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Enantioselective determination of sotalol enantiomers in biological fluids using high-performance liquid chromatography

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Abstract

A simple and sensitive high-performance liquid chromatographic (HPLC) method for the determination of (+)-(*S*)-sotalol and (−)-(*R*)-sotalol in biological fluids was established. Following extraction with isopropyl alcohol from biological samples on a Sep-Pak C_{18} cartridge, the eluent was derivatized with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC). The diastereoisomeric derivates were resolved by HPLC with UV detection at 225 nm. Calibration was linear from 0.022 to 4.41 μg/ml in human plasma and from 0.22 to 88.2 μg/ml in human urine for both (+)-(*S*)- and (−)-(*R*)-sotalol. The lower limit of determination was 0.022 μg/ml for plasma and 0.22 μg/ml for urine. The within-day and day-to-day coefficients of variation were less than 7.5% for each enantiomer at 0.09 and 1.8 μg/ml in plasma and at 0.44 and 4.4 μg/ml in urine. The method is also applicable to other biological specimens such as rat, mouse and rabbit plasma.

1. Introduction

Sotalol, 4-(2-isopropylamino-1-hydroxyethyl)-methanesulfonanilide (Fig. 1), is a potent β-blocker with class II and class III antiarrhythmic properties [1–3]. Sotalol is a racemic mixture with two optical isomers of (+)-(*S*)-solatol and (−)-(*R*)-sotalol. Both (+)-(*S*)- and (−)-(*R*)-sotalol have similar class III antiarrhythmic activities, but (+)-(*S*)-sotalol has been shown to be largely devoid of the β-blocking activity [4,5].

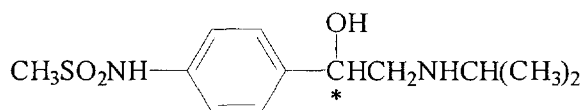

Fig. 1. Chemical structure of sotalol. The asterisk shows the asymmetric carbon atom.

Several pharmacokinetic and pharmacodynamic studies have been reported, all based on non-stereoselective assay methods [6–10]. However, considering the different pharmacological properties of (+)-(*S*)- and (−)-(*R*)-sotalol, the use of a stereoselective assay is essential for the evaluation of sotalol pharmacokinetics. Recently, three stereoselective HPLC assay methods of sotalol based on fluorimetric detection and liquid–liquid extraction were reported [11–13]. For the application of assay methods to pharmacokinetics, a recommendation on analytical

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methods validation was reported [14]. The existing assay method [11–13] poorly described several items in the recommendation.

This paper describes a new, simple, accurate and sensitive analytical method of (+)-(*S*)- and (−)-(*R*)-sotalol in human plasma and urine, using reversed-phase HPLC with UV detection and solid-phase extraction for sample preparation, and the method validation.

2. Experimental

2.1. Reagents and materials

Sotalol hydrochloride, (+)-(*S*)-sotalol hydrochloride (optical purity: 99.9%) and (−)-(*R*)-sotalol hydrochloride (optical purity: 98.4%) were synthesized in our company. Concentration of (+)-(*S*)- and (−)-(*R*)-sotalol was expressed as their free base in the text. HPLC-grade acetonitrile and methanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Milli-Q water (Millipore, Milford, MA, USA) was used as purified water throughout the study. Methylene chloride, isopropyl alcohol, sodium tetraborate, ammonium phosphate dibasic, ammonium phosphate monobasic, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) were analytical reagent grade and were also purchased from Wako. Analytical reagent grade *p*-hydroxybenzoic acid isoamyl ester was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Extraction cartridges of ODS (Sep-Pak C_{18}, 500 mg per cartridge) were purchased from Waters (Milford, MA, USA). Pooled human plasma and urine were collected from six healthy volunteers and stored below −20°C. Rat and mouse plasma were purchased from Rockland (Gilbertsville, PA, USA), and rabbit plasma was purchased from Chemicon (Temecula, CA, USA).

2.2. Apparatus

The HPLC system consisted of a Model 880-PU pump, an 850-AS autosampler, an 875-UV UV–visible detector, an 801-SC system controller, an 807-IT integrator and an 860-CO column oven (Japan Spectroscopic, Tokyo, Japan).

2.3. HPLC conditions

A reversed-phase STR ODS II column (150 × 4.6 mm I.D., 5 μm, Shimadzu Techno-Research, Kyoto, Japan) with a Guard-Pak Resolve C_{18} cartridge (Waters) was used for separation. A mixture of 0.02 *M* ammonium phosphate monobasic (pH 4.6)–acetonitrile (60:40, v/v) was used as the mobile phase at a flow-rate of 1 ml/min. The column was maintained at a constant temperature of the 25°C. Chromatograms were monitored by a UV detector at 225 nm.

2.4. Calibration

Calibration standards were prepared by adding 0.05, 0.20, 1.0, 4.0, 10.0 and 20.0 μg (for urine only) of racemic sotalol hydrochloride in 1 ml of plasma or diluted urine with 0.02 *M* sodium tetraborate buffer (pH 9.2), which resulted in 0.022, 0.088, 0.44, 1.8, 4.4 and 8.8 μg/ml solutions for both (+)-(*S*)- and (−)-(*R*)-sotalol. Calibration curves were constructed by plotting the relative peak area of each enantiomer to internal standard versus the concentration of each enantiomer in plasma and urine.

2.5. Sample preparation

Plasma

Solid-phase extraction cartridges were prepared by pre-washing with 3 ml methanol, followed by 3.0 ml of 0.2 *M* sodium tetraborate under vacuum (Vac Elut with adapters for Sep-Pak, Analytichem International, Harbor City, CA, USA). To 1.0 ml of plasma samples containing sotalol enantiomers, 0.5 ml of saturated sodium tetraborate (pH 9.3) was added. Then the samples were briefly vortex-mixed and loaded onto the pre-washed solid-phase extraction columns using vacuum displacement. After washing with 2 ml of 0.02 *M* sodium tetraborate, 2 ml of water and 1 ml of methylene chloride to

remove any residual plasma, the column was eluted with 5 ml of isopropyl alcohol. Aliquots of 0.1 ml each of *p*-hydroxybenzoic acid isoamyl ester solution (internal standard; 0.1 mg/ml in 10% acetonitrile–water, v/v), GITC (4 mg/ml in acetonitrile) and 0.01 *M* ammonium phosphate dibasic (pH 8.0) were added to the eluate and derivatized at 50°C for 3 h. The mixture was then evaporated to dryness under vacuum. The residue was dissolved in 0.2 ml of the mobile phase. An aliquot of 40 μl of the solution was injected into the HPLC system.

Urine

Urine samples were diluted ten times with 0.2 *M* sodium tetraborate and 1 ml aliquot was loaded onto the pre-washed solid–phase extraction columns. Thereafter, samples were processed as described for plasma samples.

2.6. Accuracy and precision

The within-day precision of the assay was determined by subsequential analysis of multiple spiked samples of (+)-(*S*)- and (−)-(*R*)-sotalol in human plasma and urine (3 concentrations, $n = 10$). The accuracy was expressed as the mean ratio of observed and spiked concentrations. The precision was calculated as coefficients of variation (C.V.%). The day-to-day precision of the assay in human plasma and urine samples was also evaluated by the determination at two concentrations on ten different days.

3. Results and discussion

3.1. Specificity

Fig. 2 shows typical chromatograms of (+)-(*S*)- and (−)-(*R*)-sotalol in human plasma and urine. The coefficients of variation (C.V.%) of the retention times of (+)-(*S*)-sotalol, (−)-(*R*)-sotalol and I.S. were less than 0.1% ($n = 10$) within a series of runs and less than 0.7% ($n = 10$) between series of runs. The retention time ranges were 11.3–11.6 min [(−)-(*R*)-sotalol, $n = 10$], 14.2–14.5 min [(+)-(*S*)-sotalol, $n = 10$] and 25.3–25.8 min (I.S., $n = 10$) on a day-to-day basis.

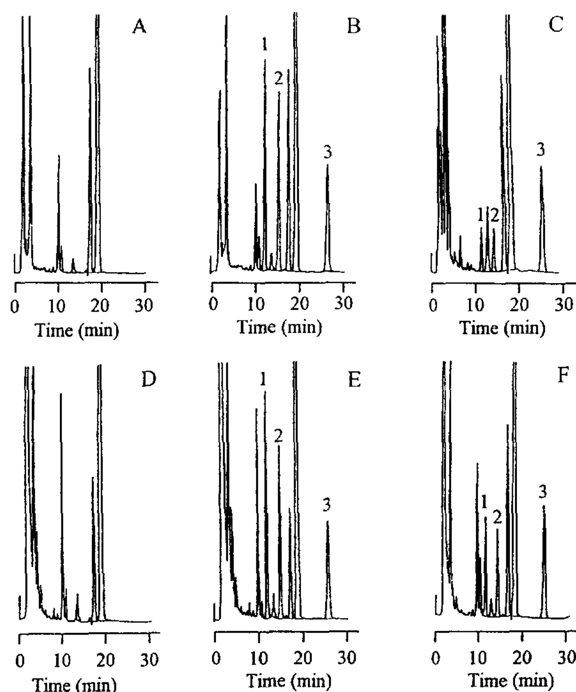

Fig. 2. Chromatograms of derivatized (+)-(*S*)- and (−)-(*R*)-sotalol in plasma extract and urine extract. Peaks 1, 2 and 3 are (−)-(*R*)-sotalol, (+)-(*S*)-sotalol and I.S., respectively. (A) Pooled plasma blank, (B) spiked plasma (2 μg/ml for both (+)-(*S*)- and (−)-(*R*)-sotalol), (C) plasma from a volunteer at 3 h after 80-mg oral administration of sotalol hydrochloride, (D) urine blank, (E) spiked urine (2 μg/ml for both (+)-(*S*)- and (−)-(*R*)-sotalol) and (F) urine from a volunteer collected between 24 and 30 h after 80-mg oral administration of sotalol hydrochloride.

3.2. Derivatization

GITC was chosen as a diastereoisomeric derivatizing agent [15]. Optimal temperature and reaction time for derivatization of (+)-(*S*)- and (−)-(*R*)-sotalol were examined. Fig. 3 shows the time course of the detector signal intensity of (+)-(*S*)- and (−)-(*R*)-sotalol derivatives, expressed as relative peak area, at 50°C. At 40°C and room temperature, the reaction was not completed with 3 h. At 50°C, the UV absorbance reached a plateau between 2 and 4 h of reaction. These reaction time courses were very similar

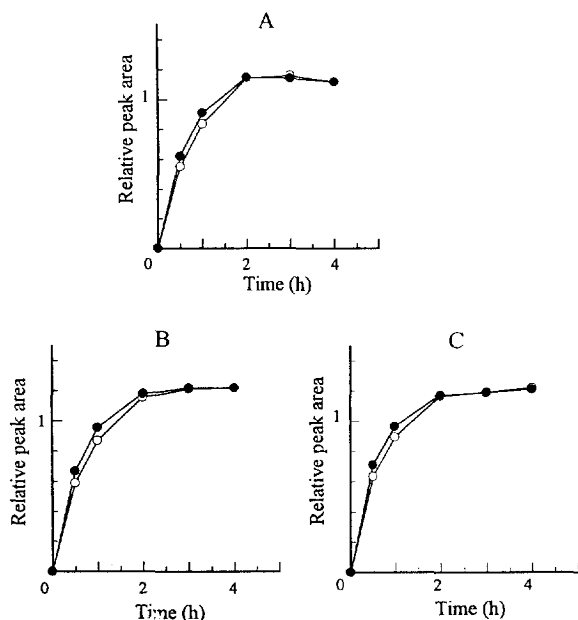

Fig. 3. Reaction time course of derivatization at 50°C. The open and the closed circles show the relative peak areas of (+)-(*S*)- and (−)-(*R*)-sotalol, respectively. (A) Non-extracted solution in water, (B) plasma extract, and (C) urinary extract.

among standard solution, plasma extract and urine extract. Thus we chose 3 h of incubation of 50°C as the reaction conditions for further study. We confirmed that no racemization had occurred under these conditions using (+)-(*S*)- and (−)-(*R*)-sotalol solutions separately.

3.3. Linearity and recovery

Plasma samples showed linear responses for both (+)-(*S*)- and (−)-*R*)-sotalol in the concentration range from 0.022 to 4.4 μg/ml (Table 1). The recoveries, which were calculated from the ratio of the slopes of plasma samples and non-extracted solutions, were about 92% for both (+)-(*S*)- and (−)-(*R*)-sotalol. Diluted urine samples showed similar results to those observed for plasma, and the recoveries were about 96% for both (+)-(*S*)- and (−)-(*R*)-sotalol (Table 2). These results indicate that (+)-(*S*)- and (−)-(*R*)-sotalol in human plasma and urine can be determined by the HPLC method using standards in plasma and diluted urine with 0.02 *M* sodium tetraborate buffer (pH 9.2). The lower limit of the quantitation (LLQ) was 0.022 μg/ml for both isomers in plasma and 0.22 μg/ml in undiluted urine. The LLQ for plasma is superior to the methods reported by Carr et al. [11] and Fiset et al. [13] and is equivalent to the method of Sallustio and Morris [12].

Table 1
Relative peak area and linearity of the response of (+)-(*S*)- and (−)-(*R*)-sotalol in pooled human plasma

Spiked concentration (μg/ml)	Relative peak area: mean (C.V.%), $n = 3$	
	(+)-(*S*)-Sotalol	(−)-(*R*)-Sotalol
0.0220	0.01287 (14.1)	0.01993 (16.2)
0.0883	0.04923 (3.17)	0.05387 (1.98)
0.4414	0.26120 (4.78)	0.26490 (4.34)
1.766	1.07547 (0.25)	1.09127 (0.20)
4.414	2.78170 (0.83)	2.81053 (0.96)
Parameters of linear regression analysis		
Slope	0.63002	0.63643
(95% confidence limits)	(0.62428–0.63742)	(0.62948–0.64337)
Y intercept	−0.01322	−0.00872
(95% confidence limits)	(−0.02726–0.00081)	(−0.02355–0.00612)
Correlation coefficient	0.99985	0.99983
Recovery (%)	91.7	91.8

The recoveries were calculated from the ratio of the slopes of plasma samples and non-extracted solutions.

Table 2
Relative peak area and linearity of the response of (+)-(S)- and (−)-(R)-sotalol in diluted human urine

Spiked concentration (μg/ml)	Relative peak area: mean (C.V.%), n = 3	
	(+)-(S)-Sotalol	(−)-(R)-Sotalol
0.0220	0.01350 (3.70)	0.01655 (13.0)
0.0883	0.05837 (5.02)	0.05997 (0.52)
0.4414	0.28367 (3.31)	0.28767 (3.67)
1.766	1.14317 (0.25)	1.15327 (0.51)
4.414	2.90283 (1.26)	2.93267 (1.10)
8.828	5.85163 (1.19)	5.89657 (1.30)
Parameters of linear regression analysis		
Slope	0.66303	0.66810
(95% confidence limits)	(0.65698–0.66906)	(0.66177–0.67442)
Y intercept	−0.01111	−0.00870
(95% confidence limits)	(−0.03657–0.01435)	(−0.03539–0.01799)
Correlation coefficient	0.99986	0.99985
Recovery (%)	96.4	96.3

The recoveries were calculated from the ratio of the slopes of urine samples and non-extracted solutions.

3.4. Accuracy and precision

The reproducibility of the method was shown to be satisfactory. The within-day accuracy was more than 96% for plasma and urine samples with coefficients of variation less than 7.5% for both (+)-(S)- and (−)-(R)-sotalol (Table 3). The day-to-day accuracy was more than 99% and C.V. values were less than 7.3% for plasma and urine samples (Table 4).

3.5. Application to clinical samples

The validated assay was used to determine the plasma level of (+)-(S)- and (−)-(R)-sotalol after administration of an 80-mg dose of sotalol

Table 3
Within-day reproducibility for the quantitation of (+)-(S)- and (−)-(R)-sotalol in human plasma and urine

Compound	Spiked concentration (μg/ml)	Observed concentration (mean ± S.D.) (μg/ml)	C.V. (%)	Accuracy (%)
Plasma (n = 10)				
(+)-(S)-sotalol	0.088	0.087 ± 0.003	3.9	98.7
	0.441	0.435 ± 0.014	3.2	98.5
	1.766	1.791 ± 0.025	1.4	101.4
(−)-(R)-sotalol	0.088	0.089 ± 0.007	7.5	100.7
	0.441	0.439 ± 0.011	2.6	99.5
	1.766	1.795 ± 0.023	1.3	101.7
Urine (n = 10)				
(+)-(S)-sotalol	0.441	0.424 ± 0.012	2.8	96.0
	1.766	1.755 ± 0.042	2.4	99.4
	4.414	4.500 ± 0.065	1.4	102.0
(−)-(R)-sotalol	0.441	0.431 ± 0.012	2.7	97.7
	1.766	1.741 ± 0.032	1.8	98.6
	4.414	4.488 ± 0.056	1.2	101.7

Table 4
Day-to-day reproducibility for the determination of (+)-(*S*)- and (−)-(*R*)-sotalol in human plasma and urine

Compound	Spiked concentration (μg/ml)	Observed concentration (mean ± S.D.) (μg/ml)	C.V. (%)	Accuracy (%)
Plasma (n = 10)				
(+)-(*S*)-sotalol	0.088	0.089 ± 0.005	5.9	101.0
	1.766	1.804 ± 0.077	4.3	102.2
(−)-(*R*)-sotalol	0.088	0.088 ± 0.006	7.3	99.8
	1.766	1.815 ± 0.040	2.2	102.8
Urine (n = 10)				
(+)-(*S*)-sotalol	0.441	0.439 ± 0.031	7.0	99.5
	4.414	4.389 ± 0.080	1.8	99.4
(−)-(*R*)-sotalol	0.411	0.444 ± 0.031	7.1	100.6
	4.414	4.431 ± 0.116	2.6	100.4

hydrochloride in volunteers. The pharmacokinetic profiles of both isomers are shown in Fig. 4. The mean peak plasma levels of (+)-(*S*)- and (−)-(*R*)-sotalol were about 0.4 μg/ml, therefore the plasma levels could be monitored for 30 h after administration until the plasma levels decreased to about 5% of the peak concentrations. The pharmacokinetics of the enantiomers revealed that (−)-(*R*)-sotalol has slightly larger plasma clearance than that of (+)-(*S*)-sotalol. This observation is consistent with the report on steady-state pharmacokinetics in human by Fiset et al. [16]. The enantiomeric difference in pharmacokinetics after administration of racemic sotalol was not observed in the rats [17].

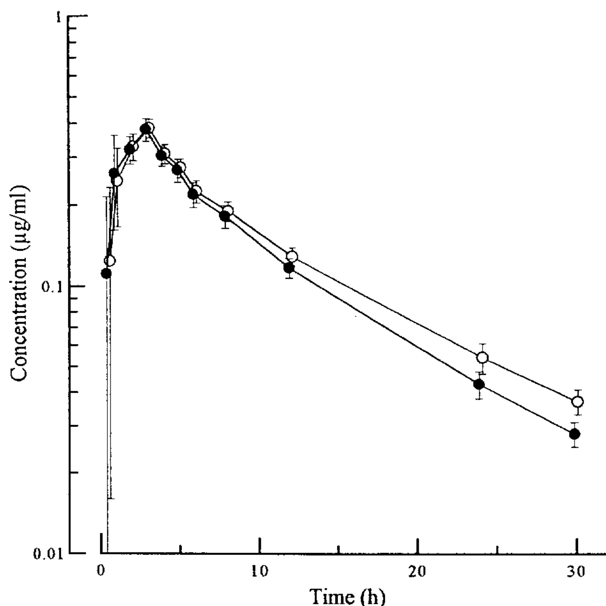

Fig. 4. Plasma levels of (+)-(*S*)- and (−)-(*R*)-sotalol after single oral administration of sotalol hydrochloride to six healthy volunteers. The open and the closed circles show the mean plasma concentration of (+)-(*S*)- and (−)-(*R*)-sotalol, respectively.

Another example of application of this method was reported by Uematsu et al. [18]. They studied pharmacokinetics of (+)-(*S*)-sotalol after oral and intravenous administration to healthy male volunteers. In the study (−)-(*R*)-sotalol was not detected in plasma and urine after both oral and intravenous administration, indicating that no biotransformation of (+)-(*S*)-sotalol to (−)-(*R*)-sotalol occurs in humans.

Stability for sample storage at −20°C was determined for three months and no significant decrease of (+)-(*S*)- and (−)-(*R*)-sotalol was observed for both plasma and urine samples. Freeze–thaw stability was also examined, and no significant change was observed during three freeze–thaw cycles in the determination of (+)-(*S*)- and (−)-(*R*)-sotalol in plasma and urine.

The matrix effects of the plasma and urine from six individuals was examined at 0.1 μg/ml for plasma and 2 μg/ml for urine. No interference was observed and the determined values

were within ±5.3% and ±1.4% of spiked concentrations for plasma and urine, respectively. Additionally, the matrix effects of the plasma from mice, rats and rabbits were also examined. No significant interference was observed, showing that the method is applicable for plasma from these animal species.

4. Conclusion

A stereoselective HPLC method was developed for the simultaneous determination of (+)-(*S*)- and (−)-(*R*)-sotalol in plasma and urine, using solid-phase extraction, diastereoisomeric derivatization and UV detection. Solid-phase extraction is more suitable for further modification to use automatic sample preparation than liquid–liquid extraction. The UV detection is also advantageous to previous methods using fluorescence, because UV detectors are more popular and convenient than fluorescence detectors. The method was validated for human plasma and urine and confirmed for the application to human pharmacokinetic studies. The validation proved that the method has satisfactorily high accuracy and precision for the determination of (+)-(*S*)- and (−)-(*R*)-sotalol in plasma and urine. The method is useful to examine the pharmacokinetics of the optical isomers.

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