A new colorimetric method for determination of procaine in pharmaceutical preparations via oxidative coupling organic reaction

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Abstract :
A new and sensitive colorimetric method for determination of procaine in pure and pharmaceutical preparations has been developed. It is based on coupling the nucleus of procaine molecule which is p-amino benzoic acid(PABA) with promathazine HCL in the presence of sodium periodate as oxidizing agent in an acidic medium. The product stable green-bluish coloured has a \( \lambda_{\text{max}} = 610 \text{nm} \). The calibration curve is liner over the range of \((0.4-18 \ \mu g . ml^{-1})\) of p-amino benzoic acid with a molar absorptivity of \(1.718 \times 10^4 \ \text{l.mole}^{-1}.\text{cm}^{-1}\), a sandal sensitivity of \(0.0079 \mu g.cm^{-2}\), a relative error of \((-1.15)-2.26\%\) and a relative standard deviation of \((0.24-1.45\%)\) depending on the concentration of p-amino benzoic acid. The proposed method is applied satisfactorily to pharmaceutical preparations containing procaine.

Introduction :
Procaine is 2-diethylaminoethyl4-aminobenzoate hydrochloride.it is a colorless crystals or a white crystalline powder and odorless.it is used as a local anesthetic available as injection procaine penicillin and has the following formula.

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{COOCH}_2\cdot\text{CH}_2\cdot\text{NET}_2\cdot\text{HCl} \\
M. \text{Wt} & = 272.8 \text{ gm. Mole}^{-1}
\end{align*}
\]

Too many various methods has been reported for the determination of procaine in biological fluids and pharmaceutical preparations. These methods are spectrophotometric(2,3), flow injection analysis(4), atomic absorption spectrophotometric(5), fluorometric(6), ion association titration(7), and micellar liquid chromatography(8). Recently oxidative coupling organic reactions seem to be the most popular methods for trace determination of many drugs such as paracetamol(9), folic acid(10),...
phenylephrine\textsuperscript{(11)}, methyl dopa\textsuperscript{(12)}. The present paper reports a new indirect colorimetric method for the determination of procaine in pharmaceutical preparations depend on using p-amino benzoic acid as nucleus for procaine molecule. The method was applied successfully for analysis of procaine in pharmaceutical preparations.

**Experimental:**

**Apparatus.**

All spectral and absorbance measurements were carried out on Shimadzu UV-Visible 260 digital double beam recording spectrophotometric using 1cm glass cells.

**Reagents.**

All chemical used were of analytical reagent grade unless otherwise stated procaine Hydrochloride standard powder materials was provided from fluka chemical company.
- procaine penicillin injection was provided from the state company for drugs industries and medical appliances sammara (SDI).
- procaine stock solution (172.3µg.ml\textsuperscript{-1}):
  0.0198 gm of procaine hydrochloride was dissolved in 5ml of ethanol and make up To 100ml with distilled water.
- p-amino benzoic acid (100µg.ml\textsuperscript{-1}):
  0.01 gm of p-amino benzoic acid was dissolved in 5ml of ethanol and make up To 100ml with distilled water.
- promethazine hydrochloride (5x10\textsuperscript{-3}M):
  Prepared by dissolving 0.1602 gm in 100 ml of distilled water.
- sodium periodate (0.01M):
  Prepared by dissolving 0.3128 gm in 100ml of distilled water.
- hydrochloric acid(1M).

**Recommended procedure.**

Into a series of 25ml volumetric flasks transfer increasing volumes of p-amino benzoic acid(100µg.ml\textsuperscript{-1}) to cover the range of calibration curve(0.4-18µg.ml\textsuperscript{-1})
Of p-amino benzoic acid.Added 2ml of 0.01M of sodium periodate and shake well, 2ml of 5x10\textsuperscript{-3}M promethazine hydrochloride and 1ml of 1M HCL.Dilute the solution To the mark with distilled water and allow the reaction mixture to stand for 15min at room temperature. Measure the absorbance at 610nm against blank solution prepared in the same way but containing no p-amino benzoic acid.The colour of the product formed is stable for More than 80min.For the optimization conditions and in all subsequent experiments 100µg.ml\textsuperscript{-1} solution of p-amino benzoic acid (procaine nucleus) was used and the final volume was 25ml.

**Procedure for pharmaceutical preparations:**

**Procaine penicillin(120.4mg procaine):**

One vial content of procaine penicillin was dissolved in 5ml of ethanol And make up to 100ml of distilled water to obtain 1204µg.ml\textsuperscript{-1} of procaine using a 100ml volumetric flasks.14.3ml from the above solution was transferred and diluted To 100ml with distilled water to obtain 172.3µg.ml\textsuperscript{-1} of procaine that is equivalent To 100µg.ml\textsuperscript{-1} of p-amino benzoic acid.Use0.5ml and 2ml aliquots of the last solution(172.3µg.ml\textsuperscript{-1}) for colour formation with promethazine hydrochloride and sodium periodate in acidic medium as described under calibration.

**Results and discussion:**

**Absorption spectra:**

When a diluted aqueous solution of p-amino benzoic acid was mixed with sodium periodate and promethazine hydrochloride in acidic medium an intense green-bluish colour was formed.
immediately, which become stable after 10 min. The green-bluish product has a maximum absorption at 610 nm, in contrast to the reagent blank, which shows at λ_{max}=610 nm. Fig(1) shows the spectra of the green-bluish formed product and of the reagent blank. The maximum absorption at 610 nm was used in all subsequent experiments.

![Absorption spectrum](image)

**Optimum reaction conditions:**

The effect of various parameters on the absorption intensity of the formed product were studies and the reaction optimized, using p-amino benzoic acid as a nucleus of procaine molecule.

**Effect of reagent concentration:**

When various concentration of promethazine hydrochloride solution were added to a fixed amount (4 µg .ml⁻¹) of p-amino benzoic acid, 2-3 ml of 5x10⁻³ M was found enough to develop the colour to its full intensity giving minimum blank value and was considered to be optimum for the concentration range of 0.4-18µg.ml⁻¹ of p-amino benzoic acid, as shown in Fig.2.

![Reagent concentration effect](image)

**Effect of sodium periodate concentration:**

When p-amino benzoic acid and promethazine hydrochloride were mixed together no colour was observed but after addition of sodium periodate and hydrochloric acid a green-bluish soluble product formed immediately and become stable after 10 min for a period of 80 min. The product formation reached a maximum with about 2-5 ml of 0.01 M of sodium periodate and remained at
this time maximum when 2.5ml of sodium periodate were added to p-amino benzoic acid. 2ml of sodium periodate (0.01M) solution was considered to be optimum and was used in all subsequent experiment. (Fig.3)

**Fig(3): Effect of oxidizing agent concentration**

**Effect of reaction time:**

The colour intensity reached a maximum after p-amino benzoic acid has been reacted with promethazine hydrochloride and sodium periodate in acidic medium for 10-15min. Therefore, a 15min development time was selected as optimum in the general procedure. The colour obtained was stable for 80min. (Fig.4)

**Fig(4): Effect of reaction time**

**Effect of order of addition:**

To obtain optimum results the order of addition should be followed as given under the procedure, otherwise a loss in colour intensity was observed.

**Effect of temperature:**
The effect of temperature ($25^0C$) on the colour intensity of the product was found enough to develop the colour to its full intensity giving minimum blank value. Otherwise a loss in the absorbance was observed when the calibration flasks was placed in an ice-bath at $0^0C$ or in a water bath at $50^0C$. (Fig.5). Therefore, it is recommended that the reaction should be carried out at room temperature.

Fig(5): Effect of temperature

**Applying calibration curve:**

The condition described under the procedure, a liner calibration curve of p-amino benzoic acid is obtained (Fig6), which shows that beer's law is obeyed over the concentration range $0.4-18 \mu g \cdot ml^{-1}$ of p-amino benzoic acid with a correlation coefficient of 0.9992 and intercept of 0.0355. The conditional molar absorptivity of the green-bluish formed product was found to be $1.718 \times 10^4 L\cdot mole^{-1}\cdot cm^{-1}$ with reference to p-amino benzoic acid and sandell sensitivity of $0.0079 \mu g\cdot cm^{-2}$. Calibration curve of procaine was prepared with the calibration curve of (PABA) in the range $0.4-18 \mu g\cdot ml^{-1}$ of PABA. Therefore this range was used for indirect determination of procaine.

Fig(6): Calibration Curve of PABA

**Accuracy and precision:**

To determine the accuracy and precision of the method, p-amino benzoic acid was determined at the different concentration. The results obtained are shown in table (1). Indicates a satisfactorily precision and accuracy could obtained with the proposed method.

<table>
<thead>
<tr>
<th>Amount of PABA ($\mu g\cdot ml^{-1}$)</th>
<th>% Recovery</th>
<th>% R.S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>1.97</td>
<td>98.84</td>
</tr>
<tr>
<td>2</td>
<td>99.86</td>
<td>1.45</td>
</tr>
<tr>
<td>8</td>
<td>102.26</td>
<td>0.41</td>
</tr>
<tr>
<td>16</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

* For five determination

**Stoichiometric of the product:**

The stoichiometric of the reaction between p-amino benzoic acid and promethazine hydrochloride usingjobs method. The results obtain (Fig7) showed that a (1:1) product is formed between p-amino benzoic acid and promethazine hydrochloride reagent at 610nm. Therefore, the formation of the product may probably occurs as follows (13).
The product formed is soluble in water. The stability constant is calculated by comparing the absorbance of a solution containing stoichiometric amount of p-amino benzoic acid and promethazine hydrochloride with that of a solution containing a five-fold excess of promethazine hydrochloride reagent. The average conditional stability constant of the product in water under the described experimental condition is $2.21 \times 10^4 \text{L.mole}^{-1}$. The result obtained are shown in table(2)\textsuperscript{14}.

\[ R = \text{COOCH}_2\cdot \text{CH}_2\cdot \text{NEt}_3. \]
Calculation the stability constant (K) and dissociation degree (α) of the formed product

<table>
<thead>
<tr>
<th>( A_m )</th>
<th>( A_s )</th>
<th>( \alpha = A_m - A_s / A_m )</th>
<th>( \alpha_{\text{Average}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.584</td>
<td>0.181</td>
<td>0.690</td>
<td></td>
</tr>
<tr>
<td>0.580</td>
<td>0.176</td>
<td>0.696</td>
<td></td>
</tr>
<tr>
<td>0.582</td>
<td>0.180</td>
<td>0.690</td>
<td></td>
</tr>
</tbody>
</table>

\( A_m \): the absorbance of solution containing a five-fold excess of reagent.

\( A_s \): the absorbance of solution containing stoichiometric amount.

\( \alpha \): dissociation degree of product.

\[ K = 1 - \alpha / (\alpha^2) C \quad (\text{Conc in final volume } 25 \text{ ml}) \]

\[ K = 2.21 \times 10^4 \text{ L.mole}^{-1} \]

Analytical application:

One vial containing (procaine penicillin) has been analyzed using the calibration curve of p-amino benzoic acid in the range 0.4-18µg.ml\(^{-1}\) giving a good accuracy and precision (table 3).

<table>
<thead>
<tr>
<th>Drug samples</th>
<th>Wt of procaine penicillin (mg)</th>
<th>Wt of procaine (mg)</th>
<th>% Recovery*</th>
<th>% R.S.D*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procaine penicillin (SDI)</td>
<td>300</td>
<td>120.4</td>
<td>118.68</td>
<td>98.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>117.94</td>
<td>97.95</td>
</tr>
</tbody>
</table>

* For five determination

Conclusions:

The present method is proposed for determination of procaine to be used in quantity analysis of the pharmaceutical preparations. The method is based on the oxidative coupling of procaine with promethazine hydrochloride in the presence of sodium periodate in an acidic medium (HCL). It has several advantages, the procedure allow elimination of the interferences of other components present in the sample, need neither temperature control or solvent extraction step. Therefore, the proposed method is considered to be suitable for the routine analysis.

References: