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Spectrophotometric determination of chloroquine and pyrimethamine through ion-pair formation with molybdenum and thiocyanate

Shaban M. Khalil[b], G.G. Mohamed[a], M.A. Zayed[a,*], H.M. Elqudaby

[a] *Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt*
[b] *National Organization of Drug Control and Research, Giza, Egypt*

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Abstract

A sensitive spectrophotometric method is developed for the determination of some antimalarian drugs such as chloroquine (CQ) and pyrimethamine (PYM). The method involves the formation of ion-pairs between the two drugs under investigation and inorganic complexes of Molybdenum(V)-thiocyanate followed by its extraction with methylene chloride. The optimum conditions for the ion-pairs formation are established. The method permits the determination of chloroquine and pyrimethamine over a concentration range of 2.0–42.0 and 2.0–43.0 $\mu g\ ml^{-1}$, respectively. The Sandell sensitivity is found to be 0.027 and 0.042 $\mu g\ cm^{-2}$ for chloroquine and pyrimethamine, respectively. The method was simple, rapid, reproducible and accurate within $\pm 1\%$. The method is applicable to the assay of the two drugs under investigation in different dosage forms and the results are in good agreement with those obtained by the official method.

Keywords: Extraction spectrophotometry; Chloroquine and pyrimethamine determination; Molybdenum(V)-thiocyanate; Pharmaceutical preparations

1. Introduction

Chloroquine and pyrimethamine are still used for the treatment of malaria, but in areas where there is no chloroquine or pyrimethamine resistance. Various spectrophotometric methods [1–6] were used for the determination of chloroquine and pyrimethamine in different pharmaceutical preparations using different reagents. Chloroquine was determined spectrophotometrically using UV derivative spectrophotometry [7] or colourimetric and atomic absorption spectropho-

* Corresponding author.
E-mail address: mazayed@chem-sci.cairo.eun.eg (M.A. Zayed)

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tometry [8]. A chloroquine membrane electrode was developed for its determination in pharmaceutical preparations [9]. A high performance liquid chromatography method was used for the determination of chloroquine and pyrimethamine in plasma [10], in antimalareous areas [11] or in Chinese men [12].

This work was undertaken in order to study the analytical aspects of the reaction between the drugs under investigation (chloroquine and pyrimethamine) with molybdenum(V)-thiocyanate. The structures of the formed ion-pairs are proposed. This study also aims to test the sensitivity, accuracy and selectivity of the ion-pair formation method and to use this method for the spectrophotometric determination of chloroquine and pyrimethamine in pure forms and in different pharmaceutical preparations.

2. Experimental

2.1. Materials and solutions

All reagents were of analytical-reagent grade and used without further purification. The water used was always doubly distilled.

CQ was supplied from Egypt Cid Company, while PYM was supplied from Egyptian Company for Chemicals and Pharmaceuticals. Their solutions (80 μg ml^{-1}) were freshly prepared by dissolving CQ in bidistilled water and PYM in ethanol. The solutions were kept at 4°C, in PVC containers, in the refrigerator. Other solutions were prepared by appropriate dilution.

Stock Mo(VI) solution (2.0% w/v) was prepared from AR grade ammonium molybdate in doubly distilled water containing a few drops of ammonia and standardized gravimetrically using 8-hydroxyquinoline [13]. The working solution was prepared by suitable dilution of the standardized stock solution.

Ammonium thiocyanate and ascorbic acid solutions (10% each) were prepared in doubly distilled water.

2.2. Apparatus

A Perkin–Elmer spectrophotometer Model 601 with matched quartz cells of 1.0-cm optical path length was used for spectrophotometric measurements at the wavelength range of 350–600 nm.

2.3. Procedure

2.3.1. Determination of chloroquine (CQ) and pyrimethamine (PYM)

Four milliliters of 80 μg ml^{-1} of ammonium molybdate, 2.0 ml of concentrated HCl, 5.0 ml of 10% each of ammonium thiocyanate and ascorbic acid were placed in a 50-ml capacity separating funnel. The mixture was left for 15 min at room temperature (20 $\pm$ 5°C). Different volumes of 200 μg ml^{-1} of CQ and PYM solutions (0.1–4.8 ml) were added and diluted with bidistilled water up to 20 ml. After another 15 min, 10 ml of methylene chloride was added (twice with 5-ml portions), the mixture was shaken well for 1 min and allowed to stand to separate into two phases. The methylene chloride extract was dried over anhydrous sodium sulfate and the absorbance of the filtered extract was measured at 467 and 471 nm for CQ and PYM, respectively, against a reagent as a blank, which was prepared similarly without the drugs.

2.4. Procedure for the tablets

The content of 20 tablets of both drugs under investigation was weighed and ground into a fine powder. A mass of powder containing approximately 100 mg of the drugs was weighed accurately, dissolved in bidistilled water for CQ and in ethanol for PYM, filtered through a Whatman no. 41 filter paper and washed with the suitable solvent. Then, the filtrate plus washings were diluted to 100 ml with distilled water or ethanol in a calibrated flask. An aliquot was used for the determination of each drug according to the procedure mentioned above.

3. Results and discussion

This work was undertaken from the point of view that ion-pairs are formed between the tertiary amine group of CQ and PYM drugs and Mo(V)-thiocyanate binary complex via the protonated nitrogen atom of both drugs [14]. Molybdenum(V), formed by the reduction of Mo(VI) with ascorbic acid, combines with ammonium thiocyanate to form a red Mo(V)-thiocyanate binary complex in hydrochloric acid solutions. On adding CQ or PYM solution, orange–red ion-pairs are formed in the same acid concentration. The ion-pair is soluble in methylene chloride while Mo(V)-thiocyanate binary complex is insoluble. A double extraction is necessary to extract the ion-pairs quantitatively into the organic phase. The absorption spectra of the ion-pairs, extracted in methylene chloride, show a maximum at 467 and 471 nm for CQ and PYM, respectively, against a reagent blank.

It was found that, the reduction probability of Mo(VI) to Mo(V) may occurs by ascorbic acid or SCN^- in acidic media [14]. However, the rapidity, sensitivity and stability of Mo(V)-thiocyanate binary complex are enhanced by using ascorbic acid. Ascorbic acid gives reproducible values and masks many interfering ions [16]. From the data shown in Fig. 1a, it is found that 3.0–5.0 ml of 10% ascorbic acid is sufficient for the reduction of 80 μg Mo(VI) to Mo(V). The addition of excess amount of ascorbic acid, more than the required volume, has no effect on the absorbance of the ion-pairs formed.

It was found that the ion-pairs were formed only in hydrochloric, sulfuric, nitric or phosphoric acid medium, but the absorbance readings of the methylene chloride extract from 1.5 to 5 M hydrochloric acid is the maximum one [15]. Hence, hydrochloric acid medium has been selected as the suitable medium for ion-pair extraction. It was found that, 10.0 ml of 2.0 M hydrochloric acid is suitable for the formation of Mo(V) thiocyanate-CQ and Mo(V)-thiocyanate-PYM ion-pairs.

The effect of ammonium molybdate on the ion-pairs formation and their extraction in methylene chloride is shown in Fig. 1b. The data show

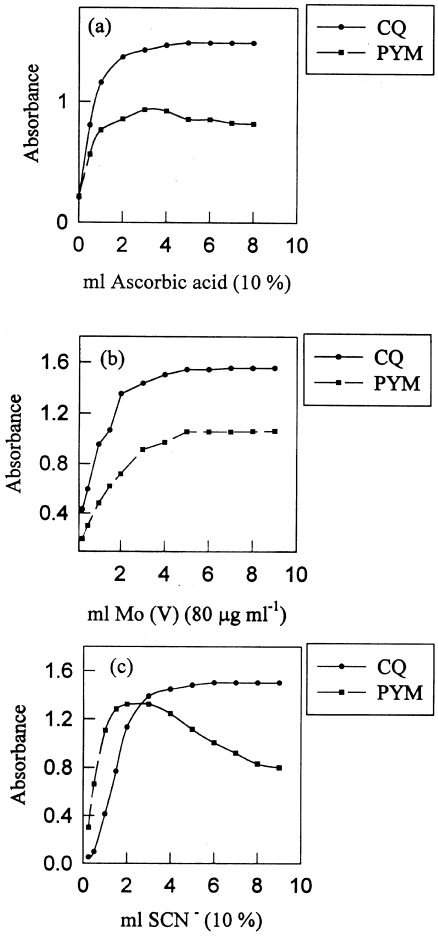


Fig. 1. The effect of variable concentrations, of different reactants, on the reaction products of the ternary complexes: (a) effect of ascorbic acid; (b) effect of Mo(V); and (c) effect of SCN^-.

that 3.0–4.0 ml of 2.0% (w/v) ammonium molybdate is required for maximum absorbance in a final volume of 20 ml aqueous solution and in the presence of 80.0 and 40.0 μg for CQ and PYM, respectively. After this, the absorbance was nearly constant. Also, it was found that 2.0 and 5.0 ml of 10% ammonium thiocyanate in a final solution of 20 ml gave the maximum pronounced effect on the absorbance in the determination of PYM and CQ, respectively (Fig. 1c).

In this method, the complete formation of the ion-pairs needs 15.0 min before extraction with methylene chloride at 25°C. The absorbance of

Table 1
Between-day precision of the determination of Mo(V)-ion pairs by the proposed method

Compound	W taken ($\mu g\ ml^{-1}$)	W found ($\mu g\ ml^{-1}$)	Percentage recovery (%)	S.D.	R.S.D.[a]
Chloroquine	10.0	9.960	99.60	0.086	0.80
	20.0	20.125	100.6	0.065	0.18
	25.0	25.165	100.6	0.085	0.19
	30.0	30.160	100.5	0.037	0.11
Pyrimethamine	10.0	10.083	100.8	0.089	0.860
	20.0	20.165	100.8	0.037	0.324
	25.0	25.14	100.6	0.049	0.340
	30.0	30.215	100.7	0.034	0.120

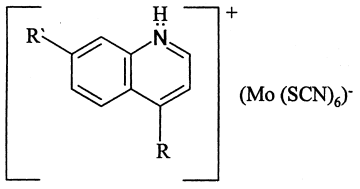
[a] The average of five replicates.

Mo(V)-thiocyanate binary complex is stable after 15.0 min while Mo(V)-thiocyanate-drugs ion-pairs need another 15.0 min for their complete formation.

Solvents like acetone, pentanol, *n*-butyl and ethyl alcohol, dioxane, acetone, dimethylformamide, carbon tetrachloride, diethyl ether and petroleum ether cannot be used for the extraction of the formed ion-pairs while methylene chloride and dichloroethane extract these ion-pairs quantitatively. The molar absorptivity values for the ion-pairs in methylene chloride and dichloroethane are 6164 and 6128 $l\ mol^{-1}\ cm^{-1}$ at $\lambda = 467$ nm for CQ, and 5802 and 5796 $l\ mol^{-1}\ cm^{-1}$ at $\lambda = 471$ nm for PYM, which is why they are selected as a suitable medium for extraction. Reproducible absorbance readings were obtained after either a single or double extraction with 10.0 ml of methylene chloride and 1-min shaking time. The studied ion-pairs are stable for more than 1 week at 25°C in the organic solvents.

In order to prove the validity and the applicability of the proposed method and the reproducibility of the results obtained, five replicate experiments at four concentrations of CQ and PYM were carried out. Table 1 shows the values of the between-day relative standard deviations for different concentrations of the drugs, obtained from experiments carried out over a period of 4 days. It was found that the within-day relative standard deviations were less than 1.0%, which indicates that the proposed method is highly reproducible and Mo(V)-thiocyanate binary complex is successfully applied to determine CQ and PYM via the formation of ion-pairs.

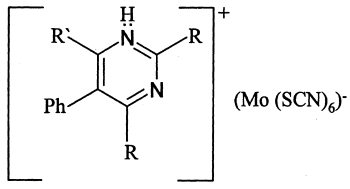
The stoichiometry of the Mo(V) to each drug in the presence of excess amounts of ammonium thiocyanate was determined by the continuous variation method. The results indicate that 1:1 (metal/drug) ion-pairs are formed through the electrostatic attraction between positive protonated CQH^+, $PYMH^+$ and thiocyanate negative



R = $NHCH(CH_3)CH_2N(C_2H_5)_2$, R` = Cl

$Mo(SCN)_6$-CQH^+ ion pair

(I)



R = NH_2, R` = C_2H_5

$Mo(SCN)_6$-$PYMH^+$ ion pair

(II)

Table 2
Analytical parameters for the determination of chloroquine and pyrimethamine by the proposed method

Drug	λ_{max} (nm)	Conc. range ($\mu g\ ml^{-1}$)	ε ($l\ mol^{-1}\ cm^{-1}$)	Sandell sensitivity ($\mu g\ cm^{-2}$)	$A = mc + z$		S.D.	R.S.D. (%)
					m	z		
Chloroquine	467	2.0–42.0	6.82×10^3	0.027	1.0196	−0.047	0.04 − 0.049	0.17–0.39
Pyrimethamine	471	2.0–43.0	10.53×10^3	0.042	1.0152	0.095	0.038–0.046	0.184–0.39

Table 3
Determination of chloroquine and pyrimethamine in pharmaceutical preparations[a]

Drug	Name of preparation	Recovery ± S.D. (%) ($n = 5$)		t-test	F-test
		Proposed method	Official method		
Chloroquine	Cidoquine	9.92	9.95	2.530	1.24
	Dagrenol	10.14	10.11	2.670	1.70
	Alexoquine	20.00	19.96	2.638	1.97
	Chloroquine phosphate ampule	23.95	23.99	2.130	1.08
Pyrimethamine	Daraprim	15.12	15.10	2.40	1.15

[a] Cidoquine tablets (250 mg), Cid Co. for Pharmaceuticals. Dagrenol tablets (250 mg), The Alexandria Co. for Pharmaceuticals, Egypt. Alexoquine tablets (250 mg), The Alexandria Co. for Pharmaceuticals, Egypt. Chloroquine phosphate ampule (250 mg), The Alexandria Co. for Pharmaceuticals, Egypt. Daraprim tablets (25 mg), The Wellcome Foundation Ltd, London, England.

complex as shown by the proposed structures I and II, respectively.

4. Interferences

Interferents are mainly basic compounds that contain heteronitrogen atoms in their aromatic nuclei. However, such compounds are not usually present with the examined antimalarials in pharmaceutical preparations and hence are not likely to cause analytical problems. On the other hand, tablet fillers such as lactose, starch and stearic acid and preservations and bacteriostatics used in parenteral preparations, which can represent a potential source of interference in other methods, do not interfere in the proposed method.

Under the optimum conditions described above, the calibration graphs were constructed for both drugs. The molar absorptivity, Sandell sensitivity and regression equation for each drug was tabulated in Table 2. The correlation coefficients of the data obtained are 0.996 and 0.997 for CQ and PYM, respectively. The standard deviations are found to be 0.40–0.049 and 0.038–0.04 and the relative standard deviations are 0.17–0.39 and 0.184–0.39 for CQ and PYM, respectively, for five replicate determinations. The low values of the relative standard deviations indicate the high accuracy, reproducibility and sensitivity of the method.

The proposed method is successfully applied to the determination of CQ and PYM in different pharmaceutical preparations and the results obtained are given in Table 3. From the calculated t- and F-values, it is clear that the results obtained by the proposed method are in good agreement with those obtained by the official method [17].

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