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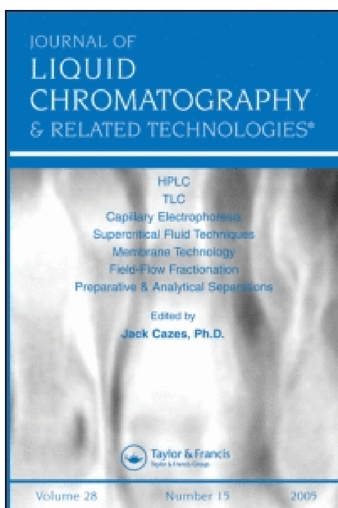

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E. M. Niazy[a]; Y. M. El-Sayed[a]; S. H. Khidr[a]
[a] Department of Pharmaceutics, College of Pharmacy King Saud University, Riyadh, Saudi Arabia

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ANALYSIS OF PRAZOSIN IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING FLUORESCENCE DETECTION

E. M. NIAZY, Y. M. EL-SAYED, AND S. H. KHIDR

Department of Pharmaceutics
College of Pharmacy
King Saud University
P.O. Box 2457
Riyadh 11451, Saudi Arabia

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ABSTRACT

A high-performance liquid chromatographic procedure using fluorescence detection has been developed for the determination of prazosin in plasma. Propylhydroxybenzoate was used as the internal standard. The chromatography was performed using adsorbsphere phenyl column; the mobile phase consisted of 30:70% acetonitrile to 0.05 M phosphate buffer and was adjusted to pH 3.3-3.4 using phosphoric acid; a flow rate of 1.5 ml/min; and the effluent was monitored at excitation and emission wavelengths of 247 and 394 nm, respectively. The retention times for prazosin and the internal standard were 4.0 and 6.0 min., respectively. The intraday coefficients of variation (CV) ranged from 1.15 to 4.96% at three different concentrations and the interday CVs varied from 0.05 to 8.99%. The mean (± SD) absolute and relative recovery of prazosin were found to be 97.4±3.14 and 100.68±2.19, respectively. Stability tests showed that prazosin is stable for at least 2 weeks in plasma after freezing. The minimum detectable concentration of prazosin by this method was

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0.5 ng/ml. The sensitivity obtained should enable the use of this method in future bioequivalency and/or pharmacokinetic studies.

INTRODUCTION

Prazosin is a quinazoline derivative with a selective $alpha_1$-adrenoceptor blocking properties (1-3) that is widely used in the treatment of hypertension and heart failure (4-7). The usual initial dose of prazosin is 0.5 mg two or three times daily. The determination of plasma drug levels after such low doses required an assay capable of measuring levels below 1 ng/ml sample.

Numerous analytical methods have been described for assaying prazosin. These include spectroflurometry (8-11) and high-performance liquid chromatography (12-14). Generally, however, prazosin assays previously reported are time consuming involved double extraction steps and some of them suffer from a lack of sensitivity.

In this report a simple, rapid, sensitive, accurate and reproducible high-performance liquid chromatographic assay for the quantitative determination of prazosin in plasma is described. The method requires only 0.2 ml of plasma and involves a single extraction step, eliminating the tedious and time-consuming procedures required by the previously reported methods.

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MATERIALS

Prazosin HCl was obtained from Sigma Chem. Co. (St. Louis, MO, USA) and propylhydroxybenzoate (internal standard) was obtained from E. Merck AG (Darmstadt, Germany). Acetonitrile and diethylether (BDH Chem. Ltd., Poole, U.K.) were HPLC grade. Sodium dihydrogen phosphate and disodium hydrogen phosphate and phosphoric acid (Riedel-De-Haen AG, Seelze, Hannover, Germany) were of analytical grade.

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METHODS

Instruments

The following instruments were used:
A model LC-10AD solvent delivery pump (Shimadzu Corporation, Koyato, Japan), a model 470 fluorescence detector (Waters Associates, Milford, MA, U.S.A.), a model S/N 206003 chart recorder (Esterline Angus-Instrument Corp., Indianapolis, IN, U.S.A.), and a model 7010 Rheodyne injector (Rheodyne Inc., Catati, CA, U.S.A.). Chromatographic separation was performed using a stainless steel adsorbsphere phenyl column, 150 mm length x 4.6 mm i.d., 5 μm particles (Alltech).

Standard Solutions

Prazosin HCl (10 mg) and propylhydroxybenzoate (10 mg) were dissolved in methanol in two separate 100 ml

volumetric flasks to give standard stock solutions of 100 μg/ml.

Chromatographic Conditions

The mobile phase consisted of acetonitrile:0.05 M phosphate buffer (30:70% v/v) adjusted to pH 3.3-3.4 with phosphoric acid. The mobile phase was degassed by passing it through a 0.45 μm membrane filter (Millipore, Bedford, MA, U.S.A.) and pumped isocratically at a flow rate of 1.5 ml/minute, at ambient temperature. The effluent was monitored at excitation and emission wavelengths of 247 and 394 nm, respectively. The chart speed was 0.25 Cm/min.

Procedure

In a screw-capped glass centrifuge tube (10 ml), 0.2 ml plasma sample, 0.2 ml of 1 N NaOH, 12.5 μl of the internal standard solution and 7 ml diethylether were added. The mixture was shaken on a vortex mixer for 1 minute, and centrifuged for 2 min, at 3000 rpm. The ether layer was transferred into another glass centrifuge tube and evaporated to dryness. The residue was reconstituted in 0.4 ml of the mobile phase. An appropriate aliquot was then injected directly into the loop injector.

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RESULTS AND DISCUSSION

The mobile phase reported herein (acetonitrile: 0.05 M phosphate buffer, 30:70% v/v, pH 3.3-3.4) was optimized for a rapid and interference-free chromatograms. The selected chromatographic conditions provided optimum resolution of prazosin and the internal standard. The retention times for prazosin and the internal standard were 4.0 and 6.0 min., respectively.

Figure 1 shows chromatograms from a drug-free blank plasma and plasma sample spiked with the drug and the internal standard.

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Quantification

The quantification of the chromatogram was performed using peak-height ratios of the drug to the internal standard. Standard curves were constructed routinely from spiked plasma samples and mobile phase containing 0, 1.0, 2.5, 5.0, 10.0, 20.0 and 40.0 ng/ml of prazosin. Four standard plots were obtained from plasma samples and six from the mobile phase. Least squares linear regression analysis of the calibration curves resulted in the following equations:

$$Y = -0.0210 + 0.1350\ X,\ r = 0.999 \text{ (Mobile phase)}$$

$$Y = 0.0070 + 0.1309\ X,\ r = 0.999 \text{ (Plasma)}$$

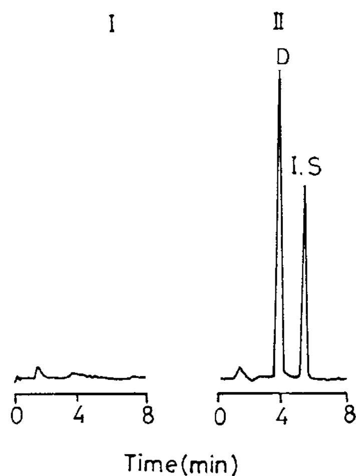

Figure 1 : Chromatograms of Blank Plasma (I) and Plasma Containing Prazosin and the Internal Standard (II)
Key : D : Prazosin
I.S. : Internal Standard.

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Standard curves of prazosin in plasma and mobile phase were constructed on different days to determine the variability of the slopes and intercepts. The results showed little day-to-day variability of slopes and intercepts as well as good linearity (r>0.99) over the concentration range studied. The coefficients of variation for the slopes were 1.33% and 4.58% for the mobile phase and plasma, respectively.

Precision

The intraday precision was evaluated by replicate analysis of pooled plasma samples containing prazosin

Table 1 : Intraday and Interday Precision of Prazosin in Plasma.

Intraday*			Interday**		
Added Conc. (ng/ml)	Measured Conc. (ng/ml)	Bias*** %	Added Conc. (ng/ml)	Measured Conc. (ng/ml)	Bias*** %
7.5			7.5		
Mean	7.4	-1.3	Mean	7.52	0.2
S.D.	0.09		S.D.	0.01	
C.V. %	1.15		C.V.%	8.99	
15			15		
Mean	14.8	-1.33	Mean	15.1	0.66
S.D.	0.71		S.D.	0.01	
C.V.%	4.96		C.V.%	0.05	
30			30		
Mean	29.7	-0.01	Mean	31	3.33
S.D.	0.91		S.D.	0.72	
C.V.%	3.03		C.V.%	2.4	

* Mean values represent eight different plasma samples for each concentration.

** Interday reproducibility was determined From 8 different runs over 15-day period for the three concentrations.

*** Bias = 100 X (measured conc. - added conc.) / added conc.

at three different concentrations (low, medium and high). The intraday precision showed a coefficient of variation (CV) of 1.15% to 4.96% (Table 1). The interday precision was similarly evaluated over a 2-week period. The interday CVs ranged from 0.05% to 8.99% (Table 1).

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Recovery

The absolute recovery of prazosin and the internal standard from plasma were assessed by comparing the peak height in plasma samples versus samples prepared in the mobile phase. The absolute recoveries ranged from 94% to 100.2% (Table 2). The relative recovery was calculated by comparing the concentrations obtained from the drug-supplemented plasma to the actual added concentrations. Eight comparisons at three different concentrations were made. As shown in Table 2, the mean relative recovery of prazosin from plasma ranged from 98.3% to 102.6%.

Stability

Stability studies of plasma spiked with prazosin (7.5, 15.0 and 30.0 ng/ml) were performed over a 15-day period (Table 3). Plasma samples were stored in the freezer at -20°C until the time of analysis. The results demonstrate that prazosin can be stored frozen in plasma for 2 weeks without degradation.

Sensitivity

The limit of quantitation for this method was found to be 0.5 ng/ml.

Table 2 : Absolute and Relative Recovery of Prazosin from Plasma* .

Conc. (ng/ml)	Mean Peak Heights (cm) Aqueous	Plasma	Absolute Recovery %	Relative Recovery % Mean ±SD	Range Relative Recovery %
7.5	1.98±0.09	1.86±0.05	94	101.15±2.1	96 - 104.16
15	3.97±0.07	3.90±0.09	98	102.6±5.1	95 - 108.1
30	7.86±0.38	7.90±0.09	100.2	98.3±2.19	95.2 - 104.3
I.S. 2.5 μg/ml	1.8±0.06	1.76±0.08	97.8		

* Eight replicate analyses of each concentration .

Table 3 : Effect of Frozen Storage on Prazosin Stability in Plasma

Added Conc. (ng/ml)	Percent Recovery * Days			
	0	5	10	15
7.5	98.0	101.0	96.0	98.0
15	95.0	98.0	101.0	101.0
30	102.0	101.0	98.0	103.0

* (Measured Conc. / (Added Conc.) X 100

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Conclusion

The developed HPLC assay in this study has the sensitivity, rapidity, simplicity and reproducibility which makes it a potentially valuable tool in many applications such as drug level monitoring, drug-drug interactions, pharmacokinetic and bioequivalence studies.

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