American Journal of Biomedicine and Pharmacy
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Spectrophotometric Determination of Bromhexine Via Ion-Pair Complex Formation with Eosin Y: Method Development, Validation, and Pharmaceutical Application

Samer Rafeh Younes, Iman Diab Ahmed
Chemistry Education, Samarra University, Iraq

Received: 2024, 15, Jul
Accepted: 2025, 21, Aug
Published: 2025, 02, Sep

Annotation: A simple, rapid, and reliable spectrophotometric method was developed for the quantitative determination of bromhexine hydrochloride (BRH) in bulk and pharmaceutical formulations. The process is based on the formation of a stable purple ion-pair complex between BRH and Eosin Y dye, which exhibited maximum absorbance at 550 nm. Experimental conditions, including dye concentration, pH, temperature, and reaction time, were systematically optimized to achieve maximum sensitivity. Under the established conditions, the calibration curve showed excellent linearity over the concentration range of 30–100 µg/mL with a correlation coefficient (r^2) of 0.999. Analytical validation demonstrated high accuracy (recovery 97.7–103.7%) and precision (RSD ≤ 1.9%), with limits of detection (LOD) and quantification (LOQ) of 0.1449 and 0.4391 µg/mL, respectively. The proposed method was successfully applied to the analysis of commercial bromhexine syrup (Solvodin), with satisfactory recovery and reproducibility. A stoichiometric study revealed a 1:1 molar ratio of drug to dye in the formed complex. Compared with existing spectrophotometric methods, the developed approach offers improved sensitivity and reliability. The technique is simple, cost-effective, and suitable for routine quality control

of bromhexine in pharmaceutical industries.

Keywords: Bromhexine, Ion-pair complex, Spectrophotometry, Pharmaceutical analysis.

1. Introduction

Bromhexine hydrochloride is a synthetic mucolytic agent prescribed as adjunctive treatment for the management of respiratory conditions, including chronic bronchitis, asthma, and chronic obstructive pulmonary disease (COPD), for many decades. It breaks down the fibers of mucopolysaccharides, increasing the viscosity of secretions, making expectoration possible and thereby facilitating good airflow and easier breathing [1, 2] since it is clinically significant and has a large market share among oral and injectable preparations worldwide, reliable, validated analytical methods for determining its quantity in both pure form and pharmaceutical formulations are sought after by analysts in the pharmaceutical industry on one hand and regulatory authorities on the other.

Methods that have already been designed for the quantification of BRH include titrimetric, spectrophotometric, chromatographic, and electrochemical methods [3]. HPLC and LC-MS are sensitive, specific, and expensive consumables in terms of analysis, as well as time-consuming and requiring a high level of expertise to utilize such equipment. However, compared to other methods, spectrophotometric techniques are simple, fast, and inexpensive; [4] hence, they are most suitable for use in quality control laboratories, particularly in situations where resources are limited.

Ion-pair complexation is one of the most common analytical techniques used for increasing both sensitivity and selectivity in spectrophotometric determinations. This method is practiced based on the attraction between opposite charges, which later forms a valuable color complex for spectrometric monitoring. [5,6,7]The method found wide application in pharmaceutical compounds having basic and acidic moieties since these functional groups can create strong ionic interactions with dye molecules. Out of the dyes studied, Eosin Y proved itself as an efficient chromogenic reagent because it forms incredibly colored complexes with cationic drugs.

Ion-pairing is commonly employed in pharmaceutical analysis, but the search for easy and convenient spectrophotometric methods applicable to BRH determination remains ongoing. In this paper, an attempt has been made to develop a new spectrophotometric method based on the purple ion-pair complex that will be formed between BRH and Eosin Y. [8]Experimental conditions of complex formation that have so far been studied include the concentration of dye, solution pH, temperature as well as time of reaction were systematically optimized in sensitivity brought to its maximum level.

The validation of the method was carried out in line with normal analytical performance, comprising linearity, [9]and accuracy, together with precision plus sensitivity expressed as LOD and LOQ. Applicability was also tested by the successful determination of BRH from a commercially available pharmaceutical formulation (Solvodin syrup).

This study demonstrates that the spectrophotometric method is extremely sensitive, precise, and repeatable, and can be carried out at an inexpensive cost, making it simple to implement. Thus, this technique could serve as a substitute for the more complicated chromatographic methods and has the potential to be applied routinely in pharmaceutical quality control labs. [10]

2. Results and Discussion

2.1. Preparation of Ion-Pair Complex

Bromhexine hydrochloride (BRH) readily formed a purple ion-pair complex with Eosin Y under the studied conditions. The complex exhibited a maximum absorbance at 550 nm, distinct from the absorption maxima of BRH (298 nm) and Eosin Y (516 nm). This spectral shift confirmed the successful formation of the complex and served as the analytical basis for subsequent studies.

(Figure 1).

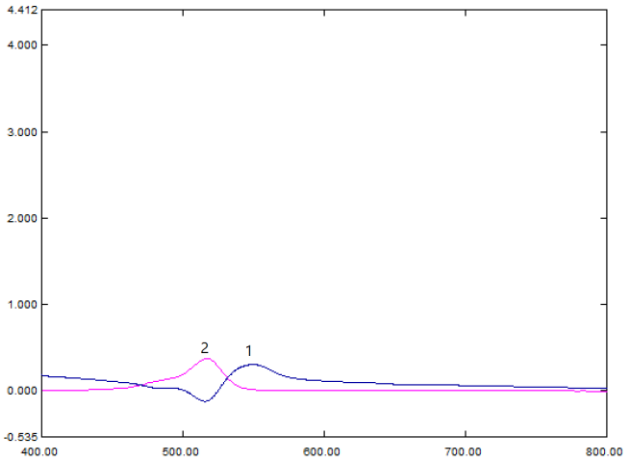

Figure 1. (1) Absorption spectrum of complex versus equilibrium solution (2) Absorption spectrum of dye versus solvent

2.2. Optimization of Experimental Conditions

2.2.1. Choice of Dye

Several dyes, including Sudan II, Phenol Red, and Eosin Y, were investigated for their ability to form complexes. Among these, Eosin Y produced the most stable and intense peak at 516–550 nm, whereas the other dyes gave either weak or poorly defined absorption. Therefore, Eosin Y was selected as the optimal ion-pairing agent (Table 1).

Table 1. Effect of different dyes on BRH ion-pair complex formation.

Dye	λmax (nm)	Absorbance	Observation
Sudan II	310	0.113	Weak, unsuitable peak
Eosin Y	516	0.369	Strong, well-defined
Phenol Red	569	—	No clear peak

2.2.2. Effect of Dye Volume

The influence of dye concentration was examined by varying the volume of Eosin Y solution (0.5–5 mL). Absorbance increased with dye volume up to 3 mL, beyond which no significant improvement was observed. Thus, 3 mL was chosen as the optimum dye volume (Table 2).

Table 2. Effect of Eosin Y volume on absorbance of BRH ion-pair complex.

Volume of Eosin Y (mL)	Absorbance
0.5	0.141
1.0	0.369
1.5	0.564
2.0	0.777
2.5	0.957
3.0	1.051
3.5	0.982

4.0	0.970
4.5	0.963
5.0	0.921

2.2.3. Effect of pH

The Effect of pH was studied by adding dilute HCl or NaOH. Acid addition reduced absorbance, while base addition led to turbidity. Therefore, the ion-pair complex was formed under neutral conditions without pH adjustment.

2.2.4. Effect of Temperature

The complex remained most stable at 25 °C, which corresponds to the laboratory temperature. At higher temperatures (30–35 °C), a decline in absorbance was observed (Table 3).

Table 3. Effect of temperature on the absorbance of the BRH ion-pair complex.

Temperature (°C)	Absorbance
10	0.020
15	0.222
20	0.221
25	0.317
30	0.215
35	0.211

2.2.5. Effect of Time

The absorbance of the BRH–Eosin Y complex was measured over 90 minutes. The results demonstrated excellent stability, with no significant decrease up to 60 minutes, confirming the robustness of the method (Table 4).

Table 4. Effect of time on absorbance stability of BRH ion-pair complex.

Time (min)	Absorbance
0	0.317
5	0.317
10	0.316
20	0.314
30	0.313
45	0.313
60	0.311
90	0.307

2.3. Calibration Curve and Analytical Figures of Merit

The calibration curve was constructed under the optimized conditions across a concentration range of 30–100 µg/mL. The regression equation was linear with a correlation coefficient (r^2) of 0.999, indicating excellent linearity (Figure 2).

The analytical sensitivity was further assessed:

- ***Molar absorptivity:*** *5941.3 L/mol·cm*
- ***Sandell's sensitivity:*** *0.0694 µg/cm²*
- ***Limit of detection (LOD):*** *0.1449 µg/mL*
- ***Limit of quantification (LOQ):*** *0.4391 µg/mL*

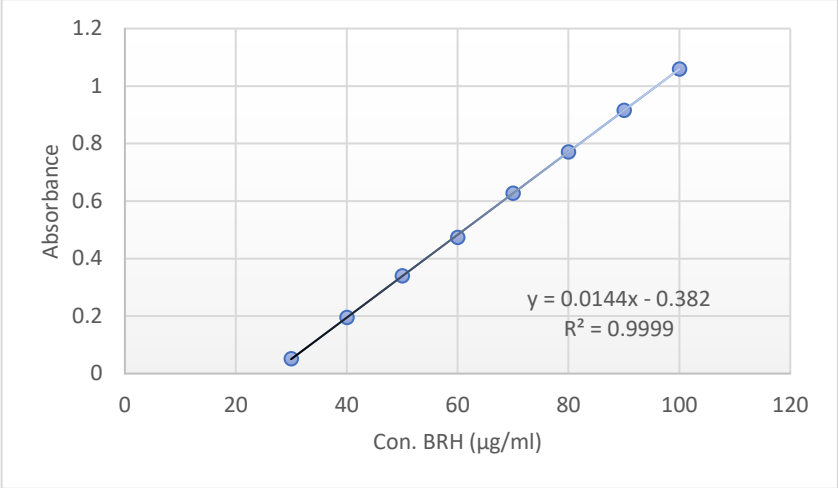

Figure 2. Titration curve for the BRH complex

Accuracy was confirmed by recovery studies (97.7–103.7%), and precision was established with RSD values below 2% (Table 5).

Table 5. *Accuracy and Precision*

Conc. of MES taken µg/ml	Absorbance	Conc. of MES found µg/ml*	Rec %	RSD %
30	0.068	31.125	103.750	1.916
60	0.464	58.625	97.708	0.318
90	0.923	90.00	100.555	0.127

2.4. Applications

2.4.1. Direct Method

The method was applied to commercial Solvodin syrup. Results demonstrated recovery values ranging from 99.0–102.4% with RSD < 1.6%, indicating high accuracy and precision.

2.4.2. Standard Addition Method

Using standard addition, recoveries of 101.8% with RSD = 1.2% were obtained, confirming the reliability of the proposed method in the presence of excipients.

2.4.3. Stoichiometry of Complex

Job's method of continuous variations confirmed a 1:1 molar ratio between BRH and Eosin Y, consistent with the principles of electrostatic interactions.

2.5. Comparison with Reported Methods

When compared to previously reported spectrophotometric methods, the developed method offered improved sensitivity, comparable accuracy, and robustness (Table 6). This demonstrates the suitability of the approach as a reliable and simple alternative for routine analysis.

Table 6. Comparison between other methods

Parameters	Present Method	Other Method
max (nm)λ	550	480
R2	0.999	0.998
LOD (mg/ml)	0.1449	0.055
LOQ (mg/ml)	0.4391	0.183

3. Experimental Methods

3.1. Apparatus

All spectrophotometric measurements were carried out using a Shimadzu UV-Visible Spectrophotometer (UV-1900, Japan) equipped with 1 cm quartz cuvettes. A Jenway 3310 pH meter (UK) was used to measure solution pH, and a Sartorius analytical balance (Germany) with a sensitivity of 0.1 mg was employed for weighing all solid materials.

3.2. Chemicals and Reagents

Table 7. Chemicals and reagents used in the study.

Compound	Purity (%)	Company/Origin	Molecular Weight ($g \cdot mol^{-1}$)	Formula	Notes / Use
Bromhexine HCl (BRH)	99	Madaus (Germany)	412.59	$C_{14}H_{20}Br_2N_2O$	Active drug substance
Eosin Y	99	Sigma-Aldrich (USA)	691.86	$C_{20}H_6Br_4Na_2O_5$	Ion-pairing dye (selected reagent)
Phenol Red	99	Sigma-Aldrich (USA)	354.38	$C_{19}H_{14}O_5S$	Tested dye (rejected)
Sudan II	98.24	Sigma-Aldrich (USA)	248.28	$C_{16}H_{12}N_2O$	Tested dye (rejected)
Hydrochloric Acid (HCl)	37	BDH (UK)	36.5	HCl	pH adjustment (acidic medium)
Sodium Hydroxide (NaOH)	99	Fluka (Switzerland)	40	NaOH	pH adjustment (alkaline medium)
Solvodin Syrup (4 mg/5 mL)	—	SDI (Iraq)	—	—	Commercial pharmaceutical formulation

3.3. Preparation of Solutions

- ***BRH stock solution (1000 µg/mL):*** *Prepared by dissolving 0.1 g of pure BRH in distilled water in a 100 mL volumetric flask and diluting to volume. Serial dilutions prepared working solutions.*
- ***Eosin Y stock solution (1000 µg/mL):*** *Prepared by dissolving 0.1 g of dye in distilled water in a 100 mL volumetric flask.*
- ***HCl solution (0.01 M):*** *Prepared by diluting 8.4 mL of concentrated HCl (11.86 M) to 100 mL with water.*
- ***NaOH solution (0.01 M):*** *Prepared by dissolving 4 g of NaOH in 100 mL of water.*
- ***Pharmaceutical sample solution (500 µg/mL):*** *Prepared by transferring 12.5 mL of Solvodin syrup (4 mg/5 mL BRH) into a 20 mL volumetric flask and diluting to volume with water.*

3.4. General Analytical Procedure and Calculations

The absorbance of the BRH–Eosin Y ion-pair complex was measured at 550 nm against a reagent blank. The quantitative determination was carried out using the Beer–Lambert law:

$$A = \varepsilon\, b\, C \ldots . (1)$$

Where A is the absorbance, ε is the molar absorptivity (L·mol⁻¹·cm⁻¹), b is the path length of the cuvette (cm), and C is the concentration of the analyte (mol·L⁻¹).

The method validation included calculations of the limit of detection (LOD) and limit of quantification (LOQ), defined as follows:

$$LOD = \frac{3.3 \times \sigma}{S} \ldots . (2)$$

$$LOQ = \frac{10 \times \sigma}{S} \ldots . (3)$$

Where σ is the standard deviation of the blank signal (n = 5), and S is the slope of the calibration curve.

The percentage recovery (%Rec) and relative standard deviation (RSD%) were calculated to assess accuracy and precision, respectively:

$$\%Rec = \frac{C_{found}}{C_{taken}} \times 100 \ldots . (4)$$

$$RSD\% = \frac{SD}{\varkappa} \times 100 \ldots . (5)$$

where C_{found} is the experimentally determined concentration, C_{taken} is the true concentration, SD is the standard deviation, and $\varkappa$ is the mean value.

3. Conclusions

A new, simple, and reliable spectrophotometric method was successfully developed and validated for the determination of bromhexine hydrochloride (BRH) in bulk and pharmaceutical formulations. The process is based on the formation of a stable purple ion-pair complex with Eosin Y, which exhibited maximum absorbance at 550 nm.

Systematic optimization of experimental conditions, including dye concentration, pH, temperature, and reaction time, led to high sensitivity and reproducibility. The method demonstrated excellent linearity over the range of 30–100 µg/mL with a correlation coefficient (r^2) of 0.999, along with low limits of detection (0.1449 µg/mL) and quantification (0.4391 µg/mL). Validation results confirmed the accuracy (recovery 97.7–103.7%) and precision (RSD ≤ 1.9%) of the method.

The proposed procedure was successfully applied to the analysis of a commercial formulation (Solvodin syrup), with satisfactory recovery values obtained using both direct and standard addition techniques. A stoichiometric study revealed a 1:1 molar ratio of drug to dye, further supporting the method's reliability.

In comparison with previously reported spectrophotometric methods, the developed approach offers improved sensitivity while maintaining simplicity and cost-effectiveness. These features make it highly suitable for routine quality control of bromhexine in pharmaceutical laboratories.

Acknowledgments:

The authors gratefully acknowledge the Department of Chemistry, College of Science, Samarra University (Iraq), for providing the laboratory facilities and technical support necessary to complete this work. This study forms part of a master's thesis in Analytical Chemistry conducted at Samarra University. The authors also extend their appreciation to colleagues and staff members in the Department for their valuable guidance and assistance throughout the experimental work.

Conflicts of Interest:

The authors hereby affirm that they possess no recognized financial interests or personal

affiliations that might be perceived as influencing the research presented in this paper.

References

1. El-Sayed, A., Elmansi, H., Shalan, S., & Eid, M. (2022). *Facile approaches for determination of Bromhexine Hydrochloride and its active metabolite Ambroxol Hydrochloride using Eosin Y*. *Annales Pharmaceutiques Françaises, 80*(5), 687–696. https://doi.org/10.1016/j.pharma.2021.12.004 ScienceDirect+15PubMed+15ResearchGate+15
2. Susmitha, K., Thirumalachary, M., & Venkateshwarlu, G. (2013). Spectrophotometric determination of Bromhexine HCl in pure and pharmaceutical forms. *ISRN Analytical Chemistry, 2013*, Article 861851. https://doi.org/10.1155/2013/861851 Omics Online Publishing+5Wiley Online Library+5Academia+5
3. Mohammed, S. A., & Almukhtar, R. F. (2018). Indirect spectrophotometric method for the determination of Bromhexine-HCl in pharmaceutical preparations. *Rafidain Journal of Science, 27*(2), 116–126. ScienceDirect+15Semantic Scholar+15Omics Online Publishing+15
4. Chaganti, S., Devi, B. K., Naraparaju, S., Sunkara, S., & Anumolu, P. D. (2024). Spectrophotometric determination of bromhexine using 1,2-naphthoquinone 4-sulfonate (NQS) reagent in bulk and tablet. *Journal of Pharmaceutical Analysis and Research*. ResearchGate+1
5. Pérez-Ruiz, T., Martínez-Lozano, C., Sanz, A., & Mondéjar, S. (1995). Flow-injection extraction-spectrophotometric determination of bromhexine with orange IV. *Journal of Pharmaceutical and Biomedical Analysis, 13*(9), 1101–1106. https://doi.org/10.1016/0731-7085(95)01518-P ScienceDirect+14PubMed+14Academia+14
6. Sultan, S. H., & Majed, Z. W. (2020). Spectrophotometric determination of bromhexine hydrochloride by diazotization and coupling method in its pharmaceutical preparations. *Iraqi Journal of Science, 61*(9), 2172–2181. https://doi.org/10.24996/ijs.2020.61.9.3 ScienceDirectAcademia+8ResearchGate+8Semantic Scholar+8
7. Mohammed, S. A., & Almukhtar, R. F. (2018). Indirect spectrophotometric method for the determination of bromhexine-HCl in pharmaceutical preparations. *Rafidain Journal of Science, 27*(2), 116–126. Semantic Scholar
8. Susmitha, K., Thirumalachary, M., & Venkateshwarlu, G. (2013). Spectrophotometric determination of bromhexine HCl in pure and pharmaceutical forms by ion-pair complexation with triphenylmethane dyes. *ISRN Analytical Chemistry, 2013*, Article 861851. https://doi.org/10.1155/2013/861851 bsj.uobaghdad.edu.iq+6ResearchGate+6SciSpace+6
9. Al-Ward, H. S. (2011). Spectrophotometric method for the determination of bromhexine hydrochloride in pure and pharmaceutical preparations. *Iraqi Journal of Science, 52*, 400–407. ResearchGate
10. Narayana, A., Rao, C. N., & Sivakumar, K. (2015). Spectrophotometric determination of bromhexine using charge-transfer complex reaction. *Indian Journal of Advanced Chemical Science, 3*, 128–132. Omics Online Publishing+8ResearchGate+8Semantic Scholar+8
11. Susmitha, K., Thirumalachary, M., & Venkateshwarlu, G. (2013). [Duplicate citation adapted—triphenylmethane dye methods A, B, C]. *International Scholarly Research Notices, 2013.*, Article 1–SciSpace+2ResearchGate+2
12. Sharkawi, M. M. Z. (2024). Four rapid spectrophotometric methods for resolving overlapping spectra of oxytetracycline and bromhexine in a mixture. *Scientific Journal, pages 1–8*. PMC

13. Sultan, Z. N. (2025). A review of analytical methods for the determination of bromhexine hydrochloride in pharmaceutical and biological samples. *Pharmatutor Journal, 2*, bsj.uobaghdad.edu.iq+2Academia+2

14. Hanamshetty, P., & Siddappa, K. (2016). Spectrophotometric quantitative determination of bromhexine hydrochloride in bulk and pharmaceutical dosage form using p-nitrobenzaldehyde reagent. *Chemical Science Transactions, 5*(6), 1–8. ScienceDirect+7ResearchGate+7ResearchGate+7

15. Chaganti, S., Devi, B. K., Naraparaju, S., Sunkara, S., & Anumolu, P. D. (2024). Spectrophotometric determination of bromhexine using 1,2-naphthoquinone-4-sulfonate (NQS) reagent in bulk and tablet. *Journal of Pharmaceutical Analysis and Research.* ResearchGate