DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF NARATRIPTAN HYDROCHLORIDE TABLET

Dissertation

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CERTIFICATE

This is to certify that the dissertation entitled **"DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF NARATRIPTAN HYDROCHLORIDE TABLET"** submitted by **Mr. P. MALLIKHARJUNA RAO (Reg. No.26101725)** in partial fulfillment of the degree of Master of Pharmacy in Pharmaceutical Analysis, at **K. M. COLLEGE OF PHARMACY,** MADURAI-625107 under **The Tamilnadu Dr. M. G. R. Medical University**, Chennai.

It is a bonafied work carried out by him under my guidance and supervision during the academic year **2011 - 2012**. This dissertation partially or fully has not been submitted for any other degree or diploma of this university or any other universities.

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GENERAL INTRODUCTION

Introduction: Analytical chemistry

Analytical chemistry [1] is the science to analyze morphologies, compositions and quantities of analytical targets. These analytical results have played critical roles from the understanding of basic science to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing and forensic science.

Modern analytical chemistry is dominated by instrumental analysis. There are so many different types of instruments today that it can seem like a confusing array of acronyms rather than a unified field of study. Analytical chemistry plays an increasingly important role in the pharmaceutical industry where, aside from QA, it is used in discovery of new drug candidates and in clinical applications where understanding the interactions between the drug and the patient are critical.

- **Types**

Traditionally, analytical chemistry has been split into two main types, qualitative and quantitative.

- **Qualitative**

  Qualitative inorganic analysis seeks to establish the presence of a given element or inorganic compound in a sample or the functional group present in a organic compound

- **Quantitative**

  Quantitative analysis seeks to establish the amount of a given element or compound in a sample.

  Analytical chemistry research is largely driven by performance (sensitivity, selectivity, robustness, linear range, accuracy, precision and speed) and cost (purchase, operation, training, time and space).

Classification of analytical methods [2]:

Generally analytical methods are classified into:

(a) Chemical analysis

(b) Instrumental methods

(a) Chemical analysis:

These methods depend upon quantitative performance of a suitable chemical reaction and either measuring the amount of reagents needed to complete the reaction or ascertaining the amount of reaction product obtained e.g. titrimetric (acid base titration, oxidation- reduction titration , non-aqueous titration, complex formation), gravimetric and volumetric methods.

(b) Instrumental methods:

These methods are based up on the measurement of physical properties of a substance such as electrical, optical and absorption and to correlate them for determination of concentration of analyte. These properties are being explained for development of analytical methods such as spectrophotometry, HPLC, GLC and polarography etc. Now a days instrumental method of analysis are widely accepted over the classical methods. These methods are extremely sensitive providing precise and detailed information from small sample materials.

Depending upon the nature and type of material either single or in combination, an appropriate method of analysis is adopted. Instrumental methods are usually much faster than chemical methods and are applicable at concentration far too small to be amenable to determination by chemical methods and find wide application in industry.

Advantages of instrumental methods:

- Small sample can be used.
- High sensitivity is obtained.
- Measurements obtained are reliable.
- The determination is very fast.

- Even complex sample can be handled easily.

CHROMATOGRAPHY [3]

Introduction:

Chromatography **(**from Greek: khromatos- colour and graphos- write) is the collective term for a family of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated.

Chromatography may be preparative or analytical. Preparative chromatography is helpful to separate the components of a mixture for further use. Analytical chromatography normally uses smaller amounts of material and seeks to measure the relative proportions of analytes in a mixture.

Chromatography techniques [4]:

Recently, the IUPAC has defined chromatography as;

"A method used primarily for the separation of the components of a sample, in which the components are distributed between two phases, one of which is stationary while other moves. The stationary phase may be a solid or a liquid supported on a solid or a gel and may be packed in a column, spread as a layer or distributed as a film. The mobile phase may be gaseous or liquid.

Classification of chromatography techniques:

1) According to the nature of stationary and mobile phase

- Gas solid chromatography
- Gas liquid chromatography
- Liquid solid chromatography
- Liquid liquid chromatography

2) According to mechanisms of separation, chromatographic methods are divided in to following general area

- Adsorption chromatography
- Partition chromatography
- Size exclusion chromatography
- Ion exchange chromatography

In adsorption chromatography, the analytes interact with solid stationary surface and are displaced with the eluent for active sites on surface.

Partition chromatography, results from a thermodynamic distribution between two liquid (or liquid like) phase. On the basis of relative polarities of stationary and mobile phase. Partition chromatography can be divided in to **normal phase and reverse-phase chromatography.**

In normal phase chromatography, the stationary bed is strongly polar in nature (e.g., silica gel) and the mobile phase is non polar (such as n-hexane or tetrahydrofuran)

Polar samples are thus retained on the polar surface of the column packing longer than less polar materials while in **reversed-phase chromatography**, the stationary bed is non polar (hydrophobic) in nature, while the mobile phase is polar liquid, such as mixtures of water and methanol or acetonitrile. Here the more non polar the materials is, the longer it will be retained.

Size exclusion chromatography involves a solid stationary phase with controlled pore size. Solutes are separated according to molecular size, with the large molecules unable to enter the pores elute first.

Ion–exchange chromatography, involves a solid stationary phase with anionic or cationic groups on the surface to which solute molecules of opposite charges are attracted.

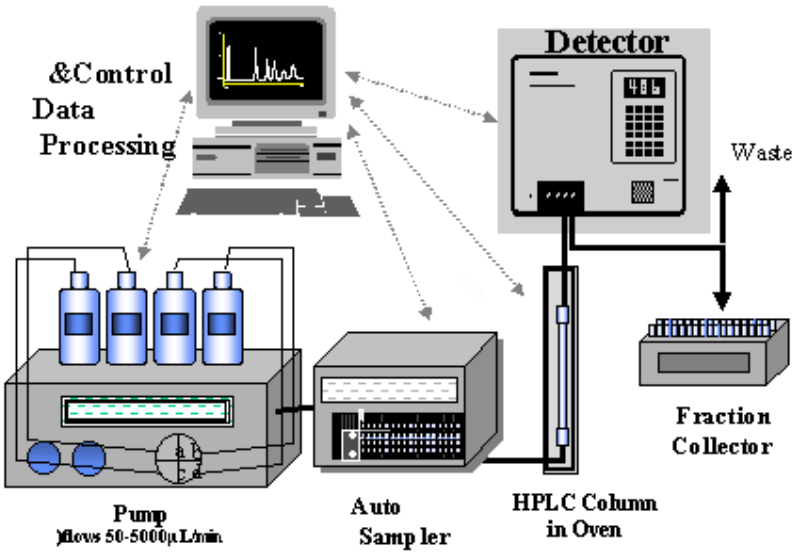
In chromatographic separation, HPLC and HPTLC methods have widely been exploited in pharmaceutical analysis because of its simplicity, precision, accuracy and reproducibility of results.

High performance liquid chromatography (HPLC) [5]:

The typical HPLC separation is based on the selective distribution of analytes between a liquid mobile phase and an immiscible stationary phase. The sample is first introduced by means of an injection port in to the mobile phase stream that is delivered by a high pressure pump. Next, the components of this sample mixture are separated on the column, a process monitored with a flow through detector as the isolated components emerge from the column.

The HPLC is classified in to two modes depending on the relative polarity of the two phases, **normal and reverse-phase chromatography.** These are two elution types in HPLC they are isocratic and gradient. In **isocratic** elution, composition of solvents is pumped through the column during complete analysis is unchanged. In **gradient** system, eluent composition and strength is steadily changed during the run.

INSTRUMENTATION [6]



Typical diagram of HPLC

The individual components HPLC and their working functions are described below.

SOLVENT DELIVERY SYSTEM

The mobile phase is pumped under pressure from one or several reservoirs and flows through the column at a constant rate. With micro particulate packing, there is a high-pressure drop across a chromatography column. Eluting power of the mobile phase is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components. For normal phase separations, eluting power increases with increasing polarity of the solvent but for reversed phase separations, eluting power decreases with increasing solvent polarity. Optimum separating conditions can be achieved by making use of mixture of two solvents. Some other properties of the solvents, which need to be considered for a successful separation, are boiling point, viscosity, detector compatibility, flammability and toxicity.

The most important component of HPLC in solvent delivery system is the pump, because its performance directly effects the retention time, reproducibility and detector sensitivity. Among the several solvent delivery systems, (direct gas pressure, pneumatic intensifier, reciprocating etc.) reciprocating pump with twin or triple pistons is widely used, as this system gives less baseline noise, good flow rate reproducibility etc.

MOBILE PHASE

Mobile phase used for HPLC typically are mixtures of organic solvents and water or aqueous buffers. Table given below lists the physical properties of organic solvents commonly used for HPLC. Isocratic methods are preferable to gradient methods. Gradient methods will sometimes be required when the molecules being separated have vastly different partitioning properties. When a gradient elution method is used, care must be taken to ensure that all solvents are miscible.

The following points should also be considered when choosing a mobile phase:

1. It is essential to establish that the drug is stable in the mobile phase for at least the duration of the analysis.

2. Excessive salt concentrations should be avoided. High salt concentrations can result in precipitation, which can damage HPLC equipment.

3. The mobile phase should have a pH between 2.5 and pH 7.0 to maximize the lifetime of the column.

4. The cost and toxicity of the mobile phase can be reduced by using methanol instead of acetonitrile.

5. Chemicals such a trifluoroacetic acid, acetic acid or formic acid should be minimized and which may prevent detection of products without chromophores at 220 nm. Carboxylic acid modifiers can be frequently replaced by phosphoric acid, which does not absorb above 200 nm.

6. Volatile mobile phases are presented to facilitate collection of products and in LC-MS analysis. Volatile mobile phases include ammonium acetate, ammonium phosphate, formic acid, acetic acid and trifluoroacetic acid.

Ionizable compounds in some cases can present some problems when analyzed by reverse phase chromatography. Two modifications of the mobile phase can be useful in reverse phase HPLC for ionizable compounds. One is called ion suppression and other is ion pairing chromatography. In both techniques, a buffer is used to ensure that the pH of the solution is constant and usually at least 1.5 pH units from a pKa of the drug to ensure that one form of the drug predominates. In ion suppression chromatography, the pH of the aqueous portion of the mobile phase is adjusted to allow the neutral form of the drug to predominate. This ensures that the drug is persistent in only one form and results in improvement of the peak shape and consistency of retention times. In ion pairing chromatography, the pH of the mobile phase is adjusted so that the drug is completely ionized. If necessary to improve peak shape or lengthen retention time, an alkyl sulfonic acid salt or bulky anion such as trifluoroacetic acid is added to the ion pair to cationic drugs or a quaternary alkyl ammonium salt is added to ion-pair to anionic drugs. Ion pairing chromatography also allows the simultaneous analysis of both neutral and charged compounds

SOLVENT DEGASSING SYSTEM

The constituents of the mobile phase should be degassed and filtered before use. Several methods are employed to remove the dissolved gases in the mobile phase. They

include heating and stirring, vacuum degassing with an aspirator, filtration through 0.45 µ filter, vacuum degassing, helium purging, ultra sonification or purging or combination of these methods. HPLC systems are also provided an online degassing system, which continuously removes the dissolved gases from the mobile phase.

GRADIENT ELUTION DEVICES

HPLC columns may be run isocratically, i.e., with constant eluent or they may be run in the gradient elution mode in which the mobile phase composition varies during run. Gradient elution over comes the problem of dealing with a complex mixture of solutes.

COLUMNS

The heart of the system is the column. The choice of common packing material and mobile phases depends on the physical properties of the drug. Many different reverse phase columns will provide excellent specificity for any particular separation. It is therefore best to routinely attempt separations with a standard C_8 or C_{18} column and determine if it provides good separations. If this column does not provide good separation or the mobile phase is unsatisfactory, alternate methods or columns should be explored. Reverse phase columns differ by the carbon chain length, degree of end capping and percent carbon loading. Diol, cyano and amino groups can also be used for reverse phase chromatography.

SAMPLE INTRODUCTION SYSTEM

Two means for analyte introduction on the column are injection into a flowing stream and a stop flow injection. These techniques can be used with a syringe or an injection valve. Automatic injector is a microprocessor-controlled version of the manual universal injector. Usually, up to 100 samples can be loaded in to the auto injector tray. The system parameters such as flow rates, gradient, run time, volume to be injected, etc. are chosen, stored in memory and sequentially executed on consecutive injections.

LIQUID CHROMATOGRAPHIC DETECTORS

The function of the detector in HPLC is to monitor the mobile phase as it emerges from the column. Generally, there are two types of HPLC detectors, bulk property detectors and solute property detectors.

a. **Bulk property detectors**: These detectors are based on differential measurement of a property, which is common to both the sample and the mobile phase. Examples of such detectors are refractive index, conductivity and dielectric constant detectors.

b. **Solute property detectors**: Solute property detectors respond to a physical property of the solute, which is not exhibited by the pure mobile phase. These detectors measure a property, which is specific to the sample, either with or without the removal of the mobile phase before the detection. Solute property detectors which do not require the removal of the mobile phase before detection include spectrophotometric (UV or UV-Visible) detector, fluorescence detectors, polarographic, electro-chemical and radio activity detectors, where as flame ionization detector and electron capture detector both require removal of the mobile phase before detection.

UV-Visible and fluorescent detectors are suitable for gradient elution, because many solvents used in HPLC do not absorb to any significant extent.

TEMPERATURE

Room temperature is the first choice. Elevated temperatures are sometimes used to reduce column pressure or enhance selectivity. Typically, temperatures in excess of 60^0C are not used.

RETENTION TIME

Due to a number of samples assayed in the course of preformulation study, it is advisable to have as short a retention time as possible. However, the retention time should be sufficiently long enough to ensure selectivity. When choosing the optimum mobile phase, considerations should be given to the retention time of degradation products, so that these compounds do not elute in the solvent front or remain in the column.

HPLC METHOD DEVELOPMENT [7]

A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modeling. During initial method development, a set of initial conditions (detector, column,

mobile phase) is selected to obtain the first "scouting" chromatograms of the sample. In most cases, these are based on reversed-phase separations on a C_{18} column with UV detection.

The important factors, which are to be taken into account to obtain reliable quantitative analysis, are

1. Careful sampling and sample preparation.
2. Appropriate choice of the column.
3. Choice of the operating conditions to obtain the adequate resolution of the mixture.
4. Reliable performance of the recording and data handling systems.
5. Suitable integration/peak height measurement technique.
6. The mode of calculation best suited for the purpose.
7. Validation of the developed method.

SAMPLE PREPARATION

Samples comes in various forms

- Solutions ready for injection
- Solutions that require dilution, buffering, addition of an internal standard or other volumetric manipulation
- Solids must be dissolved or extracted
- Samples that require pretreatment to remove interferences and/or protect the column or equipment from damage.

Most samples for HPLC analysis require weighing and /or volumetric dilution before injection. Best results are often obtained when the composition of the sample solvent is close to that of the mobile phase since this minimizes baseline upset and other problems.

Some samples require a partial separation (pre-treatment) prior to HPLC, because of the need to remove interferences, concentrate sample analytes or eliminate "column killers".

In many cases the development of an adequate sample pre-treatment can be challenging for achieving a good HPLC separation. The samples may be of two types, regular or special.

The regular samples are typical mixtures of small molecules (<2000Da) that can be separated by normal starting conditions.

CHOICE OF THE COLUMN [8]

Selection of the column is the first and the most important step in method development. The appropriate choice of separation column indicates three different approaches.

- Selection of separation.
- The particle size and nature of the column packing.
- The physical parameters of the column i.e. the length and the diameter.

Some of the important parameters considered while selecting chromatographic columns are

- Length and diameter of the column
- Packing material
- Shape of the particles
- Size of the particles
- % of Carbon loading
- Pore volume
- Surface area
- End capping

In this case, the column selected had a particle size of 5 µm and an internal diameter of 4.6 mm. The column is selected depending on the nature of the solute and the information about the analyte. Reversed phase mode of chromatography facilities a wide range of columns covering wide range of polarities by cross linking silanol groups with alkyl chains like like dimethyl silane (C_2), butylsilane (C_4), octylsilane(C_8), octadecylsilane (C_{18}),base deactivated silane (C_{18}), phenyl, cyanoproply (CN), nitro, amino etc. Silica based columns with different cross linkings in the increasing order of Polarity are as follows:

<……. Non-polar …… moderately polar……. Polar. ……>

C_{18} < C_8 < C_6 < Phenyl < Amino < Cyano<nitrile < Silica

C_{18} was choosen for this study since it is most retentive one. The sample manipulation becomes easier with this type of column.

Generally longer column as provide better separation due to higher theoretical plate numbers. As the particle size decreases the surface area available for coating increases. Columns with 5 µm particle size give the best compromise of efficiency, reproducibility and reliability. In this case, the column selected had a particle size of 5 µm and an internal diameter of 4.6 mm peak shape is equally important in method development. Columns that provide symmetrical peaks are always preferred while peaks with poor asymmetry can result in,

- In accurate plate number and resolution measurement
- Imprecise quantitation
- Degraded and undetected minor bands in the peak tail
- Poor retention reproducibility

A useful and practical measurement of peak shape is peak asymmetry factor and peak tailing factor. Peak asymmetry is measured at 10 % of full peak height and peak tailing factor at 5 %. Reproducibility of retention times and capacity factor is important for developing a rugged and repeatable method.

A column which gives separation of all the impurities and degradants from each other and from analyte peak and which is rugged for variation in mobile phase shall be selected.

SELECTION OF COLUMN TEMPERATURE

Chromatographic separations are carried out at ambient temperature. The increase in column temperature generally will result in reduction of asymmetry and peak retention. The column temperature between 30°c – 80°c is shall be adopted if necessary. If a column temperature above 80°c is required, packing material which can with stand to that temperature was preferable.

SELECTION OF SOLVENT DELIVERY SYSTEM

Chromatographic separation with isocratic elution is always preferable. However, gradient elution is powerful tool in achieving separation between closely eluting compounds or compounds with highly differing polarities.

The important future of gradient elution is that the polarity and ionic strength of the mobile phase can be changed during run. The mobile phases are introduced into column by

two different ways, low pressure and high pressure gradient systems. Low pressure gradient can be adopted when not more than 80% of organic phase is to be pumped or vice-versa. While optimizing gradient elution especially low viscous solvents like acetonitrile and phosphate buffer, it is recommended to mix about 10% aqueous portion preferably the same buffer used in mobile phase to avoid pumping problems.

The gradient programme which is rugged for organic phase up to 10% variation and up to 0.2 ml variation in flow rate was selected.

SELECTION OF MOBILE PHASE

The primary objective in selection and optimization of mobile phase is to achieve optimum separation of all impurities and degradants from each other and from analyte peak.

In liquid chromatography, the solute retention is governed by the solute distribution factor, which reflects the different interactions of the solute-stationary phase, solute-mobile phase, and mobile phase-stationary phase. For a given stationary phase, the nature and the composition of which has to be judiciously selected in order to get appropriate and required solute retention. The mobile phase has to be adapted in terms of elution strength (solute retention) and solvent selectivity (solute separation). Solvent polarity is the key word in chromatographic separations since a polar mobile phase will give rise to low solute retention in normal phase and high solute retention in reverse phase LC. The selectivity will be particularly altered if the buffer pH is close to the pKa of the analytes. The following are the parameters, which shall be taken into consideration while selecting and optimizing the mobile phase.

- Buffer and its strength
- pH of the buffer or pH of the mobile phase
- Mobile phase composition

pH of the buffer:

pH plays an important role in achieving the chromatographic separations as it controls the elution properties by controlling the ionization characteristics. Experiments were conducted using buffers having different pH to obtain the required separations.

It is important to maintain the pH of the mobile phase in the range of 2.0 to 8.0 as most columns does not withstand to the pH which are outside this range. This is due to the fact that the siloxane linkage area cleaved below pH 2.0 while pH valued above 8.0 silica may dissolve.

D. MOBILE PHASE COMPOSITION

Most chromatographic separations can be achieved by choosing the optimum mobile phase composition. This is due to the fact that fairly large amount of selectivity can be achieved by choosing the qualitative and quantitative composition of aqueous and organic portions. Most widely used solvents in reverse phase chromatography are methanol and acetonitrile. Experiments should be conducted with mobile phases having buffers with different pH and different organic phases to check for the best separations of analyte peak. A mobile phase which gives separation of analyte peak and which is rugged for variation of both aqueous and organic phase by at least ± 0.2% of the selected mobile phase composition is used.

SELECTION OF FLOW RATE

Generally flow rate shall not be more than 2.0 ml/min. the flow rate shall be selected based on the following data.

- Retention time
- Column back pressure
- Resolution between the peaks
- Peak symmetries

The flow rate which gives least retention times, good peak symmetries, least back pressures and better separation will be selected.

SELECTION OF DETECTOR

The detector was chosen depending upon some characteristic property of the analyte like UV absorbance, florescence, conductance, oxidation, reduction etc. The characteristics that are to be fulfilled by a detector to be used in HPLC determination are,

- High sensitivity facilitating trace analysis

- Negligible baseline noise to facilitate lower detection
- Large linear dynamic range
- Low dead volume
- Ease in calibration and standardization
- Inexpensive to purchase and operate

Pharmaceutical ingredients do not absorb UV light equally, so that selection of detection wavelength is important. An understanding of the UV light absorptive properties of the organic impurities and the active pharmaceutical ingredient is very helpful. Generally LC equipped with PDA detector was the first choice. UV source like mercury vapour lamp is most widely used because majority of the compounds of pharmaceutical interest absorb at 250 – 270 nm and this lamp has an intense line spectrum at this region.

For the greatest sensitivity λ_{max} should be used. Ultra violet wavelengths below 200nm should be avoided because detector noise increases in this region. Higher wavelengths give greater selectivity.

OPTIMIZATION OF HPLC METHOD

During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape, plate counts, asymmetry, capacity factor, elution time, detection limits, limit of quantitation and overall ability to quantify the specific analyte of interest.

Optimization of a method can follow either of two general approaches:

- Manual
- Computer driven

The manual approach involves varying one experimental variable at a time, while holding all other constant and recording changes in response. The variables might include flow rate, mobile or stationary phase composition, temperature, detection wavelength and P^H. This univariate approach to system is slow, time consuming and potentially expensive. However, it may provide a much better understanding of the principles and theory involved and of interactions of the variables.

In the second approach, computer driven automated method development, efficiency is optimized while experimental input is minimized. Computer driven automated approaches

can be applied to many applications. In addition, they are capable of significantly reducing the time, energy and cost of all instrumental method development.

PERFORMANCE CALCULATIONS:

Carrying out system suitability experiment does the performance calculations. System suitability experiments can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validations have been completed.

The criteria selected will be based on the actual performance of the method as determined during its validation. For example, if sample retention times form part of the system suitability criteria, their variation SD can be determined during validation.

System suitability might then require that retention times fall within a ±3 SD range during routine performance of the method.

The RSD of peak height or area of five injections of a standard solution is normally accepted as one of the standard criteria. For assay method of a major component, the RSD should typically be less than 1% for these five respective injections.

The plate number and/ or tailing factor are used if the run contains only one peak. For chromatographic separations with more than one peak, such as an internal standard assay or an impurity method expected to contain many peaks, some measure of separations such as R_S is recommended. Reproducibility of t_R or k value for a specific compound also defines system performance.

A. COLUMN EFFICIENCY& PLATE NUMBER

The number of theoretical plates or plate number is a measure of column efficiency. An efficient column produces sharp peaks and can separate many sample components in a relatively short time. Theoretical plates (N) are defined as the square of the ratio of the retention time divided by the standard deviation of the peak (σ).

$$N = \left(\frac{t_R}{\sigma}\right)^2 = \left(\frac{4t_R}{w_b}\right)^2 = 16\left(\frac{t_R}{w_b}\right)^2.$$

Another way to express efficiency of column is by calculating height equivalents of theoretical plates (HETP).

$$\mathbf{h = L/n}$$

Where h = HETP; L = Length of the column; n = number of theoretical plates.

Lower the HETP, higher is the efficiency of the column, i.e., higher the theoretical plates more efficient the column.

B. RESOLUTION

The goal of most HPLC analyses is the separation of one or more analytes in the sample from all other components present. Resolution (Rs) is a measure of the degree of separation of two adjacent analytes. Rs is defined as the difference in retention times of the two peaks divided by the average peak width. The resolution of two adjacent peaks can be calculated by using the formula

$$R_s = \frac{t_{R2} - t_{R1}}{\left(\frac{w_{b1} + w_{b2}}{2}\right)} = \frac{\Delta t_R}{w_b}$$

Where t_1 and t_2 are retention times of the adjacent peaks and

$W_{0.5,1}$ and $W_{0.5,2}$ are the width of the peaks at half height.

R_S = 2.0 or greater is a desirable target for method development.

- The retention factor k is given by the equation

$$\mathbf{k = (t_R - t_0) / t_0}$$

Where t_R is the band retention time and t_0 is the column dead time.

- The peak symmetry can be represented in terms of peak asymmetry factor and peak tailing factor which can be calculated by using the following formulae

Peak asymmetry factor = B/A

Where 'B' is the distance at 50% peak height between leading edge to the perpendicular drawn from the peak maxima and 'A' is the width of the peak at half the peak height.

According to USP (2000) Peak-tailing factor can be calculated by using the formula

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

Where $W_{0.05}$ is the width of the peak at 5% height and f is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 50% of the peak height from the base line.

ANALYTICAL METHOD VALIDATION [9, 10,11]

Method validation can be defined as per ICH "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

Method validation is an integral part of the method development; it is the process by which a method is tested by the developer or user for reliability, accuracy and precision of its intended purpose and demonstrating that analytical procedures are suitable for their intended use that they support the identity, quality, purity, and potency of the drug substances and drug products. Data thus generated become part of the method validation package submitted to Center for Drug Evaluation and Research (CDER). Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose.

Data that are generated for acceptance, release, stability or pharmacokinetic will only be trustworthy if the methods used to generate the data are reliable. The process of validation and method design also should be clearly in the development cycle before important data are generated. Validation should be on going in the form of re-validation with method changes.

Though many types of HPLC techniques are available, the most commonly used method, the reversed-phase HPLC with UV detection, is selected to illustrate the parameters for validation. The criteria for the validation of this technique can be extrapolated to other detection methods and chromatographic techniques. For acceptance, release or stability testing, accuracy should be optimized since the need to show deviation from the actual or true value is of the greatest concern.

All the variables of the method should be considered, including sampling procedure, sample preparation, chromatographic separation, and detection and data evaluation. For chromatographic methods used in analytical applications there is more consistency in validation practice. The Q (2) R1 of ICH guidelines for validation for analytical procedures includes the following parameters.

Method validation:

This process consists of establishments of the performance characteristics and the limitation of the method.

Method performance parameters are determined using equipment that is

1. Within specification
2. Working correctly
3. Adequately calibrated

Method validation is required when:

- A new method is being developed.
- Revision of the established method.
- When established method are used in different laboratories and by different analysts etc.
- Comparison of methods.
- When quality control indicates method changes.

Performance characteristics examined when carrying out method validation are:

1. Accuracy/precision
2. Repeatability/Reproducibility
3. Linearity /Range
4. Limit of detection (LOD)/Limit of quantification
5. Selectivity/Specificity
6. Robustness/Ruggedness

A) Accuracy:

The accuracy of an analytical procedure express the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found.

Determination of accuracy:

The accuracy may be determined by application of analytical method to an analyte of known purity (example: reference standard) and also by comparing the results of the method with those obtained using an alternate procedure that has been already validated.

Accuracy is calculated as the percentage of recovery by the assay of the known added amount of the analyte in the sample or the difference between the mean and accepted true value together with confidence intervals.

The ICH guidelines recommended to take minimum of 3 concentration levels covering the specified range and 3 replicate of each concentration are analysed (totally 3×3=9 determination).

B) Precision:

The precision of an analytical procedure express the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of analytical procedure is usually expressed the variance, standard deviation or co-efficient of variation of a series of measurements.

System precision:

A system precision was evaluated by measuring the peak response of naratriptan for six replicate injection of the standard solution prepared as per the proposed method.

Method precision:

The method precision was determined by preparing the sample of a single batch of naratriptan Hcl tablet formulation six times and analyzing as per the proposed method.

Determination of precision:

The procedure is applied repeatedly to separate identical sample drawn from the homogeneous batch of material and measured by the scatter of individual results from the mean and expressed as the standard deviation or as the co-efficient of variation (relative standard deviation).

C) Specificity:

ICH document divides specificity in to two categories.

- Identification tests
- Assay / impurity tests

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradeants, matrix, etc. lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

Identification test:

It is demonstrated by the ability to discriminate between compounds of closely related structures or by comparison to known reference materials. Use of positive and negative control is recommended.

Assay impurity test:

It is demonstrated by resolution of the two closest eluting compounds. If impurities are available it has to be shown that the assay is unaffected by the presence of spiked material.If impurities are not available the test results are compared to a second well characterized method.

Determination of specificity:

When chromatographic procedures are used representative chromatograms should be presented to demonstrate the degree of specificity. Samples generated by stress testing of the drug substance using acid and base hydrolysis, temperature, photolysis and mass spectrometry may be useful to show that the chromatographic peak is not attributable to more than one component.

D) Selectivity:

It is a procedure to detect qualitatively the analyte in the presence of compounds that may be expected to be present in the sample matrix or the ability of a separative method to resolve different compounds. It is the measure of the relative method location or two peaks.

Determination of selectivity:

Selectivity is determined by comparing the test results obtained on the analyte with and without addition of potentially interfering material. When such components are either unidentified or un available a measure of selectivity can be obtained by determining the recovery of a standard addition of pure analyte to a material containing a constant level of the other compounds.

E) Sensitivity:

Sensitivity is the capacity of the test procedure to record small variation in concentration. It is the slope of the calibration curve.

F) Limit of detection:

The limit of detections is the lowest concentration of the analyte in a sample that can be detected but not necessarily determined in quantitatively using a specific method under the required experiment conditions such a limit is expressed in terms of a concentration of analyte (example: µg/ml in the sample)

Measurement based on

- Signal to noise ratio
- Visual evaluation (relevent chromatogram acceptable)
- The standard deviation of the response and the slope.

$$LOD = \frac{3.3\ \sigma}{S}$$

Where

σ = The standard deviation of the response

S = The slope of the calibration curve (of the analyte)

G) Limit of quantification:

The LOQ is the lowest concentration of analyte in a sample, which can quantitatively determined that might be measured with an acceptable level of accuracy and precision under the stated operational conditions of the method. LOQ can very with the type of method employed and nature of the sample. Based on the standard deviation of the response and the quantitation limit may expressed as

$$\text{LOQ} = \frac{10\,\sigma}{S}$$

Where

σ = The standard deviation of the response

S = The slope of the calibration curve

Measurement:

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

In case of instrumental methods that exhibit back ground noise the ICH document describes to compare measured signals from samples with known concentration of analyte with those of blank samples. A typically acceptable signal to noise ratio is 10:1.

H) Linearity and range:

Linearity is the ability of the method to obtain test results that are directly proportional to the analyte concentration within a given range.

Range:

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

Measurement:

A range of standards should be prepared containing at least 5 different concentrations of analyte, which are approximately evenly spaced and span 50 -150 % of the label claim.

At least 6 replicates per concentration to be studied. Plot a graph of concentration (on x- axis) Vs mean response (on y- axis). Calculate the regression equation, y- intercept and correlation co-efficient. Plot another graph of concentration (on x-axis) Vs response ratio (replicate response divided by concentration,(on y-axis).

The range of the method is validated by verifying that the analytical method provides acceptable precision, accuracy and linearity when applied to samples containing analyte at the extreme of the range as well as within the rage.

I) Ruggedness:

Ruggedness is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of test conditions such as different laboratories, analyst, instruments, reagent lots, elapsed assay times, temperature, days etc.

It can be expressed as lack influence of the operation and environmental variable on the test results of the analytical method.

Determination:

By analysis of aliquots from homogenous lot in different laboratories by different analyst using different operational and environmental condition that may differ but are still within specified parameter.

J) Robustness:

It is measure of capacity of an assay to remain unaffected by small but deliberate variation in method parameters and provide an indication of its reliability in normal usage degradation and variation in chromatography columns, mobile phase and inadequate method development are common causes of lack of robustness.

Determination of robustness:

Method characteristics are assessed when one or more operating is varied by following certain designs. In case of liquid chromatography, examples of typical variation are

- Influence of variation of pH in a mobile phase
- Influence of variation in mobile phase
- Influence of variation in nm
- Different columns (different lots and suppliers)
- Temperature
- Flow rate

System suitability specification and tests:

It is essential for the assurance of quality performance of chromatographic system. The accuracy and the precision of HPLC data collected, which begins with a well behaved chromatographic system. The system suitability parameter and tests are the parameters that help in achieving this purpose. Suitability parameters are

1. Capacity factor
2. Precision / injection repeatability
3. Relative retention
4. Resolution
5. Tailing factor
6. Theoretical plate number

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as plate count, tailing factors, resolution and reproducibility (%RSD retention time and area for six repetitions) are determined and compared against the specifications set for the method. These parameters are measured during the analysis of a system suitability "sample" that is a mixture of main components and expected by-products. Table.no.1 lists the terms to be measured and their recommended limits obtained from the analysis of the system suitability sample as per current FDA guidelines on "Validation of Chromatographic Methods".

Table-1

System Suitability Parameters and Recommendations

Parameter	**Recommendation**
Capacity Factor (k')	k'>2.0
Repeatability	RSD $\leq$ 1% for N $\geq$ 5 is desirable.
Theoretical Plates (N)	N > 2000
Resolution (R_s)	R_s > 2 between the peak of interest and the closest eluting potential interferent (impurity, excipient, degradation product, internal standard, etc.
Tailing Factor (T)	T $\leq$ 2

DRUG PROFILE

Naratriptan Hcl

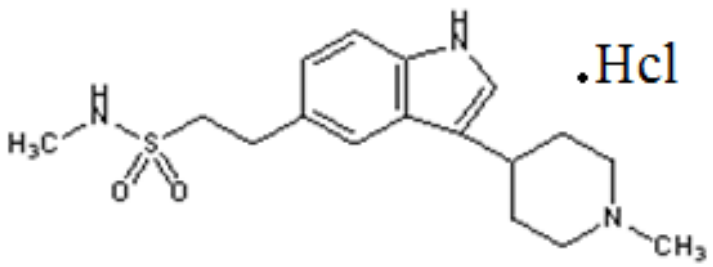
Chemical properties [12]:

IUPAC name: N-methyl-3-(1-methyl-4-piperidinyl)-1H-indole-5-ethanesulfonamide mono hydrochloride**.**

Empirical Formula: $C_{17}H_{25}N_3O_2S$•HCl

Molecular weight: 371.93

Structure:



Naratriptan Hcl

Appearance: white to pale yellow powder

Solubility: Freely soluble in water, soluble in methanol and organic solvents

Category [13a]: Anti-migraine

Mechanism of action [13b]: Naratriptan binds with high affinity to 5-HT_{1D} and 5-HT_{1B} receptors. The therapeutic activity of naratriptan in migraine is generally attributed to its agonist activity at 5-$HT_{1D/1B}$ receptors.

Pharmacokinetics [13b]:

Naratriptan tablets are well absorbed, with about 70% oral bioavailability. Following administration of a 2.5-mg tablet orally, the peak concentrations are obtained in 2 to 3 hours.

After administration of 1- or 2.5-mg tablets, the C_{max} is somewhat (about 50%) higher in women than in men. During a migraine attack, absorption was slower, with a T_{max} of 3 to 4 hours. Food does not affect the pharmacokinetics of naratriptan. Naratriptan displays linear kinetics over the therapeutic dose range.

Naratriptan is predominantly eliminated in urine, with 50% of the dose recovered unchanged and 30% as metabolites in urine. In vitro, naratriptan is metabolized by a wide range of cytochrome P_{450} isoenzymes into a number of inactive metabolites.

The mean elimination half-life of naratriptan is 6 hours. The systemic clearance of naratriptan is 6.6 ml/min/kg. The renal clearance (220 ml/min) exceeds glomerular filtration rate, indicating active tubular secretion. Repeated administration of naratriptan tablets does not result in drug accumulation.

Contraindication [12]:

• Hypersensitivity to drug or its components
• Hemiplegic or basilar headaches
• Severe renal, cardiovascular or hepatic impairment
• History of cerebrovascular or peripheral vascular conditions
• Ischemic bowel disease
• Uncontrolled hypertension
• Use of ergot-type drugs (such as dihydroergotamine) and other 5-HT_1 agonists within 24 hours
• MAO inhibitor use within past 14 days

Dose [13a]: 1 or 2.5 mg as single dose; may be repeated in 4 hours.

Drug interactions [13a]:

- **Drug-drug:** Ergot-type compounds (dihydroergotamine, methysergide): prolonged vasospastic reaction
- Hormonal contraceptives: increased naratriptan blood level and effects
- MAO inhibitors: increased systemic exposure to naratriptan, increased risk of adverse reactions
- Selective serotonin reuptake inhibitors: weakness, hyperreflexia, incoordination

- Sibutramine: serotonin syndrome
- **Drug-herbs:** S-adenosylmethionine (SAM-e), St. John's wort: increased risk of adverse serotonergic effects
- **Drug-behaviours:** Cigarette smoking: increased naratriptan metabolism

Adverse effects [13a]:

- **CNS:** dizziness, drowsiness, malaise, fatigue, paresthesia
- **CV:** coronary artery vasospasm, myocardial infarction, ventricular fibrillation or tachycardia
- **GI:** nausea, vomiting
- **Other:** pain or pressure sensation in throat or neck

REVIEW OF LITERATURE

Sneha. B. et. al[14] **.,** reported a simple, precise, rapid, and reproducible RP -HPLC method and validated for the determination of naratriptan Hydrochloride in pharmaceutical dosage form. Separation was achieved under optimized chromatographic condition on a kromasil C_{18} (ODS) column (250 X 4.6 mm i.d., particle size 5µ). The mobile phase consisted of methanolic phosphate buffer at pH 3.2 and acetonitrile in the ratio of 60: 40 v/v. An isocratic elution at a flow rate of 0.8 ml/ min at ambient temperature was used. The detection was carried out at 223 nm. The retention time of naratriptan was found to be at 3.1 min. The calibration curve was linear in the concentration range of 10–60 µg/ ml.

Madhavi. B. et. al[15] **.,** reported a simple, sensitive, precise and specific reverse phase high performance liquid chromatographic method and validated for the determination of naratriptan hydrochloride in tablet dosage forms. The HPLC separation was carried out by reverse phase chromatography on purospher star C_{18} column (150×4.6mm), 5µm particle size. A mobile phase consisted of 0.01M triethylamine buffer pH 3.0: methanol (80:20%, v/v). Buffer: acetonitrile mixture in the ratio (70:30% v/v) was used as diluent (in isocratic mode at a flow rate of 1.5ml/min). The detection was monitored at 225 nm. The calibration curve for naratriptan hydrochloride was linear from 10 to 80µg/ml. The proposed method had adequate sensitivity, reproducibility and specificity for the determination of naratriptan hydrochloride in tablet dosage forms.

Kuldeep Patel. et. al [16]**.,** reported a novel stability-indicating ultra-performance liquid chromatographic assay method and validated for naratriptan and its degradant products. An isocratic UPLC method was developed to separate the drug from the degradation products, using an acquity UPLC BEH C_{18} (50 mm x 2.1 mm) column and mixture of water: acetonitrile (pH3.4) (60:40) as mobile phase. The flow rate was kept 0.3 ml/ min and the detection was carried out at 224 nm.

Kumara Swamy. G. et. al[17]**.,** reported a simple efficient, precise and accurate spectroscopic method and validated for quantitative estimation of naratriptan hydrochloride in bulk and pharmaceutical dosage form. Naratriptan solution was scanned in the UV range (200-400 nm) in a 10 mm quartz cell in a double beam UV spectrophotometer and the λmax of naratriptan was found to be 223 nm. The method obeyed Beer's law in the concentration range from 2-12 µg/ml.

Balasekhara Reddy Challa. et. al [18]., reported a specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of naratriptan (NTP) in human plasma using naratriptan -d^3 (NPD3) as an internal standard. Chromatographic separation was performed on a Zorbax SB-C_{18}, 75×4.6 mm, 3.5 µm column with an isocratic mobile phase composed of 0.1% formic acid: acetonitrile (50:50 v/v) , at a flow rate of 0.6 ml/min.NTP and NPD3 were detected with proton adducts at m/z 336.5 and 339.4 in selected reaction monitoring (SRM) positive mode, respectively. The liquid-liquid extraction method was used to extract the NTP and NPD3.

Manish Yadav. et. al[19]., reported a simple, sensitive, selective and rapid high performance liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS) method and validated for the quantification of naratriptan, using sumatriptan as internal standard (IS). The method concerned liquid–liquid extraction of naratriptan and IS in methyl-tert-butyl ether and dichloromethane mixture from 100 µl human plasma. The chromatographic separation was achieved on ACE C_{18} (50mm X 2.1mm, 5µm) analytical column under isocratic conditions, using 0.1% acetic acid and acetonitrile (15:85 v/v) at a flow rate of 0.4 ml/min. The parent product ion transitions for naratriptan (m/z 336.10-98.06) and IS (m/z 296.09-251.06) were monitored on a triple quadrupole mass spectrometer, operating in the multiple reaction monitoring (MRM) and positive ion mode. The linearity of the method for naratriptan was ascertained in the range of 103-20690 pg/ml with the analysis time of 1.5 min

Velasco-Aguirre. C. et. al[20]., reported that naratriptan exhibited an anodic response in aqueous media over a broad pH range (pH 2–12), as determined by differential pulse voltammetry and cyclic voltammetry using glassy carbon electrodes. This response was irreversible in nature, diffusion-controlled and probably caused by the oxidation of the naratriptan indole moiety. The differential pulse voltammetry technique was performed in 0.1 mol/min Britton–Robinson buffer (pH 3), for the quantification of naratriptan in formulation.

Duléry. BD. et. al [21]., reported a liquid chromatographic-electrospray-mass spectrometric (LC-ESI-MS) assay to compare the pharmacokinetics of these three antimigraine compounds MDL–74721, sumatriptan and naratriptan. The concentration of each parent drug was determined using a solid-phase extraction and LC-ESI-MS analysis demonstrating the high sensitivity and specificity of the methods down to subnanogram levels in rabbit plasma samples.

Murthy.T.E.G.K. et. al[22]., reported a simple, sensitive, selective, accurate, precise, economical, robust and rapid UV spectrophotometric method and validated for quantification of naratriptan. The influence of three different solvents on estimation of naratriptan from bulk and pharmaceutical dosage forms was studied. The solutions of naratriptan were prepared with the water and the solvent blends containing water: acetonitrile (1:1) and water: methanol (1:1). The solutions were scanned within the ranges of 200 nm to 400nm. Naratriptan exhibited λmax at 283.5 nm in case water. However the λmax was slightly shifted to 285 nm in case of acetonitrile: water and 284.5 in methanol: water. The method obeyed Beer-Lamberts law within the range of 10 to 50 µg/ml concentration.

Vishwanathan. K. et. al [23]., developed a rapid, sensitive and selective method for the determination of antimigraine drugs from human serum to study pharmacokinetics of these drugs administered concurrently. Solid phase extraction (SPE) using Oasis HLB was used to extract the drugs (sumatriptan, naratriptan, zolmitriptan and rizatriptan) and the internal standard bufotenine from serum. A method based on liquid chromatography/tandem mass spectrometry (LC/MS/MS) was developed and validated to simultaneously quantitate these antimigraine drugs from human serum. The precursor and major product ions of the analytes were monitored on a triple quadrupole mass spectrometer with positive ion electrospray ionization (ESI) in the multiple reaction monitoring (MRM) mode.

Ramesh. C. et. al [24]., reported a three simple sensitive and reproducible visible spectrophotometric methods (A, B, and C) for the determination of naratriptan hydrochloride (NTP) in samples and pharmaceutical formulations. Method A was based on the formation of colored co-ordination complex with cobalt thiocyanate (CTC). Method B was based on the formation of colored species with citric acid - acetic anhydride (CiA-Ac O). Method C was based on the formation of colored molecular complex involving NTP and sodium nitroprusside (SNP) in the presence of hydroxylamine mono hydrochloride (HA). Regression analysis of Beer's law plots showed good concentration ranges 5-35, 2-10 and 10-60 µg/ml for methods A, B and C respectively. The applicability of the methods was examined by analyzing tablets of NTP.

AIM AND PLAN OF THE STUDY

AIM AND SCOPE:

Naratriptan Hcl is a new drug, used in the treatment of migraine headaches. From the literature survey, it was found that there are very few RP-HPLC methods available for the quantification of naratriptan Hcl in tablet formulation. Early workers have also reported the determination of the drug by hyphenated techniques such as LC-MS and also by UV spectrophotometry.

Hence the aim of present work is to develop simple and validated RP-HPLC method by isocratic mode for the quantification of naratriptan Hcl in tablet formulation.

PLAN OF WORK:

The plan of present work is as follows:

Method Development

- Selection of wavelength
- Selection of initial separation conditions
- Selection of mobile phase (pH, peak modifier, solvent strength and flow rate)
- Nature of the stationary phase
- Selection of separation method

Validation of the developed method

The developed method was validated by using the various validation parameters such as,

- Accuracy
- Precision
- Linearity, limit of detection (LOD) and limit of quantitation (LOQ)
- Selectivity / Specificity
- Robustness / ruggedness
- System suitability.

METHODOLOGY

1) MATERIALS AND INSTRUMENTS USED:

a) Chemicals and Solvents used:

S.No	Name	Grade
1.	Water	Millipore
2.	Acetonitrile	Merck (HPLC Grade)
3.	Potassium di hydrogen phosphate	AR grade
4.	Orthophosphoric acid	AR grade

b) Apparatus/ Instruments used:

S.No	Name	Model
1	HPLC	
	Pump	Waters E 2695
	Uv visible detector	Waters 2489
	Column C_{18} [250mmX 4.6mm,5µ]	Phen-s
2	Electronic balance	Mettle Toledo
3	pH meter	Elico
4	Sonicator	Sonorex dig 10 p
5	Membrane filter	AXIVA,SRP 15, 0.45 micron

2) ANALYTICAL METHOD DEVELOPMENT AND OPTIMIZATION OF CHROMATOGRAPHIC CONDITION

I. Determination of wave length:

A standard solution of the drug was prepared in water as follows in the following manner. A bout100 mg of naratriptan hydrochloride (Pure drug) was weighed accurately and dissolved in 100 ml of distilled water. From this 1ml was taken and made up to the final volume with water 10 ml in a volumetric flask so as to obtain the conc. of 100 µg/ml.

The resulting solution was scanned in the range of 200 nm to 400 nm in a UV-Vis spectrophotometer. The spectrum exhibited λ_{max} at 224 nm and 280 nm. As the absorption was high at 224 nm, this wavelength was selected for the chromatographic method development. The data for the UV absorption spectrum was shown in table no.2 and the spectrum was shown in fig.1.

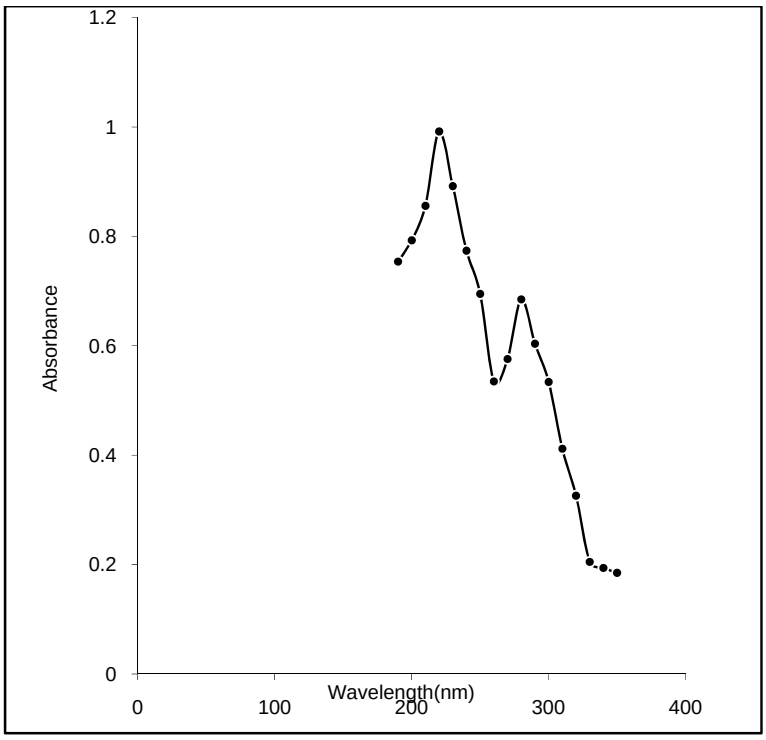
Table -2

Data for UV absorption spectrum

S. No.	Wavelength in nm	Absorbance
1.	200	0.765
2.	205	0.842
3.	210	0.856
4.	215	0.934
5.	**224**	**1.020**
6.	230	0.756
7	250	0.695
8	260	0.535
9	270	0.576

10	**280**	**0.685**
11	290	0.604
12	300	0.534
13	310	0.412
14	320	0.326
15	330	0.205
16	340	0.194
17	350	0.185

Fig 1: UV spectrum of naratriptan Hcl



II. Optimization of chromatographic parameters:

a. selection of mode of separation.

As the drug was polar in nature, RP-HPLC method was preferred.

b. Selection and standardization of mobile phase and column:

The method development of naratriptan Hcl required adequate resolution of the drug peak in the chromatogram. To obtain a resolved peak, several solvent systems and different columns were tried and finally spursil C_{18} column with Acetonitrile and phosphate buffer (30:70) as mobile phase were selected for the analysis.

III. Preparation of Mobile Phase:

Preparation of buffer:

About 1.368 gm of potassium di hydrogen phosphate was accurately weighed and transferred in to a 1 lit beaker. The salt was dissolved in water and diluted to 1 lit with water. The pH was adjusted to 7.2 with ortho phosphoric acid

Preparation of mobile phase:

A mixture of acetonitrile and buffer solution (30:70) was prepared, mixed well and filtered through 0.45 µ membrane filter and degassed.

IV. Selection of flow rate:

Different flow rates were tried in the method development for the drug. The peak shapes of drug showed fronting and tailing with the flow rate 0.6ml and 0.8ml/min respectively. Finally the flow rate with 1.0 ml per minute was selected for the analysis.

V. Determination of Retention time:

Standard stock solution of naratriptan Hcl:

About 10 mg of naratriptan Hcl working standard was accurately weighed and transferred in to a 50 ml volumetric flask, about 25 ml of water was added; sonicated to dissolve it completely and made up the volume up to the mark with the same solvent (Stock solution 200 µg/ml)

Preparation of standard solution of naratriptan Hcl:

Further 5 ml of the above stock solution was pipetted into a 10 ml volumetric flask, diluted up to the mark with water. The solution was mixed well and filtered through 0.45 µ membrane filter.

10 µl of the solution was injected in to the chromatographic system and the chromatogram was recorded. The retention time of naratriptan Hcl was found to be at 3.285 min. The chromatogram was shown in fig. 6

The experiment was repeated under different set of condition (trial 1 to trial 4) and shown as follows.

Trail 1:

Chromatographic conditions

Stationary phase	: Spursil $C_{18,}$ 5 micron particle size, 250mm × 4.6mm
Mobile phase	: Acetonitrile: methanol: Phosphate buffer (60:25:15)
Detection	: 224 nm
Flow rate	: 0.6 ml/min
Run time	: 5.8 min
Sample size	: 10 µl
Needle wash	: Water HPLC grade
Column temperature	: Ambient

Chromatogram for trail condition-1

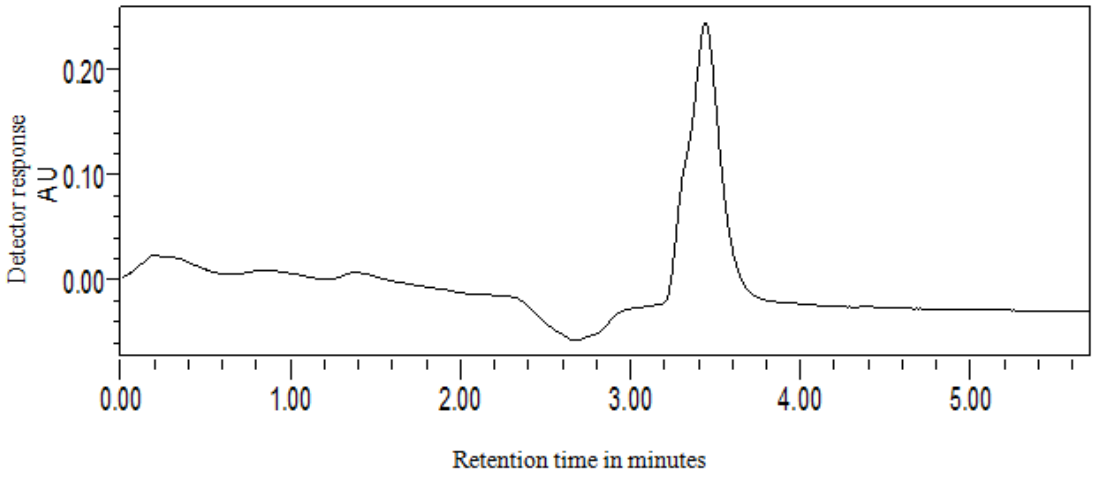


Fig: 2

Observation: Peak shape was not good and asymmetry was more than the limit.

Trail-2

Chromatographic conditions

Stationary phase	: Spursil $C_{18,}$ 5 micron particle size, 250mm × 4.6mm
Mobile phase	: Acetonitrile: Phosphate buffer (55:45)
Detection	: 224 nm
Flow rate	: 0.8 ml/min
Run time	: 6 min
Sample size	: 10 µl
Needle wash	: Water HPLC grade
Column temperature	: Ambient

Chromatogram for trail condition-2

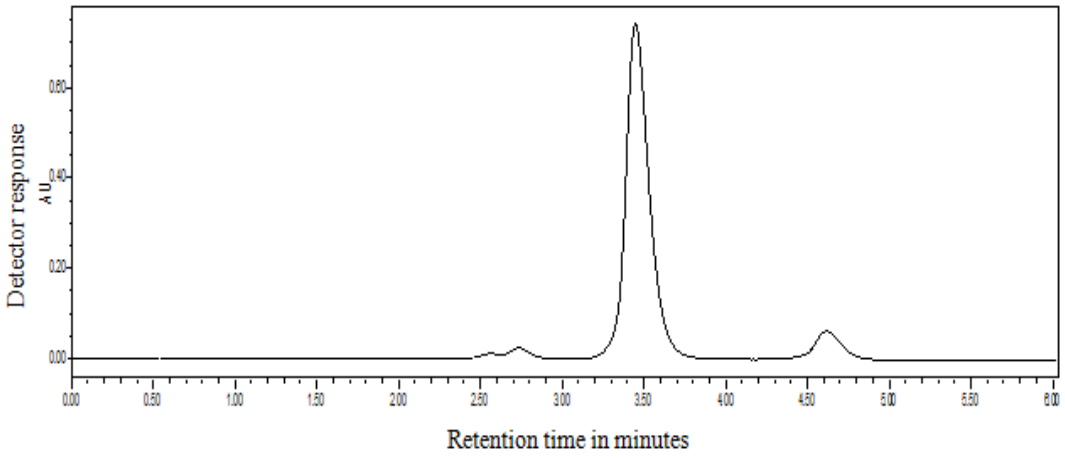


Fig: 3

Observation: Peak shape was not good, theoretical plates were too less and asymmetry was more than the limit.

Trail-3

Chromatographic conditions

Stationary phase	: Spursil $C_{18,}$ 5 micron particle size, 250mm × 4.6mm
Mobile phase	: Acetonitrile: Phosphate buffer (50:50)
Detection	: 224 nm
Flow rate	: 1 ml/min
Run time	: 5 min
Sample size	: 10 µl
Needle wash	: Water HPLC grade
Column temperature	: Ambient

Chromatogram for trail condition-3

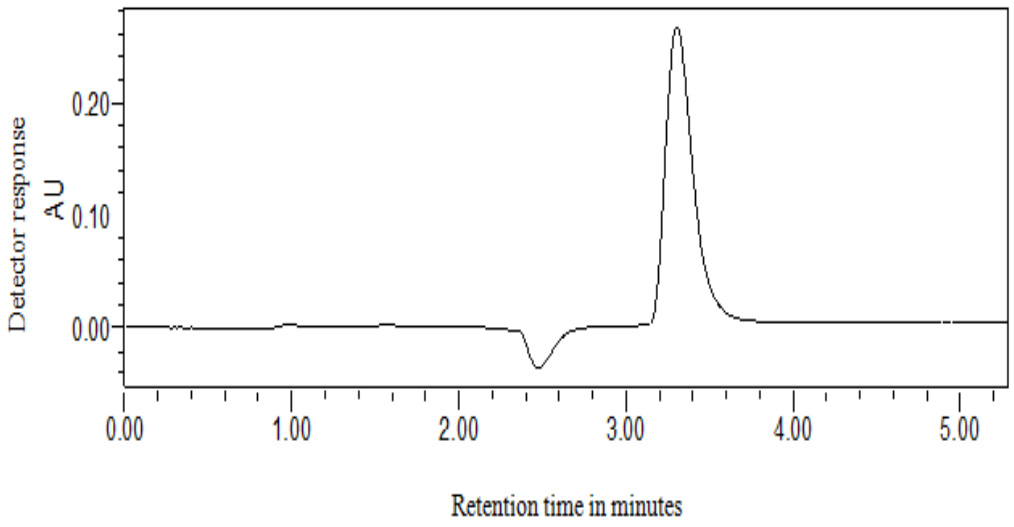


Fig: 4

Observation: Peak shape was not good; asymmetry was more than the limit.

Trail-4

Chromatographic conditions

Stationary phase	: Spursil $C_{18,}$ 5 micron particle size, 250mm × 4.6mm
Mobile phase	: Acetonitrile: Phosphate buffer (40:60)
Detection	: 224 nm
Flow rate	: 1 ml/min
Run time	: 5.4 min
Sample size	: 10 µl
Needle wash	: Water HPLC grade
Column temperature	: Ambient

Chromatogram for trail condition-4

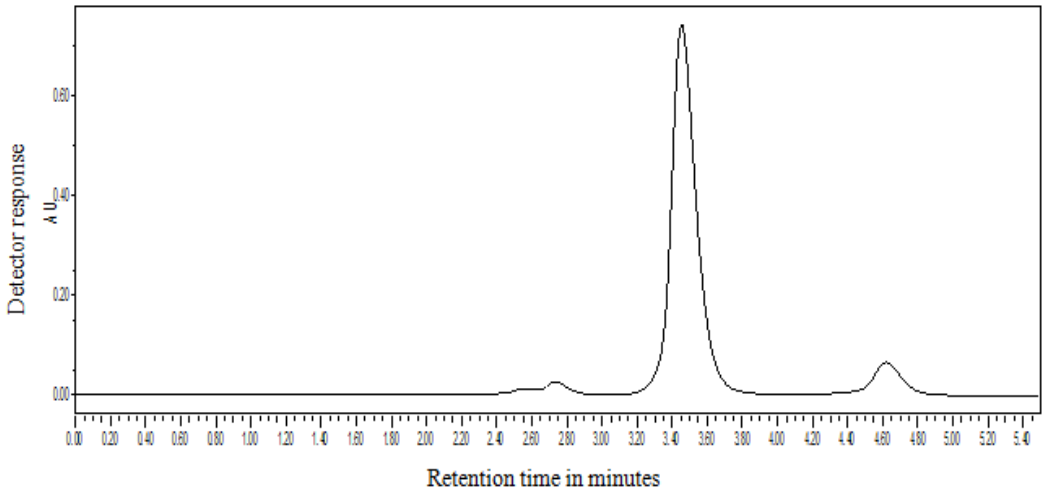


Fig-5

Observation: Peak shape was good, theoretical plates were too less and asymmetry was more than the limit.

OPTIMIZED METHOD

FIXED CHROMATOGRAPHIC CONDITION

Stationary phase	: Spursil $C_{18,}$ 5 micron particle size, 250mm × 4.6mm
Mobile phase	: Acetonitrile: Phosphate buffer (30:70)
Flow rate	: 1.0 ml/ min
Wavelength	: 224 nm
Injection volume	: 10 µl
Needle wash	: Water HPLC grade
Column temperature	: Ambient
Run time	: 5 min.

Chromatogram for fixed Chromatographic Condition

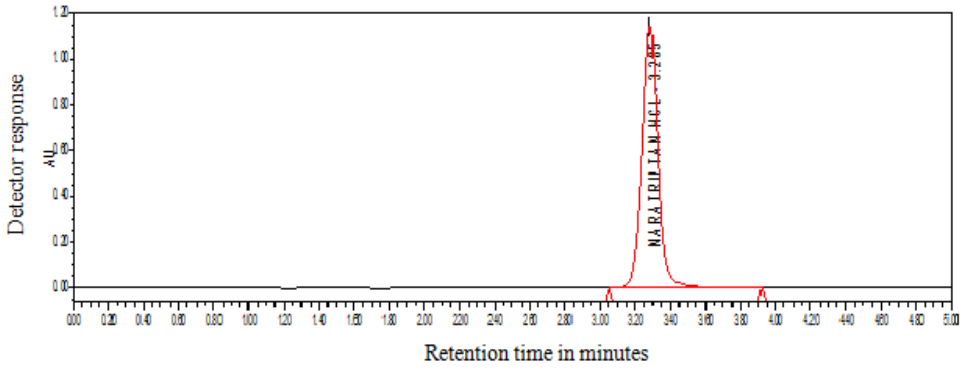


Fig6:

Observation: Peak shape was good, theoretical plates were adequate and asymmetry was within the limit.

QUANTITATIVE DETERMINATION OF THE DRUG BY USING THE DEVELOPED METHOD

Sample : Naratriptan Hcl tablet

Label claim: 1 mg

Preparation of blank: Millipore water was used as blank.

Standard solution of naratriptan Hcl:

About 10 mg of naratriptan Hcl working standard was accurately weighed and transferred in to 50 ml volumetric flask, added about 25 ml of water and sonicated to dissolve it completely and made up the volume up to the mark with the same solvent (Stock solution 200 µg/ml)

Further 5 ml of the above stock solution was pipetted into a 10ml volumetric flask, diluted up to the mark with water, mixed well and filtered through 0.45µm filter. (The final concentration of resulting solution was 100 µg/ml).

Sample solution of naratriptan Hcl:

20 naratriptan Hcl tablets were weighed and calculated the average weight. The sample equivalent to 10 mg of naratriptan Hcl was accurately weighed and transferred

in to 50 ml volumetric flask added, about 25 ml of water and sonicated to dissolve it completely and made up the volume up to the mark with diluent, mixed well and filtered through 0.45µm filter.

Further 5 ml of the above stock solution was pipetted into a 10 ml volumetric flask. The volume was made up with the diluent, mixed well and filtered through 0.45µm filter. (The final concentration of resulting solution was 100 µg/ml).

Procedure:

10 µl of the solution of each of blank, standard and sample solution were injected separately in to the chromatographic system. The chromatograms were recorded, shown in fig.7-9 and reported in table.3.

Amount of drug present in the tablet was calculated using the following formula:

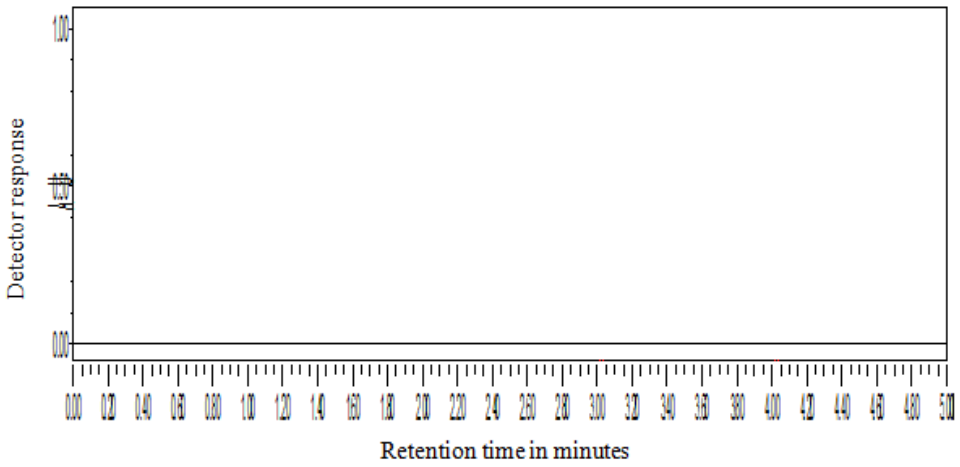
Content of the drug in a tablet of average weight

$$= \frac{\text{Sample area}}{\text{standard area}} \times \frac{\text{standarddilution}}{\text{sample diluton}} \times \frac{\text{potency}}{100} \times \text{Average weight of tablet}$$

$$\text{Percentage content} = \frac{\text{Amount present}}{\text{Label claim}} \times 100$$

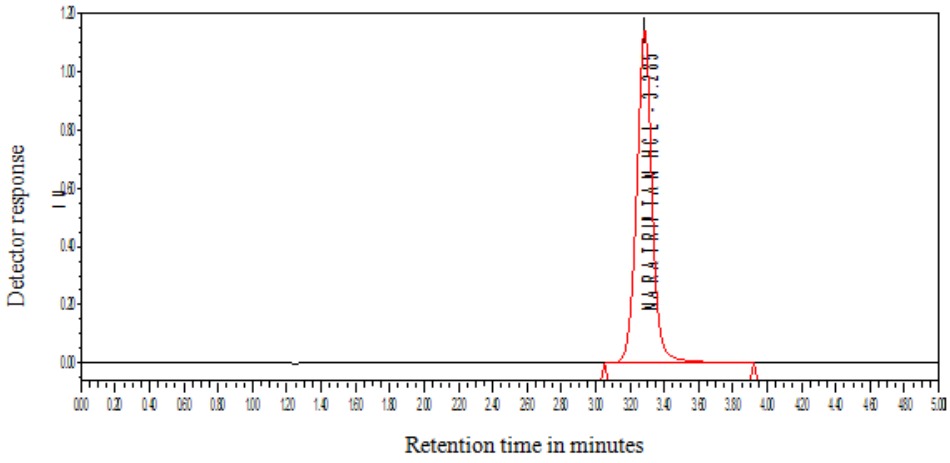
Chromatogram of Blank

Fig.7



Chromatogram of Standard preparation

Fig.8



Chromatogram of assay preparation

Fig.9

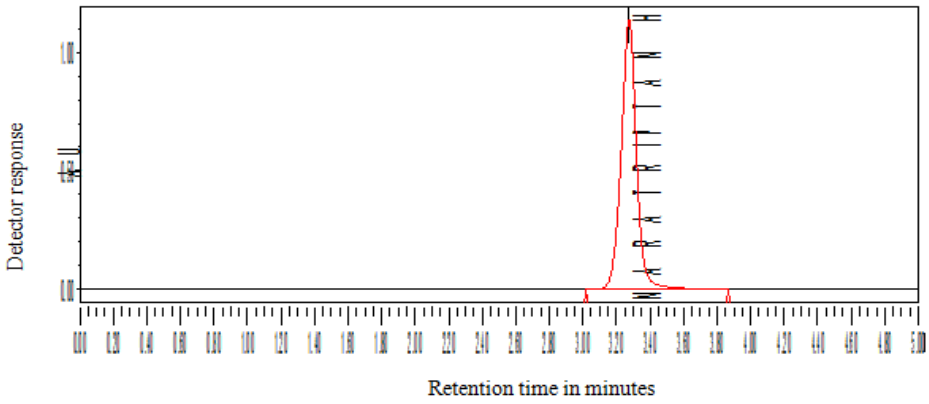


Table-3

Data for assay

SI. no	content	Label Claim(mg)	Peak area		Amount Present(mg)	Percent Content
			standard	sample		
1.	Naratriptan	1mg	7219001	7142112	0.989	98.93%

Acceptance criteria: 97.0- 103% w/v.

VALIDATION

Validation of analytical method for assay of naratriptan Hcl:

Validation of analytical method is a process to establish that the performance characteristics of the developed method meet the requirement of the intended analytical application.

Design of experiment:

Typical analytical parameters used in assay validation were,

- Accuracy
- Precision
- Linearity and range
- Limit of Detection(LOD)
- Limit of Quantitation(LOQ)
- Specificity
- Robustness
- Ruggedness
- System suitability studies
  - Theoretical plate count
  - Relative standard deviation
  - The tailing factor

1. ACCURACY

The accuracy of an analytical method is the closeness of the test result obtained by that method to the true value.

Accuracy is measured as the percentage of the analytes recovered by the assay. Spiked samples were prepared in triplicate at three intervals a range of 50-150% of the target concentration and injected in to the HPLC system.

Acceptance criteria	:	percentage recovery should be with in 97-103% w/v

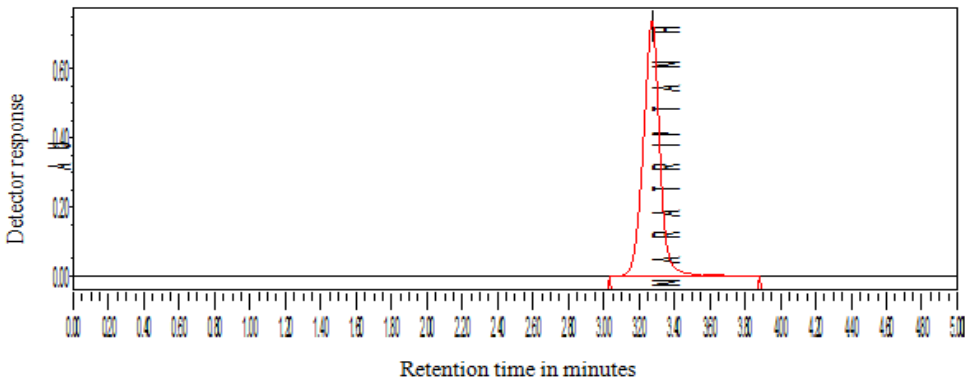
Preparation of working mixture solution:

Naratriptan working standard equivalent to 50%, 100%, 150% were weighed accurately and transferred in to three different 50 ml volumetric flask. 200 mg of placebo was weighed and transferred to each flask, dissolved with small volume of diluent and made up the volume. 5 ml of this solution was diluted to 10 ml with water. The solution was filtered through 0.45 µ membrane filter.

10 µl of each of this solution were injected and chromatograms were recorded as shown in fig. 10-12. The recovery study data for naratriptan was shown in table 4.

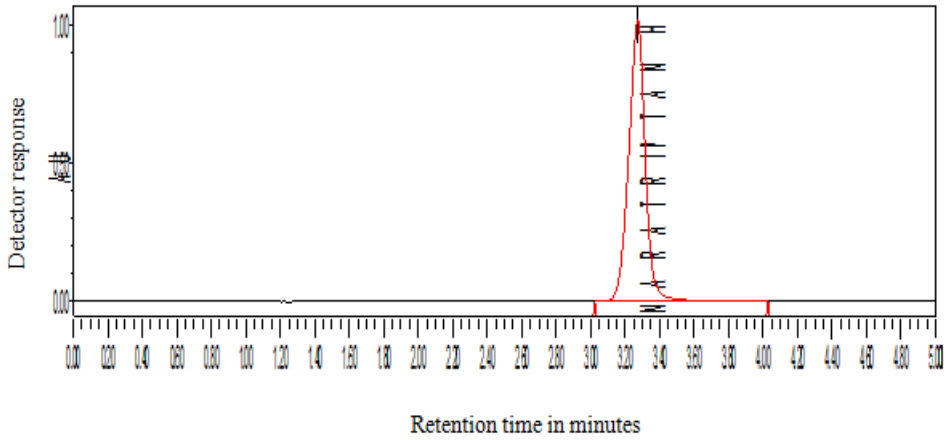
Chromatogram for accuracy at 50%

Fig.10



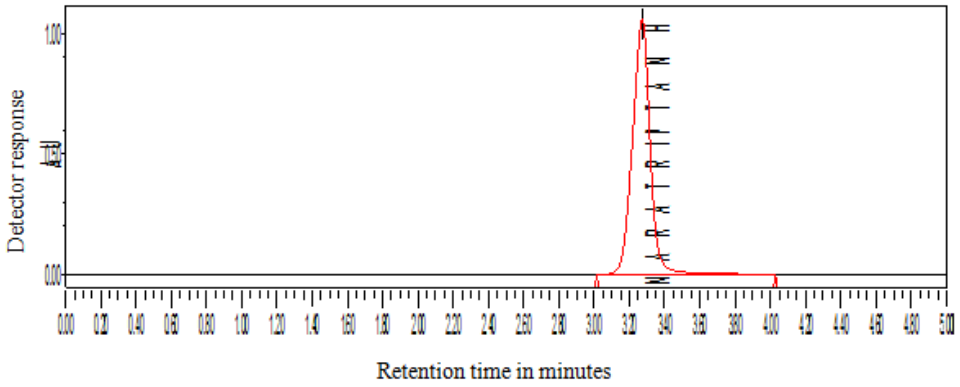
Chromatogram for accuracy at 100 %

Fig.11



Chromatogram for accuracy at 150%

Fig.12



Data for accuracy studies

Table-4

SI. No.	Recovery	Area obtained	Average area	Amount added in mg	Amount recovered in mg	% Recovery
1	**50%**	3474789	3506014	5.010	4.897	97.94%
		3558456				
		3484798				
2	**100%**	7134565	7202001	10.021	9.956	99.56%
		7125469				
		7345969				
3	**150%**	10089721	10693349	15.022	14.783	98.55%
		11092674				
		10897654				

2. PRECISION

Precision of an analytical method is the degree of agreement among individual test result when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analytical method is usually expressed as the standard deviation or relative standard deviation.

Determination:

The precision of the analytical method is determined by assaying the samples consecutively number of times and relative standard deviation was calculated.

Acceptance criteria	:	The relative standard deviation should be with in 2%

a) System precision:

Preparation of standard solution:

10 mg of naratriptan Hcl working standard was accurately weighed and transferred into a 50 ml volumetric flask added then about 25 ml of water, sonicated to dissolve it completely and made up the volume up to the mark with the same solvent (Stock solution was 200 µg/ml).

Further 5 ml of the above stock solution was pipetted into a 10 ml volumetric flask, diluted up to the mark with water. Mixed well and filtered through 0.45 µ membrane filter. (The final concentration of resulting solution was 100 µg/ml).

Method:

The system precision was evaluated by measuring the peak response of naratriptan for six replicate injections of the standard solution and chromatogram were recorded and shown in fig.13 and precision data of system was shown in table 5.

The standard deviation and relative standard deviation were calculated from the statistical formula

$$\text{Standard deviation } (\sigma) = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$$

x = sample value

$\bar{x}$ = mean value of sample

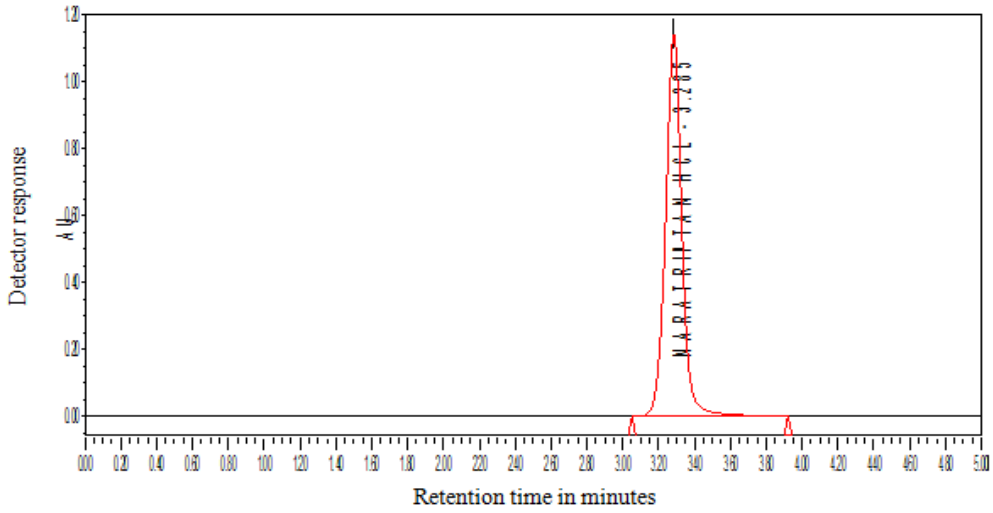
n = number of sample

$$\text{Relative standard deviation} = \frac{\sigma}{\bar{x}} x100$$

System precision

Chromatogram of standard

Fig.13



Data for system precision

Table -5

No. of injection	Peak area
Injection-1	7352659
Injection-2	7124565
Injection-3	7149579
Injection-4	7352659
Injection-5	7124565
Injection-6	7149579
Average	7208934.3
Standard Deviation	111889
%RSD	1.55

b) Method precision:

20 naratriptan Hcl tablets were weighed and calculated the average weight. The sample equivalent to 10 mg of naratriptan Hcl was accurately weighed and transferred into a 50 ml volumetric flask then added about 25 ml of water about sonicated to dissolve it completely and made up the volume up to the mark with same solvent. The solution was mixed well and filtered through 0.45µ membrane filter.

Further 5 ml of the above sample solution was pipetted into a 10 ml volumetric flask, diluted up to the mark with diluent. Mixed well and filtered through 0.45µ membrane filter.

Procedure:

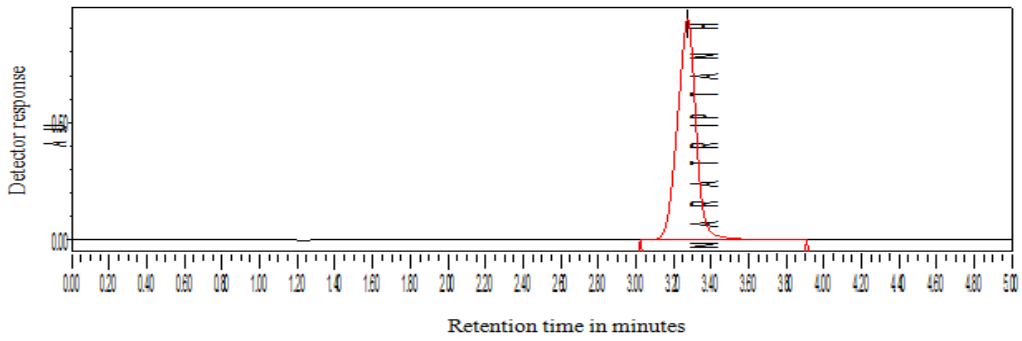
The method precision (repeatability) was determined by preparing the sample of single batch of naratriptan tablet formulation for six times and six successive injection of 10 µl of working sample solution were injected and the chromatograms were

recorded and shown in fig.14 and method precision (repeatability) data of naratriptan was shown in table 6.

Method precision

Chromatogram for method precision

Fig.14



Data for method precision

Table-6

SI.No	Tablet powder Weight in mg	Peak Area obtained	Assay value in mg	% Label claim w/v
1	2040	7149579	0.990	99.03
2	2039	7124565	0.987	98.74
3	2041	7352659	1.018	101.80
4	2038	7149579	0.991	99.13
5	2042	7124565	0.985	99.59
6	2043	7352659	0.997	99.71
	Mean			99.48
	Standard deviation			1.205
	% Relative standard deviation			1.21

3. LINEARITY AND RANGE

Linearity was assessed by performing measurement at several analyte concentrations. Minimum five concentrations were recommended for linearity studies.

The linearity of an analytical method is its ability to elicit test results that is directly proportional to the concentration of analyte in sample with in a given range. The linearity of an analytical method is determined by mathematical treatment of test result obtained by analysis of samples with analyte concentration across claimed range of peak area Vs concentration is plotted and percentage curve fitting is calculated.

Acceptance criteria	:	Correlation coefficient should not be less than 0.99%. Percentage curve fitting should not be less than 99.7%.

Method:

Preparation of working standard solution:

Naratriptan equivalent to 50%, 75%, 100%, 125% and 150% level were weighed accurately and taken in different 50 ml volumetric flask, dissolved in the diluent and the volume were made up with diluent to obtain the concentration of 50, 75, 100, 125, 150 µg/ml.

10 µl of each of working standard solutions were injected separately and the chromatogram were recorded and shown in fig.15-19.

The correlation coefficient and percentage curve fitting were calculated from the following formula,

$$R = \frac{3\left(X-\overline{X}\right)^2\left(Y-\overline{Y}\right)^2}{(n-1)\,S_x\,S_y}$$

Where,

X = concentration,

Y = instrumental response,

S_x = Std.Deviation of x

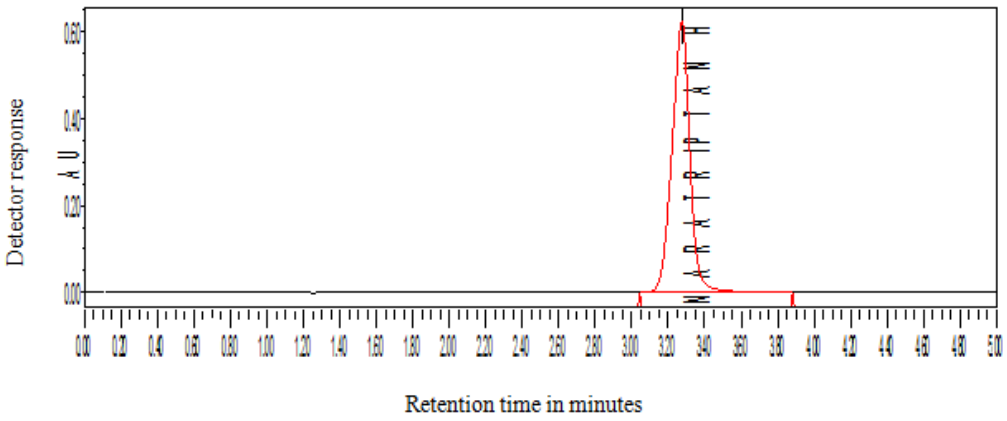
S_y = Std.Devitation of y

Percentage curve fitting = 100×correlation coefficient

The linearity data and analytical performance parameters of naratriptan was shown in table.7-8 and calibration curve of naratriptan was shown in fig.20

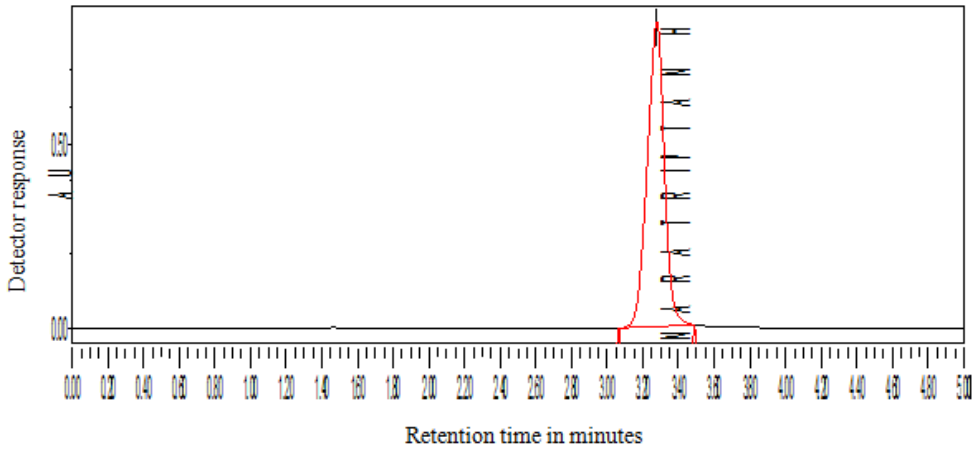
Chromatogram for linearity level at 50%

Fig.15



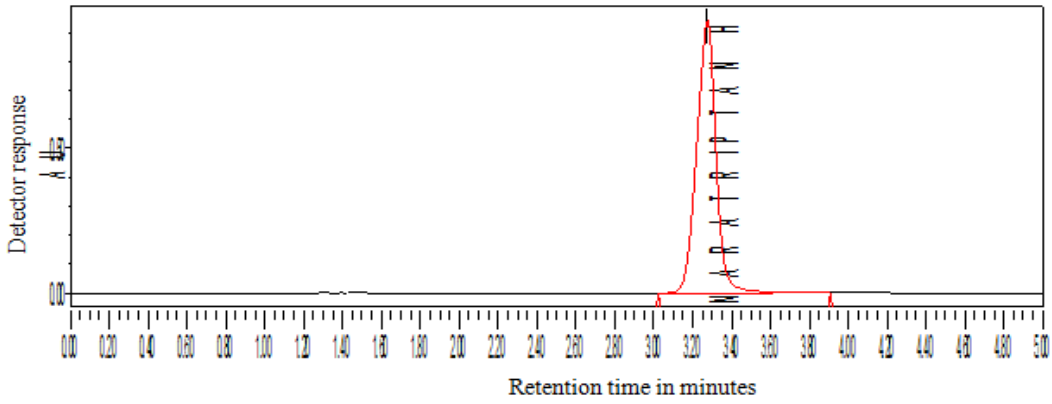
Chromatogram for linearity level at 75%

Fig.16



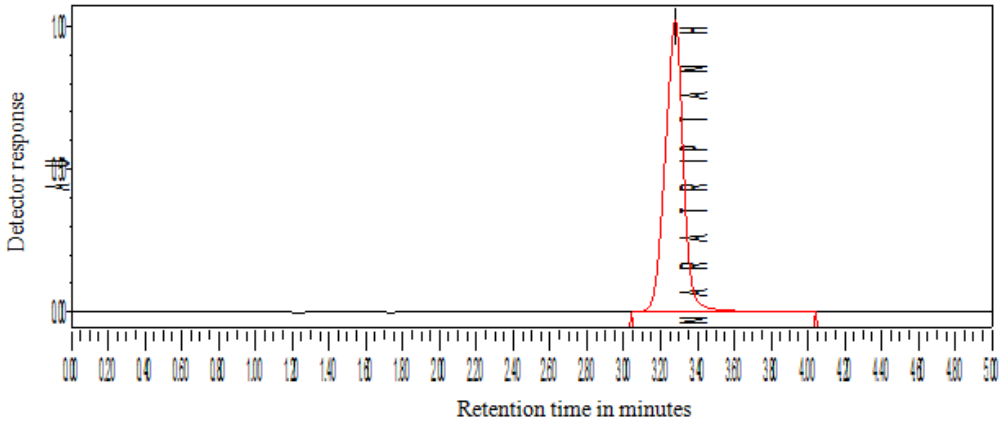
Chromatogram for linearity level at 100%

Fig.17



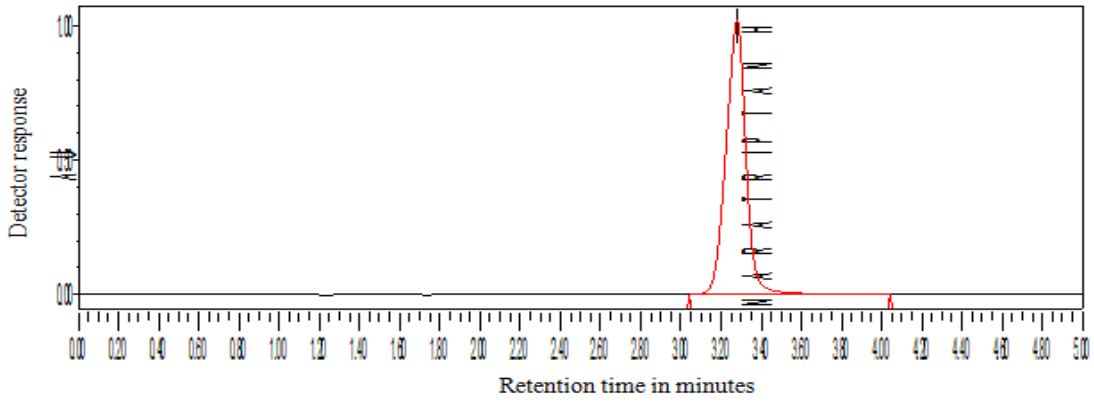
Chromatogram for linearity level at 125%

Fig.18



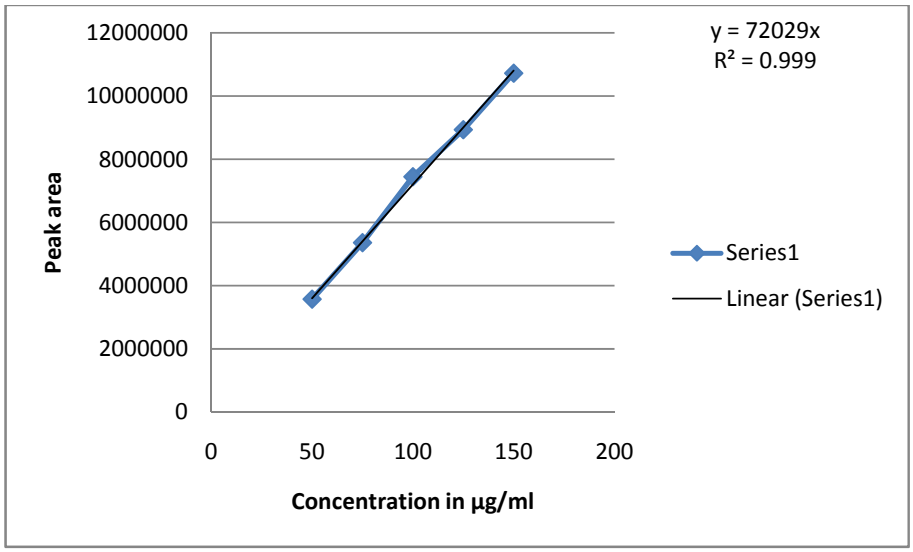
Chromatogram for linearity level at 150%

Fig.19



CALIBRATION CURVE OF NARATRIPTAN

Fig.20



Data for Linearity

Table - 7

SI. No	Concentration of naratriptan Hcl µg/ml	Average peak area
1	50µg/ml	3574789
2	75µg/ml	5362183
3	100µg/ml	7449578
4	125µg/ml	8936972
5	150 µg/ml	107243647
Correlation Coefficient		0.999

ANALYTICAL PERFORMANCE OF NARATRIPTAN HCL

Table-8

Parameters	Values
Linear dynamic range	50 -150 µg/ml
Correlation Coefficient	0.999
Percentage curve fitting of slope	99.9%
Slope	7449578

Acceptance criteria: The relationship between the concentration and response of naratriptan Hcl should be linear in the specified range and the regression coefficient should not be less than 0.999**.**

4. LIMIT OF DETECTION (LOD)

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated experimental condition. The detection limit is usually expressed as the concentration of analyte (e.g. parts per million).

It is determined by based on the standard deviation of response and the slope the detection limit may be expressed as

$$LOD = 3.3\ \sigma / S$$

Where,

σ = The standard deviation of the response

S = The slope of the calibration curve (of the analyte)

From the formula the limit of detection was found to be

LOD	Naratriptan Hcl
	0.04956 µg/ml

5. LIMIT OF QUANTITATION

The quantitation limit of an analytical procedure is the lowest amount of analytical procedure is the lowest amount of analyte in a sample, which can be quantitated with suitable precision and accuracy. Based on the deviation of the response and the slope.

Quantitation limit (QL) may be expressed as

$$LOQ = 10\ \sigma / S$$

Where,

σ = The standard deviation of the response

S = The slope of the calibration curve (of the analyte)

From the formula the limit of detection was found to be

LOQ	Naratriptan Hcl
	0.1502 µg/ml

6. SPECIFICITY

The specificity of the method was evaluated by analyzing the sample solution spiked with the excipients at appropriate levels. The assay result was unaffected by the presence of extraneous materials.

Determination:

The specificity of the analytical method was determined by injecting the placebo solution under the same experimental conditions as that of assay.

Preparation of placebo:

Placebo was prepared by mixing all the excipients without active ingredients.

Procedure:

200 mg of placebo was accurately weighed and transferred in to a 50 ml volumetric flask then added about 25 ml of water and sonicated to distribute content for 5 minutes. The volume was made up with same solvent. The solution was filtered through whatman filter paper.

Further 5ml of above solution was pipetted into 10 ml of volumetric flask diluted up to the mark with water. The solution was filtered through 0.45µ membrane filter.

Standard solution of naratriptan Hcl:

10 mg of naratriptan Hcl working standard was accurately weighed and transferred into a 50 ml volumetric flask then added about 25 ml of water, sonicated to dissolve it completely and made up the volume up to the mark with the same solvent (Stock solution 200 µg/ml)

Further 5 ml of the above stock solution was pipetted into a 10 ml volumetric flask diluted up to the mark with water. Mixed well and filtered through 0.45µ membrane filter. (The final concentration of resulting solution was 100 µg/ml).

Naratriptan standard+ placebo

200 mg of placebo and standard solution equivalent to 1mg of naratriptan Hcl were transferred in to 50 ml volumetric flask then added about 25 ml of water, sonicated to dissolve it content for 5 minutes. The volume was made up with water. The solution was filtered through 0.45µ membrane filter.

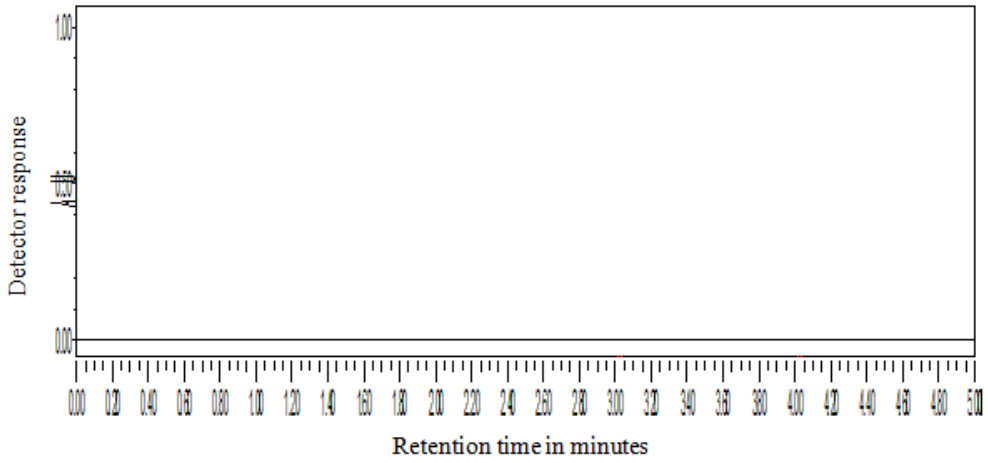
Further 5 ml of above solution was pipetted in to a 10 ml of volumetric flask diluted up to the mark with water. The solution was filtered through 0.45µ membrane filter.

10 µl 0f the solution of each of placebo, standard and standard+placebo solution were injected separately in to the chromatographic system. The chromatograms were shown in fig.21-23 and reported in table.9.

SPECIFICITY

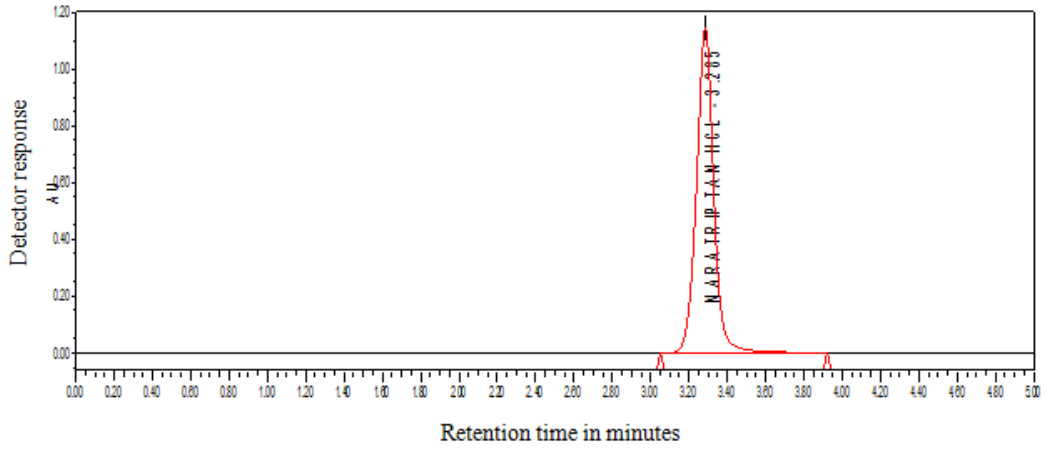
Chromatogram for placebo preparation

Fig.21



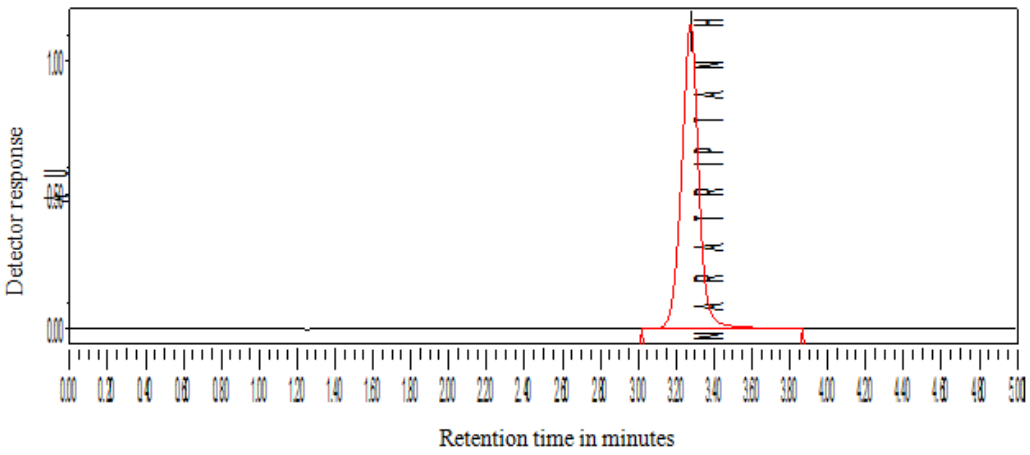
Chromatogram for standard preparation:

Fig.22



Chromatogram for Standard + placebo preparation

Fig.23



Data of specificity

Table -9

SI.No	Sample	Area obtained	Percent content of Drug
1.	Standard	7219001	98
2.	Standard+ Placebo	7124565	98.7
3.	Placebo	0	0

7. ROBUSTNESS

Robustness of an analytical method is measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage.

Determination:

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but were still within the specified parameters of the assay such as change in physical parameters like flow rate and column temperature.

Method

Preparation of Standard solution:

10 mg of naratriptan Hcl working standard was accurately weighed and transferred in to a 50 ml volumetric flask then added about 25 ml of water and sonicated to dissolve it completely and made up the volume up to the mark with the same solvent(Stock solution 200 µg/ml).

Further 5 ml of the above stock solution was pipetted in to a 10 ml volumetric flask, diluted up to the mark with water. Mixed well and filtered through 0.45µ membrane filter. (The final concentration of resulting solution was 100 µg/ml).

Procedure:

a) The flow rate was varied at 0.8 to 1.2 ml/min.

Standard solution 100 µg/ml was prepared and analysed using the varied flow rates. On evaluation of the above results, it can be concluded that the variation in flow rate do not affect the method significantly. Hence it indicates that the method is robust up on the change in the flow rate ±0.2 ml/min.

b) The temperature was varied at 28 ^{0}C to 32 ^{0}C.

Standard solution 100 µg/ml was prepared and analysed using the varied temperature. On evaluation of the above results, it can be concluded that the variation

in temperature do not affect the method significantly. Hence it indicates that the method is robust even after the change in the column temperature ±2 ^{0}C.

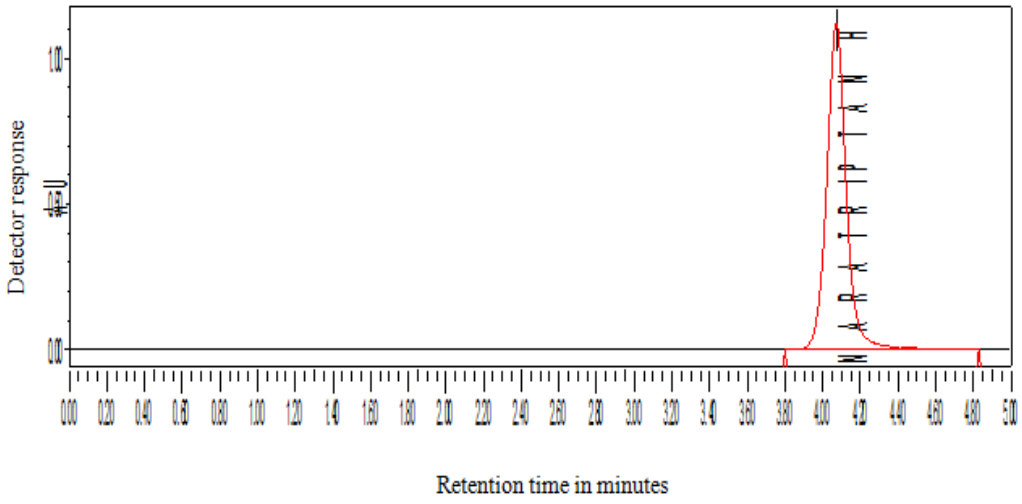
Procedure:

10 µl of various working standard solution was injected and chromatogram was recorded and shown in fig.24-29 and results are shown in table.10-13.

CHROMATOGRAM FOR ROBUSTNESS

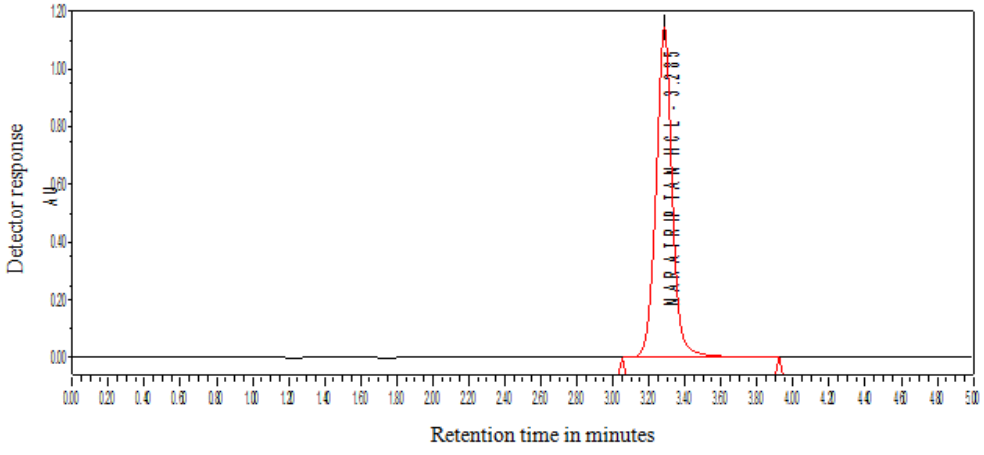
a) Effect of change in flow rate (0.8 ml/min)

Fig.24



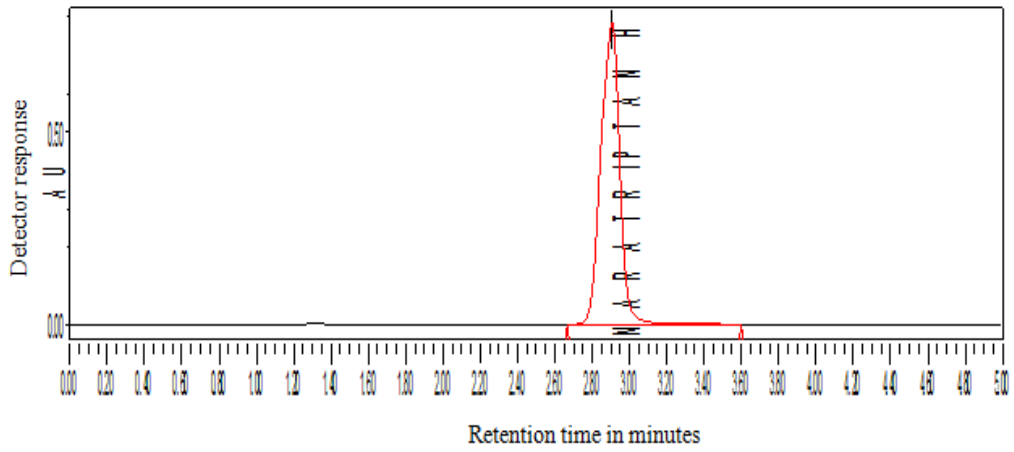
b) Effect of change in flow rate (1.0 ml/min)

Fig.25



c) Effect of change in flow rate (1.2 ml/min)

Fig.26



Report of robustness (change in flow rate 0.8 ml/min)

Table -10

Drug	**Average area (flow rate 0.8 ml/min)**	**Average area (flow rate 1.0 ml/min)**	**Standard deviation**	**% RSD**
Naratriptan Hcl	7413428	7219001	23223	0.31

Report of robustness (change in flow rate 1.2 ml/min)

Table -11

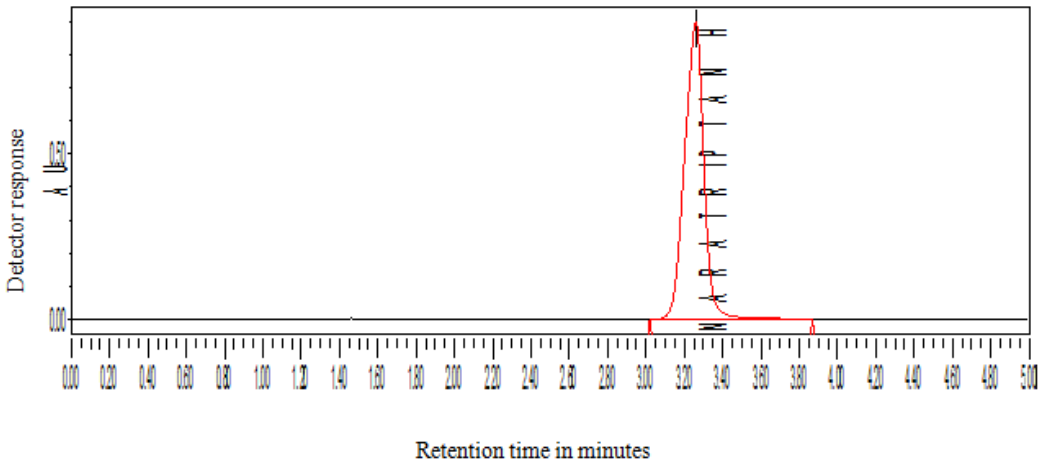
Drug	Average area (flow rate 1.2.ml/min)	Average area (flow rate 1.0 ml/min)	Standard deviation	% RSD
Naratriptan Hcl	7012141	7219001	69649	0.98

Acceptance criteria: The percentage RSD should be not more than 2.0

CHROMATOGRAM FOR ROBUSTNESS

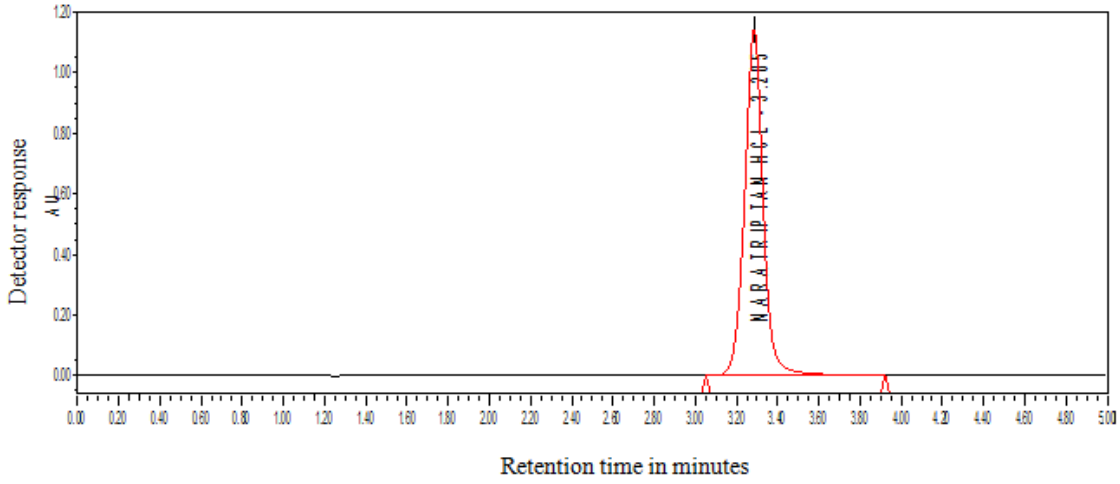
a) Effect of change in column temperature (28 ^{0}C)

Fig.27



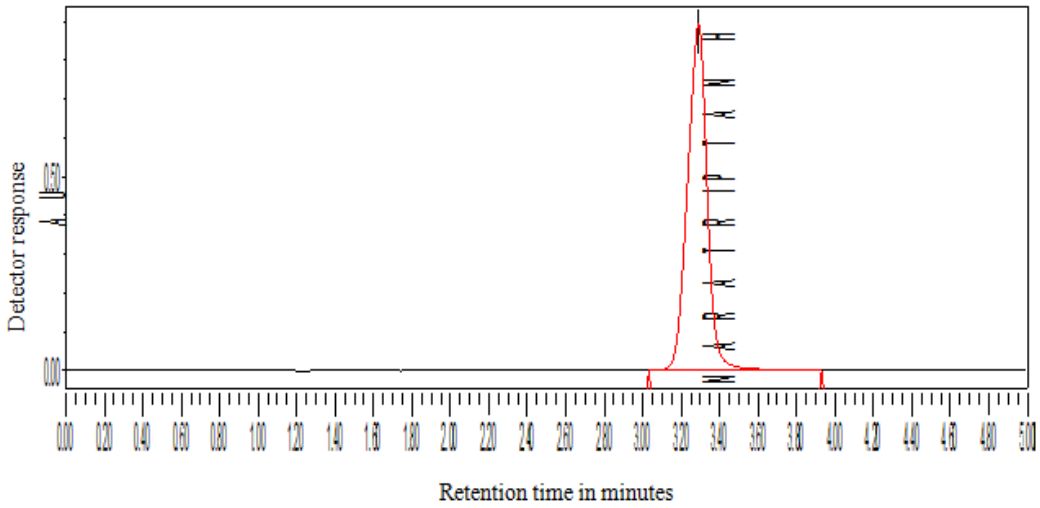
b) Effect of change in column temperature (30 ^{0}C)

Fig.28



c) Effect of change in column temperature (32 ^{0}C)

Fig.29



Report of robustness (change in column temperature 28℃)

Table -12

Drug	**Average area (column temperature 28 ℃)**	**Average area (column temperature 30 ℃)**	**Standard deviation**	**% RSD**
Naratriptan Hcl	7093821	7219001	44293	0.62 %

Report of robustness (change in column temperature 32℃)

Table -13

Drug	**Average area (column temperature 32 ℃)**	**Average area (column temperature 30 ℃)**	**Standard deviation**	**% RSD**
Naratriptan Hcl	7123740	7219001	98461	1.34 %

Acceptance criteria: The percentage RSD should not be more than 2.0

8. RUGGEDNESS

The ruggedness of an analytical method is degree of reproducibility of test result obtained by the analyst under a variety of normal test condition such as different laboratories different analysts different instruments, different lots of reagents different elapsed assay times, different temperature, different days etc.

The ruggedness of an analytical method was determined by aliquots from homogenous lots by different analyst using operational and environmental conditions that may differ but are also with in the specified parameters of the assay. The degree of reproducibility of test results is then determined as function of the assay variables. This reproducibility may be compared with the precision of the assay under normal condition to obtain a measure of the ruggedness of the analytical method. The assay of naratriptan was performed in different days.

Method:

Working standard solution and working sample solution of naratriptan were prepared by different analyst and on different days and 10 µl of working standard solution and working sample solution were injected. The chromatograms were recorded and shown in fig.30-37 and ruggedness of the method were reported in table.14.

Acceptance criteria:

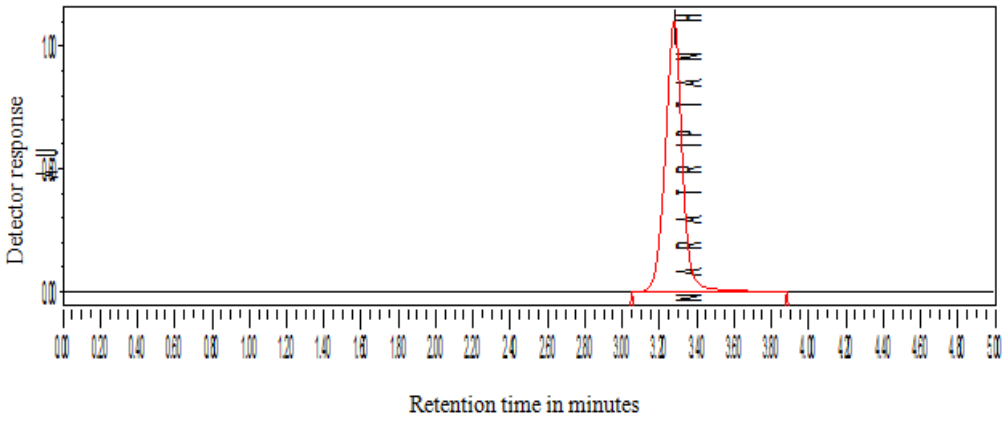
a) The relative standard deviation for the assay values of five sample preparations of same batch should not be more than 2.0%.

b) The difference in the assay of same batch of naratriptan Hcl between two analysts should not be more than 2.0%.

RUGGEDNESS FOR ANALYST-I

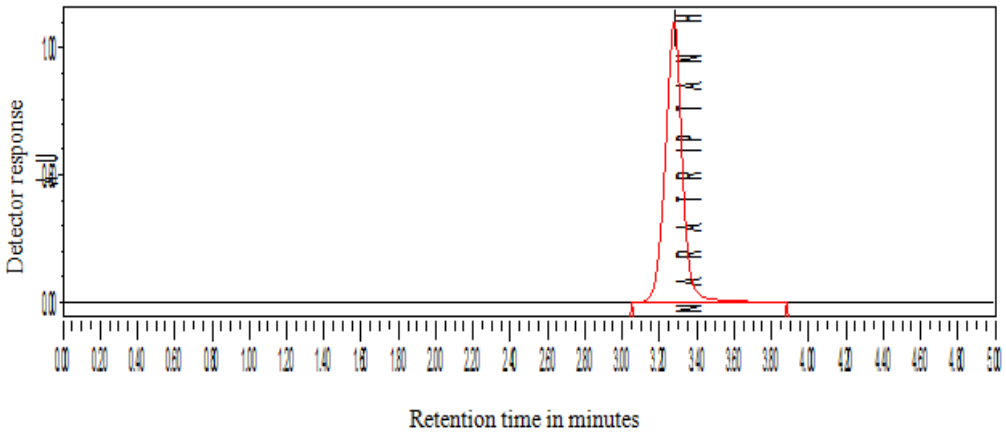
Chromatogram for standard preparation

Fig.30



Chromatogram for sample preparation

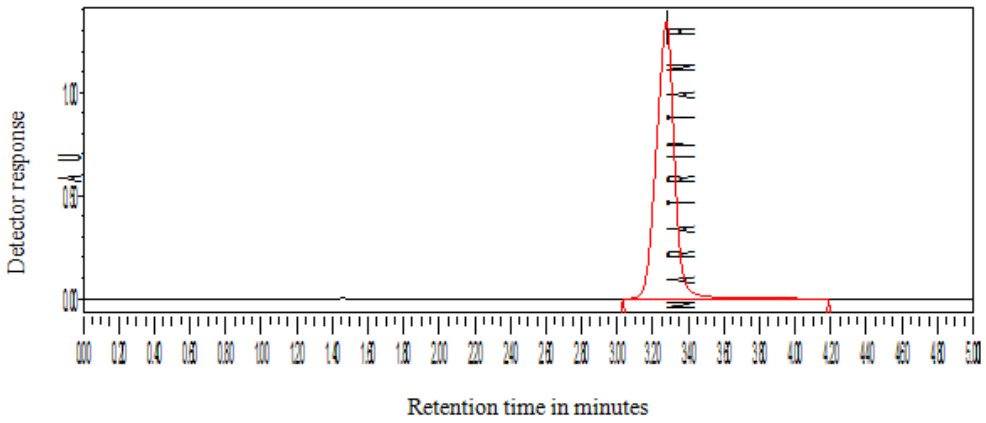
Fig.31



RUGGEDNESS FOR ANALYST-II

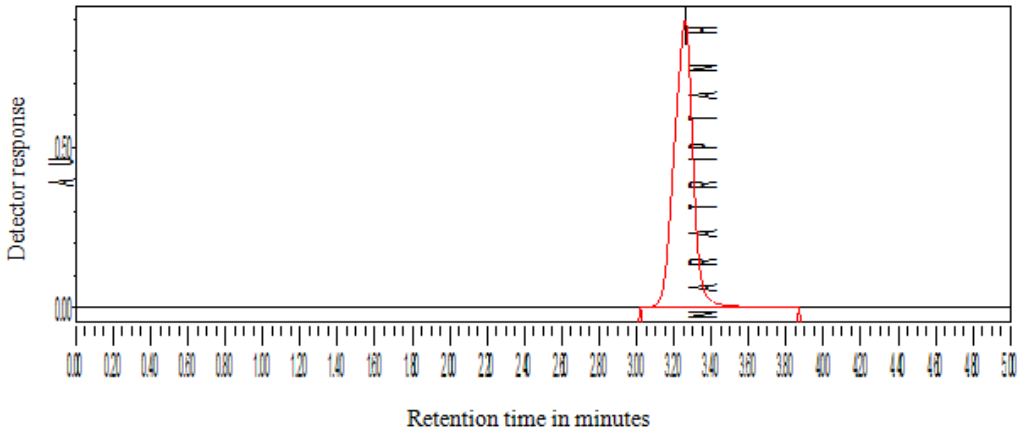
Chromatogram for standard preparation

Fig.32



Chromatogram for Sample preparation

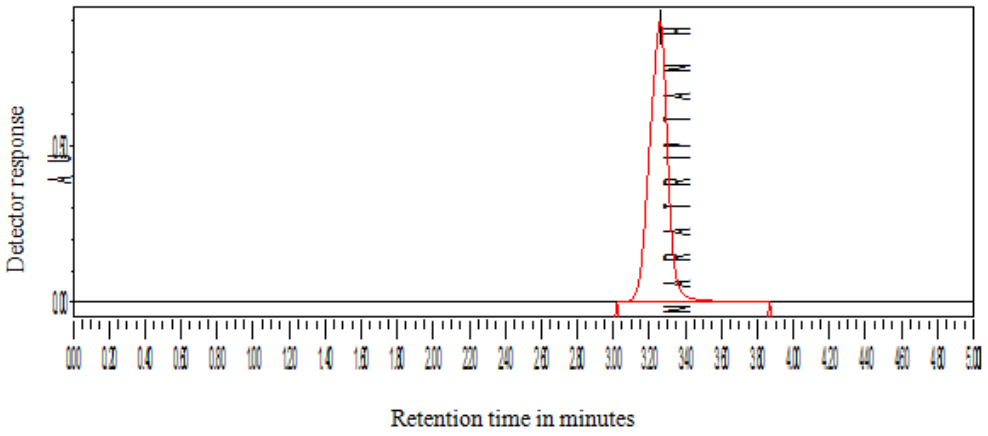
Fig.33



RUGGEDNESS FOR INSTRUMENT-I AND ANALYST-III

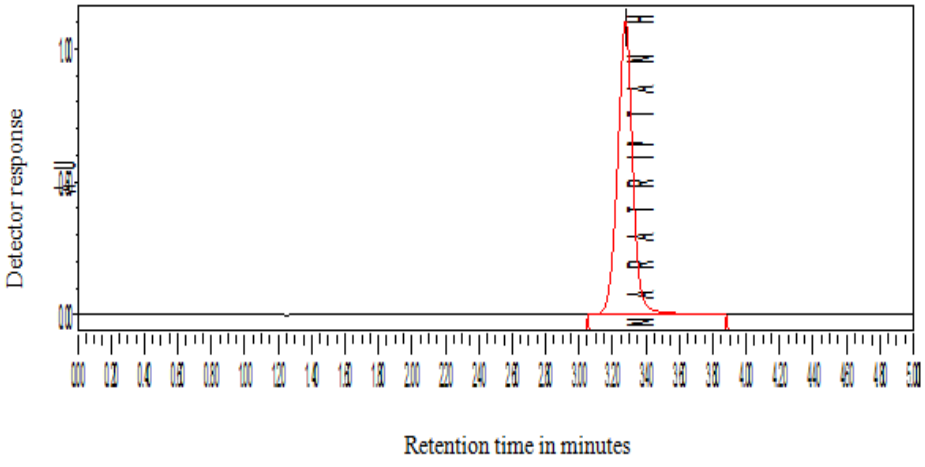
Chromatogram for standard preparation

Fig.34



Chromatogram for sample preparation

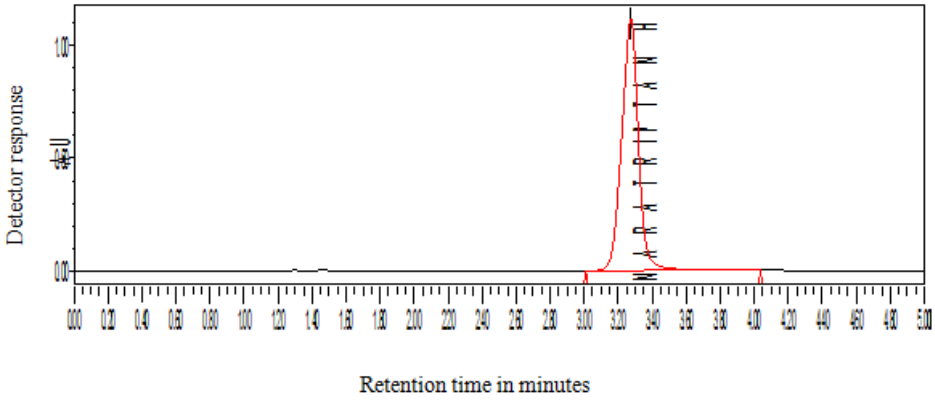
Fig.35



RUGGEDNESS FOR INSTRUMENT-II AND ANALYST-IV

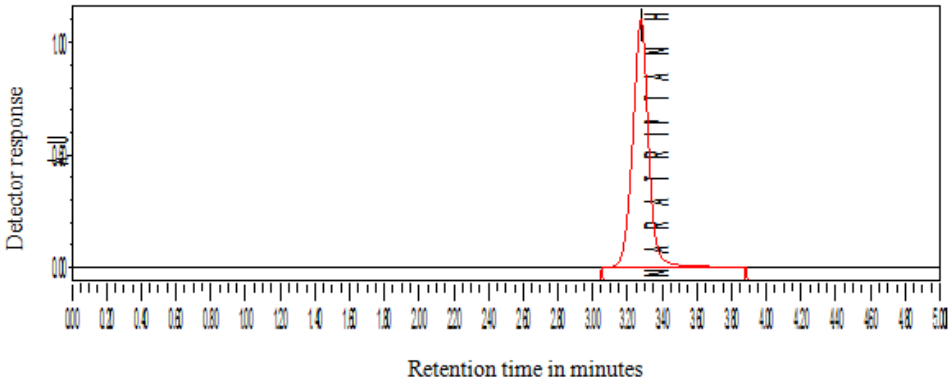
Chromatogram for standard preparation

Fig.36



Chromatogram for sample preparation:

Fig.37



Data for ruggedness

Table-14

SI.no	Instrument code	Analyst	Date of analysis	Percentage content
1	Waters-2695	I	21-11-2011	98.27%
2	Waters-2695	II	22-11-2011	98.12%
3	Waters-2695	III	23-11-2011	98.33%
4	Peak 7000	IV	24-11-2011	98.74%
	Mean			98.36%
	Standard deviation			0.2695
	% Relative standard deviation			0.27

Acceptance criteria: The percentage RSD should not be more than 2.0

9. SYSTEM SUITABILITY PARAMETERS

The system suitability studies were carried out as specified in USP. These parameters include % RSD for peak area, tailing factor and theoretical plates.

Determination

To determine the suitability of chromatographic system described for the method of analysis by establishing system suitability parameters like peak tailing factor, number of theoretical plates and the %RSD of naratriptan standard preparation on daily basis.

Method

Preparation of Standard solution:

10 mg of naratriptan Hcl working standard was accurately weighed and transferred in to a 50 ml volumetric flask then added about 25 ml of water and sonicated to dissolve it completely and made up the volume up to the mark with the same solvent (Stock solution 200 µg/ml) **.**

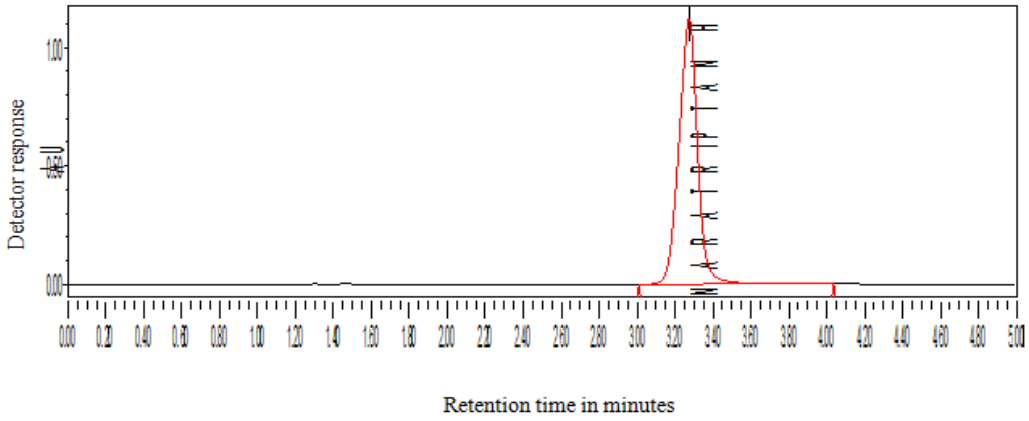
Further 5 ml of the above stock solution was pipetted in to a 10 ml volumetric flask, diluted up to the mark with water. Mixed well and filtered through 0.45µ membrane filter. (The final concentration of resulting solution was 100 µg/ml).

Acceptance criteria:

- **a.** The column efficiency is not less than 2000 theoretical plates**.**
- **b.** The tailing factor for the analyte peak is not more than 2.0**.**
- **c.** The relative standard deviation for the replicate injections is not more than 2.0%.

Chromatogram for system suitability parameters

Fig.38



Data for System Suitability Parameters

Table-15

System suitability factors	Results	Limit
%RSD	1.55	NMT 2%
Tailing factor	1.01	NMT 2
Number of Theoretical plates	6839	NLT 2000

RESULTS AND DISCUSSION

There is no official method for the estimation of naratriptan Hcl in any pharmacopoeia. Naratriptan is increasingly used in the treatment of migraine headaches. Hence it was felt necessary to develop a sensitive method for the estimation of naratriptan Hcl.

The method was developed with mobile phase system of potassium di hydrogen phosphate buffer pH 7.2 and acetonitrile in the ratio (7:3) v/v with flow rate 1.0 ml/min on spursil $C_{18,}$ 250 X 4.6 mm, 5 µm particle size with UV detection at 224 nm gave a satisfactory chromatogram with naratriptan of retention time 3.285 min. It was shown in fig.6.

The present proposed system provides shorter analysis time and conserves mobile phase system. The method was validated based on United States pharmacopoeia and ICH parameters. The parameters are accuracy, precision, linearity, specificity, LOD, LOQ, ruggedness and robustness.

The specificity of the method was found by injecting the placebo and placebo spiked with standard and observed that there was no interference due to placebo. These chromatograms were shown in fig. 21-23 and data were reported in table 9.

The data regarding the linearity is given in table 7-8 and calibration graph were represented in fig.20 and chromatograms were shown in fig.15-19. From the linearity studies, the specified range was determined for the drug. 50-150 µg/ml of naratriptan and linearity co-efficient and percentage curve fitting was found to be 0.999 and 99.9% respectively for naratriptan.

The precision of the method was determined by replicate injections of sample solution. The percentage of RSD of assay was found to be in 1.21 for naratriptan which was with in the range of acceptance criteria of 2%. Thus the proposed method was found to be providing high degree of precision and reproducibility. The chromatograms are shown in fig.14 and data are reported in table 6. The precision of the system was determined by multiple injections of a set of solution of same concentration of naratriptan. The instrument response was found to be reproducible as found from % RSD of 1.55 of naratriptan that was

well with in the acceptance criteria of 2% the chromatograms are shown in fig.13 and data are reported in table 5.

The validation of the proposed reverse phase HPLC method was further verified by recovery studies. The percentage recovery was found to be with in 97-103% w/v of naratriptan. This serve a good index of accuracy and reproducibility of the proposed method and fig was shown in 10-12 and data were reported in table 4.

The limit of detection and limit of quantitation of the drugs were calculated and found to be 0.04956 and 0.1502 mcg per ml

Robustness was determined by carrying out the assay during which the flow rate ratio and column temperature was altered slightly. The % RSD when flow rate was altered to 0.8 ml was found to be 0.31% and % RSD when flow rate was altered to 1.2 ml was found 0.98% and 0.62 %, 1.34% was obtained on slight variation in the temperature ratio, indicated that the method is robust and does not show variation in the results on slight variation in flow rate ratio and temperature also give % RSD with in acceptance criteria and chromatograms were shown in fig.24-29 and data were reported in table.10-13 indicating lack of influence on the test results by operational variable for the proposed method

The ruggedness of the method was determined by performing the same assay by different analyst, on different day to check the reproducibility. The test results were found to be 98.27%, 98.12% for naratriptan when the analysis was carried out by two different analysts on two different days. The ruggedness of the method was also determined by performing the assay by different analysts on different instrument and results were found to be 98.33%, 98.74% for naratriptan. Thus the result were found to be highly reproducible in spite of variation in the condition which could be normally expected from analyst to analyst and analysis carried out on different days and the chromatograms are shown in fig.30-37 and data were reported table 14.

The system suitability parameters were calculated to ascertain the suitability of the proposed method on the given system on C_{18} column and mobile phase of potassium di hydrogen phosphate buffer pH7.2 and acetonitrile (7:3). The number of theoretical plates was found to be 6839 for naratriptan. The tailing factor for naratriptan was 1.01. The chromatograms were shown in fig 38.

CONCLUSION

As the literature survey reveals that there are very few method have been reported for the determination of the naratriptan Hcl and so a modified RP-HPLC method was developed for the estimation of drug in tablet dosage form and validated.

HPLC waters ALLIANCE 2695 with column spursil $C_{18,}$ 250 X 4.6 mm, 5 µ AGILENT was used. A volume of 10 µl of drug solution was injected and eluted with the mobile phase of potassium di hydrogen phosphate buffer pH 7.2: acetonitrile in the ratio of 7:3 v/v, which was pumped at the flow rate of 1.0 ml and detected by UV detector at 224 nm. The peak of naratriptan was found well separated at 3.285 min.

The developed method is economical, easy and it gives sharp peak with high resolution. The developed method is applied for the determination of naratriptan Hcl. The assay results comply to the label claim of the formulation.

The developed method was validated as per ICH guidelines using parameters like Accuracy, Precision Linearity and Range, Specificity, Ruggedness, LOD, LOQ and Robustness. Hence the developed method is found to be satisfactory and it complies with all validation parameters. So this developed method can be used for the routine analysis of naratriptan Hcl in tablet dosage form.

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