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Note

Automated capillary gas chromatographic assay using nitrogen—phosphorus detection for the determination of cetiedil in plasma

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Cetiedil citrate [2-(hexahydro-1H-azepin-1-yl)ethyl α-cyclohexyl-3-thiopheneacetate—2-hydroxy-1,2,3-propanetricarboxylate (1:1)] is currently undergoing clinical evaluation for the treatment of vaso-occlusive crisis in patients with sickle cell disease [1–4]. Therapeutic doses of cetiedil citrate (0.45—0.6 mg/kg, intravenous infusion) yield plasma concentrations in the low ng/ml range. There are two assays currently available that can quantitate cetiedil in plasma in this concentration range. Wojcieszyn et al. [5] have published an assay capable of quantitating 1 ng of drug in 2 ml of plasma using a bonded-phase cartridge extraction procedure with analysis by packed-column gas chromatography—mass spectrometry (GC—MS). An additional packed-column GC method has been developed by Henderson et al. [6] using a solvent-extraction procedure to isolate the drug and a nitrogen—phosphorus ionization detector for quantitation. This assay has a quantitation limit of 5 ng/ml using 2-ml plasma samples. Since plasma concentrations of cetiedil often fall below 5 ng/ml by 6 h following drug administration (0.4 mg/kg, intravenous infusion) and a gas chromatograph—mass spectrometer is not always available for routine samples, there is a need for a sensitive and readily available assay capable of quantitating 1 ng/ml drug in plasma.

The present study reports the development of a sensitive and reproducible capillary GC assay with nitrogen-selective detection for cetiedil in plasma. As an application and validation of the assay, cetiedil concentrations were determined in plasma from dogs which had received a 0.75 mg/kg intravenous dose of the drug. The resulting data were described by an appropriate pharmacokinetic model and pertinent pharmacokinetic parameters were obtained.

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EXPERIMENTAL

Apparatus

Gas chromatography. A Hewlett-Packard 5880A capillary gas chromatograph equipped with a Hewlett-Packard 7672A autosampler and a nitrogen–phosphorus ionization detector was used. A CP Sil 8 CB fused-silica capillary column (25 m × 0.32 mm I.D.; 0.12 μm film thickness, Chrompack, Bridgewater, NJ, U.S.A.) was used with helium as carrier gas at a flow-rate of 3 ml/min at 185°C. The injector and detector temperatures were 300°C and oven temperature programming was employed from 185 to 265°C at 10°C/min. Splitless injection with a purge at 0.3 min was used in conjunction with the autosampler.

Data acquisition. A Hewlett-Packard 3354 laboratory automation system with software developed in-house was used for data acquisition and processing.

Reagents and supplies

Solvents. Nanograde methanol, toluene and hexane were obtained from Mallinckrodt (Paris, KY, U.S.A.) and used without further purification. Triply purified distilled water was obtained from Ephrata Mountain Water (Manheim, PA, U.S.A.).

Reagents. Glacial acetic acid and ammonium hydroxide (58%), analytical-reagent grade, were purchased from Mallinckrodt and HPLC-grade ammonium acetate was obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.).

Other supplies. C_{18} Bond-Elut cartridges, 500 mg capacity, and the Vac-Elut manifold were purchased from Analytichem International (Harbor City, CA, U.S.A.).

Cetiedil citrate and the internal standard bepridil hydrochloride were obtained in-house (McNeil Pharmaceutical, Spring House, PA, U.S.A.). Structures for these compounds are given in Fig. 1.

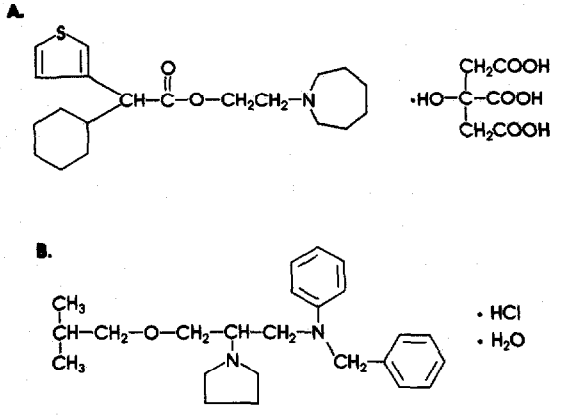

Fig. 1. Structural formulae for cetiedil citrate (A) and the internal standard bepridil hydrochloride (B).

Extraction procedure

To a 1-ml plasma sample, 2 ml of methanol containing 10 ng/ml internal standard were added to precipitate the plasma proteins. After vortexing and

certrifuging the mixture at 681 *g*, the supernatant was decanted into the reservoir of a conditioned C_{18} Bond-Elut cartridge (cartridges were conditioned by washing with 3 ml of 0.04 *M* ammonium acetate in methanol and 6 ml of methanol). Following the application of the sample, the cartridge was rinsed with 2 × 3 ml of $1.5 \cdot 10^{-3}$ *M* acetic acid and 2 × 3 ml of methanol—water (90:10).

The sample was eluted using three 0.5-ml aliquots of 0.02 *M* ammonium acetate in methanol. The eluent was transferred to a centrifuge tube containing 6 ml of hexane and 100 μl of 0.58% ammonium hydroxide, and the tube was vortexed for 10 s. After allowing the layers to separate, the hexane layer was transferred to another tube and evaporated using a gentle stream of nitrogen.

The dried residue was reconstitued with 50 μl of a toluene—methanol (90:10) solution and the sample was transferred to autoinjector vials. A 3-μl aliquot was injected into the capillary gas chromatograph for analysis.

Standard curves

To establish a calibration curve, a series of cetiedil standard solutions (1.0—100 ng/ml) containing 10 ng/ml internal standard were prepared in methanol using silylated glassware. Of these solutions, 2 ml were added to 1 ml of plasma (instead of the 2 ml of methanol containing internal standard alone) and the samples were extracted according to the procedure above. Duplicate standard curves were run on each analysis day. The peak height ratios of cetiedil and the internal standard were weighted by 1/variance and plotted against the cetiedil concentrations. Linear regression analysis gave a calibration line which was used to calculate cetiedil concentrations in unknown samples and frozen seeded controls.

As an additional control, seeded plasma pools were prepared at two concentrations (10 and 50 ng/ml cetiedil), separated into 1-ml aliquots and frozen. Two samples from each pool were analyzed with each calibration curve to assess the precision of the assay procedure.

Pharmacokinetic study in dogs

Two beagle dogs received an intravenous dose (slow bolus) of 0.75 mg/kg cetiedil (as the citrate salt). A pre-dose blood sample was collected and 5-ml blood samples were obtained at 5, 15, 30 and 45 min and 1, 2, 3, 4, 5, 6 and 24 h after dose administration. All blood samples were collected by venipuncture of the jugular vein using heparinized Vacutainer® tubes. Following collection, plasma was harvested from the blood samples by centrifugation at 681 *g* for 20 min. Plasma samples were divided into 1-ml aliquots and frozen at −10°C until analysis.

RESULTS AND DISCUSSION

Gas chromatography

Chromatograms of plasma with and without cetiedil and the internal standard are shown in Fig. 2. The retention times of cetiedil and the internal standard were 5.85 and 6.16 min, respectively. No significant interfering peaks appeared in the chromatogram of blank plasma and the two compounds were well

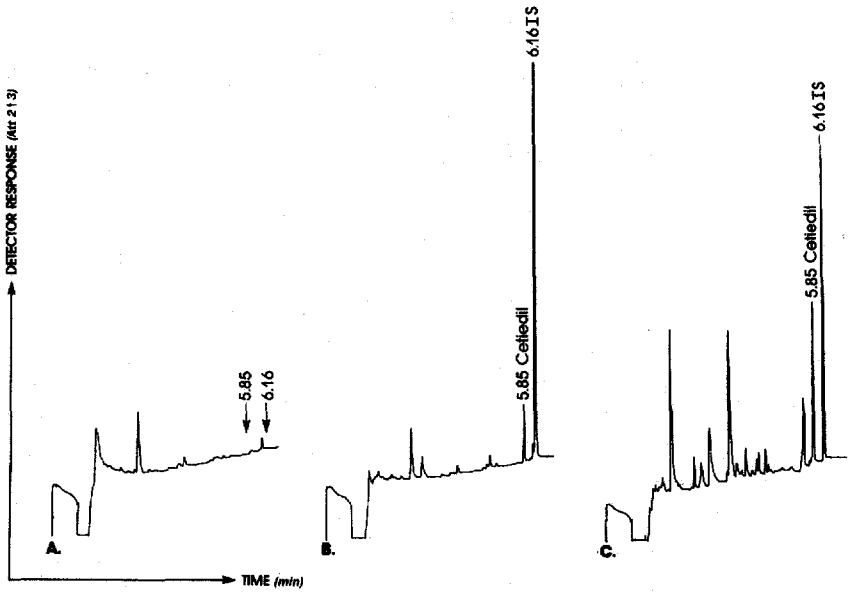

Fig. 2. Chromatograms of: blank human plasma (A); human plasma spiked with cetiedil (3.0 ng/ml) and internal standard (20 ng/ml) (B); dog plasma sample containing 10 ng/ml cetiedil (C).

separated. Additionally, owing to the selectivity of the cartridge procedure and the resolution of the capillary column, none of the following drugs interfered with the determination of cetiedil in plasma: amitriptyline, diazepam, phenobarbital, morphine, aspirin, acetaminophen, ibuprofen, chlorothiazide and propranolol.

Standard curves

Duplicate calibration curves run on three consecutive days were linear over the concentration range (1—100 ng/ml) studied here (Table I). The linear regression line which represents the best fit of the cetiedil data had an equation of $y=0.047x + 0.006$ (y=drug-to-internal standard peak height ratio; x=cetiedil concentration). The correlation coefficient for the three-day composite curve was 0.993. The relative standard deviations at each concentration were less than 13.2% except at the detection limit of 1 ng/ml (19.1%). The average back-calculated concentration was within 13% of the seeded value at each concentration. The average measured concentrations of the frozen seeded control plasma samples (n=6) were within 10% of their theoretical concentrations with relative standard deviations of less than 12% (data not shown).

Recovery and stability

The extraction efficiencies for cetiedil and the internal standard were determined using ^{14}C-labeled compounds. At cetiedil concentrations of 15 and 50 ng/ml, the extraction efficiencies were 51.8 ± 2.3% and 48.7 ± 0.4%, respectively (n=6). The extraction efficiency for the internal standard was 37.6 ± 0.4% at 20 ng/ml (n=12). Cetiedil has been reported to be stable in frozen human plasma for at least three months [6].

TABLE I

SUMMARY OF STANDARD CURVE DATA FOR THE ANALYSIS OF CETIEDIL IN PLASMA

Duplicate standard curves were analyzed on three separate days.

Concentration added (ng/ml)	n	Mean concentration found (ng/ml)	R.S.D.* (%)	Deviation from seeded value (%)
1.0	6	0.91	19.1	8.9
3.0	6	3.1	6.5	3.9
5.0	6	5.0	13.2	0.0
10.0	6	9.6	6.6	3.8
30.0	5	31.9	4.7	6.3
50.0	6	51.8	8.5	3.6
100.0	6	87.3	8.7	12.7

Day	Linear regression analysis		
	Slope ± S.D.	y-Intercept ± S.D.	Correlation coefficient
1	0.044 ± 0.000	−0.001 ± 0.006	1.000
2	0.043 ± 0.000	−0.003 ± 0.002	0.999
3	0.048 ± 0.001	0.008 ± 0.005	0.996
Three-day composite	0.047 ± 0.001	0.006 ± 0.004	0.993

*R.S.D. = Relative standard deviation.

TABLE II

SUMMARY OF PHARMACOKINETIC PARAMETERS OBTAINED FOLLOWING ADMINISTRATION OF 0.75 mg/kg EQUIVALENTS OF CETIEDIL AS THE CITRATE SALT TO TWO BEAGLE DOGS

C_{max} = Maximum concentration achieved; t_{max} = time of maximum concentration; AUC_{0-24} = area under the plasma concentration versus time curve from 0 to 24 h; estimated by trapezoidal approximation to 24 h); $t_{1/2\alpha}$ = half-life of the initial α or distribution phase. (calculated using computer program NONLIN [7]); $t_{1/2\beta}$ = half-life of the β elimination phase (calculated using computer program NONLIN [7]); $t_{1/2\gamma}$ = half-life of the γ or terminal elimination phase (calculated using computer program NONLIN [7]); Cl_s = clearance = [dose (mg/kg)]/[AUC_{0-24} (ng min/ml)].

Dog No.	Weight (kg)	C_{max} (ng/ml)	t_{max} (min)	AUC_{0-24} (ng h/ml)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	Cl_s (ml/min kg)
1	13	388	5	391	0.17	0.88	7.6	31.9
2	14	199	5	293	0.25	1.03	13.1	42.7
Mean		294	5	342	0.21	0.96	10.4	37.3

Pharmacokinetic study in dogs

Cetiedil plasma concentrations were measured in each of the dog samples obtained in the study. No interfering peaks were encountered. In cases where

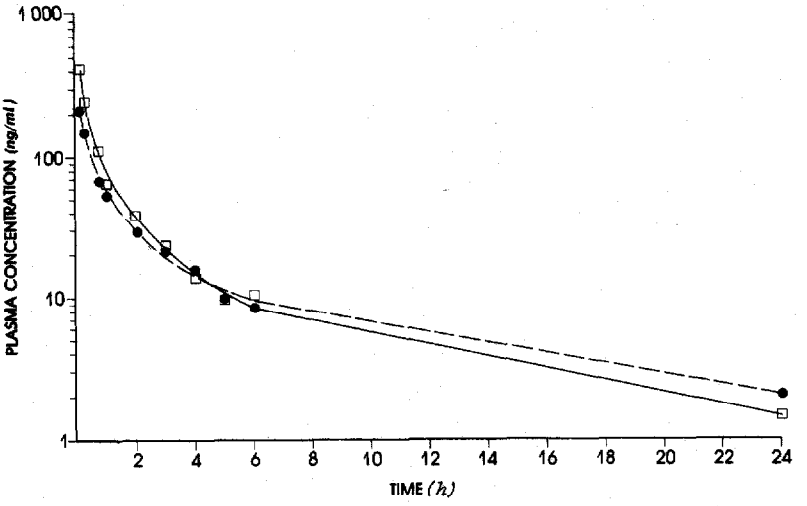

Fig. 3. Comparison of observed data points and predicted three-compartment pharmacokinetic model curves. □, Observed data points for dog No. 1; —, predicted curve for dog No. 1; •, observed data points for dog No. 2; - - - -, predicted curve for dog No. 2.

plasma concentrations exceeded the upper concentration range of the assay, smaller aliquots of plasma were diluted to 1 ml and analyzed. Table II displays the pharmacokinetic parameters that were obtained from the data. The average maximum peak plasma concentration was 294 ng/ml at 5 min following dose administration; cetiedil plasma concentrations fell to 2 ng/ml by 24 h. Cetiedil plasma concentration versus time data for each dog are presented in Fig. 3. After weighting by 1/concentration, the data were fitted to a three-compartment pharmacokinetic model using the computer program NONLIN [7]. The mean α, β and γ phase half-lives obtained using this model were 12.6 min, 58 min and 10.4 h, respectively. The appropriateness of the model is illustrated in Fig. 3, and indicated by a correlation coefficient of 0.998.

In summary, this assay procedure provides a reliable and sensitive method for determining cetiedil concentrations as low as 1 ng/ml in plasma. Application of the assay to a pharmacokinetic study in dogs indicated that following administration of cetiedil citrate to beagle dogs, cetiedil is eliminated from plasma according to a triexponential function with well defined α, β and γ phases.

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