Simple and high sample throughput LC/ESI-MS/MS method for bioequivalence study of prazosin, a drug with risk of orthostatic hypotension

Gabriel Onn Kit Loh, Emily Yii Ling Wong, Yvonne Tze Fung Tan, Hong Chin Wee, Ru Shing Ng, Haroon Khalid Syed and Kok Khiang Peh

ABSTRACT
Objective: The study aimed to develop a rapid, simple and sensitive LC/ESI-MS/MS method to measure prazosin concentration in human plasma and apply bedside sampling in bioequivalence study of two prazosin tablets to resolve the adverse effect of orthostatic hypotension.

Significance: The LC/ESI-MS/MS prazosin method was highly sensitive and selective. Bedside sampling reduced the orthostatic hypotensive incidence and subject dropout rate.

Methods: After sample preparation, prazosin and terazosin (IS) were detected on mass spectrometer operating in multiple reaction monitoring mode using positive ionization. Mobile phase flow rate was set at 0.40 mL/min with sample run time of 1.75 min. The bioanalytical method was validated as per EMEA and FDA guidelines. Bedside sampling was performed in bioequivalence study for the first 4 h after dosing. The three primary pharmacokinetic parameters, $C_{\text{max}}$, AUC$_{0-\infty}$ and AUC$_{0-\infty}$, and 90% confidence interval were determined.

Results: The small injection volume of 1 µL minimized instrumentation contamination and prolonged the analytical column lifespan. Linearity was obtained between 0.5 and 30.0 ng/mL, with coefficient of determination, $r^2 > 0.99$. The mean extraction recovery of prazosin and IS was >92%, with precision value (CV, %) ≤ 10.3%. Only two orthostatic hypotensive adverse events were reported. The two prazosin formulations were found to be bioequivalent.

Conclusion: The LC/ESI-MS/MS method has shown robustness and reliability exemplified by the incurred sample re-analysis result. Bedside sampling should be proposed for bioequivalence or pharmacokinetic studies of drugs demonstrating adverse event of orthostatic hypotension.

Introduction
Prazosin hydrochloride is an α1-adrenoceptor antagonist indicated to treat hypertension, congestive heart failure, symptoms of enlarged prostate, post-traumatic stress disorder and Raynaud syndrome [1–3]. Prazosin hydrochloride is also indicated in gestational hypertensive disorder [4,5]. One of the serious common adverse effects of prazosin was orthostatic hypotension or postural hypotension [6–8]. The adverse effect is caused by the rapid increase in hydrostatic pressure in the arterial vessels of the lower body region when standing up, causing dizziness, lightheadedness or faint [8]. Orthostatic hypotension has been identified as one of the risk factors for falls [9–12], fractures [13], myocardial ischemia [10,14], cognitive impairment [15] and mortality [10].

Several analytical methods for determination of prazosin in biological samples have been reported. These include high-performance liquid chromatography (HPLC) coupled with ultraviolet detector [16], fluorescence detector [17–22], electrochemical detector [23], photodiode array detector [24] and mass spectrometer [25]. The limitations of these methods encompass the utilization of large volume of plasma samples [17,22,24], large volume of non-environmentally friendly organic extracting solvents [17,19,21,25], lack of sensitivity [16,18,19,22–24], tedious and time-consuming sample preparation using liquid–liquid or solid-phase extraction [16–19,21,23,25]. One of the studies employed a large sample injection volume of 50 µL to attain LLOQ value of 0.5 ng/mL [21]. Table 1 highlights the salient features of reported bioanalytical methods for determination of prazosin in human plasma.

The study aims to report a simple, sensitive and high sample throughput by LC/ESI-MS/MS method for determination of prazosin concentration in plasma samples, a drug with common adverse effect of orthostatic hypotension, in Malaysian population. The small 1 µL injection volume minimized the instrumentation contamination and prolonged the analytical column lifespan. The LLOQ of 0.5 ng/mL was below 5% of the mean $C_{\text{max}}$ meeting the guideline requirement.

Materials and methods

Materials
Prazosin hydrochloride (purity: 99.7%) and terazosin hydrochloride (internal standard, IS, purity: 91.7%) reference standards were
Table 1. Summary of published bioanalytical methods for the quantification of prazosin in biological matrices.

| Analytical method | Biological matrix | Type of biological matrix volume (µL) | Calibration range | Sample preparation method | Injection volume (µL) | Run time (min) | References |
|-------------------|-------------------|--------------------------------------|-------------------|--------------------------|-----------------------|---------------|------------|
| HPLC-UV           | Human plasma      | 200                                  | 1–30 µg/mL        | SPMME                    | 5                     | 120           | [16]       |
| HPLC-RF           | Human plasma      | 5000                                 | 1–15 ng/mL        | LLE                      | 3                     | 12            | [17]       |
| HPLC-RF           | Human plasma      | NA                                   | 0.4–100 µg/mL     | LLE                      | NA                    | NA            | [18]       |
| HPLC-RF           | Human plasma      | 50                                   | 20–300 ng/mL      | LLE                      | 110                   | 8             | [19]       |
| HPLC-RF           | Human plasma      | 100                                  | NA                | PPT                      | NA                    | NA            | [20]       |
| HPLC-RF           | Human plasma      | 1000                                 | 0.5–50 ng/mL      | LLE                      | 50                    | 7.5           | [21]       |
| HPLC-UV           | Human serum       | 2000                                 | 2.5–1000 µg/mL    | PPT                      | 20                     | 9             | [22]       |
| HPLC-ECD          | Bovine serum      | 100                                  | 5–250 ng/mL       | PPT with LLE             | 50                    | 11            | [23]       |
| HPLC-PDA          | Human plasma      | 500                                  | 5–500 ng/mL       | PPT                      | 10                    | 8             | [24]       |
| LC-MS             | Human plasma      | 200                                  | 0.5–100 ng/mL     | LLE                      | 5                     | 4.5           | [25]       |
| LC-MS/MS          | Human plasma      | 250                                  | 0.5–30 ng/mL      | PPT                      | 1                     | 1.75          | Present method |

ECD: electrochemical detector; HPLC: high-performance liquid chromatography; LC-MS: liquid chromatography mass spectrometry; LC-MS/MS: liquid chromatography tandem mass spectrometry; LLE: liquid–liquid extraction; NA: not available; PDA: photodiode array; PPT: protein precipitation technique; RF: fluorescence detection; SPE: solid-phase extraction; SPMME: solid-phase micromembrane tip extraction; UV: ultraviolet–visible.

provided by Y.S.P. Industries (Selangor, Malaysia). HPLC grade formic acid, acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Blank plasma with anticoagulant of dipotassium ethylenediaminetetraacetic acid (K₂EDTA) was obtained from i-DNA Biotechnology (Kuala Lumpur, Malaysia). Purified water was taken from Thermo Fisher Scientific Ultrapure water system (Pittsburgh, PA, USA).

LC/ESI-MS/MS chromatographic analysis

Shimadzu Nexera X2 series UHPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with a degasser (DGU-20AS), a system controller (CBM-20A), an autosampler (SIL-30AC), two binary pumps (LC-30AD), a column oven (CTO-20AC) and a tandem mass spectrometer (LCMS-8040) with electrospray ionization (ESI) source. The chromatograms and analytical data were processed by LabSolution software (Version 5.91).

Chromatographic separation was carried out using an Poroshell 120 EC-C18 – Fast LC (Agilent, USA) analytical column (100 × 2.1 mm; 2.7 µm particle size) fitted with a guard (UHPLC Guard Poroshell 120 EC-C18, 5 × 2.1 mm ID, 2.7 µm particle size). The mobile phase was consisted of 0.1% formic acid and acetonitrile (35:65, v/v) run at a flow rate of 0.40 ml/min. The column oven and autosampler temperatures were 30 °C and 15 °C with the injection volume of 1 µL.

A tandem mass spectrometer with electrospray ionization (ESI) as ion source operated in positive ionization mode was used to detect prazosin and IS. The detector energy, conversion dynode voltage and interface bias/capillary voltage were 4500 V, respectively. The temperature of desolvation line (DL) and heating block were 250 °C and 400 °C. Nitrogen gas was utilized as drying gas and nebulizing gas with gas flows of 15 and 3 L/min, respectively. Collision argon gas was set at 230 kPa. Two multiple reaction monitoring (MRM) transitions for quantification and confirmation were used for determination of prazosin and IS. The optimized MRM transitions for prazosin and IS were tabulated in Supplementary data S1.

Preparation of standard and sample solutions

Prazosin and IS stock standard solutions at concentration of 20.0 µg/mL were prepared in water and acetonitrile (1:1, v/v). Stock or other working standard solutions were diluted with water and acetonitrile (1:1, v/v) to prepare prazosin (5.0, 50.0, 100.0, 200.0, 250.0 and 2000.1 ng/mL) and IS (200.1 ng/mL) working standard solutions.

The plasma calibration standards of prazosin in the range of 0.5–30.0 ng/mL were prepared in blank plasma sample using appropriate working standard solutions. Another freshly prepared prazosin stock standard solution was used to prepare QC working standard solutions at 5.0, 50.0 and 250.0 ng/mL, for preparation of QC plasma samples, comprising of lower limit of quantification (LLOQ), low QC (LQC), medium QC (MQC) and high QC (HQC), at 0.5, 1.5, 15.0 and 22.5 ng/mL, respectively.

Sample preparation

An aliquot of 250.0 µL of plasma sample was measured accurately into a 2-mL microcentrifuge tube, followed by the addition of 50 µL of terazosin IS working standard solution (200.1 ng/mL) and 750.0 µL of acetonitrile. The sample was vortexed (Heidolph REAX 200, Schwabach, Germany) for 30 s and centrifuged (Eppendorf AG Mini Spin Plus, Stevenage, UK) at 9676.8 g for 5 min. A nylon membrane syringe filter (0.2 µm, 17 mm filter, Thermo Scientific, USA) was used to filter the supernatant.

System suitability

The drift of LC/ESI-MS/MS system for all the method validation runs was monitored using system suitability testing. System suitability samples were made up of five plasma samples at 21.0 ng/mL, a solvent and an LLOQ. The conduct and acceptance criteria of system suitability test were reported in earlier studies [26,27]. Initially, the five replicates of plasma samples at 21.0 ng/mL were injected into the system to obtain a CV (%) of <6%. A solvent and LLOQ were injected right after the five replicates of these samples to assess the carryover of the method where the carryover should be less than 20% and 5% of the peak response of analyte and IS, respectively. A set of 21.0 ng/mL plasma samples were injected after every forty injections and at the end of the run. The peak area ratio of each set of these samples should be within ±15% of the mean peak area ratio of the first five plasma samples at 21.0 ng/mL. Solvent and LLOQ were injected prior to the end of each run to ensure there was no carryover during the run.

Method validation

Method validation of prazosin was conducted as per European Medicines Agency Guideline on Bioanalytical Method Validation.
except recovery which was performed as per Food and Drug Administration Bioanalytical Method Validation Guidance for Industry [29]. Specificity and selectivity parameter were evaluated using blank human plasma samples (without prazosin and IS) from six different subjects to check for interferences at the retention times of prazosin and IS. The sensitivity was determined at LLOQ level using six different subjects' blank human plasma. Calibration curve of prazosin was constructed with eight non-zero calibration standards at 0.5, 1.0, 5.0, 10.0, 16.0, 20.0, 26.0 and 30.0 ng/mL, calculated using least square linear regression with a weighting factor of $1/x^2$. Residual effect was assessed by analyzing a processed blank human plasma sample after injection of upper limit of quantification (ULLOQ) in each bioanalytical method validation run. The accuracy and precision of within-run and between-run were assessed in a single run and three different runs on three separate days using six sets of LLOQ and QC samples (0.5, 1.5, 15.0 and 22.5 ng/mL). One set of system suitability, one set of plasma standard calibration curve and 33 replicates of QC samples were injected in one single run to determine the accuracy and precision of QC samples in a size ≥ study samples in bioanalytical run, which was known as extended-run precision and accuracy. Matrix effect was compared as the peak area ratio of prazosin spiked after plasma deproteinization with acetonitrile to those prepared in water and acetonitrile (1:1, v/v) using LQC and HQC samples. For dilution integrity, prazosin plasma samples at concentrations of 25.0 ng/mL (two-fold) and 5.0 ng/mL (10-fold) were used after dilution of prazosin plasma sample at 50.0 ng/mL with blank human plasma. The recovery parameter was evaluated by comparing the peak areas of analytes and IS spiked after plasma extraction to those spiked after plasma extraction using six determinations at LLOQ and QC samples.

**Stability studies**

Prazosin plasma sample stability was evaluated at three replicates for LQC and HQC. The short-term/bench-top stability was determined at room temperature (25 ± 4 °C) for a period up to 24 h. Post-preparative stability in auto-sampler at 15 ± 3 °C was evaluated until 48 h. Freeze and thaw stability was assessed after seven freeze and thaw cycles. Long-term stability in freezer was determined at −20 ± 10 °C for 30 days. A freshly prepared plasma calibration curve was constructed to quantify the concentration of QC stability samples and the calculated concentrations were compared against the nominal concentration of the QC samples. The stability of prazosin and terazosin IS stock standard solutions was determined after storing at room-temperature (25 ± 4 °C) and in chiller (5 ± 3 °C) for 30 days. The stock solution of prazosin was appropriately diluted to LQC and HQC concentrations and assessed against new calibration curve from new stock standard solutions. IS stock solution stability was determined at 200.1 ng/mL concentration against a freshly prepared IS at the same concentration. The peak area responses of both samples were compared.

**Bioequivalence study**

A two-treatment, two-period, two-sequence, randomized, open label, single-dose, two-way crossover oral bioequivalence study of two prazosin products, Minisor tablet (1 mg prazosin, Y.S.P. Industries, Malaysia) and Minipress tablet (Pfizer Australia Pty. Ltd., Australia) in 30 healthy subjects under fasting conditions was conducted, with a washout period of 7 days. The study protocol was approved by the Medical Research and Ethics Committee (MREC) Ministry of Health, Malaysia. The subjects were informed about the possible risks and benefits of joining the study and signed written informed consent form before participating in the study. Each subject was dosed with two tablets (2 x 1 mg) of either test or reference product.

Male subjects between 18 and 55 years old, Body Mass Index (BMI) from 18.5 to 30.0 kg/m², in good health at screening, no history of hypersensitivity or allergy to prazosin or any other related drugs, willing to give written informed consent were the inclusion criteria of the study. The subjects were refrained from taking vitamin supplementation, herbal remedies, natural products or medications 7-day before admission, hospitalized 30 days before consent taking, drinking alcoholic beverages 24 h or red wine 7-day before product dosing, blood donation ≥ 500 ml 90-day before consent taking, involvement in any other clinical trial and the last drawn blood sample 60-day before taking the first dose of studied product.

The blood samples of the study were drawn before dosing (0 h) and at 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0 and 16.0 h after product dosing. Blood samples were drawn at the bedside for the first 4 h. The plasma samples were stored in freezer at −20 ± 10 °C until analysis. Adverse events were observed and recorded in the case report form throughout the study.

**Pharmacokinetic parameters and analysis**

The values of $C_{\text{max}}$ (peak plasma concentration) and $T_{\text{max}}$ (time to achieve peak plasma concentration) were obtained from the prazosin plasma concentration versus time profiles for each subject. $\text{AUC}_{0-t}$ (area under the plasma concentration–time curve) and $\text{AUC}_{0-\infty}$ (area under the curve from time zero to infinity) were calculated from prazosin plasma concentration versus time profiles. A linear-logarithmic trapezoidal method was used to calculate $\text{AUC}_{0-t}$. The last measurable prazosin plasma concentration was divided by elimination rate constant ($k_e$) to calculate the $\text{AUC}_{0-\infty}$. The $k_e$ was calculated from the slope of the prazosin plasma concentration–time profile after logarithmic transformed. The elimination half-life was calculated using 0.693/$k_e$ [30–32]. Values below LLOQ were put as zero (0) for calculation purposes.

Incurred sample re-analysis (ISR) was performed by choosing at least 10% of the total number of analyzed samples. A total of four points (two points per period) were selected from each subject, plasma samples at $C_{\text{max}}$ and elimination phase (within four times of LLOQ level). The ISR results were compared with the data obtained during the first analysis.

**Statistical analysis**

The primary pharmacokinetic parameters, $C_{\text{max}}$, $\text{AUC}_{0-t}$, and $\text{AUC}_{0-\infty}$, were transformed to natural logarithm scale. An analysis of variance (ANOVA) with geometric least square mean procedure was used to distinguish effects due to subject within sequence, period and treatment for the In-transformed values of $C_{\text{max}}$, $\text{AUC}_{0-t}$, and $\text{AUC}_{0-\infty}$ of the two products [30,33]. Bioequivalence was concluded if the 90% confidence interval (CI) of the geometric mean ratios of $C_{\text{max}}$, $\text{AUC}_{0-t}$, and $\text{AUC}_{0-\infty}$ of test/reference product fell between 0.80 and 1.25 (80.00 – 125.00%). Two one-sided t-tests were used to test for bioequivalence.

**Results**

**Method validation**

The coefficient of variation (CV, %) of first five replicates of high system suitability samples was ≤4.3%, indicating that the system
reached sufficient equilibration before the start of method validation runs. The percentage of deviation was within the acceptance criteria with the deviation value ≤ 13.6%. Carry over was not found in neat solvent after injecting high system suitability samples.

Six subjects’ blank plasma showed absence of interfering peak at or near the retention times of prazosin and IS (Figure 1). The signal to noise (S/N) ratios of prazosin and IS at LLOQ level (0.5 ng/mL) were more than 8 and 70 in the study. Prazosin and IS retention times ranged from 0.52 to 0.57 min, respectively.

Prazosin calibration curve was linear for concentration ranged from 0.5 to 30.0 ng/mL. The weighting factor of 1/prazosin concentration$^2$ was selected because it provided the most suitable approximation of variance and the highest accuracy for all non-zero calibration points. The accuracy (RE, %) of the back-calculated approximation of variance and the highest accuracy for all non-retention times ranged from 0.52 to 0.57 min, respectively.

 ISR study was done to verify the reliability of the first analyzed prazosin concentrations in subjects’ plasma samples. More than ten percent of all samples were chosen and analyzed in separate runs on different days. The ISR results were within 20% from the initial value for 92.0% of prazosin samples, proving the reproducibility of the method where the value must be ≤ 20% for at least of the plasma samples [28,29].

**Discussion**

**Optimization of chromatographic conditions and sample preparation**

The obtained precursor molecular ions [M + H]$^+$ of prazosin and IS were 384.20 and 388.20 using Q1 and Q3 scans on drug standard solutions with concentration of 2000 ng/mL. Positive ionization mode was more favored by prazosin and IS due to the molecular structures of prazosin and IS having piperazine group, a six-membered ring with two nitrogen atoms at opposite positions in the ring. A proton donor was contributed with the use of formic acid in mobile phase. In the current study, two transitions were selected as quantification and confirmation product ions for prazosin and IS, m/z 95.00 and m/z 247.05 for prazosin, while m/z 71.10 and m/z 247.10 for IS. Terazosin was selected as IS as it is easily available and not costly. It showed similar chromatographic behavior as prazosin and its use has been reported in other studies [21,34].

Methanol and acetonitrile are common organic modifiers in the preparation of mobile phase. A higher ionization efficiency was observed with the use of acetonitrile compared with methanol. An increase of ~ 40-50% in peak intensity was observed for acetonitrile due to its stronger dipole moment than methanol. The mobile phase composition with 0.1% formic acid and acetonitrile was optimized to obtain a symmetrical peak shape, sufficient sensitivity and a short sample run time. A distorted peak shape was observed when 0.1% formic acid and acetonitrile at 30:70 (v/v) was used as the mobile phase. Intensity was slightly reduced (5 – 7%) when the acetonitrile content was reduced to 60%. 0.1% formic acid and acetonitrile at 35:65 (v/v) gave the most optimum result with a short run time of 1.75 min. The short run time and the use of low flow rate reduced the consumption of organic solvents and hence the cost of analysis.
Figure 1. Mass chromatograms of (a) blank plasma samples, (b) zero sample with IS, (c) prazosin at LLOQ level with IS and (d) prazosin at ULOQ level with IS.
Figure 2. The plasma calibration curve of prazosin. Mean ± SD (n = 8).

Table 2. Within-run, between-run and extended-run precision and accuracy results.

| Nominal conc. (ng/mL) | Within-run (n = 6) | Between-run (n = 18) | Extended run (n = 33) |
|----------------------|-------------------|----------------------|----------------------|
|                      | Mean conc. obtained (ng/mL) | CV (%) | Accuracy (%) | Mean conc. obtained (ng/mL) | CV (%) | Accuracy (%) | Mean conc. obtained (ng/mL) | CV (%) | Accuracy (%) |
| 0.5                  | 0.5               | 12.5                | 96.0                | 0.5                  | 10.4              | 96.0                | –                   | –       | –         |
| 1.5                  | 1.5               | 8.7                 | 99.3                | 1.6                  | 10.9              | 104.0               | 1.5                 | 5.9     | 102.0     |
| 15.0                 | 14.8              | 3.5                 | 98.6                | 14.9                 | 5.2               | 99.3                | 16.5                | 2.6     | 110.0     |
| 22.5                 | 24.3              | 3.5                 | 108.0               | 22.8                 | 6.9               | 101.5               | 23.7                | 3.3     | 105.2     |

CV: coefficient of variation; conc.: concentration; n: number of replicates.

Table 3. Dilution integrity results of prazosin (n = 5).

| Dilution factor | Calculated conc. (ng/mL) | Mean conc. ± SD (ng/mL) | Precision (CV, %) | Accuracy (RE, %) |
|-----------------|--------------------------|-------------------------|-------------------|------------------|
| 2-Fold          | 55.8                     | 54.3 ± 1.7              | 3.0               | 8.6              |
|                 | 54.5                     |                         |                   |                  |
|                 | 55.9                     |                         |                   |                  |
|                 | 52.1                     |                         |                   |                  |
|                 | 53.2                     |                         |                   |                  |
| 10-Fold         | 52.1                     | 52.1 ± 2.7              | 5.2               | 4.2              |
|                 | 53.6                     |                         |                   |                  |
|                 | 51.4                     |                         |                   |                  |
|                 | 52.0                     |                         |                   |                  |
|                 | 48.1                     |                         |                   |                  |

CV: coefficient of variation; conc.: concentration; n: number of replicates; RE: relative error.

Table 4. Stability of prazosin in human plasma, prazosin stock standard solution and IS stock standard solution under different evaluated storage conditions (n = 3).

| Compound         | Type of stability and duration | Matrix            | QC level, concentration (ng/mL) | Accuracy (mean ± SD, %) | Precision (CV, %) |
|------------------|--------------------------------|-------------------|-------------------------------|------------------------|------------------|
| Prazosin         | Short-term (25 ± 4 °C), 24 h    | Plasma            | LQC, 1.5                      | 94.0 ± 11.3            | 12.0             |
|                  |                                |                   | HQC, 22.5                     | 98.8 ± 1.7             | 1.7              |
|                  | Postpreparative in autosampler | (15 ± 3 °C), 48 h | LQC, 1.5                      | 89.3 ± 8.7             | 9.7              |
|                  | Freeze and thaw, 7 cycles      |                   | HQC, 22.5                     | 103.2 ± 8.0            | 7.8              |
|                  | Long-term (~20 ± 10 °C), 30 days|                   | LQC, 1.5                      | 110.7 ± 5.3            | 4.8              |
|                  | Room temperature (25 ± 4 °C), 30 days | Stock standard solution | LQC, 1.5 | 101.9 ± 5.4 | 5.3 |
|                  | Chiller (5 ± 3 °C), 30 days    |                   | HQC, 22.5                     | 104.0 ± 4.7            | 4.5              |
| Terazosin, IS    | Room temperature (25 ± 4 °C), 30 days | Stock standard solution | LQC, 1.5 | 104.7 ± 7.3 | 7.0 |
|                  | Chiller (5 ± 3 °C), 30 days    |                   | HQC, 22.5                     | 105.5 ± 4.7            | 4.4              |

CV: coefficient of variation; SD: standard deviation; n: number of replicates; LQC: low-quality control, HQC: high-quality control.
A sufficiently sensitive analytical method is essential for pharmacokinetic and bioequivalence studies to accurately determine the pharmacokinetic parameters. According to FDA and EMA guidelines, LLOQ should be set to at least $C_{\text{max}}/5$% of the anticipated $C_{\text{max}}$. As there was no published pharmacokinetic data of prazosin on Malaysia’s population, pharmacokinetic data of other populations were thus used as guidance [21,25]. Gwak and Chun [21] reported a mean $C_{\text{max}}$ of 23.1 ng/mL after administration of 2 mg prazosin, while Zhu et al. [25] reported a $C_{\text{max}}$ range of 47.55–50.04 ng/mL after dosing with 4 mg prazosin. Based on these published $C_{\text{max}}$ values, 1 ng/mL was chosen as the LLOQ value for the current study.

Acetonitrile was used as a deproteinization agent in the study. Different ratios of acetonitrile to plasma at 1:1, 2:1, 3:1 and 4:1, were used. At ratios of 1:1 and 2:1, viscous and dirty samples were obtained, which were not suitable to be injected as they may damage the analytical column, clog the tubing and contaminate the system. When the acetonitrile and plasma ratios were increased to 3:1 and 4:1, clean supernatants were obtained after centrifugation. These two ratios were compared in terms of matrix effect and sensitivity at HQC level (22.5 ng/mL). Matrix factors of 0.92 and 0.93 were obtained for ratio of 3:1 and 4:1, respectively, which were close to each other. It was found that acetonitrile and plasma of ratio 3:1 gave a higher sensitivity than that obtained at a ratio of 4:1. LLOQ of 0.5 ng/mL at S/N ratio of above 5 was achieved. Therefore, acetonitrile and plasma at a ratio of 3:1 was selected for sample preparation. Based on the LLOQ of 0.5 ng/mL with 1 μL injection volume, the present analytical method is more sensitive than earlier published methods (Table 1) for the determination of prazosin in plasma samples.

In a previously published terazosin study [34], method validation was performed on terazosin with prazosin as an IS. No method validation was carried out on prazosin. In the present study, method validation was conducted on prazosin as an analyte to evaluate the reliability of the bioanalytical method [28,29]. Modification was made to the composition of mobile phase, volume of acetonitrile used as deproteinization agent and sample injection volume. Acetonitrile was reduced from 1000.0 μL to 750.0 μL as a deproteinization agent. Furthermore, a lower sample injection volume (1 μL instead of 2 μL) was used. A smaller injection volume helps to enhance peak symmetry by reducing the solvent diffusion effect and reduce any clogging issue, hence prolonging the shelf life of the analytical column.

**Comparison of prazosin pharmacokinetics with other studies**

The mean $C_{\text{max}}$ values of Minipress tablet (reference) and Minison tablet (test) were 21.1 ± 1.1 and 21.6 ± 1.1 ng/mL, respectively. Compared with the LLOQ value of 0.5 ng/mL obtained in the current study, LLOQ values >1.0 ng/mL reported in certain published studies [19,22–24] are not sufficiently sensitive for the present BE study. Quantification of the last few sampling time points at elimination phase will be very challenging with a high LLOQ value. This can affect the estimation of $k_e$, $t_{1/2}$ and extrapolated AUC. The LLOQ value of 0.5 ng/mL in the current study was sufficiently sensitive to reliably determine prazosin concentration in plasma samples of the bioequivalence study. The present bioanalytical method not only fulfilled the requirement of the LLOQ (5% of the

---

**Table 5. Pharmacokinetic parameters of prazosin (2 × 1 mg) in 26 Malaysian volunteers in fasting condition (mean ± SD, n = 26).**

| Parameters | Fasting condition |
|------------|------------------|
| $C_{\text{max}}$ (ng/mL) | Test | Reference |
| AUC0–16 (h.ng/mL) | 86.1 ± 4.9 | 86.3 ± 4.6 |
| AUC0–16 (h.ng/mL) | 89.5 ± 4.9 | 90.1 ± 4.8 |
| Extrapulated AUC16–∞ (%) | 4.0 ± 0.4 | 4.2 ± 0.4 |
| $T_{\text{max}}$ (h) | 1.3 ± 0.1 | 1.2 ± 0.2 |
| $t_{1/2}$ (h) | 2.3 ± 0.1 | 2.5 ± 0.1 |
| $k_e$ (1/h) | $3.1 \times 10^{-2} \pm 1.0 \times 10^{-2}$ | $2.9 \times 10^{-2} \pm 1.0 \times 10^{-2}$ |

---

**Figure 3.** The mean plasma prazosin concentration–time profiles of Minipress and Minison tablets. Mean ± SD (n = 26).
expected $C_{\text{max}}$ of the study) but also fulfilled the percentage of extrapolated AUC (AUC$_{\text{t1/2}}$) of below 20%, with percentage of AUC$_{10-\infty}$/AUC$_{0-\infty}$ of all subjects <10%. The results also indicate that sufficient sampling points were collected in the study. The $C_{\text{max}}$ and AUC values were similar to the results reported by Guehen et al. [20] and Gwak and Chun [21]. Guehen et al. [20] conducted a pharmacokinetic study in 12 healthy subjects using two formulations of 2 mg prazosin tablets. The study reported mean $C_{\text{max}}$ AUC$_{0-\infty}$ and $t_{1/2}$ values of 17.8 ± 4.0 ng/mL, 75 ± 16.9 ng/mL, 2.0 ± 0.9 h and 2.3 ± 0.3 h, respectively, for reference product while values of 16.6 ± 4.1 ng/mL, 72 ± 13.9 ng/mL, 1.5 ± 0.7 and 2.4 ± 0.4 h, respectively, were reported for test product. Gwak and Chun [21] reported mean $C_{\text{max}}$ AUC, $t_{\text{max}}$ and $t_{1/2}$ values of 23.1 ± 16.5 ng/mL, 108.4 ± 74.2 h ng/mL, 2.1 h and 2.5 ± 0.6 h, respectively, after administration of 2 mg of prazosin in Korean subjects. Zhu et al. [25] conducted a bioequivalence study on 4-mg prazosin. The $C_{\text{max}}$ AUC$_{0-\infty}$ and AUC$_{0-\infty}$ values of the study were 47.6 ± 12.2 ng/mL, 168.2 ± 23.5 h ng/mL and 174.4 ± 24.4 h ng/mL respectively, for reference tablet; 50.0 ± 16.6 ng/mL, 171.6 ± 33.2 h ng/mL and 177.1 ± 34.2 h ng/mL respectively, for test tablet, which were approximately two-time higher than the obtained pharmacokinetic parameters in the current study, showing that the pharmacokinetics of prazosin is linear from 2 to 4 mg.

Adverse events and orthostatic hypotension

Alpha adrenoceptor antagonists are known to cause orthostatic hypotension, which may contribute toward reduced vascular resistance [35–37]. In a study done by Graham et al. [38] in hypertensive patients, all patients (100%) showed tachycardia and developed serious orthostatic hypotension after administration of 2-mg prazosin. The side effects disappeared on day 2 after the administration of the second 2-mg tablet. Bhanu et al. [39] and van der Worp et al. [40] found that the α1-adrenoceptor antagonists were associated with up to two-fold increased orthostatic hypotension, compared with placebo. Hiremath et al. [41] demonstrated a consistently higher risk of hypotension-related adverse events with the use of alpha blockers. The study reported the proportion of hypotension and syncopal adverse events among alpha blocker users within the first 90 days were 38.4% and 40.2%, respectively. Rivasi et al. [42] stated that orthostatic hypotension risk is higher for α1-adrenoceptor antagonists which present the lowest uroselectivity and the drop in blood pressure could be more severe after the first dose (first-doses phenomenon). Therefore, bed-time administration of these alpha blockers is preferable. Other studies also reported orthostatic hypotension adverse event with the use of α1-adrenoceptor antagonist [43–45]. Peak concentration occurred between 1 and 4 h post administration [39]. High odds of drug-induced orthostatic hypotension was taken into safety consideration at the planning stage of the study. A precaution step was taken in the study by bedside taking of blood samples for the first 4 h of the study. During the first 4 h, subjects rested supinely in bed with a pillow placed under their head. All subjects underwent clinical assessment before allowing them to leave their bed for the first 4 h post dosing of 2-mg prazosin tablet. Even with the implementation of the safety policy, the study still reported a total of five adverse events post dosing of 2-mg prazosin tablet. Three of the five adverse events were dizziness while the other two were orthostatic hypotension (6.67%) where their systolic and diastolic blood pressures dropped more than 20 and 10 mmHg, respectively. However, the incidence of orthostatic hypotension from our studies was much lower compared with others [38,44]. The subjects recovered after a few h on the same day and withdrew from the study when subsequent blood samplings were not possible due to the orthostatic hypotension. Other subjects reported a drop in systolic and diastolic blood pressures of ≤ 20 and ≤ 10 mmHg, respectively, which should not be categorized as orthostatic hypotension [46,47]. The decrease in systolic and diastolic blood pressures was well tolerated and a total of 26 subjects completed the study. The findings suggest that bedside taking of blood samples reduces the occurrence and frequency of orthostatic hypotension and hence the dropout rate of the study. High dropout rate can lead to an underpowered study and fail to meet bioequivalence. This will require an increased investment in time, people and money to identify the cause of bioequivalence failure, as well as a re-run of the study [48]. This sampling approach can be applied to other α1-adrenoceptor antagonists such as terazosin, doxazosin or other medications that cause postural hypotension.

Conclusion

The study developed a sensitive, simple, accurate and rapid LC/ESI-MS/MS method for the determination of prazosin in human plasma and the method was applied to a bioequivalence study of two different formulations containing prazosin. The validated prazosin bioanalytical method was sufficiently sensitive to accurately determine the pharmacokinetic parameters. Bedside taking of blood samples is critical to reduce the occurrence of orthostatic hypotension and subject dropout rate. This practice should be recommended when conducting bioequivalence study of drugs with orthostatic hypotension risk.

Acknowledgements

The study was sponsored by Y.S.P. Industries (M) Sdn. Bhd. The Y.S.P. Industries (M) Sdn. Bhd. is also acknowledged for providing test and reference products of the study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

[1] Constantine JW, McShane WK, Scriabine A, et al. Analysis of the hypotensive action of prazosin. Progson J Med. 1975;11:18–35.
[2] Kosman ME. Evaluation of a new antihypertensive agent: prazosin hydrochloride (Minipress). JAMA. 1977;238(2):157–159.
[3] Koshy MC, Mickle D, Bourgiognie J, et al. Physiologic evaluation of a new antihypertensive agent: prazosin HCI. Circulation. 1977;55(3):533–537.
[4] Iwatsubo K, Umemura S. Alpha adrenergic receptor blockers for patients with hypertension. Nihon Rinsho. 2006;64(6):294–299.
[5] Hong B, Ding X, Lia H, et al. Combination treatment of captopril and prazosin to treat patients with gestational hypertension. Exp Ther Med. 2018;16(4):3694–3702.
[6] Bendall MJ, Baloch KH, Wilson PR. Side effects due to treatment of hypertension with prazosin. Br Med J. 1975; 2(5973):727–728.

[7] Gabriel R, Meek D, Ghosh BC. Collapse after prazosin hydrochloride. Lancet. 1975; 1(7915):1095.

[8] Rieckert H, Weidinger G, Schardt FW. Orthostatic hypotension during antihypertensive treatment with bunazosin and prazosin. Drug Invest. 1993; 6(5):327–333.

[9] Poon IO, Braun U. High prevalence of orthostatic hypotension and its correlation with potentially causative medications among elderly veterans. J Clin Pharm Ther. 2005; 30(2):173–178.

[10] Gupta V, Lipsitz LA. Orthostatic hypotension in the elderly: diagnosis and treatment. Am J Med. 2007; 120(10):841–847.

[11] Hartog LC, Cimzar-Sweelissen M, Knipscheer A, et al. Orthostatic hypotension does not predict recurrent falling in a nursing home population. Arch Gerontol Geriatr. 2017; 68:39–43.

[12] Mol A, Bui Hoang PTS, Sharmin S, et al. Orthostatic hypotension and falls in older adults: a systematic review and meta-analysis. J Am Med Dir Assoc. 2019; 20(5):589–597.e5.

[13] Juraschek SP, Lipsitz LA, Beach JL, et al. Association of orthostatic hypotension timing with clinical events in adults with diabetes and hypertension: results from the ACCORD trial. Am J Hypertens. 2019; 32(7):684–694.

[14] Frith J, Parry SW. New horizons in orthostatic hypotension. Age Ageing. 2017; 46(2):168–174.

[15] Saiedon NI, Pin TM, Frith J. The prevalence of orthostatic hypotension: a systematic review and Meta-analysis. J Gerontol A Biol Sci Med Sci. 2020; 75(1):117–122.

[16] AlOthman ZA, Alsheetaan KM, Aboul-Enein HY, et al. Applications of shun shell column and nanocomposite sorbent for analysis of eleven anti-hypertensive in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2020; 1146:122337.

[17] Dokladalova J, Coco SJ, Lemke PR, et al. Determination of polythiazide and prazosin in human plasma by high-performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 1981; 224(1):33–41.

[18] Larochelle P, du Souich P, Hamet P, et al. Prazosin plasma concentration and blood pressure reduction. Hypertension. 1982; 4(1):93–101.

[19] Bhamra RK, Flanagan RJ, Holt DW. High-performance liquid chromatographic measurement of prazosin and terazosin in biological fluids. J Chromatogr B Analyt Technol Biomed Life Sci. 1986; 380(1):216–221.

[20] Guelen PJ, Janssen TJ, Lam MH, et al. Comparative bioavailability study of two brands of prazosin-containing tablets in healthy volunteers. Pharm Weekbl Sci. 1990; 12(5):184–187.

[21] Gwak H, Chun I. Simplified HPLC method for the determination of prazosin in human plasma and its application to single-dose pharmacokinetics. Biomol Ther. 2005; 13(2):90–94.

[22] Sultana N, Shah SN, Hasan N, et al. Simultaneous determination of prazosin and NSAIDS in bulk, pharmaceutical formulations and human serum by novel RP-HPLC method. World J Pharm Res. 2015; 4(7):333–350.

[23] Rathinavelu A, Malave A. High-performance liquid chromatography using electrochemical detection for the determination of prazosin in biological samples. J Chromatogr B Analyt Technol Biomed Life Sci. 1995; 670(1):177–182.

[24] Eswarud MM, Rao AL, Vijay K. Bioanalytical method development and validation for simultaneous determination of prazosin and polythiazide drugs in spiked human plasma by RP-HPLC. Int J Pharm Chem Biol Sci. 2019; 9(1):61–70.

[25] Zhu YG, Zhang BK, Chen BM, et al. Determination of prazosin hydrochloride in human plasma by HPLC MS: application to its bioequivalence. Chin Pharm J. 2006; 41:1174–1177.

[26] Briscoe C, Stiles MR, Hage D. System suitability in bioanalytical LC/MS/MS. J Pharm Biomed Anal. 2007; 44(2):484–491.

[27] Loh GOK, Wong EYL, Tan YTF, et al. Simple and rapid LC-MS/MS method for determination of sitagliptin in human plasma and application to bioequivalence study. J Chromatogr B Analyt Technol Biomed Life Sci. 2020; 1159:122337.

[28] European Medicines Agency; Committee for Medicinal Products for Human Use (CHMP). Guideline on bioanalytical method validation. London, UK: EMA; 2012.

[29] Food and Drug Administration of the United States (US-FDA). U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM). Bioanalytical method validation (draft) in guidance for industry. Rockville, MD: US-FDA; 2018.

[30] Chow SC, Liu JP. Design and analysis of bioavailability and bioequivalence studies. New York: CRC Press, Taylor & Francis Group; 2009.

[31] Byers JP, Sarver JG. Chapter 10 – pharmacokinetic modeling. In: Hacker M, Messer W, Bachmann K, editors. Pharmacology. San Diego: Academic Press; 2009. p. 201–277.

[32] Hunderlater P, Saghir SA. Pharmacokinetics. Encyclopedia of toxicology., 3rd ed. Academic Press, Elsevier, 2014. 849–855.

[33] ASEAN Guideline for The Conduct of Bioequivalence Studies; 2015.

[34] Loh Gong WK, Wong EYL, Tan YTF, et al. Simple and fast LC-MS/MS method for quantification of terazosin in human plasma and application to bioequivalence study. J Chromatogr B Biomed Sci. 2021; 1163:122517.

[35] Wong AK, Lord SR, Sturnieks DL, et al. Angiotensin system-blocking medications are associated with fewer falls over 12 months in community-dwelling older people. J Am Geriatr Soc. 2013; 61(5):776–781.

[36] Press Y, Punchik B, Freud T. Orthostatic hypotension and drug therapy in patients at an outpatient comprehensive geriatric assessment unit. J Hypertens. 2016; 34(2):351–358.

[37] Woyisz ZB, Kasikiewicz A, Magnuszewski L. Health and functional determinants of orthostatic hypotension in geriatric ward patients: a retrospective cross sectional cohort study. J Nutr Health Aging. 2019; 23(6):509–517.

[38] Graham RM, Thornell IR, Gain JM, et al. Prazosin: the first-choice anti-hypertensive medication in geriatric ward patients: a retrospective cross sectional cohort study. J Nutr Health Aging. 2019; 23(6):509–517.

[39] Bhanu C, Nirmoms D, Petersen I, et al. Drug-induced orthostatic hypotension: a systematic review and Meta-analysis of randomised controlled trials. PLoS Med. 2021; 18(11):e1003821.

[40] van der Worp H, Jellema P, Hordijk I, et al. Discontinuation of alpha-blocker therapy in men with lower urinary tract symptoms: a systematic review and Meta-analysis. BMJ Open. 2019; 9(11):e030405.

[41] Hiremath S, Ruzicka M, Petrcich W, et al. Alpha-blocker use and the risk of hypertension and hypotension-related clinical events in women of advanced age. Hypertension. 2019; 74(3):645–651.

[42] Rivasi G, Ranaelli M, Mosello E, et al. Drug-related orthostatic hypotension: beyond anti-hypertensive medications. Drugs Aging. 2020; 37(10):725–738.

[43] Bird ST, Delaney JA, Brophy JM, et al. Tamsulosin treatment for benign prostatic hyperplasia and risk of severe
hypotension in men aged 40-85 years in the United States: risk window analyses using between and within patient methodology. BMJ. 2013;347:f6320.

[44] Dominguez-Dominguez C, Calderón-Ospina C. Postural hypotension associated with inappropriate choice of drug treatment of benign prostatic hyperplasia. Rev Colomb Cienc Quim Farm. 2015;44(3):276–281.

[45] Roehrborn CG, Cruz F, Fusco F. z1-Blockers in men with lower urinary tract symptoms suggestive of benign prostatic obstruction: is silodosin different? Adv Ther. 2017;33(12):2110–2121.

[46] Freeman R, Wieling W, Axelrod FB, et al. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. Clin Auton Res. 2011;21(2):69–72.

[47] Brignole M, Moya A, de Lange FJ, ESC Scientific Document Group, et al. Practical instructions for the 2018 ESC guidelines for the diagnosis and management of syncope. Eur Heart J. 2018;39(21):e43–e80.

[48] Rasmussen HE, Ma R, Wang JJ. Controlling type 1 error rate for sequential, bioequivalence studies with crossover designs. Pharm Stat. 2019;18(1):96–105.