Optimal Ferric Hydroxamate Method for Determination of Intact Pilocarpine

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Abstract □ The ferric hydroxamate colorimetric method for lactones has been optimized for the determination of pilocarpine in the presence of its degradation product by careful control of pH, reagent concentration, and time to yield a method which has the following advantages of similar methods previously proposed: (*a*) direct sampling of the usual 1 or 2% solutions without dilution; (*b*) a stable blank with relatively low absorbance; and (*c*) production of a color with a minimal rate of fading, suitable for quantitative determination. Analysis of 20 independent sets of calibrations by linear regression analysis yields a regression coefficient of 0.98, an absorbance (*y*) intercept of −0.007, and a slope of 0.51.

Keyphrases □ Pilocarpine solutions—analysis □ Ferric hydroxamic acid method—color formation □ Colorimetry analysis—spectrophotometer

Various analytical methods for the determination of pilocarpine hydrochloride have appeared in the literature including titrimetric (1–4), ion-exchange (5), colorimetric (6–12), and measurement of the UV absorption of the tetraphenylboride (13). For the routine quality control of pilocarpine hydrochloride ophthalmic solutions, the method of Brochmann-Hanssen *et al.* (12) appears most useful. The method consists of making the sample alkaline in the presence of hydroxylamine hydrochloride, allowing the reaction to proceed for a period of time, and adding ferric chloride to the acidified mixture to yield a colored ferric hydroxamate. After allowing the color to develop, the absorbance is read at 500 mμ in a suitable instrument. The advantages of this method are specificity for the intact lactone ring of the pilocarpine molecule and more than sufficient sensitivity.

In using the technique, this sensitivity has been found to be a burden because samples cannot be taken directly from the solutions of pilocarpine hydrochloride commonly appearing in commerce, thus entailing several dilutions during analysis of samples and preparation of calibration curves. In addition, variations in absorbance with time for treated samples, gas bubble formation on the walls of the cells, and the small volume of the final sample, making adequate rinsing of cells difficult, all serve to make this method less than ideal.

The proposed method reduces the disadvantages, allowing for direct sampling from aqueous solutions of pilocarpine hydrochloride in concentrations of 1 and 2% and yielding absorbance values which conform to the usual conditions for photometric measurement.

EXPERIMENTAL

Apparatus—The Cary model 15 recording spectrophotometer and the Gilford apparatus with the Beckman DUR monochromator were used for the absorbance measurements.

Samples—The samples consisted of aqueous solutions of pilocarpine hydrochloride USP XVII in a concentration of 0.7%.

Table I—Change in Absorbance with Time against Water

Time	Sample	Reagent Blank
	Brochmann-Hanssen Method[a]	
	Trial A	
Initial	0.732	0.205
+ 10 min.	0.844	0.320
+ 20 min.	0.894	0.392
	Trial B	
Initial	0.779	0.133
+ 10 min.	0.777	0.304
+ 20 min.	0.819	0.361
	Trial C	
Initial	0.872	0.159
+ 10 min.	0.991	0.308
+ 20 min.	1.020	0.316
	Trial D	
Initial	0.667	0.161
+ 10 min.	0.692	0.060
+ 20 min.	0.672	0.100
	Proposed Method[b]	
	Trial A	
Initial	0.504	0.005
+ 10 min.	0.489	0.007
+ 20 min.	0.472	0.007
	Trial B	
Initial	0.457	0.007
+ 10 min.	0.442	0.006
+ 24 min.	0.418	0.006

[a] $\%\Delta A$ for sample/min. for 1st 10 min. based on initial net sample value = −0.06, Trial A; −2.7, Trial B; −0.42, Trial C; and +2.49 Trial D. [b] $\%\Delta A$ for sample/min. for 1st 10 min. based on initial net sample value = −0.34, Trial A; and −0.31, Trial B.

Solutions used in the performance of the method suggested by Brochmann-Hanssen *et al.* (12) were 0.04%. The pilocarpine hydrochloride employed was assayed by the USP method (14), yielding a recovery of 99.85 ± 0.11%.

Reagents—Hydroxylamine hydrochloride, 1 *M* in water; alkaline phosphate consisting of 10 volumes of 7% trisodium phosphate and 2 volumes of 3.5 *M* sodium hydroxide; and ferric chloride solution, 0.3 *M* in 0.1 *N* hydrochloric acid.

Proposed Method—Transfer an accurately measured volume of pilocarpine hydrochloride solution containing between 10 and 20 mg. of pilocarpine hydrochloride to a 25.0-ml. volumetric flask and add water to make approximately 10 ml. Add 1.0 ml. of 1 *M* hydroxylamine hydrochloride and 3.0 ml. of alkaline phosphate solution, mixing well after each addition, and allow the solution to stand for 10 min. Add 1.0 ml. of 5.25 *M* hydrochloric acid followed by 1.0 ml. of ferric chloride solution. Dilute with water after 10 min. and read the absorbance in a suitable spectrophotometer at 480 mμ. A reagent blank is treated in the same manner. A calibration curve may be conveniently prepared by taking 1.00, 2.00, and 3.00 ml. of a 0.7% pilocarpine hydrochloride solution (which yields absorbance values equivalent to 0.35, 0.70, and 1.05% solutions, respectively) and treating in the described manner.

RESULTS

Twenty calibration curves were prepared as indicated on separate days. Each curve was prepared using fresh reagents and a fresh 0.7% pilocarpine hydrochloride standard solution. The following

Table II—Absorbance *versus* Time Obtained with Treated Sample (3.00 ml. of 0.7% Pilocarpine Hydrochloride) and Reagent Blank

Time (Minutes after Addition of Ferric Chloride Solution)	Sample Absorbance	Reagent Blank Absorbance
2	0.563	0.011
3	0.557	0.011
4	0.553	0.011
5	0.551	0.010
6	0.550	0.011
7	0.548	0.010
8	0.547	0.009
9	0.546	0.010
10	0.545	0.009
11	0.544	0.012
12	0.545	0.012
13	0.544	0.012
14	0.543	0.012
15	0.541	0.011
16	0.541	0.011
17	0.541	0.009
18	0.538	0.010
19	0.536	0.011
20	0.535	0.010
21	0.531	0.011
22	0.532	0.011

significant values were obtained upon calculation using regression analysis: regression coefficient, 0.98; absorbance (y) intercept, −0.007; and slope, 0.51.

Fading Rate—As has been pointed out (12, 15), ferric hydroxamic acid procedures, in general, yield unstable colors. Comparison of the change in absorbance with time between the proposed method and the Brochmann-Hanssen method for pilocarpine hydrochloride was rendered difficult by formation of gas bubbles on the walls of the cell immediately upon introduction of the sample when using the Brochmann-Hanssen technique. These bubbles grew larger with time. The colored solutions arising from the proposed method, however, are homogeneous and no difficulty was experienced.

Absorbance spectra of the solutions obtained with the Brochmann-Hanssen method showed a maximum at 500 mμ as reported (12), while those of solutions obtained with the proposed method showed a slightly broader maximum in the region of 480 mμ. Recorded absorbance spectra obtained with the solutions treated in accordance with the Brochmann-Hanssen technique 10 and 20 min. after the initial spectrum invariably showed a total increase in absorbance at the wavelength of maximum absorption, although the +20-min. spectrum sometimes showed a decrease in absorbance from that at +10 min. Recorded absorbance spectra of solutions obtained with the proposed method showed only a slight decrease in the absorbance maximum 10 and 20 min. after the initial spectrum.

Due to the paradoxical behavior of the solutions obtained in the Brochmann-Hanssen method and also to the fact that both the sample and blank are highly colored, the changes in absorbance with time of both samples and blanks were determined independently against water at 500 mμ. The same was done for solutions obtained with the proposed method at 480 mμ. The results are shown in Table I.

As can be seen, the method proposed by Brochmann-Hanssen *et al.* (12) yields rather large changes in absorbance with time for both the sample and blank. In addition, the overall net change in absorbance of the sample is not predictable with respect to direction or magnitude. On the other hand, the absorbance of the reagent blank used in the proposed method is constant within the limits of accuracy of the digital read-out meter of the Gilford apparatus employed. The absorbance of the sample obtained by the proposed method shows only a slight decrease over the time period studied.

In order to determine the optimal time period between combination of the sample with the reagents and determination of the absorbance, an absorbance *versus* time study for the sample and reagent blank was performed. Water was used to establish a baseline absorbance of 0. The results are shown in Table II.

As can be seen by perusal of Table II, the absorbance of the reagent blank again remains constant within the limits of accuracy of the instrument throughout the time period employed. The sample shows an initial accelerated fading, which is somewhat stabilized at approximately 10 min. following the addition of the ferric chloride solution. Thus, by following the directions given for the proposed method, the operator has sufficient time to make several solutions to volume, rinse the cells, and take absorbance readings without fear of significant fading during the time required.

CONCLUSIONS

A method has been proposed for the colorimetric analysis of aqueous solutions of pilocarpine hydrochloride which exhibits: (*a*) ease of sampling; (*b*) no change in absorbance of the reagent blank and only slight fading of the sample over the time suggested after addition of the last reagent required in sample treatment and reading of the sample absorbance; and (*c*) no gas bubble formation in the treated sample as was obtained with a previously proposed method (12).

While the proposed method has not been applied to samples of commercially available solutions, no marked deviations are expected in the results, provided that interfering substances are removed prior to sample treatment by methods similar to that prescribed by Brochmann-Hanssen *et al.* (12).

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