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HPLC Method Development and Validation for Estimation of Apremilast in Bulk and Tablet Dosage Form

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ABSTRACT:

This study presents a new RP-HPLC method for the quantification of apremilast in both bulk and pharmaceutical tablet forms. The method uses a SunQ C18 column with a mobile phase of acetonitrile: sodium acetate buffer (pH 5) in the ratio of 80:20 and a flow rate of 1.0 ml/min Detection at 230 nm, with a sharp peak at 3.692 minutes for apremilast work. The method exhibits good linearity (r2 = 0.995) over a concentration range of 5-30 µg/mL. Strict ratings follow ICH Q2 R1 guidelines, emphasizing accuracy, precision and robustness. The detection and quantitation limits were set at 0.519 µg/mL and 1.572 µg/mL, respectively. This simple and sensitive method provides a reliable tool for the production of apremilast, which is commonly used in clinical trials.

Keywords: Reverse Phase High performance liquid chromatography (RP-HPLC), Apremilast, Validation.

INTRODUCTION:

Apremilast (APR), a phthalimide derivative with the chemical name N-{2-[(1S)-1-(3-Ethoxy-4-methoxyphenyl)-2- (methyl sulfonyl) ethyl]-1, 3-dioxo-2, 3-dihydro-1H-isoindol -4-yl} acetamide (Figure 1), is a Food and Drug Administration (FDA)-approved drug for treating psoriasis and psoriatic arthritis (FDA website). Its potential also extends to other immune-related inflammatory diseases (Papp et al., 2012). APR acts as a selective phosphodiesterase 4 inhibitor and suppresses TNF-alpha production in human rheumatoid synovial cells (Kim et al., 2015).

While existing literature reports various methods for APR analysis, including UV spectrophotometry (Mondal et al., 2019; Chandra et al., 2018), HPLC (Sonawane et al., 2018; Patil et al., 2019; Chaudhari et al., 2019), HPTLC (Chaudhari et al., 2018; Bhole et al., 2019), UPLC-MS/MS (Chen et al., 2016), and stability-indicating RP-HPLC (Lonkar et al., 2017; Ravisankar et al., 2017), we aimed to develop a simpler HPLC

method for quantifying APR in both bulk and tablet forms. This method adheres to the validation criteria outlined in ICH guideline Q2 R1.

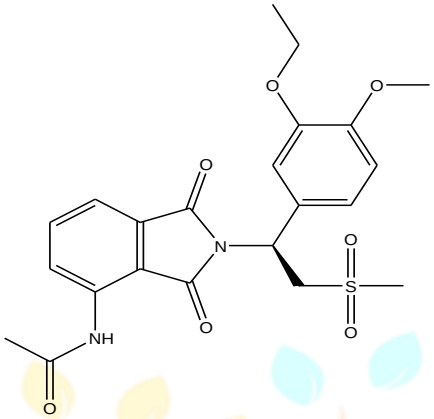

Fig.1. Apremilast structure

MATERIALS AND METHODS:

A. Chemicals and Reagents:

High-purity Acetonitrile, Sodium Acetate, Hydrochloric Acid, 3% Hydrogen Peroxide, and Sodium Hydroxide were obtained from LobaChemie Pvt. Ltd., Mumbai, India. HPLC-grade water was prepared using a PURELAB UHQ-II water purification system.

B. HPLC Apparatus and Conditions:

The analysis utilized a JASCO HPLC system equipped with a model PU 2080 Plus pump, a Rheodyne sample injector (50 µL), a JASCO UV-2075 UV-VIS detector, and Borwin chromatography software (version 1.50). Detection was set at 230 nm wavelength. The optimized mobile phase consisted of Acetonitrile: Sodium acetate buffer (pH 5) at a ratio of 80:20 (v/v) delivered at a flow rate of 1 mL/min in isocratic mode. Additional equipment involved a JASCO V-730 UV-Visible Spectrophotometer, a NEWTRONIC Photostability chamber, a JET-VAC-J1 Vacuum pump, and a Kumar Laboratory Oven.

C. Mobile Phase Optimization:

To achieve optimal chromatographic separation, various columns and mobile phase compositions were evaluated. SunQ C18 columns (No: 73, 69) were tested with different proportions of Acetonitrile: water and Methanol: water (70:30, 80:20, 90:10), but none yielded satisfactory peak shapes. Ultimately, using Sodium acetate buffer (pH 5) in combination with Acetonitrile led to successful separation with desirable system suitability parameters. The optimal conditions are summarized in Table X.

D. Mobile Phase Preparation:

The mobile phase was prepared by mixing Acetonitrile and Sodium acetate buffer (pH 5) in a 80:20 ratio, followed by filtration through a 0.45 µm membrane filter. Sonication for 15 minutes using an ultrasonic bath ensured thorough mixing.

E. Detection Wavelength Selection:

A standard stock solution of Apremilast (1000 µg/mL) was prepared in Acetonitrile. Subsequent dilutions were made using the mobile phase. Scanning the solution over the 200-400 nm range and analyzing the resulting spectra revealed that a 10 µg/mL Apremilast solution exhibited maximum absorbance at 230 nm.

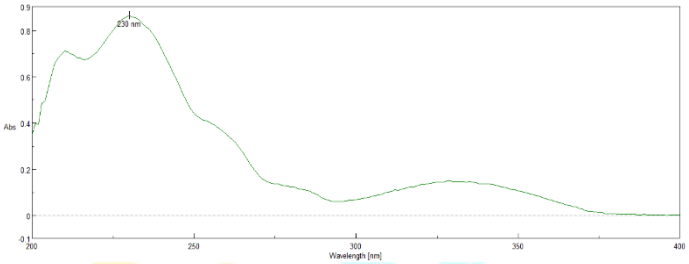

Fig. *2.*UV- Spectrum of Apremilast (10 ug*/ml*).

A. Standard Stock Solution Preparation:

A 1000 µg/mL standard stock solution of Apremilast (APR) was prepared by dissolving 10 mg of APR in 10 mL of acetonitrile. Subsequent dilutions to 100 µg/mL and lower concentrations were made using the mobile phase. The retention time of APR was observed to be 3.692 minutes.

B. Sample Solution Preparation (Tablet Formulation Analysis):

Twenty tablets (OTEZLA 30 mg) containing 30 mg of APR each were weighed and crushed. A portion equivalent to 10 mg of the drug was weighed accurately and transferred to a 10 mL volumetric flask. Acetonitrile was added to make up the volume and the solution was sonicated for 10 minutes. After filtration, 1 mL of the filtrate was further diluted to 10 mL with acetonitrile, followed by additional dilutions with the mobile phase to reach a concentration of 10 µg/mL. This procedure was repeated six times.

C. Method Validation:

The HPLC system was equilibrated with the mobile phase for 30 minutes to achieve a stable baseline. A series of standard solutions ranging from 5 to 30 µg/mL were prepared from the stock solution using the mobile phase. Each concentration was injected six times, and the peak area, retention time, asymmetry factor, and theoretical plates were recorded. A calibration curve was generated by plotting the average peak area on the Y-axis and concentration on the X-axis. The regression equation derived from the curve was used to calculate the APR content in the samples. System suitability parameters are presented in Table I.

TABLE I. Optimized chromatographic conditions.

Stationary Phase	Sun Q C18 No: 69 (250 x 4.6 mm, 5 µ)
Mobile Phase	Acetonitrile: Sodium Acetate Buffer (pH-5) (80:20 v/v)
Flow Rate	1 ml/min
Run Time	10 min
RT (min)	3.692 ± 0.02
Area	1091169.3
Asymmetry	1.08
Plates	> 3946

RESULTS AND DISCUSSION:

VALIDATION OF ANALYTICAL METHOD

The method validation was performed as per ICH Q2 (R1) guideline.

A. Linearity and Range:

From the standard stock solution (1000 µg/ml) of APR, solution was prepared containing 100 µg/ml of APR with acetonitrile. This solution was further used to prepare range of solutions containing six different concentrations. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/ml. Linearity curve is represented in Fig. 5

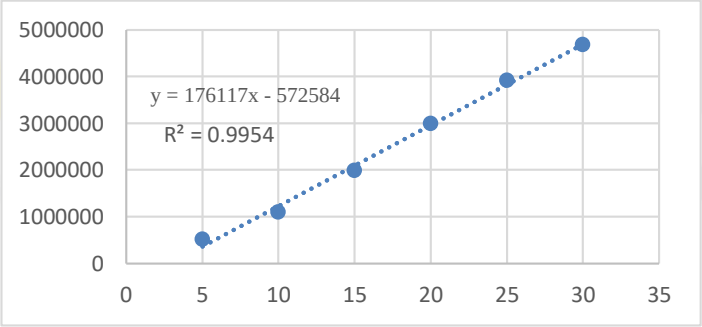

Fig. 4.Calibration curve for Apremilast (5- 30 µg/ml)

B. Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated.

For the inter day variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD were calculated.

C. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated from the formula 3.3 σ / Sand 10 σ / S, respectively, Where σ = standard deviation of y- intercept, S = slope of the calibration curve. The LOD and LOQ were found to be 0.518 µg/mland1.572 µg/ml.

D. Assay

Assay was carried out as mentioned under tablet formulation analysis at of 10 µg/ml assay concentration. The results obtained are summarized in Table III. where, X is recovered concentration.

Table III**. Assay of formulation.**

Sr. No	Conc µg/ml	Area	X	% Recovery	Mean ± RSD
1	10	1191235	10.02	100.23	101.30 ± 0.928
2	10	1228756	10.23	102.35	
3	10	1208898	10.12	101.23	
4	10	1231753	10.25	102.52	
5	10	1201992	10.08	100.84	
6	10	1198313	10.06	100.63	

E.Accuracy

Accuracy of the method was determined by method of standard addition. Known concentration of API to be analyzed was added to the formulation solution of Apremilast spiked at 50 %, 100 % and 150 % level. Percent recovery was calculated as summarized in Table IV.

Table IV. Accuracy results.

% Level	Initial amount (µg/ml)	Amount added (µg/ml)	Peak area	% Recovery	Mean ± RSD
50	10	5	2100365	101.16	101.26 ± 0.276
	10	5	2098574	101.10	
	10	5	2112232	101.61	
100	10	10	3000351	101.37	101.19 ± 0.282
	10	10	2982153	100.86	
	10	10	2998797	101.33	
150	10	15	3899563	101.48	102.18± 0.833
	10	15	3919101	101.92	
	10	15	3972146	103.13	

F.Robustness

Robustness was performed by doing the small and deliberate changes to developed system. Peak area was checked after doing the changes to mobile phase ratio, detection wavelength and flow rate. The optimized system is robust as % RSD is below 2 %.

The developed method was found to be simple, sensitive, specific, accurate, and repeatable for analysis of Apremilast in bulk and pharmaceutical dosage form without any interference from the excipients.

CONCLUSION:

A simple, accurate, precise and sensitive high-performance liquid chromatographic (HPLC) method has been developed and validated for the analysis of Apremilast in bulk and in tablet dosage form. The proposed method successfully separated the compound with degradants and estimate the active content.

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