

## DEVELOPMENT OF NEW VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF TIAPROFENIC ACID IN BULK AND FORMULATIONS

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### ABSTRACT

A simple and sensitive visible spectrophotometric method has been developed for the determination of Tiaprofenic acid from its bulk drug and formulations. This method involves charge-transfer complex formation of tiaprofenic acid with in situ oxidized form haematein obtained from haematoxylin and chloramin-T (CAT) in the presence of pH 7 buffer solution, to form purple colored species of maximum wavelength at 555 nm. Regression analysis of Beer's law plot showed good correlation in the concentration range of 16-80 µg/ml. The proposed method is 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. The method is applied successfully for the estimation of the tiaprofenic acid in formulations without the interference of excipients. The method offers the advantages of rapidity, simplicity and sensitivity and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

**Keywords:** Assay, Beer's Law, Chloramin-T, Haematoxylin, NSAID, Regression equation.

### 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 cyclooxygenase (COX), used to treat pain, especially arthritic pain. Chemically it is (RS)-2-(5-benzoyl-2-thienyl) propanoic acid.

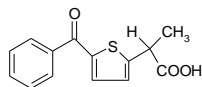


Fig.1: Chemical structure of TPA

Its empirical formula is C<sub>14</sub>H<sub>12</sub>O<sub>3</sub>S 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<sup>[1]</sup> and suggests acid-base titrimetric method for determination of TPA in bulk and tablet formulations. Some analytical methods such as HPLC, <sup>[2-3]</sup>, spectrophotometry and RP-HPLC <sup>[4]</sup>, Voltammetric and Spectrometry <sup>[5]</sup>, PMR spectrometry<sup>[6]</sup>, UV <sup>[7-8]</sup>, Differential pulse Polarography <sup>[9]</sup> and enzyme immuno assay<sup>[10]</sup> 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 method 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. In this method purple red colored charge-transfer complex formed between in situ oxidized form haematein obtained from haematoxylin-CAT <sup>[11]</sup> and sulphur in thiophene portion of TPA in the presence of (pH 7) buffer solution. The method can be extended for the routine assay of TPA formulations.

### MATERIALS & METHODS (EXPERIMENTAL)

A Milton Roy UV/Visible spectrophotometer model-1201 with 10mm matched quartz cells was used for all spectral measurements. A Systronics digital pH meter mode-362 was used for pH measurements. Pure drug sample is obtained from Tychy Industries, Hyderabad (AP) India. Its formulation Surgam tablets purchased

from the market. All the chemicals used were of analytical grade. 0.4% aqueous solution of CAT (BDH, 1.412x10<sup>-2</sup>M), 0.2% Haematoxylin (Sigma Aldrich, C.I.75290, 6.62x10<sup>-3</sup>M in methanol) and buffer solution (pH 7, by mixing 390ml of 0.067M potassium dihydrogen phosphate and 610ml of 0.067M disodium hydrogen phosphate) were prepared.

### Preparation of Standard stock solution

100mg TPA was dissolved initially in 10ml of methanol and then followed by dilution to 100 ml with distilled water to get 1mg/ml stock solution. This solution was further diluted stepwise with the same solvent to obtain working standard solution concentration of 400µg/ml.

### Sample solution

About 20 tablets were weighted to get the average tablet weight and pulverized and the powder equivalent to 100mg of TPA was weighed, dispersed in 25ml of isopropyl alcohol (IPA), sonicated for 30minutes and filtered through whatman filter paper no.41. The filtrate was evaporated and the residue was used for the preparation of working sample solution in the same way as under working standard solution.

### Assay

To a series of 25ml graduated test tubes, 1.0ml each of haematoxylin, CAT and 15ml pH 7 buffer solutions were added successively. The mixture was kept aside for 20 minutes. Then add aliquots of standard TPA solution (1.0-5.0ml, 400µg/ml) and kept in water bath at 70°C for 5 minutes. Test tubes removed and cooled to room temperature and diluted to 25ml with distilled water. The absorbance was measured at 555nm against the similar reagent blank within 30 minutes. The amount of TPA was computed from its calibration graph (Fig.2 showing Beer's law plot).

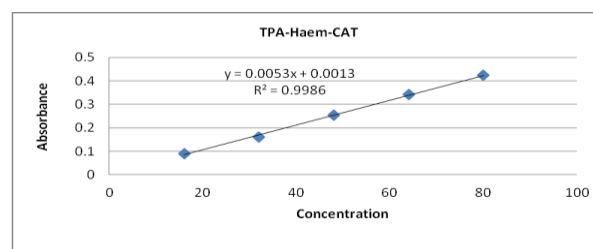


Fig.2: Beer's law plot

## RESULTS AND DISCUSSIONS

In developing a method, systematic studies of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed (OVAT method). The effect of various parameters such as time, temperature, nature and concentration of oxidant, volume and strength of haematoxylin, CAT reagents and buffer solution, order of addition of reagents on color development and solvent for final dilution on the intensity and stability of the colored species were studied and the optimum conditions were established. The various oxidants such as  $\text{NaIO}_4$ ,  $\text{K}_2\text{Cr}_2\text{O}_7$ , CAT and potassium hexacyanoferrate (III) were tried. But CAT was found to be the best by virtue of stability considerations. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile were found to provide no additional advantage. So distilled water is selected as a solvent for final dilution of the colored species. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from the six measurements containing 3/4<sup>th</sup> of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope ( $S_b$ ), standard deviation of intercept ( $S_a$ ), standard error of estimation ( $S_e$ ) and % range of error (0.05 and 0.01 confidence limits) were calculated using MS Excel software-2003 and are shown in Table-1.

Commercial formulations containing TPA were successfully analyzed by the proposed method. The values obtained by the proposed and reference method (UV method in ethanolic HCl (1:1) developed in our laboratory,  $\lambda_{\text{max}}$  305nm) for formulations were compared statistically by the t-and F-test and found not to differ significantly.

Table2: analysis of tpa in formulations by proposed and reference methods.

Method	Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method $\pm$ SD	#% Recovery by Proposed Method $\pm$ SD
			*Amount found $\pm$ SD	t	F		
TPA- Haem	Tablet-1	200	196.43 $\pm$ 1.06	2.06	1.14	197.46 $\pm$ 1.14	98.22 $\pm$ 0.53
	Tablet-2	300	294.81 $\pm$ 4.13	0.615	1.52	295.10 $\pm$ 3.35	98.27 $\pm$ 1.38

Tablet 1 & 2 Surgam tablets of Sanofi Aventis

\*Average  $\pm$  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).

Reference method (UV method) using ethanolic HCl developed in our laboratory ( $\lambda_{\text{max}}$ =305nm).

### Chemistry of colored species

Haematoxylin [7,11b-dihydroindeno-{2,1-c}chromene-3,4,6a,9,10(6H)-pentol] is a catechol derivative, extracted from the heart wood of the log wood (Haematoxylon campechianum) and is an important staining agent. When treated with an oxidizing agent (CAT), it undergoes oxidation to yield haematein (o-hydroxy quinone derivative). The extent of haematein formed depends upon conditions maintained (pH, oxidizing agent, other components present, solvent media). It has been widely used for the determination of several metal ions (aluminum, arsenic, tin, molybdenum).

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. The ingredients usually present in formulations of TPA did not interfere with the proposed analytical method.

Table 1: Optical Characteristics, Precision And Accuracy Of Proposed Method

Parameters	Results
$\lambda_{\text{max}}$ (nm)	555
Beer's law limit( $\mu\text{g}/\text{ml}$ )	16-80
Molar absorptivity (l/mol/cm)	34435.52083
Sandell's sensitivity ( $\mu\text{g}/\text{cm}^2/0.001$ abs. unit)	0.007559055
Regression equation	*Y=a+ b x
Slope (b)	0.005
Intercept(a)	0.001
Correlation coefficient	0.998
% RSD	2.05
% Range of errors (95% Confidence limits)	
0.05 significance level	2.16
0.01significance level	3.38

\*Y = a + b x, where Y is the absorbance and x is the concentration of TPA in  $\mu\text{g}/\text{ml}$

Sastry et al reported spectrophotometric methods for the determination of several organosulphur compounds (penicillin -G, cephalosporins, thiol, methionine etc.) using haematoxylin-CAT as a reagent. Based on it the method is developed. This method appears to be due to the formation of charge-transfer complex involving in situ formed haematein (oxidation product of haematoxylin with CAT, electron acceptor due to the presence of enolic form of o-quinone moiety) and drug TPA (electron donor due to existence of hetero sulphur with lone pair electrons in thiophene moiety) as in the case of quinone and organosulphur compounds [12-13] (i.e., Chloranil, Pencillin-G) (Fig.3 showing Scheme).

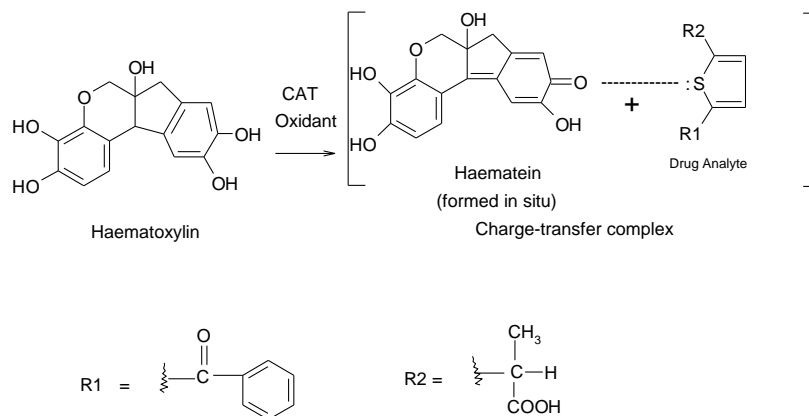


Fig.3: Probable Scheme of the Reaction

**CONCLUSIONS**

The procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed analytical method is validated as per ICH guide lines and possess reasonable precision, accuracy. The method offers the advantages of rapidity, simplicity, sensitivity and can be used as an alternative method to the reported ones for the routine determination of TPA depending on the need and situation.

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