SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF TIAGABINE HCl USING CHROMOGENIC REAGENTS

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Abstract: **Simple, accurate and reproducible UV-Visible spectrophotometric methods were established for the assay of Tiagabine HCl (TIA) based on the redox and complex reactions. Redox reaction of the TIA with NBS/PMAP-SA is proposed in method A. Method B includes complex formation of TIA with SNP-HA. 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.**

Keywords: Estimation, Tiagabine, Spectrophotometric method, Validation, Chromogenic rearents

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. **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. The aim of this study was to develop and validate two UV-Visible spectrophotometric methods for the determination of tiagabine in the presence of formulation 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 comparing the accuracy of the results obtained by the proposed methods.

II. Methods and Materials

Apparatus: An Elico, UV-Visible digital spectrophotometer (SL - 159) 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.

Reagents and standards:

The stock solution (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 concentration 200 μgmL^{-1}[, $\mathbf{M_A}$, $\mathbf{M_B}$]

Method A:

NBS solution: Prepared by dissolving 88mg of N-Bromo succinimide in 100mL of distilled water and standardized iodometrically.

PMAP solution: Prepared by dissolving 300mg of p-N-methylaminophenol sulphate in 100mL of distilled water.

SA solution: Prepared by dissolving 200mg of sulphanilamide in 2.5mL of 0.05M HCI followed by dilution to 100mL with distilled water.

Method B:

SNP solution: Prepared by dissolving 500mg of sodium nitroprusside in 100mL of distilled water.

HA solution: Prepared by dissolving 500mg of hydroxylamine hydrochloride in 100mL of distilled water.

Na_2CO_3 solution: Prepared by dissolving 10gms of sodium carbonate in 100mL of distilled water

Analysis of Pharmaceutical formulation:
Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 20 mg of Tiagabine was weighed accurately and extracted with isopropyl alcohol to eliminate any interference from excipients. It was filtered through Whatmann No. 42 filter paper and the residue was washed well with isopropyl alcohol for complete recovery of the drug. The isopropyl alcohol was evaporated to dryness and the drug was dissolved in doubly distilled water and diluted to 100 mL with doubly distilled water. It was further diluted if needed and then analyzed following the recommended procedures.

Method A:

Aliquots of standard TIA solution (1.0-5.0mL, 200µg.mL^{-1}) were transferred into a series of 25mL calibrated tubes. Then 0.5mL (8.75 x 10^{-1}M) of AcOH and 2.0mL (4.94 x 10^{-3}M) of NBS solutions were added and kept aside for 15min at room temperature. Then 1.5mL (8.71 x 10^{-3}M) of PMAP solution was added. After 2min, 2.0mL (1.16 x 10^{-2}M) of SA solution was added. The volume was made up to the mark with distilled water. The absorbance was measured after 10min. at 520nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in the absorbance and in turn the drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of **TIA** was computed from its calibration graph (Fig 3).

Method B:

Aliquots of standard TIA solution (1.0-6.0mL, 200µg.mL^{-1}) were transferred into a series of 25mL-calibrated tubes. Then 1.0mL (1.678 x 10^{-2}M) of SNP and 1.0mL (7.195 x 10^{-2}M) of HA were added successively and kept aside for 5min. Then 1.0mL (9.43 x 10^{-1}M) of Na_2CO_3 solution was added and shaken for 15min. The volume was made up to the mark with distilled water. The absorbance was measured after 10min at 580nm against a similar reagent blank. The amount of **TIA** was computed from its calibration graph (Fig4).

In the above methods, a calibration curve was prepared by plotting the absorbance versus the concentration and the unknown was read from the calibration curve or deduced using a regression equation calculated from Beer's law data.

III Results and Discussions:

Method A is an indirect spectrophotometric method which involves two steps, oxidation of the TIA with NBS (first step) and estimation of the unconsumed NBS with PMAP-SA reagent (second step).In the first step, the volume of NBS required for oxidation of drug, the time and temperature for oxidation of the drug and volume of acetic acid were established through control experiments. In the second step, the volume of PMAP and the intermittent time between additions, volume of SA and the solvent for final dilution colored species were found by varying one parameter at a time and the optimum conditions are incorporated in Table 2.

The method B involves the reaction of TIA with SNP in the presence of hydroxyl amine hydrochloride. The optimum conditions in this method were fixed based on the study of the effects of various parameters such as volume of SNP solution, volume of HA, nature and volume of base, order of addition of reagents, time and temperature of the reaction, solvent for final dilution, the intensity and stability of colored species formed. The optimum conditions developed and the actual conditions chosen for the procedure are incorporated in **Table 1.**

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 whereas the blank in each method has low or no absorption in this region.

Fig. 1: Absorption spectrum of TIA with NBS – PMAP - SA (M_A)

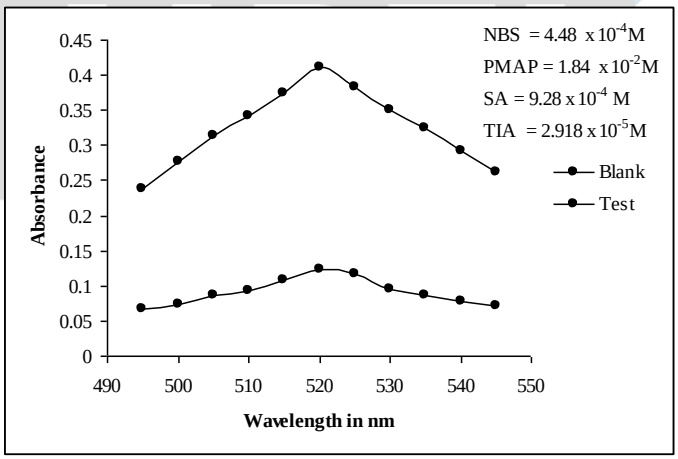


Fig. 2: Absorption spectrum of TIA with SNP – NH_2OH (M_B)

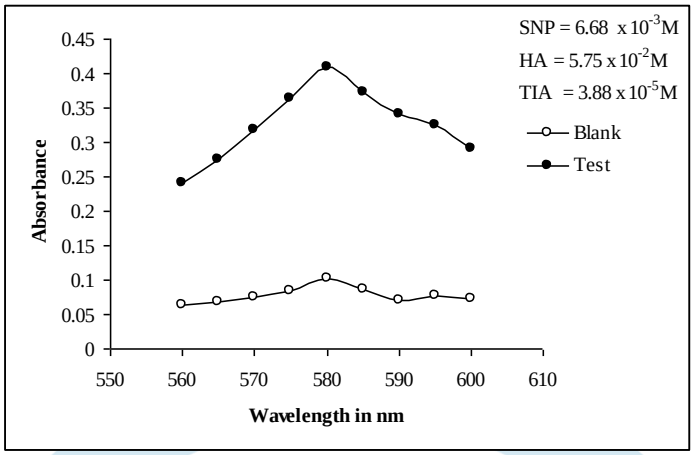


Fig. 3: Beer's Law plot of TIA with NBS – PMAP-SA (M_A)

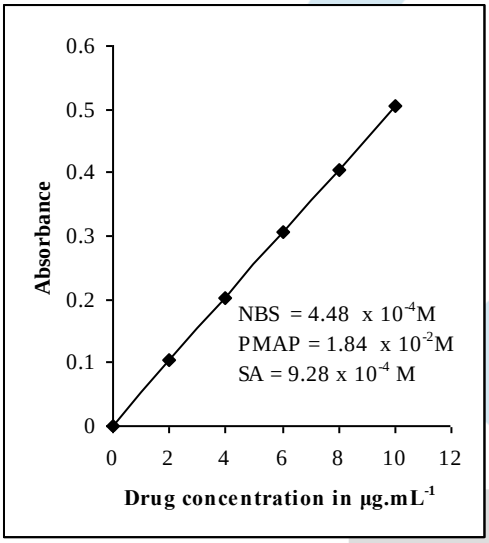
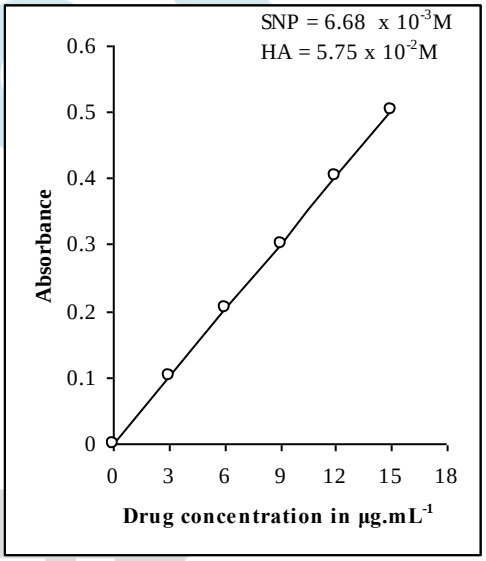


Fig. 4: Beer's Law plot of TIA with SNP-NH_2OH (M_B)



The accuracy of the methods was ascertained by comparing the results of proposed and reference methods statistically by the t-test and F-tests. The comparison shows that there is no significant difference between the results of studied methods and those of the reference ones as in Table 3. The similarity of the results is obvious evidence of the fact that during the application of these methods, the excipients are usually present in pharmaceutical formulations do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods, recovery experiments were carried out. The recovery of the added amounts of standard drug was studied at 3 different levels. Each level was repeated for 6 times. From the amount of drug found, the %recovery was calculated in the usual way. The higher λ_{max} values of the proposed methods have a advantage since the interference from associated ingredients should be generally less at higher wavelengths than at lower wavelengths. Thus the proposed visible spectrophotometric methods are simple and sensitive with reasonable precision and accuracy and used better alternatives to the existing ones to the routine determination of Tiagabine HCl in bulk forms and pharmaceutical formulations.

Table 1
Optical and regression characteristics, precision and accuracy of the proposed methods for TIA

PARAMETER	M_9	M_{19a}
λ_{max} (nm)	520	520
Beer's law limits (µg/mL)	4-24	0.8-4.8
Detection limit (µg/mL)	0.9330	0.2007
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	5.945×10^3	7.015×10^4
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.1838	3.546×10^{-2}
Optimum photometric range (µg/mL)	5-17.78	1.6-4.4
Regression equation (Y=a+bc) slope (b)	0.0137	0.1561
Standard deviation on slope (S_b)	2.980×10^{-4}	3.811×10^{-3}
Intercept (a)	9.999×10^{-4}	4.999×10^{-3}
Standard deviation on intercept (S_a)	3.953×10^{-4}	10.11×10^{-3}
Standard error on estimation (S_e)	3.769×10^{-3}	9.642×10^{-3}
Correlation coefficient (r)	0.9993	0.9992
Relative standard deviation (%)*	1.807	0.5311
% Range of error (confidence limits)		
0.05 level	2.07	0.6116
0.01 level	3.25	0.9576
% error in Bulk samples **	0.102	0.139

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

Table 2: Assay of TIA in Pharmaceutical Formulations

Formulations*	Amount taken (mg)	*Amount found by proposed Methods***		Reference method	Percentage recovery by proposed methods***	
		M_A	M_B		M_A	M_B
Tablet I	10	9.85±0.07 F=2.938 t=1.64	9.88±0.08 F=2.25 t=1.03	9.94±0.12	99.09±0.63	99.39±0.36
Tablet II	12	11.80±0.18 F=1.63 t=0.92	11.84±0.13 F=3.13 t=0.67	11.91±0.23	99.07±0.28	99.41±0.69

* Tablets from two different pharmaceutical companies.
** Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.262
*** Recovery of 10mg added to the preanalysed pharmaceutical formulations (average of three determinations).

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}**) between the methods is: **$M_A > M_B$**.These 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.

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