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BIO ANALYTICAL HPLC METHOD DEVELOPMENT AND VALIDATION OF ZOLMITRIPTAN IN RABBIT PLASMA

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ABSTRACT

A simple, sensitive and rapid high performance liquid chromatographic method was developed and validated for Zolmitriptan from rabbit plasma. Chromatographic separation and detection was carried out on a Hibar C_{18} (250 x 4.6 mm, 5µ) coloum using 10 mM di-potassium hydrogen orthophosphate buffer (pH 3.2) and methanol in the ratio of 77:23 with a flow rate of 1.1 ml/min at 231 nm. Retention times of drug and IS were found out to be 5.5 min and 7.7 min respectively. The method was linear in the concentration range of 18.73-374.6 ng/ml. The regression coefficient value was found to be 0.987. The proposed method was validated by performing linearity, recovery, specificity, robustness, LOD/LOQ and interday / intraday precision. The LOD and LOQ values were found to be 6.18 and 18.73 ng/ml. Rizatriptan was used as internal standard.

Key Words: HPLC, Zolmitriptan, Rizatriptan.

INTRODUCTION

Zolmitriptan (Fig. 1) is chemically described as (S)-4-[[3-[2-(Dimethyl amino) ethyl]-1H-indol-5-yl]methyl]-2- oxazolidinone. It is a selective serotonin receptor agonist of the 1B and 1D sub types and is used in the acute treatment of migraine attacks. Migraine is a chronic disorder characterized by recurrent moderate to severe headache often in association with a number of autonomic symptoms. Only few analytical and bio analytical methods were reported for the determination of Zolmitriptan in biological samples (Chen *et al*., 2006; Dalpiaz *et al*., 2012; Rao *et al*., 2005; Srinivasu *et al*., 2005; Vijaya Bhaskar Reddy *et al*., 2013). There were no simple, rapid and reproducible methods so far reported for the estimation of Zolmitriptan in plasma. The objective of the present investigation was to develop a new, rapid and sensitive RP-HPLC method for the estimation of Zolmitriptan in rabbit plasma using perchloric acid as a precipitating agent with C_{18} column and this method can be applied to a bioequivalence study of Zolmitriptan tablets using human volunteers. The outcome of a study depends upon the reliability, reproducibility and sensitivity of the analytical methodology employed. Therefore, the bio analytical method was validated in accordance with USFDA guidelines prior to the initiation of the study.

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MATERIALS AND METHODS

Materials

Methanol HPLC grade and Ortho phosphoric acid (Rankem), Water HPLC grade (Milli-Q RO system), Working standard of Zolmitriptan (Orchid pharmaceuticals, Chennai), Internal standard Rizatriptan (Apotex, Bangalore) and Dipotassium dihydrogen orthophosphate AR grade (SDFCL) were used. Fresh rabbit plasma used in the method development was obtained from the JSS College of Pharmacy, Ooty and was stored at 20°C until required.

Instrumentation and chromatographic conditions

Separation and detection was carried out on a Shimadzu gradient HPLC system equipped with LC-10 AT-VP solvent delivery system (pump) with UV detector. A C_{18} reverse-phase HPLC column (Hibar, 250 x 4.6 mm i.d., 5µ) was utilized for drug separation using methanol - 10 mM di potassium hydrogen orthophosphate pH 3.2 (23:77, v/v) as mobile phase. The flow rate and UV wavelength were 1.1 ml/ min and 231 nm, respectively.

Preparation of standard solution

Standard stock solution of Zolmitriptan was prepared by dissolving 10 mg of drug in 10 ml of methanol. The stock solution was diluted to suitable concentrations with mobile phase to obtain series of standard solutions. Calibration standards of Zolmitriptan (18.73, 18.95, 37.46, 74.92, 112.38, 168.57, 337.14 and 374.6 ng/ml) were used by spiking appropriate amount of the standard solution in blank plasma.

Preparation of Internal standard solution

Internal standard stock solution of Rizatriptan was prepared by dissolving 10 mg of drug in 10 ml of methanol.

Sample Preparation

Zolmitriptan plasma concentration was determined by HPLC analysis. A 200 µl plasma sample 300 µl drug and 300 µl IS were placed into a centrifuge tube and 200 µl of 10% perchloric acid was added and shaken vigorously for 30 sec at room temperature. After centrifugation at 4000 rpm for 15 min, the supernatant was separated and analyzed. Calibration curves were prepared by linear regression analysis of the plot of the peak area against concentration of Zolmitriptan. The concentration of plasma samples was determined from the area of chromatographic peak using the calibration curve.

Validation

The validation parameters (FDA 2001) such as accuracy, precision (repeatability and reproducibility), linearity and range, sensitivity (limit of detection, limit of quantification), robustness/ruggedness, stability, selectivity/specificity and system suitability were evaluated.

Specificity

HPLC-UV analysis of the blank rabbit samples showed no interference with either Zolmitriptan or Rizatriptan (IS). The standard and sample chromatograms are shown in Fig 2 indicating no interference in the sample at the retention time of 5.5 min for the drug Zolmitriptan and at the retention time of 7.7 min for the IS.

Sensitivity

The limit of detection was found to be 6.18 ng and the limit of quantification was found to be 18.73 ng and is shown in Table 1.

Linearity

A regression equation with a weighing factor of $1/\text{concentration}^2$ was judged to produce the best fit for the concentration/detector response relationship for Zolmitriptan. The linearity range for Zolmitriptan was found to be 18.73, 18.95, 37.46, 74.92, 112.38, 168.57, 337.14 and 374.6 ng/ml. The results were given in the Table 2 and shown in Figure 3 with a correlation coefficient (r^2) found to be 0.987.

Precision

The precision of the method was demonstrated by the percent coefficient of variation over the concentration range of low, middle and high quality control sample of Zolmitriptan during the course of validation. The accuracy of the assay was defined as the absolute value of the ratio of the calculated mean values of the LOQ, low, middle and high quality control samples to their respective nominal value, expressed as percentage. The results are given in Table 3a-3b.

Stability studies

The stability studies of plasma samples spiked with selected drugs were subjected to three freeze thaw cycles, short term stability at room temperature for 3 hours and long term stability at -70^{o}C over four weeks. In addition, stability of standards solutions were performed at room temperature for 6 hours and freeze conditions for four weeks. The mean concentrations of the stability samples were compared to the theoretical concentrations. The results indicated that selected dugs in plasma samples can be stored for a month without degradation in frozen state. The results of short term storage at room temperature stability and freeze thaw cycles indicate no degradation of selected drugs in plasma as well as in sample solution and hence plasma samples could be handled without special precautions. The results are given in Table 4a-4c.

Accuracy

Analyte recovery from a sample matrix (extraction efficiency) is a comparison of the analytical response from an amount of analyte to that determined from the sample matrix. The detailed results are presented in Table 5. The results indicate that the recovery of Zolmitriptan was consistent at all levels.

Ruggedness and Robustness

The ruggedness and robustness of the methods were studied by changing the experimental conditions. No significant changes in the chromatographic parameters were observed when changing the experimental conditions (operators, instruments, source of reagents and column of similar type) and optimized conditions (pH, mobile phase

ratio and flow rate etc.)

RESULTS AND DISCUSSION

In the spectral investigation by RP-HPLC method standard solution of Zolmitriptan showed peak at 5.5 min. Optimization of the method was carried out using 10 mM di-potassium hydrogen orthophosphate buffer (pH 3.2) and methanol in the ratio of 77:23 with flow rate of 1.1 ml / min. The calibration curves of Zolmitriptan were linear in the range of 18.73-374.6 ng/ml. The precision of the method was demonstrated by reproducibility studies. The % RSD values of less than 2% revealed that the methods were precise. The accuracy of the optimized method was determined by absolute recovery experiments. The percentage recovery values for Zolmitriptan were found to be between 95.99 % and 99.12 %. An analysis of the results showed that the percentage recovery values were close to 100 % thus establishing that the developed method is accurate and reliable. Detection limits and quantification limits of Zolmitriptan were found to be 6.18 ng/ml and 18.73 ng/ml respectively. No marked changes in the chromatogram occurred on changing the operator and chromatographic conditions indicating that the developed method was rugged and robust. The column efficiency, resolution and peak asymmetry were calculated for the standard solutions and are presented in Table 1.

Table 1. System suitability study

Parameters	Drug
Theoretical Plates	1483
Tailing factor	1.03
Asymmetric factor	1.07
LOD (ng/ml)	6.18 ng/ml
LOQ (ng/ml)	18.73 ng/ml

Table 2. Bio calibration linearity of Zolmitriptan

Concentration(ng/ml)	Response factor
18.73	0.0100
18.95	0.0148
37.46	0.0285
74.92	0.0691
112.38	0.1062
168.57	0.1583
337.14	0.2678
374.6	0.2778

Table 3a. Precision study for Zolmitriptan

Intra run nominal concentration (ng/ml)			
S.No	**LQC**	**MQC**	**HQC**
	56.19	187.3	337.14
1	53.01	184.97	334.05
2	53.57	185.57	334.87
3	53.62	184.13	332.37
4	53.49	184.28	334.19
5	53.84	184.90	334.92
Mean	53.506	184.77	334.08
S.D (±)	0.3061	0.5802	1.0328
C.V.(%)	0.5720	0.3140	03091
%Nominal	95.223	98.649	99.092
N	5	5	5

Table 3b. Precision study for Zolmitriptan

Inter run nominal concentration (ng/ml)			
S.No	**LQC**	**MQC**	**HQC**
	56.19	187.3	337.14
1	52.96	185.37	334.83
2	53.14	185.79	334.46
3	53.82	184.19	332.92

4	53.70	184.72	334.85
5	53.39	184.53	335.06
Mean	53.402	184.92	334.42
S.D (±)	0.3632	0.6489	0.8680
C.V.(%)	068012	0.3509	0.2595
%Nominal	95.038	98.729	99.193
N	5	5	5

Table 4a. Stability studies of Zolmitriptan

Nominal concentration (ng/ml)			
Freeze and Thaw	**LQC**	**MQC**	**HQC**
	56.19	187.3	337.14
Cycle 1	53.91	185.17	334.19
Cycle 2	53.06	185.79	334.36
Cycle 3	54.83	185.46	333.71
Mean	53.933	185.47	334.086
S.D (±)	0.8852	0.3102	0.3370
C.V(%)	1.6412	0.6223	0.10087
% Nominal	95.98	98.66	99.0
N	3	3	3

Table 4b. Stability studies of Zolmitriptan

Nominal concentration (ng/ml)			
Short Term Plasma at Room Temperature	**LQC**	**MQC**	**HQC**
	56.19	187.3	337.14
After 1 hr	53.55	185.37	334.05
After 2 hr	53.63	185.64	334.59
After 3 hr	54.03	185.81	333.97
Mean	53.736	185.606	334.203
S.D (±)	0.2571	0.2218	0.3372
C.V(%)	0.4784	0.1195	0.1008
% Nominal	95.62	99.09	99.12
N	3	3	3

Table 4c. Stability studies of Zolmitriptan

Nominal concentration (ng/ml)			
Long Term Plasma Sample at -70^{0}C	**LQC**	**MQC**	**HQC**
	56.19	187.3	337.14
After 1 week	53.96	185.74	333.82
After 2 weeks	53.85	185.42	334.25
After 4 weeks	54.14	185.06	333.89
Mean	53.98	185.40	333.98
S.D (±)	0.1464	0.3401	0.2307
C.V(%)	0.2712	0.1834	0.0690
% Nominal	96.06	98.98	99.06
N	3	3	3

Table 5. Accuracy (Recovery study)

Level	**Concentration of drug added in ng/ml**	**Amount of drug recovered in (ng/ml) in the sample**	**Recovery (%)**
Level 1	56.19	53.942 ±0.003	Mean : 95.99 CV : 0.0208 N : 6
Level 2	187.3	182.984± 0.003	Mean :97.69 CV : 0.0102 N : 6
Level 3	337.14	334.198± 0.008	Mean : 99.12 CV : 002017 N : 6

Fig 1. Structure of Zolmitriptan

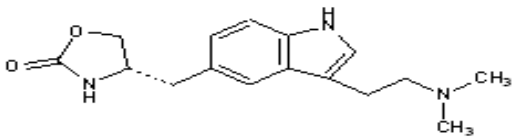


Fig 2. Standard chromatogram of Zolmitriptan and IS

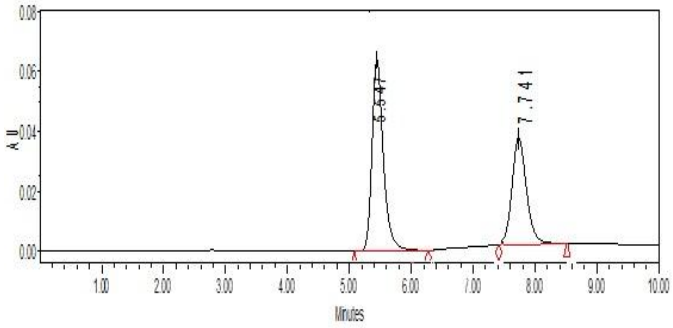
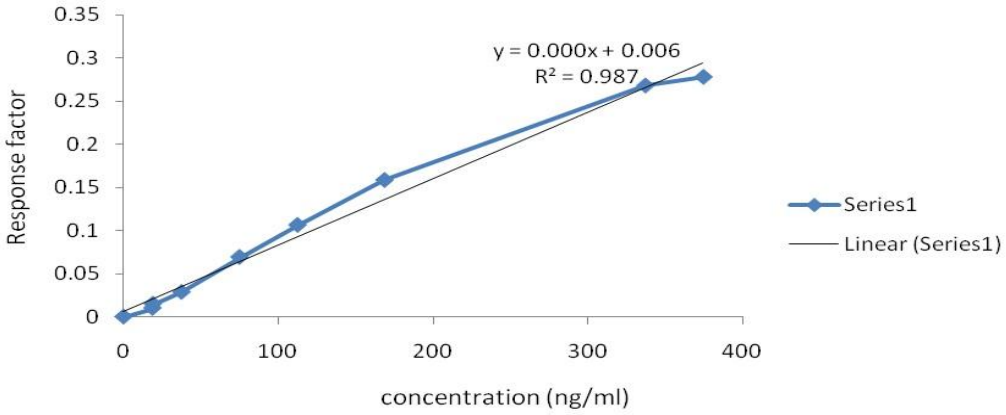


Fig 3. Bio calibration curve of Zolmitriptan



CONCLUSION

The developed RP-HPLC method in the present study for the estimation was found to be simple, rapid, accurate, precise, specific, linear and rugged. It is thus suitable for the estimation of Zolmitriptan in human blood plasma, raw materials and formulations.

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