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Analytical Method of Apremilast: A Review

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ABSTRACT

A selective method for separation and determination of potential related impurities (starting materials and by-products of synthesis, and degradants) of apremilast drug substance has been developed and validated. The separation was accomplished on a Cosmosil C-18 (250 mm × 4.6 mm, 5 µm) column connected to a photodiode array (PDA) detector using optimized mixture of 0.05% trifluoroacetic acid, methanol and acetonitrile under gradient elution. Two major degradant impurities found in force degradation study of apremilast drug substance. Both degradants were characterized preliminarily by HPLC-MS studies and synthesized in laboratory. Structure was evidenced by NMR spectroscopy, mass spectrometry and HPLC method was developed for quantification of the synthesized impurities along with starting materials. This method can be used for the quality control testing of drug substance. The performance of the method was validated according to the ICH guide lines for specificity, limit of detection, limit of quantification, linearity, accuracy, precision, ruggedness and robustness.

Keywords: Apremilast; analytical methods, adverse effects

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INTRODUCTION

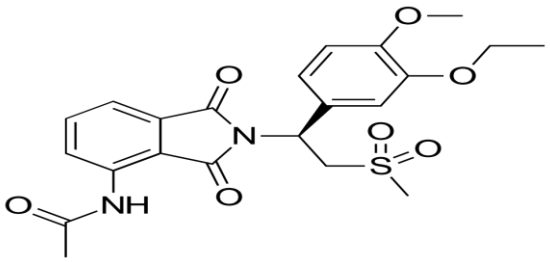
Apremilast is chemically known as *N*-[2-[(1*S*)-1-(3-ethoxy-4-methoxyphenyl) -2 (methylsulfonyl)ethyl]-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]acetamide. It has an empirical formula of C22H24N2O7S, and a molecular weight of 460.5 g mole−1.

Apremilast is a Food and Drug Administration approved drug used for treatment of psoriasis and psoriatic arthritis. It may also be useful for other immune system related inflammatory diseases. The drug acts as a selective inhibitor of the enzyme phosphodiesterase 4 (PDE4) and inhibits spontaneous production of TNF-alpha from human rheumatoid synovial cells [1, 2, 3]. 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 asankylosing spondylitis, Behcet's disease, and rheumatoid arthritis [4,5]. Several research papers have been reported in the literature for the determination of apremilast. These papers were limited to the assay of apremilast alone performed by UV spectrophotometry where impurity identification and quantification is not done [6]. New related impurities are synthesized and quantification method with HPLC is reported but the obvious degradants which studied here in present research are not reported [7]. Pharmacokinetic study of apremilast in rat plasma has been studied by using UPLC MS/MS where apremilast is quantified in the blood plasma [8, 9]. The reported related substance methods are suitable for quantification of some of related impurities but another degradants formed under the stress conditions employed were neither discussed nor characterized. Further, no monograph of apremilast is published in any of the pharmacopoeia for

compendia applications. In present research during force degradation study two major degradant impurities were observed when the drug substance is exposed to acid and base degradation. Both degradants were synthesized in laboratory and structure is elucidated using LCMS, 1H NMR and IR spectroscopy techniques. The RP-HPLC method is developed for the separation and determination of apremilast and potential related impurities i.e. raw materials, by-products and degradants. The proposed analytical method is validated as per International conference on harmonization guidelines (ICH Q2-R1) [10, 11, 12]. The manuscript describes a comprehensive investigation on isolation and characterization of a major process related impurities of Apremilast 3-(acetylamino -2-{[1-(3-ethoxy-4-

methoxyphenyl)-2 (methylsulfonyl)ethyl]carbamoyl}benzoic acid i.e. Impurity-B and 3-(acetylamino-6-{[1-(3-ethoxy-4.

Apremilast, brand name **Otezla** among others, is a medication for the treatment of certain types of psoriasis and psoriatic arthritis. It may also be useful for other immune system related inflammatory diseases. The drug acts as a selective inhibitor of the enzyme phosphodiesterase 4 (PDE4) and inhibits spontaneous production of TNF-alpha from human rheumatoid synovial cells.



Mechanism of action

Apremilast is a small molecule inhibitor of PDE4,[13] 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

Pharmacokinetics

Apremilast is absorbed from the gut well (73%) and independently of food intake, and reaches peak blood plasma concentrations after 2.5 hours. Plasma protein binding is 68%. It is metabolised in the liver, mainly via the enzyme CYP3A4, but to a minor extent via CYP1A2 and CYP2A6. The main metabolite is *O*-desmethylapremilast glucuronide.[15]

The half-life is 6–9 hours. The substance is eliminated through the kidney (58%) and feces (39%), mainly in form of its metabolites. Only 3% of the original substance are found in the urine, and 7% in the feces.

Particle size, polydispersity index (PDI), and zeta potential (ZP)

The developed APM-loaded PLGA NPs were freeze dried followed by dispersing them in the Milli-Q water (20 µg/mL). The suspension was then characterized for particle size, polydispersity index (PDI), and zeta potential (ZP) using the dynamic light-scattering technique. The Malvern Particle Size Analyzer (Malvern Instruments Ltd, Holtsville, NY, USA) was used to measure the mean particle size and the PDI of different developed NPs (F1–F3). The NP samples were diluted to 200 times with deionized water and sonicated for 10 minutes in order to obtain clear aqueous dispersion. Each sample (3 mL) was The supernatant of the sample was withdrawn at different time intervals (1, 2, 3, 4, 5, 6, 12, 24, and 48 hours). The collected sample was centrifuged at 12,000 rpm for 5 minutes and analyzed for the drug content using UV spectroscopy at 229 nm[16] The data obtained from the release study were plotted and fitted in various kinetic models to obtain the release pattern of the drug from the polymeric matrix. in transparent disposable plastic cuvettes and the mean particle size and PDI were measured. The same analyzer was utilized to measure the ZP of the NPs (F1–F3) but the measurements were done using glass electrode.

ANALYTICAL METHOD VALIDATION

Method validation is closely related to method development. When a new method is being developed, some parameters are already being evaluated during the "development stage," while in fact, this forms part of the "validation stage." Related substances method is validated as per ICH guideline [9].

Specificity and force degradation

The ability of the method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix that may be expected to be present in the sample matrix under the stated conditions. Specificity of the method was evidenced by comparing blank, apremilast and all specified impurities separate injections as well as spiking all impurities into apremilast test solution. Force degradation study is performed by exposing the sample to heat at 105^0C for 24 hours, sample treated with base 1 N sodium hydroxide and with acid 1N hydrochloric acid. Sample was exposed to ultra-violet light for 24 hours and 3% hydrogen peroxide solution. After exposure samples were tested using the proposed related substances method with photo diode array detector. The degraded samples were further analyzed to find out assay of apremilast. Mass balance is calculated by comparison of total impurities from related substances test and the assay of apremilast.

Solution stability

Drug stability in Active Pharmaceutical Ingredient is a function of storage conditions and chemical properties of the drug and its impurities. Conditions used in stability experiments should reflect situations likely to be encountered during actual sample handling and analysis. Stability data are required to show that the concentration and purity of analyte in the sample at the time of analysis corresponds to the concentration and purity of analyte at the time of sampling. The solution stability till twelve hours of apremilast API had been checked by injecting test solution and standard solution. Test solution was prepared fresh before injection and immediately injected and same solution was injected after twelve hours.

Linearity

The ability of the method to obtain test results proportional to the concentration of the analyte within a given range. It was evaluated by linear regression analysis, which was calculated by the least square regression method.

Limit of detection

The limit of detection (LOD) is the lowest concentration of analyte in a sample that can be detected but not necessary quantified. The obtained LOD values of specified impurities and API is discussed.

$LOD = 3.3 \times \sigma / S$

Where, σ = the standard deviation of the response and

S= slope of the calibration curve

Limit of quantitation

The limit of quantitation is the lowest concentration or amount of analyte that can be determined quantitatively within an acceptable level of repeatability precision and trueness.

Limit of quantitation (LOQ) = $10.0 \times \sigma / S$

Where, σ = the standard deviation of the response and

S= slope of the calibration curve

Precision at LOQ is confirmed by six replicate analyses of impurities at LOQ level.

Accuracy

Accuracy can be defined as the closeness of agreement between a test result and the accepted reference value. Accuracy of the method was determined by recovery study. Analytical method may be considered validated in terms of accuracy if the mean value is within ± 20% of the actual value. During recovery study apremilast API batch was analyzed and then all specified impurities of known concentration is spiked in the API at LOQ level, 50%, 100% and 150% with respect to the limit of specified impurity.

Ruggedness

The (intra-laboratory tested) behavior of an analytical process when small changes in environment and/or operating condition are made. The ruggedness of the method was evaluated by estimating % RSD of standard solution tested by two different analysts using different HPLC instrument and columns on different days. Three validation batches were prepared by each analyst separately. % RSD of each impurity of preparations of both analysts should not be more than 10%.

Robustness

Robustness is a measure of the capacity of the analytical procedure to remain unaffected by small but deliberate variations in method–performance parameters, which provides an indication of its reliability during normal usage. Robustness of the method was determined by analyzing the system suitability solution and batch analysis with deliberate change in the parameters like (a) flow rate of mobile phase ± 0.1ml/min and (b) column temperature ± 5°C.

Bioanalytical methods

An ultraperformance liquid chromatography coupled with tandem mass spectrometry (MS/MS) was used for the quantification of APM in rat plasma samples. Our previously reported assay was modified for this purpose.[17] To increase the sensitivity of the assay, the electrospray ionization was operated in positive mode and the calibration range was between 1 and 1,000 ng/mL with lower limit of quantification of 1 ng/mL in plasma samples. Due to change in the ionization mode, losartan was used as the internal standard (IS). The precursor to product ion transition of 461.16 &178.08 and 423.13 207.12 was used for detection and quantification of analyte (APM) and the IS (losartan), respectively, in the multiple reaction monitoring (MRM) mode. The optimized MS/MS parameters of capillary voltage 4.00 kV, source temperature 150°C, desolvation temperature 350°C, and collision gas flow rate 0.17 mL/minute were used for sample ionization. A cone voltage of 26 V (both for analyte and IS) and collision energy of 28 and 20 eV were used for the analyte and the IS, respectively, as compound-specific parameters. Due to change in ionization mode and IS, the assay was partially validated in terms of precision and accuracy following the US Food and Drug Administration 2013 guideline for bioanalytical method validation. Both intra- and interday variation in precision and accuracy was found to be within the acceptable limits of ±15%.

Adverse effects

Diarrhea and vomiting

Diarrhea occurs in about 25% of patients taking apremilast. Severe gastrointestinal symptoms, when they occur, typically start within the first few weeks of treatment.[18,19]

Psychological

Worsening depression, suicidal thoughts, and other mood changes may occur with apremilast.[20]

Weight loss

Weight loss: Weight loss has been associated with apremilast. Reports from clinical studies indicated a 5 to 10% decrease in body weight in 10% of patients taking apremilast (compared to 3.3% of patients taking placebo).[20]

Other

Common, usually mild to moderate adverse effects associated with apremilast include headache, back pain, nausea, diarrhea, fatigue, nasopharyngitis and upper respiratory tract infections.[21]

CONCLUSION

Process related impurities of apremilast are identified, synthesized and characterized. Structural elucidations of all synthesized compounds were done by using NMR, IR and mass spectral data. Impurity of RRT 0.77 is 3-(acetylamino-2- {[1-(3-ethoxy- 4-methoxyphenyl) -2-(methylsulfonyl) ethyl]carbamoyl} benzoicacid i.e. Impurity-B. Impurity at RRT 0.79 is 3- (acetylamino-6-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl] carbamoyl}

benzoicacid i.e. Impurity-C. All process related impurities and degradant impurities are quantified in the proposed method of analysis. Thus, the regulatory requirement was fulfilled by characterizing this impurity and the prepared impurity standard was used during analytical method validation studies. The above RP-HPLC analytical method satisfies all validation parameters like system suitability, precision, specificity, accuracy, linearity of detector response, ruggedness and robustness. At the same time the method satisfies the forced degradation study. It indicates that the method is stable and suitable for the apremilast and its related substances determination. Hence, the validated method can be used for routine analysis of related substances in apremilast in quality control laboratories in the pharmaceutical industry.

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