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Journal of Chromatography B, 813 (2004) 227–233
JOURNAL OF CHROMATOGRAPHY B
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Quantification of zolmitriptan in plasma by high-performance liquid chromatography–electrospray ionization mass spectrometry

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Received 10 July 2004; accepted 22 September 2004
Available online 22 October 2004

Abstract

A sensitive and specific liquid chromatography electrospray ionization mass spectrometry (LC–ESI–MS) method has been developed and validated for the identification and quantification of zolmitriptan in human plasma. After the addition of the internal standard (IS) and 1.0 M sodium hydroxide solution, plasma samples were extracted with methylene chloride:ethyl acetate mixture (20:80, v/v). The organic layer was evaporated under a stream of nitrogen at 40 °C. The residue was reconstituted with 100 μl mobile phase. The compounds were separated on a prepacked Lichrospher CN (5 μm, 150 mm × 2.0 mm) column using a mixture of methanol:water (10 mM NH_4AC, pH 4.0) = 78:22 as mobile phase. Detection was performed on a single quadrupole mass spectrometer by selected ion monitoring (SIM) mode via electrospray ionization (ESI) source. The method was proved to be sensitive and specific by testing six different plasma batches. Linearity was established for the range of concentrations 0.30–16.0 ng/ml with a coefficient of determination (r) of 0.9998 and good back-calculated accuracy and precision. The intra- and inter-day precision (R.S.D.%) were lower than 15% and accuracy ranged from 85 to 115%. The lower limit of quantification was identifiable and reproducible at 0.30 ng/ml. The proposed method enables the unambiguous identification and quantification of zolmitriptan for pharmacokinetic, bioavailability or bioequivalence studies.

Keywords: Zolmitriptan; HPLC–ESI–MS; Human plasma; Pharmacokinetics

1. Introduction

Zolmitriptan (s)-4-[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]methyl]-2-oxazolidinone (Zomig, formerly311C90) is a new 5-hydroxytryptamine (5-HT) 1B/1D receptor agonist developed for the acute oral treatment of migraine. Clinical studies have shown it to be effective and well tolerated. Zolmitriptan inhibits the peripheral trigeminovasular system and is able to access central sites in the brainstem involved in processing cranial pain. In the previously studies, Seaber et al. [1] developed a HPLC method to assay zolmitriptan and its three major metabolites with fluorescence detection and Clement and Franklin [2] established a HPLC method to quantification of zolmitriptan and its two major metaboltes with coulometric detection, the limit of quantification in plasma of the two method were both 2.0 ng/ml which was insufficiently sensitive to enable full pharmacokinetics profiling of zolmitriptan. Therefore, a more sensitive liquid chromatography–mass spectrometry (LC–MS–MS) method [3] was developed to assay zolmitriptan and its active metabolite 183C91. But the necessary of tandem mass spectrometry system was a restriction in terms of cost and general applicability. Vishwanathan et al. [4] also developed a HPLC–ESI–MS/MS method to determination of antimigraine compounds rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum, but the LLOQ of zolmitriptan was only 1 ng/ml. Recently Chen et al. [5] reported a HPLC method with fluorescence detection to analysis zolmitriptan in human plasma, the method established could quantify zolmitriptan with a LLOQ of 0.2 ng/ml. In this paper, we describes a more simple, selective and highly sensitive method

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doi:10.1016/j.jchromb.2004.09.043

by using high performance liquid chromatography coupled with electrosprsy ionization (ESI) single quadrupole mass spectrometry (MS) for the determination of zolmitriptan in human plasma.

2. Experimental

2.1. Chemicals and reagents

Zolmitriptan test tablets (batch No: 010725) and capsules (batch No: 010806), zolmitriptan reference standard (99.7% purity) and rizatriptan benzoate reference standard (99.3% purity) were identified and supplied by Nanjing Zechen S&T Development Company (Nanjing, PR China); zolmitriptan reference tablets were purchased from Astrazeneca Pharmaceuticals (LP Wilmington, DE 19850, batch No: NBD85); methanol was chromatographic pure grade and purchased from Merck (Merck Company, Germany). Other chemicals were all of analytical grade. Deionized water was distilled before using. Other reagents were used as received.

2.2. Instrumentation and operating conditions

Liquid chromatography was performed using a Shimadzu LC-10AD HPLC system consisting of an autosampler (SIL-HTc). The HPLC was coupled to a Shimadzu LCMS-2010A quadrupole mass spectrometer with an electrospray ionization (ESI) interface. Data acquisition and processing were accomplished using Shimadzu LCMS solution Software for LCMS-2010A system.

Chromatographic separation was carried out with Lichrospher CN (5 μm, 150 mm × 2.0 mm) column at 40 °C. The mobile phase consisted of methanol:water (10 mM NH_4AC, pH was adjusted to 4.0 by acetic acid) = 78:22 was set at a flow rate of 0.2 ml/min. The ESI source was set at positive ionization mode. The $[M + H]^+$, *m/z*, 288.10 for zolmitriptan and $[M + H]^+$, *m/z*, 270.10 for rizatriptan were selected as detecting ions, respectively. The quantification was performed via peak-area. The MS operating conditions were optimized as follows: drying gas 1.5 l/min, CDL temperature 250 °C, block temperature 200 °C, probe voltage +4.5 kV.

2.3. Preparation of stock solutions

The stock solutions of zolmitriptan and rizatriptan benzoate (IS) were prepared after the correcting for purity.

The primary stock solutions of zolmitriptan was prepared by dissolving 10.0 mg of zolmitriptan in 10 ml solvent (methanol:water = 78:22, v/v) producing a concentration of 1.0 mg/ml and was stored at 4 °C.

The internal standard stock solution was prepared by dissolving 10.0 mg of rizatriptan benzoate in 10 ml solvent (methanol:water = 78:22, v/v) producing a concentration of 1.0 mg/ml and was stored at 4 °C. This solution was further diluted with the same solevnt to prepare the internal standard working solution containing 400 ng/ml of rizatriptan benzoate.

Working solutions of zolmitriptan were prepared daily in solvent (methanol: water = 78:22, v/v) by appropriate dilution of the stock solution at 10.0, 100.0 and 1000.0 ng/ml.

2.4. Calibration curves

Calibration curves were prepared by spiking different samples of 1 ml blank plasma each with proper volume of one of the above-mentioned working solutions to produce the calibration curve points equivalent to 0.30 ng/ml (30 μl × 10 ng/ml), 0.50 ng/ml (50 μl × 10 ng/ml), 1.0 ng/ml (10 μl × 100 ng/ml), 2.0 ng/ml (20 μl × 100 ng/ml), 4.0 ng/ml (40 μl × 100 ng/ml), 8.0 ng/ml (80 μl × 100 ng/ml) and 16.0 ng/ml (16 μl × 1000 ng/ml) of zolmitriptan. Each sample also contained 6.0 ng (15 μl × 400 ng/ml) of the internal standard. In each run, a plasma blank sample (no IS) was also analyzed. Calibration curves were prepared by determining the best-fit of peak area ratios (peak area of analyte/peak area of internal standard) versus concentration, and fitted to the equation $R = bx + a$ by unweighted least-squares regression.

2.5. Preparation of quality control samples

Quality control samples were prepared at three different concentration levels, low limit (0.50 ng/ml), middle level (2.0 ng/ml) and a high level (8.0 ng/ml). QC samples were prepared daily by spiking different samples of 1 ml plasma each with proper volume of the corresponding standard solution to produce a final concentration equivalent to 0.50, 2.0 and 8.0 ng/ml of zolmitriptan and 6.0 ng (15 μl × 400 ng/ml) of internal standard. The following procedures were the same as describe below.

2.6. Extraction procedure

QC, calibration curve and clinical plasma samples were extracted employing a liquid–liquid extraction technique. To each tube containing 1 ml plasma, 6.0 ng (15 μl × 400 ng/ml) of internal standard, 100 μl of 1.0 M sodium hydroxide solution and 5 ml methylene chloride:ethyl acetate mixture (20:80, v/v) were added and then were vortexed for 2 min. Afterwards, samples were centrifuged for 10 min at 4000 × *g*. The organic layer was evaporated under a stream of nitrogen at 40 °C. The residue was redissolved in 100 μl mobile phase. An aliquot of 10 μl was injected into the LC–MS system.

2.7. Method validation

The method validation assays were carried out according to the currently accepted US Food and Drug Administration

(FDA) bioanalytical method validation guidance [6]. The following parameters were considered.

The specificity of the method was tested by screening six different batches of healthy human blank plasma. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic/spectroscopic conditions and compared with those obtained with an aqueous solution of the analyte at a concentration near to the LLOQ.

The matrix effect on the ionization of analytes was evaluated by comparing the peak area of analytes resolved in blank sample (the final solution of blank plasma after extraction and reconstitution) with that resolved in mobile phase. Three different concentration levels of zolmitriptan (0.50, 2.0 and 8.0 ng/ml) and 400 ng/ml of internal standard were evaluated by analyzing five samples at each level. The blank plasma used in this study were six different batches of healthy human blank plasma. If the ratio <85 or >115%, an exogenous matrix effect was implied.

Linearity was tested for the range of concentrations 0.30–16.0 ng/ml. For the determination of linearity, standard calibration curves of at least seven points (non-zero standards) were used. In addition, blank plasma samples were also analyzed to confirm absence of interferences but they were not used to construct the calibration function. Four out of seven none zero standards including LLOQ and ULOQ were to meet the following acceptance criteria: no more than 20% deviation at LLOQ and no more than 15% deviation for standards above the LLOQ. The acceptance criteria for correlation coefficient was 0.998 or more, otherwise the calibration curve should be rejected. Five replicate analyses were done. The samples should been run low to high.

The intra-day precision and accuracy of the assay was measured by analyzing five spiked samples of zolmitriptan at each QC level (0.50, 2.0 and 8.0 ng/ml). The inter-day precision and accuracy was determined over five days by analyzing 15 QC samples. The acceptable precision and accuracy deviation values should be within 15% of the actual values.

The extraction yield (absolute recovery) was determined by comparing the zolmitriptan/IS peak area ratios obtained following the outlined extraction procedure with those obtained from those that contained the same amount of zolmitriptan in extracted plasma but not be extracted after addition of drug. This procedure was repeated for the three different concentrations of zolmitriptan added, namely 0.50, 2.0 and 8.0 ng/ml. The recovery of IS was also determined.

For sensitivity determination, the lowest standard concentration in the calibration curve was considered as the lower limit of quantification (LLOQ), and was to meet the following criteria: LLOQ response should be 10 times that of noise and be identifiable, discrete and reproducible within the precision deviation of 20%. Samples at the concentration 0.30 ng/ml were investigated as the lower limit of quantification and the reproducibility and precision were also determined.

Short-term temperature stability: Stored plasma aliquots were thawed and kept at room temperature for a period of time exceeded that expected to be encountered during the routine sample preparation (around 6 h). Samples were analyzed as mentioned above.

Post-preparative stability: The autosampler stability was conducted reanalyzing extracted QC samples kept under the autosampler conditions (4 °C) for 12 h.

Freeze and thaw stability: QC plasma samples containing zolmitriptan were tested after three freeze (−20 °C) and thaw (room temperature) cycles.

Long-term stability of zolmitriptan in human plasma was studied for a period of 10 days employing QC samples at three different levels. If after the stability study the analyte was found to be unstable at −20 °C, then it should be stored at −70 °C.

The stability of zolmitriptan and internal standard working solutions were evaluated by testing their validity for 6 h at room temperature. Stability of working solutions was expressed as percentage recovery.

A calibration curve was generated to assay samples in each analytical run and was used to calculate the concentration of zolmitriptan in the unknown samples in the run. The calibration was analyzed in the middle of each run. In order to monitor the accuracy and precision of the analytical method a number of QC samples were prepared to ensure that method continues to perform satisfactorily. The QC samples in duplicate at three concentrations (0.50, 2.0 and 8.0 ng/ml) were prepared and were analyzed with processed test samples at intervals based on the total number of samples per batch.

3. Clinical study design

This was an open randomized, balanced, three-period crossover study in 24 Chinese healthy men. Each volunteer received in random order, single oral dose of 5.0 mg zolmitriptan test tablets, test capsules or reference tablets in cycle. Blood samples (5 ml) for assay of plasma concentration of zolmitriptan were collected at the time of 0, 0.5, 1, 1.5, 2, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 12 and 14 h after oral administration of the medicals. They were put into lithium heparin tubes and immediately were centrifuged at 3000 × *g* for 10 min. The plasma obtained was frozen at −20 °C in coded polypropylene tubs until analysis.

4. Results and discussion

4.1. Selection of IS

It is necessary to use an IS to get high accuracy when a mass spectrometer is used as the HPLC detector. Rizatriptan benzoate [7] was adopted in the end because of its similarity of structure (Fig. 1), retention and ionization with the analyte and the less endogenous interferences at ritriptan $[M+H]^+$, *m*/*z* 270.10.

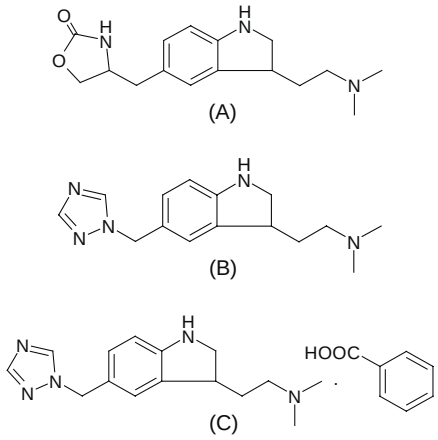

Fig. 1. Chemical structure of zolmitriptan (A), rizatriptan (B) and rizatriptan benzoate (C).

4.2. Sample preparation

Liquid–liquid extraction [8,9] was necessary and important because this technique cannot only purify but also concentrate the sample. Ethyl acetate and methylene chloride:ethyl acetate mixture (20:80, v/v) were all tested to do extraction and methylene chloride:ethyl acetate mixture (20:80, v/v) was finally adapted because of its high extraction efficiency. Sodium hydroxide (0.1 ml × 1.0 M) was added to the plasma in order to accelerate the drugs' dissociation from the plasma and reduce interference since most endogenous are of acidic nature.

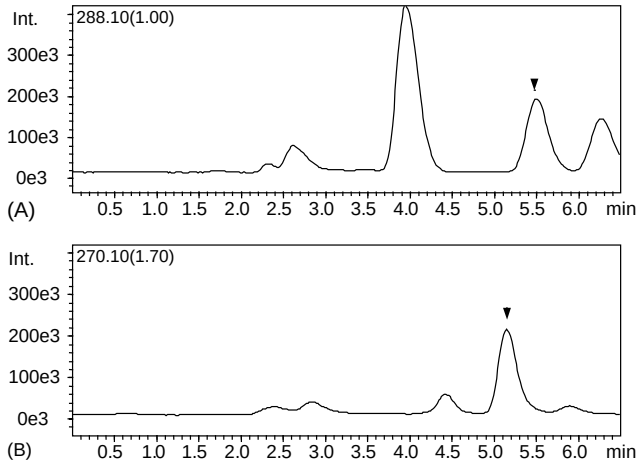

Fig. 4. The SIM(+) chromatograms extracted from supplemented plasma. Peaks were assigned with (▼). The retention times of zolmitriptan and the IS were 5.4 min (A) and 5.1 min (B), respectively.

4.3. Separation and specificity

Positive ion electrospray mass scan specta of zolmitriptan and IS are shown in Figs. 2 and 3, respectively. The major ions observed were $[M+H]^+$, $m/z = 288.10$; $[M+Na]^+$, $m/z = 310.10$; $[M+K]^+$, $m/z = 326.05$ for zolmitriptan and $[M+H]^+$, $m/z = 270.10$; $[M+Na]^+$, $m/z = 292.05$; $[M+K]^+$, $m/z = 308.05$ for rizatriptan. The ions of $[M+H]^+$, $m/z = 288.10$ for zolmitriptan and $[M+H]^+$, $m/z = 270.10$ for rizatriptan were selected for the SIM(+) due to their high stability and intensity.

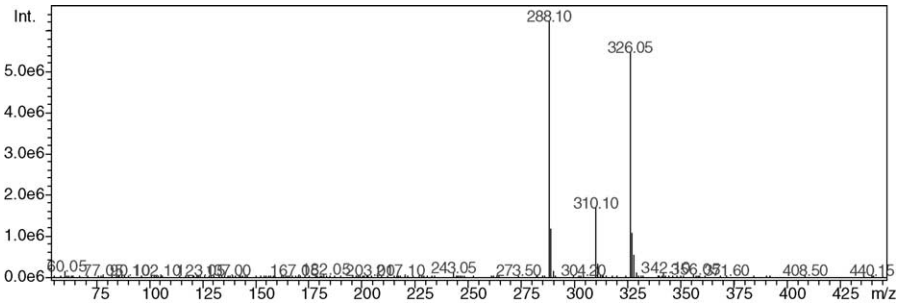

Fig. 2. Positive ion electrospray mass scan spectrum of zolmitriptan.

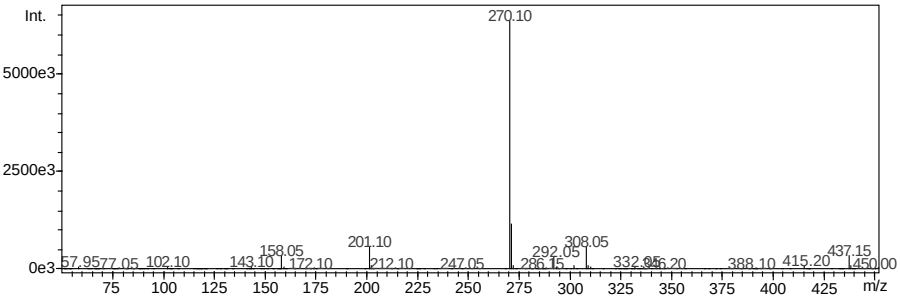

Fig. 3. Positive ion electrospray mass scan spectrum of rizatriptan.

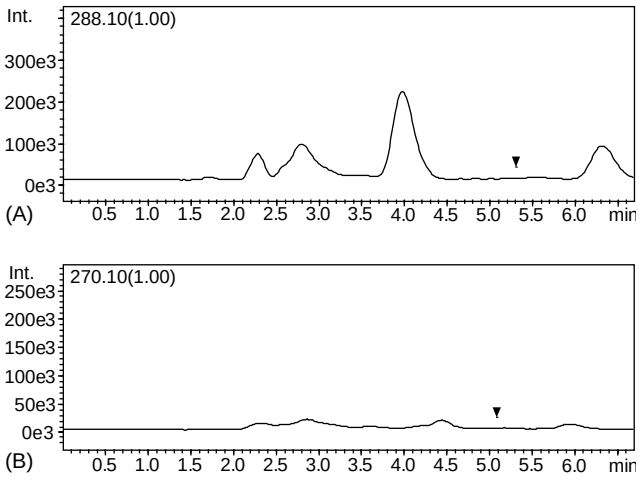

Fig. 5. The SIM(+) chromatogram for a blank plasma sample. Peaks were assigned with (▼). The retention times of zolmitriptan and the IS were 5.4 min (A) and 5.1 min (B), respectively.

The SIM(+) chromatograms extracted from supplemented plasma are depicted in Fig. 4. As shown, the retention times of zolmitriptan and the IS were 5.4 and 5.1 min, respectively.

The total HPLC–MS analysis time was 6.5 min per sample. No interferences of the analytes were observed because of the high selectivity of the SIM model. No ion suppression effects were observed under the developed sample preparation and chromatographic conditions. Fig. 5 shows an HPLC chromatogram for a blank plasma sample indicating no endogenous peaks at the retention positions of zolmitriptan or internal standard (rizatriptan). The SIM(+) chromatograms obtained from an extracted plasma sample of a healthy volunteer who participated in a bioequivalence study conducted on 24 persons are depicted in Fig. 6.

The purpose of the investigation was to develop a specific and sensitive procedure for the determination of zolmitriptan used as an antimigraine drug. HPLC–ESI–MS has several

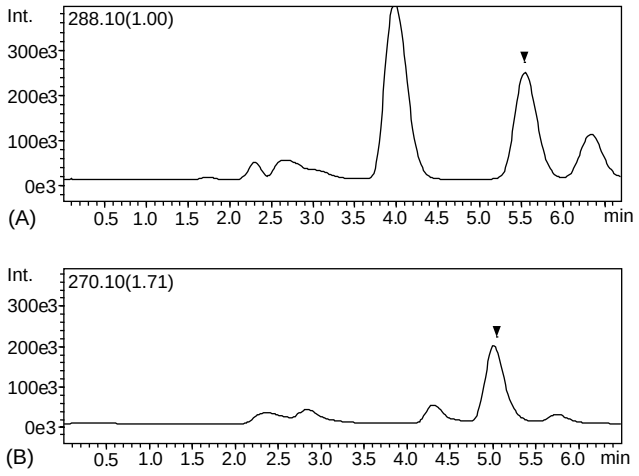

Fig. 6. The SIM(+) chromatogram for plasma sample of a healthy volunteer. Peaks were assigned with (▼). The retention times of zolmitriptan and the IS were 5.4 min (A) and 5.1 min (B), respectively.

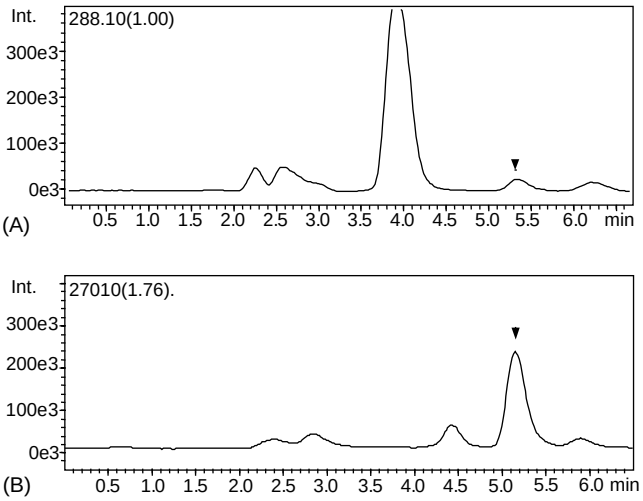

Fig. 7. The SIM(+) chromatogram of LLOQ (0.3 ng/ml). Peaks were assigned with (▼). The retention times of zolmitriptan and the IS were 5.4 min (A) and 5.1 min (B), respectively.

advantages for the analysis of zolmitriptan. The combination of HPLC (under the isocratic conditions described) with ESI–MS leads to short run time and yields both high selectivity and sensitivity. ESI is a "gentle" ionization technique that produces high mass-to-charge $[M+H]^+$ precursor ions with minimal fragmentation of the analyte.

4.4. Method validation

The method exhibited a good linear response for the range of concentrations from 0.30 to 16.0 ng/ml with a coefficient of determination of 0.9998. Results of five representative calibration curves for zolmitriptan LC–MS determination are given in Table 1.

Data for intra- and inter-day precision of the method for zolmitriptan as determined from the QC samples runs at the concentrations of 0.50, 2.0 and 8.0 ng/ml are presented in Table 2.

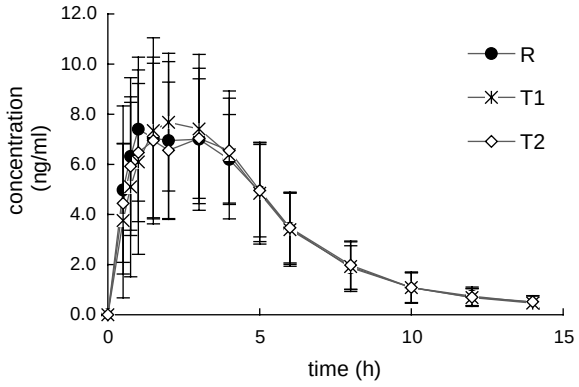

Fig. 8. Mean drug plasma concentration-time curve of zolmitriptan in 24 volunteers after oral administration (*R*: reference tablets; T1: test tablets; T2: test capsules).

Table 1
Results of five representative calibration curves for zolmitriptan LC–MS determination

Added concentration (ng/ml)	0.3	0.5	1.0	2.0	4.0	8.0	16.0
Back-caculated concentration	0.2728	0.5244	0.9534	2.1375	4.1057	7.5365	15.6968
	0.2806	0.5825	0.9587	2.0211	4.2869	7.5646	15.7410
	0.2811	0.5803	0.9983	2.0210	3.9631	7.9461	15.4225
	0.2768	0.5818	0.9761	2.1490	4.2270	7.4331	15.3881
	0.2803	0.567	1.0150	2.1411	4.0153	7.5841	15.3518
Mean	0.2783	0.5672	0.9803	2.0939	4.1196	7.6129	15.5200
R.S.D. (%)	1.26	4.36	2.67	3.18	3.33	2.56	1.18
Mean accuracy (%)	92.78	113.44	98.03	104.70	102.99	95.16	97.00

Table 2
The inter- and intra-day precision, accuracy of the method with determination of zolmitriptan ($n = 5$)

Added concentration (ng/ml)	Intra-day				Inter-day			
	Detected concentration (ng/ml)	Mean ± S.D. (ng/ml)	Mean accuracy (%)	R.S.D. (%)	Detected concentration (ng/ml)	Mean ± S.D. (ng/ml)	Mean accuracy (%)	R.S.D. (%)
0.5	0.4668	0.5172 ± 0.0449	103.44	8.68	0.4993	0.5130 ± 0.0468	102.26	9.12
	0.4895				0.5814			
	0.5106				0.4858			
	0.5832				0.5364			
	0.5361				0.4619			
2.0	2.0672	2.0588 ± 0.0481	102.29	4.41	1.9360	2.0782 ± 0.0981	103.91	4.72
	2.0268				2.1433			
	2.1455				2.0333			
	2.0740				2.1871			
	2.1279				2.0914			
8.0	8.3237	8.1475 ± 0.3412	101.8	4.19	7.4762	7.9112 ± 0.4122	98.89	5.21
	8.5747				7.6516			
	8.2119				8.4020			
	7.9285				8.2968			
	7.6986				7.7294			

Table 3
Recovery of zolmitriptan from plasma ($n = 5$)

Added (ng/ml)	Recovery (mean ± S.D.) (%)	R.S.D. (%)
0.5	91.62 ± 6.01	6.56
2.0	92.63 ± 3.65	3.94
8.0	91.49 ± 3.96	4.33

The lower limit of quantitationfor zolmitriptan was proved to be 0.30 ng/ml (LLOQ) and the lower limit of detection (LLOD) for zolmitriptan was 0.10 ng/ml. Fig. 7 shows the chromatogram of an extracted sample that contained 0.30 ng/ml (LLOQ) of zolmitriptan.

The extraction recovery determined for zolmitriptan was shown to be consistent, precise and reproducible. Data was shown below in Table 3. The extraction recovery of IS was more than 85%.

4.5. Stability

Table 4 summarizes the stability data of the short-term, freeze and thaw, long-term as well as post-preparative test of zolmitriptan.

Short-term stability indicated reliable stability behavior under the experimental conditions of the regular runs. The results of freeze and thaw stability indicated that the analyte

Table 4
Data showing stability of zolmitriptan in human plasma at different QC levels ($n = 5$)

	0.5 (ng/ml)		2.0 (ng/ml)		8.0 (ng/ml)	
	Recovery (mean ± S.D.) (%)	R.S.D. (%)	Recovery (mean ± S.D.) (%)	R.S.D. (%)	Recovery (mean ± S.D.) (%)	R.S.D. (%)
Short-term stability	94.08 ± 6.94	7.38	98.23 ± 4.05	4.12	96.63 ± 5.22	5.41
Freeze and thaw stability	90.03 ± 8.45	9.39	99.82 ± 3.98	3.99	92.08 ± 9.48	10.30
Long-term stability	87.58 ± 5.27	6.02	90.43 ± 2.39	2.64	88.51 ± 3.33	3.76
Post-preparative stability	98.91 ± 7.42	7.50	103.65 ± 11.26	10.86	94.94 ± 11.68	12.30

Table 5
Pharmacokinetic parameters of zolmitriptan in 20 men after oral administration

Parameters	T1	T2	*R*
$T_{1/2}$ (K/h)	2.85 ± 0.61	2.88 ± 0.38	2.92 ± 0.62
T_{max} (h)	2.3 ± 1.0	2.7 ± 1.2	2.2 ± 1.4
C_{max} (ng ml^{-1})	8.90 ± 3.23	8.42 ± 2.53	8.84 ± 2.86
$MRT_{0\rightarrow\infty}$ (h)	4.88 ± 0.67	4.97 ± 0.54	4.94 ± 0.72
$Auc_{0\rightarrow74}$ (ng h ml^{-1})	45.94 ± 17.56	45.73 ± 15.24	46.13 ± 15.41
$Auc_{0\rightarrow\infty}$ (ng h ml^{-1})	47.85 ± 18.22	47.91 ± 16.10	48.30 ± 15.93

was stable in human plasma for three cycles of freeze and thaw, when stored at −20 °C and thawed to room temperature. The post-preparative stability of QC samples shown that zolmitriptan was stable when kept at 4 °C in the autosampler for 12 h. The findings from long-term test indicate that storage of zolmitriptan's plasma samples at 20 °C is adequate when stored for 10 days and no stability-related problems would be expected during the samples routine analysis for the pharmacokinetic, bioavailability or bioequivalence studies.

The stability of working solutions was tested at room temperature. Based on the results obtained, these working solutions were stable within 6 h.

4.6. Results of pharmacokinetic study

The method was applied to analyze plasma samples obtained from 24 healthy volunteers which received a single dose of 5.0 mg zolmitriptan preparations each in the bioequivalence study. The procedure developed was sensitive enough to assure the quantitative analysis of zolmitriptan in plasma with acceptable accuracy over a period of 14 h after a single oral administration. The mean plasma concentration–time profiles of 24 volunteers is represented in Fig. 8. Pharmacokinetic parameters of the test tablets, test capsules and reference tablets are listed in Table 5. The test tablets and test capsule were found to be bioequivalent to the reference one.

5. Conclusion

The proposed method of analysis provided a sensitive and specific assay for zolmitriptan determination in human plasma. Simple liquid–liquid extraction procedure and short run time can curtail test's time that is important for large sample batches. It was shown that this method is suitable for the analysis of zolmitriptan in human plasma samples collected for pharmacokinetic, bioavailability or bioequivalence studies in humans.

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