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Spectrophotometric method for determination of ranitidine hydrochloride in bulk and in pharmaceutical preparation using ninhydrin

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RESEARCH ARTICLE

ABSTRACT



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A simple, sensitive and reproducible method for the determination of ranitidine hydrochloride in pharmaceutical preparations was investigated. This spectrophotometric method was based on the formation of a deep red color product with ninhydrin in basic media and the absorbance measured at $\lambda_{\text{max}} = 480$ nm. The reaction occurs at 45 °C with pH = 10 having a contact time of 38 minutes. Under the optimum conditions, Beer's Law is obeyed in the concentration range of $8.98 \times 10^3 - 9.90 \times 10^4$ µg/L. The coefficient of correlation was found to be 0.999 for the obtained method with molar absorptivity of 3.05×10^3 L/mol.cm. The calculated Sandell's sensitivity is 0.108 µg/cm². The limit of detection and limit of quantification are 0.0997 and 0.3023 µg/mL, respectively. The low values of the percentage relative standard deviation and percentage relative error indicate the high precision and the good accuracy of the proposed method. The stoichiometry of the reaction is determined and found to be 1:4 (Ranitidine hydrochloride:Ninhydrin). The initial rate method confirmed that this reaction is first order one.

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1. Introduction

Ranitidine hydrochloride (RNH) is chemically *N*-{2-[[[5-[(dimethylamino)methyl]-2-furyl] methyl]sulfonyl]ethyl}-*N'*-methyl-2-nitro-1,1-ethenediamine hydrochloride. It is a H₂-receptor antagonist and is widely used for the short-term treatment of duodenal ulcers and for the management of hypersecretory conditions [1]. Ranitidine hydrochloride has three different substitution amine group, nitro group, furan ring, and sulfur group with hydrochloride [2,3].

Several methods have been used for determination of ranitidine hydrochloride in pharmaceutical preparations such as proton magnetic resonance spectroscopy [4], near-infrared reflectance spectrometry [5], scintillation proximity assay [6], flow injection analysis [7], cyclic voltammetry [8,9], differential pulse polarography [10], capillary electrophoresis [11,12], liquid chromatography [13], gas chromatography [14] and high-performance liquid chromatography [15,16]. All the previous methods need sophisticated and advanced instruments with expensive reagents and a lot of experimental steps will be made to complete the analysis, because of these reasons; we prefer to use the spectrophotometric method for determination of ranitidine hydrochloride. The spectrophotometric

method takes a unique position because of its sensitivity, accuracy, rapidity, and simplicity. The kinetic spectrophotometer methods such as initial rate and fixed time methods have a great attention because of selectivity and because there are no interferences due to other chemical reagents during measurement the colored complex in the aqueous media for which we used the two kinetic methods in this research [17-19].

The present research aims to develop a rapid, simple and sensitive spectrophotometric method for the determination of RNH. Ninhydrin is used to make a stable colored complex with RNH under a specific condition and the absorbance of this complex will be measured at its maximum wavelength. According to our knowledge, there are no researches have been made to determine ranitidin hydrochloride by colorimetric method using ninhydrin.

2. Experimental

2.1. Instrumentation

The following instruments are used in this research, UV/Vis spectrometer (UNICAM 900) with glass cell used for all measurements and digital pH meter (Mettler Toledo, Seven Easy).

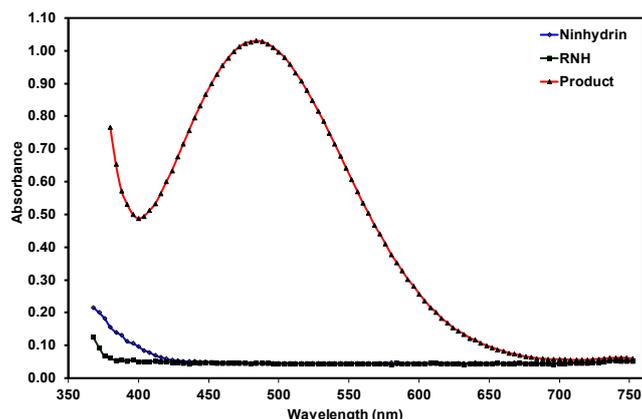


Figure 1. The absorption spectra of RNH-Ninhydrin complex. Conditions: [RNH] = 2.816×10^{-4} M, [Ninhydrin] = 3.1×10^{-3} M, pH = 10, Total volume = 25 mL, Temperature = 45 °C and contact time = 38 min.

2.2. Reagents and chemicals

All chemicals and reagents are analytical grade and obtained from Fisher Scientific and Aldrich Chemical Co. We use them as received.

2.3. Selection of the optimum wavelength

To get the optimum wavelength of the reaction between ranitidine hydrochloride and ninhydrin (98%), many experiments were done in basic media (pH = 10) using 6×10^{-3} M of sodium hydroxide. Standard solution of ranitidine hydrochloride with concentration of 2.816×10^{-4} M was mixed with 3.1×10^{-3} M of ninhydrin at 45 °C and final volume completed to 25 mL, stable deep red color of the product (ranitidine hydrochloride with ninhydrin) is obtained after 38 min. It was found after many trials that the maximum absorbance for the product was at 480 nm against the blank, the spectrum of the colored product, RNH and ninhydrin is shown in Figure 1. All the experiments were measured at 480 nm.

2.4. Experimental variables

Different parameters were applied to get the best conditions for measuring ranitidine hydrochloride, so to develop this analytical method, the following variables were investigated: temperature, pH, time, NaOH concentration, initial concentration of ninhydrin and the initial concentration of ranitidine hydrochloride. All of the kinetic investigations were done at the optimum parameters.

2.4.1. Effect of contact time

Different concentrations of RNH in the range of 2.56×10^{-5} - 2.816×10^{-4} M were prepared and mixed with fixed concentration of ninhydrin (3.1×10^{-3} M) separately at pH = 10 and at 45 °C with a final volume of 25 mL. The chosen time intervals were 2, 4, 6, 8, 22, 24, 26, ... and 60 minutes, the initial absorbance reading was obtained after about 20 minutes, then by increasing the contact time, the absorbance values increased up to 60 minutes.

2.4.2. Effect of initial concentration of ninhydrin

The effect of initial concentration of ninhydrin on the density of the color of the complex at the selected wavelength was studied by measuring the absorbance of solutions containing a specified concentration of 2.816×10^{-4} M of RNH mixed separately with different concentrations of ninhydrin

(1.8×10^{-3} - 3.1×10^{-3} M) at pH = 10, contact time is 38 minutes and temperature 45 °C with final volume of 25 mL.

2.4.3. Effect of temperature

The stability of the studied complex (RNH - Ninhydrin) was investigated at different selected temperatures which were: room temperature, 25, 30, 35, 40, 45, 50 and 55 °C, all experiments were done under the following conditions; contact time 38 min, pH = 10.0, initial concentration of RNH is 2.816×10^{-4} M, initial concentration of ninhydrin is 3.1×10^{-3} M, pH = 10 and the final volume of 25 mL, the absorbance of all experiments were measured at 480 nm.

2.4.4. Effect of pH on complex stability

Many separate experiments were done to study the effect of pH on the stability of the complex. The chosen range of the pH is 3.0-10.0 using buffer solutions and NaOH with concentration of 6×10^{-3} M. All these separate experiments done under the following constant conditions; initial concentration of RNH is 2.816×10^{-4} M, initial concentration of ninhydrin is 3.1×10^{-3} M, and the temperature is 45 °C with final volume of 25 mL, the absorbance of all experiments was measured at 480 nm.

2.4.5. Effect of initial concentration of NaOH

Many separate experiments were done to study the effect of the initial concentrations of NaOH, the range of NaOH concentration was 2×10^{-3} - 1.6×10^{-2} M. The other experimental conditions were kept constant; the initial concentration of RNH is 2.816×10^{-4} M, the initial concentration of ninhydrin is 3.1×10^{-3} M, and temperature 45 °C with final volume of 25 mL, the absorbance of all experiments was measured at 480 nm.

2.4.6. Effect of initial concentration of ranitidine hydrochloride

In order to investigate the optimum initial concentration of ranitidine hydrochloride, different concentration of ranitidine hydrochloride in the range of 5.12×10^{-5} - 2.816×10^{-4} M was used. Separate experiments were operated under the obtained optimum conditions such as contact time, pH, temperature, etc.

3. Result and discussion

3.1. Effect of contact time

It was found that low-intensity red color of the complex appeared after 20 minutes of mixing of ranitidine hydrochloride

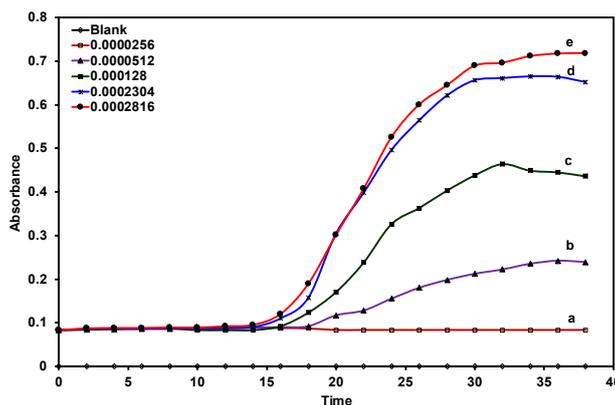


Figure 2. Absorbance-time curve for the reaction of RNH with ninhydrin. The molar concentrations of RNH were (a) 2.56×10^{-5} , (b) 5.12×10^{-5} , (c) 1.28×10^{-4} , (d) 2.304×10^{-4} and (e) 2.816×10^{-4} M.

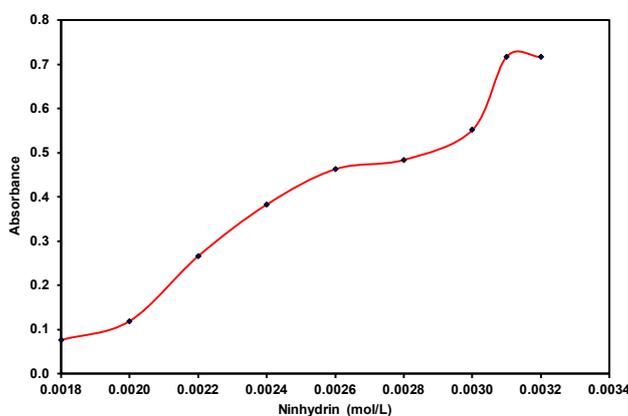


Figure 3. The effect of ninhydrin concentration on the absorbance at λ_{\max} (480 nm) of the colored product in $[\text{RNH}] = 2.816 \times 10^{-4}$ M, pH = 10, Total volume 25 mL, Temperature = 45 °C.

and ninhydrin as shown in Figure 2, this was observed for all concentrations of ranitidine hydrochloride in the range of 5.12×10^{-5} - 2.816×10^{-4} M. By increasing the contact time between the reactants increasing of the intensity of the colored product was observed and the absorbance reading increased as shown in Figure 2, this increasing keeping up to 38 min after this time constant readings of absorbance were obtained. The optimum contact time for measuring the absorbance of the complex to is 38 minutes.

3.2. Effect of ninhydrin initial concentration

The effect of concentration of ninhydrin was studied using various concentrations of ninhydrin keeping the concentration of RNH constant. Figure 3 illustrates the effect of the initial concentration of ninhydrin on the intensity of the color of the complex that formed, as we can see from Figure 3 that the concentration of ninhydrin increased when the measured absorbance increased. The maximum absorbance was achieved using 3.1×10^{-3} M of ninhydrin, using a concentration larger than 3.1×10^{-3} M of ninhydrin has no effect on the absorbance, which means that the reaction is stopped and the amount of ninhydrin was enough to made consuming of all amount of RNH.

3.3. Effect of temperature

A series of different temperature values were applied to investigate the effect of temperature on the complex that is formed between the ninhydrin and the RNH. The range was room temperature, 25, 30, 35, 40, 45, 50 up to 55 °C, the stability

of the complex was studied by measuring the absorbance as shown in Figure 4, the intensity of the color remains nearly constant in the range of room temperature to 35 °C then increasing in absorbance was noticed at 40 °C, the highest absorbance reading was at 45 °C, the maximum stability of the complex was noticed at this temperature. Figure 4 also showed that the stability of this complex decreased by increasing the temperature (50 up to 55 °C), the absorbance readings decreased. May be this reaction is an endothermic one at temperature higher than 45 °C and the dissociation of the complex will take place.

3.4. Effect of pH

Different values of pH were used to study the stability of the reaction between ninhydrin and RNH, from Figure 5, we can noticed that the absorbance readings kept constant at pH = 3 to pH = 6, then increase in the absorbance was seen at pH values higher than 6, pH = 10 has the maximum absorbance reading which means that the reaction was completed at this pH, and it is the most appropriate and desired pH for the reaction of RNH with ninhydrin. The pKa for the drug is 8.08 which is less than pH = 10, this fact can explain the increasing of absorbance of the complex at pH = 10, for which pH = 10 is applied in all other experiments.

3.5. Effect of initial concentration NaOH

Several concentrations of sodium hydroxide were used in the range of 2×10^{-3} - 1.6×10^{-2} M while keeping the other conditions

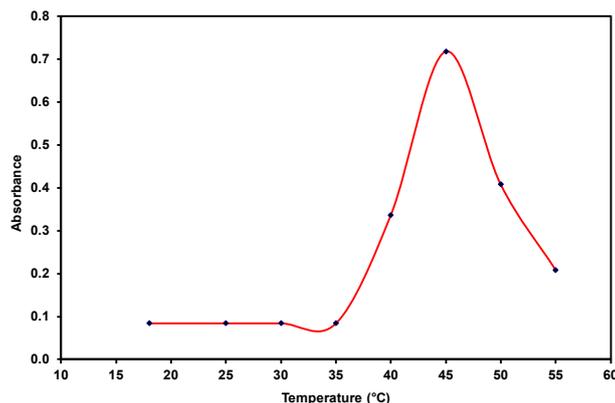


Figure 4. The effect of temperature on the abs at $\lambda_{\max} = 480$ nm of the colored product. Conditions: $[\text{RNH}] = 2.816 \times 10^{-4}$ M, $[\text{Ninhydrin}] = 3.1 \times 10^{-3}$ M, pH = 10, Total volume = 25 mL.

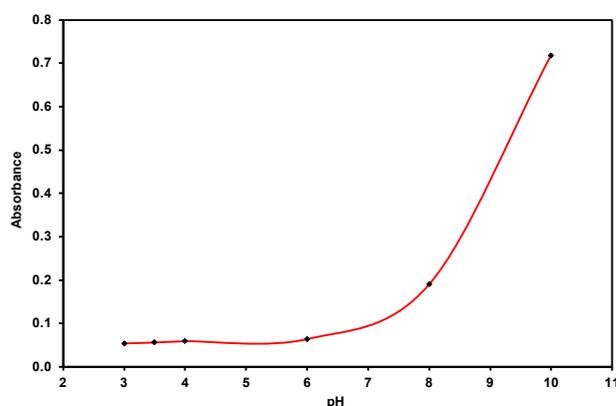


Figure 5. The effect of pH on the absorbance value of the reaction product at $\lambda_{\max} = 480$ nm. Conditions: $[\text{RNH}] = 2.816 \times 10^{-4}$ M, $[\text{Ninhydrin}] = 3.1 \times 10^{-3}$ M, Time = 38 min, Total volume = 25 mL, Temperature = 45 °C.

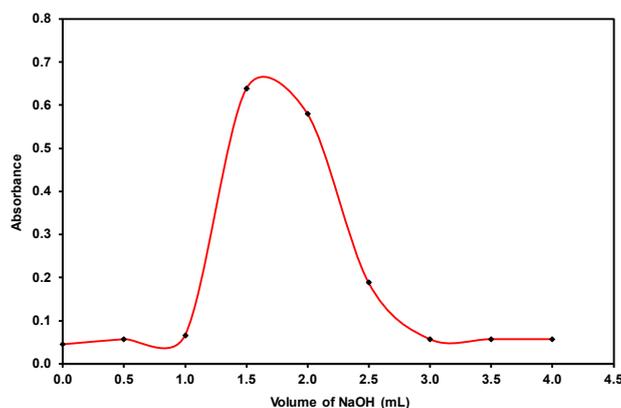


Figure 6. The effect of NaOH concentration on the reaction product at $\lambda_{\max} = 480$ nm. Conditions: $[\text{RNH}] = 2.816 \times 10^{-4}$ M, $[\text{Ninhydrin}] = 3.1 \times 10^{-3}$ M, Temperature = 45 °C.

constant to study the effect of the initial concentration of sodium hydroxide on the stability of the complex of RNH with Ninhydrin. As we see in Figure 6, the best absorbance, we got at concentration 3.1×10^{-3} M, then the absorbance value decreased by increasing the concentration of NaOH, which means the complex is completely unstable at concentration higher than 3.1×10^{-3} M, so the best used concentration for sodium hydroxide in all experiments was 3.1×10^{-3} M.

3.6. Effect of initial concentration of ranitidine hydrochloride

The effect of initial concentration ranitidine hydrochloride was studied under the optimum conditions and the results are illustrated in Figures 7 and 8, as we see from these figures, the values of absorbance increased by increasing the concentration of RNH until reaching the plateau, 2.816×10^{-4} M of RNH found to be sufficient to form stable complex with high intensity color. For values of concentration larger than $\approx 3 \times 10^{-4}$ M, the absorbance decreased slightly as shown at Figure 8.

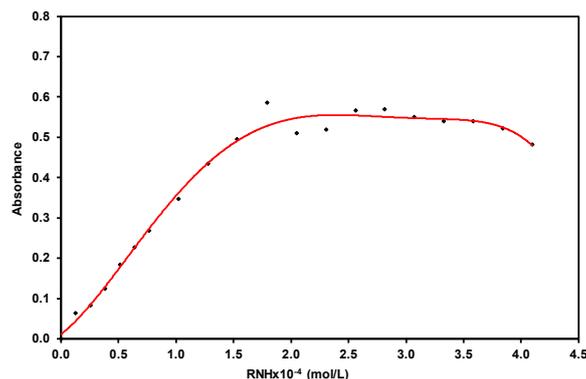


Figure 7. The effect of RNH concentration on the stability of the complex at λ_{\max} = 480 nm. Conditions: [Ninhydrin] = 3.1×10^{-3} M, pH = 10, Contact time = 38 min, Temperature = 45 °C

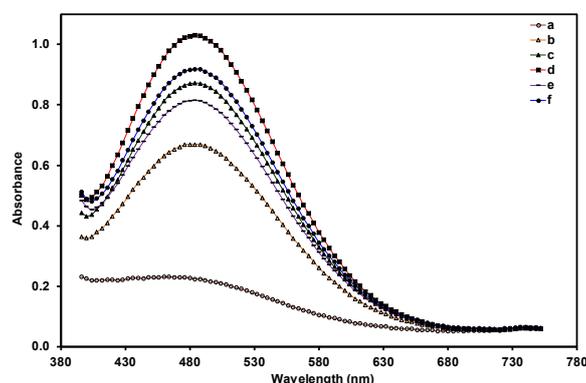


Figure 8. The absorption spectra for RNH at different molar concentrations on the absorbance at λ_{\max} = 480 nm of the colored product in [Ninhydrin] = 3.1×10^{-3} M, pH=10, Time = 38 min, Temperature = 45 °C and (a) 5.12×10^{-5} , (b) 1.28×10^{-4} , (c) 2.304×10^{-4} , (d) 3.072×10^{-4} , (e) 3.584×10^{-4} and (f) 2.816×10^{-4} .

3.7. Method validation

3.7.1. Linearity, detection and quantification limits

The construction of the calibration curve which described Beer's Lambert Law was established under the optimum conditions by plotting absorbance versus concentration of the RNH, the linear regression coefficient (r^2) is shown in Table 1. The values of the molar absorptivity (ϵ) which is the calibration sensitivity and the Sandell's sensitivity are shown in Table 1, the calibration sensitivity is high, which indicates that the probability of this transition in the visible region is high and this method is good for the detection of the RNH. The linearity range from this constructed curve was also evaluated. The limit of detection (LOD), the limit of quantification (LOQ) and the limit of linearity (LOL) [20] are shown also in Table 1.

By making comparison between our proposed method and with earlier methods [1,3,19]. We found that our method is suitable with high sensitivity for determination of RNH.

3.7.2. Precision

Real samples of ranitidine hydrochloride in injection which are created by Hikma Pharmaceuticals, Jordan, were purchased from the local market in Mafraq, Jordan, the precision and the accuracy were investigated using these real samples as references. The precision was determined in terms of intermediate precision intraday, three different concentration of ranitidine hydrochloride were prepared and mixed with ninhydrin under the optimum conditions the products were analyzed in three repeats during the same day (intraday precision), the standard deviation (SD) and the relative standard deviation (%RSD) was calculated for each concentration of

ranitidine hydrochloride and the range of %RSD for three different concentrations was 0.86-2.76% as shown in Table 2. The precision was also calculated in terms of intermediate precision interday, by choosing three different concentrations of ranitidine hydrochloride which mixed ninhydrin under the optimum conditions the products were analyzed in three repeats during three successive days (interday precision), the (%RSD) values of interday are shown in Table 2, the repeatability in three day (interday) for each concentration of ranitidine hydrochloride in terms of %RSD were acquired in the range 1.80-1.85%.

3.7.3. Accuracy

The accuracy for this analytical method is investigated; the %relative error (%RE) for each analysis (Intra- and Interday accuracy) was calculated as shown in Table 2. The small values of %RE indicate that the reference amount and the found amount are close to each other [18]. Statistical analysis was done for the Intraday and interday using F-test and the results are 2.7 and 4.4, respectively, these values indicate that there is no bias between the reference concentration and the actual concentration for ranitidine hydrochloride at 95% confidence level.

3.7.4. The effect of interfering ions on complex stability

Under the best experimental conditions; many experiment were carried out to investigate the effect of interfering ions at 45 °C. Different interfering ions (ionic salt's) such as sodium chloride, potassium chloride, potassium iodide, sodium thiocyanate, sodium nitrate, potassium nitrate with concentration of 0.1 M were added separately to 2.816×10^{-4} M of

Table 1. Analytical parameters for the developed spectrophotometric method.

Parameter	Value
Color	Deep red
λ_{\max} (nm)	480
Linearity range ($\mu\text{g/L}$)	$8.98 \times 10^3 - 9.90 \times 10^4$
ϵ ($\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$)	3.25×10^3
Sandell's sensitivity ($\mu\text{g}\cdot\text{cm}^{-2}/0.001$ Absorbance unit)	0.108
Range time for color stability (min)	38
pH	10
Temperature ($^{\circ}\text{C}$)	45
Regression coefficient (r^2)	0.999
LOD ($\mu\text{g/mL}$)	0.0997
LOQ ($\mu\text{g/mL}$)	0.3023
LOL ($\mu\text{g/mL}$)	99.0

Table 2. Evaluation of intra-day and inter-day accuracy and precision.

Method	[RNH] _{Taken}	[RNH] _{Found}	SD	%RSD	%RE
Intraday accuracy and precision	2.560×10^{-4}	1.874×10^{-4}	4.967×10^{-3}	0.86	0.26
	2.816×10^{-4}	1.781×10^{-4}	1.366×10^{-2}	2.51	0.36
	3.072×10^{-4}	1.632×10^{-4}	1.380×10^{-2}	2.76	0.46
Interday accuracy and precision	2.560×10^{-4}	1.826×10^{-4}	1.011×10^{-2}	1.82	0.28
	2.816×10^{-4}	1.748×10^{-4}	9.652×10^{-3}	1.80	0.38
	3.072×10^{-4}	1.627×10^{-4}	9.229×10^{-3}	1.85	0.47

Table 3. The effect of foreign ions on the determination of RNH.

RNH solution number	Absorbance (without interfering salts)	Interfering salts	Absorbance (with interfering salts)	% Intra
1	0.718	KCl	0.716	0.27
2	0.717	KI	0.716	0.14
3	0.716	NaCl	0.715	0.13
4	0.718	NaSCN	0.714	0.55
5	0.715	KNO ₃	0.710	0.69
6	0.717	NaNO ₃	0.718	0.13

Table 4. Initial rate constant of reaction at different concentration of RNH, [Ninhydrin] = 3.1×10^{-3} M and pH = 10.

[RNH](M)	Regression equation	Initial rate of reaction (k)	Log [RNH]	Log K
5.12×10^{-5}	Log A = $0.0099 \times C - 0.8698$	9.90×10^{-3}	-4.29	-2.005
1.28×10^{-4}	Log A = $0.0194 \times C - 0.8685$	1.94×10^{-2}	-3.89	-1.712
2.30×10^{-4}	Log A = $0.0450 \times C - 1.4036$	4.50×10^{-2}	-3.63	-1.346
2.816×10^{-4}	Log A = $0.0505 \times C - 1.5141$	5.05×10^{-2}	-3.55	-1.296

ranitidine hydrochloride and 3.1×10^{-3} M of ninhydrin at pH = 10, the final volume for each solution was in 25.0 mL, after contact time of 38 minutes the absorbance was measured at $\lambda_{\max} = 480$ nm for all solutions. The results are shown in Table 3. We can notice that both the positive and negative ions did not affect the stability of the complex and the percentage of interference (% Intra) was very low for each one for which we expect that the associated ions in the pharmaceutical preparation will not make interferences.

3.8. Stoichiometry of the reaction

The stoichiometric ratio between ranitidine hydrochloride and ninhydrin was estimated using limiting logarithmic method [21]. Two sets of experiments were performed, in the first set of experiments the concentration of ranitidine hydrochloride was varied and the concentration of ninhydrin kept constant, while in the second set of experiments the concentration of ninhydrin was varied and keeping the concentration of ranitidine hydrochloride constant. We plotted log absorbance versus log [RNH] and log absorbance versus log [ninhydrin] separately, the slopes for each linear regression line were estimated from limiting logarithmic plot, the linear regression equation for ranitidine hydrochloride reaction is $y = 0.9767x + 3.4121$, the slope is approximately equal one., and from the limiting logarithmic plot for ninhydrin reaction the linear regression equation is $y = 4.0353x + 9.9622$ and the slope is equal four. The mole ratio between ranitidine hydrochloride and ninhydrin is 1:4

3.9. Kinetic studies

3.9.1. Initial rate of reaction

Under the optimum conditions, the initial rate constant (k) of the reaction was determined, four experiments with different concentration of ranitidine hydrochloride prepared in the range of $5.12 \times 10^{-5} - 2.82 \times 10^{-4}$ M in 25 mL volumetric flask, the measured absorbance at 480 nm increased as a function of time and the limiting logarithm absorbance versus the time plots were constructed. The slope, which is the initial rate constant of the reaction (k) for each curve was estimated at different concentrations and shown in Table 4. The order of the reaction was estimated using Equation (1),

$$\text{Log K} = \log k' + n \log C \quad (1)$$

where C is the molar concentration of ranitidine hydrochloride; n is the order of the reaction, K is the initial rate of reaction, and k' is the apparent rate constant. The experimental K values in Table 4 were used to plot the linear curve; log K versus log C. The linear regression equation is: $\text{Log K} = 0.9884x + 2.224$, ($r^2 = 0.9774$), the slope value which is n is 0.9884 (≈ 1) this confirming that the order of the reaction is the first one. The data analysis was performed at 95% confidence level, the standard error of the slope is ± 0.106 and the standard error of the intercept is ± 0.179 [18,21].

3.9.2. The fixed-time method

The reaction rates were determined for various concentrations of ranitidine hydrochloride in the range of $5.12 \times 10^{-5} - 2.816 \times 10^{-4}$ M at a selected fixed time, which was precisely determined, the absorbance was measured. Calibration curves of absorbance ($\Delta A = A_t - A_{20}$) vs. initial concentration of ranitidine hydrochloride were determined at fixed times of 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 40 minutes

Table 5. Regression equations for RNH at different fixed time over the range 5.12×10^{-5} - 2.816×10^{-4} M at 480 nm.

ΔA	Time interval (min)	Regression equation	Correlation coefficient (r^2)
A22 - A20	20-22	$\Delta A = 350.10 \times C - 0.0050$	0.9820
A24 - A20	20-24	$\Delta A = 779.26 \times C - 0.0015$	0.9634
A26 - A20	20-26	$\Delta A = 1078.0 \times C - 0.0015$	0.9760
A28 - A20	20-28	$\Delta A = 1313.2 \times C + 8 \times 10^{-5}$	0.9739
A30 - A20	20-30	$\Delta A = 1475.0 \times C + 0.0025$	0.9672
A32 - A20	20-32	$\Delta A = 1606.0 \times C + 0.0056$	0.9583
A34 - A20	20-34	$\Delta A = 1678.7 \times C + 0.0035$	0.9717
A36 - A20	20-36	$\Delta A = 1801.9 \times C + 0.0014$	0.9605
A38 - A20	20-38	$\Delta A = 1685.7 \times C - 0.0024$	0.9840
A40 - A20	20-40	$\Delta A = 1720.9 \times C - 0.0012$	0.9703

where A_t is the absorbance of the complex after the selected time interval, and A_{20} is the absorbance after 20 minutes of the reaction, the regression equations were shown in Table 5. We note that the slope increases with time [21], the best acceptable values of r^2 were obtained for time interval measurement at 20-38 minutes, for which the recommended time will be 38 minutes. All of these experiments were done under the optimum conditions.

4. Conclusion

The suggested method which is the reaction of ninhydrin with ranitidine hydrochloride in aqueous media was studied and developed, our results showed that this method is simple (the reaction does not need to additional or critical conditions to occurred), sensitive, reproducible one and the used reagent which is ninhydrin is available with accepted cost with no need of expensive instruments. The validity was tested by the investigation of the precision and the accuracy for the inter- and intra-day experiments under the optimum conditions. The kinetic spectrophotometric studied was developed and validity was applied. This method is selective with wide working range of the calibration curve. We also conclude that this method is suitable for determination of ranitidine hydrochloride in pharmaceutical preparation.

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Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.

Author contributions: All authors contributed equally to this work.

Ethical approval: All ethical guidelines have been adhered.

Sample availability: Samples of the compounds are available from the author.

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