

Determination of Buspirone HCl in Commercial Dosage Forms by Extractive Spectrophotometric Method and Comparison by HPLC Method

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Abstract: An extractive spectrophotometric method has been developed for the determination of buspirone HCl in tablets. It was based on the ion-pair complex formation between buspirone and bromothymol blue in presence of disodium hydrogen phosphate and citric acid buffer solution of pH 4.0 which is extractable in chloroform. The extracted complex showed maximum absorbance at 412 nm. Beer's law was obeyed in the concentration range of 1.25-30 $\mu\text{g ml}^{-1}$. Various factors affecting the reaction conditions were carefully studied and optimized. Validation parameters based on the guidelines of International Conference on Harmonisation, USA were followed. Effect of common excipients used as additives has been tested and the tolerance limit was calculated for the determination of buspirone HCl. Limits of detection and quantitation were 0.165 and 0.499 $\mu\text{g ml}^{-1}$, respectively. Proposed method has been successfully applied for the determination of buspirone HCl in pharmaceutical formulations. High performance liquid chromatographic method was employed using 250 mm \times 4.6 mm i.d., 5- μm particle, reversed phase C18 column with 70:30 (v/v) methanol-0.01M NaH_2PO_4 buffer as a mobile phase at a flow rate of 0.8 ml min^{-1} and UV detection at 240 nm for best separation of buspirone. Results obtained by the proposed method were statistically compared with the HPLC reference method using t- and F- values and found no significant difference between the two methods. The proposed method can be used as an alternate method for routine quality control analysis of buspirone HCl in pharmaceutical formulations.

Keywords: Buspirone Hydrochloride, Bromothymol Blue, Extractive Spectrophotometry, Validation, Commercial Dosage Forms

1. Introduction

Buspirone hydrochloride is chemically known as (8-[4-(4-pyrimidin-2-ylpiperazin-1-yl) butyl]- 8-azaspiro[4.5] decane-7,9-dione, hydrochloride (CAS: 33386-08-2; M.W.: 422). It is a psychotropic drug with anxiolytic properties which belongs chemically to the class of compounds known as aza spiro decanediones. It is used primarily as an anxiolytic, specifically for generalized anxiety disorder.

In general, lower dosages of buspirone hydrochloride are recommended. Where the presenting symptoms are mild in nature, it is advisable to initiate treatment at a dose of 5-30 mg daily. At high concentrations, severe adverse effects and toxicity can appear. Therefore, the estimation of buspirone

hydrochloride is important for obtaining optimum therapeutic concentration and for quality assurance in pharmaceutical dosage forms.

The assay of buspirone hydrochloride in bulk and formulations is cited in The British Pharmacopoeia [1] which is based on liquid chromatography. In view of the great importance of the drug in terms of its optimum oral dose and wide use, various analytical methods have been reported which include high performance liquid chromatography [2-6], voltammetry [7, 8], radioimmunoassay [9], capillary GC [10], flow injection analysis with tubular membrane ion-selective electrode [11], spectrofluorimetry [12], and spectrophotometry [13, 14]. The extractive spectrophotometric method [15] has also been utilized for determination of buspirone hydrochloride based on the formation of coloured ion pair complex of the

drug with bromocresol green. The coloured ion-pair complex was quantitatively extracted into chloroform and measured spectrophotometrically at 415 nm. Analytical methods based on high performance liquid chromatography and gas chromatography for purity assay of drug provide excellent accuracy and precision but the drawback of the procedure is their high instrumentation cost and involvement of clean up procedures prior to analysis. These kinds of hurdles can be solved by utilizing extractive spectrophotometric methods in pharmaceutical analysis. Literature survey revealed only one extractive spectrophotometric method for the determination of buspirone hydrochloride in commercial dosage forms. Therefore, there is a need for the development of more sensitive and selective extractive spectrophotometric method parallel to HPLC method for the assay of buspirone hydrochloride in pharmaceutical formulations. Spectrophotometry is the most frequently used technique used for determining drug substances in the laboratories of research, hospitals and pharmaceutical industries due to its low cost, simplicity, versatility and adaptability [16]. The proposed method is based on the formation of ion-pair complex of the drug with bromothymol blue in the presence of Na_2HPO_4 -citric acid buffer solution of pH 4.0 at room temperature ($25 \pm 1^\circ\text{C}$) and subsequent extraction into chloroform which absorbs maximally at 412 nm. The reaction conditions are optimized and validated as per the International Conference on Harmonization guidelines [17].

2. Materials and Methods

2.1. Materials

Helios Alpha UV-Vis Spectrophotometer (Thermo Electron Corporation, England, UK) and Hanna pH meter (USA) were used for absorptiometric and pH measurements, respectively. IR Affinity-1 spectrophotometer (Shimadzu, Kyoto, Japan) was used for IR spectra in wave number region $4000\text{--}400\text{ cm}^{-1}$ using KBr pellet technique. Chromatography was performed with Dionex-Ultimate 3000 HPLC system equipped (Thermo Scientific, Australia) with $250\text{ mm} \times 4.6\text{ mm i.d.}$, $5\text{ }\mu\text{m}$ particle, Acclaim 120 C18 reversed phase LC column, a variable wavelength program UV-visible detector (WDM- 3000), UV- visible photometer detector, pump (HPG-3200 SD), column oven (TCC, 3000 SD), Chromeleon Data System Software version (5.80 SR11), Column temperature: ambient temperature ($25 \pm 1^\circ\text{C}$), flow rate 0.8 ml min^{-1} , mobile phase: methanol-0.01M sodium dihydrogen phosphate (70 ml: 30 ml v/v; pH 3.5), wavelength: 240 nm, injection volume: 20 μl . The mobile phase was cleaned Duopore PVDF membrane filter of $0.45\text{ }\mu\text{m}$ (Merck Millipore Ltd., Tullagreen, Ireland).

All reagents used were of analytical reagent grade. The pure buspirone HCl is gifted by National Pharmaceutical Industries Company, Oman. 0.025% buspirone HCl solution was freshly prepared by dissolving 0.025 g buspirone HCl in 100 ml volumetric flask and diluted up to the mark with distilled water. 0.01% buspirone HCl solution was prepared

by dissolving 0.01 g in 100 ml of the mobile phase (70 ml methanol: 30 ml 0.01M NaH_2PO_4 v/v). The solution was stable up to 6 h. 0.04% solution of bromothymol blue (Sigma Chemical Company; CAS: 76-59-5; M.W.: 624.38) was freshly prepared in doubly distilled water. Na_2HPO_4 – citric acid buffer solutions of pH: 2.2, 2.4, 2.6, 2.8, 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, and 6.2 were prepared by mixing appropriate volumes of 0.2 M Na_2HPO_4 and 0.1 M citric acid [18]. The commercial dosage forms of buspirone HCl such as Freeton 5 mg (National Pharmaceutical Industries Company, Oman) and Buscalm 5 mg tablets (Wochardt, India) were purchased locally from Scientific Pharmacy (Muscat, Oman).

2.2. Procedure for the Determination of Buspirone HCl by Proposed Method

Different aliquots of 0.05, 0.1, 0.2, 0.3, 0.5, 0.6, 0.7, 0.9, 1.0, 1.1, 1.2 ml of 0.025% buspirone hydrochloride were taken with 1.4 ml of 0.04% bromothymol blue and 4 ml of Na_2HPO_4 -citric acid buffer solution of pH 4 in 10 ml volumetric flasks and diluted up to the mark with distilled water. It was further transferred into separating funnel with 10 ml chloroform and shaken well for 2 min. The lower organic layer was separated and the absorbance was measured at 412 nm against blank solution. Calibration graph was plotted between absorbance versus concentration of buspirone HCl in $\mu\text{g ml}^{-1}$. The linear regression equation was generated and used to find out the concentration of buspirone HCl in tablets.

2.3. Procedure for the Reference Method [5]

A column of HPLC ($250\text{ mm} \times 4.6\text{ mm i.d.}$, $5\text{-}\mu\text{m}$ particle, C18 column) was cleaned using mobile phase (70 ml methanol: 30 ml 0.01M NaH_2PO_4 v/v) until the base line became flat. Aliquots of 0.5-2 ml of 0.01% buspirone HCl corresponding to $5\text{--}20\text{ }\mu\text{g ml}^{-1}$ buspirone HCl were taken into 10 ml standard volumetric flask and diluted up to the mark with mobile phase. The drug solution was injected into the sample port and eluted by mobile phase at a flow rate of 0.8 ml min^{-1} . The detector wavelength was fixed at 240 nm at ambient temperature and the chromatogram was recorded. The chromatographic height was plotted against the concentration of buspirone HCl in $\mu\text{g ml}^{-1}$ to get calibration graph. The linear equation was developed and used to find out the concentration of buspirone HCl in tablets.

2.4. Determination of Buspirone HCl in Pharmaceutical Formulations by Proposed and Reference Methods

The contents of commercially available tablets of Freeton and Buscalm (20 in number) of 5 mg strength of buspirone HCl were weighed and finely grounded. The powder equivalent to 25 mg (or 10 mg) buspirone HCl was taken in 60 mL of distilled water (or 60 mL of HPLC mobile phase) and kept for 10 min for complete dissolution of the drug. The mixture was filtered through Whatmann No. 42 filter paper (Whatmann International Limited, Kent, UK) in 100 ml

standard volumetric flask. The residue was washed well with 3×10 ml portions of distilled water (or mobile phase) for complete recovery of the drug and diluted up to the mark with distilled water (or mobile phase). The amount of buspirone HCl was determined following the recommended procedures.

2.5. Validation

The proposed method has been validated for linearity, limit of detection, limit of quantitation, specificity, accuracy, precision, robustness and applicability [17].

The linearity of the proposed method was evaluated by considering 0.05, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, 0.9, 1.0, 1.1 and 1.2 ml of 0.025% buspirone HCl corresponding to 1.25, 2.5, 5.0, 7.5, 12.5, 17.5, 20.0, 22.5, 25.0, 27.0 and 30 $\mu\text{g ml}^{-1}$ buspirone HCl. Each concentration level was independently analyzed for 5 times and the absorbance was recorded. The absorbance obtained at each concentration was plotted against the initial concentration of buspirone HCl in $\mu\text{g ml}^{-1}$ and the linear regression equation was evaluated by least square treatment of the calibration data. The other statistical parameters of the proposed method were calculated using OriginPro 6.1 Software.

The limit of detection (LOD) and the limit of quantitation (LOQ) for the proposed method were calculated using the following equations:

$$LOD = 3.3 \times \frac{S_0}{b} \quad (1)$$

$$LOQ = 10 \times \frac{S_0}{b} \quad (2)$$

where S_0 and b are standard deviation and slope of the calibration line, respectively.

The specificity of the proposed method was investigated by taking 1 mL of 0.025% buspirone HCl corresponding to 25 $\mu\text{g ml}^{-1}$ buspirone HCl in the presence glucose, fructose, lactose, sodium benzoate, starch, povidone, methyl cellulose, micro crystalline cellulose and mannitol. The maximum tolerance limit for each foreign species was calculated when the absorbance value did not exceed $\pm 2\%$ on addition of excipients using the following expression.

$$\text{Mass/Volume (mg/L)} = C \times \text{MW} \times 1000 \quad (3)$$

where C and MW are concentration and molecular weight of excipients, respectively.

The accuracy of the proposed method was checked by standard addition technique. In this technique, 0.5 mL of 0.25 mg ml^{-1} of the formulated tablet sample solution was spiked separately with 0, 0.05, 0.1, 0.15 and 0.2 mL of the reference drug sample solution in 10 mL standard volumetric flask and diluted up to the mark with distilled water. Each level was independently analyzed repeatedly for five times following the recommended procedure for the determination of buspirone HCl. The nominal concentration of buspirone HCl in tablet solution was determined by taking the ratio of intercept and slope. The precision of the proposed method

was investigated by intra-day and inter-day precisions. 3 aliquots of 0.2, 0.6 and 1.0 mL of 0.025% buspirone HCl corresponding to 5.0, 15.0 and 25 $\mu\text{g ml}^{-1}$ buspirone HCl were taken and independently analyzed repeatedly for five times within a day (intra-day precision) and over five consecutive days (inter-day precision).

The robustness of the proposed method was evaluated with the use of 0.8 mL of 0.025% buspirone drug solution by observing the influence of small variations of experimental variables such as concentration of bromothymol blue, volume of buffer solution of pH 4.0, shaking time and solvent.

The applicability of the proposed method was checked by direct method. The freshly prepared tablet solutions of buspirone HCl were independently analyzed in 5 replicate by considering 0.8 mL of 0.025% buspirone HCl corresponding to 20 $\mu\text{g ml}^{-1}$ buspirone HCl. The same drug solution was also tested by reference method. The results of two methods were compared statistically for their significance by calculating t - and F -values at 95% confidence level. The proposed method is considered not significantly different if the calculated t - and F -values at 95% confidence level did not exceed the tabulated values [19]. The bias was also evaluated by an interval hypothesis test based on the mean values of the proposed method and the reference method. The proposed method is considered acceptable when its true mean is within $\pm 2.0\%$ of that of the reference method. The lower (θ_L) and the upper (θ_U) acceptance limits can be calculated by the following quadratic equation [20]:

$$\theta^2(\bar{x}_1^2 - S_p^2 t_{tab}^2/n_1) + \theta \times -2\bar{x}_1^2 \bar{x}_2^2 + (\bar{x}_2^2 - S_p^2 t_{tab}^2/n_2) \quad (4)$$

where \bar{x}_1 and \bar{x}_2 are mean values at n_1 and n_2 measurements, respectively. S_p is the pooled standard deviation and t_{tab} is the tabulated one-sided t -value at 95% confidence level.

3. Results and Discussion

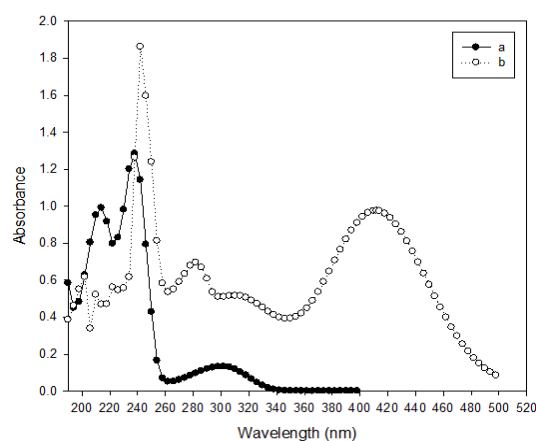


Fig. 1. Absorption spectra of (a) 0.3 mL of 0.025% buspirone HCl and (b) 0.85 mL of 0.025% buspirone HCl with 1.4 mL of 0.04% bromothymol blue and 4 mL of buffer solution in 10 mL volumetric flask and diluted up to the mark with distilled water. The mixture was extracted in 10 mL of chloroform.

Buspirone hydrochloride forms ion-pair complex with bromothymol blue in the presence of Na_2HPO_4 -citric acid

buffer solution of pH 4 which quantitatively extracted into chloroform and absorbed maximally at 412 nm. The absorption spectra of the drug and ion-pair complex are shown in Fig. 1. The reagent blank under similar conditions showed no absorption.

The stoichiometry of the reaction between buspirone and bromothymol blue was studied by mole ratio method [21]. For this purpose, different volumes (0, 0.1, 0.3, 0.5, 0.7, 0.9, 1.0, 1.1 and 1.2 ml) of 5.924×10^{-4} M buspirone were added with 1.0 ml of 5.924×10^{-4} M bromothymol blue followed by the addition of 4.0 mL of buffer solution of pH 4.0 in 10 ml standard volumetric flasks. The contents of the flasks were diluted up to the mark with distilled water and subjected to the recommended procedure for determination of buspirone HCl. The absorbance was recorded at 412 nm against the mole ratio of buspirone and bromothymol blue (5.924×10^{-4} M each). It is apparent from the result that the combining molar ratio between buspirone and bromothymol blue is 1:1. The apparent formation constant (K_f) for the complex between buspirone and bromothymol blue was calculated using the following expression:

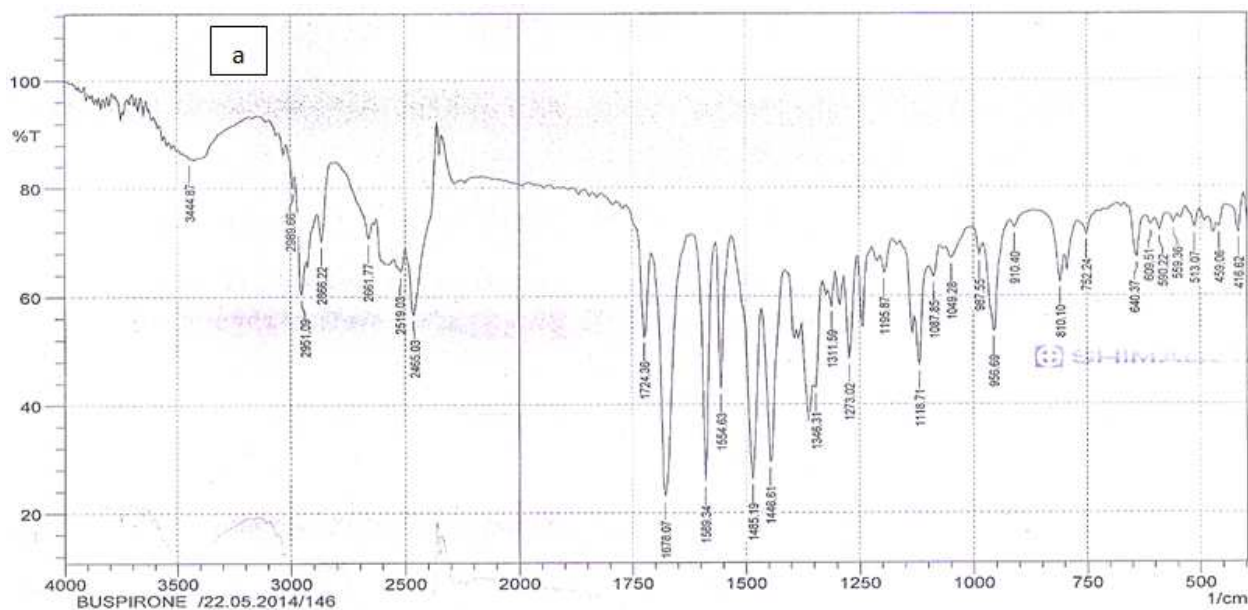
$$K_f = \frac{(A_{obs}/A_{extp})\bar{C}}{[C_D - (A_{obs}/A_{extp})\bar{C}][C_L - (A_{obs}/A_{extp})\bar{C}]} \quad (5)$$

Where $A_{obs.}$ and $A_{extp.}$ are observed and extrapolated absorbance values of buspirone-bromothymol blue complex, respectively. C_D , C_L and \bar{C} are initial concentration of buspirone, bromothymol blue and limiting concentration (bromothymol blue) in mol L⁻¹, respectively. The K_f of the complex was found to be 9.13×10^7 . The apparent Gibbs free energy (ΔG°) was calculated using $\Delta G^\circ = -2.303 RT \log K_f$ and found to be -45.427×10^3 kJ mol⁻¹, confirming the feasibility of the reaction.

The literature survey revealed an interesting result that nitrogen at position 17 in buspirone is the main atom to

accept hydrogen atom [22] and thus formed complexes with anionic exchange resins [23]. Similarly in the proposed method, the drug at pH 4 was protonated at the 17th nitrogen atom of the drug while sulphonic acid group present in bromothymol blue observed dissociation. The colour of bromothymol blue was due to the opening of the lactoid ring and subsequent formation of the quinoid group. The two tautomers are supposed to be in equilibrium but due to strong acidic nature of the sulfonic acid group, the quinoid body must predominate. Hence, the protonated drug formed an ion pair complex with bromothymol blue (quinoid body) which was quantitatively extracted into chloroform and absorbed maximally at 412 nm. This ion pair complexation reaction is exploited for the determination of buspirone in pharmaceutical formulations.

The FTIR spectra of buspirone, bromothymol blue and buspirone-bromothymol blue complex are shown in Fig. 2 a,b,c. In the FTIR spectrum of free buspirone, stretching vibrations of $-C=O$ is in the range of 1678-1724 cm⁻¹, $C=C$ stretching vibration for aromatic ring system is in the range of 1450-1600 cm⁻¹, presence of band associated with unsaturated C-H is in the range of 3000-3100 cm⁻¹, saturated C-H is in the range of 2800-3000 cm⁻¹ and N-H stretching vibration occurred at 3444.87 cm⁻¹ while for C-N stretching vibration bands are in the range of 1130-1270 cm⁻¹. The FTIR spectra of free bromothymol blue exhibited asymmetric and symmetric S-O-C stretching vibration bands at 786 cm⁻¹ and 887 cm⁻¹ [24]. The FTIR spectra of buspirone-bromothymol blue ion pair complex showed new bands due to the stretching vibration of $\geq N-H$ appeared at 2357 and $-SO_3$ exhibited at 1238 and 1014.56 cm⁻¹ which is the basis for the ion-pair complex formation between buspirone and bromothymol blue. On the basis of our experimental findings and literature background, the reaction sequence is shown in Fig. 3.



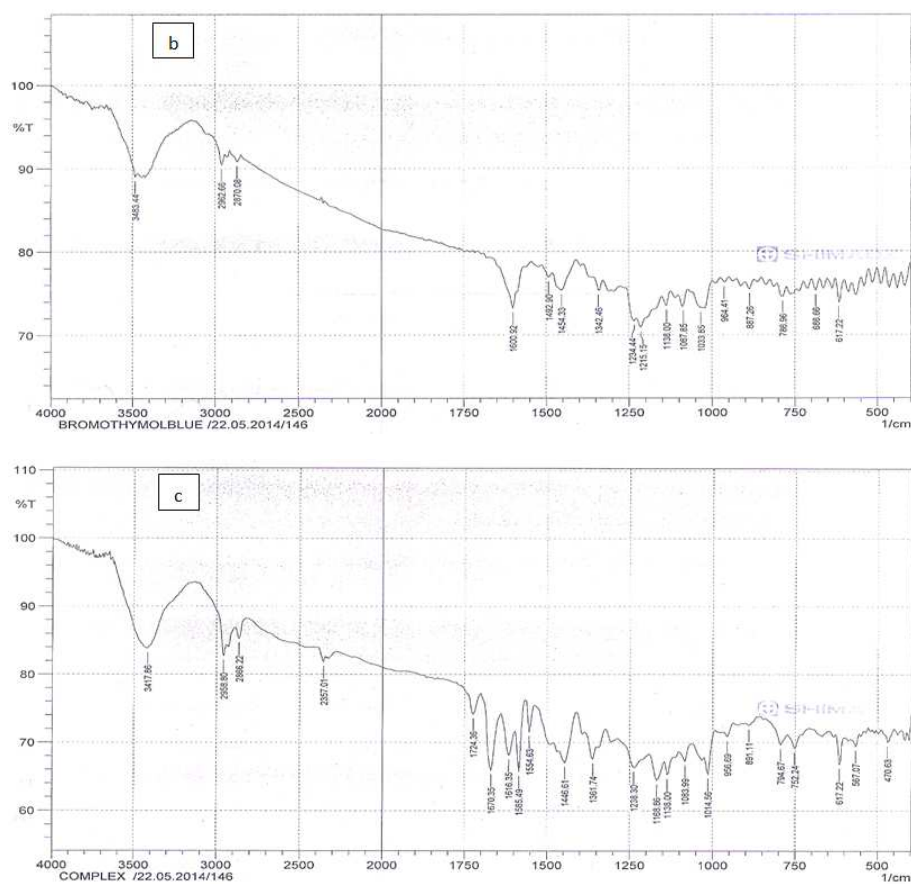


Fig. 2. Infra red spectra of (a) pure cefixime (b) free bromothymol blue and (c) buspirone-bromothymol blue ion pair complex in KBr (2 mg sample/200 mg KBr).

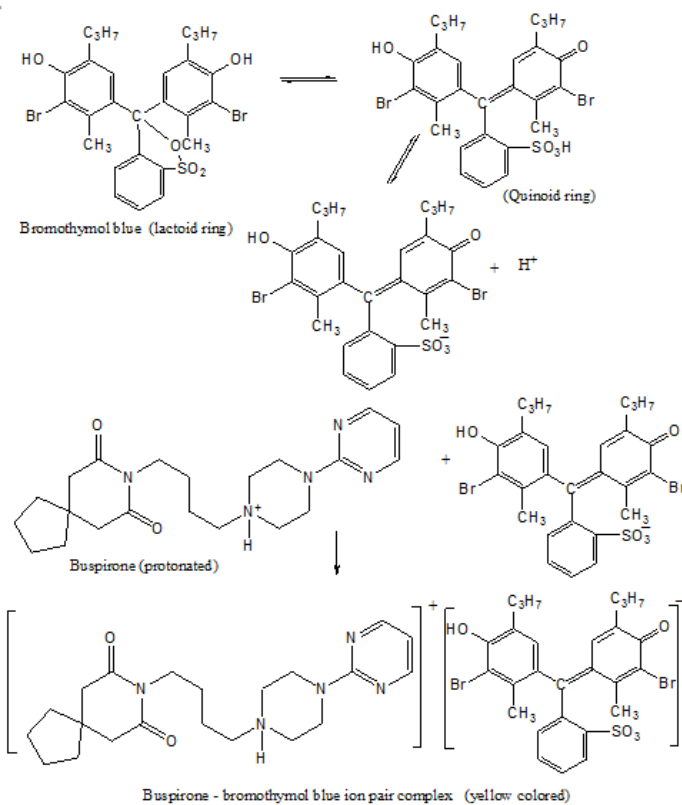


Fig. 3. Reaction sequence of buspirone-bromothymol blue ion pair complex.

3.1. Optimization of Variables

The optimization of variables for proposed spectrophotometric method was assessed by testing several parameters such as reaction time, concentration of bromothymol blue, buffer solutions of different pH, solvents and shaking time for extraction of complex.

The effect of the reaction time on the absorbance of ion-pair complex and its stability was investigated. The ion pair complex got stabilized immediately at $25 \pm 1^\circ\text{C}$ and remained stable for at least 12 hour.

The influence of pH on the absorbance of the buspirone-bromothymol blue complex was investigated using 4 ml of Na_2HPO_4 -citric acid buffer solution of different pH in the range of 2.2-6.2 with 1 ml of 0.025% buspirone HCl and 1.4 ml of 0.04% bromothymol blue. The results are recorded. The maximum absorbance of the ion-pair complex was obtained in the pH range of 2.2-5.8. Beyond pH 5.8 up to pH 6.2, the absorbance started decreasing. Therefore, 4 ml of Na_2HPO_4 -citric acid buffer solution of pH 4 was selected as the optimum pH for the determination of buspirone HCl.

The effect of the concentration of bromothymol blue was examined on the absorbance of ion pair complex between 1 ml of 0.025% buspirone and varied volume of 0.2-1.7 ml of 0.04% bromothymol blue in the presence of 4 ml of buffer solution of pH 4. The maximum absorbance was achieved at 1.1 ml of bromothymol blue and above this volume up to 1.7 ml, the absorbance remained unchanged. Therefore, 1.4 ml of 0.04% bromothymol blue was used as optimum volume for the determination of buspirone HCl.

The effect of the shaking time for the extraction of the ion pair complex was studied in the range of 0.5 – 3.0 min. The maximum absorbance of the complex was achieved at 1.5 min, above this time up to 3.0 min, the absorbance value remained constant. Therefore, 2.0 min was used as an optimum shaking time for the determination of buspirone HCl. The ion-pair complex was quantitatively recovered in one extraction only and was stable for at least 12 hour.

A number of organic solvents such as benzene, chloroform, dichloromethane, dichloroethane and ethylacetate were examined for the extraction of the ion pair complex in order to get an applicable extraction procedure and the maximum absorbance of buspirone-bromothymol blue complex. The highest absorbance of the complex was attained in chloroform and there is no extraction of the complex in ethyl acetate. Therefore, chloroform was selected as the best solvent for the extraction of the complex to determine drug in pharmaceutical formulations.

3.2. Validation

Under the optimized experimental conditions, the absorbance at 412 nm for the buspirone-bromothymol blue ion pair complex was recorded. The calibration data was obtained by recording the absorbance against the initial concentration of buspirone HCl. The linear regression equation was evaluated by statistical treatment of the

calibration data which was fitted in the form of $A = a + bC$, where A is absorbance at 412 nm, C is concentration in $\mu\text{g ml}^{-1}$, b is slope and a is intercept of calibration line and can be written as $A = 6.021 \times 10^{-4} + 4.589 \times 10^{-2} C$. Beer's law was obeyed in the concentration range of 1.25-30 $\mu\text{g ml}^{-1}$ with apparent molar absorptivity of $1.938 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and Sandell's sensitivity of $0.0217 \mu\text{g/cm}^2 / 0.001$ absorbance unit. The coefficient of correlation was calculated and found to be 0.9999, indicated excellent linearity. The experimental intercept of the calibration line was calculated for significance of deviation from the theoretical intercept i.e. zero using the relation, $t = a / S_a$ [25] and found to be 0.451, which is less than the tabulated t-value (2.262, $\nu=9$) at 95% confidence level indicated an appreciable intercept. The analytical parameters and the results of statistical analysis of the experimental data such as regression equation computed from calibration graph, linear range, correlation coefficient (r), detection limit and quantitation limit, Sandell's sensitivity, standard deviation of intercept and slope, variance, standard deviation of the calibration line of proposed and reference methods are summarized in Table I. The limit of linearity in case of the proposed method is broad as compared to reference method making the proposed method more appreciable and effective for the determination of drug in pharmaceutical formulations.

The effect of excipients added as additives on the determination of 25 $\mu\text{g ml}^{-1}$ buspirone hydrochloride was studied. The absorbance was recorded at varying concentrations of excipients such as glucose, fructose, lactose, sodium benzoate, starch, povidone, methyl cellulose, micro crystalline cellulose and mannitol with 25 $\mu\text{g ml}^{-1}$ buspirone hydrochloride, to know the concentration of buspirone hydrochloride. Table II showed the maximum tolerance limit of the studied excipients. The larger amount of tolerance limit indicated that the proposed method is more specific and selective, thus can be used to determine buspirone in pharmaceutical formulations in the presence of said additives.

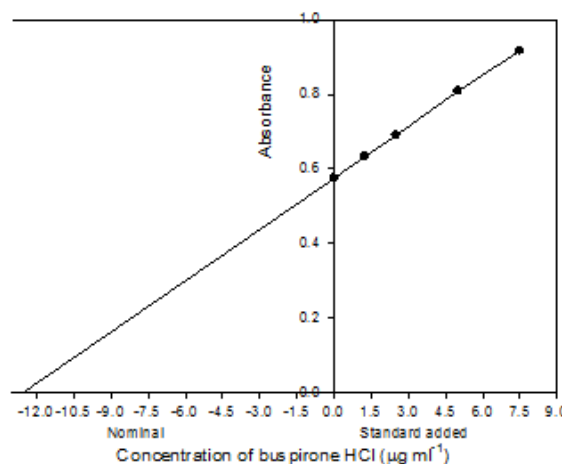


Fig. 4. Standard addition plot: 0.5 ml of 0.025% buspirone Freeton tablet solution was spiked with 0, 0.05, 0.1, 0.2 and 0.3 ml standard solution of 0.025% buspirone HCl.

The accuracy of the proposed method was judged by performing recovery experiments through standard addition technique. The absorbance of the complex for Freeton tablet sample solution spiked with standard drug solution was recorded and plotted as shown in Fig. 4. It is clear from the graph that the linearity of the regression line for tablet solution was good ($r = 0.999$) with intercept of 0.0458 and slope of 0.5759. The concentration of buspirone hydrochloride in tablet solution was calculated by taking ratio of the intercept to the slope and found to be $12.571 \mu\text{g ml}^{-1}$. The found concentration of drug in tablet solution was subjected to standard deviation, S_{x_E} , which can be calculated by the following expression:

$$S_{x_E} = \frac{S_{y/x}}{b} \sqrt{\frac{1}{n} + \frac{\bar{y}^2}{b^2 \sum (x_i - \bar{x})^2}} \quad (6)$$

The value of S_{x_E} was found to be $0.0024 \mu\text{g ml}^{-1}$. The confidence limit for the concentration of buspirone in tablet was calculated by $x_E \pm t S_{x_E}$ at $n - 2$ degrees of freedom and found to be $12.571 \pm 0.0076 \mu\text{g ml}^{-1}$. The most attractive feature of the proposed method using standard addition method is its relative freedom from pharmaceutical additives and excipients. Mostly the pharmaceutical additives and adjuvants are not forming ion pair complex with bromothymol blue and did not interfere with the proposed method. Hence the proposed method is accurate. In addition to this, it is also important to see precision of the method. Precision refers to repeatability of the determinations of the true sample mean. Intra- and inter day precisions were evaluated by determining concentration of buspirone hydrochloride at lower, middle and upper concentration levels for five repeated times within same day and on five consecutive days, respectively (Table III). Degree of precision was reported as % relative standard deviation (% RSD). It is seen from the table that RSD (%) values were in the ranges of 0.801-2.810 and 0.86-2.82 % for intraday and

inter day determinations, respectively. RSD (%) values showed that the proposed method is precise too and can be effective to analyze buspirone hydrochloride in pharmaceutical formulations.

The ruggedness of the proposed method was established by deliberately changing the reaction conditions of the proposed method as per the optimized results. To prove that the proposed method is rugged, following operational parameters were followed:

- volume of $6.406 \times 10^{-4} \text{ M}$ bromothymol blue, 1.4 ml ($\pm 0.3 \text{ ml}$)
- pH 4.0 ($\pm 1.8 \text{ pH}$)
- shaking time, 2.0 min ($\pm 0.5 \text{ min.}$)

Under these optimal conditions, buspirone hydrochloride solution containing $20.0 \mu\text{g ml}^{-1}$ (Freeton tablet) was analyzed by the proposed method. The results showed mean % recovery \pm RSD of $99.86 \pm 0.776\%$. The results indicated the ruggedness of the proposed method.

The applicability of the proposed method for the determination of buspirone hydrochloride in Freeton 5 and Buscalm 5 tablets has been tested. The results of the proposed method were statistically compared with those of the reference HPLC method [5] using point and interval hypothesis tests. Table IV showed that the calculated t-(paired) and F- values at 95 % confidence level are less than the tabulated t-value (2.036 at $\nu = 8$) and F-value (6.39 at $\nu = 4,4$), thus confirming no significant difference between the performance of the proposed method and the reference method. It is also clear from the table that the bias evaluated by interval hypothesis test by means of lower limit (θ_L) and upper limit (θ_U) are in the range of 0.98-1.02. Therefore, it is concluded that the proposed method is applicable for routine quality control analysis of buspirone hydrochloride in commercial dosage forms with acceptable recovery results which are within the acceptable limit of $\pm 2\%$.

Table 1. Optical and regression characteristics of proposed and reference methods.

Parameters	Proposed method	Reference method (HPLC)
Wavelength (nm)	412	240
Limit of linearity ($\mu\text{g ml}^{-1}$)	1.25-30	0.5-20
Sandell's sensitivity	$0.0217 \mu\text{g/cm}^2 / 0.001 \text{ absorbance unit}$	
Linear regression equation	$A = 6.021 \times 10^{-4} + 4.589 \times 10^{-2} C$	$H = 0.08865 + 2.56659 C$
Standard addition of intercept	1.33×10^{-3}	0.05598
Standard deviation of slope	8.08×10^{-5}	0.00497
Confidence limit for the intercept	3.145×10^{-3}	0.144
Confidence limit for the slope	1.83×10^{-3}	0.01278
Correlation coefficient (r)	0.9999	0.9999
Variance (S_0^2)	5.2441×10^{-6}	6.78×10^{-3}
Standard deviation of calibration line (S_0)	2.29×10^{-3}	0.08234
Limit of detection ($\mu\text{g ml}^{-1}$)	0.1646	0.0962
Limit of quantification ($\mu\text{g ml}^{-1}$)	0.499	0.3208

Table 2. Specificity and selectivity: Tolerance amount of excipients on the determination of buspirone HCl.

Excipients	Tolerance amount (mg ml ⁻¹)
Glucose	36.032
Fructose	36.032
Sodium benzoate	5.404
Lactose	27.024
Starch	0.15
Povidone	0.05
Methyl cellulose	0.05
Mannitol	0.1
Micro crystalline cellulose	0.0375

Table 3. Precision of the proposed method.

Actual Concentration (µg ml ⁻¹)	Intra day assay:	Inter day assay:
	Measured Concentration ± SD	Measured Concentration ± SD
	(µg ml ⁻¹); RSD (%) ^a	(µg ml ⁻¹); RSD (%) ^a
5.0	4.980±0.140; 2.81	5.130±0.145; 2.82
15.0	15.10±0.230; 1.523	15.197±0.243; 1.60
25.0	24.95±0.200; 0.801	25.134±0.215; 0.86

^aMean for five independent analysis

Table 4. Significance of testing: Point and interval hypothesis tests for the determination of buspirone hydrochloride in tablets at 95% confidence level.

Pharmaceutical formulations	Proposed method		Reference method		Paired t-value ^b	F-value ^b	θ _L ^c	θ _U ^c
	Recovery (%)	RSD ^a (%)	Recovery (%)	RSD ^a (%)				
Freeton 5	99.86	0.776	100.078	0.764	0.447	1.026	0.999	1.005
Buscalm 5	100.08	0.402	100.19	0.778	0.265	3.802	0.998	1.003

^aMean for 5 independent analyses

^bTheoretical *t* (*v* = 8) and *F*-values (*v* = 4, 4) at 95 % confidence level are 2.306 and 6.39, respectively

^cA bias, based on recovery experiments, of ± 2% is acceptable

4. Conclusions

Buspirone hydrochloride is a drug having nitrogen at position 17 which acts as main atom to accept hydrogen atom for protonation and thus formed complex with bromothymol blue. The proposed method was successfully applied for the determination of drug in pharmaceutical preparations in the presence of excipients. Hence the proposed method is more specific and selective. The proposed method has the advantage of having simple operation, high sensitivity, repeatability and reproducibility. In addition, the proposed method has low limit of detection and quantitation. The proposed method can be used for routine quality control analysis of buspirone hydrochloride in industries, research laboratories and hospitals.

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