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

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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 relatedfields 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

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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.

2. 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).

2.1. Instrumentation

The Young Lin (YL9100 HPLC) High Performance Liquid Chromatography (Gyeonggi-do, South Korea) Equipped with aYL9110 pump, a YL9101 degassing system, a YL9130S column oven and a YL9120 UV-Vis detector, was used for all analysis. Also, the software version 5.51 was applied for processing and data analysis.

2.2. Chromatographic conditions and sample preparation

A C8, end-capped (250 × 4.6) mm, 5µm liquid chromatography column was used for the RIA substance analysis. The mobile phase A was prepared by dissolving 2.72 g of (20Mm) PDP in water to pH=3.30 with OPA and further dilution to 1000 mL with water as solvent. The mobile phase B was (MOH:ACN)(100:500v/v).

The gradient elution program is given in Table 1. In addition, a UV detector on the wavelength of 220 nm was applied to record all of the chromatograms. The column oven temperature was maintained at 25°C and the volume of injection was 20 µL. The sample solutions were prepared as following: Test solution (a): Dissolve 10 mg of the substance dissolved and diluted in the diluent (1000 mg/L). Test solution (b): Dilute 1.0 mL of Test solution (a) to 100.0 mL with the diluent (10 mg/L) Test solution (c): Dilute 1.0 mL of Test solution (b) to 10.0 mL with the diluent (1 mg/L). The injections were 20 µL of the Blank solution, the Test solutions (a), (b), and (c), respectively.

Table 1. The gradient elution program of the analysis

No.	Time (min)	Flow (ml/min)	Mobile phase A (percent V/V)	Mobile phase B (percent V/V)
1	0	0.8	95	5
2	15	0.8	75	25
3	25	0.8	75	25
4	40	0.8	60	40

For calculation of the amount of each impurity, the peak areas of any impurity in the chromatogram obtained with Test solution (a) and the peak area of principal peak in the chromatogram obtained with Test solution (c) were required. An end-cappedC18 (5 µm, 4.6 × 250 mm) Hector chromatographic column was used for this reverse-phase HPLC system. Also, the elution program for the process was: 0/95, 15/75, 25/75 60/20 (time (min)/A%) at the flow rate of 0.8 ml/min. The solvent selected for dissolving and diluting of the sample was mobile phase A/B (50:50), and also the chromatograms were recorded at 220nm. The column oven temperature was 25°C, and volume of injection was 20 µL.

2.3. Preparation of impurities produced by stress:

Acid stress: 0.1 ml of hydrochloride 37% was introduced to 2ml of the Test solution (a) for about 2 hours Basic Stress: 0.2 ml of sodium hydroxide (25% w/w) was introduced to 2ml of the Test solution (a) for about 2 hours Oxidative stress: 0.1 ml of Hydrogen peroxide was introduced to 2ml of the Test solution (a) for about 2 hours Thermal stress: 2ml of the Test solution (a) was placed to 90°C for about 2 hours

2.

3. Results and Discussion

In the optimized chromatography condition, a C8, end-capped (250 × 4.6) mm, 5µm liquid chromatography column was applied to the RIA substance analysis. As sail above, the mobile phase A was prepared by dissolving 2.72 g of (20Mm) PDP in water to pH=3.30 with OPA and further dilution to 1000 mL with water as solvent. The mobile phase B was (MOH:ACN) (100:500v/v) The gradient elution program is given in Table 1. The chromatogram of the test solution (a) as the concentrated solution, which shows the low amounts of impurities is represented in Figure 1. Two impurities in retention times (RTs) of 12 min and 15 min are detected while the main peak of LENA appears about 17 min. The resolution of the first impurity to the second, and the second impurity to the main peak, is 8.0 and 5.3, respectively. It shows a good result in separation of the

peaks. The purity of the LENA is 99.91%. Due to high purity of the API, we stressed LENA to produce new impurities. By introducing new degrading impurities along with LENA, the method could be examined for its performance.

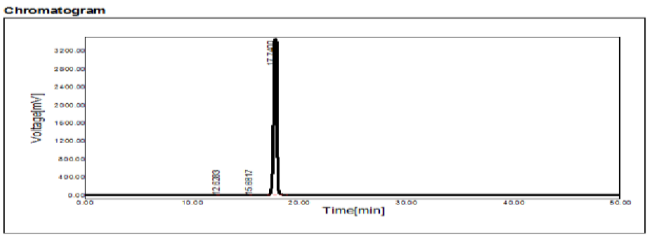

Figure 1. The chromatogram of test solution (a), by the optimized chromatographic conditions

As shown in Figure 2, the acid stress with mentioned conditions decreased the purity of the main compound to 99.81%. Compared to the fresh test solution, the acid stress led to production of total 0.1% impurities. Also, the average resolutions of the peaks are about 2.5.

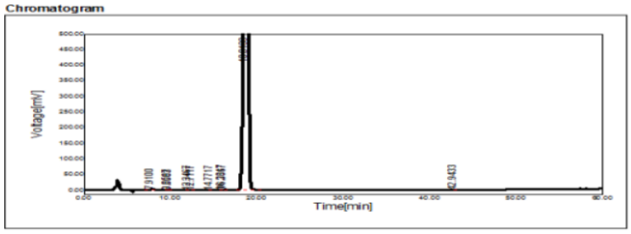

Figure 2. The chromatogram of test solution (a) after acid stress, by the optimized chromatographic conditions

Figure 3, indicates that the basic stress with given conditions decreased the purity of LENA to 32.39%. Compared to the fresh test solution, the basic stress led to production of total 67.42% impurities. It shows that the basic stress at least with the mentioned conditions has a significant effect on the destruction of LENA. Figure 4, shows that the oxidation stress caused by hydrogen peroxide (H_2O_2) with given conditions decreased the purity of LENA to 97.19%. Compared to the fresh test solution, the oxidation stress led to production of total 2.62% impurities. It shows that the oxidation stress at least with the mentioned conditions has not a significant effect on the destruction of LENA. Also, the first sharp peak at 4 min represent the existence of H_2O_2, the maximum absorbance of hydrogen peroxide occurs at about 220 nm [13], which is used to record all our grams. Figure 5, represents the thermal stress with above mentioned conditions which decreased the purity of LENA to 81.39%. Compared to the fresh test solution, the thermal stress led to production of total 18.49% impurities. It shows that the thermal stress at least with the mentioned conditions has a considerable effect on the destruction of LENA. Also, Figure 6 indicates the 10 ppm concentration of LENA (as the middle dilute solution) is detected by a high resolution, sharp, 100mV height-peak, with a tailing factor less than 1. Therefore, it was supposed that by this method, lower concentrations could be detected for a better comparison of the dilute and each impurity.

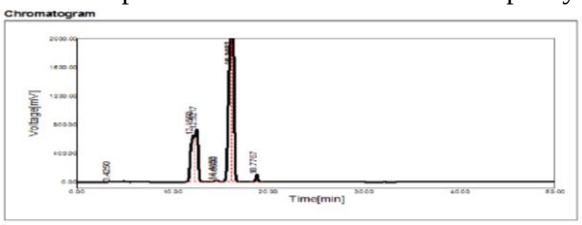

Figure 3. The chromatogram of test solution (a) after basic stress, by the optimized chromatographic conditions

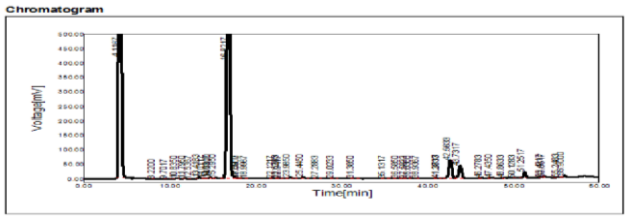

Figure 4. The chromatogram of test solution (a) after oxidative stress, by the optimized chromatographic conditions

The resolution of each peak to the next, is 3.6, 3.2, 5.3, and 4.7. It also shows that method is suitable in view of separation and resolution for the peaks produced by thermal shock.

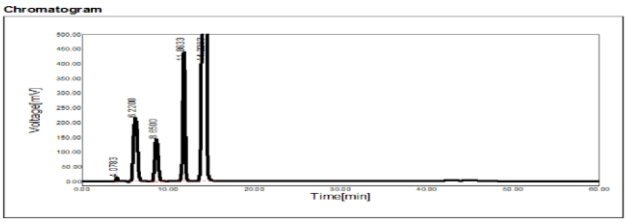

Figure 5. The chromatogram of test solution (a) after thermal stress, by the optimized chromatographic conditions

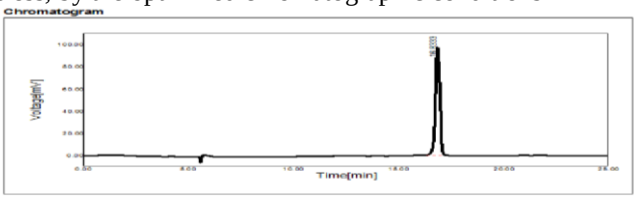

Figure 6. The chromatogram of test solution (b) with a concentration of 10 ppm (µg/L), by the optimized chromatographic conditions

As shown in Figure 7, the 1 ppm concentration of LENA (as the final dilute solution) is clearly detected by a sharp, 12mV height-peak, with a tailing factor less than 1. Thus, this method could easily detect required low concentrations.

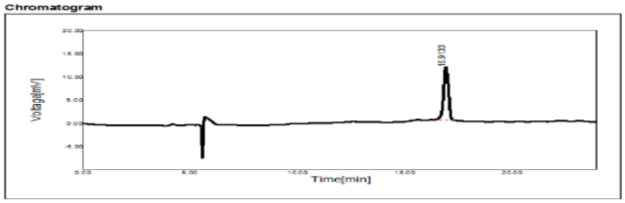

Figure 7. The chromatogram of test solution (C) with a concentration of 1 ppm (µg/L), by the optimized chromatographic conditions

4. Conclusions

In the present project, a reverse phase HPLC methodfor the fast and simple analysis of LENA was developed. The chromatogram peaks refereed to the main matter and the stressed degradants were mostly high resolutioned.The method was shown to be robust within the defined design conditions. A cheap and usual C8 liquid chromatography column was used for the process, and the results showed that the mentioned column was suitable for the high-resolution separation of the drug. The LENA sample was almost pure and thus a number of experiments were designed for production of stressed impurities and the results showed that method was able to separate the impurities. The concentrated sample was 1mg per litter; while, dilute solutions with 10 and 1 µg per litter (10 and 1 ppm), with sharp-high symmetry signals were detected. Finally, the results show that the resolution of the peaks for fresh, acid stress, and thermal stress are considerably high. For example, in the case of thermal shock, the resolution of each peak to the next, is 3.6, 3.2, 5.3, and 4.7. Thus, it indicates that the method is suitable in view of separation and resolution for the peaks produced by thermal shock. Supplementary data are available at the Journal home page.

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