Determination of ranitidine using potassium iodate and dichlorofluorescein

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Two methods for determining ranitidine hydrochloride (RNH) in pure drug and in formulations using potassium iodate and dichlorofluorescein are described. Titrimetry involves the oxidation of RNH by a known excess of potassium iodate in acidic conditions followed by iodometric determination of surplus iodate. In spectrophotometry also, the drug is oxidized by a large excess of iodate and the iodine released is oxidized to ICl$_2^-$ in the presence of chloride ions, and is used to iodinate 2,7-dichlorofluorescein dye and the amount of iodinated dye is measured. Reaction conditions of both methods have been optimized. In titrimetry, the reaction stoichiometry has been established and the reaction scheme of the spectrophotometric method is given. Titrimetry is applicable over 1-16 mg range. In spectrophotometry, the system obeys Beer’s law for 5-50 μg mL$^{-1}$. The molar absorptivity and Sandell sensitivity were calculated to be 3.88×10$^3$ L Mol$^{-1}$ cm$^{-1}$ and 5.72 ng cm$^{-2}$, respectively. The calculated limits of detection and quantification were 2.14 and 7.15 μg mL$^{-1}$, respectively. The proposed methods were applied successfully to the determination of RNH in pharmaceutical preparations with recoveries in the range of 98.28 ± 0.88 to 103 ± 1.96% (titrimetry) and 99.46 ± 1.88 to 102.58 ± 0.73% (spectrophotometry). The reliability of the assay was established by parallel determination by an established procedure and by recovery studies using standard-addition technique.

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Ranitidine, N-(2-[(5-dimethylamino)-methyl]-2-furanyl)-2-methyl-thioethyl] - N$'$-methyl-2-nitro-1,1$'$-ethylenediamine (Fig. 1), is a histamine H$_2$-blocker commonly used in clinical practice for the treatment of duodenal ulcers. It provides a new and effective therapeutic approach to gastric hypersecretory diseases$^1$. This nitroalkene has been determined by high-performance liquid chromatography$^{2-7}$, capillary electrophoresis$^8$, spectrofluorimetry$^9$, near infrared spectrophotometry$^{10}$, proton magnetic resonance spectroscopy$^{11}$, voltammetry$^{12,13}$, differential pulse polarography$^{14}$, osilopolarography$^{15}$ and gas chromatography-mass spectroscopy$^{16}$. The techniques are tedious, time consuming, and involve expensive instrumental set-up. Only two titrimetric procedures are available for the determination of RNH in dosage forms. Atkosar and Tuncel$^{17}$ have assayed RNH in tablets by titrating the drug with NaOH with pHmetric end-point detection. The method requires 300 mg of active ingredient for each titration. Very recently, Hamdan and Taha$^{18}$ have reported the use of complexation properties of RNH with metal ions, which served the basis for the spectrophotometric and conductometric titrimetric methods for the determination of the drug.

Visible spectrophotometric methods based on redox$^{19,20}$, oxidative-coupling$^{21}$, charge-transfer complex formation$^{22}$, ion-association complex formation$^{23,24}$ and nitrosation$^{25}$ reactions are reported in the literature. These reported spectrophotometric methods possess deficiencies such as extraction step$^{23,24}$, long contact time$^{21}$, and poor sensitivity$^{19,22,25}$. In the present work, an effort has been made to develop simple, sensitive, selective and cost-effective methods utilizing the thio group of the molecule.

The titrimetric procedure is based on the oxidation of the drug by a known excess of iodate in acidic conditions followed by the iodometric back titration of the surplus oxidant after the reaction is judged to be complete. However, in spectrophotometry, the drug is treated with a large excess of iodate in acidic conditions, and the iodine released is oxidized to ICl$_2^-$ in the presence of chloride ions, and is used to iodinate 2,7-dichlorofluorescein dye and the absorbance of the iodinated dye is measured at 520 nm.

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Experimental Procedure

Apparatus
A Systronics model 106 digital spectrophotometer, provided with 1-cm quartz cells was used for absorbance measurements.

Reagents and solutions
All chemicals used were of analytical reagent grade and double distilled water was used throughout the study. A 0.002 M potassium iodate solution was prepared by dissolving 0.428 g of the reagent (Sarabhai M. Chemicals, Baroda, India) in water and diluting to one litre. A ~ 0.01 M solution of sodium thiosulphate was prepared by dissolving 2.48 g of reagent in water and diluting to one litre and was standardized iodometrically. A 5 M hydrochloric acid was prepared by diluting 439 mL of concentrated acid (Qualigens Fine Chemicals, Mumbai, India) Sp. gr. 1.18 to one litre. Potassium iodide (10%) and starch solutions were prepared in the usual way and used for titrimetric work. For spectrophotometric work, potassium iodate (0.4%) and sodium chloride (6%) solutions were prepared by dissolving requisite amounts in water and diluting to definite volumes. A 0.01% dichlorofluorescein was prepared by dissolving 25 mg of reagent (May and Baker Ltd., Dagenham, England) in 0.5 mL of 1 M sodium hydroxide and diluting to 250 mL with water. Monochloroacetic acid reagent was prepared by dissolving 1 g of reagent (s.d. Fine Chem. Ltd., India) in 30 mL water, and mixing with a solution of sodium hydroxide containing 8 g in 30 mL of water and diluting to 100 mL with water. The pH of this reagent was found to be 4.35. A 1 M hydrochloric acid was prepared by appropriate dilution of 5 M acid prepared above. Pharmaceutical grade RNH was received as gift from Torrent Pharmaceuticals Ltd., Ahmedabad, India, and was used as received. A stock solution containing 2 mg mL$^{-1}$ of RNH was prepared as follows: 200 mg of RNH was weighed accurately and dissolved in 60 mL of water in a beaker and treated with 500 mg of zinc dust and 5 mL of 5 M HCl. After keeping for 30 min at room temperature, the solution was filtered through Whatman No. 41 filter paper, residue was washed with water and diluted to volume in a 100 mL calibrated flask and kept in amber coloured bottle and stored in a refrigerator (4°C). Working standard solution (200 µg mL$^{-1}$) for spectrophotometry was prepared from the stock solution on each day of analysis.

General methods

Titrimetry

Reaction stoichiometry
Different known amounts of reduced RNH were subjected to reaction with potassium iodate and the stoichiometric ratio was calculated from the amount of potassium iodate consumed.

Method
A 10 mL aliquot of pure drug solution containing 1-16 mg of reduced RNH was pipetted into a 100 mL titration flask and the solution was acidified by adding 5 mL of 5 M HCl. Then, 10 mL of 0.002 M iodate solution were added by means of pipette, the contents were mixed well and kept aside with occasional shaking. After 20 min, the solution was boiled for 2 min, 5 mL of 10% potassium iodide solution were added and the liberated iodine was titrated with 0.01 M thiosulphate to a starch end-point. A blank titration was performed simultaneously, and the amount of the drug in the aliquot was calculated from:

$$\text{Amount (mg)} = 1053.63 \left( B - S \right) R$$

where $B =$ volume of thiosulphate solution used in the blank titration, mL; $S =$ volume of thiosulphate solution used in the sample titration, mL and $R =$ molarity of iodate solution.

Spectrophotometry

Aliquots of standard reduced RNH solution (0.25-2.5 mL; 200 µg mL$^{-1}$) were delivered into a series of 10 mL calibrated flasks. The solution was acidified by adding 0.5 mL of 1 M HCl and the total volume was adjusted to 4 mL by adding water. Then, 1 mL each of 0.4% iodate and 6% sodium chloride solutions were added in succession, the contents were mixed well and the flasks were set aside for 20 min with occasional shaking. Finally, 2 mL each of 0.01% 2,4-dichlorofluorescein solution and monochloro acetic acid reagent (pH 4.35) were added to each flask and the volume was diluted to the mark with water. The absorbance of the coloured solution was measured at 520 nm against a reagent blank. A calibration graph was prepared by plotting the increase in absorbance as a function of drug concentration. The concentration of the unknown was read from the calibration graph or computed from the regression equation deduced from the Beer’s law data.
**Procedure for dosage forms**

Twenty tablets were weighed accurately and ground into a fine powder. A portion of the powder equivalent to 200 mg of RNH was weighed accurately into a 100 mL volumetric flask, 60 mL of water, 5 mL of 5 M HCl and 500 mg of zinc dust were added and shaken thoroughly for about 30 min. Then, the volume was made up to the mark with water, mixed well and filtered using Whatman No. 41 filter paper. A suitable aliquot was used for analysis by titrimetry. The solution was appropriately diluted with water to get 200 \( \mu g \) mL\(^{-1} \) solution and analysed spectrophotometrically using a convenient volume. In the case of injectable products, known volumes equivalent to 200 mg of RNH were measured accurately into a 100 mL beaker, 60 mL of water, 5 mL of 5 M HCl and 500 mg of zinc dust were added and stirred for 30 min. The insoluble mass was filtered on a Whatman No. 41 filter paper, washed with water and the filtrate plus washings were diluted to 100 mL with water in a calibrated flask. The solution (2 mg mL\(^{-1} \)) was subjected to analysis by titrimetry and spectrophotometry as described above after appropriate dilution.

**Results and Discussion**

The proposed methods are based on the oxidation of S-atom of the RNH molecule by iodate. In titrimetry, the drug was reacted with a known excess of iodate, and after oxidation, the residual iodate was determined by iodometric titration. On the other hand, in spectrophotometry, the drug was treated with a large unmeasured excess of iodate and the iodine formed was determined by an auxiliary reaction with dichlorofluorescein. In both the methods, reduction of nitro group of the molecule with zinc/HCl was the first step. Unreduced RNH was found to give erratic results.

**Titrimetry**

Potassium iodate was found to react quantitatively with reduced RNH in HCl medium. A 5.0 mL volume of 5 M acid in a total volume of 25 mL was found adequate; although 3-10 mL resulted in the same value of ‘n’. Stoichiometric study revealed that three moles of reduced RNH reacted with 1 mole of iodate. The reaction stoichiometry indicates that only S-atom of the molecule is oxidized and other sites of the molecule are unaffected. For the 1-16 mg range studied, 10 mL of 0.002 M iodate solution were found adequate for the complete oxidation of the drug. Though the oxidation was complete in 15 min, contact times up to 45 min had no effect on the stoichiometry or the results. The linearity between the amount of the drug and titration end point is apparent from the correlation coefficient of \(-0.9980\) suggesting that the reaction between reduced RNH and iodate proceeds stochiometrically in the ratio 3:1. Accurate results were obtained when iodine released in the first step of the reaction was expelled by boiling. Otherwise negative error up to 4-6% was observed.

**Spectrophotometry**

Several substances of pharmaceutical interest\(^ {27-34}\) have been determined by measuring the iodine released in the redox reaction between the substrate and iodate in acid medium. In the proposed method, oxidation of reduced RNH is effected by a large excess of iodate (1 mL of 0.4%) in the presence of 0.5 mL of 1.0 M HCl at ambient temperature, and the oxidation of the drug to sulphoxide was complete in 20 min. The liberated iodine was further oxidized by the excess of iodate in the presence of excess of chloride ions (1 mL of 6%) to ICl\(_2\). This species is a better iodinating agent than iodine and was used to iodinate 2',7'-dichlorofluorescein to 2',7'-dichloro-4',5'-diiodofluorescein (Fig. 2) (Ref. 35-37). One mL of 0.01% dye was found sufficient for the reaction. In order to observe a distinct variation in colour between dichlorofluorescein and the iodinated dye, the pH of the medium was varied using different buffer systems. The maximum colour intensity with desired-low-blank absorption was observed at 520 nm (Fig. 3) in the pH range of 3.25 \( \pm \) 0.1. This pH could be maintained by the addition of 2 mL of monochloro acetic acid of pH 4.35, and at this pH, the iodinated dye was found to be stable for more than 18 h.

The absorbance of the iodinated dye measured was found to be linearly dependent on the concentration of reduced RNH, which served as the basis of the assay. Reduced RNH, when added in increasing concentrations consumes iodate, and consequently there will be a concomitant increase in the concentration of iodine released, the reduced form of...
iodate. This is observed as a proportional increase in the absorbance of the iodinated dye on increasing the concentration of reduced RNH (Fig. 3). The increasing values of absorbance at 520 nm were plotted against increasing concentration of reduced RNH to obtain a standard graph (Fig. 4).

A linear correlation was found between absorbance \( A \) and concentration \( C \) over the range 5-50 \( \mu g \) \( mL^{-1} \). The linear regression equation was:

\[
A = 8.3 \times 10^{-4} + 0.011 C; \ r = 0.9958 \ (n = 5).
\]

The apparent molar absorptivity and Sandell sensitivity were \( 3.88 \times 10^3 \) L mol\(^{-1}\) cm\(^{-1}\) and 5.72 ng cm\(^{-2}\), respectively. The limit of detection was 2.14 \( \mu g \) \( mL^{-1} \) and the limit of quantification was 7.15 \( \mu g \) \( mL^{-1} \).

**Accuracy, precision, and ruggedness**

The accuracy of the methods was ascertained by determining the pure drug in three levels by the proposed methods. The precision was established by performing seven replicate determinations on the same solution containing the drug in three levels. The per cent error and RSD values are compiled in Table 1, and are indicative of the high accuracy and precision of the proposed methods.

The ruggedness of the methods was tested by determining the precision on day-to-day basis. Analyses were performed on the same amounts/concentrations (used to evaluate the within–day repeatability) each day for five days. Analysis of variance applied to the results showed that between–day variability, as expected, was greater than within–day variability. In terms of relative standard deviations, the within-day values were less than 2.5% and between-day values were within 3%. The latter figure probably represents the best appraisal of the precision of the procedure in routine analysis.

**Application of dosage forms**

The proposed methods were applied to the analysis of RNH in 4 brands of tablets and 4 brands of injections. The results are summarized in Table 2 and were checked by a reported method\(^{20}\). The results are in close agreement between the results obtained by the proposed and established methods as found from the Student’s t- and F-values. The results obtained by proposed methods also agreed well with the label claim values on the tablets and injections in all instances.

To further establish the validity of the methods, recovery tests were carried out by standard-addition technique. To a fixed amount of the drug in tablets

<table>
<thead>
<tr>
<th>Amount taken (mg)</th>
<th>Amount found* (mg)</th>
<th>Error (%)</th>
<th>RSD (%)</th>
<th>Amount taken (µg)</th>
<th>Amount found* (µg)</th>
<th>Error (%)</th>
<th>RSD (%)</th>
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<tr>
<td>5.00</td>
<td>5.11</td>
<td>2.20</td>
<td>2.48</td>
<td>10.00</td>
<td>10.16</td>
<td>1.60</td>
<td>1.42</td>
</tr>
<tr>
<td>10.00</td>
<td>10.08</td>
<td>0.80</td>
<td>1.06</td>
<td>20.00</td>
<td>20.25</td>
<td>1.25</td>
<td>0.71</td>
</tr>
<tr>
<td>15.00</td>
<td>14.83</td>
<td>1.13</td>
<td>1.91</td>
<td>30.00</td>
<td>30.43</td>
<td>1.43</td>
<td>2.18</td>
</tr>
</tbody>
</table>

* Average value of seven determinations.
RSD–Relative standard deviation.

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Fig. 3—Absorption spectrum; A: Reagent Blank, B: 500 \( \mu g \) of RNH + 0.5 mL 1 M HCl + 1 mL of 0.4% KIO\(_3\) + 1 mL 6% NaCl + 2 mL of 0.01% dichlorofluorescein + 2 mL of pH 4.35 buffer in 10 mL.

Fig. 4—Calibration curve (0.5 mL 1 M HCl + 1 mL of 0.4% KIO\(_3\) + 1 mL 6% NaCl + 2 mL of 0.01% dichlorofluorescein + 2 mL of pH 4.35 buffer in 10 mL.)
Table 2—Results of analysis of tablets containing RNH by the proposed methods

<table>
<thead>
<tr>
<th>Formulation brand name*</th>
<th>Claim mg per tablet or per mL</th>
<th>FoundΨ (% recovery ± SD)</th>
<th>Student’s t- value#</th>
<th>F - value$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Titrimetry (T)</td>
<td>Spectrophotometry (S)</td>
<td>Reference method T</td>
</tr>
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<td>Tablets</td>
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<tr>
<td>Ranitina</td>
<td>150</td>
<td>99.62 ± 0.36</td>
<td>99.12 ± 0.42</td>
<td>99.56±0.28</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>101.36±0.56</td>
<td>100.64±1.04</td>
<td>101.84±0.86</td>
</tr>
<tr>
<td>Histac</td>
<td>150</td>
<td>97.51 ± 0.85</td>
<td>98.26 ± 0.38</td>
<td>98.72±0.66</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>101.42±0.28</td>
<td>99.94 ± 0.66</td>
<td>100.84±0.52</td>
</tr>
<tr>
<td>Zinetac</td>
<td>150</td>
<td>98.34 ± 1.26</td>
<td>99.08 ± 0.74</td>
<td>99.26±0.48</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>99.14±0.76</td>
<td>99.62 ± 1.06</td>
<td>100.18±0.43</td>
</tr>
<tr>
<td>Acilocd</td>
<td>150</td>
<td>100.36±0.92</td>
<td>98.74 ± 1.12</td>
<td>100.44±0.63</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>99.20±1.26</td>
<td>100.62 ± 0.84</td>
<td>102.31±1.86</td>
</tr>
<tr>
<td>Injections</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranitina</td>
<td>25</td>
<td>101.21 ± 0.64</td>
<td>101.63 ± 0.97</td>
<td>100.63±1.32</td>
</tr>
<tr>
<td>Histac</td>
<td>25</td>
<td>102.64 ±0.98</td>
<td>101.96 ±1.46</td>
<td>103.10±2.42</td>
</tr>
<tr>
<td>RNHc</td>
<td>25</td>
<td>98.43 ± 1.86</td>
<td>99.72 ± 2.87</td>
<td>97.29±1.26</td>
</tr>
<tr>
<td>Acilocd</td>
<td>25</td>
<td>99.04±2.58</td>
<td>100.86 ± 0.72</td>
<td>101.30±1.36</td>
</tr>
</tbody>
</table>

Ψ Average of five determinations.
c. Glaxo Smithkline Pharm. Ltd.; d. Cadila Pharm.
# Tabulated t-value at 95% confidence level 2.77.
@ Tabulated F-value at 95% confidence level 6.39.

and injections (pre-analysed), pure drug in three different levels was added and the total was found by the proposed methods. The per cent recovery values of the pure drug added shown in Table 3, demonstrates that the excipients and additives do not interfere in the methods.

Conclusions
Two simple and economically viable methods using potassium iodate as the oxidimetric reagent are reported. Titrimetry is rapid and applicable over a long and dynamic semimicro range, whereas the pH metric method requires 300 mg of RNH for each determination. The spectrophotometric method is novel, fairly sensitive besides being accurate and precise. Only simple equipment is needed, unlike most methods previously reported, which involve expensive instrumental set-up.

Acknowledgement
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References