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A Validated Stability Indicating RP-HPLC Method Development for The Estimation of Pomalidomide In Bulk and Pharmaceutical Dosage Form

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Abstract

A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of Pomalidomide in bulk and pharmaceutical dosage form dosage form. The chromatographic method was standardized using Develosil ODS HG-5 RP C18, 5µm, 15cm x 4.6mm i.d. column with UV detection at 228nm and mobile phase composition of Methanol: Phosphate buffer = 60:40 ratio at a flow rate of 1.0 ml/ min. The proposed method was successfully applied to the determination of Pomalidomide in bulk and pharmaceutical dosage form. The method was linear over the range of 0-14µg/ml. The recovery was in the range of 98% to 102% and limit of detection was found to be 0.07 µg/ml and quantification was found to be 0.21 µg/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines.

Keywords

RP-HPLC, Pomalidomide, Method development and validation, ICH Guidelines.

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INTRODUCTION:

Pomalidomide is 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.[1]

Pomalidomide directly inhibits angiogenesis and myeloma cell growth. This dual effect is central to its activity in myeloma, rather than other pathways such as TNF alpha inhibition, since potent TNF inhibitors including rolipram and pentoxifylline do not inhibit myeloma cell growth or angiogenesis.[7] Upregulation of interferon gamma, IL-2 and IL-10 as well as downregulation of IL-6 have been reported for

pomalidomide. These changes may contribute to pomalidomide's anti-angiogenic and anti-myeloma activities. [2]

The IUPAC Name of Pomalidomide is 4-amino-2-(2, 6-dioxopiperidin-3-yl)-2,3-dihydro-1H-isoindole-1, 3-dione. [3]

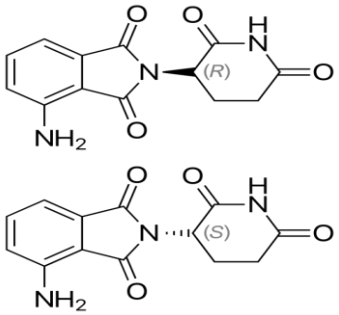


Fig 1: Chemical Structure of Pomalidomide

MATERIALS AND METHODS

HPLC Instrumentation & Conditions:

The HPLC system employed was **HPLC** with Empower2 Software with Isocratic with UV-Visible Detector.

Standard & sample preparation for UV-spectrophotometer analysis:

25 mg of Pomalidomide normal was transferred into twenty-five millilitre meter flask, dissolved to volume with mobile part. Additional dilution was done by transferring 0.1 millilitre of the higher than answer into an one0ml meter flask and conjure to volume with mobile part. Standard & sample stock solutions were ready individually by dissolving standard & sample in an exceedingly solvent in mobile part diluting with constant solvent. (After optimisation of all conditions) for ultraviolet analysis. It scanned within the ultraviolet spectrum within the vary of two hundred to 400nm. This has been performed to grasp the maxima of Pomalidomide, so constant oftenness may be utilised in HPLC ultraviolet detector for estimating the Pomalidomide. While scanning the Pomalidomide answer we have a tendency to ascertain the maxima at 228nm. The ultraviolet spectrum has been recorded on ELICO SL-159 create ultraviolet – Vis photometer model UV-2450. [4]

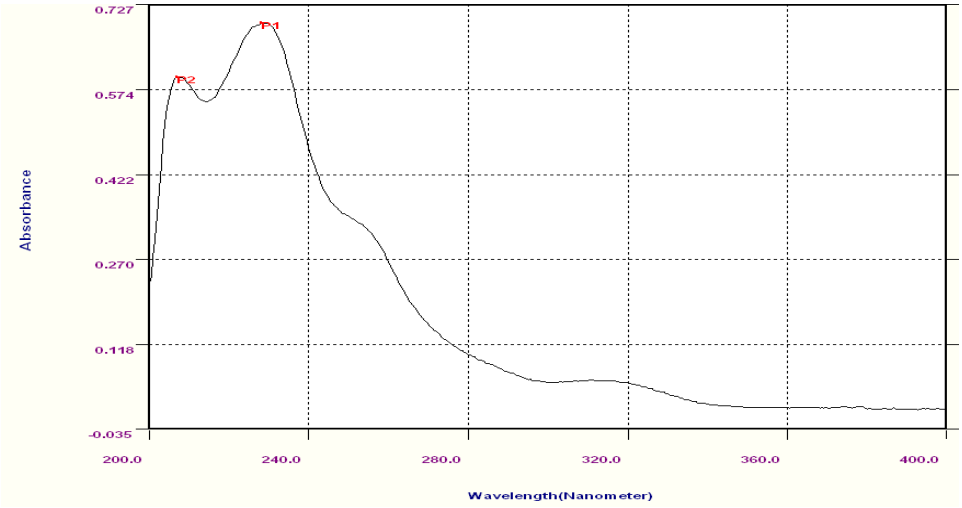


Fig 2: UV spectrum

Optimized Chromatographic Conditions:

Column: Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm.

Mobile Phase: Methanol: Phosphate buffer = 60:40 (v/v)

Flow Rate: 1.0ml/minute

Wave length: 228nm

Injection volume: 20µl

Run time: 8.0minutes.

Column temperature: Ambient

Sampler cooler: Ambient

MOBILE PHASE PREPARATION

The mobile part utilized in this analysis consists of a combination of Buffer (Potassium atomic number 1 inorganic phosphate adjusted to four.20 with orthophosphoric acid) and Acetonitrile in an exceedingly magnitude relation of 32: sixty-eight. 320 millilitres of this {buffer solution} was

supplemental and properly mixed with 680 millilitre of Acetonitrile and the same solution is achieved. This mobile part was stuffed and sonicated for quarter-hour before victimization within the experiment. [5]

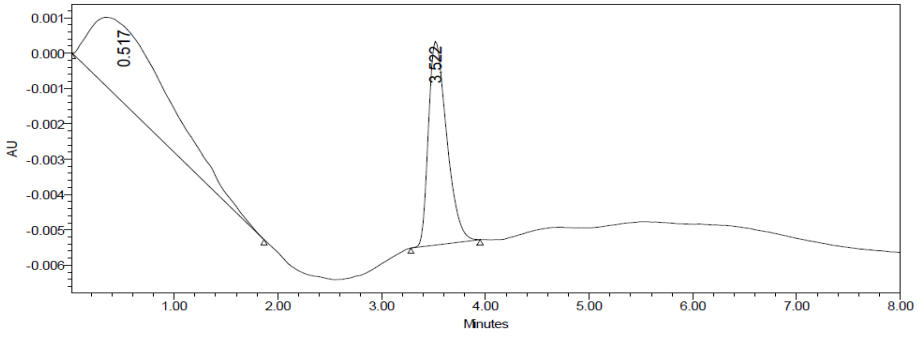
SAMPLE & STANDARD PREPARATION FOR THE ANALYSIS

Twenty-five mg of Pomalidomide normal was transferred into twenty-five millilitre meter flask, dissolved to volume with mobile part. Additional dilution was done by transferring zero.1 millilitre of the higher than answer into a one0ml meter flask and conjure to volume with mobile part. [6]

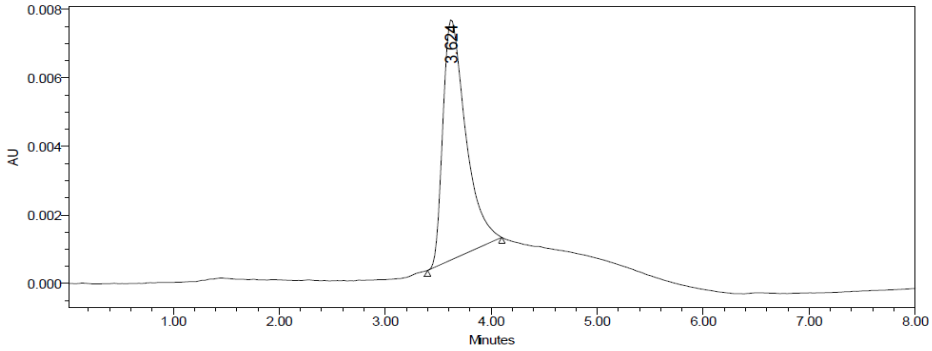
RESULT AND DISCUSSION:

Table-1: Trials for method development

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol: Water = 55 :45	0.8 ml/min	228nm	Peak broken at the end	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Acetonitrile: Water = 60 :40	1.0 ml/min	228nm	Splitting of peak	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Acetonitrile: Phosphate buffer = 70:30	1.0 ml/ min	228nm	Splitting of peak	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol: Phosphate buffer = 70:30	1.0 ml/min	228nm	Broad Peak	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol: Phosphate buffer = 60:40	1.0 ml/min	228nm	Good sharp peak	Method accepted



Trial-1



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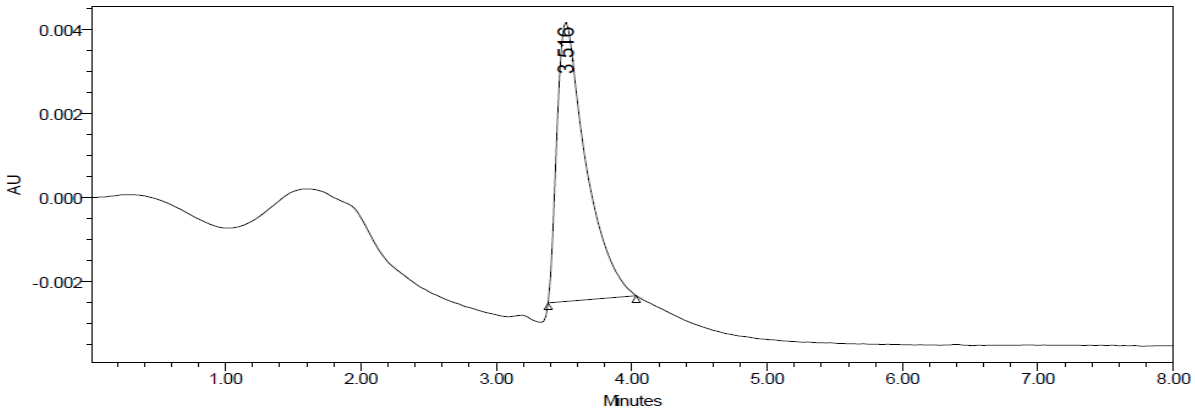

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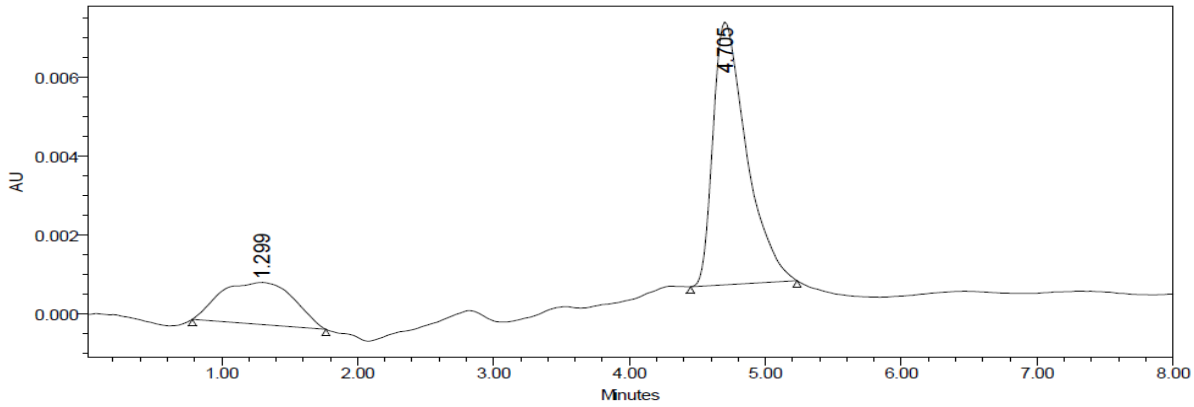

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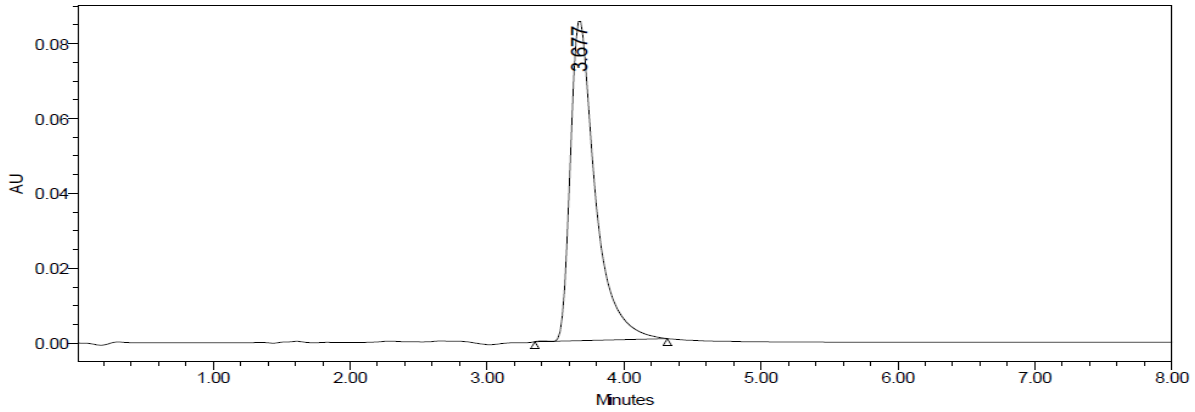

Trial-5

Table 2: Peak results

Rt	Peak Area	Theoretical Plates	Tailing Factor
3.677	725268	4126	1.28

METHOD VALIDATION:

Accuracy: *Recovery study:* To decide the precision of the proposed strategy, recuperation thinks about were done by including diverse sums (80%, 100%, and 120%) of unadulterated medication of Pomalidomide were taken and added to the pre-examined detailing of focus 10µg/ml. From that rate recuperation esteems were ascertained. [7]

Table-3: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1: 80 %	8	8.101	591625	101.262	Mean= 101.0953%
S_2: 80 %	8	8.085	590457	101.062	S.D. = 0.152753
S_3: 80 %	8	8.077	589875	100.962	% R.S.D.= 0.151098
S_4: 100 %	10	10.077	734587	100.77	Mean= 100.43%
S_5: 100 %	10	9.948	725268	99.48	S.D. = 0.833727
S_6: 100 %	10	10.104	736524	101.04	% R.S.D.= 0.830157
S_7: 120 %	12	11.989	872949	99.908	Mean= 100.6997%
S_8: 120 %	12	12.190	887456	101.583	S.D. = 0.841254
S_9: 120 %	12	12.073	878975	100.608	% R.S.D.= 0.835409

Precision:

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Pomalidomide (API) the percent relative standard deviations were calculated for Pomalidomide. [8]

Table-4: Repeatability Results of Precision

HPLC Injections Replicates of Pomalidomide	Retention Time (Minutes)	Peak Area (AUC)
Replicate – 1	3.684	725542
Replicate – 2	3.681	726334
Replicate – 3	3.678	727283
Replicate – 4	3.678	724365
Replicate – 5	3.679	728387
Replicate – 6	3.676	725342
Average		**726208.8**
Standard Deviation		**1449.807**
% RSD		**0.19964**

Intra day & Inter day: The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Pomalidomide revealed that the proposed method is precise. [9]

Table-5: Results of Intraday & Inter day

Conc. Of Pomalidomide (API) (µg/ml)	Observed Conc. Of Pomalidomide (µg/ml) by the proposed method			
	Intra day		Inter day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.92	1.05	8.05	0.98
10	10.06	0.94	9.88	1.08
12	12.09	0.95	11.96	0.97

Linearity and Range

The calibration curve showed good linearity in the range of 0-14µg/ml, for Pomalidomide (API) with correlation coefficient (r^2) of 0.999 (Fig-25). A typical calibration curve has the regression equation of y = 72353x + 5437. for Pomalidomide. [10]

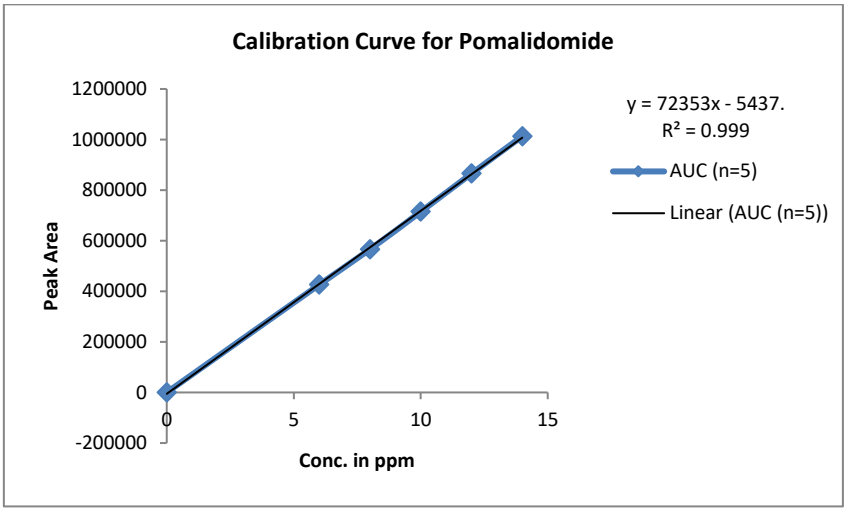

Fig-3: Calibration curve of Pomalidomide (API)

Table-6: Linearity Results of Pomalidomide

S. No.	CONC.	AUC (n=5)
1	0	0
2	6	425874
3	8	565872
4	10	714542
5	12	865632
6	14	1013121

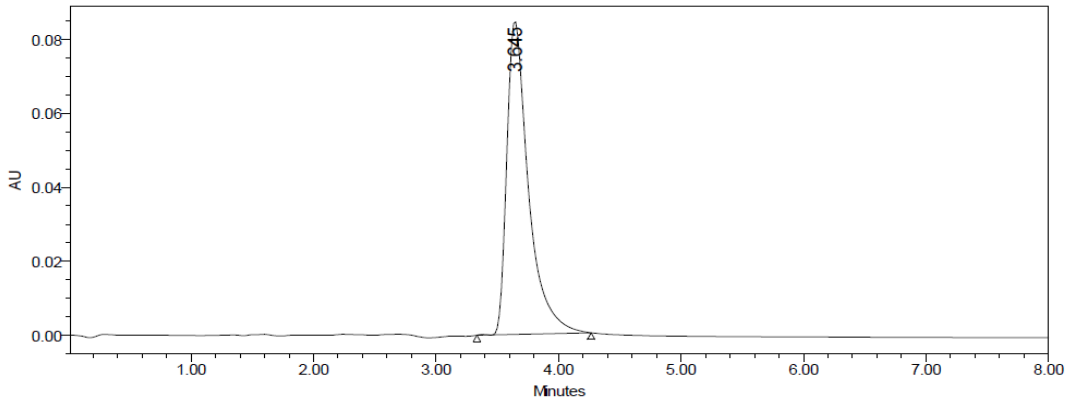

Fig 4: Calibration of Pomalidomide concentration in 6ppm

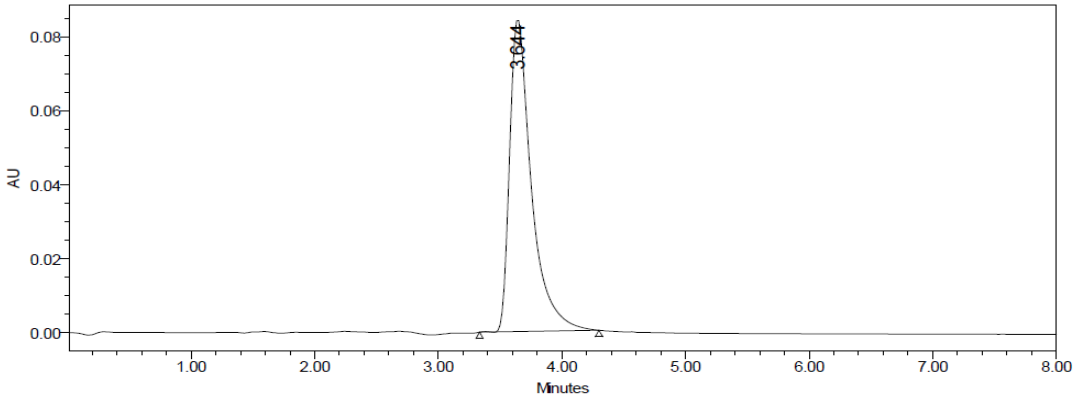

Fig 5: Calibration of Pomalidomide concentration in 8ppm

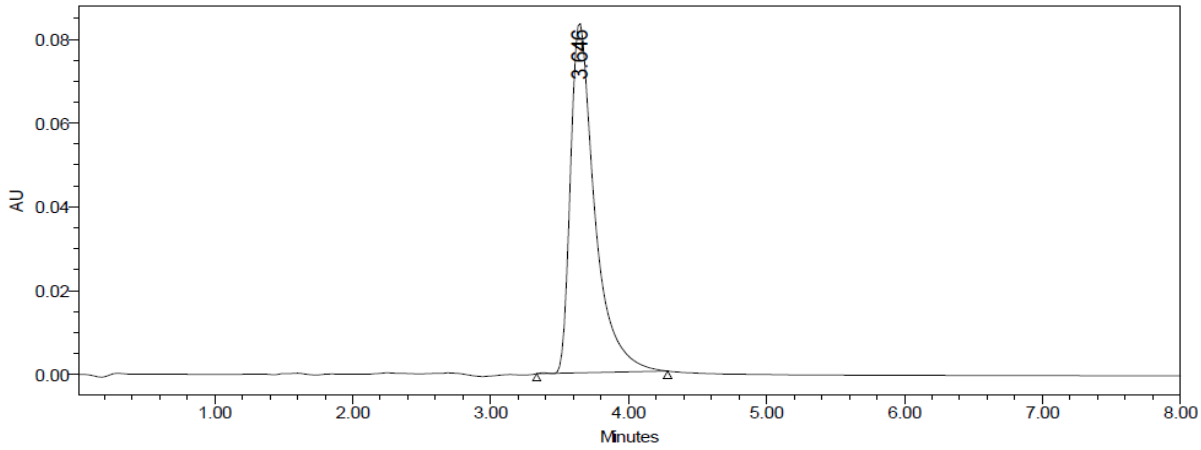

Fig 6: Calibration of Pomalidomide concentration in 10ppm

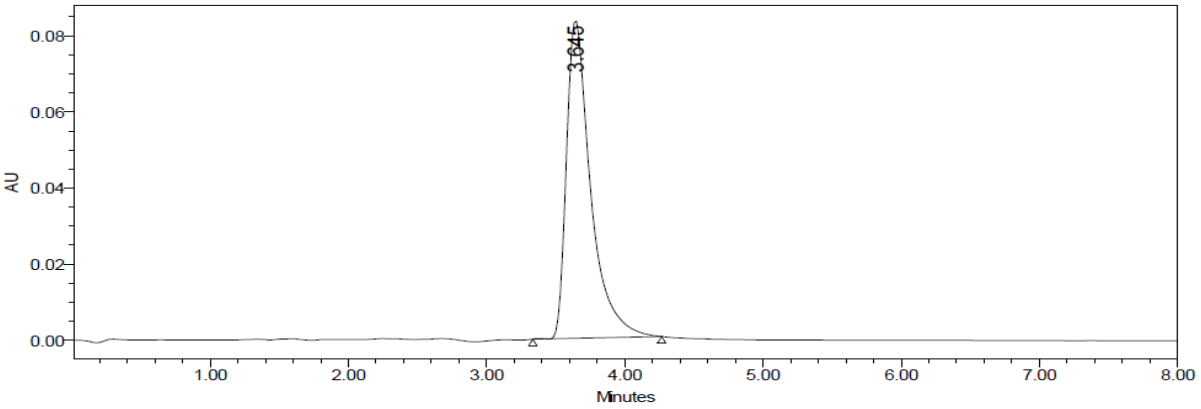

Fig 7: Calibration of Pomalidomide concentration in 12ppm

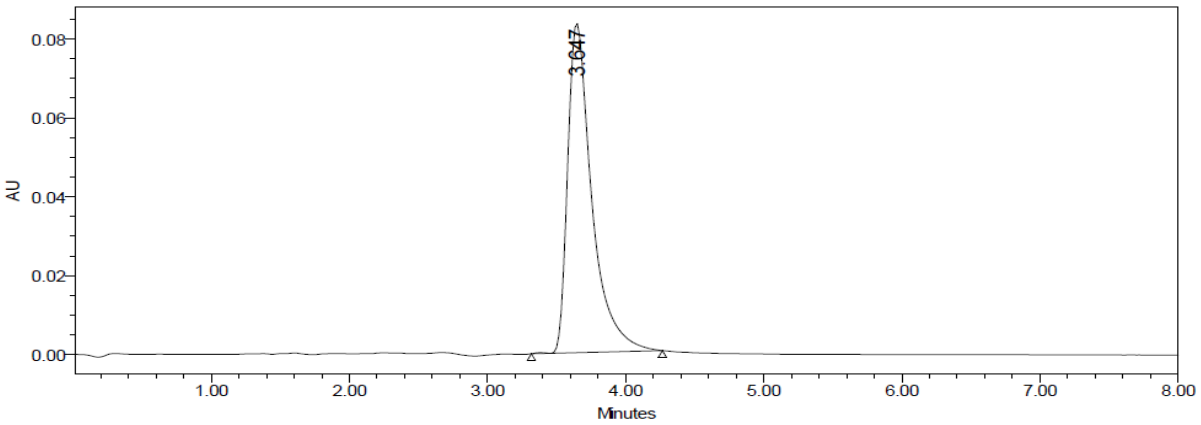

Fig 7: Calibration of Pomalidomide concentration in 14ppm

LOD & LOQ:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.07 & 0.21 µg/ml respectively. [11]

STABILITY STUDIES

ACID DEGRDATION

A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base jar. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water shower at 600C for 4 hours. Permitted to cool to room temperature. The sample was then neutralized using dilute NaOH solution &

final volume of the sample was made up to 100ml with water to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase (after optimizing the mobile phase compositions). This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile. The typical chromatogram shown below is the degradation profile of *Pomalidomide* in 0.1N HCl. [12]

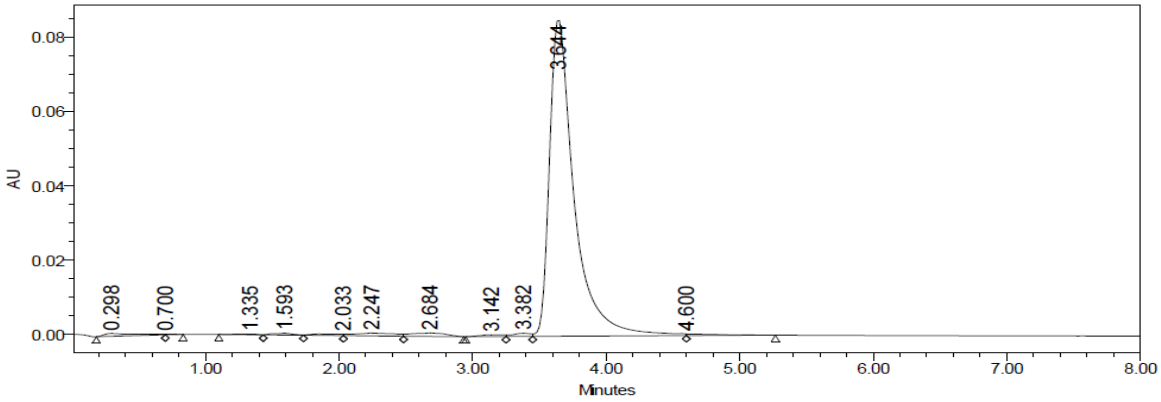

Fig-9: Acidic degradation

BASIC HYDROLYSIS:

A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base carafe. 30 ml of 0.1N NaOH was added to it. And it was refluxed in a water shower at 600C for 4 hours. Permitted to cool to room temperature. The example was then killed utilizing 2N HCl arrangement and last volume of the example was made up to 100ml to get ready 100 µg/ml arrangement. It was infused into the HPLC framework against a clear of versatile stage in the wake of enhancing the portable stage pieces. This experiment was repeated several times using same concentration of NaOH such as 0.1N to observe its degradation profile. The chromatogram shown below is the degradation profile of *Pomalidomide* in 0.1N NaOH. [13]

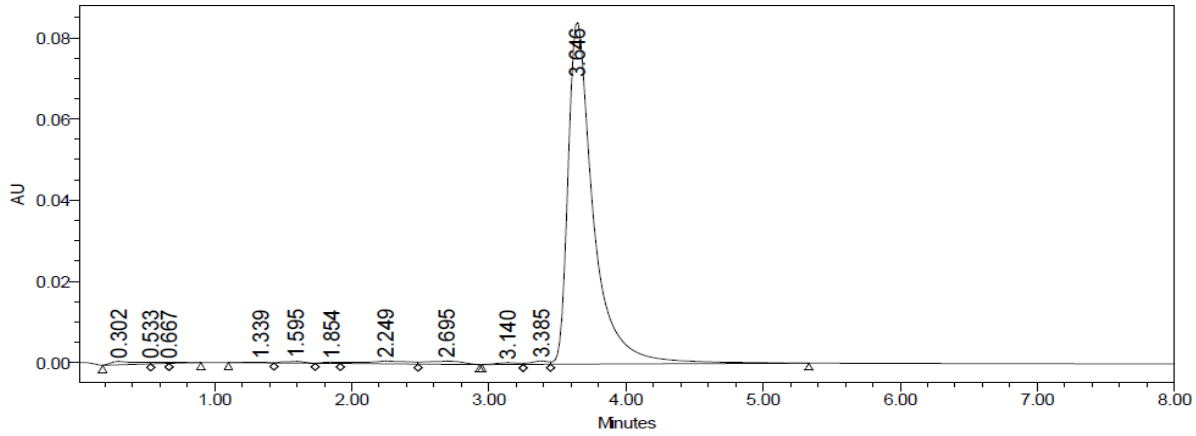

Fig-10: Basic degradation

WET HEAT DEGRADATION:

Precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base flagon. 30 ml of HPLC water was added to it. At that point, it was refluxed in a water shower at 600 c for 6 hours uninterruptedly. After the reflux was finished, the medication wound up solvent and the blend of medication and water was permitted to cool to room temperature. Last volume was made up to 100 ml with HPLC water to get ready 100 µg/ml arrangement. It was infused into the HPLC framework against a clear of versatile stage. [14]

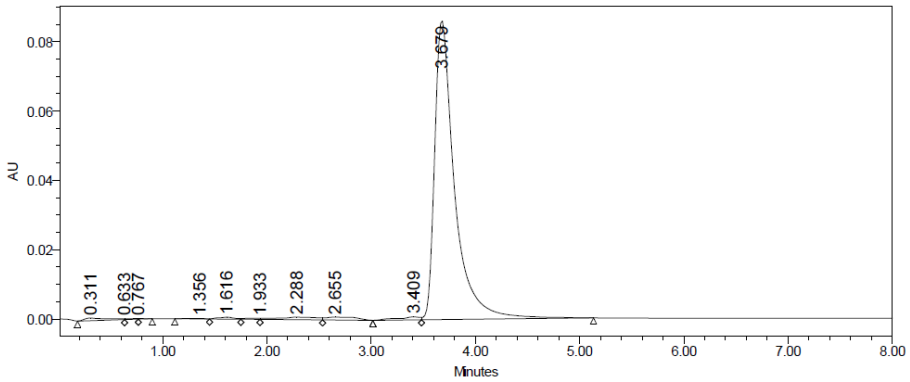

Fig-11: Wet Heat Degradation

PHOTOLYTIC DEGRADATION:

Roughly 10 mg of unadulterated medication was taken in a clean and dry Petri dish. It was kept in an UV bureau at 254 nm wavelength for 24 hours without intrusion. Precisely measured 1 mg of the UV uncovered medication was exchanged to a clean and dry 10 ml volumetric carafe. First the UV uncovered medication was broken up in methanol and made up to the stamp with portable stage to get 100 µg/ml arrangement. At last this arrangement was infused into the HPLC framework against a clear of versatile stage and chromatogram was gotten. [15]

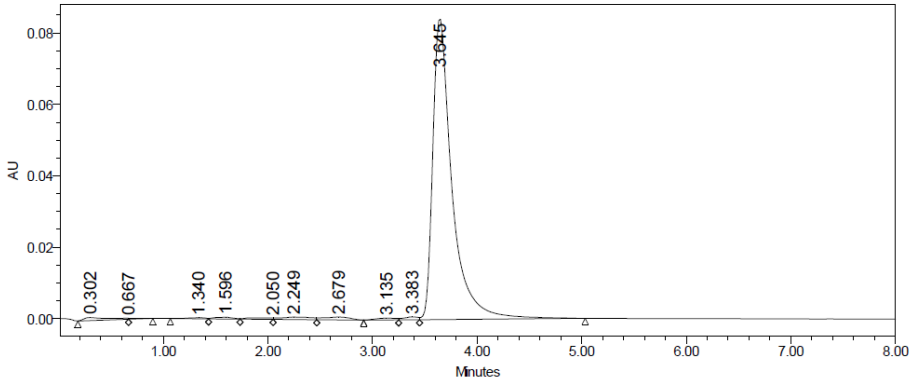

Fig-12: Photolytic degradation.

OXIDATIVE HYDROLYSIS (3% H_2O_2):

Precisely measured 10 mg. of unadulterated medication was taken in a clean and dry 100 ml volumetric cup. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it dissolvable and then kept in that capacity in dull for 24 hours. Last volume was made up to 100 ml. utilizing water to plan 100 µg/ml arrangements. The above example was infused into the HPLC framework. [16]

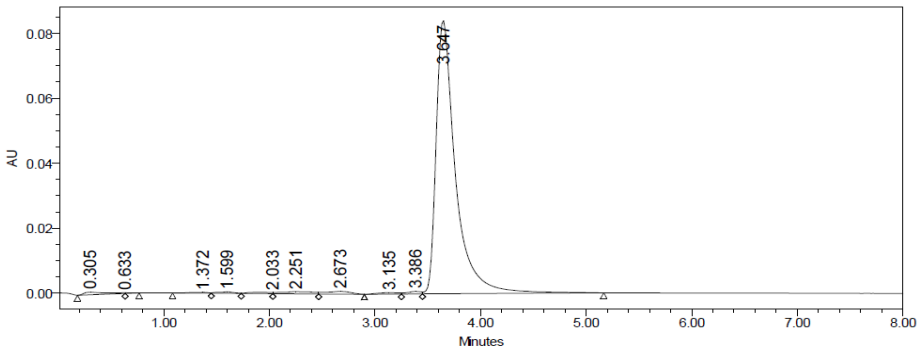

Fig-13: Oxidative degradation

Table-7: Results of forced degradation studies of Pomalidomide API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	81.31	18.69	100.0
Basic Hydrolysis (0.I M NaOH)	24Hrs.	84.54	15.46	100.0
Wet heat	24Hrs.	91.29	8.71	100.0
UV (254nm)	24Hrs.	73.48	26.52	100.0
3 % Hydrogen peroxide	24Hrs.	87.52	12.48	100.0

CONCLUSION

A sensitive & selective RP-HPLC method has been developed & validated for the analysis of Pomalidomide API. Promote the proposed RP-HPLC strategy has amazing affectability, accuracy and reproducibility. The outcome demonstrates the created technique is amazingly, one more appropriate strategy for examine, immaculateness and soundness which can help in the examination of Pomalidomide in various details.

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