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VALIDATED RP - HPLC METHOD FOR THE ESTIMATION OF ZOLMITRIPTAN IN FORMULATION

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Zolmitriptan in tablet dosage form. Isocratic elution at a flow rate of 1.0ml/min was employed on symmetry C18 (250 x 4.6 mm, 5 µm in particle size) at ambient temperature. The mobile phase consisted of 0.01% triethylamine : acetonitrile : 0.02M $NH_4H_2PO_4$; 28.2:25:46.8 (V/V/V). The UV detection wavelength was 225 nm and 20µl sample was injected. The retention time for Zolmitriptan was 3.705 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Zolmitriptan in tablet dosage form.

Keywords: Zolmitriptan, RP-HPLC, UV detection, Recovery, Precise.

INTRODUCTION

Zolmitriptan molecular formula $C_{16}H_{21}N_3O_2$ and weight 287.357 g/mol and IUPAC name (*S*)-4-({3 - [2-(dimethylamino) ethyl]- 1*H*- indol - 5-yl}methyl)-1,3-oxazolidin-2-one. Zolmitriptan is a selective serotonin receptor agonist of the 1B and 1D subtype. It is a triptan, used in the acute treatment of migraine attacks with or without aura and cluster headaches.[1,2] Zolmitriptan is marketed by AstraZeneca with the brand names Zomig, Zomigon. Zolmitriptan may increase blood pressure; it should not be given to patients with uncontrolled hypertension, should not be used within 24 hours of treatment with another 5-HT1 agonist, or an ergotamine-containing or ergot-type medication like dihydroergotamine or methysergide, and should not be administered to patients with hemiplegic or basilar migraine.[3-7]

MATERIALS AND METHODS

Chemicals and Reagents

HPLC grade acetonitrile, triethylamine and ammonium dihydrogenphosphste were purchased from Merck Specialties Pvt. Ltd.

Instrumentation and Analytical Conditions

The analysis of drug was carried out on a PEAK HPLC system equipped with a reverse phase C18 column (250x4.6mm, 5µm in particle size), a LC-P7000 isocratic pump, a 20µl injection loop and a LC-UV7000 absorbance detector and running on PEAK Chromatographic Software version 1.06. Isocratic elution with 0.01% tri ethyl amine: acetonitrile: 0.02M $NH_4H_2PO_4$ 28.2:25:46.8 (V/V) (P^H-3.2) was used at a flow rate of 1.0ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

Stock and Working Standard Solutions

Accurately weigh and transfer 10mg of Zolmitriptan working standard into a 10ml volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm nylon filter paper and finally11ppm were prepared. The calibration curve was plotted with the five concentrations of the 3ppm – 11ppm working standard solutions. Calibration solutions were prepared daily and analyzed immediately after preparation.

Assay of Zolmitriptan Tablets

Weigh 20 Zolmitriptan (ZOMIG - 5mg) tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10mg of Zolmitriptan in to a 10ml volumetric flask. Add diluent and sonicate to dissolve it completely and make volume up to the mark with diluents. Mix well and filter through 0.45um filter. Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to mark with diluents and finally 11ppm were prepared. Mix well and filter through 0.45um filter. An aliquot of this solution was injected into HPLC system. Peak area of Zolmitriptan was measured for the determination. The results are furnished in Table 3.

Validation Procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 3ppm to 11ppm prepared in triplicates to test linearity. The peak area of Zolmitriptan was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from six replicate injections of freshly prepared Zolmitriptan test solution in the same equipment at a concentration value of 100% (11ppm) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of the Zolmitriptan was determined and precision was reported as %RSD.

Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of Zolmitriptan at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Zolmitriptan recovered in the samples. Sample solution short term stability was tested at ambient temperature (20 ± 10^0C) for three days. In order to confirm the stability of both standard solutions at 100% level and tablet sample solutions, both solutions protected from light were re-injected after 24 and 48 hours at ambient temperature and compared with freshly prepared solutions.

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. The drug Zolmitriptan is nonpolar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of tri ethyl amine, acetonitrile and 0.02M $NH_4H_2PO_4$ was selected as mobile phase and the effect of composition of mobile phase on the retention time of Zolmitriptan was thoroughly investigated. The concentration of the triethylamine, acetonitrile and 0.02M $NH_4H_2PO_4$ were optimized to give symmetric peak with short run time (Fig.3).

Validation of Method

Linearity

Five points graphs was constructed covering a concentration range 3-11ppm (Three independent determinations were performed at each concentration). Linear relationships between the peak area signals of Zolmitriptan the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

Precision

The validated method was applied for the assay of commercial tablets containing Zolmitriptan. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correctly and hence the developed analytical method is highly repetitive. For the intermediate precision a study carried out by the same analyst working on the same day on two consecutive days indicated a RSD of 0.895. This indicates good method precision.

Stability

The stability of Zolmitriptan in standard and sample solutions containing determined by storing the solutions at ambient temperature (20 ± 10^0C). The solutions were checked in triplicate after three successive days of storage and the data were compared with freshly prepared samples. In each case, it could be noticed that solutions were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that Zolmitriptan is stable and standard and sample solutions for at least 2 days at ambient temperature.

System suitability

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits (Table3). The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Zolmitriptan in tablet formulation. The results are furnished in Table 3.

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Zolmitriptan in tablet dosage form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 6 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Zolmitriptan in pharmaceutical dosage form.

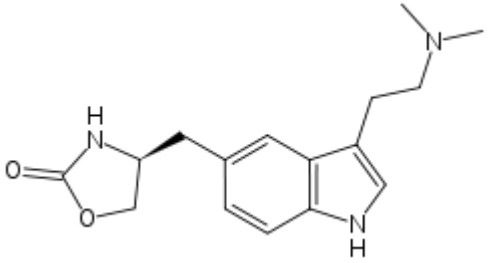


Figure1: Chemical Structure of Zolmitriptan

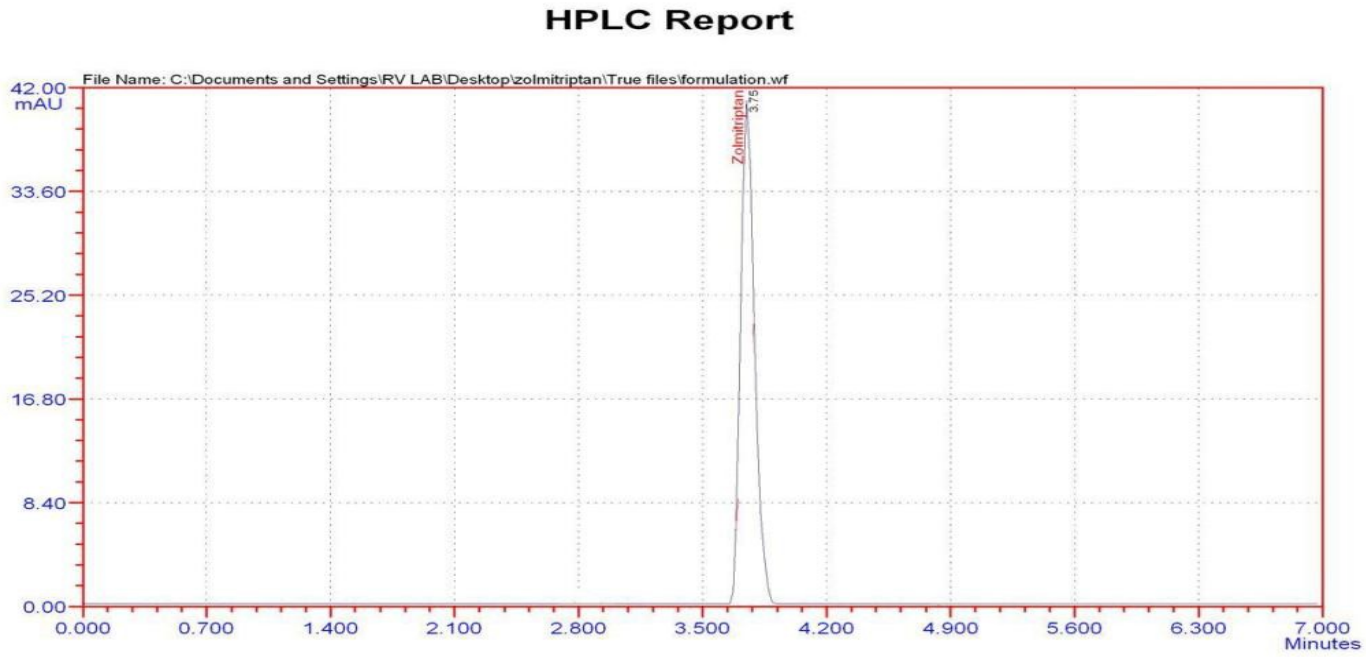
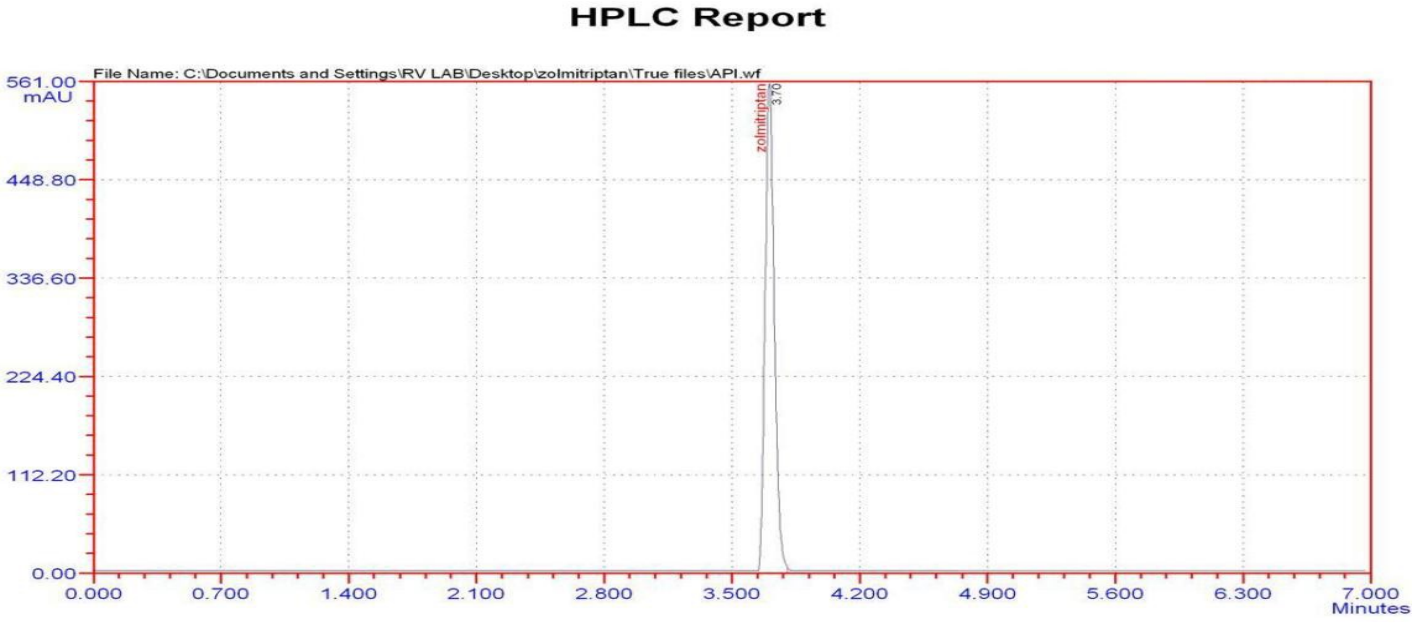


Figure2: Typical chromatogram of Zolmitriptan formulation



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	zolmitriptan	3.705	57102	210196.0	100.000	1.02	20190
	Sum:		57102	210196.0	100.0000		

Figure3: Typical chromatogram of Zolmitriptan

Table 1: Linearity of Zolmitriptan

S.No.	Linearity level	Concentration	Area
1	I	3ppm	30487.5
2	II	5ppm	55101.7
3	III	7ppm	76517.3
4	IV	9ppm	95245.1
5	V	11ppm	119727.6

Table 2: Recovery studies of Zolmitriptan

% Concentration	% Recovery	Mean Recovery
50%	98.22%	
100%	99.01%	98.62%
150%	98.65%	

Table 3: System stability parameters

Parameters	Values
λ max (nm)	225
Beer's law limit (ppm)	3-11
Correlation coefficient	0.999
Retention time	3.705
Theoretical plates	21909.00
Tailing factor	1.29
Limit of detection (ppm)	0.15
Limit of quantification (ppm)	0.5
Slope	10931.18
Intercept	-1102.42
accuracy	99.56%
R.S.D.	0.895
% of Zolmitriptan in formulation	1.17%

Table 4: Assay

Formulation	Label claim (mg)	% Amount found
Zomig	5mg	1.17%

Table 5: Chromatographic condition

Mobile phase	**0.01% triethylamine:Acetonitrile:0.02M $NH_4H_2PO_4$ (28.2:55:46.8)**
P^H	3.2
UV detection	225nm
Analytical column	C18
Flow rate	1.0ml/min
Temperature	Ambient
Injection volume	20µl
Runtime	7min
Retention time	3.705 min

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