STABILITY INDICATING RP-HPLC ASSAY METHOD DEVELOPMENT AND VALIDATION OF ELETRIPTAN HYDROBROMIDE TABLETS

A Dissertation Submitted to

THE TAMIL NADU DR.M.G.R. MEDICAL UNIVERSITY, CHENNAI

In the partial fulfillment of the requirement for

The award of degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted by

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Under the Guidance of

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DEPARTMENT OF PHARMACEUTICS

DATE:

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Date:
Place: Chennai

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CONTENTS

LIST OF ABBREVIATIONS

LC	Liquid Chromatography
UV	Ultra violet
HPLC	High performance Liquid Chromatography
IR	Infra red
API	Active pharmaceutical ingredient
FPP	Finished pharmaceutical product
Ele	Eletriptan
HBr	Hydrobromide
PDA	Photo diode array
SIAM	Stability indicating assay method
MeOH	Methanol
ACN	Acetonitrile
TEA	Triethylamine
TFA	Triflouroaceticacid
NaOH	Sodium hydroxide
H_2O	Water
H_2O_2	Hydrogen peroxide
KH_2PO_4	Potassium di hydrogen phosphate
SD	Standard deviation
RSD	Relative Standard deviation
CV	Coefficient variation
LOD	Limit of detection
LO	Limit of quantification
RH	Relative humidity
V.F	Volumetric flask
t_R	Retention time
RT	Room temperature
ICH	International conference on harmonization

EU	European Union
GMP	Good manufacturing practice
USFDA	United States food and drug administration
WHO	World health organization
Nm	Nanometer
Ml	Milliliter
µm	Micrometer
µg	Microgram
µl	Micro liter
Hrs	Hours
i.d.	Internal diameter
NMT	Not more than
NLT	Not less than
Mnths	Months
Min	Minutes
ºC	Degrees centigrade

1. INTRODUCTION

1.1 Introduction to High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is an advanced form of liquid chromatography used in separating the complex mixture of molecules encountered in chemical and biological systems, in order to understand better the role of individual molecules. Among different chromatographic methods, high performance liquid chromatography (HPLC) offers a greater variety of stationary phases, which there by allows selective interactions and more possibilities for separation. In HPLC the separation is about 100 times faster than the conventional liquid chromatography due to packing of particles in the range of 3-10 m. In HPLC mobile phase composition is changed in a programmed fashion to increase the efficiency of separation.

Depending on the unique affinity of each component (referred to as the analyte) between the mobile phase and the stationary phase, each analyte migrates along the column at different speeds and emerges from the column at different times, thus establishing a separation of the mixture. Analytes with higher affinity for the mobile phase migrate faster down the column, whereas those with higher affinity for the stationary phase migrate slower. This migration time (referred to as retention time) is unique for each analyte and can be used in its identification. With the appropriate use of a detection method after the column, each analyte can also be quantified for analysis.

Smaller column particle size can improve chromatographic resolution, but increased solvent delivery pressure is needed. Further reduction of column particle size can allow for higher solvent flow rates, reducing analysis time without sacrificing resolution.

1.1.1 Advantages and limitations of HPLC

Table 1 highlights the Advantages and limitations of HPLC. HPLC is highly efficient separation technique used for multi component analysis of real life samples and complex mixtures.

Table 1: Advantages and limitations of HPLC

Advantages

- High resolution and speed of analysis.
- Can be used for separation and analysis of complex mixtures.
- A variety of solvents and column packing's are available,

  providing a high degree of selectivity for specific analysis.
- Easy automation of instrument operation and data analysis.
- Adaptability to large scale pre operative procedures
- High sensitivity detection
- Quantitative sample recovery
- Greater accuracy and precise.

Limitations

- Less separation efficiency than capillary GC
- No universal detector
- More difficult for novices

1.1.2 Types of HPLC Techniques

- Based on modes of chromatography:
- Reverse phase chromatography
- Normal phase chromatography
- Based on principle of separation:
- Affinity chromatography
- Size exclusion chromatography
- Adsorption chromatography
- Ion exchange chromatography
- Chiral phase chromatography
- Based on the scale of operation:
- Analytical HPLC
- Preparative HPLC
- Base on elution technique:
- Gradient separation
- Isocratic separation

1.1.3 Classification of Chromatographic methods:

Table 2: classification of chromatographic methods

Mobile phase	**Stationary phase**	**Method**
Liquid	Solid	Adsorption column, thin-layer, ion exchange, High performance liquid chromatography.
Liquid	Liquid	Partition, column, thin-layer, HPLC, paper chromatography.
Gas		Gas – Liquid Chromatography.

1.2 Method development:

Developing and validating an analytical method is very expensive, before developing new method, a thorough literature survey should be conducted for existing methodologies of intended analyte or similar molecules. Survey conducted by using chemical abstracts, compendial monographs (USP, EP), journal articles, internet, manufacturer literature.

New analytical methods are required for following reasons.

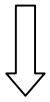
- Existing method are not available.
- Technique has better performance like easy of use, highly sensitive, rapid turnaround or new instrumentation.
- An alternate method required for regulatory compliance.

- Existing method are not sufficiently reliable, sensitive, or cost effective.

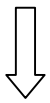
1.2.1 Strategy for method development

Commonly involved steps in strategy for method development:

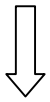
Description of method and separation goals



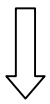
Collection of sample and analyte information



Initial method development



Method tuning – optimization



Method validation

1.2.2 Method goals

Analytical method goals in turn defined as, method acceptance criteria for peak area %RSD, linearity range, and various system suitability parameters like, resolution, tailing factor, precision of retention time, specificity, sensitivity.

Table 3: Acceptance criteria for system suitability parameters

parameter	**acceptance criteria**
retention time	analysis time 5-30 min
%RSD	< 2%
resolution	>2
tailing factor	NMT 2

Other desirable characteristics include:

- Minimal sample work up
- Robust method that does not require extensive training for execution
- Low cost per analysis

1.2.3 Method types

HPLC methods are developed for single analyte and multiple analyte assays. Methods can be divided in to three major categories.

- Qualitative
- Quantitative
- Preparative

a) Qualitative method

It is an identification test that conforms the presence or absence of analyte in the sample by matching retention time with that of reference standard.

b) Quantitative method

Quantitative method mainly gives the information related to concentration of analyte in the

sample. Quantitative method can also considered as qualitative method, an assay method can often also serve as an identification method. Developing a quantitative method is more difficult and requires greater effort for validation than developing qualitative method.

c) Preparative method

Preparative method is used to isolate the purified component in the sample. Method validation is not required in this process because, this method mainly meant for generate purified components.

1.2.4 Steps involved in development of HPLC method:

a) Literature survey

It is helpful to avoid duplication of work

It gives important information of sample to be considered during method development

- Pka value of compound
- Nature of sample matrix
- UV spectrum of compound
- Number of compounds presents
- Sample solubility
- Sample stability
- Chemical nature of sample
- Chemical structure of compound
- Concentration range of compounds in sample interest

b) Selection of chromatographic method

Primarily normal phase chromatographic method should be tried. If it is not produce reliable results then go for reverse phase chromatographic method.

For ion exchange or ion pair chromatography, first ion suppression by pH control and reversed phase chromatography should be tried.

c) Selection of stationary phase

Based on polarity and affinity of analyte towards the stationary phase the column was selected.

d) Selection of mobile phase

Reversed phase bonded packing, when used in conjunction with highly polar solvents. Mobile phase may be either single liquid or combination of liquids, which are compatible with sample, column and instrument.

Initial experimental conditions for separation in HPLC:

Table: 4 Initial experimental conditions for separation in HPLC

Separation variable		**Initial choice**
Column	Dimensions	15 (or 25) x 0.46 cm
	Particle size	5 or 3.5 μm
	Stationary phase	C – 18 or C – 8 or phenyl
Mobile Phase	Solvent A/B	Water(or aqueous buffer)/ acetonitrile
	%B	Variable
	Buffer compound,	25 mM phosphate,

	P^H Conc. Additives Flow rate	pH ≤ 3.5 25 – 50 mM Triethylamine (TEA) 1 – 2 mL/min
Temperature		40ºC
Sample size	Volume Mass	≤ 50µL ≤ 100µg

Based on Sample Characteristics different buffer solutions has been selected

Table: 5 recommended additives for reversed phase HPLC

Sample Characteristics	**Additives**
Basic compounds (e.g. amines)	50 mM phosphate buffer, 30 mM triethylamine(buffer pH-3.0)
Acidic compounds (e.g. carboxylic acids)	50 mM phosphate buffer, 1% acetic acid (buffer pH-3.0)
Mixture of acids and bases (buffer pH – 3.0)	50 mM phosphate buffer, 30 mM triethylamine, 1% acetic acid
Cationic salts (e.g. tetra alkyl quaternary ammonium compounds)	30 mM triethylamine, 50 mM sodium nitrate
Anionic salts	1% acetic acid, 50 mM sodium nitrate.

e) Selection of suitable detector

Detector is the major part of HPLC system and measures the compounds after their separation on the column.

There are basically two types of detectors.

- Bulk property detector
- Solute property detectors

UV detector is the first choice because of its convenience and applicability in case of most of the samples. The latest version of equipments is available with photo diode - array detectors (PDA or DAD).

Different variables like organic solvent, mobile phase, solvent strength, column type, column temperature, concentration of mobile phase additives may show different effect in results or response. These variables used to vary band spacing.

Table 6.Variables used to vary band spacing

Variable	**Comment**
Choice of organic solvent	A change from methanol to acetonitrile or THF often results in large changes in separation.
Mobile phase	A change in pH may result in a major effect on band spacing for samples that contain ionic or ionisable compounds.
Solvent strength	A change in percent organic often provides significant changes in retention and separation.

Column type	This refers to the choice of bonded-phases for reversed-phase LC (C – 18, C – 8, phenyl, cyano, trimethyl, etc.,)
Concentration of mobile phase additives	The most common additives for varying band spacing include amine modifiers, acid modifiers, buffers and salts
Temperature	Temperatures of 25 - 60º C are more common.

1.3 Method Validation

Guidelines provide frame work for validation of developed method.

For pharmaceutical methods guidelines prescribed by,

- United states pharmacopoeia
- World health organization
- Food and drug administration
- International conference on harmonization

1.3.1 United states pharmacopoeia (USP)

As per USP, validation is defined as "the process by which it is establish by laboratory studies the performance characteristics of the method meet the requirement for intended analytical application".

1.3.2 World health organization (WHO)

As per WHO, validation is defined as "the process of providing documented evidences that a system / procedure dose what it is supposed to do precisely and reliably".

1.3.3 Food and drug administration (FDA)

As per FDA validation is defined as "establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce meeting it pre determined specifications and quality attributes".

1.3.4 International conference on harmonization

As per ICH, validation is defined as establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce meeting it pre determined specifications and quality attributes".

1.3.5 Different regulatory validation parameters

Different regulatory bodies like ICH, FDA, GMP, USP provides validation parameters

- USP validation parameters
- FDA validation parameters
- GMP validation parameters
- ICH validation parameters

USP validation parameters

- Specificity
- Linearity& (Range)
- Accuracy
- Precision
- Limit of detection
- Limit of quantification
- Ruggedness
- Robustness

FDA validation parameters:

- Specificity
- Linearity & (Range)
- Accuracy
- Precision
- Recovery
- Ruggedness

GMP validation parameters

- Accuracy
- Specificity
- Sensitivity
- Reproducibility

ICH validation parameters:

- Specificity
- Linearity
- Range
- Accuracy
- Precision
  - Repeatability
  - Intermediate Precision
  - Reproducibility
- Limit of detection
- Limit of quantification
- Robustness

1.3.6 ICH characteristics and guidelines:

Table:7 ICH characteristics and guidelines

Analytical task	**Major qualitative analysis**	**Minor quantitative analysis**	**Minor qualitative analysis**	**Major quantitative analysis**
Specificity	Yes	Yes	Yes	Yes
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes
Accuracy	No	No	Yes	Yes
Precision				
Repeatability	No	Yes	No	Yes
Intermediate Precision	No	Yes	No	Yes
Reproducibility	No	No	No	No
Limit of detection	No	No	Yes	No
Limit of quantification	No	Yes	No	No

1.3.7 Validation of an analytical method:

Method validation parameters as per ICH guidelines are summarized below.

a) Specificity

Specificity is the ability of the method to measure accurately and specifically the analyte of interest in the presence of matrix and other components likely to be present in the sample matrix and impurities, degradation products and other related substances. If the impurities/degradation products or potential contaminants are not available, one can

apply a proposed method for strained and stressed (heat, light, humidity) samples. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

Acceptance Criteria: There should not be any interference in the assay by the spiked impurity and 1 % of initial also the assay value obtained should be within assay.

b) Linearity

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

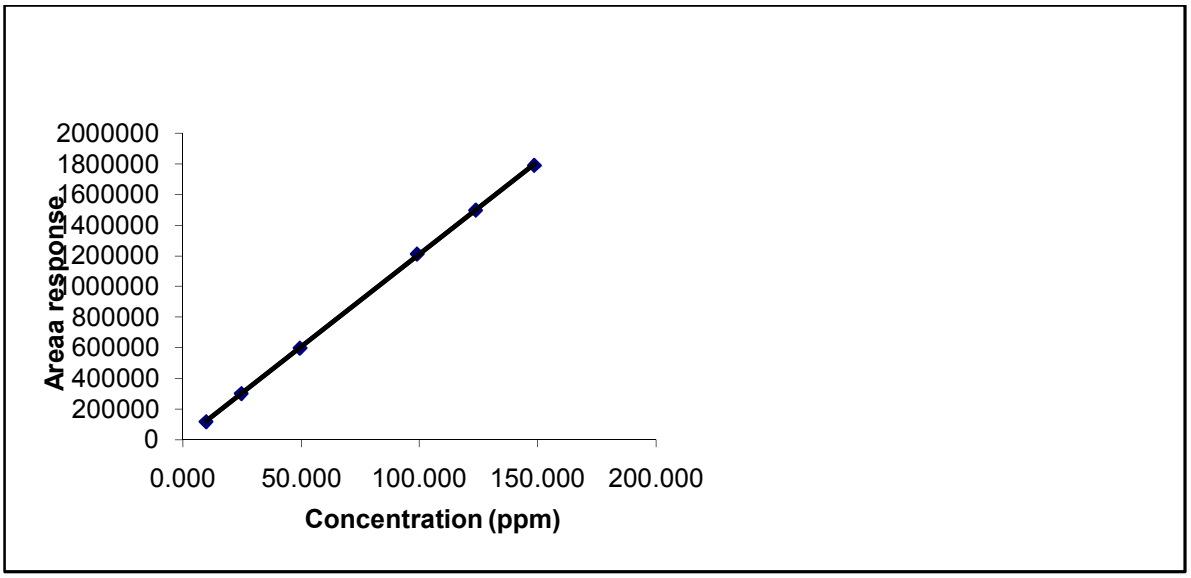


Fig.1 definition of linearity

c) Range

The range of an analytical method is the interval between the upper concentration and lower concentration of analyte with a suitable level of precision, accuracy and linearty. The ICH recommends that, for the establishment of linearity, a minimum of five concentrations normally be used.

d) Accuracy

The accuracy of an analytical method relates the closeness of the test results to true value i.e. measure of exactness of analytical method. It is expressed as % recovery by the assay of known/added amount of analyte in the linearity range. One can design experiments for recovery of known or spiked samples (usually 10% of the claim) in presence of expected matrix, keeping the matrix constant. Accuracy can also be determined by comparing the results those obtained using an alternative method, which has been validated.

e) Precision

The precision of an analytical method express 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. The precision of an analytical method is usually expressed as the variance, standard deviation or coefficient of variance of a series of measurements.

Precision may be considered at three levels,

- Repeatability
- Reproducibility
- Intermediate precision

Repeatability: (under same conditions): Precision of the method when repeated by the same analyst, same test method and under same set of laboratory conditions (reagent, equipments etc.) within a short interval of time, the only difference being the sample. The repeatability of any test Procedure is required to be assured by carrying out complete separate determination on separate sample of the same homogeneous batch of material under

normal laboratory conditions.

Acceptance Criteria: The individual assay value should not vary by more than 2 %. Relative standard deviation should not be more than 2 %.

Reproducibility: (under different conditions): Reproducibility expresses the precision when the subject method is carried out by different analysts in different laboratories, using different equipments, reagents and laboratory settings and on different days- variability of analytical results as function of analyst, day-to-day, laboratory–to-laboratory, equipment-to-equipment etc. Using the samples from same homogeneous batch.

Intermediate Precision: Intermediate precision expresses within the same laboratory variations but different days, different analysts, different equipment and reagents.

f) Limit of Detection

Limit of detection of an individual analytical method is the lowest concentration / amount of analyte in a sample that the method can detect but not necessarily quantify under the stated experimental conditions. The LOD will not only depend on the procedure of analysis but also on the type of instrument.

a) Instrumental

- S / N = 3:1
- 2-3 times of SD of blank response.

b) Non-instrumental: One has to establish the minimum level at which analyte can be reliably detected usually LOD is 2-3 times lower than LOQ.

g) Limit of Quantification

Limit of quantification of an individual analytical method is the lowest concentration/ amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy under stated experimental conditions. The quantification limit is used particularly for the determination of impurities and / or degradation products. The LOQ will not only depend on the procedure of analysis but also on the type of instrument.

a) instrumental:

- S/N = 10:1
- 3 times higher than LOD

b) Non-instrumental: One has to establish the level experimentally depending on the method of analysis.

Acceptance Criteria: The assay value obtained so should be within ± 2 % of initial assay value.

h) Robustness

Robustness is the measure of the analytical method to remain unaffected by small, but deliberate variations in method parameters. It provides an indication of its reliability during normal usage.

i) System Suitability

System Suitability is defined ability of the method produce constant response for the system parameters. If measurements are susceptible to variations in analytical conditions, a precautionary statement should be included in the method or these variations should be suitably controlled. Typical variations are the different equipment, different analysts and the

stability of analytical solutions.

Acceptance criteria: The RSD variation of the results ± 2.5 %.

System Suitability parameters and acceptance criteria for HPLC

The accuracy and precision of HPLC data collected begin with a well behaved chromatographic system. The system suitability specifications and tests are parameters that provide assistance in achieving this purpose.

- Capacity factor (k')

The capacity factor is a measure of the degree of retention of an analyte relative to an unretained peak, where TR is the retention time for the sample peak and t_0 is the retention time for an unretained peak.

$$k' = (t_R - t_0) / t_0$$

Acceptance criteria: The peak should be well-resolved from other peaks and the void volume. Generally the value of k' is > 2.

- 2. Resolution (R_s)

Ability of column to achieve baseline separation of chromatographic peaks. Resolution can be improved by increasing column length, decreasing particle size, increasing temperature, changing the eluent or stationary phase. It can also be expressed in terms of the separation of the apex of two peaks divided by the tangential width average of the peaks.

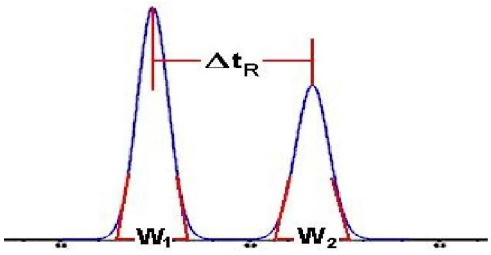


Fig.2 expression of resolution between two peaks

$$R_s = \Delta t_R / 0.5 (W_1 + W_2);$$

$$\text{Where; } \Delta t_R = t_2 - t_1$$

For reliable quantitation, well-separated peaks are essential for quantitation.

Acceptance criteria: R_s of > 2 between the peak of interest and the closest potential interfering peak (impurity, excipients, degradation product, internal standard, etc.) are desirable.

- 3. Tailing factor (T)

A measure of the symmetry of a peak, given by the following equation where $W_{0.05}$ is the peak width at 5% height and f is the distance from peak front to apex point at 5% height. Ideally, peaks should be Gaussian in shape or totally symmetrical.

$$T = W_{0.05} / 2f$$

T = Tailing factor

$W_{0.05}$ = Width of peak at 5% of height

f = Width of the peak front at peak maximum

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and

hence the calculation of the area under the peak. Integrator variables are preset by the analyst for optimum calculation of the area for the peak of interest.

Acceptance criteria: The tailing factor of peak should not more than 2.

- 4. Theoretical plate number / Efficiency (N)

A measure of peak band spreading determined by various methods, some of which are sensitive to peak asymmetry. The most common are shown here, with the ones most sensitive to peak shape shown first: 4-sigma / tangential.

$$N = 16 (t_R / W)^2 = L / H \text{ Half height}$$

$$N = 5.54 (t_R / W)^2 = L / H$$

Theoretical plate number is a measure of column efficiency, that is, how many peaks can be located per unit run-time of the chromatogram, where t_R is the retention time for the sample peak and W is the peak width. N is fairly constant for each peak on a chromatogram with a fixed set of operating conditions. H, or HETP, the height equivalent of a theoretical plate, measures the column efficiency per unit length (L) of the column. Parameters which can affect N or H include Peak position, particle size in column, flow- rate of mobile phase, column temperature, viscosity of mobile phase, and molecular weight of the analyte.

Table: 8 Acceptance criteria of validation parameters for HPLC

S.No.	**Characteristics**	**Acceptance criteria**
1	Accuracy	Recovery 98-102%
2.	Precision	%RSD < 2
3	Specificity/Selectivity	No interference

4	Detection limit	S/N > 2 or 3
5	Quantitation limit	S/N > 10
6	Linearity	r^2> 0.999
7	Range	80 - 120%
8	Stability	>24 h or < 8h

1.4 Forced degradation studies

Forced degradation studies of drug substance and products play an integral role in the development of pharmaceuticals. The results of degradation studies facilitate stability indicating method (SIM) development, the design of formulations, the choice of storage conditions and packaging, an understanding of the chemistry of the drug molecule, and stability problem solving.

1.4.1 Degradation pathways

Forced degradation or stress studies of drug substances are usually conducted in solution and the solid state at temperatures exceeding accelerated stability conditions (>40°C) . The degradation pathways investigated include hydrolysis, oxidation, thermolysis, photolysis, and polymerization.

Table: 9 degradation pathways

S.no	**Pathways**	**Mechanism**
1.	hydrolysis	exposure of drug to high relative humidity
2.	oxidation	control of exposure to molecular oxygen or addition of oxidizing agent like peroxides
3.	thermolysis	application of heat
4.	photolysis	irradiating the drug with light at 300 – 800 wavelength

1.4.2 Condition for stress testing:

Table: 10 recommended stress conditions for drug substances

Stress type	**Conditions**	**Time**
Acid hydrolysis	0.1N HCl(up to 5.0 N)	1-7 days
Base hydrolysis	0.1N NaOH/KOH (up to 5.0 N)	1-7 days
Thermal hydrolysis	70ºC	1-7 days
Oxidative solution	O_2+Initiator(AIBN) in Acetonitrile/H_2O, 80/20;40ºC	1-7 days
	0.3% (up to 3%) H_2O_2;	
Thermal	RT; protected from light	Few hrs to 7 days
	70ºC	up to 2 weeks
Thermal/Humidity	70ºC/75%RH	up to 2 weeks

Table: 11 recommended stress conditions for drug product

Stress type	**Conditions**	**Time**
Thermal	70ºC	Up to 3 weeks
Thermal/humidity	70ºC/75%RH	Up to 3 weeks
Photo-degradation	Fluorescent and	
	UV light	>2 weeks

1.4.3 Stability Guidelines

ICH: International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use.

WHO: World Health Organization.

USFDA: United States food drug administration.

GMP: Good manufacturing practices.

The ICH Topics are divided into four major categories:

- Quality (Q), i.e., those relating to chemical and pharmaceutical Quality Assurance
- Safety (S), i.e., those relating to in vitro and in vivo preclinical studies
- Efficacy (E), i.e., those relating to clinical studies in human subject
- Multidisciplinary topics (M), i.e., cross-cutting Topics which do not fit uniquely into one of the above categories.

1.4.4 Quality Guidelines – Stability, Validation & Impurities

a) Stability Q1A - Q1F

Q1A (R2) - Stability Testing of New Drug Substances and Products Q1A

Q1B- Stability Testing: Photo stability Testing of New Drug Substances and Products

Q1C - Stability Testing for New Dosage Forms

Q1D -Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and products

Q1E - Evaluation of Stability Data

Q1F - Stability Data Package for Registration Applications in Climatic Zones III & IV.

b) Q2 (R1) Validation of Analytical Procedures: Text and Methodology

c) Impurities Q3A - Q3D

Q3A (R2) Impurities in New Drug Substances

Q3B (R2) Impurities in New Drug Products

Q3C (R5) Impurities: Guideline for Residual SolventsQ3C, Q3C (M)

Q3DImpurities: Guideline for Metal Impurities

1.4.5 Stability Testing of New Drug Substances

a) Testing Frequency:

For long term studies, frequency of testing should be sufficient to establish the stability profile of the drug substance. For drug substances with a proposed re-test period of at least 12 months, the frequency of testing at the long term storage condition should normally be every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed re-test period. At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., 0, 3, and 6 months), from a 6-month study is recommended. Where an expectation (based on development experience) exists that results from accelerated studies are likely to approach significant change criteria, increased testing should be conducted either by adding samples at the final time point or by including a fourth time point in the study design.

When testing at the intermediate storage condition is called for as a result of significant change at the accelerated storage condition, a minimum of four time points, including the initial and final time points (e.g., 0, 6, 9, 12 months), from a 12-month study is recommended.

b) Storage Conditions:

In general, a drug substance should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use. The long term testing should cover a minimum of 12 months duration on at least three primary batches at the time of submission and should be continued for a period of time sufficient to cover the proposed re-test period. Additional data

accumulated during the assessment period of the registration application should be submitted to the authorities if requested. Data from the accelerated storage condition and if appropriate, from the intermediate storage condition can be used to evaluate the effect of short term excursions outside the label storage conditions (such as might occur during shipping). Long term, accelerated and where appropriate, intermediate storage conditions for drug substances are detailed in the sections below. The general case applies if the drug substance is not specifically covered by a subsequent section. Alternative storage conditions can be used if justified.

i) General case

Table: 12 Drug substances intended for storage at normal conditions

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long term*	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	12 months

*It is up to the applicant to decide whether long term stability studies are performed at 25 2°C/60% RH 5% RH or 30°C 2°C/65% RH 5% RH.

**If 30°C 2°C/65% RH 5% RH are the long-term condition, there is no intermediate condition.

If long-term studies are conducted at 25°C ± 2°C/60% RH ± 5% RH and "significant change" occurs at any time during 6 months testing at the accelerated storage condition, additional testing at the intermediate storage condition should be conducted and evaluated against significant change criteria. Testing at the intermediate storage condition should include all

tests, unless otherwise justified. The initial application should include a minimum of 6 months' data from a 12-month study at the intermediate storage condition. "Significant change" for a drug substance is defined as failure to meet its specification.

ii) Drug substances intended for storage in a refrigerator.

Table: 13 Drug substances intended for storage in a refrigerator

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long term	5°C ± 3°C	12 months

Data from refrigerated storage should be assessed according to the evaluation section of this guideline, except where explicitly noted below. If significant change occurs between 3 and 6 months testing at the accelerated storage condition, the proposed re-test period should be based on the real time data available at the long term storage condition.

If significant change occurs within the first 3 months testing at the accelerated storage condition, a discussion should be provided to address the effect of short term excursions outside the label storage condition, e.g., during shipping or handling. This discussion can be supported, if appropriate, by further testing on a single batch of the drug substance for a period shorter than 3 months but with more frequent testing than usual. It is considered unnecessary to continue to test a drug substance through 6 months when a significant change has occurred within the first 3 months.

iii) Drug substances intended for storage in a freezer

Table: 14 Drug substances intended for storage in a freezer

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long term	- 20°C ± 5°C	12 months

For drug substances intended for storage in a freezer, the re-test period should be based on the real time data obtained at the long term storage condition. In the absence of an accelerated storage condition for drug substances intended to be stored in a freezer, testing on a single batch at an elevated temperature (e.g., 5°C ± 3°C or 25°C ± 2°C) for an appropriate time period should be conducted to address the effect of short term excursions outside the proposed label storage condition, e.g., during shipping or handling.

iv) Drug substances intended for storage below -20°C

Drug substances intended for storage below -20°C should be treated on a case-by-case basis.

1.4.6 Stability Testing of Drug Product:

a) Testing Frequency:

For long term studies, frequency of testing should be sufficient to establish the stability profile of the drug product. For products with a proposed shelf life of at least 12 months, the frequency of testing at the long term storage condition should normally be every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed shelf life. At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., 0, 3, and 6 months), from a 6-month study is recommended. Where an expectation (based on development experience) exists that results from accelerated testing are likely to approach significant change criteria, increased testing should be conducted either by adding samples at the final time point or by including a fourth

time point in the study design. When testing at the intermediate storage condition is called for as a result of significant change at the accelerated storage condition, a minimum of four time points, including the initial and final time points (e.g., 0, 6, 9, 12 months), from a 12-month study is recommended. Reduced designs, i.e., matrixing or bracketing, where the testing frequency is reduced or certain factor combinations are not tested at all, can be applied, if justified.

b) Storage Conditions:

In general, a drug product should be evaluated under storage conditions (with appropriate tolerances) that test its thermal stability and, if applicable, its sensitivity to moisture or potential for solvent loss. The storage conditions and the lengths of studies chosen should be sufficient to cover storage, shipment, and subsequent use. Stability testing of the drug product after constitution or dilution, if applicable, should be conducted to provide information for the labeling on the preparation, storage condition, and in-use period of the constituted or diluted product. This testing should be performed on the constituted or diluted product through the proposed in-use period on primary batches as part of the formal stability studies at initial and final time points and, if full shelf life long term data will not be available before submission, at 12 months or the last time point for which data will be available. In general, this testing need not be repeated on commitment batches. The long term testing should cover a minimum of 12 months' duration on at least three primary batches at the time of submission and should be continued for a period of time sufficient to cover the proposed shelf life. Additional data accumulated during the assessment period of the registration application should be submitted to the authorities if requested. Data from the accelerated storage condition and, if appropriate, from the intermediate storage condition can be used to evaluate the effect of short term excursions outside the label storage conditions (such as might occur during shipping). Long term, accelerated, and, where appropriate, intermediate storage

conditions for drug products are detailed in the sections below. The general case applies if the drug product is not specifically covered by a subsequent section. Alternative storage conditions can be used, if justified.

i) General case

Table: 15 Drug products intended for storage at normal conditions

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long term*	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	12 months
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

*It is up to the applicant to decide whether long term stability studies are performed at 25 2°C/60% RH 5% RH or 30°C 2°C/65% RH 5% RH.

**If 30°C 2°C/65% RH 5% RH is the long-term condition, there is no intermediate condition. If long-term studies are conducted at 25°C ± 2°C/60% RH ± 5% RH and "significant change" occurs at any time during 6 months testing at the accelerated storage condition, additional testing at the intermediate storage condition should be conducted and evaluated against significant change criteria. The initial application should include a minimum of 6 months data

from a 12-month study at the intermediate storage condition. In general, significant change for a drug product is defined as:

A 5% change in assay from its initial value or failure to meet the acceptance criteria for potency when using biological or immunological procedures, any degradation product's exceeding its acceptance criterion.

Failure to meet the acceptance criteria for Appearance

Physical attributes: (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions.

Functionality test: (e.g., color, phase separation, re suspendability, caking, hardness, dose delivery per actuation)

Failure to meet the acceptance criterion for pH

Failure to meet the acceptance criteria for dissolution for12 dosage units.

iv) Drug products intended for storage in a refrigerator

Table: 16 Drug products intended for storage in a refrigerator

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% RH ± 5% RH	6 months

If significant change occurs within the first 3 months testing at the accelerated storage condition, a discussion should be provided to address the effect of short term excursions outside the label storage condition, e.g., during shipment and handling. This discussion can be supported, if appropriate, by further testing on a single batch of the drug product for a

period shorter than 3 months but with more frequent testing than usual. It is considered unnecessary to continue to test a product through 6 months when a significant change has occurred within the first 3 months.

Table: 17 Drug products intended for storage in a freezer

Study	**Storage condition**	**Minimum time period covered by data at submission**
Long term	- 20°C ± 5°C	12 months

For drug products intended for storage in a freezer, the shelf life should be based on the real time data obtained at the long term storage condition. In the absence of an accelerated storage condition for drug products intended to be stored in a freezer, testing on a single batch at an elevated temperature (e.g.,5°C ± 3°C or 25°C ± 2°C) for an appropriate time period should be conducted to address the effect of short term excursions outside the proposed label storage condition.

2. LITERATURE REVIEW

2.1 Drug profile of Eletriptan hydrobromide:

Non pharmacopoeial drug

Description : white to light pale colored powder

Structure :

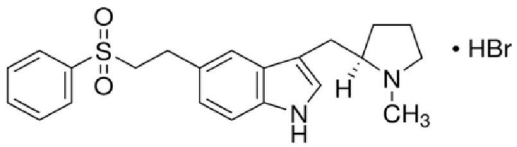


Fig.3 structure of Eletriptan hydrobromide

IUPAC name : (R)-3-[(-1-Methyl-2-pyrrolidin-2-yl)methyl]-5-[2-(phenylsulfonyl)ethyl]-1*H*-indole, monohydrobromide

Molecular weight : 463.40

Solubility : soluble in water and acetonitrile, methanol and DMSO

Melting range : 169- 171ºC

Stability : Very stable molecule under normal conditions.

Wavelength (λ_{max}) : 225nm

Category : Anti-migraine drug

Mechanism of action : Seratonin receptor agonist

Brand name : Relpax, Relert.

Pharmacokinetic Data

Bioavailability : 50%

Protein Binding : 85%

Metabolism : N-demethylation. Cytochrome P450 CYP3A4,

Half Life : 13 hrs

Excretion : >90% via faeces, 9% urine

Dosage form : Tablets

Dose : 20 mg, 40 mg.

Table: 18 List of marketed formulations of Eletriptan hydrobromide tablets:

S.no	**Brand Name**	**Dose**	**dosage form**	**Manufacturing Company**
1.	Relert	40mg	Tablet	Pfizer Pharmaceuticals Ltd.
2.	Relpax	40mg	Tablet	Pfizer Pharmaceuticals Ltd.

2.2 Literature Review of Eletriptan hydrobromide

Table: 19 previously developed analytical methods on Eletriptan hydrobromide:

S.no	Title	Column	Mobile Phase	Linearity	ref. no
1.	Development and validation of RP-HPLC method for estimation of Eletriptan hydrobromide in bulk & pharmaceutical formulation.	Phenomenax Lunac C18 (250mmx4.6 mmx5µm)	KH_2PO_4 Buffer: Acetonitrile (60:40)v/v	5-30µg/ml of Ele HBr	14
2.	RP-HPLC method for estimation of Eletriptan hydrobromide in bulk & pharmaceutical formulation.	Inertsil ODS-C18 (250mmx4.6 mmx5µm)	0.01 M KH_2PO_4 Buffer:ACN : methanol(20:40:40) v/v/v	200-1000 µg/ml of Ele HBr	13
3.	Validation of HPLC method for simultaneous determination of Eletriptan hydrobromide and UK 120.413	X-terra, C18(150mm x4.6mmx5.0 µm)	TEA :Methanol (67.2:32.8)v/v	0.05-1.00 mg/ml of Ele HBr	16

4.	Method development and estimation of eletriptan hydrobromide in pharmaceutical dosage form by RP-HPLC	Inertsil ODS-3 V C18 (250mmx4.6 mmx5µm)	0.03 M Ammonium acetate buffer, TEA (0.5%):Methanol (40:60)v/v	50-600 µg/ml of Ele HBr	22
5.	An Isocratic RP-HPLC Method development for determination of Eletriptan hydrobromide in bulk & pharmaceutical dosage form.	Zorbax SB,C18 (150mmx4.6 mmx5µm)	Ammonium acetate,Buffer:ACN (80:20)v/v	20-70 µg/ml of Ele HBr	19
6.	Development and validation of stability indicating RP-HPLC method for determination of Eletriptan hydrobromide in orally disintegrated tablets	Thermo column, C18 (150mmx4.6 mmx5µm)	Methanol;Water (35:65) v/v	5-500 µg/ml of Ele HBr	18

7.	Method development and validation for determination of Eletriptan hydrobromide in bulk & pharmaceutical dosage form by RP-HPLC	Waters symmetry column,C18 (100mmx4.6 mmx3.5µm)	KH_2PO_4,Buffer: ACN(60:40)v/v	10-50 µg/ml of Ele HBr	26
8.	Development and validation of a stability indicating RP-HPLC method for determination of Eletriptan hydrobromide in pharmaceutical formulation.	Phenomenex Chromosil C18,(250mm x4.6mmx 5µm)	Acetonitrile :TEA :THF (50:25:25) v/v/v	30-100 µg/ml of Ele HBr	27
9.	Method development and validation of eletriptan hydrobromide by UV-Visible spectrophotometry.	-	Ethanol and distilled water	1-10µg/ml of Ele HBr	20

10.	Liquid chromatographic determination of Eletriptan in pharmaceutical dosage forms	Nova-pak C18(250mm x4.6mmx5µ m)	ACN : Water(60:40)	100-4500 µg/ml of Ele HBr	17
11.	Development and application of an HPLC method for Eletriptan hydrobromide	Shim-pak VP ODS C18(250mm x4.6mmx5µ m)	KH_2PO_4,P^H 5.0 with OPA Buffer:Methanol:ACN(40:15:45)	320-20000 µg/ml of Ele HBr	26
12.	New Derivative Spectrophotometric Methods for Determination of Eletriptan hydrobromide.	-	A- 0.1N HCL B- Acetate buffer C- phosphate buffer	0.5-0.3 0.5-0.3 1-30 µg/ml of Ele HBr	25

3. AIM AND PLAN OF WORK

The drug analysis plays an essential role in the development of drugs, their manufacture and the therapeutic use. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw materials used and final product obtained meets the required specification. The number of drug formulations and drugs introduced in to the market has been increasing at an alarming rate. These drugs or formulations may be either in the new entities in the market or novel dosage forms or multi component dosage forms or partial structural modification of the existing drugs. For the present study Eletriptan Hydrobromide drug was selected. Eletriptan Hydrobromide is chemically known as Hydrochloride (R)-3-[(-1-Methyl-2-pyrrolidin-2-yl)methyl]-5-[2-(phenylsulfonyl)ethyl]-1*H*-indole,monohydrobromide. It act as an anti Migraine drug.

Objective:

Literature reveals that, various methods like HPLC, UPLC, HPTLC, RP-HPLC methods has been develop for the estimation of Eletriptan Hydrobromide alone and along with various dosage forms. Few stability indicating RP-HPLC methods were reported for estimation of Eletriptan Hydrobromide. But stability indicating assay method for determination of Eletriptan Hydrobromide was not available. So, the main objective of work is, to develop new stability indicating assay method for determination of drug Eletriptan Hydrobromide by using RP-HPLC.

Specific aim:

To achieve the above objective the study was carried out in following steps:

- To develop a simple, selective, sensitive, specific, precise stability indicating assay method by using reverse phase liquid chromatography.

- To validate the method in accordance with ICH guidance lines for the intended analytical application. The validation parameters as per ICH include specificity, system suitability, linearity, accuracy, precision, range, robustness.
- To perform stress degradation studies for Eletriptan Hydrobromide tablets.
- To apply the developed and validated method for the quantitative analysis of Eletriptan Hydrobromide tablets.

Plan of work:

- Selection of API
- Literature survey
- Method development
- Optimization of LC conditions
- Validation of developed method as per ICH guidelines
- Perform degradation studies of Eletriptan Hydrobromide

4. EXPERIMENTAL WORK

4.1 List of materials

Table: 20 Apparatus/Instruments used

S. No	Apparatus	Model	Make
1.	HPLC	LC-2010^{+}	Shimadzu
2.	Semi micro balance	CPA2P	Sartorius
3.	Sonicator	UCB 70	Spectralab
4.	Glassware	Borosilicate type-A*	-
5.	Membrane Filters	0.45 and 0.2µm	PALL life sciences

*****calibrated as per Indian Pharmacopoeia 2007*

Table: 21 Chemicals/Reagents used

S. No	Chemicals	Grade	Manufacturer
1.	Potassium dihydrogen ortho phosphate	ACS	Merck
2.	Ortho phosphoric acid	ACS	Merck
3.	Sodium hydroxide	ACS	Merck
4.	Hydrochloric acid	ACS	Merck

5.	Hydrogen peroxide	GR	Merck
6.	Acetonitrile	HPLC	Merck
8.	Methanol	HPLC	Merck
9.	Purified water	Milli-Q	Finoso pharma pvt Ltd. Hyd.

Table: 22 Reference standard, Sample used

1.	**Reference standard**	Eletriptan HBr
2.	**Sample**	Eletriptan HBr 20mg and 40mg

4.2 Instrumentation

Table: 23 Description of LC instrument used

1.	HPLC	Shimadzu 2010^{+}
2.	Software	LC Solutions
3.	Column	Waters X-terra, RP18, 250 x 4.6 mm, 5µ
4.	Pump	Gradient
4.	Detector	UV-PDA
5.	Injection System	Rheodyne Injector
6.	Injection Volume	5µl

4.3 Method development by RP-HPLC

4.3.1 Selection of Detector Wavelength:

Appropriate dilution was prepared from stock solution of Ele HBr, the solution were scanned over the range of 200- 400nm. 225nm has been selected as a detection wavelength for HPLC method.

4.3.2 Preparation of mobile phase

Preparation of mobile phase A:

Prepare a degassed mixture of Phosphate buffer p^H 7.0 and Acetonitrile in the ratio 98:2 v/v.

Preparation of mobile phase B:

Prepare a degassed mixture of Acetonitrile and Methanol in the ratio 80:20 v/v.

4.3.3 Preparation of diluting solvent:

Prepare a degassed mixture of Phosphate buffer p^H 7.0 & Acetonitrile in the ratio 55:45 v/v.

4.3.4 Preparation of standard drug solution:

60.57mg of Ele.HBr working standard was weighed and transferred in to 100ml V.F.

To above solution 50ml of diluent was added and subjected to sonication for 2minutes.

The volume was made up to the mark with diluting solvent.

From the above solution 5ml was transferred in to 50ml V.F.

The volume was made up to the mark with diluting solvent.

4.3.5 Optimization of mobile phase ratio and chromatographic conditions:

To optimize the chromatographic conditions different trails were performed by injecting the standard solution of Erl.HCL on Waters X-terra column at different ratios of mobilephase. The results of trails are shown in table no. 24

4.3.6 Optimized Chromatographic conditions:

Mobile phase A (55%) consisted of Phosphate buffer p^H 7.0 and Acetonitrile in the ratio 98:2 v/v.

Mobile phase B (45%) consisted of Acetonitrile and Methanol in the ratio 80:20 v/v.

The column temperature was 25ºC.

The eluent was monitored at 225nm.

The optimized Chromatographic conditions were shown in table no. 25

4.3.7 Calibration of standards:

Standard calibration line for the Ele.HBr was constructed by transferring different volumes of standard stock solution in to appropriate V.F and diluted up to the mark with diluting solvent to yield concentration range of 10-150 µg/ml of Ele.HBr. The calibration line was obtained by plotting peak area ratio against the concentration of drug.

4.4 Validation of developed method (RP-HPLC)

The developed method was validated as per ICH guidelines. Method validation was performed in terms of System suitability, Linearity and Range, Assay, Accuracy, Precision, Specificity and selectivity, Robustness.

4.4.1. System suitability:

It is defined as ability of the method produce constant response for the system parameters. System suitability was performed to verify the analytical system working properly and can produce accurate and precise results. The system suitability was carried out after the method development and validation have been completed. For this, parameters like plate number (N),

resolution (R_s), tailing factor, capacity factor, HETP, peak symmetry of samples were measured. The represented data was shown in table no.26

4.4.2. Linearity and Range:

Linearity defined as, ability of the method elict the test results which are directly proportional to test concentrations. The linearity of the calibration curves in pure solution of Ele.HBr was checked over the concentration range of 10-150 µg/ml of Ele.HBr. The total eluting time was less than 10 mins by using regression analysis, the regression line of standard concentrations of Ele.HBr was founded. The calibration curves were linear in the entire studied range and the equation of regression analysis was obtained.

$Y= 29,193.2694x – 16,433.0727$; $R^2 = 0.9999$ for Ele.HBr.

The mean ± standard deviation (SD) for the slope, correlation coefficient, and intercept of the standard curves (n=3) were calculated. The represented data was shown in table no.27 & 28

4.4.3. Assay:

This parameter was performed to determine the purity of the dosage form in order to see whether the method is applicable for the formulation analysis or not. Weigh and finely powdered not fewer than 20 tablets. A powder quantity equivalent to 50mg of Ele.HBr was transferred in to 100ml V.F, to this 50ml of diluting solvent was added, and subjected to sonication for 10minutes along with intermediate shaking and volume was made up to the mark with diluting solvent. The above solution was filtered through 0.45µ nylon membrane syringe. From this solution 5ml was transferred in to 50ml V.F and made up to the mark with diluting solvent. Injected under optimized chromatographic conditions and peak area was measured. The assay procedure was made triplicate and weight of the sample taken for assay was calculated. The percentage of drug found in formulation, standard deviation and mean was calculated. The results were shown in table no. 29

4.4.4 Accuracy:

Accuracy was determined by performing recovery studies at three levels in which, known amount of reference standard of the Ele.HBr at levels of 50%, 100% and 150% were added to the formulation. Recovery studies were carried out in three replicates of each concentration level and percentage recovery and percentage relative standard deviation of Ele.HBr were calculated and shown in table no.30

4.4.5. Precision:

a) Repeatability (Method precision):

Repeatability is defined as, the ability of the analytical instrument to produce reproducible results. The system precision was studied by six replicate measurements standard solution of Ele.HBr, the results were shown in table no. 31

b) Intermediate Precision:

Intermediate precision expresses within the same laboratory but variations different days, different analysts, different equipment and reagents were used. Intraday and Inter day precision studies are performed, the results were shown in table no.32 & 33.

4.4.6. Specificity and selectivity:

The specificity of method was evaluated with regard to interference due to presence of any other excipients. (or) Specificity of the method was shown by quantifying the analyte of interest in the presence of matrix and other components, like Mobile phase, placebo and diluent. Volume of 5 µl of placebo, diluent, mobile phase were injected separately, the chromatogram was recorded and Those components have shown no peaks at retention time of 5.09min, the proposed method was specific for the detection of Ele.HBr peak. The selectivity of the method was performed by injecting the impurities stock solution the impurities were well separated from the analyte peak.

4.4.7. Robustness:

The capacity of the method remains unaffected by small but deliberate variations like mobile phase, P^H, flow rate, wavelength. To evaluate Robustness, solution stability, filtration test and changes in chromatographic conditions, like variations in flow rate (± 0.2%) and wavelength (± 2%), column temperature were performed. (±5ºC)., organic phase ratio in M.P (± 10%), buffer P^H (± 0.2%),

a) Changes in chromatographic conditions:

- Change in flow rate using flow rate 0.9ml and 1.1ml, instead of 1.0ml.
- Change in wavelength, using 223nm and 227nm instead of 225nm.
- Change in column temperature, 20ºC and 30ºC instead of 25ºC.
- Change in organic phase ratio in mobile phase, +10% and -10%
- Change in buffer P^H to 6.8 and 7.2 instead of 7.0

None of alterations caused any significant changes in peak area RSD, tailing factor and theoretical plates. Results were shown in table no.34

b) Filtration test:

One portion of 100% sample solution in accuracy study was centrifuged and another portion of sample solution was filtered through 0.45 GHP filter and PVDF filter. The results were compared. Results were shown in table no.35

c) Solution stability test:

A 100% sample and standard solutions were prepared and stored in clear vials at room temperature. Those solutions were re-quantified at 4hrs, 12hrs, 24hrs, and 48hrs. The recoveries of standard and sample solution were determined against freshly prepared standard preparation.

Results were shown in table no.36

4.5 Stress degradation studies of Eletriptan Hydrobromide

Stress degradation studies were performed as per ICH guidelines. Stress degradation is defined as degradation of new drug product or drug substance at conditions more severe than accelerated conditions. Which in turn help in the structural elucidation of degradation products of drug and also it is required to establish the specificity of stability indicating assay methods. In order to demonstrate the selectivity of the proposed method, stress degradation studies carried out by using acidic, alkali, thermal, uv-light, oxidative conditions.

4.5.1 Procedure for stress degradation studies:

To determine whether the analytical method and assay are stability indicating or not Ele.HBr was stressed under different conditions. like, acidic, alkali, thermal, uv-light, oxidative conditions to conduct forced degradation studies. Degradation was attempted to stress conditions of acidic (5N HCL), alkali (5N KOH), thermal (at 105ºC), oxidative (1% H_2O_2), uv-light (In UV- cabinet at 254 nm) to evaluate the ability of proposed method to estimate the content of Ele.HBr without interference of impurities and degradants formed due to FDS.

If optimum degradation is observed under above conditions the process can be stopped at this point. If degradation is not observed under above conditions the drug should subjected to higher strengths and for longer time period. If complete degradation of drug achieved after subjecting the drug at initial conditions, the strengths of acidic, alkali, oxidative were decreased along with decrease in the reaction temperature.

a) Preparation of diluting solvent:

Mobile phase-A and Mobile phase-B in the ratio of 55:45 used as diluting solvent.

b) Preparation of Ele.HBr stock solution:

60.45 mg Ele.HBr API was weighed and transferred in to 100ml V.F

To above solution 50ml of diluent was added and subjected to sonication for 2min, the volume was made up to the mark with diluting solvent.

(Ice water is used for process of sonication)

Further 5 ml of above solution was transferred in to 50ml V.F and the volume was made up to the mark with diluting solvent.

c) Preparation of 5N HCL:

425 ml of concentrated HCL in 1000 ml of water.

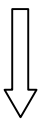
d) Preparation of 2N KOH:

280 g of potassium di hydroxide in 1000 ml of water.

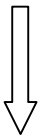
4.5.2 Stress degradation studies:

a) Acid degradation:

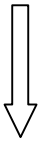
Weigh and crush 20 tablets of Eletriptan with mortar pestle.



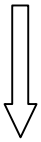
Weigh and transfer an accurately weighed portion of the powder, equivalent to about 50 mg into 100 mL volumetric flask,



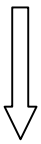
Add 30 mL of diluent and sonicate for about 20 minutes,



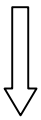
To this add about 20 mL of 5N HCl and heat the sample at 60°C for about 1 hour in water bath and cool to room temperature



Neutralize with 20 mL of 5N Sodium Hydroxide, and make up to the volume with diluents,



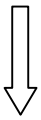
Filter through 0.45µm nylon filter. Transfer 5 mL of above solution into 50 mL of volumetric flask, make up to volume with diluent and mix well.



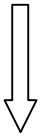
Above solution was injected under above chromatographic conditions and peak area was measured.

b) Base degradation:

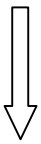
Weigh and crush 20 tablets of Eletriptan with mortar pestle.



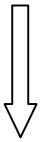
Weigh and transfer an accurately weighed portion of the powder, equivalent to about 50 mg into 100 mL volumetric flask,



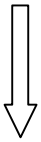
Add 30 mL of diluent and sonicate for about 20 minutes,



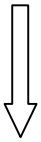
To this add about 20 mL of 5N NaOH and heat the sample at 60°C for about 1 hour in water bath and cool to room temperature



Neutralize with 20 mL of 5N HCL, and make up to the volume with diluents,



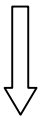
Filter through 0.45µm nylon filter. Transfer 5 mL of above solution into 50 mL of volumetric flask, make up to volume with diluent and mix well.



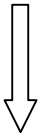
Above solution was injected under above chromatographic conditions and peak area was measured.

c) Oxidative degradation:

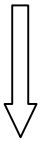
Weigh and crush 20 tablets of Eletriptan with mortar pestle.



Weigh and transfer an accurately weighed portion of the powder, equivalent to about 50 mg into 100 mL volumetric flask,



Add 30 mL of diluent and sonicate for about 20 minutes,



To this add about 20 mL of 1% Hydrogen peroxide and heat the sample at 60°C for about 1 hour in water bath and cool to room temperature

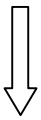
Neutralize with 20 mL of water, and make up to the volume with diluents,

Filter through 0.45µm nylon filter. Transfer 5 mL of above solution into 50 mL of volumetric flask, make up to volume with diluent and mix well.

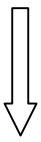
Above solution was injected under above chromatographic conditions and peak area was measured.

d) Thermal degradation:

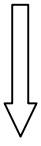
Weigh and crush 20 tablets of Eletriptan with mortar pestle.



Weigh and transfer an accurately weighed portion of the powder, equivalent to about 50 mg into 100 mL volumetric flask,

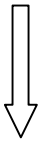


Add 70 mL of diluent and sonicate for about 20 minutes, make up the volume with diluent.

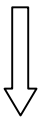


This V.F was placed in oven at temperature at 105ºC for 3 days.

Remove the flask, cooled it to room temperature. Filter through 0.45µm nylon filter.



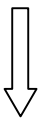
Transfer 5 mL of above solution into 50 mL of volumetric flask, make up to volume with diluent and mix well.



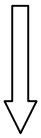
Above solution was injected under above chromatographic conditions and peak area was measured.

e) UV-light degradation:

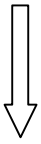
Weigh and crush 20 tablets of Eletriptan with mortar pestle.



Weigh and transfer an accurately weighed portion of the powder, equivalent to about 50 mg into 100 mL volumetric flask,

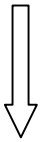


Add 70 mL of diluent and sonicate for about 20 minutes, make up the volume with diluent.



This V.F was placed in UV-cabinet at 254 nm for 3 days.

Transfer 5 mL of above solution into 50 mL of volumetric flask, make up to volume with diluent and mix well.



Above solution was injected under above chromatographic conditions and peak area was measured.

The represented data of stress degradation study was shown in table no 37

5. RESULTS AND DISCUSSIONS

5.1 Results for method development of RP-HPLC method for the determination of Eletriptan Hydrobromide tablets

Table: 24 optimization of method for the determination of Eletriptan hydrobromide.

i)Trial-1

S.NO	Parameter	Optimized Conditions
1.	Column	terra RP-18, 250x4.6mm,5µ
2.	Flow rate	ml/minute
3.	Column temperature	°C
4.	wavelength	5 nm
5.	Injection volume	µl
6.	Runtime	minutes
7.	Mobile phase ratio	M.P A 55: M.P B 45
8.	Retention time of drug	2.99 min

Discussion:

- The Eletriptan peak was eluted at 2.994 minutes
- The theoretical Plates: 5258 and tailing factor: 1.35.
- With this method the assay value is 95.1%

- In dissolution the average value is 100% result, but by observing the assay, 100 % result was not obtained, so this must be the extraction problem. Hence the diluent which is used must be changed (or) modified.

ii)Trial-2

S.NO	Parameter	Optimized Conditions
1.	Column	terra RP-18, 250x4.6mm,5µ
2.	Flow rate	ml/minute
3.	Column temperature	°C
4.	Wavelength	5 nm
5.	Injection volume	l
6.	Runtime	inutes
7.	Mobile phase ratio	M.P A 55: M.P B 45
8.	Retention time of drug	3.00 min

Discussion:

- The Eletriptan peak was eluted at 3.0 minutes
- The theoretical Plates: 5278 and tailing factor : 1.34.
- With this method the assay value is 97.1% .
- With 10 minutes sonication time the assay value is 94.6. Hence for the sample more sonication required.

- With 20 minutes sonication time and 30 minutes sonication time the assay result is are 97.1 and 97.0 respectively. Hence sonication time for sample is 20 minutes.
- By using the mobile phase as diluent,extraction of Eletriptan from Eletriptan hydrobromide tablets is improved.
- There is no blank interference at the retention time of Eletriptan peak.

iii)Trial-3

S.NO	Parameter	Optimized Conditions
1.	Column	terra RP-18, 250x4.6mm,5µ
2.	Flow rate	ml/minute
3.	Column temperature	°C
4.	Wavelength	5 nm
5.	Injection volume	l
6.	Runtime	minutes
7.	Mobile phase ratio	M.P A 55: M.P B 45
8.	Retention time of drug	4.76 min

Discussion:

- The Eletriptan peak was eluted at 4.762 minutes
- The theoretical Plates is8765 and tailing factor was 1.14.
- With this method the assay value is 98.4%.
- There is no blank interference at the retention time of Eletriptan peak.

iv)Trial-4

S.NO	Parameter	Optimized Conditions
1.	Column	X-terra RP-18, 250x4.6mm,5µ
2.	Flow rate	1.0 ml/minute
3.	Column temperature	25°C
4.	Wavelength	225 nm
5.	Injection volume	5 µl
6.	Runtime	10 minutes
7.	Mobile phase ratio	M.P A 55: M.P B 45
8.	Retention time of drug	4.76 min

Discussion:

- With 5, 10, 15 &20 minutes sonication time the assay results are99.9%, 100.2%, 100.5% and 100.7 % respectively.
- With this method the assay value is 100.7%. Hence sonication time for sample is 20 minutes.

v)Trial-5

S.NO	Parameter	Optimized Conditions
1.	Column	terra RP-18, 250x4.6mm,5µ
2.	Flow rate	ml/minute
3.	Column temperature	°C
4.	Wavelength	5 nm
5.	Injection volume	l
6.	Runtime	minutes
7.	Mobile phase ratio	M.P A 55: M.P B 45
8.	Retention time of drug	4.67 min

Discussion:

- The Eletriptan peak was eluted at 4.673 minutes and bromide peak was separated.
- The theoretical Plates is3217 and tailing factor was 1.225.
- With this method the assay value for centrifuged sample is 101.9% and with GHP filter is 100.8%.
- There is no much assay difference between centrifuged sample and GHP filtered sample
- Hence GHP filters are suitable for sample filtration

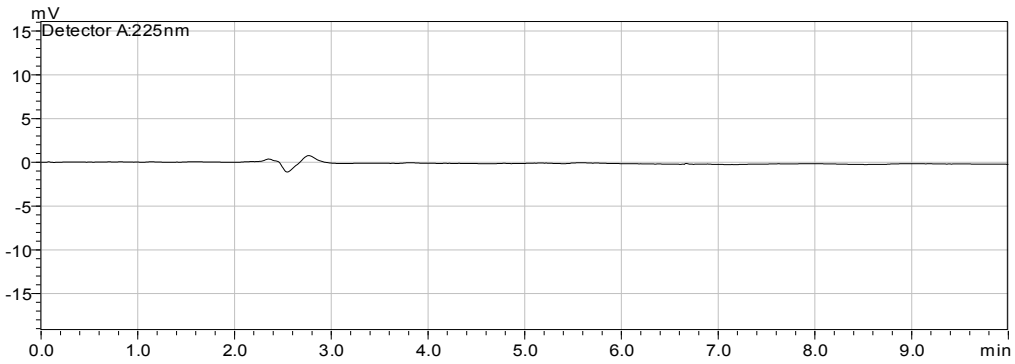
5.1.1 Optimized chromatographic conditions

Table: 25 Optimized chromatographic conditions of Eletriptan hydrobromide on C_{18} column.

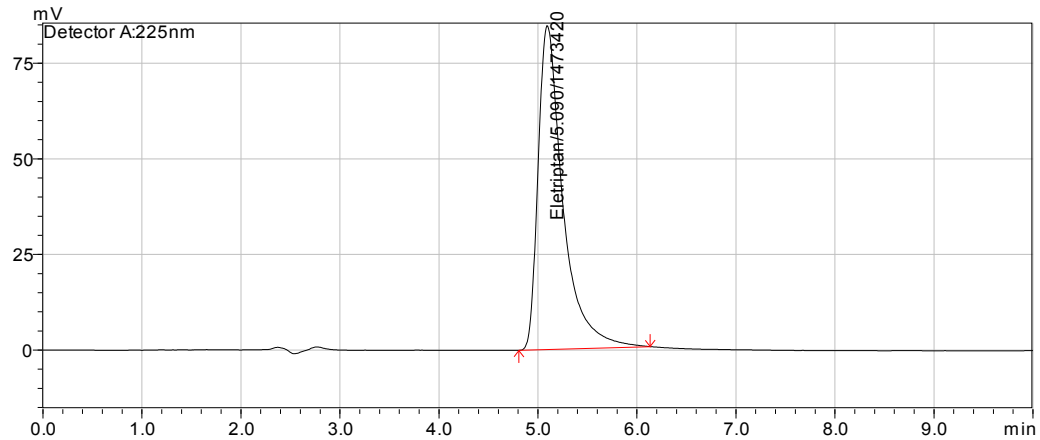
S.NO	Parameter	Optimized Conditions
1.	Column	Waters X-terra, RP18, 250 x 4.6 mm,5µ
2.	Flow rate	1.0ml / min
3.	Column temperature	25˚C
4.	Injection volume	5µl
5.	Runtime	10 min
6.	Wavelength	225 nm
7.	Mobile phase ratio	M.P A 55: M.P B 45
8.	Retention time of drug	5.09 min

5.1.2 Final optimized trail chromatograms during development process of method

Assay Diluent:



Assay Standard**:**



Assay Sample**:**

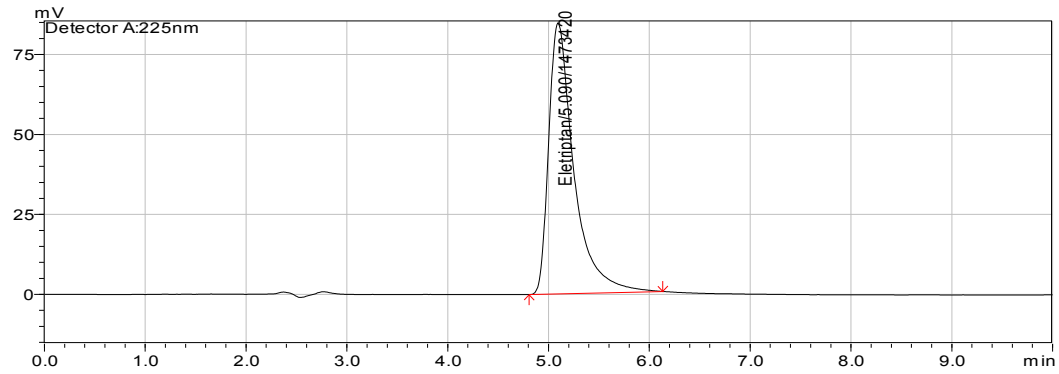


Fig.4 Final optimized chromatograms

5.2 Results for validation of analytical method for the assay of Eletriptan Hydrobromide

5.2.1 System Suitability

Table: 26 System Suitability parameters for Eletriptan Hydrobromide.

S.No	Parameter	Values obtained	Limit
1.	Retention time	5.09	<5 min
2.	Peak area	1485452	-
3.	%RSD	0.28%	<2%
4.	Tailing factor	1.52	<2%

5.2.2 Linearity and Range

Table: 27 Calibration of Eletriptan Hydrobromide

S.No	Target conc linearity levels	Vol. of linearity stock solution(ml)	Final dilution(ml)	Conc (µg/ml)	Area response
1.	Level 1 (25%)	1.3	50	13.119	374055
2.	Level 2 (50%)	2.5	50	25.229	716036
3.	Level 3 (80%)	4.0	50	40.367	1155081
4.	Level 4 (90%)	4.5	50	45.413	1308941

5.	Level 5 (100%)	5.0	50	50.459	1456551
6.	Level 6 (110%)	5.5	50	55.505	1601136
7.	Level 7 (120%)	6.0	50	60.551	1754536
8.	Level 8 (150%)	7.5	50	75.688	2196599

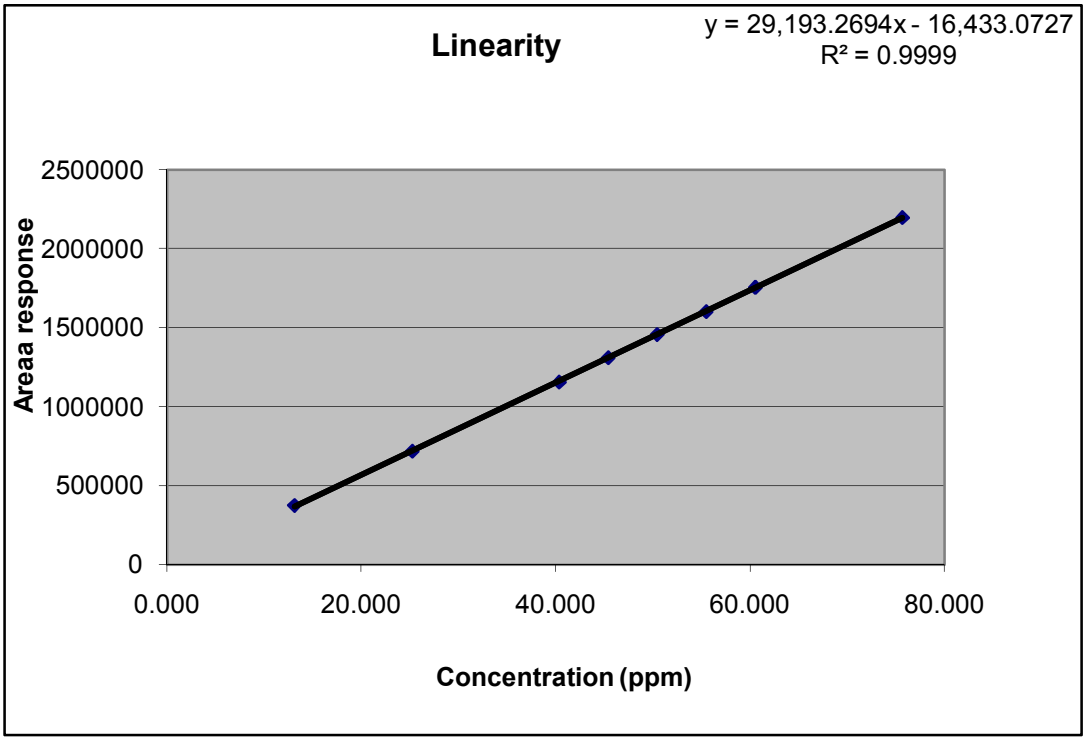


Fig. 5 Linearity of Eletriptan hydrobromide

Table: 28 Linearity report of Eletriptan hydrobromide

S.NO	**Parameter**	**Values**
1.	Linearity range	25% -150%
2.	Regression equation	y = 29,193.2694x - 16,433.0727
3.	Correlation coefficient	0.9999
4.	Intercept	16,433.0727
5.	Slope	29,193.2694

Linearity Level-1 (25%)

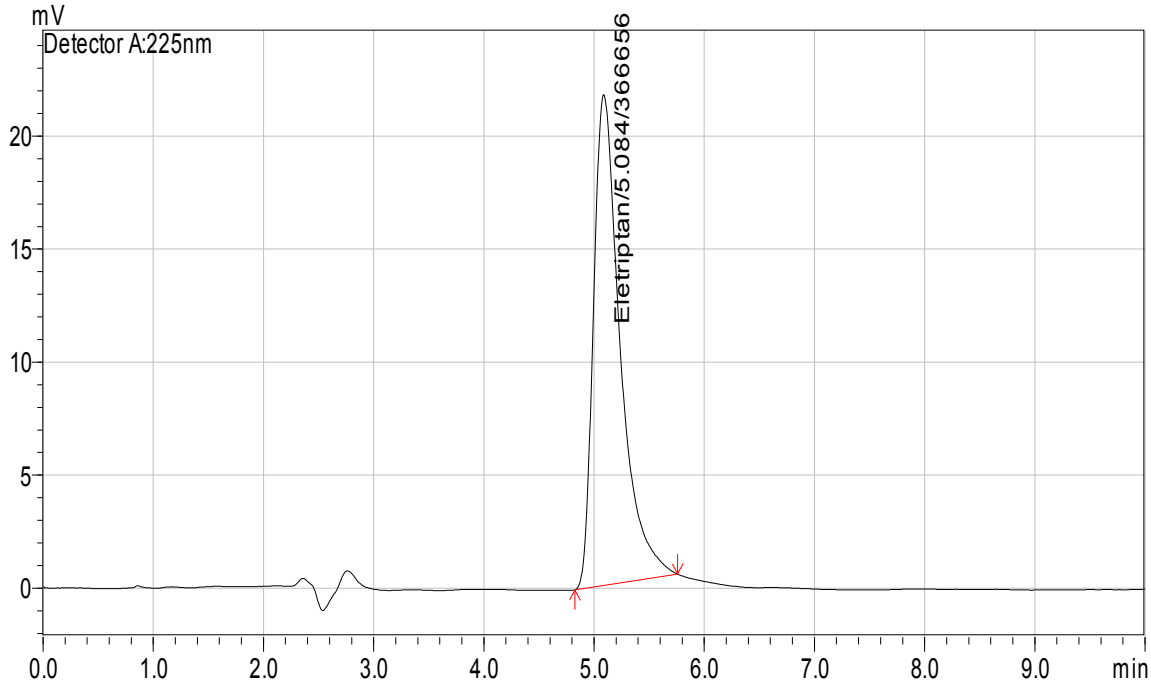


Fig. 6 Chromatogram of linearity at 25µg/ml

Linearity Level-2 (50%)

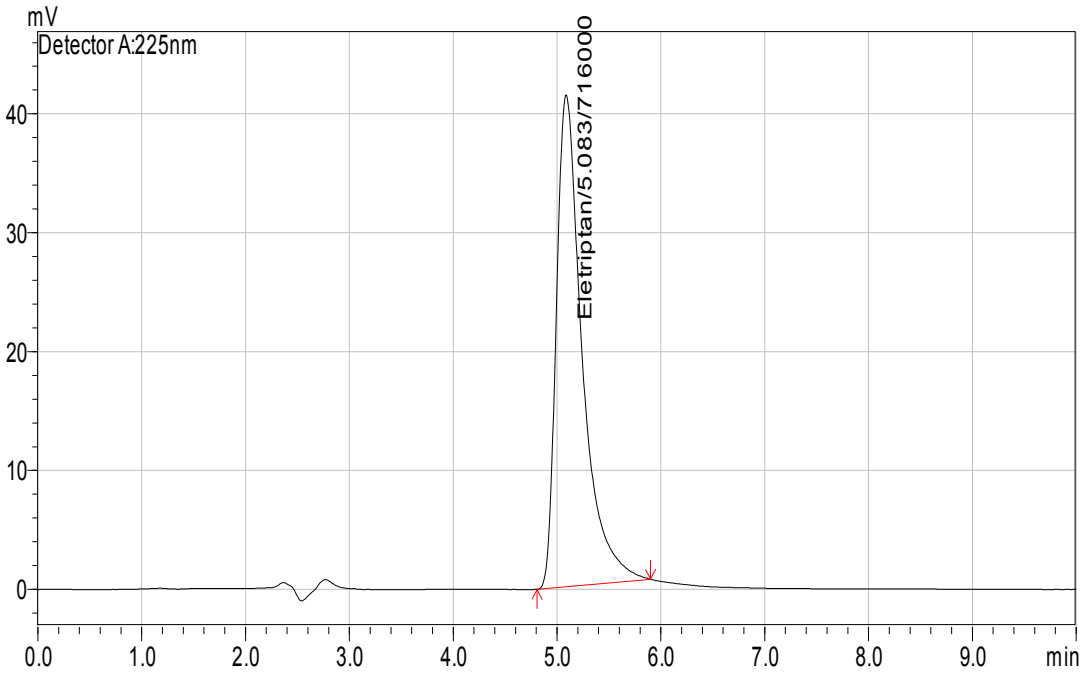


Fig. 7 Chromatogram of linearity at 50µg/ml

Linearity Level-3 (80%)

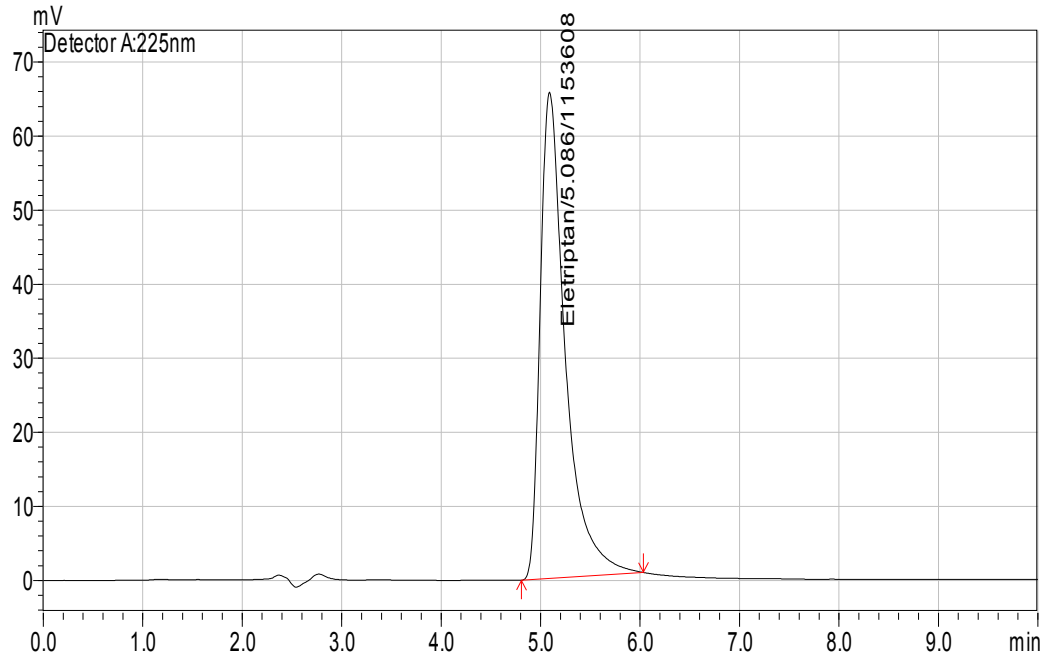


Fig.8 Chromatogram of linearity at 80µg/ml

Linearity Level-4(90%)

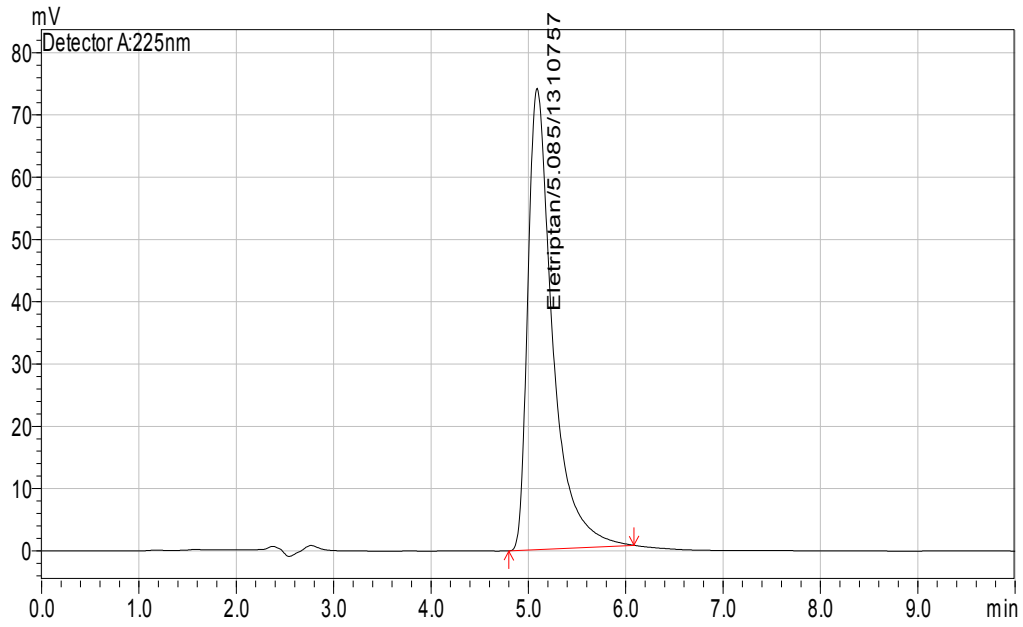


Fig.9 Chromatogram of linearity at 90µg/ml

Linearity Level-5 (100%)

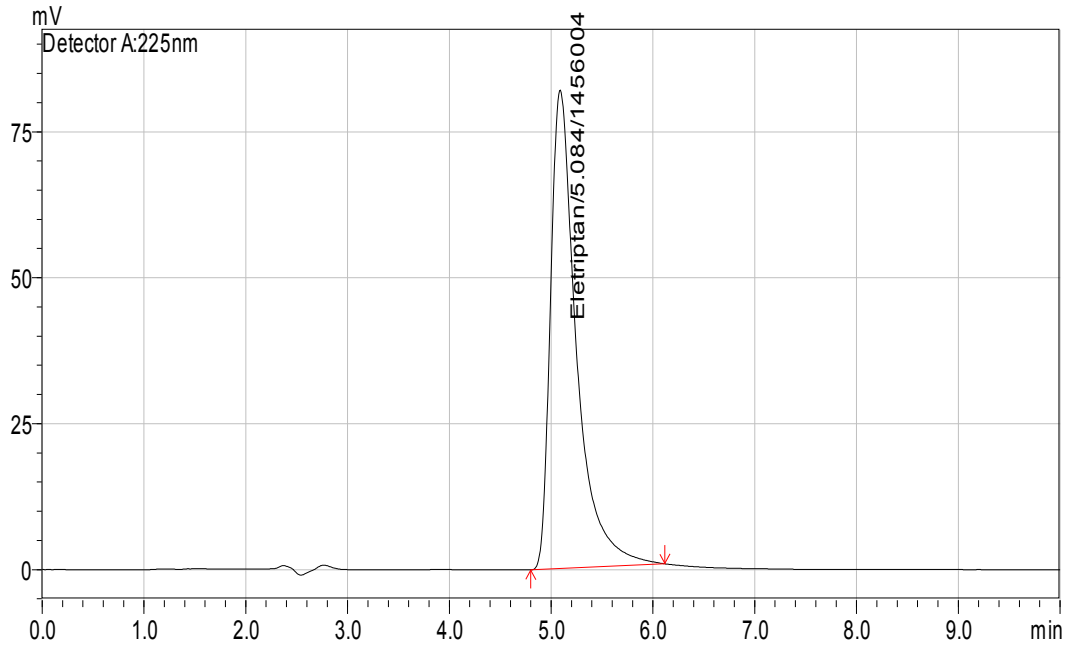


Fig.10 Chromatogram of linearity at 100µg/ml

Linearity Level-6 (110%)

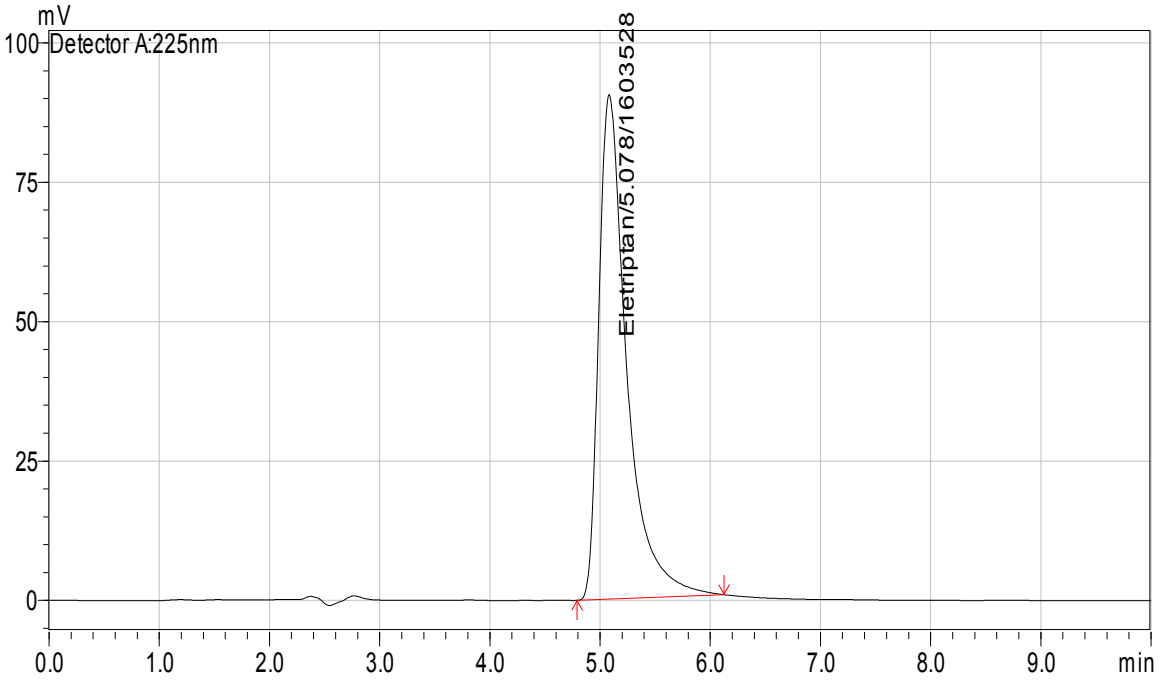


Fig.11 Chromatogram of linearity at 110µg/ml

Linearity Level-7 (120%)

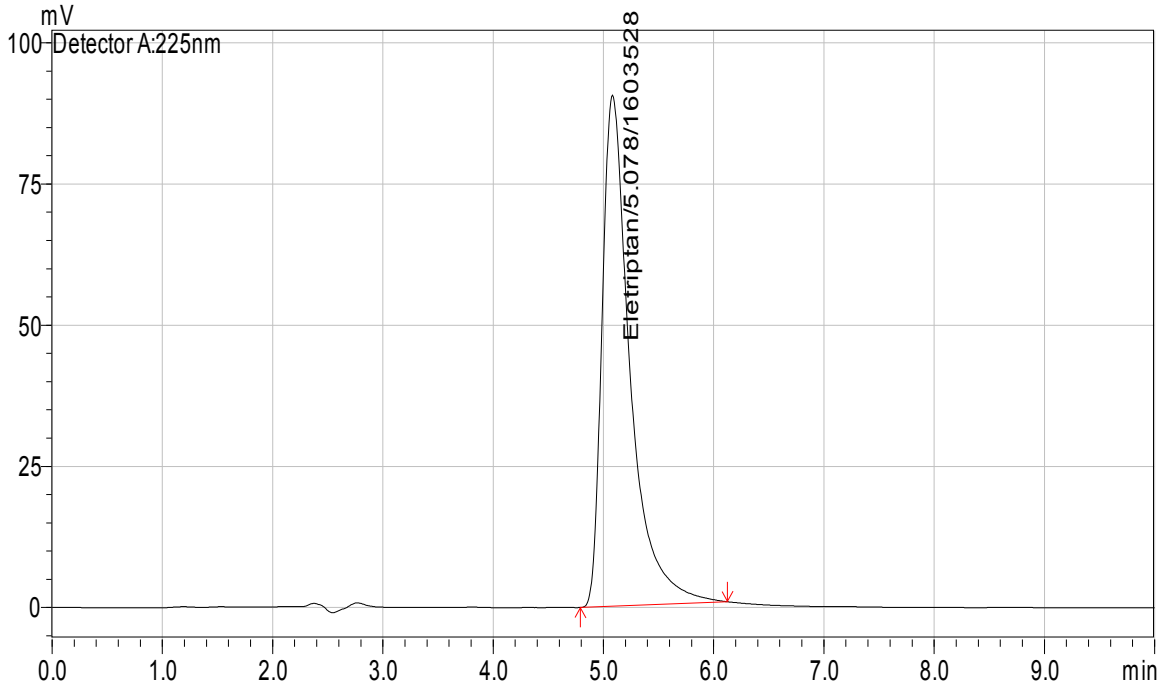


Fig.12 Chromatogram of linearity at 120µg/ml

Linearity Level-8 (150%)

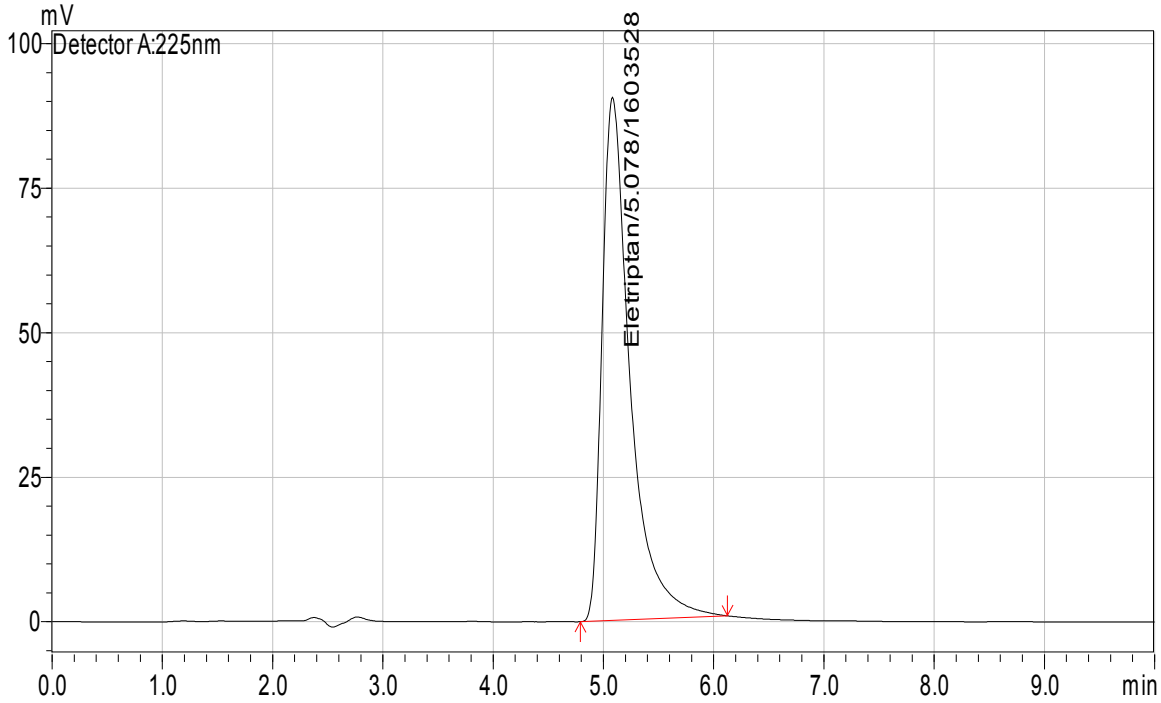


Fig.13 Chromatogram of linearity at 150µg/ml

5.2.3 Assay

Table: 29 Assay report of formulation of Ele HBr

Formulation (Tablet)	Label claimed (mg)	Peak Area Mean± SD (N=3)	Amount found (µg) Mean± SD	%Recovery	% RSD
Eletriptan hydrobromide	40mg	1485452.4±3813.6625	100.6 ± 1.633	100.6	1.62

5.2.4 Accuracy and Range

Table: 30 Recovery report of Eletriptan hydrobromide.

S.No:	Conc level	Amt added (ppm)	Amount found (ppm)	%Recovery	Mean	SD	%RSD
1	25%-1	12.37	12.29	99.4	99.5	0.231	0.23
2	25%-2	12.37	12.29	99.4			
3	25%-3	12.34	12.32	99.8			
4	50%-1	24.57	24.41	99.3	99.6	0.306	0.31
5	50%-2	24.59	24.47	99.5			
6	50%-3	24.59	24.56	99.9			
7	100%-1	49.12	49.34	100.4	100.7	0.945	0.94
8	100%-2	49.13	49.13	100.0			
9	100%-3	49.15	50.03	101.8			
10	150%-1	73.66	75.04	101.9	100.6	1.153	1.15
11	150%-2	73.68	73.48	99.7			
12	150%-3	73.67	73.79	100.2			

Recovery Level-1(25%)

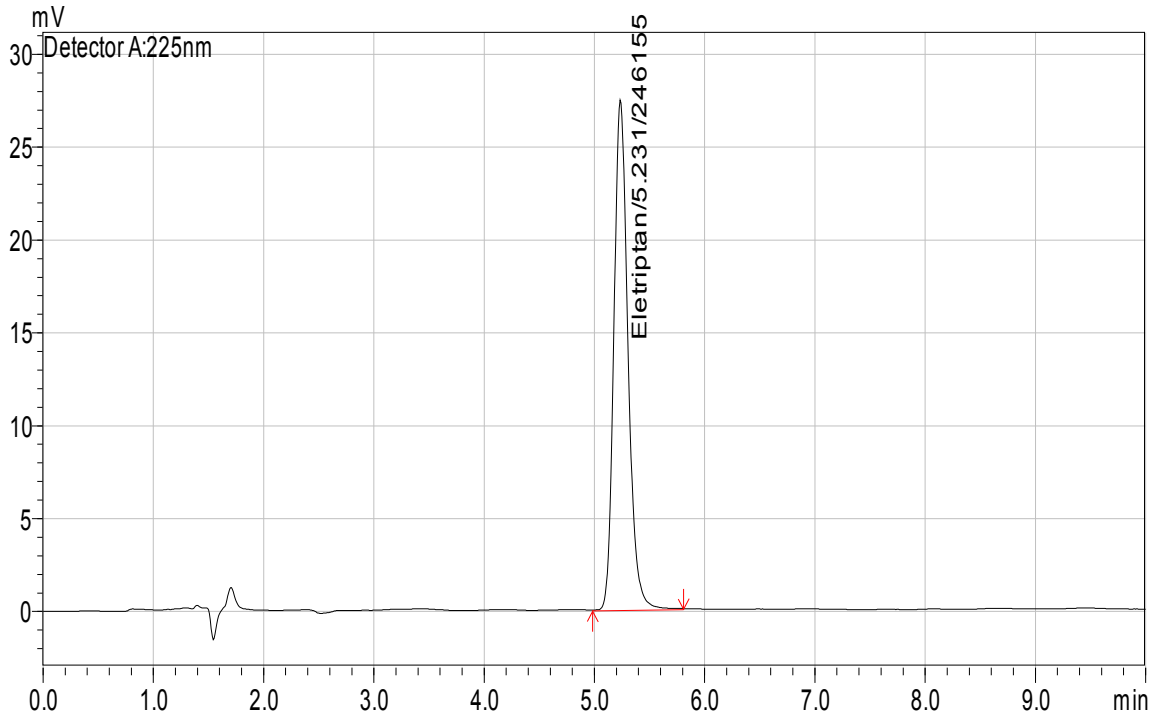


Fig.14. 25% Accuracy

Recovery Level-2(50%)

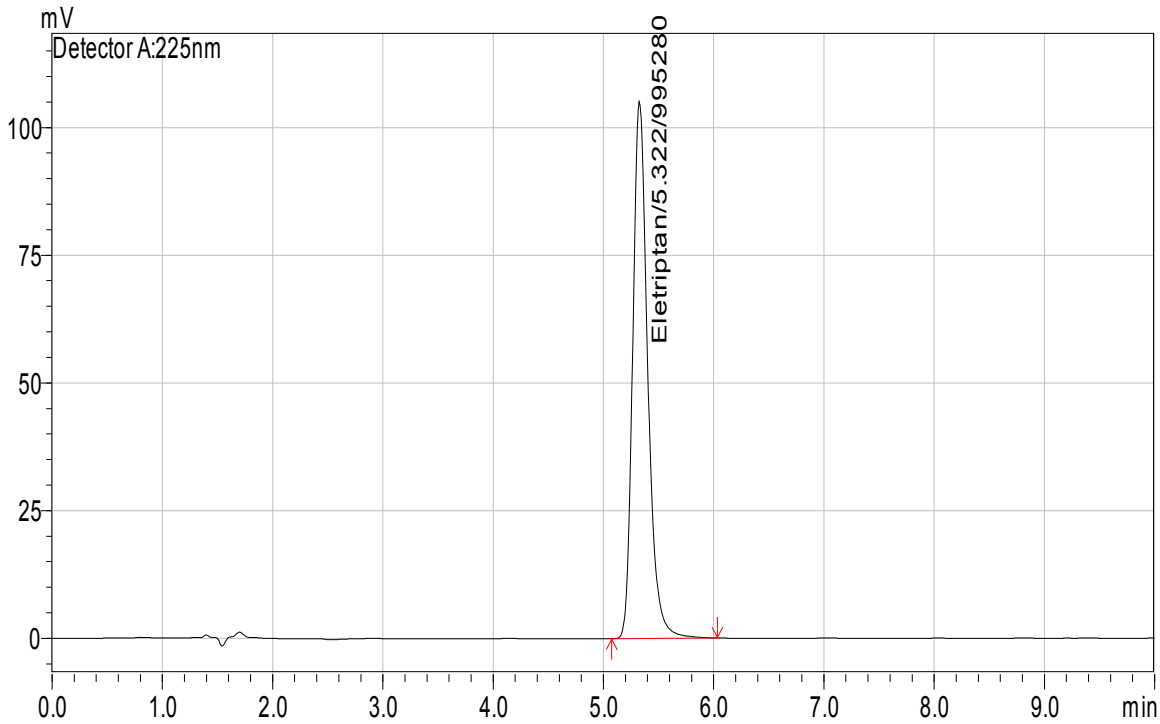


Fig.15 50% Accuracy

Recovery Level-3(100%)

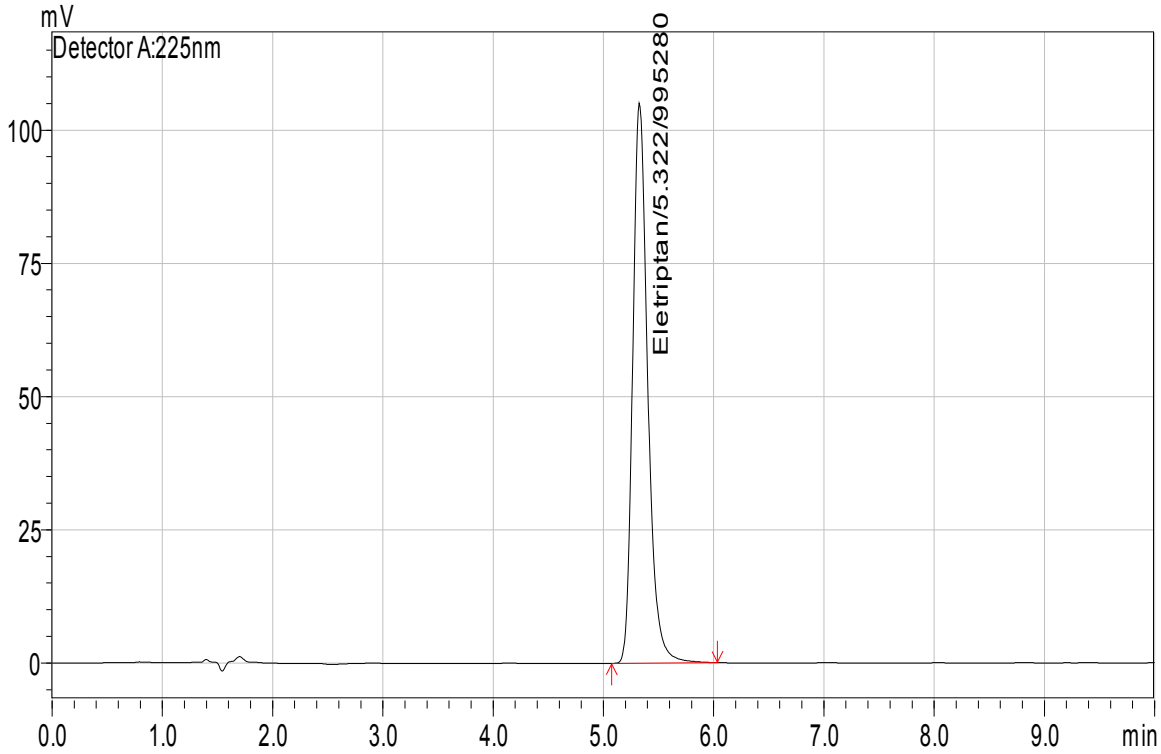


Fig: 16. 100% Accuracy

Recovery Level-4(150%)

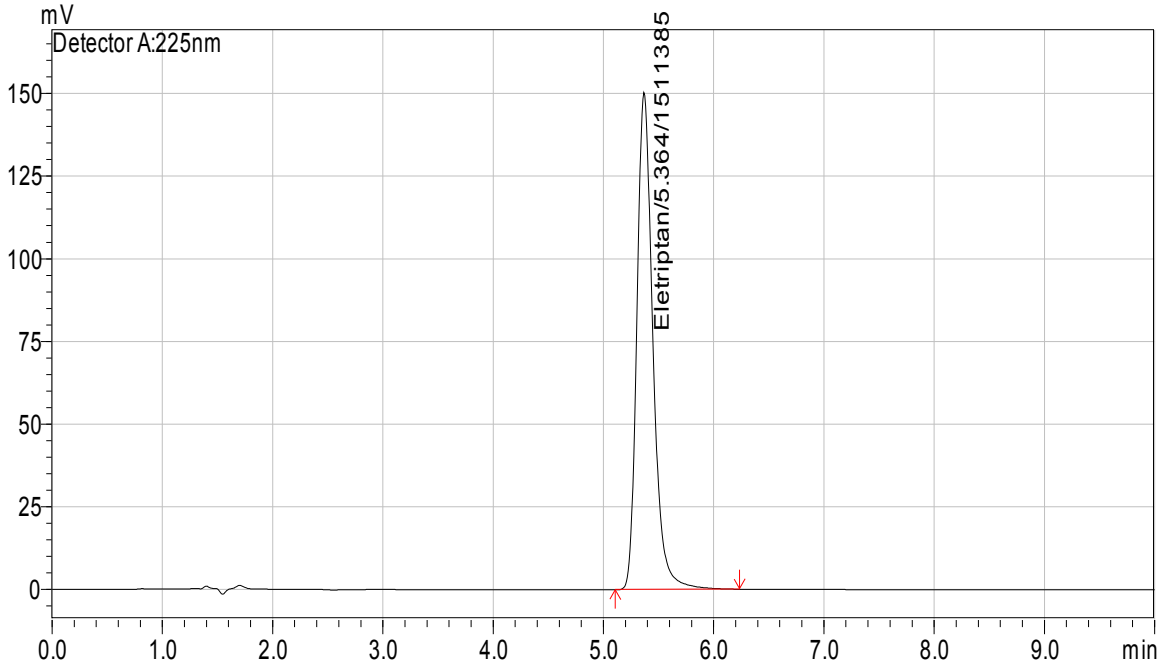


Fig: 17. 150% Accuracy

5.2.5 Precision:

a) Method Precision (Repeatability):

Table: 31 Method Precision data

Sample preparation	% Assay	Sample area
1.	98.3	1447278
2.	98.1	1446581
3.	98.7	1455803
4.	98.5	1451878
5.	98.2	1445291
6.	98.6	1452663
Mean ± SD	98.4 ± 0.237	1449916 ± 3775.45
%RSD	0.24	0.26

c) Intermediate precision:

Table: 32 Intermediate Precision data

Sample preparation	% Assay	Sample area
1.	99.0	1481560
2.	98.5	1473785
3.	98.5	1473762
4.	98.3	1470233
5.	98.7	1475723
6.	98.4	1472640
Mean ± SD	98.6 ± 0.229	1474617 ± 3511.403
%RSD	0.23	0.24

Table: 33 Report of precision for Eletriptan hydrobromide:

S.NO	Precision	%RSD	Acceptance criteria
1.	Method Precision	0.24	NMT 2%
2.	Intermediate precision	0.23	NMT 2%

5.2.6. Specificity and selectivity

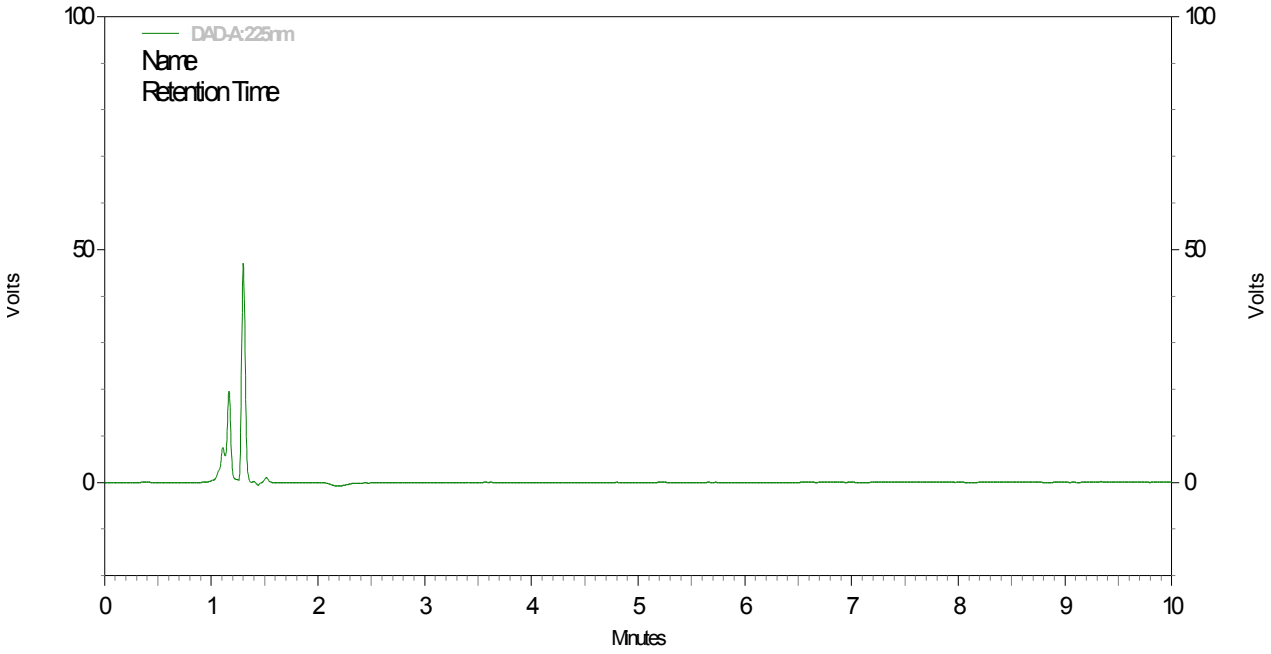


Fig.18 chromatogram of placebo

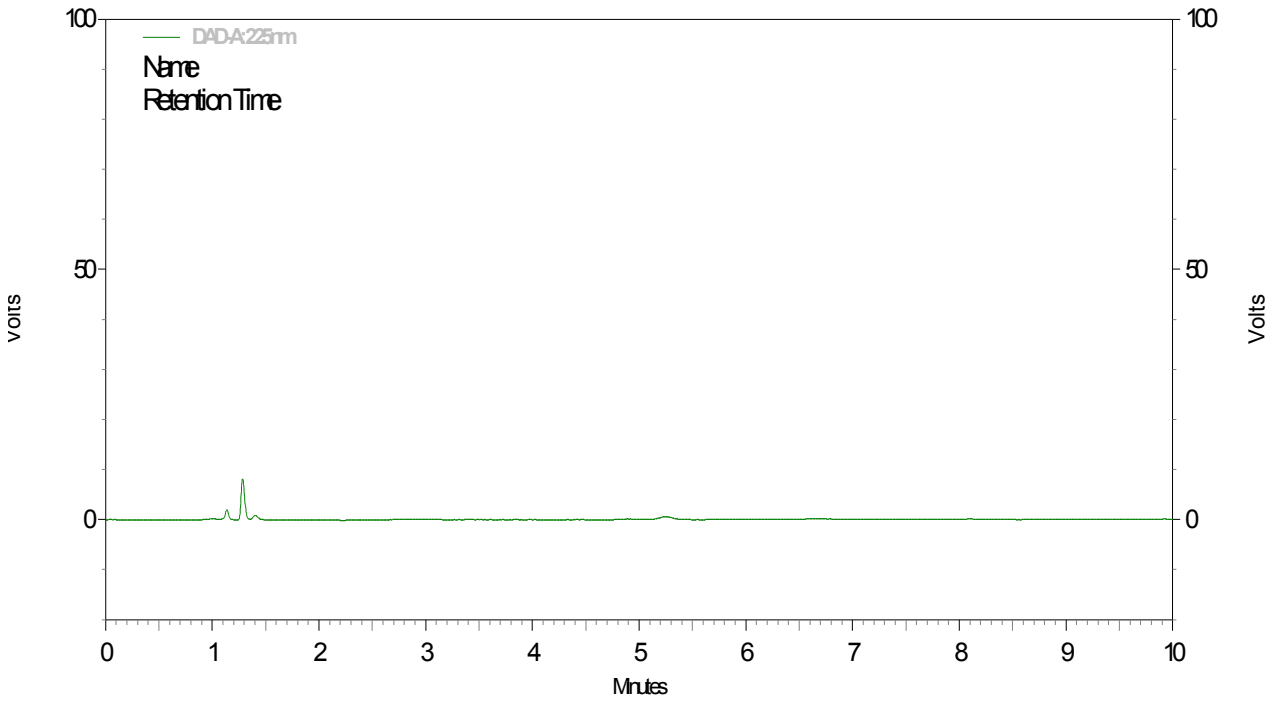


Fig.19 chromatogram of diluents

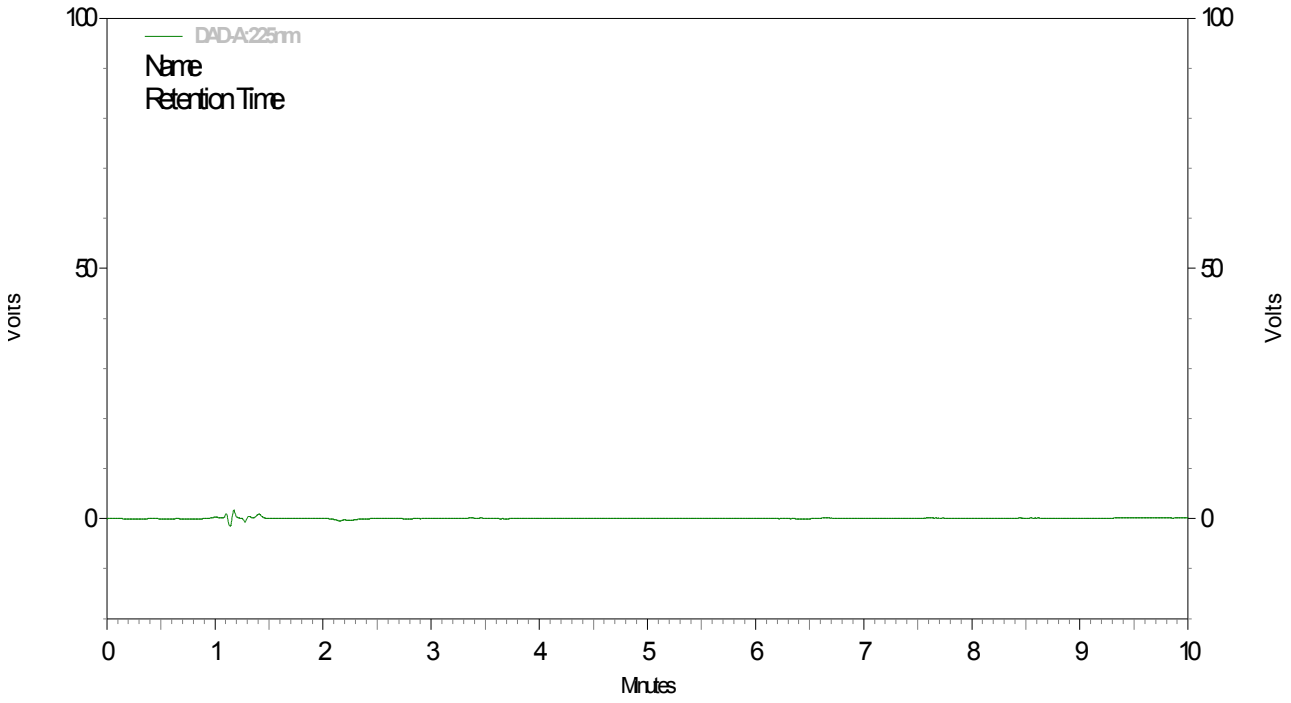


Fig.20 chromatogram mobile phase

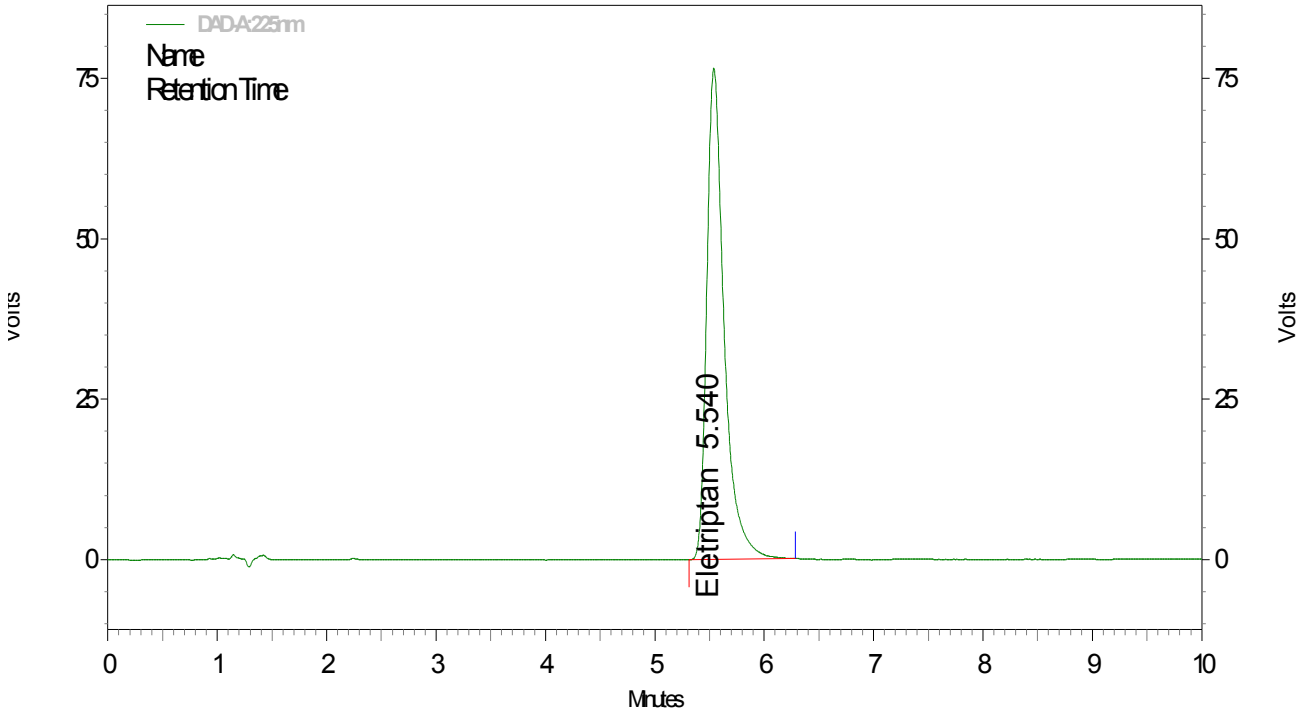


Fig.21 Standard chromatogram

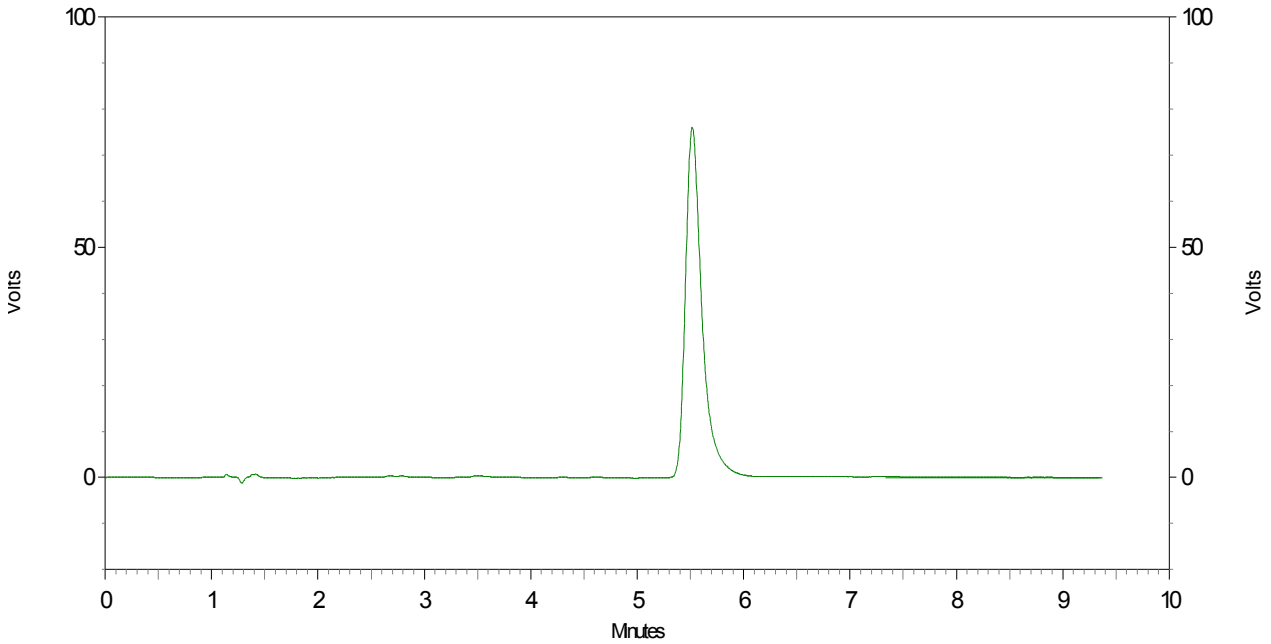


Fig.22 sample Solution chromatogram

5.2.7 Robustness

a) Changes in chromatographic conditions:

a) Flow rate

b) Wavelength

c) Column temperature

d) Buffer P^H

e) Organic solvent ratio in M.P

Table: 35 The Robustness studies of Eletriptan hydrobromide

Parameter	**Condition**	**R_t**	**Remark**
Flow rate	0.9ml 1.0ml 1.1ml	5.71 5.09 4.69	Robust
Wavelength	223nm 225nm 227nm	5.13 5.09 5.14	Robust
Column temperature	20°c 25°c 30°c	5.39 5.09 5.62	Robust
Buffer P^H	6.8 7.0 7.2	5.21 5.09 6.29	Robust
Organic solvent ratio in M.P	-10% Finalized ratio +10%	6.87 5.09 4.67	Robust

a) Flow rate

Flow Rate 0.9ml:

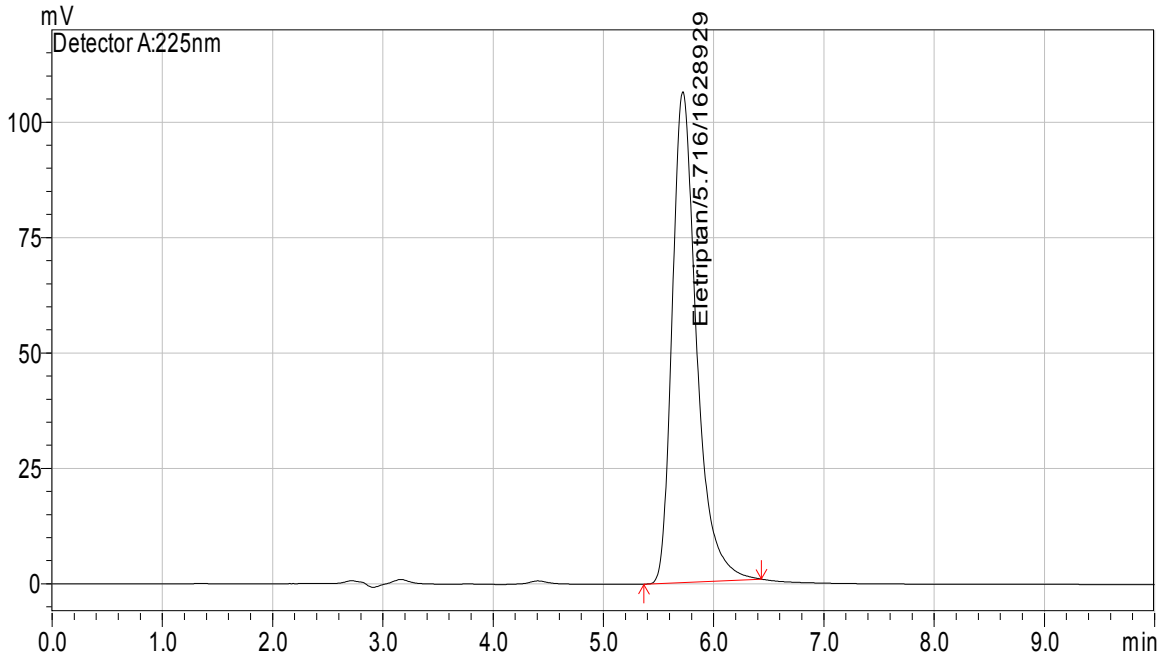


Fig.23 0. 9ml Flow

Flow Rate 1.1 ml:

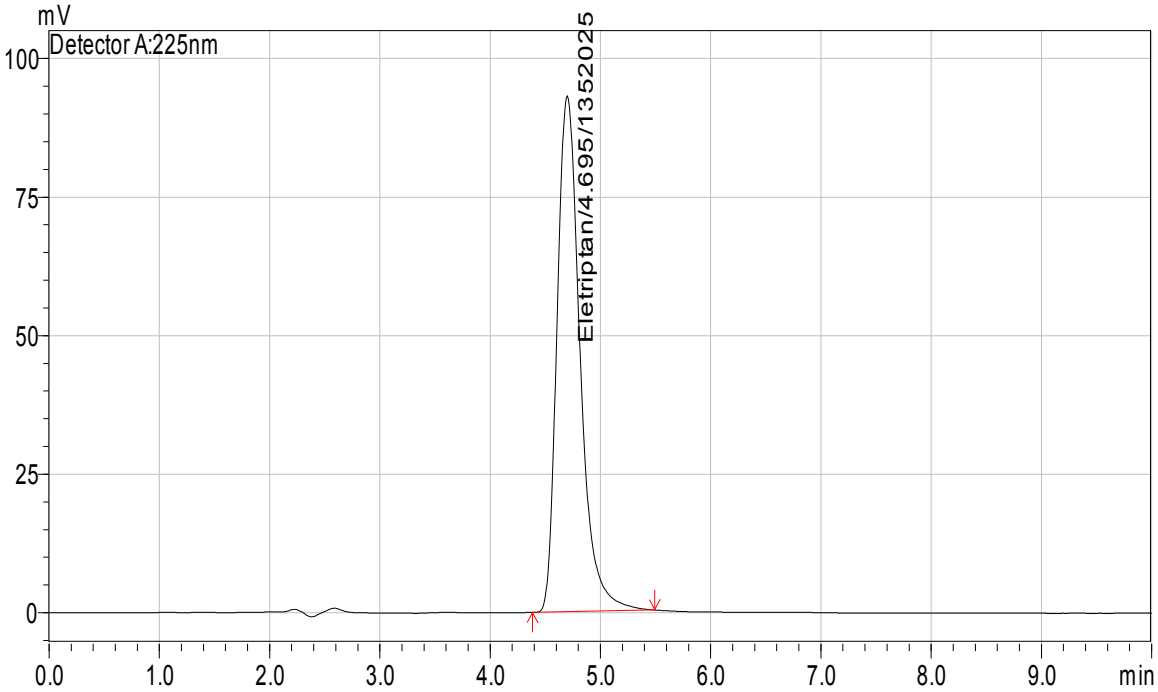


Fig.24 1.1ml Flow

b) Wavelength

Wavelength 223nm:

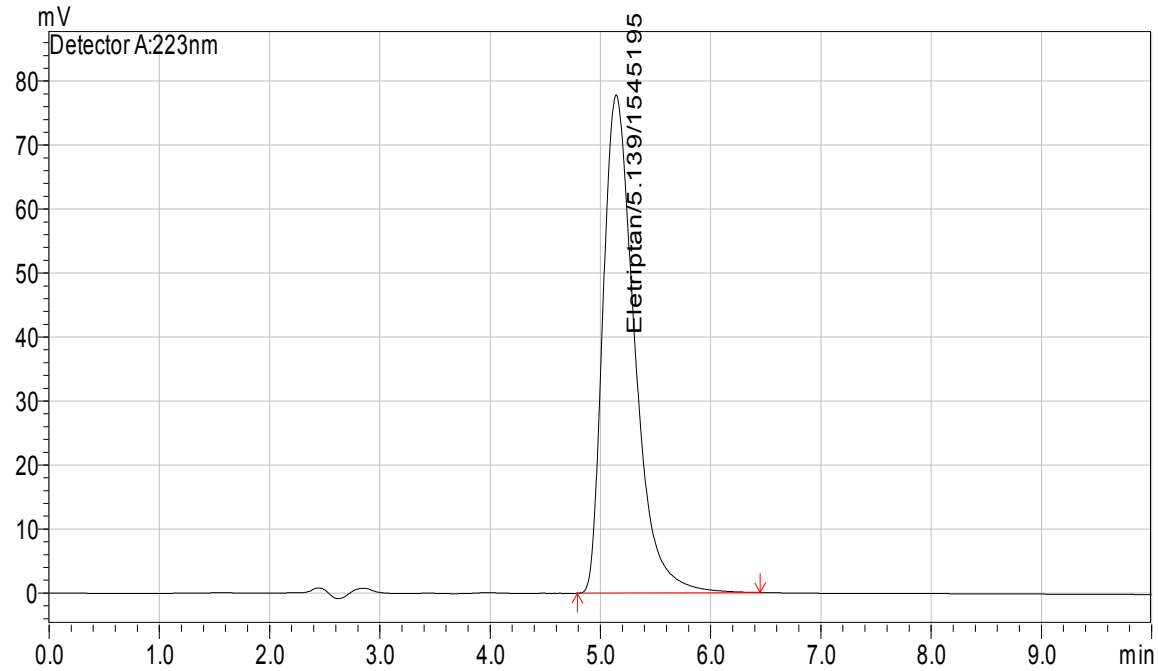


Fig.25 chromatogram at wavelength 223nm

Wavelength 227nm:

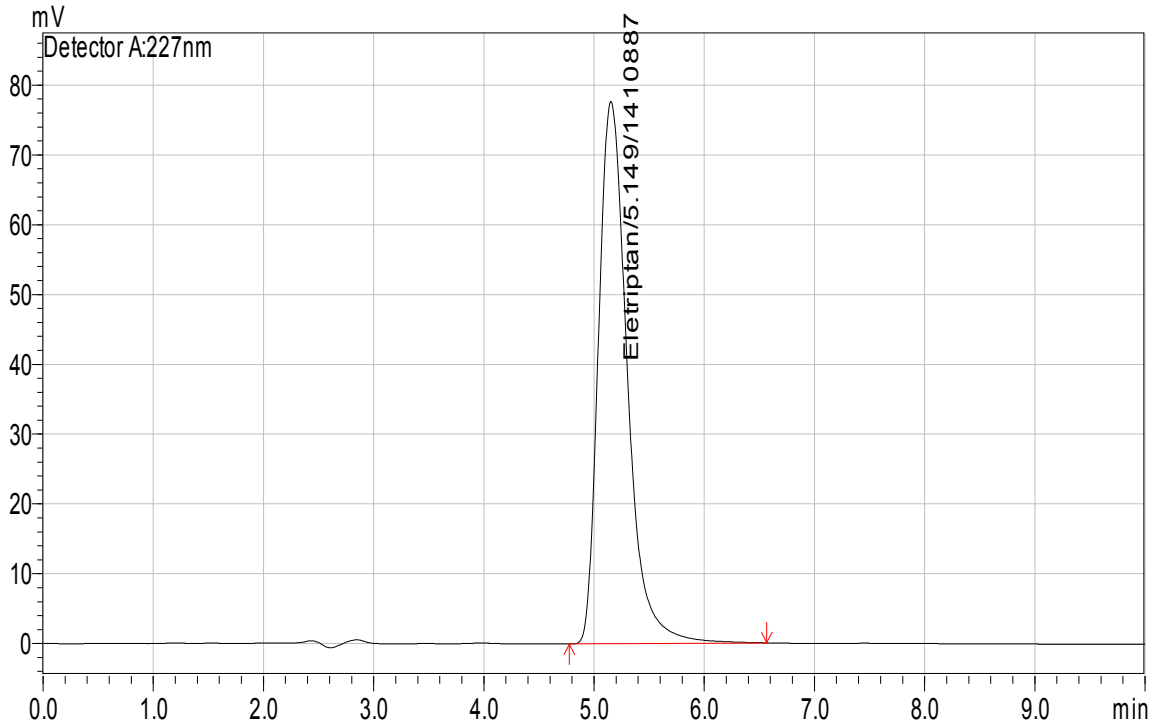


Fig.26 chromatogram at wavelength 227nm

c) Column temperature

Column temperature *20ºC*

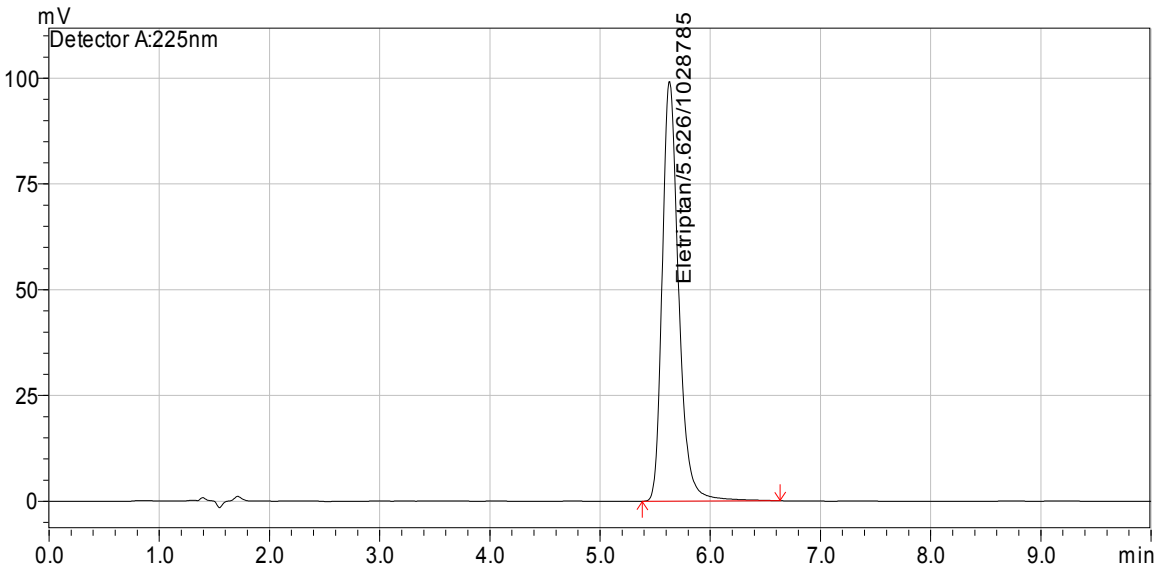


Fig.27 chromatogram at Column temperature 20ºC

Column temperature 30ºC

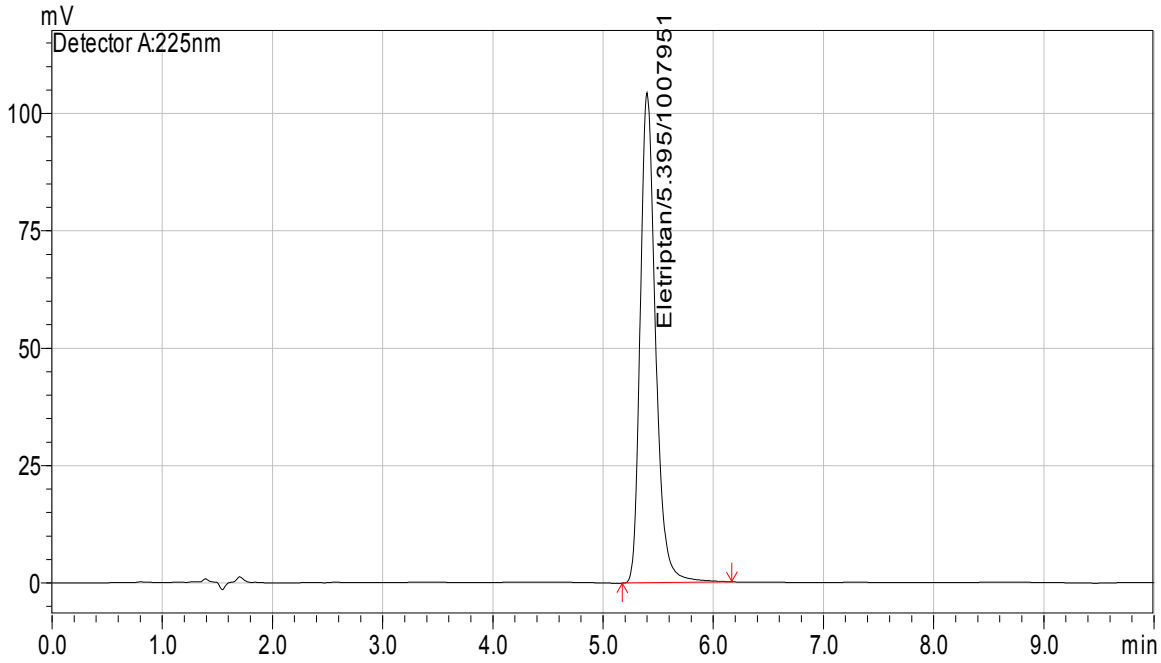


Fig.28 chromatogram at Column temperature 30ºC

d) Buffer P^H

Buffer P^H 6.8

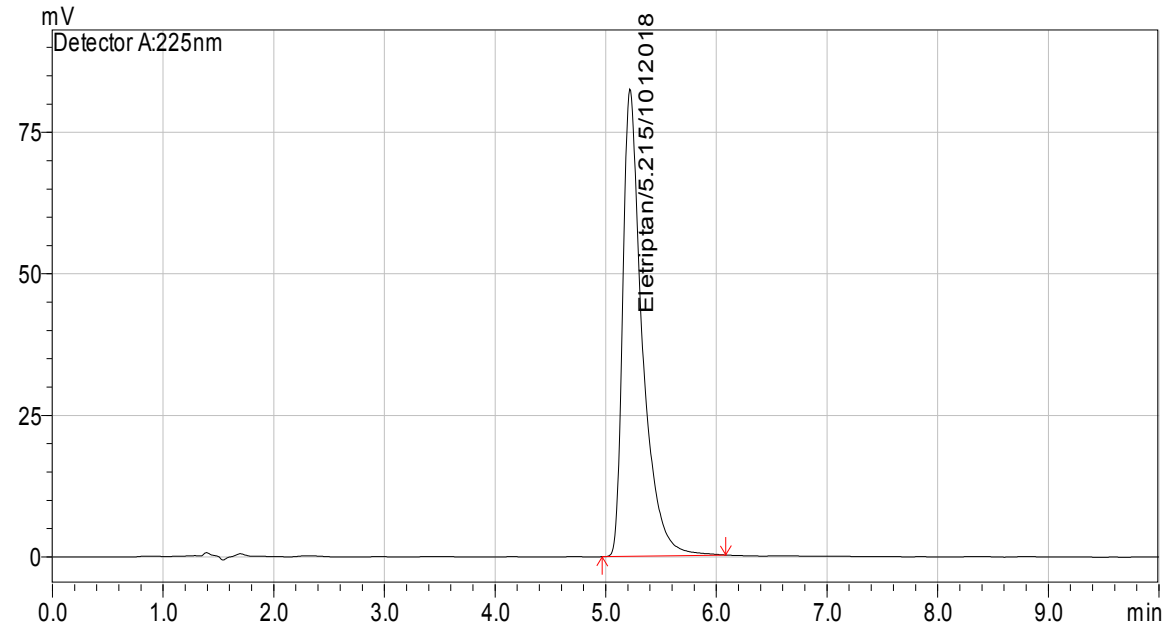


Fig.29 chromatogram of Buffer P^H 6.8

Buffer P^H 7.2

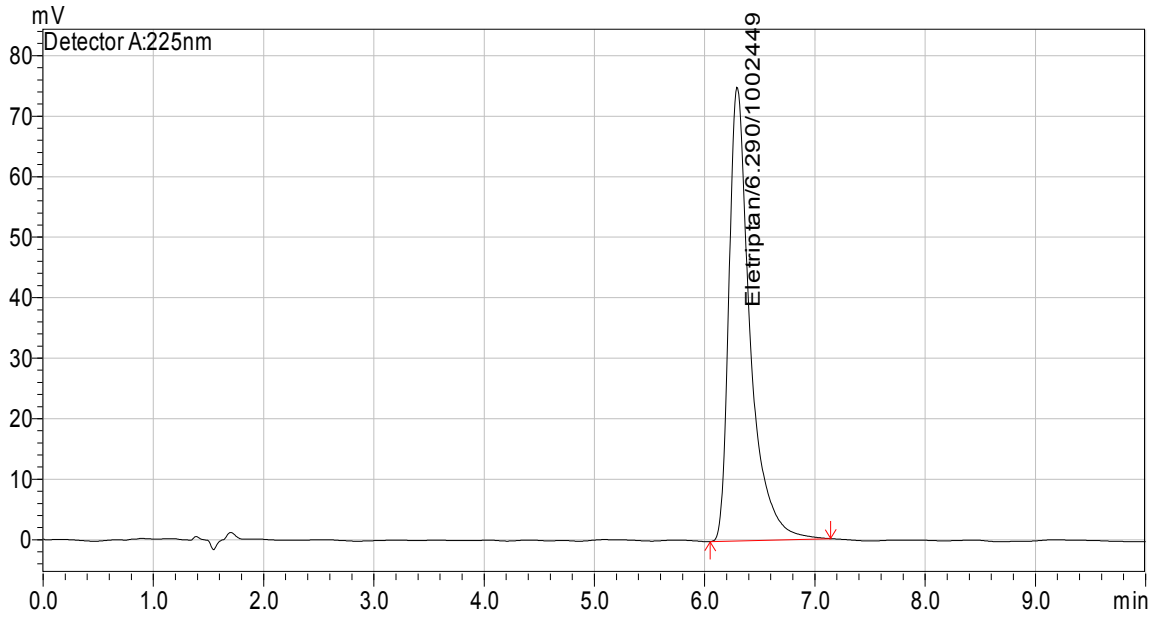


Fig.30 chromatogram of Buffer P^H 7.2

e) Organic solvent ratio in M.P

Organic solvent -10%

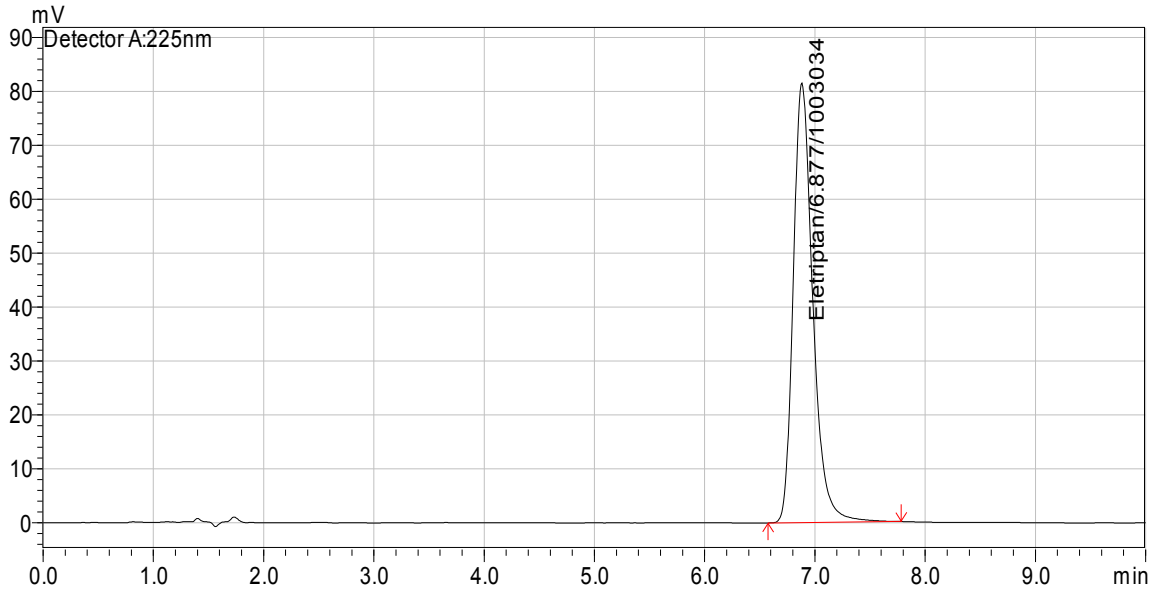


Fig.31 chromatogram of Organic solvent -10%

Organic solvent +10%

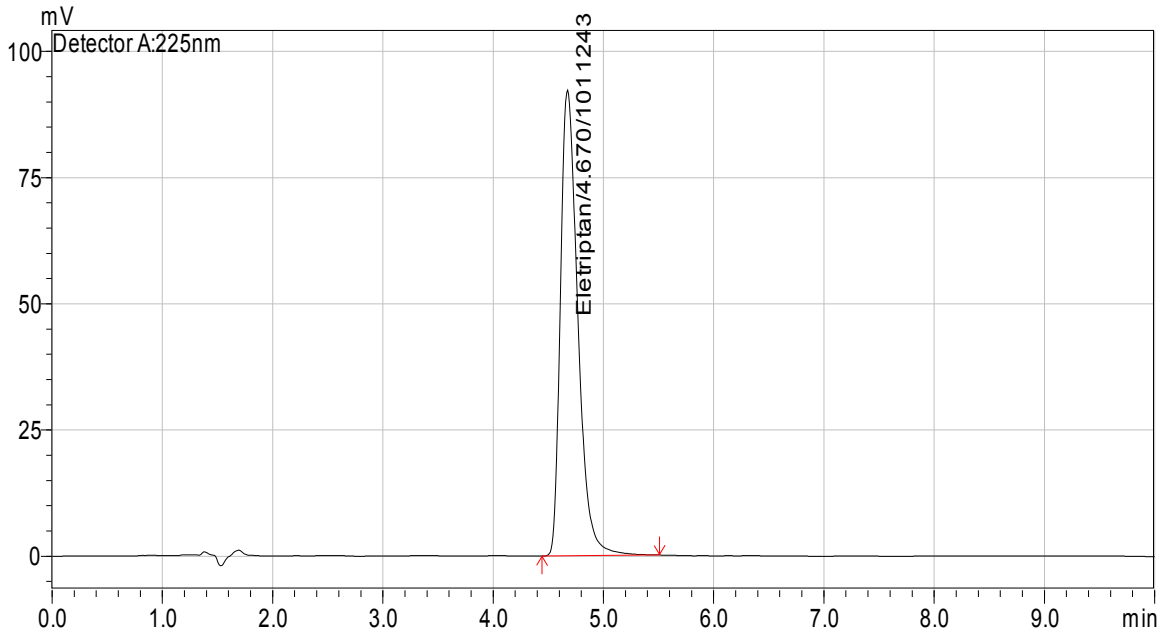


Fig.32 chromatogram of Organic solvent +10%

b) Filter Validation:

Table: 35 results of Filter Validation

S.No	**Sample type**	**% Recovery**	**% Difference**
1.	Centrifuged sample	98.3	-
2.	Nylon/GHP sample	98.1	0.2
3.	PVDF sample	99.4	1.1

Acceptance Criteria**:** The % Difference NMT 2%

Centrifuged Sample

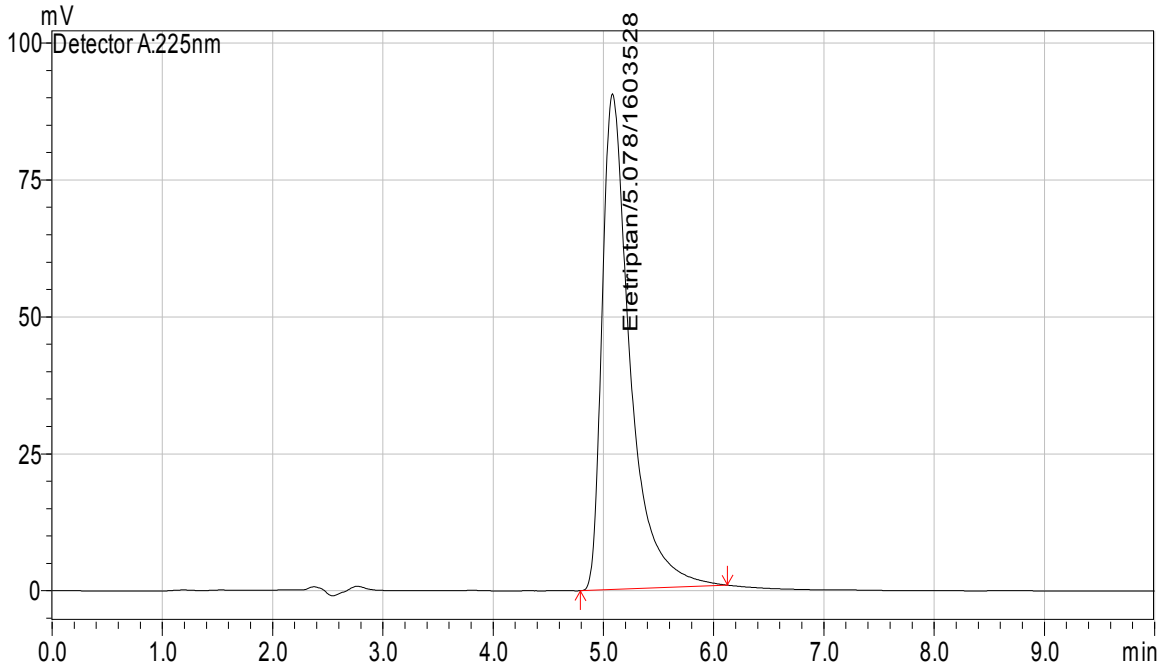


Fig. 33 Centrifuged Samples

Nylon/GHP Sample

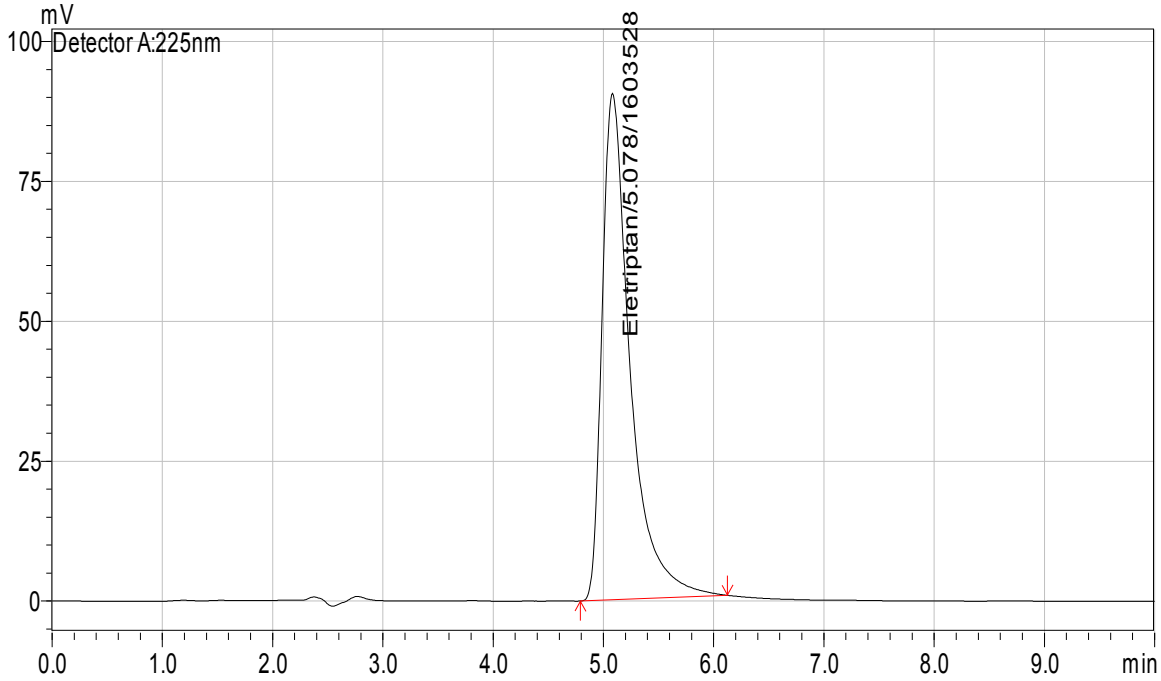


Fig. 34 . Nylon/GHP Sample

PVDF Sample

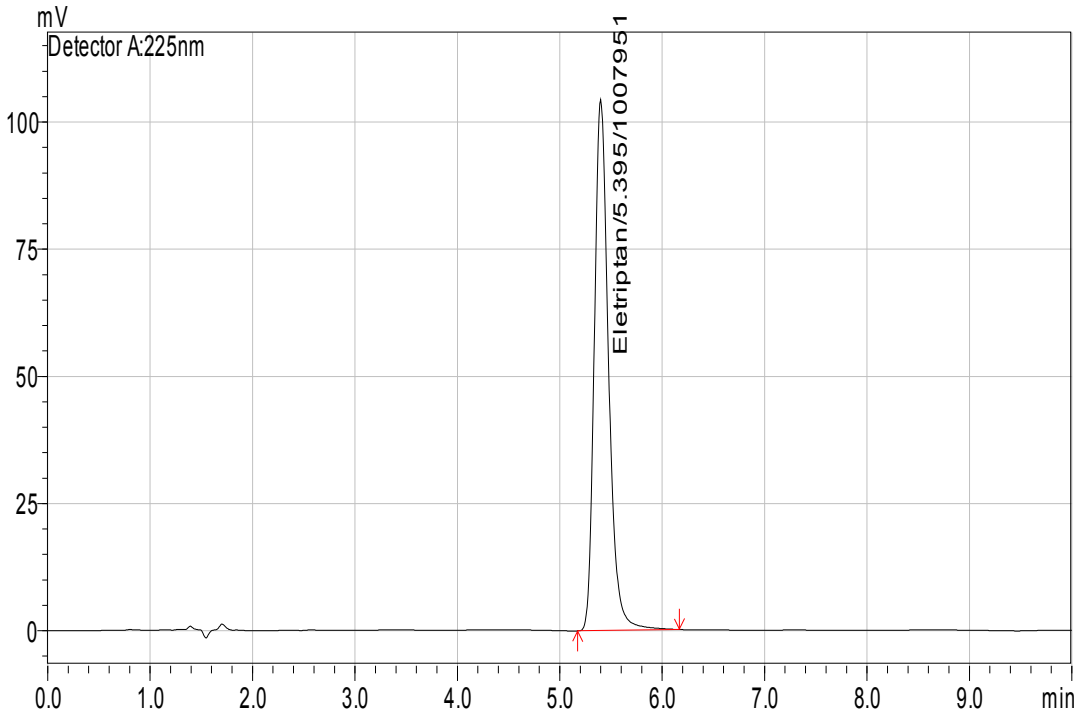


Fig. 35 . PVDF Sample

C) Solution Stability

Table: 36 Results of solution stability

S.No	Time	Type	%Recovery	%Difference
1.	Initial	standard	98.3	NA
2.	4hrs	standard	98.3	0.0
3.	12hrs	standard	98.5	0.2
4.	24hrs	standard	98.6	0.3
5.	48hrs	standard	98.5	0.2

Discussion:

The selected method was estimated by external standard method as per ICH guidelines. The method was Different trails were performed by using different ratios of mobile phase. And finally M.P ratio 55:45 was selected for proposed work. At this ratio there was no interference of analyte peak with placebo, diluent, mobile phase. The detection was carried at wavelength 245 nm with a retention time of 5.09 min and peak assymetry of 1.52.

The method was validated for all validation parameters as per ICH guidelines. No peaks were found at retention time of 5.09min and The impurities were well separated from the analyte peak. So, the proposed method was specific and selective for the detection of Eletriptan hydrobromide.

The linearity range for Erl.HCL was 25 - 150µg/ml. the value of correlation coefficient was 0.9999.

The %RSD values of inter day and intraday precision were <2 so the method was sufficiently precise.

The accuracy of the method was provide by recovery studies and was found to significant and under specification limits with % recovery 100.1 (acceptable range 98 – 102%).

The assay results were found to be 100.6% (acceptance range 95 – 105%).

The method also passes the specification limits of robustness parameters.

5.3 Stress Degradation Studies of Eletriptan Hydrobromide

5.3.1 Degradation behavior of Eletriptan Hydrobromide tablets

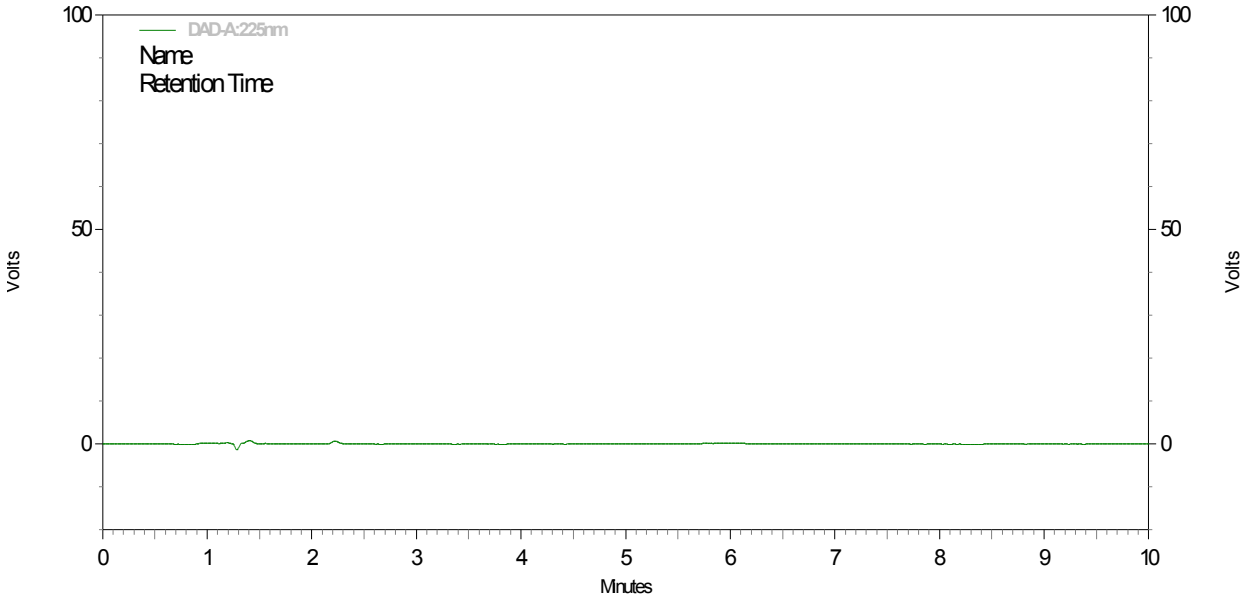


Fig. 36 chromatogram of blank

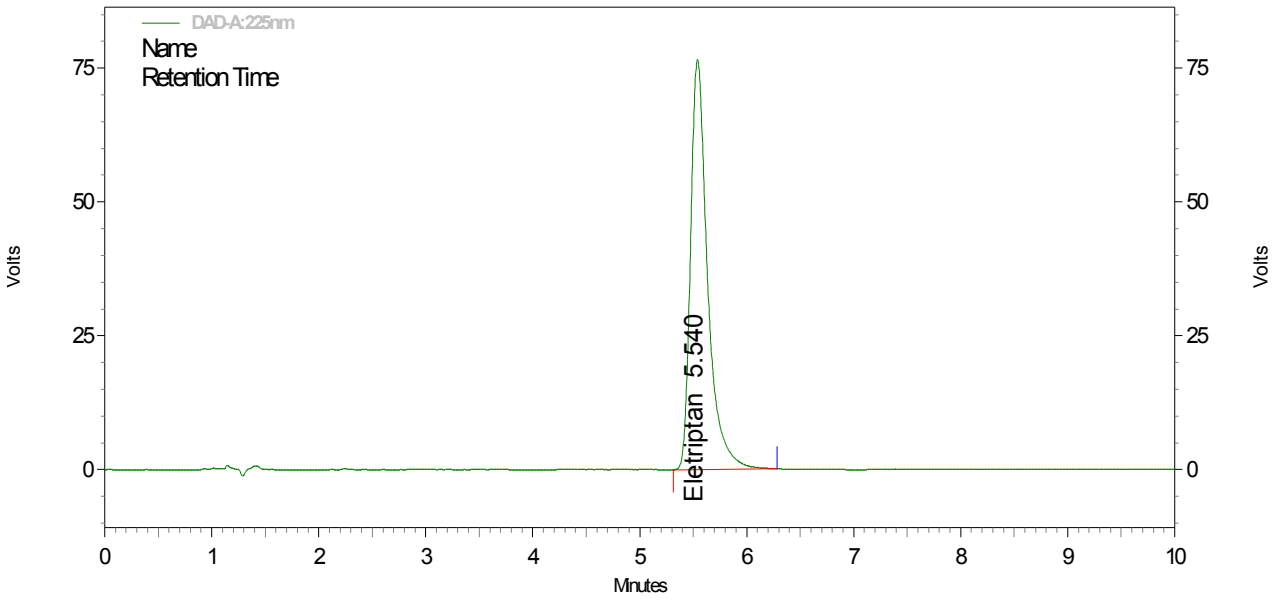


Fig.37 chromatogram of Standard

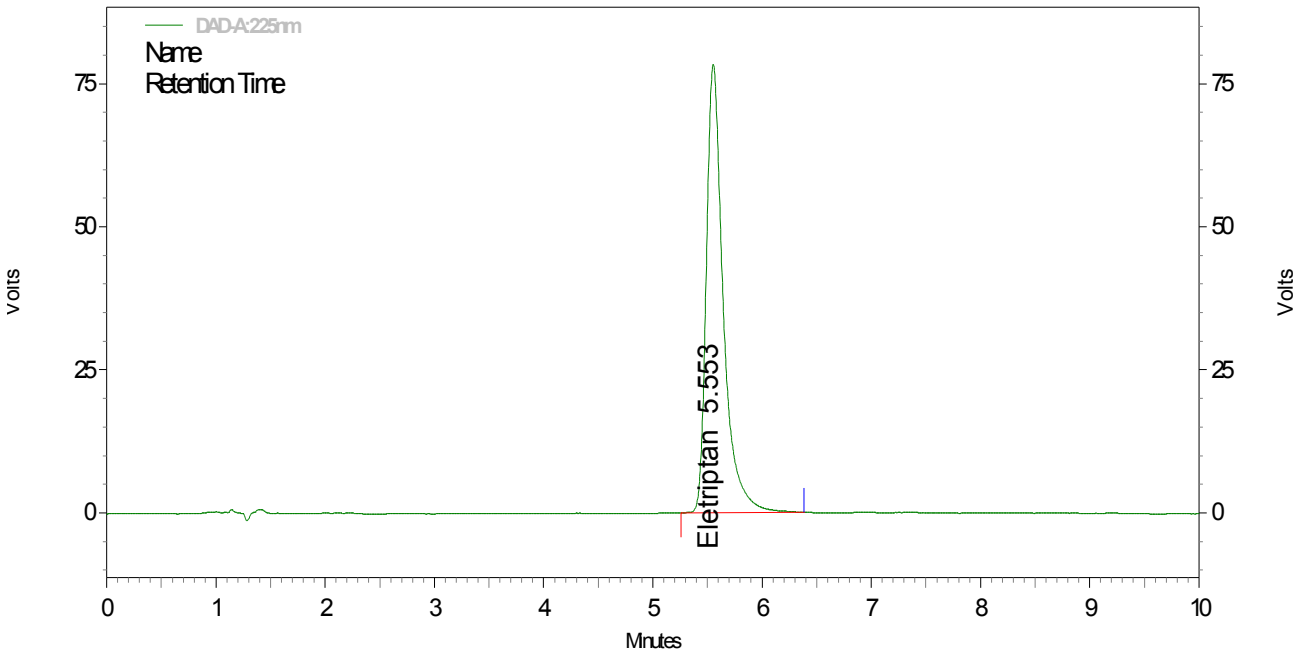


Fig. 38 chromatogram of Control sample -1

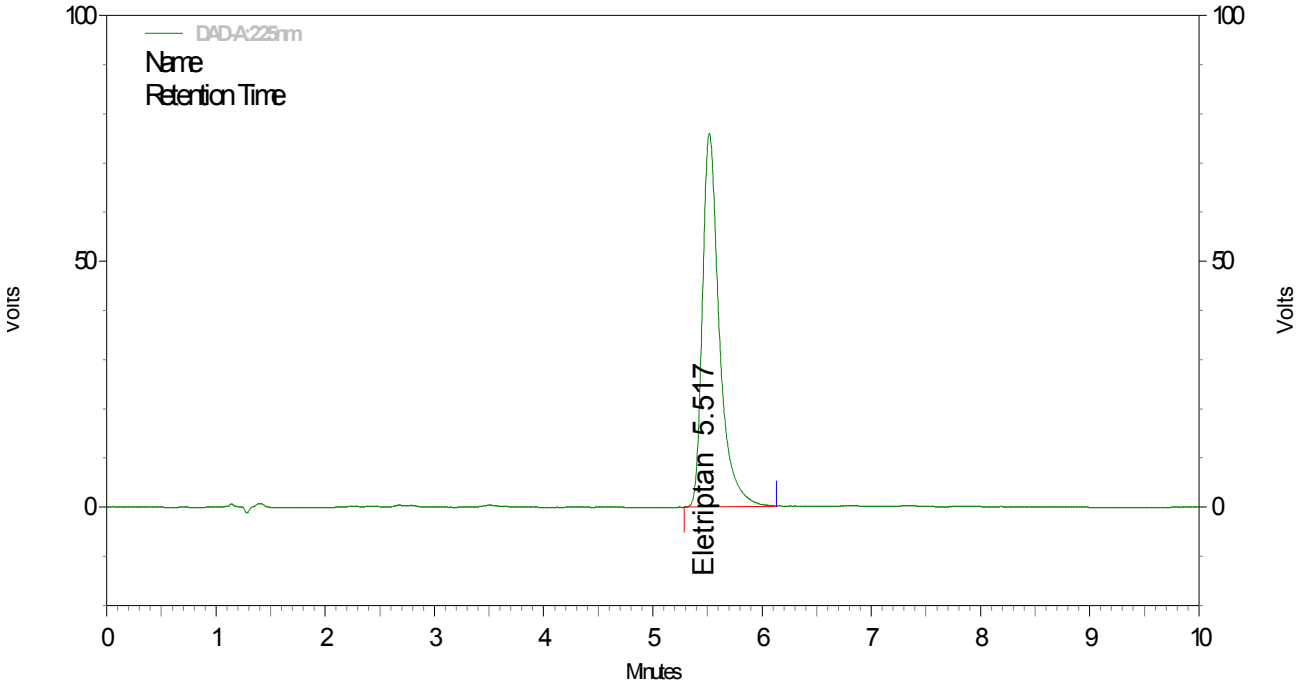


Fig. 39 chromatogram of Control sample -2

a) Acid degradation

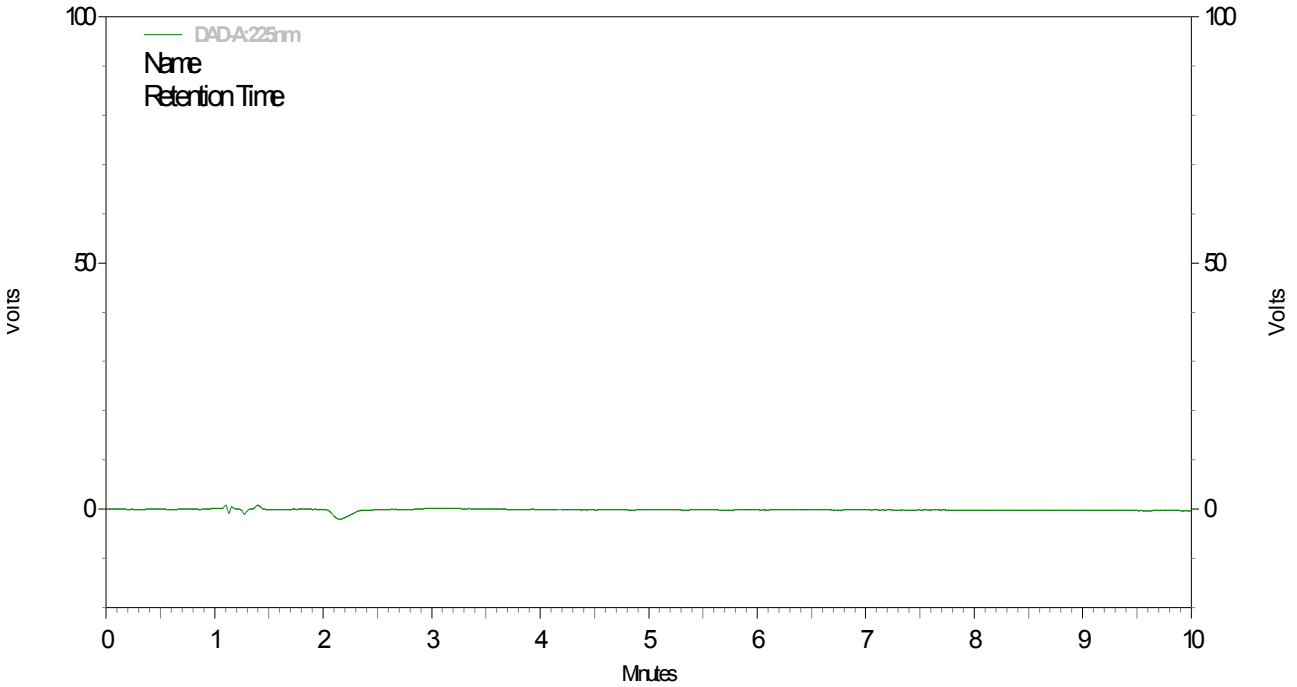


Fig. 40 chromatogram of blank of acid stress

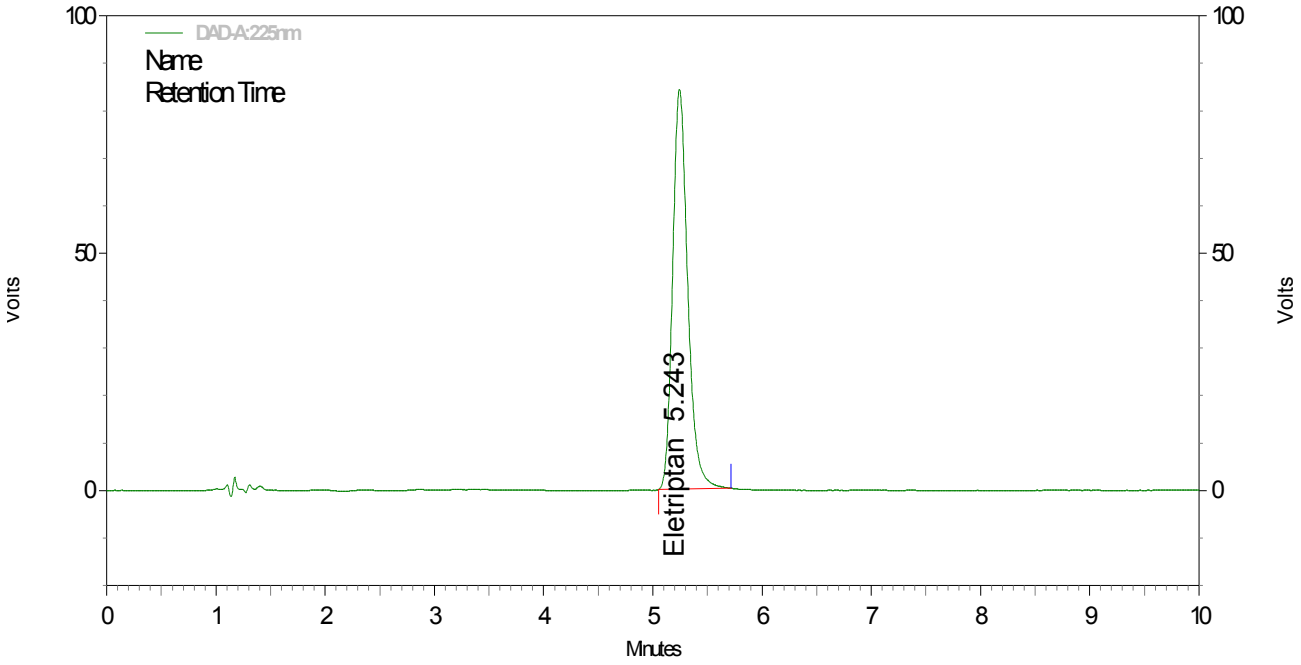


Fig. 41 chromatogram of sample of acid stress

b) Base degradation

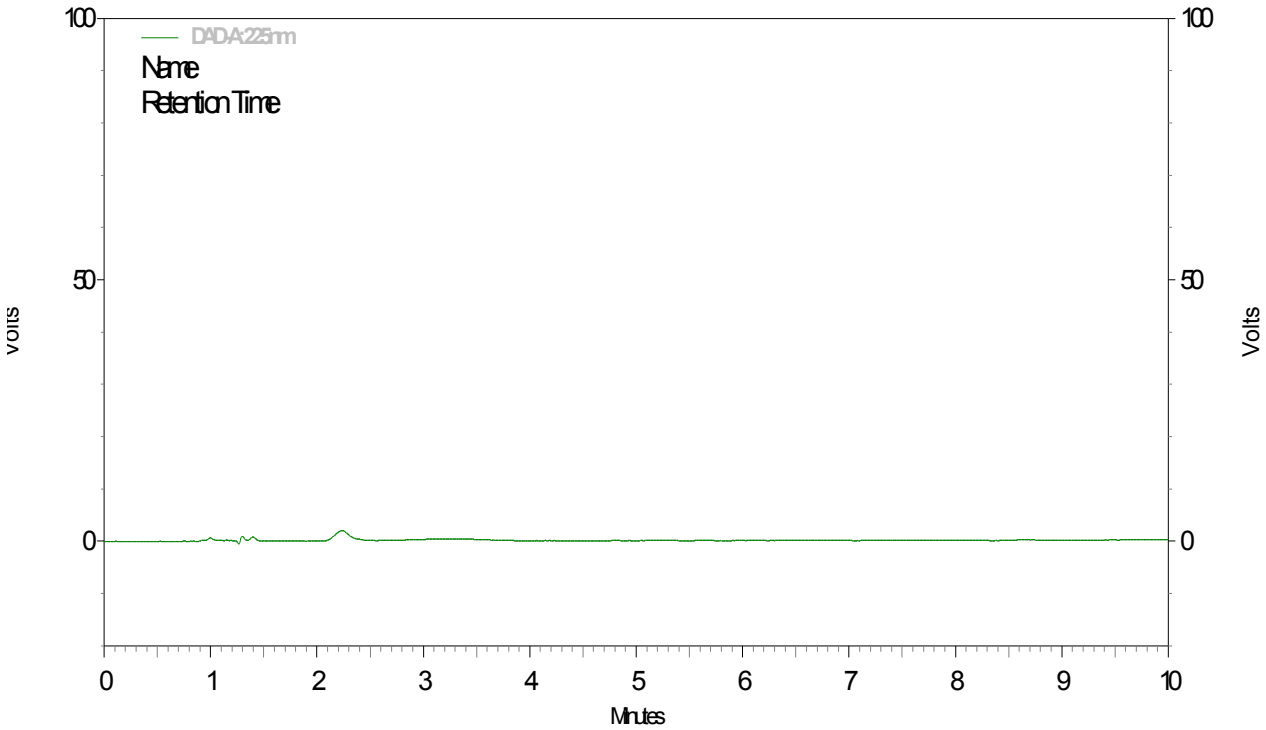


Fig. 42 chromatogram of blank of base stress

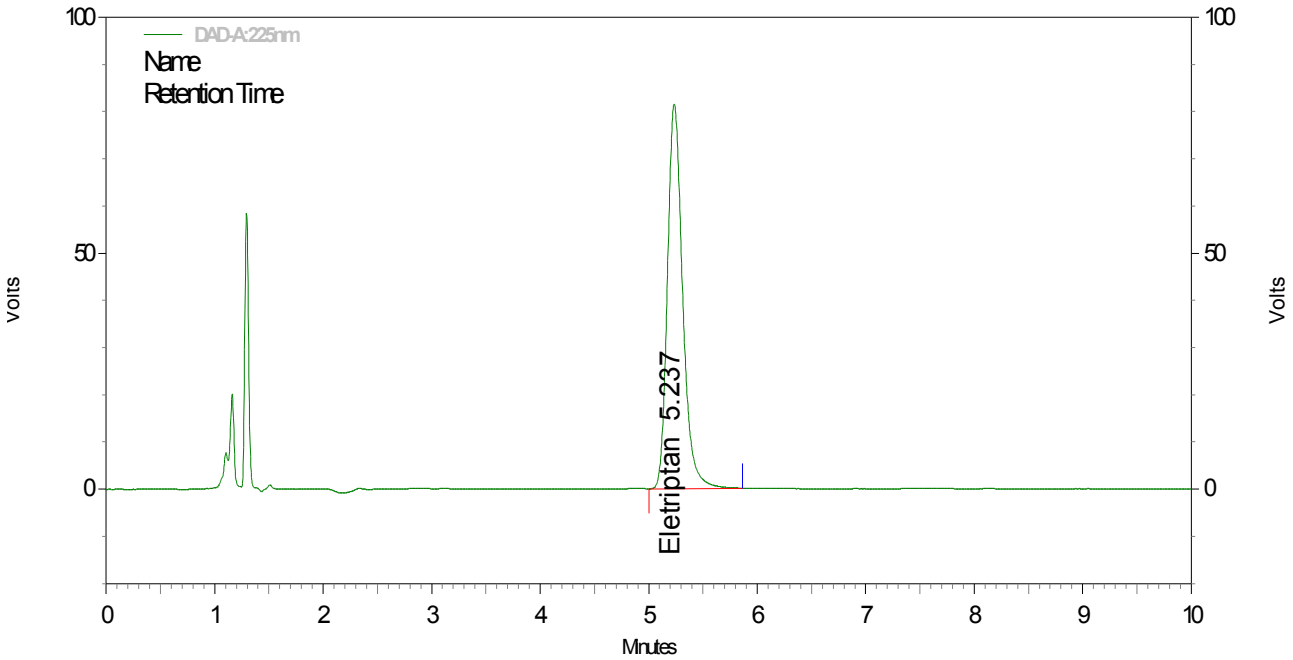


Fig.43 chromatogram of sample of base stress

c) Oxidative degradation

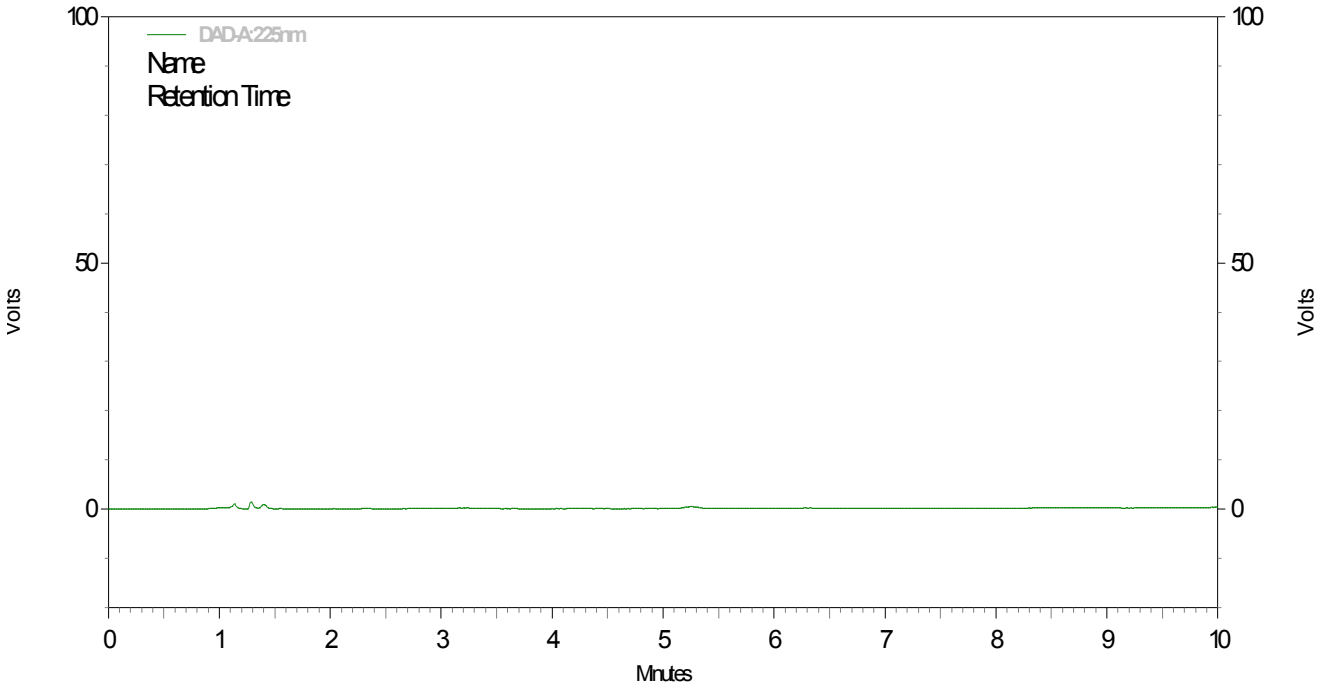


Fig.44 chromatogram of blank of oxidative stress

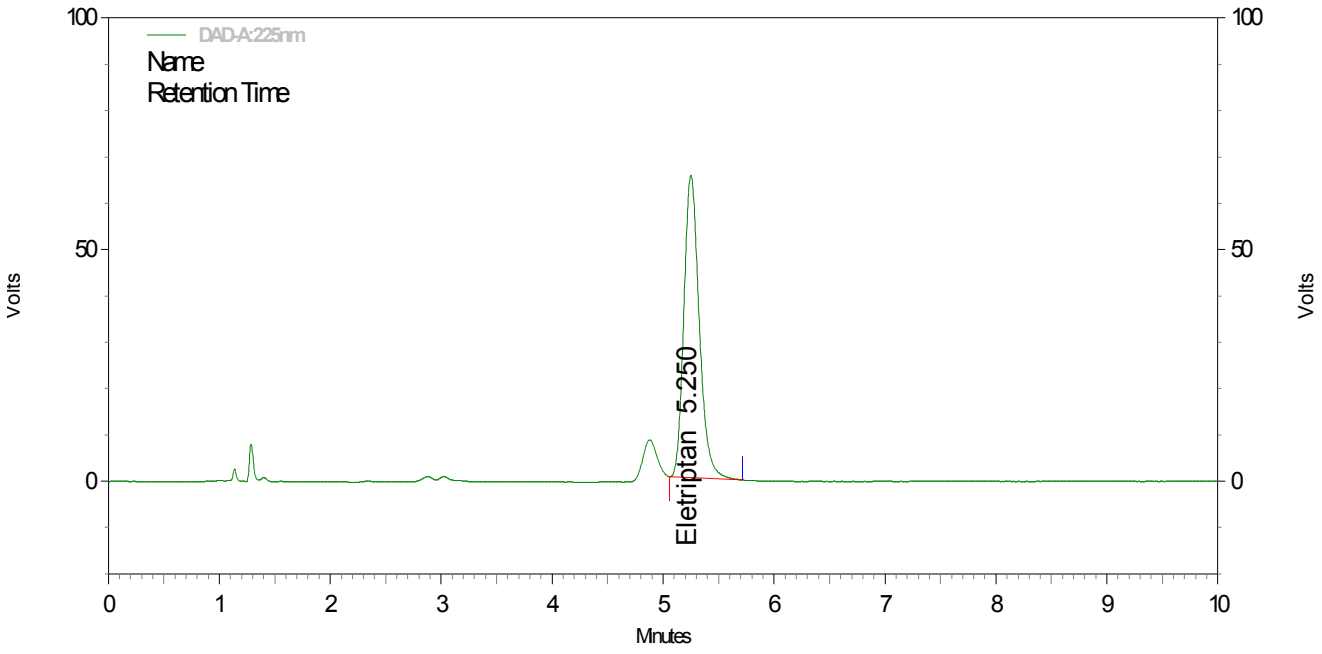


Fig.45 chromatogram of sample of oxidative stress

d) Degradation in UV- light

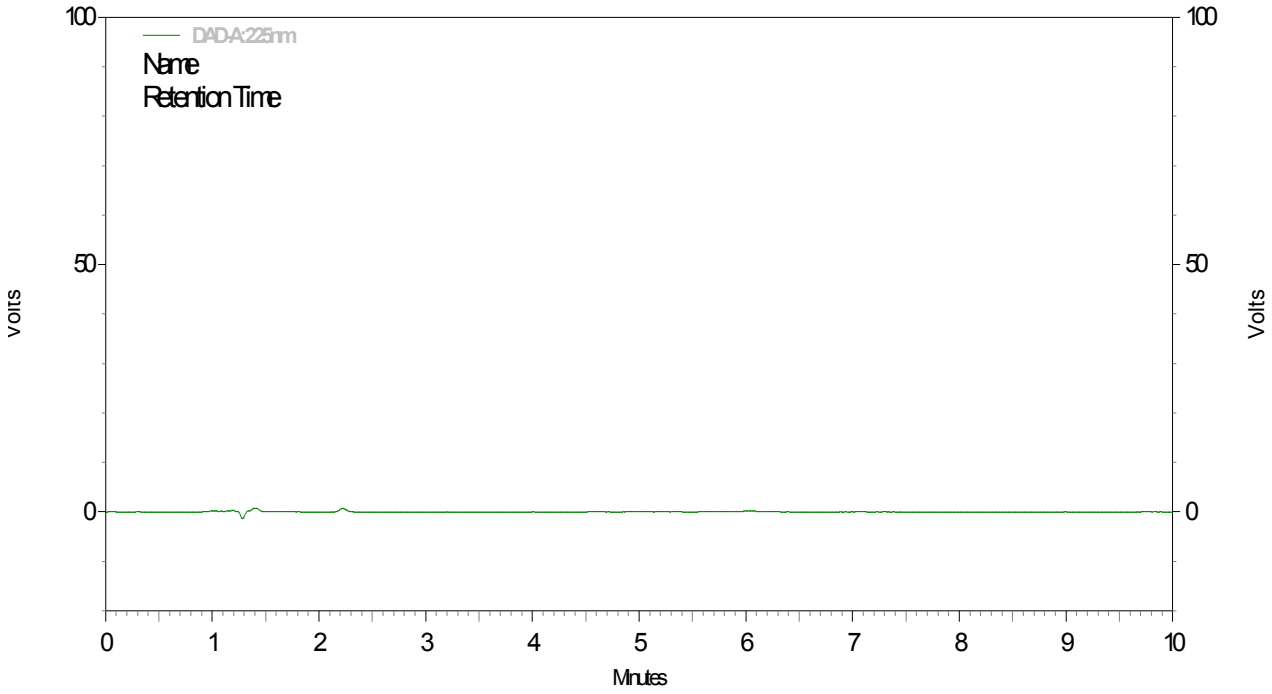


Fig.46 chromatogram of blank of UV- light stress

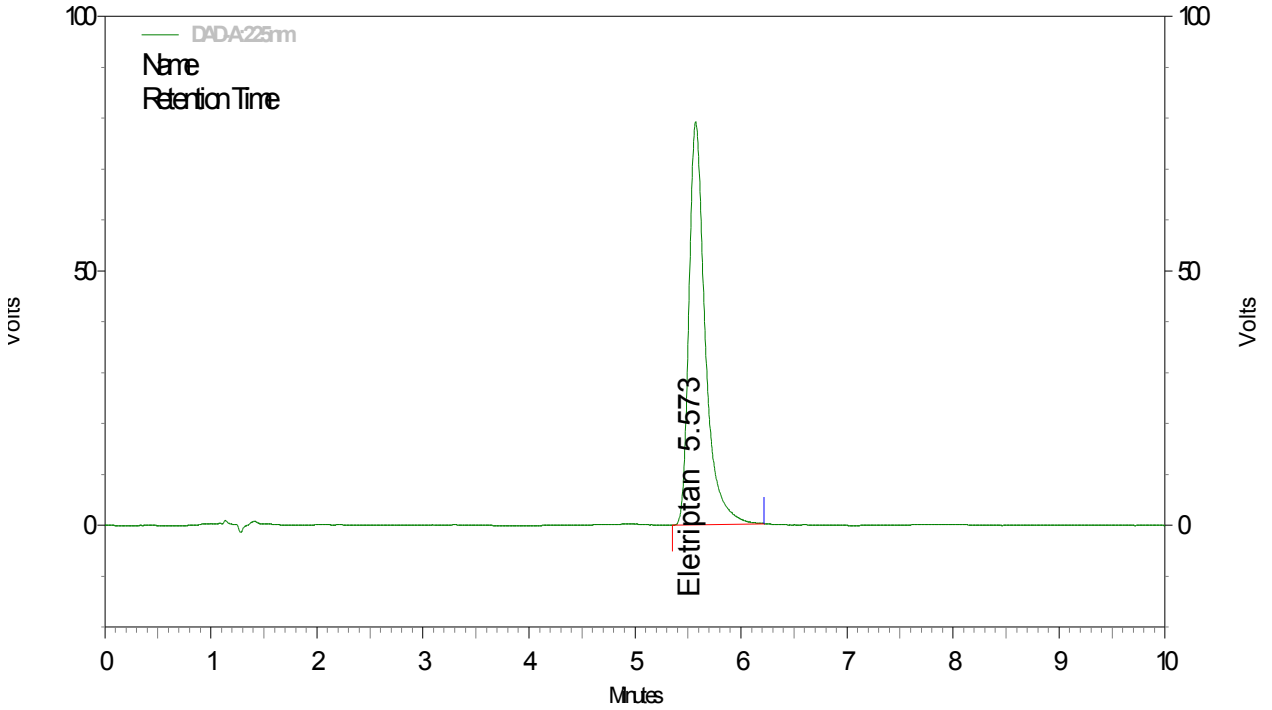


Fig.47 chromatogram of sample of UV-light stress

e) Thermal Degradation

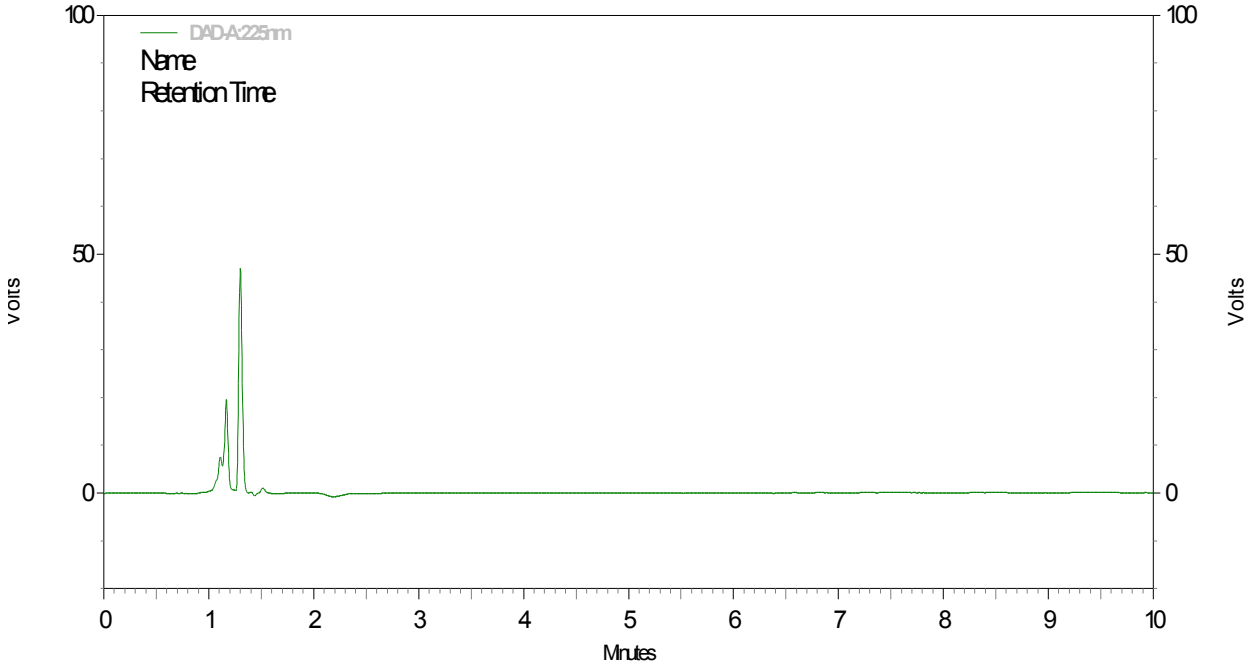


Fig.48 chromatogram of blank o thermal stress

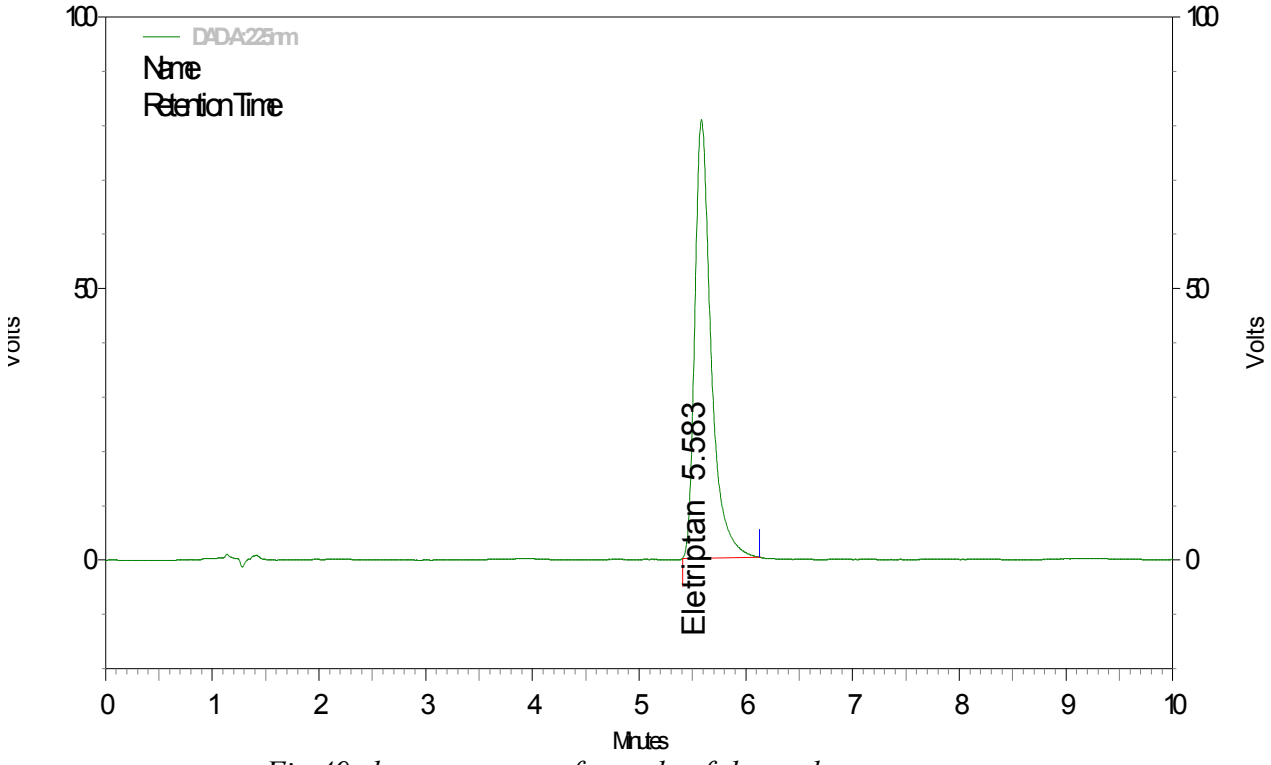


Fig.49 chromatogram of sample of thermal stress

Table: 37 Stress degradation of Eletriptan Hydrobromide at various mechanisms

S.No	mechanism	condition	% Recovery	% Degradation	Peak purity
1.	-	Control sample	98.8	-	1.00
2.	5N acid degradation	5N HCL/60^{0}C/1hr	97.7	1.1	1.00
3.	5N base degradation	5N NaOH/60^{0}C/1hr	96.2	2.6	1.00
4.	1% peroxide degradation	1% peroxide/60 min on bench top	75.9	22.9	1.00
5.	-	Control sample	101.4	-	1.00
6.	Photolytic/UV degradation	UV-254 nm/3 days	101.4	NIL	0.99
7.	Thermal degradation	105^{0}C/ 3 days	101.0	0.4	1.00

Discussion

Eletriptan Hydrobromideis highly stable molecule. It doesn't show any degradation in acidic hydrolysis, alkaline hydrolysis, thermal degradation (105ºC) and under UV-light at room temperature. But Eletriptan Hydrobromideshowed high liability to oxidative degradation by hydrogen peroxide at room temperature. It decomposed to an extent of 22.9%.

6. SUMMARY &CONCLUSION

The active pharmaceutical agent Eletriptan Hydrobromide was selected for present work, which was act as essential therapeutic agent in the treatment of migraine head ache. Even though various analytical techniques in estimation and quantification of Eletriptan Hydrobromide, HPLC method is an emerging technique reliable in vast areas of research.

Stability indicating RP-HPLC assay method for the estimation of Eletriptan Hydrobromide in marketed formulation was developed in present work. The forced degradation HPLC method was developed with mobile phase composition phosphate buffer p^H 7.0 and acetonitrile in the ratio 98:2 v/v as mobile phase A, acetonitrile and methanol in the ratio 80:20 v/v as mobile phase B. Mobile phase ratio A: B (55:45), flow rate of 1.0ml/min was used on Waters X-terra, RP18, 250 x 4.6 mm, 5µ (or) equivalent column. The retention time of Eletriptan Hydrobromide was found at 5.06 min.

The developed method was validated for all validation parameters as per ICH guidelines. The linearity range for Ele.HBr was found 25% to 150%, with regression value of 0.9999. The %RSD values for precision studies were found below 2%. The method has been validated in assay of tablet dosage form. The method was also meets the specifications for the robustness studies.

The results of validation studies demonstrated that this HPLC method is simple, specific, rapid, reliable and reproducible.

The stability study on Eletriptan Hydrobromide tablet formulation. The method is specific and unaffected by presence of degradants from stress degradation.

Table: Summary of method validation parameter results

Parameter		**For Eletriptan hydrobromide**	**Acceptance criteria**
Retention time	min	5.09 min	_
Linearity	µg/ml	25 - 150 µg/ml	R^2 = 0.9999
Accuracy	150 µg/ml	100.1	98 – 102%
Precision	Meth.precision Int.precision	0.24 0.25	< 2%
Assay	40 mg strength	100.6	95 -105%
Robustness flow	0.9ml 1.1ml	0.13 0.24	Robust
Robustness wavelength	223nm 227nm	0.11 0.18	Robust
Robustness column temperature	20ºc 30ºc	1.01 0.72	Robust
Robustness Buffer P^H	P^H 6.8 P^H 7.2	0.43 0.81	Robust

Robustness Change in organic solvent ratio in M.P	-10% +10%	0.33 0.14	Robust

Table: Summary of forced degradation report

S.No	**Condition**	**Exposure**	**Results**	**Degradants found**
1.	Acid degradation	5N HCL at 60ºC for 1 hour	No degradation	_
2.	Base degradation	5N NaOH at room temperature for 1 hour	No degradation	_
3.	Oxidative degradation	1% H_2O_2 at room temperature for 60 min on bench top	Degraded	1
4.	Thermal degradation	In hot air oven at 105ºC temperature for 3 days	No degradation	_
5.	UV-light degradation	In UV-cabinet for 3 days	No degradation	_

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