***Research Article***

Chemical Review and Letters
journal homepage: www.chemrevlett.com
ISSN (online): 2645-4947 (print) 2676-7279

# Development of a reversed-phase HPLC method for determination of related impurities of Lenalidomide

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ARTICLE INFO

*Article history:*
Received 19 March 2020
Received in revised form 28 March 2020
Accepted 3 April 2020
Available online 18 April 2020

*Keywords:*
Lenalidomide
Related Substances
HPLC
Method Development
UV detector

ABSTRACT

In this project, we have developed a reversed phase liquid chromatography method for separation and determination of lenalidomide (LENA) and related substances by using C-8 (250×4.6 mm ID, 5 µm) HPCL column. The mobile phases A and B were phosphate buffer at pH=3.30, and (methanol:acetonitrile)(1:5 V/V), respectively. The column oven temperature was 25°C, the wavelength was 220nm, and the injection volume was 20 µl. The degradation studies using basic, acidic, oxidation, and thermal stress, were performed. In addition, in the basic stress, a significant degradation for LENA, was observed.
The results showed that the resolutions of the peaks for fresh, acid stress, and thermal stress were considerably high. For example, in the case of thermal shock, the resolution of each peak to the next, was 3.6, 3.2, 5.3, and 4.7. Thus, it indicates that the method is suitable at least in view of separation and resolution for the peaks produced by thermal shock.

## 1. Introduction

Lenalidomide (LENA) with its anti-angiogenic and immunomodulatory properties, which inhibits tumor angiogenesis [1], is applied as an anticancer drug. It is the commercial name of 3-(4-amino-1-oxo 1,3-dihydro-2H-isoindol-2-yl) piperidine-2,6-dione, a biologically active molecule which shows significant effects on the treatment of Myelodys plastic Syndrome (MDS) [2]. Many medical centers all around the world try to synthesis [3] and uses this important compound as an active pharmaceutical ingredient (API) for preparation of effective drugs [4]. Thus, it is very crucial that scientists become aware about the purity of the substance as well as its assay (base on the percentage of active ingredient in the solid matter), and the amount of each impurity in the sample (*Related Impurity Analysis (RIA)*) [5]. It is obvious that each of the above-mentioned analyses need to be examined by accurate, trustable methods. That is, researchers in the related fields always study on new approaches to find better and more trustable ways for analysis of those substances.
The gas chromatography (GC) and liquid chromatography (LC) instruments are two of the most important technologies for analysis of the *Residual Solvent* (RS), and RIA *analysis* of the API, respectively [6]. In addition, development of new methods for those two instruments is a worldwide requirement for investigating the purity and the quality of the commercially released drugs [7]. As sail above, LENA is one of the most effective compounds for controlling some especial cancers, thus, some researchers examined method for synthesis and analysis this. For example, Saravanan and co-workers (2007) had developed a HPLC method for assay analysis of LENA [8]. In 2010, Raghu et al. investigated the degradation of lenalidomide by 0.01 Mm phosphate buffer at pH=2.0 and at a wavelength of 220 nm [9]. In 2012, Reddy and colleagues had developed a rapid LC procedure for assay content of LENA capsule and its related substances by using 1-octane sulfonic acid sodium salt as the modifying reagent [10]. In 2016, Alzoman developed a method for separation of enantiomeric impurities of LENA by using a LUX 5U cellulose-2 chiral column with a mobile phase containing methanol, glacial acetic acid, andtriethyl amine with a volume ratio of 100, 0.01, and 0.01, respectively [11]. Also, in 2019, Prasad and co-workers developed a HPLC method containing phosphate buffer and methanol in the ratio of (90:10 v/v) and (35:65 v/v) with X-bridge column for estimation of lenalidomide content and its organic impurities in oral solid dosage [12].
In this work, we have made attempt to develop a suitable method for analysis of the organic impurities of the synthesized LENA by using HPLC instrument. The method was able to separate the impurities from the main peak with sharp, high symmetry and high resolution peaks.

## 1. Experimental

Chemicals containing potassium dihydrogen phosphate (PDP), ortho-phosphoric acid (OPA), methanol (MOH) and acetonitrile (ACN) were prepared from Merck chemical company (Germany).LENA was provided from the Chemical Synthesis Department of Tofigh Daru Research and Engineering Company (Tehran, Iran).