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Note

High-performance liquid chromatographic determination of prazosin in human plasma, whole blood and urine

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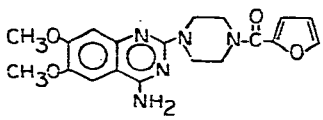
Prazosin hydrochloride (Minipress^{R}, Pfizer) is a new, effective antihypertensive agent that is currently being studied in patients with congestive heart failure for preload and afterload reduction. Previous assays of prazosin [1—4] involve spectrofluorometry after extraction of the alkaline sample with ethyl acetate and then back extraction with hydrochloric acid [4]. This technique is lengthy and requires 3—4 ml of plasma. Most recently Twomey and Hobbs [5] have reported a high-performance liquid chromatographic fluorescence detector method which also involves double extraction similar to that described above.



PRAZOSIN

A new method for the determination of prazosin in human plasma, whole blood and urine is reported here. The method is simple, rapid, sensitive, involves no extraction steps and requires only 0.2 ml of biological sample.

EXPERIMENTAL

Reagents

Prazosin hydrochloride was supplied as prazosin standard 7866-271-A from Pfizer (Groton, CT, U.S.A.). Carbamazepine (Tegretol^{R}) was obtained from Geigy Pharmaceuticals, Division of Ciba-Geigy (Ardsley, NY, U.S.A.). All other reagents were from Fischer Scientific (Fair Lawn, NJ, U.S.A.) and certified

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HPLC grade. The mobile phase was a solution of 43% methanol with 0.6 ml glacial acetic acid, pH 5.0, which was filtered through a Millipore filter and degassed prior to use. Stock solutions of prazosin were prepared by dissolving the drug in methanol and diluting with distilled water.

Chromatographic system

A Perkin-Elmer (PE) Series 2 liquid chromatograph equipped with a rotary valve injector, a PE fluorescence spectrophotometer (Model 204A), a PE fixed-wavelength (254 nm) UV detector (Model 250) and a Linear Model 300 Series dual-pen recorder was used with a μBondapak C_{18} reversed-phase column (30 cm × 3.9 mm ID, 10-μm particle size) from Waters Assoc. (Milford, MA, U.S.A.). The fluorescence detector was operated at an excitation wavelength of 340 nm and an emission wavelength of 384 nm. Due to the narrow bandwidth of the fluorometer, only prazosin and metabolite concentrations are detectable under the conditions described and as such the internal standard, carbamazepine, is measured using UV detection.

Procedures

All samples (0.2 ml biological fluid) were deproteinated by adding 0.4 ml of acetonitrile which contained the internal standard (IS) carbamazepine (2.55 μg/ml). After vortexing for 1 min and centrifuging for 10 min at 1500 *g* in an IEC HN-S centrifuge, the supernatant was transferred to a clean test tube and evaporated to 0.1 ml with nitrogen. A 40—60-μl sample is then injected onto the column

The assay limitation for prazosin is 0.1 ng/ml in plasma, urine and whole blood. In actual pharmacokinetic studies only concentrations as low as 1 ng/ml were observed in 12-h samples following 5-mg doses. Calibration graphs were constructed from spiked plasma and urine samples using the sample procedure described above. Prazosin was added to prove a standard curve concentration range of 2—76 ng/ml in actual studies, although calibration curves were shown to be linear from 1—164 ng/ml. The peak height ratios (prazosin:IS) were plotted versus drug concentration in ng/ml, and the calibration graph was used for the calculation of the plasma, whole blood or urine concentrations in human subjects. All calibration graphs were linear over the concentration ranges measured and a mean correlation coefficient for eleven calibration curves was 0.993 ± 0.004, with a coefficient of variation of 0.39%.

An acetonitrile precipitation method was used to determine the extent of prazosin recovery from plasma proteins. Three sets of six samples (sets designated A through C in Table I), with each set consisting of two samples containing 0.2 ml water (Water 1 and 2), and four samples containing 0.2 ml plasma (Plasma 1—4), were prepared at three different concentrations (17, 54, 118 ng/ml). The absolute peak height of prazosin in water and plasma was compared after injecting identical volumes (50 μl) of supernatant obtained by the procedure described above.

The within-day precision of this method was assessed by conducting replicate analyses (n = 10) of the same spiked plasma samples. Four different concentrations were used.

TABLE I

RECOVERY OF PRAZOSIN FROM PLASMA PROTEINS

Set	Concentration (ng/ml)	Sample	Prazosin peak height (cm)	Carbamazepine peak height (cm)	Peak height ratio
A	17.0	Water-1	3.7	10.5	0.35
		Water-2	3.7	10.45	0.35
		Plasma-1	3.9	10.8	0.36
		Plasma-2	3.9	10.65	0.36
		Plasma-3	3.6	10.5	0.34
		Plasma-4	3.7	11.2	0.33
B	54.0	Water-1	11.0	10.9	1.01
		Water-2	10.9	10.3	1.06
		Plasma-1	10.6	10.8	0.98
		Plasma-2	10.9	10.8	1.01
		Plasma-3	11.0	10.8	1.01
		Plasma-4	10.8	10.2	1.06
C	118	Water-1	24.0	10.9	2.20
		Water-2	24.0	9.7	2.47
		Plasma-1	24.8	10.6	2.32
		Plasma-2	23.8	10.1	2.36
		Plasma-3	23.6	10.8	2.18
		Plasma-4	25.0	10.6	2.36

RESULTS AND DISCUSSION

Representative chromatograms of spiked plasma and plasma from a human volunteer are shown in Fig. 1. Control samples of plasma, urine and whole blood show no interfering peaks. The use of the narrow bandwidth PE spectrofluorometer allows prazosin to be detected in biological fluids without interfering peaks even though no extractions are involved. However, the use of the narrow bandwidth required the IS to be determined independently using a UV detector. Although the above method requires two detectors there is an advantage in this arrangement as relatively high concentrations of IS to drug may be used and compensated for by the different sensitivity settings on the individual detectors. Under the above conditions the retention time for prazosin is 7 min and 10 min for the IS.

The results of the acetonitrile precipitation method (Table I) show that virtually all of the compound is removed from protein, that is, no difference is noted between water and plasma samples. The within-day precision (Table II) was assessed by conducting replicate analyses. The coefficient of variation (C.V.) for the four different concentrations ranged from 0.82 to 8.5%. Frozen plasma samples at various concentrations were found to be stable through the four weeks tested. Although the data suggest that the day-to-day reproducibility of this method is acceptable for analytical measurement, when patient plasma samples were being analyzed two known concentrations were included with the standard curve to insure the day-to-day reproducibility.

The method described here has been utilized in pharmacokinetic studies of prazosin in nine congestive heart failure patients and in five normal volunteers,

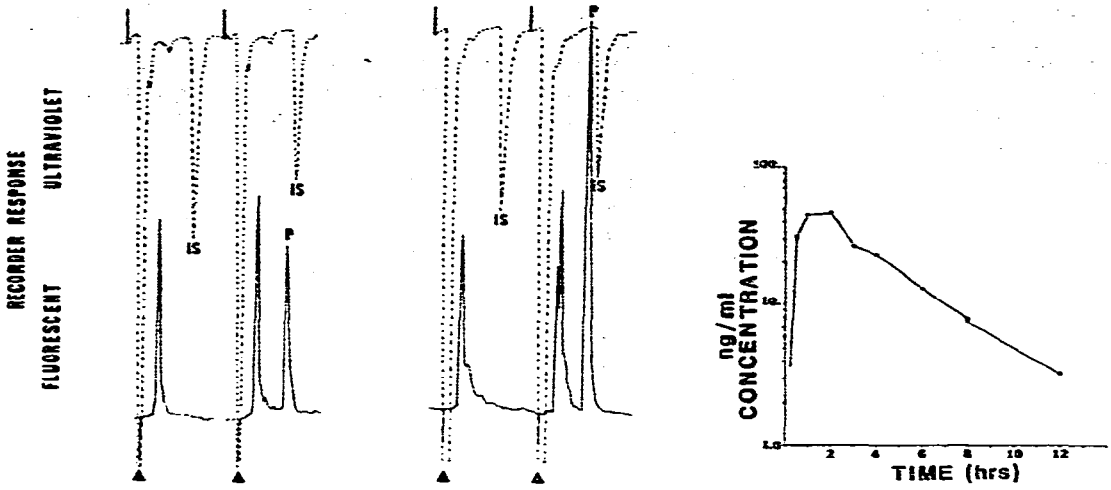

Fig. 1. (Left) dual-pen recording of chromatograms for blank plasma and plasma spiked with prazosin. In the dual-pen chromatograms the UV response is shifted to the left of the fluorescent response, such that the solid line on the UV tracing corresponds to the inject symbol on the fluorescent response. Symbols (retention times in parentheses): ▲, inject; P, prazosin (7 min); IS, internal standard, (10 min). (Right) dual-pen recording of chromatograms for blank plasma and plasma sample from a normal subject. Samples obtained 2 h after a 5-mg oral dose of prazosin. Flow-rate, 2 ml/min; UV detector sensitivity, 1.0 a.u.f.s.; input, 20 mV; fluorescent detector sensitivity, 10; PM gain, 4; input, 20 mV.

Fig. 2. Representative plasma concentration—time curve for prazosin after oral administration of 5 mg to a congestive heart failure patient.

TABLE II

PRECISION OF PLASMA PRAZOSIN ASSAY

$n = 10$.

Mean + S.D. (ng/ml)	C.V. (%)
6.5 ± 0.09	1.35
13.0 ± 0.27	1.85
19.5 ± 1.67	8.50
46.0 ± 0.37	0.82

all of whom received a 5-mg oral dose of prazosin hydrochloride (Fig. 2). When the plasma and blood samples from the subjects were analyzed for prazosin, two time-dependent chromatograph peaks were noted. To date the identities of these peaks have not been determined as no authentic samples of prazosin metabolites are available. The isolation and identification of these peaks are currently being carried out in our laboratory.

CONCLUSION

The technique described here should be useful in future pharmacokinetic studies with prazosin, as the method is sensitive, simple, rapid and requires the

use of only 0.2 ml of sample. The total time for a sample chromatogram is 12 min and no prior extraction steps are required. We are currently using this method in prazosin pharmacokinetic studies in man and animals.

ACKNOWLEDGEMENTS

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REFERENCES

1 A.J. Wood, P. Bolli and F.O. Simpson, Brit. J. Clin. Pharmacol., 3 (1976) 199.
2 I.S. Collins and P. Pek, Clin. Exp. Pharmacol. Physiol., 2 (1976) 445.
3 F.O. Simpson, P. Bolli and A.J. Wood, Med. J. Aust. (Suppl.), 2 (1977) 17.
4 R. Verbesselt, A. Mullie, T.B. Tjandramaga, P.J. de Schepper and P. Dessian, Acta Therapeutica, 2 (1976) 27.
5 T.M. Twomey and D.C. Hobbs, J. Pharm. Sci., 67 (1978) 1468.