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Development and Validation of Spectrophotometric and Chromatographic Method for the Estimation of Apremilast in Bulk and Formulations

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ABSTRACT

Objective: Objective of the present analytical research work was to develop and validate Spectrophotometric method and High Performance Liquid Chromatographic method (HPLC Method) for the Apremilast bulk and tablets dosage form.

Methods: A spectrophotometric method and a HPLC method have been developed and validated for estimation of APR in pharmaceutical oral dosage form.

Method A (UV SPECTROMETRY Method): The stock and working standard solutions of the drugs were prepared in methanol. Standard solutions were scanned over the range of 400-200 nm in spectrum mode of spectrophotometer at medium scanning speed using UV spectrophotometer. The maximum absorbance for Apremilast was found at 230 nm.

Method B (HPLC Method): The HPLC Method for Apremilast was developed using Cosmosil C18 (4.6mm x 250mm, Particle size: 5µm), as stationary particle, isocratic mode. Methanol: Water (80:20v/v) pH3 as mobile phase. Mobile phase was maintained at a flow rate of 0.8 ml/min and detection was carried out at 230 nm. Both the methods were validated in accordance with ICH guidelines

Results: Apremilast was found to be linear in the concentration range of 2-10 µg/ml for spectrophotometric method and 10-50 µg/ml for HPLC method. Retention time was found to be 4.0 min for Apremilast. The amount of Apremilast in marketed formulation by spectrophotometric method was found to be 99.82 %, the amount of Apremilast in marketed formulation by HPLC method was found to be 99.98 %.

Interpretation and Conclusion: Results of assay and validation study were found to be satisfactory. So, the methods can be successfully applied for the routine analysis of Apremilast.

Keywords: HPLC, bulk dosage form, tablets,

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INTRODUCTION

According to recent study no treatment can cure psoriasis but Apremilast can control signs and symptoms of psoriasis. Apremilast is a class of phosphodiester-4 inhibitor used in treatment of rheumatic arthritis and psoriatic arthritis. It is act as an anti-inflammatory caused by these conditions. Phosphodiesterase inhibitor is a cyclic adenosine monophosphate which is predominantly located in inflammatory cells and by inhibiting PDE-4 it increases intra-cellular level of cAMP which further inhibits pro-inflammatory mediators including interleukin-2, interferon gamma, TNF-alpha, PDE-4.

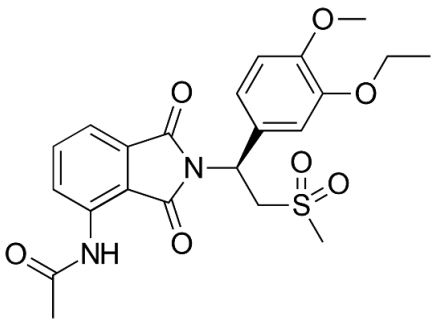

Figure 1: Structure of Apremilast

By chemically Apremilast is N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]-2,3-dihydro-1,3-dioxo-1H-isoindol-4-yl]acetamide with molecular formula and weight of C22H24N2O7S and 460.5 g/mole respectively. This analysis method follows ICH validation guidelines. This current research tries to develop new rapid, effective method for determination of Apremilast in bulk form according to ICH Q2 R1 guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Analytically pure samples of Apremilast were kindly supplied by Hetero Drugs Limited., Water (HPLC Grade) and MeOH (AR grade) Merck specialities private limited, Mumbai

Instrument Used

Electronic Weighing Balance (Shimadzu AY-220), Ultrasonicator (Wenser pvt ltd PGB-100), Cellulose Acetate Filter, 0.45 µm (Nylon 66), HPLC System (Analytical Technologies),UV VIS Spectrophotometer (Shimadzu UV-1800)

1. Spectrophotometric Method

1.1 Development of Spectrophotometric Method

Selection of Solvent

Solutions of APR (1000 µg/ml) was prepared in different solvents like methanol and water. These solutions were scanned in UV-Visible Region (200 nm to 800 nm) and intensity of absorption and wavelength of absorption were studied.

Preparation of Standard Stock Solution

Standard stock solution was prepared.

Selection of Wavelength Range

From the stock solutions, 0.1 ml of APR was transferred to 10 ml volumetric flask and the volume was adjusted to the mark with MeOH to obtain Strength 10µg/ml. The solution was scanned in the UV range 200-400 nm.

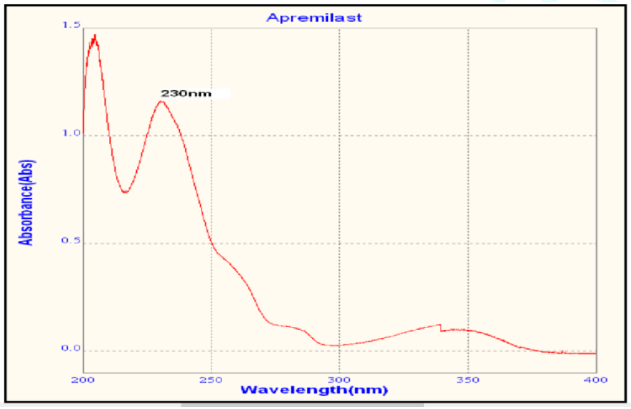

Fig 2. UV spectra of Apremilast

Preparation for Calibration Curve

Calibration curve were prepared and graph was plotted.

Analysis of Tablets

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 10 mg of APR was transferred into 10 ml volumetric flask containing 5 ml methanol, shaken manually for 10 min, volume was adjusted to mark with same solvent and filtered through Whatman filter paper. The absorbance of sample solution was recorded at recorded at 230 nm.

1.2. Validation of Spectrophotometric Method

Linearity and Range

The linearity of analytical method for APR was determined by studying standard calibration curves. The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the log curve.

Accuracy

Accuracy of the method was assessed by standard addition method at three different concentration levels i.e. 50%, 100, 150. Standard concentration of 2,4, and 6 µg/ml was added into 4 µg/ml of tablet concentration. The % recovery was then calculated by using formula

$$\% \text{ Recovery} = A - B / C,$$

Where,

A = Total amount of drug estimated

B = Amount of drug found on pre analysed basis

C = Amount of Pure drug added

Precision

The precision of an analytical method was studied by performing intermediate precision.

Intra-day Precision

Intra-day precision was determined by analyzing the 2,4,6 µg/ml of APR for three times in the same day.

Inter-day Precision

Inter-day precision was determined by measuring the the 2,4,6 µg/ml of APR for three consecutive days.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of calibration curves and average slope of calibration curves.

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

$$\text{LOD} = 10 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

Ruggedness

Ruggedness of the method was checked by two different analysts keeping same experimental and environmental conditions. An appropriate concentration 4,8 µg/ml of APR was subjected to analysis and concentration was determined. This procedure was repeated three times.

2. Chromatographic Method

2.1. Development of Chromatographic method

Description

The sample of Apremilast was observed for its color and texture.

Solubility

The sample of Apremilast was taken in test tubes and observed for solubility in various solvents like alcohol and water.

Selection of Mobile Phase

The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents as well on the basis of literature survey, Methanol and water were selected as a first choice.

Selection of Analytical Wavelength

To investigate the appropriate wavelength for determination of APR, the solution of the same in the MeOH were scanned separately by UV–Visible spectrophotometer in the range of 190-400 nm and the spectrum were recorded.

Preparation of Mobile Phase

***Mobile Phase A*:** HPLC grade MeOH was degassed in sonicator for 15 min.

***Mobile Phase B*:** HPLV grade water

Preparation of Standard Stock Solution

Standard stock solution was prepared by dissolving 10 mg of Apremilast in 10 ml methanol that gives concentration of 1000 g/ml of Apremilast and labeled as Standard stock Apremilast.

Preparation of Calibration Curve

Analysis of tablets

To determine the content of APR in conventional tablets; the twenty tablets were weighed, their mean weight determined and they were finely powered and powder equivalent 10.0 mg APR was transferred into a 10 mL volumetric flask containing 5 mL methanol, sonicated for 30 min and diluted to 1000 mL with methanol. The resulting solution was filtered, using 0.22 µm filter and 30 µg/mL was injected into system. The amount of APR was determined. The assay procedure was repeated for six times and Calculated using following equation.

$$Ct = \frac{Rt \times Cs}{Rs}$$

Where, Ct and Cs = Concentration of Sample and Standard Solution, respectively. Rt and Rs = Peak Area for Sample and Standard Solution, respectively.

2.2 Validation of HPLC Method

Linearity

The linearity of analytical method for APR was determined by studying standard calibration curves. The range of analytical method was decided from the interval between upper and lower level of calibration curves by plotting the log curve.

Accuracy

Accuracy of the method was assessed by standard addition method at three different concentration levels i.e. 50%, 100, 150%. Standard concentration of 10,20 and 30 µg/ml was added into 20 µg/ml of tablet concentration. The % Recoveries was calculated by applying regression equation.

Precision

The precision of an analytical method was studied by performing intermediate precision.

Intra-day Precision

Intra-day precision was determined by analyzing the standard solutions of APR (10,30,50 µg/ml) and at three different time intervals on same day.

Inter-day Precision

Inter-day precision was determined by analyzing the combined standard solution of Apremilast (10,30,50 µg/ml) on three consecutive days. The results were reported in terms of % RSD.

Limit of Detection and Limit of Quantitation

Detection limit and quantitation limit were determined based on the standard deviation of y-intercepts of calibration curves and average slope of calibration curves.

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

$$LOD = 10 \times \frac{\text{Standard deviation of intercept}}{\text{Slope}}$$

Robustness

Standard solution of APR (30 µg/ml)) were used and analyzed at different flow rate (0.7,0.8,0.9 ml/min) and wavelength (228,230,232 nm).

Ruggedness

Ruggedness of the method was checked by two different analysts keeping same experimental and environmental conditions. An appropriate concentration 30 µg/ml of APR was subjected to analysis and concentration was determined. This procedure was repeated three times.

System Suitability

Standard solution of APR (30 µg/ml) was prepared and analyzed. Chromatograms were studied for different parameters such as tailing factor, resolution and theoretical plates to see that whether they complies with the recommended limit or not.

RESULT AND DISCUSSION

1. UV-Visible Spectrophotometric Methods

Linearity study

Standard solution having concentration range of 2,4,6,8,10 µg/ml of APR was prepared. Absorbances of these solutions were recorded at 230 nm. Calibration curve was plotted, absorbance *vs* concentration. **Fig 3, Table 1.**

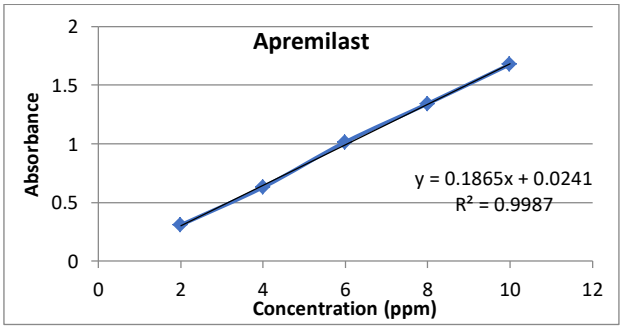

Fig 3. Calibration curve by UV

Table 1. Data of calibration curve by UV

Sr. No.	Conc. (µg/mL)	Absorbance
1	2	0.4069
2	4	0.7786
3	6	1.1225
4	8	1.4916
5	10	1.9151

Table 2. linear regression analysis by UV

Sr.No	Parameters	Zero Order spectrophotometric method
1	λmax (nm)	230
2	Beer's law limit (µg/mL)	2-10
3	Regression equation[y]	y = 0.1865x + 0.0241
4	Slope[m]	0.1865
5	Intercept [c]	0.0241
6	Correlation coefficient [r2]	0.9987
7	Limit of detection (LOD) (µg/mL)	0.0424
8	Limit of quantitation (LOQ) (µg/mL)	0.1286

Assay of Marketed Formulation

Using this method, the marketed formulation was analyzed. Sample solution containing 6 µg/ml. The amount of drug present in the marketed formulation was calculated .The mean % assay of APR was found to be 99.82%. **Table 3.**

Table 3. Result of tablet analysis

Drug Name	Mean*	SD	%RSD
Otezla	99.82	1.0586	1.06

Validation Parameters

Validation of the method was performed in accordance to ICH guidelines. Accuracy of the method was determined at 50%, 100% and 150% level by standard addition method and percentage recovery APR were found to be in the range of 99.93 – 100.29 %. Precision of the method was determined by % RSD of intra-day precision, inter-day precision. It was found to be less LOD and LOQ of APR was found to be 0.0424 and 0.1286 µg/ml, respectively.

Table 4. Result of Accuracy study

Level of addition	% Mean recovery*	SD	% RSD
50%	100.22	0.2553	0.25
100%	100.29	0.1931	0.19
150%	99.93	0.1154	0.11

Table 5A. Result of intraday precision

Sr.No	Conc. (µg/mL)	Absorbance	Mean	SD	%RSD
1	2	0.4051			
2	2	0.4069		0.0030	0.74
3	2	0.4010			
4	4	0.7769			
5	4	0.7786	0.7759	0.0032	0.42
6	4	0.7723			
7	6	1.1236			
8	6	1.1225	1.1253	0.0039	0.34
9	6	1.1298			

Table 5B. Result of interday precision

Sr.No	Conc. (µg/mL)	Absorbance	Mean	SD	%RSD
1	2	0.4036			
2	2	0.4069		0.0031	0.76
3	2	0.4098			
4	4	0.7726			
5	4	0.7786	0.7758	0.0030	0.39
6	4	0.7763			
7	6	1.1253			
8	6	1.1225	1.1239	0.0014	0.12
9	6	1.1241			

Table 6A. result of robustness study

Parameters	Change In Wavelength(±2 nm)			
	Wavelength (228nm)		Wavelength (232nm)	
	4 ppm	8 ppm	4 ppm	8 ppm
Mean(n=3)	0.7761	1.4908	0.7774	1.4902
SD	0.0007	0.0006	0.0001	0.0016
% RSD	0.1917	0.0988	0.4818	0.2634

Table 6B. result of robustness study

Parameters	Change In Solvent			
	Water		0.1N NaOH	
	4 ppm	8 ppm	4 ppm	8 ppm
Mean(n=3)	0.7765	1.4910	0.7763	1.4895
SD	0.00079	0.0015	0.0006	0.0008
% RSD	0.250	0.3614	0.2086	0.2091

Table 7. result of ruggedness study

Parameters	Change in Analyst			
	Analyst I		Analyst II	
	4ppm	8ppm	4ppm	8ppm
Mean(n=3)	0.7784	1.4932	0.7769	1.4920
SD	0.0006	0.0008	0.0011	0.0006
% RSD	0.2807	0.2076	0.5467	0.1460

2. Chromatographic Method

Selection of Analytical Wavelength

The standard solutions of APR (10 µg/ml) in mobile phase were scanned in the UV region of 190 - 400 nm and the overlain spectra were recorded. It was observed that APR drugs showed the absorbance at 230 nm. So, the wavelength of detection used was 230 nm.

Mobile phase	Methanol : Water (80:20v/v) pH3
Selection of column	Cosmosil C18 (4.6mm x 250mm, Particle size: 5µm)
Injection volume	20 µL
Flow rate	0.8 ml/min
Column temperature	Room Temperature
Detection wavelength	230nm
Retention time	4.0 min

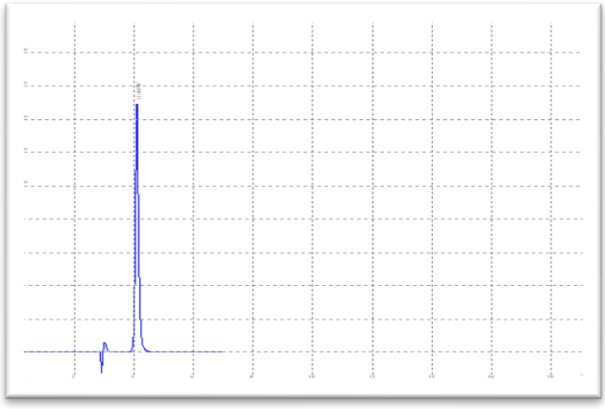

Figure 4. Typical chromatograph of Apremilast by HPLC at Optimized condition

Linearity Study

APR was found to be linear in the concentration range of 10-50 µg/ml. **Fig 5, Table 8 and Table 9**

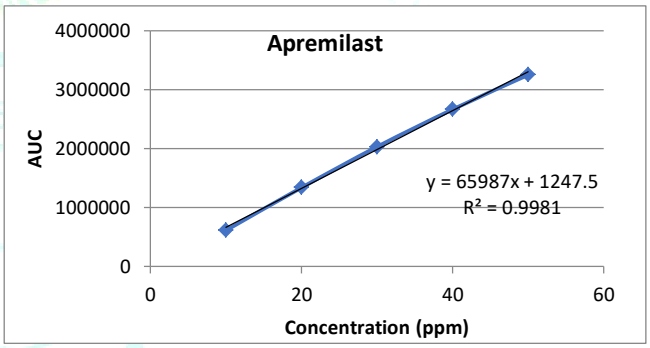

Fig 5. calibration curve by HPLC

Table 8. Result of calibration curve

Sr. No.	Conc. (µg/ml)	Area
1	10	614839
2	20	1339880
3	30	2032564
4	40	2665713
5	50	3251263

Table 9. Linear regression analysis

S.N.	Parameters	HPLC method
1	λmax (nm)	230
2	Beer's law limit (µg/mL)	10-50
3	Regression equation[y]	y = 65987x + 1247.5
4	Slope[m]	65987
5	Intercept [c]	1247.5
6	Correlation coefficient [r2]	0.9981
7	Limit of detection (LOD)	0.1646
8	Limit of quantitation (LOQ)	0.4988

Assay of Marketed Formulation

Amount of drugs present in the marketed formulation equations. Amount of APR found in the range from 99.99% and SD ± 0.015. **Table 10**

Table 10. Assay result by HPLC

Drug Name	Mean*	SD	%RSD
Otezla	99.98	0.0605	0.06

Validation Parameters

This method was validated in accordance to ICH guidelines. Percentage of recoveries of APR was found in the range from 98.65 – 101.69%. Precision of the method was determined by % RSD found among intra-day precision, inter-day precision. LOD and LOQ of APR were found to be 0.1646 and 0.4988 µg/ml, respectively. For robustness study, the effect of change in wavelength and flow rate (± 0.1 ml/min) on the Mean peak area, % RSD and % Assay were studied. Percentage RSD of each peak in all variables was found to be less than 2 %. **Table 11,12A,12B,13,14,15.**

Table 11. Result of Accuracy by HPLC

Level of addition	% Mean recovery*	SD	% RSD
50%	99.99	0.1795	0.17
100%	100.06	0.3547	0.35
150%	100.07	0.1026	0.10

Table 12A. Result of intraday precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	10	614839	615216	525.86	0.08
2	10	615817			
3	10	614993			
4	30	2032564	2033124	3313.68	0.16
5	30	2030126			
6	30	2036682			
7	50	3251263	3252790	3500.16	0.10
8	50	3256795			
9	50	3250314			

Table 12B Result of Interday precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	10	614839	614901	1283.14	0.20
2	10	613651			
3	10	616215			
4	30	2032564	2034800	3325.05	0.16
5	30	2033215			
6	30	2038621			
7	50	3253512	3254106	2445.83	0.07
8	50	3256795			
9	50	3252013			

Table 13. Result of robustness study

Sr.No	Parameter	Condition	Area	Mean	SD	%RSD
1		0.7	1333913	1337183	3024.58	0.22
2		0.8	1339880			
3		1.0	1337757			
1	Change in Wavelength	228	1340385	1340629	896.26	0.06
2		230	1339880			
3		232	1341622			

Table 14. Result of Ruggedness

Sr.No	Analyst	Conc. (µg/ml)	Mean area*	SD	% RSD
1	Analyst-I	30	2033124	3313.68	0.16
2	Analyst-II	30	2035415	1083.08	0.05

Table 15. Result of system Suitability

Sr. No.	conc. (µg/ml)	Retention Time/min	Theoretical plates	Asymmetry Factor
1	30	4.0	8988	1.25
2	30	4.0	8897	1.25
3	30	4.0	8709	1.25
4	30	4.0	8685	1.25
5	30	4.0	8479	1.24
6	30	4.0	8900	1.24
Mean		4.00	8859.66	1.246
SD		0.00	131.86	0.0051
%RSD		0.00	1.48	0.41

CONCLUSION

In the present investigation, the developed and validated, UV Spectrophotometric method were found to be simple, economical and rapid method. HPLC was found to more precise, accurate, rugged and robust for determination of Apremilast. The excipients usually present in the pharmaceutical formulation did not interfere with determination of Apremilast. Developed method can be successfully used in laboratory to measure the concentration of API in specific dosage form. This method is also beneficial for the formulation and development department. These methods are always useful for analysis, purity testing and assay. The consumption of time and chemicals is less as compare to other tedious method. This is new concept for the validation of method development and method transfer in pharmaceutical companies. The results and the statistical parameters demonstrate that the proposed UV spectrophotometric and HPLC method is simple, rapid, specific, accurate and precise.

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