

## Simple extractive colorimetric determination of buspirone by acid-dye complexation method in solid dosage form

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### Abstract

In the present study, a simple and sensitive extractive spectrophotometric method is described for determination of buspirone. The method is based on the reaction of buspirone and bromocresol green. The ion-pair complex was quantitatively extracted into chloroform at pH 2.3 followed by spectrophotometric determination at 415 nm. The complex was stable up to 2 days and obeyed Beer's law over the concentration ranges of 1.5-6 µg/ml. No significant interference was observed from the excipients, coloring and flavoring agents commonly used in the buspirone pharmaceutical preparations. The proposed method has been applied successfully for determination of buspirone in commercial pharmaceutical preparations.

**Keywords:** Spectrophotometry; Buspirone; Bromocresol green; Ion-pair complex

### INTRODUCTION

Buspirone hydrochloride (BUS-HCl), 8-[4-(4-pyrimidin-2-ylpiperazin-1-yl)butyl]-8-azaspiro[4.5]decane-7,9-dione monohydrochloride (Fig. 1), is an anti anxiety agent, a partial agonist of serotonin receptor (5HT<sub>1A</sub>) and a mixed agonist/antagonist on dopamine receptors that is not chemically or pharmacologically related to the benzodiazepines, barbiturates or other sedative / anxiolytic drugs but it has an efficacy comparable to diazepam in treating generalized anxiety disorder (GAD) (1-3). It is also effective against depression, obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD). BUS-HCl is supplied as tablets for oral administration containing 5, 7.5, 10 or 15 mg of BUS-HCl.

Several analytical methods have been reported to analyze BUS in both biological fluids and pharmaceutical preparations such as: High-Performance Liquid Chromatography (HPLC) (4-6), Gas Chromatography (GC) (7), polarography (8), voltammetry (9), radio-immunoassay (10), capillary GC (11), liquid chromatography with UV detection (12), electrochemical and mass spectrometry (13) and flow injection analysis with tubu-

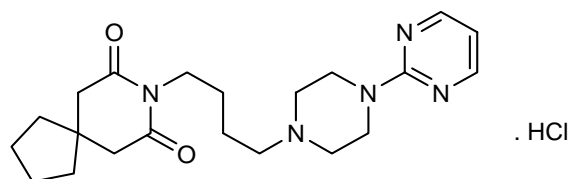


Fig. 1. Chemical structure of buspirone hydrochloride

lar membrane ion-selective electrode (14).

A revision of the literature revealed that methods that have been developed for the analysis of BUS-HCl both in pharmaceutical or biological samples (4-14) are often based on instrumental methods and there are only two reports exist in literature specifically developed for determination of BUS-HCl in pharmaceutical preparation which are based on spectrophotometric methods (15,16).

Extractive spectrophotometric procedures are popular for their sensitivity in the assay of drugs. Therefore, ion-pair extractive spectrophotometry has received considerable attention for the quantitative determination of many pharmaceutical compounds (17-21). So far, there has been no ion-pair extractive spectrophotometry method reported for an estimation of BUS-HCl. The United States

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Pharmacopoeia (USP) (22) described HPLC technique for determination of BUS-HCl in bulk drug and tablet dosage form. These methods for determination of BUS-HCl are mostly time consuming with laborious procedures or costly for routine analysis (4-16). Therefore, having a simple, fast and accurate method for determination of BUS-HCl in raw material and its dosage forms, which can be used in quality control laboratories is a necessity.

The aim of present study was to develop and validate a simple method for determination of BUS-HCl using spectrophotometric method which can be used as an alternative to the official method or other recommended procedures in quality control labs.

## MATERIALS AND METHODS

### *Apparatus*

A Shimadzu UV-160A, UV-Vis spectrophotometer (Japan) with 1 cm quartz cells was used for all absorbance measurements. The pH value of all buffers were adjusted using a Metrohm 692 pH meter.

### *Chemicals and reagents*

All chemicals were of analytical reagent grade (Merck, Germany) unless otherwise specified. Double distilled water was used to prepare all solutions. Freshly prepared solutions were always employed. USP standard buffer solution (pH 2.3) was prepared by diluting 50 ml of 0.2 M potassium hydrogen phthalate and 45.8 ml of 0.2 M HCl to 200 ml with distilled water (22). Bromocresol green solution (BCG,  $1 \times 10^{-4}$  M) was prepared in double distilled water.

BUS-HCl was obtained from Tehran Daru Pharmaceutical Company (Tehran, Iran) and tablets containing 5 mg active material (BUS-HCl) were supplied from the local pharmacy. The inactive ingredients are lactose monohydrate, magnesium stearate, microcrystalline cellulose, colloidal silicon dioxide and sodium starch glycolate (23).

### *Standard solution of the drug*

A stock standard solution of BUS-HCl

( $1 \times 10^{-3}$  M) was prepared by dissolving adequate quantity of BUS-HCl in double distilled water. Working standard solutions were prepared by suitable dilution of stock standard solution with distilled water.

### *Recommended procedure*

Into a series of 100 ml separating funnel flasks, 10 ml of buffer solution of pH 2.3 and 10 ml of BCG ( $1 \times 10^{-4}$  M) were placed. An appropriate volume of  $10^{-4}$  M standard drug solution (0.25–20 ml) was added to each funnel and mixed well for few seconds. The funnels were shaken vigorously with 2×5ml chloroform for 2 min and then allowed to stand for clear separation of two phases. Each separated organic phase was transferred to a 25 ml beaker, dried over anhydrous sodium sulfate, transferred to a 10 ml volumetric flask and were made up to the mark with chloroform and mixed well. The absorbance of the yellow color organic phase was measured at 415 nm against chloroform as a blank. The standard calibration curve was prepared to calculate the amount of the analyte drug in unknown samples.

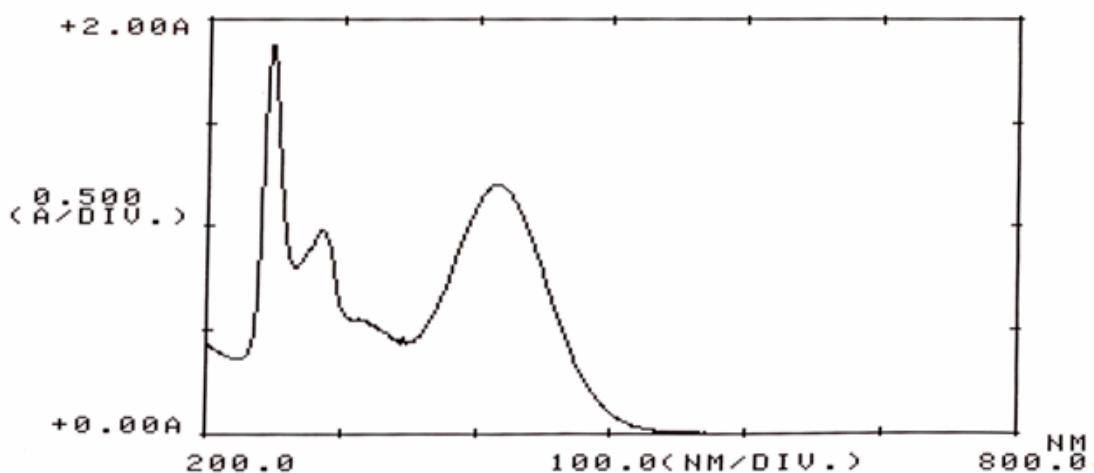
### *Procedure for the dosage form*

Ten tablets were weighted and ground to a fine powder using a pestle and mortar. The average weight of a tablet was calculated. An accurately weighed portion of the powder (109.5 mg), equivalent to 5 mg of BUS-HCl, was transferred into 1000 ml volumetric flask. The volume was adjusted to 1000 ml with distilled water and shaken well. This solution was used for determination of BUS-HCl in tablets.

## RESULTS

### *Spectral characteristics*

Absorption spectra of the yellow color BUS-BCG ion-pair complex is shown in Fig. 2 with a maximum absorbance ( $\lambda_{max}$ ) at 415 nm. The BUS-BCG ion-pair complex formation completed immediately after all reagents were added, no heating or standing time was needed. The colored complex was stable for at least 48 h at room temperature (25 °C) determined by proposed method (Fig. 3).



**Fig. 2.** Absorption spectra of BUS-HCl (4 µg/ml)-bromocresol green ( $10 \times 10^{-4}$  M) ion-paired complex in chloroform obtained through scanning at various wavelengths. The maximum wavelength ( $\lambda_{\max}$ ) was 415 nm.

### **Optimization of variables and method development**

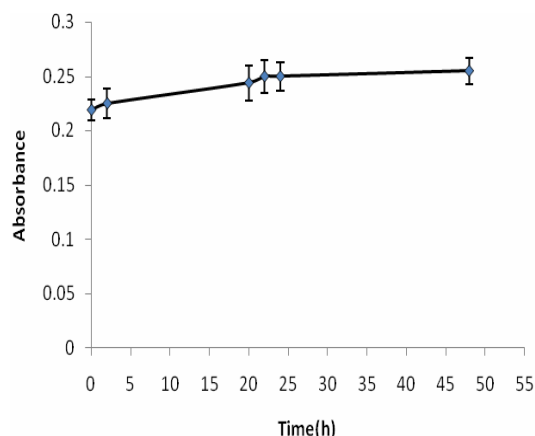
A number of preliminary experiments were performed to optimize the necessary conditions for rapid and quantitative formation of colored ion-pair complex to achieve the maximum stability and sensitivity. Optimum condition was fixed by varying one parameter at a time while keeping other parameter constant and observing its effect on the absorbance at 415 nm.

### **Effect of pH**

The influence of pH of buffer solution on the development and stability of colored complex was tested using different buffer systems as phthalate, potassium hydrogen phthalate, phosphate and acetate buffers. Potassium hydrogen phthalate-HCl buffer solution was the buffer of choice which did not interfere and gave the highest sensitivity for complex formation and extraction. The absorbance of BUS-BCG ion-pair was examined at different pH values range of 1-4. The maximum color intensity was observed at pH ranges of 1.7-2.7 and maximum absorbance was achieved with 10 ml of pH 2.3 buffer solution. This condition was applied throughout the experiment (Fig. 4).

### **Selecting the Extracting Solvent**

As it was mentioned before, chloroform was preferred to other solvents (carbon tetra-

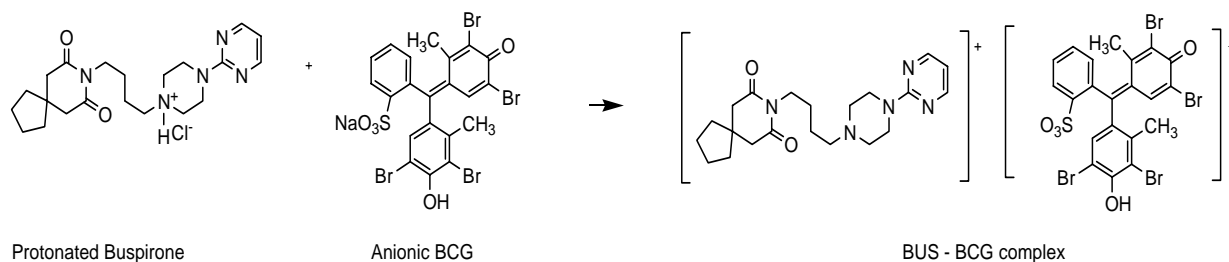


**Fig. 3.** Stability of color complex of BUS-BCG in chloroform at different times (BUS-HCl: 4.5 µg/ml).

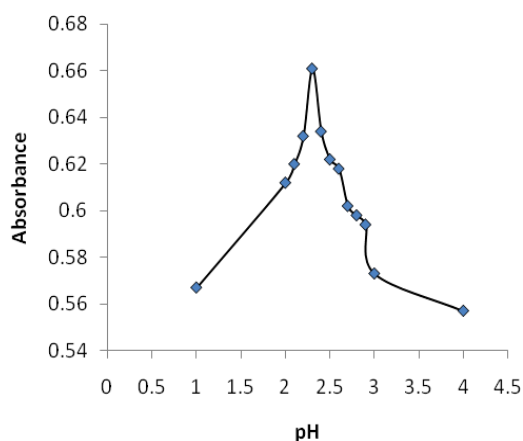
chloride, dichloromethane, and ether) because of higher stability of extracted product, almost higher efficiency on color intensity, selective extraction of the BUS-BCG complex from the aqueous phase and higher absorbance (17-19). Therefore, extraction with  $2 \times 5$  ml of chloroform had a good recovery of the complex in a short time.

### **Composition of ion-pair complexes**

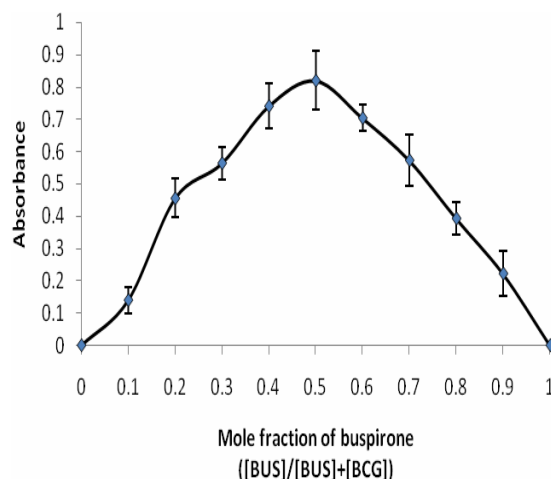
Anionic dyes such as BCG form ion-pair complex with the positively charged nitrogen-containing molecule such as BUS. Each drug-dye complex, with two oppositely charged ions, behaves as a single unit held together by an electrostatic interaction. The suggested mechanism of BUS-BCG ion-pair complex



**Scheme 1.** Structure of analyte and formed ion paired complex. Positively charged nitrogen of buspirone and negatively charged sulfonate of BCG forms an ion-pair complex soluble in organic solvents.



**Fig. 4.** Effect of pH of Potassium hydrogen phthalate-HCl buffer solution on the absorbance of BUS-HCl ( $3 \times 10^{-4}$  M)-BCG ( $10 \times 10^{-4}$  M) ion-pair complex.



**Fig. 5.** Job's method of continuous variation plot for ion-pair complexes of BUS-HCl ( $1 \times 10^{-4}$  M) with BCG ( $1 \times 10^{-4}$  M) in chloroform. Extrapolation of linear portion can be used to locate the position of break point.

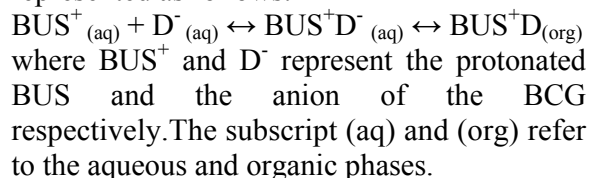
formation is displayed in Scheme 1.

The composition of the ion pairs associates was established by Job's method of continuous variation and mole-ratio method (24,25). In these methods, BUS-HCl and BCG were prepared in the same concentration ( $1 \times 10^{-4}$  M). In the present study, different amounts of BUS-HCl and BCG were added to each flask and extracted in the same manner as recommended procedure. The absorbance of formed BUS-BCG ion-pair complex was measured at 415 nm. The absorbance was plotted against  $[BUS]/[BUS]+[BCG]$  for Job's method (Fig. 5) and  $[BCG]/[BUS]$  for mole-ratio method (Fig. 6). In Job's plot (Fig. 5), the plot reached a maximum value at a mole fraction of 0.5, which indicated the formation of 1:1 (BUS-BCG) complex.

The influence of the volume of BCG ( $1 \times 10^{-4}$  M) in the ranges of 0-16 ml on the absorbance

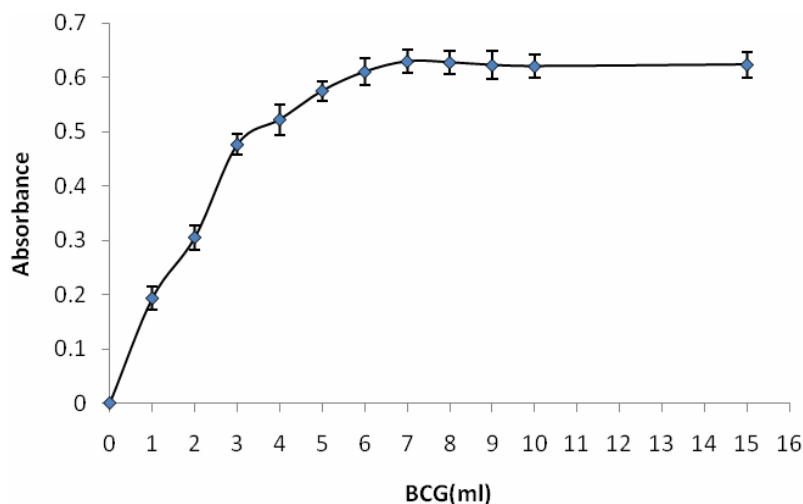
of complex was examined (Fig. 6) using mole-ratio method. The absorbance of complex increased with the change of BCG volume up to 10 ml. Above this value, absorbance remained nearly constant. Therefore, 10 ml of BCG ( $1 \times 10^{-4}$  M) was selected as optimal and used for complete ion-pair formations throughout the experiment.

The extraction equilibrium can be represented as follows:



#### Analytical data

Under the optimized experimental condition, calibration curve was constructed by plotting the absorbance at  $\lambda_{max}$  against the



**Fig. 6.** Mole-ratio plot for BUS-BCG ion-pair complex in chloroform (BCG:  $1 \times 10^{-4}$  M and BUS-HCl: 3.2  $\mu\text{g/ml}$ ). The absorbance reaches a plateau with 10 ml of BCG.

**Table 1.** Optical characteristics and quantitative parameters of the proposed method

Parameters	Results
$\lambda_{\text{max}}$ (nm)	415
Beer's law limit ( $\mu\text{g/ml}$ )	1.5-6
Molar absorptivity (1/mol cm)	$6.61 \times 10^3$
Linear regression equation <sup>a</sup> $y = mC + b$	$0.0172C + 0.004$
Slope ( $m$ )	0.0172
Intercept ( $b$ )	0.004
Correlation coefficient ( $r^2$ )	0.9991

<sup>a</sup> Where  $y$  is absorbance and  $C$  is the concentration ( $\mu\text{g/ml}$ )

concentration of BUS. Beer's law range, molar absorptivity, Sandell's sensitivity, regression equation, and correlation coefficient were determined for proposed method and are given in Table 1. A linear relationship was found between the absorbance at  $\lambda_{\text{max}}$  and the concentration of the drug in the range of 1.5-6  $\mu\text{g/ml}$  for BUS-HCl in the final measured volume of 10 ml with molar absorption coefficients of  $6.61 \times 10^3$  l/mol.cm. Regression analysis of the Beer's law plots at  $\lambda_{\text{max}}$  revealed a good correlation ( $r^2 = 0.9991$ ). The graph showed negligible intercept and were described by the regression equation,  $y = 0.0172 C + 0.004$ ; where  $y$  is the absorbance of 1 cm layer, 0.0172 is the slope, 0.004 is the intercept and  $C$  is the concentration of the

measured solution in  $\mu\text{g/ml}$  obtained by the least-squares method (Table 1). The high molar absorptivity of the resulting colored complex indicates the high sensitivity of the method.

#### Sensitivity

The limit of quantification that can be determined with RSD <3.76% was found to be 1.5  $\mu\text{g/ml}$ . The limit of detection that can be reliably detected with a S/N ratio of 3 was found to be 0.34  $\mu\text{g/ml}$ .

#### Validation of the method

Samples of pure BUS-HCl at two different concentrations were prepared and tested in 5 replicates using the proposed procedure. The

**Table 2.** Evaluation of accuracy and precision for the proposed method.

Amount taken	Amount found ( $\mu\text{g/ml}$ )	Recovery <sup>a</sup> (%)	RSD <sup>b</sup> (%)	RE <sup>c</sup> (%)
10	9.98 $\pm$ 0.010	99.85	0.10	-0.15
15	14.73 $\pm$ 0.115	98.15	0.82	-1.85

<sup>a</sup> Average of five determinations, data presented as mean  $\pm$  SD.

<sup>b</sup> relative standard deviation

<sup>c</sup> relative error

**Table 3.** Determination of BUS-HCl in tablets using the proposed and comparison to official method

Drug trade name	Label claim (mg)	% Recovery of BUS $\pm$ SD <sup>a</sup>	
		Proposed method	Official method <sup>b</sup>
Buspirone	5	97.53 $\pm$ 1.65	102.39 $\pm$ 1.63

<sup>a</sup> Average of five determination. <sup>b</sup> HPLC method for detail, see ref. 22.

**Table 4.** Determination of bupropion hydrochloride in pharmaceutical preparations in presence of excipients

Amount taken ( $\mu\text{g/ml}$ )	Amount added ( $\mu\text{g/ml}$ )	Found ( $\mu\text{g/ml}$ ) <sup>a</sup>	Recovery (%)	RSD (%)
0	10	9.93 $\pm$ 0.14	99.31	1.47
5	10	14.63 $\pm$ 0.24	97.53	1.65

<sup>a</sup> Average of five determination.

complete set of validation assays was performed. The results are given in Table 2. The accuracy of the method is indicated by the good recovery (98.15-99.85%), and the precision is supported by the low relative standard deviation <0.83%.

#### **Application to dosage forms**

The proposed method was successfully applied to the determination of BUS-HCl in commercial tablets. The applicability of the proposed method for assay of BUS-HCl in formulations was examined by analyzing various formulations and the results are tabulated in Table 3. Five replicate determinations were made. Satisfactory results were obtained and were in a good agreement with the label claims (Table 4) for different batches. The results were reproducible with low RSD values (1.65 %).

#### **Comparison with official method**

The reliability and validity of the proposed method was established by parallel

determination against HPLC method described in USP (22). The results were tested by student's t-test and no significant difference was seen between these two methods ( $P < 0.05$ ). The results were reproducible with low RSD values and accurate as indicated in Table 4.

The results of analysis of the commercial formulation and the recovery study of drug suggested that commonly used additives and excipients (23) do not interfere with the assay procedure. The proposed method is sufficiently sensitive to permit determination of low concentration of BUS-HCl (1.5  $\mu\text{g/ml}$ ).

## **DISCUSSION**

Different methods have been reported for determination of BUS-HCl in pharmaceutical preparations (5,15,16). Nevertheless, most of these techniques utilize sophisticated instruments and reagents that are not available in many laboratories or need well trained personnel. A significant advantage of the

extractive spectrophotometric technique is that it can be applied for the determination of individual compounds in a multi-component mixture.

The importance of this technique lies in the chemical reactions upon which the procedure is based rather than upon the sophistication of the instrument so it offers in the assay of a specific component in complex dosage formulations (16). Slight variations in experimental conditions such as temperature, reagent concentration or pH do not affect significantly on this method. The reaction between BUS and BCG takes place at only one site that was the nitrogen atom of piperazine ring attached to alkyl side chain.

Unlike the standard addition method, the present method is not time-consuming. It does not involve procedural steps; does not take much operator time and expertise, and there is no need for any expensive equipment and reagents unlike other methods such as HPLC. The overall advantages of the presented method is its simplicity, sensitivity, rapidity and no need for expensive instruments in comparison to reported techniques. This method can be used for routine determination of BUS-HCl in bulk drug as well as in pharmaceutical preparations.

### CONCLUSION

In the present study, an ion-pair formation method is described for determination of BUS-HCl. This method is simple, rapid, accurate, and applicable in different dosage forms in comparison to official method (22) and other time-consuming, complicated, and costly techniques such as GC and HPLC. These advantages encouraged the application of the proposed method in routine quality control laboratories for determination of BUS in bulk drug and pharmaceutical preparations.

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