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Note

Rapid and sensitive column liquid chromatographic determination of sotalol in plasma

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Sotalol, (±)-4-[1-hydroxy-2-(isopropylamino)ethyl]methane sulphonanilide hydrochloride, is a pure β-adrenergic blocking agent without intrinsic sympathomimetic activity or local anaesthetic effect [1]. The drug is one of the most hydrophilic β-blockers [2] and is excreted by the kidney as the unchanged product [3].

The original non-specific fluorimetric method for the determination of sotalol levels [4] used a three-step liquid–liquid extraction procedure. Until now, all column liquid chromatographic (LC) assays for the determination of sotalol in biological fluids [5–10] were based on that in the original publication [4] and required a long time for sample preparation. Moreover, chromatographic separations were carried out using either octadecylsilane [5–7,9,10] or phenyl [8] reversed-phase columns. Published data up to now indicated a detection limit of at least 31 nmol/l (10 ng/ml) sotalol [7,8].

The purpose of this paper is to present a simpler, more rapid and sensitive LC method for the determination of sotalol in plasma using a nitrile-bound stationary phase. This assay involves the structurally related compound bisoprolol as an internal standard and a single-step liquid–liquid extraction procedure. A spectrofluorimetric detection limit of 6 nmol/l (2 ng/ml) of sotalol and the possibility of analysing at least 50 samples over a period of 8 h are particularly useful for pharmacokinetic studies of sotalol.

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EXPERIMENTAL

Chemicals

Pure samples of sotalol and bisoprolol (internal standard) (Fig. 1) were kindly supplied by Bristol-Myers (Evansville, IN, U.S.A.) and Lederle Labs. (Rungis, France), respectively. All the solvents were of HPLC grade and were obtained from E. Merck (Paris, France). Bicine [N,N-bis(2-hydroxyethyl)glycine] was purchased from Sigma (Paris, France). The buffer solution was prepared from a saturated solution of bicine in distilled water, filtered and adjusted to pH 9.2 with 10 *M* sodium hydroxide solution.

Chromatography

The liquid chromatographic unit consisted of an M501 solvent-delivery system, a Model U6K manual injector (Millipore-Waters, Saint-Quentin en Yvelines, France) and a Shimadzu RF-535 spectrofluorimeter (Touzart et Matignon, Vitry-sur-Seine, France) operated at an excitation wavelength of 235 nm and an emission wavelength of 310 nm.

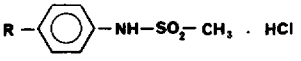
Separations were performed at ambient temperature on a LiChrosorb CN column (25 cm × 4 mm I.D., 10 μm particle size) (E. Merck) equipped with a 1-cm precolumn packed with the same material (SFCC, Neuilly-Plaisance, France). The mobile phase was methanol–2-propanol–1.16 *M* perchloric acid (75:25:0.5, v/v) pumped at a flow-rate of 2.5 ml/min, which produced a back-pressure of 83 bar.

A Servotrace one-channel recorder (Sefram, Paris, France) was used at a chart-speed of 2.5 mm/min and a sensitivity of 10 mV.

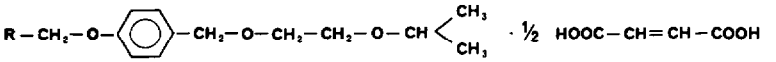
Calibration standards

Stock solutions of sotalol (4 mmol/l) and bisoprolol (0.52 mmol/l) were prepared by dissolving appropriate amounts of the pure samples in methanol.

SOTALOL :



BISOPROLOL (INTERNAL STANDARD) :



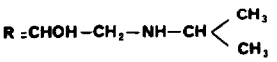


Fig. 1. Structures of sotalol and bisoprolol (internal standard).

They were stable for at least two months without observable degradation when stored at −20°C.

A working solution of sotalol at 40 μmol/l was prepared by a 1:100 dilution of the stock solution with distilled water. Plasma standards were made by appropriate dilutions of the working solution with drug-free human plasma in order to give concentrations of sotalol ranging from 16 to 4000 nmol/l (5–1280 ng/ml).

The working solution of bisoprolol was prepared by a 1:100 dilution of the stock solution with distilled water to provide a concentration of 5.22 μmol/l.

Sample preparation

To 1 ml of plasma sample in a 10-ml glass tube were added 0.1 ml of internal standard solution (200 ng), 0.2 ml of bicine (pH 9.2) and 4 ml of ethyl acetate. The tube was shaken vigorously for 5 min and centrifuged for 5 min at 2000 *g*. The upper phase was transferred into a conical glass tube and evaporated to dryness at 60°C under a stream of nitrogen. The residue was dissolved in 200 μl of methanol and an aliquot ($\leqslant$100 μl) was introduced into the LC unit.

RESULTS AND DISCUSSION

Typical chromatograms of plasma extracts are shown in Fig. 2. Assays performed on drug-free plasma samples show the absence of any endogenous interfering peaks (Fig. 2A). The retention times of the internal standard (bisoprolol) and sotalol were 3.6 and 4.4 min, respectively. Representative chromatograms of extracts of a blank plasma sample spiked with 250 nmol/l sotalol and 522 nmol/l internal standard, and of a plasma sample obtained from a patient 0.5 h after a 1.2 mg/kg intravenous dose of sotalol containing 2032 nmol/l drug, are presented in Fig. 2B and C, respectively. A least-squares linear regression was used to calculate the equation relating the peak-height ratio between the drug and the internal standard and the concentration of sotalol. The calibration graphs were linear ($r^2 \geqslant 0.999$) in the range 0–12 500 nmol/l sotalol in plasma. The daily fluctuation of the plasma calibration graphs ($n=5$) was slight, with a coefficient of variation (C.V.) of 2.8% and an intercept of 0.3 ± 0.6 nmol/l.

Within-day and day-to-day precision and accuracy data for plasma analysis were evaluated over the concentration range 16–4000 nmol/l sotalol. At the plasma level corresponding to the quantification limit (16 nmol/l) the C.V.s ($n=5$) were 7.3 and 8.0% for repeatability and reproducibility studies, respectively. The overall accuracy was 101 ± 5 and 100 ± 1% for within-day and day-to-day studies, respectively. The detection limit based on a signal-to-noise ratio of 2:1 was 6 nmol/l (2 ng/ml).

Average absolute recoveries of 48 and 78% were found for sotalol and the internal standard, respectively.

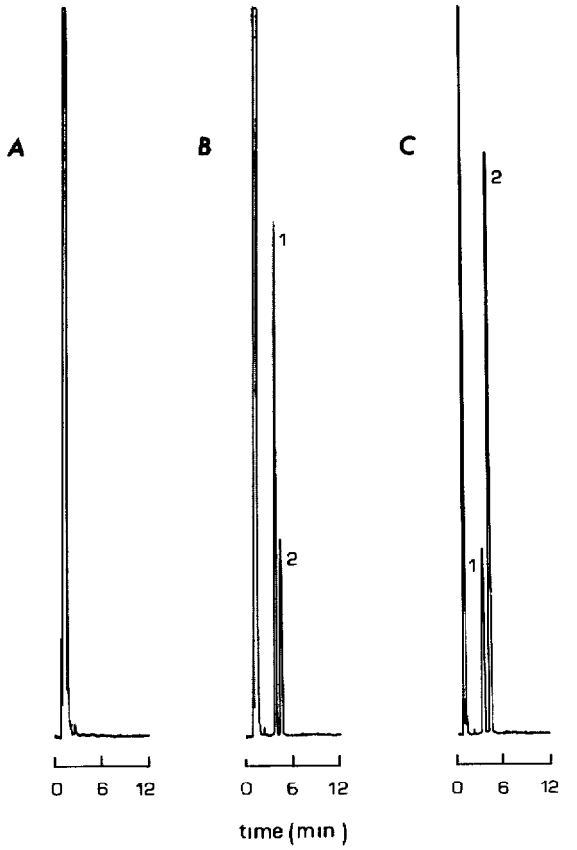


Fig. 2. Chromatograms of 1-ml plasma extracts. (A) Drug-free plasma; (B) drug-free plasma spiked with 250 nmol/l sotalol and 522 nmol/l internal standard; (C) sample obtained from a patient 0.5 h after a 1.2 mg/kg intravenous dose of sotalol containing 2032 nmol/l drug. Peaks: 1 = internal standard; 2 = sotalol.

In cases of therapeutic monitoring of sotalol for which plasma concentrations in the range 1.2–4.7 μg/ml achieved the desired antiarrythmic effect [11], the plasma volume used was lowered to 0.1 ml without altering the extraction yield.

No decrease in the measured concentration of sotalol was found when plasma extracts were kept at 4 °C for four days.

Pure samples of some commonly administered drugs used for the treatment of cardiovascular diseases (acebutolol, amiodarone, disopyramide, propafenone, hydroquinidine, quinidine and verapamil) were assayed under the described chromatographic conditions. Only verapamil could be detected at the wavelengths used for the determination of sotalol but at a retention time of 6.6 min, and did not interfere with sotalol or its internal standard.

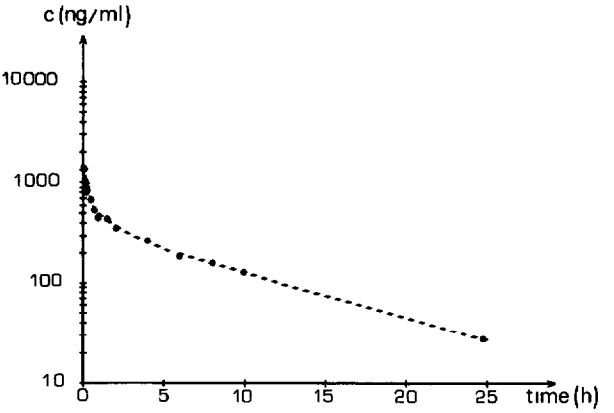


Fig. 3. Plasma sotalol concentration–time curve (log scale) obtained from one patient after a 1.2 mg/kg intravenous administration.

This LC assay has been used successfully in our laboratory and two sotalol pharmacokinetic studies can be performed over a period of 6 h, a calibration graph being constructed daily. An example of the plasma pharmacokinetic profile of sotalol obtained after a 1.2 mg/kg intravenous administration to one patient is presented in Fig. 3. The plasma levels declined in a biphasic manner and an elimination half-life of 6.8 h was found, in agreement with previously reported data after intravenous administration [3,12].

In conclusion, the proposed LC method for the determination of sotalol in plasma is simple and not time-consuming in comparison with other published assays. The reproducibility and sensitivity allow pharmacokinetic studies of sotalol.

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