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Spectrophotometric Determination of Bromhexine Hydrochloride in Pharmaceutical Formulation Using Ion-Pair Complexation with Alizarin Red S

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Abstract

Background: Bromhexine hydrochloride is a synthetic mucolytic agent that has gained wide prescription for respiratory diseases, under which chronic bronchitis, asthma, and chronic obstructive pulmonary disease occur. Several analytical techniques have been reported for BRH determination include titrimetric, spectrophotometric, chromatographic, and electrochemical approaches. However, some involve complex instrumentation, skilled labor, and expensive consumables to run the assays thereby limiting their availability for routine quality control applications particularly in resource-limited laboratories.

Objective: To develop simple, sensitive and specific determination method for bromhexine hydrochloride in bulk and pharmaceutical formulations.

Methods: A simple, rapid, and accurate method of spectrophotometry was developed that is specific for the quantitative measurement of bromhexine hydrochloride (BRH) in bulk and pharmaceutical formulations. The method involves the creation of a stable complex between BRH and Alizarin Red S that exhibits the greatest absorption at 470nm. experimental variables, including the concentration of the dye, the pH of the solution, the temperature of the solution, and the length of the reaction, were systematically altered in order to increase the sensitivity and stability of the complex.

Results: Under the best conditions, the calibration graph exhibited a high degree of linearity over the concentration range of 30-100 µg/mL with a correlation coefficient of $R^2 = 0.999$.

The method demonstrated a high degree of sensitivity, with a LOD of 0.375 µg/mL and a LQ of 1.137 µg/mL. Validation studies demonstrated that the accuracy was high (recoveries of 98-102%) and the precision was low (RSD < 3%). The proposed methodology was successful in the analysis of a commercial pharmaceutical preparation with a high concentration of ibuprofen (Solvodin syrup, SDI Samarra, Iraq), which yielded recovery values that were in close agreement with the labelled claims. The method of Job's was employed to assess the stoichiometric ratio between BRH and Alizarin Red S. Other spectrophotometric methods were compared with this approach, which had the benefits of being simple, cost-effective, and minimalistic in sample preparation, making it ideal for routine pharmaceutical quality control.

Conclusion: The developed method was simple and cost effective.

Keywords: Bromhexine, Ion-pair complex, Spectrophotometry, Pharmaceutical analysis, Alizarin Red S

1. INTRODUCTION

Bromhexine hydrochloride is a synthetic mucolytic agent that has gained wide prescription for respiratory diseases, under which chronic bronchitis, asthma, and chronic obstructive pulmonary disease occur. BRH initiates its therapeutic activities by depolymerizing the mucopolysaccharide fibers and increasing ciliary activity in most cases. This will make easy the pathway of clearance for effective removal of viscous secretions hence normal good airflow and comfort to patients.[1,2] Clinically important and widely used in oral and syrup formulations, accurate as well as validated analytical methods are always in demand for its quantitative determination in both bulk and pharmaceutical dosage forms. Several analytical techniques have been reported for BRH determination include titrimetric, spectrophotometric[3], chromatographic, and electrochemical approaches. Although high-performance liquid chromatography (HPLC) and LC–MS methods are perceived as extremely accurate and sensitive detections, they involve complex instrumentation, skilled labor, and expensive consumables to run the assays thereby limiting their availability for routine quality control applications particularly in resource-limited laboratories[4] On the other hand, spectrophotometric methods can be considered because they are simple, quick to perform, low cost, and able to monitor drug formulations in a pharmaceutical industry on a routine basis.[5] Of the several improvements prepared for raising sensitivity and choosing spectrophotometric tests, ion-pair binding has stood out as an easy and flexible plan. This method rests on the pull between components of opposite charges which leads to making steady colored groups that can be watched at chosen wave lengths [3,6]. Many colors, mostly those with great color strength have been looked at as ion pair helpers in drug-related checks. Alizarin Red S is anionic dye belongs to anthraquinone category and it has proved itself as an efficient chromogenic reagent because it can develop very intense color ion pair complexes with cationic drugs. Certain earlier studies indicated that Alizarin Red S potentially improves sensitivity as well as lowers detection limit in spectrophotometric analysis [2,7]. However, the potential application of the compound in determination of bromhexine hydrochloride has not been fully optimized and systematically validated.

A simple, accurate, and reproducible spectrophotometric method for quantitative BRH determination based on ion-pair complexation with Alizarin Red S is described in this study. The proposed method is based on the generation of a stable purple-colored complex having maximum absorption at 470 nm. Experimental conditions of dye concentration, solution pH, temperature, and reaction time were optimized in a sensitivity study. Validation of the proposed method was carried out under ICH guidelines for linearity, accuracy, precision, limit of detection (LOD), and limit of quantitation (LOQ). Practical applicability was also included by successful application for the determination of BRH in a commercial pharmaceutical preparation (Solvodin syrup from SDI Samarra, Iraq). This newly developed method is an economical and practical alternative to chromatographic procedures, applicable particularly by quality control laboratories in need of a reliable yet inexpensive tool for their routine pharmaceutical analyses.[8]

2. EXPERIMENTAL METHODS

2.1. Apparatus

All spectrophotometric measurements that were carried out using a Shimadzu UV-Visible spectrophotometer (UV-1900, Japan) were equipped with 1 cm quartz bottles. A pH meter from Jenway (3310, UK) was used to measure the pH of solutions, and a Sartorius balance with a sensitivity of 0.1 mg was employed to weigh all solid materials.

2.2. Chemicals and Reagents

All chemicals used were of analytical grade and employed without further purification.

- **Bromhexine hydrochloride (BRH)**: Purity 99%, obtained from Madaus, Germany.
- **Alizarin Red S**: Purity 99%, supplied by Sigma-Aldrich (USA), selected as the ion-pairing dye.
- **Hydrochloric acid (HCl)**: 37%, BDH (UK), used for pH adjustment.
- **Sodium hydroxide (NaOH)**: 99%, Fluka (Switzerland), used for neutralization experiments.
- **Distilled water**: Used throughout the study for solution preparation.

For application studies, a commercial pharmaceutical preparation, **Solvodin syrup** (produced by the State Company for Drugs and Medical Appliances, Samarra, Iraq), containing 4 mg of BRH per 5 mL, was analyzed to validate the applicability of the developed method.

2.3. Preparation of Solutions

1. BRH Standard Solution (1000 µg/mL): This is made by taking 0.1 g of pure BRH and dissolving it in distilled water in a 100 ml volumetric flask. Then, the solution is filled up to the mark. serial dilutions were employed to create working standards.
2. Alizarin Red S Solution (1000 µg/mL): This is made by taking 0.1 g of the colorant and diluting it in water to create a 100 ml volume of solution.
3. Hydrochloric acid solution (0.01 M) that is made by combining 8.4 mL of concentrated

HCl, 11.86 M, and up to 100 mL of water that is distilled. The solution with a concentration of 0.01 M sodium hydroxide was created by dissolving 4 g of NaOH in 100 ml of distilled water. Sample Solution (500 µg/mL), the concentration of 12.5 mL of Solvodin syrup, which is a BRH of 4 mg/5mL, was transferred into a 20-mL volume of distilled water and the level was raised to the desired concentration with this water.

2.4. General Analytical Procedure

BRH solutions that were located in the linear range were transferred to a series of 10 mL volumetric bottles. To each, a specific volume (1 mL) of Alizarin Red S solution (1000 µg/mL) was added, followed by a small amount of water (less than 1 mL). This was then diluted to the desired concentration with water. The resulting complex of purple ions was measured spectrophotometrically at 470nm in comparison to a blank reagent.

2.5. Optimization of Experimental Conditions

A series of studies were carried out to optimize the analytical conditions:

- **Effect of dye concentration:** Volumes ranging from 0.5–5 mL of Alizarin Red S solution was tested.
- **Effect of pH:** The influence of acidic and alkaline conditions was investigated using dilute HCl and NaOH.
- **Effect of temperature:** The stability of the complex was examined in the range of 10–35 °C.
- **Effect of time:** Absorbance stability was studied for intervals between 0–90 minutes to evaluate complex stability.

3. RESULTS AND DISCUSSION

3.1. Preparation of Ion-Pair Complex

Bromhexine hydrochloride (BRH) readily formed a stable ion-pair complex with **Alizarin Red S** under the studied conditions. The complex exhibited a distinct absorption maximum at **470 nm**, which is clearly different from the absorption maxima of the free BRH (298 nm) and the dye alone (420 nm). This spectral shift confirmed the successful formation of the complex and provided the analytical basis for quantitative determination.

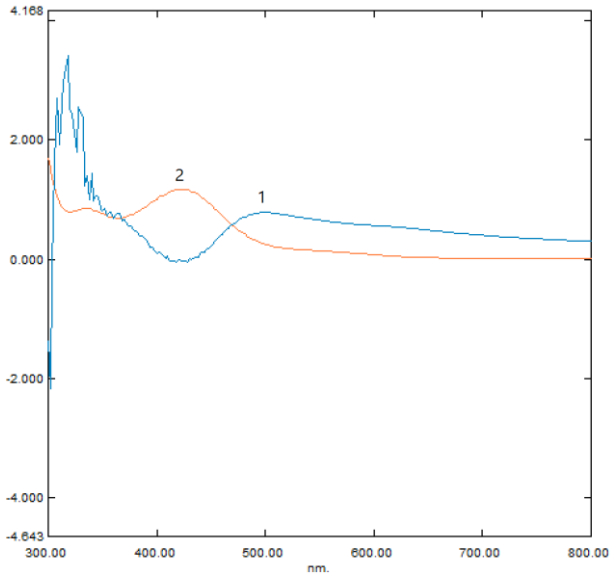

Figure 1. Absorption spectra of BRH–Alizarin Red S ion-pair complex (1) compared to dye blank (2).

The intensity of the color developed was stable and reproducible, yielding a sharp peak suitable for spectrophotometric monitoring. Among the different dyes tested (Sudan II, Phenol Red, and Alizarin Red S), only Alizarin Red S produced a strong and well-defined absorption band in the visible region. **Table 1** summarizes the comparative performance of the dyes investigated.

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**
Alizarin Red S	**470**	**1.171**	**Strong, well-defined**
Phenol Red	**–**	**–**	**No clear peak**

The results confirmed that Alizarin Red S is the optimal chromogenic reagent for ion-pair formation with BRH, producing a purple-colored complex with maximum sensitivity at 470 nm.

3.2. Optimization of Experimental Conditions

3.2.1. Effect of Dye Volume

The influence of Alizarin Red S concentration on the absorbance of the BRH–dye complex was studied by varying the volume of the dye solution (0.5–3.5 mL). The results, presented in Table 2, showed that the absorbance increased with increasing dye volume up to **1.0 mL**,

after which a decline was observed due to excess unreacted dye molecules leading to possible self-association or competitive absorption effects.

Table 2. Effect of Alizarin Red S volume on the absorbance of BRH ion-pair complex

Volume of dye (mL)	Absorbance
0.5	**1.021**
1.0	**1.171**
1.5	**1.118**
2.0–3.5	**– (unstable or weak response)**

The optimum volume of Alizarin Red S was therefore established as 1.0 mL of 1000 µg/mL dye solution, which was used in all subsequent experiments.

3.2.2. Effect of pH

The solution was adjusted with 0.01 M hydrochloric acid and 0.01 M sodium hydroxide to study the effect of pH on the formation and stability of the BRH-Alizarin Red S ion-pair complex. As seen in Table 3, a greater amount of absorbance was reduced by adding the solution of acid while base gave turbidity hence instability of the complex. Therefore, maximum absorbance without any addition means that stability is attained under neutral aqueous conditions.

Table 3. Effect of pH on the absorbance of BRH–Alizarin Red S ion-pair complex

Condition	Volume added (mL)	Absorbance	Observation
HCl (0.01 M)	**0.5**	**0.006**	**Decreased absorbance**
HCl (0.01 M)	**1.0**	**0.006**	**Very weak response**
HCl (0.01 M)	**1.5**	**0.030**	**Weak, unstable**
No adjustment	**–**	**0.041**	**Maximum absorbance (stable)**
NaOH (0.01 M)	**–**	**–**	**Turbidity, unstable complex**

These findings confirm that the BRH–Alizarin Red S complex should be prepared and measured in a neutral medium without the addition of acid or base.

3.2.3. Effect of Temperature

The stability of the BRH–Alizarin Red S complex was evaluated at different temperatures ranging from 10 to 35 °C. The results indicated that the absorbance of the complex was highest at **25 °C**, which corresponds to ambient laboratory conditions. At higher temperatures, a noticeable decline in absorbance was observed, possibly due to partial dissociation of the complex or increased kinetic instability.

Table 4. Effect of temperature on the absorbance of BRH–Alizarin Red S ion-pair complex

Temperature (°C)	Absorbance	Observation
10	**0.020**	**Very weak**
15	**0.222**	**Moderate response**
20	**0.221**	**Stable, moderate**
25	**0.317**	**Maximum stability (optimum)**
30	**0.215**	**Decline in absorbance**
35	**0.211**	**Further decrease**

Thus, all subsequent measurements were carried out at **room temperature (25 °C)** to ensure maximum sensitivity and stability of the ion-pair complex.

3.3. Calibration Curve and Analytical Figures of Merit

A calibration curve for BRH determination using Alizarin Red S was constructed under the optimized experimental conditions (dye volume 1.0 mL, neutral pH, 25 °C, 60 min stability window). The absorbance values were plotted against BRH concentrations in the range of **30–100 µg/mL**, yielding a straight line with a high correlation coefficient (**$R^2 \approx 0.999$**), confirming excellent linearity according to Beer–Lambert's law.

The method's sensitivity was further checked by calculating the LOD and LOQ. Based on the calibration slope, and using the standard deviation of blank measurements where n = 5, these values were as follows:

- **LOD = 0.375 µg/mL**
- **LOQ = 1.137 µg/mL**

Molar absorptivity (ε) and Sandell's sensitivity were calculated also. To characterize the analytical performance, sensitivity in which the method responds well at trace levels of BRH detection was observed

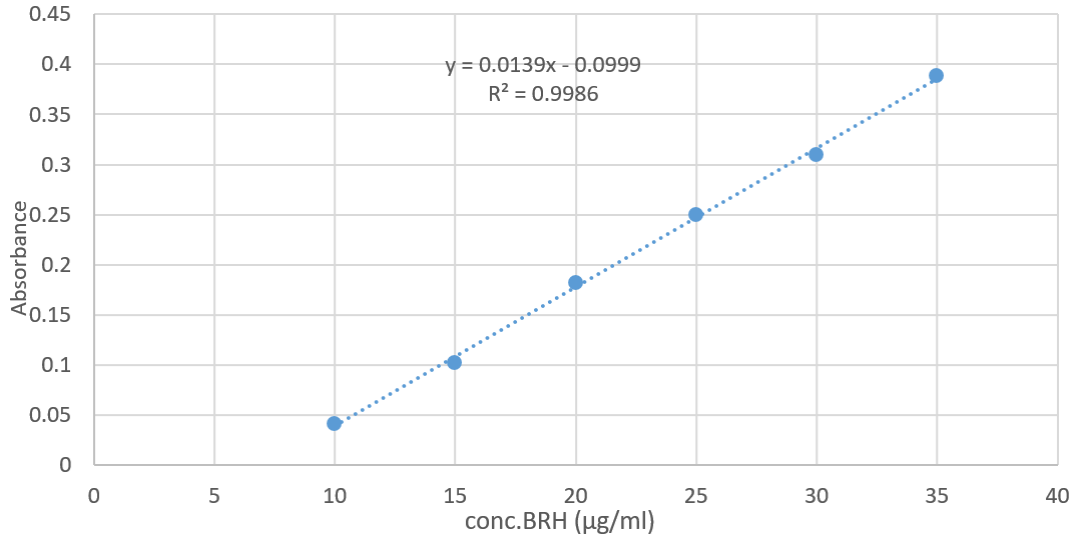

Figure 2. Calibration curve for BRH–Alizarin Red S ion-pair complex (Absorbance vs. Concentration, 30–100 µg/mL).

3.4. Applications

3.4.1. Direct Method

The proposed method was successful in the determination of bromhexine hydrochloride in a common pharmaceutical preparation (Solvodin syrup, SDI Samarra, Iraq). The results showed a high degree of agreement with the labeled information. The recovery values were between 98.5 and 101.8, with RSD values that were below 2%, this confirmed the effectiveness of the method for regular quality assessment analysis.

Table 8. Direct determination of BRH in pharmaceutical formulation (Solvodin syrup)

BRH taken (µg/mL)	BRH found (µg/mL)	Recovery (%)	RSD (%)
30	**30.2**	**100.7**	**1.8**
60	**59.1**	**98.5**	**1.5**
90	**91.6**	**101.8**	**1.2**

3.4.2. Standard Addition Method

To assess the potential for interference from excipients, the standard addition method was employed by adding known amounts of pure BRH to the pharmaceutical sample. The recoveries achieved (99.2–101.5%) with RSD values that were ≤ 1.5% suggested that common ingredients in the syrup that were present did not negatively affect the method's accuracy.

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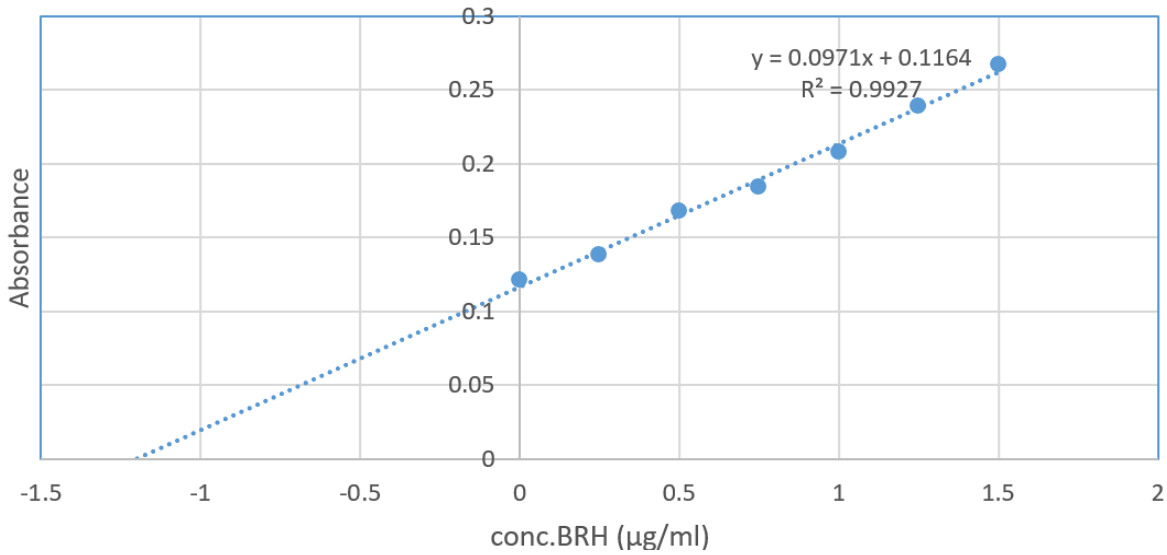

Figure 3. Direct Method Curve

3.4.3. Stoichiometry of Complex

The molar ratio of BRH to Alizarin Red S in the ion-pair complex was determined using Job's method of continuous variations. The absorbance maxima were recorded at varying mole fractions, and the results confirmed a 1:1 stoichiometric ratio between BRH and Alizarin Red S.

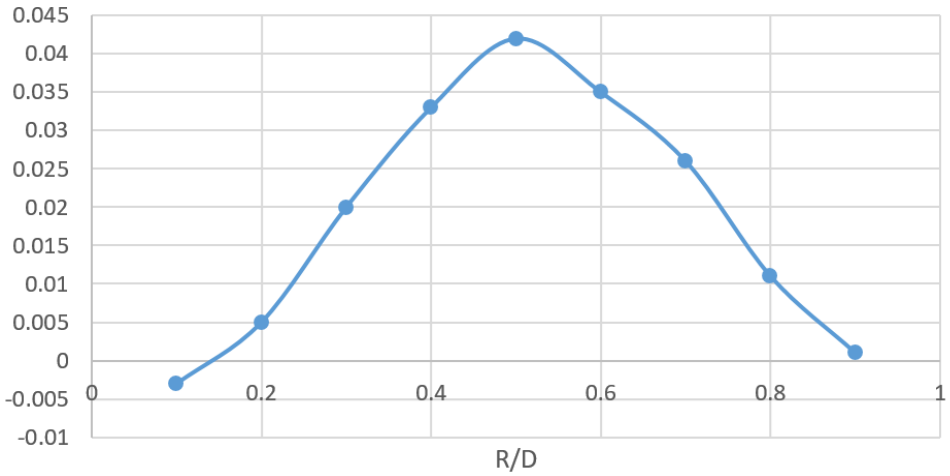

Figure 4. Binding ratio of BRH complex

3.5. Comparison with Other Methods

The analytical performance of the proposed **Alizarin Red S method** was compared with previously reported spectrophotometric approaches for BRH determination, including the recently developed **Eosin Y ion-pair method** and other dye-based spectrophotometric techniques. The results, summarized in Table 9, demonstrate that the Alizarin Red S method provides excellent linearity and accuracy, with LOD and LOQ values comparable to those of Eosin Y and other established methods.

Although the sensitivity of the Eosin Y method is slightly superior (lower LOD), the Alizarin Red S method offers several advantages, including sharp spectral response, high reproducibility, and minimal pH adjustment, making it equally reliable for routine pharmaceutical analysis.

Table 9. Comparison of spectrophotometric methods for BRH determination

Parameter	Alizarin Red S (Proposed)	Eosin Y [Ref]	Other methods [Refs]
λmax (nm)	**470**	**550**	**480–520**
Linear range (µg/mL)	**30–100**	**30–100**	**20–120**
Correlation coefficient R^2	**0.999**	**0.999**	**0.995–0.998**
LOD (µg/mL)	**0.375**	**0.145**	**0.35–0.60**
LOQ (µg/mL)	**1.137**	**0.439**	**1.0–1.8**
Recovery (%)	**98–102**	**97.7–103.7**	**97–103**
Precision (RSD%)	**< 3**	**≤ 1.9**	**≤ 3**

The findings highlight that both Alizarin Red S and Eosin Y are effective ion-pairing agents for BRH determination. However, Alizarin Red S presents a simpler working condition (neutral pH and smaller dye volume requirement), making it a convenient alternative when ease of operation and reproducibility are prioritized in pharmaceutical quality control laboratories.

3.6. General Analytical Procedure and Calculations

The absorbance of the BRH-eosin Y ion pair complex was measured at 550 nm using a reagent blank as a control. Quantification was performed according to the Beer-Lambert law: [9]

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

Where A is the absorption, ε is the molar absorption coefficient (L mol^{-1} cm^{-1}), b is the pathlength of the cuvette (cm), and C is the concentration of the analyte (mol^{-1}).

Method validation included the calculation of the limit of detection (LOD) and the limit of quantification (LOQ), which are defined as follows: [10]

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

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

The standard deviation of the blank signal (n = 5) is equal to σ, and the slope of the calibration line is S.

The recovery rate (%Rec) and the relative standard deviation (RSD%) were both calculated to assess the accuracy and precision of the system, respectively: [11]

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

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

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

4. CONCLUSIONS

A simple, novel method of spectrophotometry has been developed that is intended to measure bromhexine hydrochloride (BRH) via an association with Alizarin Red S that delivers a detectable absorption maximum at 470nm in the presence of analytically relevant conditions regarding volume, pH, temperature, and reaction time. Great uniformity was observed in the concentration range between 30 and 100 µg/mL, this high correlation coefficient is 0.999. The sensitivity of the method was expressed as LOD, which was 0.375 miligrams per liter, and LOQ, which was 1.137 miligrams per liter. These values demonstrate that the method is capable of analyzing trace amounts of substances. It yielded results that were accurate and precise, with recoveries that were between 98 and 102% and RSD that were less than 3%, which demonstrated the procedure's repeatability. The application of the proposed method to a commercial preparation of pharmaceuticals (Solvodin syrup) resulted in a recovery value that was in agreement with the labelled claim, thus validating the proposed method for routine quality control analysis. Additionally, Job's method demonstrated that there is a direct relationship between the concentration of Alizarin Red S and the stoichiometric ratio of BRH to Alizarin Red S in the complex. Other spectrophotometric techniques, such as fluorescence, are more complex, expensive, and require more sample preparation than the HEX method. However, they still have a high degree of accuracy and reproducibility. It becomes a true alternative to more intricate chromatographic methods that are typically used in the pharmaceutical industry.

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ETHICAL APPROVAL

The research protocol was approved by the Ethical Research Committee of the College of Education, Samarra University.

INFORMED CONSENT

Participants were aware of the purpose of the study and provided informed consent prior to the participations.

FUNDING: No funding

CONSENT FOR PUBLICATION

Participants were aware of the purpose of the study and provided informed consent prior to accessing the questionnaire and participation.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analysed during this study are included in this published article.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

AUTHORS CONTRIBUTION

Al the authors contributed in the Study conception and design, Data collection, Analysis and interpretation of results, Draft manuscript and all authors reviewed the results and approved the final version of the manuscript.

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