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A simple, high-throughput and validated LC-MS/MS method for determination of azithromycin in human plasma and its application to a clinical pharmacokinetic study

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Abstract

A sensitive, specific and rapid liquid chromatographic-tandem mass spectrometric (LC-MS/MS) method was developed and validated to quantify azithromycin concentrations in human plasma. Azithromycin (AZI) is the most common outpatient prescribed antibiotic in the US and clinical studies have demonstrated the efficacy and safety of AZI in many bacterial infections. To support a clinical study, we developed a high throughput LC-MS/MS method to process up to 250 samples per day to quantify AZI in human plasma. Samples were prepared by solid phase extraction. Separation was achieved with an ACE C_{18} column (2.1 x 100 mm, 1.7 µm) equipped with a C_{18} guard column. The mobile phase consisted of 0.1% formic acid and methanol/acetonitrile (1:1, v/v) at a flow rate of 0.25 mL/min. The ionization was optimized with positive electrospray source using multiple reaction monitoring transition, *m/z* 749.50>591.45 for AZI and *m/z* 754.50>596.45 for AZI-d5. Extraction recoveries were approximately 90% for AZI. The assay was linear from 0.5 to 2000 ng/mL and required only 100 µL of plasma with total analysis time of 4.5 minutes. The method was successfully applied to pharmacokinetic studies of a weight-based dosing protocol for AZI.

Keywords: Azithromycin, yaws disease, HPLC, tandem mass spectrometry, human plasma

1. Introduction

Azithromycin (AZI), a macrolide antibiotic, is the most commonly prescribed outpatient antibiotic in the United States (Durkin et al., 2018). AZI not only has a broad spectrum antimicrobial activity against gram-positive, gram-negative and atypical pathogens, but also produces immunomodulatory effects for patients with inflammatory disorders (Amsden, 2005). It is widely used for the treatment of upper and lower respiratory tract infections, skin and soft tissue infections as well as treatment of sexually transmitted diseases (Peters, Friedel, & McTavish, 1992). Alongside its clinical efficacy, AZI has a number of advantages including tolerability, simple dosage regimens, and minimal drug-drug interactions (Larson, Tavakkoli, Drane, Toskes, & Moshiree, 2010).

AZI is also widely used in global health. In particular Mass Drug Administration (MDA) with azithromycin is a cornerstone of the World Health Organization strategy for the elimination of trachoma as a public health and for the eradication of yaws(Emerson, Burton, Solomon, Bailey, & Mabey, 2006; "Eradication of yaws--the Morges strategy," 2012). Through these programs several hundred million doses of AZI are administered in low and middle income country settings. There is also increasing interest in expanding these programs due to the potential for MDA with AZI to reduce childhood mortality(Keenan et al., 2018). Considering the central role of AZI in several public health programs it is imperative to develop and validate a sensitive bioanalytical method for AZI quantification in order to support the development of efficacious drug treatment regimens.

Current analytical methods developed for quantification of AZI in human plasma or serum include high performance liquid chromatography with electrochemical detection (HPLC-ECD)(Kees, Spangler, & Wellenhofer, 1998; Raines et al., 1998; Supattanapong & Konsil, 2008), HPLC with fluorescence detection (HPLC-FD) (Bahrami, Mirzaeei, & Kiani, 2005; Sastre Toraño & Guchelaar, 1998; Wilms, Trumpie, Veenendaal, & Touw, 2005) and HPLC

with ultraviolet detection (HPLC-UV). HPLC-UV is rarely used today due to lower sensitivity (LLOQ=30 ng/mL) associated with the absence of an UV chromophore of AZI (Ebrahimzadeh, Yamini, Ara, Kamarei, & Khalighi-Sigaroodi, 2010). HPLC-ECD and HPLC-FD methods have been also largely replaced by liquid chromatography-mass spectrometry (LC-MS) due to limited sensitivity with the lowest limit of quantification of 10 ng/mL(Bahrami et al., 2005) and a large biological sample requirement (500 to 1000 µL)(Supattanapong & Konsil, 2008; Wilms et al., 2005). Liquid chromatography tandem mass spectrometry (LC-MS/MS) (Ahmed et al., 2012; Barrett et al., 2005; Chen, Qin, Ma, & Li, 2007; Filist, Buś-Kwaśnik, Ksycińska, & Rudzki, 2014; Jiang, Chen, Ruan, Lou, & Yu, 2012; Nirogi et al., 2005; Ren et al., 2008; Yüzüak, Özden, Eren, & Toptan, 2007; Zhou, Liu, Gao, Yao, & Wang, 2007) are the most frequently used methods for AZI quantification in human plasma or serum during the last decade. However, the reported methods require a large sample volume requirement (200 to 500 µL) and a relatively long total run time (up to 14 minutes), as well as complex sample pretreatment procedures.

Our goal was to develop a LC-MS/MS method for the quantitation of AZI that could detect concentrations less than 1 ng/ml with a simple extraction procedure and less total analysis time that could be used to support clinical studies on the use of AZI in MDA regimens.

2. Experimental

2.1. Chemicals and materials

AZI and AZI-d5 (internal standard) were purchased from Toronto Research Chemicals (North York, Canada). Sodium bicarbonate was purchased from Sigma Aldrich (St Louis, MO, USA). Solvents including methanol (MeOH), acetonitrile (MeCN), and formic acid (FA) were HPLC grade or better and obtained from Fisher Scientific (Fair Lawn, NJ). Waters Oasis HLB solid phase extraction (SPE) cartridges (1mL:30 mg) were purchased from Waters (Waters Inc, Massachusetts, USA). Blank human plasma was purchased from Equitech-Bio, Inc. (Kerrville,

Texas). Ultrapure water was generated by a Barnstead Genpure water purification system (ThermoFisher Scientific).

2.2. Standard solutions preparation

A stock solution of AZI was prepared in a 2 mL volumetric flask by dissolving 4.0 mg AZI in methanol and made up to 2 mL with final concentration of 2 mg/mL. The stock solution was further diluted with methanol to prepare mix calibration control standards (CCs) and quality control samples (QCs). The internal standard stock solution was prepared by dissolving 1 mg AZI-d5 in 1 mL methanol, and further diluted with methanol to prepare working IS stock solutions of 1 µg/mL.

The further ten calibration standards (CSs) were prepared by diluting stock solutions with methanol: 20, 17.5, 2, 0.5, 0.2, 0.1, 0.2, 0.1, and 0.005 µg/mL. A total of ten calibration points with a concentration range of 0.5 to 2000 ng/mL for AZI was prepared by spiking 10 µL of working standard solution into 90µL of human plasma. Four different concentrations in four replicates were prepared as QCs with a lower limit of quantification (LLOQ: 0.5 ng/mL), low quality control (LQC: 1.5 ng/mL), middle quality control (MQC: 500 ng/mL) and high quality control (HQC: 1500 ng/mL). All stock solutions, mixed working solutions, CCs and QCs and stock solutions were stored at -20 °C.

2.3. Plasma sample preparation

All CCs, QCs and patient samples were prepared via SPE utilizing Waters Oasis HLB cartridges (30 mg/1 mL; Waters, Inc, Milford, MA, USA). All CCs and QCs were prepared by the addition of working standard solution into 100 µL of blank human plasma. For study samples, 100 µL human plasma was added into a 2 mL polypropylene (PP) tube. The IS (10 µL) and 60mM sodium bicarbonate (600 µL, pH 11) were added to all CCs, QCs and study samples prior to SPE. The sample was vortexed thoroughly for 30 seconds before SPE. The SPE cartridges were conditioned by 1 mL MeOH followed by 1 mL water before loading

samples. A two-step washing procedure was utilized and included wash with water (1 mL) followed by 15% MeOH (1 mL) after loading samples. Finally, the samples were eluted into glass tubes (13 x 100 mm) using MeOH (2 mL). The eluate was dried under nitrogen at 37ºC, and the dried residue was reconstituted with 200 µL of 0.1% aqueous formic acid:MeOH/MeCN (65:35, v:v), followed by vortexing for 30 seconds and centrifugation at 2,054 x *g* for 5 minutes. Finally, the supernatant (165 µL) was transferred into auto sampler vials and a volume of 2 µL was injected onto the column.

2.4. Instrumentation

The Shimadzu Nexera UPLC system consisted of two LC-30 AD pumps, a CTO-30AS column oven, and a SIL-30AC auto sampler, together with a LC-MS/MS 8060 system (Shimadzu Scientific Instruments, Columbia, MD). An electrospray ionization source in positive ionization mode was used to ionize samples. LabSolutions LCMS software Version 5.80 (Shimadzu Scientific, Inc., Columbia, MD) was used for data acquisition.

2.5. Liquid chromatographic and mass spectrometric conditions

Sample separations were completed by an ACE C_{18} column (2.1 x 100 mm, 1.7 µm, Advance Chromatography Technologies, LTD., UK) equipped with a C_{18} guard column (Waters, Milford, MA). The mobile phase consisted of 0.1% aqueous formic acid (solvent A) and MeOH/MeCN (1:1; v/v; solvent B). The chromatographic separation was achieved using 5.1 min gradient elution. The initial mobile phase composition was 35% B, increasing to 75% B over 3.0 min, then held constant for 1 min, and finally brought back to initial condition of 35% B in 0.10 min followed by 1-min re-equilibration. Constant flow rate was maintained at 0.25 mL/min throughout the analysis.

The MS-MS conditions, including desolvation line temperature (250 °C), interface temperature (300 °C), heat block temperature (400 °C), nebulizer gas (2.0 L/min), and drying gas flow (10 L/min) were optimized accordingly. Multiple reaction monitoring (MRM) was used to detect

and quantitate AZI. The MRM transitions, voltage potential (Q1, Q3), and collision energy (CE) parameters are shown in **Table 1.**

2.6. Method validation

The developed UPLC-MS/MS method for AZI quantification in human plasma was validated according to the guidance for Bioanalytical Method Validation(FDA, May 2018) . Fundamental parameters including selectivity, specificity, sensitivity, accuracy, precision, linearity, carry-over, recovery, matrix effects, and stability were assessed to ensure the acceptability of the method performance.

Linearity was determined by plotting calibration curve with concentrations range from 0.5-2000 ng/mL. A blank sample and ten different non-zero concentrations were used as calibration points. Determination coefficient (r^2) was calculated for each calibration curve by using linear regression and was required to be 0.998 or better. Intra-assay and inter-assay accuracy and precision were assessed for the method by spiking QC samples at four known AZI concentrations (LLOQ, LQC, MQC, and HQC) with five replicates. The accuracy and precision were expressed as percent deviation from the nominal concentration (% bias) and percent relative standard deviation (% RSD), respectively. The criteria for acceptability of the data included accuracy within ± 15% (% Bias.) from the nominal values and a precision of within ± 15% relative standard deviation (% RSD.), except for LLOQ, where it should not exceed ± 20% of accuracy as well as precision.

$$\% \text{ Bias} = (\text{observed conc.} - \text{nominal conc.}) \times 100 / \text{nominal conc.}$$

$$\% \text{ RSD} = \text{Relative standard deviation, } (\text{SD}/\text{Mean} \times 100)$$

Recoveries of AZI and IS were determined by comparing the peak area of an analyte spiked before extraction to post extraction, and then multiplied by 100. The recoveries were evaluated at LQC, MQC, and HQC concentrations for total of three replicates (Matuszewski,

Constanzer, & Chavez-Eng, 2003). Matrix effects were studied by comparing the peak area of QCs in post-extraction spiked human plasma with the peak area in methanol.

Stability tests, including bench-top storage stability (20 ℃ for 4 h), long-term stability (−80 °C for 30 days), freeze/thaw stability (room temperature to -80 ℃ to room temperature for 3 cycles) and auto sampler stability (4 °C for 36 h) parameters were validated by comparing the mean peak area of AZI with the corresponding mean peak area in a fresh solution under respective conditions.

Dilution integrity was carried out by spiking the human plasma with AZI 3000 and 7500 ng/mL, which is above the upper limit of quantification (ULOQ = 2000 ng/mL). Then concentrations were diluted with pooled blank human plasma at dilution factors of 2 and 5 in five replicates and analysed. Accuracy and precision should be within the set criteria (±15%) similar to the QCs samples, to make sure dilution integrity

Incurred samples reanalysis (ISR) was investigated to confirm the reliability of the bioanalytical methods during analysis of study samples analysis (Bland & Altman, 1999; Fluhler et al., 2014). 33 clinical samples (from 3 volunteers, approximately ~10% of total study samples) were reanalyzed and assessed the reproducibility against their original concentrations. The acceptance criteria for IRS, 67% of the repeated sample results should be within ± 20% of the mean. The percentage difference of the results is determined with the following equation:

$$\% \text{ difference} = (\text{Repeat conc.} - \text{Original conc.}) \times 100 / \text{Mean}$$

Calculations and statistical evaluation

The mean value, SD, % bias, and relative standard deviation (% RSD) were calculated by using Microsoft Excel 2016. LabSolutions LCMS software Version 5.80 (Shimadzu Scientific, Inc., Columbia, MD) was used for linear regression analysis.

2.7. Clinical application

The aim of this method development was to develop a sensitive bioanalytical method utilizing a simple SPE process and requiring a total run time of less than 5 minutes while requiring 100ul of plasma for the determination of AZI concentrations in a clinical study. The study protocol was approved by the Papua New Guinea medical research advisory committee. The trial was registered at ClinicalTrials.gov (NCT03664063). Written consent forms were obtained from each participate. Equal number of men and women were included in the study arms. The azithromycin was have been generic azithromycin (500mg tablet) produced by Kern pharmaceuticals for the study. Volunteers were instructed to fast overnight and were given by direct observation AZI 30 mg/kg (maximum 2g/dose) to three treatment regimens (John et al.). Serial blood samples were collected at baseline, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 hours following the dose. Plasma samples were stored at –80°C until analysis. The plasma concentration time data were analyzed using non-compartmental methods utilizing Phoenix WinNonlin version 6.3 (Pharsight Corporation, CA, USA). The maximal concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined by direct visual inspection of the data. Half-life ($t_{1/2}$) was calculated by the following equation: $t_{1/2}= 0.693/K_{el}$. The area under the plasma concentration time curve from zero time to the last time point (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the concentration curve from zero time to infinity ($AUC_{0-\infty}$),, was calculated by the following equation: $AUC_{0-t} + C_{last} /K_{el}$, where C_{last} is the last measurable concentration. Apparent volume of distribution (Vz/F) and apparent clearance were calculated using the formulas dose/(K_{el}* $AUC_{0-\infty}$), and dose/ $AUC_{0-\infty}$ respectively.

3. Results

3.1. Method development and optimization

The precursor and product ions for analytes of interest were determined by direct infusion of 1 µg/mL stock solutions. During method development, we compared electrospray ionization (ESI) source and an atmospheric pressure chemical ionization (APCI) source to optimize the signal intensity. ESI source in positive mode provided the best signal intensity of AZI and IS. **Figure 1** The major multiple reaction monitoring (MRM) transitions for AZI m/z 749.50 > 591.45 and IS were 754.50 > 596.45, respectively. Optimized parameters including voltage potential Q1 and Q3, collision energy are shown in **Table 1**.

In order to achieve better sensitivity of this method, we further optimized the chromatographic conditions by evaluating various analytical columns, reducing the injection volume and modifying mobile phase. We found AZI and IS were resolved from extraneous peaks within 2.5 minutes by an ACE C_{18} column (2.1 x 100 mm, 1.7 µm) equipped with a C_{18} guard column (Waters, Milford, MA). By comparing various injection volumes, we found that injecting 2 µL of sample resulted in best peak shape and improved sensitivity of the method compared to a larger injection volume of 5 µL. Various mobile phase components, include MeOH, water, MeCN, and FA were evaluated by comparing peak intensity and peak shape. Finally, a gradient elution profile consisting of 0.1% FA (solvent A) and methanol/acetonitrile (1:1, v/v, solvent B) at a flow rate of 0.25 mL/min was used in our method to achieve rapid chromatographic resolution and protect the column from accumulated endogenous compounds. The resolution of AZI and IS was achieved in 2.5 minutes.

In order to accomplish this, an efficient extraction method had to be developed. We tested many extraction methods including protein precipitation, protein precipitation (PPT), liquid-liquid extraction (LLE), and solid-phase extraction (SPE). The Oasis® HLB cartridge was found to have a high extraction efficiency for the analytes of interest and with a resulting clean baseline. Conversely, SPE method provided an efficient sample clean up with improved reproducibility,

high recovery and negligible matrix effect in comparison with PPT and LLE (data not shown). Mean recoveries were greater than 90% utilizing the final SPE method.

3.2. Assay validation

3.2.1. Selectivity and specificity

Comparison of the chromatogram of blank human plasma samples from six different sources with those of analytes spiked samples was used to determine the selectivity and specificity of this method. With the final method, there were no co-eluting peaks interfering the quantification near the retention times of AZI and IS **Figure 2**. The retention times were 2.5 minutes for both AZI and IS.

3.2.2. Sensitivity

The signal-to-noise ratio was required to be greater than 3 at limit of detection (LOD) and greater than 10 at LLOQ to determine the assay limits for quantitation. The lowest concentration for quantitation in assay with %RSD <20% was taken as LLOQ and was found to be 0.5 ng/mL for AZI.

3.2.3. Intra- and Inter-assay variation

The intra-day and inter-day accuracy (expressed as %bias) and precision (expressed as % RSD) results for AZI at LLOQ, LQC, MQC and HQC samples are presented in **Table 2.** All the QCs were deemed accurate and precise, with values within ± 15% (%bias) and within ± 15% relative standard deviation (%RSD), except LLOQ, where it did not exceed ± 20% of % RSD and % bias.

3.2.4. Linearity

The assay was linear from 0.5 to 2000 ng/mL. Weighted linear regression analysis ($1/x^2$ weighting) was used while performing data analysis. Each calibration curve was achieved determination coefficient of 0.998 or better. The lowest concentration on the calibration curve was 0.5 ng/mL, which was defined as LLOQ.

3.2.5. Carry-over

No significant carry-over effect (<20%) was found in the zero samples after the HQC samples injection.

3.2.6. Recovery and matrix effects

The extraction recovery of AZI and IS are shown in **Table 3**. Calculated matrix factor were in the range of 90-110%, did not exceed 15%. Therefore, the interference of ion suppression or enhancement was negligible.

3.2.7. Stability

The effect of long-term storage and analysis stability was performed and consisted of bench-top stability, long-term stability (30 days), freeze-thaw stability and auto-sampler stability. All QCs were found to be within the range of 85-115% of the actual concentration for the stability studies **Table 4**.

3.2.8 Dilution integrity

The precision for dilution integrity of 1:2 and 1:5 dilutions were within the acceptance limits of ± 15% for precision (CV) and 85.0–115.0% for accuracy.

3.2.9 Analysis of ISR

The percentage difference in concentration at all-time points (1 h to 72 h) were within ± 20% of the average value for 90% of the samples, except for three time points out of 33 samples (Figure 3a) (Bland & Altman, 1999; Fluhler et al., 2014). In addition, there was good linear correlation (r^2= 0.9953, with slope of 0.6824) obtained between concentrations of reanalyzed and original **Figure 3b**. Thus, no significant differences were found between reanalyzed and original concentration, were within the acceptance limits of ± 20% of the mean value.

3.3. Clinical application

The developed and validated UPLC-MS/MS method was successfully applied to a clinical trial (NCT03490123) to study pharmacokinetics of AZI. This method was able to quantify AZI concentrations up to 72 hours after drug administration following oral doses of 30 mg/kg. The plasma concentration time profile of AZI in thirteen subjects are shown in **Figure 4**. The main pharmacokinetic parameters (mean ± SD) are shown in **Table 5**. C_{max} was 1,480± 798 ng/mL, occurring at the corresponding t_{max} of 4.23 ± 2.01h. Half-life ($t_{1/2}$) for AZI was 33.7 ± 8.22 h, which was similar to reported values in literatures (Ahmed et al., 2012; Liu, Xu, Huang, Gao, & Guo, 2007).The mean AUC_{0-t} and $AUC_{0-\infty}$ for AZI were 12,300 and 12,300 h*ng/mL respectively. The mean apparent Cl/Fclearance and Vz/F volume of distribution of AZI were 126.0 L/hour and 6,030 L, respectively.

4. Discussion and conclusion

LC-MS/MS is the most common bioanalytical method used in measuring AZI in human plasma. Compared to previously reported quantification methods, this method has significant advantages including the requirement of a small plasma volume, a simple SPE method with analyte recovery greater than 90%, increased sensitivity, reduced injection volume, large dynamic range, and a short total run time. To the best of our knowledge, this is the first description of a bioanalytical method for the quantitation of AZI following weight based dosing regimens (30 mg/kg) for the treatment of yaws. The linearity range from 0.5 ng/mL to 2000 ng/mL was suitable to quantitate the AZI plasma concentration up to 72 hours, allowing for the determination of pharmacokinetic parameters to describe AZI bio-distribution. Regarding to sample preparation procedure, only 100 µL human plasma was used in our method, which was the lowest requirement of bio-matrices compared to published articles which utilized 200 to 500 µL (Ben-Eltriki, Somayaji, Padwal, & Brocks, 2013; Chen et al., 2007; Choemunng & Na-Bangchang, 2010; Filist et al., 2014; Zhou et al., 2007). Moreover, the SPE method provided excellent extraction recovery of AZI without matrix effect, while avoiding methyl tertiary-

butyl ether (MTBE), which is not an environmentally friendly solvent for sample extraction. Finally, we were able to further reduce the total analysis time from 8 minutes (Filist et al., 2014) to 4.5 minutes per sample, which enabled us to measure more than 200 samples per day with acceptable accuracy and precision. The study subjects were given about two to four times higher doses of AZI (1250-2000 mg) than in previous published pharmacokinetic studies (250-500 mg). We found the C_{max} and AUC values were significantly higher as expected. C_{max} and AUC values were around three times higher than the reported values when traditional 250-500 mg dose of AZI were given (Ahmed et al., 2012; Bahrami et al., 2005; Barrett et al., 2005; Ben-Eltriki et al., 2013; Chen et al., 2007; Liu et al., 2007; Zhou et al., 2007). This highly sensitive, rapid, high-throughput bioanalytical method will provide a valuable tool to determine pharmacokinetic profile of AZI following weight-based dosing regimens.

Conflict of interest

There is no conflict of interest to disclose.

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Table 1. Summary of optimized MS/MS parameters: precursor ions, fragment ions, voltage potential (Q1), collision energy (CE), voltage potential (Q3), and retention time for analyte and internal standard.

Analytes	MRM transition m/z (Q1>Q3)	Q1 (V)	CE (V)	Q3 (V)	Retention time (min)
Azithromycin	749.50 > 591.45	-40	-29	-22	2.5
Azithromycin-d5(IS)	754.50 > 596.45	-40	-31	-22	2.5

Table 2. Intra and Inter-day precision (% RSD) and accuracy (% Bias) for azithromycin in human plasma.

Nominal (ng/mL)	Precision		Accuracy	
	% $RSD_{intra\text{-}assay}$	% $RSD_{inter\text{-}assay}$	% $Bias_{intra\text{-}assay}$	% $Bias_{inter\text{-}assay}$
0.5 (LLOQ)	10.6	12.5	3.5	13.7
1.5 (LQC)	2.0	13.3	7.0	8.3
500 (MQC)	0.6	4.5	0.9	3.7
1500 (HQC)	0.7	6.5	-11.9	-7.0

Table 3. Mean extraction recoveries of the azithromycin from human plasma.

Concentration (ng/mL)	% Extraction recoveries (Mean ± SD, n=3)
LQC	82.2 ± 0.5
MQC	93.0 ± 4.5
HQC	94.7 ± 6.7

Table 4. Mean stability recoveries of the azithromycin at different storage conditions in human plasma.

	% Stability recoveries (Mean ± SD)			
Analyte	Freeze-thaw (–80 ± 5 ℃ after three cycle)	Long-term (–80 ± 5 ℃, 60 days)	Auto-sampler (4 ℃, 36 h)	Bench-top (20 ℃, 4 h)
LQC	106.9 ± 6.8	105.2 ± 5.3	102.0 ± 9.2	106.6 ± 7.7
MQC	100.5 ± 9.5	93.2 ± 3.8	98.2 ± 5.3	89.6 ± 3.7
HQC	103.5 ± 10.3	100.5 ± 6.9	90.5 ± 7.7	97.8 ± 7.7

Table 5. Non-compartmental pharmacokinetic estimates of AZI in human subjects after received 30 mg/kg post oral administration of a single dose of AZI (mean ± SD, n=13).

PK Parameters (Unit)	Estimates (mean ± SD)
C_{max} (ng/mL)	1480± 7980
T_{max} (h)	4.22 ± 2.01
$t_{1/2}$ (h)	33.7 ± 8.22
AUC_{0-t} (h*ng/mL)	12,3006 ± 3,393
$AUC_{0-\infty}$(h*ng/mL)	14,800± 3,650
$V_{z/F}$ (L)	6030± 1870
Cl/F (L/h)	126± 30

C_{max} maximum observed plasma concentration after administration, T_{max} observed time to reach C_{max}, CL/F elimination clearance, V_z/F apparent volume of distribution, $t_{1/2}$ terminal elimination half-life, AUC_{0-t} area under the plasma concentration time curve after the last dose from zero time to last time point, $AUC_{0-\infty}$ predicted area under the plasma concentration time curve after the last dose from zero time to infinity.

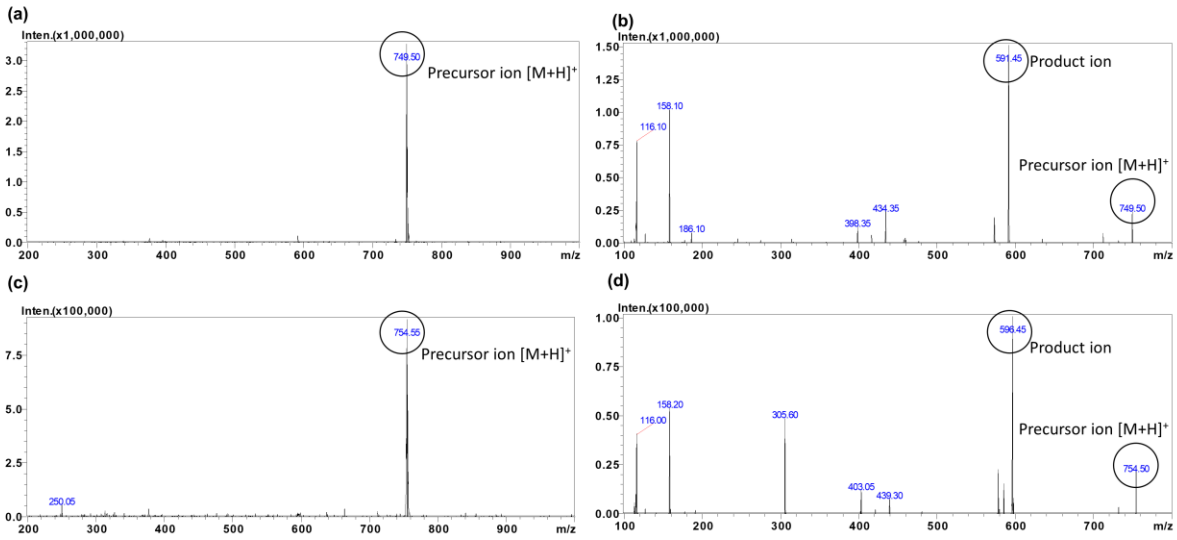

Figure 1

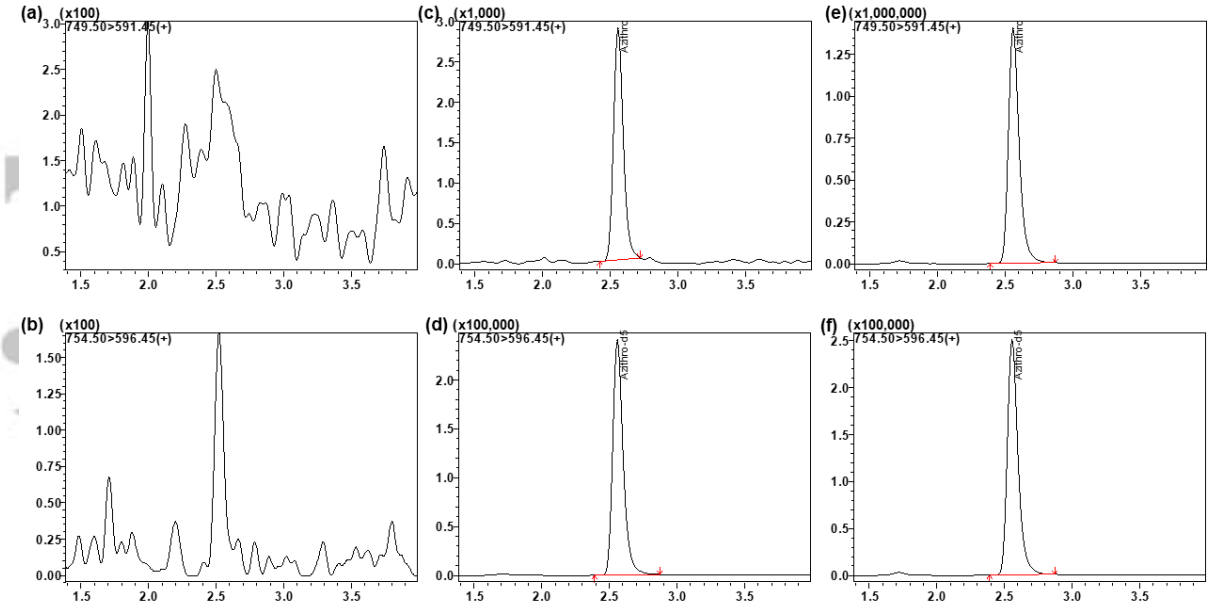

Figure 2

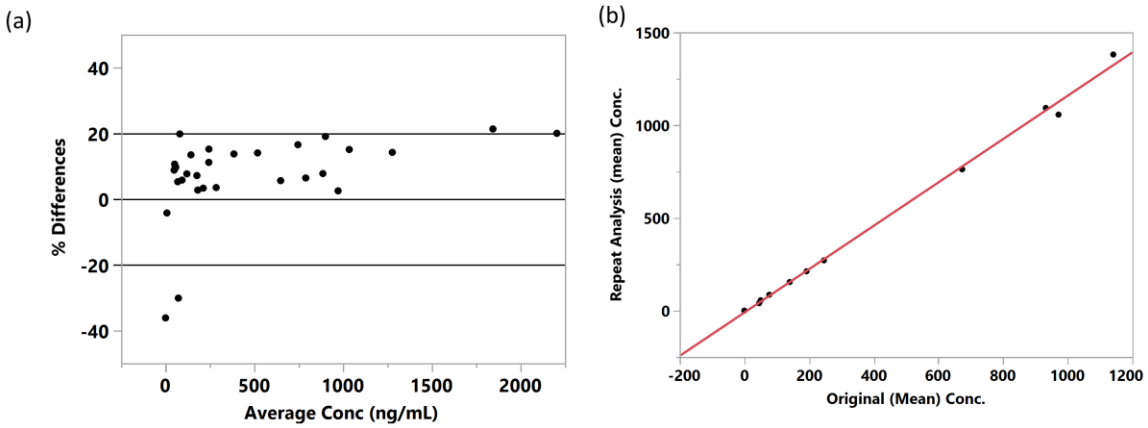

Figure 3

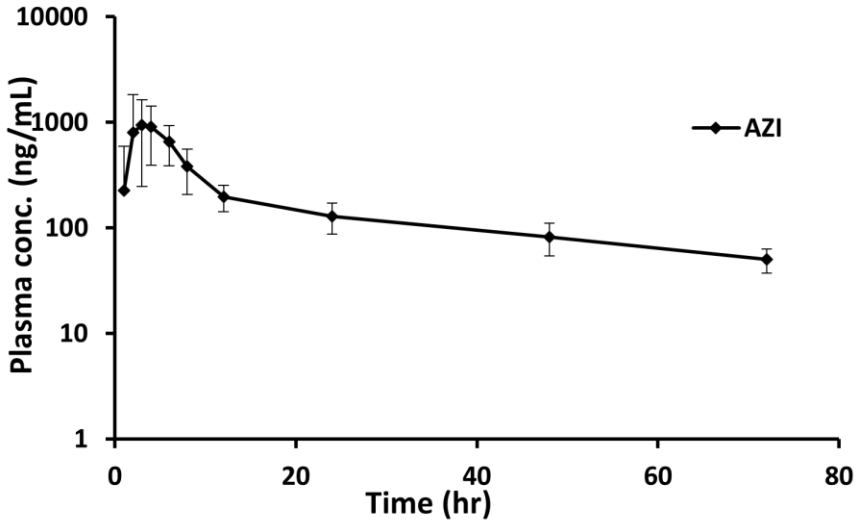

Figure 4