Liquid Chromatographic Method for the Analysis of Buspirone HCl and Its Potential Impurities

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Abstract

An accurate, reproducible, and sensitive method for the determination of buspirone HCl and its potential impurities is developed and validated. The validated liquid chromaography method is conducted to meet the Food and Drug Administration/ International Conference on Harmonization requirements for the analysis of buspirone HCl in the presence of its impurities. Five buspirone HCl potential impurities, including 1-(2-pyrimidinyl)-piperazine (I), propargyl chloride (II), 3,3'-tetramethylene glutarimide (III), propargyl glutarimide (IV), and the Mannich base-condensate of I–IV fumarate (V), are separated using a µBondapack C_{18} column by gradient elution with a flow rate 2.0 mL/min. The initial mobile phase composition is 90:10 (v/v) 10mM KH_2PO_4 (pH 6.1)–acetonitrile. After a 1-min initial hold, a linear gradient is performed in 26 min to 35:65 (v/v) 10mM KH_2PO_4 (pH 6.1)–acetonitrile. The samples are detected at 210 and 240 nm using a photo-diode array detector. The linear range of detection for buspirone HCl was between 1.25 ng/µL and 500 ng/µL, with a limit of quantification of 1.25 ng/µL. The linearity, range, peak purity, selectivity, system performance parameters, precision, accuracy, and robustness for all of the impurities were also shown to have acceptable values.

Introduction

Detection, identification, and quantitation of impurities in drug substances and formulated products is a significant part of the drug development process. The International Conference on Harmonization (ICH) has published guidelines concerning related substance impurities that consist of compounds that are structurally related to the drug substance and arise from the manufacturing process or via degradation. These guidelines require the identification of impurities that are present at levels of 0.1% or greater in the drug substance (1).

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8-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-8-azaspiro[4,5] decane-7,9-dione monohydrochloride (buspirone HCl) is a member of the azapirone group of anxiolytic drugs (2). It exhibits an anxiolytic effect similar to that of diazepam, without sedative, muscle relaxing, or anticonvulsant activity (3). 1-(2-Pyrimidinyl)-piperazine (1-PP) is a major metabolite of buspirone (4) but also a potential impurity in the raw material. There are several methods currently available for measuring buspirone and its metabolites levels in various body fluids obtained from different species.

Two capillary gas chromatography–mass spectrometry (GC–MS) methods were reported for the simultaneous determination of buspirone and 1-PP in human plasma (4,5). Capillary GC–MS techniques are excellent with respect to selectivity and sensitivity but are far more complex and expensive than liquid chromatography (LC) methods. Lai et al. (2) reported a procedure using GC with nitrogen phosphorus detection to quantitate buspirone levels in the brains of rats treated with drugs. This method is rapid, sensitive, and readily applicable to the routine analysis of buspirone (2). However, all of these GC methods require derivatization and thus further validation.

Several LC methods have been reported for the pharmacokinetic studies of buspirone in humans and experimental animals. In these various studies, the detection methods were ultraviolet

Table I. Gradient Elution Program

Time (min)	Flow rate (mL/min)	A (%)	B (%)
0–1	2.00	10	90
1–27	2.00	linear gradient to 65:35 (A:B)	
27–35	2.00	65	35
35–35.50	2.00	linear gradient to 10:90 (A:B)	

(UV) (6–8), coulometric (3,9–11), electrochemical (12), or mass spectral (13). *The United States Pharmacopoeia* (USP) (14,15) also describes an LC assay for buspirone HCl as a drug substance and buspirone HCl tablets. In this paper, a different and advantageous LC method for the determination of buspirone HCl and its potential synthetic impurities is described. This method can be used for the assay of buspirone HCl as a drug substance, quantitation of its impurities, and also quality control of finished products that contain buspirone HCl.

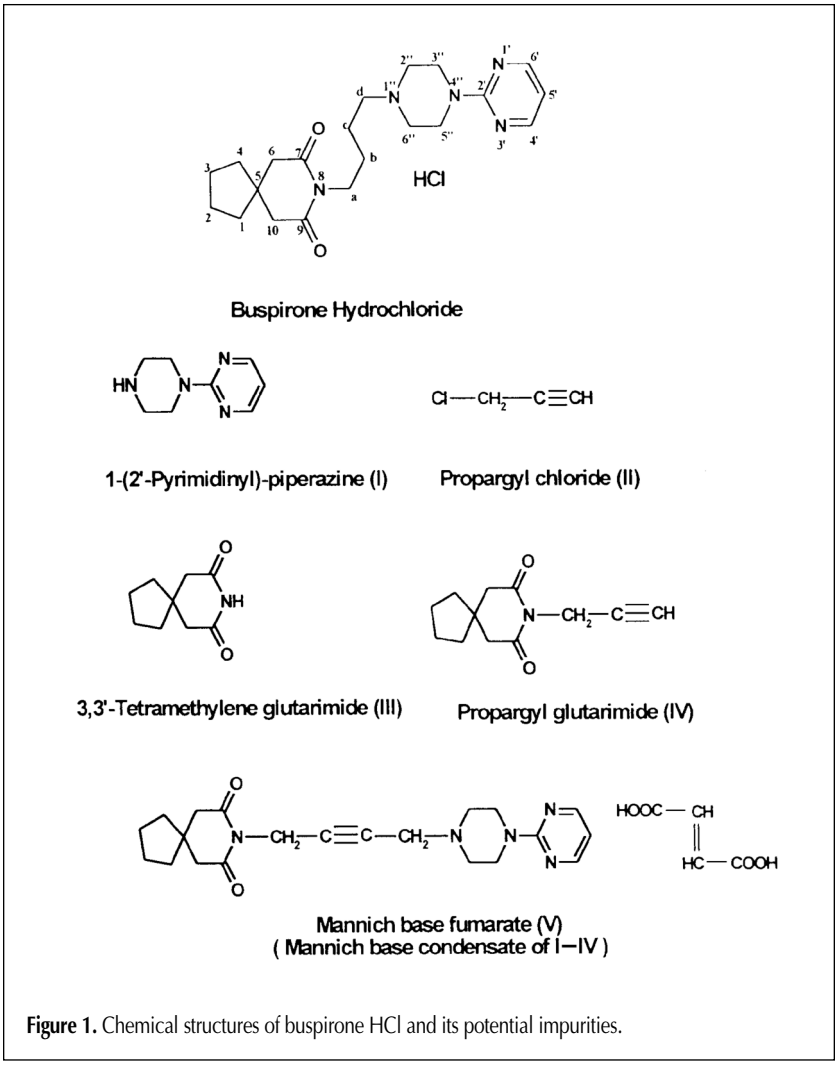

Figure 1. Chemical structures of buspirone HCl and its potential impurities.

Table II. Linearity Results, LOD, and LOQ

Compound	Standard error λ	LOD Equation*	R^2	of slope	LOQ (ng/µL)	(ng/µL)
(I)	240	$y = 63985.87x - 75648.83$	0.9999	237.97	0.312	1.248
(II)	210	$y = 1946.61x - 32497.28$	0.9984	45.44	25.20	42
(III)	210	$y = 29605.92x + 48247.45$	0.9997	273.25	0.440	2.20
(IV)	210	$y = 31897.54x + 4531.57$	0.9997	296.79	1.30	2.60
(V)	240	$y = 18263.60x + 4016.90$	0.9997	164.53	0.50	2.50
Buspirone HCl	240	$y = 31100.87x - 6246.18$	0.9999	50.56	0.125	1.250

* x, concentration (ng/µL); y, peak area.

Experimental

Chromatographic systems

The assays were performed with an LC system consisting of a Beckman (Fullerton, CA) model 126 solvent delivery system and a model 168 photodiode-array detector. Samples were injected with a Beckman 507e autosampler using a 7020-122 Rheodyne (Cotati, CA) injector system with a 20-µL sample loop. The system was controlled and data analyses were performed with Beckman Gold Nouveau software (version 1.6) on an IBM PC 350. The column, a Waters (Milford, MA) µBondapak C_{18} (10 µm, 300 × 3.9-mm i.d.), was thermostatted at 40°C in an Eppendorf (Westbury, NY) CH-30 column heater. An Upchurch (Oak Harbor, WA) guard column (30–40-µm pellicular C_{18}, 20 × 2-mm i.d.) was used to safeguard the analytical column.

Reagent and chemicals

Buspirone HCl was obtained from Agvar Chemicals (Little Falls, NJ). Five buspirone HCl potential impurities were also obtained from Agvar Chemicals, including 1-(2-pyrimidinyl)-piperazine (I), propargyl chloride (II), 3,3'-tetramethylene glutarimide (III), propargyl glutarimide (IV), and Mannich base fumarate (V): 8-[4-[4-(2-pyrimidinyl)-1-piperazinyl] but-2-yne]-8-azaspiro [4,5] decane-7,9-dione fumarate, which is the fuma-rate salt of the Mannich base condensate of I–IV (Figure 1). Analytical-grade sodium hydroxide, high-performance liquid chromatography (HPLC) -grade monobasic potassium phosphate, acetonitrile, methanol, and water were obtained from Fisher Scientific (NJ) and used as is.

Standard working solution

Standard working solutions were prepared individually in methanol for buspirone HCl and the potential impurities. Aliquots from each working solution were combined and diluted with methanol to yield a solution with final concentrations of 7.30 ng/µL (I), 124 ng/µL (II), 3.25 ng/µL (III), 4.80 ng/µL (IV), 13.52 ng/µL (V), and 10.60 ng/µL (buspirone HCl).

Calibration solutions

To establish the linear detection range for each compound, individual standard stock solutions were prepared in methanol in 100-mL volumetric flasks. Aliquots of these solutions were diluted and analyzed to determine method linearity. Limit of quantitation (LOQ) values were estimated from serial dilution and analysis for each compound. A sample was made up at that estimated concentration. This sample was then incorporated into the calibration plot as the minimum concentration.

Three standard solutions of intermediate concentrations were prepared by dilution of the stock solution once in 10-mL volumetric flasks and analyzed six times each. Calibration ranges for bispirone HCl, (I), (II), (III), (IV), and (V) were 1.25–500, 1.24–62.4, 42–420, 2.60–52, and 2.5–100 ng/µL, respectively. The mean of the peak areas at each concentration was plotted versus concentration (ng/µL), and the calibration curves were constructed. The least-square equation of regression line, correlation coefficient, and standard error of each impurity were calculated.

Procedure

LC analysis was performed using gradient elution. Bottle A was HPLC-grade acetonitrile, and bottle B was monobasic potassium phosphate buffer solution (10mM, pH 6.1). The mobile phases were filtered through a 0.45-µm nylon membrane filter and degassed in a sonicator for 10 min. The gradient elution program is given in Table I.

The volatility of propargyl chloride necessitated using the pick-up injection mode. Solutions were analyzed, and three-dimensional chromatograms (wavelength, time, absorbance) were obtained to select the optimum detector wavelength for each of the potential impurities. From the results of the three-dimensional chromatograms, it was determined that quantitation could be best achieved at 210 nm for (II), (III), and (IV) and 240 nm for (I), (V), and buspirone HCl.

Throughout the study, the suitability of the chromatographic system was monitored by calculating the capacity factor (k'), selectivity (α), resolution (R), and peak asymmetry (T). The chromatographic run time was 35.50 min, and the column void volume was 1.1 min.

Table III. System Performance Parameters of Buspirone HCl and Its Impurities

Compound	tr (n = 6, mean)	Area (n = 6, mean)	k'	R	α	T
(I)	4.300	399723	2.91			2.26
				(0.20)	(1.07)	
	5.719	1.858				
				(1.65)	(0.34)	
(II)	7.047	198291	5.41			1.72
				(0.10)	(2.20)	
	4.882	1.331				
				(1.01)	(0.17)	
(III)	9.011	143010	7.19			1.39
				(0.10)	(1.48)	
	18.239	1.923				
				(2.42)	(0.11)	
(IV)	16.319	153906	13.84			1.71
				(0.04)	(1.14)	
				5.827	1.167	
				(1.24)	(0.04)	
(V)	18.856	254007	16.14			1.66
				(0.05)	(0.94)	
				3.422	1.097	
				(1.54)	(0.06)	
Buspirone HCl	20.572 (0.04)	329353 (1.52)	17.70			2.04

* %RSD values are given in parentheses.

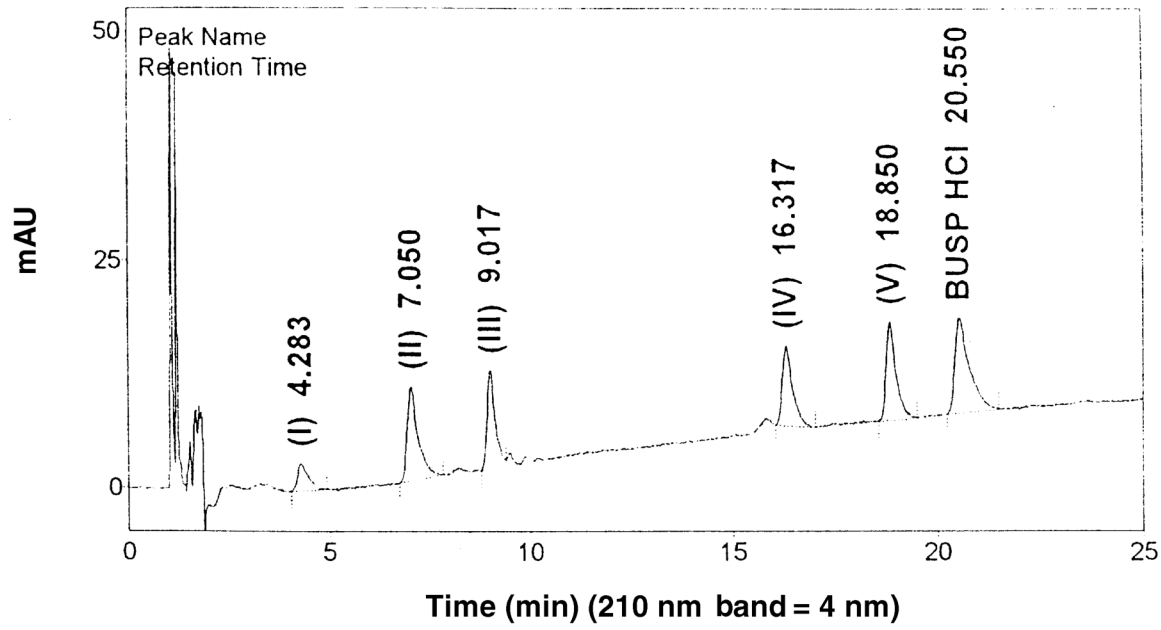

Figure 2. Chromatogram of the mixture of buspirone HCl and its potential impurities at 210 nm by the developed LC method.

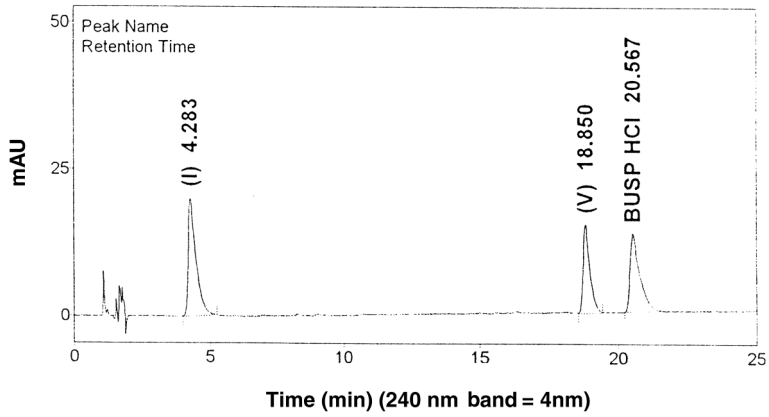

Figure 3. Chromatogram of the mixture of buspirone HCl and its potential impurities at 240 nm by the developed LC method.

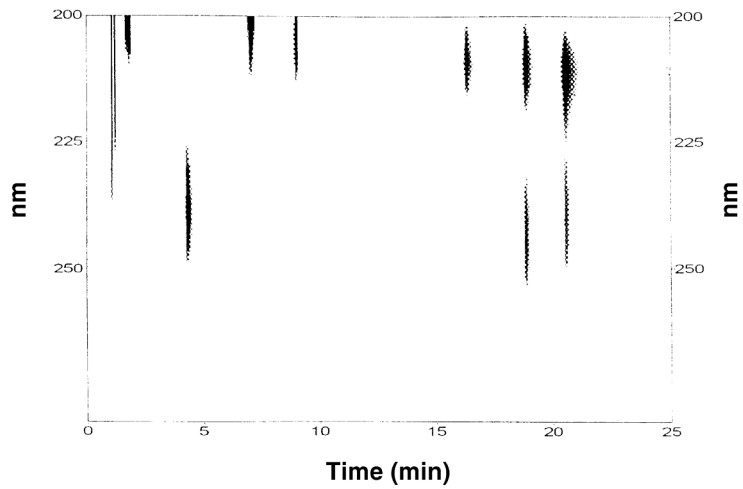

Figure 4. Contour plot chromatogram of the mixture of buspirone HCl and its potential impurities.

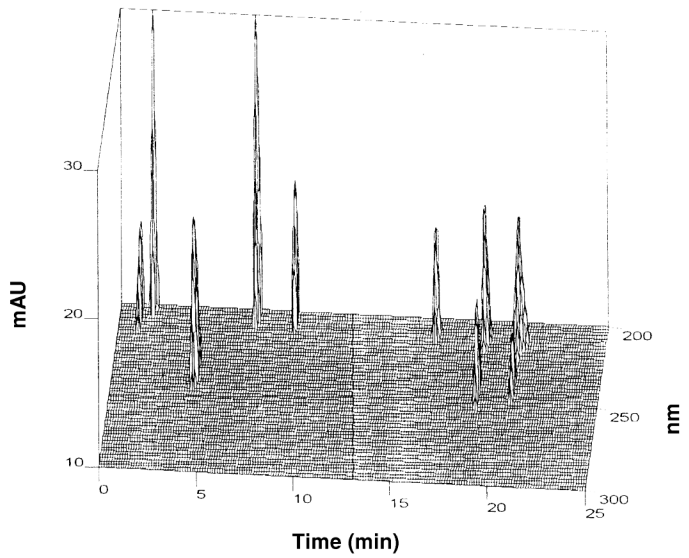

Figure 5. Three-dimensional chromatogram of the mixture of buspirone HCl and its potential impurities.

Results and Discussion

Method development

The chromatograms of buspirone HCl spiked with the five impurities in the USP 23 assay showed overlap between (I) and (IV). The USP HPLC method was not able to separate impurities (I) and (IV) from each other, and UV detection at 254 nm for the USP method was not suitable for detection and quantitation of (II), (III), and (IV). Several chromatographic parameters, including flow rate (1.8–2.2 mL/min), column temperature (25–40°C), mobile phase ratio (35:65–45:55), and pH (7.3–7.7), were changed in an attempt to resolve these peaks. However, all attempts failed. The resolution between (I) and (IV) was never more than 1.10, and the selectivity between two peaks was always less than 1.20. Also, the peak asymmetry value of (I) was greater than 2.40. Therefore, it was decided to develop a new method for the determination and quantitation of buspirone HCl and its potential impurities.

Development and validation of proposed method

Linearity and range

Each of the five different concentration standards for each analyte was injected three times. The peak areas obtained for the three analyses were averaged at each concentration. The average peak areas were plotted versus concentration. A linear response between peak area and concentration for all compounds was observed. As can be seen from Table II, the correlation coefficient R^2 values for all compounds were close to 1. The lower limit of detection (LOD) was estimated and confirmed by the analysis of a sample at that concentration based on a signal-to-noise (S/N) ratio of 3, also from the detectable chromatographic peak. The LOQ was estimated using two criteria; the first is the S/N ratio of not less than 10, and the second is the percent relative standard deviation (%RSD) not more than 5% for six replicate injections of the LOQ solution (Table II). LOD and LOQ values of propargyl chloride (II) were found to be very high because of its aliphatic structure and weak chromophore.

Peak purity

Peak purity was assessed using a peak similarity index value. All chromatographic peaks of the standard mixture were UV-scanned at the peak apex. The UV spectrum for each component in the mixture was compared to that obtained for the individual components under the same chromatographic conditions to estimate the similarity index. A similarity index of 1.000 indicates spectral

identity. Any index from 0.950 to 0.990 indicates "possible" matches, and numbers below 0.950 indicate that there is no match. All values are more than 0.984 for all of the peaks examined in this study, which were all spectrally similar and pure. The three-dimensional and contour plot view of the chromatogram also confirmed complete separation.

Selectivity and system performance parameters

The selectivity and system performance parameters of the proposed chromatographic method were tested by injecting a standard working solution containing 7.30 ng/µL (I), 124 ng/µL (II), 3.25 ng/µL (III), 4.8 ng/µL (IV), 13.52 ng/µL (V), and 10.60 ng/µL (buspirone HCl). The chromatograms at 210 nm (Figure 2) and 240 nm (Figure 3), the contour plot (Figure 4), and the three-dimensional of the chromatograms (Figure 5) showed a complete resolution of all peaks. The system performance parameters of six repetitive injections were calculated and recorded in Table III. The calculated resolution values between each peak pair were not less than 3.40, and the selectivity was not less than 1.000.

Precision

The precision of the system was checked by the analysis of six replicate injections of each impurity at the LOQ level. The %RSD for the peak areas are not more than 3.4% (Table IV).

Accuracy

Analysis of a solution spiked with known amounts of impurities and buspirone HCl showed the accuracy of the method. A standard working solution mixture containing 7.30 ng/µL (I), 124.00 ng/µL (II), 3.25 ng/µL (III), 4.80 ng/µL (IV), 13.52 ng/µL (V), and 10.60 ng/µL (buspirone HCl) was injected 9 times. The accuracy was expressed in terms of percent deviation of the measured concentration from the spiked concentration. Because the results obtained are within the acceptable range of ± 5%, the method is deemed to be accurate (Table V). The percent deviation of (II) from spiked concentration was found to be higher than other impurities. Propargyl chloride seems to be fairly reactive. It was noted that some of the small peaks were absent in a standard solution that did not include the propargyl chloride.

Method robustness

The robustness of the method was evaluated by changing the pH of phosphate buffer (5.8–6.4), column temperature (25–45°C), and flow rate (1.9–2.1 mL/min.). To determine the effects of change on results, a standard working solution containing buspirone HCl and the five impurities was injected three times after each change. Preliminary inspection of the chromatograms obtained under these various conditions suggest that the method is fairly robust, but more work needs to be done to confirm this.

Conclusion

Gradient LC methods with photodiode-array detection was successfully used for the separation and determination of impurities in buspirone HCl. The developed method allows the identification of the peaks at the LOD limits and the purity of the peaks at the LOQ limits. The standard solution of propargyl chloride should be prepared separately and then added to the mixture shortly before analysis to avoid any reaction with the other components. This paper presents an improved LC method for buspirone HCl. All five impurities and buspirone HCl exhibit good linearity, and chromatographic parameters such as capacity factor, resolution, selectivity, and peak asymmetry were found to be satisfactory for these compounds.

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Table IV. Precision of the Assay of Impurities at the LOQ Level (*n* = 6)

Compound	λ	Peak area (*n* = 6, mean)	%RSD*
(I)	240	67208	2.80
(II)	210	62925	3.40
(III)	210	79972	2.55
(IV)	210	82307	2.18
(V)	240	48156	2.29
Buspirone HCl	240	62925	1.83

* %RSD = (standard deviation/mean) × 100.

Table V. Accuracy of the Developed Method

Compound	Spiked concentration (ng/µL)	Measured concentration (ng/µL) mean ± SD	%RSD	%Deviation*
(I)	7.30	7.42 ± 0.06	0.76	1.64
(II)	124.0	118.63 ± 2.17	1.83	4.33
(III)	3.25	3.18 ± 0.08	2.41	2.15
(IV)	4.80	4.69 ± 0.05	1.05	2.29
(V)	13.52	13.66 ± 0.13	0.95	1.03
Buspirone HCl	10.60	10.74 ± 0.15	1.37	1.32

* %Deviation = [(spiked concentration – mean measured concentration) × 100] /spiked concentration.

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