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Development and Validation of Stability Indicating Rp-hplc Method for Quantitative Estimation of Lenalidomide in Lenalidomide Capsules Dosage Form

Ponnuri Bharath[1], P V Surendra Gupta[1], *Dittakavi Ramachandran[1]*

[1]Department of Chemistry, University College of Sciences, Acharya Nagarjuna University, Guntur-522 510, Andhra Pradesh, India.

Corresponding author E-mail: dittakavirc@gmail.com

Abstract

Highly sensitive RP-HPLC method developed for the quantification of Lenalidomide in Lenalidomide capsules dosage formulations. Samples are analyzed by means of reverse phase (RP-HPLC) using stationary phase an Kromasil C18 (150 x 4.6 mm, 5µm) and the mobile phase consisted of pH 2.5 phosphate buffer and acetonitrile in the ratio of (90:10 volume/volume). The flow rate is 1.0 mL/min. The column temperature was maintained at 30°C and sample cooler temperature was maintained at 5°C, injection volume 10 µL and wavelength 210 nm. The developed HPLC method was validated with respect to specificity, precision, linearity, accuracy, solution stability and filter study. Validation study compared as per ICH guideline.

Key words: Lenalidomide, Forced degradation, validation and liquid chromatography.

Introduction

Lenalidomide (3-(4-amino-1-oxo 1,3-dihydro-2H-isoindol-2-yl) piperidine-2,6-dione) is an orally available thalidomide analog, which is showing both anti-angiogenic and immunomodulatory/anti-inflammatory properties. Lenalidomide is a drug used to treat multiple myeloma, smoldering myeloma, and myelodysplastic syndromes (MDS) (1–7). It is marketed under the brand name Revlimid among other names. Figure 1 illustrates its chemical structure.

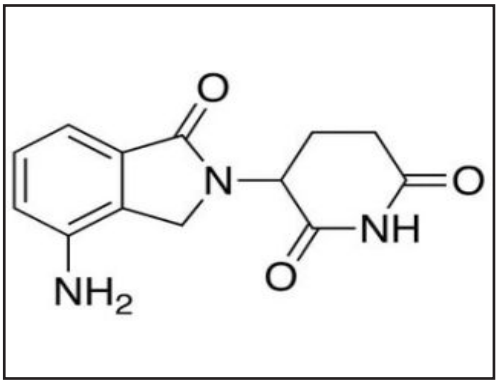

Figure 1. Chemical structure of Lenalidomide

According to an analysis of the literature, significant pharmacopeias, including the USP, EP, JP, and BP, do not include any LC procedures. Lenalidomide and its impurities have only been estimated using a few analytical techniques employing RP-HPLC and LC-MS techniques (8–11).

As a result, we worked to create an HPLC technique for quantifying lenalidomide in dosage forms for lenalidomide capsules. In

accordance with ICH recommendations (12–13), the current study presents a straightforward HPLC approach for the measurement of lenalidomide in lenalidomide capsule dose formulations.

Materials and Methods

Potassium dihydrogen orthophosphate, orthophosphoric acid, Hydrochloric acid, Sodium hydroxide and Hydrogen peroxide purchased from Merck, Mumbai, India. Acetonitrile, Methanol and Milli-Q water HPLC grade procured from Merck, India.

Preparation of pH 2.5 phosphate buffer

Potassium dihydrogen orthophosphate was carefully weighed at 1.3654 g and then added to 1000 mL of water, where it was well mixed. Then a diluted orthophosphoric acid solution was used to get the pH to 2.5. Filtered and sonicated to remove gas using a 0.45 membrane.

Preparation of mobile phase

Prepared a mixture of 900 mL of pH 2.5 phosphate buffer and 100mL of acetonitrile in the ratio of 900:100 (%v/v).

Preparation of diluent

Mobile phase used as diluent.

Preparation of standard solution

Weighed and transferred 10,418 mg of Lenalidomide standard into a 100 mL volumetric flask, added about 70 mL of diluent, and sonicated until the standard was dissolved. Dilution to volume with diluent and thorough mixing.

Preparation of sample solution for 2.5 mg

Taken 1 capsule was placed in a 25 mL volumetric vial, 15 mL of diluent was added, and the mixture was sonicated for at least 20 minutes with intermittent stirring before being diluted to volume with diluent and thoroughly mixed. 10 minutes of centrifugation at 3000 revolutions per minute.

Preparation of sample solution for 5 mg

Taken 1 capsule was placed in a 50 mL volumetric vial, 30 mL of diluent was added, and the mixture was sonicated for at least 20 minutes with intermittent stirring before being diluted to volume with diluent and thoroughly mixed. 10 minutes of centrifugation at 3000 revolutions per minute.

Preparation of sample solution for 7.5 mg

Taken 1 capsule was placed into a 100 mL volumetric vial, 75 mL of diluent was added, and the mixture was sonicated for at least 20 minutes with intermittent stirring before being diluted to volume with diluent and thoroughly mixed. 10 minutes of centrifugation at 3000 revolutions per minute.

Preparation of sample solution for 10 mg

Taken 1 capsule was placed into a 100 mL volumetric vial, 75 mL of diluent was added, and the mixture was sonicated for at least 20 minutes with intermittent stirring before being diluted to volume with diluent and thoroughly mixed. 10 minutes of centrifugation at 3000 revolutions per minute.

Preparation of sample solution for 15 mg

Taken 1 capsule was placed in a 200 mL volumetric flask along with 150 mL of diluent, which was then sonicated for at least 20 minutes with periodic shaking before being diluted to volume with diluent and well mixed. Centrifuge the mixture for ten minutes at 3000 rpm.

Preparation of sample solution for 20 mg

Taken 1 capsule was placed in a 200 mL volumetric flask along with 150 mL of diluent, which was then sonicated for at least 20 minutes with periodic shaking. The diluent was then diluted to the volume before being mixed. The solution should be centrifuged at 3000 rpm for 10 minutes.

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Preparation of sample solution for 25 mg

Taken 1 capsule was placed into a 250 mL volumetric flask, 150 mL of diluent was added, and the mixture was sonicated for at least 20 minutes with intermittent stirring before being diluted to volume with diluent and thoroughly mixed. 10 minutes of centrifugation at 3000 revolutions per minute.

Method development

Lenalidomide drug substance's maximum UV absorbance (max) was found at 210 nm, according to the UV-spectroscopic study.

Different mobile phases were used to generate an ideal peak shape in order to establish an appropriate and reliable HPLC technique for the analysis of lenalidomide in the dose form of capsules. Starting with Zodiac C18 (150x4.6mm, 3.5 m), the technique development was carried out using various mobile phase compositions, such as 0.1% orthophosphoric acid buffer and acetonitrile in an 85:15 volume/volume ratio. It was shown that greater retention times and unsatisfactory peak tailing occurred when lenalidomide was administered. The component was not suited for the stationary phase in the column. Change the column for the next experiment from Inertsil ODS-3V to Hypersil BDS. Lenalidomide was eluted at a void volume with an undesirable peak shape. Change the column for the next experiment from Hypersil BDS to Kromasil C18 (150 x 4.6mm, 5). The peak fronting of the usual Lenalidomide injection was unsatisfactory.

The mobile phase for the following experiment was composed of pH 2.5 phosphate buffer and acetonitrile in a volume-to-volume ratio of 90:10, a flow rate of 1.0 mL/min, a column temperature of 30°C, and a sampler cooler kept at 5°C. At 210 nm, UV detection was carried out. Lenalidomide was eluted at 9.50 minutes, and a satisfactory peak shape was observed. **Figure 2** displays the chromatogram of the Lenalidomide standard generated by the suggested technique. **Table 1** displays the method's system suitability results.

Optimized chromatographic conditions

A Waters 2489 UV detector/2695 Separation Module with Empower3 software was used for the analysis. A Kromasil C18 (150 x 4.6mm, 5 m) column was employed as the stationary phase. Acetonitrile and pH 2.5 phosphate buffer in the mobile phase at a 90:10 volume/volume ratio. It moves at a rate of 1.0 mL/min. The sample cooler temperature was kept at 5°C, while the column temperature was kept at 30°C. The injection volume was 10 L, and the UV detection wavelength was 210 nm.

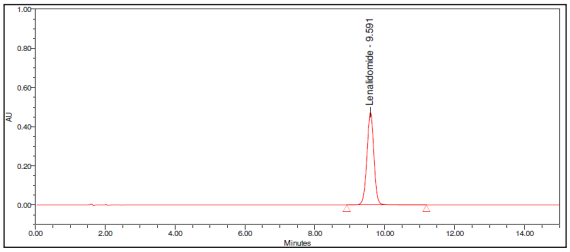

Figure 2. Typical chromatogram of Lenalidomide standard

Results and Discussion

The following parameters were used to thoroughly verify the devised RP-HPLC technique for the assay of lenalidomide in the formulation of lenalidomide capsules.

Specificity and System suitability

Blank and Placebo interference:

The research was done to see if placebo and blank effects interfered. The chromatography was injected with diluent and a placebo at the chromatographic conditions mentioned above, and the chromatograms of the two samples were recorded. The retention period of the signal for lenalidomide was not seen on the chromatogram of the blank solution in **Figure 3**. This shows that the diluent solution used to prepare the sample does not affect the estimate of lenalidomide in the dose form \

of lenalidomide capsules. Similar to this, the chromatogram of the placebo solution (**Figure 4**) did not exhibit any peaks at the Lenalidomide peak retention period. This shows that the placebo used to prepare the sample does not affect how much lenalidomide is estimated in the formulation of the lenalidomide capsules. **Table 2** displays the method's specificity findings.

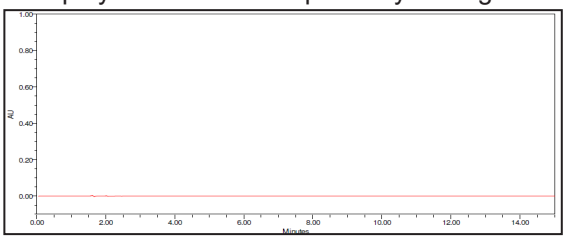

Figure 3. Typical chromatogram blank

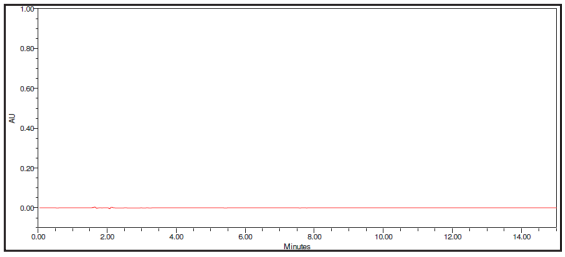

Figure 4. Typical chromatogram placebo

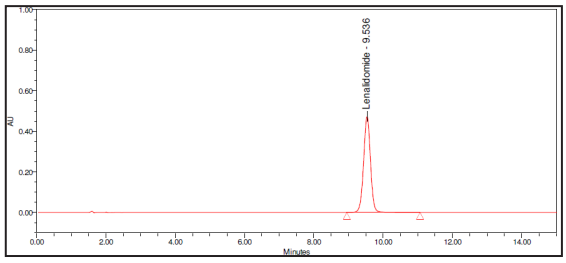

Figure 5. Typical chromatogram standard

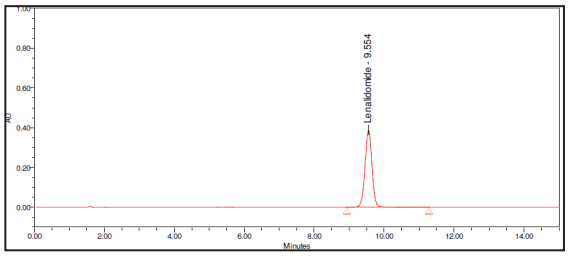

Figure 6. Typical chromatogram sample

Table 1. System suitability results

S.No.	System suitability parameters	Lenalidomide
1	Retention Time	9.591
2	Tailing factor	1.0
3	Theoretical plates	11292
4	%RSD of five replicate standard solution	0.10

Table 2. Specificity results

S. No	Name	Retention Time(min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution	9.536	No	No
4	Sample solution	9.554	No	No

Force Degradation studies

To effectively separate degradants and contaminants from lenalidomide, research was done. The following stress conditions were applied to separate parts of the sample and placebo solutions in order to cause deterioration. Samples were fed into the HPLC system using a PDA detector, both stressed and unstressed. The findings of the degradation investigation were shown in **Table 3**.

Table 3. Forced degradation results

S.No.	Stress Degradation condition	%Assay	% Degradation
1	Unstressed Sample	100.6	N/A
2	Base stress sample (0.01N) NaOH/5mL/15min/BT)	90.6	10
3	Acid stress sample (0.2N HCl/5mL/50°C/2.0 hrs.)	81.5	19.1
4	Peroxide stress sample (30%H_2O_2/5mL/4hours/ RT)	89.9	10.7
5	Thermal stress sample (80°C/48 hours)	95.3	5.3
6	Water degradation at (5mL/50°C/2Hrs)	93.3	7.3

The circumstances of acid, alkali, and peroxide stress showed significant deterioration. Thus, it may be inferred that Lenalidomide is susceptible to oxidation, alkali, and acid.

System precision

The standard solution was made in accordance with the test procedure, administered six times via the HPLC system, and the % RSD for the area responses was assessed. **Table 4** presented the information.

Table 4. System precision results

S.No.	No. of injections	Peak area
1	Inj-1	6690611
2	Inj-2	6693203
3	Inj-3	6673512
4	Inj-4	6665745
5	Inj-5	6680308
6	Inj-6	6696503
Average		6683314
STDEV		12155.4371
% RSD		0.2

Results from six replicates of the standard solution were determined to have a relative standard deviation that was within the permissible range.

Method precision

Six samples of Lenalidomide capsules (2.5, 5.0, 7.5, 10, 15, and 25mg) were assayed to determine the accuracy of the test procedure. For each test preparation, the amount of Lenalidomide in mg and percent was determined. Calculations were used to determine the six preparations' average contents and the six observations' percent RSD. Table 5 presented the information.

Assay compliance rates overall and per person are within the parameters of the test technique. Six test preparations' relative standard deviations were determined to be within the specified range.

Table 5. Method precision results (% Assay)

S. No	Preparations	2.5 mg	5 mg	7.5 mg	10 mg	15 mg	20 mg	25 mg
1	Preparation 1	99.3	99.4	99.5	99.5	99.6	100.1	100.1
2	Preparation 2	99.7	99.8	99.4	99.4	99.9	100.2	100.2
3	Preparation 3	99.1	99.9	99.9	99.5	99.5	100.6	100.6
4	Preparation 4	100.3	99.2	99.7	99.1	99.6	99.5	100.0
5	Preparation 5	100.1	100.2	100.4	99.0	99.3	99.4	100.3
6	Preparation 6	99.0	100.3	100.5	99.2	99.9	100.8	100.5
Average		99.6	99.8	99.9	99.3	99.6	100.1	100.3
SD		0.5382	0.4336	0.4604	0.2137	0.2338	0.5657	0.2317
%RSD		0.54	0.43	0.46	0.22	0.23	0.57	0.23

Linearity

By preparing solutions with concentration levels ranging from 25% to 150% of the usual concentration level, the linearity of the detector response for lenalidomide was proven. These solutions were added to the HPLC apparatus, and the results of the system's reactions were noted. It was done to plot concentration vs. peak area. It investigated how closely concentration and reaction correlated. As a result, a linear standard curve for the HPLC technique was determined and is shown in **Figure 7**. **Table 6** is a summary of the observations.

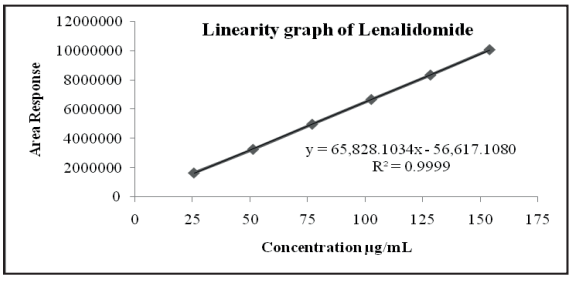

Figure 7. Calibration curve for Lenalidomide

Table 6. Linearity studies for Lenalidomide

S. No	Linearity Level	Concentration (ppm)	Area response
1	Linearity at 25%	25.6447	1668499
2	Linearity at 50%	51.2894	3283725
3	Linearity at 75%	76.9341	5001364
4	Linearity at 100%	102.5788	6693920
5	Linearity at 125%	128.2235	8366385
6	Linearity at 150%	153.8681	10097379
Correlation coefficient (r^2)			0.9999
Intercept			-56.617.1080
Slope			65828.1034
% Y-intercept			0.98

Table 7. Recovery studies for Lenalidomide

% Level	µg added	µg found	% Recovery	Mean % Recovery
50% level-1	50.3168	50.1151	99.6	99.5
50% level-2	50.1723	49.7936	99.2	
50% level-3	49.8353	49.6774	99.7	
100% level-1	101.2113	100.9433	99.7	99.5
100% level-2	101.2595	100.5417	99.3	
100% level-3	101.5484	101.0747	99.5	
150% level-1	150.7095	149.9274	99.5	99.8
150% level-2	150.6132	150.0996	99.7	
150% level-3	150.8540	151.0583	100.1	

Accuracy

By making recovery samples of Lenalidomide at concentrations ranging from 50% to 150% of the intended concentration level, the test method's accuracy was put to the test. For each concentration level of 50% and 150%, the recovery samples were made in triplicate preparations on Lenalidomide API spiked to placebo and then examined in accordance with the suggested procedure. The % recovery of each sample was computed for the quantity added after the chromatography of the aforementioned samples. By calculating the relative standard deviation of six preparations for 50% and 150% level recovery sample data, it was possible to assess the accuracy of the recovery at each level. The gathered information is shown in **Table 7**.

Solution stability

Standards for solution stability and sample solutions were created under various circumstances, including bench top at ambient temperature and in a refrigerator at 2 to 8°C. The stability of standard and sample solutions was ascertained by contrasting previously created standard and sample solutions with recently prepared standard solutions.

Table 8. Solution stability of standard

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24hrs	1.09	1.04
48hrs	1.11	1.08

Table 9. Solution stability of sample at room temperature

Time Interval	%Assay	%Assay difference
Initial	100.1	NA
24hrs	100.4	0.3
48hrs	100.6	0.5

Table 10. Solution stability of sample in Refrigerator

Time Interval	%Assay	%Assay difference
Initial	100.1	NA
24hrs	100.2	0.1
48hrs	100.2	0.1

The previously indicated solution stability parameter was determined. Both the standard and sample solutions are stable when kept on a bench top and in a refrigerator (2-8°C) for up to 48 hours.

Robustness studies

The chromatographic performance under different circumstances was assessed in comparison to the technique's nominal conditions in order to confirm the robustness of the method. At each of the aforementioned altered circumstances, the standard solution was injected.

The procedure can withstand variations in flow rate, pH, and column oven temperature.

Filter validation

One portion of the sample solution was centrifuged, while the other half was filtered through 0.45 m PVDF and 0.45 m Nylon filters as part of the filter validation process.

It was determined from the data above that 0.45 m PVDF filters are suitable. Therefore, 0.45 m PVDF filters had to be utilized for sample preparation.

Table 11. Robustness studies Results

Parameter		Retention time	% RSD of standard	Tailing factor	Theoretical plates
Flow (mL/min)	1.0	9.59	0.20	1.0	11334
	1.2	8.99	0.38	1.0	12784
	0.8	10.21	0.57	1.2	8792
pH	2.5	9.59	0.20	1.0	11334
	2.7	9.73	0.46	1.2	10981
	2.3	9.11	0.51	1.3	9924
Column temperature (°C)	30	9.59	0.20	1.0	11334
	35	9.25	0.11	1.0	12753
	25	9.64	0.36	1.1	10952

Table 12. Results for Filter validation

S.No.	Filter details	Area Response	% Assay	% difference
1	Centrifuged Sample	6595670	100.4	NA
2	0.45 µm PVDF Filtered Sample	6501024	98.3	2.1
3	0.45 µm Nylon Filtered Sample	6590786	100.1	0.3

Conclusion

The new method was validated for several criteria, including accuracy, precision, linearity, specificity, solution stability, robustness, and filter validation, in accordance with ICH guidelines. The obtained results met the acceptance criteria. As a result, it is possible to conclude that the developed approach is simple, precise, cost-effective, environmentally friendly, and safe, and that it may be successfully used for routine analysis of Lenalidomide in Lenalidomide capsule dosage forms.

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Conflict of interests

The authors claim that there is no conflict of interest.

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