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## Note

# Liquid chromatographic determination of flunarizine in human plasma

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Flunarizine, 1-cinnamyl-4-[bis(*p*-fluorophenyl)methyl]piperazine (FNZ), is a "selective" calcium channel blocker employed in some neurological and vascular diseases [1]. The monitoring of FNZ plasma levels in patients under chronic therapy is needed to evaluate the possible correlation between plasma levels and clinical (therapeutic and/or toxic) effects of the drug.

To date, two gas-chromatographic (GC) methods have been described for determining FNZ in biological samples [2, 3], both of which are highly sensitive but quite complicated and time-consuming. To our knowledge, the only liquid chromatographic (LC) procedure quoted in the literature is an internal report of a pharmaceutical company [4].

For these reasons, we developed a method that is simpler and faster than published GC procedures and sensitive enough to determine FNZ plasma concentrations at values found in patients under chronic treatment [5].

## EXPERIMENTAL

### *Reagent and standards*

Flunarizine dihydrochloride, hydroxyflunarizine and penfluridol (PFD, internal standard) were kindly supplied by Janssen Pharmaceutica (Beerse, Belgium). Methanol, water, acetonitrile (LC grade) and *n*-propylamine were purchased from Merck (Darmstadt, F.R.G.).

Stock solutions of FNZ and PFD were prepared in methanol to give a con-

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centration of 1 mg/ml (free bases) and were stored at −20°C. Working solutions of FNZ were prepared daily at a concentration of 1 μg/ml.

Calibration plasma samples containing 5, 10, 25, 50 and 100 ng/ml FNZ were prepared by adding exact volumes of working solution to test tubes and evaporating methanol to dryness under vacuum at room temperature. After reconstitution with drug-free plasma, calibration samples were handled exactly as with patients' plasma.

*Apparatus*

The chromatographic apparatus was a Series 2 pump, an LC 15 (fixed-wavelength) spectrophotometer set at 254 nm, a Model 56 recorder, a $C_{18}$ Sil-X-column (12.5 cm × 4.6 mm I.D.; particle size 5 μm), all from Perkin-Elmer (Norwalk, PA, U.S.A.).

The mobile phase was prepared by mixing 75 parts of acetonitrile with 25 parts of water containing *n*-propylamine 0.15%. The final apparent pH of the mobile phase was adjusted to 6.5 ± 0.1 with a few drops of 30% phosphoric acid. The flow-rate was 1.5 ml/min and the column temperature was 50°C.

*Extraction procedure*

To 1 ml of patients' (or calibration) plasma, 2.5 μg of PFD (as methanolic solution), 250 μl of 1 *M* hydrochloric acid and 7 ml of dichloromethane were added. The samples were carefully rotated for 15 min, then centrifuged at 4°C for 10 min at 2000 *g* and 6.0 ml of the organic phase were evaporated to dryness under vacuum at room temperature. Residues were redissolved in 100 μl of mobile phase and 20—50 μl were injected into the chromatograph.

*Recovery and reproducibility*

For recovery studies, 10, 25, 50 and 100 ng of FNZ were dissolved in 1 ml of drug-free plasma; these samples were extracted as described above but without adding PFD. A second series of standards was prepared for comparison by extracting 1-ml aliquots of drug-free plasma, adding the same amounts of FNZ in methanol to the residues and evaporating methanol. The dried residues of both series were redissolved in 100 μl of the mobile phase containing 25 μg/ml PFD. The analytical recovery was calculated by comparing peak-area ratios obtained in the two series. To calculate the absolute recovery, the analytical recovery was corrected for the fraction of added dichloromethane actually evaporated to dryness in the extraction procedure.

For precision and reproducibility studies, a drug-free plasma was spiked with FNZ and stored at −20°C. These samples were assayed eight-fold simultaneously, and six-fold in duplicate for one month.

*Plasma of patients*

Samples of blood from patients receiving FNZ for a period of one to three months (5 mg per day, in the evening) for migraine prophylaxis were collected in heparinized tubes at 8 a.m. Plasma was separated immediately and stored at −20°C until analysis. Flunarizine was found to be stable in frozen samples [4]. Patients' plasma and calibration curves were analysed in duplicate.

## RESULTS AND DISCUSSION

Flunarizine is a basic compound and extraction from an alkaline medium seems to be a logical choice; however, extraction of the drug from alkaline plasma requires lengthy, time-consuming procedures to achieve acceptable recovery. In preliminary experiments, using dichloromethane as organic solvent, we found that FNZ is easily extracted from water solutions both at high (> 10) and low (< 2) pH. (This behaviour is similar to that reported for cinnarizine, a defluorinated homologue of FNZ [6].) Our acidic extraction gave an acceptable recovery (about 85% in the range 10—100 ng/ml) with a simplified procedure.

The retention time of FNZ is strongly influenced by the pH of the mobile phase; lowering the pH reduces the retention times, allowing for a reduction of acetonitrile concentration, but increases the possibility of spurious interference from endogenous compounds. Addition of *n*-propylamine to the mobile phase was essential for the elution of FNZ from the reversed-phase

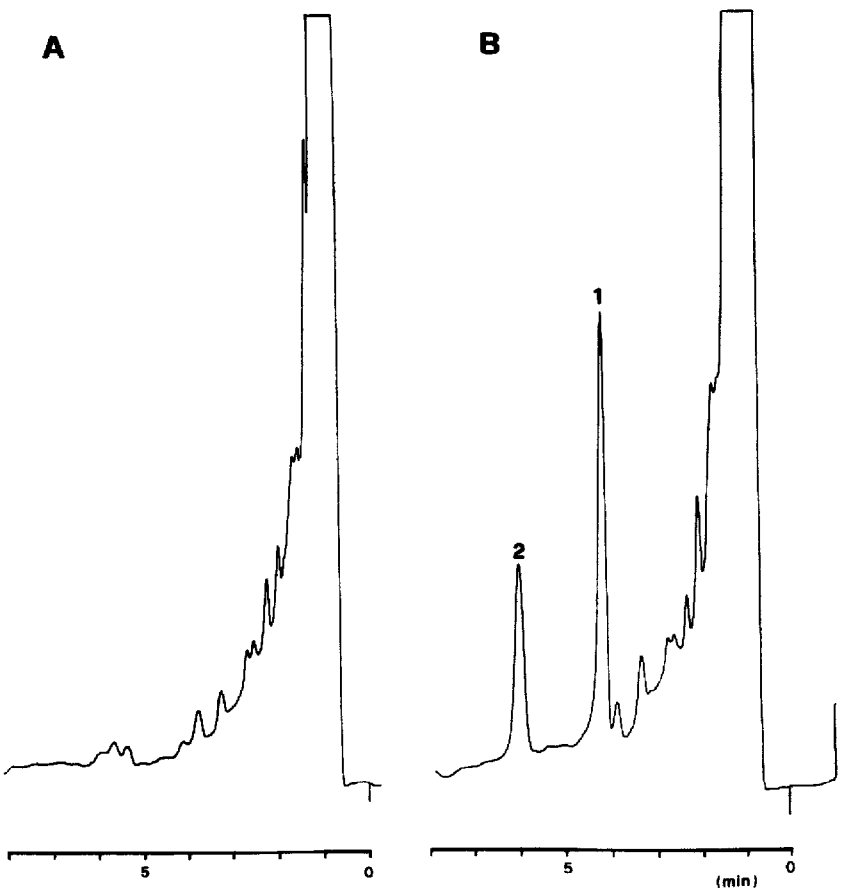


Fig. 1. Chromatograms obtained by injecting 30 μl of (A) extract from a drug-free plasma and (B) extract from a patient's plasma. Detector LC-15 at 0.032 a.u.f.s. Peaks: 1 = penfluridol; 2 = flunarizine (43.6 ng/ml).

columns tested (Hibar $C_2$ and $C_8$ from Merck and $C_{18}$ Sil-X from Perkin-Elmer). Although FNZ may be determined with all these columns, we chose to work with a $C_{18}$ column because there is less background interference.

Fig. 1 shows a chromatogram of an extract from a blank plasma and a chromatogram obtained from a patient taking FNZ. Calibration curves showed a linear correlation between FNZ concentration and FNZ/PFD peak-area ratios: the line obtained with the least-squares linear regression method is $y = (0.0115 \pm 0.0004)x + (0.0017 \pm 0.030)$, the $r$ value ranged from 0.994 to 0.999 (six curves over a two-month period). The detection limit for the methanolic solution (at a signal-to-noise ratio $> 3:1$) is ca. 500 pg of injected FNZ; owing to plasma background interference, the limit for the analysis of patients' samples is ca. 4 ng/ml, which is sufficient for monitoring purposes [5]. The within-assay coefficient of variation determined at two concentrations was 7.2% (mean concentration 41.4 ng/ml; $n = 8$) and 9.0% (10.8 ng/ml; $n = 8$); the between-assay study showed a coefficient of variation of 8.8% ($n = 6$; mean concentration 42.8 ng/ml).

Under the chromatographic conditions described in this paper, hydroxyflunarizine (a metabolite found in animals [7] showed a retention time of 2.7 min, eluting together with some endogenous impurities. Its possible presence in the plasma of patients could not be investigated with the present procedure. In a series of sixteen patients, aged from 10 to 42 years, with an FNZ mean dosage of 0.13 mg/kg per day we found concentrations of 8.4—53.2 ng/ml (24.3 ± 10.6; mean ± S.D.) in the plasma.

The proposed method is sufficiently sensitive for determining concentrations of FNZ in patients under chronic therapy. The procedure is rapid, simple and reproducible, so that it may be used to determine FNZ in clinical pharmacological trials.

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REFERENCES

1 B. Holmes, R.N. Brogden, R.C. Heel, T M. Speight and G.S. Avery, Drugs, 27 (1984) 6.
2 R. Woestenborghs, L. Michielsen, W. Lorreyne and J. Heykants, J. Chromatogr., 232 (1982) 85.
3 S. Campos Flor, J. Chromatogr., 272 (1983) 315.
4 J. Wynants, J. Hendrickx and J. Heykants, Biol. Res. Rep. R. 14950/6, Janssen Pharmaceutica, 1975.
5 J. Heykants, J De Cree and Ch. Hörig, Arzneim.-Forsch., 29 (1979) 1168.
6 H.K.L. Hundt, L.W. Brown and E.C. Clark, J. Chromatogr., 183 (1980) 378.
7 W. Meuldermans, J. Hendrickx, R. Hurkmans, E. Swysen, R. Woestenborghs, W. Lauwers and J. Heykants, Arzneim.-Forsch., 33 (1983) 1142.