Chapter-III

TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF RANITIDINE HYDROCHLORIDE
SECTION 3.0

DRUG PROFILE AND LITERATURE SURVEY

3.0.1. DRUG PROFILE

Ranitidine hydrochloride (RNH) is a new histamine H2-receptor antagonist drug having the chemical name N-(2-{{(5-dimethylamino) methyl]-2-furanil} methyl thio ethyl) N'-methyl-2-nitro-1,1'-ethylenediamine. It has the empirical formula C_{13}H_{23}N_{4}O_{3}S\cdot HCl and has the molecular weight 350.18.

It was synthesised by J. Bradshaw [1]

[Fr. Apt. 2,384, 765 & U. S. Pat. 4,128,658 (Both 1978 to Allen & Hanbury)]

It has the following structure:

\[
\begin{align*}
\text{(H}_3\text{C)}_2 \text{H}_2 \text{CN} \text{CH}_2 \text{S} \text{(CH}_2\text{)}_2 \text{NH} \text{C=CH NO}_2 \text{HCl} \\
\text{CH}_3 \text{NH}
\end{align*}
\]

RNH is marketed only as hydrochloride salt. It is a white-yellowish solid with little or no odour. A light mercapta odour may be present and freely soluble in water, acetic acid, methanol, sparingly soluble in ethanol and practically insoluble in chloroform and dioxane.

RNH competitively inhibits the action of histamine on the H2 receptors of parietal cells, reducing gastric acid secretion under daytime and nocturnal basal conditions and also when stimulated by food, insulin, histamine or pentaglandin. The drug is used for the short-term treatment of active duodenal ulcer, active and benign gastric ulcer, for the treatment of pathogenic gastrointestinal hypersecretory conditions and to provide short-term symptomatic relief of gastro esophageal reflux [2].
3.0.2. LITERATURE SURVEY ON THE TITRIMETRIC AND SPECTROPHOTOMETRIC DETERMINATION OF RNH

Ranitidine hydrochloride (RNH) has two analytically active useful functional groups, nitro and sulphydril, and all two functions have been adapted for analytical purposes. In the following paragraphs, the titrimetric and spectrophotometric methods developed for RNH is briefly reviewed.

Titrimetric methods

Literature survey revealed that, only three titrimetric procedures are available for the determination of RNH in dosage forms. Atkosar and Tuncel [3] have reported for the determination of RNH in tablets by titrating the RNH with NaOH with pH-titration end point. The method requires 300 mg of active ingredient for each titration. Recently, Hamdan and Taha [4] have reported the use of complexation properties of RNH with metal ions, which served the basis for the conductometric method. Very recently, Somashekar and Basavaiah [5] have reported for a redox titration using N-bromosuccinimide as oxidimetric reagent. In this method, the acidified drug solution was directly titrated with NBS in presence of excess potassium bromide using methyl orange as indicator. The method is applicable over 3-20 mg range.

Visible spectrophotometric methods

Several visible spectrophotometric methods has been reported for the assay of RNH in bulk drug as well as in dosage forms. Sastry et al. [6] have reported three indirect spectrophotometric methods based on the redox reaction between RNH and oxidants such as chloramine-T (CAT), N-bromosuccinimide (NBS) or potassium permanganate in acidic medium. The mixture was equilibrated for 10 min and mixed with 5-phenoxazinium-1-carboxy-7-dimethylamino-3,4-dihydroxy chloride, 5-phenoxazinium-1-(aminocarbonyl)-7-diethylamino-3,4-dihydroxy chloride or benzo [alpha] 5-phenoxazineium-5-imomp-9-aminoacetic acid salt solutions for (CAT), (NBS) or (KMnO₄) methods, respectively. The absorbance of coloured solutions was measured at 540 nm in CAT and NBS methods and 555 nm in KMnO₄ method. Beer’s law was obeyed over 0.5-4.0, 0.4-2.8 and 0.4-2.8 µg ml⁻¹ for CAT, NBS and permanganate methods, respectively. Rao et al. [7] has been reported
a method in which the Folin-Ciocalteu reagent was reduced to a blue chromogen in basic solution and the absorbance measured at 765 nm. Beer's law was obeyed in concentration range 40 - 240 μg ml⁻¹. Recently, Amin et al. [8] have reported three procedures based on the use of N-bromosuccinimide and cerium (IV) sulphate as oxidimetric reagents, and amaranth, chromotrope2R and rhodamine 6G as chromogenic agents. Though the methods are reported to be sensitive with molar absorptivity values of 1.31 x 10⁵, 1.91 x 10⁵ and 1.74 x 10⁵ respectively, the optimum experimental conditions are tedious and time consuming since they involve boiling for 30 min at 100° C for an quantitative reaction.

Oxidative coupling reaction of RNH with 3-methylbenzolin-2-one hydrazone and FeCl₃ in acid medium has been reported by Rao et al. [9]. The absorbance of the solution was measured at 615 nm after 30 min and the linearity of concentration range 0.4 - 6.0 μg ml⁻¹.

Emmaunel et al.[10] have reported a method which was based in charge-transfer complexation between RNH and 3,5-dichloro-p-benzoquinonechlorimine in alcohol medium and which required heating at 90°C for 20 min. The absorbance was measured at 520 nm and Beer's law was applicable in the concentration range 10-50 μg ml⁻¹. The charge-transfer complexation reaction between RNH with 7,7,8,8-tetracyanoquinolmethane has been reported by Belal [11] which required heating for 10 min at 70°C. The Beer's law is applicable in the concentration range 1- 6 μg ml⁻¹.

Extractive spectrophotometric method [12] which was based on the ion-pair complex formation between RNH with bromothymol blue has been advanced by Ozsoy and Guvener. The resulting complex was extracted with CH₂Cl₂ and the absorbance measured at 409 nm. The calibration graph was rectilinear for 1-20 μg ml⁻¹. Another flow-injection spectrophotometric method [13] also based on liquid-liquid extraction using bromothymol blue as the ion-pair reagent is also found in the literature. Sastry et al. [14] suggested a method based on condensation reaction involving RNH and 1, 2-natphthaquinone-4-sulfonic acid and the absorbance of coloured product was measured at 500 nm. Ahmad et al. [15] have reported a method based on condensation reaction using 1,4-benzoquinone at pH 5.6. The absorbance of resulting condensation product was measured at 502 nm and the concentration adhering to Beer's law was 20-100 μg ml⁻¹.
Recently, Hassan and Belal [16] has been reported a kinetic spectrophotometric method which involves the reaction of the RNH with alkaline potassium permanganate, where by colour peaking at 610 nm is produced. The reaction is monitored spectrophotometrically measuring the rate of change of absorbance of the resulting manganese species at 610 nm. The Beer’s law was applicable in the range 0.8 - 4.0 µg ml⁻¹.

**Other techniques**

In addition, other analytical techniques were found in the literature for determination of RNH include HPLC [17-23], polarography [24,25], differential pulse polarography [26], oscillopolarography [27], linear sweep-voltammetry [28], gas chromatography-mass spectrometry [29], proton magnetic resonance spectroscopy [30], near infrared reflectance spectrophotometry [31], UV spectrophotometry [32-35], automated fluorimetry [36], and capillary electrophoresis [37].

From the foregoing paragraphs, it can be seen that the aqueous acid-base titrimetry [3] and conductometry [4] are applicable over macro scale, and the redox method proposed by Basavaiah and Somashekar [5] requires daily standardization of NBS. Visible spectrophotometric methods, although sensitive, suffer from one or other disadvantage as indicated in Table 3.0.1. Methods based on other techniques are generally tedious and requires expensive instrumentation. In addition, some of them are applicable for combined dosage forms.

Thus, a need was felt for simple, sensitive and cost-effective methods for the determination of RNH in pharmaceuticals. The author has studied the reaction of RNH with bromate-bromide mixture, chloramine-T, iodate and Folin-Ciocalteu reagent, and has developed four titrimetric and seven spectrophotometric methods for the assay of RNH based on redox reactions. Titrimetric and spectrophotometric methods have also been developed in non-aqueous medium using perchloric acid as the titrant and crystal violet as the visual indicator or spectrophotometric reagent. The details are presented five sections in this chapter.
Table 3.0.1. Reported visible spectrophotometric methods for the assay of RNH

<table>
<thead>
<tr>
<th>Reagent/s used</th>
<th>λ_{max} nm</th>
<th>Beer's law limits, µg ml^{-1}</th>
<th>Molar absorptivity, 10^4 l mol^{-1} cm^{-1}</th>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azine dye (CI 50316)</td>
<td>600</td>
<td>5 - 30</td>
<td>0.53</td>
<td>Extraction is involved</td>
<td>6</td>
</tr>
<tr>
<td>CAT - PCDD*</td>
<td>540</td>
<td>0.5 - 4.0</td>
<td>1.83</td>
<td>Uses an expensive dye</td>
<td>6</td>
</tr>
<tr>
<td>KMnO4 - BPIA*</td>
<td>555</td>
<td>0.4 - 2.8</td>
<td>4.21</td>
<td>Uses an unstable oxidant and an expensive dye</td>
<td>6</td>
</tr>
<tr>
<td>NBS - PADD*</td>
<td>540</td>
<td>0.4 - 2.8</td>
<td>7.28</td>
<td>Uses an unstable oxidant and an expensive dye</td>
<td>6</td>
</tr>
<tr>
<td>F-C reagent NBS, Ce(IV) sulphate</td>
<td>760</td>
<td>40 - 240</td>
<td>-</td>
<td>Lacks sensitivity</td>
<td>7</td>
</tr>
<tr>
<td>Fe^{3+} - MBTH*</td>
<td>615</td>
<td>1.4 - 5.76</td>
<td>-</td>
<td>Involves heating at 100°C for 30 min; Uses an expensive reagent (MBTH); contact time 30 min</td>
<td>9</td>
</tr>
<tr>
<td>DCBC</td>
<td>520</td>
<td>10 - 50</td>
<td>-</td>
<td>Involves heating at 90°C for 20 min; less sensitive</td>
<td>10</td>
</tr>
<tr>
<td>TCQ</td>
<td>1 - 6</td>
<td>-</td>
<td>-</td>
<td>Involves heating at 70°C for 10 min; less sensitive</td>
<td>11</td>
</tr>
<tr>
<td>Bromothymol Blue</td>
<td>409</td>
<td>1 - 20</td>
<td>-</td>
<td>Critical pH; extraction is involved</td>
<td>12</td>
</tr>
</tbody>
</table>

* F - C reagent. Folin - Ciocalteu reagent;
PCDD. 5-Phenoxazinium-1-carboxy-7-dimethylamino-3, 4-dihydroxy chlorides;
BPIA. Benzo [alpha] 5-phenoxazineum-5-imino-9-amino acetic acid salt;
PADD. 5-Phenoxazinium-1- (amino carbonyl)-7-diethylamino-3, 4-dihydrochloride;
MBTH. 3-methyl-2-bezothioazolinone-hydrazone hydrochloride;
DCBC. 3,5-dichloro-p-benzoquinone chlorimine;
NBS. N-bromosuccinimide;
TCQ. 7,7,8,8-tetracyanoquinolmethane
SECTION 3.1

TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF RNH USING BROMATE-BROMIDE MIXTURE AND TWO DYES, INDIGO CARMINE AND METANIL YELLOW

3.1.1.0. INTRODUCTION

In acid solution bromate is a strong oxidant ($E^0 = 1.52$); bromide is formed in the first stage of the reaction and then reacts with excess bromate to yield free bromine.

$$\text{BrO}_3^- + 6 \text{Fr} + 6 \text{e}^- \rightarrow \text{Br}^- + 3 \text{H}_2\text{O}$$

$$\text{BrO}_3^- + 5 \text{Br}^- + 6 \text{H}^+ \rightarrow 3\text{Br}_2 + 3 \text{H}_2\text{O}$$

It is usual to add bromide to the test solution before the titration or to include in the standard bromate solution so that only the second reaction is involved.

An acidified mixture of bromate and bromide actually behaves as an equivalent solution of bromine. Thus the stable bromate-bromide solution serves for the extemporaneous preparation of a standard solution of bromine. Aqueous bromine solutions are unstable because of high vapour pressure of bromine.

The oxidizing action of bromate appears to have been noted by Balard [38], but the first application of bromate as titrimetric reagent was due to Koppeschaar [39], who used it in his well-known bromination procedure for the determination of phenol. Kratschmer [40] first recommended the use of bromate as an oxidimetric reagent. Since then, the reagent in combination with bromide has found applications in titrimetric determination of phenolic steroids [41], chlortetra cycline [42], propranolol [43], ledol [44], ascorbic acid [45-47], amino salicylic acid [48], citral [49], thamine HCl [50,51], oxyphenbutazone [52], cimetidine [53], secobarbital [54], carbimazole [55], albendazole [56], salbutmol sulphate [57], adrenergic drugs [58], sulphonamides [59], tuberculo statics [60], chloropromazine [61], frusemide [62], famotadine [63] and phenothazines [64]. And spectrophotometric determination of some pharmaceutical substances such as propranolol [43], albendazole [56], salbuatmol sulphate [57], adrenergic drugs [58], chloropromazine [61], frusemide [62], famotadine [63] and phenothazines [64].
[62], famotidine [63] and mepazine [65], has also been reported by several workers using bromate-bromide mixture in acid medium as an oxidimetric and/or brominating agent.

From the literature survey presented in Section 3.0.2 and from the above paragraphs in which the applications of bromate-bromide mixture in the titrimetric analysis of some pharmaceuticals are cited, it is evident that RNH has not been determined using bromate-bromide as a titrimetric reagent. An attempt has been made to assay RNH titrimetrically using bromate-bromide.

Many dyes are irreversibly oxidized/destroyed to colourless products by oxidizing agents in acid medium [66] and this observation has been conveniently utilized for the indirect spectrophotometric determination of many oxidisable pharmaceuticals [6,8,67-70]. Gyor [71] used methyl orange as indicator in the direct titration with bromate-bromide reagent in acid medium, the dye stuff being irreversibly bleached at the end-point. This bleaching action has successfully been used for the indirect spectrophotometric assay of a wide ranging of pharmaceuticals [43-65].

Based on the bleaching action of bromine on indigo carmine and metanil yellow, two indirect spectrophotometric methods have been developed for the assay of RNH. The details regarding the method development and method validation are presented in this section.

3.1.2.0. Experimental

3.1.2.1. Apparatus

A Systronics model 106 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements.

3.1.2.2. Reagents

All the reagents used were of analytical-reagent grade and distilled water was used throughout investigation.

Bromate - bromide: A 0.01 M KBrO₃ - 0.1M KBr solution was prepared by dissolving accurately weighed 1.67 g of the potassium bromate (S. d. Fine Chem. Ltd., Mumbai, India) and 16.6 g of KBr in water and diluting to one litre in a volumetric flask and used for
indirect titrimetry. The solution was diluted appropriately with water to get 0.005 M for use in direct titrimetric method.

For spectrophotometric methods, a 1000 µg ml⁻¹ KBrO₃ solution containing excess bromide was prepared by dissolving accurately weighed 100 mg of KBrO₃ and 1 g of KBr in water and diluted to the mark in a 100 ml volumetric flask, and then diluted appropriately to get 30 and 25 µg ml⁻¹ KBrO₃ concentrations for use in method C and method D, respectively.

**Indigo Carmine for method C:** A 1000 µg ml⁻¹ solution was prepared by dissolving 112 mg of dye (S. d. Fine Chem. Ltd., Mumbai, India; 90 % purity) in water and diluting to 100 ml in a volumetric flask. This was further diluted 10-fold to get a working concentration of 100 µg ml⁻¹.

**Metanil Yellow for method D:** A 1000 µg ml⁻¹ solution was prepared by dissolving 117 mg of dye (S. d. Fine Chem. Ltd., Mumbai, India; 85 % dye content) in water and diluting to 100 ml in a volumetric flask. This was appropriately diluted to get a working concentration of 40 µg ml⁻¹.

**Potassium iodide:** A 10 % iodide solution was prepared by dissolving 10 g of the chemical (S. d. Fine Chem. Ltd., Mumbai, India) in 100 ml.

**Sodium thiosulphate:** A 0.06 M thiosulphate solution was prepared by dissolving about 15 g of the chemical (S. d. Fine Chem. Ltd., Mumbai, India) in one litre of water, and standardized [72].

**Hydrochloric acid:** A 5 M hydrochloric acid was prepared by 443 ml of concentrated acid (S. d. Fine Chem. Ltd., Mumbai, India; sp. gr. 1.18) to one litre with water.

**Methyl orange indicator:** The indicator was prepared by dissolving 100 mg of dye (S. d. Fine Chem. Ltd., Mumbai, India) in 100 ml water.

**Starch indicator:** A paste of 1 g of soluble starch (S. d. Fine Chem. Ltd., Mumbai, India) with a little water was prepared and poured with constant stirring into 100 ml of boiling water, and boiled for 1 min and cooled.
3.1.2.3. Preparation of standard RNH solution

Pharmaceutical grade RNH was kindly gifted by Glaxo Smithkline Pharmaceuticals Ltd., Mumbai, India, and certified to be 99.8 % pure, was used as received. Stock standard solution containing 2 mg ml\(^{-1}\) drug was prepared by dissolving the weighed amount of RNH in water and kept in an amber coloured bottle and stored in a refrigerator. Working concentrations of 40 \(\mu\)g ml\(^{-1}\) for method C and 20 \(\mu\)g ml\(^{-1}\) for method D were prepared whenever required by appropriate dilution of the stock solution with water.

3.1.2.4. Procedure

Procedures of pure drug

Method A (Direct titrimetry)

A 10 ml aliquot of drug solution containing 1-10 mg RNH was pipetted out into a titration flask. One ml of 5 M hydrochloric acid and 2 drops of methyl orange indicator were added, and titrated with bromate-bromide solution (0.005 M w. r. to KBrO\(_3\)) to a colourless end point. An indicator blank titration was run and correction applied. The amount of the drug was calculated from the equation:

\[
mg = \frac{VM_wR}{n}
\]

where 
- \(V\) = volume of bromate-bromide solution consumed in the sample titration, ml
- \(M_w\) = relative molecular mass of drug
- \(R\) = strength of bromate solution,
- \(n\) = number of moles of bromate reacting with each mole of drug

Method B (Indirect titrimetry)

A 10 ml aliquot of drug solution containing 1-17 mg RNH was accurately measured and placed in a 100 ml stoppered flask. One ml of 5 M hydrochloric acid and followed by 10 ml of bromate-bromide solution (0.01 M in KBrO\(_3\)) were added by means of a pipette. The flask was stoppered and kept aside for 5 min with occasional swirling. The stopper was then washed with 5 ml of water, and 5 ml of 10 % potassium iodide were added to the flask.
The liberated iodine was titrated with 0.06 M thiosulphate using starch as indicator. A blank titration was run under identical conditions.

The amount of the drug was calculated from the equation:

$$\text{mg} = \frac{(B-S) M_n R}{n}$$

where $B =$ volume of thiosulphate consumed in the blank titration, ml

$S =$ volume of thiosulphate consumed in the sample titration, ml

$R =$ strength of sodium thiosulphate solution, mol l$^{-1}$.

**Spectrophotometric method C (Indigo Carmine)**

Aliquots of RNH solution (0.5-3.0 ml; 40 μg ml$^{-1}$) were delivered into a series of 10 ml calibrated flasks by means of a micro burette. To each flask was added 1 ml of 5 M HCl and the total volume was adjusted to 4 ml by adding water. Then, 1.5 ml of bromate-bromide solution (30 μg ml$^{-1}$ in KBrO$_3$) was added, the flasks were stoppered and set aside for 10 min with occasional shaking. Finally, 2 ml of 100 μg ml$^{-1}$ Indigo Carmine were added to each flask, diluted to volume with water and absorbance measured at 610 nm against a water blank after 5 min.

**Spectrophotometric method D (Metanil Yellow)**

Aliquots of RNH solution (0.5-3.5 ml; 20 μg ml$^{-1}$) were transferred into a series of 10 ml calibrated flasks by means of a micro burette. Two ml of 5 M HCl were added to each flask and the total volume was brought to 5.5 ml with water. Then, 1.0 ml of bromate-bromide solution (25 μg ml$^{-1}$ in KBrO$_3$) was added, the flasks were stoppered and let stand for 5 min. Finally, 1 ml of 40 μg ml$^{-1}$ metanil yellow was added to each flask, diluted to volume with water and absorbance measured at 530 nm against a water blank after 5 min.

In either spectrophotometric method, the concentration of the unknown was read from the calibration graph or calculated from the regression equation obtained from Beer's law data.
**Assay procedure for formulations**

**Tablets:** Twenty tablets were weighed and ground into a fine powder. An amount of the powder equivalent to 200 mg of RNH was weighed into a 100 ml volumetric flask, 60 ml water added and shaken thoroughly for about 20 min. The volume was diluted to the mark with water, mixed well and filtered using Whatman No 42 filter paper. First 10 ml portion of the filtrate was rejected and a convenient aliquot of filtrate (containing 2 mg ml$^{-1}$ RNH) was taken for assay by titrimetric procedures, A and B. The filtrate was diluted stepwise to get 40 and 20 µg ml$^{-1}$ of RNH for use in spectrophotometric methods C and D, respectively. A suitable aliquot was then subjected to analysis. The results are compiled in **Table 3.1.3**.

**Injections:** The contents of 20 ampoules (each containing 25 mg of RNH) were mixed and an accurately measured volume equivalent to 200 mg of RNH was transferred into 100 ml separatory funnel. Ten ml of 6 M ammonia solution added and extracted with three 10 ml portions of chloroform. The chloroform extracts were combined and evaporated to dryness. The residue was dissolved in 30 ml water and transferred into a 100 ml calibrated flask. The volume was made up to the mark with water. The solution (2 mg ml$^{-1}$) was subjected to analysis by titrimetric procedures A and B. The stock solution was diluted to 40 and 20 µg ml$^{-1}$ for spectrophotometric methods C and D, respectively.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method C</th>
<th>Method D</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda \max ) (nm)</td>
<td>610</td>
<td>530</td>
</tr>
<tr>
<td>Beer's law limits (( \mu g \text{ ml}^{-1} ))</td>
<td>2 - 12</td>
<td>1 - 7</td>
</tr>
<tr>
<td>Molar absorptivity (( 1 \text{ mol}^{-1} \text{ cm}^{-1} ))</td>
<td>( 2.06 \times 10^4 )</td>
<td>( 9.82 \times 10^4 )</td>
</tr>
<tr>
<td>Sandell sensitivity, (( \mu g \text{ cm}^{-2} ))</td>
<td>0.017</td>
<td>0.012</td>
</tr>
<tr>
<td>Limit of detection (( \mu g \text{ ml}^{-1} ))</td>
<td>0.07</td>
<td>0.12</td>
</tr>
<tr>
<td>Limit of quantification (( \mu g \text{ ml}^{-1} ))</td>
<td>0.21</td>
<td>0.40</td>
</tr>
<tr>
<td>Regression equation (Y)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Sb</td>
<td>( 3.2 \times 10^{-3} )</td>
<td>0.12</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.01</td>
<td>(-9.1 \times 10^{-3})</td>
</tr>
<tr>
<td>Sa</td>
<td>( 1.8 \times 10^{-4} )</td>
<td>( 6.2 \times 10^{-3} )</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9984</td>
<td>0.9976</td>
</tr>
</tbody>
</table>

* \( Y = a + b \times X \) where \( Y \) is the absorbance and \( X \) concentration in \( \mu g \text{ ml}^{-1} \)
Table 3.1.2 Evaluation of accuracy and precision of proposed methods

<table>
<thead>
<tr>
<th>Method*</th>
<th>RNH taken</th>
<th>RNH found</th>
<th>Relative error, %</th>
<th>Range</th>
<th>SD</th>
<th>RSD, %</th>
<th>ROE**, %</th>
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<tbody>
<tr>
<td>A</td>
<td>3.00</td>
<td>3.05</td>
<td>1.67</td>
<td>0.85</td>
<td>0.05</td>
<td>1.64</td>
<td>± 0.05</td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>5.98</td>
<td>0.33</td>
<td>0.93</td>
<td>0.04</td>
<td>0.74</td>
<td>± 0.04</td>
</tr>
<tr>
<td></td>
<td>9.00</td>
<td>8.87</td>
<td>1.44</td>
<td>1.26</td>
<td>0.11</td>
<td>1.28</td>
<td>± 0.10</td>
</tr>
<tr>
<td>B</td>
<td>5.00</td>
<td>5.12</td>
<td>2.40</td>
<td>0.36</td>
<td>0.09</td>
<td>1.86</td>
<td>± 0.08</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>10.03</td>
<td>0.30</td>
<td>0.15</td>
<td>0.08</td>
<td>0.81</td>
<td>± 0.07</td>
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<tr>
<td></td>
<td>15.00</td>
<td>15.06</td>
<td>0.41</td>
<td>0.55</td>
<td>0.16</td>
<td>1.07</td>
<td>± 0.15</td>
</tr>
<tr>
<td>C</td>
<td>4.00</td>
<td>4.08</td>
<td>1.96</td>
<td>0.08</td>
<td>0.89</td>
<td>2.18</td>
<td>± 0.86</td>
</tr>
<tr>
<td></td>
<td>8.00</td>
<td>7.91</td>
<td>1.13</td>
<td>0.12</td>
<td>0.64</td>
<td>0.93</td>
<td>± 0.63</td>
</tr>
<tr>
<td></td>
<td>12.00</td>
<td>11.82</td>
<td>1.52</td>
<td>0.19</td>
<td>1.63</td>
<td>1.67</td>
<td>± 1.56</td>
</tr>
<tr>
<td>D</td>
<td>2.00</td>
<td>2.03</td>
<td>1.48</td>
<td>0.27</td>
<td>0.34</td>
<td>1.68</td>
<td>± 0.33</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>4.05</td>
<td>1.23</td>
<td>0.33</td>
<td>0.09</td>
<td>0.24</td>
<td>± 0.08</td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>5.95</td>
<td>0.84</td>
<td>0.41</td>
<td>0.60</td>
<td>1.02</td>
<td>± 0.05</td>
</tr>
</tbody>
</table>

* In methods A & B, drug taken/found, range and SD are in mg, and in method C & D, the quantities are in μg ml⁻¹.

SD: Standard deviation

RSD: Relative Standard deviation

** At 95 % confidence level for six degrees of freedom
Table 3.1.3. Results of analysis of tablets containing RNH by the proposed methods

<table>
<thead>
<tr>
<th>Brand name and dosage Form</th>
<th>Label Claim mg per tablet or per ml</th>
<th>Found(^{\Psi}) (% of label claim ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference method</td>
<td>Method A</td>
</tr>
<tr>
<td>Ranitin(^a) 150</td>
<td>101.23±1.02</td>
<td>100.28±0.64</td>
</tr>
<tr>
<td></td>
<td>t=1.81; F=2.54</td>
<td>t=0.23; F=1.90</td>
</tr>
<tr>
<td>Histac(^b) 300</td>
<td>101.26±0.34</td>
<td>100.83±0.71</td>
</tr>
<tr>
<td></td>
<td>t=1.29; F=4.36</td>
<td>t=2.27; F=1.99</td>
</tr>
<tr>
<td>Injection Ranitin(^a)</td>
<td>25</td>
<td>98.36±0.85</td>
</tr>
<tr>
<td></td>
<td>98.64±0.38</td>
<td>t=0.73; F=5.00</td>
</tr>
</tbody>
</table>

\(^{\Psi}\) Average of five determinations


** Tabulated at t-value at 95 % confidence level 2.77
Tabulated at F-value at 95 % confidence level 6.39
Table 3.1.4. Results of recovery study by standard-addition method

<table>
<thead>
<tr>
<th>Dosage form studied</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
<th>Method D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranitin tablets (150 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNH in tablet, mg</td>
<td>Pure RNH added, mg</td>
<td>Total found, mg</td>
<td>Recovery pure RNH*, %</td>
<td>Pure RNH added, mg</td>
</tr>
<tr>
<td>4.02</td>
<td>5.0</td>
<td>9.09</td>
<td>101.40</td>
<td>10.21</td>
</tr>
<tr>
<td>4.02</td>
<td>6.0</td>
<td>10.19</td>
<td>102.80</td>
<td>10.21</td>
</tr>
<tr>
<td>4.02</td>
<td>7.0</td>
<td>11.00</td>
<td>99.71</td>
<td>10.21</td>
</tr>
<tr>
<td>Ranitin injection (25 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNH in tablet, mg</td>
<td>Pure RNH added, mg</td>
<td>Total found, mg</td>
<td>Recovery pure RNH*, %</td>
<td>Pure RNH added, mg</td>
</tr>
<tr>
<td>4.07</td>
<td>5.0</td>
<td>8.94</td>
<td>97.40</td>
<td>10.10</td>
</tr>
<tr>
<td>4.07</td>
<td>6.0</td>
<td>9.98</td>
<td>98.50</td>
<td>10.10</td>
</tr>
<tr>
<td>4.07</td>
<td>7.0</td>
<td>11.13</td>
<td>100.86</td>
<td>10.10</td>
</tr>
<tr>
<td>Ranitin tablet (150 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conc. of RNH in tablet, µg ml⁻¹</td>
<td>Conc. of RNH added, µg ml⁻¹</td>
<td>Total found, µg ml⁻¹</td>
<td>Recovery of pure RNH*, %</td>
<td>Conc. of RNH in tablet, µg ml⁻¹</td>
</tr>
<tr>
<td>9.87</td>
<td>15.0</td>
<td>25.51</td>
<td>104.3</td>
<td>9.91</td>
</tr>
<tr>
<td>9.87</td>
<td>25.0</td>
<td>35.62</td>
<td>103.0</td>
<td>9.91</td>
</tr>
<tr>
<td>9.87</td>
<td>35.0</td>
<td>45.43</td>
<td>101.6</td>
<td>9.91</td>
</tr>
<tr>
<td>Ranitin injection (25 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conc. of RNH in tablet, µg ml⁻¹</td>
<td>Conc. of RNH added, µg ml⁻¹</td>
<td>Total found, µg ml⁻¹</td>
<td>Recovery of pure RNH*, %</td>
<td>Conc. of RNH in tablet, µg ml⁻¹</td>
</tr>
<tr>
<td>9.97</td>
<td>15.0</td>
<td>24.61</td>
<td>97.58</td>
<td>10.16</td>
</tr>
<tr>
<td>9.97</td>
<td>25.0</td>
<td>24.90</td>
<td>99.74</td>
<td>10.16</td>
</tr>
<tr>
<td>9.97</td>
<td>35.0</td>
<td>45.42</td>
<td>101.3</td>
<td>10.16</td>
</tr>
</tbody>
</table>

*Mean value of three determinations
Fig 3.1.1. Beer's law curve for method C

Fig 3.1.2. Beer's law curve for method D
3.1.3.0. RESULTS AND DISCUSSION

3.1.3.1. Method Development

Titrimetry (methods A & B)

The proposed titrimetric procedures are based on the oxidation reaction between RNH and bromine generated *in situ*. Since the molar ratio of the reaction was found to be 1:1 in both methods (A & B), it is probable that the drug undergoes oxidation as per the reaction scheme given below:

\[
\text{BrO}_3^- + 5\text{Br}^- + 6H^+ \rightarrow 3\text{Br}_2 + 3\text{H}_2\text{O}
\]

\[
(H_3C)_2\text{H}_2CN\text{CHOCH}_2\text{S}(\text{CH}_2)\text{NH} - \text{C}=\text{CH NO}_2 + 3\text{Br}_2 + 3\text{H}_2\text{O}
\]

\[
(H_3C)_2\text{H}_2CN\text{CHOCH}_2\text{SOH} + \text{CH}_2\text{NH} - \text{C}=\text{CH NO}_2 + 6\text{HBr}
\]

In both direct and indirect titration of RNH, the stoichiometry of the reaction was studied by using different concentrations of HCl ranging from 0.125 to 0.50 M. One ml of 5 M hydrochloric acid in a total volume of ~25 ml was found adequate and resulted in the same value of ‘n’. In direct titrimetry 1-10 mg of RNH could be conveniently determined using 5mM KBrO₃ -50 mM KBr mixture.

For the range investigated by indirect titrmtry (1-17 mg), the reaction was found to be stoichiometric upto a contact time of 5 min beyond which slightly larger volumes of bromate solution were consumed but never yielded a definite stoichiometry up to 30 min. This is possibly due to bromination of the drug molecule. Hence, it is necessary that the back titration of the unreacted bromine should be completed immediately after 5 min to get accurate and precise results.
When different amounts of the drug (within the working range) were reacted with bromate under optimum conditions, the stoichiometric study revealed that 1 mole of each drug reacted with 1 mole of potassium bromate. The linear relation between the amount of the drug and titration end point is apparent from the correlation coefficient of 0.9986 (method A) and -0.9863 (method B) suggesting that the reaction between the RNH and bromate proceeds stoichiometrically in the ratio 1:1.

**Spectrophotometric methods C & D**

Many dyes are irreversibly destroyed to colourless species by oxidizing agents in acid medium and this observation has been exploited for the indirect spectrophotometric determination of some bioactive compounds [67-70]. In the proposed spectrophotometric methods, the ability of bromine to effect oxidation of RNH and irreversibly destroy indigo carmine and metanil yellow dyes to colourless products in acid medium has been capitalized.

Both methods are based on the oxidation of the drug by a measured excess of bromine and subsequent determination of the latter by reacting with indigo carmine or metanil yellow, and measuring the absorbance at 610 or 530 nm. In either method, the absorbance increased linearly with increasing concentration of drug.

Preliminary experiments were performed to fix the upper limits of the dyes that could be measured spectrophotometrically, and these were found to be 20 and 4 μg ml⁻¹ for indigo carmine and metanil yellow, respectively. A bromate concentration of 4.5 μg ml⁻¹ was found to irreversibly destroy the blue colour of 20 μg ml⁻¹ of indigo carmine whereas 2.5 μg ml⁻¹ of oxidant was required to destroy 4 μg ml⁻¹ of metanil yellow to a colourless product. Hence, different amounts of RNH were reacted with 1.5 ml of 30 μg ml⁻¹ bromate in method C, and with 1 ml of 25 μg ml⁻¹ bromate in method D, followed by the determination of residual oxidant as described under the respective procedures.

RNH when added in increasing amount to a fixed amount of bromine consumes the latter and there will be a concomitant decrease in its concentration. When a fixed amount of either dye is added to decreasing amounts of bromine, a concomitant increase in the concentration of dye is obtained. This is observed as a proportional increase in the
absorbance at the respective wavelengths of maximum absorption with increasing concentration of RNH. (Fig. 3.1.1 and 3.1.2)

Hydrochloric acid was the medium of choice for the oxidation of the drug by insitu-generated bromine as well as the latter's determination employing the dyes. The absorbance of the dyes was not affected in 0.125-1.25 M hydrochloric acid concentration. However, since 0.5 and 1.0 M acid concentrations were found optimum for the oxidation reaction in a reasonable time of 10 and 5 min in methods C and D, respectively, the same concentration was maintained for the determination of the unreacted bromine with the dyes, and even this reaction time is not critical. Any delay up to 30 min had no effect on the absorbance. A contact time of 5 min is necessary for the complete destruction of the dyes by the residual bromine.

Though the colour of acid form of either dye was indefinitely stable in hydrochloric acid medium, it was found to be stable up to 30 min in the presence of the oxidation product of the drug. Hence, the absorbance of the dye solution should be measured within 30 min after its addition.

3.1.3.2. Method Validation

Analytical parameters

A linear correlation was found between absorbance at \( \lambda_{\text{max}} \) and concentration ranges given in Table 3.1.1 for methods C and D. Correlation coefficients, intercepts, slopes, molar absorptivity and Sandell sensitivity values and limits of detection and determination are also given in Table 3.1.1 and indicate the high sensitivity of the methods.

3.1.3.3. Accuracy and precision of the methods

The accuracy of the proposed methods was established by analyzing the pure drug in three levels (within working concentration ranges) and the precision was ascertained by calculating the relative standard deviation (RSD) of seven replicate determinations on the same solution containing the drug in three levels. The percent error and RSD values (< 3%) indicate the high accuracy and precision of the methods (Table 3.1.2). For a better picture of reproducibility on a day-to-day basis, a series was run in which standard drug
solution in three levels was run each day for 5 days. The day-to-day RSD values were in the range of 1.5-3.5 %, and represent the best appraisal of the procedures in daily routine use.

3.1.3.4. Application to the studied drug to pharmaceutical formulations

The proposed methods were applied to the assay of RNH in tablets without any coated colour lest it should interfere with the spectrophotometric methods, and in injections. The results are compiled in Table 3.1.3 and were checked by the official method [32]. There is a close agreement between the results obtained by the proposed and official methods as found from the Student’s t- and F- values. The results obtained by the proposed methods also agreed well with the label claims in all instances.

3.1.3.5. Recovery of pure drug added to formulations

Recovery experiments were carried out by adding known amounts of pure drug to pre analysed dosage forms (tablets and injections) and determining the total amount by the proposed methods. The percent recovery values of the pure drug added as shown in Table 3.1.4 demonstrate that frequently encountered common excipients such as talc, starch, alginate, stearate, lactose and gum acacia, calcium gluconate, calcium dihydrogen orthophosphate, ammonium chloride and sodium citrate did not interfere in the methods.
SECTION 3.2

TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF RNH USING CHLORAMINE-T (CAT)

3.2.1.0. INTRODUCTION

Chloramine-T (CAT), the sodium salt of N-chloro-p-toluene sulphonamide is a versatile oxidising agent. CAT was first prepared by Chattaway [73] by treating toluene with chlorosulphonic acid when ortho and para sulphonyl chlorides are formed. The latter on treatment with ammonia gives sulphonamide which reacts with aqueous sodium hypochlorite solution forming CAT, which crystallizes out. The hydrolysis constant of CAT is very low [74] and it does not liberate chlorine in acid solutions and does not chlorinate many compounds which are attacked by chlorine water, but it liberates iodine from acidified iodide solutions. The redox potential of CAT in 1.0 N H₂SO₄ is 1.52 V; in neutral solution it is 0.94V [75]. It has the advantage that it may be used in acid or alkaline solution [76] and is said more stable than hypochlorite. Aqueous CAT solutions are very stable. If stored in dark bottles, they do not change their titre for several months; boiling for several hours likewise does not change their true titre [77].

The above properties make CAT as an useful analytical reagent. Noll [78] was the first to propose the use of CAT as an oxidimetric reagent. The behaviour of CAT as a titrimetric reagent has critically been examined by Bishop and Jennings [74] and by Jennings [79]. A large number of substances, both organic and inorganic have been estimated using CAT as an oxidimetric reagent [80,81]. The applications of CAT as an oxidimetric titrant have been reviewed by Rangaswamy [82] and Venkatachalpathy [83].

The versatility of CAT as an oxidimetric reagent can be gauged by innumerable substances, both inorganic and an organic that has been determined [80-83]. Many substances of pharmaceutical importance have also been determined by direct titration with visual end point detection. They include pencillins [84], nizatidine [85], dopamine HCl [86], penothiazine [87], famotidine [88], albendazole [89,90], atenolol [91] and sulphonamides [92].
CAT is also used as an oxidimetric reagent for the spectrophotometric determination of many pharmaceutical substances such as penothiazines [87,93,94], famotadine [88], albendazole [89,90], atenolol [91], antibiotics and anthelmentics [95], cephalalvin [96], tetracyclines [97], piroxicam [98] and bromhexine HCl [99].

It is clear that CAT has not been applied for the assay of RNH either by titrimetry or by spectrophotometry. The author used CAT successfully for the titrimetric and the spectrophotometric determination of RNH. In titrimetry, the aqueous drug solution was titrated directly with chloramine-T in hydrochloric acid medium using indigo carmine as the indicator. The indirect spectrophotometric procedures are based on the redox reaction with a known excess of chloramine-T in hydrochloric acid medium. However, here, the unreacted chloramine-T was determined by reacting with indigo carmine or methylene blue.

3.2.2.0. EXPERIMENTAL

3.2.2.1. Apparatus

A Systronics model 106 digital spectrophotometer with 1 cm quartz cells were used for all absorbance measurements.

3.2.2.2. Reagents

All chemicals used were of analytical reagent grade and double distilled water was used throughout the investigation.

Chloramine-T: A 0.04 M solution of CAT was prepared by dissolved 11.28 g of chemical in 1 litre of distilled water and standardized iodometrically [72]. The solution was diluted to 200 and 100 \( \mu g \) ml\(^{-1}\) for spectrophotometric work.

Indigo carmine indicator: The indicator solution was prepared by dissolving 50 mg of dye (S. d. Fine Chem Ltd., Mumbai, India) in 100 ml water and used for titrimetric work.

Indigo carmine (100 \( \mu g \) ml\(^{-1}\)): A 1000 \( \mu g \) ml\(^{-1}\) solution was first prepared by dissolving 112 mg of dye (S. d. Fine Chem Ltd., Mumbai, India; 90 % purity) in water and diluting to 100 ml in a volumetric flask. This was further diluted 10-fold to get a working concentration of 100 \( \mu g \) ml\(^{-1}\).
**Methylene blue (60 \( \mu \text{g ml}^{-1} \)):** A 1000 \( \mu \text{g ml}^{-1} \) solution was prepared by dissolving 117 mg of dye (S. d. Fine Chem Ltd., Mumbai, India; 85 % dye content) in water and diluting to 100 ml in a volumetric flask. This was appropriately diluted with water to get a working concentration of 60 \( \mu \text{g ml}^{-1} \).

**Hydrochloric acid:** A 5 M hydrochloric acid was prepared by diluting 443 ml of concentrated acid (S. d. Fine Chem. Ltd., Mumbai, India; sp. gr. 1.18) to one litre with water.

### 3.2.2.3. Preparation of standard RNH solution

A stock standard solution containing 2 \( \mu \text{g ml}^{-1} \) RNH was prepared as described in Chapter 3.0 Section 3.1.2.3 and used in titrimetric analysis. This was appropriately diluted to yield working concentrations of 10 and 25 \( \mu \text{g ml}^{-1} \) for spectrophotometric method B and method C, respectively.

### 3.2.2.4. Procedure

**Determination of bulk drug**

**Titrimetry (Method A)**

A 10 ml aliquot of drug solution equivalent to 1-20 mg of RNH was accurately measured into a 100 ml titration flask followed by 7 ml of 5 M HCl. One drop of indigo carmine indicator (0.05 %) was added and the solution was titrated with 0.04 M CAT to a green end point.

The amount of the drug was calculated from:

\[
\text{Amount (mg)} = \frac{V \times M_w \times R}{n}
\]

Where \( V = \) volume of CAT consumed, ml

\( M_w = \) relative molecular mass of drug

\( R = \) molarity of CAT solution

\( n = \) number of moles of CAT required to react with each mole of drug.
Spectrophotometric method B

Aliquots of RNH solution (0.2-3.0 ml; 10 μg ml⁻¹) were delivered into a series of 10 ml calibrated flasks by means of a micro burette and the total volume made upto 3 ml. To each flask, 2 ml of each 5 M HCl and 100 μg ml⁻¹ CAT solutions were added successively. The contents were mixed well and kept aside for 5 min. Then, 2 ml of 100 μg ml⁻¹ IC dye solution was added to each flask, diluted to volume with water and the absorbance was measured at 610 nm against a water blank after 5 min.

Spectrophotometric method C

Aliquots of RNH solution (0.2-4.0 ml; 25 μg ml⁻¹) were transferred into a series of 10 ml calibrated flasks by means of a micro burette and total volume brought 4 ml. Then, 2 ml of each 5 M HCl and 200 μg ml⁻¹ CAT solutions were added, mixed well and let stand for 10 min. Finally, 1 ml of 60 μg ml⁻¹ metanil yellow dye solution was added to each flask, diluted to volume with water and the absorbance was measured at 665 nm against a water blank after 5 min.

In either spectrophotometric method, the concentration of the unknown was read from the calibration graph or calculated from regression equation obtained from Beer’s law data.

Analysis of dosage forms

Pharmaceutical preparations containing studied drug was purchased from the local market. Tablet extracts and injections solutions were prepared as described in the Chapter 3.0 Section 3.1.2.3 and a suitable aliquot was taken for assay by the procedures previously described for pure drugs.
Table 3.2.1. Optical Parameters of Spectrophotometric methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$, nm</td>
<td>610</td>
<td>665</td>
</tr>
<tr>
<td>Beer's law limits, µg ml$^{-1}$</td>
<td>0.2 - 3.0</td>
<td>0.5 - 10.0</td>
</tr>
<tr>
<td>Molar absorptivity, 1 mol$^{-1}$ cm$^{-1}$</td>
<td>$6.13 \times 10^4$</td>
<td>$2.10 \times 10^4$</td>
</tr>
<tr>
<td>Sandell sensitivity, ng cm$^{-2}$</td>
<td>5.72</td>
<td>16.82</td>
</tr>
<tr>
<td>Limit of detection, µg ml$^{-1}$</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>Limit of quantification, µg ml$^{-1}$</td>
<td>0.12</td>
<td>0.22</td>
</tr>
<tr>
<td>Regression equation (Y)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.17</td>
<td>0.06</td>
</tr>
<tr>
<td>$S_{b}$</td>
<td>0.03</td>
<td>$1.3 \times 10^{-3}$</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>$5.8 \times 10^{-3}$</td>
<td>$-8.4 \times 10^{-3}$</td>
</tr>
<tr>
<td>$S_{a}$</td>
<td>$4.2 \times 10^{-4}$</td>
<td>$5.7 \times 10^{-3}$</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9958</td>
<td>0.9976</td>
</tr>
</tbody>
</table>

*Y = a + b X where Y is the absorbance and X concentration in µg ml$^{-1}$
### Table 3.2.2. Evaluation of accuracy and precision

<table>
<thead>
<tr>
<th>Method</th>
<th>RNH taken</th>
<th>RNH found</th>
<th>Relative error, %</th>
<th>Range</th>
<th>SD</th>
<th>RSD, %</th>
<th>ROE**, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.00</td>
<td>4.96</td>
<td>0.80</td>
<td>0.38</td>
<td>0.04</td>
<td>2.31</td>
<td>±0.03</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>9.86</td>
<td>1.40</td>
<td>0.51</td>
<td>0.09</td>
<td>0.91</td>
<td>±0.08</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>14.90</td>
<td>0.67</td>
<td>0.43</td>
<td>0.01</td>
<td>0.08</td>
<td>±0.01</td>
</tr>
<tr>
<td>B</td>
<td>1.00</td>
<td>1.03</td>
<td>3.00</td>
<td>0.96</td>
<td>0.01</td>
<td>0.85</td>
<td>±0.01</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>1.96</td>
<td>2.00</td>
<td>1.06</td>
<td>0.01</td>
<td>0.05</td>
<td>±0.01</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>2.95</td>
<td>1.79</td>
<td>0.89</td>
<td>0.02</td>
<td>0.79</td>
<td>±0.02</td>
</tr>
<tr>
<td>C</td>
<td>2.00</td>
<td>1.97</td>
<td>1.50</td>
<td>0.32</td>
<td>0.02</td>
<td>1.24</td>
<td>±0.02</td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>5.82</td>
<td>3.00</td>
<td>0.18</td>
<td>0.06</td>
<td>1.11</td>
<td>±0.05</td>
</tr>
<tr>
<td></td>
<td>8.00</td>
<td>7.84</td>
<td>2.00</td>
<td>0.26</td>
<td>0.07</td>
<td>0.92</td>
<td>±0.87</td>
</tr>
</tbody>
</table>

* In method A, drug taken/found, range and SD are in mg, and in method B & C, the quantities are in μg ml⁻¹.

SD: Standard deviation; RSD: Relative Standard deviation; ROE. Range of error

** At 95 % confidence level for six degrees of freedom
Table 3.2.3. Results of analysis of tablets containing RNH by the proposed methods

<table>
<thead>
<tr>
<th>Dosage form and brand name*</th>
<th>Label claim mg per tablet or per ml</th>
<th>Reference method</th>
<th>Found (% of label claim ± SD)</th>
<th>Proposed methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Method A</td>
<td>Method B</td>
</tr>
<tr>
<td>Ranitin*</td>
<td>150</td>
<td>98.64 ± 0.56</td>
<td>99.14 ± 0.26</td>
<td>98.96 ± 0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.93; F=4.64</td>
<td>t=0.72; F=2.25</td>
<td>t=2.24; F=1.47</td>
</tr>
<tr>
<td>Histac*</td>
<td>300</td>
<td>100.48 ± 0.96</td>
<td>99.74 ± 0.42</td>
<td>100.44 ± 0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.69; F=5.22</td>
<td>t=0.07; F=1.51</td>
<td>t=1.81; F=1.78</td>
</tr>
<tr>
<td>Injections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranitin*</td>
<td>25</td>
<td>102.64 ± 0.78</td>
<td>101.96 ± 0.46</td>
<td>102.18 ± 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.95; F=2.88</td>
<td>t=1.07; F=1.81</td>
<td>t=0.09; F=2.53</td>
</tr>
</tbody>
</table>

Ψ Average of five determinations
*Marketed by: a. Torrent Pharmaceuticals; b. Ranbaxy Chemicals
** Tabulated t-value at 95 % confidence level 2.77
Tabulated F-value at 95 % confidence level 6.39
Table 3.2.4. Results of recovery study using standard-addition method

<table>
<thead>
<tr>
<th>Dosage form studied</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount of RNH in tablet, mg</td>
<td>Amount of pure RNH added, mg</td>
<td>Total found, mg</td>
</tr>
<tr>
<td>Ranitin (150 mg tablets)</td>
<td>2.97</td>
<td>4.0</td>
<td>7.08</td>
</tr>
<tr>
<td></td>
<td>2.97</td>
<td>8.0</td>
<td>10.96</td>
</tr>
<tr>
<td></td>
<td>2.97</td>
<td>12.0</td>
<td>14.65</td>
</tr>
<tr>
<td>Histac (300 mg tablets)</td>
<td>2.99</td>
<td>4.0</td>
<td>7.14</td>
</tr>
<tr>
<td></td>
<td>2.99</td>
<td>8.0</td>
<td>11.06</td>
</tr>
<tr>
<td></td>
<td>2.99</td>
<td>12.0</td>
<td>14.79</td>
</tr>
<tr>
<td>Ranitin (25 mg injections)</td>
<td>3.06</td>
<td>4.0</td>
<td>7.05</td>
</tr>
<tr>
<td></td>
<td>3.06</td>
<td>8.0</td>
<td>10.93</td>
</tr>
<tr>
<td></td>
<td>3.06</td>
<td>12.0</td>
<td>14.55</td>
</tr>
</tbody>
</table>

* Mean value of three determinations
**Fig 3.2.1. Beer's law curve for method B**

**Fig 3.2.2. Beer's law curve for method C**
3.2.3.0. RESULTS AND DISCUSSION

The proposed methods are based on the oxidation of drug with CAT in acid medium.

\[
\begin{align*}
(H_3C)_2\text{-}H_2CN\text{-}\text{O-} \quad & \rightarrow \quad CH_2\text{-}S\text{-}(CH_2)_2\text{-}NH \quad \text{C\text{=}CH\text{--NO}_2} + 6 \text{CAT} \\
& \downarrow \\
(H_3C)_2\text{-}H_2CN\text{-}SO\text{-OH} + \quad & \text{CH}_2\text{-}NH\text{-}C\text{=}CH\text{--NO}_2
\end{align*}
\]

3.2.3.1. Method Development

Method A (Titrimetry)

Hydrochloric acid was found to be an ideal medium for the titration. When sulphuric or phosphoric acid was used, it was difficult to locate the endpoint and non-stoichiometric results were obtained. The reaction was found to be quantitative in HCl medium, and approximately 1.75 M acid concentration medium gave consistent and accurate results. Of the several indicators tried, indigo carmine gave very sharp endpoint. Using 0.04 M CAT, 1-20 mg of drug can be conveniently determined, and in this range the molar-ratio of the reaction was found to be always 1: 6 (RNH: CAT).

Spectrophotometric method B and method C

The ability of CAT to cause oxidation of RNH in acid medium has successfully been utilized to develop two sensitive spectrophotometric methods for the assay of RNH. These methods are based on the oxidation of the drug by a measured excess CAT in HCl medium and subsequent determination of the latter by reacting with indigo carmine or methylene blue, and measuring the absorbance at 610 or 665 nm. In either method the absorbance increased linearly with increasing concentration of drug and formed the basis for quantitation of drug.

The first step in the assay procedure was to fix the upper concentration limits of the dyes that could be determined spectrophotometrically, and these were found to be 10 and 6
μg ml⁻¹ for indigo carmine and methylene blue, respectively. A CAT concentration of 20 μg ml⁻¹ was found to convert 10 μg ml⁻¹ indigo carmine completely to a colourless product whereas 40 μg ml⁻¹ oxidant was required to destroy 6 μg ml⁻¹ of methylene blue completely to a colourless product. Hence, different amounts of RNH were reacted with 2 ml of 100 μg ml⁻¹ CAT in method B and 2 ml of 200 μg ml⁻¹ of CAT in method C, and the unreacted oxidant was determined as described under the respective procedures.

The time taken for the oxidation of the drug is not critical. Any delay up to 30 min had no effect on the absorbance. A contact time of 5 min is necessary for reaction of dyes with CAT before measuring the absorbance. Even this contact time is not critical.

RNH when added in increasing amounts to a fixed amount of CAT consumes the latter and there will be a concomitant decrease in its concentration (CAT). When a fixed amount of either dye is added to decreasing concentrations of CAT, a concomitant increase in the concentration of the dye is obtained. This is observed as a proportional increase in the absorbance with concentration of the drug (Fig. 3.2.1 and 3.2.2).

Similar to titrimetry, HCl was the medium of choice for the oxidation of drug by CAT as well as the latter's determination with the dyes. The absorbance of the dyes was not affected in 0.125-1.25 M HCl concentration. A 1.0 M concentration was found optimum for the oxidation of the drug in a reasonable time of 5-10 min, and hence the same concentration was employed for the determination of unreacted CAT with the dyes.

3.2.3.2. Method Validation

Analytical parameters

A linear correlation was found between absorbance at λmax and concentration ranges given in Table 3.2.1 for methods B and C. Correlation coefficients, intercepts, slopes, molar absorptivity and Sandell sensitivity values and detection limit and quantification limit are also given in Table 3.2.1 and indicate the high sensitivity of the methods.

3.2.3.3. Accuracy and precision of the methods

The accuracy of the methods was established by analyzing the pure drug in three levels and the precision was ascertained by calculating the relative standard deviation (RSD) of seven replicate determinations on the same solution containing the drug in three levels. The percent error and RSD values (< 3 %) indicate the high accuracy and precision of the
methods (Table 3.2.2). For a better picture of the reproducibility on a day-to-day basis, a series of experiments was performed in which standard drug solution in three levels was determined on each day for 5 days. In terms of standard deviations, the day-to-day coefficient of variation was less than 3% and represents the best appraisal of the precision of the procedures in daily routine use.

3.2.3.4. Application to the studied drug in pharmaceutical formulations

The proposed methods were applied for the assay of RNH in two brands of tablets without any coated colour and in a brand of injections. The results are summarized in Table 3.2.3 and were checked by reference method [32]. There is a 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 the proposed methods also agreed well with the claimed values on the tablets and injections in all instances.

3.2.3.5. Recovery of pure drugs added to formulations

To further establish the validity of the methods, recovery tests via standard-addition technique were performed using three different samples. To a fixed amount of the drug in tablets and in injections (pre-analysed), pure drug in three different levels was added, and the total was found by the proposed methods. Each test was repeated three times on each sample. As shown in Table 3.2.4, the recovery of pure drug added to tablets ranged from 95 to 103% indicating that the commonly encountered tablet excipients and additives such as calcium gluconate, calcium hydrogen phosphate, cellulose, lactose, gelatin, starch, talc, gumacacia, sodium alignate and magnesium stearate did not interfere in the methods.
SECTION 3.3
TITRIMETRIC AND SPECTROPHOTOMETRIC DETERMINATION OF RNH USING POTASSIUM IODATE AS AN OXIDIMETRIC REAGENT

3.3.1.0. INTRODUCTION

Potassium iodate is a powerful oxidizing agent, but the course of the reaction is governed by the conditions under which it is employed. Under acidic conditions, potassium iodate acts as a very powerful oxidizing agent. In 1903, Andrews [100] used the iodate as an oxidimetric reagent, further developed by Jamieson in 1926 [101] in which potassium iodate was used as an oxidimetric agent. Analytical applications of iodate have been reviewed by Berka, et al. [80] and Kolloff and Belcher [66]. In the past, potassium iodate has been applied as the titrimetric reagent for the determination of a wide variety of substances such as phenazone [102], penicillins [103,104], isoniazid [105], tetracyclines [106], ascorbic acid [107], hydralazine [108], captopril [109], cephalosporins [110,111], clindamycin HCl [112], methdilazine [113] and phenothiazines [114].

The formation of a coloured product by the interaction between iodate and analyte under suitable conditions has been the basis of the spectrophotometric determination of pharmaceutical substances such as methdilazine [113] phenothiazines [114,115], rimipramine and morphine [116], levapoda [117], narcotine [118], ascorbic acid [119,120], novalgin [121], dicaine [122], morphine [123], aminophenol [124], biotin [125].

From the literature survey presented in Chapter 3.0 and Section 3.0.2 and from the foregoing paragraphs, it is clear that KIO₃ has not been used as a reagent either for the titrimetric or for the spectrophotometric determination of RNH.

The investigation that led to the use of potassium iodate as an oxidimetric reagent for the titrimetric and spectrophotometric methods for the assay of RNH in bulk drug and in dosage forms were presented in this Section 3.3.
3.3.2.0 EXPERIMENTAL

3.3.2.1. Apparatus

A Systronics model 106 digital spectrophotometer with 10 mm quartz cells were used for absorbance measurements.

3.3.2.2. Reagents

All chemicals used were of analytical reagent grade and double distilled water was used throughout the investigation.

Potassium iodate: 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 in a volumetric flask and used in titrimetric method. A 0.4 % iodate solution was prepared for method B. A stock standard solution corresponding to 1000 μg ml⁻¹ KIO₃ was prepared in water and diluted stepwise to obtain 20 μg ml⁻¹ solution for method C.

Sodium thiosulphate: A 0.01 M thiosulphate solution was prepared by dissolving 2.5 g of the chemical (S. d. Fine Chem. Ltd., Mumbai, India) in one litre of water for use in titrimetric method.

Hydrochloric acid: A 5M hydrochloric acid was prepared by diluting 443 ml of concentrated acid (S. d. Fine Chem. Ltd., Mumbai, India; sp. gr. 1.18) to one litre with water. This solution (5M) was appropriately diluted to get 1 M hydrochloric acid for method B.

Sulphuric acid (1M): First, 5M sulphuric acid was prepared by adding 278 ml of concentrated acid (S. d. Fine Chem. Ltd., Mumbai, India; sp. gr. 1.83) to 722 ml of water with cooling. This stock solution was appropriately diluted to get 1M acid.

Potassium iodide: A 10 % solution was prepared by dissolving 10 g of the chemical in 100 ml of water and used in titrimetric work (method A). This was appropriately diluted to get 2 % solution for method C.

Dichlorofluorescein: A 0.01 % dichlorofluorescein solution 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.

Starch indicator: One g of the reagent was made into a paste and poured into 100 ml of boiling water, boiled for 1 min and cooled and used for methods A and C.
Monochloroacetic acid: The reagent solution was prepared by dissolving 1 g of reagent (S. d. Fine Chem. Ltd., India) in 30 ml of 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.

3.3.2.3. Preparation of standard RNH solution

Pharmaceutical grade RNH was received as gift from Torrent Pharmaceuticals Ltd., Ahmedabad, India, and was used as received. A stock standard solution containing 2 mg ml\(^{-1}\) 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.

3.3.2.4. Procedures

After preliminary investigations of several parameters, the following procedures have been recommended.

Procedures for pure drug

Titrimetry (method A)

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 was 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:

\[
\text{Amount (mg)} = 1053.63 (B - S) R
\]

where

\[
B = \text{volume of thiosulphate solution used in the blank titration, ml}
\]

\[
S = \text{volume of thiosulphate solution used in the sample titration, ml}
\]

\[
R = \text{molarity of thiosulphate solution}
\]
Spectrophotometric method B

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 % 2,7-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.

Spectrophotometric method C

In to a series of 10 ml volumetric flasks were added 0.5, 1.0, 1.5,... 4.0 ml of 200 μg ml⁻¹ drug solution, and the total volume was adjusted to 4 ml by adding requisite volume of water. Then, 1 ml each of 1M sulphuric acid and 20 μg ml⁻¹ potassium iodate solutions were added, the contents were mixed well and let stand for 20 min. Finally, 1 ml each of 2 % potassium iodide and 1% starch solutions were added to each flask at 3 min time interval and diluted to the mark with distilled water. The absorbance of coloured solution was measured at 560 nm against a reagent blank before 30 min.

A calibration graph was prepared by plotting the increase in absorbance in method B and decrease in absorbance method C as a function of drug concentration. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived 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 RNH was weighed accurately into a 100 ml volumetric flask, 60 ml water, mixed well. Then, the volume was made up to the mark with water, and filtered using Whatman No. 41 filter paper. A 5 ml of 5 M HCl and 500 mg of zinc dust were added to above prepared solution and shaken thoroughly for about 30 min and filtered. A suitable aliquot was used for analysis by titrimetry. The solution was appropriately diluted with water to get 200 μg ml⁻¹ solution and analysed spectrophotometrically using a convenient volume. In the case of injectable product, known volume equivalent to 200 mg of RNH was measured accurately into a 100 ml beaker, 60 ml 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 by spectrophotometry as described above after appropriate dilution.
### Table 3.3.1. Optical parameters of spectrophotometric methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}, \text{nm}$</td>
<td>520</td>
<td>560</td>
</tr>
<tr>
<td>Beer's law limits, $\mu g \text{ ml}^{-1}$</td>
<td>5.0 - 50.0</td>
<td>10.0 - 80.0</td>
</tr>
<tr>
<td>Molar absorptivity, $1 \text{ mol}^{-1} \text{ cm}^{-1}$</td>
<td>$3.88 \times 10^3$</td>
<td>$1.80 \times 10^3$</td>
</tr>
<tr>
<td>Sandell sensitivity, $\text{ng cm}^{-2}$</td>
<td>5.72</td>
<td>194.9</td>
</tr>
<tr>
<td>Limit of detection, $\mu g \text{ ml}^{-1}$</td>
<td>2.14</td>
<td>10.67</td>
</tr>
<tr>
<td>Limit of quantification, $\mu g \text{ ml}^{-1}$</td>
<td>7.15</td>
<td>35.55</td>
</tr>
<tr>
<td>Regression equation ($Y$)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope ($b$)</td>
<td>0.011</td>
<td>-7.4 x $10^{-3}$</td>
</tr>
<tr>
<td>$Sb$</td>
<td>$3.4 \times 10^{-3}$</td>
<td>0.012</td>
</tr>
<tr>
<td>Intercept ($a$)</td>
<td>$8.3 \times 10^{-4}$</td>
<td>0.51</td>
</tr>
<tr>
<td>$Sa$</td>
<td>$1.6 \times 10^{-3}$</td>
<td>$3.0 \times 10^{-3}$</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.9958</td>
<td>-0.9962</td>
</tr>
</tbody>
</table>

* $Y = a + b \times X$ where $Y$ is the absorbance and $X$ concentration in $\mu g \text{ ml}^{-1}$
Table 3.3.2. Evaluation of accuracy and precision of the methods

<table>
<thead>
<tr>
<th>Method*</th>
<th>RNH taken</th>
<th>RNH found</th>
<th>Relative error, %</th>
<th>Range</th>
<th>SD</th>
<th>RSD, %</th>
<th>ROE**, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.00</td>
<td>5.11</td>
<td>2.20</td>
<td>0.63</td>
<td>0.07</td>
<td>1.48</td>
<td>± 0.07</td>
</tr>
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<td></td>
<td>10.00</td>
<td>10.08</td>
<td>0.80</td>
<td>0.52</td>
<td>0.11</td>
<td>1.06</td>
<td>± 0.11</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>14.83</td>
<td>1.13</td>
<td>0.72</td>
<td>0.28</td>
<td>1.91</td>
<td>± 0.28</td>
</tr>
<tr>
<td>B</td>
<td>10.00</td>
<td>10.16</td>
<td>1.60</td>
<td>0.88</td>
<td>0.14</td>
<td>1.42</td>
<td>± 0.14</td>
</tr>
<tr>
<td></td>
<td>30.00</td>
<td>30.25</td>
<td>1.25</td>
<td>0.96</td>
<td>0.21</td>
<td>0.71</td>
<td>± 0.21</td>
</tr>
<tr>
<td></td>
<td>50.00</td>
<td>50.43</td>
<td>1.43</td>
<td>1.14</td>
<td>1.10</td>
<td>2.18</td>
<td>± 1.10</td>
</tr>
<tr>
<td>C</td>
<td>20.0</td>
<td>20.08</td>
<td>0.40</td>
<td>0.12</td>
<td>0.33</td>
<td>1.62</td>
<td>± 0.33</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>51.13</td>
<td>2.21</td>
<td>0.06</td>
<td>0.88</td>
<td>1.73</td>
<td>± 0.88</td>
</tr>
<tr>
<td></td>
<td>70.0</td>
<td>71.26</td>
<td>1.77</td>
<td>0.25</td>
<td>1.55</td>
<td>2.18</td>
<td>± 1.55</td>
</tr>
</tbody>
</table>

* In method A, drug taken/found, range and SD are in mg, and in method B & C, the quantities are in µg ml⁻¹.

SD: Standard deviation; RSD: Relative Standard deviation; ROE. Range of error

** At 95 % confidence level and for six degrees of freedom
Table 3.3.3. Results of analysis of tablets containing RNH by the proposed methods

<table>
<thead>
<tr>
<th>Dosage form and brand name</th>
<th>Label claim mg per tablet or per ml</th>
<th>Reference method</th>
<th>Found*(% of label claim± SD)</th>
<th>Proposed methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Method A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Method B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Method C</td>
</tr>
<tr>
<td>Tablets Ranitin^a</td>
<td>150</td>
<td>98.64 ± 0.56</td>
<td>99.14 ± 0.26</td>
<td>98.96 ± 0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>99.52 ± 0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.93; F=4.64</td>
<td>t=0.72; F=2.25</td>
<td>t=2.24; F=1.47</td>
</tr>
<tr>
<td>Histac^b</td>
<td>300</td>
<td>100.48 ± 0.96</td>
<td>99.74 ± 0.42</td>
<td>100.44 ± 0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>101.76 ± 1.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.69; F=5.22</td>
<td>t=0.07; F=1.51</td>
<td>t=1.81; F=1.78</td>
</tr>
<tr>
<td>Injection Ranitin^a</td>
<td>25</td>
<td>102.64 ± 0.78</td>
<td>101.96 ± 0.46</td>
<td>102.18 ± 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>102.73 ± 1.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.95; F=2.88</td>
<td>t=1.07; F=1.81</td>
<td>t=0.09; F=2.53</td>
</tr>
</tbody>
</table>

Ψ Average of five determinations

*Marketed by: a. Torrent Pharmaceuticals; b. Ranbaxy Chemical

** Tabulated t-value at 95 % confidence level 2.77
       Tabulated F-value at 95 % confidence level 6.39
Table 3.3.4. Results of recovery study using standard-addition method

<table>
<thead>
<tr>
<th>Dosage form studied</th>
<th>Method A</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Method B</th>
<th></th>
<th></th>
<th></th>
<th>Method C</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount of pure RNH added, mg</td>
<td>Amount of pure RNH added, mg</td>
<td>Total found, mg</td>
<td>Recovery of pure RNH added, %</td>
<td>Conc. of RNH in tablet, µg ml⁻¹</td>
<td>Conc. of pure RNH added, µg ml⁻¹</td>
<td>Total found, µg ml⁻¹</td>
<td>Recovery of pure RNH added, %</td>
<td>Conc. of RNH in tablet, µg ml⁻¹</td>
<td>Conc. of pure RNH added, µg ml⁻¹</td>
<td>Total found, µg ml⁻¹</td>
<td>Recovery of pure RNH added, %</td>
<td></td>
</tr>
<tr>
<td>Ranitine (150 mg tablet)</td>
<td>4.96</td>
<td>6.00</td>
<td>11.15</td>
<td>103.16</td>
<td>9.87</td>
<td>20.0</td>
<td>29.77</td>
<td>99.50</td>
<td>9.95</td>
<td>20.0</td>
<td>31.21</td>
<td>106.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.96</td>
<td>8.00</td>
<td>12.87</td>
<td>98.88</td>
<td>9.87</td>
<td>30.0</td>
<td>39.97</td>
<td>100.34</td>
<td>9.95</td>
<td>40.0</td>
<td>51.62</td>
<td>104.2</td>
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<tr>
<td></td>
<td>4.96</td>
<td>10.00</td>
<td>15.09</td>
<td>101.31</td>
<td>9.87</td>
<td>40.0</td>
<td>49.74</td>
<td>99.68</td>
<td>9.95</td>
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<td>75.05</td>
<td>108.5</td>
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</tr>
<tr>
<td>Histac (300 mg tablet)</td>
<td>3.01</td>
<td>6.00</td>
<td>8.97</td>
<td>99.33</td>
<td>5.02</td>
<td>20.0</td>
<td>25.70</td>
<td>103.40</td>
<td>10.18</td>
<td>20.0</td>
<td>31.62</td>
<td>107.2</td>
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<tr>
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<td>3.01</td>
<td>8.00</td>
<td>10.98</td>
<td>99.64</td>
<td>5.02</td>
<td>30.0</td>
<td>34.68</td>
<td>98.87</td>
<td>10.18</td>
<td>40.0</td>
<td>51.46</td>
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<td>10.00</td>
<td>13.21</td>
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<td>5.02</td>
<td>40.0</td>
<td>45.52</td>
<td>101.26</td>
<td>10.18</td>
<td>60.0</td>
<td>75.94</td>
<td>109.6</td>
<td></td>
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<tr>
<td>Ranitine (25 mg injection)</td>
<td>4.08</td>
<td>6.00</td>
<td>9.94</td>
<td>97.66</td>
<td>5.11</td>
<td>20.0</td>
<td>25.60</td>
<td>102.45</td>
<td>10.27</td>
<td>20.0</td>
<td>31.03</td>
<td>103.8</td>
<td></td>
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<tr>
<td></td>
<td>4.08</td>
<td>8.00</td>
<td>12.15</td>
<td>100.87</td>
<td>5.11</td>
<td>30.0</td>
<td>35.40</td>
<td>100.97</td>
<td>10.27</td>
<td>40.0</td>
<td>52.51</td>
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<td></td>
<td>4.08</td>
<td>10.00</td>
<td>13.93</td>
<td>98.51</td>
<td>5.11</td>
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<td>10.27</td>
<td>60.0</td>
<td>76.57</td>
<td>110.5</td>
<td></td>
</tr>
</tbody>
</table>

* Mean value of three determinations
Fig. 3.3.1. Calibration curve for method B

Fig. 3.3.2. Calibration curve for method C
3.3.3.0. 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 known excess of iodate, and after oxidation, the residual iodate was determined by iodometric titration. In spectrophotometric method B, the drug was treated with a large and unmeasured excess of iodate and the iodine formed was determined by an auxiliary reaction with dichlorofluorescein. In method C, the iodate was determined iodometrically by complexing the iodine released with starch and measuring absorbance of the resulting blue colour.

In methods A and C, the amount of iodate reacted corresponded to the amount of RNH whereas in method B the amount of iodine released in the redox reaction between iodate and RNH, corresponded to the drug content. In all the methods, reduction of nitro group of the molecule with Zn/HCl was the first step. Unreduced RNH was found to give erratic results.

3.3.3.1. Method Development

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.

At laboratory temperature (30 ± 2°C), depending on the amount of drug involved, the time required for the complete oxidation of the drug was 15 min, contact times up to 45 min had no effect on the stoichiometry or the results.

To fixed amount of the drug, different amounts of excess of iodate were added maintaining constant acid concentration and contact time. The stoichiometry of the reaction was not affected in the presence 2-3 fold excess of iodate. Under optimum conditions, 10 ml of 0.002 M iodate solution was found adequate for the complete oxidation of the drug.
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. Based on the stochiometry, the following reaction scheme is suggested.

\[
\begin{align*}
(H_3C)_2H_2CN\text{-}F\text{-}CH_2\text{-}S\text{-}(CH_2)_2\text{-}NH\text{-}C\text{=}CH\text{-}NH_2 & \quad \text{+ Iodate} \\
(H_3C)_2H_2CN\text{-}F\text{-}CH_2\text{-}SO\text{-}OH & \quad \text{+ CH}_2\text{-}NH\text{-}C\text{=}CH\text{-}NH_2
\end{align*}
\]

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.

**Spectrophotometric method B**

Several substances of pharmaceutical interest have been determined by measuring the iodine released in the redox reaction between the substrate and iodate in acid medium [113-125].

\[
\begin{align*}
\text{Reduced RNH} & \quad \text{+ Iodate} \quad \text{+ H}^+ & \quad \text{Reduced RNH sulphoxide} \\
\IO_3^- & \quad + \quad 6\text{H}^+ \quad + \quad 5\text{Cl}^- & \quad \text{5Cl}^- \quad + \quad 3\text{H}_2\text{O} \\
\text{5Cl}^- \quad + \quad 5\text{Cl}^- & \quad \text{5Cl}_2^-
\end{align*}
\]

2,7-Dichlorofluorescein 2,7-Dichloro-4,5-diodofluorescein
In the proposed method, oxidation of reduced RNH was slow in sulphuric and acid medium, HCl was chosen as the reaction medium to effect the reaction. At lower acid concentrations, oxidation was delayed and the rate increased with increased acid concentration. A 0.5 ml of 1.0 M HCl was found suitable for this reaction which was complete in 20 min. Higher concentration were avoided since the iodination of the dye required a pH of 3.25±0.1.

To a fixed amount of drug and other reagents, varying amounts of potassium iodate were added to study its effect and finally 1 ml of 0.4 % iodate was found adequate for the concentrations of the drug studied.

A 1.0 ml of 6 % sodium chloride was used as a source of Cl⁻ ions for the formation of ICl₂⁻ which in turn was used as the iodinating agent to iodinate dichlorofluorescein dye. Increase in volume up to 2.0 ml had no effect on absorbance.

To a fixed amount of drug and by keeping all the reagent concentrations constant, different volumes of dye i.e., 0.5 to 3.0 ml were added. Constant and steady absorbance values were obtained when 1.5 to 3.0 ml of 0.01 % dye solution were used. Hence 2 ml of 0.01 % dye solution were used in all experiments.

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 absorbed at 520 nm in the pH range of 3.25 ± 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.

Different combinations of addition of reactants before the oxidation of drugs had no effect on the absorbance values. But after the oxidation of the drug to sulfoxide, the order to be followed is: dye-buffer, to get maximum absorbance.

**Spectrophotometric method C**

The method is based on the oxidation of RNH by a known excess iodate in acid medium and the determination of the unreacted oxidant by treating with iodide. The
resulting iodine was determined by complexing with starch, and measuring the absorbance at 560 nm.

The optimum conditions for the colour development were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the coloured species.

One ml of 1 % starch was found adequate for the maximum absorbance at 560 nm. RNH, when added in increasing amounts to a fixed amount of iodate, consumes the latter and there will be a concomitant decrease in its concentration. When iodide is added to decreasing concentrations of iodate, there will be a concomitant decrease in the concentration of iodine released. This is observed as a proportional decrease in the absorbance of the iodine-starch complex at the wavelength of maximum absorption with increasing concentration of RNH (Fig. 3.3.2.).

Sulphuric acid medium was found to be ideal for the oxidation of the drug by iodate and latter’s determination by iodine-starch reaction. In HCl medium, colour was less stable. One ml 1M sulphuric acid in a total volume of 10 ml was found adequate for oxidation as well as subsequent steps in the analysis.

The first step in the assay procedure is the determination of the upper limit of iodate spectrophotometrically using iodine-starch reaction. In a preliminary experiment, different concentrations of iodate were reacted with fixed amount of iodide in acidic conditions and iodine released was complexed with starch and measured at 560 nm, and the upper limit was found to be 2 µg ml⁻¹. Hence, different amounts of RNH were reacted with 1 ml of 20 µg ml⁻¹ iodate.

3.3.3.2. Method Validation

Analytical parameters of spectrophotometric methods

A linear correlation was found between absorbance at λmax and concentration ranges given in Table 3.3.1 for methods B and C. Correlation coefficients, intercepts, slopes, molar absorptivity, Sandell sensitivity and detection limit are also given in Table 3.3.1 and indicate the high sensitivity of the methods.
3.3.3.3. Accuracy and precision of the methods

The accuracy and precision of the analysis were assessed by seven replicate determinations at three different concentration levels. The results are presented in Table 3.3.2. The relative standard deviation obtained for pure drug indicate reasonable accuracy and precision of the proposed methods. For a better picture of reproducibility on a day-to-day basis, a series was run in which standard drug solution in three levels was run each day for 5 days. The day-to-day RSD values were in the range of 1.0-3.0 %, and represent the best appraisal of the procedures in daily routine use.

3.3.3.4. Application to the studied drugs in pharmaceutical formulations

The proposed methods were applied to the assay of RNH in tablets without any coated colour lest it should interfere with the spectrophotometric methods and also in injections. The results are compiled in Table 3.3.3 and were checked by the reference method [32]. There is a close agreement between the results obtained by the proposed and reference methods as found from the Student’s t- and F- values. The results obtained by the proposed methods also agreed well with the label claims in all instances.

3.3.3.5. Recovery of pure drug added to formulations

Recovery experiments were carried out by adding known amounts of pure drug to already analysed dosage forms (tablets and injections) and determining the total amount by the proposed methods. The percent recovery values of the pure drug added are shown in Table 3.3.4 and demonstrate that frequently encountered common excipients such as talc, starch, alginate, stearate, lactose and gum acacia, ammonium chloride and sodium citrate did not interfere in the methods.
SECTION 3.4
SPECTROPHOTOMETRIC DETERMINATION OF RNH USING FOLIN - CIOCALTEU [F-C] REAGENT

3.4.1.0. INTRODUCTION

Folin-Ciocalteu reagent [126] is an oxidimetric reagent and its used in the determination of many phenolic compounds [127] and a large number of substances of pharmaceutical interest such as diflofenac sodium [128], salbutamol sulphate [129], omeprazole [130], gatifloxacin [131], clarimythrocin [132], lamivudine [133], isopreaaline [134], azithromycin [135], meloxicam [136], melotinin and meloxicam [137], sylimarin [138], nimodipine [139], tramadol HCl [140], cephalosporin [141] and esomeprazole [142].

From the literature survey on the spectrophotometric methods for the determination RNH presented in Section 3.0.2, it is clear that the F-C reagent has not been used for the determination of RNH. The author has used F-C reagent for the sensitive spectrophotometric method for the assay of ranitidine in its dosage forms using Folin-Ciocalteu reagent in the presence of alkali. The details are presented in this Section 3.4.

3.4.2.0. EXPERIMENTAL

3.4.2.1. Apparatus

A Systronics model 106 digital spectrophotometer with 10 mm quartz cells was used for absorbance measurements.

3.4.2.2. Reagents

All chemicals used were of analytical reagent grade and double distilled water was used throughout the investigation.

F-C reagent: Commercially available Folin-Ciocalteu (FC) reagent (Lobo Chemie, India) 2N, was suitably diluted to give 1N with water and used.

Sodium Hydroxide: A 2 M solution was prepared by dissolving 8 g of the chemical (S. d. Fine chemicals, Mumbai, India) in 100 ml of water.
3.4.2.3. Preparation of standard drug solution

The stock standard solution containing 2 mg ml\(^{-1}\) reduced RNH was prepared as described in Chapter 3.0 Section 3.3.2.3. This solution was suitably diluted to get a working standard solution of 100 \(\mu g\) ml\(^{-1}\).

3.4.2.4. Procedures

Procedure for pure drug

Aliquots of 0.5, 1.0, 2.0, ..., 6.0 ml of the standard 100 \(\mu g\) ml\(^{-1}\) drug solution were transferred into a series of 10 ml calibrated flasks by means of micro burette. The volume was adjusted to 6 ml by adding an adequate volume of water. To each flask was added 1 ml of F-C reagent (1N) followed by 1.5 ml of sodium hydroxide solution (2M). The contents were diluted to volume, mixed well and set aside for 15 min before measuring the absorbance at 760 nm against a reagent blank. A suitable aliquot of the sample solution was subjected to analysis using the above procedure and its concentration was read from the calibration graph or computed from the regression equation derived using the Beer's law data.

Procedure for formulations

Pharmaceutical preparations containing studied drug was purchased from the local market. Tablet extracts and injections solution was prepared as described in the Chapter 3.0 Section 3.3.2.3 and suitable aliquot was taken for assay by the procedure previously described for pure drugs.
Table 3.4.1. Accuracy and precision of the methods

<table>
<thead>
<tr>
<th>Amount taken, μg ml(^{-1})</th>
<th>Amount found*, μg ml(^{-1})</th>
<th>Relative error, %</th>
<th>Range, μg ml(^{-1})</th>
<th>SD, μg ml(^{-1})</th>
<th>RSD, %</th>
<th>ROE, %</th>
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</thead>
<tbody>
<tr>
<td>20.0</td>
<td>20.30</td>
<td>1.50</td>
<td>0.34</td>
<td>0.38</td>
<td>1.86</td>
<td>± 0.33</td>
</tr>
<tr>
<td>40.0</td>
<td>39.25</td>
<td>1.87</td>
<td>0.46</td>
<td>0.52</td>
<td>1.33</td>
<td>± 0.46</td>
</tr>
<tr>
<td>60.0</td>
<td>58.86</td>
<td>1.90</td>
<td>0.21</td>
<td>0.55</td>
<td>0.93</td>
<td>± 0.45</td>
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</tbody>
</table>

* Average value of seven determinations

Table 3.4.2. Results of analysis of pharmaceutical preparations containing RNH

<table>
<thead>
<tr>
<th>Brand name* and dosage</th>
<th>Label claim mg per tablet, or per ml</th>
<th>Found(^{*}) (% of label claim ± SD)</th>
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<tr>
<td></td>
<td></td>
<td>Official method</td>
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<tr>
<td>Ranitina tablet</td>
<td>150</td>
<td>98.64±0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.39; F=4.64</td>
</tr>
<tr>
<td>Histacb tablet</td>
<td>300</td>
<td>97.92±0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.05; F=1.39</td>
</tr>
<tr>
<td>Ranitin(^{a}) injection</td>
<td>25</td>
<td>100.63±0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.78; F=1.17</td>
</tr>
</tbody>
</table>

Ψ Marketed by: a. Torrent Pharmaceuticals; b. Ranbaxy Pharmaceuticals

* Average of five determinations

# Tabulated value at 95% confidence level.
Table 3.4.3. Results of recovery study using standard-addition method

<table>
<thead>
<tr>
<th>Dosage form studied</th>
<th>Conc. of RNH in tablet, µg ml⁻¹</th>
<th>Conc. of RNH added, µg ml⁻¹</th>
<th>Total found, µg ml⁻¹</th>
<th>Recovery of pure RNH added, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranitin tablet (150 mg)</td>
<td>19.83</td>
<td>10.00</td>
<td>30.15</td>
<td>103.20</td>
</tr>
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<td></td>
<td>19.83</td>
<td>20.00</td>
<td>40.19</td>
<td>101.80</td>
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<td></td>
<td>19.83</td>
<td>30.00</td>
<td>49.41</td>
<td>98.60</td>
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<tr>
<td>Histac tablet (300 mg)</td>
<td>19.95</td>
<td>10.00</td>
<td>30.22</td>
<td>102.66</td>
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<td></td>
<td>19.95</td>
<td>20.00</td>
<td>39.65</td>
<td>98.48</td>
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<td>19.95</td>
<td>30.00</td>
<td>49.30</td>
<td>97.83</td>
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<tr>
<td>Ranitin injection (25 mg)</td>
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<td>30.80</td>
<td>104.70</td>
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<tr>
<td></td>
<td>20.33</td>
<td>20.00</td>
<td>40.78</td>
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</tr>
<tr>
<td></td>
<td>20.33</td>
<td>30.00</td>
<td>50.83</td>
<td>101.67</td>
</tr>
</tbody>
</table>

* Mean value of three determinations

Fig. 3.4.1. Calibration curve
3.4.3.0. RESULTS AND DISCUSSION

The method is based on the formation of a blue coloured chromogen with absorption maximum at 760 nm following the reduction of F-C reagent and reduced RNH in the presence of sodium hydroxide. After a thorough study of the effect of different concentrations of sodium hydroxide and F-C reagent separately on the formation of colour, the optimum concentrations as recommended in procedure were fixed to get maximum sensitivity and stability.

3.4.3.1. Method Development

To find a suitable medium for the reaction, different aqueous bases were used, such as borax, sodium hydroxide, sodium carbonate or bicarbonate and sodium acetate. The best results were obtained when sodium hydroxide was used. In order to determine the optimum concentration of sodium hydroxide, different volumes of 2M sodium hydroxide solution were used with a fixed concentration of reduced RNH (100 μg ml⁻¹). 1.5 ml of 2 M NaOH solution was found optimum and larger volumes up to 5 ml had no effect on the absorbance of the coloured species.

Several experiments were carried out to study the influence of F-C reagent concentration on the colour development and one ml of reagent solution gave maximum colour hence the same quantity in a total volume of 10 ml was used throughout the work.

The colour reaction is not instantaneous. Maximum colour is developed within 15 min of mixing the reactants and the coloured species was found to be stable up to 3h thereafter.

Drug-FC reagent-NaOH gave maximum sensitivity and hence the same order was followed in the study. A contact time of 15 min was found necessary to produce maximum sensitivity.

3.4.3.2. Method Validation

Analytical parameters

Beer's law is obeyed over the concentration range 5.0-60.0 μg ml⁻¹, the equation of the line being \( A = 0.02 + 0.013 C \) (where \( A \) is the absorbance and \( C \) concentration in μgm⁻¹)
with a correlation co-efficient of 0.9878. The apparent molar absorptivity is $4.04 \times 10^4$ mol$^{-1}$cm$^{-1}$ and Sandell sensitivity is 86.94 ng cm$^{-2}$. The limits of detection and quantification are 1.37 and 4.56 µg ml$^{-1}$ respectively.

### 3.4.3.3. Accuracy and precision of the methods

To verify whether the proposed determination is accurate and reproducible, the determination was repeated seven times using the same drug solution with a final concentration of 20, 40 and 60 µg ml$^{-1}$. The results are presented in Table 3.4.1. The relative standard deviation obtained for pure drugs indicate reasonable accuracy and precision of the proposed methods.

### 3.4.3.4. Application to the pharmaceutical formulations

The proposed method was applied to the analysis tablets and injections containing RNH and the results are presented in Table 3.4.2. The products were simultaneously analysed by the reference method [32] for comparison. A close agreement between the results obtained by the two methods is apparent from the calculated Student's t-value and F-value, which are lower than the tabulated values.

### 3.4.3.5. Recovery of pure drug added to formulations

In order to justify the reliability and suitability of the proposed method, recovery tests via standard addition technique were performed. Known quantities of pure RNH in three levels were added to pre-analysed tablet and injection solutions, and the mixtures were analysed by the proposed method after carrying out the reduction step. The results of recovery experiments presented in Table 3.4.3 indicate that the commonly encountered excipients such as talc, starch, alginate, stearate and gum acacia did not interfere in the determination of the proposed method.
SECTION 3.5

TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF RANITIDINE IN NON-AQUEOUS MEDIUM

3.5.1.0 INTRODUCTION

The application of non-aqueous titrimetric and spectrophotometric methods in pharmaceutical analysis has been reviewed in Chapter 2.0 Section 2.3.

The literature survey on the titrimetric and spectrophotometric methods for the assay of RNH was presented in Section 3.0.2 from which it is evident the assay of RNH has not been accomplished in non-aqueous medium either by titrimetry or spectrophotometry. The present section describes two titrimetric methods and one spectrophotometric method which are based on neutralization reaction.

3.5.2.0. Experimental

3.5.2.1. Apparatus

A Systronics model 106 digital spectrophotometer with 1-cm matched quartz cells was used for all absorbance measurements. Potentiometric titration was performed with an Elico 120 digital pH meter provided with a combined glass-SCE system. The KCl of the salt bridge was replaced with 0.1 M lithium perchlorate in glacial acetic acid.

3.5.2.2. Reagents

All the reagents of analytical reagent grade were used throughout the investigation.

Perchloric acid (0.01 M): To 4.5 ml of 70 % perchloric acid (S.d. Fine Chem., Mumbai, India) was added 150 ml of glacial acetic acid, mixed well; added 10.5 ml of acetic anhydride and allowed the solution to cool for 30 min; finally diluted to 500 ml with glacial acetic acid and allowed to stand overnight. This perchloric acid (~0.1 M) was diluted to get 0.01 M acid with glacial acetic acid and standardized with pure potassium biphthalate and crystal violet indicator.

Crystal violet indicator: Prepared by dissolving 0.1 g of dye (S. d. Fine Chem., Mumbai, India) in 100 ml glacial acetic acid.
Perchloric acid-crystal violet mixture (1.5 mM HClO₄ - 0.25 mM crystal violet): Prepared by mixing 15 ml of 0.01 M perchloric acid and 10 ml of 1000 µg ml⁻¹ crystal violet solutions and diluting to 100 ml with glacial acetic acid.

3.5.2.4. Standard drug solution

Pharmaceutical grade ranitidine hydrochloride (RNH) was procured from Glaxo Smithkline, Nashik, India, as gift, and was used as received. A stock standard solution containing 2 mg ml⁻¹ RNH was prepared by dissolving 500 mg of pure drug in glacial acetic acid and diluted to the mark in a 250 ml calibrated flask. This solution (2 mg ml⁻¹) was used for titrimetric work, and for spectrophotometric work, the same was diluted appropriately with glacial acetic acid to get 100 µg ml⁻¹ working concentration.

3.5.2.5. Procedures

Procedure for pure drug

Visual titration (Method A)

A 10 ml aliquot of drug solution containing 1-15 mg of RNH was accurately measured and transferred into a clean and dry 100 ml titration flask, 2 drops of crystal violet indicator was added and titrated with standard 0.01 M perchloric acid to an emerald green end point. The amount of drug in the measured aliquot was calculated from:

\[
\text{Amount (mg)} = VMR
\]

where \(V\) = volume of perchloric acid required, ml

\(M\) = relative molecular mass of drug,

\(R\) = molarity of perchloric acid

Potentiometric titration (Method B)

A 10 ml aliquot of standard drug solution equivalent to 1-15 mg of RNH was pipetted out into a clean and dry 100 ml beaker and the solution was diluted to 30 ml by adding glacial acetic acid. The combined glass-SCE (modified) system was dipped in the solution. The contents were stirred magnetically and the titrant (0.01M HClO₄) was added from a micro burette. Near the equivalence point, the titrant was added in 0.2 ml increments. After each addition of titrant, the solution was stirred magnetically for 30 s and the steady potential was noted. The addition of titrant was continued until there was no significant change in potential on further addition of titrant. The equivalence point was
determined by applying the graphical method. The amount of drug in the measured aliquot was calculated as described under visual titration.

**Spectrophotometric method (Method C)**

Different aliquots (1.0 - 7.0 ml) of standard 100 µg ml⁻¹ drug solution were accurately transferred into a series of 10 ml calibrated flasks. An exactly measured volume (2 ml) of perchloric acid-crystal violet mixture was added to each flask and the volume was diluted to the mark with glacial acetic acid mixed well and absorbance was measured at 570 nm against a reagent blank. The increasing absorbance values at 570 nm were plotted against the concentration of drug to obtain the calibration graph. The concentration of the unknown was read from the calibration graph or calculated from the regression equation obtained from Beer’s law data.

**Procedure for formulations**

**Tablets.** Twenty tablets were weighed and ground into a fine powder. An amount of powder equivalent to 200 mg of RNH was weighed accurately into 100 ml calibrated flask, 70 ml of glacial acetic acid added and shaken for about 20 min. Then, the volume was made up to the mark with glacial acetic acid, mixed well and filtered using Whatman No 42 filter paper. The first 10 ml portion of the filtrate was discarded. A suitable aliquot of the subsequent portion was next subjected to analysis by titrimetry. The filtrate (equivalent to 2 mg ml⁻¹) was diluted appropriately to obtain 100 µg ml⁻¹ solution and analysed by spectrophotometry using the general procedure described earlier.

**Injections.** The contents of 20 ampoules (each containing 25 mg of RNH) were mixed and an accurately measured volume equivalent to 200 mg of RNH was transferred into 100 ml separatory funnel. Ten ml of 6 M ammonia solution added and extracted with three 10 ml portions of chloroform. The chloroform extracts were combined and evaporated to dryness. The residue was dissolved in 50 ml glacial acetic acid and transferred into a 100 ml calibrated flask. The volume was made up to the mark with the same solvent. The solution (2 mg ml⁻¹) was subjected to analysis by titrimetry, and spectrophotometry after appropriate dilution as described above.
Table 3.5.1. Evaluation of accuracy and precision of proposed methods

<table>
<thead>
<tr>
<th>Method*</th>
<th>RNH taken</th>
<th>RNH found</th>
<th>Relative error, %</th>
<th>Range</th>
<th>SD</th>
<th>RSD, %</th>
<th>ROE**, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.00</td>
<td>3.02</td>
<td>0.67</td>
<td>0.36</td>
<td>0.04</td>
<td>1.31</td>
<td>±0.04</td>
</tr>
<tr>
<td></td>
<td>8.00</td>
<td>7.92</td>
<td>1.00</td>
<td>0.61</td>
<td>0.07</td>
<td>0.91</td>
<td>±0.06</td>
</tr>
<tr>
<td></td>
<td>13.00</td>
<td>12.94</td>
<td>0.46</td>
<td>0.88</td>
<td>0.01</td>
<td>0.08</td>
<td>±0.01</td>
</tr>
<tr>
<td>B</td>
<td>3.00</td>
<td>3.03</td>
<td>0.98</td>
<td>0.13</td>
<td>0.03</td>
<td>0.85</td>
<td>±0.03</td>
</tr>
<tr>
<td></td>
<td>8.00</td>
<td>8.10</td>
<td>1.25</td>
<td>0.25</td>
<td>0.09</td>
<td>1.10</td>
<td>±0.08</td>
</tr>
<tr>
<td></td>
<td>13.00</td>
<td>12.84</td>
<td>1.23</td>
<td>0.19</td>
<td>0.10</td>
<td>0.79</td>
<td>±0.09</td>
</tr>
<tr>
<td>C</td>
<td>20.00</td>
<td>19.87</td>
<td>0.65</td>
<td>0.97</td>
<td>0.25</td>
<td>1.24</td>
<td>±0.23</td>
</tr>
<tr>
<td></td>
<td>40.00</td>
<td>39.82</td>
<td>0.45</td>
<td>1.13</td>
<td>0.44</td>
<td>1.11</td>
<td>±0.39</td>
</tr>
<tr>
<td></td>
<td>60.00</td>
<td>58.84</td>
<td>1.93</td>
<td>0.68</td>
<td>0.54</td>
<td>0.92</td>
<td>±0.48</td>
</tr>
</tbody>
</table>

* In methods A & B, drug taken/found, range and SD are in mg, and in method C, the quantities are in µg ml⁻¹.

SD: Standard deviation; RSD: Relative Standard deviation; ROE. Range of error

** At 95 % confidence level for six degrees of freedom
Table 3.5.2. Results of analysis of tablets containing RNH by the proposed methods∗

<table>
<thead>
<tr>
<th>Dosage form and brand name</th>
<th>Label claim mg per tablet or per ml</th>
<th>Reference method</th>
<th>found (% of label claim ±SD)w</th>
<th>Proposed methods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method A</td>
<td>Method B</td>
<td>Method C</td>
</tr>
<tr>
<td>Ranitina tablet</td>
<td>150</td>
<td>99.50 ± 1.25</td>
<td>98.24 ± 1.68</td>
<td>100.36 ± 1.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=0.94; F=1.88</td>
<td>t=0.65; F=3.40</td>
<td>t=1.41; F=3.66</td>
</tr>
<tr>
<td>Histacb tablet</td>
<td>300</td>
<td>100.37 ± 1.26</td>
<td>100.42 ± 1.80</td>
<td>98.63 ± 0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.16; F= 1.61</td>
<td>t=0.88; F=1.26</td>
<td>t=0.89; F=3.54</td>
</tr>
<tr>
<td>Ranitina injection</td>
<td>25</td>
<td>101.76 ± 0.62</td>
<td>100.88 ± 0.74</td>
<td>101.86 ± 0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t=1.49; F=1.98</td>
<td>t=0.88; F=2.83</td>
<td>t=1.47; F=3.73</td>
</tr>
</tbody>
</table>

Ψ Average of five determinations in methods A and C, and three determinations in method B

* Tabulated t-value at 95 % confidence level 2.77 A & C, 2.37 for method B.

Tabulated F-value at 95 % confidence level 6.39 A & C, 9.28 for method B.

* Marketed by: a. Torrent Pharmaceuticals; b. Rambaxy Pharmaceuticals,
<table>
<thead>
<tr>
<th>Dosage form studied</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranitine 150mg tablet</td>
<td>Amount of RNH in tablet, mg</td>
<td>Amount of RNH drug added, mg</td>
<td>Total found, mg</td>
</tr>
<tr>
<td>Ranitine 150mg tablet</td>
<td>4.98</td>
<td>6.0</td>
<td>11.04</td>
</tr>
<tr>
<td>Ranitine 150mg tablet</td>
<td>4.98</td>
<td>7.0</td>
<td>11.83</td>
</tr>
<tr>
<td>Ranitine 150mg tablet</td>
<td>4.98</td>
<td>8.0</td>
<td>13.02</td>
</tr>
<tr>
<td>Zinetac 300mg tablet</td>
<td>5.08</td>
<td>6.0</td>
<td>11.01</td>
</tr>
<tr>
<td>Zinetac 300mg tablet</td>
<td>5.08</td>
<td>7.0</td>
<td>12.22</td>
</tr>
<tr>
<td>Zinetac 300mg tablet</td>
<td>5.08</td>
<td>8.0</td>
<td>12.95</td>
</tr>
<tr>
<td>Histac 25mg injection</td>
<td>5.06</td>
<td>6.0</td>
<td>11.08</td>
</tr>
<tr>
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<td>7.0</td>
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<tr>
<td>Histac 25mg injection</td>
<td>5.06</td>
<td>8.0</td>
<td>12.98</td>
</tr>
</tbody>
</table>

* Mean value of three determinations
Fig 3.5.1. Potentiometric end point

Fig 3.5.2. Gran’s plot

Fig 3.5.3. Calibration curve
3.5.3.0. RESULTS AND DISCUSSION

3.5.3.1. Method Development

Titrimetric methods

The weakly basic property of RNH was enhanced due to the non-leveling effect of glacial acetic acid and titrated with perchloric acid with visual and potentiometric end point detection. Crystal violet gave highly satisfactory end point for the concentrations of analyte and titrant employed. A steep rise in the potential was observed at the equivalence point with potentiometric end point detection (Fig 3.5.1). The Gran’s plot (Fig 3.5.2) method was applied to ascertain the equivalence point. With both methods of equivalence point detection, a reaction stoichiometry of 1:1 (drug: titrant) was obtained which served as the basis for calculation. Using 0.01 M perchloric acid, 1-15 mg of RNH was conveniently determined. The relationship between the drug amount and the titration end point was examined. The linearity between two parameters is apparent from the correlation coefficients of 0.9993 and 0.9980 obtained by the method of least squares for visual and potentiometric methods, respectively. From this, it is implied that the reaction between RNH and perchloric acid proceeds stoichiometrically in the ratio 1:1 in the range studied.

Spectrophotometry

In a preliminary study, 20 \( \mu \text{g ml}^{-1} \) crystal violet in the base form was found to exhibit a convenient maximum absorbance at 570 nm. In the presence of 2 ml of 1.5 mM perchloric acid in total volume of 10 ml, this absorbance decreased to a constant minimum. Hence, different amounts of drug were treated with a fixed amount of acid-dye mixture i.e., 2 ml of 1.5 mM HClO₄ - 0.25 mM crystal violet (100 \( \mu \text{g ml}^{-1} \) w. r. t. crystal violet) to determine the concentration range of the drug that could be determined by the method of absorbance transitions of the dye accompanying the pH changes. The dye colour even in the presence of drug was found to be stable for several hours, and the order of addition of reactants was not critical.

Crystal violet (C.I. Basic violet 3) is a dye exhibiting violet colour in the base form and emerald green in the acid form. The spectrophotometric method is based on the facts that the colour of the dye is dependent on the pH of the solution and that the colour change
is not sudden but occurs continuously as the pH changes over definite range. To a fixed amount of acid-dye mixture where the dye is in the acid form (emerald green), different amounts RNH were added. This caused a progressive increase in pH of the solution because of neutralization of acid by the added drug (base), and as a result, the concentration of the base form of the dye increases. This is shown by the proportional increase in the absorbance of the solution at 570 nm (Fig 3.5.3) which is corroborated by the correlation coefficient of 0.9892.

3.5.3.2. Method Validation

Analytical parameters

Beer's law is obeyed over the concentration range 10-70 µg ml\(^{-1}\). The correlation coefficient of the calibration plot was calculated to be 0.9892 (n=7) confirming a linear increase in absorbance with increasing concentration of RNH. The calculated molar absorptivity was found to be \(2.2 \times 10^3\) \(1\) mol\(^{-1}\) cm\(^{-1}\) at 570 nm and the Sandell sensitivity was 0.16 µg cm\(^{-2}\). The limits of detection and quantification established according to ICH guidelines \[143\] were 1.07 and 3.58 µg ml\(^{-1}\), respectively.

3.5.3.3. Accuracy and precision of the methods

The accuracy and precision of the analysis were assessed by seven replicate determinations at three different/amount concentration levels. The results are presented in Table 3.5.1. The relative standard deviation obtained for pure drugs indicate reasonable accuracy and precision of the proposed methods. For a better picture of precision on a day-to-day basis, a series was run in which the standard drug solution at three levels was analysed, each day for 5 d. In terms of standard deviation, the day-to-day coefficient of variation was less than 3 per cent, and represented the best appraisal of the precision of the procedures in routine use.

3.5.3.4. Application to the formulations

The proposed methods were successfully applied to determine RNH in tablets and injections. The same batch formulations were analysed by an established procedure [32] for comparison. The results obtained by the proposed methods agree well with those of
reference method [32] and with the label claim. The results were also compared statistically by a Student's t-test for accuracy and a variance ratio F-test for precision with those of the reference method as summarized in Table 3.5.2. The results showed that the calculated t- and F- values did not exceed the tabulated values inferring that proposed methods are as accurate and precise as the reference method.

3.5.3.5. Recovery of pure drugs added to formulations

Recovery experiments were carried out by adding known amounts of pure drugs to already dosage forms tablets and injections and determining the total amount by the proposed methods. The percent recovery values of the pure drug added was showed in Table 2.6.3 demonstrate that frequently encountered common excipients such as talc, starch, alginate, stearate, lactose and gum acacia, ammonium chloride and sodium citrate did not interfere in the methods.
CONCLUSIONS ON CHAPTER 3

Four simple, accurate and sensitive titrimetric methods were developed for the assay of RNH in pure sample and dosage forms using bromate-bromide mixture, chloramine-T, and potassium iodate as oxidimetric reagents and acetic perchloric acid as titrant in acetic acid medium. The titrimetric methods ever developed for RNH are superior to many previously reported methods in terms of speed, simplicity, sensitivity and range of determination. The methods are rapid and do not involve any stringent experimental conditions which influence the sensitivity and reliability of the methods.

The spectrophotometric methods are also easier to perform and use inexpensive instrument compared to many reported techniques, and donot require any expensive or toxic reagents or organic solvents. The most striking advantage of the spectrophotometric methods is the sensitivity which surpasses the sensitivity of most of the previously reported methods. These advantages coupled with reasonable accuracy and precision render the methods suitable for routine quality control.

The drug has previously been determined using the F-C reagent in the presence of sodium carbonate. But the method is poorly sensitive with the linear range being 40-240 \( \mu g \text{ ml}^{-1} \) and the coloured species in reported to be less stable. The proposed method using F-C reagent offers the advantages of sensitivity of the reaction and stability of the measured species and shorter contact time compared to many visible spectrophotometric methods reported previously.

A significant advantages of all the proposed methods is the use of oxidisable reagents and acetic perchloric acid which are quite stable in solution than N-chlorosuccinimide used previously. In additional advantage is measured at longer wavelengths (520 or 610 nm) where the interference from the associated inactive ingredients is usually far less than at shorter wavelengths. With relative error and relative standard deviation values under 3 %, all the methods are fairly accurate and precise. Thus, while retaining the speed, accuracy and precision of other methods, the proposed methods are superior in terms of simplicity, sensitivity and convenience. However, the methods using bromate-bromide mixture are less selective and so also the spectrophotometric method using the F-C reagent.
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