Available online at www.sciencedirect.com

ScienceDirect

Journal of Taibah University for Science 10 (2016) 148–160

Journal of Taibah University for Science

www.elsevier.com/locate/jtusci

LC–MS/MS characterization of the forced degradation products of ezetemibe: Development and validation of a stability-indicating UPLC method

Prasad Kancherla [a], Venkatappaiah Velpuri [a], Pallavi Alegete [b], Saeed S. Albaseer [c,*], Mukkanti Khagga [a], Parthasaradhi Das [d]

[a] *Institute of Science &Technology, JNTUH, Kukatpally, Hyderabad 85, AP, India*
[b] *Spectrum Pharma Research Solutions, Kukatpally, Hyderabad 500072, India*
[c] *Department of Chemistry, Faculty of Education, Thamar University, Thamar, Yemen*
[d] *Medicinal Chemistry Divison, Indian Institute of Integrative Medicine, Canal Road, Jammu, 180001, India*

Received 27 June 2015; received in revised form 11 August 2015; accepted 21 August 2015
Available online 10 November 2015

Abstract

The present study reports the characterization of four degradation products from ezetemibe (EZB) through liquid chromatography–tandem mass spectrometry (LC–MS/MS) and the development of a validated and stability-indicating reversed-phase ultra-performance liquid chromatographic method for the determination of EZB in the presence of its process-related impurities in bulk drugs. The forced decomposition of EZB was carried out and studied under acidic, basic, oxidative, photolytic and thermal conditions. The degradation of EZB was observed under basic and acidic conditions, and four degradation products (DPs) were formed. Successful chromatographic separation of EZB and its degradation products were successfully separated through chromatography with a Waters Acquity HSST3 C18 stationary phase (50 × 2.1 mm, 1.7 μm). The analytes were detected with a PDA detector set at 230 nm. The figures of merit for this method were adequate. The assay remained linear from concentrations of 0.09 μg mL^{-1} to 600 μg mL^{-1} for EZB and its four DPs (r^2 = 0.99914, 0.99945, 0.99917, 0.99923 and 0.99936 for EZB, Imp-A, Imp-B, Imp-C, and Imp-D, respectively). The method precision, which was expressed as the %RSD, ranged from 0.2 to 1.0 for the four impurities. The DPs from EZB were characterized by LC–MS/MS, and the most likely degradation and fragmentation pathways for EZB and its DPs were proposed.

Keywords: Ezetimibe; Impurities; UPLC; Stress conditions; Stability-indicating; Degradation products

1. Introduction

Ezetimibe (1-(4-flurophenyl)-(3R)-[3-(4-fluorophenyl)-(3S)-hydroxypropyl]-4S-(4-ydroxyphenyl)-2-azetidinone) is a known lipid-lowering agent that selectively inhibits the intestinal absorption of cholesterol [1,2]. Previous studies of ezetimibe (EZB) include the RP-HPLC quantitation of EZB in pharmaceutical dosage forms [3,4] and stability-indicating assays utilizing

* Corresponding author. Tel.: +967 6 509131; mobile: +967 773651108.
E-mail address: sshalbaseer@yahoo.co.uk (S.S. Albaseer).
Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

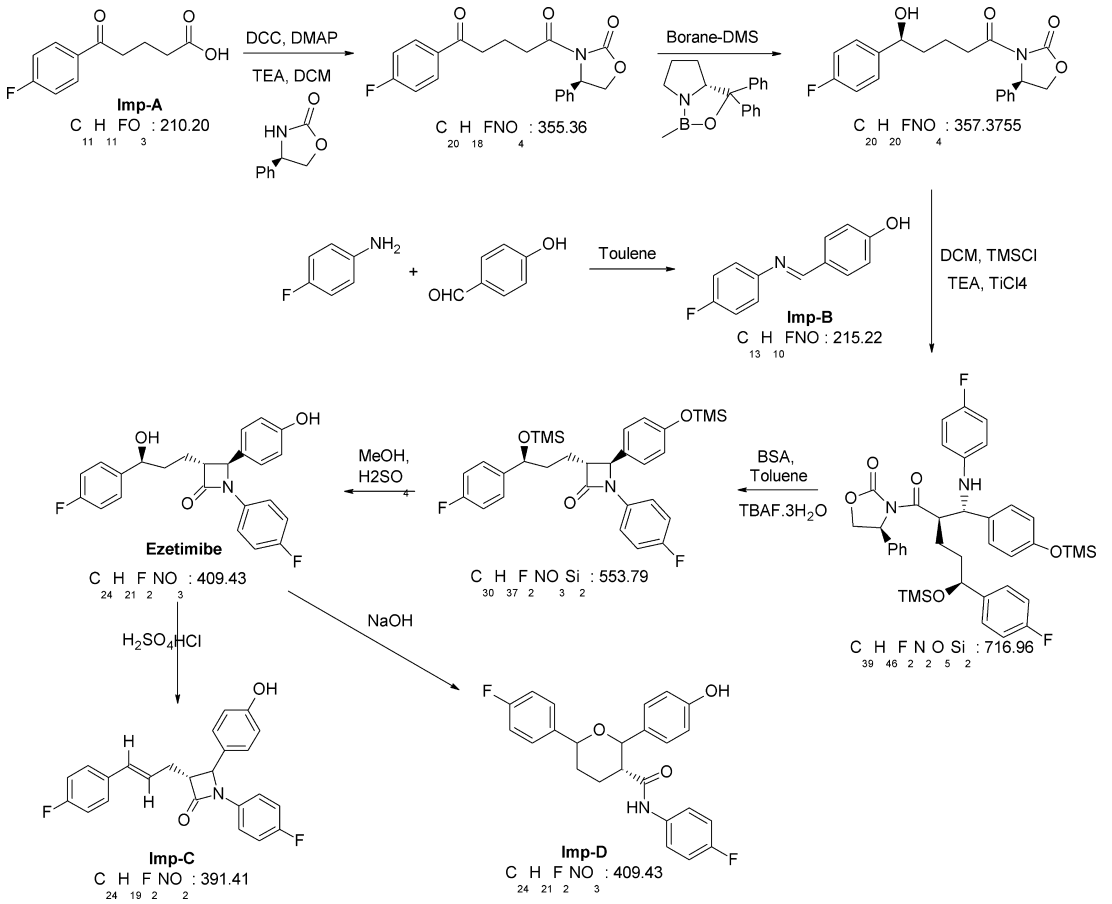
http://dx.doi.org/10.1016/j.jtusci.2015.08.001

different techniques including the HPLC, HPTLC and micellar electro kinetic chromatography of EZB in single doses or in combination with other drugs applying different techniques [5–10]. These reported stability-indicating assays have major drawbacks, such as long run times (45–60 min) and low selectivity. Therefore, the development of analytical methods that monitor the levels of every possible impurity in EZB formulations and consequently ensure the safety of these formulations has great research potential. These methods should be fast and reliable. We developed a sensitive, robust and fast UPLC method (run times below 5 min). The factors affecting the efficiency of the method were optimized, and the resultant method exhibited high sensitivity and selectivity. However, a literature survey revealed that the structural elucidation of the degradation products (DPs) of EZB has received little attention; a few attempts were made for the major impurities [11,12]. In addition, these papers have not reported the characterization of stress-related DPs while using LC–MS/MS with one exception [13]. During our study, EZB was subjected to stress degradation, producing four stress-induced DPs (referred to as Imp-A, Imp-B, Imp-C and Imp-D). These DPs were studied by LC–MS/MS, and the most probable degradation and fragmentation pathways for EZB and the DPs were proposed. EZB and Imp-D were prepared in our laboratory starting from Imp-A for method validation and characterization. The studied impurities are 4-(4-fluorobenzoyl)butyric acid (Imp-A), 4-[[(4-fluorophenyl) imino]methyl]-phenol (Imp-B), 1-(4-fluoro-phenyl)-3-[3-(4-fluoro-phenyl)-allyl]-4-(4-hydroxy-phenyl)-azetidin-2-one (Imp-C), 6-(4-fluoro-phenyl)-2-(4-hydroxy-phenyl) -tetrahydropyran-3-carboxylic acid (4-fluoro-phenyl)-amide (Imp-D).

2. Materials and methods

2.1. Chemicals and reagents

Analytical-grade reagents were used throughout the method unless stated otherwise. LC-grade acetonitrile



Scheme 1. Synthetic scheme for the degradation products of ezetimibe.