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Enantioselective liquid chromatographic-electrospray mass spectrometric assay of β-adrenergic blockers: application to a pharmacokinetic study of sotalol in human plasma

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Abstract

An enantioselective high performance liquid chromatographic-electrospray ionization mass spectrometric (HPLC-ESI-MS) method for the direct determination of several β-adrenergic blockers was developed and validated. The method is based on the direct separation of the enantiomers of drugs on a laboratory-made chiral stationary phase (CSP) containing covalently bonded teicoplanin (TE) as chiral selector. Detection of the effluent was performed by electrospray ionization mass spectrometry, run in the selected-ion recording (SIR) mode. The method was applied to the pharmacokinetic monitoring of sotalol (STL) in the plasma of five young healthy volunteers, dosed with racemic drug. The limits of quantitation (LOQ) reached 4 ng/ml for both sotalol enantiomers. Such a method, fully validated, offers a novel, fast and very efficient tool for the direct determination of sotalol enantiomers in human plasma, and can be generally applied to the β-adrenergic blockers stereoselective pharmacokinetics.

Keywords: Enantiomer separation; Pharmacokinetics; Sotalol; β-Adrenergic blockers

1. Introduction

The β-adrenergic blockers comprise a group of drugs that are mostly used in the treatment of cardiovascular disorders such as hypertension, cardiac arrhythmia or ischemic heart disease. As each of these drugs possesses at least one centre of chirality, the separation of the enantiomers is necessary both to study their biological activities and to control the enantiomeric purity of pharmaceutical formulations.

The enantioselective separation of β-adrenergic blockers has been widely described and reviewed in the literature [1] by means of two different approaches. The indirect mode requires the formation of diastereoisomeric pairs prior to analysis. In this context, several methods for stereoselective

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pharmacokinetics by HPLC are described, using different chiral derivatizing agents.

The application of the direct mode, in which no chiral derivatization prior to the separation is required, has been reported in the literature on different chiral stationary phases (CSPs): the α_1-acid glycoprotein (AGP) CSP was applied to determine the bunolol enantiomers in human urine [2] and albuterol in human plasma [3]; the (*R,R*)-diaminocyclohexane-3,5-dinitrobenzoyl (DACH-DNB) CSP [4] was utilized for the enantioselective bioassay of propranolol [5] and atenolol [6], after the previous formation of the corresponding racemic oxazolidin-2-one derivatives. The enantiomers of propranolol and its major metabolite were assayed in urine by HPLC on a cellulose tris(3,5-dimethylphenyl carbamate) CSP (Chiralcel OD) using fluorescence detection [7]. Similar applications of stereoselective determinations of propranolol in human plasma and urine were performed on a β-cyclodextrin column [8], and in human plasma and saliva on a Chiralcel OD-H column [9]. Among the protein-based CSPs, silica-immobilized cellobiohydrolase I (CBH-I) was found to be the most suitable for chiral analysis of β-receptors antagonists [10]. Very recently, two HPLC column-switching methods for the enantioselective analyses of atenolol in human urine [11] and carvedilol in human plasma [12] were developed on teicoplanin (TE) containing CSPs by polar organic mode HPLC.

In the present paper, we report on HPLC separation of the enantiomers of several β-adrenergic blockers, based on a laboratory-made CSP, containing covalently bonded teicoplanin as chiral selector. The CSP was recently developed in our laboratories [13]. In the last few years, mass spectrometric (MS) detection has been increasingly coupled to HPLC to perform bioanalytical determinations with maximum selectivity and sensitivity [14]. Thus, an electrospray ionization mass spectrometer was employed as a detector. The method was applied to the pharmacokinetic monitoring of one β-blocker (sotalol, STL) in the plasma of five young healthy volunteers. Sotalol, or *N*-[4-(1-hydroxy-(2-isopropylamino)-ethyl)-phenyl]-methanesulfonamide (Fig. 1) is a potent β-adrenoreceptors antagonist, with class II and III antiarrhythmic properties [15–17]. It is currently marketed as a racemate (abbreviated as (*R,S*)-STL or *rac*-STL) in the therapeutic treatment of hypertension and ventricular arrhythmia diseases. Although both the enantiomers are equivalent class III antiarrhythmic agents, the β-receptors blocking activity is mainly attributed to the (−)-(*R*)-enantiomer. Owing to the different pharmacological behaviour of the enantiomers of this class of drugs [18], the target was to separate and quantify them accurately in complex biological matrices.

2. Experimental

2.1. Apparatus

High performance liquid chromatography was performed using a Waters 2690 (Waters Corporation, Milford, MA, USA) chromatograph equipped with a Rheodyne Model 7725i 20 μl injector, and coupled with a Waters ZMD single quadrupole mass detector, equipped with an electrospray ionization (ESI) interface, a Model 75-72 nitrogen generator (Whatman Inc., Haverhill, MA, USA) and a model “Pump 11” microprocessor single syringe (Harvard Apparatus Inc., South Natick, MA, USA) for direct sample infusion. Chromatographic and MS data were collected and processed using the MassLynx^{TM} Version 3.4 software (Micromass UK Ltd., Manchester, UK).

Solid-phase extraction (SPE) of biological samples were performed on a vacuum extraction manifold device obtained from Waters.

The column used for the analyses was a laboratory-made (250 mm × 4.5 mm i.d.) teicoplanin containing chiral stationary phase (TE-CSP), 5 μm particle size, based on a spherical micro-particle support (LiChrospher, trademark of Merck, Darmstadt, Germany); details of the synthetic procedure and chromatographic performances of this column have been already published [13].

2.2. Chemicals and reagents

Racemic sotalol hydrochloride (*rac*-STL·HCl) and (+)-(*S*)-STL were kindly provided by Bristol (Sermoneta, Latina, Italy); racemic oxprenolol hydrochloride, metoprolol tartrate, propranolol hydrochloride, atenolol-free base and alprenolol hydrochloride were from Sigma; (1*R*,2*S*)-2-amino-1-phenyl-1-propanol or L-norephedrine (L-nor-EPH), water for HPLC, ammonium formate, 20 mM sodium tetraborate buffer, acetic

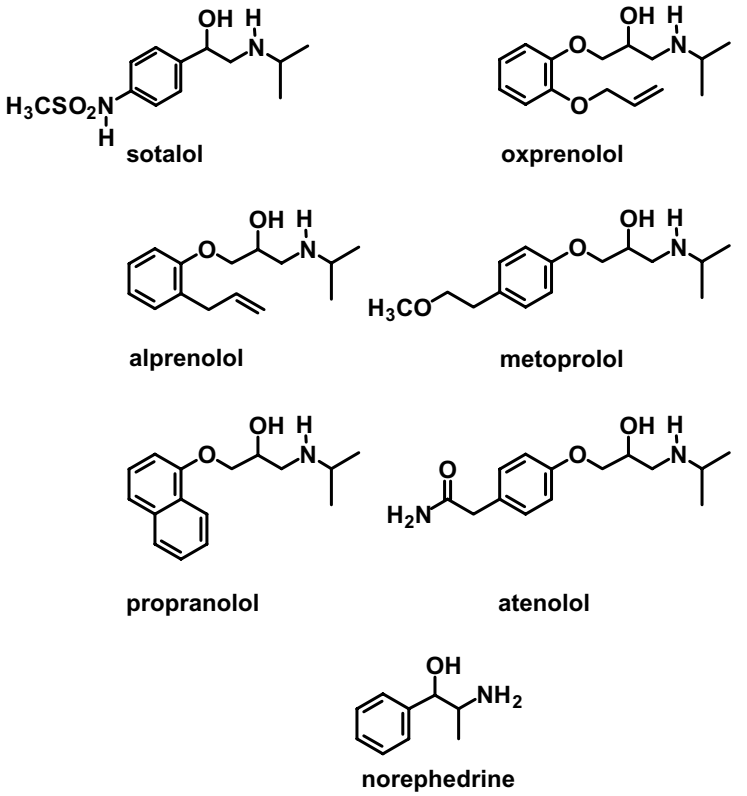

Fig. 1. Chemical structures of β-adrenergic blockers and norephedrine.

acid and triethylamine were purchased from Fluka (Sigma–Aldrich Company, Buchs, Switzerland).

HPLC-grade solvents and borate buffer (pH 9.2) were obtained from Merck (Darmstadt, Germany); trifluoroacetic acid (TFA) and ammonium acetate were from J.T. Baker (Division of Mallinckrodt Baker, Phillipsburg, NJ, USA); teicoplanin, used for the preparation of the TE-CSP [13], was kindly provided by the Lepetit Research Centre (Gerenzano, Italy). Oasis™ HLB 1 cc (30 mg) solid-phase extraction cartridges were from Waters (Milford, MA, USA). Durapore membrane 0.45 μm (type HVLP) filters were obtained from Millipore Corporation (Bedford, MA, USA).

2.3. Biological samples

Plasma samples were collected from five young healthy volunteers, before and 2, 4, 6, 8, 12, 24, 36 h after single oral doses of 80 mg (*R,S*)-STL were administrated.

After isolation, all the plasma samples were frozen below −30 °C until analysis.

2.4. Standard solutions

A stock standard solution (1 mg/ml) of *rac*-STL·HCl was prepared with water. This solution was further diluted with water to obtain the working standard solutions at given concentrations for validation tests.

The stock internal standard (IS) solution (0.25 mg/ml) of L-nor-EPH was prepared with water plus 2% methanol (v/v). The working IS solution of 2.50 μg/ml was prepared by dilution (1:100) of the stock IS solution with water. In order to avoid the eventual absorption of the amine on the glassware silanols, all the solutions were prepared by using polypropylene vials.

For the direct injection in the MS apparatus, a stock standard solution (1 mg/ml) of *rac*- STL·HCl was prepared with a mixture of methanol/aqueous ammonium acetate (10 mM, 50:50 (v/v)). This solution was diluted (1:100) with the same mixture of solvents to obtain the working solution of 10 μg/ml. A stock standard solution (1 mg/ml) of L-nor-EPH was prepared with a mixture of methanol/water (50:50 (v/v)). This solution was diluted (1:100) with the same mixture of solvents to obtain the working solution of 10 μg/ml.

All the stock solutions were stored at 5 °C; under these circumstances, they were stable for at least 1 month.

2.5. Sample preparation

A 1.0 ml aliquot of plasma sample was pipetted into a glass test tube and 50 μl (corresponding to 0.12 μg) of the working IS solution of L-nor-EPH were added. The sample was alkalinized by the addition of 1 ml of borate buffer (pH 9.2). The mixture was centrifuged at 1500 × *g* for 5 min, and the supernatant loaded onto the Oasis™ HLB cartridge, which was previously conditioned with 1 ml of methanol and equilibrated with 1 ml of 20 mM sodium tetraborate buffer (pH 8.5), under a vacuum extraction manifold device. The cartridge was washed with 2 ml of 20 mM sodium tetraborate buffer (pH 8.5) and 2 ml of water. The basic analytes, retained in the cartridge, were eluted with 3 ml of methanol containing 0.5% acetic acid and 0.5% TFA into a polypropylene test tube; the eluate was evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was directly dissolved in 200 μl of methanol containing 0.5% acetic acid and 0.5% TFA; a 20 μl aliquot of this solution was analyzed by enantioselective high performance liquid chromatographic-electrospray ionization mass spectrometric (HPLC-ESI-MS).

2.6. Chromatographic conditions

HPLC separations were performed isocratically using the enantioselective CSP, containing covalently bonded teicoplanin (TE-CSP; [13]), at a column temperature of 30 °C. A mixture of methanol/acetonitrile/acetic acid/triethylamine (70:30:0.025:0.025 (v/v/v/v)) was used as the mobile phase; the flow-rate was 1.5 ml/min. For the pharmacokinetic study on sotalol, methanol containing ammonium formate (25 mM) was used as the mobile phase. Prior to use, the mobile phases were filtered through a durapore membrane 0.45 μm filter, and degassed in situ with a helium sparge during chromatography.

The hold-up time (t_0) of the teicoplanin column was determined from the elution of an unretained marker (toluene), using methanol as eluent, at a flow-rate of 1.5 ml/min ($t_0 = 2.07$ min).

The mass spectrometric device used for the detection was operated using an electrospray ionization interface, run in the positive-ion mode (ESI+), under the following conditions: nitrogen was used as auxiliary gas, at a flow-rate of 600 l/h; the temperature of the source block was maintained at 100 °C, while desolvation temperature was set at 150 °C; the cone and capillary voltages were run at 40 V and 3.6 kV, respectively. Data were acquired at a scan rate of 1 s for all scans. To optimize ionization conditions, pure solutions (10 μg/ml) of β-adrenergic blockers in methanol/water (50:50 (v/v)) were directly infused into the ion source block, at a flow-rate of 10 μl/min with a microprocessor single syringe. When else mass range of *m/z* 50–500 was monitored with a resolution of 0.1 amu, the flow-rate of auxiliary gas was decreased to 350 l/h.

The acquisition was made in the selected-ion recording (SIR) mode, with a dwell time of 100 ms. The following protonated ions were selected: at 266 *m/z* (oxprenolol); 250 *m/z* (alprenolol); 268 *m/z* (metoprolol); 260 *m/z* (propranolol); 266 *m/z* (atenolol).

For the quantitation of sotalol, the ions at *m/z* 273 ($[M+H]^+$) and *m/z* 255 ($[M+H-18]^+$) were selected, while for L-nor-EPH (IS) the ion at *m/z* 134 ($[M + H - 18]^+$) was considered.

3. Results and discussion

3.1. Method development

The direct enantioselective separation of several β-adrenergic blockers (Fig. 1) was successfully carried out on the teicoplanin containing CSP [13] as chiral selector by polar organic mode HPLC (see Fig. 2 for representative chromatograms). In order to enhance the selectivity and sensitivity of the method, MS detection was chosen. In particular, the HPLC-MS

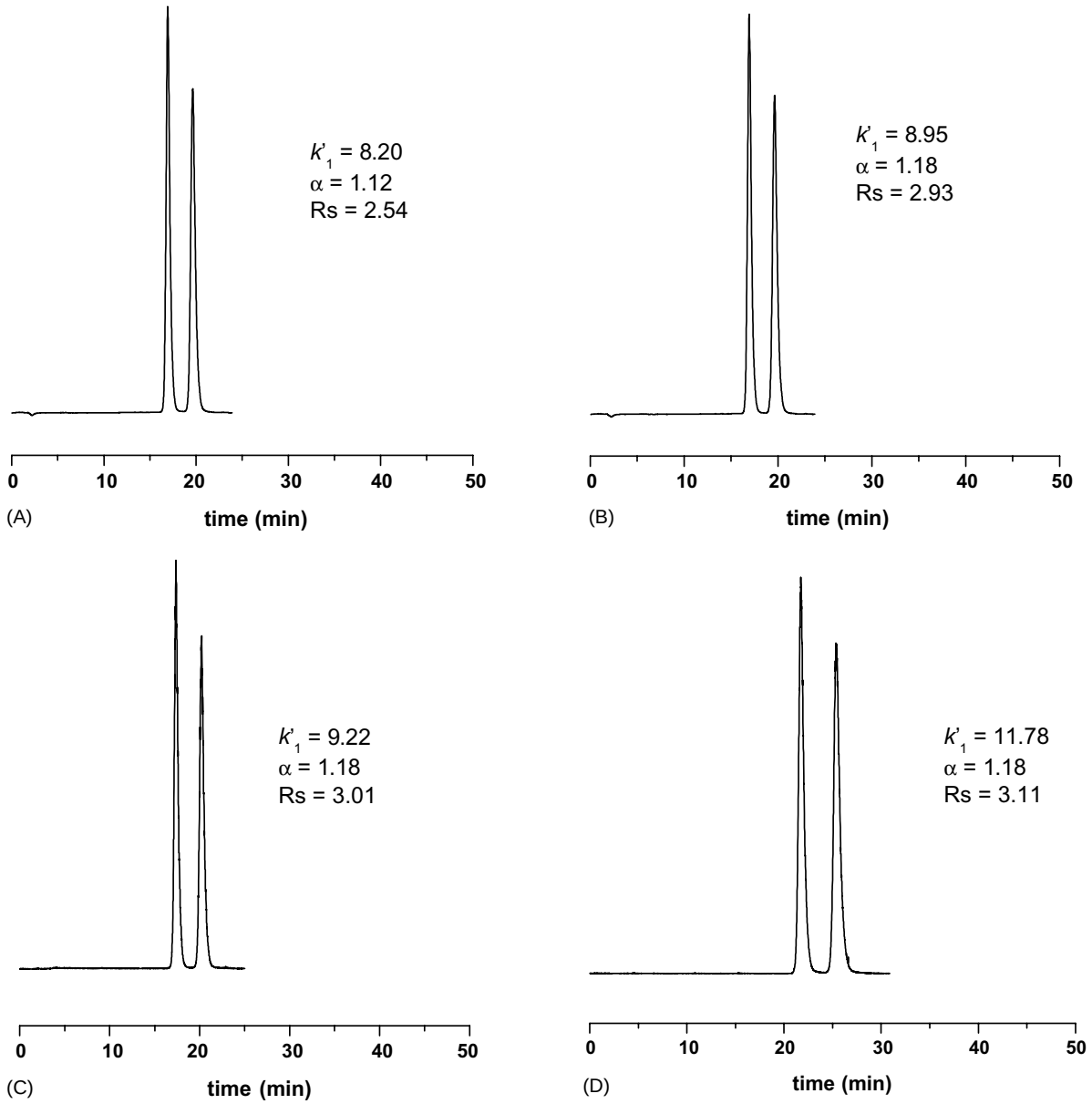

Fig. 2. Direct chromatographic resolutions of four racemic β-adrenergic blockers: (A) oxprenolol; (B) alprenolol; (C) metoprolol; (D) propranolol. For chromatographic conditions, see Section 2.

system was operated using an electrospray ionization interface, run in the positive-ion mode (ESI+). The chromatographic parameters obtained for the selected β-adrenergic blockers are summarized in Table 1. While enantioselectivity (α) approximately showed the same values for all the β-adrenergic blockers, retention of the first eluting enantiomer (k'_1) increased depending on the presence and nature of the side chain on the aromatic portion of analyte.

For the pharmacokinetic study on sotalol, L-norephedrine (L-nor-EPH, Fig. 1) was used as the internal standard.

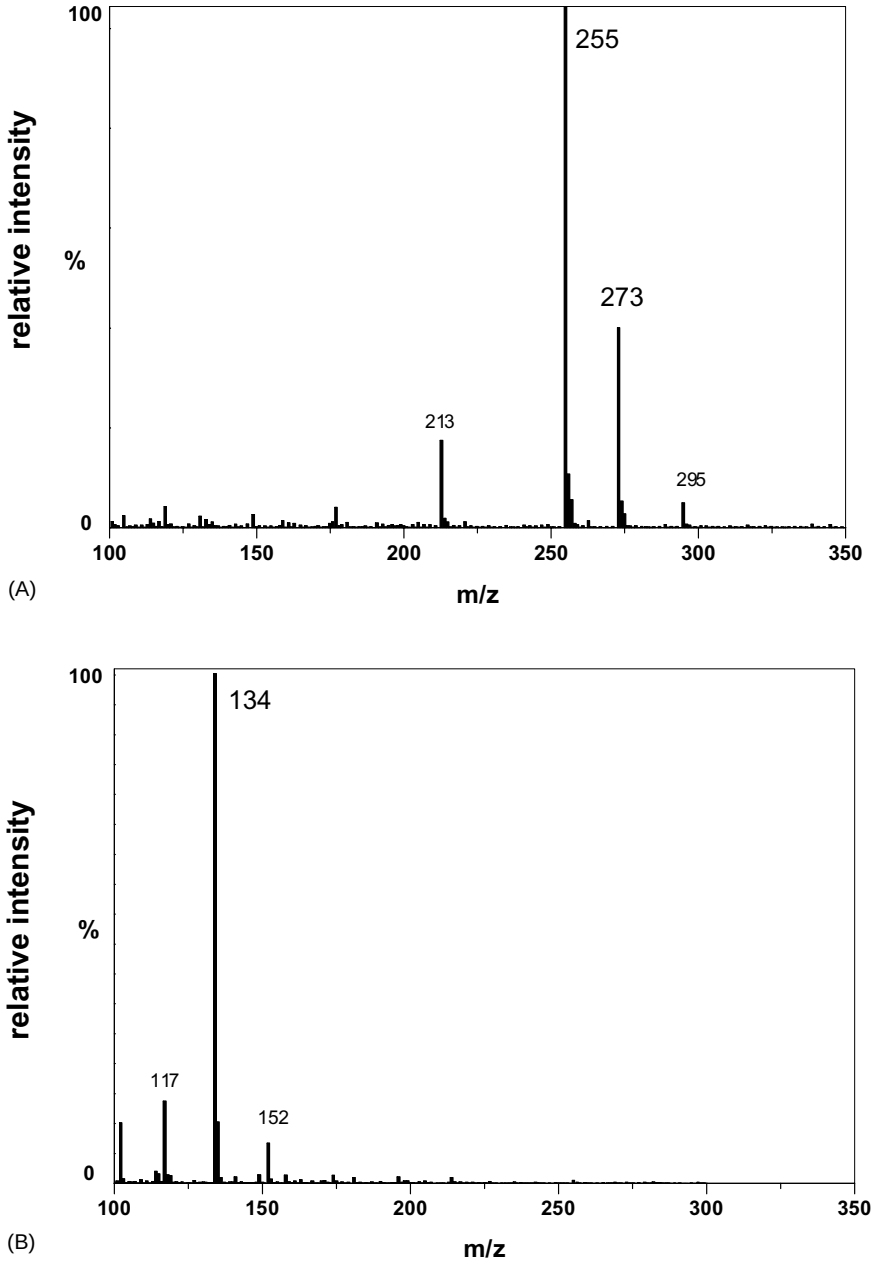

Fig. 3. ESI-positive mass spectra of (A) sotalol and (B) L-norephedrine, illustrating *m*/*z* 273 as the protonated molecule of sotalol and *m*/*z* 255 and 134 as the protonated molecules of sotalol and L-norephedrine, respectively, after the loss of water.

Mass spectra of each compound was measured, in order to investigate the predominant charge states of the analytes. Parameters such as source block temperature and cone and capillary voltages were optimized to obtain the highest possible intensity for the protonated molecule with little undesired fragmentation. Full-scan ESI-positive mass spectra of *rac*-STL and L-nor-EPH are shown in Fig. 3; the protonated

Table 1
Chromatographic data obtained for the direct resolution of some β-adrenergic blockers agents on the TE-CSP

Compound	Retention factor (k'_1)	Enantioselectivity factor (α)	Resolution factor (R_s)
Oxprenolol	8.20	1.12	2.54
Alprenolol	8.95	1.18	2.93
Metoprolol	9.22	1.18	3.01
Propranolol	11.78	1.18	3.11
Atenolol	15.87	1.18	2.61

molecule $[M + H]^+$ was identified at *m*/*z* 273 for sotalol (Fig. 3A), with a relative intensity less than 40%, while the predominant fragment corresponded to the protonated molecule after the loss of water (*m*/*z* 255). With regard to L-nor-EPH, the protonated molecule $[M + H]^+$ was identified at *m*/*z* 152 (Fig. 3B), with a relative intensity less than 10%, while the predominant fragment corresponded to the protonated molecule after the loss of water (*m*/*z* 134). The expulsion of water in both cases is not surprising, since the obtained carbocation can be stabilized by the formation of a double bond which is in conjugation to the lone pair of the amine and to the aromatic ring. For the quantitation of sotalol, the ions at *m*/*z* 273 ($[M + H]^+$) and *m*/*z* 255 ($[M + H - 18]^+$) were selected, while for L-nor-EPH (IS) the ion at *m*/*z* 134 ($[M + H - 18]^+$) was considered.

3.2. Method validation

3.2.1. Selectivity

As judged by the chromatographic parameters obtained for *rac*-STL and for IS (L-nor-EPH), investigated peaks are well resolved under our chromatographic conditions ($k'_3/k'_2 = 5.57/4.84 = \alpha_{2/3} = 1.15$; $R_s = 1.80$), and they all exhibit a notable symmetrical shape ($A_s < 1.20$), thus making their electronic integration easy and precise (Fig. 4A). The elution order of the sotalol enantiomers, determined by chromatography of racemic samples enriched with the (+)-(*S*)-STL enantiomerically pure sample, was (+)-(*S*)-STL before (−)-(*R*)-STL.

In order to verify the selectivity of the present method, a selection of five different human plasma matrices were examined. There were no interfering peaks of the biological matrix when drug-free human plasma samples were assayed (Fig. 4B).

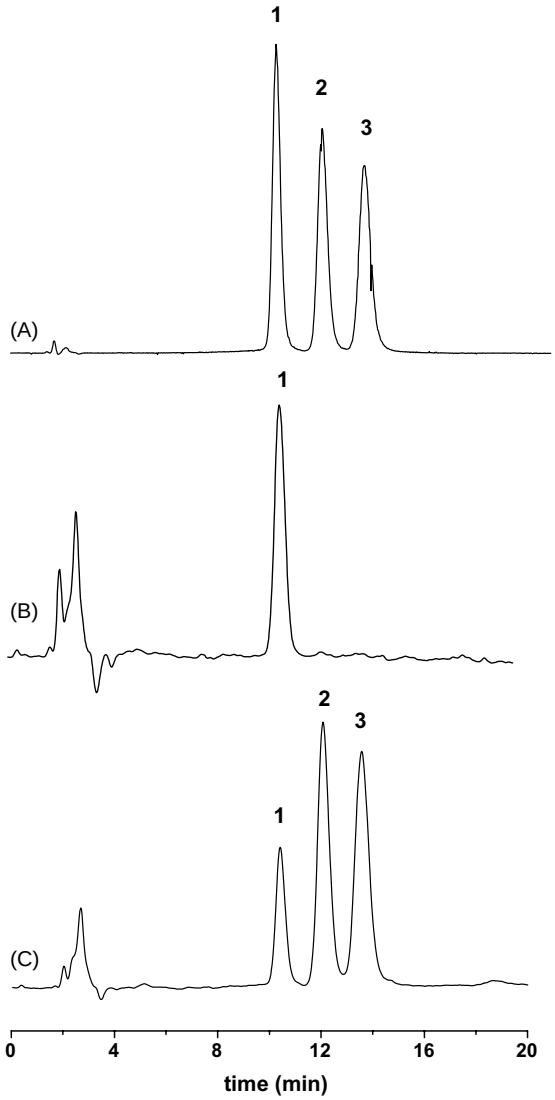

Fig. 4. Typical chromatograms of (+)-(*S*)- and (−)-(*R*)-STL. Peak 1 corresponds to IS (L-norephedrine, L-nor-EPH); peaks 2 and 3 are (+)-(*S*)- and (−)-(*R*)-STL, respectively. (A) Standard solution containing *rac*-STL and L-nor-EPH (10 μg/ml each); (B) pooled plasma blank containing IS (0.50 μg/ml); (C) plasma sample containing IS (0.50 μg/ml), from a volunteer at 4 h after 80 mg single oral administration of *rac*-STL. For chromatographic conditions, see Section 2.

Moreover, the use of MS detection, run in the selected-ion recording mode, provided a highly selective method for the determination of the analytes under investigation.

Fig. 4C shows a typical chromatogram obtained routinely from a volunteer at 4 h after 80 mg single oral administration of *rac*-STL, corresponding to plasma concentrations of 0.22 and 0.25 μg/ml for (+)-(*S*)- and (−)-(*R*)-STL, respectively.

3.2.2. Sensitivity

The limit of quantitation (LOQ), established in pooled human plasma, was 4 ng/ml (81 pg injected), based on a signal-to-noise ratio of about 9:1; in particular, for (−)-(*R*)-STL, R.S.D. = 4.2%, $n = 3$ and accuracy of 102.7%; for (+)-(*S*)-STL, R.S.D. = 3.9%, $n = 3$ and accuracy of 96.0%. The limit of detection (LOD), considered at a signal-to-noise ratio of about 3:1, was 1 ng/ml (21 pg injected).

3.2.3. Linearity

The linearity of the method was established in pooled human plasma over the concentration range of 0.004–0.620 μg/ml of each enantiomer. The correlation coefficient values for calibration curve ($n = 3$) were >0.995 for each enantiomer. The corresponding regression functions were: for (−)-(*R*)-STL, $y = 1.88 \times 10^6 x - 2659$ ($r = 0.9975$); for (+)-(*S*)-STL, $y = 1.81 \times 10^6 x + 157$ ($r = 0.9954$).

3.2.4. Recovery and accuracy

A 1.0 ml aliquots of pooled plasma sample were spiked with *rac*-STL standard solutions and L-nor-EPH standard solutions, to give final plasma concentrations of 0.50 and 0.12 μg/ml, respectively. Samples were solid-phase extracted and analyzed as described above. Peak areas of both STL enantiomers and L-nor-EPH were compared to those obtained for directly injected standard solutions of identical concentrations. The recovery was found to be 98% for sotalol and >99% for the internal standard. No differences between the two enantiomers of sotalol were observed.

The accuracy was evaluated on spiked plasma samples at three levels of concentration of each enantiomer (0.06–0.15–0.31 μg/ml), and expressed as the mean ratio of observed and spiked amount. The precision was calculated as coefficients of variation (CV%) of three sequential analyses (Table 2).

3.3. Pharmacokinetic data

The profiles of the plasma concentrations of sotalol enantiomers, obtained for five young volunteers who took single oral doses of 80 mg (*R*,*S*)-STL, are shown in Fig. 5. Among the studied volunteers, significant differences in the pharmacokinetic parameters for *rac*-STL were observed, C_{max} and T_{max} ranging from 0.47 to 0.75 μg/ml and from 2 to 6 h, respectively. The mean peak plasma levels of (+)-(*S*)- and (−)-(*R*)-STL were about 0.27 μg/ml, therefore, the plasma levels could be monitored for 36 h after administration until the plasma levels decreased to about 10% of the peak concentrations. In addition, the very high sensitivity reached allowed us to extend plasma levels monitoring to 48 and 72 h after administration of single oral doses of *rac*-STL, for one of the five volunteers. In this case, the plasma levels could be monitored for 72 h after administration until they decreased to about 3% of the peak concentration.

With regard to the enantiomers behaviour, the close correspondence in the plasma concentration–time profiles of the two enantiomers is evident (Fig. 5). No significant differences were observed for all pharmacokinetic parameters (Table 3). These findings were in agreement with previous studies [19,20] in which no

Table 2
Accuracy and precision for the quantitation of (−)-(*R*)- and (+)-(*S*)-STL in pooled human plasma

Compound	Spiked concentration (μg/ml)	Observed concentration, mean ± S.D. (μg/ml)	CV (%)	Accuracy (%)
(−)-(*R*)-STL	0.062	0.059 ± 0.001	2.2	95.4
	0.155	0.156 ± 0.007	4.3	100.9
	0.310	0.314 ± 0.014	4.6	101.3
(+)-(*S*)-STL	0.062	0.064 ± 0.003	5.0	103.9
	0.155	0.165 ± 0.006	3.4	106.1
	0.310	0.294 ± 0.011	3.7	94.7

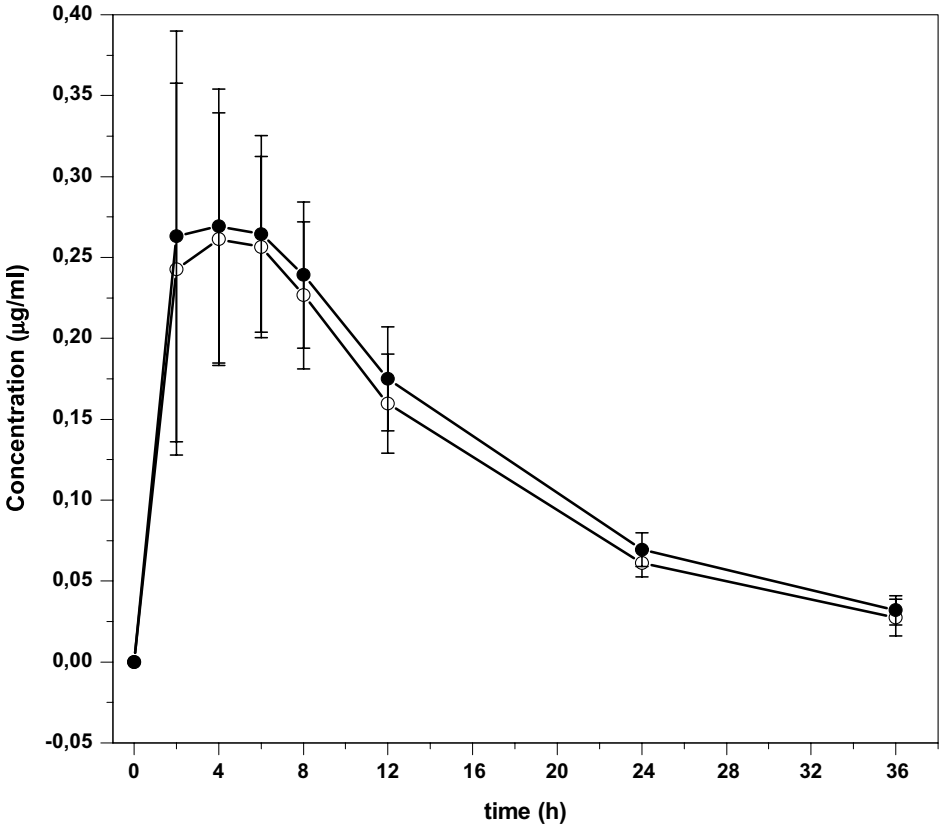

Fig. 5. Plasma levels of (+)-(*S*)- and (−)-(*R*)-STL, after single oral administration of 80 mg (*R*,*S*)-STL to five young healthy volunteers. The open and closed circles show the mean plasma concentration of (+)-(*S*)- and (−)-(*R*)-STL, respectively; ± standard deviations (S.D.), $n = 3$.

stereoselectivity was observed in the plasma concentrations of sotalol enantiomers, after single oral doses of *rac*-STL to healthy volunteers. There was, however, some stereoselectivity noted in another study [21], when repeated doses of *rac*-STL were administered to patients with supraventricular tachycardia. It is well known [22] that β-adrenergic blockers bind to both albumin and α_1-acid glycoprotein in plasma, but the binding appears to be non-stereoselective, in the case of sotalol [23]. Moreover, the reported stereoselectivity in the renal clearance of sotalol is relatively low, with (−)/(+) renal clearance ratio being 1.05 [20].

Table 3
Mean ± standard deviations (S.D.) pharmacokinetic parameters for (−)-(*R*)- and (+)-(*S*)-STL in five healthy volunteers after a single oral dose of *rac*-STL (80 mg)

Parameters	(−)-(*R*)-STL	(+)-(*S*)-STL
C_{max} (μg/ml)	0.27 (0.084)	0.26 (0.078)
T_{max} (h)	2.80 (1.79)	2.80 (1.79)
$AUC_{0-\infty}$ (μg/ml h)	5.17 (0.63)	4.68 (0.60)
$t_{1/2}$ (h)	10.36 (3.12)	9.12 (2.69)

4. Conclusions

The enantioselective HPLC-ESI-MS study described offers a novel, fast and very efficient tool for the direct determination of β-adrenergic blockers in human plasma with high sensitivity and specificity. The chiral stationary phase containing teicoplanin as selector may prove a readily available, reliable tool for future studies on β-blockers handling in different patient populations and clinical conditions. The advantages of the direct approach can be ascribed to:

(a) the not required preliminary chiral derivatization, which avoids the necessary validation of the derivatization procedure; (b) the short-time analyses; (c) the particular detection system coupled to HPLC, based on electrospray ionization mass spectrometry, run in the selected-ion recording mode, which provides a highly selective method for the determination of drugs, especially in complex biological matrices.

With regard to pharmacokinetic considerations, no significant differences were observed for the two sotalol enantiomers; these findings were in agreement with previous studies in which no stereoselectivity was observed in the plasma concentrations of STL enantiomers, after single oral doses of *rac*-STL to healthy volunteers.

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References

[1] J. Bojarski, H.Y. Aboul-Enein, Biomed. Chromatogr. 13 (1999) 197, and references cited therein.
[2] F. Li, S.F. Copper, M. Côté, C. Ayotte, J. Chromatogr. B 660 (1994) 327.
[3] H.Y. Aboul-Enein, V. Serignese, Chirality 7 (1995) 158.
[4] F. Gasparrini, D. Misiti, C. Villani, F. La Torre, J. Chromatogr. 539 (1991) 25.
[5] G. Egginger, W. Lindner, G. Brunner, K. Stoschitzky, J. Pharm. Biomed. Anal. 12 (1994) 1537.
[6] G. Egginger, W. Lindner, S. Kahr, K. Stoschitzky, Chirality 5 (1993) 505.
[7] V.L. Herring, J.A. Johnson, J. Chromatogr. 612 (1993) 215.
[8] C. Pham-Huy, B. Radenen, A. Sahui-Gnassi, J.-R. Claude, J. Chromatogr. B 665 (1995) 125.
[9] K.M. Höld, D. de Boer, J.R. Soedirman, J. Zuidema, R.A.A. Maes, J. Pharm. Biomed. Anal. 13 (1995) 1401.
[10] J. Hermansson, A. Grahn, J. Chromatogr. A 687 (1994) 45.
[11] G. Lamprecht, T. Kraushofer, K. Stoschitzky, W. Lindner, J. Chromatogr. B 740 (2000) 219.
[12] G. Lamprecht, L. Gruber, K. Stoschitzky, W. Lindner, Chromatographia 56 (2002) 25.
[13] A. Berthod, X. Chen, J.P. Kullman, D.W. Armstrong, F. Gasparrini, I. D’Acquarica, C. Villani, A. Carotti, Anal. Chem. 72 (2000) 1767.
[14] M.S. Lee, E.H. Kerns, Mass Spectrom. Rev. 18 (1999) 187.
[15] M. Laakso, P.J. Pentikainen, K. Pyorala, P.J. Neuvonen, Eur. Heart J. 2 (1981) 353.
[16] A.W. Nathan, K.J. Hellestrand, R.S. Bexton, D.E. Ward, R.A.J. Spurrel, A.J. Camm, Br. Heart J. 47 (1982) 515.
[17] I. Rizos, J. Brachmann, W. Kuler, Am. J. Cardiol. 53 (1984) 1022.
[18] T. Walle, J.G. Webb, E.E. Bagwell, U.K. Walle, H.B. Daniell, T.E. Gaffney, Biochem. Pharmacol. 37 (1988) 115.
[19] J.M. Poirier, P. Jaillon, B. Lecocq, V. Lecocq, A. Ferry, G. Cheymol, Eur. J. Clin. Pharmacol. 38 (1990) 579.
[20] R.A. Carr, R.T. Foster, R.Z. Lewanczuk, P.G. Hamilton, J. Clin. Pharmacol. 32 (1992) 1105.
[21] C. Fiset, F. Philippon, M. Gilbert, J. Turgeon, Br. J. Clin. Pharmacol. 36 (1993) 75.
[22] R. Mehvar, D.R. Brocks, J. Pharm. Pharm. Sci. 4 (2001) 185.
[23] R.A. Carr, F.M. Pasutto, R.Z. Lewanczuk, R.T. Foster, Biopharm. Drug Dispos. 16 (1995) 705.