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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 per ICH guidelines and were found to be 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]thio]ethyl}-N′-methyl-2-nitro-1,1-ethenediamine hydrochloride (Fig. 1), is a histamine H_2-receptor antagonist. Histamine is a natural chemical that stimulates the stomach cells to produce acid. The H_2-receptor antagoinsts 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[1-3]. 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 [2, 4]. 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 [5].

Several analytical methods have been reported for the determination of RNH in bulk, pharmaceutical dosage form and/or biological fluids. These methods include titrimetry [6], spectrophotometry [7, 8], spectrofluorimetry [9], near infrared reflectance spectroscopy[10], polarography [11], voltammetry [12], high-performance liquid chromatography (HPLC) [13], TLC [14], capillary electrophoresis [15], immunoassay [16], X-ray using neutral network [17]. 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 hydrazone-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 use 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 [18]. 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 [19-21]. However, application of FMOC-Cl for derivatization of carboxylic acid has also been reported [22]. 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 [23].

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.

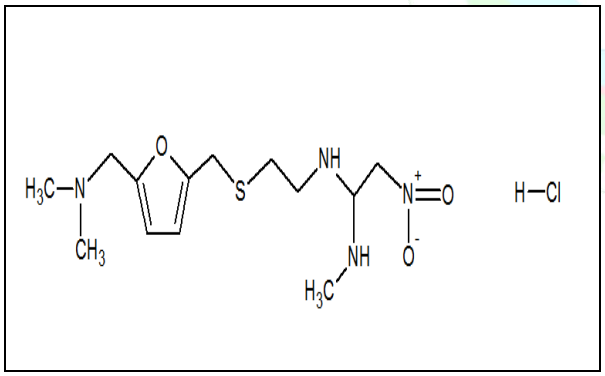


Figure 1: Chemical structure of ranitidine hydrochloride (RNH)

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 (Mumbia). 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).

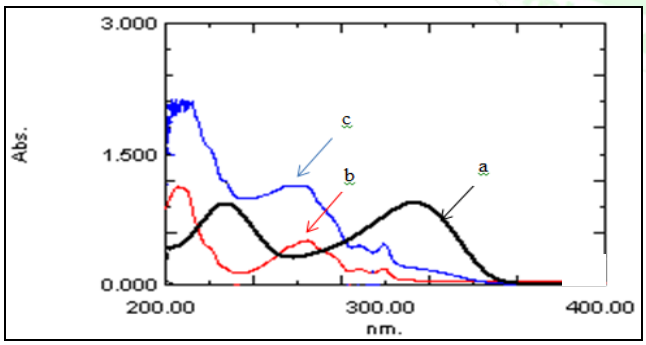


Figure 2: Absorption spectra of: (a) RNH in the reaction medium (10 µg/mL); (b) FMOC-Cl in the reaction medium (15 µg/mL); (c) the reaction product of RNH with FMOC-Cl against reagent blank.

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.

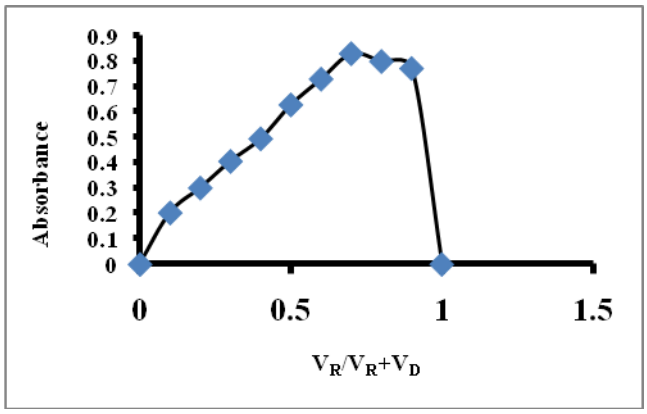
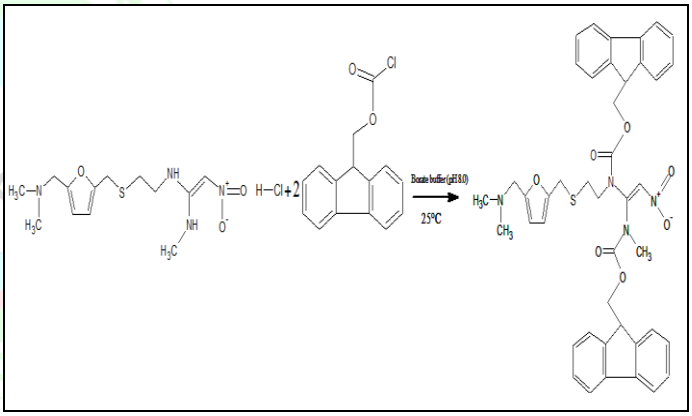


Figure 3: Determination of product formation by continuous variation method. V_R: FMOC-Cl (1×10^{-3} mol/L); V_D: RNH (1×10^{-3} mol/L); $V_R + V_D$ = 10 mL.

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 amin group in the RNH occurs by aromatic rings containing alternating double bond (24). The reaction equation is shown in Scheme 1.



Scheme 1. Reaction pathway of RNH with FMOC-Cl.

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.

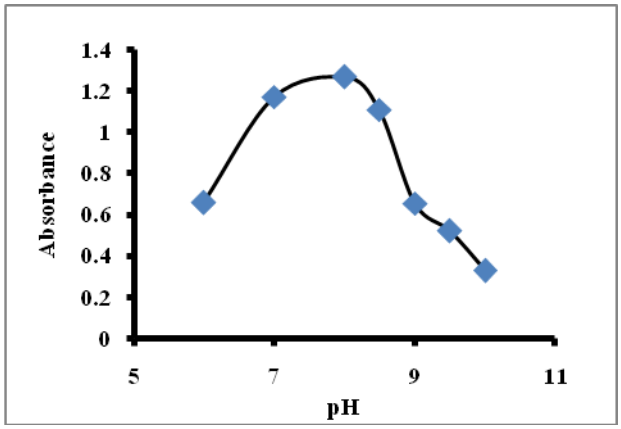


Figure 4: Effect of buffer pH on the reaction of RNH (10 µg/mL) with FMOC-Cl.

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.

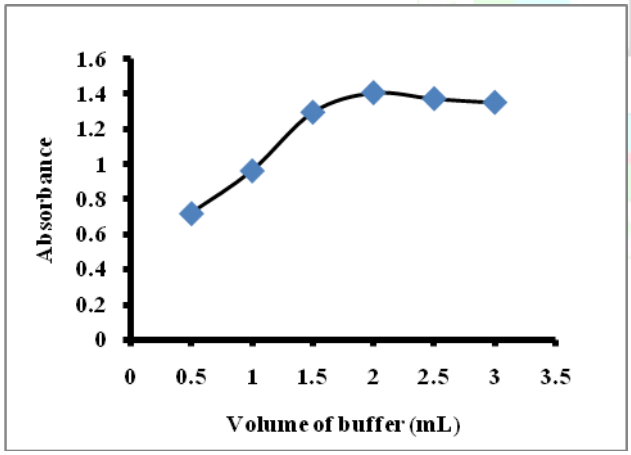


Figure 5: Effect of volume of buffer on the reaction of RNH (10 µg/mL) with FMOC-Cl, buffer solution (pH 8.0).

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.

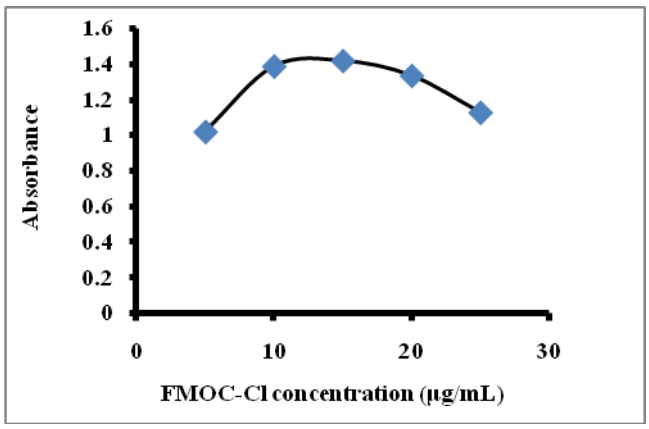


Figure 6: Effect of FMOC-Cl reagent concentration on the reaction of RNH (10 µg/mL) with FMOC-Cl, buffer solution (pH 8.0): 2.0 mL.

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.

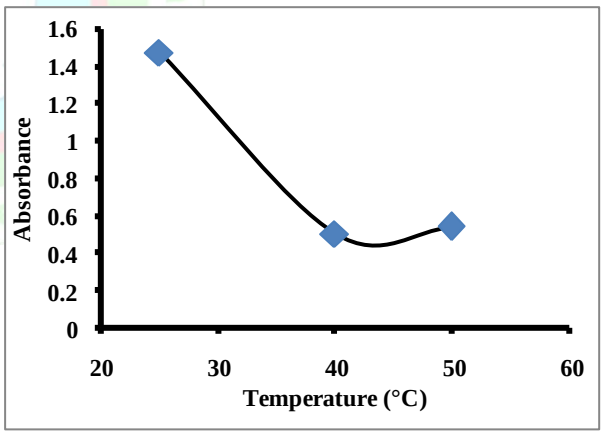


Fig. 7. Effect of heating temperature on the reaction of RNH (10 µg/mL) with FMOC-Cl (15 µg/mL), buffer solution (pH 8.0): 2.0 mL.

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.

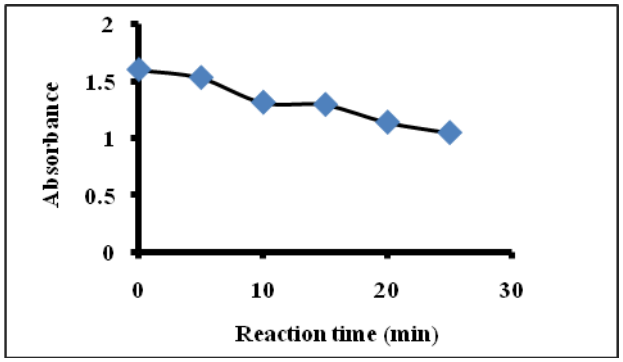


Fig. 8. Effect of time on the reaction of RNH (10 µg/mL) with FMOC-Cl (15 µg/mL), buffer solution (pH 8.0): 2.0 mL, temperature: 25 °C.

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

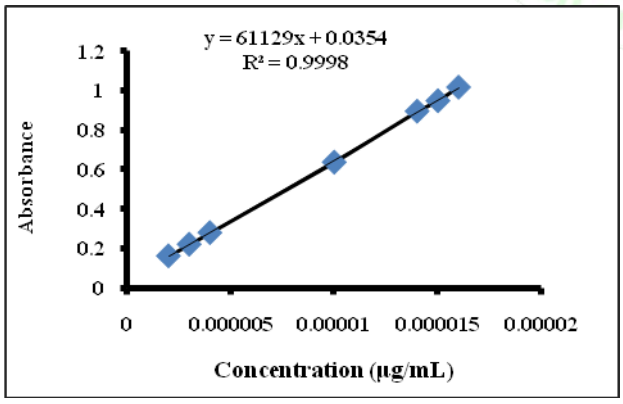


Fig. 9. Calibration curve of the reaction product between RNH and FMOC-Cl.

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2(R1) recommendation, (LOQ = 10 δ/S) where δ 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 δ/S). The results of LOQ and LOD are abridged in Table 1. Sensitivity is often described in terms of molar absorptivity (ε, L/molcm) 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 cm^2 which shows 0.001 absorbance and is expressed as µg/mL/cm^2, 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 [28-31]. As shown in Table 6, some of the reported methods have higher detection limits [28, 31]. Moreover, some of methods are alike or more sensitive, but have drawbacks such as having a narrow linear dynamic range [29], involving extraction [8] or being performed at high temperatures [29,-30].

Table 1: Parameters for the performance of the proposed method

Parameter	Value
λ_{max}, nm	255
Beer's law limit (µg/mL)	2-16
Molar absorptivity, L/mol cm	2×10^{10}
Sandell sensitivity, µg/cm^2	8.25×10^{-7}
Limit of detection (LOD) (µg/mL)	0.2219
Limit of quantification (LOQ) (µg/mL)	0.6724
Regression equation	
Intercept	0.0354
Standard deviation of intercept	0.00411
Slope	61129
Correlation coefficient (r^2)	0.9998
Standard deviation	0.00569

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

Standard (µg/mL)	Sample (µg/mL)	Found (µg/mL)	Recovery (% ± R.S.D)[a]
2	3	4.94	98.78% ± 0.1429
7	3	10.15	101.47% ± 0.0009
12	3	14.76	98.40% ± 0.0076

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

Taken (µg/mL)	Intra-day			Inter-day		
	Found (µg/mL)	R.S.D	R %	Found (µg/mL)	R.S.D	R %
3	2.92	0.0027	97.21	2.97	0.2973	98.8
10	9.83	0.0461	98.31	10.00	0.2129	100.05
15	14.63	0.0161	97.53	15.04	0.0833	100.25

Table 4: Robustness of the proposed spectrophotometric method

Parameter	Modification	Recovery (% ± R.S.D)[a]
pH	7.8	101.41% ± 0.1006
	8.0	101.63% ± 0.1537
	8.2	100.54% ± 0.1093
Volume of buffer (mL)	1.8	101.30% ± 0.2042
	2.0	101.63% ± 0.1537
	2.2	101.52% ± 0.0046
Reaction time (min.)	0	101.63% ± 0.1537
	2	101.63% ± 0.1025

[a]Values are mean of three determinations.

Table 5: Results of assay of formulations for the proposed method

Tablet brand name	Label claim mg/tablet	Proposed method Found (% ± R.S.D)[a]
Rantag	150	97.92% ± 0.0016
Ranitidine	150	99.99% ± 0.0009
Ranicid	150	97.87% ± 0.0039

[a] Values are mean of three determinations.

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

Reagents	Linear range(µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Ref.	Remarks
Nitrite Cu^{2+}/Br^-	300-12,000	100	-	29	Involves flow injection, automated assembly, least sensitive.
Dichromate-diphenylcarbazide	5-50	0.83	2.52	30	Less sensitive.
Dichromate-iron(II)-thiocyanate	5-80	1.59	4.83		
Dichromate-iron(II)-orthophenanthroline	10-100	3.07	9.28		
7,7,8,8-tetracyano quinodimethane	1-6	0.2	-	31	Requires thermostating at 70°C for 10 min and has narrow range of linear response.
$KMnO_4$/N-bromosuccinimideazine dyes	5-30	-	-	32	Involves extraction and uses unstable reagent.
7-Chloro-4-nitrobenz-2-oxa-1,3-diazole	2-20	0.13	-	33	Requires thermostating at 60°C for 25 min.
9-Fluorenyl methyl chloroformate	2-16	0.22	0.67	Present work	Simple, sensitive and selective.

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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REFERENCES

1. Narayana B, Ashwini K. Novel reagent for the spectrophotometric determination of ranitidine hydrochloride. Eurasian Journal of Analytical Chemistry. 2010; 5(2): 170-176.
2. Narayana B, Veena K, Ashwani K, et al. New reagents for the spectrophotometric determination of ranitidine hydrochloride. Eclética.Química Journal. 2010; 35(3): 109-11.
3. Darwish IA, Hussein SA, Mahmoud AM, et al. Spectrophotometric determination of H_2-receptor antagonists via their oxidation with cerium(IV). Spectrochimica Acta, Part A: Molecular and Biomolecular Spectroscopy. 2008; 69(1): 33-40.
4. Sokol A, Karpinska J, Talecka R, et al. Quantification of ranitidine hydrochloride in the presence of its decomposition product by spectrophotometric methods. Application for kinetic study. Acta Poloniae Pharmaceutica. 2011; 68(2): 169-177.
5. Kiszkiel I, Starczewska B, Leśniewska B, et al. Extraction of ranitidine and nizatidine with using imidazolium ionic liquids prior spectrophotometric and chromatographic detection. Journal of Pharmaceutical and Biomedical Analysis. 2015; 106: 85-91.
6. Basavaiah K, Nagegowda P. Determination of ranitidine hydrochloride in pharmaceutical preparations by titrimetry and visible spectrophotometry using bromate and acid dyes. II Farmaco. 2004; 59(2): 147-153.
7. Darwish IA, Hussein SA, Mahmoud A, et al. A sensitive spectrophotometric method for the determination of H_2-receptor antagonists by mean of N-bromosuccinimide and p-aminophenol. Acta Pharmaceutica. 2008; 58(1): 87-97.
8. Sastry CSP, Rao SG, Rao JSVML, et al. Application of azine dyes for the determination of ranitidine hydrochloride in pharmaceutical formulations. Analytical Letters. 1997; 30(13): 2377-2390.

9. Ahmad AS, Abdel Kawy M, Nebsen M. Spectrophotometric and spectrofluorimetric determination of famotidine and ranitidine using 1,4-benzoquinone reagent. Analytical Letters. 2008; 32(7): 1403-1419.
10. Rosa SS, Barata PA, Martins JM, et al. Development and validation of a method for active drug identification and content determination of ranitidine in pharmaceutical products using near-infrared reflectance spectroscopy: A parametric release approach. Talanta. 2008; 75(3): 725-733.
11. Richter P, Toral MI, Muñoz-Vargas F. Polarographic behavior and determination of ranitidine in pharmaceutical formulations and urine. Analyst. 1994; 119(6): 1371-1374.
12. Norouzi P, Ganjali MR, Daneshgar P. A novel method for fast determination of ranitidine in its pharmaceutical formulations by fast continuous cyclic voltammetry. Journal of Pharmacological and Toxicological Methods. 2007; 55(3): 289-296.
13. Arayne MS, Sultana N, Zuberi MH, et al. Simultaneous determination of metformin, cimetidine, famotidine, and ranitidine in human serum and dosage formulations using HPLC with UV detection. Journal of Chromatographic Science. 2010; 48(9): 721-725.
14. Novaković J. High-performance thin-layer chromatography for the determination of ranitidine hydrochloride and famotidine in pharmaceuticals. Journal of Chromatography. A. 1999; 846(1-2): 193-198.
15. Martinez-Gomez MA, Carril-Avilés MM, Sagrado S, et al. Characterization of antihistamine-human serum protein interactions by capillary electrophoresis. Journal of Chromatography. A. 2007; 1147(2): 261-269.
16. Wring SA, Kilpatrick KE, Hutchins JT, et al. Shorter development of immunoassay for drugs: application of the novel RIMMS technique enables rapid production of monoclonal antibodies to ranitidine. Journal of Pharmaceutical and Biomedical Analysis. 1999; 19(5): 695-707.
17. Agatonovic-Kustrin S, Wu V, Rades T, et al. Ranitidine hydrochloride X-ray assay using a neural network. Journal of Pharmaceutical and Biomedical Analysis. 2000; 22(6): 985-992.
18. Kataoka H. Derivatization reactions for the determination of amines by gas chromatography and their applications in environmental analysis. Journal of Chromatography. A. 1996; 733(1-2): 19-34.
19. Wróblewski K, Petruczynik A, Radzik I, et al. Determination of selected antiepileptic drugs in mouse brain homogenates by HPLC-DAD. Acta Chromatographica. 2017; 29(2): 219-234.
20. El-Enany NM, Abdelal A, Belal F. Spectrofluorimetric determination of sertraline in dosage forms and human plasma through derivatization with 9-fluorenylmethyl chloroformate. Chemistry Central Journal. 2011; 5(1): 56.
21. Farshchi A, Ghiasi G, Bahrami G. A sensitive liquid chromatographic method for the analysis of clarithromycin with pre-column derivatization: application to a bioequivalence study. Iranian Journal of Basic Medical Sciences. 2009; 12(1): 25-32.
22. Einarsson S, Josefsson B, Lagerkvist S. Determination of amino acids with 9-fluorenylmethyl chloroformate and reversed-phase high-performance liquid chromatography. Journal of Chromatography. A. 1983; 282: 609-618.
23. Danielson ND, Gallagher PA, Bao JJ. Chemical reagents and derivatization procedures in drug analysis. Encyclopedia of Analytical Chemistry. 2000; 7042-7076.
24. Catrinck TCPG, Dias A, Aquiar MCS, et al. A simple and efficient method for derivatization of glyphosate and AMPA using 9-fluorenylmethyl chloroformate and spectrophotometric analysis. Journal of the Brazilian Chemical Society. 2014; 25(7): 1194-1199.
25. Garside DM, Monteiro PMS, Orren MJ. A critical evaluation for the determination of amino acids in the marine environment by derivatization using 9-fluorenylmethyl chloroformate (FMOC-Cl) and reversed phase HPLC separation. African Journal of Marine Science. 1988; 6(1): 47-53.
26. ICH Q2B. Guidelines on validation of analytical procedure; Methodology. Federal Register. 1996; 60, 27464.
27. ICH Q2A. Guidelines on validation of analytical procedure; Definition and terminology. Federal Register. 1995; 60, 11260.
28. Basavaiah K, Somashekar BC. Quantitation of ranitidine in pharmaceuticals by titrimetry and spectrophotometry using potassium dichromate as the oxidimetric reagent. Journal of the Iranian Chemical Society. 2007; 4(1): 78-88.
29. Al-Ghannam S, Belal F. Spectrophotometric determination of three anti-ulcer drugs through charge-transfer complexation. Journal of AOAC International. 2002; 85(5): 1003-1008.
30. Walash MI, Belal F, Ibrahim F, et al. Kinetic spectrophotometric method for the determination of ranitidine and nizatidine in pharmaceuticals. Journal of AOAC International. 2002; 85(6): 1316-1323.
31. Hassan SSM, Mahmoud WH, Othman AM. Determination of ranitidine in pharmaceutical preparations using manual and flow injection potentiometry and spectrophotometry. Analaytica Chimica Acta. 1996; 332(1): 39-48.