Drug Development and Industrial Pharmacy, 33:824–829, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040601012999

informa
healthcare

Pharmacokinetics of Doxazosin Gastrointestinal Therapeutic System after Multiple Administration in Korean Healthy Volunteers

Yoon Hee Kwon and Hye Sun Gwak
College of Pharmacy, Ewha Womans University, 11-1 Daehyun-Dong, Seodaemun-Gu, Seoul, South Korea

Sun Jung Yoon and In Koo Chun
College of Pharmacy, Dongduk Women's University, Wolgok-Dong, Seongbuk-Gu, Seoul, South Korea

Doxazosin mesylate is a selective alpha-adrenoreceptor antagonist for the treatment of hypertension and benign prostatic hyperplasia. A simple high performance liquid chromatographic method has been developed and validated for the quantitative determination of doxazosin in plasma. A reversed phase C18 column was used for the separation of doxazosin and prazosin (internal standard) with a mobile phase composed of water • acetonitrile • triethylamine (68:32:0.2 v/v, pH 5.0) at a flow rate of 1.2 mL/min. The fluorescence detector was operated at 246 (excitation) and 389 nm (emission). Intra- and inter-day precision and accuracy were acceptable for all quality control samples including the lower limit of quantification of 1 ng/mL. Recovery of doxazosin from human plasma was greater than 93.4%. Doxazosin was stable in human plasma under various storage conditions. This method was used successfully for a pharmacokinetic study in plasma after oral administration of multiple 4-mg dose of doxazosin gastrointestinal therapeutic system formulation to 16 healthy volunteers. At steady state the mean area under the curve for a dosing interval and elimination half-life were calculated to be 367.0 ± 63.5 ng · hr/mL and 29.2 ± 4.5 hr, respectively. There was no difference in pharmacokinetic parameters between male and female.

Keywords doxazosin gastrointestinal therapeutic system; prazosin; high-performance liquid chromatography; pharmacokinetic parameters

INTRODUCTION

Doxazosin mesylate (4-amino-2-[4-(1,4-benzodiaxan-2-carbonyl)-piperazine-1-yl]-6,7-dimethoxyauinazoline mesylate) is a selective alpha-adrenoreceptor antagonist for the treatment of hypertension and benign prostatic hyperplasia (BPH; Daae & Westlie, 1998; Fawzy et al., 1999; Fulton et al., 1995; Lepor et al., 1997). The efficacy of alpha-adrenoreceptor antagonists in relieving BPH was attributed to inhibition of alpha-adrenoreceptors in the smooth muscle of the prostate gland and bladder neck (James et al., 1989; Shapiro et al., 1992). Pharmacologic alpha-adrenoreceptor blockade has proven to be an effective treatment of hypertension because increased peripheral vascular resistance and elevated blood pressure are mediated by postsynaptic alpha-adrenoreceptors (Davey, 1989; Fulton et al., 1995).

Although alpha-adrenoreceptor antagonist such as doxazosin is known to be effective in patients with hypertension and BPH, the first-dose effect has limited its use. Doxazosin gastrointestinal therapeutic system (GITS) formulation was developed to enhance the pharmacological profile such that the drug is more gradually absorbed and the peak-to-trough ratio is reduced (Fitzpatrick & Desgrandchan, 2005). Compared to standard formulation, doxazosin GITS formulation minimizes the possible adverse effects such as postural hypotension (Chung et al., 1999). Consequently, the initial dose of GITS formulation is 4 mg compared to the usual initial dose of 1 mg with standard doxazosin, and the GITS formulation allows titration to 8 mg, which offers an advantage to patients who require a higher therapeutic dose (De Reijke & Klarskov, 2004; Forray & Noble, 1999; Kirby et al., 2000; Kirby et al., 2003).

After oral administration, doxazosin is rapidly absorbed and peak plasma levels are achieved within 2–3 hr (Kaye et al., 1986). Doxazosin is extensively metabolized, which only 5% of the administered dose excreted unchanged in urine. For single dose, it was reported that oral bioavailability was approximately 65% and half-life 10–12 hr. In multiple-dose studies, the terminal half-life was calculated to be around 22 hr (Elliott et al., 1987).

Address correspondence to In Koo Chun, College of Pharmacy, Dongduk Women's University, 23-1 Wolgok-Dong, Seongbuk-Gu, Seoul 136-714, South Korea. E-mail: ikchun@dongduk.ac.kr Or to Hye Sun Gwak, College of Pharmacy, Ewha Womans University, 11-1 Daehyun-Dong, Seodaemun-Gu, Seoul 120-750, South Korea. E-mail: hsgwak@ewha.ac.kr

Even though it has not been reported that different genetics or ethnics have different pharmacokinetics of doxazosin, the pharmacokinetic parameters in Thai races were found to be different from those in Caucasian races. The higher C_{max} and AUC were obtained in Thais with 2 mg of doxazosin standard tablet (Chung et al., 1999; Sripalakit et al., 2006). On the contrary, there has no study conducted using Asians with doxazosin GITS. Considering that doxazosin GITS is preferred to standard dosage form due to its reduced side effect and prolonged effectiveness, pharmacokinetic study using doxazosin GITS in Asian populations is critical. Thus, in this study, we aimed to assess the pharmacokinetic parameters at steady state in Korean healthy volunteers after multiple administration of 4 mg of doxazosin GITS formulation.

EXPERIMENTAL

Equipment

The HPLC system consisted of a pump (Jasco PU-1580 Intelligent HPLC Pump, Japan) with a fluorescence detector (Jasco FP-2020 Plus, Japan) set at 246 (excitation) and 389 nm (emission) and an integrator (Model 4290, Varian, Palo Alto, CA).

Materials and Reagents

Doxazosin mesylate and prazosin hydrochloride (internal standard, IS) were obtained from Sigma Chemical Co. (St. Louis, MO). *tert*-Butyl methyl ether (Acrosorganics, New Jersey, NJ), sodium hydroxide, sulfuric acid and triethylamine (Duksan Pure Chemical, Ansan, Kyungkido, South Korea) were used. Acetonitrile was of HPLC grade and other reagents used were of analytical grade.

Preparation of Standard Solutions

Working stock solutions of doxazosin and IS were prepared in methanol at a concentration of 1 mg/mL as a base substance. Prior to use, these two stock solutions were further diluted with water to obtain working solutions at the concentration of 1 μg/mL.

Six calibration samples (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 (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

An appropriate dilution of the working solution with drug-free plasma obtained from healthy volunteers gave a concentration range between 1 and 50 ng/mL of doxazosin. To 0.5 mL of this plasma was added 50 μL of IS (300 ng/mL) and 100 μL of 2 N sodium hydroxide solution. After a brief vortex mixing, 3 mL of *tert*-butyl methyl ether was added and performed by vortex mixing for 1 min. The tubes were then centrifuged at 3000 *g* for 5 min, 2 mL of the organic phase was transferred to another set of clean conical centrifuge tubes, and back-extracted with 300 μL of 0.005 M sulfuric acid by vortex mixing for 3 min. The tubes were then centrifuged at 3000 *g* for 5 min, and 30 μL of the aqueous phase was injected into the liquid chromatograph.

Chromatographic Conditions

The reversed phase C18 column (Luna® C18, 4.6 × 250 mm, 5 μm, Phenomenex®, Torrance, CA) was eluted with a mixture of water • acetonitrile • triethylamine (68:32:0.2 v/v, pH 5.0) at a flow rate of 1.2 mL/min. The fluorescence was detected at 246 (excitation) and 389 nm (emission).

Method Validation

Specificity

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

Calibration Curve

Calibration standards at the concentrations of 1, 5, 10, 20, 30, and 50 ng/mL were extracted and assayed as mentioned above. The calibration curve was constructed based on peak area ratio of the drug to IS. Calibration curve was plotted every day.

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.

Extraction Recovery

Recovery of doxazosin at the concentrations of 5 and 30 ng/mL after the back-extraction was determined by comparing observed doxazosin peak area in extracted plasma, to those of non-processed standard solutions. Recovery of internal standard (prazosin) was determined at the level of 30 ng/mL in the plasma in the same manner as above.

Stability

Analyte stability was tested using prepared stability samples for three freeze-thaws, short-term and long-term stabilities (Sripalakit et al., 2006). The freeze-thaw stability of doxazosin in plasma was evaluated over 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 doxazosin in quality control samples stored at room temperature for 24 hr and at −70°C for 4 weeks was also assessed. The mean values of doxazosin were compared with the initial ones, which were assayed immediately after preparation of stability control plasma samples. The stability was expressed as a percentage of the initial value.

Multiple-dose Pharmacokinetic Study

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. Doxazosin mesylate (Cardura® XL Tablets, Pfizer Korea Co., Ltd., Seoul, South Korea) was administered orally with 240 mL of water in the morning (8:30 A.M.) for eight consecutive days. From day 5 through 8, the pre-dose blood sampling was conducted to examine the attainment of steady state. On day 8, 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. Five milliliters of blood samples were collected in green-top vacutainers (containing sodium heparin) via an indwelling cannula placed on the forearm before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 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.

The area under the plasma concentration-time curve from zero to end of dosing interval of 24 hr (AUC_τ) was calculated using the trapezoidal rule. At steady state, the maximum drug concentration ($C_{max(ss)}$), minimum drug concentration ($C_{min(ss)}$), the time of maximum concentration (T_{max}) and the time of minimum concentration (T_{min}) were determined directly from concentration time data for each subject. The elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$, where λ_z was determined from the slope of the terminal log-linear phase of the plasma concentration-time curve. The peak-trough fluctuation index (FI) was calculated at steady state from $(C_{max(ss)} - C_{min(ss)})/C^{ave}$, which C^{ave} is AUCτ/24.

RESULTS AND DISCUSSION

Specificity and Linearity

Figure 1 shows the well-resolved chromatographic peaks of doxazosin and IS at 7.5 and 3.0 min, respectively. The blank plasma after extraction consistently contains no significant interfering peaks.

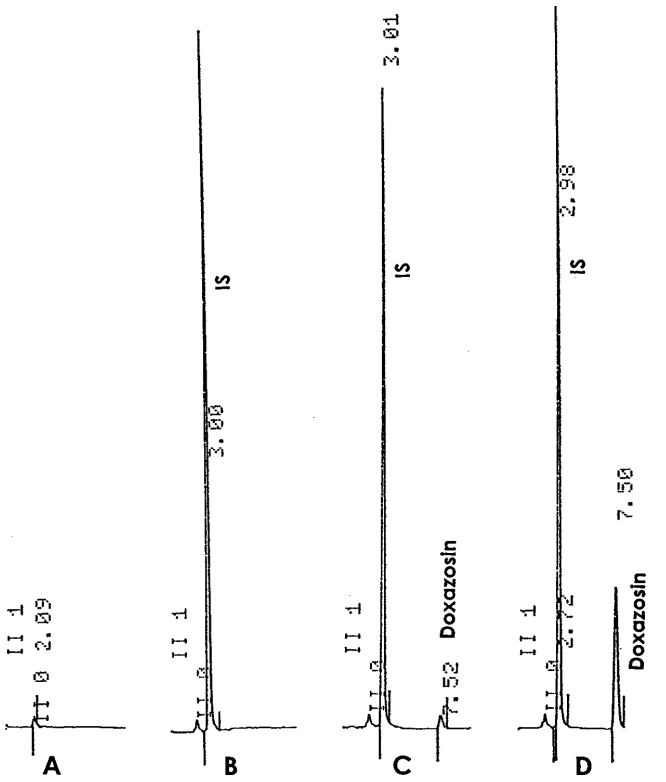

FIGURE 1. Chromatograms for control human plasma (A), control plasma spiked with 30 ng/mL of prazosin (internal standard, IS) (B), control human plasma spiked with 1 ng/mL of doxazosin and 30 ng/mL of IS (C) and control human plasma spiked with 10 ng/mL of doxazosin and 30 ng/mL of IS (D).

The relation between doxazosin concentrations and peak area ratio of doxazosin to IS was linear from 1 to 50 ng/mL ($y = 0.0225x + 0.0046$, $r^2 = 0.9998$).

In our previous bioavaiability study (Gwak & Chun, 2005) of alpha-adrenoreceptor antagonists including prazosin, we developed more simple and rapid analytical method. Based on the study, *tert*-butyl methyl ether was employed as organic phase for the first extraction of doxazosin rather than ethyl acetate mainly used in other studies (Rubin et al., 1980; Sripalakit et al., 2006). This allowed lower limit of quantitation of 1 ng/mL compared to 2 ng/mL (Chung et al., 1999) or no repeated extraction by organic solvent required in other study (Sripalakit et al., 2006). With this analytical method, linearity was obtained over the concentration range for doxazosin of 1–50 ng/mL. The slopes of the standard curves were consistent among all runs with correlation coefficients >0.999. The determination of doxazosin using back-extraction technique requires total 4 min of vortex-mixing, which was very simple and rapid.

Accuracy, Precision and Recovery

The lower limit of quantitation (LLOQ) of doxazosin was determined as the sample concentration of doxazosin resulting in peak heights of 10 times SN. The LLOQ was found to be 1 ng/mL. Based on 3 times peak height of baseline noise, the limit of detection was calculated to be 0.2 ng/mL. 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 doxazosin. As described in Table 1, the intra- and inter-day RSD (%) was within 10.8%, which were acceptable for all quality control samples including the LOQ. The accuracy of doxazosin ranged between 86.0 and 105.5%. All the batches met the quality control acceptance criteria (Karnes et al, 1991).

The extraction recovery of doxazosin at concentrations of 5 and 30 ng/mL was 93.4 ± 3.8 and 99.4 ± 4.6% (n = 3), respectively, while for IS at concentration of 30 ng/mL it was 97.2 ± 2.1% (n = 3). These results suggested that there was no difference in extraction recovery at different concentrations of doxazosin.

Stability

Knowledge of the stability of the drug in test material is a prerequisite for obtaining valuable data (Karnes et al., 1991). The stability of doxazosin under various conditions is described in Table 2. Under all conditions tested, doxazosin was stable with detected concentrations of at least 98.8% of the initial concentration.

Pharmacokinetics

The pre-dose concentrations of doxazosin are shown in Table 3. A repeated measures ANOVA of the last three pre-dose concentrations are required to check whether steady state concentrations have been reached during the study (Consensus Guideline, 1996). Even though the repeated measures ANOVA for the consecutive three pre-dose concentrations revealed that there was significant differences, the significance was due to a higher value of 6th day in the 5th-, 6th- and 7th-day pre-dose concentrations and a lower value for 7th day in 6th-, 7th- and 8th-day pre-dose concentrations. Since there was no increase in the means for the three pre-dose concentrations, steady state was assumed (Consensus Guideline, 1996).

The mean plasma doxazosin concentration-time curve at steady state is presented in Figure 2. The drug concentration decreased for the first 2 hr and then gradually increased to a mean $C_{max(ss)}$ of 21.0 ng/mL at 5.4 hr. The decrease for the first phase was attributed to the slower drug release rate, compared to the elimination rate. The AUC_τ and $t_{1/2}$ were calculated to be 367.0 ± 63.5 ng · hr/mL and 29.2 ± 4.5 hr,

TABLE 1
Intra- and Inter-day Precision and Accuracy of the Determination of Doxazosin in Human Plasma

Doxazosin Concentration (ng/mL)	RSD (%)		Accuracy (%) (n = 5)
	Intra-day (n = 5)	Inter-day (n = 5)	
1	5.46	2.22	85.97
5	6.42	5.37	95.29
10	8.32	3.90	105.53
20	5.84	2.99	101.12
30	1.65	3.21	100.41
50	1.76	5.63	99.85

TABLE 2
Stability of Doxazosin in Human Plasma

Doxazosin Concentration (ng/mL)	Treatment	Percentage of Initial Value
5	Three freeze-thaw cycles	98.8 ± 3.3
	Stored at room temperature for 24 hr	105.6 ± 1.2
	Stored at −70°C for 4 weeks	102.9 ± 4.9
30	Three freeze-thaw cycles	102.3 ± 2.7
	Stored at room temperature for 24 hr	102.8 ± 6.0
	Stored at −70°C for 4 weeks	102.1 ± 1.5

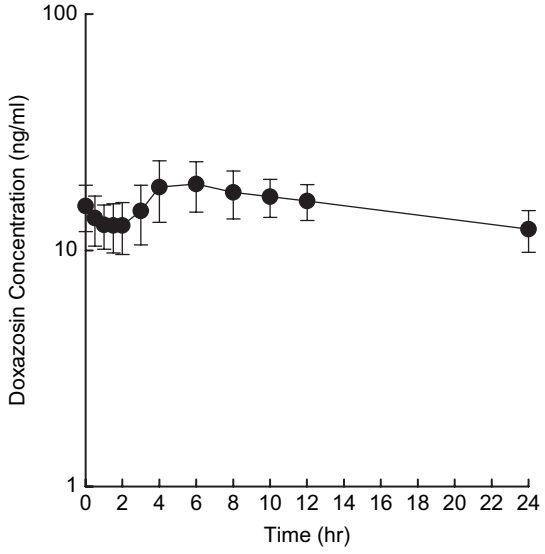

FIGURE 2. Steady state mean doxazosin plasma concentration-time profile following oral administration of doxazosin GITS 4 mg in sixteen healthy human volunteers.

TABLE 3
Pre-dose Human Plasma Concentrations of Doxazosin

Dosing Day	Mean Plasma Concn. (ng/mL)	*SD*	*P*
5th Day	11.43	3.36	
6th Day	14.84	3.79	
7th Day	12.47	3.25	$< 0.0001^{1)}$
8th Day	15.25	3.33	$< 0.0001^{2)}$

Based on [1)]5th-, 6th- and 7th-day pre-dose concentrations and [2)]6th-, 7th- and 8th- day pre-dose concentrations.

TABLE 4
The Effects of Gender on the Mean Pharmacokinetic Parameters of Doxazosin

Parameters	Male	Female
AUC_τ (ng·hr/mL)	372.9 ± 61.5	361.2 ± 69.1
$C_{max(ss)}$ (ng/mL)	21.6 ± 4.3	20.3 ± 4.9
T_{max} (hr)	5.5 ± 2.3	5.6 ± 1.5
$T_{1/2}$ (hr)	38.7 ± 9.7	26.3 ± 14.2
$C_{min(ss)}$ (ng/mL)	11.8 ± 2.2	11.5 ± 2.9
T_{min} (hr)	1.5 ± 0.6	2.1 ± 1.02
FI	0.64 ± 0.19	0.58 ± 0.13

respectively. A mean $C_{min(ss)}$ of 11.65 ± 2.5 ng/mL was obtained at 1.8 ± 0.8 hr, and the FI of the GITS formulation was 0.6 ± 0.17.

The pharmacokinetic parameters obtained in this study were significantly different from those using Caucasian male populations even though the study design was the same as our study, which employed 4 mg GITS under fasting condition using healthy volunteers and blood sampling was conducted for 24 hr at steady-state (Chung et al., 1999). Compared to our results, lower $C_{max(ss)}$, $C_{min(ss)}$ and AUC_τ with multiple 4 mg doxazosin GITS administration were reported, which were 11.3 ng/mL hr, 6.4 ng/mL and 201 ng · hr/mL, respectively. These values were 55, 54, and 55% of $C_{max(ss)}$, $C_{min(ss)}$ and AUC_τ in Korean population, respectively. This difference was also reported with 2 mg doxazosin standard tablets; C_{max} and AUC in Caucasian populations were calculated to be 74 and 63% of those in Asian populations such as Thais, respectively (Chung et al., 1999; Sripalakit et al., 2006).

Expectedly, $C_{max(ss)}$ and FI obtained in this study using 4 mg GITS were much lower than those reported in other study using 4 mg standard tablets (21.0 vs. 29.3 ± 8.4 and 0.6 vs. 1.5, respectively), suggesting that steady state concentrations remain more uniform with the GITS formulation, which was likely to result in more consistent blood pressure control (Chung et al., 1999).

The 16 healthy volunteers participating in this study included 8 young males (mean age 22.6 ± 0.9 years) and 8 young females (mean age 21.4 ± 1.1 years). The steady state plasma doxazosin-time profiles from 0 to 24 hr on day 8 were similar for both gender groups as described in Table 4. Similar conclusions have been reported in other study. In an open, multi-dose parallel group study, 11 young males and 10 young females, whose mean ages were 28 years in both genders, were employed and the steady-state plasma concentrations were obtained from 0 to 24 hr on day 7. In the study, with doxazosin 4 mg GITS formulation, no statistically or clinically significant age- or gender-related differences in pharmacokinetics have been observed at steady state, indicating that no dosage adjustments are required based on age or gender (Chung et al., 1999).

CONCLUSION

The determination of doxazosin using *tert*-butyl methyl ether as extraction solvent together 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 LLOQ of 1 ng/mL. Recovery evaluations showed that doxazosin was recovered at least 93.4%. Doxazosin 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.

In this first pharmacokinetic study of doxazosin GITS formulation using Korean population, the higher pharmacokinetic parameters such as $C_{max(ss)}$, $C_{min(ss)}$ and AUC_τ were obtained compared to those using Caucasians. This difference was thought to be possibly due to the genetic or ethnic difference, which is required to further investigate. Compared to standard dosage form, the relatively constant plasma drug concentrations at steady state with GITS were observed, which would be likely result in more consistent disease control and reduced side effects.

ACKNOWLEDGMENT

This study was supported by Grant KFDA-05132-EQI-213 from Korea Food and Dug Administration.

REFERENCES

Chung, M., Vashi, V., Puente, J., Sweeney, M., & Meredith, P. (1999). Clinical pharmacokinetics of doxazosin in a controlled-release gastrointestinal therapeutic system (GITS) formulation. *Br. J. Clin. Pharmacol., 48*, 678–687.

Daae, L. N., & Westlie, L. (1998). A 5-year comparison of doxazosin and atenolol in patients with mild-to-moderate hypertension: effects on blood pressure, serum lipids, and coronary heart disease risk. *Blood Press., 7*, 39–45.

Davey, M. J. (1989). Pharmacologic basis for the use of doxazosin in the treatment of essential hypertension. *Am. J. Med., 87*, 36S–44S.

De Reijke, T. M., & Klarskov, P. (2004). Comparative efficacy of two a^1-adrenoreceptor antagonists, doxazosin and alfuzosin, in patients with

lower urinary tract symptoms from to benign prostatic enlargement. *BJU Int., 93*, 757–762.

Elliott, H. L., Meredith, P. A., & Reid, J. L. (1987). Pharmacokinetic overview of doxazosin. *Am J. Cardiol., 59*, 78G–81G.

Fawzy, A., Vashi, V., Chung, M., Dias, N., & Gaffney, M. (1999). Clinical correlation of maximal urinary flow rate and plasma doxazosin concentrations in the treatment of benign prostatic hyperplasia. *Urology., 53*, 329–335.

Fitzpatrick, J. M., & Desgrandchamps, F. (2005). The clinical efficacy and tolerability of doxazosin standard and gastrointestinal therapeutic system for benign prostatic hyperplasia. *BJU Int., 95*, 575–579.

Forray, C., & Noble, S. A. (1999). Subtype selective alpha ^{1}-adrenoreceptor antagonists for the treatment of benign prostatic hyperplasia. *Expert Opin. Invest. Drugs, 8*, 2073–2094.

Fulton, B., Wagstaff, A. J., & Sorkin, E. M. (1995). Doxazosin: an update of its clinical pharmacology and therapeutic applications in hypertension and benign prostatic hyperplasia. *Drugs, 4*9, 295–320.

Gwak, H. S., & Chun, I. K. (2005). Simplified HPLC method for the determination of prazosin in plasma and its application to single-dose pharmacokinetics. *J. Appl. Pharmacol., 13*, 90–94.

Intl. Conf. on Harmonisation Topic Q 2B, Validation of Analytical Procedures: Methodology, Step 4, Consensus Guideline, 1996.

James, S., Chapple, C. R., Phillips, M. I., Greengrass, P. M., Davey, M. J., Turner-Warwick, R. T., Milroy, E. J., & Burnstock, G. (1989). Autoradiographic analysis of alpha-adrenoceptors and muscarinic cholinergic receptors in the hyperplastic human prostate. *J. Urol., 142*, 438–444.

Karnes, H. T., Shiu, G., & Shah, V. P. (1991). Validation of bioanalytical methods. *Pharm. Res., 8*, 421–426.

Kaye, B., Cussans, N. J., Faulkner, J. K., Stopher, D. A., & Reid, J. L. (1986). The metabolism and kinetics of doxazosin in man, mouse, rat and dog. *Br. J. Clin. Pharmacol., 21*, 19S–25S.

Kirby, R. S. (2003). A randomized, double-blind crossover study of tamsulosin and controlled-release doxazosin in patients with benign prostatic hyperplasia. *BJU Int., 91*, 41–44.

Kirby, R., Andersson, K. E., Lepor, H., & Steers, W. D. (2000). Alpha (1)-adrenoreceptor selectivity and the treatment of benign prostatic hyperplasia and lower urinary tract symptoms. *Prostate Cancer Prostatic Dis., 3*, 76–83.

Lepor, H., Kaplan, S. A., Klimberg, I., Mobley, D. F., Fawzy, A., Gaffney, M., Ice, K., & Dias, N. (1997). Doxazosin for benign prostatic hyperplasia: long-term efficacy and safety in hypertensive and normotensive patients. *J. Urol., 157*, 525–530.

Shapiro, E., Hartanto, V., & Lepor, H. (1992). The response to alpha blockade in benign prostatic hyperplasia is related to the percent area density of prostate smooth muscle. *Prostate., 21*, 297–307.

Sripalakit, P., Nermhom, P., & Saraphanchotiwitthaya, A. (2006). Validation and pharmacokinetic application of a method for determination of doxazosin in human plasma by high-performance liquid chromatography. *Biomed. Chromatogr., 20*, 729–735.

Rubin, P. C., Brunton, J., & Meredith, P. (1980). Determination of the vasodilator UK 33274 by high-performance liquid chromatography using fluorescence detection. *J. Chromatogr., 221*, 193–195.