DRUG FORMULATIONS AND CLINICAL METHODS

Colorimetric Assay of Cimetidine in the Presence of Its Oxidative Degradates

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Three simple, accurate, and sensitive colorimetric methods for the determination of cimetidine (Cim) in pure form, in dosage forms, and in the presence of its oxidative degradates were developed. These methods are indirect, involve the addition of excess oxidant [*N*-bromosuccinimide (NBS) for method A; cerric sulfate [$Ce(SO_4)_2$] for methods B and C] of known concentration in acid medium to Cim, and the determination of the unreacted oxidant by measurement of the decrease in absorbance of amaranth dye for method A, chromotrope 2R for method B, and rhodamine 6G, for method C at a suitable maximum wavelength, λ_{max}: 520, 528, and 525 nm, for the 3 methods, respectively. Regression analysis of the Beer plots showed good correlation in the concentration ranges of 0.2–4.4 μg/mL for method A, and 0.2–3.4 μg/mL for methods B and C. The apparent molar absorptivity, Sandell sensitivity, and detection and quantitation limits were evaluated. The stoichiometric ratio between the drug (Cim) and the oxidant (NBS or Ce^{4+}) was estimated. The validity of the proposed methods was tested by analyzing pure and dosage forms containing Cim with relative standard deviation ≤1.18. The proposed methods could successfully determine the studied drug with varying excess of its oxidative degradation products, with recovery between 99.2 and 101.8, 100.2 and 102.8, and 99.8 and 102.0% for methods A–C, respectively.

Cimetidine, 2-cyano-1-methyl-3-[2-[[(5-methyl imidazole-4-yl)methyl]thio] ethyl] guanidine (CAS 51481-61-9), is an effective histamine H_2-receptor antagonist which inhibits the secretion of basal and gastric acid and reduces the output of pepsin. The drug is extensively used in the treatment of duodenal and gastric ulcers, in the management of reflux esophagitis, and for the inhibition of gastric acid secretion associated with Zollinger-Ellison Syndrome (1–3). It is excreted as the unchanged drug (56–85%) and as hydroxymethyl, sulfoxide, or guanylurea metabolites (4).

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Several methods have been reported for determination of cimetidine (Cim), including chromatography (5–8), polarography (9–11), voltammetry (12), electrophoresis (13, 14), titrimetry (15–17), spectrofluorimetry (18), and spectrophotometry (19–29). Emphasis has been given to spectrophotometric and titrimetric methods, because they are simple and easily manageable. The volumetric titrations are performed by some oxidants via 0.1 *N*-bromate–bromide solution, (0.02 N); *N*-bromosuccinimide (NBS); and (0.01 M) *N*,*N*′-dibromodimethylhydantoin (DBH) for the methods described in references 14–16, respectively. All these methods determine Cim in the concentration range of 3.0–9.0 mg/mL. The spectrophotometric methods also suffer from disadvantages such as long reaction time (≥30 min) for color development (19–26), requirement for prior extraction of the colored product (21), low sensitivity and inability to accurately analyze solutions containing <50 μg/mL (20, 23, 25, 27, 28), and low stability of colored species (<15 min; 25). The UV-spectrophotometric method (29), as well as all of these spectrophotometric methods, cannot determine concentration of Cim <1.0 μg/mL. This paper describes 3 colorimetric methods, which are superior to the reported ones, for their selectivity and high sensitivity. The British Pharmacopoeia prescribes nonaqueous titration of the sample solution using 0.1 M $HClO_4$ as titrant, and the end point is detected potentiometrically (30). The present work aims to demonstrate simple, rapid, accurate, sensitive, and selective colorimetric methods suitable and convenient for the determination of Cim in pure form, in dosage forms, and in the presence of its oxidative degradates.

Experimental

Apparatus

All the absorption spectral measurements were made using JASCO V-530 (UV-Vis) spectrophotometer (JASCO, Tokyo, Japan) equipped with 10 mm matched quartz cells, a scanning speed of 400 nm/min, and a band width of 2.0 nm.

Material and Reagents

All chemicals used were of analytical or pharmaceutical grade purity, and water was doubly distilled. Pure Cim was provided by Kahira Pharmaceutical and Chemical Industries

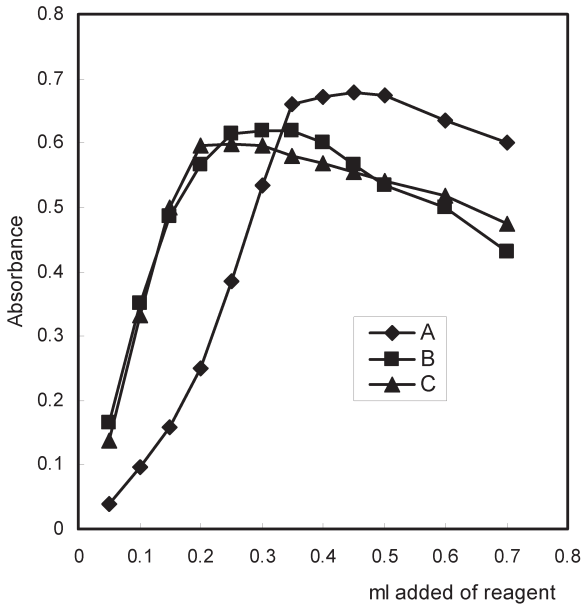

Figure 1. Effect of reagent volume on the color developed for 2.0 μg/mL Cim using methods A–C.

Co., under license from Smith, Kline & French Laboratories, Ltd, Welwyn Garden City, Herts, UK. Stock solution, 200 μg/mL, was prepared by dissolving 20 mg Cim in warm bidistilled water and diluting to the mark with water in a 100 mL calibrated flask. Working solutions of lower concentration were prepared by serial dilutions. Aqueous solutions of amaranth (AM; 2.0×10^{-3} M, Merck, Whitehouse Station, NJ), chromotrope 2R (C2R; 5.0×10^{-3} M, Sigma-Aldrich, Dorset, UK), and rhodamine 6G (Rh6G; 1.0×10^{-3} M, VWR International, Leicestershire, UK) were prepared by dissolving an appropriate weight in 100 mL bidistilled water in a calibrated flask. A solution of cerium(IV) sulfate (3.0×10^{-3} M, May and Baker, Dagenham, UK) was prepared by dissolving a known weight of $Ce(SO_4)_2$ in a small amount of warm 1.0 M H_2SO_4 in a 250 mL calibrated flask (Aldrich), and then diluting with the same acid to the mark. An aqueous solution of *N*-bromosuccinimide (100 μg/mL, Sigma-Aldrich) was freshly prepared. A solution of 5.0 M HCl (Merck) was prepared and standardized prior to use, as recommended previously (31).

Analysis of Pure Samples

(**a**) *Method A*.—To a series of 10 mL calibrated flasks, containing aliquots of Cim (2.0–44 μg/mL), 1.0 mL 100 μg/mL NBS, 1.0 mL 5.0M HCl, and 2.0 mL 1.0% KBr were transferred, and the solutions were diluted to 7.0 mL. After 5.0 min, 0.45 mL 2.0×10^{-3} M AM was added, mixed throughout, and diluted to the mark with water. The absorbance was measured at 520 nm against a blank solution prepared in the same manner without the drug. A calibration graph was prepared by plotting absorbance of the AM against the drug (Cim) concentration. The amount of Cim in unknown sample was calculated from its calibration curve.

(**b**) *Methods B and C*.—Aliquots containing 2.0–34 μg/mL Cim were transferred to a series of 10 mL calibrated flasks, and 0.5 mL 3.0×10^{-3} M $Ce(SO_4)_2$ containing 1.0 mL H_2SO_4 was added. The solution was boiled in a water bath for 10 min. The mixture was cooled, and 0.30 mL 5.0×10^{-3} M C2R was used for method B, or 0.25 mL 1.0×10^{-3} M Rh6G was mixed to hot solution and then cooled for method C. The volume was diluted to 10 mL with water. A decrease in color intensity C2R or Rh6G was measured at their corresponding λ_{max} 528 and 525 nm, for methods B and C, respectively. The concentration range was determined in

Table 1. Optical and regression characteristics

	Method		
Parameter	A	B	C
Beer's law limits, μg/mL	0.2–4.4	0.2–3.4	0.2–3.4
Ringbom limits, μg/mL	0.3–4.0	0.3–3.0	0.3–3.0
Detection limits, ng/mL	88	80	84
Quantitation limits, ng/mL	290	265	277
Molar absorptivity, L/mol cm^{-1}	9.78×10^4	7.82×10^4	7.54×10^4
Sandell sensitivity, ng/cm^{-2}	2.95	3.22	3.34
Regression equation[a]			
Slope (b)	0.339	0.310	0.299
Standard deviation of slope (S_b)	7.54×10^{-4}	7.63×10^{-4}	8.04×10^{-4}
Intercept (a)	4.29×10^{-3}	-6.67×10^{-4}	-3.62×10^{-4}
Standard deviation of intercept (S_a)	2.86×10^{-4}	9.44×10^{-4}	7.66×10^{-4}
Correlation coefficient (r)	0.9992	0.9993	0.9991

[a] With respect to $A = a + bC$, where C is the concentration of drug in μg/mL, and A is the absorbance unit.

Table 2. Evaluation of the accuracy and precision of the proposed procedures

Method	Taken, μg/mL	Recovery, %	RSD, %[a]	RE, %[b]	Confidence limits[c]
A	0.5	100.8	0.99	1.03	0.504 ± 0.0056
	1.5	100.2	0.40	0.42	1.503 ± 0.0063
	2.5	99.8	0.28	0.29	2.495 ± 0.0073
B	0.5	101.2	0.99	1.04	0.506 ± 0.0052
	1.5	99.4	0.47	0.49	1.491 ± 0.0073
	2.5	99.6	0.32	0.34	2.489 ± 0.0084
C	0.5	101.6	1.18	1.24	0.508 ± 0.0063
	1.5	100.2	0.53	0.56	1.503 ± 0.0084
	2.5	99.2	0.48	0.51	2.487 ± 0.0126

[a] RSD = Relative standard deviation for 6 determinations.
[b] RE = Relative error.
[c] 95% Confidence limits and 5 degrees of freedom.

each case by plotting concentration of Cim against absorbance at the corresponding λ_{max}.

Preparation of Degradation Products

A suitable amount (0.1 g) of Cim was dissolved in 10 mL 0.1 M HCl, and then 1.0 mL 12% H_2O_2 was added. The solution was boiled in a water bath for 45 min and then diluted in a 100 mL calibrated flask to the mark with bidistilled water. The stock solution was diluted quantitatively to obtain degraded samples of the required concentrations.

Analysis of Pharmaceutical Formulations

At least 10 tablets of the drug were weighed into a small dish, powdered, and mixed well. A portion equivalent to 200 mg was weighed and dissolved in 100 mL water, shaken well, and filtered through a sintered glass crucible G_4. A 1.0 mL aliquot of the test solution (2.0 mg/mL Cim) was diluted to 100 mL in a calibrated flask. For injection, the contents of 5 ampules (100 mg Cim) were quantitatively transferred into 250 mL calibrated flask and diluted to the mark with water. A 1.0 mL aliquot of this solution was diluted to 100 mL in a calibrated flask. Aliquots of the diluted Cim solution were then treated as described above for methods A–C.

Table 3. Analysis of cimetidine (Cim) in the presence of its degradation products

Experiment No.	Concn of degradate product added, μg/mL[a]	Recovery, %[b]		
		Method A	Method B	Method C
1	1.0	99.2	100.2	99.8
2	2.0	99.8	101.2	101.0
3	4.0	100.8	101.6	101.8
4	10	101.8	102.8	102.4

[a] Each mixture contained 2.0 μg/mL Cim.
[b] Each result is the average of 3 experiments.

Results and Discussion

This work was conducted to establish simple colorimetric methods for the determination of Cim, which contains a sulfur atom. The presence of a sulfur atom makes this compound liable to atmospheric oxidation, forming an S-oxide derivative. The structural activity relationship shows that these oxidative degradates (S-oxides) are inactive as an antipeptic ulcer treatment. For this reason the establishment of methods that quantitatively determine the pure drug in the presence of its degradation product are of great pharmaceutical value. The absorption spectra of the reaction products in methods A–C show characteristic λ_{max} value at 520, 528, and 525 nm, respectively.

Method A

NBS reacts with the drug (Cim), resulting in oxidation, substitution, or addition, depending on the functional groups present in the drug, probably a mixture of products, with reproducible data under specified experimental conditions. The excess NBS reacts with AM dye (bromination reaction) to form colorless products. Different volumes of 100 μg/mL NBS were examined, and the optimum amount was 1.0 mL; the results were highly agreeable at this concentration level. The remaining AM dye was then measured colorimetrically at λ_{max} = 520 nm.

To ascertain the optimum conditions for method A, several experiments were conducted to achieve the optimum parameters through the effects of types of acid concentration, time, KBr concentration, sequence of additions, and dye concentration. It was established that 1.0 mL 5.0 M HCl (as optimum acid), 2.0 mL 1.0% KBr, and 0.45 mL 2.0×10^{-3} M AM dye (Figure 1) are required for maximum color development and more intensive absorbance. The reaction

takes place completely in the presence of KBr after 5.0 min of mixing. Cim–NBS–HCl–KBr is the optimum sequence of addition. The effect of time after the addition of AM dye indicated that shaking for 1 min is sufficient to give reliable results. In order to investigate the molecular ratio between Cim and NBS at the selected conditions, the molar ratio method described by Yoe and Jones (32) was carried out. Experimental results showed that the molar ratio of Cim to NBS is 1:1. The excess NBS reduces the intensity of red color through disruption of the conjugation system in the dye. The remaining color of AM remains constant in absorbance for at least 48 h, and then decreases slightly afterwards.

Methods B and C

Cerium(IV) sulfate reacts with Cim, giving a number of oxidized products according to the functional groups present in Cim and the experimental conditions. The unreacted Ce(IV) oxidizes C2R or Rh6G dyes to form colorless products. The remaining C2R or Rh6G is then measured colorimetrically at its corresponding λ_{max} 528 or 525 nm, respectively.

In order to establish the optimum conditions, investigations were carried out to achieve maximum color development in the quantitative determination of Cim. The influence of each of the following variables on the reaction was tested. The most suitable acid to be used with $Ce(SO_4)_2$ was found to be sulfuric acid of 1.0 M concentration, presented as 5.0% (v/v) total volume in the reaction mixture. The oxidation process of Cim with $Ce(SO_4)_2$ is catalyzed by heat and reaches maximum at 100°C. The time required to complete the reaction is 10 min. After oxidation, the solution must be cooled at least 3.0 min before addition of C2R for method B. However, for method C, the addition of Rh6G to the hot solution gives maximum color intensity. The optimum volume of dye used for the production of maximum and reproducible color intensity is 0.30 mL 5.0×10^{-3} M C2R for method B, or 0.25 mL 1.0×10^{-3} M Rh6G for method C (Figure 1). The effect of time after the addition of dye indicated that shaking for 1 min is sufficient to give reliable results for C2R, whereas for Rh6G, the solution must be shaken for 3 min to give reliable results. The stoichiometry of the reaction between Cim and $Ce(SO_4)_2$ was investigated by the molar ratio method. Experimental results showed that the molar ratio of Cim to $Ce(SO_4)_2$ is 1:3. The excess Ce(IV) reduced the color intensity of C2R or Rh6G through disruption of the conjugation system in the dye. The color of dye remains constant in absorbance for at least 48 h, and then decreases slightly afterwards.

Analytical Data

In the proposed optimum experimental conditions, the absorbance was linearly proportional to the concentration of Cim in methods A–C, presented in Table 1. Regression analysis was performed by linear least-square treatment for intercept, slope, and correlation coefficient. The molar absorptivity, Sandell sensitivity, and regression results for each method are summarized in Table 1. The detection and quantitation limits were evaluated from the standard deviation of the absorbance measurements obtained from a series of 13 blank solutions for each procedure. The limits of detection (K = 3) and quantitation (K = 10) were established according to IUPAC definitions (33). In order to determine the accuracy and precision of the proposed methods, solutions containing 3 different concentrations of Cim were prepared and analyzed in 6 replicates. The analytical results obtained from this investigation are summarized in Table 2.

Interference

A systematic quantitative study was undertaken by measuring the absorbance of solutions containing 2.0 μg/mL

Table 4. Determination of cimetidine in pharmaceutical formulations by the proposed and official methods

			Recovery ± SD, %[a]			
Formulation	Supplier	Nominal value	Method A	Method B	Method C	Official
Tagamet (tablet)	SK & F[b]	200 mg/tablet	99.9 ± 0.8	100.5 ± 0.8	100.0 ± 1.1	99.6 ± 0.9
			t = 0.16	*t* = 1.83	*t* = 0.69	
			F = 1.26	*F* = 2.26	*F*= 1.49	
Cimetidine (tablet)	ADCO[c]	200 mg/tablet	99.5 ± 0.9	100.0 ± 0.8	100.4 ± 0.8	100.3 ± 1.0
			t = 1.46	*t* = 0.57	*t* = 0.19	
			F = 2.23	*F* = 1.56	*F* = 1.56	
Tagamet (injection)	SK & F	100 mg/ampule	99.9 ± 0.8	100.5 ± 0.8	100.3 ± 1.0	99.6 ± 1.1
			t = 0.27	*t* = 1.55	*t* = 1.15	
			F = 1.39	*F* = 1.34	*F* = 121	

[a] Average of 6 determinations ± standard deviation (SD); the *t*- and *F*-values refer to the comparison of the proposed method with the official method (tabulated *t*-value for $P = 0.05$ and 10 degrees of freedom is 2.23, and *F*-value for $P = 0.05$ and $f_1 = f_2 = 5$ is 5.05).

[b] Smith, Kline & French Labs, Ltd, Welwyn Garden City, Herts, UK.

[c] The Arab Drug Co., Cairo, Egypt.

Cim with varying excess of oxidative degradation products (1.0, 2.0, 4.0, and 10 μg/mL) using the recommended methods A–C. No significant interference was observed from the common degradation of products resulting from oxidation of Cim, which are likely to occur at normal storage condition, as shown in Table 3. Various concentrations of additives, or excipients were prepared and added to solutions containing 2.0 μg/mL Cim, following the proposed methods (A–C). If an error of ±4.0% in absorbance was found, interference was considered. Results indicated that there was no interference from the additives and excipients commonly used, such as glucose, lactose, fructose, calcium hydrogen phosphate, magnesium stearate, and starch for the examined methods A–C.

Analytical Applications

The proposed methods were successfully applied to pharmaceutical formulations. The results obtained were compared statistically by Student's *t*-test (for accuracy) and variance ratio *F*-test (for precision; 34) with the official method (29) at 95% confidence level, as recorded in Table 4. The results showed that the *t*- and *F*-values (Table 4) were less than the critical values with high percentage recoveries, indicating that no interference from additives and excipients might be found on different formulations. Consequently, the methods are simple, rapid, accurate, and stability-indicating assays. Therefore, the proposed methods can be recommended for routine analysis of Cim in pure and in dosage forms in the majority of drug quality control laboratories.

Conclusions

The proposed methods are simpler, less time-consuming, and more sensitive than the official method (depending on nonaqueous titration). All the proposed methods were advantageous over other reported visible spectrophotometric methods with respect to their high sensitivity (which permits the determination of up to 0.2 μg/mL), simplicity, reproducibility, precision, accuracy, and stability of the colored species for more than 48 h. Furthermore, the proposed methods depend on simple, readily available reagents. The proposed methods can be applied for routine analysis and in quality control laboratories for the quantitative determination of the studied drug (Cim) in raw materials, in pharmaceutical formulations, and in the presence of its oxidative degradates.

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