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Note

Gas chromatographic determination of phenazone derivatives in human plasma

II. Propyphenazone

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Propyphenazone, 4-isopropyl-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one (isopropylphenazone or 4-isopropylantipyrine), is an antipyretic, analgesic and anti-inflammatory agent. To permit the study of the bioavailability of propyphenazone, a method of measuring its concentration in plasma had to be elaborated. Several methods have been described for the assay of phenazone and aminophenazone, but few of them have been tested with propyphenazone.

Brinkmann and Hengstmann [1] determined phenazone by gas chromatography (GC) with a flame ionization detector (FID), using propyphenazone as internal standard. Lindgren et al. [2] studied the half-life of phenazone with 4-methyphenazone as internal standard, using FID—GC; Windorfer and Röttger [3] reported the GC determination of aminophenazone with docosane as internal standard, using temperature programming.

This communication describes the determination of propyphenazone in plasma by FID—GC, using hexacosane (C_{26}) as internal standard.

EXPERIMENTAL

Chemicals and reagents

Propyphenazone was supplied by Ciba-Geigy (Basle, Switzerland) and hexacosane (Kit No. 26A) was purchased from PolyScience Corporation, Niles, Ill., U.S.A. Buffer pH 12 (Merk 9882; Merck, Darmstadt, G.F.R.) is prepared by diluting the contents of 4 vials with water to a volume of 500 ml. The solvents used were all of analytical grade: chloroform (Merck 2447), isoamyl alcohol (Merck 979) and carbon disulphide (Merck 2214). 1% isoamyl alcohol is added to chloroform to make the extraction solvent.

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Equipment

GC assays are carried out on a Carlo Erba Fractovap 2400 T FID-gas chromatograph. The peak areas are given by an electronic integrator (Infotronics CRS 204). The glass column is washed with 1.0 *N* hydrochloric acid, water, acetone and benzene, silanized with a 1% solution of hexamethyldisilazane in benzene, then washed again with benzene and dried at 100°. The column (1 m × 3 mm I.D.) is packed with 3% Poly 1.110 (08264; Applied Science Labs., State College, Pa., U.S.A.) on Chromosorb W HP, 80—100 mesh. The filled column is gradually heated to 240°. The temperature is then increased 6—8 times from 140 to 240° in about half an hour and 20 µl of Silyl 8 (Pierce, Rockford, Ill., U.S.A.) is injected during every cycle. The column is operated at 220°, the injector at 230° and the manifold at 240°. The nitrogen flow is 40 ml/min. Under these conditions, propyphenazone and hexacosane have retention times of 4 and 13 min, respectively.

Standard solutions

The standard solution of propyphenazone is prepared by dissolving 5 mg of propyphenazone in 100 ml water (a few drops of methanol are necessary at the beginning to obtain a good dissolution). The standard solution of hexacosane (C_{26}) is prepared by dissolving 10 mg of hexacosane in 100 ml carbon disulphide. The dilutions required for these two solutions are prepared with the corresponding solvents.

Extraction

A 500-µl volume, corresponding to 2.5 µg of internal standard, is introduced into a stoppered glass tube and taken to dryness under a nitrogen stream. A 1-ml sample of the plasma to be analysed, 2 ml buffer (pH 12) and 5 ml chloroform—1% isoamyl alcohol are added. The tubes are shaken for 10 min at 250 rpm and centrifuged for 10 min at 4800 *g*. The aqueous phase is pipetted off and discarded. An aliquot of the organic phase is transferred to another tube and taken to dryness under a nitrogen stream in a dry bath at 70°. Evaporation must be effected very carefully and stopped just as the tubes reach dryness.

Gas chromatography

A 100-µl volume of carbon disulphide is added to the dry residue and the tube is shaken on a mixer (Vortex). A 2-µl portion of the resultant solution is injected into the gas chromatograph by the solvent-flush technique. It is necessary to raise the oven temperature after 10 consecutive injections up to 240° for half an hour to wash out plasma residues from the column.

The concentration of propyphenazone in the analysed plasma is calculated from the propyphenazone—internal standard peak-areas ratio by reference to a calibration curve. This curve is obtained by extraction of plasma spiked with increasing amounts of propyphenazone (from 0.125 µg to 10 µg/ml) and a constant amount of internal standard (2.5 µg/ml plasma). The reproducibility of the curve is checked every ten days.

RESULTS AND DISCUSSION

It was found necessary to add a sample percentage of isoamyl alcohol to

TABLE I

PRECISION AND RECOVERY OF THE DETERMINATION OF PROPYPHENAZONE APPLIED TO SPIKED HUMAN PLASMA SAMPLES

Amount added (ng/ml)	Amount found (ng/ml)	Mean	Precision reproducibility CV (%)	Recovery accuracy
125	134			107.2
125	135			108.0
125	128	129	4.9	102.4
125	121			96.8
500	524			104.8
500	502			100.4
500	500	502	3.2	100.0
500	485			97.0
2000	2071			103.6
2000	2014			100.7
2000	1916	1990	2.2	95.8
2000	1973			98.7
2000	1950			97.5
2000	2010			100.5
8000	7657			95.7
8000	7550			94.4
8000	7904	7770	2.6	98.8
8000	7985			99.8
			Mean	100.1 ± 3.9

chloroform to obtain a good extraction reproducibility. This reproducibility has been tested. Table I records the precision and recovery results for this propyphenazone GC method. It shows that a good reproducibility was obtained for 0.125 μg of propyphenazone. Unfortunately, less than 0.125 μg of propyphenazone per ml of plasma cannot be determined accurately, because the peak height is too small and the chemical background limits the detection. The limit of sensitivity of the method can consequently be taken as 0.125 μg/ml plasma.

Figs. 1 and 2 show the chromatograms obtained with a blank plasma and with the same plasma containing 0.125 μg propyphenazone per ml. Since the metabolism of propyphenazone is not known, no definitive conclusions can be drawn about the specificity of the GC assay. Nevertheless, in all bioavailability studies performed according to the technique described here, no disturbing peak appeared near propyphenazone or the C_{26} internal standard. Other hydrocarbons with a shorter chain cannot be used as internal standard; docosane (C_{22}) is not well separated from propyphenazone, and tetracosane (C_{24}) has the same retention time as a compound present in the plasma of subjects receiving propyphenazone, which could be a metabolite of the drug.

APPLICATIONS

The bioavailability of propyphenazone from different formulations was studied by this method in healthy subjects. Fig. 3 shows a curve obtained from the plasma of a subject given 220 mg of propyphenazone orally. The sensitivity

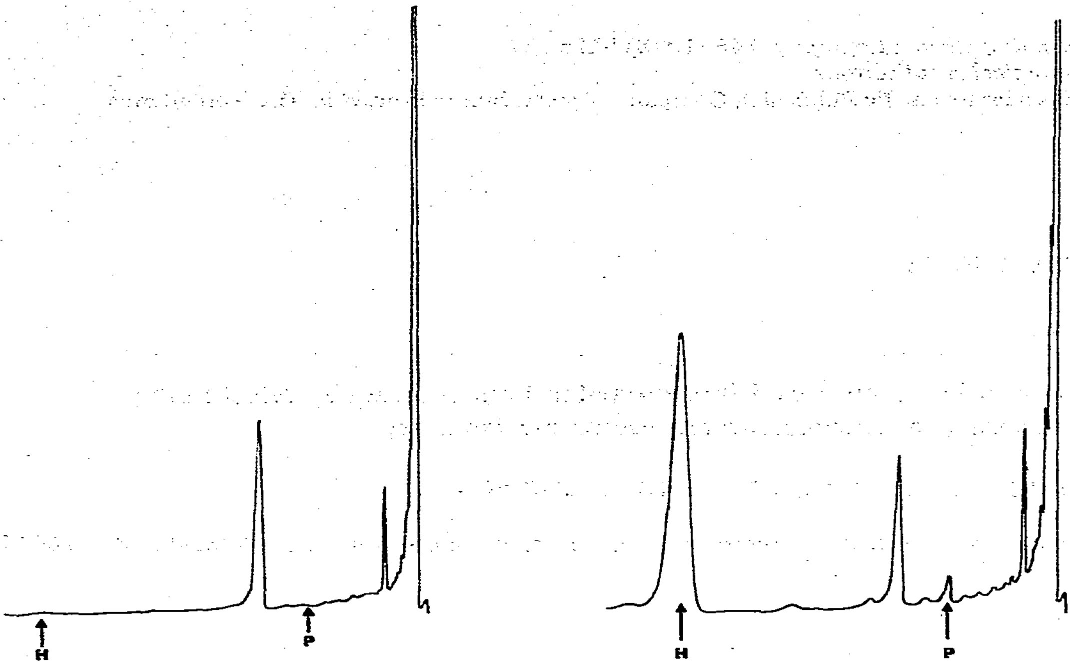

Fig. 1. Chromatogram of a plasma blank (1 ml). P = propyphenazone; H = hexacosane.

Fig. 2. Chromatogram of propyphenazone (P; 0.125 μg/ml) and hexacosane (H; 2.5 μg/ml) extracted from control plasma.

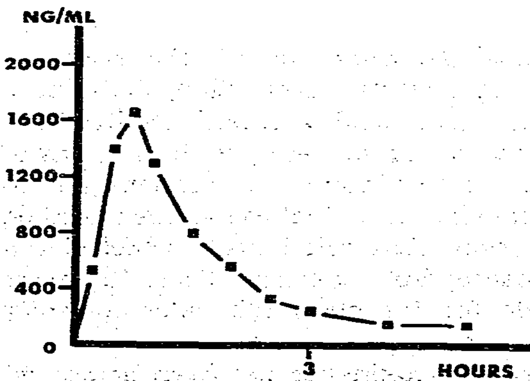

Fig. 3. Plasma concentration of propyphenazone in one subject given 220 mg of the drug orally.

of the method thus appears sufficient to determine propyphenazone in bioavailability assays.

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