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Short Communication

Validation of a solid-phase extraction high-performance liquid chromatographic assay for doxazosin

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ABSTRACT

The determination of doxazosin by high-performance liquid chromatography with fluorescence detection is described. Propanolol was used as the internal standard. Plasma samples were treated with methanol to precipitate the proteins. Doxazosin was isolated with C_{18} reversed-phase extraction columns. The determination limit is 1 ng/ml of plasma, while the extraction columns can be reused frequently. The method is applied to clinical trial samples.

INTRODUCTION

Doxazosin is a potent quinazoline α-adrenoceptor antagonist, related to prazosin but having a much longer half-life in man [1]. Previous assays for doxazosin, prazosin and related compounds have used solvent extraction prior to high-performance liquid chromatography (HPLC) with fluorescence detection [2–6]. We have simplified and speeded the procedure by the use of solid-phase extraction (SPE) columns, and have determined conditions which also permit reuse of the extraction columns in the interests of economy.

EXPERIMENTAL

Reagents

Doxazosin was a gift from Pfizer (Sandwich, U.K.). Propranolol (Inderal) used as internal standard, was obtained from ICI Australia (Melbourne, Australia). Stock solutions of 1 mg/ml in methanol were stored at −20°C and diluted to working concentrations for each assay. Control samples were prepared by dilution of stock solutions in human plasma and were also stored at −20°C. Bond-Elut C_{18} reversed-phase extraction columns were obtained from Analytichem

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International (Harbor City, CA, U.S.A.). All other reagents were of analytical or HPLC grade.

Solid-phase extraction

Plasma (1 ml) was placed into clean polystyrene tubes and internal standard (50 μl of 1 μg/ml solution) added. To each tube methanol (250 μl) was added with brief agitation and left for 10 min, after which they were centrifuged at *ca.* 3000 *g* for 10 min. In the meantime, the solid-phase columns were activated by the passage of 1 ml of methanol followed by 1 ml of water. The plasma supernatants were then passed through the columns with gentle suction and the columns washed with 1 ml of methanol–water (30:70, v/v) and 1 ml of water. The columns were then transferred to clean tubes and eluted with 1 ml of methanol containing 0.5% acetic acid. The eluate was reduced to dryness with a current of clean air and reconstituted in 150 μl of the mobile phase.

High-performance liquid chromatography

The equipment consisted of an M6000 HPLC pump and WISP automatic injector (Waters Assoc., Milford, MA, U.S.A.) and a Schoeffel FS970 fluorescence detector (Kratos Analytical, Ramsey, NJ, U.S.A.). Excitation was at 245 nm while emission wavelengths were selected with a Corning 7-60 filter (bandpass 320–390 nm). The column was a 150 mm × 4.6 mm I.D. Zorbax CN, 5 μm particle size (DuPont Instruments, Wilmington, DE, U.S.A.) and the mobile phase consisted of 10 m*M* perchloric acid, 1.8 m*M* sodium heptanesulfonate–methanol (50:50) at a flow-rate of 1 ml/min. Quantitation was by the measurement of the ratio of peak heights for doxazosin and propranolol.

RESULTS AND DISCUSSION

In any assay where components are reused, particular care must be taken to ensure that material from one assay is not carried forward to subsequent assays. This is not expected to be a problem with reversed-phase solid-phase extraction, since the materials are similar to those used in HPLC columns and guard columns, and it would be unusual to need to replace a guard column after every injection. However, conditions which lead to efficient extraction and recovery are those most likely to lead to minimal carryover of the analyte. Our first experiments were made to determine conditions for such efficient extraction. Initially we had intended to use the related compound prazosin as an internal standard, but we found difficulty in maintaining adequate resolution from doxazosin in a short chromatographic separation and so decided to use the β-blocker propranolol, which is also easily detected by fluorimetry. We found that the SPE columns were very effective at extracting both compounds from aqueous solutions, but while doxazosin could be completely recovered by elution with methanol (1 ml) propranolol eluted much more slowly. Acetonitrile was a better eluent for doxa-

zosin but completely failed to elute propranolol. Fortunately the addition of acetic acid (0.5%) to the eluent permitted complete recoveries of both compounds with 80–90% methanol–water. Recoveries from plasma were also different. Only 60–65% of doxazosin could be recovered compared with 100% of propranolol. Adjusting the pH of plasma with citric acid or ammonia reduced recoveries of both propranolol and doxazosin, but dilution of plasma with methanol to a final concentration of 20% (v/v) once more produced complete recoveries. It is possible that the effect of methanol is due to changes in protein binding, but we have no direct evidence for this. Chromatography of eluates prepared by extraction of plasma and direct elution with methanol–acetic acid showed that early eluting substances were also retained which tended to interfere with the assay, but these were readily minimised by washing the columns before elution with methanol–water (3:7, v/v). Representative chromatograms produced by this procedure are shown in Fig. 1.

The final procedure as described in Experimental produced linear, reproducible calibration curves with within-assay coefficients of variation of 4–6% from 50 to 300 ng/ml and 8% at 1 ng/ml. As expected there was no measurable carryover of doxazosin or propranolol from one assay to the next, even at the highest concentrations used, and the method was applied to the assay of samples from a clinical trial with doxazosin to assess its robustness in normal use. Over a two-month period we used *ca.* 70 SPE columns for an average of 20 extractions per column. Columns were washed and reused at random and only discarded if they became blocked. No unexpected blank values were observed, which would have indicated sample carryover, and drug recoveries averaged 75 ± 9 and 75 ± 10%

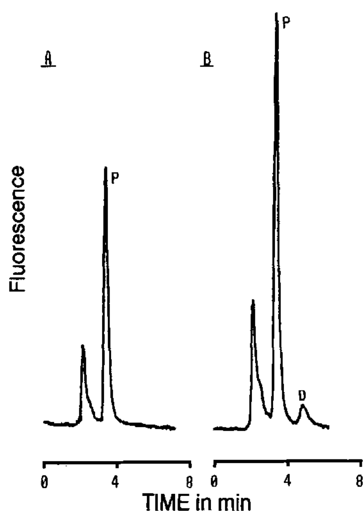

Fig. 1. Chromatograms of extracts of human plasma samples. (A) Plasma containing no doxazosin; (B) plasma containing 2 ng/ml doxazosin. Peaks: P = propranolol; D = doxazosin.

TABLE I

INTER- AND INTRA-ASSAY PRECISION AND REPRODUCIBILITY OF THE METHOD

Intra-assay measurements are the mean of 4 measurements, while the inter-assay values were obtained from the quality control samples carried through each of 24 separate assays.

Doxazosine added (ng/ml)	Intra-assay		Inter-assay	
	Found (ng/ml)	C.V. (%)	Found (ng/ml)	C.V. (%)
300	286	5.4	297	8.0
100	110	3.6	101	7.4
25	27.1	5.5		
20			18.7	11
2	2.0	5.0	2.0	17
1	0.93	8.5		

for propranolol and doxazosin, respectively, with no tendency for the recovery to fall with time. These recoveries could have been made quantitative with the use of larger elution volumes, but this was felt to be an unnecessary refinement. Inter- and intra-assay precision and reproducibility as recorded in Table I were satisfactory. With manual measurement of peak heights the limit of detection is about 1 ng/ml, but may be improved by electronic integration or optimisation of the conditions for lower concentrations. The regression equation for the calibration curves of 24 assays averaged $y = (0.033 \pm 0.006x) + (0.031 \pm 0.079)$, where y is the peak-height ratio and x is doxazosin concentration (ng/ml). The intercepts were not significantly different from zero. The correlation coefficients averaged 0.997 ± 0.004.

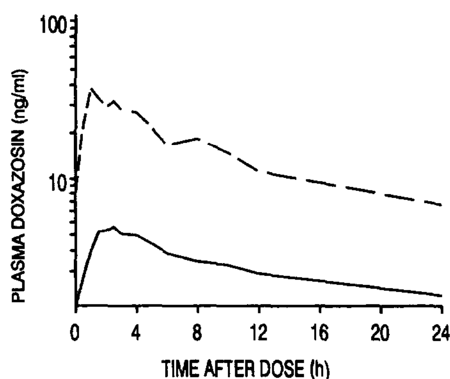

Fig. 2. Plasma doxazosin concentrations found in one subject with this assay. The solid line represents concentrations after a single oral dose of 1 mg doxazosin and the dashed line the concentrations following a single oral dose of 8 mg doxazosin in the same patient after six weeks of continuous treatment.

Results from clinical trial samples were encouraging. As can be seen in Fig. 2, the plasma half-life of doxazosin was similar after a single acute dose of 1 mg and after an 8-mg dose during chronic dosing. This suggests that the procedure is unaffected by compounds which may accumulate during chronic dosing. Overall, we conclude that solid-phase extraction techniques can be successfully applied to the economical assay of doxazosin, and although we have not specifically tested prazosin, there appears to be no reason why it and related compounds could not be assayed in this way.

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