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Note

Quantification of prazosin in plasma by high-performance liquid chromatography

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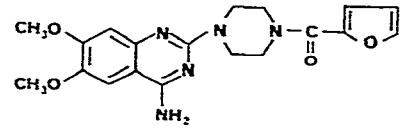
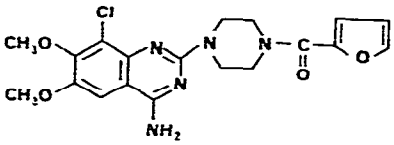
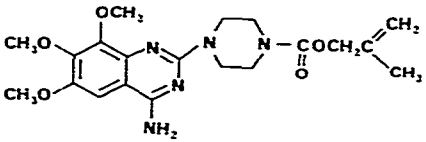
The antihypertensive drug, prazosin (I) is often administered in low doses to humans and requires sensitive methods for its detection in plasma. Of the assay methods reported the fluorometric—wet chemistry method [1] lacks sensitivity and the high-performance liquid chromatographic (HPLC) assay [2] has a high coefficient of variation and interfering plasma peaks eluting with the prazosin. The latter method also requires a lengthy extraction procedure.



I



II



III

The assay procedure presently described employs a new more suitable internal standard, a single extraction step and evaporation followed by HPLC with fluorescence detection.

EXPERIMENTAL

Reagents

All reagents were analytical grade and aqueous solutions were prepared using glass distilled water. Chloroform was Nanograde from Mallinckrodt, St. Louis, MO, U.S.A. Specially purified acetonitrile (210 nm cut-off, Unichrom from Ajax Chemicals, Melbourne, Australia) was used for the high-pressure liquid chromatography. Prazosin hydrochloride and the internal standard (III) were provided by Pfizer, West Ryde, Australia and the internal standard, 8-chloroprazosin hydrochloride (II) was synthesized from vanillin. Information regarding 8-chloroprazosin can be obtained from the author.

Standards

Stock solutions of I and II were prepared in methanol (500 nmol/l each) and stored in the dark at 4°C (stable for at least 1 week). The prazosin solution was used to prepare the appropriate plasma standards for each assay run. Peak height ratios of I to the internal standard II were determined for plasma standards and unknowns and quantification performed by reading unknown values from a plotted standard curve.

Extraction procedure

Plasma (2 ml) was pipetted into a 15-ml glass-stoppered tube. The internal standard solution (200 μl) was added, followed by 0.5 ml of 1.0 *N* potassium hydroxide and 5 ml of chloroform; the mixture was then vortexed for 30 sec. The phases were separated by centrifugation (1100 *g* for 10 min) and the aqueous layer removed by vacuum aspiration and discarded. The organic layer was poured into autosampler tubes (diSPotubes from Scientific Products, State College, PA, U.S.A.) (75 mm × 12 mm) and evaporated under a stream of pure dry nitrogen at 50°C. The residue was reconstituted in 0.5 ml of mobile phase [1.5 m*M* phosphoric acid—acetonitrile (77:23)]. One hundred microlitres of this solution were injected into the chromatograph.

High-performance liquid chromatography

A chromatograph (Spectra-Physics Model SP 8000) equipped with a ternary solvent system, helium degass and automatic data reduction facilities was used. Files for the instrument operation and integration were stored on disc (Spectra-Physics Model SP 4010 disc module). The reversed-phase column used measured 300 mm × 4 mm I.D. and was packed with alkyl phenyl bonded to 10-μm silica (μBondapak/Phenyl from Waters Assoc., Milford, MA, U.S.A.). Column oven temperature was 50°C. The mobile phase was automatically mixed by the instrument and consisted of 1.5 m*M* aqueous phosphoric acid—acetonitrile (77:23) at a flow-rate of 2 ml/min. The instrument was operated in the constant flow mode and all solvent lines from the column to the detector were carefully thermally insulated. The column effluent was monitored using a fluorescence detector (Schoeffel Model 970) at an excitation wavelength of 246 nm with an emission cut-off filter allowing 90% transmission at 389 nm.

The fluorimeter sensitivity was 3.5, range 0.05 μA full-scale and time constant 7.0 sec. Samples were injected automatically using a 100-μl sample loop and an autosampler (Spectra-Physics Model 8010).

Recovery and reproducibility

Recovery of the HPLC assay was determined at concentrations of 1.25, 2.5, 5.0, 25 and 50 nmol/l in plasma by comparison of the peak height of the prazosin peak with that obtained for a solution in mobile phase containing a known concentration of I (500 nmol/l) injected directly into the chromatograph.

Intra-assay reproducibility of the assay was determined at concentrations of 1.25, 2.5 and 50 nmole/l by assaying four plasma samples at each concentration. Inter-assay reproducibility was determined by assaying a single plasma sample containing added I (50 nmol/l) in each assay run.

Interference by other drugs

Samples of the drugs and metabolites listed in Table I were dissolved in mobile phase (500 nmol/l of each) and injected into the high-performance liquid chromatograph. The retention times were obtained if a peak was observed.

TABLE I

RETENTION TIMES OF DRUGS AND METABOLITES IN THE HPLC ASSAY FOR PRAZOSIN

Drug	Retention time (sec)
Prazosin (I)	350
8-Chloroprazosin (II)	625
Internal standard (III)	1270
Propranolol	490
4-Hydroxypropranolol*	235
N-Desisopropylpropranolol*	275
Atenolol	150
Practolol	—
Metoprolol	235
Labetolol	360
Pindolol	—
Timolol	234
3-Methyl-*s*-triazolo[3,4-*a*]phthalazine**	265
3-Hydroxymethyl-*s*-triazolo[3,4-*a*]phthalazine**	150
s-Triazolo[3,4-*a*]phthalazine**	200
Quinidine	230
Dihydroquinidine	265
3-Hydroxyquinidine***	160
Imipramine	1220
Desipramine	1070

*Propranolol metabolites.
**Hydralazine metabolites.
***Quinidine metabolite.

RESULTS AND DISCUSSION

To separate prazosin from eluted plasma peaks in the HPLC assay published [2], a lower percentage of acetonitrile in the mobile phase was required which resulted in an unacceptably long retention time for internal standard III. Internal standard II (8-chloroprazosin) had a considerably shorter retention time (Table I) and was used in preference. Chromatograms obtained for blank plasma, a plasma standard, plasma from a volunteer following a single oral dose of prazosin (2 mg) and from a patient at steady-state are shown in Fig. 1. Using the extraction procedure described here only minor plasma peaks were observed and recovery was 99.6 ± 6.8% and linear over the concentration range 1.25—50 nmol/l (0.48—19.2 ng/ml). Coefficients of variation for the assay are shown in Table II. The detection limit of the asssay was 40 pmol/l determined at a peak height of twice the noise level by injecting the entire extract from 2 ml of plasma.

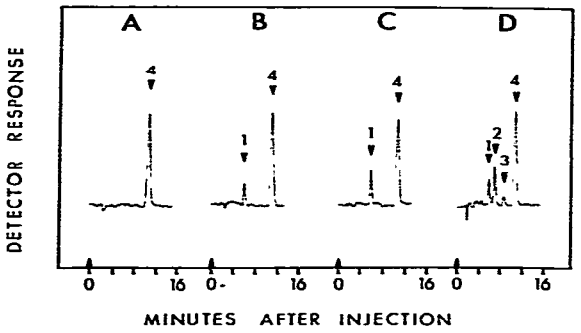


Fig. 1. Chromatograms obtained for the high-performance liquid chromatographic assay of prazosin. (A) Blank plasma, (B) plasma standard containing 1.25 nmol/l of prazosin, (C) plasma drawn from a volunteer 1.0 h after single oral dose of prazosin and containing 2.1 nmol/l of prazosin, (D) plasma sample from a patient at steady-state on prazosin and propranolol and containing 1.5 nmol/l of prazosin. Peaks: 1 = prazosin, 2 = prazosin metabolite, 3 = propranolol, 4 = 8-chloroprazosin (internal standard).

TABLE II

COEFFICIENTS OF VARIATION FOR THE PRAZOSIN ASSAY

	Concentration (nmol/l)	C.V. (%)
Intra-assay	50.0	2
	2.5	3
	1.25	3
Inter-assay	50.0	3

No interference was observed from propranolol, 4-hydroxypropranolol, N-desisopropylpropranolol and other drugs and metabolites shown in Table I. In plasma samples from patients at steady-state on prazosin an additional peak with retention time 416 sec was observed which was probably a metabolite of prazosin (peak 2, Fig. 1).

REFERENCES

1 A.J. Wood, P. Bolli and F.O. Simpson, Brit. J. Clin. Pharmacol., 2 (1967) 1293.
2 T.M. Twomey and D.C. Hobbs, J. Pharm. Sci., 67 (1978) 1468.