

Determination of zolmitriptan in human plasma by liquid chromatography–tandem mass spectrometry method: Application to a pharmacokinetic study

Xiaoyan Chen^a, Dan Liu^b, Yan Luan^c, Fengdan Jin^b, Dafang Zhong^{a,b,*}

^a Center for Drug Metabolism and Pharmacokinetics Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 646 Songtao Road, Shanghai 201203, PR China

^b Laboratory of Drug Metabolism and Pharmacokinetics, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China

^c Shenyang Center of Disease Control and Prevention, 37 Qishanzhong Road, Shenyang 110031, PR China

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Abstract

A sensitive and selective liquid chromatography–tandem spectrometry method for the determination of zolmitriptan was developed and validated over the linearity range 0.05–30 ng/ml with 0.5 ml of plasma using diphenhydramine as the internal standard. Liquid–liquid extraction using a mixture of diethyl ether and dichloromethane was used to extract the drug and the internal standard from plasma. The mass spectrometer was operated under the selected reaction monitoring (SRM) mode using the atmospheric pressure chemical ionization (APCI) technique. The instrument parameters were optimized to obtain 3.0 min run time. The mobile phase consisted of acetonitrile–water–formic acid (70:30:0.5), at a flow rate of 0.5 ml/min. In positive mode, zolmitriptan produced a protonated precursor ion at m/z 288 and a corresponding product ion at m/z 58. And internal standard produced a protonated precursor ion at m/z 256 and a corresponding product ion at m/z 167. The inter- and intra-day precision (%R.S.D.) were less than 8.5% and accuracy (%error) was less than –2.5%. The method had a lower limit of quantification of 0.05 ng/ml for zolmitriptan, which offered increased sensitivity and selectivity of analysis, compared with existing methods. The method was successfully applied to a pharmacokinetic study of zolmitriptan after an oral administration of 5 mg zolmitriptan to 20 healthy volunteers.

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1. Introduction

Migraine is a chronic, often debilitating disease that affects 12% of the general population. A working definition of migraine is benign recurring headache and/or neurological dysfunction usually attended by pain-free interludes and often provoked by stereotyped stimuli [1,2]. The introduction of the triptans in the 1990s revolutionized the treatment of migraine, bringing migraine-specific pain relief to patients with this type of headache. The triptans are believed to exert their antimigraine effects via binding to 5-HT_{1B/1D} receptors on the trigeminal nerve and dural vasculature, where they act to reverse vasodilatation of blood vessels and also to reduce neurogenic inflammation [3–5].

Currently, there are seven triptans available on the market. Zolmitriptan is a second-generation triptan [5]. Clinical research indicates that it has a better efficacy and tolerability profile at low doses of 2.5–10 mg [6]. Zolmitriptan is rapidly absorbed when given as oral tablets both in the fasting state and when given with food, and the individual C_{max} values ranged from 2.7 to 9.9 ng/ml [7,8].

High-performance liquid chromatography has been widely used for the quantitative determination of triptans in biological fluids with UV [9], fluorescence [10–13], electrochemical or coulometric detection [14,15]. More recently, as lower doses were administered for newer triptans, more sensitive liquid chromatography–tandem mass spectrometry (LC/MS/MS) methods were also developed to obtain full pharmacokinetic profiles [16–20]. Vishwanathan et al. [17] reported a LC/MS/MS method to determine four triptans rizatriptan, zolmitriptan, naratriptan and sumatriptan in human serum, but the lower limit of quantification (LLOQ) of zolmitriptan was only 1.0 ng/ml.

* Corresponding author. Tel.: +86 2150800738; fax: +86 2150800738.
E-mail address: zhongdf@china.com (D. Zhong).

Zhang et al. [18] established a LC/MS method to analyze zolmitriptan in human plasma with a LLOQ of 0.3 ng/ml. In this assay method, a chromatographic run time of 6.5 min was necessary to avoid interference from endogenous substances. In addition, a variety of LC/MS/MS methods have also been employed to determine sumatriptan [19] or rizatriptan in plasma [20].

To simplify plasma preparation procedure and reduce analysis time, a highly sensitive and rapid LC/MS/MS method was developed and validated to determine zolmitriptan in human plasma in the present study. The method was successfully applied to evaluate the pharmacokinetics of zolmitriptan after oral administration of 5 mg zolmitriptan to 20 healthy volunteers.

2. Experimental

2.1. Materials

Zolmitriptan (99.7% purity) was kindly donated by Lunan Pharmaceutical Co. Ltd. (Shandong, China) and diphenhydramine hydrochloride (internal standard, 99.4% purity) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile (Kangkede Chemical, Tianjin, China) were of analytical grade, and other chemicals used were of analytical grade. Distilled water, prepared from demineralized water, was used throughout the study. Drug-free plasma for the preparation of calibration standards was obtained from Shenyang Blood Donor Service (Liaoning, China). Before analysis, the blank samples were analyzed by the present LC/MS/MS method. No significant peaks were observed at the retention times of the analyte and IS.

2.2. Instrumentation and LC/MS/MS conditions

A Thermo Finnigan Surveyor system (San Jose, CA, USA) consisting of a vacuum degasser, a tetranary pump and an autosampler was used for solvent and sample delivery. A Thermo Finnigan TSQ Quantum Discovery triple–quadrupole tandem mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source was used for mass analysis and detection. Data were acquired using Xcalibur 1.4 software, and quantitative processing was performed using LCQuan software (Thermo Finnigan, USA).

Chromatographic separation was achieved using a Zorbax SB C₁₈ column (150 mm × 4.6 mm i.d., 5 μm; Agilent, Wilmington, DE, USA) with a 4 mm × 3.0 mm i.d. SecurityGuard C₁₈ (5 μm) guard column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile–water–formic acid (70:30:0.5, v/v) at a flow rate of 0.7 ml/min. The chromatography was carried out at room temperature (22 °C).

The mass spectrometer was operated in the positive ion detection mode with the corona discharge current set at 4.0 μA. Nitrogen was used as the sheath gas (30 Arb) and auxiliary gas (5 Arb) for nebulization. The heated capillary and vaporizer temperatures were set at 320 and 420 °C, respectively. For collision-induced dissociation (CID), argon was used as

the collision gas at a pressure of 1.2 mTorr. Quantification was performed using selected reaction monitoring (SRM) of the transitions m/z 288 → 58 for zolmitriptan and m/z 256 → 167 for the IS, respectively, with a dwell time of 0.3 s per transition. The optimized collision energy of 20 eV was used for both the analyte and the IS.

2.3. Calibration standards

A stock solution of zolmitriptan with a concentration of 400 μg/ml was prepared by dissolving 10 mg of zolmitriptan in 25 ml of methanol. Eight standard solutions of 0.25, 0.5, 1.5, 5, 15, 40, 75 and 150 ng/ml of zolmitriptan were made by further dilution of the stock solution with appropriate volumes of methanol. A working internal standard solution was prepared by diluting the 400 μg/ml stock solution of diphenhydramine in methanol with water to provide a final concentration of 50 ng/ml. The concentration of the IS was calculated as free base. All the solutions were stored at 4 °C and were brought to room temperature before use. Structural formulae of zolmitriptan and the IS are shown in Fig. 1.

2.4. Sample preparation

A 100-μl aliquot of the IS solution (diphenhydramine, 50 ng/ml in water) and 100 μl of methanol were added to 500 μl

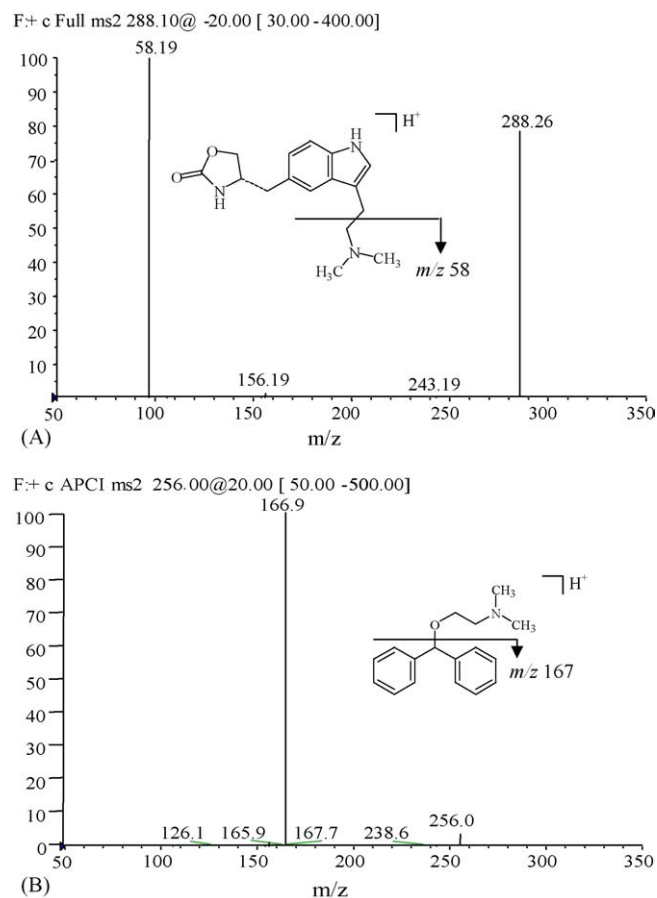


Fig. 1. Product ion mass spectra of [M+H]⁺ of zolmitriptan (A) and diphenhydramine (B).

of plasma samples, respectively. Then 100 μl of 1 M NaOH were added. The sample was vortex-mixed and extracted with 3 ml of diethyl ether–dichloromethane (2:1, v/v) by shaking for 10 min. The organic and aqueous phases were separated by centrifugation at $2000 \times g$ for 5 min. The upper organic phase was transferred to another tube and evaporated to dryness at 40°C under a gentle stream of air. The residue was reconstituted in 150 μl of the mobile phase and vortex-mixed for 1 min. A 20- μl volume of the reconstituted extract was injected onto the LC/MS/MS system by the auto-sampler for analysis.

To prepare the standard calibration samples, 100 μl of the IS solution (diphenhydramine, 50 ng/ml in water) and 100 μl of the standard working solutions were added to 500 μl blank human plasma. The following procedures were the same as described above. Calibration standards were prepared to achieve the final plasma concentrations of 0.05, 0.1, 0.3, 1.0, 3.0, 8.0, 15.0 and 30.0 ng/ml for zolmitriptan.

Quality control (QC) samples were prepared by adding zolmitriptan to drug-free plasma to make three concentrations of 0.1, 3.0 and 24.0 ng/ml. QC samples were stored at -20°C and extracted and analyzed during each analytical run. Additional standards were prepared for the determination of accuracy and precision ($n = 6$) of the method.

2.5. Application of the analytical method to a pharmacokinetic study in healthy volunteers

The LC/MS/MS procedure developed was used to determine zolmitriptan concentrations in plasma samples 0–24 h after an oral administration of 5 mg zolmitriptan tablet to 20 healthy male volunteers in a pharmacokinetic study approved by Ethic Committee. All volunteers gave written informed consent to participate in the study according to the principles of the Declaration of Helsinki. Blood (4 ml) was removed by venepuncture prior to dosage and at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h thereafter. Blood samples were collected into tubes containing sodium heparin as anticoagulant and spun at $2000 \times g$ in a centrifuge for 15 min. The plasma was separated and stored at -20°C until analysis.

The pharmacokinetic parameters of zolmitriptan were calculated by noncompartmental analysis. The maximum plasma concentrations (C_{max}) and their time of occurrence (T_{max}) were both obtained directly from the measured data. The area under the plasma concentration–time curve from time zero to the time of the last measurable concentration ($\text{AUC}_{0 \rightarrow t}$) was calculated by the linear trapezoidal method; the terminal elimination rate constant (k_e) was estimated by linear least-squares regression of the terminal portion of the plasma concentration–time curve, and the corresponding elimination half-life ($T_{1/2}$) was then calculated as $0.693/k_e$.

3. Results and discussion

3.1. Chromatography and mass spectrometry

The positive ionization mode was selected for the determination of zolmitriptan because of the presence of amino group,

which was easily protonated. The possibility of using electrospray ionization or atmospheric pressure chemical ionization sources under positive ion detection mode was evaluated during the early stage of method development. It was found that APCI could offer better linearity and reproducibility for the analyte than ESI. Therefore, APCI was chosen as ionization source in the experiment. By positive APCI mode, the analyte and internal standard formed predominantly protonated molecules $[\text{M} + \text{H}]^+$ at m/z 288 and m/z 256 in Q1 full scan mass spectra, respectively. The CID of the protonated molecule of zolmitriptan (m/z 288) resulted in the cleavage of the C–C bond in the side chain, producing the major product ion at m/z 58 $[\text{H}_2\text{C}=\text{N}(\text{CH}_3)_2]$. Other fragments showed relative intensities below 5% and are not likely to improve the sensitivity when used in SRM mode. As for the IS, the CID of the protonated molecule (m/z 256) resulted in the cleavage of the C–O bond, producing the major product ion at m/z 167 $[\text{H}^+\text{C}(\text{C}_6\text{H}_5)_2]$. Additional tuning of the APCI source and CID parameters for the transitions of m/z 288 \rightarrow 58 (zolmitriptan) and m/z 256 \rightarrow 167 (IS) further improved the sensitivity. Fig. 1 shows product ion spectra of $[\text{M} + \text{H}]^+$ ions of zolmitriptan and diphenhydramine.

The chromatographic conditions were optimized by flow injection analyses with mobile phases containing varying percentages of organic phase to achieve maximum peak responses and good reproducibility. It was found that using acetonitrile or methanol as organic additive in the mobile phase yielded similar responses, but the latter provided higher background noise in SRM mode, probably because of the small product ion of zolmitriptan. When we used methanol as organic additive in the mobile phase, the background noise was higher than 1×10^3 , whereas considerable lower background response (~ 10) was obtained when the mobile phase consisted of acetonitrile was used. The high organic solvent content shortened the chromatographic cycle time and the acidic modifier (formic acid) in the mobile phase improved sensitivity by promoting ionization of the analytes in the APCI source. A mobile phase consisting of acetonitrile–water–formic acid (70:30:0.5, v/v) was therefore chosen. With the selected chromatographic condition the chromatographic run time for each sample was completed within 3.0 min.

3.2. Preparation of plasma samples

Triptan derivatives all have basic groups substituted at the 3-position of indole ring, which makes the extraction of these compounds from alkalized biological fluids possible using different organic solvents. Zhang et al. [18] and Chen et al. [11] used methylene chloride–ethyl acetate (20:80) and methyl *t*-butyl ether to extract zolmitriptan from alkalized plasma by 1 M sodium hydroxide, respectively. Guo et al. [20] used ethyl acetate to extract rizatriptan and zolmitriptan (IS), and plasma samples were alkalized by 0.1 M sodium carbonate. Ge et al. [12] also introduced a LLE procedure using methyl *t*-butyl ether for determination of sumatriptan. These reported extraction methods could provide higher extraction recovery, but increased evaporation time due to higher boiling point solvents used. In addition, solid-phase extraction (SPE) procedures were also employed

widely to extract triptans from plasma samples in the literature [9,14–16]. The SPE methods required more than 1 ml of eluates (methanol or acetonitrile containing HCl solution or ammonia) to obtain sufficient extraction recovery. Based on our experience, large volume of eluent required a time-consuming concentration step, compared with organic extraction solvents (diethyl ether–dichloromethane, 2:1, v/v). In this experiment, the upper organic phase (3-ml of diethyl ether–dichloromethane) was evaporated to dryness at 40 °C within 8 min.

In this study, different LLE conditions were evaluated including organic extraction solvents and aqueous pH modifiers to reduce extraction time and improve recovery. Three organic extraction solvents were evaluated: diethyl ether, *n*-hexane–dichloromethane–2-propanol (20:10:1, v/v), diethyl ether–dichloromethane (2:1, v/v). The latter clearly yielded the highest recovery and the final solution was evaporated only within 8 min. According to the reported results, 1 M NaOH, 1 M NaCO₃ and phosphate buffer (pH 10) were tested during extraction procedure. No significant influence on recovery was observed, but the use of 1 M NaOH as the pH adjustment reagent could reduce unwanted interfering substances to a minimum.

3.3. Method validation

3.3.1. Selectivity and matrix effect

The selectivity of the method was demonstrated by comparing chromatograms of six independent plasma samples from volunteers, each as a blank sample and a spiked sample. Fig. 2 indicates no significant interferences at the retention times of the analyte and IS. The retention times for zolmitriptan and the IS were 2.38 and 2.49 min, respectively.

Since chromatographic conditions may cause co-elution of a number of endogenous compounds that are undetected by the MS/MS but may affect the ionization efficiency, the effect of matrix on the response of the analyte was also evaluated. To determine the possible influence of the matrix on the analysis, the response of six extracted spike matrix blank samples were compared to those of six analytical standards. No matrix effect was detected in the study.

3.3.2. Linearity of calibration curve and lower limit of quantification

Plasma samples were quantified using the ratio of the peak-area of zolmitriptan to that of IS as the assay response. To evaluate linearity, plasma calibration curves were prepared and assayed in triplicate on three consecutive days over the range of 0.05–30 ng/ml, encompassing the therapeutic range of this antimigraine drug. Calibration curves were calculated utilizing the peak-area ratio versus analyte concentration. The response was linear for zolmitriptan throughout this concentration range and the correlation coefficients (*r*) were greater than 0.99 for all standard curves using a $1/x^2$ weighted linear regression model. The typical equation was $y = -7.96 \times 10^{-4} + 0.03219x$ ($r^2 = 0.9944$). Accuracy and precision were assessed by determination of QC samples at three concentration levels on three different validation days. The accuracy was expressed by relative error (R.E.) and the precision by relative standard deviation

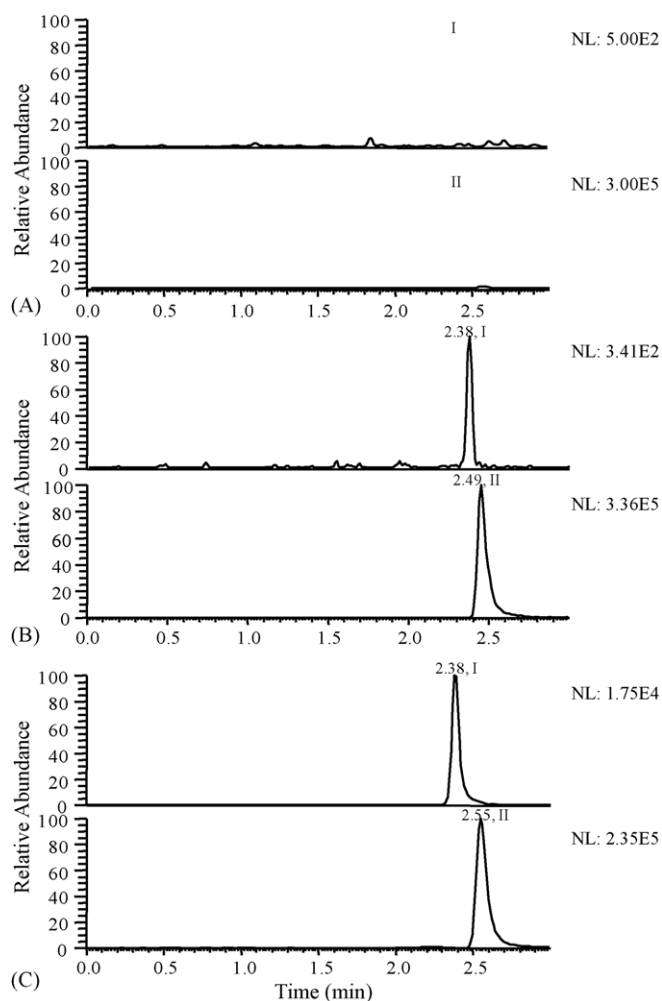


Fig. 2. Representative SRM chromatograms of zolmitriptan (I) and IS (II) in human plasma: (A) a blank plasma sample; (B) a blank plasma sample spiked with zolmitriptan (0.05 ng/ml) and IS (10 ng/ml); (C) plasma sample from a volunteer 8 h after an oral dose of 5 mg zolmitriptan.

(R.S.D.). During routine analysis each analytical run included a set of calibration samples, a set of QC samples in duplicate and unknowns.

The lower limit of quantification was 0.05 ng/ml, defined as the lowest concentration at which both the precision and accuracy were <20%. In this study, the precision and accuracy of LLOQ were <7.9% and within $\pm 0.5\%$, respectively.

3.3.3. Assay precision and accuracy

The method showed good precision and accuracy. Table 1 summarizes the intra- and inter-assay precision and accuracy for zolmitriptan from QC samples. The results were calculated using one-way analysis of variance (ANOVA). The intra- and inter-assay precisions were measured to be below 8.6% and 7.4%, respectively, with relative errors from -1.0% to -2.5% .

3.3.4. Extraction recovery and analyte stability

The extraction recoveries of zolmitriptan at three QC levels were determined by comparing peak-area ratios of the analyte to internal standard in samples that had been spiked with the ana-

Table 1
Summary of precision and accuracy of zolmitriptan from QC samples of human plasma extracts ($n = 3$ days, six replicates per day)

Added C (ng/ml)	Found C (ng/ml)	Intra-run R.S.D. (%)	Inter-run R.S.D. (%)	Relative error (%)
0.100	0.0998	8.6	2.9	-1.8
3.00	2.93	7.5	7.4	-2.5
24.0	23.8	7.3	5.3	-1.0

lyte prior to extraction with samples to which the analyte had been added post-extraction. The internal standard was added to both sets of samples post-extraction. The extraction recovery of the IS was determined in a similar way using the medium QC as a reference. The results showed that the extraction recoveries of zolmitriptan were $64.6 \pm 4.7\%$, $61.3 \pm 3.1\%$ and $64.7 \pm 6.3\%$ at concentrations of 0.1, 3.0 and 24 ng/ml, respectively. The extraction recovery of the IS was $77.5 \pm 3.2\%$.

The stability of zolmitriptan under the experimental conditions was investigated by comparison of the mean area ratios (ratios of the peak area of zolmitriptan to that of IS) obtained in each case with the mean area ratios from a control set of replicates (time zero). The concentration of the spiked plasma samples was 0.1 and 24 ng/ml. The number of replicates employed for each determination was three. Results were expressed for each concentration level as the percentage of the initial control concentration (C), which is referred to as 100%. Stability was studied for the spiked plasma after 2 h at room temperature (22°C), for up to three freeze/thaw cycles and for 3 months storage below -20°C . Stability under autosampler conditions was studied by reanalyzing the samples of the intra-day validation after 24 h at room temperature. Table 2 is the summary of stability studies for zolmitriptan.

3.4. Application of the analytical method in a pharmacokinetic study

After a single oral administration of 5 mg zolmitriptan to 20 healthy male subjects, plasma concentrations of zolmitriptan were determined by the described LC/MS/MS method. Fig. 3 shows mean plasma concentration–time curves of zolmitriptan after administration ($n = 20$).

After the oral administration, peak plasma concentrations were observed at about 3 h, and averaged 8.14 ng/ml. There was

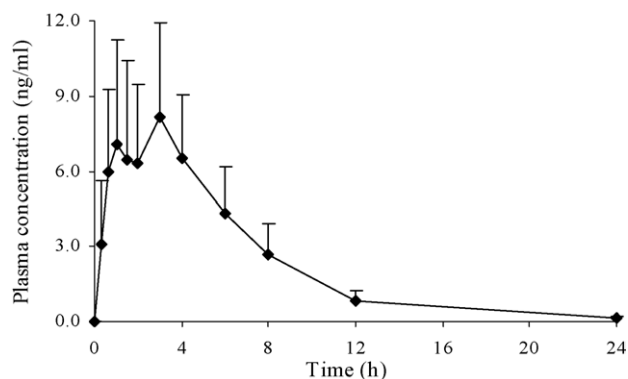


Fig. 3. Mean plasma concentration–time profile of zolmitriptan after an oral dose of 5 mg zolmitriptan to 20 healthy volunteers. Each point represents the mean \pm S.D.

at most four-fold variability in AUC and C_{\max} between subjects, which is in agreement with published pharmacokinetic studies [7,8,10]. The plasma concentration–time curves were generally best fit by monoexponential decay, in which $t_{1/2}$ averaged 3.5 h. The mean $AUC_{0 \rightarrow 24 \text{ h}}$ and $AUC_{0 \rightarrow \infty}$ values were 55.6 and 56.2 ng h/ml, respectively.

Individual profiles showed single or double peaks usually at 0.67 and 3 h with a clear elimination phase after 6 h. The T_{\max} could be at the time of the first or second peak, and hence is not a good reflection of the rate of absorption. The appearance of a second peak has also been observed in previous study [3], suggesting an erratic absorption. Compared with the pharmacokinetic results reported previously [3], it was observed that the value of $t_{1/2}$ in this study is a little longer, but that T_{\max} did not differ between them, which might be resulted from the different accuracies of the analytical methods. The high sensitivity of the present method allowed us to measure the plasma concentration 24 h after a single dose of 5 mg administration.

Table 2
Summary of stability studies of zolmitriptan in human plasma under various storage conditions ($n = 3$)

Storage conditions	Added C (ng/ml)	Found C (ng/ml)	R.S.D. (%)	Relative error (%)
Short-term (2 h at 22°)	0.100	0.0999	10.0	-0.3
	24.0	24.2	8.5	0.9
Long-term (30 days at -20°)	0.100	0.0996	9.4	-3.9
	24.0	21.9	3.9	-8.9
Three freeze/thaw cycles	0.100	0.101	12.8	5.0
	24.0	23.1	8.4	-3.7
Autosampler for 24 h (22°)	0.100	0.0995	7.5	-4.5
	24.0	23.3	9.1	-3.1

4. Conclusions

The described LC/MS/MS method is a sensitive, accurate and selective assay for the determination of zolmitriptan in human plasma with a chromatographic run time less than 3 min. The method only needed a simple liquid–liquid extraction for sample pretreatment. More than 150 samples could be assayed daily, including sample preparation, data acquisition and processing. The method had a LLOQ of 0.05 ng/ml and proved to be superior in sensitivity and speed of analysis in comparison to the reported methods. The analytical procedure was then applied as a routine method to support a pharmacokinetic study.

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