International Journal of Pharmaceutics, 76 (1991) 171–175

ADONIS 037851739100344U

IJP 02552

Stability-indicating assay method for pilocarpine nitrate in reservoirs used in the cystic fibrosis indicator system

Ooi Wong, Carter Anderson, Lori Allaben, Rama Pabmanabhan and Gary Lattin

Iontophoretic Drug Delivery Systems, Medtronic, Inc., Minneapolis, MN 55432 (U.S.A.)

(Received 18 April 1990)
(Modified version received 8 April 1991)
(Accepted 6 June 1991)

Key words: Pilocarpine nitrate; Cystic fibrosis indicator; Sweat testing; HPLC; Stability-indicating method

Summary

A stability indicating HPLC assay method was developed for analysis of pilocarpine nitrate in indicator reservoirs used in the cystic fibrosis indicator iontophoretic device for sweat induction. The method can selectively detect pilocarpine and the degradants, isopilocarpine, pilocarpic acid and isopilocarpic acid.

Introduction

Cystic fibrosis is a hereditary disorder in infants, children, and young adults whose sweat chloride content is abnormally high. The frequency of this disorder is between 0.5 and 1.0% of live births (Cystic Fibrosis Foundation, 1974). A diagnosis of this disease involves quantitative analysis of the chloride content in the sweat, which is generated by iontophoretic delivery of pilocarpine through the skin to stimulate the sweat glands. The success of this quantitative test depends on the technique used in the test, production of sufficient sweat, and the reliability of the quantitative chloride test. The pilocarpine content in the reservoir must be ascertained by a reliable quantitative analytical method. Degradation of pilocarpine has been reported in many pharmaceutical products (Mair and Miller, 1984; Balansard et al., 1986; Ibrahim et al., 1987; Sidhu et al., 1987) with the main degradants being isopilocarpine, pilocarpic acid, and isopilocarpic acid. A selective analytical method is necessary to assay quantitatively the pilocarpine nitrate content in the presence of degradation products. Ultraviolet (UV) spectrophotometric methods are unsuitable due to interference by UV absorption of nitrate ion and degradants such as pilocarpic acid, isopilocarpine and isopilocarpic acid at the lower UV region. A titration method (Official Methods of Analysis, 1984) is also expected to be interfered with by the degradants and may not be sensitive enough for our purpose. Numerous papers have been reported describing HPLC procedures for assay of pilocarpine in the presence of its degradation products (Bundgaard and Hansen,

Correspondence: O. Wong, IDDS, Medtronic, Inc., MS P207, 7000, Central Avenue N.E., Minneapolis, MN 55432, U.S.A.

1982; Szepesei et al., 1983; Rendi et al., 1984; Wood and Robinson, 1984; Popl et al., 1985; Mitra and Mikkelson, 1988; Vespake et al., 1988). However, most reported HPLC methods did not effectively separate pilocarpine and isopilocarpine. In this communication, we report an isolation method and an HPLC stability-indicating assay for pilocarpine nitrate in reservoirs. Pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid were selectively detected by the method.

Experimental

Materials and apparatus Pilocarpine nitrate reservoirs were manufactured by Promeon, a division of Medtronic, Inc., Minneapolis, MN. HPLC grade methanol (0.029% water, Baxter) was used in the mobile phase. Reference standard pilocarpine hydrochloride was obtained from U.S.P.C. Inc., Rockville, MD. Pilocarpine nitrate raw materials (USP grades) obtained from Midland Scientific, EM, and Spectrum Chemicals were dried in a vacuum oven at 105 °C for 2 h before use. Isopilocarpine nitrate (reagent grade) and pilocarpine nitrate (reagent grade) were obtained from Aldrich. Potassium phosphate and sodium choride were purchased from Mallinckrodt.

The release of pilocarpine nitrate from reservoirs was monitored by measuring the absorbance of extraction samples using a Shimadzu UV-160 spectrophotometer equipped with a Shimadzu CPS temperature controller. The temperature of the cell compartment was maintained at approx. 20.0 °C. The stability of the spectrophotometer was ascertained by recording the absorbance of the 0.9% saline solution at 230 nm over a period of 4 h. No significant change in absorbance was observed.

HPLC apparatus and conditions An HPLC stability-indicating method for the pilocarpine nitrate reservoir was developed using the following apparatus and conditions. All the pilocarpine samples were analysed using Hewlett Packard (HP) 1090 M liquid chromatography apparatus equipped with an HP 1040 M UV-VIS Diode Array detector and an HP chemstation data acquisition system, together with a reverse-phase column (Supelcosil LC-18 DB P/N 5-8358; 5 μm) of 25 cm × 4.6 mm i.d., obtained from Supelco. A guard column (Supelcosil LC-18 DB P/N 5-9565) obtained from Supelco was used in conjunction with the HPLC apparatus. The HPLC conditions were as follows: injection volume, 50 μl; flow rate, isocratic at 1.5 ml/min; column temperature, 60 °C; mobile phase, methanol (20%): monobasic potassium phosphate (0.073 M) (pH 7.7) (80%); detection wavelength, 215 nm.

Generation of degradants Pilocarpic and isopilocarpic acid were prepared by hydrolysis of pilocarpine nitrate and isopilocarpine nitrate, respectively, in 0.1 M KOH for 2 h. The alkaline solution was acidified to pH 5.5 using 85% phosphoric acid.

Treatment of the cystic fibrosis pilocarpine nitrate reservoir extracts The hydrogel began to break into pieces after 6 h of agitation. Subsequently, the debris caused problems in obtaining clear solutions for absorbance measurements. Two centrifuge test tubes were filled separately with 5–7 ml of the white milky extraction solution. The test tubes were centrifuged (Beckman model TJ-6) at 5000 rpm for 30 min. The test tubes with clear supernatant were carefully transferred to a test tube rack. A 3 ml plastic syringe equipped with a precision glide needle (BD 21G1) (both from Becton Dickinson and Co., Rutherford, NJ) was used to withdraw the clear supernatant. The needle was replaced with a Nylon Acrodisc 0.45 μm disposable filter assembly (Gelman Sciences). The solution was forced through the filter assembly. 1.0 ml of the clear filtrate was diluted to 6.0 ml with 0.9% saline solution for UV absorbance measurement or for HPLC analysis.

HPLC analysis of the pilocarpine extracts The pilocarpine nitrate was extracted according to the procedure described above. Samples were withdrawn at appropriate time points and analyzed by HPLC.

HPLC stability-indicating method The stability-indicating method was developed by considering (a) precision, (b) accuracy, (c) linearity and range, (d) selectivity and (e) limit of detection of the method. The method was applied to the anal-

ysis of some freshly manufactured and some expired pilocarpine nitrate reservoirs. A reference standard pilocarpine hydrochloride was used in the standard curves and for calibration of a pilocarpine nitrate raw material. Prior to chromatographic analysis of reservoir extraction samples, a standard curve was prepared from a series of aqueous standard solutions prepared from pilocarpine nitrate reference material. The reservoir extract samples were analysed by HPLC and the standard curve prepared. Pilocarpine concentrations in samples were calculated using the absorbance peak area in conjunction with the linear relationship derived from the standard curve.

The UV spectra of degradants (isopilocarpine, isopilocarpic acid and pilocarpic acid), nitrate, and pilocarpine as recorded on the HP diode array detector and chemstation data acquisition system were taken directly from the print out. The absorption spectra are similar for all these compounds.

Results and Discussion

The extraction method for the analysis of pilocarpine from the reservoir was developed considering two potential problems: the stability of pilocarpine under the extraction conditions, and the efficiency and the quantitation of the extraction method. Stability of pilocarpine has been extensively investigated in the past (Conners et al., 1986). Basically, in solution there are two degradative reactions: (a) the epimerization about the α-carbon to form isopilocarpine, and (b) the hydrolysis of the ester linkage of the lactone ring, resulting in production of pilocarpic acid and isopilocarpic acid. The hydrolysis reaction is susceptible to both specific acid/base and general acid/base catalyses, although the water catalytic constant has not been reported in the literature, possibly due to its very low value. Epimerization occurs predominantly at alkaline pH. From the pH-rate profile of pilocarpine, it is predicted that the pH of maximum stability is about 5.2. Under the present extraction conditions, using 0.9% saline solution or deionized water, pilocarpine is expected to be stable, as shown by the quantitative recovery of pilocarpine from some new reservoirs (discussed further below, under *Accuracy*). The efficiency and the quantitation of the extraction method were demonstrated by the release profile of pilocarpine and the quantitative recovery of pilocarpine, as discussed below.

Release of pilocarpine by HPLC analysis of the pilocarpine extracts

A release study indicates that after the reservoir began to disintegrate after about 6 h of agitation, the UV absorbance of the extracts increased at a much faster rate and, eventually, the total quantity of apparent pilocarpine nitrate exceeded the label claim. This is due to UV absorbance interference of ingredients released into the extract and the non-selectivity of the UV spectrophotometric assay at 230 nm. The similarity in the spectra makes UV spectrophotometry an unsuitable method for our purpose. The absorbance of the extract is due to the total absorbance of all ingredients present and, therefore, with a different ratio of the ingredients quantitative assay would be very difficult. In order to assay quantitatively the pilocarpine in the extract, it is necessary to employ a selective method such as HPLC to isolate pilocarpine from the impurities. An independent experiment by HPLC analysis was carried out to determine the pilocarpine equilibrium extraction time. The results shown in Fig. 1 indicate that the minimum equilibrium time is 6 h.

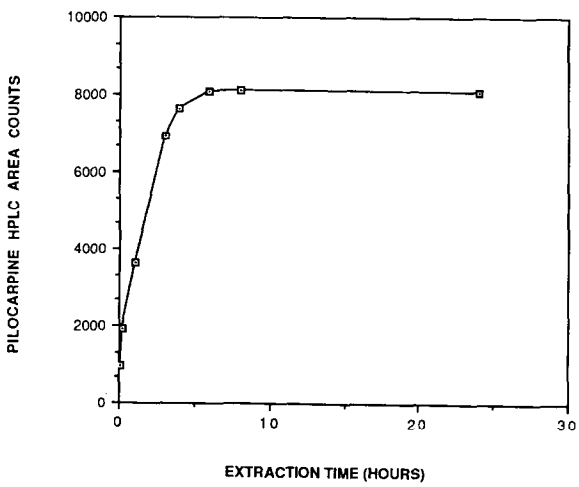

Fig. 1. Equilibration of the pilocarpine extraction.

Stability-indicating assay of pilocarpine

Precision of injection and detection system stability Six individual injections of a pilocarpine hydrochloride solution of 323.4 μg/ml were analysed by the HPLC system and the peak area counts of pilocarpine for each injection were recorded. The relative standard deviation of the six analyses was 0.23%.

Accuracy Six freshly (about 1 month old) manufactured reservoirs containing 1.0% of pilocarpine nitrate were extracted by the present extraction method and assayed for pilocarpine nitrate content by the stability-indicating HPLC method. The average recovery percentage is 99.1% with a relative standard deviation of 0.32%.

The recovery percentage of pilocarpine nitrate from the reservoirs demonstrates the accuracy of the HPLC analytical method. The HPLC method was further applied to analysis of pilocarpine nitrate raw materials obtained from three different vendors:

Vendors	*Purity (%)*
Spectrum Chemicals	99.7
EM	99.7
Midland Scientific	100.2

Linearity and range Seven standard solutions of pilocarpine nitrate were prepared and each standard solution was analysed three times by HPLC. The average peak area counts were calculated and plotted vs the concentrations (μg/ml) of the standard solutions. The slope of the regression line is 27.7 with a negative intercept, -36.3 and the correlation coefficient is 1.00. The regression analysis is based on pilocarpine nitrate concentrations ranging from 8.0 to 970.0 μg/ml.

Selectivity The selectivity of the HPLC analytical method is demonstrated by acid hydrolysis of pilocarpine and isopilocarpine to generate pilocarpic acid and isopilocarpic acid (see Experimental). To demonstrate the ability of the present HPLC method to detect selectively pilocarpine and isopilocarpine, which can be difficult to separate chromatographically, a mixture of the two compounds was analysed by the method. At pH 5.5, where pilocarpine is most stable, pilocarpine and isopilocarpine were added to the mixture of degradants in order to see whether the HPLC method could separate and detect all ingredients. The chromatogram of such a mixture is shown in Fig. 2, which shows a nitrate peak at t_R of 1.036 min, a pilocarpic acid peak at 2.722 min,

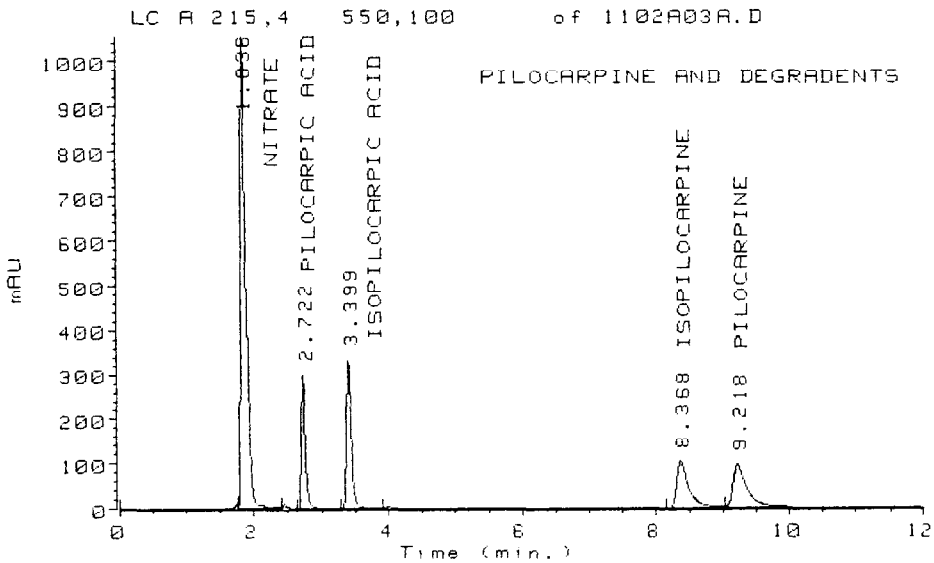

Fig. 2. Chromatogram of a pilocarpine solution containing all of its degradants.

an isopilocarpic acid peak at 3.399 min, an isopilocarpine peak at 8.368 min, and a pilocarpine peak at 9.218 min. All the peaks of the compounds are well separated.

Limit of detection The lowest concentration of pilocarpine nitrate showing a statistically significant difference from the sample blank was 1.62 μg/ml. The lowest and the highest concentrations of pilocarpine nitrate with relative standard deviation less than 5% were 8.09 and 970 μg/ml, respectively (see *Linearity and range*).

Degradants The degradants generated by acid hydrolysis were pilocarpic acid and isopilocarpic acid. This is due to instability of both pilocarpine and isopilocarpine under highly alkaline conditions (0.1 M KOH).

Chromatograms of expired reservoirs In order to detect degradants generated in reservoirs, a new (1 month old) and an expired reservoir (5 years old) were extracted and examined for degradants by the HPLC analytical method. For the new reservoir, the major peaks are for pilocarpine (t_R = 9.04 min) and nitrate (t_R = 1.8 min). However, for the old reservoir, detectable amounts of pilocarpic acid (t_R = 2.71 min), isopilocarpic acid (t_R = 3.40 min) and isopilocarpine (t_R = 8.3 min) were observed.

Conclusions

A stability-indicating HPLC assay method has been developed for analysing the pilocarpine nitrate content in reservoirs used in the Medtronic Cystic Fibrosis Indicator System.

References

Balansard, G., Vidal, E., Guigues, M., Ollivier, B. and De Meo, M., Pilocarpine and isopilocarpine determinations by normal-phase high performance liquid chromatography. Application to the study of the stability of pilocarpine based collyria and comparison of this method with a colorimetric method using hydroxylamine. *Pharm. Acta Helv.*, 61 (1986) 47–50.

Bundgaard, H. and Hansen, S.H., Hydrolysis and epimerization kinetics of pilocarpine in basic aqueous solution as determined by HPLC. *Int. J. Pharm.*, 10 (1982) 281–289.

Conners, K.A., Amidon, G.L. and Stella, V.J., *Chemical Stability of Pharmaceuticals*, 2nd Edn, Wiley, New York, 1986, pp. 675–684.

Cystic Fibrosis Foundation, Guide to diagnosis and management of cystic fibrosis, 2nd Revised Edn. Rockville, MD, Cystic Fibrosis Foundation, 1974.

Ibrahim, H., Boye, T., Wermeille, M., Gurny, R. and Buri, P., Determination of pilocarpine by HPLC in presence of isopilocarpine and a pH-sensitive polymer in ophthalmic dispersions. *J. Pharm. Biomed. Anal.*, 5 (1987) 379–382.

Mair, A.E. and Miller, J.H.McB., Effect of sterilization by autoclaving on the stability of eye drops of pilocarpine hydrochloride and physostigmine sulfate. *J. Clin. Hosp. Pharm.*, 9 (1984) 217–224.

Mitra, A.K. and Mikkelson, T.J., Mechanism of transcorneal permeation of pilocarpine. *J. Pharm. Sci.*, 77 (1988) 771–775.

Official Methods of Analysis, 14th Edn, Association of Official Analytical Chemists, Inc., 1984, p. 739.

Popl, M., Ky, L.D. and Strnadova, J., Reversed phase liquid chromatography of some alkaloids. *J. Chromatogr. Sci.*, 23 (1985) 95–100.

Rendi, M.A., Ellis, P.P. and Gal, J., High performance liquid chromatographic assay for pilocarpine in aqueous humor. *J. Chromatogr. Biomed. Appl.*, 336 (1984) 337–344.

Sidhu, A.S., Kennedy, J.M. and Deeble, S., General method for the analysis of pharmaceutical dosage forms by high performance liquid chromatography. *J. Chromatogr.*, 391 (1987) 233–242.

Szepesei, G., Gazdag, M., Ivancsics, R., Mihalyfi, K. and Kovacs, P., Investigation of degradation mechanism of pilocarpine by HPLC. *Pharmazie*, 38 (1983) 94–98.

Vespake, R., Fiedorova, M. and Subert, J., Contributions to drug analysis. XVII. Problems connected with HPLC separation of pilocarpine from its degradation products by means of silica gel and water-based mobile phase. *Pharmazie*, 43 (1988) 689–691.

Wood, R.W. and Robinson, J.R., High performance chromatographic determination of pilocarpine and pilocarpic acid in ocular tissues. *Int. J. Pharm.*, 20 (1984) 285–293.