SPECTROPHOTOMETRIC DETERMINATION OF RANITIDINE HYDROCHLORIDE (RNH) IN PHARMACEUTICAL FORMULATION USING 9-FLUORENYLMETHYL CHLOROFORMATE (FMOC-Cl)

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ABSTRACT

A new, simple and sensitive spectrophotometric method is developed for the determination of ranitidine hydrochloride (RNH). The proposed method is based upon reaction of RNH with 9-fluorenylmethyl chloroformate (FMOC-Cl) in borate buffer of pH 8.0 producing an absorption maximum at 255 nm. All parameters required for the reaction conditions are investigated. Linearity is verified with a range of 2-16 μg/mL and is described by the regression equation y = 61129 x + 0.0354 with a correlation coefficient of 0.9998 (n = 7). The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as 0.2219 and 0.6724 μg/mL, respectively. The method was successfully applied for the determination of RNH in pharmaceutical formulation. Therefore, the method can be used for routine analysis of RNH in quality control laboratories.

Keywords: Spectrophotometric, Ranitidine hydrochloride (RNH), 9-Fluorenylmethyl Chloroformate (FMOC-Cl).

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1. INTRODUCTION

Ranitidine hydrochloride (RNH), chemically N-[2-[[5-(dimethylamino)methyl-2-furanyl]methyl]thioethyl]-N′-methyl-2-nitro-1,1-ethenediamine hydrochloride (Fig. 1), is a histamine H2-receptor antagonist. Histamine is a natural chemical that stimulates the stomach cells to produce acid. The H2-receptor antagonists are used to block the action of histamine on parietal cells in the stomach, decreasing the production of acid by these cells. RNH is widely used in short term treatment of duodenal ulcer and in management of hypersecretory conditions (e.g. Zollinger-Ellison Syndrome), and it commonly used in treatment of peptic ulcer disease and gastroesophageal reflux disease. Earlier studies have indicated that RNH was oxidized by the liver microsomal oxidases and was converted to its N-oxidase, S-oxide and desmethyl metabolites. Due to the presence of thioether sulphur and amine groups in molecular structure, RNH is able to form an ion-pair complex with acidic dyes and to coordinate transition metals.

Several analytical methods have been reported for the determination of RNH in bulk, pharmaceutical dosage form and/or biological fluids. These methods include titrimetry, spectrophotometry, spectrofluorimetry, near infrared reflectance spectroscopy, polarography, voltammetry, high-performance liquid chromatography (HPLC), capillary electrophoresis, capillary electrophoresis, and immunoassay. X-ray using neutral network. These techniques were associated with some drawbacks as lack of sensitivity (titrimetry), time-consuming (TLC), laborious multiple procedures (immunoassay) and/or require sophisticated instruments and expensive reagents, and involve several manipulation steps (HPLC, capillary electrophoresis). Among these instrumental analytical techniques, spectrophotometric techniques occupy a unique position, because of its simplicity, sensitivity, accuracy and rapidity. There are few methods for the spectrophotometric determination of...
ranitidine. These are based on the reaction of ranitidine with some organic acidic dyes followed by extraction of the colored ion-pairs into organic solvents and absorbance measurements. Spectrophotometric determination of ranitidine in tablets has been also suggested through chromogenic reactions with 3-methyl-2-benzothiazoline hydrazione-iron (III), 2, 6-dichloroquinone chlorimide, Folin-Ciocalteu reagents. These methods, however, are not adaptable for use in automated systems due to the long reaction time for color development (15-30 min), they require prior extraction of the colored reaction product and involve a high reaction temperature (-90 °C) (2).

Derivatization is a process that usually involves a reaction of the analyte of interest with a particular derivatization reagent producing a new compound that has properties more amenable to a particular analytical method. There are a multitude of reagents that can be used depending on the character and functionality of the analytes. Derivatization methods have been employed to reduce the polarity of the amino group. Derivatization reactions, often selective for amine type (primary, secondary, tertiary) have also been used to improve the detection and separation of these amines. 9-Fluorenylmethyl chloroformate (FMOC-Cl) is a useful derivatizing agent used for the determination of amino acids, primary and secondary amines in alkaline conditions and the resulting derivatives are stable. However, application of FMOC-Cl for derivatization of carboxylic acid has also been reported. Both primary and secondary amines were derivatized with FMOC-Cl before separation by reversed-phase HPLC with UV detection at 254 nm. Several pharmaceutical compounds have been determined through this approach. The aim of the present study to develop and validate a simple, rapid, sensitive and selective method for determining RNH in the pharmaceutical formulation by UV-Visible spectrophotometer using FMOC-Cl as a derivatizing agent.

The colored reaction product and the resulting derivatives are stable. However, application of FMOC-Cl for derivatization of carboxylic acid has also been reported. Both primary and secondary amines were derivatized with FMOC-Cl before separation by reversed-phase HPLC with UV detection at 254 nm. Several pharmaceutical compounds have been determined through this approach.

The aim of the present study to develop and validate a simple, rapid, sensitive and selective method for determining RNH in the pharmaceutical formulation by UV-Visible spectrophotometer using FMOC-Cl as a derivatizing agent.

![Figure 1: Chemical structure of ranitidine hydrochloride (RNH)](image.png)

2. Experimental

2.1. Apparatus

All of the spectrophotometric measurements were made with a Double beam UV-1700 ultraviolet-visible spectrophotometer provided with matched 1-cm quartz cells (SHIMADZU, Japan). A Mi 150 pH / Temperature bench meter was used for adjusting pH. A rocking shaker model SK-0330-pro DLAB (USA) was used.

2.2. Chemicals and materials

Ranitidine hydrochloride (RNH) was kindly provided from General Medicine Company (Khartoum, Sudan). 9-Fluorenylmethyl chloroformate (FMOC-Cl) was purchased from Sigma-Aldrich (St.Louis, USA). Tablets containing a label claim of 150 mg RNH (Rantag (Julphar, U.A.E), Ranitidine (Medical Union Pharmaceuticals, Egypt), Ranicid (General Medicine Company, Sudan)) were purchased from local pharmacy. Acetonitrile, boric acid and sodium hydroxide were purchased from SDFCL (Mumbai). All reagents and solvents were of analytical reagent grade and used without further purification. Purified water was used throughout.

2.3. Preparation of standard and sample solutions

2.3.1. Stock standard solution of Ranitidine hydrochloride (100 μg/mL)

A stock solution (100 μg/mL) was prepared by dissolving 10 mg of RNH in 100 mL of distilled water and stored at 4 °C. Working solutions were prepared from individual stock solution at a concentration range of 2-16 μg/mL in the same solvent.

2.3.2. Preparation of 9-fluorenylmethyl chloroformate (FMOC-Cl)

A stock solution (500 μg/mL) was prepared by dissolving 50 mg of FMOC-Cl in 100 mL of acetonitrile and protected from light with aluminum foil and kept at 4 °C. Further dilution was made with the same solvent.

2.3.3. Preparation of borate buffer solutions

Borate buffer solution (0.2 mol/L) was firstly prepared by dissolving appropriate amount of boric acid and potassium chloride in distilled water and then adjusted the pH from 6.0 to 10.0 with 0.2 mol/L sodium hydroxide solution.

2.4. General recommended procedures

2.4.1. Procedure for calibration graph

Aliquot volumes of RNH working solution equivalent to 2-16 μg/mL were transferred into a series of 10 mL volumetric flasks. 2.0 mL of 0.2 mol/L borate buffers (pH 8.0) was added, followed by 0.3 mL of 500 μg/mL FMOC-Cl and mixed well for about 5 minutes at room temperature and then completed to the mark with distilled water. A blank experiment was carried out simultaneously. The absorbance of the reaction product was measured at 255 nm. The calibration graph was constructed by plotting absorbance versus concentration of drug in μg/mL, and the corresponding regression equation was derived.

2.4.2. Assay procedure for tablets

Twenty tablets were weighed and pulverized well. A weighed quantity of the powder equivalent to 150 mg of RNH was transferred to small conical flask and extracted with distilled water. The extract was filtered into 100 mL
volumetric flask and completed the volume with distilled water. Aliquots covering the working concentration range were transferred into 10 mL volumetric flasks, the procedure for calibration graph mentioned under previous section was then carried out. The nominal content of RNH within tablets was determined either from the calibration curve or by using the corresponding regression equation.

2.5. Determination of the stoichiometric ratio of the reaction (Job’s method)

The Job’s method of continuous variation was employed (Job P., 1964). Equimolar (1×10⁻³ mol/L) aqueous solutions of RNH and FMOC-Cl were prepared. Series of 10 mL portions of the master solutions of RNH and FMOC-Cl were made up comprising different complementary proportions (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0). The solution was further treated as described under the general recommended procedure.

3. RESULTS AND DISCUSSION

The derivatization reaction using FMOC-Cl is an alternative due to the high sensitivity of this compound in the ultraviolet region. RNH derivatized by FMOC-Cl with UV detection at 255 nm.

3.1. Spectral behavior in the derivatization

To confirm the effectiveness of the derivatization reaction, some procedures were performed and the results are reported in Fig. 2. The RNH has two amine groups that cause absorption in the ultraviolet at 228, 314 nm as shown in Fig. 2a. FMOC-Cl in the pure solvent had a sharp absorption band in the UV-Vis range 24. It was observed that 15 μg/mL FMOC-Cl in the reaction medium without the analyte (RNH) provided a spectrum containing two regions of high absorbance (206 and 255 nm) as shown in Fig. 2b. The analysis of RNH in the reaction medium with FMOC-Cl provides an absorption spectrum (Fig. 2c) with a similar profile to that obtained by FMOC-Cl in the reaction medium (Fig. 2b). This analysis confirms that FMOC-Cl reacts with RNH molecule, resulting in an amphipathic compound (with the polar part derived from RNH and nonpolar part derived from FMOC-Cl).

3.2. Stoichiometry of derivatization reaction

Under the optimum conditions, the stoichiometry of the reaction between RNH and FMOC-Cl was investigated by Job’s method and was found to be 1:2 because RNH contain two secondary amine groups. The result is shown in Fig. 3.

3.3. Reaction mechanism

It has been reported that FMOC-Cl could react with amino group of primary and secondary amine derivative. The reaction of RNH with FMOC-Cl has been found to take place in alkaline condition. In this process, substitution of a hydrogen atom in the amino group in the RNH occurs by aromatic rings containing alternating double bond (24). The reaction equation is shown in Scheme 1.

3.4. Optimization of the experimental conditions

Different experimental parameters affecting the derivatization reaction of RNH with FMOC-Cl were carefully studied and optimized. Such factors were changed individually, while others were kept constant. The factors included pH, volume of buffer, concentration of the reagent, temperature and reaction time.

3.4.1. Effect of pH

The buffer was used to keep the drug in dissociative form in order to retain their nucleophilic character. The pH of the samples was measured prior to the addition of FMOC-
Cl and therefore, because the reaction released protons, the final pH is likely to be slightly lower \(25\). The influence of pH on the absorbance of the reaction product was evaluated by using borate buffer with a pH ranging from 6.0 to 10.0. Maximum absorbance was obtained at pH 8.0, after which the absorbance began to decrease gradually until pH 10.0 as shown in Fig. 4. Therefore, pH of 8.0 was chosen as the optimum pH.

3.4.2. Effect of volume of buffer

It was found that increasing the volume of 0.2 mol/L borate buffer of pH 8.0 promote the reactivity of the amine function and stabilizes the solubility of the derivatizing reagent in acetonitrile, favoring the process of derivatization. The absorbance of the reaction product increased until reached 2.0 mL, and then decreased as shown in Fig. 5. Therefore, 2.0 mL of borate buffer of pH 8.0 was chosen as optimal volume.

3.4.3. Effect of concentration of FMOC-Cl reagent

The effect of FMOC-Cl concentration was evaluated in the interval of 5-25 μg/mL. It was found that increasing the concentrations of FMOC-Cl solution resulted in a consequent increase in the absorbance of the reaction product up to 15 μg/mL and then decreased as shown in Fig. 6. Therefore, 15 μg/mL of FMOC-Cl solution was chosen as the optimal concentration of the FMOC-Cl reagent.

3.4.4. Effect of temperature

The effect of reaction temperature (25-50 °C) was evaluated and it was found that increasing the reaction temperature higher than the room temperature up to 50 °C for 15 minutes, resulted in a subsequent decrease in the absorbance of the reaction product as shown in Fig. 7. Therefore, room temperature (25 °C) was chosen as the optimal temperature.

3.4.5. Effect of reaction time

The reaction time is definitive to ensure derivatization because this time period ensures the replacement of chlorine in the structure of FMOC-Cl by RNH molecule. Different time intervals were tested (zero-25 minutes) to
ascertain the time after which the solution attained its highest absorbance intensity. It was found that after 5 minutes homogenization the reaction product reached the highest absorbance intensity (at zero time), after which slight decrease in the absorbance of the reaction product was observed as shown in Fig. 8. Therefore, the reaction product was measured at zero time after homogenization take place.

**Fig. 8.** Effect of time on the reaction of RNH (10 μg/mL) with FMOC-Cl (15 μg/mL), buffer solution (pH 8.0): 2.0 mL, temperature: 25 °C.

### 3.5. Validation of the proposed method

The validity of the method was tested regarding; linearity, accuracy, precision, selectivity and robustness according to ICH Q2B recommendations.26, 27

#### 3.5.1. Linearity and sensitivity

After optimizing the conditions, the calibration graph was constructed by plotting the absorbance intensity of the reaction product (at λ = 255 nm) versus concentration of the RNH in μg/mL. The calibration plot was linear over the concentration range (2-16 μg/mL) as shown in Fig. 9. Analysis of the data gave the regression equation no. 1 with a correlation coefficient of 0.9998. The high value of the correlation coefficient of regression equation indicates good linearity and conformity to Beer’s law.

\[ Y = 61129 X + 0.0354 \]

**Equation no. 1**

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2(R1) recommendation, (LOQ = 10 \( \delta/S \) where \( \delta \) is the standard deviation of intercept of regression line of the calibration curve and \( S \) is the slope). The limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected (LOD = 3.3 \( \delta/S \)). The results of LOQ and LOD are abridged in Table 1. Sensitivity is often described in terms of molar absorptivity (\( \epsilon, \text{L/molecm} \)) and sandell’s sensitivity that can be defined as the number of micrograms of elements which are in a column of solution having a cross section of 1 cm² which shows 0.001 absorbance and is expressed as μg/mL/cm², and can be calculated from the equation no. 2 and the values are presented in Table 1.

\[ \text{Sensitivity (s)} = \frac{\text{Molecular weight} \times \text{No. of atoms in the element}}{\text{Molar absorptivity}} \]

**Equation no. 2**

#### 3.5.2. Accuracy and precision

The accuracy of the proposed method was evaluated by recovery study, by addition a known amount of standard pure drug to preanalyzed dosage form at three different concentration levels within the range of linearity. The results from study of accuracy are reported in Table 2. From these results it was clear that the method enables very accurate quantitative estimation of RNH in tablet dosage form, because all the results were within acceptable limit. The precision of the proposed method was determined by analysis of the working standard of RNH at three concentration levels. For estimation of intra-day precision, six successive replicate determinations of each concentration were carried out within the same day, using the recommended procedure. The inter-day precision was performed over three successive days, at the same concentration levels of RNH. The results of the study are summarized in Table 3. The relative standard deviations did not exceed 0.2973 indicating the good reproducibility of the proposed method. This precision level is adequate for the routine analysis of the investigated drug in quality control laboratories.

#### 3.5.3. Selectivity

The selectivity of the method was investigated by observing any interference encountered from the common tablet excipients such as sucrose, lactose, starch and magnesium stearate. The analysis of these laboratory prepared samples was carried out using the general recommended procedure, the recovery values between 98.31% to 101.63%. This indicated that the excipients did not interfere with the proposed method.

#### 3.5.4. Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not...
significantly affect the procedures; recovery values were shown in Table 4. This provided an indication for the reliability of the proposed method during its routine application for analysis of RNH drug.

3.6. Application analysis of pharmaceutical formulations

The proposed method was applied to some pharmaceutical formulations containing RNH. The results are given in Table 5. The results obtained by the proposed method were compared with other reported methods. As shown in Table 6, some of the reported methods have higher detection limits. Moreover, some of methods are alike or more sensitive, but have drawbacks such as having a narrow linear dynamic range, involving extraction or being performed at high temperatures.

### Table 1: Parameters for the performance of the proposed method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$ nm</td>
<td>255</td>
</tr>
<tr>
<td>Beer’s law limit (μg/mL)</td>
<td>2-16</td>
</tr>
<tr>
<td>Molar absorptivity, L/mol cm</td>
<td>$2\times10^{10}$</td>
</tr>
<tr>
<td>Sandell sensitivity, μg/cm$^2$</td>
<td>8.25×10$^{-7}$</td>
</tr>
<tr>
<td>Limit of detection (LOD) (μg/mL)</td>
<td>0.2219</td>
</tr>
<tr>
<td>Limit of quantification (LOQ) (μg/mL)</td>
<td>0.6724</td>
</tr>
<tr>
<td>Regression equation</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0354</td>
</tr>
<tr>
<td>Standard deviation of intercept</td>
<td>0.00411</td>
</tr>
<tr>
<td>Slope</td>
<td>61129</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.9998</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.00569</td>
</tr>
</tbody>
</table>

### Table 2: Recovery studies for determination of RNH by the proposed method

<table>
<thead>
<tr>
<th>Standard (μg/mL)</th>
<th>Sample (μg/mL)</th>
<th>Found (μg/mL)</th>
<th>Recovery (% ± R.S.D)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3</td>
<td>4.94</td>
<td>98.78% ± 0.1429</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>10.15</td>
<td>101.47% ± 0.0009</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>14.76</td>
<td>98.40% ± 0.0076</td>
</tr>
</tbody>
</table>

Recovery was calculated as the amount found/amount taken × 100. $^a$ Values are mean of three determinations.

### Table 3: Evaluation of intra-day and inter-day precision

<table>
<thead>
<tr>
<th>Taken (μg/mL)</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found (μg/mL)</td>
<td>R.S.D</td>
</tr>
<tr>
<td>3</td>
<td>2.92</td>
<td>0.0027</td>
</tr>
<tr>
<td>10</td>
<td>9.83</td>
<td>0.0461</td>
</tr>
<tr>
<td>15</td>
<td>14.63</td>
<td>0.0161</td>
</tr>
</tbody>
</table>

### Table 4: Robustness of the proposed spectrophotometric method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Modification</th>
<th>Recovery (% ± R.S.D)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.8</td>
<td>101.41% ± 0.1006</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>101.63% ± 0.1537</td>
</tr>
<tr>
<td></td>
<td>8.2</td>
<td>100.54% ± 0.1093</td>
</tr>
<tr>
<td>Volume of buffer (mL)</td>
<td>1.8</td>
<td>101.30% ± 0.2042</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>101.63% ± 0.1537</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>101.52% ± 0.0046</td>
</tr>
<tr>
<td>Reaction time (min.)</td>
<td>0</td>
<td>101.63% ± 0.1537</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>101.63% ± 0.1025</td>
</tr>
</tbody>
</table>

$^a$ Values are mean of three determinations.
Table 5: Results of assay of formulations for the proposed method

<table>
<thead>
<tr>
<th>Tablet name</th>
<th>Brand name</th>
<th>Label Claim mg/tablet</th>
<th>Proposed method Found (% ± R.S.D)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ranitidine</td>
<td>150</td>
<td>99.99% ± 0.0009</td>
<td></td>
</tr>
<tr>
<td>Ranacid</td>
<td>150</td>
<td>97.87% ± 0.0039</td>
<td></td>
</tr>
</tbody>
</table>

a Values are mean of three determinations.

Table 6: Comparison of the proposed method with other spectrophotometric reported methods for the determination of RNH.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Linear range(μg/mL)</th>
<th>LOD (μg/mL)</th>
<th>LOQ (μg/mL)</th>
<th>Ref.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite Cu²⁺/Br⁻</td>
<td>300-12,000</td>
<td>100</td>
<td>-</td>
<td>29</td>
<td>Involves flow injection, automated assembly, least sensitive.</td>
</tr>
<tr>
<td>Dichromate-diphenylcarbazide</td>
<td>5-50</td>
<td>0.83</td>
<td>2.52</td>
<td>30</td>
<td>Less sensitive.</td>
</tr>
<tr>
<td>Dichromate-iron(II)-thiocyanate</td>
<td>5-80</td>
<td>1.59</td>
<td>4.83</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Dichromate-iron(II)-orthophenanthroline</td>
<td>10-100</td>
<td>3.07</td>
<td>9.28</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>7,7,8,8-tetracyano quinodimethane</td>
<td>1-6</td>
<td>0.2</td>
<td>-</td>
<td>31</td>
<td>Requires thermostating at 70°C for 10 min and has narrow range of linear response.</td>
</tr>
<tr>
<td>KMnO₄/N-bromosuccinimideazine dyes</td>
<td>5-30</td>
<td>-</td>
<td>-</td>
<td>32</td>
<td>Involves extraction and uses unstable reagent.</td>
</tr>
<tr>
<td>7-Chloro-4-nitrobenz-2-oxa-1,3-diazole</td>
<td>2-20</td>
<td>0.13</td>
<td>-</td>
<td>33</td>
<td>Requires thermostating at 60°C for 25 min.</td>
</tr>
<tr>
<td>9-Fluorenyl methyl chloroformate</td>
<td>2-16</td>
<td>0.22</td>
<td>0.67</td>
<td>Present work</td>
<td>Simple, sensitive and selective.</td>
</tr>
</tbody>
</table>

4. CONCLUSION

In this paper, method for determination of RNH in pharmaceutical formulation was developed and validated. The method is based on the reaction of RNH with FMOC-Cl. The proposed method is simple, precise, accurate and sensitive with regard to spectrophotometric methods. It can be used in routine analysis of pharmaceutical formulations of RNH in quality control laboratories.

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