Research Article

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METHOD DEVELOPMENT AND VALIDATION OF ZOLMITRIPTAN IN BULK AND TABLET DOSAGE FORM BY RP-HPLC

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Abstract

A simple, precise, accurate, reproducible and robust HPLC method was developed for the estimation of Zolmitriptan in tablet dosage form. The method was validated as per ICH guidelines. Analysis of the drug was performed on Waters X terra C_{18} column (250 mm x 4.6 mm x 5 µ), consisting of mobile phase, employing phosphate buffer and methanol (80:20 V/V) as mobile phase at a flow rate of 1 ml/min, The retention time of drug was found to be 4.432 min. The estimation of drug was carried out at 225 nm using UV-Visible detector. The method produced good linear responses in the concentration range of 12.5 to 75 µg/ml. The % RSD for inter-day and intraday precision was found to be 0.771 and 0.7750 respectively. The proposed method was found to be applicable for determination of the drug in tablet dosage form.

Keywords: Zolmitriptan, RP-HPLC, ICH.

Introduction

Zolmitriptan, was chemically known as (4S)-4-[[3-[2-(dimethyl amino) ethyl]-1H-indol-5-yl] methyl]-2-oxazolidinone (Figure 1) is a novel serotonin 5-hydroxytryptamine receptor agonist that has shown, in an extensive clinical trial program, to be highly effective in the acute oral treatment of migraine . It works by stimulating serotonin receptors in the brain. Serotonin is a natural substance in the brain that, among other things, causes blood vessels in the brain to narrow. Zolmitriptan mimics this action of serotonin by directly stimulating the serotonin receptors in the brain. This cause the blood vessels to narrow Zolmitriptan is used to treat severe migraine headaches. The empirical formula is $C_16H_21N_3O_2$, representing a molecular weight of 287.36. Zolmitriptan is a white to almost white powder that is readily soluble in water.

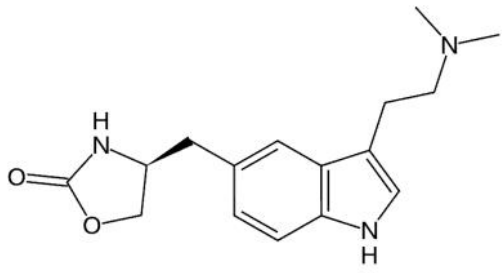

Fig. No.1: Chemical structure of Zolmitriptan

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Several studies for the estimation of the Zolmitriptan drug using various techniques have been carried out, some of them being ; dose proportionality and tolerability of single and repeat doses of a nasal spray formulation of ZLM in healthy volunteers[1]. A validated RP-HPLC method for the estimation of Zolmitriptan in Formulation[2]. Reversed-Phase high-performance liquid chromatographic Method for the estimation of Zolmitriptan in bulk and in Pharmaceutical formulations[3]. A New Stability Indicating RP-HPLC Method for Related Substances in Zolmitriptan[4]. A validated chiral LC method for the enantiomeric separation of Zolmitriptan key intermediate[5].A validated chiral LC Method for the determination of Zolmitriptan and its potential impurities[6]. A Validated RP-HPLC Method for the determination of Zolmitriptan-A Serotonin 5- HT Receptor Agonist[7]. Development and Validation of Stability-Indicating UV-Spectrophotometric Methods for the Determination of Zolmitriptan in Pharmaceuticals[8]. Development and Validation of a Simple UV Method for In-Vitro Estimation of Zolmitriptan in an Intraoral Dosage form[9].Hence the literature survey revealed that the proposed method is simple, rapid, accurate, reproducible and economical with good precision.

Materials and methods

Chemicals and reagents

Zolmitriptan working standard was obtained as gift sample from SMS Pharmaceuticals Pvt Limited, Hyderabad, India. A tablet formulation containing 5 mg of Zolmitriptan was purchased from local market. HPLC-grade Methanol and O-phosphoric acid were obtained from Merck, Mumbai Pvt limited.

Instrumentation

The HPLC instrument used was Waters ALLIANCE 2695 with Empower software was used for data acquisition. Waters X terra C_{18} (250 mm x 4.6 mm x 5 µm) column was used as a stationary phase for analysis, Denver Weighing balance and Ulta Sonicator (Fast clean). Deionized water obtained from Milli-Q Water purification system.

Chromatographic conditions

The chromatographic column, X terra C_{18} (250 mm x 4.6 mm x 5 µm) used as a stationary phase. Mobile phase consisting of O-phosphoric acid and methanol in the ratio (80:20 V/V) degassed by sonication and made up to the final volume and filtered through 0.45 µm nylon filter, Injection volume was 10 µl. The pump flow rate was 1ml/min. The eluent was detected at 225 nm at 30^0 C.

Preparation of standard stock solution

Accurately Weigh and transfer 5 mg of Zolmitriptan working standard to a 10 ml volumetric flask containing diluent, shake for 5 minutes. Then made up to the mark with diluent. The above solution was sonicated, filtered through 0.45µm nylon filter. Transfer 1 ml of the clear filtrate to 10 ml volumetric flask to obtain 50 µg/ml.

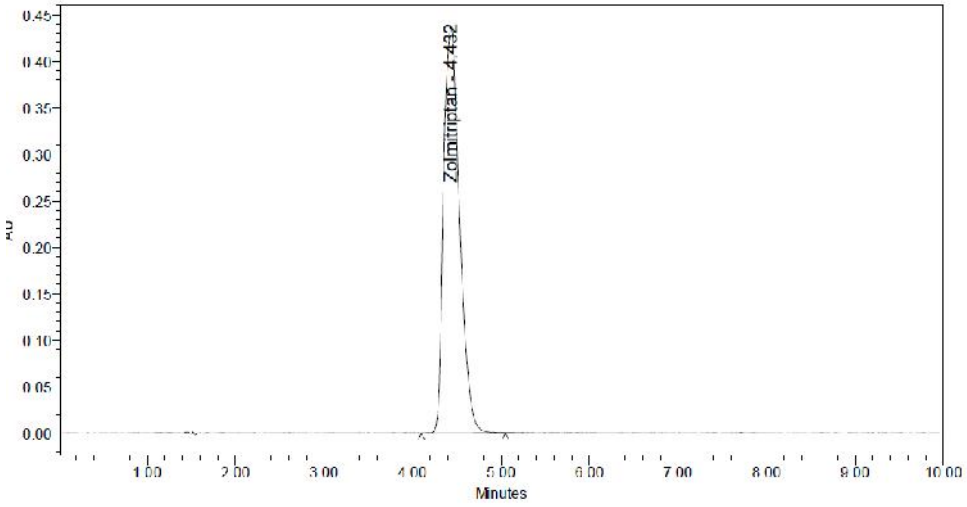

Fig. No. 02: Standard Chromatogram of Zolmitriptan

Preparation of sample solution

Accurately weigh and transfer a quantity of tablet powder equivalent to 5 mg of Zolmitriptan to 10 ml volumetric flask containing diluent, shake for 5 minutes. The above solution was sonicated, filtered through 0.45 µm nylon filter. Further 1 ml was taken in to a 10 ml volumetric flask and diluted up to the mark with diluent to obtain the final concentration 50 µg/ml. An aliquot of this solution was injected into HPLC system. Peak area of Zolmitriptan was measured for the determination.

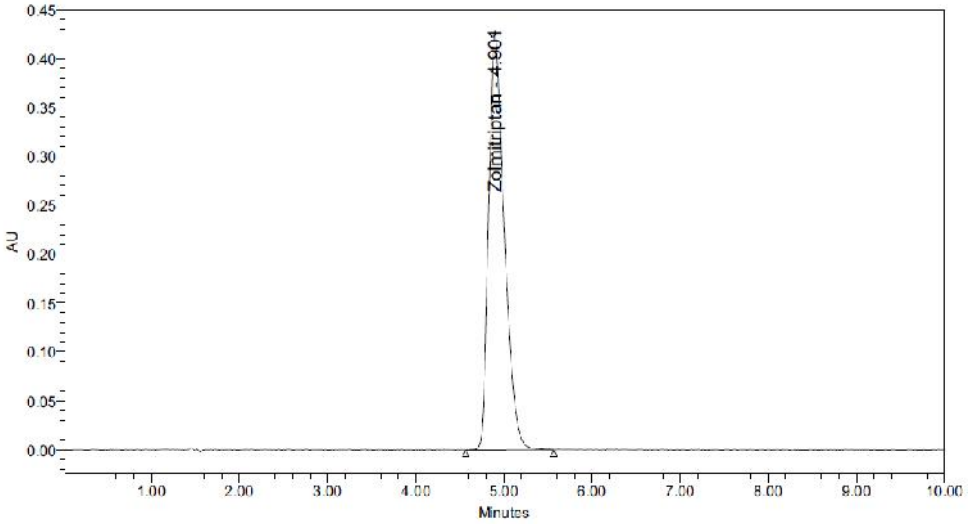

Fig. No.03: Zolmitriptan in marketed formulation

Validation parameters

Linearity

The linearity was performed from the stock solution, the 1ml was taken and transferred into a 10 ml volumetric flask and evaluated at six concentration levels by diluting the standard stock solution to give solutions over the range 12.5-75 µg ml^{-1} **(**Table 2**)**. A graph was plotted for the concentration of the corresponding drug versus area. The correlation coefficient for each drug was calculated.

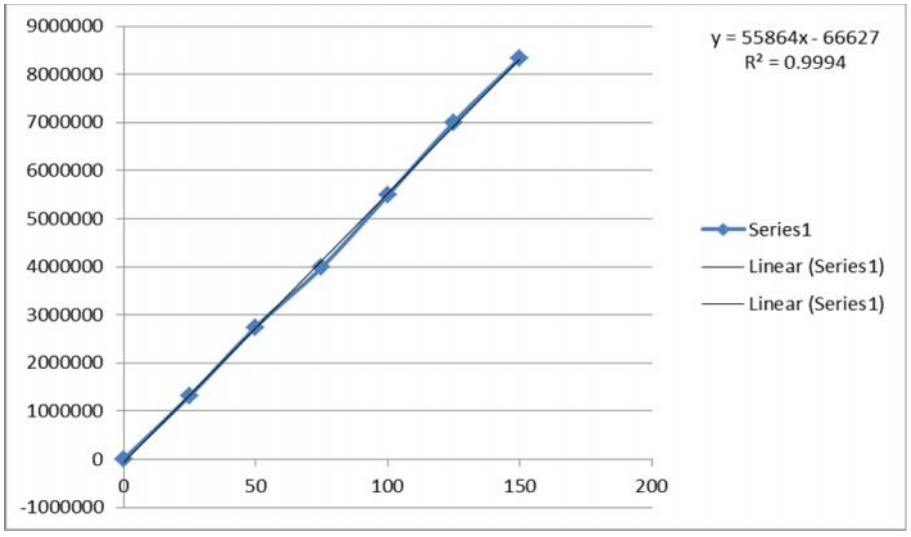

Fig. No.04: Calibration curve for Zolmitriptan

Precision

The system precision was demonstrated by preparing the standard solution at certain concentration and injected repeatedly for six times for both intraday (Table 3**)** and inter-day precision shown in (Table 4**)**. The % RSD for repeatability of

standard preparation calculated from the data obtained.

Accuracy

To determine the accuracy in sample preparation, method of standard additions was made for measuring the recovery of the drugs. To the standard solution, known concentrations of the drug (50%, and 100%, 150 %) were added. The accuracy was expressed as the percentage of the analytes recovery (Table 5).

Robustness

To verify the robustness of the method, the analysis was done under variable flow rates. The flow rate as per the developed method is 1.0 ml/ min. This has been deliberately changed to 0.8 ml/min and 1.2 ml/min and chromatogram was developed. Temperature as per the developed method is 30^0 C. This has been changed to 25^o C and 35^0 C and the chromatogram was developed (Table 6).

Results and discussion

In the initial trials, the following mobile phases were used, Methanol : O-phosphoric acid (55:45V/V) (mobile phase 1), but mobile phase 1 has been rejected due to the improper elution of eluent, so an attempt was made by changing column and mobile phase ratios gradually. The mobile phase, Methanol and O-phosphoric acid in the ratio of (80:20 V/V) gave the best results. As per developed method, the injection volume was 10 µl and the mobile phase flow rate was constant at 1.0 ml/min. The retention time of zolmitriptan was 4.432 min. The Calibration curve was linear over the range of 12.5-75 µg/ml. The linearity of method was statistically confirmed. The correlation coefficients (r^2) for calibration curve were not less than 0.999. The percentage purity for Zolmitriptan was found to be 99.40. So this method was suitable for analyzing the marketed formulations. The percentage relative standard deviation for zolmitriptan was found to be 0.771. The % RSD was found to be within the limit according to ICH guidelines.

Table No. 01: Results for Assay

S. No	Brand names	RT	Area (mean)	Label claim	Amount present	% Mean recovery
1	Zomig-5mg	4.432	4300773	5mg	4.970	99.80
2	Zomig ZMT	4.432	4300773	5mg	4.970	99.80

Table No. 02: Results for Linearity

S. No	Concentration (in µg)	Area
1	12.5	1315985
2	25	2744424
3	37.5	3945351
4	50	5484985
5	62.5	6998223
6	75	8333261

Table No. 03: Results for Intra Precision

Drug concentration	Peak area	SD	%RSD
50µg	4268993 4311997 4320848 4318671 4313102 4373499	33317	0.7716

Table No. 04: Results for Interday Precision

Drug concentration	Peak area	SD	%RSD
50µg	4268437 4311965 4320876 4318651 4313131 4373453	33466.34	0.7750

Table No. 05: Results for Accuracy

Spiked % level	Peak area	Amount added	% Amount recovery	% Recovery
50	2158807	5 mg	50.235	100.47
100	4297388	10 mg	100	100
150	6463012	15 mg	150.05	100

Table No. 06: Results for Robustness

Parameter	modification	Retention Time in min	Plate count	% RSD
Flow 1	0.8ml	5.19	3165	0.5
Normal	1ml	4.32	3645	0.4
Flow 2	1.2ml	4.97	3103	0.1
Temp 1	25	4.32	3193	0.9
Normal	30	4.32	3695	0.4
Temp 2	35	4.32	3110	0.1

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