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# NEW REAGENTS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF RANITIDINE HYDROCHLORIDE

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**Abstract:** A new spectrophotometric method is proposed for the assay of ranitidine hydrochloride (RNH) in bulk drug and in its dosage forms using ceric ammonium sulphate (CAS) and two dyes, malachite (MAG) green and crystal violet (CV) as reagents. The method involves the addition of a known excess of ceric ammonium sulphate to ranitidine hydrochloride in acid medium, followed by the determination of unreacted CAS by reacting with a fixed amount of malachite green or crystal violet and measuring the absorbance at 615 or 582 nm respectively against the reagent blank. The Beer's law is obeyed in the concentration range of 0.4-8.0 µg/ml of ranitidine hydrochloride (RNH) for RNH-MAG system and 0.2-1.6µg/ml of ranitidine hydrochloride for RNH-CV system. The molar Absorptivity, Sandell's sensitivity for each system were calculated. The method has been successfully applied to the determination of ranitidine hydrochloride in pure and dosage forms.

**Key words:** Spectrophotometry; Ranitidine hydrochloride; Ceric ammonium sulphate; Malachite green; Crystal violet

## Introduction

Ranitidine hydrochloride (RNH), chemically, is N, N-dimethyl-5-[2-(1-methylamino-2--nitrovinyl) - ethylthiomethyl] furfurylamine hydrochloride. The drug was introduced in the market in 1981. The drug is official in Indian Pharmacopoeia [1]. It is a H2- receptor antagonist and is widely used in short term treatment of duodenal and gastric ulceration, reflux oesophagitis and dyspepsia [2,3]. When used at the usual recommened dose, ranitidine has been found to be safe [4]. Earlier studies have indicated that ranitidine was oxidized by the liver micosomal oxidases and was converted to its N-oxidase, S-oxide and desmethyl metabolites [5,6,7]. Among these hepatic microsomal ranitidine metabolites, ranitidine N-oxide produced by the hepatic microsomal-flavin-containing monooxygenase (FMO) has been found to be the major metabolite excreted in human urine. Thus measuring the amount of ranitidine N-oxide present in urine after administration of ranitidine has been used as a non-invasive method of determining the human liver FMO activity in vivo [8]. Several techniques such as proton magnetic resonance spectroscopy [9], near infrared reflectance spectrometry [10], scintillation proximity assay [11], flow injection fluorimetry [12], polarography [13,14], differential pulse polarography [15], capillary electrophoresis [16], high performance liquid chromatography [17,18] 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. These methods, however, are not adaptable for use in pharmokinetic studies because of their lack of selectivity. Among these instrumental analyti-

cal techniques, spectrophotometric techniques occupies a unique position, because of its simplicity, sensitivity, accuaracy and rapidity.

Literature survey revealed that the only titrimetric method [19] reported for RNH requires 300 mg of drug for each titration. There are few methods for the spectrophotometric determination of ranitidine. These are based on the reaction of ranitidine with some organic acidic dyes followed by extraction of the colored ion-pairs into organic solvents and absorbance measurements. Spectrophotometric determination of ranitidine in tablets has been also suggested through chromogenic reactions with 3-methylbenzothiozline-2--one hydrazone [20], 3,5-dichloro-pbenzoquinone chlorimine [20], Folin-Ciocalteu [21] reagents. These methods, however, are not adaptable for use in automated systems due to the long reaction time for color development (15- 30 min) , they require prior extraction of the colored reaction product and involve a high reaction temperature (-90°C).

In this communication, we demonstrate the use of spectrophotometric techniques for the determination of RNH. The present work involves sensitive, selective and cost effective methods for the determination of ranitidine hydrochloride. The method utilizes ceric ammonium sulphate and two dyes malachite green and crystal violet. Spectrophotometric techniques are in good agreement with the reported methods. In addition, it is not susceptible to interference from common tablet excipients. The developed method has been successfully applied to the determination of ranitidine hydrochloride in pure and dosage form.

## Experimental

### Aparatus

A SHIMADZU UV-2550 UV-VIS Spectrophotometer with 1 cm matched quartz cells were used for the absorbance measurements.

### Reagents and Solutions

All reagents used were of analytical reagent grade and distilled water was used for the preparation of all solutions. A 1000 µg/ml standard drug solution of ranitidine hydrochloride was prepared in distilled water. The stock solution was diluted appropriately to get the working concentration.

Ceric ammonium sulphate (0.01 M) was prepared in 1M sulphuric acid and standardized. This was diluted stepwise to obtain the working concentrations containing 400 µg/ml (RNH--MAG system) and 900 µg/ml (RNH-CV system). Hydrochloric acid (1M), malachite green (0.05%), crystal violet (0.05%) were also used.

### Procedure

### Method A

Different aliquots (0.4- 8.0µg/ml) of RNH were transferred in to a series of 10 ml calibrated flasks by means of a micro burette. Then, 1 ml of 5M HCl was added followed by 1ml of CAS solution. The contents were shaken well and were set aside for 15 minutes with occasional shaking. Then, 1.0 ml of malachite green was added to each flask, and the volume was adjusted up to the mark with distilled water and mixed well. The absorbance of each solution was measured at 615 nm against the corresponding reagent blank. The absorbance corresponds to the bleached color, which in turn corresponds to the drug solution, was obtained by subtracting the absorbance of the blank by that of the test solution.

### Method B

Different aliquots (0.2-1.6 µg/ml) of RNH were transferred in to a series of 10 ml calibrated flasks by means of a micro burette. Then, 1 ml of 5M HCl was added followed by 1ml of CAS solution. The contents were shaken well and were set aside for 15 minutes with occasional shaking. Then, 0.5 ml of crystal violet was added to each flask, and the volume was adjusted up to the mark with distilled water and mixed well. The absorbance of each solution was measured at 582 nm against the corresponding reagent blank. The absorbance corresponds to the bleached color, which in turn corresponds to the drug solution, was obtained by subtracting the absorbance of the blank from that of the test solution.

### Analysis of dosage forms

Weighed an amount of the sample equivalent to about 168mg ranitidine hydrochloride and was dissolved in a sufficient amount of distilled water. The solution was shaken and filtered through Whatman No.1 filter paper and washed with water. The filtrate was diluted up to the mark with distilled water and made up to 100 ml. Suitable aliquots of the sample solution were analyzed by applying general procedure with no modification and the results are shown in table 2 and 3.

**Table 2.** Results of assay of formulations by the proposed method using MAG as reagent

| Sample | RNH certified | Found± SD[a] | Recovery (%) | [a]t-test |
|---|---|---|---|---|
| Zinetac | 168.0 | 168.154±0.495 | 100.090 | 0.703 |
| Rantac | 168.0 | 167. 996±0.118 | 99.900 | 0.075 |
| Zenloc | 168.0 | 168.120±0.463 | 100.070 | 0.579 |

[a]Mean ±Standard deviation (n=5) [mg/tablet], [b]Tabulated t-value at 95% confidence level is 2.78
Rantac- J.B.Chemicals Pharmaceuticals Limited, Gujarat
Zinetac- GlaxoSmithKline Pharmaceuticals Limited, Nashik
Zenloc- Relief Biotech (P) Ltd, Haridwar

**Table 3.** Results of assay of formulations by the proposed method using CV as reagent

| Sample | RNH certified | Found± SD[a] | Recovery (%) | [a]t-test |
|---|---|---|---|---|
| Zinetac | 168.0 | 168.224±0.340 | 100.130 | 1.470 |
| Rantac | 168.0 | 167. 976±0.08 | 99.980 | 0.671 |
| Zenloc | 168.0 | 168.096±0.110 | 100.057 | 1.812 |

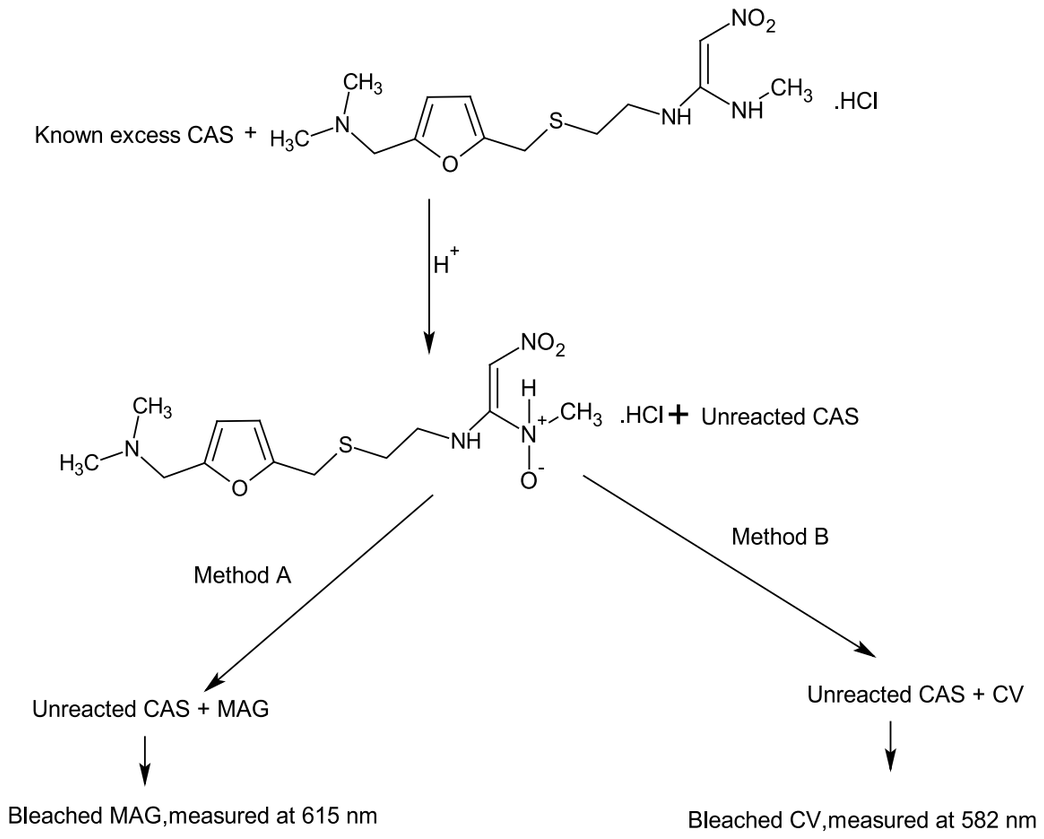
[a]Mean ±Standard deviation (n=5) [mg/tablet], [b]Tabulated t-value at 95% confidence level is 2.78
Rantac- J.B.Chemicals Pharmaceuticals Limited, Gujarat
Zinetac- GlaxoSmithKline Pharmaceuticals Limited, Nashik
Zenloc- Relief Biotech (P) Ltd, Haridwar

## Result and discussion

In this work, a method based on spectrophotometry was developed and validated for ranitidine hydrochloride in pure and dosage form. In recent years, the development of spectrophotometric methods for determinations of drugs has increased considerable, due to their importance, low cost, and simplicity. Before applying an analytical method in the quality control, it is necessary to validate it. The validation testifies that the procedure is suitable for the intended purpose. The International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH 2005) and USP 30 (USP 30, 2007) guidelines describe the analytical parameters that should be evaluated in

a method validation. The type of method and its respective use determine which parameters should be evaluated. It is the responsibility of the analyst to select the parameters considered relevant for each method [28]. The experimental conditions were chosen after testing the different parameters that influence the analysis. The methods involve the addition of a known excess CAS to ranitidine hydrochloride in acid medium, followed by determination of residual CAS by reacting with a fixed amount of either malachite green measuring the absorbance at 615 nm (RNH-MAG system), or crystal violet measuring the absorbance at 582 nm, (RNH-CV system). In the present method all parameters influencing the color development were investigated and are incorporated in the recommened procedure. When added in increasing concentrations to a fixed concentration of CAS, ranitidine consumes the latter proportionally and there is concomitant drop in the remaining concentration of CAS. When a fixed dye concentration is added to decreasing concentrations of CAS, a concomitant increase in the dye concentration results. The reaction mechanism are shown in scheme 1.

**Scheme.**1








**Analytical data**

To assess the linearity, a standard curve for ranitidine hydrochloride was constructed by plotting concentrations versus absorbance and showed good linearity in the range 0.4-8.0µg/ml of ranitidine hydrochloride for RNH-MAG system and 0.2-1.6 µg/ml of ranitidine hydrochloride for RNH-CV system. The correlation coefficients for each system were 0.9945 and 0.9903 indicating good linearity. The accuracy expresses the agreement between the accepted value and the value found. The precision and accuracy of the method was studied by analyzing the coupling solution containing known amounts of the cited reagents within Beer's law limit. Molar absorptivity, Sandell's sensitivity, slope, intercept for RNH--MAG system is found to be $1.10\times10^4$ L mol$^{-1}$ cm$^{-1}$, 0.028 µg cm$^{-2}$, 0.0096, 0.0089 while that for RNH-CV system is found to be $4.09\times10^4$L mol$^{-1}$ cm$^{-1}$ 0.007 µg cm$^{-2}$, 0.0424, 0.0372 respectively. Results of evalution of accuracy and precision for each system are shown in table 4 and 5. The specificity test demonstrated that there was no interference in the determination of the drug.

**Table 4.** Evaluation of accuracy and precision
Ranitidine hydrochloride (Using MAG as Reagent)

| Amount taken (µg mL$^{-1}$) | Amount found[a] (µg mL$^{-1}$) | Recovery (%) | SD | RSD (%) |
|---|---|---|---|---|
| 1.00 | 0.99 | 99.00 | 0.02 | 2.02 |
| 2.00 | 1.96 | 98.00 | 0.04 | 2.04 |
| 3.00 | 2.97 | 99.00 | 0.02 | 0.67 |
| 4.00 | 3.94 | 101.60 | 0.05 | 1.27 |
| 5.00 | 5.08 | 98.50 | 0.03 | 0.59 |
| 6.00 | 6.02 | 100.30 | 0.02 | 0.33 |

a- average of five determinations, SD- standard deviation, RE-relative error

**Table 5.** Evaluation of accuracy and precision
Ranitidine hydrochloride (Using CV as Reagent)

| Amount taken (µg mL$^{-1}$) | Amount found (µg mL$^{-1}$) | Recovery (%) | SD | RSD (%) |
|---|---|---|---|---|
| 0.200 | 0.203 | 101.500 | 0.002 | 0.985 |
| 0.400 | 0.405 | 101.250 | 0.002 | 0.490 |
| 0.600 | 0.607 | 101.160 | 0.002 | 0.329 |
| 0.800 | 0.810 | 101.250 | 0.007 | 0.864 |
| 1.000 | 0.982 | 98.200 | 0.008 | 0.814 |

a- average of five determinations, SD-standard deviation, RE-relative error

Artigo
Article

**Applications**

A new method is described for the spectrophotometric determination of ranitidine hydrochloride. The proposed method is applied to the determination of ranitidine hydrochloride in pure and dosage forms. The comparisons of the reported methods with earlier methods are shown in table 1. The percent recovery of added pure drug which lies between 98.0 and 101.60 reveals that the procedures are free from interference from usual tablet excipients like talc, starch, calcium gluconate, sucrose, etc.

**Table 1.** Comparison of proposed method with earlier methods

| Reagent | Remarks |
|---|---|
| F-C reagent [21] | Less sensitive |
| Bromothymol blue [22] | Involve extraction |
| Rose Bengal [23] | Involve extraction |
| $Hg(SCN)_2$-IRON(III) [24] | Less sensitive |
| KIO3-DCF [25] | Requires strict pH control and less sensitive. |
| $KMnO_4$/NBS azine dyes [26] | Involves extraction. |
| Proposed methods | Sensitive<br>Selective, no interference from usual tablet excipients. |

**Conclusions**

The method is sensitive, enabling the accurate and precise determination of the analytes over satisfactory concentration ranges without the need of special or laborious sample-pretreatment steps. The method, which is advantageously time- and cost-efficient, was successfully applied to the quantification of the analytes in commercial samples, with results being in good statistical agreement with the reported methods; therefore, it is considered useful for routine quality monitoring of pharmaceuticals.

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