Infrared Determination of Iodochlorhydroxyquin in Pharmaceutical Preparations: Analysis of Creams, Inserts, Lotions, and Ointments

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Abstract □ A specific IR absorption method for the determination of iodochlorhydroxyquin in different pharmaceutical formulations has been developed. The method is based on the measurement of the absorption band at about 700 cm.$^{-1}$ which is specific for iodochlorhydroxyquin and unaffected by the presence of other active ingredients and/or excipients commonly present in the commercial formulations under consideration. The proposed procedure allows for differentiation between the iodochlorhydroxyquin intermediates and the parent compound present in the same solution without any further separation. This method with its specificity and simplicity provides a great advantage over methods previously reported in the literature.

Keyphrases □ Iodochlorhydroxyquin formulations—analysis □ Analysis interference—iodochlorhydroxyquin determination □ UV spectrophotometry—analysis □ IR spectrophotometry—analysis

Official compendia, such as the "U. S. Pharmacopeia" and the "National Formulary" provide different assay procedures for the determination of iodochlorhydroxyquin in pharmaceutical preparations. Neither the USP UV nor the NF gravimetric methods differentiate the iodochlorhydroxyquin from its commonly present halogenated impurities.

Several methods are available in the literature for the determination of iodochlorhydroxyquin as a pure substance and in formulation; but all these procedures suffer from the lack of specificity. The UV method (1, 2), which is based on the chromophoric group, measures the absorbance of the parent compound as well as the impurities. Colorimetric methods (3–5) are generally based upon the formation of colored metal complexes with the iodochlorhydroxyquin. The colorimetric methods provide some advantages over the UV measurements and can be applied to those commercial formulations where the official method fails to work. However, colorimetric methods also suffer from the lack of specificity because the intermediates also give metal complexes. Among other methods, only the chromatographic technique (6) closely approaches the specificity requirement. This method can be applied for the semiquantitative determination of iodochlorhydroxyquin and its intermediates in pharmaceutical formulations.

Since the iodochlorhydroxyquin intermediates and the parent compound are structurally quite similar, their identification, determination, and separation by existing methods were impossible. An IR method was developed which differentiates the parent compound from its halogenated impurities and permits their quantitative determination while eliminating all the difficult separation and isolation processes previously necessary. The absorption band on which the measurement is based is specific for the intact iodochlorhydroxyquin molecule and is unaffected by the presence of the intermediates. The intermediates themselves have unique independent absorption bands in separate wavelength regions, thus making their determination in the same solution possible. Because this method was the only specific one appearing in the literature, its usefulness was extended to the determination of iodochlorhydroxyquin in various pharmaceutical preparations such as creams, inserts, lotions, and ointments; especially in cases where the official methods failed to work. Furthermore, the proposed method, with a simple extraction procedure, permits the separation and determination of other active ingredients (steroids) in the formulations while eliminating interferences from commonly present excipients. As a result of these studies a fairly rapid and a very specific IR absorption method has been developed.

EXPERIMENTAL

Apparatus and Reagents—A recording spectrophotometer[1] with 3-mm. sodium chloride cells was used to record the spectra; hydrochloric acid, 3.5 *M*; sodium hydroxide, 3.5 *M*; carbon tetrachloride, reagent grade; carbon disulfide, reagent grade.

Caution: Since the character of the compound used in this study is such that it easily forms metallic complexes, all glassware used in this study should be free from metal contamination.

General Assay Preparation for Cream, Insert, Lotion, and Ointment—Transfer accurately a quantity of iodochlorhydroxyquin preparation, equivalent to about 30 mg. of iodochlorhydroxyquin, to a centrifuge tube. Add 10 ml. of carbon tetrachloride and shake until the preparation is completely suspended. Add 3 ml. of water and shake again about 2 min. Centrifuge the suspension and filter the carbon tetrachloride layer through a pledget of cotton into another centrifuge tube. Repeat the extraction with one 10-ml. and one 5-ml. portion of carbon tetrachloride and evaporate with vacuum the combined extracts to a volume of approximately 1–2 ml. Extract the remaining carbon tetrachloride solution with one 10-ml. and three 5-ml. portions of 3.5 *M* hydrochloric acid, filtering the acid extracts through cotton into a 125-ml. separator. To the combined acid extracts add 3.5 *M* sodium hydroxide in small portions with cooling, until the solution is approximately 0.1 to 0.01 *M* with respect to hydrochloric acid (pH between 1 and 2). Extract the

[1] Perkin Elmer IR621.

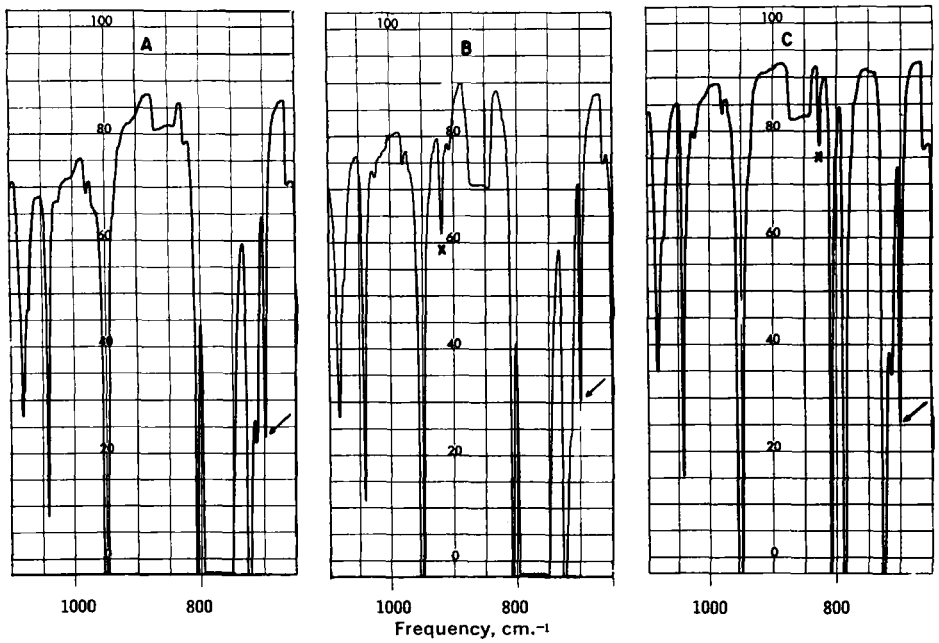

Figure 1—*IR absorption spectrum of* A*: general spectrum for cream, lotion, and ointment;* B*: ointment containing 5,7-diiodo-8-hydroxyquinoline impurity;* C*: cream containing 5-chloro-8-hydroxyquinoline impurity.*

solution with two 5-ml. portions of carbon disulfide and allow the layers to separate. Filter the carbon disulfide solution through cotton into a 10-ml. volumetric flask and dilute to volume with carbon disulfide.

Standard—Dissolve an accurately weighed portion of USP iodochlorhydroxyquin Reference Standard in carbon disulfide to obtain a solution having a concentration of about 3 mg./ml.

Procedure—Determine the absorbances of the standard and sample solutions in 3-mm. sodium chloride cells at the maximum at 695 cm.$^{-1}$ and the minimum at about 670 cm.$^{-1}$, scanning from 1,100 to 600 cm.$^{-1}$, using a suitable IR spectrophotometer and carbon disulfide as a blank.

DISCUSSION

The IR absorption spectra of iodochlorhydroxyquin in a previous publication (7) clearly demonstrate the specificity of the method in the region of measurement. Although these spectra provided accurate information about the substance alone, it seemed advisable to present additional spectra including some of pharmaceutical formulations containing steroids which might interfere in the region of measurement. Figure 1 A shows the IR spectrum of iodochlorhydroxyquin in cream, lotion, and ointment in the region 1,100 to 625 cm.$^{-1}$ obtained using the new procedure. No interference was observed due to commonly present active ingredients such as hydrocortisone, or excipients. However, a small amount of carbon tetrachloride, which was carried over into the final solution after the extraction, showed some influence on the spectra. Fortunately, these absorbances appear far from the critical region and do not affect the characteristic bands upon which the measurement is based. The usefulness of the previous method (7) was improved by the utilization of the most suitable organic solvent to be found, carbon tetrachloride, which was capable of separating iodochlorhydroxyquin (because of its much greater solubility) from the other active ingredients and impurities, as well as eliminating the formation of an emulsion as in the case of lotions. Extraction from carbon tetrachloride into 3.5 *M* hydrochloric acid was facilitated by greatly reducing the volume of the initial carbon tetrachloride extract. Although the carbon tetrachloride extraction can be avoided, it was necessary for those pharmaceutical formulations which contained other active ingredients whose separation and determination were desired. Since the protonated form of iodochlorhydroxyquin was insoluble in organic solvents, sodium hydroxide was added to bring the acid concentration to 0.1—0.01 *M* where precipitation of iodochlorhydroxyquin is completed. The precipitated iodochlorhydroxyquin was then extracted with carbon disulfide. It was observed that at about 0.4 *M* acid concentration (pH 0.4) the iodochlorhydroxyquin begins to precipitate out of solution and the precipitation is complete when the acid concentration is about 0.1 *M* with respect to hydrochloric acid (pH 1).

Table I summarizes the analytical data obtained for creams, inserts, lotions, and ointments by UV and IR methods. These data indicate that the UV method constantly gave higher assay results than the IR technique. Furthermore, the use of the UV method is limited for some formulations because of large interferences; whereas the IR method can be applied to most formulations without any modifications. Since significant differences between the results of the two assay procedures existed, the causes of these differences were investigated. An iodochlorhydroxyquin placebo cream containing all the excipients was subjected to a UV assay in a manner identical to the official method. It was found that the placebo cream itself had some absorption in the region used for the UV assay. Furthermore, the IR assay clearly showed that the analyzed sample contained 5-chloro-8-hydroxyquinoline. As this impurity has a stronger UV absorptivity than the parent compound, it is obvious that its presence would increase the UV assay of a sample considerably. Table II illustrates the agreement between the corrected UV assay results and the IR assay. A further error of 2–4% can occur, depending on the extent of heating applied to gain complete solution for the UV assay.

During the extraction procedure on the various formulations, the

Table I—Determination of Iodochlorhydroxyquin in Formulation by Ultraviolet and Infrared Methods

Manufacturer	Cream, %		Insert, %		Lotion, %		Ointment, %	
	UV	IR	UV	IR	UV	IR	UV	IR
Ciba	104.0	98.0	103.0	97.0	Inter.	97.0	101.0	99.0
A[a]	—	—	—	—	—	—	Inter.	87.0[b]
B	—	—	—	—	—	—	101.0	97.0
C	104.0	102.0	—	—	—	—	—	—
D	—	—	—	—	—	—	109.0	89.0
E	—	—	—	—	—	—	Inter.	99.0

[a] A to E are typical production samples produced by different companies. [b] Interference observed.

Table II—Interferences in Ultraviolet Assay

	UV Assay, %	IR Assay, %
Cream	104.0	98.0
Placebo (Ciba)	4.3	None
Impurity[a]	1.9	—
Total interference	6.2	—
Cream − interference	97.8	—

[a] 5-Chloro-8-hydroxyquinoline, calculated as iodochlorhydroxyquin at the wavelength of iodochlorhydroxyquin.

Table III—Iodochlorhydroxyquin Recovery in Ointment

Iodochlorhydroxyquin	Found[a]
Initial assay, mg.	28.4
Added, 9.0 mg.	37.2
Recovery, %	99.5 ± 0.5

[a] Average from triplicate assay.

final carbon disulfide solution assumes a color varying from pale yellow to pale green, in contrast to the almost colorless standard. The color variation, which did not affect the assay's validity, is due to trace metal contamination in the formulation and/or in the reagents used during the assay.

A synthetic mixture containing 16 mg. of carbon tetrachloride/10 ml. of carbon disulfide was prepared in an attempt to sustantiate the statement that the broad absorption band at 800–750 $cm.^{-1}$, due to the presence of carbon tetrachloride, (4–16 mg./10 ml. carbon disulfide) does not interfere in the assay. No appreciable differences in the iodochlorhydroxyquin assay were found when this solution was compared with a solution containing just iodochlorhydroxyquin in carbon disulfide.

Since the official USP method for the pure substance requires five scannings and recordings in the assay procedure, a typical sample was subjected to this test in the region of measurement. The results of these tests indicate that the reproducibility found is better than ±0.5%.

The recovery of iodochlorhydroxyquin in an ointment containing about 30% excess of iodochlorhydroxyquin was investigated. The results of these analyses can be seen in Table III.

Impurities—One would expect that due to structural similarities the solubilities of iodochlorhydroxyquin and its intermediates would be fairly identical. Table IV shows the solubilities of iodochlorhydroxyquin and its two intermediates in carbon disulfide and hydrochloric acid. Since the solubility of 5-chloro-8-hydroxyquinoline in both solvents is significantly greater than that of the parent and diiodo compounds, some difficulties were encountered for the quantitative determination of 5-chloro-8-hydroxyquinoline necessitating slight modification of the general method. Complete extraction of 5-chloro-8-hydroxyquinoline was obtained only when the pH of the final aqueous solution (after the carbon disulfide extraction of iodochlorhydroxyquin) was adjusted to 3 to 4. Monobasic sodium phosphate solution (10 ml. of 1.5 *M*) and sodium hydroxide were utilized to bring the solution to a suitable pH for extraction. The 5-chloro-8-hydroxyquinoline was successfully extracted from this solution with two additional 5-ml. portions of carbon disulfide. The carbon disulfide extracts containing 5-chloro-8-hydroxyquinoline were combined with the carbon disulfide extracts obtained from the previous assay and the combination was evaporated with vacuum to a suitable volume. Table V summarizes the results obtained using the modified IR method on different pharmaceutical preparations containing 5-chloro-8-hydroxyquinoline as an impurity.

Table IV—Solubility of Iodochlorhydroxyquin and Its Common Impurities at Room Temperature

Compound	Carbon Disulfide, mg./ml.	0.1 *M* Hydrochloric Acid, mg./ml.
Iodochlorhydroxyquin	12.5	0.015
5,7-Diiodo-8-hydroxyquinoline	4.1	0.002
5-Chloro-8-hydroxyquinoline	38.8	10.5

Table V—Analysis of Iodochlorhydroxyquin and 5-Chloro-8-hydroxyquinoline in Formulations by the Proposed Method

Manufacturer	Cream, % I[a]	Cream, % II[b]	Insert, % I	Insert, % II	Lotion, % I	Lotion, % II	Ointment, % I	Ointment, % II
Ciba	98.0	1.43	96.7	1.30	97.3	1.10	99.0	1.33
B	—	—	—	—	—	—	96.7	2.30
C	102.0	1.50	—	—	—	—	—	—
D	—	—	—	—	—	—	89.0	[c]
E	—	—	—	—	—	—	98.3	1.90

[a] I = Iodochlorhydroxyquin. [b] II = 5-Chloro-8-hydroxyquinoline. [c] Contains 17.5% 5,7-diiodo-8-hydroxyquinoline.

Although 5-chloro-8-hydroxyquinoline is the most common impurity in commercial formulations, a fair amount of 5,7-diiodo-8-hydroxyquinoline was detected in Sample D (see Fig. 1, spectrum B). Since this compound has a very low solubility in 0.1 *M* hydrochloric acid, preparations containing 5,7-diiodo-8-hydroxyquinoline impurities can be directly determined from the assay solution without any modification of the original procedure.

Based on experimental observations, it was necessary to develop a suitable specific analytical procedure for pharmaceutical formulations containing iodochlorhydroxyquin.

SUMMARY

A specific IR absorption method has been developed for the determination of iodochlorhydroxyquin in various pharmaceutical formulations. This technique can be applied unmodified to some formulations where the official method fails to work. With a slight modification of the general method, impurities such as 5-chloro-8-hydroxyquinoline can be quantitatively determined. Differences between the UV and IR methods are explained and the usefulness of the proposed method demonstrated.

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