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Research Article

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Method development and validation by RP-HPLC for the estimation of Zolmitriptan in bulk and its dosage form

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Abstract

Zolmitriptan is used in the acute treatment of migraine attacks. It was quantified by reverse phase HPLC equipped with Sunniest (C18, 150 x 4.6 mm, 5.0 µ) column and mobile phase using pH 8.5 buffer and methanol as organic modifiers and flow rate is adjusted to 1.0 mL/min Column temperature is maintained at 40˚C. Injection volume is 10µL. the retention time was found to be 5.7 min. The standard plots were constructed between concentrations vs. peak area a linear response of peak area was observed over the concentration range of 125-750 µg/mL for Zolmitriptan The regression equations of concentration Zolmitriptan was found to be y= 324.47x + 3.400, where y is the peak area and x is the concentrations of drugs (µg/mL).

Key words: Zolmitriptan, RP-HPLC, Estimation and validation

Introduction

Zolmitriptan is a selective serotonin receptor agonist of the 1B and 1D subtype. It is atriptan, used in the acute treatment of migraine attacks with or without aura and cluster headaches. Zolmitriptan is marketed by AstraZeneca with the brand names Zomig, Zomigon Zolmitriptan is a synthetic tryptamine derivative and appears as a white powder that is readily soluble in water. Literature survey shows that there are few methods published exclusively for this drug in bulk or formulation. Extensive literature search was done for the methods on UV- Visible spectroscopy, HPLC, LCMS/MS, TLC, & GC. Based on the methods available in single or in combination with other drugs, the chromatographic conditions are optimized and method was developed and validated

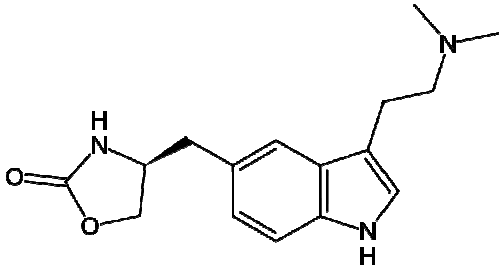

Figure. 1 Structure of Zolmitriptan

Materials and Method

Reagents and Chemicals:

The Active pharmaceutical ingredient of Zolmitriptan was obtained as gift sample from Hetero Drugs. All solvent and reagents used were of HPLC and spectroscopic grade. HPLC grades, ammonium dihydrogen orthophosphate, ammonia, acetonitrile, and methanol were obtained from Merck Pvt.ltd. Millipore water obtained from (Milli Q water) was used in all experiments.

Instrumentation:

The chromatographic separation performed using JASCO-2080 model quipped with UV-2075 detector (JASCO). JASCO-BROWIN software was used for monitored and integrate the output single at wavelength 225 nm. Sample injection was done with a Rheodye 7725 injection valve via a 20 µL loop. Digisum Electronic analytical balance

(model DI 707) was used for weighing. Drug separation achieved at room temperature with sunniest C18 (150mm × 4.6mm) with pore size 5 µm was used for method development.

Preparation of pH 8.0 buffer:

Weighed accurately and dissolved about 1.15 g of ammonium dihydrogen orthophosphate in 1000 mL of milli-Q water and mixed well. Adjusted the pH to 8.0± 0.05 with Ammonia solution and mixed.well

Preparation of mobile phase:

pH 8.0 Buffer and acetonitrile in the ratio of 85:15% v/v were taken and filtered through 0.45µm filter and sonicated for 5 min.

Preparation of Diluent:

Milli-Q water and methanol in the ratio of 30:70(v/v) was used as diluent.

Preparation of Bulk Solution:

Pipetted 5ml of diluent into a 25 ml volumetric flask diluted to volume with mobile phase and mixed. Filtered 0.45 µm membrane filter.

Preparation of Standard Stock Solution:

Weighed accurately and transferred about 50 mg of Zolmitriptan working standard into a 100 mL volumetric flask, added about 70 mL of diluent, sonicated to dissolve the material completely and allowed to cool to room temperature. Volume was made up with diluent and mixed

Preparation of Sample Solution:

Weighed 10 tablets and record the average weight of each tablet. Weighed and transferred 10 tablets into a 100 mL volumetric flask , added about 40 mL of diluent, kept in a rotary shaker at 200 rpm for 10 mins, sonicated for 30 mins with intermidate shaking and volume was made up with diluent and mixed. Centrifuged a portion of the above solution at 2500 rpm for 10 mins.

Optimization of the chromatographic conditions:

The initial literature search indicated that few HPLC methods are available for this drug. Based on literature search, attempts were made to develop a simple method which has less retention time and higher selectivity, top priority was given for determination of Zolmitriptan. Several mobile phases were tested until good resolution obtained. In the preliminary experiments, Zolmitriptan was quantified by reverse phase HPLC equipped with Sunniest (C18, 150 x 4.6 mm, 5.0 µ) column and mobile phase using pH 8.5 buffer and methanol as organic modifiers and flow rate is adjusted to 1.0 mL/min Column temperature is maintained at 40˚C. Injection volume is 10 µL. The drug was able to be separated on the chromatogram but peak shape was not good for Zolmitriptan and tailing was seen more than 1.5. The effect of pH (6.5, 7.5 and 8.0) and mobile phase composition was also checked. It improved peak shape at pH 8.0 to slight extent but the number of theoretical plates were very less. Acetonitrile was found to be better than methanol in terms of resolution and peak shapes. Initially try with mixture of buffer pH 8.0 and acetonitrile it shows good peak shape with theoretical plate. The chromatogram obtained was improved and better than the previous one in all aspects with good peak shape, tailing factor, resolution and theoretical plate as per USP requirement.

Calculations:

Amount of zolmitriptan (% label claim) = $\frac{A \times Ws \times 5 \times 200 \times 25 \times P \times 100}{B \times 100 \times 100 \times N \times 5 \times 100 \times L}$

Where

A= peak area of zolmitriptan in test preparation

B= average peak area of zolmitriptan from standard preparation.

Ws= weigh of zolmitriptan working standard taken in mg.

N= number of tablets of zolmitriptan taken for test preparation.

P= potency of zolmitriptan working standard (% /w as is basis)

L= labled amount of zolmitriptan in mg, per tablet.

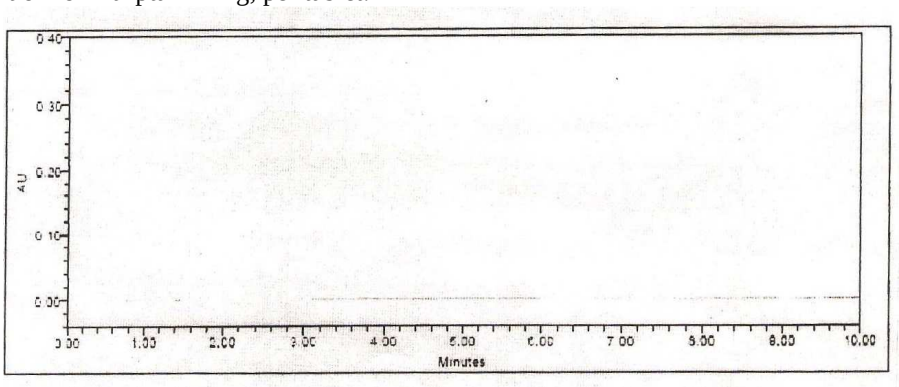

Figure: 2 Zolmitriptan Blank chromatogram

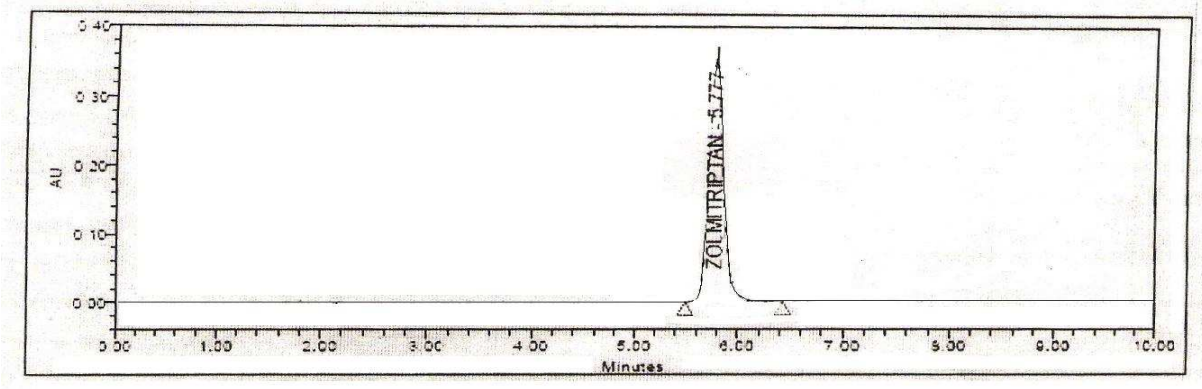

Figure: 3 Zolmitriptan standard chromatogram

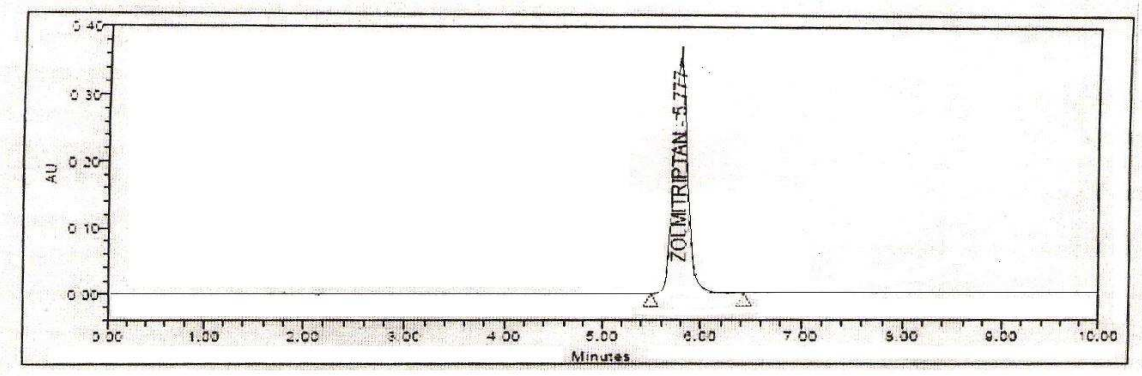

Figure: 4 Zolmitriptan formulation chromatogram

Validation:

The method was successfully validated as per ICH guideline Q2 (R1): validation of analytical procedures: text and methodology, international conference on harmonization, food and drug administration, USA, November 2005. The method was validated and parameters were linearity, range, accuracy, precision, LOQ, LOD.

Linearity and range:

The linearity of detector response to different concentration of the drug was studied with a series of working standard solutions prepared by diluting the stock solution with mobile phase. The standard plots were constructed between concentrations vs. peak area a linear response of peak area was observed over the concentration range of 125-750 µg/mL for Zolmitriptan. 10 micro liter of each sample was injected under above chromatographic conditions and peak area was measured. Keeping the values to the straight line equation of calibration curve, quantification was carried, the data of linearity curve was summarized in the table 2 and figure: 5 and it was found that correlation coefficient (R^2) and regression analysis were within the limit which is summarized in table: 1

Table: 1 Linearity data showing equation of regression line and coefficient of determination

Drug	Conc. Range (µg/mL)	Equation	R^2
Zolmitriptan	125-750	y = 324.47x + 3.400	.999

Table: 2 Linearity Data

Linearity	Conc. (µg/mL)	Average Area
L1	125	40559
L2	250	81128
L3	375	121680
L4	500	162240
L5	625	202800
L6	750	243359

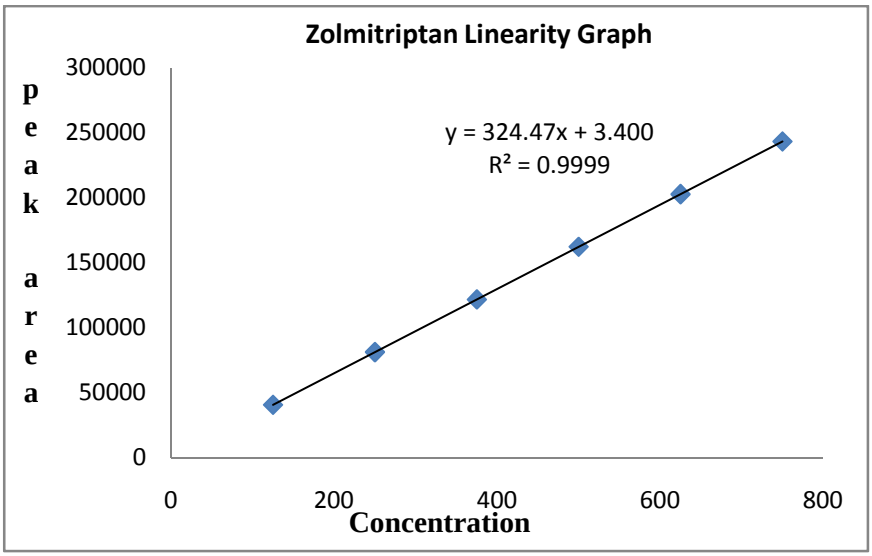

Figure: 5 Zolmitriptan Linearity Graph

Limit of Detection (LOD) and Limit of Quantification (LOQ):

A study to establish the Limit of detection and Limit of Quantification for Zolmitriptan. A series of solutions having Solifenacin and Tamsulosin were injected established by identifying the concentration which gives signal to noise ratio about 3. Limit of quantification was established by identifying the concentration which gives signal to noise ratio about 10. The LOD and LOQ were estimated in table: 3.

Table: 3 LOQ, LOD Values

Drugs	LOD µg/mL	LOQ µg/mL
Zolmitriptan	0.01	0.03

Precision:

According to ICH guidelines repeatability should be assessed by using a minimum of nine determinations covering the specified range for the procedures (i.e., three concentrations and three replicates of each concentration) precision was studied to find out intra and inter day variations of the proposed method at three different levels (50, 100 and 150% or 80, 100,120%) 250, 500, 750 µg/mL for Zolmitriptan on the same and on three different days respectively. The results were interpreted by statistical analysis by calculating % RSD values and all the results were within the acceptance criteria of not more than 2 % and the results are tabulated in the table: 4 and 5 .The % RSD values for intraday and inter day were <2%, indicating that the method was sufficiently precise.

Table: 4 Intraday precision

Conc. of the drug	Found Conc. (µg/mL)	% Assay	Statistical parameters
250	249.21	99.71	Mean= 99.787
250	249.50	99.79	SD= 0.075
250	249.10	99.86	%RSD= 0.075
500	499.80	99.98	Mean= 99.937
500	499.20	99.94	SD= 0.045
500	499.50	99.89	%RSD= 0.045
750	749.14	99.9	Mean= 99.900
750	749.16	99.9	SD= 0.000
750	749.11	99.9	%RSD= 0.000

Table: 5 Inter day precision

Conc. of the drug	Found Conc. (µg/mL)	% Assay	Statistical parameters
250	249.15	99.87	Mean= 100.000
250	249.17	99.9	SD= 0.200
250	249.49	100.23	%RSD= 0.200
500	499.21	99.97	Mean= 99.963
500	499.25	100.02	SD= 0.060
500	499.42	99.9	%RSD= 0.060
750	749.15	100.07	Mean= 99.987
750	749.13	99.91	SD= 0.080
750	749.13	99.98	%RSD= 0.080

Accuracy:

The accuracy of the HPLC method was confirmed by recovery studies by spiking 50,100 & 150% of pure drugs (250, 500, 750 µg/mL for Zolmitriptan) to the pre analyzed samples and the samples after dilution injected into the system (n=3). The peak area of drug was measured and the recovery values for Zolmitriptan were determined by using the formula. The statistical data was presented in the table: 6

Table: 6 Recovery for Zolmitriptan

Amount added (µg/mL)	Amount found (µg/mL)	% Recovery	
249.12	249.11	99.996	Mean= 99.992
249.11	249.09	99.992	SD= 0.004
249.15	249.12	99.988	%RSD= 0.004
499.11	499.08	99.994	Mean= 99.993
499.12	499.07	99.990	SD= 0.002
499.14	499.11	99.994	%RSD= 0.002
749.15	749.1	99.993	Mean= 99.994
749.52	749.47	99.993	SD= 0.002
749.17	749.14	99.996	%RSD= 0.002

System suitability

According to USP system suitability tests are an integral part of chromatographic method validation. The tests were used to verify that the reproducibility of the chromatographic system is adequate for analysis. To ascertain its effectiveness system suitability tests were carried out on freshly prepared standard stock solution containing 500 µg/mL for Zolmitriptan. 10 µL of solution was injected into the optimized chromatographic system. For system suitability 6 replicates of working standard samples were injected and the parameters like retention time (RT), plate number(N), peak area and peak asymmetry of sample were calculated these results are presented in the table: 7.

Table: 7 System Suitability of Zolmitriptan

Injection	Retention Time	Peak area	USP plate count	Tailing
1	5.77	162240	3569	0.2
2	5.76	162242	3560	0.3
3	5.77	162245	3564	0.2
4	5.75	162239	3535	0.3
5	5.76	162241	3586	0.4
6	5.77	162243	3569	0.5
Mean	**5.763**	**162241.7**	-	–
SD	**0.008**	**2.160**	-	-
%RSD	**0.142**	**0.001**	-	-

Evaluation of pharmaceutical formulation:

The validated method was applied to determination of Zolmitriptan in commercial tablet of Zolmitriptan. The result assay indicates that the method is selective for routine analysis with no chromatographic interference from excipients used. Results are shown in table 8

Table: 8 Assay of Zolmitriptan in Tablets

S.no.	%Assay Zolmitriptan (5 mg tablets)
1	99.2
2	100
Mean	99.600
SD	0.566
%RSD	0.568

Results and Discussion

To optimize the mobile phase, various proportions of buffers with methanol were tested. Zolmitriptan was quantified by reverse phase HPLC equipped with Sunniest (C18, 150 x 4.6 mm, 5.0 µ) column and mobile phase using pH 8.5 buffer and methanol as organic modifiers and flow rate is adjusted to 1.0 mL/min Column temperature is maintained at 40˚C. Injection volume is 10µL. the retention time was found to be 5.7 min. The standard plots were constructed between concentrations vs. peak area a linear response of peak area was observed over the concentration range of 125-750 µg/mL for Zolmitriptan The regression equations of concentration Zolmitriptan was found to be y = 324.47x + 3.400, where y is the peak area and x is the concentrations of drugs (µg/mL). The numbers of theoretical plates obtained were 3560, which indicates the efficiency of the column. The limit of detection and limit of quantitation were found to be 0.0.1, 0.03µg/mL, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulations did not interfere with the estimation of the drug by the proposed HPLC method.

Conclusion

A simple, specific, accurate, precise reverse phase high performance liquid chromatography method has been developed which can be used accurately quantitative estimation of Zolmitriptan for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R2) so it can be used by QC department.

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