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Short Communication

Sensitive determination of buspirone in serum by solid-phase extraction and two-dimensional high-performance liquid chromatography

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ABSTRACT

A selective and sensitive determination of buspirone in serum by high-performance liquid chromatography is described. The procedure is based on separation on a C_{18} column. A solid-phase extraction procedure is used for sample clean-up. The retention on the first column is based on the hydrophobic interaction of buspirone with the stationary phase, and the retention on the second column is based on ionic interactions due to the presence of sodium lauryl sulphate in the mobile phase as well as hydrophobic interaction. This allows for good separation of buspirone from impurities and consequently allows lower detection limits than previously reported for liquid chromatographic methods. Detection by ultraviolet absorbance gives a detection limit of 0.2 ng/ml.

INTRODUCTION

Buspirone, 8-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl}-8-azaspiro[4,5]decane-7,9-dione, is a recently introduced anti-anxiety drug, whose properties have been extensively reviewed by Goa and Ward [1]. Its molecular structure is given in Fig. 1. Buspirone serum levels in patients receiving the drug on a regular basis are low, usually less than 5 ng/ml [1,2]. Measurements of the low levels of buspirone in serum by high-performance liquid chromatography (HPLC) has proved to be a difficult task. Gammans *et al.* [2] stated that an unpublished HPLC method (UV detection) has a detection limit of 10 ng/ml. Bianchi and Caccia [3] observed

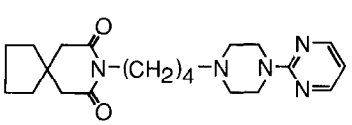

Fig. 1. Structure of buspirone.

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detection limit near 10 ng/ml when measuring plasma and brain samples from rats. Diaz-Marot *et al.* [4] reported a detection limit of 5 ng/ml in dog plasma. Gammans *et al.* [2] chose to develop a capillary gas chromatographic–mass spectrometric (GC–MS) method to obtain detection below 1 ng/ml, and found that this methodology allowed for detection limits at 0.05 or 0.2 ng/ml depending on the sample preparation procedure (liquid–liquid extraction). Later, Sciacca *et al.* [5] developed a solid-phase extraction (SPE) procedure in order to purify the samples before applying capillary GC–MS for quantitation. The capillary GC–MS technique is excellent with respect to selectivity and sensitivity, but is far more complex and expensive than HPLC methods. On the other hand, Mayol *et al.* [6] developed a radioimmunoassay (RIA) method for buspirone which furnished a detection limit of 0.1 ng/ml. Unfortunately, however, RIA methods are dependent on the supply of suitable antisera and a radioactive tracer. This paper describes the development of an HPLC method for buspirone with UV detection that gives a detection limit at 0.2 ng/ml.

EXPERIMENTAL

Materials

Analytical-grade potassium dihydrogenphosphate, sodium hydroxide, orthophosphoric acid and synthetic grade triethylamine were obtained from Merck (Darmstadt, Germany). Sodium lauryl sulphate was obtained from Sigma (Dorset, U.K.). HPLC-grade methanol and acetonitrile were obtained from Rathburn (Walkerburn, U.K.). C_{18} SPE columns (1 ml) were purchased from Supelco (Bellefonte, PA, U.S.A.). Distilled water was used throughout. Plasma was obtained from the Icelandic Bloodbank (Reykjavík, Iceland).

Buffers and mixtures

Buffer I was potassium dihydrogenphosphate (5 m*M*) with 0.1% (v/v) triethylamine, adjusted to pH 2.5 with orthophosphoric acid.

Buffer II was potassium dihydrogenphosphate (5 m*M*) with 0.2% (v/v) triethylamine, adjusted to pH 2.5 with orthophosphoric acid.

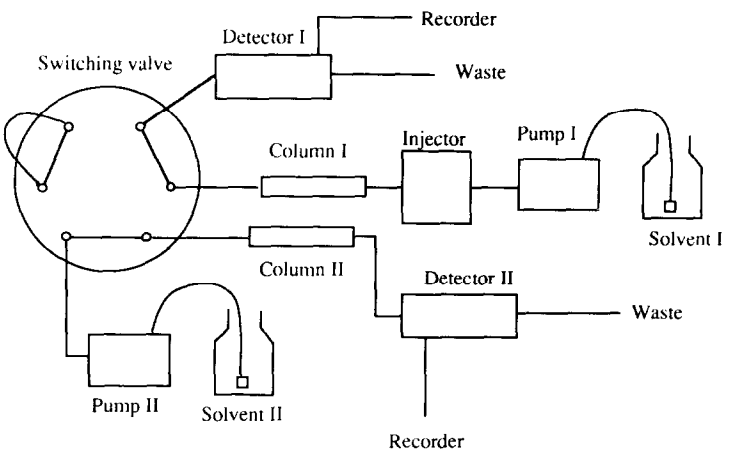
Buffer III was potassium dihydrogenphosphate (50 m*M*), adjusted to pH 7.2 with 2 *M* NaOH.

Standard solutions

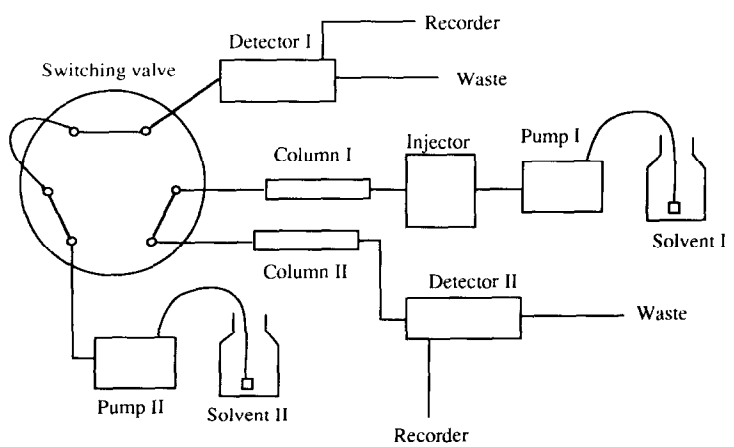
A stock solution of buspirone (1.00 mg/ml) was prepared in ethanol, and 10.0 μg/ml and 100 ng/ml solutions were made by dilution in water. Then 0.50, 1.00, 2.00, 3.00, 4.00 and 5.00 ng/ml working standards were prepared in plasma from the 100 ng/ml solution and stored at −30°C. A standard curve was elaborated daily from the working standards and blank plasma. Control samples, 0.75 and 3.75 ng/ml, were also made in plasma and stored at −30°C until measured. Control samples of each concentration were measured daily.

Chromatography

The HPLC instrument (Shimadzu, Kyoto, Japan) consisted of two LC-6A pumps, an SIL-6A autoinjector, SPD-6AV and SPD-6A variable-wavelength detectors (detection at 235 nm), a C-R4A data station and a FCV-2AH column-switching device. The columns were two 15 cm × 4.6 mm I.D. Spherisorb ODS2 columns packed with 5-μm particles from Phase Separations (Deeside, U.K.). The configuration of the HPLC unit is shown in Fig. 2. The mobile phase for column I was acetonitrile–buffer I (45:55, v/v) and that for column II aceto-



Position 0



Position 1

Fig. 2. Schematic diagram of the HPLC system. When the valve is switched from position 0 to position 1 the mobile phase from column I is transferred to column II. This allows for two-dimensional chromatography of the fraction transferred to column II.

nitrile–buffer II (55:45, v/v) with 5.0 m*M* sodium lauryl sulphate. The flow-rate was 1.2 ml/min for both pumps, and injections were performed at 20-min intervals. Peak heights were used as a measure of the buspirone concentration.

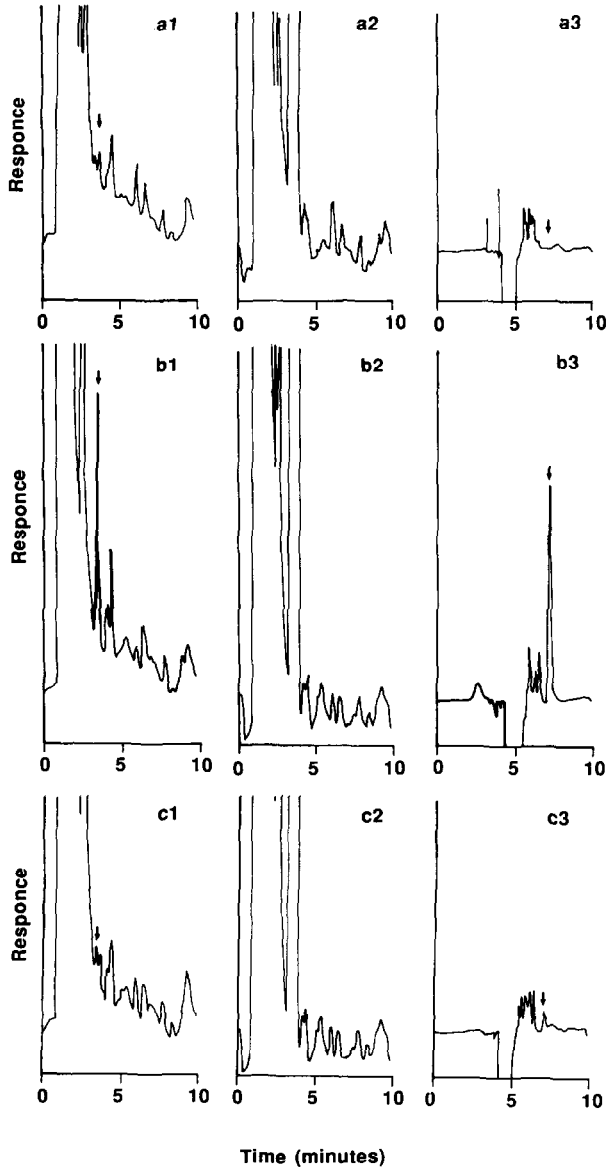


Fig. 3. (a1) Chromatogram of blank plasma from column I without column switching; (a2) chromatogram of blank plasma from column I with column switching; (a3) corresponding chromatogram from column II. (b1)–(b3) Similar chromatograms of plasma containing 5.00 ng/ml buspirone. (c1)–(c3) Similar chromatograms of a serum sample from a volunteer containing 0.55 ng/ml. The arrows indicate the retention time of buspirone.

Column switching

The fraction of the mobile phase from column I that contained buspirone was transferred to column II for further separation and quantitation. The retention time of buspirone from column I was *ca.* 3.6 min. A 0.7-min fraction was transferred by an automatic valve to column II. The timing of switching the valve from position 0 to position 1 (Fig. 2) was set to 0.3 min prior to the retention time of buspirone on column I, and the timing of the switch back to position 0 was set to 0.4 min after the retention time of buspirone on column I. The retention time was measured carefully daily to ensure that the correct fraction from column I was transferred to column II.

Sample treatment

The SPE columns were conditioned by washing with two 1-ml volumes of methanol and two 1-ml volumes of buffer III. Standards or samples (2.00 ml) were applied to the columns, and 2–5 ml of air were flushed through. The columns were washed with two 1.0-ml volume of buffer III and two 1.0-ml and one 0.5-ml volumes of methanol–water (1:1), and 2–5 ml of air were flushed through. Buspirone was eluted from the columns with 1.00 ml of acetonitrile–triethylamine (99:1, v/v). The solvent was evaporated at 37°C in a stream of air. The residue was dissolved in 0.100 ml of mobile phase I. The solution was transferred to 1.8-ml conical centrifuge vials and centrifuged for 10 min at 5000 *g*. Portions of 80 μl were transferred to 200-μl injection vials, and 70 μl were injected onto column I.

RESULTS

Typical chromatograms are shown in Fig. 3. Fig. 3.a1, a2 and a3 are chromatograms of blank plasma, Fig. 3.b1, b2 and b3 of plasma containing 5.00 ng/ml

TABLE I

ACCURACY AND PRECISION OF THE HPLC ASSAY FOR BUSPIRONE

Actual concentration (ng/ml)	Measured concentration (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	*n*
Within-day			
0.75	0.82 ± 0.06	8.1	8
3.75	3.68 ± 0.18	5.0	7
Between-day			
0.75	0.78 ± 0.16	21.4	16
0.75	0.83 ± 0.11	12.9	16
3.75	3.69 ± 0.43	11.8	16
3.75	3.88 ± 0.41	10.6	16

buspirone, and Fig. 3.c1, c2 and c3 of a serum sample from a volunteer containing 0.55 ng/ml.

Good linearity was observed for the response in the range from 0.5 to 50 ng/ml ($y = 143.6x - 15.9$). Blank samples did not give a response for buspirone, and the intercept of the linear regression line was not significantly different from zero. The standard curves were therefore forced through zero response at zero concentration. The detection limit (signal-to-noise ratio 3) was 0.2 ng/ml. The recovery of buspirone from spiked samples was close to 66% (S.D. = 2%). The recovery did not show dependence on concentration in the range 0.5–50 ng/ml. The accuracy and precision within and between days was estimated by repeatedly measuring control samples containing 0.75 and 3.75 ng/ml. The results are given in Table I. A concentration *versus* time profile in sixteen subjects after intake of four 10-mg buspirone tablets in one dose is shown in Fig. 4.

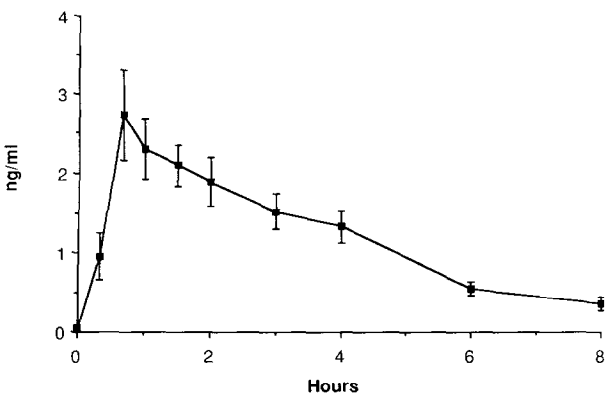


Fig. 4. Average (± S.E.M.) buspirone concentrations as a function of time from sixteen volunteers after intake of a single 40-mg dose of buspirone.

DISCUSSION

The serum or plasma concentration of buspirone following intake of therapeutic doses requires extremely sensitive methods for its measurement in order to obtain the required detection limits of *ca.* 0.1 to 0.3 ng/ml. This was attained by a twenty-fold reduction of the sample volume during the solid-phase purification of the serum (2 ml reduced to 0.1 ml), and further purification of the buspirone peak by column-switching so that the only limiting factor for detection of buspirone was the electronic noise on the baseline (Fig. 3). Following application of the samples to the SPE columns a thorough washing was necessary to eliminate interfering peaks in the HPLC traces. The resulting 66% recovery may afffect the accuracy if the samples differ substantially from the composition of the standards, therefore all working standards were made in human plasma. The column

switching was done in order to eliminate interfering peaks, often originating from previous injections. The relatively long time interval between injections (20 min) was also due to interference from late-eluting peaks.

The separation on the first column was mainly based on the hydrophobic interaction of buspirone with the stationary phase. Buspirone is an aliphatic amine that is charged in the mobile phase (the mobile phase buffer was pH 2.5). Electrostatic interaction with free silanol groups on the surface of the silica backbone of the stationary phase was therefore highly likely, and was in fact observed, but the addition of triethylamine reduced the importance of this retention mechanism substantially, most probably by competitive interaction of triethylamine with the silanol groups. The retention of buspirone on column II was based on both ionic and hydrophobic interaction due to the addition of the ion-pairing agent sodium lauryl sulphate to the mobile phase. This allows for separation of compounds from buspirone that coelute with the analyte from the first column.

As a result of the manipulation of the serum samples, this method has a detection limit of 0.2 ng/ml, which is comparable with limits observed in GC–MS [2] and RIA [6] methods, and can be used to monitor therapeutic levels of buspirone. This is two orders of magnitude lower than previously reported for HPLC methods for buspirone. Table I also shows that the accuracy and precision of this assay are acceptable and comparable with what was obtained with GC–MS methods [2].

To demonstrate the applicability of this assay for measurements in human serum, samples were collected from sixteen individuals following intake of 40 mg of buspirone (BuSpar tablets) in one dose. Fig. 4 shows the average serum concentration–time profile for the sixteen individuals. The concentration levels and pharmacokinetic parameters for these individuals are alsmost identical with those described by Gammans *et al.* [7] for a single 40-mg dose of buspirone as measured by GC–MS.

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