Development of a Visible Spectrophotometric Method for the Analysis of Clonidine Hydrochloride in Pure Form and Pharmaceutical Formulations

Wasan Najm Abdullah, Muthana Saleh Mashkour, Hanaa Kadtem Egzar

Chemistry Department, College of Science, University of Kufa, Albuhadari St., Qazwiniya, P.O. Box 21, Kufa, Najaf Governorate, 54003, Iraq; *e-mail: wasann.hassan@uokufa.edu.iq

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A spectrophotometric method was developed for the precise estimation of clonidine hydrochloride (C-HCl) in pure form and pharmaceutical formulations. This method utilizes an ion-pair interaction with 1,2-naphthoquinone-4-sulphonic acid (NQS) in a basic medium (pH 13) at 55°C, producing a peach-colored solution with maximum absorbance at 475 nm. The method demonstrated linearity over 25-225 µg/mL with a molar absorptivity of 2162 $L \cdot mol^{-1} \cdot cm^{-1}$ ($R^2 = 0.9986$) and LOD and LOQ values of 6.28 and 19.02 µg/mL, respectively. The average recovery ranged from 96.4% to 101.4%. Kinetic studies indicated pseudo-first-order kinetics, while thermodynamic analysis using Eyring and Arrhenius equations revealed the reaction to be endothermic and non-spontaneous. The method proved accurate and suitable for C-HCl analysis in pharmaceutical samples.

Keywords: clonidine hydrochloride, NQS, ion-pair interaction, kinetic spectrophotometry

Introduction

Clonidine hydrochloride, chemically known as 2-(2,6-Dichloroanilino)-2-imidazoline, is a centrally acting imidazoline derivative that reduces sympathetic tone, making it an effective antihypertensive medication [1,2]. It is commonly used in the management of hypertensive crises, prevention of migraines, cancer-related pain control, and treatment of menopausal flushing [3,4]. Quantifying clonidine hydrochloride (C-HCl) is a crucial aspect of pharmaceutical quality control, as it ensures the safety and efficacy of medications. Accurately determining the concentration of active ingredients like C-HCl is vital in pharmaceutical analysis to meet regulatory standards and guarantee product quality [3]. Several analytical methods have been developed for C-HCl quantification, including potentiometric titrimetry [5], spectrophotometry [6], high-performance liquid chromatography (HPLC) [7,8], colorimetric techniques [9-12], capillary electrophoresis [13], potentiometric assays [14], and spectrophotometric titration [15]. Among these, spectrophotometry is often preferred due to its low cost, simplicity, and availability in most clinical and quality control laboratories [17].1,2-Naphthoquinone-4-sulfonate (NQS) is a versatile reagent that forms colored complexes with primary and secondary amines under different conditions, making it suitable for spectrophotometric analysis using ultraviolet-visible techniques [18,19]. This reagent's ability to produce stable, colored derivatives allows for the sensitive and accurate determination of compounds such as clonidine hydrochloride.

The present study aims to develop a reliable and efficient spectrophotometric method for the quantification of C-HCl in bulk and tablet formulations. The proposed method utilizes an ion-pair coupling interaction between C-HCl and NQS in a basic medium (pH 13) at 55°C, resulting in a peach-colored complex with a maximum absorbance at 475 nm. Various parameters, including reagent concentration, pH, reaction time, and temperature, were optimized to achieve the best analytical performance. The method's compliance with the Beer-Lambert law, linearity, limits of detection and quantification, and recovery rates were evaluated, confirming its suitability for pharmaceutical analysis. Additionally, kinetic and thermodynamic studies were performed to provide insight into the reaction mechanism and energetics, revealing that the process is endothermic and non-spontaneous.

Experimental part

Apparatus. The instruments used in this study included an EMSLAB single-beam spectrophotometer, a Shimadzu 1800 double-beam spectrophotometer, a WTW-720 ion-lab pH meter, and a VISON water bath shaker.

Reagents and Materials. Clonidine hydrochloride with a purity of 98.0%, was sourced from Nanjing Dulai Biotechnology Co., Ltd., China. Clonidine hydrochloride tablets, each containing 100 µg of the active ingredient, were obtained from Indiamart. Additional

reagents, including 1,2-naphthoquinone-4-sulphonic acid (NQS, ≥98% purity), sodium hydroxide (NaOH, ≥99% purity), and potassium chloride (KCl, ≥99% purity), were obtained from Merck (Germany). All solutions were prepared using deionized (DI) water.

To prepare a standard solution of clonidine hydrochloride (1000 μg/mL), 0.1 g (100.0 mg) of clonidine hydrochloride was accurately weighed and initially dissolved in 3 mL of ethanol. The solution was then diluted to 100 mL with deionized water in a volumetric flask to achieve the desired concentration. To prepare the NQS reagent (0.5% w/v), 0.5 g of NQS was dissolved in 100 mL of DI water, protected from light, and used immediately. The alkaline solution (pH 13) was prepared by mixing 50 mL of 0.2 M KCl with 132 mL of 0.2 M NaOH in a 200 mL volumetric flask, and then the volume was completed to 200 mL with DI water.

Tablet sample solution preparation. Twenty tablets were carefully ground into a fine powder, and an accurately weighed portion equivalent to the average tablet weight was taken. This powder was dissolved in 3 mL of ethanol, followed by the addition of a small amount of deionized water. The mixture was then filtered to remove any insoluble particles. The resulting filtrate was diluted to 100 mL with deionized water, yielding a solution with a nominal concentration of 1000 μg/mL.

General procedure. To perform the analysis, 0.5 mL of an alkaline solution was added to a 10 mL volumetric flask, followed by 1.00 mL of the clonidine hydrochloride (C-HCl) standard solution (1000 μg/mL) and 1.0 mL of the NQS reagent solution. The mixture was then incubated in a water bath at 55±1°C for 20 minutes. After the reaction, the solution was diluted to a final volume of 10 mL with deionized water. The absorbance of the resulting solution was measured at 475 nm against a blank solution, which contained all components except the C-HCl drug.

Results and discussion

Absorption spectrum

To verify the formation of an ion-pair complex between the NQS reagent and clonidine hydrochloride (C-HCl), the absorption spectra of the reagent, the drug, and their interaction product were measured. The NQS reagent showed a maximum absorbance at 366 nm, while the C-HCl drug solution exhibited maximum absorbance at 272 nm. A significant red shift in the absorption spectrum was observed for the product of the interaction, with a new maximum absorbance at 475 nm, indicating the successful formation of the ion-pair complex (Figure 1).

Optimization of reaction conditions

To establish the optimal conditions for the spectrophotometric determination of clonidine hydrochloride, various factors influencing the color development of the solution resulting from the interaction between C-HCl and NQS were investigated.

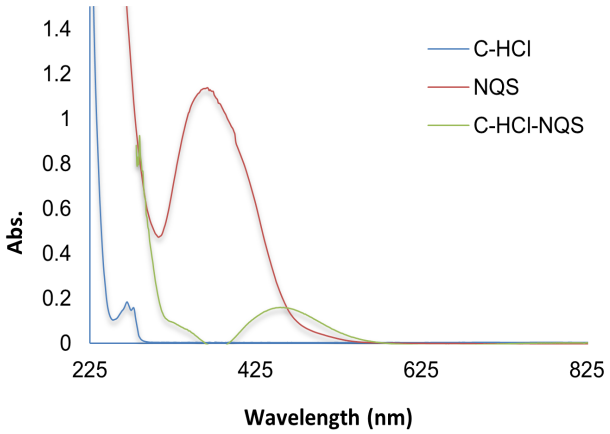


Figure 1. Comparison of absorption spectra of the NQS reagent (0.05% w/v), C-HCl drug (100 μg/mL), and the reaction product (C-HCl-NQS).

Effect of NQS concentration: The influence of NQS reagent concentration on the reaction yield was assessed. It was observed that the absorbance increased with the NQS concentration up to 0.5% (w/v), indicating an optimal concentration for maximal color development. Beyond this concentration, further increases in NQS did not result in any significant change in absorbance, suggesting that the reaction had reached a saturation point (Figure 2).

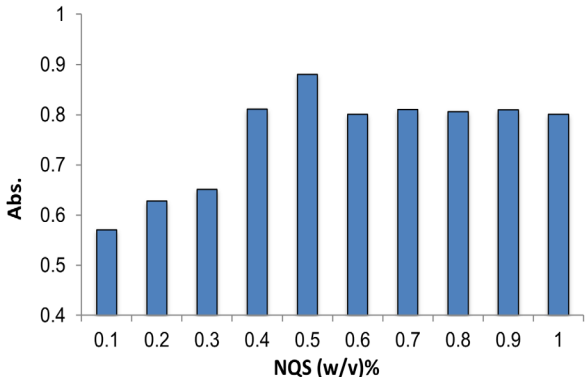


Figure 2. The relationship between NQS concentration and the absorbance of the reaction product.

Effect of reagent volume: The optimal volume of NQS reagent was determined to be 1 mL at a concentration of 0.5% (w/v), as this volume produced the highest absorption intensity for the interaction product between the reagent and clonidine hydrochloride.

Effect of C-HCl volume: The study showed that 1 mL of the C-HCl drug solution (1000 μg/mL) yielded the highest absorption intensity, indicating it as the optimal volume for the reaction.

Effect of pH: The impact of pH on the formation of the ion-pair complex between C-HCl and NQS was evaluated over a range from 7 to 13.5, as illustrated in Figure 3. At pH 7, there was no absorption, likely due to C-HCl being in its salt form, where the amino

group could not participate in nucleophilic substitution [20]. As the pH increased, the absorption intensified due to the conversion of the amino group from the hydrochloride salt form to a free amine, enhancing the reaction. The maximum absorbance was observed at pH 13, after which it decreased, possibly due to the increased concentration of hydroxide ions that interfered with the reaction.

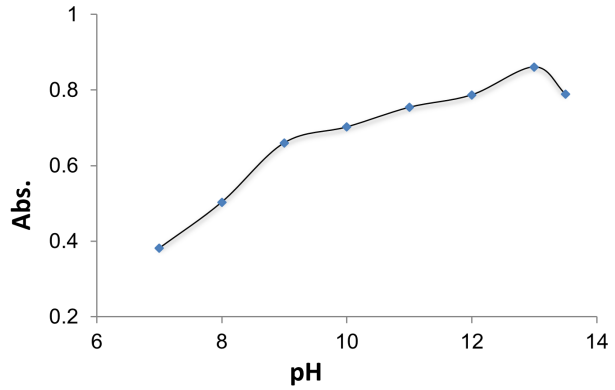


Figure 3. The relation between pH and the absorbance of the reaction product.

Effect of alkaline solution concentration: The study showed that the absorbance of the interaction product between the NQS reagent and C-HCl increased as the concentration of the alkaline solution rose, reaching a maximum at 0.01 M. Beyond this concentration, the absorbance began to decrease. Therefore, in subsequent experiments, 0.01 M was selected as the optimal concentration for the alkaline solution.

Effect of time: The influence of reaction time on the interaction between NQS and C-HCl was assessed over a range of 5 to 65 minutes under fixed conditions of pH 13, temperature 55°C, and constant reagent and drug concentrations. The absorbance increased with time, reaching a peak at 20 minutes, indicating the completion of the reaction. After 20 minutes, the absorbance decreased and then remained stable, suggesting the formation of a stable product or possible degradation of the complex.

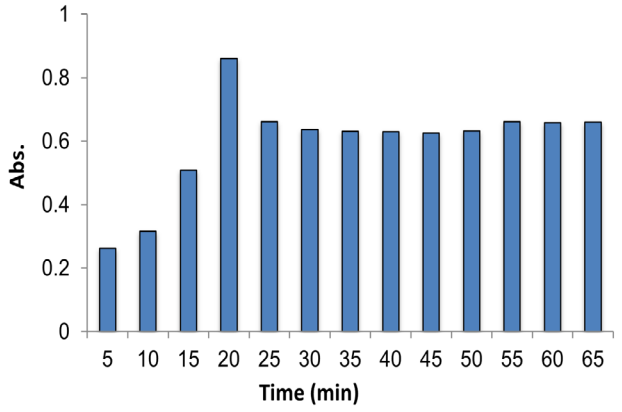


Figure 4. The relation between time and the absorbance of the reaction product.

Effect of temperature: The influence of temperature on the color development of the reaction product between the NQS reagent and C-HCl was studied over a range of 25-65°C. It was observed that the absorbance increased with rising temperature, reaching a maximum at 55°C (Figure 5). Beyond this temperature, the absorbance began to decline, indicating a potential decrease in reaction efficiency or stability of the product. Therefore, 55°C was selected as the optimal temperature for the reaction.

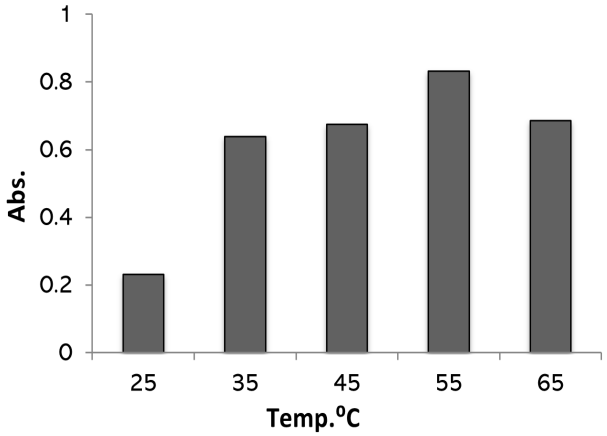


Figure 5. The relation between temperature and the absorbance of the reaction product.

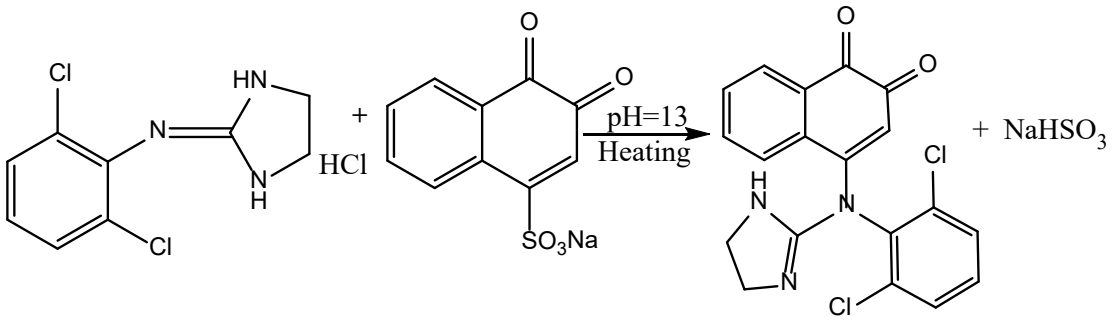
Effect of addition order: The impact of the sequence in which the reactants were added on the solution's absorbance, resulting from the ion-pair interaction between NQS and C-HCl, was examined at 475 nm. Various addition orders were tested, and it was found that the optimal sequence was to add the alkaline solution first, followed by the drug solution and then the NQS reagent. This order provided the highest absorbance, indicating the most efficient formation of the ion-pair complex.

Stoichiometry of the reaction

The stoichiometric relationship between clonidine hydrochloride (C-HCl) and the NQS reagent was investigated using two methods under optimal conditions: Job's (continuous variation) and the mole-ratio method.

Job's method (Continuous Variation): The stoichiometry of the interaction was determined using the continuous variation method (Job's method) under optimal conditions. The absorbance of the reaction mixture at 475 nm was measured while varying the molar ratios of C-HCl and NQS. The results indicated a 1:1 stoichiometric ratio between C-HCl and NQS.

Mole-ratio method: The mole-ratio method was also employed to confirm the stoichiometry by examining the product's absorbance at 475 nm as a function of the ratio between the molar concentrations of NQS and C-HCl. Using a fixed drug concentration of 3.75×10^{-4} M, the NQS concentration varied from 9.375×10^{-5} M to 9.375×10^{-4} M. The results confirmed a 1:1 ratio, consistent with Job's method findings.



Scheme 1. Proposed reaction scheme of clonidine hydrochloride with NQS.

Proposed reaction pathway: Scheme 1 illustrates the suggested mechanism for the reaction between C-HCl and NQS. The interaction involves substituting the sodium sulfonate group in NQS with an amine group from C-HCl, resulting in the release of a hydroxyl group.

Stability constants

Based on the results from Job's method and the mole-ratio analysis, the degree of dissociation (α), instability constant (K_{ins}), and stability constant (K_{st}) for the C-HCl and NQS ion-pair complex were calculated. The values are summarized in Table 1. The high stability constant indicates that the ion-pair product formed between C-HCl and NQS is highly stable.

Table 1. The stability constant for the C-HCl ion-pair complex.

α	K_{ins} (L^{-1}·Mol)	K_{st} (L·mol^{-1})	[C-HCl] (L^{-1}·mol)
0.5	1.974×10^{-6}	5.06×10^{5}	3.75×10^{-4}

Association constant

The association constant (K) for the ion-pair interaction between clonidine hydrochloride (C-HCl) and NQS was calculated using the Benesi-Hildebrand equation [21], given by:

$$([NQS])/(Abs.)=1/\varepsilon+1/(K.\varepsilon)\times1/([C\text{-}HCl]), \quad (1)$$

where: Abs is the absorbance of the ion-pair complex, ε is the molar absorptivity of the ion-pair complex, K is the association constant of the ion-pair complex.

From the linear plot, the molar absorptivity (ε) was determined to be 0.00097 $\mu g^{-1}\cdot mL\cdot cm^{-1}$, and the association constant (K) was calculated as 0.002013 $\mu g^{-1}\cdot mL$. These values indicate the strength of the ion-pair interaction and the efficiency of the complex formation under the experimental conditions.

Validation of the method

Linearity. The calibration plot (Beer's plot) for the interaction of clonidine hydrochloride (C-HCl) with the NQS reagent was linear over the concentration range of 25-225 μg/mL, with a small intercept and a high correlation coefficient (0.9987), indicating good linearity (Table 2). The limit of detection (LOD) and limit of quantification (LOQ) for C-HCl were calculated according to the International Conference on Harmonization (ICH) guidelines for the validation of analytical methods [22]. The calculations were based on the following equations: LOD = 3.3×SDyx/slope; LOQ = 10×SDyx/slope, where SDyx is the standard deviation of the intercept.

The calculated LOD was 6.28 μg/mL, and the LOQ was 19.02 μg/mL, demonstrating the method's sensitivity for detecting and quantifying clonidine hydrochloride in the specified concentration range.

Table 2. Analytical values for the calibration curve of C-HCl with NQS.

Parameters	Value
Correlation coefficient, R	0.9993
Correlation of determination R^2	0.9987
Beer's low limits (μg.ml^{-1})	25-250
Regression equation	y = 0.0086x - 0.0678
Slope (ml.μg^{-1})	0.0086
Intercept (a)	-0.0678
Absolute error	0.0268
Standard deviation of the residuals	0.0225
Standard deviation of the slop	0.0001
Standard deviation of the intercept	0.0164
Molar absorptivity Ɛ (L mol^{-1} cm^{-1})	2161.8
Sandell's sensitivity (μg cml^2)	4.625×10^{-8}
Limit of detection(μg ml^{-1})	6.28
Limit of quantification(μg ml^{-1})	19.02

Accuracy and precision. To assess the precision and accuracy of the proposed method for the determination of clonidine hydrochloride (C-HCl) using NQS, five replicate measurements of a 100 μg/mL C-HCl solution were conducted under optimal conditions. The results were used to evaluate the method's intraday precision and accuracy.

The calculated values for the standard deviation (SD) representing accuracy and the relative standard deviation (%RSD) indicating precision were found to be 7.855×10^{-3} and 0.937%, respectively. These results demonstrate that the method provides highly precise and accurate measurements for C-HCl, confirming its suitability for quantitative analysis.

Application to pharmaceutical formulation

The proposed spectrophotometric method was applied to the analysis of clonidine hydrochloride (C-HCl) in pharmaceutical tablet preparations at nominal concentrations of 25, 50, 75, 100, and 150 μg/mL. Five independent measurements were performed for each concentration to assess the method's accuracy and precision. The evaluation involved calculating the standard deviation and percentage recovery for each concentration.

The highest relative standard deviation (%RSD) observed was 1.7% at a concentration of 25 μg/mL (Table 3), indicating good precision. The recovery percentages ranged from 96.4% to 101.4%, demonstrating a high degree of accuracy and close agreement between the measured and nominal values. These results confirm that the proposed spectrophotometric method is reliable and effective for the determination of C-HCl in pharmaceutical formulations.

Table 3. Precision and recovery data for C-HCl determination in tablet formulations.

Concentration of C-HCl (μg ml^{-1})		%R	SD	RSD (%)
Present	Found*			
25	24.1	96.4	0.003536	1.73
50	50.7	101.4	0.003873	1.03
75	75.8	101.0	0.003782	0.70
100	99.8	99.8	0.001789	0.26
150	149.8	99.8	0.009343	0.93

* The drug's content was determined using the graduation graph method.

Kinetic and thermodynamic parameters

The reaction kinetics between clonidine hydrochloride (C-HCl) and the NQS reagent were investigated at various temperatures (25°C, 35°C, 45°C, and 55°C) using the initial rate method. The reaction was found to follow pseudo-first-order kinetics with respect to the concentration of C-HCl. The rate constants were determined from the first-order plot using the equation:

$$Kt = \ln A_\infty - \ln(A_\infty - At), \quad (2)$$

where At is the absorbance at time t, and A_∞ is the final absorbance.

The rate constants and half-life times for the reaction at different temperatures are presented in Table 4. The activation energy (Ea) was calculated from the Arrhenius plot, with a low Ea value indicating that the reaction requires minimal energy to proceed. Using the Eyring equation [24], other thermodynamic parameters, including entropy (ΔS), enthalpy (ΔH), and Gibbs free energy (ΔG), were calculated (Table 4). The positive enthalpy of activation (ΔH) confirms that the reaction is endothermic. The negative entropy of activation (ΔS) suggests a decrease in translational freedom, contributing to the reaction's slow rate. Furthermore, the positive Gibbs free energy of activation (ΔG = 49.09 kJ/mol) indicates that the reaction is non-spontaneous under the studied conditions.

Table 4. Kinetic and Thermodynamic Parameters for the Reaction between C-HCl and NQS.

Temp. (K)	k (min^{-1})	$t_{1/2}$ (min)	ΔH (kJ/mol)	ΔS (J/mol)	ΔG (kJ/mol)
298	0.1124	6.1	17.55	-96.71	46.36
308	0.1498	5.3			47.33
318	0.1646	5.0			48.30
328	0.2498	4.0			49.27

Conclusion

This study demonstrated that the proposed spectrophotometric method for the estimation of clonidine hydrochloride is simple, rapid, and accurate, making it suitable for the analysis of clonidine hydrochloride in both pure form and pharmaceutical formulations. The findings showed that the reaction between clonidine hydrochloride and the NQS reagent follows pseudo-first-order kinetics, with the reaction rate increasing as the temperature rises. Thermodynamic analysis indicated that the process is endothermic and non-spontaneous, as confirmed by the positive enthalpy and Gibbs free energy values. Overall, the method provides a reliable approach for the quantitative determination of clonidine hydrochloride in quality control settings.

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