Development and Validation of UV Spectrophotometric Method of Naratriptan Hydrochloride in Bulk and Pharmaceutical Formulation

Santosh Shelke[1]*, Sadhana Shahi[2], Vandana Patil[1], Suwarna Kale[1]

[1.]Department of Pharmaceutics, Yash Institute of Pharmacy, Aurangabad - 431134, Maharashtra, India.
[2.]Department of Pharmaceutics, Government College of Pharmacy, Aurangabad - 431005, Maharashtra, India

Research Article

ABSTRACT :
Naratriptan Hydrochloride (NH) is a selective $5\text{-}HT_1$ agonist developed for the acute treatment of migraine which acts by stimulating constriction of dilated cranial arteries and by inhibiting the release of neurogenic inflammatory mediators. A simple, sensitive, precise, accurate and economical UV-Spectrophotometric method has been developed for the determination of NH in bulk and pharmaceutical dosage form. Absorption for NH was measured at maximum wavelength 281nm. The percentage recovery of NH in pharmaceutical dosage form was found to be 99.43 to 98.53 %. The developed method was validated with respect to linearity, accuracy (recovery), precision and specificity. Beers law was obeyed in the concentration range of 2-10μg/ml having line equation $y = 0.019x$ with correlation coefficient of 0.99. Results of the analysis were validated according to the ICH guidelines. The results obtained were statistically evaluated and were found to be accurate and reproducible. The proposed method can be successfully applied for the estimation of Naratriptan Hydrochloride in bulk and pharmaceutical dosage form.

INTRODUCTION:

Migraine, a multifactorial primary headache disorder, is characterized by unilateral, intense, and pulsatile headaches leading to cause of medical disability.[1] Worldwide, Migraine is the fifth leading cause of disability which is three times more common among females than males, with peak prevalence occurring between early and middle adulthood [2]. NH is a $5\text{-}HT_1$ agonist used for the treatment of migraine headaches. The chemical structure of NH is shown in Figure 1. NH is available in oral form (conventional and orally disintegrating tablets) and subcutaneous formulations have been developed [3] with the recommended dose of 1 and 2.5 mg with the oral bioavailability of 50-60%. NH is an effective and well tolerated abortive antimigraine medication and has been used preventively in transformed migraine [4].

As reported in the literature very few analytical methods have been investigated for the estimation of NH in pharmaceutical dosage form and in biological fluids. UV-Vis spectrophotomeric [5-7], HPLC [8,9] and UPLC [10] are the methods used for the determination of NH. In the present investigation a simple, sensitive, precise, accurate and economical UV-Spectrophotometric method has been developed for the determination of NH in bulk and pharmaceutical dosage form. The data generated and the method validation confirms the reproducibility of the method.
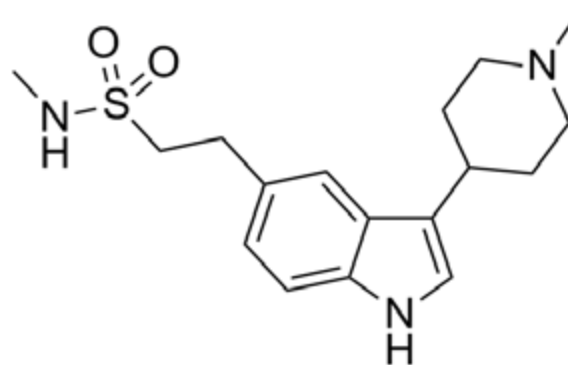

Figure 1: Chemical Structure of Naratriptan

EXPERIMENTAL

Instrument and materials:

Shimadzu 1800 double beam UV-VIS spectrophotometer provided with 1 cm matched quartz cell. and shimadzu AX200 analytical balance was used for analytical method development. NH was obtained as gift sample from Hospira Healthcare India Pvt Ltd. (Aurangabad, India). All other chemical and reagents used were of analytical grade.

Materials and Reagents

Selection of media:

Drug solubility and stability are the main decisive factors for media selection. As reported in the literature media selected for NH is water. But as the drug is lipophilic and in-

*Corresponding author:
Santosh Shelke
Yash Institute of Pharmacy, Aurangabad-431134, Maharashtra, India.
Email:santoshshel@gmail.com   Phone no: 09049996260

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tended for targeting brain, the intranasal route is preferred where drug is directly transported to the brain through the olfactory lobes [11]. Considering the nasal physiology the *in-vitro* diffusion and and *ex-vivo* permeation studies are supposed to be conducted in phosphate buffer 6.4 (preferred media for nasal formulations). Hence the proposed media selected was Phosphate buffer 6.4 for analytical method development.

Preparation of standard stock solution:

Standard drug solution of NH was prepared by dissolving 10mg of NH in 20ml ethanol and was transferred to 100ml volumetric flask and volume was made up to mark with phosphate buffer pH 6.4 to obtain stock solution of 100µg/ml concentration. Obtained solution was bath sonicated for 5 minutes to obtain the clear solution. The solution was scanned (figure 1) in the range of 200-400 nm against blank.
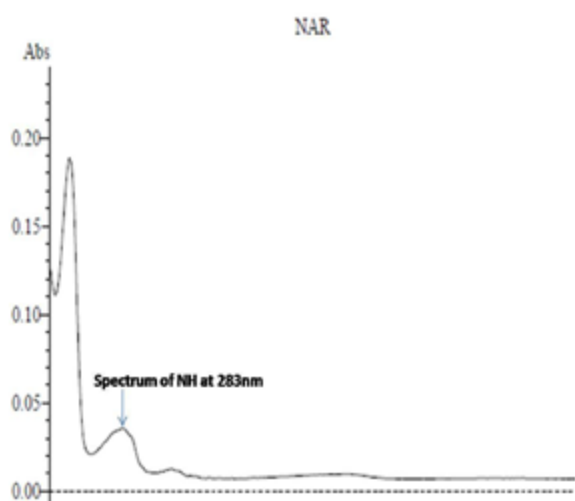

Figure 2: Spectrum of Naratriptan Hydrochloride

Validation as per ICH guidelines [12]

Linearity: The linearity of the response of the drug was verified at 2 to 10µg/ml concentration. Accurately weighed 10mg of NH was dissolved in phosphate buffer 6.4 to get the stock solution of 100µg/ml. From this stock solution aliquots of 0.1, 0.2, 0.4, 0.6, 0.8 and 1ml were withdrawn and further diluted to 10 ml with phosphate buffer pH 6.4 to obtain concentrations range of 1 to 10µg/ml. The absorbance for different concentration (Table 1) was measured at 281nm by using UV spectrophotometer. The calibration curve was obtained by plotting the absorbance versus concentration data and were treated by linear regression analysis (figure 3).

Table 1: Observation table for calibration curve of NH

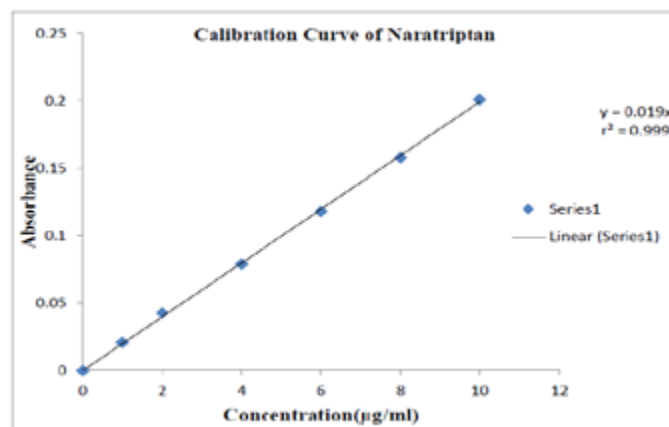| Sr. no.	Conc.(µg)	Absorbance
1	1	0.021
2	2	0.043
3	4	0.079
4	6	0.118
5	8	0.158
6	10	0.201

Figure 3: Calibration Curve of Naratriptan Hydrochloride

Precision:

Assay of method precision (intra-day precision) was evaluated for three independent assays of test samples of NH. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts, systems and different days in the same laboratory. The precision data is reported in the table 2

Table 2: Evaluation of intra-day and inter-day accuracy and precision

NH (µg/ml)	Intraday Accuracy and precision			Interday Accuracy and precision		
	NH found (µg/ml)	RE %	RSD %	NH found (µg/ml)	RE %	RSD %
4	3.753	0.2354	1.45	3.815	0.3125	1.52
6	5.795	0.3124	1.34	5.863	0.3947	1.45
8	7.859	0.4101	1.25	7.876	0.3995	1.37

Accuracy (Recovery Test): Recovery experiment was conducted to confirm the accuracy of the method. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed at three levels, 80, 100and 120% of NH standard concentration. The recovery samples were prepared in afore mentioned procedure. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The recovery values for NH are listed in the Table 3

Table 3: Recovery data

Level of addition (%)	Amount of NH added (µg)	Amount of NH found (µg)	% Recovery	% RSD
80	3.2	3.15	98.43	1.21
100	4	3.95	98.5	1.15
120	4.8	4.73	98.54	1.11

Assay: Initially ten tablets were weighed and powdered. The amount of tablet powder equivalent to 2.5 mg of NH was weighed accurately and transfer to 20 ml phosphate buffer 6.4 for 15 min with frequent shaking and volume was made up to 100 ml mark with phosphate buffer pH

6.4. The solution was then filtered through whatmann filter paper # 41. This filtrate was diluted suitably with the media to get the solution of 8µg/ml concentration .The absorbance was measured against blank. The drug content of the preparation was calculated using standard calibration curve. Amount of drug estimated by this method is given in Table 4.

Table 4: Assay Results of Tablet Dosage Form.

Formulation	Actucal amount (mg)	Amount Found (mg)	% of Drug Found
Tablet	2.5	2.51	100.4

RESULT AND DISCUSSION:

The equation of the calibration curve for NH was found to be y = 0.019 x, the calibration curve was found to be linear in the aforementioned concentrations. A linear correlation was found which obeys Beer Lambert's Law in the concentration range of 2-10 µg/ml (Figure 1). Regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and the correlation coefficient (r^2).where the slope was found to be y= 0.019x and the correlation coefficient was found to be 0.99. The maximum wavelength observed was at 222 and 283 nm(table 5), where the wavelength of 283nm was considered for the analytical method development. The value of molar extinction coefficient and sandell's sensitivity for EH estimated was 6.743 x 10^3 L/mol/cm and 0.497 µg/cm^2 respectively, as mentioned All the discussed parameters are listed in the table 5.

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices which is basically used for the determination of impurities and/or degradation products. The valve of LOD (0.323) and LOQ (0.979) are determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines [12] and are given in Table 5.Precision (Table 2), recovery data (Table 3) and assay (Table 4) confirms the reproducibility and accuracy of the method.

Table 5: Showing the maximum spectrum at different wavelenght

Peak	Start	Apex (nm)	End (nm)	Height (Abs)	Area (Abs*nm)
1	800.0	283.0	247.0	0.036	5.6.6
2	247.0	222.0	200.0	0.189	5.057

Table 6: Optical characteristics and regression data for the proposed method for NH

Parameter	Analytical data
Linearity Range (µg/ml)	2-10
λ max (nm)	283
Molar extinction coefficient	674.283
Sandell's sensitivity	0.4975
Slope	0.019
Intercept	0
Standard deviation about regression (Sy)	0.0065
Standard deviation of Slope (Sb)	0.0007
Standard deviation of intercept (Sa)	0.0039
Correlation co-efficient (r)	0.999
Limit of detection (LOD, µg/ml)	0.323
Limit of quantification (LOQ, µg/ml)	0.979

CONCLUSIONS :

From the results recorded it can be concluded that spectrophotometric method using the Phosphate buffer 6.4 as the media was found to be new, accurate, precise and economic for the determination of NH. The proposed method shows better sensitivity. In comparison with the existing visible spectrophotometric methods for the quantification of NH, the present modified method can be considered green as it demonstrates the development of spectrophotometry method without the usage of organic solvent. In addition, the proposed method employs an inexpensive instrument. Overall the proposed new and ecofriendly spectrophotometric method is economical and suitable for quality control of NH in bulk, fixed-dose combination tablets.

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