International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 4 Apr 2022, pp: 655-660 www.ijaem.net ISSN: 2395-5252

Assay of Tiagabine.Hcl (Tia) Using Chromogenic Reagents by Spectrophotometric Methods

[1] V.Nagalakshmi, [2]G.Srinivasa Rao, [3]K.Subhashini, [4]N.Madhavi, [5]NVNB SrinivasaRao, [6]A.Nirmala Jyotsna [7]N.Gayatri D Devi

[1,2,3,4,7]Department of chemistry, Ch.S.D. St. Theresa's college for women, Eluru-534003,
[6]Department of physics, Ch.S.D.St. Theresa's college for women, Eluru-534003
[5]S.Ch.V.P.M.R Government Degree College, Ganapavaram
Address:Associate Professor in Chemistry, Ch.S.D. St. Theresa's college for women(A), Eluru-534003

Submitted: 05-04-2022 Revised: 14-04-2022 Accepted: 17-04-2022

ABSTRACT
Simple, accurate and reproducible UV-Visible spectrophotometric methods were established for the assay of TIA based on the redox reaction and internal salt formation. Redox reaction of the TIA with NBS/CB.reagent is proposed in method A. Method B includes internal salt formation of the TIA with Citric acid/ Acetic anhyddride reagent .The optical characteristics such as Beers law limits, molar absorptivity and Sandell's sensitivity for the methods (A-B) are given. Regression analysis using the method of least squares was made to evaluate the slope(b), intercept(a) and correlation coefficient (r) and standard error of estimation (Se) for each system. Determination of TIA in bulk form and in pharmaceutical formulations were also incorporated

KEY WORDS : Redox, Tiagabin, Complex formation , Anticonvulsant

I. INTRODUCTION:

Tiagabine.HCl (TIA) [1-3], is an anticonvulsant drug used to help control some types of seizures in the treatment of epilepsy. This medicine cannot cure epilepsy and will only work to control seizures for as long as you continue this drug. Its official status has been presented in Table 1.The characteristics, therapeutic importance, chemical names, structure, analytically useful functional groups and commercially available formulations of TIA are presented in (Tables 1& 2).

A very few physico-chemical methods appeared in the literature for the determination of TIA in pharmaceutical formulations LC-MS[4,5]and HPLC[6,7]. As the analytically important functional groups of TIA were not fully exploited, there is a scope to develop sensitive and flexible suitable spectrophotometric and HPLC methods. Based on the above feature the author had attempted to develop new UV-Visible Spectrophotometric and HPLC methods for its determination in bulk and pharmaceutical formulations.

The methods developed by the author are based on the different chemical reactions (reactivity of functional groups) of TIA with various dyes and chromgenic reagents that produced colored species with reasonable stability paving the possibility for visible spectrophotometric determination of TIA in its bulk form and in pharmaceutical formulations. A reported spectroscopic method was chosen as reference method for compa ring the accuracy of the results obtained by the proposed methods.

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 4 Apr 2022, pp: 655-660 www.ijaem.net ISSN: 2395-5252

TABLE 1:STURCTURAL FEATURES OF TIAGABINE HCL

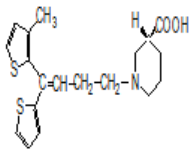
SI. No	Generic Name	Category	Chemical Name	Structure	Analytical important moieties/functional groups
1	Tiagabine Hcl	Anti convulsant	Piperdine carboxylic,1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-,3(R)		Aliphatic Carboxylic, Tertiary nitrogen and Sulphur groups.

TABLE – 2:PHYSICO CHEMICAL CHARACTERISTIC AND THERAPEUTIC IMPORTANCE OF TIAGABINE HCL

Category	Characteristics	Mode of action and therapeutic use
Anticonvulsant	Molecular formula: Formula Weight : 412.0 g/moles Appearance: White, odorless, crystalline powder. Solubility: Practically insoluble in heptane; sparingly soluble in water, and soluble in aqueous base.	It is used to help control some types of seizures in the treatment of epilepsy. This medicine cannot cure epilepsy and will only work to control seizures for as long as you continue to take it.

II. EXPERIMENTAL

2.1 Instruments used:

An Elico, UV – Visible digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

2.2 Preparation of standard drug solutions:

An 1mg/mL of TIA was prepared by dissolving 100mg of it in 100mL with distilled water. This solution was further diluted step wise with distilled water to obtain working standard solution of corresponding concentrations 200 μgmL^{-1} [M_1, M_2,]

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 4 Apr 2022, pp: 655-660 www.ijaem.net ISSN: 2395-5252

2.3 Proposed procedures:

After systematic and detailed study of the various parameters involved, the following procedures [Methods (M_1) NBS/CB; Citric Acid-acetic anhydride reagent(M_2);] were recommended for the assay of TIA in bulk samples and pharmaceutical formulations.

2.4.1 **For Bulk samples**

2.4.1.1 Method – M_1

Aliquots of standard TIA solution (0.5-3.0mL, 200μg.mL^{-1}) were transferred into a series of 25mL calibrated tubes. Then 1.25mL (5.0M) of HCI and 2.5mL (5.618x10^{-4} M) of NBS were added. The volume was brought to15mL with distilled water. After 10min, 10mL (5.50 x 10^{-4}M) of CB solution was added and mixed thoroughly. The absorbance was measured after 5min at 540nm against distilled water. The blank (omitting drug) and dye (omitting drug and oxidant) solutions were prepared in a similar manner and their absorbance's were measured at 540nm against distilled water. The decrease in absorbance corresponding to consumed NBS and in turn the drug concentration was obtained by subtracting the decrease in absorbance of the test solution (dye-test) from that of the blank solution (dye-blank). The amount of TIA was computed from its calibration graph (Fig. 3).

2.4.1.2 Method – M_2

Aliquots of standard TIA solution (0.5 - 3.0mL, 200μg.mL^{-1}) were taken into a series of 25mL graduated tubes and gently evaporated on a boiling water bath to dryness. To this 10.0mL (6.245x10^{-2}M) citric acid - acetic anhydride reagent was added and the flasks were immersed in a boiling water bath for 30 min. The tubes were cooled to room temperature and made up to the mark with acetic anhydride. The absorbance of the colored solutions was measured after 15min at 580nm against a reagent blank. The amount of TIA was calculated from the calibration graph (Fig. 4).

2.4.2 Pharmaceutical formulations:

An accurately weighed portion of tablet content equivalent to about 100 mg of TIA was transferred into a 100mL volumetric flask. Added about 80mL of warm isopropyl alcohol and shaken well for about 20min. The contents were diluted with isopropyl alcohol up to the mark and mixed thoroughly. The solution was filtered. The filtrate was evaporated to dryness. The residue was used for the preparation of formulation solutions for different methods as given under standard solutions preparations. These solutions were analyzed as under procedures described fro bulk solutions.

III. RESULTS AND DISCUSSIONS:

3.1 Spectral Characteristics:

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of TIA were taken and colors were developed separately by following the above procedures. The absorption spectra were scanned on a spectrophotometer in the wave length region of 340 to 900nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results were graphically represented in Fig. 1&2,. The absorption curves of the colored species in each method show characteristics absorption maxima where as the blank in each method has low or no absorption in this region.

3.2 Optimum conditions fixation in procedures:

3.2.1 Method – M_1[NBS/CB]

The procedure involves two steps. The first step in the procedure is the reaction of TIA with an excess of NBS giving products involving oxidation, substitution or addition and the estimation of unreacted NBS using a known excess of CB (second step). The excess dye remaining was then measured with a spectrophotometer. The effect of reagent concentration (acidity, NBS and CB), waiting period in each step with respect to maximum sensitivity, minimum blank, adherence to Beer's law, reproducibility and stability of final color were studied by means of control experiments varying one parameter at a time and the optimum conditions are incorporated in (Table 3).

3.2.2 Method – M_2 [Citric acid – Ac_2O]

This method involves the formation of internal salt between TIA and aconite anhydride (dehydration product of citric acid). The optimum conditions in this method were fixed based on the study of the effects of various parameters such as strength and volume of the reagent, heating time, solvent used for final dilution and the stability of colored species. The results are incorporated in (Table 2).

3.3 Optical Characteristics:

In order to test whether the colored species formed in the above methods, adhere to Beer's law the absorbance's at appropriate wave lengths of a set of solutions containing varying amounts of TIA and specified amounts of reagents (as given in the

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 4 Apr 2022, pp: 655-660 www.ijaem.net ISSN: 2395-5252

recommended procedures for each method) were recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded against the corresponding reagent blanks and are recorded graphically (Figs. 3to 4). Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range for TIA in each method developed. With mentioned reagents were calculated. Least square regression analysis was carried out for getting the slope, intercept and correlation coefficient values (Table 3).

3.4 Precision:

The precision of each proposal methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of TIA in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (Table 3).

3.5 Accuracy:

To determine the accuracy of each proposed method, different amounts of bulk samples of TIA within the Beer's law limits were taken any analyzed by the proposed method. The results (percent error) are recorded in (Table 3).

3.6. Interference studies:

The effect of wide range of execipients and other active ingredients usually present in the formulations for the assay of TIA in methods M_1, M_2 under optimum conditions were investigated. The commonly used execipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

TABLE 3: OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS FOR TIA

Parameter	M_1	M_2
λ_{max} (nm)	750	470
Beer's law limits (µg/mL)	2-12	4 – 24
Detection limit (µg/mL)	0.7475	2.093
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	$1.423\ 10^4$	7.205×10^3
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.1027	1.550×10^{-1}
Optimum photometric range (µg/mL)	5.0-12	12-20
Regression equation (Y=a+bc) slope (b)	0.0302	0.02075
Standard deviation on slope (S_b)	1.1335×10^{-3}	7.901×10^{-4}
Intercept (a)	4.999×10^{-12}	5.5×10^{-3}
Standard deviation on intercept (S_a)	7.519×10^{-3}	10.48×10^{-3}
Standard error on estimation (S_e)	7.169×10^{-3}	9.994×10^{-3}
Correlation coefficient (r)	0.9999	0.9966
Relative standard deviation (%)	1.557	1.222
% Range of error (confidence limits)		
0.05 level	1.79	1.405
0.01 level	2.80	2.203
% error in Bulk samples **	-0.260	-0.241

* Average of six determinations considered
** Average of three determinations

International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 4 Apr 2022, pp: 655-660 www.ijaem.net ISSN: 2395-5252

Fig. 1.: Absorption spectrum of TIA with NBS - CB (M_1)

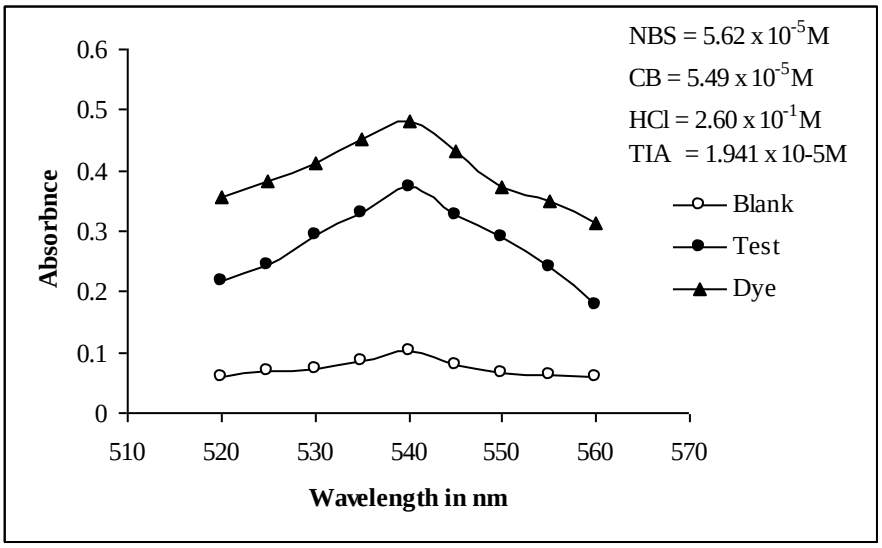
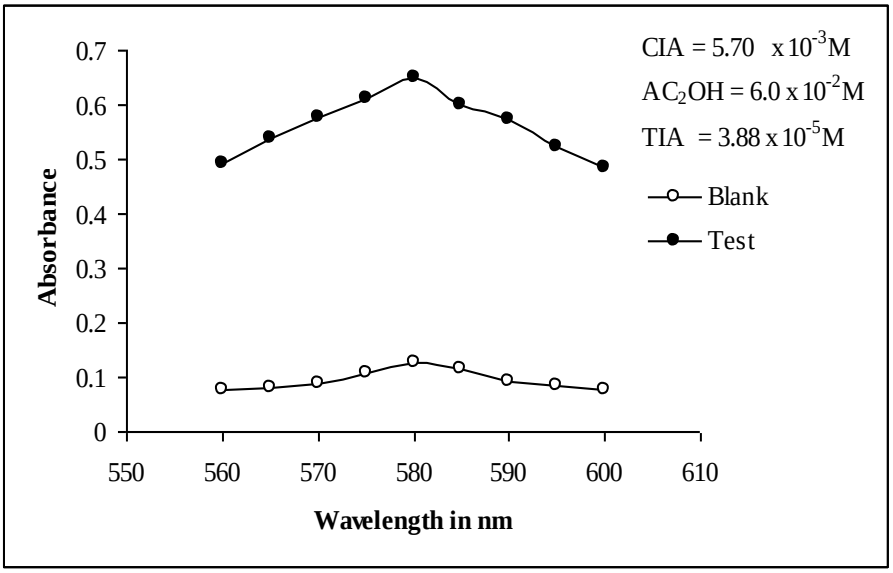


Fig. 2: Absorption spectrum of TIA with Citric acid – AC_2OH (M_2)



International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 4 Apr 2022, pp: 655-660 www.ijaem.net ISSN: 2395-5252

FIG. 3: BEER'S LAW PLOT OF TIA WITH NBS-CB (M_1)

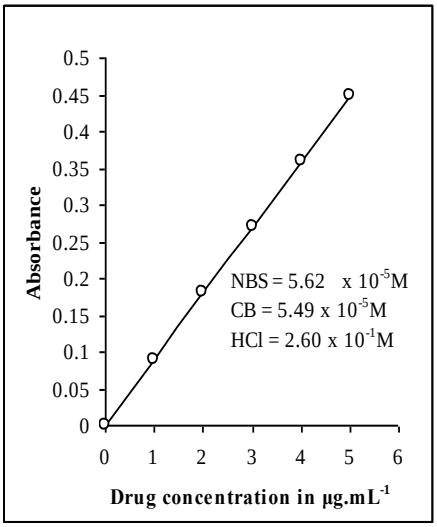
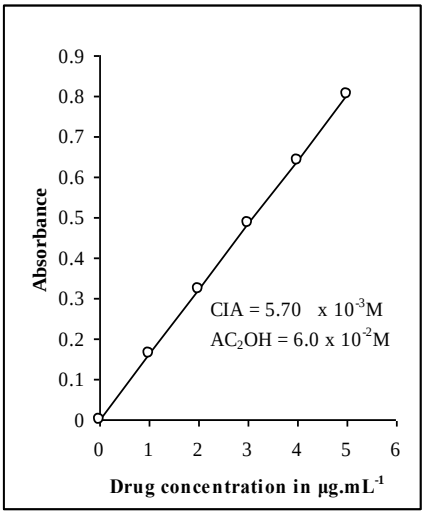


FIG. 4: BEER'S LAW PLOT OF TIA WITH Citric acid – AC_2OH (M_2)



IV. CONCLUSIONS

It can be observed from the results presented above, that the proposed methods have the good sensitivity ε_{max} and higher λ_{max}. Statistical analysis of the results sows that the proposed procedures have good precision and accuracy. Results of the analysis of pharmaceutical formulations reveal that the proposed methods are suitable for their analysis with virtually no interference of the usual additives present in pharmaceutical formulations. The order of sensitivity (ε_{max}) among the proposed methods is: $M_2 > M_1$. All the proposed methods are simple, sensitive and reliable and can be used for routine determination of TIA in bulk samples and pharmaceutical formulations depending upon the need of specific situation.

REFERENCEES

[1]. K.E.Andersen etal. J.Med.Cnem.36,1716(1993).
[2]. C.L.Faingold etal., Exp., Neurology,126, 225 (1994)
[3]. Mengel, Helle., Epilepsia, 35 (suppl.5), 581-584 (Eng) 1994
[4]. L.E.Gustavson, S.chu, J.Chromatograph . 574, 313 (1992)
[5]. J.Chromatograpgh. 503(1), 115-25(Eng)(1990)
[6]. Chollet, D.F., Castella,. E., Goumaz, L., Anderegg, G. J.Pharm. Biomed. Anal., 21(3), 641-646(Eng)1999.
[7]. Rustum , Abu M., Estrada, Victoria; J.chromatogr., B:Biomed-sci.Appl., 705(1), 111-117(Eng)1998.