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Note

High-performance liquid chromatographic measurement of prazosin and terazosin in biological fluids

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Prazosin is an orally effective post-sympathetic selective α-1 adrenoceptor antagonist widely used in the treatment of hypertension and congestive heart failure [1]. Terazosin has close structural similarities to prazosin and is under investigation for the treatment of hypertension. Both drugs are administered in low doses (prazosin 1—5 mg daily, terazosin 1—3 mg daily) and elucidation of their single- and multiple-dose pharmacokinetics has necessitated the development of selective and sensitive methodology.

Previous methods for the measurement of prazosin and terazosin (Fig. 1) in biological fluids have included methods based on spectrophotofluorimetry [2] and high-performance liquid chromatography (HPLC) with UV [3] or fluorescence [4—13] detection. The HPLC methods have the required sensitivity for studying the pharmacokinetics of either compound following single intravenous or oral dosage. However, in general these methods require a relatively large sample size (0.1—10 ml) and involve lengthy extraction procedures which either incorporate back-extraction [3, 4, 10, 11] or require

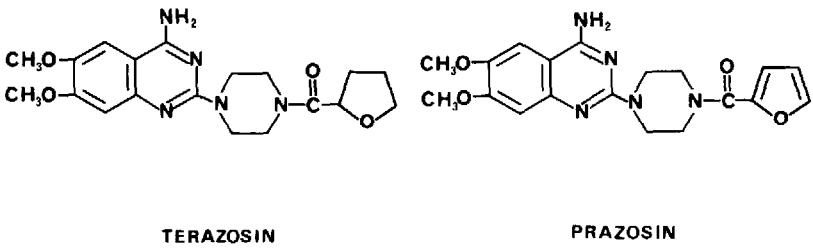

Fig. 1. Structural formulae of prazosin and terazosin.

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evaporation of the organic extract and reconstitution prior to analysis [3, 5, 7, 8, 10, 12, 13].

The method described here is based on the direct extraction of a small volume (50 μl) of sample into an organic solvent at alkaline pH followed by HPLC analysis with fluorimetric detection and has proved suitable for the measurement of both compounds following single oral dosage.

EXPERIMENTAL

Materials and reagents

Prazosin [1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-furoyl)piperazine hydrochloride] was obtained from Pfizer (Sandwich, U.K.) and terazosin [1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(2-tetrahydrofuroyl)piperazine hydrochloride] was obtained from Abbott (Queenborough, U.K.). The internal standard, dimethothiazine mesylate, was obtained from May and Baker (Dagenham, U.K.) and was used as a 2.0 mg/l solution in deionised water obtained by dilution from a methanolic solution equivalent to 1.0 g/l free base.

Methanol and methyl *tert.*-butyl ether were HPLC grade (Rathburn, Walkerburn, U.K.). Sodium hydroxide and sodium chloride were analytical-reagent grade (BDH, Poole, U.K.). Saturated sodium chloride and 4 *M* sodium hydroxide were both prepared in deionised water. Ammonium perchlorate was obtained from Aldrich (Gillingham, U.K.).

High-performance liquid chromatography

A constant-flow reciprocating pump (Applied Chromatography Systems, Model 750/04) was used with a syringe loading injection valve (Rheodyne 7125, 100-μl loop) and a stainless-steel tube (250 × 5 mm I.D.) packed with Spherisorb S5W silica (5 μm average particle size) (Hichrom, Woodley, U.K.) used at ambient temperature. The column effluent was monitored using fluorescence detection (Kratos-Schoeffel FS 970, excitation 250 nm, emission 370–700 nm, time constant 0.5 s) and integration of peak areas was performed using a Hewlett-Packard Model 3392A recording integrator. The mobile phase was 10 m*M* ammonium perchlorate in methanol adjusted to pH 6.7 by the addition of 1 ml/l methanolic sodium hydroxide (0.1 *M*) [14, 15] used at a flow-rate of 2.0 ml/min.

Sample preparation

The sample (50 μl) was pipetted into a small glass (Dreyer) test-tube (50 × 5 mm I.D.) (A.A. Service, Croydon, U.K.) and saturated sodium chloride solution (100 μl), internal standard solution (50 μl), sodium hydroxide solution (50 μl) and methyl *tert.*-butyl ether (200 μl) were added using Hamilton gas-tight syringes fitted with Hamilton repeating mechanisms. The contents of the tube were vortex-mixed for 10 s prior to the addition of the methyl *tert.*-butyl ether and subsequently for 30 s, following which the tubes were centrifuged (9950 *g*, 2 min; Eppendorf 5412). Subsequently, a portion (approximately 110 μl) of the extract was taken and used to fill the sample loop of the injection valve. Duplicate sample analyses were performed and the mean result taken.

Instrument calibration

Standard solutions containing prazosin at concentrations of 20, 50, 100, 200 and 300 μg/l analyte free base were prepared in heparinised human plasma by serial dilution from an aqueous solution of prazosin hydrochloride equivalent to 1.00 g/l free base. Standard solutions containing terazosin at concentrations of 2, 5, 10, 20, 30, 50, 70, 100, 200 and 300 μg/l analyte free base were prepared in heparinised human plasma by serial dilution from an aqueous solution of terazosin hydrochloride equivalent to 1.00 g/l free base. In addition, an internal quality-control sample containing prazosin at a concentration of 60 μg/l was prepared in heparinised human plasma by dilution from an independent stock solution of the drug. Samples containing terazosin at concentrations of 40 and 65 μg/l were prepared similarly. These solutions were stable for at least three months when stored in 250-μl portions at −20°C in the absence of visible light. On analysis of the standards the ratio of the peak area of the analyte to the peak area of the internal standard, when plotted against analyte concentration, was linear and passed through the origin of the graph in each case.

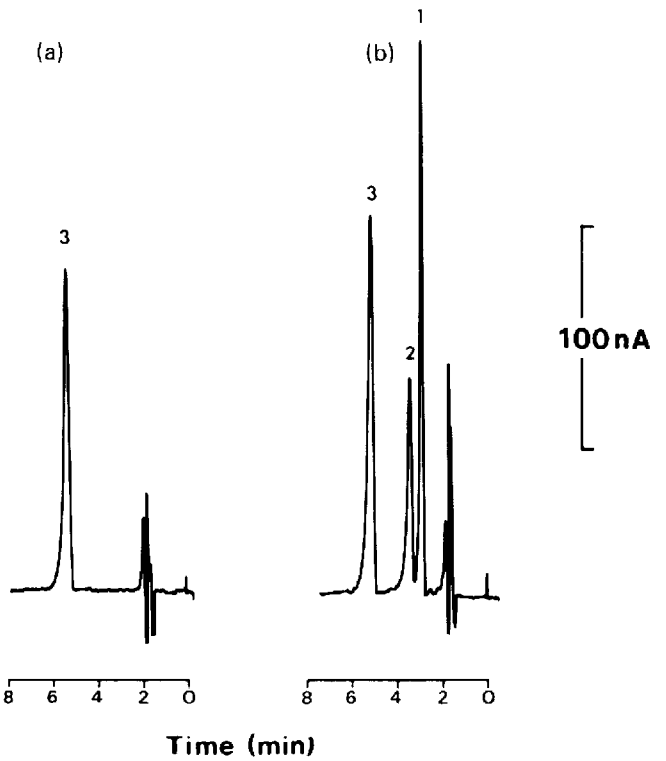

Fig. 2. (a) Chromatogram obtained on analysis of an extract of analyte free human plasma, 100-μl injection; the initial dimethothiazine (3) concentration was 2.0 mg/l. (b) Chromatogram obtained on analysis of a standard solution prepared in heparinised human plasma containing prazosin (1) and terazosin (2) (each 50 μg/l), 100-μl injection; the initial dimethothiazine (3) concentration was 2.0 mg/l.

RESULTS AND DISCUSSION

No endogenous sources of interference have been observed (Fig. 2a). The analysis of an extract of a plasma standard containing both prazosin and terazosin is illustrated in Fig. 2b and the chromatography of plasma extracts from patients receiving either prazosin or terazosin is illustrated in Fig. 3a and b. Analyses of these and other specimens performed without the addition of dimethothiazine have not revealed the presence of compound which could co-elute with the standard. No potential metabolites of either drug have been detected. (N.B. The major route of metabolism for prazosin is 6- and 7-dealkylation [1] and this route of metabolism may occur for terazosin owing to the structural similarity of the compounds although no information on the metabolism of terazosin is available.)

Interference from other drugs was minimal although some compounds which extracted under the conditions of the assay were studied further as potential sources of interference (Table I). In contrast to the other compounds studied, quinidine/quinine show good fluorescence and are only partially resolved from the internal standard. If these compounds were present, use of another internal standard such as protriptyline would be indicated. A number of additional cardioactive drugs and metabolites studied, including amiodarone, desethyl-amiodarone, atenolol, disopyramide, lignocaine, nifedipine, lorcainide, prenalterol, propafenone, labetalol, sotalol, timolol and methyldopa were not detected on this system.

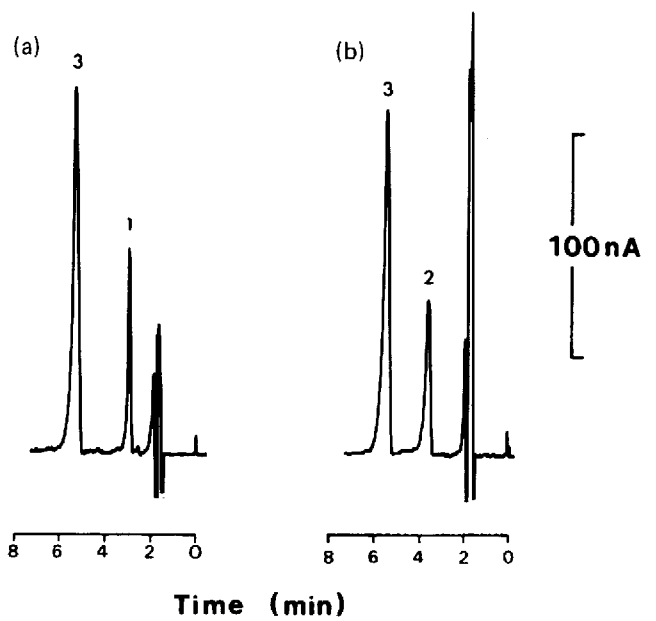

Fig. 3. (a) Chromatogram obtained on analysis of a plasma sample from a patient receiving prazosin (2 mg daily), 100-μl injection; the initial dimethothiazine (3) concentration was 2.0 mg/l and the prazosin (1) concentration was found to be 19.3 μg/l. (b) Chromatogram obtained on analysis of a plasma sample from a patient receiving terazosin (2 mg daily), 100-μl injection; the initial dimethothiazine (3) concentration was 2.0 mg/l and the terazosin (2) concentration was found to be 40.0 μg/l.

TABLE I

RETENTION TIMES RELATIVE TO DIMETHOTHIAZINE OF PRAZOSIN, TERAZOSIN AND SOME OTHER COMPOUNDS

Compound	Relative retention time	Compound	Relative retention time
Desalkylflurazepam*	0.43	Terazosin	0.65
Nitrazepam*	0.44	Prajmalium	0.67
Dipyridamole	0.46	Desipramine	0.78
Pyrimethamine*	0.48	Mianserin	0.78
Doxazosin	0.50	Norverapamil	0.78
Prazosin	0.55	Flurazepam	0.85
Nadolol	0.56	Protriptyline	0.88
Pindolol	0.56	Dimethothiazine	1.00
Triamterene	0.57	Gallopamil*	1.04
Mexiletine	0.60	Verapamil	1.05
Oxprenolol*	0.60	Quinidine/quinine**	1.09
Ketanserin	0.61	Orphenadrine**	1.10
Metoprolol	0.61	Trimipramine	1.13
Penbutolol	0.61	N-Acetylprocainamide**	1.19
Propranolol	0.61	Procainamide**	1.21
Flecainide	0.63	Imipramine	1.33
Ajmaline	0.63	Chlorpromazine**	2.02

*Relatively poor fluorescence.
**Tailing peaks.

TABLE II

INTRA- AND INTER-ASSAY REPRODUCIBILITY OF THE ASSAY FOR PRAZOSIN AND TERAZOSIN (n = 10)

Concentration (μg/l)	Coefficient of variation (%)	
	Prazosin	Terazosin
Intra-assay		
20	3.0	2.7
100	1.9	1.8
Inter-assay		
60	2.1	1.8

The addition of a saturated solution of sodium chloride improved the extraction of terazosin by approximately 20% on comparison with methanolic standard, but had a less marked effect on the extraction of prazosin. The mean recoveries from heparinised human plasma with and without saturated sodium chloride solution were 80 and 67% and 58 and 37% for prazosin and terazosin, respectively (n = 5, each at a concentration of 50 μg/l).

The intra- and inter-assay coefficients of variation (C.V.) for replicate analyses of standard solutions of prazosin and terazosin prepared in heparinised human plasma are shown in Table II. Using a sample size of 50 μl, the limit of accurate measurement for prazosin and terazosin in plasma was 1 μg/l (intra-

assay C.V. values, at this concentration were 8.3 and 9.8% for prazosin and terazosin, respectively). This assay has also been used to measure terazosin concentrations in urine samples using standards prepared in terazosin-free human urine, in the range 20—300 μg/l.

REFERENCES

1 W.J. Stanaszek, D. Kellerman, R.N. Brogden and J.A. Romankiewicz, Drugs, 25 (1983) 339.
2 A.J. Wood, P. Bolli and F.O. Simpson, Br. J. Clin. Pharmacol., 3 (1976) 199.
3 S.E. Patterson, J. Chromatogr., 311 (1984) 206.
4 Y.G. Yee, P.C. Rubin and P. Meffin, J. Chromatogr., 172 (1979) 313.
5 P.A. Reece, J. Chromatogr., 221 (1980) 188.
6 E.T. Lin, R.A. Baughman and L.Z. Benet, J. Chromatogr., 183 (1980) 367.
7 P. Larochelle, P. Du Souich, P. Hamet, P. Larocque and J. Armstrong, Hypertension, 4 (1982) 93.
8 T.M. Twomey and D.C. Hobbs, J. Pharm Sci., 67 (1978) 1468.
9 P.C. Rubin, J. Brunton and P. Meredith, J. Chromatogr., 221 (1980) 193.
10 M.K. Dynon, B. Jarrot, O. Drummer and W.J. Louis, Clin. Pharmacokinet., 5 (1980) 583.
11 V.K. Piotrovskii, V.G. Belolipetskaya, A.R. El'Man and V.I. Metelitsa, J. Chromatogr., 278 (1983) 469.
12 P. Jaillon, P. Rubin, Y.G. Yee, R. Ball, R. Kates, D. Harrison and T. Blaschke, Clin. Pharmacol. Ther., 25 (1979) 790.
13 J. Dokladalova, S.J. Coco, P.R. Lemke, G.T. Quercia and J.J. Korst, J. Chromatogr., 224 (1981) 33.
14 R.J. Flanagan and I. Jane, J. Chromatogr., 323 (1985) 173.
15 I. Jane, A. McKinnon and R.J. Flanagan, J. Chromatogr., 323 (1985) 191.