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REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF APREMILAST IN PHARMACEUTICAL FORMULATION

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ABSTRACT

In this current investigation effort, an effort was made to build up an uncomplicated, speedy, truthful as well as robust HPLC technique for the assessment of Apremilast in its tablet dosage form. Reverse phase high performance liquid chromatographic analysis was carried out on isocratic system. The column used for the investigation was Phenomenex C18 (250 mm× 4.6mm, 5µm) with ambient temperature. The optimized mobile phase was Methanol: Acetonitrile in the ratio of (20:80 %V/V). The detection was carried out at a wavelength of 230nm using a flow rate of 1ml/min. The urbanized technique was validated for validation constraints like linearity, specificity, accuracy, precision as per ICH strategy. The %RSD for all constraints was well within the limits, which indicates the validity of the technique in addition to the assay results obtained are in reasonable conformity with the label claim of the marketed formulation. Thus, the conventional scheme can be anticipated for repetitive investigation of this drug in laboratories and for superiority purposes.

Keywords: Apremilast, Acetonitrile, HPLC, Linearity

INTRODUCTION:

Apremilast is a Phosphodiesterase 4 (PDE4) inhibitors chemically named as N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-1,3-dioxoisoindol-4-yl]acetamide. It is used in the treatment of certain types of

arthritis and skin conditions **[1]**. Literature survey revealed that few analytical techniques are available for estimation of Apremilast in pharmaceutical dosage form such as UV, HPLC. Keeping this objective in mind an attempt has been made to develop and validate the RP-HPLC method for the estimation of Apremilast in which the developed method would be a highly sensitive cost effective method having good resolution and reproducible results **[2]**.

MATERIALS AND METHODS:

Equipment: Chromatographic separation was conceded on WATERS HPLC system which is outfitted with the 515 dual head reciprocating pump & a 2489 UV Visible detector. The software used is Empower-2 software and column is Phenomenex C18 column of 250mm×4.6mm i.d, 5µm.

Materials and reagents: Apremilast drug was gifted by Aurobindo Pharmaceuticals, Hyderabad, Telangana, India. Acetonitrile, methanol, HPLC grade water, sosium hydroxide, Diammonium hydrogen orthophosphate and Hydrochloric acid were collected from local manufacturers.

Preparation of standard solution: Standard stock arrangement was set up by gauging 25mg of Apremilast and moving in to 25 ml volumetric jar. At that point 25 ml weaken methanol was included and sonicated for 5 minutes to break down the medication. At that point the arrangement was weakened to check with methanol **[6]**. It was then separated through 0.45µm film channel, which gives the stock arrangement containing 1000µg/ml Apremilast. Standard arrangement was set up by moving In a 10 ml volumetric jar with a volume of 1 ml of standard stock arrangement and methanol separated through 0.45 separated layer channel; weaken the volume to provide a stock containing 100µg / ml Apremilast.

Preparation of the test solution: 20 tablets were precisely dissolved and crushed to shape fine powder. A volume powder comparable to 25 mg of Apremilast was most likely evaluated, and it was taken in 25 ml volumetric container, containing 25 ml methanol **[7]**. The container was then sonicated for 5 minutes and later made sufficient with dilute methanol. It gives stock arrangement of Apremilast with concentration of 1000µg / ml. The arrangement stock was then separated with Whatman channel paper and washed with powerless methanol; it was shed through 0.45 channel layer channel. Further weakening was built up by funneling 1ml above stock course of

action into 10ml volumetric container and weakened in volume with methanol. Around then, this game plan was isolated through 0.45 channel film channel and sounded for 2min. This was fixed test system, with obsession being around (80/g/ml).

METHOD DEVELOPMENT

Chromatographic conditions: Diverse columns enclosing octyl and octadecyl silane stationary phase were endeavored for severance in addition to resolutions. It was established that Phenomenex C18 (250mm×4.6mm i.d, 5µm) column offered additional improvement over further columns. The mobile phase was a concoction of methanol and acetonitrile (20:80 % v/v). Drug solution was introduced into column. The flow velocity of the mobile phase was accustomed to 1 ml /min **[3]**. The recognition was conceded out at wavelength 230nm. The injection capacity of the standard as well as sample solution was placed at 20µl. The preference of wavelength 230nm was well thought-out agreeable, permitting the recognition of the drugs with tolerable sensitivity. It fashioned well shaped peaks for the drug assay. A UV spectrum of the drug is given in **Figure 1** and chromatogram of the drug assayed is depicted in **Figure 2**.

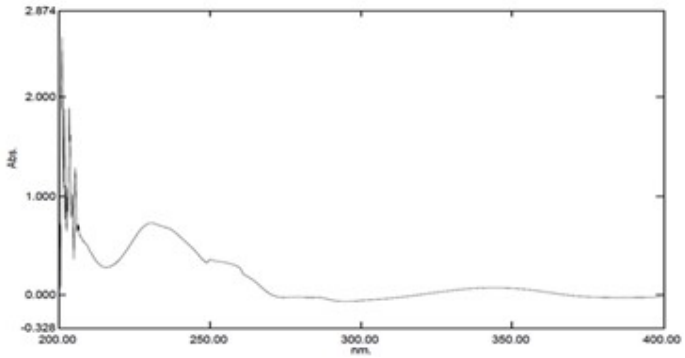

Figure 1: UV spectrum of Apremilast

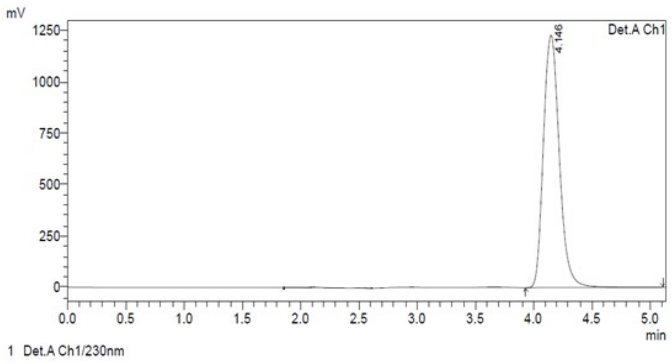

Figure 2: Chromatogram of standard

METHOD VALIDATION

System suitability test: The method under sophisticated chromatographic circumstances was performed by appropriateness testing infusing the customary arrangement 100% test fixation multiple times in to balanced out HPLC framework **[4]**. To set up System appropriateness last pinnacle six infusions was assessed. Framework appropriateness was manner by assessing parameters like number of hypothetical plates (N), peak area and tailing factor. From the results shown in **Table 1** it affirms that the system reasonableness parameters were discovered fulfilled.

Linearity: The linearity of the technique was determined at different concentration levels ranging from 10 to 500µg/ml. Each solution was injected into HPLC system and linearity was evaluated by linear regression analysis. The calibration curve was plotted against concentration and average area response **[5]** and the regression equation was given as y= 97864x-30167. The correlation coefficient was found to be 0.999 and plot was shown in **Figure 3**.

Range: Working standard arrangements were prepared and injected 6 times into the HPLC system and the upper and lower levels of analyte indicating exactness, linearity and accuracy and saw as in the fixations go 500 and 10µg/ml. The overlain chromatograms for lowest and highest concentrations of range were shown in **Figures 4** and 5 respectively and the range data was shown in **Table 2**.

Precision: Method precision was established by infusing six relaxation infusions of the standard system at 120µg/ml fixation under the same test conditions **[8]**. The relative standard deviation obtained was shown in **Table 3**.

Accuracy: Accuracy studies were completed at three unique levels of 50%, 100%, and 150% **[9]**. Each concentration solutions were prepared in three sets and injected into HPLC system and the results obtained are tabulated in **Table 4**.

Specificity: Interference of impurities was checked by injecting the blank and sample solution into HPLC system and chromatograms recorded were shown in **Figures 6 and 7**.

Robustness: Robustness study was carried out by changing the flow rate by ±0.2ml/min and wavelength by ±3nm and the relative standard deviation determined was shown in **Table 5**.

Ruggedness: Standard solution was prepared and injected into HPLC system in 6 replicate injections and system suitability criteria are checked under variety of conditions like variability due to analyst and variability due to column and the results are shown in **Table 6 [10]**.

Table 1: System suitability parameters

Name of the Peak	Rt (min)	Peak Area	Theoretical plates	Tailing factor
Apremilast	4.146	11676527	4352	1.242

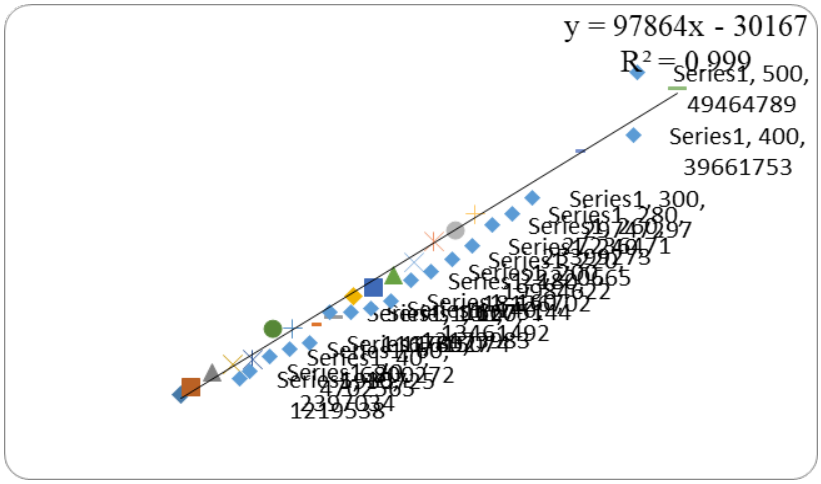

Figure 3: Linearity plot of Apremilast

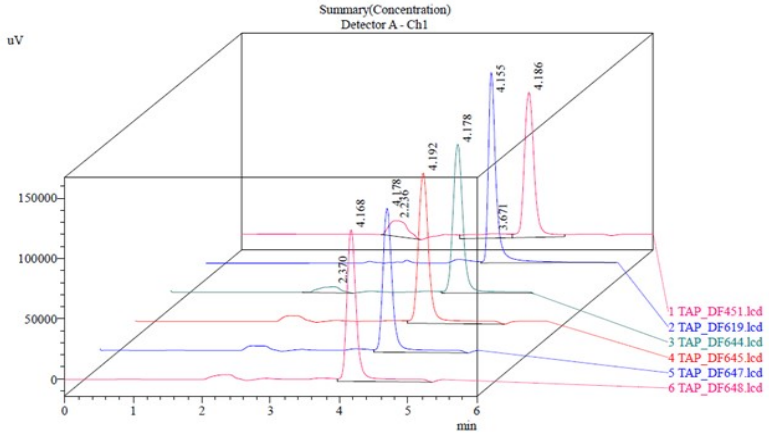

Figure 4: Overlain Chromatogram for lowest concentration of Range

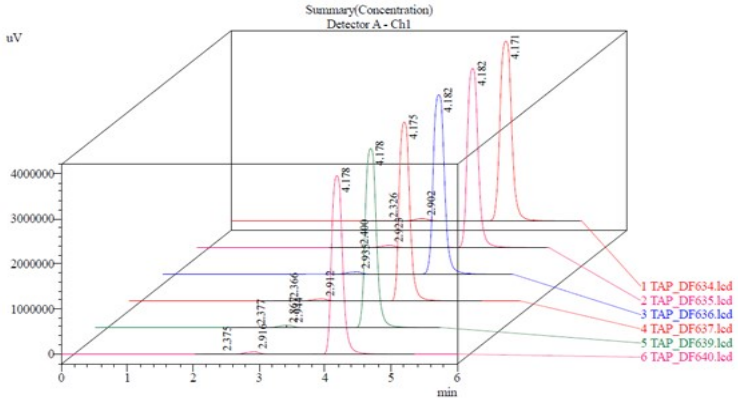

Figure 5: Overlain Chromatogram for highest concentration of Range

Table 2: Range data for Apremilast

S. No	Apremilast	
	Peak area lowest concentration (10µg/ml)	Peak area highest concentration (500µg/ml)
1	1269032	48856793
2	1253634	47041771
3	1247894	46621967
4	1255827	47302114
5	1249191	48718429
6	1240801	48552759
Mean S.D % RSD	1252730 5060.4 1.08	48856793 69937.71 1.2

Table 3: Results of Method precision

Injections	Peak area
1	111062240
2	101213784
3	10256394
4	118446342
5	111062364
6	10256376
Mean S.D % RSD	10256376 9645.2 1.24

Table 4: Accuracy Study data

Spiked Level	Standard		Mean area	Spiked		Mean area	Recovery
	Conc. (µg/ml)	Peak area		Conc. (µg/ml)	Peak area		
50%	100	10884010	10768731	180	18490895	17952012	96.1
		10387491			17215819		
		11034694			18149324		
100%	200	17817265	18075390	280	21370900	25953353	101.5
		18324653			27945221		
		18084254			28543939		
150%	300	30456484	31113633	380	39070004	37948721	97.5
		31861239			38868739		
		31023176			35907420		

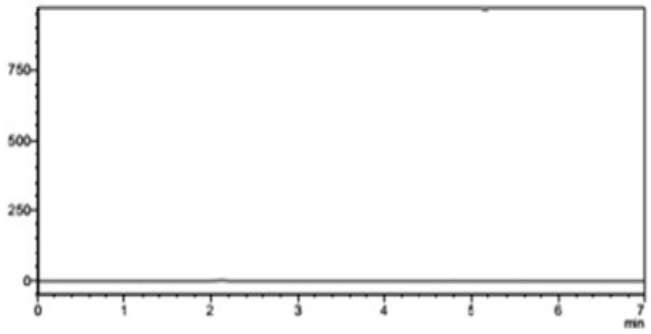

Figure 6: Chromatogram of blank

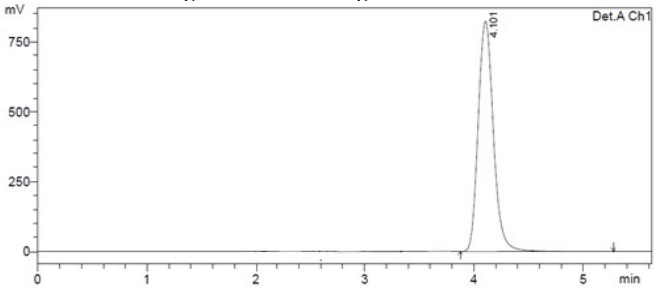

Figure 7: Chromatogram of sample

Table 5: Robustness Study (Change in Flow rate) Data

S. No.	Robustness (Flow rate)
Injections	Peak area
1	18400170
2	18775516
3	19618609
4	17285324
5	15139747
6	19706503
Mean S.D % RSD	18154311.5 23328 1.2

Table 6: Results of analyst and column variability

S. No	Analyst 1	Analyst 2	Column 1	Column 2
Injections	Peak area	Peak area	Peak area	Peak area
1	13520039	13584382	25399273	49764789
2	13432064	13506336	25611132	49760089
3	13346033	13616410	27226471	48402789
4	13376024	13464343	25312273	49763218
5	13742497	13940689	25610132	47764712
6	12324264	13914790	27446471	49701029
Mean S.D % RSD	13290153.5 15124.14 1.4	13671158.3 18231.63 1.65	26100959 2610.61 0.96	49192771 4132 0.71

RESULTS AND DISCUSSION:

Different mobile phases were tried and finally the mobile phase containing methanol: acetonitrile in the ratio of 20:80 %v/v. The Rt was achieved at 4.101 and run time was 5minutes. System suitability parameters were checked and found to be within the acceptance criteria. The method was found to be linear in the concentration range of 10 to 500µg/ml and the regression coefficient was found to be 0.999. The relative standard deviation for method precision was 1.24 which was found to be within the limits. The lowest and highest concentrations of range were found to be 10µg/ml and 500µg/ml respectively. The method was found to be more specific as there was no placebo and blank interference. The % recovery at each level was within the limits of 95% and 102% which demonstrates that the technique was accurate and furthermore uncovers that the excipients present in the pharmaceutical definition were not meddling in the proposed strategy. The robustness studies were carried by changing the flow rate and wavelength and these studies indicated that there was no effect on the study of drug. The % RSD was found to be within the limits when there was variation from analyst to analyst and column to column and the method was found to be rugged

CONCLUSION:

An effort has been made to create straightforward exact well-explicit and financially possible strategy for

estimation of apremilast by RP-HPLC in dosage form of the tablet by conducting various tests and the developed method was validated. The HPLC method is used to assay Apremilast as tablet dosage were approved regarding exactness, accuracy, linearity, particularity, robustness, ruggedness, channel approval, arrangement steadiness. The aftereffects of approval demonstrated that all the approval parameters are good. The peak territories of Apremilast peaks were straight regarding focus. The results of analysis revealed that proposed technique was appropriate for their investigation with no obstruction and recuperation was seen as satisfactory.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interests.

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