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DEVELOPMENT OF A SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF BROMHEXINE HYDROCHLORIDE IN ITS PURE FORM AND IN ITS PHARMACEUTICAL PREPARATIONS USING DIAZOTIZATION - COUPLING REACTION

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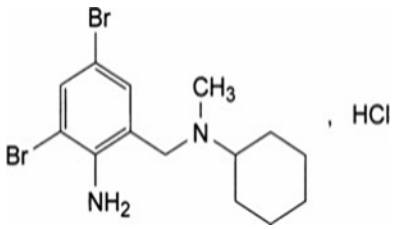
Graphical abstract



Abstract

A simple, rapid, and accurate spectrophotometric method has been developed for determining bromhexine hydrochloride (BROH) in its pure form and pharmaceutical formulations. The method is based on the reaction of diazotized bromhexine hydrochloride (DBROH) with the organic reagent 4-chlororesorcinol (4CRC) in the presence of a sodium hydroxide basic medium. This reaction produces a stable, water-soluble orange-colored azo dye, which exhibits maximum spectral absorption at a wavelength of 455 nm.The method demonstrated high sensitivity, with a molar absorptivity of 43,548 L/mol•cm. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 0.322 µg/mL and 1.073 µg/mL, respectively. The proposed method was successfully applied to the analysis of bromhexine hydrochloride in pharmaceutical preparations, including syrups and tablets, showcasing its efficacy and reliability.

Keywords: Spectrophotometric, bromhexine hydrochloride, diazotization-coupling, 4-chlororesorcinol, azodye

1.0 INTRODUCTION

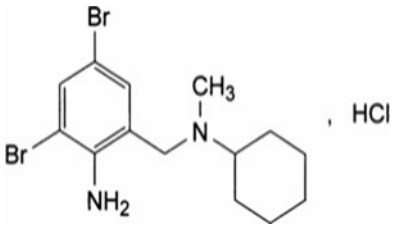
Bromhexine, a benzylamine derived cardiac depressant of vasicine, is a quinazoline alkaloid obtained from the plant Adhatoda vasica. It was developed in the research laboratories of Boehringer Ingelheim during the late 1950s as an active pharmaceutical ingredient. Bromhexine was introduced to the market in 1963 under the trademark "Bisolvon" [1].

Bromhexine is a synthetic molecule derived from vasicinone, a natural product isolated from Adhatoda vasica, an important plant in Ayurvedic medicine. It is widely prescribed as an over-the-counter (OTC) mucoactive drug for managing a variety of respiratory diseases. Recently, Bromhexine has gained attention as a potential repurposed drug candidate for managing COVID-19, given its pharmacological properties [2], [3], [4].

Due to its physiological relevance, Bromhexine has been extensively quantified using various analytical techniques that exploit its chemical and physical properties. Its structure and solubility characteristics have made it a focus of

pharmaceutical research, particularly in the development of effective dosage forms to optimize bioavailability.

Bromhexine Hydrochloride (BROH) (Figure 1) is chemically named as N-(2-Amino-3,5-dibromobenzyl)-N-methylcyclohexanamine hydrochloride. According to the International Union of Pure and Applied Chemistry (IUPAC), its systematic nomenclature is 2,4-dibromo-6-[[cyclohexyl(methyl)amino]methyl]aniline hydrochloride. It has the molecular formula C14H20Br2N2·HClC_{14}H_{20}Br_{2}N_{2} \cdot HClC14H20Br2N2·HCl and a molecular weight of 412.6 g/mol [5], [6].



M.Wt. 412.6 g/mol

Figure 1 Bromhexine hydrochloride

Numerous analytical techniques have been developed for estimating Bromhexine Hydrochloride (BROH) in pharmaceutical formulations or its pure form, either as a single active pharmaceutical ingredient or in combined formulations. techniques for estimating BROH in pharmaceutical formulations or in its pure form have been published as a single active pharmaceutical ingredient or combined formulations. These include methods such as spectrophotometry [7–12], UV spectrophotometry[13], chromatography [14–18], voltammetry [19,20], potentiometric flow injection [21], capillary electrophoresis [22], attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy [23], and chemiluminescence [24].

Among these, spectrophotometric techniques remain the most widely used due to their affordability, simplicity, and ease of implementation. These methods are particularly advantageous in resource-limited settings where more advanced analytical instrumentation may not be available.

In this study, we developed a simple and accurate spectrophotometric technique for the determination of BROH in its pharmaceutical formulations. The method involves transforming the drug's amino group into a diazonium salt, which is then coupled with 4-chlororesorcinol to produce a colored azo dye. This novel approach is characterized by its precision, sensitivity, and practicality for routine quality control of BROH in various dosage forms.

2.0 EXPERIMENTAL

Apparatus

A Jasco V-630 UV-Visible double beam (Japan) device spectrophotometer with spectra manager software and using of silica cell (1cm) have been used in spectrophotometric measurements as well as using of professional benchtop BP3001 pH meter and balance of the type ABS 120-4 by Kern and Sohn.

Chemicals

All chemicals and reagents that were used were of a high degree of analytical purity, and deionized water was used in the dissolution process and preparing solutions during the study.

Reagents

Diazotized Bromhexine HCl (DBROH) solution (250 µg/ml =6.059x10-4M)

0.0250 g of bromhexine hydrochloride BROH (provided by the General Company for Pharmaceuticals and Medical Supplies Industry SDI/Samarra/Iraq) was dissolved in a few amount of warm distilled water, followed by the addition of equivalent amounts of sodium nitrite (0.0041 g i.e. 6.059x10-4M) which supplied by BDH , then 4 ml of HCl acid (1 M) is added, and finally the final volume is supplemented with distilled water using a 100 ml volumetric flask and kept away from light.

Reagent solution 4-chlororesorcinol (0.1% 4CRC)

Prepare the reagent solution prepared from Sigma Company by dissolving 0.1 g of 4CRC in drops of absolute ethanol and stirring continuously. The volume is then supplemented with distilled water using a 100 ml volumetric flask. It is stored in an opaque bottle and remains stable for at least a week.

Solution of hydrochloric acid (1 M approx.)

Prepare this solution by taking 4.8 ml of concentrated acid HCl (11.8 molar) prepared by Fluka, and then diluting it with distilled water to a final volume of 100 ml using a volumetric flask.

Solution of sodium hydroxide (1 M)

It is prepared by taking one 100 ml ampoule prepared by BDH and then diluting it with distilled water using a 1000 ml volumetric vial.

Pharmaceutical preparation of diazotized Bromhexine HCl

Syrup solution (250 µg / ml)

31.25 ml of Mucosolove syrup (pioneer company which each 5 ml syrup contains BROH 4 mg) was prepared using the same steps and procedure used in the preparation of the standard solution of diazotized bromhexine hydrochloride, then diluted to 100 ml in distilled water by using a calibrated flask to prepare 250 µg of DBROH/ml).

Tablets solution (250 µg / ml)

10 tablets of BROH were ground well (each tablet contains 8 mg of the active ingredient, supplied by Ipca laboratories/ India). The ground material was carefully weighed, equivalent to 0.025 g of BROH. This material was dissolved in an adequate amount of warm water, filtered, and subsequently subjected to diazotization using the same procedure as employed for the standard solution.

Analytical procedure for calibration

Established in previous experiments, a series of 10 mL volumetric flasks were prepared. These flasks contained varying volumes (0.1–2.0 mL) of a standard solution of diazotized bromhexine hydrochloride (DBROH) at a concentration of 250 µg/mL (6.059×10^{-4} M), covering a concentration range of 2.5–50 µg/mL. To each flask, 1 mL of a 0.1% 4-chlororesorcinol (4CRC) coupling agent was added, followed by 1 mL of a 1 M NaOH solution. The solutions were then diluted to the mark with distilled water and allowed to stabilize at room temperature.The absorption the resulting orange azo dye solutions was measured at the maximum wavelength of 455 nm against a blank solution. The calibration curve generated by this method (Figure 2) demonstrated a linear relationship between absorbance and concentration up to 50 µg/mL. A deviation from Beer-Lambert's law was observed at concentrations higher than this range, likely due to saturation effects. The calculated molar absorptivity was 43.548 L/mol•cm. The method demonstrated a limit of quantitation (LOQ) of 1.073 µg/mL and achieved a high correlation coefficient, indicating the precision and reliability of the proposed method for BROH analysis.

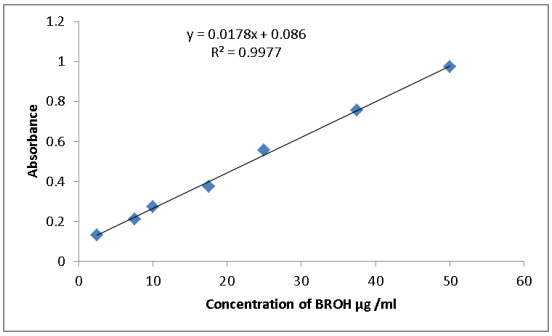


Figure 2 Calibration curve for estimating BROH according to the proposed method

3.0 RESULTS AND DISCUSSION

0.5 ml of DBROH in a final volume of 10 ml was used to investigate every factor that influences the absorbance of the colorful azo dye product that results.

The effect of various factors on the development and color stability of the azo dye formed was studied for the purpose of determining the optimal conditions for the determination of bromhexine hydrochloride (BROH), by taking 0.5 ml of the iodinated standard solution (250 µg/ml) and diluting it in a final volume of 10 ml of distilled water, and the absorption of the azo dye against the mock solution was measured. At the maximum wavelength is 455 nanometers, as shown below:

Choose the type of acid

Given the role of acid in the process of forming the diazonium salt corresponding to the amine group in BROH and in the presence of sodium nitrite, the effect of different types of acids (at a concentration of 1 molar) on the intensity of absorption of the azo dye formed was studied by adding 1 ml of these acids, followed by adding 0.5 ml of DBROH and waiting. For two minutes for the purpose of nitrification, then adding 1 ml of 0.1% of reagent 4CRC and 1 ml of NaOH (1 molar). The absorption of the azo dye was measured at a wavelength of 455 nm compared to the mock solution. Table 1 shows that HCl acid gave the highest absorption. For the azo dye formed.

Table 1 The effect of the type of acid used in diazotization

Acid used (1ml,1M)	HCl	HNO3	H2SO4	H3PO4	CH3COOH
Absorbance	0.252	0.246	0.237	0.239	0.203

Effect of HCl acid volume

Increasing volumes of 0.5 - 2.5 ml of HCl acid (1 molar) were added with the same previous additions. The results of Table 2 indicate that a volume of 1.5 ml of HCl acid is the appropriate volume and therefore it was fixed in subsequent experiments.

Table 2 Effect of the amount of acid used

x ml added HCl (1M)	0.5	1	1.5	2	2.5
Absorbance	0.245	0.2 50	0.26 1	0.243	0.237

For the purpose of choosing the best time required to complete the diazoation reaction of the drug compound BROH, absorption was measured after waiting at different time periods, and the results of Table 3 show that 3 minutes was sufficient to give the highest absorption of the azo dye formed.

Table 3 Choose the diazotization time

Time/minute	0	1	2	3	4	5
Absorbance	0.253	0.254	0.262	**0.271**	0.270	0.269

Effect of reagent quantity 4CRC

The optimal volume of the 4CRC reagent was determined by varying its volume between 0.4–1.5 mL. A volume of 1 mL was found to provide the highest absorbance and color stability of the azo dye, as indicated in Table 4 To be deleted The highest value of the estimation coefficient (R2 = 0.9964). Otherwise, a decrease in the color intensity and stability of the azo dye formed is observed, and therefore the same previous volume of the reagent was maintained.

Table 4 Choose the diazotization time

ml of 4CRC (0.1%)	Absorbance/µg BROH/ml					
	2.5	**7.5**	**12.5**	**22.5**	**30**	**R^2**
0.4	0.119	0.167	0.204	0.453	0.532	0.9862
0.7	0.126	0.187	0.255	0.521	0.610	0.9891
1	0.133	0.210	0.276	0.542	0.661	0.9964
1.5	0.122	0.175	0.245	0.473	0.513	0.9719.

Choose the type and size of the base

Different types and quantities of strong and weak bases were taken to show the extent of their effect on the intensity of absorption of the color of the azo dye. We conclude from the results of Table 5 that the highest absorption was using 1 ml of NaOH solution.

Table 5 The effect of the type and quantity of added base on the absorption of azo dye

Base added (1M)	Absorbance/ml of base added			
	0.3	**0.5**	**1**	**1.5**
NaOH	0.247	0.253	0.279	0.269
pH	3.01	8.99	11.86	12.55
KOH	0.239	0.248	0.260	0.251
pH	3.22	9.22	11.99	12.60
Na2CO3	0.201	0.216	0.233	0.228
pH	2.80	8.10	9.44	11.23
NaHCO3	0.188	0.201	0.213	0.226
pH	2.11	7.89	8.33	10.45

Temperature effect

The effect of temperature on the absorbency and stability of the formed azo dye solution was studied using different temperatures ranging between 10-50 Celsius. The results of Table 6 show that the optimum temperature was in the range of 25-30 Celsius compared to high and low temperatures. Therefore, it was adopted This degree was achieved in subsequent experiments.

Table 6 The effect of temperature on the stability of the azo dye formed

Temp. (°C)	10	15	20	25	30	35	40	45	50
Abs.	0.167	0.181	0.221	0.275	0.278	0.267	0.258	0.251	0.244

Effect of solvents

Different solvents were used in addition to water in the final dilution of the reaction solution to demonstrate the extent of the effect of these solvents on the intensity of absorption of the azo dye formed. As shown in the spectra of Figure (3) and Table 7, the highest absorption was using acetone and distilled water, so distilled water was kept as the Used, available and safe solvent.

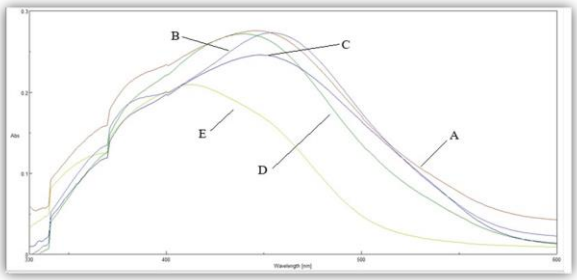


Figure 3 Absorption spectra of azo dye using different solvents

Table 7 Effect of different solvents on the intensity of absorption of azo dye

colour		Solvent	λ_{max} (nm)	Absorbance	ε(x10 ^{4}) l/mol.cm
red	A	Acetone	556	0.275	0.910
purple	B	Water	455	0.273	0.903
blue	C	Methanol	448	0.245	0.811
green	D	Ethanol	440	0.271	0.897
Pistachio	E	Acetic acid	413	0.209	0.692

The effect of time on the stability of azo dye

The stability of the azo dye formed was studied by taking two concentrations of the drug compound DBROH (12.5 and 25 µg/ml), and the absorption of the azo dye product against the solution was measured at a wavelength of 455 nm and at room temperature, and as shown in Table 8, the results indicate stability. Absorption of the azo dye for a period of time of at least 60 minutes.

Table 8 Effect of time on the stability of azo dye

Standing time, minute. minute	Absorbance/µg of BROH 12.5	25
Immediately	0.274	0.548
5	0.2 74	0.546
10	0.2 78	0.54 4
15	0.2 79	0.54 3
25	0.2 72	0.54 2
30	0.2 71	0.5 40
40	0.2 69	0.54 0
50	0.2 72	0.5 39
60	0.26 5	0.5 33

Table 9 summarizes the optimal experimental conditions that were established based on previous experiments.

Table 9 Optimal conditions for the proposed method

Variable	Optimality
Reagent used	4-Chlororesorcinol
Percentage of reagent used	0.1
Amount of reagent used (ml)	1
Based on NaOH(ml)	1
Concentration of base used (M)	1M
Amount of HCl(1M) used in diazotization, (ml)	1.5
λ max(nm)	455

Final absorption spectrum

The final absorption spectrum was measured after establishing the optimal conditions mentioned above by adding 1 ml of 4CRC reagent with a concentration of 0.1% to 0.5 ml of the prepared D-BROH solution with a concentration of 250 µg/ml, followed by 1 ml of NaOH solution with a concentration of 1 molar. Measurement of the absorption spectrum of the azo dye was recorded, confirming maximum absorption at 455 nm. The spectrum is shown in Figure 4.

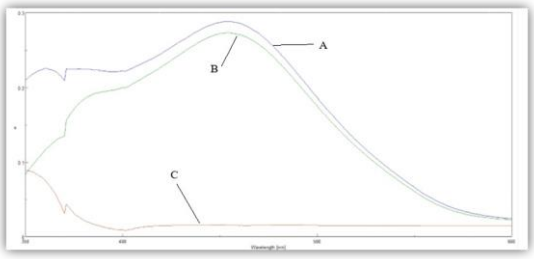


Figure 4 final absorption spectra of 12.5 µg/ml of BROH according to the optimum conditions, measured against: (A) distilled water (B) blank (C) blank vs distilled water

Accuracy and compatibility

The optimal conditions were used in the approved working method to calculate the accuracy and compatibility of the proposed method for estimating BROH by calculating the relative error and the relative standard deviation . Three concentrations of the DBROH solution were taken, which fall within the limits of the Beer-Lambert law in the standard curve, and the absorption values were measured . For solutions, with an average of five readings for each concentration , the results of Table 10 indicate that the proposed method is characterized by good accuracy and compatibility.

Table 10 Accuracy and compatibility of the proposed method

Amount of BROH µg/ml	Recovery *%	RE%	RSD%
5	98.51	-1.49	1.11
20	99.12	-0.88	1.19
40	100.20	0.20	0.97

* Average of five determinations.

Study the nature of the reaction produc of BROH with 4CRC

Both the continuous change and molar ratio methods were applied, respectively, for the purpose of studying the compositional molar ratio of the azo dye formed between BROH and the reagent 4CRC at an equal molar concentration of 6.059 x10 -4 M , and according to the Figure 5 and 6. For both the continuous change and the molar ratio, respectively, we conclude that the reaction between the drug compound and the reagent was at a ratio of 1:1.

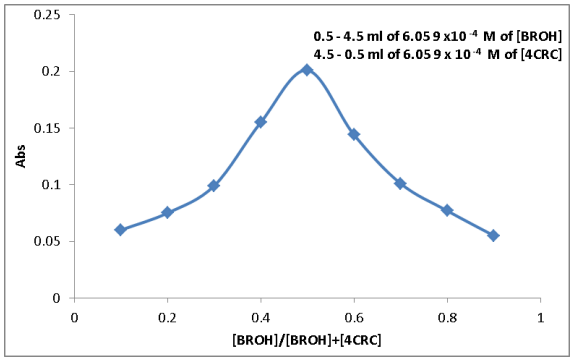


Figure 5 Continuous change method curve for BROH dye: 4CRC

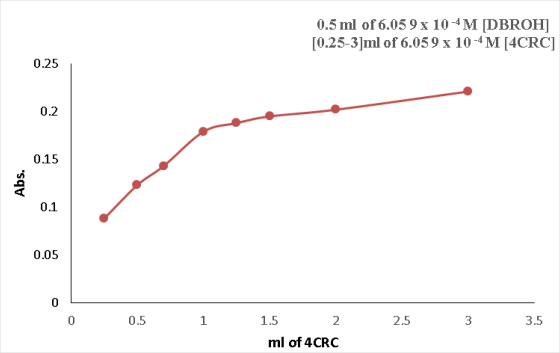


Figure 6 Molar ratio method curve for BROH dye: 4CRC

Therefore, the expected structural formula for the azo dye can be shown in Figure (7).

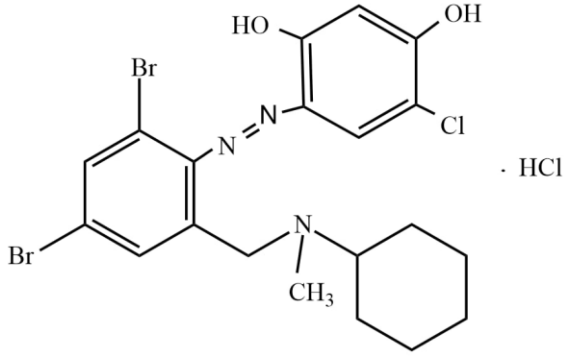


Figure 7 The expected structural formula of the azo dye formed

Apply the proposed method

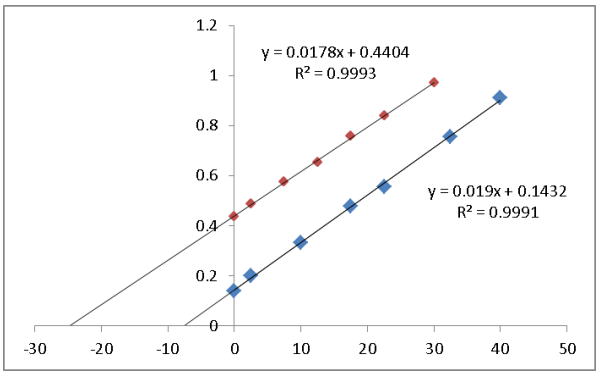
The direct method for estimating BROH was applied to some pharmaceutical preparations in the form of tablets and syrup from different origins by taking three different concentrations (5, 20, 40) of a DBROH solution with a concentration of 250 micrograms/ml. The solutions were treated with the same steps followed when preparing the calibration curve, and their absorbance was measured at the wavelength is 455 nm, and the results of Table 11 indicate the success of the proposed method in estimating BROH in the pharmaceutical preparation containing it.

Table 11 Results of BROH estimation in pharmaceutical preparations

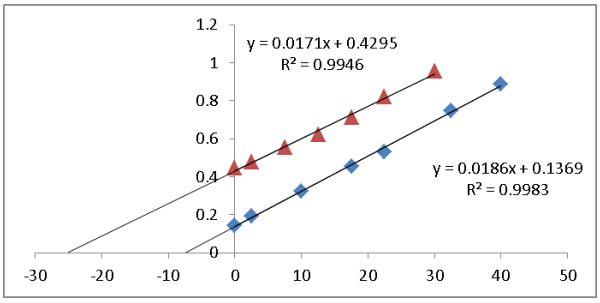
Drug content. content	BROH taken µg/ml	BROH found µg/ml	Recovery* %	RE %	RSD %
Bromhexine 8 mg/tablet Ipca /India	5	4.91	98.20	-1.80	1.01
	20	1 9.77	98.85	-1.15	1.06
	40	39.89	99.72	-0.27	0.92
Mucosolove / syrup 4 mg/5ml Pioneer/Iraq	5	4.94	98.80	-.120	1.09
	20	19.90	99.50	-0.50	0.91
	40	40.06	100.15	0.15	1.21

* Average of five determinations

Evaluate the proposed method using the standard addition method addition method was applied to determine the selectivity of the proposed method for hydrochloride determination Bromhexine In his pharmaceutical preparations , by taking two concentrations of the pharmaceutical compound with a concentration of 250 µg/ml , and through Figure 8 and the results of Table 12, it is clear to us that the standard addition method agrees well and within the acceptable error range with the results of the proposed method.



A - Tablet



B - Syrup

Figure 8 Curve of the standard addition method to estimate BROH : A - tablets B - syrup

Table 12 Results of BROH estimation in pharmaceutical preparations

Drug content. content	BROH taken µg/ml	BROH found µg/ml	Recovery*%	RE %	RSD %
Bromhexine 8 mg/tablet Ipca /India	7.5	7.53	100.40	0.40	1.10
	25	24.74	98.96	-1.04	1.02
Bromhexine/Syrup 4 mg/ 5ml Pioneer/Iraq	7.5	7.36	98.13	1.86	0.97
	25	25.11	100.44	0.44	0.66

* Average of four determinations

Compare the Proposed Method

Comparison was made of the current method with some analytical variants of other spectroscopic methods for hydrochloride determination Bromhexine

and two drug compounds, as shown in the Table 13. The proposed method was no less sensitive and important than other methods.

Table 13 Comparison with other spectrophotometric methods

Variable	Present method	Su l tan and M a jed , 2020	Hind al.,2011
Type of reaction	Diazo-coupling	Diazo-coupling	Diazo-coupling
Reagent Used	4-chlororesorcinol	Pholoroglucinol	Chromotropi c a c id
λ_{max} (nm)	455	405	507
Linearity range(µg /ml)	2.5-50	0.2 5 - 15	2-60
ε l/mol · cm	3.548×10^{4}	2.7×10^{4}	1.50×10^{4}
Sandell's Index (µg/cm ^{2})	0.0116	0.01517	0.0262
LOD	0.322	0.0121	0.51
LOQ	1.073	0.0406	1.55
Color's product	Orange	Yellow	Red
Applicatio n o f the method	T a b l e ts and Syrup	T a b l e ts, Syrup And I njec t i o n	Syrup

4.0 CONCLUSION

The proposed spectrophotometric method is simple, cost-effective, and reliable for determining bromhexine hydrochloride in both pure and pharmaceutical forms. It offers significant advantages in terms of accuracy, precision, and ease of application.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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