www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

N-Bromosuccinimide/fluorescein system for spectrophotometric determination of H_2-receptor antagonists in their dosage forms

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ABSTRACT

A simple, accurate and sensitive spectrophotometric method for analysis of H_2-receptor antagonists has been developed and validated. The method was based on the reaction of these drugs with N-bromosuccinimide (NBS) and subsequent reaction of the remaining NBS with fluorescein (FLC) to give a pink colored product that was measured at 518 nm. Different variables affecting the reaction conditions were carefully studied and optimized. Under the optimum conditions, Beer's law was obeyed in the drugs concentration range of 0.5-35 μg ml^{-1}. The assay limits of detection and quantitation were 0.13-1.32, and 0.44-4.42 μg ml^{-1}, respectively. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 2%. The reaction stoichiometry and mechanism were studied. The proposed method was successfully applied to the analysis of the investigated drugs in pure and pharmaceutical dosage forms (recovery was 98.3–102.1 ± 0.58–1.30%) without interference from the common excipients. Interference from ascorbic acid that is added as a stabilizer for the ampoule formulation was avoided by its oxidation with potassium bromate before applying the analytical procedure. The results obtained by the proposed method were comparable with those obtained by the official methods.

Keywords: N-bromosuccinimide; fluorescein; H_2-receptor antagonists

INTRODUCTION

Histamine H_2-receptor antagonists (H2-RAs) competitively inhibit the action of histamine on the H_2-receptors of parietal cells thus reduce the gastric acid secretion under daytime and nocturnal basal conditions. H2-RAs are used for the short term treatment of active duodenal ulcer, active and benign gastric ulcer, pathogenic gastrointestinal hypersecretory conditions (e.g. Zollinger-Ellison Syndrome), and short-term symptomatic relief of gastroesophageal refluxes (Souney & Matthews, 1994). H2-RAs include cimetidine (CIM), famotidine (FAM), nizatidine (NIZ), and ranitidine hydrochloride (RAN); their chemical structures are given in Fig. 1.

Because of the therapeutic importance of H2-RAs, several methods have been reported for their determination in bulk, pharmaceutical dosage forms, and/or biological fluids. These methods include titrimetry (Kumer, 1992), electrochemical methods (Ayad, 2002), TLC (Novakovic, 2000), HPLC (Carlucci, 1990; Cakir, 1997; Zarghi, 1998), capillary electrophoresis (Luo & Chen, 2001), immunoassay (Wring, 1999), fluorimetry (Badair, 1990; El-Bayomi, 1997), and spectrophotometry (Abu-Zuhri, 1999; Amin, 2003; Mohamed, 2000; Gendy, 2001; Wadood, 2002; Walash, 2002).

N-Haloimides have been used as effective oxidizing/brominating agents for the spectrophotometric determination of many pharmaceutical compounds (Krebs, 2006; Saleh, 1996). N-bromosuccinimide (NBS), being the most versatile, is the most commonly used haloimide (Rahman & Azmi, 2004; Gowda & Seetharamappa, 2002). The analysis involving NBS was based on direct measurement of the chromogenic derivative of the drug, or indirectly by measuring the remaining NBS with color-producing reagents that are susceptible for oxidation or bromination with NBS. Fluorescin (FLC) is an appropriate candidate for color development as it can react with NBS, by bromination mechanism, and produces a pink colored tetrabromoderivative (eosin). The literature review revealed that there were no at-

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Received on: 16-12-2009
Revised on: 26-03-2010
Accepted on: 28-03-2010

tempts have been made to use NBS-FLC system in the spectrophotometric determination of H2-RAs. Therefore, the aim of the present study was directed to investigate the reaction of H2-RAs with NBS, and ultimately use of the NBS-FLC system in the development of a sensitive spectrophotometric method for their determination.

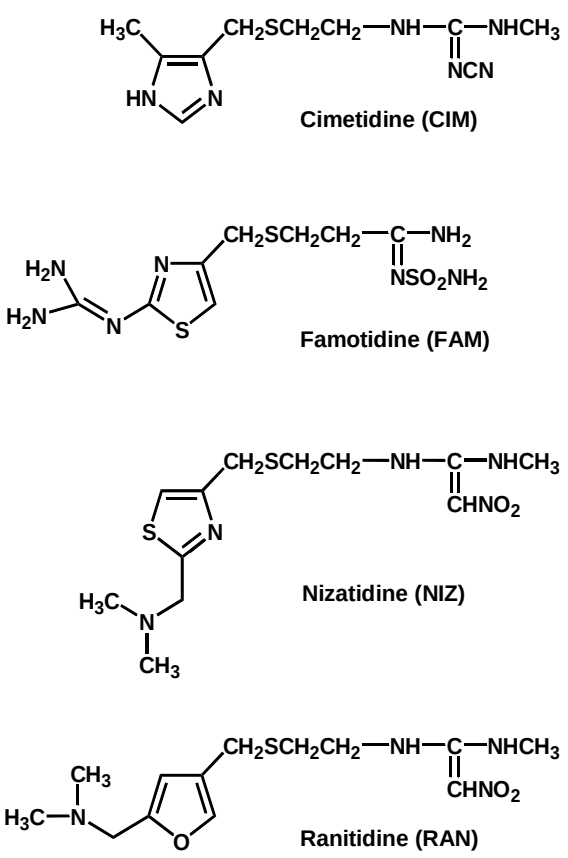

Figure 1: Chemical structures of the investigated H2-receptor antagonists (H2-RAs)

MATERIAL AND METHODS

Apparatus

UV-1601 PC (Shimadzu, Kyoto, Japan) and Lambda-3 B (Perkin-Elmer Corporation, Norwalk, USA) ultraviolet-visible spectrophotometers with matched 1-cm quartz cells were used for all measurements.

Materials and reagent solutions

Cimetidine (CIM; Sigma Chemical Co., St. Louis, MO, USA), famotidine (FAM; Sigma Chemical Co., St. Louis, MO, USA), nizatidine (NIZ; Eli Lilly Co, Indianapolis, IN, USA), and ranitidine HCl (RAN; Glaxo-Wellcome, London, UK) were obtained and used as received. The stock standard solutions (0.2 mg ml^{-1}) were prepared by dissolving an accurately weighed amount (40 mg) of the drug in 50 ml water, in a 50-ml calibrated flask. The working standard solutions were obtained by further dilution of this stock solution with water. N-Bromosuccinimde (NBS; Merck, New York, USA) was 0.15% (w/v) aqueous solution prepared fresh daily. Fluorescien (FLC; Sigma Chemical Co., St. Louis, MO, USA) was 0.015% (w/v) aqueous solution prepared fresh daily. Borate buffer solution (pH 8) was prepared and standardized (Pesez & Bartos, 1974) prior to use. All solvents, acids, and other chemicals used throughout this study were of analytical grade. Double distilled water was obtained through Nanopure II water purification system (Barnstead/Thermolyne, Dubuque, IA, USA), and used throughout the work.

Pharmaceutical formulations

Famotin® tablets (Memphis, Cairo, Egypt), Antodine® tablets (Amoun Pharmaceutical Industries, Cairo, Egypt), Servipep® tablets (Novartis Pharma, Cairo, Egypt), Peptic tablets (Julphar, U.A.E), Famotak® tablets (South Egypt Industries Company, Cairo, Egypt), Gastrodomina® tablets (Medical Union Pharmaceuticals, Ismailia, Egypt), and Antodine® ampoules (Amoun Pharmaceutical Industries, Cairo, Egypt) are labeled to contain 40 mg of FAM per tablet or ampoule. Nizatin® capsules (Hi Pharm, Cairo, Egypt) are labeled to contain 300 mg of NIZ per capsule. Ranitidol® tablets (El-Nasr Pharmaceutical Chemicals, Cairo, Egypt) are labeled to contain 150 mg of RAN per tablet. Ranitak® tablets (South Egypt Industries Company, Cairo, Egypt) are labeled to contain 300 mg of RAN per tablet. Zantac® tablets (Galaxo-Welcome Egypt S.A.E., El-Salaam City, Cairo, Egypt), and Ranitidine® tablets (Medical Union Pharmaceuticals, Ismailia, Egypt), Aciloc® tablets (Sigma, Cairo, Egypt are labeled to contain 300 mg of RAN per tablet. Zantac® ampoule (Galaxo Welcome Egypt S.A.E., El-Salaam City, Cairo, Egypt), and Ranitidine® ampoule (Medical Union Pharmaceuticals, Ismailia, Egypt) are labeled to contain 50 mg of RAN per ampoule. Cimetidine tablets simulated in the laboratory according to reported formulation labeled to contain 300 mg of CIM per tablet.

Preparation of pharmaceutical dosage form samples

Tablets and capsules

Twenty tablets or the contents of 20 capsules were weighed, and finely powdered. An accurately weighed quantity of the powdered tablet or capsule contents equivalent to 200 mg of the active ingredient was transferred into a 100-ml calibrated flask, and dissolved in about 50 ml of water. The contents of the flask were swirled, sonicated for 5 min, and then completed to the volume with water. The mixtures were mixed well, filtered and the first portion of the filtrate was rejected. A measured volume (1 ml) of the prepared solution was diluted quantitatively to 100 ml with the distilled water, and the resulting solution was used for analysis by the recommended procedure.

Ampoules

The contents of five ampoules were quantitatively transferred into a 250-ml calibrated flask, completed to

the mark with water, and the resulting solution was used for analysis by the recommended procedure.

General recommended procedure

One milliliter of the standard or sample solution containing 10-350 µg/ml of the active material was transferred into a 10-ml calibrated flask. One milliliter of NBS solution (0.15%, w/v) was added, and the reaction was allowed to proceed at room temperature (25 ± 5 °C) for 15 min. One milliliter of borate buffer (pH 8), and 1 ml of FLC solution (0.015%, w/v) were added. The contents of the flask were mixed and completed to volume with water. The decrease in the absorbances (absorbance of the blank – absorbance of the sample) was measured at 518 nm against a blank solution prepared in the same manner without drug. For direct measuring the decrease in the absorbance, the positions of the blank and sample cuvettes were exchanged. Calibration graphs were constructed by plotting the obtained absorbance values versus the corresponding drug concentration, and the amount of drug in each particular sample was calculated from its corresponding calibration curve.

Determination of molar ratio of the reactions

For H2-RAs with NBS

One-milliliter aliquots of the drug solution (6.6×10^{-3} M) were transferred into 25-ml calibrated flasks. To each flask, 1-10 ml aliquots of NBS solution (6.6×10^{-3} M) were added, and the reactions were allowed to proceed for 15 min at room temperature (25 ± 5°C).

One milliliter of FLC solution (0.015%, w/v) was added to each flask, and the reaction mixtures were completed to volume with water. The decrease in absorbance was measured at 518 nm against reagent blanks treated similarly, except the drugs were omitted. For direct measuring the decrease in the absorbance, the positions of the blank and sample cuvettes were exchanged.

For NBS with FLC

One-milliliter aliquots of NBS solution (6.6×10-3 M) were transferred into 25-ml calibrated flasks. To each flask, 1-10 ml aliquots of FLC solution (6.6×10-3 M) were added, and the reactions were allowed to proceed for 2 min at room temperature (25 ± 5 °C). The reaction mixtures were completed to volume with water, and the absorbance was measured at 518 nm against reagent blanks prepared without NBS.

RESULTS AND DISCUSSION

Reaction involved and optimization of conditions

The proposed method involved two steps; the first one was concerned with the treatment of the investigated H2-RAs drugs with known excess amount of NBS. The second step involved the determination of the remaining NBS via its reaction with FLC reagent. The addition of FLC solution to the NBS solution resulted in the formation of a pink colored tetrabromoflurescin (eosin) that showed λmax at 518 nm (Fig. 2). The decrease in the absorption intensity (ΔA) at 518 nm, caused by the presence of the drug, was directly proportional to the amount of the drug in its original sample. The investigated H2-RAs and the FLC had no absorption capability at the measuring wavelength.

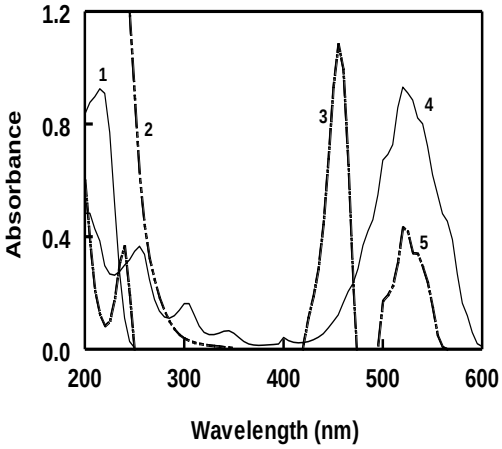

Figure 2: Absorption spectra of (1) cimetidine (20 µg ml^{-1}), (2) NBS (0.15%, w/v), (3) FLC (0.015%, w/v), (4) reaction product of NBS and FLC in presence of CIM (20 µg ml^{-1}) and (5) the reaction product in absence of CIM

Optimization of reaction variables

According to the above-mentioned reaction, NBS solution should be added in excess to react with the drug substance.

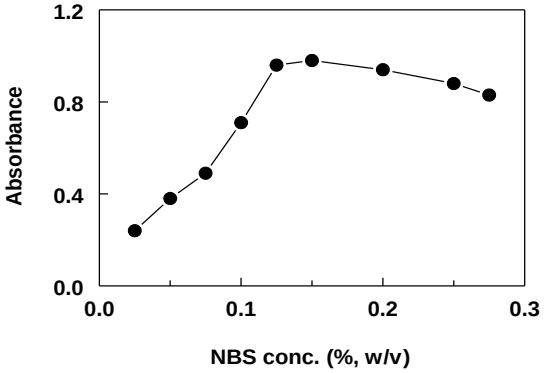

Figure 3: Effect of NBS concentration on its reaction with 20 µg ml^{-1} of each of CIM

By measuring the excess NBS reagent, the consumed reagent would correspond to the amount of the drug. The highest concentration of NBS reagent that reacts with a definite concentration of FLC reagent and gives the highest absorption value within the practical sensitivity range of absorption values (≈ 0.9) was considered as optimum. The effect of NBS reagent concentration on its reaction was studied by carrying out the reaction using 1 ml of different concentrations in the range of 0.025-0.28% (w/v). It was observed that the reaction

increase by increasing the concentration of NBS until maximum absorbance was obtained at a concentration of 0.125% (w/v), and further increase in the concentration of the reagent had a slight negative no effect on the reaction (Fig. 3). A concentration of 0.15% (w/v) was selected for the subsequent experiments. Similar series of experiments were performed to establish the optimum concentration of FLC reagent.

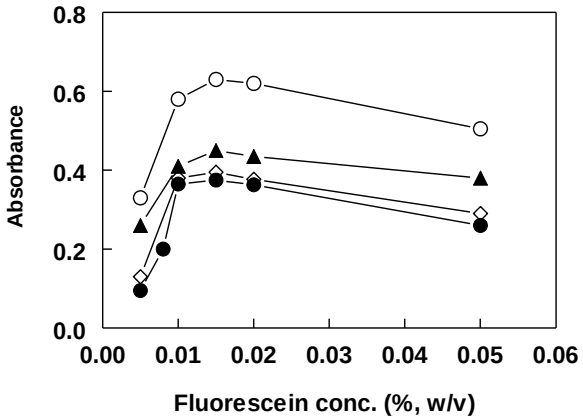

Figure 4: Effect of FLC concentration on the absorption intensity of reaction products of NBS with 20 µg ml^{-1} of each of CIM (●), FAM (▲), NIZ (◊), and RAN (o)

The results revealed that the optimum concentration was 0.015% (w/v) (Fig. 4). The reaction between NBS and FLC was a pH dependent, and maximum absorbance values and more precise readings were obtained when the pH of the reaction solution was 8 (Fig. 5). To investigate the effect of buffer solution of the reaction, different buffer solutions (phosphate, ammonia, carbonate, acetate, and borate) of pH 8 were tried. Small shifts in the position of the maximum absorption peak were observed, and the absorption intensities were also influenced (Table 1). The highest absorbance values were obtained with borate buffer, thus it was selected for subsequent work.

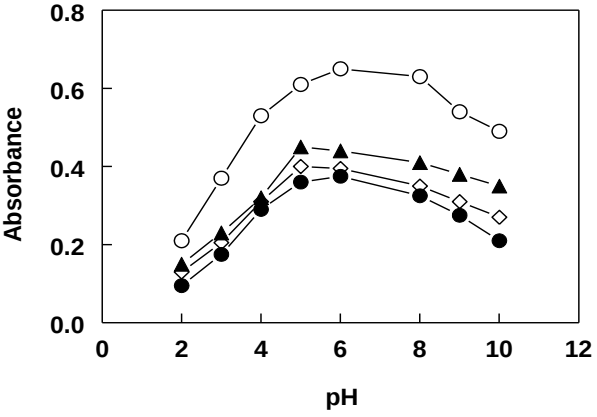

Figure 5: Effect of pH on the reaction of NBS with 20 µg ml^{-1} of each of CIM (●), FAM (▲), NIZ (◊), and RAN (o)

Under these conditions, the reaction between the investigated drugs and NBS was completed within 10 min at room temperature (25±5°C), however for more precise reading the measurements were carried out after 15 min (Fig. 6).

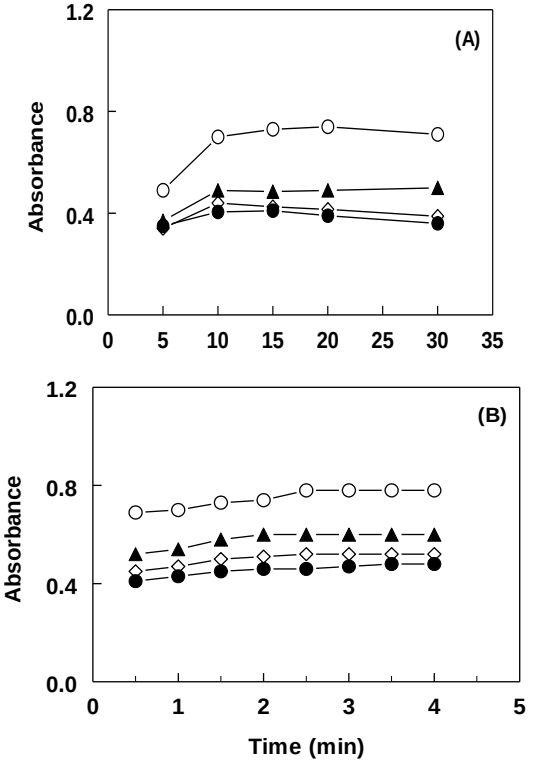

Figure 6: Effect time on the absorption intensity reaction products; (A) NBS with 20 µg ml^{-1} of each of CIM (●), FAM (▲), NIZ (◊), and RAN (o). (B) Reaction of the remaining NBS with FLC

Elevated temperatures had no significant accelerating effect on the reaction. After addition of FLC reagent, complete color development was achieved within 2 min (Fig. 7). Dilution with different solvents (Table 2) revealed that both the position of the maximum absorption peak, and the absorbance values were influenced. The highest absorbance values were obtained when methanol was used as a diluting solvent, however the difference between the values and those obtained with water were not significantly different (Table 2). For economic reasons, and safe-environment considerations, water was used dilution in the subsequent work, on expense of the sensitivity. After dilution with methanol, the absorbance values were found to be stable for at least 30 min.

Molar ratio and Reaction mechanisms

The studying of the stoichiometry for the reaction between the investigated drugs and NBS revealed that drug:NBS ratio was 1:4 (1 mole of drug reacted with 1 mole of NBS) in all cases. The molar ratio of the reaction between NBS and FLC, that gives the colored product, was found 4:1. This indicated that 4 moles of NBS reacted with one mole of FLC. Based on this finding, the reaction was postulated to proceed according to the pathway given in Fig. 7.

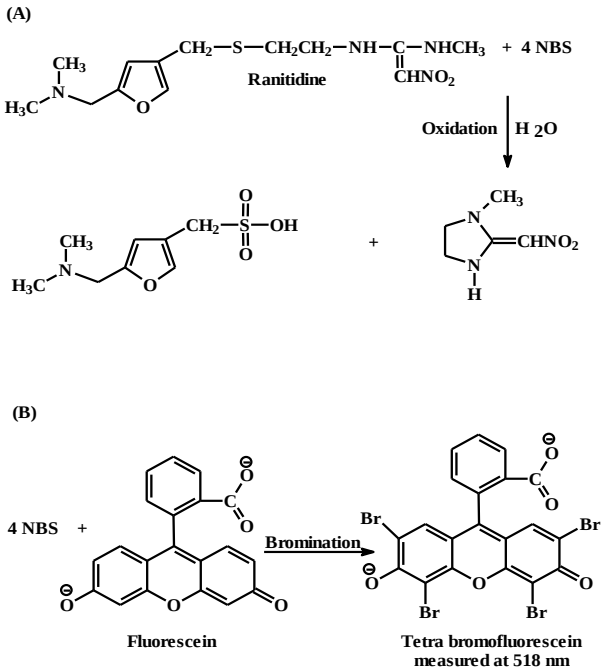

Figure 7: Suggested mechanism for the reaction of RAN with NBS (A) and NBS with FLC (B)

tration of the drug were constructed. Regression analysis for the results were as carried out using least-square method.

In all cases, Beer's law plots (n = 5) were linear with very small intercepts (-0.0015-0.0321) and good correlation coefficients (0.9982-0.9997) in the general concentration ranges of 0.5-35 μg ml^{-1} (Table 3). The limits of detection (LOD) and limits of quantitation (LOQ) were determined using the formula: LOD or LOQ = κSDa/b, where κ = 3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The LOD and LOQ values ranged from 0.13-1.32 and 0.44-4.42 μg ml^{-1}, respectively.

Precision

The precision of the proposed methods was determined by replicate analysis of five separate solutions of the working standards at three concentration levels of each drug. The relative standard deviations did not exceed 2% indicating the good reproducibility of the proposed methods (Table 4). This precision level is adequate for the precision and routine analysis of the investigated drugs in quality control laboratories.

Table 1. Effect of diluting solvent on the absorption intensity of the reaction mixtures of H2-RAs with NBS/FLC system.

Solvent	λ_{max} (nm)	Absorbance [a]			
		CIM	FAM	NIZ	CIM
Water	518	0.355	0.483	0.390	0.685
Methanol	521	0.390	0.507	0.425	0.710
Ethanol	522	0.367	0.485	0.405	0.682
Acetonitrile	515	0.355	0.465	0.405	0.655
Acetone	512	0.375	0.480	0.410	0.675
Propan-1-ol	508	0.320	0.450	0.385	0.645
Propan-2-ol	518	0.320	0.450	0.385	0.650
Butanol	500	0.360	0.450	0.390	0.635
1,4-Dioxane	515	0.275	0.475	0.410	0.625

[a] Values are mean of three determinations; the concentrations of the drugs were 20 μg ml^{-1}.

Validation of the proposed method

Linearity, limits of detection and quantization

Under the above-mentioned optimum conditions, the calibration graphs correlating the decrease in the absorption intensity (ΔA) with the corresponding concen-

Interference liabilities

Before proceeding with the analysis of the investigated drugs in their pharmaceutical dosage forms, interference liabilities were carried out to explore the effect of common excipients that might be added during

Table 2. Quantitative parameters and statistical data for the analysis of H2-RAs by the proposed spectrophotometric method

Parameter	CIM	FAM	NIZ	RAN
Linear range (μg ml^{-1})	5-35	4-25	4-28	0.5-20
Intercept (a) ± SD	-0.0015 ± 0.0110	0.0020 ± 0.0121	0.0280 ± 0.0097	0.0321 ± 0.0017
Slope (b) ± SD	0.0250 ± 0.0006	0.0353 ± 0.0095	0.0255 ± 0.0004	0.0385 ± 0.0075
Correlation coefficient (r)	0.9985	0.9987	0.9997	0.9982
$\varepsilon \times 10^3$ (L mol^{-1} cm^{-1})	6.58	10.78	9.46	13.57
LOD (μg ml^{-1})	1.32	1.03	1.14	0.13
LOQ (μg ml^{-1})	4.40	3.43	3.80	0.44

Table 3. Precision of the spectrophotometric determination of H_2-RAs by NBS/FLC method

Drug	Conc. (μg ml^{-1})	Absorbances of samples					Mean	SD	RSD (%)
		1	2	3	4	5			
Cimetidine	10	0.253	0.245	0.251	0.257	0.256	0.252	0.0048	1.892
	20	0.475	0.473	0.471	0.482	0.465	0.473	0.0062	1.306
	30	0.779	0.783	0.78	0.782	0.777	0.780	0.0024	0.306
Famotidine	8	0.262	0.252	0.263	0.256	0.255	0.258	0.0047	1.833
	15	0.479	0.497	0.486	0.483	0.492	0.487	0.0072	1.470
	25	0.836	0.84	0.83	0.853	0.846	0.841	0.0089	1.057
Nizatidine	10	0.291	0.292	0.283	0.295	0.286	0.289	0.0048	1.668
	20	0.535	0.523	0.53	0.534	0.528	0.530	0.0048	0.915
	30	0.816	0.821	0.825	0.822	0.817	0.820	0.0037	0.451
Ranitidine	5	0.21	0.217	0.211	0.21	0.215	0.213	0.0032	1.510
	15	0.575	0.579	0.585	0.581	0.584	0.581	0.0040	0.693
	20	0.815	0.803	0.806	0.81	0.816	0.810	0.0056	0.693

formulations. Samples were prepared by mixing known amount (300 mg) of the drug with various amounts of the common excipients: lactose, sucrose, starch, magnesium stearate, and ascorbic acid (added as stabilizer in the formulation of the ampoule). The analysis of these laboratory-prepared samples was carried out using the general recommended procedure, and the bromate, being mild oxidant, was used in this experiment to oxidize the ascorbic acid, however it was unable to oxidize the drug (RAN). Nevertheless, the proposed method has the advantage that the assay is performed at 518 nm in the visible region away from the UV-absorbing capabilities of interfering substances that might be co-extracted from dosage forms.

Table 4: Results of evaluation of the ruggedness of the proposed spectrophotometric method for determination of H2-RAs by NBS/FLC method.

Drug	Recovery (% ± SD)[a]				
	Instrument-to-instrument variation		Day-to-day variation		
	Shimadzu	Perkin-Elmer	Day-1	Day-2	Day-3
Cimetidine	100.5 ± 0.64	99.8 ± 0.74	100.1 ± 0.85	100.4 ±0.68	99.6 ±1.30
Famotidine	98.8 ± 0.66	99.4 ± 0.38	98.5 ± 0.82	99.2 ± 1.02	99.7 ± 0.65
Nizatidine	97.9 ± 0.86	99.2 ± 0.42	98.5 ± 0.66	99.3 ± 0.55	99.2 ± 1.33
Ranitidine HCl	98.9 ± 0.72	98.5 ± 1.18	99.4 ± 0.37	99.4 ± 0.44	98.9 ± 0.71

recovery values were determined. No interference was found from lactose, sucrose, starch, talc, gum acacia, glucose, and magnesium stearate; the recovery values were 99.3-100.7±0.86-1.44%.

This indicated the absence of interference liabilities from these excipients. Although the method is not selective, being based on oxidation reaction however, the good recoveries ensured its suitability for the analysis of the investigated drugs in their solid dosage forms without interference from the common reducing excipients. This was attributed to the high sensitivity of the method that necessitated the dilution of the sample, and consequently the excipients beyond their interference capabilities. On the other hand, ascorbic acid was found to interfere with the assay procedure. This interference could be eliminated by adding 1 ml of 0.1% (w/v) aqueous solution of potassium bromate to the ampoule samples prior to their analysis. Potassium

Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation of method variables including, concentration of analytical reagents, and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed where as the others were kept unchanged, and the recovery percentage was calculated each time. It was found that none of these variables significantly affect the method; the recovery values were 97.9-100.5±0.37-1.33%. This provided an indication for the reliability of the proposed method during its routine application for analysis of the investigated drugs. Ruggedness was tested by applying the proposed methods to the assay of the investigated drugs using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results

obtained from lab-to-lab and day-to-day variation were found to be reproducible, as RSD did not exceed 2%

Application of the proposed method to analysis of dosage forms

It is evident from the aforementioned results that the proposed method gave satisfactory results with the investigated drugs in bulk. Thus their pharmaceutical dosage forms were subjected to the analysis for their contents for the active ingredient by the proposed method and the official method (British Pharmacopoeia, 1998). The recovery, as percentages, ranged from 98.3–102.1 $\pm$ 0.58-1.30% (Table 5). These results were compared with those obtained from the official method by statistical analysis with respect to the accuracy (t-test) and precision (F-test). No significant differences were found between the calculated and theoretical values of t- and F-tests at 95% confidence level proving similar accuracy and precision in the analysis of the investigated drugs in their dosage forms.

CONCLUSIONS

The results demonstrated the useful use of NBS/FLC system in the spectrophotometic determination of H2-receptor antagonists. The proposed method was advantageous over other reported spectrophotometric methods with respect to its simplicity and wider linear range. Furthermore, the proposed method was validated for four H2-RAs, rather than most of the reported methods.

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