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Note

Determination of buspirone and 1-(2-pyrimidinyl)piperazine in plasma samples by high-performance liquid chromatography

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Buspirone, 8-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-8-azaspiro[4.5]decane-7,9-dione, a dibasic heterocyclic psychotropic agent, is a new anti-anxiety drug. 1-(2-Pyrimidinyl)piperazine (1-PP) is a major metabolite of buspirone. Studies of the pharmacokinetics and pharmacological profile are difficult owing to the low concentrations attained after oral dosing. Structures of the compounds are shown in Fig. 1.

Caccia and co-workers [1,2] reported a gas chromatographic method for determining 1-PP in rat plasma. Gammans et al. [3] reported a gas chromatographic–mass spectrometric (GC–MS) method for separately determining both buspirone and 1-PP in the range 0.05–10 ng/ml and, recently, Sciacca et al. [4] described a capillary GC–MS method for the simultaneous determination of buspirone and 1-PP in human plasma. In this work, we have evaluated the possible use of a high-performance liquid chromatographic (HPLC) technique for the simultaneous extraction and determination of buspirone and 1-PP in rat and dog plasma.

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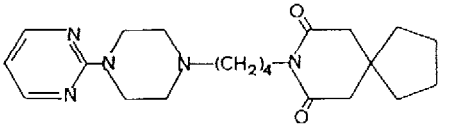
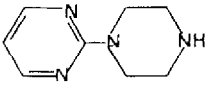
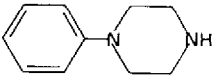


BUSPIRONE



1-(2-PYRIMIDINYL) PIPERAZINE



1-PHENYLPIPERAZINE

Fig. 1. Structures of buspirone, its metabolite (1-PP) and the internal standard used for quantification.

EXPERIMENTAL

Chemicals and reagents

Buspirone was obtained from Laboratorio FIDES (Barcelona, Spain) and the metabolite 1-PP from Aldrich (Steinheim, F.R.G.). 1-Phenylpiperazine was purchased from Fluka (Buchs, Switzerland) and methanol (HPLC-grade) from Ferosa (Barcelona, Spain).

HPLC system

The HPLC determinations were carried out on a Model 590 instrument equipped with a Model 440 spectrophotometric detector (Waters Assoc., Milford, MA, U.S.A.). Samples were injected through a Rheodyne (Cotati, CA, U.S.A.) valve injector fitted with a 20-μl loop. Detection was carried out at 254 nm and signals were recorded on a Perkin-Elmer (Norwalk, CT, U.S.A.) Model 156 recorder. A stainless-steel column (25 cm$\times$4.6 mm I.D.) packed with 5-μm Spherisorb CN (Tracer Analítica, Barcelona, Spain) was used at room temperature. A guard column (23 mm$\times$3.6 mm I.D.) was packed manually with CN/Corasil (37–50 μm). The mobile phase was methanol–monopotassium phosphate buffer (5 m*M*) at pH 7.4 (35:65) pumped at 1.7 ml/min.

Liquid–liquid extraction procedure for plasma samples

To a 2-ml volume of rat plasma were added 100 μl of an internal standard solution at a concentration of 50 μg/ml and 1 ml of borate buffer (pH 10). This mixture was extracted with two portions of 5 ml of chloroform–acetonitrile (8:2). After agitation and centrifugation, the organic phase was removed. The organic layers were combined and evaporated under reduced pressure below 40°C, the residue was dissolved in the mobile phase (100 μl) and a 20-μl aliquot was injected into the column.

RESULTS

Fig. 2 shows the separation of buspirone, 1-PP and the internal standard. The use of a cyano column was essential for a good elution of these components, otherwise the buspirone and internal standard are difficult to elute from reversed-phase columns owing to strong solute–sorbent interactions. The recovery of 100 ng/ml buspirone was 73 ± 4% ($n = 6$). Linear regression analysis of the calibration graph showed excellent correlations with $r^2 \geqslant 0.985$ over the range 5.0–500.0 ng/ml.

The between-day coefficient of variation based on duplicate calibration graphs over a two-month period at different plasma concentrations is given in Table I. The limit of detection of buspirone and 1-PP was 5 ng/ml with a signal-to-noise ratio of greater than 10:1 and an acceptable within-day coefficient of variation of 7.9%.

The application of the method to a study of the pharmacokinetic profile of

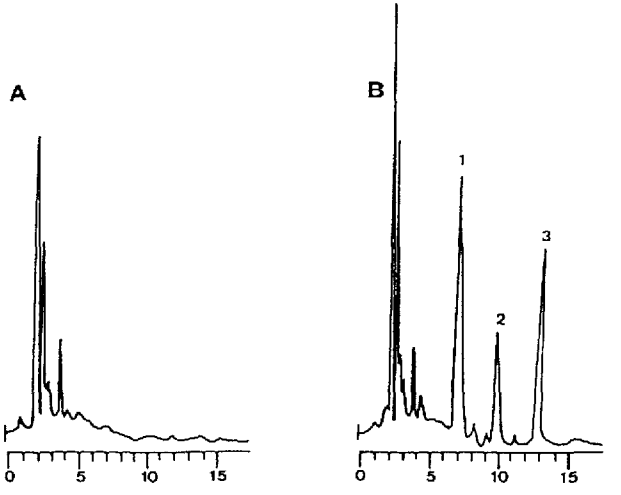


Fig. 2. Chromatograms of plasma extracts for (A) blank plasma and (B) blank plasma spiked with (1) 50 ng/ml 1-PP, (2) 100 ng/ml buspirone and (3) 50 ng/ml internal standard.

TABLE I

REPRODUCIBILITY OF THE BUSPIRONE ASSAY

The peak-area ratio of buspirone to internal standard was determined for five concentrations and the coefficient of variation (C.V.) was calculated for six duplicate calibration graphs over a two-month period (between-day) or for one triplicate calibration graph (within-day).

Concentration (ng/ml)	Between-day ($n=6$)		Within-day ($n=3$)	
	Peak-area ratio	C.V. (%)	Peak-area ratio	C.V. (%)
5	0.032 ± 0.004	12.4	0.031 ± 0.002	7.9
10	0.065 ± 0.006	9.8	0.068 ± 0.005	6.8
25	0.343 ± 0.023	6.7	0.347 ± 0.018	5.3
100	0.663 ± 0.049	7.4	0.660 ± 0.019	2.9
500	1.301 ± 0.082	6.3	1.305 ± 0.014	1.1

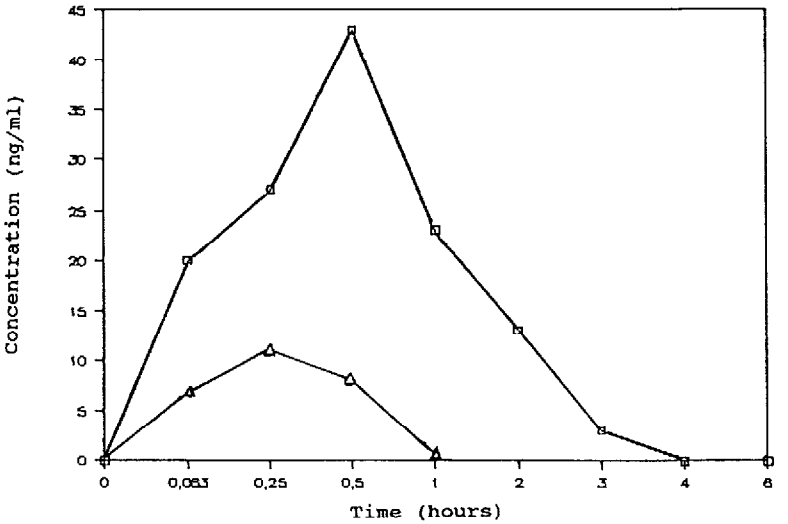


Fig. 3. Pharmacokinetic profile of (△) buspirone and (□) 1-PP in dog plasma after oral doses of 20 mg.

buspirone and 1-PP in dog plasma after oral doses of 20 mg of buspirone showed that the maximum levels of 1-PP are attained within 0.5–1 h (Fig. 3).

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