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Note

High-performance liquid chromatographic determination of diltiazem and its major metabolites, N-monodemethyldiltiazem and desacetyldiltiazem, in plasma

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Diltiazem hydrochloride is a calcium channel blocker currently used in the treatment of angina pectoris [1], and is probably effective in the treatment of hypertension [2] and cardiac arrhytmias [3]. Parent drug and a major metabolite, desacetyldiltiazem, have been measured by high-performance liquid chromatography (HPLC) [4–7], but these methods do not measure N-monodemethyldiltiazem in plasma (Fig. 1). This metabolite has recently been found to accumulate in plasma after chronic oral administration of diltiazem to humans [8]. In addition, N-monodemethyldiltiazem has been shown to have measurable pharmacological activity as a coronary vasodilator in anesthetized dogs [9]. A recent HPLC method [10] describes determination of diltiazem and several metabolites in plasma; however, reproducibility and sensitivity of the assay may not be sufficient for application to single- or multiple-dose pharmacokinetic studies in humans.

The method described here provides a sensitive, reproducible HPLC separation of diltiazem, N-monodemethyldiltiazem, and desacetyldiltiazem in plasma with ultraviolet (UV) detection. Pharmacokinetic parameters after single or multiple oral doses may be determined for diltiazem and the major metabolites by this method.

EXPERIMENTAL

Materials

Pure samples of diltiazem were generously provided by Marion Labs. (Kansas City, MO, U.S.A.). N-Monodemethyldiltiazem fumarate and desacetyldiltiazem

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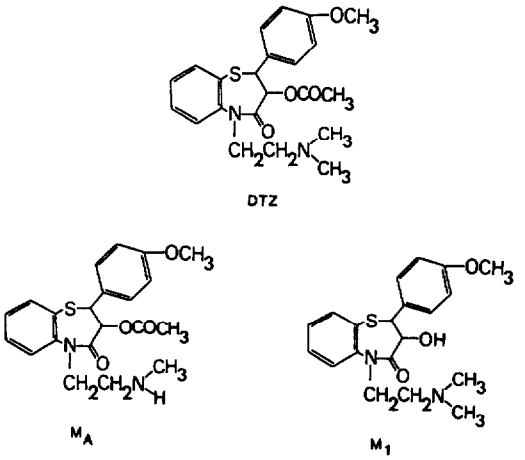


Fig. 1. Chemical structure of diltiazem (DTZ), N-monodemethyldiltiazem (M_A) and desacetyldiltiazem (M_1).

hydrochloride were generously provided by Tanabe Seiyaku (Osaka, Japan). Diphenhydramine hydrochloride, used as an internal standard, was provided by Parke-Davis (Morris Plains, NJ, U.S.A.).

All other reagents were analytical-reagent grade and were purchased from commercial sources and used without further purification. Mobile phase components (acetate buffer, acetic acid, 1-heptanesulfonic acid, methanol and acetonitrile) were filtered prior to mixing, then degassed after mixing.

Apparatus and chromatographic conditions

The HPLC system consisted of Waters Assoc. (Milford, MA, U.S.A.) Model 6000 A solvent delivery system, Model 450 variable-wavelength UV spectrophotometer operated at 230 nm, and a Model U6K sample loop. Detector output was quantitated on a Fisher (Fair Lawn, NJ, U.S.A.) Model 5000 chart recorder. The separation system was a 30 cm × 3.9 mm stainless-steel (10 μm particle size) C_{18} μBondapak reversed-phase column.

Mobile phase, 0.06 *M* acetate buffer – acetonitrile – methanol (60:35:5) containing 5 m*M* heptanesulfonic acid (glacial acetic acid was added to adjust the pH of the final solution to 6.30) was run at a flow-rate of 1.8 ml/min. All analyses were performed at room temperature.

Stock solutions

Standard solutions were prepared by dissolving the appropriate quantity of diltiazem hydrochloride to yield 10 mg diltiazem base in 100 ml methanol and similarly 10 mg N-monodemethyldiltiazem and 10 mg desacetyldiltiazem in 100 ml methanol. Sequential dilutions to 1 μg/ml were then made. For the internal standard, diphenhydramine hydrochloride was dissolved to yield 100 mg of diphenhydramine base in 100 ml methanol. Dilution to 100 μg/ml was made for use in the procedure. These solutions were stored in the dark in glass bottles at 4 °C and were stable for at least three months.

Preparation of samples

Diphenhydramine was used as the internal standard. A 20-μl volume of stock solution (100 μg/ml), containing 2 μg diphenhydramine, was added to each of a series of 14-ml (100×17 mm) conical polyethylene centrifuge tubes with tight sealing polyethylene caps (Sarstedt Scientific, St. Louis, MO, U.S.A.). A 1.0–2.0 ml aliquot of unknown plasma was added to each tube. Calibration standards for diltiazem, N-monodemethyldiltiazem, and desacetyldiltiazem were prepared by adding 5, 10, 25, 50, 100, 200, and 300 ng of drug and metabolites to consecutive tubes. Drug-free control plasma was added to each of the calibration samples.

Extraction procedure

To each tube were added 4 ml of hexane–isoamyl alcohol (98:2). The tubes were agitated gently by hand for 5 min, then centrifuged at 4°C for 5 min at 400 *g*. The organic layer was transferred to another 14-ml polyethylene centrifuge tube which contained 100 μl of 0.01 *M* hydrochloric acid. An additional 4 ml of hexane–isoamyl alcohol (98:2) was added to each tube containing plasma, and the extraction procedure was repeated. The tubes containing the both organic layers (first and second extractions) and the hydrochloric acid were then agitated gently by hand for 5 min and centrifuged at 4°C for 5 min at 400 *g*. The upper organic layer was discarded. A 20–90 μl aliquot of the lower aqueous phase was then injected directly into the sample loop.

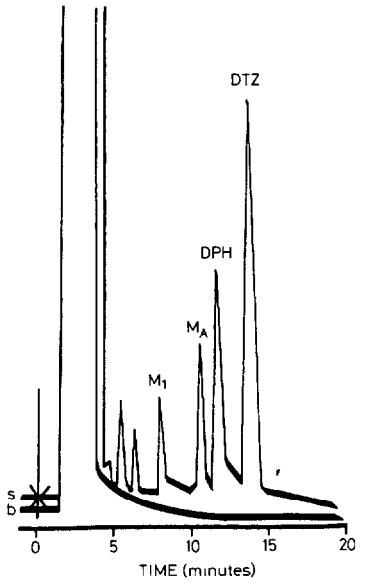
Clinical pharmacokinetic study

A 73-year-old hypertensive white male volunteer was started on 60 mg diltiazem (Marion Labs.), taken twice daily by mouth, for one week after giving written informed consent. The dose was then raised to 120 mg for an additional week. On day 14 of therapy, multiple venous blood samples were drawn into venoject heparin-containing tubes just prior to and up to 18.0 h after administration of the morning dose. The evening dose was omitted. Plasma was separated by centrifugation (400 *g* for 10 min at 4°C) and concentrations of diltiazem, N-monodemethyldiltiazem, and desacetyldiltiazem were determined by the method described above.

RESULTS

Evaluation of the method

Under the described chromatographic condition, diltiazem, N-monodemethyldiltiazem, desacetyldiltiazem, and diphenhydramine gave symmetric, well resolved chromatograpic peaks (Fig. 2). From detector wavelengths of 225–250 nm, 250 nm was optimal for diltiazem and both metabolites. Drug-free blank plasma samples were free of endogenous contaminants at the retention times corresponding to the compounds if they were used before three months of storage at −20°C. After this time, a contaminant would appear at the retention time corresponding to desacetyldiltiazem. The relation of plasma diltiazem, N-monodemethyldiltiazem, and desacetyldiltiazem concentration to the diltiazem:diphenhydramine, N-monodemethyldiltiazem:diphenhydramine, and



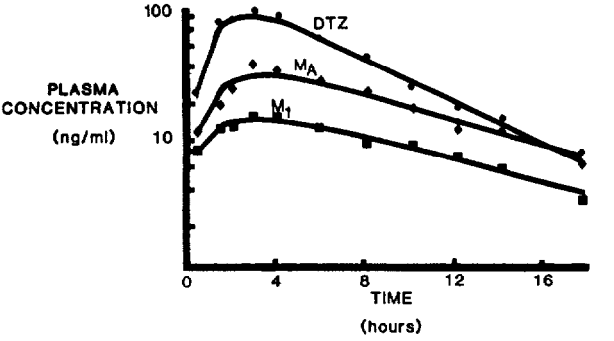


Fig. 2. Representative chromatograms of (b) blank plasma and (s) patient plasma drawn 14 h after 120 mg diltiazem taken orally during steady state. Peaks: M_1 = desacetyldiltiazem; M_A = N-monodemethyldiltiazem; DPH = diphenhydramine, internal standard; DTZ = diltiazem.

Fig. 3. Plasma levels for diltiazem (DTZ), N-monodemethyldiltiazem (M_A), and desacetyldiltiazem (M_1) in patient after 120 mg diltiazem taken orally during steady state.

desacetyldiltiazem: diphenhydramine peak-height ratios, respectively, were linear to at least 300 ng/ml. Relative slope values for diltiazem, N-monodemethyldiltiazem, and desacetyldiltiazem were 0.0134, 0.0121, and 0.0217, respectively. Extraction efficiency for diltiazem, N-monodemethyldiltiazem, desacetyldiltiazem, and diphenhydramine are 95, 96, 100 and 100%, respectively.

Day-to-day reproducibility

Analysis of four standard curves extracted and detected on different days had correlation coefficients of 0.998 or greater. The day-to-day coefficient of variation

TABLE I

WITHIN-DAY COEFFICIENTS OF VARIATION FOR IDENTICAL SAMPLES OF DILTIAZEM AND THE TWO METABOLITES ($n=6$)

Concentration (ng/ml)	Coefficient of variation (%)		
	Diltiazem	N-Monodemethyldiltiazem	Desacetyldiltiazem
5	14.3	11.1	10.6
10	7.8	10.1	5.2
25	5.4	5.3	5.9
50	2.2	2.9	2.7
100	5.3	5.0	4.3
200	3.1	3.5	4.0
300	6.1	6.6	6.0

TABLE II

PHARMACOKINETIC PARAMETERS IN A 73-YEAR-OLD HYPERTENSIVE MALE FOLLOWING THE ADMINISTRATION OF 120 mg DILTIAZEM

Compound	Area under curve (ng/ml·h)	Elimination half-life (h)	Peak concentration (ng/ml)
Diltiazem	726	7.56	95.7
N-Monodemethyldiltiazem	343	6.50	36.4
Desacetyldiltiazem	162	4.00	15.4

in the slopes of the calibration curves was 6.7% for diltiazem, 6.9% for N-monodemethyldiltiazem, and 5.2% for desacetyldiltiazem. The limit of detection of the method is 5 ng/ml for a 2-ml extracted plasma sample for diltiazem, N-monodemethyldiltiazem, and desacetyldiltiazem. Table I lists within-day coefficients of variation for diltiazem and the two metabolites.

Pharmacokinetic study

Fig. 3. shows plasma diltiazem, N-monodemethyldiltiazem, and desacetyldiltiazem concentrations for the described subject. Derived pharmacokinetic parameters are listed in Table II.

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REFERENCES

1 R. Zelis, N. Engl. J. Med., 306 (1982) 926-928.
2 K. Aoki, K. Sato, S. Kondo and M. Yamamoto, Eur. J. Clin. Pharmacol., 25 (1983) 475-480.
3 J.J. Rozanski, L. Zaman and A. Castellanos, Am. J. Cardiol., 49 (1982) 621-628.
4 C. Verghese, M.S. Smith, L. Aanonsen, E.L.C. Prichett and D.G. Shand, J. Chromatogr., 272 (1983) 149-155.
5 R.E. Wiens, D.J. Runser, J.R. Lacz and D.C. Dimmit, J. Pharm. Sci., 73 (1984) 688-689.
6 J.P. Clozel, G. Caille, Y. Taeymans, P. Theroux, P. Biron and F. Trudel, J. Pharm. Sci., 73 (1984) 771-773.
7 D.R. Abernethy, J.B. Schwartz and E.L. Todd, J. Chromatogr., 342 (1985) 216-220.
8 J. Sugihara, Y. Sugawara, H. Ando, S. Harigaya, A. Etoh and K. Khono, J. Pharm. Dyn., 7 (1984) 24-32.
9 H. Yabana, T. Nagao and M. Sato, J. Cardiovasc. Pharmacol., 7 (1985) 152-157.
10 K.J. Goebel and E.U. Kölle, J. Chromatogr., 345 (1985) 355-363.