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Short Communication

Simplified procedure for the determination of sotalol in plasma by high-performance liquid chromatography

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ABSTRACT

A simple, specific and rapid reversed-phase high-performance liquid chromatographic (HPLC) procedure for sotalol determination is described requiring small plasma volumes. The high recovery of sotalol from plasma and the high precision of measurement obviate the need for an internal standard. Plasma samples (300 μl) were deproteinised with 50 μl of 70% (w/w) perchloric acid in disposable glass tubes. After vortex-mixing and centrifugation, 30 μl of 4 *M* K_2HPO_4 were added followed by gentle shaking. A 20-μl aliquot was then injected (by autosampler) for HPLC analysis. Chromatography was performed on a glass-lined 250 mm × 4 mm 5-μm C_{18} steel column. The mobile phase was 6% (v/v) acetonitrile in 0.08 *M* KH_2PO_4 buffer (pH 4.6). The flow-rate was 0.8 ml/min. Detection was by fluorescence with excitation and emission wavelengths at 235 and 310 nm, respectively. The retention time for sotalol was 7.1 min. Calibration was linear from 0.16 to 10 μg/ml in plasma ($r > 0.999$ for detector response to sotalol). The minimum concentration for quantitation was 0.08 μg/ml [within assay coefficient of variation (C.V.) <5%]. Recovery was near quantitative (>98%) and replicate (intra-assay precision was <5% C.V.). Analysis of samples ($n = 10$) at concentrations of 0.42 and 4.2 μg/ml gave mean values of 0.44 and 4.3 μg/ml, respectively. The inter-assay C.V. values were 4.5 and 2.2%, respectively. Other clinically used antiarrhythmic drugs did not interfere. This assay can be performed using other commercial C_{18} analytical columns by suitable adjustment of mobile phase flow-rate and acetonitrile composition.

INTRODUCTION

Sotalol, *dl*-4-(2-isopropylamino-1-hydroxyethyl)methanesulphonanalide, therapeutically used as the monohydrochloride salt, is a non-selective β-adrenergic blocker without intrinsic sympathomimetic or membrane-stabilizing activity. Its actions resemble those of class III antiarrhythmic agents [1] and it is primarily indicated for prevention and treatment of supraventricular and ventricular arrhythmias. In some cases sotalol has also been used clinically because of its antihypertensive effect. The measurement of plasma or serum sotalol concentrations is necessary for pharmacokinetic studies of this drug and more routinely for establishing a relationship between blood concentration and therapeutic effect

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[2–4] or toxic effect [5]. The *d* and *l*-isomers are equipotent in the action of sotalol [6] obviating the need for a stereoselective assay.

The original fluorimetric non-chromatographic method for the determination of sotalol by Garrett and Schnelle [7] has been used extensively. Subsequent modifications utilising liquid chromatography [8–14] have continued to employ identical or near identical sample extraction procedures as used by Garrett and Schnelle [7]. All require relatively large plasma volumes (1 ml or greater) with or without protein precipitation and include extraction with organic solvent mixtures following appropriate pH adjustments. This is followed by back-extraction into aqueous acid. With some procedures the sotalol recovery is low [10] although consistent [13]. Two procedures [15,16] utilise solid-phase extraction with subsequent evaporation of relatively large volumes of extracting solvent. All methods developed use ion-pairing reagents and other mobile phase modifiers such as alkylamines, and most use an internal standard which is a β-blocker such as atenolol [9,12,13] or bisoprolol [14] or an antiarrhythmic such as procainamide [8]. This has disadvantages since these compounds are used therapeutically for indications similar to sotalol, concomitant therapy is possible and, hence, potential assay interference in the patient samples may arise.

This communication describes a simplified procedure for the determination of sotalol by high-performance liquid chromatography (HPLC); the procedure is rapid, reliable, utilizes a relatively small plasma volume (0.3 ml) and is adaptable to a variety of commercially available reversed-phase (C_{18}) columns. It has the advantages that no organic solvents are used for sample extraction, and neither ion-pairing reagents for the chromatography nor an internal standard is necessary. The precision of the quantitation using an external standard, by monitoring the fluorescence output of sotalol alone, is adequate and the sensitivity for therapeutic drug monitoring is sufficient.

EXPERIMENTAL

Materials

Sotalol hydrochloride was supplied by Astra Pharmaceuticals (North Ryde, Australia). Acetonitrile was HPLC-grade reagent (Mallinckrodt Australia, Meadowbank, Australia). Water was all-glass double-distilled and pre-filtered through a 0.45-μm Nylon filter. Potassium dihydrogenphosphate, dipotassium hydrogenphosphate and perchloric acid (70%, w/w) were analytical-grade reagents (Ajax Chemicals, Sydney, Australia).

Apparatus and chromatographic conditions

HPLC analyses were performed on a 5-μm C_{18} glass-lined stainless-steel column (250 mm × 4 mm I.D.) (Scientific Glass Engineering, Ringwood, Australia) in line with a 0.5-μm Uptight pre-column guard frit (Upchurch Scientific, Oak Harbour, WA, U.S.A.). The pump was a Millipore-Waters Model 510 (Milli-

pore-Waters, Lane Cove, Australia) and fluorescence detection was performed with an excitation wavelength of 235 nm and an emission wavelength of 310 nm (Hitachi Model F1000 variable-wavelength fluorescence spectrophotometer, Hitachi, Tokyo, Japan). The detector output was recorded either on a strip chart pen recorder (manual peak-height determination) set at 10 mV full scale detection (Servogor 120, Goerz Metrawatt, Vienna, Austria) or by an integrator printer–plotter for peak-area determination (Hewlett Packard Model 3390A integrator, Hewlett Packard, North Ryde, Australia). Sample injection was done by a Kortec K65 automatic sampler with a 20-μl sample loop (ICI Instruments, Melbourne, Australia). The mobile phase, 60 ml of acetonitrile and 940 ml of 80 mmol/l potassium dihydrogenphosphate in water (pH 4.6), was prefiltered and degassed by ultrasound sonication under reduced pressure. The chromatography was isocratic with a mobile phase flow-rate of 0.8 ml/min and was performed at ambient temperature.

Sample preparation

All blood samples were collected in heparinized tubes and, after centrifugation, the plasma was removed. A 300-μl volume of each plasma sample was added to 100 × 13 mm disposable borosilicate glass tubes (Kimble-Owens, Illinois, Toledo, OH, U.S.A.) followed by 50 μl of 70% (w/w) perchloric acid–water (1:3, v/v). Each sample was vortex-mixed for approximately 20 s, stored on ice for 5–10 min and then vortex-mixed a second time. The samples were then centrifuged at 3500 *g* for 5 min. Following centrifugation, 30 μl of 4 *M* aqueous dipotassium hydrogenphosphate were added to each tube after which the samples were gently shaken by hand (the protein precipitate was not disturbed). The tubes were allowed to stand and the potassium perchlorate precipitate was rapidly settled, or, alternatively, the tubes were re-centrifuged at 3500 *g* for 2 min. An adequate volume (approximately 100 μl) of clear supernatant was then transferred into the autosampler vials and 20 μl were injected onto the column.

Calibration and quality control samples

For the preparation of a stock solution of sotalol hydrochloride, 10 mg were dissolved in 10 ml of aqueous 80 mmol/l potassium dihydrogenphosphate–acetonitrile (2:1). This solution was stored at 4°C and was stable for at least three months. To prepare plasma calibration standards, 200 μl of the stock solution were added to drug-free plasma to make a final volume of 20 ml. This was equivalent to 10 μg/ml sotalol hydrochloride (8.8. μg/ml sotalol free base or 32.4 μmol/l). This plasma stock solution could be stored at 4°C for one month. Serial dilution (1:1) of this solution with drug-free plasma to give concentrations from 5.0 to 0.16 μg/ml sotalol hydrochloride (equivalent range of 4.41 to 0.14 μg/ml sotalol free base) was carried out and calibration of the assay was between these values.

Quantitation was done by measurement of the absolute peak-height or peak-

area responses of sotalol. Quality control samples were prepared by dissolving 10.5 mg of sotalol hydrochloride in 10 ml of water–acetonitrile (2:1). A 200-μl volume of this solution was made to 50 ml with drug-free plasma (4.2 μg/ml sotalol hydrochloride). A 2.0-ml volume of this solution was further diluted to 20 ml with plasma to give 0.42 μg/ml. Approximately 1-ml aliquots of each sample were stored frozen at –18°C and were used as internal quality control samples with each asay.

RESULTS AND DISCUSSION

Sotalol is one of the more polar compounds amongst the β-blocking drugs, the high polarity being reflected in the relatively low proportion of organic solvent (acetonitrile) in the mobile phase required to elute the drug from the C_{18} bonded phase. Fig. 1 shows representative chromatograms from plasma extracts. Under the conditions described sotalol was eluted at 7.1 min and the total run time for each analysis was 8 min. Compared with previous procedures, a mobile phase containing phosphate buffer (80 m*M* KH_2PO_4) at pH 4.4–4.6 gave a good peak shape for sotalol. In all plasma extracts an endogenous compound was eluted before sotalol but this compound did not interfere.

The extraction procedure was relatively simple to perform, the prime requirement being that of a good pipetting precision for each step. No internal standard was necessary. The extraction recovery for sotalol was near quantitative (>98%). The detector response was linear to at least 10 μg/ml sotalol hydrochloride. For the reproducibility of the calibration the following data were found.

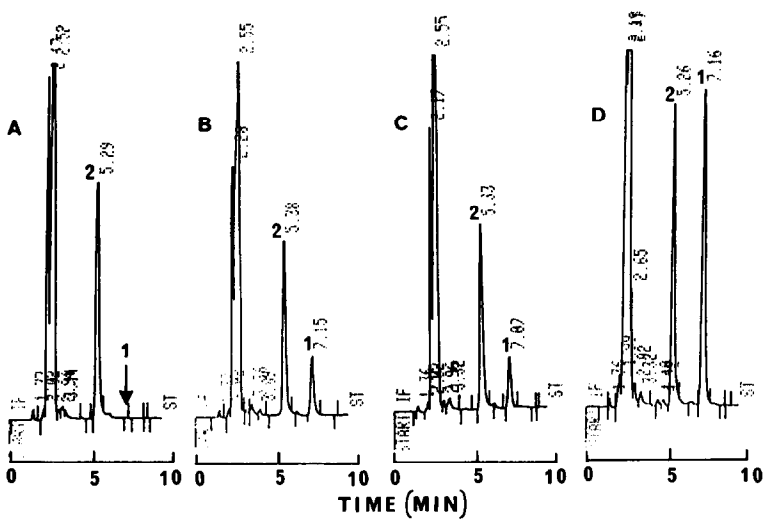

Fig. 1. Chromatograms showing (A) drug-free plasma, (B) standard plasma containing 0.625 μg/ml sotalol hychochloride, (C) 4-h post-dose sample from a volunteer (see Fig. 2) (calculated concentration 0.58 μg/ml) and (D) plasma sample from patient on sotalol therapy (calculated concentration 3.2 μg/ml). Peaks: 1 = sotalol; 2 = endogenous compound appearing in all chromatograms of plasma extracts.

Five separate calibrations from 0.16 to 10 μg/ml (total of 80 data points) performed over a period of seven days monitoring peak area (10^6–10^8 arbitrary units) gave a mean (±S.D.) slope^{-1} of 6.6494 ± 0.4537 · 10^4 [coefficient of variation (C.V.) = 6.8%] and a mean (±S.D.) intercept of 0.0463 ± 0.0318. The correlation coefficient (r) for each calibration was 0.999 or greater. The minimum concentration for quantitation was half the lowest standard concentration (0.08 μg/ml). The within-assay precision (C.V.) at this concentration was less than 5%. Depending on lamp intensity and type of detector used there is sufficient signal output to enable sotalol quantitation at even lower plasma concentrations.

The use of the quality control samples for assay reproducibility and precision gave the following results. Intra-assay: samples (n = 10) prepared at target concentrations of 0.42 and 4.2 μg/ml gave mean observed concentrations of 0.40 μg/ml (C.V. = 4.9%) and 4.10 μg/ml (C.V. = 4.10%), respectively. Inter-assay: duplicate assays performed with the five separate calibrations (see above) gave observed concentrations of 0.44 μg/ml (C.V. = 4.5%) and 4.3 μg/ml (C.V. = 2.2%).

To determine the stability of sotalol in the plasma extracts whilst awaiting analysis, extracts from three plasma concentrations (0.8, 1.6 and 3.2 μg/ml) were prepared and allowed to sit in the autosampler vials. The samples were injected onto the chromatograph at intervals to 24 h. The mean (±S.D.) sotalol response measured as peak height was for 0.8 μg/ml 21.3 ± 1.14 mm (n = 12), for 1.6 μg/ml 44.3 ± 1.16 mm (n = 17) and for 3.2 μg/ml 89.1 ± 2.17 mm (n = 17). The S.D. of each mean (C.V. = 5.4, 2.6 and 2.4%, respectively) indicated that there was no significant change in peak heights in each sample extract over 24 h; this should allow multi-sample assays to be performed, *e.g.* in pharmacokinetic stud-

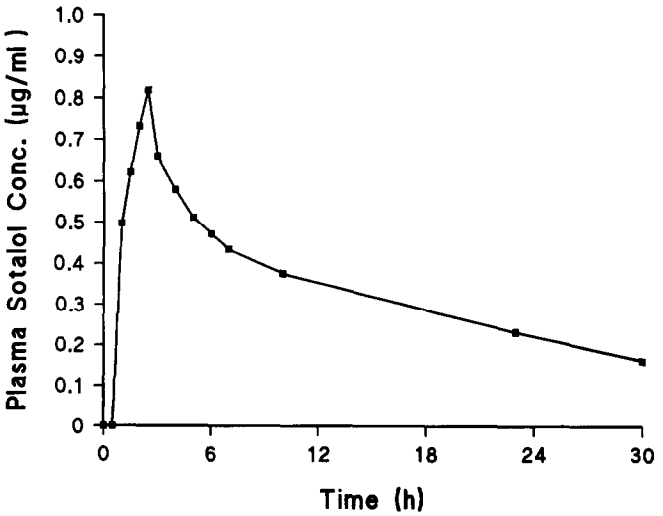

Fig. 2. Plasma sotalol concentration *versus* time profile in a single volunteer following oral ingestion of 160 mg of sotalol hydrochloride.

ies. The plasma sotalol concentration *versus* time profile in a single subject following oral administration of a 160-mg tablet (Sotacor, Astra Pharmaceuticals) is shown in Fig. 2.

There was no interference in the chromatography from the following antiarrhythmic drugs: propranolol, metoprolol, quinidine, flecainide, disopyramide, procainamide and N-acetylprocainamide. These compounds did not give a chromatographic response up to 30 min run time. Atenolol (eluted at 5.5 min) gave a response but did not interfere.

The assay described is simple to perform, rapid and reproducible. We have been utilizing this procedure for therapeutic monitoring of sotalol for some time (therapeutic range 1–3 μg/ml) but we have also used other C_{18} bonded analytical columns than described. The following columns were tested: 4-μm Nova-Pak (150 mm × 3.9 mm I.D.) (Millipore-Waters); 5-μm ExSil C_{18} Goldpak (150 mm and 250 mm × 4.6 mm I.D.) (Activon Scientific Products, Thornleigh, Australia); and 10-μm Parisil ODS-3 (250 mm × 4.6 mm I.D.) (Whatman, Clifton, NJ, U.S.A.). By adjustment of the acetonitrile composition (6–13%, v/v) in the mobile phase and the flow-rate (0.8–1.2 ml/min) essentially the same results in terms of chromatography, linearity, reproducibility and sensitivity were obtained.

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