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## Note

# Rapid and sensitive high-performance liquid chromatographic determination of bisoprolol in plasma and urine

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Bisoprolol, (±)1-(4-(2-isopropylethoxy)methylphenoxy)-3-isopropylamino-2-propanol hemifumarate is a new highly selective β1-adrenoreceptor antagonist lacking intrinsic sympathomimetic activity and with low anaesthetic potency [1–4]. In humans, the drug is excreted in urine partly (50%) unchanged and partly in the form of pharmacologically inactive polar metabolites [5].

A high-performance liquid chromatographic (HPLC) method for the determination of bisoprolol in biological samples has been described [6]. In this assay, bisoprolol was chromatographed either on a normal-phase column with an aqueous mobile phase containing only a few percent of organic modifier, or on a reversed-phase column after derivatization of the compound.

The purpose of this paper is to present a more simple and rapid HPLC method for the quantification of bisoprolol in plasma and urine samples. This assay involves a two-step liquid–liquid extraction procedure without the need for derivatization, prior to reversed-phase column chromatography, and is of sufficient sensitivity to be easily applicable to pharmacokinetic studies.

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## EXPERIMENTAL

### *Chemicals*

Pure samples of bisoprolol and internal standard (Fig. 1) were kindly supplied by Lederle Labs. (Pearl River, NY, U.S.A. and Oullins, France, respectively). All the other reagents and solvents were of analytical grade and were obtained from Prolabo (Paris, France).

### *Chromatography*

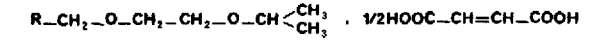
The liquid chromatographic unit consisted of an M45 solvent-delivery system, a Model U6K injector (Millipore-Waters, Saint-Quentin en Yvelines, France) and an RF-535 Shimadzu spectrofluorimeter (Touzart et Matignon, Vitry-Sur-Seine, France). The fluorescence detector was operated at an excitation wavelength of 232 nm and an emission wavelength of 300 nm. The column, which was used at ambient temperature, was a reversed-phase μBondapak $C_{18}$ (30 cm × 3.9 mm I.D., 10 μm particle size; Millipore-Waters) equipped with a 1-cm pre-column packed with the same material (SFCC, Gagny, France). A one-channel Servotrace recorder (Sefram, Paris, France) was used at a chart-speed of 2.5 mm/min and a sensitivity of 10 mV. The mobile phase was acetonitrile–methanol–0.09 *M* phosphoric acid–water (24:20:6:50, v/v), vacuum-degassed before use and at a flow-rate of 1.0 ml/min.

### *Calibration standards*

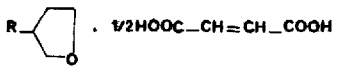
Stock solutions of bisoprolol (100 μg/ml) and internal standard (30 μg/ml) were prepared by dissolving appropriate amounts of pure samples in methanol. They were stable for at least two months without observable degradation when stored at −20°C.

Working solutions of bisoprolol at 0.5 and 2.0 μg/ml were prepared by appropriate dilutions in methanol, precise volumes being introduced into 10-ml glass tubes and evaporated to dryness in a stream of nitrogen. A blank plasma sample of 1 ml was added to give concentrations of bisoprolol ranging from 1 to 100 ng/ml.

BISOPROLOL



INTERNAL STANDARD:



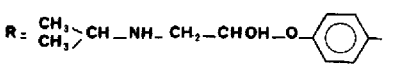


Fig. 1. Molecular structures of bisoprolol and internal standard.

The working solution of internal standard was prepared by a 1:100 dilution in distilled water of the stock solution to provide a concentration of 300 ng/ml.

*Extraction procedure*

To 1 ml of plasma sample in a 10-ml glass tube, were added 0.1 ml of internal standard (30 ng), 0.1 ml of 1 *M* sodium hydroxide and 4 ml of diethyl ether. The tube was shaken vigorously for 5 min and centrifuged for 5 min at 2000 *g*. The upper phase was transferred to another 10-ml glass tube, containing 0.2 ml of 1 *M* acetic acid. After shaking (5 min) and centrifugation (5 min, 2000 *g*), the organic phase was removed by vacuum aspiration and an aliquot of the acidic phase ($\leqslant 100\ \mu l$) introduced into the HPLC unit.

## RESULTS AND DISCUSSION

Typical chromatograms of plasma and urine extracts are shown in Figs. 2 and 3, respectively. Assays performed on drug-free plasma and urine samples show the absence of any endogenous interfering peaks (Figs. 2A and 3A, respectively). The retention times of internal standard and bisoprolol were 4.4 and 6.6 min, respectively. Representative chromatograms of extracts of a blank plasma sample spiked with 10 ng/ml bisoprolol and 30 ng/ml internal standard, and of a plasma sample obtained from a patient 30 h after a single 20-mg oral dose of bisoprolol containing 8.7 ng/ml drug, are presented in Figs. 2B and 2C, respectively. Figs. 3B and 3C are the chromatograms of extracts of a blank urine sample spiked with 1.0 $\mu$g/ml bisoprolol and 1.2 $\mu$g/ml internal standard, and of a urine sample collected in the period 5–10 h after administration of the drug and containing 5.4 $\mu$g/ml bisoprolol, respectively.

A least-squares linear regression was used to calculate the equation relating the peak-height ratio between drug and internal standard, and the concentration of bisoprolol. Calibration curves were linear ($r^2 \geqslant 0.999$) in the range 1–200 ng/ml for bisoprolol in plasma. The daily fluctuation of plasma standard curves ($n=5$) was slight, with a coefficient of variation (C.V.) of 1.3% and an intercept of $-0.04 \pm 0.03$ ng/ml.

Within-day and day-to-day precision and accuracy data for plasma analysis were evaluated over the concentration range 1–80 ng/ml. The results, expressed as the mean $\pm$ S.D. of five determinations, are presented in Tables I and II, respectively. At the plasma level corresponding to the quantification limit (1 ng/ml) the C.V. was 8% for both repeatability and reproducibility studies and less than 3% at plasma levels higher than 5 ng/ml. The overall accuracy was $102 \pm 4$ and $101 \pm 4$% for within-day and day-to-day studies, respectively. The detection limit, based on a signal-to-noise ratio of 2:1, was 0.5 ng/ml.

Estimated absolute recoveries are listed in Table I, indicating quantitative extraction of bisoprolol from plasma. For determination of bisoprolol in urine, the same extraction procedure and chromatographic conditions were used, but with a urine sample of only 0.1 ml. The determination limit for bisoprolol in urine was 10 ng/ml, but for urine pharmacokinetic studies calibration standards were pre-

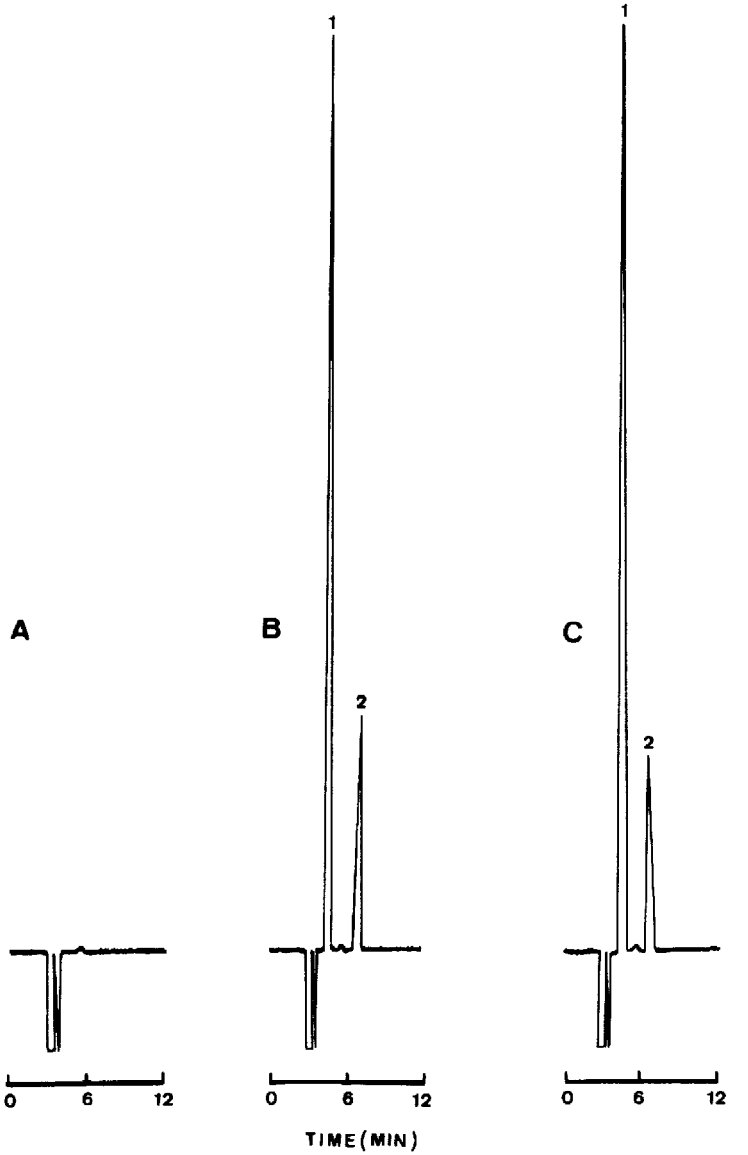



Fig. 2. Chromatograms of 1-ml plasma extracts. (A) Drug-free plasma; (B) drug-free plasma spiked with 10 ng/ml bisoprolol and 30 ng/ml internal standard; (C) sample obtained from a patient 30 h after a 20-mg oral dose of bisoprolol containing 8.7 ng/ml drug. Peaks: 1 = internal standard; 2 = bisoprolol.

pared between 0.5 and 25 $\mu$g/ml, owing to the large amounts of unchanged bisoprolol excreted in urine.

No decrease in the measured concentration of bisoprolol was found when plasma or urine extracts were kept at ambient temperature for four days.

Pure samples of some commonly administered drugs (with fluorescent properties) used for the treatment of cardiovascular diseases (acebutolol, quinidine, hydroquinidine, propranolol, sotalol and verapamil) were assayed under the de-

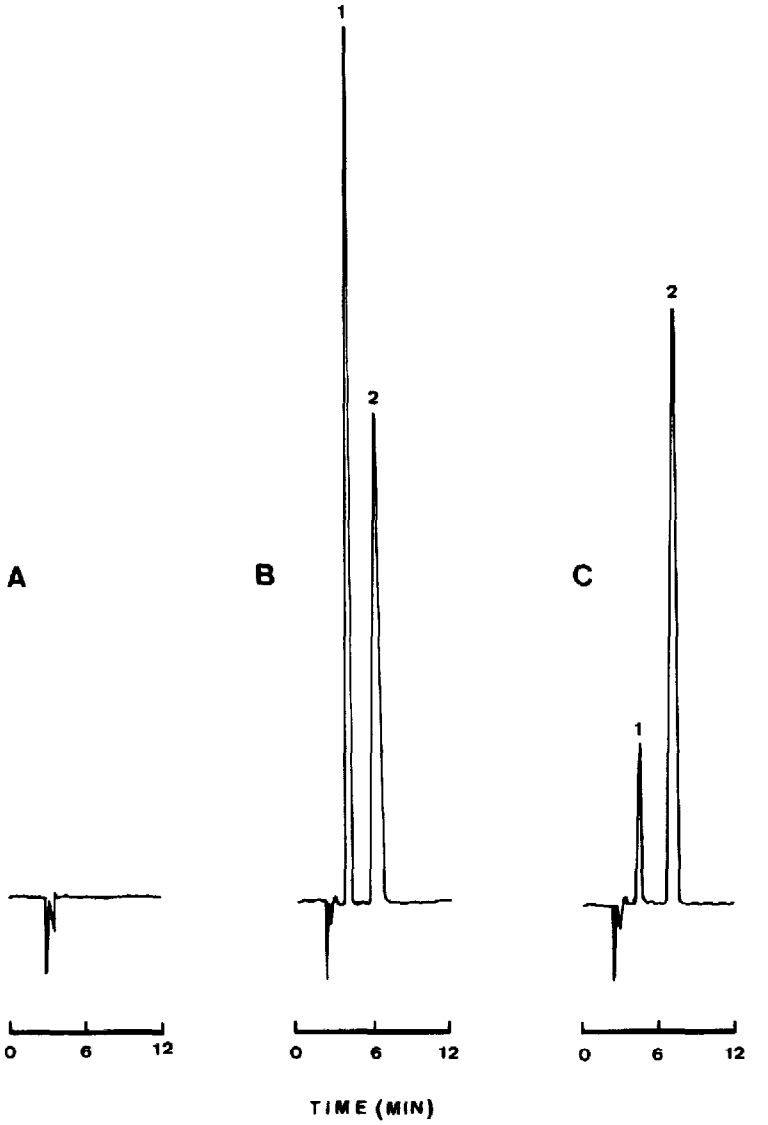



Fig. 3. Chromatograms of 0.1-ml urine extracts. (A) Blank urine; (B) blank urine spiked with 1.0 μg/ml bisoprolol and 1.2 μg/ml internal standard; (C) urine collected from a patient between 5 and 10 h after a 20-mg oral dose of bisoprolol containing 5.4 μg/ml of the drug. Peaks: 1 = internal standard; 2 = bisoprolol.

scribed chromatographic conditions. None was found to interfere with bisoprolol and its internal standard.

An example of the plasma pharmacokinetic profile of bisoprolol obtained after oral administration of 20 mg of bisoprolol to one healthy volunteer is presented in Fig. 4. The assay enabled accurate determination of plasma concentrations of bisoprolol over at least 48 h following oral administration; at 72 h the plasma level was close to the detection limit (i.e. 0.5 ng/ml). The peak plasma concentration was observed 2.0 h after administration, plasma levels of bisoprolol then

TABLE I

WITHIN-DAY PRECISION, ACCURACY AND ABSOLUTE RECOVERY DATA FOR BISOPROLOL IN PLASMA

| Amount added (ng/ml) | Amount found (mean ± S.D., $n=5$) (ng/ml) | C.V. (%) | Accuracy (%) | Absolute recovery (mean ± S.D., $n=4$) (%) |
|---|---|---|---|---|
| 1 | 1.1 ± 0.1 | 7.6 | 110.0 | |
| 2 | 2.1 ± 0.1 | 2.4 | 105.0 | |
| 5 | 5.1 ± 0.1 | 1.6 | 102.0 | 100.9 ± 1.4 |
| 20 | 19.8 ± 0.2 | 0.8 | 99.0 | 98.9 ± 4.7 |
| 80 | 79.7 ± 0.3 | 0.4 | 99.6 | 98.9 ± 0.9 |

TABLE II

DAY-TO-DAY PRECISION AND ACCURACY DATA FOR BISOPROLOL IN PLASMA ($n=5$)

| Amount added (ng/ml) | Amount found (mean ± S.D.) (ng/ml) | C.V. (%) | Accuracy (%) |
|---|---|---|---|
| 1 | 1.1 ± 0.1 | 8.1 | 110.0 |
| 2 | 2.0 ± 0.1 | 4.2 | 100.0 |
| 5 | 5.0 ± 0.1 | 1.7 | 100.0 |
| 20 | 19.8 ± 0.2 | 1.2 | 99.0 |
| 80 | 79.7 ± 0.6 | 0.8 | 99.6 |

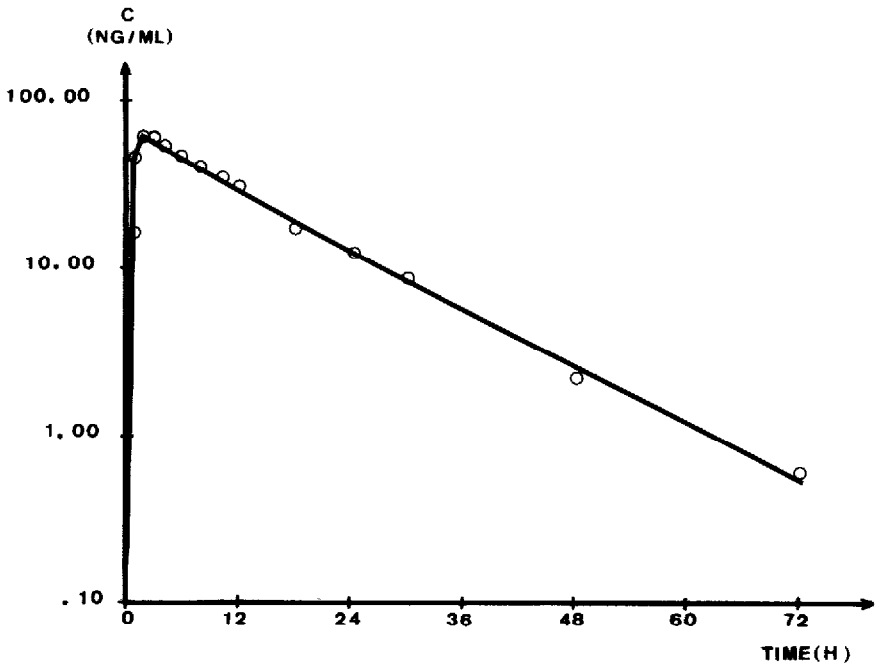



Fig. 4. Plasma bisoprolol concentration–time curve (log scale) obtained from one healthy volunteer after a 20-mg oral dose of bisoprolol.

declining in a monophasic manner. An elimination half-life of 9.5 h was found, in agreement with previously reported data [7,8].

About 50% of the administered dose of bisoprolol was excreted in 72 h as unchanged drug in urine, as reported previously [5,7]. In agreement with the finding of Bühring and Garbe [6], metabolites of bisoprolol were not recovered in urine samples of patients treated with bisoprolol, after extraction.

In conclusion, an HPLC assay for quantitative analysis of bisoprolol in plasma and urine is presented. The reproducibility and the sensitivity of the method allow investigation of pharmacokinetics of bisoprolol. The procedure is also quite simple and rapid in performance.

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