STABILITY DEMONSTRATING VALIDATED HIGH PRESSURE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF POMALIDOMIDE AND DEXAMETHASONE

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Abstract: *An accurate, rapid, inexpensive, and straightforward, reliable simultaneous assay technique was developed and demonstrated to evaluate Pomalidomide and Dexamethasone using RP-HPLC. The suggested approach achieved efficient chromatographic separation at a flow rate of 1 mL/min with a wavelength of 246 nm using an inertsil ODS column (150 mm x 4.6 mm, 3.5µ), acetonitrile and 0.1% ortho phosphoric acid (OPA) as the mobile phase. The retention time for pomalidomide was 3.161 minutes, whereas that for dexamethasone was 3.956 minutes. Isocratic chromatography was carried out in around six minutes at room temperature. Using a mathematical technique, we were able to determine that the system's suitability parameters fall within reasonable bounds. Stages with regression coefficients of 0.999 or above were considered in the linear analysis. The drug recovery rate was between 98% - 102%, which is within acceptable range. The method was determined to be appropriate for bulk and formulation analysis, and the validation findings were positive. In accordance with ICH recommendations, the suggested action is justified.*

Key words: *Pomalidomide, Dexamethasone, Development, Validation, RP-HPLC, Stability studies.*

1. INTRODUCTION

Pomalidomide (INN; sold as Pomalyst in the United States and Imnovid in the European Union and Russia) is a Celgene-marketed thalidomide derivative (Piechotta et al., 2019; Vargesson, 2015). It has anti-angiogenic properties (Elice & Rodeghiero, 2012; Kimura et al., 2001), serves as an immune modulator (Rizk et al., 2020; Fuge et al., 2015), and has been used to treat relapsed (Breese et al., 2011) and refractory multiple myeloma (Rajkumar & Vincent, 2019; Willrich et al., 2018). It is authorised for use in persons who have undergone at least two previous treatments, including lenalidomide and bortezomib, and have shown disease progression on or within 60 days after the last therapy's finish. Pomalidomide suppresses angiogenesis and myeloma cell proliferation directly. This dual action is critical to its effectiveness in myeloma rather than other mechanisms such as TNF alpha inhibition (Kalinowska et al., 2020; Kemanetzoglou & Andreadou, 2017), as shown with rolipram (Krause et al., 1990) and pentoxifylline (Salhiyyah et al., 2015; Broderick et al., 2020). Experimental

evidence suggests that pomalidomide augments gamma interferon, IL-2, and IL-10 while suppressing IL-6. Changes like these may improve pomalidomide's anti-angiogenic and anti-myeloma effects.

Dexamethasone is a glucocorticoid (Banuelos et al., 2016; Gelber, 2017) medication that is used to treat rheumatic problems (Conigliaro et al., 2019; Peres et al., 2017), a variety of skin diseases (Alfageme et al., 2015), severe allergies, asthma (May & Dolen, 2017), chronic obstructive lung disease (Herath et al., 2018), croup (Baiu & Melendez, 2019; Marchessault, 2001), brain swelling (Leinonen et al., 2018; Koenig, 2018), eye pain [after eye surgery, superior vena cava syndrome (Kent & Port, 2007) (a complication of some forms of cancer), and tuberculosis (Grace et al., 2019). It may be used in conjunction with a mineralocorticoid drug such as fludrocortisone (Walker & Chang, 2014) to treat adrenocortical (Lodish, 2017) insufficiency. It may be used to enhance infant outcomes in premature labour. It can be administered orally, intravenously, as a topical cream or ointment for the skin, or as a topical ophthalmic solution for the eyes. Long-term dexamethasone usage can cause thrush, bone loss, cataracts, easy bruising (Kinnaman et al., 2015), and muscular weakening. Dexamethasone possesses anti-inflammatory (Alemi et al., 2012) and immunosuppressive (Gillett & Chan, 2000) properties. Chemical structures of Pomalidomide and Dexamethasone were shown in Figure 1.

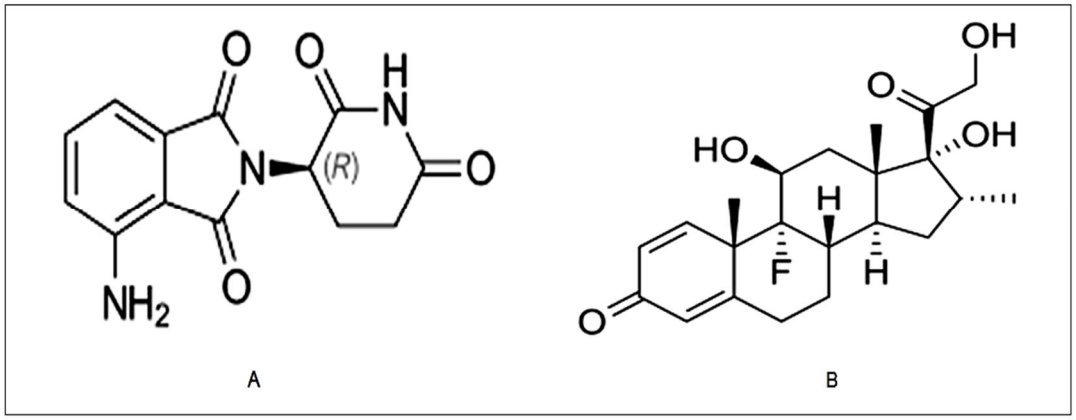

Figure 1: Chemical structures of (A) Pomalidomide and (B) Dexamethasone

Pomalidomide and dexamethasone simultaneous quantification using HPLC techniques does not exist at this time. The purpose of this research is to use RP-HPLC to make predictions about the medicinal ingredients Pomalidomide and Dexamethasone.

2. EXPERIMENTAL STUDY

Chemicals and Reagents

Acetonitrile (HPLC grade), Ortho phosphoric acid (OPA) (purity-99.9 percent), and water were acquired from Merck (India) Ltd., Mumbai, India (HPLC grade). APIs (purity-99.9%) of Pomalidomide and Dexamethasone were given by Glenmark Pharmaceutical Private Ltd., Andheri (E), Mumbai, India.

Equipment

We used a quaternary pump and a PDA detector in conjunction with an e-2695 chromatographic system. The chromatographic information was analysed using Empower software version 2.0.

Chromatographic Conditions

Conditions for Chromatography An inertsil ODS (150 mm x 4.6 mm, 3.5µ) column was used for the separation, and it was carried out in isocratic mode at room temperature. In this study, the mobile phase consisted of a mixture of acetonitrile and orthophosphoric acid (50:50 v/v) flowing at a rate of 1 mL/min. It was determined that 246 nm was the optimal injection wavelength for both Pomalidomide and Dexamethasone, and a volume of 10 µL was used for the injection. Therefore, 246 nm was chosen as the appropriate wavelength.

Preparation of standard solution

Working standards of Pomalidomide and Dexamethasone, each at 50 mg, were added to a 100 mL flask and diluted with the diluent to the desired amount. A final volume of 50 mL should be achieved by diluting 5 mL of the produced solution using diluents.

3. RESULTS AND DISCUSSION

Method validation

After adjusting the parameters of the chromatographic run for specificity, plate count, tailing factor, and retention time, we found that the best isocratic condition for eluting Pomalidomide and Dexamethasone was with an Inertsil ODS Column and a mobile phase of 0.1% OPA and Acetonitrile in a ratio of 50:50. If the proportion of mobile phase utilised was high, the resultant chromatogram will show background noise or peaks that indicate the tailing phenomenon. The chromatographic settings used in the procedure are shown in Table 1.

Table 1: Optimized chromatographic conditions

Parameter	Proposed method
Stationary Phase	Inertsil ODS (150 x 4.6 mm, 3.5 µ)
Mobile Phase	0.1% OPA : Acetonitrile (50:50)
Injection Volume	10 µl
Flow Rate	1.0 mL/min
Column Temperature	Ambient
Wave Length	246 nm
Run Time	6.0 min
Retention time of Pomalidomide	3.161 min
Retention time of Dexomethasone	3.956 min

Specificity

Prior to the retention of the molecules for the allotted duration, Pomalidomide and Dexamethasone were not covered. Figure 2 depicts a blank chromatogram.

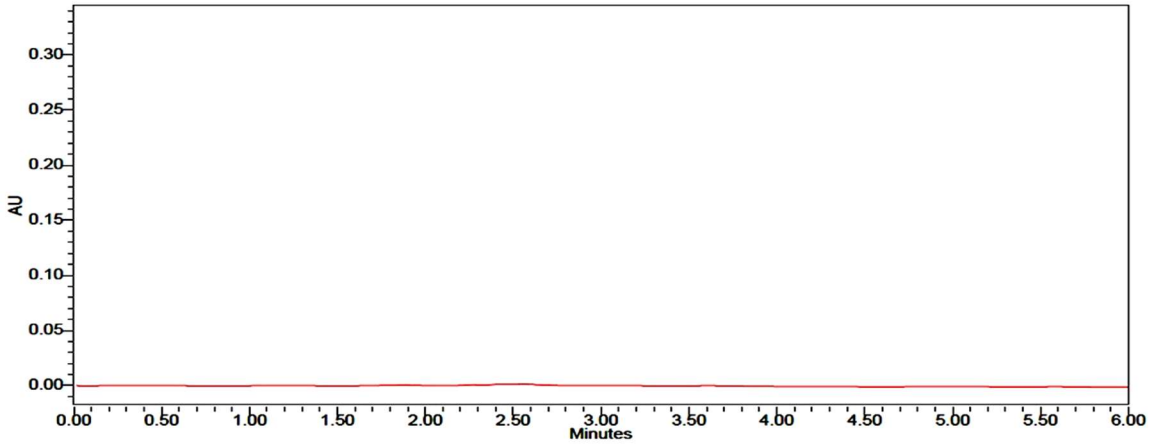

Figure 2: Chromatogram of blank

System suitability

A 60-minute period of stabilisation was carried out to establish a stable baseline. Six injections of Pomalidomide and Dexamethasone each containing 50 µg/ml, were administered to test the system's compatibility. After accounting for tailing, the theoretical plate counts for Pomalidomide and Dexamethasone were calculated to be 3985 and 4288 respectively and the tailing factors were 1.23 and 1.28. It was decided that these values were suitable. chromatographic software will be used to collect all of the data (Empower 2.0). Figure 3 shows a standard chromatogram, and Table 2 details the system accuracy achieved.

Table 2: Results of system suitability

Parameter	Pomalidomide	Dexomethasone
Theoretical plate count	3985	4288
Tailing factor	1.23	1.28
Resolution	-	3.29
Retention time	3.161 min	3.956 min

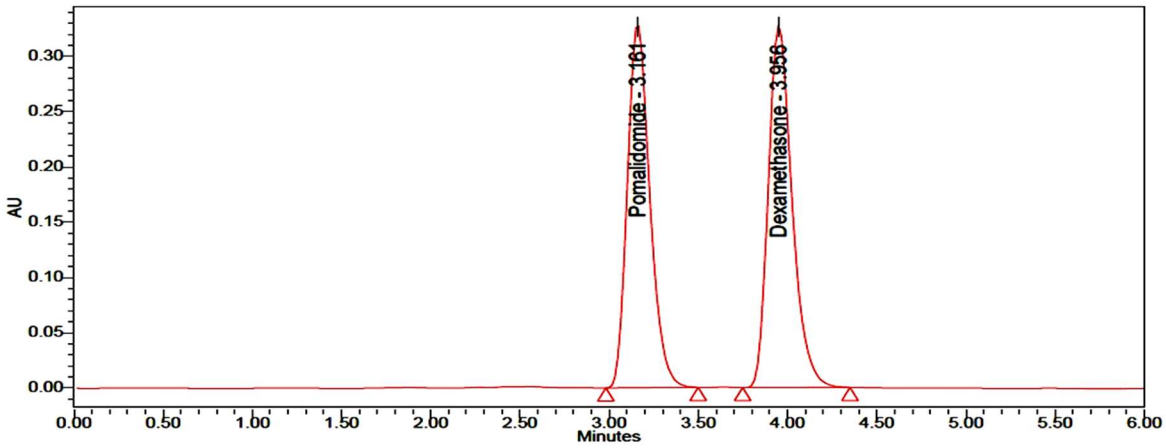

Figure 3: Chromatogram of standard

Linearity

To evaluate the linearity of the strategy, we made a reference solution comprising Pomalidomide and Dexamethasone at 50 µg/mL each (100 percent of the targeted level of the assay concentration). This required a series of dilutions of the provided solutions from 25% down to 50% down to 75% down to 100% down to 125% down to 150% of the desired concentration. Because the peaks were artificially inflated, they may be used to plot calibration curves over the actual data. This pair of analytes was found to have a correlation value of 0.999. Calibration plots for Pomalidomide and Dexamethasone are presented in Figure 4, and Table 3 presents the results of the linearity testing. A linearity calculation sheet was used to get the values for slope, intercept, and correlation coefficient.

Table 3: Results of linearity

S. No	Pomalidomide		Dexamethasone	
	Concentration (µg/mL)	Area	Concentration (µg/mL)	Area
1	12.50	730457	12.50	771304
2	25.00	1460914	25.00	1542608
3	37.50	2191371	37.50	2313912
4	50.00	2921829	50.00	3085217
5	62.50	3552286	62.50	3756521
6	75.00	4382743	75.00	4607825
Slope	7142.64		10714.07	
Intercept	57865.15		60961.48	
Correl Coeff	0.9998		0.9998	

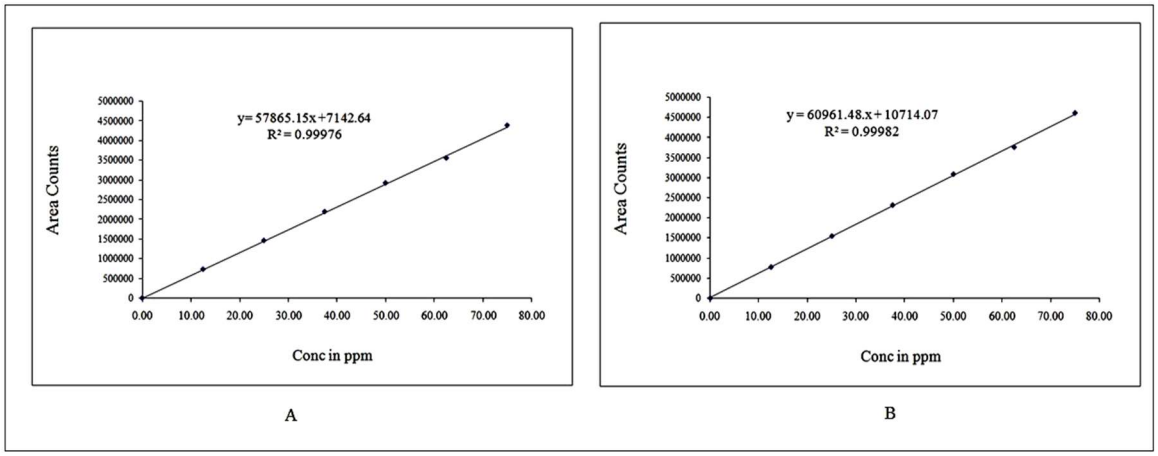

Figure 4: Calibration plots of (A) Pomalidomide and (B) Dexamethasone

Limit of detection and quantification

The limit of detection and quantification is the lowest concentration at which an analyte can be identified and measured accurately. The lowest detectable doses (LODs) of pomalidomide and dexamethasone were respectively 0.15 µg/ml and 0.15 µg/ml, with corresponding S/N values of 3, 3 respectively. The LOQ concentrations of Pomalidomide and Dexamethasone were 0.5 µg/ml, 0.5 µg/ml and S/N values of 10 and 10 respectively. Here S/N is the signal-to-noise ratio.

Precision

To gauge the accuracy of the procedure, six replicates of the same batch were made. Maximum responses from each of these six independent standards were injected with a solution, so we can compute their mean and % RSD values. A relative standard deviation (RSD) of less than 2% was obtained using this approach, demonstrating its precision. Results, including chromatograms and table 4 for the accuracy of the procedure, are shown in table 4. (Figure 5).

Table 4: Results of method precision

S. No.	Area of Pomalidomide	Area of Dexamethasone
1	2990284	3035648
2	2970877	3075480
3	2985782	3044571
4	2975879	3086427
5	2981136	3096427
6	2965435	3023487
Mean	2978232	3060340
Std. dev	9317.53	29760.79
% RSD	0.313	0.972

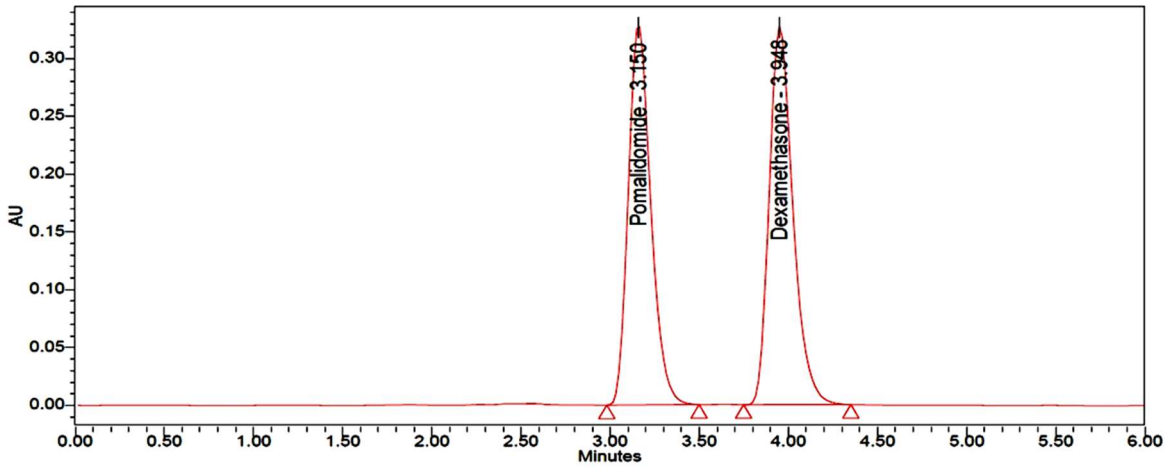

Figure 5: Chromatogram of sample

Accuracy

Recoverability tests in three different concentrations confirmed the treatment's efficacy (50 percent, 100 percent and 150 percent). API was concentrated to 25, 50, and 75 µg/ml. The assay was carried out by injecting the solution into three solutions of increasing concentration, as per the prescribed test procedure. Values for recovery ranged from 98.3 to 100.6 percent for Pomalidomide and from 98.0 to 101.3 percent for Dexamethasone. The results for accuracy are shown in Table 5.

Table 5: Results of accuracy

Accuracy	Pomalidomide		Dexamethasone	
	Amount	% Recovery	Amount	% Recovery
50*	25	99.5	25	99.3
100*	50	99.3	50	99.0
150*	75	99.2	75	99.2

* Results are mean recovery of three sample preparations

Ruggedness

In order to determine whether there was a discernible difference in the chromatographic patterns, effects of changing the HPLC technique, observer and column. The fact that the RSD rate was less than 2% is a testament to the reliability of the time-tested procedure. Table 6 shows the results of the ruggedness tests.

Table 6: Results of intermediate precision

S.No.	Area of Pomalidomide	% Relative standard Deviation	Area of Dexamethasone	% Relative standard Deviation
1	2971782	0.55	3068921	0.96
2	2985955		3099357	
3	2996490		3056870	
4	2962187		3070945	
5	2982458		3037874	
6	2952547		3013895	

Robustness

Tests conducted by RSD showed that just 2% of RSD were attracted by the strategy despite its apparent durability. Modest adjustments were made to the flow rate (±0.1 mL/min) and the organic concentration of the mobile phase (±10%) before injection. Table 7 shows the findings of the robustness analysis.

Table 7: Results of robustness

Parameter	% RSD of Pomalidomide	% RSD of Dexamethasone
Flow (0.9 mL/min)	0.51	1.32
Flow (1.1 mL/min)	0.50	0.80
Organic phase (45:55)	0.76	0.95
Organic phase (55:45)	0.47	1.10

Stability

Stability procedures were analysed on both the control and test solutions from the first to the 24th hour of storage at RT and 2-8°C. The assay percentage at the time of the first injection was roughly 3% lower than that made 24 hours later. Injections were administered at varying intervals. Pomalidomide and dexamethasone are unaffected by storage conditions. In Tables 8 and 9, we can see the results of our stability tests.

Table 8: Stability results at RT

Time intervals	Pomalidomide		Dexamethasone	
	% assay	% Deviation	% assay	% Deviation
Initial	99.9	0.00	99.9	0.00
6 Hrs	99.5	-0.40	99.3	-0.60
12 Hrs	99.2	-0.70	99	-0.90
18 Hrs	98.8	-1.10	98.6	-1.30
24 Hrs	98.5	-1.40	98	-1.90

Table 9: Stability results at 2-8°C

Time intervals	Pomalidomide		Dexamethasone	
	% assay	% Deviation	% assay	% Deviation
Initial	99.9	0.00	99.9	0.00
6 Hrs	99.8	-0.10	99.2	-0.70
12 Hrs	99.4	-0.50	98.7	-1.20
18 Hrs	98.8	-1.10	98	-1.90
24 Hrs	98	-1.90	97.7	-2.20

Forced degradation

This suggested method is an advancement over existing methods since it permits simultaneous release and stability tests. Forced degradation studies, as specified by the ICH rules, include acid, base, oxidation, reduction and heat degradation. Degraded peaks are depending on the chromatographic technique used, although it seems that the drugs under evaluation were stable. Outcomes of forced degradation (Table 10).

Acid degradation: In a volumetric flask with a 10-milliliter capacity, 1 millilitre of the standard stock solution was added to 1 millilitre of 1N HCl and the mixture was let to stand for 15 minutes. After 15 minutes, dilute to the appropriate concentration by adding 1 millilitre of 1N sodium hydroxide. Inject the solution, after having filtered it with a syringe into a high-performance liquid chromatography (HPLC) apparatus.

Alkali degradation: In a volumetric flask with a 10-milliliter capacity, 1 millilitre of the standard stock solution was transferred, 1 millilitre of 1N NaOH was added and the combination was allowed to stand for 15 minutes. Once the 15 minutes have passed, add 1 mL of 1N HCl to get the concentration up to where it needs to be. This solution will be injected into an HPLC system after being filtered using a syringe filter.

Peroxide degradation: In a 10-milliliter volumetric flask, 1 millilitre of standard stock solution was transferred, and then 1 millilitre of 30% hydrogen peroxide solution was added and the volume was adjusted accordingly. Filter the solution using syringe filter and injected into HPLC system.

Reduction degradation: Place 1 mL of standard stock solution and 1 mL of 30% hydrogen peroxide solution in a 10-mL volumetric flask, mix, and dilute to the desired concentration. This solution will be injected into an HPLC system after being filtered using a syringe filter.

Thermal degradation: The temperature of the reference solution was maintained at 105°C during the 6-hour heating period. An HPLC system was then used to analyse the solution.

Hydrolysis degradation: To prepare the working solution, 1 mL of standard stock solution was added to 1 mL of HPLC water in a 10-mL volumetric flask and the remaining

volume was adjusted using diluents to get the desired concentration. This solution will be injected into an HPLC system after being filtered using a syringe filter.

Table 10: Results of forced degradation

Stress Parameter	% of Degradation Pomalidomide	% of Degradation Dexamethasone
Acid degradation (1N HCl)	13.7	12.1
Alkali degradation (1N NaOH)	14.2	13.8
Peroxide degradation (30% Peroxide)	15.7	15.5
Reduction degradation (30% sodium bi sulphate)	11.5	12.4
Thermal (sample, 70°C, 6 Hrs)	9.4	2.4
Hydrolysis (1 ml HPLC water)	1.7	1.9

CONCLUSION

Table 11: Summary and conclusion for RP-HPLC method

Parameter	Limit	Result		Conclusion
		Pomalidomide	Dexamethasone	
Linearity	R^2>0.999	0.99976	0.99982	Method was linear
System Precision	RSD<2	0.12	0.96	Method was repeatable
LOD	-	0.15 µg/mL	0.15 µg/mL	-
LOQ	-	0.5 µg/mL	0.5 µg/mL	-
Method Precision	RSD<2	0.31	0.97	Method was precise
Ruggedness	RSD<2	0.55	0.96	Method was precise
Accuracy	% Recovery 98-102%	99.2-99.5%	99.0-99.3%	Method was accurate
Robustness	RSD<2	0.47-0.76	0.8-1.32	Method was robust

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