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Note

Assay of a calcium antagonist (PU 122) in plasma by gas chromatography

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PU-122 (I, Fig. 1) is a new difluorinated piperazine derivative with a structural resemblance to flunarizine and cinnarizine, and possessing very interesting calcium antagonist activity [1–4]. As these drugs are administered in low doses (10–50 mg), are characterized by high values of distribution volume and are pharmacologically active at low plasma concentrations, very sensitive analytical methods are required for studies of their pharmacokinetics and bioavailability [1]. These were carried out by gas chromatography with thermoionic specific detection [5–8] or by high-performance liquid chromatography with UV detection at 250–258 nm [9–12].

This paper describes an analytical method that has been standardized to investigate the pharmacokinetics of I in animals and humans.

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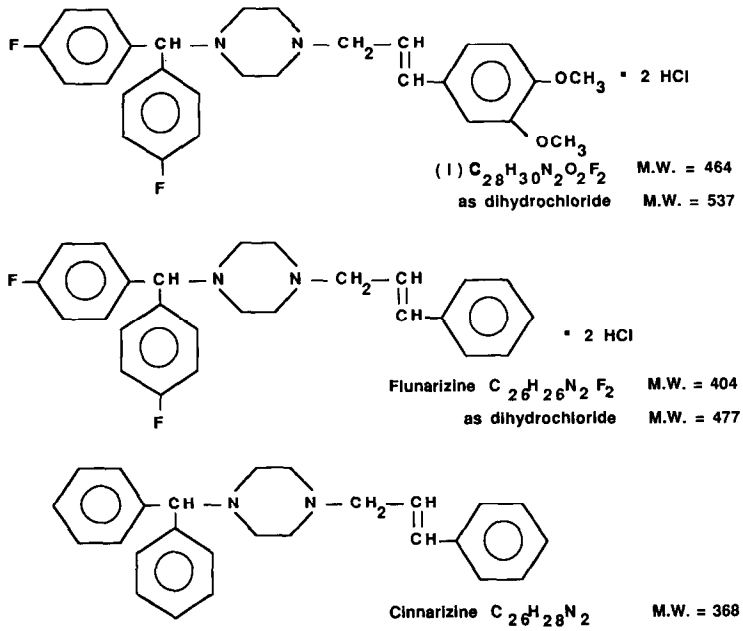

Fig. 1. Structures of I, flunarizine and cinnarizine.

EXPERIMENTAL

Chemicals and instruments

Solvents and reagents of analytical grade were supplied by Carlo Erba (Milan, Italy), Merck (Bracco, Milan, Italy) and Supelco (Supelchem, Milan, Italy). Gases of very high purity were supplied by SIAD (Cinisello Balsamo, Italy). The working standard of I was supplied by Medea Research (Milan, Italy) and that of penfluridol by Studio Chimica (Milan, Italy).

A Varian 3700 gas chromatograph equipped with flame ionization, thermoionic specific and electron-capture detectors was used. Another similar gas chromatograph connected to a VG Instruments Model VG 70/70 mass spectrometer enabled the mass spectrum of the analyte to be recorded. Statistical and pharmacokinetic evaluations were carried out using a Hewlett-Packard HP 86A personal computer.

Extraction

A 1-ml sample of plasma (or urine), 0.1 ml of 10 *M* sodium hydroxide and 5 ml of diethyl ether–*n*-hexane (8:2, v/v) were placed in a glass-stoppered test-tube. The mixture was vigorously stirred for 10 min and then centrifuged for 10 min at 2400 *g*. An aliquot of supernatant was transferred to another test-tube, and evaporated to dryness. The residue was redissolved in 50 μl of the

same extraction solvent. Amounts of 0.5–1 μl of this solution were injected into the gas chromatograph. An internal standard solution was prepared by dissolving 10 μg/ml penfluridol in diethyl ether–*n*-hexane (8:2, v/v), and 2–10 μl of this solution were added to the sample after extraction when the recovery of I was investigated and before the extraction in routine analysis.

Gas chromatographic conditions

A fused-silica Megabore DB-1 column (15 m × 0.53 mm O.D., 15 μm particle size) was used. The column temperature was 230°C for 1 min, increased at 25°C/min to 295°C, then held for 2 min. The injector temperature was 300°C, and thermoionic specific detector temperature was 330°C (hydrogen flow-rate, 4.5 ml/min; air flow-rate, 175 ml/min). Nitrogen was used as the carrier gas at 20 p.s.i. The retention times were 3.89 min for penfluridol and 4.50 min for I.

Preliminary pharmacokinetic investigation

A hard gelatine capsule containing 45 mg of I was administered orally to a fasting male volunteer. Plasma concentrations were evaluated in timed samples using this method.

RESULTS AND DISCUSSION

Linearity

The linearity of the detector response was verified from the constant value of the detector response factor, with both fixed (1:1) and variable (4:1 to 1:4) ratio of analyte to internal standard in the 1–50 ng range of I injected (corresponding to 50–2500 ng/ml).

Extraction recovery

The recovery of I from plasma proved to be linear throughout the range investigated, i.e. 10–1000 ng/ml. The mean recovery was 98.6%.

The linear regression method gave the following relationship between I added (x) and that found (y): $y = -0.90 + 0.984x$ with $r^2 = 0.9999$.

Reproducibility

In the analysis of I extracted from plasma, the intra-assay coefficient of variation (C.V.) ranged from 4.1 to 5.3% in the 50–1000 ng/ml concentration range. With 20 ng/ml, it was 7.1% and with 10 ng/ml it was 8.5%. The inter-assay C.V. was 2.0% throughout the investigated range, 10–1000 ng/ml.

Limit of detection

The lowest detectable plasma concentration of I associated with a C.V. of less than 10% proved to be 5 ng/ml. A concentration of 2 ng/ml was also detected, with a C.V. of 14%.

Specificity

The method allows analysis to be carried out with good specificity, in that analytical peaks were well separated and no interfering endogenous peaks were

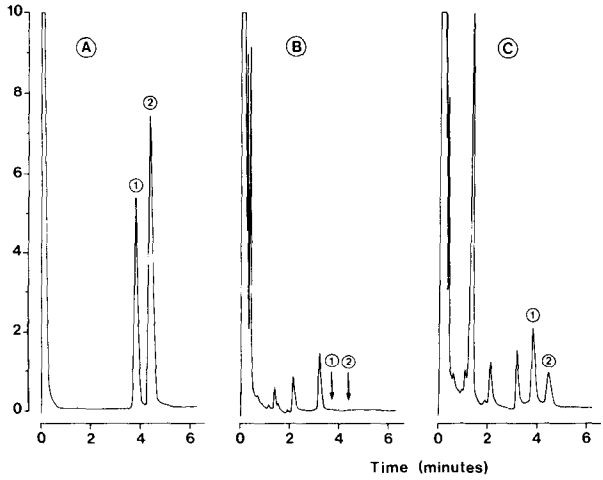

Fig. 2. Typical gas chromatograms of I and penfluridol (internal standard). (A) I (PU 122) and penfluridol; (B) a plasma blank; (C) a plasma extract from the male volunteer treated orally with I (PU 122) at a dose of 45 mg. Peaks: 1 = penfluridol; 2 = PU 122.

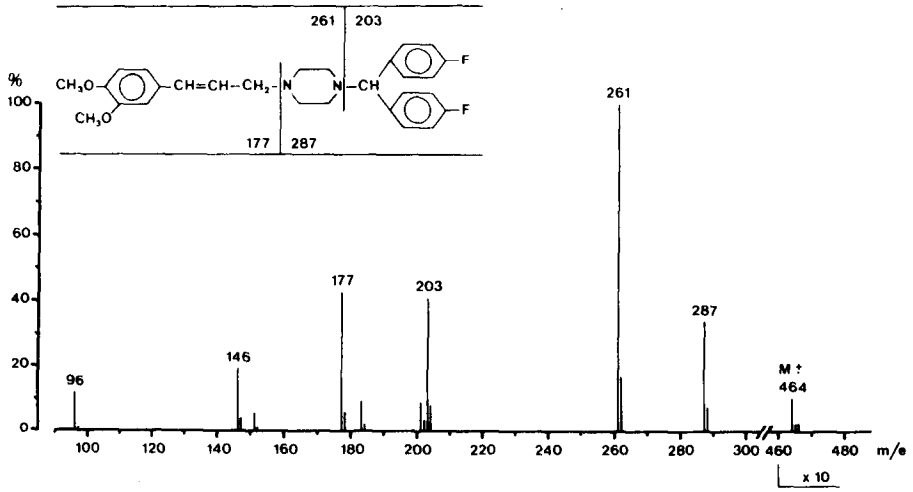

Fig. 3. Mass spectrum of I obtained by GC-MS in the electron-impact ionization mode (70 eV).

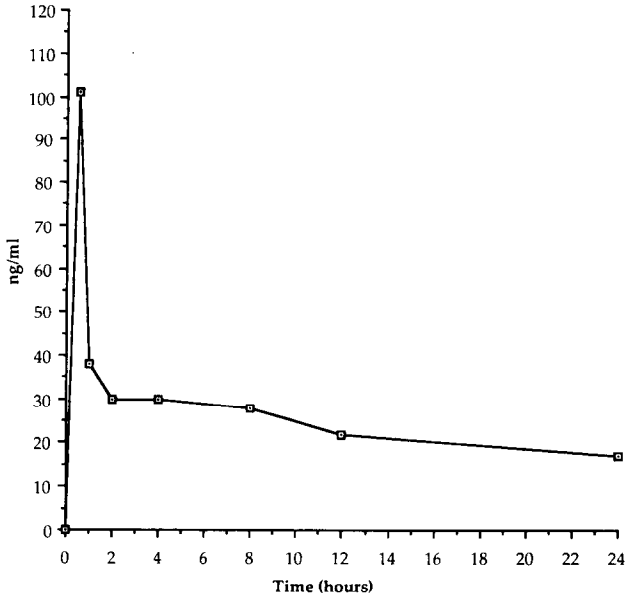

Fig. 4. Plasma concentration-time course of I in a volunteer treated orally with 45 mg.

detected (Fig. 2). Gas chromatography–mass spectrometry (GC–MS) allowed the peak attributed to I to be clearly identified (Fig. 3).

Preliminary pharmacokinetic results

Fig. 4 shows the plasma concentration–time behaviour of I in a male volunteer orally treated with the drug at a dose of 45 mg. The plasma concentration reached a peak after 30 min, then decreased, showing a long-lasting plateau in the elimination phase.

CONCLUSION

Calcium antagonists belonging in the same class as flunarizine and cinnarizine require very sensitive methods for pharmacokinetic and bioavailability investigations. Plasma concentrations as low as a few ng/ml must be detected [1,5–12]. We did not use electron-capture detection in GC analysis because despite the presence in I of two fluorine atoms, it was not sufficiently sensitive. The thermoionic specific detector enabled a very low plasma concentration of I to be evaluated, i.e. 2 ng/ml, a detection limit that is required for a calcium antagonist having a very high distribution volume, as in the case with I, flunarizine and cinnarizine.

Cinnarizine (MW 368) and flunarizine (MW 404) were also well evaluated with this method, but with retention times markedly shorter than that of I

(MW 464). This militated against the use of these substances as internal standard; penfluridol proved to be more suitable.

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