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Research Article

A Developed and Validated Method for the Determination of Zolmitriptan in Human Plasma Through Liquid Chromatography Mass Spectrometric (LC-MS/MS)

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Abstract

A selective, rapid and sensitive liquid chromatography-mass spectrometry (LC-MS/MS) method was developed and validated for the quantitative estimation of zolmitriptan in plasma where Rizatriptan as internal standard and pretreatment of sample was involved a one-step extraction with ethyl acetate from plasma. Method: The sample was analyzed using Methanol: Buffer Solution in a ratio of 60:40, v/v as mobile phase at ambient condition and the volume of the sample was injected at 5 **µl** with run time of 2.5 mins. The chromatographic separation was achieved on a Chromolith® Speed ROD RP-18e 50 -4.6 mm column which was followed by detection with mass spectrometry. Results: The Retention time of the Zolmitriptan and Rizatriptan both have approximately 1.5 minutes with the Flow rate at 0.500 ml/minute. Linear calibration curves were obtained in the concentration range of 0.201 ng/ml to 14.873 ng/ml. The inter- and intra-day accuracy values were below 15% in the least internal control levels. Lipemic plasma ranged from 104.09% (HQC) to 104.97% (LQC) with a precision ranging from 3.79% (HQC) to 5.53% (LQC). The percent nominal of Zolmitriptan in Haemolysed plasma ranged from 105.03% (HQC) to 109.35% (LQC) with a precision ranging from 1.99% (LQC) to 3.06% (HQC). The mean% recovery of Drug A was 53.620% with a precision of 14.44%. The mean% recovery of internal standard Rizatriptan was 43.620% with a precision of 9.46%. Ruggedness was ranged from 91.38% (LLOQ QC) to 105.92% (LQC) with a precision ranging from 3.40% (MQC) to 7.24% (LLOQ QC). Therefore, all the validated parameters showed that the the method is specific, stable, accurate and precise. Conclusions: The results demonstrated that the above developed and validated LCMS method is simple, rapid, precise and accurate, which is useful for the estimation of Zolmitriptan in for the support to the pharmacokinetic study as well as pharmaceutical applications in the industry.

Keywords: Liquid Chromatography Mass Spectroscopy; Antimigraine; Plasma; Zolmitriptan

Introduction

Migraine is a neurovascular disorder caused by severe headache, dysfunction of autonomic nervous system and in case of some patients, its involving neurological symptoms. The current treatment for migraine has some advantages, but it has certain limitations, therefore alternative treatment is required for minimal side effects. [1] Migraine triggers can be divided into two groups, one is internal (like hormonal fluctuations, stress, fasting, and sleep disturbance) and another is external (like weather, odour, alcohols and heat). [2-3] Out of these some factors triggers the premonitory symptoms of migraine, for example Photophobia, as a premonitory symptom, which is trigger by flickering or bright light. [4] All Antimigraine drugs are mainly selective serotonin receptor agonists and they work by narrowing blood vessels in the brain and stopping pain signals to the brain or stopping the release of certain natural substances that cause pain, nausea, and other symptoms of migraine. [5] Now a days Zolmitriptan is a triptan class of drug which is used for treatment of acute migraines. Zolmitriptan is a selective 5-hydroxytryptamine 1B/1D receptor agonist and it has weak affinity for the 5-HT 1A receptor sub-types. This activity can indicate Zolmitriptan as a valuable agent for the treatment of acute migraines. [6]

More recently, some newer triptans were administrated with as low doses and more sensitive liquid chromatography–tandem mass spectrometry (LC/MS/MS) methods were also developed to obtain full pharmacokinetic profiles. [7-11] The LCMS/ MS has high sensitivity and selectivity. Now a days, compare with other techniques the Mass spectrometry can achieve low limits of detection, hence Mass spectrometry is widely used for quantification of trace constituents in biological as well as environmental samples. [12] Therefore, to simplify plasma preparation procedure and reduce the time of analysis, a highly sensitive and rapid LC/MS/MS method was developed and validated to determine zolmitriptan in human plasma in the present study.

Materials and Methods

Pure Zolmitriptan (99.2% w/w on as is basis) was obtained from VARDA Biotech (P) Limited and Rizatriptan Benzoate (Internal standard, 99.5% w/w) obtained as gift samples from Natco Pharma Limited, India. Methanol and ethyl acetate were used as HPLC grade and obtained from Merck, Mumbai, India. The chemicals, ammonium acetate and sodium citrate were of analytical grade purchased from S. D. Fine Chemicals. High purity water was prepared through a Milli-Q water purification system. Blank sodium citrate pooled plasma was stored at -80° prior to use.

Instrumentation and chromatographic conditions

The liquid chromatographic system Analyst Software version 1.4.2 – LC-Shimadzu LC10 from Shimadzu (BE/LC/03) – MS/MS (API 3200) from SCIEX (BE/MS/06) equipped with binary solvent manager, column manager, auto sampler with turbo spray positive polarity detector. The separation of the compounds was made on a Chromolith® Speed ROD RP-18e 50 -4.6 mm column. The sample was analyzed using Methanol: Buffer Solution: 60:40, v/v as mobile phase and filtered through 0.45 µ membrane filters before use and degassed in an ultrasonic bath. All analysis was performed under isocratic condition at a flow rate of 0.500 ml/ minute without splitter (Binary flow) at ambient temperature and the sample volume injected was 5 µl with run time of 2.5 min.

Preparation of reagents and quality control

A mixture of methanol and buffer solution in the ratio 60:40, v/v was prepared. The mobile phase solution was mixed well, sonicated and degassed in an ultrasonicator bath. The mobile phase solution was stored at room temperature and the solution was used within 7 days from the date of preparation.

Preparation of stock solution

A stock solution of Drug (approximately 1 mg/ml) was prepared in diluent solution. The prepared stock concentration was corrected with respect to its potency on as is basis and the actual amount weighed. The stock solution was stored at a temperature 2-8 °C in refrigerator. The stock solution was used within 11 days from the date of its preparation. The further dilutions from the stock solutions were prepared using diluent solution (Methanol: Milli Q water: 40:60, v/v) for spiking in plasma to obtain calibration curve (CC) standards and quality control (QC) samples.

A stock solution of Rizatriptan (approximately 1 mg/ml) was prepared in diluent solution. The prepared stock concentration was corrected with respect to its potency on as is basis,

molecular weight and the actual amount weighed. The stock solution was stored at a temperature 2-8 °C in refrigerator. The stock solution was used within 11 days from the date of its preparation. The stock solution of internal standard was diluted to suitable concentration of (approximately 1000.00 ng/ml) using diluent solution (Methanol: Milli Q water: 40:60, v/v).

Calibration Curve Standard Dilutions were prepared the for-calibration curve standards by using diluent solution (Methanol:Milli-Q Water = 40:60 v/v) from the above prepared calibration curve stock solution. Spiked Calibration Curve Standards were prepared by transferring 5% of aqueous Calibration Curve Standard dilution (0.5 ml of stock dilution for 10 ml of spiking volume) in pooled plasma to achieve the desired concentration of calibration curve standards.

Bio-analytical method

A set of calibration curve standards and/or quality control samples were withdrawn from the deep freezer and allowed them to thaw at room temperature. 25 µl of Rizatriptan as an internal standard dilution (approximately 1000 ng/ml) was added into ria vials. 600 ml of plasma from the pre-labelled polypropylene tubes was aliquoted and vortexed. 50 ml of 5% formic acid solution was added and vortexed. Then 400 µl of Milli- Q water was added and vortex.

The samples were processed using Solid Phase Extraction Method, given below.

Solid phase extraction method

Step 1: Conditioned the HLB cartridge (30 mg/1cc) with 1.0 ml methanol followed by 1.0 ml of Milli-Q water. Step 2: Loaded the samples. Step 3: Washed the cartridges with 1 ml Milli-Q grade water. Step 4: Washed the cartridges with 1 ml of washing solution. Dried the cartridges for approximately 3 minutes. Step 5: Eluted the samples with 1 ml elution solution. Step 6: Evaporated the eluate to dryness at 40 °C & at constant pressure in nitrogen evaporator. Step 7: Reconstituted the samples in 200 µl mobile phase and transferred into HPLC vials for analysis.

Procedure for method validation

Selectivity-Plasma samples from a minimum of six different batches were screened. Aqueous mixture of analyte (s) and internal standard (IS) was prepared and injected to check the retention time (RT) and mass transition (in case of LC-MS/MS) of all peak of interest using proposed chromatographic conditions. Blank matrix from at least six different batches and LLOQ (Lower Limit of quantization) spiked singly in each batch using proposed extraction procedure was prepared. The interference at the RT of an analyte was evaluated by comparing the response in the blank matrix against the mean response of the extracted LLOQ. The interference at the IS RT was evaluated by comparing the response in the blank matrix against the mean response of the extracted internal standard.

Sensitivity

The lowest standard was accepted as the lower limit of quantification of the method, if the between batch precision at the LLOQ is ≤ 20% and accuracy is 80-120%.

Matrix effect

The effect of any co extracted materials on the assay was determined by the extraction and analysis of replicate (n = 6) aliquots of the matrix, spiked after extraction with analyte (low, and high QC concentrations) and the internal standard. The detector responses for analyte and the internal standard for the replicate samples was compared with those of replicate (n=6) non-extracted samples prepared at the same concentrations.

Linearity

Linearity was assessed by analyzing a minimum of three analytical batches and back- calculating the concentrations of injected calibration curve standards. A regression equation with a suitable weighting factor was used for determining the concentration/detector response relationship.

Precision and accuracy

Both within and between run precision and accuracy was performed by a minimum of three validation batches. Each batch consisted of the containing the following samples.

- Aqueous mixture (with the Internal Standard, if applicable)
- Standard blank matrix in duplicate (Blank)
- Standard Zero sample in duplicate (Blank Internal Standard)
- Spiked calibration curve standards (at least 6 non-zero concentrations)
- Carry over blank-1
- Carry over blank-2,
- Six Low Limit of Quantitization samples
- Six Low QC Concentration samples
- Six High QC Concentration samples
- Six Mean (Medium) QC Concentration samples

QC sample concentrations were calculated from the respective calibration curve. The mean concentration, standard deviation, coefficient of variation (%precision) and percent nominal (accuracy) values for all calibration standards and at each LLOQ, Low, Medium, High QC concentration levels were calculated. 'Within Batch Precision and Accuracy' was determined by calculating%CV and%Nominal, respectively at each QC concentration level of an analytical batch. 'Between Batch/Inter Day Precision and Accuracy' was determined by calculating%CV and%Nominal respectively, at each QC concentration level of all analytical batches.

Recovery

Six sets each of quality control samples (low, middle and high) were withdrawn from deep freezer and allowed to thaw. Quality control samples were extracted and injected. Six sets each of unextracted quality control comparison samples (Low, Middle and High) were prepared as given below.

Solid phase extraction

Aqueous recovery comparison samples were prepared taking 25 µl aqueous dilution of Zolmitriptan (LQC, MQC and HQC respectively), 25 µl of Internal standard dilution and 250 µl of mobile phase (representing 100% extraction). The aqueous comparison samples (LQC, MQC and HQC) were compared against 6 sets of each extracted samples of LQC, MQC and HQC. Recovery of internal standard was also compared at LQC, MQC and HQC levels. The absolute percent recovery of analyte and internal standard for each spiked quality control samples were determined. The mean percent recovery, standard deviation (SD) and coefficient of variation (%CV) for each concentration level of the quality control samples were calculated. Finally, the overall recovery, SD and % CV was calculated.

Freeze and thaw stability

The freeze and thaw stability in matrix was assessed by assaying freshly prepared calibration standards samples against six replicates of QC samples at low and high concentrations previously frozen and thawed over multiple cycles. Freeze and thaw cycle consists of first freezing at or below -50°C for 24 hours and other cycles for a minimum of 12 hours followed by thawing unassisted at room temperature. The freeze and thaw stability was evaluated at the end of first and third cycle. The QC concentrations were tabulated and the mean concentration, SD,%CV and%nominal values was determined at low and high QC levels.

Long-term stability in matrix

Long-term stability evaluations were performed following a period of storage that equals or exceeds the period of time between the date of first sample collection and the date of last sample analysis. 28 days stability was performed. Following an appropriate storage period, six replicates of the stored low and high concentration of QC samples were removed from the freezer and permitted to thaw. Concurrent with or prior to the removal of the QC samples from the freezer storage, calibration standards were freshly prepared by spiking in the appropriate matrix lot. The samples were processed. The long-term stability duration was calculated as the difference between the date of analysis of QC samples and the date of preparation of QC samples (in days). The concentration of long-term stored QC samples was read against the freshly spiked calibration curve. The QC concentrations were tabulated and the mean concentration, SD,% CV,% nominal values of samples at low and high QC values was determined.

Dilution integrity

Six quality control samples for dilution integrity were prepared by spiking approximately 8.5 times (214.366 ng/

ml) of ULOQ of drug. Six-dilution integrity samples were processed by diluting all the samples by 10 times using pooled plasma. These quality control samples were analyzed against a calibration curve standard. The quality control sample concentrations were calculated using 10 times dilution factor. Results demonstrated acceptable dilution integrity for 10 times dilution. Six sets each of quality control samples (low, middle and high) were withdrawn from deep freezer and allowed to thaw. Quality control samples were extracted and injected. Six sets each of un-extracted quality control comparison samples (Low, Middle and High) were prepared.

Result and Discussion

Selectivity

The selectivity of the method was demonstrated by comparing chromatograms of independent plasma samples from volunteers, each as a blank sample and a spiked sample. Figure 2 indicates no significant interference at the retention times of the analyte and IS. Retention time of Zolmitriptan & Rizatriptan both have approximately 1.5 minutes with the injection volume of 25 µl and the Flow Rate is 0.500 ml/minute, which makes the process more economic. The short analysis time may meet the requirement for high sample throughout in bio-analysis.

Linearity of calibration curve

It was observed that the optimized methods were linear within a specific concentration range for Zolmitriptan. The calibration curves were plotted between response factor and concentration of the standard solutions and the linearity was found to be from 0.201 ng/ml to 14.873 ng/ml for Zolmitriptan with regression coefficients (R^2) of 0.9981 in Table 1 and Figure 1.

	Concentration ng/ml								Slope	Intercept	r- value	r^2- value
CCID MV107/ZOL (CC 2)	**STD A**	**STD B**	**STD C**	**STD D**	**STD E**	**STD F**	**STD G**	**STD H**				
Nominal	0.201	0.402	0.803	1.487	2.975	5.949	11.898	14.873				
Observed Concentration	0.201	0.385	0.888	1.386	3.016	6.060	10.985	15.548	0.0364	0.00239	0.9974	0.9948
% Nominal	100.00	95.77	110.59	93.21	101.38	101.87	92.33	104.54	-	-	-	-

Table 1: Calibration Curve Data of Zolmitriptan for Sensitivity.

Figure 1: Representative Calibration Curve of Zolmitriptan for Regression Analysis.

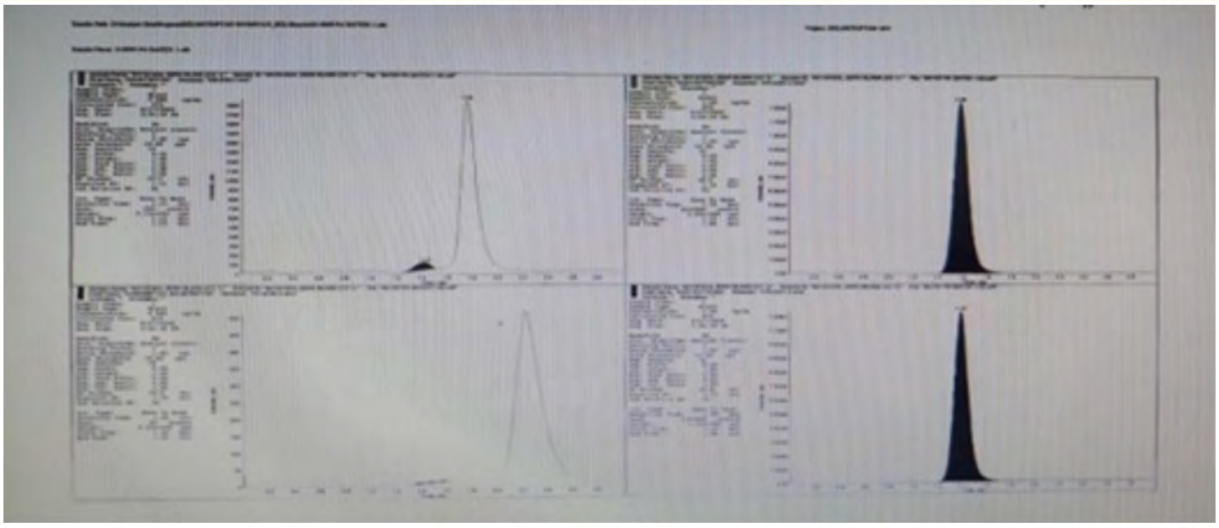

Figure 2: Representative Chromatogram of Calibration Curve of Zolmitriptan for Regression Analysis.

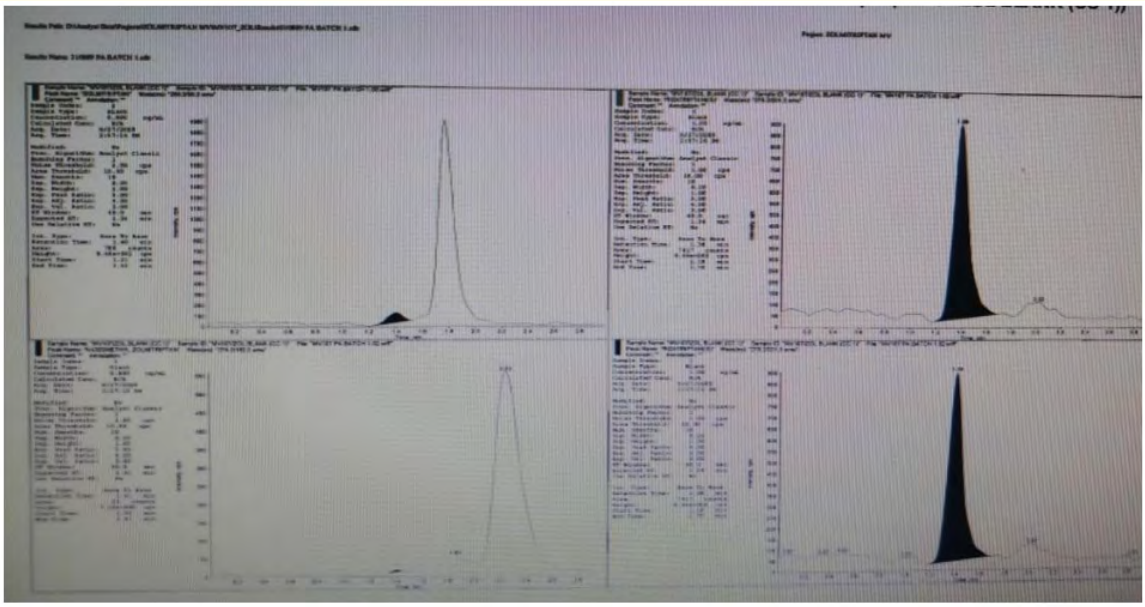

Figure 3: Representative Chromatogram of Calibration Curve of Zolmitriptan for Regression Analysis.

Assay precision and accuracy

Inter day Precision ranged was found to be 4.56% (HQC) to 7.32% (LLOQ QC) and Accuracy (Table 2, 3) ranged was found to be from 97.51% (LLOQ QC) to 106.02% (LQC). Whereas, Intra-day Precision ranged was found to be from 3.70% (HQC) to 11.55% (LLOQ QC) and Accuracy ranged was found to be from 96.69% (LQC) to 102.82% (HQC). The results indicated that there was no significant inter-day and Intra-day variability of slopes and intercepts over the optimized concentration range.

Day- 1 27/ AUG/2009	QC ID MV107/ ZOL LLOQ QC/LQC/ MQC/HQC	LLOQ QC		LQC		MQC		HQC	
Batch		0.201 ng/ml		0.574 ng/ml		7.177 ng/ml		11.961 ng/ml	
		Observed Concentration	% Nominal	Observed Concentration	% Nominal	Observed Concentration	% Nominal	Observed Concentration	% Nominal
PA Batch 1	1	0.189	94.03	0.651	113.41	7.554	105.25	12.300	102.83
	2	0.219	108.96	0.665	115.85	7.131	99.36	13.129	109.77
	3	0.200	99.50	0.577	100.52	6.567	91.50	13.115	109.65
	4	0.194	96.52	0.660	114.98	7.538	105.03	12.499	104.50
	5	0.188	93.53	0.608	105.92	7.170	99.90	13.286	111.08
	6	0.214	106.47	0.599	104.36	6.520	90.85	13.051	109.11
	N	6	-	6	-	6	-	6	-
	Mean	0.2007	-	0.6267	-	7.0800	-	12.8967	-
	SD	0.01308	-	0.03675	-	0.45209	-	0.39779	-
	%CV	6.52	-	5.86	-	6.39	-	3.08	-
	Mean% Nominal	99.83	-	109.18	-	98.65	-	107.82	-
Day- 1 27/ AUG/2009									
PA Batch 2	7	0.180	89.55	0.618	107.67	6.476	90.23	13.111	109.61
	8	0.178	88.56	0.601	104.70	7.594	105.81	12.365	103.38
	9	0.187	93.03	0.588	102.44	7.201	100.33	13.357	111.67
	10	0.179	89.05	0.602	104.88	6.735	93.84	13.177	110.17
	11	0.167	83.08	0.601	104.70	7.671	106.88	12.396	103.64
	12	0.197	98.01	0.598	104.18	7.042	98.12	13.216	110.49
	N	6	-	6	-	6	-	6	-
	Mean	0.1813	-	0.6013	-	7.1198	-	12.9370	-
	SD	0.01001	-	0.00967	-	0.46987	-	0.43863	-
	%CV	5.52	-	1.61	-	6.60	-	3.39	-
	% Nominal	90.22	-	104.76	-	99.20	-	108.16	-

Table 2: Precision and Accuracy Batch Quality Control Data for Zolmitriptan.

Day- 2 28/ AUG/2009	QC ID MV107/ ZOL LLOQ QC/LQC/ MQC/HQC	LLOQ QC		LQC		MQC		HQC	
Batch		0.201 ng/ml		0.574 ng/ml		7.177 ng/ml		11.961 ng/ml	
		Observed Concentration	% Nominal	Observed Concentration	% Nominal	Observed Concentration	% Nominal	Observed Concentration	% Nominal
PA Batch 3	13	0.210	104.48	0.624	108.71	7.390	102.97	12.432	103.94
	14	0.208	103.48	0.626	109.06	7.299	101.70	11.446	95.69
	15	0.208	103.48	0.569	99.13	7.458	103.92	12.149	101.57
	16	0.204	101.49	0.622	108.36	7.445	103.73	12.401	103.68
	17	0.209	103.98	0.600	104.53	7.272	101.32	11.651	97.41
	18	0.197	98.01	0.545	94.95	7.424	103.44	12.177	101.81
	N	6	-	6	-	6	-	6	-
	Mean	0.2060	-	0.5977	-	7.3813	-	12.0427	-
	SD	0.00486	-	0.03373	-	0.07818	-	0.40466	-
	% CV	2.36	-	5.64	-	1.06	-	3.36	-
	% Nominal	102.49	-	104.12	-	102.85	-	100.68	-

Table 3: Precision and Accuracy Batch Quality Control Data for Zolmitriptan.

Freeze thaw stability and dilution integrity

The freeze thaw stability (Table 4, 5) of Drug is percent nominal ranged was found to be from 107.67% (LQC) to 108.24% (HQC) and precision ranged was found to be from 2.51% (HQC) to 4.76% (LQC) for Drug. The long-term stability (Table 6-7) the percent nominal ranged was found to be from 106.54% (HQC) to 109.67% (LQC) and precision ranged was found to be from 3.87% (LQC) to 5.57% (HQC) for Drug. Six quality control samples for dilution integrity were prepared by spiking approximately 8 times (118.616 ng/ml) and (97.512 ng/ml) of ULOQ concentration of Zolmitriptan. The within batch precision and accuracy with a dilution factor of 10 for Zolmitriptan (Table 8) was 5.29% and 98.13%, respectively.

04/SEP/2009	MV107/ZOL FS LQC (1-6)		MV107/ZOL LQC (25-30)		Correction Factor
Nominal	0.572 ng/ml		0.574 ng/ml		1.00
	Observed Conc.	% Nominal	Observed Conc.	% Nominal	Corrected Concentration of FTQC
Freeze Thaw Stability	0.593	103.67	0.664	115.68	0.662
	0.598	104.55	0.594	103.48	0.592
	0.602	105.24	0.610	106.27	0.608
	0.556	97.20	0.613	106.79	0.611
	0.665	116.26	0.641	111.67	0.639
	0.622	108.74	0.586	102.09	0.584
Count	6	-	6	-	6
Mean	0.6060	-	0.6180	-	0.6158
SD	0.03602	-	0.02944	-	0.02934
% CV	5.94	-	4.76	-	4.76
Mean% Nominal	105.94	-	107.67	-	-
% Change	-1.61				

Table 4: Freeze Thaw Stability of LQC for Zolmitriptan.

Correction Factor: Concentration of Fresh QC/ Concentration of FT QC Corrected Concentration of FT QC= Concentration of FT QC x Correction Factor.

% Change= (Mean of QC Fresh QC -Mean of corrected QC FT QC) / Mean of (Mean of QC Fresh QC and Mean of corrected QC FT QC)*100.

04/SEP/2009	MV107/ZOL FS HQC (1-6)		MV107/ZOL HQC (25-30)		Correction Factor
Nominal	11.922 ng/ml		11.961 ng/ml		1.00
	Observed Con	% Nominal	Observed Con	% Nominal	Corrected Concentration of FT QC
Freeze Thaw Stability	11.813	99.09	12.920	108.02	12.878
	14.149	118.68	13.180	110.19	13.137
	12.635	105.98	13.460	112.53	13.416
	13.062	109.56	12.798	107.00	12.756
	12.643	106.05	12.551	104.93	12.510
	12.135	101.79	12.767	106.74	12.725
Count	6	-	6	-	6
Mean	12.7395	-	12.9460	-	12.9038
SD	0.81667	-	0.32545	-	0.32439
% CV	6.41	-	2.51	-	2.51
Mean % Nominal	106.86	-	108.24	-	-

Table 5: Freeze Thaw Stability of HQC for Zolmitriptan.

04/SEP/2009	MV107/ZOL FS LQC (1-6)		MV107/ZOL LQC (63-68)		Correction Factor
Nominal	0.572 ng/ml		0.574 ng/ml		1.00
	Observed Conc.	% Nominal	Observed Conc.	% Nominal	Corrected Concentration of LT QC
Long Term Stability - 86 °C	0.593	103.67	0.662	115.33	0.660
	0.598	104.55	0.635	110.63	0.633
	0.602	105.24	0.650	113.24	0.648
	0.556	97.20	0.599	104.36	0.597
	0.665	116.26	0.624	108.71	0.622
	0.622	108.74	0.607	105.75	0.605
Count	6	-	6	-	6
Mean	0.6060	-	0.6295	-	0.6273
SD	0.03602	-	0.02439	-	0.02430
% CV	5.94	-	3.87	-	3.87
Mean% Nominal	105.94	-	109.67	-	-
% Change	-3.46				

Table 6: Long Term Stability – 86 °C of LQC for Zolmitriptan (9 days).

04/SEP/2009	MV107/ZOL FS LQC (1-6)		MV107/ZOL HQC (63-68)		Correction Factor	
Nominal	11.922 ng/ml		11.961 ng/ml		1.00	
	Observed Conc.	% Nominal	Observed Conc.	% Nominal	Corrected Concentration of LT QC	
Long Term Stability - 86 °C	11.813	99.09	12.923	108.04	12.881	
	14.149	118.68	13.076	109.32	13.033	
	12.635	105.98	13.528	113.10	13.484	
	13.062	109.56	12.871	107.61	12.829	
	12.643	106.05	12.637	105.65	12.596	
Count		12.135	101.79	11.426	95.53	11.389
6		-	6	-	6	
Mean		-	12.7435	-	12.7019	
12.7395						
SD		-	0.71016	-	0.70784	
0.81667		-	5.57	-	5.57	
% CV						
6.41		-	106.54	-	-	
Mean% Nominal						
106.86						
% Change		0.30				

Table 7: Long Term Stability – 86 °C of HQC for Zolmitriptan (9 days).

	(DF 10 Times)	
QC ID MV107/ZOL DI	118.616 ng/ml	
	Observed Concentration	% Nominal
1	110.492	93.15
2	121.991	102.85
3	109.880	92.64
4	120.880	101.91
5	112.194	94.59
6	122.977	103.68
N	6	-
Mean	116.4023	-
SD	6.15941	-
% CV	5.29	-
% Nominal	98.13	-

Table 8: Dilution Integrity for Zolmitriptan.

The all above observation demonstrated that the developed and validated methods have adequate sensitivity and no significant changes of the parameters were observed even with changing the experimental conditions like instruments, source of reagents and column type and optimized conditions such as pH, mobile phase ratio and flow rate etc.

Conclusions

The developed and validated LC/MS/MS method is a sensitive, accurate, precise, reproducible and selective for the determination of Zolmitriptan in human plasma with a run time less than 3 min and with a narrow linearity range. The LLOQ of this method was found to be 0.201 ng/ml which proved that the method is highly in sensitivity and speed of analysis in compare to other reported methods. This analytical technique can be applied as a regular method for the support to the pharmacokinetic study as well as pharmaceutical applications.

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