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Spectrophotometric Determination of Bromhexine Hydrochloride by Diazotization and Coupling Method in Its Pharmaceutical Preparations

Saad Hasani Sultan[*1], Zainab Walid Majed[2]

[1]Department of Chemistry, College of Science ,University of Mosul , Mosul , Iraq .
[2]Department of New and Renewable Energies, College of Science , University of Mosul , Mosul , Iraq .

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Abstract

A simple, fast, and sensitive spectrophotometric method was suggested for the determination of Bromhexine Hydrochloride (BHH) in its pharmaceutical formulations. The method depends on the diazotization of BHH by sodium nitrite in acidic medium to produce the corresponding diazonium salt. The latter is coupled with phloroglucinol reagent in alkali medium to form a yellow water soluble azo-dye which has a maximum absorption at 405 nm with a molar absorptivity of 2.7×10^4 $l.mol^{-1}.cm^{-1}$ and Sandell's sensitivity of 0.01517 $\mu g.cm^{-1}$. Beer's low is obeyed within a concentration range of 0.25-15 $\mu g.mL^{-1}$ of BHH. The LOD and LOQ values of the proposed method were 0.087 $\mu g.mL^{-1}$ and 0.293 $\mu g.ml^{-1}$, respectively. The proposed method was validated with standard methods and successfully applied to the determination of Bromhexine in its pharmaceutical formulations as tablets, syrup, and injections.

Keywords: Bromhexine hydrochloride , diazotization and coupling, phloroglucinol , spectrometry.

التقدير الطيفي للبرومهكسين هيدروكلورايد بطريقة الأزوتة والاقتران في مستحضراته الصيدلانية

سعد حساني سلطان[1*]،زينب وليد ماجد[2]

[1]قسم الكيمياء، كلية العلوم، جامعة الموصل،الموصل، العراق

[2]كلية العلوم، جامعة الموصل،الموصل، العرق

الخلاصة

يتضمن البحث اقتراح طريقة سهلة وسريعة وحساسة لتقدير البرومهكسين هيدروكلوريد في مستحضراته الصيدلانية . تعتمد الطريقة على أزوتة المركب الدوائي قيد الدراسة بوساطة نتريت الصوديوم في الوسط الحامضي لتكوين ملح الدايزونيوم المقابل الذي يقترن مع الكاشف فلوروكلوسينول في الوسط القاعدي لأعطاء صبغة آزوية صفراء ذائبة في الماء و تعطي أعلى امتصاص عند الطول الموجي 405 نانوميتر وبمعامل امتصاص مولاري 2.7×10^4 لتر.مول$^{-1}$. سم$^{-1}$ ودلالة ساندل 0.01517 مايكروغرام.سم$^{-1}$. انطبق قانون بير في مدى التراكيز 0.25− 15 مايكروغرام مللتر$^{-1}$ من البرومهكسين هيدروكلورايد. بلغت قيمتي حد الكشف وحد التقدير للطريقة المقترحة 0.087 مايكروغرام مللتر$^{-1}$ و 0.293 مايكروغرام مللتر$^{-1}$ على التوالي .قورنت نتائج الطريقة المقترحة مع الطريقة القياسية وكانت متقاربة ، وقد تم تطبيق الطريقة بنجاح لتقدير البرومهكسين هيدروكلوريد في مستحضراته الصيدلانية بشكل اقراص وشراب و حقن .

Introduction

Primary aromatic amines react with nitrous acid to yield diazonium salts which are coupled with

*Email: saad.hasani@uomosul.edu.iq

phenols in alkali medium to produce a colored azo-dyes; this is one of the most important reactions in organic chemistry [1]. From the point of view of analytical chemistry, this reaction is considered as highly sensitive because of the intense colors of the formed azo dyes. Thus, this reaction was used widely to assay the primary aromatic drugs [2-6] and for the synthesis of indicators [7].

Bromhexine Hydrochloride (BHH) (Fig.1.) is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus [8]. It exhibits its action by increasing bronchial secretion and reducing their viscosity. Also, this agent was recently recommended as a new therapy for pathological conditions, such as alcoholic chronic pancreatitis where increased viscosity is involved [9]. It was developed in the research laboratory of Boehringer Ingelheim in the late 1950s as an active ingredient for pharmaceutical use. It was then introduced in 1963 under the trademark of Bisolvon® [10]. BHH is chemically known as N-(2-Amino-3,5-dibromobenzyl)-N-methylcyclohexanamine hydrochloride, and it is a white or almost white crystalline powder [11].

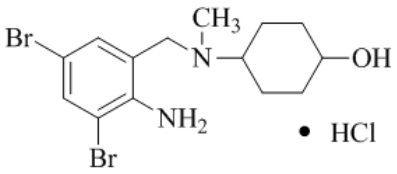


Figure 1-Bromhexine Hydrochloride

Many methods have been reported for the estimation of BHH in its pure form or pharmaceutical preparations. These methods include spectrophotometry [12-14], chromatography [15-18], voltammetry [19,20] and capillary electrophoresis [21]. However , spectrophotometric methods are still more spread than other techniques due to their simplicity and inexpensive equipment. In this work, we developed an easy and sensitive spectrophotometric method for assaying BHH in its pharmaceutical formations by converting the amino group in the drug to a diazonium salt, which is coupled with phlologlucinol to produce a colored azo dye, the concentration of which is related with the original concentration of the studied drug.

Experimental Part

Instruments

The spectrophotometric measurements were performed on CE CILL 2700 UV-Vis spectrophotometer using 1.0 cm plastic cell .

Chemicals and samples. All chemicals used in this work were of analytical grade.

Bromhexine hydrochloride solution (100 µg.mL^{-1})

Pure Bromhexine hydrochloride (0.0100 g, State Company for drug industries and medical appliances, Samarra, Iraq, SDI) was dissolved in 100 mL of distilled water with gentle heating.

Hydrochloric acid solution (1N)

This solution was prepared by diluting 8.6 mL of the concentrated acid (11.6 N, (Thomas Baker) to 100 mL by adding distilled water.

Sodium nitrite solution (10000 µg.mL^{-1})

of sodium nitrite (1.0 g, BDH) was dissolved in a sufficient amount of water and the volume was completed to 100 mL.

Sulphamic acid solution (30000 µg.mL^{-1})

This solution was prepared by dissolving 3.0 g of sulphamic acid (BDH) in 100 mL distilled water.

Phloroglucinol solution (1000 µg.mL^{-1})

A weight of 0.1 g of this reagent (Fluka) was dissolved in 100 mL distilled water.

Sodium hydroxide solution (1 M)

This solution was prepared by the appropriate dilution of the concentrated solution of 10 M (BDH) with distilled water to 1 L using volumetric flask.

SDS (1×10^{-3} M)

This solution was prepared by dissolving 0.0288 g of sodium dodecyl sulfate in 100 mL distilled water.

CPC (1×10^{-3} M)

This solution was prepared by dissolving 0.0339 g of cetylpyridinium chloride in 100 mL distilled water.

Triton X-100 (1.5×10^{-2}M)

This solution was prepared by dissolving 1.0 g of pure reagent in 100 mL distilled water.

Tablets solution (100 µg.mL^{-1})

Ten tablets (each tablet contains 8 mg of BHH) were crushed well and an accurate weight of the powder (equivalent to 0.01 g of BHH) was dissolved in a sufficient amount of water with gentle heating. The residue was filtered into 100 mL volumetric flask and the volume was completed to the mark by repeated washing with distilled water.

Syrup solution (100 µg.mL^{-1})

A volume of 12.5 mL of syrup solution (each 5mL contains 4 mg of BHH) was transferred into 100 mL volumetric flask and the volume was completed to the mark with distilled water .

Injections solution (100 µg.mL^{-1})

The content of three ampoules (each 2 mL ampoule contains 8 mg of BHH) was mixed, 2.5 mL of the resulting solution was transferred into 100 mL volumetric flask, and the volume was made up to the mark with distilled water .

Preliminary Investigations

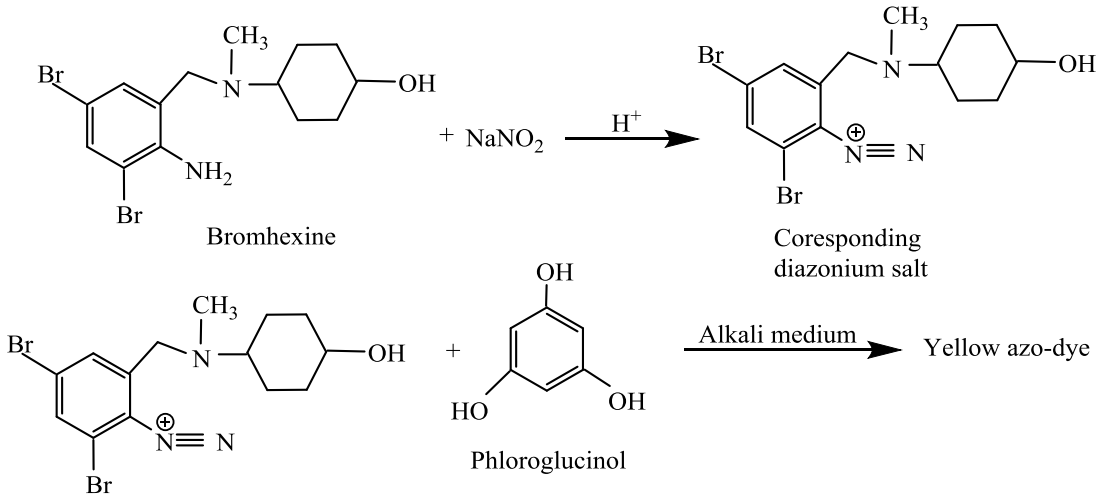
A volume of 1.0 mL of the standard BHH solution (100 µg.ml^{-1}) was added to a 20 mL volumetric flask, followed by 2.0 mL of 1 N HCl solution and 1.0 mL sodium nitrite solution (10000 µg.ml^{-1}). The contents were left for one min, then 1.0 mL of sulphamic acid solution (30000 µg.ml^{-1}) was added with occasional shaking for one min. After that, 1.0 mL of phloroglucinol reagent (1000 µg.ml^{-1}) and 3.0 mL of 1.0 M sodium hydroxide were added and the volume was diluted to the mark by distilled water. A yellow colored product was obtained with λ_{max} 405 nm against the blank solution.

Results and Discussion

100 µg of BHH with 20 mL as a final volume was used for the next investigations.

Principle of the method

The first step in the proposed method involves diazotization of BHH in aqueous solution to form the corresponding diazonium salt, which is coupled with phloroglucinol in basic medium to produce a colored azo dye, as follows.



Optimization of reaction circumstances

The influence of different parameters on the absorption intensity of the produced azo dye was studied. These included the quality and quantity of acids, the amount of sodium nitrite used for diazotization process, the amount of sulphamic acid needed for destruction of excess sodium nitrite, the amount of phloroglucinol reagent, and finally the quality and quantity of suitable bases that showed maximum color development.

Effect of acids used

The influences of the amount and type of acids on the diazotization process were investigated by adding various volumes of acids. The results listed in the Table-1 and Figure-2 demonstrate that 2.5 mL of 1N HCl is the typical volume since it showed the highest absorbance .

Table 1-Effect of acids on absorbance

Acid type 1 N	Absorbance / mL of acid used				
	1.0	**1.5**	**2.0**	**2.5**	**3.0**
HCl	0.376	0.396	0.407	0.415	0.414
HNO_3	0.366	0.375	0.371	0.386	0.413
H_2SO_4	0.409	0.401	0.319	0.339	0.347
CH_3COOH	0.070	0.061	0.053	0.058	0.057

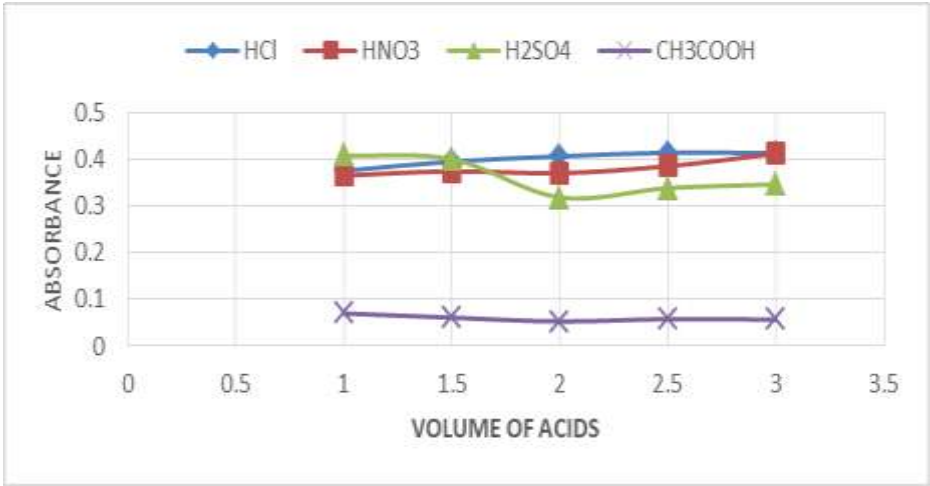


Figure 2-Effects of acids on absorbance

Effect of sodium nitrite amount and time reaction

The quantity and the reaction time of sodium nitrate was examined by adding different amounts of sodium nitrite solution for different times. The obtained results in Table- 2 indicate that 250 µg.ml^{-1} of $NaNO_2$ solution for 2 min was sufficient for complete diazotization of BHH and, therefore, it was selected for next experiments.

Table 2-Effects of sodium nitrite quantity and time reaction

$NaNO_2$ (µg.mL^{-1}) sol.	Absorbance/ standing time (min.)			
	1	**2**	**3**	**5**
50	0.301	0.331	0.347	0.312
125	0.404	0.411	0.410	0.340
250	0.421	0.425	0.422	0.355
375	0.420	0.399	0.418	0.363
500	0.415	0.389	0.412	0.301

Effects of sulphamic acid

The excess of sodium nitrite should be removed due to its undesirable reactions [22]. According to previous studies, the most suitable agent for destruction of sodium nitrite is sulphamic acid. Therefore, the influence of sulphamic acid quantity and time reaction was investigated and the results listed in Table- 3 indicate that 1125 µg.ml^{-1} of sulphamic acid solution with 3 min time reaction was typical for the complete removal of excess sodium nitrite.

Table 3-Effects of sulphamic acid amount with the time

Sulfamic (μg.mL^{-1}) sol.	Variable	Absorbance/ standing time (min.)			
		1	2	3	5
150	S	0.280	0.121	0.182	0.221
	B	1.401	1.390	1.382	1.342
375	S	0.404	0.228	0.304	0.392
	B	0.586	0.073	0.077	0.026
750	S	0.398	0.363	0.340	0.407
	B	0.013	0.017	0.022	0.024
1125	S	0.405	0.406	0.426	0.424
	B	0.012	0.014	0.008	0.003
1500	S	0.410	0.382	0.415	0.422
	B	0.007	0.032	0.036	0.029

Effects of coupling agent amount

The effects of phloroglucinol solution amount on the absorbance of the produced azo dye was studied by adding various amounts of the coupling agent to increase the amount of BHH. The results in Table-4 show that the concentration of 50 μg.ml^{-1} of phloroglucinol solution was suitable for high sensitivity and, therefore, it was chosen for next experiments.

Table 4-Effects of coupling agent amount

Phl. (μg.mL^{-1}) sol.	Absorbance/μg of BBH				R^2
	25	50	100	150	
12.5	0.170	0.201	0.442	0.629	0.9835
25	0.194	0.264	0.452	0.668	0.9952
50	0.190	0.273	0.445	0.651	0.9975
75	0.198	0.286	0.450	0.661	0.9964
100	0.225	0.283	0.535	0.658	0.9843

Effect of base

The preliminary experiments revealed that the produced azo dye had the maximum color development in basic medium. Thus, the effects of different bases on color intensity of the formed azo dye was studied. The results in Table-5 indicate that 3 ml of 1 M NaOH solution showed a high absorbance and, therefore, it was selected for the next experiments.

Table 5-Effects of type and amount of base

Type of media (1M)	Variable	mL of base added					
		1.0	1.5	2.0	2.5	3.0	3.5
NaOH	Abs.	0.278	0.271	0.300	0.417	0.448	0.442
	$\lambda_{max\ (nm)}$	417	407	405	408	405	402
	pH	1.46	1.57	1.71	12.15	12.45	12.75
KOH	Abs.	0.290	0.336	0.344	0.381	0.403	0.411
	$\lambda_{max\ (nm)}$	4019	413	409	410	409	406
	pH	1.58	1.63	1.67	7.05	8.57	12.14
Na_2CO_3	A	0.238	.273	0.296	0.353	0.375	0.384
	$\lambda_{max\ (nm)}$	419	413	409	410	409	406
	pH	5.47	7.16	8.45	9.12	9.50	10.03
$NaHCO_3$	Abs.	0.270	0.275	0.279	0.281	0.283	0.296
	$\lambda_{max\ (nm)}$	427	421	419	417	416	412
	pH	1.68	2.33	5.77	6.16	6.47	6.99

Effects of surfactants

The effects of 3 types of surfactants was investigated by the addition of 2 ml of sodium dodecyl sulphate (SDS), cetylpyridinium chloride (CPC) and Triton X-100 with various orders to the medium reaction. The obtained results showed that there is no improvement in absorbance and, thus, the studied surfactants were excluded in the subsequent experiments.

Effects of reaction time and stability

The influence of the reaction time on the formation and stability of the colored product was investigated and the results in Table- 6 reveal that the colored azo dye was formed as soon as the base was added and that it stayed stable for minimum 2 hrs.

Table 6-Effects of reaction time and stability of the azo dye

Time(min.)		After addition	10	20	30	40	50	1 hrs.	2 hrs.
Absorbance/ µg of BHH per 20 mL	25	0.120	0.118	0.118	0.118	0.116	0.118	0.117	0.115
	200	0.665	0.648	0.646	0.641	0.636	0.635	0.641	0.632

Final absorption spectra

The final absorption spectra of the colored azo dye, formed from the reaction between diazotized bromhexine and phloroglusinol reagent in basic medium, versus its reagent blank revealed a maximum absorption at 405 nm, in contrast to the phloroglusinol reagent blank which showed a slight absorption at the same wavelength (Figure-3).

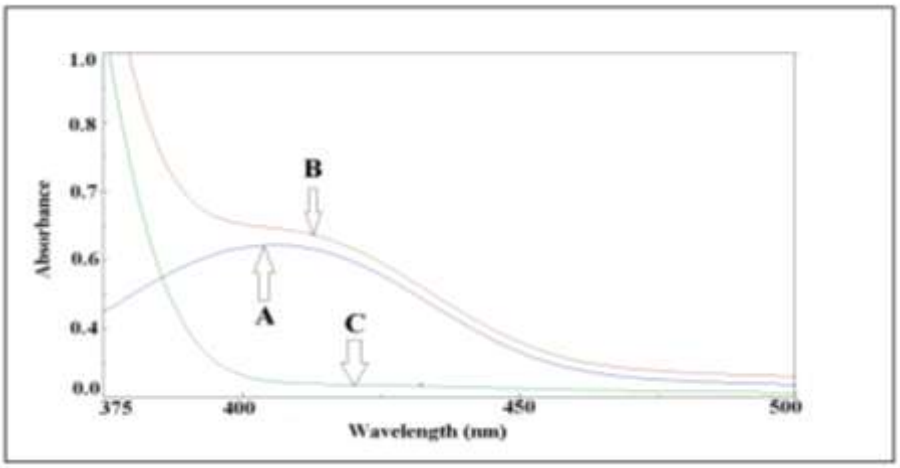


Figure 3-Final absorption spectra of 100 µg of BHH/20 mL according to the optimum conditions, measured against: (A) blank (B) distilled water (C) blank against distilled water

General procedure and calibration curve

Increasing volumes of BHH covering concentrations from 5 to 300 µg were transferred to a series of 20 ml calibrated flasks, followed by the addition of 2.5 ml of 1M HCl and 0.5 mL of 10000 $\mu g.ml^{-1}$ sodium nitrite solution. The solution was left for a period of two min, then a 0.75 mL of 30000 $\mu g.ml^{-1}$ sulphamic acid solution was added with discontinuous shacking for 3 min to destroy the excess sodium nitrite. After that, 1 ml of 1000 $\mu g.ml^{-1}$ phloroglucinol and 3 ml of 1 M sodium hydroxide were added and the volumes were completed with distilled water. The absorbance was measured at 405 nm versus the reagent blank solution. Beer's low was obeyed within concentrations ranging from 5 to 300 µg of bromhexine hydrochloride in final a volume of 20 mL (0.25-15 $\mu g.ml^{-1}$), as shown in Figure- 4. The molar absorptivity and Sandell's sensitivity were 2.7×10^4 $l.mol^{-1}.cm^{-1}$ and 0.01517 µg. cm^{-1}, respectively.

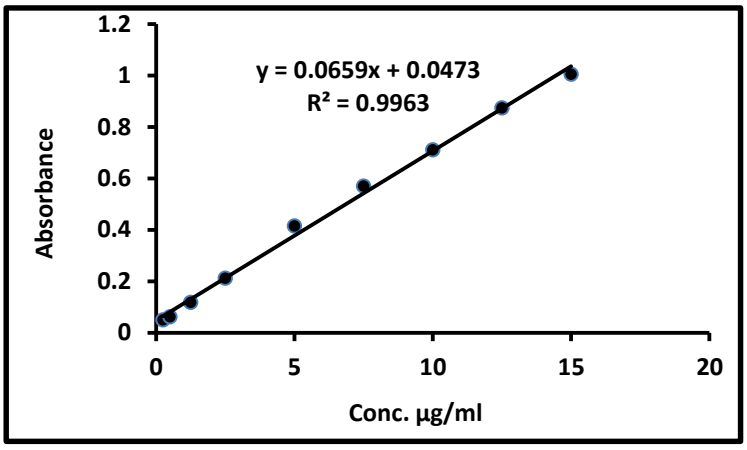


Figure 4-Calibration graph for bromhexine hydrochloride determination

Nature of the produced azo dye

To establish the composition of the produced azo dye, Job's and mole-ratio methods were adopted. The obtained results indicated that the dye has a composition of 1:1 of diazotized bromhexine to phloroglusinol Figures-(5 and 6), revealing that a mono azo dye was formed.

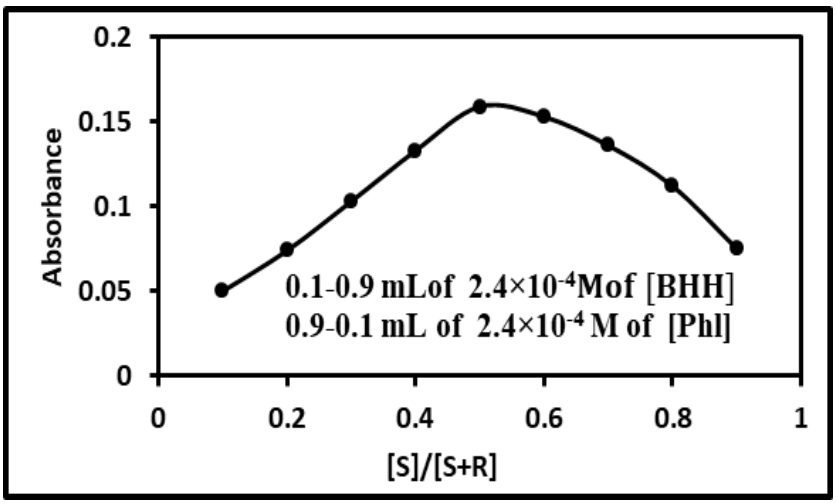


Figure 5-Job's plot for BHH-phloroglucinol dye

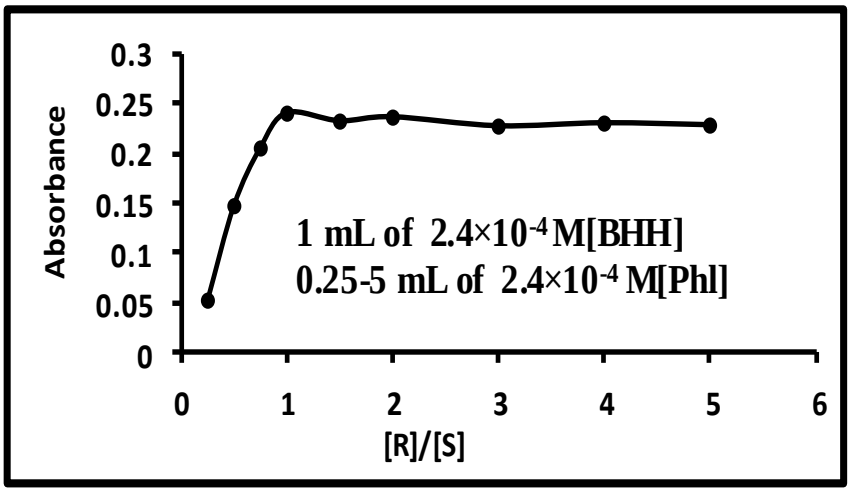
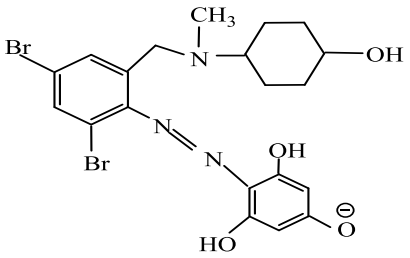


Figure 6-Mole ratio plot for BHH-phloroglucinol dye

Hence, the structure of the produced azo dye may be drawn as follows :



The effects of interferences

The effects of some foreign compounds which are expected to exist in pharmaceutical formulation of bromhexine were studied by adding various amounts of additives using the recommended procedure. The results in Table-7 show that there is no significant interference in the determination of BHH in the presence of these additives.

Table 7-Study the effect of interferences

Additives	Recovery (%)of 100 µg BHH/µg of added compound		
	100	**500**	**1000**
Glucose	96.3	95.8	95.5
Lactose	100.2	96.7	95.8
Starch	100.0	98.5	96.7
Arabic gum	98.7	99.2	96.3

Accuracy and precession of the proposed method

Under the optimum conditions , the accuracy and precision of the proposed method were investigated. The results are listed in Table- 8 and indicated good accuracy and precision.

Table 8-Accuracy and precession

Amount taken (µg)	Amount measured (µg)	Recovery* (%)	Relative error*(%)	Relative standard deviation*(%)
50	49.98	99.96	-0.04	±2.05
100	100.84	100.84	+0.84	±0.47

***** Average of five determinations

Application of the proposed method

To test the applicability of the suggested method, it was performed to test BHH in its pharmaceutical preparations. The results in Table-9 reveal that satisfactory results were obtained for the applied pharmaceutical forms which were in a good agreement with the label claims.

Table 9-Application of the proposed method

Pharmaceutical preparation	Amount taken (µg)	Present Method		Standard addition Method	
		Amount measured(µg)	Recovery* (%)	Amount measured(µg)	Recovery (%)
Tablet(Bisolvon), Nile Co.,Egypt	**50**	**49.0**	**98.0**	**49.3**	**98.6**
	100	**97.8**	**97.8**	**98.1**	**98.1**
Syrup(Solvodin), SDI ,Iraq	**50**	**47.6**	**95.2**	**48.0**	**96.0**
	100	**95.7**	**95.7**	**96.3**	**96.3**
Ampules(Bisolvon), Nile Co.,Egypt	**50**	**49.8**	**99.6**	**50.1**	**100.2**
	100	**99.2**	**99.2**	**102.5**	**102.5**

*Average of five determinations

Also, the performance of the proposed method was evaluated by t-test and F-test compared with the standard method (British Pharmacopoeia, 2013) for a 95% confidence level and eight degrees of

freedom. The results listed in Table-10 indicate no significant differences between the suggested and standard methods for BHH analysis.

Table 10-t-test analysis of the proposed method

Pharmaceutical preparation	Recovery*, %		t-test	F-test
	Present method	Standard method		
Tablet (Bisolvon), Nile Co.,Egypt	98.1	97.9	± 0.54	3.06
Syrup (Solvodin), SDI ,Iraq	95.6	95.2	± 0.79	4.51
Ampules (Bisolvon), Nile Co.,Egypt	99.5	99.3	± 0.37	2.33

Comparison of the proposed method

To make a comparison between the present method and the reported spectrophotometric method, some analytical variables were listed in Table 11, which indicate that the present method has the highest sensitivity, in addition to the fact that the proposed method was applied for thee forms of pharmaceutical drugs.

Table 11-Comparison with another spectrophotometric method

Analytical parameters	Present method	Literature method [5]
Reagent	Pholoroglucinol	Chromotropic acid
pH	12.45	Acidic
Temperature(C°)	R.T	R.T
Development time(min.)	After dilution	10
λ max(nm)	405	507
Beer's law range(μg.ml^{-1})	0.25-15	2-60
Molar absorptivity (L.mol^{-1}.cm^{-1})	2.7×10^4	1.5×10^4
Stability of azo-dye(hr.)	2	2
Color of the dye	Yellow	Red
Nature of the dye	1:1	1:1
Application of the method	Tablets, Syrup and Injection	Syrup

Conclusions

A simple, rapid, and sensitive spectrophotometric method was described for the estimation of BHH in aqueous solution .The proposed method depends on the diazotization of the studied drug by sodium nitrite in the presence of hydrochloric acid and coupling with phloroglucinol reagent in basic medium to produce a colored azo dye, which is stable for at least 2 hrs. The proposed method has simple a procedure and was applied successfully for testing BHH in three forms of pharmaceutical preparations; tablets, syrup and injections.

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