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THE ESTIMATION OF POMALIDOMIDE IN CAPSULE DOSAGE FORMS BY RP-HPLC

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Pomalidomide in Capsule dosage form. Zorbax Eclipse XDB-C18, (150x4.6mm, 5µm particle size) with mobile phase consisting of Solvent-A (Buffer) is 3.48 gms of Di Potassium hydrogen *ortho*-phosphate (0.02M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute orthophosphoric acid and Solvent-B as acetonitrile in gradient mode of elution was used. The flow rate was 1.0 mL/min and the effluents were monitored at 262 nm. The retention time was 4.4 min and run time was set for 8 minutes. The detector response was linear in the concentration of 5-60 mcg/mL. The respective linear regression equation being Y= 271612.284x+180103.1388. The limit of detection and limit of quantification was 0.5 mcg/mL and 1.5 mcg/mL respectively. The percentage assay of Pomalidomide was 99.3 %. The method was validated by determining its accuracy, precision and linearity. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Pomalidomide in bulk drug and in its pharmaceutical Capsule dosage form.

KEY WORDS: Pomalidomide, RP-HPLC and Capsules.

INTRODUCTION

Pomalidomide is an immune-modulatory antineoplastic agent. The chemical name is (RS)-4-Amino-2-(2,6-dioxo-piperidin-3-yl)-isoindoline-1,3-dione. The empirical formula for

pomalidomide is $C_{13}H_{11}N_3O_4$ and the gram molecular weight is 273.24. Pomalidomide is a yellow solid powder. It has limited to low solubility into organic solvents and it has low solubility in all pH solutions (about 0.01 mg/mL). Pomalidomide has a chiral carbon atom which exists as a racemic mixture of the R (+) and S(-) enantiomers. Pomalidomide (CC-4047), a derivative of thalidomide, is a member of the immune-modulatory drugs (IMiDs) family. Pomalidomide is 5000 fold more potent than thalidomide and 10 fold more potent than lenalidomide in anti-TNFα effects.[1, 2] Its other immunomodulatory properties, e.g., enhancing T-cell response, increasing natural killer cells activity and augmenting endothelial progenitor cells differentiation, make it a potential reagent for sickle cell disease, hematologic neoplasms, like multiple myeloma (MM), as well as some solid tumors, such as metastatic melanoma, prostate and colorectal cancers.[3–6] In February 2013, the U.S. Food and Drug Administration (FDA) gave accelerated approval for pomalidomide for the treatment of patients with relapsed or refractory MM who have received at least two prior therapies. Pomalidomide is also under review by the European Medicines Agency for approval in treating MM.

To date, published studies of pomalidomide have included effectiveness and activity in different neoplasms[7–10], molecular mechanisms[6, 11–14], a pharmacokinetics study of [^{14}C] pomalidomide in humans[15], clinical trials and reviews. Most published clinical trials are phase I studies aimed at determining the maximum tolerated dose, dose limiting toxicity and response with pomalidomide alone or in combination with other therapeutic drugs.[12, 14] Only one quantitative assay of pomalidomide has been published, which measured the chiral inversion of pomalidomide in phosphate-buffered saline and human plasma in the μg/mL range using high-performance liquid chromatography with ultraviolet absorbance detection. In the supplementary material of a separate study that focused on pomalidomide augmentation of fetal hemoglobin production in transgenic sickle cell mice, the authors presented limited mouse plasma pharmacokinetic (PK) data generated with a mass spectrometry method with a reported linear range of 0.5–500ng/mL. A LC-MS method was also used in the absorption, metabolism and excretion study of [14C] pomalidomide in humans[15]; however, as in the previous fetal hemoglobin study, no method validation details were presented. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Pomalidomide in pharmaceutical formulations. The method was validated by determining its accuracy, precision and linearity as per ICH guidelines.

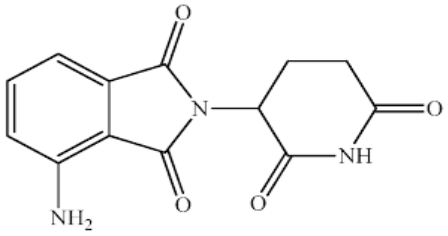

Fig. 1: Structure of Pomalidomide.

EXPERIMENTAL

Materials and Methods

Pomalidomide was obtained as a gift sample from M/s. Vishnu Chemicals Ltd, Hyderabad. Acetonitrile, Potassium Di hyrogenphosphate and *ortho*-phosphoric acid and water used were of HPLC grade (Qualigens). Commercially available Pomalidomide Capsules 200mg (Dificid® 200 mg, Cubist Pharmaceuticals Inc, USA) were procured from local market.

Instrument

Quantitative HPLC was performed on the Waters Alliance 2695 Separations Module is a high performance liquid chromatographic system with a quaternary, low-pressure mixing pump and inline vacuum degassing. Flow rates from50 uL/min to 5 mL/min can be generated for use with 2.1 mm ID columns and larger. The auto-sampler has a maximum capacity of 120 vials (12x32, 2-mL) with programmable temperature control from 4 to 40°C. A heated column compartment provides temperatures from 5 degrees above ambient to 65°C. The detector is a photodiode array (model 2996) with a wavelength range of 190-800 nm and sensitivity settings from 0.0001-2.0000 absorbance units.X-Terra RP-C18 Column (250x4.6 mm i.d; particle size 5 µm) was used. The HPLC system was equipped with LC solution software.

HPLC Conditions

Column- Zorbax Eclipse XDB-C18, (150x4.6mm, 5µm particle size)

Mobile phase— Buffer: Acetonitrile. Solvent-A (Buffer) is 3.48 gms of Di Potassium hydrogen *ortho*-phosphate(0.02M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute orthophosphoric acid. Solvent-B: Acetonitrile in gradient mode of elution shown in Table-1.

Diluent- Water.	Mode of Separation: Gradient
Flow rate – 1.0 ml/min.	Run time—8 min
Temperature- Ambient.	Injection volume--20 µl

Detection wavelength--260 nm Retention time— 3.166min.

	Time	Mobile phase-A	Mobile phase-B
Gradient programme	0	70	30
	2	70	30
	5	20	80
	10	20	80
	11	70	30
	15	70	30

Preparation of Standard drug solution: A standard stock solution of the drug was prepared by dissolving 50 mg of Pomalidomide in 100 ml volumetric flask containing 50 ml of water, sonicated for about 15 min and then made up to 100 ml with water to get approximately 500µg/mL.

Working Standard Solution: 5ml of the primary standard stock solution of 500µg/mL was taken in 50 ml volumetric flask and thereafter made up to 50 ml with mobile phase to get a concentration of 50µg/ml.

Preparation of Sample solution: 20 soft gelatine capsules of Pomalidomide (Pomolyst® 2 mg, Celgene Corporation, Capsules,) were and then powdered. A sample of the powdered capsules, equivalent to 10 mg of the active ingredient, was mixed with 70 ml of mobile phase in 100 ml volumetric flask. The mixture was allowed to stand for 1 hr with intermittent sonication for complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by addition of mobile phase up 100 ml to obtain a stock solution of 100µg/mL. The resultant solution was further diluted by taking 5 ml of the stock solution with 10ml of mobile phase to get the concentration of 50µg/mL.

Preparation of Mobile phase: The contents of the mobile phase were 3.48 gms of Di Potassium hydrogen Orthophosphate(0.02M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute *ortho*-phosphoric acid (mobile phase solvent-A) and acetonitrile (mobile phase solvent-B) in a gradient mode of elution was used to resolute the Pomalidomide. They were filtered before use through a 0.45 µm membrane filter and degassed by sonication.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of Pomalidomide. The parameters studied to evaluate the suitability of the system are given in table III**.**

Linearity: **Preparation of stock solution:** A standard stock solution of the drug was prepared by dissolving 50 mg of Pomalidomide in 100 ml volumetric flask containing 30 ml of water, sonicated for about 15 min and then made up to 100 ml with water to get 500 µg/ml standard stock solution. Take 5 ml of the resulting solution is further diluted with water: acetonitrile in the ratio of 45: 55 v/v to get 50 µg/ml solutions. Further pipette out appropriate volume of resulting solution of working standard solution as taken in 10ml volumetric flask Dilute up to the mark with diluents it contains the concentrations from 5,10, 20, 30, 40, 50, and 60 µg/ml respectively. Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels (5–60 µg/mL) of the assay analyte concentration and 10 µL of each solution was injected in to the HPLC system and the peak area of the chromatogram obtained was noted. The calibration curve was plotted by taking the concentration on the x-axis and the corresponding peak area on the y-axis. The data was treated with linear regression analysis method.. Evaluation was performed with Diode Array detector at 260 nm and a Calibration graph was obtained by plotting peak area versus concentration of Pomalidomide (Fig 3).

The plot of peak area of each sample against respective concentration of Pomalidomide was found to be linear in the range of 5–60 mcg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in table I. The respective linear regression equation being y=2228.4x-2227.3. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in table I.

Assay: To find out the suitability of the proposed method for the assay of Pomalidomide in pharmaceutical dosage forms (Pomolyst® 4 mg, Film coated capsules) the sample solutions from capsules containing Pomalidomide were analyzed by the proposed method. A homogenized powder of Pomolyst® capsules of Pomalidomide equivalent to 10mg of the active ingredient was mixed with 50 ml of diluentin100 ml volumetric flask. The mixture was allowed to stand for 30 minutes with intermittent sonication for complete solubility of the bulk drug, and then filtered through a 0.45 µm membrane filter, followed by addition of mobile phase up 100 ml to obtain a stock solution of 100µg/mL as the working sample solution. The resultant solution was further diluted by taking 5 ml of the stock solution with 10 ml of mobile phase to get the concentration of 50µg/mL. The results are recorded in Table 1.7. The data are presented in table II.

Recovery Studies: Recovery studies were conducted by analyzing pharmaceutical formulation in the first instance for the active ingredient in the concentration of 80% of the working standard (contains 40 µg/mL of Pomalidomide); 100% of the working standard solution (contains 50 µg/mL of Pomalidomide) and 120% of the working standard solution (contains 60 µg/mL of Pomalidomide) by the proposed method. Each concentration was injected 3 times and the peak area was recorded. Known amounts of pure drug [10% of the working standard solution contains 5 µg/mL of Pomalidomide for 80% of the working standard, for 100% of the working standard, for 120% of the working standard] was then added to each 3 previously analyzed formulation and the total amount of the drug was once again determined by the proposed method (each concentration was again injected 3 times) after keeping the active ingredient concentration within the linearity limits.

Table: Recovery Peak areas of Pomalidomide by Accuracy studies.

S.No	Recovery at 80% dilution level Peak areas		Recovery at 100% dilution level Peak areas		Recovery at 120% dilution level Peak areas	
	Standard	Spiked	Standard	Spiked	Standard	Spiked
1	11085657	12450351	13910908	15361992	17070634	18190333
2	11062914	12461202	13855482	15340595	17079835	18167831
3	11062876	12383217	13835789	15369199	17064215	18143173
Avg	11070482.3	12431590.0	13867393.0	15357262.0	17071561.3	18167112.3
Std.Dev	13141.7	42242.1	38950.2	14877.1	7851.2	23588.2
%RSD	0.1	0.3	0.3	0.1	0.0	0.1
% Recovery	102.20		112.3%		82.90	

Robustness: A method is robust if it is unaffected by small changes in operating conditions. To determine the robustness of this method, the experimental conditions were deliberately altered at two different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the mobile phase flow rate was varied by ±10%) and different column had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. When the chromatographic conditions were deliberately altered, system suitability results remained within acceptance limits and selectivity for individual substance was not affected. The results of the study prove the robust nature of the method.

Table: **Robustness study of Pomolyst®-2 mg capsules solution at 100 % level (50µg/mL).**

Parameter	Peak areas of Pomalidomide in Flow increase study		Peak areas of Pomalidomide in Flow decrease study		Peak areas of Pomalidomide in Variable column Study	
	Run time	Peak Area	Run time	Peak Area	Run time	Peak Area
Injection-1	4.05	11976336	4.78	14828243	4.42	13317464
Injection-2	4.06	11852294	4.79	14897290	4.42	13270012
Injection-3	4.05	11978216	4.80	14833574	4.41	13284434
Mean	4.1	11935615.3	4.8	14853035.6	4.4	13290636.6
% RSD	0.0	72164.4	0.0	38418.0	0.0	24326.5
Std.Dev	0.1	0.6	0.2	0.3	0.1	0.2

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection [LOD] and Limit of Quantification [LOQ]: The detection limit of the method was investigated by injecting standard solutions Pomalidomide into the HPLC column. By using the signal-to-noise method the peak-to-peak noise around the analyte retention time is measured, and subsequently, the concentration of the analyte that would yield a signal equal to certain value of noise to signal ratio is estimated. A signal-to-noise ratio (S/N) of 3 is generally accepted for estimating LOD and signal-to-noise ratio of 10 is used for estimating LOQ. This method is commonly applied to analytical methods that exhibit baseline noise. Chromatograms illustrating the LOD are shown in figure 2.10. The limit of detection (LOD) and limit of quantification (LOQ) for Pomalidomide were found to be 0.5 µg/ml and 0.1 5 µg/ml respectively.

Table I: Linear Regression Data for Calibration curves.

Parameter	Results of HPLC Method
Detection wavelength (nm)	252
Linearity range (µg/mL)	5-60
Regression Equation (y=mx + c)	Y= 271612.284x+ 180103.1388
Slope (m)	271612.284
Intercept (c)	180103.1388
Correlation coefficient	0.9999
Relative Standard deviation*	Standard solution-0.4 Sample solution-0.3
% error in bulk samples	0.87

Table II: Results of HPLC Assay and Recovery studies.

Sample	Amount claim (mg/Capsule)	% found by the proposed method	% Recovery*
1.	2	99.7	99.2
2.	2	99.4	98.9
3.	2	99.1	99.1

*Average of three different concentration levels.

Table III: Validation Summary.

Parameter	Results of the proposed HPLC method	
	Pomalidomide Standard solution	Pomalidomide Sample (Pomolyst®-2 mg capsules) Solution
Retention time (min)	4.437	4.438
Theoretical plates (n)	2175.94	2192.77
Plates per meter (N)	14506.33	14618.466
HETP	$6.893x10^{-5}$	$6.84x10^{-5}$
Peak asymmetry (T)	1.68	1.65
Linearity range (µg/mL)	5-60	
Limit of Detection (µg/mL)	0.5	
Limit of Quantification (µg/mL)	0.15	

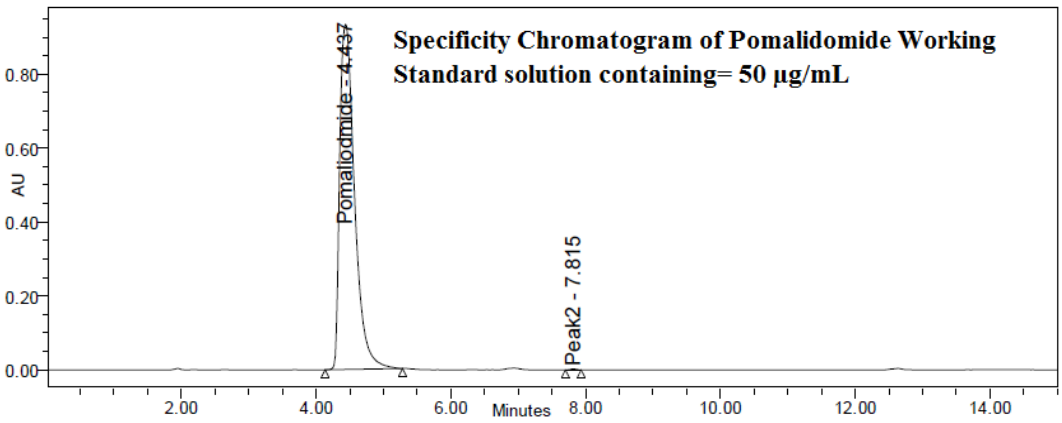

Fig. 2: Typical Chromatogram of Pomalidomide by HPLC.

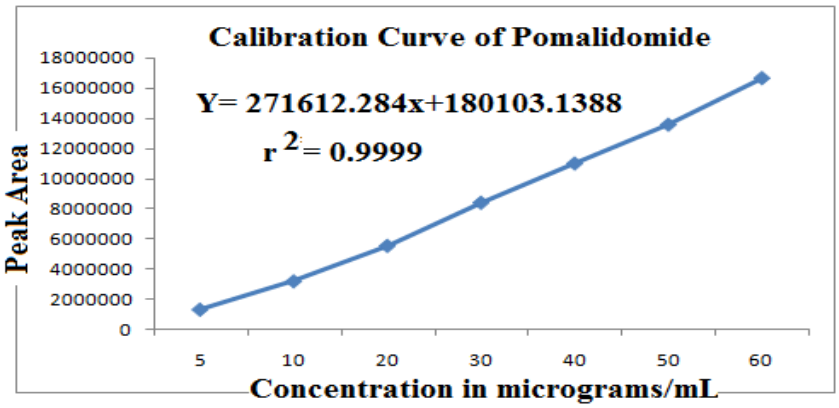

Fig. 3: Calibration curve of the Pomalidomide by RP-HPLC.

CONCLUSION

There are no reports on the HPLC determination of Pomalidomide in pharmaceutical formulations in the literature prior to commencement of this work. The author has developed a sensitive, accurate and precise HPLC for the estimation of Pomalidomide in bulk drug and in capsules dosage form. From the typical chromatogram of Pomalidomide as shown in fig 3.1.2, it was it found that the retention time was 3.166 min. The contents of the mobile phase were Buffer: Acetonitrile 45: 55 (v/v). Solvent-A (Buffer) is 3.48 gms of Di Potassium hydrogen *ortho*-phosphate (0.03M) in 1000 ml of water and by adjusting the pH to 2.5 with dilute *ortho*-phosphoric acid and Solvent-B is Acetonitrile in a gradient mode of separation was used to resolute the Pomalidomide at a flow rate of 1.0 ml/min and eluents were monitored at 252 nm, was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r^2=0.9998) was observed between the concentration range of 5-60µg/mL. The assay of Pomalidomide in bulk was found to be 99.74%. From the recovery studies it was found that about 108.18 % on average of Pomalidomide was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the film coated capsules. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and sterile powder for injection dosage form of Pomalidomide within a short analysis time.

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