# Enantioselective Quantitation of (*R*)- and (*S*)-Alprenolol by Gas Chromatography–Mass Spectrometry in Human Saliva and Plasma

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## Abstract

**An enantioselective gas chromatographic–mass spectrometric assay is developed for alprenolol and its metabolite, 4-hydroxy-alprenolol, in saliva and plasma. The procedure is based on a two-step derivatization technique with *N*-heptafluorobutyryl-*l*-prolylchloride and *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide, followed by a gas chromatographic separation with mass spectrometric detection of the diastereomeric derivatives. A selected ion chromatogram extracted from full scan data shows that the respective enantiomers of alprenolol, 4-hydroxy-alprenolol, and the internal standard (ions at *m/z* 481) are well-separated in saliva and plasma. Linear and reproducible calibration curves are obtained over the concentration ranges 1.67–13.33 ng/mL and 2.50–20.00 ng/mL enantiomer in saliva and plasma, respectively. The performance of the method for alprenolol, in terms of accuracy and precision, fits well within the generally accepted criteria for validation. The enantioselective assay is successfully used in a study involving a single oral dose of alprenolol administered to two healthy volunteers. Stereo-selective differences are observed in the saliva and plasma concentrations following an oral dose of 50 mg (*R,S*)-alprenolol hydrochloride.**

## Introduction

Alprenolol is a nonpolar, nonselective $\beta_1$- and $\beta_2$-blocking drug with intrinsic sympathomimetic activity and membrane stabilizing properties. It has been used in the treatment of hypertension, angina pectoris, and arrhythmia (Figure 1) (1). Like most β-blockers, alprenolol is marketed as a racemic mixture. However, the principal β-blocking activity can be mainly attributed to the (*S*)-enantiomer of the aryloxy propanol amine type compounds (2). As with many β-blockers, the pharmacokinetics of the alprenolol enantiomers differ significantly (3). In order to perform enantioselective pharmacokinetic studies, validated methods for resolution and quantitation of the single enantiomers in biological fluids are needed. Various enantioselective high-performance liquid chromatographic (HPLC) methods for the separation of (*R,S*)-alprenolol have been published, but only a few methods have been applied to biological samples (4). Hermansson and von Bahr (3) developed an HPLC method for the separation and quantitation of (*R*)- and (*S*)-alprenolol in human plasma using *N-tert*-butoxcarbonyl-*l*-leucine as a chiral reagent. The plasma concentration of (*S*)-alprenolol was much higher than that of (*R*)-alprenolol after the administration of a single oral dose of the racemic drug. Alprenolol is extensively metabolized, and the 4-hydroxylated enantiomers, which are the major metabolites, may reach even higher concentrations in plasma than the parent drug after oral doses (5,6). To date, there are no validated assays available that allow simultaneous determination of alprenolol enantiomers and their 4-hydroxylated metabolites.

Previously, Caccia and co-workers (7) compared the derivatizing performance of the reagents *N*-trifluoroacetyl-*l*-prolylchloride (TPC) and *N*-heptafluorobutyryl-*l*-prolylchloride (HPC) with that of β-blockers using gas chromatography (GC). The *N*-heptafluorobutyryl-*l*-prolyl (HFBP) derivatives gave superior resolution for alprenolol, oxprenolol, atenolol, pindolol, and propranolol enantiomers, but the *N*-trifluoroacetyl-*l*-prolyl (TFAP) derivatives gave better separation of nifenalol and pronethalol enantiomers (8). HFBP derivatives have shorter retention times and higher sensitivities than TFAP derivatives. In addition, the commercial *l*-TPC reagent is not enantiomerically pure because it contains 8.2% *d*-TPC (9). Quantitation of propranolol enantiomers as *N*-HFBP,*O*-trimethylsilylether derivatives in biological specimens from rats was reported by Caccia and co-workers (10). This article reports the development of an enantioselective gas chromatographic–mass spectrometric (GC–MS) assay for the separation of (*R,S*)-alprenolol and its active metabolite (*R,S*)-4-hydroxy-alprenolol in saliva and plasma using the chiral reagent HPC. The method is applied to the study of plasma and saliva concentration–time profiles after oral administration of a single dose of racemic alprenolol hydrochloride to human adults.

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## Experimental

### Materials and reagents

(*R*)- and (*S*)-alprenolol hydrogentartrate, (*R,S*)-alprenolol hydrochloride, and (*R,S*)-4-hydroxy-alprenolol were donated by AB Hässle (Mölndal, Sweden). (*R,S*)-Toliprolol was donated by Boehringer Ingelheim (Haarlem, The Netherlands). *N*-Heptafluorobuteric anhydride (HFBA), phosphorous pentoxide ($P_2O_5$), and *l*-proline (99%) were purchased from Aldrich Chemicals (Bornem, Belgium). Diethyl ether, thionyl chloride, dichloromethane, ethylacetate (all reagent grade), potassium hydroxide (KOH), sodium hydroxide, sodium acetate, and borate buffer (pH 9.0) were obtained from Merck (Darmstadt, Germany). HPLC-grade methanol was purchased from J.T. Baker Chemicals BV (Deventer, The Netherlands). Cyclohexane (reagent grade) was obtained from Janssen (Beerse, Belgium). Acetonitrile (HPLC grade) was obtained from Biosolve (Barneveld, The Netherlands). Toluene (reagent grade) was obtained from Mallinckrodt (St. Louis, MO). *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) was obtained from Macherey-Nagel (Düren, Germany). Helix Pomatia enzyme solution in water (no. 23867), containing β-glucuronidase and arylsulfatase, was obtained from Serva (Heidelberg, Germany).

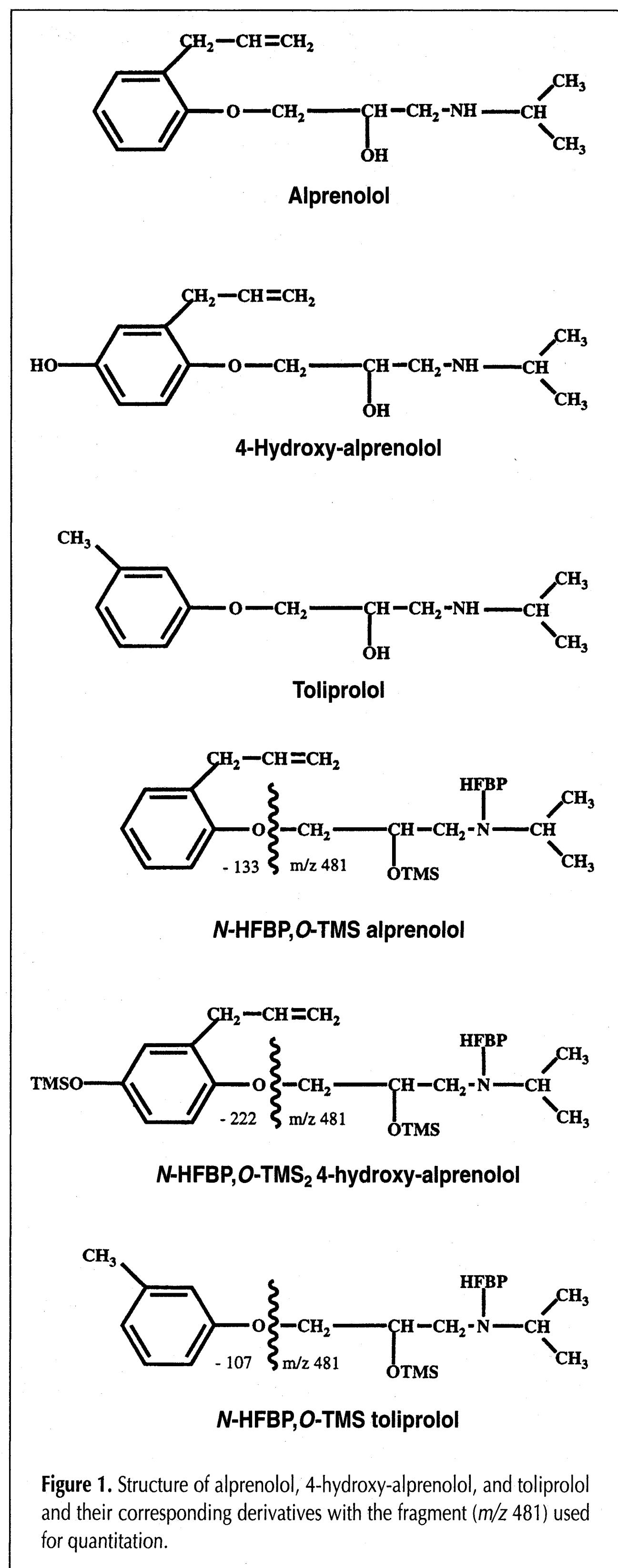


**Figure 1.** Structure of alprenolol, 4-hydroxy-alprenolol, and toliprolol and their corresponding derivatives with the fragment (*m/z* 481) used for quantitation.

Bond-Elut $C_2$ solid-phase extraction (SPE) columns (100 mg/1.0 mL) were obtained from Varian (Harbor City, CA). The vacuum manifold was purchased from J.T. Baker. Neutral Salivettes were obtained from Sarstedt (Etten-Leur, The Netherlands). Drug-free saliva was obtained from healthy subjects with the Salivette. Drug-free plasma was obtained from a local hospital. Acidified methanol was prepared by mixing 0.3 mL of 96% acetic acid (Merck) with 50 mL methanol.

Standard solutions of (*R,S*)-alprenolol (0.5 µg/mL), (*R*)-alprenolol (1.0 µg/mL), (*S*)-alprenolol (1.0 µg/mL), (*R,S*)-4-hydroxy-alprenolol (1.0 µg/mL), and (*R,S*)-toliprolol (1.0 µg/mL) (internal standard) were prepared in methanol and kept at 4°C.

The chiral derivatizing reagent, HPC was synthesized as described previously, dissolved in toluene to give a concentration of 0.02M, and stored in 2-mL portions under nitrogen at –20°C (11).

### Animals

Two male Wistar rats (Unilever, Utrecht, The Netherlands), weighing 470 g were orally given a solution of 10 mg/kg (*S*)-alprenolol as free base. Alprenolol was prepared in demineralized water (5 mg/mL as a free base). The rats had free access to food and water at all times and were housed together in a room with controlled lighting (12 h light/dark cycle) and temperature (21°C). The blood of the rats was collected 1 or 2 h after administration, respectively. Blood was taken in heparinized collection tubes and centrifuged at 1200 × *g* for 10 min. Plasma samples were stored at –20°C until assayed. To 2 mL plasma, 1 mL sodium acetate buffer (0.2M; pH 5.2) and 50 µL of enzyme solution were added to hydrolyze the conjugated metabolites. Hydrolysis was performed at 37°C overnight. After hydrolysis, the solution was made basic by adding five drops of sodium hydroxide (1M). The samples were extracted after the addition of 50 µL of the internal standard solution of toliprolol (1.0 µg/mL) according to an SPE procedure that will be described later.

### Clinical study

Two healthy volunteers (one 22-year-old male and one 30-year-old female), who were not receiving any form of medica-

tion, took a single gelatine capsule containing 50 mg (*R,S*)-alprenolol hydrochloride. The study was approved by an ethics committee, and informed written consent was obtained from the subjects. Saliva samples were obtained with the Salivette after the participants were given written instructions. The subjects had to rinse their mouths two times with water. After the subjects had swallowed the freshly formed saliva, a dental cotton roll was placed in their mouths. After chewing on the cotton roll for about 45 s, it was placed in a container that was closed with a plastic stopper. The container was put into a polystyrol tube that was centrifuged for 5 min at about 1000 × *g*. During centrifugation, about 1.5 mL of nonviscous, easily pipettable saliva passed from the cotton roll into the lower part of the tube. In order to facilitate blood sampling, a catheter was placed in the forearm. Before ingestion of the alprenolol capsule, samples of saliva and blood were obtained for assay blanks. Blood samples (10 mL) and saliva samples were taken 15 and 30 min after administration, as well as 1, 2, 4, and 8 h after administration. Blood samples were taken in heparinized collection tubes and centrifuged at 1200 × *g* for 10 min. Plasma and saliva samples were stored at –20°C until assayed.

### Extraction procedure for saliva and plasma

After thawing, 1 mL of the saliva or plasma sample was diluted with 1 mL of the borate buffer and extracted after the addition of 50 µL of the internal standard solution of toliprolol (1.0 µg/mL) according to the following SPE procedure. The column was conditioned by eluting twice with 1 mL methanol, once with 1 mL demineralized water, and once with 1 mL borate buffer. The sample was added to the column and suction was applied. The column was washed with 500 µL acetonitrile. Elution of the analytes was carried out in test tubes using acidified methanol (2 × 500 µL). The eluted aliquots were evaporated to dryness under a gentle stream of nitrogen at 60°C.

### Derivatization with HPC and MSTFA

The residue was dissolved in 1 mL borate buffer (pH 9.0) and then allowed to stand in an ice bath for 10 min. HPC (40 µL) was added, and the solution was mixed for 20 s. The tube was incubated for another 20 min in the ice bath. Subsequently, cyclohexane (4 mL) was added to the mixture, and the tube was shaken for 10 min. After centrifugation at 500 × *g* for 5 min, the organic layer was transferred into another tube. The solvent was evaporated under a gentle stream of nitrogen at 60°C. Before silylation, the residue was dried further in a vacuum exsiccator over $P_2O_5$–KOH for at least 60 min. Next, 20 µL MSTFA was added, and the solution was mixed for 20 s. The tube was incubated for 5 min at 60°C. Then, 80 µL ethylacetate was added and mixed. An aliquot of 1 µL was analyzed by GC–MS using an ion-trap mass spectrometer, unless specified otherwise.

### Calibration curves and preparation of quality control samples

Calibration samples were prepared in 1.5 mL of drug-free saliva by adding aliquots of the stock solutions of (*R,S*)-alprenolol (0.5 µg/mL) and (*R,S*)-4-hydroxy-alprenolol (1.0 µg/mL) to drug-free saliva. The spiked saliva was allowed to soak into the cotton roll, which was then replaced in the container and centrifuged for 5 min at about 1000 × *g*. Eight calibration samples containing 1.67–13.33 ng/mL of each enantiomer of alprenolol and 3.33–26.67 ng/mL of each enantiomer of 4-hydroxy-alprenolol were prepared. Plasma samples were prepared in 1 mL of drug-free plasma by adding aliquots of the stock solution of (*R,S*)-alprenolol (0.5 µg/mL) and (*R,S*)-4-hydroxy-alprenolol (1.0 µg/mL) to drug-free plasma. Eight calibration samples containing 2.5–20 ng/mL of each enantiomer of alprenolol and 5.0–40 ng/mL of each enantiomer of 4-hydroxy-alprenolol were prepared. After the addition of 50 µL of the internal standard solution of (*R,S*)-toliprolol (1 µg/mL), the calibration samples were extracted according to the previously described SPE procedure. Because no reference substances of the separate enantiomers of toliprolol were available, the exact elution order of the two enantiomers of this compound was uncertain. The first chromatographic peak of the racemic toliprolol was used as an internal standard for (*S*)-alprenolol and (*S*)-4-hydroxy-alprenolol, and the second chromatographic peak of toliprolol was used as an internal standard for (*R*)-alprenolol and (*R*)-4-hydroxy-alprenolol. Calibration curves for the (*R*)- and (*S*)-enantiomers of alprenolol and 4-hydroxy-alprenolol were constructed by plotting the ratios of the peak areas of the ion at *m/z* 481 of the (*R*)- and (*S*)-enantiomers of alprenolol and 4-hydroxy-alprenolol to the second and first peak areas of the ion at *m/z* 481 of toliprolol versus the concentrations of the enantiomers. This procedure was followed by regression analysis of the calibration curves. Intra-assay validation was conducted by analyzing on the same day six sets of quality control samples containing the enantiomers of alprenolol at three different concentrations. An inter-assay validation was carried out by analyzing the quality control samples containing the enantiomers of alprenolol at three concentrations on each of six days. The quality control samples were prepared on the same day of the analysis. In each study, the concentrations were calculated from the regression calibration curves. Routinely, eight calibration samples, a drug-free sample, an (*S*)-alprenolol sample, and three quality control samples were analyzed along with samples from the volunteers.

### GC–MS (Saturn II) analysis

Ion trap mass spectrometric analysis was performed using the Saturn II mass spectrometer, which was equipped with a Model 3400 GC and a Model 8100 autosampler (Varian). The GC was equipped with a Model 1093 injector (SPI) and a DB-5 MS fused-silica capillary column (15 m × 0.25-mm i.d.; 0.25-µm film thickness) (J&W Scientific; Folsom, CA). The following injector temperature program was used: the initial temperature was maintained at 90°C for 2 min, increased to 280°C at 100°C/min, and maintained for 1 min. A multilevel column program was used: the initial temperature was maintained at 90°C for 1 min, increased to 230°C at 8°C/min and maintained for 3 min, increased to 245°C at 10°C/min and maintained for 1.0 min, and increased to 290°C at 30°C/min and maintained for 4 min. High-quality helium (Hoekloos; The Netherlands) was used as a carrier gas with a flow rate of 1 mL/min. Full scan spectra were taken in the electron-impact (EI) mode with the automatic gain control (AGC) on. The scan range was 70–650 amu with a scan rate of 1 scan/s.

### GC–MS (TSQ-45) analysis

Quadrupole mass spectrometric analysis was performed on a Triple Stage Quadrupole (TSQ-45, Finnigan MAT; San Jose, CA) in the EI and cation-impact (CI) mode to determine major ions. Helium was used as the carrier gas with a flow of 1 mL/min. Methane was used as reagent gas in the CI mode with a source pressure of 0.40 mm Hg. The temperatures of the injection port, interface and transferline, manifold, and ionizer were 250, 275, 135, and 120°C, respectively. Emission current, electron energy, and multiplier were set at 0.20 mA, 70 eV, and 1300 V, respectively. Other characteristic MS conditions were selected specifically for the derivative at optimal response. The GC was equipped with a DB-5 fused-silica capillary column (30 × 0.32-mm i.d.; 0.25-µm film thickness) (J&W Scientific). The following oven temperature program was used: the initial temperature was maintained at 90°C for 1 min, increased to 280°C at 40°C/min, and maintained for a total run time of 15 min. EI and CI spectra were taken with a scan rate of 1 scan/s and a scan range of 100–850 amu.

### Effect of amount of HPC on derivatization

Samples of saliva spiked with (*R,S*)-alprenolol at a concentration of 20.00 ng/mL were allowed to soak into the cotton roll. After centrifugation, the samples were extracted and reacted with various volumes of HPC separately (20, 40, 60, 80 µL) as described previously.

### Effect of reaction time on diastereomer formation

Samples of saliva spiked with (*R,S*)-alprenolol at a concentration of 20.00 ng/mL were allowed to soak into the cotton roll. After centrifugation, the samples were extracted and reacted with 40 µL HPC as described previously. The reaction was stopped at various times (10, 20, 30 min). Because the reaction takes place at reduced temperatures, it can be stopped by removing the sample from the ice bath and extracting it with cyclohexane.

### Recovery

Recoveries of the extraction of the enantiomers of alprenolol and 4-hydroxy-alprenolol from saliva and plasma were determined at two concentrations (5.0 and 17.5 ng/mL, respectively). The spiked saliva samples were soaked into the cotton roll. The plasma and saliva samples were processed and derivatized as previously described except that 80 µL derivatized racemic toliprolol was added after the sample was derivatized with HPC and MSTFA. The peak area ratios of the ion at *m/z* 481

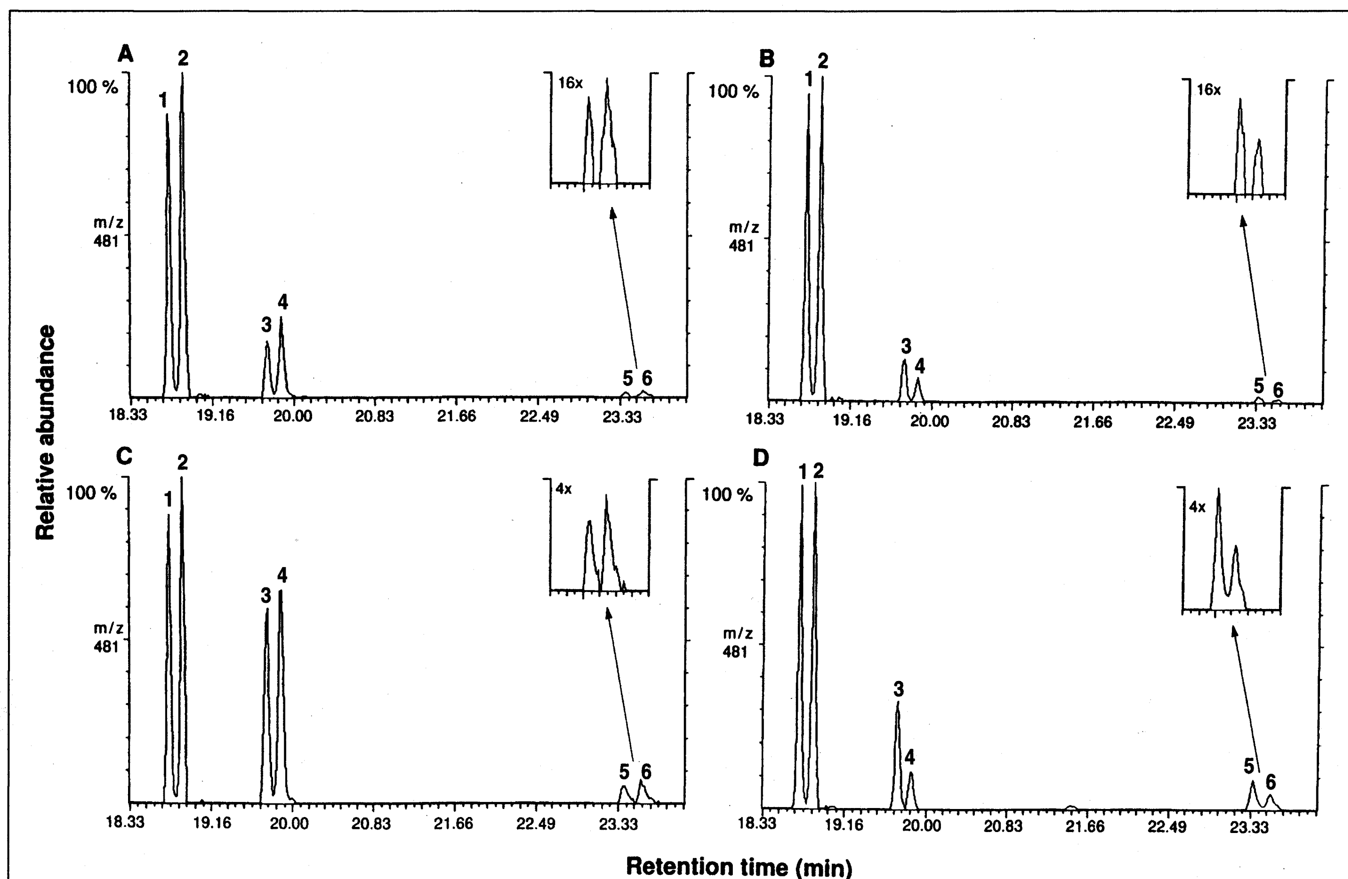


**Figure 2.** Representative extracted mass chromatogram of a Salivette sample spiked with 6.67 ng/mL of each enantiomer of alprenolol (A) and a real saliva sample (B). Concentrations in the real sample were 1.88 and 4.21 ng/mL for (*R*)-alprenolol and (*S*)-alprenolol, respectively. Also shown are the extracted plasma sample spiked with 10 ng/mL of each enantiomer of alprenolol (C) and a real plasma sample (D). Concentrations in the real plasma sample were 1.76 and 5.52 ng/mL for (*R*)-alprenolol and (*S*)-alprenolol, respectively. Peaks: 1, first enantiomer of toliprolol (internal standard); 2, second enantiomer of toliprolol; 3, (*S*)-alprenolol; 4, (*R*)-alprenolol; 5, (*S*)-4-hydroxy-alprenolol; and 6, (*R*)-4-hydroxy-alprenolol.

obtained from these samples were compared with those obtained when an equal amount of racemic alprenolol or 4-hydroxy-alprenolol was derivatized without prior extraction.

## Results and Discussion

### Derivatization

Initial efforts to develop an enantioselective HPLC assay for racemic alprenolol using a commercially available chiral column (Chiralcel OD-H) and a fluorescence detector were not successful because the method lacked sensitivity. Therefore an assay was developed. It was based on derivatization with a chiral reagent and separation of the diastereomeric pairs on an achiral capillary column using GC prior to MS analysis.

It was found that 40 µL HPC was adequate for the acylation of (*R,S*)-alprenolol in the nanogram-per-milliliter range without undue interference from reagent peaks. The use of more than 40 µL HPC, however, led to interference peaks in the chromatograms. The acylation reactions were optimized within 20 min. The tendency of the peak areas to decline after 20 min may have been due to slow hydrolysis of the ester function under alkaline conditions (11).

### Chromatography and peak identification

Figure 2 shows gas chromatograms of derivatized enantiomers of toliprolol, alprenolol, and 4-hydroxy-alprenolol extracted from spiked control samples of saliva and plasma. It also shows chromatograms that were obtained after extraction of saliva and corresponding plasma from a healthy adult volunteer dosed with racemic alprenolol hydrochloride. Near baseline separation of all the diastereomeric pairs was achieved. The elution order of (*R*)- and (*S*)-enantiomers of 4-hydroxy-alprenolol was established from analysis of plasma samples from rats dosed with optically pure (*S*)-alprenolol hydrochloride. The (*S*)-enantiomer of each diastereomeric pair eluted earlier than the (*R*)-enantiomer. The toliprolol, alprenolol, and 4-hydroxy-alprenolol derivatives (without extraction) were evaluated on the TSQ in the CI and EI modes to confirm the MS data of the compounds that were obtained with the Saturn. The mass spectral data of the compounds that gave rise to the six peaks are shown in Table I. The fragmentation pattern of the corresponding enantiomers was almost equal. The EI spectra obtained with the Saturn were in agreement with the spectra obtained with the TSQ. The CI spectra of 4-hydroxy-alprenolol obtained with the TSQ could not be obtained with the Saturn because the Saturn can only measure up to 650 amu; after derivatization with HPC and MSTFA, 4-hydroxy-alprenolol shows a molecular weight of 703 amu.

**Table I. Partial CI and EI Mass Spectrometric Data of Toliprolol, Alprenolol, and 4-Hydroxy-Alprenolol After Derivatization with HPC and MSTFA**

| | | *m/z* Values (normalized on the base peak) | |
|---|---|---|---|
| Compound | MW | CI-mode | EI-mode |
| 1st Toliprolol | 588 | 589(100); 499(8.2); 572(4.4); 481(3.2) | 266(100); 481(39.7); 204(47.9); 294(11.0); 322(11.0) |
| 2nd Toliprolol | 588 | 589(100); 499(11.4); 572(4.4); 481(5.7) | 266(100); 481(43.2); 204(50.0); 294(11.4); 322(9.1) |
| (*S*)-Alprenolol | 614 | 615(100); 525(11.0); 481(15.1); 599(4.1) | 266(100); 481(72.6); 230(27.4); 348(8.2) |
| (*R*)-Alprenolol | 614 | 615(100); 525(8.2); 481(15.1); 599(2.7) | 266(95.9); 481(100); 230(27.4); 348(8.2) |
| (*S*)-4-OH-Alprenolol | 703 | 704(100); 481(43.8) 409(16.4) | 481(100); 266(45.4) |
| (*R*)-4-OH-Alprenolol | 703 | 704(100); 481(49.3) 409(16.4) | 481(100); 266(50.0) |

### Statistical data of the assay

The recoveries (after SPE and derivatization) of spiked plasma and saliva samples of each enantiomer of alprenolol and 4-hydroxy-alprenolol are summarized in Table II. No differences in the recoveries between the single enantiomers were observed. The spiked saliva samples showed a recovery around 40%

**Table II. Recoveries of (*R*)- and (*S*)-Enantiomers of Alprenolol and 4-Hydroxy-Alprenolol from Spiked Saliva and Plasma (*n* = 4)**

| Compound | Conc. (ng/mL) | Mean recovery (%) | Coefficient of variation (%) | Compound | Conc. (ng/mL) | Mean recovery (%) | Coefficient of variation (%) |
|---|---|---|---|---|---|---|---|
| *Saliva* | | | | *Saliva* | | | |
| (R)-Alprenolol | 5.0 | 41.6 | 19.6 | (R)-4-OH-Alprenolol | 10.0 | 19.9 | 36.9 |
| (S)-Alprenolol | 5.0 | 39.3 | 12.3 | (S)-4-OH-Alprenolol | 10.0 | 8.4 | 47.6 |
| (R)-Alprenolol | 17.5 | 40.1 | 8.8 | (R)-4-OH-Alprenolol | 35.0 | 17.6 | 22.3 |
| (S)-Alprenolol | 17.5 | 37.4 | 4.8 | (S)-4-OH-Alprenolol | 35.0 | 18.9 | 18.8 |
| *Plasma* | | | | *Plasma* | | | |
| (R)-Alprenolol | 5.0 | 86.8 | 19.4 | (R)-4-OH-Alprenolol | 10.0 | 100.8 | 28.6 |
| (S)-Alprenolol | 5.0 | 79.7 | 8.2 | (S)-4-OH-Alprenolol | 10.0 | 87.0 | 14.0 |
| (R)-Alprenolol | 17.5 | 66.8 | 2.7 | (R)-4-OH-Alprenolol | 35.0 | 96.6 | 25.5 |
| (S)-Alprenolol | 17.5 | 68.6 | 7.5 | (S)-4-OH-Alprenolol | 35.0 | 97.4 | 20.0 |

for alprenolol, whereas the plasma samples showed a recovery around 76%. The loss of alprenolol of the spiked saliva samples is caused by the Salivette because the cotton absorbs a significant amount of this compound. The recovery from the Salivette appeared to be correlated to the log of the partition coefficient of the drug (12): the more lipophilic a drug, the lower the recovery from the Salivette. However, this explanation does not account for the low mean recovery of about 16% for 4-hydroxyalprenolol in saliva samples. This low recovery has to be caused by adsorption to the Salivette because the mean recovery from the plasma samples is almost 100%.

### Linear regression

The computer-generated regression lines of the calibration curves for (*R*)- and (*S*)-alprenolol in saliva and plasma were linear over the concentration range used and gave a correlation coefficient of at least 0.990 for each enantiomer of analyte. The mean regression equations for plasma were $y = -0.032 + 0.099x$ and $y = -0.081 + 0.092x$, respectively, for (*R*)-alprenolol and (*S*)-alprenolol. The equations for saliva were $y = -0.048 + 0.064x$ and $y = -0.048 + 0.060x$, respectively, for (*R*)-alprenolol and (*S*)-alprenolol. The calibration curves for 4-hydroxyalprenolol in saliva and plasma gave poor correlations, probably because of the low recoveries from the Salivette and the variable recoveries for plasma and saliva. Therefore, the concentrations of this metabolite were not used quantitatively.

The reproducibility of the method for alprenolol was studied at three different concentrations. The results are summarized in Table III. Between-run precision ranged from 3.4–15.3% in saliva and 5.3–9.0% in plasma, expressed as coefficients of variation. Within-run precision ranged from 2.5–20.0% in saliva and 2.1–7.5% in plasma. The results of the method validation showed that the accuracy and between-run and within-run precision were in agreement with accepted validation procedures (13). The lower limit of quantitation (LOQ) was defined as the quantity of the sample after preparation and extraction that was quantitated with a deviation and precision less than 20%. The LOQs of each of the (*R*)- and (*S*)-enantiomers were below 2.5 ng/mL in plasma and below 1.67 ng/mL in saliva.

**Table III. Precision and Accuracy for Quantitation of (*R*)- and (*S*)-Enantiomers of Alprenolol in Spiked Saliva and Plasma**

| Biological specimen | Compound | Conc. (ng/mL) | Accuracy (%) | Between-run precision (%CV) | Within-run precision (%CV) |
|---|---|---|---|---|---|
| Saliva | (*R*)-Alprenolol | 1.67 | 95.5 | 15.3 | 15.3 |
| | | 6.67 | 90.9 | 13.7 | 2.5 |
| | | 13.33 | 96.7 | 4.4 | 5.3 |
| | (*S*)-Alprenolol | 1.67 | 99.0 | 12.4 | 20.0 |
| | | 6.67 | 94.4 | 14.1 | 4.9 |
| | | 13.33 | 94.6 | 3.4 | 3.6 |
| Plasma | (*R*)-Alprenolol | 2.50 | 106.9 | 6.3 | 7.0 |
| | | 10.00 | 99.2 | 6.7 | 3.9 |
| | | 20.00 | 104.1 | 9.7 | 2.1 |
| | (*S*)-Alprenolol | 2.50 | 106.9 | 6.4 | 7.5 |
| | | 10.00 | 98.8 | 5.3 | 7.5 |
| | | 20.00 | 102.9 | 9.0 | 3.4 |

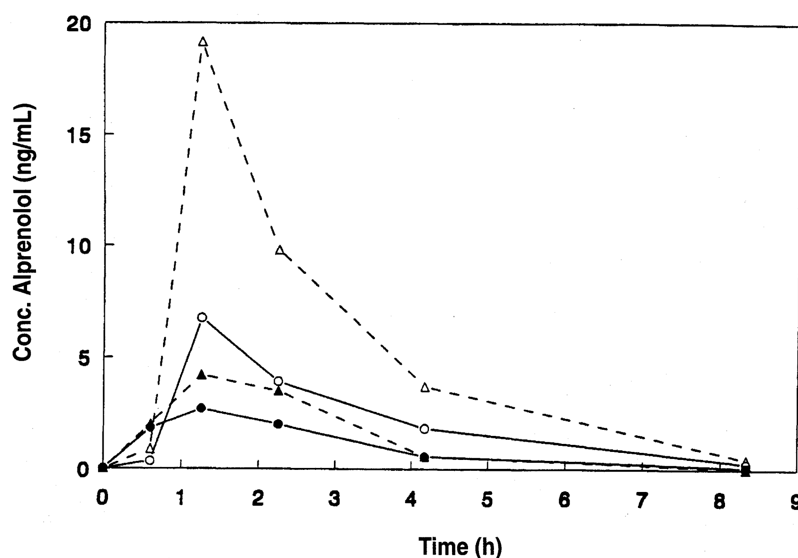


**Figure 3.** Saliva concentrations of (*R*)-alprenolol (●) and (*S*)-alprenolol (▲) and plasma concentrations of (*R*)-alprenolol (○) and (*S*)-alprenolol (△) from one subject receiving a single gelatine capsule of 50 mg (*R,S*)-alprenolol hydrochloride.

### Clinical application

The saliva and plasma samples from two subjects taking racemic alprenolol hydrochloride were analyzed to demonstrate the applicability of the method to measure saliva and plasma concentrations of the enantiomers. A sample from one subject taken after 30 min is shown in Figure 2. The pharmacokinetic disposition of salivary alprenolol enantiomers over the 8-h dosing interval is seen in Figure 3. The concentrations in plasma reached maximums of 7.8 and 21.8 ng/mL, respectively, for (*R*)-alprenolol and (*S*)-alprenolol after 1 h. The concentrations in saliva reached maximums of 2.8 and 4.7 ng/mL, respectively, for (*R*)-alprenolol and (*S*)-alprenolol after 1 h. The concentrations of the (*S*)-enantiomer were consistently found to be higher than those of the (*R*)-enantiomer in plasma as well as in saliva. Only the (*S*)-enantiomer in plasma was above the LOQ after 4 h.

## Conclusion

The method presented is based on a two-step derivatization reaction of (*R,S*)-alprenolol and its metabolite with HPC and MSTFA. The

assay is reproducible and very sensitive for monitoring alprenolol in saliva and plasma. The performance of the method for alprenolol, in terms of accuracy and precision, fit well within the generally accepted criteria for validation. However, the method must still be optimized to measure the concentrations of the metabolite simultaneously. The assay was successfully applied to a human study that involved orally administering a single dose of 50 mg (*R,S*)-alprenolol hydrochloride.

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## References

1. R. Janknegt and A. Steenhoek. Bètablokkers. Een preparaatkeuze volgens de SOJA-methode. *Pharm. Weekbl.* **126:** 4–14 (1991).
2. T. Walle, J.G. Webb, E.E. Bagwell, U.K. Walle, H.B. Daniell, and T.E. Gaffney. Stereoselective delivery and actions of beta receptor antagonists. *Biochem. Pharmacol.* **37:** 115–24 (1988).
3. J. Hermansson and C. von Bahr. Determination of (*R*)-alprenolol and (*S*)-alprenolol and (*R*)- and (*S*)-metoprolol as their diastereomeric derivatives in human plasma by reversed-phase liquid chromatography. *J. Chromatogr.* **227:** 113–27 (1982).
4. C.L. Davies. Chromatography of β-adrenergic blocking agents. *J. Chromatogr. Biomed. Appl.* **531:** 162–75 (1990).
5. P. Collste, K.O. Borg, H. Aström, and C. von Bahr. Contribution of 4-hydroxy-alprenolol to adrenergic beta receptor blockade of alprenolol. *Clin. Pharmacol. Ther.* **25:** 416–22 (1979).
6. N.O. Bodin. Identification of the major urinary metabolite of alprenolol in man, dog and rat. *Life Sci.* **14:** 685–92 (1974).
7. S. Caccia, C. Chiabrando, P. De Ponte, and R. Fanelli. Separation of beta adrenoceptor antagonist enantiomers by high resolution capillary gas chromatography. *J. Chromatogr. Sci.* **16:** 543–46 (1978).
8. A.M. Dyas. The chiral chromatographic separation of β-adrenoceptor blocking drugs. *J. Pharm. Biomed. Anal.* **10:** 383–404 (1992).
9. J. Hermansson and C. von Bahr. Simultaneous determination of *d*- and *l*-propranolol in human plasma by high-performance liquid chromatography. *J. Chromatogr. Biomed. Appl.* **221:** 109–17 (1980).
10. S. Caccia, G. Guiso, M. Ballabio, and P. De Ponte. Simultaneous determination of the propranolol enantiomers in biological samples by gas–liquid chromatography. *J. Chromatogr.* **172:** 457–62 (1979).
11. H.K. Lim, J.W. Hubbard, and K.K. Midha. Development of enantioselective gas chromatographic quantitation assay for *d,l*-threomethylphenidate in biological fluids. *J. Chromatogr. Biomed. Appl.* **378:** 109–23 (1986).
12. K.M. Höld, D. deBoer, J. Zuidema, and R.A.A. Maes. Evaluation of the Salivette as sampling device for monitoring β-adrenoceptor blocking drugs in saliva. *J. Chromatogr. Biomed. Appl.* **663:** 103–10 (1995).
13. V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, et al. Analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies. *J. Pharm. Sci.* **81:** 309–12 (1992).

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