Spectrophotometric Determination of Paracetamol by Coupling Reaction

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ABSTRACT

Simple, rapid, accurate and sensitive spectrophotometric method has been developed for the quantitative determination of paracetamol in both pure and its dosage forms. The method is based on diazotization of primary amine group of procaine hydrochloride with sodium nitrite and hydrochloric acid followed by coupling with paracetamol in alkaline medium of sodium hydroxide to form coloured azo dye shows a maximum absorption at 420 nm against reagent blank solution.

Beer's law is obeyed over the concentration range of 0.5-20 ppm with a determination coefficient ($R^2=0.9907$) and molar absorptivity $1.31 \times 10^6$ L.mol$^{-1}$.cm$^{-1}$ and a relative error in the range of 0.4% and a relative standard deviation from $\pm 0.456 \%$. The method is suitable for the determination of paracetamol in the presence of other ingredients that are usually present in dosage forms.

The composition of the resulting product has also been worked out and it is found to be 1:2 paracetamol: procaine hydrochloride. The method has been successfully applied to the determination of paracetamol in its pharmaceutical preparations.

Keywords: Spectrophotometric Determination, Paracetamol, Coupling Reaction.
1. Introduction

From an analytical point of view, methods for pharmaceutical analysis are considerably less complex than methods for analysis of drugs and their metabolites in biological samples as blood, plasma, hair or urine. However, the unequivocal determination of a drug in pharmaceutical formulations is as important as determination in complex matrices, because the pharmaceutical product quality is directly related to patient health (Suntornsuk et al., 2010). In the drug development and pharmaceutical control, chemical analysis plays a key role to ensure a high efficacy and safety for patients. The common availability of the instrumentation, the simplicity of procedures, economy, speed, precision and accuracy of the technique still make spectrophotometric methods attractive (Rojas et al., 2009).

Paracetamol is a pharmaceutical compound widely used as analgesic and antipyretic (Martindale, 1996). It belongs to the class of drugs, known as aniline analgesics. It is commonly used for the relief of headache, other minor aches, pains, inflammation and a major ingredient in numerous cold and flu remedial combination drugs (British Pharmacopoeia, 1999). While generally safe for use at a recommended dose, toxicity of paracetamol is the foremost cause of acute gastrointestinal problems (Michael et al., 2009).

Paracetamol is considered to be the inhibitor of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2. While it has analgesic and antipyretic properties comparable to those of aspirin or other NSAIDs, its peripheral anti-inflammatory activity is usually limited by several factors, one of which is high level of peroxides present in inflammatory lesions (Tripathi, 2004). It could be considered as one in Non-Steroidal Anti Inflammatory Drugs (NSAID). Many methods for its determination have been described in literature, including chromatography (RP-HPLC) (Suzen et al., 1998; Sa’sa et al., 1984; Joshi et al., 2008; Carnevale, 1983), chemometric-assisted spectrophotometric (Wafaa, 2008), spectroscopy (Garg et al., 2007; Narayan et al., 2009; Karla et al., 2009), spectrophotometry (Bouhsain et al., 1996; Xu et al., 2004), Titrimetry (Kumar et al., 1997) and electrochemistry (Altricia et al., 1994). In the standard method, paracetamol is determined titrimetrically with Ce (IV) in acidic medium, using ferroin as indicator. The titration is performed in cold conditions and hence the estimation takes long time with limited accuracy, hence a quicker and accurate method needed. The objective of the present work is to develop a simple, rapid and reliable method to assay paracetamol and to determine the paracetamol in medical paracetamol tablets.

2. Experimental

2.1. Apparatus

A shimadzu 1650 PC/Japan, (Double beam) and UV-visible spectrophotometer, lipra UV 560, England (Single beam) and quartz cells were used for all absorbance measurements.

2.2. Reagents and Standards

All chemicals used were of analytical reagent grade. Distilled water was used to prepare all solutions.

- Standard solution of paracetamol (250 ppm) was prepared by dissolved 0.5g of pure paracetamol (SDI) in 50mL volumetric flask in distilled water.
• Procaine hydrochloride solution (4.6 ppm) was prepared by dissolve 0.1160 g of pure procaine (Sigma) in distilled water and diluting to the marked in 50 mL volumetric flask.
• Hydrochloric acid solution (3M) was prepared by diluted 6.3 mL of concentrated hydrochloric acid (BDH) with distilled water and diluting to the marked in 50 mL volumetric flask.
• Sodium hydroxide solution (6M) also prepared by dissolving 2.4 g of NaOH(BDH) in deionized water and diluting to the marked in 50 mL volumetric flask.

2.3. Pharmaceutical preparations of paracetamol

• Paracetamol tablets (SDI, Iraq): 500 mg paracetamol for each tablet.
• Paracetamol tablets (Ajanta, India): 500 mg paracetamol for each tablet.
• Paracetamol tablets (GSK, UK): 500 mg paracetamol for each tablet.
• Paracetamol tablets (julfar, UAE): 500 mg paracetamol for each tablet.

2.4. General procedure and calibration graph

In 10 mL volumetric flask, 1mL of paracetamol (0.5-20 ppm), 1mL of procaine azotized and 1mL of (6M) hydrochloric acid were mixed and completed to mark by distilled water in cold bath 15 °C and allowed to stand for 20 min. The absorbance of colored product was measured at 420 nm against the reagent blank, a linear calibration graph was obtained over the concentration range of (0.5-20 ppm), a concentration above 20 ppm paracetamol gave a negative deviation from Beer's law (Fig. 1). The molar absorptivity had been found to be $1.31 \times 10^6$ L/mol.cm.

![Fig.1. Calibration curve for determination of paracetamol.](image-url)
2.5. Procedure for the assay of tablets solution (250ppm)
The average tablet weight was calculated from the contents of 20 tablets that had been finely
powdered and weighed. A portion of this powder (0.025 mg) of paracetamol was accurately
weighed. The sample was dissolved in distilled water and filtered into a 100 mL volumetric
flask, the residue was washed and diluted to the marked with distilled water. Further
appropriate solution (100 ppm) was made by using distilled water. Two different
concentrations of this tablets solution were analyzed in five replicate by analytical
spectrophotometric procedure.

3. Results and Discussion

The most generally applicable method for the determination of phenol and primary
aromatic amine is coupling with diazotized aromatic amine ordiazonium salts, yielding a
colored azo dye. The azo compounds formed are intensely colored because the diazenedyl
linkage –N=N– brings two aromatic rings in conjugation. This gives an extended system of
delocalized– electrons and allows absorption of light in the visible region (i.e. Bathochromic
shift).

3.1. Effect of acids
It was found experimentally that the colored products were formed stable by using the amount
of best acid chosen was HCl having higher absorbance optimized. The maximum intensity
reached at 3 mL HCl (1M) (Fig. 2).

![Graph](image)

Fig.2. Effect the Vml of (1M) HCl

3.2. Effect of Bases
It was found that the presence of a base led to increasing the intensity of the product that was
choice many bases. NaOH was selected and was responsible of leading to increasing the
intensity of product. So (6.0 M) of NaOH was selected which is the best volume equal to 1.0
mL giving high sensitivity (Fig.3).
3.3. **Effect of sodium nitrite**

The optimum concentration of sodium nitrite solution that gave maximum absorption was found to be 3mL of (0.1M) of sodium nitrite solution (Fig.4).

![Fig.3. Effect of Vml of (6M) NaOH](image)

![Fig.4. Effect of Vml of (0.1M) NaNO₃](image)
3.4. Sequence of addition
Different orders of addition of reagents were examined and it was found that the order of addition of reagents by mixing paracetamol and procaine with sodium nitrite \((P + R + B)\) gave the highest absorbance used in all subsequent experiments (Table 1).

<table>
<thead>
<tr>
<th>Sequence</th>
<th>(P+R+B)</th>
<th>(B+P+R)</th>
<th>(R+B+P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs.</td>
<td>0.840</td>
<td>0.093</td>
<td>0.026</td>
</tr>
</tbody>
</table>

3.5 Effect of time required for diazotization process
The effect of time on the development and stability of the dye obtained from 5-60 min has been investigated under the optimum experimental conditions described. The formation of colored dye being complete immediately 20min before dilution was least time to forms azo days (Fig.5).

![Fig.5. Effect of time required for diazotization process](image)

3.6 Stability of the colored product
The effect of the time needed to complete the formation of colored dye had been studied. The results indicated that maximum intensity of the colored product at 420 nm occurred after 20min (Fig.6)
3.7. **Temperature effect on the final product**
The effect of temperature on diazotization and coupling were studied. It was found that diazotization at (15-20) °C gives maximum color intensity.

3.8. **The stoichiometry study**
The stoichiometry of the product was studied applying the continuous variation method. Different volumes of 0.5, 1, 1.5, 2….4.5 mL of paracetamol solution (20 ppm) were transferred to 25mL volumetric flask containing 0.5, 1, 1.5, 2….4.5 mL of the diazotized solution, respectively. The contents were diluted to the mark with distilled water and mixed well. The results obtained in Fig. (7) shows that the 2 : 1 azo dye was formed between diazotized paracetamol and procaine.

![Fig.6. Effect of Stability of the colored product](image-url)
Fig. 7. Continuous variation method

Scheme 1 Reaction sequence

3.9. Accuracy and precision
The accuracy and precision of the calibration curve are checked by determining paracetamol at three different concentrations. The results shown in Table 2 indicate that the method is quite satisfactory.

Table 2 accuracy and precision

<table>
<thead>
<tr>
<th>Conc. of paracetamol</th>
<th>Experimental Conc.</th>
<th>Relative error%</th>
<th>R%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.89</td>
<td>2.2</td>
<td>102.2</td>
</tr>
<tr>
<td>10</td>
<td>11.2</td>
<td>-1.2</td>
<td>98.8</td>
</tr>
<tr>
<td>20</td>
<td>19.2</td>
<td>4</td>
<td>104</td>
</tr>
</tbody>
</table>
3.10. Study of interferences
In order to assess the possible analytical applications of the spectrophotometric method described above, the effect of concomitant species on the determination of paracetamol in real samples was studied by analyzing synthetic sample solution that contain paracetamol and various excess amount of the common interferes (Table 3).

Table 3 Effect of interference

<table>
<thead>
<tr>
<th>Interference</th>
<th>Without</th>
<th>Lactose</th>
<th>Starch</th>
<th>NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs.</td>
<td>0.179</td>
<td>0.171</td>
<td>0.169</td>
<td>0.172</td>
</tr>
<tr>
<td>E%</td>
<td>----</td>
<td>4.4</td>
<td>5.58</td>
<td>3.91</td>
</tr>
<tr>
<td>R%</td>
<td>----</td>
<td>104.4</td>
<td>105.5</td>
<td>103.9</td>
</tr>
</tbody>
</table>

4. Application of the method
To test the applicability of the present method, it has been applied to the determination of paracetamol in pharmaceutical preparation. On applying the proposed procedure, good recovery is obtained as shown in Table 4.

Table 4 Determination of paracetamol in pharmaceutical preparation

<table>
<thead>
<tr>
<th>Tab</th>
<th>Conc.mg</th>
<th>Error%</th>
<th>Recovery%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ParacetolSDI.Iraq</td>
<td>present</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>496.7</td>
<td>97.3</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>498.5</td>
<td>98.5</td>
</tr>
<tr>
<td>ParacetolAjenta.India</td>
<td>500</td>
<td>497.9</td>
<td>97.9</td>
</tr>
<tr>
<td>PanadolGSK.Ireland</td>
<td>500</td>
<td>501.1</td>
<td>101.1</td>
</tr>
<tr>
<td>AdolJulfar.UAE</td>
<td>500</td>
<td>498.5</td>
<td>98.5</td>
</tr>
</tbody>
</table>
5. Analytical characteristics of the proposed method

The calibration graph was obtained by the analytical procedure described previous and series of standard solutions were analyzed in triplicates to test the linearity. The molar absorptivity ($\epsilon$), the Sandell sensitivity (S), the intercept (a), the slope (b), the correlation coefficient (r), the correlation of determination ($r^2$), were evaluated by a least-squares regression analysis and are included in Table (5).

<table>
<thead>
<tr>
<th>Table 5 Analytical parameters for spectrophotometric determination</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters of determination paracetamol</strong></td>
</tr>
<tr>
<td>Beer’s law limit (ppm)</td>
</tr>
<tr>
<td>Molar absorptivity (L mol$^{-1}$ cm$^{-1}$)</td>
</tr>
<tr>
<td>Sandell’s sensitivity(Ng cm$^{-2}$)</td>
</tr>
<tr>
<td>Correlation coefficient [r]</td>
</tr>
<tr>
<td>Slope [b]</td>
</tr>
<tr>
<td>Intercept [a]</td>
</tr>
<tr>
<td>Standard deviation (S.D)</td>
</tr>
<tr>
<td>Relative Standard deviation (R.S.D)</td>
</tr>
<tr>
<td>$E_{rel}$ %</td>
</tr>
<tr>
<td>Re %</td>
</tr>
<tr>
<td>LOD($\mu$g ml$^{-1}$)</td>
</tr>
<tr>
<td>LOQ($\mu$g ml$^{-1}$)</td>
</tr>
</tbody>
</table>

The results obtained by the proposed method were compared with BP method (British Pharmacopoeia on CD-ROM., 2001) (Table 6).

<table>
<thead>
<tr>
<th>Table 6 Comparison of the proposed method with BP method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmaceutical tablets</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Paracetamol pure</td>
</tr>
<tr>
<td>Paracetamol SDI</td>
</tr>
<tr>
<td>Paracetamol GSK</td>
</tr>
</tbody>
</table>
6. **Conclusions**
   - The proposed spectrophotometric method has proved to be simple, rapid, precise, low-cost and sensitive for paracetamol determination.
   - The usually required time-consuming stages of the paracetamol hydrolysis and its transformation in colored substance were excluded by use of the proposed reagent.
   - The determinations can be performed at room temperature and do not require heating step.
   - Compared with the majority of HPLC methods developed for the determination of paracetamol, this method is simpler, shorter in time and lower cost.

**References**


