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COMMUNICATION

Stability of Pilocarpine Ophthalmic Solutions

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ABSTRACT

The stability of pilocarpine and pilocarpine-timolol eyedrop preparations available on the Argentine market was studied. A high-performance liquid chromatographic method that allows the estimation of pilocarpine in the presence of degradation products was used for the study according to the preestablished design. It was found that pilocarpine solutions are stable, while pilocarpine in association with timolol shows significant degradation.

INTRODUCTION

Ophthalmic solutions containing pilocarpine as the only active principle and those with pilocarpine hydrochloride plus timolol maleate are available on the Argentine market for the treatment of glaucoma. The two possible pathways of degradation of pilocarpine in aqueous solution involve the lactone ring (Scheme 1): the ester hydrolysis to pilocarpic acid, which is a reversible process, catalyzed by both hydrogen ion and hydroxide ion; and epimerization at the alpha carbon to form isopilocarpine.

Hydrolysis in this environment is the prevailing degrading reaction. Pilocarpine shows relative stability in acidic pH. As the pH increases, it progressively becomes unstable. Some epimerization would occur at alkaline pH, but at a rate slower than that of hydrolysis.

Both degradative pathways lead to a loss of pharmacological activity because pilocarpic acid and isopilocarpine lack parasympathomimetic activity (1). As suggested above, the pilocarpine degradation rate in aqueous solution depends, among other factors, on the formulation pH, quoted in Ref. 2 as the pH range 4–5 to achieve suitable stability and physiological availability. This would be similar to that already cited by Baeschklin and Etter (3), who differentiated pH ranges of good stability, action, and ocular tolerance.

As regards the active principle timolol maleate, it has

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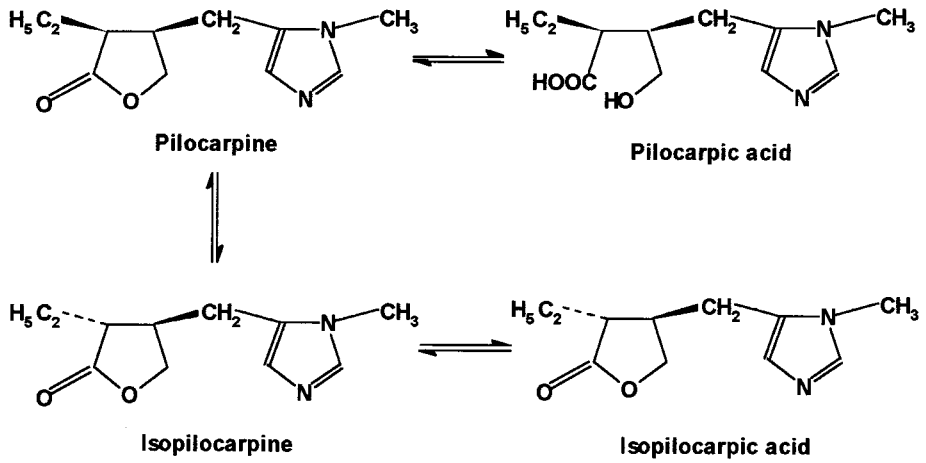

Scheme 1. Degradative pathways of pilocarpine.

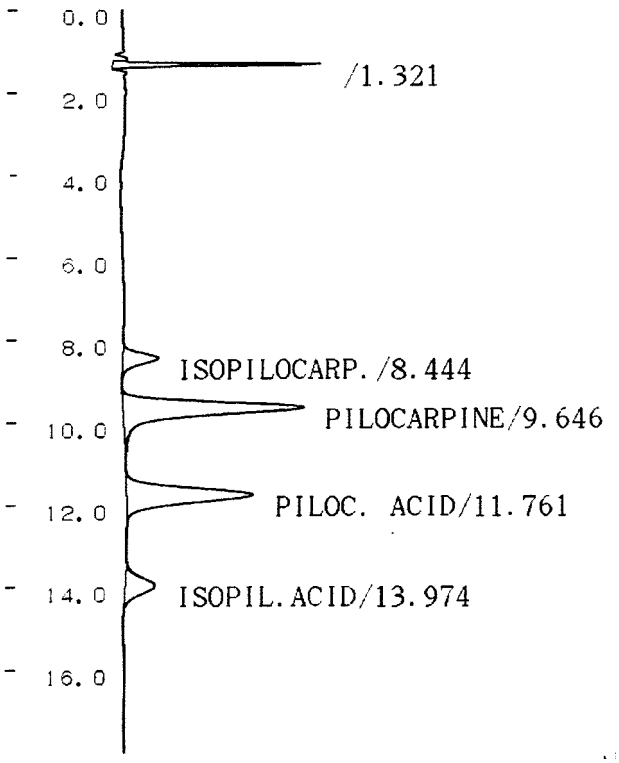

Figure 1. Method specificity.

been pointed out that it is very stable in a solution protected from light (4) and at pH 6.8 for the optimum maximum stability (5).

Many different methodologies have been developed and proposed for the quantification of pilocarpine in the presence of its degradation products in ophthalmic solutions (1,6–12). In this report, a pilocarpine active principle follow-up has been carried out for pilocarpine alone and in association with timolol maleate, the latter because it is considered the period-limiting factor for shelf life.

MATERIALS AND METHODS

A batch of products (A–H) was sampled. The samples were taken in sufficient quantity to carry out a survey of stability according to the preestablished design, which was necessary to adapt to the analytic results that were obtained.

An example of what was exposed is the case of the product F, for which, due to the data found in the active principle titration, it was found convenient to carry out additional tests, taking samples for certain quality controls.

Reagents and solvents used were bidistilled water and gradient-grade methanol. Phosphoric acid and triethylamine were analytical grade.

The pH measurements were made with a 716 DMS Titrino Metrohm (Herisau, Switzerland). A Shimadzu high-performance liquid chromatographic system was equipped with a multiple wavelength detector, a sample

injector fitted with a 20-mcl loop (Rheodyne, Cotati, CA), an integrator (model C-R7A plus), and a Merck Lichrocart (Darmstadt, Germany) 125 × 4.6 mm Lichrospher 100-C18 (5 μm) column.

For the chromatographic conditions, the buffer of the mobile phase was prepared by diluting 13.6 ml of phosphoric acid in 700 ml of bidistilled water; 3 ml of triethylamine were added, and it was taken to a final volume of 1000 ml with bidistilled water. The pH was adjusted to 3.0 with a 20% sodium hydroxide solution. The solution was filtered immediately prior to use through 0.45-μm filters. The buffer and the methanol were desgasified in an ultrasonic bath for 15 min. The mobile phase was 98% pH 3 buffer and 2% methanol. The flow rate was 1 ml/min. The detection wavelength was 215 nm.

To demonstrate the specificity of the chosen method, which was adapted from Ref. 13, the pilocarpine hydrochloride contaminated with isopilocarpine was degraded in the following conditions. About 30 mg of the drug substance was weighed and diluted to 50 ml with distilled water; 25 ml of this solution were transferred to a balloon; 5 ml of sodium hydroxide was added. It was taken to reflux for 1 hr and allowed to cool; it was then neutralized with 0.25 M orthophosphoric acid. The solution was poured into a 50-ml volumetric flask and diluted to volume with water. The 25 ml remaining of the original solution were diluted to 50 ml with water. Then, 1 ml was taken from both the degraded and nondegraded solutions, and both were brought to 10 ml with distilled water.

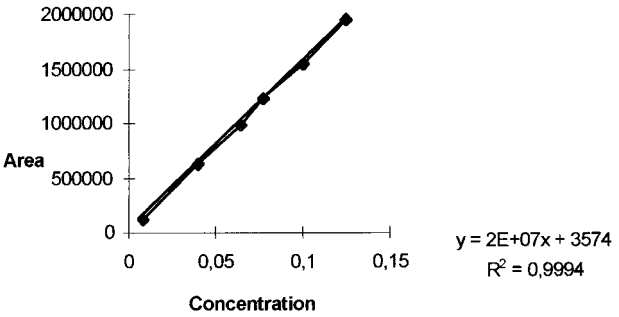

Figure 2. Linearity.

From this solution, 20 mcl were injected, creating the chromatogram in Fig. 1, from which it was determined that the chosen method was suitable for the study since it allows the separation of pilocarpine from isopilocarpine and pilocarpic acids. In addition, it was proved that the timolol peak does not interfere with the quantification of pilocarpine as it appears with a relative retention time of 0.5.

For the calibration curve (Fig. 2), 20 mcl of the solutions were injected in a range of concentrations, 0.008–0.125 mg/ml, of pilocarpine hydrochloride. The linear

Table 1

Results for Solutions Containing Pilocarpine

Product	January 1996	May 1996	October 1996	February 1997	June 1997
Product A: pilocarpine HCl, 2 g/100 ml; manuf. 11/95; exp. 11/97.					
% of labeled amount of pilocarpine (90–110%)	92.9	92.8	93.1	91.9	—
pH (3.5–5.5)	3.5	3.5	3.2	3.3	
Product B: pilocarpine HCl, 2 g/100 ml; manuf. 11/95; exp. 11/97					
% of labeled amount of pilocarpine (90.0–110.0%)	97.3	94.6	90.0	92.0	91.5
pH (3.5–5.5)	4.4	4.4	4.1	4.0	4.0
Product C: pilocarpine nitrate, 2.5 g/100 ml; manuf. 12/95; exp. 12/97.					
% of labeled amount of pilocarpine (90.0–110.0%)	102.0	99.1	93.6	93.0	92.7
pH (3.5–5.5)	4.9	5.0	5.0	4.8	4.8
Product D: pilocarpine HCl, 2 g/100 ml; manuf. 2/95; exp. 2/97.					
% of labeled amount of pilocarpine (90.0–110.0%)	96.5	96.2	94.9	92.1	
pH (3.5–5.5)	3.4	3.5	3.5	3.4	—
Product E: pilocarpine HCl, 1 g/100 ml; manuf. 1/95; exp. 1/97.					
% of labeled amount of pilocarpine (90.0–110.0%)	91.7	91.4	89.4	91.5	
pH (3.5–5.5)	4.3	4.2	4.1	4.1	—

Specifications correspond to USP 23.
Manuf., manufacture date; exp., expiration date.

Table 2

Results for Solutions Containing Pilocarpine and Timolol

	January 1996	May 1996	October 1996
Product F, batch 1: Pilocarpine HCl 2.5 g, timolol maleate 0.684 g/100 ml; manuf. 11/95; exp.: 11/97.			
% of labeled amount of pilocarpine (90–110%)	83.5	79.1	78.6
pH (5.2–5.6)	5.1	4.96	4.8
Product G: Pilocarpine HCl 2.35 g, timolol maleate 0.684 g/100 ml; manuf. 10/95; exp. 10/97.			
% of labeled amount of pilocarpine (90.0–110%)	87.2	84.9	83.3
pH (4.8–5.2)	4.7	4.6	4.4
Product H, batch 1: pilocarpine HCl 1.0 g, timolol maleate 0.684 g/100 ml; manuf. 11/95; exp. 5/97.			
% of labeled amount of pilocarpine (90.0–110.0%)	105.1	97.1	90.3
pH (5.4–6.1)	5.6	5.4	5.3

The specifications correspond to those of the respective manufacturer's laboratory.
Manuf., manufacture date; exp., expiration date.

regression coefficient was 0.9994 ($n = 6$). The concentration of 0.08 mg/ml was chosen. As to accuracy, six consecutive injections were given of a concentration standard of 0.08 mg/ml, with dsr% = 1.2. Aqueous solutions of an 0.08 mg/ml concentration of pilocarpine hydrochloride were prepared using the respective drug substance. The solutions were prepared from each of the eyedrop preparations studied and diluted to 0.08 mg/ml with water.

RESULTS AND DISCUSSION

From the follow-up carried out on products in the home market, the following findings were obtained. First, the formulations containing only pilocarpine showed (Table 1) acceptable stability during shelf life. In the case of product C, the laboratory has begun to study if the active principle drop is due to some problems with the formulation or if it is an isolated case. Second, when the active principle was associated with timolol maleate, it was observed (Tables 2 and 3) that all formulations showed a stressed drop in the pilocarpine active principle titer that fits with the appearance of pilocarpic acids and isopilocarpine and with the decrease in pH titers.

As a result of these findings, the laboratories involved in the manufacture of products F, G, and H, to provide optimum stability of their products, proposed a change in presentation (extemporaneous preparation), formulation, and storage conditions. These changes will be evaluated to check if acceptable stability of the products has been achieved.

Table 3

Results for Data Obtained from File Samples and Sales Outlet of Product F

Product F	Origin	Pilocarpine Hydrochloride (%)	pH
Batch 2: manuf. 11/94; exp. 11/96	File samples	50.2	5.5
	Sales outlet	53.4	[a]
Batch 3: manuf. 3/95; exp. 3/97	File samples	73.8	5.2

manuf., manufacture date; exp., expiration date.
[a] Given the origin of the sample, the quantity was not sufficient to determine pH.

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