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A validated spectrophotometric method for the determination of ranitidine hydrochloride in pharmaceutical pure and dosage forms

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ABSTRACT

A rapid, simple and sensitive spectrophotometric method has been developed for the determination of ranitidine hydrochloride in pharmaceutical pure and dosage forms. The method depends on the charge-transfer complex formation between ranitidine bases as n-electron donor with chloranil as -acceptor to give a colored complex that absorbs maximally at 550 nm. Beer's law is obeyed in the concentration ranges 2-40μg/mL with molar absorptivity of 2.510^4 L $mole^{-1}$ cm^{-1}. The proposed method is precise, accurate and specific for the quantitative determination of drug in bulk and dosage forms. The results of analysis of commercial formulations and the recovery study (standard addition method) of ranitidine suggested that there is no interference from any excipients, which are present in pharmaceutical formulations of ranitidine. Statistical comparison of the results was performed with regard to accuracy and precision using student's t-test and F-ratio at 95% confidence level. There is no significant difference between the reported and proposed methods with regard to accuracy and precision.

KEYWORDS

Spectrophotometric methods;
Ranitidine hydrochloride;
Pharmaceutical pure;
Dosage forms.

INTRODUCTION

Ranitidine hydrochloride (RNH), chemically is N,Ndimethyl-5-[2-(1-methylamine-2-nitrovinyl)-ethylthiomethyl] furfurylamine hydrochloride . It is a H2-receptor antagonist and is widely used in short term treatment of duodenal ulcer and in the management of hypersecretory conditions[1]. Several techniques such as proton agnetic resonance spectroscopy[2], near infrared reflectance spectrometry[3], scintillation proximity assay[4], flow injection fluorimetry[5], polarography[6,7], differential pulse polarography[8], capillary electrophoresis[9], liquid chromatography[10], and high performance liquid chromatography[11-15], have been used for the determination of RNH in pharmaceuticals. These techniques require sophisticated instruments and expensive reagents, and involve several manipulation steps and derivatization reactions.

Use of these methods is justified when sample matrix is rather complex and the drug concentration is low, as is usually the case with clinical samples and in biological fluids, e.g., human plasma. However, in pharmaceutical analysis, where the sample matrix is usually less complex and analyte concentration levels are fairly high, the main aim is to develop fast, simple, inexpensive methods that can readily be adapted for routine

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analysis at relatively low cost to the different requirements of analytical problems.

The literature survey revealed that there are also several spectrophotometric methods involving the use of cerium (IV) in presence of perchloric acid[16], excess of mercury (II) thiocyanate and iron (III) nitrate[17], Diphenylcarbazide[18], Perchloric acid-crystal violet[19], Sodium azide and iodine in an aqueous solution[20], DDQ[21], Indigo carmine[22], Alkaline potassium permanganate[23], Hydrogen peroxide[24], TCNQ[25], 1, 4 Benzoquinone reagent[26], Wool fast blue[27] and 3-methyl-2-benzothiazolinone hydrazone[28]. But the these spectrophotometric methods suffer from one or more disadvantage including poor sensitivity, a complicated and time-consuming procedure, oxidation that involve degradation of the drug, extraction step and some procedures are pH dependent and require stringent reaction conditions (TABLE 1).

The aim of this study was to develop simple, fast, sensitive, accurate and validated spectrophotometric method that can be used in pharmaceutical quality control laboratories where modern and expensive apparatus, such as that required for GLC or HPLC is not available.

EXPERIMENTAL

Equipment

All spectrophotometric measurements were carried out using a spectrophotometer (U 1100 Hitachi, Japan) with silica glass cell of 1 cm thickness. Officially calibrated Pyrex glassware was used throughout this study.

Reagents and standard solutions

First, 0.5 % (w/v) ranitidine hydrochloride (Indus pharma Pvt. Ltd., Karachi, Pakistan) in water, 0.5 % (w/v) chloranil in 1,4-dioxan and 0.1 m aqueous sodium bicarbonate was prepared. All chemicals used were of Analytical Reagent Grade. Double distilled water was used for aqueous solution preparation.

Preparation of ranitidine base solution

A ranitidine base solution was prepared by transferring 100 mL of 0.5% (w/v) ranitidine hydrochloride solution into a 250 mL separating funnel, followed by 25 mL 0.1 N sodium bicarbonate solution. The contents of the separating funnel were mixed well and shaken for two minutes. The two phases were allowed to sepa-

TABLE 1: Comparison of existing spectrophotometric methods with proposed method

λ_{max} (nm)	Beers range µg/mL	Molar absorptivity $1molL^{-1}$ cm^{-1}	Reagent	Remarks	Ref.
464	1-20	6.11×10^4	Cerium (IV) in presence of perchloric acid	Involves extraction, uses unstable reagent and has narrow range of linear response	16
470	5 - 7	3.27×10^3	Excess of mercury (II) thiocyanate and iron (III) nitrate	Involve heating and stringent conditions	17
540	5-50	3.4×10^4	Diphenylcarbazide	Involve heating and stringent conditions	18
570	10 - 70	2.2×10^3	Perchloric acid-crystal violet	Involve heating and require non aqueous medium	19
348	4-24	1.55×10^4	Sodium azide and iodine in an aqueous solution	This is kinetic method and involve degradation of the drug	20
467	20-140	2.43×10^3	DDQ	Involve extraction and less sensitive	21
610	2 -12	2.06×10^4	Indigo carmine	Involve extraction and pure dyes required	22
610	8- 40	1.97×10^4	Alkaline potassium permanganate	Involves oxidation and extraction	23
313	2-20	-	Hydrogen peroxide	Involves oxidation	24
840	1- 6	-	TCNQ	Involve heating	25.
508	20 - 100	-	1, 4 Benzoquinone reagent at pH 5.6	pH dependent and involve stringent conditions	26
600	5.0 - 30.0	5.26×10^3	Wool fast blue	Involves extraction	27
313	5- 18	-	3-methyl-2-benzothiazolinone hydrazone	Involve heating and stringent conditions	28
560	4-80	1.42×10^5	Chloranil	Highly sensitive, greater molar absorptivity and reaction occur at RT.	Present work

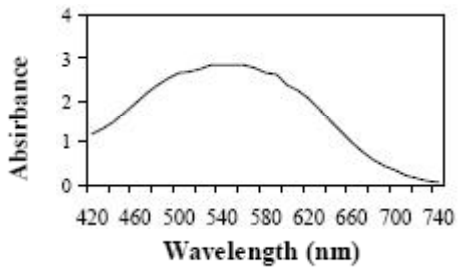

Figure 1: Absorption spectra of ranitidine and chloranil complex

rate, and the chloroform layer was dried over anhydrous sodium sulphate.

Proposed procedure

Aliquots of 2-40 μg/ mL of the ranitidine base solution were pipetted into a series of 10 mL standard volumetric flasks. Then, 2 mL chloranil solution was added to each flask, and the reaction mixtures were heated at 60^0C in water bath. The volume was made up to the mark with chloroform. The absorbance was measured within the stability period of 2 hours after dilution at 550 nm against a reagent blank.

Procedure for determination of dosage forms

For tablets: An accurately weighed portion of powdered tablet equivalent to 100 mg of ranitidine hydrochloride was stirred well with 20 mL water and left standing for five minutes. The residue was filtered on Whatman filter no. 42 paper and washed with water. The filtrate and washings were diluted to the volume in 50 mL measuring flask with water. The ranitidine hydrochloride solution was converted into ranitidine base following the procedure given under the head "preparation of ranitidine base solution", and subjected to the recommended procedure for the determination.

For injections: Five ampoules were mixed; a volume equivalent to 100 mg of ranitidine hydrochloride was stirred well with 50 mL distilled water. The ranitidine hydrochloride solution was converted into ranitidine base following the procedure given under the head "preparation of ranitidine base solution", and subjected to the recommended procedure for the determination.

Determination of the molar ratio

The Job's method of continuous variation[29] was employed. Master equimolar solutions of the drug and chloranil were prepared. The concentration of the drug solution was 20μg/mL. A series of 10-mL portions of the master solutions of the drug with chloranil reagent were made up comprising different complementary proportions (0 : 10, 1 : 9, …, 9 : 1, 10 : 1) in 10-mL volumetric flasks. After the reaction was allowed to proceed at room temperature (25±5°C), the absorbance of the solutions was measured at 550 nm against the reagent blank.

RESULTS AND DISCUSSION

Many drugs are easy to be determined by spectrophotometric method based on formation of colored charge-transfer complexes between electron acceptors, either δ or π acceptors and drugs as electron donors either n or δ donors[30–34]. Chloranil is a π-electron acceptor and has been used for determination of amino acids[35], aliphatic and aromatic amines[36] and tertiary amines but not their salts[37]. Chloranil in dioxanchloroform exists in unionized form and acts as a π-acceptor in a manner similar to quinonone[38]. Some hydrochloride salts of amines do not react with σ or π-acceptors because they do not possess a lone pair of electrons. Similarly, ranitidine hydrochloride, for the same reason, is unable to react with chloranil. To determine ranitidine hydrochloride, it was dissolved in water and shaken with chloroform and 0.1 M sodium bicarbonate, resulting in the formation of ranitidine base in the chloroform layer. Therefore, the addition of chloranil to ranitidine base possessing a lone pair of electrons results in the formation of a charge-transfer complex of n-π type that show absorption maximum at 550 nm as shown in figure 1.

Effect of solvent

Different solvents have been tried in order to achieve maximum sensitivity and product stability. Dichloro methane, acetonitrile, chloroform, carbon tetrachloride and 1,4-dioxane are suitable solvents for CT complexes. However dioxan-chloroform is the best solvent for complex formation with regard to molar absorptivity and color stability.

Stoichiometric relationship and reaction mechanism

The composition of the charge-transfer complex was established by the molar ratio and Job's method of con-

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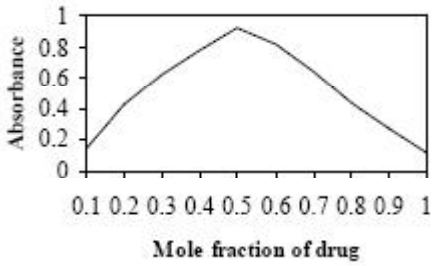
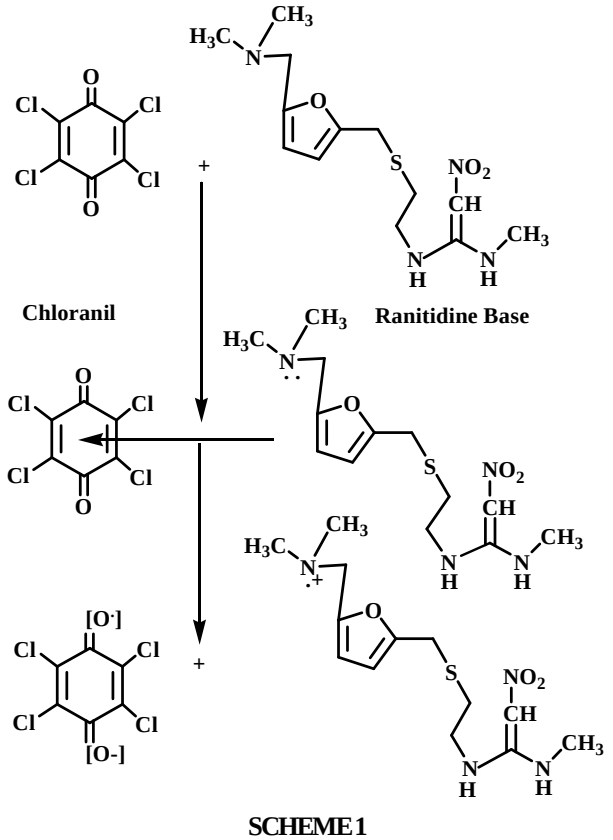


Figure 2: Continuous variation plot for the reaction of ranitidine and chloranil

TABLE 2: Percent recovery of the ranitidine hydrochloride in the presence of possible excipients used in its formulation

Excipients	Amount taken (μm/mL)	% Recovery±RSD (n =5)
Microcrystalline cellulose	300	100.5? 0.56
Magnesium stearate	200	99.58? 0.41
Hypromellose	100	101.5? 0.75
Titanium dioxide	80	99.1? 0.41
Sodium chloride	200	99.48? 0.33
Dibasic sodium phosphate	200	99.15? 0.72
Citric acid	100	100.3? 0.19



SCHEME 1

tinuous variation using equimolar solutions of the drug and reagent. The results obtained are shown in figure 2 and indicate that the composition of charge-transfer complex was (1:1) drug to reagent. This finding supports that the interaction of the studied drug and the reagent used takes place at only one site, which was the more sterically free terminal basic amino group.

The colored complex is formed by the lone pair of electrons donated by the ranitidine base as *n*-donor and the charge transfer reagent (chloranil) as an electron acceptor. The proposed reaction mechanism between ranitidine base and chloranil is shown in reaction SCHEME 1.

Interference studies

More than 101.5 % recovery of ranitidine hydrochloride was obtained in the presence of possible excipients and other additives in tablet formulations such as microcrystalline cellulose, magnesium stearate, hypromellose, titanium dioxide and in injection formulation such as sodium chloride, dibasic sodium phosphate and citric acid. Under the experimental conditions employed, to a known amount of drug (ranitidine hydrochloride 20μg/mL), excipients in different concentrations were added and analyzed. Results of the recovery analysis are presented in TABLE 2. Excipients up to the concentrations shown in the Table 2 do not interfere with the assay. In addition recoveries in most cases were 100.5 % and the lower values of the RSD indicate the good precision of the method.

Optimization of reaction conditions

To optimize the reaction conditions different parameters such as temperature, time, reagent concentration, and color stability have been investigated. It was observed that complete color development was attained at room temperature. The optimum reaction time was determined by keeping the reaction mixture at room temperature, and absorbance measurement was taken at different intervals of time. It was noted that complete color development was attained in five minutes at room temperature (25°C) and remain stable up one hour which was considered sufficient time for an analyst to carry out analysis.

Analytical data, method validation and applications

Optical characteristics and statistical data for the regression equation of the proposed method are given in TABLE 3. The proposed method was found to give

TABLE 3: Spectral data for the reaction of ranitidine with chloranil

Parameters	Values
λ max (nm)	550
Beer's law limits (μg/mL)	2-40
Molar absorptivity (L mole^{-1} cm^{-1})	2.5×10^4
Detection limit (μg/mL)	1.01
Quantification limit (μg/mL)	3.33
Sandle sensitivity (μg cm^{-2})	1.4×10^{-2}
Slope	7.2×10^{-2}
Intercept	-4.4×10^{-3}
Correlation coefficient	0.9999

TABLE 4: Results of analysis of commercial formulations containing ranitidine and statistical comparison with reference method

Brand name and dosage form	Label claim	% Found±SD		t-test	F-test
		Proposed method	reference method		
Tablets					
Acedonil[a]	150 mg	100.1±3.2	99.98±2.1	1.85	3.11
Anzol[b]	150 mg	101.5±2.5	100.9±1.8	1.61	2.17
Chase[c]	150 mg	99.6±1.8	99.6±2.1	1.11	4.10
Altal[d]	150 mg	100.8±2.3	100.2±3.2	1.72	1.13
Injections					
Anzol[b]	25 mg/ml	100.4±2.3	101.1±1.9	2.40	3.25
Aspar[e]	25 mg/ml	101.2±1.7	100.8±2.1	0.50	1.48
Ranidin[f]	25 mg/ml	99.8±2.8	100.2±1.3	0.46	2.13

Percent found is the mean value of five determinations Marketed by, [a]Global pharmaceuticals, [b]Indus Phrama, [c]Sharooq Pharmaceuticals, [d]Alson Pharmaceuticals, [e]Star Labortaries, [f]Ferozsons Labortaries.

TABLE 5: Results of recovery study by standard addition method

Formulation and dosage form	Cefaclor in sample	Pure cefaclor added(mg)	Total cefaclor found(mg)	Pure cefaclor recovered (%)
Tablets	150mg	10	159.25	99.53
Anzol	150mg	20	170.2	100.11
(150mg)	150mg	30	179.25	99.58
Injections	25mg/ml	5	29.91	99.70
Anzol	25mg/ml	10	35.08	100.22
(25mg/ml)	25mg/ml	15	39.25	98.12

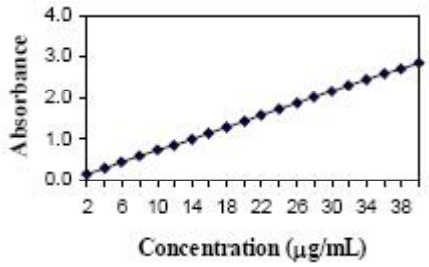


Figure 3: Beer's law verification range

linear calibration curves over the concentration ranges of 2- 40μg/mL with a regression coefficient (r) of 0.9999, indicating good linearity figure 3. Assays were performed in triplicate at different levels. This was repeated with a second instrument, standard and sample preparation on different days. These results of accuracy and precision show that the proposed method has good repeatability and reproducibility. Also the assay results are unaffected by the presence of excipients, this establish specifity of the method. The proposed method was applied for the determination of the ranitidine in commercial preparations. Five replicate determinations were made. Satisfactory results were obtained for all of them (TABLE 4). Moreover, to check the validity of the proposed method, the standard addition method was applied by adding pure ranitidine to the previously analyzed tablets and injections. The recovery of drug was calculated by comparing the concentration obtained from the spiked mixtures with those of the pure drug. The results of analysis of commercial dosage forms and the recovery study standard addition method) of the cited drugs(TABLE 5) suggested that there is no interference from any excipients, which are present in tablets or capsules. The results of determination of the ranitidine in commercial dosage forms were compared with the reported method[39].

Statistical comparison of the results was performed with regard to accuracy and precision using students t-test and F-ratio at 95% confidence level (TABLE 4). There is no significant difference between the reported and proposed method with regard to accuracy and precision.

CONCLUSIONS

The proposed spectrophotometric method is rapid, simple, precise, accurate and is comparable in sensitivity to many of the existing methods and is superior to many HPLC procedures. The proposed procedure is free from tedious steps like extraction or heating and involves least number of experiment variables, which is reflected in high precision. An additional advantage of this method is its specificity. Since basic nitrogen is the reaction site, the method is specific to RNH since none of the excipients normally used in dosage forms contains basic nitrogen. Furthermore, all the analytical reagents are inexpensive, have excellent shelf life, and are

easily available in any analytical laboratory. The proposed method can be applied in quality control laboratories for the routine analysis of the ranitidine in raw materials and pharmaceutical formulations.

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