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Spectrophotometric Determination of Amodiaquine Hydrochloride in Pharmaceutical Dosage Forms

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A simple, sensitive and selective spectrophotometric method has been developed for the determination of amodiaquine hydrochloride and its dosage forms, which is based on a colour reaction with chloramine-T in the pH range 7.4–8.0. The chromogen, after extraction into chloroform, is measured at 442 nm. Beer's law is obeyed in the concentration range 0–200 μg ml^{-1} of amodiaquine hydrochloride. The identity of the chromogen is deduced from the infrared and nuclear magnetic resonance spectral data.

Keywords: *Spectrophotometry*; *amodiaquine hydrochloride*; *pharmaceutical preparations*; *chloramine-T*; *chloroform extraction*

Amodiaquine hydrochloride is an official drug in the BP,[1] PI[2] and USP.[3] Attenuated total reflectance infrared spectroscopy[4] has been used to detect amodiaquine hydrochloride in capsules or tablets. It has also been reported that the formulation of amodiaquine hydrochloride with primaquine phosphate has better efficacy as an antimalarial than the individual formulations.[5] Primaquine phosphate can be determined[6] in the presence of amodiaquine hydrochloride using 1,2-naphthaquinone-4-sulphonic acid or 2,6-dichloroquinone chlorimide. A spectrophotometric method using an acid dye technique[7] is also available for the determination of amodiaquine hydrochloride in drug and dosage forms; however, no method for amodiaquine determination has been reported when present in combination with primaquine phosphate. Berthelot and Michel[8] reported the colour reactions of phenolic substances with chloramine-T. The reaction of chloramine-T with phenol can still be utilised in the presence of ammonia[9] and copper.[10] A spectrophotometric method making use of the reaction of chloramine-T with amodiaquine hydrochloride is reported in this paper.

Experimental

Apparatus

A Unicam SP1700 ultraviolet spectrophotometer and 10-mm cells were used.

Reagents

Amodiaquine hydrochloride solution, 1000 p.p.m. Weigh out 500.0 mg of amodiaquine hydrochloride (99.5% pure), dissolve them in water and dilute the solution to volume in a 500-ml calibrated flask.

Chloramine-T solution, 0.05 M. Weigh out 7.5 g of purified[11] chloramine-T (BDH Chemicals Ltd., Poole, Dorset), dissolve them in water and dilute to 500 ml.

Buffer solution. Britton - Robinson buffer, pH 7.6, was prepared by adding 0.2 M sodium hydroxide solution to a mixed acid solution 0.04 M with respect to phosphoric acid and 0.04 M with respect to boric and acetic acids, with the help of a pH meter.

Solvents. Chloroform, carbon tetrachloride, methanol, amyl acetate, butyl acetate and 4-methylpentan-2-one were all BDH laboratory-reagent grade. The solvents were distilled before use.

Procedure

Preparation of standard graph

Place 0.5-, 1.0-, 2.0-, 4.0-, 6.0,- 8.0- and 10.0-ml portions of amodiaquine hydrochloride solution in individual 250-ml separating funnels, then add 5 ml of buffer solution, 5 ml of chloramine-T reagent and dilute each to 50 ml. Extract with four portions of chloroform (10 ml) after thorough mixing, and dry the extracts over anhydrous sodium sulphate. Dilute

to 50 ml in calibrated flasks with chloroform and measure the absorbance at 442 nm against a reagent blank.

A graph of absorbance against amodiaquine hydrochloride concentration was a straight line over the range 0–200 μg ml^{-1} and passed through the origin.

Tablets

Tablets of amodiaquine hydrochloride and composite tablets of amodiaquine hydrochloride with primaquine phosphate were analysed. Transfer tablet powder equivalent to 100.0 mg of amodiaquine hydrochloride into a 100-ml calibrated flask, shake for 10 min with 50 ml of water, filter the extract into a 100-ml calibrated flask and dilute it to volume with water. Take 4.0 ml of this solution, extract the filtrate as described under *Preparation of standard graph* and determine the amodiaquine hydrochloride content from the standard graph, again as described under *Preparation of standard graph*. The results of some commercial batches are shown in Table I.

TABLE I

APPLICATION OF THE PROPOSED METHOD TO THE DETERMINATION OF AMODIAQUINE HYDROCHLORIDE IN DOSAGE FORMS

Code No.*	Amodiaquine hydrochloride content/mg		Recovery of standard additions of amodiaquine hydrochloride	
	BP method	Proposed method	Added/mg per tablet	Recovery, %
A	244.5	246.2	60	102.2
B	250.2	248.4	60	101.8
C	236.5	234.8	60	101.6
D	250.7	249.7	60	102.5
E	242.0	239.6	60	100.3
A_1	—	154.2	60	102.0
B_1	—	153.7	60	102.5
C_1	—	152.5	60	102.0
D_1	—	150.9	60	102.2
E_1	—	155.0	60	102.5

* A–E, 240 mg per tablet; A_1–E_1, 150 mg per tablet of amodiaquine hydrochloride + 15 mg of primaquine.

Results and Discussion

Selection of pH and Extractant

Several experiments were carried out to study the influence of pH on the colour development and the results are shown in Fig. 1. The optimum pH range for colour development is between 7.4 and 8.0. Above pH 8.0 the absorbance decreases.

Keeping all other parameters constant, different solvents (chloroform, carbon tetra-

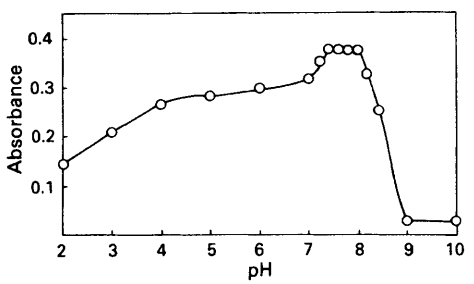

Fig. 1. Influence of pH on the reaction of amodiaquine hydrochloride with chloramine-T. Amodiaquine hydrochloride concentration, 80 μg ml^{-1}.

chloride, butyl acetate, amyl acetate and 4-methylpentan-2-one) were tried as extractants and the results are plotted in Fig. 2. The absorption maxima and absorbances were influenced by the solvent (Fig. 2) and chloroform and carbon tetrachloride were found to be suitable solvents. Chloroform was utilised as the extractant by us.

Further, a series of experiments revealed that the variation of volume of buffer (up to 10 ml) had no effect on the colour development. Any variation in the volume of the aqueous phase between 20 and 70 ml also did not affect the determination.

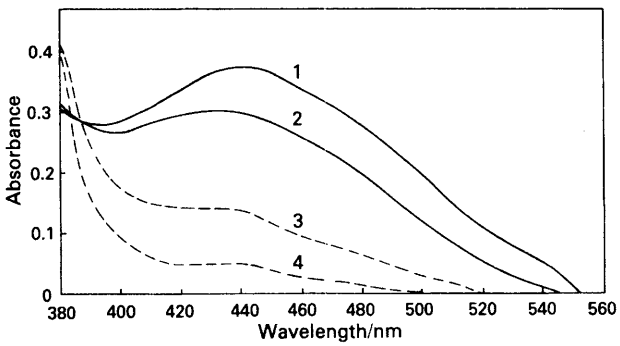

Fig. 2. Effect of different organic solvents on the absorption maximum and absorbance. Amodiaquine hydrochloride concentration, 80 μg ml^{-1}; pH = 7.6. 1, Chloroform and carbon tetrachloride; 2, 4-methylpentan-2-one; 3, amyl acetate; and 4, butyl acetate.

Effect of Reagent Concentration

Different volumes of reagent (0.5, 1, 2, 3, 4, 5, 6, 8 and 10 ml) were added to a constant amount (4.0 mg) of amodiaquine hydrochloride and the results of the observations are plotted in Fig. 3. From Fig. 3 it is evident that the minimum molar ratio of amodiaquine hydrochloride to chloramine-T should be 1:8 for completion of the reaction.

Effect of Order of Addition of Reagents

After fixing all of the other parameters, a few further experiments were carried out to ascertain the influence of order of addition of reagents on the determination of amodiaquine hydrochloride and the results are presented in Table II. The order of addition at serial numbers 1, 2 or 3 is recommended.

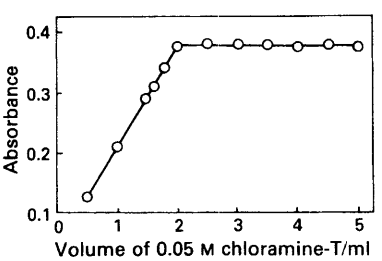

Fig. 3. Effect of reagent concentration on the reaction of amodiaquine hydrochloride with chloramine-T. Amodiaquine hydrochloride concentration, 80 μg ml^{-1}; pH = 7.6.

TABLE II

EFFECT OF ORDER OF ADDITION OF THE REAGENTS ON THE COLOUR DEVELOPMENT

Serial No.	Order of addition	Absorbance
1	Amodiaquine hydrochloride + buffer + chloramine-T	0.382
2	Buffer + amodiaquine hydrochloride + chloramine-T	0.381
3	Chloramine-T + buffer + amodiaquine hydrochloride	0.382
4	Buffer + chloramine-T + amodiaquine hydrochloride	0.373
5	Amodiaquine hydrochloride + chloramine-T + buffer	0.321
6	Chloramine-T + amodiaquine hydrochloride + buffer	0.330

Interferences

The commonly used tablet excipients, such as starch, talc and magnesium stearate, did not interfere with the present method.

Effect of Chloroquine Phosphate or Primaquine Phosphate

It was further observed that neither chloroquine phosphate nor primaquine phosphate interfere in this method of determining amodiaquine hydrochloride (Table III).

TABLE III

EFFECT OF CHLOROQUINE PHOSPHATE AND PRIMAQUINE PHOSPHATE

Absorbance without chloroquine phosphate or primaquine phosphate = 0.382 (amodiaquine hydrochloride 80 μg).

Drug	Amount added/mg	Absorbance
Chloroquine phosphate	2.0	0.383
	4.0	0.383
	6.0	0.382
	8.0	0.384
	10.0	0.382
Primaquine phosphate	0.1	0.382
	0.2	0.383
	0.4	0.381
	0.6	0.382
	0.8	0.386

Precision and Accuracy

The precision of the present method was evaluated by determining the same amount (80 μg ml^{-1}) of amodiaquine hydrochloride in ten experiments. The coefficient of variation was found to be 0.64%. Further, from the recovery studies (Table I) it was observed that recoveries ranged between 100.3 and 102.5%.

Stability and Sensitivity

The colour of the chloroform extract was stable for more than 4 h. The Sandell sensitivity and molar extinction coefficient were 0.2105 μg cm^{-2} and 2036 l mol^{-1} cm^{-1}, respectively.

Nature of the Product

The chloroform extract, after stripping off the solvent, left dark red crystals. Thin-layer chromatographic separation over silica gel G (eluent: methanol - chloroform, 7 + 3) revealed the presence of two products. The less polar component was identified as *p*-toluenesulphonamide by its mixed melting-point and superimposable infrared spectrum.

The more polar component, purified by preparative thin-layer chromatography (R_F 0.81; solvent, methanol - chloroform, 7 + 3), had a melting-point of 110 °C, an empirical formula of $C_{27}H_{29}Cl.N_4O_3S$ and absorbed at ν_{KBr} 3350 cm^{-1} (>NH), 1340 cm^{-1} and 1160 cm^{-1} (sulphonamide). The chloroformic solution of the component was found to show maximum absorbance in the range 440–444 nm, as did the original chloroform extract.

The nuclear magnetic resonance (NMR) spectrum (90 MHz in $CDCl_3$) of the product indicated the presence of a $-CH_2N(C_2H_5)_2$ group [δ 1.33 (t), 6H; δ 3.25 (q), 4H; and δ 4.27 (s), 2H]. The infrared bands at 3350 and 1370 cm^{-1} indicate the presence of a secondary amino group in the molecule.

From the spectral characteristics of the product it is expected that the phenolic hydroxyl of amodiaquine hydrochloride is involved in the reaction with chloramine-T. The compound obtained by the reaction of amodiaquine hydrochloride with potassium hexacyanoferrate(III), with the same time parameter and at pH 7.6, after extraction into chloroform and evaporation, absorbed at ν_{KBr} 1650 cm^{-1}, thus indicating the presence of a quinonoid type structure for this compound. The absence of absorbance in the 3400–3440 cm^{-1} range indicates that the phenolic hydroxyl group in amodiaquine hydrochloride is oxidised. The absence of any absorption in the 1650–1700 cm^{-1} region obtained by the present method rules out the possibility of a quinonoid type structure for the product. The elemental analysis, coupled with infrared absorption bands at 1340 and 1160 cm^{-1}, suggests the presence of a sulphonamide grouping in the chromogen. From the NMR, infrared and elemental analysis data it can be inferred that the sulphonamide group is attached either to the oxygen of the phenolic hydroxyl, as in structure **I** in Fig. 4, or to the nitrogen, as in structure **II**. However, the presence of a one-proton singlet at δ 5.23 in the NMR spectrum of the product, which is also observable in the NMR spectrum of the amodiaquine (δ 5.23, D_2O exchangeable), indicates the presence of an $-NH$ proton in the reaction product. Also, oxidant reacts at the end of the conjugated system. For these reasons structure **I** is suggested by us for the reaction product.

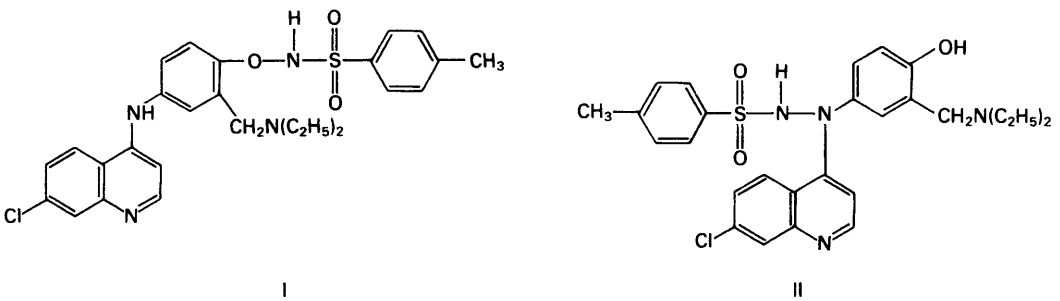

Fig. 4. Probable structure of the product formed.

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