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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING DENSITOMETRIC METHOD FOR ESTIMATION OF APREMILAST IN BULK DRUG AND TABLET DOSAGE FORM

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ABSTRACT

The current work describes development and validation of a simple, accurate and precise stability-indicating high performance thin layer chromatographic (HPTLC) method for determination of Apremilast as bulk drug and in tablet dosage form. As stability testing is major step in the development of new drug as well as formulation, stress degradation studies were carried out according to ICH guidelines. Apremilast was found susceptible to all the analyzed stress conditions except photolysis. The separation was performed on pre-coated silica gel 60 F_{254} plates (10 cm×10 cm) utilizing Toluene: Ethyl acetate (5: 5, v/v) as mobile phase with densitometric scanning at 230 nm. The retention factor was found to be 0.53 ± 0.02.Results were found to be linear in the range 100-600 ng $band^{-1}$. The developed method has been effectively applied for the drug estimation in tablet dosage form. The % drug content was found to be 100.03. The method was found to be precise as % R.S.D. values were less than 2. The developed method is stability indicating and can be successfully employed for quantification of drug in tablet dosage form. The results clearly indicated that there was no interference of the related substance for Apremilast which demonstrated that the developed method was stability-indicating and can be used for drug stability studies and quality control monitoring of Apremilast.

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INTRODUCTION

Apremilast, chemically, N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methyl sulfonyl) ethyl]-2, 3-dihydro-1, 3-dioxo-1 H-isoindol-4-yl] acetamide (Fig. 1) is a phosphodiester-4 inhibitor that is used to treat rheumatoid and psoriatic arthritis ad also works as an anti-inflammatory to treat the inflammation brought on by these illnesses [1].

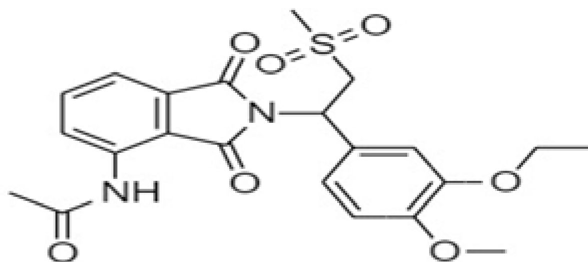

Figure 1: Chemical structure of Apremilast.

Apremilast was approved by the Food and Drug Administration (FDA) in 2014 as the first selective inhibitor of phosphodiesterase 4 (PDE4) for individuals with active Psoriatic arthritis [2].Anextensive literature survey revealed that different analytical methods has been reported for quantitative analysis of Apremilast. UV spectrophotometric methods for determination of Apremilast either as a single drug or in combination with other drugs in bulk and its tablet formulation has been reported [3-6]. Development and validation of stability indicating RP-HPLCmethod for the estimation of Apremilast was also reported [7]. UPLC-MS-MS for determination of Apremilast in human rat plasma is also reported [8].One HPTLC method involvingapplication of Box-Behnken design for validation of high-performance thin-layer chromatography/densitometry method for robustness determination of Apremilast in Bulk and inhouse Tablets is also found in the literature [9].

To best of our information, no reports were found in the literature for determination of Apremilast in pharmaceutical tablet dosage form by stability-indicating high performance thin layer chromatographic (HPTLC) method. High performance thin layer chromatography (HPTLC) is the most powerful analytical version of thin layer chromatography which is used for the analysis of pharmaceuticals to determine the purity of the drugs available from various sources by detecting the related impurities. The technique is simpler, provides more flexibility than HPLC and used as cost-effective quality-control tool for analysis of pharmaceuticals. Hence the purpose of present work was to develop and validate a simple, sensitive, precise and accurate stability indicating HPTLC procedure for determination of Apremilast as bulk drug and in tablet dosage form in accordance with International Conference on Harmonisation Guidelines [10, 11].

MATERIALS AND METHODS

Chemicals and reagents

Analytically pure working standard Apremilast was obtained as a gift sample from AjantaPharma Pvt Ltd., Aurangabad, India. The pharmaceutical tablet dosage form used in this study was Aprezo labeled to contain 20mg of Apremilast was procured from the local pharmacy. Toluene, Ethyl Acetate (both AR grade) was purchased fromLobaChemiePvt Ltd., India.

Instrumentation and chromatographic conditions

Chromatographic separation of the drug was achieved by use of Merck TLC plates precoated with silica gel 60 F_{254} (10 cm × 10 cm with 250 µm layer thickness) from E. MERCK, Darmstadt, Germany, using a CAMAG Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band with a 6 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland). Linear ascending development was carried out in 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using toluene: ethyl acetate (5: 5, v/v) as mobile phase. The chamber was saturated with mobile phase for 20 min prior to development. The length of chromatogram run was 70 mm. Densitometric scanning was performed at 230 nm using Camag TLC scanner III operated by winCATS software version 1.4.2.

Preparation of standard stock solution

For preparation of working standard solution, accurately weighed 10 mg of the drug was dissolved 10 ml of methanol to attain concentration of 1000 µg mL^{-1} which was diluted further with methanol to acquire final concentration 100 ng μL^{-1}.

Analysis of tablet dosage form

Analysis of tablet formulation was performed to estimate the content of Apremilast by using commercial brand of tablet namely Aprezo. Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalentto 10 mg of Apremilast was weighed and transferred to a 10 ml volumetric flask. The mixture was sonicated for 5 min and diluted to volume with methanol. The solution was filtered and one milliliter of the above solution was further diluted with methanol to obtain the concentration 100 ng μL^{-1}. Two µL volume of this solution was applied on TLC plate to obtain final sample concentration of 200 ng $band^{-1}$. After chromatographic development the peak areas of the bands were measured at 230 nm and the amount of each drug in each sample was determined from the respective calibration plots. The analytical procedure was repeated six times for the homogenous powder sample. The percent drug content (Mean±S.D.) was found to be 100.03± 0.59.

Stress degradation studies of bulk drug

Stress degradation experiments were carried out for the bulk drug to confirm the stability by subjecting it to ICH-recommended physical stress conditions. The degradation studies were conducted at a concentration of 1000 ngμL^{-1}. The acid and base hydrolytic experiments were carried out by treatment of stock drug solution with 1 N HCl and 1 N NaOH separately at room temperature for 2 h. The acid and alkali stressed samples were neutralized with HCl and NaOH, respectively to provide the final concentration of 100ng $band^{-1}$. To perform oxidative degradation, standard drug solution was treated with 3 % H_2O_2 at room temperature for 30 min and then diluted with methanol to obtain 100 ng $band^{-1}$ solution. Thermal stress degradation was achieved by placing the powdered bulk drug in an oven at 60°C for 18 h. The solid drug powder was exposed UV light up to 200-watt hour square $meter^{-1}$ to check photolytic degradation. Thermal and photolytic samples were diluted with methanol to get concentration of 100 ng $band^{-1}$.

RESULTS AND DISCUSSION

Method optimization

The objective of present research work was to develop stability indicating HPLTC method which would be capable to give the acceptable resolution between Apremilast and other excipients present in formulation. To attain the necessary chromatographic separation, varied solvent systems containing different ratios of methanol, toluene, benzene, chloroform and ethyl acetate were examined (data not shown) to separate and resolve spot of Apremilast. Finally, the mobile phase comprising of Toluene: Ethyl acetate (5:5, v/v) was chosen as optimum for achieving well defined and resolved peak with densitometric detection at 230 nm. The retention factor was found to be 0.53±0.03. Representative densitogram of standard solution of Apremilast is represented in Fig. 2.

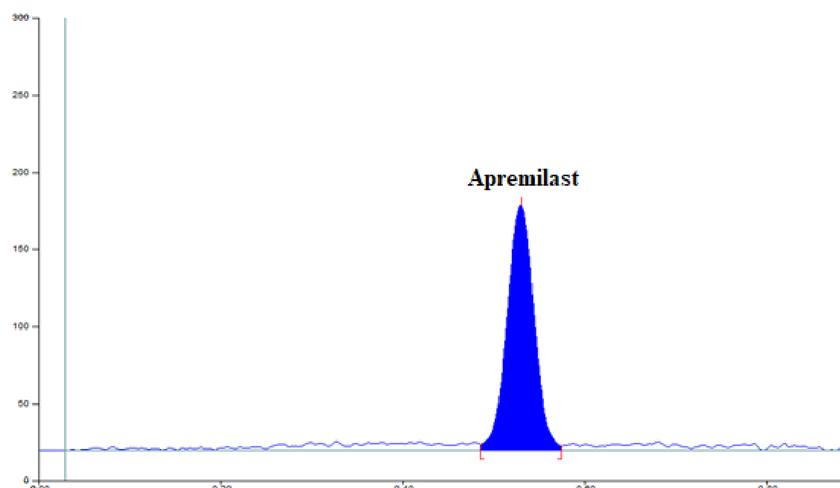

Fig. 2: Densitogram of standard solution of Apremilast (400 ng $band^{-1}$, Rf= 0.53±0.03).

The stress degradation results demonstrated susceptibility of drug to all the analyzed stress conditions except photolysis. Apremilast after exposure to hydrolytic stress conditions showed considerable degradation without appearance of any degradation product but there was decrease in the area of drug as compared to initial area. Apremilast after exposure to oxidative stress condition showed significant degradation with product of degradation at Rf values 0.82 (D1) and 0.87 (D2) (Fig. 3). The outcomes of degradation studies for Apremilast along with % degradation and % of drug recovered are summarized in Table 1.

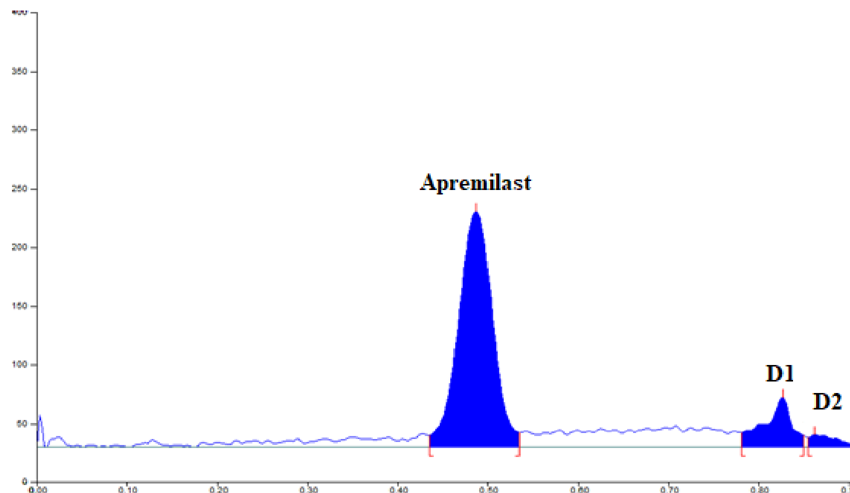

Fig.3: Densitogram after treatment with 3% H_2O_2.

Table 1: Stress Degradation Studies.

Stress conditions	% Degradation	% Recovery
Acid hydrolysis (1 N HCl, Kept at RT for 2 h)	14.57	85.42
Base hydrolysis (1 N NaOH, Kept at RT for 2 h)	19.86	80.13
Oxidative degradation (3 % H_2O_2, Kept at RT for 30 min)	16.92	83.07
Thermal degradation (70º C for 18 h)	15.16	84.83
Photolytic degradation (UV light, 200 watt h) square meter^{-1})	----	99.02

Method Validation

In accordance with ICH Q2 (R1) criteria, the developed procedure was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantitation, and robustness.

Linearity

The linearity of proposed method was checked by spotting volumes 1, 2, 3, 4, 5 and 6 µL of standard solution of Apremilast (100 µg mL^{-1}) onto the TLC plates, developed and scanned under optimized chromatographic conditions. The method was found to be linear in the concentration range 100-600 ng band^{-1} with high correlation coefficient. The linear regression equation was found to be y = 2.7474x + 1512.7 with correlation coefficient (R^2) value of 0.995 (Fig. 4). A 3D densitogram obtained in the concentration range 100-600 ng band^{-1}is shown in Fig. 5.

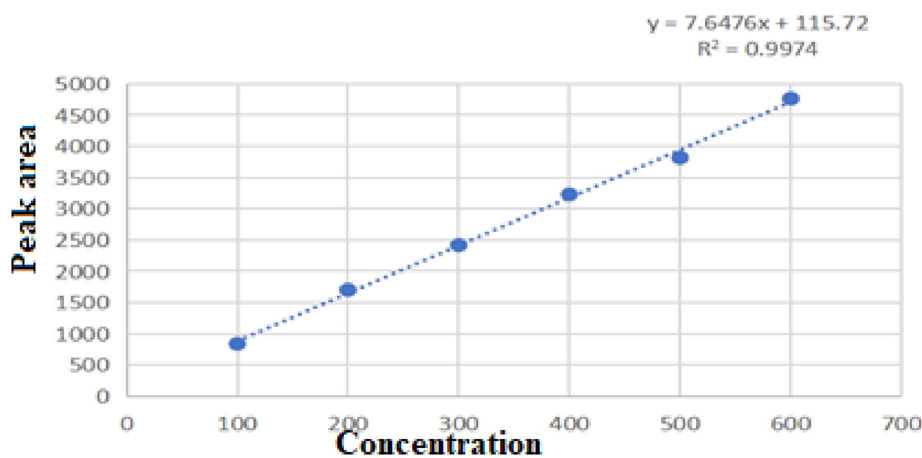

Fig. 4: Calibration curve for Apremilast.

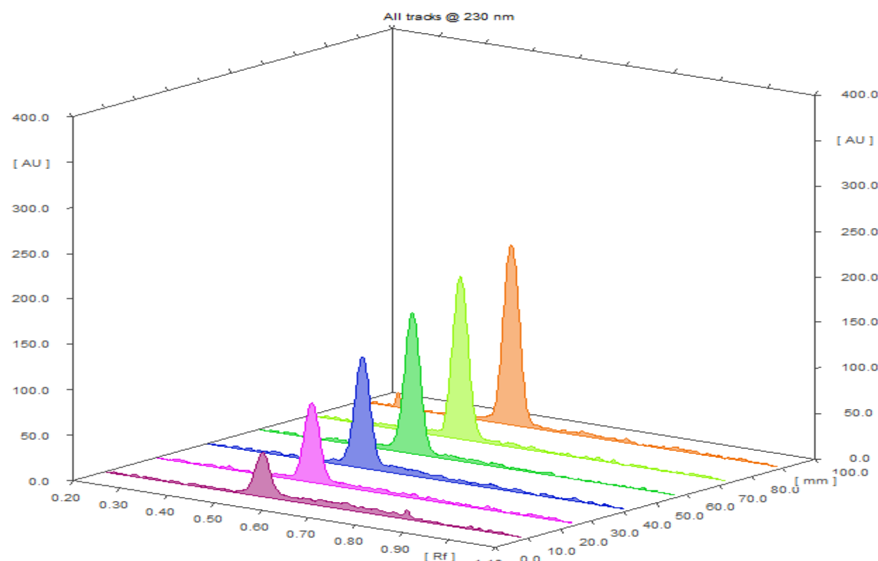

Fig. 5: 3D densitogram in concentration range 100-600 ng band^{-1}.

Precision

The precision of the method was demonstrated by intraday and interday variation studies in which three replicates of three concentrations (200, 300, 400 ng band^{-1}) within the linearity range were analyzed on the same day and on three consecutive days, respectively and percentage R.S.D. was calculated. The % R.S.D. for intraday and interday variation was found to be in the range of 0.56 to 0.96 and 0.46 to 0.82, respectively.The % R.S.D. values obtained for intraday and interday variations were found to be < 2 which indicated that method is precise.

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were calculated using the formula LOD= $3.3\ \sigma / S$ and LOQ = $10\ \sigma / S$; Where σ = standard deviation of lowest concentration and S = slope of the calibration curve. The LOD and LOQ was found to be 10.71ng band^{-1}and 32.47 ng band^{-1}, respectively.

Accuracy

Accuracy of developed method was carried out by recovery studies by standard addition method which involved addition of known quantity of standard drug to pre-analysed sample solution at three different levels 80, 100 and 120 %. Basic concentration of sample chosen was 200 ng band^{-1} from tablet solution. The drug concentrations were calculated from linearity equation. The results of the recovery studies indicated the accuracy of method for estimation of drug in tablet dosage form. The results obtained are represented in Table 2.

Table 2: Recovery studies.

Drug	Concentration taken (ng band^{-1})	Concentration added (ng band^{-1})	Total Concentration found (ng band^{-1})	% Recovery±R.S.D.*
Apremilast	200	160	361.54	100.53±0.68
	200	200	401.15	101.16±0.92
	200	240	437.70	99.47±0.17

*Average of three determinations

Robustness

Robustness of the method was determined by introducing intentional variations in method parameters during which mobile phase composition (± 2 % ethyl acetate), wavelength (± 1 nm) was altered and the effect on the area of drug was noted. The areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

CONCLUSION

Stability-demonstrating HPTLC-densitometric method without interference from the excipients or from degradation products has been developed and validated for the determination of Apremilast as bulk drug and in tablet dosage form. Compared with HPTLC method reported by Chaudhari S R et al., the established method is more sensitive as the range for the method developed starts from 100ng band^{-1}, whereas range starts from 250 ng band^{-1} for reported method. The developed method is simple, sensitive, precise, accurate, and reproducible. The developed method can be used for quantitative analysis of drug in pharmaceutical dosage form. As the method is stability indicating, it may be extended to study the degradation kinetics of drug.

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Conflict of Interest

No Conflict of Interest

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Authors' Contribution

All authors contributed in the research work

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