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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTIFICATION OF ZOLMITRIPTAN

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ABSTRACT

A simple, rapid, sensitive, reverse phase isocratic RP-HPLC method was developed for determination of Zolmitriptan. The method was carried out using SYMMETRY C18 packed column [3.5µm, 2.1x50mm] with mobile phase comprised of Acetonitrile: water (70:30 v/v) with 0.2 ml / min flow rate was quite robust. The optimum wavelength for detection was 225 nm at which better detector response for the drug was obtained. The run time was set at 5 min and the retention time was 1.04 minutes. The method was validated for specificity, accuracy, precision, linearity, and limit of detection, limit of quantification, robustness, solubility and stability. LOD and LOQ were found to be 0.5 ng/ml, 1ng/ml respectively. The calibration curve was linear in the concentration range of 0.03-1µg/ml with coefficient of correlation 0.994.

Keywords: Zolmitriptan, RP-HPLC, Chromatography, Validation.

INTRODUCTION

Migraine is a recurrent incapacitating neurovascular disorder characterised by attacks of debilitating pain associated with photophobia, phonophobia, nausea and vomiting. Neurogenic theory considers migraine to be a spreading depression of cortical, electrical activity followed by vascular phenomenon. [1, 2]

Attacks of migraine typically last from several hours to 2 to 3 days, and many patients suffer one or more attacks a month [3]. Against this background the triptans, selective serotonin 5-HT (1B/1D) agonist are very effective acute migraine drugs with a well-developed scientific rationale.[4] Zolmitriptan is a second generation triptan developed to provide improved pharmacokinetic and optimised trigeminovascular targeting of both the peripheral and central trigeminal terminals.[3]

Zolmitriptan (s) – 4 – [3-[2-(dimethylamino) ethyl] – 1H – indol – 5 – yl] methyl] – 2 – oxazolidinone (figure-1). Clinical research indicates that it has a better efficacy and tolerability profile at low doses of 2.5 – 10mg. Zolmitriptan is rapidly adsorbed when given as oral tablets both in fasting state and when given with food.

In the previous studies, Seaber et al.[5] developed a HPLC method to assay zolmitriptan and its three major metabolites with fluorescence detection and Clement and Franklin[6] established a HPLC method for quantification of zolmitriptan and its two major metabolites with coulometric detection in plasma. Srinivasu developed liquid chromatographic method for its enantiomereic separation as well as its potential impurities [7, 8]. A few HPLC, LC-MS/MS and colorimetric methods were reported[9,10,11,12]. In the present study, a new RP-HPLC method was developed which shows high reproducibility and sensitivity. The developed method was validated as per ICH guidelines[13].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Zolmitriptan (purity >99%) was a gift sample from a local manufacturing unit in Hyderabad, India. HPLC grade acetonitrile was purchased

from sigma Aldrich, and all other chemicals were of analytical grade.

2.2 Apparatus

High Performance Liquid chromatography was performed on a SYMMETRY C18 packed column (3.5µm, 2.1×50mm particle size, WATERS, Ireland) maintained at 60^0 c. The HPLC system consists of Shimadzu LC-20 AT liquid chromatographic pump, Rheodyne injection port (Rheodyne, cotati, CA, USA) with a 20µl sample loop and SPD-M20A photo diode array (PDA) detector (Shimadzu, Kyoto, Japan). SYMMETRY C – 18 packed column (2.1 × 50 mm, 3.5 µm particle diameter) manufactured by WATERS (Ireland) was used. The mobile phase consisted of HPLC Acetonitrile: milli-Q water (70: 30 v/v) delivered at 0.2ml/min.

2.3 Calibration Standards

Stock solutions of zolmitriptan with a concentration of 1000µg/ml was prepared by dissolving 25mg of zolmitriptan into 25ml volumetric flask and adds about 25ml of diluents (80:20 of acetonitrile and water) and sonicate to dissolve completely, make volume up to the mark with the same diluent. Seven standard solutions of 30, 50, 70, 100, 500, 700 and 1000ng/ml of zolmitriptan were prepared by further dilution of the stock solution with appropriate volumes of diluents.

3. RESULTS AND DISCUSSION

The objective of this study was to develop a rapid and sensitive HPLC method for the analysis of zolmitriptan in bulk drug using the most commonly employed C-18column with UV-detection. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor), run time, resolution.

The system with Acetonitrile: Phosphate buffer (70:30 v/v) with 1.0 ml / min flow rate was quite robust. The optimum wavelength for detection was 273 nm at which better detector response for the drug was obtained. The run time was set at 7 min and the retention time for was 3.48 min.Chromatogram was shown in the following figure 2.

3.1. Linearity

To evaluate linearity, plasma calibration curve were prepared over the concentration range of 30 – 100 ng/ml, encompassing the therapeutic range of this antimigraine drug. Calibration curve was calculated utilizing the peak area v/s analyte concentration. The response was linear for zolmitriptan throughout this concentration range and the correlation coefficient was 0.994. The results were presented in Table-1 and standard graph is shown in Figure-3.

3.2. 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 (interday precision) involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts.

The intra-day repeatability was investigated using three separate sample solutions each at three different levels (50, 100 and 600 ng/ mL. Each solution was injected in six replicates and the peak areas obtained were used to calculate mean and RSD% values. The inter-day reproducibility was checked on three different days, by preparing and analyzing six replicates of three sample solutions at the same concentration level of intraday repeatability, the mean and RSD% values were calculated from peak areas (Table **2**).

3.3. Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed Standard solution. The standard addition method was performed at 50%, 100% and 150% level of 50 ng/ml. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table.3.Satisfactory recoveries ranging from 98% to 102% were obtained by the proposed method (Table 2). This indicates that the proposed method was accurate.

3.4. Limit of Detection and Limit of Quantification

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 quantification, defined as lowest concentration of analyte that can be quantified with suitable precision and accuracy, is estimated as 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 0.5 and 1ng/ ml respectively

3.5. System Suitability Testing

Standard solution for the determination of system suitability contained 225 ng/ml which was prepared by diluting the corresponding standard stock solution. It was determined from 3 replicate injections of standard solution before sample analysis. The % RSD was found to be 4.42.

3.6. Robustness

ICH defines robustness as a measure of the methods capability to remain unaffected by small but deliberate variations in method parameters. As a part of determining robustness, deliberate change in the flow rate (± 0.01 ml/min) and temperature (± 5^{0}C) were carried out with a solution of 100 ng/ml of Zolmitriptan. Results are tabulated in Table (3, 4).

CONCLUSION

The developed RP-HPLC method for the determination of Zolmitriptan is simple, sensitive and precise. More than 150 samples could be assayed daily, including sample preparation data acquisition and processing. Further the method can be used in routine quality control studies of zolmitriptan in pure state and its formulations.

Table 1: Linearity data of Zolmitriptan

Concentration (ng/ml)	Peak area
30	1480.17
50	5345.56
70	8737.38
100	12111.20
500	71865.74
700	94819.89
1000	125249.50

Table 2: Precision and accuracy for the determination of Zolmitriptan[a]

Nominal Concentration (ng/ml)	Calculated Concentration (ng/ml)	Intra-day Precision RSD (%)	Inter-day Precision RSD (%)	Accuracy (%) recovery
50	50±2.46	2.25	2.56	104.93
100	100±5.72	1.4	2.06	95.72
600	600.±8.91	3.27	4.52	101.48

[a] Data are based on assay of 6 replicates on 3 different days.

Table 3: Robustness at different flow rates [100ng/ml]

Flow rate (ml/min)	RT	Mean Peak Area	Mean Concentration	SD Concentration	RSD Concentration
0.19	1.02	11486	96.35	1.84	1.91
0.20	1.04	11337	95.25	2.64	2.77
0.21	0.99	10798	92.36	1.58	1.71

Table 4: Robustness at different temperatures [100ng/ml]

Temperature ^{0}C	RT	Mean Peak Area	Mean Concentration	SD Concentration	RSD Concentration
55	1.03	11351	95.30	1.89	1.93
60	1.01	11337	95.25	2.64	2.77
65	1.00	10329	95.23	2.01	2.36

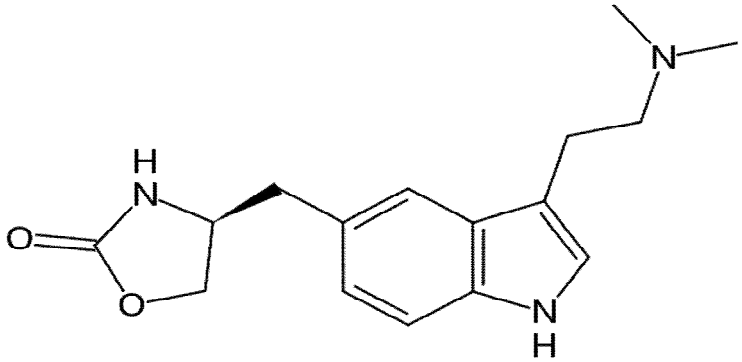

Fig. 1: Structure of Zolmitriptan

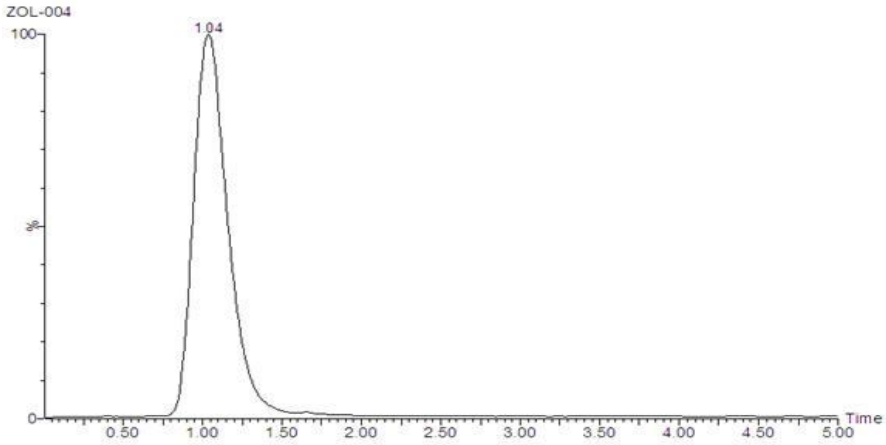

Fig. 2: Chromatogram of Zolimitriptan

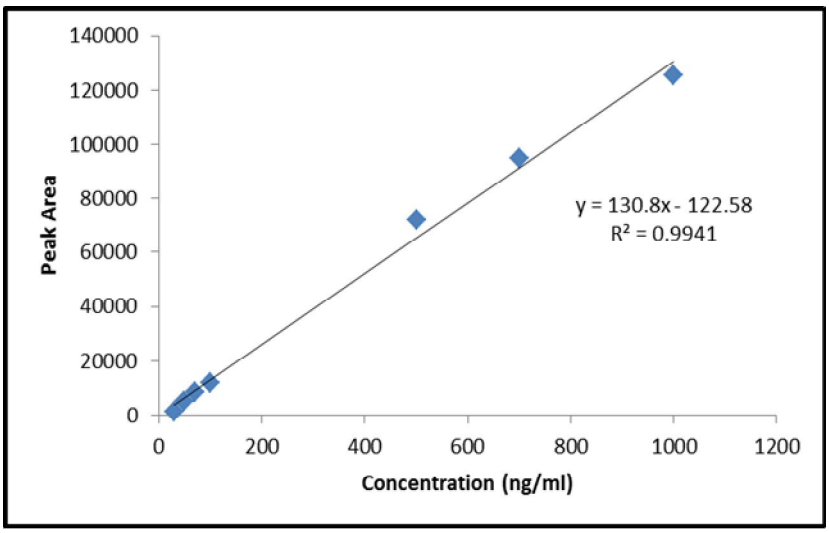

Fig. 3: Standard calibration curve of Zolmitriptan

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