Development of Spectrophotometric Method for Determination of Dopamine Hydrochloride in Bulk and Injectable Forms

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ABSTRACT

A precise, simple, cost-effective, specific and sensitive spectrophotometric method was developed for the assay of Dopamine HCl. The proposed method is based on generation of a colored complex through utilizing the known reaction of 2,6-dichloroquinone 4-chloroimide (DCQ) with phenols, primary and secondary amines. Dopamine HCl reacts with 2,6-dichloroquinone 4-chloroimide (0.12% solution in ethanol), in aqueous media at room temperature to produce a colored product that absorbs light at $\lambda_{\text{max}}$ 470 nm. All reaction conditions were optimized and standardized. The absorbance intensity of the colored product was linear with Dopamine HCl concentration in the range of (5 to 45 µg/ml). The correlation coefficient was found to be ($r=0.999$). The limit of detection was 2.5µg/ml. The stoichiometry of the reaction between Dopamine HCl and 2,6-dichloroquinone 4-chloroimide was studied, and revealed a 1:4 ratio of Dopamine HCl: DCQ respectively. The added recovery and standard addition approaches’ results were 99.67%±2.25 (n=6) and 99.09% for the former and later respectively, indicating the absence of interference.

Keywords: Spectrophotometry, Dopamine HCl, DCQ, injectable form

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INTRODUCTION

Dopamine HCl (DA HCl) structure is shown in figure 1. It is chemically named 4-(2-aminoethyl) benzene-1,2-diol hydrochloride. It is indicated for cardiogenic shock in infarction or cardiac surgery [1]. It is ineffective orally because it is a substrate for both monoamine oxidase (MAO) and Catechol O-methyl transferase (COMT). Thus, it is used intravenously. Methods used for analysis of Dopamine HCl injection include liquid Chromatography in official compendia B.P & USP [2-3], and flow injection analysis techniques [4-5-6]. Several other spectrophotometric methods were reported for determination of Dopamine HCl in dosage forms [7-8-9-10]. Most of these methods are either sophisticated, material or time consuming.

![Dopamine HCl molecular structure](image)

**Figure 1** Dopamine HCl molecular structure \([C_8H_{11}NO_2, HCl, M.Wt. 189.6]\)

Gibbs reagents (2,6-dichloroquinone-4-chloroimide and 2,6-dibromoquinone-4-chlorimide) are extensively used in colorimetric determination by generation of colored products. They react with phenols to produce dichloroindophenols or dibromoindophenols[11]. DCQ has also been used for detection of antioxidants, including phenolic types and a few primary and secondary amines [12].

Applications of these reactions using DCQ include determination of; Prenalterol hydrochloride [13], Leflunomide in the presence of its degradates [14], Propofol in bulk, dosage forms, and biological fluids [15], Captopril [16], and Methyldopa [17].

In this study DCQ was used for the colorimetric determination of Dopamine HCl in bulk and injectable form.

MATERIALS & METHODS

Reagents

Dopamine HCl working standard (assigned purity 99.83%), was supplied by the Central Medicines Supply (C.M.S-Sudan).

Distilled water is used all through, together with chemicals & reagents of analytical grade. Phosphate buffers of pH 3 and 7, and borate buffer were prepared according to the B.P.2010 procedure. DCQ reagent was freshly prepared by dissolving appropriate amount in absolute ethanol to prepare 0.1, 0.12, 0.24 % w/v solutions.
Pharmaceutical Formulation

Sterile Dopamine HCl concentrate BP [each ml contains Dopamine HCl BP 40 mg, water for injection BP q.s], manufactured by Claris Lifesciences Limited, Batch No. A080473, Mfg. date 07-2008, Exp. Date 06-2010, India.

Preparation of Standard Solution:

A weight of 0.0125 g of Dopamine HCl RS were dissolved in distilled water, transferred into 50 ml volumetric flasks, diluted to volume and mixed. Serial dilutions were made to obtain 0.18 mg/ml, 0.1 mg/ml and 0.09 mg/ml working solutions.

Experimental

All absorbance readings were measured using UV-1800 Shimadzu Spectrophotometer connected to hp Laserjet 1300 printer or Perkin Elmer UV/VIS Spectrometer lambda 2 connected to Perkin Elmer Ex-800 printer.

Standard Calibration Curves

To 0.5, 1.0, 1.5, 2.0, and 2.5 ml of the Dopamine HCl (0.18 mg/ml working solution), 2.0, 1.5, 1.0, 0.5, 0.0 ml of distilled water was added respectively. One ml of DCQ (0.12 %) was added, mixed, and reaction mixture allowed standing for 60 minutes. The volume was then completed to 10 ml using distilled water. Absorbance was measured at 470 nm against reagent blank prepared similarly. Calibration curves were constructed by plotting the absorbance readings at 470 nm versus concentrations of Dopamine HCl.

Pharmaceutical Preparation

4.5 ml (180 mg) from injection were pipetted and diluted to 50 ml with distilled water to obtain a solution of 3.6 mg/ml concentration (solution A). Solutions of concentrations of 1.8 mg/ml and 0.18 mg/ml were prepared from solution A through appropriate dilutions. Aliquots of the sample solutions were taken and prepared similarly as for standard calibration curves.

RESULTS AND DISCUSSION

The chromophoric system of the dopamine structure shows absorption maximum at 280 nm (Fig. 2). Sometimes it is useful to develop a colored species of the drug molecule containing reactive functional groups so as to produce a bathochromic shift. The bathochromic shift of the formed colored species will eliminate any irrelevant absorption exerted by pharmaceutical excipients. The selectivity of such a reaction could be a means of assaying the drug in presence of another non-reaction drug molecule or even a non-reacting structurally related substance (stability indicating property). Hence, in an attempt to increase the selectivity and prevent the interference effect, indirect spectrophotometric assay was proposed. Scanning of the colored
product obtained from the reaction of Dopamine HCl with DCQ (chromogen) was done against reagent blank in the range of 350 nm- 650 nm. The spectra showed a maximum absorption at 470 nm for Dopamine HCl and DCQ product (Fig.3). The value of A_{1%}^{1cm} calculated at 470 nm was found to be 104.36±4.15 (n=5). The absorbance intensity of the colored product was linear in the concentration range of 5 µg/ml to 45µg/ml (Fig.4). The correlation coefficient was found to be (r =0.999). The limit of detection was 2.5µg/ml.

Figure 2 UV spectrum of Dopamine HCl (36 µg/ml)

Figure 3 Visible spectrum of Dopamine HCl –DCQ colored product (45 µg/ml), showing the maximum absorbance at 470 nm

\[ y = 10204x + 0.030 \]
\[ r= 0.999 \]
Figure 4 Calibration curve for Dopamine HCl-DCQ complexes, at room temperature

<table>
<thead>
<tr>
<th>Regression data (n=5):</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A1% (slope)</td>
<td>102.04</td>
</tr>
<tr>
<td>$\varepsilon$ (slope)</td>
<td>1938.76</td>
</tr>
<tr>
<td>r (correlation Coefficient)</td>
<td>0.999</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.03</td>
</tr>
<tr>
<td>Range</td>
<td>5-45µg/ml</td>
</tr>
</tbody>
</table>

Application

The results of good linearity ($r=0.999$) and repeatability obtained, allowed the application of the proposed method for determination of Dopamine HCl in dosage form. The result of assay of Dopamine HCl injection (MYOTIL) was found to be 98.56±0.89% (mean± SD n=3) using double point standardization method, and was 99.5±4.4% (mean± SD n=3) using $A_{1\%}^{1cm}$ of standard and sample comparison. These results comply with the B.P 2010 and USP 30-NF25 specifications of content % of Dopamine HCl in injection (95-105%).

Influence of experimental variables

In order to find the best combination of factor levels to give the optimum response, several experiments were conducted.

Solvent effect

For studying the solvent effect, three solvents; protic (distilled water, absolute ethanol) and aprotic (acetonitrile) were used. The spectra showed absorbance maxima at 467, 469, and 470nm for acetonitrile, ethanol, and water respectively. Through analyzing the results, slight red shift on absorbance maxima with increase in polarity occurred indicating that the absorbance is mainly due to $\pi$ to $\pi^*$ transitions (the absorption maximum changed to a longer wavelength in a polar solvent (Red shift) [18]),while the intensities of the absorbance were affected to a little extent. In terms of safety, availability, and cost, distilled water was selected as the reaction’s solvent in this study.

pH effect

Another experimental factor is the pH of the reaction media. A concentration of 18 µg/ml of Dopamine HCl and three buffer solutions were used for this study. These were phosphate buffer of pH 3, and 7 and borate buffer of pH 9. These buffers gave either less sensitivity or distorted undefined peak with high blank reading. It was concluded that, the buffer solutions were not suitable for use in this reaction.
Concentration effect

The optimal concentration of DCQ that can give maximum color development was studied. A concentration of Dopamine HCl (36 µg/ml) was kept constant while reacting with three different concentrations of DCQ, 1 ml each of 0.1%, 0.12 %, or 0.24% DCQ solutions. The results show that, no significant changes of intensity of the colored product (absorbance reading) were observed upon increase of the DCQ concentration from 0.12% to 0.24%. The lowest concentration (0.1%) gave the lowest reading. The DCQ concentration of 0.12% was selected as an optimum chromogen’s concentration during generation of the colored product.

Temperature effect

Series of experiments were performed, in each of which the concentrations of the Dopamine HCl and the DCQ (at its optimum value 1 ml of 0.12%) were kept constant while the temperature was varied. In each case the absorbance readings were obtained for two concentrations of Dopamine HCl (18, and 36 µg/ml) at room temperature and at 90 °C. The results are shown in table 1.

**Table 1: Effect of temperature on the absorbance of the complex using constant concentration of DCQ (1 ml of 0.12% solution)**

<table>
<thead>
<tr>
<th>Tube’s no.</th>
<th>Concentration of DA HCl µg/ml</th>
<th>Temperature</th>
<th>Absorbance mean (n=3)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>90 °C</td>
<td>0.4235</td>
<td>± 0.074</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>90 °C</td>
<td>0.794</td>
<td>± 0.124</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>Room temperature</td>
<td>0.215</td>
<td>±0.0028</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>Room temperature</td>
<td>0.395</td>
<td>±0.0071</td>
</tr>
</tbody>
</table>

Despite the higher SD values obtained when applying heat at 90°C for 5 min, compared to those at room temperature, the results were considered encouraging for further investigation in order to increase the sensitivity, and speed up the reaction. Absorbance readings over the range of 4.5 to 22.5 µg/ml were studied. However, the results were not satisfactory, and imprecise (the absorbance of the lowest concentration 4.5 µg/ml showed an RSD of 20.14(n=4) compared to an RSD value of 2.69 (n=5) for 9 µg/ml. The correlation coefficient (r) at 90°C were 0.979 and 0.974 for standard and sample’s curves respectively (Fig.5), compared to 0.999 and 0.998 for standard and sample curves obtained at room temperature (Fig. 4).

Stability of the complex

The stability of the colored product was monitored for 90 min time using Dopamine HCl concentration of 18 µg/ml. The reaction rate was fast during the first 15 min. At zero time the absorbance was 0.047 and at 15 min it was 0.176. Absorbance increased slowly up to 45 min, and the readings were then stable during 45, 60, and 90 min. There was no significant difference on absorbance readings between 45, and 60 min. At 45 min absorbance was 0.192,
and at 60 min it was 0.215 (Fig. 6). The results at 60 min gave more reproducible results with RSD of about 1.32%. Therefore the time of 60 min was considered the optimal reaction time at room temperature.

Figure 5 Calibration curve for dopamine HCl –DCQ complexes, at 90˚C.

Figure 6 Profile of absorption Vs time at room temperature

Accuracy of the method

The accuracy of the method was evaluated through calculation of the Student-t-value at 95% confidence limit utilizing the formula [19];

\[ t = \frac{(\bar{x} - \mu) \sqrt{n}}{SD} \]

\[ t = \frac{(98.9-100) \sqrt{3}}{0.83} = 2.295 \]

Where;

- \( t \) = Student-t-value
- \( \mu \) = known mean (considering injection content as 100%)
- \( \bar{x} \) = mean content of the sample
- \( n \) = number of samples
- SD = standard deviation of the assay results

Results of evaluation of the accuracy of the method are presented in table 2.
Comparing the calculated t value with that obtained from the table [20], indicates that the developed method is accurate.

**Precision (Repeatability)**

The precision is given by the standard deviation of the repeatability of the method. It is determined from the results of 5 determinations of 3 different concentrations under the same analytical conditions. Results are listed in table 3.

<table>
<thead>
<tr>
<th>Conc. Of DA HCl µg/ml</th>
<th>Absorbance mean (n=5)</th>
<th>Mean ± SD</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.1218</td>
<td>0.1218±0.0033</td>
<td>2.69</td>
</tr>
<tr>
<td>27</td>
<td>0.303</td>
<td>0.303±0.0029</td>
<td>0.96</td>
</tr>
<tr>
<td>36</td>
<td>0.3996</td>
<td>0.3996±0.0069</td>
<td>1.75</td>
</tr>
</tbody>
</table>

The minimal SD of the mean of the 3 concentrations indicates that the results obtained by the present method are satisfactory.

**Interference**

The matrix effect was studied using two approaches namely standard addition method, and added recovery. The results obtained by both methods indicated absence of matrix effect. For standard addition method, the following regression equation was obtained; $y=0.0117x+0.2087$ ($r=0.998$) (Actual sample concentration= A/B= 17.84 µg/ml and recovery % = 99.09%) (Fig.7).The added recovery results (Table 4) further confirmed the absence of matrix effect.
Table 4: Results of added recovery

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Sample</th>
<th>Mixture</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance mean (n=6)</td>
<td>0.2037±0.0052</td>
<td>0.2185±0.0063</td>
<td>0.4215±0.0061</td>
<td>99.65</td>
</tr>
</tbody>
</table>

**Molar Ratio Method**

In order to study the stoichiometry of the reaction and determine the number of moles of the reactants consumed for Dopamine-DCQ complex formation, molar ratio method was used. In this experiment a concentration of 20.131X10^{-5} (M), 0.998X10^{-3} (M) of Dopamine HCl and DCQ respectively, were used and the absorbance readings were plotted against the molar ratio of DA HCl/ DCQ. (Fig.8). The molar ratio was found to be 1/4.

![Molar ratio curve (fixed DCQ concentration) showing 1 to 4 ratio (DA HCl / DCQ)](image)

Based on this ratio, the following scheme is suggested for the possible pathway of the reaction of Dopamine HCl with DCQ.
CONCLUSION

A simple, sensitive, cost-effective, and accurate, spectrophotometric method is described for the assay of Dopamine HCl in bulk and injectable form. The method is based on the generation of a brownish-orange colored product that absorbs at λmax 470 nm through the reaction of Dopamine HCl with DCQ in aqueous media at room temperature. The simplicity and cost-effectiveness of the developed method make it suitable for routine quality control analysis of Dopamine HCl especially for colored coated tablets.

ACKNOWLEDGEMENT

The authors would like to convey their thanks to Dr. Salah Abd Elrazeig for his valuable assistance and appreciable help, and to senior technician Mr. Ibrahim Ismail for technical support. Thanks are also extended to the Central Medicine Supply-Sudan for supplying Dopamine HCl standard and MYOTIL injections.

REFERENCES