Spectrophotometric Determination of Dopamine in Pharmaceutical Formulations by Reaction with Tyramine.

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INTRODUCTION

Dopamine (DA) \([ 2–(3,4\text{–} \text{dihydroxy} \ \text{phenyl}) \ \text{ethyl amine} ]\) is one of the catecholamines drugs that discovered in 1960 and was used as anti Glucoma and agonist \((1)\). Various methods have been reported for the determination of (DA). Rami Reddy. N and et al\((2)\) was developed spectrophotometric method for (DA) estimation based on the bromination of the (DA) with a solution of excess brominating mixture, after bromination, the excess brominating mixture is treated with potassium iodide to produce a yellow solution. Tayyebeh .M et al\((3)\) determination of catecholamines based on their oxidation reaction followed by coupling with 4-aminobenzoic acid. Markovic \((4)\) S and Amrain. S determined of (DA) with thiosemicarbazide .Nagaraja\((5)\) P, determined (DA) by reaction with chloramind T,and many other spectro -photometric methods\((6)\) .Wang and et al\((7)\) determined (DA) Fluorimetrically in pharmaceutical products and urine by using ethylene -diamine as fluorogenic reagent. Flow-injection spectrophotometric method use for determining (DA), Berzas Nevado, and et al \((8)\) used A
flow-injection spectrophotometric method for determining (DA) via reaction with metaperiodate. Nalewaja, E., and et al. (9) determined (DA) by flow injection analysis coupled with luminol-hexacyanoferrate III via chemiluminescence detection. Al-Abachi, M. Q., and Da, amy, determined Adrenaline and (DA) in pharmaceutical preparation via oxidative coupling reaction with thiourea and ferric nitrate. Also determined of (DA) with 3-Amino pyridine and sodium periodate. (DA) Injection was determined using flow injection-spectrophotometric by reaction of (DA) with P-toluidine and sodium Periodate. (12) Chromatographic methods have been reported for the determination of (DA) in various matrices. (DA) could be determined by liquid chromatography (LC) (13-15), gas chromatography (GC) (16), C-Mass (17) and Capillary Electrophoresis-Mass Spectrometry (18-19). Electro-analytical Techniques have been used extensively for the determination of (DA), Voltammetric (20), Electrochemical (21, 24) and Amperometric (25). In the objective of the investigation reported in this paper is to evaluate a spectrophotometric batch method for the determination of a (DA) based on its reaction with tyramine in the presence of potassium metaperiodate in neutral medium. A stable-soluble-orange color product was formed which can be measured at 475 nm. The method does not require temperature control or solvent extraction step. No previous published reports on the reaction mechanism have been appeared. The reaction scheme may be proposed for the (DA)-tyramine in the present potassium metaperiodate. The method was successfully applied to determination of (DA) in pharmaceutical formulations.

**MATERIALS AND METHODS**

The Dopamine pure drug was obtained from biological – Italy Company.

Dopamine stock solution (1000 µg.ml⁻¹):

0.1000 gm of Dopamine was dissolved in 10 ml of ethanol and completed the volume to 100 ml with deionized water in a volumetric flask of 100 ml.

Tyramine reagent (0.1 M):

Tyramine reagent standard was purchased from Samara Drug Company (Iraq).

Was prepared by dissolving 1.2108 gm in 100 ml of deionized water.

Potassium metaperiodate (0.1 M):

Potassium metaperiodate from Merck (Germany)

Solutions were prepared by dissolving 2.3000 gm of KIO₄ in 100 ml of deionized water. More dilute solutions were prepared by suitable dilutions.
Apparatus used
All spectral and absorbance measurements were carried out on a Shimadzu UV–visible 260 digital double beam recording spectrophotometer using 1 cm silica cell.

Into a series of 25 ml calibrated flask, transfer increasing volumes of Dopamine (10 µg.ml⁻¹). Add 1.5 ml of 1x10⁻¹ M of potassium metaperiodate solution, flowed by 3.5 ml of 5x10⁻² M of tyramine solution. Dilute the solution to the mark with deionized water and allow the reaction mixture to stand for 25 min at room temperature. Measure the absorbance at 472 nm against a reagent blank prepared in the same way but containing no Dopamine. The color of the formed dye is stable for about 120 min. For the optimization of conditions and in all subsequent experiments, a solution of 10 µg.ml⁻¹ Dopamine was used and the final volume was 25 ml.

RESULTS AND DISCUSSION
When a diluted aqueous solution of Dopamine and tyramine are mixed in the present of potassium metaperiodate in neutral medium, an intense orange color forms immediately and become stable after 25 min. The color has a maximum absorption at 472 nm. Fig (1) shows the spectra of the orange color formed and of the reagent blank. The above reaction can be utilized for the determination of Dopamine using spectrophotometric system. Initial studies were directed toward optimization of the experimental conditions, in order to establish the most favorable parameters for the determination of Dopamine. The influence of various reaction variables such as concentration of reactants, temperature, order of addition, and time of reaction were investigated. Experimental result showed that there was no effect in color intensity and stability on using different order of addition and the order of addition of reagents cited under recommended procedure was in further experiments. The effect of reagent (tyramine) concentration from 1x10⁻⁴ to 1x10⁻² M was studied and found the concentration of 6x10⁻³ M enough to developed the color to its full intensity and was chosen for subsequent studies. The effects of oxidant (potassium metaperiodate) concentration from 1x10⁻⁴ to 8x10⁻³ M was studied, the results obtained indicated that a concentration of 6x10⁻³ M gave the highest absorption and give a minimum blank value and was considered to be optimum for the further studies. The effect of reaction time indicated that the color intensity reached a maximum after a mixture of Dopamine solution containing 10 µg.ml⁻¹ in 0.0004 M potassium metaperiodate and 0.006 M tyramine in a neutral medium in final volume of 25 ml, had been reacted, the color develops during the first 25 min. and remains stable for more than 120 min.
The effect of temperature on the color intensity of the dye was studied. In practice, high absorbance was obtained when the color was developed at room temperature (25°C) than when the calibrated flask were placed in an ice–bath at (0°C) or in a water bath at (45°C). The calibration graph Fig(2) for the determination of Dopamine was constructed under the optimum conditions listed in Table (1). The regression equation have been obtained from a series of Dopamine standards, the analytical figures of merit of this procedure are summarized in Table 2.

The stoichiometry of the reaction was investigated using molar ratio method. The result obtained (Fig. 3) show that a 1:1 product was formed between Dopamine and tyramine reagent at 472 nm. Therefore the formation of the product probably occurs as follows:

\[
\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{OH} + \text{HO-}\text{C}_6\text{H}_4-\text{NH}_2 \rightarrow \text{Orangic soluble dye}
\]

The product formed was soluble in water. The apparent stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amount of Dopamine and tyramine in a neutral medium, with that of a solution containing a five-fold excess of tyramine reagent. The stability constant of the product in water under the describe experimental conditions were 3.177x10^3 L. mol\(^{-1}\)

**Analytical application**

The developed methodology is very adequate for the determination of Dopamine in aqueous solution and in pharmaceutical preparation samples at a concentration level of traces (ppm), and without requiring neither any previous separation step nor a temperature or pH control. Moreover the proposed procedures are very economical when compared to other methods such as those based on the use of another
instrumental analysis such as GC, HPLC and etc. Sample preparation was done by diluting the ampoules with deionized water.

**Accuracy and precision**

The accuracy and precision of the method was evaluated by analyzing pure sample of Dopamine. A good recovery was obtained (Table 3). Finally the proposed method was applied successfully to the analysis of some ampoules containing Dopamine. The results in Table 3 are in accordance with those obtained by the official method (26).

**Table -1: Optimum conditions for the determination of Dopamine.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. of tyramine</td>
<td>6x10^{-3}M</td>
</tr>
<tr>
<td>Conc. of potassium metaperiodate</td>
<td>6x10^{-3} M</td>
</tr>
<tr>
<td>Time on the stability of complex</td>
<td>120 min.</td>
</tr>
<tr>
<td>Temperature</td>
<td>Room temp.</td>
</tr>
<tr>
<td>wavelength</td>
<td>472 nm</td>
</tr>
</tbody>
</table>

**Table -2: Analytical feature of the procedures developed for the determination of Dopamine.**

<table>
<thead>
<tr>
<th>parameter</th>
<th>Batch method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression equation</td>
<td>Y=0.0161X-0.001</td>
</tr>
<tr>
<td>Linear range (µg.ml^{-1})</td>
<td>0. 5 – 20</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9999</td>
</tr>
<tr>
<td>Limit of detection (s/n=3) (µg.ml^{-1})</td>
<td>0.28</td>
</tr>
<tr>
<td>RSD% for 10 µg.ml^{-1}</td>
<td>1.03</td>
</tr>
<tr>
<td>Recovery % for 10 µg.ml^{-1}</td>
<td>99.65</td>
</tr>
<tr>
<td>Molar absorptive (L.mol^{-1}.cm^{-1})</td>
<td>3.177x10^{3}</td>
</tr>
<tr>
<td>λ_{max} (nm)</td>
<td>472</td>
</tr>
</tbody>
</table>
Table -3: Application of the proposed methods to the determination of Dopamine in ampoules

<table>
<thead>
<tr>
<th>Drug sample</th>
<th>Amount of drugs</th>
<th>Batch method</th>
<th>OFFICIAL Method(^{(26)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Recovery(^*) %</td>
<td>RSD(^*) %</td>
</tr>
<tr>
<td>Pure Dopamine</td>
<td>10 µg.ml(^{-1})</td>
<td>98.84</td>
<td>1.09</td>
</tr>
<tr>
<td>Ampoules Dopamine</td>
<td>10 µg.ml(^{-1})</td>
<td>101.34</td>
<td>1.16</td>
</tr>
<tr>
<td>Ampoules Dopamine</td>
<td>25 µg.ml(^{-1})</td>
<td>98.74</td>
<td>1.24</td>
</tr>
</tbody>
</table>

* Average of five determination

Fig -1: Absorption spectra of Dopamine treated as described under procedure and measured against reagent blank and B the reagent blank measured against deionized water.
A Practical, reliable, Simple analytical procedures using Spectrophotometer has been described for the quantitative determination of pharmaceutical injections contain (DA). The procedures described in this research no needs the elaborate treatment and tedious extraction or pH control.

REFERENCES
3-Tayyebeh Madrakian, Abbas Afkhami; Lida Khalafi; Massoumeh Mohammad nejad Spectrophotometric determination of catecholamines based on their oxidation reaction followed by coupling with 4-aminobenzoic acid. Journal of the Brazilian Chemical Society 17 (7) So Paulo Nov./Dec. 2006


