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Development And Validation Of Rp-Hplc Method For Estimation Of Apremilast In Bulk And Pharmaceutical Dosage Form

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ABSTRACT

In this study, a straightforward, reliable, precise, and stable RP-HPLC method for estimating Apremilast in the tablet dosage form. Among the method's many benefits are its straightforward and mobile phase, inexpensive solvents, quick analysis. The method uses a Zodiac C18 (150 x 4.6mm) 5µ column with a mobile phase of Water: Acetonitrile: OPA (buffer) in the ratio of (25:75:0.2 v/v) flow rate of 1.0 ml/min Detection at 230 nm, with a sharp peak at 3.60 minutes for Apremilast work. The method exhibits good linearity (r2 = 0.9999) over a concentration range of 2.50-7.50 µg/ml. The % RSD values for method precision and intermediate precision studies were found to be less than 2%. The % recovery was found to be within an acceptable limit 98%-102%.Thus, the created method was described as robust, accurate, exact, and linear. Because the process eliminates the need for costly reagents and also It takes less time and can be used frequently in the business for a standard analysis of the marketed Apremilast tablet dosage form.

Keywords: *Reverse Phase High performance liquid chromatography (RP-HPLC), Apremilast, Validation, Mobile phase, Method Development, Orthophosphoric acid, Acetonitrile*

1. INTRODUCTION

The novel therapeutic medication Apremilast (APL) has been approved for the treatment of psoriasis and psoriasis arthritis . APL is a medication that is taken orally and works by blocking intracellularly inhibited phosphodiesterase 4. It causes inflammation by reducing the amount of cAMP; essential for the synthesis of mediators that promote and inhibit inflammation [(1).] Psoriasis and a few other dermatological conditions have responded well to APREMILAST treatment [(2)] because of its safety record and ability to modulate numerous cells. RCT data was used to report a case study. Loss of hair areata, atopic dermatitis, hidradenitis suppurativa, psoriasis of the nails and scalp, and palmoplantar psoriasis. Rosacea, lichen planus, discoid lupus erythematosus, and cutaneous sarcoidosis were among the open-label study case studies that were published, exhibiting both positive and negative results. Vitiligo, generalized pustular psoriasis, Hailey-Hailey illness, and pityriasis rubra pilaris have all been documented in a number of case studies [(3-4)].

The chemical name of apremilast is N-{2-[(1S)-1-(3-Ethoxy-4-methoxyphenyl)-2- (methylsulfonyl) ethyl]-1, 3-dioxo-2, 3-dihydro-1H-isoindol -4-yl} acetamide. It is an authorized medication by the Food and Drug Administration that is used to treat psoriatic arthritis and psoriasis. For other inflammatory illnesses linked to the immune system, it might also be helpful[(5)] mol. Formula C22H24N2O7S and Mol. Wt. 460.5 g/mol [(6)] High Performance Liquid Chromatography (HPLC), High Performance Thin Layer Chromatography (HPTLC), Liquid Chromatography-Mass Spectrometry (LC-MS), and other techniques are helpful for the analytical process while analyzing these big or small substances. Usually, mass spectrometry and the other methods indicated are employed to detect substances using these analytical techniques. High performance liquid chromatography (HPLC), a crucial and improved method for drug analysis, is a very helpful technique [(7-8).]

Chromatographic techniques have surpassed traditional analysis methods because of their higher precision. High sensitivity to even trace amounts of degradation products generated outside of component separation. Numerous chromatographic techniques have been employed, including thin-layer chromatography (TLC), high performance thin-layer chromatography

(HPTLC), gas chromatography (GC), and high-performance liquid chromatography (HPLC) and newer technique like ultra performance liquid chromatography (UPLC). High Performance Liquid Chromatography (HPLC) [9-13] is a method of analysis that is widely used to separate and identify organic and inorganic solutes in any sample, particularly those that are industrial, environmental, food, medicinal, biological, etc. It has becoming more often used to determine stability studies of polar/ionic, thermally unstable, or non-volatile chemicals because has great sensitivity, specificity, and resolution capabilities. Given the previously mentioned data and the literature review, a unique RP-HPLC technique has been created and approved for the estimation of Apremilast in formulation and in bulk [14-16].

Chemical structure of Apremilast:

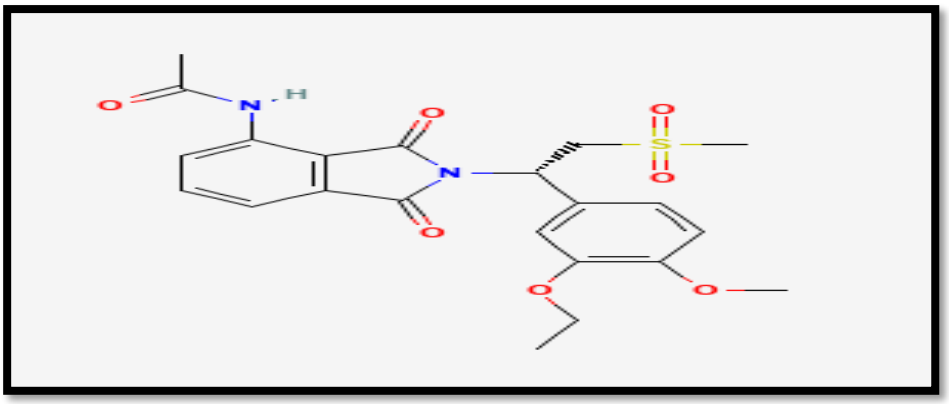

Figure 1: structure of apremilast

2. MATERIALS AND METHODS

Materials And Reagents:

Apremilast was supplied by Vidisha Analytical in the Indian state of Maharashtra. Each tablet, produced by Glenmark Pharmaceuticals Ltd., contains 30 mg of apremilast. It was purchased from a nearby drugstore. Methanol, water, acetonitrile, and orthophosphoric acid were all HPLC-grade reagents employed in the current study. HPLC analysis was performed using the pharmaceutical formulation Apremilast (label claim includes 30 mg). Rankem's HPLC-grade water was used in the HPLC study.

Instrumentation And Software:

An Agilent 1260 Infinity II HPLC system equipped with an autosampler and a UV detector was used as the chromatographic system. Chromatograms were recorded using the Open Lab EZ Chrome Workstation on a Windows-based computer for data collection and processing. Apremilast concentrations were measured using Zodiac C18 (150 x 4.6mm) 5µm.

Experimental Work:

Chromatography

After several trials with the different combination and ratio of solvents, the mobile phase Water: Acetonitrile: OPA (25:75:0.2 v/v)and Stationary phase Zodiac C18 (150 x 4.6mm) 5µ was used for the analysis at a flow rate of 1 ml/min, Column temperature30°C, injection volume of 10 µL, run time of 6 mins and detection wavelength of 230 nm and Retention time (Rt) 3.60 min for Apremilast.

Preparation of mobile phase

Prepare mixture of Water, Acetonitrile and Orthophosphoric acid in the ratio of 25:75:0.2 v/v respectively, mix well. Filter through 0.45µ nylon membrane disc filter. Sonicated for 15 min to degas the mobile phase.

Preparation of Standard solution

Weighed and transferred accurately about 50 mg of Apremilast working standard into 100 mL clean and dry volumetric flask. Added about 80 mL of diluent, sonicate to about 30 minutes to dissolve and dilute up to the mark with diluent and mix. Further dilute above stock 3.0 mL of this stock solution to 50 mL with diluent and mix well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.

(Concentration of Apremilast standard solution: 30 ppm)

Preparation of sample solution

Weighed and transferred 2 Apremilast tablets in to 200 mL clean and dry volumetric flask. Added about 150 mL of diluent, sonicate for 60 minutes with intermittent shaking, at control room temperature and make volume up to mark with diluent and mix. Further diluted above stock solution 5.0 mL of this sample stock solution to 50 mL volumetric flask make up with Diluent and mixed well. Filter the sample solution through 0.45µ membrane PVDF filter. Discard first 4.0 mL of filtrate and then collected the sample.

(Concentration of Sample Solution: 30 ppm)

Selection of analytical wavelength

UV-visible 2487 detector was selected, as it is reliable and easy to set at the correct wavelength and 230 nm wavelengths was selected as detection wavelength

3. RESULT AND DISCUSSION

Table 1: Final reversed phase High performance liquid Chromatographic Conditions

Column	Zodiac C18 (150 x 4.6mm) 5µ
Mobile Phase	Water: Acetonitrile: OPA (25:75:0.2 v/v)
Flow Rate	1 mL/min
Injection Volume	10 µL
Wavelength	230 nm
Column Temp.	30°C
Auto sampler Temp.	25°C
Run time	6.0 min.
Needle wash	Water : Methanol (90:10 v/v)
Seal wash	Water : Methanol (10:90 v/v)

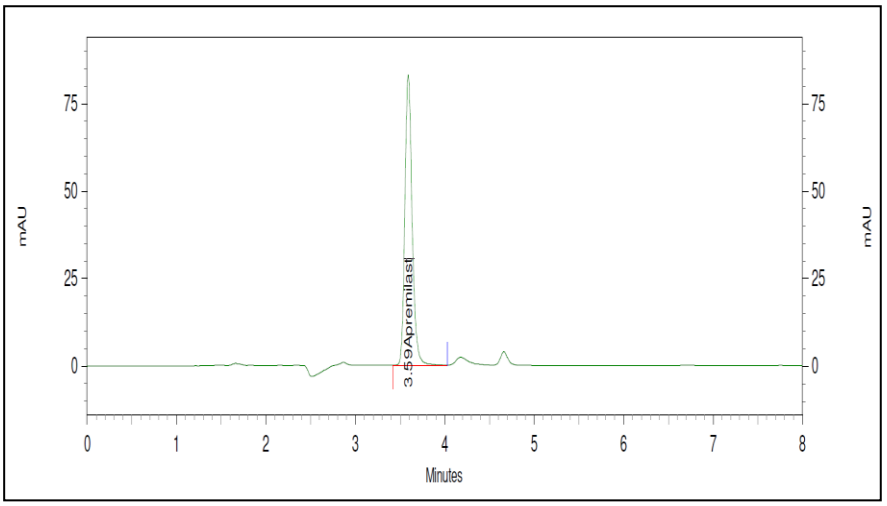

Figure 2 : Typical chromatogram for apremilast

Observation: Apremilast eluted at 3.60 minutes with acceptable chromatography (Asymmetry: 1.17 and Theoretical plates 9885)

Conclusion: Method can be used for further analysis and will be subjected for validation.

Method Development

The proposed chromatographic method was found to be suitable for effective separation of Apremilast with good resolution, peak shape given in the figure. The mobile phase composed of Methanol: water in ratio of Water: Acetonitrile: OPA (25:75:0.2 v/v), at a flow rate of 1.0 ml/min was selected as it gave well resolved peaks of standard Apremilast.

Analyatical Method Validation of RP-HPLC

The established method for estimating Apremilast for the following parameters was validated using ICH Q2(R1) recommendations. Specificity, linearity, accuracy, precision, and robustness were among these criteria.

System Suitability :

System suitability is the evaluation of the components of an analytical system to show that the performance of a system meets the standard required by a method. System suitability study was performed before each validation run. Area, Retention time (RT), Tailing factor and Theoretical plates were determined. Tailing factor for the Apremilast in standard solution should not be more than 2.0. Theoretical plates for the Apremilast peaks in standard solution should not be less than 2000.

Table 2: System suitability data for Apremilast

Parameter	Apremilast	Acceptance criteria
Retention Time	3.60	±10
Theoretical Plate	9742	>2000
Tailing Factor	1.22	< 2.00
% RSD	0.04	< 2.00

Linearity:

The calibration curves were found to be linear for the concentration range of 2.50-7.50 µg/mL. The standard working curve equation of drug was found to be y = 257215x + 83413 with correlation coefficient value r^2 = = 0.9999. The results of linearity are given in table and figure.

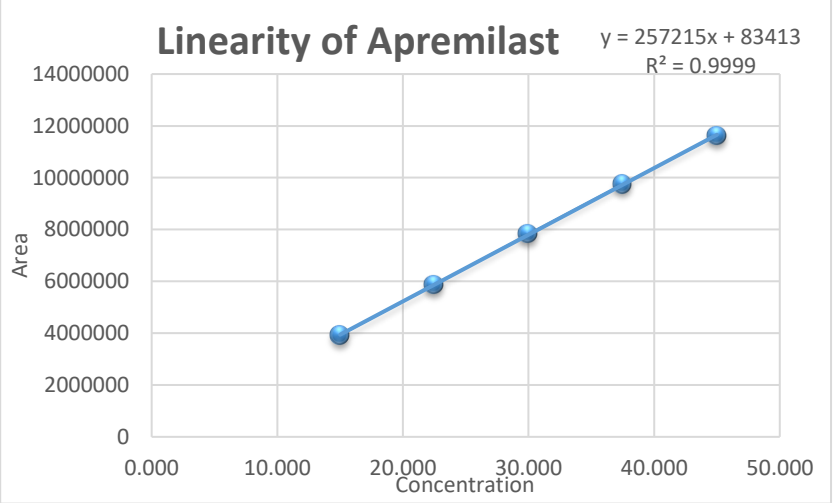

Figure 3: Linearity curve of standard Apremilast

Table 3: Linearity Data of Apremilast

Level	Conc (µg/mL)	Area	Mean
50%	2.50	3918307	3921287
		3925410	
		3920143	

75%	3.75	5873105	5871162
		5870498	
		5869883	
100%	5.00	7827989	7827825
		7825964	
		7829522	
125%	6.25	9752791	9753204
		9756558	
		9750264	
150%	7.50	11622664	11625826
		11615232	
		11639581	
Corr. Coeff			0.9999
Intercept			83413
Slope			257215
% Y-intercept			1.07

Recovery Studies:

Accuracy was determined from recovery studies. The mean % recovery at 50, 100, 150 % of the test concentration along with its statistical validation for drug Apremilast given in table. The % recovery at 50, 100, 150 % is given below. It was confirmed that the developed method was accurate as the percent recovery was in the range of 100 %.

Table 4: Recovery data of Apremilast

Level (%)	Apremilast Added Conc (µg/mL)	Apremilast Recovered conc	Area	% Recovery	Mean % Recovery
50	2.53	2.53	3960961	100.202	99.35
	2.60	2.56	4012593	98.58	
	2.58	2.56	4001529	99.263	
100	5.03	5.09	7969521	101.306	100.34
	4.98	5.00	7830529	100.539	
	5.00	4.96	7763943	99.186	
150	7.53	7.43	11629563	98.718	99.35
	7.48	7.50	11746529	100.377	
	7.55	7.47	11696410	98.956	

Method Precision:

Single injection of blank (Diluent), Standard solution (six replicates) and sample solution (six preparations) was injected on the system. The % assay, average and % RSD was calculated and tabulated in the Table.

Table 5: Method Precision of Apremilast

Sample	Area	% Assay
Sample 1	7750126	99.01
Sample 2	7659864	97.95
Sample 3	7736529	99.02
Sample 4	7602593	96.94
Sample 5	7712423	98.95
Sample 6	7686415	98.43
Mean		**98.38**
STD DEV		**0.8247**
% RSD		**0.838**

Intermediate precision:

six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Table 6: Intermediate Precision of Apremilast

Sample	Area	% Assay
Sample 1	7636525	97.88
Sample 2	7752419	98.85
Sample 3	7642109	97.30
Sample 4	7762593	99.17
Sample 5	7805634	99.77
Sample 6	7956371	101.40
Mean		**99.06**
STD DEV		**1.4494**
% RSD		**1.463**

Specificity: (Identification, Interference & Peak Purity)

Inject Blank (Diluent), standard solution, placebo solution and sample solution. The data obtained is summarized in Table .

Table 7: Specificity (Identification and Interference

Solution	Specificity data		
	Retention time (min)	Purity Match	
Blank solution	NA	NA	
Placebo solution	NA	NA	
Standard solution	3.59	Purity angle	Purity threshold
		1.64	3.33
Sample solution	3.59	1.40	2.87

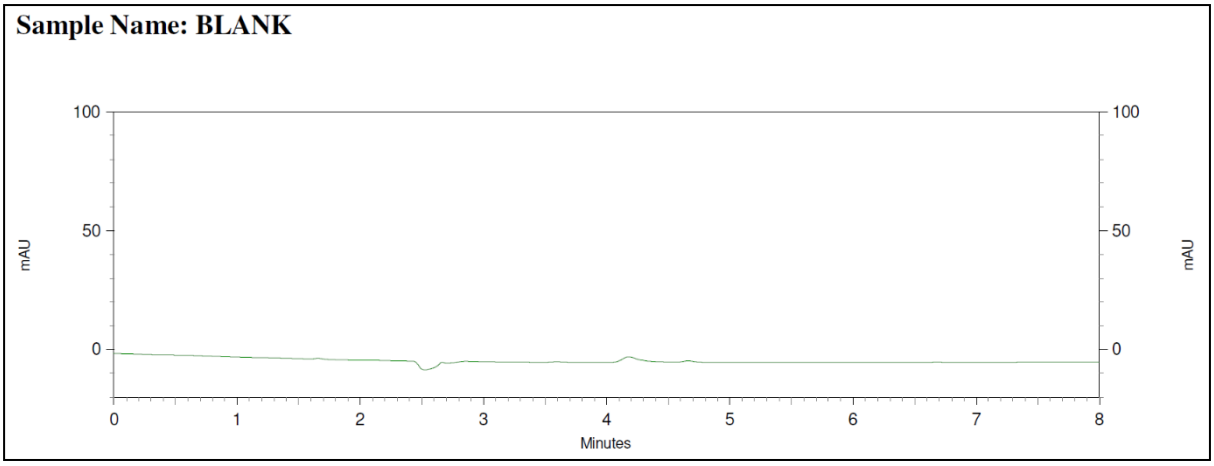

Figure 4 : Chromatogram of Blank

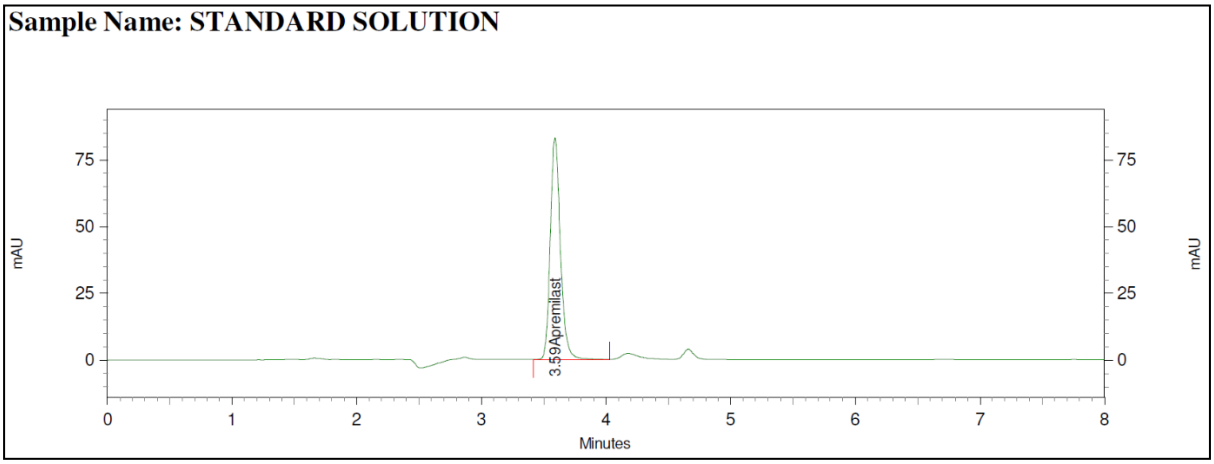

Figure 5: Chromatogram of Standard

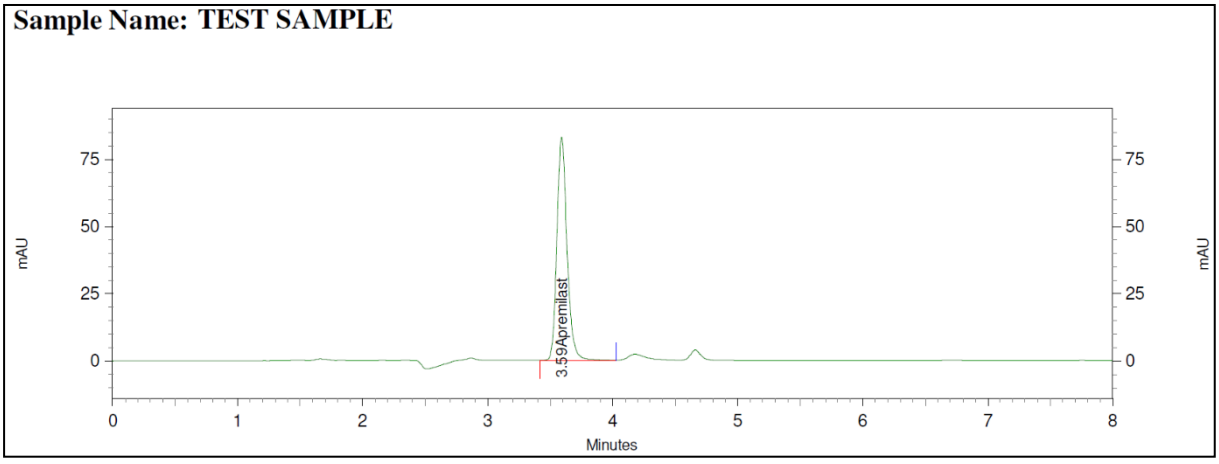

Figure 6: Chromatogram of Sample

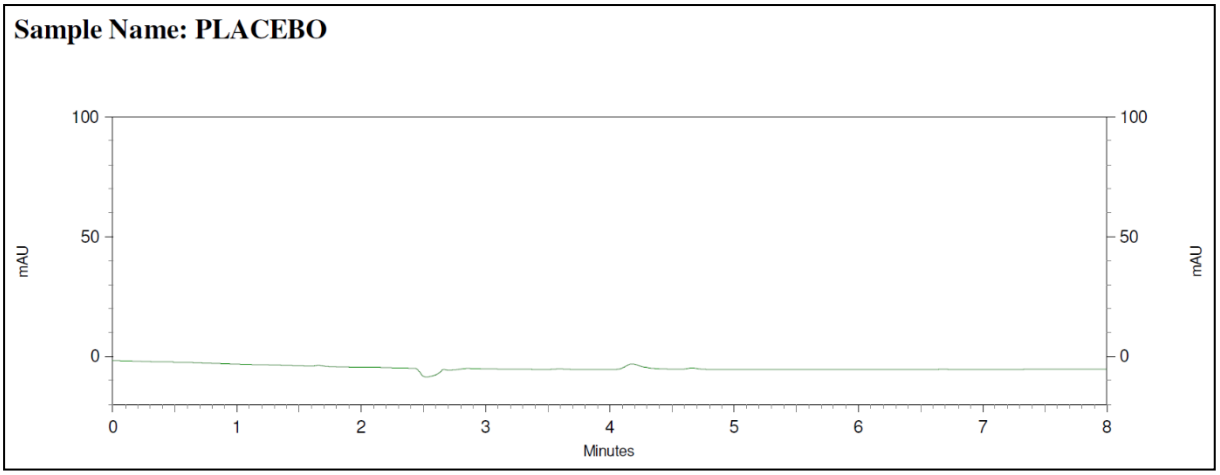

Figure 7: Chromatogram of Placebo

Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

Table8: Robustness for Apremilast

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	7750126	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.1 ml/min	7820182	0.9
	0.9 ml/min	7693883	-0.7
Change in wavelength (±2 nm)	232 nm	7598578	-1.9
	228 nm	7702288	0.2

Conclusion:

The method shows good reproducibility; moreover the RP-HPLC method is accurate, precise, specific, reproducible and sensitive. The analysis of single dose formulation of Apremilast bulk and tablet can also be successfully performed by the RP-HPLC method. The analysis time can be reduced due to less retention time and also cost-effective mobile phase is useful for analysis.

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