Journal of Chromatography, 533 (1990) 291–296
Biomedical Applications
Elsevier Science Publishers B.V Amsterdam

CHROMBIO 5476

Note

Sensitive method for the determination of amsulosin in human plasma using high-performance liquid chromatography with fluorescence detection

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(Received June 6th, 1990)

Amsulosin hydrochloride (YM-12617-1), (−)-(*R*)-5-[2-[[2-(*o*-ethoxyphenoxy)-ethyl]amino]propyl]-2-methoxybenzenesulphonamide hydrochloride, is structurally a new type of extremely potent and highly selective α_1-adrenoceptor antagonist [1,2]. Recently, it has been reported that urinary obstruction in benign prostatic hypertrophy was decreased effectively by administration of α_1-adrenoceptor antagonists, such as phenoxybenzamine [3] and prazosin hydrochloride [4,5]. These drugs may increase urinary flow-rate, possibly by reducing the prostatic urethral resistance. The α_1-adrenoceptor antagonist activity of amsulosin hydrochloride has been found to be more potent than that of prazosin [6]. A sensitive method for the determination of plasma concentrations is required for pharmacokinetic studies in the preclinical and clinical evaluation of this drug. In this report, a sensitive and simple method using high-performance liquid chromatography (HPLC) with fluorescence detection is described.

EXPERIMENTAL

Chemicals

Amsulosin hydrochloride and the internal standard (I.S.), (±)-5-[2-[[2-(*o*-ethoxyphenoxy)propyl]amino]propyl]-2-methoxybenzenesulphonamide hydrochloride, were supplied by our Central Research Laboratories. Their structures are shown in Fig 1. All other chemicals were analytical grade and were obtained commercially and used without further purification.

Apparatus and chromatographic conditions

The HPLC system consisted of an L-6200 pump (Hitachi, Tokyo, Japan), a KSP-600 autoinjector (Kyowaseimitsu, Tokyo, Japan), a stainless-steel column

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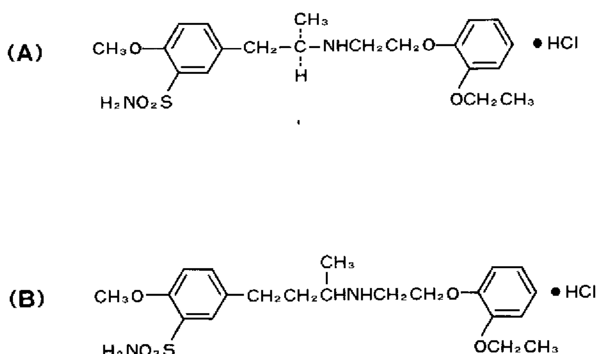

Fig 1 Structures of amsulosin hydrochloride (A) and the internal standard (B)

(15 cm × 4.0 mm I.D.) packed with Nucleosil $5C_{18}$ (Macherey-Nagel, Düren, F.R.G.), an RF-535 fluorescence detector and a C-R2AX integrator (Shimadzu, Kyoto, Japan). The column was maintained at 26°C; the mobile phase was 0.2 *M* potassium biphosphate–0 2 *M* phosphoric acid–acetonitrile (7:7:5, v/v) at a flow-rate of 1 0 ml/min. The column eluate was monitored fluorometrically at excitation and emission wavelengths of 275 and 325 nm, respectively.

Standard solutions

Stock solutions of amsulosin and the I S. were prepared at a concentration of 1 mg/ml in 0.05 *M* hydrochloric acid. Standard solutions containing 5, 10, 30, 50, 100 and 150 ng/ml amsulosin hydrochloride were prepared by diluting the stock solution with 0.05 *M* hydrochloric acid A solution containing 1 μg/ml I.S. was also prepared using 0.05 *M* hydrochloric acid.

Sample preparation

To 1 5 ml of plasma sample in a centrifuge tube, 0.1 ml of the I S. solution, 1 ml of a saturated sodium bicarbonate solution and 5 ml of ethyl acetate were added, and the resulting mixture was shaken for 15 min on a mechanical shaker. After centrifugation for 10 min at 1200 *g*, the organic layer was transferred to another centrifuge tube. To the organic layer, 2.5 ml of 0.4 *M* hydrochloric acid were added and the resulting mixture was shaken for 15 min. After centrifugation for 5 min at 400 *g*, the organic layer was discarded. To the aqueous layer, 2 ml of the saturated sodium bicarbonate solution were added and the resulting mixture was stirred. After equilibration for 20 min at room temperature, 5 ml of ethyl acetate were added to the mixture followed by shaking for 15 min. After centrifugation for 5 min at 400 *g*, the organic layer was transferred to a test-tube and evaporated to dryness under reduced pressure. The residue was dissolved in 100 μl of the mobile phase, and 70 μl of the solution were injected into the HPLC system

Calibration curve

To 1.5 ml of control human plasma, a 0.15-ml aliquot of each standard solution was added, and the mixture was processed as described above. A calibration curve was constructed by plotting the peak-height ratios of amsulosin to the I.S. against amsulosin hydrochloride concentrations in plasma.

Human pharmacokinetics

Twelve healthy male volunteers received 0.2 mg of amsulosin hydrochloride as a controlled release formulation after overnight fasting. Blood samples (9 ml) were drawn at appropriate intervals and centrifuged to obtain plasma samples.

RESULTS

Extraction procedure for sample preparation

Amsulosin has a basic secondary amino and a weakly acidic sulphamoyl group in its molecule, therefore it will not be extracted into organic solvents from highly acidic or basic aqueous solution. Fig. 2 shows extraction profiles for amsulosin added to aqueous solutions with various pH values and extracted with ethyl acetate or diethyl ether. The extraction efficiencies, in the pH range 8–11, were 93.2–95.4% with ethyl acetate and 73.9–88.3% with diethyl ether. For the extraction of the drug in plasma, ethyl acetate was selected because of the higher extraction efficiencies, and the pH of the aqueous layer was adjusted to *ca.* 8.5 by

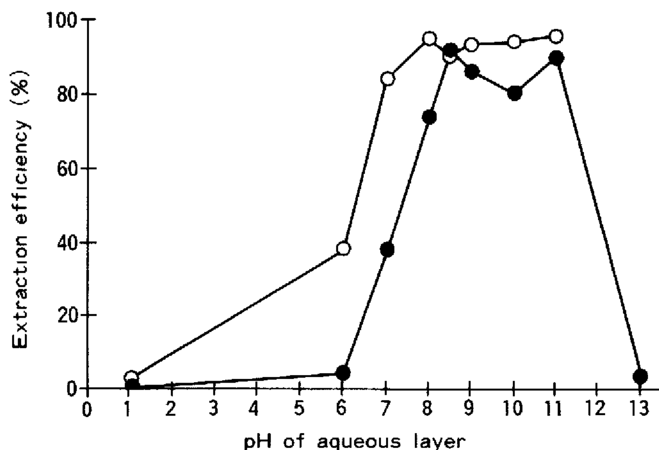

Fig. 2 Effect of pH of the aqueous layer on the extraction of amsulosin. In this determination, 200 ng of amsulosin hydrochloride were added to 4 ml each of aqueous solutions with different pH and extracted with diethyl ether or ethyl acetate The aqueous solutions were 0.1 *M* hydrochloric acid (pH 1), 0.1 *M* phosphate buffer (pH 6–8); saturated sodium bicarbonate solution (pH 8 5), 0 1 *M* disodium hydrogenphosphate (pH 9), 0 1 *M* Tris–HCl buffer (pH 10), 0 1 *M* sodium carbonate (pH 11), 0 1 *M* sodium hydroxide (pH 13). (●) Diethyl ether, (○) ethyl acetate

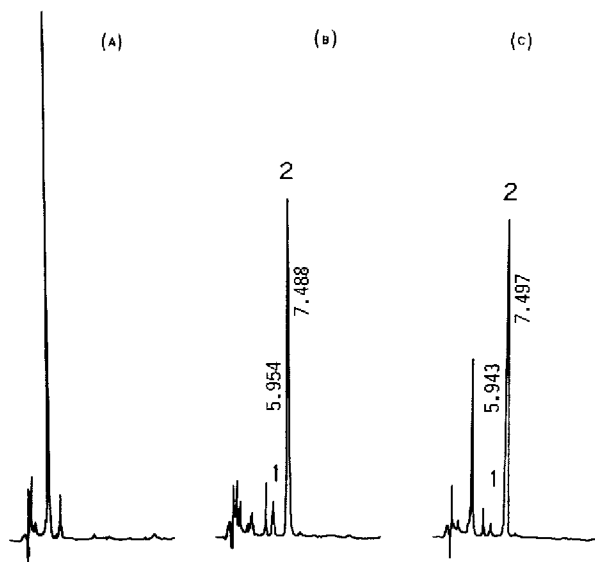

Fig 3 Chromatograms of (A) control human plasma, (B) control human plasma spiked with 5 ng/ml amsulosin hydrochloride and (C) a plasma sample obtained from a volunteer dosed with amsulosin hydrochloride Peaks: 1 = amsulosin; 2 = I S The amsulosin concentration of the plasma sample shown in (C) was estimated to be 2.3 ng/ml of plasma

adding the saturated sodium bicarbonate solution. To remove endogenous substances that interfered with the HPLC analysis, the drug was then back-extracted into hydrochloric acid solution followed by re-extraction with ethyl acetate. The overall recovery for amsulosin hydrochloride (5 ng/ml in human plasma) was *ca.* 70%.

Separation of amsulosin, I.S. and endogenous substances

Fig 3 shows chromatograms of (A) control human plasma, (B) control human plasma spiked with 5 ng/ml amsulosin hydrochloride and appropriate amounts of the I.S. and (C) a plasma sample obtained from a subject dosed with amsulosin hydrochloride as a controlled release formulation. The retention times of amsulosin and the I.S. were *ca.* 5 9 min and 7 5 min, respectively. Both peaks completely separated from those of endogenous substances in plasma of humans and laboratory animals such as dogs, rats and mice.

Calibration curve and sensitivity

The calibration curves prepared from the control plasma of humans and laboratory animals were linear in the range 0.5–15 ng/ml amsulosin hydrochloride. The determination limit was 0.5 ng/ml when a 1.5-ml plasma sample was used.

TABLE I

ACCURACY AND PRECISION OF THE METHOD FOR THE DETERMINATION OF AMSULOSIN HYDROCHLORIDE IN HUMAN PLASMA

Concentration prepared (ng/ml)	Concentration measured (mean ± S.D., $n=6$) (ng/ml)	Percentage difference (%△)[a]	Coefficient of variation (%)
0.5	0.543 ± 0.066	8.6	12.2
1	0.967 ± 0.077	−3.3	8.0
5	5.02 ± 0.07	0.4	1.4
15	14.8 ± 0.2	−1.3	1.4

[a] $\% \triangle = \frac{\text{mean of concentration measured} - \text{concentration prepared}}{\text{concentration prepared}} \times 100$

Accuracy and precision

The accuracy and precision of this method were examined using human plasma samples prepared at amsulosin hydrochloride concentrations of 0.5, 1, 5 and 15 ng/ml. Six samples were determined for each concentration. As shown in Table I the method had sufficient accuracy and precision, although percentage difference and coefficients of variation were both slightly greater at a concentration of 0.5 ng/ml.

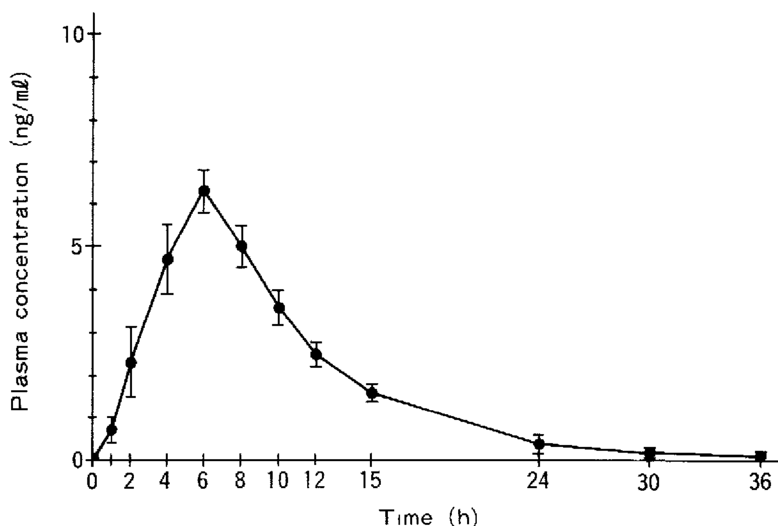

Fig. 4 Plasma concentrations of unchanged drug after oral administration of 0.2 mg of amsulosin hydrochloride to humans as a controlled release formulation. Each point represents the mean ± S.E. of twelve volunteers.

Application of the method

The utility of the method was demonstrated by monitoring the plasma concentration of unchanged drug after oral administration of amsulosin hydrochloride to healthy male volunteers. Plasma concentrations could be determined up to 36 h after administration of 0.2 mg of amsulosin hydrochloride as a controlled release formulation. The mean plasma concentration–time curve is shown in Fig. 4 The maximum concentration of 7.0 ± 0.5 ng/ml was achieved 5.3 ± 0 3 h after administration, and then the concentration decreased with a half-life of 7.8 ± 1.7 h

DISCUSSION

The pharmacokinetics of amsulosin in humans and laboratory animals, which has been presented by Soeishi *et al.* [7], was investigated by HPLC with fluorescence detection. The method had a determination limit of 0.5 ng/ml amsulosin hydrochloride in plasma. The sensitivity was high enough to evaluate the pharmacokinetics in humans of amsulosin hydrochloride administered at a dose of 0.1 mg, corresponding to the minimum therapeutic dose. Amsulosin and the I.S. were extracted from plasma and derivatized by reaction with dansyl chloride followed by the extraction of both derivatives before HPLC. The HPLC analysis took longer than 30 min per sample to separate peaks of dansylated amsulosin and the I.S. from those caused by the reagent and endogenous substances In the present method, these complicated and time-consuming processes have been excluded by detecting amsulosin directly from its own fluorescence. The sensitivity of the method is same as that of the dansylation method To examine the relationship between the two methods, 40 human plasma samples were determined by both methods. The results from the two methods correlate very well (r = 0.992) The present method has sufficient accuracy and precision and is applicable to routine measurements of plasma concentrations, not only in humans but also in laboratory animals such as dogs, rats and mice.

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