



Trade Science Inc.

ISSN : 0974-7419

Volume 12 Issue 11

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 12(11) 2013 [439-442]

Quantitative determination of tiaprofenic acid by visible spectrophotometry using diazotization and coupling reactions

K.Raghubabu¹, N.Mohan¹, B.Kalyana Ramu^{2*}

¹Department of Engineering Chemistry, AU College of Engineering (A), Andhra University, Visakhapatnam-530003, AP, (INDIA)

²Department of Chemistry, Maharajah's College (Aided & Autonomous), Vizianagaram-535002, AP, (INDIA)
E-mail : Kalyanaramu23566@gmail.com; drraghualways@yahoo.co.in

ABSTRACT

A simple and sensitive visible spectrophotometric method for the determination of tiaprofenic acid in pure and dosage forms based on the formation of colored azodye under specified experimental conditions are described. The colored species exhibits absorption maxima at 470nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges 5-25 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. This method is applied successfully for the estimation of the tiaprofenic acid in the presence of other ingredients that are usually present in dosage forms. The method offers 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.

© 2013 Trade Science Inc. - INDIA

KEYWORDS

Assay;
NSAID;
Diazotisation;
Coupling;
Regression equation.

INTRODUCTION

Tiaprofenic acid (TPA) (Figure 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 C₁₄H₁₂O₃S 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 lit-

Full Paper

erature 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 sophisticated equipments which are costly and pose problems of maintenance. Hence they are not in the reach of most laboratories and small scale industries. So the authors have made some attempts in this direction and succeeded in developing the method using SA- NaNO_2 - α -NA⁽¹¹⁾ reagents based on the formation of colored azo dye. The method can be extended for the routine quality control analysis of pharmaceutical products containing TPA.

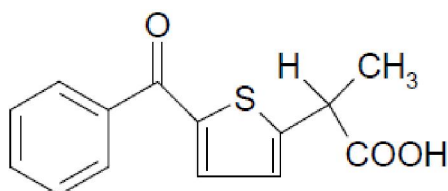


Figure 1 : Chemical structure of TPA.

MATERIALS & METHODS (EXPERIMENTAL)

Apparatus and chemicals

A Milton Roy UV/Visible spectrophotometer model-1201 with 10mm 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. Sulphanilamide (SD-fine, 0.5%, $7.25 \times 10^{-2}\text{M}$ prepared by dissolving 500mg of SA in 25ml of acetone), NaNO_2 (E.Merck, 2%, 0.29M prepared by dissolving 2.0g of sodium nitrite in 100ml distilled water), α -Naphthyl Amine (BDH, 0.2% $1.40 \times 10^{-2}\text{M}$ prepared by dissolving 200mg of α -NA in 100ml methanol) were prepared.

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 (100 $\mu\text{g}/\text{ml}$) 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.

Determination of wavelength maximum (λ_{max})

2.5ml of Standard TPA solution was transferred into 10ml calibrated tube. To this 0.1ml each of sulphanilamide, sodium nitrite, α -naphthyl amine solutions were added successively. Then total volume was brought to 5ml with distilled water and heated for 5 min at 70 $^{\circ}\text{C}$. After immersing the tube a water bath at 20 $^{\circ}\text{C}$ for 2 min, 2ml of ethanol was added and the volume in the calibrated tube was made up to the mark with distilled water. In order to investigate the wavelength maximum, the above colored solution was scanned in the range of 400-660 nm UV-Visible spectrophotometers against a reagent blank. From the absorption spectra (Figure 2), it was concluded that 470nm is the most appropriate wavelength for analyzing TPA with suitable sensitivity.

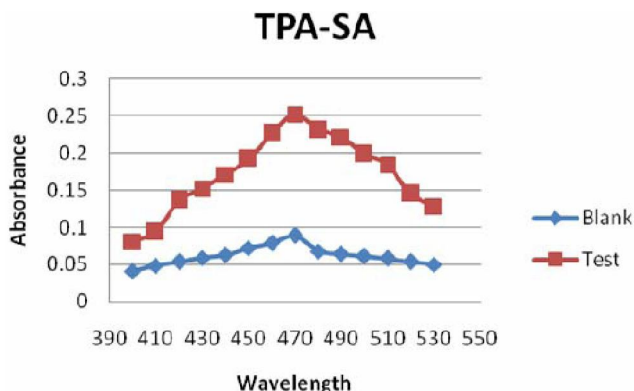


Figure 2 : Absorption spectra of TPA-SA-NANO₂- α -NA system.

Recommended procedure

Aliquots 0.5ml-2.5ml, 100 $\mu\text{g}/\text{ml}^{-1}$) of the standard TPA solution was transferred into a series of 10ml calibrated tubes. To this 0.1ml each of sulphanilamide, so-

dium nitrite, α -naphthyl amine solutions were added successively. Then total volume was brought to 5ml with distilled water and heated for 5 min at 70°C. After immersing the tube a water bath at 20°C for 2 min, 2ml of ethanol was added and the volume in the calibrated tube was made up to the mark with distilled water. The absorbance of the colored azo dye solutions were measured after 5 min at 470nm against a reagent blank prepared similarly. The content of the drug was computed from the calibration graph (Figure 3).

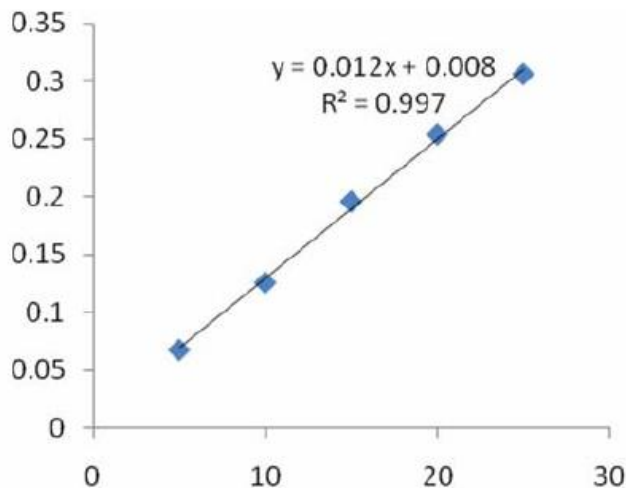


Figure 3 : Calibration graph of TPA-SA-NANO₂- α -NA system.

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 reagents, volume and strength of reagents and 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. 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 3/4th 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.

TABLE 1 : Optical characteristics, precision and accuracy of proposed method.

Parameters	Results
λ_{max} (nm)	470
Beer's law limit($\mu\text{g/ml}$)	5-25
Molar absorptivity (l/mol/cm)	34012.53333
Sandell's sensitivity ($\mu\text{g/cm}^2/0.001$ abs. unit)	0.007653061
Regression equation	
*Y=a+ b x	
Slope (b)	0.012
Intercept(a)	0.008
Correlation coefficient	0.997
% RSD	1.11
% Range of errors (95% Confidence limits)	
0.05 significance level	1.16
0.01significance level	1.82

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

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, λ_{max} 305nm) 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. The ingredients usually present in formulations of TPA did not interfere with the proposed analytical method.

Chemistry of colored species

Alkanols are generally determined by converting them into alkylnitrites. The latter upon hydrolysis liberates nitrous acid stoichiometrically. The liberated nitrous acid is used for diazotizing a primary aryl amine (SA). The diazo compound so produced is coupled to an amine (NA) in the usual manner to yield a dye. In the present

Full Paper

TABLE 2 : 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	197.81 \pm 1.03	0.62	1.22	197.46 \pm 1.14	98.90 \pm 0.51
	Tablet-2	300	296.91 \pm 5.27	1.02	2.47	295.10 \pm 3.35	98.97 \pm 1.75

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 (λ_{\max} =305nm).

investigation, the free carboxyl group present in the drug is involved for the release of nitrous acid from NaNO_2 . The formed diazotizes SA, which is then coupled with α -naphthylamine to give a colored azo dye (Figure 4-Scheme).

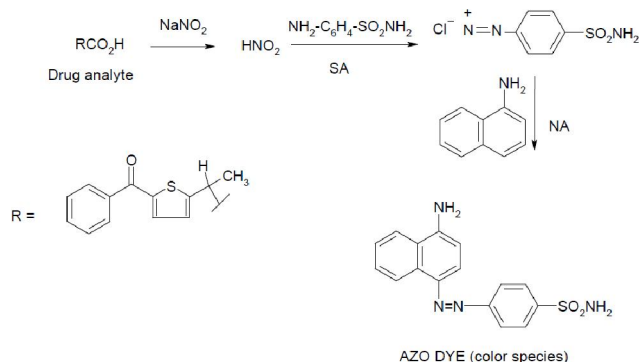


Figure 4 : Scheme of the reaction.

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.

ACKNOWLEDGEMENTS

The authors are very much thankful to m/s Tychy Industries., R&D division, Hyderabad, AP (India) for providing gift sample of the drug.

REFERENCES

- [1] EP, European Pharmacopoeia, 5th Edition, 2578, (2005).
- [2] F.Jamali, A.S.Russell, B.W.Berry, C.Lehmann; Journal of Chromatography B: Biomedical Sciences and Applications, **310**, 327-333 (1984).
- [3] G.Geisslinger, S.Menzel, K.Brune; Journal of Chromatography B: Biomedical Sciences and Applications, **675(1)**, 77-81 (1996).
- [4] M.Sayeed Arayne, Najma Sultana, M.Hasim Zuberi; Urooj Haroon; J.Chil.Chem.Soc., **55(2)**, 206-211 (2010).
- [5] Ali, M.M.Azza, M.Emara Kamla, Khodari Mahmoud; Analyst, **119**, 1071-1074 (1994).
- [6] M.A.Mahmoud, Hassan, Said E.Ibrahim; Spectroscopy Letters, **19(7)**, 719-724 (1986).
- [7] Tariq Ali, Omair Anwar Mohiuddin; Journal of Chemical and Pharmaceutical Research, **4(6)**, 2911-2918 (2012).
- [8] Beata, Zabrazewska, Anna Bogdan, Anna Kaniewskw, Ewa Duszak, Elzbieta Zelazowska; Acta Poloniac Pharmaceutica-Drug Research, **55(3)**, 179-188 (1998).
- [9] M.A.Mohamed, A.A.Wahbi, E.R.Gad-Kariem, M.M.Hassan; Journal Association of Anal. Chem., **67(4)**, 684-687 (1984).
- [10] B.Unterhalt, S.Baudner, J.M.Degenhardt; Pharmazie, **50(8)**, 571-572 (1995).
- [11] W.Nileeb, M.G.Boltz; Mettal (Berlin), **8**, 374 (1954).