ISSN: 0975-766X
CODEN: IJPTFI
Available Online through *Research Article*
www.ijptonline.com

DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR DETERMINATION OF CANCERS DRUG LENALIDOMIDE IN BULK

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Received on: 14-05-2021 *Accepted on: 22-06-20216*

Abstract:

Lenalidomide is used to treat various types of cancers. It works by slowing or stopping the growth of cancer cells. It is also used to treat anemia in patients with certain blood/bone marrow disorders (myelodysplastic syndromes-MDS).

Lenalidomide may lessen the need for blood transfusions and class of medications called immunomodulatory agents. It works by helping the bone marrow to produce normal blood cells and by killing abnormal cells in the bone marrow.[1]

Keywords: Lenalidomide, Cancer, Blood Cell, HPLC.

Introduction:

Lenalidomide is an analogue of thalidomide and has been shown to be more potent than thalidomide. Both drugs have direct cytotoxic effects on myeloma cells and are capable of inducing apoptosis. They are also capable of reducing angiogenesis through the inhibition of the secretion of vascular endothelial growth factor. Inhibition of vascular endothelial growth factor leads to alterations in the microvasculature of the bone marrow environment and inhibits myeloma cell growth and proliferation.

Unlike lenalidomide has almost no sedative or constipative properties and induces only minimal neurotoxicity; however, there is concern about lenalidomide's teratogenic potential. Phase I, II, and III trials have been carried out with lenalidomide in patients with relapsed or refractory multiple myeloma, and the drug has shown impressive response rates in relapsed disease. Lenalidomide efficacy in newly diagnosed multiple myeloma is currently being studied.[2]

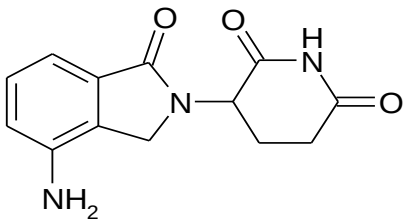

Fig-1: Linalidomide.

Information of Linalidomide:

Molecular formula: $C_{13}H_{13}N_3O_3$

Molecular weight: 259.26

Chemical Name: 3-(4-Amino-1-oxo 1,3-dihydro-2H-isoindol-2-yl)piperidine-2,6-dione.

MATERIALS & METHOD DETAILS:

Chemicals:

Potassium dihydrogen phosphate	: AR grade or equivalent
Orthophosphoric acid (88%)	: AR grade or equivalent
Acetonitrile	: HPLC grade or equivalent
Methanol	: HPLC grade or equivalent
Water	: Milli‘Q’

Chromatographic Conditions:

Column	: Zorbax SB C18, 150 x 4.6 mm, 5.0 µm
Flow rate	: 0.8 mL/minute
Detector wavelength	: 210 nm
Injection volume	: 10 µL
Column temperature	: 27°C
Elution mode	: Gradient
Run time	: 60 minutes
Diluent	: Prepare a mixture of solvent-A and solvent-B in the ratio of 50:50 (v/v).

Note: The column temperature should maintain at 27°C to get the exact retention time.

Preparation of Buffer: Dissolve about 1.36 g of potassium dihydrogen phosphate in 1000 mL of water and mix. Adjust the pH of this solution to 3.5± 0.05 with orthophosphoric acid and mix. Filter and degas through

the 0.45 µm membrane filter paper.

Preparation of Solvent-A: Use buffer as Solvent-A.

Preparation of Solvent-B: Prepare a mixture of methanol and acetonitrile in the in the ratio of 90:10 (v/v). Filter and degas through 0.45µm membrane filter paper.

Gradient program:

Time (Minutes)	Solvent-A (%)	Solvent-B (%)
0.01	90	10
15.0	90	10
40.0	45	55
52.0	45	55
53.0	90	10
60.0	90	10

Preparation of Reference solution:

Weigh about 6.0 mg of Related Compound-01 and 4.0 mg of Lenalidomide standard into a 100 mL volumetric flask, dissolve in 10 mL of acetonitrile and dilute to the volume with diluent and mix.

Dilute 0.25 mL of above solution to 20 mL with diluent and mix.

Preparation of Test solution: Weigh about 50.0 mg of the test sample into a 100 mL volumetric flask, dissolve and dilute to the volume with the diluent and mix. Prepare in duplicate.

Note: The weights and volumes given are the recommended amounts for routine quantitative analysis. Alternative amounts may be used, provided that the final concentrations remain the same.[4]

Procedure: Equilibrate the column for at least 60 minutes. Inject diluent as blank solution into the system and record the chromatogram. Program the data processor to inhibit the integration of peaks due to blank and perform blank correction if necessary. Inject Reference solution into the system in duplicate and record the chromatograms. Check for the system suitability criteria, if met the requirement, proceed further.

System suitability acceptance criteria:

The resolution between Related Compound-01 and Lenalidomide peaks from reference solution should be not less than 2.0. The tailing factor for Lenalidomide peak from reference solution should be not more than

2.0. Separately inject the test solutions into the system and record the chromatograms. Calculate the amount of all known and unknown impurities in each test solution separately by using the following formulae and report the average result.[3]

Calculations:

$$\% \text{ of Known Impurity} = \frac{\text{AU}}{\text{AS}} \times \frac{\text{CS}}{\text{CU}} \times \text{P}$$

$$\% \text{ of Unknown Impurity} = \frac{\text{AU1}}{\text{AS1}} \times \frac{\text{CS1}}{\text{CU}} \times \text{P1}$$

Where,

AU = Area response of corresponding known impurity in the test solution.

AU1 = Area response of unknown impurity in the test solution.

AS = Average area response of corresponding known impurity peak in reference solution.

AS1 =Average area response of Lenalidomide peak in reference solution

CU = Concentration of corresponding known impurity in reference solution (mg/mL).

CS1 = Concentration of Lenalidomide (Form-H1) standard in reference solution (mg/mL).

CT = Concentration of test solution(mg/mL)

P = Potency /Purity of the corresponding known impurity standard(%w/w).

P1 = Potency of the Lenalidomide (Form-H1) standard (%w/w).

Total impurities = Sum of the Known and unknown impurities.

Sample information:

S. No.	**Name of the component**	**~ RT (minutes)**	**RRT**	**Specification (%)**
1.	H-LNDRC01	24.1	1.21	NMT 0.15
2.	Lenalidomide	19.8	1.00	--
3.	Maximum single unknown impurity (MSUI)	--	--	NMT 0.10
4.	Total impurities (TI)	--	--	NMT 0.50

VALIDATION PARAMETERS & PROCEDURE:

The following parameters should be considered for the validation.

Follow the procedure and establish the system suitability as per the method for the validation study.

Specificity, SST and Reference Solution's:

Preparation of RELATED COMPOUND-01 stock solution:

Weigh about 6.0 mg of RELATED COMPOUND-01 standard into a 100 mL volumetric flask, dissolve in 10 mL of acetonitrile and dilute to the volume with diluent and mix.

Preparation of RELATED COMPOUND-01 (0.15%) solution:

Take 0.25 mL of RELATED COMPOUND-01 stock solution into a 20 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of LMN (H-LNDRC02) stock solution:

Weigh about 6.0 mg of LMN **(**H-LNDRC02) into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of LMN (H-LNDRC02) (0.15%) solution:

Take 0.25 mL of LMN (H-LNDRC02) stock solution into a 20 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of BME (H-LNDRC03) stock solution:

Weigh about 6.0 mg of BME (H-LNDRC03) into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent and mix.

Preparation of BME (H-LNDRC03) (0.15%) solution:

Take 0.25 mL of BME (H-LNDRC03) stock solution into a 20 mL volumetric flask, dilute to the volume with diluent and mix.

Preparation of Lenalidomide (Form-H1) standard stock solution:

Weigh about 5.0 mg of Lenalidomide (Form-H1) standard into a 100 mL volumetric flask, dissolve and dilute to the volume with diluent and mix well.

Preparation of Lenalidomide (Form-H1) standard (0.10%) solution:

Take 0.2 mL of Lenalidomide (Form-H1) standard stock solution into a 20 mL volumetric flask, dilute to the volume with diluent and mix well.

Preparation of Blend solution:

Weigh about 50.0 mg of the test sample into a 100 mL volumetric flask, add 1.25 mL of Related Compound-01 from individual stock solution, dissolve and dilute to the volume with the diluent and mix.[5]

Procedure:

1. Perform the specificity using photo diode array detector (PDA).
2. Establish the system suitability as per the method.
3. Inject Related Compound-01(0.15%) solution, Related Compound-02 (0.15%) solution, Related Compound-03 (0.15%) solution, Lenalidomide (Form-H1) standard (0.10%) solution and blend solution into the system and record the chromatograms.
4. Compare the relative retention times of known impurity obtained from individual solution and from blend solution.
5. Determine the peak purity for each component obtained from individual solution and known impurity peak from blend solution.

Acceptance criteria:

1. System suitability should comply as per the method.
2. There should not be any interfering peak at the retention time of known impurity and Lenalidomide due to the blank peaks and other impurities.
3. The elution order and the relative retention time obtained from individual solution and blend solution should be comparable for known impurity.

Note: *Relative retention times may vary as the retention time increases as a general limit it can be ± 0.1 below 30 minutes and ± 0.2 above 30 minutes.*

4. The peak purity should comply for each component obtained from individual solution and each component obtained from blend solution. The purity angle should be less than the purity threshold value.

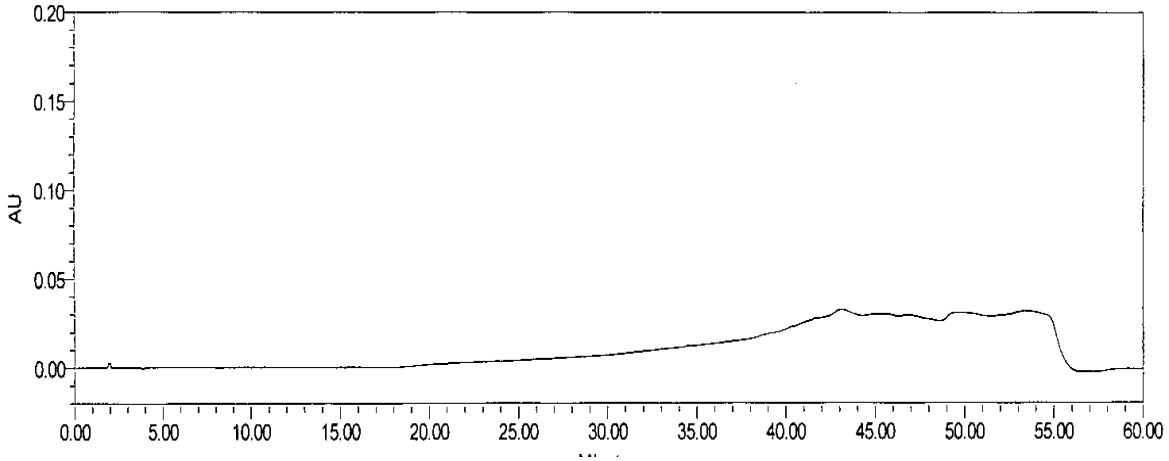

Fig-2: Blank Preparation.

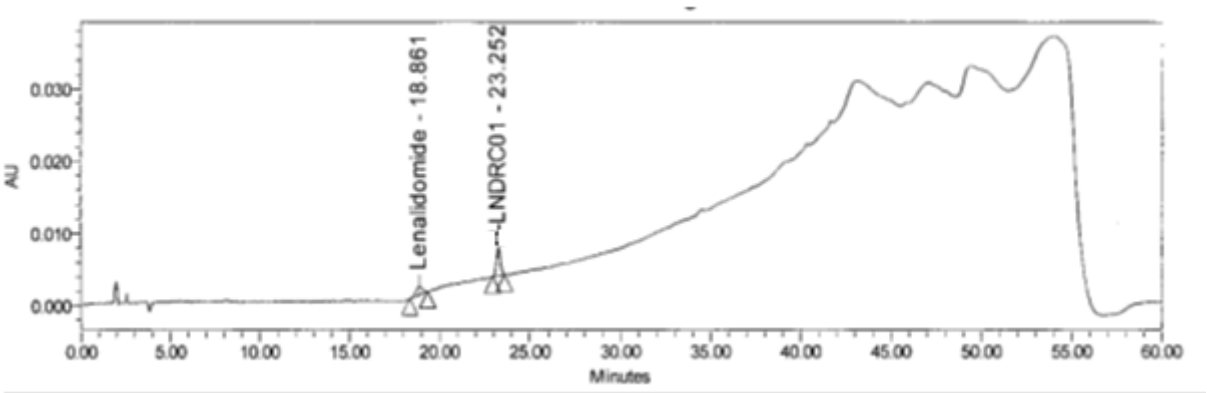

Fig-3: Specificity Preparation.

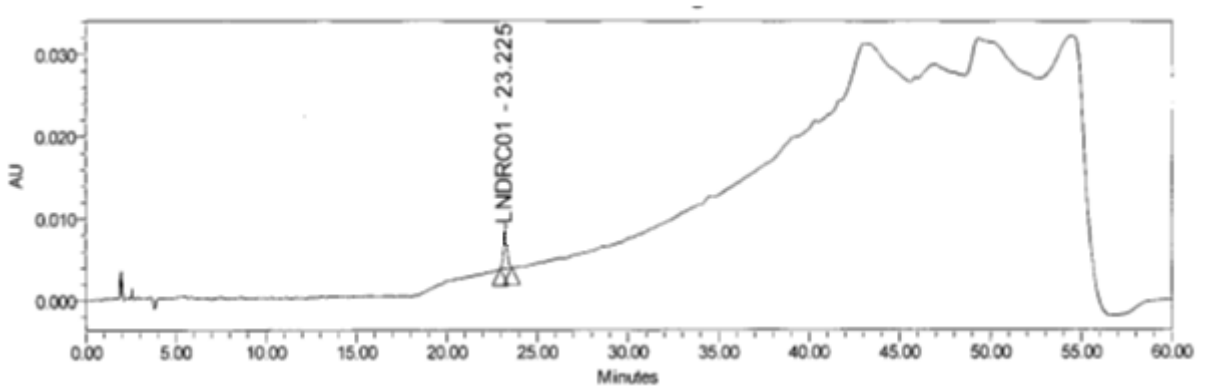

Fig-4: RC01 at 0.15% level Preparation.

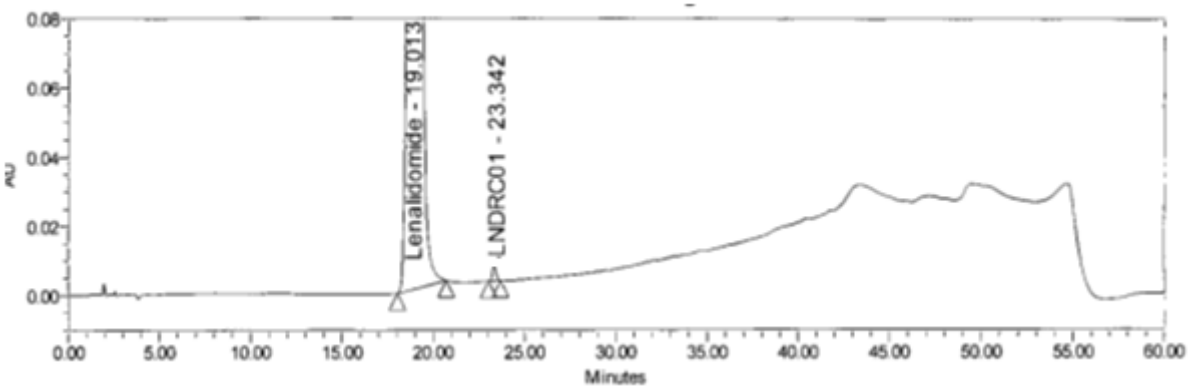

Fig-5: Blend solution Preparation.

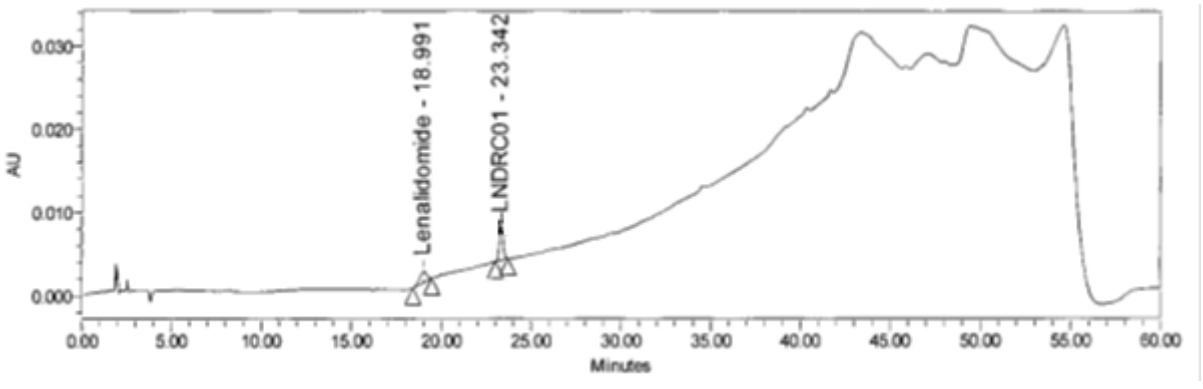

Fig-6: Reference Solution.

Conclusion: In the present research, a fast, accurate, precise, and linear stability-indicating HPLC method has been developed and validated for the content in final active pharmaceutical ingredient of lenalidomide, and hence it can be employed for routine quality control analysis. The analytical method conditions and the mobile phase solvents provided good resolution for impurity sample and final sample. In addition, the main features of the developed method robust enough to reproduce accurate and precise results under different chromatographic conditions based on ICH guidelines. Conclude from the above studies whether the method is valid and suitable for the determination of lenalidomide by HPLC.

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