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Note

Quantitative determination of cardiotonic agent MDL 17,043 in plasma by reversed-phase high-performance liquid chromatography

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MDL 17,043, 1,3-dihydro-4-methyl-5-[4-(methylthio)benzoyl]-2H-imidazol-2-one, is a new noncatechol, nonglycoside cardiotonic agent. Pharmacological studies in laboratory animals suggest that the drug will be useful in the treatment of congestive heart failure since both oral and intravenous (i.v.) administration results in dose-dependent increase in cardiac contractile force, slight increases in heart rate and small decreases in systemic blood pressure [1—3]. In order to facilitate the studies of bioavailability and pharmacokinetics of this compound, a sensitive and reliable analytical procedure was needed to measure plasma concentrations of MDL 17,043. This communication describes a reversed-phase high-performance liquid chromatographic (HPLC) method developed for this purpose and the application of the assay to a feasibility study using two male beagle dogs given single oral and i.v. doses of MDL 17,043.

EXPERIMENTAL

Materials

Ethyl acetate, acetonitrile and methanol were glass-distilled grade obtained from commercial suppliers and used as received. Sodium phosphates (monobasic and dibasic) were standard reagent grade. Glass-distilled water was used in the preparation of aqueous solutions.

MDL 17,043 and the internal standard [MDL 18,763, 1,3-diethyl-1,3-dihydro-4-(4-methoxy-benzoyl)-5-methyl-2H-imidazol-2-one] were supplied by the Merrell Dow Research Center.

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Standards

Standard solutions of MDL 17,043 were prepared in methanol. For use, 0.1 ml of the appropriate concentration of standard solution was mixed with 1.9 ml human plasma (heparin anticoagulant) to give the plasma concentrations shown in Table I. Internal standard solution was 4.16 μg/ml in methanol. To test the precision and accuracy of the assay a six-day validation study was carried out. A six-point standard curve in duplicate together with six duplicate plasma samples with concentrations unknown to the analyst were analyzed each day.

Feasibility experiment

To investigate the sensitivity and specificity of the assay, it was applied to a limited dog bioavailability study. In a crossover experiment, two male beagle dogs (A & B) were given single oral and i.v. doses of MDL 17,043 at 3 mg/kg. Periodic plasma samples were taken from each dog and the plasma concentrations of MDL 17,043 were determined by this analytical method. Two weeks separated the two legs of the experiment.

Extraction procedure

To each 2-ml standard or unknown plasma sample, 100 μl internal standard solution were added. Then 3 ml of acetonitrile were added to each tube and vortexed to precipitate plasma protein. Two ml of 0.1 *M* sodium phosphate buffer, pH 7.5, were mixed with each sample which was then centrifuged (900 *g*, 20 min). A 6-ml aliquot of the supernatant was transferred to a 25-ml screw-cap extraction tube which contained 9 ml of ethyl acetate. The compounds were extracted into the organic phase by mixing in a horizontal Eberbach reciprocating shaker for 20 min. After centrifugation, 10 ml of the supernatant were transferred to a 15-ml conical tube and evaporated (50—55°C) to dryness under a slow stream of nitrogen gas. For HPLC analysis, the residue was redissolved in 200 μl of methanol, and a 25-μl aliquot was injected into the column.

Apparatus

HPLC analyses were performed on equipment manufactured by Waters Assoc. (Milford, MA, U.S.A.); solvent delivery systems: Model 6000A; UV detection system: Model 440 (fixed wavelength at 313 nm). Samples were processed with a Waters Autoinjector (WISP Model 710A). The HPLC column used for this analysis was a DuPont Zorbax C-8, 25 cm × 4.6 mm I.D., particle size 6 μm; mobile phase: methanol—water (60:40); flow-rate: 1.0 ml/min. HPLC peaks were integrated by an Automated Laboratory Data System (Computer Inquiry Systems, Englewood Cliffs, NJ, U.S.A.).

Calibration and calculation

On each day, calibration equations were determined for the standardization samples, using linear regression analysis. These equations were used to calculate the MDL 17,043 concentrations in the coded unknown samples.

RESULTS AND DISCUSSION

Using the extraction procedures described previously, there was no endogenous material found in dog or human plasma that would interfere with the assay. Retention times for MDL 17,043 and internal standard were 6.0 and 11.5 min, respectively. A typical chromatogram is shown in Fig. 1.

Composite results for the six validation days are tabulated in Table I. The assay was linear over the range of 25—1000 ng/ml and had acceptable precision. The day-to-day variation as judged by the slope had a coefficient of variation of 1.0%.

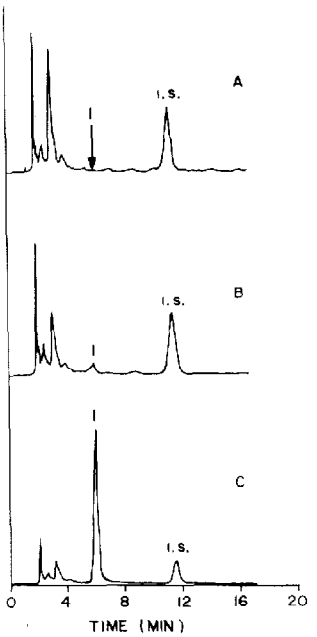

Fig. 1. Chromatograms of extracted plasma standards: (A) blank, (B) 25 ng/ml, (C) 1000 ng/ml. Peaks: 1 = MDL 17,043; I.S. = internal standard.

TABLE I

MDL 17,043 STANDARDIZATION RESULTS FOR SIX DAYS

Standard concentration of MDL 17,043 (ng/ml)	*n*	Mean peak height ratio* (%)	S.D.
0	12	0	—
25	11	26.0	2.3
100	12	102.8	3.0
300	11	308.2	6.7
600	12	627.3	10.4
1000	11	1031.5	24.8
Slope	6	1.0333	0.0102

*(Peak height of MDL 17,043/peak height of internal standard) × 100.

The accuracy of the assay was assessed by preparing and analyzing 36 unknowns in a randomly coded fashion. The mean results are shown in Table II.

Dog plasma concentrations found after i.v. and oral dosing are given in Fig. 2. MDL 17,043 was found to be readily absorbed and could be followed up to 8 h post dose. At 24 h post dose no drug was detected. Based upon area under the plasma concentration versus time curve, the absorption of MDL 17,043 was about 50% and 85% for dogs A and B, respectively. From the program CSTRIP [4], the $t_{1/2}$ values for the two dogs were 1.75 h and 2.25 h in the β-phase after i.v. dosing.

In conclusion, the validity of the method to measure plasma level was demonstrated; the method is sensitive enough to apply to pharmacokinetic and bioavailability studies of the drug in animals and humans.

TABLE II

ANALYSIS OF PLASMA CONTAINING UNKNOWN ADDED CONCENTRATIONS OF MDL 17,043

Concentration added (ng/ml)	n	Mean concentration found (ng/ml)	S.D.
0	4	0	—
25	3	24.8	2.4
50	2	47.8	—
100	6	96.7	1.9
200	2	194.6	—
300	6	304.9	3.8
500	2	505.0	—
600	6	605.2	7.8
800	2	806.2	—
1000	3	1014.9	13.6

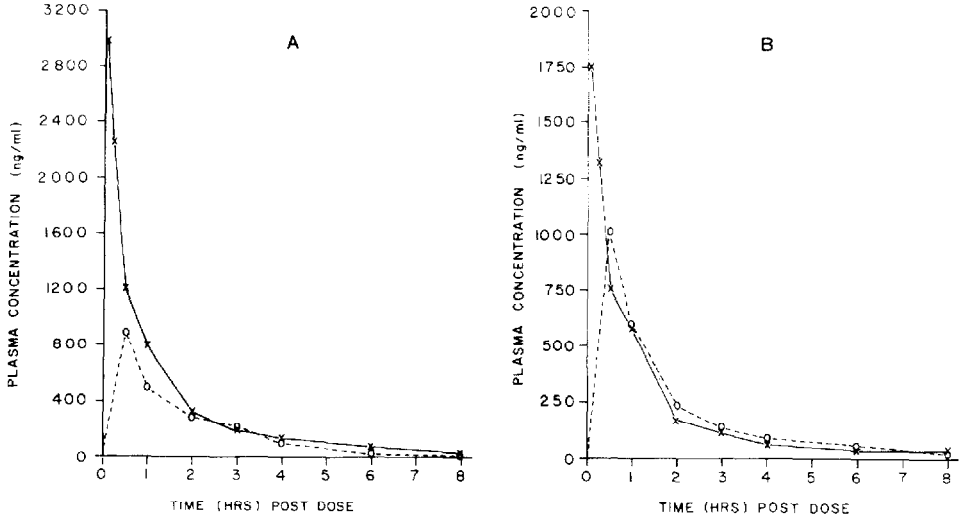

Fig. 2. Plasma concentrations of MDL 17,043 in (A) dog A and (B) dog B after oral (○— — —○) and i.v. (×——×) administration of MDL at 3 mg/kg.

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