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

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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.

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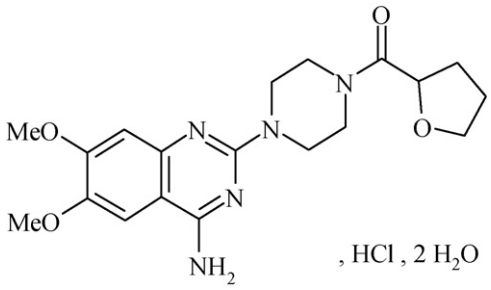

Fig. 1. Structure of terazosin monohydrochloride dehydrate (TER).

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-