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Journal of Pharmaceutical and Biomedical Analysis
35 (2004) 41–50
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Development and validation of a high-performance liquid chromatographic method for the determination of buspirone in pharmaceutical preparations

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Received 6 August 2003; received in revised form 15 December 2003; accepted 18 December 2003

Abstract

A stability indicating, reversed-phase high-performance liquid chromatographic method was developed and validated for the determination of buspirone (Bsp) in pharmaceutical dosage forms. The use of a semi-micro XTerra MS C18 (150 mm × 3.0 mm i.d., 5 μm particle size) analytical column, results in substantial reduction in solvent consumption and increased sensitivity. The mobile phase consisted of a mixture of 0.010 M ammonium acetate (pH 4.0) and methanol (55:45, v/v), pumped at a flow rate 0.30 ml min^{-1}. The UV detector was operated at 245 nm. The retention times for lidocaine (Ldc), which was used as internal standard, and buspirone were 4.57 and 7.72 min, respectively. The calibration graph was ranged from 1.00 to 5.00 μg ml^{-1}, while detection and quantitation limits were found to be 0.22 and 0.67 μg ml^{-1}, respectively. The intra- and inter-day relative standard deviation (% R.S.D.) values were less than 1.94%, while the relative percentage error (% E_r) was less than 4.0% ($n = 5$). The method was applied to the quality control of commercial tablets and content uniformity test and proved to be suitable for rapid and reliable quality control.

Keywords: Buspirone; Liquid chromatography; Semi-micro columns; Pharmaceutical dosage forms; Stability indicating

1. Introduction

Buspirone hydrochloride, 8-azaspiro[4,5]decane-7, 9-dione,8-{4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl} monohydrochloride, is a psychotropic drug with anxiolytic properties that belongs chemically to the class of compounds known as azaspirodecanediones [1] and it is widely used for the treatment of anxiety and depression [2]. Buspirone (Bsp) is a non-benzodiazepine drug that it was found to be equally effective as diazepam for the treatment of anxiety [3]. However, it has a pattern of pharmacological properties that is distinct from that of benzodiazepines, including an inability to influence the binding of benzodiazepines or GABA and a lack of anticonvulsant activity and muscle relaxant effects [4]. In the central nervous system it is believed to affect the 5-HT_{1A} subtype of the serotonin receptors [5,6]. The drug also lacks the

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doi:10.1016/j.jpba.2003.12.014

prominent sedative effect that is associated with more typical anxiolytics; there also appears to be little risk of producing tolerance or dependence.

Several methods have been reported in literature for the determination of buspirone in biological fluids. Thus, high-performance liquid chromatography coupled with UV [7] or electrochemical detection [8–10] has been applied to determine buspirone and metabolites. Furthermore, a gas–liquid–electron-capture detection chromatographic (GLC-ECD) procedure [11] and a gas chromatographic mass spectrometric (GC-MS) procedure [12] have been applied to the quantitation of buspirone and its main metabolite in human plasma. The pharmacokinetic analysis of the drug has been carried out by means of brain microdialysis coupled to high-performance liquid chromatography with electrochemical detection in rat plasma [13]. The metabolite structure profile of buspirone has also been assessed by liquid chromatography mass spectrometry [14,15].

Colorimetric methods have been reported for the determination of buspirone in tablets, with laborious derivatization procedures: by means of a reaction with 2,4-dinitrophenyl hydrazine in hydrochloric acid, and a reaction with Folin Cio–Calteu reagent in the presence of sodium carbonate [16]. Thin layer chromatography has also been applied to the detection of buspirone and its degradation products in tablet formulation under forced degradation conditions (85 °C, 76% relative humidity) [17]. A gradient high-performance liquid chromatographic procedure with photo-diode array detection has been evaluated for the determination of buspirone impurities in bulk drug [18]. Moreover, four impurities of buspirone were fractionated using liquid chromatography electrospray ionisation mass spectrometry [19]. Official reports for the determination of buspirone hydrochloride in bulk material and tablets have been published in the United States Pharmacopeia [20,21].

The focus of the present study was to develop and validate a rapid, stability indicating, and environmentally friendly high-performance liquid chromatographic method for the quality control of buspirone in pharmaceutical preparations. A semi-micro (3.0 mm i.d.), XTerra MS C18, analytical column was chosen for the experiments; lidocaine (Ldc) hydrochloride was used as the internal standard. A very practical aspect of the use of semi-micro columns is the reduction of solvent usage, as they operate at lower flow rates (0.10–0.50 ml min^{-1}) along with the much reduced peak volumes which are related to their low volumetric flow rates. In consequence, for an equal injected mass, the solute concentration is greater for semi-micro columns due to decreased volumetric dilution, which results in increased sensitivity [22]. The proposed method is applicable as well for routine analysis and content uniformity test of buspirone in tablets and complies well with the validation requirements in the pharmaceutical industry.

2. Experimental

2.1. Equipment

The chromatographic apparatus consisted of a Spectra Series Model P100 isocratic pump, SP thermo separation products and a Rheodyne model 7725i injector with a 5 μl loop which were coupled to a Waters Model 486 UV-Vis detector operated at 245 nm. Data acquisition was performed using a Hewlett-Packard Model HP-3394A integrator. All pH measurements were performed on a pH meter Metrohm, model 654 Herisau, while dilutions were accomplished using Hamilton precision pipettes.

2.2. Materials and reagents

All solvents used were of analytical-reagent grade and were purchased from E. Merck, Darmstadt, Germany. Ammonium acetate (pro analysis) and glacial acetic acid (pro analysis) were also purchased from E. Merck, Darmstadt, Germany. Water was deionised and further purified by means of a Milli-Q Plus water purification system, Millipore Ltd., Molsheim, France. Buspirone hydrochloride of pharmaceutical purity grade was kindly provided by Bristol–Myers Squibb, Athens, Greece, while lidocaine hydrochloride of pharmaceutical purity grade was kindly provided by Uni-Pharma Hellas A.E., Athens, Greece. All substances were used without any further purification. Buspirone tablets are products of Bristol–Myers Squibb Co., USA; each tablet was labelled to contain 10.0 mg of buspirone hydrochloride. The excipients present in tablets are: colloidal silicon dioxide, lactose,

magnesium stearate, microcrystalline cellulose, and sodium starch glycolate.

2.3. Chromatographic conditions and measurement procedure

Chromatographic separation was performed on a reversed-phase XTerra MS C18 column (150 mm × 3.0 mm i.d., 5 μm particle size) Waters Corporation (Milford, Massachusetts, USA). The mobile phase consisted of 0.010 M ammonium acetate, adjusted to pH 4.0 with glacial acetic acid, and methanol (55:45, v/v). The mobile phase was filtered through a 0.22 μm nylon-membrane filter (GelmanSciences Ltd.) and degassed under vacuum prior to use. A flow rate of 0.30 ml min^{-1} with a column inlet pressure of 1250 psi was used in order to separate buspirone and the internal standard lidocaine. Peak areas were measured and HPLC analysis was conducted at ambient temperature.

2.4. Stock and working standard solutions

Stock standard solutions of buspirone, 1.0 mg ml^{-1}; lidocaine, 1.0 mg ml^{-1} were prepared by dissolving appropriate amounts of the compounds in methanol. These solutions were stored in the dark under refrigeration at 4 °C and were found to be stable for several weeks.

A series of working standard solutions of Bsp were prepared by the appropriate dilution of the above mentioned stock standard solution in the mobile phase to reach concentration ranges of 1.00–5.00 μg ml^{-1} for Bsp. In each sample 25.00 μg ml^{-1} of the internal standard Ldc was added. Standard solutions were found to be stable during the analysis time.

2.5. Assay of pharmaceutical preparations

Twenty tablets were weighed and finely pulverised. An appropriate portion of this powder, equivalent to 10.0 mg of Bsp was placed in a 50 ml volumetric flask with 40 ml of methanol. The solution was sonicated for 45 min and diluted to volume with methanol. A portion of this solution was centrifuged at 4000 rpm (2890 g) for 15 min. A 150 μl aliquot was transferred to a 10 ml volumetric flask and diluted to 10 ml of a mobile phase solution containing 25.00 μg ml^{-1} of the internal standard Ldc; 5 μl sample was injected into the HPLC system. Peak area ratios of buspirone to that of the internal standard were then measured for the determinations. The same procedure was followed for the content uniformity test, using one tablet per sample.

2.6. Calibration procedure

Calibration curve of Bsp was conducted using the series of working standard solutions described previously. The concentration range of Bsp was 1.00–5.00 μg ml^{-1}. All solutions were analysed immediately after their preparation. Triplicate 5 μl injections were made of each solution and the peak area ratio of Bsp to that of the internal standard was plotted against the corresponding concentration to obtain the calibration graph.

The over-all precision and accuracy of the HPLC assay was evaluated by analysing five series of standard solutions of Bsp, at three concentration levels (1.00, 3.00, and 5.00 μg ml^{-1}). The percentage recovery along with the relative percentage error (% E_r) were determined so as to assess the accuracy, while precision was expressed by the relative standard deviation (% R.S.D.).

In order to determine the effect of the excipients used in the formulation of tablets on the determination of Bsp, the standard addition method was used. Therefore, five equal amounts of powdered tablets equivalent to 10.0 mg of Bsp were spiked with different amounts of the reference standard of Bsp. The samples were analysed as mentioned in the assay procedure, while in each sample, 25.00 μg ml^{-1} of the internal standard Ldc was added. Peak area ratios of Bsp to that of the internal standard were measured for the quantitative determination of buspirone.

The proposed method was further compared with an HPLC procedure proposed by the United States Pharmacopeia for the quantitative determination of buspirone [20]. The USP chromatographic conditions were as follows: the mobile phase consisted of 0.010 M potassium dihydrogen phosphate adjusted to pH 7.5 with 10% sodium hydroxide (w/v) and acetonitrile (60:40, v/v), the column was ODS C18 (250 mm × 4.6 mm i.d., 5 μm particle size); the flow rate, 1.6 ml min^{-1}; the UV absorbance was measured at 254 nm.

To assess the proposed HPLC method as a stability indicator for Bsp, chromatograms were recorded under various stress conditions where degradation was stimulated by heat, acidic, or basic environment.

3. Results and discussion

3.1. Chromatographic characteristics

The chromatographic separations were performed on a semi-micro XTerra MS C18 column with a 3.0 mm internal diameter, offering the advantage of lower solvent consumption and increased sensitivity [23]. The effect of composition and pH of the mobile phase on the retention time of Bsp and the internal standard, Ldc, were investigated.

Results of the effect of methanol in the mobile phase are presented in Fig. 1. An increase in the percentage of methanol decreases the retention of both of the compounds. When methanol concentration increased to more than 55% Bsp and Ldc peaks eluted pretty closely. At methanol concentration lower than 35%, the compounds are well separated, but the retention time of Bsp peak is increased dramatically. The optimum methanol concentration was found to be 45%.

The effect of pH in the chromatographic elution of both compounds was also investigated. Thus, the pH values of the aqueous component of the mobile phase was changed from 2.8 to 5.0 using ammonium acetate (0.010 M) and glacial acetic acid for the adjustment of the pH value. For all experimental pH values, the internal standard, Ldc, was eluted first followed by the elution of Bsp (Fig. 2). The effect of pH in the

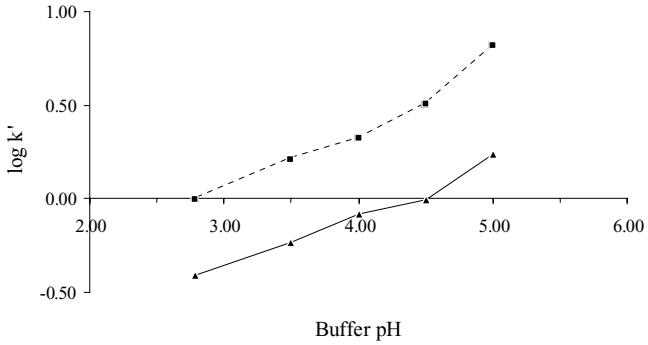

Fig. 2. Effect of mobile phase pH on the capacity factor (k') of buspirone hydrochloride (broken line (■)) and lidocaine hydrochloride (solid line (▼)). Column, XTerra MS C18 (150 mm × 3.0 mm, 5 μm i.d.); 0.010 M ammonium acetate–methanol (55:45, v/v); flow rate, 0.30 ml min^{-1}; detection wavelength, 245 nm.

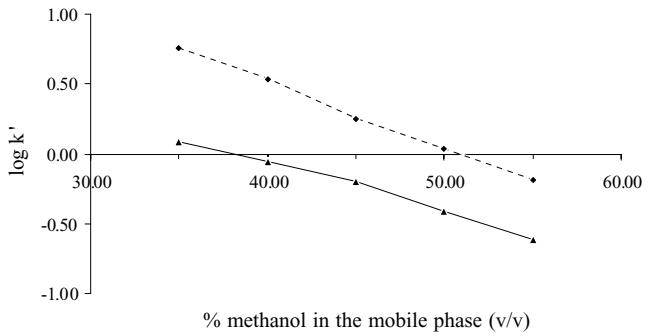

Fig. 1. Effect of methanol concentration on the capacity factor (k') of buspirone hydrochloride (broken line (■)) and lidocaine hydrochloride (solid line (▼)). Column, XTerra MS C18 (150 mm × 3.0 mm, 5 μm i.d.); 0.010 M ammonium acetate (pH 4.0) and methanol; flow rate, 0.30 ml min^{-1}; detection wavelength, 245 nm.

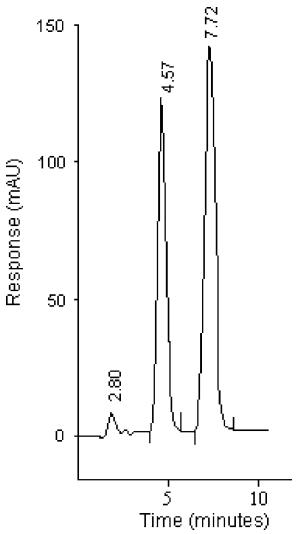

Fig. 3. Representative chromatogram of a mixture of the internal standard, lidocaine hydrochloride (25.0 μg ml^{-1}) and buspirone hydrochloride (2.50 μg ml^{-1}) at retention times, 4.57 and 7.72 min, respectively. Chromatographic conditions: reversed-phase HPLC on a small-bore XTerra MS C18 (150 mm × 3.0 mm, 5 μm i.d.); mobile phase: 0.010 M ammonium acetate (pH 4.0) and methanol (55:45, v/v); flow rate, 0.30 ml min^{-1}; detection wavelength, 245 nm.

chromatographic elution of both compounds is related to the degree of ionisation. The internal standard, Ldc, is a basic substance (pK_a = 7.9; 25 °C), a decrease of pH values from 5.0 to 2.8 causes concomitant decrease of its retention time due to an increase in protonation of the nitrogen atom in the diethylaminoaceto-group. The same decrease in pH decreases also the retention time of Bsp, due to ionisation of its basic site (pK_a = 7.2; 25 °C). A pH value of 4.0 was chosen for the optimum separation of the compounds, as at this pH the analyte peaks were well defined and resolved. The optimum wavelength for detection was at 245 nm, at which the best detector responses for all substances were obtained.

The specificity of the HPLC method is illustrated in Fig. 3 where complete separation of the compounds was observed. Bsp was eluted at 7.72 min, while the internal standard Ldc was eluted at 4.57 min.

3.2. Linearity and reproducibility

Seven points calibration graphs were constructed covering a concentration range from 1.00 to 5.00 μg ml^{-1} for Bsp; three independent determinations were performed at each concentration (n = 3). Linear relationship was obtained between the peak area ratio of Bsp to that of the internal standard Ldc and the corresponding concentration, as shown by the equation presented in Table 1. The correlation coefficient (r) and the standard error of the estimate (S_r) of the calibration line are also given, along with the standard deviations of the slope and intercept.

In order to further evaluate the linearity of the proposed method, five calibration equations were constructed over a period of 4 weeks. The average regression equation is also presented in Table 1, along with the R.S.D. values of the slopes and intercepts, the correlation coefficient invariably exceeded 0.9990.

Intra-day data for the accuracy and precision of the method given in Table 2, indicate E_r = −2.0 to 3.0% and R.S.D. = 0.20–1.94%. Moreover, the inter-day % R.S.D. values (Table 2) for the determination of Bsp were ranged from 0.58 to 1.92%.

The limit of detection (D_L) and the limit of quantitation (Q_L) attained, as defined by IUPAC [24] and

Table 1
Calibration equations for the determination of buspirone by high-performance liquid chromatography

Bsp sample (μg ml^{-1})[a]	Regression equations[b]	r[c]	S.D.[d]		S_r[e]
			Slope	Intercept	
1.00–5.00	R_{Bsp} = 0.462C_{Bsp} − 0.007	0.9998	0.011	0.031	0.030
Mean of five calibration curves over a period of 1 month					
1.00–5.00	R_{Bsp} = 0.459C_{Bsp} − 0.022	>0.9990	0.030	0.112	<0.065

[a] Bsp, buspirone.
[b] Ratios of peak area of buspirone to that of the internal standard (R) vs. the appropriate concentration (C) in μg ml^{-1}; seven standards.
[c] Correlation coefficient.
[d] Standard deviation of slope (μg ml^{-1}) and intercept.
[e] Standard error of the estimate.

Table 2
Accuracy and precision of within and between run analysis for the determination of buspirone by high-performance liquid chromatography

Concentration level ($\mu g\,ml^{-1}$)	Assayed concentration ($\mu g\,ml^{-1}$)			
	Mean ± S.D.	Accuracy		Precision (% R.S.D.)[b]
		Recovery (%)	E_r (%)[a]	
Intra-day ($n = 5$)				
1.00	1.03 ± 0.02	102.7 ± 1.5	3.0	1.94
3.00	2.94 ± 0.02	97.6 ± 0.5	−2.0	0.68
5.00	5.05 ± 0.01	101.0 ± 0.2	1.0	0.20
Inter-day ($n = 5$)				
1.00	1.04 ± 0.02	103.7 ± 1.5	4.0	1.92
5.00	5.12 ± 0.03	102.4 ± 0.6	2.4	0.58

[a] Relative percentage error.
[b] Percentage relative standard deviation.

ICH Topic Q2B [25] using the slope (b) of the calibration graph and the standard deviation (S_α) of the intercept (α). The limit of detection, calculated from $y - \alpha = 3.3S_\alpha$ and $y - \alpha = bD_L$ it was found to be $0.20\,\mu g\,ml^{-1}$. The limit of quantitation, calculated from $y - \alpha = 10S_\alpha$ and $y - \alpha = bQ_L$ it was found to be $0.67\,\mu g\,ml^{-1}$.

In order to evaluate the robustness of the proposed method, the influence of small deliberate variations of the method parameters in the retention times of Bsp and Ldc was examined thoroughly. The factors selected to examine were the pH of the buffer, the flow rate and the percentage of methanol in the mobile phase; each factor was charged at three levels (−1, 0, and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections ($n = 3$) of a mixed standard solution containing $3.00\,\mu g\,ml^{-1}$ of Bsp and $25.0\,\mu g\,ml^{-1}$ of Ldc were performed under small changes of three chromatographic parameters (factors). Results, presented in Table 3, indicate that the selected factors remained unaffected by small variations of these parameters.

The statistical evaluation of the proposed HPLC method revealed its good linearity and reproducibility and led us to the conclusion that it could be used for the rapid and reliable determination of Bsp in tablets.

3.3. Label claim recoveries from buspirone tablets

The proposed method was evaluated in the assay of commercially available tablets containing 10.0 mg of buspirone hydrochloride. Ten replicate determinations were carried out on an accurately weighted amount of the pulverised tablets equivalent to 10.0 mg of Bsp. The percent label claim found was to be 97.79 ± 0.88 mg ($n = 10$, R.S.D. = 0.90%) or 9.77 mg per tablet.

The method proved to be suitable for the content uniformity test, where a great number of assays on individual tablets are required. Two different lots of commercially available tablets containing Bsp were analysed using the proposed procedure and the results are summarised in Table 4. Recoveries achieved were in accordance with the declared content of Bsp in tablets.

In order to assess the specificity of the proposed method, recoveries studies were also performed, by spiking sample powders with appropriate amounts of the reference standard of Bsp. A calibration curve was constructed by plotting the amount of the drug found (mg) versus the amount of the drug added (mg). The following linear regression equation was obtained through regression analysis of data:

$$C^{f}_{Bsp} = 0.9948(\pm 0.024)C^{a}_{Bsp} + 10.35(\pm 0.25),$$

$$r = 0.9991, \qquad S_r = 0.187$$

where C^{f}_{Bsp} and C^{a}_{Bsp} are the amounts in (mg) found and added, respectively, for Bsp; r the correlation coefficient of the regression equation; S_r is the standard error of the estimate. The y-axis intercept of the above mentioned linear regression equation indicates the amount (mg) of the drug found in the powdered tablets, while the percentage recoveries were calculated as:

Table 3
Robustness evaluation of the HPLC method for the determination of buspirone hydrochloride

Chromatographic changes		Buspirone hydrochloride			Lidocaine hydrochloride		
Factor[a]	Level	t_r[b]	k'[c]	T[d]	t_r[b]	k'[c]	T[d]
A: pH of the buffer							
3.90	−1	7.63	1.72	1.26	4.34	0.55	1.23
4.00	0	7.72	1.76	1.25	4.57	0.63	1.20
4.10	1	7.89	1.82	1.24	4.65	0.66	1.32
Mean ± S.D. ($n = 3$)		7.75 ± 0.13	1.77 ± 0.05	1.25 ± 0.01	4.52 ± 0.16	0.61 ± 0.06	1.25 ± 0.06
B: flow rate (ml min^{-1})							
0.28	−1	7.82	1.79	1.28	4.71	0.68	1.32
0.30	0	7.72	1.76	1.25	4.57	0.63	1.20
0.32	1	7.79	1.78	1.29	4.25	0.52	1.23
Mean ± S.D. ($n = 3$)		7.78 ± 0.05	1.78 ± 0.02	1.27 ± 0.02	4.51 ± 0.23	0.61 ± 0.08	1.25 ± 0.06
C: percentage of methanol in the mobile phase (v/v)							
44	−1	7.84	1.80	1.29	4.75	0.70	1.19
45	0	7.72	1.76	1.25	4.57	0.63	1.20
46	1	7.61	1.72	1.23	4.35	0.55	1.24
Mean ± S.D. ($n = 3$)		7.72 ± 0.12	1.76 ± 0.04	1.26 ± 0.03	4.56 ± 0.20	0.63 ± 0.08	1.21 ± 0.03

[a] Three factors (A, B, and C) were slightly changed at three levels (1, 0, and −1); each time a factor was changed from level (0) the other factors remained at level (0).
[b] Retention time.
[c] Capacity factor.
[d] Tailining factor.

recovery (%) = slope × 100. The results presented in Table 5 indicate that there is no interference from the excipients used in the formulation of the tablets.

The proposed method was further evaluated by comparison with an HPLC method, proposed by the USP–NF Beta 19 [20]. Seven samples prepared according to the standard addition method that described in the experimental session were analysed in duplicate by the USP and the proposed method. Data pairs were plotted on a scatter diagram, with the abscissa for the USP procedure (assumed to be more precise) and the ordinate for the proposed semi-micro HPLC procedure. Linear regression analysis gave the following equation:

$$^1C_{Bsp} = 1.007(\pm 0.064)^2C_{Bsp} - 0.145(\pm 1.223),$$

$$r = 0.991, \qquad S_r = 0.66$$

where $^1C_{Bsp}$ and $^2C_{Bsp}$ are the concentrations of Bsp determined by the proposed and the USP method, respectively. A Student's *t*-test was performed to

Table 4
Determination of buspirone in commercial formulations by high-performance liquid chromatography

Lot (commercial formulation: bespar, 10 mg)	Buspirone hydrochloride found (mg per tablet)[a]	
	Mean ± S.D. ($n = 10$)	Recovery (%)
1	9.78 ± 0.09	97.8
2	9.76 ± 0.08	97.6

[a] The indicated values are the mean of 10 different analyses of the same commercial batch.

Table 5
Recoveries of buspirone in spiked commercial samples

Drug	Amount added[a] (mg)	Amount found (mg)	m[b]	Recovery[c] (%)
Buspirone	5.0	15.1	0.9948	99.48
	7.5	18.0		
	10.0	20.4		
	12.5	22.9		
	15.0	25.1		

[a] Amount of buspirone reference standard added to sample powders equivalent to 10.0 mg buspirone hydrochloride.
[b] m is the slope the linear regression analysis of the amount found vs. the amount added.
[c] Recovery (%) = m × 100.

determine whether the experimental intercept of the above regression line was significantly different from the theoretical value of zero. The test is based on the calculation of the quantity $t = \alpha/S_\alpha$, where α is the intercept of the regression line and S_α is the standard deviation of α, and their comparison with tabulated data for the *t*-distribution. The absolute value calculated for *t* is 0.12 (it does not exceed the 95% criterion of $t_p = 2.78$), so the intercept is not significantly different from zero. Another Student's *t*-test was carried out in order to determine whether the slope differs significantly from unity. The test is based on the calculation of the quantity $t = (1.0 - b)/S_b$, where *b* is the slope of the regression line and S_b is the standard deviation of *b*. The absolute value calculated for *t* is 0.10 (it does not exceed the 95% criterion of $t_p = 2.78$), so the slope is not significantly different from unity.

3.4. Degradation studies

In order to assure the selectivity and provide an indication of the stability indicating properties of the proposed method, forced degradation studies were performed under various stress conditions. Thus, appropriate amounts of powdered tablets equivalent to the average tablet weight (approximately 200.6 mg), were stressed with 1.0N HCl, 1.0N NaOH, and 5% (v/v) H_2O_2 at room temperature. After the degradation treatments were completed, the samples were analysed according to assay sample preparation.

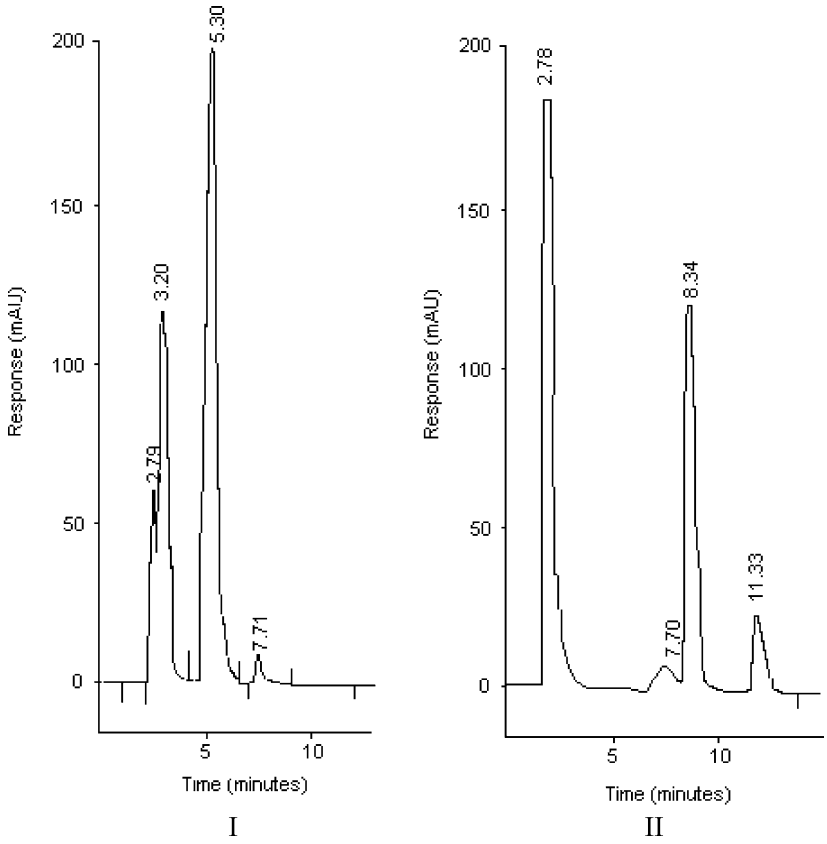

Fig. 4. Typical chromatograms obtained from the analysis of powdered tablets of buspirone hydrochloride: under (I) basic stressed conditions (1.0N NaOH) and (II) acid stressed conditions (5.0%, v/v, sodium peroxide solution) for 4 h. Chromatographic conditions: reversed-phase HPLC on a small-bore XTerra MS C18 (150 mm × 3.0 mm, 5 μm i.d.); mobile phase: 0.010 M ammonium acetate (pH 4.0) and methanol (55:45, v/v); flow rate, 0.30 ml min^{-1}; detection wavelength, 245 nm.

The acid stressed samples, in 1.0N HCl, degradation proceeded very slowly. The sample showed only 0.20% of degradation within the first 24 h. Almost 18.7% of degradation was observed after 7 days and one unknown degradation peak (t_r = 8.34 min) was appeared.

On the other hand, the acid stressed samples, with a (5.0%, v/v) sodium peroxide solution, two unknown degradation products appeared at retention times 8.34 and 11.33 min in the first 5 min of degradation. A strong interaction peak at 2.78 min was also observed due to the presence sodium peroxide in the sample. The samples, degraded under these conditions, showed approximately 95.5% of degradation in 4 h.

In the base stressed samples, in 1.0N NaOH, showed approximately 93.6% degradation in less than 5 min, while one unknown degradation peak (t_r = 5.30 min) appeared. Within the first 4 h another degradation peak (t_r = 3.20 min) appeared, and almost complete degradation of Bsp was occurred.

A typical chromatogram obtained from the analysis of powdered tablets of Bsp under basic stressed (1.0N NaOH) and acid stressed (5.0%, v/v, sodium peroxide solution) for 4 h is presented in Fig. 4.

Moreover, blistered tablets of Bsp were stored for 4 weeks at 50 ± 2 °C and 75 ± 5% relative humidity, and at 50 ± 2 °C and 15 ± 5% relative humidity. Approximately 69.4 and 67.8% of Bsp recovered after 4 weeks of degradation under high humidity (75%) and low humidity (15%) conditions, respectively [26]. In both cases, one degradation peak was observed and eluted at 5.10 min.

The percentage recoveries of buspirone, along with the retention times of the degradation peaks are presented in Table 6. From the above degradation data it can be deduced that stressed degradation studies on sample preparations did not exhibit any degradation peaks that could interfere with the elution of Bsp.

Table 6
Degradation of buspirone in 10 mg bespar tablets

Storage conditions	Time	Recovered (%)	Retention time of degradation products
Acid 1.0N HCl; 25 °C	24 h	99.8	–
	2 days	98.4	–
	7 days	81.3	8.34
H_2O_2, 5% (v/v); 25 °C	5 min	10.0	8.34, 11.33
	4 h	4.5	8.34, 11.33
Base 1.0N NaOH;	5 min	6.4	5.30
25 °C	4 h	2.5	5.30, 3.20
50 ± 2 °C/15 ± 5%	4 days	97.1	–
relative humidity	9 days	91.1	–
	20 days	79.2	5.10
	28 days	69.4	5.10
50 ± 2 °C/75 ± 5%	4 days	99.8	–
relative humidity	9 days	87.1	–
	20 days	77.6	5.10
	28 days	67.8	5.10

4. Conclusions

The proposed high-performance liquid chromatographic method has been evaluated over the linearity, precision, accuracy, and specificity and proved to be convenient and effective for the quality control of buspirone in pharmaceutical dosage forms. The measured signal was shown to be precise, accurate, and linear over the concentration range tested (1.0–5.0 μg ml^{-1}) with a correlation coefficient better than 0.9990. Most of the reported methodologies for the determination of buspirone in pharmaceuticals require time consuming procedures with derivatization reaction for colorimetric detection and thin layer liquid chromatographic procedures. The proposed method was further compared with an HPLC procedure proposed by the United States Pharmacopeia and proved to be equally accurate and precise. Moreover, the lower solvent consumption along with the short analytical run time of 8.0 min leads to a cost effective and environmentally friendly chromatographic procedure. Forced degradation studies revealed that possible degradation products do not interfere with the determination of buspirone. Thus, the proposed methodology is rapid, selective, requires a simple sample preparation procedure, and represents a good alternative for the quality control of buspirone in tablets.

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