World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

A METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF ZOLMITRIPTAN CONTENT IN ZOLMITRIPTAN PHARMACEUTICAL DRUGS PRODUCT

Sushil H. Jaiswal*[1], Dr. M. V. Katariya[1], Dr. V. R. Katariya[2] Dr. G. S. Karva[3] and Dr. U. R. Maniyar[1]

[1]Department of Chemistry, Deogiri College, Aurangabad-431004, Maharashtra, India.
[2]Kamala Nehru Pharmacy college, Aurangabad-431004, Maharashtra, India.
[3]Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431004, Maharashtra, India.

***Corresponding Author: Sushil H. Jaiswal**
Department of Chemistry, Deogiri College, Aurangabad-431004, Maharashtra, India.

Article Received on 26/05/2017 **Article Revised on 11/06/2017** **Article Accepted on 26/06/2017**

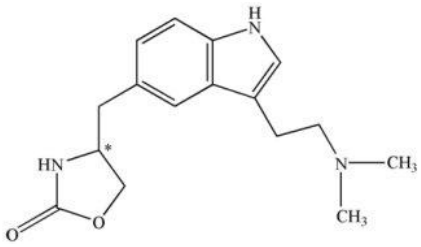
ABSTRACT

A reversed-phase high performance liquid chromatography (RP-HPLC).[1] method was developed and validated for the estimation of Zolmitriptan in pharmaceutical drugs dosage forms. The separation was achieved on Inertsil C8 – 3, (150 mm X 4.6 mm), 5µm using the gradient composition of phosphate buffer pH 7.5 as mobile phase A and mixture of methanol and buffer pH 7.5 in the ratio of 750:250 v/v as mobile phase B at flow rate 1.5mL per minute and detection wavelength 285 nm. The retention time of Zolmitriptan was about 7.2 min. The method for the quantitative determination of Zolmitriptan in Zolmitriptan tablets was validated. The method was evaluated for its specificity, precision, solution stability, accuracy, linearity and range, and robustness. The method was developed and validated under the light of International Conference on Harmonization (ICH) guidelines.[2,3,4] And for the statistical evaluation of results, standards guidelines were followed.[5]

KEYWORDS: RP HPLC, ICH guidelines, validation, assay.

INTRODUCTION

Zolmetriptan[6] is a synthetic tryptamine derivative[7] and appears as a white powder that is partially soluble in water. Zolmitriptan is used for the acute treatment of migraines. The molecular formula is $C_{16}H_{21}N_3O_2$ IUPAC name is (4S)-4-[[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]methyl]-1,3-oxazolidin-2-one and has the following chemical structure



MATERIALS AND METHODS

Instrumentation

HPLC method development,[8,9,10] was carried out by using Shimadzu HPLC, Series LC2010 autosampler system equipped with UV and UV/PDA detector and consisted Inertsil C-8 3 (150 mm X 4.6mm) column with 5µ particle.

Chemicals & Reagents

HPLC grade Sodium dihydrogen phosphate monohydrate (Merck India), Potassium Hydroxide (Merck India), Methanol (Rankem), Hydrochloric acid (Rankem), Sodium hydroxide (Thomas Baker), Hydrogen peroxide (Rankem) were used throughout as it is unless and until stated the experiment and was purchased from reliable commercial source.

Zolmitriptan Drug substances and Drug product Zolmitriptan tablet 5 mg, Zolmitriptan N-Oxide Impurity, Zolmitriptan Amino Alcohol, were kindly gifted by Macleods Pharmaceutical Ltd. India.

Standard Preparation

Weighed accurately and transferred about 50 mg of Zolmitriptan to a 100 ml volumetric flask. Added about 60 ml of diluent and sonicated to dissolve. Allowed to equilibrate to room temperature and diluted to volume with diluent. Further dilute 10 ml of this solution to 50 ml with diluent.

Sample preparation

Weighed 10 intact tablets and transfer to a dry 500mL volumetric flask. Added about 300mL of diluent and sonicated for 30 min with intermittent shaking. Allowed

equilibrating to room temperature and diluted to volume with diluent. Filtered the solution through 0.45 µm nylon filter (25mm) discarding first few ml of the filtrate.

Impurity solutions (For specificity study)
N-oxide impurity preparation
Weighed accurately about 3.75 mg of N-oxide impurity to 50 ml volumetric flask added 30 ml of diluent, sonicated to dissolved equilibrated to room temperature and diluted to volume with diluent. Further diluted the 1 ml of the solution to 50 ml with diluent.

Amino Alcohol impurity preparation
Weighed accurately about 2.52 mg of Amino Alcohol impurity std to 500 ml volumetric flask and added 360 ml of diluent Sonicated to dissolve and equilibrated to room temperature and diluted to volume with diluent. Further diluted 2.5 ml of this solution to 25 ml with diluent.

Mobile phase
Prepared mixture of potassium dihydrogen orthophosphate buffer pH 7.5 and methanol (750:250v/v) and degassed.

Chromatographic condition
The experimental condition further optimized to get the desired separation and sensitivity.[11] The final chromatographic conditions are as given in Table 1

Table 1: Final chromatographic condition.

Chromatographic Mode	Isocratic
Column	Inertsil C8 –3, (150 mm X 4.6 mm), 5µm
Wavelength	285 nm
Flow rate	1.5 mL / min.
Injection volume	10 µL
Column oven temperature	Ambient
Sample temperature	Ambient
Run Time	15 min

RESULT AND DISCUSSION

The validation[12] of any developed method ensures credibility of analysis. It demonstrates the scientific soundness of the measurement or characterization. In the present study, following parameters were studied for validation.

Specificity
The solutions prepared for identification of Zolmitriptan and each impurities solutions were injected. The Zolmitriptan eluted at 6.712 min. and well separated from impurity. Chromatogram of sample solution (Figure1) and impurities retention time point is as given in Table 2.

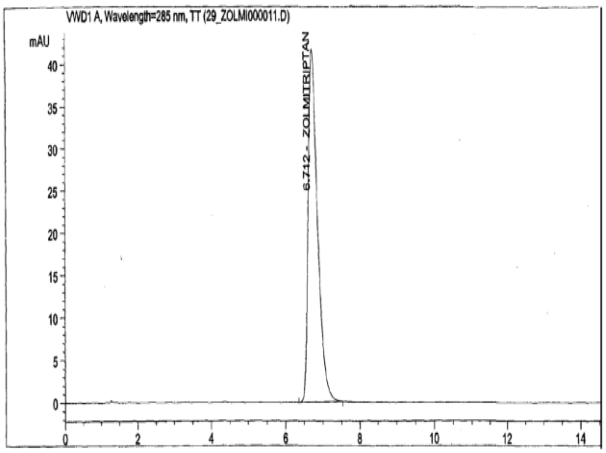


Figure 1: Chromatogram of Sample solution of Zolmitriptan.

Table 2: The retention time of impurity solution.

Name of solution		Retention Time (minutes)
Blank		No interference
Placebo		No interference
Impurities of Zolmitriptan	N-oxide	4.370
	Amino alcohol	1.972

Solution stability
The standard and sample solutions were prepared as described in the methodology and stored at controlled room temperature 20°C – 25°C. The stored solutions were injected at initial, 8 hours, 16 hours and 24 hours. The absolute difference in assay of peak due to Zolmitriptan peaks at different time interval, with respect to initial assay are as in table 3 for standard solution and sample solution.

Table 3: Zolmitriptan solution stability.

Time (hours)	Zolmitriptan at Controlled room temperature (20°C–25°C) for standard solution		Zolmitriptan at Controlled room temperature (20°C–25°C) for sample solution	
	% Assay	Absolute difference	% Assay	Absolute difference
Initial	100.5	NA	99.6	NA
8 hours	100.4	0.1	99.3	0.3
16 hours	100.1	0.4	99.8	0.2
24 hours	100.5	0.0	100.1	0.5

Linearity and Range
To determine Linearity, a series of solutions were prepared by quantitative dilutions of the stock solution of Zolmitriptan standards to obtain solutions at 50 %, 80 %, 100 %, 120 % and 150 % of the working concentration of Zolmitriptan. This corresponds to a concentration range of 50 ppm to 150 ppm for Zolmitriptan.

Each solution was injected in duplicate and the peak areas were recorded. Slope, intercept, correlation

coefficient of the regression line and residual sum of squares were calculated and observed result presented in table 4. Figure 2 shows linearity graph for Zolmitriptan.

Table 4 : Linearity result for Zolmitriptan.

Level	Concentration (ppm)	Corrected Concentration	Mean area
50 %	50	49.68	352.91000
80 %	80	79.49	562.67750
90 %	90	89.42	641.84850
100 %	100	99.36	694.90350
110 %	110	109.30	777.81050
120 %	120	119.23	845.14150
150 %	150	149.04	1054.99250
Slope			7.06404
Intercept			2.44289
Correlation coefficient			0.99973
Residual sum of squares			161.51583
RANGE: 50 % to 150 % of target concentration 100ppm (i.e 50 ppm to 150 ppm)			

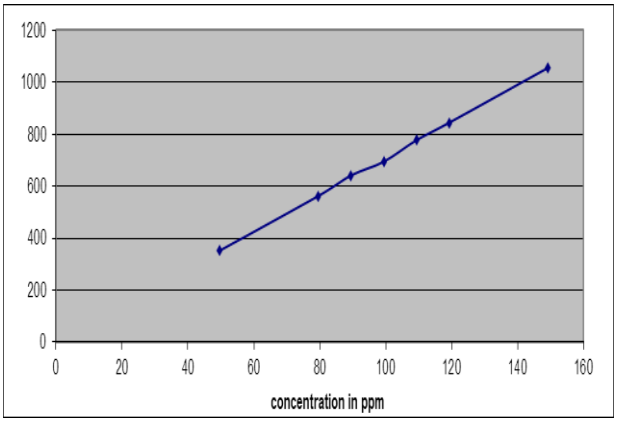


Figure 2: Linearity graph for Zolmitriptan.

The correlation co-efficient was found to be 0.99973 which is well within the acceptance criteria of not less than 0.999. Hence it is concluded that the method is linear in the range of 50 % to 150 % i.e 50 ppm to 150 ppm for Zolmitriptan.

Precision

System precision was established with the Zolmitriptan standard solution, peak response were measured, each reading in six replicates were taken and presented in table 5. The relative standard deviations found 0.11.

Table 5: System precision result of Zolmitriptan.

Injection No.	Peak Area
1	721.83100
2	723.49100
3	723.36000
4	724.15000
5	723.77700
6	723.52900
Mean	723.35633
% RSD	0.11

Intermediate Precision

A different analysis carried out on a different day, using a different HPLC and different lot of column. The overall comparative data of Intraday and Interaday percentage assay of Zolmitriptan are presented in the table 6

Table 6: Intraday and Interaday results of Zolmitriptan.

Parameter	% Assay of Zolmitriptan	
	Intraday	Interaday
% Assay of Zolmitriptan	98.6	98.8
% RSD	0.42	0.38

Accuracy / Recovery

The percentage recovery of Zolmitriptan was calculated for each of the recovery solution and the mean recovery was determined. The percentage recovery for Zolmitriptan at 50 %, 100 % and 150 % of target concentration in triplicate.

Table 7: Percentage recovery study of Zolmitriptan.

Level	Zolmitriptan spiked (mg)	Wt of placebo (mg)	Mean Area	Zolmitriptan recovered (mg)	% Recovery	% RSD
50 %	26.20	1951.0600	375.15050	25.77	98.4	0.92
	26.57	1950.2900	374.36350	25.72	96.8	
	26.06	1950.8600	373.14900	25.63	98.3	
100 %	51.76	1950.6400	749.54650	51.49	99.5	0.06
	51.95	1949.8000	751.72650	51.64	99.4	
	51.80	1949.6300	749.66000	51.50	99.4	
150 %	74.50	1950.8400	1098.03600	75.43	101.2	0.32
	74.76	1951.2500	1102.38800	75.73	101.3	
	74.83	1950.0000	1096.69850	75.34	100.7	
Mean % Recovery					99.4	
Overall % RSD					1.49	

Robustness

Robustness of method is evaluated by making the following alterations in the chromatographic conditions

1. Change in the mobile phase buffer pH (pH = 7.3, pH = 7.7)
2. Change in the mobile phase composition (730:270), (770:230)
3. Flow Rate change (1.7 ml) (1.3 ml)

The absolute difference in assay results of Zolmitriptan obtained in the normal condition (Repeatability mean result) and altered conditions were calculated and monitored the system suitability, the observed result are reported in table 8.

Table 8: Robustness study result and system suitability of Zolmitriptan.

Altered condition	Area (Mean)	% Assay	Absolute Difference (w.r.t. unaltered)	System suitability			
				Retention time (min)	Tailing factor	Theoretical plates	% RSD(five replicate injections)
Unaltered (Repeatability Mean)	717.58708	98.6	NA	6.7	1.3	3462	0.11
Mobile phase buffer pH 7.3	716.90450	99.7	1.1	6.2	1.6	3329	0.24
Mobile phase buffer pH 7.7	709.65750	98.9	0.3	8.7	1.7	3551	0.16
Mobile phase Composition 730:270	720.89750	99.0	0.4	5.8	1.3	3527	0.17
Mobile phase Composition 770:230	717.79850	99.3	0.7	7.9	1.6	3757	0.05
Flow rate 1.3	829.76900	98.8	0.2	7.8	1.3	3858	0.08
Flow rate 1.7				6.0	1.3	3190	0.08

CONCLUSION

The method was evaluated for its specificity, precision, solution stability, accuracy, linearity and range, and robustness and the method meets all the acceptance criteria. Hence, it has been concluded that the method is suitable for its intended use, i.e. to determine the assay of Zolmitriptan in Zolmitriptan tablet dosage form.

REFERENCES

1. Reversed-phase high-performance liquid chromatography: theory, practice, and biomedical applications, Ante M. Krstulović, Phyllis R. Brown, Wiley Publication, 1982.
2. International Conference on the Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Validation of Analytical Procedures:Text and Methodology Q2 (R1). Available from http://www.ich.org, 2005.
3. International Conference on Harmonization (ICH) Q8(R2): Pharmaceutical Development (August), 2009.
4. International Conference on Harmonization (ICH) Q11: Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities) (May 2011).
5. United States Pharmacopeia/National Formulary, General Chapter <621> Chromatography, General Chapter <1010> Analytical Data-Interpretation and Treatment, General Chapter <1224> Transfer of Analytical Procedures, General Chapter <1225> Validation of Compendial Procedures, General Chapter <1226> Verification of Compendial Procedures.
6. C.Sean Sweetman, Martindale-The Complete Drug Reference, 2009; 36.
7. http://www.drugs.com/availability/generic.
8. Soven P., et al, Assay method development and validation of Ibuprofen tablets by HPLC, Pelagia Research Library, 2013; 4(4): 91-96.
9. Satinder Ahuja Henrik Rasmussen, HPLC Method Development for Pharmaceuticals, 8.
10. A. K Gupta, P.K Patel, Analytical Method Validation of Stability-Indicating HPLC Method for Determination of Assay of Carbamazepine CR Tablets, Global Research Analysis, Nov 2013, 2(11).
11. Sun H, Qin X, Ge X, Wang L., Effective separation and sensitive determination of cyanuric acid, melamine and cyromazine in environmental water by reversed phase high-performance liquid chromatography, Pub Med. gov, 2011 Feb-Mar; 32(3-4): 317-23.
12. Gohil K., Spectrophotometric analysis of Amlodipine besylate in bulk and in tablet dosage forms. Indian J.Pharm. Sci., 2005; 67(3): 376.