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Enantioselective determination of (*R*)- and (*S*)-sotalol in human plasma by on-line coupling of a restricted-access material precolumn to a cellobiohydrolase I-based chiral stationary phase

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Abstract

A liquid chromatographic column-switching method for the enantioselective determination of (*RS*)-sotalol in plasma was developed and validated. The method is based on the on-line coupling of a precolumn filled with the restricted access material LiChrospher ADS to a cellobiohydrolase I-based chiral stationary phase (CSP). The plasma samples were injected onto the precolumn using a mobile phase containing 1% methanol in 10 m*M* phosphate buffer at pH 7.4 for 10 min for the removal of matrix components. The analytes were transferred to the CSP for their enantiomeric separation by backflushing the precolumn with 15% 2-propanol in 10 m*M* phosphate buffer (pH 7.0) including 0.05 m*M* EDTA. The quantitative determination of the sotalol enantiomers was possible upon addition of the internal standard (*S*)-atenolol. The method was validated showing a good linearity in the concentration range from 25 to 1000 μg l^{-1} for each enantiomer. The average values of the intra- and inter-day variability were 1.17% and 3.42%, respectively, for (*R*)-sotalol and 1.24% and 1.99%, respectively, for (*S*)-sotalol. The applicability of the method to real world samples has been proven by means of two pharmacokinetic studies. They revealed that the pharmacokinetic properties of the sotalol enantiomers do not differ significantly neither for healthy young volunteers after single dose application nor for elder patients in the steady state.

Keywords: Enantiomer separation; Restricted-access material; Chiral stationary phases, LC; Sotalol

1. Introduction

Sotalol, 4-[1-hydroxy-2-(isopropylamino)ethyl]-methanesulfonanilide, is a chiral β-adrenoceptor antagonist marketed in racemic form for the treatment of hypertension, angina pectoris and cardiac arrhythmia [1,2]. Apart from its β-blocking activity, which is mainly attributed to its (*R*)-(−)-enantiomer (Fig. 1) [1], both (*S*)-(+)- and (*R*)-(−)-sotalol are equipotent class III antiarrhythmic agents [1,2]. Due to this stereodifferentiating pharmacological profile

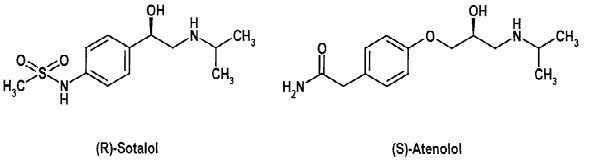

Fig. 1. Structures of (*R*)-sotalol and the internal standard (*S*)-atenolol.

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the therapeutic drug monitoring of sotalol requires an analytical method which enables the determination of both enantiomers in biofluids.

Several chromatographic methods for the achiral determination of sotalol in plasma and urine are described in the literature. Liquid–liquid extraction [3–5], plasma protein precipitation [6,7] or solid-phase extraction (SPE) [8–12] prior to the injection into a high-performance liquid chromatographic (HPLC) system have been used as sample pretreatment. Two automated procedures with on-line coupling of a precolumn for sample clean-up and an analytical column for the separation from endogenous components and co-medications have been established [13,14]. Since sotalol is rather polar, it is poorly retained on reversed-phase (RP) material. Therefore the addition of an ion-pairing reagent [13] or the use of a cation-exchange precolumn [14] was necessary.

The hitherto established enantioselective determination methods for sotalol in biofluids all comprise a time-consuming sample preparation removing disturbing plasma components by liquid–liquid extraction [15–18] or SPE [19] and a derivatisation of sotalol with a chiral reagent such as (−)-menthyl chloroformate [17], 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate [19] or asymmetric isocyanates [15,16,18]. Finally, the resulting diastereomers were separated on achiral columns. Moreover, apart from the usual drawbacks of indirect chiral methods concerning the enantiomeric purity of the reagent or the preferential derivatisation of one enantiomer, both the removal of plasma components and the derivatising step cannot be automatised and are therefore less suited for a high sample throughput. We reasoned that an on-line coupling of a precolumn for sample preparation and a chiral analytical column allowing the direct enantioselective determination of sotalol from biological samples should solve these problems.

In principle, the precolumn should selectively retain the analytes whereas the protein matrix should pass without precipitation. For enantioselective chromatography the interferences must be efficiently removed by the precolumn since the chiral analytical columns are very sensitive. Recently, Boos et al. introduced a new kind of restricted access material alkyl-diol silica (ADS) [20]. This bimodal phase contains pores with apolar inner surface by means of which small molecules (<15 kDa) can be retained. Since the outer surface covered with diol groups does not adsorb or denaturate proteins, these matrix components are washed off the precolumn. The ADS material therefore fulfils the demands for a precolumn and has proven suitable for an on-line coupling to a chiral stationary phase (CSP) [21–24].

The separation of the sotalol enantiomers on a cellobiohydrolase I (CBH) column was previously described by our group [25]. In this paper, we will focus on the method development and validation for the combination of the LiChrospher ADS and the CBH chiral stationary phase. Furthermore, the applicability of the method to real world samples will be evaluated by means of two pharmacokinetic studies.

2. Experimental

2.1. Chemicals and reagents

(*S*)-(+)-Sotalol hydrochloride, racemic sotalol hydrochloride and Sotalex® mite tablets containing each 80 mg of (*RS*) sotalol hydrochloride were kindly provided by Bristol-Myers Squibb (Regensburg, Germany). (*S*)-(−)-Atenolol (99% ee) was purchased from Sigma–Aldrich Chemie (Deisenhofen, Germany), HPLC grade methanol, 2-propanol and disodium EDTA puriss. p.a. from Fluka Chemie (Seelze, Germany) and sodium dihydrogenphosphate p.a. from Merck (Darmstadt, Germany). The quantitative protein determination (see Section 3.1.2) was performed with standardised human serum Qualitrol® HS N, copper(II) sulfate pentahydrate p.a., the Folin-Ciocalteus phenol reagent equivalent to the molybdate–wolframate reagent according to Ph. Eur., disodium carbonate p.a., and disodium tartrate dihydrate p.a. from Merck.

2.2. Solutions

Stock solutions were prepared by dissolving 11.34 mg (*RS*)-sotalol hydrochloride (corresponding to 10.0 mg (*RS*)-sotalol) in 100.0 ml of solvent A (Sot.A.1) and solvent B (Sot.B.1), respectively, and by dissolving 10.0 mg (*S*)-atenolol in 100.0 ml of

solvent A (At.A.1). Thus, all stock solutions contained the analytes in a concentration of 100.0 μg ml^{-1}. Sample solutions without plasma were obtained by diluting the stock solutions with the corresponding solvent to the desired concentrations of 10 μg ml^{-1} (*RS*)-sotalol in solvent B (Sot.B.2) and 1 μg ml^{-1} (*RS*)-sotalol in solvent A (Sot.A.2).

The plasma samples for the method development were prepared by spiking 1.25 ml plasma with 250 μl of a solution containing 6 μg ml^{-1} (*RS*)-sotalol and 3 μg ml^{-1} (*S*)-atenolol in solvent A resulting in final concentrations of 0.5 μg ml^{-1} for each of the three analytes. For the calibration curve, 1.25 ml plasma were spiked with 250 μl of a solution containing 3 μg ml^{-1} (*S*)-atenolol and 0.3, 0.6, 1.5, 3, 6 and 12 μg ml^{-1} (*RS*)-sotalol, respectively, in solvent A. The method validation was performed with the optimised solvent A consisting of 1% methanol in 10 m*M* phosphate buffer at pH 7.4. The sample solutions for the method development were prepared in dependence on the actually investigated mobile phases with either the solvent A consisting of methanol in the given concentration in 10 m*M* phosphate buffer at the given pH value or solvent B consisting of 2-propanol in the given concentration in 10 m*M* phosphate buffer (pH 7.0) including 0.05 m*M* EDTA.

The plasma samples for the pharmacokinetic studies were prepared by spiking 1.25 ml plasma with 250 μl of a solution containing 3 μg ml^{-1} (*S*)-atenolol in solvent A resulting in a final concentration of 0.5 μg ml^{-1} for the internal standard.

2.3. Apparatus

The chromatographic system consisted of two Waters 501 HPLC Pumps (Waters, Eschborn, Germany), a Rheodyne Model 7725i injector equipped with a 200-μl loop and a Rheodyne Model 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, USA). A Rheodyne Model 7000 was used as switching valve. Detection was performed with an FD-500 programmable fluorescence detector (Groton Technology, Concord, MA, USA) and a Waters 486 UV-detector. Eluents were degassed by passing an In-Line Degasser, the column temperature was controlled by a Jetstream 2 Peltier-Column-Thermostat (VDS optilab, Montabaur, Germany). The software used for recording the chromatograms was ChromStar light Version 4.05 (SCPA, Stuhr, Germany). The precolumn was a LiChroCart (25×4 mm I.D.) column packed with LiChrospher RP-18 ADS (25 μm) from Merck. The chiral stationary phase was a Chiral-CBH® column (150×4 mm I.D., 5 μm) (Chromtech, Hägersten, Sweden) coupled with a corresponding guard column (Chiral-CBH®, 10×3 mm I.D., 5 μm).

The instrumentation set-up of the apparatus for on-line coupling of the precolumn and the analytical column is identical to literature instructions [20]. During the sample pretreatment step, solvent A passes the precolumn. The transfer of the analytes and their separation is started by switching the valve at the time t_{V1}. In this valve position, solvent B runs the opposite direction through the precolumn and thereafter through the analytical column. The injector with the 200-μl loop is located before the precolumn and is used for the injection of plasma samples, whereas the 20-μl loop injector is directly connected to the analytical column for analysis circumventing the precolumn. The UV detector is in the waste line of the precolumn used for the evaluation of the elution profiles of the analytes (see Section 3.1.1) whereas the fluorescence detector is passed by the analytes after their separation on the CSP.

2.4. Chromatographic conditions

The mobile phases, solvent A and solvent B, were prepared by dissolving the indicated amount of organic modifier in 10 m*M* phosphate buffer (v/v). The solvent B which passes the analytical column additionally contained 0.05 m*M* EDTA since the CSP is sensitive to metal cations. The pH of the mobile phases were adjusted with NaOH to the given value. Prior to chromatography, the mobile phases were filtered through a 0.45 μm regenerated cellulose filter (Schleicher & Schuell, Dassel, Germany). The flow-rate was 0.5 ml min^{-1} for the sample preparation and 0.9 ml min^{-1} for the analytical separation. The CSP was thermostated at 10 °C. The UV detection was carried out at λ 230 nm and the fluorescence detection at λ 250 nm (excitation wavelength, λ_{ex})/312 nm (emission wavelength, λ_{em}). The enantiomeric elution order was determined by injecting samples of pure (*S*)-(+)-sotalol.

3. Results and discussion

3.1. Method development

The method development for the on-line coupling of a precolumn to a chiral analytical column with a switching valve is a multi-step procedure. Two mobile phases and two valve-switching times have to be optimised. Furthermore the quantitative determination from biofluids demands an internal standard which should possess spectroscopic and hydrophilic properties similar to those of the analytes. Therefore (*S*)-atenolol (Fig. 1), which has already been used in several quantitative chromatographic determination methods for sotalol, was chosen as internal standard [3,5,17].

Solvent A passes the precolumn and should elute the matrix components completely from the precolumn while the analytes are retained. After this fractionation step the switching valve is turned (first valve-switching time, t_{V1}) and the precolumn then backflushed with solvent B. This mobile phase must possess a higher elution capacity than solvent A to transfer the analytes as fast as possible to the analytical column in order to minimize the peak broadening. When the analyte transfer is completed, the switching valve is turned back to its initial position (second valve-switching time, t_{V2}) in order to reequilibrate the precolumn with solvent A. Solvent B does not only transfer the analytes to the chiral stationary phase but must also enable their separation. Furthermore, the two solvents should not differ too much in order to avoid interfering signals upon switching the eluents and to maintain the equilibration of the CSP.

3.1.1. Elution profiles of the analytes

The first step consisted in the determination of the elution profiles of the analytes in order to deduce the delay with which the analytes start to elute from the precolumn. This time obviously depends on the composition of the washing liquid (solvent A). The elution profiles were determined by monitoring the UV absorbance of sotalol and atenolol which were injected on the precolumn directly connected to the UV detector. The washing liquid must not possess denaturating properties to avoid precipitation of the proteins on the precolumn. Therefore the pH value of solvent A should be close to physiological pH. The addition of small amounts of organic modifier is known to prolong the lifetime of the precolumn when analysing samples with lipidic components.

The optimisation was started with a washing liquid consisting of 2% methanol in a 10 m*M* phosphate buffer at pH 7.0 at a flow-rate of 0.5 ml min^{-1}. Since sotalol is rather polar, its elution already began after 8.1 min. This period is not long enough for a complete removal of the matrix components. Since sotalol has two ionisable functionalities, the sulfonamide (pK_S 8.3) and the amino group (pK_S 9.8), sotalol exists to a larger extent in its zwitterionic form with a decreased polarity at higher pH values. The results for pH 7.0, 7.2 and 7.4 are presented in Table 1 showing a minor effect of the mobile phase pH. However, by decreasing the methanol content of the washing liquid from 2 to 1% a remarkable gain in elution time was achieved. A further decrease to 0.5% methanol only led to a slight additional improvement. Since the organic modifier should protect the precolumn from contamination with hydrophobic endogenous compounds, a methanol concentration of 1% was chosen for sample pretreatment. Due to its lower polarity, atenolol was always better retained on the precolumn compared to sotalol and its elution was therefore uncritical.

3.1.2. Elution profile of the matrix components

In the next step the elution profile of the matrix components was evaluated to set the first valve-switching time. For this purpose blank plasma samples were injected on the precolumn and the time needed for a complete elution of the proteins was

Table 1
Influence of the composition and the pH value of the washing liquid on the elution profiles of sotalol and atenolol

Methanol (%)	pH value	Sotalol (min)	Atenolol (min)
2.0	7.0	8.1	9.7
2.0	7.2	8.8	10.6
2.0	7.4	9.6	11.5
1.0	7.4	12.1	15.9
0.5	7.4	13.2	16.7

Precolumn, LiChrospher ADS RP-18; mobile phase, methanol in the given concentration in 10 m*M* phosphate buffer at the given pH value; flow-rate, 0.5 ml min^{-1}; UV detection, 230 nm; samples, sotalol (1 μg ml^{-1}) and atenolol (1 μg ml^{-1}) in the mobile phase; injection, 200 μl.

determined. The eluate was collected every 30 s and the protein content was quantified according to Lowry [26]. This method is based on the molybdan blue reaction and the limit of detection was evaluated to 5 μg ml^{-1} with a standardised human serum with a defined protein content. With the optimised solvent A, no more protein could be detected after 7 min. The first valve-switching time was set at 10 min to ensure a complete elution of the matrix without washing off the analytes.

3.1.3. Transfer profile of the analytes

Switching the valve initiates the transfer of the analytes. The required period of time corresponds to the duration of the quantitative backflush of the analytes from the precolumn to the CSP and determines the time t_{V2} when the valve can be turned back in its initial position allowing the reequilibration of the precolumn with solvent A. This time is measured with the detector directly connected to the switching valve. The completion of the analyte transfer was hardly recognisable due to a strong peak tailing. Therefore the peak area was calculated at a certain elution time in ratio to the whole peak area.

Solvent B is used for both the transfer of the analytes and their separation on the chiral column. Since the separation of the sotalol enantiomers on the Chiral-CBH is best with 2-propanol [25], different concentrations of this organic modifier were evaluated. The mobile phases consisted of 10%, 12.5% or 15% 2-propanol in 10 m*M* phosphate buffer at pH 7.0. More than 99% of sotalol were eluted after 5 min with 10% 2-propanol, after 4 min with 12.5% 2-propanol and after 3.5 min with 15% 2-propanol. Due to its lower polarity, atenolol is transferred more efficiently than sotalol under any condition. Since the faster elution of the analytes from the precolumn leads to higher and sharper peaks, 15% 2-propanol was chosen. The second valve-switching time was set at 5 min to achieve a nearly complete elution of the analytes.

3.1.4. Coupling to the chiral column

Preliminary investigations have shown that the column temperature has a distinct effect on the chiral separation of sotalol on Chiral-CBH [25]. A low temperature is recommended with 2-propanol-containing mobile phases. In fact, at 10 °C a satisfactory resolution of sotalol could be achieved with solvent B. A further decrease in temperature created too high a back pressure due to the viscosity of 2-propanol–water mixtures.

The following separations were performed with 15% 2-propanol in 10 m*M* phosphate buffer (pH 7.0) including 0.05 m*M* EDTA at 10 °C. First sotalol was injected directly on Chiral-CBH without sample pretreatment (Fig. 2I). In the next step, sotalol dissolved in the mobile phase was separated on the analytical column after HPLC-integrated sample preparation (Fig. 2II) in order to ensure a sufficient resolution despite the peak broadening caused by the precolumn. Furthermore, both the sotalol-spiked plasma (Fig. 2III) and the blank plasma (Fig. 2IV) were injected the same way in order to investigate whether matrix components interfere with the sotalol peaks.

The resolution factor of (*RS*)-sotalol on the Chiral-CBH without sample pretreatment is 2.9 (Fig. 2I). Since the analytes have to be backflushed from the precolumn prior to their separation, the retention times of (*R*)- and (*S*)-sotalol are prolonged for 68 and 85 s, respectively, by sample preparation. The selectivity factor α is not influenced by the sample pretreatment, whereas the resolution factor is decreased to 1.6 due to the peak broadening on the precolumn (Fig. 2II). The retention times and the resolution remain unchanged for sotalol-spiked plasma samples compared to the separation of sotalol dissolved in the mobile phase (Fig. 2III). In fact, the plasma components do not disturb the determination of (*RS*)-sotalol since no interfering signals appear in the range of the sotalol peaks (Fig. 2IV). The elution of matrix components from the precolumn leading to peaks at the beginning of the chromatogram can be stopped by turning back the switching valve after 5 min (t_{V2}).

3.1.5. Addition of the internal standard (S)-atenolol

In the last step of the method development the question whether (*S*)-atenolol can be used as internal standard had to be addressed. The fluorescence properties of atenolol and sotalol dissolved in the mobile phase are very similar. The absorption and emission maxima for atenolol are λ_{ex} 247 nm and λ_{em} 304 nm and for sotalol λ_{ex} 250 nm and λ_{em} 312

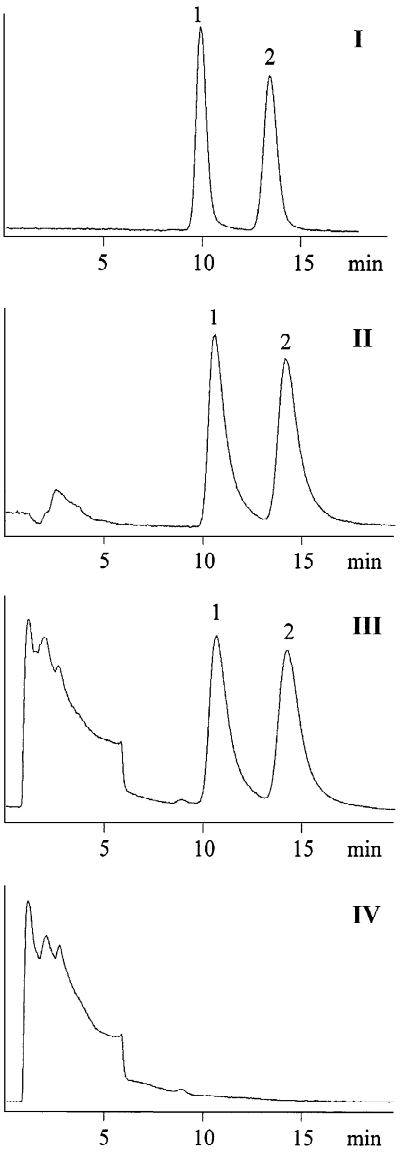

Fig. 2. Chiral analysis of (*RS*)-sotalol without and with HPLC-integrated sample preparation. Precolumn, LiChrospher RP-18 ADS (25×4.0 mm I.D.); solvent A, 1% methanol in 10 m*M* phosphate buffer at pH 7.4; flow-rate, 0.5 ml min^{-1}; t_{V1}, 10 min; t_{V2}, 5 min; analytical column, Chiral-CBH (150×4.0 mm I.D.); solvent B, 15% 2-propanol in 10 m*M* phosphate buffer (pH 7.0) including 0.05 m*M* EDTA, 10 °C; flow-rate, 0.9 ml min^{-1}; detection, fluorescence at 250 nm (λ_{ex})/312 nm (λ_{em}); injection of (I) 20 μl of (*RS*)-sotalol (10 μg ml^{-1} in solvent B) (Sot.B.2) without sample pretreatment, (II) 200 μl of (*RS*)-sotalol (1 μg ml^{-1} in solvent A) (Sot.A.2) with sample pretreatment, (III) 200 μl of (*RS*)-sotalol (1 μg ml^{-1} in plasma) with sample pretreatment and (IV) 200 μl of blank plasma with sample pretreatment. 1, (*R*)-sotalol; 2, (*S*)-sotalol.

nm, respectively. Furthermore the chromatographic separation of (*RS*)-sotalol and (*S*)-atenolol is possible with the optimised mobile phases and valve-switching times as depicted in Fig. 3.

The overall analysis time including the sample preparation is 38 min consisting of 10 min fractionation and 28 min transfer and analytical separation periods. Since the reequilibration of the precolumn and the fractionation step can be performed parallel to the analytical separation on the CSP, the time delay between two injections is only 29 min.

3.2. Method validation

Unless otherwise indicated, the method validation was performed throughout with sotalol- and atenolol-spiked plasma samples which were prepared as described in the Experimental.

3.2.1. Linearity

The calibration curve was evaluated in the concentration range from 25 to 1000 μg l^{-1} for each sotalol enantiomer with the internal standard (*S*)-atenolol added at a constant concentration of 500 μg l^{-1}. The peak areas of the sotalol enantiomers were

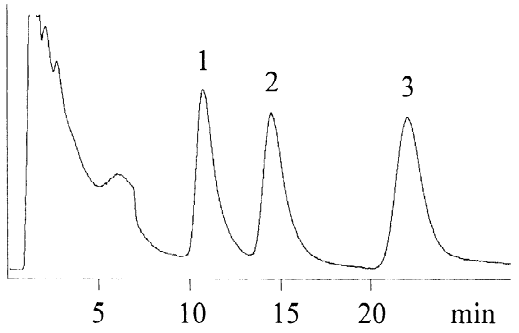

Fig. 3. Chromatogram of (*RS*)-sotalol and the internal standard (*S*)-atenolol after HPLC-integrated sample preparation. Precolumn, LiChrospher RP-18 ADS (25×4.0 mm I.D.); solvent A, 1% methanol in 10 m*M* phosphate buffer at pH 7.4; flow-rate, 0.5 ml min^{-1}; t_{V1}, 10 min; t_{V2}, 5 min; analytical column, Chiral-CBH (150×4.0 mm I.D.); solvent B, 15% 2-propanol in 10 m*M* phosphate buffer (pH 7.0) including 0.05 m*M* EDTA, 10 °C; flow-rate, 0.9 ml min^{-1}; detection, fluorescence at 250 nm (λ_{ex})/312 nm (λ_{em}); injection of 200 μl of (*RS*)-sotalol (1 μg ml^{-1}) and (*S*)-atenolol (0.5 μg ml^{-1}) dissolved in plasma. 1, (*R*)-sotalol; 2, (*S*)-sotalol; 3, (*S*)-atenolol.

divided by the peak area of the internal standard. This ratio (y) was plotted versus the analyte concentration (x) in $\mu g\ l^{-1}$ leading to the linear regression analysis for (R)- and (S)-sotalol. The following equations were obtained:

(R)-sotalol: $y = 0.15632x - 0.55718$, $r^2 = 0.9999$

(S)-sotalol: $y = 0.16941x - 1.15519$, $r^2 = 0.9999$

Both calibration curves show an excellent linearity demonstrated by the correlation coefficients r^2. The standard deviations of the slopes are 0.00011 for (R)- and 0.00031 for (S)-sotalol and the standard deviations of the intercepts are 0.05007 for (R)- and 0.14783 for (S)-sotalol, respectively.

3.2.2. Detectability

The limits of detection (LODs) and quantitation (LOQs) were determined as analyte concentrations corresponding to three and 10 times the standard deviation of the noise signal, respectively. For both enantiomers the LODs are 18 $\mu g\ l^{-1}$ and the LOQs are 37 $\mu g\ l^{-1}$.

3.2.3. Recovery rate

The recovery rate was determined by means of the peak areas after injection of (R)- and (S)-sotalol and (S)-atenolol at concentrations of 500 $\mu g\ l^{-1}$ each dissolved in plasma after sample preparation divided by the peak areas at the same concentrations dissolved in the mobile phase directly injected on the analytical column. These injections were performed three times each and the recovery rates are 94.0±2.1% for (R)-sotalol, 100.1±2.2% for (S)-sotalol and 99.4±1.1% for (S)-atenolol.

3.2.4. Accuracy

The accuracy of the method was determined by injecting four times spiked plasma samples containing 500 $\mu g\ l^{-1}$ of each (R)-,(S)-sotalol and (S)-atenolol. The accuracy was calculated as the relative difference of the mean from the true value and is −3.58±2.86% for (R)-sotalol and −3.76±2.77% for (S)-sotalol. The corresponding t values are 2.50 for (R)-sotalol and 2.71 for (S)-sotalol and are both smaller than the critical t value of 3.18 [27]. Therefore the accuracy of the method is guaranteed with a probability of more than 95%.

3.2.5. Precision

The precision of a bioanalytical method characterises the reproducibility of the results. The intra-day variability was calculated as the relative standard deviation from four injections of (RS)-sotalol at concentrations of 50, 100, 250, 500 and 1000 $\mu g\ l^{-1}$ of each enantiomer. The inter-day variability was determined at the same sample concentrations on 4 different days and corresponds to the relative standard deviation of the mean values of the daily performed injections. The results are shown in Table 2. The average values of the intra- and inter-day variability are 1.17% and 3.42%, respectively, for (R)-sotalol and 1.24% and 1.99%, respectively, for (S)-sotalol. The high precision of this method is based on the simple handling of the sample preparation keeping experimental errors to a minimum.

Table 2
Precision of the method as intra- and inter-day variability

Concentration ($\mu g\ l^{-1}$)	Intra-day variability (%)		Inter-day variability (%)	
	(R)-sotalol	(S)-sotalol	(R)-sotalol	(S)-sotalol
1000	0.28±0.20	0.28±0.18	3.80	2.89
500	0.73±0.26	0.83±0.60	3.10	1.99
250	1.17±0.58	1.03±0.33	3.85	1.44
100	1.39±0.82	1.57±0.56	2.72	0.47
50	2.27±0.51	2.49±0.69	3.63	3.15

3.2.6. Column stability

Lambrecht et al. reported that the ADS precolumn performance is drastically reduced after several injections of 50 μl plasma [24]. They reasoned that components of the plasma matrix below 15 kDa exclusion limit can penetrate into pores and undergo absorption. Thus the property of the ADS material is made more polar. Since sotalol is rather hydrophilic its retention on the precolumn might become incomplete. However, no significant loss in retention of sotalol on the ADS material could be observed after more than 200 injections of 200 μl spiked plasma samples (1.25 ml plasma and 0.25 ml internal standard in solvent A). This could be deduced from the unchanged high recovery rate determined after ~80 and 200 injections, respectively. Small matrix components which are retained on the ADS material during the washing step might be eluted from the precolumn by the less polar solvent B and might be transferred to the CSP. This explanation for the surprising long lifetime of the precolumn is supported by the appearance of interfering signals caused by matrix components in the beginning of the chromatograms which can be stopped by turning back the switching valve (Fig. 2IV). To ensure high reproducibility it is nevertheless recommended to replace the precolumn after 200 injections. This procedure is still less expensive than the use of solid-phase extraction columns.

Protein-based chiral stationary phases are usually not as stable as other CSPs. Eluents passing the Chiral-CBH should contain EDTA since this CSP is sensitive to metal cations. However, in the course of the method development for the chiral separation of sotalol on the Chiral-CBH [25] a slight deterioration of the column selectivity and efficiency could be observed after more than 300 runs. This method development procedure comprised frequent changes of the mobile phase composition and of the column temperature up to 40 °C resulting in a continuous stress of the CSP. The herein developed and validated method performed with a new analytical column uses the same mobile phase at a low temperature of 10 °C throughout. Therefore no loss in selectivity and resolution could be observed after more than 200 injections. It is expected that the lifetime of the analytical column exceeds 500 runs under these gentle conditions.

3.3. Pharmacokinetic studies of the sotalol enantiomers

3.3.1. Pharmacokinetics of sotalol in young healthy volunteers after single dose application

The pharmacological profile of the sotalol enantiomers applied as a single dose of 80 mg (*RS*)-sotalol hydrochloride, corresponding to 35.28 mg (*R*)- and (*S*)-sotalol, respectively, to three healthy volunteers aged between 29 and 31 years was evaluated. The plasma concentration of the sotalol enantiomers was determined after 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h following oral application of the drug. The maximum plasma concentrations c_{max} were 404±82 μg l^{-1} for (*S*)-sotalol and 394±69 μg l^{-1} for (*R*)-sotalol and were reached after t_{max} 2.1±0.9 h for both enantiomers. The half-life $t_{1/2}$ was determined as 6.64±1.02 h for (*S*)-sotalol and 6.65±1.18 h for (*R*)-sotalol. The area under the curve values $AUC_{0-\infty}$ were 3.53±0.58 mg h l^{-1} for (*S*)-sotalol and 3.48±0.65 mg h l^{-1} for (*R*)-sotalol. Fig. 4 shows the plasma concentration–time curve of one volunteer. In accordance with the pharmacokinetic study on healthy volunteers by Carr et al. [28] the sotalol enantiomers show no significant pharmacokinetic discrimination. The slightly bigger $AUC_{0-\infty}$ value for (*S*)-sotalol can be explained by the smaller renal clearance of (*S*)-sotalol due to the

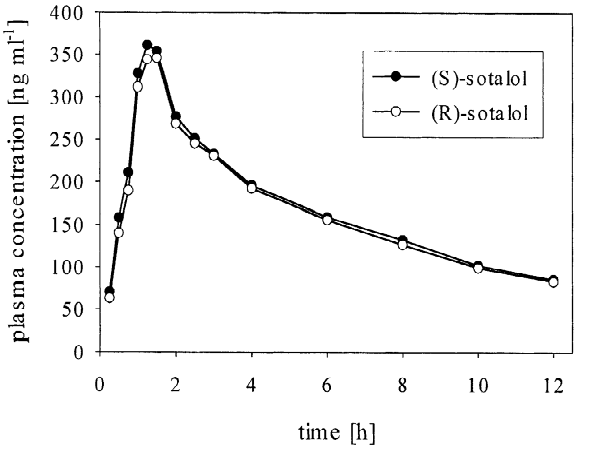

Fig. 4. Plasma concentration–time curve of (*R*)- and (*S*)-sotalol for a healthy volunteer after oral single dose application of 80 mg (*RS*)-sotalol hydrochloride. Symbols: ○, (*R*)-sotalol; ●, (*S*)-sotalol.

active renal secretion process via a tubular base carrier which is subject to a weak chiral recognition [29]. These stereoselective differences in the secretion process should be more perceptible with patients with limited renal function.

3.3.2. Pharmacokinetics of sotalol in elder patients in the steady state

For the pharmacokinetic study of the sotalol enantiomers in the steady state, plasma levels of patients with an average age of 65 suffering from arrythmia were determined. The results for 15 patients are shown in Table 3 and a typical chromatogram of plasma (patient no. 8) spiked with the internal standard is depicted in Fig. 5. The developed method is suitable for nine from 15 plasma samples. In four cases the plasma concentrations exceed the evaluated linear range, but the ratio of (*R*)- versus (*S*)-sotalol reveals that the linear range could be expanded up to concentrations of 2000 μg l^{-1}. In only two cases the method is not sensitive enough to determine the plasma concentrations.

Sotalol is mainly eliminated by renal excretion. The glomerular filtration rate is often decreased for elder patients. Furthermore, they have an increased production of α_1-acid glycoproteins which can lead to a stereodifferentiating distribution due to a stronger plasma protein binding of the sotalol enantiomers. Therefore stereoselective differences in excretion and distribution should be more remarkable in elder patients as observed by Fiset et al. [30] for such patients upon long-term treatment with (*RS*)-sotalol. We could not confirm these findings, but we

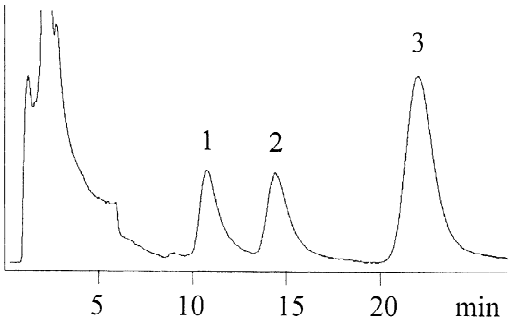

Fig. 5. Chromatogram of a patient plasma sample with addition of the internal standard (*S*)-atenolol after HPLC-integrated sample preparation. Precolumn, LiChrospher RP-18 ADS (25×4.0 mm I.D.); solvent A, 1% methanol in 10 m*M* phosphate buffer at pH 7.4; flow-rate, 0.5 ml min^{-1}; t_{V1}, 10 min; t_{V2}, 5 min; analytical column, Chiral-CBH (150×4.0 mm I.D.); solvent B, 15% 2-propanol in 10 m*M* phosphate buffer (pH 7.0) including 0.05 m*M* EDTA, 10 °C; flow-rate, 0.9 ml min^{-1}; detection, fluorescence at 250 nm (λ_{ex})/312 nm (λ_{em}); injection of 200 μl of plasma spiked with (*S*)-atenolol (0.5 μg ml^{-1}). 1, (*R*)-sotalol; 2, (*S*)-sotalol; 3, (*S*)-atenolol.

Table 3
Plasma concentration of (*R*)- and (*S*)-sotalol of patients in the steady state

Patient no.	Concentration (μg l^{-1}) of		Percentage (%) of	
	(*R*)-sotalol	(*S*)-sotalol	(*R*)-sotalol	(*S*)-sotalol
1	316.6	306.9	50.77	49.23
2	1330.6[a]	1332.4[a]	49.97	50.03
3	328.9	325.6	50.25	49.75
4	1129.6[a]	1108.8[a]	50.46	49.54
5	210.6	218.3	49.11	50.89
6	682.3	658.2	50.90	49.10
7	38.1	29.1[b]	(56.70)	(43.30)
8	268.7	249.2	51.89	48.11
9	489.2	486.0	50.16	49.84
10	114.4	116.5	49.55	50.45
11	38.0	28.9[b]	(56.81)	(43.19)
12	548.4	554.8	49.71	50.29
13	1413.9[a]	1445.3[a]	49.45	50.55
14	1145.8[a]	1127.7[a]	50.40	49.60
15	445.5	459.4	49.23	50.77
Mean			50.14	49.86

[a] The plasma concentrations are outside the evaluated calibration range.
[b] The plasma concentrations are below the limit of quantitation of 37 μg l^{-1}.

observed that the plasma concentrations of the two enantiomers do not differ significantly independent of the duration of the therapy, the dosage and the time of sample withdrawal.

4. Conclusions

In this paper, the analytical method development for the stereoselective determination of sotalol in plasma is described. For this purpose a LiChrospher ADS precolumn for sample pretreatment was coupled with a Chiral-CBH column for the separation of the sotalol enantiomers. The composition of the washing liquid was optimised by increasing the pH value and by decreasing the content of the organic modifier to 1% methanol in 10 m*M* phosphate buffer at pH 7.4. The first valve-switching time was set at 10 min for a quantitative removal of the proteins. With a mobile phase containing 15% 2-propanol in 10 m*M* phosphate buffer (pH 7.0) including 0.05 m*M* EDTA the transfer to the CSP and the chiral separation of the analytes were performed at 10 °C. The second valve-switching time was set at 5 min guaranteeing a complete analyte transfer.

The quantitation of sotalol was possible by means of addition of the internal standard (*S*)-atenolol. The method was validated showing a good linearity in the concentration range from 25 to 1000 $\mu g\ l^{-1}$ of each enantiomer. Due to the on-line coupling of the two columns a high sample throughput can be achieved with an effective analysis time of 29 min. This is by far faster than the hitherto established methods for the stereoselective determination of sotalol in plasma except one [15,16,18,19] with which the analytical separation alone devoid of the sample preparation step requires 30 min and more. Furthermore, the herein described method can be fully automatised allowing overnight runs.

The suitability of this method for a therapeutic drug monitoring of the sotalol enantiomers was shown by two pharmacokinetic studies with healthy young volunteers after single dose application and elder patients in the steady state. The results revealed that the sotalol enantiomers do not differ in their pharmacokinetic properties.

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