Spectrophotometric determination of clioquinol via complex formation with Fe(III)in pharmaceutical preparations (creams) and environmental wastewater samples

Nief Rahman Ahmad*   and   Ayad Mohamad Ali**

*Department of Environmental Technology, College of Environmental University of Mosul, Mosul-Iraq

**AL-Hokamaa Company for Drug Industries, Mosul-Iraq

Abstract:

To develop spectrophotometric method for the determination of clioquinol in pharmaceutical preparations and industrial wastewater samples. The method is based on the chelating of the drug with Fe(III) to form blue- green colored metal - chelate at room temperature which absorbs maximally at 639 nm. Beer' s law is obeyed over the concentration range of 2.00-20.00 µg/ml (6.5×10^{-6} - 6.5×10^{-5} M) with molar absorptivity and Sandell' s sensitivity of 0.611×10^4 l/mol.cm and 5×10^{-5} mg/cm respectively, relative standard deviation (RSD) is less than 2.00% (n=10.00). The method is applied successfully for determination of clioquinol in some pharmaceutical formulations (creams) and industrial wastewater samples.

التقدير الطيفي لكليوكوينول عن طريق تكوينه معقد مع ايون الحديد الثلاثي في بَعْض المستحضراتِ الصيدلانيةِ (كريم) وعينات من المياه ١ الصناعية المطروحة.

نايف رحمان احمد*   **إياد محمد علي

*جامعه الموصل-كليه البيئة-قسم التقانات   ** شركه ادويه الحكماء –موصل-العراق

الخلاصة :

تم تَطوير طريقةٍ طيفيةٍ لتقديرِ كليوكوينول في مستحضراته الصيدلانية وعيناتٍ من المياه الصناعيةِ المطروحة. تعتمد الطريقةَ على تكوين معقد كليتي بين الدواء وايون الحديد الثلاثي لتَشكيلِ معقد ازرق مخضر اللَّوَّن في درجةِ حرارة الغرفةِ والذي له أقصى امتصا ص   عند 639 نانوميتر. حيث أن قانون بير ينطبق على مدى تركيزَ2.00-20.00 مايكروغرام \مل (6.5×10^{-5} M) (6.5×10^{-6} -)ووجد بان قيمة معامل الامتصاص المولاري ودلالة ساندل للطريقة كانا 0.611×10^4 لتر\مول. سم و5×10^{-5} ملغرام\سم   على التوالي،إن الانحراف القياسي النسبي للطريقة أقل مِنْ 2.00 % (n=10.00). تم تطبيق الطريقةَ بنجاح لتقدير كليوكوينول في بَعْض المستحضراتِ الصيدلانيةِ (كريم) وعينات من المياه ١ الصناعية المطروحة.

Introduction:

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline; CQ;[1] [Fig. 1]

C9H5ClINO   305.5
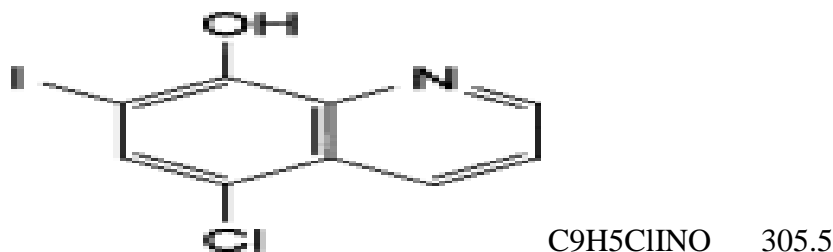
Fig[1]: Chemical Structure of Clioquinol

It was first prepared in Germany in the early part of the last century [2] and has been widely used as an antibiotic for the treatment of diarrhea and skin infection. The metal-binding properties of clioquinol led to its use in a mouse model of Alzheimer's disease in which it was shown to reduce or prevent the formation of amyloid plaques in the brain [3]. It was also shown to have efficacy in an animal model of Parkinson's disease [4] . Biochemical analysis revealed that clioquinol induced cancer cell death through apoptotic pathways that require caspase activity. Although clioquinol induced modest inhibition of SOD1 activity in treated cells, comparable inhibition by a known SOD1 inhibitor, diethyldithiocarbamate, did not result in cytotoxicity [5] . Few reports have been described for the determination of clioquinol , these include titrimetric methods [6,7],spectrofluorimetric method [8], spectrophotometric method[9] extractive alkylation and gas-liquid chromatography[10], high-performance liquid chromatography[11-12],and gas chromatography[13] . These methods are not simple for routine analysis and required expensive or sophisticated instruments.The purpose of this work was to develop rapid accurate ,procedure for the determination of pure clioquinol, pharmaceutical preparations and environmental water samples . The method is based on the reaction of drug with Fe^{+3}ion at pH_2 resulting in the formation of blue complex which absorbs maximally at 639 nm.

Experimental:

Apparatus :

Shimadzu UV- 1700 pharmaspec (double beam) spectrophotometer with 1.00 cm quartz cells was used for absorption measurements, and Jen way 3310 pH meter was used.

Reagents:

All chemical used were of analytical or pharmaceutical grade and clioquinol standard material was provided from state company of drug industries and medical appliance (NDI) Nineveh - Iraq .

Clioquinol standard solution (100ppm):

This solution was prepared by dissolving 0.01 gm of clioquinol in 100 ml of ethanol(96%) in calibrated flask.

Ferric ammonium sulfate 1%(0.02 M):

This solution was prepared by dissolving 1 gm of $NH_4Fe(SO_4)_2.12\ H_2O$ in distilled water containing 3.0 ml of concentrated H_2SO_4 and make up to 100ml in a calibrated flask.

Buffer solution(pH 2):

This solution was prepared by mixing 25.0 ml of 0.2 M KCl with 6.5 ml of 0.2M HCl , then the volume is completed to 100 ml with distilled water in a volumetric flask[14].

General procedure :

Different aliquots of standard solution of clioquinol equivalent 50-500 µg were transferred into a series of 25.0ml volumetric flasks, 3.0 ml of buffer solution pH2 , and 3.0 ml of (1%) Ferric ammonium sulfate solution were added. The content was mixed and let stand for 5min with occasional shaking. The volume was diluted to the mark with distilled water and mixed well. The absorbance of each solution was measured at 639 nm against a reagent blank.

Procedures for pharmaceutical preparations(creams):

The amount of cream equivalent to 10.0 mg of clioquinol was transferred quantitatively into a conical flask. The drug content of the cream extracted with three successive 30 ml of dimethylformamide and collected in a 100 ml calibrated flask after filtering through a whatman no.1 filter paper. The solution was made up to the mark with dimethyl formamide. Treat 3.0 ml of this solution as mentioned under general procedure. .

Procedure for wastewater sample:

To demonstrate the practical applicability of the proposed method, real water samples were analyzed by this method. Industrial wastewater from the state company for drug industries and medical appliances Mosul-Iraq, were filtered and analyzed for clioquinol. They tested negative, the samples were fortified with the concentrations in the range of 4.0, 12.0, 20.0 µg/ml of clioquinol. The fortified water samples were analyzed as described above for recommended procedure and the concentration was calculated by using the calibration curve of this method.   .

Results and Discussion:

Clioquinol was found to react with Fe (III) at room temperature resulting in formation of blue-green colored complex which absorbed at 639 nm [Fig. 2].The various experimental affecting the development and stability of the reaction product

was optimized by changing each variable in turn while keeping all other variables constant.
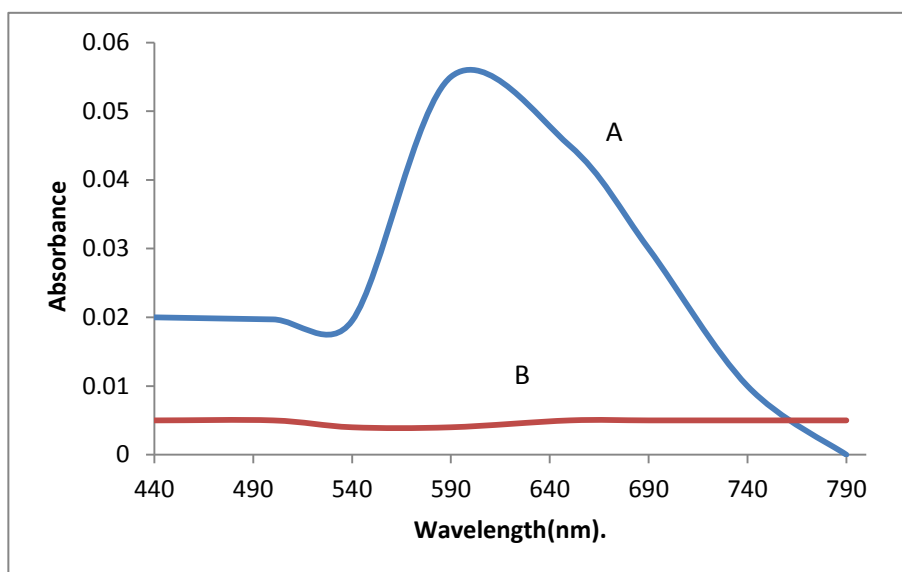
Fig[2]:Absorption spectra of A- clioquinol (12.0 μg/ml) with Fe (III) against reagent blank. B- clioquinol (12.0 μg/ml) against blank
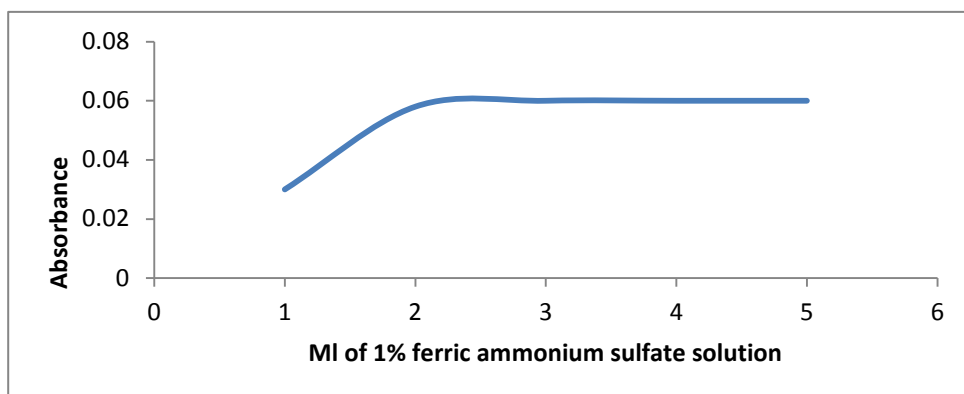
Effect of pH:

The effect of pH was investigated in the range of 1.0- 4.0 . The results indicated that the product remained maximum and constant over the pH range 1.5-2.5, fig (3). There for a 3.0 ml of pH2 was selected for further study.
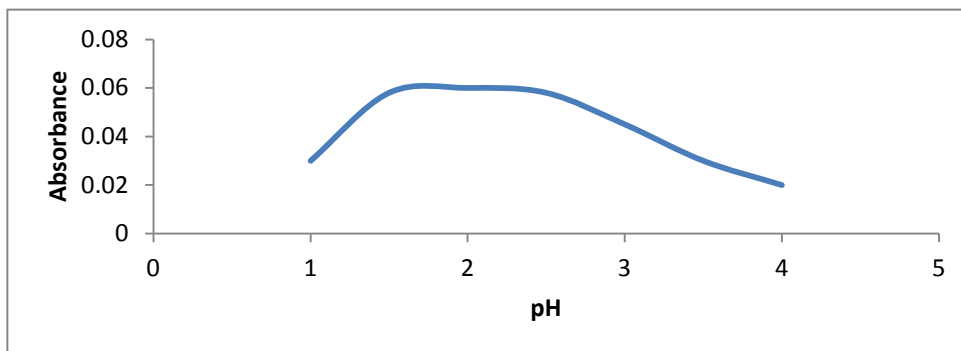
Fig(3):Effect of pH

Effect of ferric ammonium sulfate solution:

The amount of ferric ammonium sulfate solution (1%) for maximal color intensity was examined the maximum constant intensity was reached at 2.0 ml of reagent solution and remained constant up to 5.0 ml ,fig(4). However 3.0 ml of the reagent solution was selected for the subsequent work.

Fig(4):Effect of the amount of ferric ammonium sulfate solution.

Effect of temperature and time:

The results   obtained indicated that complete color formation occurred immediately and not effected by temperature therefore, room temperature was selected as suitable temperature. The absorbance remained constant for 6.0 hours at least, and 5.0 min was selected as a suitable time , the effect of heating time and temperature is shown in Table(1).

Table 1 : Effect of temperature and heating time

Temp C^o	15.0			25.0			40.0		
Time(mint)	5.0	60.0	360.0	5.0	60.0	360.0	5.0	60 .0	360.0
Absorbance	0.059	0.58	0.55	0.58	0.57	0.057	0.059	0.059	0.058

Effect of order of addition:

To test the effect of order of the addition of the reagents on the absorbance of the product, different order were tested. The selected order was sample solution (S), buffer solution pH2 (B) followed by ferric ammonium sulfate solution(R) which was gave high absorbance value.

Table 2 : Effect of order of addition

Reaction components	Absorbance
R+ S+B	0.053
R+B+S	0.052
S+B+R	0.060
S+R+B	0.055

Calibration graph:

Employing the conditions described in the general procedure   a linear calibration graph of clioquinol was obtained [Fig.5.0], which shows that Beer's law was obeyed over the concentration range of 2.0-20.0 µg/ml with correlation coefficient of 0.9990, intercept of 0.000 and slope of 0.020 . The conditional molar

absorptivity of the product formed and sandelĺ' s sensitivity were found to be 0.611×10^4 L/ mol .cm and 5×10^{-5} µg/cm^2 respectively. The limit of detection (LOD) and quantification (LOQ) was calculated using the formula LOD= $3.3 \sigma/s$ and LOQ $10 \sigma/s$ where σ is the standard deviation of seven reagent blank determination and s is the slope of the calibration curve [15].The results presented in table[3] and reveal the high sensitivity of the present method
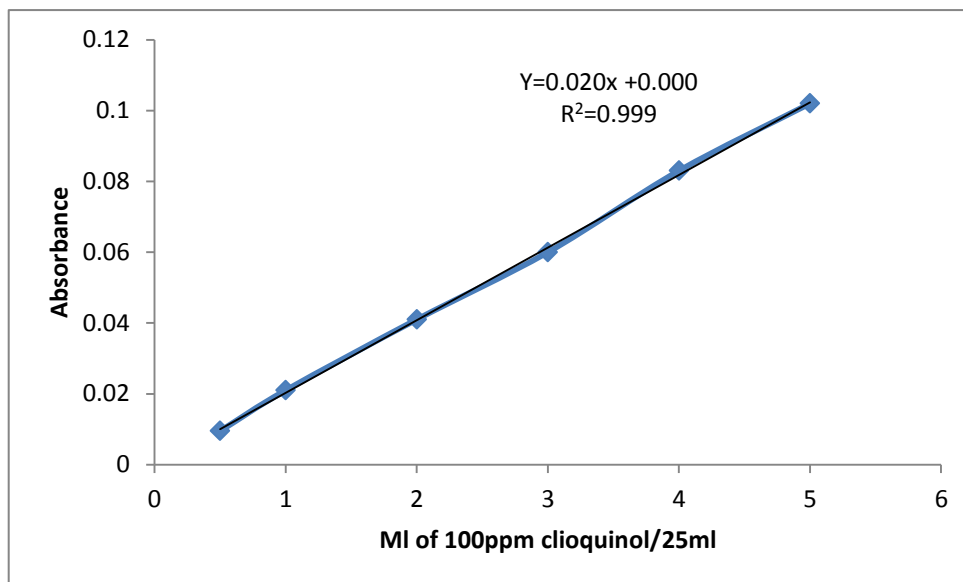
Fig[5]:Calibration curve of clioquinol.

Accuracy and precision:

The accuracy and precision of the method was established by analyzing the pure drug solution at three different levels. each determination being repeated ten times. The average recovery which is a measure of accuracy is 100 ± 0.95 revealing high accuracy of the method . The relative standard deviation (RSD%) , which is an indicator of precision is less than 2.0% . The results are complied in Table[3] .

Table [3]: Optical characteristics and statistical data for regression equation of the proposed method

| Parameters	Value
λ max (nm)	639
Beer´s law limits (µg /ml)	2.0-20.0
Molar absorpitivity (1/mol.cm)	0.611×10^4
Sandell' s Sensitivity (mg/cm)	5×10^{-5}
Correlation coefficient (R^2)	0.9990
Regression equation (Y= a $\times$ + b)	Y=0.020x+0.00
Slope (a)	0.020
Intercept (b)	0.000
Recovery %	100 ± 0.95
Relative standard deviation (%)	< 2.0
LOD (µg /ml)	0.06
LOQ (µg /ml)	0.18

Stoichiometry of reaction:

The stoicheiometry of the reaction between clioquinol and Fe(III) was investigated using job's method of continuous variation and mole ratio methods of equimolar solution(0.2×10^{-3}M), the result obtained show that 1:3 Fe(III) to drug at 639 nm fig(6) .
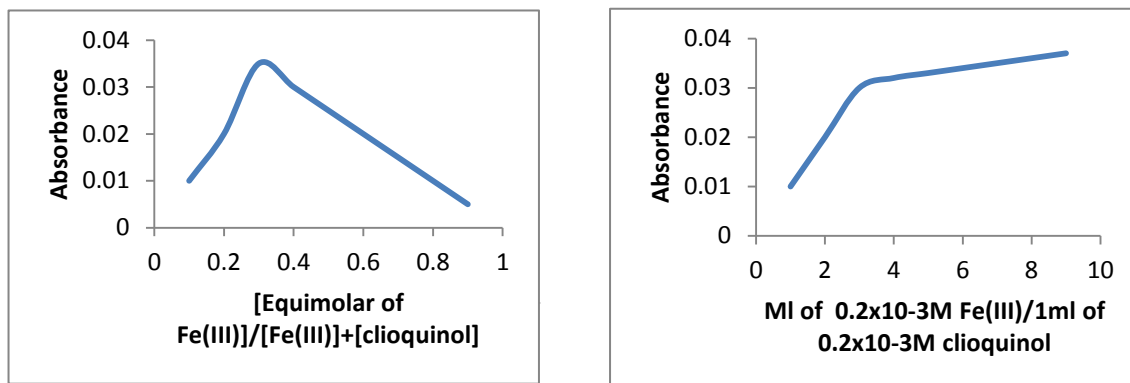
Fig(6) :Continuous variation and mole ratio plots for reaction of Fe(III) with clioquinol

The suggested reaction and structure of the product might be written as :
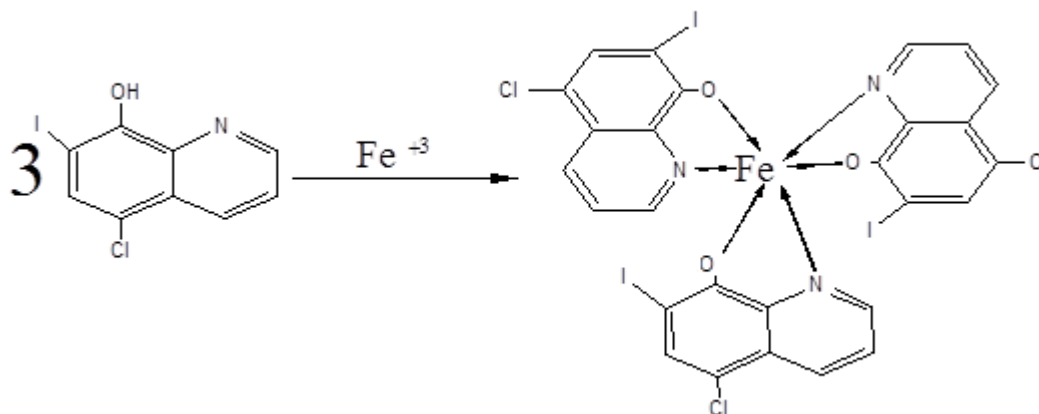
Application of the proposed method:

The proposed method was successfully applied to the analysis of clioquinol in creams and industrial wastewater sample. The result of analysis for pharmaceutical formulations revels that there is close agreement between the results obtained by the proposed method and the label claim Table[4] , and the results of water samples Table [5] show that the recovery values obtained were close to 100%. .

Table[4]: Assay of clioquinol in pharmaceutical formulations.

Pharmaceutical formulation supplied by NDI	Amount of clioquinol* Proposed method(mg/gm)	Label claim (mg/gm)	Recovery%
Quadrim Cream	9.99	10.0	99.9

*Mean of ten determinations.

Table[5]: Determination of clioquinol in spiked industrial wastewater sample.

Water samples	Clioquinol (µg/ml) *		Recovery%
	Added	Found	
Industrial wastewater	5.0	5.0	100.0
	10.0	9.95	99.5
	20.0	20.1	100.5

*Mean of ten determinations

Conclusion:

In this work, a simple, rapid, precise and accurate spectrophotometric method was developed and validated for the determination of clioquinol in pharmaceutical preparations and industrial waste water samples. The method free from such experimental variables such as heating or solvent extraction step. The method rely on the use of simple and cheap chemicals and techniques and can be used for rapid routine determination and quality control of clioquiminol in pure form, bulk sample ,pharmaceutical preparations and real industrial waste water sample.

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Acknowledgments:

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