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Quantitative determination of azithromycin in human plasma by liquid chromatography–mass spectrometry and its application in a bioequivalence study

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Abstract

A sensitive, rapid liquid chromatographic–electrospray ionization mass spectrometric method for determination of azithromycin in human plasma was developed and validated. Azithromycin in plasma (0.2 mL) was extracted with methyl *tert*-butyl ether–hexane (50:50, v/v), organic phase was transferred to another clear 1.5 mL Eppendorf tube and evaporated to dryness at 40 °C and dissolved in mobile phase, samples were separated using a Thermo Hypersil HyPURITY C18 reversed-phase column (150 mm × 2.1 mm i.d., 5 μm), together with a mobile phase containing of 20 mM ammonium acetate (pH 5.2)–acetonitrile–methanol (50:40:10, v/v/v) and was isocratically eluted at a flow rate of 0.2 mL/min. Azithromycin and its internal standard, clarithromycin, were measured by electrospray ion source in positive selective ion monitoring mode. The method demonstrated that good linearity ranged from 2 to 1000 ng/mL with $r = 0.9977$. The limit of quantification for azithromycin in plasma was 2 ng/mL with good accuracy and precision. The higher mean extraction recovery, say 81.2% and 75.5% for azithromycin and internal standard (IS), respectively, was obtained in this work. The intra-day and inter-day precision ranged from 4.8% to 8.6% and 6.4% to 10.7% (R.S.D.), respectively. The established method has been successfully applied to bioequivalence study of 2 azithromycin formulations for 24 healthy volunteers.

Keywords: Azithromycin; LC–MS; Electrospray ionization; Bioequivalence

1. Introduction

Azithromycin is a semisynthetic macrolide antibiotic of the erythromycin group with a 15-membered azalactone ring. It has the chemical name (2*R*,3*S*,4*R*,5*R*,-8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-L-ribo-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-tride-oxy-3-(dimethylamino)-*b*-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one. Azithromycin is derived from erythromycin. However, it differs chemically from erythromycin in that a methyl-substituted nitrogen atom is incorporated into the lactone ring. Fig. 1 shows the chemical structure of azithromycin. Azithromycin seems to be more acid stable than erythromycin and therefore has greater oral bioavailability [1]. Azithromycin appears to bind to the same receptor as erythromycin and has a very high tissue-to-blood concentration ratio with a half-life of 2–4 days in most tissues, which may partly explain its outstanding antibiotic performance [2,3]. Azithromycin has been used in treatment of infections of the skin, the respiratory system, and sexually transmitted diseases [4,5]. It has also been demonstrated to be more effective than erythromycin in vitro against several respiratory pathogens, including *Legionella pneumophila*, *Haemophilus influenzae* and *Branhamella catarrhalis* [1,6]. Azithromycin represents a significant improvement in the treatment of selected community-acquired infections [7].

Generally, the determination of antibiotics, including macrolide antibiotics, is mainly carried out by microbiological

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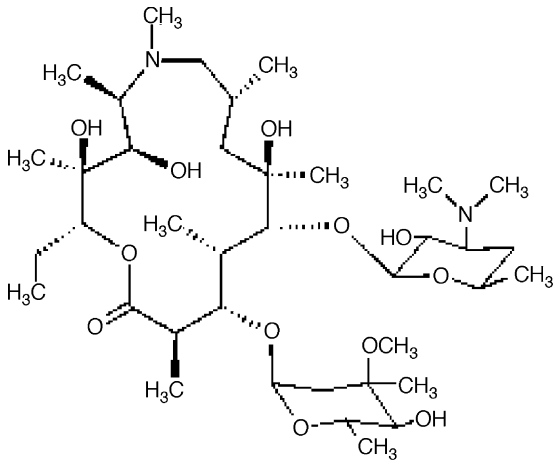

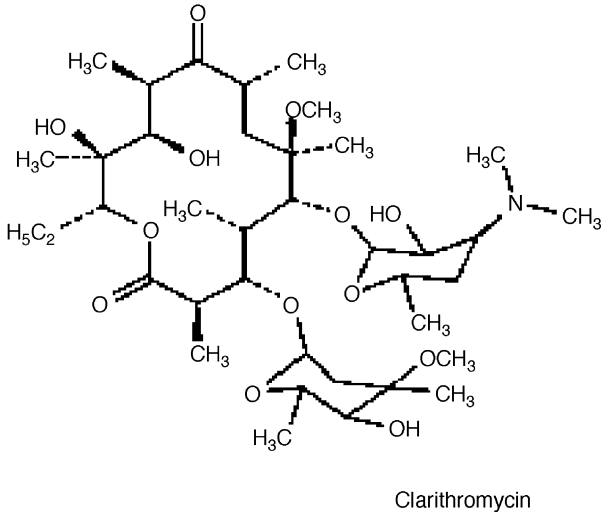

Fig. 1. Chemical structures of azithromycin and clarithromycin (IS).

assays in bioavailability and bioequivalence study [8–11]. However, the assays tended to lack specificity and their use involves difficulty in confirming what kinds of drugs remain in biological sample. In order to overcome these problems, chemical analyses such as high-performance liquid chromatographic (HPLC) techniques with UV spectrophotometer detection [12,13], electrochemical detection [14–21], and fluorescence detection after derivatization [22–25], have been used for the determination of azithromycin. However, azithromycin does not have a specific UV chromophore. UV detection is only of low sensitivity for determination of azithromycin in biological matrix and fluorescence detection requires complex sample pretreatment involving pre-column derivatization of the analyte. Thus, liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) seem to be the most promising technique for separation and quantitative analysis of drugs and have recently been used in the determination of azithromycin [26–30]. Koch et al. [26] determined azithromycin in the wastewater using LC/MS/MS with basic mobile phase (pH 10.8). Ten millilitres of sample was extracted in their method, which was not suitable to the analysis of biological sample. Bioavailability of azithromycin was also studied by HPLC–APCI–MS, in which the mobile phase was not adjusted with acetic acid and the limit of quantification of 5 ng/mL was accomplished by using 0.5 mL plasma [27]. Barrett et al. used ultrafilter to pretreat 0.5 mL of human plasma and the limit of quantification of 2.55 ng/mL was obtained by LC–MS/MS [29]. Nirogi et al. used solid-phase extraction to prepare 0.5 mL of human plasma and the limit of quantification of 5 ng/mL was achieved by LC/MS/MS [30]. It is worthy noting that the pH values of mobile phases reported so far were all higher than 6.6 in the published LC–MS and LC–MS/MS methods. Since azithromycin is more acid stable than erythromycin [1], it seems possible for us to further investigate the experimental conditions to see if we could detect the double protonated molecule $[M+2H]^{2+}$ directly. If so, it will be possible for us to find a much simple and rapid analytical method with higher sensitivity for bioavailability study.

The aim of the work reported in this paper was to develop and validate a simple, highly sensitive and selective LC–MS method for quantitative analysis of azithromycin in human plasma based on acidic mobile phases. The results obtained in this investigation showed that the acidic mobile phase was really suitable for LC–MS method and double charged ion (*m*/*z* 375) was directly detected with higher sensitivity in the method. The established method was successfully applied to the bioavailability study with satisfactory results.

2. Experimental

2.1. Reagents and chemicals

Azithromycin (purity > 99.5%) reference standard and clarithromycin (internal standard, IS, purity > 98.9%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). Acetonitrile was supplied by Caledon Laboratories Ltd. (Georgetown, Ont., Canada). HPLC grade methanol was obtained from TEDIA company, Inc. (Fairfield, OH, USA). Other reagents were of analytical grade, and all water used was Milli-Q grade.

2.2. Equipment

The HPLC system consisted of a Shimadzu LC-10Advp pump, an SCL-10Advp system controller, a CTO-10Avp column oven, an FCV-10ALvp low pressure gradient unit, a DGU-14A degasser (Shimadzu, Kyoto, Japan). The mass spectrometer was an LCMS-2010 single quadrupole equipped with electrospray ionization interface (Shimadzu, Kyoto, Japan). The data was collected and processed using LCMSsolution software. The DZF-6020 Model vacuum drier (Shanghai, China) was used in the preparation of samples.

2.3. Chromatographic conditions

Chromatographic separations were performed using a Thermo Hypersil HyPURITY C18 (150 mm × 2.1 mm i.d.,

5 μm) analytical column. The oven temperature was set at 40 °C. The mobile phase consisted of 20 mM ammonium acetate (adjusted pH to 5.2 with acetic acid)–acetonitrile–methanol (50:40:10, v/v/v) and was isocratically eluted at a flow rate of 0.2 mL/min.

2.4. Mass spectrometer conditions

An LCMS-2010 quadrupole mass spectrometer was interfaced with electrospray ionization (ESI) probe. The temperatures were maintained at 250, 250 and 200 °C for the probe, CDL and block, respectively. The voltages were set at 4.5 kV, −30 V, 25 V, 150 V, and 1.5 k V for the probe, CDL, Q-array 1, 2, 3 bias, Q-array RF and detector, respectively. The flow rate of nebulizer gas and dried gas were set at 1.5 and 10 L/min, respectively. The ions of selection monitoring were decided by positive scanning from *m/z* 100 to 1000. For the quantification of Azithromycin, the positive protonated molecule ions of azithromycin at $m/z = 375$ $[M + 2H]^{2+}$ and clarithromycin (IS) at $m/z = 749$ $[M + H]^{+}$ were monitored. Tuning of mass spectrometer was performed with the help of autotuning function of LCMSsolution software (Version 2.02) using tuning standard solution (polypropylene glycol). Optimization and calibration of mass spectrometer were achieved with autotuning.

2.5. Analytical procedure

2.5.1. Preparation of stock solutions, calibration standard and quality control samples

A stock solution of azithromycin at concentration of 100 μg/mL was prepared in methanol. The internal standard (clarithromycin) was also prepared as a stock solution (125 μg/mL) in methanol and was further diluted with methanol to give a concentration of 500 ng/mL and used for all analyses. A serial calibration curve samples at concentration of 2, 5, 10, 50, 100, 200, 500 and 1000 ng/mL of azithromycin were freshly prepared by serially diluting stock solution with drug-free plasma. Firstly, plasma sample contained 1000 ng/mL of azithromycin was prepared by spiking with azithromycin stock solution to drug-free plasma. Then the prepared plasma sample contained 1000 ng/mL of azithromycin was serially diluted with drug-free plasma to form calibration samples. The quality control (QC) samples were prepared by in the same way at concentrations of 2 ng/mL (LLOQ), 5 ng/mL (low), 50 ng/mL (medium) and 800 ng/mL (high) of azithromycin. All plasma samples were stored at −20 °C. Fifty microliters of IS (500 ng/mL) was added to 0.2 mL of calibration curve samples and quality control samples, respectively. The further processing both calibration curve samples and quality control samples were the same as described in the following section (Section 2.5.2.) for collection and preparation of the samples. All standard stock solutions were prepared once a month and stored at −20 °C.

2.5.2. Collection and preparation of the samples

Twenty-four male healthy volunteers received the investigation. The average age of volunteers was 29 years old within the range of 26–35. The mean of body weights was 70.4 kg (63–75.5 kg) and the mean of body heights was 172.5 cm (165–177 cm). Subjects were included based on their medical history, clinical examination results and routine laboratory test results. All eligible subjects provided written informed consent for participation in the study. A 2 × 2, crossover, randomized, open-label design was used. Subjects were randomly assigned to receive reference formulation followed by test formulation with a 2-week washout period between doses. After a 12-h (overnight) fast, subjects received a single, 500-mg oral dose (tablet) of azithromycin with 200 mL of water. Blood samples were collected in heparinized tubes pre-dose (0 h) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120 h post-dose. Plasma was immediately separated by centrifugation at 4000 rpm and stored −20 °C until analysis. A plasma sample (0.2 mL) was placed in a 2 mL Eppendorf tube. After the addition of 50 μL of a 500 ng/mL solution of internal standard and 50 μL of solution of saturated sodium carbonate, the tube was briefly vortexed and 1 mL of methyl *tert*-butyl ether–hexane (50:50, v/v) was added into the tube. After vortexing for 3 min, the tube was centrifuged at 14,000 rpm for 5 min at room temperature and the organic phase was transferred to another clear 1.5 mL Eppendorf tube. The extract was evaporated to dryness in a vacuum drier at 40 °C. The residue was redissolved in 200 μL of mobile phase, vortexed for 30 s and centrifuged at 14,000 rpm for 5 min, and 5 μL of supernatant was injected onto the analytical column.

3. Results and discussion

3.1. Mass spectrometry

Azithromycin consists of a 15-membered macrocyclic lactone ring onto which 2 sugar moieties are linked. An aminosugar, D-desosamine, is attached through a β-glycosidic bond to the C5 position of the lactone ring. A neutral sugar, L-cladinose, is attached via a α-glycosidic linkage to the C3 position of the lactone ring. The molecule of azithromycin contains 2 nitrogen atoms, 1 locating the 15-membered ring, and the other attaching to the sugar. These two nitrogen atoms could be simultaneously protonated easily during ionization process in the acidic condition. In the present study, an appropriate ionization mode in LC–MS analysis was firstly selected and then azithromycin and clarithromycin (IS) were scanned with ESI and APCI positive and negative ion modes using injection standard solutions. In different ionization modes, the base peak intensity of positive ion was higher than those of negative ion, and the efficiency of ionization in ESI was higher than APCI. The molecular ions with an *m/z* 375 $[M + 2H]^{2+}$ and *m/z* 750 $[M + H]^{+}$ for azithromycin were produced. The ion *m/z* 375 was more sensitive than *m/z* 750. The ion *m/z* 375 was not found in the published LC–MS and LC–MS/MS methods [26–30] because higher pH mobile phase was used. Clarithromycin (IS) molecule was protonated to form molecule ion with *m/z* 749 $[M + H]^{+}$ because of one nitrogen in the molecule. Fig. 2 showed the positive ion mass spectra of azithromycin and clarithromycin (IS) by ESI scanning from *m/z* 100 to 1000. Selected ion monitoring was chosen for the method because of its high sensitivity and high selectivity.

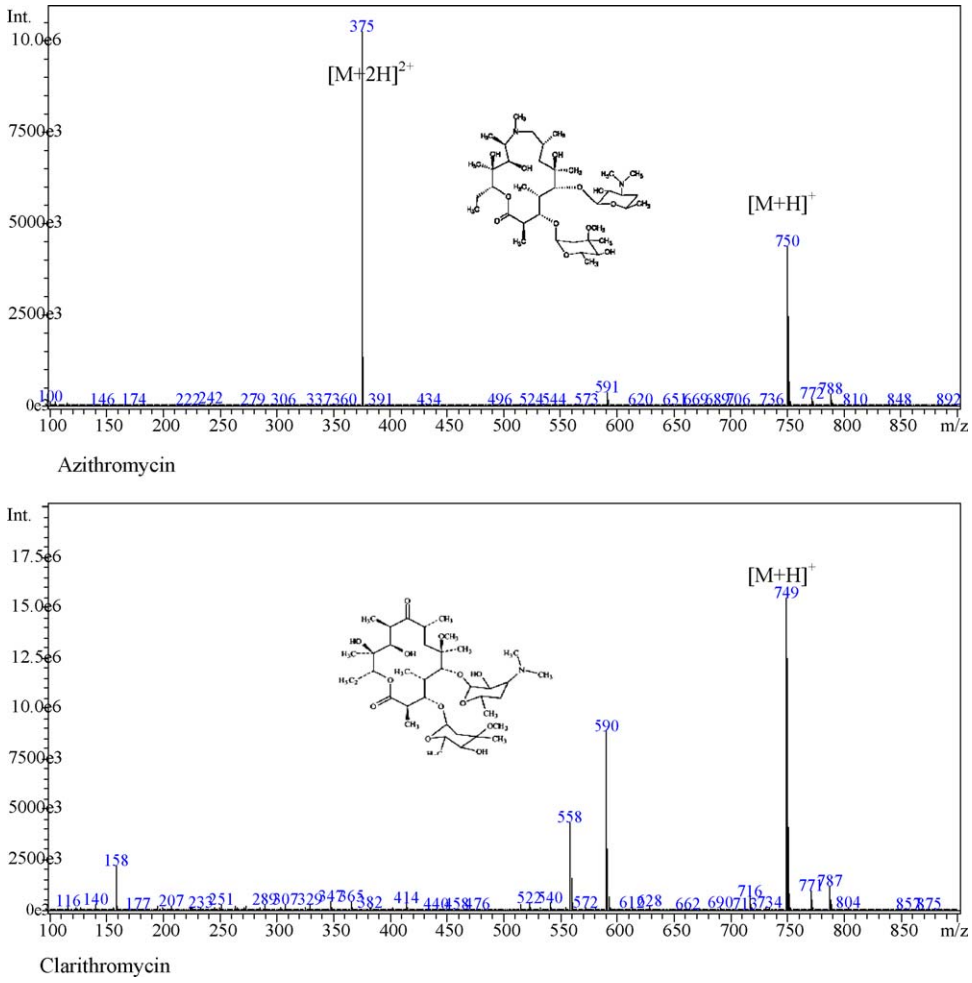

Fig. 2. ESI–MS positive ion scanning spectra of azithromycin and clarithromycin (IS). Chromatographic conditions: column: thermo Hypersil HyPURITY C18 (150 mm × 2.1 mm, 5 μm), oven temperature: 40 °C, mobile phase: 20 mM ammonium acetate (adjusted pH to 5.2 with acetic acid)–acetonitrile–methanol (50:40:10, v/v/v), flow rate: 0.2 mL/min. Mass spectrometer: electrospray ionization (ESI) source; positive scan *m/z*: 100–1000; temperature, probe: 250 °C, CDL: 250 °C, block: 200 °C; voltage, capillary: 4.5 kV, CDL: −30 V, Q-array 1, 2, 3 bias: 25 V, Q-array RF: 150 V, detector: 1.5 kV. Nebulizer gas flow rate: 1.5 L/min.

3.2. Selection of HPLC conditions

Azithromycin is a lipophilic compound with basic property. Two different types of column (Thermo Hypersil HyPURITY C18 and Shim-Pack ODS) were tested to obtain optimized response of suitable retention time and good peak shapes for azithromycin and clarithromycin. The Thermo Hypersil HyPURITY C18 column was selected for all the analyses since it provided symmetrical peak shape and the highest intensity to azithromycin. The separation and ionization of azithromycin and clarithromycin were affected by compose of mobile phase. The mobile phase pH affected not only the retention time, but also the ionization efficiency of azithromycin and clarithromycin. The retention time prolonged with the increasing of mobile phase pH, especially azithromycin. The acidity of mobile phase benefited to the ionization of azithromycin and clarithromycin. Thus, the sensitivity of azithromycin was improved by increasing acidity of mobile phase because of raising the ionization efficiency. In this study, we also observed that increasing the concentration of ammonium acetate in mobile phase would prolong the retention time of analyte under the same organic solvent percentage. Thus, increasing the concentration of ammonium acetate in mobile phase resulted in the higher percentage of organic solvent to maintain the same retention time for azithromycin and clarithromycin. Increasing the proportion of methanol could increase the ionization efficiency of azithromycin and the peak shape was also improved by acetonitrile.

3.3. Validation of the method

3.3.1. Matrix effect and extraction recovery

Three sets of eight calibration standards and blank spiked with internal standard were prepared for evaluation of recovery and ionization suppression or enhancement. The standard solutions were diluted with mobile phase to reach the concentration of 2, 5, 10, 50, 100, 200, 500 and 1000 ng/mL to construct the first set of calibration standards. Set 2 consisted of eight plasma samples spiked with standard solutions after extraction to the same concentration series as set 1. Plasma samples spiked with standard solution before extraction and S0 (blank plasma spiked with internal standard) were processed and analyzed in order to obtain the third set of calibration standards. Three replicates of

Table 1
Recovery and matrix effects of azithromycin and clarithromycin extraction from human plasma

Nominal concentration (ng/mL)	Mean peak area			ME (%)	RE (%)
	Set 1	Set 2	Set 3		
2	39542	38761	31927	98.0	82.4
5	103175	97532	83637	94.5	85.6
10	417634	402713	327829	96.4	81.5
50	1027545	1029374	853748	100.2	82.9
100	2176543	2086352	1684738	95.9	80.8
200	3976324	3887623	3023878	97.8	77.8
500	9954304	10328323	8137462	103.8	78.8
1000	20256785	19873625	15827463	98.1	79.6
S0 (IS)	584652	573649	432839	98.1	75.5

each set were conducted for determination of recovery and absolute matrix effect. Internal standard was not added to standards. The matrix effect (ME), i.e. the possibility of suppression or enhancement of ionization and the effect of the matrix on recovery (RE), were evaluated by comparing results from analysis of three sets of samples as follows:

$$\mathrm{ME}\,(\%) = B/A \times 100, \qquad \mathrm{RE}\,(\%) = C/B \times 100$$

where A is the mean peak area of set 1, B the mean peak area of set 2 and C is the mean peak area of set 3.

By comparing peak areas of standard and IS of samples spiked with standards after extraction with the analogous peak areas obtained by injecting neat standard and IS directly, the extent of the absolute matrix effect was estimated (Table 1). The values >100% indicated ionization enhancement in plasma versus neat standards, whereas values <100% indicated ionization suppression. The data presented in Table 1 indicated that the mean "absolute" matrix effect for azithromycin and clarithromycin (98.08% and 98.12%, respectively) was negligible. In addition, the coefficients of variation (CVs, %) of the mean peak areas of azithromycin at eight calibration standard concentrations and clarithromycin in five different plasma lots were small (<11%, Table 2), strongly indicating little or no difference in ionization efficiency and consistent recovery of standard and internal standard from different plasma lots. Direct precipitation protein with acetonitrile, perchloric acid or trichloroacetic acid was used for processing plasma samples and obtained low recovery. Various liquid–liquid extraction methods were investigated for the extraction of azithromycin from plasma. Comparison of extraction efficiency of different organic solvents including diethyl ether, ethyl acetate, methyl *tert*-butyl ether, hexane and dichloromethane, showed that methyl *tert*-butyl ether–hexane (50:50, v/v) gave the highest recoveries (81.2% and 75.5%) for azithromycin and the IS (Table 1) in this work. The other organic solvents used gave lower extraction recoveries (data was not shown). The pH of plasma sample was adjusted to basic (pH 10–11) with the saturate solution of sodium carbonate during extraction. Barrett et al. [29] applied ultra-filtration method to process sample and significant matrix effect was observed. Nirogi et al. [30] selected solid-phase extraction (SPE) for the sample preparation and obtained extraction recovery of 71.1%. In our study, the recoveries of azithromycin and IS were calculated by comparing the areas obtained from spiked blank plasma ($n = 5$) with those obtained from injecting directly standard solutions with the same concentrations in mobile phase. The mean extraction recoveries were higher than 81% and 75% for azithromycin and IS, respectively.

Table 2
Intermediate precision, accuracy and linear regression parameters of azithromycin determination in human plasma

Added concentration (ng/mL)	Mean measured concentration ($n = 5$) (ng/mL)	Precision (R.S.D., %)	Mean relative error[a] (%)
2.0	2.0	10.9	2.0
5.0	4.9	7.3	2.6
10.0	9.7	5.6	3.4
50.0	48.9	6.5	2.1
100.0	104.3	4.6	4.3
200.0	192.6	5.8	3.7
500.0	488.3	5.5	2.3
1000.0	985.3	4.9	1.5

Calibration curve: slope 0.0337; intercept −0.1734; correlation coefficient: 0.9977.

[a] Mean relative error = |mean measured concentration − added concentration| × 100/added concentration.

3.3.2. Selectivity

Six lots of blank plasma extracts from different sources were analyzed. Interference peaks from endogenous substances in free-drug human plasma at the retention time of azithromycin and clarithromycin were not observed in any of the plasma lots. In addition, azithromycin and clarithromycin were separately injected and selective ions were monitored. Fig. 3(A) showed one of representative chromatogram of six lots of blank plasma extracts. Fig. 3(B) showed the selective ion chromatogram of the plasma sample at the lowest limit of detection (0.5 ng/mL). No cross-talk was observed.

3.3.3. Sensitivity and linearity

The limit of quantitation (LOQ) using 0.2 mL plasma with acceptable accuracy and precision (<20%) is 2 ng/mL. A good signal-to-noise ratio was observed at the LOQ indicating that the corresponding value could be reached. The lowest limit of detection (LLOD) was estimated as the amount of azithromycin that gave a signal three times the noise (S/N ≥ 3); it was

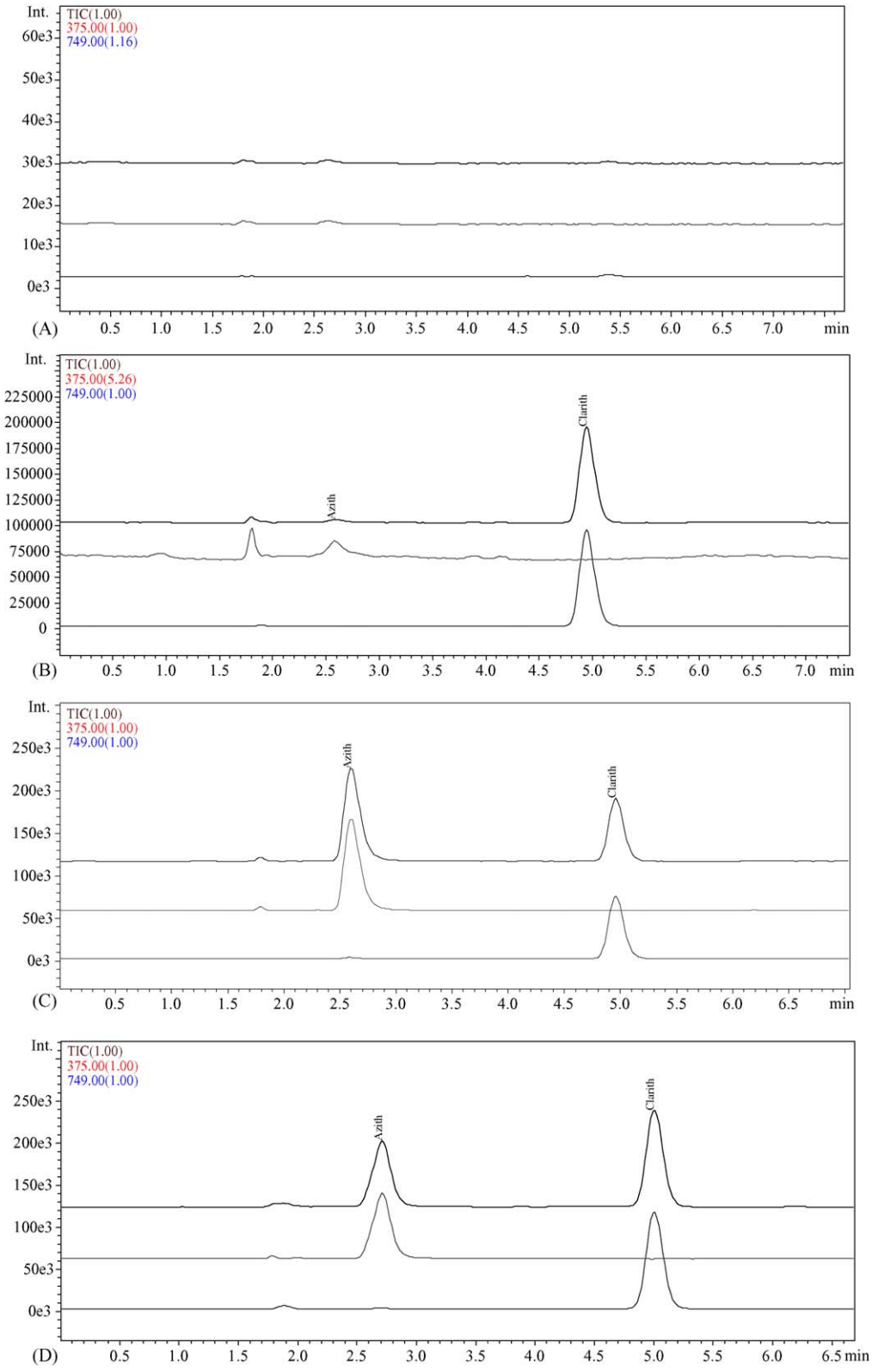

Fig. 3. Selective ion chromatograms of azithromycin and clarithromycin (IS). Positive ion monitored at *m/z* 375 (azithromycin), 749 (clarithromycin, IS), (A) blank plasma, (B) blank plasma spiked with 0.5 ng/mL (the lowest limit of detection) of azithromycin and IS, (C) blank plasma spiked with standard (50 ng/mL) and IS, and (D) human plasma sample after administration of azithromycin and spiked with IS.

Table 3
Reproducibility and accuracy for azithromycin of quality control sample in human plasma ($n = 5$)

Nominal concentration (ng/mL)	Mean found concentration (ng/mL)	Precision (R.S.D., %)	Mean relative error[a] (%)
Intra-day			
5	4.8	8.6	4.0
50	48.3	5.7	3.4
500	476.4	4.8	4.9
Inter-day			
5	4.7	10.7	6.0
50	54.4	7.2	2.2
500	464.8	6.4	7.0

[a] Mean relative error = |mean measured concentration − added concentration| × 100/nominal concentration.

Table 4
Stability of azithromycin in human plasma

Nominal concentration (ng/mL; $n = 5$)	Concentration found (ng/mL)	Precision (%)	Accuracy (%)
Short-term stability for 24 h in plasma at room temperature (RT)			
5	4.9	9.2	98.0
50	47.3	7.3	94.6
500	484.5	5.1	96.9
Four freeze-thaw cycles			
5	4.8	7.9	96.0
50	46.5	6.4	93.0
500	475.9	5.8	95.2
Storage in plasma at −20 °C for 2 months			
5	5.2	8.2	104.0
50	53.2	7.5	106.4
500	491.8	4.6	98.4
Storage in processed plasma extract at RT for 24 h			
5	4.8	6.5	96.0
50	52.5	4.6	105.0
500	467.4	4.2	93.5

calculated to be 0.5 ng/mL. The eight-point calibration plots obtained by weighted linear regression were highly linear over the range from 2 to 1000 ng/mL with the correlation coefficient of 0.9977. The calibration curve had the regression equation of $y = 0.0337x - 0.1734$, where y is the peak area ratio of azithromycin to IS and x is the concentration of azithromycin. Representative calibration curve parameters for the method from intra-day standard curve replicates are showed in Table 2. Intra-assay precision and accuracy were very satisfactory for all the concentrations tested. R.S.D. values were less than 10.9% at all concentrations.

3.3.4. Reproducibility and accuracy

The intra- and inter-day reproducibility of the method for plasma is summarized in Table 3 by analysis of replicates ($n = 5$) of QC samples containing known concentrations of 5.0, 50 and 500 ng/mL of azithromycin. The precision of the method was described as relative standard deviation (R.S.D.) among each assay. The intra-day R.S.D.s were always below 8.6% and the inter-day R.S.D.s were within 10.7%. The accuracy of the method was evaluated by analysis the quality control samples spiked with standard solutions and expressed as a percentage error of measured concentrations versus nominal concentrations. Precision and accuracy were calculated at each concentration. The results of the precision and accuracy of the proposed method were acceptable for bioequivalence.

3.3.5. Stability

The stability of azithromycin and IS in human plasma under different storage conditions was evaluated as follows: QC samples were subjected to short-term room temperature conditions, to four freeze-thaw cycles stability studies, to long-term (2-month) storage conditions (−20 C) and to processed sample kept at room temperature. All the stability studies were conducted at three concentration levels (5, 50 and 500 ng/mL) with five determinations for each. For short-term stability determination, stored plasma aliquots were thawed and kept at room temperature for a period of time (around 24 h) during routine sample preparation. Samples were extracted and analyzed as described above, and results were shown in Table 4. These results indicated reliable stability behavior under the experimental conditions of the regular analytical procedure. Azithromycin is stable at room temperature for at least 24 h. The analyte is also stable in human plasma when stored at −20 °C for at least 2 months and at room temperature for at least 24 h. It is stable under the influence of four freeze-thaw cycles. Table 4 showed the stability data of azithromycin subsequent to various storage conditions and freeze-thaw cycles.

3.4. Bioequivalence study

The developed and validated method has been successfully used to determination of azithromycin concentrations in human plasma samples after the administration of a 500 mg oral dose

Table 5
Pharmacokinetic properties of two oral formulations of single-dose azithromycin 500 mg in healthy subjects ($n = 24$)

Property	Test formulation (T)	Reference formulation (R)	T/R
C_{max} (ng/mL)	454.3 (66.7)	447.6 (101.5)	1.05 (0.20)
T_{max} (h)	2.06 (0.5)	2.17 (0.3)	0.98 (0.21)
AUC_{0-t} (ng/mL h)	4354.7 (1381.2)	4308.4(1235.5)	1.01(0.15)
$AUC_{0-\infty}$ (ng/mL h)	4936.6 (1668.3)	4986.5[509.6] (1552.6)	0.99 (0.20)
$T_{1/2}$	41.9 (12.6)	42.3 (12.6)	

Values are mean (S.D.).

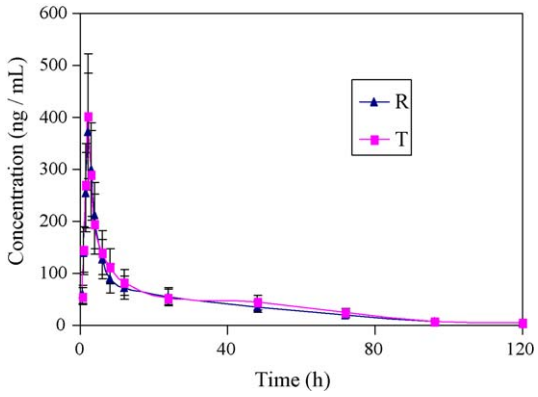

Fig. 4. Mean plasma concentration–time profile of azithromycin from 24 healthy volunteers following a single oral dose of 500 mg. *T*—test formulation; *R*—reference formulation.

of azithromycin. Table 5 showed the pharmacokinetic parameters of test and reference formulations. In this study in healthy volunteers, a single, 500-mg dose of test formulation was found to be bioequivalent to reference formulation based on the rate and extent of absorption. Representative concentration versus time profile for a subject, receiving a single dose, is presented in Fig. 4.

4. Conclusion

In conclusion a sensitive, rapid and specific LC–MS method has been described for the determination of azithromycin in human plasma. The acidic mobile phase was used and double charged ion $[M+2H]^{2+}$ for azithromycin was selectively monitored. Compared with the previously reported methods [26–30], lower limit of quantification (2 ng/mL) using 0.2 mL of plasma and simpler procedure for pretreatment were obtained. The method has been successfully applied to the bioequivalence studies and demonstrated that the method is reproducible.

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