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Short Communication

Determination of fendiline in human plasma by means of capillary gas chromatography and nitrogen–phosphorus selective detection

ANDREAS LOHMANN* and NORBERT DIEKMANN

R & D, Referat Pharmakokinetik, Thiemann Arzneimittel GmbH, Im Wirrigen 25, D-4355 Waltrop (Germany)

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ABSTRACT

A sensitive and specific method for the determination of fendiline in human plasma is presented. Fendiline was extracted from human plasma after the addition of phosphate buffer two times with 4 ml of *n*-hexane. The organic phase was separated and evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved and an aliquot was injected into the gas chromatograph. Chromatographic separation was performed with a DB-1 column with helium as carrier gas. Nitrogen-selective detection was performed. Quantification was performed with the signal output. The limit of detection was 1 ng/ml of plasma.

INTRODUCTION

Fendiline, N-(3,3-diphenylpropyl)-(1-phenylethyl)amine, pharmacologically belongs to the group of calcium antagonists and it has calmodulin antagonistic properties, too. The mode of action is a specific and dose-dependent inhibition of

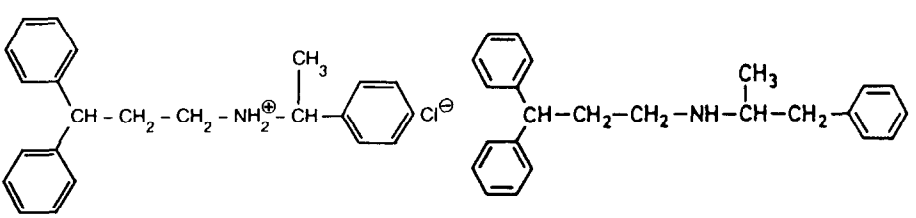

Fig. 1. Molecular structures of fendiline and prenylamine.

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the calcium influx across the membrane into the cells of excited myocard and smooth muscles, plus the inhibition of the calmodulin-mediated contraction [1,2].

This paper describes the first quantitative assay for this drug. The molecular structures of fendiline and the internal standard (I.S.) prenylamine are shown in Fig. 1.

EXPERIMENTAL

Instruments and instrumental conditions

The assay was performed on a Hewlett Packard 5890 gas chromatograph, equipped with a nitrogen–phosphorus selective detector and a 7673 A autosampler. A DB-1 fused-silica capillary (30 m × 0.315 mm I.D.) with cross-linked methyl silicone and a film thickness of 0.25 μm was used. An HP injector was used in the split–splitless mode at 220°C, with a purge delay of 30 s and an injected volume of 1 μl. The detector temperature was 270°C. Helium (purity 99.9999%) was used as carrier gas at a flow-rate of 2.4 ml/min (column flow). The column flow-rate (plus auxiliary gas) was 34 ml/min helium, the hydrogen flow-rate 3.5 ml/min, and the synthetic air flow-rate 75 ml/min. All gas flows were measured at an oven temperature of 120°C. Temperature programme: start, 120°C; initial time, 0.0 min; increase rate, 50°C/min; final temperature, 250°C; stable over 7.4 min; equilibration time, 3.0 min. The injection amplitude in the automatic process was 18 min.

Chemicals and reagents

Fendiline · HCl and prenylamine were from Thiemann Arzneimittel (Waltrop, Germany). Human pool plasma was obtained from the blood bank of the University Hospital Düsseldorf (Düsseldorf, Germany). *n*-Hexane of reagent purity for the analysis of residues was from Merck (Darmstadt, Germany), as was methanol of reagent purity. The pH 8 buffer contained 1 *M* dipotassium hydrogenphosphate, 1 *M* potassium dihydrogenphosphate and methanol of reagent purity LiChrosolv (Merck). Double-distilled water was prepared in our laboratory. The fendiline stock solution was prepared from 5 mg of fendiline base weighed into a 100-ml volumetric flask and dissolved in 100.0 ml of methanol. This corresponds to a concentration of 50 μg/ml. The solution was stored at room temperature and protected from light.

The prenylamine (I.S.) stock solution was prepared from 5 mg of prenylamine base weighed into in a 100-ml volumetric flask and dissolved in 100.0 ml of methanol. This corresponds to a concentration of 50 μg/ml (solution A). The solution was stored at 20°C and protected from light. The I.S. working solution was prepared from 5 ml of solution A diluted up with methanol to 50 ml. The concentration corresponds to 5 μg/ml. Of this solution, 20 μl (100 ng) were used.

Analytical procedure

The assay was performed as described previously [3]. For the calibration curve the following concentrations were used: 1, 5, 10, 25, 50, 100, 250 and 500 ng/ml of plasma. For plasma extraction, two 4-ml volumes of *n*-hexane were used. After centrifugation and evaporation to dryness, the residue was dissolved in 20 μl of methanol, and 1 μl of this solution was injected into the gas chromatograph.

Application of the assay

To demonstrate the applicability of the method, plasma samples from two pharmacokinetic trials were analysed. In one trial, twelve healthy male volunteers ingested 75 mg of fendiline in period A and 100 mg of fendiline in period B as a single dose. Up to 24 h after dosing, serial blood samples were taken at certain times. In a second trial, twelve healthy male volunteers ingested the same doses per day over a period of six days. From day 5 to day 6, serial blood samples were taken. The blood samples were centrifuged to prepare plasma, and the plasma samples were stored at $-20°C$ until required for analysis.

RESULTS AND DISCUSSION

The selectivity of the assay was verified by comparing the chromatograms of a spiked plasma sample and a human blank plasma. As can be seen quite clearly, there are no interfering peaks (Fig. 2).

The detection limit (defined as three times the baseline noise) for the plasma sample examined was 1 ng/ml of human plasma. As a practical level of determination we chose the 5 ng/ml human plasma value.

The linearity of the calibration curve was examined and shown to be good over the concentration range 5–500 ng/ml of human plasma. In general, the r^2 values obtained were above 0.9999.

The absolute analytical recoveries of fendiline were 100% over all concentrations. The recovery of the I.S., determined at the concentration examined, was also 100%.

The intra-assay reproducibility was determined by assaying five different spiked plasma pools. A calibration curve established beforehand was used as reference. All analyses were performed in randomized order. Over the concentration range the intra-assay coefficient of variation (C.V.) was better than 5.9% at 5 ng/ml and 6.4% at 500 ng/ml (Table I).

The inter-assay reproducibility was determined by comparing the ratios of the calibration curves of fourteen different days. Over the concentration range the inter-assay C.V. was better than 5.5% at 5 ng/ml and 5.0% at 500 ng/ml (Table II).

Two examples of the time-course of fendiline plasma levels are shown in Fig. 3. Fig. 3A shows the plasma concentrations after the administration of 75 mg or 100 mg as a single dose to one volunteer; Fig. 3B shows the concentration–time

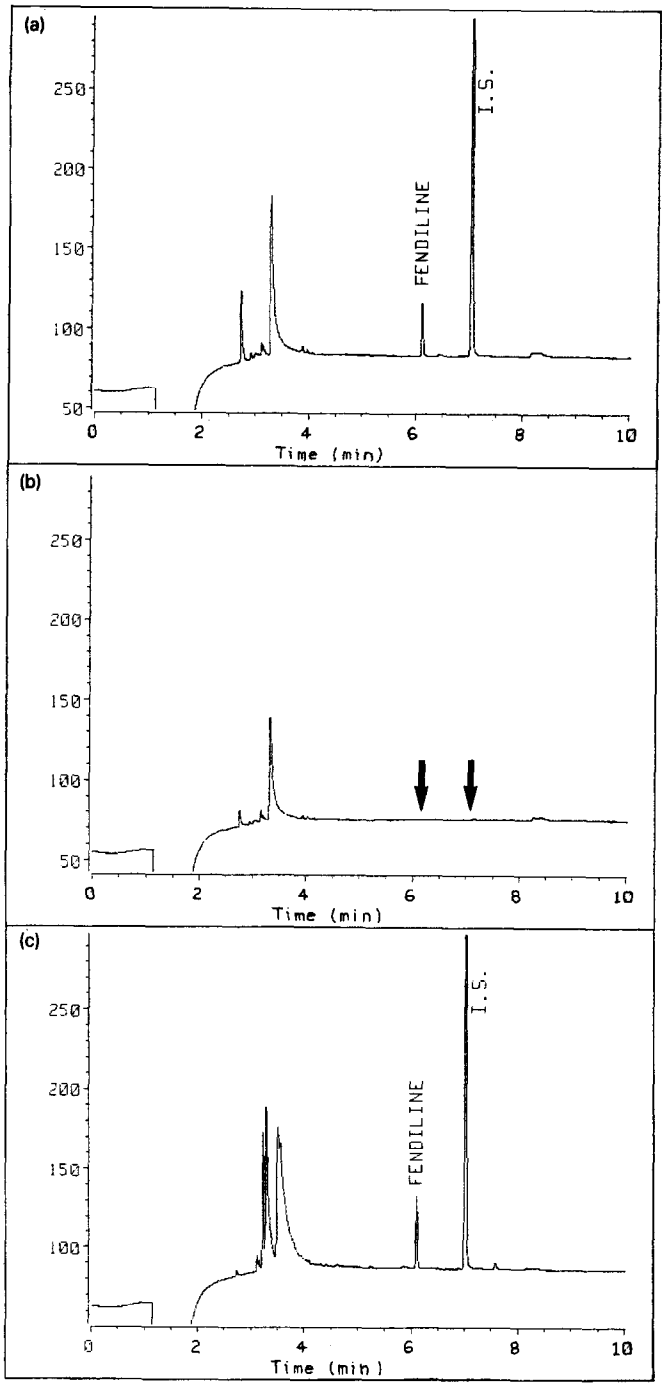

Fig. 2. Chromatograms of a spiked plasma sample (a), a blank human plasma (b) and a real sample (c).

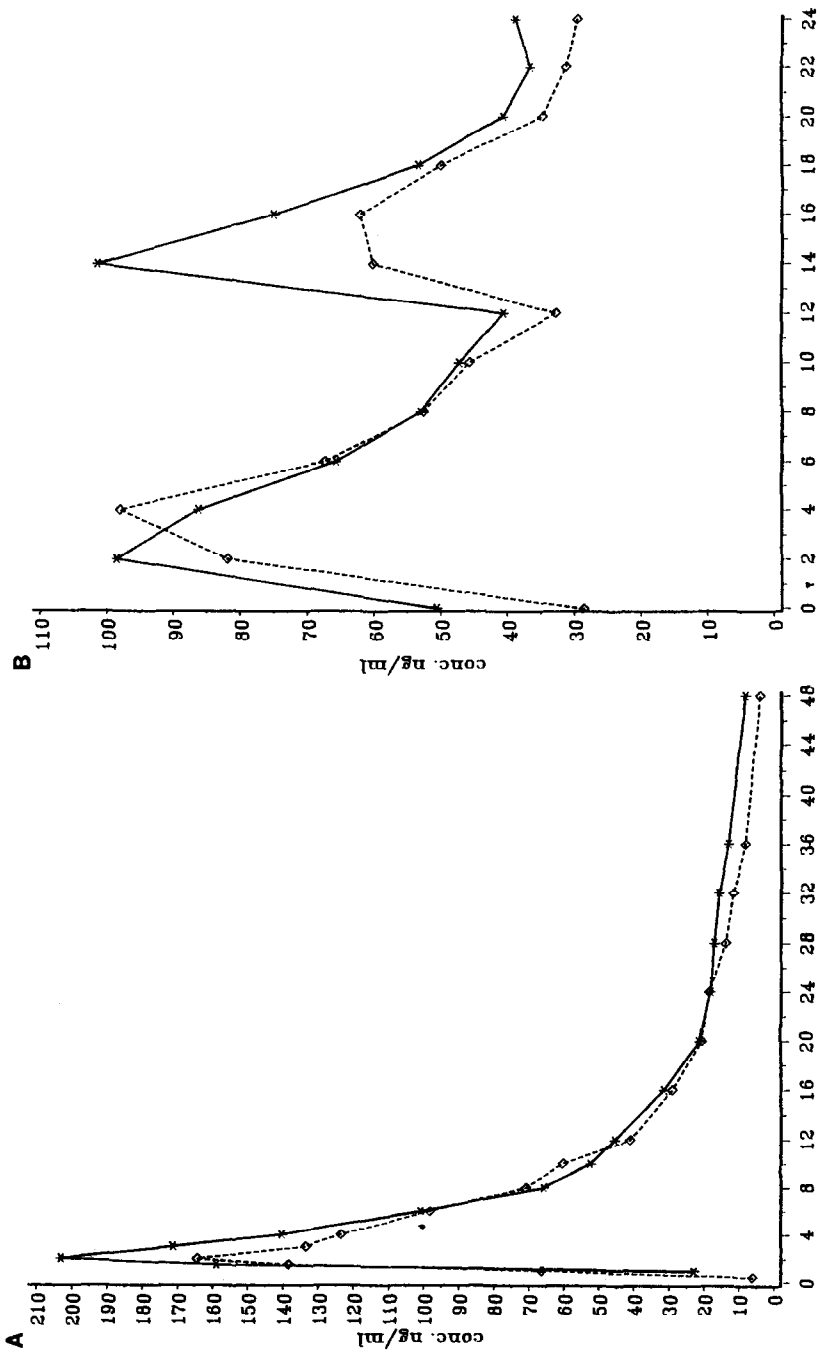

Fig. 3. Time-course of fendiline plasma levels after oral administration of 75 mg or 100 mg (single-dose kinetic) (A) and a steady-state kinetic (B).

TABLE I

INTRA-ASSAY REPRODUCIBILITY AND ACCURACY OF THE METHOD

Actual concentration (ng/ml)	Concentration determined (mean ± S.D., $n=11$) (ng/ml)	Accuracy (%)	C.V. (%)
5	5.19 ± 0.3	96.3	5.87
10	10.01 ± 0.6	99.0	5.93
25	25.1 ± 1.5	99.6	5.78
50	50.9 ± 3.1	98.2	6.02
100	99.5 ± 6.4	100.5	6.46
250	247.9 ± 15.7	100.8	6.33
500	500.9 ± 32.0	99.8	6.38

course of the same volunteer in the steady state. Over more than eight months, neither the plasma samples nor the calibration samples showed any sign of deterioration. The stock solutions of fendiline and the I.S. were stable for at least fourteen days.

Neither pool plasma nor plasma samples from more than 100 different volunteers and patients showed any interferences in the chromatograms.

The method described is well suited for the analysis of fendiline in human plasma owing to its selectivity, its sensitivity and its reproducibility. It is therefore useful for routine analyses, as required for pharmacokinetic studies.

TABLE II

INTER-ASSAY REPRODUCIBILITY AND ACCURACY OF THE METHOD

Actual concentration (ng/ml)	Concentration determined (mean ± S.D., $n=14$) (ng/ml)	Accuracy (%)	C.V. (%)
5	5.3 ± 0.3	106.0	5.47
10	10.2 ± 0.8	102.0	7.87
25	27.2 ± 0.4	108.0	1.63
50	48.7 ± 1.6	97.4	3.23
100	94.7 ± 3.5	94.7	3.71
250	250.2 ± 5.9	100.0	2.34
500	500.9 ± 24.9	100.1	4.97

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