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 r² 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 degradation 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 of degradant product. The above method was validated as per ICH guideline.

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

INTRODUCTION:

Apremilast is a phosphodiesterase 4 (PDE4) inhibitor, which mediates the activity of cyclic adenosine monophosphate (cAMP), a secondary messenger. The chemical name of Apremilast is N-[2-([1S]-1-[3-Ethoxy-4-methoxyphenyl]-2-[(methyl sulfonfyl) ethyl]-1, 3-dioxo-2, 3-dihydro- 1H-isindol -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 UV Spectrophotometric method(s)[3], HPLC methods[4] and Stability indicating RP-HPLC[5] are reported for determination of Apremilast but there were no reported stability indicating RP-HPLC method along with degradation kinetic study for Apremilast. Accordingly it was found that present research study has some extra advantages to develop and validate Stability indicating method for determination of Apremilast 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:

| Parameter       | Chromatographic conditions |
|-----------------|----------------------------|
| Instrument      | Shimadzu LC20A             |
| Column          | Analytical Technologies Limited C18 column (250 mm x 4.6 mm, 5 µm) |
| Flow rate       | 1 mL/min                   |
| Detection wavelength | 360 nm                   |
| Injection volume | 20 µl                     |
| 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 (50 mM) prepared by dissolving about 6.8 g 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 0.45 µm Nylon-6, 6 membrane filter followed by 5 min of sonication.

Preparation of standard stock solution: The standard stock solution of APR was prepared by dissolving about 10 mg APR in 10 mL volumetric flask and dissolving in diluent and make up the mark with diluent. Further, dilute 1 mL of standard stock solution in 10 mL 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, 8, 9].

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present [9]. 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 50-400 µg/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 (300 µg/mL) with different standard stock solution (500 µg/mL).

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

- INTRADAY PRECISION: Intraday precision can be defined 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 defined 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 100 µg/mL in triplicate at 80%, 100% and 120% by standard addition method.

LOD: The limit of detection is determined 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 [7-9].

Degradation Study: Degradation study was carried out in acidic, alkaline, oxidative condition. The standard stock solution for forced degradation study 1000 µg/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 50ºC. Then 0.5 mL Stock solution in 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 30ºC 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 to achieve the concentration of 300 µg/mL. Solution was then 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.
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.

| 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.

| 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 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    |

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               | 360 nm       |
| Range                    | 50-400 µg/mL |
| Regression Equation      | $y = 8.0275x + 4.2493$ |
| Slope (m)                | 8.0275       |
| Intercept (c)            | 4.249        |
| Correlation coefficient  | 0.9999       |

**Precision:**

Precision of analytical method was estimated by Repeatability, intraday precision and interday precision of the APR standard solution. The Repeatability %RSD was 0.77 and intraday precision %RSD was 0.92 and that of interday 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 (100 µg/mL) | Area   |
|---------------------------|--------|
| Replicates                |        |
| 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                    | 394.04              | 0.83 |
| 100                   | 801.39              | 1.25 |
| 300                   | 2479.05             | 1.20 |

Table: 8 Interday Precision data of APR

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.

Linearity of APR

\[ y = 8.0275x + 4.2493 \]

\[ R^2 = 0.9999 \]
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.

| 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                 | 99.48±0.51    |
| 80%          | 100                            | 80                 | 180.13                 | 99.86±0.59    |
| 100%         | 100                            | 100                | 198.72                 | 99.48±0.51    |
| 100%         | 100                            | 100                | 198.09                 | 99.48±0.51    |
| 120%         | 100                            | 120                | 221.13                 | 99.86±0.59    |
| 120%         | 100                            | 120                | 219.26                 | 99.86±0.59    |
| 120%         | 100                            | 120                | 218.64                 | 99.86±0.59    |

| Level of change | Average peak Area | %RSD |
|-----------------|-------------------|------|
| KH2PO4:ACN=58:42 | 779.7             | 0.72 |
| KH2PO4:ACN=60:40 | 777.9             | 0.46 |
| KH2PO4:ACN=56:44 | 778.3             | 0.52 |
| 0.8             | 776.2             | 0.51 |
| 1               | 777.9             | 0.46 |
| 1.2             | 777.7             | 0.45 |
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.

Table: 11 Assay data of APR

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

Figure: 7 Alkaline Degradation of APR

Figure: 8 Acidic Degradation of APR
Degradation kinetic study:

Degradation kinetic study of bulk drug in alkaline condition at 30°C and 50°C 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 alkali degradation kinetic study of APR mention in Table 13.

| 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% H2O2 NaOH   | 20min  | 74.3    | 25.7                     |
| Photolysis degradation    | 5382 LUX and 144UW/cm2 | 10 days | 96.1    | 3.9                      |

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