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Method Development and Validation for Apremilast by RP-HPLC

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ABSTRACT

The present work involves the development of simple, accurate, precise and stable RP- HPLC method for the estimation of Apremilast in the tablet dosage form. The method has several advantages, including simple and mobile phase, low cost solvents, rapid analysis, and simple sample preparation. In developed method, the analyte was resolved by using isocratic method and mobile phase was used methanol: acetonitrile: water in proportion of (35v:38v:27v), at a flow rate 1.0 ml/min, the detection was carried out at 230 nm. The results of analysis in the method were validated in terms of accuracy, precision, linearity, robustness. Linearity for Apremilast was found in the linear concentration range of 1-6µg/ml with regression coefficient r2 = 0.9998. The % RSD values for intra-day and inter-day precision studies were found to be less than 2%. The % recovery was found to be within an acceptable limit 98%-102%. Therefore the developed method said to be linear, precise, accurate, and robust. Since the method does not require use of expensive reagent and also less time consuming, it can be performed routinely in industry for a routine analysis of marketed product of Apremilast in tablet dosage form.

Key-words: Apremilast, HPLC, validation, Method Development

I. INTRODUCTION

Apremilast, also known as Otezla, is a phosphodiesterase 4 (PDE4) inhibitor used to treat various types of symptoms resulting from certain inflammatory autoimmune diseases. It belongs to the same drug class as Roflumilast and Crisaborole. Initially approved in 2014, it is marketed by Celgene. In July 2019, apremilast was granted a new FDA approval for the treatment of oral ulcers associated with Behcet's disease, an autoimmune condition that causes recurrent skin, blood vessel, and central nervous system inflammation. [1]

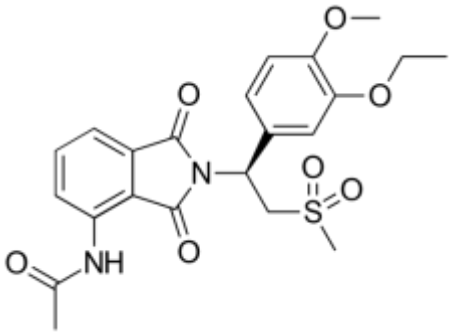

Fig. No. 01: Structure of Apremilast

Chromatographic methods have taken precedence over the conventional methods of analysis due to the advantage of greater accuracy and sensitivity for even small quantities of degradation products produced other than separation of multiple components. Various chromatographic methods that have been used are 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)[2-6] is an analytical technique used extensively for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial, etc. It has gained popularity in determining the stability studies of non-volatile, thermally unstable or polar/ionic compounds due to its high-resolution capacity, sensitivity and specificity. In the view of above fact and as per the literature survey a novel RP-HPLC method had been developed and validated for the estimation Apremilast from bulk and formulation. [7-9]

II. EXPERIMENTAL

HPLC Method Development and Validation[10-14]

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The quantitation of Apremilast from bulk and formulation was carried out by HPLC method. The LC20AD Prominence Liquid Chromatograph SPD20-A Shimadzu, Japan with UV-Vis detector and C18 column with dimension on 25 x 0.6 cm was used for the method development with flow rate 1.0 ml/min at wavelength 230 nm. The methanol: acetonitrile: water in proportion of (35v:38v:27v) as a mobile phase, for development of chromatogram. The method was validation for synthesized compound and various parameters according to ICH guidelines (Q2B) were studied.

Table No.1: Optimized chromatographic condition for RP-HPLC

Chromatographic Conditions	**SHIMADZU HPLC System**
Mobile phase	Methanol: Acetonitrile: Water(35:38:27)
Column	ARP-C18 (250 mm X 4.6 mm), 5µ column
Flow rate	1 ml/min
Wavelength detection	236 nm
Injection volume	20µl
Temperature	Ambient
Retention time	10.5 min
Run time	15 min

Analytical Method Validation

A suitable analytical method was developed and validated for identification. New drug development requires meaningful and reliable analytical data to be produced at various stages of development.

Preparation of Mobile phase

The selection of mobile phase was according to polarity and non-polarity of solvents. The methanol: acetonitrile: water was selected as mobile phase in ratio of 35:38:27 and was filtered on membrane filter (0.45 µ) to remove degassing and were stirred for 10-15 min.

Preparation of sample solution (formulation)

Stock solution of bulk Apremilast, 2 different batches of Apremilast marketed formulation of 100 ppm in 100 ml volumetric flask were prepared. Dissolve 10 mg of test sample in 100 ml diluents. 1ml of this stock was diluted to 10 ml to prepare 10 ppm stock solution. For the tablet formulation 20 tablets from each 2 tablet batch were crushed respectively. The powder of this formulation equivalent to 10 mg of the drug was used to prepare the stock solution. Further dilute to 1 ppm, 2 ppm, and so on, were prepared by taking 0.1 ml, 0.2 ml and so on of standard test solution and diluting it to 10 ml.

Validation experiment was performed to demonstrate system suitability, linearity, precision, accuracy study, ruggedness and robustness as per ICH guidelines.

System Suitability Parameters:

The area of respective concentrations, theoretical plates, number of theoretical plates per height and the peak symmetry was recorded.

Linearity

Dilution of standard impurity in the range of 1-6 µg/ml were prepared by taking suitable aliquots of working standard solution in different 10 ml volumetric flasks and diluting upto the mark with mobile phase. 20 µl was injected from it each time into the column at flow rate of 1 ml/min. The standard from elute was monitored at 236 nm and corresponding chromatogram were obtained from these chromatograms peak area were calculated. A plot of peak area over concentration was constructed. Regression of the plot was computed by least square regression method.

Precision

Precision of analytical method was studied by multiple injections of homogenous samples. 6 replicate of 4 ppm solution were prepared and injected for precision at the same flow rate of 1ml/min. The intra-day and inter-day precision was used to study the variability of the method. SD and RSD were calculated for both.

Accuracy

Accuracy of the method was studied using the method of standard addition. Standard Apremilast solutions were added to the unknown bulk and tablet formulation of Apremilast. The percent recovery was determined at three different levels (50%, 75% and 100%). Impurity content was determined and the percent recovery was calculated.

Robustness

Robustness was studied by changing parameters like change in flow rate. The SD and RSD between the change parameter were calculated.

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LOD and LOQ

Limit of detection and limit of quantitation of the method was calculated by formula given below

LOD= 3.3xSD/Slope

LOQ= 10xSD/Slope

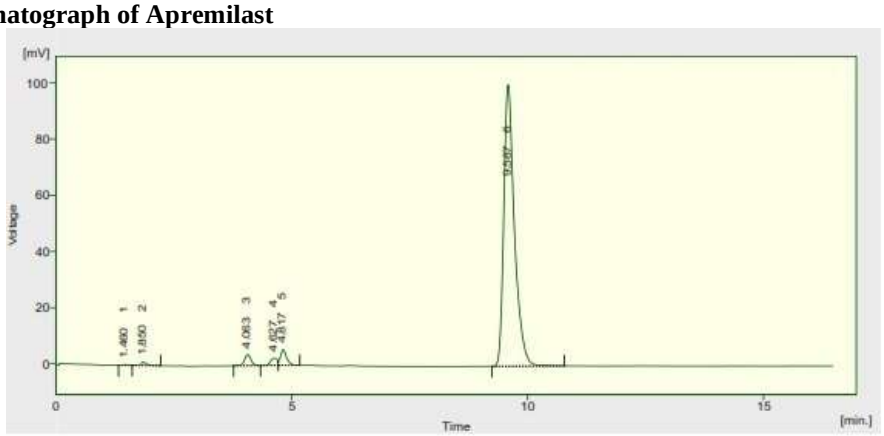
III. RESULT AND DISCUSSION

HPLC Method Development and Validation

The ICH Q2B guidelines discuss the analytical method validation on HPLC. Currently the vast majority of process-related impurity determinations are performed by HPLC. It offered the desired sensitivity for trace level determinations with a high degree of automation. A wide variety of stationary phases and operation modes make HPLC applicable to all drug classes. The typical detection limits for process-related impurities by HPLC are 0.1% or lower and this can be routinely met in the majority of circumstances using conventional UV detectors. These methods involved the prediction of likely impurities within the synthetic process, their isolation and identification by suitable analytical techniques.

The last step of the present study was to develop, validated HPLC method for detection and quantification of Apremilast in bulk and tablet formulations.

HPLC Chromatograph of Apremilast



Result Table

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.460	2.424	0.289	0.1	0.3	0.13
2	1.850	11.705	1.278	0.7	1.1	0.11
3	4.063	37.267	3.919	2.2	3.4	0.15
4	4.627	25.732	2.608	1.5	2.3	0.17
5	4.817	52.839	5.630	3.1	4.9	0.14
6	9.587	1568.718	100.231	92.3	88.0	0.23
	Total	1698.684	113.955	100.0	100.0	

Figure No.2: HPLC Chromatogram of Apremilast

TheRetention time of Apremilast was 9.5 min.

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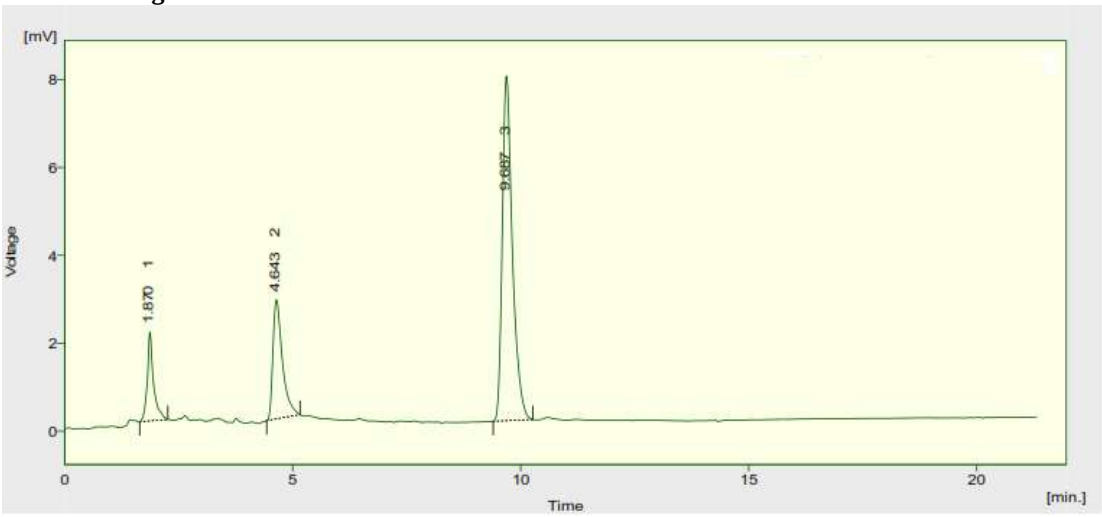
HPCL Chromatogram of Tablet



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.870	19.233	2.024	10.7	16.1	0.12
2	4.643	39.227	2.710	21.9	21.6	0.22
3	9.687	120.869	7.840	67.4	62.4	0.23
	Total	179.330	12.573	100.0	100.0	

Figure No.3: HPLC Chromatogram of Apremilast Tablet formulation

The retention time of Apremilast tablet was found at 9.6 min.

Optimized chromatographic condition

1. Linearity

Table No. 2: Result of Linearity by HPLC (Peak area vs. Conc.)

Sr. No	Concentration (ppm)	Area (mill volts) at 230 nm
1.	1	124.35
2.	2	216.34
3.	3	311.34
4.	4	418.76
5.	5	514.35
6.	6	619.45

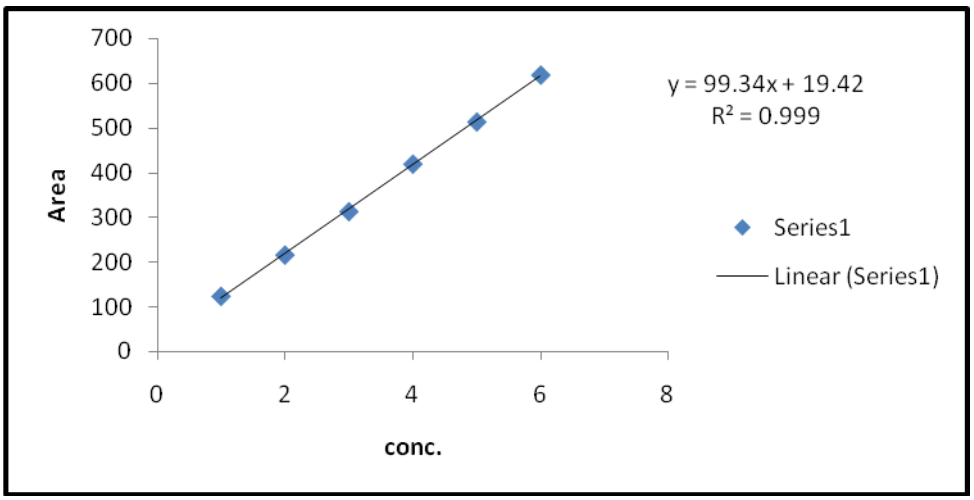


Figure No.4: Graph of linearity of synthesized compound by HPLC

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The linearity of the proposed method was estimated by regression analysis at six concentration levels in the range of 1-6 µg/ml for intermediate. The correlation coefficient (R^2) was found to be 0.999 and intercept Y= 99.34x + 19.42 was linear.

2. Precision

Table No.3: Precision by HPLC

Sr.No	Concentration (ppm)	Peak area (mV)at 236nm	Mean	SD	% RSD
1.	4	419.19	419.89	1.331	0.317
2.	4	418.98			
3.	4	421.90			
4.	4	419.90			
5.	4	418.39			
6.	4	421.01			

The precision of the intermediate was quantified for repeated concentration of 4 µg/ml in range and was reliable with their area of chromatogram as shown in above table. The Standard deviation (SD) and Relative standard deviation (RSD) was found to be 1.331 and 0.317 respectively.

a. Intraday precision after 4 hours

Table No. 4: Result of Intraday precision after 4 hours

Sr. No	Conc. (ppm)	Peak area after 4 hour at 230 nm	Mean	S.D	%RSD
1.	4	417.68	418.23	2.267	0.545
2.	4	412.35			
3	4	419.45			
4.	4	416.58			
5.	4	414.78			
6.	4	416.78			

b. Interday precision after 24 hours

Table No.5: Intraday precision after 24 hours

Sr. No	Conc. (ppm)	Peak area after 24 hour at 230 nm	Mean	S.D	%RSD
1.	4	424.12	425.36	1.789	0.425
2.	4	423.60			
3.	4	427.66			
4.	4	425.43			
5.	4	424.42			
6.	4	427.54			

The intra and interday precision was carrying out and difference in % RSD was found not much varies and remains less than 2% indicate preciseness of method.

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3.Robustness

Table No. 6:Results of Robustness study by change in flow rate.

At flow rate of 0.8 ml/min

Sr. No	Conc. (ppm)	Peak area (mV) 0.8 ml/min	Mean	S.D	%RSD
1.	4	786.34	789.86	2.897	0.415
2.	4	785.89			
3.	4	794.32			
4.	4	787.89			
5.	4	792.58			
6.	4	790.07			

The robustness of the Intermediate was performed for change in flow rate upto 0.8 ml/min and method was robust with standard deviation 2.897 and relative standard deviation 0.415

Table No.7: Summary of Precision

Sr .No	Parameter	SD	%RSD
1.	Precision	1.331	0.317
2.	Intraday precision	2.267	0.545
3.	Interday precision	1.789	0.425
4.	Robustness	2.897	0.415
5.	Ruggedness	1.436	0.341

The summary of the precision is given in the above table and % RSD was found to be ≤ 2.

5. Accuracy

Table No. 8: Result of recovery study by HPLC

Sr.No.	Drug / Formulation	Percentage recovery			Mean	S.D.	%RSD
		50%	75%	100%			
1.	Bulk	98.18	99.07	99.89	99.04	0.852	0.863
2.	Tablet I	99.22	101.30	103.79	101.43	2.288	2.255
3.	Tablet II	99.25	101.68	103.13	101.30	1.969	1.934

Accuracy study was performed by the recovery method. The results demonstrate that the percentage recovery in tablet was more than bulk due to the presence of impurity in the tablet. Percentage recovery was found to be more at higher concentration level a compare to lower concentration level.

6. Limit of detection

$$LOD = \frac{3.3 \times \text{Standard deviation}}{\text{Slope}}$$

$$LOD = \frac{3.3 \times 1.3313}{98.44}$$

LOD = 0.4462

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7. Limit of quantitation

$$LOQ = \frac{10 \times \text{Standard deviation}}{\text{Slope}}$$

$$LOQ = \frac{10 \times 1.3313}{98.44}$$

LOQ = 0.1352

The LOD by HPLC was 446.2 ng and that of LOQ 135.2 ng the method is more sensitive and selective.

IV. CONCLUSION

The current method developed and validated for the estimation of Apremilast in bulk as well as pharmaceutical tablet. None of the usual excipients employed in the formulation of Apremilast dosage forms interfered in the analysis of Apremilast by the developed method. Validation parameters are found within the limits. It was observed that all the statistical analysis results of % RSD values particularly precision, accuracy are observed below two which speaks that the method is precise and accurate. The results of pharmaceutical formulations assert that the proposed method of Apremilast feasible for their determination without interfering the additives and excipients. Therefore this method was simple, precise, accurate and cost effective and in actual fact feasible for routine sample analysis of Apremilast in bulk and formulations.

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