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Determination of pilocarpine by HPLC in presence of isopilocarpine and a pH-sensitive polymer in ophthalmic dispersions

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Abstract: Quantification of pilocarpine in the presence of the epimer isopilocarpine in polymeric dispersions is reported. The method describes a technique using reversed-phase HPLC and UV detection of pilocarpine in the presence of a pH-sensitive polymer. As this method does not require prior sample preparation it will be of special interest for process control and development.

Keywords: *Reversed-phase HPLC; UV detection; pilocarpine; isopilocarpine; drug analysis.*

Introduction

The introduction of polymeric dispersions as drug carriers [1] has brought with it a new problem to the drug analyst. Several such dispersions of great pharmaceutical interest are in the submicroscopic size range and the pharmacologically active compounds cannot easily be separated routinely from the macromolecules because the former are incorporated at the molecular level throughout the polymeric network.

Recently, a new submicroscopic dispersion, known as pseudo-latex, containing pilocarpine for the treatment of glaucoma has been reported [2, 3]. These preparations have a prolonged period of action and should therefore induce better patient compliance. It is known that pilocarpine under certain conditions may readily form isopilocarpine, a thermodynamically more stable form [4–6]. This epimer differs from pilocarpine only in the position of the ethyl group on the α-carbon of the lactone ring. Isopilocarpine as well as isopilocarpic acid and pilocarpic acid, both obtained by ring opening, have no pharmacological activity [7, 8]. While pilocarpine can be easily detected by various methods [9–11], a number of procedures have also been developed for detection of the epimer. The simultaneous detection of pilocarpine, isopilocarpine and one [18, 19] or two [6, 20–23] degradation products has been investigated. However, these methods are not applicable in the presence of large amounts of a pH-sensitive polymer without an extraction step.

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Moreover, we were unable to reproduce the method suggested by Noordam *et al.* [22, 23]. After consideration of all the methods reported in recent literature and summarized by Dunn [14] and Van Ackeren [19] in excellent review papers, as well as the attempts to dose pilocarpine in biological fluids [24–26], it was concluded that none of these techniques are applicable in the present situation.

In the present work, the ophthalmic dispersion containing pilocarpine (maximum 4% w/w) is based on a pH-sensitive polymeric carrier (maximum 30% w/w). Before application in the cul-de-sac the nanoparticulate dispersion shows very low viscosity but gels immediately after application. One of the pH-sensitive polymers used in this study is cellulose acetate phthalate, CAP, (Eastman Kodak, Rochester, NY) which dissolves at pH $\geq$5. Historically, for the development of an appropriate analytical technique, a HPLC method mentioned in a technical report from the Swiss Intercantonal Office of the Control of Medicaments, was selected as a starting point [27]. This binary solvent system also was used in an attempt by Van Ackeren [19] in 1984.

Experimental Methods

The solvents and reagents were of analytical grade. Only diethylamine was distilled prior to use. For the high-performance liquid chromatography (HPLC), a solvent delivery system Model 850 and a gradient pump (Du Pont Instruments, USA) with an injector Model 7010 (Rheodyne, Berkley, USA) and a 20 μl loop were used. A radial compression separation system (Z-Module) with an analytical cartridge Resolve C18 (10 cm × 8 mm i.d., Radial-PAK, particle size 10 μm) and a variable wavelength detector operated at 235 nm (Model 481), connected to a data station (Data Module), all from Waters Associates (Milford, MA, USA), were used. The mobile phase consisted of acetonitrile–diethylamine–water (5:1:94, v/v/v), freshly prepared before use and protected from light. The flow rate was 3 ml/min. Chromatography was performed at room temperature. The sample solution contained a maximum of 2.5 mg/ml of pilocarpine hydrochloride (Siegfried, Zofingen, Switzerland) and/or isopilocarpine nitrate (Ega Chemie, Steinheim, Germany) and 18.8 mg/ml CAP (Eastman Kodak, Rochester, USA). Aliquot volumes of these solutions were injected.

Results and Discussion

The HPLC analysis of pilocarpine in the presence of nanodispersed pH-sensitive polymers could be carried out without a clean-up step (Fig. 1). The samples containing between 0.5 and 50 μg of pilocarpine hydrochloride and a maximum of 375 μg of CAP were injected directly into the 20 μl sample loop. If necessary, the samples were diluted with distilled water before injection. The mobile phase immediately converted the polymeric dispersion into a polymeric gel, due to salt formation. The UV absorption maxima of the main components, i.e. CAP, the surfactant Pluronic® F127 (BASF, Wyandotte, USA) and pilocarpine hydrochloride, are in the same region and only a separation technique would enable the quantitative determination of pilocarpine. An external standard curve for pilocarpine hydrochloride was prepared to determine the concentration of pilocarpine hydrochloride by peak area measurements. Figure 1a shows that the constituents of the polymeric dispersion are eluted with the solvent front and that the bioactive material and its epimer (Fig. 1b) are clearly separated. The other degradation products (i.e. pilocarpic acid and isopilocarpic acid) cannot be identified by

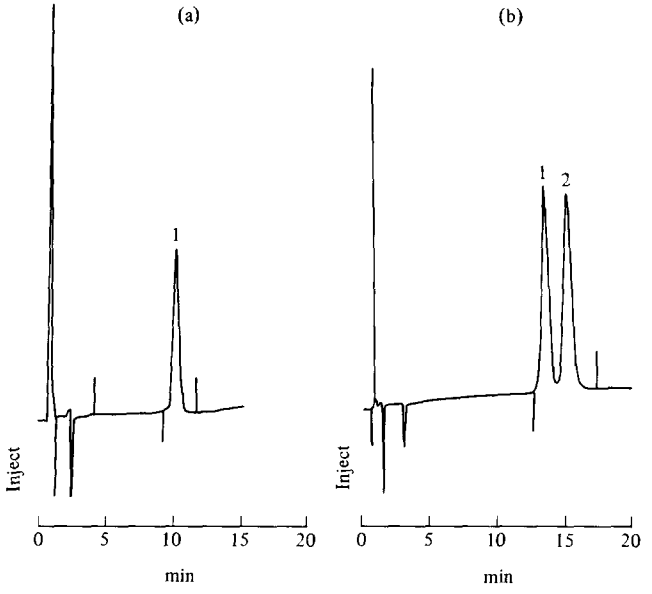

Figure 1
HPLC chromatograms obtained by injection of: (a) polymeric dispersion of CAP containing 4% (w/w) pilocarpine hydrochloride and 30% polymer; peak 1 = pilocarpine; (b) pilocarpine hydrochloride and isopilocarpine nitrate 0.1% (w/v); peak 1 = isopilocarpine; peak 2 = pilocarpine.

this method as they are eluted with the solvent front. The standard curve was found to be linear over the concentration range of 0.125–2.500 mg/ml. A typical standard curve determined prior to a test is described by the following equation which is obtained by linear regression: $y = 0.067 + 0.320x$ ($n = 42$ and $r = 0.9997$), where y is the area under the curve and x the amount injected in μg.

The limit of detection was determined following the recommendation of the International Union of Pure and Applied Chemistry and was set at a signal-to-noise ratio of 2:1 [28]. The limit found was 0.25 μg per injection. Due to partial hydrolysis of pilocarpine in the alkaline mobile phase there was concern about reproducibility. It was found that the rate of hydrolysis is 0.077 min^{-1} at 25°C. It has been shown that the degradation of pilocarpine is a first order reaction: r of 0.9987 ($n = 10$). The reproducibility is adequate under these conditions and the quantitative determination of pilocarpine is feasible. The confidence interval was calculated according to Linder [29] and Fieller [30] for a 10 μg injection of pilocarpine hydrochloride. It was found that the limits using the calibration curve of a typical assay are between 10.27 and 9.65 μg.

The method described has been used successfully for the development of ophthalmic drug delivery systems. A shift in retention time and a change in the characteristics of the reversed phase column packing have to be taken into account owing to the use of an alkaline mobile phase.

Conclusion

The HPLC method described allows the determination of pilocarpine in the presence of the epimer isopilocarpine in an ophthalmic preparation containing a high amount of a

pH-sensitive polymer. The short time of analysis, the absence of the need for sample preparation and the low cost of the mobile phase enable the use of the method for routine quality control as well as for development purposes.

References

[1] R. Gurny, in *Topics in Pharmaceutical Sciences* (D. D. Breimer and P. Speiser, Eds), pp. 277–288. Elsevier, Amsterdam (1983).
[2] R. Gurny, T. Boye, H. Ibrahim and P. Buri, *Proc. Int. Symp. Control. Rel. Bioact. Mater.* **12**, 300–301 (1985).
[3] T. Boye, R. Gurny and P. Buri, *Proc. Eur. Cong. Biopharm. Pharmacokin.* **2**, 188–197 (1984).
[4] M. A. Nunes and E. Brochmann-Hanssen, *J. Pharm. Sci.* **63**, 716–721 (1974).
[5] G. Szepesi, M. Gazdag, R. Ivancsics, K. Mihalyfi and P. Kovacs, *Pharmazie* **38**, 94–98 (1983).
[6] H. Bundgaard and S. H. Hansen, *Int. J. Pharm.* **10**, 281–289 (1982).
[7] R. A. Anderson and J. B. Cowle, *Br. J. Ophthalmol.* **52**, 607–611 (1968).
[8] R. K. Hill and S. Barcza, *Tetrahedron* **22**, 2889–2893 (1966).
[9] S. K. W. Khalil, *J. Am. Chem. Soc.* **66**, 1625–1626 (1977).
[10] M. Kneczke, *J. Chromatogr.* **198**, 529–533 (1980).
[11] A. E. Mair and J. H. McB. Miller, *J. Clin. Hosp. Pharm.* **9**, 217–224 (1984).
[12] B. S. Scott, D. L. Dunn and E. D. Dorsey, *J. Pharm. Sci.* **70**, 1046–1048 (1981).
[13] T. Urbanyi, A. Piedmont, E. Willis and G. Manning, *J. Pharm. Sci.* **65**, 257–260 (1976).
[14] D. L. Dunn, *J. Chromatogr.* **264**, 264–271 (1983).
[15] M. V. Drake, J. J. O'Donnell and R. P. Sandman, *Invest. Ophthalmol. Visual Sci.* **19S**, 68 (1980).
[16] A. K. Mitra, C. L. Baustian and T. J. Mikkelson. *J. Pharm. Sci.* **69**, 257–261 (1980).
[17] D. L. Dunn, B. S. Scott and E. D. Dorsey, *J. Pharm. Sci.* **70**, 446–449 (1981).
[18] J. D. Weber, *J. Assoc. Off. Anal. Chem.* **59**, 1409–1415 (1976).
[19] J. L. Van Ackeren, R. M. Venable and I. W. Wainer, *J. Assoc. Off. Anal. Chem.* **67**, 924–926 (1984).
[20] J. M. Kennedy and P. E. McNamara, *J. Chromatogr.* **212**, 331–338 (1981).
[21] J. J. O'Donnell, R. Sandman and M. V. Drake, *J. Pharm. Sci.* **69**, 1096–1097 (1980).
[22] A. Noordam, L. Maat and H. C. Beyerman, *J. Pharm. Sci.* **70**, 96–97 (1981).
[23] A. Noordam, K. Waliszewski, C. Olieman, L. Maat and H. C. Beyerman, *J. Chromatogr.* **153**, 271–273 (1978).
[24] R. W. Wood and J. R. Robinson, *Int. J. Pharm.* **20**, 285–293 (1984).
[25] M. A. Rendi, P. P. Ellis and J. Gal, *J. Chromatogr.* **336**, 337–344 (1984).
[26] M. Francoeur, I. Ahmed, S. Sitek and T. F. Patton, *Int. J. Pharm.* **16**, 203–213 (1983).
[27] H. Gold and A. Hösli, Technical Report, Intercantonal Office for the Control of Medicaments, Berne, Switzerland (1982).
[28] Nomenclature, symbols, units and their usage in spectrochemical analysis, *Anal. Chem.* **48**, 2294–2295 (1976).
[29] A. Linder, in *Statistische Methoden*, Chap. 6, p. 161. Birkhäuser, Basel (1964).
[30] E. C. Fieller, *Q. J. Pharm. Pharmacol.* **17**, 117–120 (1944).

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