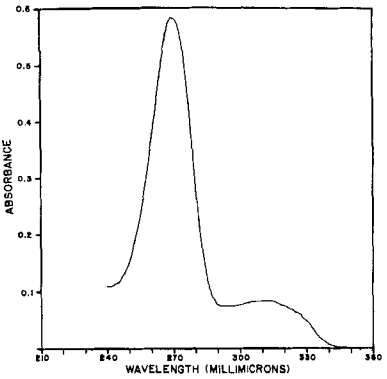

Fig. 1—Ultraviolet absorption spectrum of cyclothiazide in methanol (10 mcg./ml.); Beckman model DK-2A spectrophotometer.

conditions in which increased urinary excretion of sodium chloride and water is desirable. It is also effective for reduction of hypertension, alone or in combination with other antihypertensive agents.

Identity Tests—The identity tests included for the drug substance are satisfactory for distinguishing cyclothiazide from official compounds of similar structure. The ultraviolet absorption spectrum of cyclothiazide is significantly different from the spectrum of chlorothiazide in methanol (single absorbance maximum at 280 mμ) for identification purposes. The color and fluorescence observed when cyclothiazide is warmed in hydrochloric acid solution are not characteristic of chlorothiazide or hydrochlorothiazide and constitute a differentiating test.

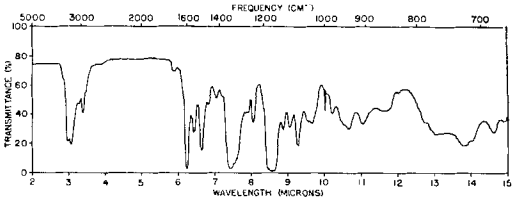

Fig. 2—Infrared spectrum of cyclothiazide in potassium bromide disk (0.5%); Perkin-Elmer model 21 spectrophotometer, sodium chloride prism.

Quantitative Tests—The nonaqueous titration of cyclothiazide with sodium methoxide using *o*-nitroaniline as the indicator gave an average value of 99.4 ± 0.3%.[2] The assay recoveries are calculated on an equivalent based on two titratable groups in the molecule for the conditions specified. Cyclothiazide dissolved in dimethylformamide and titrated with 0.1 *N* sodium methoxide to a thymol blue end point exhibits only one acidic group and a corresponding equivalent of 38.99 mg./ml. of titrant. Analysis of commercial tablets by the spectrophotometric method gave an average value of 99.2 ± 1.2%[2] of the labeled amount of cyclothiazide. The suitability of the procedure was verified by an average recovery of 100.3 ± 0.6%[2] of a standard cyclothiazide solution carried through the extractive steps as included in the tablet assay.

[2] Maximum deviation from the mean value.

Colorimetric Determination of Iodochlorhydroxyquin and Diiodohydroxyquin

By JOHN J. WINDHEUSER and DIAN Y. CHU

A method has been developed for the extraction and determination of 8-hydroxyquin derivatives utilizing cupric ion as a chelating agent. The method is highly sensitive and simple to carry out. The effect of time, pH, and cupric ion concentration has been investigated.

ALTHOUGH introduced into use as medicinal agents more than a half century ago, the halogenated derivatives of 8-hydroxyquinoline are still of significance today. The analysis of these drugs has been carried out by a number of methods. Fresenius (1) reported a colorimetric method by formation of a colored complex with ferric ion in a glacial acetic acid. The system suffered from a marked sensitivity to variation in the moisture content of the system. Haskins and Luttermaser (2) modified the system by dissolving the drugs or dried extracts from urine in 2-methoxymethanol[1] forming ferric complexes which were reported to be less sensitive to moisture variations. Both systems had the problem of interference by other phenolic compounds which might be present in the systems. Official compendia have utilized extraction techniques followed by halogen determination (3), precipitation as a metal chelate (4), and ultraviolet absorption (5). The halogen determination and metal chelate precipitation methods lack the sensitivity of the spectrophotometric methods. On the other hand, the direct spectrophotometric method suffers from interference by other absorbing species.

In this communication the development of an analytical procedure based on the formation of a

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[1] Marketed as Methyl Cellusolve by Union Carbide Corp., New York, N.Y.

copper chelate which is readily extractable into chloroform yielding a stable and highly reproducible yellow color, proportional to the 8-hydroxyquinoline derivative present, is reported. This highly sensitive procedure is simple and rapid and readily adaptable to unit dose analysis

EXPERIMENTAL

Reagents—Iodochlorhydroxyquin U.S.P. and diiodohydroxyquin U.S.P. Cupric sulfate solution, 0.1% $CuSO_4 \cdot 5H_2O$ reagent grade, was dissolved in distilled water. All other reagents used were of analytical reagent grade. Spectrophotometric measurements were carried out using 5-cm. cells in a Cary model 14 or 15 spectrophotometer.

Standard Solutions—Twenty milligrams of iodochlorhydroxyquin or 20 mg. of diiodohydroxyquin are dissolved in sufficient 3 *N* hydrochloric acid to make 100.0 ml.

Analytical Procedure—Prepare a solution of the drug to be assayed in such a manner in 3 *N* hydrochloric acid so that 5.0 ml. contain approximately 1 mg. of the active substance. In the case of tablets, the drug can be extracted from a powdered aliquot of a single tablet or a composite sample of the desired number with 3 *N* hydrochloric acid. In the case of a cream, the required amount of cream equivalent to 20 mg. of the drug is weighed and treated in the manner described below. A sample containing the equivalent of 20 mg. of the drug is placed into a glass-stoppered conical flask and 75 ml. of 3 *N* HCl is added and the mixture shaken for 30 min. on a mechanical shaker. The solution is filtered into a 100-ml. volumetric flask and the flask rinsed with sufficient 3 *N* HCl and passed through the filter to make 100.0 ml. of test solution.

Pipet 5 ml. of the test solution (in the case of diiodohydroxyquin a 1-ml. sample is used) into a 125-ml. separator and add enough 1 *N* sodium hydroxide to make 50 ml.; add 0.5 ml. of the 0.1% copper sulfate solution, mix well, and extract with 25-ml., 15-ml., and 10-ml. fractions of water-saturated chloroform. Adjust the volume of the chloroform extract to 50 ml. and determine the absorbance of the solution at 430 mμ in a 5-cm. cell against a water-saturated chloroform blank. The concentration of the sample can be calculated as follows:

$$\left(\frac{A_{\text{sample}}}{A_{\text{std.}}}\right) \times (\text{concn. of the std.}) = \text{concn. of the test soln.}$$

From this the concentration in the dosage form can be readily calculated.

Figure 1 shows the absorption curve of the iodochlorhydroxyquin–copper complex and the diiodohydroxyquin complex showing essentially the same characteristics with maxima at 430 mμ.

DISCUSSION

Figure 2 depicts the adherence to Beer's law of the absorbance of the complex as a function of drug concentration. At values of greater than 0.6 mg./50 ml. of diiodohydroxyquin, there is a negative deviation due to the insolubility of the copper complex. The method was applied to solutions of the pure drugs, oral tablets, vaginal tablets, and creams and showed a standard deviation of ± 1.1%.

Investigation of Variables—*Effect of Time on the Color Stability*—Samples of iodochlorhydroxyquin were assayed and the absorbances determined immediately and at varying time intervals up to 96 hr. The results as shown in Fig. 3 indicated no change in absorbance over the time of the observations.

Effect of pH of the Test Solution—A 5.0-ml. sample containing 1.035 mg. of iodochlorhydroxy-

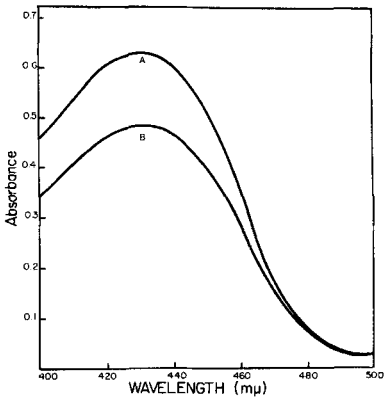

Fig. 1—Absorption spectra of the copper (II) chelate of iodochlorhydroxyquin (A) *and diiodohydroxyquin* (B) *in chloroform. Different concentrations were used to allow ready comparison of the curves.*

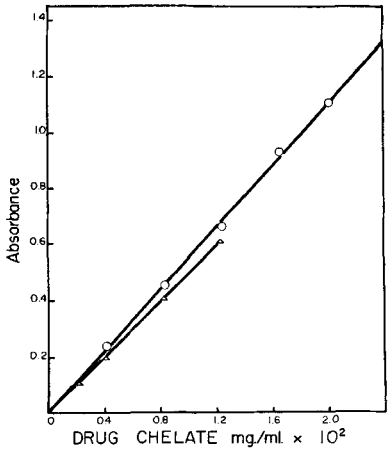

Fig. 2—Beer's law plot for iodochlorhydroxyquin and diiodohydroxyquin copper (II) chelates in chloroform at 430 mμ using 5-cm. cells. Key: ○, *iodochlorhydroxyquin;* △, *diiodohydroxyquin.*

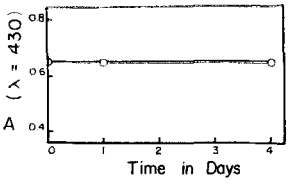

Fig. 3—The time-stability of the absorbance of the copper (II) chelate of iodochlorhydroxyquin in chloroform.

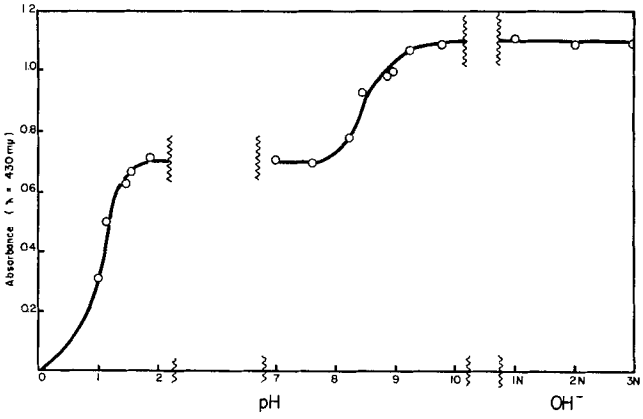

Fig. 4—pH profile of the extraction of iodochlorhydroxyquin–copper (II) chelate by chloroform.

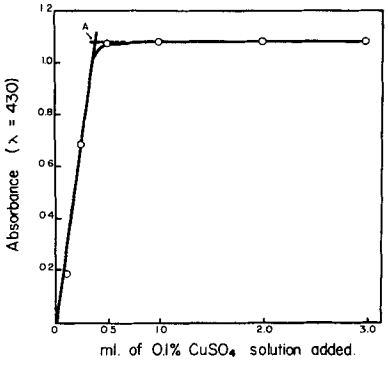

Fig. 5—The effect of cupric ion concentration on the formation and extraction of the iodochlorhydroxyquin–copper (II) chelate at constant drug and hydroxyl ion concentration.

quin in 3 *N* HCl was diluted to 50 ml., the pH being adjusted by the addition of 1 *N* NaOH, and then extracted as under the general procedure. The absorbance was determined at 430 mμ. Figure 4 shows the results, indicating that the system is highly pH dependent with increasing absorbance at higher pH. Attempts were made to find invariant absorbance at higher hydroxyl ion concentration. As shown in Fig. 4 trials using 1, 2, and 3 *N* sodium hydroxide to dilute the test sample prior to extraction produced invariant results. Based upon this, 1 *N* sodium hydroxide was used for all dilutions prior to extraction. From the pH-extraction profile, it would appear that only the ionized form of the drug formed the copper chelate. In the intermediate pH range, the profile is not presented, due to the marked variation of results which might possibly be attributed to the insolubility of the drug at these pH values. It might be suggested that in this region the neutral form of the drug itself can also be extracted into the chloroform layer. At low pH the absorbance of the chloroform layer goes to zero due to nonextractability of the protonated drug. It is obvious from the profile that the most accurate and reproducible results are obtained at high hydroxyl ion concentration.

Effect of Copper Concentration—To determine the dependence of the extractable chelate formation on the molar ratio of cupric ion to iodochlorhydroxyquin, an experiment was conducted at constant drug concentration and varying the copper concentration.

As shown in Fig. 5, the absorbance of the extracted chelate reaches a maximum and further addition of copper sulfate does not result in increased absorbance as might be anticipated. Since all work was carried out with concentrations of iodochlorhydroxyquin less than 1 mg./50 ml. of aqueous sample, 0.5 ml. of 0.1% copper sulfate solution was utilized in this study. It is obvious that if higher concentrations were utilized, the copper sulfate concentration would have to be increased.

The stoichiometry of the complex can be estimated from Fig. 5 by extending the horizontal and vertical lines to point A, the point at which equivalance is reached. It was found that at this point 1.6×10^{-6} moles of Cu^{++} ion had been added to the system containing 3.3×10^{-6} moles of iodochlorhydroxyquin. On the assumption that the complexation reaction is essentially complete, the ratio of Cu^{++} to drug would be 1:2.06 which is in good agreement with the 1:2 complex suggested by others (6) for the interaction of cupric ion with 8-hydroxyquinoline.

SUMMARY

Based upon the formation of an extractable chelate between 8-hydroxyquinoline derivatives and cupric ion, an assay has been developed for several medicinally important drugs. The method is simple, rapid, and highly sensitive. Interference from other drugs or dosage form adjuvants has been minimized. The method is suitable for unit dosage form analysis.

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