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Enantioselective analysis of metoprolol in plasma using high-performance liquid chromatographic direct and indirect separations: applications in pharmacokinetics

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Abstract

Direct enantioselective separation on chiral stationary phases and indirect separation based on the formation of diastereomeric derivatives were developed and compared for the HPLC analysis of *R*(+) and *S*(−)-metoprolol in human plasma. Plasma samples prepared using solid-phase extraction columns or liquid–liquid extraction were directly analyzed on a Chiralpack® AD or on a Chiralcel® OD-H columns, respectively. *S*-(−)-menthyl choroformate was also used to yield diastereomeric derivatives resolved on a RP-8 column. The methods were employed to determine plasma concentrations of metoprolol enantiomers in a pharmacokinetic study of single dose administration of racemic metoprolol to a healthy Caucasian volunteer phenotyped as extensive metabolizer of debrisoquine. The correlation coefficients among enantioselective metoprolol plasma concentrations (5–223 ng/ml) obtained by the three methods were equal or higher than 0.99. The direct method that employed the chiral column Chiralpak® AD may be considered the most sensitive, although the three methods demonstrated interchangeable use in the pharmacokinetic investigation. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: Enantiomer separation; Metoprolol

1. Introduction

Metoprolol (1-isopropylamine-3-[4-(2-metoxy-ethyl)phenoxy]-2-propanol) is a β_1-adrenoceptor selective antagonist clinically used in the racemic form for the treatment of hypertension and ischaemic heart disease. Like most β-blockers, the affinity for β_1-adrenoceptor is significantly higher for the *S*-(−)metoprolol [1,2].

Metoprolol is mainly eliminated by hepatic oxidative metabolism. In humans, the main metabolic pathways are *O*-demethylation, oxidative deamination and α-hydroxylation. The *O*-demethylated metabolite undergoes further oxidation to the corresponding carboxylic acid, relatively the major metabolic pathway, which is responsible for the elimination of 65% of the dose in humans. The oxidative deamination process is less important quantitatively

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in metoprolol elimination (<10% of the dose). The α-hydroxylation pathway contributes to the elimination of only 10% of the dose but it is polymorphic, depends on the genetically determined activity of CYP2D6 and results in the formation of the pharmacologically active metabolite α-hydroxymetoprolol, with 1/10 of the pharmacological activity of metoprolol. CYP2D6 also participating in metoprolol *O*-demethylation, although other isoforms of CYP are also involved in this process [2]; Fig. 1.

In vivo and in vitro studies using human liver microsomes have suggested enantioselectivity in the α-hydroxylation and *O*-demethylation pathways, favoring the *R*-(+)-metoprolol enantiomer. Extensive metabolizers have greater capacity to eliminate the *R*-(+)-metoprolol enantiomer and therefore higher clearance for this enantiomer. However, poor metabolizers have an equal or even lower clearance for the *R*-(+)-metoprolol than for its antipode *S*-(−). Consequently, the oxidation phenotype depending on CYP2D6 activity and enantioselectivity represent the major causes of interindividual variability in the pharmacokinetics and pharmacodynamics of this betablocker [2–4].

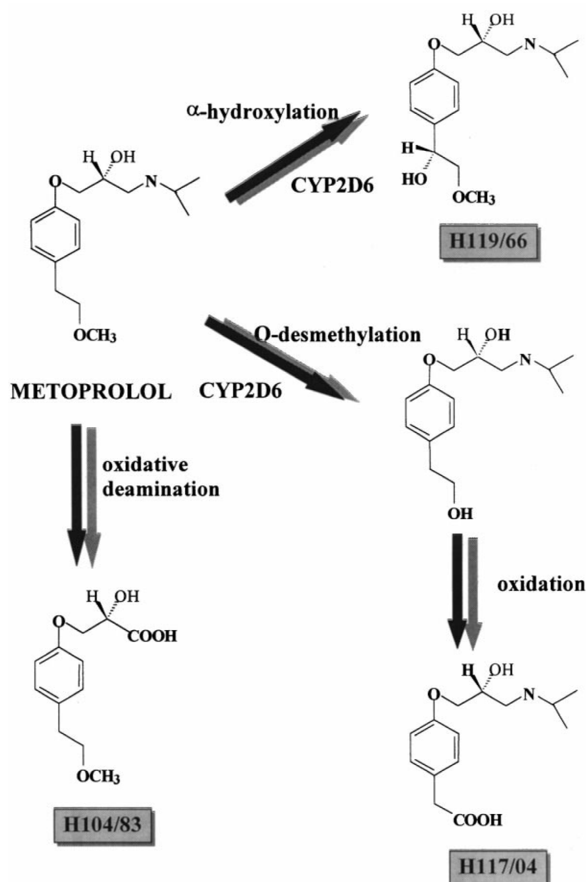

Fig. 1. Oxidative metabolism of metoprolol.

Enantioselective analysis of metoprolol in standard solutions or in plasma or urine samples for pharmacokinetic application can be performed by direct or indirect methods [3,5–28].

Direct analysis is described by using chiral additives in the mobile phase [3,5] or with the use of chromatography columns based on chiral stationary phases [6–17]. (+)-10-Camphor-10-sulfonic acid is the only mobile phase chiral additive described for the separation of metoprolol enantiomers in biological fluids [3,5]. Only two types of chiral stationary phases have been described for the direct enantioselective analysis of unchanged metoprolol in biological fluids with application in studies of kinetic disposition: cellulose tris(3,5-dimethylphenylcarbamate) polymer coated on 10 μm macroporous silica [7–12] and silica bonded α_1-acid glycoprotein [13–16]. Yang et al. [17] used a fluorogenic reagent, 4-(*N*-chloroformylmethyl-*N*-methyl) amino-7-*N*,*N*-dimethylaminosulfonyl-2,1,3-benzoxadiazole, to yield metoprolol derivatives separated enantiomerically on a Chiralcel® OJ-R (cellulose tris(4-methylbenzoate) coated on silica gel) column.

Chiral derivatization is described as an indirect method for optical isomer resolution through the conversion of the enantiomers to their corresponding diastereomeric derivatives. Metoprolol enantiomers have been resolved as the derivatives formed with several chiral reagents as (+)- and (−)-4-(6-methoxy-2-naphthyl)-2-butyl chloroformate [18], *S*-(−)-menthyl chloroformate [19,20], *S*-*tert*.-butyl-3-(chloroformoxy) butyrate [20], *S*-(+)-l-(l-naphthyl)-ethyl isocyanate [21], (*R*)(+)-phenylethyl isocyanate [22], 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) [23,24], *S*-(+)-benoxaprofen chloride [25], (*S*)-(−)-phenylethyl isocyanate [26] and *tert*.-butoxycarbonyl-L-leucine anhydride [27,28]. *S*-(+)-benoxaprofen chloride and *S*-(−)-menthyl chloroformate were employed for the resolution of metoprolol enantiomers in urine. The other chiral

reagents were used for the determination of metoprolol enantiomers in plasma.

In the present study we develop and compare procedures for the enantioselective analysis of metoprolol in human plasma using direct (Chiralpak AD® and Chiralcel OD-H® columns) and indirect HPLC methods (chiral derivatization with *S*-(−)-menthyl chloroformate). We also report a preliminary investigation of enantioselectivity in the kinetic disposition of metoprolol administered in a single dose to a healthy Caucasian volunteer phenotyped as extensive metabolizer of debrisoquine.

2. Experimental

2.1. Chemicals

(±)-Metoprolol tartrate was obtained from Sigma (St. Louis, MO, USA), *S*-(−)metoprolol hydrochloride (H 150/65), *R*-(+)-metoprolol hydrochloride (H 150/64) and the metoprolol metabolites H 117/04 hydrochloride (acid metabolite), H 119/66 as p-OH benzoate (α-OH metoprolol), H 105/22 as p-OH benzoate (*O*-demethylmetoprolol) and H 104/83 were kindly donated by Astra Hässle AB, Sweden. The derivatization reagent *S*-(−)-menthyl chloroformate was obtained from Sigma (St. Louis, MO, USA). Solvents used as mobile phases were HPLC-grade from Merck (Darmstadt, Germany). Other reagents were of analytical-reagent grade. Water was purified with a Milli-Q Plus ultra-pure water system (Millipore, Bedford, USA).

2.2. Apparatus

HPLC system consisted of a model 7125 manual loop injector (Rheodyne, Berkeley, CA, USA), a pump for isocratic elution, a model RF 551 fluorescence detector and a model C-R6A integrator (Shimadzu, Kyoto, Japan). The chiral columns used were an amylose tris(3,5-dimethylphenylcarbamate) coated on a 10 μm silica-gel substrate (Chiralpak® AD, 250×4.6 mm) and a cellulose tris-(dimethylphenyl carbamate) on a 5 μm silica-gel substrate (Chiralcel® OD-H, 150×4.6 mm), both from Daicel Chemical Industries, LO, California, USA. The diastereomeric derivatives of metoprolol were resolved on a Lichrospher® 100 RP-8 column (125×4 mm I.D., 5 μm particle size) with a similar 4×4 mm precolumn from Merck, Darmstadt, Germany.

2.3. Standard solutions

Standard solutions of (±)-metoprolol tartrate were prepared in methanol at 0.2 mg free base/ml. The diluted solutions were prepared at concentrations of 0.4, 0.8, 2.0 and 4.0 μg/m1 methanol. Solutions of the individual metoprolol enantiomers as well as of metoprolol metabolites were prepared to a concentration of 0.1 mg/ml free base in methanol. All of the standard solutions were stored at −20°C in the dark.

S-(−)-menthyl chloroformate was dissolved daily in dichloromethane to a concentration of 2% and stored at 4°C when not in use. Sodium chloride (Merck, Darmstadt, Germany) and sodium hydroxide solutions were washed twice with diisopropyl ether–dichloromethane 1:1, v/v.

2.4. Sample preparation

2.4.1. Liquid–liquid extraction (LLE)

Plasma samples (1000 μl) were alkalinized with 100 μl of a 1 *M* NaOH aqueous solution. After shaking in a mixer for 30 s and a 5 min rest, 50 mg sodium chloride and 6.0 ml dichloromethane–diisopropyl ether (1:1, v/v) were added. The samples were extracted for 30 min in a horizontal shaker (220±10 cycles/min) and centrifuged at 2000 *g* for 5 min and the organic phases (5.0 ml) were transferred to conical tubes and evaporated to dryness under an air flow at room temperature.

2.4.2. Solid-phase extraction (SPE)

Plasma samples (1000 μl) were supplemented with 500 μl of a saturated aqueous solution of disodium tetraborate. The mixtures were applied to LC-18 SPE columns (Supelclean® LC-18 SPE, 3.0 ml tubes, Supelco, Bellefonte, PA, USA) preconditioned by sequential washing with methanol (2.0 ml) and a saturated aqueous solution of disodium tetraborate (2.0 ml). Cartridges were washed successively with 2.0 ml 0.02 *M* disodium tetraborate

solution and 2.0 ml water. Excess of water was removed by leaving the cartridges in a vacuum system for 20 min, washing with 1.0 ml acetonitrile and maintenance in a vacuum system for an additional 4 min. The analytes were eluted from the cartridges with 6.0 ml methanol. The eluates were evaporated to dryness under an air flow at room temperature.

2.5. Direct analysis

2.5.1. Chiralcel® OD-H separation

The residue obtained in the LLE procedure was dissolved in 100 μl of the mobile phase consisting of a mixture of hexane–isopropanol–diethylamine (95:5:0.1, v/v/v). The samples were injected with a 20 μl injection loop and the separation of the *R*-(+) and S(−)-metoprolol enantiomers was performed on a Chiralcel® OD-H column with a 4×4 mm Licrospher® 100 RP18 precolumn, 5 μm particle size (Merck, Darmstadt, Germany). The flow-rate was 1.0 ml/min. Detection of the individual enantiomers was achieved at 229 nm (λ_{exc}) and 298 nm (λ_{em}).

2.5.2. Chiralpak® AD separation

The residue obtained in the SPE procedure was dissolved in 100 μl of the mobile phase consisting of a mixture of hexane–ethanol–isopropanol–diethylamine (88:10.2:1.8:0.2, v/v/v/v). The separation of the enantiomers was performed on a Chiralpak® AD column with a 4×4 mm Licrospher® 100 CN precolumn, 10 μm particle size (Merck, Darmstadt, Germany). The flow-rate was 1.2 ml/min. HPLC system operated with a 50 μl loop and a fluorescence detector at 229 nm (λ_{ex}) and 298 nm (λ_{em}).

2.6. Indirect analysis

The residue obtained in the LLE procedure was dissolved in 200 μl of the 0.1 *M* NaOH aqueous solution. The derivatization reaction was performed by the addition of 200 μl of the chiral reagent (2% *S*-(−)-MCF solution in dichloromethane) followed by shaking in a mixer for 2 min. After the addition of 1 ml water, the diastereomeric derivatives were extracted with 3.0 ml chloroform for 2 min in a mixer shaker. The samples were centrifuged at 1800 *g* for 5 min and the organic phases (2.5 ml) were transferred to conic tubes and evaporated to dryness under an air flow at room temperature. The residues obtained were taken up in 50 μl of the mobile phase and centrifuged in order to obtain the separation of the aqueous mobile phase from the *S*-(−)-MCF excess. The aqueous layers were injected with a 20 μl-injection loop and the diastereomers were separated on a RP-8 column with a similar 4×4 mm precolumn (Merck, Darmstadt, Germany). The mobile phase consisted of a mixture of 73% methanol–0.25 *M* acetate buffer (pH 3.0) and was utilized at a flow-rate of 1.0 ml/min. The peaks were monitored with the fluorescence detector operating at 228 nm (λ_{ex}) and 306 nm (λ_{em}).

2.7. Calibration curves and validation of the methods

The human plasma pool employed for the validation of the analytical methods was initially used to determine the absence of interference peaks with retention times equal or close to those of metoprolol enantiomers or its diastereomeric derivatives.

The calibration curves were constructed from 1000 μl samples of blank plasma spiked with 50 μl of each diluted standard solution of metoprolol. The linear regression equations and the correlation coefficients were obtained from the areas (direct methods) or heights (indirect method) of the peaks plotted against their respective plasma concentrations (10–100 ng/ml for each metoprolol enantiomer).

The recovery of metoprolol enantiomers was evaluated by comparing the areas or heights of the peaks obtained after plasma extraction (or after the extraction and derivatization procedures for the indirect method) with the areas or heights of the peaks obtained after direct injection of the standard solutions (or after the derivatization procedure for the indirect method).

The quantitation limit was determined as the lowest intra-assay concentration ($n=5$) analyzed with a coefficient of variation of less than 15%.

The linearity of each method was studied by the analysis of plasma samples spiked with increasing metoprolol concentrations in relation to those employed for the construction of the calibration curve

(>100 ng/ml). The method was considered to be linear up to the highest concentration studied having a linear relation with the detector response.

The precision of each method was evaluated by the analysis of blank plasma samples spiked with two different metoprolol concentrations (10 and 100 ng of each enantiomer/ml plasma) and the results were reported as within-day ($n = 8$) and between-day ($n = 5$) coefficients of variation.

The interference of the metoprolol metabolites and of other drugs possibly coadministered with the antihypertensive agent was evaluated by the analysis of blank plasma spiked with drug concentrations similar to those observed at therapeutic doses.

2.8. Clinical sampling

The direct and indirect methods were applied for the investigation of the enantioselectivity in the kinetic disposition of metoprolol administered in the racemic form in a single p.o. dose. Volunteer F.H.M. (a 22-year-old man weighing 68 kg and 175 cm tall) was included in the study after giving written consent to participate and after hepatic, renal and cardiac functions were found to be normal by clinical examination and on the basis of laboratory data. After a 12 h fast, the volunteer received two 100 mg metoprolol tartrate tablets (Seloken®, Astra, Brazil). Blood samples were removed via an intravenous catheter at zero, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h after metoprolol administration, with heparin (Liquemine® 5000 IU, Roche) used as anticoagulant. The blood samples were centrifuged at 1800 *g* for 10 min and the plasma stored at −20°C until chromatographic analysis.

The enantioselective kinetic disposition of metoprolol was analyzed on the basis of the open bicompartmental model [29]. The absorption ($t_{1/2}$a) and the distribution ($t_{1/2}\alpha$) half-lives were determined after correction of the respective phases by the residue method. The elimination half-life ($t_{1/2}\beta$) was determined directly by the graphic method (log c vs. t). The absorption (Ka), distribution (α) and elimination (β) rate constants were calculated by the $0.693/t_{1/2}$ equation. Maximum plasma concentration (C_{max}) and the time needed to reach C_{max} (t_{max}) were directly calculated from the plasma enantiomer concentrations obtained. The area under the plasma concentration vs. time curve ($AUC^{0-\infty}$) was calculated by the trapezoid method with extrapolation to infinity. This parameter was used to calculate apparent total clearance ($Cl/f = dose/AUC^{0-\infty}$) and apparent distribution volume $(Vd)/f = Cl/\beta.f$.

3. Results and discussion

In the present study we developed and compared three methods for the analysis of metoprolol enantiomers in human plasma. The direct analysis was carried out on the chiral columns Chiralcel® OD-H and Chiralpak® AD, whereas the indirect analysis was based on the formation of diastereomeric derivatives with the enantiomerically pure chiral reagent *S*(−)-menthyl chloroformate.

S(−)-menthyl chloroformate reacts with amines and alcohols forming carbamate and carbonate derivatives, respectively [19,20,30,31]. Considering that metoprolol has both the secondary amine and the aliphatic hydroxyl groups in its side chain, theoretically chloroformate may react with the two groups. However, considering that in the present study the reaction occurred in alkaline conditions and in the presence of water, only the amino group reacted, resulting in the formation of a carbamate derivative, as suggested in Fig. 2 [30,31]. Only the study by Li et al. [19] reported the reaction of urinary metoprolol with the chiral agent *S*(−)-menthyl chloroformate, although the conditions of that study involved the total absence of water. The elution order of the diastereomeric derivatives of metoprolol was defined by injecting the chloroformate derivatives of the pure enantiomers. Under the experimental conditions adopted, the first peak eluted from the RP-8 column corresponded to the *S*(−)-metoprolol, Fig. 3C.

The analysis of the blank plasma pool collected from different healthy volunteers did not show interference of the endogenous components with the metoprolol enantiomers eluted from the chiral stationary phase columns (Figs. 3A and 3B) or with their diastereomeric derivatives (Fig. 3C).

The study of recovery (Table 1) revealed values higher than 70% and independent of the concentrations for both metoprolol enantiomers analyzed by liquid–liquid extraction or solid-phase extraction procedures. The absolute recoveries obtained by

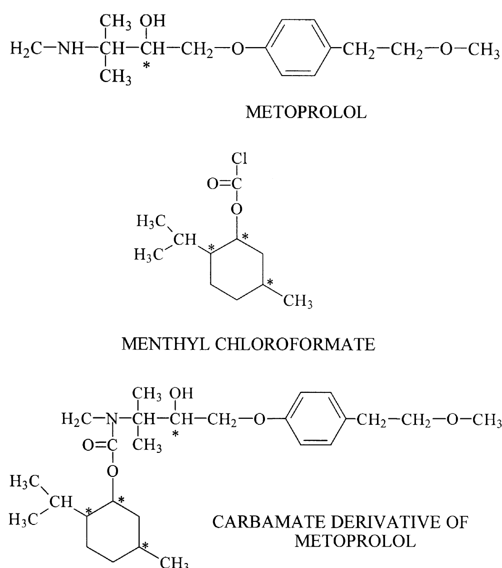

Fig. 2. Structures of metoprolol, $S(-)$-menthyl chloroformate and a diastereomeric derivative of metoprolol.

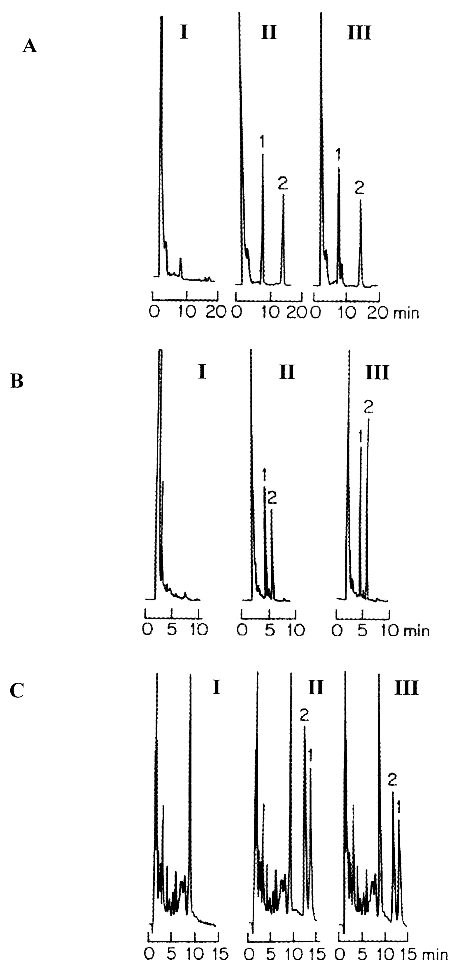

Fig. 3. (A) Direct method-Chiralcel® OD-H; (B) direct method-Chiralpak® AD; (C) Indirect method. Chromatograms of (I) blank plasma; (II) blank plasma spiked with $R(+)$-metoprolol (1), and $S(-)$-metoprolol (2), and (III) plasma from a volunteer treated with racemic metoprolol tartrate.

liquid–liquid extraction were slightly higher than those obtained with solid-phase extraction (83.7 versus 74.4%). Previously reported methods of enantioselective analysis of plasma metoprolol preferentially employ procedures of liquid–liquid extraction with the use of the solvents ethyl ether [6,8,10,11,28], hexane [7], chloroform [21], dichloromethane [23,27], diisopropyl ether [18,26] or a mixture of diethyl ether and dichloromethane [3,14], with recoveries normally higher than 80% for both enantiomers. Ching et al. [12] used a combination of solvent extraction (dichloromethane) and solid-phase extraction (CN Bond Elut column) in order to eliminate interfering peaks in the chromatograms from plasma samples. Solid-phase extraction alone (C_{18} column) was reported by Herring et al. [9]. This last method presented high extraction (more than 99.0%) but poor detection limit (4 ng/ml for each enantiomer).

The peak area or height versus plasma concentration curves for both metoprolol enantiomers were constructed in the 10–100 ng/ml range, with correlation coefficients higher than 0.99 for the direct and indirect methods. The quantitation limits determined by the analysis of 1000 μl plasma samples were 5.0 ng/ml for both metoprolol enantiomers analyzed on a Chiralcel® OD-H column. These data are compatible with the sensitivity of previously reported methods (2.5–10.0 ng/ml) with the use of the cellulose tris(3,5-dimethylphenylcarbamate) as chiral stationary phase [7–12]. The indirect analysis resulted in higher sensitivity for the $S(-)$-metoprolol enantiomer than for its corresponding antipode, i.e., 2.5 versus 5.0 ng/ml. The Chiralpak® AD permits the quantitation of metoprolol concentrations as low as 1.0 ng of each enantiomer/ml plasma. The sensitivity of 1.0 ng/ml was obtained only by

Table 1
Confidence limits of the methods for the analysis of metoprolol enantiomers in plasma samples

	Recovery (%)	Quantitation limit (ng/ml)	Linearity (ng/ml)
Direct method-Chiralcel OD-H[a]			
S(−)-metoprolol	83.0	5.0	10–500
R(+)-metoprolol	86.3	5.0	10–500
Direct method-Chiralpak AD[b]			
S(−)-metoprolol	76.0	1.0	10–500
R(+)-metoprolol	72.7	1.0	10–500
Indirect method			
S(−)-metoprolol	83.0	2.5	10–500
R(+)-metoprolol	85.3	5.0	10–500

[a] LLE was employed in the direct method-Chiralcel OD-H and in the indirect method.
[b] SPE was employed in the direct method-Chiralpak AD.

Persson et al. [14] with the use of a gradient system and a silica bonded α_1-acid glycoprotein column, relatively less stable than the Chiralpak® AD column.

The coefficients of variation obtained in the study of intra- and inter-assay precision were less than 15% (Tables 2 and 3), assuring the reproducibility and repeatability of the results in the 10–100 ng/ml plasma range. The data obtained in the precision and accuracy study also demonstrated that the internal standard is not required in the analysis.

The analysis of selectivity (Table 4) permitted the application of the direct and indirect methods to the study of kinetic disposition in hypertensive patients submitted to chronic therapy with metoprolol in combination with other drugs. The metoprolol metabolites also did not interfere with the direct and indirect methods.

The direct and indirect methods developed and validated were employed in the investigation of enantioselectivity in the kinetic disposition of metoprolol administered p.o. in the racemic form in a single 200 mg dose of the tartrate salt to volunteer F.H.M., phenotyped as an extensive metabolizer of debrisoquine [27] (urinary recovery of debrisoquine/4-hydroxydebrisoquine = 1.8). The correlation coeffi-

Table 2
Analysis of the precision and accuracy of the methods for analysis of metoprolol enantiomers. Within-day precision ($n = 5$)

Concentration added (ng/ml)	Concentration observed (ng/ml) (mean)	Precision (C.V. %)[a]	Accuracy (systematic error %)[b]
Direct method-Chiralcel OD-H			
10 ng/ml			
S(−)-metoprolol	10.2	5.8	2.0
R(+)-metoprolol	9.7	3.8	−2.9
100 ng/ml			
S(−)-metoprolol	95.6	6.7	−4.4
R(+)-metoprolol	98.2	6.4	−1.7
Direct method-Chiralpak AD			
40 ng/ml			
S(−)-metoprolol	41.5	4.7	3.8
R(+)-metoprolol	43.1	5.3	7.8
Indirect method			
10 ng/ml			
S(−)-metoprolol	10.6	6.8	−4.0
R(+)-metoprolol	9.7	8.2	−3.0
100 ng/ml			
S(−)-metoprolol	104.3	8.0	4.3
R(+)-metoprolol	107.1	7.3	7.1

[a] C.V. = coefficient of variation [(SD/mean)×100].
[b] Systematic error = (concentration observed-concentration added/concentration added)×100.

Table 3
Analysis of the precision and accuracy of the methods for the analysis of metoprolol enantiomers. Between-day precision ($n=5$)

Concentration added (ng/ml)	Concentration observed (ng/ml) (mean)	Precision (C.V. %)[a]	Accuracy (systematic error %)[b]
Direct method-Chiralcel OD-H			
10 ng/ml			
S(−)-metoprolol	9.5	5.9	−4.7
R(+)-metoprolol	9.6	12.5	3.6
100 ng/mI			
S(−)-metoprolol	103.0	14.3	3.1
R(+)-metoprolol	103.1	11.9	3.2
Direct method-Chiralpak AD			
40 ng/ml			
S(−)-metoprolol	37.0	12.0	−7.5
R(+)-metoprolol	38.3	12.8	−4.3
Indirect method			
10 ngl/ml			
S(−)-metoprolol	10.9	9.3	9.5
R(+)-metoprolol	10.7	7.8	7.0
100 ng/ml			
S(−)-metoprolol	109.9	8.3	9.9
R(+)-metoprolol	108.6	7.4	8.6

[a] C.V.=coefficient of variation [(SD/mean)×100].
[b] Systematic error=(concentration observed-concentration added/concentration added)×100.

Table 4
Study of the selectivity of the methods for the analysis of metoprolol enantiomers using direct and indirect separation[a]

Drug	Concentration (μg/ml)	Retention time (min)		
		Direct method-Chiralcel OD-H	Direct method-Chiralpak AD	Indirect method
S(−)-metoprolol	0.05	18.9	7.8	13.1
R(+)-metoprolol	0.05	6.3	6.0	14.6
N-dealkylmetoprolol	0.1	15.5/21.1	ND[b]	ND
O-demethylmetoprolol	0.1	22.3	10.4/12.2	6.6/7.3
Acidic metabolite[c]	0.1	ND	32	ND
α-Hydroxymetoprolol	0.1	21.9/33.3/42.4/45.0	15.8/16.6/20.1/24.3	6.4/7.0
Atenolol	0.39	ND	19.9	4.6/5.1
Benzidamine	0.80	ND	4.2	ND
Captopril	0.14	24.6/24.8	NP	ND
Lidocaine	5.00	ND	4.0	ND
Pindolol	0.20	ND	8.5/10.4	7.3/8.1
Propranolol	1.00	ND	–[d]	23.4/26.8
Sotalol	6.00	ND	12.6/20.3	4.9/5.1
Verapamil	0.45	12.0	5.8/6.3	ND

[a] Drugs not detected (0–45 min): acetaminophen, aminopyrine, amiodarone, carbamazepine, clobazam, clomipramine, clonazepam, chlorpromazine, dapsone, digoxin, disopyramide, ethidocaine, metoclopramide, nitrazepam, propafenone, quinidine, theophylline, trimipramine.
[b] ND: Not detected (0–45 min).
[c] 2-hydroxy-3-(4-methoxyethylenoxy)-propanoic acid.
[d] – Not injected.

cients obtained between the enantioselective plasma concentrations of metoprolol determined by the direct and indirect methods were higher than 0.99 (Fig. 4) and permitted the quantitation of both enantiomers up to 12 h after administration of the betablocker.

The plasma concentration versus time curves (Fig. 5 and Table 5) demonstrated more prolonged elimination half-lives and a lower apparent clearance for the *S*(−)-metoprolol enantiomer compared to its *R*(+) antipode. The enantiomer ratio of the areas under the plasma concentration versus time curve ($AUC^{0-\infty}_{S(-)}/AUC^{0-\infty}_{R\text{-}(+)}$) was 1.16 for volunteer F.H.M. The plasma accumulation of the *S*(−)-metoprolol

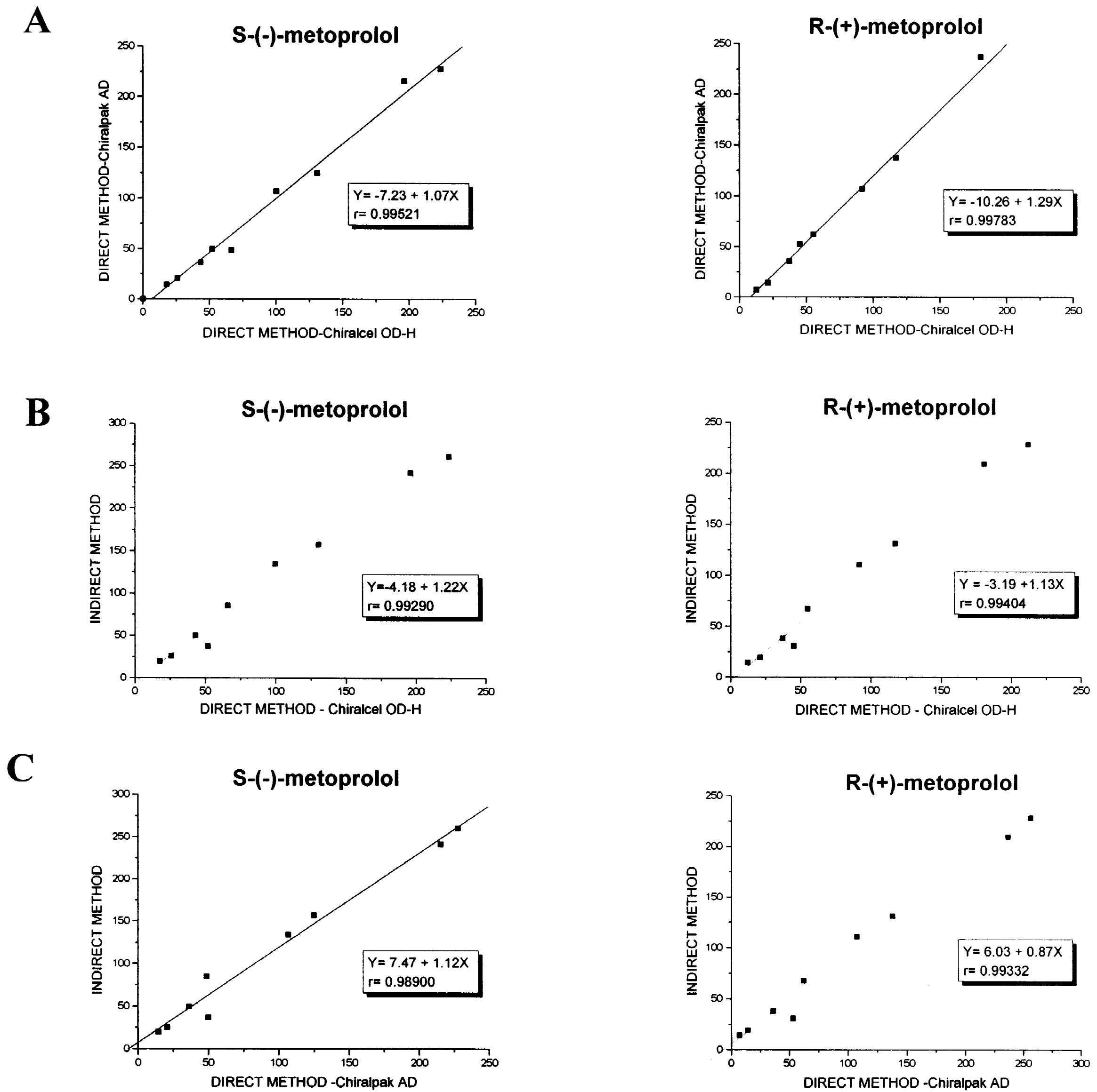

Fig. 4. Plasma concentrations of *S*(−)-metoprolol and *R*(+)-metoprolol (ng/ml) obtained by direct methods-Chiralcel® OD-H versus Chiralpak® AD (A); direct method-Chiralcel® OD-H versus indirect method (B) and direct method-Chiralpak® AD versus indirect method (C). Plasma samples were obtained during the investigation of volunteer F.H.M.

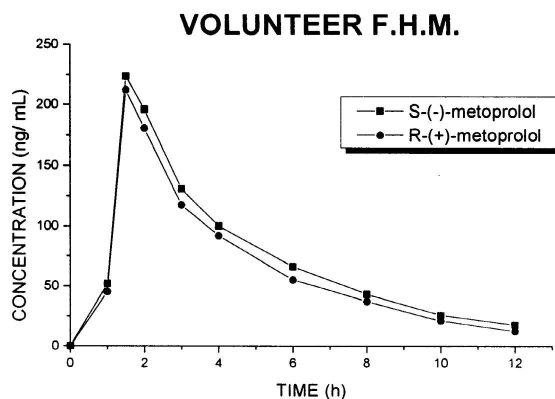

Fig. 5. Plasma concentration versus time curves for $R(+)$-metoprolol and $S(-)$-metoprolol in volunteer F.H.M. treated with a 200-mg dose of racemic metoprolol.

Table 5
Kinetic disposition of $S(-)$ and $R(+)$-metoprolol in a EM of debrisoquine after a 200-mg oral dose of metoprolol

Parameters	Volunteer F.H.M.	
	$S(-)$-Metoprolol	$R(+)$-Metoprolol
C_{max} (ng.ml^{-1})	223.84	212.19
t_{max} (h)	1.50	1.5
AUC$^{0-\infty}$ (ng h ml^{-1})	951.04	820.25
$t_{1/2}$ a (h)	0.72	0.45
Ka (h^{-1})	0.96	1.54
$t_{1/2}$ α (h)	0.90	0.70
α (h^{-1})	0.77	0.99
Vd/f (l kg^{-1})	5.75	5.65
$t_{1/2}$ β (h)	3.30	2.80
β (h^{-1})	0.21	0.25
Cl/f (l min^{-1})	1.37	1.59
$\text{AUC}^{0-\infty}_{S(-)/R(+)}$	1.16	

enantiomer agrees with previously reported data [3,27].

4. Conclusion

The direct (Chiralcel® OD-H; Chiralpak AD®) and indirect (derivatization with the chiral reagent $S(-)$-menthyl chloroformate) methods presented sensitivity, selectivity and precision suitable for application in studies of enantioselective kinetic disposition of metoprolol administered in a single p.o. dose. The indirect method introduces the chiral $S(-)$-menthyl chloroformate reagent as an option to the enantioselective analysis of plasma metoprolol. The direct method (Chiralpak AD®) may be considered the most sensitive (quantitation limit of 1.0 ng of each enantiomer/ml plasma).

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