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Development and Validation of Stability Based Hplc Method for Apremilast Tablets

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ABSTRACT: The goal of this research is to develop and validate a sensitive, specific, fast, and precise high-performance liquid chromatography (HPLC) method for quantifying apremilast process-related and degrading contaminants. With a runtime of 55 minutes, the chromatographic separation was obtained using a YMC Pack ODS-A; 250 x 4.6 mm; 5m HPLC column. The mobile phase-A (buffer and acetonitrile, 85:15 v/v) and mobile phase-B (buffer, acetonitrile, and formic acid, 30:70:0.025 v/v/v) were thoroughly mixed. The UV detector was tuned to 230 nm, and the column oven temperature was set at 25 °C. In a test sample of apremilast, the newly discovered approach was used to detect process-related impurities (N-acetyl amine impurity and Deacetyl impurity). To verify the stability-indicating character of the devised HPLC technology, it was subjected to a forced degradation investigation under acidic, alkaline, oxidative, photolytic, and thermal conditions. According to ICH criteria, the devised approach was determined to be specific, precise, sensitive, and robust. Finally, the HPLC method was successfully developed and verified, and it was successfully used to analyze both the apremilast medicinal substance and its product.

KEYWORDS: Apremilast, impurity, HPLC, Validation, Filter

I. INTRODUCTION:

Apremilast is an antirheumatic medication with the molecular name N-{2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl}ethanimidic acid (Figure 1).

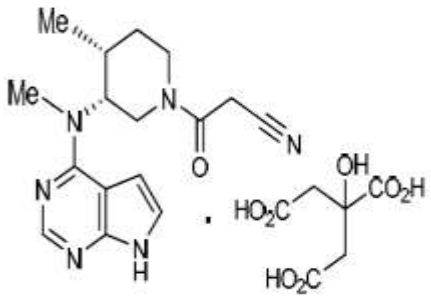

Figure 1: Molecular Structure of Apremilast

Apremilast is a medicine used to treat psoriasis and psoriatic arthritis. It is offered under the brand names Otezla and others [1-2]. It could also help with other immune-related inflammatory illnesses. Water is virtually insoluble, ethanol is marginally soluble, and acetone is soluble [3-4]. The medication reduces the spontaneous generation of TNF-alpha from human rheumatoid synovial cells by acting as a selective inhibitor of the enzyme phosphodiesterase 4 (PDE 4) [5-7]. The creation of an accurate and efficient analytical method to determine the product's quality is a critical step in the development of any medicinal substance or product in the pharmaceutical sector [8-9]. The current study focused on the development of a cost-effective quantitative analysis method. The goal of this research is to develop and validate a sensitive, specific, fast, and precise high-performance liquid chromatography (HPLC) method for quantifying apremilast process-related and degrading contaminants [10-11]. With a runtime of 55 minutes, the chromatographic separation was obtained using a YMC Pack ODS-A; 250 x 4.6 mm; 5m HPLC column. The mobile phase-A (buffer and acetonitrile, 85:15 v/v) and mobile phase-B (buffer, acetonitrile, and formic acid, 30:70:0.025 v/v/v) were thoroughly mixed [12-13]. The UV detector was tuned to 230 nm, and

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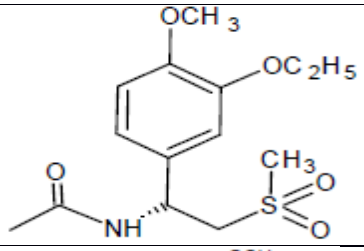
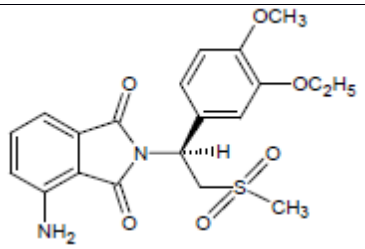
the column oven temperature was set at 25 °C. In a test sample of apremilast, the newly discovered approach was used to detect process-related impurities (N-acetyl amine impurity and Deacetyl impurity) [14]. To verify the stability-indicating character of the devised HPLC technology, it was subjected to a forced degradation investigation under acidic, alkaline, oxidative, photolytic, and thermal conditions [15]. According to ICH criteria, the devised approach was determined to be specific, precise, sensitive, and robust [16].

II. MATERIALS AND METHODS:

Glenmark Pharmaceuticals provided Apremilast as a complimentary sample (CAS No. 608141-41-9). All of the supplemental chemicals and reagents used were of HPLC grade and did not require further purification. During the experiment, double distilled water was used.

Table 1: Possible process impurities details

Name	Structure	Chemical name	As per API Classification
N-acetyl impurity		N-[(1R)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl] acetamide	This impurity will produced by the reaction of Stage-IV free amine with acetic acid during the Stage-V reaction.
Deacetyl impurity		(S)-4-amino-2-(1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl) isoindoline-1,3-dione	This impurity will produced by degradation of Stage-V in acetic acid during the Stage-V reaction.

Preparation of Solutions:

1. https://medlineplus.gov/druginfo/meds/a614022.html
2. Preparation of Buffer solution: Transferred 0.6543 g of Ammonium format and 0.7932 g Ammonium Bicarbonate into 2000 mL of purified watersonicated to dissolve. Adjusted the pH 5.2 ±0.05 with Formic acid. Filter this Solution through 0.45 µm filter paper.
3. Preparation of Mobile phase-A: Mixed Buffer and Acetonitrile in the ratio 85:15 v/v, mixed well.
4. Preparation of Mobile phase-B: Mixed Buffer, Acetonitrile and Formic acid in the ratio 30:70:0.025 v/v/v, mixed well.
5. Preparation of Diluent: Mobile phase-B used as diluent.
6. Preparation of N-acetyl amine impurity Stock solution: Weighed accurately 2 mg of N-acetyl amine impurity and transferred into a 20 mLvolumetric flask, dissolved and diluted up to the mark with diluent. Mixed well.(Concentration of N-acetyl amine impurity stock solution: 0.1 mg/mL)
7. Preparation of N-acetyl amine impurity solution: Transferred 0.8 mL of N-acetyl amine impurity stock solution into 100 mL flask diluted with diluent and mixed well. (Concentration of N-acetyl amine impurity: 0.0008 mg/mL)
8. Preparation of Deacetyl impurity Stock Solution: Weighed accurately 2 mg of Deacety1 impurity and transferred into a 20 mL volumetricflask, dissolved and diluted up to the mark with diluent. Mixed well. (Concentration of Deacetyl impurity stock solution: 0.1 mg/mL)
9. Preparation of Deacetyl impurity solution: Transferred 0.8 mL of Deacetyl impurity stock solution into 100 ml flask diluted with diluent and mixed well. (Concentration of Deacetyl impurity: 0.0008 mg/mL)
10. Preparation of Apremilast Standard Stock Solution: Weighed accurately about 25 mg of Apremilast Standard and transferred into a 50

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mL volumetric flask,dissolved and made up to the mark with diluent. Mixed well. (Concentration of Apremilast stock solution: 0.5 mg/mL)

11. Preparation of System suitability solution: Transferred accurately 0.8 mL of all above impurities solution and 1.6 ml of Apremilast Standard Stock Solution into a 100 mL volumetric f1ask, diluted up to the mark with and mixed well. (Concentration of Apremilast and impurities: 0.0008 mg/mL)
12. Preparation of standard solution: Transferred 2 mL of standard stock solution into 50 ml flak diluted with diluent and mixed well. Further, 2 mL of this solution into 50 ml flak diluted with diluent and mixed well. (Concentration of standard and impurities: 0.0008 mg/mL)
13. Preparation of Test solution: Determined the Average weight of 20 tablets,crushed the tablets. Weighed the tablet powderequivalent to 40 mg of Apremilast and transferred into 100 mL volumetric flask, added about 60 mL of diluent sonicated it for 20 minutes with intermediate shaking. Made the volume with diluent, mixed well. Filtered the solution through 0.45µm Nylon membrane filter, discard first 2 mL of filtrate. (Concentration of test solution: 0.4 mg/mL)
14. Preparation of Placebo solution: Weighed placebo powderequivalent to 40 mg of Apremilast and transferred into 100 mL volumetric flask, added about 60 mL of diluent sonicated it for 20 minutes with intermediate shaking. Made the volume with diluent, mixed well. Filtered the solution through 0.45µm Nylon membrane filter, discard first 2 mL of filtrate.

Chromatographic Procedure:

There were injections of blank solution, standard solution, system suitability solution, placebo solution, individual impurities and test solution of Apremilast tablets used for analysis. The chromatographic conditions are mentioned in following table.

Table 2: Chromatographic conditions

Column	YMC Pack ODS-A; 250 x 4.6 mm;5µm		
Pump mode	Gradient		
Column Temperature	25°C		
Flow rate	0.8 mL/ min		
Detection	230nm		
Injection volume	20µL		
Run time	55.0 minutes		
Sample cooler temperature	5°C		
Gradient	Time (min)	MP-A	MP-B
	0	100	0
	8	100	0
	15	60	40
	25	50	50
	30	40	60
	35	25	75
	42	25	75
	45	100	0
	55	100	0

Forced degradation:

a) Photolytic degradation:

Sample preparation: Transferred 10 Tablets in a two separate petri plates (One open petri plate and another wrapped with aluminum foil) and exposed it under UV and white light for 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt/square. After exposure, crushed the 10 tablets & Weighed the tablets powder equivalent to 50 mg of Apremilast and transferred into 100 mL of volumetric flask. Added about 60 mL of diluent, sonicate for 20 minutes with intermittent shaking. Allowed it to cool to room temperature and made up to volume with diluent and mixed well. Centrifuge the sample solution at 5000 rpm for 10 minutes.

Placebo preparation: Prepared and exposed the placebo solution under UV and white light for 1.2 million lux same as sample solution.

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b) Heat degradation

Sample preparation: Transferred 10 Tablets in a petri plate kept in oven at about 100°C for 24 h. After exposure, crushed the tablets. Weighed the tablets powder equivalent to 50 mg of Apremilast and transferred into 100 mL of volumetric flask. Added about 60 mL of diluent and sonicated for 20 minutes with intermittent shaking. Allowed it to cool to room temperature and made up to volume with diluent and mixed well. Centrifuge the sample solution at 5000 rpm for 10 minutes.

c) Humidity degradation:

Sample preparation: Transferred 10 Tablets in a petri plate and exposed it at 25°C & 90% RH chamber for 48 h. After exposure, crushed the tablets & weighed the tablets powder equivalent to 50 mg of Apremilast and transferred into 100 mL of volumetric flask. Added about 60 mL of diluent & sonicated for 20 minutes with intermittent shaking. Allowed it to cool to room temperature and made up to volume with diluent and mixed well. Centrifuge the sample solution at 5000 rpm for 10 minutes.

d) Acid degradation:

Sample preparation: Crushed the 10 tablets to fine powder. Weighed the tablets powder equivalent to 50 mg of Apremilast and transferred into 100 mL of volumetric flask. Added about 60 mL of diluent, sonicate for 20 minutes with intermittent shaking. Added 5 mL of 2 N Hydrochloric acid solution and heated the solution on the water bath at 70°C for 7 h. Diluted up to the mark with diluent and mixed. Centrifuge the sample solution at 5000 rpm for 10 minutes.

e) Base degradation

Sample preparation: Crushed the 10 tablets to fine powder. Weighed the tablets powder equivalent to 50 mg of Apremilast and transferred into 100 mL of volumetric flask. Added about 60 mL of diluent, sonicate for 20 minutes with intermittent shaking. Added 0.5 mL of 1N Sodium hydroxide solution and heated the solution on the water bath at 70°C for 7 hr. Diluted up to the mark with diluent and mixed well. Centrifuge the sample solution at 5000 rpm for 10 minutes.

f) Peroxide degradation

Sample preparation: Crushed the 10 tablets to fine powder. Weighed the tablets powder equivalent to 50 mg of Apremilast and transferred into 100 mL of volumetric flask. Added about 60 mL of diluent, sonicate for 20 minutes with intermittent shaking. Added 1 mL of 3% Hydrogen peroxide and kept the solution at room temperature for 5 minutes. Diluted up to the mark with diluent and mixed. Centrifuge the sample solution at 5000 rpm for 10 minutes.

(Placebo preparation: Prepared and treated the placebo solution same as sample solution)

Filter Compatibility: Filter compatibility tests were carried out in order to provide a particle-free solution that would also duplicate a 100 percent response as per the content. The centrifuged sample was compared to the filtrate from each filter. For further research, the filter that met all of the criteria and showed the same concentration as the centrifuged sample was chosen. The equivalence of standard and sample solutions filtered through several types of filters (Nylon 0.45 and PVDF 0.45 filter) as well as centrifuged sample solutions was tested.

Validation Studies:

Accuracy: Calculating percent recovery was used to determine the accuracy of the procedure. The amounts of apremilast were approximated by measuring the peak area after a known amount of apremilast was introduced to a placebo. The amount of apixaban was calculated by measuring the peak area ratios in these investigations, which were conducted in triplicate over the prescribed concentration range. The percentage recovery was calculated, as well as the standard deviation of the percentage recovery.

Precision: The precision of the method was determined in terms of Intra-day and inter-day precision.

Specificity: The ability to assess the analyte definitively in the presence of components, etc. is referred to as specificity.

Linearity and Range: The method's linearity was tested at various drug concentration levels. Peak area vs. concentration was shown as a regression line. The regression line's correlation coefficient and equation were calculated. The procedure's linearity range is the range from the lowest to the highest measured concentration.

LOD (Limit of Detection) and LOQ (Limit of Quantification): The detection limit of an individual analytical process is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an accurate value. The lowest amount of analyte in a sample that can be quantitatively measured with sufficient precision and accuracy is the quantitation limit of a particular analytical process.

Robustness: The developed method's robustness was investigated by varying the flow rate and

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column temperature. All chromatographic settings were kept the same except for the flow rate to investigate the effect of flow rate.

System Suitability: Retention duration, the number of USP theoretical plates, USP tailing, peak area, and peak height were all evaluated as system suitability criteria. System suitability acceptance criteria:

1. The % RSD for the peak area of Apremilast in standard solution for six replicate injection should not be more than 10.0
2. The Tailing factor of Apremilast in standard solution should not be more than 2.0.
3. The Theoretical plates of Apremilast in 0 .15 % Reference solution should not be less than 3000.

III. RESULTS AND DISCUSSION:

Specimen chromatogram: The specimen chromatogram is given below.

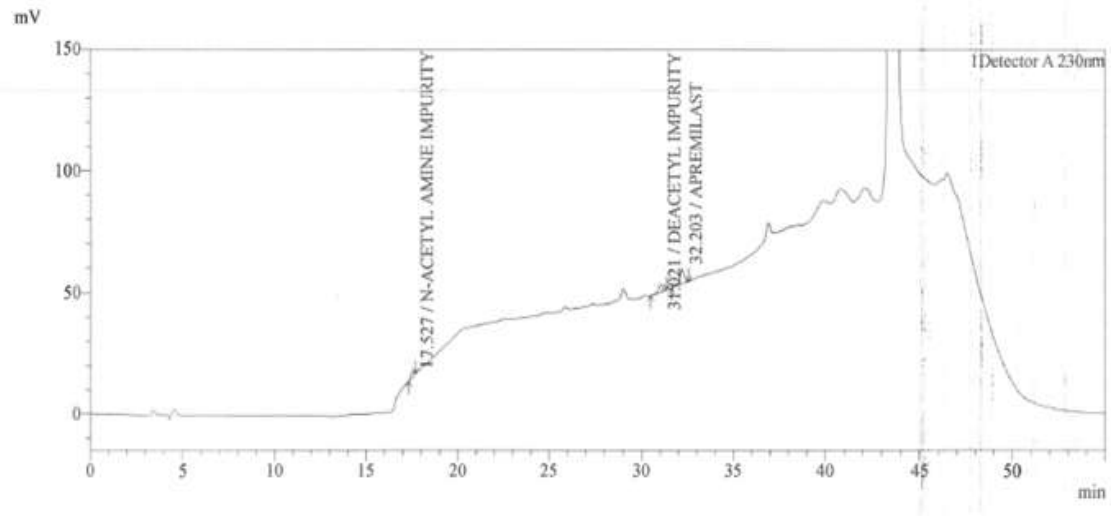

Figure 2: Chromatogram of standard specimen

Forced Degradation Studies: The modified analytical method with a run time of 55.0 min was used for the determination of impurities generated in the apremilast API sample after conducting forced degradation using various stressors. The results obtained in Table 3 indicated that the developed method was able to resolve all the degradation peaks from the apremilast, which indicted in Figure 3.

Table 3: Mass balance

Conditions	N-acetyl amine impurity (%)	Deacetyl impurity (%)	Unknown max impurity (%)	% Total impurities	% Assay	% Mass Balance
Control	ND	ND	0.01	0.01	99.4	NA
Acid degradation 5 mL 2N HCl_30min_70°C	ND	10.51	ND	10.51	88.9	100.0
Base degradation 0.5 mL 1N NaOH	ND	0.02	0.42	1.02	94.6	96.2
Peroxide degradation 1 mL 3% H_2O_2	ND	ND	0.01	0.00	98.8	99.4
Heat degradation 24 h 100°C	ND	0.04	0.01	0.05	101.5	102.2
Humidity 25°C/90% RH for	ND	ND	0.01	0.01	101.0	101.6

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48 h						
Photolytic study Open 1.2 million Lux HR and 200 watt/Sq.meter	ND	0.01	0.01	0.02	99.6	100.2

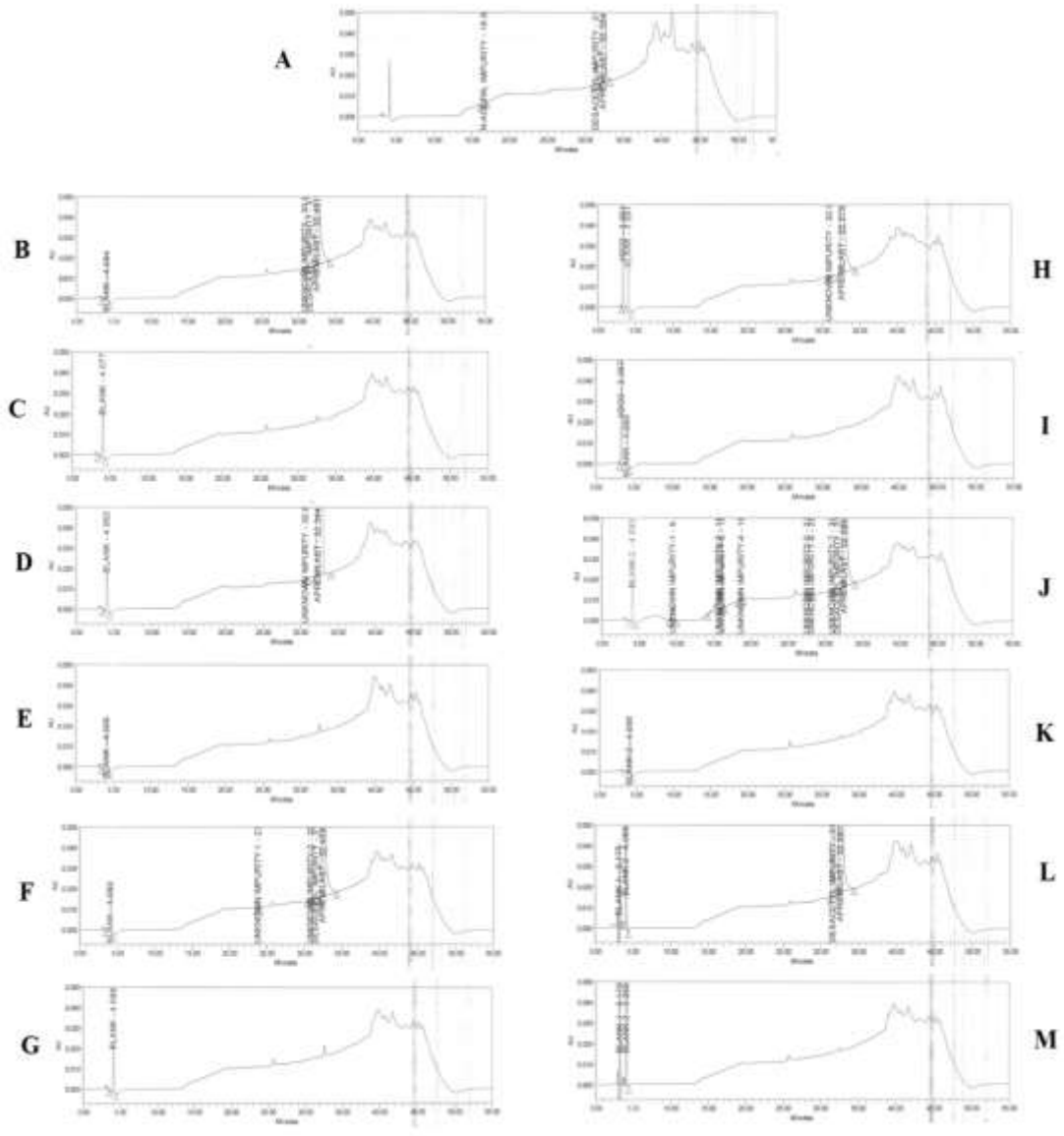

Figure 3: Forced degradation chromatograms of A) Spiked sample, B) Sample photolytic, C) Placebo photolytic, D) Sample humidity, E) Placebo humidity, F) Sample heat, G) Placebo heat, H) Sample peroxide, I) Placebo peroxide, J) Sample base, K) Placebo base, L) Sample acid & M) Placebo acid

Filter Compatibility: The data shows that absolute difference between centrifuged sample solution and filtered sample solution with PTFE 0.45µ, Nylon 0.45µ (Millipore), and PVDF 0.45µ filter is within the acceptance criteria (should be within ± 2). Hence all tested filters are suitable for sample solution filtration.

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Accuracy:

Table 4: Accuracy for N-acetyl impurity

Accuracy Level	Conc added(µg/mL)	Peak Area	Conc recovered (µg/mL)	% Recovery
LOQ	0.084	4000	0.084	100.0
50%	0.847	39687	0.832	98.2
100%	1.693	76268	1.598	94.
150%	2.540	107396	2.251	88.6
Mean recovery %				**95.3**

Table 5: Accuracy for Deacetyl impurity

Accuracy Level	Conc added(µg/mL)	Peak Area	Conc recovered (µg/mL)	% Recovery
LOQ	0.084	10719	0.106	126.2
50%	0.747	80511	0.794	106.3
100%	1.493	147403	1.454	97.4
150%	2.240	212951	2.100	93.8
Mean recovery %				**105.9**

Linearity:

Table 6: Linearity for Apremilast

Level (%)	Concentration (µg/mL)	Mean Area
10	0.00506	6719
25	0.25314	37235
50	0.50629	66923
75	0.75943	96394
100	1.01257	128889
150	1.51886	190760
Correlation coefficient (R)		**1.000**
Slope		**121372.1691**
Working Level (Area)		**128888.50**
% Limit Of Y-Intercept On Working Level		**4.49**
RRF		**1.00**

Table 7: Linearity for N-acetyl amine impurity

Level (%)	Concentration (µg/mL)	Mean Area
10	0.10001	7380
25	0.41669	16366
50	0.83338	32199
75	1.25008	49232
100	1.66677	66261
150	2.50015	100553
Correlation coefficient (R)		**0.999**
Slope		**39351.309**

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Working Level (Area)	**66260.50**
% Limit Of Y-Intercept On Working Level	**1.43**
RRF	**0.32**

Table 8: Linearity for Deacetyl impurity

Level (%)	**Concentration (µg/mL)**	**Mean Area**
10	0.08795	5624
25	0.36647	32358
50	0.73293	61744
75	1.09940	94576
100	1.46586	125337
150	2.19879	180382
Correlation coefficient (R)		**0.999**
Slope		**82867.1607**
Working Level (Area)		**125337.00**
% Limit Of Y-Intercept On Working Level		**0.91**
RRF		**0.68**

LOD and LOQ: The results of signal to noise ratio was compared with the response of Apremilast standard. The LOD and LOQ were found to be 0.016 ppm and 0.050 ppm, respectively.

Robustness Studies: The changes were applied, and system suitability parameters were checked, found to be within the acceptable limits. It was noted that trivial changes in temperature and flow rate does not affect the method and produces results, which passes system suitability. Hence, the method was robust.

System Suitability: System suitability tests were performed using Apremilast standard and test solutions to check for compliance with specified parameters.

Table 9: Relative retention times

Impurity Name	**RRT**
N-acetyl amine impurity	0.52
Deacetyl impurity	0.97
Apremilast	1.00

IV. CONCLUSION:

A new HPLC method was developed and validated for determining apremilast. For the quantitative examination of medicines in bulk and tablets, the established approach proved accurate, precise, specific, and sensitive. Validation experiments revealed that the approach is unaffected by slight changes in chromatographic parameters. In this research endeavour, no attempt was made to measure product degradation. This method can be used to monitor the quality of a medicine and its tablets on a regular basis. This method may be used in LC-MS for quantitative measurement of known and unknown contaminants created during forced degradation and related compound investigations for apremilast API and finished products.

CONFLICTS OF INTEREST: The authors make no mention of any potential conflicts of interest.

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