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Simultaneous quantitation of buspirone and its major metabolite 1-(2-pyrimidinyl)piperazine in human plasma by high-performance liquid chromatography with coulometric detection

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Abstract

Buspirone is a member of the azapirone group of anxiolytic drugs and has one major metabolite, 1-(2-pyrimidinyl)piperazine (1-PP). The analyte, its metabolite and the internal standard were extracted from plasma utilizing solid-phase extraction columns. Chromatography was performed using isocratic reversed-phase high-performance liquid chromatography with coulometric end-point detection. The calibration graph was linear over the range 0–50 ng ml^{-1} of plasma. The lower limits of quantitation for buspirone and 1-PP were 0.5 and 2 ng ml^{-1}, respectively, when 1 ml of plasma was extracted. The intra-assay relative standard deviations (RSD) over the range of the calibration graph varied from 4 to 12.5% for buspirone and 1-PP. The inter-assay RSD was 6.9% for 1-PP and 9.6% for buspirone. The recovery averaged 96% for buspirone and 66% for 1-PP. Plasma profiles of buspirone and 1-PP following oral dosing are presented.

Keywords: Buspirone; 1-(2-Pyrimidinyl)piperazine metabolite; HPLC; Coulometric detection

1. Introduction

Buspirone, 8-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione, an azapirone, is a relatively new anxiolytic drug whose major metabolite, 1-(2-pyrimidinyl)piperazine (1-PP), may contribute to the overall therapeutic effects of the drug. Its pharmacological and therapeutic properties have been extensively reviewed [1–3]. The structures of both buspirone and 1-PP metabolite are shown in Fig. 1.

Gammans and co-workers [4,5] have reported gas chromatographic–mass spectroscopic (GC–MS) methods for determining separately buspirone and 1-PP in the ranges 0.05–10 ng ml^{-1} and 0.2–15 ng ml^{-1}, respectively. Sciacca et al. [6] also described a capillary GC–MS method that simultaneously determined buspirone and 1-PP. Although these methods are highly sensitive with very low detection limits, they involve extensive

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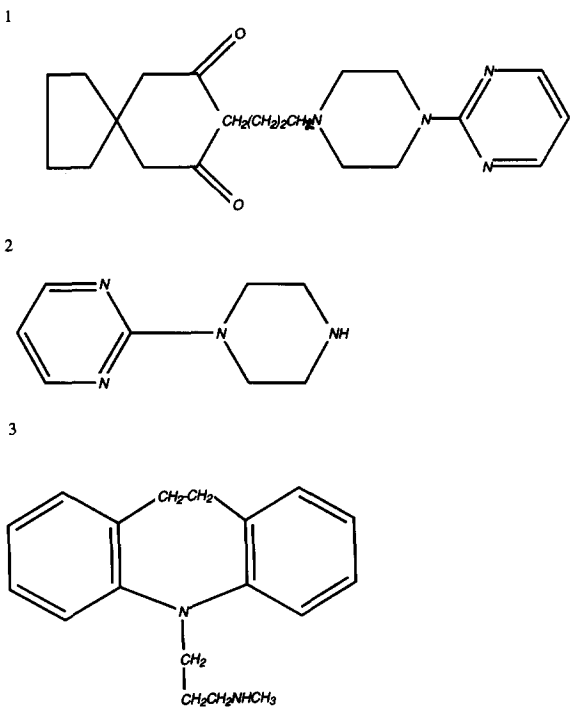

Fig. 1. Structures of buspirone (1), its major metabolite 1-PP (2) and the internal standard DMI (3).

sample preparation procedures and, more importantly, cannot be used for everyday clinical use because of their speciality requirement and cost.

Diaz-Marot et al. [7] reported an HPLC method for the simultaneous determination of buspirone and 1-PP with ultraviolet (UV) end-point detection. The main drawback with this particular methodology was the lack of sensitivity for buspirone (5–500 ng ml^{-1}), since peak plasma concentrations in pharmacokinetic studies have revealed, after oral dosing (10 mg of buspirone·HCl), analyte concentrations of 2–3 ng ml^{-1} [1]. Frankin [8] reported a sensitive and selective HPLC method for buspirone, but it failed to include the determination of 1-PP.

Betto et al. [9] reported an HPLC method with coulometric detection for the simultaneous measurement of buspirone and 1-PP in mouse plasma. They claimed limits of detection of 50 and 35 pg ml^{-1} for buspirone and 1-PP, respectively, despite quoting acceptable reliability factors over the range 25–600 ng ml^{-1}. The extraction recovery and detector variation responses were monitored without the use of an internal standard. This method may be suitable for the measurement of buspirone and 1-PP in mouse plasma, but on the above evidence it is suggested that this procedure would not be good enough to be used for determining plasma levels of these compounds in human plasma.

Therefore, there remains a requirement for a simple, selective, inexpensive and highly sensitive assay procedure with a short preparation time for the everyday clinical measurement of buspirone and its metabolite. Described here is a simple, fast, sensitive and robust method for the determination of buspirone and 1-PP in plasma, involving HPLC with coulometric end-point detection, solid-phase extraction (SPE) and an internal standard, desmethylimipramine (DMI), for monitoring both analyte recovery and detector response variations during analysis.

2. Experimental

2.1. Materials

The hydrochlorides of buspirone and 1-PP were kindly donated by Bristol-Myers (Uxbridge, UK). The internal standard, desmethylimipramine hydrochloride (Fig. 1), was kindly donated by Ciba Laboratories (West Sussex, UK). All solvents and reagents used were of the highest grade available. Drug-free plasma for the preparation of standards for extraction was obtained from normal volunteers in the laboratory. All water was glass distilled and deionized prior to use. Carboxypropyl (CBA) solid-phase columns (Isolute SPE) for the extraction were purchased from IST (Mid-Glamorgan, UK). Stock standard solutions of both buspirone and 1-PP were prepared at concentrations of 100 ng μl^{-1} in methanol and stored at 4°C. The stock standard solutions were serially diluted (100-fold) with distilled water to obtain working standard solutions of 1 ng μl^{-1} for each analyte. Stock standard solutions in methanol of buspirone, 1-PP and the internal standard were stable for 1 month, 1 week and 1 month, respectively.

2.2. Apparatus and conditions

The HPLC system comprised of a Milton Roy Constametric 3000 pump (LDC, Stone, UK), a manual Rheodyne 7125 injection valve fitted with a 50 μl loop, a 250 mm × 4.6 mm i.d. cartridge column system (Capital HPLC, Edinburgh, UK) packed with a 5 μm Spherisorb ODS/CN (mixed mode (a combination of a C_{18}- and cyano-bonded material). End-point detection was achieved with a Model 5100A coulometric detector, consisting of a Model 5010 high-sensitivity cell and a Model 5020 guard cell (ESA, Bedford, MA, USA). Selected operating potentials for the guard cell and detectors 1 and 2 were 0.98, 0.75 and 0.93 V, respectively (see Fig. 2). The guard cell pre-oxidizes any electroactive impurities which may be present in the mobile phase. Detector 1 is utilized to pre-oxidize any extractable compounds that are not of interest but that might interfere with the analytes of premier interest at detector 2, the analytical electrode. The response time was set at 5 s. The detector was linked to a chart recorder or intergrator as required.

The mobile phase consisted of 0.04 M potassium orthophosphate buffer (adjusted to pH 4.5 with 2 M potassium hydroxide solution) and methanol (26:74, v/v). This was filtered through a 0.2 μm filter and degassed under vacuum for 15 min prior to use. The flow rate was 1.00 ml min^{-1}. Peak heights rather than peak areas on the chromatograms were normally measured. Concentrations of buspirone and 1-PP were assessed by interpolation of the calibration graph using the general linear equation $y = ax + b$. All chromatography was performed at ambient temperature.

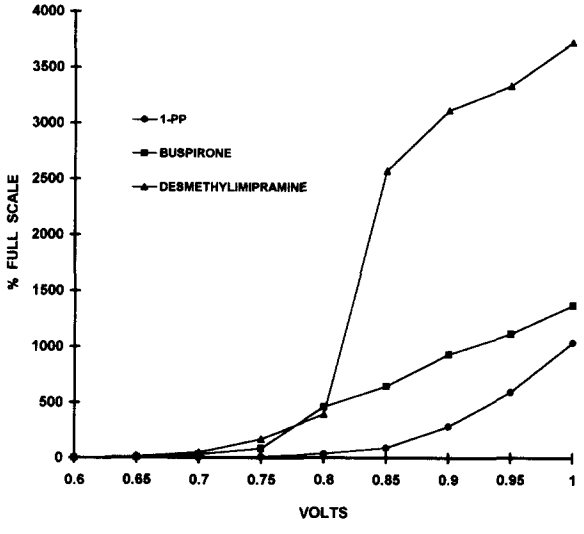

Fig. 2. Voltammograms for buspirone, 1-PP and the internal standard DMI for detector 2 at different potentials. The voltammograms were determined when the guard cell and detector 1 were at zero potential.

2.3. Procedures

Blood samples were collected in lithium heparin tubes and centrifuged. The plasma was separated and stored at −20°C until required for assay. A 1 ml volume of plasma was added to a 5 ml disposable polypropylene tubing containing 20 μl of the internal standard solution (1 ng μl^{-1}). After brief vortex mixing, the mixture was transferred into a CBA SPE column supported by a sample vacuum manifold system. Calibration graphs were prepared fresh daily and consisted of five concentration points over the range 0–50 ng μl^{-1} of buspirone and 1-PP in drug-free plasma. CBA SPE columns (50 mg ml^{-1}) were initially conditioned with successive column volumes of methanol and distilled, deionized water under vacuum. The vacuum was diverted to keep the columns from drying out and to allow transfer of standards and samples on to the columns. This was followed by washing with two column volumes of water. The vacuum was again diverted, the manifold needle wiped dry and a collection tray containing 12 × 75 mm glass tubes inserted into the manifold system. Isolates were eluted with one column volume of 1% ammonia solution in methanol. The eluates were evaporated to dryness under vacuum at 40°C. Samples and standards were reconstituted in 200 μl of mobile phase, vortexed mixed and made ready for injection into the HPLC system.

3. Results

Assay resolution and sensitivity were determined by injection of extracted plasma standard (Fig. 3). The linearity of both the extraction pro-

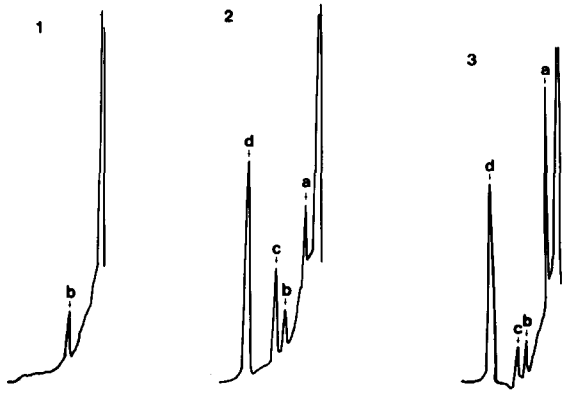

Fig. 3. Chromatograms of (1) blank drug-free plasma, (2) drug-free plasma spiked with 5 ng ml^{-1} each of buspirone and 1-PP and 20 ng ml^{-1} of the internal standard. (3) Chromatogram from a sample from a volunteer following an oral dose of 30 mg buspirone·HCl, 1-PP peak equivalent to 22.1 ng ml^{-1} and the buspirone peak equivalent to 2.3 ng ml^{-1}. Peaks a, b, c and d represent 1-PP, an endogenous compound, buspirone and the internal standard, respectively. The retention times for 1-PP, buspirone and the internal standard were 5.5, 8.0, and 11.0 min, respectively, with a total run time of 13 min. The ordinate represents a full-scale deflection of 4 nA.

cedure and the detector response (determined from the peak height) was verified over the range of the assay calibration graph. This was determined by measuring pooled drug-free plasma spiked with known amounts of buspirone and 1-PP. Calibration graphs were calculated for both buspirone and 1-PP and the peak-height ratio over the concentration range studied. The mean slope ± the standard error of the mean (SEM) and intercept ± SEM were 0.067 ± 0.009 and −0.001 ± 0.018 for buspirone and 0.031 ± 0.008 and −0.014 ± 0.006 for 1-PP. The correlation coefficients for both compounds were 0.999. The intra-assay precision data are presented in Table 1. The mean inter-assay relative standard deviations (RSDs) for buspirone and 1-PP were 6.9% ($x = 9.19 \pm 0.63$) and 9.6% ($x = 9.68 \pm 0.93$), respectively, for a spiked plasma quality control pool containing 10 ng ml^{-1} of each analyte ($n = 10$) and 16% ($x = 2.7 \pm 0.423$) for a spiked plasma pool containing 2 ng ml^{-1} of buspirone ($n = 10$). Recoveries for buspirone and 1-PP from spiked drug free plasma are given in Table 2.

Table 1
Intra-assay RSDs and precision data for the determination of buspirone and its metabolite 1-PP in plasma ($n = 6$)

Actual value (ng ml^{-1})	Observed value (ng ml^{-1})		RSD (%)[a]	
	Buspirone	1-PP	Buspirone	1-PP
2	2.10 ± 0.25	–[b]	11.9	–[b]
5	4.87 ± 0.59	4.53 ± 0.51	12.1	11.2
10	10.35 ± 0.51	9.81 ± 1.10	4.9	11.2
25	24.89 ± 1.02	25.17 ± 2.99	4.1	11.9

[a] The RSDs were calculated from results for pooled drug-free plasma spiked with known amounts of buspirone and 1-PP. For precision data and the results were compared with externally prepared standards.
[b] 1-PP was not determined at this level because the assay limit of detection was only 2 ng ml^{-1}.

Table 2
Recoveries of buspirone and 1-PP from spiked drug-free plasma

Amount (ng ml^{-1})	Recovery ± SEM (%) ($n = 6$)	
	Buspirone	1-PP
2	87.4 ± 3.2	–[a]
5	82.2 ± 4.0	64.9 ± 1.9
10	102.5 ± 4.4	65.1 ± 2.4
25	111.0 ± 1.3	67.2 ± 4.0

[a] 1-PP was not determined at this level because the assay limit of detection was only 2 ng ml^{-1}.

A number of psychotropic agents were tested for assay interference, and of these only haloperidol (relative retention time with respect to buspirone = 0.9) would be expected to cause problems. Sample extracts were stable for up to 1 week when stored in the dark at 4°C.

Plasma profiles following oral administration of buspirone·HCl to 15 normal volunteers are shown in Fig. 4.

4. Discussion

A simple, sensitive and selective HPLC assay has been described for the measurement of buspirone and its major metabolite 1-PP in plasma. It utlilzes HPLC with coulometric detection and an internal standard, DMI, for monitoring extraction and coulometric detector variations. The detec-

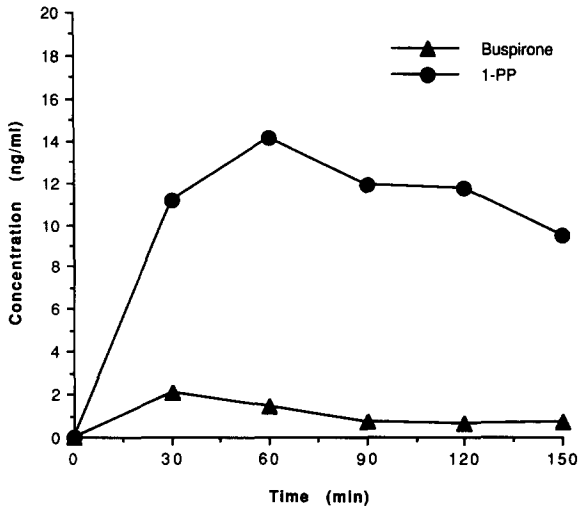

Fig. 4. Mean plasma concentrations of buspirone and 1-PP from 15 male volunteers following oral dosing with buspirone hydrochloride (30 mg).

tion limits for buspirone and 1-PP were 0.5 and 2 ng ml^{-1}, respectively when 1 ml of plasma was extracted. It was established that the ratio between the analytical recoveries of buspirone, 1-PP and that of the internal standard submitted to the same operations was constant over a wide concentration range. The requirements for an internal standard assay procedure were therefore satisfied. Franklin [8] suggested that his method could be further improved by alternative sample preparation techniques (i.e. SPE) because of the lower recovery (50–55%) attained for buspirone in this assay methodology, and the fact that isolating 1-PP using a three-step solvent extraction process was not possible. An analytical cell potential of 0.93 V was utilized because full oxidation of 1-PP (as demonstrated by the voltammograms in Fig. 2) required a potential much greater than that for buspirone or the internal standard.

This method has demonstrated significant improvements in comparison with other assay systems. Higher recoveries for both buspirone and 1-PP, lower sample volumes and reduced sample preparation time have all been attainable by utilizing SPE technology. It has also facilitated both the superior resolution of these compounds from other psychotropic drugs that might possibly interfere chromatographically and a greater detector response, thus allowing for lower levels of buspirone and, in particular, 1-PP to be detected.

5. Conclusions

A novel technique using HPLC with coulometric detection has been described for the measurement of buspirone and its major metabolite 1-PP in plasma. It is quick, sensitive and relatively cheap to operate compared with alternative GC–MS methods currently available. It is therefore suitable for routine clinical analysis and research purposes.

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