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Indirect Spectrophotometric Method for Determination of Bromhexine-Hydrochloride in Pharmaceutical Preparations

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ABSTRACT

A sensitive indirect spectrophotometric method is developed for the determination of bromhexine-HCl in pure and dosage forms. The method is based on the oxidation of bromhexine-HCl with iron(III) in acidic medium, then reaction of iron(II) with 1,10-phenathroline to produce a ferroin complex which is water-soluble, stable, and has a maximum absorption at 510 nm against the reagent blank with a molar absorptivity of 3.51×10^{4} $l.mol^{-1}.cm^{-1}$. Beer's law is valid over the concentration range of 10 to 220 μg bromhexine-HCl in a final volume of 25 ml. The proposed method has been applied successfully to determine bromhexine-HCl in different pharmaceutical preparations.

طريقه طيفيه غير مباشره لتقدير البرومهكسين – هيدروكلورايد في مستحضراته الصيدلانيه

الملخص

تم تطوير طريقة طيفية غير مباشره حساسة لتقدير البرومهكسين بشكله الحر وفي مستحضراته الصيدلانية. تعتمد الطريقة على أكسدة البرومهكسين بوساطة الحديد الثلاثي في الوسط ألحامضي ومفاعله الحديد الثنائي الناتج مع الكاشف 1،10-فيناثرولين لينتج معقد الفيرون الذي يكون ذائباً في الماء ومستقراً ويعطي أعلى شدة امتصاص عند الطول الموجي 510 نانوميتر مقابل المحلول الصوري، وكانت قيمة الامتصاصية المولارية 3.507×10^{4} مول.لتر$^{-1}$.سم$^{-1}$. وينطبيق قانون بير في مدى التركيز من 10 إلى 220 مايكروغرام برومهكسين في حجم نهائي 25 مل. تم تطبيق الطريقة بنجاح في تقدير البرومهكسين في مستحضراته الصيدلانية المختلفة.

INTRODUCTION

Bromhexine is widely used in medicine as a mucoltic drug. It works through decreasing the amount of respiratory tract fluid and reduced its viscosity by activating enzymes that hydrolyze mycopoly saccharides. Bromhexine is used for respiratory infections, such as cold, influenza and respiratory tract infections (Bub and Friedrich, 2002). Many spectrophotometric methods have been proposed for the determination of bromhexine-HCl, these included derivative spectrophotometric and multi-wavelength spectrophotometric methods for simultaneous determination of terbutaline sulphate (I), bromexine-HCl (II) and guaiphenes (III) in three-component tablet dosage forms. Methanolic hydrochloric acid (0.1N) is used as a solvent and the absorbance measured at 279 nm for(I), II shows two absorbance maxima, one at 279 nm and another at 248 nm and (III) shows absorbance maximum at 174 nm (Gangwal and Trivedi, 1999). Linear regression at zero-crossing wavelengths of the first derivative spectra or by application of multiple linear partial least squares regression method are used in determination of salbutamol sulphate and bromhexine-HCl encounterd in tablets (Habib et al., 2005).

Three simple and sensitive spectrophotometric methods (A-C) for the assay of bromhexine-HCl in pure and dosage forms are proposed; based on the formation of chloroform soluble ion-associates under specified experimental conditions, three acidic dyes namely, tropacolin 00 (method A), naphthalene blue 12BR (method B) and azocarmin G (method C). The extracts of ion-associates exhibit absorption maxima at 420, 620 and 540 nm for methods A, B and C, respectively (Murali et al., 2005). Other spectrophotometric methods included diazotization of bromhexine-HCl then coupling with different coupling agents such as: N-(1-naphthyl)ethylenediamine which gives aviolet azo dye which has a maximum absorption at 555nm (Shingbal and Rao, 1985),naphthylamine in stead of Bratton Marshall reagent, the resulting dye gives maximum absorption at 478 nm and Beer's law is obeyed in the concentration range of 2.0-8.0 mg/L (Buitrago et al., 2005). Another method used resorcinol in an alkaline medium, the azo dye gives maximum absorption at 435 nm and Beer's law obeyed in the concentration range of 1-7mg/L (Shingbal and Sardesai, 1987). Another method used 2-naphthol in an alkaline medium, the red azo dye gives maximum absorption at 490 nm and Beer's law is obeyed in the concentration range 1-7mg/L (Shingbal and Kudchadkar, 1987).

Bromhexine-HCl is also determined by coupling with p-dimethylaminobenzaldehyde in highly acidic medium to yield Schiff's base. The yellow chromophore obtained showed maximum absorbance at 425 nm and Beer's law is obeyed in the concentration range 2-16 mg/L (Choinani et al., 1986).

Another colorimetric method based on the reaction of bromhexine-HCl with an excess amount of p-dimethylaminobenzaldehyde in acidic medium (pH=1.6) and in the presence of sodium dodecyl sulphate. The yellow product has been followed spectrophotometrically at absorbance maximum of 430 nm. Beer's law is obeyed in the concentration range 0.41-82.5 mg/l (Khalil and Saeed, 2007).

The proposed work aims mainly to develop a sensitive spectrophotometric method for the determination of bromhexine-HCl in different pharmaceutical preparations. The method based on the oxidation of bromhexine-HCl by using iron(III) and the librated iron(II) reacts with 1,10-phenathroline in aqueous solution to form a highly coloured dye that has been applied successfully to the assay of bromhexine-HCl in pharmaceutical preparations.

EXPERIMENTAL

All spectrophotometric measurements are performed on Shimadzu UV-visible recording spectrophotometer UV-160 using 1-cm silica cells. pH meter type Philips PW 9420 is used for pH reading.

Thermostatic controlled water-bath type Memmert 854 Schwa back is used.

Reagent

All chemicals used are of analytical-reagent grade.

Standared solution of bromhexine-HCl, 100 μg.ml^{-1}.

This solution is prepared by dissolving 0.01g of bromhexine hydrochloride in distilled water (heating is necessary to increase solubility) and then the volume is made up to 100 ml in a volumetric flask with distilled water.

Iron (III) nitrate nanohydrate solution, 0.02 M

This solution is prepared by dissolving 0.808g of ferric nitrate monohydrate in 8 ml of nitric acid (1M) and then the volume is completed to 100ml in a volumetric flask with distilled water.

1,10-phenanthroline monohydrate solution, 0.02 M

This solution is prepared by dissolving 0.3964g of 1-10-phenathroline monohydrate in 10 ml of ethanol and the solution is made up to 100 ml in a volumetric flask with distilled water.

Acetic acid solution, 0.05 N.

This solution is prepared by appropriate dilution of concentrated solution.

Bromhexine-HCl tablets solution, 80 μg.ml^{-1}.

Ten tablets of bromhexine-HCl (each tablet contains 8 mg bromohexine-HCl) are finely powdered, an accurately weighed of the powder equivalent to 0.008 g is dissolved in 2 ml hydrochloric acid (1N) and the residue is filtered into 100 ml calibrated flask and then the volume completed to mark by repeated washing with distilled water.

Bromhexine-HCl injection solution, 80 μg.ml^{-1}.

A 4 ml of bromhexine-HCl injection solution (2 ml contain 4 mg bromehexine-HCl) is diluted to 100 ml with distilled water in a calibrated volumetric flask.

Bromhexine-HCl syrup solution, 80 μg.ml^{-1}.

A 10 ml of bromhexine-HCl syrup (each 5ml contains 4 mg bromohexine-HCl) is diluted to 100 ml with distilled water in a calibrated volumetric flask.

Procedure and calibration graph:

To a series of 25-ml calibrated flasks, 0.1-2.6 ml of 100 μg.ml^{-1} bromhexine-HCl solution are transferred, then 0.2 ml of 0.02 M ($Fe(NO_3)_3.9H_2O$ solution is added, followed by 3 ml of 0.02 M 1,10-phenanthroline monohydrate solution and 0.1 ml of 0.05 N acetic acid solution. After the volumes are completed to the mark with distilled water, the solutions left for 25 minutes in water bath adjusted at 80°C, then the solution. left to stand for 20 minutes at room temperature before the absorbances of the red coloured product are measured at 510 nm against the reagent blank. A linear calibration graph is obtained over the concentration range of 10-220 μg bromhexine-HCl/25ml and a concentration above 220 μg/25ml gives a negative deviation (Fig. 1). The molar absorptivity has been found to be 3.51×10^4 l.mol^{-1}.cm^{-1}.

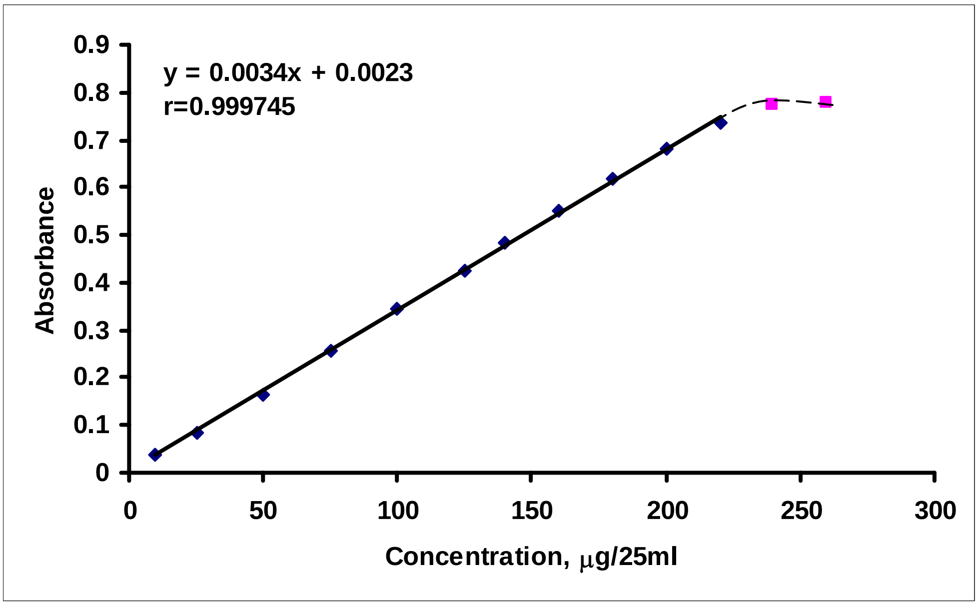


Fig. 1: Calibration graph of bromhexine-HCl determination

RESULTS AND DISCUSION

Principle of the method

Iron (III) ion can act as an oxidant and reacts with bromhexine-HCl to produce quantitatively iron(II) ion . The amount of iron(II) ion can be determined by using 1,10-phenanthroline monohydrate reagent and the intensity of the red complex can be used to develop a spectrophotometric method for the determination of bromhexine-HCl in different pharmaceutical preparations.

Effect of pH

The effect of pH on colour intensity is examined firstly. The extent of complex formation, and hence the absorbance of the final solution, is often a function of hydrogen ion concentration. Different acids have been used and the results indicate that 0.1ml of acetic acid solution (0.05N) is the most suitable acid solution (pH=3.04) to produce the highest colour intensity (Table 1).

Table 1: Effect of pH on absorbance.

Acid used, (1N)	Absorbance/ml acid added					pH-range
	0.1	0.2	0.4	0.5	0.7	
HCl	0.223	0.229	0.227	0.227	0.222	2.81-2.48
HNO_3	0.235	0.229	0.223	0.220	0.210	2.86-2.54
H_2SO_4	0.236	0.234	0.232	0.197	0.188	2.83-2.51
CH_3COOH	0.283	0.267	0.265	0.264	0.264	3.04-2.91

Effect of temperature

The effect of temperature on the colour intensity of the resulting complex is investigated. The results indicated that absorbance of the complex increased with increasing temperature (Table 2).

Table 2: Effect of temperature.

Temperature, C°	30	50	60	70	80	90
Absorbance	0.021	0.182	0.246	0.289	0.297	0.299

The result in Table 2 indicated that practically there is no significant difference in intensity when the colour is developed at 70°C or 90°C; 80°C is recommended.

Effect of ferric nitrate nanohydrate concentration

The effect of changing the ferric nitrate nanohydrate amount on the absorbance of the red complex formed has been investigated (Table 3).

Table 3. The effect of ferric nitrate nanohydrate amount on absorbance.

Ml ferric nitrate (0.02M) solution	Absorbance/µg of bromhexine-HCl in 25ml					r
	25	50	75	100	125	
0.05	0.067	0.084	0.087	0.090	0.103	0.9524
0.1	0.078	0.155	0.233	0.283	0.328	0.9919
0.2	0.088	0.169	0.258	0.346	0.429	0.9999
0.3	0.086	0.159	0.254	0.316	0.413	0.9981
0.4	0.079	0.150	0.246	0.311	0.393	0.9987

The results illustrated in Table (3) indicated that 0.2 ml of 0.02 M ferric nitrate nanohydrate solution gives the highest absorbance with a correlation coefficient (r=0.999879) over a range of determinand concentration of 25-125 µg bromohexine-HCl/25ml, therefore it is recommended for the subsequent experiments.

Effect of time on oxidation-reduction reaction

The effect of time needed to complete the oxidation-reduction reaction is studied by allowing the solutions to stand in water bath for different times,after adding the component of reaction(bromhexine-HCl, ferric nitrate monohydrate and 1-10-phenathroline monohydrate), then the absorbances measured against the reagent blank (Table 4).

Table 4. Effect of time on oxidation- reduction process.

Time (min.)	0	5	10	15	20	25	30	35	40	45	50
Absorbance	0.000	0.177	0.273	0.298	0.310	0.323	0.324	0.324	0.325	0.327	0.328

The results indicate that high absorbance of complex at 510nm occurred after 25 minutes and there is no significant differences in the intensity of complex above 25 minutes. Therefore, the standing time 25 minutes is recommended for the subsequent experiments.

Effect of 1,10-phenanthroline monohydrate reagent amount

The effect of different amounts of 1.10-phenanthroline monohydrate reagent on the absorbance of solution containing different amounts of bromhexine-HCl (25-125 μg/25ml) is studied. The results indicated that the absorbance increases with increasing reagent concentration and reached maximum on using a volume of 3 ml of 0.02 M 1,10-phenanthroline monohydrate which also gives the highest value of correlation coefficient (Table 5).

Table 5. The effect of reagent amount on absorbance.

Ml of 1-10,phenanthroline (0.02M) solution	Absorbance/μg of sample					r
	25	50	75	100	125	
2.0	0.063	0.148	0.226	0.297	0.378	0.9996
3.0	0.086	0.165	0.255	0.344	0.426	0.9998
3.5	0.079	0.147	0.249	0.330	0.419	0.9987
4.0	0.032	0.125	0.228	0.310	0.400	0.9994

Effect of time

The effect of time on the development and stability period of the red complex is investigated under optimum experimental conditions cited above. The absorbance measured after each 5 minutes for 24 hours (1440 minutes) after emerging the flasks from water bath (Table 6).

Table 6. Effect of time on absorbance.

Bromhexine-HCl (μg/25ml)	Absorbance/minutes									
	0	5	10	20	30	40	50	60	120	1440
50	0.155	0.159	0.164	0.167	0.167	0.167	0.167	0.166	0.164	0.169
100	0.323	0.338	0.342	0.345	0.347	0.348	0.349	0.350	0.345	0.351
150	0.490	0.509	0.514	0.517	0.519	0.519	0.521	0.521	0.523	0.537

The results in Table 6 indicate that the red complex is stable for at least 24 hours.

Final absorption spectrum

When bromohexine-HCl is treated according to the recommended procedure, the absorption spectrum shows a maximum absorption at 510 nm, characteristics of the ferroin chromophore, in contrast to the reagent blank which shows a slight absorption at 510 nm (Fig. 2).

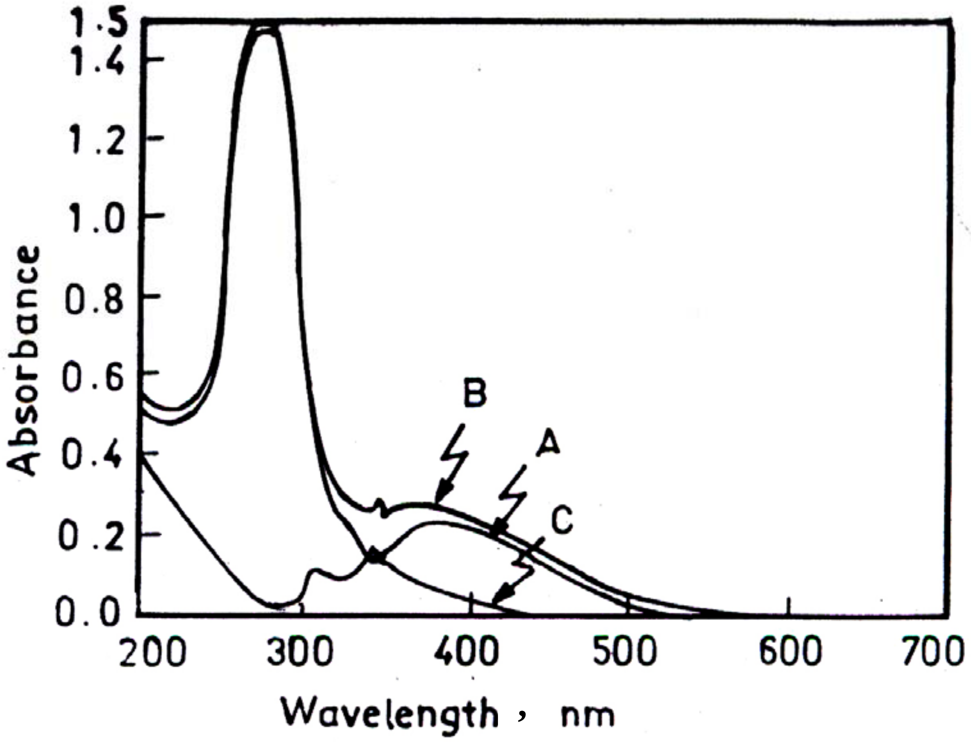


Fig. 2: Absorption spectra of 100μg bromohexine-HCl/25ml treated according to the recommended procedure and measured against (A) reagent blank, (B) distilled water and (C) reagent blank measured against distilled water.

Interference

The effect of some foreign compounds which often accompanied pharmaceutical preparations were studied by adding three different amounts (100, 500 and 1000μg) to 100μg bromhexine-HCl in a final volume 25 ml (Table 7).

Table 7:Effect of foreign compounds for assay of bromhexine-HCl.

Foreign compound	Recovery (%) of 100μg bromhexine-HCl per μg foreign compound added		
	100	500	1000
Glucose	102.95	102.41	100.26
Glycerin	101.88	100.26	99.73
Lactose	102.41	101.34	102.15
Starch	98.65	100.8	102.15

The results in Table 7 indicated that the studied foreign compounds do not interfere in the determination of bromhexine-HCl by using the proposed method. An error not more than of +2.95% or-1.35% in the absorbance readings is considered tolerable.

Accuracy and precision

To check the accuracy and precision of the method, bromhexine-HCl is determined at three different concentrations. The results illustrated in Table 8 indicated that the method is satisfactory.

Table 8. The accuracy and precision.

µg bromhexine-HCl/25ml	Relative error, %*	Relative standard deviation, %*
50	-1.63	±1.20
100	-0.89	±0.66
150	-0.97	±0.76

*Average of five determinations

Stoichiometry of the reaction

The stoichiometry of the product formed from the reaction of bromhexine [BH] with ferric ion [Fe^{+3}] was investigated by applying the continuous variations method (Job's method). The results indicate that the product was formed in the ratio of 1 bromhexine: 1 Fe^{+3} (Fig.3).

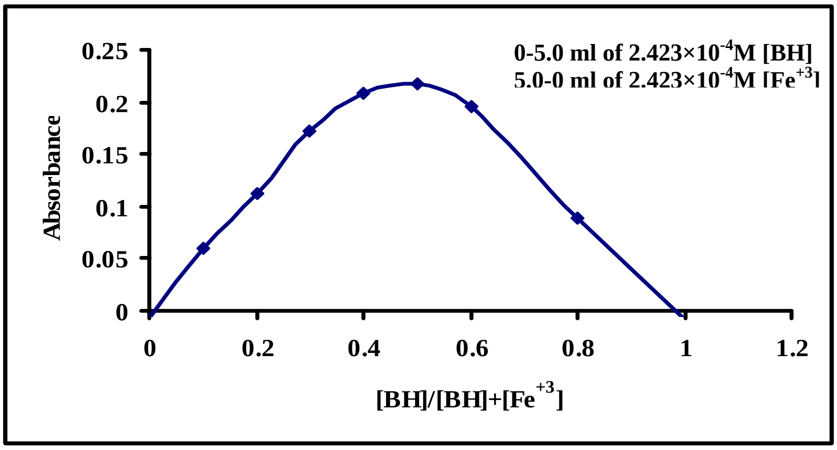
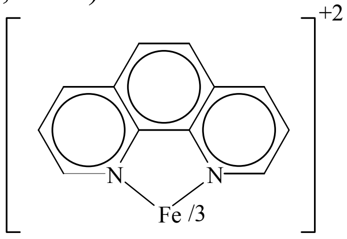


Fig.3: Job's plot for bromhexine-Fe^{+3}

The ferrous ion produced is chelated by 1,10-phenathroline to form the well-known red chelate(Christian, 1986):



Red complex

Analytical application

The proposed method is applied to determine bromhexine-HCl in different pharmaceutical preparations on appling proposed procedure, good recovery is obtained as shown in Table 9.

Table 9. Analytical applications of the proposed method.

Pharmaceutical preparation	μg bromhexine-HCl present/25ml	μg bromhexine-HCl found/25ml	Recovery * %
Solvodin tablet 8.0mg bromhexine-HCl/ tablet, (S.D.I-Iraq)	80 120 160	80.80 118.62 153.96	101.00 98.85 96.22
Solvodine syrup, 4mg bromohexine-HCl/5 ml (S.D.I. Iraq)	80 120 160	81.52 120.00 154.88	101.90 100.00 96.80
Bromohexine-HCl injection, 4mg/2ml (Ibn Hayan, Syria)	80 120 160	83.76 126.24 166.24	104.70 105.20 103.90

*Average of three determinations

Evaluation of the proposed method

According to the difficulties of using the standard method for determination of bromhexine-HCl in its pharmaceutical preparation, so the standard addition method has been used in order to prove that the proposed method is applied to the determination of bromhexine-HCl without interferences (Table 10 and Fig.4)

Table 10. The results of standard addition method.

Pharmaceutical preparation	μg bromhexine-HCl present/25ml	μg bromhexine-HCl found/25ml	Recovery*,%
Solvodin tablet 8.0mg bromhexine-HCl tablet, (S.D.I-Iraq)	40 80	39.00 78.50	97.50 98.12
Solvodine syrup, 4mg bromhexine-HCl/5 ml (S.D.I. Iraq)	40 80	41.50 82.00	103.75 102.50
Bromhexine-HCl injection, 4mg/2ml (Ibn Hayan, Syria)	40 80	40.50 81.00	101.25 101.25

* Average of three determinations.

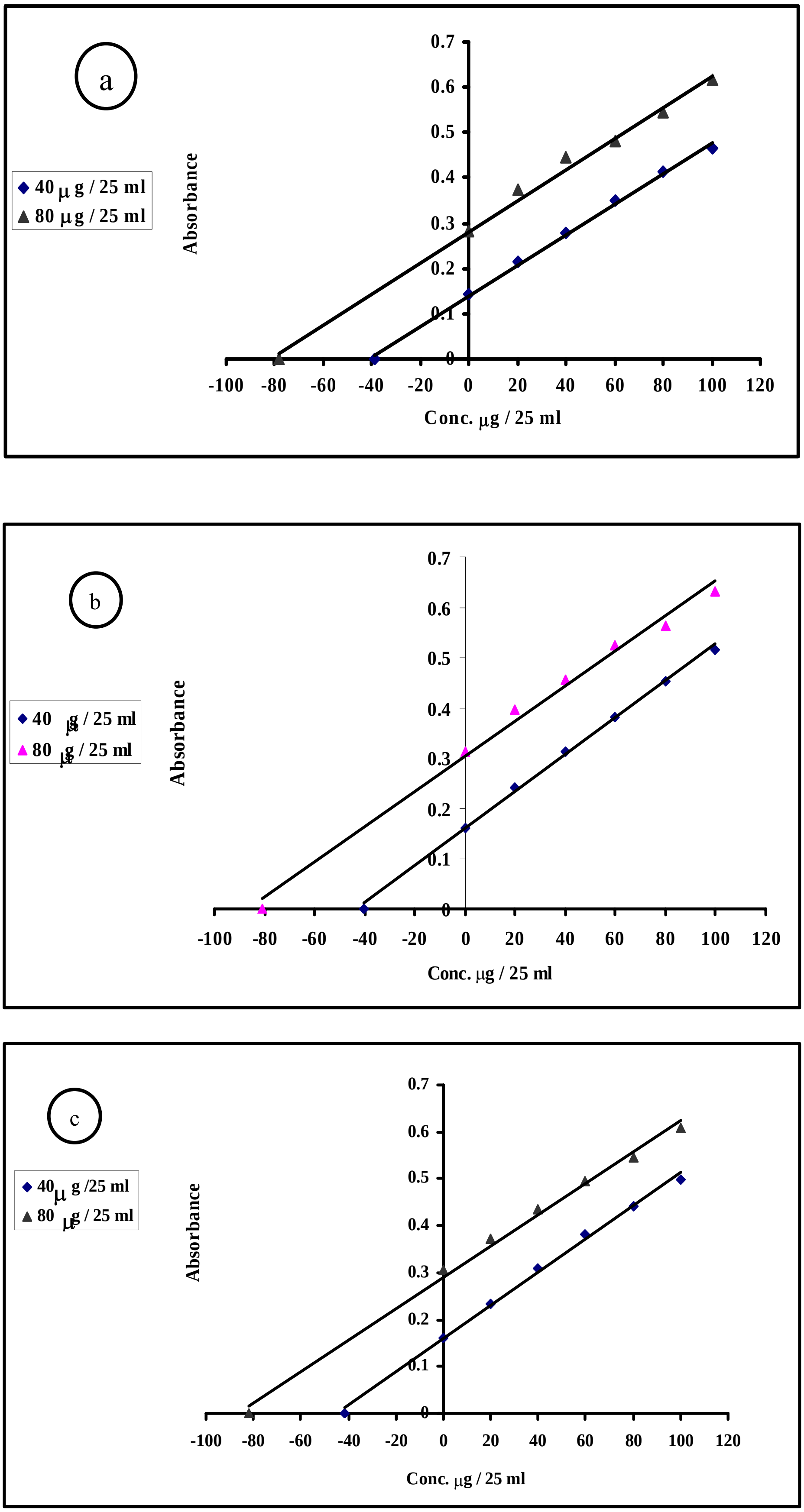


Fig.4: Graphs of standard addition method for the determination of bromhexine- HCl in tablet(a), injection(b) and syrup(c) pharmaceutical preparations.

The results in Table (10) and Fig. (4) indicated that the proposed method can be used to determine bromhexine-HCl in different pharmaceutical preparations with satisfactory results.

Comparison of the methods

Table (11) shows the comparison between some of analytical variables for the present method with that of another literature spectrophotometric method.

Table 11: comparison of the methods.

Analytical parameters	Present method	Literature method*
pH	3.04	1.6
Temperature (°C)	80^oC	Room temperature
λ_{max} (nm)	510	430
Reagent	1,10-phenanthroline -monohydrate	*p*-dimethylamino -benzaldehyde in presence of sodium dodecyl sulphate
Beer's law range (ppm)	0.04-8.8	0.41-82.5
(ε, $l.mol^{-1}.cm^{-1}$)	3.507×10^4	0.36×10^4
Application of the method	Determination of bromhexine-HCl in tablet, syrup and injection	Determination of bromhexine-HCl in tablet and syrup

* Khalil R. and Saeed Ab. 2007. , J. Chin. Chem. Soc.,54, 1099-1105.

The results indicate that the proposed method is more sensitive than the literature method and has a wide application part in determination of drug under investigation in its pharmaceutical preparations,but the present method needs longer time of analysis.

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