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Determination of a buspirone metabolite in plasma samples*

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Abstract: A high-performance liquid chromatographic (HPLC) method is described for the assay of the active metabolite [1-(2-pyrimidinyl)piperazine] of buspirone, an anxiolytic agent, in rat plasma.

The method is based on the use of ion-pair HPLC coupled to a liquid–solid extraction scheme. Samples of rat plasma (2 ml) with internal standard (1-phenylpiperazine), adjusted to pH 10.5 with borate buffer, were loaded on to a preactivated C-18 cartridge. The metabolite and the internal standard were eluted with 5 ml of methanol and injected on to a reversed-phase 10-μm Spherisorb ODS-2 column. The column was eluted with a mobile phase of 0.005 M sodium lauryl sulphate in citrate buffer (pH 3.6)–acetonitrile (65:35, v/v) at 2 ml min^{-1}. Detection was carried out at 248 nm. The recovery of the metabolite was 55%. The method was applied to the determination of the metabolite in rat plasma after oral dosing (25 mg kg^{-1}) of the parent compound.

Keywords: *Buspirone; [1-(2-pyrimidinyl)piperazine]; reversed-phase HPLC; solid-phase extraction; pharmacokinetics.*

Introduction

Buspirone (8-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-8-azaspiro [4,5]-decane-7,9-dione), a lipophilic and dibasic heterocyclic psychotropic agent, has been shown to be clinically effective in primary anxiety. However, little is known about its exact mechanism of action and studies on its bioavailability, and its pharmacological profile in human and animal fluids are scarce owing to the low concentrations attained after oral dosing. Most of the determinations carried out to date have been based on two gas chromatographic (GC) methods. The first GC method reported by Caccia *et al.* [1, 2] used flame-ionisation detection to measure buspirone at concentrations greater than 200 ng ml^{-1} and electron capture detection of the heptafluorobutyrate derivative of its main metabolite 1-(2-pyrimidinyl)piperazine (1-PP) (Fig. 1). Later Gammans *et al.* [3] reported on a capillary GC–MS method capable of quantifying plasma buspirone levels in the range of 0.05–10 ng ml^{-1}.

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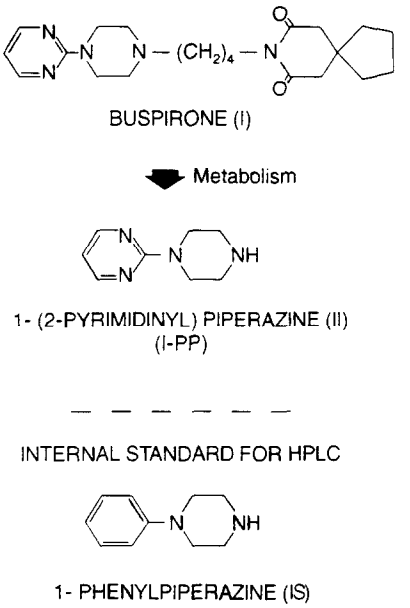

Figure 1
Chemical structures of buspirone, its metabolite (1-PP) and the internal standard (IS) used for quantification.

Because of the availability and extended use of modern HPLC procedures in pharmacological studies, the possible use of this technique for the study of the disposition and metabolism of the buspirone metabolite in rat plasma has been evaluated.

Experimental

Chemicals and reagents

Buspirone was synthesised in the laboratory and the metabolite (1-PP) was obtained from Janssen Chimica (Beerse, B). Methanol (analytical grade) and 1-phenylpiperazine were obtained from Fluka AG (Buchs, CH). Sep-Pak C-18 cartridges were from Waters Assoc. (Milford, MA, USA).

Pharmacokinetic study

Male Wistar rats (100–200 g) were maintained in controlled conditions with free access to food and water. Each animal received an oral dose of buspirone (25 mg kg^{-1}) administered via a gastric probe in distilled water (10 ml kg^{-1}). Animals were fasted 18 h before administration of the drug. Blood from the carotid artery was collected in heparinised tubes and was centrifuged at 1500 rpm for 10 min to separate plasma.

HPLC system

The HPLC determinations were carried out on a Series 2 HPLC instrument equipped with a LC-75 spectrophotometric detector, all from Perkin-Elmer (Norwalk, PA, USA). Samples were injected through a Rheodyne valve injector model 7125 (Cotati, CA, USA) fitted with a 50 μl loop. Detection was carried out at 248 nm and the detector output was recorded on a Hitachi Perkin-Elmer recorder model 156. The 250 × 4.6 mm i.d. column was packed with 10-μm Spherisorb ODS-2 (Tracer Analítica, S.A. Barcelona, Spain). Guard columns were hand packed with 37–50 μm C18/Corasil. The

mobile phase was 0.005 M sodium lauryl sulphate in citrate buffer (pH 3.6) acetonitrile (65:35, v/v) pumped at 2 ml min^{-1}.

Liquid–solid extraction procedure for plasma samples

To 2 ml of rat plasma were added 150 μl of internal standard solution (Fig. 1) (30 μg ml^{-1}) and 400 μl of borate buffer (pH 10.5). The sample was then loaded on to a pre-activated reversed-phase C-18 cartridge. The cartridge was washed with water (5 ml) and then with water–methanol (50:50, v/v) (1 ml). Finally, the retained metabolite was eluted with MeOH (5 ml). The dry residue obtained from evaporation of the MeOH was dissolved in 400 μl of HPLC eluent and 50 μl was injected on to the HPLC column.

Results

The HPLC chromatograms (Fig. 2) show the separation of 1-PP and internal standard both in a standard solution (Fig. 2A) and in a plasma extract (Fig. 2B). Buspirone is also separated under these conditions although it takes longer to be eluted. The use of an ion-pairing reagent in the mobile phase was important for efficient and quantitative elution of the pyrimidinyl moiety present in both buspirone and 1-PP; otherwise these components are difficult to elute from reversed-phase columns owing to strong solute–sorbent interactions. The limit of detection for 1-PP in plasma was about 22 ng ml^{-1}. The mean extraction recovery for five replicate samples run on two different days was 54.5% with relative standard deviations of 4.4–10.8% (intra-assay) and 4.7% (inter-

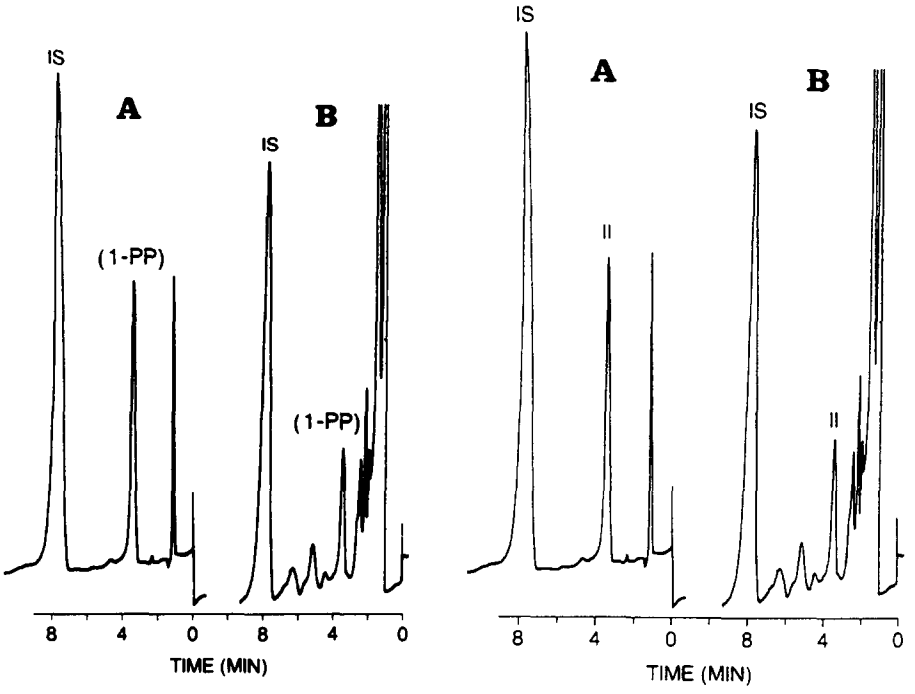

Figure 2
HPLC ion-pair reversed-phase separation of 1-PP (Peak II) and the internal standard (IS). See text for chromatographic conditions.

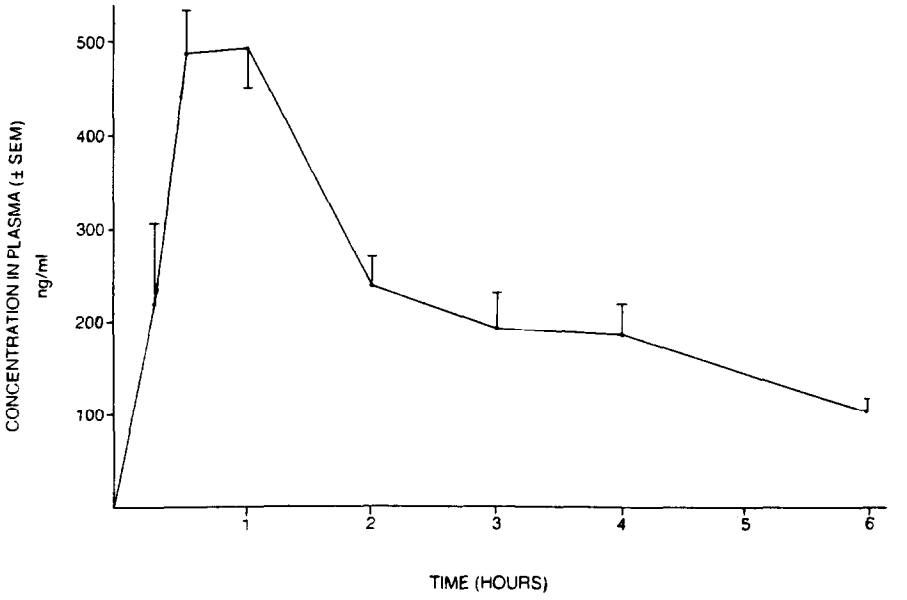

Figure 3
Pharmacokinetic profile of 1-PP in rat plasma ($N = 3$) after oral doses of 25 mg kg^{-1}.

assay). This recovery is an improvement over the 33% achieved by the authors of the present work using the benzene extraction procedure previously described [2]. Although quantitative recovery has been claimed with the use of a benzene extract, the authors were unable to achieve an acceptable consistency of results by that method.

Application of the newly developed method to a study of the pharmacokinetic profile of 1-PP in rat plasma after oral doses of 25 mg kg^{-1} shows that after the maximum level is attained within 0.5–1 h (Fig. 3) the plasma metabolite concentration decreases rapidly in a mono-exponential manner; the area under the curve is estimated as 1444.6 ng ml^{-1} h from 0 to 6 h. This is in line with values reported by Caccia *et al.* [2]. A detailed pharmacokinetic account of this work will be given elsewhere.

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