Journal of Chromatography A, 1216 (2009) 5385–5390

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Direct high-performance liquid chromatography enantioseparation of terazosin on an immobilised polysaccharide-based chiral stationary phase under polar organic and reversed-phase conditions

Rosella Ferretti, Bruno Gallinella, Francesco La Torre, Leo Zanitti, Luciana Turchetto, Antonina Mosca, Roberto Cirilli*

Istituto Superiore di Sanità, Dipartimento del Farmaco, Viale Regina Elena 299, I-00161 Rome, Italy

ARTICLE INFO

Article history:
Received 19 March 2009
Received in revised form 12 May 2009
Accepted 14 May 2009
Available online 21 May 2009

Keywords:
Enantioselective analysis
Chiral stationary phase
Chiralpak IC
Reversed-phase
Enantiomeric excess
Terazosin

ABSTRACT

High-performance liquid chromatography (HPLC) enantioseparation of terazosin (TER) was accomplished on the immobilised-type Chiralpak IC chiral stationary phase (CSP) under both polar organic and reversed-phase modes. A simple analytical method was validated using a mixture of methanol–water–DEA 95:5:0.1 (v/v/v) as a mobile phase. Under reversed-phase conditions good linearities were obtained over the concentration range 8.76–26.28 μg mL^{-1} for both enantiomers. The limits of detection and quantification were 10 and 30 ng mL^{-1}, respectively. The intra- and inter-day assay precision was less than 1.66% (RSD%). The optimised conditions also allowed to resolve chiral and achiral impurities from the enantiomers of TER. The proposed HPLC method supports pharmacological studies on the biological effects of the both forms of TER and analytical investigations of potential drug formulations based on a single enantiomer. At the semipreparative scale, 5.3 mg of racemic sample were resolved with elution times less than 12 min using a mobile phase consisting of methanol–DEA 100:0.1 (v/v) and both enantiomers were isolated with a purity of ≥99% enantiomeric excess (ee). The absolute configuration of TER enantiomers was assigned by comparison of the measured specific rotations with those reported in the literature.

1. Introduction

Terazosin, 2-[4-(tetrahydro-2-furanyl)carbonyl]-1-piperazinyl-6,7-dimethoxy-4-quinazolinamine monohydrochloride dihydrate (TER) (Fig. 1), is a representative of a group of drugs that block the α_1-adrenoceptor (α_1-antagonists) and it is used in the treatment of hypertension and benign prostatic hypertrophy. TER presents a single stereogenic centre in the tetrahydrofuran residue which gives origin to two enantiomeric forms. The racemic TER and its enantiomers show similar affinity for any of the three α_1-adrenoceptor subtypes, α_{1A}, α_{1B} and α_{1D}, whereas the (*R*)-TER enantiomer was significantly less potent than the (*S*)-TER enantiomer at α_{2B}- and α_{2b}-adrenoceptor subtypes [1,2] (the upper case subscript letter is referred to tissue-sourced receptors and the lower case letter defines cloned receptors). This suggests a possible use of the (*R*)-TER as a pharmacological probe to discriminate the heterogeneity of the α_2-adrenoceptors and to study the functional responses mediated by the α_2 receptors in tissues expressing multiple subtypes.

Nowadays, TER is only commercially available as a racemic mixture. Nevertheless, since receptor selectivity may have an important pharmacological impact on biological activity, side effects and safety, the stereoselective synthesis of (*R*)-TER has been patented by a pharmaceutical manufacturer for its potential therapeutic application [3].

To the best of our knowledge, there are only a few reports on the enantioseparation of TER by high-performance liquid chromatography (HPLC) on the analytical scale [4] whereas there are no reports in the literature on its resolution at the semipreparative scale.

The aim of this study was to develop a simple HPLC method capable of separating the enantiomers of the title compound. Our research aimed to evaluate the enantiomeric resolving capability of the immobilised-type Chiralpak IC chiral stationary phase (CSP) in the polar organic phase and reversed-phase conditions. First, we selected a mobile phase capable of giving a baseline enantioseparation of TER and in which the racemic form was well soluble so as to allow a larger amount of sample to be loaded onto the column per run. Then, the best analytical reversed-phase condition was validated in terms of linearity, repeatability and limits of detection (LOD) and quantification (LOQ) in order to quantify both enantiomers in biological fluids and in potential drug formulations. The validated method was also applied to the enantioselective analysis of TER in the presence of its main chiral and achiral impurities reported in the European Pharmacopoeia (EP) monograph.

* Corresponding author. Fax: +39 06 49387100.
E-mail address: rcirilli@iss.it (R. Cirilli).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.05.034

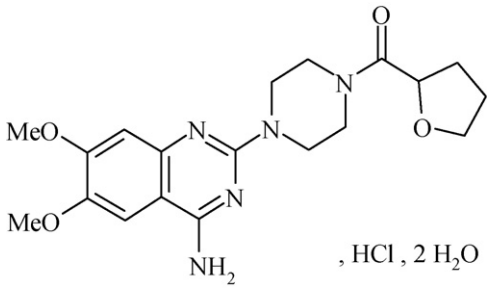

Fig. 1. Structure of terazosin monohydrochloride dehydrate (TER).

2. Experimental

2.1. Chemical and reagents

TER and available impurities were obtained by the European Directorate for the Quality of Medicines & Healthcare (EDQM) (France). HPLC-grade solvents were supplied by Carlo Erba (Milan, Italy). HPLC enantioseparations were performed by using stainless-steel Chiralpak IC (250 mm × 4.6 mm I.D., 150 mm × 4.6 mm I.D. and 250 mm × 10 mm I.D.) columns (Chiral Technologies Europe, Illkirch, France).

2.2. Instruments and chromatographic conditions

Analytical HPLC apparatus consisted on a Dionex P580 LPG pump, an ASI-100 T autosampler, a STH 585 column oven, a PDA-100 UV detector; data were acquired and processed by a Chromeleon Datasystem (Dionex Corporation, Sunnyvale, CA). For semipreparative separation a PerkinElmer (Norwalk, CT, USA) 200 LC pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 500 μL sample loop, a PerkinElmer LC 101 oven and Waters 484 detector (Waters Corporation, Milford, MA, USA) were used. The signal was acquired and processed by Clarity software (DataApex, Prague, The Czech Republic).

Mobile phases were filtered and degassed by sonication shortly before using. Experimental conditions for analytical enantioseparations are indicated in Table 1.

In analytical separations, fresh standard solution of TER and single impurities were prepared shortly before using by dissolving 1–3 mg of each analyte in the mobile phase. The injection volume was of 20 μL.

In semipreparative enantioseparations, standard solutions were prepared by dissolving the racemic sample in the mobile phase. The sample concentration was 10.7 mg mL^{-1}. The injection volumes were 0.2, 0.3 and 0.5 mL. The column temperature and the flow-rate were set at 25 °C and 4 mL min^{-1}, respectively. After semipreparative separation, the collected fractions were pooled, evaporated and analyzed by a chiral analytical column to determine their enantiomeric excess (ee).

The column hold-up time (t_0 = 3.0 min for 250 mm × 4.6 mm I.D. column) was determined from the elution of an unretained marker (toluene), using methanol as eluent, delivered at a flow-rate of 1.0 mL min^{-1}.

Specific rotations of the enantiomers of TER, dissolved in 3N HCl, were measured at 589 nm by a PerkinElmer (Norwalk, CT, USA) polarimeter model 241 equipped with a Na lamp. The volume of the cell was 1 mL and the optical path 10 cm. The system was set at a temperature of 20 °C using a Neslab RTE (Manasquan, New Jersey, USA) 740 cryostat.

Table 1
Retention factor (k_1) for the first eluting enantiomer, enantioseparation (α) and resolution (R_s) factors of TER.

Mobile phase	k_1 (S)[a]	α	R_s
Methanol–DEA 100:0.1	1.38	1.30	3.77
Ethanol–DEA 100:0.1	0.95	1.35	2.04
Acetonitrile–DEA 100:0.1	1.56	1.36	3.12
Methanol–H_2O–DEA 95:5:0.1	1.76	1.35	4.12
Methanol–H_2O–DEA 90:10:0.1	2.00	1.37	4.22
Methanol–H_2O–DEA 80:20:0.1	2.99	1.41	4.25
Methanol–H_2O–DEA 70:30:0.1	5.39	1.42	4.13

Column, Chiralpak IC (250 mm × 4.6 mm I.D.); flow-rate, 0.5 mL min^{-1}; temperature, 25 °C; detection, UV at 238 nm.
[a] Absolute configuration of the first eluted enantiomer.

2.3. Absolute configuration and enantiomeric elution order determination

The absolute configuration of the collected enantiomers was assigned by comparing their specific rotations values with those reported in literature [3]. The enantiomeric elution order on the Chiralpak IC CSP was established by analysing non-racemic samples enriched by the (*R*)-TER enantiomer.

2.4. Method validation

2.4.1. HPLC operating conditions

Analytical chromatographic separations were carried out on a Chiralpak IC column (250 mm × 4.6 mm I.D.) with a mobile phase consisting of MeOH–H_2O–DEA in the ratio 95:5:0.1 (v/v/v) at a flow-rate of 0.5 mL min^{-1} and maintaining the column at 25 °C.

The injection volume was 20 μL, sampler temperature was set at 5 °C, and the detection wavelength was set at 238 nm.

2.4.2. Specificity

The selectivity of the analytical method was evaluated by the analysis of a solution containing TER enantiomers and its main related substances.

2.4.3. Preparation of stock and standard solutions

Standard solutions of TER, carefully protected from light, were prepared and used daily for calibration purpose. Standard solutions were examined in the range from about 50% to about 150% relative to the working concentration of about 17.52 μg mL^{-1} (100%) of each enantiomer of TER. Stock solutions of racemic TER were prepared by dissolving about 20 mg in 20 mL volumetric flasks with the mobile phase and kept at −20 °C. Aliquots of 1, 1.5, 2, 2.5 and 3 mL of these solutions were transferred into 50 mL volumetric flasks and diluted with the mobile phase. The final concentrations of standard solutions were 8.76, 13.14, 17.52, 21.92 and 26.28 μg mL^{-1}. The vials containing the solutions for the injections were put in the autosampler set at 5 °C before the analyse.

2.4.4. Recovery

Commercially available tablets containing 5 mg of TER were pulverized with a pestle in a porcelain mortar. The powder was added with methanol and the suspension was sonicated for 20 min and filtered. The methanol was evaporated under reduced pressure.

2.4.5. Linearity

The linearity evaluation was performed with the standard solutions of racemic TER at the concentrations described ranging from 8.76 to 26.28 μg mL^{-1} for each enantiomer. Three injections of each solution were made under the chromatographic conditions described above, using an injection volume of 20 μL. The peak area response corresponding to the first eluted (*S*)-TER enantiomer and the second eluted (*R*)-TER enantiomer was plotted against the cor-

responding concentration and the linear regression equation was computed.

2.4.6. LOD and LOQ

Limit of detection and limit of quantitation represent the concentration of the analyte that would yield a signal/noise (S/N) of 3 and 10, respectively, following the EP. LOD and LOQ of (*S*)-(−)-TER were determined by injecting a series of dilute solutions.

2.4.7. Precision and repeatability

Method precision was determined by measuring the repeatability (intra-day precision) and intermediate precision (inter-day precision) of retention times and peak areas for TER enantiomers. The intra-day variability was performed by the same analyst over one day, while inter-day precision was carried out by another independent analyst over three days. In order to determine the repeatability of the method, replicate injections ($n = 6$) of 17.52 μg mL^{-1} of TER enantiomers were carried out. The intermediate precision was evaluated over three days by performing six consecutive injections each day. Precision was reported as % of relative standard deviation (%RSD).

2.4.8. Accuracy

Accuracy of the method was tested by analyzing samples of racemic TER at various concentration levels. Both enantiomers were considered for the evaluation.

Solutions of TER extracted from commercially available tablets were also analyzed. In this case the accuracy was evaluated by using three samples at the nominal concentration of 20 μg mL^{-1}.

3. Results and discussion

3.1. Influence of the mobile phase composition on enantioselectivity and resolution

Besides the already available Chiralpak IA [5] and Chiralpak IB [6,7] CSPs, a new immobilised-type CSP for HPLC, the Chiralpak IC CSP [8–10], has been recently launched by Daicel Chemical Industries Ltd. The Chiralpak IC uses cellulose tris(3,5-dichlorophenylcarbamate) immobilised onto silica 5-μm particles as a chiral selector. The procedure of anchoring of the semisynthetic polymer to the silica matrix gives a high chemical stability without any drawbacks in terms of enantioselectivity and efficiency [8]. In the first part of our study, the resolving ability of the Chiralpak IC CSP towards TER was investigated in the polar organic mode using pure methanol, ethanol and acetonitrile with a 0.1% addition of DEA. The chromatographic data are shown in Table 1. The resolving power of the IC CSP was sufficiently high to achieve a baseline enantioseparation in each of the used condition. The best resolution value was achieved by using the methanol–DEA 100:0.1 (v/v) eluent with enantioselectivity factor (α) and resolution factor (R_s) values of 1.30 and 3.77, respectively.

3.2. Semipreparative enantioseparation and absolute configuration assignment

As a matter of fact, in order to load a greater amount of sample on the column per run it is preferable to use a mobile phase in which the analyte is appreciably soluble [11–16]. TER is soluble

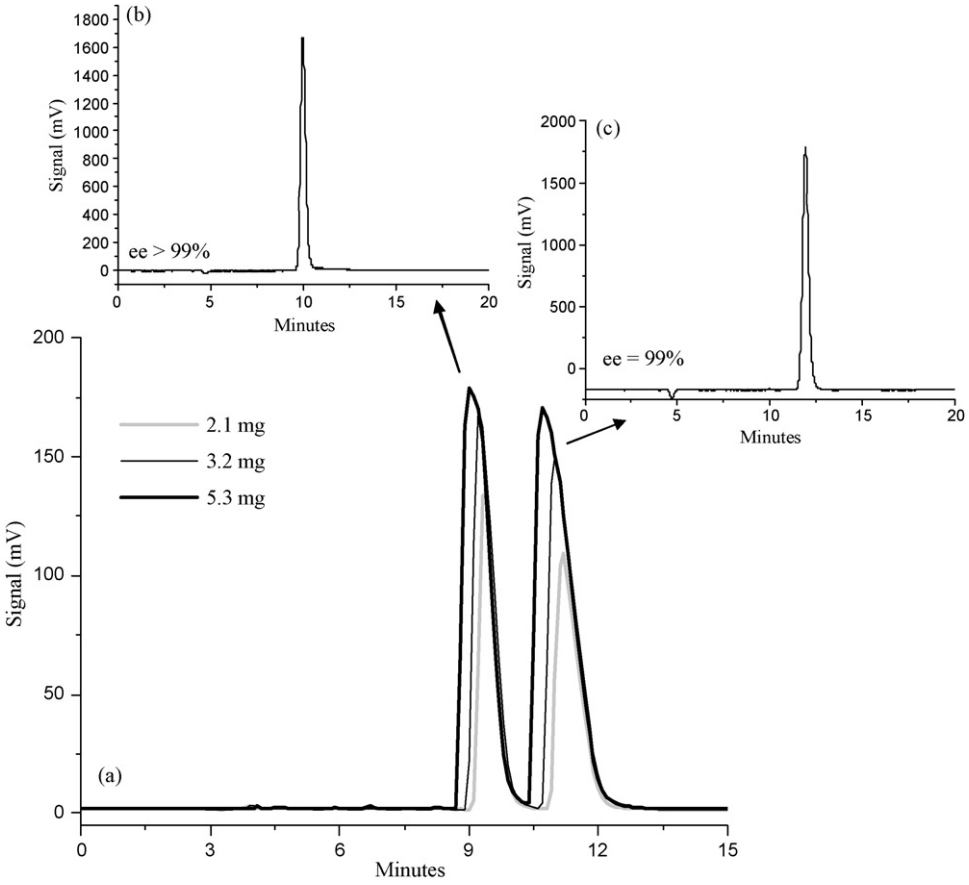

Fig. 2. (a) Typical chromatograms illustrating the resolution of 2.1, 3.2 and 5.3 mg of TER and (b, c) analytical check of the collected enantiomers. Column: (a) Chiralpak IC (250 mm × 10 mm I.D.), (b, c) Chiralpak IC (150 mm × 4.6 mm I.D.); detection: (a) UV at 305 nm, (b, c) UV at 238 nm; eluent: methanol; flow-rate: (a) 4 mL min^{-1}, (b, c) 0.5 mL min^{-1}; column temperature: 25 °C.

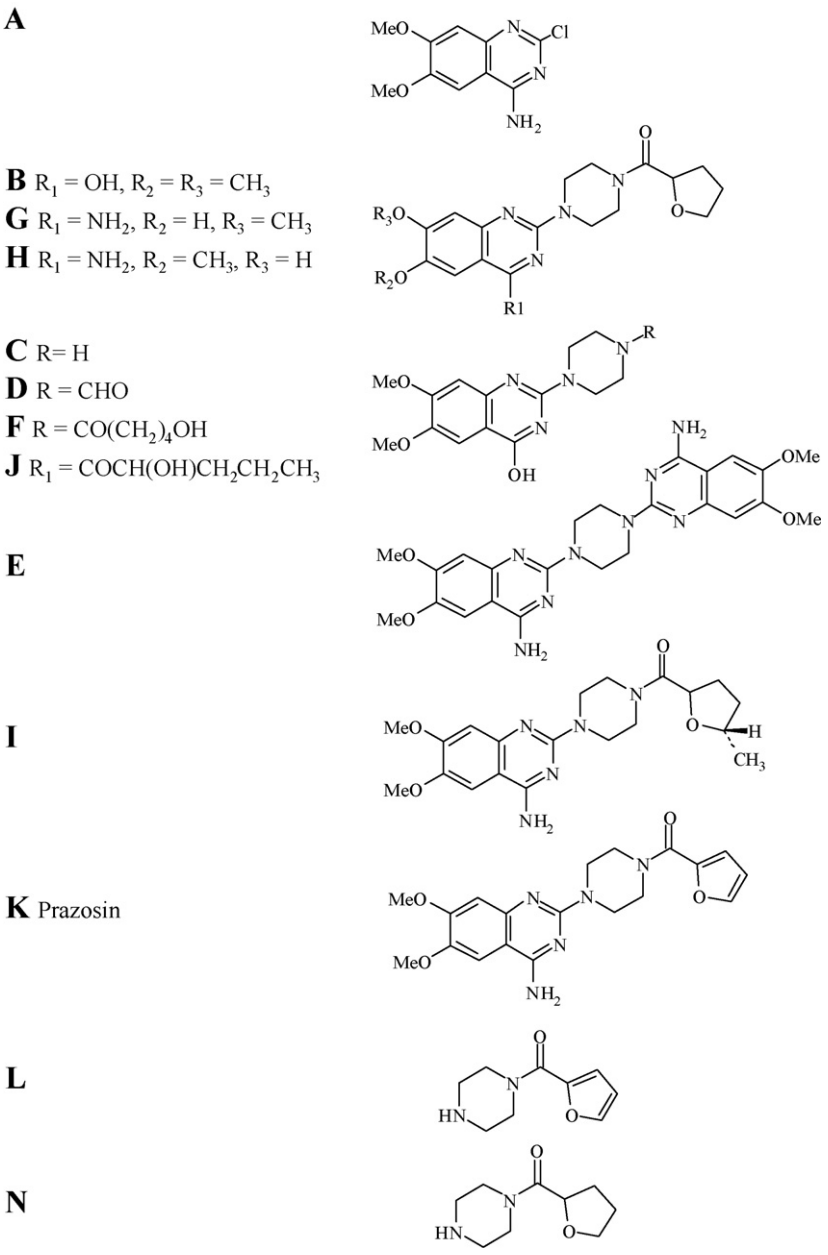

Fig. 3. Structure of the main impurities of TER.

in methanol and water, slightly soluble in ethanol and practically insoluble in acetone and hexane. As a consequence, methanol–DEA 100:0.1 (v/v) eluent was selected to shortly resolve mg-amounts of the racemic sample at the semipreparative level. An injection of 2.1, 3.2 and 5.3 mg of racemic sample dissolved in the mobile phase (methanol–DEA, 100:0.1, v/v) at a flow-rate of 4.0 mL min^{-1} afforded complete enantioseparation (Fig. 2). Assays of both fractions collected from the 5.3 mg run gave ee (enantiomeric excess) ≥99%. Total time for this run was 12 min. The specific rotation values of the two enantiomers, isolated as free bases, were as follows: first elueted enantiomer: $[\alpha]_D^{20} - 35.0$ (c 0.2, 3N HCl); second eluted enantiomer: $[\alpha]_D^{20} + 38.0$ (c 0.2, 3N HCl). On comparing the polarimetric data with those reported in the literature [3] {$[\alpha]_D^{22} + 34.83$ (c 1, 3N HCl) (ee not reported) for the (*R*)-TER enantiomer and $[\alpha]_D^{22} - 26.9$ (c 1, 3N HCl) (ee not reported) for the (*S*)-TER enantiomer, it was possible to establish the following absolute configuration and the enantiomeric elution order: (*S*)-configuration was assigned to the levorotatory enantiomer, first eluted on the Chiralpak IC CSP, and (*R*)-configuration to the dextrorotatory second eluted enantiomer.

3.3. Reversed-phase enantioseparation

The immobilised polysaccharide-derived CSPs have been used under reversed-phase conditions for the resolution of many classes of chiral substances [5,17–19]. In this work, the enantiorecognition ability of the IC CSP was evaluated by using aqueous eluents consisting of a methanol–DEA mixture and an increasing percentage of water (5%, 10%, 20% and 30%) was added. From inspection of Table 1, it can be seen that the enantioseparation of TER increased slightly but progressively as the water percentage increased up to a maximum of between 20% and 30%. The addition of only 5% of water in volume to methanol increased the enantioseparation and resolution factors to 1.35 and 4.12, respectively, without excessively

Table 2
Retention factor (k_1) for the first eluting enantiomer, separation (α) and resolution (R_s) factors of the chiral impurities H, G, N, B, J and I.

Impurity	k_1	α	R_s
H	0.22	1.17[a]	1.30
G	0.43	1.30[a]	2.28
N	0.66	1.11[a]	1.45
B	0.75	1.41[a]	3.45
J	1.39	1.23[a]	2.92
I	1.73	1.40[b]	5.53

Column, Chiralpak IC (250 mm × 4.6 mm I.D.); flow-rate, 0.5 mL min^{-1}; temperature, 25 °C; detection, UV at 251 nm.
[a] Enantioseparation factor.
[b] Diastereoseparation factor.

prolonging the time of analysis. The transition from polar organic to reversed-phase conditions or *vice versa* did not significantly affect the performance of the IC CSP.

3.4. Chemoselective analysis

A critical aspect in the development of an analytical method to determine the enantiomeric purity of a chiral drug is the presence of impurities from the processes of synthesis, degradation or manufacturing either in the drug substance or in the finished product. The main organic impurities of TER reported in the EP monograph are shown in Fig. 3. Stability studies, chemical development studies, routine batch analyses and quality control of the commercial product require a chemo- and enantioselective method capable of discriminating the enantiomeric API (Active Pharmaceutical Ingredient) from the related substances. Consequently, we turned our attention to examine the chemical selectivity of the Chiralpak IC using the methanol–water–DEA 95:5:0.1 (v/v/v) mixture as a mobile phase.

As can be seen from the chromatograms in Fig. 4, in these conditions, the TER enantiomers were well separated and there was no overlapping with potential organic impurities.

From an inspection of Table 2 and Fig. 4 it can be seen that all the racemic impurities were resolved with an α-value ranging from 1.11 to 1.41. The diastereomeric mixture I was separated with a separation factor of 1.40. In addition, the observed enantiomeric elution order (*S*)-TER < (*R*)-TER was beneficial to the quantitative determination of the (*R*)-enantiomer, considered as a potential API, since the minor (*S*)-enantiomer was eluted first.

3.5. Validation of the method

3.5.1. System suitability

Impurity A and TER enantiomer were chosen to determine the system suitability parameters. Six consecutive runs were performed and the results are showed in Table 3.

3.5.2. Linearity

The linearity of the HPLC method was evaluated by injecting standard concentrations of racemic samples with a concentration ranging from 8.76 to 26.28 μg mL^{-1} (50–150%) for each single enantiomer. The peak area response of the both enantiomers was plotted versus the nominal concentration of the enantiomer. The linearity was evaluated by linear regression analysis, which was calculated by least squares regression method. The obtained calibration curve showed correlation coefficient greater than 0.9997. For (*S*)-(−)-TER the following regression equation was obtained $y = 0.0031x + 0.0034$ ($r^2 = 0.9997$) where y is the peak area of (*S*)-(−)-TER and x is the concentration. For (*R*)-(+)-TER the following regression equation was obtained $y = 0.0031x + 0.0031$ ($r^2 = 0.9998$).

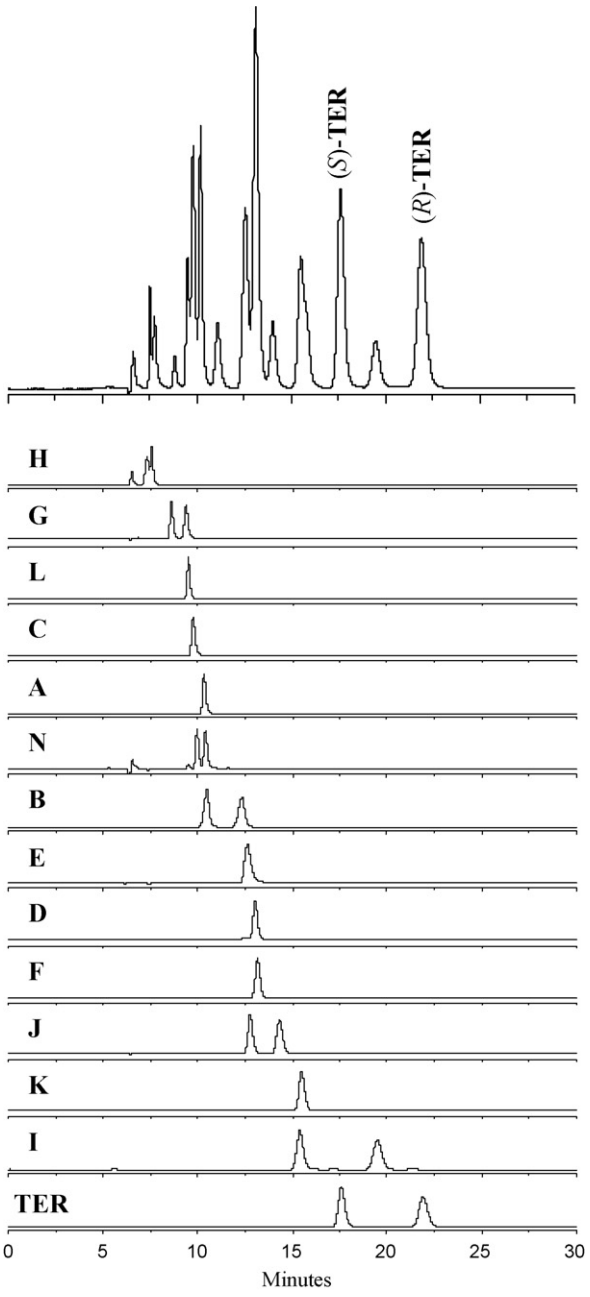

Fig. 4. Typical HPLC chromatograms of TER spiked with impurities (top) and single impurities.

Table 3
System suitability parameters of the chromatographic method for impurity A and TER enantiomers.

Compound	t_r[a] (RSD%)	R_s[a] (RSD%)	Theoretical plates[a] (RSD%)	Asymmetry factor[a] (RSD%)
A	5.88 (0.07)	–	7942 (0.28)	1.16 (0.77)
(*S*)-(−)-TER	10.16 (0.10)	11.47 (0.13)	7053 (0.31)	1.07 (0.38)
(*R*)-(+)-TER	12.36 (0.16)	4.02 (0.20)	6593 (0.38)	1.06 (0.52)

[a] Mean value of six replicates. Chromatographic condition as reported in the test.

3.5.3. LOD and LOQ

LOD and LOQ were estimated to be 10 and 30 ng mL^{-1} for each enantiomer of (*S*)-(−)- and (*R*)-(+)-TER, when S/N of 3 and 10 were used as criteria.

3.5.4. Precision and repeatability

The precision of the HPLC method was determined by repeatability (intra-day) and intermediate precision (inter-day). Method precision had a relative standard deviation (RSD%) below 2.0% for repeatability (1.26% for retention times and 0.78% for peak area) and for the intermediate of precision (1.37% for retention times and 0.80% for peak area), which comply with the proposed acceptance criteria (RSD%: not more than 2.0%).

3.5.5. Accuracy

Accuracy for the determination of enantiomeric composition was determined by preparing three drug substance samples at 50%, 100% and 150% of the target concentration (8.80–26.40 μg mL^{-1}). Apparent recovery ranged from 101.13% to 103.59%. Overall percent recovery was 102.33 (RSD% 0.90) for the first eluted (*S*)-(−)-TER enantiomer and 100.51 (RSD% 0.95) for the second eluted (*R*)-(+)-TER enantiomer. Solutions of extracted TER were also analyzed. Accuracy evaluated from commercially available tablets was 100.38% (RSD% 0.96) for (*S*)-(−)-TER and 101.12% (RSD% 1.07) for (*R*)-(+)-TER.

4. Conclusions

In conclusion, a simple, sensitive, chemo- and enantioselective HPLC method for the quantitative determination of the enantiomers of TER was developed and validated using the immobilised-type Chiralpak IC CSP in reversed-phase conditions. The proposed method meets the requirements of the EP guidelines and seems to be a reliable way to determine the enantiomeric purity of TER in bulk drugs and pharmaceutical preparations. We have also demonstrated the applicability of the Chiralpak IC CSP for semipreparative enantioseparations using a simple mobile phase consisting of methanol–DEA 100:0.1 (v/v).

The easy availability of both enantiomeric forms of TER supports pharmacological studies aimed at assessing the α_1-/α_2-adrenergic receptor selectivity of this drug.

References

[1] L.M. Ireland, J.C. Cain, G. Rotert, S. Thomas, S. Shoghi, A.A. Hancock Jr., J.F. Kerwin, Eur. J. Pharmacol. 327 (1997) 79.
[2] A.A. Hancock, S.A. Buckner, L.M. Ireland, S.M. Knepper Jr., J.F. Kerwin, J. Recept. Signal. Transduct. Res. 15 (1995) 863.
[3] J.J. Kyncl, B.W. Horrom, Patent No. WO/1992/000073.
[4] A.P. Zavitsanos, T. Alebic-Kolbah, J. Chromatogr. A 794 (1998) 45.
[5] T. Zhang, C. Kientzy, P. Franco, A. Ohnishi, Y. Kagamiharb, H. Kurosawa, J. Chromatogr. A 1075 (2005) 65.
[6] T. Zhang, D. Nguyen, P. Franco, T. Murakami, A. Ohnishi, H. Kurosawa, Anal. Chim. Acta 557 (2006) 221.
[7] A. Ghanem, J. Sep. Sci. 30 (2007) 1019.
[8] T. Zhang, D. Nguyen, P. Franco, Y. Isobe, T. Michishita, T. Murakami, J. Pharm. Biomed. Anal. 46 (2008) 882.
[9] T. Zhang, D. Nguyen, P. Franco, J. Chromatogr. A 1191 (2008) 214.
[10] C. Wang, D.W. Armstrong, C.D. Chang, J. Chromatogr. A 1194 (2008) 172.
[11] T. Zhang, M. Schaeffer, P. Franco, J. Chromatogr. A 1083 (2005) 96.
[12] R. Cirilli, A. Simonelli, R. Ferretti, A. Bolasco, P. Chimenti, D. Secci, E. Maccioni, F. La Torre, J. Chromatogr. A 1101 (2006) 198.
[13] R. Cirilli, R. Ferretti, B. Gallinella, L. Turchetto, A. Bolasco, D. Secci, P. Chimenti, M. Pierini, V. Fares, O. Befani, F. La Torre, Chirality 16 (2004) 625.
[14] R. Cirilli, R. Ferretti, B. Gallinella, F. La Torre, G. La Regina, R. Silvestri, J. Sep. Sci. 28 (2005) 627.
[15] M. Biava, R. Cirilli, V. Fares, R. Ferretti, B. Gallinella, F. La Torre, G. Poce, G.C. Porretta, S. Supino, C. Villani, Chirality 20 (2008) 775.
[16] E.R. Francotte, J. Chromatogr. A 906 (2001) 379.
[17] R. Cirilli, R. Ferretti, E. De Santis, B. Gallinella, L. Zanitti, F. La Torre, J. Chromatogr. A 1190 (2008) 95.
[18] R. Cirilli, R. Ferretti, B. Gallinella, E. De Santis, L. Zanitti, F. La Torre, J. Chromatogr. A 1177 (2008) 105.
[19] R. Cirilli, R. Ferretti, B. Gallinella, A.R. Bilia, F.F. Vincieri, F. La Torre, J. Sep. Sci. 31 (2008) 2206.