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A new rapid HPLC method for the analysis of lenalidomide related substances in bulk drug samples

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ABSTRACT

Lenalidomide is a dicarboximide that consists of 1-oxoisoindoline bearing an amino substituent at position 4 and a 2, 6-dioxopiperidin-3-yl group at position 2. It inhibits the secretion of TNF-alpha. It has a role as an angiogenesis inhibitor, an antineoplastic agent, and an immuno modulator. It is a member of isoindoles, a dicarboximide, a member of piperidones, and an aromatic amine. A new chromatographic method was established for the determination of lenalidomide and related substances using Phenomenix C18 (250 × 4.6 mm, 5 µm) HPCL column with gradient method at ambient temperature. The chromatographic separation was clear at a flow rate of 0.8 ml/min is maintained and all degradation studies are performed at 210 nm. Method Validation is carried out according to International Council for Harmonization (ICH) guidelines and the parameters namely; precision, accuracy, specificity, stability, robustness, linearity, the limit of quantitation (LOQ), and limit of detection (LOD) are evaluated. The present developed RP-HPLC method shows the purity angle of peaks is less than their threshold angle, signifying that it to be suitable for stability studies. Hence, the developed method can be used for the successful separation of LLM and its impurities in the pharmaceutical dosage formulations.

Keywords: Lenalidomide, related substances, validation, RP-HPLC.

INTRODUCTION

Lenalidomide is an immuno modulatory drug with potent antineoplastic, anti-angiogenic, and anti-inflammatory properties. It is a 4-amino-glutamyl analogue of thalidomide and like thalidomide, lenalidomide exists as a racemic mixture of the active S (-) and R (+) forms. However, lenalidomide is much safer and potent than thalidomide, with fewer adverse effects and toxicities.[1-5] Thalidomide and its analogues, including lenalidomide, are referred to as immuno modulatory imides drugs (also known as cereblon modulators), which are a class of immuno modulatory drugs that contain an imides group. Lenalidomide works through various mechanisms of actions that promote malignant cell death and enhance host immunity. Available as oral capsules, lenalidomide is approved by the FDA and EU for the treatment of multiple myeloma, myelodysplastic syndromes, mantle cell lymphoma, follicular lymphoma, and marginal zone lymphoma in selected patients. Lenalidomide is available only under a special restricted distribution program.[6-11] It is soluble in organic solvent/water mixtures, and buffered aqueous solvents. Lenalidomide is more soluble in organic solvents and low pH solution. Solubility was significantly lower in less acidic buffers, ranging from about 0.4 to 0.5 mg/mL. (Fig.1)

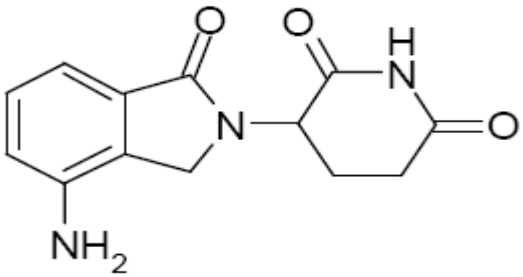


Fig.1: Structure of Lenalidomide

Only few methods were reported for estimation of Lenalidomide related substances by HPLC.[12-14]Hence we had made an attempt to develop a simple, accurate and precise RP-HPLC method for the estimation of Lenalidomide related substances.

METHODOLOGY

Gift samples of Lenalidomide and its related substances were received from Startech lab, Hyderabad, whereas water, methanol for HPLC, acetonitrile for HPLC and phosphoric acid were purchased from Merck.

Instrumentation: Waters HPLC was used for the separation of Lenalidomide and its related substances. UV/VIS spectrophotometer (LABINDIA UV 12.500^{+}) was used for detection. Instruments such as; pH meter used was of Adwa — AD 10100 and weighing machine was of Afcoset ER-1000A.

Method development: The objective of this experiment was to optimize the Related substances method for estimation of lenalidomide based on the literature survey.

Chromatographic conditions

Column : Phenomenix C18 (250 × 4.6 mm, 5 µm) or equivalent
Flow rate : 0.8 ml/min
Detection : 210 nm by UV
Injection volume : 20 µL
Run time : 65 minutes
Elution : Gradient
Column Temperature: Ambient

Diluents: Mobile phase A and Mobile phase B in the ratio of 60:40 (v/v)

Buffer: Weigh about 1.36 g of KH_2PO_4 and transfer it into a 1.0 liter volumetric flask and dissolve in 1000 ml water. Adjust pH of the solution to 3.5 $\pm$ 0.05 with dilute phosphoric acid. Filter the solution through 0.45 microns porosity membrane filter.

Mobile phase preparation:

Mobile phase solution A- Use buffer as mobile phase solution

Mobile phase solution B- Use mixture of Methanol and Acetonitrile (50:50 (v/v)) (Table 1)

Table 1: Gradient Time program

Time (min.)	Solution A (%v/v)	Solution B (v/v)
0	80	20
10	80	20
20	60	40
55	60	40
55	60	40
56	80	20
65	80	20

Observation: Desired elution achieved and Peaks resolution is good.

Reference solution: Dissolved 5mg of Lenalidomide standard and impurity- An in 50ml of diluents.

Preparation of Standard: Accurately weighed about 50mg working standard and transferred into a 50ml volumetric flask, added 25ml of diluents, and sonicated to dissolve and diluted to volume with diluents. Dilute 100µl of this solution to 100ml with diluents.

Preparation of Sample: Accurately weighed about 50mg Sample and transferred into a 50ml volumetric flask, added 25ml of diluents, and sonicated to dissolve and diluted to volume with diluents.

Preparation of impurity blend stock solution: Accurately weighed about 10mg each of Impurity-A, Impurity-B and Impurity-C transferred into a 10ml volumetric flask, added 5ml of diluents and diluted to volume with diluents.

Evaluation of System Suitability: Inject 10 µl of the blank, resolution solution and standard solution in six replicate injections, into the chromatograph and record the chromatograms. The resolution between impurity –A and Lenalidomide peaks should be not less than 3.0. %RSD for six replicate injections in standard solution should be not more than 2.0%.

Procedure: Equilibrated the column for 15 minutes at the initial composition.10 µl of diluents as Blank, Standard, Blend solution and Sample preparation were injected separately into the HPLC system and record the chromatogram for 65 minutes.

Calculation

$$\text{known impurity} = \frac{A_T \times D_S \times P}{A_S \times D_T \times RRF}$$

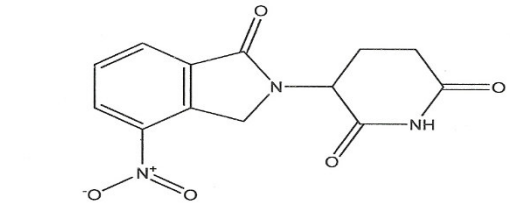
At : Area due to sample preparation As : Area due to standard preparation
Ds : Dilution factor of the standard Dt : Dilution factor of the sample
P : Potency of the standard RRF : Relative response factor
The Assay content on dried basis was calculated by using the formula

$$\textbf{Assay} = \frac{At \times Ds \times P \times 100}{As \times Dt \times (100\text{-}LOD)}$$

Where,
At : Area due to sample preparation, As : Area due to standard preparation
Ds : Dilution factor of the standard, Dt : Dilution factor of the sample
P : Potency of the standard and LOD : Loss on drying of the sample

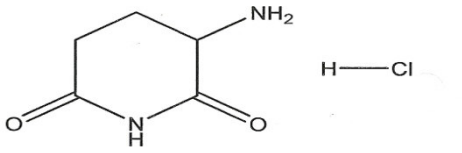
Table 2: Impurity Specifications

S.no	Impurity Name	Specification
1	Impurity-A	Not more than 0.15%
2	Impurity-B	Not more than 0.15%
3	Impurity-C	Not more than 0.15%



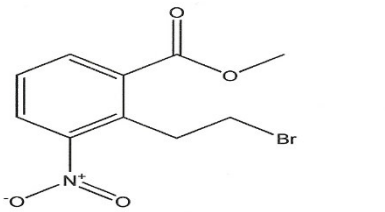
3-(4-nitro-1-oxo-1,3-dihydro-isoindol-2-yl)-piperidine-2,6-dione

(Impurity-C)



3-amino-piperidine-2,6-dione hydrochloride

(Impurity-A)



2-bromethyl-3-nitro -benzoic acid methyl ester

(Impurity-B)

Fig.2: Impurity Chemical Names and structures

RESULTS AND DISCUSSION

Method Validation: The optimized method for determination of Lenalidomide and its related substances has been validated as per International Conference of Harmonization (ICH) guidelines Q3A(R2) for evaluating system suitability, specificity, precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.[15](Fig 3, 4,5)

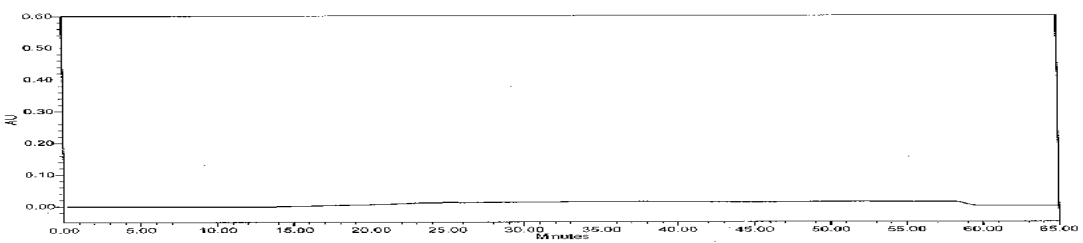


Fig.3: Typical blank chromatogram of Lenalidomide

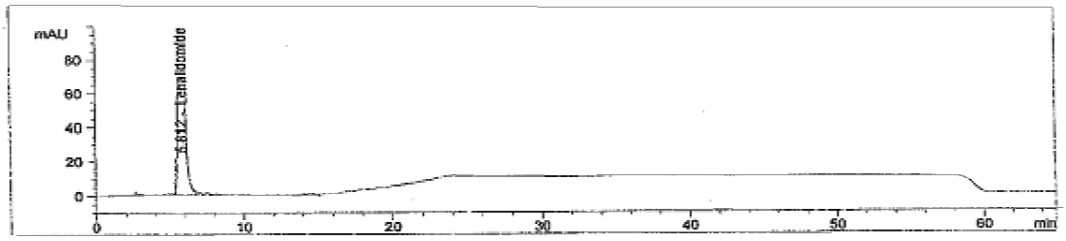


Fig.4: Typical standard chromatogram

Table 3

Peak	Retention time	Area	%Area
Lenalidomide	5.815	7675.607	100

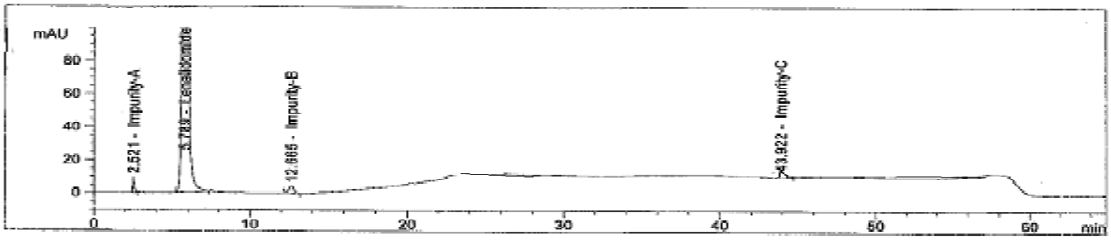


Fig.5: Typical spiked chromatogram

Table 4

Peak	Name	Retention time	Area	Area%
1	Impurity-A	2.521	55.88	0.0982
2	Lenalidamide	5.789	56746.0	99.7002
3	Impurity-B	12.665	87.906	0.1544
4	Impurity-C	43.922	26.847	0.472
	Total		56916.6	100.00

Range for related substances (Accuracy, precision & Linearity): Recovery samples were prepared using solutions at concentrations spanning from LOQ,25%,50%, 75%, 100%,125%,150% of the all impurities and analyte with respect to the specification limit. Three preparations were made at each level except the 100% level, where six preparations are made. Each solution was injected once and analyzed. The solution preparation of Lenalidomide samples used in this study is described in Table No: 5.

Table 5: Solution preparation for Range study (Related substance)

S.no	Stock	Conc.(mg/mL)
	Impurity stock solution	**1 mg/ml**
	Lenalidomide **API stock solution**	**1.0 mg/ml**
1	LOQ solution	spiked solution
2	25% solution	0.0375 mg/mL spiked solution
3	50% solution	0.075 mg/mL spiked solution
4	100% solution	0.15 mg/mL Spiked solution
5	150% solution	0.225 mg/mL spiked solution

The percentage recovery was calculated for Lenalidomide related compound A,B and C for the individual preparation at each level and a mean of the recovery was determined at each level and %RSD was calculated. (Table 6, 7, 8)

Table 6: System suitability results of range study (Related substance)

Injection No	Area
1	76.62131
2	76.75607
3	76.66734

4	77.09001
5	77.0146
6	76.86369
Average	**76.8355**
SD	**0.1885**
% RSD	**0.25**

Table 7: System suitability results and acceptance criteria

Parameter	Result	Acceptance criteria
Resolution between impurity-A and lenalidomide peak	15.83	NLT 3.0
% RSD	0.25	NMT 2.0%

Table 8: %Recovery

Impurity Names	% Recovery			Acceptance criteria
	50%	**100%**	**150%**	
Impurity-A	108.0	104.9	105.2	50%,100% and 150% NLT 85.0 and NMT 115.0
Impurity-B	95.6	97.3	97.6	
Impurity-C	104.4	100.5	100.5	

Precision (Repeatability): Repeatability was determined by analyzing six different sample preparations prepared from same drug substance. The % relative standard deviation for the assay results and % related standard deviation for related substances were determined. (Table 9, 10)

Table 9: Precision results for Assay

Preparation	Assay(%w/w)
1	99.9
2	99.5
3	99.9
4	99.7
5	99.6
6	99.4
Average	**99.7**
SD	**0.21**
% RSD	**0.21**

Table 10: Precision results for related substances (impurity contents)

Preparation	Imp. –A	Imp -B	Imp-C
1	0.15	0.17	0.15
2	0.15	0.16	0.14
3	0.15	0.16	0.14
4	0.15	0.16	0.14
5	0.15	0.16	0.14
6	0.15	0.16	0.14
Average	0.15	0.161	0.14
%RSD	0.0	2.53	2.88

Linearity: Linearity was performed to assess whether a linear relationship is obtained between the response and the concentration of Lenalidomide assay & related substances over the intended operating range of the method. (Fig 6, 7, 8)

Table 11: Linearity results of Impurity-A (related substance)

Level%	Concentration (%)	Area
LOQ	0.0007	1271

25	0.0375	15587
50	0.0750	34834
75	0.1125	51728
100	0.1500	67987
125	0.1875	84067
150	0.2250	100747
Correlation		0.999
Slope		447131.2
Y-intercept		541.74
Y-intercept at 100% level		0.8

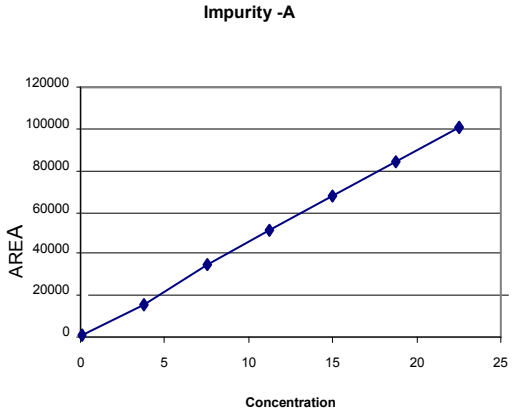


Fig.6: Linearity plot

Table 12: Linearity results of Impurity B

Level%	Concentration (%)	Area
LOQ	0.0028	1256
25	0.0375	25037
50	0.0750	44148
75	0.1125	67982
100	0.1500	93922
125	0.1875	113064
150	0.2250	136600
Correlation		0.999
Slope		606566
Y-intercept		377.16
Y-intercept at 100% level		0.4

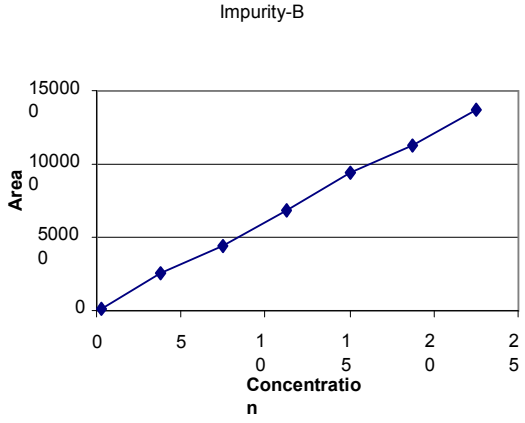


Fig.7: Linearity plot

Table 13: Linearity results of Impurity C

Level%	Concentration (%)	Area
LOQ	0.0282	5105
25	0.0375	7105
50	0.0750	12515
75	0.1125	19103
100	0.1500	24128
125	0.1875	30906
150	0.2250	37558
Correlation		0.999
Slope		162137
Y-intercept		594.96
Y-intercept at 100% level		2.5

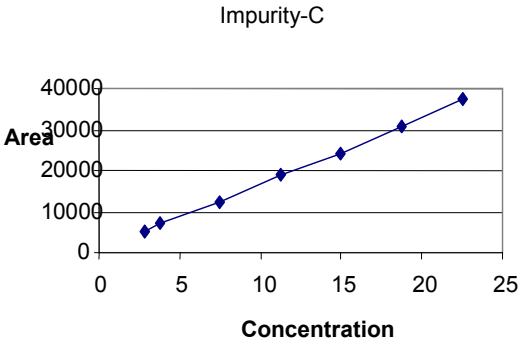


Fig.8: Linearity plot

Relative response Factor: The relative response factor for Lenalidomide related compound A, B, and C (Lenalidomide impurities) was established. (Table 14)

Table 14: RRT, RRF, CF results

Name of the impurity	RRT	RRF	CF
Impurity-A	0.44	0.48	2.08
Impurity-B	2.18	0.70	1.43
Impurity-C	7.57	0.18	5.56

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Emtricitabine, Bictegravir and Tenofovir Alafenamide was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

REFERENCES

1. Vallet S, Palumbo A, Raje N, Boccadoro M, Anderson KC. Thalidomide and lenalidomide: mechanism-based potential drug combinations. Leuk Lymphoma. Jul 2008; 49(7):1238-45. doi: 10.1080/10428190802005191, PMID 18452080.
2. Zhu YX, Braggio E, Shi CX, Bruins LA, Schmidt JE, Van Wier S, Chang XB, Bjorklund CC, Fonseca R, Bergsagel PL, Orlowski RZ, Stewart AK. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. Blood. Nov 2011; 118(18):4771-9. doi: 10.1182/blood-2011-05-356063, PMID 21860026.
3. Stewart AK. Medicine. How thalidomide works against cancer. Science. Jan 2014; 343(6168):256-7. doi: 10.1126/science.1249543, PMID 24436409.
4. Chang DH, Liu N, Klimek V, Hassoun H, Mazumder A, Nimer SD, Jagannath S, Dhodapkar MV. Enhancement of ligand-dependent activation of human natural killer T cells by lenalidomide: therapeutic implications. Blood. 2006 Jul 15; 108(2):618-21. doi: 10.1182/blood-2005-10-4184, PMID 16569772.
5. Anderson KC. Lenalidomide and thalidomide: mechanisms of action--similarities and differences. Semin Hematol. 2005 Oct; 42(4); Suppl 4:S3-8. doi: 10.1053/j.seminhematol.2005.10.001, PMID 16344099.
6. Kotla V, Goel S, Nischal S, Heuck C, Vivek K, Das B, Verma A. Mechanism of action of lenalidomide in hematological malignancies. J Hematol Oncol. 2009 Aug 12; 2:36. doi: 10.1186/1756-8722-2-36, PMID 19674465.
7. Qiao SK, Guo XN, Ren JH, Ren HY. Efficacy and safety of lenalidomide in the treatment of multiple myeloma: A systematic review and meta-analysis of randomized controlled trials. Chin Med J (Engl). 2015 May 5; 128(9):1215-22. doi: 10.4103/0366-6999.156134, PMID 25947406.
8. Chen N, Wen L, Lau H, Surapaneni S, Kumar G. Pharmacokinetics, metabolism and excretion of [(14)C]-lenalidomide following oral administration in healthy male subjects. Cancer Chemother Pharmacol. 2012 Mar; 69(3):789-97. doi: 10.1007/s00280-011-1760-3, PMID 22037879.
9. Fink EC, Ebert BL. The novel mechanism of lenalidomide activity. Blood. 2015 Nov 19; 126(21):2366-9. doi: 10.1182/blood-2015-07-567958, PMID 26438514.
10. Galustian C, Dalgleish A. Lenalidomide: a novel anticancer drug with multiple modalities. Expert Opin Pharmacother. 2009 Jan; 10(1):125-33. doi: 10.1517/14656560802627903, PMID 19236186.
11. Kiaei M, Petri S, Kipiani K, Gardian G, Choi DK, Chen J, Calingasan NY, Schafer P, Muller GW, Stewart C, Hensley K, Beal MF. Thalidomide and lenalidomide extend survival in a transgenic mouse model of amyotrophic lateral sclerosis. J Neurosci. 2006 Mar 1; 26(9):2467-73. doi: 10.1523/JNEUROSCI.5253-05.2006, PMID 16510725.
12. MAHESWARA REDDY L, JANARDHAN REDDY K, REDDY LB, RAVEENDRA REDDY P. Development of A rapid and sensitive HPLC assay method for lenalidomide capsules and its related substances. E-journal of chemistry. 2012; 9(3):1165-74.
13. Isaq M et al. New RP-HPLC method development and validation for the estimation of assay and related substances of lenalidomide in bulk and dosage, Indo-. Am J Pharmacol Sci. 2015; 2(8).
14. Siva Prasad S, Krishna Mohan GV, Naga Babu A. Development and validation of stability-indicating RP-HPLC method for the estimation of lenalidomide and its impurities in oral solid dosage form. Orient J Chem. 2019; 35(1):140-9. doi: 10.13005/ojc/350115.
15. ICH. Guideline"IMPURITIES IN NEW DRUG SUBSTANCES Q3A (R2)" International conference on harmonization, Geneva, Switzerland. Vol. 4; 2005. p. 1-13.