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SENSITIVE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF CINNARIZINE AND FLUNARIZINE IN BIOLOGICAL SAMPLES

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SUMMARY

A sensitive method has been developed for the determination of the vasoactive compounds cinnarizine and flunarizine in plasma, urine and milk samples from man and animals. The procedure involves the extraction of the drugs and their internal standard from the biological samples at alkaline pH, back-extraction into sulphuric acid and re-extraction into the organic phase (heptane—isoamyl alcohol).

The analyses were carried out by gas chromatography using a nitrogen-selective thermionic specific detector. The detection limit was 0.5 ng/ml of biological fluid and extraction recoveries were sufficiently high (87—94%).

The method was applied to plasma samples from bioavailability studies of both cinnarizine and flunarizine in healthy volunteers, and to plasma, urine and milk samples from flunarizine-treated dogs.

INTRODUCTION

Cinnarizine, (*E*)-1-(diphenylmethyl)-4-(3-phenyl-2-propenyl)piperazine (I, Fig. 1), and its difluoro analogue flunarizine, (*E*)-1-[bis(4-fluorophenyl)-methyl]-4-(3-phenyl-2-propenyl)piperazine (II, Fig. 1) are Ca^{2+}-entry blockers, both widely used in the treatment of cerebral and peripheral vascular insufficiency [1].

Gas chromatographic (GC) methods [2—4] and a high-performance liquid chromatographic (HPLC) procedure [5] have been described for determining cinnarizine in biological samples with detection limits ranging from 2 to 10 ng/ml. No determination method for flunarizine has been described thus far.

The present paper describes a more sensitive GC method for both cinnarizine and flunarizine in biological samples. The method was used to obtain more

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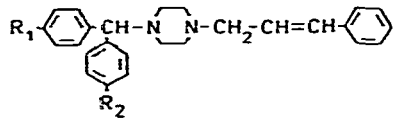

Compound	R_1	R_2
I	H	H
II	F	F
III	F	Cl

Fig. 1. Chemical structure of cinnarizine (I), flunarizine (II) and the internal standard (III).

detailed information about the pharmacokinetics of the drugs in man and animals.

EXPERIMENTAL

Reagents

Cinnarizine (R 516 base), flunarizine hydrochloride (R 14 950) and the internal standard (R 13 415), (*E*)-1-[(4-chlorophenyl)(4-fluorophenyl)methyl]-4-(3-phenyl-2-propenyl)piperazine dihydrochloride (III, Fig. 1), were obtained from the Life Sciences Products Division of Janssen Pharmaceutica (Beerse, Belgium) and were of analytical grade.

Spectrophotometric grade *n*-heptane and methanol were used (Uvasol; E. Merck, Darmstadt, G.F.R.). All the other reagents were of analytical grade. The inorganic reagents were prepared in double-distilled water. A borate buffer (pH 8.5) was prepared by adding 15.2 ml of 0.1 *M* hydrochloric acid to 50 ml of 0.025 *M* sodium borate decahydrate (borax). A final volume of 100 ml was prepared.

Standard solutions

Stock solutions, corresponding to 1 mg of the free base per ml of methanol, were prepared for compounds I, II, and III. Standard solutions were obtained by diluting the stock solution of I and II to concentrations ranging from 0.01 to 20 μg/ml of methanol.

To spike the samples with the internal standard, the stock solution of III was further diluted to 1 μg/ml.

Extraction procedure

Plasma. Two millilitres of plasma (unknown samples, drug-free plasma, or plasma standards containing known amounts of the drugs) were transferred to 15-ml glass centrifuge tubes, spiked with 0.1 μg of the internal standard and buffered with 2 ml of the borate buffer solution (pH 8.5). After addition of 4 ml of a heptane—isoamyl alcohol mixture (98.5:1.5, v/v), the tubes were carefully rotated for 10 min (10 rpm, Cenco rotary mixer) and then centrifuged (5 min, 1000 *g*). The upper organic layer was transferred to a second

centrifuge tube by means of a disposable pasteur pipet and the plasma was extracted again with 4 ml of the heptane—isoamyl alcohol mixture. The combined organic layers were back-extracted with 3 ml of 0.05 *M* sulphuric acid and removed after centrifugation. The remaining acidic phase was made alkaline with 0.15 ml of concentrated ammonia and extracted twice with 2-ml aliquots of the heptane—isoamyl alcohol mixture. The combined organic layers were finally evaporated to dryness under a gentle stream of nitrogen in a water bath at 55°C.

Urine and milk samples. Volumes of 1 ml were processed in the same way as the plasma samples.

Calibration procedure

Using the cinnarizine and flunarizine standard solutions, separate series of blank control plasma samples (2 ml) were spiked with either I or II at concentrations ranging from 0.0005 to 2 μg/ml, and with the internal standard at a fixed concentration of 0.05 μg/ml. These calibration plasma samples were then taken through the extraction procedure described above.

In the same way, standard curves for both cinnarizine and flunarizine were prepared in blank control milk and urine samples.

Apparatus

The instrument used was a Varian Model 3700 gas chromatograph equipped with the thermionic specific detector containing an electrically heated ceramic-alkali bead. The glass column (100 × 0.2 cm) was packed with 3% OV-17, coated on 80—100 mesh Supelcoport (Supelco, Bellefonte, PA, U.S.A.). The column, injector and detector temperatures were maintained at 275°C, 300°C and 340°C, respectively. Nitrogen was used as a carrier gas at a flow-rate of 25 ml/min. The detector was operated at a bias voltage of −4 V and the bead heating current was adjusted to 3 A, corresponding to a bead temperature of about 800°C. To ensure optimal detectability of the investigated compounds, the detector bead was in the path of a gas stream comprising hydrogen and air at flow-rates of 4.5 and 175 ml/min, respectively. A Spectra-Physics Model 4000 data system was used for the integrations, calculations and plotting of the chromatograms.

Gas chromatography

The various extraction residues were redissolved in 50 μl of methanol by vigorous vortexing. Sample volumes of 0.5—2 μl were then directly injected into the gas chromatograph.

Calculations

Ultimate sample concentrations were calculated by determining the peak area ratios of cinnarizine or flunarizine, related to the internal standard, and comparing these ratios with the standard curves obtained after analysis of the calibration samples.

TABLE I

EXTRACTION RECOVERIES FOR CINNARIZINE AND FLUNARIZINE FROM BIOLOGICAL SAMPLES

Compound	Percentage recovery* (mean ± S.D., $n = 5$)		
	Plasma	Milk	Urine
Cinnarizine (I)	93 ± 3	91 ± 4	94 ± 3
Flunarizine (II)	89 ± 2	91 ± 4	87 ± 5

*Percentage recovery = recovery of the extraction procedure, obtained after analysis of 100 ng of the appropriate compound added to 2 ml of control plasma.

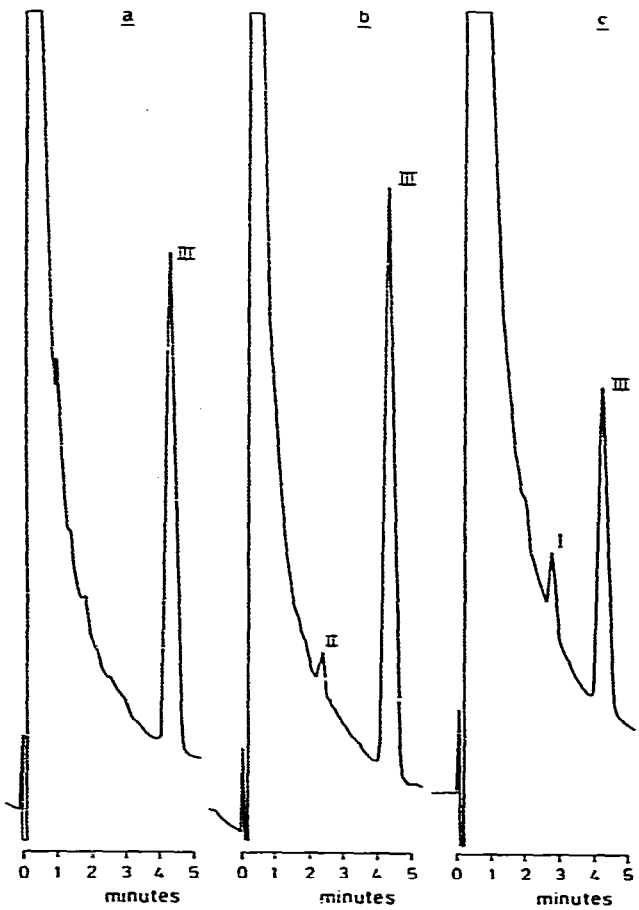

Fig. 2. Chromatograms of extracts from (a) blank control plasma, (b) plasma from a volunteer, seven days after oral intake of 30 mg of flunarizine, and (c) plasma from a volunteer, 30 min after oral intake of 20 mg of cinnarizine. I = cinnarizine (1.67 ng/ml); II = flunarizine (1.02 ng/ml); III = internal standard (50 ng/ml for chromatograms a and b; 40 ng/ml for chromatogram c). Chromatographic conditions were as indicated in the text.

RESULTS

The recoveries of the extraction procedure for cinnarizine and flunarizine (100 ng) from 2-ml control plasma, urine and milk samples are summarized in Table I.

Fig. 2 shows that no interfering peaks occurred at the retention times of cinnarizine, flunarizine or their internal standard under the described chromatographic conditions. All compounds were eluted as completely separated symmetrical peaks. The retention times for compounds I, II, and III were 2.8, 2.3, and 4.3 min, respectively. A linear relationship (r = 0.9999) was found when the ratios of the peak area of cinnarizine and flunarizine to the peak area of their internal standard (50 ng/ml) were plotted on the y-axis against various concentrations of either cinnarizine or flunarizine (in ng/ml) on the x-axis. Equations by the least-squares method were $y = 0.037\,x + 0.007$ for cinnarizine and $y = 0.035\,x - 0.020$ for the flunarizine calibration samples.

The accuracy and precision of the procedure was ascertained by adding different amounts of I and II to drug-free plasma and analyzing four samples of each concentration with the method described. The results are summarized in Tables II and III.

The detection limit was 0.5 ng/ml for both investigated compounds.

The method described has been used in a bioequivalence study of two flunarizine formulations (a 5-mg capsule and a 10-mg tablet) in healthy volunteers. Mean plasma levels for six subjects receiving oral doses of 30 mg of flunarizine in either dosage form are shown in Fig. 3. The method allowed the quantification of the plasma levels up to 28 days in all but two subjects and thus enabled the accurate measurement of the plasma elimination half-life ($t_{1/2\beta}$) which appeared to be 18.3 ± 8.47 and 17.3 ± 5.75 days for the capsule and tablet formulation, respectively. The method has also proved to be valuable for the determination of flunarizine in plasma, milk and urine of dogs orally treated with a single 20 mg/kg doze of flunarizine [6, 7].

TABLE II

ACCURACY AND PRECISION OF THE GC METHOD FOR THE DETERMINATION OF CINNARIZINE IN PLASMA SAMPLES

Theoretical cinnarizine plasma concentration (ng/ml)	Observed cinnarizine plasma concentration (ng/ml, mean ± S.D., n = 4)	C.V. (%)*	Accuracy
0.5	0.59 ± 0.05	9.0	118.0
1	0.89 ± 0.07	7.4	89.0
2.5	2.71 ± 0.15	5.4	108.4
5	4.85 ± 0.30	6.1	97.0
10	9.86 ± 0.38	3.9	98.6
25	24.5 ± 1.0	4.0	98.0
50	51.3 ± 1.1	2.2	102.6
100	100.6 ± 2.8	2.8	100.6
250	247.0 ± 4.7	1.9	98.8

*C.V. = coefficient of variation.

TABLE III

ACCURACY AND PRECISION OF THE GC METHOD FOR THE DETERMINATION OF FLUNARIZINE IN PLASMA SAMPLES

Theoretical flunarizine plasma concentration (ng/ml)	Observed flunarizine plasma concentration (ng/ml, mean ± S.D., $n = 4$)	C.V. (%)	Accuracy
0.5	0.56 ± 0.08	14.7	111.3
1	1.16 ± 0.15	12.6	116.0
2.5	2.64 ± 0.34	12.9	105.5
5	4.77 ± 0.40	8.4	95.4
10	10.7 ± 0.8	7.7	106.5
25	23.5 ± 2.2	9.3	93.9
50	48.5 ± 3.4	7.0	97.0
100	97.4 ± 6.3	6.5	97.4
250	229.4 ± 14.7	6.4	91.8
500	517.7 ± 31.6	6.1	103.5
1000	992.9 ± 4.0	0.4	99.3

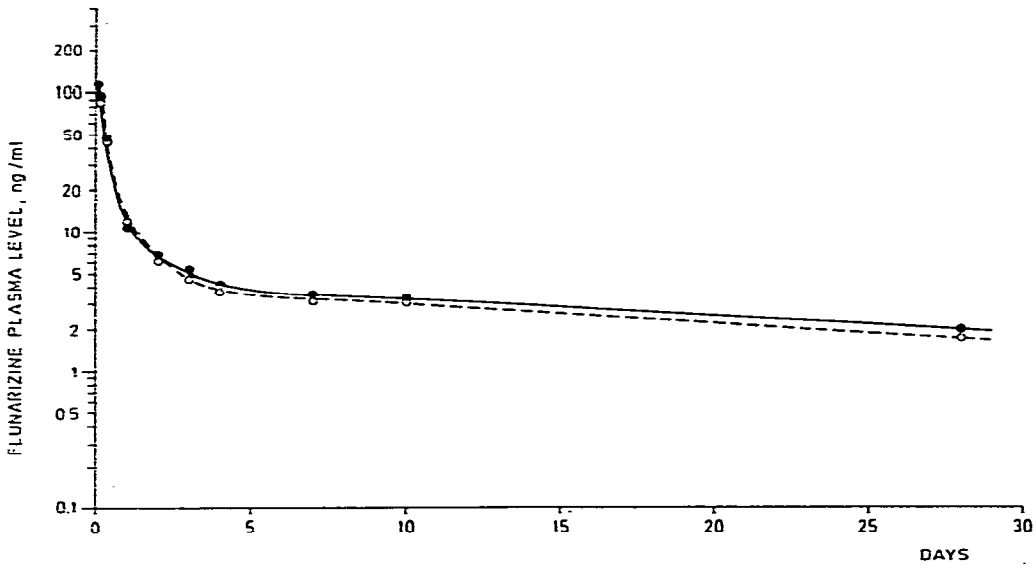

Fig. 3. Mean plasma levels of flunarizine up to 28 days after 30-mg doses either as 5-mg capsules (●) or as 10-mg tablets (○).

Cinnarizine plasma levels were measured in five healthy subjects up to 8 h after oral intake of 20 mg of the drug. The results are presented in Table IV.

DISCUSSION

The solvent system heptane—isoamyl alcohol (98.5:1.5, v/v) was selected for the extraction of cinnarizine and flunarizine since it is efficient, and also since the amount of co-extractants and water which interfere in the chromato-

TABLE IV

CINNARIZINE PLASMA LEVELS IN FIVE HEALTHY VOLUNTEERS AFTER ORAL ADMINISTRATION OF A 20-mg CINNARIZINE TABLET

Time (h)	Cinnarizine (ng/ml, mean ± S.D.)
0.25	1.7 ± 0.9
0.50	2.4 ± 1.2
1	10.4 ± 6.0
2	31.6 ± 14.3
4	36.6 ± 15.7
6	27.1 ± 9.5
8	17.8 ± 6.9

graphic step are minimal. Recovery experiments with different alkaline buffer systems indicated a maximum extraction efficiency at pH 8.5. The application of the selected buffer system enabled the use of standard extraction conditions for plasma, milk and urine samples. A recovery above 85% was reached for all samples. Standardization of the method was also obtained by the use of the same internal standard and the application of the more universal thermionic specific detector.

Although one can expect some benefit from the use of the electron capture detector for the detection of flunarizine, the absolute lower limit of detection proved to be about 1 ng, being about 50 times the absolute lower limit of detection of the thermionic specific detector which was less than 20 pg.

The application of the method to the assay of a few hundred plasma, urine and milk samples demonstrated its suitability; interferences were not observed and the GC column proved to be stable under the conditions used for at least 2—4 weeks.

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