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NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ASSAY AND RELATED SUBSTANCES OF LENALIDOMIDE IN BULK AND DOSAGE

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Abstract:

Lenalidomide, a thalidomide analogue, is an immune-modulatory agent with antiangiogenic and antineoplastic properties. The chemical name is 3-(4-amino-1-oxo 1,3-dihydro-2*H*-isoindol-2-yl) piperidine- ,6-dione. Lenalidomide is indicated for the treatment of patients with transfusion-dependent anemia due to Low- or Intermediate-1-risk myelodysplastic syndromes associated with a deletion cytogenetic abnormality with or without additional cytogenetic abnormalities. Lenalidomide possesses antineoplastic, immuno- modulatory and antiangiogenic properties. The present work explains the development and validation of a simple and reliable liquid chromatographic method for the quantitative determination of Lenalidomide in bulk and in tablet formulation. Chromatography was carried out by reversed phase technique on a Waters X-terra RP 18 (250mm x 4.6mm x 5µ) with a mobile phase composed of Methanol and Acetonitrile in the ratio of 60:40 (v/v) pumped at a flow-rate of 1.0 ml/min. The detection was carried out at 210 nm at the ambient column temperature of. The method was evaluated according to ICH guidelines for the various validation parameters, such as linearity, accuracy, precision, LOD, LOQ, specificity, and Forced degradation studies. The results of intraday and inter-day validation (n = 3) showed the method to be efficient and the same was applied in the assay of lenalidomide in tablet formulation. In conclusion, this was a simple and effective method using HPLC to detect lenalidomide in tablet formulation, which may be useful for routine quality control analysis.

Keywords: Lenalidomide, antineoplastic, Waters X-terra, Methanol, Acetonitrile, HPLC.

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INTRODUCTION:

Lenalidomide (LND) is a potent novel thalidomide analog which demonstrated remarkable clinical activity in treatment of multiple myeloma disease [1-5] via a multiple-pathways mechanism [6-9]. The strong evidences-based clinical success of LND in patients has led to its recent approval by US-FDA under the trade name of Revlimid capsules by Celgene Corporation [10]. LND has an improved side effects profile than its parent compound thalidomide [11]. These side effects can be managed by combination therapy and/or careful dose adjustment [12]. The therapeutic benefits profile of LND is anticipated to encourage the development of new pharmaceutical preparations for LND. As a consequence, there is an increasing demand for proper analytical technologies for quality assurance of LND formulations.

Few methods have been reported for the determination LND in bulk material and in capsules. These methods included two spectrophotometric methods [13]. The first method was based on diazo-coupling reaction with N-(1-napthyl) ethylenediamine dihydrochloride and the second method was based on the formation of a colored condensation product with p-dimethyl amino cinnamaldehyde. In addition, two HPLC methods have reported for analysis of bulk material of LND and its related impurities [14] and capsules [15]. These methods were associated with some major drawbacks such as lack of selectivity, time-consumption and/or use of expensive instruments.

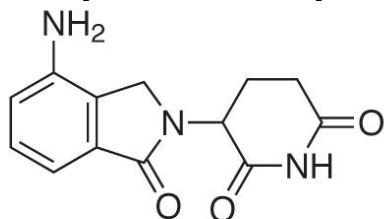

Fig 1: Chemical Structure of Lenalidomide

MATERIALS AND METHODS:

Shimadzu High Performance liquid chromatography with auto sampler, Model LC. Potassium dihydrogen orthophosphate, HPLC grade, Ortho Phosphoric acid, Acetonitrile, HPLC grade, and Methanol HPLC grade were purchased from Merck, Mumbai. Lenalidomide (API) and related substance were kindly gifted by Dr. Reddy's Laboratories, Hyderabad.

Preparation of 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.

Preparation of Mobile Phase

Methanol and Acetonitrile in the ratio of 60:40 (v/v). Mobile phase was used as diluent. A summary of chromatographic conditions were shown in table 1.

Table 1: Summary of HPLC Parameters

Parameters	Conditions
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	Methanol and Acetonitrile in the ratio of 60:40 (v/v)
Column	Waters X-terra RP 18(250mm x 4.6mm x 5µ) or equivalent
Flow rate	1.0 ml/min
Wavelength	210 nm by UV
Column temperature	Ambient
Auto sampler tray temperature	Off mode
Injection volume	10 µL
Run time	65 minutes
Diluent	Mobile phase A and Mobile phase B in the ratio of 50:50 (v/v)
Sample concentration	0.1 mg/ ml

RESULTS AND DISCUSSION:

The developed method was validated as per ICH guidelines for range of related substances (precision, accuracy and linearity), precision, intermediate precision, linearity for related substances, Impurity content, limit of detection and quantification, precision at limit of quantification, Accuracy at limit of quantification, solution stability and robustness.

Precision (Repeatability):

Repeatability was determined by analyzing six different sample preparations prepared from same drug substance. A single injection of each sample preparation was performed. The % related substances were determined for each of the sample preparation, as per the method. The % related standard deviation for related substances was determined. The precision results are presented in Table 2.

Table 2: The precision Results.

S. No.	Impurity-A		Impurity-B		Impurity-C	
	Area	Impurity content	Area	Impurity content	Area	Impurity content
1	100747	0.236	136600	0.219	37558	0.234
2	101246	0.237	135533	0.217	35531	0.222
3	101375	0.237	139099	0.223	35591	0.222
4	108182	0.253	143894	0.231	35859	0.224
5	102726	0.240	144284	0.231	35663	0.222
6	102762	0.240	137344	0.220	36085	0.225
Average	102839.6	0.2405	139459	0.224	36047.8	0.225
STDEV	2743.12	0.0063	3772.00	0.0061	767.014	0.0047
% RSD	2.67	2.64	2.70	2.74	2.13	2.08
Acceptance criteria: The % RSD for the area and impurity content should not be more than 10.0						

Accuracy (Recovery):

Accuracy is a measurement of exactness of the analytical method, which is determined by adding the known amounts of impurities at 50%, 100% and 150% of specification level to sample solution. The accuracy is calculated in terms of % recovery of analyte. The % Recovery results were shown in table 3.

Linearity

Linearity was performed to assess whether a linear relationship is obtained between the response and the concentration of Lenalidomide related substances over the intended operating range of the method. For related substances, linearity was performed for Lenalidomide related compound A, B and C (Lenalidomide impurities) from LOQ level to 150.0% level with respect to individual specification of Lenalidomide and Lenalidomide impurities. A single injection of each linearity solution was analyzed. A plot of response vs. concentration for Lenalidomide is presented below and the individual data points were reported and also a plot of response vs. concentration for related compound A, B and C is presented. A linear regression analysis (without forcing through the origin) was performed on the data (concentration and peak response). A linearity sample preparations are presented in the table 4.

Table 3: Recovery of impurity- A, B and C:

Preparation	50%			100%			150%		
	Imp-A	Imp-B	Imp-C	Imp-A	Imp-B	Imp-C	Imp-A	Imp-B	Imp-C
1	109.3	94.7	102.7	106.0	100.7	100.0	104.9	97.3	104.0
2	109.3	94.7	105.3	104.7	95.3	103.3	105.3	96.4	98.7
3	105.3	97.3	105.3	104.0	96.0	101.3	105.3	99.1	98.7
Average	108.0	95.6	104.4	104.9	97.3	101.5	105.2	97.6	100.5
Acceptance criteria: The % recovery of impurities should be between 85.0 and 115.0									

Table 4: Impurity-A, B and C Linearity Data:

% Level	Impurity A		Impurity B		Impurity C	
	% Concentration	Area	% Concentration	Area	% Concentration	Area
LOQ	0.0007	1271	0.0028	1256	0.0282	5105
25	0.0375	15587	0.0375	25037	0.0375	7105
50	0.0750	34834	0.0750	44148	0.0750	12515
75	0.1125	51728	0.1125	67982	0.1125	19103
100	0.1500	67987	0.1500	93922	0.1500	24128
125	0.1875	84067	0.1875	113064	0.1875	30906
150	0.2250	100747	0.2250	136600	0.2250	37558
Correlation		0.999		0.999		0.999
Slope		447131.2		606566		162137
Y-Intercept		541.74		377.16		594.96
%Y-Intercept at 100% level		0.8		0.4		0.4

Table 5: LOD and LOQ results

Analyte	LOD		LOQ	
	Concentration (µg/ml)	S/N ratio	Concentration (µg/ml)	S/N ratio
Lenalidomide	0.0002	2.8	0.008	10.1
Impurity-A	0.0015	2.4	0.007	10.0
Impurity-B	0.005	2.2	0.028	10.4
Impurity-C	0.101	2.6	0.28	10.2

Detection and Quantification limit:

The limit of detection and limit of quantification for Lenalidomide and Lenalidomide impurities A, B and C was established using the data obtained from the linearity verification solution by analyzing the solutions prepared at different concentrations, viz., from 0.005 % level – 1.0 % level of the test method concentration of 1.0 mg /ml. A single injection at each concentration level was performed. The limit of detection and limit of quantification for Lenalidomide and Lenalidomide related compound A, B and C (impurities) was determined using the slope method.

The limit of detection was calculated using the following formula.

LOD (limit of Detection) = <u>3.3 x Standard Deviation (error)</u>

Slope

The limit of quantification was calculated using the following formula.

LOQ (limit of Quantification) = <u>10.0 x Standard Deviation (error)</u>

Slope

The LOD and LOQ results for Lenalidomide and Lenalidomide related compound A, B and C were presented in Table 5.

CONCLUSION:

The proposed analytical method was simple, rapid, selective, precise, accurate, and economic. The developed analytical HPLC method was robust, rugged, and efficient and represents specific procedure for quantitative determination of imatinib in bulk as well as in pharmaceutical dosage form and this HPLC method successfully applicable for regular analysis of imatinib in quality control laboratories.

REFERENCES:

1.Tariman JD: Lenalidomide: a new agent for patients with relapsed or refractory multiple myeloma. *Clin. J. Oncol. Nursing* 2007, 11:569-574.

2.Sonneveld P, Palumbo A: Lenalidomide: a new therapy for multiple myeloma. *EJHPP* 2008, 14:58-61.

3.Shah SR, Tran TM: Lenalidomide in myelodysplastic syndrome and multiple myeloma. *Drugs* 2007, 67:1869-1881.
4.Falco P, Cavallo F, Larocca A, Liberati AM, Musto P, Boccadoro M, Palumbo A: Lenalidomide and its role in the management of multiple myeloma. *Expert Rev Anticancer Ther* 2008, 8:865-874.
5.Hideshima T, Richardson PG, Anderson KC: Current therapeutic uses of lenalidomide in multiple myeloma. *Expert Opinion on Invest. Drugs* 2006, 15:171-179.
6.Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, Ocampo CJ, Patterson RT, Stirling DI, Kaplan G: Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. *J Immunol* 1999, 163:380-386.
7.Anderson KC: Lenalidomide and thalidomide: mechanisms of action-similarities and differences. *Seminars Hematol.* 2005, 42:S3-S8.
8.Richardson P, Anderson K: Immunomodulatory analogs of thalidomide: an emerging new therapy in myeloma. *J Clin Oncol* 2004, 22:3212-3214.
9.Verhelle D, Corral LG, Wong K, Mueller JH, Parseval LM, Pergakes KJ, Schafer PH, Chen R, Glezer E, Ferguson GD, Lopez-Girona A, Muller GW, Brady HA, Chan KWH: Lenalidomide and CC-4047 inhibit the proliferation of malignant B cells while expanding normal CD34^{+}progenitor cells. *Cancer Res* 2007, 67:746-755.
10.Celgene Corporation: *Revlimid® in combination with dexamethasone sNDA granted approval by FDA for treatment of multiple myeloma*.
11.Rao KV: Linalidomide in the treatment of multiple myeloma. *Am. J. Health System Pharmacists* 2007, 64:1799-1807.
12.Palumbo A, Falco P, Corradini P, Falcone A, Di Raimondo F, Giuliani N, Ciccone CCG, Omedè P, Ambrosini MT, Gay F, Bringhen S, Musto P, Foà R, Knight R, Zeldis JB, Boccadoro M, Petrucci MT: Melphalan, prednisone, and lenalidomide treatment for newly diagnosed myeloma: a report from the gimema-Italian multiple myeloma network. *J. Clin. Oncol* 2007, 25:4459-4465.
13.Sastry BS, Gananadhamu S, Prasad SVS, Venu GRK: New spectrophotometric methods for estimation of lenalidomide in pharmaceutical formulations. *Int. J. PharmTech Res.* 2009, 1:416-419.
14.Saravanan G, Rao BM, Ravikumar M, Suryanarayana MV, Someswararao N, Acharyulu PVR:Development of an HPLC assay method for lenalidomide. *Chromatographia* 2007, 66:287-290.
15.Maheswara RL, Janardhan RK, Bhaskar RL, Raveendra Reddy P: Development of a rapid and sensitive HPLC assay method for lenalidomide capsules and its related substances. *E-J. Chem* 2012, 9:1165-1174.