ELSEVIER

Journal of Chromatography B, 710 (1998) 137–142

JOURNAL OF CHROMATOGRAPHY B

Determination of terazosin in human plasma, using high-performance liquid chromatography with fluorescence detection

E. Chandra Sekhar, T.R.K Rao, K. Ravi Sekhar, M.U.R. Naidu*, J.C. Shobha, P.U. Rani, T. Vijay Kumar, V. Praveen Kumar

Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Panjagutta, Hyderabad 500 082, India

Received 23 July 1997; received in revised form 25 February 1998; accepted 26 February 1998

Abstract

A selective, sensitive, rapid and reproducible high-performance liquid chromatographic method for the determination of terazosin in plasma is described. The structurally related compound prazosin was used as an internal standard. The method comprises extraction with methylene chloride followed by chromatography on a C_{18} reversed-phase column. The compounds were detected using spectrofluorimetry. The absolute recoveries were more than 90% with a minimal detection of 1 ng/ml and calibration curve was linear between 1 and 80 ng/ml.

Keywords: Terazosin

1. Introduction

Terazosin hydrochloride dihydrate (THD) 2-[4-(2-tetra hydrofuranyl carbonyl]-1-piperazinyl-6,7-dimethoxy-4-quinazolinamine monohydro- chloride dihydrate (Fig. 1) is a highly selective potent alpha-1 adrenoreceptor antagonist used in the treatment of hypertension [1]. The effectiveness of THD as an antihypertensive is reported in the literature [2–7]. THD pharmacokinetics were shown to be linear in the range 0.1–7.5 mg orally and 1–5 mg intravenously [8,9]. It undergoes extrahepatic metabolism and major route of elimination is via bile [8,9]. The high potency of THD necessitated the development of a very sensitive assay in order to quantify the low plasma concentrations following a therapeutic dose of 1–3 mg. Different analytical methods have been used for the determination of THD in biological fluids following intravenous and oral dosage [8] and also to separate the minor impurities and degradation products from bulk THD [24]. These include spectrofluorimetry [10], high-performance liquid chromatography (HPLC) with UV [11] or fluorescence [12–20,23] detection. These methods involve lengthy extraction procedures which either incorporate back-extraction [11,12,17,18] or direct extraction of small sample volume into an organic layer [21,22] which leads to column deterioration. Though different analytical methods were published for THD estimation the relative standard deviation was reported to be >16% [22] at a concentration of 1 ng/ml. The HPLC method described here is rapid, sensitive, selective and reproducible for THD determination with low background interference and may be suit-

*Corresponding author.

0378-4347/98/$19.00
PII: S0378-4347(98)00120-0

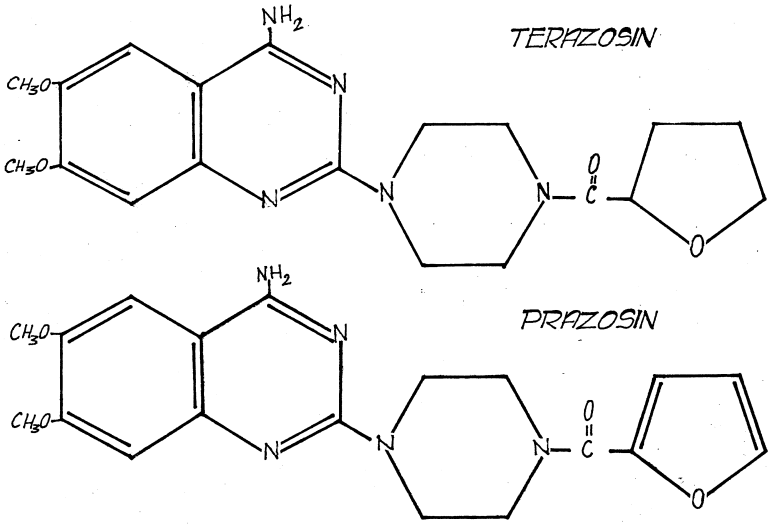

Fig. 1. Chemical structures of terazosin and prazosin.

able for routine pharmacokinetic studies following therapeutic doses.

2. Experimental

2.1. Chemicals and equipment

Terazosin was obtained from Cheminor Drugs (Hyderabad, India). The internal standard (I.S.) prazosin [1,(4-amino-6,7-dimethoxy-2-quinazolinyl-4-(2-furoyl) piperazine hydrochloride was a generous gift from Sun Pharmaceuticals (Bombay, India). Methanol, acetonitrile and methylene chloride were HPLC grade and sodium hydroxide disodiumhydrogen orthophosphate were analytical grade obtained from Qualigens (India). A vortex mixer (Model 2MLH), a 16-tube centrifuge (R8C from Remi Instruments, India) and a metabolic shaker (NSW133) from NSW (New Delhi, India) were used for sample preparation. All aqueous solutions were prepared using penta distilled water.

2.2. Instrumentation and conditions

Chromatographic separations were performed using a Shimadzu (Shimadzu, Kyoto, Japan) Model LC-6A a constant-flow reciprocating pump. The samples were injected using an autosampler SIL-6B (Shimadzu). The column dimensions were 250×4.6 mm I.D. packed with 5-μm ODS material (Shimpack, Shimadzu). The mobile phase was methanol–acetonitrile–0.04 *M* disodiumhydrogen orthophosphate (22:22:56, v/v) adjusted to pH 5.0 with 0.9 *M* phosphoric acid. All separations were performed isocratically at a flow-rate of 1.2 ml/min and the column temperature was maintained at 40°C with a column oven CT0-6A (Shimadzu). The column effluent was monitored for fluorescence using Shimadzu RF535 fluorescence detector operated at λ_{ex} of 250 nm and λ_{em} of 370 nm. For data recording and integration a Shimadzu CR4A integrator was used.

2.3. Standard solutions

A stock solution of THD and prazosin equivalent to 1 mg/ml was prepared separately in methanol. Appropriate volumes of stock THD were diluted with methanol to give different concentrations of 1, 5, 10, 20, 40 and 80 ng/ml, respectively. Working I.S. (prazosin) solution giving a concentration of 1 ng/μl was also prepared.

2.4. Sample preparation

2.4.1. Extraction procedure

To 1 ml of plasma in a 10-ml screw-capped test tube, 50 μl of internal standard (50 ng prazosin) were added. After the addition of 100 μl of 1 *M* sodium hydroxide and 8 ml of methylene chloride the test tubes were stoppered tightly with a PTFE seal and extraction was carried out on a mechanical shaker for 30 min to ensure complete extraction of THD particularly at higher concentrations (>60 ng/ml). The test tubes were centrifuged for 10 min, 6–7 ml of the organic layer was transferred to a 10-ml conical glass tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was dissolved in 125 μl methanol and a 50-μl volume was injected onto the reversed-phase chromatographic system.

2.4.2. Calibration curves

Calibrators were prepared by the addition of THD ranging from 1 to 80 ng/ml to 1 ml of drug-free plasma. These calibrators were stable for at least 2 months when stored at −20°C [22]. On analysis of the standards the ratio of the peak height of the THD to the peak height of prazosin was plotted against THD concentrations and linear regression analysis was performed. The resulting slope was used to calculate the concentration of THD. The calibration curve was linear between 1 and 80 ng/ml with correlation coefficient $r \geq 0.999$.

3. Results and discussion

3.1. Chromatography

Fig. 2 shows typical chromatograms of extracted samples of blank human plasma (A), plasma spiked with THD equivalent to 20 ng/ml and 50 ng/ml of the I.S. (B), and plasma sample from a healthy volunteer 3 h after the oral administration of 5 mg of terazosin (C). All the peaks were well resolved without endogenous interference. No potential metabolites of the drug have been detected. Thus the method has been shown to be specific for THD determination. The retention times of terazosin and prazosin were 3.8 and 4.9 min, respectively. Variation of the pH of the mobile phase by ±0.2 units was not found to have significant effect on the resolution of the peaks. The selection of prazosin as the I.S. was based on chemical structure and its chromatographic and extraction behaviour. The number of theoretical plates from these chromatograms, according to our calculations, was found to be >1500. Under the conditions described the equilibration between stationary phase and the mobile phase could readily be achieved within 30 min and no deterioration of the column efficiency was observed after everyday use for several months and at least 300 injections.

3.2. Linearity, accuracy and precision

The calibration curve was linear over the range tested (1–80 ng/ml) with correlation coefficient $r \geq 0.999$. 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 1–80 ng/ml. The intra-day and inter-day variations for THD were calculated using data accumulated over a period of 6 days. Intra-day precision was evaluated by replicate analysis of a pooled plasma containing THD at various concentrations ranging between 1 and 80 ng/ml ($n=6$) and the I.S. Inter-day precision was similarly evaluated over 1 week ($n=6$). The results of intra- and inter-day are presented in Table 1. The intra-day coefficient of variance (C.V.%) was 2.3–10.6% and the inter-day C.V.% varied from 1.7 to 12.5%. High accuracy and precision was achieved by the use of the I.S. and relatively large injection volume (50 μl) thus minimizing liquid transfer and sampling errors. The solvent chosen and the time taken for extraction helped to attain better yield.

3.3. Recovery and detection limit

The extraction recovery of THD was assessed in the range 1–80 ng/ml. Three samples of each concentration of THD in methanol containing I.S. were directly injected. The resulting peak height ratios represent the maximum recovery from the extraction procedure. Plasma samples ($n=3$) containing THD (1–80 ng/ml) and I.S. were analysed after extraction. The absolute recovery was calculated by comparing peak height ratios of direct

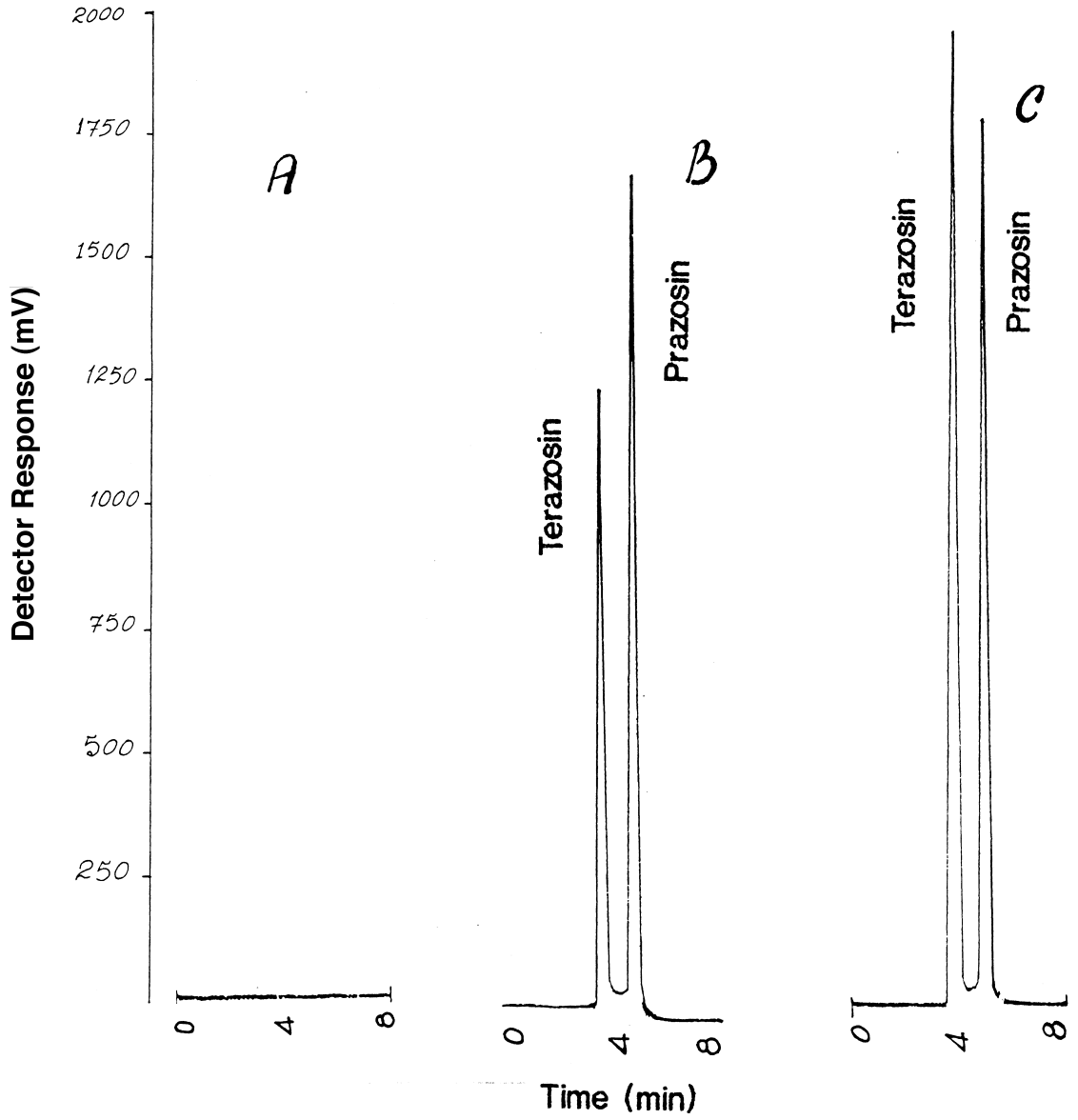

Fig. 2. Chromatograms of human plasma extracts: (A) tracing of human blank plasma; (B) human plasma spiked with 20 ng/ml terazosin and 50 ng/ml prazosin (I.S.); (C) 3-h postdosing plasma from human volunteer who received 5 mg of terazosin orally.

injections of pure THD with those of plasma samples containing equivalent amounts of THD. The absolute recoveries of THD ranged from 94% to 107%. Based on a signal-to-noise ratio of 4:1 (3 times to basal line) the lower limit of detection was 1 ng/ml Table 2.

3.4. Pharmacokinetic study

The described method has been applied to measure the plasma levels of THD following a single oral dose of 5 mg terazosin in 24 healthy human volunteers. Fig. 3 shows the typical plasma concen-

Table 1
Precision accuracy and reproducibility of the analysis at each concentration daily over a period of 6 days

Conc. (ng/ml)	Inter-day (n=8)			Intra-day (n=6)		
	Peak height ratio Mean	±S.D.	C.V. (%)	Peak height ratio Mean	±S.D.	C.V. (%)
0	0	0	–	0	0	–
1	0.04	0.005	12.5	0.047	0.005	10.6
5	0.21	0.02	9.5	0.22	0.01	4.5
10	0.41	0.02	4.9	0.44	0.025	5.7
20	0.81	0.03	3.7	0.87	0.035	4.2
40	1.56	0.07	4.5	1.56	0.07	4.5
80	3.03	0.05	1.7	3.09	0.07	2.3

Table 2
Absolute recovery of terazosin in plasma (n=6)

Added known concentration terzosin (ng/ml)	Mean calculated concentration of terazosin (ng/ml)	Absolute recovery of terazosin (%)	Relative standard deviation (%)
1	1.07	107.0	12.8
5	5.16	103.0	8.1
10	10.00	100.0	5.4
20	19.80	99.0	4.0
40	38.00	95.0	4.2
80	75.00	94.0	1.8

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

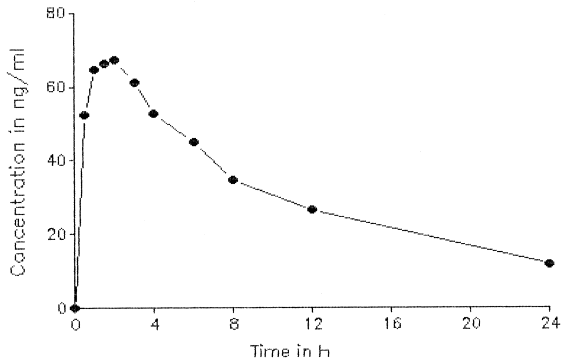

Fig. 3. Mean plasma concentrations of terazosin after oral administration of single dose of 5 mg terazosin, (N=40).

tration–time profiles of THD. The mean C_{max} of THD in these volunteers was 69.00±10.2 ng/ml achieved at t_{max} 1.48±0.79 h. These results were in good agreement with the reported values [8]. During method development several solvent systems were investigated for extraction of the THD from plasma. They include dichloromethane, diethyl ether, ethyl acetate, chloroform, benzene, methanol and acetonitrile. It was found that both acetonitrile and methylene chloride offered complete extraction of THD. The use of acetonitrile as extraction solvent [22] did not give good recovery of THD at lower concentrations and also resulted in increased column pressure. In conclusion, the method described is an accurate, precise, sensitive and reproducible analytical procedure for the estimation of THD in human plasma using a structurally related compound prazosin as I.S. Due to its ability to detect low

concentrations the assay is appropriate for routine pharmacokinetic studies following therapeutic doses.

References

[1] J.J. Kynel, Am. J. Med. 80 (1986) 12–19.
[2] H. Tsuchihashi, T. Nagatomo, J. Pharmacobiol. Dyn. 12 (1989) 170–174.
[3] T. Nagatomo, A. Tajiri, T. Nakamura, R. Hokibara, Y. Tanaka, J. Aono, H. Tsuchihashi, Chem. Pharm. Bull. 35 (1987) 1629–1632.
[4] S. Chrsant, I. Bal, B. Johnson, M. McPherson, Clin. Cardiol. 8/9 (1985) 486–489.
[5] R. Graham, Am. J. Cardiol. 53 (1984) 16A–20A.
[6] E. Nelson, J. Pool, A. Taylor, J. Mitchell, Clin. Pharmacol. Ther. 31 (1982) 255.
[7] P. Abraham, C. Halstenson, G. Matzke, J. Napier, W. Keane, Pharmacotherapy 5 (1985) 285–289.
[8] R.C. Sonders, Am. J. Med. 80 (1986) 20–24.
[9] S.E. Patterson, Clin. Pharmacol. Ther. 38(4) (1985) 423–427.
[10] A.J. Wood, P. Bolli, F.O. Simpson, Br. J. Clin. Pharmacol. 3 (1976) 199.
[11] Y.G. Yee, P.C. Rubin, P. Meffin, J. Chromatogr. 172 (1979) 313.
[12] P.A. Reece, J. Chromatogr. 221 (1980) 188.
[13] E.T. Lin, R.A. Baughman, L.Z. Benet, J. Chromatogr. 183 (1980) 367.
[14] P. Larochelle, P. DuSouich, P. Hamet, P. Larocque, J. Armstrong, Hypertension 4 (1982) 93.
[15] T.M. Twomey, D.C. Hobbs, J. Pharm. Sci. 67 (1978) 1468.
[16] P.C. Rubin, J. Brunton, P. Meredith, J. Chromatogr. 221 (1980) 193.
[17] M.K. Dynon, B. Jarrot, O. Drummer, W.J. Louis, Clin. Pharmacokinet. 5 (1980) 583.
[18] V.K. Piotrovskii, V.G. Belolipetskaya, A.R. El'Man, V.I. Metelista, J. Chromatogr. 278 (1983) 469.
[19] P. Jaillon, P. Rubin, Y.G. Yee, R. Ball, R. Kates, D. Harrison, T. Blaschke, Clin. Pharmacol. Ther. 25 (1979) 790.
[20] J. Dokladalova, S.J. Coco, P.R. Lemke, G.T. Quercia, J.J. Korst, J. Chromatogr. 224 (1981) 33.
[21] R.K. Bhamra, R.J. Flanagan, D.W. Holt, J. Chromatogr. 380 (1986) 216–221.
[22] S.E. Patterson, J. Chromatogr. 311 (1984) 206–212.
[23] I. Jane, A. McKinnon, R. Flanagan, J. Chromatogr. 380 (1986) 216–221.
[24] J.F. Bauer, S.K. Krogh, Z.L. Chang, C.F. Wong, J. Chromatogr. 323 (1993) 175–181.