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Stereoselective high-performance liquid chromatographic assay for the determination of sotalol enantiomers in biological fluids

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ABSTRACT

A high-performance liquid chromatographic (HPLC) assay for determination of sotalol enantiomers in biological fluids was developed to assess the stereoselective disposition of the drug in man. Following extraction at pH 9.0 with a mixture of chloroform–isopropanol (3:1, v/v), the organic phase was evaporated to dryness and the residue derivatized with (−)-menthyl chloroformate. Diastereoisomeric derivatives were resolved by HPLC (C_8 column) with fluorescence detection (λ_{ex} = 235 nm and λ_{em} = 300 nm). Retention times of *l*- and *d*-sotalol derivatives were 13 and 15 min while that of the internal standard, *S*-(−)-atenolol, was 12.3 min. The detection limit of each enantiomer was 12.5 ng/ml using 1 ml of plasma or urine. Intra-day and inter-day coefficients of variation were less than 10% for each enantiomer in the range 0.125–2.5 μg/ml in plasma and 0.25–2.5 μg/ml in urine.

INTRODUCTION

Sotalol, 4-(2-isopropylamino-1-hydroxyethyl)-methanesulfonanilide, is a non-cardioselective β-blocker with class III antiarrhythmic properties [1–3]. The drug is used clinically as a racemic mixture (Fig. 1) and both enantiomers exhibit different pharmacological properties [4–6]. In fact, *l*-sotalol is an up to 50 times more potent β-adrenergic antagonist than *d*-sotalol. In contrast, both enantiomers and the racemic mixture possess similar class III antiarrhythmic properties.

Although these stereoselective differences in the pharmacology of both enantiomers are well established, data on complete characterization of the disposition of sotalol enantiomers are still lacking. Therefore, it was our objective to develop a sensitive stereoselective assay for sotalol in order to assess disposition of both enantiomers following administration of the racemate.

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EXPERIMENTAL

Reagents and standards

dl-Sotalol hydrochloride and its enantiomers were kindly provided by Bristol-Myers Squibb (Wallingford, CT, USA). *S*-(−)-Atenolol and (−)-menthyl chloroformate were purchased from Aldrich (Milwaukee, WI, USA) while HPLC-grade methanol and acetonitrile were obtained from Fisher Scientific (Montreal, Canada). All other reagents and solvents were of analytical grade.

Tris buffer (2 *M*) was adjusted to pH 9.0 with 12 *M* hydrochloric acid and kept at 4°C. Stock solutions of *dl*-sotalol (concentrations ranging

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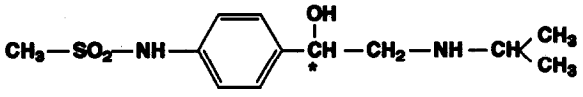
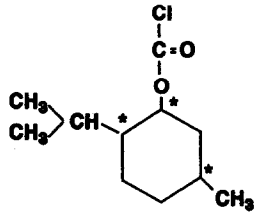
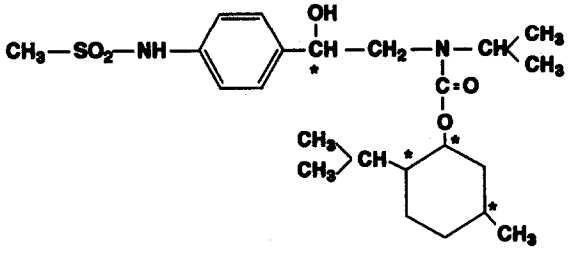


dl-SOTALOL



(-)-MENTHYL CHLOROFORMATE



CARBAMATE DERIVATIVE OF *dl*-SOTALOL

Fig. 1. Structures of *dl*-sotalol and (−)-menthyl chloroformate, and proposed structure of diastereoisomeric derivatives; the asterisk depicts the location of asymmetric carbons.

between 12.5 and 250 μg/ml) and of *S*-(−)-atenolol (2.5 μg/ml) were prepared and stored at −20°C for seven weeks without degradation. Chiral reagent solution was prepared daily by dissolving 400 μl of (−)-menthyl chloroformate in 10 ml of acetonitrile [7].

Standard curve

Aliquots of *dl*-sotalol stock solutions were added to blank plasma or urine to obtain final concentrations of each enantiomer ranging from 0.125 to 2.5 μg/ml in plasma and from 0.250 to 2.5 μg/ml in urine. Calibration curves were constructed by plotting the peak-area ratio of both diastereoisomeric derivatives of sotalol to the internal standard *versus* the concentration of sotalol enantiomers added to plasma or urine.

Sample preparation

Plasma. To 1.0 ml of plasma containing sotalol enantiomers were added 200 μl of *S*-(−)-atenolol stock solution (2.5 μg/ml) and 660 μl of 2 *M* perchloric acid. The samples were shaken briefly (Thermadyne 37600 mixer) and centrifuged (IEC CENTRA-7R centrifuge) for 5 min at 2000 *g*. A 1-ml volume of supernatant was transferred into glass tubes and the pH adjusted to 9.0 with 1 ml of 2 *M* Tris buffer and 250 μl of 2 *M* sodium hydroxide. The resulting aqueous medium was extracted twice with a 5-ml mixture of chloroform–2-propanol (3:1, v/v). Following vortex-mixing for 1 min and centrifugation at 2000 *g* for 10 min, organic fractions were collected and dried with anhydrous sodium sulfate (≈ 3 g). The organic phase was transferred and evaporated to dryness under a nitrogen stream. To the residue were added 200 μl of a saturated sodium carbonate solution followed by 200 μl of (−)-menthyl chloroformate solution; samples were vortex-mixed for 30 s. Water (1 ml) and 2 ml of chloroform were added to samples which were again vortex-mixed for 1 min. Following centrifugation at 1500 *g* for 5 min, the aqueous layer was discarded and the organic phase was evaporated to dryness. The residue containing diastereoisomeric derivatives of sotalol enantiomers and of *S*-(−)-atenolol was reconstituted with 100 μl of mobile phase and centrifuged for 5 min. A 40-μl aliquot was injected into the HPLC column.

Urine. After diluting urine samples twenty times with blank urine, 200 μl of *S*-(−)-atenolol stock solution (7.5 μg/ml) were added, and the pH adjusted to 9.0 with 1 ml of 2 *M* Tris buffer. The resulting aqueous medium was extracted twice with 5 ml of a chloroform–2-propanol mix-

ture (3:1, v/v). Thereafter, samples were processed as described for plasma samples.

Chromatography

Analyses were performed at ambient temperature using a Water Model 510 pump, a SIL-9A Shimadzu autoinjector (100-μl loop) and a Shimadzu RF-535 fluorescence detector with excitation and emission wavelengths set at 235 and 300 nm, respectively [8]. A Shimadzu CR501 Chromatopac integrator was coupled to the system. Resolution of diastereoisomeric derivatives of sotalol was achieved on a Jones (Lakewood, CO, USA) C_8 5-μm column (25 cm × 4.6 mm I.D.). The mobile phase consisted of methanol–water–acetonitrile (50:35:15) and was pumped at a flow-rate of 2.0 ml/min.

RESULTS AND DISCUSSION

Typical chromatograms of diastereoisomeric derivatives of sotalol enantiomers and of *S*-(−)-atenolol in plasma and urine are shown in Figs. 2 and 3, respectively. Retention times of the derivatives of *l*-and *d*-sotalol were 14 and 15 min, respectively, while that of the internal standard, *S*-(−)-atenolol, was 12.3 min. These retention times were identical to those obtained when individual enantiomers were analyzed separately.

As shown in Figs. 2 and 3, complete baseline resolution of sotalol enantiomers was achieved following derivatization with the homochiral agent (−)-menthyl chloroformate. This in contrast to Mehvar [9] who previously reported partial resolution of sotalol enantiomers with the same chiral reagent. In our assay, changes performed in the mobile phase and the use of a Jones C_8 column significantly improved peak separation and elution.

Resolution of sotalol enantiomers with GITC (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate) and AITC (2,3,4-tri-O-acetyl-α-D-arabinopyranosyl isothiocyanate) has also been reported [10]. Although resolution of diastereoisomeric sotalol derivatives was achievable with GITC, several attempts to resolve interfering peaks from endogenous products co-extracted with sotalol were unsuccessful. In contrast, in our assay, no peaks from endogenous compounds or reagents interfered with either sotalol enantiomers or internal standard diastereoisomeric peaks when derivatization was performed with (−)-menthyl chloroformate. This is not only due to the derivatization reagent used but also to the high selectivity of fluorescence detection since interfering peaks are noticed when UV detection is used.

Other reported assays for resolution of sotalol enantiomers include derivatization with *S*-(−)-α-methylbenzyl isocyanate and with *S*-(+)-1-(1-napthyl)ethyl isocyanate [11,12]. In the first case, elution of sotalol enantiomers was very close to interfering peaks which impairs accuracy of the assay at low concentrations. Moreover, derivatization with *S*-(−)-α-methylbenzyl isocyanate has

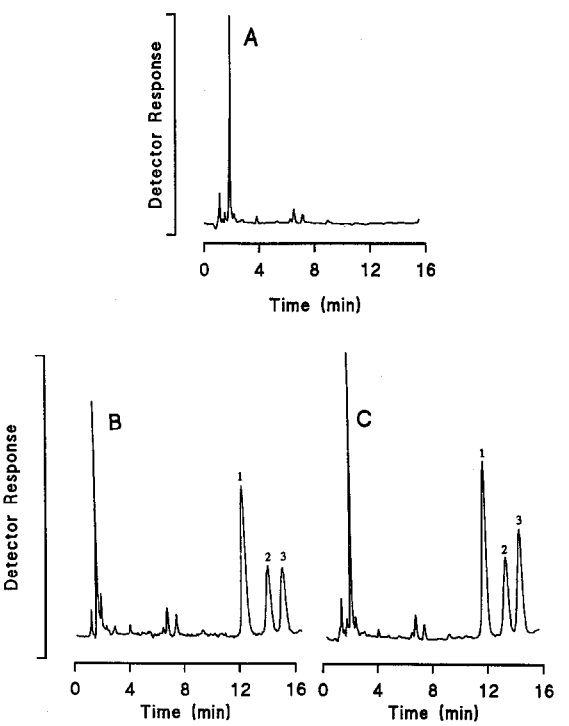


Fig. 2. Chromatograms of (A) blank plasma, (B) blank plasma spiked with 2 μg/ml racemic sotalol and (C) patient plasma sample containing 525 ng/ml *d*-sotalol and 411 ng/ml *l*-sotalol after oral administration of 80 mg of racemic sotalol · HCl (Sotacor). Peaks: 1 = diastereoisomeric derivative of *S*-(−)-atenolol; 2 = diastereoisomeric derivative of *l*-sotalol; 3 = diastereoisomeric derivative of *d*-sotalol.

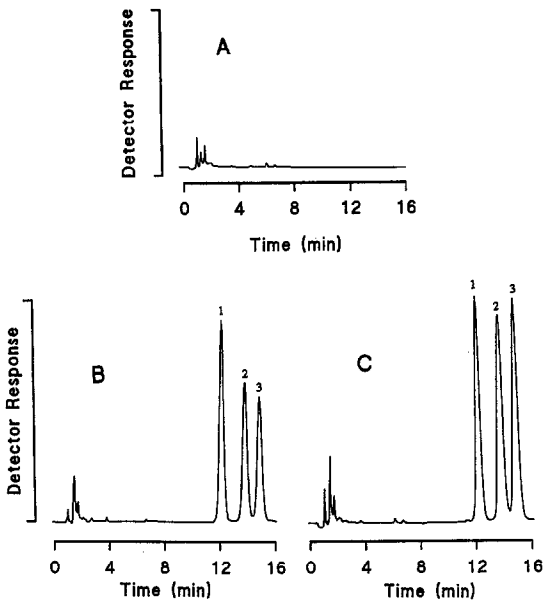


Fig. 3. Chromatograms of (A) blank urine, (B) blank urine spiked with 5 mg/l racemic sotalol and (C) patient urine sample containing 2.88 mg/l *d*-sotalol and 2.79 mg/l *l*-sotalol after oral administration of 160 mg of racemic sotalol · HCl (Sotacor). Peaks: 1 = diastereoisomeric derivative of *S*-(−)-atenolol; 2 = diastereoisomeric derivative of *l*-sotalol; 3 = diastereoisomeric derivative of *d*-sotalol.

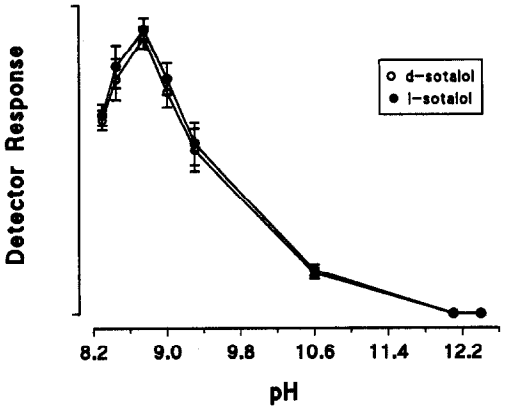


Fig. 4. Variation in pH extraction profile of sotalol enantiomers in plasma. Values are reported as mean ± S.E.M. (n = 5).

to be performed overnight [12]. In the second assay, derivatization with *S*-(+)-1-(1-naphthyl) ethyl isocyanate yields baseline resolution of sotalol enantiomers. However, reported sensitivity was four times less than that obtained in our assay [11].

The presence in the sotalol molecule of a methanesulfanilide and a secondary amine confers amphoteric properties to the drug. Therefore, it is anticipated that maximal extraction could be effected into an organic solvent at maximum concentration of the neutral form in equilibrium with the zwitter ion, half-way between the two pK_a values [13]. Potentiometric pK_a values of sotalol are 8.30 and 9.80; thus, maximum extractability should be observed around pH 9.0 [13–16]. Evaluation of *d*- and *l*-sotalol extractability over a wide range of pH (8.3–12.4) demonstrated that maximal extraction was indeed achieved at pH 9.0 (Fig. 4) as demonstrated previously [13,14]. With the procedure used in our assay, the extraction efficiency for both enantiomers of sotalol from 1 ml of plasma was > 90% (*d*-sotalol: 94.8 ± 0.3%; *l*-sotalol: 92.3 ± 0.4%). In urine, extraction was complete for both enantiomers when 1 ml of urine was used (*d*-sotalol: 102.0 ± 6.0%; *l*-sotalol: 100.2 ± 5.2%) and equal to 100% from 1-ml urine samples. Although extractability was not stereoselective, the pH should be carefully adjusted for quantitative extraction of sotalol from aqueous medium.

S-(−)-Atenolol represents an appropriate internal standard in this assay because (1) like sotalol it is a hydrophilic β-blocker, (2) under the chromatographic conditions used with this method, it is appropriately separated from diastereoisomeric derivatives of sotalol and (3) it elicits suitable fluorescence intensity. Moreover, Mehvar [7] has shown that formation of derivatives of atenolol with (−)-menthyl chloroformate is successful.

Chloroformates react almost instantaneously with amines and alcohols to produce carbamates and carbonates, respectively [7]. Since sotalol contains both a secondary amine and an aliphatic alcohol group in its side-chain, (−)-menthyl chloroformate may have reacted with both moieties. However, since selective derivatization with primary and secondary amines can be achieved under mild alkaline conditions in presence of water [17] the reaction was performed in the presence of a saturated sodium carbonate aqueous

solution so that only derivatization with the amine moiety was anticipated in the present study (Fig. 1). Derivatization under uncontrolled pH conditions gave two derivatives for each enantiomer whereas only a single peak could be detected under alkaline conditions [7,17].

We performed studies where derivatization time and temperature were varied in order to assess completeness of the reaction between sotalol and (−)-menthyl chloroformate. Efficiency of the reaction was independent of time (30 s to 1 h) and temperature (25–50°C). Unfortunately, with the chromatographic conditions used, underivatized sotalol could not be detected even 90 min after injection. Therefore, whether the reaction is complete or not is not known. However, data from Mehvar [7] have shown that derivatization of atenolol with (−)-menthyl chloroformate is complete after 30 s [7]. Since sotalol is a closed structural analogue of atenolol, complete derivatization may be expected.

No decrease in fluorescence intensity and no change in peak-area ratio of either diastereoisomers were noticed following repeated analysis of the same derivatized sample for up to 72 h. Furthermore, no racemization was observed with time when pure *l*- or *d*-sotalol were derivatized. This stability of the derivatives at room temperature allowed the use of an HPLC autoinjector.

Use of fluorescence detection over UV detection improved the detection limit of the assay significantly (signal-to-noise ratio of 3) which was 12.5 ng/ml for each enantiomer using 1-ml of plasma or urine [18]. On the other hand, the limit of quantification of the assay (five times the standard deviation of the baseline noise) was 20 ng/ml for each enantiomer [18].

Day-to-day and within-day coefficients of variation were less than 10% as shown in Tables I and II. Typical regression lines of *d*- and *l*-sotalol in plasma were $y = (1.2925 \pm 0.1401)x + (0.0347 \pm 0.0559)$ ($r > 0.99$, $n = 9$) and $y = (1.3113 \pm 0.1406)x + (0.0341 \pm 0.0492)$ ($r > 0.99$, $n = 9$), respectively. In urine, typical regression line were $y = (0.4289 \pm 0.0232)x$ ($r > 0.99$, $n = 8$) and $y = (0.4386 \pm 0.0240)x$ ($r > 0.99$, $n = 8$), for *d*-sotalol and *l*-sotalol, respectively.

TABLE I

DAY-TO-DAY AND WITHIN-DAY COEFFICIENTS OF VARIATION FOR *d*- AND *l*-SOTALOL PLASMA CONCENTRATIONS

Concentration (μg/ml)	Coefficient of variation (mean ± S.D.) (%)	
	d-Sotalol	*l*-Sotalol
Intra-day variability (n = 6)		
0.25	4.0 ± 0.01	4.5 ± 0.02
1.0	7.4 ± 0.10	7.7 ± 0.11
2.0	7.0 ± 0.17	5.8 ± 0.15
Inter-day variability (n = 6)		
0.25	4.6 ± 0.02	4.1 ± 0.01
1.0	3.7 ± 0.05	5.4 ± 0.07
2.0	5.4 ± 0.01	6.6 ± 0.14

These calibration curves performed with racemic sotalol were shown to appropriately quantify sotalol enantiomers since individual enantiomers exhibit similar regression lines: $y = 1.3077x - 0.0047$ ($r > 0.99$) for *d*-sotalol and $y = 1.3527x + 0.0080$ ($r > 0.99$) for *l*-sotalol. We did not observe racemization in these calibration curves neither did we when blank plasma spiked with different proportions of both enantiomers was analyzed (Table III). Mean accuracy of the assay, (difference between the measured and added so-

TABLE II

DAY-TO-DAY AND WITHIN-DAY COEFFICIENTS OF VARIATION FOR *d*- AND *l*-SOTALOL URINE CONCENTRATIONS

Concentration (μg/ml)	Coefficient of variation (mean ± S.D.) (%)	
	d-Sotalol	*l*-Sotalol
Intra-day variability (n = 6)		
0.25	7.9 ± 0.01	8.4 ± 0.01
1.0	7.4 ± 0.03	6.5 ± 0.03
2.5	4.4 ± 0.05	4.0 ± 0.04
Inter-day variability (n = 5)		
0.25	10 ± 0.01	9.4 ± 0.01
1.0	4.7 ± 0.02	4.6 ± 0.02
2.5	6.3 ± 0.07	6.4 ± 0.07

TABLE III

ASSESSMENT OF POTENTIAL RACEMIZATION OF SOTALOL ENANTIOMERS THROUGHOUT THE ASSAY PROCEDURE

Proportion of sotalol enantiomers added in plasma (%)		Measured proportion of sotalol enantiomers in plasma (%)	
d-Sotalol	*l*-Sotalol	*d*-Sotalol	*l*-Sotalol
50	50	49.8	50.2
75	25	75.9	24.1
25	75	24.7	75.3

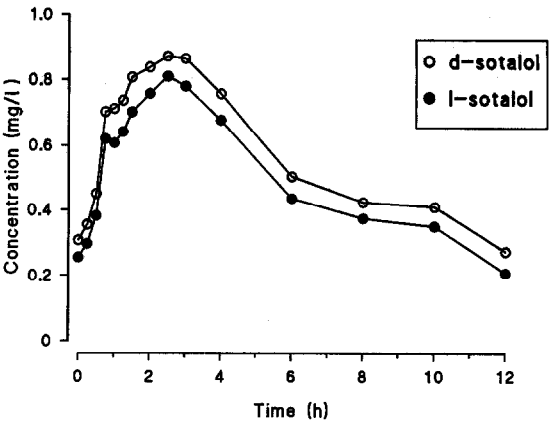


Fig. 5. Plasma concentration of sotalol enantiomers *versus* time profile in a patient during treatment (160 mg, 12-hourly) with racemic sotalol · HCl (Sotacor).

talol concentrations) calculated in the range of concentrations measured in the plasma was estimated at 99 ± 6%. In urine, accuracy of the assay was estimated at 101 ± 3%.

Our objective was to develop a stereoselective assay suitable for pharmacokinetic studies after administration of racemic sotalol in patients. As shown in Fig. 5, concentrations of sotalol enantiomers could easily be determined in plasma of a patient undergoing chronic therapy with the drug (80 mg twice daily). In this patient, minor stereoselectivity in the disposition of the *l*-enantiomer was observed. This is in agreement with data reported in other assay development studies for sotalol enantiomers. The assay described herein should also be suitable for single-dose pharmacokinetic studies since the sensitivity limit is lower than the plasma concentrations usually measured 48 to 72 h after oral administration of a single dose of the drug [19].

TABLE IV

DRUGS THAT DID NOT INTERFERE WITH THE CHROMATOGRAPHY OF *l*- AND *d*-SOTALOL

t_R = retention times.

Drug	t_R (min)	Drug	t_R (min)	Drug	t_R (min)
Acetaminophen	>30	Heparin	>30	Oxazepam	>30
Acetylsalicylic acid	>30	Hydralazine	>120	Pindolol	46–52
Alginic acid	>30	Hydrochlorothiazide	>30	Procainamide	>90
Alprenolol	31	Indapamide	>30	Propafenone	130–140
Amiloride	>60	Lidocaine	>60	Propranolol	>60
Amoxicilline	>30	Lorazepam	>30	Propoxyphen	>30
Captopril	26	Lovastatin	>30	Quinidine	>60
Diazoxide	>60	Metoprolol	102	Ranitidine	>30
Digoxine	>30	Mexiletine	>60	Timolol	>120
Domperidone	>30	Nifedipine	>30	Triamterene	>90
Flurazepam	>30	Nitroglycerine	>30	Verapamil	>90
Furosemide	>30	Nortriptylline	>30	Warfarin	>30
Glyburide	>30	Omeprazole	>30		

As stated earlier, we were interested to study steady-state stereoselective pharmacokinetics of *dl*-sotalol in patients undergoing chronic therapy with the drug. Therefore, it was important to develop a highly selective assay since cardiac patients often receive multi-drug therapy regimen. Table IV lists a series of drugs which do not interfere with the assay. Several of those drugs had been administered concomitantly with sotalol to patients while others were tested since they possess similar pharmacological properties or structures.

CONCLUSION

We have described a highly selective and sensitive stereoselective HPLC assay for the determination of sotalol enantiomers in biological fluids. The assay is suitable for pharmacokinetic studies after administration of single or multiple doses of the drug as a racemate in patients undergoing multi-drug therapy regimens.

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