Sapporo Medical Journal
Volume 56, Issue 08, August 2022

Thermodynamic study for spectrophotometric determination of anti-viral drug (hydroxychloroquine) by azo coupling with 2, 4-Dinitro phenyl hydrazine

HamidaIdan Salman[1], Maryam Ali Abdulsamad[1], MuthanaSalehMashkour[2]
[1]University of Kerbala ,college of Education for Pure Science, Department of Chemistry.
[2]University of kufa, College of Science, Department of Chemistry.

Abstract— Asimple, accurate and controlled spectrophotometric methodis studied for the determination of hydroxychloroquine (HCQ) both pure and in pharmaceutical preparations. The drug's reaction with the 2,4-dinitrophenylhydrazine (2,4-DNPHz) reagent is the foundation of this mathod. The optimum conditions were studied from the effect of the reagent,oxidized agent and NaOH, the reaction time and the optimum temperature for the formation of the color of azoproduct and it was in compliance with Beer's law in the range 2.5 to 22.5 µg/mL at the wavelength 620 nm. the molar absorptivity, Sandell's sensitivity, detection limit and quantitation limit were 1048.28L.mol-1 .cm-1, 0.3204µg.cm^{-2},0.243µg.ml^{-1},, and 0.738 µg.ml^{-1} respectively. The proposed method has been successfully applied to determination of HCQ in its pharmaceutical preparations. The thermodynamic functions such as enthalpy ΔH, entropy ΔS, and Gibbs free energy ΔG were calculated, and it was found that the drug absorption not Spontaneous, endothermic process and more regular of the system.

Introduction

In combination therapy for RA, hydroxychloroquine (HCQ), a good anti-rheumatism infectious disease drug (DMARD), is more frequently used than chloroquine.(1) , HCQ($C_{18}H_{26}ClN_3O$) is known by the IUPAC designation (RS)- 2-[4-(7-Chloro-4-quinolylamino) pentyl(ethyl)amino]. -ethanol(2),Chloroquine (CQ), an antimalarial medication, was used to create HCQ in 1946 after incorporating a hydroxyl group into its structure. With the discovery of HCQ, which was found to be a two- to three-times safer alternative than CQ (3). Hydroxychloroquine figure 1 is 4-aminoquinolin where it possesses the activities described above. These medications also boost resistance, HCQ symptoms treated less frequently than CQ, and acute liver lesions are also related(4).

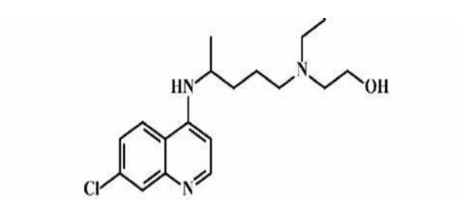

Figure1 Chemical structures of HCQ.

2,4-Dinitrophenylhydrazine (2,4-Dnphh) is substituted hydrazine. It is structure contains hydrazine which is the main active functional group.2,4-DNPHz is an important reagent in analytical chemistry it is used firstly in the identification (qualitative analysis) of carbonyl groups (aldehyde and ketones compounds)(5). and it is used in quantitative spectrophotometric determination of different compounds via different types of reactions(6),The reagent was used to determination drugs (Propranolol Hydrochloride and isopropramid as iodide)(7).Various analytical methods have been reported to identify drugs in the form of a commercial dose and biological fluids such as high-performance liquid chromatography (HPLC)(8)spectral method, LC method, electrochemical , and electrophoretic method. Derivatization or the requirement for time-consuming extraction operations are the main issues that arise while adopting such approaches. [9,10]. And the methods used to determination HCQ drug.liquid chromatography–tandem mass spectrometry (LC-MS/MS)[11], fluorescence chromatographic detectiontechnique (HPLC-FLD)[12] , differential pulse voltammetry(DPV)[13] and square wave voltammetry(SWV)[14].

Experimental

Apparatus

pH measurements were taken using a pH meter, analytical balance, a water bath, and a heated plate with a magnetic stirrer. Absorbance was performed using an 1800 PC UVvisible spectrophotometer Shimadzu Japan (Double Beam), with quartz cells of 1 cm optical path length.

Material and Reagent:

All of the chemicals were of analytical quality, and all reagent and sample dilutions were done with double-deionized water.

Preparation of solutions

1-Standard hydroxychloroquine solution,(1000 µg.mL^{-1})

The accurate weighted 0.1g of the pure medication was diluted in 10 mL of ethanol to produce the stock solution for (HCQ), and the volume was adjusted to the correct level in a volumetric flask using 100 mL of deionized water.

2-Hydroxychloroquine Working Solution (100 µg.mL-1)

Prepared by adding deionized water to a volume bottle and dissolving 10 ml of stock solution to 100 ml.

3-2,4-dinitro phenylhydrazine reagent solution (5 ×10^{-4}M)

It was prepared through dissolution 0.01 g of 2,4-DNPHz in 2.5 mL of concentrated sulfuric acid, then completing this volume to the mark in a 100 mL volumetric flask with deionized water.

4- Potassium periodate oxidized agent solution (6x10^{-4}M)

prepared through dissolution 0.014 g of KIO4 in the appropriate volume of deionized water, and then filling the flask to the specified volume.

5- Sodium hydroxide solution (1M)

Four g of sodium hydroxide were dissolved in a practical amount of deionized water, completed with deionized water and transferred to a 100 mL volumetric flask.

6- Inter ference solutions 10000 - µg.ml^{-1}

In a volumetric flask with deionized water, 1.0g of each foreign substance was dissolved before the volume was increased to 100 mL.

Initial investigations

A 10 mL volumetric flask is used to create a blue-colored product by mixing 1 mL of 2,4-DNPH reagent (359nm) with1mL of potassium periodate solution, and 1ml of standard HCQ solution

(332nm), than 0.5 mL of sodium hydroxide. The colored dye's highest absorption, in comparison to the blank reagent's, may be seen in the absorption spectrum at 620 nm.

Results and evaluation

The Experimental Conditions' Optimization

To establish the ideal conditions, the influence of various factors on the absorption intensity of 1 mL of (2,4-DNPH) and 1 mL of KIO4 than 1 mL of hydroxychloroquine solution (100 g.mL-1) in alkaline medium (0.5 ml), of(1M)NaOH) was examined.

Optional oxidizing agent selection

The study was carried out by adding 1 mL of various oxidizing agents (6×10^{-4} M) to 2,4-dinitro phenyl hydrazine solution (5×10^{-4} M) than added 1ml of drug solution and 0.5mLof sodium hydroxide solution.The results showed that potassium periodate solution gives a higher intensity for coloredazo dye at 620 nm than other oxidizing agents used as show in table 1. so this oxidizing agent was chosen in subsequent experiments.

Table 1 Effect of oxidizing factor type

Type of oxidizing factor	absorption
2,4-DNPHz + KIO_4+drug+NaOH	0.422
2,4-DNPHz + KIO_3+drug+NaOH	0.312
2,4-DNPHz + $NaIO_4$+drug+NaOH	0.305

Absorption Spectra

Hydroxychloroquine's (HCQ) absorption spectra was measured in comparison to ethanol. The greatest absorption peak for HCQ was discovered to be at 332nm. After performing the reaction between HCQ and 2,4-DNPHz, the product's absorption spectra was recorded in comparison to the reagent blank. figure 2. The product was discovered to be colored, displaying (λmax) at 620 nm and 2,4-DNPHz (λ max) at 359 nm. Any potential interference was removed by blue shifting the (λmax) of the HCQ 2,4-DNPHz derivative. As a result, 620 nm was chosen for the measurements.

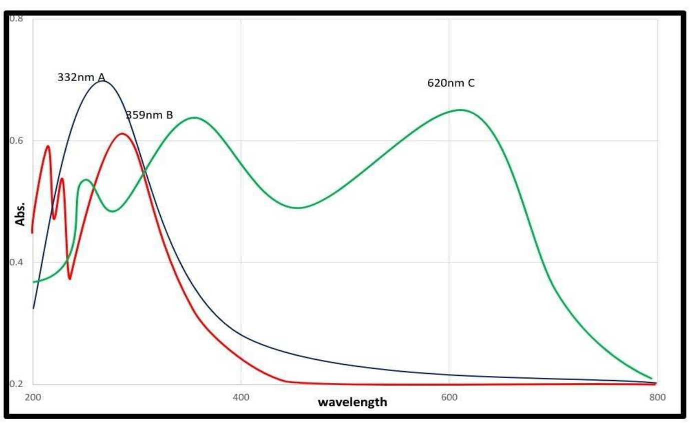

Figure (2) Absorption spectra of **(A)** HCQ (100 µg.ml^{-1}). **(B)** 2,4-DNPHz (0.005 M) **(C)** azo dye product of HCQ with 2,4-DNPHz.

Effect of 2,4-DNPHzconcentration

The analysis of (2,4-DNPHz) concentrations ($5\times10^{-5}$to 1.5×10^{-3})M showed that the reagent (2,4-DNPHz) was necessary for the reaction.Thegreatest absorption obtained when concentrating (7.5×10^{-4} M) of the reagent for HCQ .The absorption results were unaffected by 2,4-DNPHz concentrations up to (1.5×10^{-3}M) figure 3.

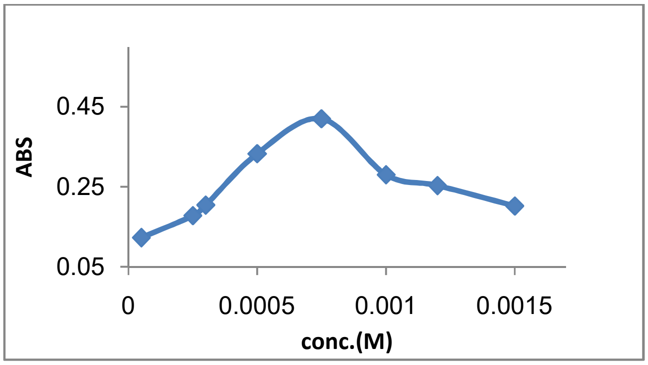

Figure 3 Effect of 2,4-DNPHz concentration on the azo coupling with HCQ .

Effect the amount of 2,4-DNPHz

By using various volumes (0.25 to 2.5)ml of the reagent at a concentration of (7.5×10^{-4}M) 2,4-DNPHz for HCQ, it was possible to determine the impact of the reagent volume on the degree of absorption of the dye that resulted. At 1 mL, the maximum absorption intensity was found figure 4.

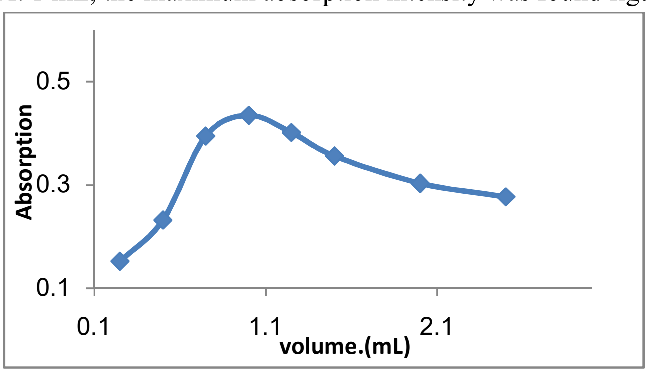

Figure 4 Effect of volume of 2,4-DNPHz on the azo coupling with HCQ.

Effect of potassium periodate concentration

By applying different concentrations of KIO_4 ($3\times10^{-4}$to 2.4×10^{-3})M and calculating the HCQ wavelength-dependent absorption intensities for each solution mixture,the study examined the impact of oxidizing agent concentration. The greatest amount of absorption intensity was found. KIO_4 is present in the best concentration at (9×10^{-4} M). and the figures for the absorption were unaffected by the higher concentration of the oxidizing agent (2.4×10^{-3} M)figure 5.

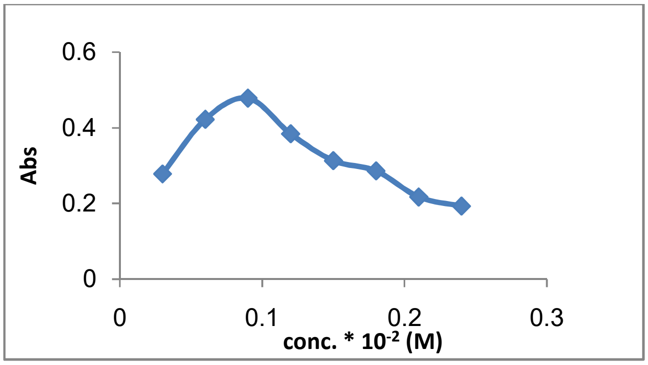

Figure 5 Effect of concentration (KIO_4) on theazo coupling with HCQ.

Effect the volume of KIO_4

In order to determine the optimal concentration of the oxidizing agent KIO_4 (9×10^{-4} M), volumetric flasks containing 1.0 ml of the 2,4-DNPH reagent (7.5×10^{-4} M) and different volumes of KIO_4 (0.25 to 2.5)ml than 1.0 ml of HCQ (100 g/ml) were used in the study. After that, 0.5 ml of sodium hydroxide was added, and then deionizd water was used to finish the volume to 10 ml. The results shown in the figure 6. It was decided in later studies that 0.75 ml of the oxidizing agent KIO_4 was the ideal volume because it had the maximum absorbance.

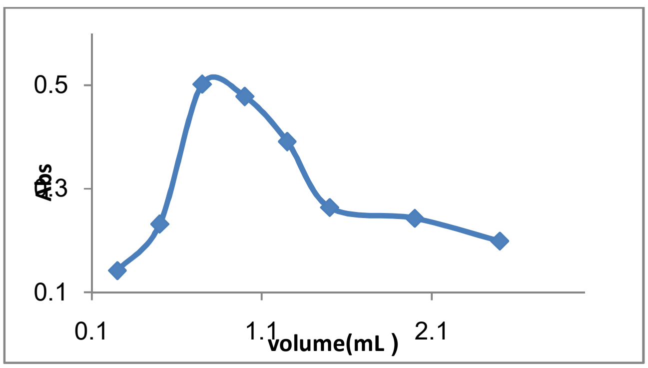

Figure 6 Effect of the volume of (KIO_4) on the azo coupling with HCQ

Effect of volume of HCQ

Using various volumes of the medicine and a wavelength of HCQ ($100\mu g.ml^{-1}$) to measure the intensity of the solution's absorption, the study looked at how much HCQ was effective. At 1.25 mL, the ideal amount of HCQ, the maximum absorption intensity was found figure 7.

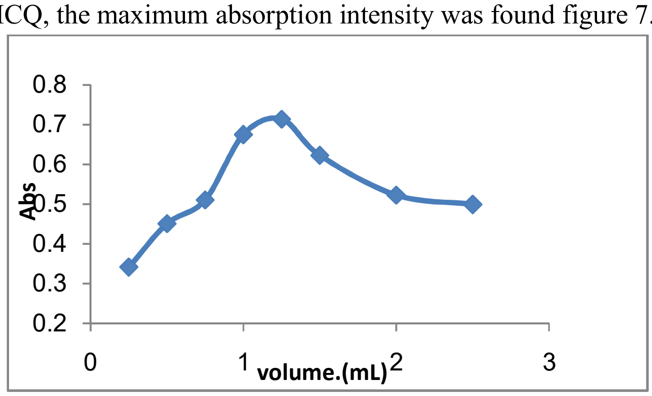

Figure 7 Effect of volume of HCQ

Effect of sodium hydroxide concentration

After the installation of condition of the best in the past experiences were study effect of base solution concentration on the reaction HCQ. The study was investigated using different concentrations of Sodium Hydroxide (0.25 to 1.25)M . As shown in Figure 8, by measuring of the intensity of each solution mixture's absorption.the best concentration of NaOH at (0.6M) , and the higher concentration of Sodium Hydroxide (1.25M) had no effect on the absorption values.

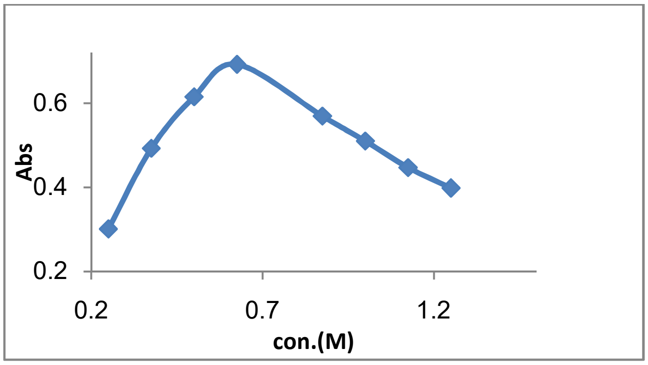

Figure 8 Effect of concentration (NaOH) on the coupling with HCQ.

Effect of the volume ofNaOH

Additionally, the effect of the volume of sodium hydroxide of HCQ on the absorbance of the reaction product was also studied. By using different volume (0.1 to 2)ml of base solution (NaOH) figure 9 shows that. The highest absorption intensity was detected. The best volume of NaOH at (0.5ml). and the higher volume of base solution (2ml) had no effect on the absorption values.

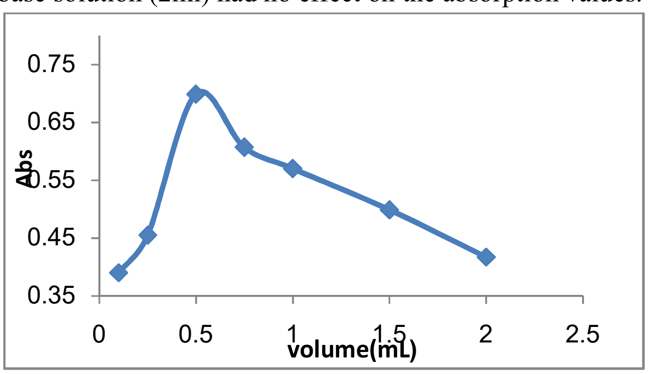

Figure 9 Effect of the volume of (NAOH) on the azo coupling with HCQ.

Effect of reaction time

The color intensity peaked after adding 2,4-DNPH and KIO4 with to HCQ for 10 minutes in basic medium; as a result, 10 minutes is enough time for the oxidation to finish, thus it is used in the studies that follow.The hue was retained for 65 minutes.

Table 2 Effect of reaction time

Time (min)	5	10	20	30	40	50	60	70
Absorbance	0.589	0.725	0.721	0.720	0.721	0.722	0.722	0.714

Effect of temperature on reaction

Temperature (5-45°C) and the absorbed hue of the product were also examined.The results are shown on Figure10.show that (25°C) is the ideal temperature because it produced the best absorption. Therefore, it is utilized in later experiments.

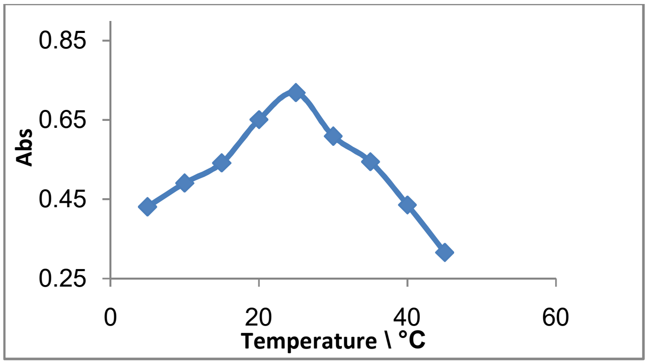

Figure 10 Effect of temperature on reaction

Sequence of additions

The impact of various addition orders on the product's ability to absorb color was investigated.The results are shown in table.3indicate that the addition (2,4−DNPH + KIO4+ HCQ + OH-) ensures that colored product is absorbed more fully. Consequently, it was used in later research.

Table 3 Addition order of HCQ**.**

NO.	Addition	Abs
1	**2,4-DNPHZ + KIO_4+Drug + NaOH**	**0.725**
2	**Drug + NaOH + 2,4-DNPHZ + KIO_4**	**0.287**
3	**2,4-DNPHZ + drug + KIO_4 + NaOH**	**0.415**
4	**Drug+2,4-DNPHZ + KIO_4 + NaOH**	**0.50**

Calibration curve of HCQ

The standard calibration curve for the determination of HCQ, which was found to follow Beer's law, is shown in Figure 11. Under ideal circumstances, at a maximum wavelength of 620 nm, and the R = 0.9978 correlation value. 1048.28 L/mol/cm was the value of molar absorptivity.

The following relationship's coefficient of specific absorption was used to compute Sandell's sensitivity, which came out to 3.204 g mL^{-2}.This analytical approach preferred HCQ measurement at low concentrations due to Sandell's sensitivity and the high value of molar absorptive.

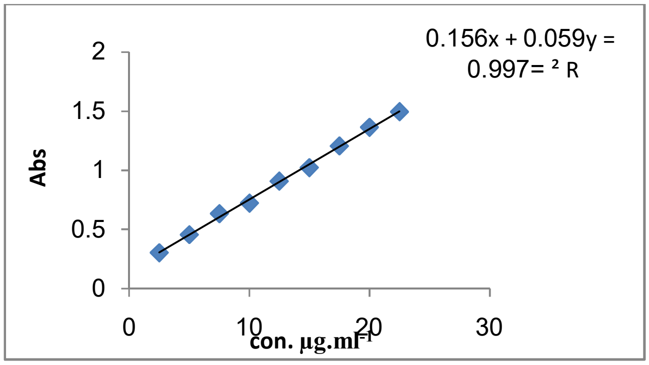

Figure 11 Calibration curve of HCQ by azo coupling with 2,4-DNPHz in presence KIO_4.

Limit of detection (LOD), linearity, and limit of quantification (LOQ)

By creating nine HCQ concentrations, The linearity was evaluated using a linear regression analysis. By creating nine HCQ concentrations, To evaluate the linearity, a linear regression analysis was employed(15).The calibration equation and correlation coefficient were estimated using the least square regression approach to be in the range of (2.5to22.5)g/mL. Using linear regression analysis, The calibration curves were made by plotting concentration vs absorbance. A = 0.0596x + 0.1568 served as the regression equation for the outcomes,where R is the correlation coefficient(table4). According to the ICH guidelines, the limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the following formulas: LOD = 3.3 SDa/b, and LOQ = 10 SDa/b. SDa stands for standard deviation of the blank, and b for slope. (16).

Table 4 Analytic parameter for HCQ determinationby azo coupling with 2,4-DNPHz in presence KIO_4.

Parameter value	Value\HCQ
Beer's law limit ($\mu g.ml^{-1}$)	(2.5-22.5)
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	1048.28
Sandell's sensitivity($\mu g.cm^{-2}$)	0.3204
Detection limit ($\mu g.ml^{-1}$)	0.243
Quantitation limit ($\mu g.ml^{-1}$)	0.738
Determination coefficient(R^2)	0.9978
Slope(b)	0.0596
Intercept(a)	0.1568

% R.S.D	**%1.44**
%Error	**%-0.012**

Accuracy and Precision for this method

The accuracy and precision of the proposed approach were assessed using five repetitions at a concentration of 5 g/mL HCQ mixture. Relative standard deviation (RSD), which was used in the computation, was applied to the suggested ways to calculate HCQ, as indicated in table 5.

Table 5 Relative errorand recovery as parameters to expressthe accuracyof methodsdeterming HCQ

Values	HCQ
Wavelength (nm)	624
Conc. ($\mu g.ml^{-1}$)	5
X^-	0.456
%R.S.D	1.288%
%Error	0.334%
D.L(µg.ml-1)	0.326
%Recovery	100.33%

The derivatization reaction's stoichiometry

To ascertain the ideal conditions' stoichiometric ratio of the formation reaction between the reagent and HCQ, HCQ was employed in a 1:1 mole ratio with a wavelength of 620nm. continuous variation (Job's method) was also applied. (figure. 12 & 13)

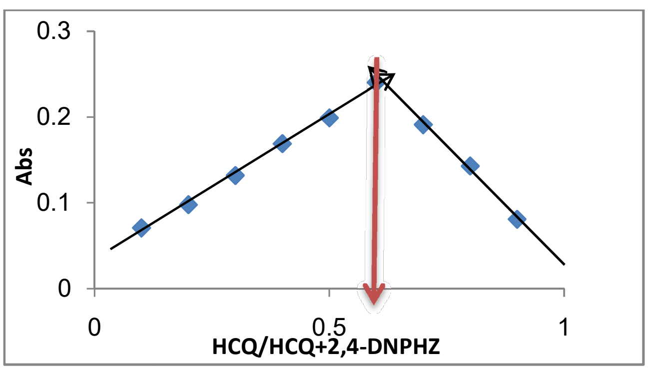

Figure 12the continuous variation method of the reaction between 2,4-DNPHz in presence KIO_4 with HCQ.

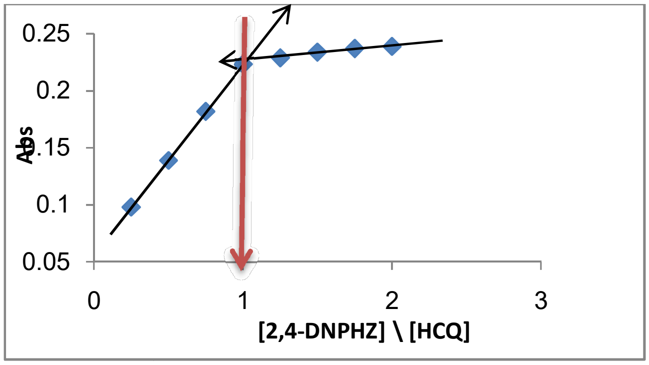

Figure 13 The mole ratio of the reaction between 2,4-DNPHz in presence of KIO_4 with HCQ.

The suggested path way reaction the 2,4-DNPHz in presence KIO_4 with HCQ is illustrated in Scheme 1.

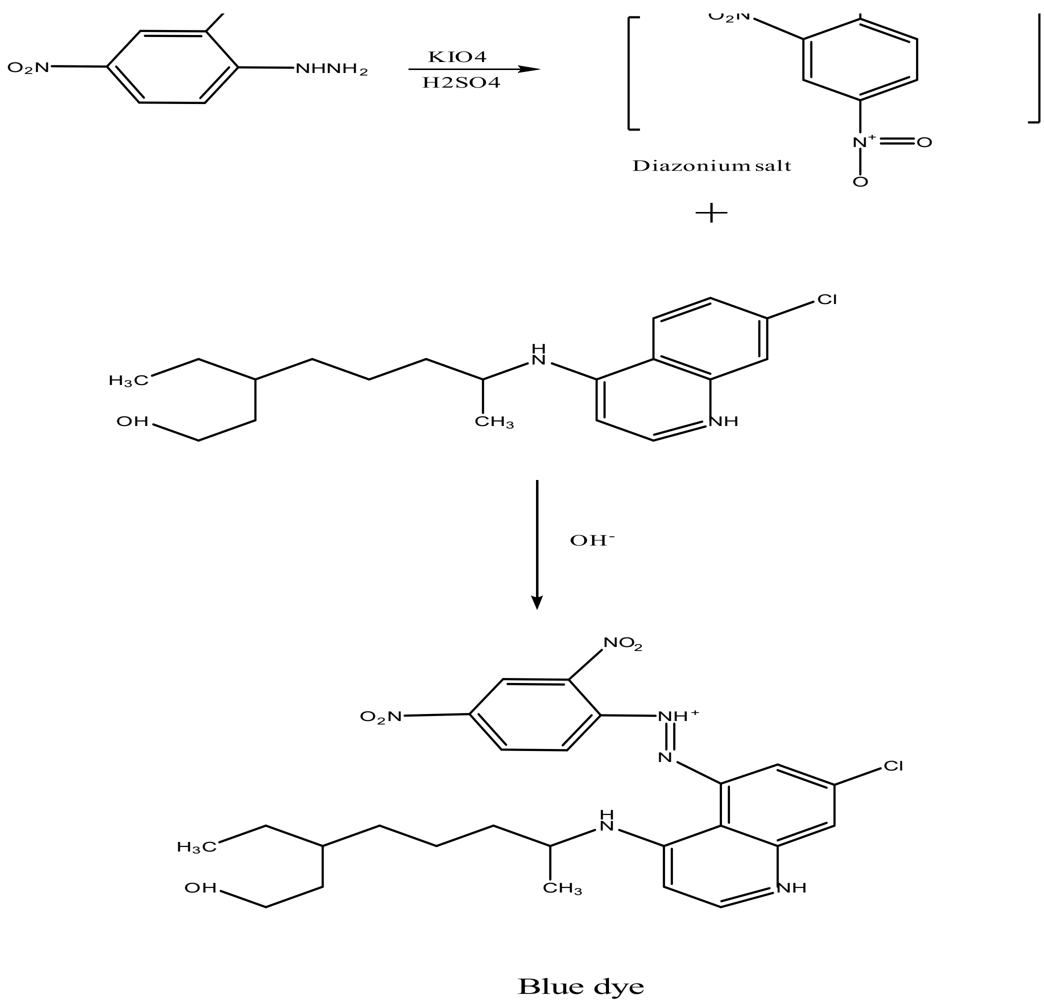

Figure 14 Scheme for the reaction pathway for determination of HCQ with 2,4-DNPHz.

Effect of interferences

To be sure the method of permeability in the studies of pharmacological application ,was conducted to obtain the effect of foreign chemical materials found in pharmacological formula.The results are shown in table 6.

Table 6 Effect of interferenceson the reaction of 2,4-DNPHz in presence KIO_4 with HCQ.

interferences	Abs
Magnesium stearate	0
Starch	0
Polyethylene glycol	-0.005
Dibasic calcium phosphate	0
Polysorbate	0.002
Hydroxypropylmethylcellulose	0

Applications

This technique was used to determine the presence of HCQ in the tablets used in its pharmaceutical preparation (20mg).

In a series of 10 mL volumetric flasks. added 1 mL of reagent 2,4-DNPHz ($5x10^{-4}$ M), and 0.75 mL of KIO_4 ($6x10^{-4}$ M) than 1 mL of HCQ solution (0.25-2.25) $\mu g.ml^{-1}$was added to the mixture after that is to add 0.5 ml ofNaOH , then dilute the contents with deionized water to the mark, and then measure the absorbance after 5 min at the wavelength 620nm against the blank solution, The resultsare shown in table 6.

Table 7analytical applications.

Sample	Conc. mg/ml		%Error	%Recovery	%R.S.D
	Present	Found			
Quenil tablets (France)	20	19.9	0.1	100.01	0.381%
Quinoric tablets (British)	20	20.1	-0.9	99.1	0.455%
Dolquine tablets (Spain)	20	20.4	-1.6	98.4	0.663%

Thermodynamic study

Studying the impact of temperature allowed us to calculate the thermodynamic parameters (free energy change [ΔG], enthalpy [ΔH], and entropy [ΔS]). ΔH was calculated by drawing Vant – Hoff

Arrhenius equation(18) for absorption of HCQ .In this work the thermodynamic parameters were calculated as shown in figure15 and table 8

Thermodynamic parameters: (ΔG), (ΔH) and (ΔS) were found by applying this equations :

$\Delta G = - RT \ln Keq$ ………..……….....… (1)

$Keq = \frac{Qe\ m}{Ce\ V}$ …………………………...… (2)

$\ln Keq = -\frac{\Delta H}{RT} + Constant$ .... (3)

$\Delta G = \Delta H - T\Delta S$ ………………...…...... (4)

Slop =ΔH…………………………..……(5)

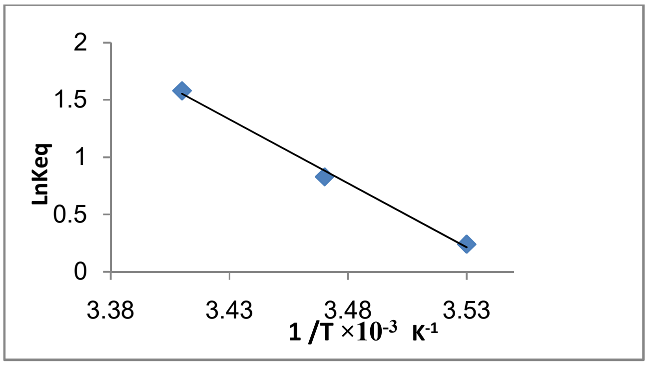

Figure 15 The relationship between lnKeq and 1/T for determination HCQ by azo coupling with 2,4-DNPHz.

Table 7 thermodynamic parameters of HCQ

	T (K)	ΔG (kJ/mol.K)	ΔH (kJ/mol.K)	ΔS (J/mol)
HCQ	288	-0.56939	0.0927	-0.5694
	293	-1.98738		-1.9874
	298	-3.84888		-3.8489

The results of the thermodynamic parameters, The positive values of (ΔH) show that hydroxychloroquine's absorption is an endothermic process. [19],negative values of (ΔG)indicate,thatthe process is Spontaneous[20],and the negative values of (ΔS) indicate that the system is regular[21].

Conclusions

The results show that the suggested approach for determining HCQ is straightforward, quick, and sensitive. The process relies on the oxidative coupling of HCQ with the 2,4-dinitrophenylhydrazine reagent in the presence of potassium periodate in a basic media to create a blue dye that is stable, water soluble, and has a maximum absorption at 620nm. This approach can be used successfully to identify HCQ in pharmaceuticals with a recovery of at least 100.01 per cent without having to control temperature or use organic solvents. HCQ absorption is a spontaneous, more regular and heat absorbent process, according to thermal dynamic properties.

REFERENCES

[1] Cannella AC, O'Dell JR. Traditional DMARDs. Kelley and Firestein's Textbook of Rheumatology; 2017. p. 958–82. DOI:10.1016/b978-0-323-31696-5.00061-9.

[2] British Pharmacopoeia. Available from: https://www. pharmacopoeia.com/ban-2012/ban-2017.pdf. [Last assessed on 18 Apr 2020].

[3] Jordan P, Brookes JG, Nikolic G, Le Couteur DG. Hydroxychloroquine overdose: toxicokinetics and management. J ToxicolClinToxicol 1999;37:861-4.

[4] National Centre for Biotechnology Information. PubChem Database. Hydroxychloroquine, CID=3652. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Hydroxychloroquine [Last accessed on 18 Apr 2020].

[5] Zakaria, S. A., Talal, Z., & Othman, N. S. (2022). Using 2, 4-dinitrophenylhydrazine in spectrophotometric determination. Samarra Journal of Pure and Applied Science,4 (2), 107-117.

[6] Habeeb, E. D., &Sulaiman, I. D. Spectrophotometric determination of propranolol hydrochloride via oxidative coupling reaction with 2, 4-dinitrophenyl hydrazine. InternationalJournalofDrugDeliveryTechnology, 11(1), 29-35.

[7] Habeeb, E. D., &Sulaiman, I. D. Spectrophotometric determination of propranolol hydrochloride via oxidative coupling reaction with 2, 4-dinitrophenyl hydrazine. InternationalJournalofDrugDeliveryTechnology, 11(1), 29-35.

[8] G. Carlucci, "Analysis of fluoroquinolones in biological fluids by high-performance liquid chromatography," J. Chromatogr. A, vol. 812, no. 1–2, pp. 343–367, 1998.

[9] V. F. Samanidou, E. A. Hapeshi, and I. N. Papadoyannis, "Rapid and sensitive high-performance liquid chromatographic determination of four cephalosporin antibiotics in pharmaceuticals and body fluids," J. Chromatogr. B, vol. 788, no. 1, pp. 147–158, 2003.

[10] J. C. Abbar and S. T. Nandibewoor, "Development of electrochemical method for the determination of chlorzoxazone drug and its analytical applications to pharmaceutical dosage form and human biological fluids," Ind. Eng. Chem. Res., vol. 51, no. 1, pp. 111–118, 2012.

[11] Y. Qu, K. Brady, R. Apilado, T. O'Malley, S. Reddy, P. Chitkara, T. Dervieux, J. Pharm. Biomed. Anal. 2017, 140, 334–341.

[12] Y. Qu, G. Noe, A. R. Breaud, M. Vidal, W. A. Clarke, N. Zahr, B. Blanchet, Future sci. OA 2015, 1(3), FSO26.

[13] M. L. P. Arguelho, J. F. Andrade, N. R. Stradiotto, J. Pharm. Biomed. Anal. 2003, 32(2), 269–275.

[14] P. B. Deroco, F. C. Vicentini, G. G. Oliveira, R. C. Rocha-Filho, O. Fatibello-Filho, J. Electroanal. Chem. 2014, 719, 19–23.

[15] Singh, A., Singh, C. L., Gupta, R., Kumar, S., & Kumar, M. (2015). Development and validation of reversed-phase high performance liquid chromatographic method for hydroxychloroquinesulphate. Indian Journal of Pharmaceutical Sciences, 77(5), 586.

[16] H. Rajak, Validation of analytical procedure: Methodology. ICH harmonised tripartite guideline. CPMP, 1996: 1450.

[17] Johnson, A. E., Jeppsson, F., Sandell, J., Wensbo, D., Neelissen, J. A., Juréus, A., ... &Svensson, S. P. (2009). AZD2184: a radioligand for sensitive detection of β-amyloid deposits. Journal of neurochemistry, 108(5), 1177-1186.

[18] Richards, O. W. (2002). A nomogram for the van'thoff-arrhenius temperature equation. The Journal of Physical Chemistry, 30(9), 1219-1221.

[19] Omer, R. A., Ahmed, L. O., Koparir, M., &Koparir, P. (2020). Theoretical analysis of the reactivity of chloroquine and hydroxychloroquine. Indian Journal of Chemistry-Section A (IJCA), 59(12), 1828-1834.

[20] Ejuh, G. W., Fonkem, C., Assatse, Y. T., Kamsi, R. Y., Nya, T., Ndukum, L. P., &Ndjaka, J. M. B. (2020). Study of the structural, chemical descriptors and optoelectronic properties of the drugs Hydroxychloroquine and Azithromycin. Heliyon, 6(8), e04647.

[21] Powell, G. E., & Percival, I. C. (1979). A spectral entropy method for distinguishing regular and irregular motion of Hamiltonian systems. Journal of Physics A: Mathematical and General, 12(11), 2053.