CHAPTER IV

Sensitive Spectrophotometric Assay of Piroxicam in Bulk and Pharmaceutical Formulations

This chapter is concerned with the development of indirect and direct spectrophotometric methods for the assay of piroxicam in bulk and pharmaceutical preparations. In the proposed methods, the optimum reaction conditions such as nature of acid, reagents concentration, sensitivity, stability of the reaction products etc., are discussed.

Abstract:

Two indirect and one direct spectrophotometric methods for the assay of piroxicam (PX) in bulk and pharmaceutical formulations have been proposed. The indirect methods are based on the oxidation of PX by a known excess of standard ceric ammonium sulphate (CAS) in an acidic medium followed by the reaction of excess oxidant with promethazine hydrochloride (PMH) and methdilazine hydrochloride (MDH) to yield red-coloured products. The absorbance values decreased linearly with increasing concentration of the drugs. The direct method was based on oxidation of PX by Fe (III) followed by bluish-green coloured complex formation with potassium ferricyanide (PFC). The systems obeyed Beer's law over the concentration ranges of 0.4-7.5, 0.2-10 and 0.1-5.0 ppm with PMH, MDH and PFC respectively. Molar absorptivity values, as calculated from Beer's law data were found to be 2.08 X 10^4, 2.05 X 10^4 and 4.51 X 10^4 l mol^{-1} cm^{-1} with PMH, MDH and PFC respectively. The common excipients and additives did not interfere with their determinations. The proposed methods have been successfully applied to the determination of PX in various dosage forms. The results obtained by the proposed methods compare favorably with those of official methods.

The results obtained by using PMH and MDH as reagents were published in: Analytical Sciences (Japan), 2002, 18, 671-674.
GENERAL DRUG PROFILE

Chemical name 4-Hydroxy-2-methyl-3-[pyrid-2-yl-carbamoyl]-2H-1,2-benzothiazine 1,1-dioxide

Structure

Molecular formula C\textsubscript{15}H\textsubscript{13}N\textsubscript{3}O\textsubscript{4}S

Molecular weight 331.4

Melting point 199-200°C

Description A white or slightly yellow crystalline powder

Solubility Practically insoluble in water, soluble in methylene chloride, dimethyl sulphoxide, sparingly soluble in ethanol.

Category Analgesic; anti-inflammatory; antipyretic

Piroxicam (PX) was prepared\textsuperscript{1} by treating iso-propanol, 3,4-dihydro-4-hydroxy-2-methyl-1,2-benzothiazine-3-carboxylate with 2-aminopyridine in xylene as a single solvent, instead of a mixture of solvents to increase the yield and to avoid secondary reactions. The 2-aminopyridine in xylene is added continuously for 4 h during which the azeotrope iso-propanol/xylene is dissolved off and recrystallisation takes place at 95-100°C in 2.5 parts DMF. It is a non-steroidal anti-inflammatory drug (NSAID) belonging to a class of compounds called oxicams\textsuperscript{2,3}. Its anti-inflammatory action is caused by its inhibition to prostaglandin synthetase. It is widely used in the treatment of

Chapter IV
rheumatological disorders. It may be administered systematically or topically and its once-daily administration has made it widely appreciated when NSAID treatment is required for a chronic condition. It is official in USP4.

LITERATURE SURVEY

In view of widespread use of PX, there is a need for the development of simple, sensitive and easily adoptable analytical methods for its assay in bulk and pharmaceutical formulations. Chemical literature reports analytical methods such as H1-NMR spectrometry, voltammetry, high-performance liquid chromatography and polarography for the assay of PX, which are expensive. Spectrophotometric technique continues to be the most preferred method for routine quality assurance because of its simplicity and reasonable sensitivity with significant economical advantages. The spectrophotometric methods, which are reported for its assay are given below:

Haematoxylin and chloramine-T in phosphate buffer of pH 7.0 were utilised by Sastry et al12 for the assay of PX. The hematein formed in situ from these reagents reacts with PX to give a red coloured complex having maximum absorption at 555 nm. In this method, the reagent mixture was kept for 20 min and then heated at 70°C for 5 min on a water bath. The method has Beer’s law range, molar extinction coefficient and Sandell’s sensitivity of 5-40 ppm, 4.86 X 103 l mol⁻¹ cm⁻¹ and 0.068 µg cm² respectively.

Co(II) and Cu(II) ions in neutral medium were employed by Kumar et al13 for the determination of PX. The method was based on the measurement of absorbances of chloroform soluble chromophores at 495 nm and at 378 nm for Co(II) and Cu(II) respectively. The methods have linear ranges of 50-300 ppm and 2-25 ppm for Co(II) and Cu(II) respectively.

The spectrophotometric method reported by Emmanuel et al14 involved the measurement of absorbances of pinkish red coloured product of PX with 2,6-dichloroquinone chlorimide in an alkaline medium (pH 7.2) at 540 nm. The method required 25 min for colour development. The Beer’s was valid over the concentration range of 10-50 ppm.

Chapter IV
The spectrophotometric method developed by Agarwal et al\textsuperscript{15} involved the formation of yellow coloured complex with Cu (II) in neutral medium. The product was extracted into chloroform and the absorbance measurements were carried out at 404 nm. The method obeyed Beer's law in the concentration range of 1.5-20 ppm.

Emara et al\textsuperscript{16} suggested a spectrophotometric method for the determination of PX using 2,2-diphenyl-1-picrylhydrazyl. The method was based on the decolourisation of violet coloured reagent on reaction with the drug. The method has a linear range of 2-20 ppm.

Phosphomolybdic acid in alkaline medium was utilised by Emmanuel et al\textsuperscript{17} for the spectrophotometric determination of PX. The absorbance measurements were carried out at 715 nm at room temperature. The method obeyed Beer's law in the concentration range of 10-30 ppm.

Apart from the above, literature also mentions some other spectrophotometric methods\textsuperscript{18-24} for the assay of PX (Table 4.1). But, most of these suffer from limitations such as extraction\textsuperscript{18,20}, long standing for colour formation\textsuperscript{19,22} and low sensitivity\textsuperscript{21,23,24}.

Owing to the growing concern with the applications of PX in pharmacology and the limitations of the reported analytical methods, it was thought worthwhile to develop simple and reasonably sensitive spectrophotometric methods for the assay of PX.

In this chapter, the investigator reports two indirect (Method A) and a direct (Method B) spectrophotometric methods for the assay of PX in bulk and pharmaceutical formulations.

**EXPERIMENTAL**

*Preparation of drug solution:*

A stock solution of pure PX was prepared by dissolving a suitable amount in 5 ml of acetone followed by its dilution with DMSO. It was further
standardized. The solution was stable at room temperature. It was diluted as
and when required.

**Reagent solutions:**

Aqueous solutions of 0.1 % each of promethazine hydrochloride (PMH) and
methdilazine hydrochloride (MDH) were prepared and stored in amber
coloured bottles in a refrigerator. \( \text{H}_2\text{SO}_4 \) (1.5 M) was used in the study.
Solutions of 0.1 % ceric ammonium sulphate (CAS) and 0.02 M ferric
ammonium sulphate (FAS) were prepared separately by dissolving appropriate
amounts of CAS and FAS initially in a few drops of dilute sulphuric acid and
then diluting to the mark with distilled water in 100 ml calibrated flasks. An
aqueous solution of 0.02 M potassium ferricyanide (PFC) was prepared and used
in the study.

**Recommended procedures**

After a systematic and detailed study of various parameters involved in
the formation of coloured products (as described under results and discussion)
the following procedures were recommended for the determination of PX in pure
and pharmaceutical formulations.

**Analysis of pure drug:**

**Method A:**

Suitable amounts of aliquots of standard PX (4.0-75 \( \mu \text{g} \) for PMH and
2.0-100 \( \mu \text{g} \) for MDH) were transferred in to a series of 10 ml calibrated flasks.
To these were added sulphuric acid (2.5 ml for PMH and 1.75 ml for MDH) and
CAS (1.0 ml for PMH and 0.8 ml for MDH). The contents of the flasks were
kept for 5 min at room temperature. Then 1.5 ml of PMH or 1.25 ml of MDH
were added. The contents were shaken well and the absorbances were measured
at 515 nm for PMH and at 513 nm for MDH.

A blank experiment was also carried out omitting PX. The decrease in
absorbance corresponding to PX was obtained by subtracting the absorbance of
the drug solution from the corresponding reagent blank. The amount of PX was computed from the calibration curves.

**Method B:**

Aliquots of standard solution containing 1.0-50 μg of PX were transferred in to a series of 10 ml calibrated flasks. A volume of 3.0 ml of FAS followed by 5.0 ml of PFC was added to each of the flask. The contents of the flask were diluted to the mark with distilled water and kept for 30 min at room temperature. The absorbances of bluish-green coloured complex were measured at 742 nm against the reagent blank.

**Analysis of pharmaceutical formulations:**

Twenty tablets of PX were finely powdered or mixed contents of ten capsules were taken. An amount equivalent to 20 mg of the drug was weighed accurately and transferred into a 100 ml beaker. Using a mechanical stirrer the powder was completely disintegrated in acetone for PX. The solution was filtered through a Whatman filter paper No. 42 and the filtrate was made up to 100 ml with DMSO. An aliquot of the solution was analyzed as described earlier for pure drug as in methods A and B.

**RESULTS AND DISCUSSION**

It is known that CAS oxidizes PX.\textsuperscript{26,27} It is also known that phenothiazine derivatives undergo reversible one electron oxidation by various oxidants in acidic medium at room temperature to yield coloured radical cations.\textsuperscript{28-34} These analytical aspects have been successfully utilized by the investigator to develop sensitive indirect spectrophotometric methods (method A) for the assay of PX in bulk and pharmaceutical formulations. This method is based on the oxidation of PX by CAS in sulphuric acid medium and subsequent formation of red coloured radical cations due to the oxidation of PMH or MDH by unused CAS. The coloured species exhibited absorption maxima at 513 nm and at 515 nm for MDH and PMH respectively (Fig. 4.1).
In method B, the PX undergoes oxidation by FAS, and Fe (II) thus formed in the reaction combines with PFC to give Prussian blue complex, \( \text{Fe}_4[\text{Fe(CN)}_6]_3 \), having absorption maximum at 742 nm (Fig. 4.2).

**Fixation of optimum reaction conditions for the formation of coloured products:**

In order to establish the optimum conditions necessary for the rapid and quantitative formation of the coloured products with maximum stability and sensitivity, the investigator ran several trials before he could achieve success.

**METHOD A:**

**Effect of acid on the sensitivity and stability of the coloured species:**

The medium plays an important role in the proposed methods. Various acids (HCl, H₂SO₄, H₃PO₄ and CH₃COOH) were tried for this purpose. Sulphuric acid was found to be the most suitable for the oxidation of PX by CAS and for latter’s determination with PMH and MDH. The stability of the coloured species obtained by the interaction of CAS with PMH and MDH also depends upon the strength of sulphuric acid. It was further noticed that the stable and intense red coloured products were formed with 1.5 M H₂SO₄ (2.0-3.0 ml for PMH and 1.5-2.0 ml for MDH). Below these volumes of acid, less intense coloured products were observed while at higher volumes of acid, the coloured species formed were found to be unstable (Fig. 4.3). Hence, 2.5 ml and 1.75 ml of H₂SO₄ for PMH and MDH respectively were used in a total volume of 10 ml for subsequent study.

**Effect of CAS:**

The formation of radical cations of PMH and MDH depends on the concentration of CAS. So, the effect of CAS on the oxidation of PMH and MDH was studied by measuring the absorbances of the radical cations at their respective \( \lambda_{\text{max}} \) values. It was found that 1.0 ml and 0.8 ml of 0.1 % CAS for PMH and MDH respectively was necessary for complete colour development (Fig. 4.4). At above or below the optimum volumes of CAS, the coloured
products formed were found to be either less sensitive or unstable and hence, the above mentioned volumes were strictly used in subsequent studies.

**Effect of PMH/MDH:**

The optimum volume of PMH/MDH required for the formation of radical cations of maximum stability and sensitivity was determined by measuring the absorbances at the respective $\lambda_{\text{max}}$ value for a series of solutions containing fixed concentration of CAS and sulphuric acid, and varying amounts of PMH/MDH. It was observed that a volume of 1.75 ml of 0.1 % PMH or 1.25 ml of 0.1 % MDH was sufficient for maximum absorbance (Fig. 4.5). Below these volumes, less intense coloured species were observed.

**METHOD B:**

**Effect of FAS on sensitivity:**

It was found that a volume of 2.5-3.5 ml of 0.02 M FAS was necessary for quantitative oxidation of PX (Fig. 4.6). However, less intense coloured species was observed when the volume of FAS was less than 2.5 ml or more than 3.5 ml. Hence, a volume of 3 ml in a total volume of 10 ml was used to ensure complete reaction.

**Effect of PFC on absorbance and stability of the species:**

A volume of 1.0 ml to 1.5 ml of 0.02 M PFC was found to be sufficient for optimum colour development of the coloured product (Fig. 4.6). Below or above these volumes of PFC, less sensitive species was obtained. Therefore, a volume of 1.25 ml of PFC was used for the study.

**Order of addition of reagents:**

From the preliminary experiments, it was found that the sequence of addition of reagents in both methods (A and B) was critical and hence the order suggested in the respective procedure was followed.
Precision and accuracy of the proposed methods:

The precision and accuracy of the proposed methods were checked by analyzing known amounts of PX (five replicates within Beer's law range). The low percentage of relative standard deviation and percentage error values (Table 4.2) highlighted excellent precision and accuracy of the proposed methods.

Optical characteristics of method A and method B:

In order to find whether the coloured products formed in the proposed methods are in accordance with Beer's law or not, the absorbances of a series of solutions containing varying amounts of PX were measured under optimized conditions. Beer's law limits (Fig. 4.7), optimum photometric range, molar absorptivity and Sandell's sensitivity values were evaluated and are recorded in Table 4.2. Least-square regression analysis was also carried out for evaluating the slope, intercept and correlation coefficient. The results are summarized in Table 4.2.

Stability of the coloured species:

Within the experimental time domain the drug solutions and the reagent solutions are stable. The coloured species formed were found to be stable at room temperature for more than 30 min and 1 h in methods A and B respectively. The intensity of the coloured species decreased slowly after 35 min in method A while in method B, precipitate was formed slowly after 60 min.

Studies of interferences of excipients and additives:

In order to assess the possible analytical applications of the proposed methods, the effects of excipients and additives that often accompany PX in various pharmaceutical formulations were studied by adding different amounts of these to known amounts of PX. It was observed that the talc, glucose, dextrose, magnesium stearate, lactose, starch and gelatin did not interfere in the determination of PX in synthetic mixtures at the following levels (mg):

1. PX (20), talc (400), glucose (300), dextrose (350), magnesium stearate (400), lactose (250), starch (300) and gelatin (250).
2. PX (20), talc (450), glucose (250), dextrose (375), magnesium stearate (425), lactose (275), starch (350) and gelatin (275).

A suitable amount of each mixture was analyzed using the procedure described for “Analysis of pharmaceutical formulations”. The percentage of PX found by the proposed methods was found to be in the range of 98.74 - 99.68 with RSD values less than 1.1 for five replicates.

**Statistical analysis of the results in comparison with the official method**:4

The results of the analysis of tablets and capsules were compared statistically by the Student t-test and by the variance ratio F-test with those obtained by the official method4. The Student t-values at 95% confidence level did not exceed the theoretical value, indicating that there was no significant difference between the proposed and official methods. It was also noticed that the variance ratio F-values calculated for p = 0.05 did not exceed the theoretical value, indicating that there was no significant difference between the precision of the proposed and official methods. The results are given in Table 4.3.

**CONCLUSIONS**

The proposed spectrophotometric methods are demonstrated as inexpensive alternatives to the most of the expensive existing procedures for the analysis of PX. The method involving Fe(III) and PFC as reagents is found to be more sensitive compared to other two proposed methods. The utility of the methods for the determination of PX in dosage forms has been well demonstrated. The assay methods do not involve any stringent experimental conditions and also free from interferences by the excipients and additives in levels found in dosage forms. The statistical and the recovery studies data clearly indicate the reproducibility and accuracy of the proposed methods. Hence, the proposed methods could be safely recommended for routine quality control in pharmaceutical industries.
REFERENCES


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1987, 20, 349.
27. H. G. Daabees, M. S. Mohamed, and Y. A. Beltagy, Alexandriya 
2001, 17, 979.
Fig. 4.1 Absorption spectra of radical cations of selected phenothiazine derivatives

1. PMH + \( \text{H}_2\text{SO}_4 \) + CAS
2. MDH + \( \text{H}_2\text{SO}_4 \) + CAS
Fig. 4.2 Absorption spectra of Prussian blue complex and reagent blank

1. PX (4 ppm) + FAS + PFC
2. Reagent blank
Fig. 4.3 Effect of volume of 1.5 M H₂SO₄ on the formation of radical cations of<br>\((\rightarrow)\) PMH and \((\leftarrow)\) MDH

Fig. 4.4 Effect of volume of 0.1 % CAS on absorbances of radical cations of<br>\((\rightarrow)\) PMH and \((\leftarrow)\) MDH
Fig. 4.5 Effect of volume of 0.1 % of (→) PMH and (←) MDH on the formation of radical cations.

Fig. 4.6 Effect of volume of 0.02 M (→) FAS and (←) PFC on the formation of coloured products of 4 ppm PX.
Fig. 4.7 Beer's law plots of PX with (—) PMH, (—*- ) MDH and (—*-) PFC.
Table 4.1 Literature survey on spectrophotometric determination of PX

<table>
<thead>
<tr>
<th>Reagent/s used</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Beer's law limits (ppm)</th>
<th>Remarks</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene violet</td>
<td>-</td>
<td>-</td>
<td>Involved extraction</td>
<td>18</td>
</tr>
<tr>
<td>p-Nitroaniline, NaNO$_2$ and NaOH in methanol</td>
<td>490</td>
<td>0.0 - 50</td>
<td>Absorbances were measured after 30 min</td>
<td>19</td>
</tr>
<tr>
<td>FeCl$_3$</td>
<td>528</td>
<td>32-192</td>
<td>The method involved extraction</td>
<td>20</td>
</tr>
<tr>
<td>FeCl$_3$ in 0.1 N HCl</td>
<td>530</td>
<td>50-400</td>
<td>Applicable to higher concentration of drug</td>
<td>21</td>
</tr>
<tr>
<td>NaOH and Folin-Ciocalteu’s phenol reagent</td>
<td>730</td>
<td>1.0 - 8.0</td>
<td>Required 15 min for completion of the reaction</td>
<td>22</td>
</tr>
<tr>
<td>Diazotised p-nitroaniline</td>
<td>490</td>
<td>0-50</td>
<td>Less sensitive</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Applicable at higher concentration of drug</td>
<td>24</td>
</tr>
<tr>
<td>Parameter</td>
<td>Values of</td>
<td></td>
<td></td>
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<tr>
<td>---------------------------------------</td>
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<tr>
<td></td>
<td>PMH</td>
<td>MDH</td>
<td>PFC</td>
<td></td>
</tr>
<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>515</td>
<td>513</td>
<td>742</td>
<td></td>
</tr>
<tr>
<td>Beer's law limits (ppm)</td>
<td>0.4-7.5</td>
<td>0.2-10.0</td>
<td>0.1-5.0</td>
<td></td>
</tr>
<tr>
<td>Molar absorptivity ($X \times 10^4$ mol$^{-1}$ cm$^{-1}$)</td>
<td>2.08</td>
<td>2.05</td>
<td>4.51</td>
<td></td>
</tr>
<tr>
<td>Sandell’s sensitivity (ng cm$^{-2}$)</td>
<td>15.92</td>
<td>16.12</td>
<td>5.45</td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.9995</td>
<td>0.9981</td>
<td>0.9995</td>
<td></td>
</tr>
<tr>
<td>Regression equation ($Y$)$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope, $b$</td>
<td>0.0607</td>
<td>0.0611</td>
<td>0.1866</td>
<td></td>
</tr>
<tr>
<td>Intercept, $a$</td>
<td>0.0151</td>
<td>0.0002</td>
<td>-0.0222</td>
<td></td>
</tr>
<tr>
<td>Relative standard deviation (%)$^d$</td>
<td>0.84</td>
<td>0.90</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>% Range of error$^d$ (95% confidence limit)</td>
<td>0.77</td>
<td>0.63</td>
<td>0.76</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ $Y=a+bX$, where $X$ is the concentration of drug in ppm.

$^d$ Average of five determinations.
Table 4.3 Analysis of PX in pharmaceutical formulations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim mg/cap mg/tab</th>
<th>Recovery* ± SD, % and their comparison with the official method³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USP³ method</td>
<td>Proposed methods</td>
</tr>
<tr>
<td></td>
<td>PMH</td>
<td>MDH</td>
</tr>
<tr>
<td>PX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsule 1</td>
<td>20 19.56 ± 0.67</td>
<td>19.48 ± 0.88 1.72; t = 1.23</td>
</tr>
<tr>
<td>Capsule 2</td>
<td>10 9.84 ± 0.75</td>
<td>9.79 ± 0.92 1.50; t = 1.13</td>
</tr>
<tr>
<td>Tablet 1</td>
<td>10 9.93 ± 0.52</td>
<td>9.89 ± 0.38 1.87; t = 1.22</td>
</tr>
<tr>
<td>Tablet 2</td>
<td>10 9.91 ± 0.93</td>
<td>9.87 ± 0.95 1.04; t = 1.12</td>
</tr>
</tbody>
</table>

* Average of five determinations.