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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC ASSAY METHOD OF ZOLMITRIPTAN IN PURE AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, accurate and stability-indicating reversed phase high performance liquid chromatographic method was developed and validated for the determination of Zolmitriptan and using a Altima C_{18} (150 x 4.6 mm), 5µm column using a mobile phase of phosphate buffer pH 2.5 and acetonitrile in the proportion of 85:15. The retention time of zolmitriptan were found to be 4.522min respectively. Linearity was established for Zolmitriptan in the range of 5.104 to 30.626µg/ml. The percentage recovery of Zolmitriptan was found to be in the range of 98.50-100% respectively. The selected drug were subjected to acid, alkali, oxidation, dry heat and UV degradation and these degradation studies revealed that this method can be successfully employed for stability indicating method for routine analysis of Zolmitriptan in pure and formulations.

KEYWORDS: Zolmitriptan, Stability indicating method, RP-HPLC.

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INTRODUCTION

Zolmitriptan (**Figure 1**),(S)-4-{[3-(2-dimethylaminoethyl)-1H-indol-5-yl]methyl}-1,3-oxazolidin-2-one is an oral, selective serotonin receptor agonist used for the treatment of acute migraine attacks[1–3].

Literature survey revealed several analytical HPLC methods were reported in literature for the quantitative determination of zolmitriptan and its metabolites in drug substance human plasma, other biological fluids[4–11] and in drug substance[12–17]. Yet no simple stability indicating analytical method was reported for its estimation by reverse phase HPLC. Therefore, it made the author to develop and validate the stability of zolmitriptan under acidic, alkaline, oxidative, UV and photolytic conditions using RP-HPLC technique. This present paper aimed in reporting sensitive, precise, accurate, robust and rugged validated RP-HPLC method for the assay of zolmitriptan in pure as well as dosage forms.

MATERIALS AND METHODS

Zolmitriptan (99.65% pure) of pharmaceutical grade was supplied as gifted sample by Sun Pharmaceuticals, India. Sodium dihydrogen mono phosphate (AR grade), o-phosphoric acid(AR grade), Acetonitrile(HPLC grade)and water(Mill Q) were used in the present study. The tablet formulation of Zolmitriptan (strength 5.0mg) was procured from local pharmacy and used for analysis for marketed formulation.

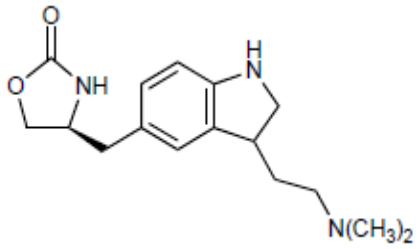

Fig.1. Chemical structure of Zolmitriptan

Instruments: The liquid chromatographic system was of Waters (USA), series consisting of Waters 2695 Separation module, Waters 2996 Photo diode array Detector equipped with Waters Empower2 software. The chromatographic analysis was carried on an Altima C_{18} (150 x 4.6 mm), 5µm column. In addition, an electronic balance (Shimadzu AX200), a pH meter (Systronics model EQMK VI), a sonicator (Spectra Lab, model UCB 40), a hot air oven (Remi) were used in this study.

Buffer Preparation: Weighed 7.8g of Sodium dihydrogen mono phosphate dissolved in 1000 ml of water. The buffer pH is maintained at 2.5 with o-phosphoric acid. This solution was filtered through 0.45µ filter and degassed before use.

Mobile phase Preparation: Buffer solution and acetonitrile were mixed in the ratio of 85:15% and degassed**.**

Standard preparation: Weigh accurately about 25mg of Zolmitriptan into 100ml volumetric flask. Add 70ml of diluent and swirl gently to dissolve and make-up the volume with diluent. The calibration curve was plotted over the concentration range 5.104 to 30.626µg/ml prepared by dissolving aliquots of standard working solution of zolmitriptan with mobile phase. Aliquots (20µl) of each solution were injected under the operating chromatographic condition described above.

Formulation preparation: 20tablets of brand Skelat (manufactured by Sun Pharmaceuticals Limited) containing 50mg of zolmitriptan were weighed, average weight determined and finely crushed to powder. Weigh accurately about 25mg of zolmitriptan into 100 ml volumetric flask. Add 60 ml of diluent and swirl gently to dissolve and make-up to the volume with diluent, use this solution for degradation. Transfer 2.0ml of this solution to 50ml with diluent andwas then filtered through 0.45 µ membrane filter. Use this solution for assay.

Chromatographic Conditions: The HPLC analysis was performed on reversed-phase high-performance liquid chromatographic system with isocratic elution mode using the mobile phase pumped at a flow rate of 1.0 ml/min through the column (C_{18}; 4.6 X 150 mm, 5µ, Altima column) at 30ºC, and injection volume is 20µl, wavelength used was 225nm,and runtime was 7mins.

RESULTS AND DISCUSSION

Method development: This involves the optimization studies of various RP-HPLC parameters that include the selection of mobile phase compositions, selection of appropriate detector wavelength and selection of column etc. A satisfactory separation and good peak symmetry for zolmitriptan was obtained with a mobile phase consisting of buffer solution (pH-2.5) and acetonitrile in the ratio of 85:15v/v. Quantification was achieved with UV detection at 225nm based on peak area. At this wavelength complete resolution of the peak with clear baseline of zolmitriptan was obtained (**Figure 2**). System suitability parameters was calculated and compared with the standard limit as per ICH standards.

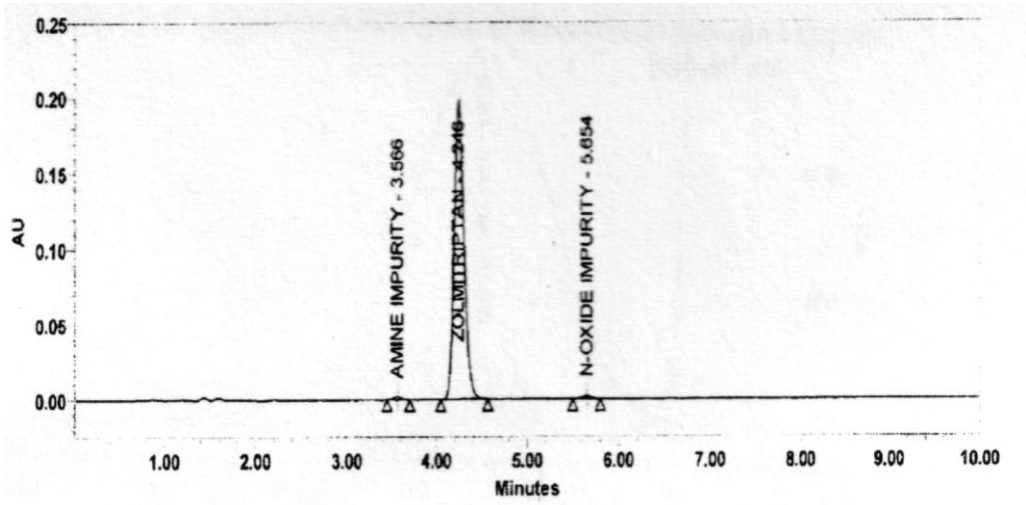

Fig.2.Validative Chromatogram of Zolmitriptan

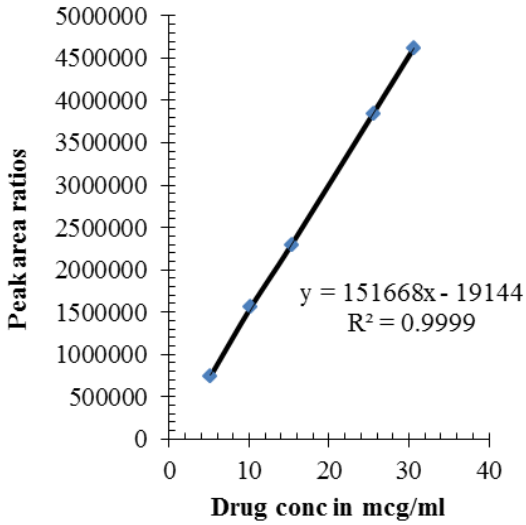

Fig.3.Calibration curve of Zolmitriptan

Method Validation: The method of analysis was validated as per the recommendations of ICH for the parameters like linearity, detection limit, quantitation limit, accuracy, precision and robustness.

System Suitability: System suitability study of the present method was carried out by six replicate analysis of solution containing 100% target concentration of zolmitriptan. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters. The results of this study are given in **Table.1**.

Table.1: Results of system suitability parameters

System suitability parameters	Results
Ret Time	4.52
Area	2299529
% Area	100.0
USP Tailing	1.2
USP Plate Count	7094

Table.2: Results of Specificity studies

Stress conditions	%Degradation	Purity angle
5N HCl at 60°C for 2hrs	2.97	0.194
1N NaOH at 60°C for 2hrs	2.57	0.289
30% H_2O_2 for 8hrs	14.01	0.403
Heat at 105°C temp for 120hrs	2.0	0.342
UV light for 120hrs	No degradation	0.334

Table.3: Results of Linearity of the proposed method

Concentration (µg/ml)	Peak area
5.104	735037
10.209	1557888
15.313	2299529
25.522	3848202
30.626	4624443
Slope	28990.73
Intercept	-3606.23
Correlation Coefficient (r)	0.9997

Table.4: Results of Method Precision

Preparation	*% Assay (on dried basis)
1	101.9
2	99.9
3	100.3
4	101.5
5	101.7
6	101.3
Mean	101.1
% RSD	0.801

*Average of three readings

Table.5. Results of Accuracy

%Spiked level	mg added	*mg found	%Recovery	Mean %recovery	%RSD
40	10.13	9.95	98.2		
40	10.13	9.98	98.6	98.5	0.3
40	10.11	9.99	98.7		
60	15.16	15.06	99.3		
60	15.14	14.97	98.9	99.0	0.2
60	15.15	14.99	99.3		
80	20.27	20.24	100.0		
80	20.27	20.23	99.9	100.0	0.1
80	20.24	20.27	100.0		
100	25.34	25.14	99.2		
100	25.32	25.15	99.3	99.5	0.4
100	25.31	25.30	100.0		

*Average of three readings

Table.6. Results of robustness

Results of effect of variation in flow rate	Observed value with flow rate		
	0.9ml/min	1.0ml/min	1.1ml/min
Theoretical plates	7640	7828	7659
Tailing factor	1.2	1.2	1.2
%RSD	0.2	0.2	0.1
Results of effect of variation in column temperature	Observed value with column temperature		
	25°C	30°C	35°C
Theoretical plates	7828	6767	7643
Tailing factor	1.2	1.2	1.2
%RSD	0.2	0.1	0.1

Table.7. Results of stability studies

System suitability parameters	Observed value		
	At initial	After 1 day	After 3 days
Theoretical plates	7828	7293	7562
Tailing factor	1.2	1.2	1.2
%RSD	0.2	0.1	0.6

Table.8. Analysis of marketed tablets by the proposed method

Drug	Label Claim	Quantity Found*	Reference method	Statistical Results	%Assay
Zolmitriptan (Zomig)	**5.0**	4.92±0.04	4.97±0.08	F=4.00 t=1.44	99.49

* Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.262

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Intentional degradation was attempted under stress conditions of UV light (225nm), acid (5N HCl), base (1N NaOH), oxidation (30% H_2O_2) and thermal (105°C) to evaluate the ability of the proposed method to separate zolmitriptan from its degradation products. Peak purity test results obtained from DAD confirm that the zolmitriptan peak is homogenous and pure in all the analyzed stress samples confirming the stability-indicating power of the proposed method. The summary of forced degradation studies is given in Table.2.

Linearity: Linearity of the method was determined by constructing calibration curves. Standard solutions of zolimitriptan of different concentration range 5.104 to 30.626µg/ml were used for this purpose. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients were 0.999 for the drugs which prove that the method is linear. The graph is represented in the Figure.3 and the results of linearity data for zolmitriptan are shown in Table.3.

Limit of detection (LOD) and Limit of Quantification (LOQ): The lowest concentration of the drug that can be detected is expressed as a concentration that gives signal to noise ratio of 3:1. The lowest amount of analyte that can be determined with acceptable precision and accuracy under the experimental condition or LOQ was measured in terms of signal to noise ratio of 10:1. The LOD and LOQ were found to be 0.01µg/ml and 0.036µg/ml respectively.

Precision: Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proved that proposed method is precise. The values are shown in Table.4.

Accuracy: The degree of accuracy of the proposed method, were performed in triplicate by standard addition method at 40%, 60%,80%,100% and 120%.Known amounts of standard zolmitriptan were added to pre-analyzed samples and were subjected to the proposed RP-HPLC method. The percentage recovery of zolmitriptan in bulk drug samples ranged from 98.5% to 100.0% (Table.5) indicating the high accuracy of the proposed method.

Robustness: To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. In the present study the effect of change in flow rate of mobile phase and temperature were studied and the values are shown in Table.6. The values are shown in Table.6 revealed that the proposed method was found to be unaffected by the above said deliberate varied chromatographic conditions illustrating its robustness.

Stability: The stability of zolmitriptan was checked by determining the concentration of the solution after 48hrs at room temperature. The % RSD of the assay of zolmitriptan during solution stability experiments were within 1.0% confirming that the sample solutions and mobile phases used during assay were stable over a period of 48hrs at room temperature (Table.7).

CONCLUSIONS

In the present paper a stability-indicating RP-HPLC method was developed for the determination of zolmitriptan and validated as per ICH guidelines. Statistical analysis proved that developed RP-HPLC method was accurate, precise, repeatable, sensitive and selective for analysis of zolmitriptan without any interference from the excipients. The developed method is proved to be stability-indicating and can be very useful for quality monitoring of regular production samples and can also be employed to check the quality during stability studies.

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REFERENCES

[1]. Oldman AD, Smith LA, McQuay HJ, Moore RA. A systematic review of treatments for acute migraine. Pain. 2002;97:247–57.
[2]. Yates R, Nairn K, Dixon R, Seaber E.. J Clin Pharmacol. 2002;42:1237–43.
[3]. Yates R, Nairn K, Dixon R, Kemp JV, Dane AL.. J Clin Pharmacol. 2002;42:1244–50.
[4]. Chen X, Liu D, Luan Y, Jin F, Zhong D. J Chromatogr B. 2006;832:30–5.
[5]. Yu L, Wen Y, Song Z, Mu D, Su L, Yang Y.. Fenxi Ceshi Xuebao. 2006;25:67–70.
[6]. He H, Meng H, Zhou Y, Li B, Li X. Yaowu Fenxi Zazhi. 2005;25:323–5.
[7]. Yao J, Qu Y, Zhao X, Hu L, Zhu R, Li H. J Chinese Pharm Sci. 2005;14:25–8.
[8]. Zang Z, Xu F, Tian Y, Li W, Mao G. J Chromatogr B Analyt Technol Biomed Life Sci. 2004;813:227–33.
[9]. Chen J, Jiang X, Jiang W, Mei N, Gao X, Zhang Q. J Pharm Biomed Anal. 2004;35:639–45.
[10]. Clement EM, Franklin M. J Chromatogr B Analyt Technol Biomed Life Sci. 2002;766:339–43.
[11]. Kilic B, Ozden T, Toptan S, Ozilhan S. Chromatographia. 2007;66:129–33.
[12]. Hu Y, Yao T, Wang X. Yixueban. 2004;33:37–40.
[13]. Srinivasu MK, Mallikarjuna Rao B, Sridhar G, Rajender Kumar P, Chandra Sekhar KB,Aminul I. J Pharm Biomed Anal. 2005;37:453–60.
[14]. Mallikarjuna Rao B, Srinivasu MK, Sridhar G, Rajender Kumar P, Chandra Sekhar KB, Aminul I. J Pharm Biomed Anal. 2005;39:503–9.
[15]. Vijayakumar EKS, Samel MA, haleka SB, Pakhale SM. Indian J Pharm Sci. 2010; 72(1): 119–122.
[16]. Navaneeswari R, RaveendraReddy P. African Journal of Scientific Research. 2011; 4:230-243.
[17]. Mathrusri Annapurna M, Bidyut Nanda. Journal of Pharmacy and Nutrition Sciences, 2011; 1:, 9-14.