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Note

Rapid and sensitive analysis of terazosin in plasma, peritoneal dialysis solution, and urine using high-performance liquid chromatography with fluorescence detection

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Terazosin hydrochloride dihydrate {2-[4-(2-tetrahydrofuranyl)carbonyl]-1-piperazinyl-6,7-dimethoxy-4-quinazolinamine monohydrochloride, dihydrate, Fig. 1} is an antihypertensive agent [1, 2] under current development at Abbott Laboratories under the registered trademark Vasocard. The high potency of this drug necessitated the development of a very sensitive assay in order to quantify the low plasma levels which follow a therapeutic dose (1—3 mg).

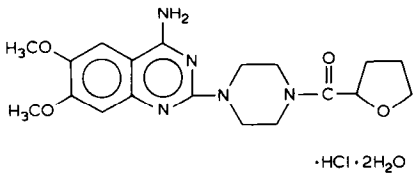

Fig. 1. Chemical structure of terazosin hydrochloride dihydrate.

The high-performance liquid chromatographic (HPLC) method reported here utilizes extraction of plasma, dialysis solution, or urine samples for lowest background interference and highest assay sensitivity. A much faster alternative sample preparation method for small volumes of plasma or serum (0.1—0.2 ml) using acetonitrile precipitation of proteins is also presented. Following extraction, the lower quantifiable concentration of terazosin was about 0.25 ng/ml using a 1-ml plasma or dialysis solution sample, and was about 1 ng/ml using a 0.2-ml urine sample. The assay was reproducible in the range of 1—50 ng/ml with a mean relative standard deviation of about 7% and 4% for plasma

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and dialysis solution, respectively. The urine assay was reproducible within the range of 10—500 ng/ml with a mean relative standard deviation of about 3%. Following acetonitrile precipitation, the serum assay was reproducible within the range of 50—1000 ng/ml with a mean relative standard deviation of about 1%.

EXPERIMENTAL

Chemicals and equipment

Terazosin hydrochloride dihydrate (THD) and prazosin hydrochloride (PH), the internal standard, were obtained from Abbott Labs. (North Chicago, IL, U.S.A.). Peritoneal dialysis solution (Dianeal PD-2 with 2.5% dextrose) was obtained from Travenol Labs. (Deerfield, IL, U.S.A.). Reagent-grade phosphoric acid, sodium hydroxide, and buffer salts were obtained from Mallinckrodt (St. Louis, MO, U.S.A.). All solvents were HPLC grade from J.T. Baker (Phillipsburg, NJ, U.S.A.). A Vortex Genie mixer or SMI Multi-tube Vortex mixer from American Scientific Products (Division of American Hospital Supply, McGaw Park, IL, U.S.A.) was used for sample preparation.

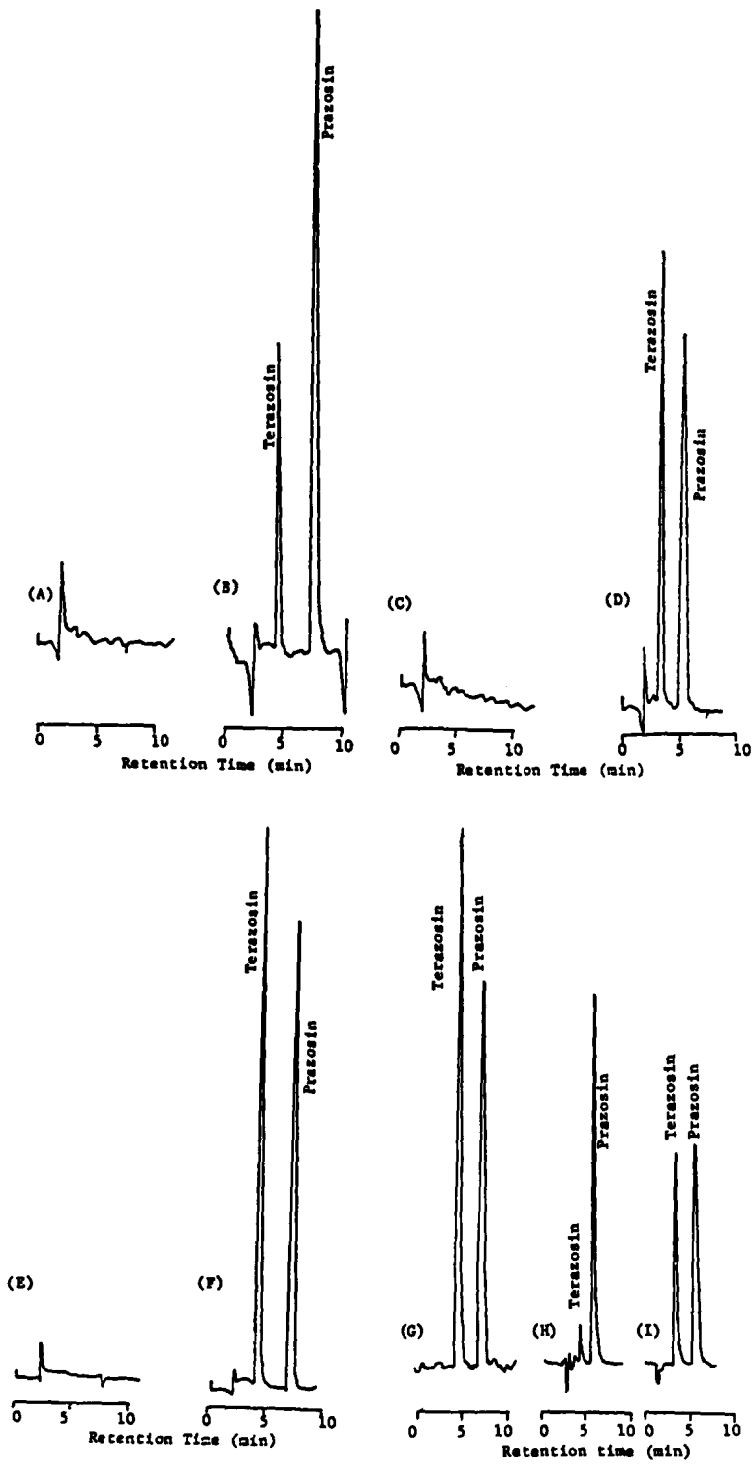
Stock solutions

For convenience in the preparation of eluents for HPLC, stock solutions of 0.1 *M* sodium phosphate buffer, pH 7.0 and 5.0, were usually prepared. Eluents were filtered through a Nuclepore (Pleasanton, CA, U.S.A.) 400-nm polycarbonate membrane after preparation, and degassed by vacuum sonication just before use.

Stock solutions of 1 *M* sodium hydroxide and 0.1 *M* phosphoric acid were prepared in distilled water. Stock solutions of THD equivalent to 200 μg/ml terazosin (11.9 mg of THD is equivalent to 10.0 mg of terazosin) and PH (80 μg/ml), the internal standard, were prepared in methanol. Standard solutions of terazosin at 20 μg/ml in methanol, and 2, 0.2, and 0.01 μg/ml in methanol—water were prepared by serial 1:10 dilutions of the 200 μg/ml terazosin stock solution. Standard solutions of PH at 200 ng/ml in acetonitrile and 4 ng/ml in 20% (v/v) ethyl acetate in benzene were prepared.

Chromatographic systems and conditions

HPLC separations were performed using a Waters Assoc. (Milford, MA, U.S.A.) Model M6000 or M6000A or Beckman (Fullerton, CA, U.S.A.) Model 110A reciprocating pump at a flow-rate of 1—2 ml/min for solvent delivery. The reversed-phase HPLC columns used in this study were Waters Assoc. 300 or 150 × 3.9 mm I.D. columns which were repacked with IBM C 1 (Danbury, CT, U.S.A.) 5-μm particle size or Spherisorb ODS (Phase Separation, Queensbury, U.K.) 10-μm particle size packing by Analytical Sciences (Santa Clara, CA, U.S.A.). The eluents were composed of 22—25% (v/v) acetonitrile and 6% (v/v) tetrahydrofuran in 0.004—0.02 *M* sodium phosphate, pH 5—7.0 (see legends of Figs. 2 and 3 for specific columns and conditions). A Waters Assoc. WISP 710B or Perkin-Elmer (Norwalk, CT, U.S.A.) Model ISS-100 automatic sampler was used for sample processing. The HPLC system was operated at ambient temperature, and the effluent was monitored for fluorescence with a


(A)
(B)
Terazosin
Prazosin
(C)
(D)
Terazosin
Prazosin
0 5 10
Retention Time (min)
(E)
(F)
Terazosin
Prazosin
(G)
Terazosin
Prazosin
(H)
Terazosin
Prazosin
(I)
Terazosin
Prazosin
Retention time (min)

Schoeffel (Westwood, NJ, U.S.A.) Model 970 LC fluorometer using a 370-nm emission filter after excitation at 250 nm.

Sample preparation procedures

Extraction. A suitable volume of plasma, dialysis solution, or urine up to 1 ml was combined with 100 μl of 1 *M* sodium hydroxide in a culture tube and mixed well. Five ml of 20% (v/v) ethyl acetate in benzene containing 2—50 ng/ml of PH (as appropriate) was added to each tube and mixed for 5 min on a vortex-type mixer to extract terazosin into the organic (upper) phase. Following centrifugation to separate phases (if necessary), 4—4.5 ml of the organic phase were transferred to a second culture tube and evaporated to dryness in a water bath at 40—50°C under a gentle stream of air. The residue was redissolved in 300 μl of 0.1 *M* phosphoric acid or mobile phase and an aliquant was injected into the chromatograph.

Acetonitrile precipitation. A small volume (100—200 μl) of plasma was combined with 2 vols. of cold (5°C) acetonitrile containing 200 ng/ml of PH, the internal standard, and mixed immediately to precipitate proteins. Following centrifugation at 5°C for 5—10 min to sediment the precipitate, the supernatant was decanted into a second culture tube and evaporated to dryness in a water bath at 40—50°C under a gentle stream of air. The residue was redissolved in 400 μl of HPLC eluent and an aliquant was injected into the chromatograph.

Calibration curves for plasma, serum, dialysis solution, and urine

Sets of standard plasma, serum, dialysis solution, and urine samples were prepared by the addition of known amounts of THD to blank plasma, serum, dialysis solution, or urine. The chromatographic peak height ratios of terazosin/prazosin were subjected to linear regression versus the corresponding terazosin concentrations. The resulting equation was used to calculate the concentration of terazosin in the test samples. The terazosin levels, sample size, and the PH levels added as internal standard to the samples may be varied to suit the concentration ranges of the intended analyses.

Recovery

Recovery of terazosin from the extraction procedures was determined by comparing the peak height ratios of test samples to blank samples which were spiked with terazosin at the same concentration following extraction.

Fig. 2. Chromatograms from human plasma (1 ml), dialysis solution (1 ml), and human urine (0.2 ml) samples prepared by the extraction method. (A) Blank plasma; (B) blank plasma spiked with THD equivalent to 5 ng/ml terazosin and 20 ng/ml PH; (C) blank dialysis solution; (D) blank dialysis solution spiked with THD equivalent to 10 ng/ml terazosin and 20 ng/ml PH; (E) blank urine; (F) blank urine spiked with THD equivalent to 50 ng/ml terazosin and 100 ng/ml PH; (G) 0.5-h postdosing plasma spiked with 20 ng/ml PH; (H) 3—6 h postdosing peritoneal dialysate spiked with 20 ng/ml PH; (I) 8—12 h postdosing urine spiked with 50 ng/ml PH from a patient with renal insufficiency who received an oral dose of THD (1 mg free base). Chromatographic conditions were as follows: column, 150 × 3.9 mm I.D., IBM C1; mobile phase, 22% acetonitrile and 6% tetrahydrofuran, pH 7.0; flow-rate, 1 ml/min; detector sensitivity, 0.2 μA; injection volumes, 100 μl (A, B); 60 μl (C, D, G, H); 15 μl (E, F).

RESULTS AND DISCUSSION

Fig. 2 shows typical chromatograms for extracted samples of blank human plasma (A), blank human plasma spiked with THD equivalent to 5 ng/ml terazosin and 20 ng/ml of PH, the internal standard (B), blank dialysis solution (C), blank dialysis solution spiked with THD equivalent to 10 ng/ml terazosin and 20 ng/ml PH (D), blank human urine (E), and blank human urine spiked with THD equivalent to 50 ng/ml terazosin and 100 ng/ml PH (F). Fig. 2 also shows 0.5 h postdosing plasma spiked with 20 ng/ml PH (G), 3—6 h postdosing peritoneal dialysate spiked with 20 ng/ml PH (H), and 8—12 h postdosing urine spiked with 50 ng/ml PH (I) from a patient with renal insufficiency who received an oral dose of THD (1 mg free base). Fig. 3 shows typical chromatograms of dog serum prepared by the acetonitrile precipitation method: blank (A), blank spiked with THD equivalent to 50 ng/ml terazosin and 100 ng/ml PH (B), and a 2-h postdosing serum sample from a dog which was administered an oral dose of THD (0.6 mg/kg free base) and methyclothiazide (0.3 mg/kg), spiked with 100 ng/ml PH (C).

To determine the precision and accuracy of the assay methods, replicate samples (3—4) were analyzed at five concentrations of the plasma, serum, dialysis solution, and urine assays. The results of these analyses are summarized in Table I for extraction data, and Table II for acetonitrile precipitation data.

A comparison of various weighting factors showed that 1/response-squared ($1/R^2$) weighting was most similar to 1/variance weighting for the plasma

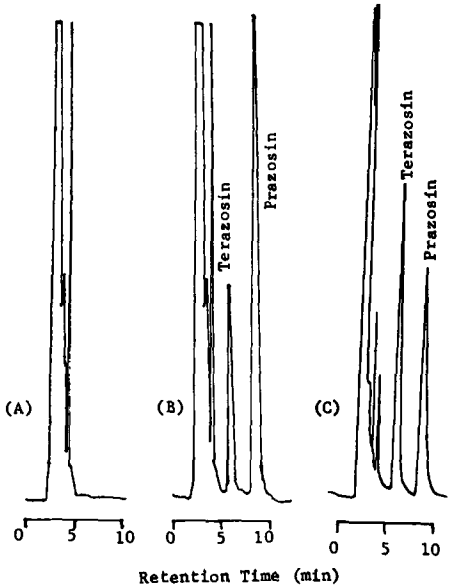


Fig. 3. Chromatograms from dog serum samples (0.2 ml) prepared by the acetonitrile precipitation method. (A) Blank; (B) blank spiked with THD equivalent to 50 ng/ml terazosin and 100 ng/ml PH; (C) 2-h postdosing sample from a dog which was administered an oral dose of THD (0.6 mg/kg free base) and methyclothiazide (0.3 mg/kg), spiked with 100 ng/ml PH. Chromatographic conditions were as follows: column, 300 × 3.9 mm I.D., Spherisorb ODS; mobile phase, 25% acetonitrile and 6% tetrahydrofuran, pH 5.0; flow-rate, 1.6 ml/min; detector sensitivity, 0.2 μA; injection volumes, 100 μl (A, B); 50 μl (C).

standard curve, while 1/concentration-squared ($1/C^2$) weighting was most similar to 1/variance weighting for the dialysis solution and urine standard curves (Table I). Using $1/R^2$ weighting (Table I), the mean predicted plasma concentrations ranged from 99% to 101% of the calculated concentrations. The relative standard deviations (R.S.D.) of the peak height ratios ranged from 2.8% to 17.8% (mean = 6.9%). This large range resulted from the 1 ng/ml concentration. If this point were excluded, the R.S.D. would range from 2.8% to 6.6% (mean = 4.2%). The mean predicted dialysis solution concentrations ranged from 94% to 105% ($1/C^2$ weighting), with an R.S.D. range of 2.9—6.6% (mean = 4.4%). The mean predicted urine concentrations ranged from 91% to 128% ($1/C^2$ weighting). This large range resulted from the 50 ng/ml point. If this point were excluded, the mean predicted concentrations would range from 91% to 96% of the calculated concentrations. The urine assay had an R.S.D. range of 2.5—5.1% (mean = 3.3%). The data from Table I were subjected to linear regression analysis, and standard curves were constructed which were linear from 1 to 50 ng/ml terazosin in plasma or dialysis solution (r = 0.999) or from 10 to 500 ng/ml terazosin in urine (r = 0.985).

A comparison of various weighting factors for the acetonitrile precipitation method showed that $1/C^2$ weighting was most similar to 1/variance weighting

TABLE I

PRECISION AND ACCURACY DATA OF THE PLASMA, DIALYSIS SOLUTION, AND URINE STANDARD CURVES FOR THE EXTRACTION METHOD

Calculated concentration of terazosin (ng/ml)	Observed mean peak height ratio	Predicted mean concentration of terazosin [Percent of theory] (ng/ml)	Relative standard deviation (%)
Plasma			
1.00	0.087	0.999 [100]	17.8
5.00	0.408	5.029 [101]	3.9
10.00	0.803	10.001 [100]	6.6
30.00	2.401	30.095 [100]	2.8
50.00	3.954	49.632 [99]	3.6
		Mean =	6.9
Dialysis solution			
1.00	0.116	0.994 [99]	6.6
5.00	0.596	5.066 [101]	2.8
10.00	1.233	10.462 [105]	2.9
30.00	3.560	30.179 [101]	5.5
50.00	5.550	47.042 [94]	4.2
		Mean =	4.4
Urine			
10.00	0.232	9.552 [96]	2.5
50.00	1.255	64.163 [128]	5.1
100.00	1.783	92.349 [92]	3.3
300.00	5.273	278.656 [93]	2.7
500.00	8.569	454.607 [91]	2.8
		Mean =	3.3

TABLE II

PRECISION AND ACCURACY DATA OF THE SERUM STANDARD CURVE FOR THE ACETONITRILE PRECIPITATION METHOD

Calculated concentration of terazosin (ng/ml)	Observed mean peak height ratio	Predicted mean concentration of terazosin [Percent of theory] (ng/ml)	Relative standard deviation (%)
50.00	0.443	50.17 [100]	0.3
100.00	0.902	99.61 [100]	0.8
250.00	2.273	247.27 [99]	2.6
500.00	4.641	502.33 [100]	1.1
1000.00	9.325	1006.84 [101]	0.8
			Mean = 1.1

for the serum standard curve. Using $1/C^2$ weighting for the serum assay (Table II), the mean predicted concentrations ranged from 99% to 101% of the calculated concentrations with an R.S.D. range of 0.3—2.6% (mean = 1.1%). Linear regression analysis of these data showed that the curve was linear (r = 0.999) from 50 to 1000 ng/ml of terazosin.

Mean recoveries of terazosin from the extraction procedure were as follows: plasma, 94% and 95% at 5 and 50 ng/ml, respectively; dialysis solution, 99% and 91% at 5 and 50 ng/ml, respectively; urine, 88% and 85% at 50 and 500 ng/ml, respectively. Recovery of terazosin from the acetonitrile precipitation procedure averaged 102% at 1000 ng/ml.

Results of plasma stability experiments have shown quantitative recovery of terazosin from samples stored at room temperature (about 23°C) for at least 30 days and from refrigerated (5°C) or frozen (—20°C) plasma samples stored for at least 60 days.

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