Journal of Chromatography, 575 (1992) 117–121
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6201

Simultaneous high-performance liquid chromatographic analysis of buspirone and its metabolite 1-(2-pyrimidinyl)-piperazine in plasma using electrochemical detection

P. Betto*, A. Meneguz, G. Ricciarello and S. Pichini

Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome (Italy)

(First received August 8th, 1991; revised manuscript received October 24th, 1991)

ABSTRACT

A selective and sensitive high-performance liquid chromatographic method with coulometric detection is described for the quantitation of buspirone and its active metabolite, 1-(2-pyrimidinyl)piperazine, in plasma samples of mice treated orally with buspirone (10 mg/kg body weight). The analytes are extracted with a carboxylic acid solid-phase extraction column before chromatography. A dual-electrode electrochemical detector is used. The limit of detection is 50 pg for buspirone and 35 pg for 1-(2-pyrimidinyl)piperazine.

INTRODUCTION

Buspirone, 8-[4-[(2-pyrimidinyl)piperazinyl]-butyl]-8-azaspiro[4.5]decane-7,9-dione, is a novel and effective anxiolytic agent [1–3]. It is equipotent with the benzodiazepines but it does not exhibit adverse side-effects such as sedation, muscle relaxation, motor impairment and anticonvulsion [4–7]. Buspirone is biotransformed in rats and humans, with major groups of metabolites [8,9]. Of the various metabolites, 1-(2-pyrimidinyl)piperazine (1-PP), appears in significant amounts in rat plasma and tends to concentrate in the brain, reaching high concentrations [10]. This observation, together with the fact that 1-PP, like buspirone, is active in the conflict test for anxiolytic activity [11], indicates that this metabolite may contribute significantly to the pharmacological effects of the parent drug. Pharmacokinetic and pharmacological studies require an assay tecnique with a very low limit of detection for the low concentrations of buspirone and its active metabolite, attained after oral dosing [12].

Bianchi and Caccia [13] reported a high-performance liquid chromatographic (HPLC) method for the measurement of buspirone and 1-PP in rat plasma and brain tissue with UV detection. This method did not present sufficient data to ensure a good sensitivity. Gammans *et al.* [14] reported a method that involved the use of selected-ion monitoring gas chromatography–mass spectrometry. This method is highly sensitive and specific, but it cannot be used for clinical routine because it requires a high degree of speciality and expensive equipment. Franklin [15] reported an HPLC method with coulometric detection, which involved sample purification by organic extraction of buspirone from plasma. The major metabolite 1-PP was not extracted and the analytical recovery was only 50%. Recently, Kristiansson [16] described another HPLC method with UV detection and solid-phase extraction for plasma samples. The metabolite 1-PP was not detected.

The method described in this paper can detect both buspirone and 1-PP. It combines simplicity, specificity and sensitivity, using an extraction of

0378-4347/92/$05.00

plasma samples with cation-exchange chromatography followed by reversed-phase HPLC and dual-electrode electrochemical detection. To obtain preliminary information on the significance of 1-PP formation in the pharmacological effect of this drug, plasma concentrations of the parent drug and its metabolite, after oral administration of buspirone (10 mg/kg body weight) to mice, were determined and compared.

EXPERIMENTAL

Reagents

Buspirone was a gift of Bristol Meyers (Uxbridge, UK). 1-PP was purchased from Aldrich-Chemie (Steinheim, Germany). All other chemicals were obtained from Carlo Erba (Milan, Italy).

Chromatographic system

The HPLC system consisted of a Series 4 liquid chromatograph (Perkin-Elmer, Norwalk, CT, USA) and a Rheodyne Model 7125 injector (Rheodyne, Berkeley, CA, USA) equipped with 100-μl loop. The column used was a Supelcosil LC-CN (3.3 cm × 4.6 mm I.D., 3 μm particle size; Supelco, Bellefonte, PA, USA) protected by a cyanonitrile guard column (5 μm particle size). A Model 5100 A Coulochem detector (ESA, Bedford, MA, USA) was equipped with a Model 5011 analytical cell containing two porous graphite working electrodes with associated palladium reference electrodes. The potentials were set at +0.55 V for the first electrode and +0.70 V for the second electrode. Chromatograms were analysed with a Chromatopac-R4A data processor (Shimadzu, Kyoto, Japan) monitoring both signals. The signals generated by the second electrode were used for the quantitation. The mobile phase was 20 m*M* potassium phosphate buffer (pH 7) and HPLC-grade acetonitrile (57:43, v/v) adjusted to pH 7.34 with 0.5 *M* potassium hydroxide. The flow-rate was set at 0.4 ml/min. During analysis the mobile phase was recycled.

Animal treatment

Male WS mice (Charles River, Como, Italy, body weight 20 g) were given oral doses (10 mg/kg) of buspirone hydrochloride and were killed by decapitation at various times (1–15 min). Each sample was a pool of four or five mice. Blood samples were collected in heparinized tubes and centrifuged, and the plasma was stored at −20°C until analysis.

Preparation of plasma samples

A 5-ml volume of distilled water was added to 500 μl of plasma. The mixture was well mixed and aplied to an SPE carboxylic acid column (3 ml) (Baker, Phillipsburg, NJ, USA). Before the use the column was conditioned with 5 ml of 2 *M* hydrochloric acid and 10 ml of distilled water. The compounds retained in the column were washed out with 5 ml of distilled water, and buspirone and 1-PP were eluted with 2 ml of 1 *M* formic acid. The eluate was evaporated to dryness under vacuum, and the residue was dissolved in 200 μl of mobile phase. An aliquot (20 μl) of the resulting solution was injected into the HPLC system.

RESULTS AND DISCUSSION

The specific and sensitive method reported here for the determination of buspirone and its metabolite 1-PP employs HPLC with the use of coulometric detection with two working electrodes. The two electrodes were used in the oxidative screen mode. For the determination of optimum potentials for the two electrodes, current–voltage curves for buspirone and 1-PP were investigated. Fig. 1 shows the hydrodynamic voltammograms of buspirone and 1-PP standard solutions. The first electrode was set at a potential near the low end of oxidation current–voltage curves for buspirone and its metabolite, to remove compounds with a lower oxidation potential than the analytes. Current responses for both compounds had a maximum at +0.75 V, but an operating oxidation potential of +0.70 V for the second electrode was chosen to avoid the background noise induced by higher potentials.

Representative chromatograms illustrating the resolution of a standard mixture and a plasma

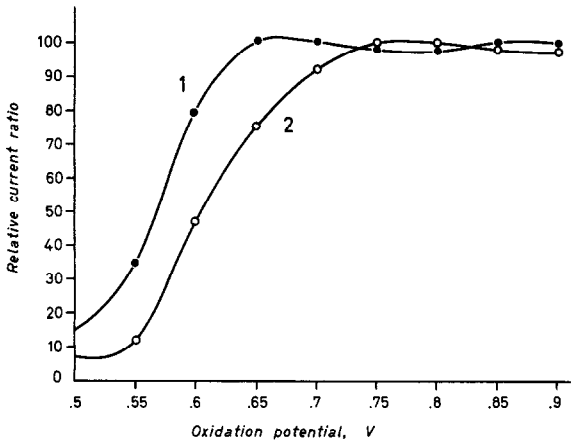

Fig. 1. Hydrodynamic voltammograms for standard substances obtained under the conditions described in the text. The response (current) at several potentials was recorded, and the ratios of the current at any given potential to that of the average response at plateau level were plotted as a function of oxidation potential. The injected amounts of 1-PP and buspirone were both 2.9 ng. Each point represents the mean of three determinations. Curves: 1 = 1-PP; 2 = buspirone.

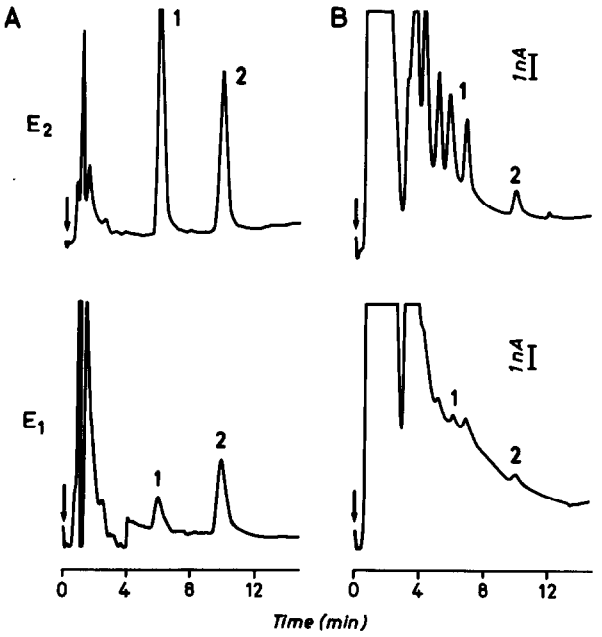

Fig. 2. (A) Chromatograms of standard 1-PP (2.9 ng) and buspirone hydrochloride (2.0 ng) (E_1 = +0.55 V, E_2 = +0.70 V). (B) Chromatograms from plasma of mice treated orally with buspirone (10 mg/kg), estimated to contain 1.87 ng of 1-PP and 0.3 ng of buspirone. Peaks: 1 = 1-PP; 2 = buspirone.

sample from treated mice are shown in Fig. 2A and B, respectively.

Recovery, reproducibility and detection limit

The analytical recovery was determined by comparing the peak heights of known amounts of buspirone and 1-PP standards (60, 120 and 480 ng/ml) added to a pool of mouse plasma carried through the complete assay procedure, with those resulting from the analysis of the same amount of standard stock solutions (Table I). The calibration curves for the two compounds were prepared and checked daily from spiked plasma; they were linear over the range 25–500 ng/ml (regression equations: $y = 10.263x - 0.091$ for 1-PP and $y = 4.506x - 0.294$ for buspirone). The correlation coefficients for both compounds were higher than 0.999. The reproducibility of the method, evaluated from multiple analyses of pooled plasma samples, was satisfactory. Table II gives the between- and within-assay re-

TABLE I

RECOVERY OF 1-PP AND BUSPIRONE FROM PLASMA SAMPLES

The values were calculated by analysing plasma samples spiked with standards at three different concentrations. Each value is the mean of five analyses ± standard deviation.

Concentration (ng/ml)	1-PP		Buspirone	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
60	99.7 ± 4.8	4.8	105.3 ± 2.6	2.5
120	98.3 ± 5.2	5.3	99.5 ± 6.1	6.1
480	103.2 ± 6.6	6.4	101.5 ± 4.9	4.8

TABLE II

PRECISION

Plasma samples were spiked with known amounts of 1-PP and buspirone.

Compound	Concentration (ng/ml)	R.S.D. (%)	
		Within-assay (n = 6)	Between-assay (n = 20)
1-PP	50	4.1	5.5
Buspirone	60	3.7	4.8

producibilities. The detection limit of the HPLC system was assessed by injecting decreasing amounts of the two compounds onto the column after extraction from spiked plasma. A 50-pg amount of buspirone can be reliably detected by system, and 35 pg of 1-PP could be measured. The signal-to-noise ratio was more than 5:1 in these experiments.

Peak identification

The peak of buspirone and 1-PP were identified by a combination of methods, initially on the basis of chromatographic retention times and simultaneous injection of a standard. Also the ratios of the second detector response (E_2, +0.70 V) *versus* the first detector response (E_1, +0.55 V) were calculated for the standard solutions and compared with those obtained from plasma samples. The peak-height ratios of reference compounds and those obtained from plasma samples are reported in Table III.

TABLE III

REVERSIBILITY RATIOS

The values represent the ratios of the detector response (oxidation current at +0.70 V to oxidation current at +0.55 V). The ratios were determined and averaged for standards and plasma samples over a ten-day period. Standards were analysed in amounts equivalent to 50–500 ng/ml for buspirone and 60–600 ng/ml for 1-PP.

Compound	Standard (n = 10)	Plasma (n = 10)
1-PP	7.76 ± 0.15	8.08 ± 0.26
Buspirone	2.49 ± 0.18	2.71 ± 0.21

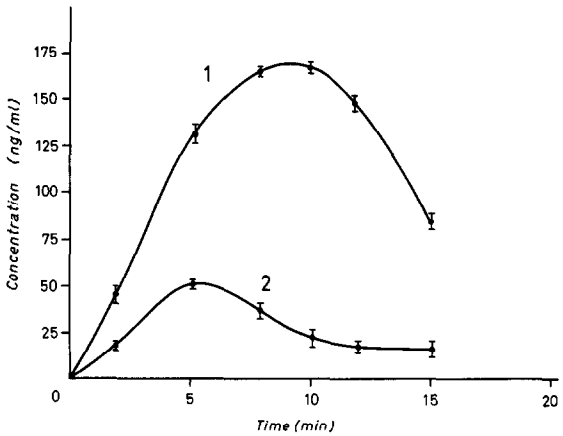

Fig. 3. Plasma concentration–time curves of 1-PP (1) and buspirone (2) after oral administration of buspirone to mice. The results are means of three determinations.

Clinical applications

Plasma analysis of mice treated orally with buspirone (10 mg/kg body weight) showed (Fig. 3) that, at this oral dose, concentration of 1-PP rose rapidly to a peak at a mean of 167.7 ng/ml within 10 min after administration, and the parent compound reached a mean peak concentration of 51.3 ng/ml within 5 min; thereafter the two concentrations decreased to the mean values of 83.6 and 16.1 ng/ml, respectively, after 15 min.

CONCLUSION

The combination of solid–liquid extraction using a cation-exchange column for sample preparation with HPLC and coulometric detection has proved to be a useful and reliable tool for determination of buspirone and its metabolite in plasma.

REFERENCES

1 A. S. Eison and D. L. Temple, *Am. J. Med.*, 80 (1986) 1.
2 K. L. Goa and A. Ward, *Drugs*, 32 (1986) 114.
3 D. P. Taylor, L. E. Becker, J. A. Crane, D. K. Hyslop and L. A. Riblet, *Drug Dev. Res.*, 4 (1984) 95.
4 H. L. Goldberg, *Pharmacotherapy*, 4 (1984) 315.
5 R. E. Newton, J. D. Marunycz, M. T. Alderdice and M. J. Napoliello, *Am. J. Med.*, 80 (3B) (1986) 17.
6 M. A. Schuckit, *Psychopathology*, 17 (1984) 61.
7 L. A. Riblet, D. P. Taylor, M. S. Eison and H. C. Stanton, *J. Clin. Psychiatry*, 43 (1982) 605.

8 S. Caccia, M. H. Fong, A. Mancinelli and S. Garattini, *J. Pharm. Pharmacol.*, 34 (1982) 605.
9 D. L. Temple, J. P. Yevich and J. S. New, *J. Clin. Psychiatry*, 43 (1982) 4.
10 S. Caccia, M. Muglia, A. Mancinelli and S. Garattini, *Xenobiotica*, 13 (1983) 147.
11 R. E. Gammans, R. F. Mayol and M. S. Eison, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 42 (1983) 377.
12 A. Diaz-Marot, E. Puigdellival, C. Salvatella, L. Comellas and M. Gassiot, *J. Chromatogr.*, 490 (1988) 477.
13 G. Bianchi and S. Caccia, *J. Chromatogr.*, 431 (1988) 477.
14 R. E. Gammans, E. H. Kerns and W. W. Bullen, *J. Chromatogr.*, 345 (1985) 285.
15 M. Franklin, *J. Chromatogr.*, 526 (1990) 590.
16 E. Kristiansson, *J. Chromatogr.*, 566 (1991) 250.