# Quantitative Determination of Pilocarpine in Ophthalmic Solutions

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**A method is described for the quantitative determination of pilocarpine in ophthalmic solutions. It is based on reaction with hydroxylamine in an alkaline medium to give the hydroxamic acid, which is complexed with ferric ion. Although the color produced is relatively unstable, the method has a high degree of precision if the experimental conditions are observed carefully. Certain preservatives which interfere in the reaction can be removed easily by extraction prior to the determination of pilocarpine.**

In spite of the importance of pilocarpine in ophthalmology, relatively little work has been done to develop a satisfactory method for the determination of pilocarpine salts in aqueous solution. A color reaction developed by Helch (1) and used in the U.S.P. (2) as a test for identification has been modified and applied to quantitative determination of pilocarpine (3–5). However, little is known about its specificity. In fact, since the method makes use of strong oxidizing agents such as hydrogen peroxide and potassium dichromate, it seems reasonable to assume that the method will not differentiate between pilocarpine and its primary decomposition products. Pilocarpine is a lactone with two asymmetric centers. Therefore, it presents several possible pathways of degradation, including hydrolysis to pilocarpic acid and epimerization. The most important of these is the opening of the lactone ring. As long as the lactone is intact, the alkaloid maintains its stereochemical configuration (6). A quantitative determination based on this functional group would, therefore, be expected to exhibit a high degree of specificity.

Most lactones and esters react with hydroxylamine to form hydroxamic acids which produce red to purple complexes with ferric ion. This reaction, developed as a spot test by Feigl and co-workers (7), has been used extensively for estimation of a large number of esters, lactones, anhydrides, amides, and nitriles.

## EXPERIMENTAL

**Samples.**—The alkaloids used in this work were pilocarpine hydrochloride and pilocarpine nitrate, both conforming to the specifications in U.S.P. XVI. The ophthalmic solutions were commercial samples produced by several manufacturers.

**Reagents.**—Hydroxylamine hydrochloride, 1 *M* in water; hydrochloric acid, 3.5 *M*; sodium hydroxide, 3.5 *M*; and ferric chloride solution, 0.3 *M* in 0.1 *N* hydrochloric acid were utilized.

**Effect of pH on Hydroxylaminolysis.**—To 4 ml. of pilocarpine salt solution, containing about 40 mg./100 ml., were added 1 ml. of hydroxylamine hydrochloride solution and 3 ml. of an alkaline phosphate buffer; the solution was mixed thoroughly after each addition. At the end of a predetermined time interval, 1 ml. of 3.5 *M* hydrochloric acid was added, followed by 1 ml. of ferric chloride reagent. After exactly 10 min., the absorbance was determined against a reagent blank at 500 mμ. Figure 1 illustrates the formation of hydroxamic acid at three different pH values. At pH 10.6, the rate of hydroxylaminolysis is slow but increases rapidly with an increase in pH. Furthermore, the yield of color is the same at pH 11.4 as it is at pH 11.2. It may be concluded from this that the reaction with hydroxylamine is faster than the base-catalyzed hydrolysis of the lactone ring.

**Stability of Color.**—The instability of the color produced is one of the main problems of the ferric hydroxamic acid procedures. Hill improved color stability by the addition of nitric acid (8) or hydrogen peroxide (9) which destroyed the excess of hydroxylamine. Goddu *et al.* (10) and Siggia (11) used a freshly prepared alkaline solution of hydroxylamine in methanol and an alcoholic solution of ferric perchlorate and obtained solutions which were stable for several hours. When the methods of Hill (8) and Siggia were used for pilocarpine, they showed no advantage over a simple aqueous system (Table I).

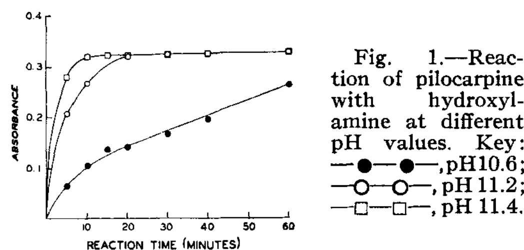


Fig. 1.—Reaction of pilocarpine with hydroxylamine at different pH values. Key: —●—●—, pH 10.6; —○—○—, pH 11.2; —□—□—, pH 11.4.

Table I.—Stability of Color Formed in Various Ferric Hydroxamic Acid Methods Applied to Pilocarpine

| Time, min. | Fading Rate, % per 10 min. Method of Hill | Method of Siggia | Proposed Method |
|---|---|---|---|
| 10 | 1.5 | 2.7 | 1.0 |
| 20 | 1.3 | 2.7 | 1.0 |
| 30 | 1.3 | 3.0 | 0.8 |
| 40 | 1.1 | 2.6 | 0.9 |
| 50 | 0.8 | 2.8 | 1.3 |
| 60 | 1.0 | 2.5 | 1.0 |

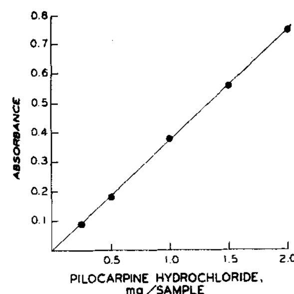


Fig. 2.—Standard curve for pilocarpine hydrochloride.

Received December 22, 1964, from the School of Pharmacy, University of California Medical Center, San Francisco.
Accepted for publication January 27, 1965.

TABLE II.—ANALYSIS OF PILOCARPINE SOLUTIONS CONTAINING INTERFERING SUBSTANCES

| Soln. Analyzed | Absorbance |
|---|---|
| Pilocarpine hydrochloride | 0.620 |
| Pilocarpine hydrochloride with chlorobutanol, 0.04% | 0.618 |
| Pilocarpine hydrochloride with methylparaben, 0.015% | 0.622 |
| Chlorobutanol solution, 0.04% | 0.170 |
| Methylparaben solution, 0.015% | 0.100 |

TABLE III.—ANALYSIS OF COMMERCIAL PREPARATIONS OF PILOCARPINE OPHTHALMIC SOLUTIONS

| | Pilocarpine Salt | | |
|---|---|---|---|
| Mfr. | Labeled Amt., % w/v | Found,[a] % w/v | Other Constituents |
| *A* | 1 | 0.85 | Chlorobutanol, methylparaben, benzoic acid |
| | 2 | 1.50 | |
| *B* | 1 | 0.92 | Chlorobutanol |
| | 2 | 2.01 | |
| *C* | 1 | 1.01 | Chlorobutanol, phenylephrine |
| | 2 | 2.02 | |
| *D* | 1 | 1.04 | Benzalkonium chloride, sodium edetate |
| | 2 | 2.12 | |
| *E* | 1 | 1.06 | Benzalkonium chloride, phenylmercuric nitrate |
| | 2 | 2.10 | |

[a] Average values based on two determinations.

The color formation in the proposed method is proportional to the concentration of pilocarpine. (See Fig. 2.)

**Procedure.**—To 4.0 ml. of pilocarpine salt solution, containing about 0.4 mg./ml., are added 1.0 ml. of hydroxylamine hydrochloride solution and 1.0 ml. of 3.5 *M* sodium hydroxide; the solution is mixed after each addition. After 10 min., 1.0 ml. of 3.5 *M* hydrochloric acid and 1.0 ml. of ferric chloride reagent are added, and the absorbance is determined against a reagent blank after exactly 10 min. at 500 m$\mu$. A standard solution of the appropriate pilocarpine salt is prepared and treated in the same way.

**Precision.**—The method described above gave a precision of 1.0%, calculated as the coefficient of variation based on 21 independent determinations. The procedure described by Siggia (11) gave a precision of 2.2% when applied to pilocarpine.

**Interfering Substances.**—Certain substances used as preservatives in ophthalmic solutions tend to interfere with the determination of pilocarpine. This is the case with chlorobutanol and esters of *p*-hydroxybenzoic acid. These substances can be removed readily by a single extraction with ether as follows.

A solution was prepared containing about 50 mg. of pilocarpine hydrochloride in 100 ml. of solution. A 20-ml. aliquot was diluted with water to 25 ml. and assayed as described above. Another 20-ml. aliquot was pipeted into a 125-ml. separator, 8 mg. of powdered chlorobutanol was added and allowed to dissolve, and the solution was shaken for about 1 min. with 40 ml. of ether. The aqueous layer was transferred to a 25-ml. volumetric flask, the ether phase was shaken with 4 ml. of water, which was added to the pilocarpine solution in the flask, and the volume was adjusted to the mark. After mixing, the content of pilocarpine was determined as previously. Similar experiments were performed in which methylparaben was added to the pilocarpine solution in a concentration commonly found in ophthalmic solutions. Aqueous solutions of the preservatives were subjected to the ferric hydroxamic acid reaction without prior extraction. The results are given in Table II.

## Analysis of Pilocarpine Ophthalmic Solutions

**Preparation of Assay Solution.**—(*a*) If the sample contains no chlorobutanol or *p*-hydroxybenzoic acid esters, 2.0 ml. of the solution is diluted with water in a volumetric flask to give a concentration of about 40 mg./100 ml. of pilocarpine salt. This solution is analyzed as described under *Procedure.*

(*b*) If the ophthalmic solution contains chlorobutanol or one of the parabens, 2.0 ml. is placed in a 125-ml. separator, and the pH is adjusted to about 4 with 0.1 *N* hydrochloric acid if necessary. After addition of 10 ml. of water, the solution is extracted once with 40 ml. of ether. The aqueous phase is transferred to a volumetric flask and the ether layer washed with 5 ml. of water, which is added to the solution in the flask. The volume is adjusted to the mark to give a concentration of about 40 mg./100 ml. of pilocarpine salt. This solution is analyzed as described under *Procedure.*

The method has been applied to a number of commercial samples. The results are given in Table III.

Table III shows that most of the samples gave results which agreed well with the concentrations declared on the label. The products of manufacturer *A*, however, must be considered as substandard.

## REFERENCES

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