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Development and validation of few UV Spectrophotometric methods for the determination of Apremilast in bulk form and pharmaceutical dosage form

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ABSTRACT

Apremilast is an analog of thalidomide and used as a medication for the treatment of certain type of psoriasis and psoriatic arthritis. It is pale yellow to white colour powder which is non-hygroscopic and practically insoluble in water and buffer solutions of wide pH range, but is soluble is lipophilic solvents like acetone , acetonitrile , butanone , dichloromethane and tetrahydrofuran. Validation study was performed to develop novel, simple, precise, sensitive and accurate UV spectrophotometric method w for the estimation of Apremilast. Double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) with a pair of 10mm path length matched quartz cells were used for the study. Method A(methanol), Method B(ethanol), Method C(DMSO), Method D(acetonitrile) were developed foe estimation of Apremilast by zero-order and first-order derivative. Linearity was carried out in the concentration range of 0.2-1.0µg/ml and correlation coefficients were found to be 0.999. The relative standard deviation was found to be <2%. The LOD and LOQ were found to be 0.120µg/ml and 0.7810µg/ml respectively. Hence, the methods were validated according to ICH to guidelines and can be adopted for the routine analysis of Apremilast in pure and table dosage form.

Keywords: Apremilast, UV visible spectrophotometer, zero-order, first-order derivative, ICH guidelines.

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1. INTRODUCTION

Apremilast is a pthalimide derivative indicated for the treatment of certain types of psoriasis and psoriatic arthritis in adults and also used to treat moderate to severe plaque psoriasis. Apremilast, a specific PDE-4 antagonist, acts by specifically targeting a central pathogenic mechanism, binding directly to the PDE-4 enzyme and bypassing complex antigen-receptor interactive immunoregulatory mechanisms. Once drug-enzyme binding occurs, a series of events follow, the foremost being increased levels of cAMP, which in turn plummets the levels of pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-23, IL-12, [1] and leukotriene B4, and also increases the levels of anti-inflammatory cytokines such as IL-10.[2] In addition, Apremilast also binds to toll-like receptor 4 in peripheral blood mononuclear cells, further reducing the production of pro-inflammatory cytokines. [3] Apremilast also reduces the activity of nitric oxide synthase, [4, 5] an enzyme responsible for the synthesis of nitric oxide which is an important pro-inflammatory mediator, thereby preventing trafficking of macrophages and myeloid dendritic cells to the dermis and epidermis in psoriatic skins. In this way, Apremilast plays a noteworthy anti-inflammatory role.

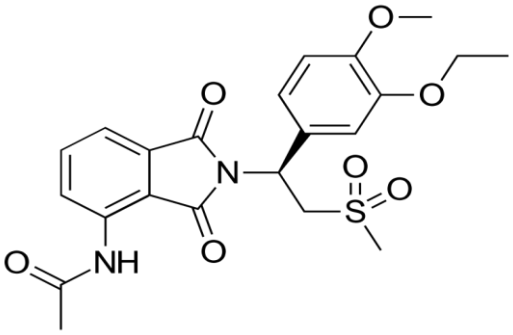


Figure 1: Chemical structure of Apremilast

Apremilast is an orally administered drug. Post intake, it is rapidly absorbed by the body reaching its peak plasma concentration after 2-3 h. The bioavailability of Apremilast is around 73% and its mean apparent volume of distribution is 87 L. Apremilast has a t 1/2 of 6-9 h. [6] Metabolism of Apremilast occurs through a cytochrome (CYP) 3A4-

mediated oxidative metabolism, followed by glucuronidation, nonenzymatic hydrolysis, and a non-CYP 3A4-mediated metabolism. [7] Apremilast is eliminated mainly by the renal route, though some of the drug is also excreted through the feces. [8]

Literature survey revealed that Apremilast was determined by UV-Visible spectroscopy. In the present study, the authors have proposed four simple validated spectrophotometric methods for the determination of Apremilast in pharmaceutical dosage forms.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Methanol (Merck), Ethanol (Merck), Dimethyl sulfoxide (Merck), Acetonitrile (Rankem) was used. Apremilast, obtained as a gift sample from MSN Life Sciences Pvt. Ltd., (India), was used.

2.2 Preparation of stock solution

The standard solution of Apremilast was prepared by dissolving accurately about 25 mg of the Apremilast with methanol in a 25 ml volumetric flask. From the standard solution dilutions are made using methanol and they are scanned in the range of 200-400nm to determine the wavelength of maximum absorption.

2.3 Validation Procedure

The proposed method was optimised using methanol as stock solvent. Methanol (Method A) and Ethanol, DMSO and Acetonitrile (method B, C and D) as solvents. The present method was validated for the various parameters as per ICH guidelines. [9]

2.4 Linearity

Different aliquots were taken from working solution and diluted with Methanol (Method A) Ethanol (Method B), DMSO (Method C), Acetonitrile(Method D) separately to prepare series of concentrations from 0.2-1.0µg/ml.[10] Absorbance was measured at 340 nm. Finally, the calibration curve was plotted between concentration and absorbance.

2.5 Accuracy

For assay methods, samples are prepared in triplicate at three concentration levels covering the specified three levels over a range of 50–150% of the target concentration. [11]

2.6 Precision

2.6.1 Intraday Precision

It is determined by analysing the drug at a 3 different concentration and each concentration for three times on a same day and calculated the value of Mean, SD, and %RSD.

2.6.2 Inter day Precision

It is determined similarly, but the analysis being carried out daily for three consecutive days and calculated the value of Mean, SD, and %RSD.

2.7 Limit of Detection and Limit of Quantification

ICH guideline describes several approaches to determine the detection and quantification limits. These include visual evaluation, signal to- noise ratio and the use of standard deviation of the response and the slope of the calibration curve. The LOD and LOQ were based on the third approach and were calculated according to the 3.3σ/S and 10 σ/S criterions, respectively, where σ is the standard deviation of the S-intercepts of the regression lines and σ is the slope of the calibration curve. [12]

2.8 Assay of marketed dosage form

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to10 mg of VGC was transferred to 100 ml volumetric flask and dissolved in methanol and final volume was made up with the same. The sample solution was then filtered through Whatman filter paper no. 41. This is stock solution of 100 ug/ml. From the above stock solution 0.5, 1, 2, 3, 4, 5, 6, ml of solution was transferred in 10 ml volumetric flask and was diluted with methanol up to 10 ml. This gives solution of 0.2to 1.0 ug/ml concentration of VGC. These solutions were scanned under entire UV region (400 nm to 200 nm) and area of it between the wavelength range 340 nm to 345 nm was calculated to get the concentration of drugs.

3. RESULTS

New spectrophotometric methods were developed for the determination of Apremilast in pharmaceutical preparations. Apremilast has shown absorption maxima (λ_{max}) at 342 nm in Methanol (Method A) and Ethanol (Method B) and DMSO (Method C) and Acetonitrile (Method D) at the absorption maxima (λ_{max}) at 345 nm and the corresponding absorption spectra were shown in Figures.

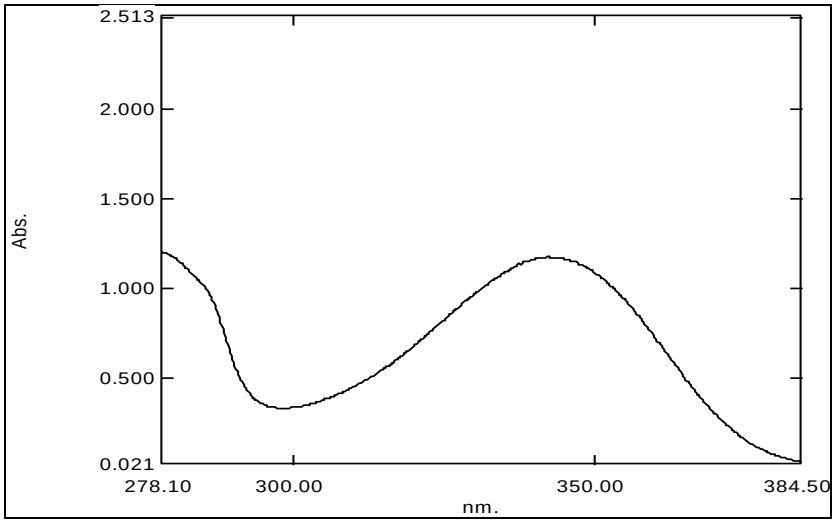


Figure 2: Absorption spectrum of Apremilast (0.6µg /mL) in Methanol

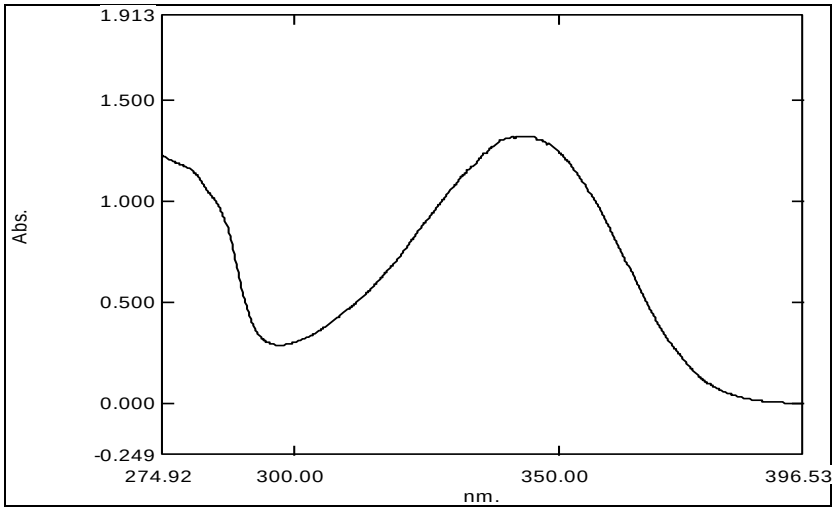


Figure 3: Absorption spectrum of Apremilast (1.2µg /mL) in Ethanol

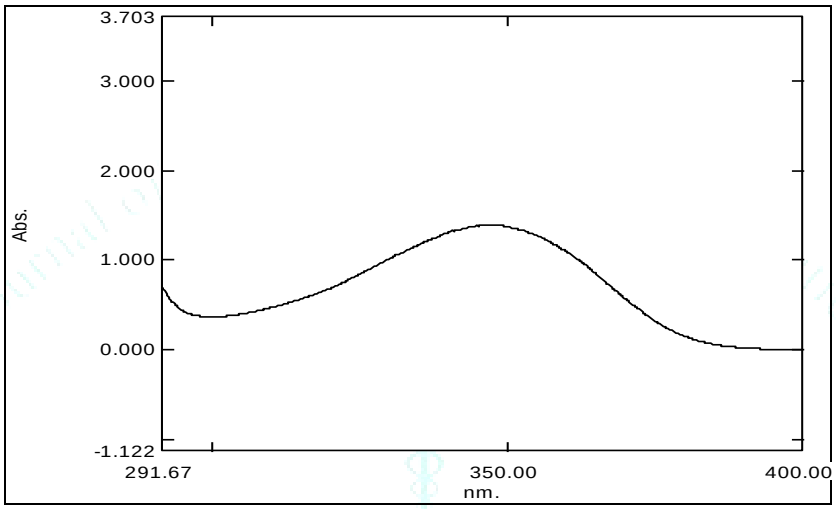


Figure 4: Absorption spectrum of Apremilast (1.0µg /mL) in DMSO

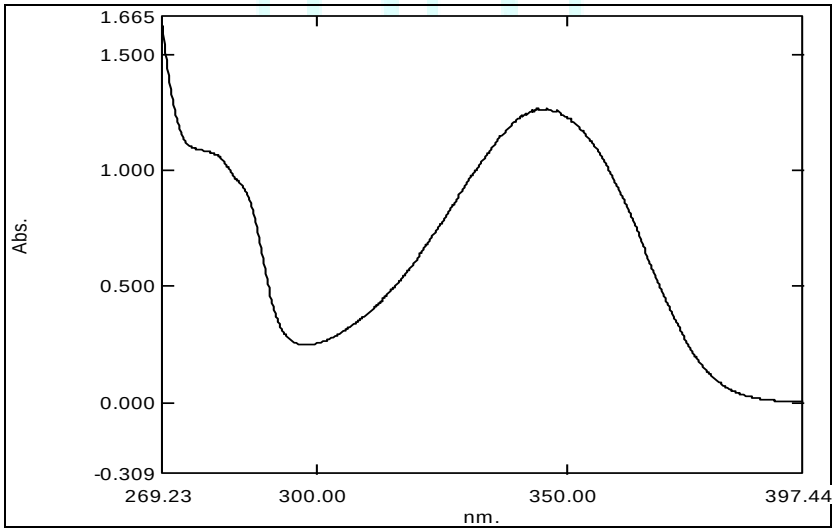


Figure 5: Absorption spectrum of Apremilast (0.8µg /mL) in Acetonitrile

In method A, Apremilast has shown zero crossing points at 316.48, 342.22, and 394.43 nm with maxima at 322.66 nm and minima at 363.57 nm in Figure 7 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve. In method B, Apremilast has shown zero crossing point at 277.31, 296.54, 343.07, and 388.45 with maxima at 325.55 nm and minima at 363.27 nm in Figure 8 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve. In method C, Apremilast has shown

zero crossing points at 300.00, 347.55, and 392.475 nm with maxima at 326.17 nm and minima at 367.88 nm in Figure 9 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve. Similarly, in method D, Apremilast has shown zero crossing point at 277.19, 296.78, 345.56, and 390.08 with maxima at 324.79 nm and minima at 365.88 nm in Figure 10 and therefore the amplitude has been taken against the concentration for the construction of the calibration curve.

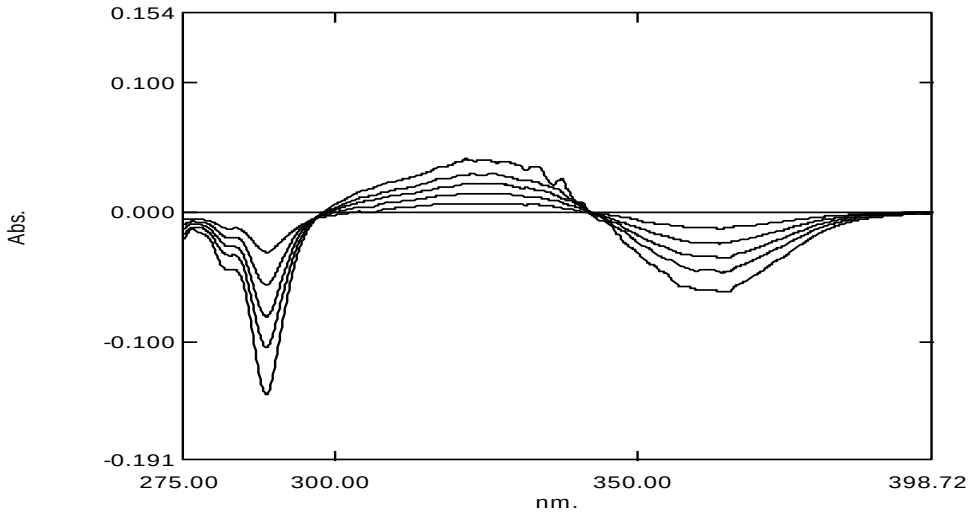


Figure 6: Overlay first derivative spectra (D_1) of Apremilast in Methanol

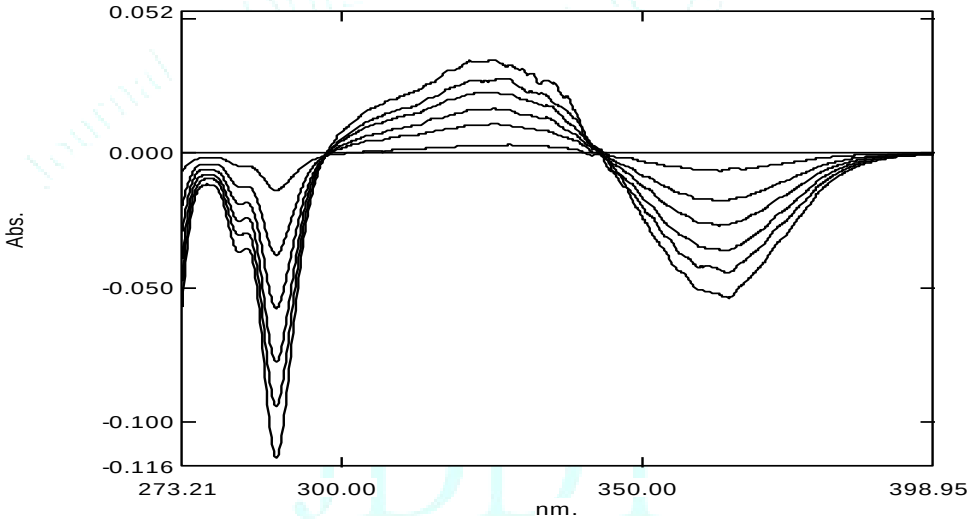


Figure 7: Overlay first derivative spectra (D_1) of Apremilast in Ethanol

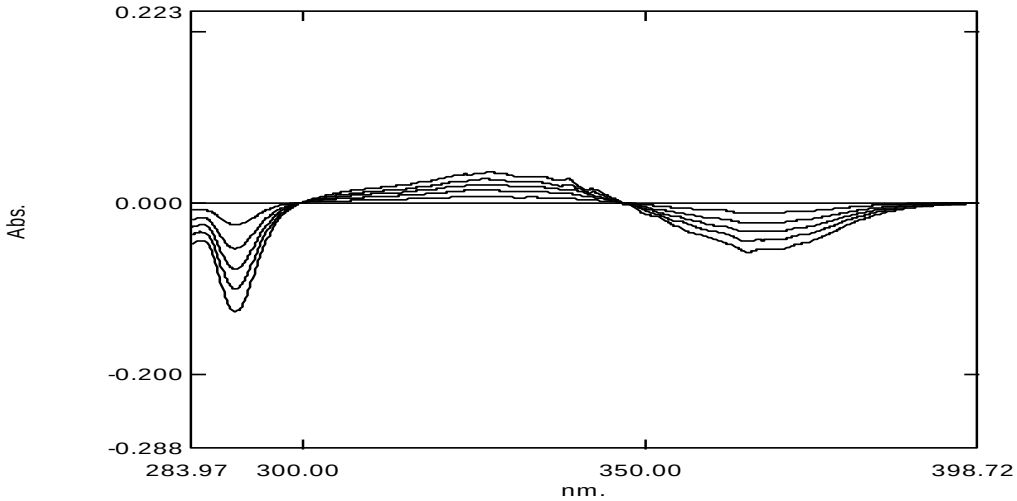


Figure 8: Overlay first derivative spectra (D_1) of Apremilast in DMSO

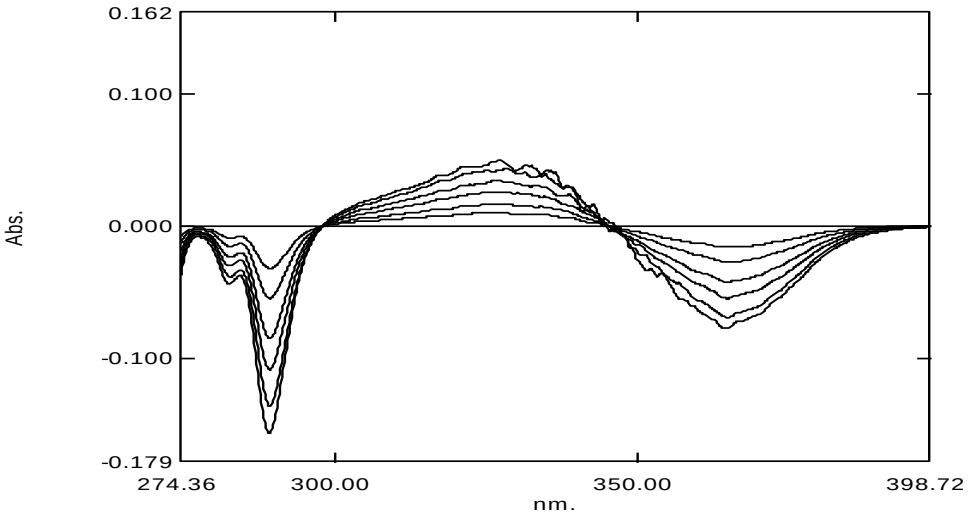


Figure 9: Overlay first derivative spectra (D_1) of Apremilast in Acetonitrile

Beer's law was obeyed in the concentration range of: (0.2-1.0 µg/mL) for method A, (0.2-1.6 µg/mL) for method B, (0.2-1.6 µg/mL) for method C and (0.2-1.2 µg/mL) for method D. The linear regression equations were found to be: y = 0.1548x, y = 0.1095x, y = 0.1383x, y = 1.589x for method A, B, C and D respectively with correlation coefficient 0.9994, 0.9990.9997 and 0.9997 respectively.

Table 1: Optical Characteristics of Apremilast for Methanol in Zero order

Parameters	Zero Order
λ_{max}	342
Regression equation	0.1555x-0.0046
Slope	0.1555x
Intercept	0.0046
Correlation coefficient (R^2)	0.9994
Sandell's Sensitivity (µg/cm²/0.001absorbance unit)	$6.52*10^{-5}$µgcm^{-2}

Table 2: Optical Characteristics of Apremilast for Ethanol in Zero order

Parameters	Zero Order
λ_{max}	342
Regression equation	0.1092x+0.0032
Slope	0.1092x
Intercept	0.0032
Correlation coefficient (R^2)	0.9993
Sandell's Sensitivity (µg/cm²/0.001absorbance unit)	$8.96*10^{-5}$µgcm^{-2}

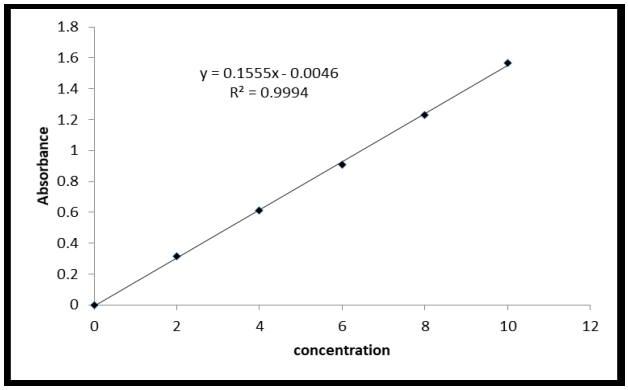
Table 3: Optical Characteristics of Apremilast for DMSO in Zero order

Parameters	Zero order
λ_{max}	345
Regression equation	0.1372x+0.0097
Slope	0.1372x
Intercept	0.0097
Correlation coefficient (R^2)	0.9998
Sandell's Sensitivity (µg/cm²/0.001absorbance unit)	$7.01*10^{-5}$µgcm^{-2}

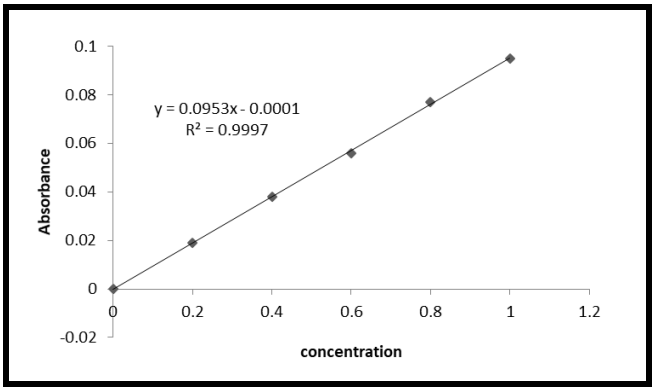
Table 4: Optical Characteristics of Apremilast for Acetonitrile in Zero order

Parameters	Zero order
λ_{max}	345
Regression equation	0.1596x-0.0063
Slope	0.1596x
Intercept	0.0063
Correlation coefficient (R^2)	0.9997
Sandell's Sensitivity (µg/cm²/0.001absorbance unit)	$6.25*10^{-5}$µgcm^{-2}

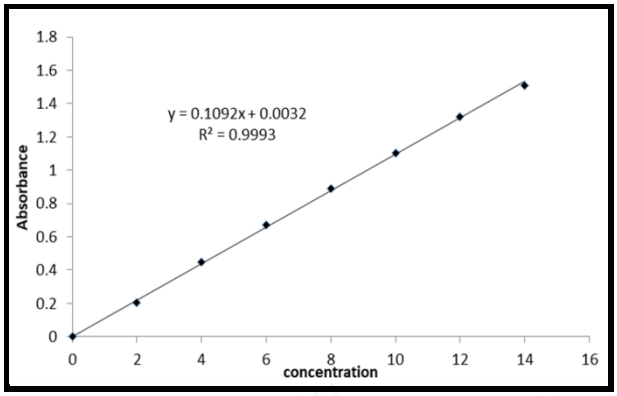
Beer's law was obeyed in the first derivative of concentration range of: (0.2-1.0 µg/mL) for method E, (0.2-1.6 µg/mL) for method F, (0.2-1.6 µg/mL) for method G and (0.2-1.2 µg/mL) for method H. The linear regression equations were found to be: y = 0.094x, y = 0.727x, y = 0.0899x, y = 0.1096x for method E, F, G and H respectively with correlation coefficient 0.9995, 0.9998, 0.9984 and 0.999 respectively.



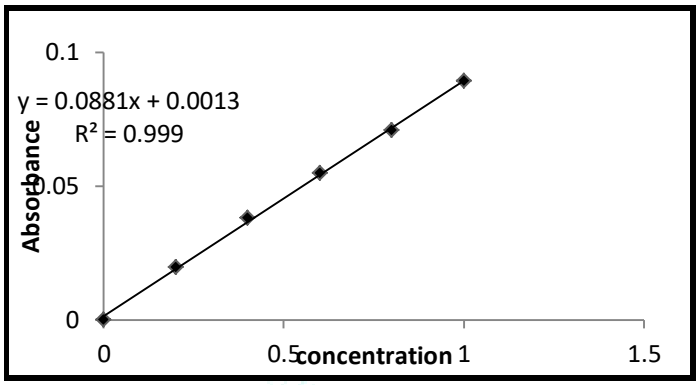
[A]



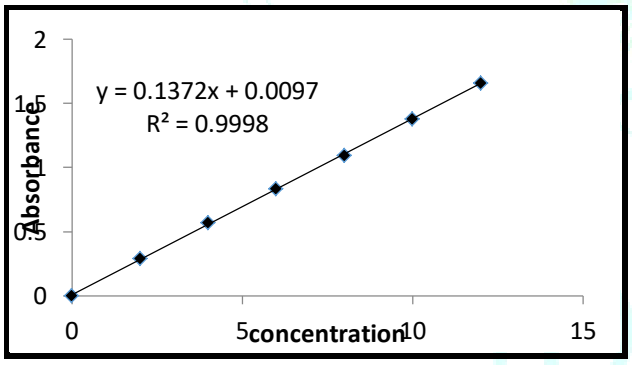
[E]



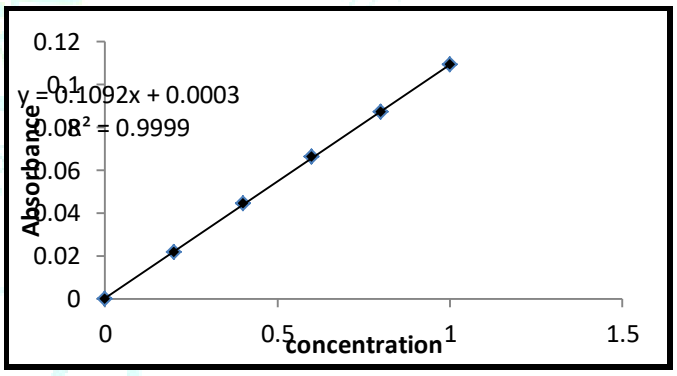
[B]



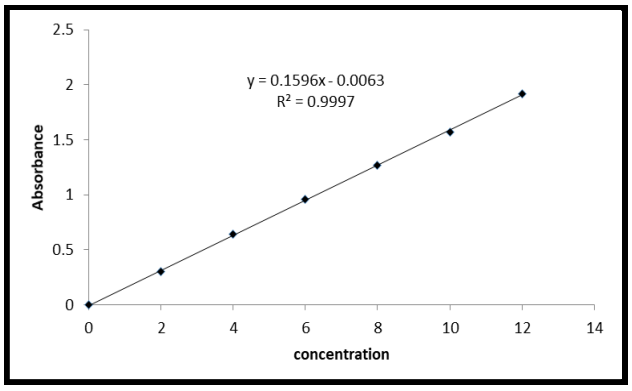
[F]



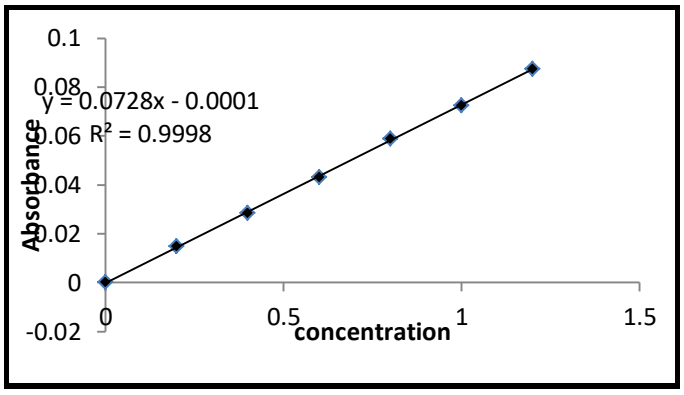
[C]



[G]



[D]



[H]

Figure 10: Calibration curves of Apremilast in method A, B, C, D, E, F, G and H

Table 5: Linearity of Apremilast for method A and B

Concentration (µg/ml)	Methanol Absorbance at 342nm	Ethanol Absorbance at 342nm
20	0.316	0.202
40	0.613	0.446
60	0.908	0.672
80	1.230	0.888
100	1.569	1.104

Table 6: Linearity of Apremilast for method C and D

Concentration (µg/ml)	DMSO Absorbance at 345nm	Acetonitrile Absorbance at 354nm
20	0.291	0.298
40	0.570	0.640
60	0.834	0.960
80	1.095	1.269
100	1.380	1.572

The % RSD values in precision studies were found to be 0.0109 – 0.0136(RSD <2%) indicating that the method is more precise (Table 3,4). The % RSD values in accuracy studies were found to be 1.19-1.67 (RSD <2%) indicating that the method is more accurate (Table 5).

Table 7: Results of system precision

S.No	Absorbance
1	1.8990
2	1.8995
3	1.8996
4	1.8991
5	1.8996
6	1.8992
Mean	1.8993
Standard deviation	0.0002
% Relative Standard deviation	0.0109

Table 8: Results of method precision for intra - day precision

Concentration (µg)	Sample Absorbance	Mean Absorbance ± S.D	% RSD (n=3)
4	0.7991 0.7989 0.7998	0.7992 ± 0.0001	0.0375
6	1.1341 1.1344 1.1348	1.1344 ± 0.0004	0.0352
8	1.4677 1.4676 1.4672	1.4675 ± 0.0002	0.0136

Table 9: Result of accuracy

Recovery level%	Absorbance	% Recovery	Mean% recovery	%RSD (n=3)
80 80 80	0.7888 0.7990 0.7899	98.6 99.87 98.73	99.0	1.9
100 100 100	1.1347 1.1343 1.1349	100.2 93.80 97.99	97.33	1.67
120 120 120	1.4677 1.4676 1.4678	97.26 101.1 97.01	98.45	1.74

CONCLUSION

In this work, an attempt made to develop a spectrophotometric method and for the determination of Apremilast which is validated and applied for the determination of Apremilast in pharmaceutical dosage form (API form).

Apremilast in bulk as well as pharmaceutical tablet, none of the usual excipients employed in the formulation of Apremilast dosage forms interfered in the analysis of Apremilast by the developed method. Validation parameters are found within the limits.

The proposed method was validated per ICH guidelines. The accuracy and precision are within the acceptable limits. Consistent recoveries are observed. The method is precise and accurate enough.

% RSD of the precision and accuracy batch by different analysts were found to be within the acceptable limits (≤2.0%) indicating the method was rugged.

The method was shown to be free of interference from any components of pharmaceutical dosage form or degradation products indicating that the method is highly specific.

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AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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