

Simplified HPLC Method for the Determination of Prazosin in Human Plasma and Its Application to Single-dose Pharmacokinetics

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Abstract – Prazosin hydrochloride is an antihypertensive drug with selective α_1 -adrenoreceptor blocking effects. A simple high performance liquid chromatographic method has been developed and validated for the quantitative determination of prazosin in human plasma. A reversed-phase C18 column was used for the separation of prazosin and terazosin (internal standard) with a mobile phase composed of water, acetonitrile and triethylamine (75 :25:0.1, v/v; pH 5.0) at a flow rate of 1.5 ml/min. The fluorescence detector was set at excitation and emission wavelengths of 250 and 370 nm, respectively. Intra- and inter-day precision and accuracy were acceptable for all quality control samples including the lower limit of quantification of 0.5 ng/ml. Good recovery (> 80%) was seen in plasma. Prazosin was stable in human plasma under various storage conditions. This method was used successfully for a pharmacokinetic study in plasma after oral administration of a single 2-mg dose as prazosin base to 16 healthy volunteers. The maximum plasma concentration of prazosin was 23.1 ± 16.5 ng/ml at 2.1 h, and the mean area under the curve and elimination half-life were calculated to be 108.4 ± 74.2 ng · hr/ml and 2.5 ± 0.6 h, respectively.

Keywords □ Prazosin, terazosin, high-performance liquid chromatography, pharmacokinetic study

INTRODUCTION

Prazosin (Fig. 1), 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furoyl)piperazine, is a quinazoline derivative used in the treatment of hypertension and congestive heart failure. It is known to be a selective antagonist of α_1 -adrenoreceptors (Constantine, 1975; Hess, 1975; Hobbes *et al.*, 1978). It has been reported that prazosin does not possess significant effects on β -receptors or α_2 -receptors, and although it has direct vasodilator activity at high concentrations, this is unlikely to contribute to its therapeutic action (Vincent *et al.*, 1985).

Prazosin is well absorbed after oral administration; it is required 1-2 hours to reach peak plasma concentrations. It has a short half-life of 2.5 hours and consequently a relative short duration of action, requiring that it be administered 2-3 times a day. It is eliminated mainly by hepatic metabolism and renal insufficiency and aging do not affect the pharmacokinetics of prazosin (Akduman and Crawford, 2001).

Even though many analytical methods for terazosin (Patterson, 1985; Sekhar *et al.*, 1998; Cheah *et al.*, 2000; Kang *et al.*, 2001; Cho *et al.*, 2002), another α_1 -adrenoreceptor antagonist, have been reported, few studies have been conducted for prazosin analysis from blood samples (Rathinavelu and Malave, 1995). Since prazosin is often used to treat hypertensive patients, a selective, sensitive and fully validated analytical method is required for pharmacokinetic studies of prazosin. In the present study, we developed a validated HPLC method to analyze prazosin from plasma samples, and applied it to a pharmacokinetic study on Korean healthy volunteers.

MATERIALS AND METHODS

Equipment

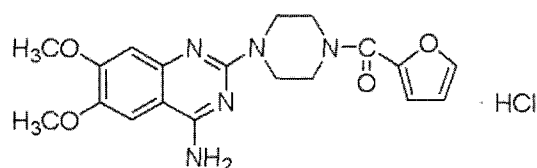


Fig. 1. Chemical structure of prazosin hydrochloride.

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The HPLC system consisted of an isocratic pump (PU-1580, Jasco, Tokyo, Japan), a sample injector (Rheodyne 7125, Cotati, CA, USA), a 50 μ l-loop, a fluorescence detector (FP-2020 Plus, Jasco, Tokyo, Japan) and an integrator (Model 4290, Varian, Palo Alto, CA, USA). A reversed-phased C18 column (10 μ m particles, μ Bondapak C18, 3.9×300 mm, Waters) equipped with a precolumn insert (μ Bondapak C18 10 μ m 125 Å C18 Guard-Pak insert, Waters Corp., Milford, MA, USA) was used.

Materials and reagents

Prazosin hydrochloride, terazosin hydrochloride (internal standard, IS) and *tert*-butylmethyl ether were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and methanol (J.T. Baker, Inc., Phillipsburg, NJ, USA) used were of HPLC grade. All water (18 M Ω) used in this method was obtained by passing purified water through a Water Purification System (Elgastat UHQ PS, Elga Ltd., UK). Other reagents were of analytical grade.

Preparation of standard plasma samples

Working stock solutions of prazosin hydrochloride and terazosin hydrochloride (IS) were prepared in methanol at a concentration of 1 mg/ml. Prior to use, these two stock solutions were further diluted with 50% methanol to obtain working solutions at the concentration of 10 μ g/ml. An appropriate dilution of the working solution with drug free plasma obtained from healthy volunteers gave a concentration range between 0.5 and 50 ng/ml of prazosin hydrochloride. To 1 ml of the prepared plasma were added 100 μ l of IS solution at a concentration of 500 ng/ml in 50% methanol.

Seven calibration samples (0.5, 1, 5, 10, 20, 30 and 50 ng/ml) were prepared by spiking blank plasma with appropriate volumes of the working solutions. Quality control samples (0.5, 1, 5, 10, 20, 30 and 50 ng/ml) and stability samples (5 and 30 ng/ml) were independently prepared in the same manner.

Sample preparation

To 1 ml of plasma were added 100 μ l of IS solution and 200 μ l of 2.0 M sodium hydroxide solution. After a brief vortex mixing, 7 ml of *tert*-butylmethyl ether was added and performed by vortex mixing for 5 min. The tubes were then centrifuged at 3000 g for 5 min. Six ml of the organic phase were transferred to another set of clean conical centrifuge tubes and back-extracted with 200 μ l of 0.05% phosphoric acid by vortex mixing for 3 min. The tubes were then centrifuged at 3000 g for

5 min, and 50 μ l of the aqueous phase was injected onto the liquid chromatograph.

Chromatographic conditions

The reversed-phase C18 column was eluted with a mixture of water, acetonitrile and triethylamine (75 : 25 : 0.1, v/v; pH 5.0 adjusted with phosphoric acid) at a flow rate of 1.5 ml/min. The mobile phase was degassed before use. The fluorescence detector was set at excitation and emission wavelengths of 250 and 370 nm, respectively. All analyses were performed at room temperature.

Method Validation

Specificity

The degree of interference by endogenous plasma constituents with prazosin and IS was evaluated by inspection of chromatograms derived from processed blank and spiked plasma samples, and also from processed blank samples injected during each analytical run.

Calibration curve

Calibration standard plasmas at the concentrations of 0.5, 1, 5, 10, 20, 30 and 50 ng/ml including the lower limit of quantification ($n = 5$ for each concentration point) were prepared and assayed as mentioned above. Blank samples were included in the calibration curves to assure no interfering components were co-eluted. The calibration curves were plotted using peak area ratios of prazosin to IS versus known prazosin concentrations.

Accuracy and precision

Intra-day accuracy and precision of the method were estimated by assaying five replicate plasma samples at five different concentrations, in five analytical runs. The overall mean precision was defined by the percentage of relative standard deviation (RSD) of five standards at five different concentrations analyzed on the same day. Inter-day variability was estimated from the analysis of the five standards on five separate days during method validation.

Recovery

Recovery of prazosin was determined by comparing observed prazosin peak area in extracted plasma samples ($n = 3$) with the mean peak area obtained from direct injection of the corresponding non-processed standard solutions. The recovery was measured at four different concentrations (0.5, 10, 30 and 50 ng/ml) over the concentration range used. Regarding the IS, recovery was only calculated at the working concentration (50 ng/ml).

Stability

The freeze-thaw stability of prazosin hydrochloride in plasma was evaluated after three freeze-thaw cycles. Stability control plasma samples in triplicate at the levels of 5 and 30 ng/ml were immediately frozen at -70°C , and thawed at room temperature three consecutive times. After that, the samples were processed and assayed. The stability of prazosin in stability control samples stored at room temperature for 24 h and at -70°C for 4 weeks was also assessed. The mean values of prazosin were compared with the initial ones, which were assayed immediately after preparation of stability control plasma samples. The stability of the samples was expressed as the residual concentration related to the freshly prepared samples.

Single-dose pharmacokinetic study

The assay was applied to a pharmacokinetic study of prazosin following 2-mg single dose. According to the Guidance for Bioequivalence Test of Korea Food and Drug Administration, eight male and eight female healthy volunteers aged between 19 and 25 years were selected for the study. All subjects gave their written informed consents, and the clinical protocol was approved by the Ethics and Review Committee. The volunteers were judged to be healthy by physical examination and were not receiving any medications one week before and during the study period. Prazosin hydrochloride (Minipress[®] Tablets, lot No. 3362-11105, Pfizerkorea Inc., Seoul, Korea) was administered orally with 240 mL of water in the morning (8:00 A.M.) after 12-hr overnight fast. Any food and drink were withheld for at least 4 hr after dosing. Lunch and dinner of beef soup with rice were served 4 and 10 hr after dosing. Ten milliliters of blood samples were collected in green-top vacutainers (containing sodium heparin) via an in-dwelling cannula placed on the forearm before (predose) and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 hr after drug ingestion. The blood samples were centrifuged at 3000 g for 15 min at room temperature, and the plasma was transferred to separate plasma tube. The separated plasmas were stored at -70°C until analysis. Comparison of peak area ratios from the unknown samples with those from the calibration curve permitted quantitation of the samples.

RESULTS AND DISCUSSION

Prazosin hydrochloride, one of quinazoline derivatives, is a lipophilic molecule with an amine group. Its determination in plasma samples using a HPLC method requires a previous extraction. The extraction procedure with *tert*-butylmethyl

ether described by Patterson (1984) and Cho et al. (2002) was modified for the extraction of prazosin and terazosin (IS). A final back-extraction step using 0.05% phosphoric acid was introduced to remove any endogenous substances such as residual plasma lipids and to increase drug concentrations in the aqueous extract. This procedure affords a longer column life and cleaner chromatograms (Fig. 2). Terazosin hydrochloride was adopted as the internal standard since it showed a similar chromatographic behaviour as prazosin and at the same time it was clearly separated from prazosin and its degradation products. The chromatographic conditions were based on those used by Cho et al. (2002).

Specificity

Fig. 2 shows the well-resolved chromatographic peaks of prazosin and terazosin at 6.3 and 5.1 min, respectively. The chromatogram of blank plasma after extraction consistently contains no significant interfering peaks. Interference of prazosin with internal standard or other metabolites was not observed for the samples obtained at different time intervals from healthy volunteers orally given prazosin at a 2 mg-dose and samples of the calibration curve. It can be concluded that the proposed method is specific and selective for prazosin.

Calibration and linearity

The relation between prazosin hydrochloride concentrations and peak area ratio of prazosin to IS was linear over the entire

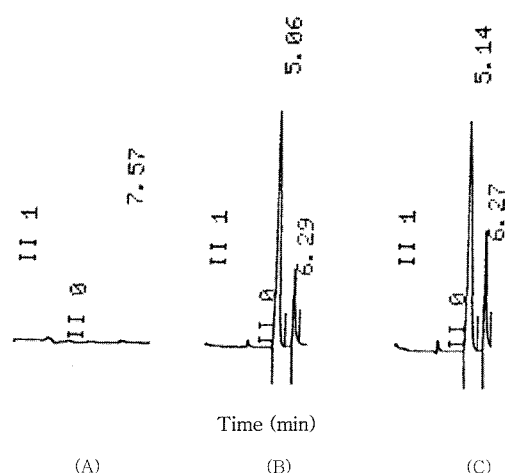


Fig. 2. Chromatograms for (A) control human plasma, (B) human plasma spiked with prazosin hydrochloride (10 ng/ml) and internal standard (IS), terazosin hydrochloride (50 ng/ml), and (C) plasma samples obtained from a healthy volunteer 2.5 hr (C) after 2-mg oral dose of prazosin.

Table I. Intra-day and inter-day precision and accuracy of the determination of prazosin hydrochloride in plasma

| Concentration (ng/ml) | RSD (%) | | Accuracy (%) |
|--------------------------|---------------|---------------|--------------|
| | Intra (n = 5) | Inter (n = 5) | |
| 0.5 | 4.4 | 2.5 | 114.2 |
| 1 | 7.9 | 9.6 | 111.0 |
| 5 | 0.56 | 7.4 | 102.6 |
| 10 | 0.85 | 4.7 | 103.3 |
| 20 | 2.4 | 1.7 | 103.2 |
| 30 | 0.88 | 3.3 | 102.2 |
| 50 | 3.0 | 3.5 | 104.0 |

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

concentration range 0.5 - 50 ng/ml, including the LOQ. Linearity was assessed by a weighted least squares regression coefficient. Calibration curve was found to have a correlation coefficient (r^2) of 1, slope of 0.0272 and intercept of 0.0006.

Limit of detection and limit of quantification

The limit of detection (LOD) is the lowest concentration of an analyte that the analytical process can reliably differentiate from the background levels. The LOQ is the lowest amount of an analyte which can be quantitatively determined with defined precision and accuracy under the given experimental conditions. Both values are calculated as concentrations. The LOQ of prazosin hydrochloride was determined as the sample concentration of prazosin hydrochloride resulting in peak heights of 10 times baseline noise. The LOQ was found to be 0.5 ng/ml. Based on 3 times peak height of baseline noise, the limit of detection was calculated to be 0.1 ng/ml.

Accuracy, precision and recovery

The intra- and inter-day precisions of the methods were determined by the assay of five samples of drug-free plasma containing known concentrations of prazosin hydrochloride. As described in Table I, the intra- and inter-day coefficients of variation (C.V., %) were within 10%, which were acceptable for all quality control samples including the LOQ. The accuracy of prazosin hydrochloride ranged between 102.1 and 114.2%. For the criteria of intra- and inter-day accuracy and precision, accuracy values should be within 85-115% over the calibration range, except at the LOQ, it should be between 80-120%, and the C.V. values should be not more than 15% over the calibration range, except at the LOQ, it should not exceed 20% (Kamas et al., 1991; FDA, 2001). All the batches met the quality control acceptance criteria.

The extraction recovery of prazosin hydrochloride at con-

Table II. Stability of prazosin hydrochloride in plasma

| Concentration (ng/ml) | Treatment | Percentage of initial value |
|--------------------------|-------------------------------------|--------------------------------|
| 5 | Three freeze-thaw cycles | 108.0 \pm 4.9 |
| | Stored at room temperature for 24 h | 93.3 \pm 1.0 |
| | Stored at -70°C for 4 weeks | 103.8 \pm 3.7 |
| 30 | Three freeze-thaw cycles | 97.1 \pm 3.5 |
| | Stored at room temperature for 24 h | 94.6 \pm 9.4 |
| | Stored at -70°C for 4 weeks | 97.6 \pm 6.2 |

Each data represents the mean \pm S.D. (n = 3).

centrations of 0.5, 10, 30 and 50 ng/ml was 83.9 ± 3.9 , 84.0 ± 2.8 , 82.3 ± 2.8 and 80.4 ± 2.3 % (n = 3), respectively. These results suggested that there was no significant difference in extraction recovery at different concentrations of prazosin hydrochloride. For the IS, a recovery of 57.1 ± 2.0 % was obtained. These results were responded to the acceptance criteria of recovery which should be consistent, precise and reproducible with near 100% or not lower than 50-60% (FDA, 2001).

Stability

It is well known that drugs may be degraded in plasma during storage or analytical run due to temperature, light, air and enzymes. Knowledge of the stability of the drug in test material is a prerequisite for obtaining valuable data. The stability of prazosin hydrochloride under various conditions is described in Table II. Under all conditions tested, prazosin hydrochloride was stable with detected concentrations of at least 93.3% of the initial concentration. Results of the study indicated the good stability of prazosin in plasma samples when stored over one month period at -70°C, protected from light. Stability data obtained were responded to the acceptance criteria of stabilities that the deviation compared to the freshly prepared standard should be within $\pm 15\%$ (FDA, 2001).

Pharmacokinetics

This analytical method was applied to the quantitation of plasma prazosin concentrations in around 200 samples from healthy volunteers in pharmacokinetic studies. A typical chromatogram of a plasma sample in a subject at 2.5 hr after drug administration are shown in Fig. 2. The application of the method to determine the plasma level in humans is depicted in Fig. 3. After oral administration of prazosin hydrochloride tablets at 2 mg dose as the base, the maximum plasma concentration of prazosin was 23.1 ± 16.5 ng/ml at 2.1 h. The mean area under the curve and elimination half-life were calculated to be 108.4 ± 74.2 ng \cdot hr/ml and 2.5 ± 0.6 h, respectively.

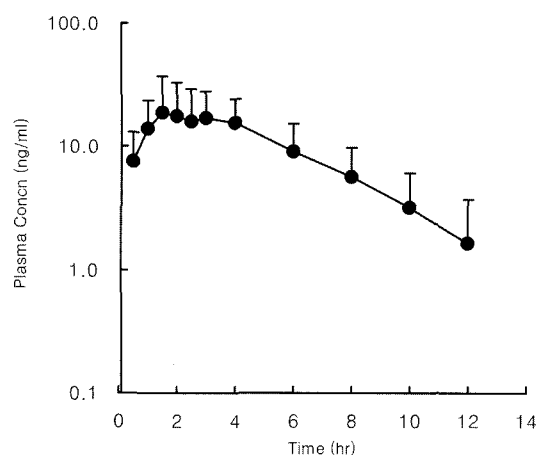


Fig. 3. Mean plasma concentration of prazosin vs. time after oral administration of Minipress® tablet at the dose of 2 mg prazosin base. Each point represents the mean \pm S.D. of 16 healthy subjects.

In conclusion, the determination of prazosin with HPLC has proven to be simple, rapid, sensitive, specific, accurate and reproducible. The intra- and inter-day precision and accuracy were acceptable in all quality control samples including the LOQ of 0.5 ng/ml. Recovery evaluations showed that there was no difference in extraction recovery at different concentrations of prazosin hydrochloride. Prazosin was stable in human plasma under various storage conditions including three freeze-thaw cycles. The applicability of this method for pharmacokinetic and bioequivalence studies in human has also proved to be suitable. Therefore, this simple and validated assay could readily be used in any pharmacokinetic studies using humans.

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