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LC–MS–MS Determination of Zolmitriptan In Human Plasma

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ABSTRACT

A specific and sensitive liquid chromatography-electrospray ionization tandem mass spectrometry method was developed for the determination of zolmitriptan in human plasma. Zolmitriptan and the internal standard (IS) rizatriptan were extracted by liquid–liquid extraction and were separated on a sunfire C18 column (100 x 2.1mm and 3.5µm) with isocratic elution of mobile phase consisting of 10mM ammonium formate containing 0.1% formic acid and acetonitrile. Electrospray ionization in multiple reaction monitoring mode was used to monitor the parent and the daughter ions of the analyte and the internal standard. The response is linear over a range of 0.5 to 100.0 ng/ml concentration with a correlation coefficient (r^2) greater than 0.99 and an extraction efficiency of about 95%. The validated method can be applied to pharmacokinetic, toxicokinetic and bioequivalence studies.

Keywords : Zolmitriptan, tandem-mass spectrometry, multiple reaction monitoring, method validation

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INTRODUCTION

Zolmitriptan is used in the treatment of migraines and headaches. It is a triptan which is a selective 5-hydroxytryptamine$_{1B/1D}$ (5HT1B/1D) receptor agonist. Zolmitriptan is chemically designated as (S)-4-[[3-[2-(dimethylamino) ethyl]-1H-indol-5yl] methyl]-2-oxazolidinone and the chemical structure of zolmitriptan is shown in the figure.1.

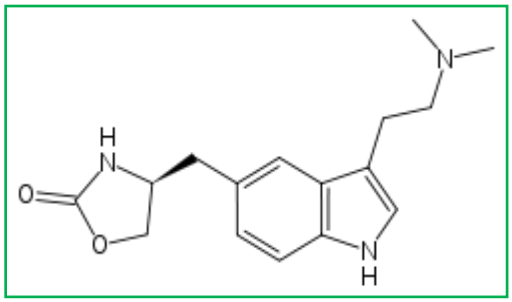

Figure.1 Chemical structure of Zolmitriptan

Zolmitriptan shows relatively high oral bioavailability (about 40%) and an in vivo plasma half-life of about 3 h. Clinical studies show that zolmitriptan half-life and bioavailability after nasal administration do not significantly differ from those obtained after oral intake of the drug [1-3]. Quantification of zolmitriptan in human plasma by coulometric[4], UV [5] and fluorescence [6] methods was used but the hyphenated technique, liquid chromatography with mass detection is the preferred method of analysis because of its sensitivity and selectivity. An LC/MS/MS method was described by Vishwanathan et al[7] for the determination of antimigraine drugs rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum using SPE technique. In another study, zolmitriptan and its active metabolite N-desmethylzolmitriptan were determined by liquid chromatograpy mass spectrometry but this method requires larger volume of plasma[8]. Another LC/MS/MS method was described by Chen et al[9] for the determination of zolmitriptan in human plasma by APCI method. Selected ion monitoring mode was used for the determination of zolmitriptan by Zhang et al [10].The aim of the present work was to develop a sensitive, convenient and cost effective bioanalytical method with a simpler extraction procedure that requires low volumes of biological fluid. Thus, the proposed method has the following advantages viz., avoiding the expensive SPE procedure, use of simple extraction procedure that minimizes errors, low volumes of plasma that helps to include more number of time points during sample collection and adoption of universally used ESI in multiple reaction monitoring mode to increase sensitivity as well as selectivity.

MATERIALS AND METHODS

Chemicals and Reagents

The reference standards of zolmitriptan and rizatriptan were obtained from Chemical Division, Natco Pharma Limited, India. Analytical grade acetonitrile, and methyl tertiary butyl ether were purchased from JT Bakers, ammonium acetate, acetic acid from Fluka and water used was of double distilled grade.

Instrumentation and operating conditions

The LC/MS/MS analysis was performed using a Waters Quattro-Micro API mass spectrometer equipped with 2695 LC separation module connected to a triple quadrupole analyzer. The separation was carried out using sunfire C18 column (100 x 2.1mm and 3.5µm) equipped with an ODS guard column. An isocratic elution of 10 mM ammonium acetate containing 0.1% acetic acid - acetonitrile (10:90 v/v) was adopted with a run time of 3.0 min and an injection volume of 10 µl. A constant flow rate of 0.3 ml/min was used. The mass spectral analysis was carried out by electron spray ionization in positive ion detection mode with MRM using parent-daughter transition of 288.4>182.2 for zolmitriptan and 270.3>201.3 for rizatriptan. The voltages, temperatures, desolvation gas and collision gas are optimized to produce maximum intensity of the ions. The source parameters of the mass spectrometry are summarized in the table.1.

Table.1 LC/MS/MS source parameters

Source parameters	Value
Capillary voltage (KV)	3.5
Cone voltage (V)	25
Extractor voltage (V)	5
RF voltage(V)	0.2
Source temperature (^{o}C)	120
Desolvation temperature (^{o}C)	350
Desolvation gas flow (l/hr)	650
Cone gas flow (l/hr)	50
Resolution	12
Collision enery (eV)	20

Primary stock solutions and working standard solutions

The primary stock solution of zolmitriptan and rizatriptan (internal standard) were prepared by dissolving appropriate amounts in acetonitrile. The working standard solutions for calibration curve and quality control and internal standard were prepared by dilution of the primary stock solutions. All the stock solutions were stored at 4^{o}C, previously tested for stability.

Spiking of calibration curve and quality control samples

Spiking was done by adding appropriate amounts of working standard solutions to drug-free human plasma. The calibration curve samples at concentrations of 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0ng/ml and quality control samples at concentrations of 0.5, 1.5, 35.0 and

85.0ng/ml were spiked. After spiking aliquots each of 100 µl were transferred into eppendorf tubes and were stored at -70^{o}C until further analysis. Three successive validation batches were done and on each validation the aliquots were thawed, 20 µl of internal standard were added and extracted by liquid-liquid extraction method.

Plasma sample extraction procedure

To 100µl plasma, 20µl of internal standard (0.5µg/ml) were added and vortexed for about 30 sec. To this, 4ml of methyl tertiary butyl ether extraction solvent was added, vortexed for 5 min using Multipulse vortexer (Glas-Col) then 2 ml of the supernatant clear organic layer were transferred to a 7.5 ml test tube and evaporated to dryness using Speedovap at 40°C under a stream of nitrogen. Then the dried extract was reconstituted with 100µl of diluent (Water: Acetonitrile-1:1) and a 10µl aliquot was injected into the chromatographic system. During each run a plasma blank sample and a zero standard sample (IS) were also analyzed.

Assay validation

The linearity was tested for the concentration range of 0.5 to 100ng/ml. Eight different concentrations 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, 50.0 and 100.0ng/ml were used for constructing the calibration curve by spiking in blank human plasma. A least square linear regression analysis was performed to determine the intercept, slope and the correlation coefficient.

The selectivity was established by checking blank human plasma samples obtained from different sources. Blank plasma and blank plasma spiked with zolmitriptan and internal standard were analyzed to check for potential interferences.

The concentration at which the response is greater than 5 times compared to the response of blank plasma sample is the limit of quantification. The lower limit of quantification was set at 0.5ng/ml with an accuracy of 80-120% and precision ±20%.

The accuracy of the assay is defined as the ratio of the mean of the assay values to the actual values expressed in percentage. The accuracy and precision was checked by analyzing six replicates of all the three quality control samples (1.5, 35.0 and 85.0ng/ml) against a single linearity curve on three different days.

The extraction efficiency expressed as the percentage recoveries was determined by measuring the peak area of the extracted plasma quality control and compared to the peak area of extracted blank plasma spiked with standards containing the same concentrations.

Autosampler stability/post preparative stability was determined for ~24 hrs to cover the anticipated run time for analytical batch and also to allow for delayed injection owing to

unforeseen circumstances like instrument malfunction. The extracted six replicate QC samples at three different concentrations were kept at autosampler temperature of 5^{o}C for ~24 hrs and analyzed against fresh standards. The concentrations of the stability samples and fresh samples were determined from a calibration curve prepared on the same day.

Bench-top stability/short-term stability was measured to cover the duration of the time taken to extract the samples. Bench-top stability was checked by analyzing six replicate QC samples at three different concentrations. The spiked QC samples were kept for 6 hrs at ambient temperature and processed thereafter. The concentration of the stability samples were compared against freshly spiked and processed standards.

Freeze thaw stability of the spiked quality control samples was determined during three freeze thaw cycles. Low, medium and high QC samples were analyzed in six sets. The percentage degradation was determined by comparing the concentration of zolmitriptan from the freshly prepared plasma validation samples at the same concentrations.

RESULTS AND DISCUSSION

Selectivity

The plasma samples analyzed showed that there is no interference due to any endogeneous components. A representative chromatogram of blank plasma and blank plasma spiked with IS and analyte are shown in Figure.2,.3 and.4 respectively.

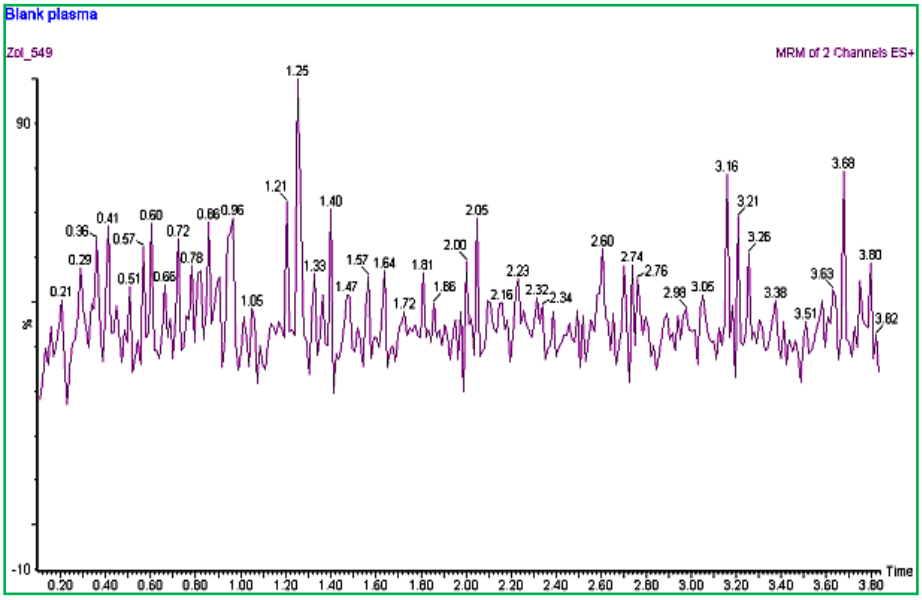

Figure: 2 Representative MRM chromatogram of blank plasma

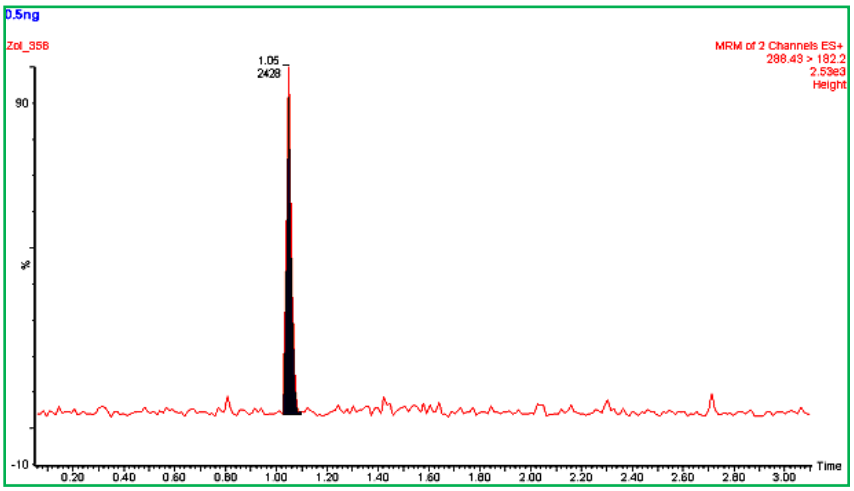

Figure: 3 Representative MRM chromatogram of blank plasma spiked with zolmitriptan at LOQ level

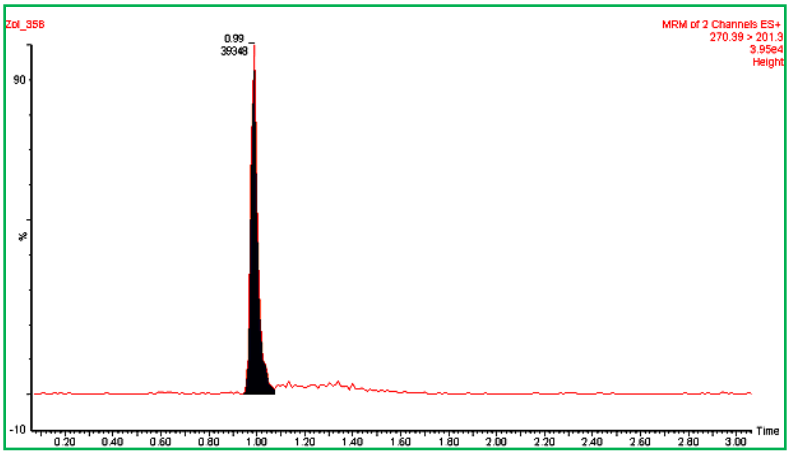

Figure: 4 Representative MRM chromatogram of blank plasma spiked with internal standard

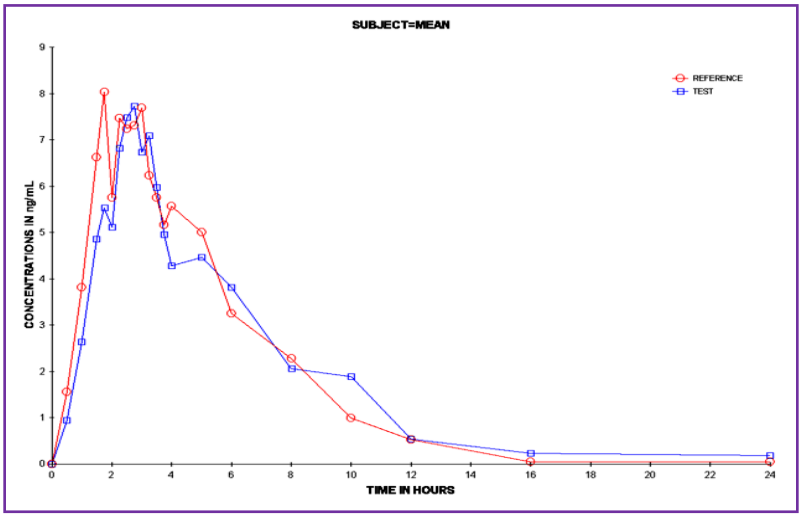

Figure.5 Mean plasma concentration curves of test and reference product of zolmitriptan

Linearity

Five different calibration curves were analyzed and the correlation coefficients were found to be greater than 0.99. This confirms that the calibration curves are linear over the range of 0.5 to 100.0ng/ml. The curve parameter summary of five calibration curves are given in the table.2

Table.2 Curve parameter summary of five calibration curves

Curve code	Slope(a)	y-intercept (b)	Correlation coefficient (r^2)
1	0.002855	0.004234	0.999
2	0.002875	0.002945	0.992
3	0.003138	0.002974	0.998
4	0.002918	0.002555	0.996
5	0.002847	0.008440	0.997

Lower limit of quantification

The lower limit of quantification was set to be as 0.5ng/ml with a response greater than 5 times to the response of blank plasma sample. The accuracy obtained was 102.0% and precision of 12.7%.

Accuracy & Precision

The accuracy and precision were checked by analyzing six replicates of all the three quality control samples (1.5, 35.0 and 85.0ng/ml) against a single linearity curve on three different days. The intra-day/within run accuracy ranged from 92.8% to105.4% and the intra-day/within run precision ranged from 4.31% to 7.34%. The inter-day/between run accuracy ranged from 101.3% to 105.7% and the inter-day/between run precision ranged from 6.06% to 9.73%. The intra-day and inter-day accuracy and precision are given in table.3

Table.3 Precision of zolmitriptan in human plasma

Spiked concentration (ng/ml)	Intra-day (n=6)	precision	Inter-day (n=6)	precision
	Mean±SD	RSD (%)	Mean±SD	RSD (%)
1.5	1.59±0.15	6.65	1.52 ±0.23	7.71
35.0	32.5±1.40	4.31	37.05±3.60	9.73
85.0	85.73±5.35	7.34	89.68 ±5.44	6.06

Recovery (Extraction Efficiency)

The extraction efficiency or the recovery of the analyte and the internal standard were consistently greater than 90%.The recovery of zolmitriptan and rizatriptan were 94.4% and 91.9% respectively.

Stability

The concentrations of the stability samples kept at autosampler temperature of 5^{o}C for ~24 hrs and the concentrations of the freshly prepared samples were determined from a calibration curve

prepared on the same day. The accuracy and precision of the stability samples were very much within the limits.

The concentration of the bench top stability samples were compared with freshly spiked and processed standards. Zolmitriptan was found to be stable even after 6 hrs. It was found that after three successive freeze thaw cycles the concentrations of zolmitriptan were nearly the same with the original concentrations and the percentage remained at 99.7% to 103.8% after the end of the three cycles.

Application of the method:

The proposed method was applied to determine the concentrations of zolmitriptan in real plasma samples collected periodically up to 24 hrs after oral administration of 5 mg tablet to 12 healthy male volunteers during the development of a conventional formulation. The mean plasma concentration curves of test and reference are represented in Fig.5. The AUC measured from 0 hour to the last collection point is greater than 90% sof the AUC extrapolated from 0 hour to infinity.

CONCLUSION

The proposed bioanalytical LC/MS/MS method for zolmitriptan is simple, economical, sensitive and accurate to quantify the concentrations in human plasma in a small volume of 100 ul. A simple liquid-liquid extraction procedure which is cheaper compared to the solid phase extraction procedure and with a run time of only 3.0 min is very useful to analyze large number of samples in a short time period. This method is suitable for pharmacokinetic, toxicokinetic and bioequivalence studies in human plasma.

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