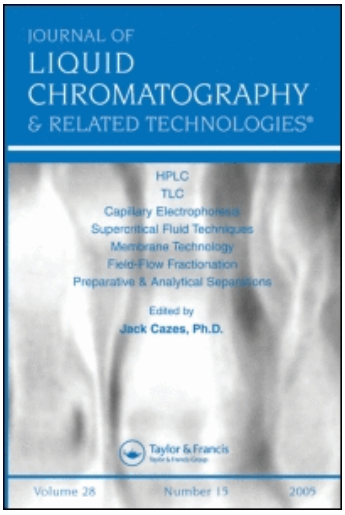

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Determination of Alfuzosin in Human Plasma by High-Performance Liquid Chromatography with Column-Switching

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DETERMINATION OF ALFUZOSIN IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH COLUMN-SWITCHING

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ABSTRACT

A high - performance liquid chromatographic method for the determination of alfuzosin in human plasma has been developed and validated. A column-switching procedure without extraction was used to isolate the drug from biological matrix prior to the quantitative analysis. The lower limit of detection for the analyte was 1 ng/ml. The method was linear from 2 to 150 ng/ml for human plasma. Within- and between-assay precision and accuracy were all found to be <5.2% at the eight concentrations evaluated. This procedure, simple and rapid, is suitable for pharmacology studies on alfuzosin.

INTRODUCTION

Alfuzosin is a selective antagonist of α_1-adrenergic receptors [1], active as antihypertensive agent [2], recently introduced in therapeutics for the treatment

of benign prostatic hypertrophy [3]. Its chemical name is N-[3-[(4-amino-6,7-dimethoxy-2-quinazolinyl) methylamino] propyl] tetrahydro-2-furancarboxamide; its formula is shown in Fig. 1.

Some HPLC assays for alfuzosin have been recently described. Three of these methods [4-6] were developed for the determination of the enantiomers of the drug in rat and human plasma on a chiral protein column of human α_1-acid glycoprotein; one used a liquid-liquid extraction and a large volume injection technique for the quantitation of alfuzosin in biological fluids [7].

This paper describes a direct injection method, using column- switching without extraction, for the determination of alfuzosin in human plasma, providing accurate and precise results. The analysis of plasma samples from volunteers was carried out using this procedure.

EXPERIMENTAL

Chemicals and Materials

HPLC-grade acetonitrile and methanol were obtained from Farmitalia-Carlo Erba (Milan, Italy). Orthophosphoric acid (85% m/m) and potassium dihydrogenphosphate (analytical grade) were purchased from Fluka Chemika - BioChemika (Buchs, Switzerland). Alfuzosin hydrochloride was kindly supplied by LIRCA S.p.A. (Milan, Italy). Water (HPLC-grade) was obtained by distillation in glass and purification through a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA). Phosphate buffer was filtered through an HA 0.45 μm filter, while acetonitrile and methanol were filtered through an FA 0.5 μm filter (Millipore, Bedford, MA, USA).

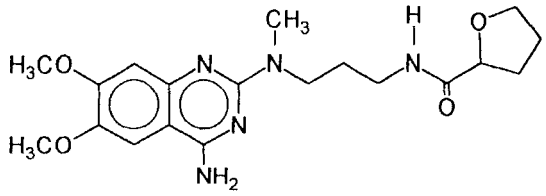

Figure 1 Chemical structure of alfuzosin.

Chromatographic System and Conditions

The chromatographic apparatus consisted of a model 510 and a model M-45 solvent delivery systems (Waters Associates, Milford, MA, USA). The luminescence detector model LS 30 (Perkin-Elmer, Rome, Italy) was set at 265 nm (excitation wavelength) and 400 nm (emission wavelength). The chromatograms were recorded on a model HP-3296-II integrator (Hewlett-Packard, Rome, Italy). A model 7125 sample injector and a six-port switching valve model 7000 (Rheodyne, Cotati, CA, USA) were used. The chromatographic arrangement (Fig.2) included a clean-up column (50 x 4.6 mm i.d.) filled with LiChrosorb C_{18} (10 µm) reversed-phase material (Merck, Darmstadt, Germany) and a guard column (20 x 4.6 mm i.d.) (not shown in Fig.2), packed with Pellicular-CN (40 µm) (Supelco, Bellefonte, PA, USA), placed between the switching valve and the analytical column. This was a cyanopropyl column Spherisorb S5W (25cm x 4.6 mm i.d., 5 µm particle size) (Phase Separations, UK). The clean-up solvent from pump A consisted of methanol-water (5:95, v/v). The analytical solvent from pump B was a mixture of acetonitrile-methanol-0.05M phosphate buffer (38:2:60, v/v/v). The pH of the phosphate buffer was adjusted to 2.5 with orthophosphoric acid. The mobile

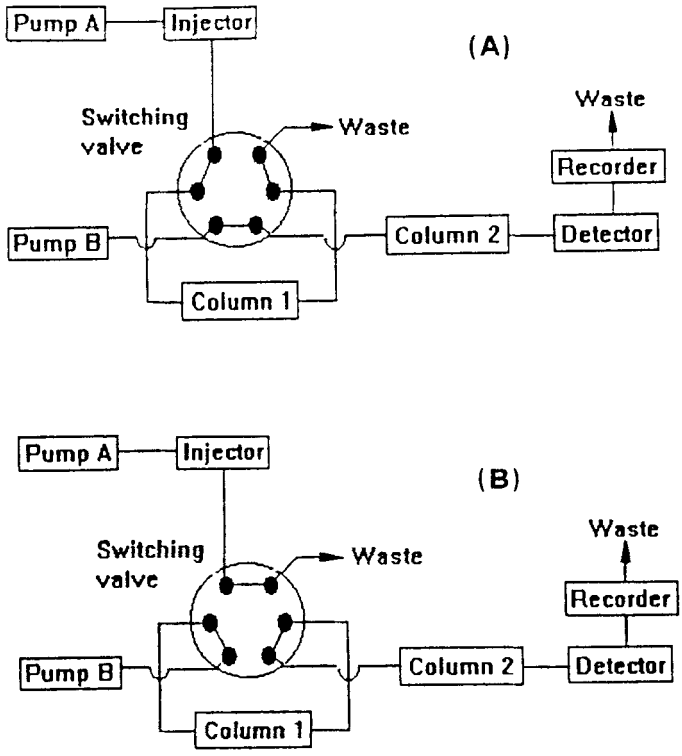

Figure 2 Column-switching system for direct injection of plasma and on-line HPLC analysis of alfuzosin: (A) Sample application; (B) Sample elution.

phase was prepared daily, filtered, sonicated before use, and delivered at a flow-rate of 1.0 ml/min.

Standard Solutions

A stock solution of alfuzosin hydrochloride was prepared by dissolving 10 mg of this compound in 10 ml of water. This solution could be stored at -20°C for over two weeks with no evidence of decomposition. Standard solutions, containing alfuzosin in the concentration range 2-150 ng/ml, were prepared by

diluting the stock solution with control human plasma. Calibration curve was obtained by plotting the peak-area of alfuzosin against the drug concentration.

Analysis of Samples

After injection of 50 µl of plasma sample into the clean-up column, which had been previously equilibrated with clean-up solvent (methanol-water 5:95, v/v), the column was washed for 1 min with this solvent at a flow-rate of 1.0 ml/min. The substance adsorbed on the clean-up column was then introduced into the analytical column with the analytical mobile phase, by switching the six-port valve to back-flush mode for 2 min. The six-port valve was then returned to its initial position. The analytical column was disengaged from the clean-up column, and the latter was equilibrated with clean-up solvent ready for the next injection. The separation was carried out with analytical solvent at a flow-rate of 1.0 ml/min, and the analysis was achieved by using the above calibration curve.

RESULTS AND DISCUSSION

Under the chromatographic conditions described, the alfuzosin peak is well resolved. Fig. 3 shows typical chromatograms of blank plasma, blank plasma spiked with alfuzosin, and plasma of a volunteer treated with alfuzosin. No endogenous component or metabolite was observed near the retention time corresponding to alfuzosin. The retention time for alfuzosin was 6.2 min. The equation obtained through regressional analysis of data for the above standard solutions was $y=6\cdot10^4 + 1.5\cdot10^4x$ with correlation coefficient $r=0.998$, where y= peak-area in the arbitrary units of the HP-3396-II system used and x=alfuzosin concentration (ng/ml). The assay was validated by analysing seven alfuzosin

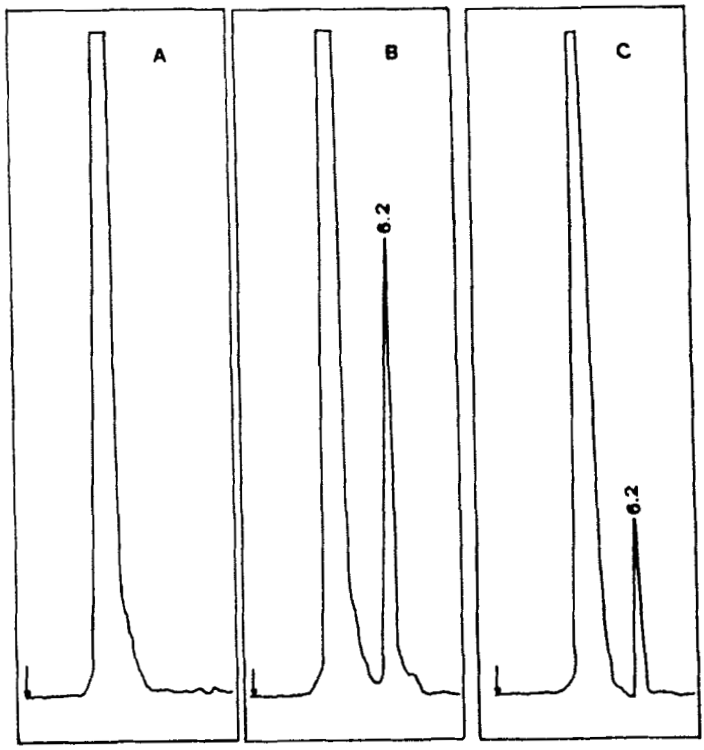

Figure 3 Chromatograms after column-switching: (A) Blank plasma; (B)Blank plasma spiked with alfuzosin (2.0 ng/ml); (C) Plasma of a volunteer treated with 5 mg of alfuzosin (4.4 ng/ml).

standards. Each datum was the average of a minimum of five determinations. The calibration curve for alfuzosin in human plasma was linear over the range 2.0 -150ng/ml. Reproducibilities for witin-day and between-day were evaluated to assess the precision and accuracy of this analytical method. The results are shown in Tables 1-2. The coefficients of variation (CVs) of the five independent samples at each concentration in the within-day assay were between 2.0 and 5.2% with relative errors (REs) of 2.1 -4.1% and in the between-day assay were

TABLE 1
Within - day precision and accuracy in the calibration standard of alfuzosin in human plasma.

Theoretical concentration (ng/ml)	Measured concentration* (ng/ml)	CV (%)	RE (%)
2.0	1.92±0.10	5.2	4.1
3.0	2.90±0.18	5.2	3.4
5.0	4.89±0.22	4.4	2.2
10	9.70±0.48	4.9	3.0
20	19.20±0.67	3.5	4.1
50	48.30±1.50	3.1	3.5
100	97.50±1.96	2.0	2.5
150	149±0.8	2.0	2.1

* Mean of five assays±SD; CV = Coefficient of Variation; RE = Relative Error.

TABLE 2
Between - day precision and accuracy in the determination of alfuzosin in human plasma

Theoretical concentration (ng/ml)	Measured concentration* (ng/ml)	CV (%)	RE (%)
2.0	1.92±0.64	3.3	3.7
3.0	2.84±0.12	2.8	3.4
5.0	4.95±0.05	2.1	2.0
10	9.7±0.04	4.1	3.1
20	19.50±0.70	3.6	2.5
50	49.15±1.50	3.0	2.7
100	98.30±1.30	3.3	2.7
150	148±1.70	4.2	3.4

*Mean of five assays±SD; CV = Coefficient of Variation; RE = Relative Error.

between 2.1 and 4.2% with relative errors of 2.0-3.7% in the concentration range 2.0-150ng/ml. Using a signal-to-noise ratio of 3, the detection limit of alfuzosin in human plasma was 1.0 ng/ml. The alfuzosin extraction efficiency, determined by comparing peak-area of plasma extracts versus the corresponding aqueous standards, was approximately 87%. This simple HPLC method should be of value for monitoring the alfuzosin concentration in plasma in patients, for assessing the patient compliance in assuming prescribed alfuzosin regimes and for examining the relationship between alfuzosin concentration in plasma and its efficacy in patients with benign prostatic hypertrophy. The HPLC method described in this paper is rapid, sensitive, and allows accurate and precise results, using a simple column switching extraction technique.

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