*	Slope (b)	0.004	0.043
	Intercept (a)	0.008	-0.012
Variance		0.832	0.244
Correlation coefficient		0.999	0.999
Molar absorptivity** (L Mol^{-1} cm^{-1})		1.498x10^{3}	1.441x10^{4}
Sandell's sensitivity (μg cm^{-2})		1.73x10^{-1}	1.8x10^{-2}
Limit of detection; LOD (μg ml^{-1})		7.44	0.692
Limit of quantification; LOQ (μg ml^{-1})		22.55	2.09

*A = a + bC where A is absorbance, C is the concentration of the drug in μg ml^{-1}
**Calculated in the basis of molecular weight of the drug.

Table (2). Statistical data for determination of ranitidine HCl and famotidine using the proposed method compared with reference method

Statistics	Ranitidine HCl		Famotidine	
	Reference method[Ola, 2011]	Proposed method	Reference method[Walash *et al*, 205]	Proposed method
Mean recovery*±SD	100.8±0.722	101.54±0.91	101.33±0.752	101.78±0.49
RSD	0.716	0.898	0.742	0.485
N	5	5	5	5
Variance	0.521	0.832	0.566	0.244
S.E.	0.323	0.409	0.337	0.221
*t-test***		1.427		1.122
F-test**		1.588		2.355

* Average of three experiments; **Theoretical *t* and F values are 2.306 and 5.05, respectively at p=0.05.

Table (3): Statistical data for the determination of pharmaceutical preparations of ranitidine HCl and famotidine through the proposed method compared with the reference method.

Statistics	Ranitidine HCl			Famotidine	
	Reference method[28]	Proposed method		Reference method[29]	Proposed method
		Aciloc® tablet	Zantac® ampoule		Antodine® tablets
Mean recovery*±SD	100.8±0.722	101.44±0.422	101.29±0.469	101.33±0.752	101.21±1.2
N	5	5	5	5	5
Variance	0.521	0.195	0.277	0.566	1.88
S.E.	0.323	0.189	0.210	0.337	0.535
*t-test***		1.715	1.272		0.190
*F-test***		2.927	2.369		2.504

* Average of three experiments; **Theoretical *t* and F values are 2.306and 5.05, respectively at p=0.05.

Table (4). Application of standard addition technique for the determination of ranitidine HCl and famotidine in pharmaceutical formulations through the proposed method

Items	Ranitidine HCl						Famotidine		
	Aciloc® tablets			Zantac® ampoules			Antodine® tablets		
	Conc. added form pure drug (µg/ml)	Conc. taken from Aciloc® (µg/ml)	Recovery*%	Conc. added form pure drug (µg/ml)	Conc. taken from Zantac® (µg/ml)	Recovery*%	Conc. added form pure drug (µg/ml)	Conc. taken from Antodine® (µg/ml)	Recovery*%
	24	0	101.04	24	0	101.04	6	0	100.37
	24	24	101.56	24	24	101.04	6	6	101.85
	24	48	101.73	24	48	100.69	6	10	100.69
	24	72	100.78	24	72	101.56	6	12	101.48
	24	96	101.66	24	96	101.87	6	14	100.89
Mean*			101.44			101.29			101.23
N			5			5			5
S.D.			0.422			0.469			0.600
R.S.D.			0.416			0.463			0.593
V			0.195			0.277			0.284
S.E.			0.189			0.21			0.269

* Average of three experiments.

Sensitivity

The limit of detection (LOD) for the spectrophotometric method was calculated using the following equation:

LOD = 3.3S/K

The limit of quantification, LOQ is defined as;

LOQ = 10S/K

Where S is the standard deviation of the three replicate determination values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely, the slope of calibration graph.

According to these equations, the limits of detection and the limits of quantification were calculated and are listed in table (1).

Accuracy

The accuracy of the proposed method was checked by performing recovery.

experiments through standard addition technique. The results are shown in table (3). No interference from the excipients was observed.

Intraday precision was evaluated by calculating standard deviation (SD) of five replicate determinations using the same solution containing pure drug. The SD values were 0.230 and 0.218 for rantidine HCl and famotidine, respectively. The small values of SD revealed the precision of the method.

For interday reproducibility on a day-to-day basis, a series was run, in which the standard drug solutions were analyzed each for five days. The interday SD values were 0.193 and 0.268 for ranitidine HCl and famotidine, respectively.

The standard analytical errors, relative standard deviations (RSD) and recoveries obtained by the proposed method were found to be acceptable.

Robustness

Robustness of the method was examined by small changes in the method variables such as change reagent concentration (±0.2ml), volume of 0.05M NaOH (±1.5ml) and standing time (±5 minutes).

The minor changes that may take place during the experiment didn't affect the absorbance of the reaction products.

Conclusion

The proposed spectrophotometric method provided simple, sensitive, specific and inexpensive analytical procedures for the determination of the two cited drugs either in pure forms or in their pharmaceutical formulations without interference from common excipients. The satisfactory sensitivity and reproducibility as well as the convenience and simplicity, make the two proposed methods suitable for routine analysis in quality control laboratories.

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القياس الطيفي للرانيتيدين هيدروكلوريد باستخدام ٢،١ ـنافثوكينون-٤ ـسلفونات

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يتم هذا البحث باستنباط طريقة بسيطة، دقيقة و حساسة لتعيين كلا من الرانتيدين هيدروكلوريد و الفاموتيدين باستخدام التقدير الطيفي و ذلك في صورتهما النقية و في أشكالهما الصيدلية. و تعتمد هذه الطريقة على تفاعل كلا الدوائين مع ٢،١ ـ نفتوكينون سلفونات في وسط قاعدي حيث أن اللون الناتج يمكن قياسه عندالطول الموجي ٤٨٨ و ٤٦٨ نانومتر لكل من الرانتيدين هيدروكلوريد و الفاموتيدين على التوالي . و بالدراسة وجد أن قانون بير يقع خلال التركيزات الآتية (٢٤-١٢٠ ميكروجرام/ ميللتر) في حالة الرانتيدين هيدروكلوريد و (٦-٢٠ ميكروجرام/ ميللتر) في حالة الفاموتيدين .
و قد تم تطبيق هذه الطريقة في تحليل كلا الدوائين في مستحضراتهما الصيدلية و التي جاءت نتائجها متفقة مع النتائج المرجعية .