

# Validated RP-HPLC Method for the Determination of Zolmitriptan - A Serotonin 5-HT Receptor Agonist

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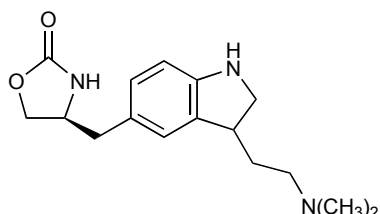
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**Abstract:** A simple precise, accurate RP-HPLC method has been developed and validated for analysis of Zolmitriptan (ZLM). The separation and quantization were achieved on a 250 mm reversed phase column with a hydrophilic linkage between silica particles and hydrophobic alkyl chains. The mobile phase was constituted (flow rate 0.8 ml min<sup>-1</sup>) of eluant A (CH<sub>3</sub>OH) and eluant B (aqueous tetra butyl ammonium hydrogen sulphate) (pH 3.4; 10 mM) using isocratic elution with UV detection at 224 nm. The method showed good linearity for ZMT in the 1–100 µg mL<sup>-1</sup> range with regression equation 15576x ± 99401 and correlation coefficient 0.999 respectively. The limit of quantitation (LOQ) and limit of detection (LOD) were found to be 0.8134 and 0.2687 µg mL<sup>-1</sup> respectively. Finally the applicability of the method was validated according to ICH guidelines and can be applicable for the analysis of commercial dosage forms.

**Keywords:** Zolmitriptan, RP-HPLC, ICH, LOD, LOQ

## INTRODUCTION

Zolmitriptan, (4S)-4-[[3-[2-(dimethylamino) 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 [1]. 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 causes the blood vessels to narrow. Zolmitriptan is used to treat severe migraine headaches. The empirical formula is C<sub>16</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>, representing a molecular weight of 287.36. Zolmitriptan is a white to almost white powder that is readily soluble in water. Xu *et al.* developed A convenient synthesis ZLM from (S)-glyceraldehyde acetonide [2].



**Figure 1:** Chemical structure of Zolmitriptan (ZLM).

Literature review revealed that studied the dose proportionality and tolerability of single and repeat doses of a nasal spray formulation of ZLM in healthy volunteers [3] and Vishwanathan *et al.* [4] and Chen [5] determine ZLM in human fluids by liquid chromatography/electrospray tandem mass spectrometry. Zhang [6] and Yao [7] quantified ZLM by high-performance liquid chromatography-electrospray mass spectrometry in plasma. Srinivasu developed liquid chromatographic method for its enantiomeric separation as well as its potential impurities [8, 9]. Yu *et al.* [10] and Chen [11] determined zolmitriptan enantiomers in rat liver microsomes by chiral high performance liquid chromatography with fluorescence detection. Hu *et al.* [12] and Vijayakumar *et al.* [13] determined ZLM and its related substances. Clement EM (2002) did simultaneous measurement of ZLM and its major metabolites by HPLC. Rao *et al.* [15] and Induri [16] developed HPLC methods and Raza *et al.* [17] and Aydogmus *et al.* [18] spectrophotometric methods for the determination of ZLM. Pang *et al.* [19] studied the interaction between the enantiomers of ZLM and hydroxypropyl-beta-cyclodextrin by capillary electrophoresis. The reported methods in the literature suffer from one or the other disadvantage such as poor sensitivity, very narrow linearity range, scrupulous control of experimental variables and the present study reports the development and validation of a liquid chromatographic method with better detection ranges in pure form and its dosage forms. HPLC method was validated ICH guidelines [20].

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## EXPERIMENTAL

### Chemicals and Reagents

Zolmitriptan was obtained from NOSCH Labs Pvt. Ltd. as gift sample. Zomig Tablets are available as 2.5 mg (yellow) and 5 mg (pink) as film coated tablets for oral administration. Zomig (Nasal spray, 5mg) and Zomig ZMT tablets (2.5 mg, orally disintegrating) are also available in the local market. HPLC grade Methanol (Merck) and Tetra butyl ammonium hydrogen sulphate (Merck) were used for the entire work.

### Instrumentation

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC-10AT and LC-10AT VP Series HPLC pumps, with a 20 $\mu$ L sample injection loop (manual) and SPD 10AT series UV-Visible detector. The output signal was monitored and integrated using Shimadzu Class-VP Version 6.12 SP1 Software. A Hypersil ODS C<sub>18</sub> column (250mm  $\times$  4.6mm, 5 $\mu$ m) was used for separation. Afcoset analytical electronic balance was used.

### Preparation of TBAHS and Standard ZLM Stock Solutions

To prepare (10mM) tetra butyl ammonium hydrogen sulphate (TBAHS) solution about 3.3954 grams was accurately weighed and transferred into a 1000ml volumetric flask and dissolved in HPLC grade water. The solution was sonicated, filtered and used for the mobile phase. The solution has pH of 3.4.

About 50 mg of zolmitriptan reference standard was exactly weighed and dissolved in a 50 mL volumetric flask with the mobile phase i.e. mixture of eluants A and B (50:50 v/v) to prepare the stock solution and was further diluted with the mobile phase according to the requirement.

### Validation Procedure

#### Linearity

Linearity of the method was evaluated at five equispaced concentration levels by diluting the standard ZLM solutions to give solutions over the range 1–100  $\mu$ g mL<sup>-1</sup> (Table 1). 20  $\mu$ L of these solutions were injected in triplicate in to HPLC system and the peak areas were recorded A representative chromatogram is shown in Figure 2.

#### Precision

The precision of an analytical procedure expresses the degree of scatter between a series of

measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The repeatability (intra-day precision) refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment. Intermediate precision (inter-day precision) involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts.

**Table 1: Calibration Data of Zolmitriptan**

Conc. ( $\mu$ g mL <sup>-1</sup> )	Mean peak area (n = 3)	RSD (%)
1	190640	0.151
2	364635	0.172
5	864689	0.183
10	1665531	0.432
20	3260907	0.381
40	6457603	1.061
50	8037955	0.843
80	12684228	0.734
100	15441934	1.023

Each value is the average of three determinations

The intra-day repeatability was investigated using three separate sample solutions each at three different levels (10, 20 and 50  $\mu$ g mL<sup>-1</sup>) prepared as reported above, from the freshly reconstructed formulations. Each solution was injected in triplicate and the peak areas obtained were used to calculate means and RSD% values (Table 2).

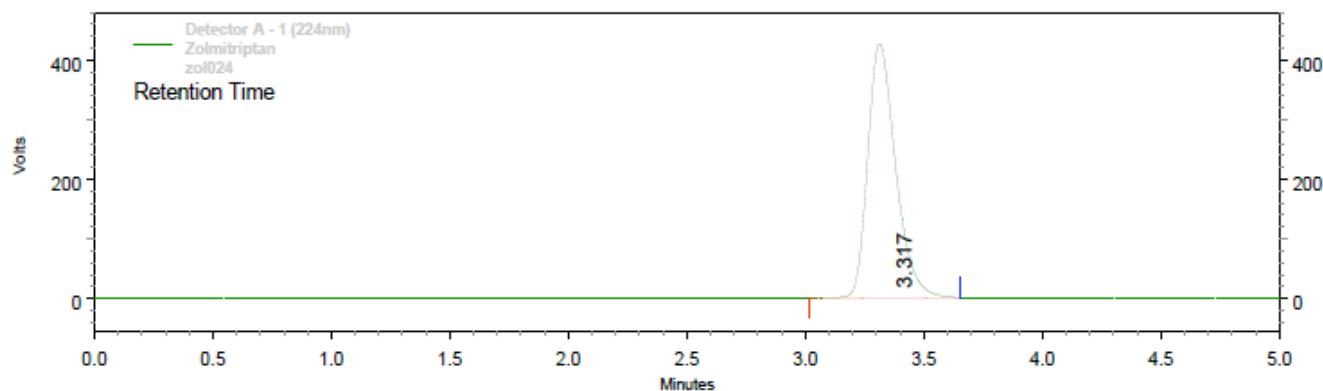
The inter-day reproducibility was checked on three different days, by preparing and analyzing in triplicate four separate sample solutions from the reconstructed formulations at the same concentration level of intra-day repeatability; the means and RSD% values were calculated from peak areas.

#### Accuracy

The accuracy of an analytical method is the closeness of the test results to the true value. To assess accuracy, freshly prepared placebo of the ZLM pharmaceutical formulations were spiked with various amounts of pure ZLM at 80, 100 and 120%. Each solution was injected in triplicate and the peak areas

Table 2: Intra-Day and Inter-Day precision for Zolmitriptan (n = 3)

Conc. ( $\mu\text{g mL}^{-1}$ )	Intra-day precision		Inter-day precision	
	Mean peak area $\pm$ SD (n = 3)	RSD (%)	Mean peak area $\pm$ SD (n = 3)	RSD (%)
10	1646871 $\pm$ 2120.617	0.1288	1645673 $\pm$ 2254.72	0.1370
20	3212514.3 $\pm$ 3219.51	0.1002	3232523.43 $\pm$ 3786.09	0.1171
50	8052344.7 $\pm$ 8571.71	0.1065	8142541.26 $\pm$ 8331.93	0.1023

Figure 2: Representative chromatogram of Zolmitriptan (20 $\mu\text{g/ml}$ )

were used to calculate means and RSD% values (Table 3) and compared with those obtained with standard ZLM solutions.

#### Assay of Commercial Formulations

Zomig® tablets (5 mg) and Zomig® nasal spray (5 mg) were purchased from the local market and the contents of tablets / nasal spray equivalent to 50 mg

sonicated for 30 min and filtered through membrane filter. The results are given in (Table 4).

#### RESULTS

A mobile phase composed of 10 mM tetra butyl ammonium hydrogen sulphate: methanol (50:50 v/v) was chosen with flow rate 0.8 mL min<sup>-1</sup> for the

Table 3: Accuracy - Recovery data for Zolmitriptan (n = 3)

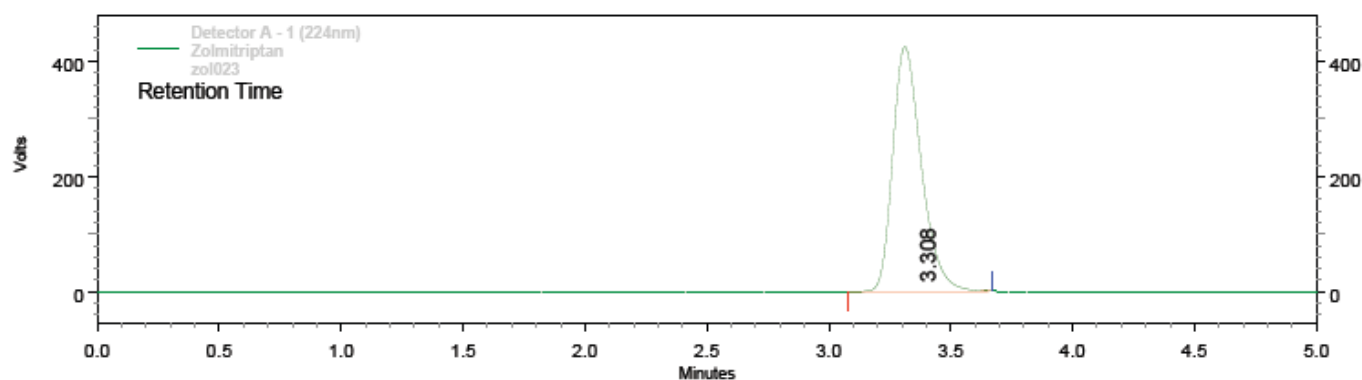
Amount (%) of drug added to analyte	Theoretical content ( $\mu\text{g mL}^{-1}$ )	Mean Conc. found ( $\mu\text{g mL}^{-1}$ ) $\pm$ SD	% Recovery	% RSD
80	36	36.13 $\pm$ 0.137	100.36	0.3792
100	40	39.43 $\pm$ 0.162	98.58	0.4109
120	44	43.83 $\pm$ 0.174	99.61	0.3969

were accurately weighed and transferred to 50 mL volumetric flask and diluted with the mixture of eluants A and B (50:50, v/v). The resultant mixture was

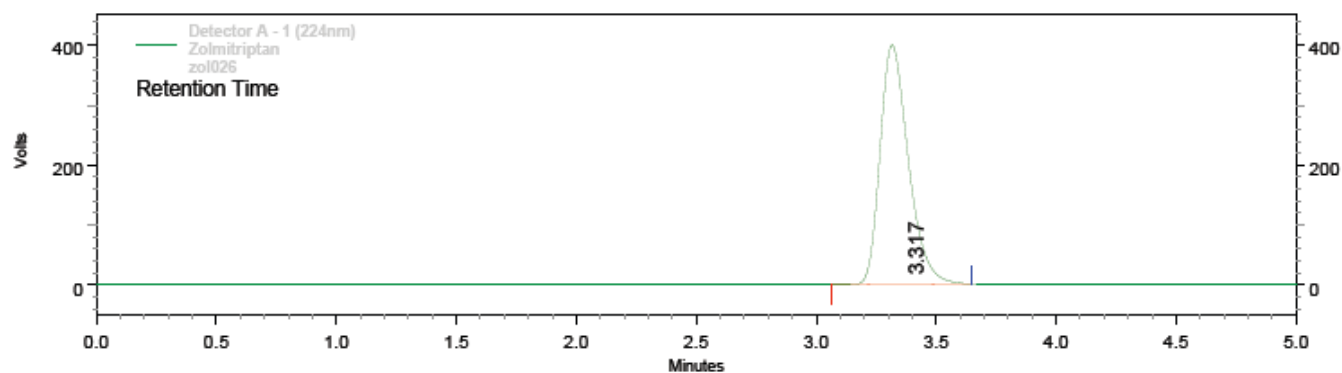
determination of Zolmitriptan. The UV detection wavelength was 224 nm.

Table 4: Analysis of Commercial Formulations

Commercial Formulation	Labeled amount (mg)	Amount found (mg)	% Recovery
Brand I	5	4.9866	99.731 $\pm$ 0.05
Brand II	2.5	2.4980	99.923 $\pm$ 0.03
Brand III	5	4.9932	99.864 $\pm$ 0.09



**Figure 3:** Representative chromatogram of Zolmitriptan (20 µg/ml) Formulation (Zomig tablets, 5 mg).



**Figure 4:** Representative chromatogram of Zolmitriptan (20 µg/ml) Formulation (Zomig ZMT tablets, 2.5 mg)

## Validation of the Method

### Selectivity

This method was selective for the Zolmitriptan (Retention time about 3.308 min). The typical excipients included in the drug formulation do not interfere with selectivity of the method (Figures 3 and 4). The analysis of the chromatogram of Zolmitriptan revealed the following efficiencies of the column: for Zolmitriptan  $N = 3696$  (where  $N$  represents theoretical plate number) and asymmetry 1.32.

### Precision and Accuracy

The method is precise [0.1002-0.1288 (Intraday) and 0.1023-0.1370 (Intraday)] and accurate (0.3792-0.4109) as the RSD values were less than 2 %.

### Linearity

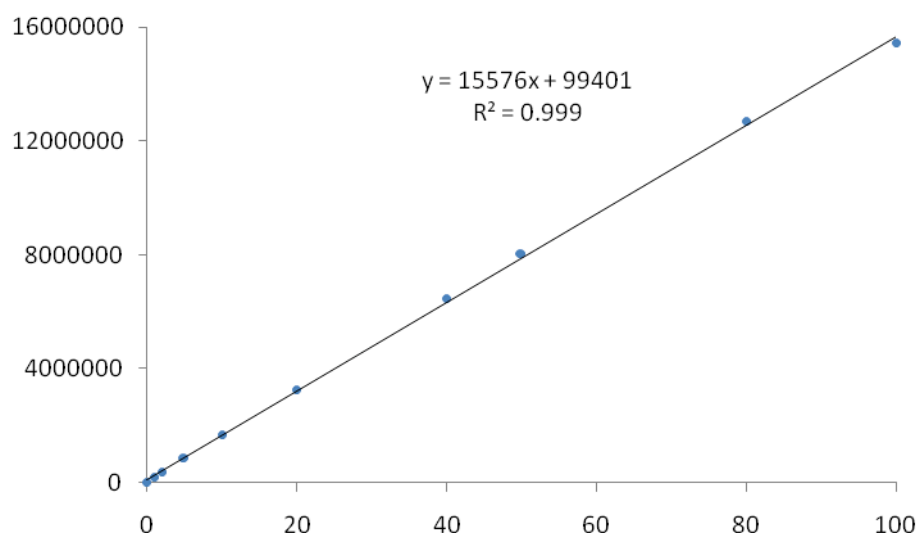
The linearity of the method was determined in terms of the correlation coefficient between concentration of Zolmitriptan and the peak normalization of Zolmitriptan. The calibration data of Zolmitriptan was given in Table 1. The linearity range was between 1–100 µg mL<sup>-1</sup> presented with the equation of  $15576x + 99401$  (Figure 5) with correlation coefficient ( $r^2 = 0.999$ ) closed to unity.

### Sensitivity

The limit of detection, defined as lowest concentration of analyte that can be clearly detected above the baseline signal, is estimated as three times the signal-to-noise ratio. The limit of quantitation, defined as lowest concentration of analyte that can be quantified with suitable precision and accuracy, is estimated at 10 times the signal-to-noise ratio. The limit of detection (LOD) and limit of quantification (LOQ) were achieved by injecting the series of dilute solutions of ZLM and are found to be 0.2687 and 0.8134 µg mL<sup>-1</sup> respectively.

## DISCUSSION

In the initial trials the following mobile phases were used: acetonitrile and water (20:80, v/v) (mobile phase 1) and acetonitrile and water (50:50 v/v) (mobile phase 2) as the mobile phases. Mobile phase 1 has been rejected due to a lack of ZLM signal on chromatogram. When samples of ZLM were analyzed using mobile phase 2, peaks shape were not good and retention time was ~12 min, therefore organic modifier concentration was changed but no improvement was observed. Subsequent attempts were made by lowering the pH of the mobile phase with various buffers including phosphate buffer but the peak shape



**Figure 5:** Calibration curve of Zolmitriptan.

was disturbed and therefore finally 10mM tetra butyl ammonium hydrogen sulphate (TBAHS) (pH 3.4) was chosen and marked improvement was observed. Eventually, a mobile phase composed of 10 mM tetra butyl ammonium hydrogen sulphate: methanol (50:50 v/v) gave the best results. During these studies injection volume was 20  $\mu$ L and the mobile phase flow rate was constant at 0.8 mL min<sup>-1</sup>. The analytical wavelength was 224 nm.

## CONCLUSION

The developed method for the determination of Zolmitriptan is simple, sensitive and precise. Further the method is suitable for estimation of drug in commercial formulations.

## ACKNOWLEDGEMENT

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