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Note

Determination of the ophthalmic therapeutic pilocarpine and its degradation products by reversed-phase high-performance liquid chromatography

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Pilocarpine [(1), Fig. 1], an imidazole alkaloid, is important because of its pharmacological properties. The main source of (+)-(2*S*, 3*R*)-pilocarpine is *Pilocarpus microphyllus* Stapf, where it is found together with its diastereoisomer isopilocarpine[1] (2).

1: $R^1 = H$, $R^2 = C_2H_5$
2: $R^1 = C_2H_5$, $R^2 = H$
3: $R^1 = H$, $R^2 = C_2H_5$
4: $R^1 = C_2H_5$, $R^2 = H$

Fig. 1. Structures of (1) pilocarpine, (2) isopilocarpine, (3) pilocarpic acid and (4) isopilocarpic acid.

The properties of pilocarpine include a miotic action, and its chief application is in ophthalmology for lowering the intraocular pressure. For this purpose, buffered isotonic solutions of 0.5–6% pilocarpine nitrate or hydrochloride are used[2]. In aqueous solution, however, pilocarpine may be hydrolysed to pilocarpic acid (3) and epimerized to isopilocarpine (2), which, in turn, is hydrolysed to isopilocarpic acid (4) [3,4]. Both hydrolysis and epimerization result in a decrease in pharmacological activity.

To measure the extent of degradation, many methods have been described for the quantitative determination of pilocarpine, but they all suffer from the disadvantage that they do not distinguish between the four compounds (1)–(4). Recently, several separations of pilocarpine from isopilocarpine, based on the use of high-performance liquid chromatography (HPLC) on a cation-exchange resin, were published[5–7], but pilocarpic acid and isopilocarpic acid could not be determined. We report here that a convenient and accurate separation of the four compounds can be achieved by HPLC on a reversed-phase column.

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EXPERIMENTAL

Pilocarpine (Dutch Pharmacopoeia, 6th ed.) and isopilocarpine were obtained in the form of the hydrochlorides from Verenigde Pharmaceutische Fabrieken (Apeldoorn, The Netherlands). Pilocarpic acid and isopilocarpic acid were obtained by hydrolysis of pilocarpine and isopilocarpine in 0.1 *N* sodium hydroxide solution. A Waters Model 6000 A pump with a Model U6K injector was used in combination with a Waters R401 differential refractometer. The column (30 × 0.4 cm I.D.) was packed with LiChrosorb RP-18 (10 μm) (Merck, Darmstadt, G.F.R.). The flow-rate was set at 1.5 ml/min. The column and the differential refractometer were maintained at 22° by a thermostat.

RESULTS AND DISCUSSION

A suitable method for the determination of pilocarpine in the presence of its degradation products in ophthalmic solution requires a technique with a high specificity and sensitivity, which can operate under mild conditions. The existing methods (nuclear magnetic resonance spectroscopy[4,8], gas–liquid chromatography[9,10] and thin-layer chromatography[11]) lack at least one of these requirements. A new method, using HPLC on a cation-exchange resin[5–7], suffers from the following disadvantages: (i) the retention time increases because of a gradual increase in operating pressure with time; (ii) the use of these resins implies operating conditions (pH 9) where pilocarpine is known to undergo alkali-catalysed hydrolysis; and (iii) the method does not separate pilocarpic acid from isopilocarpic acid.

We separated pilocarpine, isopilocarpine, pilocarpic acid and isopilocarpic acid within 30 min on a reversed-phase column with a mixture of water and methanol (97:3) containing 5% of potassium dihydrogen orthophosphate. The pH was adjusted to 2.5 with orthophosphoric acid (Fig. 2 and Table I). An increase in the methanol–water ratio in the mobile phase resulted in a decrease in the retention times, but the selectivity remained the same. Replacement of methanol with acetonitrile also resulted in a bad separation between pilocarpine and isopilocarpine. The selectivity was improved when higher salt concentrations and a lower pH of the mobile phase were

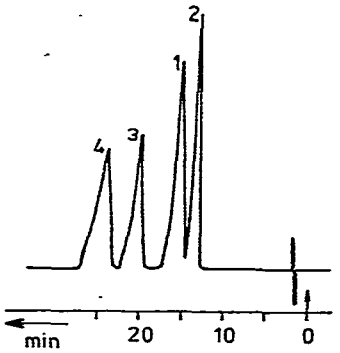

Fig. 2. Separation of pilocarpine (1), isopilocarpine (2), pilocarpic acid (3) and isopilocarpic acid (4) on RP-18 with water–methanol (97:3) containing 5% of potassium dihydrogen orthophosphate, pH 2.5. Detection with a differential refractometer (R401) at 22°.

TABLE I

CAPACITY FACTORS (k') AND DETECTION LIMITS

Compound	k'	*Detection limit (μg)*
Pilocarpine	11.3	6.6
Isopilocarpine	9.7	5.5
Pilocarpic acid	15.5	6.3
Isopilocarpic acid	18.7	9.1

used. These conditions also resulted in less tailing of the peaks. A concentration of 5% potassium dihydrogen orthophosphate and pH 2.5 are optimal conditions as regards the life of the apparatus and the column. Increasing the temperature results in shorter retention times, with almost no improvement in column performance and consequently insufficient resolution.

This HPLC determination of the four compounds seems to be suitable for the analysis of pharmaceutical preparations. In addition, the method offers an opportunity for studying the mechanism of hydrolysis, epimerization and lactonization in the degradation of pilocarpine.

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