Improved Gas Chromatographic Determination of Diltiazem and Deacetyldiltiazem in Human Plasma

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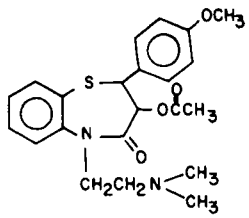
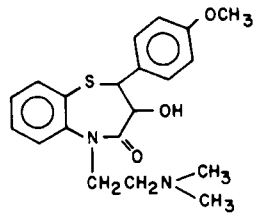
Abstract □ This study describes an improved, simple, and specific gas chromatographic method for the determination of diltiazem (I) and deacetyldiltiazem (II) in human plasma using loxapine (III) as an internal standard. After extraction at pH 7.5 with anhydrous ether–ethyl acetate (1:1), II was silylated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide. The gas chromatograph, equipped with an electron-capture detector, allowed measurements as low as 2 ng/mL for I and 3 ng/mL for II. Recoveries of III, I, and II were 95, 85, and 79%, respectively. There were no interferences with endogenous substances in plasma or with common cardiovascular drugs. This method was used to measure plasma concentrations of two patients who received 20 mg iv of I. The areas under the curve for these two patients were 275 and 273 ng·h/mL, respectively. The apparent volumes of distribution were 493.6 and 288.6 L, and the elimination half-lives were 4.70 and 2.73 h. No deacetyldiltiazem could be detected in the blood after the single-dose diltiazem administration.

Keyphrases □ Diltiazem—human plasma, GC with electron-capture detection, concurrent assay with deacetyldiltiazem □ Deacetyldiltiazem—human plasma, GC with electron-capture detection, concurrent assay with diltiazem

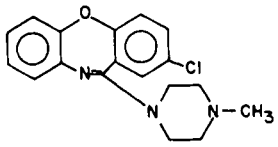
Diltiazem hydrochloride, *cis*-(+)-3-acetoxy-5-(2-dimethylaminoethyl) -2,3-dihydro-2- (4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one hydrochloride (I), is a new calcium antagonist used in the treatment of clinical manifestations of variant angina (1–5). Several methods have been used to determine I and its metabolites in biological media including spectrophotometry (8), gas chromatography (GC) (7), and more recently HPLC (6). The latter technique (6) enables the determination of I and six of its metabolites in urine, but UV detection is not sensitive enough for pharmacokinetic studies involving the determination of I and its principal metabolite, deacetyldiltiazem (II), in human plasma. On the other hand, a GC method involving the use of a nitrogen-sensitive detector has been described (7). To achieve adequate sensitivity while maintaining high reproducibility and specificity, a GC technique using electron-capture detection with ^{63}Ni was chosen. The present study describes an assay for I and II in plasma, and some pharmacokinetic parameters obtained after intravenous administrations of 20 mg of I in two patients are given.



DILTIAZEM
I



DEACETYLDILTIAZEM
II



LOXAPINE
III

EXPERIMENTAL

Equipment—A gas chromatograph[1], equipped with an ^{63}Ni electron-capture detector and a computer for peak area integration[2], was used. The chromatographic column was a coiled glass tube, 2 m × 2-mm i.d., packed with 3% OV 7 on GasChrom Q, 80–100 mesh. The following conditions were used: oven temperature, 245°C; injection port temperature, 300°C; detector temperature, 300°C; argon–methane carrier gas at 60 mL/min.

Reagents and Chemicals—All solvents were certified grade. The hydrochloride salts of diltiazem (I), deacetyldiltiazem (II), and loxapine (III) were used. Standard solutions of I, II, and III were prepared in distilled water at concentrations of 0.5 mg%.

Assay Procedure—To 1 mL of plasma, contained in a culture tube, were added 100 μL of internal standard solution (500 ng) and 3 mL of phosphate buffer, pH 7.5. This mixture was vortexed for 30 s. Following the addition of 6 mL of 50% ether in ethyl acetate, the mixture was agitated for 10 min on a reciprocating shaker and then centrifuged at 800×*g*. The aqueous phase was discarded, and the organic phase was transferred to a second culture tube and dried under a nitrogen stream. *N*-Methyl-*N*-(trimethylsilyl)trifluoroacetamide (25 μL) and benzene (100 μL) were

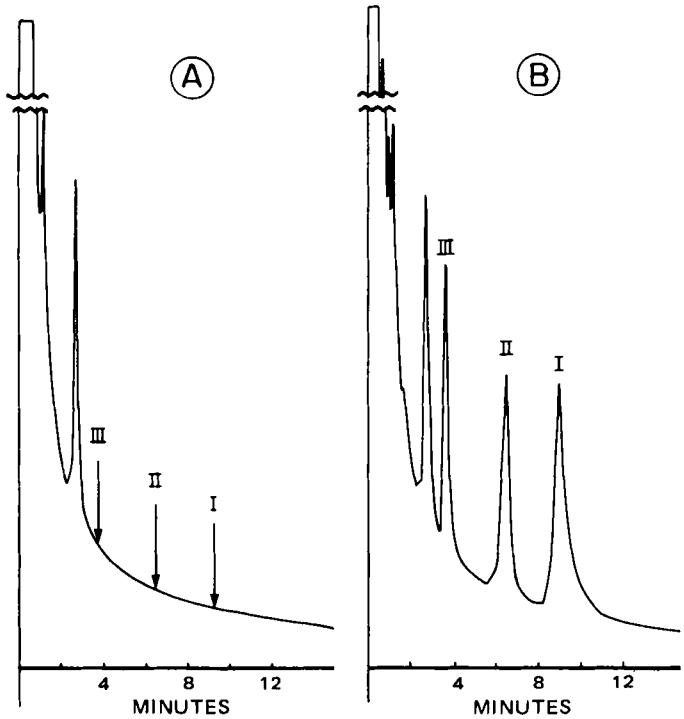


Figure 1—*Typical chromatograms of plasma extracts. Key: (A) blank; (B) containing I, II, and III.*

[1] Hewlett Packard model 5700.
[2] Hewlett Packard model 3300.

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Table I—Peak Height Ratios of Diltiazem (I) and Deacetyldiltiazem (II) in Plasma

Diltiazem			Deacetyldiltiazem		
Amount in Plasma, ng/mL	Peak Height Ratio	*CV*, %	Amount in Plasma, ng/mL	Peak Height Ratio	*CV*, %
10	0.049	3.9	7.5	0.031	8.5
20	0.100	4.9	15	0.065	5.4
50	0.245	4.1	30	0.126	2.6
100	0.490	4.5	60	0.242	5.3
200	1.000	2.0	120	0.497	3.0
400	2.020	3.1	240	0.985	3.5

added, and the tube was sealed and heated at 70°C for 1 h for the silylation of II. The solution was dried again under a nitrogen stream, 50 μL of methanol was added, and 5 μL of the resulting solution was injected into the chromatograph.

Standard samples were prepared by spiking human plasma with increasing concentrations of I (10, 25, 50, 100, 200, and 400 ng/mL) and of II (7.5, 15, 30, 60, 120, and 240 ng/mL). Four spiked samples were measured for each concentration, and calibration curves were obtained by plotting the ratios of the peak areas of I and II to the area of the internal standard *versus* the concentrations of I and II. Plasma recoveries of I and II were determined by comparing peak areas obtained by direct injection of I or silylated II with those obtained after the extraction procedure.

Clinical Study—Two fully informed patients with unstable angina received a bolus 20-mg iv dose of I. Blood samples were withdrawn into heparinized tubes at 0, 5, 10, 15, 20, 30, 45, 60, 90, 160, 180, 240, 360, and 480 min after administration. The blood samples were immediately centrifuged at 800×*g* and the plasma was removed and kept at −14°C until analysis (within 2 weeks).

Pharmacokinetic Data—Areas under the curve (AUC) were calculated by the trapezoidal rule:

$$AUC_{0\to t} = \sum_{i=1}^{n} 0.5(C_1 + C_2)\Delta t \qquad \text{(Eq. 1)}$$

The total area under the curve (AUC_∞) has been obtained by adding C_p/β:

$$AUC_\infty = \sum_{i=1}^{n} 0.5(C_1 + C_2)\Delta t + C_{p,n}/\beta \qquad \text{(Eq. 2)}$$

where C_p is the plasma concentration at 8 h and β the elimination constant calculated by logarithmic linear regression. The elimination half-life ($t_{1/2}$) and the apparent volume of distribution (Vd) were calculated by the following:

$$t_{1/2} = \frac{0.693}{\beta} \qquad \text{(Eq. 3)}$$

and

$$Vd = \frac{D_{iv}}{AUC \cdot \beta} \qquad \text{(Eq. 4)}$$

where D_{iv} is the intravenous dose.

RESULTS AND DISCUSSION

Assay Method—Before silylation, I and II had the same retention time in the assay. After silylation of II, the retention times for III, II, and I were 3.70, 6.55, and 9.15 min, respectively. Typical chromatograms of a plasma blank and a plasma extract spiked with the three standards are presented in Fig. 1. The extent of II silylation was 95%.

The calibration curves were linear throughout the range of the concentrations measured in this study ($r^2 = 0.9998$ for I and $r^2 = 0.9999$ for II). The equations of the calibration curves for I and II were, respectively, $y = 0.005x - 0.013$ and $y = 0.0041x + 0.00137$, where y is the peak height ratio and x is the plasma concentration of the drug.

The analytical recoveries of I, II, and III were 95, 79, and 85%, respectively. The sensitivity of the method was 2 ng/mL for I and 3 ng/mL for II; amounts as low as 0.5 ng/mL of I or II could be detected. Table I shows the peak area ratios and their coefficients of variation (*CV*) for the spiked samples.

Digoxin, propranolol, procainamide, nitroglycerin, isosorbide dinitrate, furosemide, flurazepam, and diazepam did not interfere with the plasma assay. Even if the frozen plasma was analyzed after 4–8 weeks, there were no interference peaks. The present study shows that the use of an electron-capture technique permitted a large increase in the sensitivity of the assay. Rovei *et al.*, with a nitrogen–phosphorus detector, could measure 10 ng/mL with 2 mL of plasma (5); in the present method, 2 ng/mL of I could be measured with 1 mL of plasma. Moreover, the plasma extraction with anhydrous ether–ethyl acetate permitted recoveries of 85 and 79%, respectively, for I and II, compared with 70 and 55% with the hexane-extraction method of Rovei *et al.* This improvement in recovery permits determination of lower concentrations of these compounds.

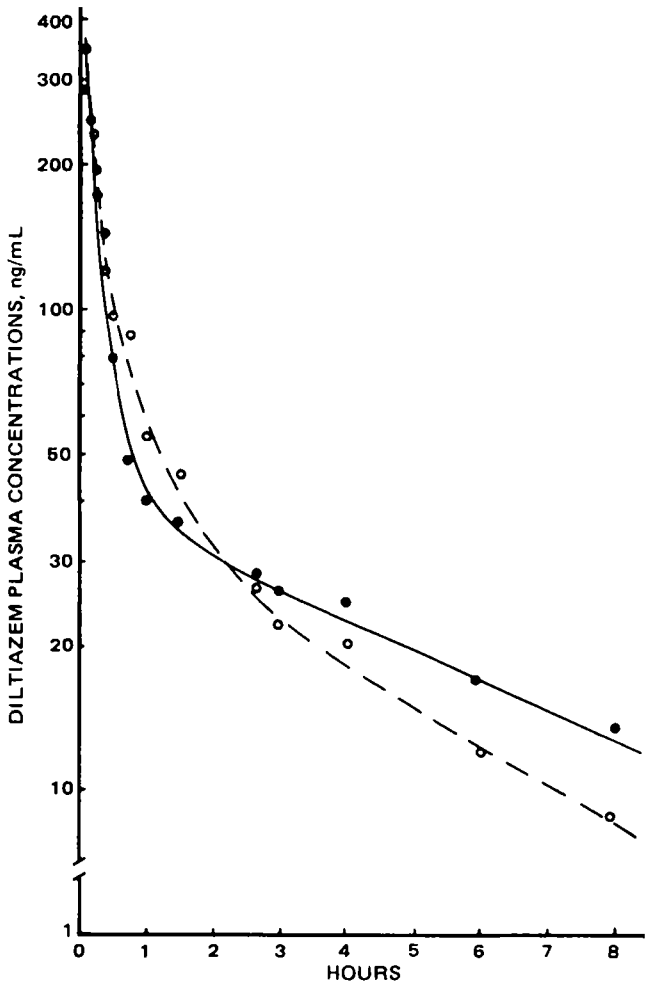

Figure 2—*Diltiazem plasma concentrations in two patients after a 20-mg iv dose. Key: (●) patient 1; (○) patient 2.*

Clinical Study—Figure 2 shows the plasma concentrations of I in two patients who were injected intravenously with 20 mg. Five minutes after the administration, the plasma concentrations were 289 and 344 ng/mL in the two patients. The distribution phase in both patients was rapid, and after 1 h the plasma concentrations were 40 and 59 ng/mL. The elimination half-lives were 4.70 and 2.73 h ($\beta = 0.1873$ and 0.2535, respectively). The areas under the curve were 275 and 273 ng·h/mL, and the apparent volumes of distribution were 493.6 and 288.6 L. The clearance values were 72.71 and 73.24 L/h, respectively.

The results are difficult to compare with those of previous investigations using oral administration. Rovei *et al.* found a half-life of 6 h after oral administration of 120 mg of the drug (7), and Kinney observed half-lives of 4.1, 5.1, and 5.6 h, with oral doses of 60, 90, and 120 mg of I, respectively (8). In the latter study, the AUC obtained after an oral dose of 40 mg was 514 ng·h/mL; in the present study a mean AUC of 274 ng·h/mL was found after a 20-mg iv dose. According to these results, the absolute bioavailability of I can be estimated by the following:

$$\text{Absolute bioavailability (\%)} = \frac{AUC_{po}/D_{po}}{AUC_{iv}/D_{iv}} \times 100 \qquad \text{(Eq. 5)}$$

By substituting into the equation the experimental values obtained for each term in the aforementioned studies, the absolute bioavailability of diltiazem is (514/50)/(274/20) = 62.5%.

No deacetyldiltiazem was detected in the plasma of the two patients after a 20-mg iv administration of I. More detailed studies are required for a better understanding of pharmacokinetics of diltiazem.

In summary, the present study has shown that the use of GC with electron-capture detection improves, to a large extent, the sensitivity of the previous HPLC and GC methods. This procedure is appropriate for monitoring plasma levels during single-dose pharmacokinetic studies.

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Desorption of Carbonate from Aluminum Hydroxycarbonate Gel by Nitrogen Purging

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Abstract □ Carbonate was completely desorbed from amorphous aluminum hydroxycarbonate gel by purging with nitrogen. The reversibility of carbonate adsorption suggests that aluminum hydroxycarbonate particles are composed of planes of aluminum hydroxide with carbonate adsorbed at edge aluminum sites. A slow-reacting phase which was identified as a precursor of gibbsite, formed when the carbonate/aluminum molar ratio was <0.20.

Keyphrases □ Desorption—carbonate from aluminum hydroxycarbonate gel, nitrogen purging □ Carbonate—desorption from aluminum hydrocarbonate gel, nitrogen purging □ Nitrogen purging—desorption of carbonate from aluminum hydroxycarbonate gel □ Aluminum hydroxycarbonate gel—desorption of carbonate, nitrogen purging

The coordination of carbonate by aluminum in aluminum hydroxide has been demonstrated by Raman and IR analyses (1–3) as well as by the effect of carbonate adsorption on the point of zero charge of aluminum hydroxide (4). The term specific adsorption describes the adsorption of carbonate by aluminum hydroxide as it refers to the adsorption of anions by the formation of a partial covalent bond with structural metal atoms and the concomitant displacement of hydroxyl or water groups from the surface (5, 6).

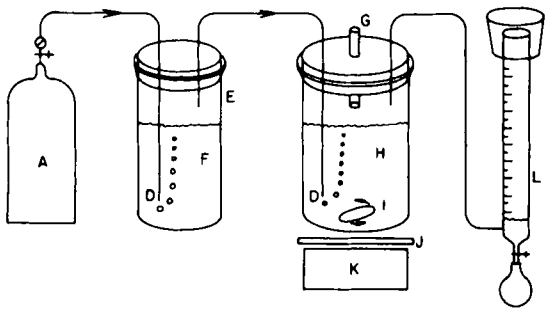

Figure 1—*Apparatus for purging aluminum hydroxycarbonate gel with nitrogen gas. Key: (A) nitrogen gas cylinder; (E) glass cylinder; (D) fritted glass gas disperser; (F) water; (G) sealable sampling port; (H) aluminum hydroxycarbonate gel; (I) magnetic stirring bar; (J) heat shield; (K) magnetic stirrer; (L) soap bubble flow meter.*

The importance of specifically adsorbed carbonate in maintaining the structure and antacid properties of aluminum hydroxide gel has been demonstrated by numerous kinetic studies (3, 7–11). Insight into adsorption mechanisms may be gained by studying desorption reactions. Hingston *et al.* (12) found that specific adsorption of phosphate, selenite, and fluoride by gibbsite or goethite varied between complete reversibility and almost complete irreversibility. The reversibility of the specifically adsorbed anion was related to the nature of the adsorption complex. Adsorption was reversible when only unidentate ligands formed between the anion and metal, *i.e.*, fluoride anion. However, multidentate ligands, bridging, or the formation of ring structures at the surface led to irreversibility. McBride (13) concluded from spin probe studies that easily desorbed anions were adsorbed on aluminum hydroxide by ligand exchange of a single surface hydroxyl while anions that could not be easily desorbed were adsorbed by bidentate bonds. Gast (6) also suggested that readily desorbed anions have one coordinated bond to the surface while those more strongly retained have two coordinated bonds. Therefore, the desorption of specifically adsorbed carbonate from aluminum hydroxycarbonate gel was studied as part of efforts to improve the antacid properties and stability of antacid products.

EXPERIMENTAL

The two aluminum hydroxycarbonate gels (CG1 and CG2) studied were obtained commercially[1] as 9.5% equivalent Al_2O_3 pourable gels and were diluted with distilled water to 5% equivalent Al_2O_3. IR analysis[2] was

[1] Reheis Chemical Co., Berkeley Heights, N.J. and Chattem Chemical, Chattanooga, Tenn.

[2] Model 180; Perkin-Elmer Co., Norwalk, Conn.

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