SPECTROPHOTOMETRIC METHOD FOR RANITIDINE DETERMINATION IN DRUGS USING RHODAMINE B

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ABSTRACT

A new spectrophotometric method has been developed for the determination of ranitidine hydrochloride (R-HCl) in bulk drug and in tablet formulation. The proposed method involves the addition of a measured excess of bromate-bromide reagent in hydrochloric acid medium to R-HCl, followed by determination of residual bromine by reacting with a fixed amount of Rhodamine B (RB) and measuring the absorbance at $\lambda_{\text{max}}=557$ nm. Beer's law was found in the range of 0.3-3.2 $\mu\text{g ml}^{-1}$ of R-HCl, with a good correlation coefficient ($r=0.9997$). The limits of detection and quantification were calculated to be 0.08 $\mu\text{g ml}^{-1}$ and 0.24 $\mu\text{g ml}^{-1}$, respectively. The different experimental parameters affecting the development and stability of the proposed method were carefully studied and optimized. The validity of the proposed method tested by analyzing the pure and pharmaceutical formulations and compared well with those obtained by the official method, demonstrated good accuracy and precision.

Keywords: Spectrophotometry; Ranitidine hydrochloride; Rhodamine B; Redox reaction; Drug.

INTRODUCTION

Ranitidine, N-(2-[[5-dimethylamino)methyl]-2-furanyl]-methylthioethyl)-N'-methyl-2-nitro-1,1'-ethane diamine hydrochloride (abbreviated as R-HCl), is a potent inhibitor of gastric acid secretion effective in the treatment and prophylaxis of gastrointestinal lesions aggravated by gastric acid secretion. In order to assure the quality of R-HCl containing pharmaceutical formulations, several methods have been developed for its determination, including high-performance liquid chromatography (HPLC)¹⁻⁶, high-performance thin-layer chromatography and scanning densitometry⁷, differential pulse polarography⁸, amperometry⁹, capillary electrophoresis¹⁰, conductometry¹¹, coulometry¹², voltammetry¹³, potentiometry¹⁴, fluorimetry¹⁵, chemiluminescence¹⁶, AAS¹⁷. The official method described in the United States Pharmacopeia¹⁸ is based on potentiometric titration of R-HCl with 0.1 mol l⁻¹ NaOH, requiring 300 mg of drug for each titration. Although precise and exact, the procedure is time-consuming. HPLC procedures usually used for R-HCl determination need expensive and complex equipment, also consuming significant amounts of solvents. For these latter reasons, spectrophotometric methodologies, because of their inherent simplicity, low cost and wide availability were extensively proposed for R-HCl determination¹⁹⁻³⁰. These methods are based on redox, coupling, charge-transfer complexation, and ion pair complexation reactions.

The present study describes a simple, rapid, cost-effective, precise, sensitive and accurate spectrophotometric method that is ideal for routine analysis of R-HCl in pharmaceuticals. The proposed spectrophotometric method involves treating a fixed amount of bromate-bromide solution in acid medium with R-HCl solution and determining the unreacted bromine by treating with a fixed amount of RB dye solution and measuring the absorbance at $\lambda_{\text{max}}=557$ nm.

EXPERIMENTAL

Reagents and materials

Pharmaceutical grade ranitidine hydrochloride obtained from Biotend Chemikalien GmbH, Cologne, Germany) was used as received. Pharmaceutical products containing R-HCl were purchased from local commercial sources. Rhodamine B (RB), 9-(2-Carboxyphenyl)-3,6-bis(diethylamino)xanthenium chloride, was provided by Chemos GmbH-Germany. All solutions were prepared in bidistilled water. A stock standard solution containing 35 $\mu\text{g ml}^{-1}$ R-HCl was prepared by dissolving 35 mg of pure drug in water and diluted to the mark in 100 ml calibrated flask. This solution was used for preparation of working standard solutions. A 6×10^{-4} mol l⁻¹ stock solution of RB was prepared by dissolving 28.7 mg of dye (99% purity) in water and diluting to 100 ml in a calibrated flask. A bromate-bromide solution equivalent to 1000 $\mu\text{g ml}^{-1}$ NaBrO₃ and 10-fold excess of NaBr was prepared by dissolving accurately

weighed 100 mg of NaBrO₃ (Merck, Darmstadt, Germany) and 1 g of NaBr (Merck, Darmstadt, Germany) in water and diluting to the mark in a 100 ml calibrated flask. This solution was diluted to obtain working concentration of 30 $\mu\text{g ml}^{-1}$ NaBrO₃. An aqueous solution of hydrochloric acid (5 mol l⁻¹) was prepared by diluting 41.8 ml of concentrated acid (Merck, Darmstadt, Germany, sp. gr. 1.18, 37%) to 100 ml with water.

Apparatus

All absorbance measurements were made with a Jasco V-530 molecular absorption spectrometer provided with matched 1-cm quartz cells.

Potentiometric measurements were carried out using an INOLAB 740 potentiometer (WTW GmbH, Weilheim, Germany). A glass electrode as the indicating electrode was used in conjunction with a double-junction saturated calomel electrode as reference electrode (WTW-GmbH).

Recommended procedure for calibration graph

In each of a series of 10 ml calibrated flasks were placed 0.08-0.9 ml of 35 $\mu\text{g ml}^{-1}$ R-HCl solution by means of a micro burette. To each of the nine flasks, 1 ml of 5 mol l⁻¹ HCl and 1 ml bromate-bromide mixture (30 $\mu\text{g ml}^{-1}$ in KBrO₃) were added and the total volume was adjusted to 4 ml by adding water. The flasks were stoppered and allowed to stand for 10 min, with occasional shaking. Later, 0.5 ml of 6×10^{-4} mol l⁻¹ RB solution was added and the contents were diluted to volume with water and mixed well. The absorbance was measured at 557 nm, after 10 min, against water as reference. Blank experiments were also performed by adding bidistilled water instead of R-HCl solutions. The difference between the absorbance of samples in the presence and in the absence of R-HCl was taken into consideration.

Precision and accuracy

The accuracy and precision of the proposed method was established by determining the content of R-HCl in pure form at three different concentration levels (0.70, 1.75 and 2.81 $\mu\text{g ml}^{-1}$). The intra day precision of the proposed method was performed by carrying out five independent analyses at each concentration level. The precision and accuracy of the spectrophotometric method were compared with those obtained by applying the reference method.

Procedure for recovery tests

The validity of the proposed method was presented by recovery studies using the standard addition method. For this purpose, known volumes (0.1; 0.2 and 0.3 mL) of the stock standard solution of pure R-HCl (35 $\mu\text{g mL}^{-1}$) were added to known volumes (0.2 mL, containing 10 μg R-HCl) of the sample solution and the general procedure applied. Percent recovery of the added pure drug was calculated from: %Recovery = $[(A_1 - A_2)/A_2] \times 100$ where A_1 is the total amount of R-HCl found, A_2 is the amount of R-HCl present in the formulation and found by the proposed method; A_3 is the amount of the pure

R-HCl added to formulation and found by the proposed method.

Analysis of R-HCl in pharmaceutical formulations

The applicability of the proposed method for the determination of R-HCl has been tested on three brands of commercially available pharmaceutical formulations. For this purpose, five tablets of each pharmaceutical product were accurately weighed and ground into fine powder. An accurately weighed portion of the resulting powder, equivalent to 5 mg of R-HCl was dissolved in water. Then it was filtered through a Whatman no. 42 filter paper directly into a 100 ml standard volumetric flask. The residue was washed three times with water for complete recovery of the drug. The washings were added to the volumetric flask which was then filled to the mark with the same solvent, to obtain a solution of $50 \mu\text{g ml}^{-1}$ R-HCl. Aliquots of this solution (0.2 ml), equivalent to $10 \mu\text{g}$ R-HCl were analyzed as described earlier.

Procedure for recovery tests

The validity of the proposed method was presented by recovery studies using the standard addition method. For this purpose, known amounts of pure R-HCl were spiked to its formulated preparation and the total amount of the drug was estimated by applying the proposed procedure.

RESULTS AND DISCUSSION

The proposed spectrophotometric method is indirect and is based on the determination of the residual bromine (*insitu* generated) after allowing the reaction between R-HCl and a measured amount of bromine to be complete. The surplus bromine was determined by reacting it with a fixed amount of Rhodamine-B dye. The method relies on the bleaching action of bromine on the dye, the decoloration being caused by the oxidative destruction of the dye. Various experimental parameters such as dye and bromine concentration, acidity and reaction time were optimized, and the optimum conditions were specified in the proposed procedure.

Effect of dye concentration

The first step in the assay procedure was the determination of the upper limit of the dye that could be determined spectrophotometrically. For this purpose, different volumes of $6 \times 10^{-4} \text{ mol l}^{-1}$ RB solution were acidified with 1 ml of 5 mol l^{-1} HCl and diluted to 10 ml with water. The absorbance was measured at 557 nm. The upper limit of RB that could be measured spectrophotometrically was found to be $3 \times 10^{-5} \text{ mol l}^{-1}$.

Effect of bromate concentration on the oxidation of RB

Preliminary experiments revealed that by adding bromate-bromide mixture in increasing concentrations of bromate, to a fixed concentration of RB, the oxidation and thus the concomitant decrease in the absorbance of RB was observed (Fig. 1). A bromate concentration of $2 \times 10^{-5} \text{ mol l}^{-1}$ was found to irreversibly destroy the colour of $3 \times 10^{-5} \text{ mol l}^{-1}$ RB. The composition of the reaction product between RB and bromine, generated *in situ*, was established by adopting the limiting logarithmic method³¹. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments was carried out using increasing bromate concentrations ($8 \times 10^{-6} - 2 \times 10^{-5} \text{ mol l}^{-1}$) at fixed reagent concentration ($3 \times 10^{-5} \text{ mol l}^{-1}$ RB in a total volume of 10 ml).

Fig. 1 – Absorption spectra of RB in the presence of bromate-bromide mixture.

Without oxidizing agent (a); with oxidizing agent, [Bromate], mol l^{-1} : (b) 0.5×10^{-5} ; (c) 1×10^{-5} ; (d) 1.5×10^{-5} ; (e) 2×10^{-5} ; [RB] = $3 \times 10^{-5} \text{ mol l}^{-1}$; [HCl] = 0.5 mol l^{-1} .

Fig. 2 – Limiting logarithmic plots to evaluate the stoichiometry of Bromate-RB reaction.

(A): variation of [Bromate]; (B): variation of [RB].

The second set of experiments were carried out using increasing RB concentrations ($4 \times 10^{-5} - 7 \times 10^{-5} \text{ mol l}^{-1}$) at fixed bromate concentration ($2 \times 10^{-5} \text{ mol l}^{-1}$). The $\log$ absorbance values were plotted as a function of the $\log$ of the bromate concentration and RB concentration in the first and second sets of experiments, respectively (Fig. 2). The ratio of the slope of two straight lines was 0.98. This means that the reaction proceeds in 1:1 (bromate:dye) stoichiometric ratio. Taking into account that one mole of bromate generates *in situ*, by the action on bromide and in acid medium, three moles of bromine (1):

we assume that, in the experimental conditions, the bromination of RB takes place as is shown in Scheme 1.

Scheme 1 – Reaction mechanism between RB and bromine

Effect of time on the oxidation of RB

The reaction time was determined by following the color decreasing

of RB. After mixing the stoppered cell containing the reaction mixture, the absorbance was measured at 2 min intervals against a blank reagent. The absorbance measurement started at 30 s after adding the bromate solution. The absorbance-time profile of the RB-bromine reaction showed full color decreasing after 10 min. Therefore, the reaction time of 10 min was chosen for color decreasing of RB.

Effect of acid concentration on the oxidation of R·HCl

Hydrochloric acid was the medium of choice for the oxidation of R·HCl by bromine as well as its determination employing RB. The effect of HCl concentration on the R·HCl oxidation was studied from 0.83 to 2 mol l⁻¹ hydrochloric acid solution and 3×10⁻⁵ mol l⁻¹ RB solution. For this purpose, the recommended procedure was applied as follows: in each of a series of 10 ml calibrated flasks were placed 0.5 ml of 10⁻⁴ mol l⁻¹ R·HCl solution by means of a micro burette. To each flask, 1 ml bromate-bromide mixture (30 µg ml⁻¹ in KBrO₃, equivalent to 2×10⁻⁴ mol l⁻¹), 1 ml of 5 mol l⁻¹ HCl and variable volumes of water (0 to 3.5 ml) were added to adjust the acidity in the range 0.83-2 mol l⁻¹. The flasks were stoppered and allowed to stand for 10 min, with occasional shaking. Later, 0.5 ml of 6×10⁻⁴ mol l⁻¹ RB solution was added and the contents was diluted to volume with water and mixed well.

Fig. 3 – Effect of acid concentration on the oxidation of R·HCl.

Acidity of the mixture R·HCl – Bromate, mol l⁻¹ HCl: (1) 0.83; (2) 1; (3) 1.25; (4) 1.67; (5) 2; [Bromate]=2×10⁻⁵ mol l⁻¹; [RB]=3×10⁻⁵ mol l⁻¹.

As is shown in Figure 3, the analytical signal increased with increase of the HCl concentration up to 1.25 mol l⁻¹ and decreases for higher concentrations. Thus, the 1.25 mol l⁻¹ HCl was selected for further work.

Effect of time on the oxidation of R·HCl

According to the "Recommended procedure", two steps are required for samples preparation: in the first one, the mixture R·HCl - bromate - bromide - HCl was set aside for a period (t₁); after oxidation of R·HCl, in the second step, RB was added and the mixture was set aside (t₂) for oxidation reaction between RB and excess of bromine. The oxidation time of R·HCl by bromine was determined using the following conditions: by varying the time of R·HCl-bromine reaction (t₁=5-20 min, at 5 min intervals), at a fixed time of RB-bromine reaction (t₂=10 min). The absorbance time profile of R·HCl-bromine-RB mixture showed maximum color increasing after 10 min. Hence this reaction time of R·HCl with bromine was chosen for further experiments.

Stoichiometry of the reaction between R·HCl and bromine

Stoichiometry of the reaction between R·HCl and bromine was established by adopting the limiting logarithmic method³¹. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments was carried out using increasing bromate concentrations (1.2×10⁻⁵ – 2×10⁻⁵ mol l⁻¹) at fixed R·HCl concentration (5×10⁻⁶ mol l⁻¹ R·HCl in a total volume of 10 ml). The second set of experiments were carried out using increasing R·HCl concentrations (2×10⁻⁶ – 6×10⁻⁶ mol l⁻¹) at fixed bromate concentration (2×10⁻⁵ mol l⁻¹). The log absorbance values were plotted as a function of the log of the bromate concentration and R·HCl concentration in the first and second sets of experiments, respectively (Fig. 4). The ratio of the slope of two straight lines was 0.99. This means that the

reaction proceeds in 1:1 (bromate:R·HCl) stoichiometric ratio. According to literature data²⁵, this behavior confirms that three moles of bromine, generated in situ by the action of bromate-bromide mixture (see equation (1)), reacts with one mole of R·HCl as shown in Scheme 2.

Fig. 4 – Limiting logarithmic plots to evaluate the stoichiometry of Bromate-RB reaction.

(A): variation of [Bromate]; (B): variation of [R·HCl].

Scheme 2 – Reaction mechanism between R·HCl and bromine

Analytical Parameters

Analytical characteristics obtained under the optimum experimental conditions are shown in Table 1. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as follows³²: $LOD = 3.3s_0/b$; $LOQ = 10s_0/b$, where b is the slope and s_0 is the standard deviation of the regression line. In order to apply these formulas, firstly, it was represented the calibration graph, in the concentration range 0.3-3.2 µg ml⁻¹ R·HCl, by using the program Origin. Thus, it was plotted the x_i, y_i points (corresponding to the nine standard solutions) on a xy graph (where x_i =concentration of the standard solution i ; y_i =absorbance of the standard solution i). Then, a linear fit was applied.

Automatically, the calibration line and its parameters appeared on the Origin file. These parameters are:

- Linear regression equation ($A=0.3815 \times [R \cdot HCl]^a - 0.127$)
- Correlation coefficient, r (0.9997)
- Standard deviation of the regression line, s_o (0.009).

These parameters are shown in Table 1.

A comparison between the proposed method and other reported methods is shown in Table 2. As shown in this table, some of the reported procedures^{20,27} have higher detection limits. Moreover, some of the reported methods are alike or more sensitive but have drawbacks such as having a narrow linear dynamic range²⁶, involving extraction^{19,21} or being performed at high temperatures^{22,23,26}.

Table 1 – Analytical data for the regression equation of the proposed method.

Parameter	Value
$\lambda_{\max}$, nm	557
Linear range, $\mu\text{g ml}^{-1}$	0.3-3.2
Correlation coefficient, r	0.9997
Linear regression equation	$A=0.3815 \times [R \cdot HCl]^a - 0.127$
Slope	0.3815
Standard deviation of the regression line, s_o	0.009
LOD, $\mu\text{g ml}^{-1}$	0.08
LOQ, $\mu\text{g ml}^{-1}$	0.24

^a Concentration of $R \cdot HCl$ ($\mu\text{g ml}^{-1}$).

Table 2 – Comparison of the proposed method with existing spectrophotometric methods for the determination of ranitidine.

Reagents	Linear range, $\mu\text{g ml}^{-1}$	LOD $\mu\text{g ml}^{-1}$	LOQ $\mu\text{g ml}^{-1}$	Ref.	Remarks
Bromothymol blue	1-20	-	-	19	Involves extraction and strict pH control
Nitrite- $\text{Cu}^{2+}/\text{Br}^-$	300-12000	100	-	20	Involves flow injection automated assembly, least sensitive
KMnO_4/N -bromosuccinimide-azine dyes	5-30	-	-	21	Involves extraction and uses unstable reagent
7-chloro-4-nitrobenz-2-oxa-1,3-diazole	2-20	0.13	-	22	Requires thermostating at 60°C for 25 min
Tetracyanoquinodimethane	1-6	-	-	23	Requires thermostating at 70°C for 10 min and it is less sensitive
Ce(IV)-chromotrope 2R	0.1-2.8	0.03	0.07	26	Narrow range of linear response. Involve boiling for 5 min and has narrow range of linear response
Ce(IV)-rhodamine 6G	0.1-2.6	0.03	0.06		
Dichromate-diphenylcarbazide	5-50	0.83	2.52	27	Less sensitive
Dichromate-iron(II)-thiocyanate	5-80	1.59	4.83		
Dichromate-iron(II)-orthophenanthroline	10-100	3.07	9.28		
Rhodamine B-bromate-bromide	0.3-3.2	0.08	0.24	Present work	Simple and sensitive

Accuracy and precision

To determine the accuracy and precision of the method, pure $R \cdot HCl$ solutions containing three different concentrations within the linearity range were analysed in five replicates. The results obtained from this investigation are summarised in Table 3. The relative standard deviation ($\leq 1.26\%$) and the percent recovery from 99.67% to 100.36% can be considered to be satisfactory.

Table 3 – Precision and accuracy of the proposed method.

R·HCl, $\mu\text{g ml}^{-1}$		S, $\mu\text{g ml}^{-1}$	RSD, %	R, %
Taken	^a Found $\pm tS/N^{0.5}$			
0.70	0.70 $\pm$ 0.01	0.01	1.06	99.67
1.75	1.76 $\pm$ 0.03	0.02	1.26	100.36
2.81	2.80 $\pm$ 0.01	0.03	0.78	99.70

^aMean $\pm$ 95% confidence limit, for $N=5$; $t=2.77=t$ -distribution for confidence level of 95 % with $N-1$ degrees of freedom S =Standard deviation; RSD=Relative standard deviation; $R(\%)$ =percent recovery.

Recovery study

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. The purpose of the recovery tests was also to verify the accuracy of the proposed method, applied on

real samples. Pre-analysed tablet powder was spiked with pure $R \cdot HCl$ at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The results were reproducible with low standard deviation and the mean recovery ranged from 99.44 - 100.65% (Table 4). Hence, the recovery of the pure drug added was quantitative and revealed that co-formulated substances such as which are commonly presented in pharmaceutical formulations such as talc, starch, gelatin, magnesium stearate, calcium gluconate and calcium dihydrogen orthophosphate, did not interfere with the determination.

Statistical analysis of the results

The performance of the proposed method was judged further by the Student's t -test for accuracy and F -test for precision. At the 95% confidence level, the calculated t - and F -values did not exceed the tabulated values ($t = 2.18$ and $F = 3.63$), indicating that there is a close agreement between the results obtained by the proposed and official methods. Thus, statistical analysis of the results³³, obtained by the official¹⁸ and the proposed method using Student's t -test and variance ratio F -test, show no significant difference between the performance of the two methods regarding the accuracy and precision, respectively (Table 5).

Table 4 – Results of recovery study using standard-addition method.

Tablet brand name ^a	Amount of pure R·HCl in tablet, µg	Amount of pure R·HCl, µg		Amount of pure R·HCl added ± S ^b , µg	Recovery of R·HCl added, %
		added	found		
Ranitidina LPH 150 mg	10	-	9.54	-	-
	10	3.5	13.07	3.53±0.2	100.50
	10	7.0	16.60	7.06±0.13	100.65
	10	10.5	19.98	10.44±0.31	99.44
Ulcoran 150 mg	10	-	9.61	-	-
	10	3.5	13.14	3.53±0.27	100.62
	10	7.0	16.60	6.99±0.13	99.65
	10	10.5	20.14	10.53±0.25	100.33
Zantac 150 mg	10	-	9.75	-	-
	10	3.5	13.27	3.52±0.24	100.40
	10	7.0	16.76	7.01±0.15	99.78
	10	10.5	20.14	10.39±0.19	100.30
^a Ranitidina LPH - LABORMED PHARMA Romania; Ulcoran - EUROPHARM Romania; Zantac - GLAXO WELLCOME SA - Spain ^b Mean of three measurements ± standard deviation (S).					

Table 5 – Application of the proposed method and official methods for the determination of R·HC in pure pure form.

Parameters		
No. of experiments	5	10
Mean found ^a ±SD, %	99.70±0.78	99.93±0.73
RSD, %	0.78	0.73
Variance	0.61	0.53
Student's t-value ^b	0.56 (2.18)	-
F-test value ^b	1.14 (3.63)	-
^a Results obtained at a concentration level of 2.81 µg ml ⁻¹ R·HCl (see Table 3). ^b Values in parantheses are the tabulated values of t and F respectively at confidence level P=95%.		

Application

The proposed method was applied to the determination of R·HCl in three brands of tablets. The results obtained by the proposed method were compared statistically with that of the reference method¹⁸. There is a close agreement between the results obtained by the proposed method and by the official method regarding accuracy (according to t-test) and precision (according to F-test) as shown in Table 6.

Table 6 – Determination of R·HCl content in pharmaceutical tablets using the proposed and the official methods.

Drug formulation	R·HCl Found ^a , %±SD		t-Value ^b	F-Value ^c
	Proposed method	Official method ¹⁸		
Ranitidina LPH, 150 mg R·HCl/tablet	98.25±0.82	99.57±1.02	2.22	1.64
Ulcoran, 150 mg R·HCl/tablet	98.95±1.36	99.68±0.75	1.05	3.29
Zantac, 150 mg R·HCl/tablet	101.36±1.05	100.68±0.94	1.08	1.25
^a Values are mean of five determinations. ^b Tabulated t- value for 95% confidence level for four degrees of freedom is 2.77; ^c Tabulated F- value for 95% confidence level for four degrees of freedom is 6.39.				

CONCLUSIONS

The proposed method is more sensitive than other reported methods, permitting the determination of a concentration down to 0.3 µg ml⁻¹ R·HCl. Also, it is devoid of undesirable heating or extraction step involved in other methods. Therefore, the ranitidine determination not requires extensive preliminary

sample treatment and expensive reagents and can be implemented easily in pharmaceutical laboratories. The method presents accuracy and precision necessary to laboratories quality control. The simplicity of the procedure and reliability of the results recommend the proposed spectrophotometric method to be used for the determination of ranitidine in pharmaceutical formulations.

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