High-Performance Liquid Chromatographic Analysis of Methadone Hydrochloride Oral Solution

THOMAS H. BEASLEY, Sr., and HOWARD W. ZIEGLER [x]

Abstract □ A direct and rapid high-performance liquid chromatographic assay for methadone hydrochloride in a flavored oral solution dosage form is described. A syrup sample, one part diluted with three parts of water, is introduced onto a column packed with octadecylsilane bonded on 10-μm porous silica gel (reversed phase). A formic acid–ammonium formate-buffered mobile phase is linear programmed with acetonitrile. The absorbance is monitored continuously at 280 or 254 nm, using a flow-through, UV, double-beam photometer. An aqueous methadone hydrochloride solution is used for external standardization. The relative standard deviation was not more than 1.0%. Drug recovery from a syrup base was better than 99.8%.

Keyphrases □ Methadone hydrochloride—high-performance liquid chromatographic analysis, liquid pharmaceutical preparation □ High-performance liquid chromatography—analysis, methadone hydrochloride in liquid pharmaceutical preparation □ Narcotic analgesics—methadone hydrochloride, high-performance liquid chromatographic analysis in liquid pharmaceutical preparation

Methadone hydrochloride is used widely in drug maintenance treatment programs. Although tablet and injection formulas are available, the preferred dosage form is a flavored oral solution. A rapid and simple analysis of methadone hydrochloride in a proprietary dosage form[1] was sought. The USP GLC method for methadone hydrochloride injection and tablets (1) is cumbersome and lengthy and often lacks precision. Also, the GLC column can become overloaded with thermal decomposition products readily.

The British Pharmacopoeia specifies an extraction–acid titration procedure for methadone injection and tablets (2). A simplified gravimetric method for methadone hydrochloride in flavored syrup formulations was described (3). The alkaloid was precipitated as the phosphomolybdate complex and weighed. Choulis and Papadopoulous (4) developed a GLC assay of methadone in sustained-release tablets after dissolving the drug with dissolution fluids used for *in vitro* evaluation. Most methadone hydrochloride assays are trace methods after extraction of the drug from human urine, blood, or serum.

Some advantages of reversed-phase high-performance liquid chromatography (HPLC) are: (*a*) aqueous samples can be diluted and chromatographed without extraction, concentration, or derivatization; (*b*) samples can be run at ambient temperature; (*c*) analyses can be completed in about 20 min; and (*d*) a formulation can be analyzed for preservative, flavor, or another drug if present.

EXPERIMENTAL

Apparatus—A modular liquid chromatograph equipped with 280- and 254-nm UV detectors[2] was used along with a 10-mv recorder, a piston pump[3], suitable pulse dampening, a stainless steel sampling valve[4], a pressure gauge, valves, tubing, and fittings necessary to complete a closed system.

The stainless steel column was 3.2 × 250 mm, slurry packed with octadecylsilane bonded on 10-μm porous silica gel[5]. A stainless steel precolumn, 3.2 × 75 mm, containing porous silica coated with a monomolecular layer of octadecylsilane[6] (37 μm, nominal particle size) was placed in the HPLC system ahead of the sample injection valve.

The gradient elution cell consisted of a 25-ml glass bulb with fittings compatible with the instrument. The gradient cell and valving were described previously (5).

Reagents—*Mobile Phase A*—Distilled formic acid, 100 ml, and 1000 ml of water were transferred to a 2-liter flask or vessel and mixed. After 2.5 ml of concentrated ammonium hydroxide was added, the solution was diluted to 2000 ml with water and mixed.

Mobile Phase B—Spectrophotometric grade acetonitrile[7], which shows little or no absorbance in the UV from 220 to 350 nm, was used.

Gradient Elution—A programmed linear gradient elution from 100% Mobile Phase A to 80% Mobile Phase B in Mobile Phase A in about 20 min was used.

Chromatographic Conditions—The column temperature was ambient. The electrometer was set at 0.32 absorbance unit full scale (aufs) with a recorder chart speed of 5.08 mm/min. The flow rate was 2 ml/min, and column pressure was about 1500 psi. The volume of the sample and standard sample introduced onto the column was 50 μl. Water, pumped through the system, was used to rinse the sugars, *etc.*, from the column. Mobile Phase A can be stored in the system.

Methadone Hydrochloride Standard Solutions—*Stock*—Methadone hydrochloride USP[8] was used as the working standard. Approximately 1.0 g of the standard was accurately weighed, transferred to a 100-ml volumetric flask, and dissolved in and diluted to volume with water.

Diluted—A 25.0-ml aliquot of stock methadone hydrochloride standard solution was transferred to a 100-ml volumetric flask and diluted to volume with water. This solution contained 2.5 mg/ml of standard.

Both solutions were freshly prepared.

Sample Preparation—Water, 75 ml, was transferred to a dry 100-ml volumetric flask. The sample syrup formulation, delivered from a 30-ml syringe fitted with a long needle, was used to dilute to the mark. Then the solution was mixed thoroughly.

Analysis—Aliquots of 50 μl of diluted sample solution were injected into a prepared chromatograph operated at the designated flow rate of Mobile Phase A. The gradient system and recorder were started; the run was 20 min. Reequilibration to Mobile Phase A required about 10 min. Diluted standard solution, 50 μl, was injected under the same conditions used for the sample. Replicate sample and standard solutions were generally run to test for system stability.

Table I—Replicate Assay Results from a Production Lot of a Proprietary Methadone Hydrochloride Oral Solution

	Methadone Hydrochloride, mg/ml		
Sample[a]	1	2	3
A	10.01	10.14	10.07
B	10.05	10.23	10.15
C	10.14	10.10	10.06
D	10.12	10.14	10.07
E	10.20	10.09	10.09

[a] Statistically sampled from the lot. Each individual sample run using triplicate sample injections.

[1] Methadose, Mallinckrodt, St. Louis, Mo.
[2] Model 151, Altex, Berkeley, Calif.
[3] Model 3366-460, Glenco, Houston, Tex.
[4] Valco CV6-UHPa-N60, 7000 psi, Glenco, Houston, Tex.
[5] LiChrosorb S160, reversed phase, 10 μm, Altex (5-μm material may also be used).
[6] C_{18} reversed phase, Waters Associates, Milford, Mass.
[7] SpectrAR, Mallinckrodt, St. Louis, Mo.
[8] Mallinckrodt, St. Louis, Mo.

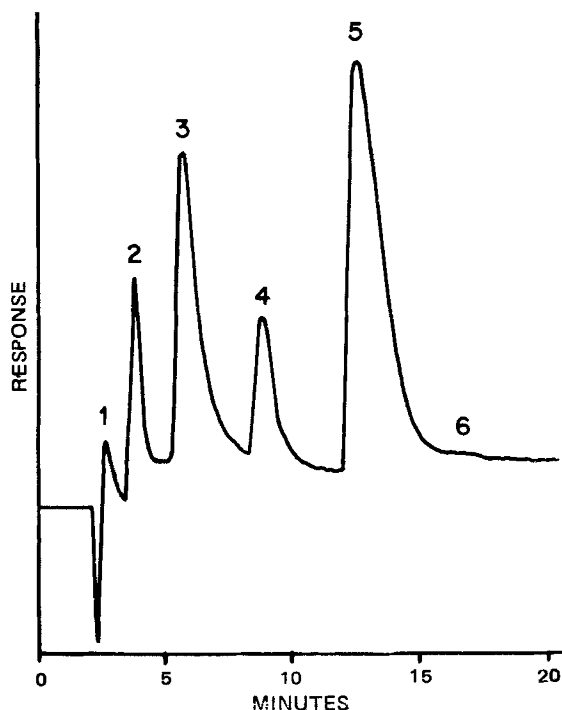

Figure 1—*Typical chromatogram of a proprietary methadone hydrochloride oral solution. Key: 1, solvent front; 2, preservative (sodium benzoate); 3 and 4, flavor components; 5, methadone hydrochloride; and 6, dye.*

Calculations—Peak areas were measured by electronic methods, and peak heights were measured manually. The methadone hydrochloride concentration was calculated using:

$$\frac{(A_1 \text{ or } H_1)(4C)}{A_2 \text{ or } H_2} = \text{mg/ml} \quad \text{(Eq. 1)}$$

where A_1 is the average area of peaks from the diluted sample solution, A_2 is the average area of peaks from the diluted standard solution, H_1 is the average height of peaks from the diluted sample solution, H_2 is the average height of peaks from the diluted standard solution, C is the concentration in milligrams per milliliter of methadone hydrochloride in the diluted standard solution, and 4 is the dilution factor.

RESULTS AND DISCUSSION

A typical chromatogram of a diluted sample of a proprietary methadone hydrochloride oral solution is shown in Fig. 1. Diluted methadone hydrochloride standard solution is displayed in the chromatogram in Fig. 2. The standard solution is unstable and must be freshly prepared and diluted for each analysis. About 1 mg of sodium benzoate/ml can be added to the solutions to stabilize the standards for at least 1 month if it is necessary to retain them.

No chromatographic interference is encountered with the preservative. Some laboratory distilled water contains trace impurities that display varying chromatographic baselines. For this reason, both the sample syrup and stock standard solution aliquots should be diluted with identical amounts of the same water before chromatographic analysis. The rather unconventional reverse technique of using sample syrup as the volumetric diluent was selected because the formulation is very viscous. More precise sample measurements were made using this procedure.

Table II—Recovery of Methadone Hydrochloride from Synthetic Formulation Syrup Base

Methadone Hydrochloride Added, mg/ml					
4.96		9.78		14.96	
Recovered, mg	Recovered, %	Recovered, mg	Recovered, %	Recovered, mg	Recovered, %
5.16	104.0	9.85	100.7	14.93	99.8
5.01	101.0	9.82	100.4	14.98	100.1
5.05	101.8	9.77	99.9	14.90	99.6
4.98	100.4	9.67	98.9	14.97	100.1
—	—	9.68	99.0	14.96	100.0
—	—	9.78	100.0	14.91	99.7
—	—	9.76	99.8	—	—

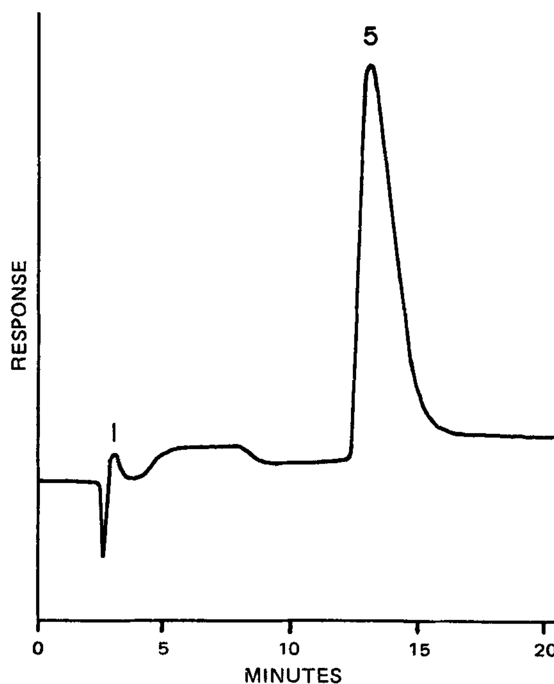

Figure 2—*Chromatogram of an aqueous methadone hydrochloride reference material solution (50 μl of a 2.5-mg/ml solution). Key: 1, solvent front; and, 5, methadone hydrochloride.*

HPLC results of replicate analyses are displayed in Table I. The relative standard deviation was not more than 1.0%. Recovery data of weighed amounts of methadone hydrochloride added to laboratory prepared formulation base are shown in Table II. The average recovery was not less than 99.8%. In no case could it be proven statistically, at the 95% confidence level, that the recovery was different from 100%. All data were calculated by an on-line computer[9] that calculates from integrated areas. With the computer interface, chromatographic analysis time can be cut in half. The procedure recommended allows for manual computations using either peak area or peak height.

HPLC parameters require that methadone hydrochloride be chromatographed as the protonated amine in an acid system. Methadone hydrochloride in an acidified mobile phase attacks some types of stainless steel, necessitating the use of chloride-resistant types. Stainless steel No. 304 or 316 cannot be used.

The formic acid used for Mobile Phase A had to be distilled to remove UV absorbers, which are apparently leached from the plastic containers in which the acid is supplied.

The method described is a unique application of *direct* chromatography of a sample with little or no sample preparation, extraction, or derivatization. The technique may be applicable to other aqueous dosage forms such as cough–cold preparations.

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(2) "The British Pharmacopoeia," Her Majesty's Stationery Office, Cambridge, England, 1973, pp. 294, 295.

(3) S. P. Loucas, R. L. Feinberg, P. A. Gunning, F. F. Hartman, and B. Mehl, *Am. J. Hosp. Pharm.*, **31,** 1193 (1974).

(4) N. H. Choulis and H. Papadopoulous, *J. Chromatogr.*, **106,** 180 (1975).

(5) H. W. Ziegler, T. H. Beasley, Sr., and D. W. Smith, *J. Assoc. Offic.*

[9] Model 3354 Lab Data System, Hewlett-Packard.

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ˣ To whom inquiries should be directed.

Prednisolone Bioavailability in the Dog

FRANCIS L. S. TSE and PETER G. WELLING ˣ

Abstract ☐ With a fasted dog as an animal model, the bioavailability and pharmacokinetics of prednisolone were studied following rapid intravenous injection and oral dosing of a prednisolone sodium phosphate solution and also following oral doses of prednisolone as tablets and a slurry. Hydrolysis of the phosphate ester to prednisolone in the body is extremely rapid and complete, thus permitting accurate calculation of the distribution volume of prednisolone. Enteral absorption of prednisolone from a slurry is superior to that from prednisolone tablets and from a prednisolone sodium phosphate solution. Reduced absorption from tablets, compared to the slurry, is probably due to tablet disintegration characteristics; reduced absorption from the solution is probably due to poor membrane permeability of the ionized drug. Information obtained from a single animal may indicate the need for expanded studies in humans.

Keyphrases ☐ Prednisolone—bioavailability and pharmacokinetics, various dosage forms compared, dogs ☐ Bioavailability—prednisolone, various dosage forms compared, dogs ☐ Pharmacokinetics—prednisolone, various dosage forms compared, dogs ☐ Glucocorticoids—prednisolone, bioavailability and pharmacokinetics, various dosage forms compared, dogs

Prednisolone is used primarily for its anti-inflammatory activity in several diseases. It is cleared from the body predominantly by hepatic metabolism; only about 10% of orally dosed compound is excreted unchanged in urine (1).

Peak serum prednisolone concentrations of 160 ng/ml at 0.5 hr and 298 ng/ml at 1 hr were reported following 2.5- and 5.0-mg oral doses of prednisolone, respectively, to male beagle dogs (2). Studies in humans yielded peak serum prednisolone levels at 1–2 hr following oral doses and a serum half-life of about 3 hr (3–8).

Although the influence of *in vitro* disintegration and dissolution characteristics on prednisolone pharmacokinetics was reported (5–8), no information is available on the effect of the dosage form on bioavailability characteristics.

With the beagle dog as a model system, the present study was undertaken to compare the bioavailability and pharmacokinetics of prednisolone from two different doses of a commercial tablet, a slurry, and a solution of a water-soluble prednisolone salt.

EXPERIMENTAL

A 3-year-old male beagle dog, 17.5 kg, was given 30- and 60-mg doses of prednisolone tablets[1] (oral), oral prednisolone slurry[1], and oral and intravenous prednisolone sodium phosphate solution[2] in separate experiments. All experiments were done in duplicate.

Food was withheld at least 12 hr before and throughout each experiment. During an experiment, the dog was placed in a restraining apparatus so that it could stand normally but could not disturb indwelling catheters.

In the intravenous dosing experiments, an infusion set was positioned in each front leg. Each set consisted of a 19-gauge needle with 30 cm of flexible plastic tubing with a total volume of 0.6 ml. Clotting of blood in infusion sets was prevented by infusing 2 ml of saline-diluted heparin sodium solution (10 units/ml) into the set each hour it was in position. Prednisolone sodium phosphate was administered *via* one infusion set, with injection being completed in about 30 sec. The set was flushed with 10 ml of normal saline solution and was then removed.

Blood samples (8 ml) were collected through the other infusion set shortly before dosing and at 5, 15, and 30 min and 1, 2, 4, and 6 hr after dosing. Before each sample was taken, 5 ml of residual fluid was withdrawn to remove the heparin solution in the infusion set. The blood sample was then drawn, and the volume was replaced by an 8-ml injection of normal saline. The residual fluid was reinjected followed by 2 ml of heparin solution.

Tablets were administered by placing them on the posterior portion of the tongue so that they were not fractured or chewed before being swallowed; then 50 ml of water was immediately given. The slurry was prepared by triturating prednisolone tablets to a powder and dispersing this powder in 50 ml of water. The slurry was administered directly into the dog's stomach by means of a 120-cm × 3-mm (i.d.) stomach tube. Water, 25 ml, was used to flush residual drug from the syringe and stomach tube into the stomach. The oral solution was administered directly into the stomach in identical fashion to the slurry. With all oral dosing experiments, blood samples were collected *via* an infusion set as in the intravenous case.

Blood samples were placed in heparinized tubes[3] and centrifuged at 2300 rpm for 10 min. Plasma was removed and frozen until analyzed, usually within 1 week.

Experiments were performed 3 weeks apart to avoid changes in drug pharmacokinetic parameters due to prior exposure and to ensure complete drug washout from a previous dose.

Assay of Plasma Samples—The assay used to measure prednisolone in plasma was a modification of the GLC method described by Bacon and Kokenakes (9). In the purification step by column chromatography, the volume of the first eluting solvent [benzene–acetone (9:1)] was reduced from 70 to 30 ml and the volume of the second eluting solvent [benzene–acetone (1:3)] was increased from 20 to 30 ml. This change improved separation of prednisolone from other plasma components[4].

A solution containing the silylating agent[5] was prepared by combining 0.3 ml of pyridine, 37 μl of dotriacontane (as the internal standard) in carbon tetrachloride (0.95 μg/μl), and 0.5 ml of the silylating agent. A 20-μl aliquot of this solution was added to the extracted and purified prednisolone sample in a 1-ml vial[6]. This vial was immediately sealed with a polytef-lined cap, agitated on a vortex-type mixer, and allowed to stand at room temperature for 18 hr before assay.

GLC was carried out on an instrument[7] fitted with dual flame-ionization detectors. The column was a 1.8-m × 2-mm (i.d.) U-shaped glass

[1] Meticortelone, Schering Corp., Bloomfield, N.J.
[2] Hydeltrasol, Merck Sharp and Dohme, West Point, Pa.

[3] Vacutainer, Becton-Dickinson, Rutherford, N.J.
[4] G. E. Bacon, University of Michigan Medical Center, Ann Arbor, Mich., personal communication.
[5] Tri-Sil TBT, Pierce Chemical Co., Rockford, Ill.
[6] Reacti-vial, Pierce Chemical Co., Rockford, Ill.
[7] Nuclear Chicago Selecta System model 5000, Searle, Des Plaines, Ill.