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SHORT COMMUNICATION

Isolation and characterization of a degradation product in leflunomide and a validated selective stability-indicating HPLC–UV method for their quantification

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Abstract Leflunomide (LLM) is subjected to forced degradation under conditions of hydrolysis, oxidation, dry heat, and photolysis as recommended by International Conference on Harmonization guideline Q1A(R2). In total, four degradation products (I–IV) were formed under different conditions. Products I, II and IV were formed in alkaline hydrolytic, acidic hydrolytic and alkaline photolytic conditions. LLM and all degradation products were optimally resolved by gradient elution over a C_{18} column. The major degradation product (IV) formed in hydrolytic alkaline conditions was isolated through column chromatography. Based on its ^{1}H NMR, IR and mass spectral data, it was characterized as a British Pharmacopoeial impurity B. The HPLC method was found to be linear, accurate, precise, sensitive, specific, rugged and robust for quantification of LLM as well as product IV. Finally, the method was applied to stability testing of the commercially available LLM tablets.

1. Introduction

Leflunomide (LLM), 5-methyl-N-(4-(trifluoromethyl)phenyl)isoxazole-4-carboxamide (Fig. 1), is a pyrimidine synthesis inhibitor belonging to disease-modifying antirheumatic drugs. It is used to relieve joint inflammation and pain in rheumatoid arthritis [1]. It is official in British Pharmacopoeia (BP) wherein eight impurities are reported in its monograph (Fig. 1) [2]. International Conference on Harmonization (ICH) guideline Q1A(R2) requires characterization of all degradation products formed during forced degradation of a drug substance under varied chemical environments like hydrolysis, oxidation, dry heat and photolysis [3]. A few HPLC and LC–MS methods are reported in literature for determination of LLM, and its active metabolite [4–6]. A few specific stability-indicating HPLC methods for LLM are also available in literature [7,8]. In addition, one method is known for determination of its related substances [9]. However, none of these reported studies have attempted to isolate and/or characterize

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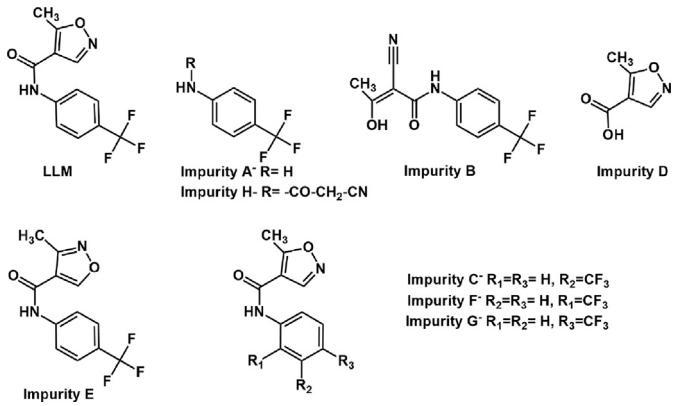

Fig. 1 Structures of leflunomide and its pharmacopoeial impurities.

degradation products of LLM. Moreover, no method has afforded simultaneous quantification of LLM and its degradation product(s). In the present study, we (i) conducted systematic forced degradation study on LLM under the ICH prescribed conditions, (ii) isolated and characterized a degradation product, and (iii) developed and validated a simple, sensitive and selective stability-indicating RP-HPLC–UV method for simultaneous quantification of LLM and the isolated degradation product.

2. Experimental

2.1. Drug and chemicals

LLM was procured from Cadila Healthcare Ltd. (Goa, India) as a generous gift sample. Sodium hydroxide (NaOH), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2, 30%) and anhydrous ammonium acetate were purchased from Loba Chemical Pvt. Ltd. (Mumbai, India). Methanol and acetic acid (HPLC grade) were purchased 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. LLM tablets (Lefumide-10, Batch No. J95242, Cipla Ltd., Goa, India) were purchased from a local pharmacy store.

2.2. Equipments

Hydrolytic and thermal forced degradations were carried out in high precision water bath and a hot air oven equipped with digital temperature control capable of controlling temperature within a range of ± 1 °C and ± 2 °C (Narang Scientific Works, New Delhi, India). Photo-degradation was carried out in a photostability chamber (KBF 240, WTB Binder, Tuttlingen, Germany) capable of controlling temperature and relative humidity (RH) within a range of ± 2 °C and ± 5%. The chamber was equipped with an illumination bank of light source as described in Option 2 in the ICH guideline Q1B [10], and was set at a temperature of 25 °C and 55% RH. 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 Empower 2 software. The chromatographic separation was achieved on Kromasil C_{18} (250 mm × 4.6 mm; 5 μm) column. LC–MS–TOF studies were carried out in positive mode of electrospray ionization (+ESI) on micrOTOF-Q mass spectrometer (Bruker Daltonics GmbH, Germany) controlled by microTOF control software ver. 2.0. The LC components comprised Agilent 1100 series LC system (Agilent Technologies Inc., CA, USA), controlled by Hystar (Ver. 3.1) software. The column in LC–MS study was the same as that used for LC-UV study. For characterization of product IV of LLM, the ^{1}H NMR spectra were recorded on a Bruker FT-NMR spectrophotometer (Bruker India Scientific Pvt. Ltd., New Delhi, India, 400 MHz) in DMSO-d_6. Chemical shifts were reported in ppm (δ) relative to tetramethylsilane as an internal standard. FT-IR spectra were recorded using KBr disc with FTIR-spectrophotometer (Perkin Elmer, England).

2.3. Forced degradation study

For hydrolytic degradation, about 0.1 g of LLM was mixed separately with 100 mL of water, HCl solutions of varied strengths (0.1, 1, 2 and 5 M), and 0.1 M NaOH solution in volumetric flasks. Each flask was placed in the high precision water bath maintained at 85 °C for 8 h. For oxidative degradation, about 0.1 g of LLM was dispersed in 100 mL of 30% H_2O_2 and placed at room temperature under dark for 24 h. Thermal degradation was carried out on solid LLM sealed in amber colored vials at 50 °C for 30 days. For photolytic degradation, 2 mL of a 0.1% (w/v) solution of LLM in acetonitrile was mixed separately with 3 mL of water, 0.1 M HCl and 0.1 M NaOH in transparent glass vials. These vials as well as the solid drug, spread as a thin layer, in a petri-dish, were exposed to the light in the photostability chamber. The samples were placed at a distance of about 23 cm from the light source for 14 days during which the total UV and white light exposure equaled about 200 Wh/m^2 and 1.2 million lx h, respectively. A parallel set of solid drug and drug solutions was kept in dark under the same temperature and humidity for the same period of time to serve as dark control.

2.4. HPLC method and sample preparation

LLM and its degradation products were optimally resolved on a Kromasil C_{18} (250 mm × 4.6 mm, 5 μm) column at ambient temperature (30 °C) with methanol (mobile phase A) and ammonium acetate (pH 5.0, 10 mM) (mobile phase B) flowing at a rate of 0.7 mL/min in gradient mode (0–21 min, A:B; 25:75%→21–22 min, A:B from 25:75% to 60:40%→22–50 min, A:B; 60:40%→50–51 min, A:B from 60:40% to 25:75%, 51–55 min, A:B; 25:75%). The injection volume and detection wavelength were fixed at 20 μL and 260 nm, respectively. Each degraded drug solution was diluted up to 10 times with a diluent. The acid and alkali hydrolyzed solutions were neutralized before dilution. The