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Research Article

A NOVEL RP-HPLC METHOD FOR THE ESTIMATION OF NARATRIPTAN HCL IN BULK AND DOSAGE FORM

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Abstract: A new simple, precise, sensitive and validated RP-HPLC was developed for the estimation of Naratriptan Hcl in pharmaceutical dosage form. The chromatographic conditions used for the separation was spursil $C_{18,}$ 250 X 4.6 mm, 5 µm particle size and mobile phase comprised of potassium di hydrogen phosphate buffer pH 7.2 and acetonitrile in the ratio (70:30) v/v. The flow rate was 1 ml/min with detection at 224 nm. The retention time was found to be 3.825 min. The linearity was found to be in the range of 50-150 µg/ml with correlation coefficient of 0.999. The proposed method is accurate with 99.55% - 99.56 % recovery for Naratriptan Hcl and precise (%RSD of system and method precision were 1.55,1.21). The limit of detection (LOD) and limit of quantitation (LOQ) were found to be 0.04956 and 0.1502 µg/ml respectively. The method was successfully applied to pharmaceutical formulation because no chromatographic interferences from tablet excipients were found.

Key words: Naratriptan Hcl, RP-HPLC, Validation, Acetonitrile.

INTRODUCTION[1-10]

Naratriptan Hcl(N-methyl-3-(1-methyl-4-piperidinyl)-1H-indole-5-ethanesulfonamide mono hydrochloride) is a new drug, used in the treatment of migraine headaches. 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.

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.

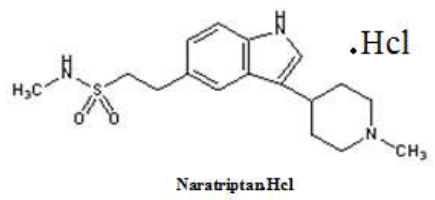


Fig 1: Structure of naratriptan Hcl

MATERIALS AND INSTRUMENTS USED:

HPLC Waters - E 2695 , Uv visible detector - Waters 2489, Electronic balance- Mettle Toledo, pH meter- Elico, Sonicator- Sonorex dig 10 p were used for the experiment. Acetonitrile - HPLC Grade (Merck specialities private ltd, Mumbai). Potassium di hydrogen phosphate, Orthophosphoric acid- AR grade (Qualigens pvt limited ,Mumbai) were used for the method development.API was procured from yarrow chem. pvt ltd,Mumbai.Formulation was procured from local market.

Selection of Mobile Phase:

Based on sample solubility, stability and suitability various mobile phase compositions were tried to get a good resolution and sharp peaks. The standard solution was run in different mobile phases. From the various mobile phases tried, mobile phase containing potassium di hydrogen phosphate buffer pH 7.2 and acetonitrile in the ratio (70:30) v/v with flow rate 1.0 ml/min with UV detection at 224 nm was selected, since it gave sharp peak with good symmetry within limits for the drug.

Chromatographic conditions:

The optimized parameters which were used as a final method for the estimation of Naratriptan Hcl represented in the Table 1.

Table 1: Optimized Chromatographic Conditions

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

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

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).

Assay Procedure:

10 µl of the solution of each of standard and sample solution were injected separately in to the chromatographic system. The chromatograms were recorded and peak areas were calculated.

METHOD VALIDATION

The optimized Chromatographic method was completely validated according to the procedures described in ICH guidelines Q2 (R1) for the validation of analytical methods (ICH, 2005) [(11).]

System suitability test

20 µL of the standard solution (3µg/ml) was injected under optimized chromatographic conditions to evaluate the suitability of system. The values of system suitability parameters were shown in Table 2.

Specificity:

Specificity of the HPLC method was demonstrated by the separation of the analytes from other potential components such as impurities, degradants or excipients. A volume of 20µl of working placebo sample solution was injected and the chromatogram was recorded. No peaks were found at retention time of 3.285 min. Hence, the proposed method was specific for naratriptan Hcl.

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.

System precision:

The system precision was evaluated by measuring the peak response of naratriptan for six replicate injections of the standard solution and chromatogram were recorded.

Method precision

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.

Linearity

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.

Robustness

The robustness of the HPLC method was evaluated by analyzing the system suitability parameters after varying the Flow rate (±0.2), column temperature (± 2 0c). None of these alterations caused a significant change in peak area RSD, tailing factor and theoretical plates. Although the changes in the retention time were significant, yet quantitation was possible.

Limit of detection and Limit of quantification:

Limit of detection (LOD) and Limit of Quantification (LOQ) were determined by using the formula based on the standard deviation of the response and the slope.LOD and LOQ were calculated by using equations, LOD=3.3 x□ /s and LOQ=10 x □ /s, where □ = standard deviation, S= slope of the calibration curve.

Ruggedness

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 .

RESULTS AND DISCUSSION

The method was developed with mobile phase system of potassium di hydrogen phosphate buffer pH 7.2 and acetonitrile in the ratio (70:30) 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. 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. 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 percentage of RSD of assay was found to be in 1.21 for naratriptan which was within the range of acceptance criteria of 2%. Thus the proposed method was found to be providing high degree of precision and reproducibility. 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.

The validation of the proposed reverse phase HPLC method was further verified by recovery studies. The percentage recovery was found to be within 97-103% w/v of naratriptan. These serve a good index of accuracy and reproducibility of the proposed method. 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 indicating lack of influence on the test results by operational variable for the proposed method

The ruggedness 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.

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.

Table 2: Data for System Suitability Parameters

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

Table 3: Data for Linearity

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

Table 4: Validation parameters of the proposed method.

Parameter	Results
Linearity (µg/ml)	50 -150 µg/ml
Slope(b)	7449578
Correlation co efficient (r)	0.999,
System precision(%RSD n=6)	1.55
Method precision(%RSD ,n=6)	1.21
% Recovery	97.94%-99.56
Robustness	Robustted
LOD(µg/ml)	0.04956
LOQ(µg/ml)	0.1502

Table 5: Data for accuracy studies

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

Table 6: Data for ruggedness

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

Table 7.1: Report of robustness (change in column temperature 28°C)

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

Table 7.2: Report of robustness (change in column temperature 32°C)

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

Table 7.3: Report of robustness (change in flow rate 0.8 ml/min)

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

Table 7.4: Report of robustness (change in flow rate 1.2 ml/min)

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

Table 8: 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%

Fig 2: Chromatogram for Naratriptan API

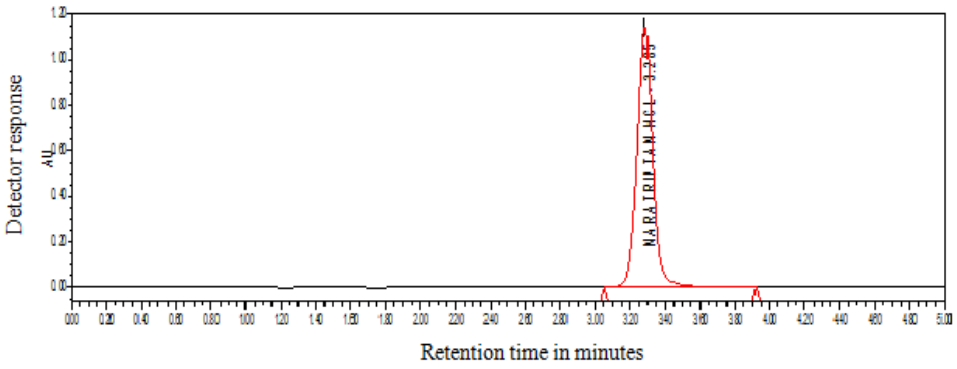


Fig 3: Chromatogram for Naratriptan formulation

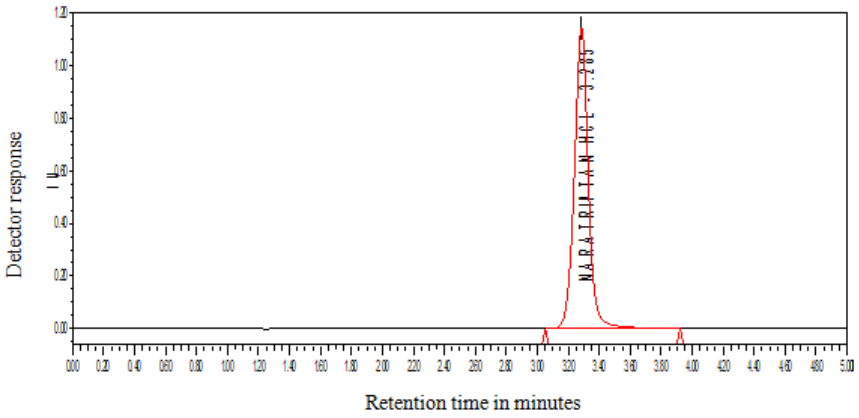
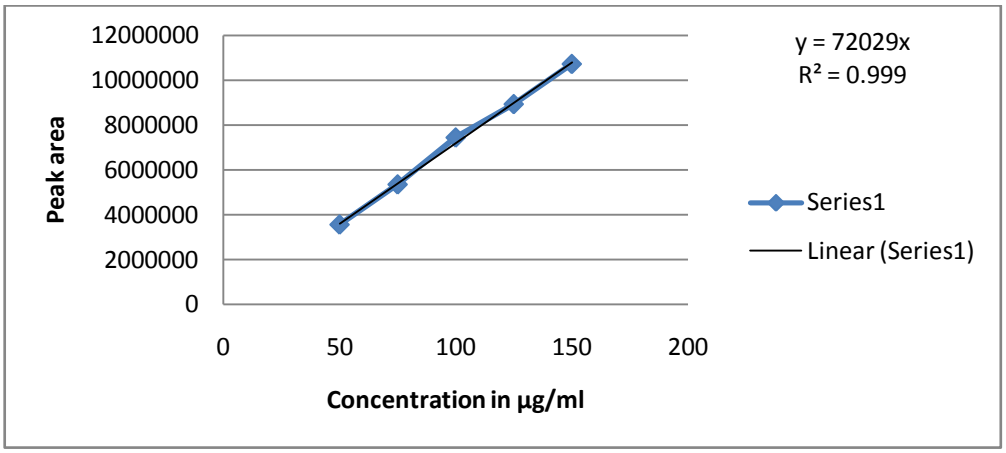


Fig 4: CALIBRATION CURVE OF NARATRIPTAN



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.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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