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Technical Note

High-Performance Liquid Chromatographic Determination and Pharmacokinetics of Methyl *N*-[5[[4-(2-Pyridinyl)-1-piperazinyl]carbonyl]-1H-benzimidazol-2-yl] Carbamate (CDRI Compound 81-470), a New Anthelmintic Agent in Rats[1]

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INTRODUCTION

Methyl *N*-[5[[4-(2-pyridinyl)-1-piperazinyl]carbonyl]-1H-benzimidazol-2-yl] carbamate (CDRI Compound 81-470; 1; Fig. 1), is a new broad-spectrum anthelmintic agent (1,2). The compound is also effective against developing and adult forms of various helminth parasites (e.g., hook worms, trichostrongylus, filariids, and cestodes), when administered orally or parenterally (3). It has also been found to be effective for treatment of some skin dwelling parasites by topical application (4). Additionally, 1 possesses prophylactic action against experimental nematode infections (5).

Compound 1 is currently undergoing preclinical evaluation as a potential anthelmintic drug. This report describes a simple and specific high-performance liquid chromatographic (HPLC) method for the rapid determination of 1 in rat plasma and its application to pharmacokinetics evaluation of 1 after a single bolus dose of 5 mg/kg in normal young albino rats by the intraperitonial route.

MATERIALS AND METHODS

Reagents

Methyl *N*-[5[[4-)(-pyridinyl)-1-piperazinyl]carbonyl]-1H-benzimidazol-2-yl] carbamate (CDRI Compound 81-470; 1) was obtained from the Pharmaceutics Division of this institute. Stock standard solution of 1 was prepared at a concentration of 1.6 μg/ml in methanol. The stock solutions were stable for at least 3 months when stored at 4°C. The working standards were prepared by diluting the stock solution with methanol. For intraperitonial administration, 1 (5 mg/ml) was dissolved in dimethyl sulfoxide. Acetonitrile, AR grade, was distilled before use. All other reagents were analytical grade and used without further purification.

Chromatographic Conditions

The apparatus used for this work was a high-performance liquid chromatograph (Model 600, Kontron AG, Switzerland), coupled with a fluorescence detector (Model RF-530; Shimadzu, Japan) and UV detector (Model UVIKON-730S LC, Kontron AG, Switzerland). The fluorescence detector was set at excitation 300 nm and emission 365 nm, while UV detection was at 240 nm. Peak area was determined by an integrator-plotter (Model CR1B Chromatopac, Shimadzu, Japan) using either of the detectors. Samples of 20 μl were introduced by a loop injector (Model 7125, Rheodyne, USA) under ambient conditions onto a reverse-phase Nucleosil 100-5 C_{18} column (200 × 4-mm I.D.; 5-μm particle size; Machery Nagel, Duren, FRG), protected with a stainless-steel guard column (50 × 4.6-mm I.D.) packed with C_{18} 40-μm pellicular material (Supelco Inc., USA). A mixture of acetonitrile and 0.05 *M* phosphate buffer 70:30 (pH 6) was used as the mobile phase at a flow rate of 2 ml/min. The retention time of 1 was 8.0 ± 0.2 min.

Assay Procedure

To 0.1 ml of plasma, an equal volume of acetonitrile was added in a 3-ml conical glass centrifuge tube and vortexed. After 15 min the tubes were centrifuged at 3000 rpm for 10 min. For low concentrations of 1, the supernatant was analyzed as such, while for higher concentrations the supernantant was diluted 1:5 or 1:20 with acetonitrile. External calibration curves were prepared with the procedure by adding varying concentations of 1 to drug-free rat plasma.

Animal Protocol

Healthy albino Wistar rats weighing 200 ± 10 g were

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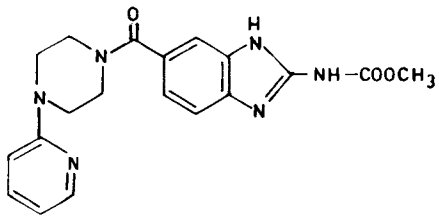

Fig. 1. Compound 81-470.

housed in groups of five in plastic cages and fed ad libitum standard pelleted food and water throughout the study. A 5 mg/ml solution of 1 in dimethyl sulfoxide (6) was administered intraperitonially at a dose of 5 mg/kg to all the animals. Five animals were used for each time point. Terminal blood samples from rats were collected at 0.25, 0.5, 1.0, 2, 4, 8, 12, 24, 36, and 48 hr after dosing from the inferior vena cava under light ether anesthesia. Plasma was separated by centrifugation and stored at −20°C till analyzed.

Pharmacokinetic analysis of the plasma concentration–time data was done on an IBM microcomputer using STATIS2, a nonlinear regression program (7).

RESULTS AND DISCUSSION

The HPLC method described here overcomes the problems associated with extraction and tedious sample preparations. Among the several analytical columns tested, the reverse-phase Nucleosel 100-5 C_{18} (200 × 4-mm I.D.) was the most favorable column for efficient separation of 1 and endogenous interfering substances in plasma samples. Representative chromatograms of rat plasma with fluorescence and UV detectors are presented in Fig. 2. There were no interfering peaks from blank plasma samples at the retention time of 1 with either detector (Figs. 2A and C). Figures 2B and D show the chromatograms obtained from rat plasma after administration of 1, using the fluorescence and UV detector, respectively. Fluorescence detection was slightly more sensitive (quantitation limit, 40 ng/ml) compared to UV

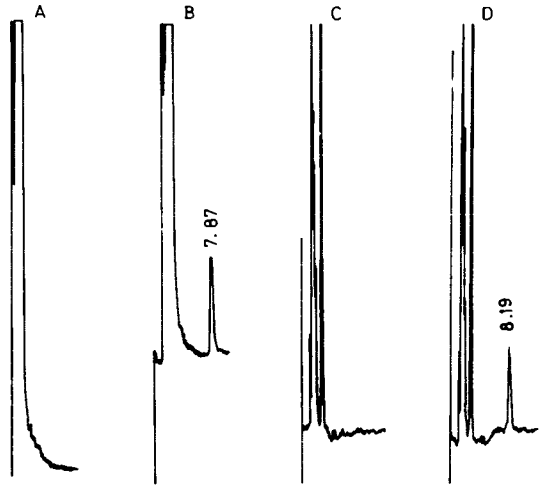

Fig. 2. High-performance liquid chromatogram of (A) blank human serum using fluorescence detector, (B) compound 81-470 in serum using fluorescence detector, (C) blank human serum using UV detector, and (D) compound 81-470 in serum using UV detector.

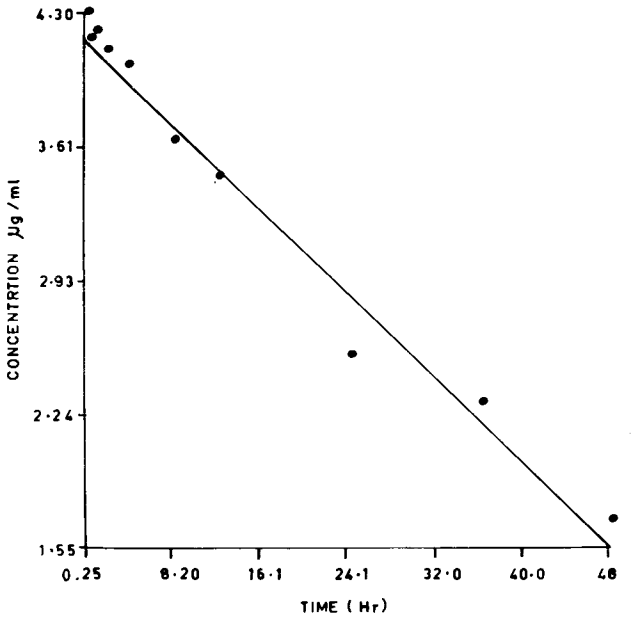

Fig. 3. Semilogarithemic plot of serum levels of 81-470 in rats after a single intraperitoneal dose of 5 mg/kg.

detection (quantitation limit, 50 ng/ml at 240 nm), and it was therefore employed for the present study.

Calibration curves were obtained by plotting peak areas of 1 against its concentration in spiked plasma. Linear correlations were observed over the concentration range of 40–800 ng/ml.

$$y = 29.14 \times -131.6,\ r = 0.9982$$

Reproducibility and accuracy of the procedure were determined by analyzing replicate ($n = 5$) samples of spiked plasma at five concentration levels (40, 100, 200, 400, and 800 ng/ml). The coefficient of variation was less than 10% at all concentrations, with 9.7% at 40 ng/ml concentration.

The plasma concentration–time profiles of 1 after 5 mg/kg intraperitoneal administration on semilogarithmic scale are given in Fig. 3. The data were best described by a one-compartment open model with first-order elimination kinetics, on the basis of Akakis information criteria and Schwarz values (8,9). The biological half-life of the parent compound was 4.3 hr. Pharmacokinetic parameters as calculated by STATIS 2 are given in Table I.

In summary, we have developed an HPLC method for the determination of 1 in plasma samples and have evaluated its pharmacokinetic parameters in rats.

Table I. Pharmacokinetic Parameters of CDRI Compound 81-470 After a Single 5 mg/kg Intraperitonial Dose in Rats

Parameter	Estimated value
A	18.8 µg · ml^{-1}
K_e	0.163 hr^{-1}
$T_{1/2}$	4.26 hr
AUC (0–infinity)	115 µg · hr · ml^{-1}
$V_{d_{ss}}$	266 ml · kg^{-1}
CL	43.3 ml · kg^{-1} · hr^{-1}

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