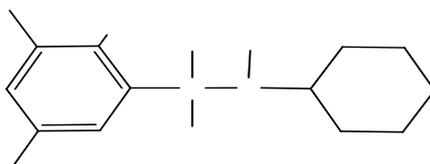




## INTRODUCTION

Bromhexine-HCl, (BH) is widely used in medicine as a mucolytic drug. It works through decreasing the amount of respiratory tract fluid and reduces its viscosity by activating enzymes that hydrolyze mucopolysaccharides (Ribone *et al.*, 2000). BH is chemically known as [N-(2-amino-3,5-dibromophenylmethyl)-N-methylcyclohexylamine hydrochloride] with a molecular weight of  $412.6 \text{ g.mol}^{-1}$  and has the following structure (British Pharmacopeia, 2000 ).



Bromhexine-HCl

Different techniques used to estimate BH individually and also in combined forms along with other drugs which include: potentiometric flow Injection analysis using conventional and coated wire ion-selective electrodes (Abdel-Ghani *et al.*, 2006), reverse phase-HPLC (Nalini *et al.*, 2014) (Satyanarayana *et al.*, 2012), TLC (Dave *et al.*, 2010), high performance thin layer chromatography (HPTLC) (Dhoka *et al.*, 2010), voltammetry (Turch'an *et al.*, 2007) and HPLC-ICP-MS compared with radiochemical detection (Jensen *et al.*, 2005). However, most of them suggest that quantification of BH in any matrix require elaborate and sophisticated instruments which may or may not be available in every laboratory, others are time consuming or required solvent extraction, as well as the most potentiometric methods used a bromhexine-selective electrode or other ion-selective electrodes which are either expensive or not readily available in the market, or involve difficult methods of fabrication.

Many UV-Visible spectrophotometric methods have been reported in the literature for analytical determination of BH. Most of them included diazotization of BH and then coupling with different coupling agents such as: chromotropic acid (Al-Ward, 2011), pyrogallol (Othman and Omer, 2009). Other methods were either based on the formation of Schiff's base reaction with p-dimethylaminobenzaldehyde in the presence of SDS (Khalil and Saad, 2007), reduction of  $\text{Fe}^{3+}$  into  $\text{Fe}^{2+}$  by the drug followed by the complex formation of  $\text{Fe}^{2+}$  with 2, 2' bipyridyl to form cherry red colored chromogen having maximum absorbance at 510 nm (method A) and oxidation of 3-methylbenzothiazolinone-2-hydrazone by  $\text{FeCl}_3$  followed by its coupling with the drug in acidic medium forming an intense green colored chromogen with absorbance maxima at 630 nm (method B) (Raja *et al.*, 2010) or ion pair complexation of BH, in acidic buffers, with triphenylmethane dyes (Susmitha *et al.*, 2013). Also, oxidation-reduction (Othman and Omer, 2008) and first derivative spectrophotometric methods have been used for determination of BH (Vishal *et al.*, 2011). However, some of these procedures suffer from various difficulties such as, low stability of the colored product formed, laborious, poor sensitivity, have high detection limit and others require extraction, non-aqueous medium or long time for the reaction to complete, or applicable to higher concentrations of the drug.

The proposed work aims mainly to develop a sensitive spectrophotometric method for the determination of BH in different pharmaceutical preparations. The method based on the oxidation of BH by using cerium (IV) and the liberated cerium (III) reacts with arsenazo III in aqueous solution to form a highly colored dye that has been applied successfully to the assay of BH in pharmaceutical preparations.

## EXPERIMENTAL

### Apparatus

All absorption spectra and absorbance measurements are carried out by a JASCOV- 630 double beam UV-visible spectrophotometer (Japan) with 1.0-cm quartz cells. The pH measurements are made with a professional HANNA pH meter 212. Thermostatic controlled water-bath type Memmert 854.

### Reagents and chemicals

All chemicals used are of analytical grade.

*Bromhexine-HCl solution (100 $\mu$ g/ml).* It is prepared by dissolving 0.0100g of BH in amount of distilled water and the volume is completed to 100 ml with the same solvent in a 100 ml volumetric flask. Working solution of BH is prepared by appropriate dilution of the stock solution with distilled water.

*Cerium (Iv)ion solution ( $4.5 \times 10^{-4}$  M).* This solution is prepared daily by dissolving 0.0155 g of cerium sulphate in 3 drops of concentrated sulphuric acid and the solution is made up to 100 ml with distilled water using a 100 ml volumetric flask.

*ArsenazoIII (AzIII) solution ( $7 \times 10^{-4}$  M).* This solution is prepared by dissolving 0.0543 g of AzIII (Fluka) in amount of distilled water and the volume is completed to 100 ml with the same solvent in a volumetric flask.

*Formate buffer solution (pH3.4).* It is prepared by mixing 6 ml of formic acid and 2.8 g of NaOH in water, and the solution is diluted to 100 ml with distilled water using a volumetric flask (Marczenko and Blacerczak, 2004).

### General procedure

To a series of 20 ml volumetric flasks, 0.1-2 ml of 100 ppm BH solution are transferred, then 5 ml of  $4.5 \times 10^{-4}$  M Ce(IV) ion solution is added, followed by 0.8 ml of buffer solution (pH3.4) and mixed thoroughly. After 25 minutes, 1 ml of  $7 \times 10^{-4}$  M AzIII solution is added. The solutions are heated in a water bath adjusted at 60°C for 5 min, then cooled, diluted up to the mark with distilled water and mixed well. The absorbance of the colored complex is measured at 651 nm against the corresponding reagent blank.

### Procedure for the assay of pharmaceutical preparations

*For tablets (100 $\mu$ g/ml).* Five tablets of BH (each tablet contains 8 mg BH) are finely powdered, an accurately weighed of the powder equivalent to 0.0100g is dissolved in 2ml of 1N hydrochloric acid and the solution is filtered into 100 ml calibrated flask and then the volume is completed to the mark by repeated washing with distilled water. Each ml of this solution containing 100  $\mu$ g of BH.

*For syrup solution (100 $\mu$ g/ml).* A 12.5 ml of syrup (each 5ml contain 4 mg BH) is transferred into a 100 ml calibrated flask and the total volume is diluted with distilled water.

*For injection solution (100 $\mu$ g/ml).* This solution is prepared by diluted 5 ml of BH injection solution (each 2 ml contains 4 mg BH), with distilled water in 100 ml calibrated flask.

## RESULTS AND DISCUSION

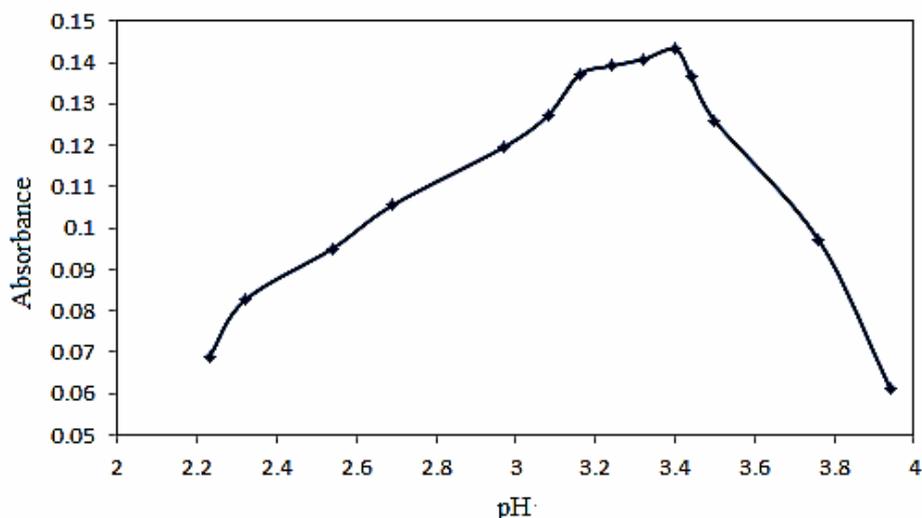
Throughout the preliminary study cerium (Iv) ion solution reacts with 100 $\mu$ g BH in acid medium to produce quantitatively cerium (III) ion. The amount of cerium (III) ion is then determined by using AzIII reagent to form an intensely greenish-blue water soluble chromogen that showed maximum absorption at 651 nm in contrast to the reagent blank.

The intensity of the formed colored complex has been found to be proportional to the amount of BH originally present in the solution. The effect of various parameters on the absorption intensity of the colored complex is investigated and the optimum reaction conditions have been selected.

### Effect of pH

The effect of pH on [Ce(III)-AzIII] complex is investigated and showed pH dependent maximum absorption at 651 nm, whereas the reagent blank solution showed maximum absorption

at 433 nm. The optimum pH range for complex formation is 3.21-3.4 ( Fig.1). pH3.4 is considered the optimum because of the high absorbance intensity and good color contrast ( $\Delta\lambda=218$  nm).

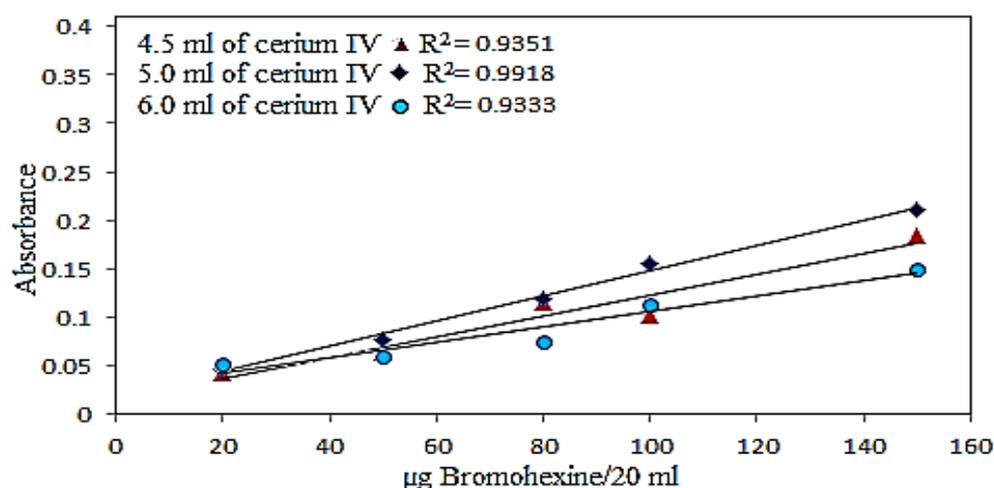


**Fig. 1: Effect of pH on absorbance of (Ce(III)-AzIII) complex**

Therefore, various buffer solutions of pH 3.4 are prepared and their efficiencies are also tested on the absorbance of the [Ce(III)-AzIII ] complex. The experimental data of this investigation indicated that 0.8 ml of format buffer solution of pH 3.4 is the optimum and it has been selected for the subsequent experiments.

#### Effect of cerium (IV) sulfate concentration

The effect of cerium (IV) sulfate amount on the absorbance of the colored complex formed has been investigated (Fig. 2).



**Fig. 2: The effect of cerium sulfate amount on absorbance**

The results in Fig. 3 shows that 5ml of  $4.5 \times 10^{-4}$ M cerium(IV) sulfate solution gives the highest absorbance with a determination coefficient ( $R^2 = 0.9918$ ) over a concentration range 20-150 µg BH /20 ml, therefore it is recommended for the subsequent experiments.

### Effect of time on oxidation-reduction reaction

The effect of time required to complete the reduction of cerium(IV) ion to cerium(III) ion is studied by allowing the solutions after adding cerium(IV) ion solution to stand at room temperature for different times then the other reagents are added and the absorbance are measured at 651nm against the reagent blank (Fig. 3).

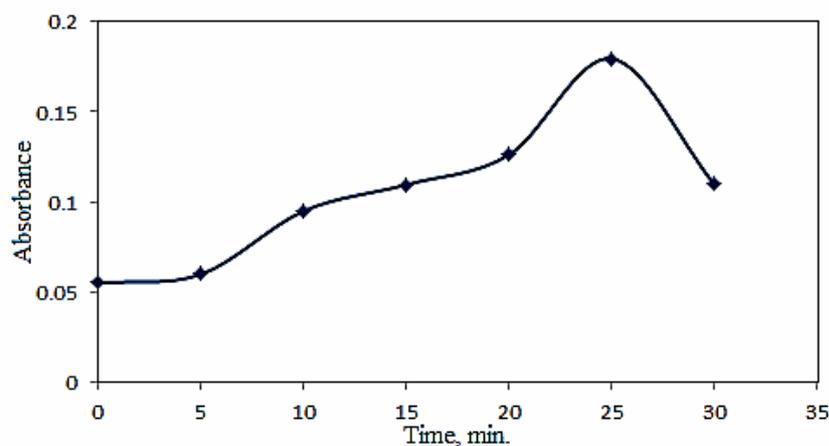


Fig. 3: Effect of time on reduction process

The results in (Fig.3) indicate that complete reduction of cerium(IV) ion occurred at 25 minutes and the intensity decreased above 25 minutes, because the intensity of reagent blank solution is increased (weak color contrast). Therefore, the standing time 25 minutes is recommended for the subsequent experiments.

### Effect of AzIII reagent amount

The effect of different amounts (0.5, 1 and 1.5 ml) of  $7 \times 10^{-4}$  M AzIII on the absorbance of the resulting complex have been also studied. The results in Fig.4 indicate that 1ml of  $7 \times 10^{-4}$  M of AzIII solution show better absorbance and give a good determination coefficient ( $R^2 = 0.9869$ ), therefore it is recommended for the subsequent experiments.

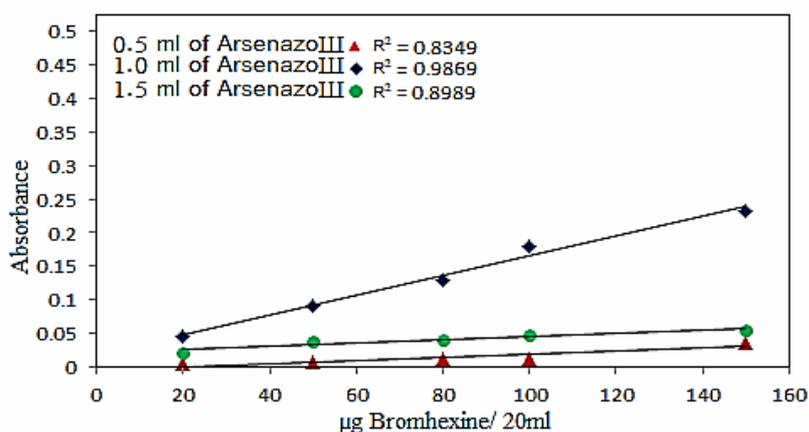


Fig. 4: Effect of reagent amounts on absorbance

### Effect of temperature and reaction time

The reaction time is determined by following the colour development at room temperature and in a thermodynamically controlled water-bath at different temperatures. The absorbance is measured at 5 min intervals against reagent blank treated similarly. The formation of coloured complex (Ce(III)-AzIII) was slow at room temperature and required longer time for completion. Hence, efforts are made to accelerate the reaction by carrying out the reaction at higher temperatures. It was observed that the maximum absorbance is obtained when heating the reaction

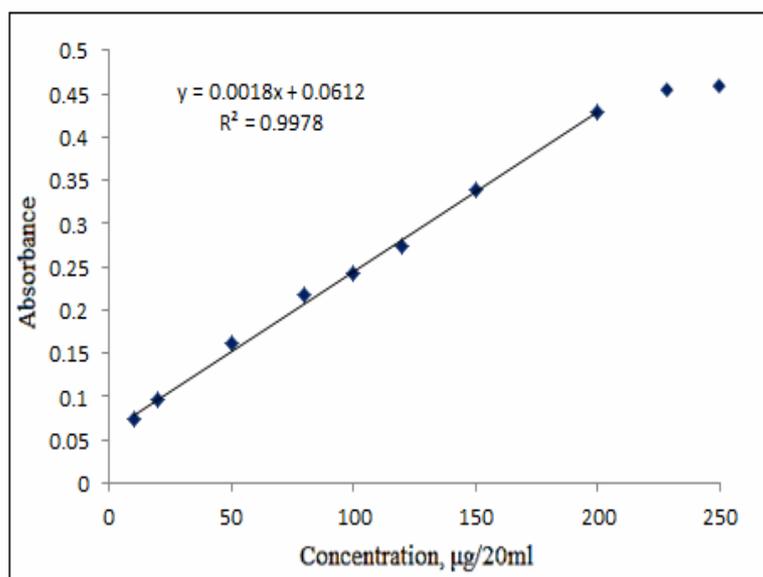
mixture to 60 °C for 5min (Table 1). The stability of complex was found constant after cooling to room temperature for at least 1 hr.

**Table 1: Effect of temperature on the colour development of the complex**

Temperature (C°)	Variable	Absorbance / minutes				
		0	2	5	10	15
R.T.	S	0.1558	0.1600	0.1721	0.1681	0.1651
	B	0.0821	0.1089	0.0933	0.0911	0.0901
45	S	0.1588	0.1604	0.2094	0.1802	0.1766
	B	0.0812	0.0785	0.0593	0.0730	0.0559
60	S	0.1621	0.1804	0.2302	0.2156	0.2118
	B	0.0709	0.0662	0.0374	0.0359	0.0332
70	S	0.1593	0.1428	0.1091	0.0905	0.0882
	B	0.0738	0.0331	0.0245	0.0211	0.0210

### Quantification

In order to investigate the range in which the colored complex adheres to Beer's law, the absorbance of the complex is measured at 651 nm after developing the colour by following the suggested procedure for a series of solutions containing increasing amounts of BH drug (Fig. 5).



**Fig. 5: Calibration curve for BH determination**

The Beer's law limits, molar absorptivity, Sandell's sensitivity, (LOD) and (LOQ) values (ICH Harmonized, 2005) are evaluated and are given in Table 2 which indicates that the method is sensitive. Linearity is represented by the regression equation and the corresponding determination coefficient for BH determined by the proposed method represents excellent linearity ( $R^2=0.9978$ ). The relative standard deviation (RSD) and accuracy (relative error %) for the analysis of five replicates of each of the three different concentrations of BH indicates that the method is precise and accurate.

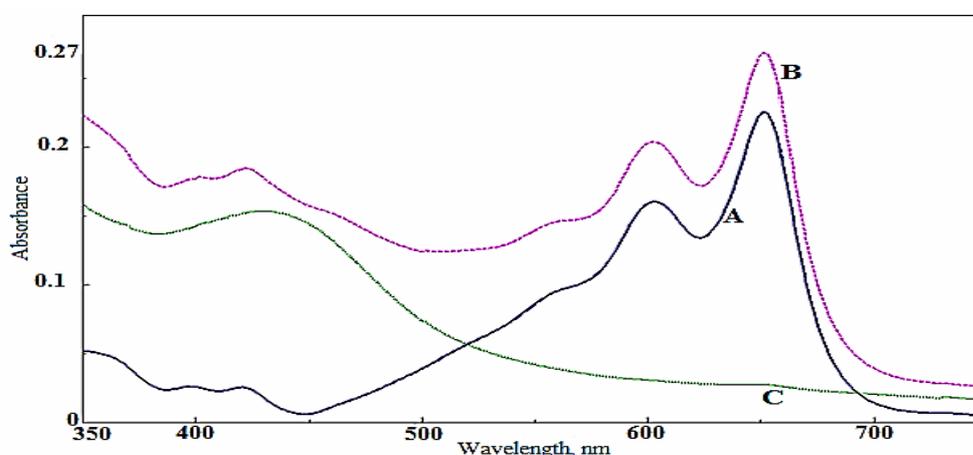
**Table 2: Summary of optical characteristics and statistical data for the proposed method**

Parameter	Values of method
Beer's law limits (ppm)	0.5-10
Molar absorptivity ( $\text{l.mol}^{-1}.\text{cm}^{-1}$ )	$1.48 \times 10^4$
LOD ( $\mu\text{g ml}^{-1}$ )	0.408
LOQ ( $\mu\text{g ml}^{-1}$ )	1.394
Range of relative error * (%)	0.354 to 1.93
Determination coefficient	0.9978
Slope	0.0018
Intercept, <i>b</i>	0.0612
RSD* (%)	No more than 1.033

\* Average of five determinations.

**Absorption spectrum**

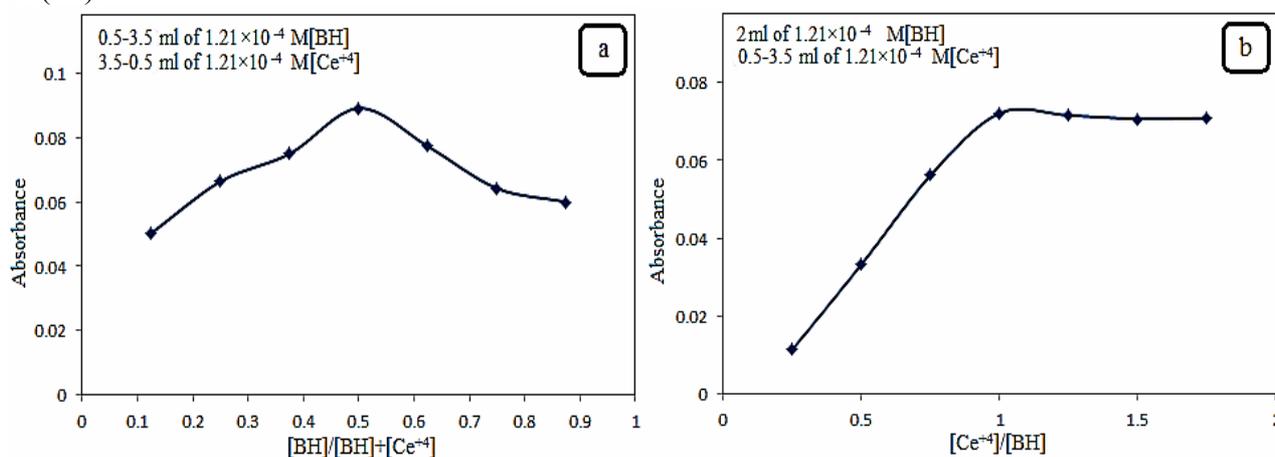
Absorption spectrum of the colored complex formed from the reaction between cerium(III) ion with AzIII at pH3.4 against its corresponding reagent blank shows maximum absorption at 651 nm in contrast to the AzIII reagent blank (Fig. 6).



**Fig. 6: Absorbance spectra of 100 $\mu\text{g}$  BH / 20 ml treated according to proposed method and measured against (A) reagent blank (B) distilled water and (C) blank measured against distilled water.**

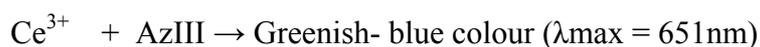
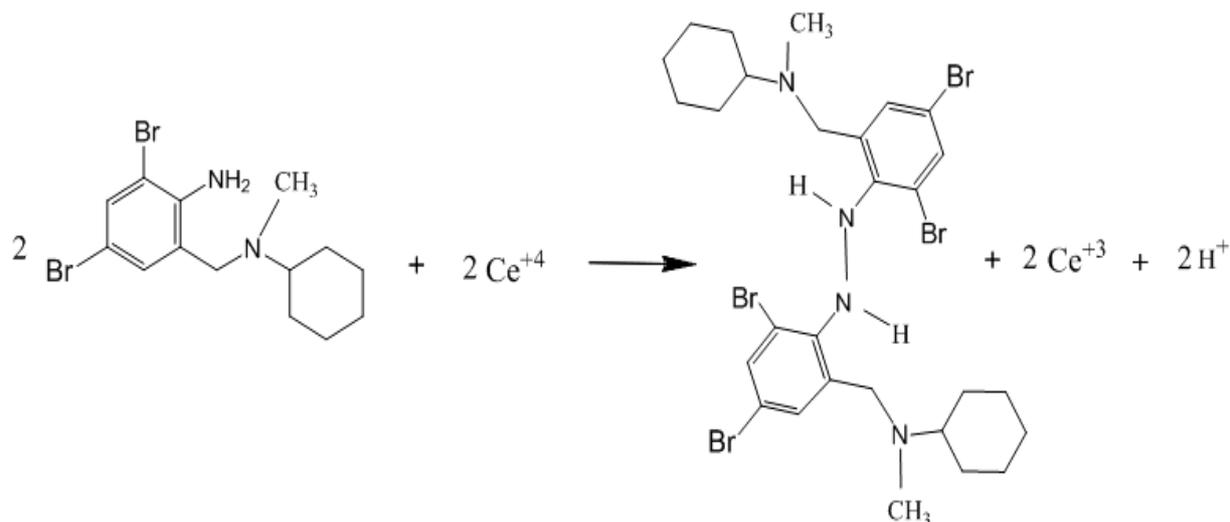
**Stoichiometry of the reaction**

The stoichiometry of the reaction of BH with cerium ion  $\text{Ce(IV)}$  is studied under the established conditions using both continuous variation and molar ratio methods. The results obtained in both methods (Fig.7) reveal that the product is formed by a 1:1 combining ratio of BH:  $\text{Ce(IV)}$ .



**Fig. 7: (a) Continuous variations and (b) molar-ratio plots for BH- [Ce(IV)]**

The Cr(III) ion produced is chelated by AzIII to form the well-known greenish-blue complex. (Marczenko and Balcerzak, 2004). Therefore, the probable reaction path might be written as follows:



### Interference

In order to test the efficiency and selectivity of the proposed method, the effect of the presence of some common pharmaceutical additives such as: starch, glucose, lactose, sorbitol and gum Arabic that are usually present in dosage forms is studied by adding different amounts of foreign substances to 100  $\mu\text{g}$  of BH. The results in Table 3 indicate that there are no significant interferences produced by these foreign substances on the proposed procedure.

**Table 3: Effect of foreign compounds for assay of BH**

Foreign compound	Recovery (%)* of 100 $\mu\text{g}$ BH / $\mu\text{g}$ foreign compound added		
	100	250	500
Glucose	99.13	101.52	102.09
Starch	98.72	101.52	101.39
Lactose	98.55	98.47	99.42
Sorbitol	100.69	99.34	100.32
Gum Arabic	102.18	102.51	103.25

\* Average of three determinations

### Application of the method

The proposed method is applied to assay six different pharmaceutical preparations containing BH (tablets, syrups and injection), Table 4 shows that good recoveries are obtained .

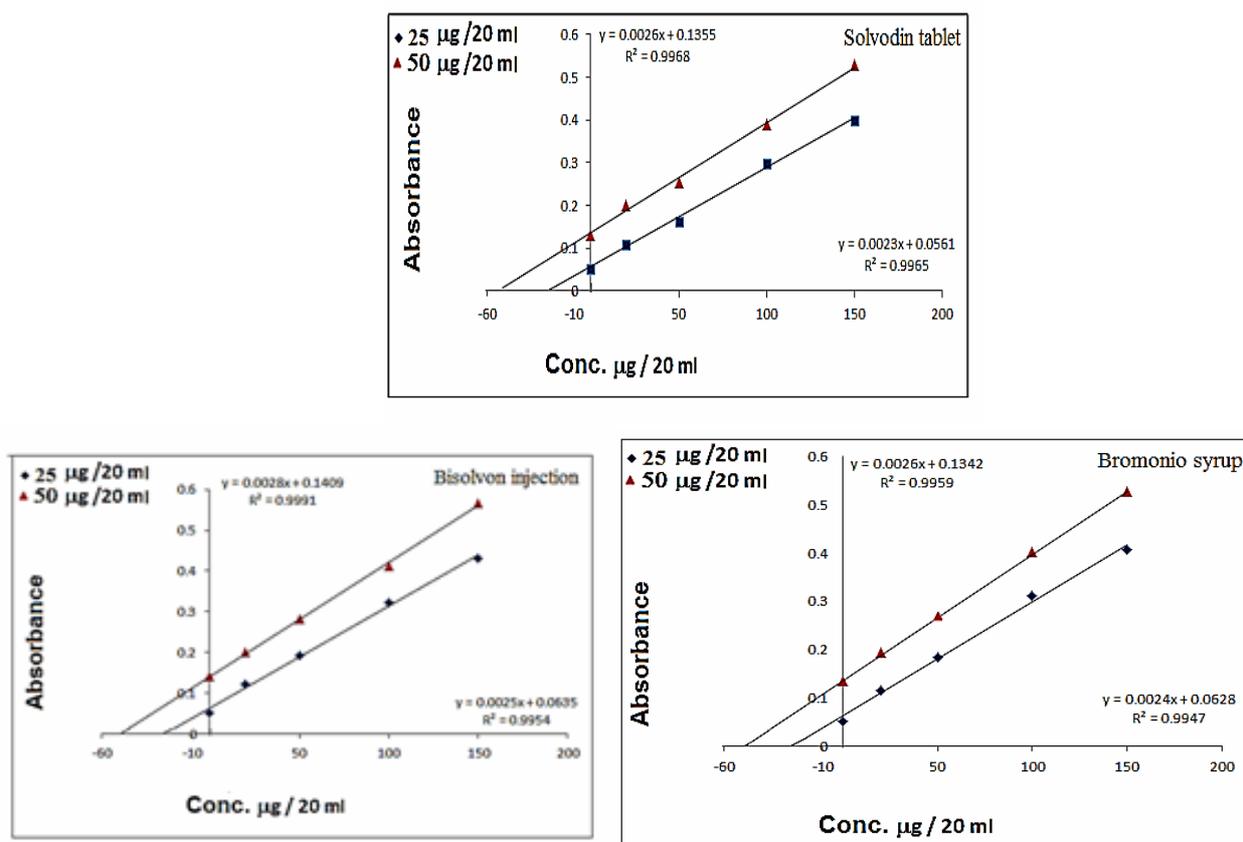
**Table 4: Determination of BH in pharmaceutical preparations**

Pharmaceutical preparation	$\mu\text{g}$ BH present per 20 ml	$\mu\text{g}$ BH found per 20 ml*	Relative error (%)*	Recovery (%)*
Solvodin tablet 8.0 mg BH / tablet (S.D.I.- Iraq)	50	51.16	2.32	102.32
	100	102.30	2.30	102.30
	150	150.63	0.42	100.42
Bisolvon injection 4mg /2ml Boehringer Ingelheim, (Germany)	50	50.04	0.09	100.08
	100	99.45	- 0.55	99.45
	150	150.07	0.05	100.05
Solvodin syrup 4mg BH /5ml (S.D.I.- Iraq)	50	50.02	0.04	100.04
	100	98.98	- 1.01	98.98
	150	148.59	- 0.93	99.06
Bromonio syrup 4mg BH /5ml Medpharma (United Arab Emirates)	50	50.40	0.80	100.80
	100	100.08	0.08	100.08
	150	150.16	0.11	100.11
Salbid syrup 4mg BH /5ml Micr Labs Limited (INDIA)	50	49.73	- 0.54	99.46
	100	99.53	- 0.47	99.53
	150	150.63	0.42	100.42
Bisolvon tablet 8.0 mg BH / tablet Boehringer Ingelheim, (Germany)	50	50.26	0.52	100.52
	100	100.70	0.70	100.70
	150	150.88	0.59	100.59

\* Average of three determinations

**Evaluation of the proposed method**

According to the difficulties of using the standard method in our laboratory for determination of BH in its pharmaceutical preparations, so that a standard addition method has been used for its simplicity which proves that the proposed method is applied successfully for the determination of BH without interferences (Fig. 8 and Table 5)



**Fig. 9: Graphs of standard addition method for the determination of BH in pharmaceutical preparations (tablet, syrup and injection)**

**Table 5: The results of standard addition method**

Pharmaceutical preparation	BH taken µg /20 ml	BH measured µg /20 ml	Recovery, %
Solvodin tablet (8.0 mg BH /tablet) (S.D.I. Iraq)	25	24.39	97.56
	50	52.11	104.22
Bisolvon injection (4 mg/2ml) (Boehringer Ingelheim) Germany	25	25.40	101.60
	50	50.32	100.06
Bromonio syrup (4mg BH /5ml) (Medpharma) United Arab Emarates	25	26.16	104.64
	50	51.61	103.22

### CONCLUSION

It could be concluded that the developed method for bromhexine hydrochloride assay is simple, sensitive (microgram amount can be determined), relatively precise, accurate and can be satisfactorily applied to the analysis of bromhexine hydrochloride in bulk and pharmaceutical formulations.

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