Table IV—Precision and Accuracy in Assay of Added Aprindine to Human Plasma

	In Vitro Pools, μg/ml			
	0.050	0.200	0.400	0.800
Number of replicates	5	5	5	5
Mean, μg/ml	0.048	0.200	0.390	0.796
CV, %	9.32	3.54	4.80	2.45
Number of replicates	5	5	5	5
Mean, μg/ml	0.046	0.202	0.400	0.796
CV, %	11.91	2.21	1.77	1.43
Number of replicates	5	5	5	5
Mean, μg/ml	0.058	0.210	0.408	0.814
CV, %	7.71	3.37	2.05	2.55
Overall precision				
Between-day *CV*, %	17.4	3.40	2.07	1.54
Within-day *CV*, %	9.50	3.10	3.14	2.21
Total *CV*, %	19.9	4.60	4.26	2.69
Overall accuracy				
Mean, μg/ml	0.051	0.204	0.399	0.802
Total relative error, %	+1.33	+2.00	−0.07	+0.25

spectrograms of aprindine. The protonated $[M + H]^+$ ion at *m/e* 323 and fragmentation ions at *m/e* 113, 117, and 207 confirmed the presence and integrity of aprindine in the plasma of subjects administered the drug.

The accuracy and precision of the method were determined by preparing four plasma pools of known aprindine concentrations. The same analyst assayed five replicates from each pool on each of 3 days (Table IV). Good reproducibility was indicated by the within-day coefficients of variation of 9.50, 3.10, 3.14, and 2.21% for 0.05, 0.20, 0.40, and 0.80 μg of aprindine/ml, respectively, as well as by the between-day variations of 17.4, 3.40, 2.07, and 1.54% at the same concentrations. The total coefficients of variation were 19.9, 4.60, 4.26, and 2.69%, respectively. The overall relative errors at 0.05, 0.20, 0.40, and 0.80 μg of aprindine/ml were +1.33, +2.00, −0.07, and +0.25%, respectively.

Because of the specificity, sensitivity, precision, and accuracy of the reported method for the determination of aprindine in plasma, bioavailability and pharmacokinetic studies of aprindine can be undertaken at therapeutic doses. These studies will be reported later.

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NOTES

Measurement of Pilocarpine and Its Degradation Products by High-Performance Liquid Chromatography

JAMES J. O'DONNELL ˣ, ROBERT SANDMAN, and MICHAEL V. DRAKE

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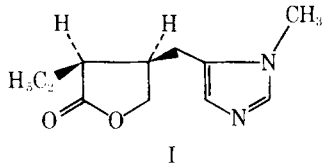
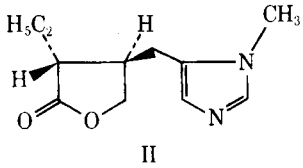
Abstract □ An assay method for pilocarpine using reversed-phase high-performance liquid chromatography is presented. This method also measures isopilocarpine, the stereoisomer of pilocarpine, and the degradation products pilocarpic acid and isopilocarpic acid. Maximum sensitivity was obtained with optical absorbance at 216 nm.

Keyphrases □ Pilocarpine—reversed-phase high-performance liquid chromatographic analysis □ High-performance liquid chromatography—analysis, *pilocarpine*, isopilocarpine, pilocarpic acid, and isopilocarpic acid □ Ophthalmic drugs—pilocarpine, high-performance liquid chromatographic analysis

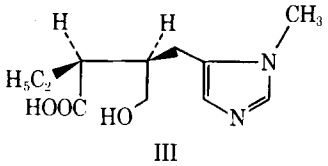
Pilocarpine (I) is a natural alkaloid used for the parasympathomimetic treatment of glaucoma. Isopilocarpine (II), the therapeutically inactive stereoisomer of pilocarpine, is present in nature and in commercial preparations of pilocarpine (1, 2). The degradation products,



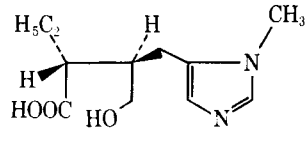
I



II



III



IV

pilocarpic acid (III) and isopilocarpic acid (IV), also are found in commercial products (3). This report presents an efficient and sensitive assay for I–IV using reversed-phase high-performance liquid chromatography (HPLC). This method is a modification of a procedure described recently (4).

EXPERIMENTAL

Pilocarpine hydrochloride USP[1] and isopilocarpine hydrochloride[2] were obtained commercially. Pilocarpic acid and isopilocarpic acid were prepared by hydrolysis of pilocarpine and isopilocarpine in 0.1 *N* NaOH. Isocratic reversed-phase chromatography was accomplished by pumping[3]

[1] Mallinckrodt, St. Louis, Mo.
[2] Aldrich, Milwaukee, Wis.
[3] Model 310 high-performance liquid chromatograph, Altex Scientific Inc., Berkeley, Calif.

0022-3549/80/0900-1096$01.00/0

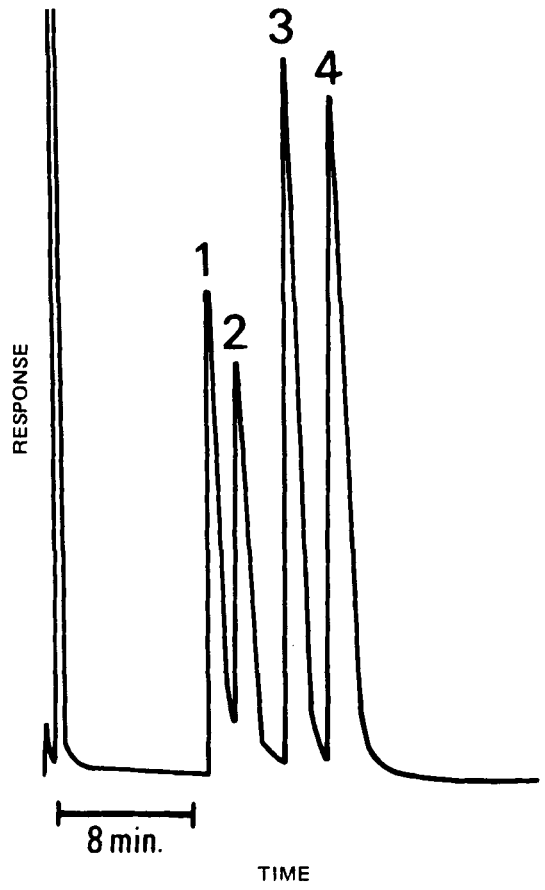


Figure 1—*HPLC separation of isopilocarpine (1), pilocarpine (2), pilocarpic acid (3), and isopilocarpic acid (4).*

97% H_2O–3% CH_3OH containing 5% KH_2PO_4 (pH 2.5) through a C_{18} column[4] at 1.5 ml/min at ambient temperature. The compounds were detected[5] by their optical absorbance at 216 nm.

RESULTS AND DISCUSSION

The chromatographic separation of pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid is shown in Fig. 1. The maximum sensitivity obtainable in this system with a peak height signal of twice the short-term baseline noise is 300 ng. Detection of isopilocarpine in pilocarpine dosage forms is a problem in pilocarpine analysis. Since the isopilocarpine and pilocarpine peaks overlap, the lower limit of detection of isopilocarpine in the presence of pilocarpine by this method is one part of isopilocarpine in 100 parts of pilocarpine.

Despite long ophthalmic use, sensitive measurement of pilocarpine and its metabolites has been possible only recently. The USP method uses extraction and an insensitive colorimetric determination (5). Polarographic (6), GLC (7, 8), and NMR (3) assays have been reported. HPLC offers the convenience of speed, selectivity, sensitivity, mild and nondestructive conditions, and direct measurement. Ion-exchange HPLC (1, 2, 9) has the disadvantages of variable retention time, poor resolution, and destructive alkaline pH. The method used here was adapted from a reported reversed-phase HPLC method that used refractive index detection (4). Optical absorbance detection at 216 nm was used for increased ease and sensitivity. External standards can be used for pilocarpine analysis.

Sensitive measurement of pilocarpine and its metabolites will allow studies of the purity of dosage forms and investigation of the pharmacokinetics of pilocarpine.

[4] Lichrosorb RP C_{18} (10 μm) in 4.6 × 250-mm column, Altex Scientific Inc., Berkeley, Calif.

[5] Model 785 variable-wavelength detector, Micromeritics Instrument Corp., Norcross, Ga.

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