Estimation of Naratriptan Hydrochloride by using RP-HPLC method.

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Abstract:

Naratriptan Hydrochloride is a 5-hydroxytryptamine 1 receptor subtype agonist used to treat migraines. Naratriptan was purified using an RP-HPLC technique using a 25:75 v/v potassium di-hydrogen phosphate (KH2PO4):acetonitrile mobile phase. The detecting wavelength was 224nm, and the flow rate was kept constant at 1.0 ml/min. The linearity of the suggested Naratriptan technique was tested in the 5-25mg/ml range. The quantitation limit was 0.75 mg/ml, and the detection limit was 0.25 mg/ml. The % recovery of nariptan ranged from 98.22 to 99.04. The percent R.S.D. value for intraday precision research was 1% and 2% for interday precision study, demonstrating that the procedure was exact enough. The validation investigations were conducted in compliance with ICH guidelines.The approach was discovered to be particular, linear, exact, accurate, and resilient.

Keywords:Naratriptan; HPLC; Assay, Migraine

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Introduction

Naratriptan is a selective serotonin 5-HT1B/1D agonist and a second generation membrane of a drug class known as triptans. In many countries, Naratriptan is used to treat acute migraines. It is not intended for migraine prophylaxis or the treatment of hemiplegic, basilar, or opthalmoplegic migraine. Naratriptan's biological and pharmacokinetic profiles, as well as its excellent tolerability profile, unlike other selective 5-HT (1B/1D) agonists, it has a long duration of action. Naratriptan has a long duration of action. Naratriptan is a selective serotonin (5-HT1) agonist (N-Methyl-3-(1-methyl4-piperidinyl)-1H-indole-5-ethanesulphonamide mono hydrochloride). It is used to treat migraine attacks during the acute headache phase. It is not intended to be used as a preventative measure. It is taken orally as the hydrochloride salt, and doses are calculated based on the base. 1.11 mg of NAR is approximately equivalent to 1.00 mg of NAR base. The recommended dose of NAR in the United Kingdom is 2.5 mg, while in the United States it is 1.0 or 2.5 mg. The study's goal was to develop a simple, sensitive, selective, and repeatable analytical method for quantitative drug estimation.

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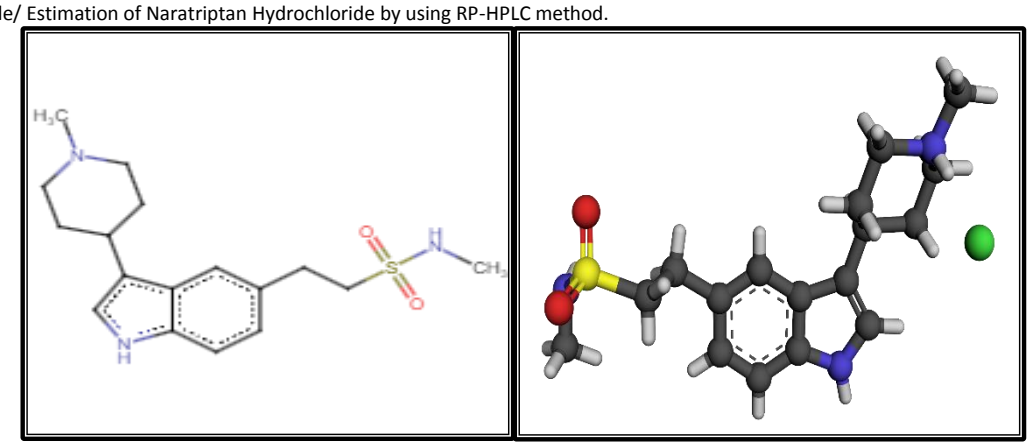


Fig.1: Structure of Naratriptan

Materials and Method

Naratriptan of pharmaceutical grade was supplied as a gift sample by USV limited Mumbai, potassium dihydrogen phosphate, acetonitrile have been bought from SD fine Mumbai India.

Determination of Wavelength Maxima (λ_{max})

In a 10 ml volumetric flask, 10 mg of drug was accurately weighed and dissolved in 10 ml of 6.8 pH phosphate buffer. Pipette 0.1 ml of this stock solution into a 10 ml volumetric flask and top up with 6.8 pH phosphate buffer. Using a UV/Vis double beam spectrophotometer, the resulting solution was scanned between 200 and 400 nm.

Preparation of calibration curve of Naratriptan

Calibration curve in 6.8 pH phosphate buffer: In a 10 ml volumetric flask, 10 mg of drug was accurately weighed and dissolved in 5 ml of 6.8 pH phosphate buffer, and the volume was made up to 10 ml with 6.8 pH phosphate buffer. The resulting solution has a concentration range of 5-25 g/ml Naratriptan and 1 ml pipette out and transfer into a 10 ml volumetric flask and volume make up with 6.8 pH phosphate buffer to prepare a suitable dilution. The spectrum of this solution was measured using a UV spectrophotometer (Labindia-3000+) in the 200-400 nm range. The absorbance of these solutions was measured in comparison to a 6.8 pH phosphate buffer.Plotting absorbance versus concentration yielded the calibration curve. The best-fit straight line was obtained using the linear regression analysis programmed.

Selection of Diluent

The diluent used to prepare the sample was compatible with the mobile phase and had no significant effect on analyte retention and resolution. Following several trials, 0.1 N HCl was chosen as a diluent.

Chromatographic Condition

Selection of Mobile Phase

Initially, different ratios of mobile phase were tried to estimate Naratriptan in fixed dosage form. Table shows the outcome.

Taking into account system suitability parameters such as RT, tailing factor, number of theoretical plates, and HETP, the mobile phase discovered to be most suitable for analysis was a 25:75v/v ratio of 20mM KH2PO4: Acetonitrile (pH adjust with 3.5 with OPA). The mobile phase was filtered through 0.45m filter paper and then degassed using sonication to remove particulate matter. The analysis flow rate was 1.0 ml/min.

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Selection of separation variable

Table 1: Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5µ
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
20mM KH_2PO_4	25
Acetonitrile	75
Diluent	0.1 N HCl
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 µl
Detection wavelength	224nm
Retention time	
Naratriptan	9.726 ± 0.002min

Calibration of standards

Preparation of Stock Solution:

10 mg API of NARA was accurately weighed and transferred into a separate 10 ml volumetric flask, and 5ml of 0.1 N HCl was added as diluents, sonicated for 20 minutes, and volume was made up to 10ml with 0.1 N HCl to obtain a solution concentration of 1000mg/ml (Stock-A)

Preparation of Sub Stock Solution:

5 ml of each drug's stock-A solution was transferred separately into a 50ml volumetric flask and diluted up to 50 ml with diluent (0.1 N HCl) to give a concentration of 100g/ml of NARA (Stock-B).

Preparation of Different Solution

In a 10 ml volumetric flask, 0.5ml, 1.0ml, 1.5ml, 2.0ml, and 2.5ml of stock-B were taken separately and volume was made up to 10ml with water (0.1 N HCl). This yields NARA solutions of 5g/ml, 10g/ml, 15g/ml, 20g/ml, and 25g/ml.

Method Validation of Naratriptan Hydrochloride

Linearity, Specificity and selectivity, LOD, LOQ, accuracy, precision and robustness of validated method were performed

Linearity

The drug Naratriptan HCL has been tested in pure solution for linearity of calibration curves (i.e. peak area Vs concentrations) over a concentration range of about 5-25 g/ml. There was only about 15 minutes of eluting time. Using regression analysis, the regression lines associated with normal drug levels. In the studied range, the calibration curves were linear, and a regression equation was obtained.

Specificity and Selectivity

The ability of a method to distinguish between the intended analyte and other sample elements from other potential components such as impurities, degradants, or excipients is referred to as specificity.

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Precision

Repeatability

The precision of the method was determined by computing the percentage RSD of peak areas from six standard concentration replicate injections. The average RSD for naratriptan hydrochloride was found to be 0.09percent.

Reproducibility

The RSD of peak areas was calculated from eight replicate injections of standard concentrations. Naratriptan Hydrochloride had an average RSD of 0.12 percent. Intermediate **Precision (Intra and Inter-Day)**

The precision shown on different days of analytical results variability within the same laboratory. For three distinct normal levels, the RSD of peak regions from three replicate injections was calculated. For intra-day research, the average RSD of nariptan hydrochloride was found to be 0.50 percent. The average RSD of Naratriptan Hydrochloride was 0.06 percent in inter-day studies.

Accuracy

Recovery tests were used to determine the method's accuracy. The drug's reference requirements were added to the formulation at 80%, 100%, and 120% concentrations. The recovery studies were repeated three times, and the percentage of recovery and relative standard deviation of recovery were calculated and displayed in Tables 10-12.

% Recovery = (Amount found/Amount added) X 100.

Assay of Naratriptan Hydrochloride

Transferred exactly 10 mg of pure Naratriptan Hydrochloride to a 10 ml volumetric flask. Methanol (1000 g/ml) was used to the limit. 1 ml was transferred to a volumetric flask of 10 ml, and the volume was measured against the portable stage mark (100 g / ml). A volumetric flask of 10 ml was filled with 0.5 ml of the above solution, and the volume was added to the mark. Under the chromatographic conditions described above, the solution was injected and the maximum region was measured. The assay procedure was repeated three times, and the drug weight used in the assay was calculated each time. Table 18 shows the percentage of drug detection, as well as the mean and standard deviation.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

According to ICH guidelines, the procedure's Limit of Detection (LOD) and Limit of Quantitation (LOQ) were determined on samples containing very low concentrations of analytes. LOD was expressed using the mathematical formula method by determining the lowest level at which the analyte can be reliably detected. The lowest concentration of analyte in standards that can be measured reproducibly and with acceptable precision was defined as the LOQ. Table 10 shows the LOD and LOQ values for Naratriptan Hydrochloride.

Robustness

The optimum HPLC requirements for this technique were mildly modified in order to evaluate the technique's robustness for samples of Naratriptan hydrochloride dissolved in the drug matrix. Minor changes include flow rate (0.1 ml/min), mobile phase composition (two millilitres), and wavelength detection (three nanometers).

System suitability

Trials are used to evaluate techniques that can produce results with acceptable accuracy and precision. The system suitability was performed after the method development and validation were completed. Five replicate drugs were tested at concentrations of 10 g/ml of Naratriptan hydrochloride to assess the system's suitability and parameters such as plate number (n) and tailing.

Forced degradation studies of Naratriptan Hydrochloride

Degradation studies were undertaken in compliance with ICH guidelines. The goal of this study was to identify degradation products, which would aid in determining degradation pathways and the intrinsic stability of the therapeutic molecule. To validate the selectivity of the proposed

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approach, degradation studies were carried out under acidic, alkaline, oxidative, picture, and heat conditions.

Procedure for forced degradation studies

Naratriptan Hydrochloride API was stressed under various conditions to conduct forced degradation experiments to establish whether the analytical method and assay were stability indicated or not. Intentional degradation was attempted under acidic (0.1N HCl, 1N HCl, 1N HCl heated at 600C), alkali (0.1N NaOH, 1N NaOH, 1N NaOH heated at 600C), oxidative (0.3% H2O2, 3% H2O2, and 10% H2O2), photo (sunlight), and thermal (heated at 1050C) conditions to assess the ability of the proposed method to separate Naratriptan Hydrochloride

If reasonable degradation is observed under the key parameters, the test can be discontinued at this point. However, if no degradation occurs under the conditions tested, the medicine should be treated to higher strengths and a longer duration. If total deterioration is detected after starting circumstances, the intensity of acid / alkali / oxidative resistance may be reduced with a drop in reaction temperature.

Acid degradation

25 milligrammes of pure Naratriptan Hydrochloride was precisely weighed and put into a 25 mL clean dry volumetric flask. The volume was topped up with 1N HCl and kept at 600°C (1000 g/ml). Periodically (0, 1, 3, 6 hours), 1 ml was transferred to a 10 ml volumetric flask and topped up with mobile phase (100 g/ml). 1 ml of the aforementioned solution was transferred to a 10 ml volumetric flask, 5 ml of mobile phase was added, and the pH was adjusted to between 3-4 by adding 1N NaOH and diluting with mobile phase up to the mark (10 g/ml). The solution was injected under the chromatographic conditions described above, and the peak area was measured. Figure 12 shows the represented data.

Alkaline degradation

25 milligrammes of pure Naratriptan Hydrochloride is accurately weighed and put to a 25 ml clean dry volumetric flask. The volume was increased to 1N NaOH and held at 600C (1000 g / ml). 1 ml was taken from a 10 ml volumetric flask at intervals of 0, 3, 6, and 9 hours and made up to the mobile phase mark (100 g / ml). 1 ml of the aforementioned solution was transferred to a 10 ml volumetric flask, 5 ml of mobile phase was added, and the pH was adjusted to between 3-4 by adding 1N HCl and diluting with mobile phase up to the mark (10 g/ml). The solution was injected under the chromatographic conditions described above, and the peak area was measured.Figure 12 shows the represented data.

Oxidative degradation

25 milligrammes of pure Naratriptan Hydrochloride is accurately weighed and put to a 25 ml clean dry volumetric flask. The flask was filled to capacity with 10% H2O2 (1000 g / ml) and set aside at room temperature. Periodically (0, 1, 24, 48, 72, 120 hours), 1 ml of mobile phase (100 g / ml) was added to a 10 ml volumetric flask. 1 ml of the aforementioned solution was placed in a 10 ml volumetric flask and built up to the mobile phase mark (10 g / ml). The solution was injected under the chromatographic conditions described above, and the maximum region was measured.Figure 12 shows the represented data.

Photo degradation

25 mg of pure Naratriptan Hydrochloride were accurately weighed and placed to a 25 ml clean dry volumetric flask. The volume was adjusted with HPLC water (1000 g / ml). The flask was exposed to sunlight for 6 hours. Periodically (0, 1, 3, 6 hours), 1 ml was collected from a 10 ml volumetric flask and built up to the portable stage mark (100 g / ml). 1 ml of the aforementioned solution was placed in a 10 ml volumetric flask and built up to the mobile phase mark (10 g/ml). The solution was injected under the chromatographic conditions described above, and the maximum region was

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measured.Figure 12 shows the represented data.

Thermal degradation of solid sample at 105^{0}C

300 mg of pure Naratriptan Hydrochloride was precisely weighed, placed in a petri dish, and stored at 105^{0}C in a temperature-controlled oven. Periodically (0, 1, 3, 6 hours), 10 mg of material was weighed, transferred to a 10 ml volumetric flask, and topped up with HPLC water (1000 g/ml). 1 ml of the aforementioned solution was placed in a 10 ml volumetric flask and topped up with mobile phase (100 g/ml). 1 ml of the aforementioned solution was extracted and transferred to a 10 ml volumetric flask before being brought up to the mark with mobile phase (10 g/ml). The solution was injected under the chromatographic conditions described above, and the peak area was measured. Figure 12 shows the represented data.

Results for the development and validation of RP-HPLC method for determination of Naratriptan Hydrochloride

Determination of wavelength maxima in 5.5 pH phosphate buffer: The wavelength maxima for Naratriptan were found 226nm.

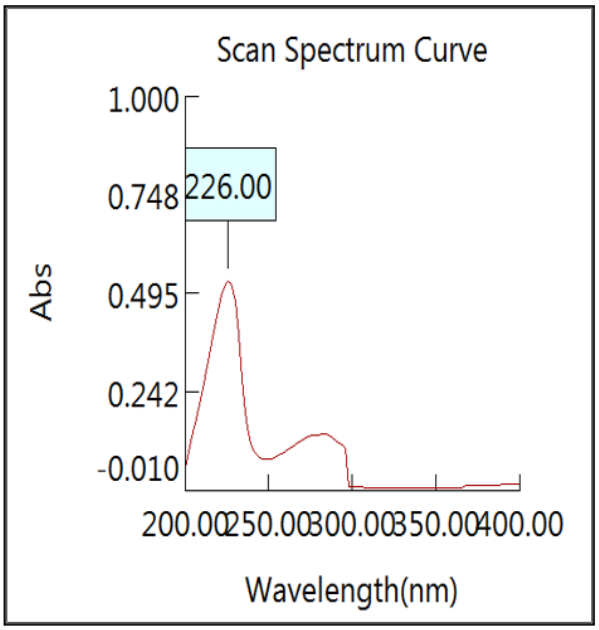


Fig. 2: Wavelength maxima of Naratriptan 5.5 pH phosphate buffer

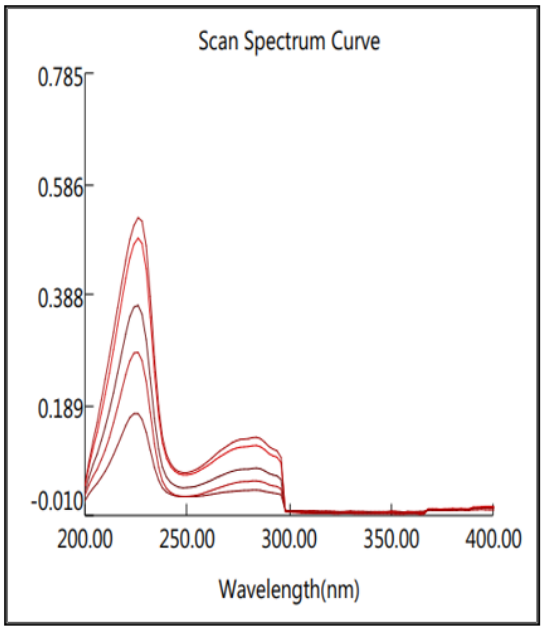


Fig.3: Preparation of calibration curve of Naratriptan 5.5 pH phosphate buffer

Calibration curve of Naratriptan in Phosphate Buffer5.5 pH

The calibration curve was calculated by plotting concentration vs absorbance. In the concentration range of 5-25 g/ml in Phosphate buffer 5.5 pH, naratriptan demonstrated a linear connection with a

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correlation coefficient of 0.9964.

SN	Conc. (µg/ml)	Absorbance* Mean ± SD
1.	0	0
2.	5	0.136±0.001
3.	10	0.246±0.001
4.	15	0.355±0.003
5.	20	0.451±0.004
6.	25	0.551±0.001

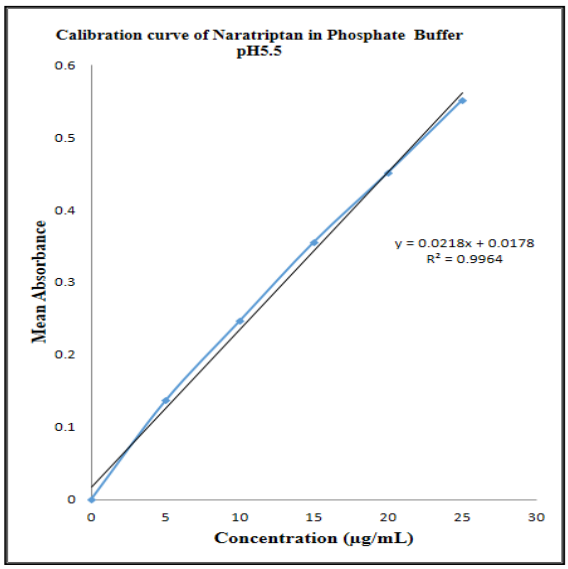


Fig 4: Calibration curve of Naratriptan in at 226nm Phosphate Buffer 5.5 pH

Drug	Parameters	Values
Naratriptan	Beer's Law Range	5-25 µg/ml
	Regression Coefficient	0.9964
	Regressed line equation (y = mx + c)	Y = 0.0218x + 0.0178

Where y is the response, x is the concentration, m is the slope and c is the intercept of a best fit line to the data.

Table 2: Statistical parameters related to standard curve of Naratriptan

Determination of wavelength maxima in 6.8 pH phosphate buffer: The wavelength maxima for Naratriptan were found 226nm.

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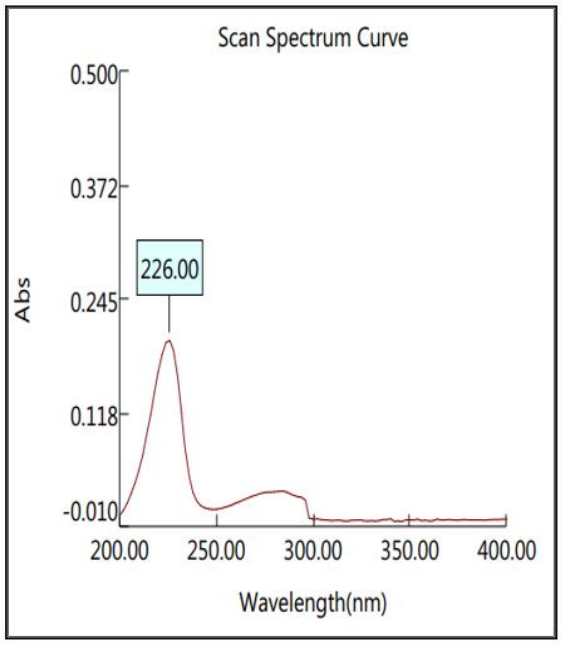


Fig. 5: Wavelength maxima of Naratriptan phosphate buffer 6.8 pH

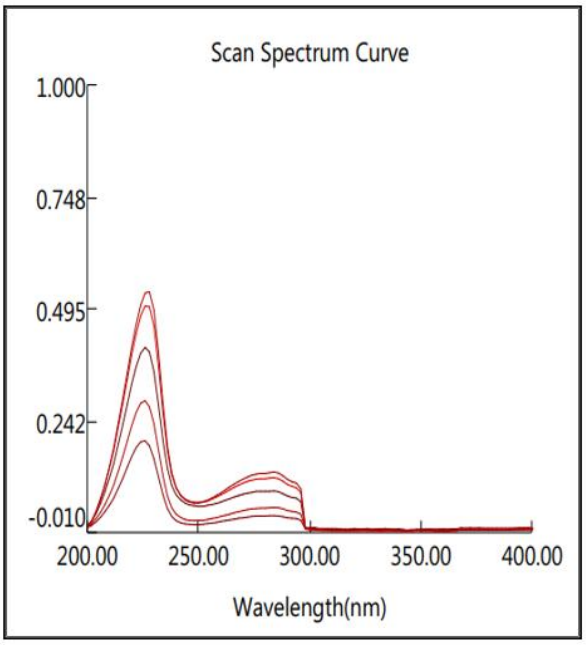


Fig. 6: Preparation of calibration curve of Naratriptan phosphate buffer 6.8 pH

Calibration curve of Naratriptan in 6.8 pH Buffer

The calibration curve was calculated by plotting concentration vs absorbance. In the concentration range of 5-25 g/ml in 6.8 pH buffer, naratriptan demonstrated a linear connection with a correlation coefficient of 0.996.

SN	Conc. (µg/ml)	Absorbance* Mean ± SD
1.	0	0
2.	5	0.145±0.006
3.	10	0.248±0.001
4.	15	0.361±0.006
5.	20	0.460±0.010
6.	25	0.562±0.01

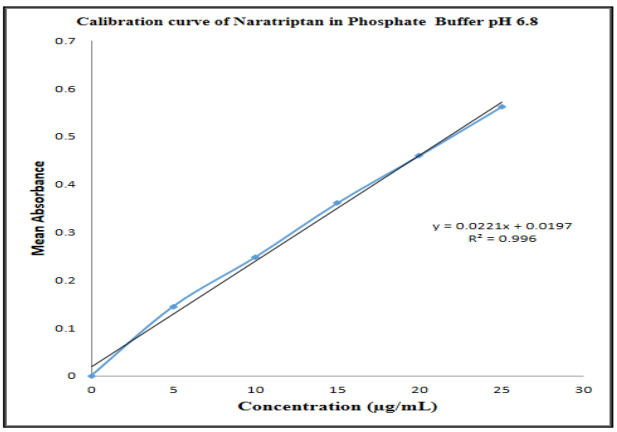


Fig. 7: Calibration curve of Naratriptan in at 226nm of Phosphate Buffer pH 6.8

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Drug	Parameters	Values
Naratriptan	Beer's Law Range	5-25 µg/ml
	Regression Coefficient	0.996
	Regressed line equation (y = mx + c)	Y=0.0221+0.0197

Where y is the response, x is the concentration, m is the slope and c is the intercept of a best fit line to the data.

Table 3: Statistical parameters related to standard curve of Naratriptan

Method development and optimisation of Naratriptan hydrochloride

Selection of wavelength

A double beam UVVIS spectrophotometer (Lab India) was used to get the absorption spectrum of Naratriptan hydrochloride in water. It indicates that Naratriptan hydrochloride has a maximum methanol content at 226 nm. The same wavelength was employed in the creation of the HPLC process, where contaminants can also be detected.

Selection of Mobile Phase

Initially, numerous ratios of mobile phase were explored to estimate Naratriptan in fixed dosage form. Taking into account system appropriateness parameters such as RT, Tailing factor, number of theoretical plates, and HETP, the mobile phase discovered to be best acceptable for analysis was 20mM KH2PO4: Acetonitrile in a 25:75v/v ratio (pH adjust with 3.5 with OPA). To remove particulate debris, the mobile phase was filtered through 0.45m filter paper and then degassed using sonication. The flow rate used for analysis was 1.0 ml/min.

Mobile Phase	Ratio	Retention Time
		NARA
	Remark	
20mM KH_2PO_4: Acetonitrile	25 : 75 v/v	Most Suitable

Table 4: Mobile Phase Selection

Selection of Diluent

The diluent used to prepare the sample was compatible with the mobile phase and had no noticeable effect on analyte retention and resolution. Through numerous studies, 0.1 N HCl was chosen as a diluent.

1. Preparation of Stock Solution:

Accurately weighed 10 mg API of NARA was placed into a separate 10 ml volumetric flask and 5ml of 0.1 N HCl was added as diluents, sonicated for 20 minutes, and volume was brought up to 10ml with 0.1 N HCl to obtain a solution concentration of 1000mg/ml (Stock-A)

2. Preparation of Sub Stock Solution:

5 ml of each drug's stock-A solution was put into a 50ml volumetric flask individually and diluted up to 50 ml with diluent (0.1 N HCl) to give a concentration of 100g/ml of NARA (Stock-B).

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3. Preparation of Different Solution

In a 10 ml volumetric flask, 0.5ml, 1.0ml, 1.5ml, 2.0ml, and 2.5ml of stock-B were taken separately and volume was brought up to 10ml with water (0.1 N HCl). This yields NARA solutions of 5g/ml, 10g/ml, 15g/ml, 20g/ml, and 25g/ml.

4. Linearity and Calibration Graph

A series of dilutions ranging from 5-25mg/ml for NARA were developed to establish the linearity of the analytical technique. After filtering the solution via a 0.45mm membrane filter and injecting it, chromatograms were recorded at 224 nm and the process was repeated five times. A calibration graph was drawn between the mean peak area and the corresponding concentration, and a regression equation was developed.

4. Linearity and Calibration Graph

A series of dilutions ranging from 1-5 mg/ml for NARA were made to establish the linearity of the analytical technique. After filtering the solution via a 0.45mm membrane filter and injecting it, chromatograms were recorded at 303 nm and the process was repeated five times. A calibration graph was drawn between the mean peak area and the corresponding concentration, and a regression equation was developed.

Standard Concentration μg/ml	Area under Curve (AUC)						Mean
	Rep-1	**Rep-2**	**Rep-3**	**Rep-4**	**Rep-5**	**Rep-6**	
5	250.658	255.658	245.478	243.325	240.159	240.658	245.989
10	515.458	525.654	510.321	515.458	520.365	523.478	518.456
15	756.458	765.589	769.985	764.412	752.478	745.522	759.074
20	1020.365	1025.458	1015.478	1020.365	1018.854	1022.145	1020.444
25	1254.478	1245.587	1235.658	1247.745	1285.658	1245.658	1252.464
Correlation coeff. Coeff (r^2)							0.999
Slope (m)							50.43
Intercept (c)							2.287

Table 7: Linearity of NARA

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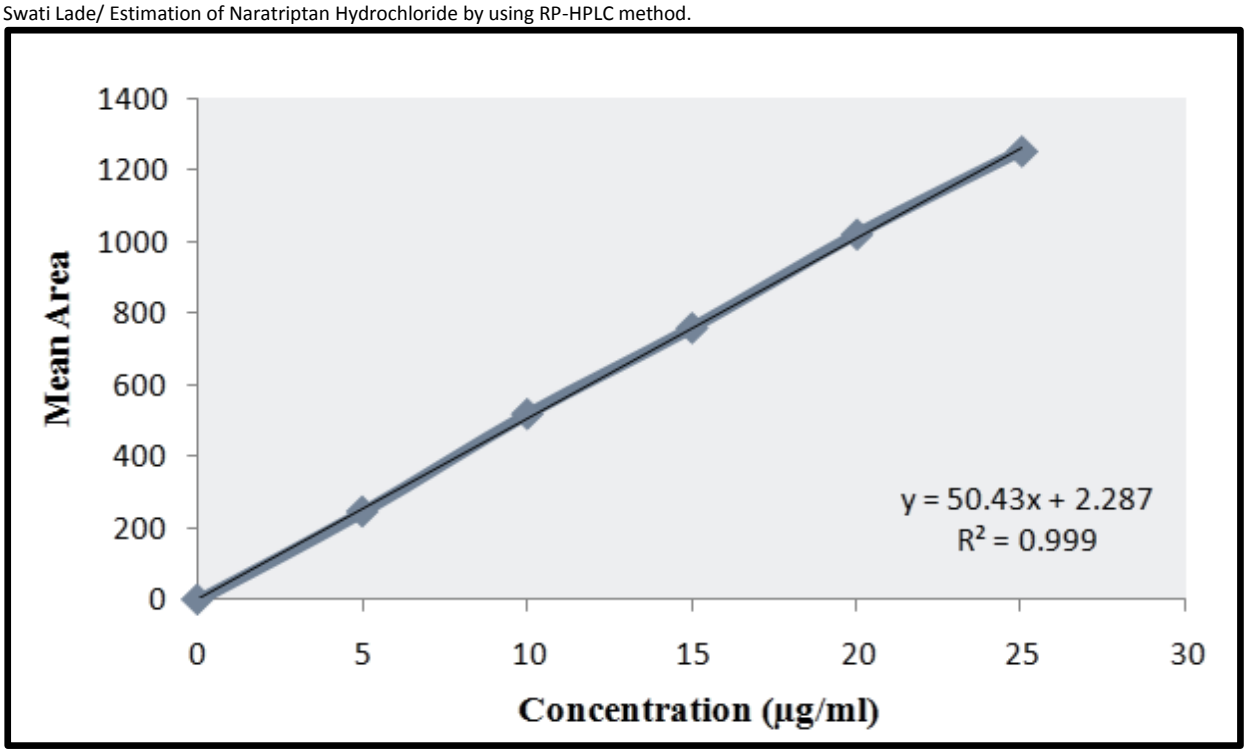


Fig 8: Calibration Curve of NARA

4.1System Suitability Parameters

The separation variables were set, and the column was allowed to saturate with mobile phase at 1.00 ml/min. Six replicates of the working standard of NARA 10 mg/ml for NARA were injected separately after the column had been completely saturated. For each chromatogram, a peak report and a column performance report were generated.

System suitability Parameter →	RT	AUC	No. of theoretical plates	Tailing factor
Rep-1	9.726	515.458	2650	1.12
Rep-2	9.725	525.654	2645	1.15
Rep-3	9.728	510.321	2630	1.14
Rep-4	9.723	515.458	2678	1.16
Rep-5	9.728	520.365	2652	1.17
Rep-6	9.726	523.478	2630	1.14
Mean	9.726	518.456	2647.500	1.147
S.D.	0.002	5.742	17.751	0.018

Table 8: System Suitability Parameters of NARA

Validation of developed Method

The validation of analytical methods is done as per ICH guidelines:

Linearity

The ability of an analytical process (within a specific range) to produce results that are directly proportional to the area of analyte in the sample is referred to as linearity. After analysing five different concentrations (from 5 to 25 g/ ml for NARA), the calibration plot was constricted, and areas for each concentration were recorded three times, and the mean area was computed. The regression equation and correlation coefficient of the curve are provided, and the drug's standard calibration curve is depicted in the picture. The response ratio (response factor) was calculated by dividing the AUC by the concentration.

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Concentration (µg/ml)	Mean AUC	Response Ratio
5	245.9893	49.198
10	518.4557	51.846
15	759.074	50.605
20	1020.444	51.022
25	1252.464	50.099
Mean		50.668
SD		1.107
%RSD		2.185

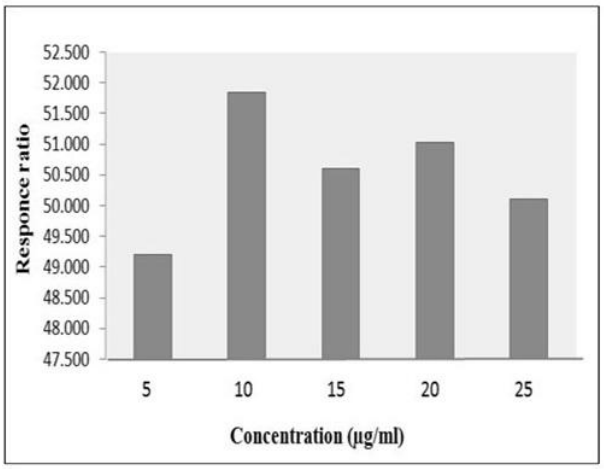


Fig. 9: Response ratio of Naratriptan for Linearity and specificity

The method's specificity was tested in order to determine unequivocally the analyte presence of the components that would be predicted to be present, such as contaminants, degradationproducts, and matrix components.

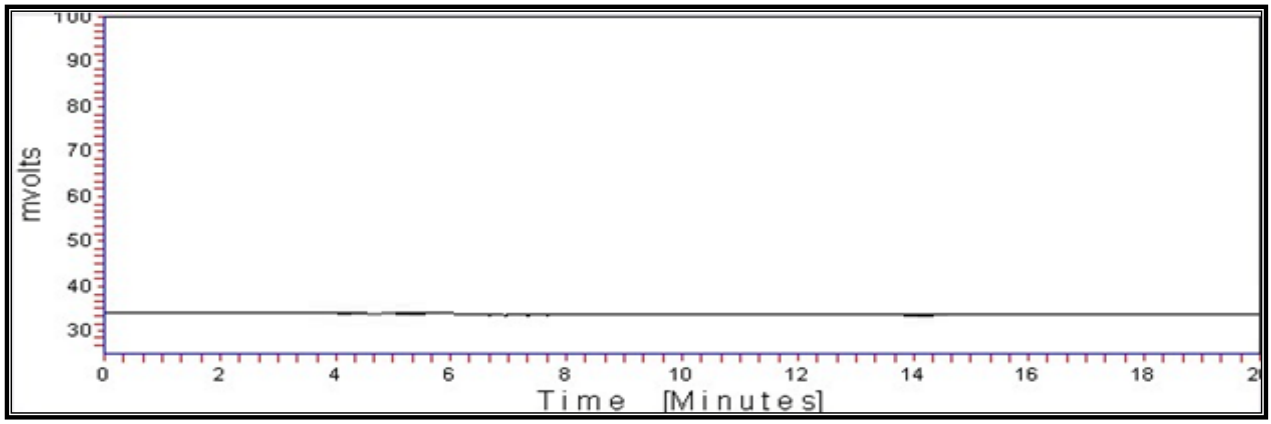


Figure 10: Chromatogram of blank

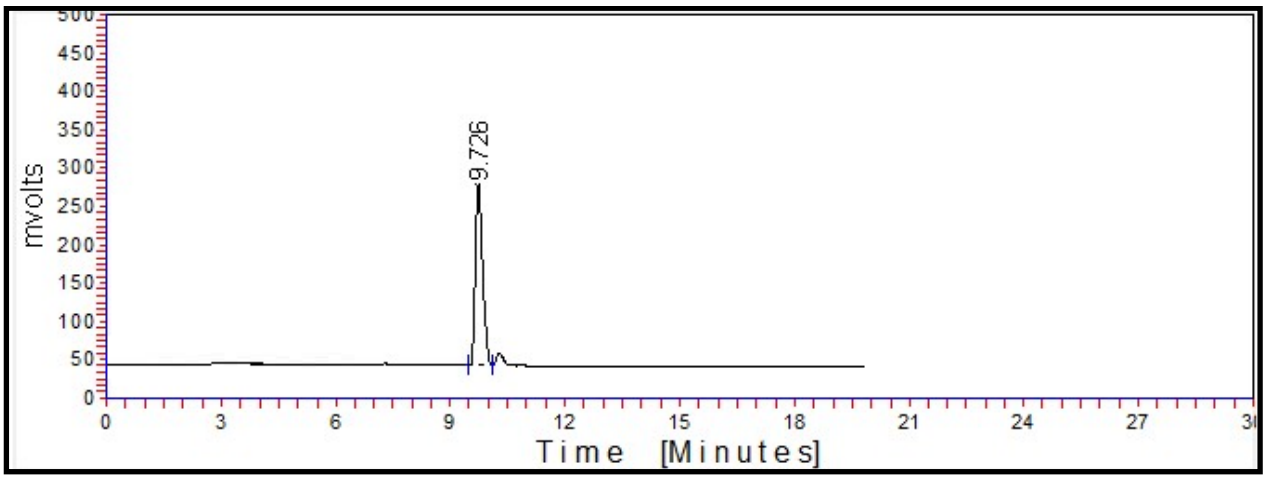


Fig.11: Graph of Naratriptan in 20mM KH_2PO_4: Acetonitrile (pH adjusts 3.5 with OPA)

Accuracy

To calculate the accuracy of the devised approach to preanalysed sample solution, a certain concentration of standard medication (80%, 100%, and 120%) was added and its recovery was measured.

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Conc. of sample (mg)	Amt. Added (mg)	Conc. Found. (µg/ml)			% conc. Found			Mean % conc.
		Rep-1	**Rep-2**	**Rep-3**	**Rep-1**	**Rep-2**	**Rep-3**	
5	4	3.95	4.01	3.87	98.75	100.25	96.75	**98.58**
10	8	7.95	7.85	7.96	99.38	98.13	99.50	**99.00**
15	12	11.85	11.78	11.65	98.75	98.17	97.08	**98.00**
							Mean	**98.53**
							SD	**0.502**
							% RSD	**0.510**

Table 10: Recovery Study of NARA (80% Level)

Conc. of sample (mg)	Amt. Added (mg)	Conc. Found. (µg/ml)			% conc. Found			Mean % conc.
		Rep-1	**Rep-2**	**Rep-3**	**Rep-1**	**Rep-2**	**Rep-3**	
5	5	4.95	4.85	4.92	99.00	97.00	98.40	**98.13**
10	10	9.98	9.95	9.97	99.80	99.50	99.70	**99.67**
15	15	14.85	14.75	14.95	99.00	98.33	99.67	**99.00**
							Mean	**98.93**
							SD	**0.769**
							% RSD	**0.777**

Table 11: Recovery Study of NARA (100% Level)

Conc. of sample (mg)	Amt. Added (mg)	Conc. Found. (µg/ml)			% conc. Found			Mean % conc.
		Rep-1	**Rep-2**	**Rep-3**	**Rep-1**	**Rep-2**	**Rep-3**	
5	6	5.85	5.96	5.87	97.50	99.33	97.83	**98.22**
10	12	11.65	11.78	11.92	97.08	98.17	99.33	**98.19**
15	18	17.85	17.65	17.98	99.17	98.06	99.89	**99.04**
							Mean	**98.48**
							SD	**0.479**
							% RSD	**0.486**

Table12: Recovery study of NARA (120% Level)

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Precision

The stock solution was prepared. The precision are established in three differences:

Repeatability

The repeatability was tested for five replicates at five concentrations in the linearity ranges of 5, 10, 15, 20, and 25mg/ml for NARA to determine precision under the same operating conditions during short time intervals.

Conc. / Rep.	Concentration Found (μg/ml)					
	5	**10**	**15**	**20**	**25**	
Replicate-1	4.82	9.98	14.85	19.96	24.85	
Replicate-2	4.89	9.95	14.98	19.85	24.95	
Replicate-3	5.02	9.87	14.85	19.78	24.85	
Replicate-4	4.78	9.92	14.74	19.96	24.65	
Replicate-5	4.99	9.98	14.86	19.86	24.88	
Mean	4.90	9.94	14.86	19.88	24.84	
% Mean	98.00	99.40	99.04	99.41	99.34	**99.04**
SD	0.104	0.046	0.085	0.078	0.112	**0.08**
% RSD	**0.106**	**0.047**	**0.086**	**0.078**	**0.112**	**0.09**

Table13: Repeatability of NARA

Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for NARA reported in table.

Conc. / REP.	Concentration found (μg/ml)					
	5	**10**	**15**	**20**	**25**	
Day 1	4.95	9.98	14.65	19.95	24.78	
Day 2	4.98	9.95	14.78	19.98	24.55	
Day 3	4.96	9.89	14.96	19.65	24.98	
Mean	4.96	9.94	14.80	19.86	24.77	
% Mean	99.27	99.40	98.64	99.30	99.08	**99.14**
SD	0.015	0.046	0.156	0.182	0.215	**0.12**
% RSD	**0.015**	**0.046**	**0.158**	**0.184**	**0.217**	**0.12**

Table 14: Day-to-Day variation of NARA

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Analyst to Analyst

Conc. / Rep.	Concentration found (µg/ml)					
	5	**10**	**15**	**20**	**25**	
Analyst 1	4.95	9.95	14.78	19.96	24.98	
Analyst 2	4.88	9.87	14.92	19.98	24.87	
Mean	4.92	9.91	14.85	19.97	24.93	
% Mean	98.30	99.10	99.00	99.85	99.70	**99.19**
SD	0.049	0.057	0.099	0.014	0.078	**0.06**
% RSD	**0.050**	**0.057**	**0.100**	**0.014**	**0.078**	**0.06**

Table 15: Analyst to analyst variation of NARA

Robustness

Small but controlled fluctuations in the concentration of the mobile phase were made in accordance with ICH standards to test the method's ability to stay unaffected. The mobile phase ratio was altered from 20mM KH2PO4:Acetonitrile (25:75% v/v) to (20:80% v/v). The robustness results are shown in the table.

Conc. / Rep.	Concentration Found (µg/ml)					Mean
	5	**10**	**15**	**20**	**25**	
Replicate-1	5.01	9.95	14.85	20.01	24.78	
Replicate-2	4.98	9.98	14.96	19.85	24.65	
Replicate-3	4.78	9.85	14.95	19.78	24.78	
Replicate-4	4.65	9.78	14.78	19.95	24.65	
Replicate-5	4.88	9.65	14.86	19.67	24.85	
Mean	4.86	9.84	14.88	19.85	24.74	
% Mean	**97.20**	**98.42**	**99.20**	**99.26**	**98.97**	98.61
SD	**0.148**	**0.134**	**0.075**	**0.135**	**0.089**	0.120
% RSD	0.152	0.136	0.076	0.136	0.090	0.120

Table 16: Robustness of NARA

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Name	LOD (µg/ml)	LOQ (µg/ml)
NARA	**0.25**	**0.75**

Table17: LOD and LOQ of NARA

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ASSAY OF NARATRIPTAN

SN	Conc. (μg/ml)	Conc. Obs,(n=3)	%Assay
1	**5**	**4.034±0.025**	**99.68**

Table 18:Assay of NARA

SN	Time in hr	% degradation
1	0	0
2	2	6.59
3	4	10.29
4	6	16.40

Acid degradation of Naratriptan HCl in 1N HCl at 60^{0}C

SN	Time in hr	% degradation
1	0	0
2	2	4.46
3	4	8.99
4	6	11.40

Alkaline Degradation of Naratriptan HCl in 1 N NaoH at 60^{0}C

SN	Time in hr	% degradation
1	0	0
2	12	0.89
3	24	2.98
4	36	7.63

Oxidative degradation of Naratriptan hydrochloride in 10%h2O2 at Room Temp.

SN	Time in hr	% degradation
1	0	0
2	2	6.49
3	4	12.40
4	6	17.63

Photo degradation of Naratriptan Hydrochloride

SN	Time in hr	% degradation
1	0	0
2	2	5.36
3	4	7.75
4	6	16.21

Thermal degradation of Naratriptan hydrochloride

Fig. 12: Degradation study of Naratriptan Hcl.

Discussion

According to ICH criteria, the method was validated for all validation parameters. Naratriptan Hydrochloride had a linearity range of 5-25 g/ml. The correlation coefficient was equal to 0.999. In the Precision studies, the % RSD results were 2%. This confirmed that the procedure was accurate enough. The method's accuracy was validated using recovery trials at (80%,100%, and 120%) and was determined to be significant and within specification limitations, with percent recovery of 98.53,98.93, and 99.04 (within acceptable range 98-102%).The assay result was 99.68% (between 95 and 105%). The approach also meets the requirements for robustness parameters. Naratriptan Hydrochloride LOD was found to be 0.25 g/ml, and Naratriptan Hydrochloride LOQ was found to be 0.75 g/ml. For the measurement of Naratriptan Hydrochloride API, a forced degradation HPLC technique with a mobile phase solution of 20mM KH2PO4: Acetonitrile in the ratio of 25: 75 v/v was devised. On a C18 column (250 x 4.6 mm, particle size 5 m), a flow rate of 1ml/min was utilised. Naratriptan Hydrochloride retention time was 9.726 minutes and was determined to be linear, exact, accurate, specific, and robust. As a result, the method can be utilised on a regular basis to estimate Naratriptan Hydrochloride API.The findings of the API stress testing, conducted in accordance with ICH norms, demonstrated that deterioration occurred under acidic, alkaline, oxidising, photolytic, and thermal conditions. The medication was highly light sensitive, as well as susceptible to thermal, acid, alkaline, and oxidative deterioration. The essential therapeutic agent in the treatment of

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migraine is naratriptan hydrochloride. Among the analytical methods available for estimate and quantification, the HPLC method stands out as trustworthy in a wide range of applications, prompting the author to create and validate the method in accordance with ICH requirements.According to ICH criteria, the method was validated for all validation parameters. Naratriptan Hydrochloride had a linearity range of 5-25 g/ml, with r2 = 0.999. The percentage RSD for intra- and inter-day precision was 2%. The test has been completed. Recovery experiments validated the method's accuracy, which was determined to be considerable and within specification limitations, with % recovery 98.53-99.04% (within acceptable range 98-102%). The approach also matched the requirements for robustness parameters. A stability research on Naratriptan Hydrochloride was performed, as well as an efficient HPLC method for Naratriptan Hydrochloride measurement. The findings of the API stress testing, conducted in accordance with ICH norms, demonstrated that deterioration occurred under acidic, alkaline, oxidising, photolytic, and thermal conditions.The drug was highly light sensitive, as well as susceptible to thermal, acid, alkaline, and oxidative deterioration. In methanol, Naratriptan Hydrochloride has a maximum absorbance of 226 nm. Because the molecule has fewer chromophoric groups, the degradants generated may lack chromophores at the desired wavelength. As a result, the degradants may not have been detected at the chosen wavelength.

Conclusion

The proposed HPLC methods for estimating NARATRIPTAN drug are simple, accurate, and reproducible, and have been validated in accordance with ICH recommendations. HPLC methods were used to determine three samples of pure Naratriptan, and the results were correlated. Statistical tests show that the proposed HPLC methods save analytical time and are appropriate for regular quantification of Naratriptan in pharmaceutical formulations and bulk drugs.

Conflict of interest: Author Declare That There Will Be No Conflict of Interest

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