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Validation of Stability Indicating Method and Degradation Kinetic Study of Apremilast

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ABSTRACT

A novel stability indicating RP- HPLC method was developed for the estimation of Apremilast in bulk and marketed formulation. Separation was achieved by using Shimadzu HPLC Analytical Technologies Limited C18 (250 mm x 4.6 mm, 5µm) as stationary phase. The optimized mobile phase consist of potassium dihydrogen ortho phosphate (pH-3.2): acetonitrile in ratio of 40:60 %v/v with flow rate of 1mL/min by using methanol as diluent. Retention time of Apremilast was found to be 5.4 min which was estimated at wavelength 360nm. Linearity of Apremilast was observed in the concentration range of 50-400µg/mL with r^2 value of 0.9999. Assay of Apremilast tablet was found to be 99.14-100.75%. Stability indicating nature of RP- HPLC method was estimated by conducting degradation kinetic study. The forced degradation of Apremilast bulk indicate that degradation in acidic, alkali, oxidative and photolysis condition were found to be 21%, 6.5%, 25.7% and 3.9% respectively. The kinetic study of apremilast in alkali degradation followed first order kinetic study. The result indicate that the developed RP-HPLC method is suitable for estimation of Apremilast in presence ofdegradant product. The above method was validated as per ICH guideline.

Keywords: Apremilast, RP-HPLC, Validation, Forced Degradation Study, Alkali Degradation Kinetic study

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Abbreviation:

APR : Apremilast

ICH : International Conference of Harmonization

RP-HPLC : High-performance liquid chromatography

%RSD : Relative standard deviation

INTRODUCTION:

Apremilast is a phosphodiesterase 4 (PDE4) inhibitor, which mediates the activity of cyclic adenosine monophosphate (cAMP), a secondary messenger. The chemical name of APR is N- 2-[(1S)-1-(3-Ethoxy-4-methoxyphenyl)-2- (methyl sulfonyl) ethyl]-1, 3-dioxo-2, 3-dihydro- 1H-isoindol -4-yl acetamide. Apremilast is indicated for the treatment of active psoriatic arthritis in adults, for the treatment of active moderate to severe psoriatic arthritis [1]. 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 [2]. This method aimed to validate the developed RP- HPLC method for determination of apremilast drug as per the ICH guideline. There are many Analytical method like U.V Spectrophotometric methods[3], HPLC methods[4] and Stability indicating RP-HPLC[5] are reported for determination of APR but there were no reported stability indicating RP-HPLC method along with degradation kinetic study for APR. Accordingly it was found that present research study has some extra advantages to develop and validate Stability indicating method for determination of APR in the presence of degradant products and to perform degradation kinetic study of APR.

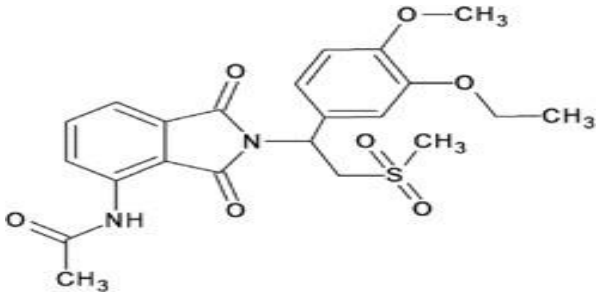

Figure: 1 Chemical structure of APR [6]

MATERIALS AND METHODS

Instrumentation: Shimadzu HPLC system with UV Detector, UV Visible spectrometer, pH meter, electronic balance.

Chromatographic condition:

Table: 1 Chromatographic Condition

Parameter	Chromatographic conditions
Instrument	Shimadzu LC20A
Column	Analytical Technologies Limited C18 column(250 mm x 4.mm,5µm)
Flow rate	1 mL /min
Detection wavelength	360nm
Injection volume	20µl
Run time	10 min
Temperature	Ambient
Mobile phase	potassium dihydrogen orthophosphate: Acetonitrile(40:60)
Diluent	90:10(methanol:DMSO)

Mobile phase preparation:

Buffer for mobile phase: Phosphate buffer (50mM) prepared by dissolving about 6.8g potassium dihydrogen orthophosphate (pH-3.2)in 1000 mL double distilled water.

Mobile phase preparation: Phosphate buffer (pH-3.2): acetonitrile mixing in ratio of 40:60%v/v. Before use the mobile phase was filtered through 0.45µm Nylon-6, 6 membrane filter followed by 5min of sonication.

Preparation of standard Stock solution: The standard stock solution of APR was prepared by weighed about 10mg APR in 10 ml volumetric flask and dissolving in Diluent and make up the mark with diluent. Further, dilute 1mL of standard stock solution in 10mL volumetric flask and volume was made up to mark with diluent.

Method Validation: The developed method was validated by different parameters like specificity, linearity, precision, accuracy, ruggedness, robustness, LOD and LOQ as per ICH Q2 (R3) guidelines [7-9, 12]

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present [8]. Typically these might include impurities, degradants, matrix etc. Specificity was estimated by injecting the APR standard solution, sample solution and blank.

Linearity: The linearity of an analytical procedure is its ability (within given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. A graph of peak area versus concentration was plotted.

Linearity was prepared at 6 independent levels from 50-400µg/mL.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions [7]

- REPEATABILITY: Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision [7]. It was performed at 6 levels of same target concentration (300µg/mL) with different standard stock solution (500 µg/mL).

  Intermediate precision: (within laboratory variation) Different days/Different equipment.

- INTRADAY PRECISION: Intraday precision can be define as within day precision .It was performed at 3 levels of 3 different concentration (50,100,150 µg/mL) of APR within a day from standard stock solution (500 µg/mL).

- INTER-DAY PRECISION: Inter-day precision can be define as within a day precision. It was performed at 3 levels of 3 different concentration (50,100,150 µg/mL) of APR on different day from same standard stock solution (500 µg/mL).

Accuracy: The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness [7]. Accuracy of method was performed at 100µg/mL in triplicate at 80%, 100% and 120% by standard addition method.

LOD: The limit of detection is determine by the individual analytical procedure of samples with known concentration of drug and by establishing the lowest level of analyte in a sample which can be detected, but not necessarily quantitated the specific value.

The Actual lowest concentration of analyte in sample detected is compared with blank result and which is based on standard deviation of the response and the slope [7].

LOD=3.3 σ/S

Where, S = the slope of calibration curve

σ = the standard deviation of the response

LOQ: The limit of quantitation is determined by the individual analytical procedure of samples and establishing the lowest amount of analyte in a sample, which can be determined with appropriate precision and accuracy [7].

LOQ=10 σ/S

Where, S=the standard deviation of response

σ = Mean of slopes of the calibration curves

Robustness: The robustness of an analytical procedure is an estimation of its capacity to remain unaffected by small, but deliberate variations in method parameters.

- Flow rate
- Concentration of acetonitrile

Ruggedness: The ruggedness is an analytical method of the degree of reproducibility of samples results obtained by analysis of the same samples under a different conditions for example in different pH, different temperature and different mobile composition [7-8].

Degradation Study: Degradation study was carried out in acidic, alkali, oxidative condition. The standard Stock solution for forced degradation study 1000µg/mL of APR

was prepared. Kinetic study of Apremilast was performed to determine the order of degradation kinetic under different stress condition [11].

Acidic Degradation:

From the standard stock solution 3mL was taken in a 10-mL volumetric flask. Further 0.5mL of 0.05N HCl was added in the flask. The mixture was kept at room temperature for 20 min. Solution was neutralized with 0.5 mL of 0.05N NaOH and the volume was made up to mark with diluent to achieve the concentration of 300µg/ mL. Solution was then filtered with 0.45-µm Nylon syringe filter and injected in the system.

Alkaline Degradation:

From the standard stock solution 3mL was taken in a 10-mL volumetric flask. Further 0.5mL of 0.05N NaOH was added in the flask. The mixture was kept at room temperature for 20 min. Solution was neutralized with 0.5 mL of 0.05N HCl and the volume was made up to the mark with diluent to achieve the concentration of 300µg/ mL. Solution was then filtered with 0.45-

µm Nylon syringe filter and injected in the system.

Oxidative Degradation:

From the standard stock solution 3mL was taken in a 10-mL volumetric flask. Further 1mL of 0.2% Hydrogen peroxide was added in the flask and the volume was made up to the mark with diluent to achieve the concentration of 300µg/ mL. Solution was then filtered with 0.45-µm Nylon syringe filter and injected in the system.

Photolytic Degradation:

Photolytic degradation about 10 mg of bulk drug was weighed and added in the Petri dish. Petri dish exposed to 5382 LUX and 144UW/cm2 for 10 days. Degradation samples were subjected to analysis after suitable dilutions with diluent

Degradation Kinetics Study:

The Degradation kinetic study was done in alkaline condition. The conditions selected for the kinetic study was 0.05 N and 0.1N of NaOH at 30ºC and 50°C .Then 0.5 mL Stock solutionin 10 mL volumetric flask and 2 mL of 0.05N and 0.1N NaOH was further added to the flask. The solution was subjected to two different conditions at 30°C and 50ºC.The solution was neutralized with 0.5 mL of 0.05N and 0.1N HCl respectively and made up to mark with diluent and make the concentration 300 µg/mL. Solution was filtered with 0.45-µm Nylon syringe filter and injected in the system. Percentage degradation of APR was estimated at above mentioned conditions.

RESULT AND DISCUSSION:

Specificity:

The specificity of the analytical method of APR is established by injecting the sample solution into the HPLC System.

- Diluent solution is used as blank (methanol)
- Standard solution of APR(100µg /mL)
- Test solution(APR marketed formulation)

The specificity data of APR mention in **table 2**.

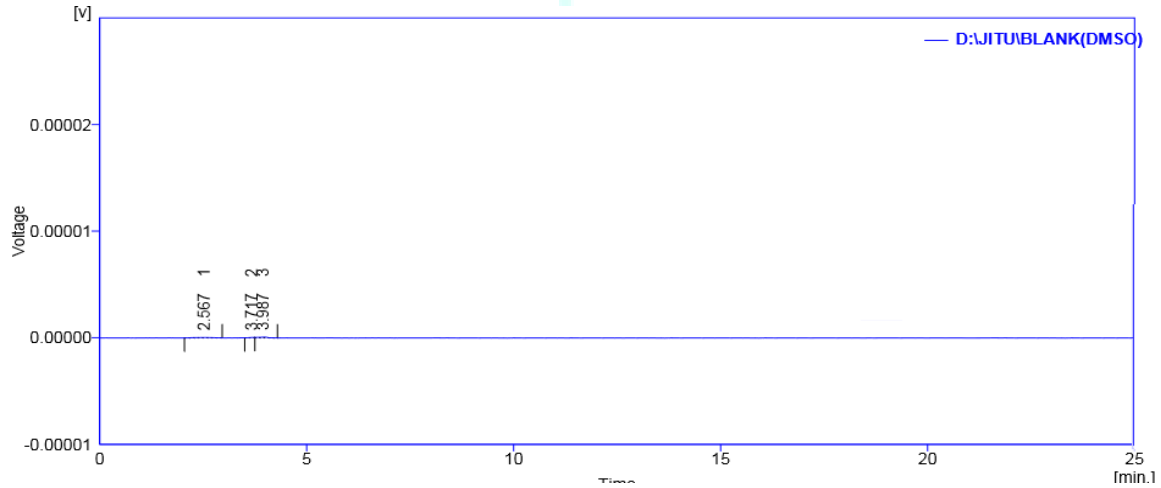

Figure.2 Chromatogram of blank methanol

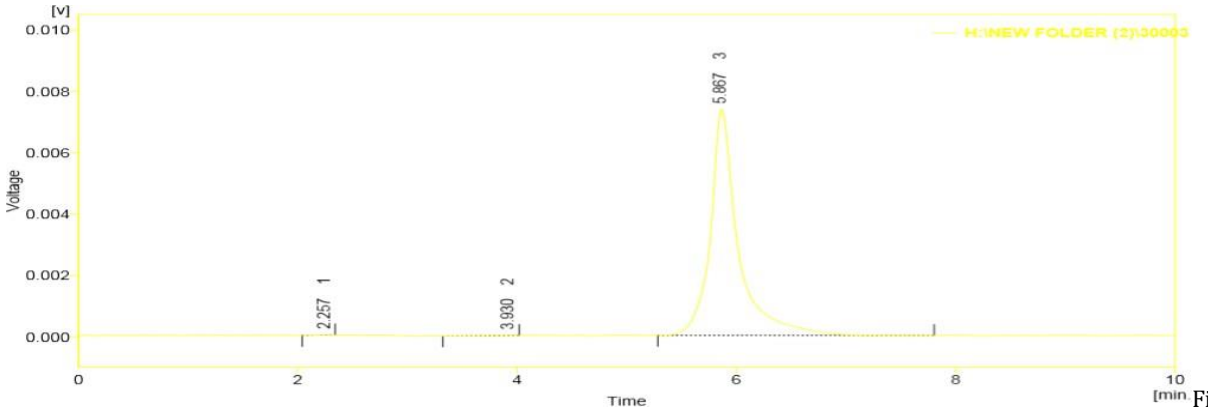

Figure.3 Chromatograph of standard APR

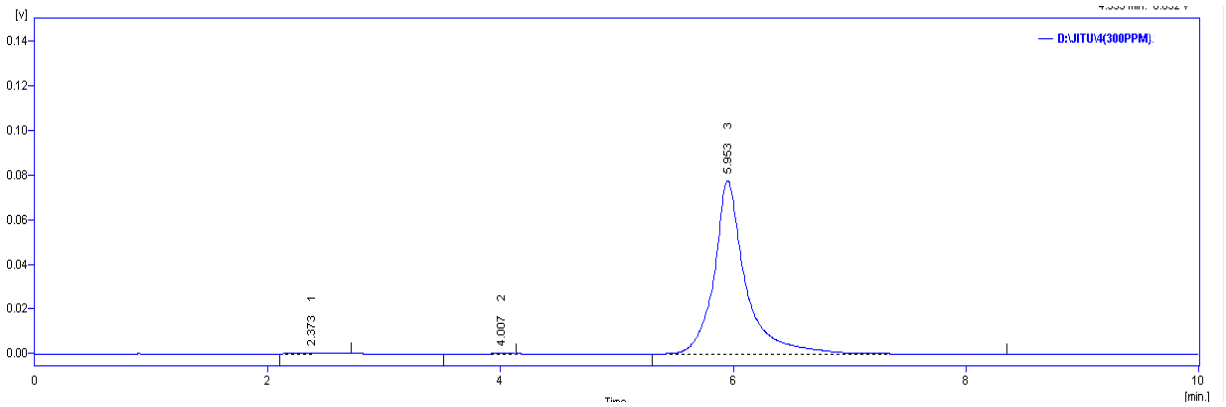

Figure.4 chromatograph of Test solution APR Table: 2 Specificity data of APR

Sr. no.	Sample Name	Drug Name	Specificity
1	Blank	No Peak	-
2	Standard	APR	Specific
3	Test	APR	Specific

System Suitability:

The prepared 100µg/mL standard solution of APR was measured for parameters like retention time (RT), Theoretical plate and Tailing Factor by injecting the solution at three replicate level. Below mentioned values are within acceptable limit of chromatographic condition. The system suitability data of APR mention in **table3**.

Table: 3 System Suitability data of APR

Parameters	Mean(n=3)	%RSD
RT(min)	5.41	1.51
Theoretical plate	4958.67	1.68
Tailing factor	1.306	0.93

Linearity:

The calibration curve of APR is linear in the concentration range of 50-400µg/mL. The regression data analysis of RP-HPLC method is mention in **table 4.**

Table: 4 linearity data of APR

Concentration (µg/mL)	Area(mv) Mean(n=6)	%RSD
50	393.844	0.45
100	806.067	0.69
200	1633.766	0.47
300	2408.507	0.33
400	3207.949	0.42

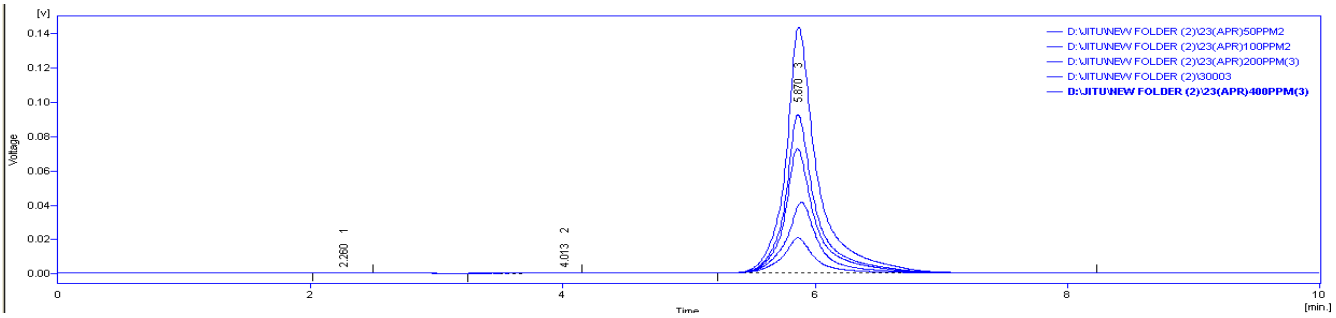

Figure: 5 chromatograph of APR Linearity Conc. (µg/mL)

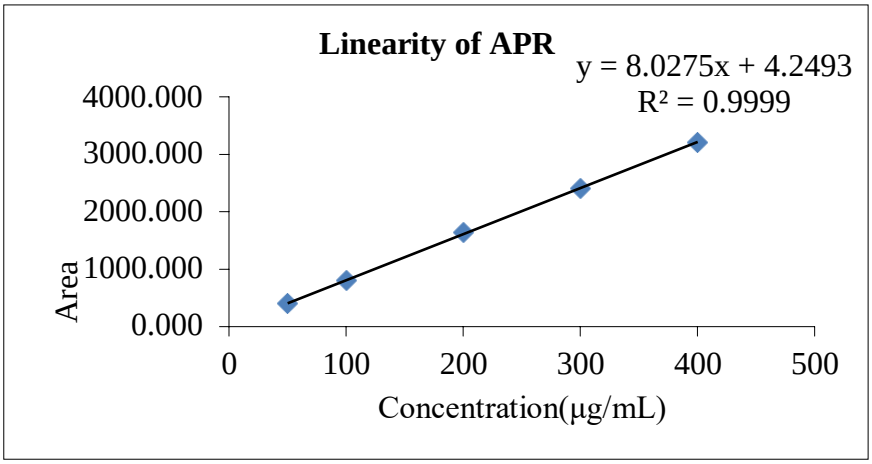

Figure: 6 Calibration curve of APR Table: 5 Regression data analysis RP-HPLC

Parameter	APR
Wavelength	360nm
Range	50-400µg/mL
Regression Equation	Y=8.0275x+4.2493
Slop(m)	8.0275
Intercept(c)	4.249
Correlation coefficient	0.9999

Precision:

Precision of analytical method was estimated by Repeatability, intraday precision and inter day precision of the APR standard solution. The Repeatability %RSD was 0.77 and intraday precision %RSD was 0.92 and that of inter day precision was 1.25. The results of repeatability data, intraday and interday precision data of APR are shown in **table 6**, **table 7 and table 8** respectively**.**

Table: 6 Repeatability data of APR

Repeatability(100µg/mL)	
Replicates	**Area**
1	804.56
2	796.31
3	810.72
4	802.2
5	794.32
6	806.3
mean	802
%RSD	0.77

Table: 7 Intraday Precision data of APR

Concentration(µg/mL)	Average area (n=3)	%RSD
50	394.74	0.49
100	803.77	0.92
300	2433.62	0.42

Table: 8 Interday Precision data of APR

Concentration(µg/mL)	Average area (n=3)	%RSD
50	394.04	0.83
100	801.39	1.25
300	2479.05	1.20

Accuracy:

Accuracy was calculated by performing the recovery study. A standard known quantity of APR was added into aliquots of sample solutions at the three different levels in 80%, 100%, 120% and then diluted with the solvent. The percentage recovery obtained in range 99.71%, 99.48%, and 99.86% respectively as mention in **table 9**.

Table: 9 Accuracy data of APR (n=3)

Spiked level	Conc present in mixture(µg/mL)	Conc added (µg/mL)	Conc recovered ((µg/mL)	%Recovery ±SD
80%	100	80	179.54	99.71±0.37
80%	100	80	178.79	
80%	100	80	180.13	
100%	100	100	200.08	99.48±0.51
100%	100	100	198.72	
100%	100	100	198.09	
120%	100	120	221.13	99.86±0.59
120%	100	120	219.26	
120%	100	120	218.64	

Limit of Detection and Limit of Quantification:

Limit of Detection was 0.04 and the Limit of Quantification was 0.13 was calculated.

Robustness:

The developed method was found to be robust when changes were made in parameters as mentioned in table like change in mobile phase, change in flow rate, and change in pH. Average peak area and %RSD was noted. The Robustness data of APR mention in **table 10.**

Assay of Marketed formulation:

The assay was estimated by taking twenty tablets of APR weighted and crushed. The solution was prepared by weighed 10mg APR in 10mL volumetric flask and making volume up to the mark with diluent. From stock solution take 1mL in 10mL volumetric flask and making volume up to mark with diluent. The Assay result of APR mention in **table 11.**

Table: 10 Robustness data of APR (n=3)

Factor	Level of change	Average peak Area	%RSD
Mobile phase	KH_2PO_4:ACN=58:42	779.7	0.72
	KH_2PO_4:ACN=60:40	777.9	0.46
	KH_2PO_4:ACN=56:44	778.3	0.52
Flow rate	0.8	776.2	0.51
	1	777.9	0.46
	1.2	777.7	0.45

Table: 11 Assay data of APR

Sr.No	Recovered (n=6)	ean % Drug Recovered	RSD of Drug Recovered
1	99.14	99.78	0.68
2	100.20		
3	99.90		
4	100.75		
5	99.91		
6	99.84		

Degradation Study: The degradation was observed in acidic condition (21%) and in alkaline condition (6.5%), in 0.2% v/v Hydrogen peroxide condition (25.7%) and in photolysis degradation (3.9). Degradation behaviour in different condition mention in **table 12**.

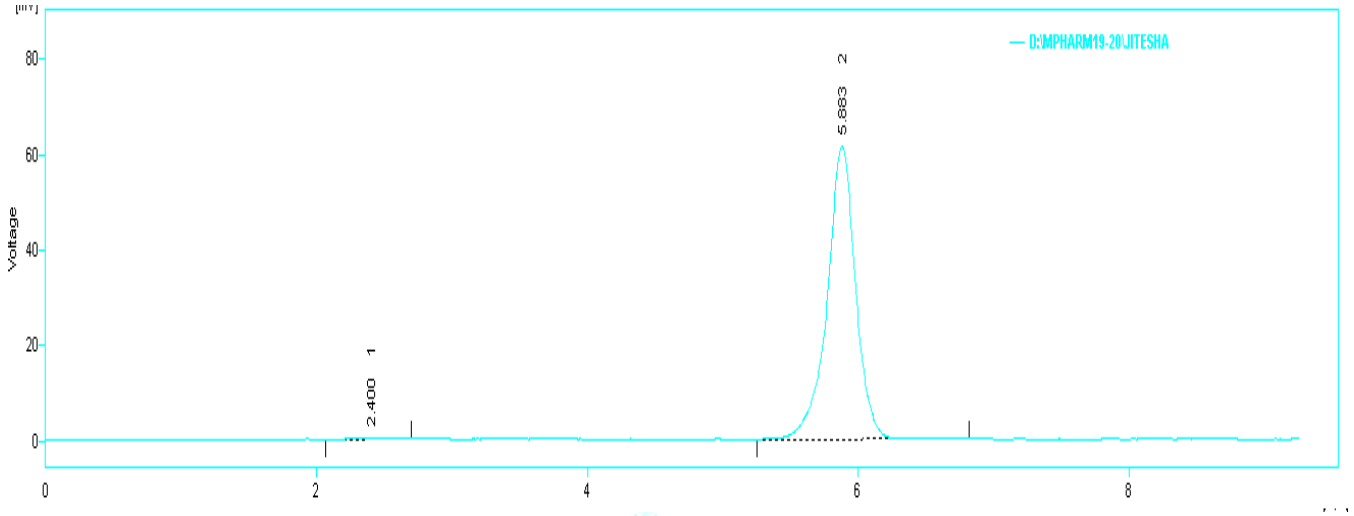

Figure: 7 Alkaline Degradation of APR

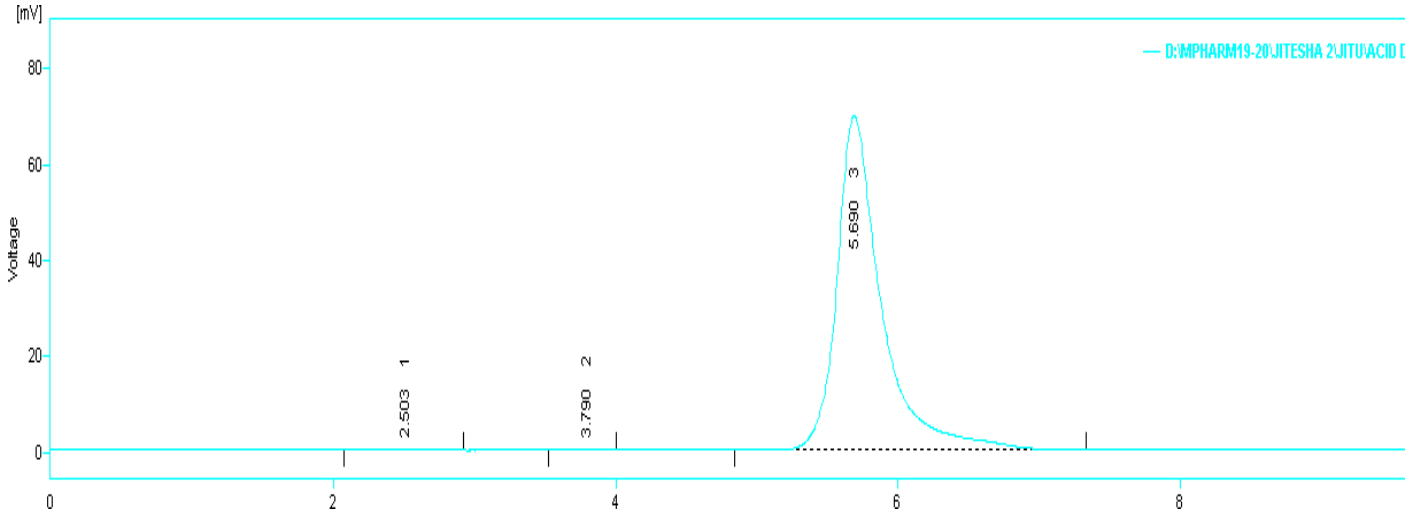

Figure: 8 Acidic Degradation of APR

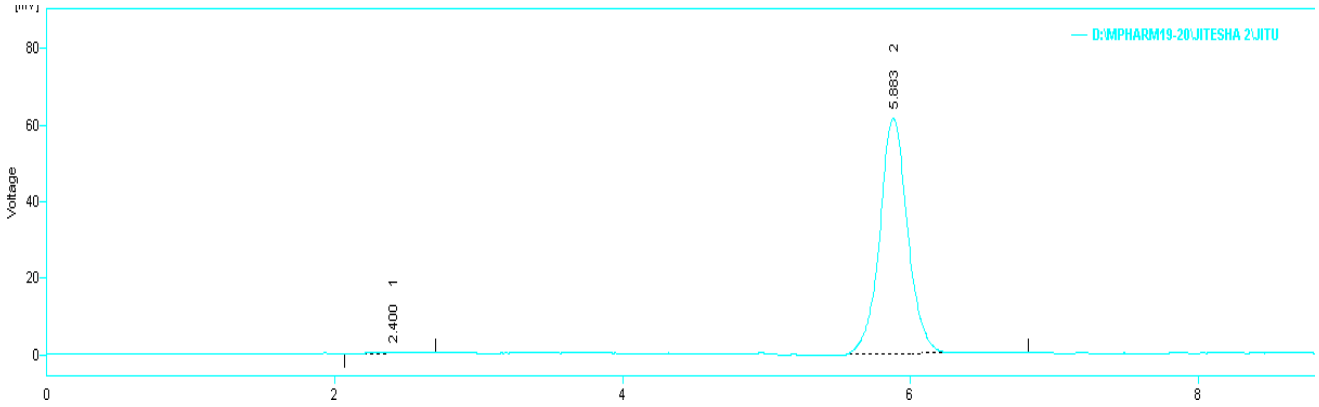

Figure: 9 oxidative Degradation of APR

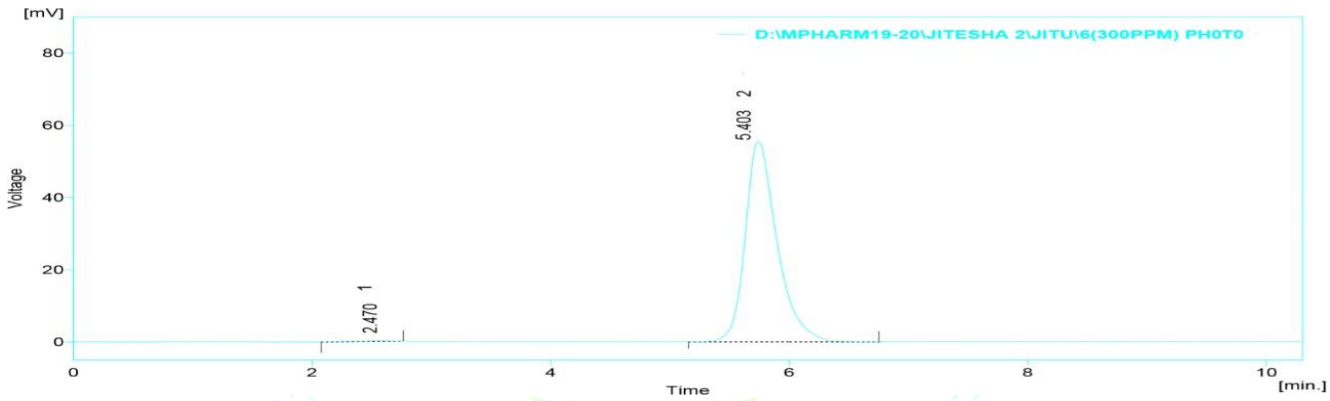

Figure: 10 Photolytic degradation Table: 12 Stability studies of APR

Degradation Type	Stress condition	Time	%Assay	% of Degradation Product
Control sample	As Such	-	100	-
Acidic degradation	0.05M MeoH HCl	20min	79	21
Alkaline degradation	0.05M MeoH NaOH	20min	93.5	6.5
Oxidative degradation	0.2% H_2O_2	20min	74.3	25.7
Photolysis degradation	5382 LUX and 144UW/cm2	10 days	96.1	3.9

Degradation kinetic study:

Degradation kinetic study of bulk drug in alkaline condition at 30ºC and 50ºC that result show that the decrease in area with increasing the time. The plots of zero order (%drug remaining vs time), first order (log c value vs time) and second order (1/log c vs time) were plotted individually. From the result obtain it was concluded that the r^2 value of first order appeared high as compared to the r^2 value of zero order and second order. So, alkali degradation of APR followed first order kinetic. The result of alkaline degradation kinetic study of APR mention in **table 13.**

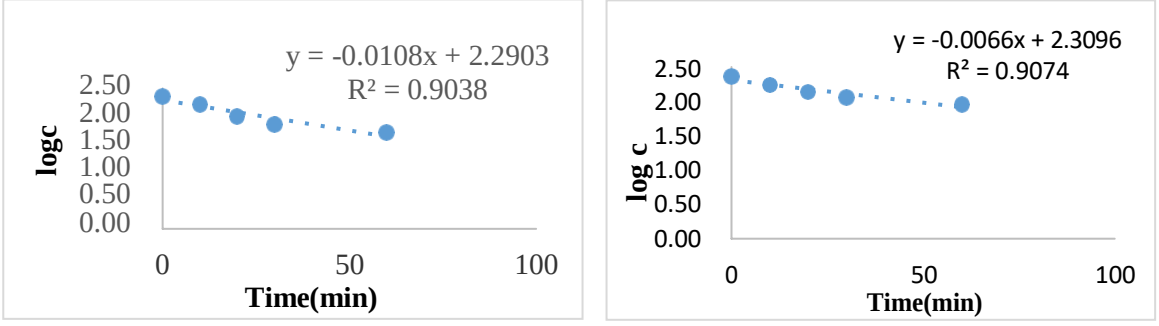

Figure: 11 Log C vs Time Graph for alkaline condition in 0.05N NaOH at 30ºC (a) and 50ºC (b)

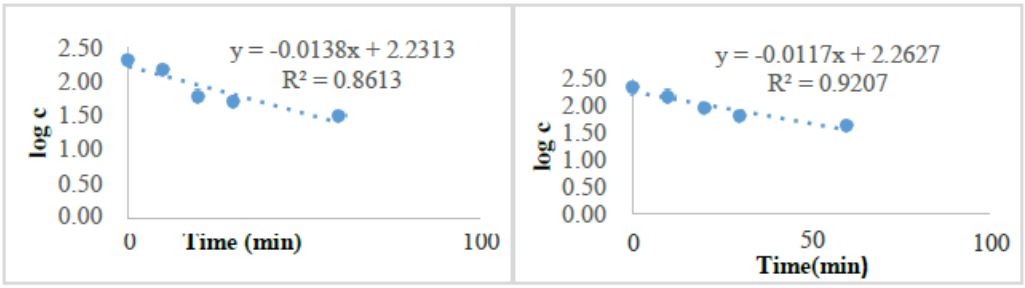

Figure: 12 Log C vs Time Graph for alkaline condition in 0.1N NaOH at 30ºC (a) and 50ºC (b) Table: 13 Degradation Kinetic study of APR in alkaline condition

Table: 13 Degradation Kinetic study of APR in alkaline condition

As such Area	2385.286	Degradation kinetic study			
Target conc(µg/mL)	300				
Degradation condition	**Temperature**	**Time(min)**	**%Assay**	**Degradation rate constant k**	**t ½(min)**
0.05N NaOH	30°C	0	77.15	0.000	0.00
		10	62.84	0.004	180.31
		20	47.82	0.009	75.36
		30	41.11	0.012	56.38
		60	28.96	0.020	34.93
	50°C	0	77.15	0.000	0.00
		10	57.43	0.006	124.26
		20	37.35	0.014	48.45
		30	28.11	0.021	33.77
		60	17.23	0.032	21.51
0.1N NaOH	30°C	0	71.05	0.000	0.00
		10	50.17	0.007	103.22
		20	30.61	0.017	40.55
		30	21.42	0.023	30.11
		60	14.13	0.036	19.33
	50°C	0	71.05	0.000	0.00
		10	47.41	0.008	88.32
		20	25.60	0.021	32.81
		30	16.76	0.031	22.07
		60	10.52	0.044	15.73

CONCLUSION:

The developed RP-HPLC method is simple, specific, accurate, precise and stability indicating which can be useful in routine analysis laboratories for the determination of APR in bulk drug and pharmaceutical formulation without any interference from excipient, impurity and degradation product. This method have been validated as per ICH guidelines, and it meets all the acceptance criteria given in ICH guidelines. Degradation kinetic study shows that APR follows first order kinetic in alkaline condition.

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