(2) M. A. Loutfy and H. A. Abu-Shady, *ibid.*, **31**, 573 (1976).
(3) M. A. Loutfy, M. A. Hassan, A. I. Jado, and H. A. Abu-Shady, *ibid.*, **31**, 819 (1976).
(4) M. A. Loutfy, Ph.D. thesis, University of Riyadh, Riyadh, Saudi Arabia, 1976.
(5) M. A. Loutfy and H. A. Abu-Shady, *Pharmazie*, **32**, 240 (1977).
(6) L. Musajo and G. Rodighiero, "Photophysiology," vol. VII, A. C. Giese, Ed., Academic, New York, N.Y., 1972, p. 115.
(7) A. Mustafa, "Furopyrans and Furopyrones," Interscience, London, England, 1967, p. 14.
(8) F. Dall'Acqua, S. Marciani, D. Vedaldi, and G. Rodighiero, *Biochim. Biophys. Acta*, **353**, 267 (1974).
(9) M. A. Pathak, J. H. Fellman, and K. D. Kaufman, *J. Invest. Dermatol.*, **35**, 165 (1960).
(10) L. R. Worden, A. W. Bergstahler, K. D. Kaufman, J. A. Weis, and T. K. Schaaf, *J. Heterocycl. Chem.*, **6**, 191 (1969).
(11) A. Schonberg and A. Sina, *J. Am. Chem. Soc.*, **72**, 4827 (1950).

ACKNOWLEDGMENTS AND ADDRESSES

Received October 12, 1976, from the *Pharmaceutical Chemistry Research Laboratory, Pharmaceutical Chemistry Department, College of Pharmacy, University of Riyadh, Riyadh, Saudi Arabia.*

Accepted for publication January 11, 1977.

The authors gratefully acknowledge the assistance of Dr. G. Rodighiero and Dr. F. R. Baccichetti, Pharmaceutical Chemistry Institute, University of Padova, Padova, Italy, in securing the pharmacological data and for help and advice. The authors are also indebted to Dr. G. Schwenker, College of Pharmacy, University of Heidelberg, Heidelberg, West Germany, for carrying out some spectral analyses.

* Present address: Department of Pharmaceutical Chemistry, University of Cairo, Cairo, Egypt.

ˣ To whom inquiries should be directed.

High-Speed Liquid Chromatographic Determination of Pilocarpine in Pharmaceutical Dosage Forms

S. K. WAHBA KHALIL

Abstract □ A specific method for the direct determination of pilocarpine in aqueous pharmaceuticals in the presence of decomposition products, methylcellulose, and other ingredients usually present in pharmaceuticals is described. The method involves separation by high-speed liquid chromatography using, in series, octadecylsilane bonded to silica and cyanopropylsilane bonded to silica columns and a tetrahydrofuran–pH 9.2 borate buffer (3:7) eluant. Quantitation is achieved by monitoring the absorbance of the effluent at 254 nm and using a pyridine internal standard and a calibration curve prepared from known concentrations of pilocarpine nitrate. The reproducibility of the retention time and peak area was better than 2.0%.

Keyphrases □ Pilocarpine—high-speed liquid chromatographic analysis, pharmaceutical preparations □ High-speed liquid chromatography—analysis, pilocarpine in pharmaceutical preparations □ Ophthalmic cholinergics—pilocarpine, high-speed liquid chromatographic analysis, pharmaceutical preparations

Many methods for estimating pilocarpine and its salts are available. Several are based on the titration of the extracted base with standard acid, with bromphenol blue (1–3), methyl red (4, 5), methyl orange (6), or dimethyl yellow (7) as the indicator. Several gravimetric (8–10), colorimetric (11), and iodometric (12) methods were developed. Recently, a GLC method using electron-capture detection was employed (13).

The USP XIX monographs for pilocarpine hydrochloride (14) and pilocarpine nitrate (15) incorporate a nonaqueous titration. However, the monographs on pilocarpine hydrochloride ophthalmic solution (15) and pilocarpine nitrate ophthalmic solution (16) require an extraction procedure, followed by colorimetric determination. Most of these methods lack the specificity and simplicity desired in routine analysis, especially in the presence of degradation products and/or other ingredients. In addition, the extraction of pilocarpine base from pharmaceuticals followed by colorimetric determination leads to erroneous results[1].

Recently, high-speed liquid chromatography (HSLC) was used to analyze alkaloids (17, 18). This paper describes an HSLC method that permits the quantitative determination of pilocarpine in the presence of its degradation products and other chemicals commonly included with pilocarpine salts in pharmaceutical products.

Table I—Calibration Data[a] for HSLC Pilocarpine Analysis

Concentration Added, mg/ml	Peak Area of Pilocarpine / Peak Area of Standard
5	0.141 ± 0.0065[b]
10	0.286 ± 0.0104
20	0.562 ± 0.0084
30	0.830 ± 0.0080

[a] Average of 10 replicate injections. [b] Standard deviation.

EXPERIMENTAL

Apparatus—A high-speed liquid chromatograph[2] equipped with a pump[3], a single-wavelength UV monitor (254 nm), and a liquid chromatograph injector[4] was used.

Columns—An octadecylsilane bonded to silica[5] column and a cyanopropylsilane[6] column were used in series. Both columns were 300-mm long × 4-mm i.d. stainless steel tubes.

Reagents—Sodium borate, tetrahydrofuran, and pyridine were reagent grades. Pilocarpine nitrate USP was used without further purification.

[1] U.S. Pharmacopeia, Drug Standards Division, personal communication.
[2] Model 202, Waters Associates.
[3] M-6000.
[4] U6K Universal.
[5] μBondapak C_{18}, Waters Associates.
[6] μBondapak CN, Waters Associates.

Table II—Analysis and Recovery of Pilocarpine from Pharmaceutical Preparations by HSLC

Preparation	Labeled Amount, mg/ml	Amount Added, mg/ml	Amount Found, mg/ml: Before Addition	Amount Found, mg/ml: After Addition	Recovery, %
Ophthalmic buffered solution	20	20	19.6	39.4	99.0 ± 1.0[a]
Ophthalmic solution containing pilocarpine hydrochloride and hydroxypropyl methylcellulose	10	20	10.3	20.2	99.5 ± 0.5
Ophthalmic solution containing pilocarpine hydrochloride and methylcellulose	100	50	97.5	148.2	101.4 ± 1.4
Ophthalmic solution containing pilocarpine nitrate, polyvinyl alcohol, chlorobutanol, and volatile oil constituents	10	10	10.1	20.1	100.0 ± 0.0

[a] Standard deviation.

Mobile Phase—The mobile phase, consisting of 30% tetrahydrofuran and 70% pH 9.2 borate buffer, was degassed by applying a water aspirator vacuum for 1 hr.

Internal Standard Solution—Pyridine, 50 μl/100 ml of water, was used.

Standard Pilocarpine Nitrate Solution—A 10-g/100 ml pilocarpine nitrate aqueous solution was used.

Conditions for Chromatographic Analysis—The degassed mobile phase was pumped through the columns at 1.0 ml/min (3000–3500 psi) at ambient temperature until a stable baseline was obtained.

Preparation of Standard Curve—Replicate 10-, 15-, and 20-μl injections of pyridine–pilocarpine nitrate (1:4) solutions were made using a 25-μl syringe[7]. The chromatograms were recorded[8] at a chart speed of 5 mm/min. The output from the UV monitor at 0.04 absorbance unit was fed into an electronic integrator[9] where the peak areas were integrated.

Interferences—The possible interferences from hydroxypropyl methylcellulose and benzalkonium chloride were studied by adding different amounts to a standard pilocarpine nitrate solution and performing the analysis.

The interference of decomposition products was studied also by rendering a sample of pilocarpine nitrate solution strongly alkaline with sodium hydroxide solution and refluxing for 30 min; the pH was adjusted to 7.0, the solution was diluted to volume, and the analysis was performed.

[7] Precision Sampling Corp., Baton Rouge, La.
[8] Perkin-Elmer model 56.
[9] Hewlett-Packard model 3370 B with digital printout.

Sample Preparation and Analysis—An aliquot of the pilocarpine preparation containing 0.5–1.0 g of pilocarpine as the free base was measured into a 10-ml volumetric flask, and 2 ml of the standard pyridine solution was added. Then 20 μl of the mixture was injected, and the amount of pilocarpine was calculated by comparison with a standard or the standard curve.

RESULTS AND DISCUSSION

The utilization of a mobile phase consisting of 30% tetrahydrofuran and 70% pH 9.2 borate buffer gave well-resolved, sharp peaks for pilocarpine and pyridine, with retention times of 5.0 and 9.3 min, respectively. The use of the columns in series afforded a better separation, especially between pilocarpine and hydroxypropyl methylcellulose. The ratio of the area of the pilocarpine peak to the area of the internal standard (pyridine) was calculated (Table I). The data show excellent linearity and reproducibility.

The method was applied to four pharmaceutical preparations containing pilocarpine salts to determine its suitability. These preparations were analyzed before and after the addition of known quantities of standard pilocarpine nitrate solution (Table II).

The proposed method allows for direct sampling from aqueous solutions of pilocarpine salts and eliminates the disadvantages of extraction. Hence, the method is suited for the routine quality control of pilocarpine salts in ophthalmic solutions.

REFERENCES

(1) J. M. Kolthoff, *Biochem. Z.*, **162,** 289 (1925).
(2) H. Wales, *Ind. Eng. Chem.*, **18,** 390 (1926).
(3) F. Reimers, K. R. Gottlieb, and V. A. Christensen, *J. Pharm. Pharmacol.*, **20,** 99 (1947).
(4) I. S. Shupe, *J. Assoc. Offic. Agr. Chem.*, **24,** 757 (1941).
(5) G. R. Lynch and 12 collaborators, *Analyst*, **73,** 311 (1948).
(6) V. Wurtzen, *Dan. Tidsskr. Farm.*, **13,** 160 (1939).
(7) J. M. Kolthoff, *Biochem. Z.*, **162,** 289 (1925).
(8) H. Matthes and O. Rammstedt, *Arch. Pharm.*, **245,** 131 (1907).
(9) G. Heikel, *Chem. Centr.*, **80,** 949 (1909).
(10) M. Mascre and J. Loiseau, *Bull. Sci. Pharmacol.*, **48,** 272 (1941).
(11) I. S. Gibbs and M. M. Tuckerman, *J. Pharm. Sci.*, **59,** 395 (1970).
(12) G. N. Thomis and D. Jatrides, *J. Pharm. Chem.*, **21,** 585 (1935).
(13) S. W. Dziedzic, S. E. Gitlow, and D. L. Krohn, *J. Pharm. Sci.*, **65,** 1262 (1976).
(14) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 384.
(15) *Ibid.*, p. 385.
(16) *Ibid.*, p. 386.
(17) C.-Y. Wu and S. Sigga, *Anal. Chem.*, **44,** 1499 (1972).
(18) J. N. Done, J. H. Knox, and J. Loheac, "Applications of High-Speed Liquid Chromatography," Wiley, London, England, 1974, p. 164.

ACKNOWLEDGMENTS AND ADDRESSES

Received November 26, 1976, from the *College of Pharmacy, North Dakota State University, Fargo, ND 58102.*

Accepted for publication January 11, 1977.