SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF BROMHEXINE HYDROCHLORIDE IN PURE AND PHARMACEUTICAL PREPARATIONS

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Abstract

A new simple and sensitive spectrophotometric method has been developed for the determination of Bromhexine Hydrochloride in pure form and pharmaceutical preparations. The method is based on the diazotization reaction of Bromhexine Hydrochloride with sodium nitrite in hydrochloric acid medium to form diazonium salt, which is coupled with chromotropic acid to form a red water-soluble azo dye, that has a maximum absorption at λmax = 507 nm. Beer's law is obeyed over the concentration range (2-60 $\mu g.ml^{-1}$) with RSD less than 2.050% and molar absorptivity of 1.569x104 $l.mol^{-1}.cm^{-1}$ and Sandell sensitivity of 0.0262 $\mu g.cm^{-2}$. The method was applied successfully for the determination of Bromhexine Hydrochloride in pharmaceutical preparations.

طريقة للتقدير الطيفي لهيدروكلوريد البرومهكسين بصورته النقية وفي المستحضرات الصيدلانية

هند صادق الورد

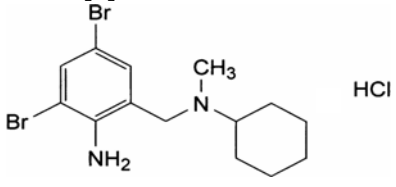
قسم الكيمياء، كلية العلوم، جامعة بغداد، بغداد– العراق.

الخلاصة

يتضمن البحث تطوير طريقة طيفية جديدة للتقدير المايكروي الـسريع والبـسيط والحـساس لـدواء هيدروكلورايد البرومهكسين بصورته النقية وفي المستحضرات الصيدلانية, تعتمد الطريقة علـى تفاعـل الازوتة لهيدروكلورايد البرومهكسين مع كمية مكافئة من نتريت الصوديوم في وسط حامض الهيدروكلوريك لتكوين ملح الديازونيوم الذي يزدوج مع كاشف حامض الكروماتروبيك لتكوين صبغة الازو حمراء اللـون اللتي اعطت اعلى امتصاص عند الطول الموجي الاعظم 507 نانوميتر. كان مدى الخطية الذي يطيع قانون بير ضمن مدى التراكيز من 2-60 مايكروغرام.مل-1 وكانت قيمة الامتـصاصية المولاريـة مـساوية لـ(1.569 x 10 4) لتر . مول$^{-1}$. سم$^{-1}$ و قيمة حساسية ساندل 0,0262 مايكروغرام. سم$^{-2}$ والانحراف القياسي النسبي اقل من 2,05% اعتمادا" على مستوى التركيز المراد تحديده. تمت دراسة الظروف المثلى للتفاعل, وطبقت الطريقة بنجاح على المستحضرات الصيدلانية الحاوية على هيدروكلوريد البرومهكسين.

Introduction

Bromhexine Hydrochloride, *N*-(2-Amino-3,5-dibromobenzyl)-*N*-methyl cyclohexanamine hydrochloride is a white crystalline powder with molecular weight of 412.6. Its chemical structure[1]:



It is a expectorant drug, while it works through decreasing the amount of respiratory tract fluid and reduces its viscosity by activating enzymes that hydrolyze mucopolysaccharides [2]. Several methods have been reported in the literature for analytical determination of this substance, it has been determined by different techniques including spectrophotometry [2-5],

HPLC [6,7], Colorimetry [8,9], TLC[10], Flow-Injection-Spectrophotometry[11], Ion-Selective Electrode [12], Hybrid Linear Analysis [13], Capillary Isotachophoresis [14], Absorption Spectrophotometry and Electrophoresis[15], Reverse Phase Liquid Chromatography [16,17], HPLC-ICP-MS compared with Radiochemical Detection[18] and Flow Injection Analysis using conventional and coated wire ion-selective electrodes[19] are described.

The presence of an aromatic primary amino group in Bromhexine HCl enables the use of diazotization-coupling, according to the classic Bratton-Marshall method, Bromahexine Hydrochloride was diazotized with sodium nitrite and dilute hydrochloric acid and the excess of nitrite was destroyed with sulfamic acid. A soluble pink color dye was noticed by adding of N-(1-naphthyl) ethylenediamine dihydrochloride (NED) and the color intensity was measured spectrophotometriclly [20].

In this work a rapid and sensitive method using spectrophotometric detection at 507 nm was proposed for the determination of Bromhexine Hydrochloride in pure and pharmaceutical preparations. The method is based on the diazotization reaction of Bromhexine Hydroc-hloride with sodium nitrite in hydrochloric acid medium; the formed diazonium salt is then coupled with chromotropic acid to form a red water soluble azo dye. The proposed method has been applied successfully to the determination of Bromahexine Hydrochloride in pharma-ceutical preparations.

Experimental

Apparatus:

All spectral and absorbance measurements were carried out on a Shimadzu UV-Visble-260 digital double-beam recording spectro-photometer (Tokyo-Japan), using 1-cm quartz cells.

Reagents:

All chemicals used were of analytical reagent grade. Bromhexine Hydrochloride standard material was provided from the state company for drug industries and medical appliances (SDI) Sammara-Iraq

1- Bromhexine HCl, stock standard solution (500 µg.ml^{-1} = 1.211 x 10^{-3}M), prepared by dissolving 0.05 gm of pure Bromhexine HCl in amount of distilled water and made up to 100 ml with the same solvent.

2- Sodium nitrite solution (5 x 10^{-3}M), prepared by dissolving 0.0690 gm of $NaNO_2$ (Merck) in distilled water and diluting to the mark of 200 ml volumetric flask, then (1.211 x 10^{-3}M) was prepared by diluting 24.2 ml of sodium nitrite solution (5 x 10^{-3}M) with distilled water to 100 ml in a volumetric flask.

3- Hydrochloric acid 0.8M, prepared by diluting 34.4 ml of 11.64M of concentrated hydrochloric acid (BDH) with distilled water in a 500 ml volumetric flask. More dilute hydrochloric acid solutions were prepared by suitable dilution of concentrated hydrochloric acid with distilled water.

4- Chromotropic acid (0.1% W/V = 3.122 x 10^{-3} M, M.wt= 364.26), prepared by dissolving 0.1 gm of chromotropic acid (BDH) in distilled water and diluting to the mark with the same solvent in 100 ml volumetric flask. A solution of (1.211 x 10^{-3} M) of chromotropic acid solution was prepared by diluting 38.8 ml of chromotropic acid solution (0.1 %) with distilled water in a 100 ml volumetric flask.

Pharmaceutical preparation of Bromahexine HCl.

1- Pectomed-syrup (Medica Labs. Homs-Syria) each 100 ml of syrup containing 20 mg of Bromhexine HCl.

2-Solvoden –syrup (Sammara-Iraq), each 5 ml of syrup containing 4 mg of Bromhexine HCl.

Analytical procedure for calibration

Into a series of 25 ml volumetric flask, transfer increasing volumes of standard stock solution (500 µg.ml^{-1} = 1.211 x 10^{-3}M) containing (0.1-3.0 ml) of Bromhexine HCl to cover the range of the calibration graph (50-1500 µg in a final volume of 25 ml) i.e; 2-60 µg.ml^{-1}, to this solution added (0.1-3.0 ml) of $NaNO_2$ (1.211 x 10^{-3}M) and the acidity was adjust with 3 ml of 0.8M HCl. The solution was shaking thoroughly and 2 ml of 0.1% chromotropic acid was added. The contents was diluted to the mark with distilled water and shake well, after 10 min the absorbance of the azo dye was measured at 507 nm against a reagent blank containing (0.1-3.0 ml) of $NaNO_2$, 3 ml of 0.8 M Hydrochloric acid and 2 ml of 0.1% chromotopic acid. For the optimization of conditions and in all subsequent experiments, a solution of 500 µg.ml^{-1} of the drug in a final volume of 25 ml was used.

Procedures for pharmaceutical preparations

For each type of syrup transfer 25 ml of the syrup into a 50 ml volumetric flask and dilute it to the mark with distilled water to obtain (100 µg.ml^{-1}) and (400 µg.ml^{-1}) respectively.

Results and Discussion

Preliminary studies

Throughout the preliminary study on the diazotization reaction of Bromhexine HCl, with sodium nitrite in hydrochloric acid medium; the formed diazonium salt is then coupled with chromotropic acid, a red water-soluble azo dye was obtained with a maximum absorbance at 507 nm (figure 1). The absorbance of the azo dye solution measured versus reagent blank which has a negligible absorbance at this wavelength.

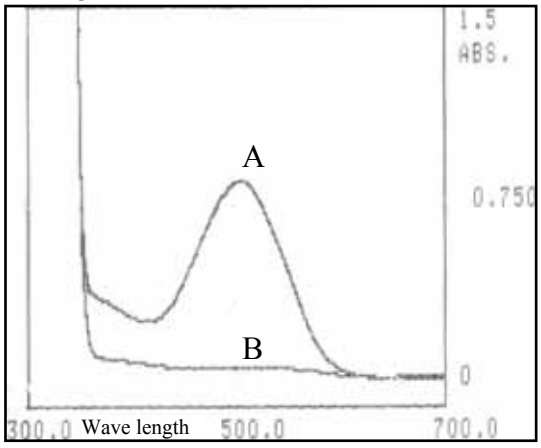


Figure 1: Absorbance spectra of the azo dye (1 ml of 500 µg.ml^{-1} =1.211 x 10^{-3}M of Bromhexine HCl, 1ml of 1.211 x 10^{-3}M $NaNO_2$, 3 ml of 0.8 M hydrochloric acid and 2 ml of 0.1% chromotopic acid) against reagent blank (A) and blank (1ml of =1.211x10^{-3}M $NaNO_2$, 3 ml of 0.8 M hydrochloric acid and 2 ml of 0.1% chromotopic acid) against distilled water (B).

Optimization of the experimental conditions

The effect of various parameters on the color development was studied to establish the optimum conditions for the determination of Bromahexine HCl.

In the subsequent experiments, 1 ml of Bromahexine HCl solution (500 µg.ml^{-1} = 1.211 x 10^{-3}M) with equimolar of sodium nitrite solution (1 ml of 1.211 x 10^{-3}M), 3 ml of 0.8 M hydrochloric acid and 2 ml of 0.1% chromotopic acid, was taken in to 25 ml final volume and the absorbance of the series of solutions were measured by varying one and fixing the other parameters at 507 nm. versus reagent blank.

1- Effect of acid

In practice, the addition of acid to the diazonium reaction was necessary for the formation of diazonium salt between the amine group (Bromhexine HCl) and sodium nitrite, which couples with phenolic reagent (chromotropic acid) to give an azo dye therefore, various acids were studied (acetic acid, hydrochloric acid, nitric acid, phosphoric acid and sulfuric acid), hydrochloric acid seems to be the most suitable acid through the high absorbance under the reaction condition.

When various concentrations of hydrochloric acid (0.1-3.0M) were added to the solution of 1 ml of 500 µg.ml^{-1}=1.211 x 10^{-3}M Bromhexine HCl, 1 ml of 1.211 x 10^{-3}M sodium nitrite and 2 ml of 0.1% chromotopic acid, the concentration of 0.8M seems to be the suitable concentration, and was considered to be optimum as shown in (figure 2).

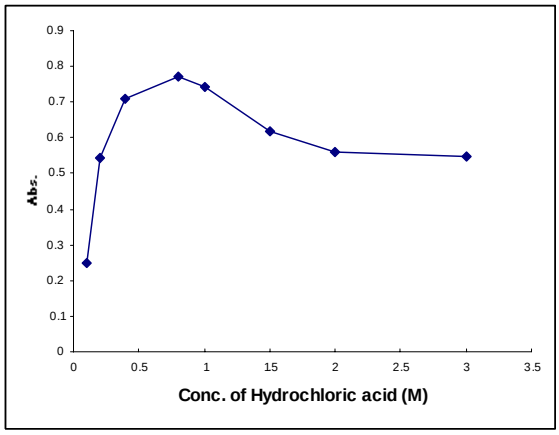


Figure 2: Effect of concentration of Hydrochloric acid (M) with 1 ml of 500 µg.ml^{-1}=1.211 x 10^{-3}M Bromhexine HCl, 1 ml of 1.211 x 10^{-3}M sodium nitrite and 2 ml of 0.1% chromotopic acid.

The effect of different volumes of 0.8M hydrochloric acid (0.1-5.0 ml) was studied, and 3 ml of hydrochloric acid was found optimum as shown in (figure 3).

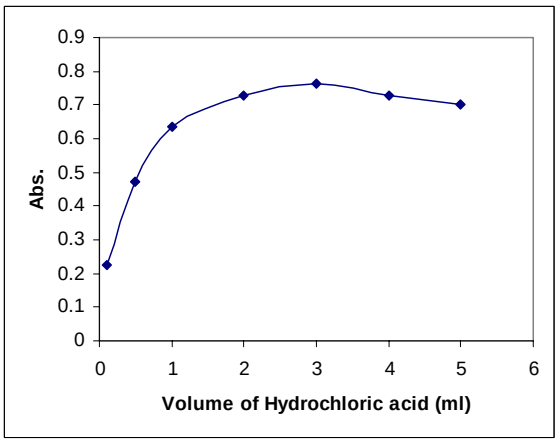


Figure 3: Effect of volume of 0.8M hydrochloric acid with 1 ml of 500 µg.ml^{-1}=1.211 x 10^{-3}M Bromhexine HCl, 1 ml of 1.211 x 10^{-3}M sodium nitrite and 2 ml of 0.1% chromotopic acid

2- Effect of reagent concentration

When various concentrations of chromotropic acid solutions were added to affixed amount of diazonium salt 0.1% (w/v) to develop the color of reaction to its full intensity and sensitivity and to ensure a quantitative determination at the upper limit of calibration graph, 2 ml volume of 0.1% chromotropic acid was gave the optimum value as shown in figure 4.

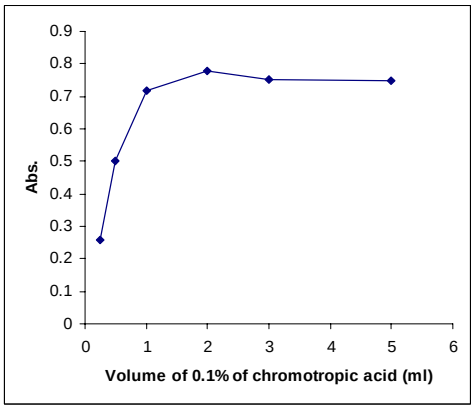


Figure 4: Effect of volume of 0.1% chromotropic acid with 1 ml of 500 µg.ml^{-1}=1.211 x 10^{-3}M Bromhexine HCl, 1 ml of 1.211 x 10^{-3}M sodium nitrite and 3 ml of hydrochloric acid.

3-Effect of order of addition

To optimum results, the order of addition of reagents should be followed as: 1ml of 500 µg.ml^{-1}=1.211 x 10^{-3}M Bromhexine HCl +1 ml of 1.211 x 10^{-3}M sodium nitrite + 3 ml of hydrochloric acid + 2 ml of 0.1% chromotropic acid, otherwise a loss in color intensity and stability was observed.

4- Effect of temperature

The effect of temperature on the diazotization and coupling reaction show that the absorbance of the azo dye (which contains 1ml of 500 µg.ml^{-1}=1.211 x 10^{-3}M Bromhexine HCl, 1 ml of 1.211 x 10^{-3}M sodium nitrite, 3 ml of hydrochloric acid, 2 ml of 0.1% chromotropic acid), remains constant at room temperature (25 C^{o}) for more than 120 min., and decrease at higher than 45 C^{o}. Cooling of reaction the mixture to (0-5C^{o}) made the dye formed precipitate with decreasing in absorbance after 10 mins.

5- Effect of time on the stability of the dye

The stability of the dye was studied for 2h. following the mixing of the reagents (which contains 1ml of 500 µg.ml^{-1}=1.211 x 10^{-3}M Bromhexine HCl, 1 ml of 1.211 x 10^{-3}M sodium nitrite, 3 ml of hydrochloric acid, 2 ml of 0.1% chromotropic acid). The absorbance of the dye became intense and sharp after 10 mins., after mixing the diazonium salt with chromotropic acid and remained stable for at least 2h.

Calibration graph

Employing the conditions described under procedure, a linear calibration graph (figure 5) for Bromhexine HCl was obtained, and Beer's law was obeyed over the concentration range of 50-1500 µg at a final volume 25 ml, or (2-60 µg.ml^{-1}) with a correlation coefficient of 0.9992 and an intercept of 0.0329. The conditional molar absorptivity of the red dye formed with reference to Bromhexine HCl. was found to be 1.569 x 10^{4} L.mol^{-1}.cm^{-1} and a Sandell sensitivity of 0.0262 µg. cm^{-1}, the limit of detection (LOD) was found to be 1.642 µg. ml^{-1} and the limit of quantification (LQD) equal to 5.471 µg.ml^{-1}.

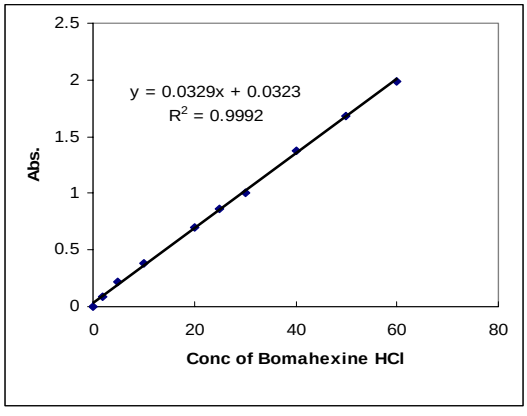


Figure 5: Calibration graph for Bromhexine HCl.

Accuracy and precision

To determine the accuracy and precision of the metho[illegible] Conc of Bromhexine HCl [illegible]rmined in three diffe[illegible]ılts shown in table (1), indicate that a satisfactory precision and accuracy could be obtained with the proposed method.

Table 1: Accuracy and precision of the proposed method

Concentration of Bromhexine HCl. µg.ml^{-1}		Error %	Recovery %	Relative standard deviation (R.S.D) %
Present	Found*			
10.00	10.016	+0.16	100.16	2.049
25.00	25.036	+0.14	100.14	1.632
40.00	40.016	+0.04	100.04	0.805

* for five determinations.

Structure of the dye

The stoicheiometry of the reaction between the diazotized drug and chromotropic acid was investigated using both continuous variation and molar ratio methods respectively, in continuous variation method, volumes 1-5 ml of (1.211 x 10^{-3} M) portions of Bromhexine HCl (V_D) were diazotized using equimolar of sodium nitrite (1.211 x 10^{-3} M) and 3 ml of 0.8 hydrochloric acid and coupled according to analytical procedure with the corresponding complementary volume of (1.211 x 10^{-3} M) of chromotropic acid solution (V_R) to give a total volume of 5 ml for (V_R+V_D) then dilute to 25 ml with distilled water, and for the molar ratio method, increased volumes 0.1-2 ml of (1.211 x 10^{-3} M) chromotropic acid (V_R) were added to a 1 ml of (1.211 x 10^{-3} M) Bromhexine HCl (V_D) were diazotized using 1 ml of sodium nitrite (1.211 x 10^{-3} M) and 3 ml of 0.8 hydrochloric acid and dilute to 25 ml with distilled water, the results obtained (figure 6 and 7) shows that a (1:1) azo dye was formed between diazotized Bromhexine HCl and chromotropic acid.

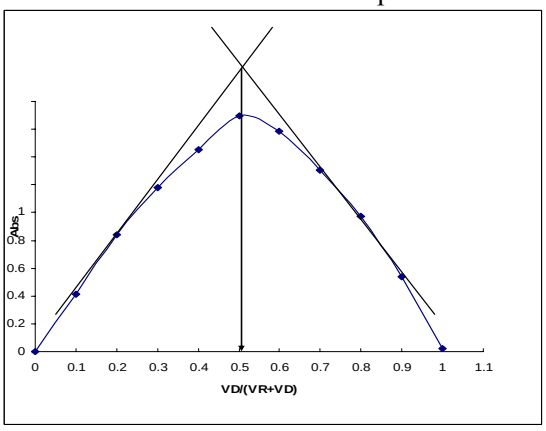


Figure 6: Continuous variation plot

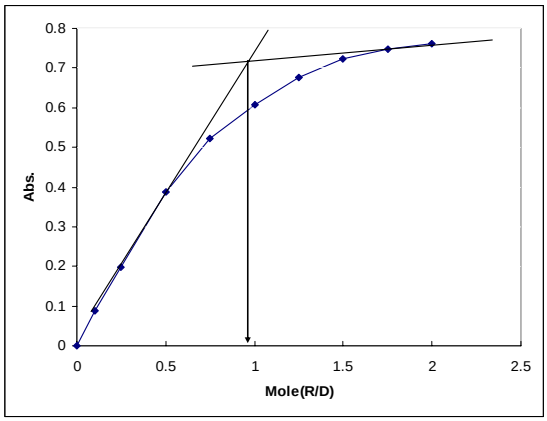
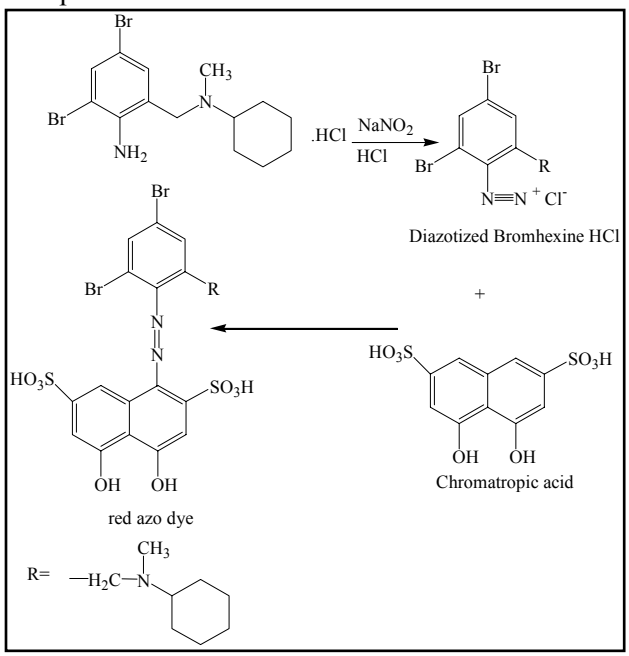


Figure 7: Mole ratio plot

A reaction subsequent based on the above results is shown in Scheme (1).

Scheme 1: reaction sequence

The product formed was soluble in water. The



apparent stability constant was calculated by comparing the absorbance of a solution containing stiochiometric amount of Bromhexine HCl (500 µg.ml^{-1}) (1.211 x 10^{-3} M) (A_S) with that of a solution containing a five – fold excess of chromotropic acid reagent (A_m) and according to analytical procedure. The average stability constant (K)[21] = 1.924x10^5 L.mol^{-1} as shown in table 2, where is [K=(1-α)/ α^{2}C] and α=A_m-A_s/A_m.

Table 2: The stability constant

Volume of drug ml	Conc. of drug µg.ml^{-1}	C (M) x10^{-6}	A_s*	A_m*	α	K (L.mol^{-1}) x10^5
0.25	5	12.118^{-6}	0.103	0.164	0.371	3.789
1	20	48.473^{-6}	0.230	0.409	0.437	0.606
1.5	30	72.709^{-6}	0.929	1.273	0.270	1.377

*** for five determinations**

Pharmaceutical applications

Two types of syrup containing Bromhexine HCl have been analyzed using the proposed procedure(1.25 ml of 400 µg.ml^{-1} for solvodien drug and 5 ml 0f 100 µg.ml^{-1} for pictomed drug , and the equimolar volumes for sodium nitrite, 3 ml of hydrochloric acid and 2 ml of 0.1% chromotropic acid), and they gave the results shown in table (3).

Table 3: Application of the proposed method of Bromhexine HCl. In pharmaceutical preparations

Drug sample	Concentration of Bromhexine HCl µg.ml^{-1}		Error %	Recovery %	R.S.D %
	Present	Found*			
Solvodien syrup 4mg/5ml	20.00	20.015	+0.075	100.075	0.618
Pictomed syrup 20mg/100 ml	20.00	18.540	-7.30	92.700	0.993

***for five determinations**

The proposed method was compared successfully with Bratton Marshall's method[20] for both pure Bromhexine HCl and Solvodien syrup but the pictomed syrup gave a low recovery value in comparison with the classic Bratton Marshall's method (table 4), therefore, the standard addition method[21] was applied to determine the Bromahexine HCl in Pictomed syrup and a good recovery was obtained as shown in figure 8.

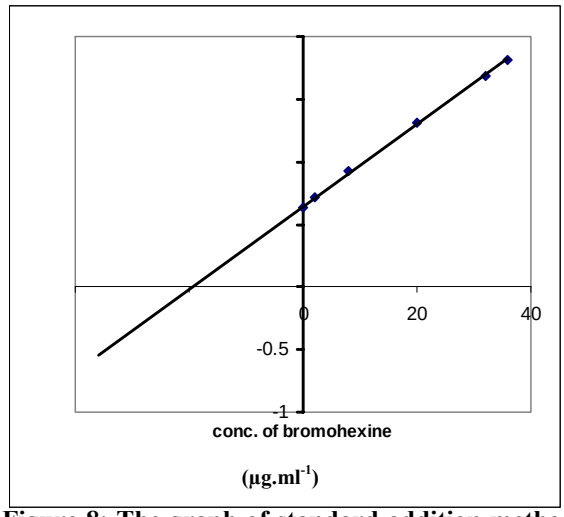


Figure 8: The graph of standard addition method for the determination of Bromhexine HCl in Pictomed syrup with R.S.D % = 0.175%.

Table 4: Comparison of the proposed method with standard method.

Drug sample 20 µg.ml^{-1}	Recovery%*		
	Proposed method	Standard addition method	Standard method
Pure Bromhexine HCl	99.68	-	98.00
Solvodien syrup	100.075	-	101.50
Pictomed syrup	92.700	100.00	100.80

***for five determinations**

Evaluation of the proposed method

For evaluating the competence and the success of the proposed method, the results obtained were compared with those by popular Bratton-Marshall's method (standard method), the same pharmaceutical preparations for Bromahexine HCl were analyzed by the standard method. The results obtained were statistically compared, using the Student t-test and variance ratio F-test at 95% confidence level [22], the calculated t- and F-values did no exceed the theoretical values, which indicate that there is no significant difference between the methods in the determination of Bromahexine HCl in pharmaceutical preparations (table 5).

Table 5: The comparison of the proposed method with standard method using t- and F-statistical tests.

The pharmaceutical preparations for 20 µg.ml^{-1}	The proposed method		The standard method	
	Rec.%	$(Xi-\bar{Xi})^2_1$	Rec.%	$(Xi-\bar{Xi})^2_2$
Pure Bromhexine HCl	99.68	0.0529	98.00	4.41
Solvodien syrup	100.07	0.0256	101.50	1.96
Pictomide syrup	100.0	0.0081	100.80	0.49
	$(\bar{Xi})_1$= 99.91	$\sum(Xi-\bar{Xi})^2_1$= 0.0866	$(\bar{Xi})_2$= 100.1	$\sum(Xi-\bar{Xi})^2_2$= 6.68

$F_{calculated} = S_1^2 / S_2^2 = 0.0433/3.34 = 0.0129$

$F_{theoretical} = 19.0$ $F_{theoretical} > F_{calculated}$ at 95% confidence level,

$T_{calculated} = 0.178$, $T_{theoretical} = 3.812$

$T_{theoretical} > T_{calculated}$; at 95% confidence level.

Table (6) shows a comparison between the developed method and some spectrophotometric methods with various organic reagents. Some of these methods needed organic solvents for the extraction of the dye [3,8], or have a low linearity range that obeyed Beer's law also needed a high temperature to develop the

reaction [8]. The proposed method has a wide linearity range (2-60 $\mu g.ml^{-1}$) also it didn't need organic solvents or cooling and has a good accuracy and precision.

Table 6: A comparison between the proposed method and some spectrophotometric methods.

No	Type of reaction	Coupling agent	λ_{max} (nm)	Linearity (ppm)	Molar absorptivity ($l.mol^{-1}.cm^{-1}$)	Ref.
1	Ion association	Tropaleolin Naphthalene Azocamine	420 620 540	2-10 5-25 5-25	$2.36x10^4$ $1.06x10^4$ $1.23x10^4$	3
2	Oxidative-coupling	2,2¯bipyridyl Methyl benzothiozolinone hydrazone	510 630	2-10 5-25	$1.113x10^4$ $1.128x10^4$	8
3	Diazotiziation and coupling	Chromotropic acid	507	1-60	1.569×10^4	*

*** Proposed method.**

Conclusions

A simple, accurate and sensitive spectrophotometric method has been proposed for the determination of trace amount of Bromhexine HCl in aqueous solution based on the diazotiziation reaction and coupling witchromotropic acid at room temperature, the proposed method has some advantages like the fast determination of the drug on its pure form and in pharmaceutical preparations also it did not require temperature control, solvent extraction and expensive reagents and solvents.
The wide linear rang that obeyed Beer' low of the proposed method gave a good application for the pharmaceutical preparation.

References

1. The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA). **2007**. British Pharmacopoeia on CD-Rom. Fifth Edition, London.
2. Ribone,M.E; Pagani,A.P. and Olivier, A.C. **2000**. Determination of the minor component Bromhexine in cotrimoxazole-containing tablets by absorption spectrophotometry and partial least-squares (PLS-1) multivariate calibration. *J. Pharm. Biomed. Anal*., **23**: 591-595.
3. Murali Mohan Rao,S.V; Nageswara Rao. I; Rama Subba Reddy ,T. and Sastry, C. S. P. **2005**. Assay of Bromhexine Hydrochloride in pharmaceutical formulations by extraction spectrophotometry, *Indian J. Chem. Technol.*, **12**: 170-174.
4. Dave, N.H; Mashru, R.C. and Thakkar, A.R. **2007**. Simultaneous determination of salbutamol sulphate, Bromhexine hydrochloride and etofylline in pharmaceutical formulations with the use of four rapid derivative spectrophotometric methods. *Anal Chim Acta*., **1**: 113-120.
5. Dias,A.C.B; Santos,J.L.M; Lima,J.L. F.C. and Zagatto,E.A.G. **2003**. Multi-pumping flow system for spectrophotometric determination of Bromhexine. *Anal Chim Acta*., **499** : 107-113.
6. Packert Jensen, B; Gammelgaard, B; Honore Hansen, S. and Vanggaard Andersen, J. **2005**. HPLC-ICP-MS compared with radiochemical detection for metabolite profiling of 3H-Bromohexine in rat urine and faeces. *J. Anal. At. Spectrom*., **20** : 204-209.
7. Chu, K. O. and Tin, K. C. **1998**. Analysis of Antihistamines in Cough Syrup. *Anal. Lett*., **31**: 1879-1890.
8. Vijaya Raja, G; Venu gopal, G; Mounik, V; Satyavathi, S and Lavanya, Ch. **2010**. Simple colorimetric assay for microgram determination of Bromhexine Hydrochloride with MBTH and2, 2' Bipyridyl, *IJPSR*, **1**: 90-94.
9. Sanghavi,N.M; Samart,M.h; Singh,R and Matharu,P.S. **1990**. Colorimetric Analysis of Bromhexine. *Indian Drugs*, **27**: 486-488.
10. Sumarlik, E and Indrayanto, G. **2004**. TLC Densitometric Determination of Bromhexine Hydrochloride in Pharmace-uticals, and Its Validation. *J. Liq. Chromatogr. Relat. Technol*., **27**: 2047-2056.
11. Perez-Ruiz,T; Martinez-Lozano,C; Sanz,A and Mondejar,S. **1995**. Flow-injection extraction-spectrophotometric determination of Bromhexine with orange IV. *J. Pharm. Biomed.Anal*., **13**: 1101-1106.
12. Khalil, S. and Elrabiehi, M. **1999**. Bromhexine-Selective PVC membrane electrode based on Bromhexinium Tetraphenylborate. *Microchem. J*., **62**: 237-243.
13. Goicoechea, H. C and Olivieri, A. C. **1999**. Determination of Bromhexine in cough-cold syrups by absorption spectrophotometry and multivariate calibration using partial least-squares and hybrid linear analyses. Application of a novel method of wavelength selection. *Talanta*, **49**: 793-800.

14. Pospisilova,M; Polasek,M. and Jokl, V. **2001**. Determination of ambroxol or Bromhexine in pharmaceuticals by capillary isotachophoresis. *J.Pharm.Biomed.Anal*., **24**: 421-428.
15. Rodriguez, V. G; Lucangioll, S. E; Otero, G. C. F. and Carducci, C. N. **1996**. Purity testing of drugs by capillary electrophoresis. *J. High Resol. Chromatogr.*, **19**: 703-705.
16. Pai, P. N. S; Rao, G. K; Murthy, M. S; Agarwal, A. and Puranik, S. **2009**. Simultaneous determination of salbutamol sulphate and Bromhexine Hydrochloride in tablets by reverse phase liquid chromatography. *Ind. J. pharma. sci*., **71**: 53-55.
17. Shaikh, K. A; Patil, S. D. and Devkhile, A. B. **2008**. Development and validation of a reversed-phase HPLC method for simultaneous estimation of ambroxol hydrochloride and azithromycin in tablet dosage form. *J. Pharm. Biomed. Anal*., **48**: 1481-1484.
18. Packert Jensen, B; Gammelgaard, B; Honore Hansen, S. and Vanggaard Andersen, J. **2005**. HPLC-ICP-MS compared with radiochemical detection for metabolite profiling of 3H-Bromohexine in rat urine and faeces. *J. Anal. At. Spectrom*., **20**: 204-209.
19. Abdel-Ghani, N. T; Issa, M. Y. and Ahmed, M. H. **2006**. Potentiometric flow injection analysis of Bromohexine Hydrochloride and its pharmaceutical preparation using conventional and coated wire ion-selective electrodes. *Sci. Pharma.*, *74*: 121-135.
20. Santoro, M. I; dos Santos, M. M. and Maqalhaes, J. F. **1984**. Spectrophotometric determination of Bromhexine Hydrochloride in pharmaceutical preparations.*J Assoc Of Anal Chem*. , **67** :532-540.
21. Al-Abachi, M. Q. and Al-Ghabsha, T. S. **1983**. *Fundamentals of Analytical Chemistry*. Press of Mousl University. pp. 414, 346.
22. Farrant, T. J. **1997**. *Practical Statistics for the Analytical Scientist*. L.G.G. pp. 415.