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Original Research Paper

DEVELOPMENT AND VALIDATION OF LC METHOD FOR THE ESTIMATION OF ZOLMITRIPTAN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed and validated for the estimation of Zolmitriptan in tablet dosage form. A chromosil C-18, 5µm column having 250 x 4.6 mm internal diameter in isocratic mode with mobile phase containing acetonitrile: methanol: orthophosphoric acid in the ratio of 69:29:02 (v/v/v) was used. The flow rate was 1.0 ml/min and effluents were monitored at 210 nm. The retention time for Zolmitriptan was 1.9 min. The method was validated for linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness. Limit of detection and limit of quantification were found to be 0.005 ppm and 0.0165 ppm respectively and recovery of Zolmitriptan from tablet formulation was found to be 99.85%. The proposed method was successfully applied for the quantitative determination of Zolmitriptan in tablet formulation.

Keywords: Zolmitriptan, HPLC, Linearity, Validation, Robustness.

INTRODUCTION

Zolmitriptan is a selective serotonin receptor agonist of the 1B and 1D subtypes. It is a triptan, used in the acute treatment of migraine attacks with or without aura and cluster headaches. IUPAC name is (S) -4-({3-[2-(dimethylamino) ethyl]-1*H*-indol-5-yl}methyl)-1,3-oxazolidin-2-one. Zolmitriptan is used for the acute treatment of migraines with or without aura in adults. Zolmitriptan is not intended for the prophylactic therapy of migraine or for use in the management of hemiplegic or basilar migraine. Molecular formula of Zolmitriptan $C_{16}H_{21}N_3O_2$ and Molecular weight 287.357.

Literature survey revealed that numerous methods have been developed and reported for estimation of Zolmitriptan in pharmaceutical formulations.

Present study involves development of LC method using simple mobile phase which is

sensitive and rapid for quantification of Zolmitriptan in tablet dosage forms as well as subsequent validation of developed method according to ICH guide lines.

EXPERIMENTAL

Instrument

The liquid chromatographic system consisted of Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20 µl fixed loop. Chromatographic analysis was performed using Intersil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing purpose.

Reagents and Materials

Methanol, acetonitrile and tetrahydrofuran of HPLC grade was purchased from E. Merck, Mumbai, India.

Preparation of Standard Stock Solution

A stock solution of Zolmitriptan was prepared by accurately weighing 10mg of drug into 100 ml of volumetric flask and dissolved in the chosen solvent. Appropriate aliquot of this solution was further diluted with solvent to obtain final standard solution of 25 ppm of Zolmitriptan. Resultant solution was filtered through Ultipor N_{66} Nylon 6, 6 membrane sample filter paper.

Preparation of Sample Solution

The formulation tablets of Zolmitriptan were crushed to give finely powdered material. Powder equivalent to 10 mg of drug was taken in 10 ml of volumetric flask containing 5 ml of mobile phase and was shaken to dissolve the drug and then filtered through Ultipor N_{66} Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent to obtain concentration of 20 ppm.

Chromatographic Conditions

The mobile phase consisting of acetonitrile : methanol : Ortho phosphoric acid were filtered through 0.45µm Ultipor N_{66} Nylon 6,6 membrane solvent filter, degassed and were pumped from the solvent reservoir in the ratio of 69:29:2(v/v/v),and was pumped into the column. The flow rate of mobile phase was maintained at 1.0ml/min and detection wavelength was set at 210nm with a run time of 10min. The volume of injection loop was 20µl. Prior to injection of the drug solution, the column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

Calibration Curve

Appropriate aliquots of standard Zolmitriptan stock solution were taken in different volumetric flasks and resultant solution was diluted up to the mark with mobile phase to obtain final concentration of 0.1959, 0.3918, 0.7839, 1.5675, 3.125, 6.25, 12.5, 25, 5, 0 ppm of Zolmitriptan. These solutions were injected into chromatographic system. Chromatograms were obtained and peak area ratio was determined for each concentration of drug solution. Calibration curve of Zolmitriptan was constructed by plotting peak area ratio versus applied concentration of Zolmitriptan and regression equation was computed. Similarly the sample solution was chromatographed and concentration of Zolmitriptan in tablet sample was found out using regression equation.

Method Validation

The method was validated for accuracy, precision, linearity, specificity, limit of detection, limit of quantification and robustness by following procedures.

Accuracy

The accuracy of the method was determined by calculating recovery of Zolmitriptan by the method of standard addition. Known amount of Zolmitriptan (10 ppm, 5 ppm and 15 ppm) was added to a pre quantified sample solution and the

amount of Zolmitriptan was estimated by measuring the peak area ratios and by fitting these values to the straight line equation of calibration curve. The recovery studies were carried out three times over the specified concentration range and amount of Zolmitriptan was estimated by measuring the peak area ratios by fitting these values to the straight line equation of calibration curve. From the above determination, percentage recovery and standard deviation of percentage recovery were calculated.

Precision

The intra-day precision study of Zolmitriptan was carried out by estimating the correspondence responses six times on the same day with 6.25 ppm concentration and inter-day precision study of Zolmitriptan was carried out by estimating the correspondence responses six times next day with 6.25 ppm concentration.

Linearity and range

The linearity of the method was determined at seven concentration levels ranging from 0.19-50 ppm for Zolmitriptan.

Specificity

Commonly used excipients (colloidal silicon dioxide, lactose, magnesium stearate, povidone, starch and talc) were spiked into a pre-weighed quantity of drug. The chromatogram was taken by appropriate dilutions and the quantity of drug was determined.

Limit of detection and limit of quantification

Limit of detection = 0.005 ppm

Limit of quantification = 0.0165 ppm

Stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both the solutions were analyzed over a period of 8 hours at room temperature.

Robustness

Robustness of the method was studied by changing the composition of organic phase by ±5% and the p^H by ± 0.2, and also by observing the stability of the drugs for 24 hours at ambient temperature in the mobile phase.

RESULTS AND DISCUSSION

The UV spectra of Zolmitriptan showed that the drug absorbs appreciably at 210 nm was selected as the detection wave length in liquid chromatography. Optimization of mobile phase was performed based on asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase acetonitrile: methanol: Ortho phosphoric acid in the ratio of 69:29:02 (v/v/v) was used. The retention time of Zolmitriptan was found to be 1.9 min, which indicates a good base line.

The number of theoretical plates was found to be 5036, which indicates efficient performance of the column. The asymmetric factor was found to be 1.21, which indicates asymmetric nature of the peak. The calibration curve for Zolmitriptan was obtained by plotting the peak area ratio versus the concentration of Zolmitriptan over the range of 0.19-50 ppm, and it was found to be linear with regression coefficient of 0.9953. The regression equation of Zolmitriptan concentration over its peak area ratio was found to be y = 185.83+ 211807.72 x, where x is the concentration of Zolmitriptan (ppm) and Y is the respective peak area. The data of regression analysis of the calibration curve was shown in Table 1. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The limit of detection and limit of quantization for Zolmitriptan was found to be 0.005 ppm and 0.0165 ppm, indicating the sensitivity of the method. The system suitability and validation parameters were given in Table 1. The high percentage of recovery of Zolmitriptan was found to be 99.25% indicating that the

proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Zolmitriptan in tablet formulation. The result for Zolmitriptan was comparable with a corresponding labelled amount. The absence of additional peaks indicates no interference of the excipients used in the tablets.

CONCLUSION

Proposed study describes new LC method for the estimation of Zolmitriptan in tablet formulation and serum. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis of estimation of Zolmitriptan in its tablet formulation and serum.

Table 1: Regression analysis of the calibration curve

Parameters	Values
Calibration range (ppm)	0.1959-50
Slope	11807.72
Intercept	185.83
Correlation coefficient	0.9953

Table 2: System suitability and validation parameters

Parameters	Results
Theoretical plates	5036
Retention time (min)	1.9
Asymmetric factor	1.21
LOD (ppm)	0.005
LOQ (ppm)	0.0165
Accuracy (%)	99.25%
R.S.D. (%)	0.877%

Table 3: Assay results of formulation

Formulatio	Labelled claim	% of Zolmitriptan in
ZOMIG	2.5	99.85 %

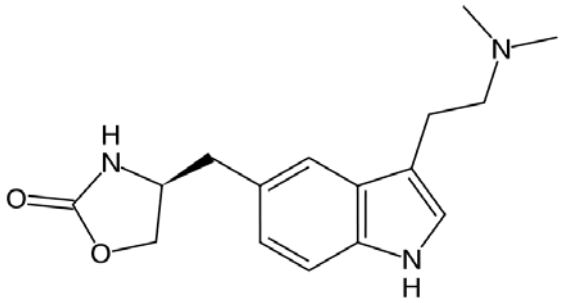
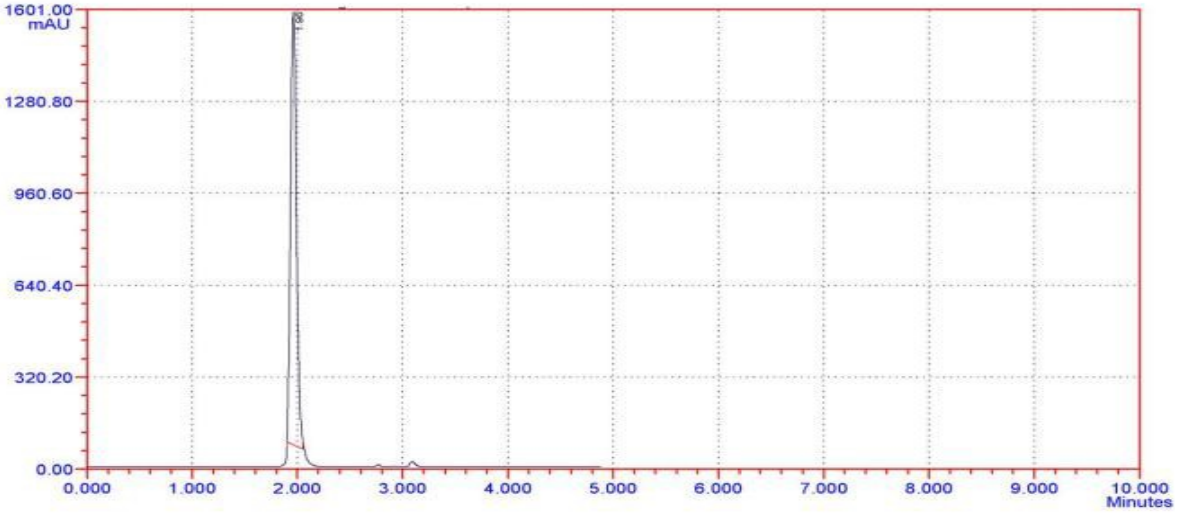
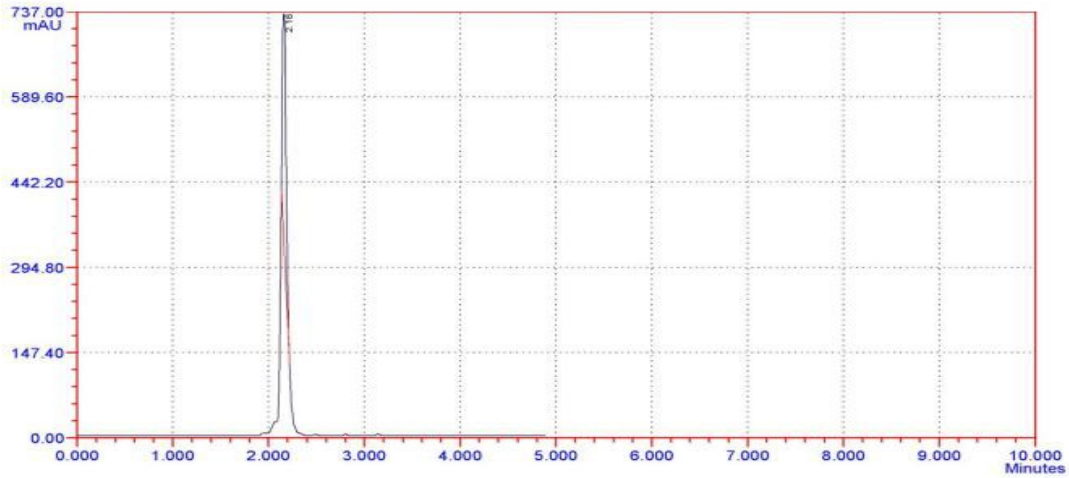


Figure1: Molecular structure of Zolmitriptan



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		1.962	151762	592372.6	100.000	1.21	5036
	Sum:		151762	592372.6	100.0000		

Figure2: HPLC chromatogram of Zolmitriptan formulation



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		2.160	41632	112041.6	100.000	1.10	12838
	Sum:		41632	112041.6	100.0000		

Figure3: HPLC chromatogram of Zolmitriptan

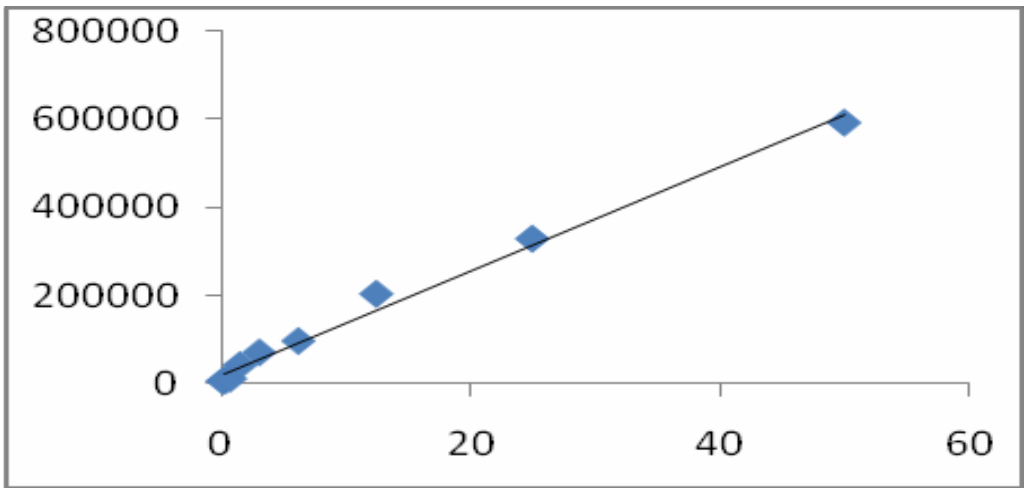


Figure3: Calibration curve

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