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Note

Determination of tiodazosin in plasma and whole blood by high-performance liquid chromatography

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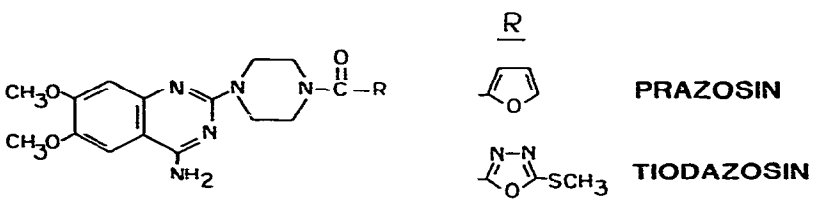
Tiodazosin levulinate, a chemical and pharmacological analogue of prazosin (Minipress, Pfizer), is a new antihypertensive agent currently in clinical trials. The structures for these two drugs are indicated below.



Although there are no published assays of tiodazosin, the assay procedure currently in use at Bristol Laboratories [1] is based on the procedure for prazosin analysis developed by Twomey and Hobbs [2]. This technique involves extraction of the alkaline sample with ethyl acetate, followed by back extraction with a sulfuric acid solution. After a second alkaline extraction with ethyl acetate, the organic layer is evaporated to dryness with a stream of nitrogen gas, then reconstituted with the mobile phase and analyzed by high-performance liquid chromatography (HPLC). This procedure is lengthy and requires 2—4 ml of biological sample to obtain the desired sensitivity. We report here a method for the determination of tiodazosin in plasma and whole blood which is based on our previously published assay of prazosin [3]. The method is simple, reliable and sensitive; it involves no extraction steps and requires only 0.2 ml of biological sample.

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EXPERIMENTAL

Reagents

Tiodazosin levulinate (BL-5111R), 1-(4-amino-6,7-dimethoxy-2-quinazolinyl-4-(5-methylthio-1,3,4-oxadiazol-2-carbonyl)-piperazine levulinate, was obtained as tiodazosin standard 77F655 (Bristol Laboratories, Syracuse, NY, U.S.A.). Prazosin, used as internal standard, was a generous gift of Pfizer (Groton, CT, U.S.A.). All other reagents were obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.) and certified HPLC grade.

Chromatographic system

A Varian 5000 liquid chromatograph equipped with a Waters Intelligent Sample Processor, a Perkin-Elmer fluorescence spectrophotometer (Model 650-10S) and a Linear Model 300 series dual-pen recorder was used with a C18 reversed-phase column (25 cm × 4.6 mm I.D., 10 μm particle size, Alltech, Arlington Heights, IL, U.S.A.). The fluorescence detector was operated at an excitation wavelength of 340 nm and an emission wavelength of 384 nm. The mobile phase was a solution of 21% (v/v) acetonitrile in water with 0.1% (v/v) phosphoric acid. After adjusting the pH of the mobile phase to 3.60 with a sodium hydroxide solution, the mobile phase was filtered and degassed prior to use.

Procedures

Plasma and whole blood samples (0.2 ml) were deproteinated by adding 0.4 ml of acetonitrile which contained the internal standard prazosin (184 ng/ml). After vortexing for 30 sec and centrifuging for 10 min at 1500 *g* in an IEC HN-S clinical centrifuge, the supernatant was transferred to a clean test tube and evaporated to approximately 100 μl under a gentle stream of nitrogen gas. Typically, 15—30 μl of sample were then injected onto the column.

Tiodazosin was quantitated by comparison of the peak height ratio of drug to internal standard with a calibration curve. Calibration graphs were prepared from spiked plasma samples using the sample preparation procedures described above. Stock solutions were prepared by dissolving the drug and internal standard in a small volume of methanol and diluting with distilled water. Tiodazosin was added to provide a standard curve concentration range of 6—868 ng/ml. Peak height ratios (tiodazosin:prazosin) were plotted versus drug concentration. The inter-day variability was assessed by the reproducibility of the slope of the standard curves (n = 6). The intra-day precision of this method was estimated by performing replicate analyses (n = 6) of the same spiked plasma samples at concentrations of 14.5, 43.4 and 217 ng/ml.

The acetonitrile precipitation method was used to determine the extent of tiodazosin recovery relative to prazosin from plasma proteins. Sets of water and plasma samples were spiked with drug at 15, 50 and 100 ng/ml and the samples were prepared and analyzed as described above. A comparison of peak height ratios yielded an estimate of relative recovery from plasma proteins.

The stability of tiodazosin in frozen (−20°C) plasma and whole blood was assessed by comparing measured tiodazosin concentration after 0, 29 and 51 days of storage. Means, standard deviations, linear regressions and correlation

coefficients were calculated using the subroutines available on a Hewlett-Packard 33C calculator.

RESULTS AND DISCUSSION

Representative chromatograms of plasma spiked with tiodazosin and plasma from a beagle dog which received tiodazosin are shown in Fig. 1. The chromatographic conditions utilized yield baseline separation of tiodazosin from internal standard. Under the above conditions the retention time was 12 min for tiodazosin, and 8 min for the internal standard, prazosin. As shown in Fig. 1A, control samples of plasma show no interfering peaks. The use of the narrow bandwidth spectrofluorometer allows detection of tiodazosin in plasma and whole blood without interfering peaks even though no extraction step is involved. HPLC traces of whole blood samples (Fig. 1C and D) were essentially identical to those of plasma samples. Analysis of urine samples from beagle dogs by the method described herein yielded chromatograms with large interfering peaks. However, when urine samples were prepared using the extraction procedures described by Twomey and Hobbs [2], clean chromatograms were obtained.

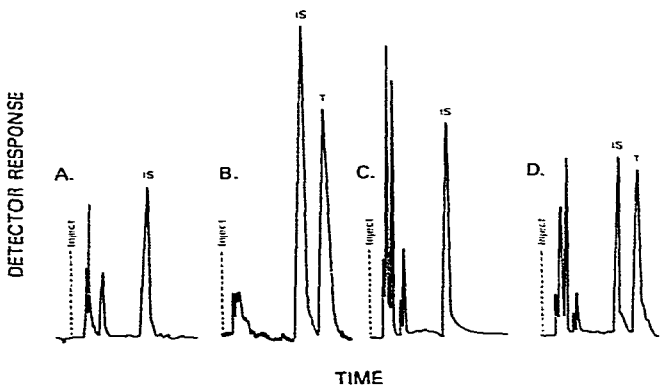

Fig. 1. HPLC—fluorescence chromatograms of (A) blank plasma with internal standard prazosin (184 ng/ml), (B) plasma spiked with tiodazosin (217 ng/ml) and internal standard, (C) blank whole blood with internal standard (184 ng/ml), (D) whole blood spiked with tiodazosin (290 ng/ml) and internal standard. Peaks (retention time in parenthesis): T, tiodazosin (12 min); IS, internal standard (8 min). Conditions: flow-rate, 2 ml/min; detector sensitivity, 1 a.u.f.s.; PM gain, normal; input, 10 mV.

The slopes of calibration curves constructed with spiked plasma samples over the range of 6—868 ng/ml were linear and highly reproducible. The mean slope of six calibration curves was 0.003145 with a coefficient of variation of 7.6%. The mean correlation coefficient of six standard curves was 0.9933 with a coefficient of variation of 0.70%. These data indicate the assay procedure is highly reproducible.

The within-day variation was estimated by conducting replicate analyses (n = 6) of spiked plasma samples. At 14.5, 43.5 and 217 ng/ml of tiodazosin, the coefficients of variation of tiodazosin content were 9.4, 4.2 and 4.9%, respectively. The recovery of tiodazosin from plasma proteins was essentially quanti-

tative. The recoveries at 15, 50 and 100 ng/ml were 104, 103 and 101%, respectively. The concentration of tiodazosin in frozen plasma and whole blood samples did not change after 29 and 51 days of storage demonstrating the stability of tiodazosin in biological samples.

The method described here has been utilized in oral bioavailability studies in five beagle dogs (Fig. 2). In these studies, tiodazosin could be measured at 1 ng/ml (signal-to-noise ratio = 4:1). Although this sensitivity was acceptable for our present pharmacokinetic studies, considerably greater sensitivity could be achieved by further concentration of the sample, use of larger plasma samples and/or use of a 5-μm particle size HPLC column. The assay described herein is currently being used to determine prazosin content in biological samples with similar sensitivity and reliability.

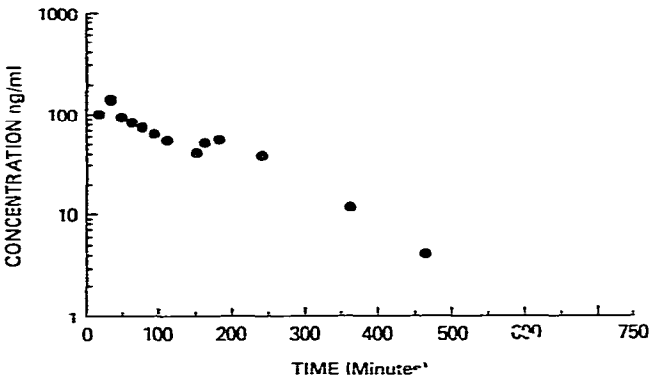

Fig. 2. Plasma concentration—time profile of tiodazosin after oral administration of a 1 mg/kg solution to a beagle dog.

ACKNOWLEDGEMENTS

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