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Note

High-performance liquid chromatographic analysis of flordipine in human plasma

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Flordipine {diethyl-1,4-dihydro-2,6-dimethyl-1-[2-(4-morpholinyl)ethyl]-4-[2-(trifluoromethyl)phenyl]-3,5-pyridinedicarboxylate} is a new cardiovascular agent that is orally active in animal models [1, 2]. It is currently undergoing clinical trials in humans for use in essential hypertension [3]. This paper describes a procedure, consisting of solvent extraction and reversed-phase high-performance liquid chromatography (HPLC), for the measurement of flordipine in human plasma. This general approach has been used by Pietta et al. [4] for another dihydropyridine compound, nifedipine.

EXPERIMENTAL

Apparatus

A liquid chromatograph (Model 8000B, Spectra-Physics, Santa Clara, CA, U.S.A.), equipped with a variable-wavelength UV detector (Model 770, Schoeffel Instruments, Westwood, NJ, U.S.A.) was used for the assay. The column was a Zorbax ODS, 25 cm × 4.6 mm, 5 μm particle size (DuPont, Wilmington, DE, U.S.A.).

Reagents

Flordipine was synthesized by Revlon Health Care Group, Research and Development Division (Tuckahoe, NY, U.S.A.). Hexane (Burdick and Jackson, Muskegon, MI, U.S.A.), was glass-distilled. Acetonitrile (Fisher Scientific, Fairlawn, NJ, U.S.A.) was HPLC grade. Water was deionized and glass-distilled. All other reagents used were of analytical grade.

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Chromatographic conditions

The mobile phase consisted of acetonitrile—0.005 *M* potassium phosphate buffer (pH 7.0) (60:40, v/v). Its flow-rate was 1.5 ml/min. The column oven temperature was set at 55°C. The wavelength of detection was 238 nm.

Preparation of standards

Flordipine standards, ranging between 25 ng/ml and 200 ng/ml, were prepared by adding appropriate aliquots of a 1.0 μg/ml solution of flordipine in acetonitrile to glass culture tubes (16 × 150 mm) with PTFE-lined screw caps. After evaporation of the acetonitrile to dryness with a stream of nitrogen, 1.0 ml of heparinized human plasma was added to each tube and the tube contents were mixed by vortexing. A tube containing 1.0 ml of plasma served as a blank. Equivalent standards were prepared without addition of plasma to provide a comparison for recovery of drug extracted from plasma.

Extraction

To each plasma standard, or 1 ml of plasma sample, were added 100 μl of distilled triethylamine. The use of triethylamine was found to yield more reproducible extractions of flordipine. The tubes were mixed by vortexing and the samples were extracted with 10 ml of hexane by shaking for 10 min in a reciprocating shaker (Eberbach, Ann Arbor, MI, U.S.A.). The phases were separated by centrifugation at 4°C and the tubes placed in a dry ice—acetone bath to selectively freeze the lower aqueous layer. The hexane extracts were then transferred to conical tubes and evaporated to dryness with a stream of nitrogen. The residues were reconstituted with 75 μl of mobile phase and a 50-μl aliquot was injected onto the column.

Calculation of results

The height of the flordipine peak was measured and calibration curves were constructed by plotting the peak height vs. the flordipine concentration of the standards. The linear regression line was determined and flordipine sample concentrations were calculated from their peak heights using the calibration curve.

RESULTS AND DISCUSSION

Chromatographic properties

Representative chromatograms of extracted blank and standard plasmas are shown in Fig. 1. Chromatograms obtained from human plasma drawn before and after an oral dose of flordipine are presented in Fig. 2. The retention time of flordipine was 9.1 min which was identical to that of a solution of the pure compound injected directly. The peak at 7.6 min in Fig. 2B appears to be a metabolite, yet to be identified, since it was present in post-dose, but not pre-dose, samples. There were no interfering peaks in control or pre-dose human plasma samples with the same retention times as flordipine or the possible metabolite. The possible interference of other drugs, such as β-blockers, diuretics and digitalis glycosides, which could be co-administered with flordipine, has not yet been investigated.

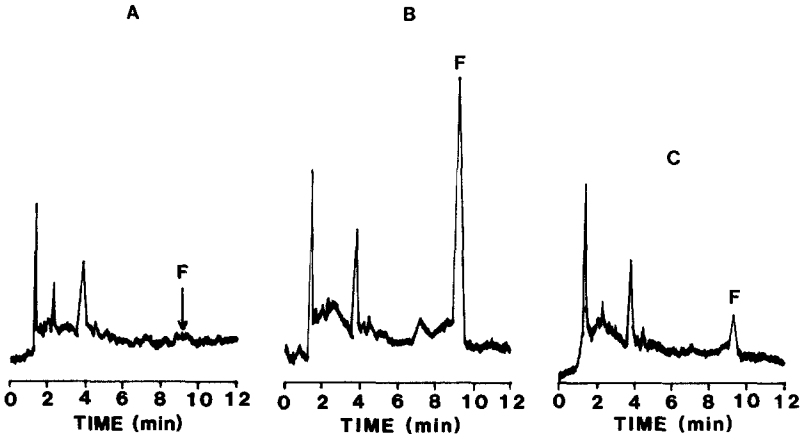

Fig. 1. Chromatograms of extracts of plasma. (A) Blank plasma (F indicates expected retention time of flordipine); (B) and (C) plasma containing 200 ng/ml and 25 ng/ml flordipine (F), respectively.

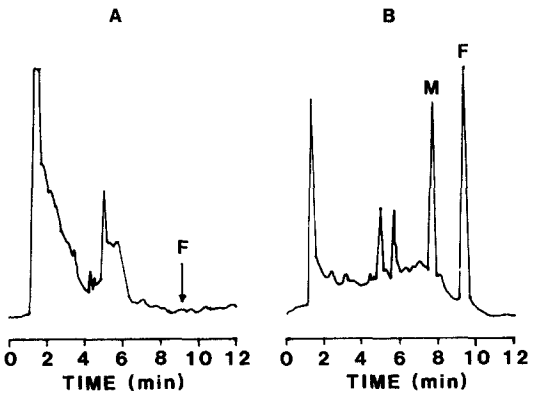

Fig. 2. Chromatograms of extracts of human plasma samples obtained before (A) and 2 h after (B) administration of 200 mg flordipine. F indicates location of the flordipine peak, which was not present in (A) and was determined equivalent to 145 ng/ml in (B). M indicates a possible metabolite peak, since it was present in (B) but not (A)

Precision and reproducibility

Standards of 25, 50, 100 and 200 ng/ml flordipine were assayed in duplicate on three different days to determine the precision and reproducibility of the assay. The composite coefficients of variation (% C.V.) for the peak heights ranged between 6.0% at 25 ng/ml and 6.3% at 200 ng/ml (Table I).

Linearity of calibration curves

Calibration curves obtained by plotting the peak height of flordipine vs. the flordipine concentration were linear over the concentration range of 25 ng/ml to 200 ng/ml. For the composite calibration curve (n = 3) the correlation coefficient was 0.996 and the regression equation was $y = 0.0201x + 0.136$, where y = peak height (cm) and x = concentration (ng/ml).

TABLE I

REPRODUCIBILITY OF THE METHOD

Concentration (ng/ml)	*n*	Mean peak height (cm)	Coefficient of variation (%)
25	6	0.633	6.0
50	5	1.13	6.2
100	5	2.17	6.0
200	5	4.15	6.3

Accuracy and lower limit of quantitation

The accuracy of the method was determined by assaying plasma flordipine standards of 25, 50, 100 and 200 ng/ml. Table II shows that the assay was accurate to 1% or better within the concentration range studied. The coefficient of variation of the calculated concentrations ranged from 15% at 25 ng/ml to 1% at 200 ng/ml. The lower limit of quantitation of the assay was considered to be 25 ng/ml, a level at which the assay was accurate and fairly precise.

TABLE II

ACCURACY OF MEASUREMENT OF FLORDIPINE ADDED TO PLASMA

Theoretical concentration (ng/ml)	*n*	Calculated concentration (Mean ± S.D.) (ng/ml)	C.V. (%)	Difference from theoretical (%)
25	6	24.8 ± 3.6	15	—0.8
50	5	49.8 ± 4.8	10	—0.4
100	5	101 ± 2	2	+1.0
200	5	199 ± 2	1	—0.5

Extraction efficiency

Comparison of the peak height measurements of extracted plasma standards with those of standards dissolved in mobile phase gave an estimate of the extraction efficiency for flordipine. The recovery over three days for the four concentrations averaged 89.4% with a 1.2% relative standard deviation.

Clinical samples

The HPLC procedure described herein has been used for the assay of human plasma samples obtained from subjects who received oral doses of flordipine in an ascending dose tolerance study. Fig. 3 shows the plasma flordipine concentration as a function of time after dose in a subject who received a 200-mg oral dose. The HPLC method presented for the assay of flordipine in plasma is precise and linear and is suitable for the analysis of clinical samples.

ACKNOWLEDGEMENTS

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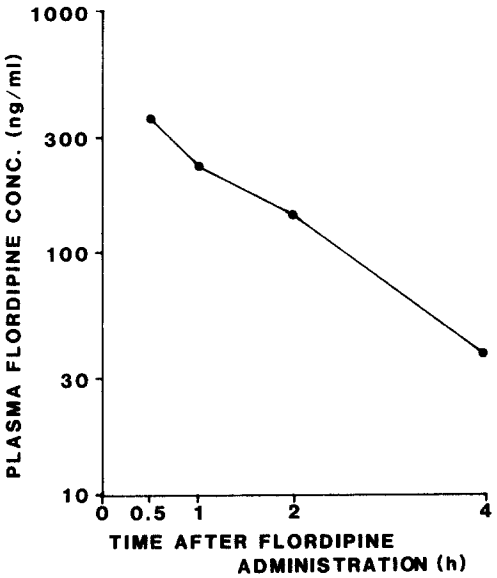

Fig. 3. Plasma concentrations of flordipine in a human subject who received a 200-mg oral dose.

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