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Estimation of zolmitriptan by a new RP-HPLC method

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ABSTRACT

A Simple, Sensitive and specific reverse phase high performance liquid chromatographic method has been developed for the determination of Zolmitriptan tablet Dosage Forms. Chromatographic separation was achieved on a Kromasil C18 (150×4.6 mm), 5.0 µm column with a 750mL of 0.01M anhydrous dipotassium hydrogen orthophosphate, added 2mL Triethylamine then adjusted the pH to 7.5 with orthophosphoric acid, added 250mL of methanol and mixed as mobile phase, detection was at 230 nm. Response was a linear function of concentration in the range 2-0.01 µg/mL for Zolmitriptan. LOD and LOQ for Zolmitriptan were found 0.01µg/mL and 0.03µg/mL. Accuracy (recoveries 90-97%) and reproducibility were found to satisfactory.

Key Words: Zolmitriptan, RP-HPLC method, method validation.

INTRODUCTION

Zolmitriptan (Figure 1) 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. Chemically it is (S)-4-({3-[2-(dimethylamino) ethyl]-1H-Indol-5-yl} methyl)-1, 3-oxazolidin-2-one.

In this paper, we describe a simple, sensitive, and validated RP-HPLC method for determination of Zolmitriptan in tablet Dosage Forms. The method has been successfully used for quality control analysis of the drugs and other analytical purposes.

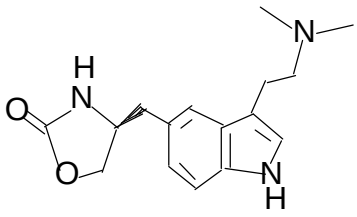

Figure I: The structure of Zolmitriptan

APPARATUS AND CHROMATOGRAPHIC CONDITIONS

Chromatographic separation was performed on a Shimadzu chromatographic system equipped with a LC-20AT pump and SPD-20A UV-VIS detector with 20µL fixed loop and analyzed by using LC-Solution software.

Kromasil C18 (150×4.6 mm), 5.0 µm column with a 750ml of 0.01M anhydrous dipotassium hydrogen orthophosphate, added 2ml Triethylamine then adjusted the pH to 7.5 with orthophosphoric acid, added 250ml of methanol and mixed as mobile phase was filtered through 0.45µ membrane filter and sonicated for 10min. An external standard method was used. UV detection was performed at 230nm and column oven temperature is 40°C. Peak was confirmed by comparison of retention time with standard.

MATERIALS AND METHODS

Preparation of standard solution

Accurately weighed 5.03mg of reference standard of Zolmitriptan in 100ml volumetric flask and the volume was brought upto the mark using Mobile phase.

Preparation of sample solution

The commercial samples of tablet containing the drug namely zomig, 5 mg (Astra Zeneca) chosen for this purpose. One tablet, containing 5 mg of Zolmitriptan was weighed accurately and transferred to a 100 ml volumetric flask with 30ml acetonitrile, shaken for 5min, and then diluted to volume with acetonitrile to furnish a solution containing 50 µg/mL Zolmitriptan. After filtration, the solution the solution was diluted with diluent as an acetonitrile to give a final concentration of 1 µg/mL Zolmitriptan.

METHOD VALIDATION

Once the HPLC method development was over, the method was validated in terms of parameters like specificity, press ion, accuracy, linearity and range, LOD,LOQ, raggedness, robustness, stability etc. For all the parameters percentage relative standard deviation values ware calculated. The proposed HPLC method was validated as per ICH guidelines.

Linearity and range

Different known concentrations of Zolmitriptan (2.0 µg/mL – 0.01 µg/mL) were prepared in diluent by diluting the stock solution. Injected the standard solutions and measured the peak area. A calibration curve has been plotted for concentration of the standards injected versus area observed and the linearity of the method was determined from the correlation coefficient. The results were shown in Table: 2. the slope, intercept and correlation coefficient values were found to be 31320, 84.75 and 0.9996.

Precision

Precision was evaluated by carrying out three independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in the sample preparation. Percentage relative standard deviation (% RSD) was found to be less than 1% for within a day and day to day variations, which proves that that method is precise. Results were shown in Table 3-4.

Accuracy

To study the reliability, suitability and accuracy of the method recovery experiments were carried out. A known quantity of the pure drug was added to the preanalysed sample formulation at the level of 50%, 100% and 200%, dissolved in diluents and made up to 100ml with same solvent. Further dilutions were made so that the each aliquot contained 0.03µg/mL of Zolmitriptan. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The recovery procedure was repeated 10 times and % RSD was calculated by using the following formula. The contents of Zolmitriptan tablet found by proposed method are shown in Table 3; the lower values of RSD of assay indicate the method is accurate. The mean recoveries were in range of 92-98 % which shows that there is no interference from excipients. Table: 5.

$$\% \text{ recovery} = \frac{b-a}{c}$$

Where,

a-The amount of drug found before the addition of standard drug
b-The amount of drug found after the addition of standard drug
c- The amount of standard drug added

Repeatability of solution

A standard solution of drug substance was injected ten times and corresponding peak areas were recorded. The % RSD was found to be less than 1%.Table:6.

Specificity
Condition of HPLC method like percentage of organic solvent in mobile phase, ionic strength, pH of buffer flow rate etc, was changed. In spite of above changes no additional peaks were found, although there were shift retention times or little changes in peaks shapes.

Assay
20µl of standard and sample solutions were injected into an injector of RP-HPLC, from the peak area of standard amount of drug in sample were computed. The values are given in Table: 7

Limit of detection and limit of quantification
The limit of Detection (LOD) and limit of Quantification (LOQ) of the development method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Zolmitriptan found to be 0.01µg/mL The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10) The LOQ was 0.03µg/mL for Zolmitriptan. It was concluded that the developed method is sensitive.

Ruggedness and robustness
The ruggedness of the method was determined by carrying out the experiment on different instruments like shimadzu HPLC and Agilent HPLC by different operators using different columns of similar types. The % RSD values with two different instruments shimadzu HPLC and Agilent HPLC, analyst and columns were 0.5-0.5, 0.6-0.5 and 0.4-0.3% respectively.

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as changes in mobile phase, flow rate and column temperature. It was observed that ther were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method is rugged and robust. The robustness limit for mobile phase variation, flow rate variation, and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%.

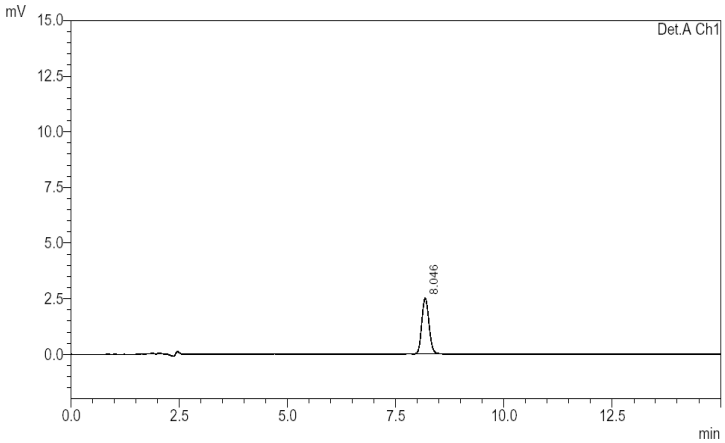

Figure II: Chromatogram of standard (1.0 µg/mL)

RESULTS AND DISSECTION

UV spectrum of Zolmitriptan was recorded from which 230nm was selected as wavelength. Flow rate of 1.0mL/min was selected. 0.01M anhydrous dipotassium hydrogen orthophosphate added 2ml Triethylamine then adjusted the pH to 7.5 with orthophosphoric acid, added 250ml of methanol as mobile phase. The retention time was found to be 8.1min. Zolmitriptan shown linearity in the range of 0.01-2µg/mL, and the co-efficient was found to be 0.9998. Recovery studies were performed at 50%, 100% and 200%, levels. The sensitivity of method LOD and LOQ is shown in Table 2. The stability at room temperature and refrigeration was found to be 3 and 8.5 hrs respectively. Hence the proposed method is simple, accurate, and rapid and can be employed for routine analysis. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method.

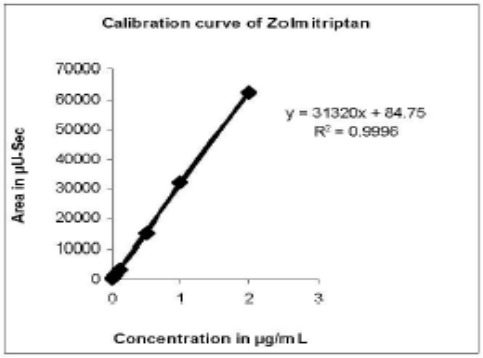

Figure III: Linearity curve of Zolmitriptan

Regression analysis of the calibration curve for Zolmitriptan showed a linear relationship between the concentration and peak area with correlation coefficients higher than 0.9998 in all curves assayed.

Table I Optimized chromatographic conditions

Parameter	Optimized condition
Chromatograph	HPLC (Shimadzu system equipped with LC-20 AT pump and SPD-20A interfaced with LC Solution software
Column	Kromasil C18 (150×4.6 mm), 5.0 µm column
Mobile Phase	750ml of 0.01M anhydrous dipotassium hydrogen orthophosphate, added 2ml Triethylamine then adjusted the pH to 7.5 with orthophosphoric acid, added 250ml of methanol and mixed as mobile phase
Flow Rate	1.0mL/min
Detection	UV at 230nm
Injection Volume	20µL
Column oven temperature	40ºC

Table II Validation Parameters

Parameters	Zolmitriptan
Linearity range	0.01-2 µg/mL
Correlation coefficient	0.9996
Slope	31320
Y Intercept	84.75

Table III Intraday Precession

Concentration (µg/mL)	Area	%RSD
0.03	969	0.87
	979	
	986	
0.3	9609	0.67
	9545	
	9481	
1	32387	0.63
	32658	
	32789	

The intraday precision was found to be within 1% RSD for conc.0.03, 0.3, 1.0µg/mL

Table IV Interday Precision

Concentration (µg/mL)	Day	Area	% RSD
0.03	1	902	
	2	913	1.33
	3	889	
0.3	1	9175	
	2	9069	1.18
	3	9286	
1	1	32087	
	2	32184	1.02
	3	31575	

Intraday precision was performed for con. Of 0.03, 0.3 and 1.0 µg/mL. For about three days and their peak, areas are shown in the table. The %RSD for con. 0.03, 0.3, and 1.0 µg/mL was found to be within 2%

Table V Recovery studies

Level (µg/mL)	% Recovery	% RSD
0.03	90	0.74
0.3	96	0.53

Recovery studies were performed at 0.03µg/mL and 0.3 µg/mL levels and the results were found to be within the limits mentioned as per ICH guidelines.

Table VI Repeatability of injection

Con (µg/mL)	Peak area	%RSD
0.3	9688 9574 9513 9298 9366 9478 9523 9603 9555 9385	1.25

Repeatability of injection was performed using 0.3µg/mL sample for 10 times and corresponding peak areas were recorded. The % RSD peak was reported.

Table VII Analysis of formulation

Amount of drug (mg)		% Label claim	%RSD (n=6)
Labeled	Estimated	97	0.41
5.0	4.86		

Analysis of formulation was performed using Zolmitriptan 1.12 mg of injections and the claim was found to be 96.

CONCLUSION

A convenient and rapid RP-HPLC method has been developed for estimation of Zolmitriptan in tablet dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. The proposed method is simple, fast, accurate and precise for the simultaneous quantification of Zolmitriptan in dosage form, bulk drugs as well as for routine analysis in quality control.

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REFERENCES

[1] P. Vivek Sagar, Dhiraj Kumar, Suddhasattya Dey and Himansu Bhusan Samal. *Journal of Pharmacy Research* **2010**; 3(12): 2930-2933.
[2] Mallikarjuna Rao, M.K. Srinivasu, G. Sridhar, P. Rajender Kumar, K.B. Chandrasekhar, Aminul Islam. *Journal of Pharmaceutical and Biomedical analysis*, **2005**; 39: 503-509.
[3] Moira Dunne and Peter Andrew. *Journal of Pharmaceutical and biomedical Analysis* **1996;** 14: 721-726.
[4] The *British Pharmacopoeia*; British Pharmacopoeial Commission, London, UK, (**2008**).
[5] I.C.H Guidelines, *Analytical Method Validation (Q3).* Geneva, (**2000**).
[6] Jun Chem, Xinguo Jiang, Wenming, Ni Mel, Xiaoling Gao, Qizhi Zhang. *Journal of chromatography B,* **2004;** 805 169-173.
[7] A. Femen ya-Font, V. Merino, V. Rodilla, A. L opez-Castellano, *Journal of Pharmaceutical and Biomedical Analysi,s* **2005;** 37: 621-626.