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Development and Validation of RP-HPLC method for the assay of Zolmitriptan

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ABSTRACT

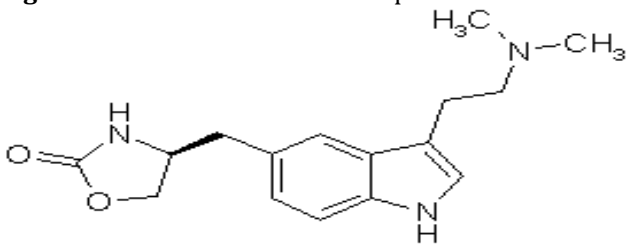
A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for ZOLMITRIPTAN. Chromatographic analysis was performed on Column Symmetry C18 (4.6 x 150mm, 5 µm, Make: Thermosil) mobile phase employed was a mixture of buffer and organic solvent at a specific flow rate and identified by UV detector. The method was validated for accuracy, precision, specificity, linearity, and robustness. The retention time of Zolmitriptan was found to be 2.460±0.137 respectively. Linearity was observed in concentration ranges of 30–70 µg/ml. The limit of detection and the quantification limit were found to be within limits. The accuracy of the proposed method was determined by recovery studies and found to be 99.4%.

Keywords: Zolmitriptan, Triptans, RP-HPLC, Linearity, LOD & LOQ

INTRODUCTION

Zolmitriptan[(4R)-4-({3-[2-(diethyl amino) ethyl]-1H-indol-5-yl} methyl)-1, 3- Oxazolidin-2-one] (see Figure 1) is a prescription drug approved by the U.S. Food and Drug Administration for the acute treatment of migraine with aura or migraine without aura in adults.

Figure 1. 2-D structure of Zolmitriptan



It is not approved to prevent migraines or for treatment of hemiplegic migraines or basilar migraines. The drug's chemical name is Zolmitriptan and it is not sold as a generic. The quick dissolving tablets ZMT (Zolmitriptan) hit the market in 2001 while the nasal spray became available in 2003. It belongs to vasoconstrictor agents, anti inflammatory agent, anti-migraine agents, and selective serotonin agonists. Zolmitriptan is a synthetic tryptamine derivative and appears as a white powder that is readily soluble in water. The therapeutic activity of Zolmitriptan for the treatment of migraine headache can most likely be attributed to the agonist effects at the 5HT1B/1D receptors on intracranial blood vessels (including the arterio-venous anastomoses) and sensory nerves of the trigeminal system which result in cranial vessel constriction and inhibition of pro-inflammatory neuro peptide release. It helps to relieve headaches, pain, and other symptoms of migraines, including nausea, vomiting, and sensitivity to light and sound. Zolmitriptan belongs to a group of drugs called "triptans." Migraines are thought to occur when certain blood vessels in the brain become swollen (dilated).

MATERIALS AND METHODS

Chemicals and reagents

Zolmitriptan has been collected from Suven Life Pharmaceuticals Ltd, Hyderabad. All used reagents were HPLC grade as; "Methanol, Sodium di hydrogen Phosphate, Ortho Phosphoric acid" were purchased from Rankem India. All other chemicals were of analytical reagent grade unless specified. All glassware

were washed with detergent, rinsed thoroughly with distilled water, and dried prior to use.

Chromatographic (HPLC) conditions

Chromatographic separation was performed on a Waters HPLC with alliance with Auto sampler, Empower 2.0 software, Symmetry C18 (4.6 x 150mm, 5 µm, Make: Thermosil), and UV- detection of 240 nm at ambient temperature. The injection volume was 20µl with a flow rate of 0.8 ml/min per minute and a run time of 5 minutes.

Mobile phase and solutions

Mixed a mixture of above buffer preparation 350ml (35%) and 650 ml of Methanol HPLC (65%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Standard Solution Preparation

Accurately weigh and transfer 10mg of Zolmitriptan working standard into a 10 ml volumetric flask add about 7 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.5 ml of the above prepared solution into a 10ml volumetric flask and dilute up to the mark with diluents. Mix well and filter through 0.45µm filter.

Sample Solution Preparation

Accurately weigh and transfer 10mg of Zolmitriptan sample powder into a 10 ml volumetric flask add about 7 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.5 ml of the above prepared solution into a 10ml volumetric flask and dilute up to the mark with diluents. Mix well and filter through 0.45µm filter.

System suitability

Tailing factor for the peak due to Zolmitriptan in Standard solution should not be more than 1.7 Theoretical plates for the Zolmitriptan peak in Standard solution should not less than 4000.

VALIDATION PARAMETERS:

Accuracy

Preparation standard solution:

Accurately weigh and transfer 10 mg of Zolmitriptan Working standard into a 10 ml volumetric flask add about 7 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.5 ml of the above solution into a 10ml volumetric flask and dilute up to the mark with diluents. Mix well and filter through 0.45µm filter.

Preparation Sample solutions:

For preparation of 50% solution: Accurately weigh and transfer 5.0 mg, 10 mg & 15mg of Zolmitriptan API samples into a 10 ml volumetric flask add about 7 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.5ml of the above solutions into a 10ml volumetric flasks and dilute up to the mark with diluents. Mix well and filter through 0.45µm filter and prepare for 50%, 100%, 150% solutions respectively.

Procedure: Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Zolmitriptan and calculate the individual recovery and mean recovery values. The % Recovery for each level should be between 98.0 to 102.0%.

Precision

Procedure**:** The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The % RSD should not be more than 2%.

Linearity

Working dilutions of Zolmitriptan in the range of 50-90µg/ml was prepared by taking suitable aliquots of working standard solutions of drug in different 10ml volumetric flask and diluting up to the mark with mobile phase. 20µl quantity of each dilutions was injected into the column at a flow rate of 0.8ml/min. the drug in the elute was monitored at 240nm and the corresponding chromatogram were recorded. From these the mean peak areas were calculated and a plot of concentration vs peak areas was constructed and acceptance Criteria**:** Correlation coefficient should be not less than 0.999.

Limit of Detection

Preparation of 0.15% solution At Specification level (0.075µg/ml solution):

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluents. Further pipette 0.15ml of above diluted solution into a 10 ml of volumetric flask and dilutes up to the mark with diluents and the acceptance Criteria**:** S/N Ratio value shall be 3 for LOD solution.

Limit of Quantification

Preparation of 0.5% solution At Specification level (0.25µg/ml solution):

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluents. Further pipette 0.5ml of above diluted solution into a 10 ml of volumetric flask and diluted up to the mark with diluents and the acceptance Criteria**:** S/N Ratio value shall be 10 for LOQ solution.

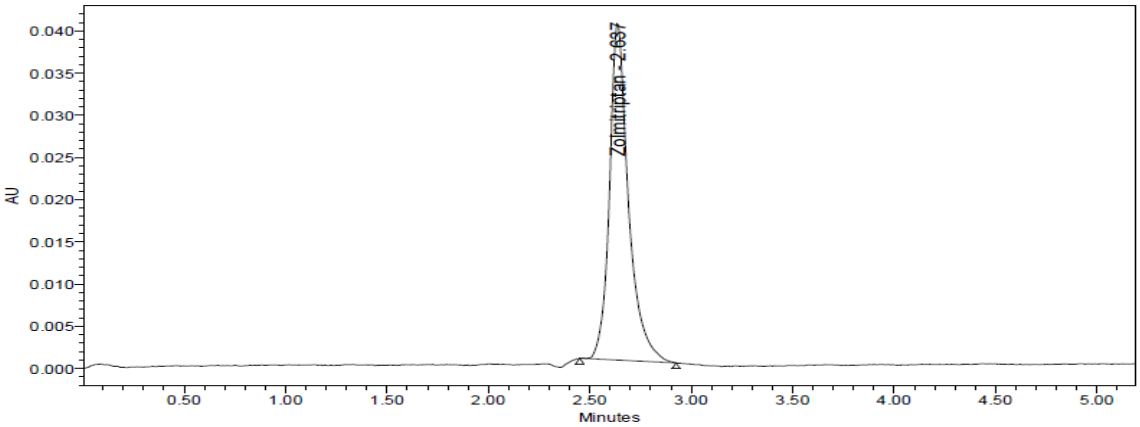
Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. . It was observed from the chromatograms that the results were within the limits. This indicates that the method developed is robust.

RESULTS AND DISCUSSION

An Analytical method development by HPLC carried out in this work resulted in sharp peak of zolmitriptan with negligible fronting and tailing factors. The sharp peak resembles the purity of the sample.

Figure 2. Standard chromatogram of zolmitriptan showing sharp peak at 2.637min at 240nm.



Chromatogram obtained with a mixture of Sodium phosphate buffer: Methanol (35:65v/v).

Table 1. Accuracy

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	597447	5.0	4.96	99.3%	
100%	1196950	10.0	9.95	99.5%	99.4%
150%	1794278	15.0	14.9	99.4%	

Mean Recovery was found to be 99.4%.

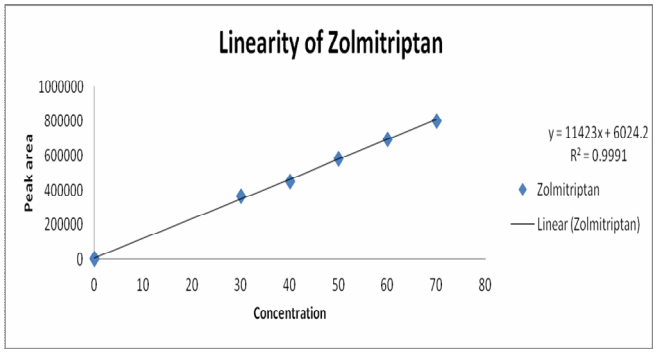
Table 2. Precision

S. No.	R_T	Peak area	Average peak area	Standard deviation	% RSD
1.	2.600	587151			
2.	2.605	58569			
3.	2.611	587072	586891	1466.05	0.249
4.	2.612	585191			
5.	2.621	589072			

The %RSD of the drug was found to be 0.249**.**

Table 3. Linearity

S.no.	Peak name	Concentration	Peak Area
1.	Zolmitriptan	30	363774
2.	Zolmitriptan	40	454659
3.	Zolmitriptan	50	580444
4.	Zolmitriptan	60	691834
5.	Zolmitriptan	70	801299



The regression co-efficient of the drug was found to be **0.999** under the concentration of ranges of 30-70µg/ml.

Table 4. Limit of Detection

S. No.	Peak name	R_T	Area	Height
1.	Zolmitriptan	2.596	927	146

Average Baseline Noise obtained from Blank: 48µV
Signal Obtained from LOD solution (0.15% of target assay concentration) : 146 µV
S/N = 146/48= 3.04

The limit of detection was found to be **3.04µg/ml.**

Table 5. Limit of Quantification

S. No.	Peak name	R_T	Area	Height
1.	Zolmitriptan	2.596	3194	496

Average Baseline Noise obtained from Blank: 48µV
Signal Obtained from LOD solution (0.5% of target assay concentration) : 496 µV
S/N = 496/48 = 10.3

The limit of quantification was found to be**10.3 µg/ml.**

Table 6. Robustness

S. No.	Peak name	Condition	R_T	Area	Height	USP Plate count	USP Tailing
1.	Zolmitriptan	Increased organic phase	2.197	586666	80521	4152	1.688
2.	Zolmitriptan	Decreased organic phase	2.943	579118	80713	4263	1.665
3.	Zolmitriptan	Increased flow rate	2.303	516989	91863	4167	1.660
4.	Zolmitriptan	Decreased flow rate	3.008	653207	90776	4285	1.650

CONCLUSION

Through the modern analytical study, it can be concluded that more rapid, precise, specific, sensitive, economic, reproducible, isocratic reverse phase HPLC method was developed and validated for quantitative determination of Zolmitriptan. The run time around 2.637min allows the analysis of a large number of samples in short period of time. The method was validated successfully using parameters like accuracy, precision, linearity and robustness. This approach will unquestionably build an innovative way out on behalf of maintaining the quality, consistency as well as. These efforts will ensure therapeutic functionality of the drugs. The developed RP-HPLC method presented here is more advantageous as the method was robust with low retention times and sharp peak with reduced fronting and tailing.

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