INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF POMALIDOMIDE BY UV SPECTROSCOPIC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The UV Spectrophotometric estimation was done by using Shimadzu 1700- UV Visible spectrophotometer. The estimation of Pomalidomide is done by using Water as a solubilising agent and distilled water as the solvent and the λ_{max} was found to be 223nm for calibration curve method and first order derivative. The proposed UV-Spectrophotometric methods were suitable method for the determination of Pomalidomide dosage form. All the parameters of developed methods met the criteria of ICH guidelines for method validation. The developed UV methods for the estimation of Pomalidomide are said to be rapid, simple, precise, accurate, sensitive and cost effective and reproducible within the specified method parameter and can be effectively applied for the routine analysis of Pomalidomide in bulk and formulations.

Key Words: Pomalidomide, UV-Spectrophotometric

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INTRODUCTION

Various authors defined the term drug in various stages. The most widely accepted definition of a drug is as any chemical agent that affects the living process. Further the term drug includes all chemicals and medicines meant for treatment, mitigation or prevention of diseases in human beings or animals for internal or external use. Pharmaceutical chemistry is a science that makes use of the general laws of chemistry to study drugs i.e., their preparation, chemical nature, composition, structure, influence on an organism and studies the physical and chemical properties, the methods of quality control and conditions of their storage. According to their chemical structure or therapeutic action, the drugs may be classified as Antibacterial agents, Anti hypertensive drugs, Antidiabetic drugs, Pharmaco dynamic agents, gastrointestinal agents and Prokinetic drugs etc. **Active pharmaceutical ingredient** is defined as any component that provides pharmacological activity or other effect in the diagnosis, cure, mitigation, treatment or prevention of disease or to affect the structure or any function of the body.

Pharmaceutical Analysis is defined as the application of analytical procedures used to determine the purity, safety and quality of the drugs and chemicals. Pharmaceutical Analysis plays a vital role in the quality assurance and quality control of bulk drugs and their formulations. It is a branch of chemistry that deals with identification of compounds and mixtures (qualitative analysis) or the determination of the proportions of the constituents (quantitative analysis). Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Pharmaceutical analysis is a specialized branch of analytical chemistry, which involves separating, identifying and determining the relative amounts of components in a sample of matter. It is concerned with the chemical characterization of matter both quantitative and qualitative.

Spectroscopy is the study of the interaction of electromagnetic radiation with matter. When matter is energized (excited) by the application of thermal, electrical, nuclear or radiant energy, electromagnetic radiation is often emitted as the matter relaxes back to its original (ground) state. The spectrum of radiation emitted by a substance that has absorbed energy is called an emission spectrum and the science is appropriately called emission spectroscopy. Another approach often used to study the interaction of electromagnetic radiation with matter is one whereby a continuous range of radiation (e.g., white light) is allowed to fall on a substance; then the frequencies absorbed by the substance are examined. The resulting spectrum from the substance contains the original range of radiation with dark spaces that correspond to missing, or absorbed, frequencies. This type of spectrum is called an absorption spectrum. In spectroscopy the emitted or absorbed radiation is usually analyzed, i.e., separated into the various frequency components, and the intensity is measured by means of an instrument called a spectrophotometer. Absorption of visible and ultraviolet (UV) radiation is associated with **excitation of electrons**, in both atoms and molecules, from lower to higher energy levels. Since the energy levels of matter are quantized, only light with the precise amount of energy can cause transitions from one level to another will be absorbed[38]. UV-visible spectrometers can be used to measure the absorbance of ultra violet or visible light by a sample, either at a single wavelength or perform a scan over a range in the spectrum. The UV region ranges from 190 to 400 nm and the visible region from 400 to 800 nm. The technique can be used both quantitatively and qualitatively.

Pomalidomide (INN; marketed as Pomalyst in the U.S and Imnovid in the EU and Russia) is a derivative of thalidomide marketed by Celgene. It is anti-angiogenic and also acts as an immunomodulator. Pomalidomide was approved in February 2013 by the U.S. Food and Drug Administration (FDA) as a treatment for relapsed and refractory multiple myeloma. It has been approved for use in people who have received at least two prior therapies including lenalidomide and bortezomib and have demonstrated disease progression on or within 60 days of completion of the last therapy. Promalidomide is an immunomodulatory agent with antineoplastic activity. It is shown to inhibit the proliferation and induce apoptosis of various tumour cells. Furthermore, promalidomide enhances T cell and natural killer (NK) cell-mediated immunity and inhibited the production of pro-inflammatory cytokines, like TNF-alpha or IL-6, by monocytes. The primary target of promalidomide is thought to be the protein cereblon. It binds to this target and inhibits ubiquitin ligase activity. It is also a transcriptional inhibitor of COX2 (1-6).

Aim is to develop and validate calibration curve method and 1st order derivative method for the determination of Pomalidomide in bulk and capsule dosage forms.

MATERIALS AND METHOD

Determination of λ_{max}

Method A: A solution of 10µg/mL was scanned against Water blank in the range of 200-400 nm. The λ_{max} was found to be 223nm**.**

Preparation of standard solution

The pure drug of about 10 mg was weighed and transferred in to a 100mL volumetric flask. The drug was dissolved completely in Water and made up to the final volume with the same solvent to get a stock solution of concentration 100µg/mL. Aliquots of standard stock solution were pipette out 1ml to 10ml and diluted suitably with Water to get the final concentration of standard solutions

Selection of analytical concentration range

Appropriate aliquots were pipetted out from the standard stock solution in to a series of 100mL volumetric flasks.

The volume was made up to the mark with water to obtain a series of dilutions of concentration range, ranging from 1, 2.5, 5, 10, 12.5, 15µg/mL of Pomalidomide. Absorbances of the above solutions were measured at 223 nm and converted to zero order spectra calibration curve of absorbance against concentration were plotted.

The regression equation and correlation coefficient was determined. Beer Lambert's law was obeyed in the concentration range of 1-15 µg/mL for both the methods.

Analysis of capsule formulation (7)

2 capsules were weighed and powdered. The amount of capsule powder equivalent 10mg of Pomalidomide was taken in a 100mL volumetric flask and it was dissolved in water and made up to the mark with same solvent. Then the solution was filtered using Whatmann filter paper No.40. From this filtrate, dilute 1ml to 10ml volumetric flask was made with water to obtain the desired concentration (10µg/mL). These solutions were analyzed in UV and the result was indicated by % assay.

RESULTS AND DISCUSSION

Determination of λ_{max}

The λ_{max} was found to be 223nm (Fig-1)

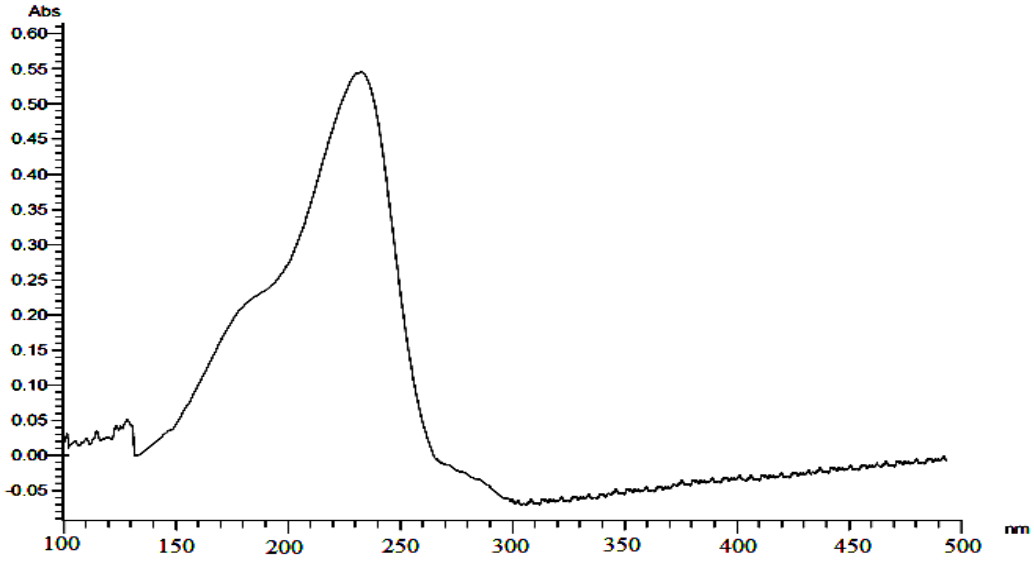

Fig-1 UV spectrum of Pomalidomide

The regression equation and correlation coefficient was determined. Beer Lambert's law was obeyed in the concentration range of 1-15 µg/mL for both the methods.

Assay of Pomalidomide Capsules

These solutions were analyzed in UV and the result was indicated by % assay (Table-1).

Table-1 Results of analysis of Formulation by UV-Spectrophotometry

Wave Length nm	Label claim (mg/tab)	Standard absorbance	Test absorbance	Amount found (mg/mL)	% recovery
223	1mg	0.3133	0.3140	10.2	100.3

Linearity

The drug shows linearity between 1-15 µg/mL for method. The slope obtained was 0.029 (for method). The correlation co efficient was found to be 0.999 for method (Table-2 and Fig-2).

Table-2 Linearity of Pomalidomide

S.No	Pomalidomide	
	Conc.(µg/mL)	Absorbance
1	1	0.0412
2	2.5	0.0909
3	5	0.1618
4	10	0.3133
5	12.5	0.3844
6	15	0.4653
Regression equation	y = 0.029X+0.006	
Slope	0.029	
Intercept	0.008	
R^2	0.999	

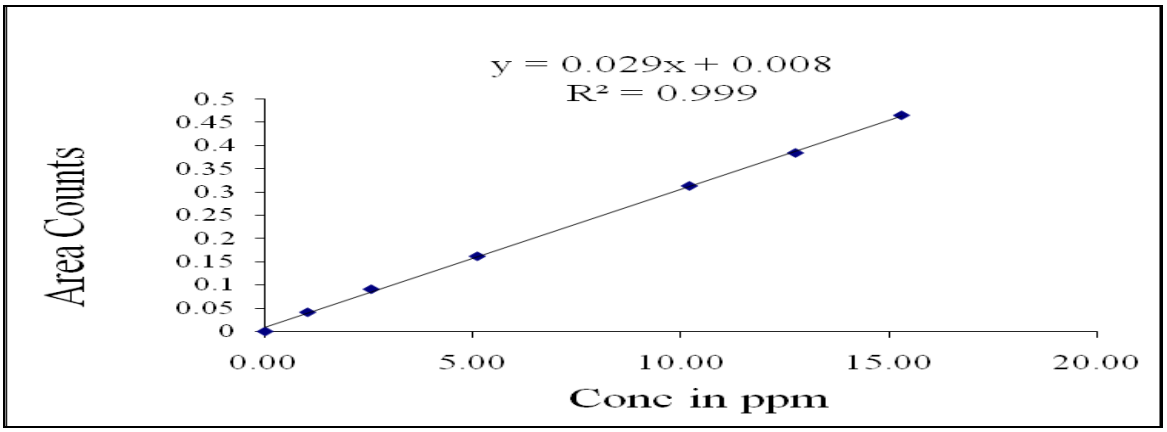

Fig-2 Calibration curve for first order

Accuracy

Accuracy of the developed method was confirmed by performing recovery studies at three different concentration ranges 50%, 100%, 150% each one in triplicate and the accuracy was indicated by % recovery. The %RSD for accuracy of Pomalidomide in the method was found to be less than 2. The % recovery was in the range of 100.4 (for zero order method). According to ICH guidelines the statistical results were within the acceptance range

Table-3 Accuracy data of UV Methods

Method	Amount of µg/mL		% of drug added	%recovered	% RSD
	Tablet	Pure drug			
Method-A	18.6	5	50	100.34	0.46
	37.2	10	100	99.9	
	55.8	15	150	100.23	

Stability of Analytical Solution

10µg/mL Pomalidomide was prepared and stability study was carried out at different time intervals and the results were recorded (Table-4).

Table-4 Stability results

Analytical method	Stability	Absorbance	% Assay
METHOD A	Initial	0.3123	100.1
	6 Hr	0.3171	99.9
	12 Hr	0.3172	99.9
	18 Hr	0.3132	100.2
	24 Hr	0.3118	99.8

CONCLUSION

The UV Spectrophotometric estimation was done by using Shimadzu 1700- UV Visible spectrophotometer. The estimation of Pomalidomide is done by using Water as a solubilising agent and distilled water as the solvent and the λ_{max} was found to be 223nm for calibration curve method and first order derivative.The UV Spectrophotometric estimation uses Water as solubilising agent, and water is used in major proportions and validated according to ICH guidelines for linearity, sensitivity parameters, precision, accuracy, ruggedness and robustness and all the validation results were found well within the limits, indicating that the developed method was simple, rapid, accurate, precise, robust and economical. The proposed UV-Spectrophotometric methods were suitable method for the determination of Pomalidomide dosage form. All the parameters of developed methods met the criteria of ICH guidelines for method validation.The developed UV methods for the estimation of Pomalidomide are said to be rapid, simple, precise, accurate, sensitive and cost effective and reproducible within the specified method parameter and can be effectively applied for the routine analysis of Pomalidomide in bulk and formulations.

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