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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF NARATRIPTAN IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

An isocratic RP-HPLC method was developed and validated for the quantitation of Naratriptan in tablet dosage form. Quantitation was achieved using a reversed-phase sunfire, $C_{18,}$ 5 µ (250 x 4.6 mm) column at ambient temperature with mobile phase consisting of Ammonium acetate Buffer (pH 3) and Acetonitrile in the ratio (50:50). The flow rate was 1.0 mL/min. Measurements were made at a wavelength of 225nm. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay method was found to be linear from 2.5-15.5µg/mL was within the acceptable range. The developed method was successfully applied to estimate the amount of of Naratriptan in tablet dosage form.

Key Words:- Naratriptan, Method development, Validation, RP-HPLC.

INTRODUCTION

Naratriptan is Antimigrainic agent. It is chemically, N-methyl-2-[3-(1-methylpiperidin-4-yl)-1H-indol-5-yl]ethane-1- Sulfonamide. Its Molecular Formula is C17H25N3O2S (Anonymous 1). This paper now describes an HPLC method for the determination of Naratriptan in tablets. Literature review revealed that only few methods have been reported for the quantification of Naratriptan. So there is a need of a simple, economical and proper method for estimation of Naratriptan in tablet dosage form. The method is rapid, accurate and precise. The method was validated by following the analytical performance parameters suggested by the International Conference on Harmonization. The aim of the present study was to develop and validate a simple and fast LC method, through evaluation of the parameters of linearity, precision, accuracy, detection and quantitation limits, robustness, and to determine Naratriptan in pharmaceutical formulations. The structure of Naratriptan is shown in Fig 1.

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Chemicals and Reagents

Naratriptan reference standard (assigned purity, 99.8%) was obtained from Bio Leo Pvt Ltd (Hyderabad, India). The purity was evaluated by obtaining their melting point and ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification. Tablets were purchased from local market and the labeled amount was 2.5 mg. HPLC-grade acetonitrile was from Merck. Ammonium Acetate

(AR grade) and Glacial Acetic acid (AR grade) were from Merck. Solvents were filtered through a 0.45µm membrane filter and degassed by using ultrasonicator.

Instrumentation and Chromatographic Conditions

The developed method used a Waters 2695 Prominence 2010, Detector 2487 with Empower 2 software. The separation was carried out at ambient temperature by using a sunfire, C_{18} (250 x 4.6 mm, 5 µ particle size) column. The mobile phase consisting of Ammonium acetate buffer (pH was adjusted to 3.0 with 10% glacial acetic acid) and Acetonitrile (50:50, v/v) was used. The flow rate was 1.0 mL/min. The injection volume was 20 µL. For all standards and samples, triplicate injections were made. External standards with measurement of peak areas were used for quantitation.

Preparation of Standard Solutions

Stock solution of Naratriptan (100µg/mL) was prepared by weighing 2.5 mg and dissolving in the 25ml mobile phase i.e. Ammonium acetate buffer (pH.3): Acetonitrile (50:50%v/v). Standards solutions of Naratriptan were prepared in the desired concentration range (10µg/ml) by diluting the stock solution with mobile phase (Jayshri S *et al*., 2012).

Preparation of Sample Solutions

An accurately weighed amount of powdered tablets equivalent to 2.5 mg of Naratriptan transferred into 100 mL volumetric flask, 60 mL of diluent was added and sonicated to dissolve for 10 minutes and diluted to volume with diluent. Further filtered the solution through 0.45µ membrane filter.

METHOD DEVELOPMENT AND VALIDATION

The RP HPLC procedure was optimized with a view to develop an effective method for the estimation of Naratriptan in tablet dosage form. Preliminary tests were performed in order to select the adequate and optimum chromatographic conditions. Sunfire c18, (250 mm X 4.6 mm, 5 µm)column was used as a stationary phase and the separation was achieved by using mobile phase consisting of Ammonium acetate Buffer(PH 3) and Acetonitrile in the ratio(50:50)% v/v in isocratic mode. Chromatogram of standard solution containing Naratriptan is shown in Fig 2. The developed HPLC method for the estimation Naratriptan was validated as per the ICH guideline in terms of linearity, accuracy, precision, ruggedness and robustness, limit of detection and limit of quantification (Balasekharareddy Challa *et al* ., 2011; Sneha B *et al* ., 2008; Ramu G *et al* ., 2012).

System suitability

The system suitability of the method was determined by six replicate analysis of the standard solution containing Naratriptan to check the reproducibility of the chromatographic system. In this method the reproducibility of peak area, retention time, theoretical plate and tailing factor of the peaks of Naratriptan were checked.

Linearity

The linearity of the method was assessed by analyzing the standard solution containing Naratriptan at 6 different levels ranging 2.5, 5.0, 7.5, 10, 12.5 and 15µg/ml of its working concentration. The calibration curve of peak area (Vs) concentration was plotted and correlation coefficient and regression line equation were determined. The calibration curve of Naratriptan is shown in Fig 3.

Accuracy

Accuracy of the method was assessed by analyzing the solutions containing Naratriptan at three different levels 50%, 100% and 150% of its working concentration. Standard solutions were spiked with placebo and the percentage recovery of the drugs from the placebo was calculated.

Precision

System precision was determined by measuring 5 successive injections of 20 µl of standard solution. The peak responses were measured from the chromatograms. The standard deviation and relative standard deviation were calculated from the statistical formula. The method precision was determined by preparing the sample of single batch of Naratriptan from the tablet formulation for five times and five successive injections of 20 µl of sample solution were injected and the chromatograms were recorded. The % RSD of the obtained results was calculated.

Ruggedness and robustness

The ruggedness of the method was ascertained by carrying out the assay of the sample on different instrument by different analyst using different column of similar types. The chromatogram which is recorded. Robustness of the method was determined by analyzing the sample by deliberately changed chromatographic conditions such as change in mobile phase composition (Acetonitrile: Buffer, 68: 32 and 72: 28), flow rate (±0.1 ml/min).

LOD and LOQ

The limit of detection and limit of quantification of Naratriptan were calculated by using standard deviation of the responses and the slope of the calibration curve of Naratriptan. LOD and LOQ were estimated by using the following formula,

LOD = (3.3 X σ) / S

LOQ = (10 X σ) / S

Where σ is the standard deviation of the response

S is the slope of the calibration curve.

Analysis of Naratriptan in Tablet formulation

For the assay of Naratriptan in tablet formulations, twenty tablets were weighed and the average weight of the tablets was calculated. The weighed tablets were crushed in to fine powder. A quantity of powder equivalent to 2.5 mg of Naratriptan was transferred in to 100 ml volumetric flask, 60 mL of diluent (mobile phase) was added. The content of the flask was sonicated for 10 minutes and the volume was then made up to 100 ml with diluent. This solution was filtered through 0.45µ filter. From the resulting solution 20 µl was injected in to the column and response was recorded under the same chromatographic conditions. The amount of Naratriptan present in the sample was determined by comparing the peak area of sample with that of standard.

RESULT AND DISCUSSION

A simple, accurate and precise RP HPLC method was developed for the simultaneous estimation of Naratriptan in Pharmaceutical dosage forms. System suitability was determined by performing the assay with the same sample repeatedly. The number of theoretical plates was found to be 2478. The tailing factor was found to be 1.738 with well-defined base line. Linearity of the drug was obtained in the range of 2.5-15.0 µg/ml. The linearity coefficient and percentage curve fitting slope was found to be 0.999 and 99.99%. The limit of detection of Naratriptan was found to be 1.40700 µg/ml. The limit of quantification of Naratriptan was found to be 4.39 µg/ml. Accuracy of the method was determined through recovery studies of the drug. Recovery of the drug is well within acceptance limits (Table 2). Precision of the method was determined by assays of drug formulations by replicate injection and precision of system was determined by using standard solution. %RSD of the assays was found to be within the limits of 2 % (Table 3 and 4). Thus the developed method is found to provide high degree of precision and reproducibility.

Ruggedness was determined by performing the same assay on different days, assay being carried out by different analyst. The test results were within the limits. The result is found to be reproducible. In spite of variation in conditions which could be normally expected from analyst to analyst. Robustness was determined by carrying out the assay by changing flow rate and mobile phase composition. The test results were within the limits. The standard drug purity of Naratriptan is 99.8%. The sample drug purity is 100.318%.The values of percentage purity obtained with the change in flow rate; mobile phase composition makes it possible to carry out the method for Naratriptan with a small variation in flow rate and mobile phase. This indicates the lack of influence on test results by operational and environmental variables for developed method. The solvents which had been used in the mobile phase were cost effective than the solvents used in the other HPLC methods which are reported in the literatures (Madhavi B *et al*., 2009; Kumara Swamy G *et al*., 2011).

Fig 1. Structure of Naratriptan

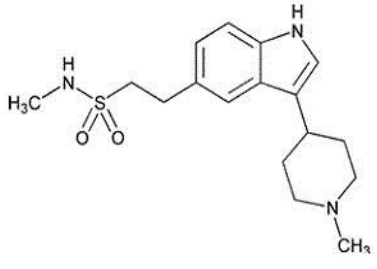

Fig 2. Chromatogram of Naratriptan standard

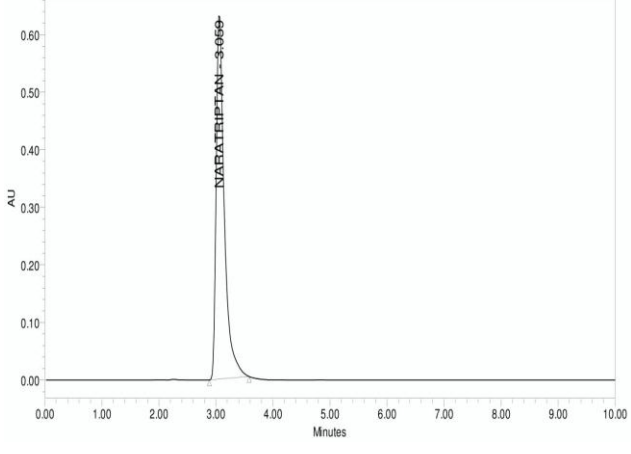

Fig 3. Calibration curve of Naratriptan

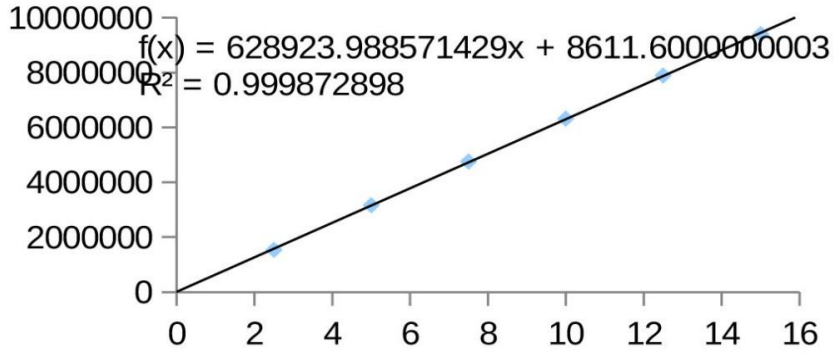

Table 1. System suitability data

Injection	Rt	Peak Area	USP Plate count	USP Tailing
1	3.059	6238592	2507	1.76
2	3.059	6262663	2455	1.73
3	3.058	6245779	2465	1.71
4	3.058	6246772	2465	1.71
5	3.059	6253363	2499	1.76
6	3.059	6253543	2478	1.73
Mean	3.059	6249470.2	2478.2	1.738
SD	0.00054	9082.003	23.1775	0.021
% RSD	0.017	0.145	0.93	0.12

Table 2. Recovery Results for Naratriptan

Sample No	Spike Level	Amount (µg / ml) added	Amount (µg / ml) found	% Recovery	Mean % Recovery
1	50 %	50	49.58	99.16	99.1666
	50 %	50	49.55	99.10	
	50 %	50	49.62	99.24	
2	100 %	100	99.65	99.65	99.6933
	100 %	100	99.74	99.74	
	100 %	100	99.69	99.69	
3	150 %	150	148.96	99.306	99.306
	150 %	150	148.95	99.30	
	150 %	150	148.97	99.313	

Table 3. System precision data

Injection No	Retention time	Peak Area	Plate count	Tailing factor
1	3.065	6220955	2639	1.76
2	3.064	6210630	2638	1.76
3	3.064	6221970	2706	1.74
4	3.062	6211664	2619	1.76
5	3.063	6218682	2677	1.74
6	3.062	6223204	2676	1.76
Mean	3.06333	6217850	2655.8	1.752
SD	0.001211	5410.5075	35.08	0.010
% RSD	0.04	0.11	0.132	0.57

Table 4. Method Precision data

Injection No	Retention time	Peak Area	Plate count	Tailing factor
1	3.061	6205118	2667	1.75
2	3.062	6218180	2692	1.74
3	3.061	6194688	2685	1.75
4	3.062	6186350	2698	1.75
5	3.061	6199264	2666	1.75
6	3.061	6177404	2714	1.76
Avg	3.06133	6196834	2681.6	1.748
SD	0.0005164	14298.43	14.5386	0.0044
% RSD	0.02	0.23	0.54	0.25

CONCLUSION

As the literature survey reveals there are only few methods have been reported for the estimation of Naratriptan. So there is a need of a simple, economical and proper method for estimation of Naratriptan in tablet dosage form. The developed method is cheap, easy and it gives sharp peak with high resolution. The developed method is applied for the determination of Naratriptan. The assay results are with the label claim of the formulation. The developed method is validated as per ICH guidelines using parameters like Accuracy, Precision Linearity and 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 in tablet dosage form.

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