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Note

Determination of plasma buspirone by high-performance liquid chromatography with coulometric detection

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Buspirone, 8-{4-[4-[2-pyrimidinyl)-1-piperazinyl]butyl}-8-azaspiro[4.5]-decane-7,9-dione, is one of a new group of anti-anxiety drugs. Its structure is given in Fig. 1a. Its properties, both pharmacological and therapeutic, have been recently reviewed [1–3].

Buspirone has been shown to be effective in oral doses of 15–30 mg per day for the treatment of generalised anxiety, the maximum recommended dose being 45 mg per day. Plasma levels of buspirone following on oral dose of 5 mg reach a maximum of around 5 ng/ml [4]. Hence an assay technique with a low limit of detection is required. A gas chromatographic method using flame ionization detection was reported to be capable of measuring 200 ng/ml [5]. Bianchi and Caccia [6] report a method for the measurement of buspirone, gepirone, isapirone and their common metabolite 1-(2-pyrimidinyl)piperazine (1PP, Fig. 1c) in rat plasma and brain tissue. This method utilizes high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. No mention is made of its sensitivity. Gammans et al. [4] reported a highly sensitive and specific method which involved the use of selected-ion monitoring gas chromatography–mass spectrometry utilizing fused-silica capillary columns. This had a quantification range of 0.05–10 ng/ml. However good this latter method may be, it cannot be used for everyday clinical use because of its high degree of speciality and its high cost.

The simple method described here is based on organic extraction of buspi-

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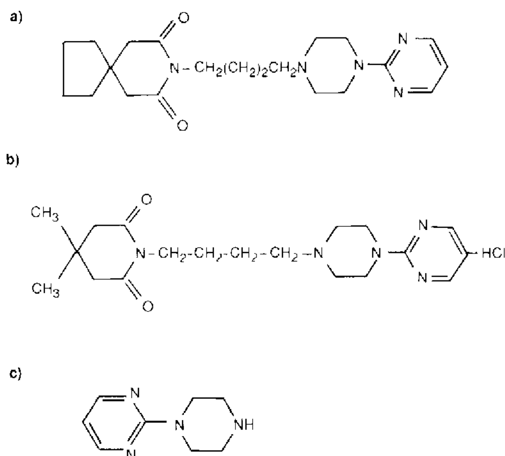

Fig. 1 Structures of buspirone (a), the internal standard, gepirone (b), and their common metabolite, 1PP (c).

rone from plasma or serum followed by isocratic reversed-phase HPLC with coulometric detection. The method uses gepirone (Fig. 1b) as an internal standard.

EXPERIMENTAL

Materials

The hydrochlorides of buspirone and gepirone were kindly donated by Bristol Myers (Uxbridge, U.K.). Acetonitrile (HPLC grade), ethyl acetate (AR grade) and *n*-butyl acetate (AR grade) solvents were obtained from Fisons (Crawley, U.K.). Potassium dihydrogen orthophosphate, hydrochloric acid, 0.88 S.G. ammonia, sodium hydroxide, potassium hydroxide and ethanol were all AR-grade reagents purchased from BDH (Poole, U.K.). Plasma for the preparation of standards for extraction was obtained from the Blood Transfusion Unit (Oxford, U.K.). All water was deionised and glass-distilled prior to use.

Stock standard solutions of both buspirone and gepirone, the internal standard, were prepared at concentrations at 100 μg/ml in ethanol. The buspirone and gepirone solutions were further diluted in ethanol to a concentration of 1 μg/ml and stored at 4°C. From these, standard solutions of buspirone were prepared in 50-μl volumes of ethanol, for each assay run. Similarly a final solution of gepirone in ethanol was prepared.

Chromatographic apparatus and conditions

The HPLC system comprised of a Kratos Spectraflow 400 pump (Severn Analytical, Shefford, U.K.), a Showa Denko KK Shodex solvent degasser

(Severn Analytical), a manual Rheodyne 7125 injection valve equipped with 50-μl loop, a Brownlee MPLC cartridge system with a 5 μm particle size cyanonitrile analytical column (10 mm×4.6 mm I.D.) protected by a 5 μm particle size cyanonitrile guard column (Anachem, Luton, U.K.). The detection system consisted of a Model 5100A Coulochem detector and a Model 5020 guard cell (ESA, Bedford, MA, U.S.A.). The detector was linked to an LDC CI-4000 integrator (LDC, Stone, U.K.).

The potentials for detectors 1 and 2 were selected after injection of fixed amounts of buspirone and gepirone, the internal standard, over the range 0.35–0.8 V for each detector (Fig. 2). The potentials for the guard cell and detectors 1 and 2 were 0.3, 0.55 and 0.7 V, respectively. The response time was 2 s.

The mobile phase was prepared freshly every day. It consisted of 0.04 *M* potassium phosphate buffer (adjusted to pH 6.6 with 2 *M* potassium hydroxide) and HPLC-grade acetonitrile (330:170, v/v). The mobile phase was initially degassed under vacuum before use. It was also continually degassed for baseline stability purposes by the in-line solvent degasser. The flow-rate was set at 1.5 ml/min.

The cyanonitrile columns were conditioned before the mobile phase was run through. This was done by initially flushing the columns with 50 ml of distilled, deionised water, then with 0.005 *M* sodium acetate buffer (pH 4.8) followed by acetonitrile in acetate buffer (2:3, v/v). Finally, the column was washed with 0.005 *M* potassium phosphate (pH 4.8) before equilibration with the mobile phase.

Peak heights rather than peak areas in the chromatograms were normally measured. Concentrations of buspirone were assessed by using the slope of the standard curve for peak-height ratios for the analyte and the internal standard.

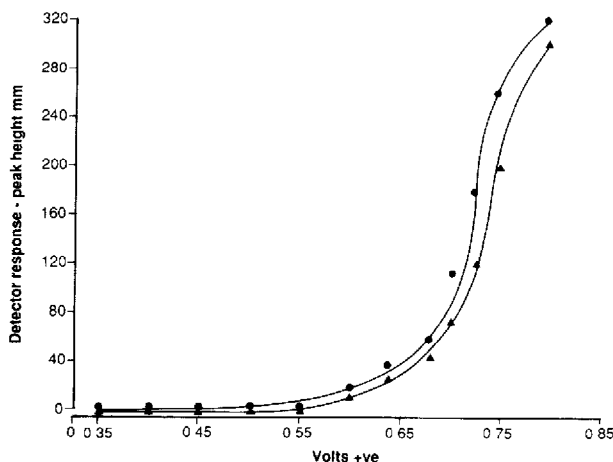

Fig. 2. Voltammogram of buspirone (▲) and gepirone (●), the internal standard, for detector 2 at different potentials. The voltammogram was determined whilst the other electrodes were set at zero potential.

Procedures

Blood samples were collected into tubes containing lithium heparin as anticoagulant, and centrifuged, and the plasma was separated and stored at $-20°C$ in a freezer until required for assay.

To 2 ml of plasma contained in a 15-ml glass-stoppered round-bottomed centrifuge tube, 20 ng of gepirone in 0.05 ml of ethanolic solution, 0.5 ml of 1 *M* sodium hydroxide and 4 ml of ethyl acetate were added. The tube was shaken mechanically (slowly to avoid emulsion formation) for 10 min and centrifuged (400 *g* for 10 min). After freezing the lower plasma layer in a solid carbon dioxide–acetone freezing mixture, the organic phase was decanted into a 15-ml glass-stoppered tapered centrifuge tube containing 2 ml of 0.05 *M* hydrochloric acid. The tube was shaken for 10 min and centrifuged (400 *g* for 10 min). The organic phase was aspirated with a Pasteur pipette and discarded. A 0.5-ml volume of 4 *M* ammonia solution and 400 μl of butyl acetate were added, and the tube mixed for 15–20 s followed by centrifugation at 400 *g* for 10 min. The lower aqueous phase was aspirated with a Pasteur pipette, leaving a small residual amount in the bottom of the tube. The complete organic layer was transferred to a clean glass-stoppered tapered tube and then placed in a Buchler vortex-evaporator (Orme Scientific, Manchester, U.K.) at 30°C under vacuum and evaporated to dryness. The residue was reconstituted in 90 μl of mobile phase with mixing.

Standards and drug-free samples were extracted in the same manner as were the unknown samples, except that freshly prepared buspirone was added in known amounts to drug-free plasma.

RESULTS

Resolution and sensitivity were determined by injection of an extracted plasma standard (Fig. 3). The retention times of buspirone and the internal standard, gepirone, were 5.4 and 7.3 min, respectively. The linearity of both the extraction procedure and the detector response (determined from the peak height) was verified over the anticipated range of the assay (1–25 ng/ml). The linearity was determined by assying pooled blood bank plasma (which had been previously screened for extraneous peaks) spiked with known amounts of buspirone. A calibration curve was calculated for buspirone concentration and the peak-height ratio over the concentration range studied. The equation for the calibration curve was $y = 0.059x + 0.0276$. Each point on the curve was calculated from the means of the inter-day assay variation data (Table I).

The actual recovery of buspirone over the anticipated concentration range was 50–55%. The absolute recovery of buspirone (5 ng/ml) was $87 \pm 3.8\%$ (mean $\pm$ SD, $n = 10$). Sample extracts were stable for up to ten days when stored in mobile phase at 4°C.

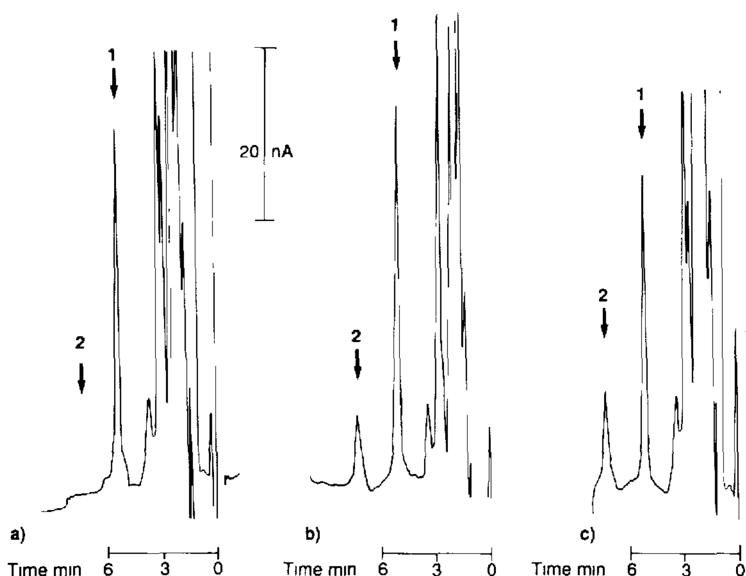

Fig. 3. Representative chromatograms from (a) extracted drug-free plasma, (b) extracted drug-free plasma spiked with 2 ng/ml buspirone and (c) plasma extracted from volunteer subjects 1 h after oral administration of 15 mg buspirone hydrochloride (estimated to contain 3.2 ng/ml buspirone). Peaks: 1 = gepirone, internal standard, 2 = buspirone.

TABLE I

INTRA-ASSAY PRECISION AND ACCURACY OF THE DETERMINATION OF BUSPIRONE IN HUMAN PLASMA ($n=6$)

Actual value (ng/ml)	Observed value (ng/ml)	Coefficient of variation[a] (%)
2	2.16	8.3
5	5.12	6 3
10	9.95	6.0
25	25 37	5.8

[a]The precision (coefficient of variation) of the method was calculated from results for pooled normal plasma containing known concentrations of added buspirone.

TABLE II

CHROMATOGRAPHIC MOBILITY OF SOME PSYCHOTROPIC DRUGS RELATIVE TO BUSPIRONE (50 ng OF EACH INJECTED)

Drug	Retention relative to buspirone[a]	Drug	Retention relative to buspirone[a]
Buspirone	1	Amitriptyline	2.36
Gepirone	0 74	Fluphenazine	0.84
1PP	0 41	Clomipramine	2.13
Valium	N.R	Mianserin	N.R.
Haloperidol	0.95	Zimelidine	N.R
Imipramine	1.4	Chloropromazine	1 75
Desimipramine	N.R.	Caffeine	N.R.

[a]N R = no response after 20 min

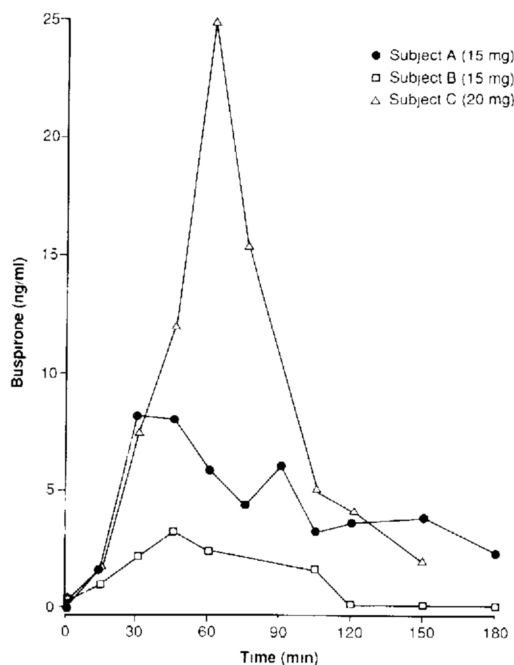

Fig 4. Plasma concentrations of buspirone in three male volunteer subjects following oral dosing with buspirone hydrochloride.

Some chromatographic mobility data for several psychotropic drugs together with the major metabolite of buspirone, is given in Table II.

Buspirone plasma profiles following oral administration of the drug to three normal volunteers are shown in Fig. 4.

DISCUSSION

Described here is a simple and highly selective HPLC assay which utilizes coulometric detection and an internal standard, gepirone, for monitoring solvent extraction recovery and coulometric detector variation. The detection limit (i.e. peak height equal to three times baseline noise) was 0.5 ng, allowing routine measurements of 0.5–1.0 ng/ml in a 2-ml plasma sample.

It has been established that the ratio between the analytical recovery of buspirone and that of the internal standard submitted to the same operations was constant over a wide range of concentrations. Furthermore, the detector response was linear for both compounds. The requirements for an internal standard assay procedure were therefore satisfied.

Prior conditioning of the cyanonitrile columns as described earlier in the text was found to be an important and essential pre-requisite for the avoidance of column blockage.

The major metabolite of buspirone, 1PP, which is said to have only 20% of the pharmacological activity of its parent [3] is not extracted to any significant degree by the technique described here. Measurement of 1PP would therefore require a separate or secondary treatment of the same plasma sample extracted for buspirone. The analytical recovery of buspirone itself is 50%, which is recognizably low; however, with the high sensitivity attained with the coulometric detector this is somewhat overcome. Extracted samples have shown little or no deterioration for up to ten days when stored in the dark at 4°C,

The technique described here may be further improved. Firstly the extraction process can be speeded up by the use of micro-columns such as the Bond-Elut type rather than using the three-stage extraction process described earlier. Some preliminary work on this has already been carried out; unfortunately extractions using cyanonitrile, C_2 and combinations of these columns have so far in general been found to give wide solvent fronts upon injection into the HPLC system. Further work along these lines is obviously worthwhile. Secondly the HPLC injection throughout can be speeded up with the use of an automatic injector.

Using this method plasma concentrations of buspirone found in volunteer subjects given oral doses of 15 and 20 mg were in the same range as those reported by others [4].

CONCLUSIONS

A novel technique using HPLC with coulometric detection has been described for the measurement of buspirone in plasma. It is quick, simple and relatively cheap to run compared to the only relevant alternative gas chromatographic–mass spectrometric method. It is therefore suitable for routine clinical analysis and research purposes. Suggestions for simplifying this procedure further have been described.

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