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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF LENALIDOMIDE IN CAPSULE

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Abstract:
A UV spectrophotometric method for the estimation of lenalidomide in the capsule is developed and validated. The method involved estimation of lenalidomide at 246 nm using acetonitrile as a solvent. The developed method is validated as per ICH guidelines for linearity, range, accuracy, precision, and robustness parameters. Lenalidomide showed a linear response in the concentration range of 08-40 µg/ml with a correlation coefficient of 0.9993. The developed method is accurate (100.26 % recovery), precise (%RSD < 2) and robust (%RSD < 2). Hence, the proposed method can be applied for routine analysis of lenalidomide in bulk and formulations.

Keywords- *lenalidomide, UV spectrophotometric, UV method*

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INTRODUCTION:

Lenalidomide (LND) is an immunomodulatory drug with potent antineoplastic, anti-angiogenic, and anti-inflammatory properties. Lenalidomide is a 4-amino-glutamyl analogue of Thalidomide [($C_{13}H_{13}N_3O_3$, 259.26) 3-(4- amino-1-oxo 1, 3-dihydro-2H-isoindol-2-yl) piperidine-2,6-dione] with better biological activity [1]. It is soluble in organic solvent/water mixtures, buffered aqueous solvents, and is more soluble at low pH [2]. It acts through three main mechanisms viz; direct anti-tumour effect, inhibition of angiogenesis, and immunomodulatory activity. Lenalidomide is available as oral capsules. Lenalidomide is approved for use in India in the treatment of multiple myeloma, myelodysplastic syndromes, follicular lymphoma, and marginal zone lymphoma [1].

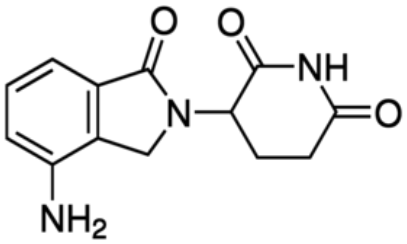

Fig.1 Lenalidomide

A literature survey reveals that only a single method is reported for estimation of lenalidomide by UV spectrophotometry using methanol as solvent and at λmax 250 nm [3]. Another reported method involves the determination of lenalidomide by carrying out diazotization followed by coupling with B.M. reagent and Schiff's base and spectrophotometric determination at 530 nm [4]. Some other analytical methods reported are LC using Mass detector [5-7], PDA detector [8-11], UV detector [3,12-16], and Fluorescence detector [17]. The proposed study aims to develop a simple, accurate, and precise UV spectrophotometric method for the estimation of lenalidomide using acetonitrile as solvent at 246 nm in the capsule.

MATERIALS AND METHODS:

Instruments

A Shimadzu UV-1800 UV spectrophotometer equipped with Shimadzu UV probe 2.43 software was used for the estimation of lenalidomide. Shimadzu-AY220 Electronic balance and Microclean-103 Ultra Sonicator were used for method development.

Reagents and material

Lenalidomide was obtained as a gift sample from Biocon Ltd, Bangalore. Acetonitrile for HPLC and spectroscopy was purchased from Research Lab Fine Chem Industries, Mumbai. Lenalid capsules (Lenalidomide 5 mg) of Natco Pharma Ltd. were purchased from the local pharmacy.

Method Development

Selection of solvent

The solvent selection was done based on the solubility of lenalidomide in water, methanol, ethanol, and acetonitrile.

Preparation of standard stock solution

10 mg of lenalidomide was weighed and transferred to a 10 ml volumetric flask, to which 5 ml of acetonitrile was added, sonicated for 15 minutes, and volume was made up to the mark using acetonitrile to obtain LND stock solution of 1000 µg/ml concentration. Further dilution was made to obtain a LND standard solution of 80 µg/ml.

Determination of Absorption maxima

0.2 ml of the stock solution (1000 µg/ml) was diluted to 10 ml with acetonitrile to obtain a 20 µg/ml solution. It was then scanned in the range of 200-400 nm in a UV spectrophotometer to determine the wavelength of maximum absorption.

Method Validation

The developed UV spectrophotometric method for lenalidomide was validated as per ICH guidelines [18] for the following parameters.

Linearity

8, 16, 24, 32, 40 µg/ml standard LND solutions were prepared by transferring 1, 2, 3, 4, and 5 ml of LND standard solution (80 µg/ml) into a series of 10 ml volumetric flasks respectively and making the volume up to 10 ml using acetonitrile. The absorbance of each solution was measured at 246 nm against acetonitrile as blank and a calibration curve of absorbance v/s concentration was plotted. The regression coefficient and regression equation were obtained.

Range

The range of analytical methods was determined from the interval between upper and lower concentration levels of the analyte in the calibration curve.

Precision

Precision was performed as repeatability and intermediate precision.

Repeatability- Repeatability was determined by analyzing six standard LND solutions of the same concentration of drug (24 µg/ml), recording their

absorbance, and calculating the percent relative standard deviation.

Intermediate precision- Intermediate precision was determined by repeating the study for three consecutive days using 24 µg/ml standard LND solution and calculating percent relative standard deviation.

Accuracy

The accuracy was determined by calculating the % recovery of lenalidomide by the standard addition method. A known amount of standard lenalidomide solution was spiked to the pre-analyzed sample at 3 levels i.e. 80%. 100% and 120%. The percent recovery was calculated which should fall in the range of 98 - 102%.

Robustness

The robustness was performed by deliberately changing the wavelength of analysis by ±1 nm of λmax. The result was reported as % RSD.

Preparation of sample solution

The content of 10 capsules was collected and mixed. The powder equivalent to 10 mg of lenalidomide was transferred into a 10 ml volumetric flask and a sufficient volume of acetonitrile was added, sonicated for 15 minutes and volume was made up to 10 ml using acetonitrile. The solution was filtered through Whatman filter paper. 0.8 ml of the above solution was diluted to 10 ml to obtain a solution of 80 µg/ml concentration (Sample stock solution). From this, 3 ml solution was pipetted out and diluted up to 10 ml with acetonitrile to obtain a solution of 24 µg/ml concentration. The absorbance of this solution was measured at 246 nm.

RESULTS AND DISCUSSION:

Method Development

Selection of solvent

It was found that lenalidomide is soluble in acetonitrile and methanol and insoluble in water and ethanol. So in the present work, Acetonitrile was used as a solvent.

Determination of Absorption maxima

LND solution (20 µg/ml) showed λmax at 246 nm as shown in fig. 3.

Method Validation

Linearity and Range

Table 1 shows absorbance values obtained for linearity solutions. The linearity was found to be ranging from 8 – 40 µg/ml of LND with a regression coefficient (r^2) of 0.9993 and the regression equation as y = 0.0443x + 0.0278 as shown in the calibration curve (Fig. 2). Fig. 3 shows the overlay of UV spectra of 8 – 40 µg/ml standard LND solutions.

Table 1 Linearity results

Sr. No.	Concentration (µg/ml)	Absorbance
1.	8	0.400
2.	16	0.722
3.	24	1.077
4.	32	1.450
5.	40	1.809

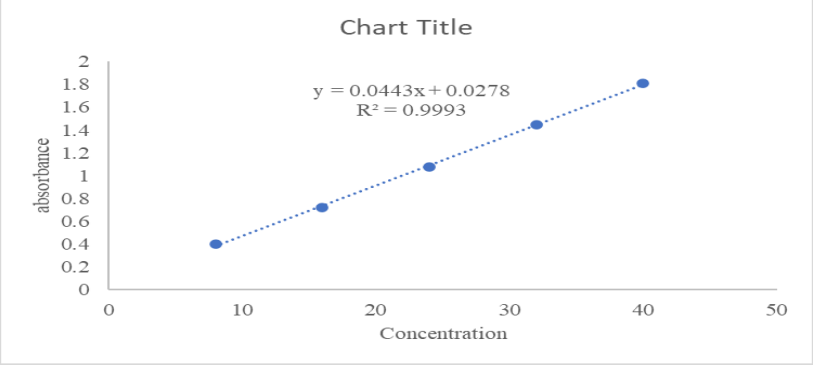

Fig. 2 Calibration curve of lenalidomide

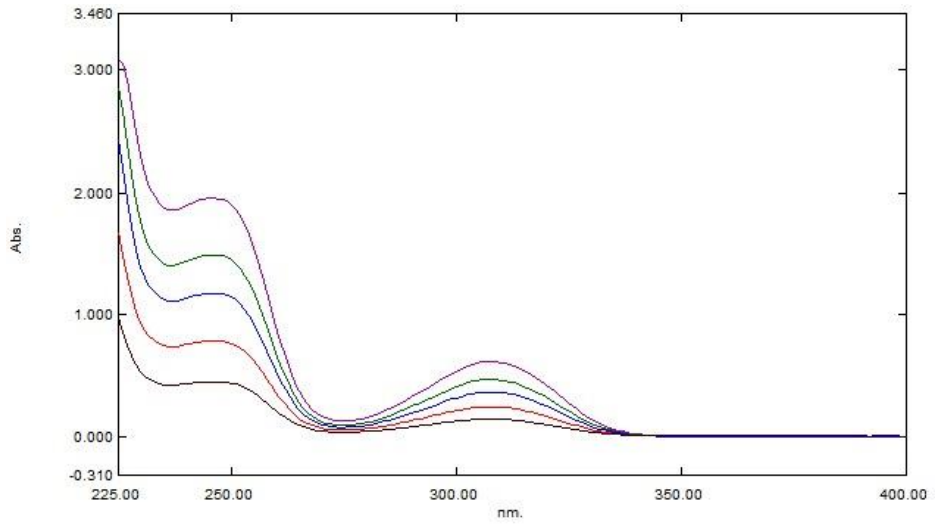

Fig. 3 Overlain UV spectra of 8-40 µg/ml of LND

Precision

The % RSD values for both the studies, repeatability and intermediate precision, were found to be less than 2 (Table 2). Hence the developed method is precise.

Table 2 Precision results

Sr. No.	Repeatability Absorbance	Intermediate precision Absorbance		
		Day 1	Day 2	Day3
1.	1.077	1.077	1.083	1.097
2.	1.078	1.078	1.087	1.099
3.	1.078	1.078	1.087	1.101
4.	1.079	1.079	1.087	1.102
5.	1.082	1.082	1.088	1.103
6.	1.082	1.082	1.088	1.103
Mean	1.079	1.079	1.087	1.101
SD	0.002	0.002	0.002	0.002
% RSD	0.185	0.185	0.184	0.182

Accuracy

The mean percent recovery was found to be 100.26 % which is between 98-102 %. So, the proposed method is accurate. (Table 3)

Table 3 Results of accuracy

Sr. No.	% Spiking	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery
1.	80	12.8	13.048	101.93
2.	100	16	16.072	100.45
3.	120	19.2	18.894	98.41

Robustness

The % RSD value was found to be less than 2 (Table 4) suggesting that the method is robust for change in wavelength by ±1 nm of λmax.

Table 4 Robustness results

Sr. No.	Wavelength	Absorbance	Mean ± SD	% RSD
1.	245	1.076	1.075 ± 0.002	0.186
2.	246	1.077		
3.	247	1.073		

Determination of LND in capsule

The developed method was applied to the assay of the capsule and the result is shown in Table 5. Lenalidomide capsule contains 98.025 % of stated amount of Lenalidomide, $C_{13}H_{13}N_3O_3$ (Table 5).

Table 5 LND content in capsule

Capsule	Amount taken (µg/ml)	Amount found (µg/ml)	% Content
LENALID	24	23.52	98

Summary

Table 6 Summary of UV spectrophotometric method for lenalidomide

Sr.No.	Parameters	Values
1	Beer's law limit (µg/ml)	8 – 40 µg/ml
2	Regression equation (y=mx+c)	y = 0.0443x + 0.0278
3	Correlation coefficient (r^2)	0.9993
4	Slope (m)	0.0443
5	Intercept (c)	0.0278
6	Precision (% RSD)	
	Repeatability	0.185
	Intermediate	0.184
7	Accuracy (% recovery)	100.26
8	Robustness (% RSD)	0.186

CONCLUSION:

In the present study, a simple, rapid, precise, and accurate UV spectrophotometric method for the determination of lenalidomide is developed and validated according to ICH guidelines. All the parameters meet the specific acceptance criteria. The UV spectrophotometric method does not involve lengthy sample preparation and is inexpensive, convenient, and effective for quality control. Hence, the method can be applied to routine analysis of lenalidomide in the bulk and pharmaceutical dosage forms.

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