ORIGINAL ARTICLE

Development and Validation for Determination of Apremilast in Bulk and in Tablets by UV Spectrophotometer

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Abstract

Background: Pharmaceutical analysis plays a key role in the quality assurance and quality control of bulk drugs. A documented validation program offers a high level of confidence that an operation or facility will consistently deliver a product that satisfies a set standard. A premilast is an aphosphodiesterase-4 inhibitor used in the treatment of psoriasis. **Objective:** The main objective is to develop a simple, rapid, accurate, and economical spectrophotometric method for the determination of apremilast in bulk and its tablet dosage form as per ICH guidelines. **Materials and Method:** The estimation was carried out using HPLC-grade acetonitrile as a solvent and quantitation was achieved using a double-beam UV spectrophotometer at 230 nm. The present method was suitable for its intended purpose as prescribed in ICH Q2 guidelines. The analytical method was validated to determine the linearity, precision, accuracy, robustness, ruggedness, LOD, and LOQ of the method. **Results:** The λ_{max} of apremilast in acetonitrile was found to be 230 nm. The drug has a correlation coefficient (r^2) value of 0.999 and exhibits linearity in the concentration range of 2–10 µg/mL. The proposed method was applied to pharmaceutical formulation and % the amount of drug estimated 99% was found in good agreement with the label claim. The repeatability, intraday, and interday changes of accuracy were examined. The approach is considered exact if the percentage RSD value is <2. **Conclusion:** The above method was a rapid and cost-effective quality control tool for routine analysis of apremilast in bulk and in pharmaceutical dosage form. The method can be useful for the day-to-day routine analysis in the quality control departments of bulk and pharmaceutical formulations industries.

Key words: Apremilast, phosphodiesterase-4 inhibitor, psoriasis, UV-spectrophotometry, validation

INTRODUCTION

Pharmaceutical analysis plays a key role in the quality assurance and quality control of bulk drugs. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. Analytical chemistry is the science of obtaining, processing, and communicating information about the composition and structure of matter. In other words, it is the art and science of determining what matter is and how much it exists.[1,2] Analytical chemistry also is concerned with developing the tools used to examine chemical compositions. It is concerned with the chemical characterization of matter both qualitatively and quantitatively. Quantitative analysis

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establishes the quantity of one or more of these components, whereas qualitative analysis indicates the identification of the chemical species present in the sample.[3,4]

Apremilast is a medication primarily used for the treatment of certain inflammatory conditions, specifically psoriasis and psoriatic arthritis. It belongs to a class of drugs known as phosphodiesterase-4 (PDE-4) inhibitors. Apremilast works by inhibiting the activity of the PDE-4 enzyme, which plays a role in the inflammatory response. PDE-4 is an enzyme that is involved in the breakdown of cyclic adenosine monophosphate (cAMP), a messenger molecule that regulates inflammation. By inhibiting PDE-4, apremilast increases cAMP levels, leading to a reduction in the inflammatory response. Apremilast is typically administered orally in tablet form, making it convenient for patients to take at home.

Apremilast chemically N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-methylsulfonylethyl]-1,3-dioxoisoindol-4-yl] acetamide. The apremilast molecular formula is $C_{22}H_{24}N_2O_7S$, and molecular weight is 460.501 g/mol. Apremilast is a white to pale yellow non-hygroscopic powder with a melting point range of approximately 150–152°C. It is practically insoluble in water, slightly soluble in ethanol, and soluble in methanol, acetone. Apremilast is the S-enantiomer with a specific rotation of +28.1° in acetonitrile at a concentration of 20 mg/mL. The molecular structure of apremilast is shown in Figure 1. Marketed available trade names are otezla, aprezo, aprenext, and apxenta.[5,6]

Apremilast was approved by the United States Food and Drug Administration (US-FDA) in March 2014 for the treatment of adults with active psoriatic arthritis. Apremilast is the first oral agent that is FDA-approved for the treatment of psoriatic arthritis and offers the convenience of oral dosing compared to treatment with biopharmaceuticals. In September 2014, the US FDA approved apremilast for the treatment of moderate-to-severe plaque psoriasis. It is also being tested for its efficacy in treating other chronic inflammatory diseases such as ankylosing spondylitis, Behcet's disease, and rheumatoid arthritis.[7-9]

Literature survey reveals that various analytical methods have been reported for the estimation of apremilast; they are HPLC, LC-MS/MS, HPTLC, FTIR, DSC, NMR, and XRD in bulk with its impurities, laboratory mixture, pharmaceutical dosage form, as well as biological fluids. Spectrophotometry is still one of the most widely used drug identification techniques due to its affordability, ease of use, and high specificity. The objective of the present research work was to develop a new analytical UV spectrophotometric method and its validation parameters for the proposed method according to ICH Q2 guidelines for the estimation of apremilast in bulk and tablet formulations.[10-13]

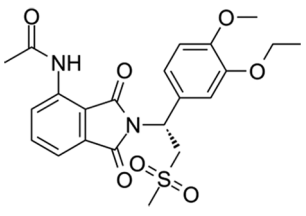

Figure 1: Chemical structure of apremilast

In the present study, for the 1st time, a simple new spectrophotometric method for quantification of sitagliptin has been developed and validated according to the International Conference on Harmonization (ICH) guidelines and successfully applied for assay of pharmaceutical formulations.

MATERIALS AND METHODS

Instruments used

The analysis was performed on a double-beam UV-visible spectrophotometer (PG Instruments, T-60 UV, India) connected to a computer loaded with spectra manager software UVW was employed with a spectral bandwidth of 1 nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. In addition, an electronic balance was used for weighing purpose, and an ultra-sonicator was used in this study.

Chemicals and reagents used

All reagents such as HPLC grade methanol, HPLC grade water, and HPLC grade acetonitrile purchased from Hyderabad, Telangana were utilized throughout the experiment. All chemicals and reagents used were of analytical grade.

Apremilast API

The pure API sample of apremilast was procured as a gift sample from Aizant Drug Research Solutions Pvt. Ltd., Dulapally, Hyderabad.

Pharmaceutical dosage form

The marketed pharmaceutical dosage form of apremilast tablets (aprezo 30 mg) was purchased from local Apollo Pharmacy, Hyderabad, Telangana, India.

Selection of solvent[2,14]

Many experiments were conducted to determine the ideal solvent system for the drug's dissolution. Depending on the solubility of the apremilast, several solvents were investigated, including acetonitrile, methanol, ethanol, chloroform, distilled water, different phosphate buffers with varying pH values, and dimethyl sulfoxide. Various solvents, including acetonitrile, methanol, ethanol, and double-distilled water, were tested in relation to the drug's solubility.

Preparation of standard stock solution[15]

Apremilast 10 µg/mL standard stock solution was done by transferring precisely weighed 10 mg of standard apremilast to 10 mL volumetric flask and dissolved in acetonitrile. Working standard solutions of apremilast was prepared by suitable dilution of the stock solution (1000 µg/mL) with the acetonitrile. These stock solutions were used to prepare further dilutions throughout the experiment.

Selection of wavelength (λ_{max})[16]

The appropriate volume of 1 mL of standard stock solution of apremilast was transferred into a 10 mL volumetric flask, and diluted to a mark with acetonitrile to give a concentration of 10 µg/mL. The resulting solution was scanned in the UV range (200–400 nm).

Assay for pharmaceutical formulation (Tablets)

The solution was filtered through Whatman filter paper No. 41. 0.5 ml of this solution was transferred to 10 mL volumetric flask and the final volume was made with acetonitrile. It gives 0.5 µg/mL. It was scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 230 nm against the blank solution of acetonitrile. Determine the amount of % apremilast in the tablet according to the following formula:

$$\%\,Assay = \frac{WS\ X\ AT\ X\ Sample\,D.F.\,X\ Avg.wt.}{AS\ X\ S\tan dard\ D.F.\,X\,WT\ X\ LC} \times PS$$

Where,
WS = Weight of standard,
WT = Weight of sample,
AT = Absorbance of apremilast in the test solution,
AS = Absorbance of apremilast in the standard solution,
Standard D.F. = Standard dilution factor,
Sample D.F. = Sample dilution factor,
PS = Purity of working standard [%],
LC = Label claim of apremilast.

Analytical method validation developed[2,17,18]

The aim of method validation was to confirm that the present method was suitable for its intended purpose as prescribed in ICH Q2 guidelines. The method was validated to determine the linearity, precision, accuracy, ruggedness, robustness, LOD, and LOQ of the method.

Linearity

Linearity and range of different concentrations of apremilast solutions were prepared. The range of the solutions varies from 2 to 10 µg/mL of standard concentration (µg/mL) of 1 mg. The absorbance of these solutions is noted. The absorbance of the lower-level linearity solution (10%) and the higher-level linearity solution (60%) in six replicates were recorded. The graph of concentration versus absorbance of linearity solutions was plotted.

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. Intraday and interday precision study was carried out using 6µg/ml concentration of apremilast and analyzing it at three different times in a day, that is, morning, afternoon, and evening.

Accuracy

The accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated.

Ruggedness

The robustness of the proposed technique was evaluated by means of two independent analysts analyzing sections from uniform slots under identical operational and environmental conditions. The findings were reported appropriately.

Robustness

Robustness was obtained by performing the analysis at two different wavelengths (±5 nm), that is, 225 nm and 235 nm. The results were reported.

Limit of detection (LOD) and limit of quantitation (LOQ)

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The LOD and the LOQ of the drug were derived by calculating using the following equations designated by ICH Q2 guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where,
σ = Standard deviation of the response, and
S = Slope of the calibration curve.

RESULTS AND DISCUSSION

Validated analytical methods are aimed for the estimation of apremilast in API and its formulation. The validated method

Table 1: Assay of apremilast tablets

Drug	Labeled amount	Amount found	SD	Assay	% RSD
Apremilast	30 mg	99.89%	0.0054	99.89%	0.0054

was applied for the analysis of a tablet containing 30 mg of apremilast drug as the label claimed. The method developed was simple, accurate, and rapid.

In the case of the UV-spectroscopic method, solubility is the important parameter. The solubility parameter was studied and acetonitrile was selected as the solvent since it gave a maximum absorbance and a good spectral pattern when compared with other solvents. The marketed formulation was extracted and diluted to get the concentration in the linearity range. The solution was scanned and measured at 230 nm. Percentage recovery, linearity, and stability studies were also carried out. The above method gave a satisfactory recovery value and found to be stable, linear. Hence, it can be used for routine analysis of the drug formulation (tablets). Solutions of apremilast and its marketed product were prepared using acetonitrile and the UV spectrum of each was recorded by scanning between 200 and 400 nm.

Selection of solvent

Apremilast was found to be freely soluble in acetonitrile. Throughout the experiment, acetonitrile was chosen based on its solubility. A spectrum of apremilast and the marketed product was prepared in a solvent such as HPLC-grade acetonitrile. Better absorbance was observed for both the API and formulation when acetonitrile is used as a solvent.

Absorbance maxima (λ_{max})

It was observed that the drug showed maximum absorbance at 230 nm in acetonitrile which was chosen as the detection wavelength for the estimation of apremilast in the present research. The UV spectrum for apremilast is depicted in Figure 2.

Assay for pharmaceutical formulation (aprezo 30 mg)

The percentage recovery for apremilast tablets was found to be 95–101% enlisted in Table 1. The results for the assay are within acceptable limits.

Method validation

The proposed method was validated as per ICH Q2 guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experimental work.

Table 2: Linearity

Concentration in µg/mL	Absorbance
2	0.134
4	0.248
6	0.343
8	0.443
10	0.546
Regression equation	y = 0.051× + 0.0371
r^2	0.9992

Table 3: Intraday precision

Parameter	Conc. in µg/mL	Intraday precision Mean±SD	% RSD
Morning	6	0.343333±0.00216	0.629198
Afternoon	6	0.342167±0.002317	0.67704
Evening	6	0.340667±0.001211	0.355497

Table 4: Interday precision

Parameter	Conc. in µg/mL	Interday precision Mean±SD	% RSD
Morning	6	0.344833±0.002787	0.80818
Afternoon	6	0.343667±0.003011	0.876166
Evening	6	0.345833±0.002927	0.846329

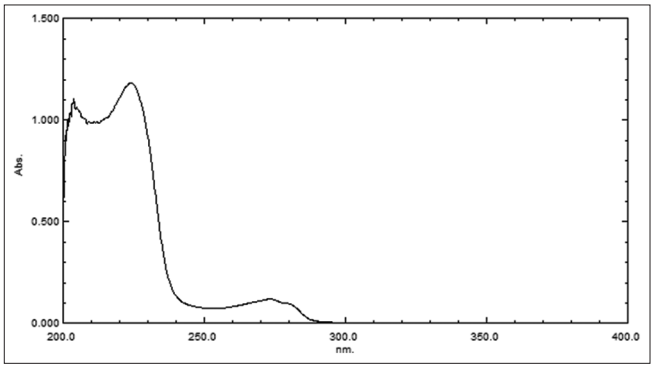

Figure 2: Absorption spectrum of apremilast in acetonitrile

Linearity

Standard solutions of apremilast in the concentration range of 2–10 µg/mL were observed in UV-spectroscopy. The absorbance values are depicted in Table 2.

A graph of absorbance (on Y-axis) versus concentration in µg/ml (on X-axis) was plotted and calibration graph

Table 5: Accuracy studies

Sample spiked	Sample (Tablet)	Standard (6 ppm)	Mean	SD	% RSD	% Recovery
50%	1 mL	0.3	0.497	0.000577	0.11601	101.02
	1 mL	0.3				
	1 mL	0.3				
100%	1 mL	0.6	0.651	0.001528	0.23452	100.72
	1 mL	0.6				
	1 mL	0.6				
150%	1 mL	0.9	0.811	0.811	0.1233	101.93
	1 mL	0.9				
	1 mL	0.9				

Table 6: Ruggedness

Parameter	Conc. (µg/mL)	Absorbance	Mean±SD	% RSD
Different Analyst-1	6	0.344	0.343±0.001	0.291545
		0.342		
		0.343		
Different Analyst-2	6	0.34	0.341±0.002646	0.77588
		0.339		
		0.344		

is shown in Figure 3. The regression equation was found to be y=0.051× + 0.0371, correlation coefficient (r^2) was 0.9992.

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show the reproducibility of the assay. The % RSD values found to be < 2 that indicate this method precise for the determination of the pure form. The intraday and interday precision results are mentioned in Tables 3 and 4, respectively.

Accuracy

Accuracy shall be determined by performing recovery studies at three levels in which a known amount of analyte shall be added and recovery shall be carried out in three replicates of each concentration level and the % recovery was calculated. The mean recovery was found between 98 and 102% and % RSD between 0.7 and 1.0. The results are illustrated in Table 5.

Ruggedness

This study was performed by analyzing 6 µg/ml of apremilast by two different analysts and on two instruments, results of the study are given in Table 6 and % RSD obtained was < 2 which is within the acceptance limits.

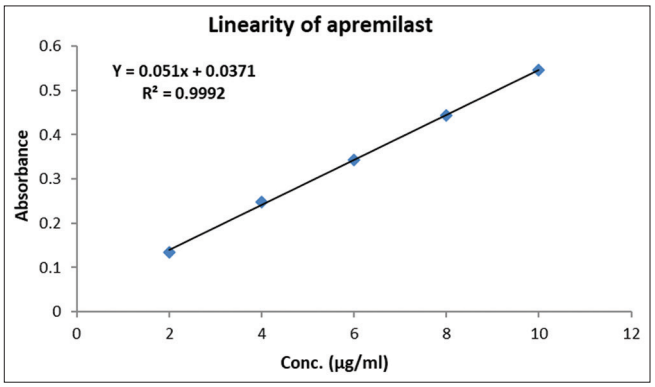

Figure 3: Linearity graph

Robustness

Applying a modest wavelength variation (±5 nm) to the approach described, the results showed that the %RSD was between 0.44621 and 0.294118, which were judged to be within the limitations. Table 7 presents the study's findings.

LOD and LOQ

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. The LOD and LOQ values were found as 0.155 µg/mL and 0.471 µg/mL, respectively. The results are depicted in Table 8. The summary of validation parameters is illustrated in below Table 9.

Table 7: Robustness

Conc. (µg/ml)	Parameter	Wavelength in nm	
		225	235
6	Mean	0.342	0.34
6	SD	0.001	0.001
6	%RSD	0.44621	0.294118

Table 8: LOD and LOQ

Drug	LOD	LOQ
Apremilast	0.155 µg/ml	0.471 µg/ml

Table 9: Summary of optical characteristics and validation parameters

Parameter	Method
λ_{max}	230 nm
Beer's law limit	2–10 µg/mL
Correlation coefficient (r^2)	0.9992
Regression equation (y=mx+c)	y = 0.051× + 0.0371
Slope (m)	0.051
Intercept (c)	0.0371
Accuracy	98–102%
Precision	<2
LOD	0.155 µg/mL
LOQ	0.471 µg/mL

CONCLUSION

From the current research work, it is concluded that the method developed and validated is economical and reproducible. The approach was designed and approved in accordance with ICH Q2 (R1) requirements. The suggested techniques can be used for regular examination of apremilast in tablet form from pharmaceutical dosage forms. It is inferred that the methods were found to be simple, accurate, precise, and linear. The methods were found to be having suitable application in routine laboratory analysis with a high degree of accuracy and precision. The precision was measured in terms of repeatability, which was determined by a sufficient number of aliquots of a homogeneous sample. The results showed that the recovery of marketed product by the proposed method was satisfactory. The validation procedure confirms that this is an appropriate method for their quantification in the bulk and pharmaceutical formulation. It is also used in routine quality control of the raw materials as well as formulations containing this entire compound. The result obtained from the validation parameters met the ICH Q2 and USP requirement as well as obeys Beer's law. The recommended method for determining apremilast in bulk and pharmaceutical formulations is straightforward, sensitive, accurate, repeatable, and reasonably priced, according to the previously described data.

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DECLARATIONS

Author contributions

All authors contributed to the literature review, experimental work, data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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Ethical approval

Not required.

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