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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF TIAGABINE HYDROCHLORIDE IN TABLET

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Abstract:
A simple, rapid, economical, precise and accurate Stability indicating RP-HPLC *method for estimation of* ***Tiagabine Hydrochloride*** *in their dosage form has been developed.*
A ***reverse phase high performance liquid chromatographic method*** *was developed for the simultaneous estimation of Tiagabine Hydrochloride. in their dosage form has been developed. The separation was achieved by* ***column C_{18} (250mm x 4.6 mm) Hypersil BDS and Buffer (pH 4.5): Methanol (85:15 % v/v) as mobile phase, at a flow rate of 1 ml/min. Detection*** *was carried out at* ***260 nm. Retention time****s of Tiagabine Hydrochloride were found to be* ***4.210 min****, respectively. The method has been validated for linearity, accuracy and precision.* ***Linearity*** *observed for Tiagabine Hydrochloride* ***5-15 µg/ml.*** *Developed method was found to be accurate, precise and rapid for estimation of Tiagabine Hydrochloride In Their Dosage Form.*
The drug was subjected to stress condition of hydrolysis, oxidation, photolysis and Thermal degradation.The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial combined dosage form.

Keywords: *Tiagabine Hydrochloride, Stability indicating RP-HPLC Method, Validation.*

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1. INTRODUCTION:

1.1 Introduction to Seizure Disorder [1]:

Definition:

- A seizure is an abnormal electrical discharge that occurs in your brain. Usually brain cells, or neurons, flow in an organized fashion along the surface of your brain. A seizure occurs when there is an excess of electrical activity.

Types of Seizure:

1. Partial Seizures

Partial, or focal, seizures begin in a specific part of your brain. If they originate on one side of your brain and spread to other areas, they are called simple partial seizures. If they begin in an area of your brain that affects consciousness, they are called complex partial seizures.

Simple partial seizures have symptoms including:

- involuntary muscle twitching
- vision changes
- dizziness
- sensory changes
- Complex partial seizures can cause similar symptoms, and may also lead to loss of consciousness.

2. Generalized seizures

Generalized seizures begin on both sides of your brain at the same time. Because these seizures spread quickly, it can be difficult to tell where they originated. This makes certain kinds of treatments more difficult.

There are several different types of generalized seizures, each with their own symptoms:

- Absence seizures are brief episodes that may make you stare off while remaining motionless, as though you are daydreaming. They typically occur in children.
- Myoclonic seizures can cause your arms and legs to twitch on both sides of your body

Partial, or focal, seizures begin in a specific part of your brain. If they originate on one side of your brain and spread to other areas, they are called simple partial seizures. If they Tonic-clonic seizures can go on for a long time, sometimes up to 20 minutes. This type of seizure can cause more serious symptoms, such as loss of bladder control and loss of consciousness, in addition to uncontrolled movements.

3. Febrile seizures

- Another type of seizure is a febrile seizure that occurs in infants as the result of a fever.
- About one in every 25 children, between the ages of 6 months to 5 years, has a febrile seizure, according to the National Institute of Neurological Disorders and Stroke. Generally, children who have febrile seizures don't need to be hospitalized, but if the seizure is prolonged, your doctor may order hospitalization to observe your child.

Etiology:

Neurons use electrical activity to communicate and transmit information. Seizures occur when brain cells behave abnormally, causing neurons to misfire and send wrong signals.

Seizures are most common in early childhood and after age 60. Also, certain conditions may lead to seizures, including:

- Alzheimer's disease or dementia
- heart problems, such as stroke or heart attack
- head or brain injury, including injury before birth
- lupus
- meningitis

Some newer research investigates possible genetic causes of seizures.

Treatment

There is no known treatment that can cure seizures or seizure disorders, but a variety of treatments may help to prevent them or help you avoid seizure trigger

Medicines

Your doctor may prescribe medicines called antiepileptics, which aim to alter or reduce excess electrical activity in your brain. Some of the many kinds of these medicines include phenytoin and carbamazepine.

Surgery

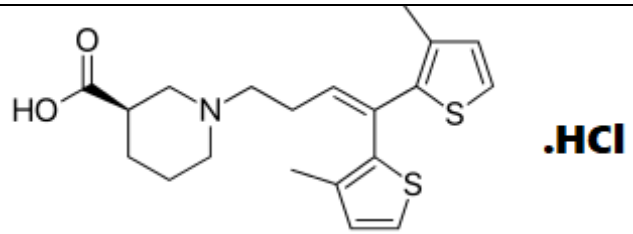
Surgery may be another treatment option if you have partial seizures that aren't helped by medicine. The goal of surgery is to remove the part of your brain where your seizures begin.

Diet changes

Changing what you eat can also help. Your doctor may recommend a ketogenic diet, which is low in carbohydrates and proteins, and high in fats. This eating pattern may change your body's chemistry and may result in a decrease in your frequency of seizures.

1.2. DRUG PROFILE [2-4]

1.2.1. Drug Profile Tiagabine HCl [2,4]

INTRODUCTION	
Name	**Tiagabine HCl, Tiagabne**
Official in	USP32
Description	Tiagabine is an anti-convulsive medication. It is also used in the treatment for panic disorder as are a few other anticonvulsants. Though the exact mechanism by which tiagabine exerts its effect on the human body is unknown, it does appear to operate as a selective GABA reuptake inhibitor.
Structure	
Chemical Formula	$C_{20}H_{26}ClNO_2S_2$
Mol. Weight	412.0 gm/mol
IUPAC Name	(−)-(3R)-1-[4,4-bis(3-methyl-2-thienyl)-3-buten-1-yl]-3-piperidinecarboxylic acid, Hydrochloride
Categories	• Antiepileptic
Solubility	• Soluble in water and Dimethyl sulfoxide
PHARMACOLOGY	
Mechanism of action	Tiagabine does appear to operate as a selective GABA reuptake inhibitor.
PROPERTIES	
State	Solid.
CAS NO.	145821-59-6
Melting point	190-200 ° C
Experimental properties	**Property** / **Value**: Log P — 2.6; PKa — Strongest acidic 4.14, Strongest basic 9.26

1.3. Introduction to analytical method [5-9]

Classical Liquid Chromatography, the term chromatography meaning "color writing," was first dis covered by Mikhail Tswett, a Russian botanist who separated plant pigments on chalk (CaCO3) packed in glass columns in 1903. Since the 1930s, chemists used gravity fed silica columns to purify organic materials and ion-exchange resin columns to separate ionic compounds and radio nuclides.

In the late 1960s, Liquid Chromatography turned "high performance" with the use of small-particle columns that required high pressure pumps.

The first generation of HPLC was developed by researchers in the 1960s, including Horvath Kirkland, and Huber. Commercial development of in-line detectors and reliable injectors allowed HPLC to become a sensitive and quantitative technique leading to an explosive growth of applications. In the 1980s, the versatility and precision of HPLC rendered it

virtually indispensable in pharmaceuticals as well as other diverse industries.

Today, HPLC continues to evolve rapidly toward higher speed, efficiency, and sensitivity, driven by the emerging needs of life sciences and pharmaceutical applications.

Figure 1.1.(a) depicts the classical technique of Liquid Chromatography with a glass column that is packed with coarse adsorbents and gravity fed with solvents.

Fractions of the eluent containing separated components are collected manually. This is contrasted with the latest computer-controlled HPLC, depicted in Figure 1.1.(b), operated at high pressure and capable of very high efficiency.

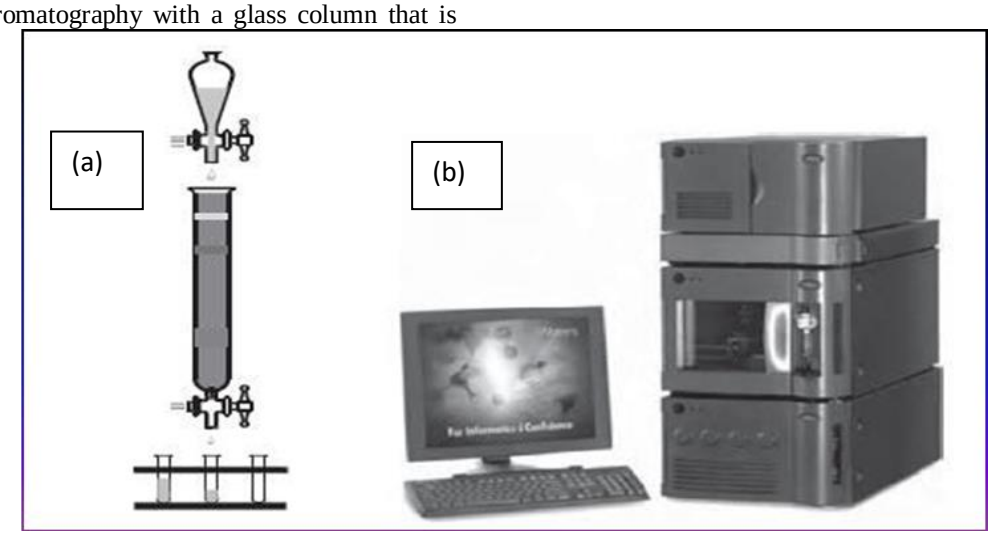


Fig.1.1. (a) The traditional technique of low-pressure liquid chromatography using a glass column and gravity-fed solvent with manual fraction collection.
(b) A modern automated HPLC instrument capable of very high efficiency and pressure.

1.3.1. INTRODUCTION TO HPLC METHOD [5,6]

Liquid chromatography (LC) is a physical separation technique conducted in the liquid phase. A sample is separated into its constituent components (or analytes) by distributing between the mobile phase (a flowing liquid) and a stationary phase (sorbents packed inside a column). For example, the flowing liquid can be an organic solvent forced through the column at high speed and the stationary phase can be porous silica particles packed in a column. The modern form of column chromatography has been called high performance, high Pressure, high-resolution and high-speed liquid chromatography. HPLC is a modern form of LC that uses small-particle columns through which the mobile phase is pumped at high pressure.

High-performance liquid chromatography (HPLC), sometimes called high-pressure liquid chromatography, is a separation technique based on a solid stationary phase and a liquid mobile phase.

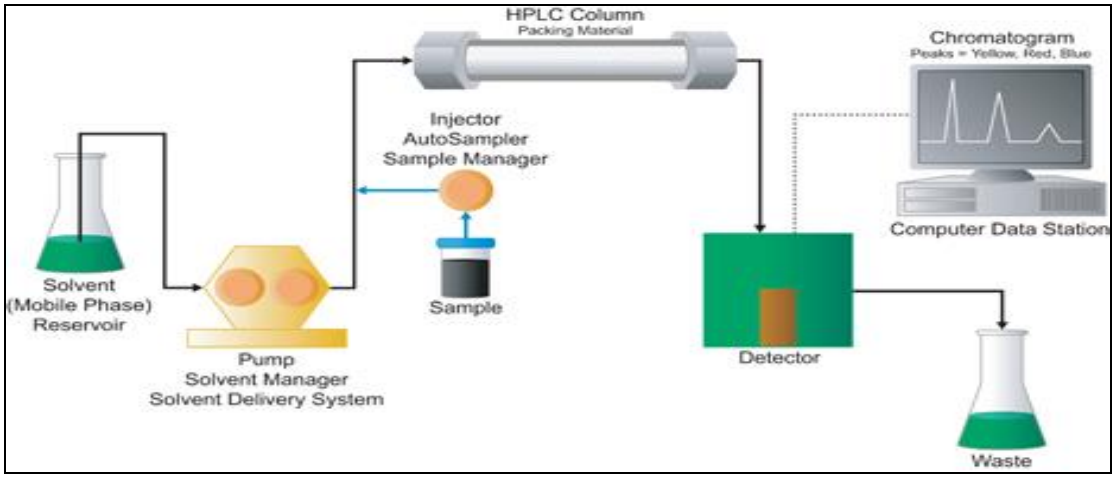


Fig.1.2. Block diagram of HPLC.

Fig.1.2. It describes the work out flow about High performance liquid chromatography (HPLC).

Principle of separation [5,6]

The principle of separation in normal phase mode and reverse phase mode is adsorption. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.

An in-line detector monitors the concentration of each separated component band in the effluent and generates a trace called the "Chromatogram," shown in Figure1.3.c.

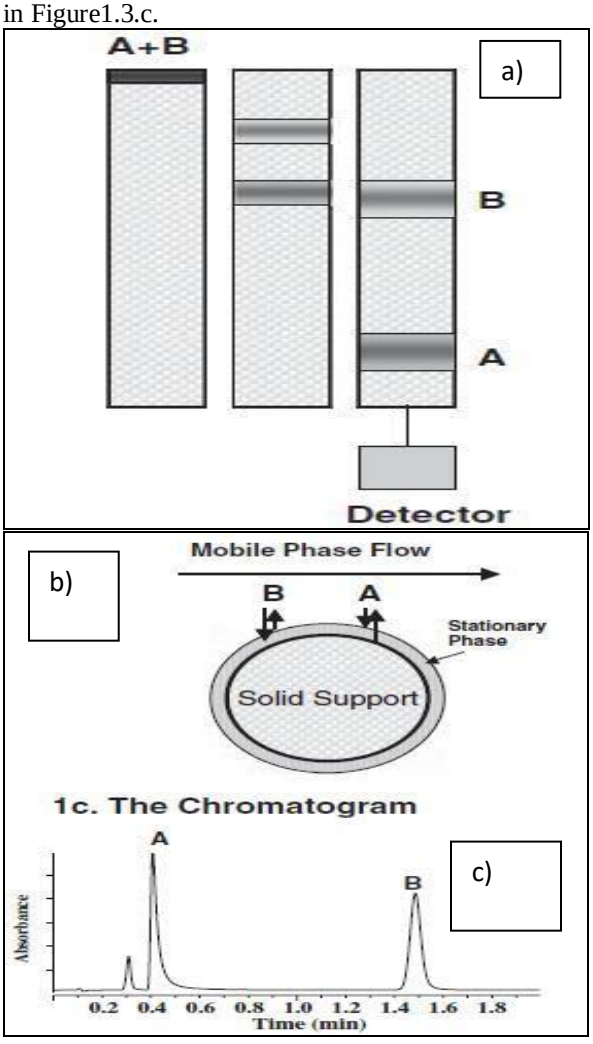


Fig.1.3.(a). Schematic of the chromatographic process showing the migration of two bands of components down a column

(b). Microscopic representation of the partitioning process of analyte molecules A and B into the stationary phase bonded to a spherical solid support.

(c). A chromatogram plotting the signal from a UV detector displays the elution of components A and B.

There are different modes of separation in HPLC:

1) Normal phase mode.
2) Reversed phase mode.
3) Ion exchange chromatography.
4) Reverse phase ion pair chromatography.
5) Affinity chromatography and
6) Size exclusion chromatography.

1) Normal phase:

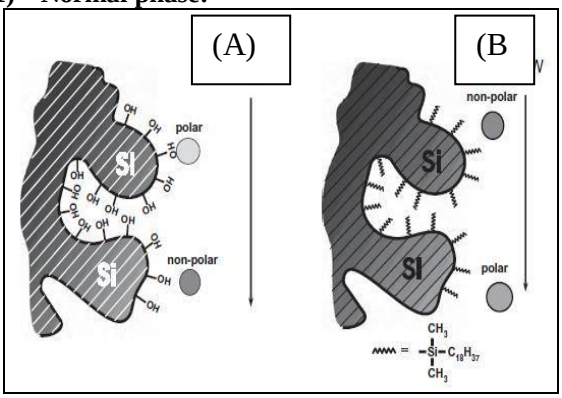


Fig.1.4. Schematic diagrams depicting separation modes of
(a) Normal-phase chromatography (NPC) and
(b) Reversed-phase chromatography (RPC).

In the normal phase mode,The stationary phase is polar and the mobile phase is non polar in nature. In this technique, non polar compounds travel faster and are eluted first. This is because of the lower affinity between the non polar compounds and the stationary phase. Polar compounds are retained for longer times because of their higher affinity with the stationary phase. These compounds, therefore take more times to elute. Normal phase mode of separation is therefore, not generally used for pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

2) Reversed phase mode:

Reversed phase mode is the most popular mode for analytical and preparative separations of compound of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non polar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization

control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and non polar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are octa decyl silane (ODS) or C_{18}, C_8, C_4, etc., (in the order of increasing polarity of the stationary phase).

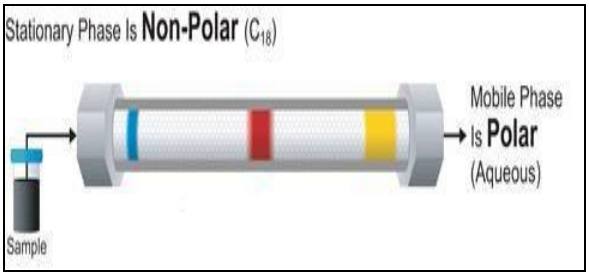


Fig.1.5. Reversed-Phase Chromatography

3) Ion exchange chromatography:

In ion exchange chromatography, the stationary phase contains ionic groups like NR_3^+ or SO_3^-, which interact with the ionic groups of the sample molecules. This is suitable for the separation of charged molecules only. Changing the pH and salt concentration can modulate the retention.

4) Reverse phase ion pair chromatography:

Ion pair chromatography may be used for the separation of ionic compounds and this method can also substitute for ion exchange chromatography. Strong acidic and basic compounds may be separated by reversed phase mode by forming ion pairs (coulumbic association species formed between two ions of opposite electric charge) with suitable counter ions. This technique is referred to as reversed phase ion pair chromatography or soap chromatography.

5) Affinity chromatography:

Affinity chromatography uses highly specific biochemical interactions for separation. The stationary phase contains specific groups of molecules which can absorb the sample if certain steric and charge related conditions are satisfied. This technique can be used to isolate proteins, enzymes as well as antibodies from complex mixtures.

6) Size exclusion chromatography:

Size exclusion chromatography separates molecules according to their molecular mass. Largest molecules are eluted first and the smallest molecules last. This method is generally used when a mixture contains compounds with a molecular mass difference of at least 10%. This mode can be further subdivided into gel permeation chromatography (with organic solvents) and gel filtration chromatography (with aqueous solvents).

Parameters that are affected by the changes in chromatographic conditions:

1. Resolution (Rs).
2. Capacity factor (k').
3. Selectivity (α).
4. Column efficiency (N).
5. Peak asymmetry factor (As).

1) Resolution (Rs):

Resolution is the parameter describing the separation power of the complete chromatographic system relative to the particular components of the mixture.

The resolution (Rs), of two neighboring peaks is defined as the ratio of the distance between two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of Rs is 1.5.

It is calculated by using the formula,

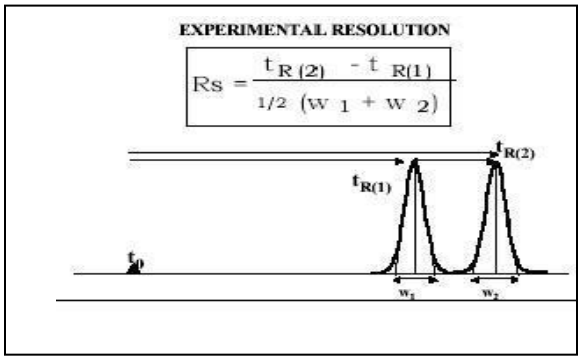


Fig.1.6. Resolution Between two peaks.

Where, $t_{R(1)}$ and $t_{R(2)}$ are the retention times of components 1 and 2 and

W_1 and W_2 are peak width of components 1 and 2.

Baseline resolution is achieved when R = 1.5

It is useful to relate the resolution to the number of plates in the column, the selectivity factor and the retention factors of the two solutes;

$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{1 + k'_B}{k'_B}\right)$$

To obtain high resolution, the three terms must be maximized. An increase in N, the number of theoretical plates, by lengthening the column leads to an increase in retention time and increased band broadening which may not be desirable. Instead, to increase the number of plates, the height equivalent to a theoretical plate can be reduced by reducing the size of the stationary phase particles.

It is often found that by controlling the capacity factor (k'), separations can be greatly improved. This can be achieved by changing the temperature (in Gas Chromatography) or the composition of the mobile phase (in Liquid Chromatography).

2) Capacity factor (k'):

Capacity factor is the ratio of the reduced retention volume to the dead volume. Capacity factor (k'), is defined as the ratio of the number of molecules of solute in the stationary phase to the number of molecules of the same in the mobile phase. Capacity factor is a measure of how well the sample molecule is retained by a column during an isocratic separation. The ideal value of k' ranges from 2-10. Capacity factor can be determined by using the formula.

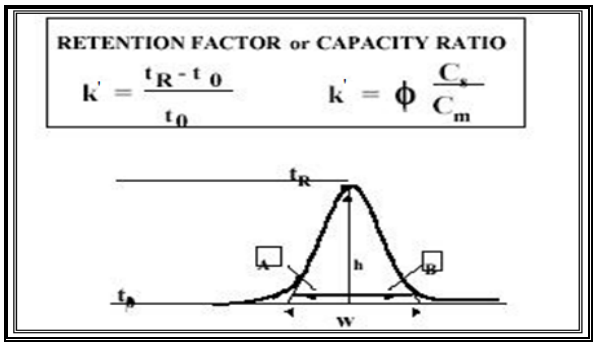


Fig.1.7. Capacity Factor.

Where, t_R= retention volume at the apex of the peak (solute).

t_0 = void volume of the system

3) Selectivity factor (a):

It can also be manipulated to improve separations. When is close to unity, optimizing k' and increasing N is not sufficient to give good separation in a reasonable time. In these cases, k' is optimized first, and then it is increased by one of the following procedures:

1. Changing mobile phase composition.
2. Changing column temperature.
3. Changing composition of stationary phase.
4. Using special chemical effects (such as incorporating a species which complexes with one of the solutes into the stationary phase).

4) Column Efficiency (N):

Efficiency (N), of a column is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Similar the band spread, higher is the number of theoretical plates, indicating good column and system performance. Columns with N ranging from 5,000 to 1,00,000 plates/meter are ideal for a good system.

Efficiency is calculated by using the formula,

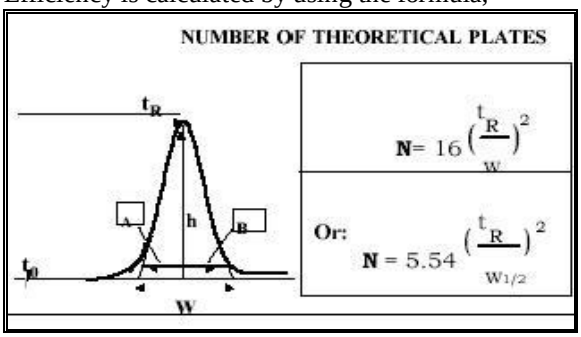


Fig.1.8. Number of Theoretical Plates.

Where, t_R is the retention time.

W is the peak width.

5) Peak asymmetry factor (T_f):

Peak asymmetry factor, (T_f) can be used as a criterion of column performance. The peak half width (b), of a peak at 10% of the peak height, divided by the corresponding front half width (a), gives the asymmetry factor.

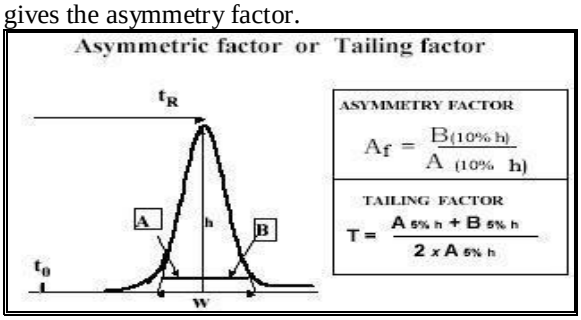


Fig.1.9. Asymmetric Factor.

For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

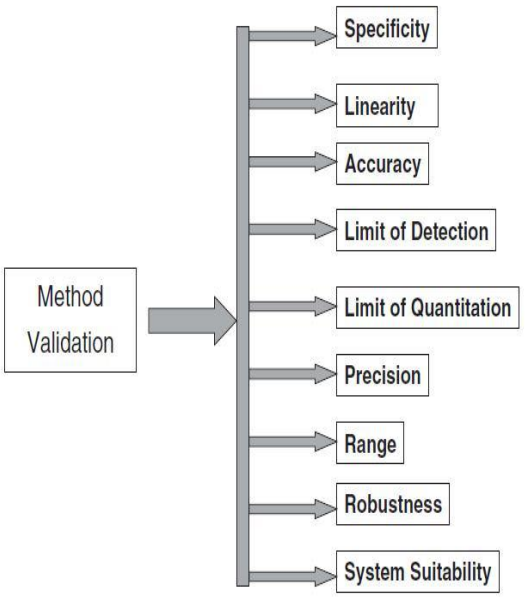
1.3.2Analytical Method Validation [10, 11]

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

Analytical methods need to be validated or revalidated.

- Before their introduction into routine use.
- Whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix).
- Whenever the method is changed and the change is outside the original scope of the method.

The USP has published specific guidelines for method validation for compound evaluation.



Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

The accuracy of an analytical method should be established across its range. In the case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte have been added within the range of the method. Minimum of test concentrations from 50% to 120% are normally used, for establishment of accuracy in assay of drug substance (or a finished product). Average recovery should be 98 to 102% of drug at each level.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. In the precision results of all samples should not have RSD > 2%.

Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Determination of Reproducibility:

Reproducibility can be assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Identification: to ensure the identity of an analyte.

Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay: To provide an exact result this allows an accurate statement on the content or potency of the analyte in a sample

Determination of specificity:

ICH document state that when chromatographic procedure used, representative chromatograms should be used to demonstrate specificity and individual components should be appropriately detected. Peak purity tests may be useful to show that the analyte chromatographic peak is not attributable to more than one component (e.g., diode array, mass spectrometry).

Limit of Detection

The limit of detection 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.

Determination of limit of detection:

For instrumental and non-instrumental methods detection limit is generally determined by the analysis of samples of known concentration of analyte and by establishing the minimum level at which the analyte can be reliability detected.

The limit of detection (LOD) may be expressed as:

$$LOD = 3.3\ \sigma/s$$

Where, σ = the standard deviation of the response.

S = the slope of the calibration curve.

The slope S may be estimated from the

calibration curve of the analyte.

Limit of Quantitation

The limit of quantitation of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Determination of limit of quantitation:

For instrumental and non-instrumental methods quantitation limit is generally determined by the analysis of samples of known concentration of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

The limit of quantitation (LOQ) may be expressed as:

$$LOQ = 10\ \sigma/s$$

Where, σ = the standard deviation of the response.

S = the slope of the calibration curve.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Determination of Linearity and Range:

For the determination of linearity, a minimum of 5 concentrations is recommended. Linearity can be determined by a series of sample whose concentrations span 80-120% of the expected concentration range. Linearity is evaluated by graphically.

Ruggedness

Degree of reproducibility of test results obtained by the same samples under a different condition such as, different analysts, different laboratories condition, different instrument etc. normally expressed as the lack of influence on test results of operational &environmental variables of the analytical method. Ruggedness is a measure of reproducibility of test results under the variation in the condition normally expected from laboratory to laboratory and from analyst to analyst.

Determination of Ruggedness:

By analysis of aliquots from homogenous lots in different laboratory, by different instrument and using operational and environmental condition that may differ but still with the specified parameters of the assay. Degree of reproducibility of test results is then determined as a function of the assay variables.

- Different operator in same laboratory, Different equipment in same laboratory.
- Different source of segment and solution, Different source of column.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination of robustness:

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

Examples of typical variations are:

- Stability of analytical solutions.
- Extraction time.

In the case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in a mobile phase.
- Influence of variations in mobile phase composition.
- Different columns (different lots and/or suppliers).
- Temperature and flow rate.

Applications and Advantages

i. An ideal method for separation of various compounds in plant extracts which resemble in structure and thus demand specific and very sensitive method.

ii. A premier separation technique capable of multi component analysis of real life samples and complex mixtures.

iii. This method is used for ascertaining of various pharmaceuticals. The analysis of the various degradation products can be done and thus stability indicating HPLC systems and method has developed.

iv. Highly automated, using sophisticated auto-samplers and data systems for unattended analysis and report generation. Few techniques can match its versatility and precision of ±0.5% RSD.

v. A host of highly sensitive and specific detectors extend detection limits to nanogram, picogram, and even femtogram levels. As a preparative technique, it provides quantitative recovery of

many labile components in milligram to kilogram quantities.

vi. Having Rapid and precise quantitative analysis. Quantitative sample recovery and amenable to diverse samples. Most importantly, It is amenable to 60% to 80% of all existing compounds.

1.4 Introduction to Stability Degradation Method [12]:-

According to an FDA guidance document, a stability-indicating method (SIM) is "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substances and drug product. A stability indicating method accurately measures the active ingredients, without interference from degradation products, process impurities, excipients, or other potential impurities.

The ICH guidelines Q1A(R2) (2003) elaborate on stability testing of API's and drug products in order to determine storage conditions, retest period, maximum expiring dating period of drug products, correct packaging to protect the product and transport conditions.

➢ **Forced degradation study**:

- The major routes of degradation of any drug substance include hydrolysis, oxidation, heat and photolysis.

1. Hydrolytic degradation
2. Oxidative degradation
3. Thermal degradation
4. Photolytic degradation

1. **Hydrolytic**:

- Hydrolytic study under acidic and basic condition involves catalyzation of ionisable functional groups present in the molecule. HCl and NaOH are employed for generating acidic and basic stress samples, respectively.

2. Oxidative Condition:

- Many drug substances undergo autoxidation i.e. oxidation under normal storage condition and involving ground state elemental oxygen.
- Therefore it is an important degradation pathway of many drugs. Auto- oxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. Hydrogen peroxide, metal ions, or trace level of impurities in a drug substance act as initiators for auto-oxidation
- The mechanism of oxidative degradation of drug substance involves an electron transfer mechanism to form reactive anions and cations.
- Amines, sulphides and phenols are susceptible to electron transfer oxidation to give N-oxides, hydroxylamine, sulphones and sulphoxide.
- Hydrogen peroxide is very common oxidant to produce oxidative degradation products which may arise as minor impurities during long term stability studies.

3. Thermal Condition:

- In general, rate of a reaction increase with increase in temperature. Hence, the drugs are susceptible to degradation at higher temperature.
- Many APIs are sensitive to heat or tropical temperatures. For example, vitamins, peptides, etc. Thermal degradation involves different reactions like pyrolysis, hydrolysis, decarboxylation, isomerisation, rearrangement and polymerization.

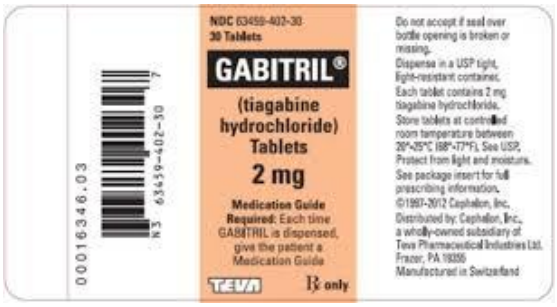
4. Photolytic Condition:

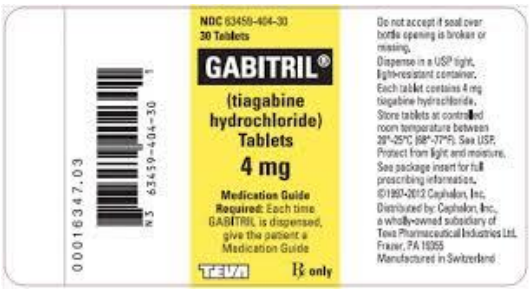
- The rate of photodegradtion depends upon the intensity of incident light and quantity of light absorbed by the drug molecule. The photolytic degradation can occur through non-oxidative or oxidative photolytic reaction.
- Photolytic degradation is carried out by exposing the drug substance or drug product to a combination of visible and UV light.

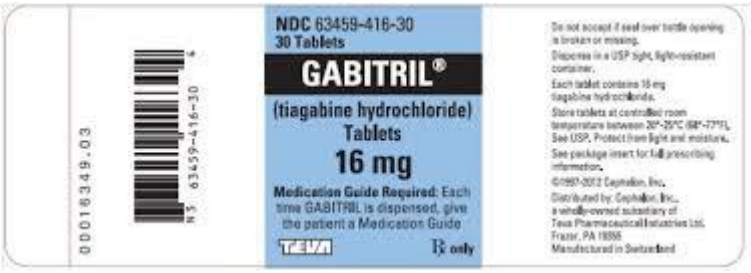
The non-oxidative photolytic reaction include isomerization, dimerization, cyclization, rearrangements & decarboxylation etc. and while oxidative photolytic reaction occur through either singlet oxygen ($1O_2$) or triplet oxygen ($3O_2$) mechanism.

1.5: Introduction to Dosage form:

Brand Name	Contents	Manufacturer	Formulation
Gabitri Tablet	Tiagabine HCl 2mg/4mg/16mg	Teva Pharmaceuticals	Tablet







2. Literature Review [13-19]

2.1. Official Method for Tiagabbine HCl [13]

Sr. No.	Official in	Method	Brief Introduction	Ref. No
1	USP32-NF27 (Tiagabine HCl API)	**HPLC (Assay Method)**	**Mobile phase**:- Phosphate Buffer, pH 2.0: Acetonitrile (65:35) **Column**:- Packing L_1 (150 mm × 4.6 mm, 5µ,) **Flow rate**:- 1.0 ml/min **Detection:-** 254nm	14

2.2. Reported Method for Tiagabbine HCl [14-19]

Sr. No.	Drug	Method	Brief Introduction	Ref. No
1	Tiagabine HCl (API form)	**HPLC (Assay Method)**	**Mobile phase**:- Acetonitrile: Methanol: Water (37:10:53) **Column**:- Ultrasphere C_{18} (250 mm × 4.6 mm, 5µ,) **Flow rate**:- 1.2 ml/min	14

			Detection:- Graphite Electrode +0.76V (monitored), Guard cell 0.95V (before injector)	
2	Tiagabine HCl {S(+) and R(-)-enantiomers}	**Chiral Chromatography**	**Mobile phase**:- 0.1M Ammonium Acetate: Acetonitrile (69:31) **Column**:- Pirkle type Phenyl Glycine Column	15
3	Tiagabine HCl (Stability of two concentrations of tiagabine in an extemporaneously compounded suspension)	**HPLC (Assay Method)**	**Mobile phase**:- Acetonitrile: 0.1% Phosphoric acid (37:53)ss **Column**:- Phenomenex C_{18} (75 mm × 4.6 mm, 5µ) **Flow rate**:- 2.75 ml/min **Detection:-** 254nm	16
4	Tiagabine HCl (Stability of Tiagabine in Two Oral Liquid Vehicles)	**HPLC (Assay Method)**	**Mobile phase**:- 5mM Octane Sulfonic acid: Acetonitrile (50:50) **Column**:- Zorbax CN (150 mm × 3.0 mm, 5µ) **Flow rate**:- 0.4 ml/min **Detection:-** 240nm	17
5	Tiagabine HCl (API Form)	**UV Spectrophotometer (Assay Method)**	**Wavelength:-** 257nm **Concentration range :-** 10-50 µg / mL **Solvent**: Water	18
6	Tiagabine HCl (API Form)	**RP-HPLC (Stress Degradation study)**	**Mobile phase**:- Phosphate Buffer, pH 2.0: Acetonitrile (50:50) **Column**:- HT C_{18} (150 mm × 4.6 mm, 5µ) **Flow rate**:- 1.0 ml/min **Detection:-** 254nm	19

3. RATIONALE

- Tiagabine is an anti-convulsive medication. It is also used in the treatment for panic disorder as are a few other anticonvulsants.
- The precise mechanism by which tiagabine exerts its antiseizure effect is unknown although it is believed to be related to its ability,documented in vitro experiments, to enhance the activity of gamma aminobutyric acid(GABA),the major inhibitory neurotransmitter in the central nervous system.Tiagabine blocks GABA uptake into presynaptic neurons.

4. AIM AND OBJECTIVE

4.1 AIM

Literature review reveals that There are a HPLC and Stability methods are reported, but the Method for stress degradation reported is Risky to Column, Because the pH of Mobile phase is 2 pH, Which cause the hydrolysis in Column leads to column more polar.

So Aim of present work is to develop stability indicating RP-HPLC Method for the Estimation of Tiagabine HCl in its Pharmaceutical Dosage form.

4.2 OBJECTIVE

1) To develop HPLC method for estimation of Tiagabine HCl in its Pharmaceutical Dosage form.

2) To perform Stability study on the developed method

2) Applying the newly developed, validated analytical method for the estimation of Tiagabine HCl in its Pharmaceutical Dosage form.

5.1 Standards and Reagents:

❖ **Standard**

Standard	Source
Tiagabine HCl	Gitar Laboratpries

❖ **Sample**

Sample	Source
Gabitril tablet (Tiagabine 4mg)	Teva Pharmaceuticals

❖ **REAGENTS USED IN EXPERIMENT:**

Chemical/ Reagent	Grade	Manufacturer
Methanol	HPLC Grade	Merck specialties pvt, Ltd., Mumbai
Potassium Dihydrogen Phosphate	AR	Merck specialties pvt, Ltd., Mumbai
Water	HPLC Grade	Merck specialties pvt, Ltd., Mumbai
Ammonium Acetate	AR	Merck specialties pvt, Ltd., Mumbai

5.2 Apparatus and Equipments used in experiment:

Apparatus / Equipments:

Components	Volume	Type
Volumetric flasks	10 ml, 25 ml, 50 ml,100 ml	Borosilicate glass type I
Pipettes	1 ml, 2 ml, 5 ml, 10 ml	Borosilicate glass type I
Measuring cylinder	100 ml	Borosilicate glass type I
Beaker	100 ml, 250 ml, 500 ml	Borosilicate glass type I
Whatmann Filter	-	Filter Paper No.42

5.3 Instrumentation:

➢ **Instrumentation for HPLC**

Component	Brand / Model / Software	Manufacturer/ Supplier
HPLC	Shimadzu LC-20 AT2000	Shimadzu
HPLC Column	C18 (25cm x 0.46 cm) Hypersil BDS	-
Detector	UV detector	-
Ultrasonic Water Bath	Fast Clean	Ultrasonic cleaner
pH meter	-	Electroquip's Digital pH meter
Analytical Balance	AUX-200	-

➢ **Instrumentation for UV spectrophotometer**

Component	Brand / Model / Software	Manufacturer/ Supplier
UV Visible spectrophotometer	Shimadzu 1800 double beam UV visible spectrophotometer, UV probe 2.33	Shimadzu Corporation, Kyoto, Japan
Cuvette	Quartz cuvette	Shimadzu Corporation, Kyoto, Japan
Analytical Balance	AUX-200	-

➢ **Instrumentation for Melting Range**

Component	Brand / Model / Software	Manufacturer/ Supplier
Melting point Apparatus	Thermocal	Analab

5.3 Identification of Drugs

❖ **Determination of Solubility**

Drug	Solubility
Tiagabine HCl	Soluble in methanol and water

❖ **Determination of Melting Point:**

Drug	Melting Point
Tiagabine HCl	192-195^{0}C

❖ **Identification by IR:**

(A) IR spectra of Tiagabine HCl

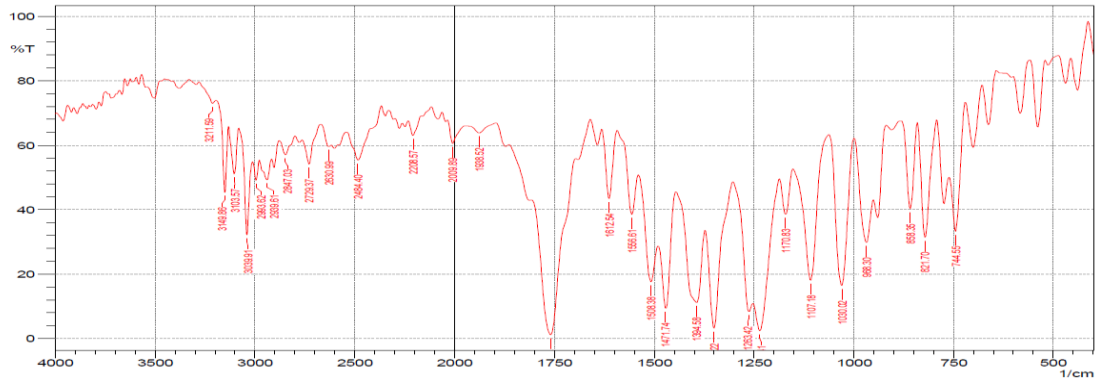


Figure 5.1: IR spectra of sample

Frequency (cm^{-1})	Assignment
1759	C=O (S)
1612	C=C (S)
1360	C-N (S)
3099	O-H (S)

Table 5.1: Interpretation of IR of Tiagabine HCl

5.4 Development of RP-HPLC Method

5.4.1 Selection of wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study drug solutions of Tiagabine HCl (10 ppm) was prepared in Methanol. This drug solution was than scanned in UV region of 200-400 nm and maximum Absorbance was recorded.

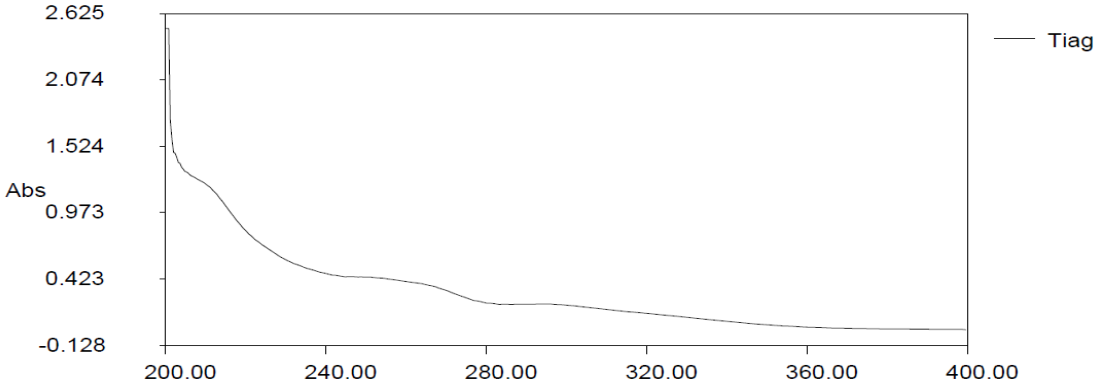


Figure 5.2: UV Spectra of Tiagabine HCl (10 ppm) (Maximum Absorbance 254 nm)

Tiagabine HCl solution: 10 mg-→100ml with methanol. Further 1ml to a 10ml and make up with methanol (10µg/ml in methanol)

solutions was scanned between 200 - 400 nm.
Wavelength What Gives maximum Absorbance was selected from the above Spectra.

5.4.2 Selection of Mobile Phase

Trail contains various mobile phase which are considered of Methanol, Water and Acetonitrile in different proportions and different volumes at different flow rate were tried. On the basis of various trails the mixture of Water: Acetonitrile (20:80) at 1.0 mL/min flow rate, proved to be better than the other mixture in terms of peak shape, theoretical plate and asymmetry.

Trials are summarizes in following table.

Table 5.2: **List of Mobile Phase trials**

Sr. No	Mobile Phase	Remark
1	Water: Methanol (50:50)	One peak Observed with irregular shape
2	Water: Acetonitrile (50:50)	Fronting Observed
3	Water (pH 6.0): Acetonitrile (30:70)	Peak shape became good but run time is high
4	Water (pH 6.0): Acetonitrile (80:20)	Run time decreased and Peak follow all SST Parameters

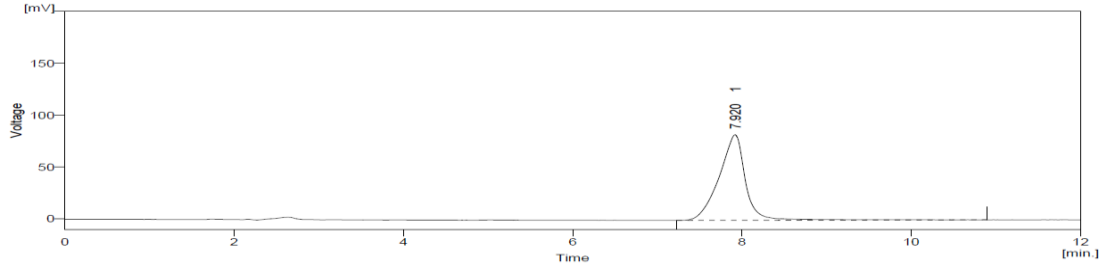


Figure 5.3: HPLC Chromatogram of Tiagabine HCl 10 ppm in Water: Methanol (50:50)

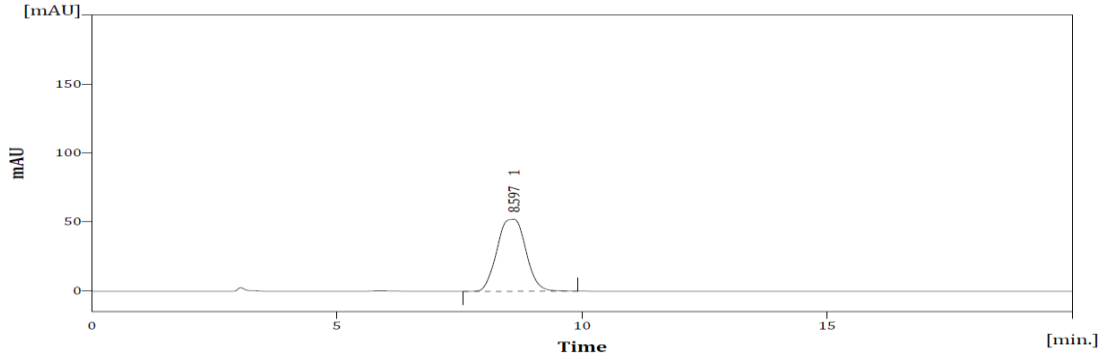


Figure 5.4: HPLC Chromatogram of Tiagabine HCl 10 ppm in Water: Acetonitrile (50:50)

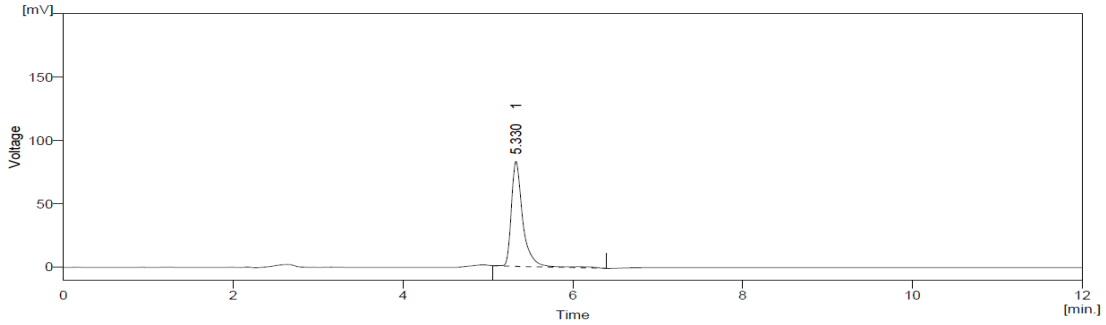


Figure 5.5: HPLC Chromatogram of Tiagabine HCl 10 ppm in Water (pH 6.0): Acetonitrle (30:70)

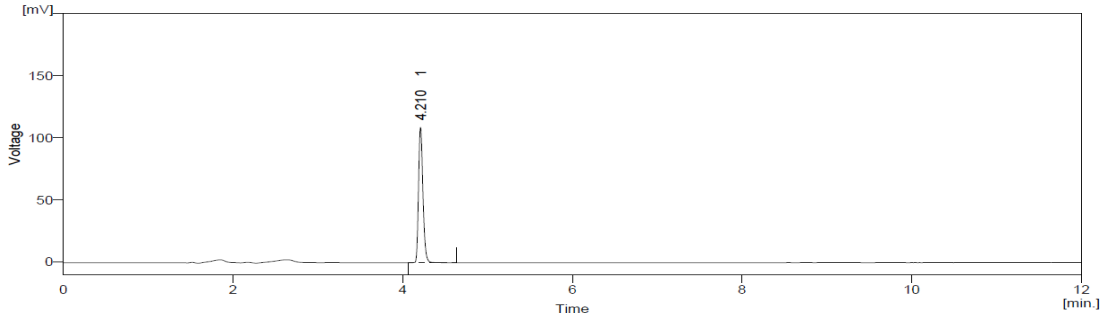


Figure 5.6: HPLC Chromatogram of Tiagabine HCl 10 ppm in Water (pH 6.0): Acetonitrile (20:80)

Mobile Phase was selected based on the review of literature. Various mobile phases were tried. Trial contains various mobile phases which consisted of Methanol, Water, and Acetonitrile in different proportions with various pH and different volumes at flow rate 1 ml/min were tried. On the basis of various trials the mixture of water (pH 6.0): Acetonitrile (20:80)

Parameters	Tiagabine HCl
Retention Time	4.210
Theoretical Plates	3579
Asymmetry	1.357

5.4.3 Optimization of flow rate

1ml/min flow rate, proved to be better than the other in terms of resolution, peak shape and shorter retention time.

5.4.4 Preparation of standard solution of mixtures of Tiagabine HCl (10 ppm).

(A) Tiagabine HCl standard stock solution: (100 µg/mL)

A 10 mg of Tiagabine HCl was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with mobile phase.

(B) Preparation of Working standard solution of Tiagabine HCl (10 µg/mL)

Take 1 mL from the Tiagabine HCl stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Table 5.3: RP-HPLC optimized chromatographic conditions

Parameters	Chromatographic Condition
Mode of elution	Isocratic
Mobile Phase	Water (pH 6.0): Acetonitrile (80:20)
Column	C18 (25cm x 0.46 cm) Hypersil BDS
Flow rate	1ml/min
Runtime	6 min
Injection volume	20 µL
Detection wavelength	254 nm

5.5 Validation of RP-HPLC method:

5.5.1 Specificity:

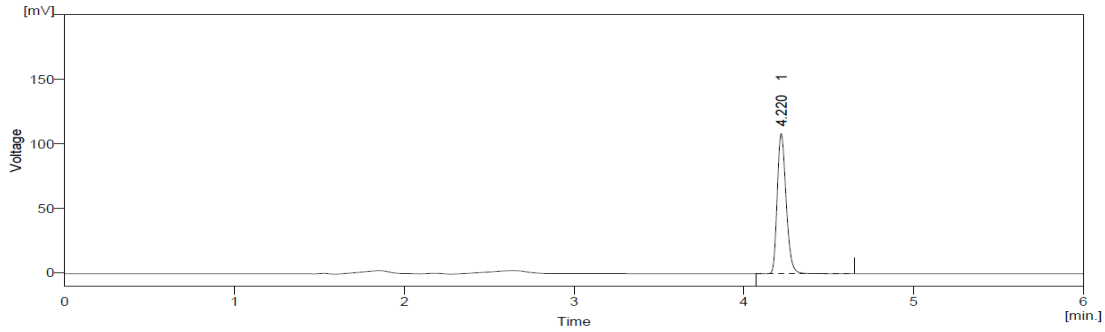


Fig. 5.7:- Chromatogram of Tiagabine HCl std

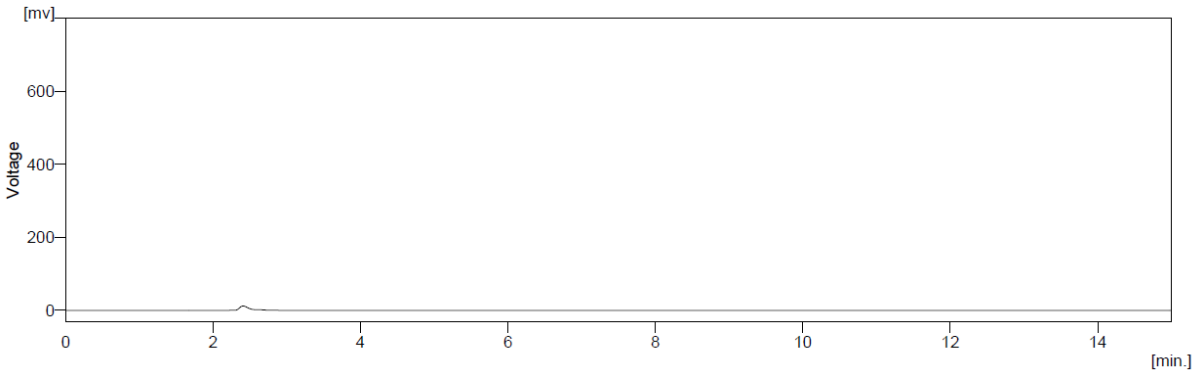


Fig. 5.8:- Chromatogram of Blank

The Chromatograms of Tiagabine HCl standards shows no interference with the Chromatogram of Blank, so the Developed method is Specific.

5.5.2 Linearity:

The linearity for Tiagabine HCl was assessed by analysis of combined standard solution in range of 5-15 µg/ml.

5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Tiagabine HCl (100 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 5,7.5,10,12.5 and 15 µg/ml for Tiagabine HCl.

In term of slope, intercept and correlation co-efficient value, The graph of peak area obtained verses respective concentration was plotted. Correlation co-efficient for calibration curve for Tiagabine HCl was found 0.999.

The regression line equation for Tiagabine HCl is

For Tiagabine HCl **y = 45.523x - 17.315**

Table 5.4: Linearity data for Tiagabine HCl

Sr. No	Concentration (µg/ml)	Area
1	5	210.847
2	7.5	325.093
3	10	431.885
4	12.5	558.613
5	15	663.120

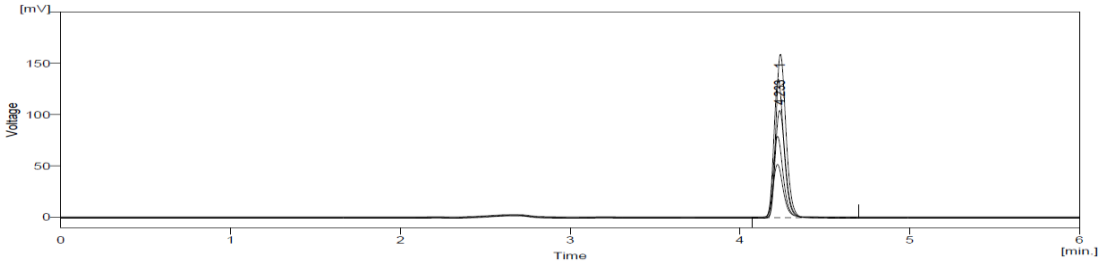


Fig. 5.9: Overlay chromatogram of different concentrations of Tiagabine HCl

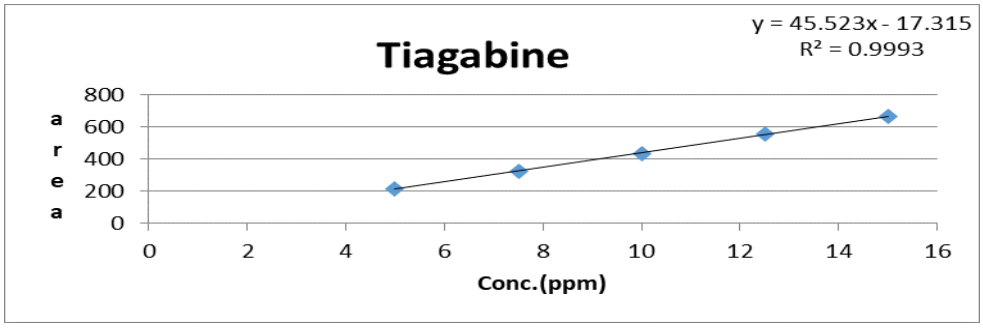


Fig. 5.18: Calibration Curve of Tiagabine HCl (5-15 µg/ml).

5.5.3 Precision

I. Repeatability

The data for repeatability of peak area measurement for Tiagabine HCl (10 µg/ml) based on six measurements of same solution of Tiagabine HCl (10 µg/ml).The % RSD for Tiagabine HCl was found to be 0.392

Table 5.5: Repeatability data for Tiagabine HCl

Tiagabine HCl				
Sr. No.	Conc. (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
1.	10	395.698	394.286±1.548	0.393
		393.713		
		391.742		
		396.053		
		394.061		
		394.449		

II. Intraday precision

Standard solution containing (5,10,15 µg/ml) of Tiagabine HCl were analyzed three times on the same day and % R.S.D was calculated

Table 5.6: Intraday precision data for estimation of Tiagabine HCl

	Tiagabine HCl		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	198.017 ± 1.221	0.611
2	10	393.582 ± 3.026	0.769
3	15	595.030± 2.240	0.376

III. Interday precision

Standard solution containing (5,10,15 µg/ml) of Tiagabine HCl were analyzed three times on the different day and % R.S.D was calculated

Table 5.7: Interday precision data for estimation of Tiagabine HCl

	Tiagabine HCl		
SR. NO.	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**% R.S.D**
1	5	588.026 ± 4.949	0.841
2	10	394.3783± 3.909	0.991
3	15	588.428± 4.911	0.834

5.5.4 Accuracy:

For Tiagabine HCl

5 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80%, 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 222 nm. The amount of Nebivolol HCl was calculated at each level and % recoveries were computed

Table5.8: Recovery data for Tiagabine HCl

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	5	4	4.019	100.477	101.001 ± 0.564
2		5	4	4.064	101.599	
3		5	4	4.037	100.927	
4	100 %	5	5	5.037	100.736	99.512 ± 1.147
5		5	5	4.967	99.339	
6		5	5	4.923	98.462	
7	120 %	5	6	6.029	100.488	99.569 ± 0.918
8		5	6	5.974	99.567	
9		5	6	5.919	98.652	

5.5.5 LOD and LOQ:

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where, SD = Standard deviation of intercepts

Table 5.9: Limit of Detection and Limit of Quantitation data for Tiagabine HCl

Tiagabine HCl	
Limit of Detection	**Limit of Quantitation**
LOD = 3.3 x (SD / Slope) = 3.3 x (5.505/45.523) = 0.399 µg/ml	LOQ = 10 x (SD / Slope) = 10 x (5.505/45.523) = 1.209 µg/ml

5.5.6 Robustness:

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.
2. pH of Mobile phase was changed (± 0.2) 5.8 and 6.2
3. Ratio of Mobile phase was changed (±2) Water: Acetonitrile (18:82) and Water: Acetonitrile (22:78)

Table 5.10: Robustness data for Tiagabine HCl

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (- 0.2)	Area at pH (+ 0.2)	Area at Mobile phase(-2)	Area at Mobile phase(+2)
1	410.695	380.832	403.914	388.092	418.596	373.130
2	410.279	381.569	406.743	387.333	417.752	372.763
3	407.744	380.823	404.703	383.451	420.441	370.866
% R.S.D	0.390	0.112	0.360	0.644	0.328	0.326

5.5.7: Analysis of marketed formulation by developed method

Sample Stock Solution (Tiagabine HCl 100 µg/mL):

Take Tablet powder equivalent to 10 mg of Tiagabine HCl was transferred to a 100 ml volumetric flask, Add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

Working Sample Preparation (Tiagabine HCl 10 µg/mL):

Take 1 mL from standard stock solution and transferred to 10 ml volumetric flask and made up volume up to the mark with the mobile phase

Inject above Solution 20 µl for Assay Analysis.

Table5.11: Analysis on marketed formulation

Tablet	Gabitril Tablet
Label claim	**Tiagabine HCl (4mg)**
Assay (% of label claim*) Mean ± S. D.	97.387±2.206

The assay results were comparable to labeled value of drug in dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

5.1 Stability indicating method for simultaneous estimation of Tiagabine HCl by RP-HPLC

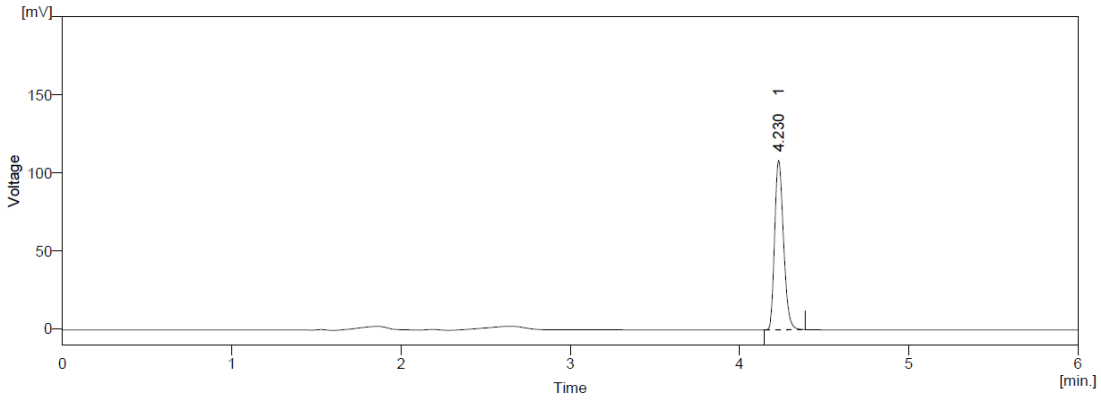


Fig. 5.11: Tiagabine HCl Standard for stability

I. Acid degradation

Acid degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and put for 3 hrs at RT. Then the volume was adjusted with diluent to get 10µg/ml for Tiagabine HCl

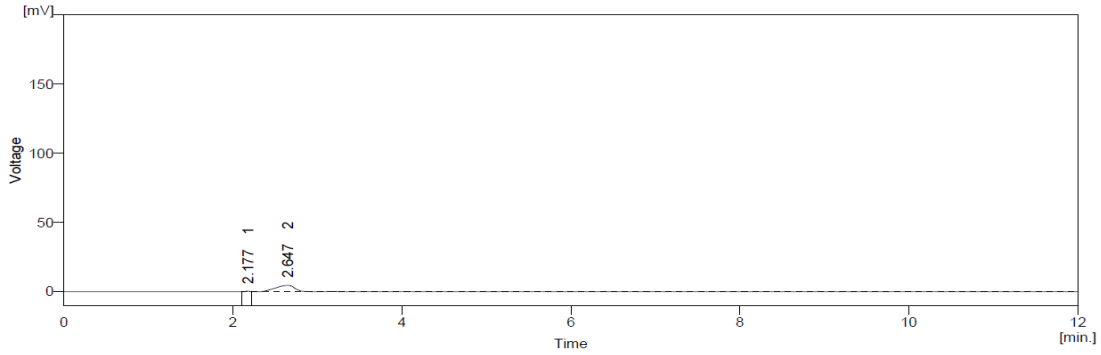


Fig. 5.12: Tiagabine HCl Acid Degradation Blank

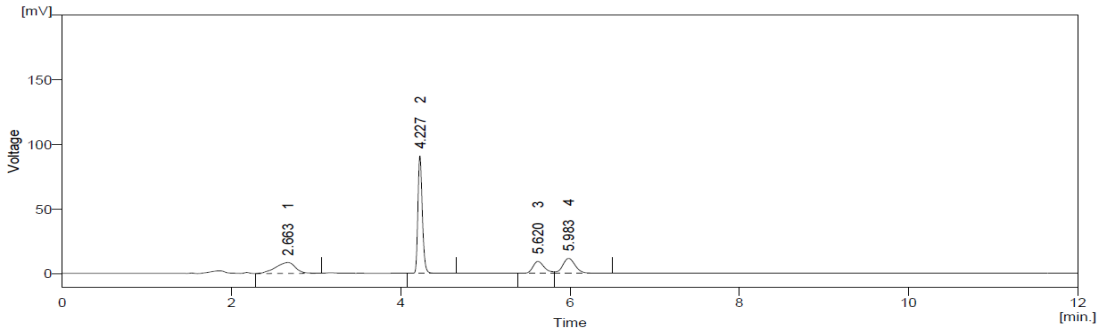


Fig. 5.13: Tiagabine HCl Acid Degradation Standard

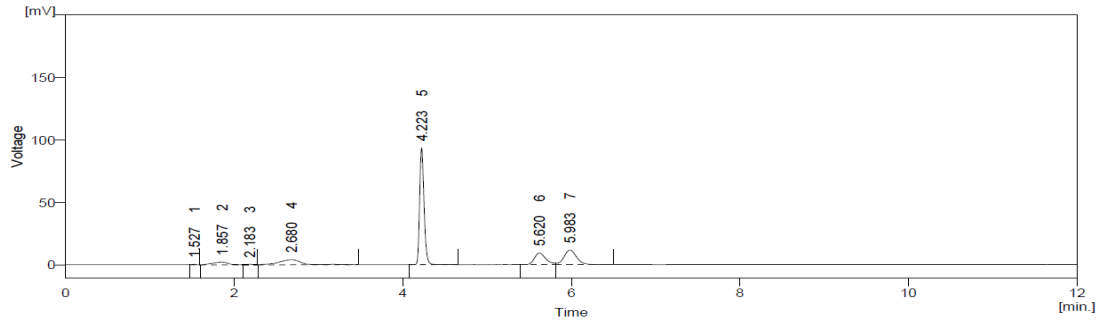


Fig. 5.14: Tiagabine HCl Acid Degradation Sample

II. Base degradation

Base degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 6 hrs at RT. Then the volume was adjusted with diluent to get 10µg/ml for Tiagabine HCl.

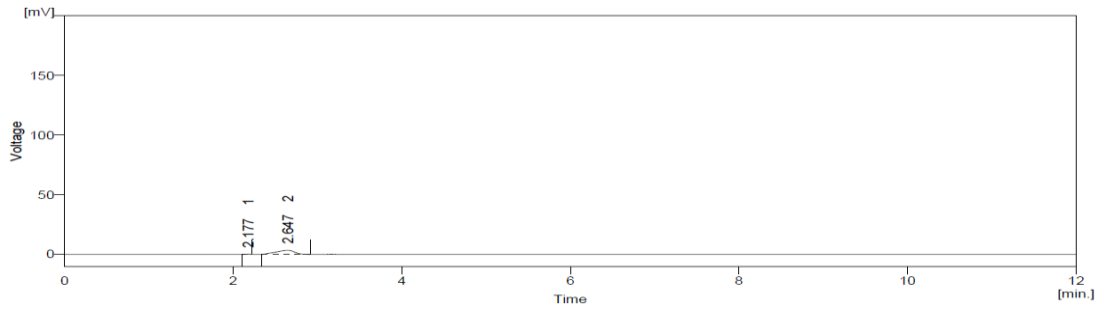


Fig. 5.15: Tiagabine HCl Base Degradation Blank

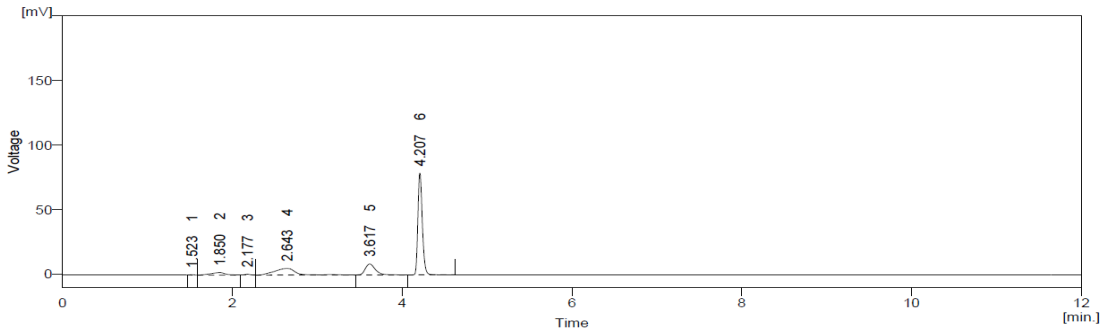


Fig. 5.16: Tiagabine HCl Base Degradation standard

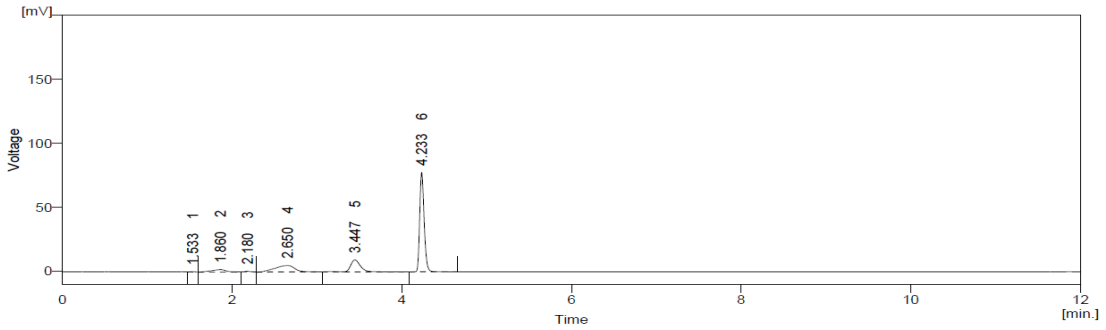


Fig. 5.17: Tiagabine HCl Base Degradation Sample

III. Oxidative degradation

Oxidation degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and put for 4 hrs at RT. Then the volume was adjusted with diluent to get 10µg/ml for Tiagabine HCl.

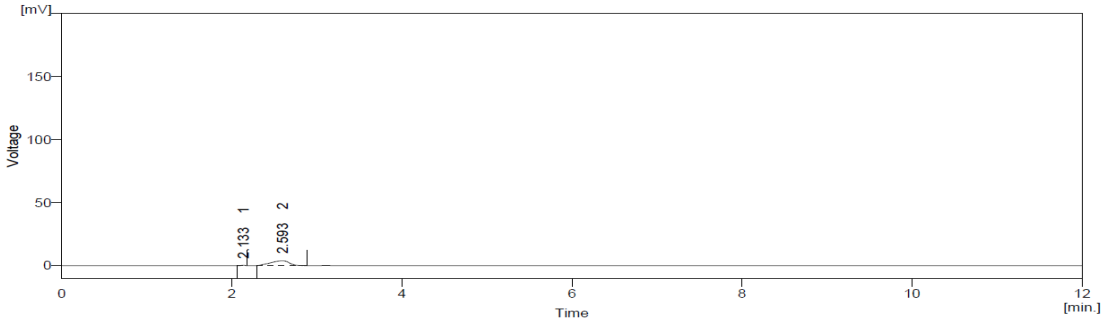


Fig. 5.18: Tiagabine HCl Oxidation Degradation Blank

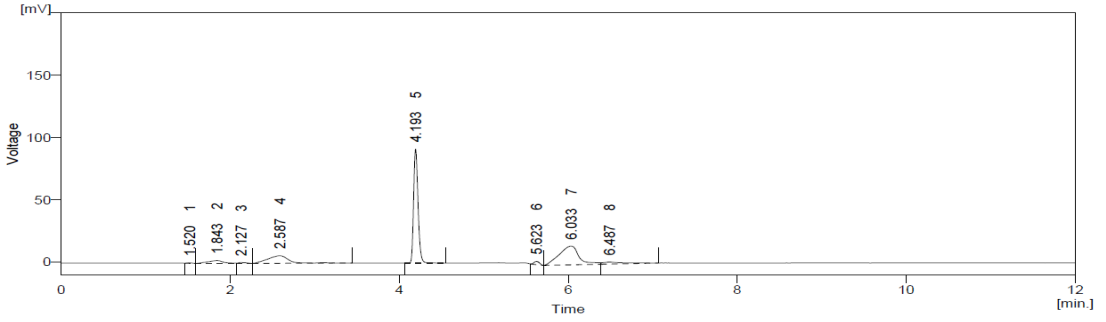


Fig. 5.19: Tiagabine HCl Oxidation Degradation standard

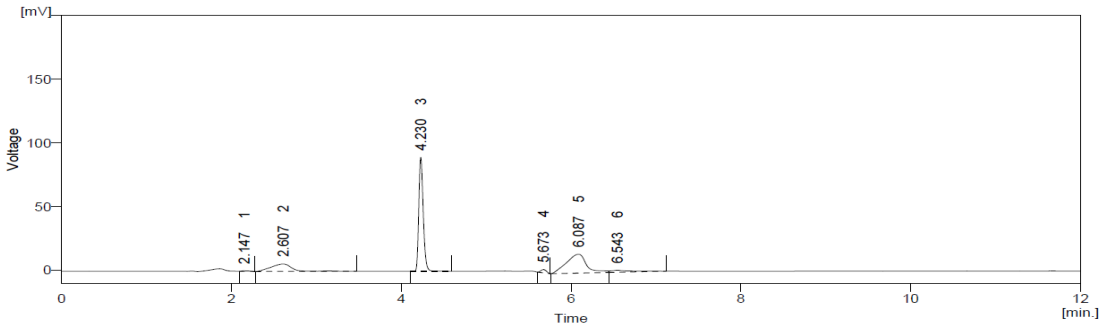


Fig. 5.20: Tiagabine HCl Oxidation Degradation Sample

IV. Photo degradation

Photo degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Then the volumetric flask was kept in UV chamber for 18 hrs. Then the volume was adjusted with diluent to get 10µg/ml for Tiagabine HCl.

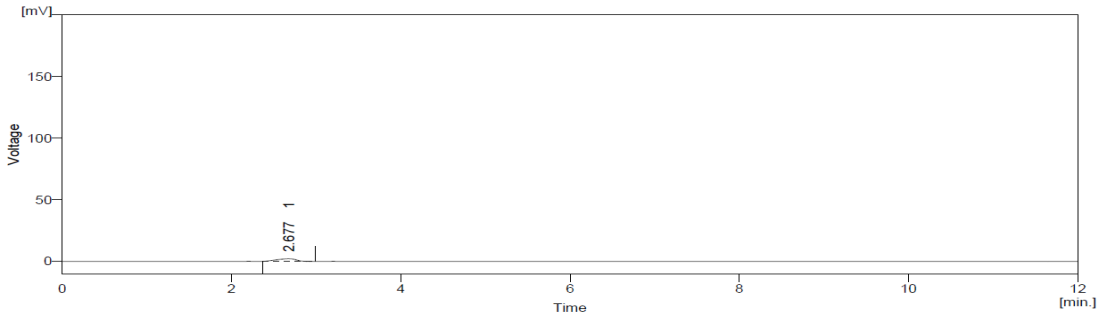


Fig. 5.21: Tiagabine HCl Photo Degradation Blank

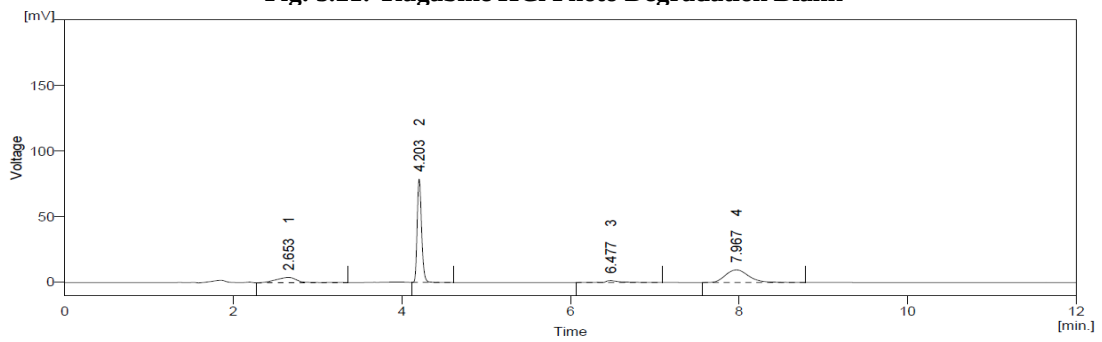


Fig. 5.22: Tiagabine HCl Photo Degradation standard

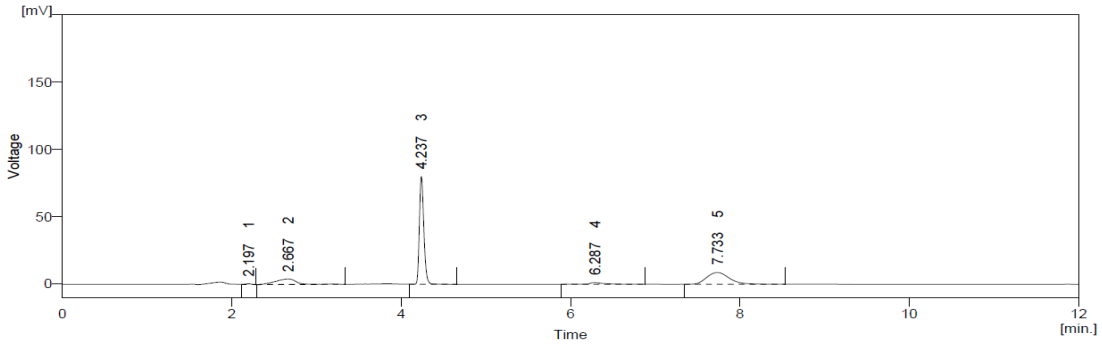


Fig. 5.23: Tiagabine HCl Photo Degradation Sample

V. Thermal degradation

Photo degradation studies were performed by transferring one ml of stock solution to 10 ml of volumetric flask. Then the volumetric flask was kept in a oven at 80^0C Temperature for 7 hrs. Then the volume was adjusted with diluent to get 10µg/ml for Tiagabine HCl.

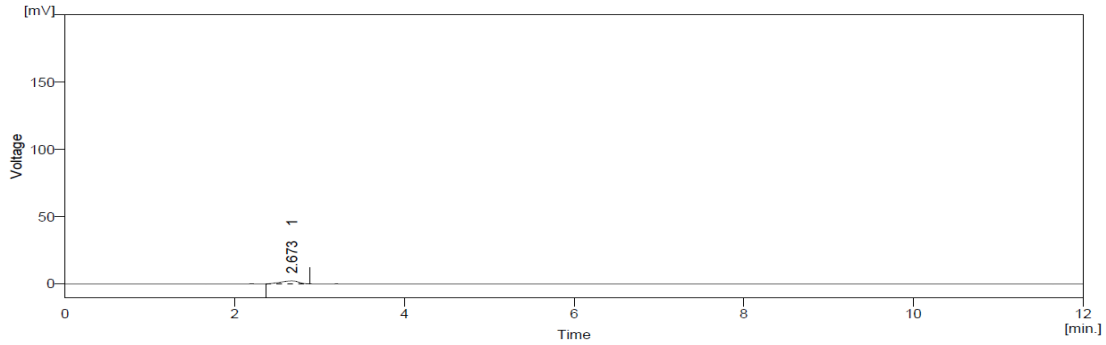


Fig. 5.24: Tiagabine HCl Thermal Degradation Blank

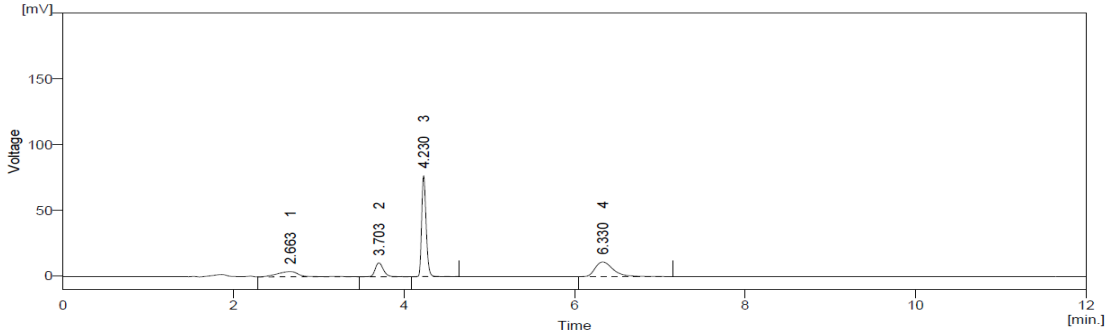


Fig. 5.25: Tiagabine HCl Thermal Degradation standard

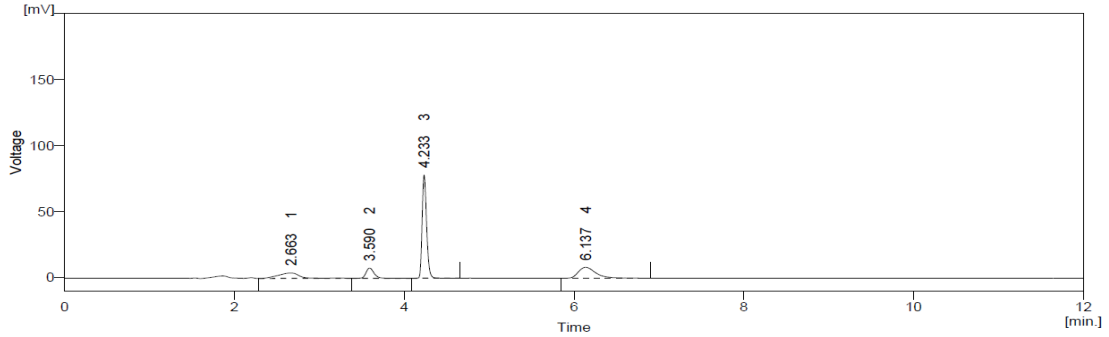


Fig. 5.26: Tiagabine HCl Thermal Degradation Sample

Table 5.12: Tiagabine HCl std for stability

Calculation for Stability:

Drugs	Area
Tiagabine HCl	394.911

Table 5.13: Tiagabine HCl % Degradation

Parameter	Standard		Sample	
	Area	% Degradation	Area	% Degradation
Acid	330.714	16.256	338.591	14.261
base	287.092	27.302	284.866	27.866
oxidation	332.024	15.924	327.455	17.081
Photo	286.049	27.566	293.456	25.691
Thermal	281.394	28.745	286.457	27.463

CONCLUSION:

Tiagabine is an anti- convulsive medication. It is also used in treatment for panic disorder as are a few other anticonvulsants. Tiagabine HCL is operate as GABA reuptake inhibitor**.**

- It enhance the activity of gamma aminobutyric acid,the major inhibitor neurotransmitter in the CNS.Tiagabine binds to recognition sites associated with the GABA uptake carrier.
- RP-HPLC method was developed for estimation of Tiagabine Hydrochloride. In RP-HPLC method, good resolution and separation of two drugs was achieved. Water (pH 6): Acetonitrile (80:20) was used as mobile phase. Retention time of Tiagabine Hydrochloride were found to be 4.210 min respectively with a flow rate of 1 ml/min. The proposed method was accurate and precise. Therefore proposed method can be used for routine analysis of Tiagabine Hydrochloride in tabletDosage form.
- Forced degradation study of Tiagabine Hydrochloride was performed by RP-HPLC method which includes Acid, Base, Oxidative, Photo and Thermal degradation. Results of degradation were found within limit

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Appendix: A
LIST OF ABBREVIATIONS

ABBREVIATION USED	FULL FORM
Symbols	
%	Percentage
Mcg or µg	Microgram
µL	Micro liter
cm	Centimeter
Fig	Figur
Abs	Absorbance
gm	Gram
L	Liter
Min.	Minute
nm	Nanometer
r^2	Correlation coefficient
Sec.	Second
Temp.	Temperature
tR	Retention time
Others	
IP	Indian Pharmacopoeia
BP	British Pharmacopoeia
USP	United State Pharmacopoeia
ICH	International Conference on Harmonization
UV	Ultra Violet
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IUPAC	International Union of Pure and Applied Chemistry
LOD	Limit of Detection
LOQ	Limit of Quantitation
SD	Standard Deviation
RSD	Relative Standard Deviation
% RSD	Percentage Relative Standard Deviation
ODS	Octyldecylsilane
BDS	Base deactivated silanone