ejpmr, 2018,5(12), 310-315

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

RP-HPLC METOD DEVELOPMENT AND VALIDATION ON TIAGABINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Article Received on 02/10/2018 **Article Revised on 22/10/2018** **Article Accepted on 12/11/2018**

ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Tiagabine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6×250mm) 5µ column using a mixture of Methanol and Water (50:50 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 247nm. The retention time of the Tiagabine was 2.187 ±0.02min. The method produce linear responses in the concentration range of 30-150ppm of Tiagabine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Tiagabine, RP-HPLC, validation.

INTRODUCTION

Tiagabine is used primarily as an anticonvulsant for the adjunctive treatment of epilepsy. The precise mechanism by which Tiagabine exerts its antiseizure effect is unknown, although it is believed to be related to its ability to enhance the activity of gamma aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system. Tiagabine binds to recognition sites associated with the GABA uptake carrier. It is thought that, by this action, Tiagabine blocks GABA uptake into presynaptic neurons, permitting more GABA to be available for receptor binding on the surfaces of post-synaptic cells. It is chemically known as (−)-(3R)-1-[4,4-bis(3-methyl-2-thienyl)-3-buten-1-yl]-3-piperidinecarboxylic acid. A literature survey revealed that very few analytical methods for this drug are available in pharmaceutical formulations. These include Uv spectrophotometry,[1] chiral chromatography,[2] and only few methods were reported for RP-HPLC,[3,4] for the estimation of this drug in bulk and in its formulation. Hence the present work targeted to develop a new precise, accurate and sensitive RP-HPLC,[5-10] method for the determination of Tiagabine in API and formulation. The developed method validated as per ICH guidelines.[11,12]

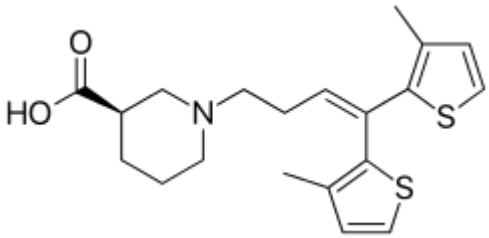

Figure 1: Structure of Tiagabine.

MATERIALS AND METHODS

Chemicals and reagents used

Tiagabine as pure standard reference drug was obtained from SURA LABS, Hyderabad, India. Acetonitrile, Methanol and Water used were of HPLC grade and purchased from MERCK specialties Private Limited, Mumbai, India.

Apparatus

HPLC analysis was performed on chromatographic system of water 2695 separation module with empower software liquid chromatography comprising water 996 photo diode array detector, Phenomenex Gemini C18 (4.6×250mm) 5µ was used and an equipped with auto sampler.

Preparation of standard solution

Accurately weigh and transfer 10 mg of Tiagabine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.9ml of the above Tiagabine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization
Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Water in proportion 50:50 v/v respectively.

Optimization of Column
The method was performed with various columns like Xterra and C18 column. Phenomenex Gemini C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape.

Preparation of Mobile Phase
Accurately measured 500ml (50%) of HPLC Methanol and 500ml of Water (50%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation
The Mobile phase was used as the diluent.

Experimental conditions
Chromatographic separation achieved using an analytical Phenomenex Gemini C18 (4.6×250mm) 5µ. Mobile phase consisted of Methanol: Water (50:50% v/v). The elution was achieved isocratically at a flow rate of 1.0ml/min with injection volume of 10µl. the column temperature was set at ambient temperature and chromatograph was recorded at wavelength 247nm.

Method development
Trials showed that mobile phase with reverse phase Phenomenex Gemini C18 (4.6×250mm) 5µ column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Tiagabine as carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Tiagabine was recorded in Figure 2. Now the sample solution was injected separately and chromatogram (Figure 3) was recorded until the reproducibility of the peak areas were satisfactory.

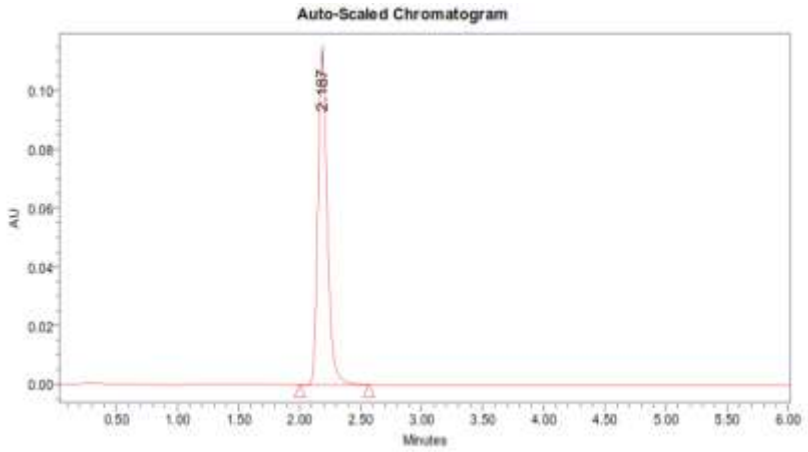

Figure 2: Standard Chromatogram of Tiagabine.

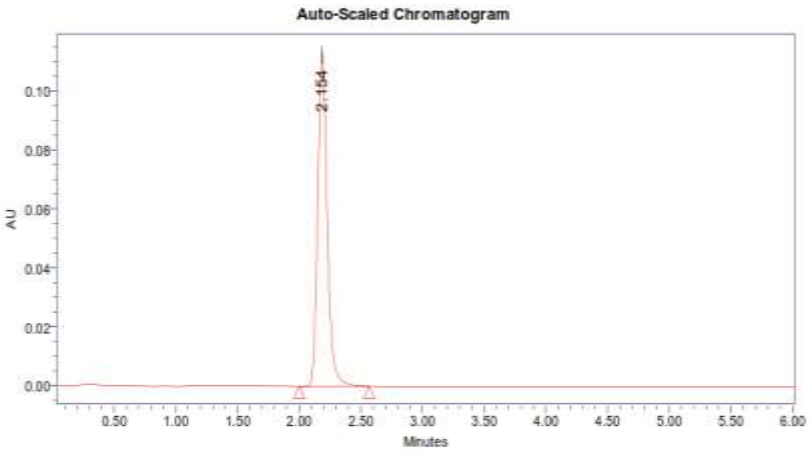

Figure 3: Sample Chromatogram of Tiagabine.

Analytical method validation

HPLC method was validated[13,14] according to the International Conference on Harmonization guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as linearity, precision, accuracy, system suitability limit of detection, limit of quantification and robustness.

Linearity

Inject each level (30, 60, 90,120 and 150µg/mL) solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area. in the concentration range of 30 – 150µg/mL. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients.

Precision

Repeatability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was calculated.

Intermediate precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different analysts by maintaining same conditions. For intermediate precision % RSD was calculated from repeated studies.

Accuracy

Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Tiagabine and calculate the individual recovery and mean recovery values.

Robustness

Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for injections peak area values of each change in condition.

System suitability

This parameter used to know whether the HPLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting five standard solutions of Tiagabine and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected.

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ=10×σ/S

RESULTS AND DISCUSSION

Linearity and range

Linearity and range estimated by constructing the calibration curve by taking concentration on X-axis and peak area on Y-axis of (30, 60, 90,120 and 150µg/mL) solutions (prepared from standard stock solution) and calculate the correlation coefficient. Correlation Coefficient (r) is 0.99, and the intercept 3299. These values meet the validation criteria as shown in Figure 4 and linearity values tabulated in Table 1.

Table 1: Chromatographic data for linearity study.

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	30	201932
80	60	368071
100	90	577859
120	120	740654
140	150	950396

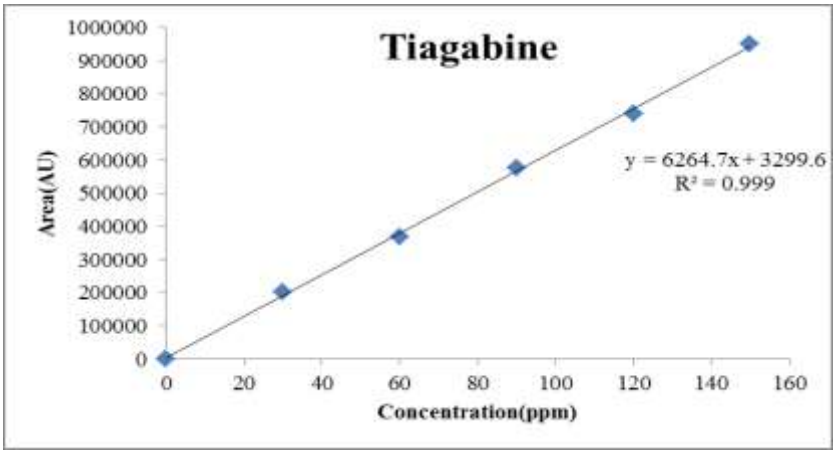

Figure 4: Calibration curve of Tiagabine.

Precision

Intermediate precision

Analyst 1

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits. The results were reported in table 2.

Analyst 2

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits. The results were reported in table 3.

Table 2: Results of Intermediate precision analyst 1 for Tiagabine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	**Tiagabine**	**2.188**	**584681**	**109050**	**5837**	**1.2**
2	**Tiagabine**	**2.186**	**589281**	**109497**	**5927**	**1.2**
3	**Tiagabine**	**2.165**	**596719**	**108559**	**5653**	**1.2**
4	**Tiagabine**	**2.181**	**597658**	**110359**	**5294**	**1.2**
5	**Tiagabine**	**2.181**	**597800**	**110294**	**5201**	**1.2**
6	**Tiagabine**	**2.198**	**597386**	**109235**	**5572**	**1.2**
Mean			**593920.8**			
Std. Dev.			**5581.291**			
% RSD			**0.939737**			

Table 3: Results of Intermediate precision Analyst 2 for Tiagabine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Tiagabine	2.142	597362	118272	5182	1.2
2	Tiagabine	2.201	589726	117393	6192	1.2
3	Tiagabine	2.154	592515	119173	6183	1.2
4	Tiagabine	2.165	592161	117382	5716	1.2
5	Tiagabine	2.132	589761	119028	5729	1.2
6	Tiagabine	2.174	592183	117463	5938	1.2
Mean			592284.7			
Std. Dev.			2784.732			
% RSD			0.470168			

Repeatability

Multiple sampling from a sample solution was done and five working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas Standard Deviation and % Relative Standard Deviation are mentioned in Table 4.

Table 4: Results of repeatability for Tiagabine.

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Tiagabine	2.182	591196	109171	5237	1.2
2	Tiagabine	2.177	594056	109020	5928	1.2
3	Tiagabine	2.196	594419	108157	5826	1.2
4	Tiagabine	2.178	596875	109096	5364	1.2
5	Tiagabine	2.191	598538	109581	5173	1.2
Mean			595016.8			
Std.dev			2816.504			
%RSD			0.473349			

Accuracy

Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. The accuracy results for Tiagabine are recorded in Table 5.

Table 5: The accuracy results for Tiagabine.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	288773.7	45	44.8	99.5	99.6%
100%	566573.3	90	89.7	99.6	
150%	842321.7	135	134.8	99.8	

Robustness

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Tiagabine. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Tiagabine were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results were recorded in Table 6.

Table 6: Results for Robustness.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	604374	2.187	5372	1.2
Less Flow rate of 0.9 mL/min	642411	2.4	5837	1.24
More Flow rate of 1.1 mL/min	521472	2.0	5381	1.1
Less organic phase	576249	2.4	5374	1.2
More organic phase	575829	2.0	4261	1.2

System suitability

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The results were cited in table 7.

Table 7: Results of system suitability for Tiagabine.

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Tiagabine	2.177	593722	104839	5827	1.2
2	Tiagabine	2.145	593028	105833	5933	1.2
3	Tiagabine	2.154	593029	108371	5284	1.1
4	Tiagabine	2.113	596981	105832	5018	1.2
5	Tiagabine	2.108	590863	104857	5283	1.2
Mean			593524.6			
Std. Dev.			2211.448			
% RSD			0.372596			

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Tiagabine in drug product. The percentage purity was found to be 99.7%. The results for specificity of Tiagabine were cited in Table 8 and Table 9.

Table 8: Peak results for assay standard.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Tiagabine	2.187	601821	116341	1.2	5837	1
2	Tiagabine	2.187	592711	112635	1.2	5039	2
3	Tiagabine	2.182	593726	119483	1.2	5492	3

Table 9: Peak results for Assay sample.

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Tiagabine	2.184	597282	112398	1.2	5038	1
2	Tiagabine	2.182	592732	110382	1.2	5873	2
3	Tiagabine	2.180	593627	117361	1.2	5928	3

Limit of detection

Limit of detection is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. It is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected and it was found to be 7.4µg/ml of Tiagabine.

Limit of quantification

Limit of quantification is the concentration that can be quantified reliably with a specified level of accuracy and precision. LOQ was found to be 22.6µg/ml of Tiagabine.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Tiagabine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Tiagabine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Tiagabine in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

Authors express their sincere thanks to our principal Dr. M. Dhanalakshmi, M.Pharm, Ph.D. KLR Pharmacy College, Palvoncha and the Management of KLR Pharmacy College for providing necessary facilities to carry out the research work.

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