PART-III

RESULTS & DISCUSSION
CHAPTER-5

STUDIES ON MEDICINALLY IMPORTANT COMPOUNDS
Paracetamol

Paracetamol (N-acetyl P-amino phenol) is well known for its analgesic and antipyretic action. Many methods for its determination have been described. These include titrimetry\textsuperscript{194-197}, chromatography\textsuperscript{198-201}, spectrophotometry\textsuperscript{104-114} and electrochemical\textsuperscript{202-204} methods. The majority of these are spectrophotometric, most of which require lengthily treatment and therefore lack suitability for routine analysis. For instance, the official pharmacopoeia method\textsuperscript{205} requires a 60 minute reflection in acidic media. The other methods also suffer from various disadvantages like high acid concentration requirement longer reaction time, lesser sensitivity and complicated procedures. The author has made an attempt to develop much simpler methods for the spectrophotometric determination of paracetamol which are less time consuming and more sensitive. The methods described here make use of:

a) The reduction of vanadium (V) to vanadium (IV) by paracetamol.
b) Reaction with alkaline potassium ferricyanide; and
c) Reaction with nitrous acid
a) **Method based on the reduction of vanadium (V) to vanadium (IV) by paracetamol**

7ml of concentrated sulphuric acid and 2ml of 0.1m vanadium (V) solution and a known aliquot of paracetamol solution are taken in a 25ml volumetric flask. The contents are made up to the mark with distilled water and the solution is cooled to room temperature. The absorbance of the resulting blue solution is measured against blank at 760nm. A calibration curve is constructed between the absorbance and the amount of paracetamol.

Organic compounds are known to reduce the metal ions such as vanadium\textsuperscript{206,207}, molybdenum\textsuperscript{208,209}, chromium\textsuperscript{210,211}, iron\textsuperscript{212} etc. It is noticed by the author that in acid solutions vanadium (V) is reduced to vanadium (IV) with much ease by paracetamol. An instantaneous blue colour corresponding to, vanadium (IV) formation is obtained on the addition of paracetamol to an orange coloured acidic solution of vanadium (V). The spectrum of the resulting solution against blank (titration) containing the metal ion and the acid showed an absorbance maximum at 760nm (Fig. 3.5.1). Experiments are conducted to establish the optimum conditions for the rapid development of blue colour. Different acids [HCl, H\textsubscript{2}SO\textsubscript{4}, H\textsubscript{3}PO\textsubscript{4}, CH\textsubscript{3}COOH, HClO\textsubscript{4}] were employed to select the proper medium for the reaction (Table 3.5.1). It is found that 5M sulphuric acid (Table 3.5.2) and 8 x 10\textsuperscript{-3}M vanadium (V) (Table 3.5.3) are suitable. The absorbance values are measured at 760nm at different concentrations of paracetamol to know whether the drug can be determined by employing this method. It was found that a linear relationship exists between the amount of paracetamol and absorbance in the range 9.06 – 90.6 µg/ml (Fig. 3.5.2).
Fig. 3.5.1. Absorption spectrum of V(V) + paracetamol

\([V(V)] = 12 \times 10^{-4} \text{M}; \ [\text{Paracetamol}] = 3.6 \times 10^{-4} \text{M},\]
\([\text{H}_2\text{SO}_4] = 5 \text{M}\)
Fig. 3.5.2. Effect of paracetamol on absorbance

\[ [V(V)] = 12 \times 10^{-4} \text{M}; \ [H_2SO_4] = 5 \text{M} \]

Table 3.5.1

Effect of different acids on absorbance

\[ [V (V)] = 2 \times 10^{-2} \text{M}; \ [Paracetamol] = 6 \times 10^{-4} \text{M} \]

<table>
<thead>
<tr>
<th>Acid (4M)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>0.389</td>
</tr>
<tr>
<td>HNO_3</td>
<td>0.177</td>
</tr>
<tr>
<td>H_2SO_4</td>
<td>0.696</td>
</tr>
<tr>
<td>H_3PO_4</td>
<td>0.211</td>
</tr>
<tr>
<td>HClO_4</td>
<td>0.135</td>
</tr>
</tbody>
</table>

Table 3.5.2

Effect of Sulphuric acid on absorbance

\[ [V (V)] = 2.0 \times 10^{-2} \text{M}; \ [Paracetamol] = 6.2 \times 10^{-4} \text{M} \]

<table>
<thead>
<tr>
<th>[H_2SO_4] M</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4</td>
<td>0.154</td>
</tr>
<tr>
<td>3.6</td>
<td>0.299</td>
</tr>
<tr>
<td>5.0</td>
<td>0.506</td>
</tr>
<tr>
<td>7.2</td>
<td>0.451</td>
</tr>
<tr>
<td>8.6</td>
<td>0.460</td>
</tr>
<tr>
<td>10.8</td>
<td>0.491</td>
</tr>
</tbody>
</table>
Table 3.5.3

Effect of vanadium (V) on absorbance

\[ [\text{H}_2\text{SO}_4] = 5 \text{ M} ; [\text{Paracetamol}] = 6.2 \times 10^{-4}\text{M} \]

<table>
<thead>
<tr>
<th>([\text{V(V)}] \times 10^3\text{M})</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.382</td>
</tr>
<tr>
<td>4.0</td>
<td>0.526</td>
</tr>
<tr>
<td>6.0</td>
<td>0.734</td>
</tr>
<tr>
<td>8.0</td>
<td>0.805</td>
</tr>
<tr>
<td>10.0</td>
<td>0.777</td>
</tr>
<tr>
<td>12.0</td>
<td>0.661</td>
</tr>
<tr>
<td>14.0</td>
<td>0.557</td>
</tr>
<tr>
<td>16.0</td>
<td>0.330</td>
</tr>
</tbody>
</table>

Therefore, this observation can be advantageously exploited for the determination of paracetamol. The solutions are stable for 24 hours. The method provides satisfactory results if the following order of addition is adopted - \(\text{H}_2\text{SO}_4\), Vanadium (V), paracetamol and water. The mechanism of paracetamol oxidation with vanadium is probably similar to that of cerium (IV) deacetylation to P-aminophenol and then oxidation to p-benzoquinone

\[
\text{NHCOCH}_3 \quad \text{NH}_2 \quad \text{O} \\
\text{OH} \quad \text{OH} \quad + \text{NH}_3 \\
\text{paracetamol} \quad \text{para aminophenol} \quad \text{benzoquinone}
\]

The method is rapid, no heating is necessary and it does not involve any rare chemicals and complicated equipment.
(b) Reaction with alkaline potassium ferricyanide

Different known amounts of paracetamol solution are drawn through a pipette into a series of 25ml volumetric flasks each containing $1.6 \times 10^{-5}$M ferricyanide and $6 \times 10^{-4}$M sodium hydroxide. The resulting red coloured solutions are made up to the volume with water and absorbances are measured against reagent blank at 475nm.

Potassium ferricyanide in alkaline medium produces an intense red species on the addition of paracetamol. The formation of the red colour is instantaneous and it shows an absorbance maximum at 475nm against ferricyanide blank (Fig 3.5.3). The optimum concentration of ferricyanide and sodium hydroxide required for the development of stable red colour are established as $1.8 \times 10^{-3}$M ferricyanide and $6 \times 10^{-3}$M sodium hydroxide. The linear relation obtained between paracetamol and the absorbance provides a method for the determination of the drug in the range 3.824 – 21.6 $\mu$g/ml (Fig 3.5.4). The intense red colour formation of paracetamol – potassium ferricyanide mixture in alkaline medium may be attributed to a ligand exchange reaction. Such reactions involving ligand exchange and resulting in intense colours and reported in literature²²⁰,²²¹,²²¹a.
Fig. 3.5.3. Absorption spectrum of $K_3Fe(CN)_6 +$ paracetamol

$[Fe(CN)_6] = 1.6 \times 10^{-3}M; [\text{Paracetamol}] = 6 \times 10^{-3}M,$
$[\text{NaOH}] = 1.2 \times 10^{-3}M$

Fig. 3.5.4. Effect of paracetamol on absorbance

$[Fe(CN)_6] = 1.6 \times 10^{-3}M; [\text{NaOH}] = 6 \times 10^{-4}M$
(c) Reaction with nitrous acid

5ml each of 5 M HCl and 5M NaNO₂ are taken in a 25ml volumetric flask and a known aliquot of paracetamol stock solution was added and the contents were made upto the mark with water. The flask with the contents is cooled to 0°C by keeping it in an ice bath for about 30 minutes. The contents of the flask are then allowed to attain room temperature and the absorbance is measured at 420nm against a reagent blank (Fig. 3.5.5). The procedure is repeated with different known aliquots of paracetamol stock solution and a calibration plot is made as usual.

Organic compounds containing hydroxy groups are known to undergo nitrosation to produce nitroso compounds. The author has studied the nitrosation reaction of paracetamol using sodium nitrite and hydrochloric acid. The nitrosated product is yellow in colour and has maximum absorbance at 420nm against nitrous acid blank. An optimum concentration of 1M hydrochloric acid and 5M sodium nitrite are found to be suitable for the stable yellow colour development. 5ml of methanol is added to destroy excess of nitrous acid. Paracetamol can be determined employing this method in the range 18 - 180 μg/ml as linear relationship is obtained between absorbance and the amount of the drug (Fig. 3.5.6).

Interferences

The common excipients such as lactose, starch, glucose added during the preparation of tablets do not interfere in any of the methods. The presence of caffeine, codeine, pheniramine in paracetamol drug formulation do not cause any interference in the determination.
Fig. 3.5.5. Absorption spectrum of nitrosated paracetamol

\([\text{NaNO}_2] = 1 \text{ M}; [\text{HCl}] = 1 \text{ M}; [\text{Paracetamol}] = 1 \times 10^{-3} \text{M}\)

Fig. 3.5.6. Effect of paracetamol on absorbance

\([\text{NaNO}_2] = 1 \text{ M}; [\text{HCl}] = 1 \text{ M};\)
Table 3.5.4

Assay of paracetamol in drug formulations

Average of five determinations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labeled amount mg/Tab (or) Cap</th>
<th>Amount Found mg/Tab or cap</th>
<th>Recovery %</th>
<th>Molar absorptivity x 10^3 (1mol^-1 cm^1)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calpol&lt;sup&gt;a&lt;/sup&gt;</td>
<td>500</td>
<td>498</td>
<td>99.6</td>
<td>2.04</td>
<td>0.278</td>
</tr>
<tr>
<td>Paracin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>500</td>
<td>499</td>
<td>99.8</td>
<td>1.96</td>
<td>0.278</td>
</tr>
<tr>
<td>Paramet&lt;sup&gt;c&lt;/sup&gt;</td>
<td>500</td>
<td>497</td>
<td>99.4</td>
<td>1.22</td>
<td>0.292</td>
</tr>
<tr>
<td>Fortagetic&lt;sup&gt;d&lt;/sup&gt;</td>
<td>500</td>
<td>496</td>
<td>99.2</td>
<td>2.10</td>
<td>0.428</td>
</tr>
<tr>
<td>Beserol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>500</td>
<td>498</td>
<td>99.6</td>
<td>1.84</td>
<td>0.388</td>
</tr>
<tr>
<td>Foracet&lt;sup&gt;e&lt;/sup&gt;</td>
<td>500</td>
<td>499</td>
<td>99.8</td>
<td>2.15</td>
<td>0.212</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Duphar – Interfran Ltd., Mumbai, India  
<sup>b</sup> = Stadned Private Ltd., Kolkatta, India  
<sup>c</sup> = Wallace Pharmaceuticals Ltd., Mumbai, India  
<sup>d</sup> = Win – Medicare Ltd., New Delhi, India  
<sup>e</sup> = Ranbaxy Laboratories Ltd., New Delhi, India.

The proposed methods are tested for their applicability in certain drug formulation containing paracetamol. The results are satisfactory and are shown in the Table 3.5.4.
Ascrobic acid

Ascorbic acid is present in large amounts in citrus fruits and tomatoes. It is one of the most important vitamins in the human body. A prolonged lack of ascorbic acid results in scurvy and less severe deficiency of the acid produces alterations in connective tissue structure and may also cause decreased resistance to some infections. Many methods have been described in the literature for the determination of ascorbic acid based on its reducing property and its ability to produce coloured compounds. These methods have been reviewed\textsuperscript{222–225}. The titrimetric methods are limited to higher concentrations where as the spectro photometric\textsuperscript{115–130,226,227} methods can be used to determine ascorbic acid down to microgram level. The sensitivity of HPLC methods depends on the type of detection system employed. The author describes simple, rapid and sensitive methods for the spectrophotometric determination of ascorbic acid based on its reduction properties, bleaching action and complexation reaction. The methods are based on the following reactions involving ascorbic acid.

a) Reduction of vanadium (V) to vanadium (VI)

b) Reaction with iron (III)–ammonium thiocyanate complex.
c) Reaction with alkaline potassium ferricyanide
d) Reaction with potassium permanganate
e) Reaction with molybdo phosphoric acid, and
f) Reaction with oxime complexes of vanadium(V), uranium (VI) and chromium (VI).

a) Reduction of vanadium (V) to vanadium (IV)

Different known aliquots of ascorbic acid solution are pipetted out into a series of 25ml volumetric flasks each containing 3.0M H₂SO₄ and 2 x 10⁻³M vanadium (V). The solutions are made up to the volume with distilled water. The absorbances of the resulting blue coloured solutions are measured after cooling to room temperature at 760nm against reagent blank.

Transition metals in their higher oxidation state get reduced to lower oxidation state by organic compounds. In the present case, the author has noticed an instantaneous blue colour formation due to the reduction of vanadium (V) to vanadium (VI) on the addition of ascorbic acid. The blue solution has a maximum absorbance at 760nm. The optimum concentration of vanadium (V) and sulphuric acid for the instantaneous development of blue colour are established as 0.1 M and 3.0M respectively. Experiments carried out with different known aliquots of ascorbic acid show linearity between the amount of ascorbic acid and absorbance in the range 0.354 – 2.464 µg/ml (Fig. 3.5.7). The blue colour development can also be used to determine the vanadium in the range of 8 – 32 x 10⁻³M (Fig. 3.5.8) as linear relationship exists between the metal ion concentration and the absorbance.
Fig. 3.5.7. Effect of ascorbic acid on absorbance

\[ [\text{V(V)}] = 2 \times 10^{-3} \text{M}; [\text{H}_2\text{SO}_4] = 3.0 \text{ M} \]

Fig. 3.5.8. Effect of vanadium(V) on absorbance

\[ [\text{Ascorbic acid}] = 2 \times 10^{-5} \text{M}; [\text{H}_2\text{SO}_4] = 3.6 \text{ M}; \]
(b) Reaction with iron (III) – ammonium thiocyanate complex

In a 25ml volumetric flask, 1 ml of 0.01 M HClO₄ 6.4 x 10⁻⁶M iron (III) and 3.2 x 10⁻⁴M thiocyanate is taken. A known amount of ascorbic acid is added. Absorbance of the solution is measured after making the solution to the volume with distilled water against water as blank.

Iron (III) is known to form a blood red coloured complex with ammonium thiocyanate. Different acids were employed to find out whether this bleaching action takes place with same speed resulting in maximum (λ_max 460nm). Therefore, HClO₄ has been used at the medium for carrying out further experiments. It is also found by the author that a concentration of 0.1 HClO₄ is sufficient to result in appreciable absorbance. Higher concentrations of acid cannot be employed.

Ascorbic acid bleaches the solution in perchloric acid medium quantitatively. This fact has been successfully employed for the indirect determination of ascorbic acid with the successive addition of known aliquots of ascorbic acid, the absorbance values progressively decreases. The range of determination is 35.2 to 246 μg/ml (Fig. 3.5.9) employing this indirect method.

<table>
<thead>
<tr>
<th>Table 3.5.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of different acids on λ_max and absorbance</td>
</tr>
<tr>
<td>[NH₄ SCN] = 1.2 x 10⁻²M</td>
</tr>
<tr>
<td>[Fe (III)] = 2.0 x 10⁻⁴M</td>
</tr>
<tr>
<td>[Ascorbic acid] = 4.0 x 10⁻⁵M</td>
</tr>
<tr>
<td>[Acid] M</td>
</tr>
<tr>
<td>HCl</td>
</tr>
<tr>
<td>H₂SO₄</td>
</tr>
<tr>
<td>CH₃COOH</td>
</tr>
<tr>
<td>HClO₄</td>
</tr>
</tbody>
</table>
Fig. 3.5.9. Effect of ascorbic acid on absorbance

\[\text{Absorbance} \]

\[
\begin{align*}
[\text{Fe(III)}] &= 6.4 \times 10^{-6} \text{M} \\
[\text{SCN}] &= 3.2 \times 10^{-4} \text{M} \\
[\text{HClO}_4] &= 4.0 \times 10^{-3} \text{M}
\end{align*}
\]
(c) **Reaction with alkaline potassium ferricyanide**

In a series of 25ml volumetric flasks each containing 0.16M NaOH and $1.6 \times 10^{-5}$M ferricyanide, known amounts of ascorbic acid are drawn through a pipette. The solutions are made up to the volume with water and the absorbances of the resulting red solutions are measured at 475nm against blank containing no ascorbic acid. Calibration plot is constructed as usual.

Alkaline potassium ferricyanide has absorbance maximum at 418nm. On the addition of ascorbic acid the yellow colour of the ferricyanide decreases. This fact can be suitably employed for the determination of ascorbic acid. It is found by the author that the optimum concentration of the alkali (NaOH) and ferricyanide are 0.1M and 0.01M respectively. The absorbance in this case should be measured within 15 minutes after the addition of ascorbic acid. The linear plot obtained between absorbance and the amount of ascorbic acid indicates the suitability of the method for the determination of ascorbic acid in the range 1.78 to 24.64 µg/ml (Fig 3.5.10)

![Absorbance vs Amount of Ascorbic Acid](image-url)
(d) Reaction with potassium permanganate

1 x 10^{-3}M potassium permanganate and 0.1 ml of concentrated perchloric acid are taken in a 25ml volumetric flask. Known amount of ascorbic acid is transferred into the flask. The solution is made upto the volume with water. The absorbance is measured immediately at 545 nm against water as blank.

The pink colour of K_mnO_4 is found to be bleached on the addition of ascorbic acid. The fact has been utilized to develop a method for the determination of ascorbic acid. Among the acids tested for the purpose, phosphoric acid is found to be most suitable because it results in maximum absorbance (Table 3.5.6). Therefore, phosphoric acid is used in further experiments. Since, the bleaching action is fast absorbance measurements are made immediately after the addition of ascorbic acid. The fall in intensity is proportional to the concentration of the added ascorbic acid in the range 7.76 – 28.16 µg/ml (Fig. 3.5.11).

(e) Reaction with molybdo phosphoric acid

In a 25ml volumetric flask, 1 x 10^{-3}M molybdenum (VI) 4 x 10^{-2}M HCl and 1.6 x 10^{-3}M Sodium dihydrogen phosphate are taken. Then a known aliquot of ascorbic acid is added. The solution develops blue colour instantaneously. However the solution becomes stables after 15 minutes. Therefore, the absorbance of the solution is recorded after 15 minutes against a blank at 348 nm.
Table 3.5.6
Effect of different acids on $\lambda_{\text{max}}$ and absorbance

$[\text{KmnO}_4] = 6.0 \times 10^{-4}\text{M}$

$[\text{Ascorbic acid}] = 4.0 \times 10^{-5}\text{M}$

<table>
<thead>
<tr>
<th>Acid</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$SO$_4$</td>
<td>545</td>
<td>0.265</td>
</tr>
<tr>
<td>H$_3$PO$_4$</td>
<td>545</td>
<td>0.554</td>
</tr>
<tr>
<td>HClO$_4$</td>
<td>545</td>
<td>0.535</td>
</tr>
<tr>
<td>CH$_3$COOH</td>
<td>525</td>
<td>0.485</td>
</tr>
</tbody>
</table>

Fig. 3.5.11. Effect of ascorbic acid on absorbance

$[\text{KMnO}_4] = 1 \times 10^{-3}\text{M}$

$[\text{HClO}_4] = 4 \times 10^{-2}\text{M}$
Ascorbic acid reacts with molybdophosphoric acid and gives a blue coloured species instantaneously probably molybdenum blue. The solution shows maximum absorbance at 348nm against the reagent blank (Fig.3.5.12). The optimum concentrations at molybdenum (VI), hydrochloric acid, sodium phosphate are established as $1.0 \times 10^{-3}$M, $4.0 \times 10^{-3}$M in a final volume of 25ml respectively. The following order of addition must be followed to get stable colour – (Mo (VI) + HCl + H$_3$PO$_4$ + ascorbic acid. The absorbance measurements of the solutions are made after 15 minutes, since the solution shows maximum absorbance and stability only after a lapse of time. The method can be successfully employed for the determination of ascorbic acid in the range 0.176 – 0.881 µg/ml (Fig. 3.5.13).

<table>
<thead>
<tr>
<th>Method</th>
<th>Medium</th>
<th>Max (nm)</th>
<th>Range of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanadium (V) method</td>
<td>Reported 227</td>
<td>680</td>
<td>40 µg – 0.8 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>H$_2$SO$_4$</td>
<td>760</td>
</tr>
<tr>
<td>Molybdenum blue method</td>
<td>Reported 117</td>
<td>760</td>
<td>2 – 32 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Present</td>
<td>HCl</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.04M)</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3.5.12. Absorption spectrum of molybdophosphoric acid + ascorbic acid

\[ [\text{Mo(VI)}] = 1 \times 10^{-2} \text{M}; [\text{NaH}_2\text{PO}_4] = 1.6 \times 10^{-3} \text{M}; \]
\[ [\text{HCl}] = 8 \times 10^{-2} \text{M}; [\text{Ascorbic acid}] = 4 \times 10^{-4} \text{M} \]

Fig. 3.5.13. Effect of ascorbic acid on absorbance

\[ [\text{Mo(VI)}] = 1 \times 10^{-3} \text{M} \]
\[ [\text{NaH}_2\text{PO}_4] = 1.6 \times 10^{-3} \text{M} \]
\[ [\text{HCl}] = 4 \times 10^{-2} \text{M} \]

(f) Reaction with oxime complexes of vanadium(V), uranium(VI) and chromium (VI)
Ascorbic acid reacts with salicyladoxime (SAO) complexes of the metals giving characteristic colours in an acetate buffer of pH 5.0. The methods are described below.

(i) Reaction with vanadium (V) – SAO Complex

8 x 10^{-3} M vanadium (V) and 2 x 10^{-3} M oximes are taken in a 25ml volumetric flask and known aliquots of ascorbic acid added. 10ml of acetate – acetic acid buffer solution of pH 5 is added. The solution is made upto the mark with distilled water. Reagent blank is prepared similarly without the drug.

In the presence of vanadium (V) complex of SAO, ascorbic acid produces an yellowish brown coloured solution and it shows maximum absorbance at 360nm against reagent blank (Fig 3.5.14). The absorbance of the solution increases proportionately with the addition of ascorbic acid. The optimum concentrations of the oxime and vanadium (V) are established for the stable and instantaneous colour development and these are found to be 8 x 10^{-3} M vanadium (V) and 2 x 10^{-3} M salicylaloxime. Studies with different known aliquots of ascorbic reveal that a linear relationship exists between the amount of ascorbic acid and absorbance in the range 17.6 – 88 μg/ml. The data is shown in the Fig 3.5.15.
Fig. 3.5.14. Absorption spectrum of V(V) + SAO + ascorbic acid

[V(V)] = 4 \times 10^{-3} \text{M}; [SAO] = 2 \times 10^{-3} \text{M};
[Ascorbic acid] = 2 \times 10^{-4} \text{M}; \text{pH} = 5.0;

Fig. 3.5.15. Effect of ascorbic acid on absorbance

[V(V)] = 8 \times 10^{-3} \text{M}
[SAO] = 2 \times 10^{-3} \text{M}
\text{pH} = 5.0

(ii) Reaction with uranium complex of SAO
8 x 10\(^3\)M uranium (VI) and 2 x 10\(^{-2}\)M SAO are taken in a 25ml volumetric flask. A known aliquot of ascorbic acid is added and 10ml of buffer solution of pH5.0 is added. The solution is mixed well and the absorbance of the resulting orange red solution is measured against the reagent blank at 345nm (Fig 3.5.16).

Addition of ascorbic acid to uranium complex of SAO produces an orange red coloured solution which absorbs maximum at 345nm against the blank in an acetate buffer of pH5.0. Optimum concentration of the uranium (VI) and oxime established for the stable orange red colour are 8 x 10\(^3\)M uranium (VI) and 2 x 10\(^{-2}\)M oxime. Studies with various amount of ascorbic acid shows the existence of linearity in the range 8.70 µg/ml (Fig 3.5.17)

(iii) Reaction with chromium (VI) oxime Complex

A known amount of ascorbic acid is transferred into a 25ml volumetric flask containing 4 x 10\(^{-3}\)M each of chromium (VI) and salicyaldoxime and 10ml of acetate buffer of pH4.0. The solution is made upto the volume with distilled water. The solution is shaken well for homogeniety and the spectrum is recorded against the reagent blank (Fig 3.5.18).
Fig. 3.5.16. Absorption spectrum of U(VI) + SAO + ascorbic acid
\[ [\text{U(VI)}] = 8 \times 10^{-3}\text{M}; [\text{SAO}] = 2 \times 10^{-2}\text{M}; \]
\[ [\text{Ascorbic acid}] = 4 \times 10^{-5}\text{M}; \text{pH} = 5.0; \]

Fig. 3.5.17. Effect of ascorbic acid on absorbance
\[ [\text{U(VI)}] = 8 \times 10^{-3}\text{M} \]
Organic compounds are capable of reducing chromium (VI) to chromium (III) resulting in green coloured species. In the present investigations, ascorbic acid reduces chromium (VI) complex to chromium (III) in a buffer of pH 5.0. 4 x 10^{-3} M Cr (VI), 4 x 10^{-3} M oxime are found to be suitable for the formation of stable pale green solution, probably chromium (III). The reaction mixture is kept aside for 30 minutes and the absorbance was measured at 580 nm against the blank. Linear relationship exists between the amount of the drug and absorbance in the range 17.6 – 123 μg/ml (Fig 3.5.19).

Interferences

Species such as ferrous fumarate, vitamins B_{1}, B_{2}, B_{6}, B_{12}, nicotinamide and calcium pentothenate, that are commonly present in the pharmaceutical preparations do not effect the determinations.

The analysis of ascorbic acid in pharmaceutical formulations is carried out by using the above mentioned methods and the assay of the tablets are evaluated and the data are presented in Table 3.5.7.
Fig. 3.5.18. Absorption spectrum of Cr(VI) + SAO + ascorbic acid
[Cr(VI)] = 4 \times 10^{-4} M; [SAO] = 4 \times 10^{-3} M;
pH = 5.0;

Fig. 3.5.19. Effect of ascorbic acid on absorbance
[Cr(VI)] = 4 \times 10^{-3} M
[SAO] = 4 \times 10^{-3} M
pH = 5.0
Table 3.5.7
Assay of ascorbic acid in drug formulations
(Average of five determinations)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labeled amount mg/Tab (or) Cap</th>
<th>Amount Found mg/Tab or cap</th>
<th>Recovery %</th>
<th>Molar absorptivity x 10^3 (1 mol^-1 cm^-1)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sukcea^a</td>
<td>500</td>
<td>497</td>
<td>99.4</td>
<td>2.88</td>
<td>0.101</td>
</tr>
<tr>
<td>Chewceeb^b</td>
<td>500</td>
<td>498</td>
<td>99.6</td>
<td>3.10</td>
<td>0.207</td>
</tr>
<tr>
<td>Citravite^c</td>
<td>100</td>
<td>97</td>
<td>97.0</td>
<td>1.92</td>
<td>0.432</td>
</tr>
<tr>
<td>Cibion^d</td>
<td>500</td>
<td>497</td>
<td>99.4</td>
<td>1.89</td>
<td>0.325</td>
</tr>
<tr>
<td>Limceae^e</td>
<td>500</td>
<td>498</td>
<td>99.6</td>
<td>1.92</td>
<td>0.256</td>
</tr>
<tr>
<td>Redoxon^f</td>
<td>500</td>
<td>499</td>
<td>99.8</td>
<td>2.34</td>
<td>0.239</td>
</tr>
</tbody>
</table>

a = IDPL, Hyderabad, India  
b = Cyanamid India Ltd., Mumbai, India  
c = Pharmed Ltd., Mumbai, India  
d = E.Merck (India), Mumbai, India  
e = Sarabhai Chemicals, Vadodara, India.  
f = Roche Products Ltd., Mumbai, India.
Aspirin

Aspirin is an acetylene derivative of salicylic acid

The increased interest in non-steroidal anti-inflammatory drugs in the past has been the results of attempts to discover the compounds that possess therapeutic effectiveness, but lack undesirable side effects. The most important compounds in current clinical use sodium salicylate, salicylamide and acetylsalicylic acid. Many derivatives of salicylic acid have been prepared and tested for their anti-inflammatory effect although as yet a substitute for aspirin has not been introduced. Aspirin is a non-narcotic analgesic. It is more active than salicylic acid as an analgesic. Methods for the determination of aspirin\textsuperscript{228} are not very many, even though for salicylic acid there are some\textsuperscript{100-103, 163, 171, 229, 230}.

The author proposes three simple spectrophotometric methods for the determination of aspirin. The methods are based on the reactions of aspirin with.

a) Sodium molybdate
b) Uranyl acetate; and
c) Nitration mixture
A) Reaction with sodium molybdate

Different known aliquots of aspirin are pipetted out into a series of 25ml volumetric flask. Containing 3ml of 0.01m sodium molybdate, 10ml of buffer solution of pH 4.0. The solutions are shaken for homogeneity and then made up to the volume with distilled water. The flasks are heated for 10 minutes at 80°C. An yellow coloured solution results. The contents the flasks are cooled to room temperature and the absorbance is measured at 310nm against a reagent blank treated similarly having no aspirin (Fig 3.5.20).

Experimental conditions are optimized for the appropriate colour development. It is found that an acetate buffer of pH 4.0 and $1.2 \times 10^{-3}$M ammonium molybdate are suitable. Experiments repeated with different amount of aspirin gave a linear plot in the range 7.2 – 43.2 µg/ml (Fig 3.5.21). The author assumes that a complex formed between the metal ion aspirin is responsible for the colour.

![Fig. 3.5.20. Absorption spectrum of Mo(VI) + aspirin](image-url)

$$[\text{Mo(VI)}] = 1.2 \times 10^{-3}\text{M}; [\text{Aspirin}] = 4 \times 10^{-3}\text{M};$$
$$pH = 5.0;$$
B) Reaction with uranyl acetate

5ml of 0.001M uranyl acetate and 0.5ml of and 0.01M of hydrocholoric acid, 12ml of methanol and a known aliquot of aspirin are taken in a 25ml volumetric flask. The contents are made up to mark with distilled water and heated at 80°C for 10 minutes. The solution is cooled and the absorbance of the resulting orange red solution is measured at 305nm against reagent blank (Fig 3.5.22).

Uranium (VI) reacts with aspirin giving an orange red coloured species. To establish the optimum conditions for the stable colour and reproducible results, the effect of various parameters is studied thoroughly. It was found that 5ml of 0.001M uranium (VI), 0.5ml of 0.01M HCl and 12ml of methanol are suitable. The absorbance values measured at 305nm with different known amounts of aspirin show the existence of linearity in the range 7.2 – 57.6 µg/ml (Fig 3.5.23)
Fig. 3.5.22. Absorption spectrum of U(VI) + aspirin

\[ [\text{U(VI)}] = 2 \times 10^{-4} \text{M}; [\text{HCl}] = 2 \times 10^{-4} \text{M}; \]
\[ [\text{Aspirin}] = 1.4 \times 10^{-5} \text{M} \]

Fig. 3.5.23. Effect of aspirin acid on absorbance

\[ [\text{U(VI)}] = 2 \times 10^{-4} \text{M} \]
\[ [\text{HCl}] = 2 \times 10^{-4} \text{M} \]

\[ \text{Amount of aspirin (\mu g/ml)} \]

\[ \text{Absorbance} \]

\[ \text{Wavelength (nm)} \]

c) Reaction with nitration mixture
2ml each of concentrated nitric acid and concentrated sulphuric acid are taken in a 25ml volumetric flask and a known volume of aspirin is added. The contents of the flask are heated on a water – bath maintained at 80°C for 10 minutes. Absorbance of the resulting nitrated product is measured at 330nm (Fig 3.5.24), after cooling the contents to room temperature against a blank solution containing no aspirin.

It is well known that organic compounds containing pH group are known to form yellow coloured nitrated product when they are made to react with nitration mixture. 2ml each of concentrated nitric and concentrated sulphuric acid are found to be adequate for the stable colour development. Experiments are carried with different known amounts of aspirin show the existence of linearity with absorbance in the range 1.44 – 11.5 μg/ml (Fig 3.5.25)

The methods are tested for their applicability with certain real samples containing aspirin. The results are presented in the Table 3.5.8.

**Interferences**

Caffeine, noscapine, codeinephos, present in tablet formulations do not cause interference in the determination.
Fig. 3.5.24. Absorption spectrum of nitrated aspirin
\[ [\text{HNO}_3] = 0.8; [\text{H}_2\text{SO}_4] = 1.4\text{M}; \]
\[ [\text{Aspirin}] = 1.6 \times 10^{-5}\text{M} \]

Fig. 3.5.25. Effect of aspirin acid on absorbance
\[ [\text{HNO}_3] = 0.8; [\text{H}_2\text{SO}_4] = 1.4\text{M} \]
Table 3.5.8

Assay of aspirin in drug formulations

(Average of five determinations)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labeled amount mg/Tab (or) Cap</th>
<th>Amount Found mg/Tab or Cap</th>
<th>Recovery %</th>
<th>Molar absorptivity $x , 10^3 \left( \text{1 mol}^{-1} \text{cm}^{-1} \right)$</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disprin$^a$</td>
<td>350</td>
<td>352</td>
<td>100.5</td>
<td>3.33</td>
<td>0.270</td>
</tr>
<tr>
<td>Megore$^b$</td>
<td>750</td>
<td>71</td>
<td>94.6</td>
<td>2.88</td>
<td>0.421</td>
</tr>
<tr>
<td>Topul$^c$</td>
<td>500</td>
<td>496</td>
<td>99.2</td>
<td>3.25</td>
<td>0.380</td>
</tr>
<tr>
<td>Micropyrin$^d$</td>
<td>350</td>
<td>349</td>
<td>99.7</td>
<td>2.90</td>
<td>0.291</td>
</tr>
<tr>
<td>Biospirin$^e$</td>
<td>900</td>
<td>895</td>
<td>99.4</td>
<td>2.95</td>
<td>0.362</td>
</tr>
</tbody>
</table>

$^a$ = Rickit and Colman of India Ltd., Kolkata, India
$^b$ = CTC Pharmaceuticals Ltd., Mumbai, India
$^c$ = Win – Medicare Pvt. Ltd., New Delhi, India
$^d$ = Nicholas Laboratories India Ltd., Mumbai, India
$^e$ = Biochem Pharmaceuticals Industries, Mumbai, India.
Pencillins

The pencillins are commonly named as penams, a designation in which the sulphur atom is given the top priority. Pencillins are containing β–lactane ring. Pencillins are used for the chemotheraphy of bacterial infections. They are excellent gram positive potency against susceptible staphylococci, streptococci. Many spectrophotometric methods available in literature for determination of pencillins. The author proposes a simple and selective method for the determination of pencillin. The details are discussed herein. In this method, pencillin is converted to pencillamine on acid hydrolysis followed by treatment with paracetamol and chromium(VI).

Recommended procedure

100 mg of pencillin is exactly weighed and the sample is hydrolysed with 10 ml of 5M hydrochloric acid. The solution is cooled and made up to the mark with water in a 100 ml volumetric flask. After hydrolysis excess of hydrochloric acid is removed by adding 10 ml of 5M sodium hydroxide to 10 ml of the hydrolysed pencillin. The resulting solution is diluted to 100 ml with water.

Aliquots (1.0 – 4.0 ml) of the drug prepared above are transferred into a 25ml volumetric flask and 1.5 ml of paracetamol of 3 x 10^{-2}M and 2 ml of 0.01 M potassium dichromate are added to it. The solutions are made up to the mark with distilled water. The resulting solution is heated on water bath for about 10 minutes at 80°C. The absorbance of the green coloured solution is measured at 585nm against a blank solution prepared similarly but with out pencillin (Fig. 3.5.26).
### Table 3.5.9

**Effect of paracetamol on absorbance**

\[
\text{[Pencillin]} = 1.6 \times 10^{-4} \text{M}; \quad \text{[Cr(VI)]} = 4.0 \times 10^{-4} \text{M}
\]

<table>
<thead>
<tr>
<th>\text{[Paracetamol]} \times 10^{4} \text{M}</th>
<th>\text{Absorbance}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>0.071</td>
</tr>
<tr>
<td>1.8</td>
<td>0.099</td>
</tr>
<tr>
<td>2.4</td>
<td>0.087</td>
</tr>
<tr>
<td>3.6</td>
<td>0.091</td>
</tr>
<tr>
<td>6.0</td>
<td>0.046</td>
</tr>
</tbody>
</table>

### Table 3.5.10

**Effect of Cr(VI) on absorbance**

\[
\text{[Paracetamol]} = 1.8 \times 10^{-4} \text{M}; \quad \text{[Pencillin]} = 1.6 \times 10^{-4} \text{M}
\]

<table>
<thead>
<tr>
<th>\text{[Cr(VI)]} \times 10^{4} \text{M}</th>
<th>\text{Absorbance}</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>0.051</td>
</tr>
<tr>
<td>4.0</td>
<td>0.101</td>
</tr>
<tr>
<td>6.0</td>
<td>0.137</td>
</tr>
<tr>
<td>8.0</td>
<td>0.139</td>
</tr>
<tr>
<td>10.0</td>
<td>0.139</td>
</tr>
<tr>
<td>12.0</td>
<td>0.140</td>
</tr>
</tbody>
</table>
Fig. 3.5.26: Absorption spectrum of Cr(VI) + paracetamol + penicillin

\[ [\text{Cr(VI)}] = 4 \times 10^{-4} \text{M}; \]
\[ [\text{Paracetamol}] = 4 \times 10^{-2} \text{M}; \]
\[ [\text{Penicillin}] = 1.6 \times 10^{-4} \text{M} \]

Fig. 3.5.27: Effect of penicillin on absorbance

\[ [\text{Cr(VI)}] = 8 \times 10^{-4} \text{M}; \]
\[ [\text{Paracetamol}] = 4 \times 10^{-2} \text{M}; \]
The effect of possible variables is studied in order to establish the optimum conditions for the determination of pencillin. It is observed that $1.8 \times 10^{-4}$M paracetamol and $8.0 \times 10^{-4}$M chromium(VI) are adequate for complete colour development (Tables 3.5.9 and 3.5.10). In the absence of paracetamol 3 to 4 peaks are noticed. Studies pertaining to the effect of temperature revealed that no reaction takes place in cold. After heating the reaction mixture for 1 minutes at $80^\circ$C complete stable colour development takes place. Heating to higher temperature causes no change in the absorbance. The solutions are stable for at least 24 hours.

The linear relationship is obtained between the amount of pencillin and the absorbance in the range $14.88 - 119.04$µg/ml (Fig. 3.5.27). The author believes that a ternary intermediate involving chromium (VI) – paracetamol and pencillin is responsible for the appearance of a single absorbance maximum. Similar ternary intermediate formation was reported by Sastry et al.,\textsuperscript{218} in the case of metal – pencillin chromium(VI).

The method is successfully applied for the pharmaceutical formulations containing pencillin (Table 3.7.10). The excipients and diluents do not interfere in the assay of pencillins. Extraction of the antibiotic from the pharmaceutical preparation is found unnecessary.