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CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION OF ASSAY OF APREMILAST IN BULK AND TABLET DOSAGE FORM

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ABSTRACT
The present work describes development and validation of a simple, sensitive, precise, accurate and specific reverse phase high performance liquid chromatographic method for analysis of Apremilast, as bulk drug. Apremilast is used to treat psoriatic arthritis. The separation was achieved by using a mobile phase of Methanol: Water (80:20, v/v) on a Grace C18 column (250mm x 4.6ID, 5µm) at flow rate of 0.8 ml/min. The detection was done at 231 nm. The retention time of Apremilast was 4.80 minutes. Calibration plot was linear (r^2=0.9998) over a range of 2-5µg/ml. The method was validated for linearity, accuracy, precision, robustness and recovery. The proposed method was successfully used for quantitative determination of Apremilast. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Apremilast in tablet dosage form.

KEYWORDS: Apremilast, RP-HPLC, Validation.

1. INTRODUCTION
Apremilast is chemically N-{2-[(1S)-1-(3-Ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl}acetamide (fig. 1) is a drug for the treatment of certain types of psoriasis and psoriatic arthritis.[1] Apremilast is a phthalimide derivative. It is a white to pale yellow, non-hygroscopic powder that is practically insoluble in water and buffer solutions in a wide pH range, but is soluble in lipophilic solvents such as acetone, acetonitrile, butanone, dichloromethane, and tetrahydrofuran. It is manufacture in India by Glenmark Pharmaceutical under the brand name of Otezla and Aprezo. Several analytical methods have been described in the literature for the determination of Apremilast in Pharmaceutical dosage form and in biological fluids. A LC-UV method was developed for the determination of process-related impurities in Apremilast. The present work is to carry out the simple RP-HPLC method development for the determination of Apremilast. The method was validated according to the ICH guidelines.

Structure

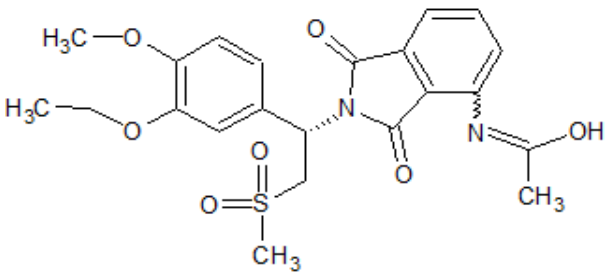

Fig.1. Structure of Apremilast.

2. EXPERIMENTAL
2.1 CHEMICAL AND REAGENTS
Apremilast was procured from Modern Science Apparatus Pvt.Ltd, Nashik. The purity of drug was evaluated by obtaining its melting point and by ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification.

Methanol (HPLC- grade), Water and other reagents were of standard quality. Potassium dihydrogen Phosphate (AR grade) was obtained from Merk.

A tablet formulation of Apremilast (30mg) was procured from local market.

2.2 INSTRUMENTATION

The chromatographic technique was performed on shimandzu UV 2450 Double Beam UV-Visible spectrometer with software UV probe, reversed phase Grace C18 column (250mm×4.6ID, Particle size:5 micron) as stationary phase Ultrasonic Cleaner, Wenser High Precision Balance PGB 100, Wenser Ultra Sonicator WUC-4L, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study.

2.3 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.

2.4 PREPARATION OF CALIBRATION STANDARDS

Stock solution was prepared by dissolving 10mg of drug into a 10ml of methanol in 10ml volumetric flask. From this pipette 1ml of solution dissolve in a methanol and make up volume up to 10ml with methanol. From this pipette 0.2ml of solution dilute with methanol and make up the volume up to 10ml with methanol. further dilutions of 2,4,6,8,10ppm were prepared with same solvent.

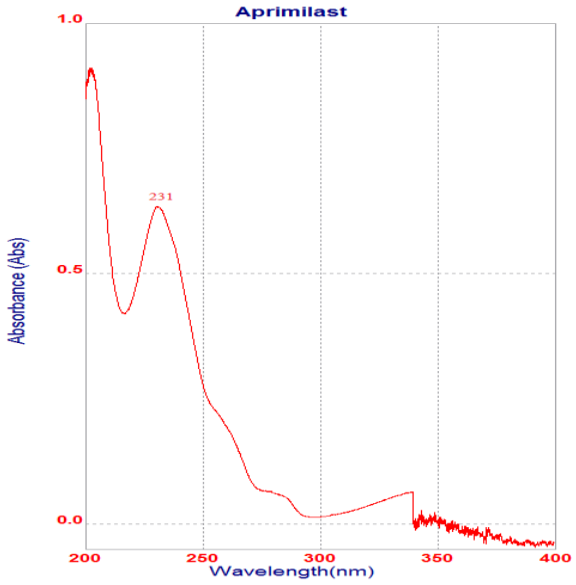

Fig. 2: UV absobance spectra for Apremilast.

Table 1: Calibration data of Apremilast for absorbance maxima.

Sr.No.	Concentration (µg/ml)	Absorbance (nm)
1	2	0.223
2	4	0.459
3	6	0.679
4	8	0.814
5	10	1.234

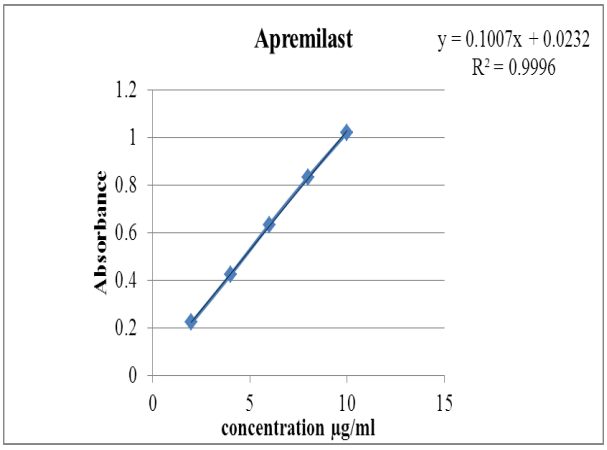

Fig.3: UV-calibration curve for Apremilast.

2.5 Sample Preparation

- **Standard stock 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).
- **Working stock solution:** 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.

2.6 METHOD VALIDATION

The method was validated for the following parameters: linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, robustness and ruggedness.

A. 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 10µg/ml to 50µg/ml for Apremilast. 20µL of each concentration injected and calibration curve was constructed by plotting the peak area versus the drug concentration. Correlation coefficient should not be less than 0.999.

B. 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.

1. Mean recovery should be in the range of 98-102%
2. The relative standard deviation should not be more than 2.0%

C. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Prepare six different test solutions. Inject duplicate injection of each test solution. Pipette 0.3ml of stock solution in 10ml volumetric flask and dilute with solvent in sufficient quantity and make volume up to mark with solvent to get concentration of Apremilast 30ppm.

D. 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. 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 in detection wavelength 231, 229, 233nm and flow rate 0.7, 0.8, 0.9ml/min.

E. Analysis of marketed tablet formulation (Assay)

Twenty tablets of each brand (Aprezo) were purchased from the local market, weighed and crushed to a fine powder. Accurately weigh and transfer a quantity of powder sample equivalent to 10 mg of Apremilast into a 10ml clean volumetric flask, add sufficient 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 out 0.3 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluents for 30µg/ml solution.

2 RESULT AND DISCUSSION

The present work was aimed to develop a RP-HPLC method for the determination of Apremilast in its tablet formulations. A reversed-phase chromatographic technique was developed to determine Apremilast at 231 nm and Grace C18 column was adopted for the analysis.

HPLC method development and optimization

In the literature survey of Apremilast no suitable assay method was available for the determination of Apremilast using RP-HPLC. A mixture of Methanol: Water was selected as mobile phase. Initially the Apremilast drug sample solutions were analyzed using a mobile phase consisting of Methanol: Water (60:40, v/v) at a flow rate of 1 ml/min. Under these conditions, a broad peak was observed at 6.44 min. Then the mobile phase ratio was changed to 70:30, v/v and flow rate also changed to 0.9 and 0.7 ml/min resp. but as a result retention time was 5.66 and 5.63min. Another mobile phase also used of Methanol: Potassium dihydrogen Phosphate buffer (80:20 v/v) at the flow rate of 0.8ml/min but retention time was not optimised, So the mobile phase of Methanol : Water (80:20 v/v) with flow rate of 0.8 ml/min which gives a sharp peak was eluted with good symmetry and retention time 4.808 ± 0.46 min. Hence a mobile phase containing Methanol: Water (80:20, v/v) with flow rate of 0.8 ml/min was chosen as the best chromatographic condition for the entire study.

METHOD VALIDATION

A. Linearity

The calibration curve for Apremilast was linear over the concentration range 10-50µg/ml. Calibration curve was constructed by plotting the peak area versus the drug concentration. The data for the peak area of the drug corresponding to the concentration was treated by linear regression analysis (Table 2 and fig. 3) and the regression equation for the calibration curve was found to be y =125424x +159410 with correlation coefficient of 0.9998 which is nearly equals to unity.

Table No. 2: Linearity of Apremilast.

SR.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	

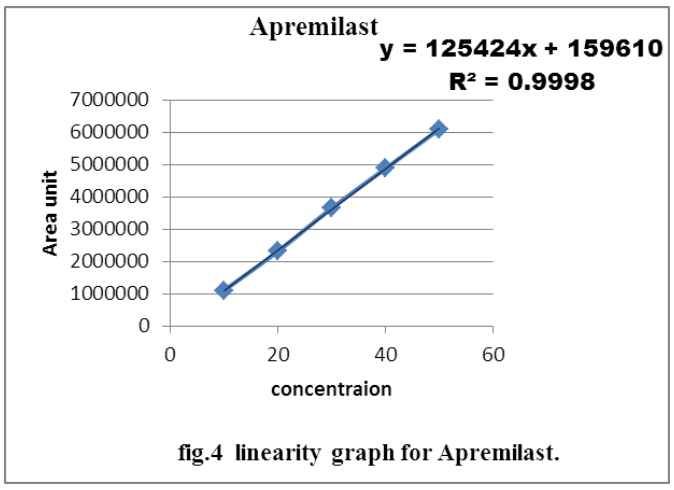

fig.4 linearity graph for Apremilast.

B. 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 (<2.0 %) with recovery 99.49-99.80% (Table 3 and 4).

Table 3: Accuracy data for Apremilast.

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

Accuracy results were expressed in terms of percent recovery.

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

C. 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 (30µg/ml) on the same day. The inter-day precision was calculated by assaying three samples of each at three concentration levels (30µg/ml) on two different days. The % RSD in precision studies was found to be 0.19% for both intra-day and Interday which is <2.0 % (Table 5).

Table No. 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		

D. Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate change in method parameter and provide an indication of its reliability for routine analysis. The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changing from the original condition. The detection wavelength was set at 231, 229, 233nm. The flow rate was set at 0.8, 0.7, 0.9ml/min. The results obtained (Table 6) from assay of the test solutions were not affected by varying the conditions and were in accordance with the results for original conditions. The %SD value of assay determined for the same sample under original conditions and robustness conditions was less than 2.0% indicating that the method is robust (Table 6).

Table No. 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	

E. Analysis of Marketed Tablet Formulation (Assay)

The proposed method was applied for the determination of Apremilast in marketed formulations (tablets) (Figure 4) and the % recovery is found to be 99.47% (Table 7). Accepted range is 98-102%.

Table No. 7: Assay Result of Apremilast.

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

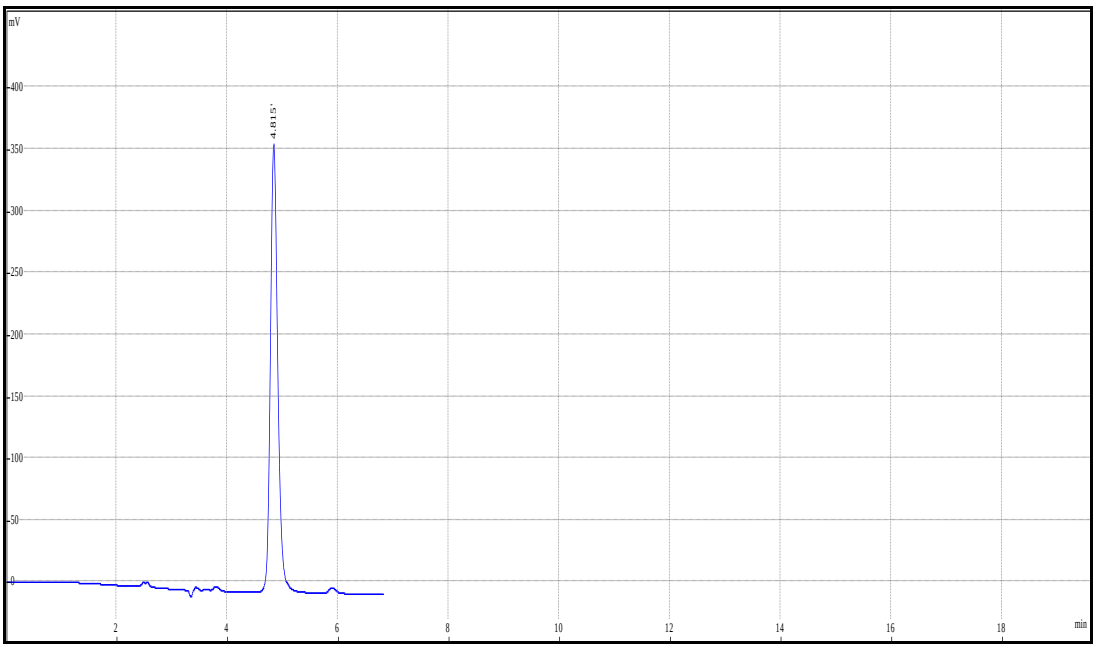

Fig.5: Chromatogram for assay of Apremilast.

3 CONCLUSION

The present work involved the development of simple, accurate, precise, cost effective and suitable RP-HPLC method for estimation of drug in pharmaceutical dosage forms.

The method has several advantages, including simple mobile phase, low cost solvent, rapid analysis. The regression coefficient (r^2) for analyte is not less than 0.9998 which shows good linearity. The % RSD in precision, accuracy and robustness studies was found to be less than 2 % indicating that the proposed method is precise, accurate and robust.

The proposed method was validated as per ICH guidelines and can be successfully applied to perform RP-HPLC of Apremilast even in pharmacokinetic studies. Since the method does not require use of expensive reagent and also less time consuming, it can be performed routinely in industry for routine analysis of marketed product of Apremilast tablet.

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