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Analytical Method Development and Validation of Stability Indicating RP-HPLC method for Related Substances of Apremilast in Tablet Dosage Form

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Abstract: *The present work involves the development of simple, accurate, precise and stable RP- HPLC method for the estimation of Apremilast in the tablet dosage form. The method has several advantages, including simple and mobile phase, low cost solvents, rapid analysis, and simple sample preparation. In developed method, the analyte was resolved by using isocratic method and mobile phase was used methanol: acetonitrile: water in proportion of (35v:38v:27v), at a flow rate 1.0 ml/min, the detection was carried out at 230 nm. The results of analysis in the method were validated in terms of accuracy, precision, linearity, robustness. Linearity for Apremilast was found in the linear concentration range of 1-6µg/ml with regression coefficient r2 = 0.9998. The % RSD values for intra-day and inter-day precision studies were found to be less than 2%. The % recovery was found to be within an acceptable limit 98%-102%. Therefore the developed method said to be linear, precise, accurate, and robust. Since the method does not require use of expensive reagent and also less time consuming, it can be performed routinely in industry for a routine analysis of marketed product of Apremilast in tablet dosage form*

Keywords: Apremilast, HPLC, Validation, Method Development

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).

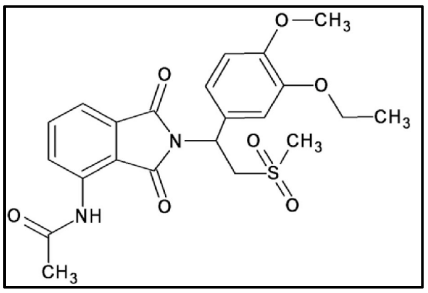

Figure 1: Molecular Structure of Apremilast

Apremilast is a medicine used to treat psoriasis and psoriatic arthritis. It is offered under the brand names Otezla and others. It could also help with other immune-related inflammatory illnesses. Water is virtually insoluble, ethanol is marginally soluble, and acetone is soluble. 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).The creation of an accurate and efficientanalytical method to determine the product's quality is a critical step in the development of any medicinal substance or product in the pharmaceutical sector. 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.

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Drug profile:

Drug name	**Apremilast**
Chemical name	N-[2-[(1S)-1-(3ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-1,3dioxoisoindol-4-yl] acetamide
Molecular weight	C22H24N2O7S
Melting point	460.401g/mol
Solubility	156-158℃
Half life	6-9hrs
Pak value	Strongest acid- 14.42 Strongest base- 8.91

Mechanism of Action:

Apremilast is a small molecule inhibitor of PDE413, an enzyme that breaks down cyclic adenosine monophosphate (CAMP). In inflammatory cells, PDE4 is the dominant enzyme responsible for this reaction. The resulting increase in cAMP levels down-regulates expression of a number of pro-inflammatory factors like tumor necrosis factor alpha (TNFα), interleukin 17, interleukin 23, and many others, and up-regulates the anti-inflammatory interleukin 10. In ex vivo models of arthritis, IL- 12/IL-23p40 was specifically identified as a downstream target of apremilast[14] The importance of these individual factors for the clinical effect of apremilast is not clear.

II. EXPERIMENTAL STUDY:

HPLC Method Development and Validation

The quantitation of Apremilast from bulk and formulation was carried out by HPLC method. The LC20AD Prominence Liquid Chromatograph SPD20-A Shimadzu, Japan with UV-Vis detector and C18 column with dimension on 25 x 0.6 cm was used for the method development with flow rate 1.0 ml/min at wavelength 230 nm. The methanol: acetonitrile: water in proportion of (35v:38v:27v) as a mobile phase, for development of chromatogram. The method was validation for synthesized compound and various parameters according to ICH guidelines (Q2B) were studied.

Chromatographic Conditions	**SHIMADZU HPLC System**
Mobile phase	Methanol: Acetonitrile: Water(35:38:27)
Column	ARP-C18 (250 mm X 4.6 mm), 5µ column
Flow rate	1 ml/min
Wavelength detection	236 nm
Injection volume	20µl
Temperature	Ambient
Retention time	10.5 min
Run time	15min

Table 1: Optimized chromatographic condition for RP-HPLC

Analytical Method Validation

A suitable analytical method was developed and validated for identification. New drug development requires meaningful and reliable analytical data to be produced at various stages of development.

Preparation of Mobile phase

The selection of mobile phase was according to polarity and non-polarity of solvents. The methanol: acetonitrile: water was selected as mobile phase in ratio of 35:38:27 and was filtered on membrane filter (0.45 µ) to remove degassing and were stirred for 10-15 min.

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Preparation of sample solution (formulation)

Stock solution of bulk Apremilast, 2 different batches of Apremilast marketed formulation of 100 ppm in 100 ml volumetric flask were prepared. Dissolve 10 mg of test sample in 100 ml diluents. 1ml of this stock was diluted to 10 ml to prepare 10 ppm stock solution. For the tablet formulation 20 tablets from each 2 tablet batch were crushed respectively. The powder of this formulation equivalent to 10 mg of the drug was used to prepare the stock solution. Further dilute to 1 ppm, 2 ppm, and so on, were prepared by taking 0.1 ml, 0.2 ml and so on of standard test solution and diluting it to 10 ml.

System Suitability Parameters:

The area of respective concentrations, theoretical plates, number of theoretical plates per height and the peak symmetry was recorded.

Linearity

Different levels of standard solution were prepared by diluting out known volumes of intermediate stock solution with the diluent to get the required analyte concentrations. A graph of Concentration (ppm) vs.area was plotted and the regression coefficient 'r2 ', yintercept and slope of the regression were calculated

Precision

Precision of analytical method was studied by multiple injections of homogenous samples. 6 replicate of 4 ppm solution were prepared and injected for precision at the same flow rate of 1ml/min. The intra-day and inter-day precision was used to study the variability of the method. SD and RSD were calculated for both.

Accuracy

Accuracy of the method was studied using the method of standard addition. Standard Apremilast solutions were added to the unknown bulk and tablet formulation of Apremilast. The percent recovery was determined at three different levels (50%, 75% and 100%). Impurity content was determined and the percent recovery was calculated.

Robustness

Robustness was studied by changing parameters like change in flow rate. The SD and RSD between the change parameter were calculated.

LOD and LOQ

A limit of detection (LOD) and a limit of quantification (LOQ) were calculated according to the formula:

LOD = 3.3 σ/s

LOQ = 10 σ/s

Where, 'σ' is the standard deviation of 'y' intercept of regression line and 's' is the slope of the calibration curve

III. RESULT AND DISCUSSION:

HPLC Method Development and Validation

The ICH Q2B guidelines discuss the analytical method validation on HPLC. Currently the vast majority of process-related impurity determinations are performed by HPLC. It offered the desired sensitivity for trace level determinations with a high degree of automation. A wide variety of stationary phases and operation modes make HPLC applicable to all drug classes. The typical detection limits for process-related impurities by HPLC are 0.1% or lower and this can be routinely met in the majority of circumstances using conventional UV detectors. These methods involved the prediction of likely impurities within the synthetic process, their isolation and identification by suitable analytical techniques. The last step of the present study was to develop, validated HPLC method for detection and quantification of Apremilast in bulk and tablet formulations.

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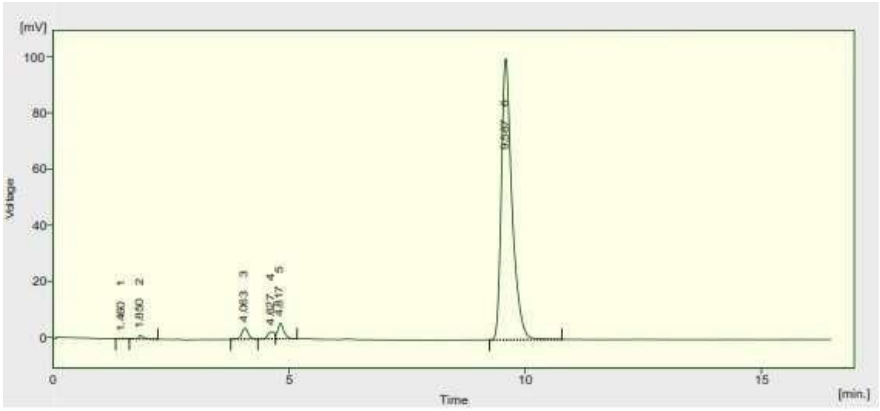
HPLC Chromatograph of Apremilast

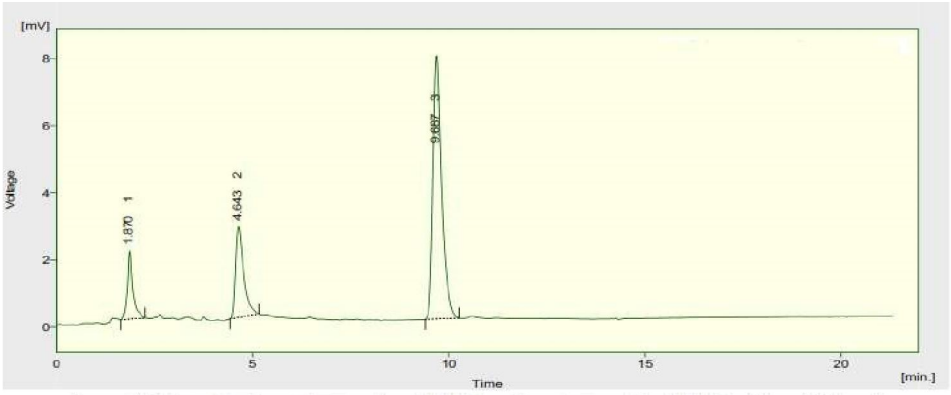


Result Table

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.460	2.424	0.289	0.1	0.3	0.13
2	1.850	11.705	1.278	0.7	1.1	0.11
3	4.063	37.267	3.919	2.2	3.4	0.15
4	4.627	25.732	2.608	1.5	2.3	0.17
5	4.817	52.839	5.630	3.1	4.9	0.14
6	9.587	1568.718	100.231	92.3	88.0	0.23
	Total	1698.684	113.955	100.0	100.0	

The Retention time of Apremilast was 9.5 min.

HPLC Chromatogram of Tablet:



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.870	19.233	2.024	10.7	16.1	0.12
2	4.643	39.227	2.710	21.9	21.6	0.22
3	9.687	120.869	7.840	67.4	62.4	0.23
	Total	179.330	12.573	100.0	100.0	

Figure No.3: HPLC Chromatogram of Apremilast Tablet formulation

The retention time of Apremilast tablet was found at 9.6 min

Linearity

Table No. 2: Result of Linearity by HPLC (Peak area vs. Conc.)

Sr. No	Concentration (ppm)	Area (mill volts) at 230 nm
1.	1	124.35

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2.	2	216.34
3.	3	311.34
4.	4	418.76
5.	5	514.35
6.	6	619.45

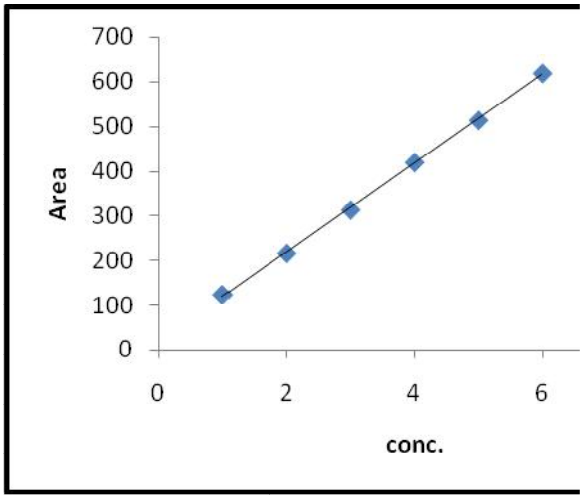

Figure No.4: Graph of linearity of synthesized compound by HPLC

The linearity of the proposed method was estimated correlation coefficient (R^2) was found to be 0.999 by regression analysis at six concentration levels in and intercept Y= 99.34x + 19.42 was linear. the range of 1-6 µg/ml for intermediate.

Precision

The precision of the intermediate was Standard deviation (SD) and Relative standard quantified for repeated concentration of 4 µg/ml in deviation (RSD) was found to be 1.331 and 0.317 range and was reliable with their area of respectively.

Robustness

The robustness of the Intermediate was performed for change in flow rate upto 0.8 ml/min and method was robust with standard deviation 2.897 and relative standard deviation 0.415

Accuracy

Sr. No.	Drug / Formulation	Percentage recovery			Mean	S.D.	%RSD
		50%	75%	100%			
1.	Bulk	98.18	99.07	99.89	99.04	0.852	0.863
2.	Tablet I	99.22	101.30	103.79	101.43	2.288	2.255
3.	Tablet II	99.25	101.68	103.13	101.30	1.969	1.934

Accuracy study was performed by the Percentage recovery was found to be more at recovery method. The results demonstrate that the higher concentration level a compare to lower percentage recovery in tablet was more than bulk concentration level. due to the presence of impurity in the tablet.

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Limit of detection

$$LOD = \frac{3.3 \times \text{Standard deviation}}{\text{Slope}}$$

$$LOD = \frac{3.3 \times 1.3313}{98.44}$$

LOD = 0.4462

Limit of quantitation

$$LOQ = \frac{10 \times \text{Standard deviation}}{\text{Slope}}$$

$$LOQ = \frac{10 \times 1.3313}{98.44}$$

LOQ = 0.1352

IV. CONCLUSION

RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique was employed in the present investigation for estimation of Apremilast tablet formulation.The results of pharmaceutical formulations assert that the proposed method of Apremilast feasible for their determination without interfering the additives and excipients. Therefore this method was simple, precise, accurate and cost effective and in actual fact feasible for routine sample analysis of Apremilast in bulk and formulations

REFERENCES

[1]. European Medicines Agency (EMA). Assessment report: Otezla. International non-proprietary name: apremilast.
[2]. Papp, K., Cather, JC., Rosoph, L., et al. Lancet, 2012. 380:738–746
[3]. Mendham j., denny r. C., thomas m.; vogel's text book of quantitative chemical analysis; pearson education limited; 6th edition, 2008, 29-39.
[4]. Chatwal g. R., anand s. K.; instrumental methods of chemical analysis; himalaya publishing house, mumbai; 11th edition, 2005, 1.1-1.2, 2.108-2.109, 2.151-2.153.
[5]. Hemaraj R. Patil, Dr. S. T. Patil, V. H. Jain AndDr. S. P. Pawar. Devlopment And Validation of UV-Spectrophotometric and Hplc Method for Apremilast In Bulk and Tablet Dosage Form. Ejpmr, 2019,6(8), 233-239
[6]. Gupta V, Jain AD, Gill NS, Gupta K. Development and validation of HPLC method-a review. Int. Res J Pharm. App Sci. 2012;2(4):17-25
[7]. Gupta V, Jain AD, Gill NS, Gupta K. Development and validation of HPLC method-a review. Int. Res J Pharm. App Sci. 2012;2(4):17-25
[8]. Kasture a. V., wadodkar s. G., mahadikk.r., more h.n.; pharmaceutical analysis instrumental methods; niraliprakashan; 12th edition, 2005; 148-156.
[9]. Bhardwaj SK, Dwivedia K, Agarwala DD. A review: HPLC method development and validation. International Journal of Analytical and Bioanalytical Chemistry. 2015;5(4):76-81.
[10]. ICH, Specifications, International Conference on Harmonization, IFPMA, Geneva, 1999
[11]. International Conference on Harmonization (ICH), Guidance for Industry, Q1A (R2): Stability Testing of New Drug Substances and Products, IFPMA, Geneva,2003.

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[12]. Rina Mohan Sonawane, Rutuja PrabhakarSonare, Snehal Ganpat TekawadeAndDr. Ashok Pandurang Pingle. Chromatographic Method Development and Validation of Assay of Apremilast In Bulk and Tablet Dosage Form Ejbps, 2018, Volume 5, Issue 8, 412-417.

[13]. Schett, G., Sloan, VS., Stevens, RM., et al. Ther Adv Musculoskelet Dis, 2010. 2: 271–278.

[14]. Paul, C., Cather, J., Gooderham, M., et al. Br J Dermatol, 2015.173:1387–1399.

[15]. Skoog d., holler f., timothy a., nieman n.; principles of instrumental analysis; saunders college publications, london; 4th edition, 1992; 1-2, 338-340.