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Research Article

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Reversed Phase High Performance Liquid Chromatography Method for Determination of Assay and Forced Degradation Study of Apremilast from Active Pharmaceutical Dosage Form

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ABSTRACT

A new and accurate high performance liquid chromatography method is developed for determination of apremilast from active pharmaceutical ingredients. An inertsil C8 (250 X 4.6 mm) 5µ column is used for assay of apremilast. The mixture of buffer and methanol (47:53 % v/v) is used as mobile phase. The buffer made up of 0.1% orthophosphoric acid in water adjusted pH 2.8 with potassium hydroxide. The detector wavelength set at 230 nm. The mixture of buffer and acetonitrile (70:30% v/v) was used as a diluent. As per ICH guidelines, the method was validated containing parameter of system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze apremilast from active pharmaceutical ingredients.

Keywords: Apremilast; RP-HPLC; Orthophosphoric acid; Acetonitrile; Methanol

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INTRODUCTION

In this study, new HPLC method was developed for assay and forced degradation study of apremilast from pharmaceutical dosage form. The chemical name of apremilast is N-{2-[(1S)-1-(3-Ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl]-1, 3-dioxo-2, 3-dihydro-1H-isoindol -4-yl} acetamide. It is a Food and Drug Administration approved drug used for treatment of psoriasis and psoriatic arthritis. It may also be useful for other immune system related inflammatory diseases. The drug acts as a selective inhibitor of the enzyme phosphodiesterase 4(PDE4) and inhibits spontaneous production of TNF –alpha from human rheumatoid synovial cells. The US FDA approved Apremilast for the treatment of moderate to severe plaque psoriasis. It is also being tested for its efficacy in treating other chronic inflammatory diseases such as ankylosing spondylitis, Behcet's disease, and rheumatoid arthritis [1]. The existing available literature reports HPLC [2-6], LC-MS [7], UV spectrometry [8-9] and HPTLC [10] methods. The present study aims is to develop a new, simple, suitable and accurate HPLC method for the determination of apremilast.

MATERIALS AND METHODS

Chemical and reagents

Standard and sample of apremilast was obtained from reputed firm with certificate of analysis. A analytical grade, orthophosphoric acid was used. The HPLC grade water was used from Merck.

Instrumentation

The liquid chromatographic system of Shimadzu make containing LC 2010 C series and diode array detector was used. The LC solution software was used for determination of apremilast peak.

A SHIMADZU analytical balance was used.

Standard solution preparation:

Standard solution

The 500 µg /ml standard solution of apremilast was prepared. The working standard solution of concentration 50 µg /ml was prepared from above solution.

Sample preparation

The 500 µg /ml sample solution of apremilast was prepared. The working sample solution of concentration 50 µg /ml was prepared from above sample solution.

Chromatographic condition

An inertsil C8 (250 X 4.6 mm) 5µ column with 40°C temperature was used for chromatographic separation. The mixture of buffer and methanol (47:53 % v/v) was used as mobile phase. The buffer was 0.1% orthophosphoric acid in water adjusted pH 2.8 with potassium hydroxide. The pump flow rate was set at 1.5 ml /min. The detector wavelength was set at 230 nm. (Fig.1). The injection volume was 20 µl.

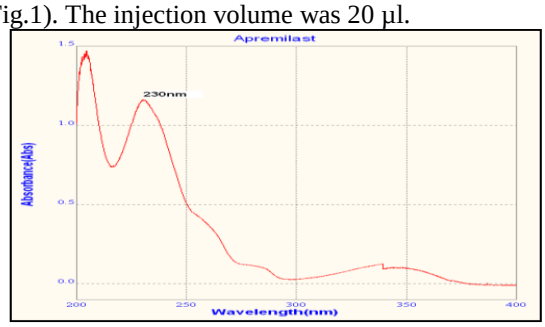

Figure 1: UV spectra of apremilast

Method validation

System suitability

The system suitability test was used to verify the parameter such as theoretical plates (N), tailing factor, and relative standard deviation by injecting standard solution. The result was indicates a good performance of system as shown in table 1.

Table 1: System suitability results

Retention Time	Area	% Area	USP theoretical plates	USP Tailing factor
8.3 minutes	3467600	100	3830	1.1

Specificity

Specificity is used to measure analytical response in the presence of its impurities. Hence this parameter was performed by injecting blank and standard apremilast to prove the specificity. The typical chromatogram of the standard and sample are given in figure 2 and 3 respectively.

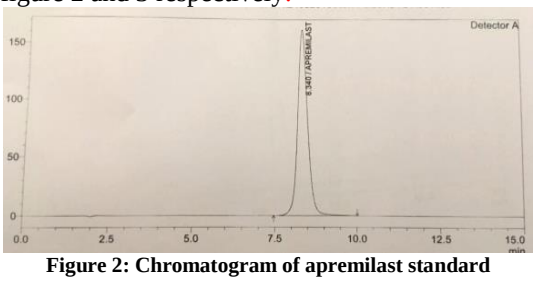

Figure 2: Chromatogram of apremilast standard

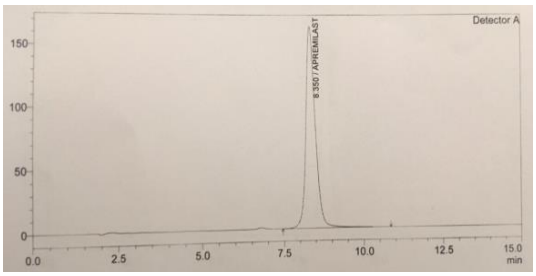

Figure 3: Typical chromatogram of apremilast (sample)

Linearity

Linearity parameter was performed by preparation different concentration of solutions and the linear calibration curve was obtained throughout the concentration range. Regression analysis was done on the peak area (y) v/s concentration (x). The obtained results are tabulated in table 2.

Table 2: Results of linearity parameter

Parameters	Results
Correlation Coefficient (r)	1
% Intercept (y)	2301.8
Slope (m)	68263

Accuracy

The accuracy parameter was determined by preparation of mixture containing know amount of drug corresponding to 50 %, 100 % and 150 %. The accuracy was expressed as percentage of analyte recovered. The results of the recovery analysis are shown in table 3.

Table 3: Results of accuracy

No. of Injection	Level (%)	Amount spiked (µg/ ml)	Area	Amount found (µg/ ml)	% Recovery
Change 1	50%	**25.06**	1720693	24.88	99.41
2			1721526	24.97	99.46
3			1722086	24.9	99.49
1	100%	**50.15**	3433021	49.79	99.17
2			3434000	49.61	99.2
3			3435148	49.84	99.23
1	150%	**75.11**	5138435	74.34	98.96
2			5141170	74.37	99.14
3			5144553	74.42	98.99
			Mean		**99.23**
			Standard Deviation		**0.1927**
			% RSD		**0.19**

Precision

The method precision test was carried out of the drug using analytical method in six replicates. The value of relative standard deviation shows method is precise. The results of the same are tabulated in the table 4.

Table 4: Method precision of apremilast

Sr. No.	Test samples	Assay (%)
1	Test preperation-1	98.21
2	Test preperation-2	98.44
3	Test preperation-3	98.91
4	Test preperation-4	98.58
5	Test preperation-5	98.52
6	Test preperation-6	98.35
Mean		**98.5**
Standard Deviation		**0.238**
% RSD		**0.24**

Forced degradation study:

The forced degradation study was performed using condition of acid, base, oxidation, thermal and photolysis.

Acid degradation:

Forced degradation in acidic condition was performed by adding 5 ml of 1N hydrochloric acid to 100 ml flask containing 50 mg of apremilast test, kept this mixture at room temperature for 30 mins. About 50 ml of diluent was added to this acidic mixture and sonicated till dissolve The volume was make up to mark to obtained concentration 500 µg /ml. Diluted 5 ml of 500 µg /ml solution to 50 ml volumetric flask with diluent to get final concentration 50 µg /ml. The study indicates that the drug was degraded and well separated from degradation product

Base degradation:

Forced degradation in basic condition was performed by adding 10 ml of 0.01N sodium hydroxide to 100 ml flask containing 50 mg of apremilast test, kept this mixture at room temperature for 1 hr. About 50 ml of diluent was added to this alkaline mixture and sonicated till dissolve. The volume was make up to mark to obtained concentration 500 µg /ml. Diluting 5 ml of 500 µg /ml solution to 50 ml volumetric flask with diluent to get final concentration 50 µg /ml. the chromatogram shows that the drug was degraded and separated from degradation product.

Oxidative degradation:

50 mg of apremilast test was taken in 100 ml volumetric flask, added 3ml of 3% hydrogen peroxide solution and kept this mixture at room temperature for 2 hrs. About 50 ml of diluent was added to this mixture and sonicated till dissolve. The volume was make up to mark to obtained concentration 500 µg /ml. Diluting 5 ml of 500 µg /ml solution to 50 ml volumetric flask with diluent to get final concentration 50 µg /ml. From the observation it was concluded that in oxidative degradation the drug was found stable. Degradation was not observed in above condition.

Thermal degradation:

The drug substance was kept at 105oC for 24 hrs to study the effect of temperature. A 50 mg of degradable drug substance was weighted accurately and transferred in 100 ml volumetric flask. About 50 ml of diluent was added and sonicated for 1 minute. The volume was make up to mark to obtained concentration 500 µg /ml. Diluting 5 ml of 500 µg /ml solution to 50 ml volumetric flask with diluent to get final concentration 50 µg /ml. From the results of assay it was shows that the drug was stable at thermal degradation.

Photolysis degradation:

To study the effect of photolysis, the drug substance was exposed to UV light (254 nm) for 24 hrs. A 50 mg of degradable drug substance was weighted accurately and transferred in 100 ml volumetric flask. About 50 ml of

diluent was added and sonicated for 1 minute. The volume was make up to mark to obtained concentration 500 µg /ml. Diluting 5 ml of 500 µg /ml solution to 50 ml volumetric flask with diluent to get final concentration 50 µg /ml. From the result of photolysis condition, the drug was found stable.
The results on degradation study were given in table 5.

Table 5: Statistical evaluation of the data subjected to degradation study of apremilast

Stress condition	Assay (%) observed	% Degradation observed
Acidic degradation	95.4	4.6
Base degradation	85.1	14.9
Oxidative degradation	98.4	No degradation
Thermal degradation	98.6	No degradation
Photolysis degradation	98.5	No degradation

Robustness
The robustness of the method was determined by changing small but deliberate change in method parameter.
The typical variations are given below:
The flow rate changed by + 0.02 ml /min
The pH of buffer changed by + 0.2
Under the condition of above variation the results was recorded and indicates that method is robust.
Method application
The present HPLC method was validated and applied for the determination of apremilast from its formulation. About twenty tablets were used and crushed to get a powder form. Accurately 50 mg apremilast, sample was weighed and transferred in 100 ml volumetric flask. It was sonicated with 50 ml of diluent for 1 minute. The volume was make up to mark to obtained concentration 500 µg /ml. From this solution 50 µg /ml was prepared. Under the specified condition the sample was injected and compared analyte peak with that of respective standard. The (%) assay results were expressed in table 4. It is observed that the apremilast product meets requirement.

RESULT AND DISCUSSION

The results of method validation parameter containing specificity, linearity, accuracy, method precision and robustness of proposed method shows low value of standard deviation as well as low values of relative standard deviation. All the parameter satisfactory meets criteria as per ICH guideline.
Using standard addition method, the results of accuracy and reproducibility of the proposed method was determined. The forced degradation studies of apremilast was done on stress conditions under acidic condition by using 1N hydrochloric acid was found to be 4.6% for 30 mins. The product degradation under base condition using 0.01N sodium hydroxide was found to be 14.9% for 1 hr. There is no degradation observed under the condition of oxidation degradation, thermal degradation and photolysis degradation.
Thus the developed method is used for assay of apremilast from active pharmaceutical ingredient. The new, precise, specific and accurate method was developed for degradation and assay for the determination of apremilast. Hence the proposed RP-HPLC method is recommended for routine evaluation of quality of apremilast in active pharmaceutical ingredient and formulation.

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