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Chromatographic Method Development and Validation Assay of Apremilast in Tablet Dosage Form

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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: Water (80:20 v/v), at a flow rate 0.8 ml/min, the detection was carried out at 231nm. 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 10-50 µg/ml with regression coefficient r^2= 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: RP-HPLC, Apremilast, Validation, Chromatographic Method, Tablet Dosage Form

Introduction

Apremilast is a novel, orally available small molecule inhibitor of type-4 cyclic nucleotide phosphodiesterase (PDE-4).[1,2] PDE-4 is a cyclic adenosine monophosphate (cAMP)-specific phosphodiesterase that is predominantly located in inflammatory cells.[3,4,5] By inhibiting PDE-4, apremilast increases intracellular levels of cAMP and thereby inhibits the production of multiple proinflammatory mediators including PDE-4, TNF-alpha, interleukin-2 (IL-2), interferon-gamma, leukotrienes, and nitric oxide synthase.[6]

A through literature survey reveals that not many analytical methods published to describe the quantification of Apremilast by UV-Spectrophotometric method[7], RP-HPLC[8,9] and UPLC.[10] Infect the published UV method utilizes methanol as solvent. But there is no stability indicating UV method with acetonitrile solvent. These stability indicating methods would be helpful in establishing the stability data of these drugs in bulk and tablet dosage forms. Generally this UV technique is less expensive and with inherent simplicity. Rapid development in the pharmaceutical industries, producing more number of new drugs and formulations in different parts of world has been increasing. For providing effective and safe drug formulation to consumers direly needed.

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So innovative new analytical methods necessary for controlling their quality and amount of drug in pharmaceutical dosage forms especially it plays a vital role in the case of powerful drugs. So the author selected this method as it has several advantages, including simple and mobile phase, low cost solvents, rapid analysis, and simple sample preparation.

Material and Methods

Instrument used was UV-Visible Spectrophotometer of Shimadzu-2450 and High Performance Liquid Chromatography having UV-3000-M as detector. Wenser High Precision Balance was used for weighing and Wenser Ultra Sonicator of sonication.

Materials

Apremilast was procured from Modern Science Apparatus Pvt. Ltd., Methanol and o-Phosphoric Acid was purchased from Finnar. Potassium Dihydrogen phosphate was purchased from Sigma-Aldrich and Water was purchased from Merck. Aprezo tablet 30mg was purchased from Glenmark.

Chromatographic condition

Chromatographic separation was performed on Grace C_{18} analytical column. Isocratic mobile phase consisting of Methanol: Water (80:20) was delivered at the flow rate 0.9ml/min, injection volume was 20µL.

Methods

Selection of solvent

Methanol was selected for as solvent for dissolving Apremilast

Preparation of standard solutions

An accurately weighed about 10.0mg quantity of Apremilast was transferred in a 10.0mL of volumetric flask, dissolved in a sufficient quantity of methanol and volume was made up to the mark with methanol. (Conc. 1000µg/mL of Apremilast). A 1.0mL of standard stock solution was transferred in 10.0mL of volumetric flask and volume was made up to the mark with methanol. (Conc. 100µg/mL of Apremilast). A 0.2mL of working stock solution was transferred in 10.0mL of volumetric flask and volume was made up to the mark with methanol. (Conc. 2µg/mL Apremilast). Then further dilution of drug like 2, 4, 6, 8, 10µg/mL from stock solution was prepared in the same solvent.

Selection of wavelength

Standard solution was scanned between 400nm to 200nm.Overlain spectra of the drug was recorded. From the spectra 231nm (λ max of Apremilast) were selected for the method development.

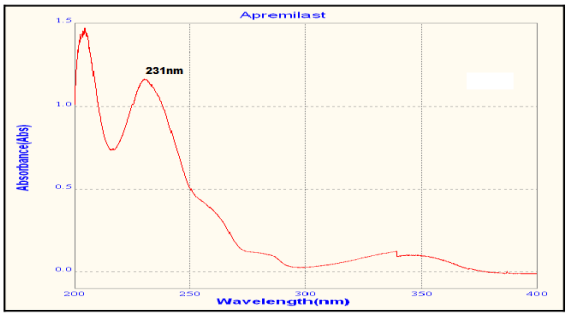

Fig. 1: UV absorption spectra for Apremilast

Table 1: Calibration data of Apremilast for absorbance maxima

Conc. (µg/ml)	Absorbance (nm)
2	0.223
4	0.459
6	0.679
8	0.814
10	1.234

Method Development by RP-HPLC

Selection of mobile phase: Mobile was selected on the basis of solubility of the drug and trials.

Preparation of standard solution:

An accurately weighed about 10.0mg Apremilast was transferred in a 10.0mL volumetric flask, dissolved in sufficient quantity and volume was made upto the mark with mobile phase. (Conc. 1000µg/mL). A 0.1mL of stock solution was transferred in 10.0mL volumetric flask and volume was made up to the mark with mobile phase. (Conc. 10µg/mL)

Potassium dihydrogen phosphate solution: Solutions of 1 M may be prepared by dissolving 136.09g of potassium dihydrogen phosphate in sufficient water to produce 1000ml. pH adjust Upto 3 with the OPA.

Preliminary optimization of mobile phase and other chromatographic conditions

In order to achieve the optimized chromatographic condition, one or two parameters modified at each

trial and chromatograms were recorded with all specified chromatographic conditions. Various mobile phases were tried by permutation and combination and also by change in flow rate, buffer and its pH. The standard solution of drug was prepared in different mobile phases and various trials were taken out.

The different solvents were used for mobile phase such as Potassium di-hydrogen phosphate buffer, Methanol, Water and their composition also changed as 70:30; 60:40; and 80:20 v/v respectively for the method optimization. Flow rate also changed to 1, 0.9 and 0.8 mL/min respectively. From above observation the RP-HPLC Method has been developed for Apremilast by using Methanol: Water (80:20 v/v) as mobile phase with flow rate of 0.8mL/min and Retention Time 4.808.

Preparation of sample solution:

Weigh finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 10 mg of Apremilast into a 100 ml clean volumetric flask, add about sufficient ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the diluents. Filter the solution through 0.45µm membrane filter. Pipette 3 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents.

Procedure: Equal volumes (10µl) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded, and the response i.e. peak area of major peaks were measured. The content of Apremilast was calculated by comparing a sample peak with that of standard.

Validation of proposed method[11-14]:

Linearity: Linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range.

Linearity was performed by diluting standard stock solution to give final concentration in the range of 2µg to10 µg/ml for Apremilast. 10 µg of each concentration injected and calibration curve was constructed by plotting the peak area versus the drug concentration. The plot should be linear passing through the origin. Correlation coefficient should not be less than 0.999.

Accuracy: The accuracy of an analytical method is closeness of test results obtained by that method to the true value. Accuracy is calculated from the test result as the percentage of analyte recovered by the assay. Accuracy was performed in triplicates and compares the results. % recovery was performed by spiked known quantity of drug at 50%, 100% and 150% to a pre-quantified sample solution and analyses sample. From the result % recovery was calculated. Mean recovery should be in the range of 98-102% the relative standard deviation should not be more than 2.0%

Precision: Precision of an analytical method is the degree of agreement among individual test result when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation. A powder sample equivalent to 10 mg of Apremilast into 100 ml clean dry volumetric flask, add about sufficient ml of diluents and sonicate to dissolve it completely and make volume up to the mark with the diluents. Pipette out 3 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents. RSD of assay were calculated.

Prepare six different test solution of the 100% test concentration from the same sample matrix. Inject duplicate injection of each test solution. The relative standard deviation should not be more than 2.0%.

Robustness: It is the measure of capacity of the method to remain unaffected by small but deliberate change in the method parameter and provide an indication of its reliability under normal usage. Determination: the robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. Carry out the following procedure individually by changing following variation in chromatographic conditions like change in flow rate and change in detection wavelength.

Results and Discussion

According to the literature survey of Apremilast no suitable assay method was available for the determination of Apremilast using RP-HPLC. So the mixture of Methanol and water was selected as mobile phase. Initially the trail was performed by taking different ratios of methanol: water and then

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finally the Methanol: Water (80:20v/v) method was selected with flow rate of 0.8ml/min.

Validation of RP-HPLC

Linearity

The linearity of an analytical procedure is its ability (within given range) to obtain test results that are directly proportional to concentration of analyte in sample. The linearity of measurement was evaluated by analyzing different concentrations of Apremilast such as 10, 20, 30, 40, 50 ppm. The acceptance criteria should be less than 0.999 and the correlation coefficient was found to be 0.9998. Therefore, development method is said to be linear.

Table 2: Linearity of Apremilast

S/No.	Conc. (µg/ml)	Area
1	10	1099357
2	20	2310222
3	30	3644309
4	40	4872029
5	50	6089664
Correlation coefficient (r^2)	0.9998	
Slope(m)	125424	
Intercept(c)	159610	

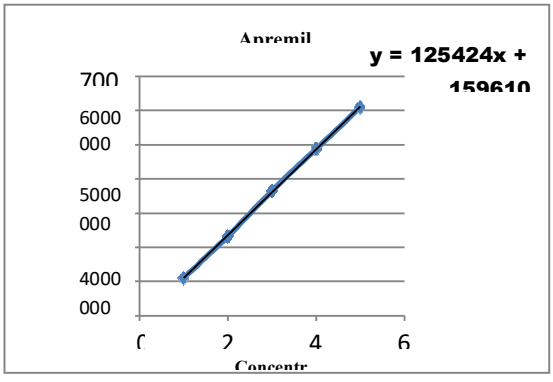

Fig. 2: Linearity graph for Apremilast

Accuracy

Accuracy was performed in three different levels for Apremilast. Analysis was done in triplicate for each level. The resultant % SD was in the range 0.10-0.42 which is less than 2% with recovery 99.49-99.80%. The acceptance criteria form percent recovery should be within the range of 98-102 %. Percent recovery for the drug from tablet dosage form in different composition was found to be within range 98-102 %.

Table 3: Accuracy data for Apremilast

			Standard Deviation		Accuracy
Conc	Conc.	Area	Mean	SD	%SD
	10	1099357			
1	10	1092282	1094064	4664.680804	0.4263627
	10	1090553			
	30	3644309			
	30	3635167	3639610.667	4576.317552	0.12573646
2	30	3639356			
	50	6089664			
3	50	6084774	6090645.333	6418.512704	0.10538313
	50	6097498			

Table 4: Recovery study for Apremilast

Sr. No.	% Composition	Area of Standard	Area of Sample	% Recovery
1	50%	3644309	3637340	99.80877033
2	100%	4872029	4862263	99.79954963
3	150%	6089664	6058796	99.49310832

Precision

The precision of the method was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision). Repeatability was calculated by assaying three samples of each at three concentration levels by using 30µg/ml on the same day. The inter-day precision was calculated by assaying three samples of each three concentration levels by

using 30µg/ml on two different days. The acceptance criterion of %RSD is less than 2. The %RSD was found to be 0.19% which is less than 2 %. Therefore, developed method said to be precise.

Table 5: Precision studies of Apremilast

Intraday				
Concentration	Area		Mean	% RSD
30 µg/ml	Morning	Evening	3641668	0.19
	3644309	3645806		
	3635167	3633338		
	3639356	3652032		
Inter day				
Concentration	Area		Mean	%RSD
30 µg/ml	Day 1	Day 2	3639356	0.19
	3645806	3644309		
	3633338	3635167		
	3652032	3639356		

Robustness

Two parameters were considered for robustness determination such as change in flow rate and change in detection wavelength. The %SD value of assay determined for the same sample under original conditions and robustness conditions was less than 2.0% indicating the method is robust.

Table 6: Robustness study of Apremilast

Parameters	**Variation**	**Rt.(min)**	**Tailing Factor**	**Area**	**% SD**
Flow rate	0.8	4.844	1.21	2310222	0.29282464
	0.7	5.508	1.21	2323162	
	0.9	4.372	1.20	2320240	
Wavelength	231	4.844	1.21	2310222	0.20359639
	229	4.856	1.20	2316607	
	233	4.851	1.21	2307426	

Analysis of Marketed Tablet Formulation:

Preparation of sample solution:

Weigh finely powder not fewer than 20 tablets. Accurately weigh and transfer a quantity of powder sample equivalent to 10 mg of Apremilast into a 10ml clean volumetric flask, add diluents and sonicate to dissolve it completely and make up to the mark with diluents. Filter the solution through 0.45 µm membrane filter. Pipette out 0.3 ml of above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents.

Table 7: Assay of Apremilast

Drug	**Label claim (mg/tab)**	**Concentration taken**	**Area**	**Amount found(mg/tab)**	**% Assay**
Apremilast	30mg	30ppm	3624363	29.8	99.47

Conclusion

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: Water (80:20 v/v), at a flow rate 0.8 ml/min, the detection was carried out at 231nm. 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 10-50 µg/ml with regression coefficient r^2= 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.

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