ELSEVIER

Journal of Chromatography B, 745 (2000) 439–443

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Improved high-performance liquid chromatographic analysis of terazosin in human plasma

Phaik Yeong Cheah[a], Kah Hay Yuen[a,*], Men Long Liong[b]

[a]School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Penang, Malaysia
[b]Lam Wah Ee Hospital, 11600 Penang, Malaysia

Received 10 January 2000; received in revised form 19 May 2000; accepted 22 May 2000

Abstract

A simple, sensitive and reproducible high-performance liquid chromatography (HPLC) method was developed for the determination of terazosin in human plasma. The method involves a one-step single solvent extraction procedure using dichloromethane with a 0.25 ml plasma sample. Recovery values were all greater than 90% over the concentration range 0.25–100 ng/ml. Terazosin was found to adsorb to glass or plastic tubes, but this could be circumvented by using disposable plastic tubes. Also, rinsing the injector port with methanol after each injection helped to prevent any carry-over effect. The internal standard, prazosin, did not exhibit this problem. The method has a quantification limit of 0.25 ng/ml. The within- and between-day coefficient of variation and accuracy values were all less than 7% over the concentration range 0.25–100 ng/ml and hence the method is suitable for use in pharmacokinetic studies of terazosin. © 2000 Elsevier Science B.V. All rights reserved.

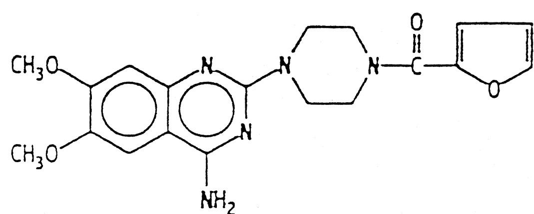
Keywords: Terazosin

1. Introduction

Terazosin hydrochloride dihydrate {2-[4-(2-tetrahydrofuranyl)carbonyl]-1-piperazinyl-6,7-dimethoxy-4-quinazolinamine monohydrochloride dihydrate} (Fig. 1) is an effective drug for hypertension [1–3] and benign prostate hyperplasia [4–6]. Various analytical methods have been reported for the determination of terazosin in human plasma following intravenous or oral dosing. Zavitsanos and Alebic-Kolbah [7] reported a normal-phase high-performance liquid chromatographic method with electrospray mass spectrometry. While the method was reported to be sensitive with a limit of quantification of approximately 65 pg/ml, such a detector system may not be available in most laboratories. Moreover, the precision and accuracy of the method were not reported. A radioreceptor assay method has also been reported by Taguchi et al. [8], but the method is too complex to be applied in routine pharmacokinetic studies and may not be selective to terazosin due the possibility of receptor binding by its metabolites. Other reported methods are based on a high-performance liquid chromatography system with fluorescence detection [9–11]. In the method described by Patterson [9], a sample volume of 1 ml was used and the sensitivity was only 1 ng/ml. Sekhar et al. [10], on the other hand, used dichloromethane as an extracting solvent, but the method

*Corresponding author. Fax: +60-4-659-6517.
E-mail address: khyuen@usm.my (K.H. Yuen).

0378-4347/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0378-4347(00)00313-3



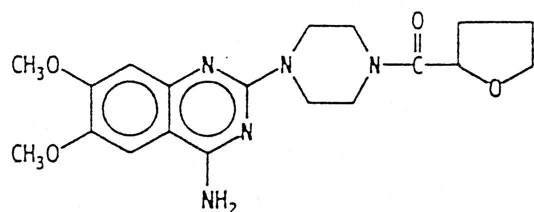


Fig. 1. Structures of terazosin and prazosin.

involved the use of a relatively large volume of solvent (8 ml) and the HPLC column was required to be maintained at 40°C to achieve adequate peak resolution. In addition, the accuracy of the method was not reported. Bhamra et al. [11] described a method involving a relatively simple sample preparation, but the mean recovery was only 67% and the accuracy of the assay method was not determined.

In this paper, we report a simple, sensitive and reproducible HPLC method using fluorescence detection for terazosin in human plasma and demonstrate the pharmacokinetic application of the method.

2. Experimental

2.1. Materials

Disodium hydrogen phosphate and sodium hydroxide pellets, AR grade, were purchased from Merck (Darmstadt, Germany), and phosphoric acid 85% was purchased from BDH Chemicals (Poole, UK). Prazosin hydrochloride standard (Fig. 1) was obtained from United States Pharmacopoeia (Rockville, MD, USA) and terazosin monohydrochloride standard was obtained from Abbott Laboratories (North Chicago, IL, USA). All other solvents were of AR or HPLC grade purchased from Mallinckrodt (Paris, KY, USA). The purity of terazosin monohydrochloride and prazosin hydrochloride was 99.8 and 99.9%, respectively, whilst that of all other chemicals was >99.8%.

2.2. Standards preparations

Stock solutions of terazosin and the internal standard, prazosin, both in distilled water, at a concentration of 10 μg/ml were prepared separately. From the stock solution, working concentrations of terazosin at 100, 50, 25, 12.5, 1, 0.5 and 0.25 ng/ml were prepared by serial dilution with distilled water. A working solution of prazosin internal standard at a concentration of 100 ng/ml was also prepared by appropriate dilution of stock solution with distilled water. A stock solution of terazosin in plasma at a concentration of 200 ng/ml was prepared by spiking drug-free human plasma with a suitable volume of the terazosin stock solution in water. Working concentrations of terazosin in plasma at 100, 50, 25, 12.5, 1, 0.5 and 0.25 ng/ml were then prepared by serial dilution of the plasma stock solution with drug-free human plasma.

2.3. Instrumentation

The HPLC system comprised a Jasco PU-980 pump (Hachioji, Tokyo, Japan), a Jasco 821-FP fluorescence detector equipped with an Hitachi D-2500 Chromato-integrator (Hitachi, Tokyo, Japan) and a Rheodyne 7161 sample injector fitted with a 50-μl sample loop. A Metaphase KR100-5-C18 (Bioscience Instrumentation, Kuala Lumpur, Malaysia) column packed with spherical silica gel particles chemically bonded with octadecyl groups (5 μm, 150×4 mm I.D.) fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA) packed with Perisorb RP-18 (30–40 μm, pellicular) powder (Upchurch Scientific) was used for chromatographic separation. The mobile phase, which was similar to that reported by Patterson [9], consisted of 0.01 *M* disodium hydrogen phosphate–acetonitrile–tetrahydrofuran (76:22:2, v/v) adjusted

to pH 6.5 using 85% w/w phosphoric acid solution and delivered at a flow-rate of 1 ml/min. The detector was operated at an excitation wavelength of 250 nm and an emission wavelength of 370 nm.

2.4. Sample preparation

A 0.25-ml volume of human plasma sample was accurately measured into a new 2-ml Eppendorf microcentrifuge tube, followed by addition of 100 μl of 100 ng/ml prazosin hydrochloride internal standard solution, 100 μl of 1 *M* sodium hydroxide solution and 1.25 ml dichloromethane extraction solvent. The mixture was vortex-mixed for 1 min and centrifuged at 12 800 *g* for 10 min. The organic layer was transferred into a new 1.5-ml Eppendorf tube and evaporated to dryness at 40°C under a gentle stream of nitrogen gas. The residue was reconstituted with 100 μl of mobile phase and 50 μl was injected into the column. Samples were quantified using the peak height ratio of terazosin over the internal standard.

2.5. Recovery, accuracy and precision

The terazosin plasma working standards prepared above, at concentrations of 0.25, 0.5, 1.0, 12.5, 25, 50 and 100 ng/ml, were divided into two portions, one portion used for constructing calibration curves, the other used to determine the extraction recovery and the within- and between-day precision and accuracy ($n=6$) of the method. The recovery of the extraction procedure for terazosin and the internal standard was calculated by comparing the peak height obtained after extraction with that of an aqueous drug solution of corresponding concentration without extraction.

3. Results and discussion

Chromatograms obtained with blank plasma, plasma spiked with 25 ng/ml terazosin and 100 ng/ml prazosin, as well as a plasma sample from a healthy volunteer containing 1.2 ng/ml terazosin 36 h after oral administration of 1 mg terazosin, are shown in Fig. 2A–C. It can be seen that the terazosin and prazosin peaks were well resolved and free of

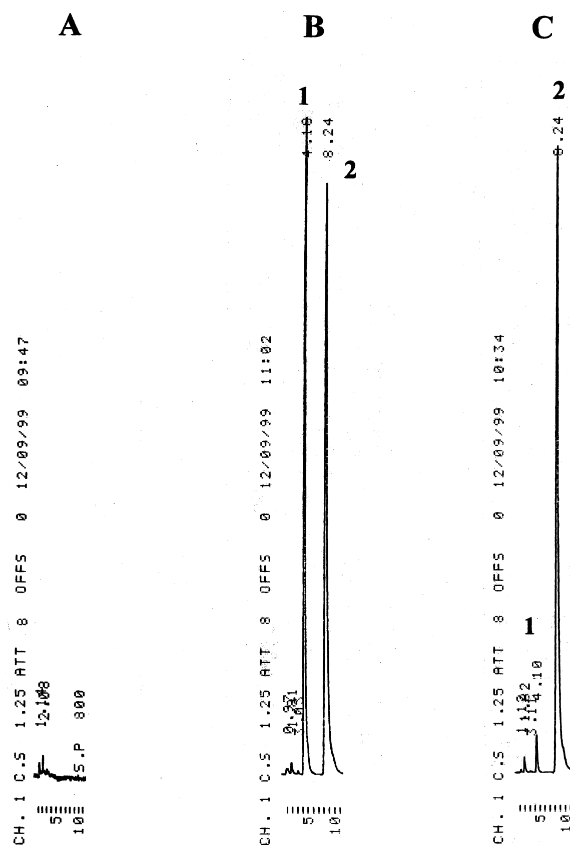


Fig. 2. Chromatograms for the analysis of terazosin in plasma. (A) Blank plasma. (B) Plasma spiked with 25 ng/ml terazosin and 100 ng/ml prazosin. (C) Volunteer plasma containing 1.2 ng/ml terazosin 36 h after oral administration of 1 mg terazosin. *Y*-axis, attenuation 8; *X*-axis, chart speed 1.25 mm/min; 1 = terazosin, 2 = prazosin.

interference from endogenous compounds in the plasma, with retention times of 4.10 and 8.24 min, respectively. The total run time for each sample was 11 min.

Calibration curves were obtained by plotting the peak height ratio of terazosin to that of prazosin versus plasma terazosin concentration. The standard calibration curves ($n=6$) were linear over the concentration range used, with a correlation coefficient of 0.9999, slope of 0.0513 and intercept of 0.0086. The extraction recovery, within- and between-day precision and accuracy values are presented in Table 1. The coefficient of variation (C.V.) and percent error values were all $<7\%$.

Table 1
Extraction recovery, within- and between-day precision and accuracy ($n = 6$)

Conc. (ng/ml)	Recovery		Within-day		Between-day	
	Mean (%)	C.V.%	Precision (C.V.%)	Accuracy (% error)	Precision (C.V.%)	Accuracy (% error)
0.25	107.7	6.9	4.0	5.4	5.4	1.7
0.5	108.4	7.2	2.4	2.7	5.0	3.0
1	105.3	2.5	2.2	1.9	3.0	2.9
12.5	91.5	4.1	2.8	2.2	3.7	2.8
25	93.2	4.9	2.1	1.8	3.9	2.6
50	97.7	8.5	2.2	3.2	2.7	2.3
100	93.6	4.9	2.8	6.1	2.7	2.4

During development of the assay method, poor linearity was initially observed in the calibration curves. This phenomenon was thought to be attributed to the drug being adsorbed onto the glass and plastic tubes used in the analysis and was confirmed by extracting glass and plastic tubes pre-exposed to terazosin with the extracting solvent. A drug peak was observed when these extracts were injected into the HPLC system, but not with extracts from tubes not previously exposed to the drug. An attempt to quantify the percentage adsorbed showed highly variable results.

Such adsorption of drugs to glassware has previously been reported for fluorouracil [12], calcitonin [13] and some antibiotics [14], but not for terazosin. Indeed, this may be the reason for the relatively poor reproducibility of the methods described in previous reports [9–11]. In the present study, silanization or vigorous cleaning of the glassware did not solve the problem, but it could be circumvented by using disposable Eppendorf microcentrifuge tubes. Also, rinsing the injector port with methanol after each injection helped to prevent any carry-over effect. While adsorption could not be totally or completely eliminated, there was no cross-contamination, and the linearity of the standard curves and the precision and accuracy of the assay method were not affected.

The present method was applied to analyze plasma

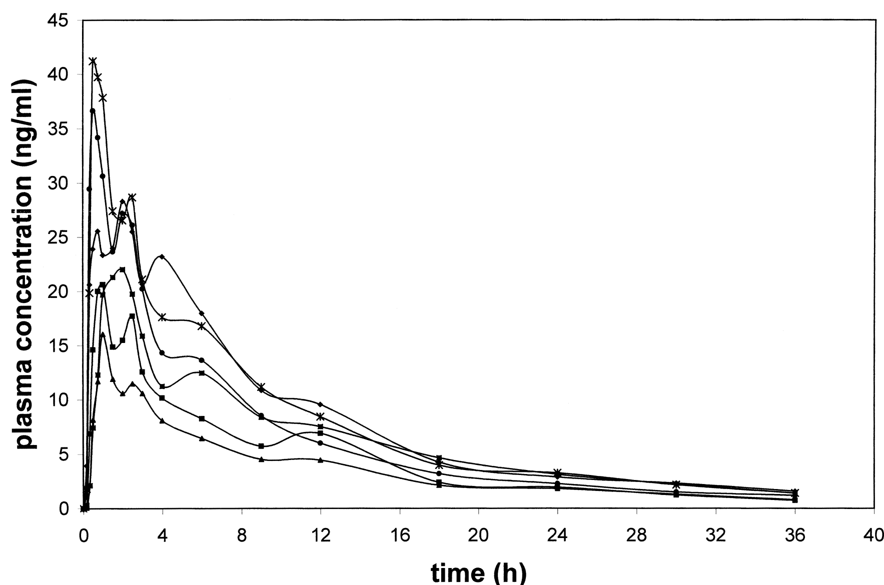


Fig. 3. Plasma terazosin concentration versus time profiles for six volunteers following oral administration of 1 mg terazosin.

samples obtained from six healthy adult male volunteers who participated in a pharmacokinetic study of terazosin. Fig. 3 shows the individual plasma terazosin concentration versus time profiles from the volunteers after oral dosing with 1 mg terazosin. It can be seen that terazosin is absorbed rapidly with peak concentrations (16.9–41.2 ng/ml) attained within 2 h. It appears to have a long terminal elimination phase ($t_{1/2} = 10.8$ h). The development of the present assay enabled the accurate quantification of terazosin at 36 h post dosing.

In summary, the HPLC method described here is simple, sensitive, reproducible, requires only a small sample volume and is applicable to pharmacokinetic studies of terazosin.

Acknowledgements

The authors wish to acknowledge the support of Abbott Laboratories (Malaysia).

References

[1] P.A. Abraham, C.E. Halstenson, G.R. Matzke, Pharmacotherapy 5 (1985) 285.
[2] R.A. Graham, Am. J. Cardiol. 53 (1984) 16A.
[3] J.J. Kyncl, R.E. Hollinger, K.W. Oheim, M. Winn, Pharmacologist 3 (1980) 272.
[4] P.G. Fabricus, P. Weizert, U. Dunzendorfer, J.M. Hannaford, C. Maurath, Prostate 3 (Suppl.) (1990) 85.
[5] U. Dunzendorfer, Urology 32 (Suppl. 6) (1988) 21.
[6] H. Lepor, Urology 45 (1995) 406.
[7] A.P. Zavitsanos, T. Alebic-Kolbah, J. Chromatogr. A 794 (1998) 45.
[8] K. Taguchi, R.F. Schafers, M.C. Michel, Br. J. Clin. Pharmacol. 45 (1998) 49.
[9] S.E. Patterson, J. Chromatogr. 311 (1984) 206.
[10] E.C. Sekhar, T.R. Rao, K.R. Sekhar, M.U. Naidu, P.U. Rani, T.V. Kumar, V.P. Kumar, J. Chromatogr. B 710 (1998) 137.
[11] R.K. Bhamra, R.J. Flanagan, D.W. Holt, J. Chromatogr. 380 (1986) 216.
[12] O. Driessen, D. de Vos, P.J.A. Timmermans, J. Pharm. Sci. 67 (1978) 1495.
[13] S.L. Law, C.L. Shih, Drug Dev. Ind. Pharm. 25 (1999) 253.
[14] T. Mizutani, J. Pharm. Pharmacol. 34 (1982) 608.