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Determination of Diiodohydroxyquinoline Using UV-Visible and Atomic Absorption Spectrometry

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Three novel methods were developed for the determination of diiodohydroxyquinoline based on UV-Visible and atomic absorption spectrometry (AAS). Two UV-Visible sensitive methods based on the oxidation of diiodohydroxyquinoline by iron(III) and the produced iron(II) reacted with potassium ferricynide or 1,10-phenanthroline forming Prussian blue colour (Method I) or red colour complex [method(II)], respectively. The AAS method is based on the extraction of the excess iron(III) in the oxidation reaction of diiodohydroxyquinoline/iron(III). The iron(II) in the aqueous layer was aspirated into air-acetylene flame. The reactions have been evaluated to obtain optimum experimental conditions. Linear responses were exhibited over the ranges 2.0-20.0, 1.0-10.0 and 0.2-2.0 μg mL^{-1} for method I, method II and AAS, respectively. A high sensitivity is recorded as 0.091, 0.074 and 0.028 μg mL^{-1} for the proposed methods I and II and AAS, respectively. The limit of detection of diiodohydroxyquinoline by method I and II and AAS method were 0.2, 0.17 and 0.07 μg mL^{-1}, respectively. The developed methods were applied for the assay of the drug substance in its commercial formulation. The results were statistically compared with official method using t- and f- tests at $p < 0.05$. The intra and inter-assay coefficients of variation were less than 6 % and the recoveries ranged from 94.0 to 102.5 %.

Key Words: Diiodohydroxyquinoline, Spectrophotometry, Atomic absorption spectrometry, Potassium ferricyanide, 1,10-Phenanthroline, Prussian blue.

INTRODUCTION

Diiodohydroxyquinoline (iodoquinol, Fig. 1) is widely used as anti-amebic agent[1]. Several methods were reported for the quantitative determination of diiodohydroxyquinoline including colorimetry[2-7], cyclic voltammetry[8], gravimetry[9], differential thermal analysis[10], microbiology[11] and AAS[12] methods.

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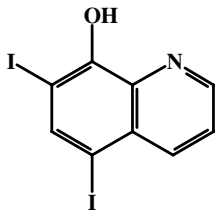

Fig. 1. Diiodohydroxyquinoline

The reduction of iron(III) by some certain drugs such as paracetamol[13], diclofenac sodium[14], salbutamol sulfate[15], captopril[16], amoxicillin and ciprofloxacin[17] was used to determine these drugs by detecting the produced iron(II) colorimetrically.

According to our knowledge, diiodohydroxyquinoline has not been investigated before basing on the oxidation with iron(III). In the present study, three methods were proposed for the determination of diiodohydroxyquinoline by spectrophotometric and AAS techniques. Two proposed spectrophotometric methods based on the reduction of iron(III) by diiodohydroxyquinoline to iron(II) which reacted with potassium ferricyanide to form Prussian blue colour (method I) or with 1,10-phenanthroline to form red colour complex (method II). The method is simple since its a single step procedure and low costing without loss of accuracy.

The atomic absorption measurement [method (III)] is accomplished, after suitable extraction of the excess iron(III) into diethyl ether[18] from 6 M hydrochloric acid, probably as the solvated complex [$H_3O(R_2O)^+$, $FeCl_4^-$] and then iron(II) in aqueous layer was determined by AAS. Therefore the aim of the present work is to develop a more sensitive, simple and rapid methods compared to USP method (Oxygen Flask Combustion)[19] which are suitable for application in quality control laboratories.

EXPERIMENTAL

Atomic absorption measurements were performed using A Perkin-Elmer AAS, Model A, Analyst 100 spectrophotometer equipped with an iron hollow-cathode lamp, under the following condition: 302.1 nm wavelength, 30.0 mA lamp current, 0.2 nm slit width and 3.5:1.5 air: acetylene ratio.

A UV/Visible spectrophotometer SPECTRO 23 labomed Inc USA, with 1 cm quartz cell, was used for the absorbance measurements. A Hanna pH meter was used.

All chemicals used were of analytical reagent or chemically pure grade and deionized water was used through out the study. Pharmaceutical grade drug substance was obtained from the Middle East Pharmaceuticals and Cosmetics Lab., Palestine. Pharmaceutical formulation of diiodohydroxy-

quinoline was obtained commercially. The formulation contains only one drug content and not in combination with other drug. Other chemicals were supplied as the following: hydrochloric acid 32 % (BDH), diethyl ether (Carlo Erba), ferric sulfate (Sigma)

A standard stock solution of diiodohydroxyquinoline was prepared in 0.1 M HCl to make the final concentration (10 μg mL^{-1}, 2.5×10^{-5} M). Ferric sulfate (40 μg mL^{-1}, 1×10^{-4} M) was also prepared in 0.1 M HCl. The 1,10-phenanthroline (1×10^{-2} M) was prepared by dissolving 0.04 g in 20 mL 10 % ethanol. Potassium ferricyanide 1×10^{-3} M was used. A buffer solution of pH 4 was prepared by adding 15 mL of 1.0 M NaOH to 85 mL of 1.0 M acetic acid.

Procedure for determination of diiodohydroxyquinoline: Method (I): Six 2.5 mL aliquots of the standard solution (2-20 μg mL^{-1}) were transferred into a series of 10 mL calibrated flasks. To each flask, 1.5 mL of 1×10^{-4} M ferric sulfate was added. Mixtures were heated using a boiling water bath at 100 ºC for 10 min and after cooling, 1.0 mL of potassium ferricyanide was added and diluted with water. The absorbance was measured at λ 700 nm after 24 min[16].

Method (II): Six 2.5 mL aliquots of the standard solution (1-10 μg mL^{-1}) were transferred into a series of 10 mL volumetric flasks. To each flask, 1.5 mL of 1×10^{-4} M Ferric sulfate was added. The mixtures were heated using a boiling water bath at 100 ºC for 10 min. After cooling, 1.0 mL of 1,10-phenanthroline was added and diluted with acetate buffer. The absorbance was measured at λ 515 nm after 3 min[17].

AAS Method (III): Six 2.5 mL aliquots of the standard solution (0.2-2.0 μg mL^{-1}) were transferred into a series of 10 mL volumetric flasks. To each flask, 1.5 mL of 1×10^{-5} M ferric sulfate was added. The mixtures were heated using a boiling water bath at 100 ºC for 10 min. After cooling, 4.0 mL of 12 M HCl was added and the excess iron(III) was extracted with three portions of 10 mL of diethyl ether in a separatory funnel. Then the aqueous layer was aspirated into the air-acetylene flame. The absorbance of iron was measured at 302 nm and the drug concentration was determined from a calibration curve, previously constructed.

RESULTS AND DISCUSSION

At room temperature, diiodohydroxyquinoline is considered a weak reducing agent and the oxidation process is not quantitative because the reaction is slow and required longer time for completion. However after using the boiling water bath at 100 ºC, diiodohydroxyquinoline immediately reduced iron(III) to iron(II). The amount of iron(II) was determined using the proposed methods which correspond to the drug concentrations.

Response characteristics of the proposed methods: Typical calibration curves were constructed for the three proposed methods based on linear regression analysis of absorbance *versus* concentration. Linear relations were obtained between drug concentration and absorbance values in the three methods. The analytical characteristics of the three methods are shown in Table-1.

TABLE-1
ANALYTICAL CHARACTERISTICS OF THE THREE DIFFERENT METHODS

Parameter	Method I	Method II	AAS Method
λ (nm)	700	515	302
pH	1	4	6.0 M HCl
Molar absorptivity (L $mol^{-1}cm^{-1}$)	2.005×10^4	1.667×10^4	–
LOD (μg mL^{-1})	0.2	0.17	0.07
Linearity (μg mL^{-1})	2.0-20.0	1.0-10.0	0.2-2.0
RSD (n =5)	< 6	< 6	< 6
Regression:			
Slope	0.0480	0.040	0.1575
Intercept	0.006	0.027	-0.002
Correlation coefficient (R)	0.9998	0.9965	0.9985

The determination of diiodohydroxyquinoline as raw material and in drug formulations was achieved using method I and II and AAS method. The results summarized in Table-2 indicated a high recovery of diiodohydroxyquinoline as raw material and in the drug formulation (iodoquinol tablets). The results obtained by the three proposed methods demonstrated high accuracy and precision as the official as found through t- and f- statistical tests ($p < 0.05$). The intraday, interday analysis of precisions and recovery are summarized in Tables 3-5. These data indicated that the three proposed methods were reproducible within and between days. The mean percentage recovery ranged from 94.0 to 102.5 % with RSD values less than 6 %.

Effect of temperature: The effect of temperature was optimized by subjecting the reaction to a water bath at different temperatures and constant heating time of 15 min. The absorbance was found to increase while increasing the reaction temperature. The temperature 100 ºC gave the maximum absorbance as shown in Fig. 2. Time of heating is also important factor to ensure a complete reaction. Different heating time intervals were investigated at constant optimum temperature (100 ºC); 10 min was found to be enough and suitable for the reaction completion as shown in Fig. 3.

TABLE-2
ASSAY OF RAW MATERIAL AND COMMERCIAL TABLET FROMULATIONS BY THREE PROPOSED METHODS

Sample	Parameters	Spectrophotometric		AAS	Official
		Method I	Method II	method III	method[1]
Raw material	Recovery (%) ± SD[a]	99.2 ± 1.2	98.9 ± 1.4	100.4 ± 2.7	100.6± 3.1
	t-test	2.1	1.9	1.6	-
	F-test	3.4	3.1	3.7	-
Iodoquinol tablet	Recovery (%) ± SD[a]	101.7 ± 1.7	99.8 ± 2.1	102.1 ± 2.3	101.7 ± 2.8
	t-test	1.5	1.1	0.9	-
	F-test	2.2	2.7	1.6	-

[a] An average of five determinations; t_{tab} (n=5) = 2.776; F_{tab} (5, 5) = 6.39.

TABLE-3
INTRADAY AND INTERDAY ASSAY BY METHOD I

Concentration (μg mL^{-1})	Found ± SD[a]	Recovery (%)	SAE[b]	RSD (%)	CL[c]
		Intraday assay			
4.00	4.1 ± 0.12	102.5	0.054	2.9	4.1 ± 0.149
12.0	11.9 ± 0.41	99.2	0.183	3.4	11.9 ± 0.509
20.0	19.7 ± 0.73	98.5	0.326	3.7	19.7 ± 0.906
		Interday assay			
4.00	3.9 ± 0.22	97.5	0.098	5.6	3.9 ± 0.273
12.0	11.8 ± 0.55	98.3	0.246	4.7	11.8 ± 0.683
20.0	19.4 ± 1.1	97.0	0.492	5.7	19.4 ± 1.366

[a]An average of five determination; [b]SAE, standard analytical error;
[c]Confidence limits (CL), 95 % and 4 degree of freedom.

TABLE-4
INTRADAY AND INTERDAY ASSAY BY METHOD II

Concentration (μg mL^{-1})	Found ± SD[a]	Recovery (%)	SAE[b]	RSD (%)	CL[c]
		Intraday assay			
2.00	1.9 ± 0.11	95.0	0.045	5.5	1.9 ± 0.124
6.00	6.1 ± 0.25	101.6	0.111	4.1	6.1 ± 0.310
10.0	9.4 ± 0.49	94.0	0.219	5.2	9.4 ± 0.608
		Interday assay			
2.00	1.9 ± 0.10	95.0	0.045	5.3	1.9 ± 0.124
6.00	6.0 ± 0.30	100.0	0.134	5.0	6.0 ± 0.372
10.0	9.5 ± 0.42	95.0	0.188	4.4	9.5 ± 0.521

[a]An average of five determination; [b]SAE, standard analytical error;
[c]Confidence limits (CL), 95% and 4 degree of freedom.

TABLE-5
INTRADAY AND INTERDAY ASSAY BY AAS METHOD III

Concentration ($\mu g\ mL^{-1}$)	Intraday assay				
	Found ± SD[a]	Recovery (%)	SAE[b]	RSD (%)	CL[c]
0.4	0.40 ± 0.02	100.0	0.009	5.0	0.40 ± 0.025
1.20	1.21 ± 0.04	100.8	0.018	3.3	1.21 ± 0.050
2.00	2.02 ± 0.09	101.0	0.040	4.5	2.02 ± 0.111
	Interday assay				
0.4	0.39 ± 0.02	97.5	0.009	5.1	0.39 ± 0.025
1.20	1.20 ± 0.05	100.0	0.022	4.2	1.20 ± 0.062
2.00	2.04 ± 0.15	102	0.051	5.6	2.04 ± 0.142

[a]An average of five determination; [b]SAE, standard analytical error;
[c]Confidence limits (CL), 95% and 4 degrees of freedom.

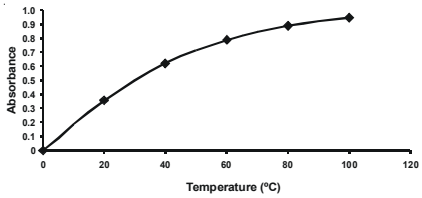

Fig. 2. Effect of temperature on oxidation reaction; 20 µg mL; pH 1; heating time (15 min)

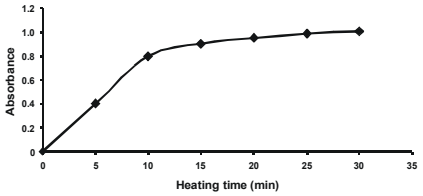

Fig. 3. Effect of heating time on oxidation reaction; 20 µg mL; pH 1; temp. (100 ºC)

Effect of pH: The effect of the pH on the reaction was studied by changing the pH values from 1-4 through the addition of different volumes of HCl. The optimum temperature 100 ºC and the suitable heating time interval of 10 min were kept steady through the pH study. Constant and maximum absorbance values were obtained at the pH = 1 as shown in Fig. 4.

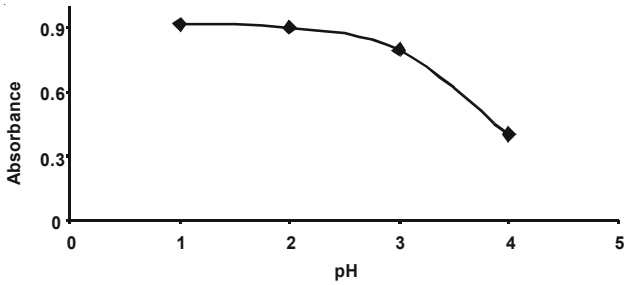

Fig. 4. Effect of pH on oxidation reaction; 20 µg mL; Heating time (10 min); temperature (100 ºC)

Iron(III)/drug mole ratio: To study the stoichiometry of iron(III)/ drug mole ration in the reaction, the three methods were investigated using 2.5 mL aliquot of drug solution 5×10^{-5} M, 2.5×10^{-5} M and 5×10^{-6} M for method I, method II and AAS method, respectively where several increments of iron(III) were added. The maximum absorbance was observed with two fold iron(III) of diiodohydroxyquinoline (Fig. 5). A 1.5 mL of 1×10^{-4} M and 1.5 mL of 1×10^{-5} M of ferric sulfate were used to get a constant and maximum absorbance for the spectrophotometric and AAS methods, respectively.

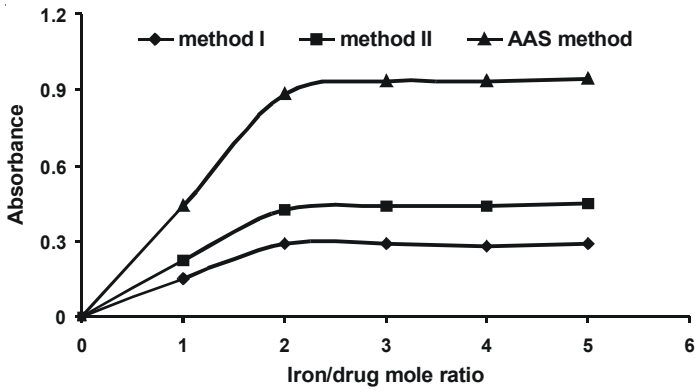

Fig. 5. Effect of iron(III) concentration on oxidation reaction

Extraction efficiency for AAS method: Atomic absorbance measurements were recorded after the extraction of Fe-drug complex. Four different solvents, 1,2-dichloroethane, chloroform, acetone and ether were tested to run an efficient extraction. Diethyl ether was the only solvent which has the ability to extract only iron(III) excluding iron(II). The amount of metal extracted depends upon the concentration of the acid where the maximum absorbance was observed at about 6.0 M HCl[18].

Standard aliquots of iron(III) were dissolved in 6.0 M HCl solutions. Each sample was extracted with three portions of 25 mL diethyl ether. The unreacted iron(III) in the aqueous layer was determined using KSCN at 480 nm by spectrophotometric methods or by AAS method at 302 nm. The overall extraction efficiency was found to be 100 and 99.99 % based on spectrophotometry or AAS methods, respectively.

Effect of interferences: The effect of the presence of common excipients (dextrose, glucose, saccharine sodium, starch talc and magnesium stearate) were evaluated. There were no significant interferences occurred in the determination of diiodohydroxyquinoline due to the presence of these excipients. Most of drugs (ascorbic acid, caffeine, salbutamol, diclofenac

sodium, amoxicillin, furazolidone and ciprofloxacin) were oxidized by iron(III) and therefore diiodohydroxyquinoline should be extracted and separated before applying these methods.

Conclusion

The proposed methods are simple, highly sensitive and more over are rapid since its a single step procedure. The methods can be used for routine analysis of diiodohydroxyquinoline drug as raw materials and in pharmaceutical formulations. No interferences were occurred from the excipients found normally in the commercial drug preparations. The statistical parameters and recovery tests data clearly indicate the reproducibility and accuracy of the proposed methods.

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