DEVLOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF APREMILAST IN TABLET DOSAGE FORM

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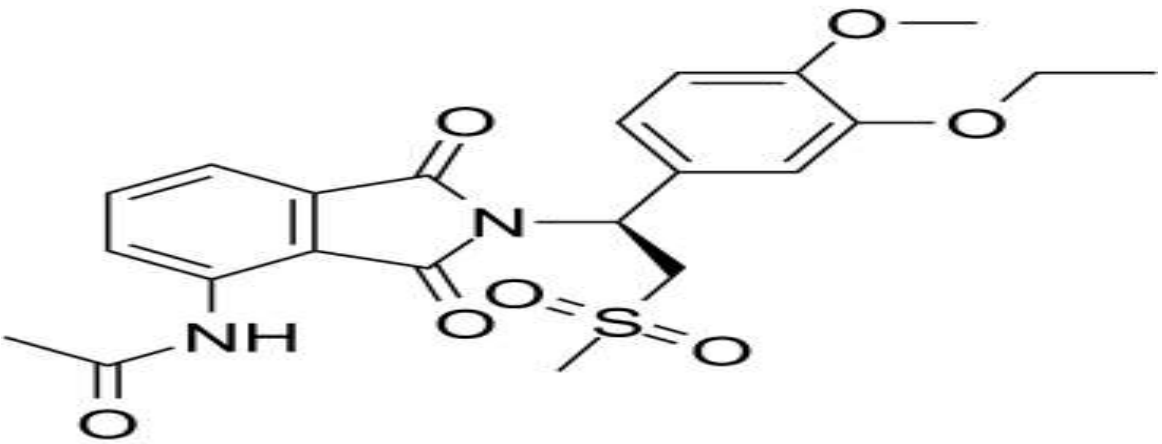
Abstract- RP-HPLC for the determination of apremilast tablet dosage form the chromatographic s9999on was carried out UV 1700 detector, utilising C18 column (based on 99.999% ultra high purity silica) 150 mm ×4.6 mm ,5µm particle size, utilising water methanol, triethylamine, at flow rate 1ml/min within injection vol. 20µl was selected for this study. The sepreation was carried out at a room temperature and the eluentant werw observed by photodiode array detector set a 264nm. The retention time of apremilast obtained was at 3.9 min. thus the propose method for APR was found to be feasible for estimation of APR in pharmaceutical dosage form.

Keyword- HPLC, Mobile phase,

I. Introduction- Apremilast used to certain type of arthritis (Psoriatic Arthritis). Apremilast is a class phosphodiester-4 inhibitor used in rheumatic arthritis and psoriatic Arthritis phosphodiestarase inhibitor is a cyclic adenosine monophosphate which is predominantly located in inflammatory cells and by inhibiting PDE-4. It increases of CAMP which further inhibit proinflammatory mediatory including interleukin-2 chemically name of apremilast is {N-{2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl}acetamide and mol. Formula $C_{22}H_{24}N_2O_7S$ and Mol. Wt. 460.5 g/mol.



II. EXPERIMENTAL STUDY

A. Reagents and chemicals-

Water, methanol, triethylamine were used as solvents to prepare mobile phase. All the chemicals used were HPLC grade (Merck Ltd. Mumbai) used without further purification.

B. Selection of solvents -

Water, methanol and Triethylamine (30:70:2 *v/v*) was selected as the common solvent for dissolving Apremilast.

C. Selection of stationary phase-

On the basis of reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with C18 bonded phase i.e YMC pack C18 (150 mm X 4.6 mm), 5µm was selected.

D. Selection of Mobile Phase:

The selection of mobile phase was done after assessing the solubility of drug in different solvent as well on the basis of literature survey and finally mobile phase was selected for is the mixture of Water, methanol and Triethylamine in the ratio 30:70:2 *v/v*,

E. Selection of Detector and Detection wavelength:

UV-visible 2487 detector was selected, as it is reliable and easy to set at the correct wavelength and 234 nm wavelengths was selected as detection wavelength.

F. Optimization of Chromatographic Parameters:

Optimization in HPLC was the process of finding a set of conditions that adequately separate and enable the quantification of the analyte from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

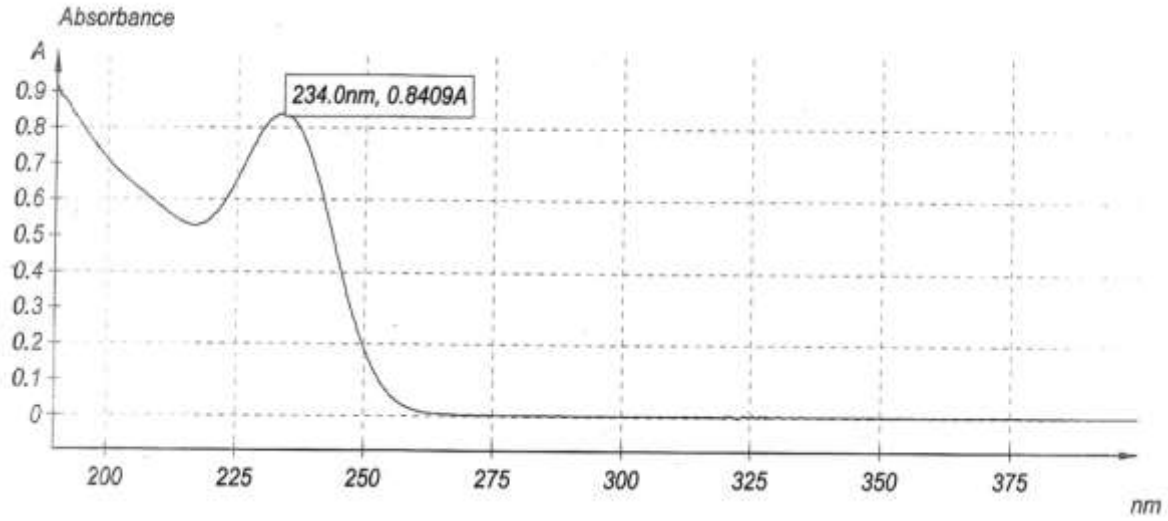
G. Optimization of mobile phase strength:

The mobile phase chosen after several trials with water, methanol in various proportions which is shown in Table 6.3 finally the mobile phase consisted mixture of Water, methanol and Triethylamine in the ratio 30:70:2 *v/v*,. which resolved the tailing of peak.

The flow rate of 1.0 mL/min was selected as it gave good result, system suitability parameters and reasonable retention time. The retention time of Apremilast was observed 3.3 min at 234 nm wavelength with total run time of 8 min.

H. Optimization of Detection Wavelength:

A fixed concentration of analyte was analyzed at different wavelengths. As per the response of analyte, wavelength of 234 nm was selected. This is the optimum wavelength as it shows maximum absorption at this wavelength.



Stability indicating RP-HPLC development and optimization

I. Praparation of Standard solution:

Accurately weighed 75 mg of Apremilast as working/reference standard was transferred into 100 mL volumetric flask. About 70 mL of diluent added and sonicated to dissolve. The solution was cooled to room temperature and made up to mark with diluent.

Further 4 mL of stock solution of Apremilast was pipette out and transferred to 50 mL volumetric flask and made volume up to mark with Diluent.

J. Linearity studies :

Different levels of standard solution were prepared by diluting out known volumes of intermediate stock solution with the diluent to get the required analyte concentrations. A graph of Concentration (ppm) *vs.*area was plotted and the regression coefficient 'r^2', y-intercept and slope of the regression were calculated. weigh accurately about 100 mg of Apremilast standard was taken and transferred to 200 mL volumetric flask, 150 mL of diluent was added, sonicated to dissolve and made up to volume with diluent and mixed.

Linearity

Level (%)	Concentration (ppm)	Response		
		1	2	Mean
50	30.13	823983	827014	825498
75	45.80	1222695	1263714	1243205
100	60.01	1681080	1642317	1661699
125	75.03	2088000	2042610	2065305
150	90.12	2462694	2483770	2473232
Co-relation coefficient (r^2)				**0.999**
SLOPE				**16472.5304**
Y-INTERCEPT				**6763.5826**
WORKING LEVEL AREA				**1661700**
%LIMIT OF Y-INTERCEPT (± 5 OF WORKING LEVEL)				**0.60**

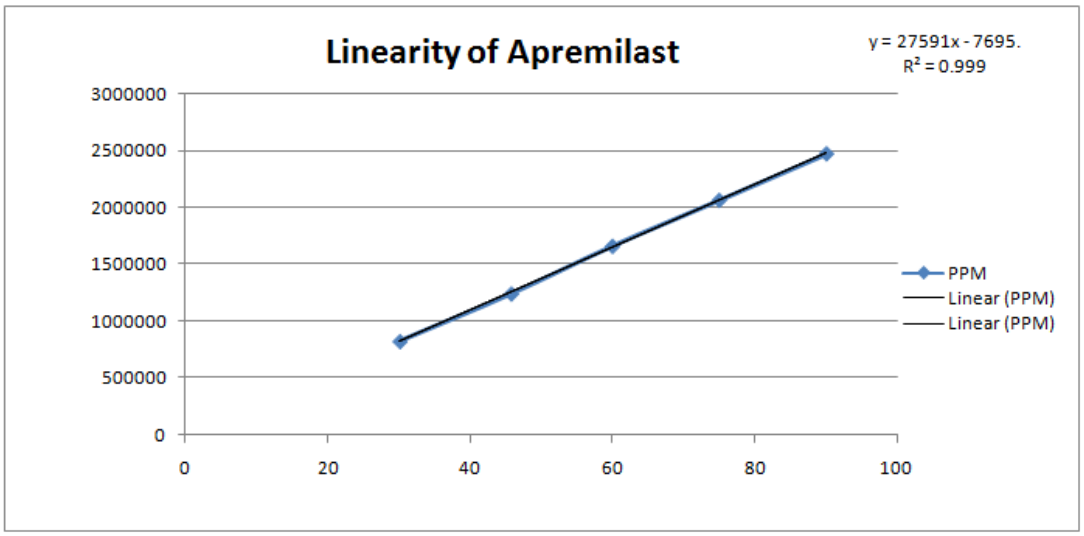


Figure A : Calibration curve of Apremilast

K. Preparation of Test Solution:

Weighed and Transfered 5 intact tablet of Apremilast into 250 mL volumetric flask. Added about 200 mL of diluent, sonicated for 25 minutes with intermittent shaking cool and dilute up to the mark with diluent and mix, **Allowed** to settle for 15 min. centrifuge this solution at 5000 RPM for 5 min. Further tranfered 5 ml of stock solution into 50 ml volumetric flask dilute up to the mark with diluent and mix, **Filtered** through 0.45µ Nylon membrane syringe filter-mdi or equivalent and **injected** (Concentration of Apremilast: About 60 ppm)

L. METHOD VALIDATION-

The developed method was validated as per International Conference on Harmonization (ICH) guidelines with respect to system suitability, Specificity, Linearity, Accuracy, Precision and robustness.

SYSTEM SUITABILITY :

System suitability test is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.

system suitability test:-

Tailing factor	**1.0**
Theoretical plates	**7402**
S. No.	**Area**
1	1666335
2	1661183
3	1660260
4	1670799
5	1669835
6	1666117
Mean	1665755
% RSD	0.3

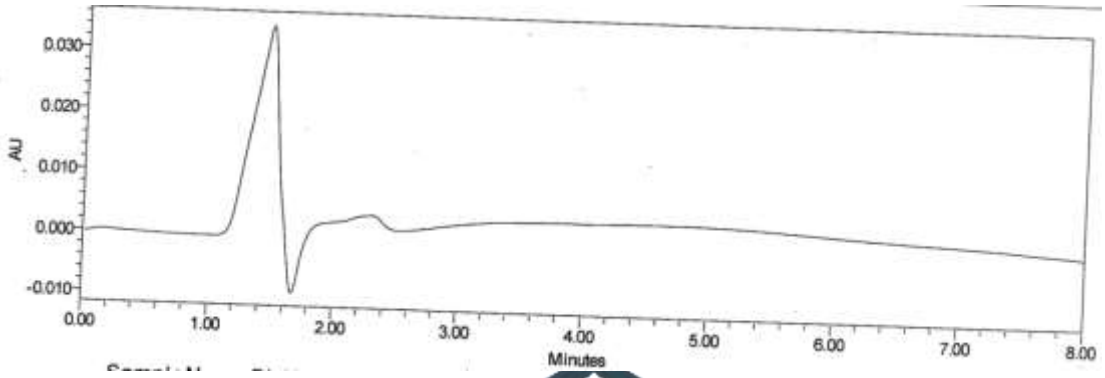
Specificity: (Identification, Interference & Peak Purity)

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

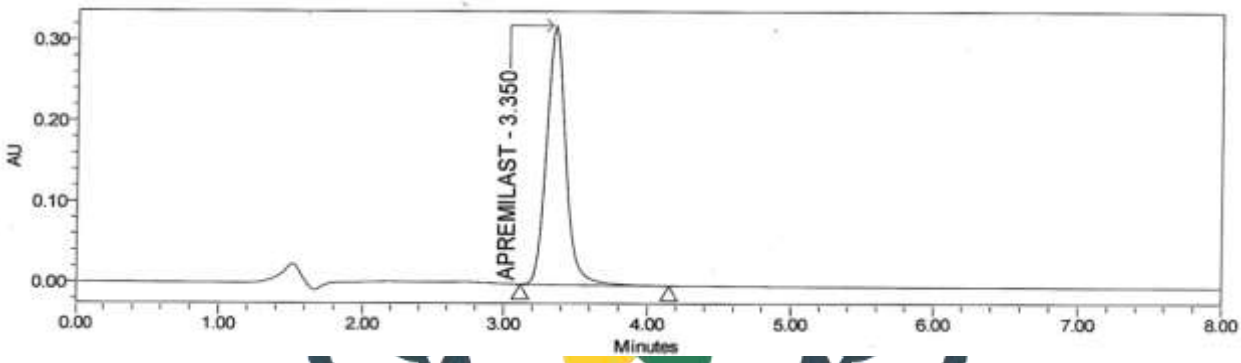
Specificity (Identification and Interference)

Component	**Retention time (min)**	**Tailing factor**	**Theoretical plates**	**Purity angle**	**Purity threshold**
Blank	-	-	-	-	-
Placebo solution	-	-	-	-	-
Standard solution	3.350	1.2	7254	0.06	0.86
Sample solution	3.432	1.01	7027	0.07	0.93
Spike solution					
Sample solution	3.351	1.02	7166	0.6	0.84
N-Acetyl Amine	1.96	1.2	3258	0.41	1.45
Des-Acetyl	2.927	1.0	2704	0.90	1.72
Individual Impurity Solution					

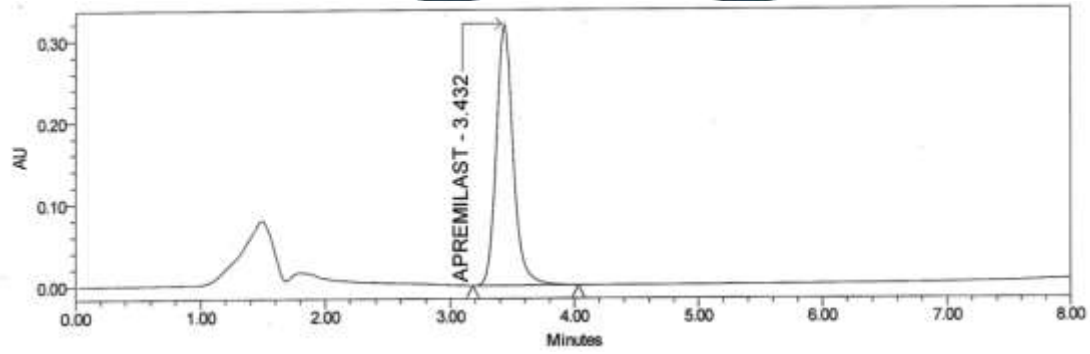
N-Acetyl Amine	1.98	1.09	3542	0.39	1.56
Des-Acetyl	2.885	1.25	2687	0.82	1.86



Chromatogram of Blank



Chromatogram of Standard



Chromatogram of Sample

Accuracy:- Accuracy (Recovery):

Accuracy was evaluated three levels 50%, 100% and 150% of the working concentration level for Apremilast. As the working concentration level of Apremilast, Each level prepared in triplicates.

Level (%)	Theoretical concentration (mcg/mL)	Area	% Recovery	Mean recovery%
50	30.517	856280	100.8	100.6
	30.295	856890	100.9	
	30.298	851653	100.1	
100	60.360	1706792	100.3	100.2
	60.691	1703069	100.2	
	60.366	1699177	100.1	
150	90.283	2529184	99.3	99.3
	90.963	2527490	99.1	
	90.950	2537997	99.5	
	Mean recovery			**100**
	%RSD			**0.6**

Precision:

System precision:

Tailing factor	**1.12**
Theoretical plate	**7034**
Sr. No.	**Area**
1	1696043
2	1695295
3	1695560
4	1697190
5	1695465
6	1695346
Mean	**1695817**
% RSD	**0.1**

Method Precision: Single injection of blank (Diluent), Standard solution (five replicates) and sample solution (six preparations) was injected on the system.

Method precision

Sample No.	Response	% Assay
1	1635907	99.4
2	1638839	99.8
3	1638810	99.4
4	1636847	99.0
5	1633176	98.6
6	1638572	99.2
Mean		**99.2**
% RSD		**0.4**

Intermediate Precision :-Five independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

Table 7.9 Intermediate Precision

Parameter	Method Precision(Analyst-I)	Intermediate Precision(Analyst-II)
HPLC Instrument No.	**AD/HPLC-031**	**AD/HPLC-052**
HPLC column No.	**C18-134**	**C18-063**
Sample No.	**% Assay**	
1	99.4	100.1
2	99.8	99.6
3	99.4	99.2
4	99.0	99.9
5	98.6	99.5

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.

Parameters	Values	Retention Time	Tailing factor	Theoretical plates	%Assay	Absolute difference
Control	As per method	3.5	1.1	7125	100.5	-
Flow rate (± 0.1 mL/min)	1.1mL/min	3.4	1.2	7090	100.4	0.1
	0.9mL/min	3.6	1.2	7006	100.3	0.2
Change in Wavelength(± 5 nm)	239nm	3.5	1.1	7039	100.5	0.0
	229 nm	3.5	1.2	7107	99.1	1.4
Column temperature (± 5°C)	35°C	3.4	1.2	7092	99.5	1.0
	25°C	3.6	1.1	7203	99.9	0.6

CONCLUSION

RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique was employed in the present investigation for estimation of Apremilast tablet formulation. HPLC Water2469 with YMC pack C18 (150 mm X 4.6 mm), 5µm column and UV/PDA detector with empower pro Software was used for the study. The standard and sample solution of Apremilast were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

The mobile phase that was found to be most suitable was Water and Methanol, Triethylamine the wavelength 234nm were selected for the evaluation of the chromatogram of Apremilast respectively. The selection of the wavelength was based on the λmax obtained by UV scanning of standard laboratory mixture in water: methanol. This system gave good resolution and optimum retention time with appropriate tailing factor (<2).

After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by procedure described under Materials and methods. It gave accurate, reliable results and was extended for estimation of drugs in tablet formulation.

The results from table clearly indicate that the RP-HPLC technique can be successfully applied for the estimation of above-mentioned drugs in their formulation.

SUMMARY

The results of analysis in this method were validated in terms of accuracy, precision, ruggedness, linearity. The method was found to be sensitive, reliable, reproducible, rapid and economic also.

Table 9.1 Summary of System suitability

S. No.	Parameters	Acceptance criteria	Result obtained		
1.0	System suitability The relative standard deviation of six replicate injections	NMT 2.0%	0.3		
	Tailing factor	NMT 1.5	1.0		
	Theoretical plates	NLT 2000	7402		
2.0 2.1	Specificity Identification	Results should be comparable with respect to the retention time.	RT of Standard: 3.35		
			RT of Sample	3.432	
2.2	Interference	Blank (Diluent), Placebo and known Impurities should not show any peak at the retention time of Apremilast peak	Complies		
2.3	Peak purity	Standard and Sample peak should be pure at working concentration level. Purity angle should be less than purity threshold.	**Apremilast**	**Purity angle**	**Purity Threshold**
			Standard	0.06	0.86
			Sample	0.07	0.93

Sr.No	Parameters	Acceptance criteria	Result obtained	
3.0	Linearity and Range Correlation coefficient	NLT 0.990	0.999	
	Y- intercept	Intercept y <± 2.0% of standard response	1.60	
4.0	Accuracy (Recovery)	Mean and Individual recovery for 25% to 150% should be in the range of 95.0%- 105.0%.	Level %	% Mean Recovery
			50	100.6
			100	100.2
			150	99.9
5.0 5.1	Precision System Precision	System suitability criteria should be fulfilled.	Complies	
5.2	Method precision	The RSD for % assay of six independent samples preparations: NMT 2.0%.	% Mean Assay	99.2
			% RSD	0.4

Sr.No	Parameters	Acceptance criteria	Result obtained	
5.3	Intermediate Precision (Ruggedness)	The RSD for % Assay of six independent samples preparation should not be more than 2.0%.		
		The cumulative % RSD for % assay of twelve independent samples preparation of two analysts should not more than 2.0%.	% Mean Assay	99.4
			% RSD	0.4

Sr.No	Parameters	Acceptance criteria	Result obtained
6.0	Robustness Change in Flow rate (± 0.1 mL/min) Change in Column temperature (± 5°C) Change in Wavelength (±5 nm)	System suitability criteria should be fulfilled. The cumulative % RSD for % assay obtained in each modified condition should not be more than 2.0 when compared to the method precision.	Complies

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