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ESI-MS^n and LC–ESI-MS studies to characterize forced degradation products of bosentan and a validated stability-indicating LC–UV method

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ABSTRACT

The present study reports the characterization of forced degradation products of bosentan and a validated stability-indicating HPLC method for the stability testing of bosentan tablets. The forced degradation was carried out under the conditions of hydrolysis, oxidation, dry heat and photolysis. The drug was found unstable in acid, alkali and oxidative media whereas stable to the hydrolysis in water, to dry heat and to photolysis. In total, six degradation products were formed in all conditions which were resolved in a single run on a C-18 column with gradient elution using ammonium acetate buffer (pH 4.5, 5.0 mM), methanol and acetonitrile. Structures of all the degradation products were characterized through +ESI-MS^n and LC–ESI-MS spectral data of bosentan as well as LC–ESI-MS spectral data of the products. The products **II–VI** were characterized as 6-amino-[2,2′]bipyrimidinyl-4,5-diol, 6-amino-5-(2-methoxyphenoxy)-[2,2′]-bipyrimidinyl-4-ol, 2-[6-amino-5-(2-methoxyphenoxy)-[2,2′]-bipyrimidinyl-4-yloxy]-ethanol, 4-*tert*-butyl-*N*-[6-(1-methoxyethoxy)-5-(2-methoxyphenoxy)-[2,2′]-bipyrimidinyl-4-yl]-benzenesulfonamide and 4-*tert*-butyl-*N*-[6-hydroxy-5-(2-methoxyphenoxy)-[2,2′]bipyrimidinyl-4-yl]-benzenesulfonamide, respectively. The peak of the product **I** was found to be due to two secondary degradation products which co-eluted and were characterized as β-hydroxyethyl *p*-*tert*-butylphenylsulfonate (**Ia**) and 2-[2-(2-hydroxyethoxy)-phenoxy]-ethanol (**Ib**). These products were formed due to hydrolysis of sulfonamide and alkylaryl ether and the diaryl ether linkages as well as dehydration of the primary alcohol group. The most probable degradation mechanisms were proposed. The HPLC method was found to be stability-indicating, linear (2–100 μg ml^{-1}), accurate, precise, sensitive, specific, rugged and robust for quantitation of the drug. The method was applied to the stability testing of the commercially available bosentan tablets successfully.

1. Introduction

Bosentan, 4-*tert*-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-[2,2′]-bipyrimidinyl-4-yl]-benzenesulfonamide is a non-peptide endothelin (ET) receptor antagonist, which is used for the treatment of pulmonary hypertension, chronic heart failure, sub-arachnoid hemorrhage and Raynaud's syndrome. It acts by antagonizing the binding of endothelins to ET_A as well as ET_B receptors [1,2]. It is one of the latest cardiovascular drugs and now into Phase IV clinical trials [3]. The drug is not official in any pharmacopeia. Several analytical methods have been reported in the literature for the determination of bosentan alone, along with its derivatives, metabolites or other drugs in various sample matrixes [4–7]. Recently, Niphade et al. [7] have identified two process related impurities formed during synthesis of the drug (Fig. 1). However, there is no report on degradation products of the drug available so far. Moreover, no analytical method is claimed to be stability-indicating. Hence, the aims of the present study are to (i) conduct the forced degradation study on bosentan under the ICH prescribed conditions to identify the possible degradation products arising under various stress conditions like hydrolysis, photolysis and oxidation; (ii) characterize the degradation products through MS^n and LC–MS studies; (iii) develop and validate a stability-indicating HPLC method and finally (iv) establish the degradation pathways and the intrinsic stability characteristics.

2. Experimental

2.1. Drug and chemicals

Bosentan was procured from MSN Laboratories Ltd. (Hyderabad, India) as a gift sample with a batch number BST/A138/V(H)/01.

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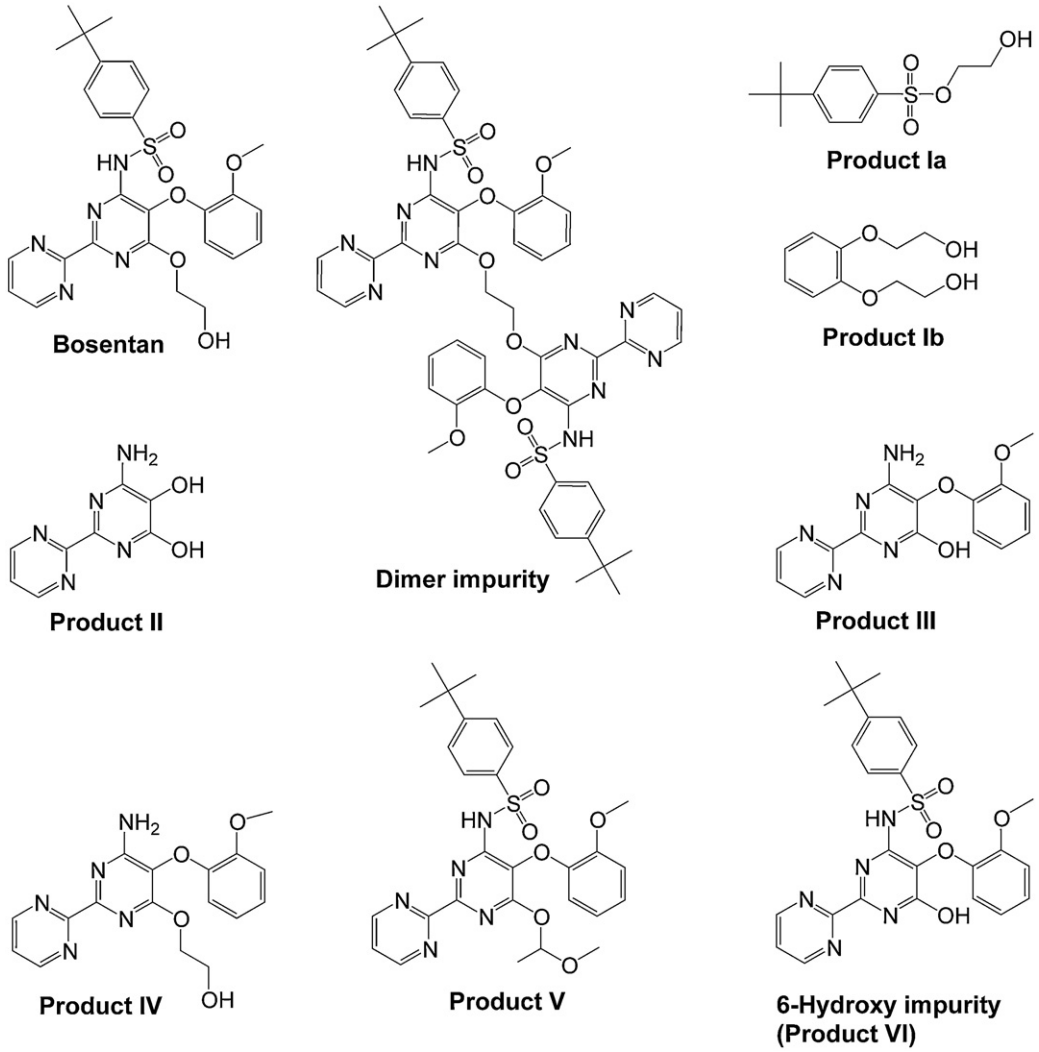

Fig. 1. Structures of bosentan, its known impurities and degradation products.

Lupibose tablets of bosentan with a label claim of 62.5 mg/tablet, batch number 041011049 (MSN Laboratories Ltd, Hyderabad, India) were used for the stability studies. Sodium hydroxide (NaOH), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2 30%) and ammonium acetate were purchased from Loba chemical Pvt. Ltd. (Mumbai, India). Methanol, acetic acid and acetonitrile (HPLC grade) were purchased from Merck Specialist Pvt. Ltd. (Mumbai, India). HPLC grade water was obtained from the DirectUltra water purification system in the laboratory (Bio-Age Equipment and Services, SAS Nagar, India).

2.2. Equipments

The hydrolytic and thermal forced degradation studies were carried out using a high precision water bath and a hot air oven equipped with digital temperature control capable of controlling the temperature within range of ±1 °C and ±2 °C, respectively (Narang Scientific Works, New Delhi, India). The photodegradation was carried out in a photostability chamber (KBF 240, WTB Binder, and Tuttlingen, Germany) capable of controlling the temperature and humidity within a range of ±2 °C and ±5%RH, respectively. The chamber was equipped with illumination bank made of light source as described in Option 2 in the ICH guideline Q1B [8]. The chamber was set at a temperature of 25 °C and humidity of 55%. The forced degradation samples were analyzed on a Waters HPLC system consisting of binary pumps (515), dual wavelength detector (2487) and Rheodyne manual injector (Milford, MA, USA). The data were acquired and processed in the Empower 2 software. Kromasil C-18 (250 mm × 4.6 mm; 5 μm) and Zorbax C-18 (150 mm × 4.6 mm; 5 μm) columns were employed in the HPLC method development process. The mobile phase was filtered through nylon membrane (0.45 μm) and then degassed using a ultrasonication bath (570/H ELMA, Germany). The UV absorption spectrum of the drug was recorded on UV spectrophotometer (Model DU® 640 B series, Beckman, U.S.A). The LC–MS and MS^n studies were carried out using positive mode of electrospray ionization (+ESI) on a quadrupole mass spectrometer (LTQ XL, Thermo Scientific, Germany). The LC component equipped with an autosampler and a PDA detector was of Thermo Scientific make (Germany). The column in the LC–MS study was same as that used for the LC–UV study.

2.3. Forced degradation study

For hydrolytic degradation, about 0.1 g of the drug was mixed separately with 100 ml of water and each of 0.1, 1, 2 and 5 M HCl as well as NaOH solutions in separate volumetric flasks. Each flask was kept in the high precision water bath at 85 °C for 12 h. For oxidative degradation, about 0.1 g of the drug was dispersed in 100 ml of 30% H_2O_2 and the suspension was kept at the room temperature in dark condition for 24 h. The thermal degradation was carried out on the