ISSN : 0974-7419 Volume 12 Issue 9

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 12(9) 2013 [338-346]

Sensitive and selective extractive determination of Citalopram hydobromide in pure solutions, pharmaceutical dosage form and urine samples

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ABSTRACT

Simple, rapid, sensitive, precise and accurate spectrophotometeric methods for the determination of antidepressant drug Citalopram Hydrobromide (C-HBr) 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 (C-HBr) with Picric acid (PA), Bactophenol red (BPR), Alizarin red (AR), Bromothymol blue (BTB) reagents. Abuffer solution had been used and the extraction was carried out using chloroform, the ion associates exhibit absorption maxima at 410, 403, 432 and 415 nm for PA,BPR, AR and BTB respectively. (C-HBr) can be determined up to 35, 58, 85 and 40 μg mL^{-1}, respectively. The optimum reaction conditions for quantitative analysis were investigated. In addition the molar absorptivity, Sandell sensitivity were determined for the investigated drug. The correlation coefficient was ≥ 0.995 (n =6) with a relative standard deviation (RSD) ≤ 1.15 for five selected concentrations of the reagents. Therefore the concentration of C-HBr drug in its pharmaceutical formulations and spiked urine samples had been determined successfully.

KEYWORDS

Citalopram hydrbromide;
Bactophenol red;
Bromothymol blue;
Alizarin red;
Picric acid.

INTRODUCTION

Citalopram hydrobromide has a wide range of applications in pharmaceutical chemistry; it is white crystalline powder that is easily soluble in water.

Citalopram hydrobromide 1-[3-(Dimethylamino) propyl]-1-(4–fluorophenyl)-1,3–dihydro–5–isobenzofurancarbonitrile, is a selective and potent serotonin reuptake inhibitor with a very broad spectrum of therapeutic activity against depression, anxiety, obsessive and impulse control disorder.

Several analytical methods had been applied to determine Citalopram hydrobromide (C -HBr) quantitatively in their dosage forms including spectrophotometric method[2-6], liquid chromatography mass spectrometry LC/MS[7-13] High Performance Liquid Chromatogrphy HPLC[14-25], gas chromatography mass spectrometry[26], gas chromatography with flame ionization detector[27] capillary electrophoresis[28-31], ion selective electrode[32] and flourimetry[33-34].

EXPERIMENTAL

Apparatus

The electronic absorption spectral measurements

of C -HBr (Figure 1) with selected reagents were recorded on Agilent 8543 UV-Vis 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 were obtained Millipore distillation apparatus model Direct Q3, France.

Materials

Citalopram hydrobromide and citalo tablets (20 mg/tablet) were obtained from delta pharma, Egypt. All chemicals used through the work were of analytical reagents grade and solutions were made with doubly distilled water. They included sodium sulphate anhydrous (BDH); highly purified solvents as chloroform (labscan), methanol (BDH), methylene chloride (BDH), carbon tetrachloride, benzene (Prolabo), petroleum ether, diethyl ether, toluene, ethyl acetate, acetone (Merck), ammonium reineckate (Merck), chlorophyllin coppered trisodium salt (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 g/L) and 0.1 M sodium acetate trihydrate (13.6 g/L) On the other side Phosphate buffer solutions were made of a mixture of 0.1M disodium hydrogen phosphate (14.2g/L), 0.1M HCl and 0.1M NaOH.

General procedure

Into 125 mL separating funnel, 5.0 mL (2.0 x10^{-3} M) PA, BPR, AR and BTB were added to different volumes of solution containing (2.0 x10^{-3} M) C-HBr. 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, by shaking 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 (λ_{max}) as recorded in (TABLE 1) The blank solutions were prepared using the same method in absence of the examined drug.

Application to various dosage forms

Four tablets of Citalo (20 mg/tab) C -HBr drug were weighed into a small dish, powdered and mixed well, then dissolved in 100 mL distilled 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 distilled water in a 100 mL calibrated measuring flask. The drug content of this solution was obtained by applying the general procedure to aliquot containing different volumes of solution drugs as described above.

Application to spiked urine samples

For spiked human urine five milliliters of Citalopram free urine taken in a 125 mL separating funnel was spiked with different volumes of solution containing (2.0 x10^{-3} M) C -HBr, 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 drugs as described above.

Composition of ion-associates

To investigate the molecular composition of C-HBr ion-associates with PA, BPR, AR and BTB 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][35].

Job's method of continuous variation method[36] was employed; 2.0 x10^{-3} M solution of C-HBr 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 (5.0 mL). The reagents were mixed with each drug in various proportions along with the chosen buffer solution, which then diluted in 10.0 mL calibrated flask with the appropriate solvent following the above mentioned procedures.

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RESULTS

Several parameters such as selection of optimum wavelength of maximum intensity, effect of pH, effect of extracting solvent, sequence of addition, effect of time, effect of temperature, stoichiometry of ion associate, and conditional stability constant were investigated to attain the optimum conditions to achieve maximum colour development, for the quantitative determination of Citalopram by using the reported methods.

Optimization of the reaction conditions

In order to determine the most favorable conditions to achieve maximum color intensity of C-HBr drug, the effects of pH, solvent and its polarity, sequence of mixing, time and temperatures were investigated to achieve the optimum conditions to aid in accurate quantitative analysis for this drug. The optimum wavelengths of maximum intensity (λ_{max}) of C-HBr (TABLE 1) and its ion associates with PA, BPR, AR and BTB reagents are recorded at the choozen optimum conditions. The absorption band of C-HBr with PA, BPR, AR and BTB ion–associates are located at 410, 403, 432 and 415 nm, respectively. It worth mentioning that, the maximum absorbencies (λ_{max}) were recorded and tested against reagent blank (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 drug and reagents (Figure 1).

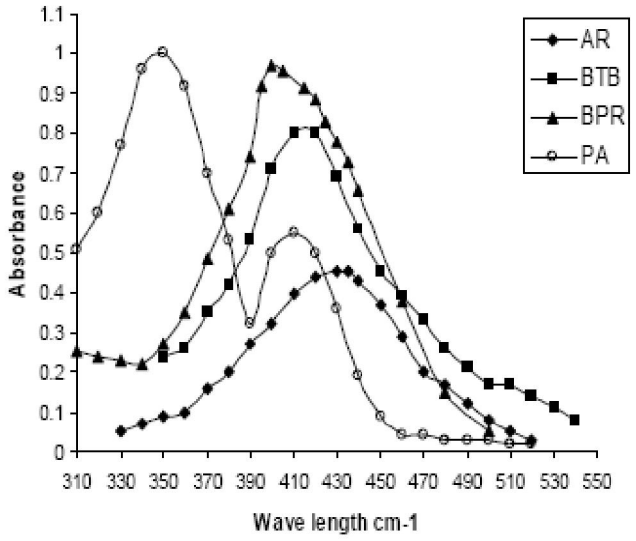

Figure 1 : Absorption spectra of (C-HBr) ion-associates with AR, BTB, BPR and PA.

(a) Effect of pH

Different stock of acetate buffer solutions were prepared with pH values of 3, 4, 5 and 6 to account for the effect of pH on the formation of ion associates. Initially 5.0 mL of $2x10^{-3}$ M of reagent was mixed with 1.0 mL ($2x10^{-3}$ M) of the drug solution, then 2.0 mL of Acetate buffer was added to adjust the pH followed by dilution with distilled water in 10.0 mL calibrated measuring flask. The optimum pH range for complete formation of the ion-associates, showed highest absorbance values, at their respective λ_{max} were found to be in the ranges (3-5), (3-5), (3-5) and (4-6), for PA, BPR, AR and BTB, respectively as shown in (Figure 2).

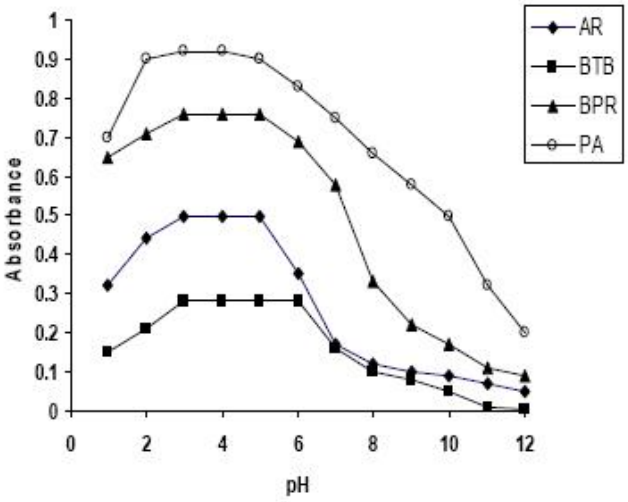

Figure 2 : Effect of pH on (C-HBr) ion-associates with AR, BTB, BPR and PA.

(b) Effect of the extracting solvent

The polarity of the solvent affects both extraction efficiency and absorpitivity of the ion-associates. Several water-immiscible organic solvents including benzene, toluene, carbon tetrachloride, methylene chloride, ether, nitrobenzene, chloroform, and ethyl acetate, had been tried. The most convenient solvent found to produce the highest absorbance, extraction power and stability of color of the formed ion-associates was chloroform as shown in Figure 3. The aqueous to organic phase ratio of 1:1.5 was the most suitable ratio for the ion-associate extraction. (Figure 3).

(c) Effect of mixing sequence

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 maxi-

mum absorbance. On the other hand, other sequences rather the one given above requires longer time in addition to lower stability of the ion associates.

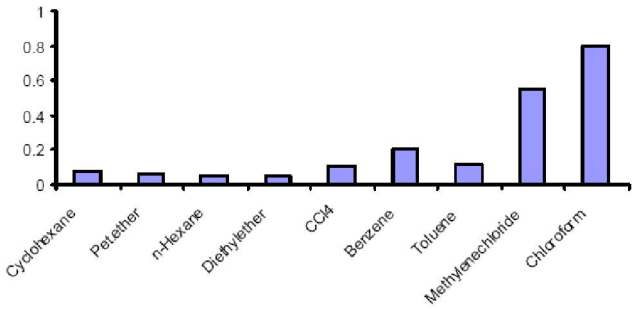

Figure 3 : The most suitable solvents for extraction of drugs - coloring reagents ion-associates.

(d) Effect of time

The effect of time on the formation of the ion-associates was studied by measuring the absorbance of the extracted ion-associates at increasing time intervals. The results showed that the ion-associates are formed almost instantaneously and the developed color remained stable for 6, 7, 9 and 6 hours for PA, BPR, AR and BTB, 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 4).

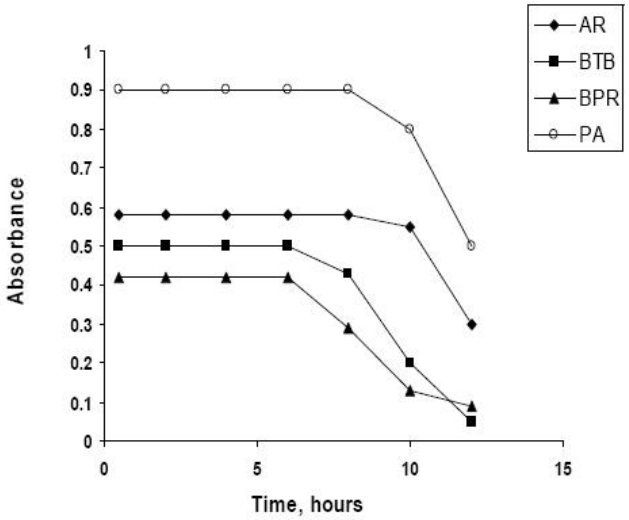

Figure 4 : Effect of time on the stability of C-HBr ion-associates with AR, BTB, BPR and PA.

(e) 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 was studied by measuring absorbance of the extracted ion-associates at temperature range 25-100°C. The results showed that the ion-associates are formed almost instantaneously in all cases at room temperature 25+ 5 °C and remain constant up to 55°C, 55°C, 50°C and 60°C for PA, BPR, AR and BTB, respectively as shown by their absorptivities at the recommended (λ_{max}). The effect of temperature on the stability of ion–associates is shown in (Figure 5).

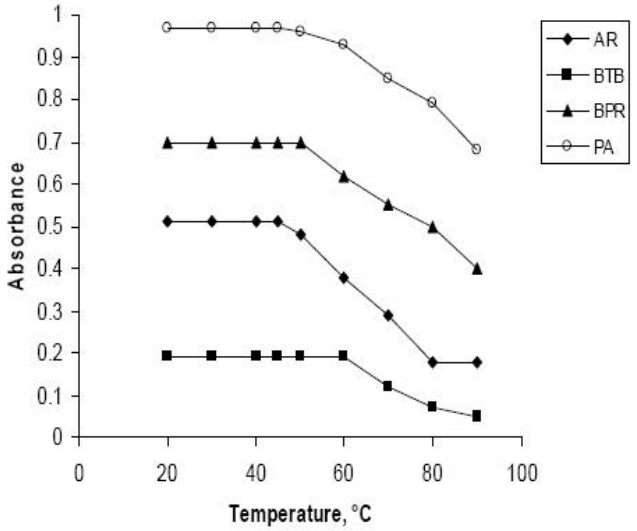

Figure 5 : Effect of temperature on the stability of C-HBr ion-associates with AR, BTB, BPR and PA.

The stoichiometry of the ion–associates

The stoichiometric ratio of the C-HBr ion–associates formed between drug of interest and the selected reagents has been determined by implementing the molar ratio method as shown in (Figure 6) and continuous variation method as shown (Figure 7). The results indicate the existence of 1:1 in case of PA, BPR, AR and BTB, at a definite λ_{max} for each reagent as $_{recorded}$ in (TABLE 1).

Conditional stability constant (k_f) of the ion- associate complexes

The stability of the ion- pair complexes was evaluated. The formation of the ion- pairs was rapid and the colored extracts were stable at least 8 hours for drug - reagent ion pair without any change in color intensity and the maximum absorbance at room temperature. The conditional stability constant (k_f) of the ion- pair complexes for the studied drugs was calculated from the continuous variation data using the following equation :

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

Where A and A_m 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[37]. In accordance with the formula the conditional stability constants were found to be 3.01, 2.63, 3.85and 2.31 for PA, BPR, AR and BTB respectively.

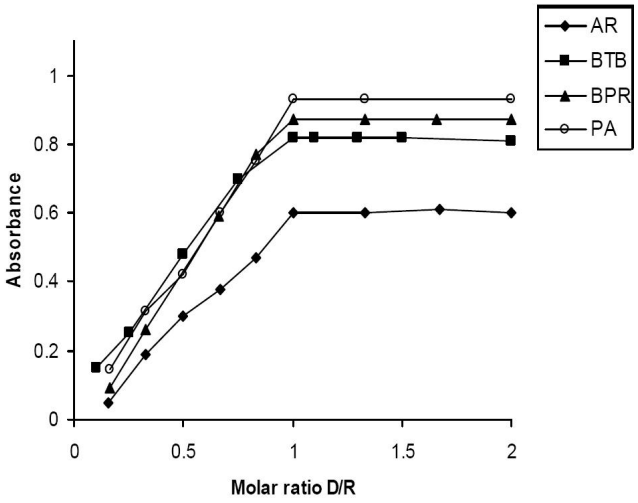

Figure 6 : Molar ratio of C-HBr ion-associates with BTB, BPR, AR and PA.

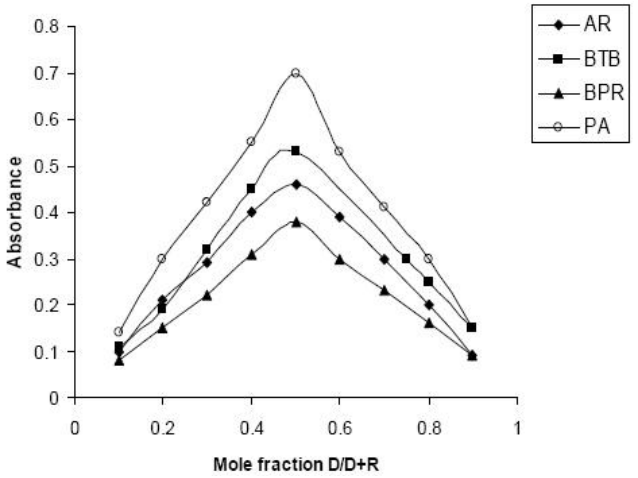

Figure 7 : Continuous variation of C-HBr ion-associates with AR, BTB, BPR and PA.

Method validation

(a) Linearity

Beer's law is obeyed in the concentration range 1.78-35, 3.37-58, 4.0-85 and 1.5-40.0 μg mL^{-1} (Figure 8) for PA, BPR, AR and BTB respectively. The optical characteristics; Beer's law limits, molar absorptivities, Sandell's sensitivities are summarized in (TABLE 1) along with the results of regression analysis using the method of least square was made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations.

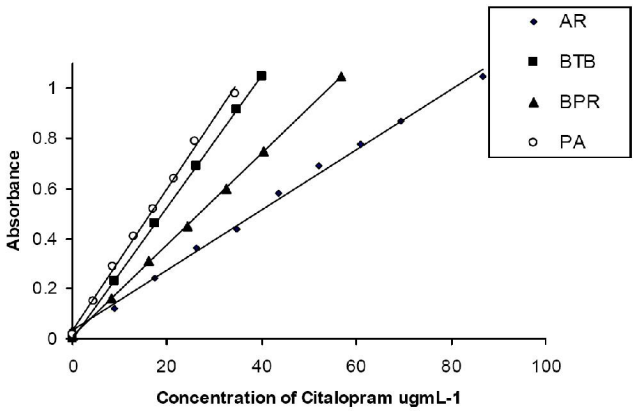

Figure 8 : Standard curves of C-HBr ion-associates with AR, BTB, BPR and PA.

(b) Specificity

No interference was observed during the quantitative determination of C-HBr drug with all reagents in presence of different additives such as lactose, glycerol, propylene glycol, sugar, magnesium stearate, methyl paraben, propyl paraben and starch which are present in its pharmaceutical preparations as excipients.

(c) 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 analyte 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, is defined as:

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

According to these equations, the limit of detection and quantification were calculated and listed in (TABLE 1).

TABLE 1 : Characteristics and analytical data of (C-HBr) ion-associates with PA, BPR, AR and BTB.

Parameter	C/PA	CBPR	C /AR	C /BTB
λ max (nm)	410	403	432	415
Beer's law up to (μgmL^{-1})	35	58	85	40
Molar absorpitivity (ε) ($Lmol^{-1}$ cm^{-1})	1. $3x10^4$	0.75 $x10^4$	0.54 $x10^4$	1.32 $x10^4$
Ringbom ($\mu g\ mL^{-1}$)	3.38	6.93	7.63	5.11
Sandell sensitivity ($\mu g\ cm^{-2}$)	0.0329	0.054	0.0748	0.038
Limit of detection(μgmL^{-1})	0.23	0.63	0.72	0.49
Limit of quantification (μgmL^{-1})	1.29	2.08	2.39	1.59
Color of ion- associate	Yellow	Yellow	Yellow	Yellow
Intercept	0.034	0.011	0.037	0.002
Slope	0.028	0.018	0.013	0.026
Correlation Coefficient	0.998	0.999	0.998	0.999

(d) Precision and accuracy

In order to determine the accuracy and precision of the method, solution containing four different concentrations of the studied drug were prepared and analyzed in quintuplicate. Percentage relative standard deviation (R.S.D. %) as precision indicating reasonable repeatability of the selected methods and percentage relative error (Er %) as accuracy of the suggested methods were calculated. Precision was carried out by six determinations at four different concentrations in these spectrophotometric methods. The percentage relative error was calculated using the following equation:

$$\text{Er \%} = \left[\frac{\text{Found - added}}{\text{Added}}\right] \text{X } 100$$

The inter–day and intra-day precision and accuracy results are shown in (TABLE 2). The results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

(e) Robustness and ruggedness

For the evaluation of the method robustness, some parameters were interchanged ; pH, reagent concentration, wavelength range, and shaking time. The capacity remain unaffected by small deliberate variations. Method Ruggedness was expressed as R.S.D. % of the same procedure applied by two analysts and in 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).

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

method	Intra-day					Inter-day			
	Added $\mu g\ mL^{-1}$	Recovery %[a]	Precision RSD%	Accuracy Er%	Confidence limit[b]	Recovery %	Precision RSD%	Accuracy Er%	Confidence limit[b]
BTB	10	99.2	1.41	-0.8	9.92+0.16	99	1.31	-1	9.90+0.15
	20	99.25	0.81	-0.75	19.85+0.18	98.9	0.6	-1.1	19.78+0.14
	30	99.18	0.64	-0.82	29.75+0.22	99.33	0.27	-0.67	29.79+0.09
	40	99.05	0.33	-0.95	39.62+0.15	98.78	0.43	-1.22	39.51+0.20
BPR	10	99.38	1.21	-0.62	9.94+0.14	99.11	2.22	-0.89	9.91+0.25
	20	99.11	1.06	-0.89	19.82+0.24	99.05	0.66	-0.95	19.81+0.15
	30	99.15	0.64	-0.85	29.75+0.64	99.23	0.57	-0.77	29.77+0.20
	40	98.99	0.58	-1.01	39.60+0.20	98.67	0.38	-1.33	39.47+0.17
PA	5	99.10	3.43	-0.9	4.96+3.43	99	3.43	-1	4.95+0.20
	10	99.20	1.61	-0.8	9.92+0.18	98.70	1.42	-1.3	9.87+0.16
	15	98.87	0.87	-1.13	14.83+0.15	98.99	1.28	-1.1	14.85+0.22
	20	98.99	0.56	-1.01	19.80+0.13	99.20	1.16	-0.8	19.84+0.26
AR	10	99.13	0.81	-0.87	9.91+0.09	99.0	2.73	-1	9.90+0.31
	20	99.45	0.60	-0.55	19.89+0.14	99.33	0.40	-0.67	19.84+0.09
	30	99.05	0.57	-0.95	29.75+0.20	99.11	0.47	-0.89	29.73+0.16
	40	98.97	0.58	-1.03	39.59+0.26	98.77	0.3	-1.23	39.51+0.14

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

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TABLE 3 : The results of analysis of C-HBr obtained by two different analysts and instruments (n = 6).

Ion associate	Different instrument			different analyst		
	X	± S.D.	R.S.D	X	± S.D.	R.S.D%
C- BTB Pure C -HBr (20 µg mL^{-1})	19.83	0.16	0.81	19.73	0.17	0.86
Citalo 20 mg per tablet (20 µg mL^{-1})	20.02	0.18	0.90	20.09	0.22	1.10
Spiked urine sample(20 µg mL^{-1})	19.62	0.23	1.17	19.83	0.25	1.26
C-BPR Pure C -HBr (20 µg mL^{-1})	19.76	0.21	1.06	19.87	0.18	0.91
Citalo 20 mg per tablet (20 µg mL^{-1})	20.18	0.16	0.79	20.05	0.17	0.85
Spiked urine samples(20 µg mL^{-1})	19.85	0.09	0.46	19.71	0.12	0.61
C – PA Pure C -HBr (20 µg mL^{-1})	19.91	0.19	0.95	19.80	0.13	0.66
Citalo 20 mg per tablet (20 µg mL^{-1})	19.98	0.11	0.55	20.11	0.09	0.45
Spiked urine samples(20 µg mL^{-1})	18.98	0.11	0.58	19.19	0.09	0.47
C –AR Pure C -HBr (20 µg mL^{-1})	18.99	0.21	1.10	19.27	0.18	0.93
Citalo 20 mg per tablet (20 µg mL^{-1})	19.95	0.17	0.85	20.07	0.13	0.65
Spiked urine samples(20 µg mL^{-1})	18.67	0.12	0.64	19.0	0.14	1.73

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

TABLE 4 : Spectrophotometric determination of (C-HBr).

Reagent	Pure solution			Citalo 20 mg / tablet			Spiked urine samples		
	Taken µg mL^{-1}	Found µg mL^{-1}	Recovery %	Taken µg mL^{-1}	found µg mL^{-1}	Recovery %	taken µg mL^{-1}	found µg mL^{-1}	Recovery %
PA	5	4.94	99.15	5	5.00	100.00	5	4.94	98.87
	10	9.92	99.23	10	9.99	99.89	10	9.87	98.69
	15	14.82	98.87	15	15.01	100.05	15	14.85	99.02
	20	19.80	99.00	20	19.99	99.93	20	19.82	99.11
	25	24.78	99.11	25	25.01	100.04	25	24.70	98.81
	Mean recovery ± RSD* 99.27 ± 1.01			Mean recovery ± RSD* 99.98 ± 1.15			Mean recovery ± RSD* 98.90 ± 1.00		
AR	10	9.91	99.13	10	9.99	99.88	10	9.80	97.99
	20	19.79	98.93	20	20.01	100.05	20	19.63	98.13
	30	29.71	99.02	30	30.00	100.00	30	29.51	98.37
	40	39.95	98.97	40	39.97	99.93	40	39.39	98.47
	50	49.43	98.86	50	50.02	100.04	50	49.32	98.63
	Mean recovery ± RSD* 98.9 8± 0.71			Mean recovery ± RSD* 99.98 ± 0.80			Mean recovery ± RSD* 98.31 ± 0.92		
BPR	10	9.94	99.40	10	9.99	99.99	10	9.9	99.02
	20	19.82	98.12	20	20.04	100.20	20	19.83	99.13
	30	29.74	99.13	30	30.04	100.13	30	29.62	98.73
	40	39.57	98.93	40	39.89	99.73	40	39.54	98.86
	50	49.62	99.24	50	49.91	99.81	50	49.45	98.90
	Mean recovery ± RSD* 99.14 ± 0.95			Mean recovery ± RSD* 99.94 ± 1.05			Mean recovery ± RSD* 98.93 ± 0.96		
BTB	5	4.96	99.10	5	5.01	100.1	5	4.95	99.00
	10	9.92	99.19	10	10.03	100.3	10	9.91	99.11
	20	19.85	99.24	20	20	100.00	20	19.82	99.08
	30	29.75	99.18	30	29.96	99.87	30	29.74	99.13
	40	39.6	99.00	40	39.91	99.78	40	39.57	98.93
	Mean recovery ± RSD* 99.14 ± 0.81			Mean recovery ± RSD* 100.01 ± 0.95			Mean recovery ± RSD* 99.05 ± 1.11		

Analytical applications

Results obtained were compared with those of the official methods along with the statistical outcomes. The comparison ensures that there is no significant difference between the current study and the official methods as shown in (TABLE 4). Six replicate determination at different concentration levels were carried out to test the precision and accuracy of the method. The recoveries were ranged from (99.0 to 100.1) % which reflect the high accuracy of the results, with reliable precision as indicated by very low values of standard deviation (TABLES 5). The performance of the proposed method was assessed by calculation of t and F tests[38,39] compared with the official method[40,41]. Mean values were obtained with t and Ftestes at 95% confidence level for five degrees (n-1) = (6-1; i.e., six replicate minus 1) of freedom were in the accepted values.

TABLE 5 : Statistical treatment of data obtained for determination of (C-HBr) applying the proposed methods in comparison with the reference method.

Parameters	Official method	C-BTB	C-BPR	C- PA	C-AR
Pure solution					
20 µg m L^{-1}	99.37	99.25	99.12	99.0	98.93
X ± SD	±0.15	±0.16	±0.18	±0.20	±0.14
n	6	6	6	6	6
t-value*		0.22	0.52	0.69	0.96
F-value		1.14	1.44	1.77	0.87
Citalo (20 mg /tablet)					
20 µg m L^{-1}	100.10	100.00	100.02	99.93	100.05
X ± SD	±0.18	±0.19	±0.21	±0.23	±0.16
n	6	6	6	6	6
t-value*		0.20	0.09	0.25	0.10
F-value		1.11	1.36	1.63	1.27
Spiked urine					
20 µg m L^{-1}	99.24	99.08	99.11	99.11	98.13
X ± SD	±0.17	±0.22	±0.19	±0.19	±0.18
n	6	6	6	6	6
t-value*		0.09	0.29	0.19	2.18
F-value		1.67	1.25	1.38	1.12

DISCUSSION

Determination of Citalopram hydrobromide (C-HBr) in bulk sample and in dosage form were investigated. The implemented procedures are based on the formation of a yellow colored ion-associates due to the interaction between the examined drug C-HBr with Picric acid (PA), bactophenol red (BPR), alizarine red (AR) and bromothymol blue (BTB) reagents. the suitable recommended buffer solution has been used and the extraction was carried out using chloroform, then recording the optimum wavelength using visible spectrophotometer. Moreover, the optimum reaction conditions were carefully investigated whereas Beer's law is obeyed within a concentration range of 1.78-35, 3.37-58, 4.0-85 and 1.5-40.0 µg mL^{-1} (Figure 8) for PA, BPR, AR and BTB respectively. In addition the molar absorptivity, Sandell sensitivity and the optimum conditions for quantitative analysis of the investigated drugs were determined. The correlation coefficient was ≥ 0.995 (n =6) with a relative standard deviation (RSD) ≤ 0.65 for five selected concentrations of the reagents. Therefore the concentration of C-Hbr drug in its pharmaceutical formulation and in spiked urine has determined successfully up 35, 52, 90 and 42.5 µg mL^{-1} for PA, BPR, AR and BTB respectively.

CONCLUSIONS

The proposed methods are made by using of simple reagents, which most ordinary analytical laboratories can afford. The methods are sufficiently sensitive to permit determinations for Citalopram hydrobromide (C-HBr) drug at the given optimum conditions. Unlike GC and HPLC procedures, the spectrophotometer is relatively simple to be handled and affordable. The proposed methods are simple, precise, accurate and convenient. Hence, the proposed methods should be useful for routine quality control purposes.

REFERENCES

[1] K.Parfitt (Ed); Martindale, the extra pharmacopoeia, The Pharmaceutical Press, London, 32nd Edition, **(1999)**.

[2] B.Narayana, K.Veena, J.Mex; Chem.Soc., **54**, 98-102 **(2010)**.

[3] A.Raza, T.M.Ansari, S.B.Nazi; J.of the Chem.Society Pakis., **30**, 241-250 **(2008)**.

[4] J.Menegola, M.Steppe, E.E.S.Schapoval; J.AOAC Int., **91**, 52-59 **(2008)**.

[5] S.V.Gandhi, N.D.Dhavale, V.Y.Jadhav, S.S.Sabnis;

J.AOAC Int., **91**, 33-38 **(2008)**.
[6] A.Raza; Chem.Pharm.Bull (Tokyo)., **54**, 432-436 **(2006)**.
[7] J.Hasselstrom; J.Chromatogr., **879**, 123-128 **(2011)**.
[8] A.R.Chaves, F.Z.Leandro, J.A.Carris, M.E.C.Queiroz; J.Chromatogr., **878**, 2123- 2129 **(2010)**.
[9] N.Unceta, M.C.Sampedro, N.K.Abu-Bakar, A.Gómez-Caballero, M.A.Goicolea, R.J.Barrio; J.Chromatogr., **1217**, 3392-3399 **(2010)**.
[10] T.Jiang, Z.Rong, L.Peng, B.Chen, Y.Xie, C.Chen, J.Sun, Y.Xu, Y.Lu, H.Chen; J.Chromatogr., **878**, 615- 619 **(2010)**.
[11] B.Raman, B.A.Sharma, P.D.Ghugare, P.P.Karmuse, A.Kumar; J.Pharm.and Biomed.Anal., **50**, 377-388 **(2009)**.
[12] C. Pistos, I. Panderi and J. Atta-Politou. J. Chromatogr., 810, 235- 244, 2006.
[13] Müller, S.Vogt, R.Goerke, A.Kordon, W.Weinmann; Forensic Sci.Int., **113**, 415- 421 **(2000)**.
[14] L.P.Melo, A.M.Nogueira, F.M.Lanças, M.E.C.Queiroz; Analytica Chimica Acta, **633**, 57-64 **(2009)**.
[15] H.Bagheri, F.Khalilian, E.Babanezhad, A.Es-haghi, M.Rouini; Analytica Chimica Acta, **610**, 211-216 **(2008)**.
[16] N.Unceta, A.Gómez-Caballero, A.Sánchez, S.Millán, M.C.Sampedro, M.A.Goicolea, J.Sallés, R.J.Barrio; J.Pharm.and Biomed.Anal., **46**, 763-770 **(2008)**.
[17] T.Vasskog, T.Anderssen, S.Pedersen-Bjergaard, R.Kallenborn, E.Jensen; J.Chromatogr., **1185**, 194-205 **(2008)**.
[18] B.J.G.Silva, F.M.Lanças, M.E.C.Queiroz; J.Chromatogr., **862**, 181-188 **(2008)**.
[19] W.R.Malfará, C.Bertucci, M.E.C.Queiroz, S.A.D.Carvalho, M.L.P.Bianchi, E.J.Cesarino, J.A.Crippa, R.H.C.Queiroz; J.Pharm.and Biomed.Anal., **44**, 955-962 **(2007)**.
[20] A.R.Chaves, S.M.Silva, R.H.C.Queiroz, F.M.Lanças, M.E.Costa Queiroz; J.Chromatogr., **850**, 295-302 **(2007)**.
[21] C.Greiner, C.Hiemke, W.Bader, E.Haen; J.Chromatogr., **848**, 391- 394 **(2007)**.
[22] R.N.Rao, A.N.Raju, D.Nagaraju; J.Pharm.and Biomed.Anal., **41**, 280-285 **(2006)**.
[23] Qing H.Meng, Donna Gauthier; Clinical Biochemistry, **38**, 282-285 **(2005)**.
[24] P.Molander, A.Thomassen, L.Kristoffersen, T.Greibrokk, E.Lundanes; J.Chromatogr., **766**, 77-87 **(2002)**.
[25] J.Macek, P.Ptaceka, J.Klima; J.Chromatogr., **755**, 279-285 **(2001)**.
[26] J.J.B.Nevado, M.J.V.Llerena, C.G.Cabanillas, V.R.Robledo; J.Chromatogr., **1123**, 130- 133 **(2006)**.
[27] J.J.Berzas, C.Guiberteau, M.J.Villaseñor, V.Rodríguez; Analytica Chimica Acta, **519**, 219-230 **(2004)**.
[28] R.Skibinski, G.Misztal; Acta Pol Pharm., **62**, 331-335 **(2005)**.
[29] -J.J.B.Nevado, C.G.Cabanillas, M.J.V.Llerena, V.R.Robledo; J.Chromatogr., **1072**, 249- 257 **(2005)**.
[30] S.Andersen, T.Gr.Halvorsen, S.Pedersen-Bjergaard, K.E.Rasmussen, L.Tanum, H.Refsum; J.Pharm.and Biomed.Anal., **33**, 263-273 **(2003)**.
[31] T.G.Halvorsen, S.Pedersen-Bjergaard, K.E.Rasmussen; J.Chromatogr., **909**, 87- 93 **(2001)**.
[32] L.E.E.Eibak, A.Gjelstad, K.E.Rasmussen, S.Pedersen-Bjergaard; J.Chromatogr., **1217**, 5050-5056 **(2010)**.
[33] S.Vasantharaju, S.Prabu,A.Jacob; Ind.J.Pharm.Sci., **70**, 647-648 **(2008)**.
[34] Sherbiny, El-T.J.Dina; AOAC Intern., **89**, 1288-1295 **(2006)**.
[35] J.H.Yoe, A.L.Jones; Ind.Eng.Chem.Anal.Ed., **16**, 14 **(1944)**.
[36] **(a)** P.Job; Camp.Rend.(Paris), **180**, 928 **(1925)**; **(b)** Ann.Chim.(Paris), **9**, 133 **(1928)**; **(a)** Ann Chim.(Paris), **6**, 97 **(1936)**.
[37] J.Inczedy; Analytical application of complex equilibria, Ellis Horwood Ltd, England, 137 **(1976)**.
[38] S.Dowdy, S.Weardern; Statistics for research, Wiley, NY, **(1983)**.
[39] J.C.Miller, J.N.Miller; Significance tests in statistics for analytical chemistry, 3rd Edition, Chap.3, Ellis Hardwood, Chichester, UK, **(1993)**.
[40] British Pharmacopoeia, HMSO, International Edition, Cambridge, **2**, 6317 **(2009)**.
[41] United States Pharmacopia, Twentieth Review, the National Formulary, Nineteenth Review, the United States Pharmacopeial Convention, Rockville, MD, **(2000)**.