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Modified HPLC analysis of diltiazem in plasma for pharmacokinetic studies

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Summary

A simple procedure for determination of diltiazem in plasma using high-performance liquid chromatography (HPLC) is described. The method consists of extraction of the drug and the internal standard (loxapine) by using hexane-isopropanol (98 : 2) followed by solvent evaporation and redissolution of the residue in acetonitrile/0.5 M potassium dihydrogen phosphate (pH 2.5, 30 : 70). The procedure has a sensitivity limit of 2 ng/ml and a standard curve that is linear to 200 ng/ml. The method was applied to a pharmacokinetic study of diltiazem tablets.

Diltiazem is a calcium channel blocker widely prescribed for the treatment of angina pectoris, arrhythmia and hypertension (Rosanski et al., 1982). A number of analytical techniques using thin-layer chromatography (Kohno et al., 1977), gas chromatography (Rovei et al., 1977; Clozel et al., 1984a) and HPLC (Verghese et al., 1983; Clozel et al., 1984b; Wiens et al., 1984; Abernethy et al., 1985, Abdel-Hamid et al., 1988; Zhao and Chow, 1989) have been reported. Although the HPLC techniques are simpler and more sensitive, they still suffer from problems encountered during the extraction process which affect both reproducibility and sensitivity.

The present study describes a rapid, simple, sensitive and suitable HPLC method for pharmacokinetic studies for the determination of diltiazem in plasma.

All solutions were prepared in deionized water from analytical reagent grade materials. Diltiazem (Lavipharm, Greece), loxapine (Lederle Lab., Pearl River, NY) and other materials were obtained from commercial sources. The HPLC system consisted of a Waters model U6K universal injector, a model 590 solvent delivery system, and a model 481 LC variable-wavelength UV detector set at 239 nm. A Spherisorb ODS2 5 μm reversed-phase column (15 cm $\times$ 4.6 mm) thermostated at 40 °C was used. The mobile phase consisted of 65% potassium dihydrogen phosphate buffer (0.05 M, pH 3.9) and 35% acetonitrile. The flow rate was 1.75 ml/min.

Three buffer solutions of pH 2.5, 3.9, and 9.0 were prepared as follows. The pH of a 0.05 M potassium dihydrogen phosphate buffer was adjusted with orthophosphoric acid (85%) to pH 2.5.

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The dropwise addition of triethylamine to this solution was used to prepare the phosphate buffer solution of pH 3.9. The buffer of pH 9.0 was prepared by dissolving 3.73 KCl and 3.1 g boric acid using 500 ml of water followed by adjustment of the pH to 9.0 by addition of 0.1 N NaOH and dilution to 1 l with water. Stock solutions of diltiazem (100 μg free base/ml) and loxapine (100 μg free base/ml) were prepared by dissolving the appropriate weight of pure substance in methanol. A working standard solution (1 μg/ml for diltiazem) in phosphate buffer pH 9.0 was prepared daily by dilution of the stock solution. A working standard solution (1 μg/ml for loxapine) was prepared in methanol by dilution of the stock solution. All solutions were stored in total darkness at 4 °C.

The extraction of drug from plasma was accomplished as follows. To 1.0 ml of plasma, contained in a culture tube, were added 100 μl of internal standard solution (1 μg/ml) and 6 ml of hexane-isopropanol (98 : 2, v/v). The mixture was agitated for 15 min on a home-made shaker (rotation angle 120 °) at 25 strokes per min and then centrifuged for 10 min at 2000 rpm. The aqueous phase was decanted and the organic phase was evaporated to dryness at 40 °C under a nitrogen stream. The residue was dissolved in 200 μl of a

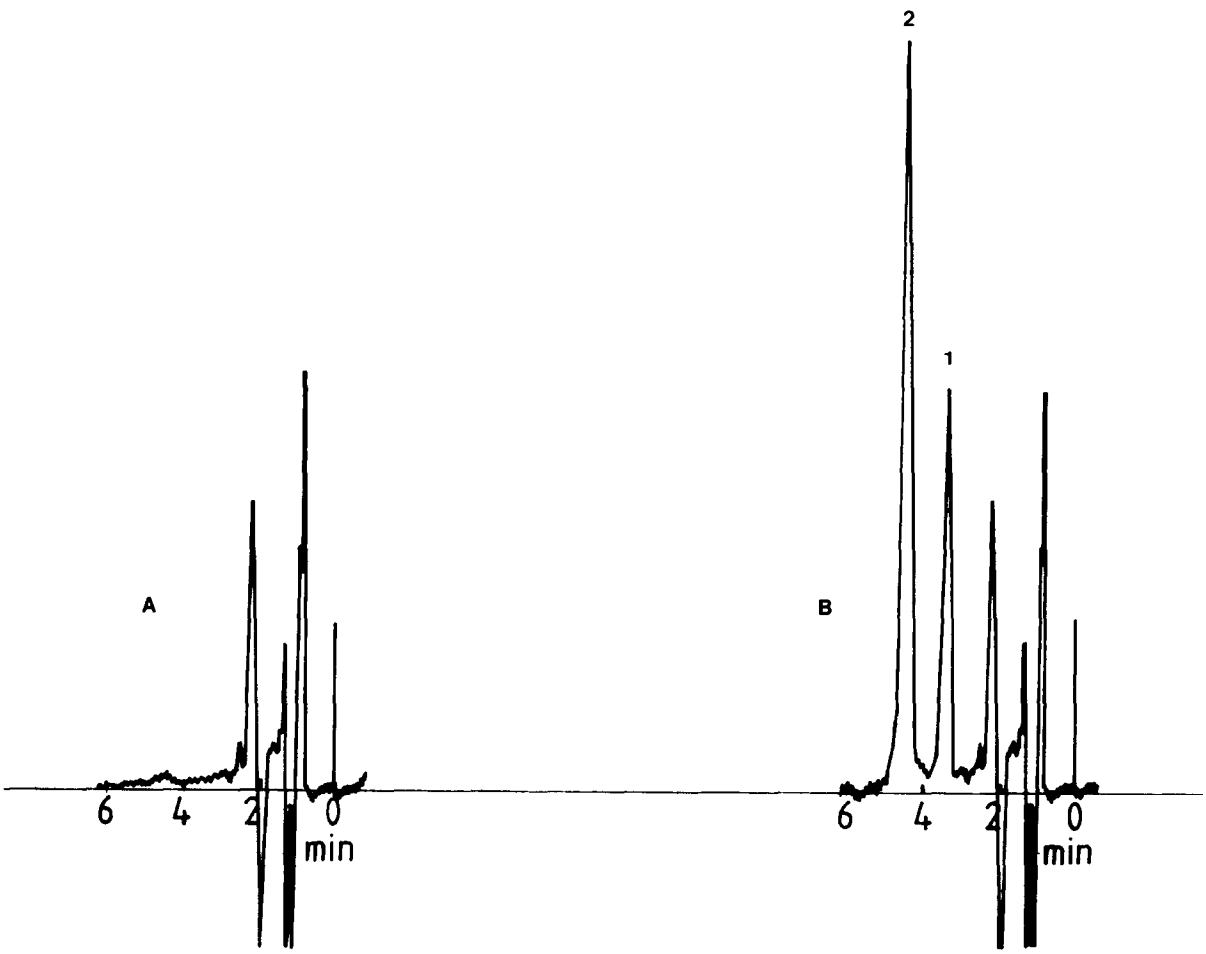

Fig. 1. Chromatograms from (A) blank human plasma and (B) human plasma following the oral administration of diltiazem (two 60 mg tablets, 0.5 h post-dose). 1, diltiazem; 2, loxapine.

30 : 70 (v/v) mixture of acetonitrile, 0.05 M potassium dihydrogen phosphate buffer pH 2.5 and a 100 μl aliquot was then injected.

The method developed was applied to a diltiazem bioavailability study. A single dose of two diltiazem tablets (Tildiem, Lavipharm, Greece) (2 × 60) mg was administered to 12 healthy informed volunteers after a light breakfast. Samples of venous blood were drawn at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0 and 24.0 h after taking the medication. The plasma was separated and stored at −70 °C for later analysis.

The present assay procedure modifies the recently reported method of Zhao and Chow (1989) in two ways. First, the back-extraction with 100 μl of 0.025 M sulfuric acid is not employed. Instead, the organic solvent is evaporated and the residue is dissolved in 200 μl ACN-buffer (pH 2.5, 30 : 70, v/v). By doing so, the technical difficulty of collecting a volume of 20 μl of the aqueous layer is avoided while ensuring the preservation of column performance. Moreover, the injection of a 100 μl sample is easily accomplished. Second, a Spherisorb ODS2 (5 μm, 4.6 mm, 15 cm) column thermostated at 40 °C was used in this assay while the mobile phase consisted of ACN/phosphate buffer (pH 3.9, 35 : 65) with a small quantity of triethylamine. These conditions gave shorter retention times and clear chromatograms. Fig. 1 shows typical chromatograms of extracted plasma. Blank samples of plasma without internal standard resulted in no interfering peaks. The chromatographic conditions gave a retention time of 3.6 min for diltiazem and 4.5 min for loxapine (as internal standard).

Calibration curves, using a 1 ml plasma sample spiked with diltiazem (10–200 ng/ml), were constructed. In all cases, good linearity was observed; a typical calibration equation with $n = 7$ and $r = 0.999$ is

$$Y = -0.00949(\pm 3.07 \times 10^{-2}) + 0.0103(\pm 1.99 \times 10^{-4}) \cdot C$$

where y is the peak height ratio of diltiazem/loxapine, and C the concentration in ng/ml of diltiazem. Analysis of replicate samples ($n = 5$) for the variation of precision gave a coefficient of variation ±6.47 to 0.38 for the range 10–200 ng/ml. The assay developed has a sensitivity limit of 2 ng/ml utilizing 1 ml plasma and a 100 μl injection volume. The large injection volume in conjunction with the clear chromatograms (low noise-to-signal ratio) resulted in a lower sensitivity limit for diltiazem as compared to previously reported methods.

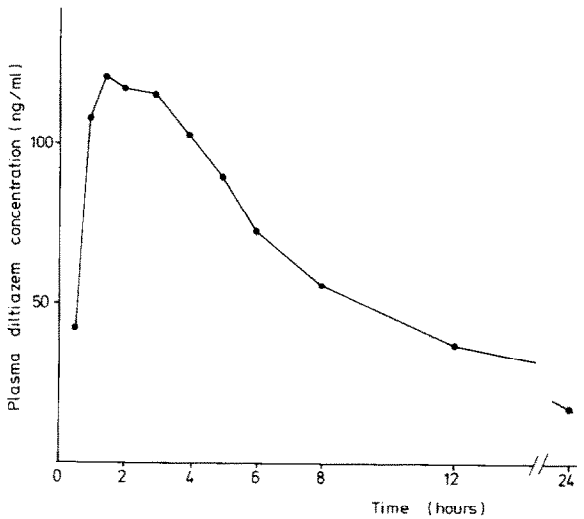

Fig. 2. Mean plasma levels of 12 healthy volunteers administered two 60 mg diltiazem tablets.

This procedure was found to be appropriate for monitoring plasma levels during the bioavailability study; the results are presented in Fig. 2. Since the method developed was found to be accurate, reproducible and rapid, these characteristics make the present method suitable for human pharmacokinetic studies.

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