Spectrophotometric Determination of Bromhexine Hydrochloride by Azo-Dye Formation Reaction

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الملخص

يتضمن البحث طريقة طيفية لتقدير كميات متناهية في الصغر من البرومهكسين هيدروكلورايد . تعتمد الطريقة على أزوتة البرومهكسين هيدروكلورايد وذلك بمفاعلته مع ايون النتريت بوجود حامض الهيدروكلوريك ثم اقتران ملح الدايازونبوم الناتج مع كاشف الا قتران البايروكالول لتكوين صبغة آزوية صفراء مستقره وذائبة في الماء ، تم قياس شدة الامتصاص للصبغة الناتجة عند الطول الموجي الأعظم ٣٨٧ نانوميتر وكانت حدود قانون بير في مدى التركيز من ٢٠ إلى ٢٨٠ مايكروغرام من البرومهكسين هيدروكلورايد /٢٥ مل (٠.٨–١١.٨ جزء بالمليون) وبلغت قيمة الامتصاصية المولارية ١.٠٣١× ١٠ ٤ لتر . مول$^{-١}$. سم$^{-١}$، والخطأ النسبي تراوح بين 0.36- و 0.72+ % والانحراف القياسي النسبي بين 1.07 ± و 1.72± % اعتمادا على مستوى تركيز البرومهكسين هيدروكلورايد . تم تطبيق الطريقة بنجاح في تقدير البرومهكسين هيدروكلورايد في مستحضراته الصيدلانية المختلفة.

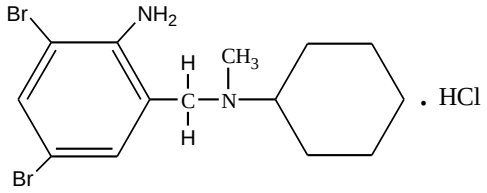
ABSTRACT

A spectrophotometric method for the assay of trace amounts of bromhexine-HCl based on the reaction of bromhexine-HCl with nitrite ion to form the corresponding diazonium salt followed by coupling reaction with pyrogallol in a acidic medium to form a stable and a soluble yellow azo dye with maximum absorption at 387 nm. Beer's law is obeyed in the concentration range of 20-280µg of bromhexine-HCl in a final volume of 25 ml (0.8- 11.2 ppm) with a molar absorptivity of 1.031×10^4 $L.mol^{-1}.cm^{-1}$, a relative error of -0.36 to +0.72 % and a relative standard deviation of ±1.07 to ±1.72 %, depending on the concentration level of bromhexine-HCl. The method has been

successfully applied for the assay of bromhexine-HCl in various pharmaceutical preparations.

INTRODUCTION

Bromhexine is used for respiratory infections, such as the cold and influenza [1]. Bromhexine hydrochloride, N-(2-amino-3,5-dibromophenylmethyl)-N-methyl-cyclohexylamine hydrochloride has the following structure[2]:



Bromhexine hydrochloride

Several spectrophotometric methods have been reported for the estimation of bromhexine–HCl using different reagents such as, rose Bengal in sulphuric acid medium [3], citric acid-AC_2O in chloroform medium [4], *p*-dimethylamino benzaldehyde in highly acidic medium [5], bromocresol purple [6], tropaeoline, naphthalene blue 12Br and azocarmon Greact acidic dyes in non aqueous medium (chloroform)[7], *p*-dimethylaminobenzaldehyde in the presence of sodium dodecyl sulphate[8], after diazotisation, the corresponding bromhexin diazonium salt is coupled with a suitable agent such as, N-(1-naphthyl) ethylenediamine[9], 1-naphtylamine [10], resorcinol [11], 2-naphtho [12], orcinol [13]. Also, derivative and mults wavelength [14] and first derivative spectrophotometric methods [15] have been used in determination bromhexine-HCl. However, some of these proced-ure suffer from one or another disadvantage such as poor sensitivity [5] or require non- aqueous medium [4,7,14].

The objective of the investigation reported in this paper is to evaluate a simple spectrophotometric method for the determination of bromhexine-HCl. The method involves the diazotisation of bromhexine-HCl and subsequent coupling with pyrogallol reagent as a coupling agent to form a highly coloured dye. that has been proved successfully for the assay of bromhexine-HCl in its different pharmaceutical preparations.

Experimental

Instruments

All spectrophotometric measurements are performed on Shimadzu UV-Visible Recording Spectrophotometer UV-160 by using 1 cm silica cell, pH meter type Philips PW 9420 is used for pH reading.

Reagents

All chemicals used in this investigation are of analytical – reagent grade, and bromhexine-HCl standard material is provided from general establishment for medical appliance and drugs / NDI – Mosul / Iraq.

Solutions

Bromhexine-HCl, 200 μg.ml^{-1}. This solution is prepared by dissolving 0.2 g of pure bromhexine-HCl in 50 ml of warmed distilled water to increase the solubility and then the solution is diluted to 100 ml in a volumetric flask with distillated water.

Pyrogallol, 0.1% (w/v). This solution is prepared freshly daily by dissolving 0.1 g of pyrogallol in 100 ml distilled water.

Sodium nitrite solution, 1% (w/v). This solution is prepared by dissolving 1 g of sodium nitrite (BDH) in 100 ml distilled water.

Sulphamic acid solution, 3% (w/v). This solution is prepared by dissolving 3 g of sulphamic acid (Fluka) in 100 ml distilled water.

Hydrochloric acid solution, 2N. This solution is prepared by diluting 17ml of concentrated acid (11.8 N) to 100 ml with distilled water.

Bromhexine-HCl tablets solution, 80μg.ml^{-1}. Weighted and finely powdered 10 tablets (each one contain 8 mg bromhexine-HCl), an accurately weighed amount of powder equivalent to 0.008g bromhexine-HCl is dissolved in 2 ml hydrochloric acid solution (1N), then 50 ml of warm distilled water is added and the solution is shaking to increase the solubility, filtered into 100 ml calibrated flask, then the solution is completed to the volume with distilled water.

Bromhexine-HCl syrup solution, 80μg.ml^{-1}. A 10 ml of syrup (each 5ml contain 4 mg bromhexine-HCl) is transferred into a 100 ml calibrated flask and the total volume is diluted with distilled water.

Bromhexine-HCl injection solution, 80μg.ml^{-1}. This solution is prepared by diluted 4 ml of bromhexine-HCl injection solution (each 2ml contains 4 mg bromhexine-HCl), with distilled water in 100 ml calibrated flask.

Procedure and calibration graph

To a series of 25-ml calibrated flasks transfer 0.2 – 3.2 ml of bromhexine-HCl solution (equivalent to 100 μg.ml^{-1} bromhexine-HCl), then 5 ml of 2N hydrochloric acid and 0.4 ml of 1% (w/v) sodium nitrite solution are added and the mixture is allowed to stand for 3 minute and then 0.3 ml of 3% (w/v) sulphamic acid solution is added with occasional

shaking for 5 minute. After that a 4 ml of 0.1% (w/v) pyrogallol was added. Then the solutions let to stand for 10 minutes at room temperature before the volumes are completed to the mark with distilled water, the absorbance is read at 387 nm against the reagent blank. A linear calibration graph is obtained over the concentration range of 20 – 280 µg bromhexine-HCl / 25 ml
(0.8-11.2 ppm) and a concentration above 280 µg / 25 ml gives a negative deviation (Fig. 1). The molar absorptivity has been found to be 1.031×10^{4} l. mol^{-1}. cm^{-1}.

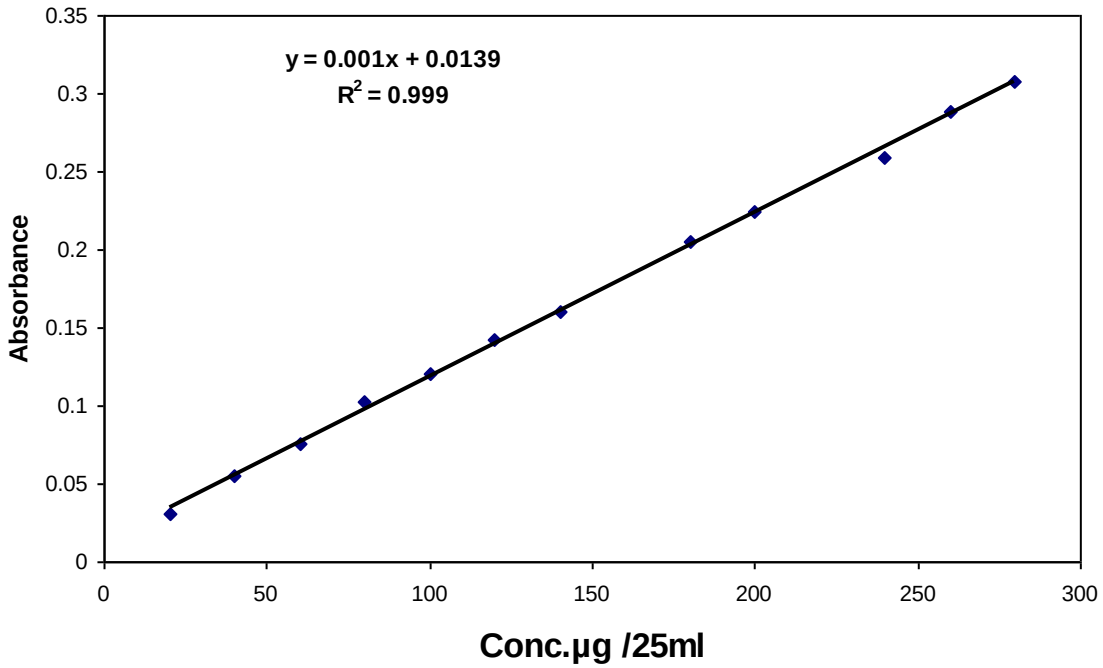


Fig. 1. Calibration graph of bromhexine-HCl determination

Results and Discussion

During the investigation, bromhexine-HCl solution equivalent to 200 µg.ml^{-1} bromhexine-HCl is taken and the final volumes are brought to 25 ml with distilled water.

Effect of acid

Different amounts and types of acids have been used in diazotisation of bromhexine-HCl, the results show that the diazotization and then the formation of the dye needs highly acidic medium due to the presence of two bulky bromo group neighbouring the amino group,hence 5 ml of 2N HCl has been selected for subsequent experiments (Table 1).

Table 1. Effect of acids on absorbance and colour contrast

2N Acid solution used	Absorbance and colour contrast / ml of acid used							
	0.5		2.5		5.0		6.0	
	A*	Δλ**,nm	A	Δλ,nm	A	Δλ,nm	A	Δλ,nm
HCl	0.130	108	0.205	111	0.215	112	0.203	106
HNO_3	0.124	108	0.192	112	0.162	109	0.157	111
H_2SO_4	0.106	104	0.160	102	0.169	110	0.163	113
CH_3COOH	0.097	29	0.108	30	0.088	29	0.090	30

*$\Delta\lambda = \lambda_{max}S - \lambda_{max}B$ Where S = The dye , B = Blank

Effect of sodium nitrite amount and time

The maximum absorbance reading is obtained by adding 0.4 ml of 1% sodium nitrite with 3 minutes of reaction time (Table2).

Table 2. Effect of sodium nitrite amounts and time on the absorbance of bromhexine-HCl

ml of 1% $NaNO_2$ solution	Absorbance / minute standing time						
	0	1	2	3	4	5	7
0.1	0.165	0.145	0.173	0.175	0.178	0.174	0.172
0.2	0.195	0.188	0.178	0.175	0.182	0.180	0.168
0.3	0.200	0.197	0. 198	0.190	0.177	0.175	0.171
0.4	0.220	0.216	0.218	0.222	0.208	0.205	0.203
0.5	0.189	0.187	0.213	0.215	0.187	0.199	0.200

Effect of sulphamic acid amounts and time

The excess of nitrite can be removed by the addition of sulphamic acid solution [16]. The effect of sulphamic acid amount and time has been studied. (Table3)

Table 3. Effect of sulphamic acid amounts and time on the absorbance of bromhexine-HCl

ml of 3% Sulphamic acid solution	Variable	Absorbance/minute standing time						
		0	1	2	3	4	5	7
0.0	Sample = S	0.075	0.093	0.072	0.104	0.095	0.020	0.068
	Blank = B	0.554	0.589	0.657	0.617	0.667	0.794	0.945
0.2	S	0.194	0.182	0.180	0.176	0.174	0.173	0.160
	B	0.098	0.078	0.045	0.039	0.038	0.037	0.034
0.30	S	0.172	0.200	0.202	0.203	0.216	0.225	0.195
	B	0.099	0.051	0.042	0.040	0.034	0.027	0.034
0.4	S	0.179	0.199	0.187	0.202	0.199	0.187	0.158
	B	0.088	0.049	0.033	0.026	0.025	0.023	0.020

The results in the table 3 indicate that 0.3 ml of sulphamic acid solution (3%, w/v) with 5 minute as standing time for the reaction give the most suitable effect on the intensity of the azo-dye.

Effect of pyrogallol amount on absorbance

The effect of pyrogallol amount on the absorbance of the dye has been studied. From the results, it can be observed that 4 ml of 0.1% pyrogallol is the more suitable amount which gives the highest value of intensity for the azo-dye formed and the highest value of correlation coefficient (Table 4).

Table 4. Effect of coupling agent amount on absorbance

ml of Pyrogallol solution (0.1%)	Absorbance/µg bromhexine-HCl in 25 ml						
	40	80	120	180	200	240	r
2	0.048	0.084	0.130	0.175	0.192	0.219	0.99674
3	0.052	0.100	0.138	0.201	0.216	0.248	0.99793
4	0.055	0.102	0.142	0.205	0.228	0.259	0.99877
5	0.051	0.092	0.140	0.198	0.215	0.242	0.99662

Effect of time

µg of Bromhexine-HCl present	Absorbance / minute standing time									
	0	**5**	**10**	**15**	**25**	**30**	**35**	**45**	**55**	**60**
80	0.107	0.114	0.120	0.119	0.118	0.118	0.117	0.115	0.114	0.114
140	0.157	0.162	0.160	0.159	0.155	0.154	0.154	0.152	0.151	0.151
200	0.223	0.229	0.230	0.230	0.228	0.228	0.228	0.224	0.223	0.222

The coloured azo dye developed rapidly after addition of pyrogallol and the stability period (within the first hour of stability) is sufficient to perform several measurements and the results are given in table 5.

Table 5. The effect of time and bromhexine-HCl amount on absorbance

Final absorption spectra

The absorption spectra of the yellow azo dye formed by coupling of diazotised bromhexine-HCl with pyrogallol in acidic medium shows a maximum absorption at 387 nm. The reagent blank gives very weak absorption at this wavelength (Fig. 2).

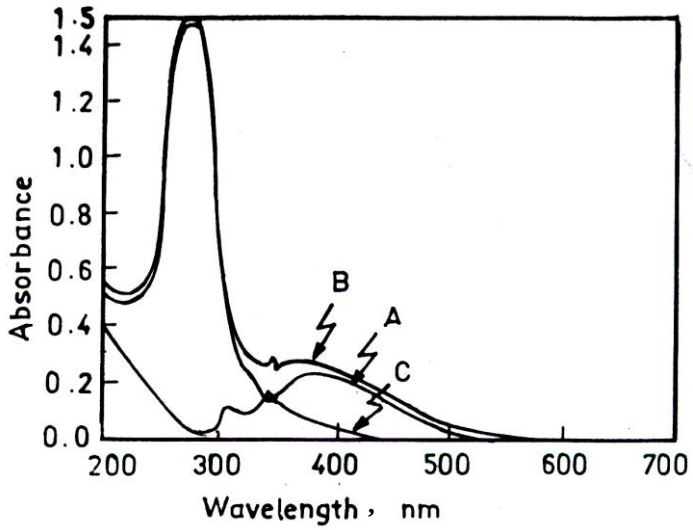


Fig.2: Absorption spectra of 200µg bromhexine-HCl / 25ml treated according to the recommended procedure and measured against (A)

reagent blank, (B) distilled water and (C) reagent blank measured against distilled water.

Nature of the dye

The stoichiometry of the azo dye thus formed by reaction of diazotised bromhexine [BH]with pyrogallol [PY] is investigated by applying the continuous variations method (Job's method)and mol ratio method. The results indicate that the azo-dye was formed in the ratio of 1:1diazotised bromhexine to pyrogallol (Fig.3 and Fig.4).

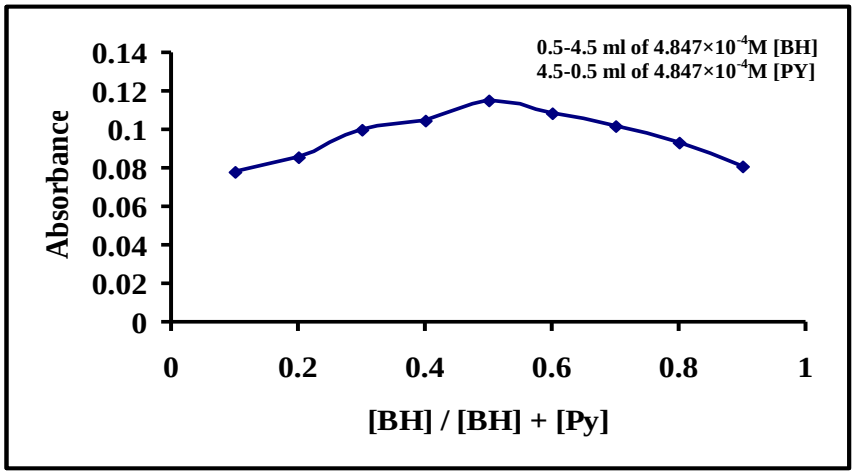


Fig.3: Job's plot for bromhexine-pyrogallol

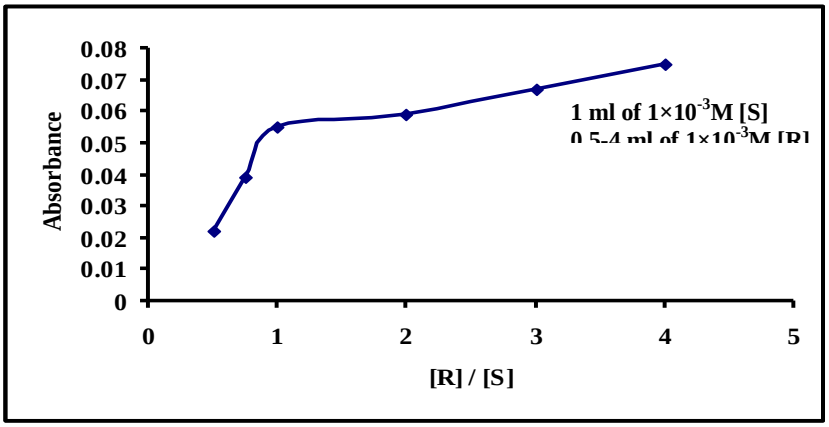
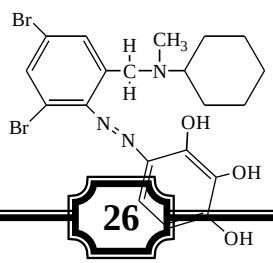


Fig.4: Mole ratio plot for bromhexine-pyrogallol

Therefore the azo-dye may have the following suggested structure**:**



Yellow azo-dye

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 25ml (Table 6).

Table 6: Effect of foreign compounds for assay of bromhexine-HCl

Foreign compound	Recovery (%) of 200μg bromhexine-HCl per μg foreign compound added		
	100	**500**	**1000**
Glucose	99.56	97.39	97.82
Glycerin	99.13	96.52	95.65
Lactose	98.78	98.26	97.39
Starch	96.93	92.17	91.73

The results in table 6 indicated that the studied foreign compounds do not interfere in determination of bromhexine-HCl by using the proposed method. An error not more than of ±3**%** in the absorbance readings is considered tolerable from that of the bromhexine-HCl alone.

Accuracy and precision.

To check the accuracy and precision of the method, bromhexine-HCl is determined at three different concentrations. The relative error% and relative standard deviation% results indicate the high accuracy and precision of the proposed method (Table 7).

Table 7. Accuracy and precision

Amount of bromhexine-HCl taken, μg	Relative error, %*	Relative standard deviation, %*
80	+0.72	± 1.72

140	+0.58	± 1.42
200	-0.36	± 1.07

* Average of five determinations

Analytical application

The proposed method is applied to assay bromhexine-HCl in different pharmaceutical preparations (syrup, tablet and injection). On applying proposed procedure, good recovery is obtained as shown in table 8.

Table8. Analytical applications

Pharmaceutical preparation	μg bromhexine-HCl present/25ml	μg bromhexine-HCl measured/25ml	Recovery* (%)
Solvodine syrup, 4mg bromohexine-HCl/5 ml (S.D.I. Iraq)	112	112.88	100.78
	160	160.88	100.55
	194	190.43	98.16
Solvodin tablet 8.0mg bromhexine-HCl/ tablet, (S.D.I-Iraq	112	109.28	97.57
	160	158.19	98.87
	194	192.19	99.07
Bromohexine-HCl injection, 4mg/2ml (Ibn Hayan, Syria)	112	114.57	102.29
	160	160.00	100.00
	194	195.70	100.88

*Average for five determinations

Evaluation of the proposed method

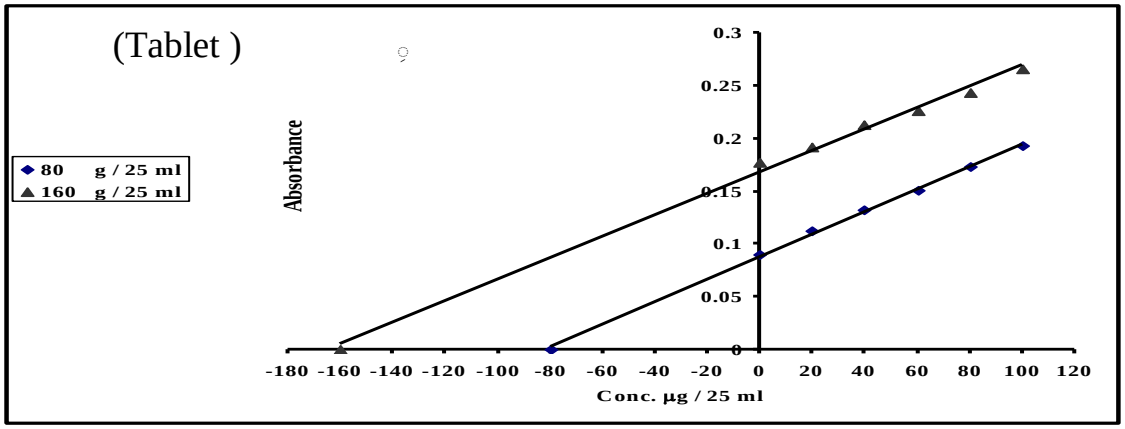
According to the difficulties of using the standard method for determination of bromhexine-HCl in its pharmaceutical preparation, so that standard addition method has been used in order to prove that the proposed method is applied in the determination of bromhexine-HCl without interferences (Table 9 and Fig.5)

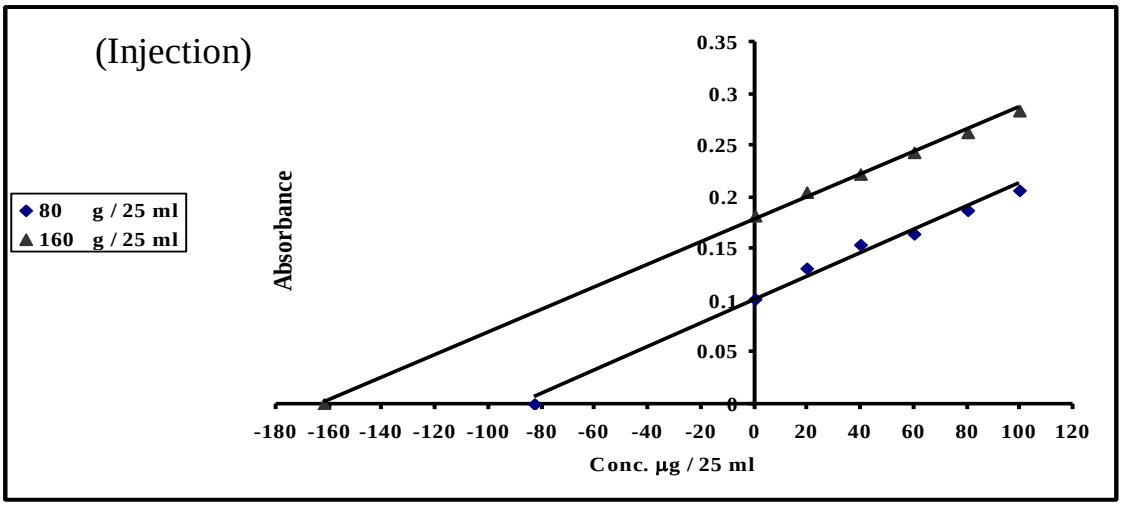
Table 9: The results of standard addition method

Pharmaceutical preparation	μg paracetamol present/25ml	μg paracetamol measured/25ml	Recovery* (%)
Solvodine syrup, 4mg bromohexine-HCl/5 ml (S.D.I. Iraq)	80	82.50	103.12
	160	164.00	102.50
Solvodin tablet 8.0mg bromhexine-HCl/ tablet, (S.D.I-Iraq)	80	80.00	100.00
	160	161.00	100.65

Bromohexine-HCl injection, 4mg/2ml (Ibn Hayan, Syria)	80	83.00	103.75
	160	162.00	101.25

*Average of three determinations





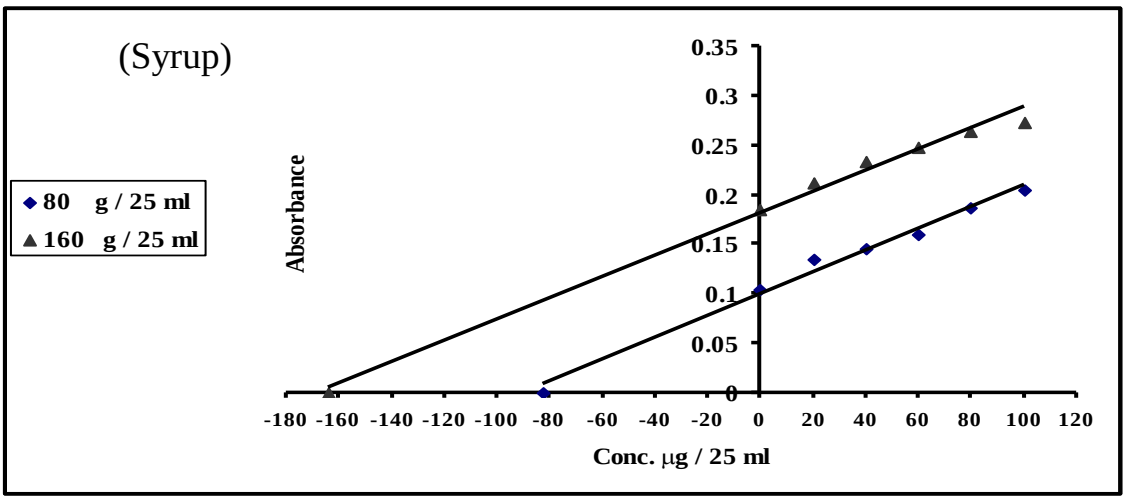


Fig. 5: Graphs of standard addition method for the determination of bromhexine- HCl in pharmaceutical preparations

The results in table 9 and fig.5 indicated that the proposed method can be used in determination of bromhexine-HCl with satisfactory results.

Comparison of the methods

Table 10 shows the comparison between some of analytical variables obtained from the present method with those of the recent spectrophotometric methods.

Table 10: The comparison of the methods

Analytical parameters	Present method	Literature method[8]	Literature method[10]
pH	1.34	1.6	…
Temperature (°C)	Room temperature	Room temperature	Room temperature
λ_{max} (nm)	387	430	478
Medium of reaction	Aqueous	Aqueous	Aqueous
Reagent	Pyrogallol	*p*-Dimethyl amino benzaldehyde in presence of sodium dodcyl sulphate	1-Naphthyl amine
Beer's law range (ppm)	0.8-11.2	0.41-82.5	2-8
Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	1.031×10^4	0.36×10^4	…
RSD (%)	≤1.72	≤3.5	…
Stability of the colour (minute)	60	…	1440
Colour of the product	Yellow	Yellow	Orange
Application of the method	Has been applied to the assay of bromhexine hydrochloride	Has been applied to the assay of bromhexine hydrochloride	Has been applied to the assay of bromhexine hydrochloride

	in pharmaceutical preparations (tablets, injection and suspensions)	in pharmaceutical preparations (tablets and suspensions)	in pharmaceutical preparations

The results in table 10 shows that the suggested method for the determination of bromhexine-HCl is the more sensitive.

Conclution

The proposed method for the determination of bromhexine-HCl in pharmaceutical preparations is simple and sensitive. The azo-dye formed is fairly soluble in aqueous solution. The statistical analysis of the result indicates that the method has good accuracy (average relative error between -0.36 to+ 0.72 %) and good precision(average relative standard deviation not more than 1.72 %).

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