Spectrophotometric Assay of Bromhexine hydrochloride in Pure and in Pharmaceutical Dosage Forms by Diazometry Coupling Reaction

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ABSTRACT

A simple, accurate and sensitive spectrophotometric method has been developed for quantitative determination of bromhexine hydrochloride (BH) in both pure form and in its pharmaceutical preparations. The proposed method is based on the diazotization of BH with sodium nitrite in hydrochloric acid medium to form diazonium salt, and the reaction with 6,4,2-trihydroxybenzoic acid in a basic medium to form a stable yellow azo dye with maximum absorption at 408 nm. The calibration graph passed through the origin and the method is found to be accurate with correlation coefficient of 0.9973 and molar absorptivity of 3.053 × 10^4 l mol⁻¹ cm⁻¹. The relative error (RE) for bromhexine was found to be between 0.19 and 1.42% with relative standard deviation between 0.11± to 0.47± depending on the level of concentration. The detection limit (LOD) and quantitation limit (LOQ) of the method were found to be 0.0156 and 0.0520 µg ml⁻¹ respectively.

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which is coupled with 2,4,6-trihydroxy benzoic acid in alkaline medium of sodium hydroxide to form a stable and water-soluble azo dye exhibited absorption maximum at 408 nm against reagent blank. Beer’s law is obeyed over the concentration range of 5 to 750 μg of BH/20 ml with a good determination coefficient ($R^2=0.9973$) and apparent molar absorptivity $3.053 \times 10^4$ 1.mol$^{-1}$.cm$^{-1}$. The limit of detection (LOD) and limit of quantification (LOQ) are 0.0156 and 0.0520 μg ml$^{-1}$, respectively. The relative errors and relative standard deviations are found as $0.19 – 1.42$ % and $\pm 0.11$ to $\pm 0.47$% respectively, depending on the concentration level. The method is suitable for the determination of BH in the presence of other ingredients that are usually present in dosage forms. The composition of the resulting product has been also worked out and it is found to be (1:1) BH : 2,4,6-tri-hydroxybenzoic acid. This procedure is applied successfully for the analysis of BH in pharmaceutical preparations (tablets, syrup and injection) without prior separation and with acceptable errors.

**Keywords**: Bromhexine hydrochloride ; Diazotization ; 2,4,6-Trihydro-xybenzoic acid ; Spectrophotometry

**INTRODUCTION**

Bromhexine hydrochloride (BH), a synthetic benzyl amine derivative of vasicine, is a mucolytic used in the treatment of respiratory disorders associated with productive cough such as, cold and influenza infections. BH works through rendering the sputum less viscous thereby facilitating easy expulsion of it from the respiratory tract$^{1,2}$. It is chemically known as [N-(2-amino-3,5-dibromophenylmethyl)-N-ethylcyclohexylaminehydrochloride] with a molecular weight of 412.6 g/mol and a molecular formula C$_{14}$H$_{20}$Br$_{2}$N$_{2}$·HCl. BH has the following structure$^3$.

![Bromhexine hydrochloride](image)

A number of methods have been developed for the estimation of BH individually and also in combined forms along with other drugs which include reverse phase-HPLC$^4$, HPLC$^5$, Ion-selective electrode$^6$, TLC$^7$, capillary electrophoresis$^8$, voltammetry$^9$, potentiometric flow Injection analysis using conventional and coated wire ion-selective electrodes$^1$, and
high performance thin layer chromatography (HPTLC)\textsuperscript{10}, liquid chromatography electro spray ionization mass spectrometry\textsuperscript{11}, HPLC-ICP-MS compared with radiochemical detection\textsuperscript{12}. However, most of them suggest that quantification of BH in any matrix require elaborate and sophisticated instruments which may or may not be available in every laboratory, others were time consuming or required solvent extraction have high detection limit, as well as the most potentiometric methods used a bromhexine-selective electrode or other ion-selective electrodes which are either expensive or not readily available in the market, or involve difficult methods of fabrication.

Many UV-Visible spectrophotometric methods have been reported in the literature for analytical determination of BH. Most of them included diazotization of BH and then coupling with different coupling agents such as : pyrogallol\textsuperscript{13}, chromotropic acid\textsuperscript{14}, 1-naphthylamine\textsuperscript{15}. Other methods were either based on the formation of Schiff’s base reaction with p-dimeth-ylaminobenzaldehyde in the presence of SDS\textsuperscript{16}, ion pair complexation of BH, in acidic buffers, with triphenylmethane dyes\textsuperscript{17} or oxidation of 3-methylbenzothiazolinone-2-hydrazone (MBTH) by FeCl\textsubscript{3} followed by its coupling with BH in acidic medium\textsuperscript{18}. Also, oxidation-reduction\textsuperscript{19} and first derivative spectrophotometric methods have been used for determination of BH\textsuperscript{20}. However, some of these procedure suffer from various limitations such as , low stability of the coloured product formed\textsuperscript{15,poor sensitivity\textsuperscript{16,18}, laborious\textsuperscript{20}, have high detection limit\textsuperscript{14} and others require heating\textsuperscript{18,19} , extraction or require non-aqueous medium\textsuperscript{17}, long time for the reaction to complete , or applicable to higher concentrations of the drug\textsuperscript{13}.

In order to overcome the above limitations, it was thought worthwhile to develop simple, sensitive and accurate spectrophotometric method for the determination of BH based on the coupling of diazotized BH with a new coupling agent 2,4,6-trihydroxybenzoic acid to form a yellow coloured product in alkaline medium that has been proved successfully for the determination of BH in both pure form and its pharmaceutical preparations.

**EXPERIMENTAL**

**Apparatus**

All absorption spectra and absorbance measurements are carried out by a JASCOV- 630 double beam UV-visible spectrophotometer (Japan) with 1.0-cm quartz cells. The pH measurements are made with a professional HANNA pH meter 212.
Reagents

Bromhexine.HCl solution (100 μg / ml), is prepared by dissolving 0.01 g of BH in amount of distilled water and the volume is completed to 100 ml with the same solvent in a 100 ml volumetric flask. Working solution of BH is prepared by appropriate dilution of the stock solution with distilled water.

2,4,6-Trihydroxybenzoic acid (0.1%, w/v) solution, is prepared by dissolving 0.1 g of 2,4,6-tri hydroxybenzoic acid (Fluka) in 100 ml distilled water using a 100 ml volumetric flask.

Sodium nitrite (1%, w/v) solution, is prepared by dissolving 1.0 g of sodium nitrite (BDH) in 100 ml distilled water.

Sulphamic acid (Fluka) (3%, w/v) solution, is also prepared.

General procedure and calibration graph

To a series of 20 mL volumetric flasks in ice water, BH 5-750 μg and 1 ml of 1N HCl solution are transferred, followed by 0.8 ml of 0.1% sodium nitrite solution and mixed thoroughly. After 3 minutes, 1 ml of 3% sulphamic acid is added with occasional shaking for 1 minute, followed by 1.5 ml of 0.1% 2,4,6-trihydroxybenzoic acid solution and 2.5 ml of 1N sodium hydroxide solution are added. The flasks are kept at room temperature for 1 minute and the contents are diluted to the marks with distilled water and mixed well. The absorbance of the solutions are measured at 408 nm against the corresponding reagent blank.

Procedure for the assay of pharmaceutical preparations

For tablets. Five tablets of BH (each tablet contains 8 mg BH) are finely powdered, an accurately weighed of the powder equivalent to 0.01 g is dissolved in 2 ml of 1N hydrochloric acid and the residue is filtered into 100 ml calibrated flask and then the volume is completed to mark by repeated washing with distilled water. Each ml of this solution containing 100 μg of BH.

For syrup solution. A 12.5 ml of syrup (each 5 ml contain 4 mg BH) is transferred into a 100 ml calibrated flask and the total volume is diluted with distilled water.

For injection solution. This solution is prepared by diluted 4 ml of BH injection solution (each 2 ml contains 4 mg BH), with distilled water in 100 ml calibrated flask.

RESULTS AND DISCUSSION

Throughout the preliminary study on the reaction of BH with sodium nitrite in hydrochloric acid medium and after removal of the residual nitrite (as nitrous acid) with sulphamic acid, the formed diazonium salt is then coupled with 2,4,6-trihydroxybenzoic acid in a basic medium, an
intensely coloured water soluble azo dye is obtained which showed absorption maximum at 408 nm in contrast to the colourless reagent blank (Fig.1). The absorbance of the azo dye solution [it produce from mixing 1 ml of BH (100 ppm), 2 ml of 1N HCl, 0.8 ml of 1% sodium nitrite, 1 ml of 3% sulphamic acid, 1.5 ml of 2,4,6-trihydroxybenzoic acid and sodium hydroxide solution and diluted to 20 ml with distilled water] measured versus reagent blank.

Fig.(1) Absorbance spectra of the azo dye against (A) reagent blank (B) distilled water and (C) blank measured against distilled water.

The intensity of the formed dye has been found to be proportional to the amount of BH originally present in the solution.

The effect of various parameters on the absorption intensity of the coloured azo dye is investigated and the optimum reaction conditions have been selected.

The diazotization reaction of BH is formed in acidic medium. Therefore, the effect of different amounts of various acids (1N), such as: HCl, HNO₃, H₂SO₄, HCOOH and CH₃COOH is studied in diazotization of BH for the purpose of producing intense coloured dye and lower blank value. The results in fig.2 show that 2 ml of 1N HCl solution is the most suitable acidic because it gives high intensity for the dye with corresponding to the low reagent blank absorbance and is selected for the reaction.
Fig.(2) Effect of different amounts of various acids on the absorbance of azo dye

The effect of different amounts (0.2-1.2 ml) of 1% sodium nitrite and (0.0-2 ml) of 3% sulphamic acid on the absorbance of the resulting azo dye have been also studied. (Figures 3&4) show that 0.8 ml of 1% sodium nitrite solution and 1ml of 3% sulphamic acid with occasional shaking for 3 and 1 minutes for sodium nitrate and sulphamic acid respectively, are enough to obtain the maximum absorbance and they are recommended for the all subsequent experiments.

Fig.(3) Effect of the amount of NaNO₂ and time on the absorbance of azo dye

Fig.(4) Effect of sulphamic acid amounts and time on the absorbance of azo dye
Effect of 2,4,6-Trihydroxybenzoic acid amount and time

The effect of 2,4,6-trihydroxybenzoic acid amount on the absorbance of the azo dye has been studied. The results in table 1 indicate that 1.5 ml of 0.1% 2,4,6-trihydroxybenzoic acid is the more suitable to give the highest intensity value for the azo dye.

Table 1. Effect of 2,4,6-trihydroxybenzoic acid amount and time

<table>
<thead>
<tr>
<th>ml of coupling agent (1%)</th>
<th>Absorbance / µg of BH present in 20 ml</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>0.5</td>
<td>0.1178</td>
<td>0.2359</td>
</tr>
<tr>
<td>0.8</td>
<td>0.1011</td>
<td>0.2619</td>
</tr>
<tr>
<td>1.0</td>
<td>0.1390</td>
<td>0.2239</td>
</tr>
<tr>
<td>1.2</td>
<td>0.0712</td>
<td>0.1516</td>
</tr>
<tr>
<td>1.5</td>
<td>0.1848</td>
<td>0.3235</td>
</tr>
<tr>
<td>2.0</td>
<td>0.1332</td>
<td>0.2991</td>
</tr>
</tbody>
</table>

Effect of base

The coupling reaction of diazotized BH with 2,4,6-trihydroxybenzoic acid is formed in alkaline medium. Therefore, the effect of different amounts (1-5 ml) of 1N solutions of various bases such as: sodium hydroxide, sodium carbonate, sodium bicarbonate, potassium hydroxide and ammonium hydroxide have been examined for the purpose of producing intense coloured dye with lower blank values. The results in fig.5 indicate that sodium carbonate, sodium bicarbonate and ammonium hydroxide exhibit weak colour contrast which is apparently due to pH variation. Therefore, 2.5 ml of 1N sodium hydroxide at (pH=11.03) is recommended for the subsequent experiments.

![Fig.5](image_url)

Fig.(5) The effect of time and BH amount on absorbance

The development time and stability period on the absorbance of the coloured dye at 408 nm under the optimal experimental conditions have
been investigated. The experimental results showed that the coloured azo dye developed immediately after mixing for 1 min and the absorbance remained maximum and constant for at least 60 minutes at room temperature.

**Validity of Beer's law and reproducibility**

Under the optimum operating conditions, a linear calibration curve is obtained over the concentration range of 5-750 µg of BH in a final volume of 20 ml (Fig.6).

![Fig.(6) Calibration curve for BH determination](image)

Higher concentrations show a negative deviation from Beer's law. The apparent molar absorptivity of the azo dye has been found to be $3.053 \times 10^4$ l.mol$^{-1}$.cm$^{-1}$.

The reproducibility of the procedure is studied by the analysis of three series of solution (five identical samples for each series) having final BH concentration of 1.5, 5, 15 µg/ml. The results showed a relative standard deviation of 0.11, 0.47 and 0.3 %, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were found to be 0.0156 and 0.0520 µg/ml, respectively.

**Composition of the azo dye**

The stoichiometry of the reaction between the diazotized drug and 2,4,6-trihydroxybenzoic acid is studied under the established conditions using both continuous variation and molar ratio methods. The results obtained in both methods (Fig.7) reveal that the azo dye is formed by a 1:1 combining ratio of diazotized BH to 2,4,6-trihydroxybenzoic acid. The conditional stability of the azo dye formed is also studied, and it was found to be $1.11 \times 10^7$ M$^{-2}$.
R: 2,4,6-trihydroxybenzoic acid

Fig.(7). (a) Continuous variations and (b) molar-ratio plots for BH–2,4,6-
Trihydroxybenzoic acid

A reaction subsequent based on the above results is shown in scheme (1).
In order to test the efficiency and selectivity of the proposed method, the effect of the presence of some common pharmaceutical additives such as: starch, glucose, lactose, sorbitol and gum arabic that are usually present in dosage forms is studied by adding different amounts of foreign substances to 100 μg of BH. The results in table 2 indicate that there are no significant interferences produced by these foreign substances on the proposed procedure.

<table>
<thead>
<tr>
<th>Foreign compound</th>
<th>Recovery (%)* of 100 μg BH / μg foreign compound added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>Glucose</td>
<td>98.51</td>
</tr>
<tr>
<td>Starch</td>
<td>98.27</td>
</tr>
<tr>
<td>Lactose</td>
<td>98.87</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>98.75</td>
</tr>
<tr>
<td>Gum Arabic</td>
<td>97.84</td>
</tr>
</tbody>
</table>

* Average of five determinations

**Application of the method**

The proposed method is applied to assay six different pharmaceutical preparations containing BH (tablets, syrups and injection), table 3 show that a good recoveries are obtained.
Table 3. Determination of BH in pharmaceutical preparations.

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>μg BH present per 20 ml</th>
<th>μg BH found per 20 ml</th>
<th>Relative error (%)</th>
<th>Recovery (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvodin tablet</td>
<td>50</td>
<td>50.98</td>
<td><strong>-1.96</strong></td>
<td>101.96</td>
</tr>
<tr>
<td>8.0 mg BH / tablet (S.D.I.- Iraq)</td>
<td>100</td>
<td>95.99</td>
<td><strong>4.01</strong></td>
<td>95.99</td>
</tr>
<tr>
<td>Bisolvon injection</td>
<td>50</td>
<td>49.35</td>
<td><strong>1.30</strong></td>
<td>98.70</td>
</tr>
<tr>
<td>4mg /2ml</td>
<td>100</td>
<td>99.82</td>
<td><strong>0.18</strong></td>
<td>99.82</td>
</tr>
<tr>
<td>Boehringer Ingelheim, (Germany)</td>
<td>300</td>
<td>304.8</td>
<td><strong>-1.6</strong></td>
<td>101.60</td>
</tr>
<tr>
<td>Solvodin syrup</td>
<td>50</td>
<td>50.07</td>
<td><strong>-0.14</strong></td>
<td>100.14</td>
</tr>
<tr>
<td>4mg BH /5ml (S.D.I.- Iraq)</td>
<td>100</td>
<td>97.19</td>
<td><strong>2.81</strong></td>
<td>97.19</td>
</tr>
<tr>
<td>Bromonio syrup</td>
<td>50</td>
<td>50.05</td>
<td><strong>-0.10</strong></td>
<td>100.10</td>
</tr>
<tr>
<td>4mg BH /5ml (MEDPHARMA) Unated Arab Emarates</td>
<td>100</td>
<td>96.88</td>
<td><strong>3.12</strong></td>
<td>96.88</td>
</tr>
<tr>
<td>Salbid syrup</td>
<td>50</td>
<td>48.84</td>
<td><strong>2.32</strong></td>
<td>97.72</td>
</tr>
<tr>
<td>4mg BH /5ml (MICR LABS LIMITED INDIA)</td>
<td>100</td>
<td>97.68</td>
<td><strong>2.32</strong></td>
<td>97.68</td>
</tr>
<tr>
<td>Boehringer Ingelheim, (Germany)</td>
<td>300</td>
<td>289.20</td>
<td><strong>3.60</strong></td>
<td>96.40</td>
</tr>
</tbody>
</table>

* Average of five determinations.

Evaluation of the proposed method

According to the difficulties of using the standard method for determination of BH in its pharmaceutical preparation, so that a standard addition method has been used for its simplicity which proves that the proposed method is applied successfully for the determination of BH without interferences (Table 4 and Fig.8)

Table 4. The results of standard addition method

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>BH taken μg /20 ml</th>
<th>BH measured μg /20 ml</th>
<th>Recovery*, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Solvodin tablet (8.0 mg BH /tablet) (S.D.I. Iraq)</td>
<td>50</td>
<td>51.54</td>
<td>103.08</td>
</tr>
<tr>
<td>-Bisolvon injection (4 mg/2ml) (Boehringer Ingelheim) Germany</td>
<td>150</td>
<td>145.80</td>
<td>97.20</td>
</tr>
<tr>
<td>-Bromonio syrup (4mg BH /5ml) (MEDPHARMA) Unated Arab Emarates</td>
<td>50</td>
<td>50.70</td>
<td>101.40</td>
</tr>
<tr>
<td>8.0 mg BH / tablet (S.D.I.- Iraq)</td>
<td>150</td>
<td>147.85</td>
<td>98.57</td>
</tr>
</tbody>
</table>
Figs.(8) Graphs of standard addition method for the determination of BH in pharmaceutical preparations (tablet, syrup and injection)

CONCLUSION

It could be concluded that the developed methods for bromhexine hydrochloride assay is simple, sensitive (microgram amount can be determined), relatively precise, accurate and can be satisfactorily applied to the analysis of bromhexine hydrochloride in bulk and pharmaceutical formulations.

REFERENCES