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Short communication

Analytical and semipreparative high performance liquid chromatography enantioseparation of bicalutamide and its chiral impurities on an immobilized polysaccharide-based chiral stationary phase

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ABSTRACT

Direct HPLC separation of enantiomers of Bicalutamide (BCT), a non-steroidal antiandrogen used for the treatment of prostate cancer, was performed by using the immobilized amylose-based Chiralpak IA chiral stationary phase (CSP). Enantioselective conditions were achieved using standard normal phase mixtures *n*-hexane-alcohol (ethanol or 2-propanol) and a "non-standard" mobile phase containing ethyl acetate (EA). The chromatographic behaviour of the IA CSP under these elution modes was evaluated and compared at different temperatures. The eluent mixture *n*-hexane-EA-ethanol 100-30-5 (v/v/v) and the column temperature of 40 °C were identified as the best operational conditions to carry out semipreparative enantioseparations on a 1-cm I.D. IA column. Using this protocol, about 960 mg of (*R*)-BCT, which is the enantiomer with the almost entire anti-androgenic activity of BCT, per day could be isolated.

The analytical and semipreparative HPLC resolution of chiral impurities of BCT, and their empiric absolute configuration assignment by circular dichroism correlation method are also presented.

1. Introduction

Bicalutamide (BCT) (Fig. 1), *N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-propanamide, is a selective nonsteroidal anti-androgen drug used for treatment of prostate cancer [1]. It binds to the androgen receptors in the prostate and other androgen sensitive tissues and competitively inhibits the action of testosterone and dihydrotestosterone. Although literature surveys have revealed that the anti-androgenic activity of BCT lies almost exclusively in the (*R*)-enantiomer [1], BCT is currently available as a racemic mixture under the trade name of Casodex. Compared to the corresponding racemic drug, the (*R*)-BCT has also more favourable pharmacokinetic and toxicological properties and its administration could lead to a reduction of the dosage and the demand on the liver function. In 2003, the enantiopure (*R*)-BCT active pharmaceutical ingredient (API) has been patented [2]. Accordingly, there is a clear need for methods able to obtain BCT in enantiomerically pure form and to check the stereochemical course of pharmacological, analytical and synthetic investigations.

High-performance liquid chromatography (HPLC) on chiral stationary phase (CSP) has been successfully employed to separate the enantiomers of this drug at an analytical level [3–6]. In particular, enantioselective methods based on polysaccharide-based CSPs seem to be efficient routs for checking the enantiomeric purity of BCT. However, to the best of our knowledge, there were no reports about the development and application of HPLC methods for separation of enantiomers of BCT on a semipreparative scale.

Our objective in this study was to find the best conditions to separate the enantiomers of BCT on mg-scale using as enantioselective packing material the amylose-based Chiralpak IA CSP. Chiralpak IA has the same amylose tris(3,5-dimethylphenylcarbamate) selector as Chiralpak AD and Lux Amylose-1CSPs but immobilized, and not coated, onto porous silica particles [7]. The advantage gained by use of Chiralpak IA CSP with respect to non-immobilized CSPs in optimization of enantioselective conditions at semipreparative level lies on its total solvent compatibility [8–12]. As a rule of thumb, a mg- or g-scale enantioseparation is practically feasible and economically attractive if the racemic sample shows an appreciable solubility in the eluent. Non-standard solvents such as ethyl

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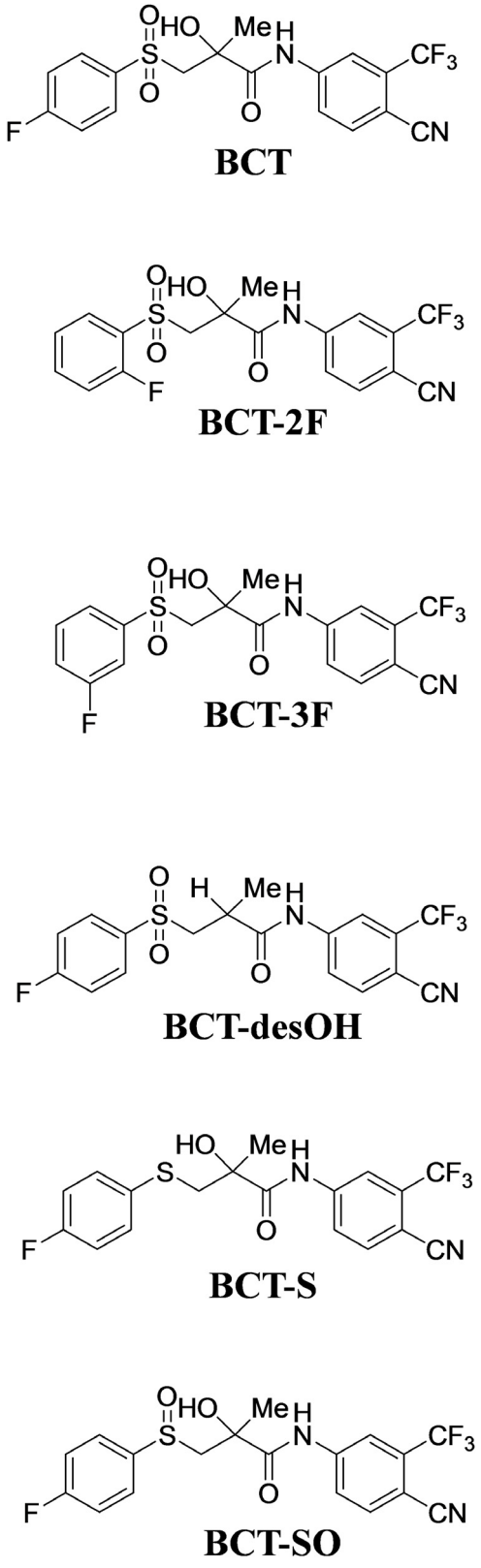

Fig. 1. Structure of BCT and its potential chiral impurities.

acetate (EA) or tetrahydrofuran (THF), whose use is precluded in the preparation of eluent and sample with coated-type CSPs, can easily dissolve BCT and its impurities. Soma Raju et al. [5] have reported that the Chiralpak IA 250 mm × 4.6 mm column in combination with mobile phase containing non standards solvents such as THF and EA provided an appreciable recognition ability towards BCT. Under the mobile phase *n*-hexane-EA-methanol 15:30:55 (v/v/v) the retention factor values for two enantiomers of BCT were 3.3 and 3.6 and the resolution factor was 1.5.

So, although the HPLC method showed a satisfactory behaviour for analytical applications, it cannot be considered productive in terms of sample throughput.

In the present investigation, an improved way of achieving high sample loading in semipreparative enantioselective HPLC of BCT under EA-based conditions is described. Because the availability of single enantiomers of chiral impurities and their stereochemical characterization are topic issues in the drug research and development of APIs in enantiopure form, a further objective of this study was to resolve the chiral related substances of BCT at semipreparative level and determine their absolute configuration.

2. Experimental

2.1. Chemical and reagents

BCT and the impurities showed in Fig. 1 were obtained by the European Directorate for the Quality of Medicines & Healthcare (EDQM, France). HPLC enantioseparations were performed by using stainless-steel Chiralpak IA (250 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 or a Jasco (Jasco, Tokyo, Japan) Model CD 2095 Plus UV/CD detector; data were acquired and processed by a Chromeleon Datasystem (Dionex Corporation, Sunnyvale, CA). For semipreparative separation a Perkin-Elmer (Norwalk, CT, USA) 200 LC pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 500 μL sample loop, a Perkin-Elmer 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).

In semipreparative enantioseparations of BCT and its chiral impurities, standard solutions were prepared by dissolving the racemic sample in a mixture EA/mobile phase C (2:3). After semipreparative separation, the collected fractions were pooled, evaporated and analyzed by a chiral analytical column to determine their enantiomeric excess (e.e.).

The circular dichroism (CD) spectra of enantiomers BCT and chiral impurities (Fig. 1), dissolved in ethanol (concentration about 0.2 mg/mL), in a quartz cell (0.1 cm-path length) at 25 °C, were measured by using a Jasco Model J-700 spectropolarimeter. The spectra are average computed over three instrumental scans and the intensities are presented in terms of ellipticity values (mdeg).

2.3. Absolute configuration and enantiomer elution order determination

The absolute configuration of the collected enantiomers of BCT was assigned by comparing the enantiomer elution order on the Chiralpak AD CSP with that reported in literature [4]. The stereochemical characterization of the enantiomers of the chiral impurities of BCT was established by comparing their CD spectra with those of the enantiomers of BCT (Fig. S1 of Supporting information, SI).