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Research Article

Analytical Method Development and Validation of RP-HPLC Method for Determination of Eletriptan HBr.

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ABSTRACT

The aim of recent study was to develop highly precise RP-HPLC method for estimation of eletriptan hydrobromide in pharmaceutical formulation, RP-HPLC method was developed using Alligent-1100 C18 column (Chemstation-32 software) at 30^0c with flow rate of 1.0ml/min. at detection wavelength of 234nm with UV visible detector. The method was validated as per ICH guidelines and various validation parameters like accuracy, precision, limit of detection (LOD), Limit of Quantification (LOQ), recovery study and range were determined. The proposed method was simple, rapid, precise, and accurate, and can be used for routine analysis of eletriptan hydrobromide in bulk and combinations the method was found to be linear in the range of 5-30 mcg/ml with coefficient relation of 0.9998.

KEYWORDS

Eletriptan HBr, RP-HPLC Method, Validation, Accuracy, Precision, LOD, LOQ.

1. INTRODUCTION

Eletriptan chemically designated as 3-[[(R)-1-Methyl-2-pyrrolidinyl] methyl]-5-[2-(phenylsulfonyl) ethyl] indole, monohydrobromide, is selective 5-hydroxytryptamine 1B/1D (5-HT1B/1D) receptor agonist. Eletriptan HBr is generally used in the treatment of the migraine headache, because the eletriptan HBr activate the 5-HT1 receptors located on intracranial blood vessels, including those on the arteriovenous anastomoses, leads to vasoconstriction, which is correlated with the relief of migraine headache.

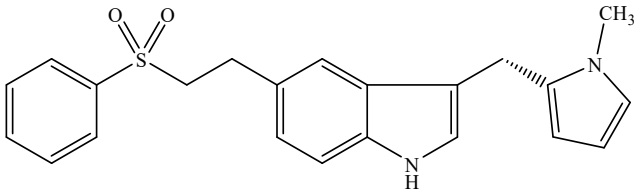

Fig. 1. Structure of Eletriptan HBr.

Analysis is the most important aspect of any drug development whether in bulk or in combination, a suitable method must be developed so as to ensure that any drug either in dosage form or bulk form can be pointed out. The method development ensures that amount of particular drug can be easily determined. The validation parameters confirm that the developed method is precise, accurate and reproducible and can be used for routine evaluation of eletriptan in bulk and combined dosage form. [1-10]

In the present study suitable RP-HPLC method was developed with the aim of making the detection of eletriptan more accurate and precise with addition of validation parameters it was convenient for determining the concentrations of eletriptan in various dosage forms.

2. MATERIALS & METHODS

2.1. Reagents & chemicals

Eletriptan HBr was received as gift sample from WOKHARDT Research Centre, Aurangabad. Tablet formulation manufactured by Pfizer limited was purchased from local market RELPAX containing eletriptan hydro bromide 40mg per tablet.

2.2. Instrumentation: [9-14]

Agilent 1100 Series HPLC Value System by Agilent Technologies Hewlett-Packard-Strasse 8 76337 Waldbronn Germany with UV detector was used for metoprolol with chemostation-32 software; the analytical column used to achieve chromatographic separation was a stainless steel Agilent C18 RP column at 30^0c with flow rate of 1.0ml/min.

2.3. Preparation of buffer solution (10 mM Potassium di-hydrogen phosphate)

Potassium di-hydrogen phosphate (KH_2PO_4) 1.3609 gm was accurately weighed and transferred to 1000ml volumetric flask by dissolving in HPLC grade water and final volume was adjusted using HPLC grade water upto 1000ml.

2.4. Mobile Phase

Mixture of buffer solution and acetonitrile in the ratio of 65:35 (650ml of buffer solution and 350 ml of acetonitrile) was made in 1000ml volumetric flask mixed and sonicated for 5 minutes, degassed and filtered through 0.45um membrane filter.

2.5. Preparation of standard solution

Eletriptan hydrobromide 10 mg was weighed accurately and transfer to 100ml volumetric flask and volume was made with mobile phase

2.6. Sample Preparation

Accurately weighed 10mg of sample eletriptan hydrobromide was transferred in 100ml volumetric flask. Mixed, sonicated and diluted to volume with mobile phase from this solution 0.5, 1, 1.5, 2, 2.5 & 3ml was taken, transferred to 10ml of volumetric flask and diluted with buffer solution to make 5, 10, 15, 20, 25 & 30mcg/ml concentrations respectively.

2.7. Chromatographic System

Chromatographic system was selected as per parameters in Table 1.

Table 1. Chromatographic system parameters.

Column	**C18**
Column temperature	**30^0 C**
Flow rate	**1mL per minute**
Wavelength	**234nm**
Injection volume	**20uL**

2.8. Validation Parameters [12 & 13]

2.8.1. Linearity

The linearity of the response of the drug was verified at 5 to 30µg/ml concentrations. The calibration curve was obtained by plotting the peak area Vs. the concentration data and was treated by linear regression analysis. The equation of the calibration curve for eletriptan was obtained.

2.8.2. Sensitivity

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined at least concentration with accuracy method was determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method LOD and LOQ were calculated by the equations;

$LOD = 3.3\sigma / S$ and $LOQ = 10\sigma/S$ ---Equation 1

Where S is the slope of the calibration curve and σ is the residual standard deviation

2.8.3. Precision

The accuracy of the method was determined by recovery experiments. Each solution was repeated in triplicate and the percentage recovery was calculated. The precision of the method was demonstrated by intra-day and inter-day variation studies.

2.8.4. Accuracy [Recovery studies]

Recovery study carried out for the drug was performed by spiking the known standard drug in powdered formulations. The assay procedure was repeated for standard and sample six times and mean peak area ratio and concentration of drug was calculated. The percentages of individual drug found in formulation, mean, standard deviation in formulation were calculated.

3. RESULTS AND DISCUSSION

Several tests were performed in order to get satisfactory separation-resolution of Eletriptan hydrobromide in different mobile phases with various ratios by using C18 column. The ideal mobile phase used was phosphate buffer and acetonitrile in the ratio of 65:35 v/v to obtain satisfactory and good resolution. The retention of Eletriptan hydrobromide on analytical column was evaluated at a flow rate of 1.0 mL.min-1. The injection volume was 20µL. The typical chromatogram of Eletriptan hydrobromide is shown in Fig. 2. The retention time of standard and sample for Eletriptan hydrobromide was satisfactory with good resolution.

3.1. Method validation

3.1.1. Linearity

The linearity for HPLC method was determined at eight concentration levels ranging from 5-30 mcg/ml for Eletriptan hydrobromide. The calibration curve was constructed by plotting response factor against concentration of Eletriptan hydrobromide (Figure.3). The slope and intercept for calibration curve were y=164818x+223.13 with a correlation coefficient (R^2 = 0.9998) for Eletriptan hydrobromide, where Y represents the ratio of peak area ratio of analyte and X is the analyte concentration. From the results it was found that shown that significant correlation exists between response factor and concentration of drug in the range shown on Y-axis.

Table 2. Calibration Curve Data.

Sr. No	Conc. mcg/ml	Peak Area
1.	5	825770
2.	10	1651502
3.	15	2489756
4.	20	3256789
5.	25	4128790
6.	30	4954620

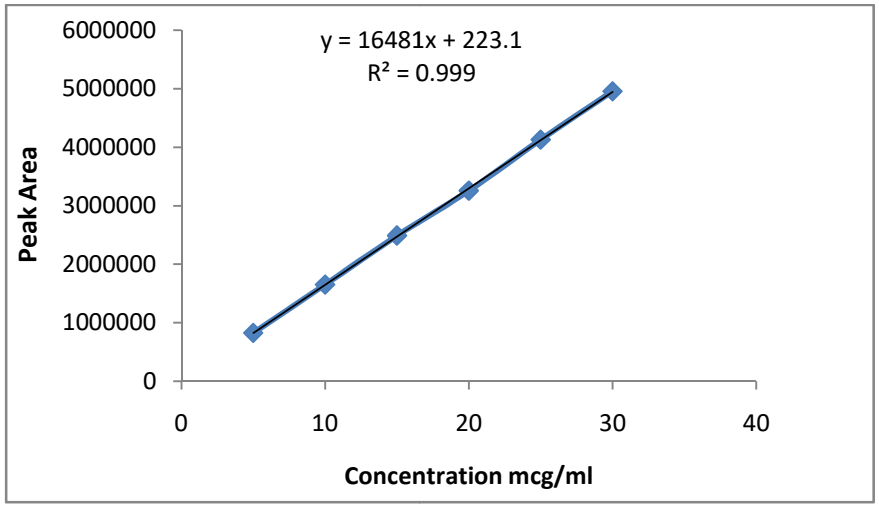

Fig. 2. Calibration Curve.

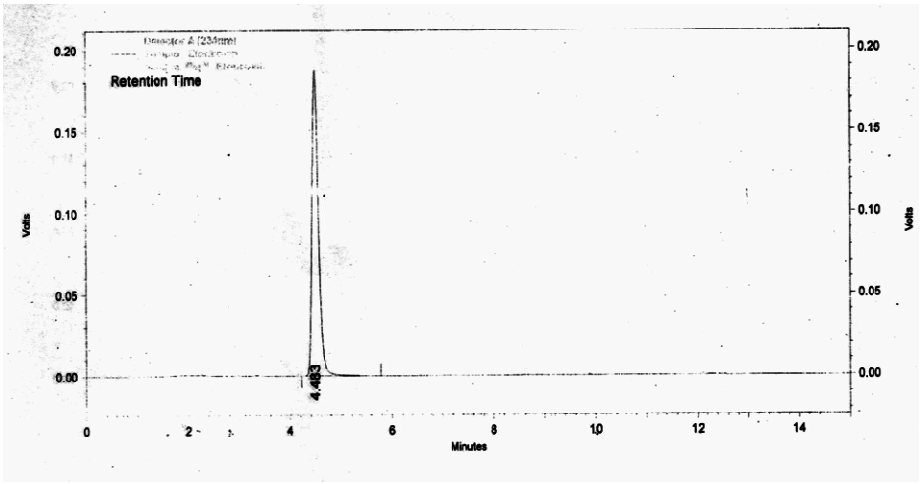

Fig. 3. A Typical Chromatogram of Eletriptan HBr.

3.1.2. Sensitivity

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined at least concentration with accuracy method was determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) for Eletriptan hydrobromide was found to be 0.081969 mcg/ml and 0.248392 mcg/ml respectively.

3.1.3. Precision

The precision of the method was obtained by interday and intraday variation studies. In the intraday studies, samples were injected same day from which the response factor of drug peaks and percentage RSD were calculated. In the interday variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in Table 3 from the data obtained, the developed RP-HPLC method was found to be precise.

Table 3. Precision.

Precision	Intra-Day Precision*	Inter-Day Precision*
Result	0.458828654	0.45059381

3.1.4. Accuracy [Recovery studies]

Recovery study carried out for the drug was performed by spiking the known standard drug in powdered formulations. The assay procedure was repeated for standard and sample six times and mean peak area ratio and concentration of drug was calculated .The percentage of individual drug found in formulation, mean, standard deviation in formulation were calculated. The results of the recovery analysis were found to be 98.9506 ± 0.890186 with %RSD of 0.90666 reported in Table.03. The results of analysis (Table 04) show that the amounts of drug were in good agreement with the labeled claim of the formulation.

Table 4. Recovery Study.

Formulation stock	Standard Conc.	Total Conc.	Drug Recovered	% Recovery	Mean % Recovery	SD	%RSD
8	10	18	17.88	99.3333	97.537	1.8644	1.91
8	10	18	17.21	95.6111			
8	10	18	17.58	97.6666			
10	10	20	19.99	99.95	99.65	0.26457	0.27
10	10	20	19.89	99.45			
10	10	20	19.91	99.55			
12	10	22	22	100	99.6648	0.54159	0.54
12	10	22	21.79	99.04			
12	10	22	21.99	99.9545			
				Mean	98.9506	0.890186	0.90666

4. CONCLUSION

A Precise and sensitive RP-HPLC method for Eletriptan HBr was developed and validated as per ICH guidelines for its determination in dosage formulations. It was shown above that, the proposed method was linear, accurate, reproducible, repeatable, precise, selective, specific and cost effective proving the reliability of the method. More over same solvent was used throughout the experimental work and it was found that no interference from any excipients was observed in

the method. Hence, the proposed method was successfully applied to routine analysis of Eletriptan HBr in bulk and combined formulations.

5. ACKNOWLEDGEMENT

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