CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com Research Article

A SIMPLE UV-SPECTROPHOTOMETRIC METHOD DEVELOPMNT AND VALIDATION FOR THE ESTIMATION OF POMALIDOMIDE IN ACTIVE PHARMACEUTICAL INGREDIENT

D. SUCHITRA[1*], DR. K. SHRAVAN KUMAR[2], SWAPNA VEMIREDDY[3], E. CH SUDHA BHARGAVI[4]

[1*]PhD Research scholar, University College of Technology, Osmania University.
[2]Department of Pharmaceutics, Trinity college of pharmaceutical sciences, peddapalli, Karimnagar, Telangana, India.
[3] Assistant Professor, Department of Pharmaceutical Analysis and Quality Assurance, Chilkur Balaji college of pharmacy, RVS Nagar, Aziz Nagar, Hyderabad, Telangana, India.
[4]Assistant Professor, Department of Pharmacology, Sankara Academy of Vision Bangalore, India.

Abstract:
The present research work was mainly focussed on establishing a novel, rapid, accurate and simple Ultraviolet-Spectrophotometric method by using LABINDIA double beam 3000+ UV- Visible Spectrophotometer. The simple analytical method was established using potassium dihydrogen phosphate buffer (3.5 pH, adjusted using ortho phosphoric acid) and Acetonitrile in the ratio 30:70v/v. The absorbance was measured over wavelength range of 200-400nm and from the spectrum the λmax was found to be at 389nm. Later, the method was proceeded for validation. The developed method was obeyed Beers-Lamberts law showing a good linearity over a concentration range of 5-25µg/ml. The developed method was also proved to be accurate and precise showing good percentage recovery and having %Relative standard deviation with in the acceptable criteria. The obtained Limit of Detection and Limit of Quantitation values of 2.004µg/ml and 6.072µg/ml respectively proved the sensitivity of the established method. Thus, the developed method can be used routinely for quality control analysis of pomalidomide drug in bulk.

Keywords: *Method development and validation, UV- Spectrophotometric method, %Relative Standard deviation, LOD and LOQ.*

Corresponding author:
DR. K. SHRAVAN KUMAR,
Department of Pharmaceutics,
Trinity College of pharmaceutical sciences, Karimnagar.
Phone No.-9959489191
E-mail: shravankonda@yahoo.com

QR code

Please cite t h i s article in press ***D. Suchitra*** *et al.,* ***A Simple Uv-Spectrophotometric Method Developmnt And Validation For The Estimation Of Pomalidomide In Active Pharmaceutical Ingredient.,*** *Indo Am. J. P. Sci, 2018; 05(12).*

INTRODUCTION:

At present, maximum pharmaceutical industries are mainly giving importance to reduce the cost for the development of a new method and to improve the sensitivity and rapidity. The present research was carried out on Pomalidomide drug which is chemically 4-amino-2-(2,6-dioxopiperidin-3-yl) isoindole-1,3-dione is an orally bioavailable thalidomide derivative having immunomodulatory, anti-angiogenic and anticancer activities [1]. FDA granted approval to Pomalidomide drug for treating multiple myeloma on Feb 8, 2013. It is also approved by European commission in Aug, 2013 [2]. On May 14, 2020, FDA also accelerated approval to Pomalidomide for treating AIDS related Kaposi Sarcoma. It is available in market as 1mg, 2mg, 3mg, and 4mg capsules [3]. Pomalidomide inhibits TNF-alpha production, increases the activity of T cells and natural killer (NK) cells and antibody dependent cellular cytotoxity. It also inhibits tumour angiogenesis arrest the cell cycle in susceptible tumour cell populations and stimulate erythropoiesis [1].

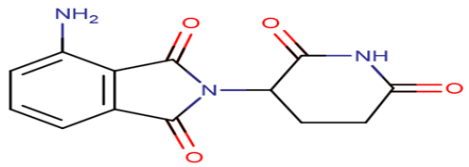

Figure 1: Chemical structure of Pomalidomide

After thorough literature survey, it was noticed that many authors reported new methods on RP-HPLC [4] [5] [6], Stability indicating RP-HPLC [7], Stability indicating RP-UPLC [8], UPLC MS/MS [9] [10], LC-MS [11] [12] [13], and Spectro-fluorometry [14] techniques but till today no simple analytical method was available for regular quality control analysis of Pomalidomide drug in the bulk. Thus, the present method was aimed to develop and validate a novel, rapid, sensitive and cost-effective simple UV-Visible Spectrophotometric method.

MATERIALS & METHODS:

Materials: Pomalidomide active pharmaceutical ingredient, water, acetonitrile, methanol, orthophosphoric acid, & potassium dihydrogen ortho phosphate.

Equipment: Double beam UV-Visible Spectrophotometer (Make- LABINDIA, Model- UV 3000+), Vacuum filtration kit, pH meter & weighing balance.

METHODOLOGY:

- **Preparation of Stock solution(1000µg/ml):** Weighed about 25mg of Pomalidomide API, transferred into a 25ml of volumetric flask, diluted to volume using diluent, sonicated for 10 mins and filtered.
- **Preparation of working stock solution(10µg/ml):** From stock solution about 0.25ml solution was pipetted out into 25 ml of volumetric flask, diluted to volume, mixed thoroughly, sonicated and filtered.
- **Preparation of 0.01M buffer:** About 0.136g of KH_2PO_4 was accurately weighed and dissolved in 100ml of distilled water and the pH was adjusted to 3.5 using 0.1% ortho phosphoric acid.
- **Preparation of diluent:** Accurately measured 300ml (30%) 0.01M Potassium dihydrogen ortho phosphate buffer (adjusted to pH 3.5 using 0.1% diluted Orthro phosphoric acid) and 700ml of Acetonitrile (70%) were mixed thoroughly degassed and filtered.

METHOD DEVELOPMENT

For developing the present method, the prepared Pomalidomide(10µg/ml) working stock solution's absorbance was measured by scanning in UV-Visible Spectrophotometer in the wavelength range of 200-400nm against Buffer (pH 3.5) and Acetonitrile in the ratio of 30:70v/v as blank. The sample solution showed maximum absorbance(λmax) at 389nm.

METHOD VALIDATION

The developed method was validated as per ICH Guidelines. The parameters checked were- Linearity, Accuracy, Precision, LOD and LOQ.

i) Linearity:

Preparation of Pomalidomide working standard solutions for Calibration

- **5%working standard solution:** 0.125ml of standard stock solution was pipetted out into a 25ml volumetric flask and made up to 25 ml to

obtain 5µg/ml Pomalidomide working stock solution.

- **10%working standard solution:** 0.25ml of standard stock solution was pipetted out into a volumetric flask and made up to 25ml to obtain 10µg/ml Pomalidomide working stock solution.
- **15%working standard solution:** 0.375ml of standard stock solution was pipetted out into a volumetric flask and made up to 25ml to obtain 15µg/ml Pomalidomide working stock solution.
- **20% working standard solution:** 0.5ml of standard stock solution was pipetted out into a volumetric flask and made up to 25ml to obtain 20µg/ml Pomalidomide working stock solution.
- **25% working standard solution:** 0.625ml of standard stock solution was pipetted out into a volumetric flask and made up to 25ml to get 25µg/ml Pomalidomide working stock solution.

Procedure: The method's linearity over the concentration range of 5-25µg/ml was determined by taking the prepared solutions into quartz cuvettes and measuring the absorbance of each solution against diluent as blank in UV-Visible Spectrophotometer. Plotted a standard curve of concentration vs Absorbance and calculated correlation coefficient by regression analysis.

ii) Accuracy: Accuracy was confirmed by calculating the recovery of the samples at the concentration level of 50% ,100% and 150%.

Procedure: For this assessment, 5µg/ml, 10µg/ml and 15µg/ml working standard solutions were prepared and their absorbances were measured at 389nm against blank and finally calculated the amount found, percentage recovery and mean percentage recovery values.

iii) Precision:

Intra-day precision: Intra-day precision was carried out by measuring the absorbance of 10µg/ml for 5 repititive times under the same operating conditions for a short period of time. Finally calculated the %RSD value.

Inter-day precision: Inter -day precision was performed by measuring the absorbance of 10µg/ml concentration solution of Pomalidomide for 5 repititive times on the different days under identical operating conditions and calculated the %of RSD value.

iv) Limit of Detection (LOD): The lowest amount of analyte that can be detected was calculated by using the formula-

Limit of detection(LOD) : 3.3×SD of Intercept /Slope

v) Limits of Quantitation(LOQ) : Limits of quantitation was calculated by using

the formula- **Limits of Quantification LOQ = 10×SD of intercept / slope**

RESULTS AND DISCUSSION:

METHOD DEVELOPMENT: After few trial and errors, using the mobiles phase 0.1M Potassium dihydrogen ortho phosphate (adjusted to pH 3.5 using 0.1% orthro phosphoric acid : Acetonitrile (30:70) mobile phase composition, has shown highest absorbance at λmax of 389nm. So it was finalized and proceeded for method validation. The spectrum of Pomalidomide and blank was showed in "Figure 2" & "Figure 3" respectively.

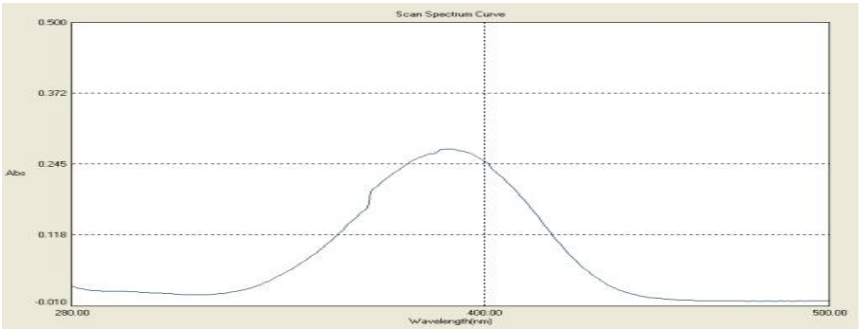

Figure 2: λmax Spectrum of Pomalidomide

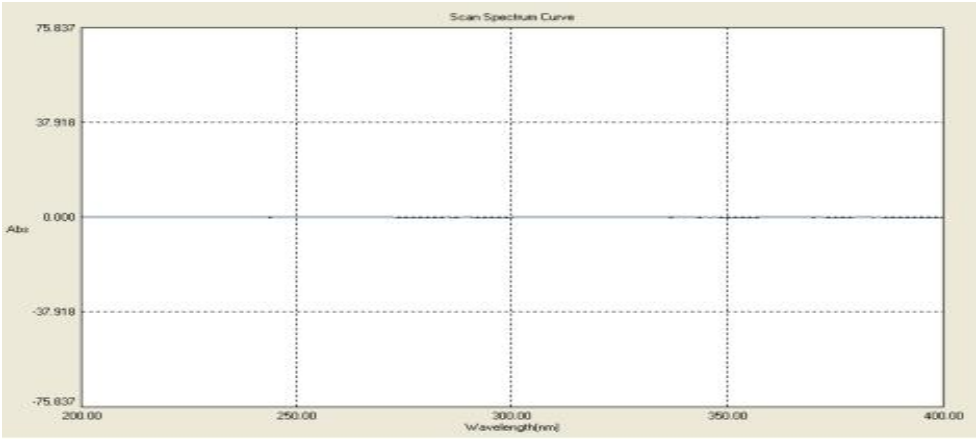

Figure 3: Blank spectrum of Pomalidomide

METHOD VALIDATION :

i) **Linearity:**

From the linearity graph, it was confirmed that the method is exhibiting Linearity over the range of 5-25µg/ml . The correlation coefficient is 0.999 which is meeting the validation criteria. The plotted graph and linearity data are provided in the "Figure 4" and "Table 1" and the spectrum of 5-25µg/ml are shown in "Figures- 5, 6, 7, 8 and 9 respectively. The overlaid spectrum of Linearity were shown in "Figure 10".

Table 1: Linearity data

At λmax of 389nm	
Concentration(µg/ml)	**Absorbance**
5	0.130
10	0.254
15	0.366
20	0.495
25	0.627

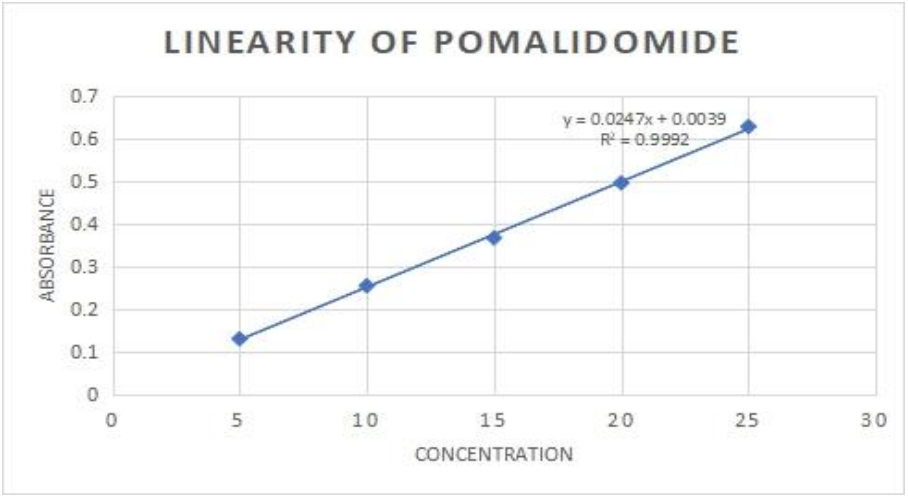

Figure 4: Calibration curve of Pomalidomide

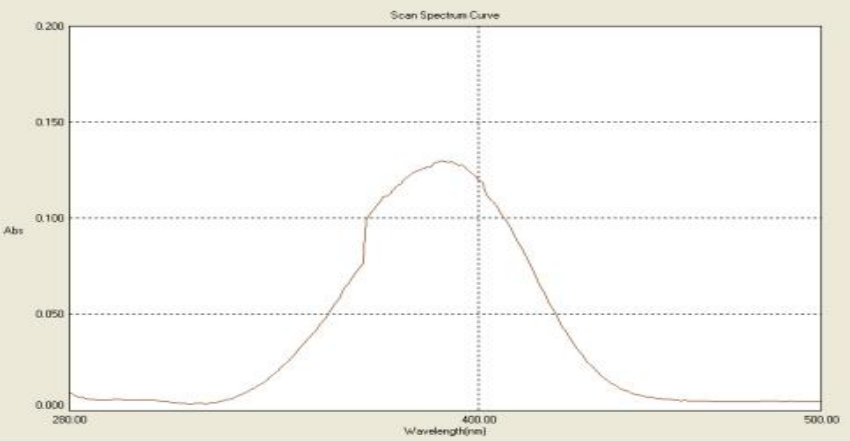

Figure 5 : Spectrum showing linearity level -1(5µg/ml)

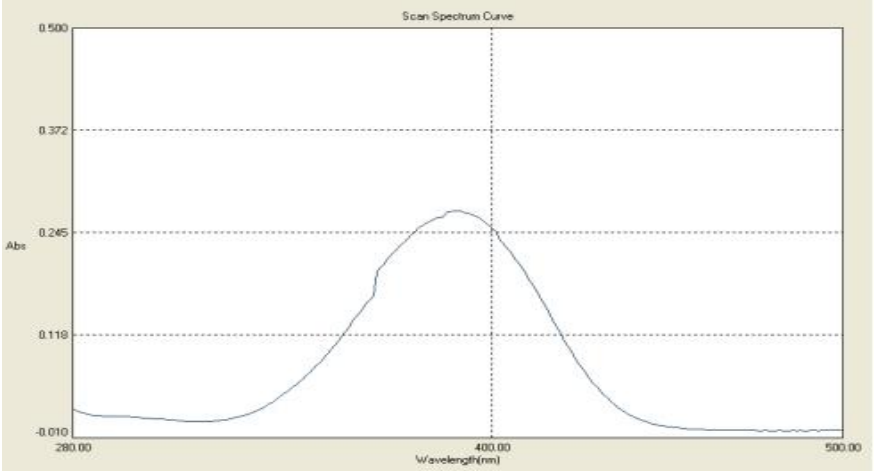

Figure 6: Spectrum showing linearity level-2(10µg/ml)

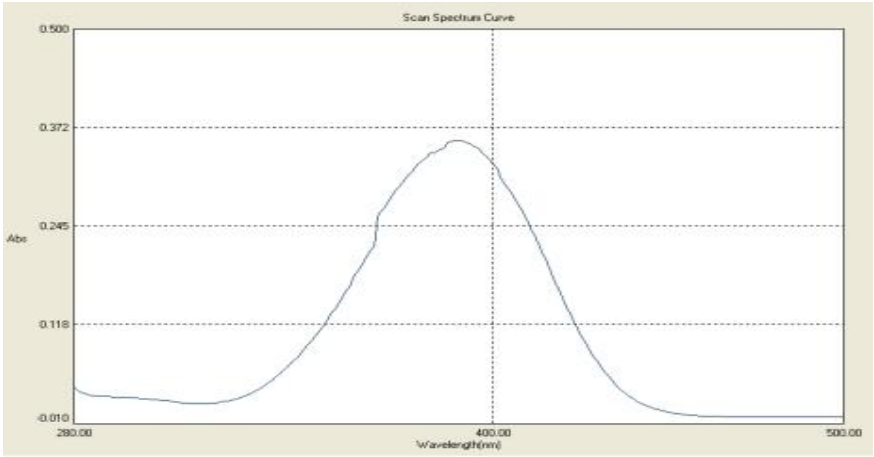

Figure 7: Spectrum showing linearity level-3 (15µg/ml)

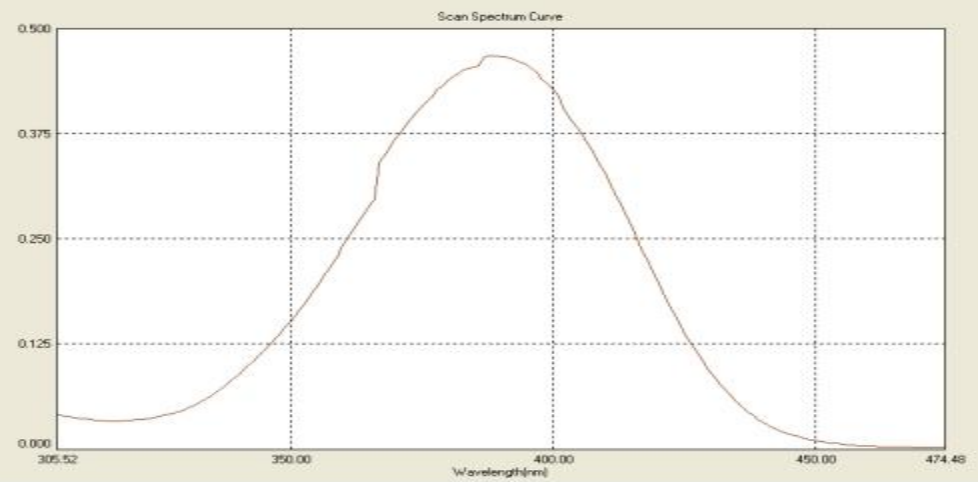

Figure 8: Spectrum showing linearity level-4 (20µg/ml)

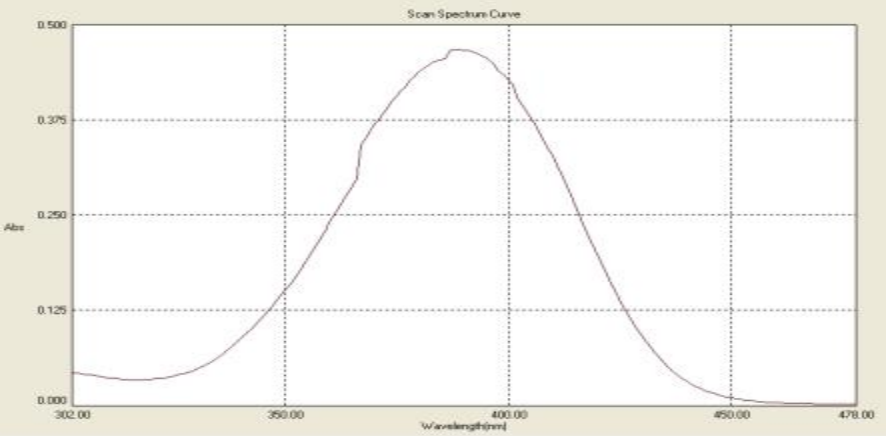

Figure 9: Spectrum showing linearity level-5 (25µg/ml)

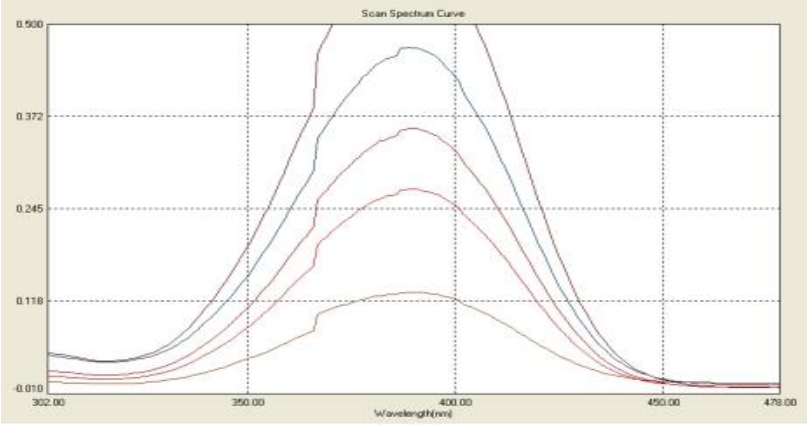

Figure 10: Overlaid spectrum of linearity

ii)Accuracy : The measured percentage average recovery at the levels of 50%,

100% & 150% was found to be 99.69%, 99.13% and 100.66% respectively. The Accuracy data is provided in "Table 2".

Table 2: Accuracy data

Spiking level	Absorbance	Amount added	Amount found	Percentage recovery	Mean %recovery
	0.127	5	4.98	100.04%	99.69%
	0.127	5	4.98	100.04%	
50%	0.128	5	5.02	99%	
	0.249	10	0.99	99.2%	99.13%
	0.249	10	0.99	99.2%	
100%	0.251	10	1.00	100%	
	0.369	15	1.009	100.99%	100.66%
150%	0.369	15	1.009	100.99%	

iii) Precision : The measured percentage relative standard deviation of Intermediate precision and Repeatability was found to be 0.431 and 0.351 respectively which are within the specified limits. Accordingly, it confirmed the method precision. The Intermediate precision data Repeatability data is provided in "Table 3" and "Table 4" respectively.

Table 3: Inter-day Precision data

S.NO	ABSORBANCE
1	0.254
2	0.254
3	0.253
4	0.255
5	0.254
MEAN	0.2538
SD	0.001095
%RSD	0.43144208%

Table -4: Intra-day precision data

S.NO	ABSORBANCE
1	0.254
2	0.254
3	0.253
4	0.252
5	0.254
MEAN	0.2534
SD	0.000894427
%RSD	0.351%

iv) LOD & LOQ : The detection limit and the quantification limit values are found to be 2.004µg/ml and 6.072µg/ml.

Standard deviation of intercept = Standard deviation error of intercept × √n
= 0.0068 × √5
=0.0068×2.236
= 0.01520

Limits of detection (LOD)= 3.3 × SD of Intercept /Slope

The LOD value was analysed by using the formula

= 3.3×0.015/0.0247

= 0.0495/ 0.0247

= 2.004µg/ml

Limits of Quantification LOQ = 10 × SD of intercept / slope

The LOQ value was analysed by using the formula

= 10×0.015/0.0247

= 0.15/0.0247

=6.072µg/ml

SUMMARY
The main objective of the present research work is mainly focussed on development of a novel , rapid, accurate and UV- Spectrophotometric method by using an LABINDIA double beam UV- Visible Spectrophotometer. The analytical method was developed using potassium dihydrogen phosphate buffer (3.5 adjusted using ortho phosphoric acid) and Acetonitrile in the ratio 30:70v/v. The absorbance was measured Over a range 200-400nm and the λmax was found to be 389. The develop method obeyed beers lamberts law showing good linearity over a range of 5-25µg/ml. The developed method was found to be Accurate and precise showing good recovery value and having %RSD with in the acceptance criteria. Thus the developed method can be used routinely for quality control analysis of pomalidomide drug.

CONCLUSION:

A simple, rapid, precise and accurate UV-Spectrophotometric method was developed & validated for the estimation of pomalidomide in Active and pharmaceutical Ingredient. All the validation parameters was found to be within the acceptance criteria. Thus the present developed method can be applied for routine quality control analysis.

REFERENCES:

1. https://pubchem.ncbi.nlm.nih.gov
2. https://en.m.wikipedia.org/wiki/pomalidomide
3. https://www.fda.gov/drugs/Pomalidomide
4. Sasidhar B, Girija sankar G, 2017. High performance liquid chromatographic method for the determination of pomalidomide in pharmaceutical dosage forms. Journal of Global Trends in Pharmaceutical Sciences. 8(1), Pages - 3599 – 3608.
5. Swetha P, Rajendra Prasad V.V.S, Bhagavan Raju M. and Siva Jyothi B, 2013. Estimation of Pomalidomide in capsule dosage form by RP-HPLC. Der Pharm Lett. 5, Pages- 397-404.
6. Dhananjaya G, Naveen Reddy M, Janaki Pathi P, et, al, 2018. The estimation of Pomalidomide in capsule dosage form by RP-HPLC. World Journal of Pharmacy and Pharmaceutical Sciences. 7, Pages-1452-1462.
7. Santhosh I, Sanjana Muthyam, Shirisha V, Rao, K.N.V, and Rajeshwar Dutt, 2019. A Validated stability indicating RP-HPLC method development for the estimation of Pomalidomide in bulk and pharmaceutical dosage form. International Journal of Pharmacy and Biological Sciences. 9, Pages - 63-72. 10.21276/ijpbs.2019.9.1.8

8. Suchitra D, Satyanarayana Battu, 2022. Development and Validation of Stability indicating RP-HPLC method for the estimation of Pomalidomide in Bulk and Pharmaceutical dosage form. YMER. 21(2), Pages - 248-263.
9. Atul Vasanth D, Rajkamal B, 2017. A UPLC-MS/MS method development and validation for the estimation of pomalidomide from human plasma. International journal of applied pharmaceutics. 9, Pages - 37-43. https://doi.org/10.22159/ijap.2017v9i1.15653
10. Mujaffar Iqbal, Ezelddin E, Khalid A, et al, 2015. A validated UPLC-MS/MS assay using negative ionization mode for high-throughput determination of Pomalidomide in rat plasma. Journal of Chromatography B. 983-984, Pages-76-82. https://doi.org/10.1016/j.jchromb.2014.12.036.
11. Ping Lu, Wang L, Song M, Hang T, 2015. Identification and characterization of related substances in pomalidomide by hyphenated LC–MS techniques. Journal of Chromatography B. 114, Pages - 159-167. https://doi.org/10.1016/j.jpba.2015.05.018.
12. Jiang Y, Jiang Wang, Darlene Rozewski M, Shamalatha K, et al, 2013. Sensitive liquid chromatography/Mass spectrometry methods for quantification of Pomalidomide in mouse plasma and brain tissue. Journal of pharmaceutical and biomedical analysis. 88, Pages -262-268. 10.1016/j.jpba.2013.08.036.
13. Komarov T.N, Shohin I.E, Tokareva M.N, Archakova O.A, et al, 2020. Development and validation of Pomalidomide determination in human plasma by HPLC-MS/MS method. Drug development and Registration. 9, Pages-146-154. https://doi.org/10.33380/2305-2066-2020-9-4-146-154
14. Aydogmus Z, Yilmaz E.M, Yildiz G, 2019. A Simple and rapid spectrofluorometric determination of Pomalidomide in spiked plasma and urine. Application to degradation studies. Wiley Analytical Science. 35, Pages - 466-477. https://doi.org/10.1002/bio.3748
15. ICH, Q2B, International Conference on Harmonization, Validation of Analytical Procedures, Methodology 1996.
16. International Conference on Harmonization, ICH Q1A(R2), Stability Testing of New Drug substances and Products, 2002.
17. Snyder L.R, Kirkland J.J. and Glanch J.L. Practical HPLC Method Development. Second Edition. 1997.