



Determination of Doxazosin in Human Plasma by Reversed Phase Ion Pair High Performance Liquid Chromatography with Fluorescence Detection

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A high performance liquid chromatographic method was developed for the estimation of doxazosin in human plasma. It employs a simple and rapid method of sample preparation. Doxazosin and an internal standard (prazosin) were chromatographed as ion pairs with heptane sulphonic acid. Sample preparation involved liquid-liquid extraction with diethyl ether, evaporation of the organic layer, reconstitution of the residue into mobile phase and injection of 20 μ l into the chromatograph. Chromatography was performed using ODS C_{18} , 5 μ reverse-phase column with fluorescence detection. Mobile phase consisted of heptane sulphonic acid buffer and methanol. At a flow rate of 1.2 ml/min, one analysis was completed in less than 10 min. The method was sensitive and reproducible with accurate detection as low as 0.1 ng/ml in plasma. The method was linear for doxazosin concentrations in the range of 0.5-30 ng/ml ($r^2 = 0.9976$). Recoveries for the same drug concentrations from spiked human plasma ranged from 90-95% ($n=5$). The mean RSD values for interday and intraday assay reproducibility ($n=5$) were 4.47% and 4.52% respectively. Peak area ratios were fit to a least squares linear regression algorithm with a 2/x weighting. LOD and LOQ of the method were found to be 0.1 ng/ml and 0.5 ng/ml, respectively.

Doxazosin is a potent antihypertensive agent, which is effective when administered either orally or intravenously¹. Doxazosin is slowly eliminated in man and its relatively long half-life provides the basis for once-daily dosing, a therapeutic advantage. Few HPLC methods are reported to estimate doxazosin in human plasma^{2,3}. These methods involve solid phase extraction⁴ and liquid-liquid extraction techniques for sample preparation. The reported methods lack the sensitivity and involve lengthy extraction procedures, which involve back-extraction^{5,6}. The method described here is based on direct extraction of sample into an organic solvent at alkaline pH, evaporation of organic extract and reconstitution prior to analysis.

MATERIALS AND METHODS

Doxazosin mesylate and prazosin hydrochloride refer-

ence standard were obtained from Sun Pharma, Vadodara as gift samples. Sodium heptane sulphonic acid monohydrate AR, 20% aqueous solution of tetra-n-butyl ammonium hydroxide AR, sodium hydroxide AR, glacial acetic acid AR, methanol HPLC grade and ethyl ether HPLC grade were purchased from Spectrochem.

Chromatographic conditions:

The HPLC system consisted of a dual wavelength detector (Waters 2487), a separation module (Waters 2690), and the Waters Millennium version 2.15.01 software. The column used was HYPERSIL, 5 μ , ODS, C_{18} , 250 x 4.6 mm. A 30 x 4.6 mm guard column of the same material is used. The mobile phase used was methanol:heptane sulphonic acid buffer (pH 3.4, 0.02 M) in the ratio 55:45 v/v. Heptane sulphonic acid buffer was prepared by dissolving 2.02 g of heptane sulphonic acid sodium salt in 900 ml of HPLC grade water, to this 25 ml of tetra-n-butyl-ammonium hydroxide (20% solution in aqueous medium) was added. The pH of

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the above solution was adjusted to 3.4 with glacial acetic acid and volume adjusted to 1000 ml. Flow rate was 1.2 ml/min, detection wavelengths were λ_{ex} 246 and λ_{em} 370, and the injection volume was 20 μ l.

Sample extraction:

To a 15 ml borosilicate glass tube 980 μ l of drug free plasma, 20 μ l of prazosin (1.5 μ g/ml) were added and vortexed for 10 s. To this, 200 μ l of 2 N sodium hydroxide was added and vortexed for 30 s. The above solution was extracted with 5 ml of diethyl ether by vortexing for 5 min. The organic layer was then transferred into another tube and the procedure was repeated. The pooled organic layers were evaporated under nitrogen at 40°. The residue was dissolved in 200 μ l of mobile phase and 20 μ l of this solution was injected into HPLC.

Method validation:

The linearity of the method was investigated by serially diluting a stock solution of doxazosin (in methanol; 0.5 mg/ml) with drug free plasma to concentrations in the range 25-1500 ng/ml and subjecting 20 μ l of each of these solutions to the proposed assay method. Calibration curves were constructed by plotting the ratio of peak areas of doxazosin to prazosin (IS) against the concentration of doxazosin added.

Analyte recovery was determined by comparing the ratio of peak areas of doxazosin to internal standard for the standard preparations against those of same preparations in mobile phase. Interday assay reproducibility was assessed over a period of 5 d at 7.5, 15 and 25 ng/ml concentration. Intraday analysis was determined upon replicate analysis of 3 check samples at same concentrations.

RESULTS AND DISCUSSION

The precision of the retention times observed for doxazosin and IS (n=25), expressed as RSD were less than 0.5 %. The representative overlay chromatograms of blank plasma and plasma spiked with doxazosin and IS shown in fig. 1 indicate that there are no endogenous interfering components co-eluting with doxazosin or the IS. Linear regression results for calibration curves performed on 5 different days showed mean correlation coefficients (r^2) of more than 0.99, slope of $3.54 \times 10^4 \pm 29.71$ and intercept of $8.0 \times 10^3 \pm 183.1$.

Table 1 summarizes the assessment of both interday and intraday reproducibility of the method. Data presented in Table 1 are the coefficients of variability (CV%) for each check sample processed. Absolute recovery of doxazosin

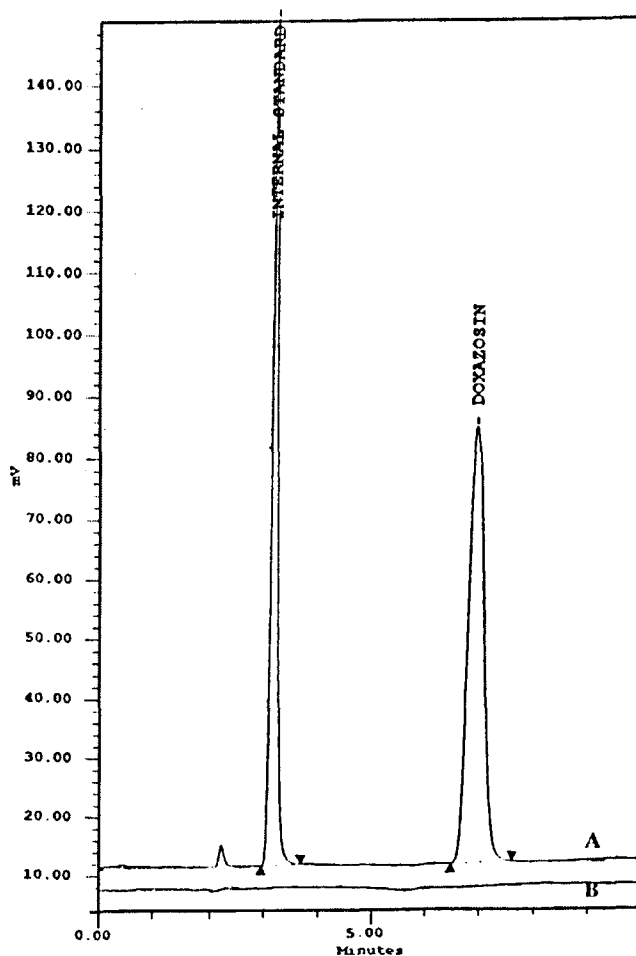


Fig. 1: Overlay chromatogram of doxazosine and blank. A represents the chromatographic run with sample and internal standard while B represents the blank run.

from plasma was greater than 90%. Evaluation of the effect of short-term storage of extracted plasma samples on the standard curve characteristics and chromatographic behaviour of doxazosin and IS were also performed. Regression analysis of the standard curve data gave correlation coefficients and values for the slope and Y-intercept within the same order of magnitude following storage of samples at -20° from 1-25 d. The chromatographic behaviour was also unaffected by storage of extracted plasma samples in auto sampler at 20° for 36 h. Freeze-thaw analysis of 3 cycles did not show any effect on the recovery of doxazosin. The results are shown in Table 2.

The method described here for the quantitative determination of doxazosin has the advantage of being a simple and rapid two-step extraction procedure without loss of sen-

TABLE 1: INTRADAY AND INTERDAY VARIABILITY OF THE ASSAY OF QUALITY CONTROL SAMPLES AT THREE CONCENTRATION LEVELS.

Concentration added (ng/ml)	Intra Day			Inter Day		
	Mean analysed±s.d. ng/ml (n=30)	%CV	% Bias	Mean analysed±s.d. ng/ml (n=30)	%CV	% Bias
7.5	7.63±0.42	5.50	1.74	7.61±0.40	5.26	1.47
15.0	15.15±0.65	4.29	1.00	15.09±0.64	4.29	0.60
25.0	25.30±0.98	3.79	3.20	25.30±0.97	3.86	1.20

TABLE 2: FREEZE-THAW EFFECT ON DOXAZOSIN RECOVERY.

Concentration (ng/ml)	Mean (n=3)	% CV
7.5	91.14	1.08
15.0	92.83	1.83
25.0	93.61	0.97

sitivity. The present method is sensitive, rapid and specific for doxazosine and can be applied for routine therapeutic monitoring of the drug.

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