International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 1, 2016

Original Article

STRESS DEGRADATION STUDIES AND DEVELOPMENT OF A VALIDATED RP-HPLC METHOD FOR DETERMINATION OF TIAGABINE IN PRESENCE OF ITS DEGRADATION PRODUCTS

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Received: 05 Jan 2015 Revised and Accepted: 25 Nov 2015

ABSTRACT

Objective: To develop a simple and rapid isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) method and validate as per ICH and USP guidelines for analysis of tiagabine in the presence of its degradation products.

Methods: The chromatographic separation was achieved on a Vision HT C_{18} column (150 mm × 4.6 mm, 5 µm) with mobile phase comprising of 11.5 mM of sodium dihydrogen phosphate (adjusted to pH 2.0 with orthophosphoric acid):acetonitrile (50:50,v/v) at a flow rate of 1.0 ml/min and the UV detection wavelength set at 254 nm. Stress degradation studies were performed as stated in ICH guidelines on tiagabine bulk drug using acid, base, oxidation, heat and light.

Results: Tiagabine was found to degrade under acidic, photolytic, oxidative and thermal conditions, but stable under basic hydrolysis condition. The developed method was found to be linear in the concentration range of 50-150 µg/ml (r^2= 0.9983) and the percentage recovery for the accuracy was 98.86%-99.35%. The LOD and LOQ obtained were 31.93 µg/ml and 96.76 µg/ml, respectively while the %RSD for precision, robustness, and stability studies were less than 2%. The degradation products formed from the stress study were well separated from tiagabine and hence the method could be regarded as stability indicating.

Conclusion: A simple and rapid RP-HPLC method was developed and validated for analysis of tiagabine in the presence of its degradation products and thus, the proposed method can be used in the analysis of tiagabine bulk drug and pharmaceutical formulation in quality control laboratories.

Keywords: Tiagabine, RP-HPLC, Stress degradation studies, Validation.

INTRODUCTION

Tiagabine (fig. 1) is chemically known as [R-(-)-(R)-1-(4,4-Bis(3-methyl-2-thienyl)-3-butenyl] nipecotic acid hydrochloride [1,2,3]. Generally, tiagabine possesses a chiral center in the nipecotic acid moiety of the molecule with the R-(-) enantiomer form of tiagabine found to be more pharmacologically potent than that of the S-(+) enantiomer [4]. Tiagabine is an AED that has been approved by the Food and Drug Administration (FDA) to be used as an adjuvant therapy in adults and children above 12 y of age with partial seizure disorders [5]. Tiagabine is known to be an effective AED for the treatment of epilepsy due to its lipophilic anchor to the amino acid nitrogen that enables this drug to pass through from systemic circulation across the blood-brain barrier to the central nervous system [4, 6].

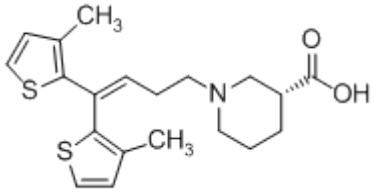

Fig. 1: Chemical structure of tiagabine

There are only a few analytical methods that have been reported in the analysis of tiagabine using HPLC and other chromatographic methods, such as chiral HPLC, gas chromatography-mass spectrometry (GC-MS) and UV spectrophotometry in pharmaceutical formulations, bulk drug, human plasma and serum [7-10]. As for stress degradation studies, there is one study that has been reported so far that involves the use of liquid chromatography with electro spray ionization multistage mass spectrometry (LC/ESI-MS^n) and ultra-performance liquid chromatography/high-resolution mass spectrometry (UPLC/HRMS), but this study shows some limitations whereby it involves using expensive instruments and it lacks stress degradation studies [4].

According to the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), drug stability testing guidelines Q1A (R2), stress testing is being performed in order to identify the presence of possible degradation products which eventually helps in determining the intrinsic stability of the molecules, establish the degradation pathways and also to validate the stability indicating procedures used [11].

Hitherto, no stress degradation study of tiagabine following the ICH guidelines has been reported, hence, the main objective of the present work is to develop an RP-HPLC method for determination of tiagabine in presence of its degradation products and to validate the method as per ICH and USP guidelines so that it can be successfully used in quality control of tiagabine in bulk drug and pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and reagents

The reference standard of tiagabine hydrochloride (≥98%, HPLC) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and methanol (HPLC grade), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Fischer Scientific (Leics, UK). Hydrogen peroxide (H_2O_2) was purchased from Merck

(Darmstadt, Germany). Sodium dihydrogen phosphate anhydrous was purchased from Friedemann Schmidt (Park wood, WA, Australia). Orthophosphoric acid was purchased from R & M chemicals (Essex, U. K.). 18 MΩ purity grade water was obtained from Mili-Q Water system (Milipore, Bedford, USA). All other chemicals and reagents were analytical grade.

Instruments

The HPLC system used for method development and validation was Agilent 1200 series LC system equipped with a binary pump, solvent degasser, UV detector (Waldbronn, Germany) and Rheodyne manual injector model 7725i with 20 µl sample loop (Cotati, CA, USA). Data was acquired and processed using Agilent Chem Station software. A B-220C analytical balance (Fischer scientific, Dietikon, Switzerland) was used to measure the mass of standard and bulk drug of tiagabine. UV-Vis spectrophotometer (Lambda 25, Perkin Elmer, Massachusetts, USA) was used to determine the maximum wavelength of tiagabine. The pH of the mobile phase was checked using a pH meter (Mettler Toledo, Greifensee, Switzerland). As for stress degradation studies, the acidic, basic, oxidative and thermal degradation studies were performed using a forced air circulation oven (Model UFB 400, Memmert, Germany) while the photolytic degradation studies were performed using a handheld UV lamp (UVGL-58, Upland, CA, USA).

Methods

Chromatographic conditions

The chromatographic analysis was performed using Vision HT C_{18} column, (150 mm × 4.6 mm, 5 µm particle size) (Grace, Illinois, USA) as the stationary phase. The mobile phase used is based on isocratic elution mode that comprised of 11.5 mM sodium dihydrogen phosphate buffer-acetonitrile (50:50, v/v) and the buffer solution was adjusted to pH 2.0 with orthophosphoric acid. The mobile phase was pumped at the flow rate of 1 ml/min throughout the analysis. All the mobile phase solutions were filtered through a 0.22 µm nylon membrane filter (Millipore, Bedford, USA) using a vacuum pump (Vacuubrand, Wertheim, Germany) and degassed using an ultrasonic bath (FB 15061, Fisher Scientific, Germany) before use. The eluent was monitored using UV detection at a wavelength of 254 nm. The column was maintained at ambient temperature, and the injection volume of 20 µl was used.

Preparation of standard and bulk drug solution

Tiagabine stock solution (1 mg/ml) was prepared by accurately weighing 10 mg of tiagabine(reference standard and bulk drug sample)to a 10 ml volumetric flask followed by addition of 5 ml diluent (water: methanol, 50:50 v/v). The solution was sonicated for five minutes and further diluted up to the volume with the diluent. The stock solution was then kept at 4 °C prior to use. Series of working solutions were prepared through serial dilution of the stock solution with diluent in order to produce working solutions of tiagabine in concentrations of 50 µg/ml, 80 µg/ml, 100 µg/ml, 120 µg/ml and 150 µg/ml respectively. The working solutions were then filtered using a 25 mm, 0.45 µm nylon syringe filter prior to injection.

Stress degradation studies

In order to evaluate the stability indicating a property of the developed HPLC method, stress degradation studies were carried out following the ICH guidelines, Q1A (R2) [11]. The bulk drug of tiagabine was exposed to acidic, basic, oxidative, thermal and photolytic stress conditions in order to evaluate the ability of the developed method to separate tiagabine from its degradation products. The stress degradation studies were performed until about 5-20% degradation is achieved [12, 13].

Acidic hydrolysis

Stress degradation in acidic media was performed by adding an aliquot of stock solution (1 mg/ml) of tiagabine to 10 ml volumetric flask, followed by addition of 2 ml of diluent and 3 ml of 0.5 N HCl. The mixture was then kept in an oven at 50 °C for 24 h. The solution was left to reach room temperature, neutralized to pH 7 through the addition of 0.5 N NaOH, and further diluted with diluent in order to obtain a final concentration of 100 µg/ml.

Basic hydrolysis

Stress degradation in basic media was performed by adding an aliquot of stock solution (1 mg/ml) of tiagabine to 10 ml volumetric flask, followed by addition of 2 ml of diluent and 3 ml of 0.5 N NaOH. The mixture was then kept in an oven at 50 °C for 24 h. The solution was left to reach room temperature, neutralized to pH 7 by the addition of 0.5 N HCl and further diluted with diluent in order to obtain a final concentration of 100 µg/ml.

Oxidative degradation

Stress degradation in oxidizing condition was performed by adding an aliquot of stock solution (1 mg/ml) of tiagabine to 10 ml volumetric flask, followed by addition of 2 ml of diluent and 3 ml of 3% H_2O_2. The solution was then kept in an oven at 50 °C for 24 h. The solution was left to reach room temperature and further diluted with diluent in order to obtain a final concentration of 100 µg/ml.

Thermal degradation

To study the effect of heat, dry heat degradation study was performed, in which approximately 2 mg of tiagabine was stored at 50 °C in an oven for 48 h. It was then dissolved with 2 ml of diluent and transferred into a 10 ml volumetric flask. The above solution was further diluted with diluent, to give a final concentration of solution equivalent to 100 µg/ml of tiagabine.

Photolytic degradation

To study the effect of light, approximately 2 mg of tiagabine was exposed to UV light at 365 nm for 24 h. It was then dissolved with 2 ml of diluent and transferred into a 10 ml volumetric flask. The above solution was further diluted with diluent, to give a final concentration of solution equivalent to 100 µg/ml of tiagabine.

The final concentration of each stressed solution was filtered using a 25 mm, 0.45 µm nylon syringe filter, and 20 µl of each respective solution were then injected into the HPLC system.

Method validation

The optimized analytical method was validated as per ICH and USP guidelines with respect to parameters such as system suitability, specificity, linearity and range, accuracy, precision, robustness, limit of detection (LOD), and limit of quantitation (LOQ) [14].

System suitability

The system suitability was assessed by six replicate analysis of tiagabine standard at a concentration of 100 µg/ml. Parameters such as capacity factor (k'), theoretical plate number (N) and tailing factor were determined.

Specificity

The specificity of the method was accessed through exposure of drug substance to acidic (0.5 N HCl), basic (0.5 N NaOH), oxidation (3% H_2O_2), thermal (50 °C) and photolytic (UV light at 365 nm) stress conditions. The resulting solution was then used to study the resolution factors of the substance drug peak from the nearest resolving peak and among other peaks.

Linearity and range

The linearity was evaluated at five concentration levels by diluting the tiagabine standard stock solution (1 mg/ml) to give solutions in the range of 50 µg/ml to 150 µg/ml, corresponding to 50 to 150% of the targeted working concentration (100 µg/ml). Each solution was then injected in triplicate, and a calibration curve was constructed. The linear correlation coefficient (r^2), Y-intercept and slope of the calibration curve were used to determine the linearity of the curve.

Accuracy

To confirm the accuracy of the developed method, recovery experiments were carried out using standard addition method. A known quantity of drug substance that corresponds to 50%, 100%

and 150% were added to the 100% working standard in triplicate. The % recovery at each level and % relative standard deviation (RSD) was then calculated.

Precision

In this study, precision is basically determined based on two different levels which are the repeatability and intermediate precision.

Repeatability

The repeatability or also known as intra-assay precision was evaluated using tiagabine drug substance at three different concentrations (50 µg/ml, 100 µg/ml and 150 µg/ml) in three replicates for each concentration on the same day. The results were expressed as % RSD.

Intermediate precision

Intermediate precision or also known as inter-day precision was assessed using tiagabine drug substance in three different concentrations (50 µg/ml, 100 µg/ml and 150 µg/ml) with three replicates for each concentration over three consecutive days and the results were expressed as % RSD.

LOD and LOQ

Both LOD and LOQ were evaluated based on the standard deviation of the response and slope using the linearity curve. The formula used for LOD and LOQ was 3.3 σ/S and 10 σ/S respectively, whereby σ is the standard deviation of the response while S is the slope of the calibration curve.

Robustness

The robustness of the method was investigated by varying the chromatographic conditions such as changing the organic solvent composition of the mobile phase (±2%) and the flow rate (±0.1 ml/min). When the effect of changing one set of conditions was tested, the other conditions were kept constant at optimum values. For all changes in the chromatographic conditions, the tiagabine drug substance (100 µg/ml) was analysed in six replicates. The robustness of the developed method was determined by the retention time, k', N, tailing factor and the % RSD at each variable condition.

Stability studies

The stability of tiagabine was analysed by storing the tiagabine drug substance (50 µg/ml, 100 µg/ml and 150 µg/ml) in tightly capped volumetric flasks at room temperature (25±2 °C) and at 4 °C for 24 h. These solutions were then compared after 24 h against a freshly prepared standard. The % RSD was calculated in order to determine its stability.

RESULTS AND DISCUSSION

HPLC method development and optimization

The main aim for the development of successful RP-HPLC method in this study was to ensure that tiagabine is eluted and well separated from its degradation products and also, the developed method should be simple, accurate, reproducible, robust and stability indicating so that it can be applied for routine use in quality control laboratories.

The USP 36 monograph [3], has stated a method for determination of tiagabine using a RP-HPLC method with C_{18} column. The stated isocratic mobile phase comprised of sodium dihydrogen phosphate buffer (pH 2.0): acetonitrile (65:35, v/v), the flow rate of 1 ml/min with UV detection at 254 nm and injection volume of 20 µl. By using the above-reported method, it was found that the retention time of tiagabine was 8.50 min. The peak shape was narrow but severe peak tailing was observed. The reported UV wavelength was found to be equivalent to the maximum absorbance (λ_{max}) obtained when tiagabine reference standard was scanned over a wavelength range of 200 nm to 400 nm against a blank using a UV-Vis spectrophotometer.

Thus, the attempt done to modify the method stated in the monograph was by varying the solvent strength. By using the same mobile phase in the ratio 60:40, v/v, it was found that the retention time of tiagabine was 4.76 min and the tailing factor obtained was more than two, although other parameters such as k' and N was within the acceptable range.

The proportion of the mobile phase was further changed to 50:50, v/v. Under this condition, it was found that tiagabine eluted at 2.65 min and the peak shape was narrow with acceptable peak tailing. Besides that, the system suitability parameters (k', N and tailing factor) were within the normal range. The typical chromatogram obtained based on the proposed RP-HPLC method is shown in fig. 2.

The method developed initially using tiagabine standard was then extended to the various stressed samples of tiagabine, in order to identify whether the proposed method would be able to separate tiagabine from its degradation products. The results obtained as shown in fig. 3 to fig. 7, indicate that tiagabine is well resolved from its degradation products although the degradation products seem to be overlapping and not well separated among other degrading products.

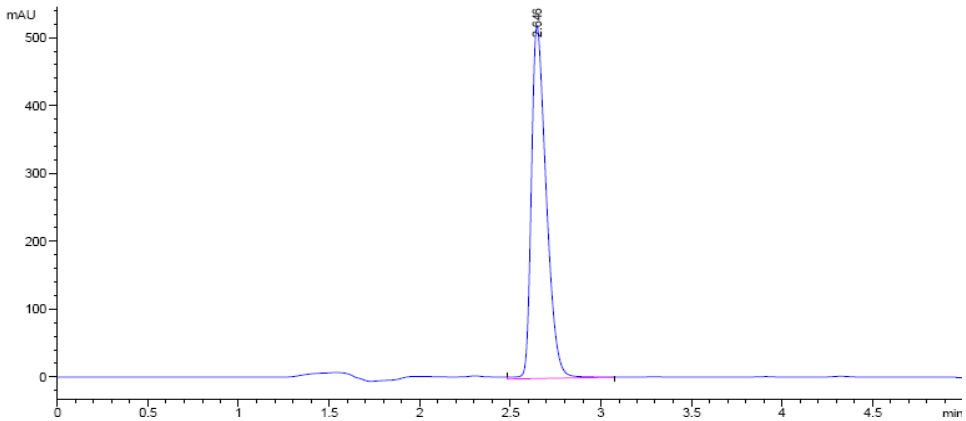

Fig. 2: Chromatogram of tiagabine standard (100 µg/ml)

Stress degradation studies

After the chromatographic condition had been determined, stress degradation studies were performed on tiagabine bulk drug. In general, many degradation products were detected to be present when the drug was exposed to various stress conditions. The degradation behavior of tiagabine bulk drug under various stress conditions are shown in fig. 3 to fig. 7 while the results are shown in table 1.

Acidic hydrolysis

Tiagabine was found to be labile under acidic hydrolysis condition with the presence of maximum degradation of about 20% when the drug was exposed to 0.5 N HCl at 50 °C for 24 h. Also, four unknown degradation products were formed at a retention time of 1.43 min, 1.54 min, 1.93 min and 3.47 min respectively as shown in fig. 3.

Generally, the first three degradation products formed are polar as they elute out faster than tiagabine while the degradation product peak that elutes at 3.47 min is slightly less polar as it elutes later than tiagabine.

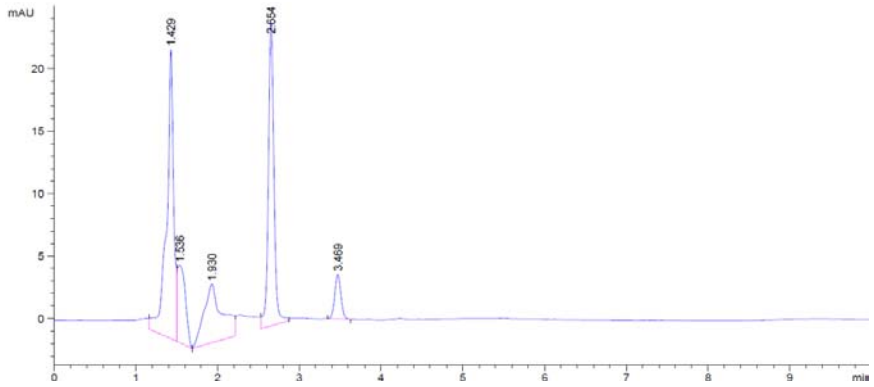

Fig. 3: Chromatogram showing degradation of tiagabine bulk drug after exposure to 0.5 N, HCl at temperature of 50 °C for 24 h

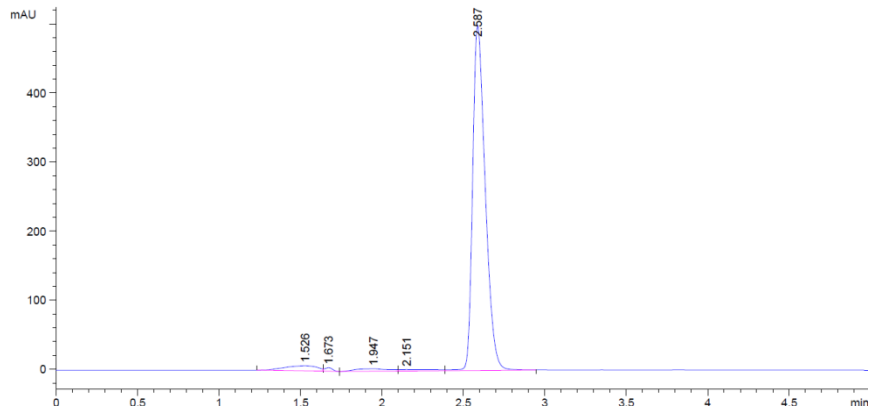

Fig. 4: Chromatogram showing degradation of tiagabine bulk drug after exposure to 0.5 N NaOH at temperature of 50 °C for 24 h

Basic hydrolysis

Tiagabine was found to be stable under basic hydrolysis condition as it was found to be degraded by approximately 6% when the bulk drug was exposed to 0.5 N NaOH at 50 °C for 24 h. Only minor degradation products were observed to be present at a retention time of 1.53 min, 1.67 min, 1.95 min and 2.15 min respectively as shown in fig. 4.

Oxidative degradation

Tiagabine was found to degrade around 16% when the bulk drug was exposed to 3% H_2O_2 at 50 °C for 24 h. This form of degradation can be classified as mild degradation with the presence of unknown degradation products at 1.54 min, 1.67 min, 1.93 min and 2.26 min respectively (fig. 5). The formation of these degradation products may be explained through oxidative pathways involving the attack of oxygen at various centers mainly at the double bond of tiagabine [4].

Thermal degradation

Tiagabine was found to be susceptible to heat as it degraded to about 13% when the bulk drug was exposed to heat at 50 °C for 48 h. When the bulk drug was exposed to heat for 24 h, the drug was found to be stable as no degradation product was formed. Thus, the bulk drug was exposed to 50 °C for another 24 h. At this time, the drug was found to degrade with the presence of four unknown degradation products at 1.46 min, 1.54 min, 1.66 min and 1.95 min respectively. The chromatogram of thermal degradation of tiagabine is shown in fig. 6.

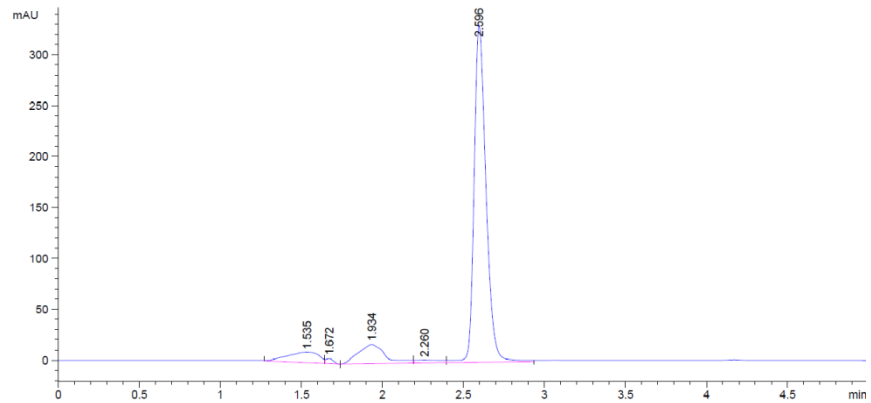

Fig. 5: Chromatogram showing degradation of tiagabine bulk drug after exposure to 3%, H_2O_2 at temperature of 50 °C for 24 h

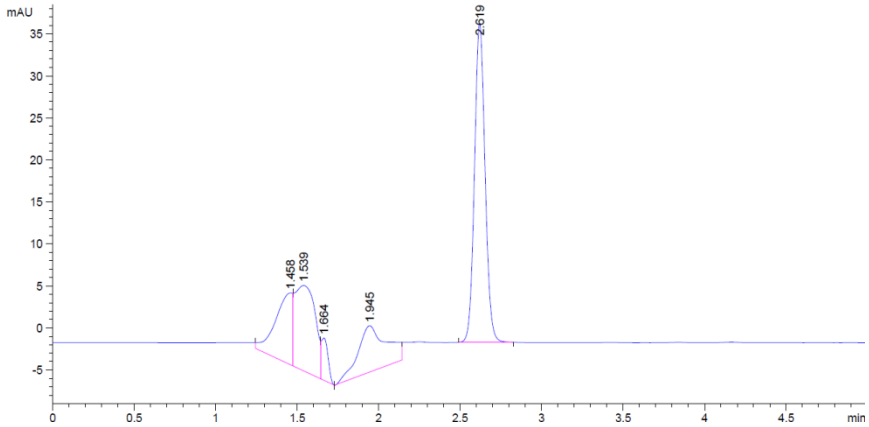

Fig. 6: Chromatogram showing degradation of tiagabine bulk drug after exposure to heat at 50 °C for 48 h

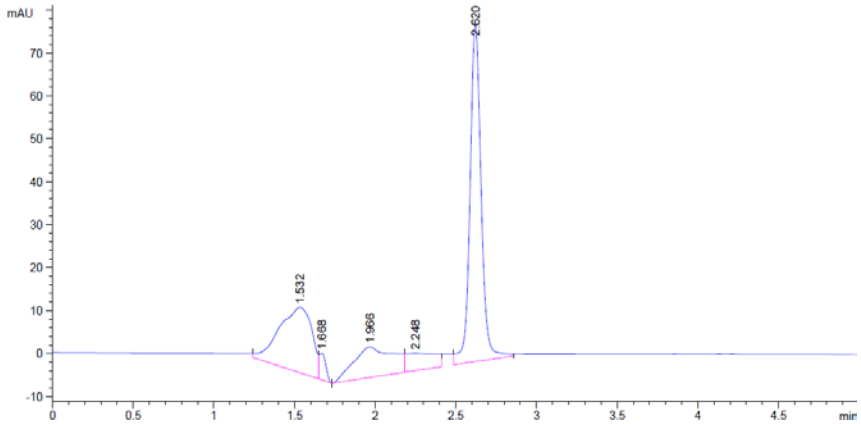

Fig. 7: Chromatogram showing degradation of tiagabine bulk drug after exposure to UV light (365 nm) for 24 h

Table 1: Percentage assay and percentage degradation of tiagabine under various stress conditions

Stress condition	Duration (h)	% assay of drug after exposure to stress condition	% Degradation
Standard drug	-	98	No degradation
Acidic hydrolysis (0.5 N HCl, 50 °C)	24	73.20	25.31
Basic hydrolysis (0.5 N NaOH, 50 °C)	24	92.13	5.99
Oxidative degradation (3% H_2O_2, 50 °C)	24	82.40	15.91
Thermal degradation (50 °C)	48	84.98	13.29
Photolytic degradation (UV light at 365 nm)	24	80.47	17.88

Table 2: System suitability parameters of optimized HPLC method

Parameter	Value
Retention time (min)	2.64
Capacity factor (k')	1.64
Theoretical plate number (N)	5184
Tailing factor	1.50

Acceptance criteria: 1<k'<10; N>2000; Tailing factor ≤ 2

Photolytic degradation

Tiagabine was found to show mild degradation (18%) under the photolytic condition when the bulk drug was exposed to UV light at 365 nm for 24 h. Under this condition, four unknown degradation products were present at 1.53 min, 1.67 min, 1.97 min and 2.25 min, similar to those present in oxidative degradation condition. This probably indicates that the degradation products formed under photolytic degradation condition may be similar to that formed under oxidative degradation condition. The chromatogram of photolytic degradation of tiagabine is shown in fig. 7.

Method validation

System suitability

A system suitability test was conducted in order to evaluate the k', N and tailing factor before the validation runs. The results obtained for the system suitability test are within the acceptance criteria [15] and are shown in table 2.

Specificity

All peaks corresponding to the degradation products that were formed under various stress conditions were well separated from

the peak of tiagabine as shown in fig. 3 to fig. 7 with the resolution factor for tiagabine drug peak being>2 from the nearest resolving peak. This indicates that the developed and optimized method was specific to tiagabine.

Linearity and range

The results of the linearity and peak response of tiagabine are shown in table 3.

Table 3: Linearity and peak response of tiagabine

Calibration range (%)	Concentration (µg/ml)	Mean peak area (n=3)	Standard deviation (SD)	%RSD
50	50	2176.51	9.39	0.43
80	80	2678.96	6.61	0.28
100	100	2948.68	15.31	0.52
120	120	3310.21	33.02	0.99
150	150	3725.99	18.03	0.48

Based on the calibration curve (fig. 8), tiagabine showed a linear relationship between the peak area and the concentration in the range between 50 and 150 µg/ml. Besides that, it was also noted that tiagabine showed good correlation coefficient (r^2 = 0.9983) in the given range. The results of the r^2value obtained from this study meet the acceptance limit of the USP guidelines, whereby, it is stated that the r^2 value must be higher than 0.997 for five points over 50-150% of the target analyte concentration [16]. This shows that the developed method was linear within the given range.

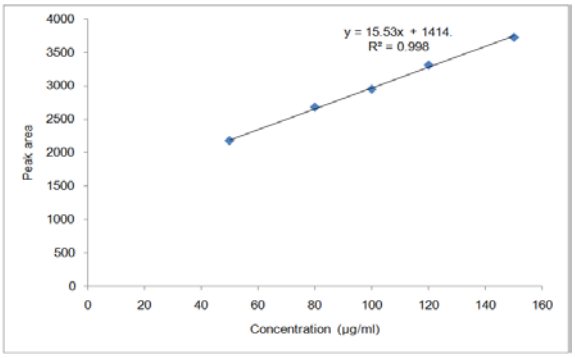

Fig. 8: Calibration curve of tiagabine in the range of 50-150 µg/ml. The regression analysis parameters for the linearity of the method are shown in table 4

Table 4: Regression analysis of the linearity data of tiagabine

Parameter	Tiagabine
Linearity range (µg/**ml**)	50-150
Slope	15.534
Y-intercept	1414.6
r^2	0.9983

Accuracy

The developed method had good accuracy with the % recovery obtained in the range of 98.86%-99.35% and the %RSD obtained was less than 2%. According to the USP guidelines, the acceptable value for accuracy obtained through calculating % recovery value is 98%-102% [16]. Since the % recovery calculated is within the acceptable range, the developed method is found to be accurate and suitable for its intended use. The results of the accuracy study are shown in table 5.

Precision

The %RSD obtained for repeatability, and intermediate precision as shown in table 6 was less than 2% and thus shows that the developed method is precise.

LOD and LOQ

The minimum concentration levels at which tiagabine drug substance can be detected and quantified are 31.93 µg/ml and 96.76 µg/ml respectively.

Robustness

The results obtained through modification of organic solvent strength of the mobile phase composition (±2%) and flow rate (±0.1 ml/min) as shown in table 7, indicate that the developed method is robust as all the system suitability parameters (k', N and tailing factor) tested, are within the acceptable range and the %RSD obtained is less than 2%. This indicates that the developed method can be used even when there is slight variation in the organic solvent strength of the mobile phase composition and flow rate.

Stability studies

The results obtained (table 8) for all the three different concentrations of drug substance showed that the %RSD obtained was less than 2% relative to the freshly prepared standard. This indicates that tiagabine drug substance is stable within the indicated period when it is kept at room temperature or at 4 °C.

Table 5: Evaluation of accuracy study

% concentration	Amount added (µg/ml)	Amount recovered (µg/ml) (n = 3)	% recovery	%RSD
50	50	49.43	98.86	0.43
100	100	99.17	99.17	1.06
150	150	149.02	99.35	0.69

Table 6: Evaluation of repeatability and intermediate precision through intra-day and inter-day precision

Concentration (µg/ml)	Intra-day precision			Inter-day precision		
	Mean retention time (min) (n = 3)	Mean peak area (n = 3)	%RSD	Mean retention time (min) (n = 9)	Mean peak area (n = 9)	%RSD
50	2.62	2137.68	0.64	2.63	2138.81	1.53
100	2.63	2986.29	1.42	2.63	2964.61	1.53
150	2.66	3725.99	0.48	2.62	3745.49	1.90

Table 7: Evaluation of robustness for tiagabine by varying the mobile phase composition and flow rate

Chromatographic condition	Optimized	Modification	k'	N	Tailing factor	Mean retention time (min)	Mean peak area (n=6)	%RSD (n=6)
Mobile phase composition	Buffer: Acetonitrile (50:50, v/v)	Buffer: Acetonitrile (52:48, v/v)	1.86	7272	1.31	2.86	2943.26	1.22
(±2% organic solvent)		Buffer: Acetonitrile (48:52, v/v)	1.45	7019	1.24	2.44	2939.34	0.63
Flow rate	1 ml/min	0.9 ml/min	1.61	7506	1.31	2.89	2974.57	0.58
(±0.1 ml/min)		1.1 ml/min	1.63	6609	1.28	2.39	2957.00	1.12

Table 8: Evaluation of stability studies of tiagabine drug substance at room temperature (25±2 °C) and at 4 °C over a period of 24 h

Actual concentration (µg/ml)	Calculated concentration (µg/ml), (%RSD)			
	Room temperature (25±2 °C)		4 °C	
	Fresh solution (µg/ml) (n =3)	Stored solution (µg/ml) (n =3)	Fresh solution (µg/ml) (n =3)	Stored solution (µg/ml) (n =3)
50	49.05, 1.23	44.39, 0.43	49.05, 1.23	48.33, 1.62
100	98.76, 1.00	99.25, 1.66	98.76, 1.00	99.57, 0.82
150	148.80, 0.78	150.71, 0.80	148.80, 0.78	155.11, 1.62

CONCLUSION

A simple and rapid stability indicating RP-HPLC method was developed for the determination of tiagabine in the presence of its degradation products. In this study, tiagabine was exposed to various stress conditions as stated in the ICH guidelines and the results of the stress degradation studies show that tiagabine is labile to acidic hydrolysis followed by photolytic degradation, oxidative degradation, and thermal degradation. On the other hand, it was found that tiagabine was stable to basic hydrolysis. Based on the proposed method, it was found that all the degradation products formed under various stress conditions were well separated from tiagabine. The developed method was then validated, and it proved to be linear, specific, accurate, precise, and robust and stability indicating. Thus, the proposed method can be used to determine tiagabine in bulk drug and pharmaceutical dosage forms in quality-control laboratories.

ABBREVIATION

AED, Antiepileptic drugs; FDA, Food and Drug Administration; GC-MS, Gas chromatography-mass spectrometry; H_2O_2, Hydrogen peroxide; HCl, Hydrochloric acid; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; k', Capacity factor; LC/ESI-MS^n, liquid chromatography with electro spray ionization multistage mass spectrometry; LOD, Limit of detection; LOQ, Limit of quantitation;

N, Theoretical plate number; NaOH, Sodium hydroxide; r^2, Correlation coefficient; RP-HPLC,

Reversed phase High-Performance Liquid Chromatography; RSD, Relative standard deviation; SD, Standard deviation; UPLC/HRMS, ultra-performance liquid chromatography/high-resolution mass spectrometry; USP, United States Pharmacopeia.

ACKNOWLEDGEMENT

This work was supported by the International Medical University (Project ID NO.:MAPC | 1/2014 (02)).

CONFLICT OF INTERESTS

Declared none

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