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ORIGINAL ARTICLE

Method Development and Validation of Pomalidomide Bulk Drug by Using UV Visible Spectrophotometer

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ABSTRACT
The aim of investigation was to develop a simple, accurate, precision and rapid UV visible spectrophotometric method for the determination of pomalidomide in its pure. Pomalidomide was estimates using UV-visible double beam spectrophotometer at the wavelength maxima are 217.40nm in 0.1N NaOH solvent. The drug was characterized by UV-visible spectrophotometry, Fourier transform infra-ray (FTIR) and melting point techniques. The analysis of pomalidomide was carried out by UV– visible method which was validated analytical parameters like precision, linearity, accuracy, limit of detection (LOD), limit of quantitation (LOQ) as per guidelines laid down by ICH.The melting point of pomalidomide was found to be 302.2°c .The FTIR spectra of pomalidomide with potassium bromide was found the sharp and accurate peak.
Key words: *Pomalidomide, FTIR, UV-visible spectrophotometry*

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INTRODUCTION

Method development is a process which proving the analytical method is use to measuring the concentration of bulk drug or active pharmaceutical ingredient in a dosage form. The method validation is process of provides a documented process demonstrating that the analytical procedure is suitable for its intended purpose, provides evidence of the method performance and ensure quality and reliability [1].

UV visible spectrophotometry is the one of the most frequently use technique in pharmaceutical analysis. It involves measuring the amount the visible radiation absorbs by a drug substance in solution. The basic principle of UV visible spectrophotometers is based on Beer-Lambert law [1].

The name of active pharmaceutical substance is pomalidomide. The category of pomalidomide is immunomodulation agent and also use in anti-myeloma tumouricidal activity. These drugs are soluble in 0.1 N NaOH and insoluble in water. Pomalidomide is chemically (RS)-4-amino-2-(2,6-dioxo-piperidin-3-yl)-isoindoline -1,3-dione [2-6]. Pomalidomide has a chiral carbon atom and exist as a racemic mixture of the R (+) andS(-) enantiomers.

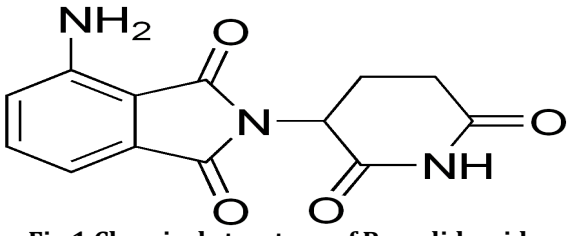

Fig.1 Chemical structure of Pomalidomide

Pomalidomide is a yellow solid powder. The molecular weight of pomalidomide is 273.24gm and molecular formula is C13H11N3O4.The pharmacokinetic of pomalidomide is maximum absorbed in a

plasma concentration (Cmax) occurring in between 2 and 3 hrs. The plasma half -life is approximately 9.5hrs.In the healthy person and 7.5hrs in a multiple myeloma patient. Pomalidomide demonstrated anti-antigenic activity in a mouse tumor model and the in vivo umbilical cord model [6-12].

MATERIAL AND METHODS

Instruments and material

A double beam UV-visible spectrometer (SHIMADZU 1800) in these instruments is connected to PC control loaded with the UV probe software where use for the analysis. Quartz cells are use as sample caveat. FTIR spectrometer (PERKIN ELMER) spectrum 65 is use for analysis and software is spectra. Melting point apparatus (VEEGO) VMP-D/S is use for the to check physical constant.

The pomalidomide was purchasedfrom Vishal institute of pharmaceutical education and research, Ale. NaOH was purchased from Vishal institute of pharmaceutical education and research, Ale.

Solution preparation

0.1N NaOH solution (Solvent)

Weights accurately 4 gm. NaOH transferred into 1000ml volumetric flask and add the 200ml purified water and shake it for 2min.After NaOH dissolve in 200ml water then make up volume with water up to 1000ml.

Preparation of stock solution

Weighed accurately 25mg pomalidomide powder drug and these drugs carefully transferred into 25ml volumetric flask. Then add 10ml 0.1 N NaOH in volumetric flask and shake it. After the shaking volume make up with 0.1N NaOH solution .These solution is make 1000 µg/ml.

Preparation of sample solution

From stock solution (1000 ppm) withdraw 2.5 ml and transferred into 25ml volumetric flask and after transferring solution volume make up with 0.1 N NaOH up to 25ml and to get 100µm/ml .From 100µm/ml withdraw 0.5ml, 1ml, 1.5ml, 2ml and 2.5ml solution with the help of pipette and dilute with 0.1 N NaOH diluent up to 25ml and then to achieve 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml dilutions.

Determination of wavelength of maximum absorbance

The prepared 100µm/ml solution was taken and withdraws 2.5ml and dilute with 0.1N NaOH with diluent up to 25ml and make 10µm/ml dilution. This solution was use for scanning in UV visible spectrophotometer in range 200nm to 400nm. These scanning are carried out in a spectra mode of UV visible spectrophotometer. The wavelength maxima are found to be 217.40nm.

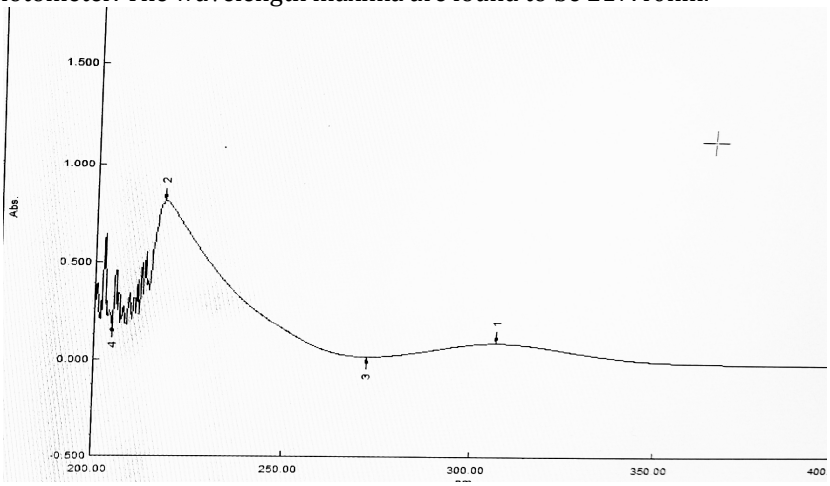

Fig .2 Lambda maximum of Pomalidomide

RESULTS AND DISCUSSION

The all dilution is scan at the wavelength217.20nm by using 0.1N NaOH as a blank solution according to following manner and to record the reading means absorbance.

Linearity

Linearity is also called as a calibration curve method. In the linearity concentration of solution is directly proportional to the absorbance of solution. To assess linearity of analytical method of standard solution of drug concentration 2-10µm/ml was prepared in 25ml volumetric flask by using 0.1N NaOH as solvent. Absorbance of all solution was giving and recorded at wavelength of maximum is 217.20nm using 0.1N NaOH as blank.

Table no 1: Linearity data of pomalidomide

Sr. No	Concentration (µg/ml)	Absorbance
1	Blank	0.000
2	2	0.197
3	4	0.348
4	6	0.473
5	8	0.621
6	10	0.788

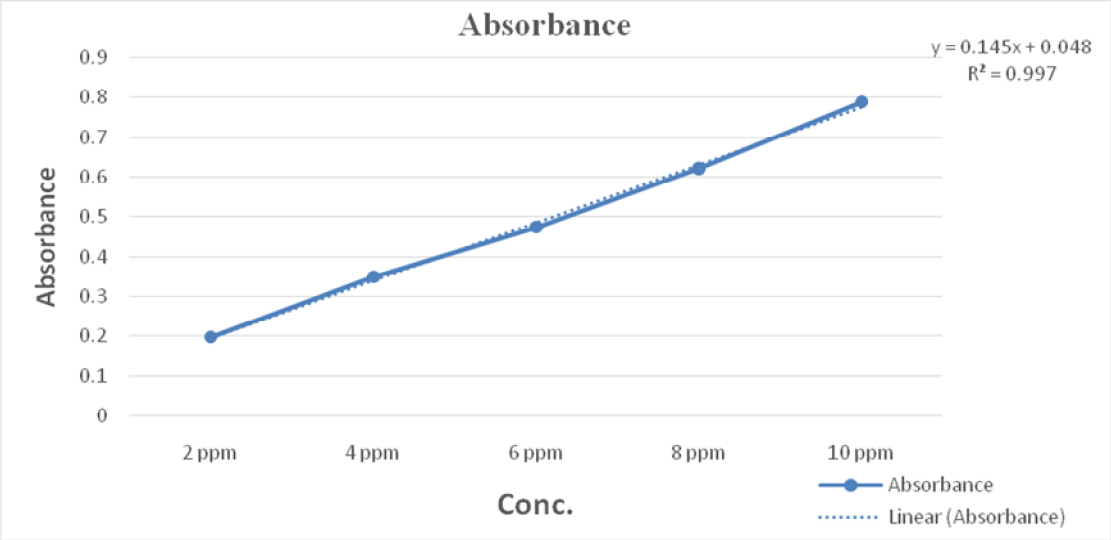

Fig.3 Calibration curve of pomalidomide

Table no 2: Linearity of Pomalidomide

Sr. No	Concentration (µg/ml)	Absorbance
1	2	0.197
2	4	0.348
3	6	0.473
4	8	0.621
5	10	0.788
Regression Data	M	0.145
	C	0.048
	R^2	0.997

Limit of detection

LOD is the analytical method in which to determine lowest amount of sample that can determine but not necessary quantitation.

LOD=3.3×SD/slope

Table no 3: Limit of detection of Pomalidomide

Sr. No	Concentration (µg/ml)	Absorbance
1	2	0.197
2	4	0.348
3	6	0.473
4	8	0.621
5	10	0.788
SD	0.2170	
Slope	0.1455	
LOD	4.9232	

Limit of quantitation

LOQ is the analytical method in which lowest amount of sample that can be quantities but not determine detection

LOQ=10×SD/Slope

Table no 4: Limit of quantitation of Pomalidomide

Sr. No	Concentration (µg/ml)	Absorbance
1	2	0.197
2	4	0.348
3	6	0.473
4	8	0.621
5	10	0.788
SD	0.2170	
Slope	0.1455	
LOQ	14.918	

Precision

Precision is an analytical process in which to determine the absorbance of drug solution after specific time period. Precision expresses the reproducibility of the absorbance. It is expected that an analytical method should generate input that are reproducible. The importance of reproducibility is accurate result. In the precision intraday and interday reading was recorded.

Table no5: Intra-day precision

SR. No	Concentration (µg/ml)	Absorbance	Average	SD	RSD	%RSD
1	4	0.367	0.3616	0.0101	0.0279	2.7970
2	4	0.350				
3	4	0.368				
4	6	0.412	0.4036	0.0073	0.0182	1.8260
5	6	0.398				
6	6	0.401				
7	8	0.537	0.5333	0.0063	0.0119	1.1907
8	8	0.537				
9	8	0.526				

Table no 6: Inter-day precision

SR. No	Concentration (µg/ml)	Absorbance	Average	SD	RSD	%RSD
1	4	0.368	0.3656	0.0049	0.0134	1.3490
2	4	0.360				
3	4	0.369				
4	6	0.423	0.4113	0.0110	0.0268	2.6889
5	6	0.401				
6	6	0.410				
7	8	0.540	0.5376	0.00208	0.00387	0.3871
8	8	0.537				
9	8	0.536				

Ruggedness

Ruggedness of an analytical process is the degree of reproducibility of sample result obtain by the analysis of the same sample under a various condition like laboratories different analyst, different instrument, different reagent, time, temperature, day etc.

Table no 7: Ruggedness of Pomalidomide

Sr. No	Concentration (µg/ml)	Analysis 1	Analysis 2	Analysis 3
1	10	0.788	0.788	0.789
2	10	0.787	0.785	0.781
3	10	0.768	0.786	0.784
4	10	0.785	0.782	0.785
5	10	0.779	0.781	0.786
Average		0.781	0.784	0.785
SD		0.00826	0.00288	0.00291
RSD		0.01057	0.00367	0.00371
%RSD		1.05763	0.36728	0.37139
Average %RSD		1.79630		

Robustness

Robustness is the validation parameter in which to measure the performance of a method when small but deliberate change. In that, measure is the reliability of a method.

Table no 8: Robustness of Pomalidomide

Sr. No	Concentration (µg/ml)	216nm	217.4nm	2218nm
1	8	0.611	0.621	0.628
2	8	0.613	0.624	0.629
3	8	0.611	0.622	0.629
4	8	0.614	0.621	0.630
5	8	0.612	0.621	0.631
6	8	0.614	0.623	0.627
Average		0.612	0.622	0.629
SD		0.00137	0.00126	0.00141
RSD		0.00225	0.00203	0.00224
%RSD		0.22504	0.20336	0.22483
Average %RSD		0.65317		

FTIR spectrometer

IR spectroscopy is an absorption spectroscopy. IR radiation is situated in between 0.78µm to 200µm wavelength. The med IR region is used to detect various functional group regions. A graph is a plot of percentage transmission V/S wave number and is used to determine various functional groups present in compound and to find out the starching or bending vibration occurring in compound.

Table no 9: IR observation of Pomalidomide

U(cm^{-1}) experimental	U(cm^{-1}) Theoretical	Functional group
3378.71	3741,3602	Aniline N-H stretching
3481.80	3590	ImideN-H stretch
3247.11	3217,3189	Aromatic C-H stretch
3116.30,2983.66,2898.21	3145,3129,3077,3063	Aliphatic C-H stretch
1478.22	1505,1428,1456	Aromatic C=C stretching
1360.26	1362	Aniline C-N stretch

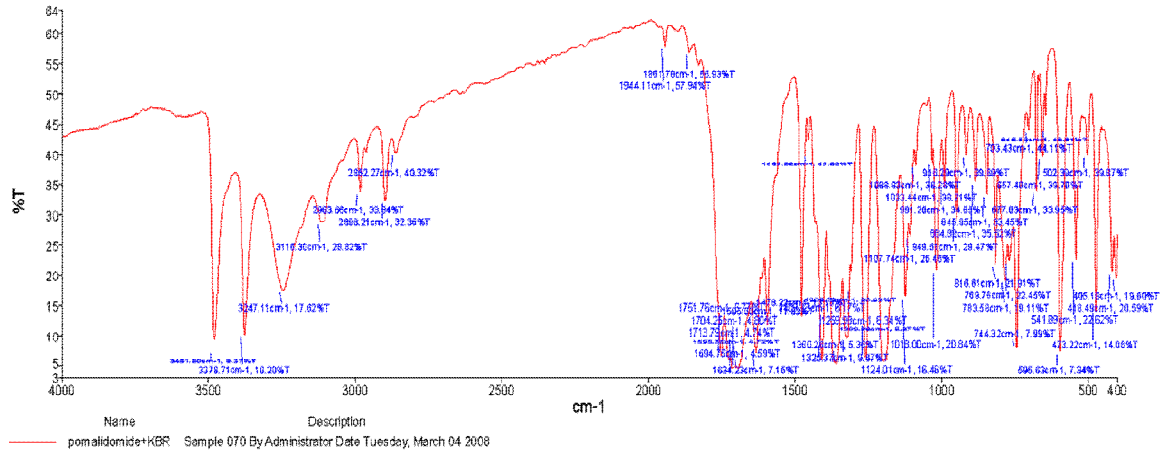

Fig 4. FTIR Spectroscope of pomalidomide

Melting point

Melting point is a physical property of a compound. The melting point is a point at which the particular solid sample changes into liquid state this point is known as a melting point. The melting point of pomalidomide was **302.2°c** recorded.

DISCUSSION

The proposed method was found to be simple, accurate, sensitive, precision and economic for routine quality control analysis. Data for validation parameters are given in tables 1to9.The detection wavelength maxima at 217.40.From the calibration curve it was found that it shows linearity in range 2-10µg/ml with regression coefficients 0.997.The melting point of pomalidomide was detected in range 302.2°c.The FTIR spectra was detected as per its standard spectra.The results of validation tests were found to be satisfactory and therefore this method can be applied successfully for the estimation of pomalidomide in it's pure form.

CONCLUSION

The pomalidomide is an immunomodulatory drug and to treat the multiple myeloma. The Pomalidomide bulk drug was studied by using UV visible Spectrophotometer, FTIR and melting point apparatus. This technique is accurate, precise and reliable for the analysis. The developed spectrophotometric method was validated for pomalidomide using parameter like precision, linearity, LOQ, LOD and robustness, ruggedness. The IR spectra and melting point are accurate observed.

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References

1. Rahul Kumar Garg, Indrajeet Singhvi, (2015). UV Spectrophotometer method development and validation for Quantitative estimation of Nizatidine. JIPBS,Vol (3)333-336.
2. Muller GW, Chen R, Huang SY, CorralLG, WongLM, PattersonRT, et al,(1999). Amino-substituted thalidomide analogs: potent inhibitor of TNF-alpha production. Bioorganic and medicinal chemistry letter. 9(11); 9:1625-1630.
3. Corral LG, Haslett PA, MullerGW, ChenR, WongLM, and Ocampo CJ, et al. (1999). Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha Journal of immunology.163(1):380-386.
4. Sasidhar Bhimana, Girija Sankar Guntuku (2017). High performance liquid chromatographic method for the determination of pomalidomide in pharmaceutical dosage forms, Journal of global treads in pharmaceutical science, 8(1):3599-3608.
5. Crane E, List A. (2005). Lenalidomide: an immunomodulatory drug. Future oncology. 1(15):575-583.
6. Reza Ghiasi, Rahimeh Rasouli, Farrzaneh Zaaeri, Fatemen Ebrahimi Shaghaghi, (2017).Structure Exploring, IR and UV Spectroscopic Properties of Pomalidomide as a Second-generation of Immunomodulatory Agent. Journal of appliedChemical Research 11,2,23-38(2017).
7. Gertz MA, (2013). Pomalidomide and myeloma meningitis. Leukemia & lymphoma. 54(4):681-682.

8. Mussetti A, DaltoS, MontefuscoV. (2011).Effective treatment of pomalidomide in central nervous system myelmatosis. Leukemia &lymphoma.13 Apr; 54 (4):864-866.
9. Moutouh-de Parseval LA, Verhelle D, GlazerE, Jensen-Pergakes K, Ferguson GD, Corral LG. (2008).Pomalidomide and lenalidomide regulate erythropoiesis and fetal hemoglobin production in human Cd 34+ cells. The Journal of Clinical Investigation. 118 (1):248-258.
10. Dispenzieri A, Buadi F, Laumann K, LaPlant B, Hayman SR, Kumar SK, et al. (2012). Activity of pomalidomide in patient with immunoglobulin light-chain amyloidosis. Blood, 119 (23):5397-5404
11. Streetly MJ, Gyertson Daniel Y, Zeldis JB, Kazmi M, Schley SA. (2008). Alternate day pomalidomide retains anti-myeloma effect with reduced adverse events and evidence of *in vivo* immunomodulation. British journal of hematology. 141(1):41-51.
12. Zhu D, CorralLG, FlemingYW, SteinB. (2008). Immunomodulatory drug Revlimid (lenalidomide)and CC-4047 induce apoptosis of both hematological and solid tumor cells through NK cell activation. Cancer immunology, immunotherapy: CII;57(12):1849-1859.
13. Weingarten S, Zerr P, TomcikM, Palumbo-Zerr K, Distler A, Dees C, BeyerC, et al. (2012). Pomalidomide is effective for prevention and treatment of experimental skin fibrosis. Annals of the rheumatic diseases. 71(11);1895-1899.
14. Cooney MM, Nock C, Bokar J, Krishnamurthy S, Gibbons J, Rodal MB, Ness A, et al. (2012). Phase I trial of pomalidomide given for patients with advanced solid tumor. Cancer chemotherapy and pharmacology. Nov;70 (5):755-761.

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