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Analytical Determination of Buspirone Hydrochloride in Pure Solutions, Pharmaceutical Preparation and Urine Samples

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Abstract

Simple, rapid, sensitive, precise and accurate spectrophotometeric methods for the determination of Buspirone hydrochloride (Bu-HCl) in bulk samples, dosage form and in spiked urine samples were investigated. The methods are based on the formation of a yellow colored ion-associates due to the interaction between the examined drug with Picric acid (PA), Bromothymol blue (BTB), Alizarin red (AR) and Bactophenol red (BPR) reagents. A buffer solution had been used and the extraction was carried out using organic solvent, the ion associates exhibit absorption maxima at 410, 410, 430 and 423 nm of (Bu-HCl) with PA, BBT, AR and BPR respectively. (Bu-HCl) could be determined up to 42.5, 29.5, 73.8 and 105.5 μg mL^{-1}, using PA, BBT, AR and BPR respectively. The optimum reaction conditions for quantitative analysis were investigated. In addition, the molar absorptivity and Sandell sensitivity were determined for the investigated drug. The correlation coefficient was ≥ 0.995 (n=6) with a relative standard deviation (RSD) ≤ 2.66 for five selected concentrations of the reagents. Therefore the concentration of Bu-HCl in its pharmaceutical formulations and spiked urine samples had been determined successfully.

Keywords: Buspirone hydrochloride; Bromothymol blue; Bactophenol red; Alizarin red; Picric acid

Introduction

Buspirone hydrochloride 8-[4-[4-(2-Pyrimidinyl)-1–piperazinyl] butyl]-8–azaspiro[4.5]decane–7,9–dione hydrochloride, is an anti anxiety agent, partial agonist of serotonin receptors and a mixed agonist /antagonist on dopamine receptors. It is also effective against depression, obsessive compulsive disorder, attention deficit hyperactivity disorder [1].

Several analytical methods have been applied to determine Buspirone hydrochloride (Bu-HCl) quantitatively in their dosage forms including spectrophotometric method [2-5], liquid chromatography mass spectrometry LC/MS [6-9], High Performance Liquid Chromatogrphy HPLC [10-18], gas chromatography [19] gas chromatography mass spectrometry GCMS [20-22], ion selective electrode [20-23] and potentiometry [24-26].

In the present work simple, rapid, sensitive, precise and accurate spectrophotometeric methods for the determination of Buspirone hydrochloride (Bu-HCl) in bulk samples, dosage form and in spiked urine samples were suggested.

The proposed methods are useful for routine quality control laboratory and are suiTable to be used in forensic Chemical laboratories and drug control laboratories as identification, confirmation and quantitative determination methods.

Experimental

Apparatus

The electronic absorption spectral measurements of Bu–HCl (Figure 1) with selected reagents were recorded on Agilent 8543 UV-V is spectrophotometer equipped with quartz cell of 1 cm optical path length with a resolution of 0.1 nm. The pH measurements of the prepared solutions were adjusted using Jenway 3510 pH meter. All spectrophotometric measurements were carried out at room temperature (25 ± 2°C). Moreover, doubly distilled water was obtained Millipore distillation apparatus model Direct Q3, France.

Materials

Buspirone hydrochloride (Bu-HCl) and Buspar tablets (10 mg/tablet) (provided from Bristol-Myers Squibb, Egypt): All chemicals used through the work were of analytical reagents grade and solutions

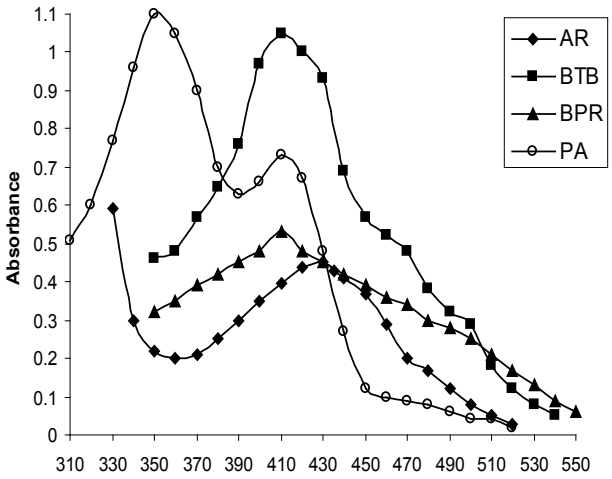

Figure 1: Absorption spectra of (Bu-HCl) ion-associates with AR, BTB, BPR and PA

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were made with doubly distilled water. They included sodium sulphate anhydrous (BDH), highly purified solvents as chloroform (Sigma-Aldrish), methanol (Merck), methylene chloride (BDH), carbon tetrachloride, benzene (Prolabo), petroleum ether, diethyl ether, toluene, ethyl acetate, acetone (Merck), Bromothymol blue (Merck), Bactophenol red (Aldrich), picric acid (Arablab) and alizarin red S (Fluka).

Preparation of Stock and Standard Solutions of 2.0 x 10^{-3} M were prepared with doubly distilled water: Acetate buffer solutions were made of a mixture of 0.1 M acetic acid (1050 gL^{-1}) and 0.1 M sodium acetate trihydrate (13.6 gL^{-1}). On the other side Phosphate buffer solutions were made of a mixture of 0.1M disodium hydrogen phosphate (14.2 gL^{-1}), 0.1 M HCl and 0.1M NaOH.

General procedure

Into 125 ml separating funnel, 5.0 mL (2.0 x10^{-3} M) of reagents (PA, BBT, AR and BPR) were added to different volumes of solution containing (2.0 ×10^{-3} M) of (Bu-HCl), and 2.0 mL of buffer solution were added and the volume was made up to 10 mL with distilled water. The formed ion–associates were extracted using the separating funnel with 10 mL chloroform, the ion-associates were shaked for two minutes and allowed to separates into two phases. The organic layer was collected and dried with anhydrous sodium sulphate then completed to 10 mL chloroform. The absorbance of the extract was measured at the recommended wavelength (λmax).The blank solutions were prepared using the same method in absence of the examined drug. Linear curves were obtained by plotting absorbance versus concentration at respective λ_{max} for each reagent. The calibration graphs were constructed and the concentrations of unknown samples were determined by using these graphs.

Procedure for dosage forms

For the analysis of (Bu-HCl) in tablets [Buspar tablets (10 mg / tablet), five tablets were weighed into a small dish, powdered and mixed well, then dissolved in 100 mL bidistilled water, a turbid solution was shaken well and filtered through a filter paper to obtain a clear solution. Then, the clear solution was diluted with bidistilled water in a 100 mL calibrated measuring flask. Successive dilutions were prepared for carrying out the subsequent studies. The drug content of these solutions was obtained by applying the general procedure to aliquot containing different volumes of drug solutions as described above.

Procedure for spiked urine samples

For spiked human urine five milliliters of investigated drugs free urine taken in a 125 mL separating funnel was spiked with different volumes of solution containing (2.0 x10^{-3} M), Bu-HCl then 2.0 mL of buffer solution were added and the volume was made up to 10 mL with distilled water. The drug content of this solution was obtained by applying the general procedure to aliquot containing different volumes of solution drug as described above.

Composition of ion-associates

To investigate the molecular composition of (Bu-HCl) ion-associates with (PA, BBT, AR and BPR) reagents, a series of solutions was prepared in which the reagent contents was kept constant, while that of the drug was regularly varied and the method was accomplished as previously mentioned in the general procedure. The absorbances of the resultant extracts were measured at the respective λ_{max} of the ion-associates. The absorbance values were plotted against the molar ratio [drug]/ [reagent] [27].

Job's method of continuous variation method [28] was employed, 2.0×10^{-3} M solution of investigated drugs was mixed with 2.0 x10^{-3} M solution of each selected reagent. A series of solutions were prepared in which the total volume of drug and reagent was kept constant. The reagents were mixed with each drug in various proportions along with the chosen buffer solution, which then diluted in calibrated flask with the appropriate solvent following the above mentioned procedures.

Results

Optimization of the reaction conditions

In order to determine the most favorable conditions to achieve maximum color intensity of investigated drug, the following parameters were investigated to achieve the optimum conditions to aid in accurate quantitative analysis for these drugs.

Selection of suiTable wavelength: The absorption spectra of Bu-HCl ion associates with (PA, BBT, AR and BPR) were recorded as shown in (Figure 2). The optimum wavelengths of maximum intensity (λ_{max}) of investigated drug - coloring reagents, ion- associates were 410, 410, 430 and 423 nm for Bu-HCl ion associates with PA, BBT, AR and BPR respectively. The wavelengths maximum absorbencies (λ_{max}) of the drug-coloring reagent ion-associates were recorded and tested against reagent blanks (prepared in the same manner without the addition of drug) to study the influence of each of the following variables on the formed ion associates between drugs and reagents.

Effect of pH: To elucidate the most suiTable medium for the quantitative determination of investigated drug ion associates with PA, BBT, AR and BPR the effect of pH was studied to reach to the optimum pH values using acetate buffer solutions for each ion-associate. The optimum pH ranges for complete formation of the ion-associates which showed the highest absorbance values, at their respective λ_{max} were (3-5), (4-5), (3-6) and (3-6), for Bu-HCl ion associates with PA, BBT, AR and BPR respectively as shown in (Figure 2). From the results shown in (Figure 2) we can observe that there are suiTable ranges of stability of the absorbances of the drug- coloring reagent ion- associates which indicates that small change in pH will not affect on the efficiency of the formation of ion associates. So the proposed methods are suiTable for determination of investigated drugs at different pH values.

Effect of extracting solvents: The polarity of the solvent affects

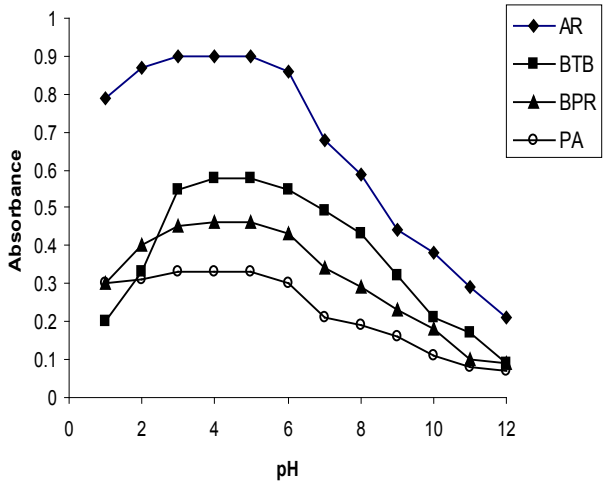

Figure 2: Effect of pH on (Bu-HCl) ion-associates with AR, BTB, BPR and PA

both extraction efficiency and absorpitivity of the ion-associates. Several water-immiscible organic solvents including benzene, toluene, carbon tetrachloride, methylene chloride, ether, nitrobenzene, chloroform, ethyl acetate, and mixture of chloroform: acetone either 9:1 or 4:1 had been checked to select the most convenient solvent of the highest absorbance. The aqueous to organic phase ratio of 1:1.5 was the most suiTable for the ion-associate extraction. Complete extraction was attained by using one portion, of 10 mL solvent on using the above reagents. It was found that chloroform is the most suiTable solvent for the extraction of drug- coloring reagent ion -associates in all cases.

Effect of reagent concentration: The effect of reagent concentration was tested by using various amount (1-6) mL of 2×10^{-3} M solution of each reagent with 1 mL of 2×10^{-3} M of tested drugs. The results showed that 5 mL of of 2×10^{-3} M of PA, BBT, AR and BPR regent solutions were sufficient for the production of maximum and reproducible color intensity of the investigated ion-associates at their λ_{max}. Further excess of the reagents have no considerable effect on the extracted fraction of the ion associates.

Effect of sequence of mixing: Several different possible sequences of addition were checked to select the most suiTable one for developing the most sTable concerned ion-associates showed the highest absorptivity. The optimum sequence of mixing was found to be drug, reagent, buffer, and then solvent, which allow the highest color intensity and shortest time to obtain maximum absorbance. On the other hand, other sequences rather the one given above requires longer time in addition to lower stability of the formed ion associates.

Effect of time: Under the above mentioned conditions the effect of time on the formation of the ion-associates was studied by measuring absorbance of the extracted ion-associates formed between its investigated drugs and the coloring reagents at increasing time intervals. The results showed that the ion-associates were formed almost instantaneously and the developed color remained sTable for several hours which are 9, 8, 7 and 9 hrs for Bu-HCl ion associates with PA, BBT, AR and BPR respectively. After these intervals, a decrease in color intensity occurred. The effect of time on the stability of the ion–associates is represented graphically in (Figure 3).

The ranges of the stability of the investigated drugs- coloring reagents ion- associates are arranged between 7 and 9 hours which gives the proposed methods the chance for undergoing all available studies without change in absorbance due to time factor.

Effect of temperature: Under the above mentioned conditions (pH, solvents, reagent concentration, sequence of mixing and time) the effect of temperature on the formation of the ion-associates of investigated drugs with coloring reagents was studied at temperature range 25-100°C. The results showed that the ion-associates were formed almost instantaneously in all cases at room temperature 25 ± 5 °C and remained constant up to different temperature ranges which are 50, 60, 55 and 50°C for Bu-HCl ion associates with PA, BBT, AR and BPR respectively. The effect of temperature on the stability of ion–associates is shown in (Figure 4), the results show that the ion associates of investigated drugs with coloring reagents are sTable in the temperature range (25°C- 60°C) which is suiTable to apply the proposed methods in different temperature ranges i.e. the proposed methods are suiTable to be applied in hot and cold places and at different weather.

The stoichiometry of the ion-associates: The stoichiometry of the ion-associates formed between investigated drug and reagents was investigated by the aid of the following recommended methods.

The molar ratio method: To investigate the molecular composition of investigated drug ion-associates with coloring reagents, serieses of solutions were prepared in which the reagents contents were kept constant, while that of the drugs regularly varied and the method was accomplished as previously mentioned in the general procedure. The absorbance of the resultant extracts was measured at the respective λ_{max} of the ion-associates. The absorbance values were plotted against the molar ratio [drug]/[reagent](D/R) as shown in (Figure 5), where straight lines were obtained intersecting at 1 that means the molar ratio of 1:1 and (drug: reagent). Results showed that the existence of 1:1 in all cases with the investigated drug.

The continuous variation method: Series of solutions were prepared by mixing equimolar solutions of the drug and reagent in varying proportions while keeping the total molar concentration constant and the method was accomplished as previously mentioned in general procedure. The absorbance of the resultant extracts was measured at the respective λ_{max} of the ion-associates. A plot of the absorbance versus

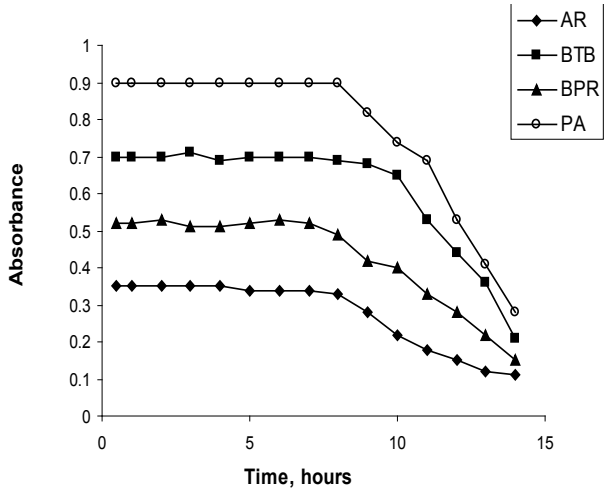

Figure 3: Effect of time on the stability of Bu-HCl ion-associates with AR, BTB, BPR and PA

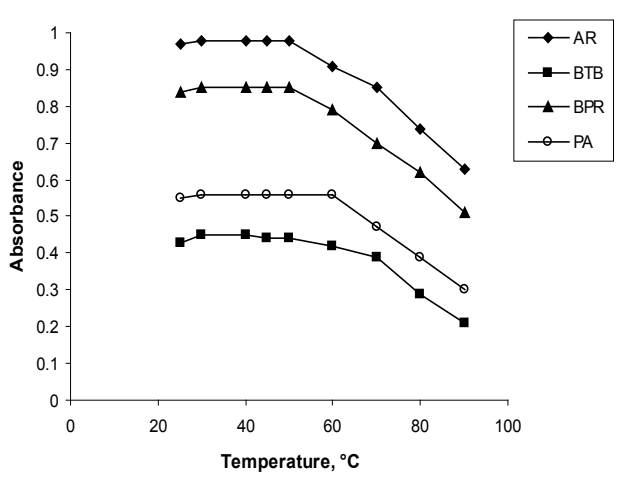

Figure 4: Effect of temperature on the stability of Bu-HCl ion-associates with AR, BTB, BPR and PA

the mole fraction of the drug is represented graphically in (Figure 6). The curves exhibit a maximum at mole fraction at 0.5 indicating that formation of 1:1 (drug: reagent), maximum at mole fraction at 0.66 indicating that formation of 2:1 (drug: reagent), while maximum at mole fraction at 0.75 indicating that formation of 3:1 (drug: reagent). Results showed that the existence of 1:1 in all cases with the investigated drug. It was found that the stoichiometry of investigated drugs- coloring reagent ion-associates obtained from molar ratio results matched with those obtained from continuous variation results which confirm the obtained stoichiometry of the reactions by both methods.

Conditional stability constant (k_f) of the ion- associates: The stability constants of the ion associates were evaluated. The formation of the ion- associates was rapid and the colored extracts were sTable at least 7 hours for drug -reagent ion associates without any change in color intensity and the maximum absorbance at room temperature. The conditional stability constant (k_f) of the complex species of the ion- associates for the studied drugs with the coloring reagents were

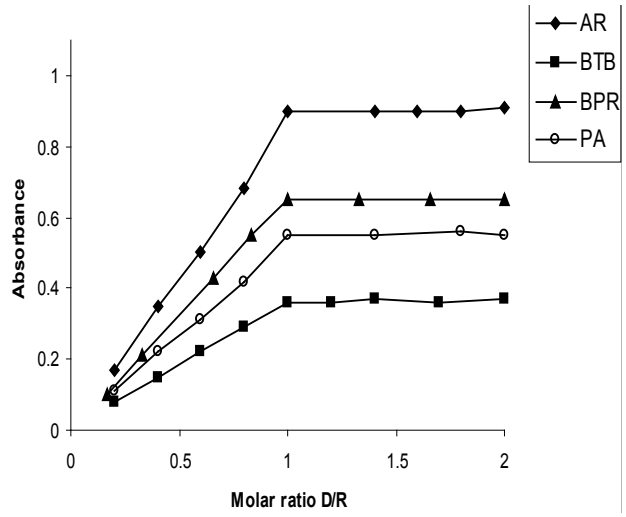

Figure 5: Molar ratio of Bu-HCl ion-associates with BTB, BPR, AR and PA

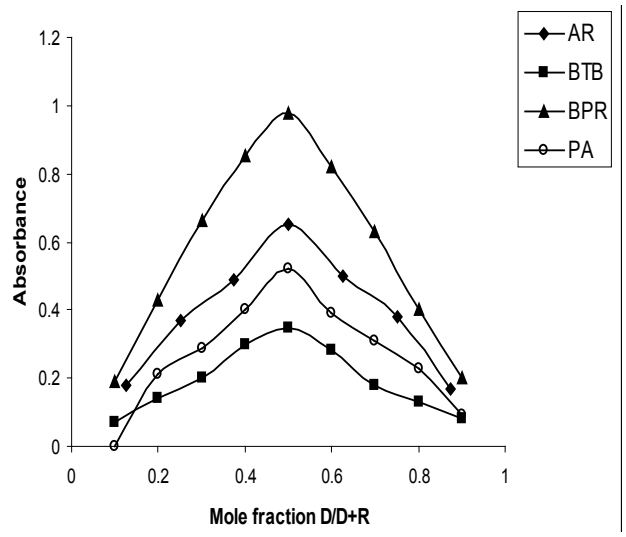

Figure 6: Continuous variation of Bu-HCl ion-associates with AR, BTB, BPR and PA

calculated from the continuous variation data using the following equation :

$$k_f = \frac{A / A_m}{[1-(A / A_m)]^{n+2} C_M (n)^n}$$

Where A and A_m are the observed maximum absorbance and the absorbance value when all the drug is associated, respectively. C_M is the mole concentration of the drug at the maximum absorbance and n is the stoichiometry with which dye ion associates with drug [29]. Calculated conditional stability constants were 3.28, 2.87, 2.95 and 2.67 for Bu-HCl ion associates with PA, BBT, AR and BPR respectively. In accordance with the formula the conditional stability constants reflected high stability of ion associates.

Influence of foreign ions: The effect of presence of foreign ions was studied by measuring the absorbance of the solutions containing 1 mL of 2×10^{-3} M drug solution together with varying excess of different additives and excipients which may be present in the pharmaceutical preparations using the recommended methods of such reagents for Bu-HCl. No significant interference was observed from excipients commonly used such as glucose, lactose, starch, sucrose, magnesium stearate, methyl paraben and propyl paraben. This shows that the method is highly selective and applicable safely in the case of pharmaceutical preparations of the investigated drug.

Effect of other variables: To study the effect of the shaking time for complete extraction of the ion-associates, the general procedure was applied after shaking the mixture for different time intervals (0.5-5.0 min). It was found that two min. is quite enough to complete the ion associate formation with highest absorptivity.

Number of extraction cycles was checked by applying the general procedure for single, double and triple extraction (each of two minutes) of the formed ion–associates. Complete extraction was attained by using one step of 10 mL of solvent using selected reagent within the usable concentration range.

The amount of water-immiscible organic solvent was tested by using the general procedure in the sequence of addition of reagent-drug- solvent and different amount of organic solvent. The results revealed that a volume ratio of 1:1.5 (aqueous phase: organic phase) was enough for the quantitative extraction of ion-associates of the investigated drugs with the selected reagents.

Obedience to Beer's law and method validation

Linearity: The obedience of absorbance of the ion-associates of the investigated drugs with coloring reagents to Beer's law is shown in (Figure 7) The linear concentration ranges , the molar absorpitiveties (Є) were calculated and tabulated in (Table 1) indicating high sensitivity of the reagents under investigation for the determination of investigated drugs.

The regression equation (A = a + bc, where A= absorbance, a = intercept, b = slope and c = concentration in µg mL^{-1}), calculated from the calibration graph according to the Kaliedgraph program, were evaluated and recorded in (Table 1). The intercepts of the lines were very small indicating that there is no systematic difference between the determined and expected concentrations within the investigated range using the present methods (Figure 7). For more accurate results, ringbom concentration range was determined by plotting log [drug] in µgmL^{-1} against % transmittance from which the linear portion of the curve gives accurate range for the drug under investigation (Table 1).

Sensitivity: The detection limit (LOD) for the proposed method was calculated using the following equation

$$LOD = \frac{3S}{k}$$

Where S is the standard deviation of replicate determination values under the same conditions as the sample analysis in the absence of the drug and k is sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits obtained for the absorbance were calculated and listed in (Table 1). The limit of quantitation, LOQ, defined as:

$$LOQ = \frac{10S}{k}$$

According to this equation, the limits of quantification were calculated and listed in (Table 1).

precision and accuracy: In order to determine the accuracy and precision of the method, solutions containing four different concentrations of the studied drugs were prepared and analyzed in quintuplicate. Low values of Percentage relative standard deviation (R.S.D. %) indicating good precision and reproducibility (repeatability) of the proposed methods (Table 2). Precision was carried out by six determinations at four different concentrations using these spectrophotometric methods. Percentage relative error (Er %) as accuracy of the suggested methods was calculated using the following equation:

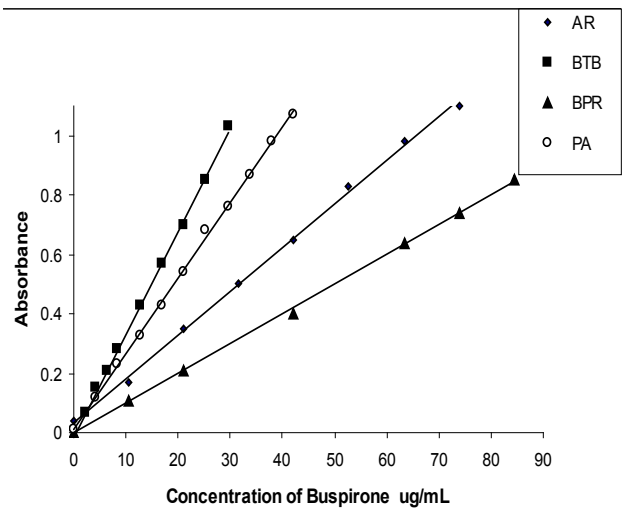

Figure 7: Standard curves of Bu-HCl ion-associates with AR, BTB, BPR and PA

Parameter	Bu /PA	Bu /BPR	Bu /AR	Bu /BTB
λ max (nm)	410	430	435	430
Beer's law up to (μgmL^{-1})	42.5	105.5	73.8	29.5
Molar absorpitivity (ε) (Lmol^{-1} cm^{-1})	1.8 x10^3	4.26 x10^3	6.67 x10^3	14.33 x10^3
Ringbom (μg mL^{-1})	2.68	8.06	8.20	1.61
Sandell sensitivity (μg cm^{-2})	0.039	0.099	0.063	0.0294
Limit of detection(μgmL^{-1})	0.53	2.24	0.70	0.21
Limit of quantification (μgmL^{-1})	1.67	0.96	2.34	0.699
Color of ion- associate	Yellow	Yellow	Yellow	Yellow
Intercept	0.013	- 0.002	0.033	- 0.017
Slope	0.025	0.01	0.015	0.035
Correlation Coefficient	0.999	0.999	0.999	0.998

Table 1: Characteristics and analytical data of (Bu-HCl) ion-associates with PA, BPR, AR and BTB

$$Er\% = [\frac{Found - added}{Added}] \times 100$$

Found - added

Added

The inter–day and intra-day precision and accuracy results are shown in (Table 2) indicate that the proposed methods are precise. The average percent recoveries were in range (96.5%-100.1%) indicating good accuracy of the proposed methods. These results showed that the proposed methods have good repeatability and reproducibility.

Robustness and ruggedness: For the evaluation of the method robustness, some parameters were interchanged, pH, reagent concentration, wavelength range, and shaking time. The capacity remains unaffected by small deliberate variations. Method ruggedness was expressed as R.S.D. % of the same procedure applied by two analysts and using two different instruments on different days. The results showed no statistical difference between different analysts and instruments suggesting that the developed methods were robust and rugged (Table 3).

Analytical applications: The proposed methods were successfully applied to dosage forms and spiked urine samples of the investigated drugs. Six replicate determinations, using reported coloring reagents at different concentration ranges, were carried out for pure Bu-HCl, Buspar tablets and their spiked urine samples. The recovery values almost reach 100%, revealing a high accuracy of the results (Table 4). The mean values obtained and the calculated standard deviations are compared with those obtained by the official methods [30,31], by applying the t- and F- tests [32,33] (Table 5). Such comparison showed that there is no significance difference between proposed and the Official methods. This indicates that high accuracy and precision of the proposed methods. So the present methods are accurate, precise, highly sensitive, rapid, and simple and their results are in good agreement with those of the official methods. Table 5 shows the results of the successful analytical applications of the suggested methods to the dosage forms of the investigated drugs and their spiked urine samples using the reported coloring reagents.

Conclusion

The proposed methods were made by using simple reagents which most ordinary analytical laboratories can afford. The methods are sufficiently sensitive to permit determination of the investigated drugs at given optimum conditions in pure solutions, pharmaceutical preparations and spiked urine samples. Unlike gas chromatography and high performance liquid chromatography, Spectrophotometry is relatively simple to handle and affordable.

The proposed methods are simple, precise, accurate, robust, rugged and low coast. So the proposed methods should be useful for routine quality control purposes and pharmaceutical industries. The proposed methods are economical in the determination of the investigated drugs in pure solutions, pharmaceutical preparations (which may be used as illicit drugs) and spiked urine samples (which may be help in detection of drug abusers, overdoses and suicidal cases) . So the proposed methods are suiTable to be used in forensic Chemical laboratories and drug control laboratories as identification, confirmation and quantitative determination methods.

PA gave good results in determination of Bu-HCl Low limits of detection, limits quantifications, high accuracy, high recovery, and high precision values indicate the suitability of this reagent in determination of investigated drug. AR is recommended in determination of Bu -HCl due to its high recovery.

Inter-day				Intra-day					
Confidence limit[b]	Accuracy Er%	PrecisionRSD%	Recovery %	Confidence limit[b]	Accuracy Er%	Precision RSD%	Recovery %[a]	Added µgmL^{-1}	
4.89 ± 0.14	- 2.20	2.66	97.80	4.98 ± 0.15	- 0.40	2.81	99.60	5	RK
9.89 ± 0.20	- 1.10	1.92	98.90	9.97 ± 0.17	- 0.30	1.6	99.70	10	
14.90 ± 0.17	- 0.77	1.08	99.23	14.81 ± 0.13	- 1.30	0.81	98.70	15	
19.30 ± 0.19	- 0.92	1.09	99.08	19.8 ± 0.20	- 0.79	0.96	99.21	20	
19.90 ± 0.22	- 0.70	0.91	99.30	19.8 ± 0.16	- 1.00	0.76	99.00	20	CLPH
39.70 ± 0.16	- 0.70	0.38	99.29	39.7 ± 0.13	- 0.70	0.30	99.30	40	
59.27 ± 0.1	- 1.21	0.15	98.79	59.5 ± 0.10	- 0.80	0.17	99.20	60	
79.40 ± 0.14	- 0.80	0.16	99.20	79.10 ± 0.18	- 1.08	0.21	98.92	80	
9.88 ± 0.23	- 1.24	2.23	98.76	9. 96 ± 0.12	- 0.39	1.1	99.61	10	PA
19.60 ± 0.17	- 2.01	0.82	97.99	19.90 ± 0.19	- 0.69	0.91	99.31	20	
29.70 ± 0.16	-0.87	0.50	99.13	29.70 ± 0.14	- 0.89	0.44	99.11	30	
39.70 ± 0.15	- 0.73	0.35	99.27	39.80 ± 0.13	- 0.62	0.30	99.38	40	
14.80 ± 0.24	- 1.22	1.55	98.78	4.98 ± 0.15	- 0.79	1.41	99.21	15	AR
29.60 ± 0.18	-1.38	0.57	98.62	4.98 ± 0.15	- 1.03	0.47	98.97	30	
44.60 ± 0.17	- 1.00	0.36	99.00	4.98 ± 0.15	-0.90	0.25	99.10	45	
59.64 ± 0.20	-0.60	0.32	99.40	4.98 ± 0.15	- 0.68	0.25	99.32	60	

n-number of determination, R.S.D. %, percentage relative standard deviation, Er%, percentage error.
[a]mean of five determination
[b]confidence limit at 95% confidence level and five degrees of freedom

Table 2: The intra-day and inter-day precision and accuracy of data obtained for determination of (Bu-HCl) by the proposed methods (n = 6)

	Different instrument		Different analyst	
	R.S.D.%	X±S.D	R.S.D.%	X ±S.D
Bu- BTB	-	-	-	-
Pure Bu -HCl (10 µg mL^{-1})	1.62	9.90±0.16	1.84	9.79±0.18
Buspar 10 mg per tablet(10 µg mL^{-1})	2.10	10.1±0.21	1.90	9.99±0.19
Spiked urine sample(10 µg mL^{-1})	1.73	9.83±0.17	2.04	9.79±0.20
Bu-BPR	-	-		-
Pure Bu -HCl (10 µg mL^{-1})	1.11	9.92±0.11	1.42	9.87±0.14
Buspar 10 mg per tablet(10 µg mL^{-1})	1.29	10.05±0.13	1.57	10.20±0.16
Spiked urine samples(10 µg mL^{-1})	1.23	9.73±0.12	1.84	9.79±0.18
Bu - PA	-	-		-
Pure Bu -HCl (10 µg mL^{-1})	1.01	9.87±0.10	0.82	9.73±0.08
Buspar 10 mg per tablet(10 µg mL^{-1})$_{-1}$	1.70	10.0±0.17	1.50	9.97±0.15
Spiked urine samples(10 µg mL)	1.42	9.85±0.14	1.21	9.93±0.12
Bu –AR	-	-	-	-
Pure Bu -HCl (10 µg mL^{-1})	0.91	9.89±0.09	2.12	9.91±0.21
Buspar 10 mg per tablet (10 µg mL^{-1})	1.29	10.11±0.13	1.09	10.07±0.11
Spiked urine amples(10 µg mL^{-1})	1.92	9.9±0.19	1.52	9.84±0.15

*: theoretical value at 95% confidence level.
n: number of replicates

Table 3: The results of analysis of Bu-HCl obtained by two different analyst and instrument (n = 6)

reagent	Pure solution			Buspar tablets 10 µg / tablet			Spiked urine samples		
	Taken µgmL-1	found µgmL-1	Recovery%	Taken µgmL-1	found µgmL-1	Recovery%	Taken µgmL-1	found µgmL-1	Recovery%
PA	5	4.93	98.60	5	5.10	102.00	5	4.60	92.00
	10	9.96	99.61	10	9.80	98.00	10	9.30	93.00
	15	14.6	97.33	15	16.00	106.67	15	14.10	94.00
	20	19.86	99.31	20	19.80	99.00	20	19.20	96.00
	25	24.8	99.20	25	25.10	100.40	25	24.30	97.20
	Mean recovery ± RSD* 98.81 ± 1.1			Mean recovery ± RSD* 101.21 ± 1.3			Mean recovery ± RSD* 94.44 ± 1.55		
AR	10	9.80	98.00	10	10.00	100.00	10	9.60	96.00
	20	19.70	98.50	20	19.90	99.50	20	19.00	95.00
	30	29.50	98.33	30	31.00	103.33	30	29.10	97.00
	40	39.60	99.00	40	40.20	100.50	40	39.20	98.00
	50	49.30	98.60	50	49.70	99.40	50	49.10	98.20
	Mean recovery ± RSD* 98.48 ± 1.81			Mean recovery ± RSD* 100.55 ± 1.40			Mean recovery ± RSD* 96.84 ± 1.97		

BTB	5	4.98	99.60	5	4.95	99.00	5	4.80	96.00
	10	9.97	99.70	10	10.20	102.00	10	9.70	97.00
	15	14.81	98.70	15	14.80	98.67	15	14.80	98.67
	20	19.84	99.21	20	19.80	99.00	20	19.60	98.00
	25	24.70	98.80	25	26.00	104.00	25	24.70	98.80
	Mean recovery ± RSD* 99.20 ± 1.6			Mean recovery ± RSD* 100.53± 1.6			Mean recovery ± RSD* 97.69 ± 2.06		
BPR	20	19.80	99.00	20	21.00	105.00	20	19.00	95.00
	40	39.70	99.25	40	39.80	99.50	40	39.00	97.50
	60	59.52	99.20	60	59.70	99.50	60	59.00	98.33
	80	79.14	98.92	80	81.00	101.25	80	78.00	97.50
	100	99.00	99.00	100	101.00	101.00	100	98.00	98.00
	Mean recovery ± RSD* 99.07 ± 1.10			Mean recovery ± RSD* 101.25 ± 1.79			Mean recovery ± RSD* 97.27± 1.75		

Table 4: Spectrophotometric determination of (Bu-HCl)

Parameters	Official method	Bu-BTB	Bu-BPR	Bu-PA	Bu -AR
Pure solution 10 µg m L^{-1} X±SD n t-value* F-value	99.93±0.13 6	99.70±0.16 6 0.24 1.51	99.83±0.11 6 0.14 1.40	99.61±0.11 6 0.14 1.40	99.38±0.18 6 0.61 1.92
Buspar 10mg / tablet 10 µg m L^{-1} X±SD n t-value* F-value	100.55±0.12 6	102.0±0.16 6 1.77 1.78	100.40±0.18 6 0.17 2.25	100.24±0.13 6 0.43 1.17	100.10±0.14 6 0.60 1.36
Spiked urine samples 10 µg m L^{-1} X±SD n t-value* F-value	98.10 ± 0 .18 6	97.0±0.20 6 1.0 1.23	97.30±0.17 6 0.79 1.12	96.8±0.15 6 1.36 1.44	96.5±0.19 6 1.50 1.11

*: theoretical value at 95% confidence level.
n: number of replicates

Table 5: Statistical treatment of data obtained for determination of (Bu-HCl) applying the proposed methods in comparison with the reference method.

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