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High-performance liquid chromatographic method with coulometric detection for the determination of buspirone in human plasma by means of a column-switching technique

K. Ary[a,*], K. Róna[a], S. Ondi[b], B. Gachályi[a]

[a]Haynal Imre University of Health Sciences, Department of Medicine, Unit of Clinical Pharmacology, P.O. Box 112, H-1389 Budapest, Hungary

[b]ICN Alkaloida Co., P.O. Box 1, H-4440 Tiszavasvári, Hungary

Abstract

A reversed-phase high-performance liquid chromatographic method with electrochemical detection has been developed for the determination of buspirone from human plasma. The separation was carried out by using a Supelcosil ABZ+plus C_{18} reversed-phase column and 0.05 *M* potassium dihydrogenphosphate (pH 6.5)–acetonitrile (7:3, v/v) as the mobile phase. The compounds were detected by coulometry. Buspirone and the internal standard were extracted from the human plasma using Bond-Elut C_{18} solid-phase extraction cartridges. Following removal of the highly lipophilic plasma components we applied a column-switching technique which reduced the duration of HPLC measurement from 60 min to 15 min. The limit of quantitation was found to be 100 pg/ml plasma. © 1998 Elsevier Science B.V.

Keywords: Column-switching; Buspirone; Prazosin

1. Introduction

Buspirone, 8-{4-[4-(2-pyrimidinyl)-1-piperazinyl] butyl}-8-azaspiro[4,5]decane-7,9-dione, is a dibasic heterocyclic psychotrophic agent, relatively new anti-anxiety drug.

The aim of our study was to develop a high-performance liquid chromatographic (HPLC) method for the bioequivalence investigation of buspirone-containing drugs.

Because of the low plasma concentration levels of buspirone (the plasma concentration values after oral administration of 20 mg buspirone are about 0.9–2.4 ng/ml [1]) the pharmacokinetic studies require a sensitive analytical method for the quantification of buspirone with a low limit of detection.

The previous determinations of buspirone from biological fluids were described by the authors with different techniques.

Gammans et al. described a gas chromatographic–mass spectrometric (GC–MS) method [2], Caccia et al. [3] and Kristjánsson [4] reported a HPLC method with UV detection for the determination of buspirone. Kristjánsson used a solid-phase extraction for sample preparation. Franklin [5] and Betto et al. [6] applied electrochemical detection during the HPLC analysis, Odontiadis and Franklin measured buspirone and 1PP by coulometric detection [9].

In this paper, the authors describe a selective, sensitive and simple method for the analysis of buspirone, using solid-phase extraction followed by

*Corresponding author.

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reversed-phase HPLC with coulometric detection. The quantification limit was 100 pg/ml, the detection limit was 60 pg/ml.

2. Experimental

2.1. Materials

Buspirone (BUS) was provided by ICN Alkaloida (Tiszavasvári, Hungary), the prazosin as internal standard (I.S.) was obtained from Bristol-Myers (Germany). The structural formula of the compounds are described in Fig. 1.

Methanol and acetonitrile (LiChrosolv grade), ammonium hidroxide solution, (25%) and phosphoric acid (85%) were purchased from Merck (Darmstadt, Germany). Potassium dihydrogenphosphate, sodium hydroxide and hydrochloric acid solution (1 *M*) were from Reanal (Budapest, Hungary). Bond-Elut C_{18} cartridge of 1 ml was the product of Varian (Harbor City, CA, USA).

2.2. Chromatographic conditions

The HPLC system consisted of Shimadzu (Kyoto, Japan) equipments (LC-6A pump, SIL-6B autosampler, SCL-6B system controller and a FCV-2AH 2 position channel selection valve). For the detection a Coulochem II electrochemical detector (ESA, Bedford, MA, USA) was used, equipped with a 5011 model analytical cell. The potentials for the guard cell and the first and second electrode were 950, 600 and 900 mV, respectively.

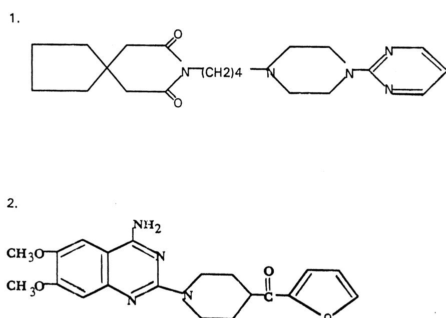

Fig. 1. Structural formula of buspirone (BUS, 1) and internal standard (I.S., 2).

The separation was accomplished at ambient temperature (air-conditioned room with temperature 22±2°C), on a Supelcosil ABZ+plus C_{18} (250×4.6 mm) analytical column equipped with a Supelguard ABZ+plus C_{18} (30×4.6 mm) guard column (Supelco, Bellefonte, PA, USA). The mobile phase consisted of 0.05 *M* potassium dihydrogenphosphate buffer (pH 6.5)–acetonitrile (70:30, v/v). The flow-rate was 1 ml/min. The eluent was filtered through a Supelco nylon (0.2 μm) membrane (Supelco) and degassed by ultrasonocation. For removal of the highly lipophilic plasma components, we applied a column-switching technique reducing the duration of HPLC measurement from 60 min to 15 min. The time program was: column-switching at 3 min, current range change at 10 and 15.9 min, stop time at 16 min.

Chromatograms were analysed with Knauer HPLC software (Ver. 1.1), (Knauer, Germany).

2.3. Solutions

Stock solutions (1 mg/ml) of both BUS and I.S. were prepared in methanol. When stored at −20°C, both stock solutions were stable for at least 8 weeks. Working standard solutions of 10 μg/ml, 1 μg/ml, 100 ng/ml and 10 ng/ml of BUS and 10 μg/ml and 500 ng/ml of I.S. were obtained by diluting the stock solution with 0.01 *M* potassium dihydrogenphosphate buffer (pH 2.5).

The working standard solutions were prepared fresh every week and stored at 4°C.

Blank human plasma was prepared from blood of drug-free volunteers obtained by venipuncture from the cubital vein. Disodium-EDTA was used as anticoagulant, at a concentration of 1 mg/ml of whole blood. The anticoagulant was dissolved in distilled water (100 mg/ml) and 0.1 ml of the solution was added to 10 ml of blood. Whole blood was centrifuged at 1500 *g* for 10 min and the resulting plasma was stored at −20°C until processing.

2.4. Sample processing

To 1 ml of plasma, 25 ng of the I.S. was added, using a 500 ng/ml concentration working solution (50 μl with a 100 μl Hamilton syringe), and then

homogenised with a vortex mixer for 5 s. 1 ml of 0.05 *M* potassium dihydrogen phosphate (pH=7.2) solution was added to the mixture, and vortexed for 5 s. BUS was extracted from the plasma with solid-phase extraction (SPE), using vacuum manifold (Supelco, Bellefonte, PA, USA). 1-ml volume Bond-Elut C_{18} (100 ng) (Varian, USA) cartridges were activated with 2×1 ml of methanol and 2×1 ml of 0.05 *M* potassium dihydrogen phosphate (pH=7.2) solution. Homogenised plasma samples were then applied onto the cartridge. The column was not allowed to dry before sample application. However prior to washing the cartridge, it was dried in an air stream. The column was consecutively washed with 2×1 ml of 0.05 *M*, pH=7.2 buffer, and 0.5 ml of 50% methanol. The column was then dried in an air stream, and allowed to stand for 15 min at room temperature. The sample was then eluted with 1 ml of acetonitrile–ammonium hydroxide (25%) 99:1 solution. The eluates were evaporated to dryness in a stream of nitrogen, and dissolved in 200 μl of the mobile phase.

2.5. Method validation

2.5.1. Quality control (QC) samples

For the method validation, QC samples were prepared from pooled drug-free human plasma, in advance 0.2, 5 and 25 ng/ml BUS levels. QC samples were stored deep-frozen at −20°C. The I.S. was added to each QC samples just prior to sample processing.

2.5.2. System suitability test

For examination of system suitability, five injections were made from the same biological sample containing 5 ng/ml BUS and 25 ng/ml I.S.

2.5.3. Summary of calibration curve parameters

To 1 ml of drug-free pooled human plasma, 0.1, 0.2, 1, 5 and 25 ng of BUS were added, respectively. To each sample, 25 ng I.S. was also added. The extraction and liquid chromatography were carried out as described above. At each concentration level five replicate determinations were made.

Calibration curves were constructed from the peak area ratio of BUS and I.S. calculated by the $1/y^2$ weighting method using the KALIB (Egis, Hungary) computer program. The *F* test for linearity and linear regression analysis were chosen for testing linearity.

2.5.4. Within-day precision of the method

The within-day precision and accuracy of the method were determined using quality control (QC) samples at three different concentration levels (0.2, 5, 25 ng/ml). Five replicate determinations were made at each concentration level.

2.5.5. Between-day precision of the method

The between-day precision and accuracy of the method were determined by the analysis of QC samples at three different concentration levels (0.2, 5, 25 ng/ml) on five different days.

2.5.6. Determination of absolute recovery

1-ml plasma samples containing 0.2, 5 and 25 ng of BUS but no I.S. were processed by the SPE. 25 ng of the I.S. was added to the sample after extraction when the dry residue was dissolved in the mobile phase. Three replicate determinations were made at each concentration. The peak area ratios obtained with the extracted samples were compared to those of a methanolic solution containing the same concentration of BUS and the I.S.

2.5.7. Stability test

The stability of BUS in plasma samples of three different concentrations (0.2, 5 and 25 ng BUS/ml of plasma), stored at −20°C was examined after two and four weeks of storage. The I.S. was added immediately prior to extraction.

At all three concentration levels and for each storage period (0, two and four weeks) three replicate determinations were made. The stability of QC plasma samples during three freeze/thaw cycles were determined. Triplicate samples at concentrations of 0.2, 5 and 5 ng/ml QC samples were frozen for 24 h at −20°C. The samples were thawed at room temperature for 2 h and subsequently returned to the freezer for 24 h. The samples were thawed, processed and injected. The mean measured concentrations of the stability samples were compared to the nominal concentrations.

The stability of the extracted and dissolved samples in the autosampler were measured by comparing the ratios of the original standard curve to the ratios

of the standard curve injected 0, 6, 12, 24, 36, 48, 72 h later.

3. Results and discussion

A liquid chromatographic method with coulometric detection has been developed for the quantitative determination of buspirone in human plasma.

In the present method the mobile phase, together with Supelcosil ABZ+plus C_{18} stationary phase ensured symmetrical, close to optimum, peak shape both for the study compound and the I.S.

Fig. 2 demonstrates the chromatogram of a typical blank plasma extract obtained from the described SPE procedure. Fig. 3 shows a typical chromatogram of a volunteers plasma sample after oral administration of 30 mg BUS. Endogenous compounds interfering with retention times of BUS or that of the I.S. cannot be seen in the chromatogram. This was evidenced by the lack of interfering endogenous peaks in the plasma samples for BUS and I.S.

Based on the system suitability test, the mean retention times and S.D. for BUS were 11.24 (0.21) min, and for the I.S. 6.14 (0.018) min. On the basis of five replicate determinations, the reproducibility (R.S.D.) of the retention time was 0.14% for BUS and 0.19% for I.S., whereas the reproducibility of the peak area values was 1.35% and 1.73% for BUS and I.S., respectively.

Tables 1–3 contain the detailed results of method validation.

The precision of the method was always within the ±20% R.S.D level according to international accepted criteria [7,8] for biological samples. Validation data indicated that the limit of quantitation (LOQ) was 100 pg/ml and the limit of detection (LOD) was 60 pg /ml.

The calibration curve showed good linearity over the range of the standard curve (Table 1). In the range of 0.1–25 ng/ml the equation of curve was: $y=0.0641+0.1788x$ (y represents the ratio of the area under the chromatographic peak of BUS and that of the I.S., and x represents the concentration of BUS).

As indicated by the result of within-day and between-day precision and accuracy examinations with QC samples, the R.S.D values always remained below ±15% and the accuracy of the determination

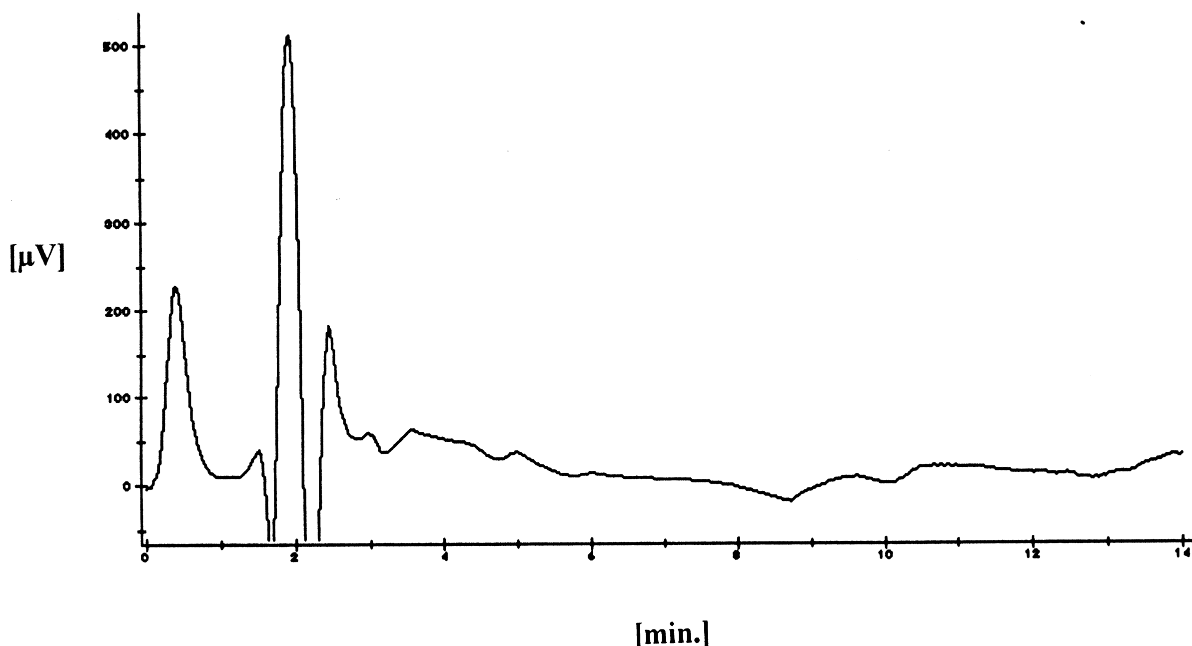

Fig. 2. Chromatogram of a typical blank plasma extract after the solid-phase extraction.

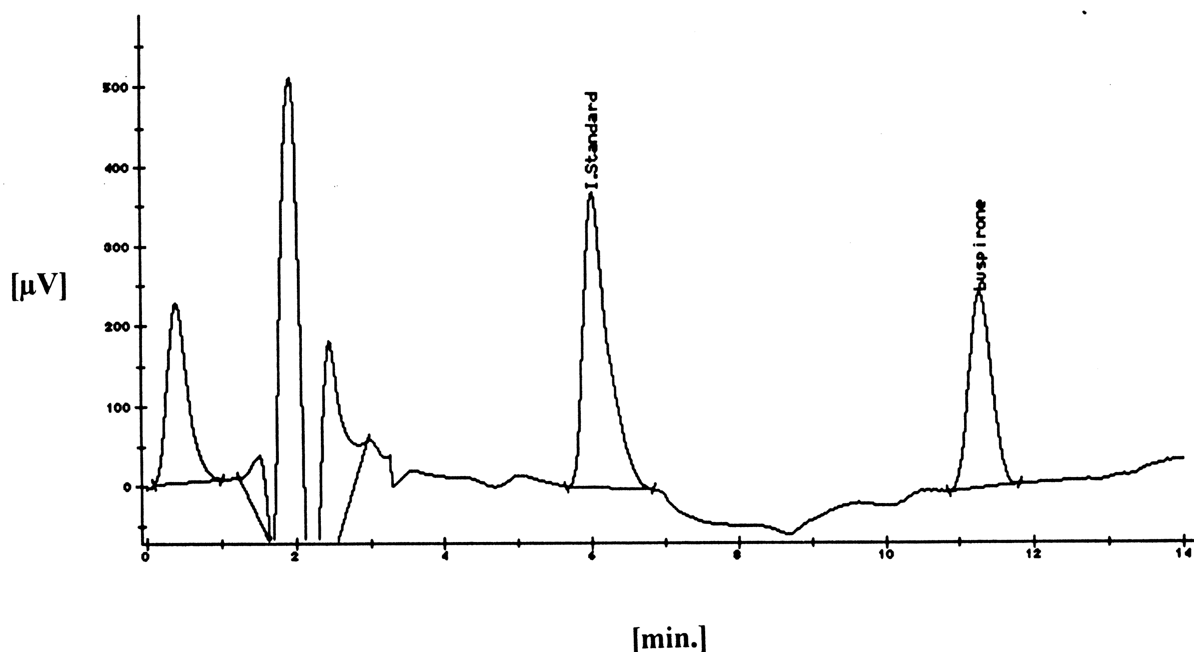

Fig. 3. Chromatogram of a human plasma sample after oral administration of 30 mg of BUS (0.7 ng BUS/ml of plasma).

Table 1
Summary of the calibration curve of buspirone

Nominal conc. (ng/ml)	BUS/I.S. peak area ratio means±S.D.	R.S.D. (%)	Measured conc. (ng/ml) means±S.D.	Accuracy (%)	n
0.1	0.081±0.011	13.4	0.093±0.061	92.8	5
0.2	0.104±0.009	8.6	0.221±0.049	110.4	5
1	0.264±0.005	1.9	1.115±0.029	111.5	5
5	1.067±0.046	4.3	5.608±0.256	112.2	5
25	4.057±0.164	4.0	22.328±0.916	89.3	5

Equation of calibration curve: $y=0.0641+0.1788x$; $R=0.998$.

Table 2
Within-day and between-day precision and accuracy of the method

Nominal conc. (ng/ml)	Measured conc. (ng/ml) mean±S.D.	R.S.D. (%)	Accuracy (%)	n
Within-day precision and accuracy of the method				
0.2	0.218±0.012	5.7	109.2	5
5	5.350±0.232	4.3	107.0	5
25	21.862±1.028	4.7	87.1	5
Between-day precision and accuracy of the method				
0.2	0.227±0.029	13.1	113.3	5
5	5.501±0.388	7.1	110.0	5
25	23.542±1.947	8.3	94.2	5

Table 3
Stability data for buspirone in human plasma following 3 freeze/thaw cycles (−20°C for 24 h and room temperature for 2 h three times consecutively)

Nominal conc. (ng/ml)	Measured concentrations (ng/ml) Mean±S.D.			*n*
	Cycle I	Cycle II	Cycle III	
0.2	0.231±0.034	0.229±0.041	0.235±0.043	3
5	5.435±0.354	5.445±0.422	5.607±0.532	3
25	23.913±1.314	24.214±0.982	23.832±1.497	3

did not deviate from 100% by more than ±15% (Table 2).

The concentration dependence of recovery was negligible, the average extraction efficiency was 85.1%.

Stability tests proved that BUS and the I.S. remained stable for at least four weeks in stock solution. BUS did not show significant decomposition in human plasma during two and four weeks of storage at −20°C. The accuracy of BUS in the plasma after storage was between 94.5 and 111.7%.

The data in Table 3 indicate that freezed/thawed samples remained stable under the applied conditions.

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