Simple and Economical Visible Spectrophotometric Methods Development for Determination of Tiaprofenic Acid From its Solid Dosage Forms Based on Ion Association Complex Formation

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Two simple and sensitive extractive visible spectrophotometric methods (M1 and M2) for the determination of tiaprofenic acid in pure and dosage forms based on the formation of colored chloroform soluble ion-association associates under specified experimental conditions are described. Two basic dyes namely methylene blue (MB, method M1) and safranin-O (SAF-O, method M2) are utilized. The extracts of the ion-associates exhibit absorption maxima at 650nm and 520nm for methods M1 and M2 respectively. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 8-24 µg/ml for method M1 and 8-40 µg/ml for method M2. The proposed methods are applied to commercial available tablets and the results are statistically compared with those obtained by the UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the tiaprofenic acid in the presence of other ingredients that are usually present in dosage forms. These methods offer the advantages of rapidity, simplicity and sensitivity and normal cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

Keywords: Assay, NSAID, Ion-association complex, Methylene blue, Safranin-O

INTRODUCTION

Tiaprofenic acid (TPA) (Fig.1) is a non-steroidal, anti-inflammatory, analgesic chiral compound that belongs to the 2-aryl propionic acid (2-APA) class and also a potent inhibitor of prostaglandin biosynthesis in vitro and in vivo, due to the inhibition of cyclo-oxygenase (COX), used to treat pain, especially arthritic pain. Chemically it is (RS)-2-(5-benzoyl-2-thienyl) propanoic acid.

Its empirical formula is C14H12O2S representing molecular weight of 260.3. It is a white microcrystalline powder that is soluble in alcohol, acetone, methylene chloride and sparingly soluble in water and dilute HCl (<0.5%). The drug is available as the racemate and the S-enantiomer possessing most of the beneficial anti-inflammatory activity. The drug is absorbed well orally, with an absolute bioavailability of around 90%. TPA binds extensively to plasma albumin. The drug is listed in European Pharmacopoeia-5.0 (1) and suggests acid-base titrimetric method for determination of TPA in bulk and tablet formulations. Some analytical methods such as HPLC (2-3), spectrophotometry and RP-HPLC (4), Voltammetric and Spectrometry (5), PMR spectrometry (6), UV (7-8), Differential pulse Polarography (9) and enzyme immuno assay (10) have been reported in the literature for the determination of TPA in pharmaceutical preparations. The main purpose of the present study was to establish a relatively simple, sensitive and validated visible spectrophotometric methods for the determination of TPA in pure form and in pharmaceutical dosage forms, since most of the previous methods involve critical reaction conditions or tedious sample preparations and less specificity. So the authors have made some attempts in this direction and succeeded in developing these methods based on the reaction between the drug and basic dyes namely MB or SAF-O under specified experimental conditions. These methods can be extended for the routine quality control analysis of pharmaceutical products containing TPA.

As the extraction spectrophotometric procedures are popular for their sensitivity and selectivity in the assay of drugs, the extractive spectrophotometric technique (11) was therefore, utilized in the present work for the estimation of TPA. The present paper describes two simple and sensitive extraction visible spectrophotometric methods for the determination of TPA, based on its tendency to form chloroform extractable ion-associates with basic dyes belonging to Thiazine category dye methylene blue (CI No.52015) (Method M1) or safranin-O belonging to phenazine category dye (C.I.50240) (Method M2) under experimental conditions by exploiting acidic nature (carboxylic group) of the drug molecule.

MATERIALS AND METHODS

Apparatus and chemicals
A Milton Roy UV/Visible spectrophotometer model-1201 with 10nm matched quartz cells was used for all spectral measurements. Systronics model-362 pH meter was used for...
all the pH measurements. A pure drug sample of TPA was provided as a gift sample by Tychy industries, Hyderabad (AP) India. Surgam Tablets purchased from market. All the chemicals used were of analytical grade. Methylene blue (Fluka, 0.01%, 3.12x10⁻⁹ M prepared by dissolving 10mg of methylene blue in 100ml distilled water and subsequently washed with chloroform to remove chloroform soluble impurities), Safranin-O (Fluka, 0.01%, 2.857x10⁻⁴ M prepared by dissolving 10mg of Safranin-O in 100ml distilled water and subsequently washed with chloroform to remove chloroform soluble impurities), pH 9.8 buffer solution (prepared by mixing 7g of ammonium chloride with 6.8 ml of liquor ammonia and diluted to 100ml with distilled water and pH was adjusted to 9.8) were prepared for methods M₁ & M₂.

Table 1: Analysis of TPA in pharmaceutical formulations by proposed and reference methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Formulations</th>
<th>Labeled Amount (mg)</th>
<th>Found by Proposed Methods</th>
<th>Found by Reference Method</th>
<th>% Recovery by Proposed Method ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tablet-1</td>
<td>200</td>
<td>199.87 ± 2.53</td>
<td>197.46 ± 1.14</td>
<td>99.93 ± 1.26</td>
</tr>
<tr>
<td></td>
<td>Tablet-2</td>
<td>300</td>
<td>296.96 ± 1.54</td>
<td>295.10 ± 3.35</td>
<td>98.99 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>Tablet-1</td>
<td>200</td>
<td>197.3 ± 1.66</td>
<td>197.46 ± 1.14</td>
<td>98.6 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>Tablet-2</td>
<td>300</td>
<td>294.7 ± 4.49</td>
<td>295.10 ± 3.35</td>
<td>98.2 ± 1.49</td>
</tr>
</tbody>
</table>

* Tablet 1&2 Surgam Tablets of Sanofi Aventis**Average ± Standard deviation of six determinations, the t- and F-values refer to comparison of the proposed method with reference method (UV). Theoretical values at 95% confidence limits t = 2.57 and F = 5.05. # Recovery of 10mg added to the pre-analyzed sample (average of three determinations).

Preparation of Standard stock solution:
The standard stock solution (1mg/ml) of TPA was prepared by dissolving 100mg of TPA initially in 10ml of ethanolic HCl (1:1) and followed by dilution to 100 ml with distilled water. The working standard solution of TPA (200µg/ml) was obtained by appropriately diluting the standard stock solution with the same solvent.

Sample solution: About 10 tablets were pulverized and the powder equivalent to 100mg of TPA was weighed, dispersed in 25ml of IPA, sonicated for 30 minutes and filtered through Whatman filter paper No 41. The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparations.

Fig. 2. Beer’s Law Plot of TPA-MB

Recommended procedure:
Aliquots of the standard TPA solution [1.0-3.0ml, 200µg/ml (method M₁) and 1.0-5.0ml, 200µg/ml (method M₂)] were placed in a series 125ml separating funnels. Then 1.0 ml of pH 9.8 buffer solution and 0.5 ml of methylene blue solution (3.12x10⁻⁹ M) (for method M₁) or 1.0 ml of SAF-O (2.85x10⁻⁴ M) (for method M₂) were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0ml with distilled water. Then 10.0ml of chloroform was added to each separating funnel and the contents were shaken for 2 minutes. The two phases were allowed to separate. The absorbances of the separated chloroform layer were measured at 650nm (Method M₁) or 520nm (Method M₂) against a reagent blank within the stability period (5 minutes to 1 hour). The amount of drug was computed from its calibration graph (Fig. 2&3 showing Beer’s Law Plot).

Fig. 3. Beer’s Law Plot of TPA-SAF-O

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established by adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of methylene blue and safranin-O reagents and pH buffer solutions and solvent for final dilution of the colored species were studied. The water immiscible solvents tested for the extraction of colored complex into organic phase include chloro benzene, dichloromethane, carbon tetra chloride, benzene, n-butanol or
chloroform. Chloroform was preferred for its selective extraction of colored drug-dye complex into organic layer from the aqueous phase. The stoichiometric ratio of the dye-drug was determined by the slope ratio method and was found to be 1:1 for both methods (M1 and M2). The optical characteristics such as Beer’s law limit, Sandell’s sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing 3/4th of the amount of the upper Beer’s law limits), Regression characteristics like standard deviation of slope (Sb), standard deviation of intercept (Sa), standard error of estimation (S), and % range of error (0.05 and 0.01 confidence limits) were calculated using MS Excel software-2007 version and the results are summarized in Table-1. Commercial formulations containing TPA were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre-analyzed formulations at three different concentration levels. These results are summarized in Table-2.

Table 2: Optical characteristics, precision and accuracy of proposed methods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method M1</th>
<th>Method M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>λmax(nm)</td>
<td>650</td>
<td>520</td>
</tr>
<tr>
<td>Beer’s law limit (µg/ml)</td>
<td>8-24</td>
<td>8-40</td>
</tr>
<tr>
<td>Sandell’s sensitivity</td>
<td>0.002735043</td>
<td>0.003918367</td>
</tr>
<tr>
<td>Molar absorptivity</td>
<td>95172.1875</td>
<td>66430.72917</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = a + b c</td>
<td></td>
</tr>
</tbody>
</table>

Confidence limits

0.05 significance level

% Range of errors (95% confidence limits)

0.01 significance level

where Y = absorbance, c = concentration of TPA in µg/ml, a = intercept (calculated from the six measurements containing 3/4th of the amount of the upper Beer’s law limits), b = slope (calculated from the six measurements containing 3/4th of the amount of the upper Beer’s law limits), S = standard deviation of slope (Sb), Sa = standard deviation of intercept (Sa), S = standard error of estimation (S), and % range of error (0.05 and 0.01 confidence limits) were calculated using MS Excel software-2007 version and the results are summarized in Table-1. Commercial formulations containing TPA were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre-analyzed formulations at three different concentration levels. These results are summarized in Table-2.

**CONCLUSION**

A significant advantage of an extraction spectrophotometric determination is that it can be applied to the determination of individual compounds in a multi-component mixture. This aspect of spectrophotometric analysis is of major interest in analytical chemistry, since, it offers distinct possibilities in assay of a particular component in a complex dosage formulation. In the present study, TPA was determined successfully as pure compound as well as a component in representative dosage formulations. The proposed methods applicable for the assay of drug and the advantage of wider range under Beer’s law limits. The proposed extractive visible spectrophotometric methods are validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive and can be used as alternative methods to the reported ones for the routine determination of TPA depending on the need and situation.

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**REFERENCES**


**Fig.4.** Showing the probable scheme of the reactions

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