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High-performance liquid chromatographic determination of sotalol in plasma

I. Application to the disposition of sotalol enantiomers in humans

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ABSTRACT

Two high-performance liquid chromatographic analytical methods have been developed for the measurement of *dl*-sotalol or *d*-sotalol and *l*-sotalol in plasma, using *dl*-atenolol as internal standard. Quantitation of *dl*-sotalol was carried out, following solid-phase extraction, on a 5-μm C_{18} reversed-phase column, with a mobile phase containing acetonitrile, ion-pairing reagent and distilled water, using ultraviolet detection at 235 nm. Quantitation of *d*-sotalol and *l*-sotalol was based on derivatisation with the chiral agent *S*-(−)-α-methylbenzyl isocyanate, followed by chromatographic separation on a 3-μm C_{18} reversed-phase column, with a mobile phase containing methanol, glacial acetic acid and distilled water, with fluorimetric detection at 220 nm excitation and 300 nm emission. A preliminary application of the latter method suggests that the disposition of sotalol in humans is not enantioselective.

INTRODUCTION

Like all β-adrenoreceptor antagonists, sotalol contains a chiral centre, thus existing as the two optical isomers *d*-sotalol and *l*-sotalol, which are currently administered clinically as a racemic mixture. Racemic *dl*-sotalol is a non-selective β-antagonist with no intrinsic sympathomimetic or membrane-stabilising properties, however, it differs from other β-antagonists in that it is also a class III anti-arrhythmic agent, prolonging the duration of the cardiac action potential and increasing ventricular repolarization time [1,2]. Both *d*- and *l*-sotalol have similar class III anti-arrhythmic activities, but *d*-sotalol has been shown to be largely devoid of the β-blocking activity [1,3].

The effects of *dl*-sotalol on conduction intervals and arrhythmogenesis have been shown to correlate with plasma concentrations of *dl*-sotalol with a postulated therapeutic range between 1.2 and 4.7 mg/l, whilst clinical toxicity is generally associated with plasma concentrations greater than 5 mg/l [4–6]. Thus therapeutic drug monitoring may assist in optimising the clinical treatment of patients with *dl*-sotalol. In the future it

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may also be possible to further enhance the clinical effectiveness of sotalol by dissociating its β-antagonist and class III anti-arrhythmic activities using an enantiomerically pure *d*-sotalol dose form [7–9].

Enantioselectivity, not only in pharmacological activity but also in the pharmacokinetics of *d*- and *l*-sotalol, is therefore a relevant factor in both the therapeutic drug monitoring of *dl*-sotalol as well as research applications which may lead to the clinical development of *d*-sotalol as a therapeutic agent. This paper describes two analytical methods using reversed-phase high-performance liquid chromatography (HPLC): (i) for the routine measurement of *dl*-sotalol in plasma; and (ii) for the enantiospecific measurement of *d*-sotalol and *l*-sotalol in plasma, including a preliminary application to examine the pharmacokinetics of *d*- and *l*-sotalol in humans.

EXPERIMENTAL

Reagents

dl-Sotalol, *d*-sotalol and *l*-sotalol were kindly provided by Bristol-Myers (Evansville, IN, USA). *S*-(–)-α-Methylbenzyl isocyanate was purchased from Aldrich (Milwaukee, WI, USA). Solid-phase extraction columns (Extra Sep C_{18}, 200 mg, 3.0 ml) were manufactured by Lida Manufacturing (Kenosha, WI, USA). Heptanesulphonic acid (Sigma, St. Louis, MO, USA) and anhydrous sodium sulphate (May and Baker, Dagenham, UK) were of analytical grade. All other reagents were of analytical grade and aqueous solutions were prepared in glass-distilled water. Ion-pairing solution was prepared by adding 5.0 g of heptanesulphonic acid to 70 ml of distilled water followed by 50 ml of glacial acetic acid. Derivatising solution was prepared fresh each day and consisted of 0.2% (v/v) *S*-(–)-α-methylbenzyl isocyanate in cloroform, which had previously been dried with anhydrous Na_2SO_4.

Measurement of dl-sotalol in plasma

Stock solutions of *dl*-sotalol (100 and 10 mg/l) and *dl*-atenolol (50 mg/l, internal standard) were prepared in distilled water. Sotalol calibration standards spanning a concentration range of 0.1–5.0 mg/l were prepared by making appropriate dilutions of the stock solutions in drug-free plasma. A separate stock solution of *dl*-sotalol (200 mg/l) in distilled water was diluted 1:100 in drug-free plasma to prepare a quality control standard (2.0 mg/l) that would be used to monitor inter-assay accuracy and reproducibility.

Solid-phase extraction cartridges were prepared by pre-washing with 3.0 ml of methanol, followed by 3.0 ml of mobile phase (see below) and 3.0 ml of distilled water under vacuum (Vac-Elut, Analytichem International, Harbor City, CA, USA). To a 5-ml disposable borosilicate glass culture tube were added 1 ml of plasma sample or calibration standard and 50 μl of internal standard solution. Samples were briefly vortex-mixed and loaded onto the pre-washed solid-phase extraction columns using vacuum displacement. After complete passage of the plasma mixture, the columns were washed with 500 μl of distilled water to remove any residual plasma. Sotalol and atenolol were then eluted with 1.0 ml of mobile phase into clean 5-ml disposable glass culture tubes. This eluate was vortex-mixed and 70 μl were injected directly onto the HPLC column.

Chromatographic separation was carried out on a C_{18} column (5 μm, 220 mm × 4.6 mm I.D., Brownlee Labs., Santa Clara, CA, USA, Part No. OD-224) with guard column (5 μm, 30 mm × 4.6 mm I.D., Brownlee Labs., Part No. OD-GU) maintained at a temperature of 40°C with a mobile phase consisting of acetonitrile, ion-pairing solution and distilled water (38.0:0.5:61.5, v/v/v), pumped (Millipore Waters Model 510) at a flow-rate of 1.0 ml/min. Detection was carried out using a variable-wavelength UV detector (Jasco Uvidec-100-V, Japan Spectroscopic, Tokyo, Japan) at 235 nm, at a sensitivity setting of 0.02 a.u.f.s., with a dual-pen chart recorder set at 10 and 50 mV.

Quantitation of *dl*-sotalol concentrations in unknown samples was based on a calibration curve constructed, for each analytical run, by plotting the peak-height ratio (PHR) of *dl*-sotalol to internal standard against the spiked concen-

tration of the calibration standard. Each calibration standard was then used as an estimate of the slope of the line of best fit through the origin by dividing PHR by the spiked concentration to obtain a normalised PHR, and a mean normalised PHR was calculated. The concentration of *dl*-sotalol in unknown samples was calculated as the PHR divided by the calibration mean normalised PHR. Calibration curves were accepted only if the coefficient of variation (C.V.) for the mean normalised PHR was less than 10% and if the quality control sample was within ± 10% of the spiked value.

Intra-assay reproducibility was determined by assaying five replicates of three standards at 0.1, 0.5 and 5.0 mg/l *dl*-sotalol. Inter-assay reproducibility was assessed over six analytical runs by comparing the mean normalised PHRs for the six calibration curves, and the concentrations calculated for the quality control sample.

Measurement of d-sotalol and l-sotalol in plasma

Stock solutions of *dl*-sotalol (100, 10 and 1 mg/l) and *dl*-atenolol (10 mg/l, internal standard) were prepared in distilled water. Sotalol calibration standards spanning a concentration range of 0.025–2.5 mg/l *d*-sotalol and *l*-sotalol were prepared by making appropriate dilutions of the stock solutions in drug-free plasma.

To a 15-ml screw-capped disposable borosilicate glass culture tube were added 1.0 ml of plasma sample or calibration standard, 50 μl of internal standard solution and 2.0 ml of 1.0 *M* borate buffer (pH 9.0). Samples were vortex-mixed briefly and 5.0 ml of dichloromethane–2-propanol (3:1, v/v) added [10]. The tubes were capped (PTFE-lined) and mixed gently on a horizontal shaker (80 oscillations/min) for 20 min followed by centrifugation at 1000 *g* for 20 min. The aqueous layer was discarded, and approximately 1.0 g of anhydrous Na_2SO_4 was added to each tube to remove any residual water. Samples were recentrifuged at 1000 *g* for 5 min, and the organic layer was transferred into a clean 5-ml disposable glass culture tube and dried under a stream of nitrogen at 40°C. To each tube, 200 μl of derivatising solution were added. Samples were vortex-mixed briefly, capped and allowed to stand overnight at 4°C. The chloroform was evaporated to dryness under a stream of nitrogen at room temperature, and the samples were reconstituted in 200 μl of mobile phase (see below). These tubes were centrifuged to separate any particulate matter prior to injection onto the HPLC column.

Chromatographic separation of the derivatives of *d*-sotalol, *l*-sotalol, *d*-atenolol and *l*-atenolol was carried out using a C_{18} column (Velosep, 3-μm, 100 mm × 3.2 mm I.D., Brownlee Labs., Part No. V18–103) at a temperature of 40°C with a mobile phase of methanol–glacial acetic acid–distilled water (40.0:0.5:59.5, v/v/v) at a flow-rate of 0.4 ml/min. Detection was carried out using a fluorescence detector (Perkin Elmer, Model LS40) at an excitation wavelength of 220 nm and an emission wavelength of 300 nm, a sensitivity factor of 256 and chart recorder settings of 50 and 100 mV.

Quantitation of *d*-sotalol and *l*-sotalol in unknown samples was based on the PHR of *d*-sotalol and *l*-sotalol to internal standard (routinely the *d*-atenolol peak) using calibration curves to calculate mean normalised PHR as described above. Calibration curves were only accepted if the C.V. for the mean normalised PHR was less than 10% and if the quality control sample was within ± 10% of the spiked value.

The derivatisation efficiency was determined by comparing the peak heights of underivatised *dl*-sotalol and *dl*-atenolol recovered from a 5.0 mg/l distilled water standard "derivatised" using 200 μl of derivatising solution or 200 μl of chloroform.

Intra-assay reproducibility was assessed using five replicates of three standards at 0.025, 0.1 and 2.5 mg/l *d*-sotalol and *l*-sotalol. Inter-assay reproducibility was assessed over four analytical runs by comparing the mean normalised PHRs, and the concentrations calculated for the quality control sample. Standards containing pure *d*-sotalol or *l*-sotalol were also analysed to positively identify the elution order of the two sotalol enantiomers, and a calibration standard (0.25 mg/l) containing excess quantities of one enantiomer was also analysed to ensure that cali-

bration curves remained linear over a range of enantiomeric ratios.

Pharmacokinetics of d-sotalol and l-sotalol in patients

The pharmacokinetics of *d*-sotalol and *l*-sotalol were examined over a dosing interval in two patients admitted to the cardiology ward of The Queen Elizabeth Hospital. Patient A.H. was a 54-year old male who had been taking 80 mg twice daily (b.d.) *dl*-sotalol (Sotacor, Astra Pharmaceuticals) for two days prior to the study. Patient D.W. was a 66-year-old male who had been taking 80 mg b.d. *dl*-sotalol for several years and whose dose had been increased to 80 mg three times daily (t.d.s.) on the day before the study.

A total of eighteen trough samples drawn from a further ten patients receiving *dl*-sotalol were also analysed to further consider if there was any enantioselectivity in sotalol pharmacokinetics.

All samples were analysed using both HPLC methods desribed above.

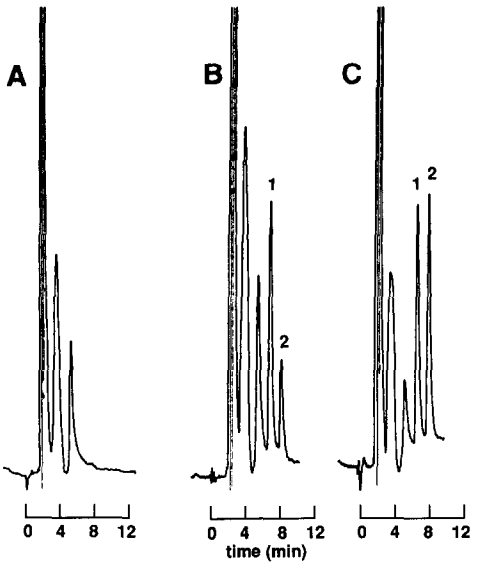

Fig. 1. Chromatogram showing *dl*-atenolol (1) and *dl*-sotalol (2) in (A) blank plasma, (B) a 0.5 mg/l plasma standard and (C) a plasma sample from a patient taking *dl*-sotalol.

RESULTS

Measurement of dl-sotalol in plasma

Using solid-phase extraction the mean (±S.D., $n = 6$) elution recoveries of *dl*-sotalol (5 μg) and *dl*-atenolol (2.5 μg) were 75.6 ± 4.1 and 71.4 ± 2.8%, respectively. The extraction recovery of sotalol remained constant over the concentration range 0.1–5.0 mg/l. Example chromatograms are shown in Fig. 1. Using the chromatographic conditions described, the retention times of *dl*-atenolol and *dl*-sotalol were 7.1 and 8.5 min, respectively. There was no chromatographic interference in samples of drug-free plasma.

Calibration curves for *dl*-sotalol, from analyses of inter- and intra-assay reproducibility and patient specimens, were linear over the concentration range 0.1–5.0 mg/l [r^2 (mean ± S.D.) = 1.000 ± 0.0003, $n = 9$]. Intra-assay reproducibility, as measured by PHR C.V.s at 0.1, 0.5 and 5.0 mg/l *dl*-sotalol were 1.2, 1.2 and 2.4%, respectively ($n = 5$ for each concentration). The mean percentage bias in the calculated concentration of the three standards were −0.8, +2.3 and −0.6%, respectively. The intra-assay C.V.s for each calibration mean normalised PHR ranged from 1.0 to 3.9%. Inter-assay reproducibility was determined using the individual mean normalised PHRs of six assays, which had a C.V. of 1.5% and mean slope of 0.800. The mean calculated concentration of the quality control standard (2.0 mg/l) over six assays was 2.04 mg/l with a C.V. of 2.1%.

Measurement of d-sotalol and l-sotalol in plasma

Example chromatograms are shown in Fig. 2. Under the chromatographic conditions described, the retention times of *d*-sotalol, *l*-sotalol, *d*-atenolol and *l*-atenolol derivatives were 21.2, 23.5, 25.1 and 29.4 min, respectively. There was no chromatographic interference in samples of drug-free plasma, however, there was a late-eluting peak at approximately 35–40 min (Fig. 2). Underivatised *dl*-sotalol and *dl*-atenolol had retention times of 7.1 and 8.0 min, respectively. By comparing the amount of underivatised *dl*-sotalol and *dl*-atenolol remaining in a 5.0 mg/l standard following derivatisation in the presence or absence of *S*-(−)-α-methylbenzyl isocyanate, it

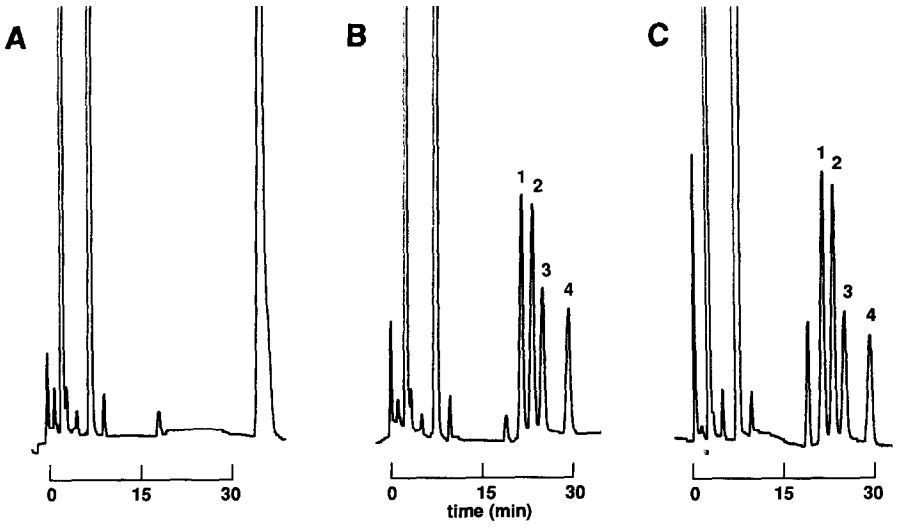

Fig. 2. Chromatogram showing the methylbenzyl isocyanate derivatives of *d*-sotalol (1), *l*-sotalol (2), *d*-atenolol (3) and *l*-atenolol (4) in (A) blank plasma, (B) a 0.5 mg/l plasma standard and (C) a plasma sample from a patient taking *dl*-sotalol.

was calculated that >95% of extracted *dl*-sotalol and *dl*-atenolol reacted to form methylbenzyl isocyanate derivatives.

Calibration curves for *d*-sotalol and *l*-sotalol, from analyses of intra-assay reproducibility and patient specimens, were linear over the concentration range 0.025–2.5 mg/l (*d*-sotalol: r^2 (mean ± S.D.) = 0.997 ± 0.002, n = 4; *l*-sotalol: r^2 = 0.997 ± 0.001, n = 4). Intra-assay reproducibility as measured by PHR C.V.s at 0.025, 0.1 and 2.5 mg/l were 2.4, 2.2 and 2.9% for *d*-sotalol and 5.0, 5.3 and 2.2% for *l*-sotalol, respectively (n = 5 for each concentration). The mean percentage bias in the calculated concentrations of the same three standards were −1.6, −7.4 and −4.8% for *d*-sotalol and −3.2, −5.4 and 6.8% for *l*-sotalol. The intra-assay C.V.s for each calibration mean normalised PHR ranged from 6.3 to 8.6% for *d*-sotalol and 4.3 to 7.7% for *l*-sotalol.

A sample containing 0.25 mg/l of either *d*- or *l*-sotalol in the presence of excess quantities of its antipode gave mean concentrations of 0.240 and 0.233 mg/l for *d*- and *l*-sotalol respectively, with corresponding C.V.s of 4.2 and 9.1%, respectively (n = 3).

Inter-assay reproducibility was determined us-

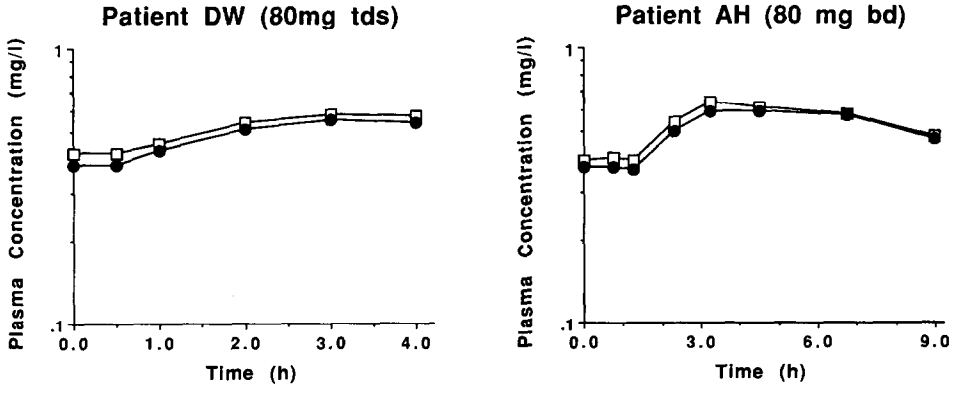

Fig. 3. Plasma concentration *versus* time profiles of *d*-sotalol (□) and *l*-sotalol (●) in two patients following oral administration of 80 mg *dl*-sotalol.

ing the individual mean normalised PHRs of four assays, which had C.V.s of 9.0 and 7.2% and mean slopes of 3.571 and 3.372 for *d*- and *l*-sotalol, respectively. The mean calculated concentrations of the quality control standard (1.0 mg/l of each enantiomer) over four assays were 1.02 and 1.02 mg/l for *d*- and *l*-sotalol, with C.V.s of 10.8 and 10.8% for *d*- and *l*-sotalol, respectively.

Pharmacokinetics of d-sotalol and l-sotalol in patients

In a pilot study, *d*- and *l*-sotalol did not appear to show any enantioselectivity in either the plasma concentration *versus* time profiles of two patients receiving 80 mg *dl*-sotalol orally (Fig. 3) or in eighteen trough plasma samples obtained from ten patients for which the mean (± S.D.) ratio of *d*/*l* sotalol was 0.98 ± 0.04 ($n = 18$).

For all samples, the sum of the two sotalol enantiomer concentrations was not significantly different from the *dl*-sotalol concentration obtained using the non-enantiospecific assay, varying by less than 10%.

DISCUSSION

Several methods are available for the quantitation of *dl*-sotalol in plasma [10–14]. However, all these methods employ a time-consuming liquid–liquid extraction step followed either by drying of the organic solvent or a back-extraction into an acidic aqueous phase before injection onto the HPLC column. Although quite satisfactory for research applications the methods were considered to be too labour-intensive for routine therapeutic drug monitoring. The method described in this paper for quantitation of *dl*-sotalol offers a rapid sample processing step (typically 1 min per sample) with sensitivity, accuracy and reproducibility suitable for both therapeutic drug monitoring and pharmacokinetic research applications. The chromatographic conditions were adapted from a method previously used in this laboratory [11]. Use of the HPLC mobile phase to elute samples from the solid-phase extraction columns allowed injection of relatively large volumes onto the HPLC column without any distortion of the chromatogram as previously reported [10].

Solid-phase extraction could not be used for the enantioselective HPLC assay as the samples needed to be dried prior to derivatisation with *S*-(−)-α-methylbenzyl isocyanate, a reaction which is sensitive to the presence of water. For the enantiospecific method the liquid–liquid extraction described by Urech *et al.* [10] was employed. The dichloromethane–isopropanol solovent was easily dried at 40°C and resulted in good extraction recoveries and derivatisation efficiencies. Dried chloroform was found to be the best solvent in which to carry out the derivatisation step. In preliminary studies, acetonitrile, methanol, diethyl ether and dichloromethane showed poorer reproducibility and derivatisation efficiencies. Overnight derivatisation also improved reproducibility and efficiency, and was carried out at 4°C to minimise evaporation of the chloroform. Derivatisation at room temperature also gave similar results, however, increasing temperatures to above 30°C resulted in poorer efficiency and reproducibility.

Derivatisation with the chiral *S*-(−)-α-methylbenzyl isocyanate has previously been used for HPLC resolution of pindolol, atenolol, acebutolol, metoprolol and propranolol [15–17]. The reaction is based on the formation of diastereomeric urea derivatives [15], which, unlike the parent enantiomers, have sufficiently different physical and chemical properties allowing resolution by conventional reversed-phase HPLC.

In the very early stages of the method development UV detection at 235 nm was used. However, there was a great deal of chromatographic interference from endogenous compounds, including the presence of very late-eluting chromatographic peaks (> 1 h). Fluorescence detection greatly improved sensitivity and removed all endogenous chromatographic interference, the only late-eluting chromatographic peak being at approximately 35–40 min. If injections onto the HPLC column were routinely made every 30 min the late peak appeared within the first 10 min of the next chromatogram thus not affecting the sotalol and atenolol derivative peaks.

The method presented describes the first plasma assay for sotalol enantiomers and has sensitivity, accuracy and reproducibility suitable for pharmacokinetic research applications.

The apparent lack of enantioselectivity in sotalol disposition observed in this study is consistant with predominant renal glomerular filtration and lack of any significant metabolism, renal secretion or plasma protein binding of sotalol [1,2]. A previous study that examined sotalol kinetics, using a non-enantiospecific assay, following oral doses of *d*-sotalol and *dl*-sotalol in healthy volunteers, also reported a lack of enantioselectivity in sotalol pharmacokinetics [18]. Atenolol which is similarly cleared predominantly by glomerular filtration has been reported to show negligible enantioselectivity in the plasma concentration profiles of its two enantiomers [19,20], whilst pindolol, which is highly secreted by the proximal tubules of the kidney, exhibits clearly enantioselective renal clearance in healthy human volunteers [15,17]. In contrast, β-blockers that are highly bound to plasma proteins or more extensively metabolised show more marked enantioselective pharmacokinetics in humans [21,22]. Recently Bagwell *et al.* [23] reported that the uptake of atenolol into cytosolic storage granules of rat isolated PC12 cells and bovine adrenal chromaffin ghosts was enantioselective for *l*-atenolol, and proposed that the transport of β-adrenoreceptor antagonists in general into adrenergic storage granules is enantioselective, providing a further mechanism by which the active β-antagonist *l*-enantiomer is delivered to the site of action after membrane depolarization [23].

In conclusion, both assays described provide accurate, reproducible measurements of sotalol in plasma, suitable for either therapeutic drug monitoring or research applications. Due to the apparent lack of enantioselectivity in the disposition of sotalol in humans, the non-enantiospecific assay would be sufficient for therapeutic drug monitoring purposes, especially given the very simple and rapid sample preparation described.

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