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UTILITY OF N-BROMOSUCCINIMIDE AS A GREEN CHEMICAL REAGENT FOR DETERMINATION OF H_2-RECEPTOR ANTAGONISTS IN THEIR PHARMACEUTICAL DOSAGE FORMS

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Abstract: An environmentally safe, simple and robust spectrophotometric method has been developed for determination of H_2-receptor antagonists namely: cimetidine (CIM), famotidine (FAM), nizatidine (NIZ), and ranitidine hydrochloride (RAN). The method was depend on the reaction of the studied drugs with N- bromosuccinimide (NBS) , environmentally friendly reagent, and the excess NBS was measured by its reaction with phloroglucinol to give a yellow chromogenic product (λ_{max} at 435 nm). The absorption intensity decrease (ΔA) was correlated with drug concentrations in the sample solutions. By using of the optimum conditions, linear calibration curves with good correlation coefficients (0.9958–0.9998) were found between the measured ΔA values and the corresponding drugs concentrations in the range of 12-80 μg mL^{-1}. Limits of detection were in the range 1.31-2.21 μg mL^{-1}. The proposed method was validated and successfully applied for the analysis of the above mentioned drugs in their bulk and pharmaceutical dosage forms with good recoveries (98.5 ± 0.98 to 102.5 ± 0.79%). No interferences were obtained from the common excipients. The proposed method was successfully applied for the analysis of H_2RAs in their dosage forms and the results were comparable with that obtained by the official methods.

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Keywords: H_2-receptor antagonists; N-bromosuccinimide; phloroglucinol; spectrophotometry; pharmaceuticals

Introduction

Histamine H_2-receptor antagonists (H_2RAs) are competitively inhibits the action of histamine on H_2-receptors of parietal cells and thus reduces the gastric acid secretion under daytime and nocturnal basal conditions.. Therefore, these drugs are useful in treatment of active duodenal ulcer, gastric ulcer and Zollinger- Ellison Syndrome.[1] Cimetidine (CIM), famotidine (FAM), nizatdine (NIZ) and ranitidine (RAN) are commonly used H_2-receptor antagonists in our community. The chemical structures of these drugs are given in **(**Figure 1**).** An extensive literature survey revealed that H_2RAs drugs have been estimated in their bulk and pharmaceutical dosage forms. These methods include titrimetry,[2] electrochemical methods,[3] TLC,[4] HPLC,[5,6] capillary electrophoresis[7] and fluorimetry.[8,9] These techniques were associated with some drawbacks as lack of sensitivity (titrimetry), time-consuming (TLC), requires expensive instruments that are not available in all quality control laboratories (HPLC and capillary electrophoresis). Spectrophotometric techniques provided practical (less-time consuming, simple, and more convenient) and significant economic advantages over other methods; therefore, they are a frequent choice for pharmaceutical analysis.[10–16] Therefore, the present work was directed to the development of new simple, accurate, and economic spectrophotometric method that overcomes these drawbacks. N-bromosuccinimide (NBS) is an environmental-friendly reagent which used as effective oxidizing/brominating agents. The spectrophotometric determinations involving NBS were based on direct measurement of the chromogenic derivative of the drug, or indirectly by measuring the

remaining NBS with color-producing reagents.[17-22] Phloroglucinol is easily susceptible to bromination with NBS and gives a yellow chromogenic product. The bromination of phloroglucinol occurs very rapidly with NBS due to presence of substitution sites activated by hydroxy-groups.[23] The purpose of the present work is to describe, for the first time, the use of a NBS/ phloroglucinol combination for determination of H_2RAs. The analytical procedure based on oxidation of H_2RAs with excess NBS and subsequent measurement of unreacted NBS by its reaction with phloroglucinol to give a yellow colored product that was measured at 435 nm. The decrease in the absorption intensity (ΔA) at 435 nm was directly proportional to the amount of the drug in the sample solution.

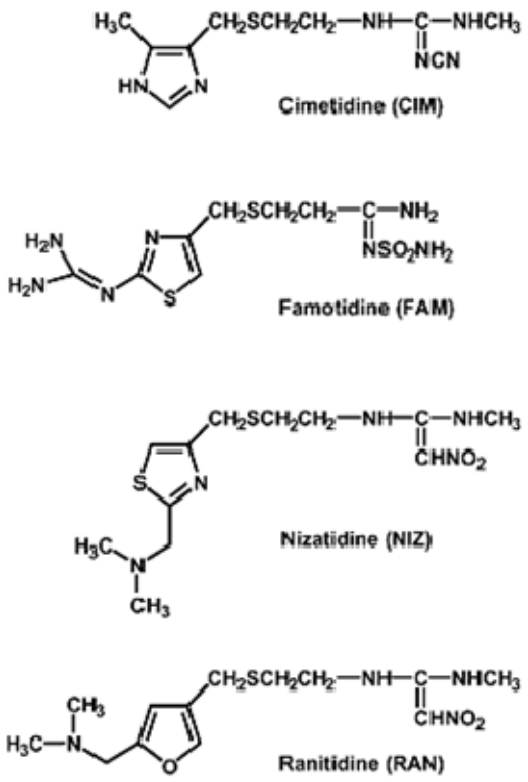

Figure 1. Chemical structures of the investigated H_2RAs.

Results and discussions

The use of molecular bromine as oxidizing and brominating agent has several drawbacks as it is harmful and there are difficulties in handling and maintaining the stoichiometric ratio during the reaction. From the green chemistry point of view, the replacement of such harmful reagents with non-toxic, inexpensive, commercially available and non-polluting reagents is an important goal. Recently, NBS has gained much attention as oxidation and bromination agent in determination of a variety of organic compounds including those of pharmaceuticals.[17-22]

NBS can be considered a convenient source of molecular bromine, or it can also act as a source of hypobromous acid which is the actual oxidizing agent.

Method Development

The proposed method using NBS and phloroglucinol is based on oxidation of the investigated H_2RAs with a known excess amount of NBS and determination of the residual NBS by the reaction with phloroglucinol. The decrease in the absorption intensity (ΔA) at λ_{max} 435 nm was corresponding to the drug content in its sample solution (Figure 2).

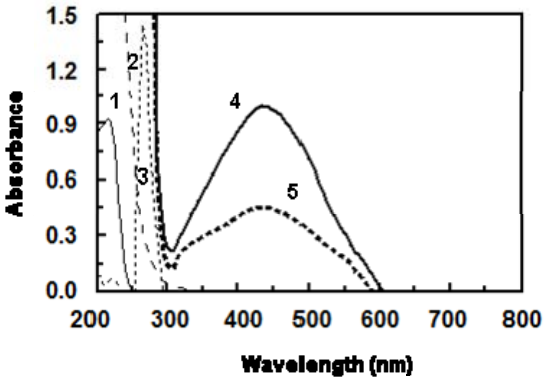

Figure 2. Absorption spectra of 20 µg/ml of CIM (**1**), 0.45% (w/v) NBS (**2**) and 0.2% (w/v) Phloroglucinol (**3**)**,** and their reaction product in the absence (**4**) and in the presence (**5**) of 50 µg /ml CIM.

It was observed that the reaction efficiency rise by increasing the concentration of NBS. The optimum absorbance (≈0.9) was obtained at a concentration of 0.45% (w/v) (Figure 3), and thus this concentration was used in all further experiments. Similar series of experiments were performed to establish the optimum concentration of phloroglucinol reagent. The results revealed that the optimum concentration was 0.2% (w/v) (Figure 4). For the reaction between NBS and phloroglucinol, the reaction was found to be rapid yielding a constant absorbance with maximum stability in presence of hydrochloric acid.[15, 17] Therefore, the optimum concentration of phloroglucinol solution, obtained by dissolving the reagent in diluted hydrochloric acid (2.5%; v/v), was found to be 0.2% w/v. The reaction was completed within 10–20 min at room temperature (25 ± 5 °C); therefore measurements were carried out after 15 min in order to achieve high precision (Figure 5). Distilled water was preferred as a solvent for economic reasons and safe- environment considerations (green solvent). In water, the absorption values, at λ_{max} 435 nm were found to be stable for at least 30 min.

Molar ratio and the reaction mechanism

The molar ratio of the reaction between the investigated H_2RAs and NBS in ratio 1:4 (1 mole of drug reacted with 4 mole of NBS) as reported previously.[14, 15] Similarly, the reaction study of NBS with phloroglucinol revealed that NBS/phloroglucinol ratio was 4:1 (Figure 6).

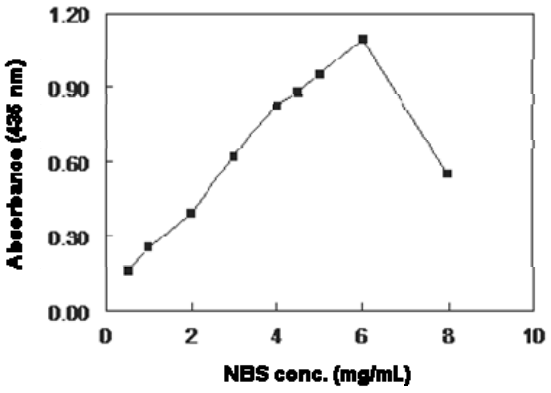

Figure 3. Effect of NBS concentration on its reaction with phloroglucinol.

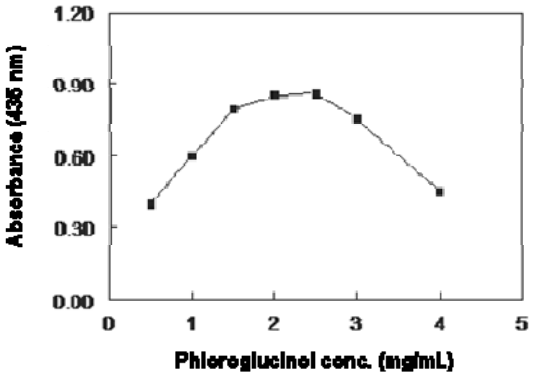

Figure 4. Effect of phloroglucinol concentration with NBS (0.45 % w/v).

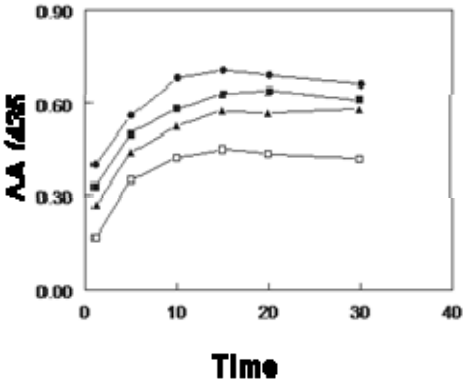

Figure 5. The kinetic colorimetric assay of NBS with CIM (-□-), FAM (-■-), NIZ (-▲-), and RAN (-●-). The concentrations of drugs were 50 µg mL^{-1}.

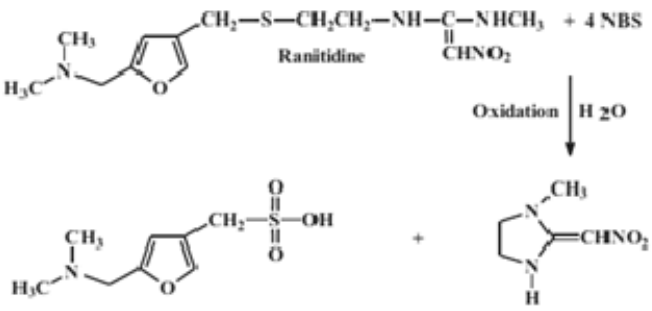

Figure 6. The proposed reaction mechanism of RAN with NBS.

Method validation

Linearity, limits of detection and quantitation

The calibration graphs were linear with good correlation coefficients (0.9958–0.9998) between ΔA at λ_{max} 435 nm, and various concentration ranges of drugs as indicated in Table 1. The molar absorptivity and together with the limits of detection and quantification were also summarized in Table 1. The limits of detection (LOD) and limits of quantitation (LOQ) were determined [24] using the formula: LOD or LOQ = kSDa/b, where k = 3.3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope.

Accuracy and Precision

Evaluation of the accuracy of the proposed method was carried out by using the standard addition method. The obtained recovery values were in the range 98.8-101.1% (Table 2). This indicated high accuracy of the proposed method. Five separate solutions of the working standards at three concentration levels were analyzed to evaluate the precision (repeatability) of the proposed method. Relative standard deviations were not exceeded 2.5 % indicating good precision (Table 3).

Interferences

Recoveries obtained from the interferences study of the proposed method showed that; there is no interference was found from the commonly used excipients (starch, sucrose, lactose, and magnesium stearate). Ascorbic acid (added as stabilizer in the formulation of the ampoule) 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.[18]

Robustness and ruggedness

Robustness was examined by changing one parameter of the method whereas the others were kept unchanged, and the recovery percentage was

observed each time. It was found that small variations in these variables did not affect the method significantly (Table 4). Results obtained from lab-to-lab and day-to-day variations in order to evaluate the ruggedness of the proposed method were found to be reproducible and RSD values were less than 2.5%.

Application to dosage forms

The validation of proposed method gave satisfactory results in determination of the investigated drugs in their pure forms. The pharmaceutical dosage forms of H_2RAs were subjected to the analysis by the proposed method and the official titrimetric method stated in the British Pharmacopoeia.[25] The recovery values ranged from 98.5–102.5%. A statistical comparison of the results obtained from proposed and the common used methods[25] are presented in Table 5. When the results were statistically compared with those of the reference method by applying the t-test and F-test, the calculated values at 95% confidence level did not exceed the tabulated values. Hence, no significant difference exists between the proposed and reference method with respect to accuracy and precision in the analysis of the investigated drugs in their dosage forms.

Experimental

Instrumentation

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

Materials and reagent solutions

Cimetidine and famotidine (Sigma Chemical Co., USA), nizatidine (Eli Lilly Co, USA), and ranitidine hydrochloride (Glaxo-Wellcome, UK) were obtained and used as received. Stock standard solutions (0.2 mg mL^{-1}) were prepared using distilled water as a green solvent. Working standard

solutions were obtained by further dilution of the stock solution with water. N-bromosuccinimde (Merck, USA) was 0.45% (w/v) aqueous solution prepared fresh daily. Phloroglucinol (Laba Chemie PVT Ltd., India) was 0.2% (w/v) prepared fresh daily by dissolving phloroglucinol in 2.5% (v/v) hydrochloric acid. All solvents, acids, and other chemicals used throughout the study were of analytical grade. Doubly distilled water was used throughout the work.

Pharmaceutical formulations

Famotin® tablets (Memphis, Egypt), Antodine® tablets (Amoun Pharmaceutical Industries, Egypt), Peptic tablets (Julphar, UAE), Famotak® tablets (South Egypt Industries Company, Egypt), and Antodine® ampoules (Amoun Pharmaceutical Industries, Egypt) are labeled to contain 40 mg of FAM per tablet or ampoule. Nizatin® capsules (Hi Pharm, Egypt) are labeled to contain 300 mg of NIZ per capsule. Ranitidol® tablets (El-Nasr Pharmaceutical Chemicals, Egypt) are labeled to contain 150 mg of RAN per tablet. Ranitak® tablets (South Egypt Industries Company, Egypt) are labeled to contain 300 mg of RAN per tablet. Zantac® tablets (Glaxo-Simthkline), and Aciloc® tablets (Sigma, Egypt) are labeled to contain 300 mg of RAN per tablet. Zantac® ampoules (Glaxo-Simthkline) are labeled to contain 50 mg of RAN per ampoule. Cimetidine tablets were simulated in the laboratory according to the reported formulation and were labeled to contain 300 mg of CIM per tablet.

General recommended procedure

One milliliter of the standard or sample solution containing 80–800 μg mL^{-1} of the active material was transferred into a 10-mL volumetric flask. One milliliter of NBS solution (0.45%, w/v) was added, and the reaction was allowed to proceed at room temperature (25 ± 5 °C) for 15 min. One milliliter of phloroglucinol (0.2%, w/v) was added. The contents of the flask were mixed and allowed to stand at room temperature (25 ± 5

°C) for 5 min. The reaction mixtures were made up to volume with water and absorbances were measured at 435 nm against blank solutions prepared in the same manner without the drug. Direct measurements of the decrease in the absorbance intensity (ΔA values) were achieved by exchange the positions of the blank and sample cuvettes. Calibration curves were constructed by plotting the ΔA values versus the corresponding drug concentration.

Determination of accuracy

Standard addition method was used for determination of the accuracy of the proposed method. Three concentrations of the authentic materials were added for each analyte; 20.0, 40.0 and 60.0 mg in case CIM, NIZ and 10.0, 20.0 and 50.0 mg in case of FAM and RAN were added to a dosage form containing a fixed amount of the active ingredient, and the recovery percentage was determined for the added amount. Famotin®, Ranitidol® and Cimetidine tablets as well as Nizatin capsules were used.

Interferences study

The general recommended procedure of the proposed method was used for determination of CIM samples prepared by mixing a known amount (300 mg) of CIM with common excipients: starch, sucrose, lactose, magnesium stearate, and ascorbic acid (added as stabilizer in the formulation of the ampoule). The recovery values were determined in order to determine the effects of these excipients.

Analysis of dosage forms

Tablets and capsules

Twenty tablets or contents of 20 capsules were weighed accurately and ground into a fine powder. A quantity of powder equivalent to 200 mg

of the active ingredient was accurately into a 100-mL volumetric flask and dissolved in about 50 mL of water. The content was shaken for about 20 min; the mixture was diluted to the mark with the water, mixed, and filtered. Firstly, a 10 mL portion of the filtrate was discarded, and a convenient aliquot was taken. Secondly, the assay was completed according to an earlier described procedure.

Ampoules

The contents of five ampoules were quantitatively transferred into a 250-mL volumetric flask, the volume was diluted to the mark with water, mixed, and the resulting solution was used for analysis according with the recommended procedure.

Determination of molar ratio of the reaction of NBS and phloroglucinol

One-milliliter aliquots of NBS solution (6.6 x 10^{-3} mol L^{-1}) were transferred into 25-mL volumetric flasks. To each flask, 1–10-mL aliquots of phloroglucinol solution (6.6 x 10^{-3} mol L^{-1}) were added. The reactions were allowed to proceed for 5 min at room temperature and completed to the volume with water. The absorbance was measured at λ_{max} 435 nm against reagent blanks prepared without NBS.

Conclusions

The results presented in this study demonstrate that it is possible to use a NBS/phloroglucinol coupled assay for spectrophotometric determination of H_2-receptor antagonists. The proposed method was advantageous over the previously reported spectrophotometric methods in terms of simplicity, cost-effectiveness and applicability to analysis of four H_2RAs.

Table 1. Quantitative parameters and statistical data for the analysis of H_2RAs by the proposed spectrophotometric method.

Drug	ε ($L.M^{-1}.cm^{-1}$)	Range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)	Slope (SE)	Intercept (SE)	Correlation coefficient (r)
CIM	2457	20-80	2.21	6.52	0.009 (0.0003)	0.0053 (0.0140)	0.9958
FAM	4515	15-65	1.43	4.31	0.017 (0.001)	0.002 (0.0004)	0.9998
NIZ	3972	20-75	1.8	5.52	0.018 (0.0001)	-0.003 (0.006)	0.9991
RAN	5001	12-60	1.31	3.91	0.015 (0.007)	0.0071 (0.005)	0.9992

Table 2. Results of standard addition method for the proposed NBS/phloroglucinol method for determination of H_2RAs.

Drug	Dosage form	Declared amount (mg)	Added amount (mg)	% Recovery ± SD
CIM	Simulated (Tablets)	10	20	100.52 ± 0.88
			40	99.8 ± 1.25
			60	101.1 ± 0.75
FAM	Famotine (Tablets)	10	10	99.61 ± 0.90
			20	100.7 ± 1.35
			50	101.5 ± 1.02
NIZ	Nizatin (Capsules)	10	20	99.75 ± 0.97
			40	100.25 ± 1.03
			60	100.51 ± 0.85
RAN	Ranitidol (Tablets)	10	10	99.82± 1.25
			20	99.55 ± 1.10
			50	98.71 ± 0.67

Table 3. Precision of the spectrophotometric proposed NBS/phloroglucinol method for determination of H_2RAs.

Drug	Conc. (µg/ml)	Absorbance difference					Mean	SD	RSD
		Sample number							
		1	2	3	4	5			
	30	0.250	0.253	0.257	0.254	0.256	0.255	0.0027	1.06
CIM	60	0.569	0.565	0.567	0.562	0.560	0.565	0.0036	0.64
	90	0.844	0.837	0.840	0.835	0.842	0.840	0.0040	0.48
	20	0.273	0.270	0.279	0.275	0.277	0.275	0.0034	1.23
FAM	30	0.408	0.406	0.405	0.400	0.413	0.405	0.0045	1.11
	60	0.811	0.804	0.806	0.808	0.800	0.805	0.0042	0.53
	20	0.235	0.237	0.242	0.240	0.244	0.240	0.0032	1.34
NIZ	50	0.595	0.604	0.611	0.590	0.603	0.600	0.0075	1.25
	70	0.807	0.821	0.810	0.806	0.813	0.810	0.0080	0.99
	20	0.279	0.290	0.286	0.295	0.296	0.295	0.0043	1.46
RAN	40	0.570	0.573	0.572	0.588	0.556	0.570	0.0113	1.98
	60	0.840	0.861	0.853	0.846	0.851	0.850	0.0080	0.94

Table 4. Results of evaluation of robustness of the proposed NBS/phloroglucinol method determination of H_2RAs.

Variation	% Recovery* ± SD			
	CIM	**FAM**	**NIZ**	**RAN**
NBS conc.				
0.40 % w/v	97.8 ± 1.2	97.8 ± 1.2	97.8 ± 1.2	97.8 ± 1.2
0.45 % w/v	100.1 ± 0.68	100.1 ± 0.68	100.1 ± 0.68	100.1 ± 0.68
0.50 % w/v	102.5 ± 1.32	102.5 ± 1.32	102.5 ± 1.32	102.5 ± 1.32
Phloroglucinol conc.	97.03 ± 1.57	97.03 ± 1.57	97.03 ± 1.57	97.03 ± 1.57
0.15 % w/v	100.6 ± 1.04	100.6 ± 1.04	100.6 ± 1.04	100.6 ± 1.04
0.20 % w/v	99.1 ± 0.66	99.1 ± 0.66	99.1 ± 0.66	99.1 ± 0.66
0.25 % w/v				
HCl acid conc.				
2.0 % v/v	98.06 ± 0.58	98.5 ± 1.10	98.8 ± 0.46	99.2 ± 0.53
2.5 % v/v	100.5 ± 1.36	99.42 ±0.87	100.6 ± 1.52	100.6±1.27
3.0 % v/v	101.1 ± 1.11	100.2 ± 1.25	98.88 ± 1.08	99.8± 0.95
Reaction time				
10 min.	99.2 ± 1.12	97.22± 0.85	98.52 ± 2.00	99.22 ± 1.05
15 min.	100.3 ± 0.46	100.7 ± 0.57	100.5 ± 0.68	100.7 ± 0.76
20 min.	99.5 ± 1.23	100.5 ± 1.02	99.3 ± 1.00	99.6 ± 1.45

N.B: The concentrations of NBS and phloroglucinol variations were carried out in absence of drugs.

Table 5. Analysis of H_2RAs-containing dosage forms by the proposed and official methods.

Product	Ingredient (Content, mg)	% Recovery ± SD[a]		F-value[c]	t-value[c]
		Proposed method	Official method[b]		
Cimetidine (Tablets)	Cimetidine (300)	100.5±0.96	98.9 ±0.64	2.25	2.45
Famotine (Tablets)	Famotidine (20)	100.2 ±0.78	99.3±0.69	1.27	1.60
Peptic (Tablets)	Famotidine (20)	98.5 ± 0.98	97.2 ± 1.5	2.34	1.42
Famotak (Tablets)	Famotidine (20)	100.7 ± 1.03	99.4± 0.69	2.22	2.03
Antodine (Tablets)	Famotidine (20)	99.5 ± 0.76	98.6± 0.73	1.08	1.29
Antodine (Ampoule)	Famotidine (20)	102.5 ± 0.79	101.5± 0.57	1.88	1.62
Nizatin (Capsules)	Nizatidine (300)	100.3± 1.33	98.1± 1.25	1.13	2.66
Zantac (Tablets)	Ranitidine (300)	99.5 ±1.12	97.3±0.79	2.00	2.53
Ranitak (Tablets)	Ranitidine (300)	98.5±1.03	97.6 ± 0.69	2.22	1.56
Ranitidol (Tablets)	Ranitidine (150)	99.5±1.49	97.2 ± 1.5	1.15	1.09
Aciloc (Tablets)	Ranitidine (300)	99.7±1.11	98.7± 0.82	1.83	1.38
Zantac (Ampoul)	Ranitidine (50)	101.6 ± 1.63	100.1± 1.25	1.71	1.92

[a] Values are the mean of five determinations ± S.D.
[b] Previous studies.[25]
[c] Theoretical values for *t*- and *F*-values at 95% confidence limit (n = 5) were 2.78 and 6.39, respectively.

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