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VALIDATED SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF TIAGABINE HCl IN BULK AND FORMULATIONS

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Abstract : Simple, accurate and reproducible UV-Visible spectrophotometric methods were established for the assay of Tiagabine HCl(TIA) based on ion association complex reactions. Ion association complex reaction of the TIA with SFNO and MB is proposed in method 1 and 2. The optical characteristics such as Beers law limits, molar absorptivity and Sandell's sensitivity for the methods (1-2) 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.

INTRODUCTION

Tiagabine.HCl (TIA) (K.E.Andersen etal., 1993; C.L.Faingold etal., 1994; Mengel and Helle, 1994) 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(L.E.Gustavson and S.chu, 1992)and HPLC (Chollet, D.F etal., 1999, Rustum etal., 1998). 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.

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 100 μgmL^{-1}[, **M_1 , M_2]**

Method 1& 2: SFNO solution :

Prepared by dissolving 200mg of safranin O in 100mL of distilled water and subsequently washed with chloroform.

MB solution : Prepared by dissolving 200mg of MB in 100mL of distilled water and subsequently washed with chloroform.

Buffer solution (pH 9.8)NH_4OH – NH_4 Cl: 7gms of NH_4Cl and 6.8mL of liquid Ammonia solutions were mixed and diluted to 100mL with distilled water and pH was adjusted to 9.8.

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 1 & 2:Aliquots of standard drug solution for method M_{22a} & M_{22b} (0.5-3.0mL, 100µg.mL^{-1}) and 1.0mL of pH 9.8buffer solution were placed separately in a series of 125mL separating funnels. A volume of 1.0mL of Safranin o (for method M_{22a}) and 0.5mL of MB (for method M_{22b}) was added respectively. The total volume of aqueous phase in each funnel was adjusted to 10.0mL with distilled water. Then 10.0mL of chloroform was added in each separating funnel and the contents were shaken for 2min and allowed to separate. The organic layer was collected through cotton plug and the absorbance was measured immediate at 520nm (for method M_{22a}) and at 650nm (for method M_{22b}) against reagent blank. Both the colored species were stable for 2 hours. The amount of drug (**TIA**) in a sample was obtained from the Beer's Lambert plot. (**Fig. 3** for M_1 ;, M_2 for **Fig.4**).

Results and Discussions:

The optimum conditions in these methods were fixed based on the study of the effects of various parameters such as type of acid for buffer, conc. of acid, conc. of dye SFNO (M_1) or MB (M_2), choice of organic solvent, ratio of organic phase to aqueous phase, shaking time, temp, intensity and stability of the colored species in organic phase. The author performed controlled in pediments by measuring absorbance at λmax 530nm (M_1) or 655nm (M_2) of a series of solutions varying one and fixing the other parameter and the results are recorded 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 where as the blank in each method has low or no absorption in this region.

Fig. 1: Absorption spectrum of TIA with SFNO (M_1)

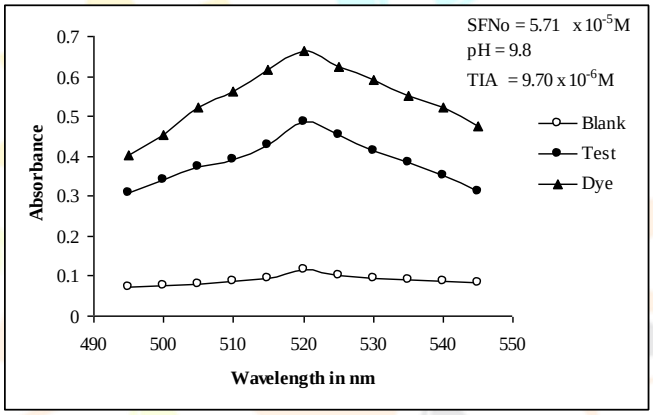


Fig. 2: Absorption spectrum of TIA with TIA with MB (M_2)

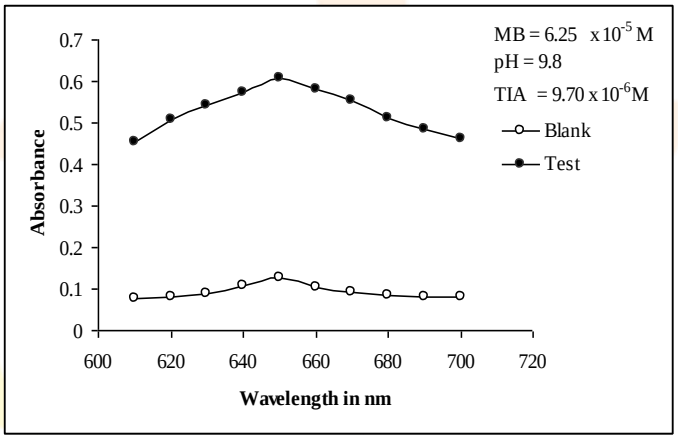


Fig. 3: Beer's Law plot of TIA with SFNO (M_1)

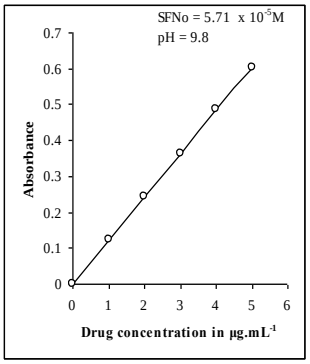
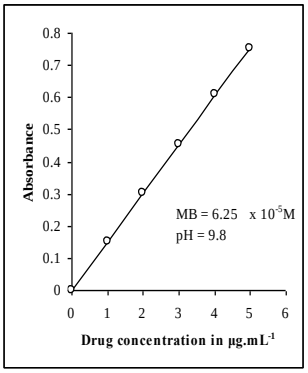


Fig. 4: Beer's Law plot of TIA with MB (M_2)



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_1	M_2
λ_{max} (nm)	540	620
Beer's law limits (µg/mL)	20 – 120	5 – 30
Detection limit (µg/mL)	5.994	3.158
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	1.664×10^3	5.295×10^3
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.4296	0.1907
Optimum photometric range (µg/mL)	45-105	12.6 – 30
Regression equation (Y=a+bc) slope (b)	9.209×10^{-3}	0.0143
Standard deviation on slope (S_b)	1.026×10^{-3}	7.654×10^{-3}
Intercept (a)	5.749×10^{-3}	2.25×10^{-3}
Standard deviation on intercept (S_a)	6.806×10^{-2}	1.269×10^{-1}
Standard error on estimation (S_e)	6.490×10^{-2}	1.210×10^{-1}
Correlation coefficient (r)	0.9993	0.9998
Relative standard deviation (%)*	0.9905	1.584
% Range of error (confidence limits)		
0.05 level	1.1389	1.822
0.01 level	1.7860	2.856
% error in Bulk samples **	0.348	-0.143

* 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_{22a}	M_{22b}		M_{22a}	M_{22b}
Tablet I	10	9.90±0.07 F=2.93 t=0.72	9.90±0.08 F=2.25 t=0.69	9.94±0.12	99.59±0.91	99.59±0.38
Tablet II	12	11.82±0.11 F=4.37 t=0.91	11.84±0.15 F=2.35 t=0.63	11.91±0.23	99.24±0.39	99.41±0.84

* Tablets from four 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).

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: $M_2 > M_1$.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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