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A validated RP-HPLC method for the quantification of Zolmitriptan in tablet dosage form

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ABSTRACT

A simple and rapid RP-HPLC method was developed for quantification of zolmitriptan in tablet dosage form. The chromatography system used a reversed phase C_{18} column with dual wavelength absorbance detection at 229 nm. The mobile phase consisted of acetonitrile and phosphate buffer (pH adjusted to 3.5 using ortho phosphoric acid) in the ratio of 10:90 % v/v at flow rate of 1.5 mL/min. The linearity range was found to be 10-50 µg/mL. The method was validated and it was concluded that the developed method was accurate, sensitive, precise, robust and useful for the quality control of zolmitriptan in pharmaceutical preparations.

Keywords: Zolmitriptan, RP-HPLC, Validation, Serotonin.

INTRODUCTION

Zolmitriptan (ZOL), 4(S)-4-[3-(2-dimethyl amino ethyl)-1H-5-indolyl-methyl]-1, 3-oxazolan-2-one (Figure 1) is a selective serotonin 5-HT $_{1B}$ and 5-HT$_{1D}$ receptor agonist. Migraine headache is believed to result from dilation of the blood vessels in the brain. ZOL causes constriction of the blood vessels and thereby relieves the pain of a migraine headache**.** It is therapeutically used in the acute treatment of migraine attacks with or without aura and cluster headaches. While ZOL is very effective in relieving migraine headaches, it does not prevent or reduce the number of headaches if taken prophylactically [1-2].

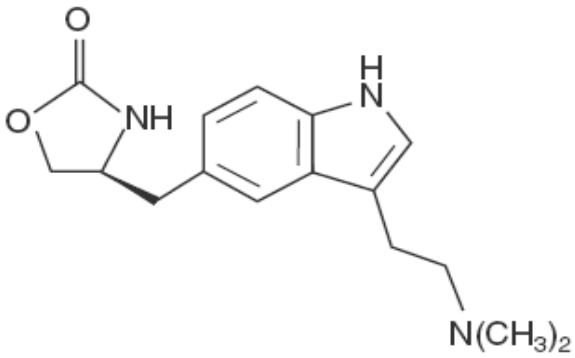

Fig. 1: Structure of Zolmitriptan

A detailed literature survey for ZOL revealed that several analytical methods were reported for the determination of zolmitriptan by spectrophotometric [3] and HPLC [4-7] in pharmaceutical formulations, chiral HPLC [8] in rat liver microsomes, HPLC [9] in human plasma and HPLC/MS [10] in plasma. The principle objective of this study was to develop a new, simple, economical, selective, accurate and precise reverse phase high-performance liquid chromatographic method with good sensitivity for assay of ZOL in tablet dosage form.

MATERIALS AND METHODS

Reagents and chemicals

Zolmitriptan was generous gift from Suven Life Sciences Limited, Hyderabad (INDIA). ZOL tablets containing 5 mg of active substance from Astra Zeneca Pharmaceutical Ltd. (ZOMIG and ZOMIG-ZMT) were obtained from commercial market and used within their self life period. HPLC grade acetonitrile, ortho phosphoric acid and potassium dihydrogen phosphate were obtained from Rankem, New Delhi, India. High purity deionized water was obtained from a Millipore, Milli-Q (Bedford) purification system.

Instrumentation

A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2487 dual wavelength absorbance detector (DAD) was employed throughout the analysis. The data was acquired using Empower 2 software. The column used was XTerra symmetry C_{18} (150×4.6 mm, 5µm). A Bandline sonerex sonicator was used for enhancing dissolution of the compounds. A Digisum DI 707 digital pH meter was used for pH adjustment.

Chromatographic conditions

The chromatographic elution was carried out in isocratic mode using a mobile phase consisting of acetonitrile and phosphate buffer (pH 3.5, pH adjusted with ortho phosphoric acid) in a ratio of 10:90 v/v. The analysis was performed at ambient temperature using a flow rate of 1.5 mL/min with a run time of 9 min. The eluent was monitored using DAD at wavelength of 229 nm. The mobile phase was filtered through whatmann filter paper No.41 prior to use.

Preparation of stock and standard solutions

A stock solution of ZOL (1000 µg/mL) was prepared by taking accurately weighed 100 mg of ZOL reference standard into 100 mL volumetric flask containing 50 mL deionized water and then volume was made up to the mark with deionized water. The stock solution is protected from light using aluminum foil. Aliquots of the standard stock solution of ZOL ware transferred using

A-grade bulb pipette into 100 mL volumetric flasks and solutions were made up to the mark with mobile phase to give the final concentrations of 10-50 µg/mL.

Estimation of zolmitriptan from tablet dosage form

To determine the content of ZOL in tablets (label claim: 5 mg), 20 tablets were taken and contents were weighed and mixed. An aliquot of powder equivalent to the weight of one tablet was accurately weighed and transferred to 50 mL volumetric flask containing 25 mL of deionized water and then volume was made up to the mark with deionized water. The flask was sonicated for 25 min to affect complete dissolution. The solution was filtered through whatmann filter paper No. 41. Suitable aliquot of the filtered solution was transferred into a 100 mL volumetric flask and made up to the volume with mobile phase to yield the concentration of 20µg/mL. The experiment was performed six times under the chromatographic conditions described above. The peak areas were measured at 229 nm and concentration in the sample was determined by comparing the area of sample with that of the standard.

Method validation

Linearity

By appropriate aliquots of the standard ZOL solution with mobile phase, five working solutions ranging between 10-50 µg/mL were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions. The peak area of the chromatogram was plotted against the concentration of ZOL to obtain the calibration curve.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Standard ZOL was added to previously analyzed samples in according to 50, 100 and 150% of label claim. The accuracy was expressed as the percentage of analyte recovered by the proposed method. [11]

Precision

Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines [11]. The intra-day and inter-day precision were determined by analyzing the samples of ZOL at concentration of 20, 30 and 40µg/mL. Determination was performed with three replicates on the same day as well as on three consequent days.

Reproducibility

The reproducibility of the method was checked by determining precision on a same instrument, analysis being performed by another person in same laboratory. It was determined by analyzing the samples of ZOL at different concentration (30, 40, 50 µg/mL) in triplicate and the percentage of drug present in the sample was calculated. [11]

Limit of detection and limit of quantification

Limit of detection (LOD) and limit of quantification (LOD) were calculated based on the ICH guidelines [11].

Robustness

The robustness of the method was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from1.5 to 1.4 mL/min and 1.6 mL/min. The organic strength of the mobile phase was varied by ±2%. [11]

System suitability tests

System suitability parameters like tailing factor, number of theoretical plates and retention time were evaluated by injecting the working standard solution (20µg/mL) ten times into HPLC system. [11]

RESULTS AND DISCUSSION

A RP-HPLC was proposed as a suitable method for the quantification of ZOL in tablet dosage forms. The best chromatographic conditions were adequately selected. The selection of mobile phase and flow rate were made on the basis of peak shape, baseline drift, time required for analysis and economical. The mobile phase consisted of acetonitrile and phosphate buffer (pH 3.5, adjusted pH with ortho phosphoric acid) in the ratio of 10:90 v/v at flow rate of 1.5 mL/min and analyzed at 229 nm. The retention time observed (5.601) allows a rapid determination of the drug. In Figure 2, a typical chromatogram obtained under these conditions is shown. [11, 12]

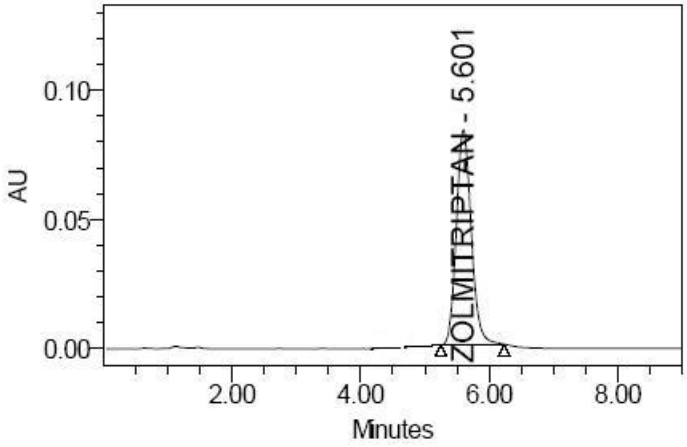

Fig. 2: Typical chromatogram of Zolmitriptan

The calibration plot of peak area against concentration was linear in the range of 10-50 µg/mL. Calibration data with their % relative standard deviation (%RSD) and linear regression equation are listed in Table 1. The range of reliable quantification was set at 10-50 µg/mL as no significant difference was observed in the slope of the standard curve in this range. The linear regression data for the calibration curve is indicative of a good linear relationship between peak area and concentration over a wide range. The correlation coefficient was indicative of high significance. The LOD and LOQ were determined based on a signal-to-noise ratio and were based on analytical responses of 3 and 10 times of the background noise, respectively. The LOD was found to be 0.08µg/mL. The LOQ was found to be 0.26µg/mL. [11, 12]

Table 1: Linearity regression data for the calibration plot of zolmitriptan

Analyte	Conc. (µg/mL)	Mean area ±SD (n=3)	RSD (%)	Linear regression equation
ZOL	10	637059±2489	0.391	y = 68384x - 51228 r^2=0.9996
	20	1333974±3264	0.245	
	30	1974885±4529	0.229	
	40	2664689±5444	0.204	
	50	3390926±8623	0.254	

The accuracy was assessed from three replicates containing concentration of 15, 20 and 25µg/mL. The recovery of the method was determined by spiking a previously analyzed test solution with addition of standard ZOL solution and was found to be in the range of 99.28-100.6%. The values of % recovery and %RSD are listed in Table 2, indicates that the method is accurate.

Table 2: Results of recovery studies

Analyte	Amount (%) of drug added to analyte	Theoretical content (µg/mL)	Conc. found (µg/mL) ± SD (n=3)	RSD (%)	Recovery (%)
ZOL	50	15	15.06±0.02	0.134	100.39
	100	20	20.12±0.01	0.05	100.6
	150	25	24.82±0.11	0.442	99.28

Precision of the method was measured in accordance with ICH guidelines. Repeatability of the method was determined as intra-day variation while intermediate precision was determined by measuring inter-day variation for triplicate determination of ZOL at three different concentrations. The results of repeatability and intermediate precision are listed in Table 3. The low %RSD values indicate that the method is precise. Reproducibility of the method was performed in the same laboratory on a same instrument which was performed by another analyst. The results for reproducibility are listed in Table 4. The assay values and low %RSD values indicate that the method is reproducible.

Table 3: Intra-day and Inter-day precision of the method

Analyte	Conc. (µg/mL)	Repeatability precision		Intermediate precision	
		Mean area ±SD (n=3)	RSD (%)	Mean area±SD (n=3)	RSD (%)
ZOL	20	1336495±14385	1.076	1353101±11185	0.827
	30	1920963±16611	0.865	1906391±15930	0.836
	40	2596872±12005	0.462	2570176±10992	0.428

Table 4: Reproducibility of the proposed method

Analyte	Conc. (µg/mL)	Conc. found (µg/mL) ± SD (n=3)	RSD (%)	Amount found (%)
ZOL	30	29.71±0.032	0.108	99.02
	40	39.82±0.031	0.077	99.55
	50	50.16±0.355	0.708	100.32

The robustness was determined by analyzing the same sample under a variety of conditions. The factors considered were variations in the flow rate (±0.1) and percentage of acetonitrile (±2%). The results and the experimental range of the selected variable are given in Table 5, together with the optimized conditions. There were no significant changes in the chromatographic pattern when the above modifications were made in the experimental conditions, showing that the method is robust. [11, 12]

Table 5: Results from testing the robustness of the method

Conc. of analyte (µg/mL)	Flow rate		% Amount found ±SD (n=3)	RSD (%)	% Organic		% Amount found ±SD (n=3)	RSD (%)
	Original	used			Original	used		
20	1.5	1.4	99.61±0.239	0.2401	10	9.8	99.79±0.668	0.6695
		1.5	100.37±0.428	0.4266		10	100.18±0.529	0.5382
		1.6	100.84±0.764	0.7575		10.2	100.81±0.091	0.0898

The system suitability tests were also carried out to evaluate the reproducibility of the system and the results are given in Table 6, showing that the parameters are within the suitable range. [11, 12]

Table 6: Results of system suitability tests

Parameters	Results of ZOL
Retention time (min)	5.608
Tailing factor	1.15
Theoretical plates (N)	2922

The proposed method was applied to the analysis of marketed formulations and the results obtained are given in Table 7. The blank solution was prepared containing the components indicated in tablet dosage form except the active ingredient. No interference was observed from the tablet excipients. The ZOL content was found to be 99.58% and 100.08 % for ZOMIG and ZOMIG-ZMT, respectively. The low %RSD indicated the suitability of this method for routine analysis of ZOL in pharmaceutical dosage forms, shown in Table 7. [11-13]

Table 7: Analysis of zolmitriptan in tablets

Tablet Formulation	Label Claim per Tablet (mg)	% Drug found ± SD (n=6)	RSD (%)	SEM
ZOMIG	5	99.58±0.1999	0.201	0.0816
ZOMIG-ZMT	5	100.08±0.2038	0.204	0.0832

CONCLUSION

The proposed RP-HPLC method is rapid, specific, accurate and precise for the quantification of ZOL from its tablet dosage form. The method has been found to be better than previously

reported methods, because of its wide range of linearity, use of readily available mobile phase, lack of extraction procedures and low t_R. All these factors made this method suitable for quantification of ZOL in tablet dosage forms. The method can be successfully used for routine analysis of ZOL in bulk drugs and pharmaceutical dosage forms without interference.

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