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Ocular Delivery of Pilocarpine from Erodible Matrices

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Abstract □ The present study examined the feasibility of sustaining the release of a water-soluble drug, pilocarpine, to the tear film. Both gels and dried films were utilized as drug delivery systems. *In vitro* studies demonstrated significant prolongation of drug release from these systems as compared with simple aqueous or viscous solutions. The *in vitro* results were supported by *in vivo* miosis studies in albino rabbits.

Keyphrases □ Pilocarpine—sustained release, polymeric delivery systems, *in vitro* release studies, *in vivo* miosis in rabbits □ Drug delivery systems—polymeric gels and films, pilocarpine, sustained release to the cornea, rabbits □ Polymeric matrices—sustained release of pilocarpine to the cornea, *in vivo* miosis in rabbits, *in vitro* release studies

It is well established that ocular drug bioavailability from topical dosing is dependent on several factors, one of the most prominent being the contact time of drug with tissues in the precorneal area of the eye (1). Several investigators (2, 3) have reported that the relatively low-viscosity ophthalmic solutions, while able to somewhat improve the amount of drug that penetrates the cornea to the anterior chamber, generally do not provide a sustaining effect. Conversely, Schoenwald *et al.* (4) and Patton and Robinson (2) have demonstrated that moderately viscous systems, such as gels, can sustain drug delivery to the cornea and anterior segment of the eye. Furthermore, it is known that slowly dissolving lamellae remain in the eye for extended periods of time. Therefore, it is of interest to examine and assess these systems for their potential in ocular drug delivery.

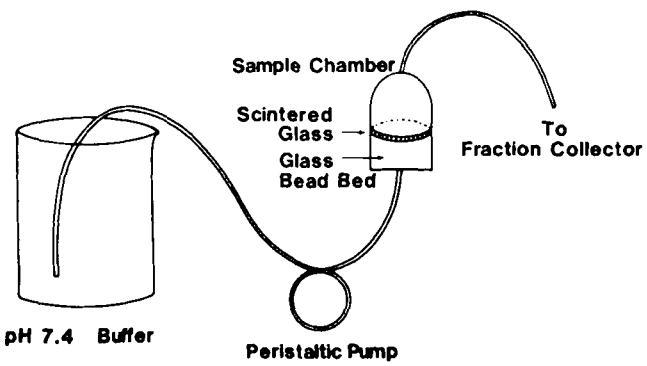

Figure 1—In vitro *dissolution apparatus used to measure appearance of pilocarpine from solutions and lamellae.*

A chronic problem with dissolving hydrogels is their usual high water content, which allows low molecular weight substances to diffuse quickly out of the gel, leaving a "ghost" without active ingredients. Extensive cross-linking of polymers, or dehydration of the gel to a dry film state, could measurably improve the release characteristics of the system.

Due to the need for frequent dosing of pilocarpine from commercial solution preparations, the expense of currently marketed controlled-release devices, and because extensive work has been published regarding ocular absorption of pilocarpine from both solutions and various gel systems, this drug was selected as a model compound for release from several types of ocular polymeric systems.

EXPERIMENTAL

Materials—Reagent-grade polyvinyl alcohol (PVA)[1], with an average mol. wt. of 16,000, and carboxyvinyl copolymer (carbomer 934)[2] were used as received. Tritiated pilocarpine[3], with a specific activity of 20 mCi/mg, was purified by vacuum evaporation immediately prior to use (5). All other chemicals were reagent or analytical grade.

Male albino rabbits[4], weighing 2.5–3.5 kg, were used throughout the study. No special dietary restrictions were used for the animals, and they were maintained in a normal lighting and auditory environment.

Methods—*Preparation of Solutions and Films*—A 4% w/v solution of pilocarpine nitrate, made isotonic with sodium chloride, was prepared in pH 7.4 Sorensen's phosphate buffer. To 10 mL of this solution, tritiated pilocarpine (10 μL) was added to yield a specific activity of ~1.5 μCi/mL. A negligible change in pilocarpine solution concentration occurred through this spiking procedure.

Pilocarpine, 2% in 10% PVA in phosphate buffer, was prepared with a specific activity of ~0.9 μCi/mL. Solutions were refrigerated between uses and were discarded within 7 d.

Erodible polymer films containing drug were prepared in the following manner. The 10% PVA solution in distilled water (40 mL) was poured into

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Carbopol 934; B. F. Goodrich, Cleveland, Ohio.
[3] New England Nuclear, Boston, Mass.
[4] Sasko, Omaha Neb.

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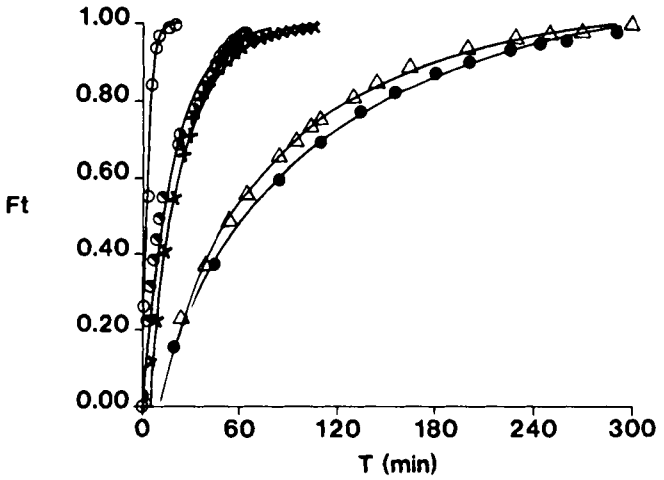

Figure 2—*Plots representing appearance of pilocarpine from solutions and lamellae. Points represent data from* in vitro *experiments. Curves were fit by nonlinear regression, r >0.99 for all formulations. Standard error of the mean is less than point size for all formulations. Key: Ft is fraction of drug released at t; (○) 25-μL solution (1.0 cps); (◑) 25-μL solution (60 cps); (×) erodible lamellae (both); (△) nonerodible lamella (no carbomer 934); (●) nonerodible lamella (with carbomer 934).*

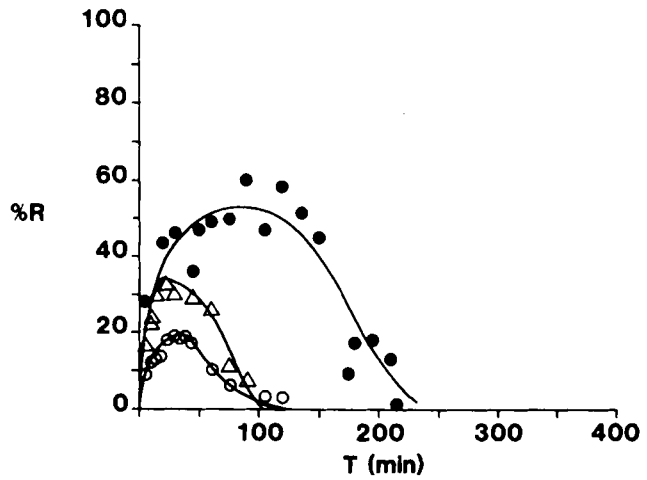

Figure 3—In vivo *comparison of miotic response to a 70-μg dose of pilocarpine nitrate administered as solutions and erodible lamellae. Key: (○) 25-μL solution (1.0 cps); (△) 25-μL solution (60 cps); (●) erodible lamellae (both).*

a glass petri dish and allowed to gel for 1 h at 4°C. A 40-mL volume of 2% pilocarpine (specific activity of ~2.75 μCi/mL) in 4% boric acid solution was layered on the PVA gel, covered[5], and allowed to remain at room temperature for 2 h before being refrigerated for an additional 24 h. At this point the excess fluid was drained away, and the dish was covered and stored in the refrigerator (4°C) until use.

Samples were punched from the gel film using a No. 4 cork borer and were weighed on an analytical balance. The gel buttons were employed in dissolution tests within 15 min of punching.

Nonerodible dried films were prepared by adding 1.125 g of glycerol to 40 mL of 0.875% pilocarpine solution (specific activity of ~1.0 μCi/mL) and 40 mL of 20% PVA solution. This solution was poured into a 100 × 50-mm glass petri dish and allowed to air dry for 7 d at ambient conditions. It was assumed that any remaining labile tritium would exchange with water and evaporate with the solvent. Stability of the tritium label was determined after 10 d by dissolving film samples in water and flash evaporating the solvent. Analysis of this solvent by scintillation counting revealed <7% loss of tritium. Samples were punched out as described for the gel matrix.

Carboxyvinyl copolymer (carbomer 934) films were also prepared. Films were prepared as described above (erodible and nonerodible films) with the exception that 100 mg of carbomer 934, neutralized with 2.4 mL of 20% NaOH, was added to the PVA (10% or 20%) solution prior to the addition of other ingredients. Preparation of the sample lamellae and storage was as previously described.

In Vitro *Dissolution*—A pictorial representation of the dissolution apparatus is shown in Fig. 1. The apparatus utilized a peristaltic pump which delivered phosphate buffer (Sorensen's pH 7.4, isotonic) from a reservoir through a flow cell containing the sample lamella and emptied into a fraction collector at a rate of 15 mL/h. The dissolution cell chamber (1 mL) was separated into two compartments by a scintered glass disk. A glass bead bed was placed below the disk to prevent a "tunnel current" through the center of the cell.

The lamella was placed on the scintered glass disk and buffer was pumped through the chamber exiting at a central opening above. Buffer effluent was collected[6] at either 2- or 5-min intervals, depending on the duration of the experiment. The collected samples were assayed by liquid scintillation[7] using liquid scintillation cocktail[8]. Based on standards, counts were converted to concentration of drug. A minimum of six runs was conducted for each experiment.

In Vivo *Studies*—Rabbits, previously acclimated to the testing procedure, were positioned in restraining cages and placed in a room with controlled lighting. A television camera with a macrolens was focused on the rabbit's eye. A television monitor and an observer were physically outside the room containing the rabbit during the experiment to minimize observer interference. To measure pupillary diameter, the observer used a transparent grid placed on the television monitor.

[5] Parafilm M.
[6] Fraction Collector; ISCO, Lincoln, Neb.
[7] Beckman model 230; Fullerton, Calif.
[8] Aquasol II; New England Nuclear, Boston, Mass.

In an attempt to assess the biological performance of these drug delivery systems, the miotic response in albino rabbits was determined. The percent miotic response in the rabbit (%R) was calculated with reference to the diameter of the pupil after prolonged exposure to a high-intensity light source (D_{min}). The appropriate equation is given as:

$$\%R = 100\,[(D_0 - D_t)/(D_0 - D_{min})] \qquad \text{(Eq. 1)}$$

where D_t is the observed pupillary diameter at time t and D_0 is the initial pupillary diameter. D_{min} was measured before dosing, and drug treatment was not initiated until pupil diameter had returned to preexposure values.

Pilocarpine concentration in the solution was selected to be identical to the amount of drug in the gel/film, *i.e.*, 70 μg/25-μL dose as compared with 70 μg/25-mg dose of lamella. In the dried film cases, the dose of drug was 700 μg and, thus, an appropriate level of drug in the solution dosage form was prepared. This method gave reproducible results such that pupillary diameter changes of 0.1 mm (~2%) could be observed easily.

The lamella or solution was then instilled into the eye by pulling the lower eyelid away from the globe and placing the dose in the lower portion of the conjunctival sac. The eyelid was immediately returned to its normal position. During each experimental run, only one eye of each animal was dosed with drug. After instillation of the dose, the observer left the testing room.

Measurements of pupillary diameter were made at 5-min intervals for the first 15 min and every 15 min thereafter. A minimum of three runs was conducted for each experiment. Significance of results was determined by comparison of response values by t test.

RESULTS

***In Vitro* Dissolution**—Pilocarpine solution data fit by nonlinear regression (NONLIN) were found to be cleared by first-order kinetics as given by the following expression:

$$W_r = W_f e^{-k_d t} \qquad \text{(Eq. 2)}$$

where W_r is the weight of drug remaining to be cleared at time t, W_f is the final weight of drug cleared from the chamber, and k_d is the rate constant for clearance. Typical release profiles are shown in Fig. 2. It was found that the half-life ($t_{1/2}$) for clearance of a pilocarpine solution with a viscosity of 1.0 cps was 2.0 min and for a 60-cps PVA solution it was 12.7 min. To account for the delay in appearance of drug from the dissolution chamber to the sample collection (physical distance), a lag time ($t = t_0 - t_{lag}$) was required for all solutions and gel/films.

Pilocarpine release from gel/films is also shown in Fig. 2. Release from these systems, as compared with simple aqueous solutions, is sustained for a significantly longer time.

Release of drug from the gel/films could be either by a dissolution- or diffusion-controlled process or, in some cases, by a combination of both mechanisms. The data were fit by nonlinear regression (NONLIN) to both the Hixon–Crowell dissolution cube root equation (6, 7) and to a diffusion-controlled dissolution equation (8, 9):

$$F_t = 3k_r t^{1/2} - 3(k_r t^{1/2})^2 + (k_r t^{1/2})^3 \qquad \text{(Eq. 3)}$$

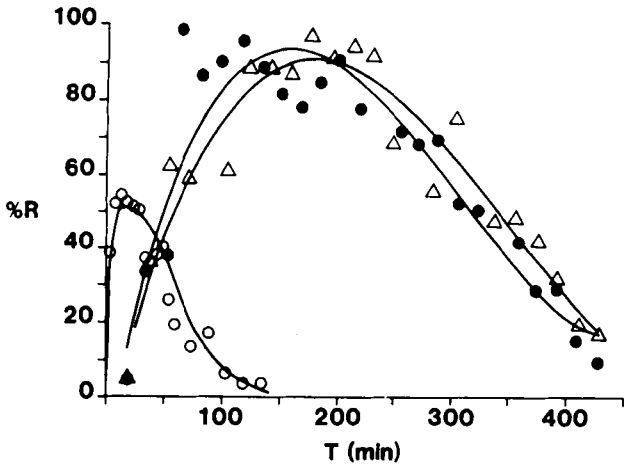

Figure 4—In vivo *comparison of miotic response to a 700-μg dose of pilocarpine nitrate administered as solutions and nonerodible lamellae. Key: (○) 25-μL solution (1.0 cps); (●) nonerodible lamella (no carbomer 934); (△) nonerodible lamella (with carbomer 934).*

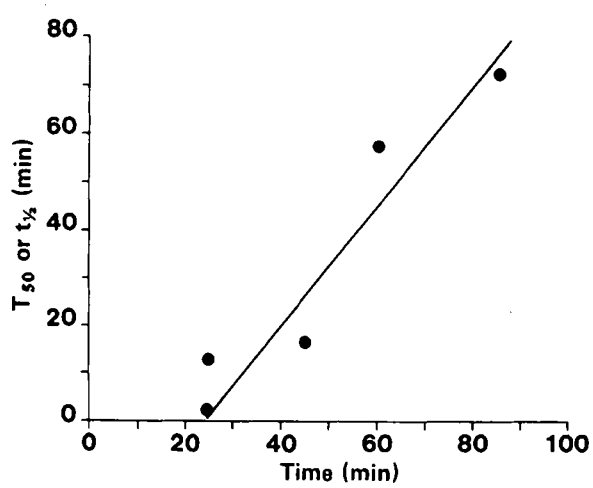

Figure 5—In vivo–in vitro *correlation utilizing* $t_{1/2}$ *for solutions and* T_{50} *for lamellae. Key: (●)* T_{50} *or* $t_{1/2}$. *The time indicated on the* x-*axis is the time at which 90% of the observed maximum response is first obtained.*

where F_t is the fraction of drug released to time t and k_r is the release rate constant.

Both erodible cross-linked lamella types (with and without carbomer 934) displayed identical release characteristics which were best fit by the diffusion equation (Eq. 3). The time to half dissolution (T_{50}) from these cross-linked systems was 16.2 min, calculated from the following equation:

$$T_{50} = (T_0^{1/2} + 0.206/k_r)^2 \qquad \text{(Eq. 4)}$$

where $T_0^{1/2}$ is the root lag time (9).

The cross-linked lamella samples dissolved during the testing procedure, and no visible swelling of the sample was noted during the test period. Although it was anticipated that an ionic-exchange release could retard the release of the cationic pilocarpine, the presence or absence of anionic copolymer in these preparations made no difference in the release rate of pilocarpine from the samples.

Nonerodible dried films were also tested, and the release of drug was best described by the diffusion expression (Eq. 3). Dried films without carbomer 934 did not appear to swell during the time course of testing. These films gave a T_{50} of appearance of 57.5 min. Conversely, the copolymer-containing films swelled to two or three times their original size, but did not undergo appreciable dissolution. These films gave a T_{50} of pilocarpine appearance of 72.4 min.

***In Vivo* Miotic Response**—The low-dose case results are shown in Fig. 3. Note that the peak response times and the 25–30-min postdosing intervals for the 1.0- and 60-cps solutions appear identical. However, the slowly eroding lamellae show a longer time to reach a maximum. The 60-cps solution shows approximately a 1.7 times greater response than the 1.0-cps solution. These results are in agreement with published data on the bioavailability of low- and medium-viscosity ocular pilocarpine solutions (5). The erodible lamellae also show a statistically greater response ($p < 0.001$) as compared with the solution dosage forms. The most significant aspect is the extended duration of effect of the erodible lamellae as compared with the solutions. Bearing in mind that although measurement of miosis allows continuous monitoring of pharmacological response, it is not as sensitive an indicator of drug bioavailability as direct sampling of ocular tissues or aqueous humor for drug concentration (5, 10). Therefore, this extended duration is significant.

The high-dose dried film systems (Fig. 4) show a number of significant differences between the nonerodible systems and the solution dosage form. These lamellae show a greater miotic response ($p < 0.001$) and a longer period of response, as compared with the solutions. There is no statistically significant difference between the lamellae with carbomer 934 as compared with the system without this copolymer.

It has been established previously that there is a linear relationship between dose and bioavailability for pilocarpine over six orders of magnitude of dose (6). The effect of different doses with the solution is clearly different: 20 and 50% of maximum to the 70- and 700-μg doses, respectively. This differential effect is expected on the basis of published drug bioavailability data (11).

***In Vivo–In Vitro* Correlation**—Since a strictly definable peak from miotic response is not discernable, especially for the lamellae formulations, the time at which 90% of the observed maximum response was first obtained was selected. This value correlates reasonably well with the time for 50% release ($t_{1/2}$ or T_{50}) of drug in the *in vitro* studies (Fig. 5). Note that for both drug solutions, the half-life of drug clearance corresponds to the time for removal of drug from the chamber, whereas in the case of lamellae, the dissolution–diffusion process is assumed to be rate limiting. It is recognized that other parameters such as duration of effect or area under the response curve may allow correlation with *in vitro* performance, but these were not examined.

DISCUSSION

To assess the merits of the gel/film pilocarpine systems, it is first necessary to comment on the performance of aqueous and viscous pilocarpine solutions. These systems, therefore, serve as a baseline.

The nonviscous (1.0 cps) solutions provide drug to the absorbing tissue, *i.e.*, the cornea, readily so that the amount of drug absorbed is proportional to the concentration instilled (5). However, the system is not very efficient and ~98% of the applied dose is lost. Significant dilution with tears occurs within 5 min of instillation, thus effectively stopping the flux of drug across the cornea (1). This effect coupled with nonproductive absorption of drug accounts for most of the drug loss.

Conversely, viscous solutions (60 cps) increase the amount of drug absorbed by a factor of 2 or 3 and exhibit a correspondingly greater biological response. This positive influence of viscosity on drug bioavailability can be due to several factors. The better wetting of the cornea by the polymer solution would allow more intimate contact of drug with the absorbing tissue. The increased viscosity of the solution would decrease drainage loss of the drug solution and thereby promote a greater ocular drug bioavailability. Finally, some adsorption of the polymer to the corneal tissue would favor greater absorption of the drug.

The gel/film system would have several of the positive attributes of the viscous solution and should be more dramatic in influence on drug bioavailability. The most obvious expectation is a decreased drainage loss of drug and slow availability of drug from the gel/film to the corneal surface. Both the *in vitro* and *in vivo* results support these postulates.

In an attempt to decrease appearance of drug from the films, the carbomer 934 salt of pilocarpine was prepared and used. No noticeable improvement in the system was evident. This may be explained on the basis that dissolution of the gel itself in the cross-linked systems was prompt and, thus, there was insufficient time for dissolution and subsequent diffusion of the drug from the film. In the dried film case, substantial swelling was noted which would increase both water content and channels for diffusion.

It is evident from this preliminary study that gels/films can sustain the release of water-soluble drugs to the precorneal area. Moreover, it appears that the *in vitro* assessment of drug release provides at least a qualitative yardstick on *in vivo* performance.

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Electrochemical Chromatographic Determinations of Morphine Antagonists in Biological Fluids, with Applications

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Received December 17, 1982, from *The Beehive, College of Pharmacy, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610.* Accepted for publication January 27, 1983.

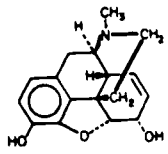
Abstract □ The morphine antagonists naltrexone and naloxone were extracted from plasma and urine, separated on a chromatographic column, and assayed by electrochemical detection. Optimum oxidation potentials were 0.65 V for morphine and 0.75 V for naloxone and naltrexone. Assay sensitivities were 2–5 ng/mL for plasma and 10 ng/mL for urine. The assays were applied to determine red blood cell partition coefficients of 1.83 ± 0.15 (*SD*) for naltrexone and 1.49 ± 0.27 (*SD*) for naloxone in a concentration range of 10–3500 ng/mL. No significant time dependence for the partitioning could be observed. Plasma protein binding in the same concentration range, determined by ultracentrifugation, was 27.7% ± 2.5% (*SD*) for naltrexone and 30.1% ± 5.1% (*SD*) for naloxone. The degree of protein binding did not change in the presence of morphine for morphine–antagonist ratios between 1:10 and 10:1. No concentration dependencies of red blood cell partitioning or protein binding were observed.

Keyphrases □ Naltrexone—HPLC with electrochemical detection, plasma and urine, applications to protein binding and red blood cell partitioning □ Naloxone—HPLC with electrochemical detection, plasma and urine, applications to protein binding and red blood cell partitioning □ Protein binding—naltrexone and naloxone in the presence of morphine, HPLC with electrochemical detection, red blood cell partitioning □ Red blood cell partitioning—naltrexone and naloxone in the presence of morphine, HPLC with electrochemical detection, protein binding

The classical detection methods for high-performance liquid chromatography (HPLC) by spectrophotometric and fluorescence detection are not useful for some drugs with low absorptivities or low fluorescence. Electrochemical detection has been a method of recent choice in selected instances. It combines amperometric titration and liquid chromatography (1) and has been applied to the assays of catecholamines (2), phenolic compounds (3), phenothiazines (4, 5), and morphine (I) (6–8).

The present study describes sensitive and accurate HPLC assays for the antagonists of morphine, naloxone (II) and naltrexone (III) in biological fluids with electrochemical detection. Studies on possible pharmacokinetic interactions between morphine and its antagonists are needed since naltrexone or naloxone are given to morphine-overdosed subjects. Prerequisites for such studies are specific and sensitive assays that allow simultaneous determinations of agonist and antagonist as well as their metabolites.

A facile procedure for the simultaneous determination of I and III or II is described using HPLC with electrochemical detection. The assay was applied to the determination of the protein binding of II and III and the influence of I on their protein binding. The red blood cell partition properties of both antagonists were also investigated.



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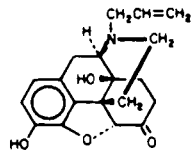
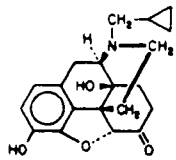


II



III

EXPERIMENTAL

Materials—The following analytical-grade materials were used: sodium bicarbonate[1], monobasic potassium phosphate[1], volumetric concentrates of hydrochloric acid and sodium hydroxide[2], 1-butanol[3], and benzene[4]. Acetonitrile[4] was HPLC grade. Morphine (I)[5], naloxone (II)[5], and naltrexone (III)[5] were used as received. Sodium chloride injection USP[6], sodium heparin injection[7], and disposable syringes[8] were used in the preparation of red blood cell suspensions.

Apparatus—For the HPLC assay the following instruments were used: high-pressure pump[9], a six-port injector[10], an electrochemical detector[11] with a working electrode of glassy carbon[12], a strip-chart recorder[13], and an octadecylsilane column[14] with a guard column of the same material.

Plasma protein binding was determined with an ultracentrifuge[15]. A laboratory centrifuge[16] was used in the separation of organic extracts from aqueous phases.

Chromatographic Conditions—The mobile phase was a mixture of 0.04 M monobasic potassium phosphate and acetonitrile (90:10). The flow rate was

[1] Mallinckrodt, Paris, Ky.
[2] Ricca Chemical Co., Arlington, Tex.
[3] Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Fisher Scientific Co., Fair Lawn, N.J.
[5] National Institute on Drug Abuse, Research Technology Branch, Rockville, Md.
[6] McGaw Laboratories, Irvine, Calif.
[7] The Upjohn Co., Kalamazoo, Mich.
[8] Monoject, Division of Sherwood Medical, A. Brunswick Co., St. Louis, Mo.
[9] Series 3B Microcomputer controlled pump module; Perkin-Elmer, Norwalk, Conn.
[10] Model 7105; Rheodyne, Cotati, Calif.
[11] LC 4A; Bioanalytical Systems, West Lafayette, Ind.
[12] TLS; Bioanalytical Systems, West Lafayette, Ind.
[13] Series 5000, Fisher Recordall; Fisher Scientific Co., Fair Lawn, N.J.
[14] C_{18} μBondapak column; Waters Associates, Milford, Mass.
[15] Ultracentrifuge Model LS-50 with rotor Ti 50; Beckman Instruments, Norcross, Ga.
[16] Lab centrifuge; International Centrifuge Equipment Co., Needham, Mass.

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