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DETERMINATION OF PIRIBEDIL IN BIOLOGICAL MATERIALS BY GAS–LIQUID CHROMATOGRAPHY–MASS FRAGMENTOGRAPHY

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SUMMARY

A sensitive and specific method for the quantitative determination of piribedil in brain tissue and plasma is described. After extraction, piribedil is detected by gas–liquid chromatography using a flame ionization detector or the mass fragmentographic technique, and these two procedures allow 250 and 10 ng/ml or ng/g, respectively, of piribedil present in biological samples to be determined.

INTRODUCTION

Piribedil [ET 495; 1-(3,4-methylenedioxybenzyl)-4-(2-pyrimidinyl)piperazine] is a drug which, in addition to its known vasodilator activity[1,2], has recently been shown to possess the capacity to stimulate dopaminergic receptors[3–6] and to increase the levels of acetylcholine in the striatum[7]. As piribedil may also be a useful drug for the treatment of Parkinson's disease[3], it was of interest to develop a specific and sensitive method for measuring piribedil concentrations in blood and tissues. This aim has been achieved by means of gas–liquid chromatography and by using for detection either a flame ionization detector or a mass spectrometer[8–10].

MATERIALS AND METHODS

Standards and reagents

Piribedil was obtained from Servier Labs., Paris, France, and 2-N-benzylamino-5-chlorobenzophenone (used as an internal standard) was supplied by U. Ravizza, Muggiò, Italy.

The following reagents were used: absolute ethanol R.P.E*. (Carlo Erba, Milan, Italy), hydrochloric acid, sodium hydroxide (E. Merck, Darmstadt, G.F.R.), diethyl ether R.P.E. (Carlo Erba) and acetone (Carlo Erba).

Apparatus

Gas–liquid chromatography (GLC). The gas chromatograph was a Carlo Erba Fractovap Model G1, equipped with a flame ionization detector (FID). The gas chromatographic conditions were as follows. The stationary phase was 3% OV-17 on Gas-Chrom Q, 60–80 mesh (Applied Science Labs., State College, Pa., U.S.A.), packed into a 2-m glass column. The flow-rate of the carrier gas (nitrogen) was

* R.P.E. = Reagente Puro Erba.

30 ml/min. The column and the injector port temperatures were 240° and 270°, respectively.

Gas–liquid chromatography–mass fragmentography (GLC–MF). An LKB (Stockholm, Sweden) Model 9000 mass spectrometer equipped with a gas chromatograph and an accelerating voltage alternator (A.V.A.) was used. The gas chromatographic conditions were as given above, except that helium at a flow-rate of 25 ml/min was used as the carrier gas. The mass spectrometer was operated under the following conditions: molecular separator temperature, 280°; ion source temperature, 290°; trap current, 60 μA; electron energy, 30 eV; accelerating voltage, 3.5 kV; filters, 20 Hz.

By means of the A.V.A., the mass spectrometer was focused alternately upon the ions at $m/e=298$ for piribedil and at $m/e=321$ for 2-N-benzylamino-5-chlorobenzophenone; the switching frequency of the A.V.A. was twice per second and the filters were ± 3 Hz.

Fig. 1 shows typical gas chromatograms and mass fragmentograms of piribedil and the internal standard.

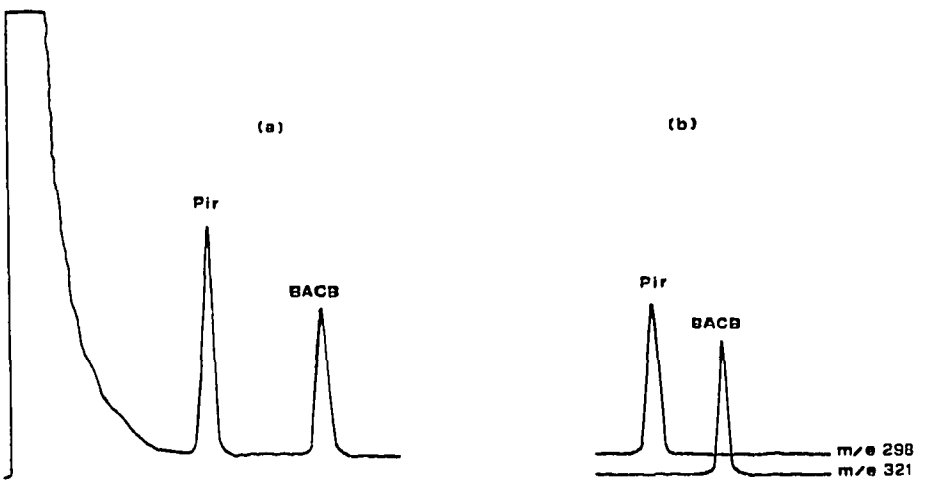

Fig. 1. (a) Gas chromatogram and (b) mass fragmentogram of piribedil (Pir) and the internal standard, 2-N-benzylamino-5-chlorobenzophenone (BACB).

Construction of the calibration graphs and quantitative analysis of piribedil

For calibration and quantitative analysis, the internal standard technique was used. Piribedil can be quantitated by GLC or by GLC–MF when the relative peak area is used as an index of concentration, as a linear relationship exists between relative peak area and piribedil concentration in the range 5–20 ng with the FID and 0.25–1.5 ng with mass fragmentographic detection (Fig. 2).

Extraction of piribedil from plasma

To 1 ml of plasma, made alkaline with 0.5 ml of 6 *N* sodium hydroxide solution, 4 ml of water were added and the mixture was extracted twice with 10 ml of diethyl ether. The combined ether phases were extracted with 5 ml of 6 *N* hydrochloric

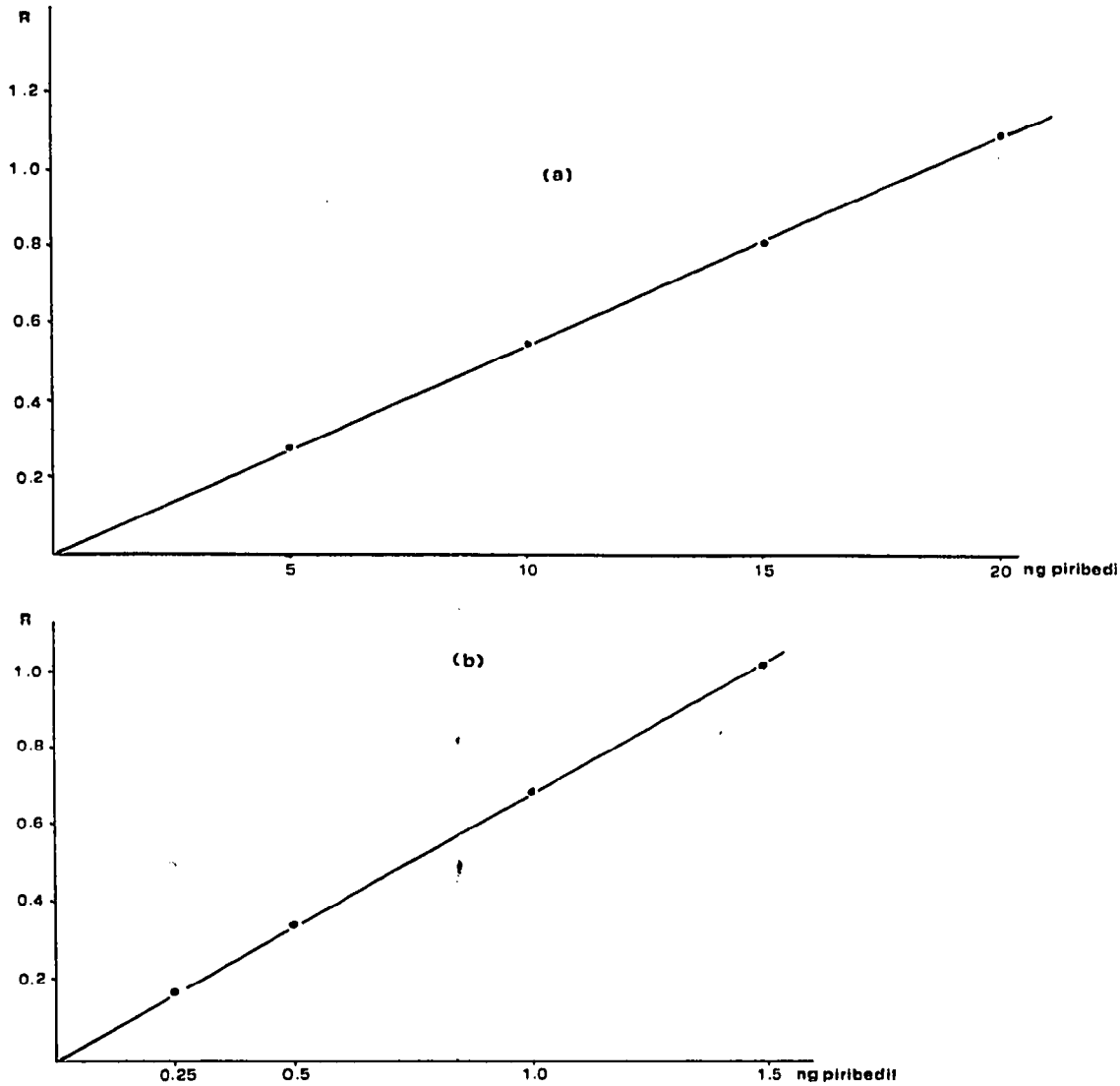

Fig. 2. Calibration graph for piribedil using (a) the flame ionization detector and (b) the mass fragmentographic technique. R = Ratio of the peak areas of piribedil and internal standard.

acid; the ether phase was discarded and the acidic phase was washed three times with 10 ml of diethyl ether. The acidic phase was then neutralized with 6 *N* sodium hydroxide solution and extracted twice with 10 ml of diethyl ether. The ether extracts were evaporated to dryness on a hot water-bath; the dry residue was redissolved in a suitable amount of acetone containing 10 μg/ml of the internal standard for GLC and 1 μg/ml for GLC–MF, and a volume of 1–3 μl was injected into the GLC column.

Extraction of piribedil from brain tissue

The brain tissue was homogenized in cold absolute ethanol (1:10, w/v) and the homogenate was centrifuged at 9000 *g* for 30 min. The alcoholic phase was separated from the precipitate and evaporated to dryness in a rotating evaporator, the residue was dissolved in 5 ml of 6 *N* hydrochloric acid and the acidic phase was submitted to the procedure described above for plasma. In Table I, the recoveries obtained by adding different amounts of piribedil to plasma and brain tissue are reported.

TABLE I

RECOVERY OF PIRIBEDIL FROM BIOLOGICAL SAMPLES BY UTILIZING A FLAME IONIZATION DETECTOR (FID) OR THE MASS FRAGMENTOGRAPHIC TECHNIQUE (MF)

Amount of drug added (μg/ml or μg/g)	*Detection*	*Recovery (%)*	
		From rat plasma	*From rat brain*
0.010	MF	96.1	80.3
0.020	MF	95.0	81.7
0.050	MF	97.8	78.5
0.100	MF	93.5	77.8
1	FID	96.0	75.8
10	FID	98.1	82.1
50	FID	97.1	83.4
	Mean ± S.E.	96.2 ± 0.61	79.9 ± 1.01

RESULTS AND DISCUSSION

2-N-benzylamino-5-chlorobenzophenone was chosen as the internal standard because of its suitable retention time and the fact that it and piribedil after electron impact give rise to two molecular ions at $m/e=321$ and $m/e=298$, respectively (Fig. 3), which are characteristic, intense and within a mass range of 10%, in accordance with the technical requirements of the instrument employed.

No interference from endogenous substrates was noted in a series of plasma and brain samples obtained from different animal species (rat, mouse and guinea pig). Typical mass fragmentograms of rat brain samples obtained from a control animal and from an animal treated intraperitoneally with 30 mg/kg of piribedil 4 h before examination are shown in Fig. 4.

Sensitivity studies indicate that the minimum amount of piribedil that can be detected by using GLC with an FID is about 250 ng/ml or ng/g, while with the GLC–MF technique it is possible to measure 10 ng/ml or ng/g of the drug present

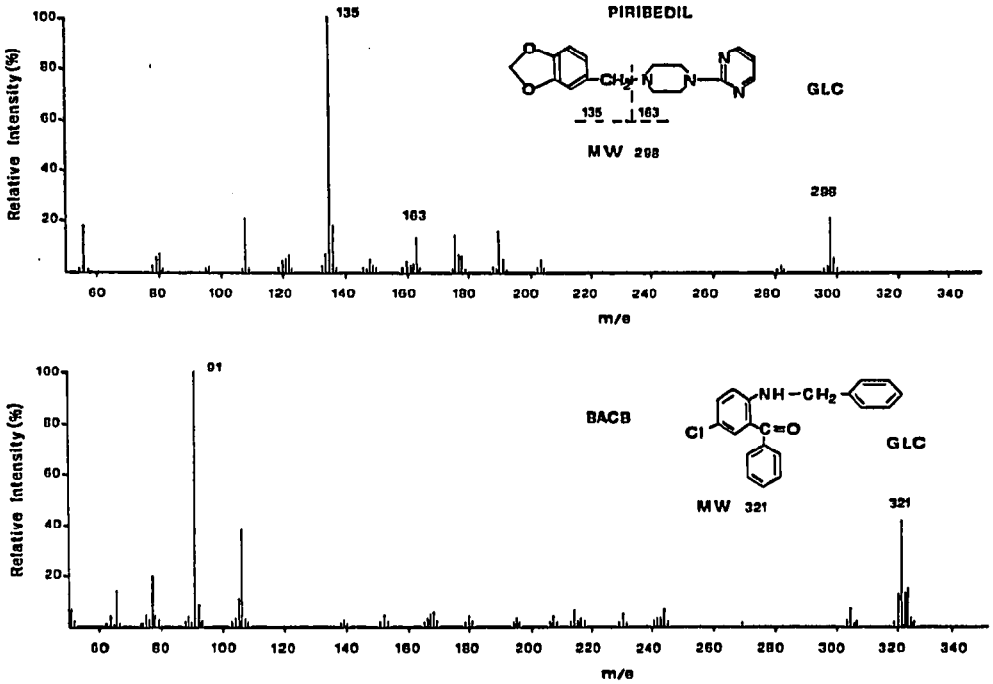

Fig. 3. Mass spectra of piribedil and 2-N-benzylamino-5-chlorobenzophenone (BACB).

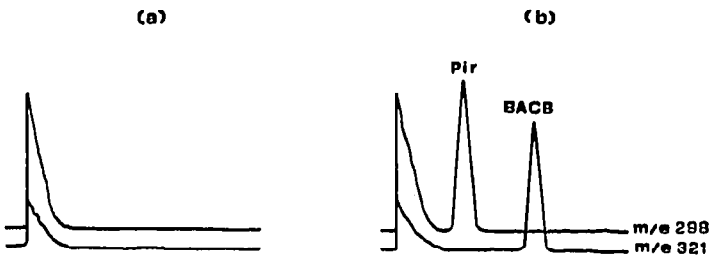

Fig. 4. Mass fragmentograms obtained from rat brain: (a) control animal; (b) 4 h after treatment with piribedil (Pir) (30 mg/kg. i.p.) with the addition of the internal standard, 2-N-benzylamino-5-chlorobenzophenone (BACB).

in biological samples. The results indicate that the method is suitable for measuring piribedil levels in plasma and tissue in experimental animals and for monitoring piribedil levels in human plasma.

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