Research Article

A New Spectrophotometric Method for Determination of Bromhexine Hydrochloride (BX.HCL) in Pure and Dosage Forms using Prussain Blue Complex Reaction

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ABSTRACT

A fast, rapid, sensitive and stable spectrophotometric method is developed and validate for quantitative determination of Bromhexinehydrochloride (BX.HCl) in pure and dosage forms . The purpose method was based on the reduction of Fe^{3+} to Fe^{2+} by the bromhexine HCl, and the produced Fe^{2+} ion react with $K_3Fe(CN)_6$ to yield a blue color product measured at 800nm. The current method demonstrates a good linearity over the range (1-12µg.ml^{-1}) with correlation coefficient (r^2) of 0.9985. The average recovery of the method (97.66-98.70%). The limit of detection and limit of quantitative were found to be 0.481 and 1.6046 µg.ml^{-1} respectively.The purposed method was found to be accurate and precise for routine estimation of (BX.HCl) in bulk and tablets formulations.

Keywords: Spectrophotometric method, quantitative determination, bromhexine HCl.

INTRODUCTION

Bromhexinehydrochloride (BX.HCl) is a mucolytic s agent used in the treatment of respiratory disorder with viscid or excessive mucous; chemically the molecular formula is $C_{14}H_{20}Br_2N_2$ .HCl as shown in figure (1) and the molecular weight is 376.13 g/mol. It is off-white crystalline powder .it is freely soluble in water and alcohol[1]. Various analytical methods for BX.HCl determination have been described these were including reverse phase-high performance liquid chromatography[2-6], spectrophotometery[7-13],A literature survey shows that several HPLC method have been reported for it is determination alone and in combination in pharmaceutical[14-18].

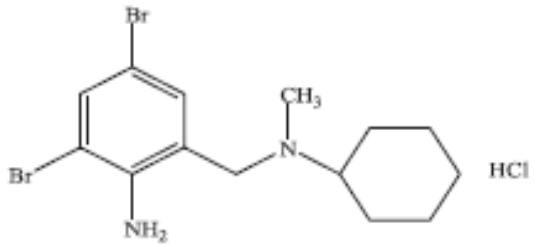


Figure 1: Chemical Structure of BromhexineHCl.

Charge transfer complexes (electron donor - acceptor complexes) may be formed when an electron donor group is adjacent to an electron acceptor group. In this situation, experimental evidences suggest that the donor may transfer a portion of its charge to the acceptor. As a result, one compound becomes partially positively charged with respect to the other and a weak electrostatic bond is formed[19]. Prussian blue complex is one of the most known charge transfer complex [Fe4 [Fe (CN) 6]3] and was probably synthesized for the first time by the paint maker Diesbachin Berlin around the year 1706.

Prussian blue is produced by oxidation of ferrous ferrocyanide salts. These white solids have the formula M_2Fe [Fe $(CN)_6$] where M^+ = Na^+ or K^+. The iron in this material is all ferrous, hence the absence of deep color associated with the mixed valency. Oxidation of this white solid with hydrogen peroxide or sodium chlorate produces ferricyanide and affords Prussian blue. A "soluble" form of PB, K [FeIIIFeII (CN) 6], which is really colloidal, can be made from potassium ferrocyanide and iron (III):

$$K^+ + Fe^{3+} + [Fe^{II}(CN)_6]^{4-} \rightarrow KFe^{III}[FeII(CN)_6]$$

The similar reaction of potassium ferricyanide and iron (II) results in the same colloidal solution, because $[Fe^{III}(CN)_6]^{3-}$ is converted into ferrocyanide."Insoluble" Prussian blue is produced if in the reactions above an excess of Fe^{3+} or Fe^{2+}, respectively, is added. In the first case[20]:

$$4Fe^{3+} + 3[Fe^{II}(CN)_6]^{4-} \rightarrow Fe^{III}[Fe^{III}Fe^{II}(CN)_6]_3$$

$$Antibiotic + Fe^{3+} \rightarrow Antibiotic+ + Fe^{2+}$$

In this present work a Prussian blue charge transfer complex reaction was developed for the determination of BX.HCl in pure and pharmaceutical preparations. The method was based on reduction of ferric ion (Fe^{+3}) to ferrous (Fe^{+2}) ion by bromhexine HCl and the produced Fe^{+2} ion react directly with potassium hexacynoferrate (K_3Fe $(CN)_6$) to produce a blue-greenish color complex as follow.

$$BX.HCl + Fe^{III} \rightarrow BX^+ + Fe^{II}$$

$$Fe^{II} + K_3FeIII\ (CN)_6 \rightarrow Fe_4[Fe(CN)_6]_3\ Blue\ colored\ product$$

MATERIALS AND METHODS

Instruments Used

A double beam spectrophotometer

A Shimadzu-UV-1800 using 10mm path length for quartzes cell.

Four digit electronic balances

Electronic balance Sartorius CE (Germany) four digits ewas used throughout this research work.

pH meter

Ametrohm E.632 pH meter (Switzerland) fitted with an Ametrohm combined glass electrode.

Chemicals and Reagents Used

All chemical used were of analytical grade reagents while highly pure Bromohexane-HCl was provided from Samarra drug factory – Iraq(SDI) as gift .

Preparation of BX.HCl stock solution

A standard solution of Bromhexine-HCl (Bx.HCl) was prepared by dissolving 1000mg of (Bx.HCl) in 100ml of distilled water and further dilution to get 100 µg/ml.

Ferric chloride ($6.165x10^{-3}$ M) solution

This was prepared by dissolving 1000mg of $FeCl_3$ in 1 ml of concentrated HCl and diluting to the mark with distilled water.

Potassium hexacyanoferrate (III) ($3.04x10^{-2}$ M) stock solution

This was prepared by dissolving 1.002g of $K_3[Fe(CN)_6]$in distilled water and diluting to the mark in 100ml volumetric flask .

Methodology

Into series of 10 ml volumetric flask, increasing volumes of (100 µg.ml^{-1}BX.HCl) were transferred to cover the range of standard curve (1-12.0µg/ml) . To each flask 1ml of $6.165x10^{-3}$ M of $FeCl_3$ was added and mixed well followed by addition of 1ml of $3.04x10^{-2}$ M $K_3[Fe(CN)_6]$,dilute the solution to the mark with distilled water and allow the reaction to stand for 20minutes. After then the absorbances of the above mixtures were measured against reagent blank.

Analysis of Pharmaceutical Formulations

Twenty tablets were weighed; average weight was calculated and grinding into a fine powder. An average weight equivalent to 8 mg of BX.HCl was taken from the powder then dissolve in distilled water, sonecated and make up to the mark in 100 ml volumetric flask to obtained 80µg.ml-1 , then filtered through Whitman's filter paper no.41 and the filtrate was collected in the flask . 8 and 2 µg.ml^{-1} solutions were prepared from the stepwise dilution of above solution. These solutions were measured to evaluate the validity of the proposed method.

RESULTS AND DISSCUSION

Absorption Spectra

The absorbance of blue colored Complex was scanned from 400 - 900 nm against reagent blank prepared by the same way but containing no BX.HCl. A maximum absorbance was obtained at 800nm (λ_{max}), therefore 800nm was used in the subsequence measurements. Figure (2) shows the spectrum of the colored product measured against reagent blank.

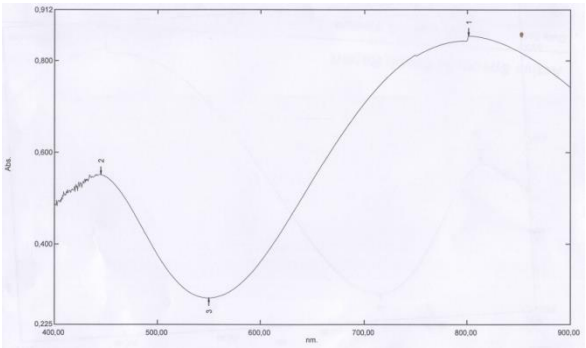


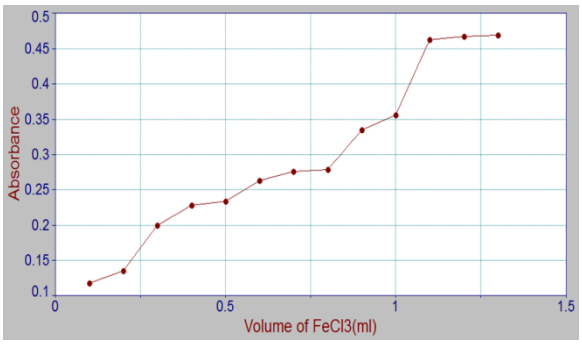
Figure 2: Absorption spectrum of Bromhexine. HCl treated with $FeCl_3$ ($6.165x10^{-3}$ M) and K_3 [Fe $(CN)_6$ ($3.04x10^{-2}$ M)at 30 C^0 and measured against blank solution.

The influence of the reaction variables such as concentration of the reactants, order of addition, temperature and time of reaction were studied.

Effect of Iron (III) Concentration

In order to study the effect of the amount of Ferric chloride ($FeCl_3$), different volumes in the range (0.1-1.3ml) of $6.165x10^{-3}$M $FeCl_3$ were examined. The obtained results are shown in figure (3) and indicated that 1.1ml of $FeCl_3$ was enough amount to obtain a maximum optical density with lowest absorbance value of blank. Therefore 1.1ml was used in all subsequent experiments.

Figure 3: Effect of concentration of $FeCl_3$.



Effect of potassium hexacynoferrate (K_3Fe $(CN)_6$)

The optimum volume of potassium hexacynoferrate (K_3Fe $(CN)_6$) was study. This study was carried out using $3.04x10^{-2}$M in the range (0.1-1.3ml).Figure (4) shows the

obtained results. An increase in the absorbance of the colored product was obtained up to 1.1ml of $K_3Fe(CN)_6$ and beyond this volume the absorbance was found at study state. Therefore 1.1ml from $3.04x10^{-2}$M was chosen as an optimum volume.

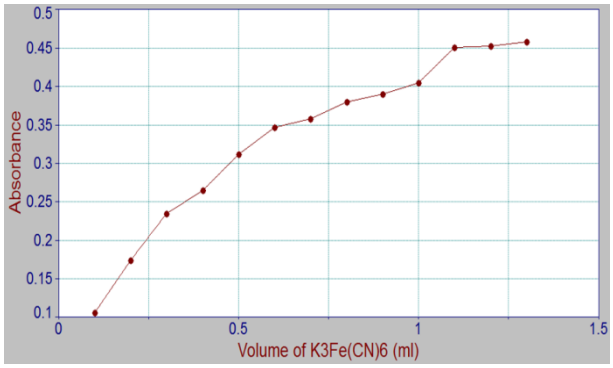


Figure 4: Effect of concentration of$K_3Fe(CN)_6$.

Effect of temperature

To obtain suitable temperature to complete the reaction and give highest absorption a deferent temperature were studied (20-60°C). It was observed as in table (1) that the maximum absorbance was obtained at 30 C and thus this temperature was taken in subsequent studied.

Table 1: Effect of temperature on optical density of color product.

Temperature(C^0)	Absorbance
20	0.290
25	0.295
30	0.300
35	0.301
40	0.302
45	0.303
50	0.303
55	0.305
60	0.305

Reaction and stability time

A study was carried out to optimized the effect of reaction and stability time on the absorbance of the colored complex at 800nm under the optimal conditions that were achieved in previous. The absorbance was measured at different intervals of time up to 60 min. The experimental results indicated that the colored complex developed at 15 minutes and the absorbance remains constant for at least 60 minutes. Therefore, after 15min was used to measure the absorbance of product in all subsequent experiments.

Order of addition

The effect of order of addition was also studied under the obtained optimum results. The results shows that the best order of addition of reactants were as drug + $FeCl_3$ + $K_3Fe(CN)_6$ gave the maximum optical intensity and stability in measurements as shown in table(2).

Table 2: Effect of order of addition of the reagents.

Order of addition	Absorbance
DR. + $FeCl_3$ + $k_3Fe(CN)_6$	0.445
$FeCl_3$+ DR + $k_3Fe(CN)_6$	0.432
$FeCl_3$ + $k_3Fe(CN)_6$ +DR	0.345
DR. + $k_3Fe(CN)_6$ + $FeCl_3$	0.376

Validate of the proposed method

After optimized all the experimental conditions, a calibration curve was constructed between the measured optical density and the corresponding concentration of BX.HCl. A regression analysis and statistical variables were calculated from the calibration curve using least-square method. The intercept, slope and correlation coefficient for the calibration curve and the sensitivity parameters such as molar absorptivity and sandell sensitivity were tabulated in table (3). While figure (5) shows the calibration curve of the proposed method.

Table 3: Summery of optical characteristics and statistics of the proposed method.

Parameters	Value
Λ_{max}(nm)	800
Color	Blue-greenish
Regression equation Y=bX + a	Y= 0.0685 X + 0.0092
Correlation coefficient (r^2)	0.9985
Linearity percentage (r^2%)	99.85%
Linear dynamic range(µg/ml)	1 - 12
Molar absorptivity, ξ (L/mol. cm)	2.607×10^4
Slope , b (ml/µg)	0.0685
Intercept, a (a=y-bx)	0.0092
Standard deviation of slope, S_b	0.00087
Standard deviation of intercept, S_a	0.0061
Sandell sensitivity , S(µg/cm)	$1.04 x10^{-5}$
Standard deviation of the residual, $S_{y/x}$	0.00967

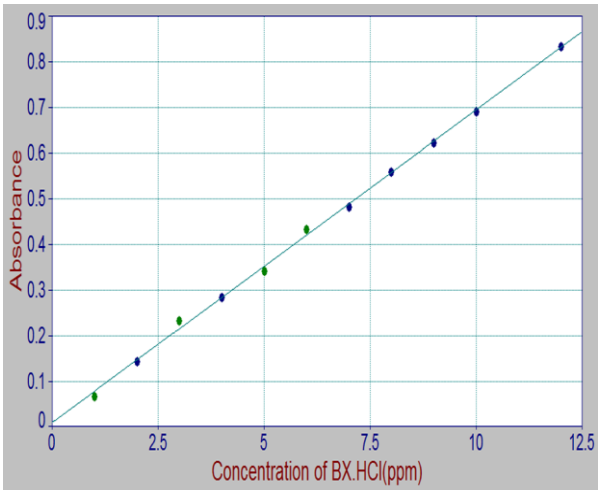


Figure 5: The calibration curve of BX.HCl.

Senstivity

Limit of detection (LOD) and limit of quantification (LOQ) were calculated according to the equations 3 S.D / k and 10 S.D / k respectively, where S.D(0.011), is the standard deviation of the absorbance of the blank solution measured at 800nm and k is the sensitivity, which represent the slope of the calibration curve. The LOD and LOQ values were 0.481 and 1.604µg/ml respectively.

Accuracy and precision

To evaluate the accuracy and precision of the proposed method, three different concentrations from BX.HCl were chosen from the range of the calibration curve and analyzed in five replicates. The obtained results were shown in table (4) indicated that the low values of percentage relative standard deviation (%RSD) and percentage of relative errors (%E); the proposed method with high precision and accuracy.

Table 4: Accuracy and precision of proposed method.

Concentration of Bromhexine HCl (µg.ml^{-1})		%Recovery*	%R.S.D*	%Error*
Taken	Found			
2.00	1.97	98.70	0.471	-1.3
6.00	5.86	97.66	0.544	-2.3
10.00	9.67	96.80	0.509	-3.3

***Average of five determinations.**

Analytical Applications

The developed method was successfully applied to determination of BX.HCl in different pharmaceutical formulations through the analysis of two different concentrations of pharmaceutical preparations using the recommended analytical procedure. The obtained results were tabulated in table (5). The result shows the high recoveries and low percentage errors and based on that the new proposed method can be used as an alternative analytical method for the determination of BX.HCl in pure and dosage forms.

Table 5: Application of the proposed method for the determination of BromhexineHCl in pharmaceutical tablets.

Drug Sample	Concentration of BromhexineHCl (µ g. ml^{-1})		%Recovery*	%R.S.D*	%Error*
	Taken	Found			
BromhexineHCl .8mg SDI	2.00	1.94	97.00	0.465	-3.00
	8.00	7.88	98.50	0.720	-1.50
BromhexineHCl .8mg Bisolvon	2.00	1.98	99.00	0.11	-1.00
	8.00	8.054	100.67	0.86	0.67

*Average of three replicate.

CONCLUSION

Different parameter affecting the reaction were thoroughly studied also this method were applied to pharmaceutical preparation and the results were satisfactory. The validates of the method were a ascertained by the standard curve revealed five result in consideration to mean recovery and standard deviation. The proposed method was sensitive, accurate and precise than many spectrophotometric methods proposed earlier. The stability of the color system is an advantage over the earlier methods. The results of analysis of authentic samples and bulk drugs reveal that the method is both accurate and precise. Commonly encountered excipients and additives do not interfere.

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