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Isolation and characterization of process related impurities and degradation products of bicalutamide and development of RP-HPLC method for impurity profile study

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Abstract

A reversed-phase high-performance liquid chromatographic method was developed for determination of process impurities and degradation products of bicalutamide in bulk drug and pharmaceutical formulations. The separation was accomplished on a Symmetry C_{18} (4.6 mm × 250 mm; particle size 5 μm) column under isocratic mode. The mobile phase was 0.01 M KH_2PO_4 (pH 3.0):acetonitrile (50:50 v/v) and a PDA detector set at 215 nm was used for detection. Forced degradation of bicalutamide was carried out under thermal, photo, acidic, alkaline and peroxide conditions. The unknown process impurities and alkaline degradation products were isolated and characterized by ESI-MS/MS, ^{1}H NMR and FT-IR spectral data. Under alkaline conditions bicalutamide was degraded in to an acid and an amine. The kinetics of degradation was studied. The proposed method was validated and successfully applied to the analysis of commercial formulations. Thus, the developed method can be used for process development as well as quality assurance of bicalutamide in bulk drug and pharmaceutical formulations.

Keywords: Bicalutamide; Forced degradation; Process impurities; Kinetics of degradation; Isolation and characterization

1. Introduction

Bicalutamide (BCT) (Fig. 1) (*RS*)-*N*-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methyl-propanamide, under the trade name of Casodex® is one of the leading non-steroidal anti-androgens used for treatment of prostate cancer. It competes with testosterone and dihydrotestosterone for binding on to the prostate and other androgen-sensitive tissues. It binds preferentially to receptors located outside the central nervous system and causes little increase in testosterone levels with little agonist activity [1–3]. The pharmacokinetics of BCT was studied thoroughly [4,5].

A thorough literature search has revealed that, there are only a few HPLC methods reported for determination of BCT in plasma [6–8]. A few chiral HPLC methods have been reported for the determination of enantiomers of BCT [9–12]. Recently we have also reported chiral HPLC method for the determination of BCT enantiomers in bulk drug and pharmaceutical formulations [13]. Torok et al. have evaluated different chiral stationary phases for separation of enantiomers of BCT and its impurities [14]. Recently Saravanan et al. have reported a stability indicating HPLC method for separation of two of the process impurities and one of the degradation products of BCT in gradient elution mode using tetra *n*-butyl ammonium hydrogen sulphate as an ion pair reagent [15]. The method has several draw backs, viz.; (i) only one of the degradation products was separated where as more than one product were formed on degradation under alkaline conditions, (ii) use of ion pair reagent decreases not only the column life time but also increases the time required for equilibration, (iii) use of an ion pair reagent in a gradient elution mode is not preferred because of system artifacts, and (iv) the process impurities and degradation products were not characterized. Further, BCT is not yet official in any of the pharmacopoeia and no method for determination of its impurities has been reported. The present work describes the development of simple isocratic reversed-phase high-performance liquid chromatographic (RP-HPLC) method for determination of BCT, degradation products and process related impurities in bulk drug and pharmaceutical formulations. Forced degradation of BCT was carried out

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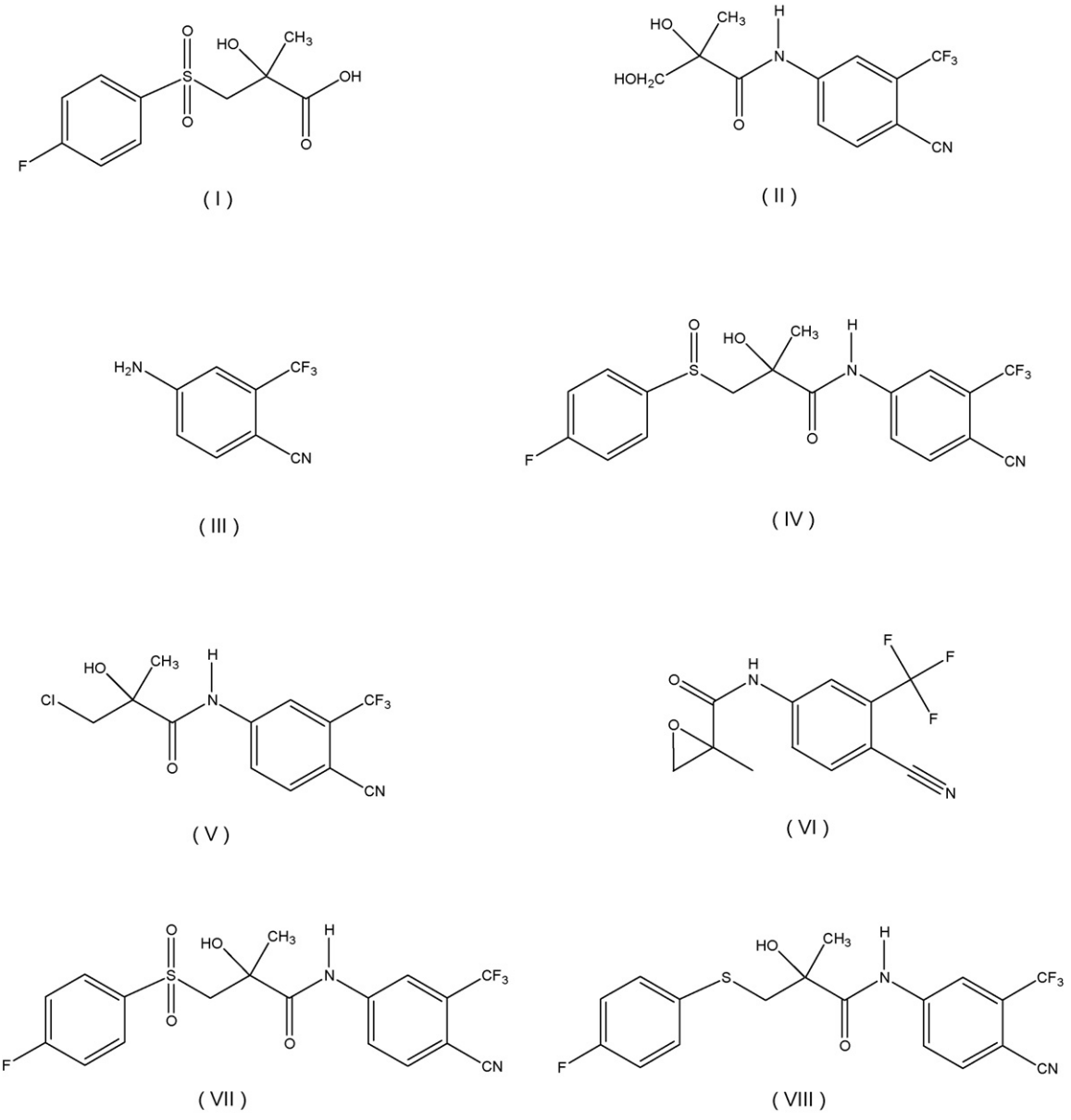

Fig. 1. Chemical structures of BCT (VII), degradation products (I and III) and its process-related impurities (II, IV, V, VI and VIII).

under thermal, photo, acidic, alkaline and peroxide conditions. Two degradation products were formed under alkaline conditions where as Saravanan et al. [15] have reported only one of them. In the present study we also discussed the kinetics of degradation. While studying the synthetic process in our laboratory, we observed (II), (IV), V, VI and (VIII) (Fig. 1) as process related impurities which are likely to be present in the finished products. Two are unknown impurities (II and IV) and detected consistently in almost all the batches. The area percentage of impurities was in the range from 0.03 to 0.1%. The impurity profile study has to be carried out for any final product to identify and characterize all the unknown impurities that are present at a level of even below 0.05% [16]. The requirement of identifying and characterizing the impurities in the final product is extremely important in the wake of stringent requirements from the regulatory authorities. Two process impurities (II and IV) and the alkaline degradation products (I and III) were isolated and characterized by ESI-MS/MS, ^{1}H NMR and FT-IR spectral data. The ESI-MS/MS profiles of BCT and all impurities were discussed during characterization.

2. Experimental

2.1. Materials and reagents

All the reagents were of analytical-reagent grade unless stated otherwise. Glass-distilled and de-ionized water (Nanopure, Barnsted, USA), HPLC-grade acetonitrile, methanol, ammonium acetate, potassium dihydrogen phosphate, phosphoric acid, hydrochloric acid, sodium hydroxide, hydrogen peroxide, chloroform, ethyl acetate and hexanes (S.D. Fine Chem., Mumbai, India) were used. $CDCl_3$ and DMSO were purchased from