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# Enantioselective analysis of atenolol in biologic fluids: comparison of liquid–liquid and solid-phase extraction methods

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**Abstract**

In this study we evaluated a liquid–liquid extraction procedure and a solid-phase extraction procedure for sample preparation for the enantioselective analysis of atenolol in plasma and urine by high-performance liquid chromatography. A Chiralcel OD-H column was used for the resolution of atenolol enantiomers with hexane–ethanol (85:15, v/v) plus 0.1% diethylamine as the mobile phase. In the liquid–liquid extraction procedure, atenolol was extracted from alkalinized body fluids with 5 ml chloroform–2-propanol (4:1, v/v). In the solid-phase extraction procedure, atenolol was isolated from plasma using a $C_8$ column and methanol. Both extraction procedures were efficient in recovering atenolol and removing endogenous interferents. The RSDs and deviation from nominal values were lower than 10% for both within-day and between-day assays. The results show that there were no statistically significant differences in between-day variation. The *t*-test showed that there were no significant differences between the real concentrations and the determined concentrations. The limit of quantitation was 10 ng/ml and the linear range was 10–5000 ng/ml for both methods. These methods can be used in pharmacokinetic studies. 

*Keywords:* Enantiomer separation; Chiral stationary phases, LC; Atenolol

## 1. Introduction

Atenolol (AT) is a cardioselective β-blocker. It is reported to lack intrinsic sympathomimetic activity and membrane-stabilising properties. This drug is used in the management of hypertension, angina pectoris, cardiac arrhythmias, and myocardial infarction [1]. In different studies Clementi et al. [2] and Stoschitzky and co-workers [3,4] observed that only (−)-(*S*)-AT exerted β-blockade when the currently used racemic drug was administered, and that the full β-blocking effect could be obtained with the half-dosed optically pure (*S*)-enantiomer.

The kinetic disposition of AT is also stereoselective, resulting in higher plasma and urinary concentrations of (+)-(*R*)-AT. Clementi et al. [2] suggested that the higher (+)-(*R*)-AT concentration may be the result of a smaller volume of distribution of

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(+)-(*R*)-AT compared to (−)-(*S*)-AT, in addition to stereoselective uptake of (−)-(*S*)-AT into sympathetic nerve terminals.

In order to perform enantioselective pharmacokinetic studies, methods for the resolution and quantitation of the single enantiomers in biological fluids are required. High-performance liquid chromatography (HPLC) on chiral stationary phases is currently the most widely used direct method for the analysis of chiral compounds because of sufficient sensitivity and availability of a wide variety of commercial columns [5].

Egginger et al. [6] used an (*R*,*R*)-diaminocyclohexane-dinitrobenzoyl chiral stationary phase for the resolution of AT enantiomers after achiral derivatization with phosgene. This procedure resulted in limits of quantitation of 0.5 and 0.6 ng/ml for (+)-(*R*)-AT and (−)-(*S*)-AT, respectively, although the procedure for sample preparation was complex and time consuming. Fornstedt et al. [7] reported the resolution of AT enantiomers in plasma samples using a chiral stationary phase based on cellobiohydrolase I immobilized on silica (Chiral CBH), with a limit of quantitation of 0.2 μg/ml. The $\alpha_1$-AGP column was employed by Berthault et al. [8] for the determination of (+)-(*R*)-AT and (−)-(*S*)-AT in plasma samples after liquid–liquid extraction (LLE) with ether–dichloromethane (80:20, v/v), with a limit of detection of 25 μg/l and a recovery of 79.5%. Lamprecht et al. [9] used an on-line sample preparation procedure for the analysis of (+)-(*R*)- and (−)-(*S*)-AT in urine. AT was separated from most matrix components using an RP-18 column and AT enantiomers were then resolved on a Chirobiotic T (Teicoplanin) column using acetronitrile–methanol–acetic acid–triethylamine (55:45:0.3:0.2, v/v) as eluent. More than 500 urine samples were analyzed using this column-switching system, with only slight deterioration of column performance. However, the RP-18 column performance was drastically reduced after injection of plasma samples. He et al. [10] used an achiral/chiral coupled column HPLC system for the analysis of AT enantiomers in plasma; the system consisted of a size-exclusion column, an ODS silica column and a β-cyclodextrin perphenylcarbamate-bonded silica column connected in series via two switching valves. Kofahl et al. [11] developed a method using a Chiralcel OD column in which the extraction with dichlorometane–2-propanol led to mean rates of recovery of 89.1% for (+)-(*R*)-AT and 89.0% for (−)-(*S*)-AT from plasma.

A sample preparation step is often necessary to isolate the component of interest from a sample matrix, as well as to purify and concentrate the analytes. The analysis of AT enantiomers using a solid-phase extraction (SPE) procedure has not been previously described until now. Thus, the objective of the present study was to compare two extraction procedures for the analysis of AT enantiomers in human plasma and urine using LLE and SPE.

## 2. Experimental

### 2.1. Standard solutions and chemicals

A stock solution of (*R*,*S*)-AT, kindly supplied by Laboratório Biossintética, Brazil, was prepared in methanol at a concentration of 1.0 mg/ml. Working solutions (0.8–400.0 μg/ml) were prepared by appropriate dilution in methanol. The internal standard solution (propranolol) was prepared in methanol at concentrations of 20 and 40 μg/ml. The solutions were stored at 4°C and were stable for at least 3 months.

Hexane, 2-propanol, chloroform (EM Science, USA), ethanol and methanol (Merck, Germany) were HPLC grade. All other chemicals were analytical-reagent grade and were used without further purification. Sulpelclean LC-8 SPE columns (500 mg) were obtained from Supelco (USA).

Pooled drug-free human plasma and urine were obtained from healthy volunteers, stored at −20°C and allowed to thaw at ambient temperature prior to use. Water was purified in a Milli-Q system (Millipore, USA).

### 2.2. Instruments and chromatographic conditions

The HPLC system consisted of an LC10AS solvent pump, an RF 535 fluorescence detector ($\lambda_{ex}$= 235 nm or 240 nm, $\lambda_{em}$=300 nm), a CR6-A integrator (all from Shimadzu Instruments, Japan) and a 7125 Rheodyne injector with a 50-μl loop (Rheodyne, USA). Separations were carried out in a climatized room (22±2°C) on a Chiralcel OD-H

column (5 μm particle size, 150×4.6 mm) purchased from Chiral Technologies, USA. A CN guard column (4×4 mm I.D., Merck) was used to protect the analytical column. The mobile phase consisted of hexane–ethanol (85:15, v/v) plus 0.1% diethylamine and the flow-rate was 1.0 ml/min, with a column inlet pressure of 30 kg/cm$^2$.

### 2.3. Extraction procedure

#### 2.3.1. Liquid–liquid extraction

Aliquots of 1 ml of plasma samples were spiked with 25 μl of internal standard solution [(*R*,*S*)-propranolol, 20 μg/ml] and alkalinized with 25 μl of 8 mol/l NaOH solution. After the addition of 5 ml chloroform–2-propanol (4:1, v/v), the tubes were capped, shaken horizontally for 20 min and then centrifuged for 5 min at 1800 *g*. The organic phases were transferred to clean tubes and the solvent was evaporated to dryness. The residues were dissolved in 100 μl mobile phase and 50 μl was chromatographed. The standard curves were prepared by adding 25 μl of the internal standard (20 μg/ml) and 25 μl of the working solutions [0.8–100.0 μg/ml (*R*,*S*)-AT] to 1 ml of drug-free plasma in order to obtain a concentration range of 10 to 1250 ng/ml of each enantiomer. The samples were assayed in duplicate by the described procedure.

Urine samples were similarly prepared as follows: urine aliquots of 100 μl were added to 900 μl water, alkalinized with 25 μl of 8 mol/l NaOH solution and extracted with chloroform–2-propanol (4:1, v/v) as indicated above. The standard curves were prepared in the concentration range of 0.25 to 25.0 μg/ml.

#### 2.3.2. Solid-phase extraction

The extraction columns were preconditioned by passing 3 ml of methanol followed by 1 ml of water aliquots applied twice. Plasma samples (1 ml) containing 25 μl of internal standard solution [(*R*,*S*)-propranolol, 40 μg/ml] were eluted through the solid-phase columns and the loaded columns were washed with 5×1 ml of water. AT and the internal standard were eluted from the column using 2 ml of methanol. The solvent extracts were evaporated to dryness under a stream of dry air. The residue was reconstituted with 100 μl of mobile phase, sonicated for 5 min, and centrifuged at 1800 *g* for 3 min, and 50 μl of the supernatant was injected. The standard curves were similarly prepared in the 10 to 1250 ng/ml range, using 25 μl of the internal standard solution (40 μg/ml).

### 2.4. Recovery and linearity

The analytical recovery of AT enantiomers for plasma samples was determined at concentrations of 25, 125 and 1250 ng/ml of each enantiomer ($n=4$). Drug-free plasma samples were spiked with known amounts of the drug to achieve the concentrations previously specified. These samples were submitted to the extraction procedures and the organic phases were supplemented with the internal standard solution. After solvent evaporation the residues were dissolved in 100 μl mobile phase and 50 μl was chromatographed. Peak height ratios (AT height/internal standard height) were compared with the peak height ratio obtained by the direct injection of the drugs in the mobile phase.

The linearity study was carried out in the range of 10–5000 ng/ml and of 0.25 to 25.0 μg/ml of each enantiomer in plasma and urine, respectively.

### 2.5. Precision and accuracy

Precision and accuracy were determined by analyzing aliquots of three spiked plasma or urine samples. Within-day precision and accuracy were determined by analyzing four replicates of spiked human plasma or urine and between-day precision and accuracy were determined over a 1-week period ($n=5$). The precision of the method was calculated as the relative standard deviation (RSD) and the analysis of variance (ANOVA). The accuracy was calculated as the percent deviation of observed concentrations from theoretical concentration and by the *t*-test comparing determined concentrations to the real value. All the statistics test have been performed with a 5% significance level.

### 2.6. Specificity

Interference of commonly used drugs was evaluated by injecting solutions of drugs prepared in the mobile phase into the chromatographic system and recording their retention time. When the retention

time was similar to the retention times of AT enantiomers or internal standard, a plasma sample spiked with the drug in the upper limit of the therapeutic range was submitted to the extraction procedure and to chromatographic analysis.

### *2.7. Preliminary human experiment*

In order to evaluate the applicability of the methods, several plasma and urine samples collected from a healthy volunteer after administration of a single dose of (*R*,*S*)-AT (Atenol, 50 mg of AT) were analyzed under the conditions established in the present study. Venous blood was drawn into heparinized tubes immediately before and 0.5, 1, 1.5, 2, 4, 6, 8 and 10 h after drug administration. After centrifugation for 10 min at 1800 *g*, plasma samples were transferred to clean tubes and stored at −20°C until analysis. Urine samples were collected into glass containers at intervals of 0–1, 1–3, 3–5, 5–7, 7–9, 9–10 h after AT administration. For each urine collection interval, the total urine volume was measured and recorded and a 10-ml aliquot was removed and frozen for HPLC analysis. The volunteer gave informed written consent to participate in the study, which was approved by the Ethics Committee of Escola de Enfermagem de Ribeirão Preto-USP (0081/2000).

## 3. Results and discussion

Although AT enantiomers have already been resolved using other column types [6–10], the Chiralcel OD-H column has high optical resolving ability, affording a practically useful chiral stationary phase [12,13]. Using the hexane–ethanol (85:15, v/v) plus 0.1% diethylamine mixture as the mobile phase it was possible to obtain a separation factor of 1.26 and elution times shorter than 11 min.

The elution order of AT enantiomers through the Chiralcel OD column has already been established by Kofahl et al. [11], and was confirmed here by the analysis of the individual enantiomers according to the method described by Webb et al. [14]. (+)-(*R*)-AT and (−)-(*S*)-AT were separated and collected from the Chiralcel OD-H column into tubes and the mobile phase was evaporated to dryness under an air flow. The enantiomers were derivatized with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) and chromatographed on a $C_8$ column for separation of the diastereomers (+)-(*R*)-AT-GITC and (−)-(*S*)-AT-GITC. The mobile phase consisted of acetonitrile–acetate buffer, pH 4 (40:60), at a flow-rate of 1 ml/min. The eluate from the column was monitored by UV (280 nm) detection.

The procedures employed for the extraction of AT enantiomers from plasma and urine samples were simple and efficient in removing endogenous interferents. Representative chromatograms obtained from drug-free plasma and urine, standard calibration plasma and urine spiked with AT and internal standard, and treated subject plasma and urine samples obtained are shown in Figs. 1–3, respectively. The peaks eluting at retention times closer to 4.3 and

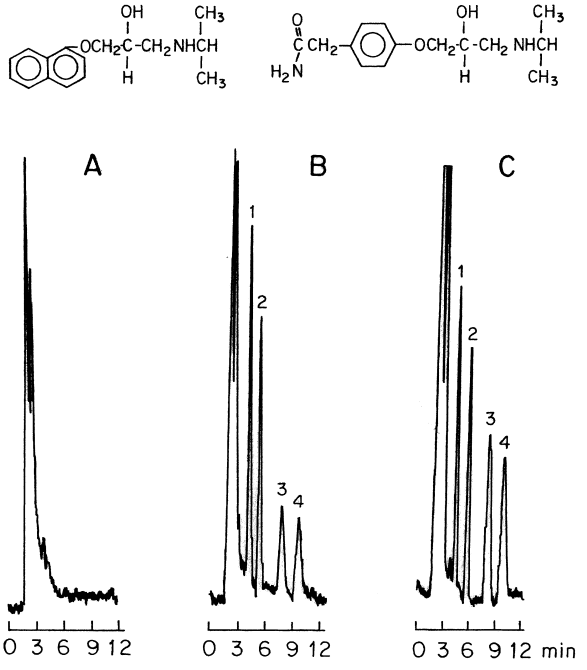


Fig. 1. Chromatograms referring to the analysis of AT enantiomers in plasma after LLE. (A) Blank plasma; (B) plasma spiked with 25 ng/ml of AT enantiomers; (C) plasma sample from a healthy volunteer collected 1.5 h after administration of 50 mg of AT. Propranolol (1, 2); (+)-(*R*)-AT (3); (−)-(*S*)-AT (4). Chromatographic conditions: Chiralcel OD-H column; mobile phase: hexane–ethanol (85:15, v/v) plus 0.1% diethylamine; flow-rate, 1 ml/min; fluorescence detection ($\lambda_{ex}$=235 nm, $\lambda_{em}$=300 nm).

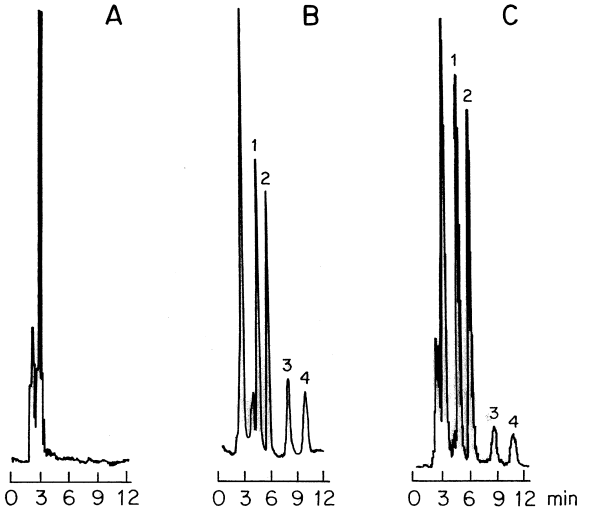


Fig. 2. Chromatograms referring to the analysis of AT enantiomers in plasma after SPE. (A) Blank plasma; (B) plasma spiked with 25 ng/ml of AT enantiomers; (C) plasma sample from a healthy volunteer collected 1.5 h after administration of 50 mg of AT. Propranolol (1, 2); (+)-(*R*)-AT (3); (−)-(*S*)-AT (4). Chromatographic conditions: Chiralcel OD-H column; mobile phase: hexane–ethanol (85:15, v/v) plus 0.1% diethylamine; flow-rate, 1 ml/min; fluorescence detection ($\lambda_{ex}$=240 nm, $\lambda_{em}$=300 nm).

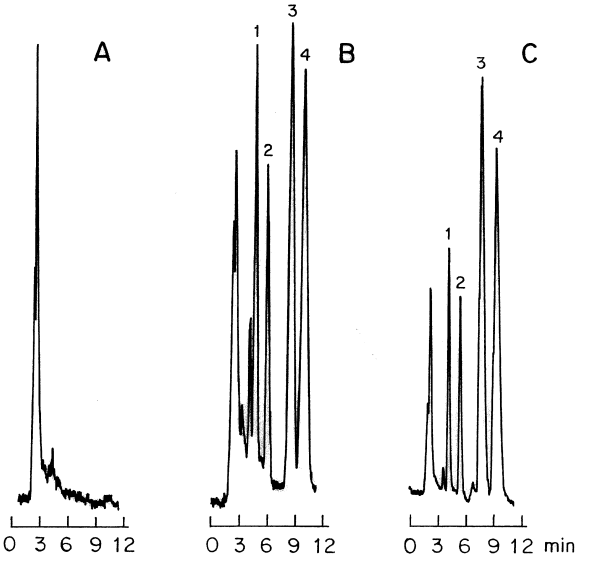


Fig. 3. Chromatograms referring to the analysis of AT enantiomers in urine after LLE. (A) Blank urine; (B) urine spiked with 1.25 μg/ml of AT enantiomers; (C) urine sample from a healthy volunteer collected 9–10 h after administration of 50 mg of AT. Propranolol (1, 2); (+)-(*R*)-AT (3); (−)-(*S*)-AT (4). Chromatographic conditions: Chiralcel OD-H column; mobile phase: hexane–ethanol (85:15, v/v) plus 0.1% diethylamine; flow-rate, 1 ml/min; fluorescence detection ($\lambda_{ex}$=235 nm, $\lambda_{em}$=300 nm).

5.5 min correspond to propranolol enantiomers (internal standard) which are also resolved on the Chiralcel OD-H column. The second peak was used in the quantitation to avoid the interference of an endogenous compounds with the first eluted propranolol enantiomers.

Tables 1–5 summarize the data obtained in the validation of the methods. The standard curves (Tables 1 and 2) obtained by least-squares linear regression were linear up to 5000 ng/ml and 25.0 μg/ml of plasma and urine, respectively, for the two enantiomers with correlation coefficients of 0.9935 or better. The ratio detector response/concentration was constant all over these ranges, within a tolerance of ±10% [15,16]. Tables 1 and 2 also show reproducible recovery of the enantiomers using the proposed procedures. In addition, no racemization of AT was observed during sample work-up for both methods.

To assess the precision and accuracy of the methods, plasma samples containing AT at different concentrations were analyzed repeatedly. The within-day and between-day precision data are summarized in Tables 3 and 4. RSD values of less than 10% were obtained for all samples analyzed by both methods and the analysis of variance ($\alpha$=5%) showed that there was no statistically significant difference in the results of the analysis over the 5 days.

In addition, the methods were accurate since deviation from the theoretical value was also in the 10% range (Tables 3 and 4). The *t*-test ($\alpha$=5%) showed that there were no significant differences between the real concentrations and the determined concentrations.

The limit of quantitation was established as the lower concentration used in the construction of the standard curve, i.e., 10 ng/ml for each enantiomer studied. This value is low enough for the application of the method to clinical pharmacokinetic studies.

The proposed methods proved to be highly selective. Among approximately 40 drugs and metabolites (Table 5) evaluated for interference, only procainamide showed an elution time close to those of the

Table 1
Recovery and linearity of the method for the analysis of AT enantiomers in plasma and urine after LLE

| | Plasma | | Urine | |
|---|---|---|---|---|
| | (+)-(*R*)-AT | (−)-(*S*)-AT | (+)-(*R*)-AT | (−)-(*S*)-AT |
| Recovery (%) (mean±SD) | | | | |
| 25 ng/ml | 100.2±6.2 | 102.7±7.2 | | |
| 125 ng/ml | 108.2±2.3 | 106.8±1.6 | | |
| 1250 ng/ml | 101.6±4.3 | 100.4±3.7 | | |
| Linearity | | | | |
| Concentration range | 10–5000 ng/ml | 10–5000 ng/ml | 0.25–25.0 μg/ml | 0.25–25.0 μg/ml |
| Slope | 0.011 | 0.010 | 0.001 | 0.001 |
| Intercept | 0.42 | 0.40 | −0.43 | −0.36 |
| Correlation coefficient | 0.9986 | 0.9987 | 0.9935 | 0.9938 |

AT enantiomers or to the second peak of propranolol (internal standard). This interference could not be avoided by the extraction procedure.

Figs. 4–6 show the plasma concentration–time curves and the cumulative urinary excretion curves for AT enantiomers obtained from a healthy subject treated with a single dose of (*R*,*S*)-AT (Atenol 50 mg). Although our data were obtained from only one subject, our results are in agreement with the literature [2,6,10,11], i.e., higher $C_{max}$ and area under the curve (AUC) for (+)-(*R*)-AT and similar $t_{max}$ for both enantiomers. The preliminary results of this pharmacokinetic investigation suggest that the assay is sensitive enough to be used in pharmacokinetic studies of AT.

Table 2
Recovery and linearity of the method for the analysis of AT enantiomers in plasma after SPE

| | Enantiomers | |
|---|---|---|
| | (+)-(*R*)-AT | (−)-(*S*)-AT |
| Recovery (%) (mean±SD) | | |
| 25 ng/ml | 109±2.9 | 103.1±5.7 |
| 125 ng/ml | 98.4±1.2 | 102.3±1.8 |
| 1250 ng/ml | 101.2±1.5 | 104.5±2.7 |
| Linearity | | |
| Concentration range (ng/ml) | 10–5000 | 10–5000 |
| Slope | 0.00206 | 0.00185 |
| Intercept | 0.07641 | 0.0692 |
| Correlation coefficient | 0.99883 | 0.99867 |

### *3.1. Comparison of the liquid–liquid and solid-phase extraction methods*

Both extraction techniques used for sample preparation were efficient in recovering AT and removing endogenous interferents. The advantages of LLE are simplicity and selectivity depending on the choice of solvent, and the advantages of SPE include no formation of emulsions, reduced solvent use and greater rapidity and reproducibility with automation. The orthogonal regression with correlation coefficients of 0.98122 and 0.98409 for (+)-(*R*)-AT and (−)-(*S*)-AT, respectively, shows that the methods have good correlation. These results indicated that both methods are well suited for the enantioselective analysis of AT in biological samples.

The methods developed in the present study show higher recuperation and linearity than the previous methods reported in the literature [6–11]. In addition, they are more sensible, except for the method reported by Egginger et al. [6], in which the limit of quantitation was 0.6 ng/ml due to a non chiral derivatization procedure included in the sample preparation step.

## 4. Conclusion

In this paper we describe two stereoselective methods for the determination of AT enantiomers in plasma and urine, in the concentration ranges of 10–5000 ng/ml and 0.25–25.0 μg/ml, respectively.

Table 3
Analysis of precision and accuracy of the method for the analysis of atenolol enantiomers in plasma and urine after LLE

| | Within-day | | | | Between-day | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration | *n* | RSD (%) | *E* (%) | Concentration | *n* | RSD (%) | *E* (%) |
| Plasma, 16 ng/ml | | | | | | | | |
| (+)-(*R*)-AT | 15.42 ng/ml | 4 | 7.3 | −3.6 | 16.34 | 5 | 3.8 | 2.1 |
| (−)-(*S*)-AT | 15.67 ng/ml | 4 | 7.8 | −2.0 | 16.64 | 5 | 6.2 | 4.00 |
| Plasma, 120 ng/ml | | | | | | | | |
| (+)-(*R*)-AT | 120.55 ng/ml | 4 | 6.6 | 0.5 | 113.18 | 5 | 4.6 | −5.68 |
| (−)-(*S*)-AT | 124.32 ng/ml | 4 | 5.4 | 3.6 | 117.59 | 5 | 4.1 | −2.01 |
| Plasma, 800 ng/ml | | | | | | | | |
| (+)-(*R*)-AT | 797.08 ng/ml | 4 | 4.0 | −0.4 | 796.48 | 5 | 4.1 | −0.44 |
| (−)-(*S*)-AT | 800.40 ng/ml | 4 | 3.9 | 0.1 | 817.33 | 5 | 5.1 | 2.17 |
| Urine, 0.5 μg/ml | | | | | | | | |
| (+)-(*R*)-AT | 0.53 μg/ml | 3 | 6.66 | 5.4 | | | | |
| (−)-(*S*)-AT | 0.52 μg/ml | 3 | 9.24 | 3.4 | | | | |
| Urine, 5.0 μg/ml | | | | | | | | |
| (+)-(*R*)-AT | 5.43 μg/ml | 3 | 4.81 | 8.5 | | | | |
| (−)-(*S*)-AT | 5.48 μg/ml | 3 | 4.49 | 9.6 | | | | |
| Urine, 20.0 μg/ml | | | | | | | | |
| (+)-(*R*)-AT | 21.48 μg/ml | 3 | 4.19 | 7.40 | | | | |
| (−)-(*S*)-AT | 21.49 μg/ml | 3 | 4.62 | 7.43 | | | | |

*n*, Number of determinations; RSD, relative standard deviation; *E*, deviation from nominal value.

Table 4
Analysis of the precision and accuracy of the method for analysis of atenolol enantiomers in plasma after SPE

| Enantiomers, concentration | Within-day | | | | Between-day | | | |
|---|---|---|---|---|---|---|---|---|
| | Concentration | *n* | RSD (%) | *E* (%) | Concentration | *n* | RSD (%) | *E* (%) |
| 16 ng/ml | | | | | | | | |
| (+)-(*R*)-AT | 16.90 ng/ml | 4 | 5.5 | 5.5 | 16.84 | 5 | 2.7 | 5.2 |
| (−)-(*S*)-AT | 17.13 ng/ml | 4 | 4.6 | 7.1 | 16.89 | 5 | 3.3 | 5.6 |
| 120 ng/ml | | | | | | | | |
| (+)-(*R*)-AT | 122.99 ng/ml | 4 | 1.6 | 2.5 | 118.99 | 5 | 2.7 | −0.8 |
| (−)-(*S*)-AT | 125.03 ng/ml | 4 | 2.4 | 4.2 | 119.41 | 5 | 3.6 | −0.5 |
| 800 ng/ml | | | | | | | | |
| (+)-(*R*)-AT | 773.62 ng/ml | 4 | 1.3 | −3.3 | 808.45 | 5 | 3.3 | 1.1 |
| (−)-(*S*)-AT | 792.9 ng/ml | 4 | 1.5 | −0.9 | 813.36 | 5 | 3.5 | 1.7 |

*n*, Number of determinations; RSD, relative standard deviation; *E*, deviation from nominal value.

Table 5
Drugs studied as possible interferents in the determination of atenolol enantiomers, in plasma

| Drug | $t_R$ (min) | Drug | $t_R$ (min) |
|---|---|---|---|
| (+)-(*R*)-AT | 8.3 | Lidocaine | ND |
| (−)-(*S*)-AT | 10.3 | Lorazepam | ND |
| Propranolol | 4.3 | Metaprolol | 2.9; 3.9 |
| Propranolol (PI) | 5.6 | Mexiletine | 3.4 |
| Alprazolam | ND | Phenobarbital | ND |
| Amitriptyline | ND | Phenylbutazonum | ND |
| Bromazepam | ND | Phenylephrine | 3.6 |
| Carbamazepine | ND | Phenytoin | ND |
| Chloramphenicol | ND | Pindolol | 6.5 |
| Chlordiazepoxide | ND | Piracetam | ND |
| Chlormezanone | 21.7 | Praziquantel | ND |
| Chlormezanone | 22.5 | Primidone | ND |
| Chlorpromazine | ND | Procainamide | 8.0 |
| Dexamethasone | ND | Propiophenone | ND |
| Diazepam | ND | Propoxyphene | ND |
| Diclofenac sodium | ND | Salbutamol | 3.0 |
| Dipyrone | ND | Salicylic acid | ND |
| Disopyramide | ND | Thioridazine | ND |
| Fenproporex | 3.8 | Triazolam | ND |
| Fenfluramine | ND | Trimethoprim | 15.1 |
| Flunitrazepam | ND | Valproic acid | ND |
| Fluoxetine | 2.8 | Verapamil | 4.1 |
| Haloperidol | ND | Warfarin | ND |
| Levomepromazine | ND | | |

$t_R$, Retention time; ND, not detected, drug concentration=1 mg/ml.

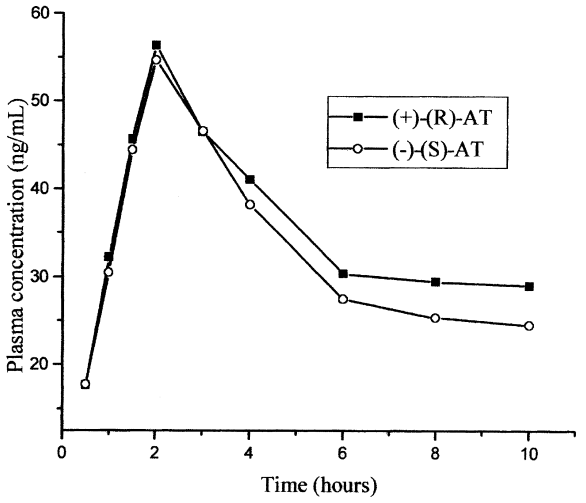


Fig. 4. Time–concentration profiles of AT enantiomers after oral administration of racemic AT to a healthy volunteer. Sample preparation by LLE.

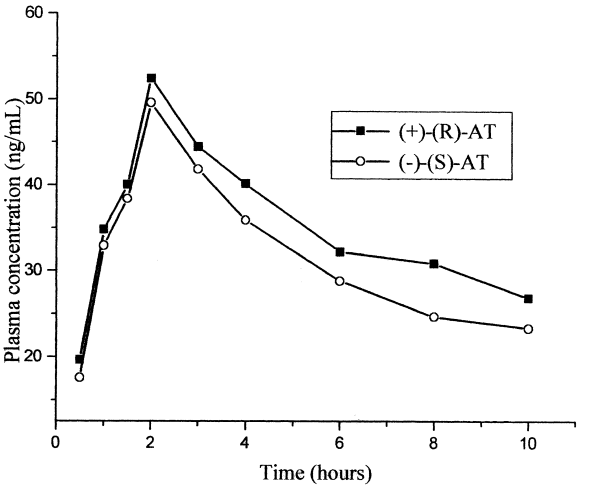


Fig. 5. Time–concentration profiles of AT enantiomers after oral administration of racemic AT to a healthy volunteer. Sample preparation by SPE.

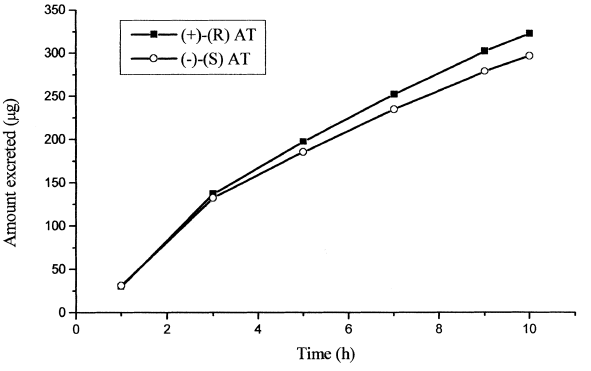


Fig. 6. Cumulative urinary excretion–time curves for AT enantiomers after oral administration of racemic AT to a healthy volunteer.

Both methods showed RSDs and deviation from nominal values lower than 10% and were, rapid, selective, simple and sensitive enough to be used in clinical pharmacokinetic studies of the enantioselective disposition of AT in humans.

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