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Original Article

Four new degradation products of doxorubicin: An application of forced degradation study and hyphenated chromatographic techniques ☆

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ABSTRACT

Forced degradation study on doxorubicin (DOX) was carried out under hydrolytic condition in acidic, alkaline and neutral media at varied temperatures, as well as under peroxide, thermal and photolytic conditions in accordance with International Conference on Harmonization (ICH) guidelines Q1(R2). It was found extremely unstable to alkaline hydrolysis even at room temperature, unstable to acid hydrolysis at 80 °C, and to oxidation at room temperature. It degraded to four products (O-I–O-IV) in oxidative condition, and to single product (A-I) in acid hydrolytic condition. These products were resolved on a C_8 (150 mm × 4.6 mm, 5 μm) column with isocratic elution using mobile phase consisting of $HCOONH_4$ (10 mM, pH 2.5), acetonitrile and methanol (65:15:20, v/v/v). Liquid chromatography–photodiode array (LC–PDA) technique was used to ascertain the purity of the products noted in LC–UV chromatogram. For their characterization, a six stage mass fragmentation (MS^6) pattern of DOX was outlined through mass spectral studies in positive mode of electrospray ionization (+ESI) as well as through accurate mass spectral data of DOX and the products generated through liquid chromatography–time of flight mass spectrometry (LC–MS–TOF) on degraded drug solutions. Based on it, O-I–O-IV were characterized as 3-hydroxy-9-desacetyldoxorubicin-9-hydroperoxide, 1-hydroxy-9-desacetyldoxorubicin-9-hydroperoxide, 9-desacetyldoxorubicin-9-hydroperoxide and 9-desacetyldoxorubicin, respectively, whereas A-I was characterized as deglucosaminyl doxorubicin. While A-I was found to be a pharmacopoeial impurity, all oxidative products were found to be new degradation impurities. The mechanisms and pathways of degradation of doxorubicin were outlined and discussed.

1. Introduction

Drug regulatory agencies such as United States Food and Drug Administration (USFDA) and ICH have laid down very stringent guidelines for the control of impurities in drug substances and products [1–6]. ICH Q3A(R2) and Q3B(R2) guidelines specifically require the identification of impurities (process or degradation related) in any drug substance and product [3,4]. Identification of degradation related impurities (degradation products) remains a major challenge during product development. These arise due to chemical susceptibility of a drug molecule to varied chemical environments during product development, transportation and shelf life. Moreover, these are formed generally in minute amounts, which may not be sufficient to facilitate their characterization. Hence, ICH Q1A(R2) guidelines recommend forced degradation study (stress testing) on drug substance under different chemical environments to facilitate isolation and/or characterization of all possible/major degradation products of the drug [2].

Anthracyclines constitute an important class of anti-tumor chemotherapeutic drugs with a broad spectrum of anticancer activity. DOX (also known as adriamycin) is a naturally anthracycline derivative and is a hydroxylated analog of daunorubicin (Fig. 1). It is particularly used for the treatment of disseminated neoplastic conditions such as acute lymphoblastic leukemia, acute myeloblastic leukemia, Wilm's tumor, neublastoma, soft tissue and bone sarcomas, breast carcinoma, ovarian carcinoma, transitional cell bladder carcinoma, thyroid carcinoma, Hodgkin's and non-Hodgkin's lymphomas, bronchogenic carcinoma and gastric carcinoma [7,8]. DOX is an important component of multi-chemotherapeutic drug regimen, and is usually given combined with cyclophosphamide, vincristine, bleomycin or prednisone. It is official in British Pharmacopoeia, United States Pharmacopoeia, Indian pharmacopoeia and Martindale [9–12]. The monograph about DOX in British Pharmacopoeia lists four impurities (Imp A–Imp D) [9] (Fig. 1). Some studies have reported on degradation and stability of DOX under varied conditions such as aqueous, photolytic and biological environments [13–20]. Of these, only Cielecka-Piontek et al. [13]

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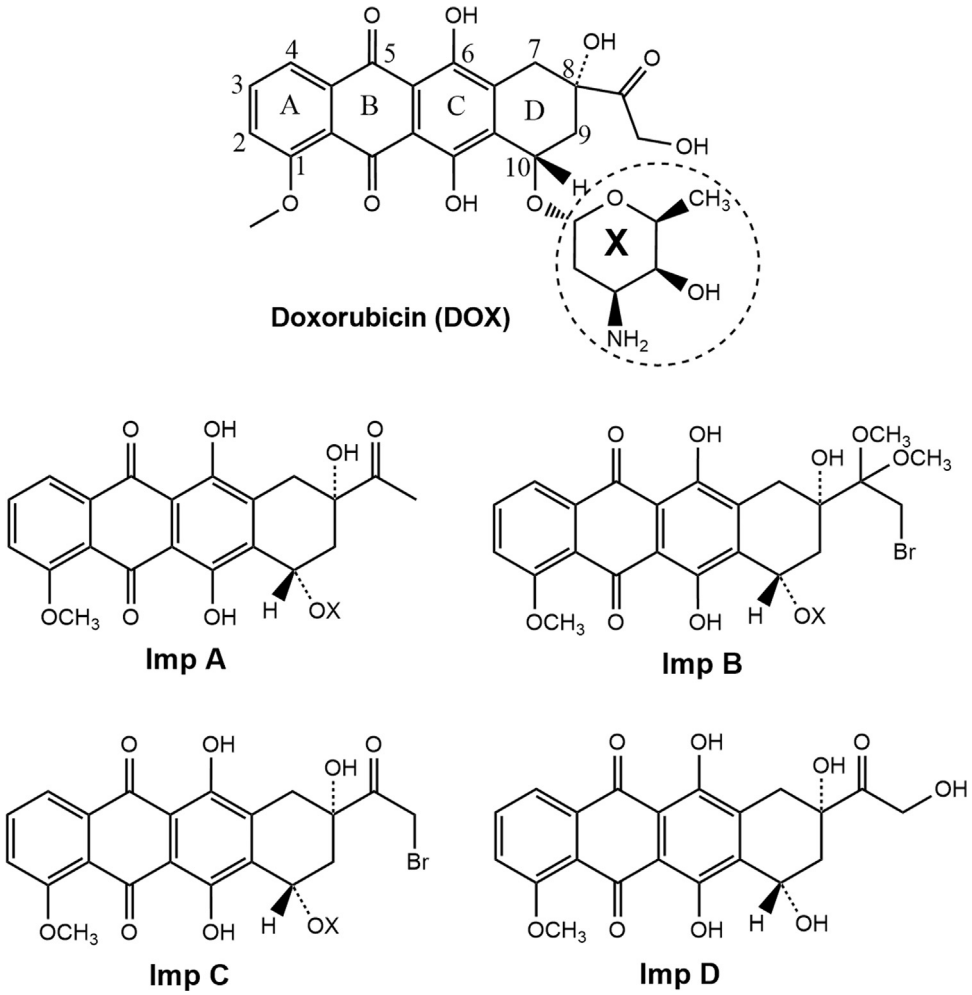

Fig. 1. Structure of doxorubicin (DOX) and its known impurities.

have proposed that on the basis of limited data, DOX and daunorubicin in solid state degrade to apolar degradation products that behave like 7-deoxyaglycons. None of other studies has characterized the degradation products formed under different conditions. Herein, we report (i) a comprehensive forced degradation study on DOX under hydrolytic, photolytic, oxidative and thermal degradation conditions as prescribed in ICH Q1(R2) guidelines; (ii) structural characterization of degradation products through mass fragmentation and accurate mass spectral analyses; and (iii) most plausible mechanisms of DOX degradation.

2. Experimental

2.1. Drug and chemicals

Doxorubicin hydrochloride (DOX) was generously provided by Strides Arcolabs Pvt. Ltd. (Bangalore, India) as a gift sample. All analytical grade chemicals (sodium hydroxide, hydrochloric acid, hydrogen peroxide (30%) and ammonium formate) were supplied by Loba Chemical Pvt. Ltd. (Mumbai, India). The high performance liquid chromatography (HPLC) grade solvents and chemicals (methanol, acetonitrile and formic acid) were procured from Merck Specialist Pvt. Ltd. (Mumbai, India). HPLC grade water was obtained from Direct Ultra water purification system (Bio-Age Equipment and Services, SAS Nagar, India) in the laboratory.

2.2. Equipments

A high precision water bath and a hot air oven digitally controlling temperature variation of 1 and 2 °C, respectively (Narang Scientific Works, New Delhi, India) were employed for hydrolytic and thermal degradation studies. Photostability chamber which is equipped with digital controller capable of maintaining temperature and relative humidity (RH) within ± 2 °C and ± 5%, respectively (KBF 240, WTB Binder, Tuttlingen, Germany) was used for photodegradation studies. The light sources fixed in the chamber provided an illumination bank in compliance with ICH guideline Q1B [21]. Liquid chromatography–ultraviolet (LC–UV) analyses of the forced degradation samples were carried out on a a binary HPLC system (515 pumps) equipped with a Rheodyne manual injector and a 2487 dual wavelength detector controlled by Empower 2 software (Waters, Milford, MA, USA). An Agilent C_8 (150 mm × 4.6 mm, 5 μm) column was used for chromatographic separation of the drug and degradation products. Purity of peaks of DOX and products in the degradation samples were ascertained by LC–PDA analysis using a binary HPLC system consisting of a 2707 auto-injector and a 2998 PDA detector (Waters, Milford, MA, USA). LTQ-XL ion trap quadrupole mass spectrometer (Thermo Scientific, Germany) was used to record multi-stage mass spectral (MS^n) data of DOX in positive mode of electrospray ionization (+ESI). For LC–MS–TOF analyses of DOX and degradation products, an Agilent 1100 series LC system (Agilent Technologies Inc., CA, USA) controlled by Hystar (Ver.3.1) software coupled with a microTOF-Q11 mass spectrometer (Bruker Daltonics GmbH, Germany) controlled