Improvement of Doxazosin Determination in Human Plasma Using High-Performance Liquid Chromatography with Fluorescence Detection

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Abstract

A simple, sensitive, rapid, and reproducible high-performance liquid chromatographic method is developed and validated for the determination of doxazosin in human plasma without a solvent extraction procedure. This method involves plasma protein precipitation using methanol. The structurally related compound prazosin is used as an internal standard. Doxazosin is detected with high sensitivity using spectrofluorimetry. Over the concentration range 0.5–20 ng/mL, the absolute recovery values are all greater than 98%. The method has a quantitation limit of 0.5 ng/mL. The intra- and interday coefficient of variation and inaccuracy values are all less than 8% and 7%, respectively. Therefore, the method has been applied in pharmacokinetic studies of doxazosin.

Introduction

Doxazosin mesylate (4-amino-2-[4-(1,4-benzodiaxan-2-carbonyl)-piperazin-1-yl]-6,7-dimethoxyquinazoline myselate), a quinazoline derivative, is effective and well known for treatment of hypertension and benign prostatic hyperplasia by selectively blocking α-1 adrenoreceptor (1–3). It is structurally similar to prazosin (Figure 1). Different analytical methods have been reported for determination of doxazosin in biological fluids (4–6) and pharmaceutical formulations (7–10). For analysis of human plasma, almost all of these methods were based on a high-performance liquid chromatographic (HPLC) system with fluorescence detection (4–6). Fouda et al. reported the method using an alumina-based column and glass-bead guard column (4); both column systems were expensive and may not be available for purchase. In addition, the latter technique used a high-pH mobile phase, which may deteriorate the HPLC instrument. Moreover, in the previous studies, either the usage of solvent extraction procedure (4–6) or solid-phase extraction (SPE) (6) prior to HPLC determination seems to be a more complicated and lengthy method. Generally, sensitive HPLC methods with fluorescence detection that have been reported for doxazosin are stated to detect levels no lower than 1 ng/mL (5–6).

The HPLC method described here is simple, sensitive, and reproducible for doxazosin determination in serum samples with low background interference and may be suitable for routine clinical application following therapeutic doses. The assay method of doxazosin is also validated to provide enough selectivity and sensitivity in pharmacokinetic and bioequivalence studies.

Experimental

Chemicals

Doxazosin mesylate was purchased from Euresian (Mumbai, India), and prazosin hydrochloride was purchased from and Aldamex AG (Schweiz, Switzerland). The purity of doxazosin mesylate and prazosin hydrochloride was 99.99% and 99.9%, respec-

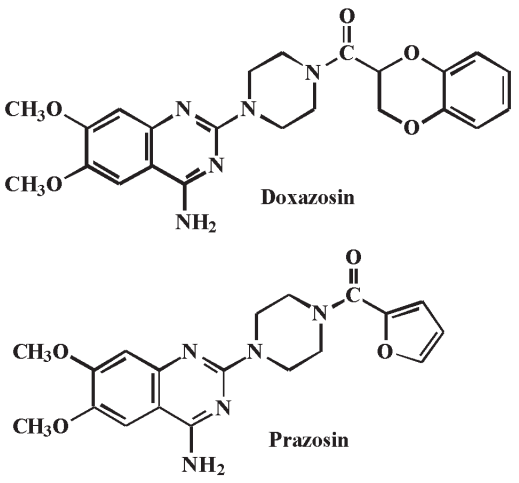

Figure 1. Chemical structures of doxazosin and prazosin.

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tively. All other solvents were of HPLC grade and obtained from various sources. Analytical-grade disodium hydrogen orthophosphate was obtained from Fisher Chemicals (Fairlawn, NJ).

Instrumentations and conditions

The HPLC system consisted of a pump (LC-10ATVP, Shimadzu, Kyoto, Japan), fluorescence detector (RF-10AXL, Shimadzu) equipped with system controller (SCL-10AVP, Shimadzu), and Rheodyne 7725 sample injector (Rohnert Park, CA) fitted with a 20-µL sample loop. The chromatographic separations were carried out on Apollo C18 column (250- × 4.6-mm i.d., 5 µm, 250A) (Alltech, Deerfield, IL) fitted with a refillable guard cartridge (Alltech) packed with Apollo C18 (7.5- × 4.6-mm i.d., 5 µm). The mobile phase was methanol–acetonitrile–0.04M disodium hydrogen orthophosphate (22:22:56 v/v) adjusted to pH 5 with 0.9M phosphoric acid. All separations were performed isocratically at a flow rate of 1.2 mL/min, and the column temperature was maintained at room temperature. The detector was operated with an excitation wavelength of 246 nm and an emission wavelength of 389 nm

Standard solutions

Stock solution of doxazosin and the internal standard, prazosin equivalent to 1 mg/mL, was prepared separately in methanol. Working concentrations of doxazosin in plasma at 20, 10, 5, 2, 1, and 0.5 ng/mL were prepared by serial dilution of the stock solution with drug-free human plasma. Working internal standard solution giving a concentration of 1 ng/µL was prepared in the mobile phase. From the stock solution, appropriate volumes of stock doxazosin were also diluted with the mobile phase to give different concentrations of 20, 10, 5, 2, 1, and 0.5 ng/mL

Sample preparation

Each 150-µL volume of various concentrations of working doxazosin solution in plasma was accurately measured into a new 1.5-mL Eppendrof microcentrifuge tube after being rinsed with methanol, followed by addition of 300 µL methanol precipitation solvent and 350 µL of a 1-ng/mL prazosin internal standard solution. The mixture was stepwise vortex-mixed for 5 s and then centrifuged at 12,000 rpm for 10 min. The supernatant was filtered through 0.45-µm nylon disposable filter (Alltech), and 20-µL was then injected onto the reversed-phase chromatographic system. Samples were quantitated using the peak area ratio of doxazosin over the internal standard.

Accuracy, precision, and recovery

The doxazosin plasma working standards prepared as described previously (at concentrations of 0.5, 1, 2, 5, 10, and 20 ng/mL) were divided into two portions. First portion used for constructing calibration curves. On analysis of the standards, the ratios of the peak area of doxazosin to the peak area of prazosin were plotted against doxazosin concentrations and linear regression analysis was also performed. The resulting slope and *y*-intercept of the standard curve was used to calculate the concentration of doxazosin. The second portion used to determine accuracy, inter- and intraday precision, and the recovery. The recovery of the protein precipitation procedure for doxazosin and the internal standard was calculated by comparing the peak area ratios obtained after precipitation with that of a drug solution in the mobile phase of corresponding concentration without precipitation.

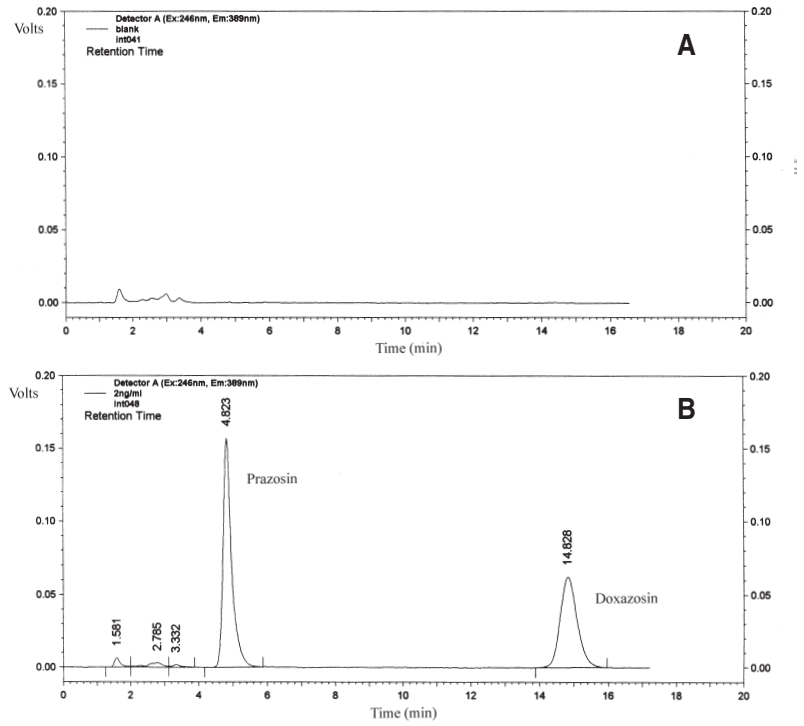

Figure 2. Chromatograms for the analysis of doxazosin in drug-free human plasma. (A) Blank plasma and (B) human plasma spiked with 1 ng/mL prazosin and 2 ng/mL doxazosin.

Results and Discussion

Chromatography

Figure 2 shows typical chromatograms of precipitated samples of blank human plasma (A) and plasma spiked with doxazosin and prazosin equivalent to 2 and 1 ng/mL, respectively (B). This method used a simple C18 reversed-phase chromatographic column, as compared with the previous reports (4–6). It can be seen that the doxazosin and prazosin peaks were well resolved and free of interference from endogenous compounds in the plasma. The selection of prazosin as the internal standard was based on the chemical structure and its chromatographic and precipitation behavior. Thus the method has been shown to be specific for doxazosin determination. The retention time of prazosin and doxazosin were 4.8 and 14.8 min, respectively. The total run time for each sample was 17 min. Under the described conditions, the equilibration between stationary phase and mobile phase could be readily be achieved within 30 min. For long-term column efficiency and consistent drug peaks, it should be noted that, after daily use, the overnight cleansing of the column with methanol was required.

Linearity, accuracy, and precision

Calibration curves were obtained by plotting the peak area ratio of doxazosin to that of prazosin versus plasma doxazosin concentrations. The standard calibration curve (n = 6) was linear between 0.5 and 20 ng/mL, with a correlation coefficient (r^2) ≥ 0.999, slope of 0.434, and intercept of –0.0336. The accuracy (difference between the amount added to blank plasma and the amount found) and precision of the method was assessed by spiked plasma standards in the range 0.5–20 ng/mL. The intra- and interday variations for doxazosin were calculated using data accumulated over a period of 6 days. The intra- and interday results are presented in Table I. The percentage of inaccuracy values were all < 7% in both intra- and interday accuracy. The intraday coefficient of variance (%CV) was 0.5–2.8%, and the interday %CV varied from 0.3% to 7.8%.

Recovery and detection limit

The recovery of doxazosin was assessed in the range 0.5–20 ng/mL (Table II). Each concentration of doxazosin in the mobile phase containing the internal standard was directly injected. The resulting peak area ratios represent the maximum recovery from the precipitation procedure. Plasma samples (n = 6) containing doxazosin (0.5–20 ng/mL) and the internal standard were analyzed after precipitation. The absolute recovery was calculated by comparing peak area ratios of direct injections of pure doxazosin with those of plasma samples containing equivalent amounts of doxazosin. The absolute recoveries of doxazosin ranged from 98.4% to 102.6%. This high recovery eliminated the need for large volumes of serum samples. Glassware silylation, reportedly required to eliminate drug losses (5), was not necessary in the present procedure. Based on a signal-to-noise ratio of 5:1 (5 times to basal line) the lower limit of quantitation was 0.5 ng/mL and the lower limit of detection was 0.125 ng/mL. Although the doxazosin molecule is highly sensitive to fluorescence detection, the obtained data was incorrect over the concentration of 25 ng/mL, because of the limitation of the instrumental system.

Table I. Intra- and Interday Accuracy and Precision of the Analysis of Doxazosin in Human Plasma at each Concentration Daily Over a Period of 6 Days

	Intraday (n = 6)		Interday (n = 6)	
Concentration (ng/mL)	Accuracy (%inaccuracy*)	Precision (%CV)	Accuracy (%inaccuracy*)	Precision (%CV)
0.5	2.1	2.8	6.2	7.8
1	2.5	1.2	4.4	6.2
2	1.3	0.5	3.5	4.3
5	3.3	1.0	2.6	2.6
10	0.5	0.6	1.5	1.8
20	0.6	0.9	0.2	0.3

$$* \%\text{Inaccuracy} = \frac{[\text{measured concentration} - \text{targeted concentration}] \times 100}{\text{targeted concentration}}$$

Table II. Absolute Recovery of Doxazosin in Human Plasma (n = 6)*

Added known concentration (ng/mL)	Mean calculated concentration (ng/mL)	Absolute recovery (%)	Relative standard deviation (%)
0.5	0.51	102.6	3.8
1	0.98	98.4	6.0
2	1.97	98.5	8.3
5	4.88	97.5	12.8
10	9.91	99.1	17.5
20	20.00	100.0	6.6

* Result based upon triplicate injections of each concentration over a period of 6 days.

Conclusion

A specific method for the determination of doxazosin in human plasma is described. The improved chromatography of the present assay simplified the determination method of doxazosin by replacing the solvent extraction procedure or usage of SPE with the precipitation step. The method utilizes the related drug prazosin as an internal standard and is based on a simple precipitation scheme followed by analysis by HPLC with fluorescence detection. In this work, methanol was used as precipitating agent. This method is suitable for the pharmacokinetic studies because of the relative simplicity of the sample treatment. In addition, the validation results were accepted under the United States Pharmacopoeia Bioanalytical Method (11).

Acknowledgments

The authors thank Assistant Prof. Manupat Lohitnavy (Naresuan University, Phitsanulok, Thailand) for his valuable suggestions. The reference standards, HPLC column equipment, and financial support were provided by Umeda Company Limited (Bangkok, Thailand). The authors also thank all staffs of Bioequivalence Test Center, Faculty of Pharmaceutical Sciences, Naresuan University.

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Manuscript received April 30, 2004.