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RESEARCH ARTICLE

STABILITY-INDICATING RELATED SUBSTANCES METHOD OF APREMILAST BY HPLC AND SYNTHESIS AND CHRACTERIZATION OF RELATED IMPURITIES USING MASS AND NMR SPECTROSCOPY

Ajit Anerao*., Vihar Telange., Nitin Bondre., Satish John., Thaksen Gadhave and Nitin Pradhan

R&D centre (API), Wanbury Ltd., EL-16, TTC Industrial Estate, Mahape, Navi Mumbai 400710, India

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ABSTRACT

A selective RP-HPLC 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 FT-IR. 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.

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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 $C_{22}H_{24}N_2O_7S$, 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 LC-MS, 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-

methoxyphenyl)-2- (methylsulfonyl)ethyl] carbamoyl}benzoic acid i.e. Impurity-C. Apart from these impurities as per the route of synthesis of apremilast two starting materials (S)-1-(3-ethoxy-4-methoxyphenyl) -2-(methyl sulfonyl)ethanamine i.e. KRM-A and N-(1,3-dioxo-1,3-dihydroisobenzofuran-4-yl)acetamide i.e. KRM-B are quantified by the proposed method. As well as the desethoxy process impurity (S)-4-amino-2-(1-(3-ethoxy-4-methoxyphenyl) -2-(methylsulfonyl)ethyl) isoindoline-1,3-dione i.e. Impurity-A is also quantified in the same method. All five impurities and apremilast structures are given in figure I to VI.

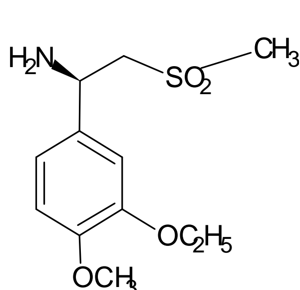


Figure-I (S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methyl sulfonyl)ethanamine i.e. KRM-A

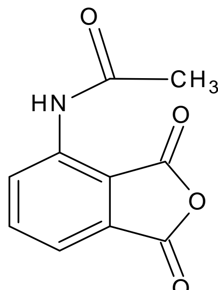


Figure-II N-(1,3-dioxo-1,3-dihydroisob- enzofuran-4-yl)acetamide i.e. KRM-B

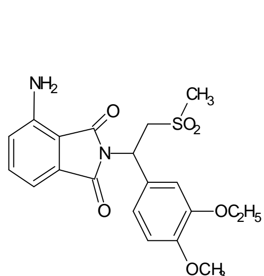


Figure-III (S)-4-amino-2-(1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl) isoindoline-1,3-dione i.e. Impurity-A

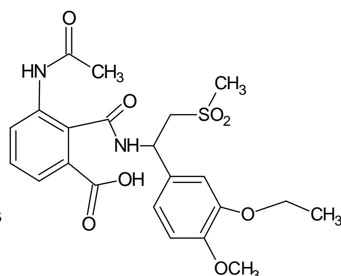


Figure-IV 3-(acetylamino-2-{[1-(3- ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]carbamoyl} benzoic acid i.e. Impurity-B

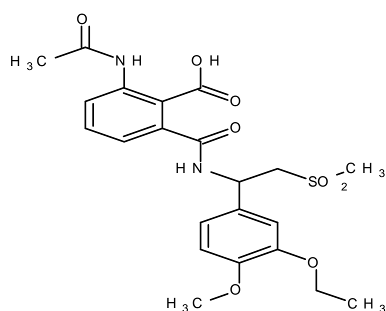


Figure-V 3-(acetylamino-6-{[1-(3-ethoxy-4- methoxyphenyl)-2-(methylsulfonyl)ethyl] carba- moyl}benzoic acid i.e. Impurity-C

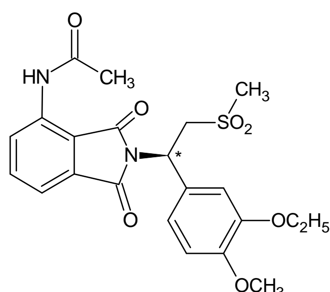


Figure-VI Apremilast

MATERIALS AND METHODS

Manufacturing process

Preparation and isolation of apremilast impurity B and impurity C

A solution of Apremilast (5 gm) in water (50 ml) was added sodium hydroxide (2.0 eq.) and then reaction mixture was heated to 55-60°C and stirred for 2 hours. The resulting reaction mixture cooled to 25-30°C and washed with methylene dichloride (15 ml x 3). The resulting aqueous layer was added aqueous hydrochloric acid up to pH 2 to obtain reaction mixture containing impurity B and impurity C in (60:40%). These impurities were separated from the reaction mixture by column chromatography (Silica gel: 60-120 mesh, 0.25% Hexane/Ethyl acetate) and was characterized by mass and ^{1}H-NMR techniques.

The related substances method is developed and made suitable for unspecified and specified impurities (S)-4-amino-2-(1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl) isoindoline-1,3-dione i.e. Impurity-A; 3-(acetylamino-2-{[1-(3-ethoxy-4-methoxyphenyl) -2-(methylsulfonyl)ethyl]carbamoyl}benzoicacid i.e. Impurity-B; 3-(acetylamino-6-{[1-(3- ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]carbamoyl}benzoicacid i.e. Impurity-C; (S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethanamine i.e. KRM-A and N-(1,3- dioxo-1,3-dihydroisobenzofuran -4- yl)acetamide i.e. KRM-B.

Related substances by HPLC

Shimadzu HPLC system LC-2010 CHT with UV detector with LC solutions software or its equivalent was used. The analysis was carried out on Cosmosil C-18 column 250 mm x 4.6 mm, 5.0 µm with Guard cartridge: Make; Phenomenex C18, 4.0 mm x 3.0 mm ID. Column temperature is 35°C and auto sampler temperature 5°C. Separation was achieved with the mixture of mobile phase-A and mobile phase-B in gradient elution with timed programme T_{min}/A:B: T_0/98:02; T_{20}/98:02; T_{30}/60:40; T_{40}/50:50; T_{50}/40:60; T_{60}/25:75; T_{62}/98:02; T_{70}/98:02. The flow rate was 1.0 mL/min and sample injection volume was 15 µL. Detector wavelength is 230 nm.

Diluent: Acetonitrile: Water (25:75 v/v)

Buffer-1: preparation (for Mobile phase)
Add 1.0 mL of Trifluoroacetic acid in 2000 mL of HPLC grade water.

Buffer-2: (for KRM-B solution preparation)
Weigh accurately about 1.36 gm of potasium dihydrogen phosphate in 1000 mL of water.Adjust pH of the solution to 3.0 ± 0.05 with dilute orthophosphoric acid.

Mobile Phase-A: Buffer-1: Methanol (90:10) v/v

Mobile Phase-B: Buffer-1: Acetonitrile (10:90) v/v

Preparation of solutions

Standard Stock Solution-A

Weigh and transfer accurately about 15.0 mg of KRM-B reference standard in a 100 mL volumetric flask. Add about 2.0 ml of Acetonitrile and sonicate for 2 minutes. Add about 90 mL of Buffer-2 and sonicate for 2 minutes. Make up to the mark with buffer-2 and mix well. Keep the solution at room temperature for 30 minutes.

Standard Stock Solution-B

Weigh and transfer accurately about 15.0 mg of KRM-A, Impurity-A, Impurity-B, Impurity-C reference standard respectively and 10.0 mg of Apremilast reference standard in a 100 mL volumetric flask. Add about 25 mL of Acetonitrile and sonicate for 2 minutes. Make up to the mark with water. Solution to be prepared immediately before analysis.

Standard Stock Solution-C

Transfer accurately 5.0 ml of Standard Stock Solution-A and 5.0 mL of Standard Stock Solution-B in a 100 mL volumetric flask and dilute up to the mark with diluent. Solution to be prepared immediately before analysis.

Standard Solution

Transfer accurately 10.0 mL of Standard Stock Solution-C to 50 mL volumetric flask and dilute up to the mark with diluent. Solution to be prepared immediately before analysis.

Test solution

Weigh and transfer accurately about 50 mg of sample in 50 mL volumetric flask, add diluent sonicate to dissolve and dilute to the mark with diluent. Solution to be prepared immediately before analysis.

Procedure

a) Inject Blank (diluent) in duplicate
b) Standard Solution six replicate
c) Test Solution in duplicate.

The retention time for Apremilast about 39.0 minutes and Relative Retention time (RRT) are as follows:

Name	RRT
N-(1,3-dioxo-1,3-dihydroisobenzofuran -4- yl)acetamide i.e. KRM-B	0.29
(S)-1-(3-ethoxy-4-methoxyphenyl) -2-(methyl sulfonyl)ethanamine i.e. KRM-A	0.42
3-(acetylamino-2-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]carbamoyl}benzoic acid i.e. Impurity-B	0.78
3-(acetylamino-6-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]carbamoyl}benzoic acid i.e. Impurity-C	0.79
(S)-4-amino-2-(1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl) isoindoline-1,3-dione i.e. Impurity-A	0.97

System Suitability Criteria

a) The Resolution between Impurity-A and Apremilast should not be less than 2.5

b) % RSD for Average Areas for Apremilast and related impurities in six replicate injections of standard solution should be not more than 5.0.

Calculation

Disregard peaks due to blank and report specified impurities i.e. KRM-A, KRM-B, Impurity-A, Impurity-B, Impurity-C, unspecified impurity and Total impurities by following formula.

% specified impurity=

$$\frac{\text{Area}_{sample}}{\text{Area}_{std}} \times \frac{W_{Std}}{100} \times \frac{5.0}{100} \times \frac{10.0}{50} \times \frac{50}{W_{sample}} \times P$$

Where,
Area sample: Area due to specified impurity in Test Solution.
Area std: Average Area specified impurity in Standard Solution.
W Std: Weight of specified impurity reference standard in mg.
W sample: Weight of Test Sample in mg.
P: Potency of specified impurity Reference Standard.

% Unspecified Impurity =

$$\frac{\text{Area}_{sample}}{\text{Area}_{std}} \times \frac{W_{Std}}{100} \times \frac{5.0}{100} \times \frac{10.0}{50} \times \frac{50}{W_{sample}} \times P$$

Where,
Area sample: Area of unspecified impurity in Test Solution.
Area std: Average Area of Apremilast in Standard Solution.
W Std: Weight of Apremilast Reference Standard in mg.
W sample: Weight of Test Sample in mg.
P: Potency of Apremilast Reference Standard.

Total Impurities = Specified impurities + Total unspecified impurities

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°C 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 × / S
Where, = 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 × / S

Where, = 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%.

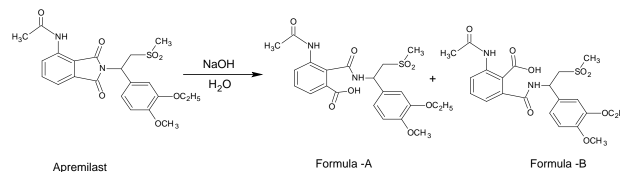
Robustness

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 ± 0.1 ml/min and (b) column temperature ± 5°C.

RESULTS AND DISCUSSION

During impurity profiling of apremilast hydrochloride drug substance by HPLC, an unknown impurity at RRT 0.78 and RRT 0.79 was observed about 31.13% and 27.55% respectively in base degraded sample during force degradation study. Both impurities are synthesized and characterized and structure elucidation data is discussed below.



Formula-A-Impurity B: 3-(acetylamino-2-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyyl)ethyl]carbamoyl}benzoic acid
MS (ESI positive ion) m/z 479.3 (M + 1)
1H NMR ($CDC1_3$, 400 MHz) figure-VII: 1.41-1.45 (t, 3H), 1.99 (s, 3H), 2.80-2.82 (s, 3H), 3.40-3.76 (dd, 2H), 3.83 (s, 3H), 4.06-4.11 (q, 2H), 5.66-5.72 (t, 1H), 6.81-6.83 (d, 1H), 6.92-6.94 (d, IH), 7.28 (s, lH). 7.70-7.72 (d, 1H), 8.11-8.13 (d, 1H), 8.11-8.24 (dd, 1H).

Formula-B-Impurity C:
3-(acetylamino-6-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyyl)ethyl]carbamoyl}benzoic acid
MS (ESI positive ion) m/z 479.0 (M + 1)
1H NMR ($CDC1_3$, 400 MHz) figure-VIII: 1.31-1.35 (t, 3H), 2.04 (s, 3H), 2.92 (s, 3H), 3.57-3.65 (dd, 2H), 3.75-3.77 (s, 3H), 4.01-4.06 (q, 2H), 5.44-5.50 (t, 1H), 6.90-6.97 (dd, 2H) 7.08 (d, 1H), 7.34-7.36 (d, IH), 7.47-7.51 (dd, lH). 7.80-7.82 (d, 1H), 9.03-9.05 (s, 1H), 9.72 (s, 1H)

From the experimental data of method validation following are observations

Specificity

There are no interfering peaks at the retention times of apremilast and specified impurities are observed from the chromatogram. All specified impurities are well resolved without any interference is observed from the spiked chromatogram. Peak purity of all peaks is checked with photo diode array detector (PDA). It is found that peak purity index is more than 0.99 which proves that all peaks are pure without any interference. Refer Table-I for relative retention time (RRT) and peak purity which is found passing. Refer figure IX, X and XI of Blank, system suitability solution and test solution chromatograms.

Force degradation

It was observed that apremilast is stable when exposed to heat, UV light and oxidation exposure. Impurity profile is matching with freshly injected test solution and mass balance is also found within 98.0% to 102.0%. In acid degradation two degradants were observed at relative retention time (RRT) 0.77 is about 0.13% and RRT 0.79 is about 0.08%. Similarly in base degradation impurity at RRT 0.77 is 31.13% and at RRT 0.79 is 27.55% (refer figure XII). Both impurities were synthesized and characterized with mass, NMR and IR. Impurity of RRT 0.77 is identified as 3-(acetylamino-2-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl]carbamoyl}benzoic acid i.e. Impurity-B and impurity at RRT 0.79 is 3-(acetylamino-6-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]carbamoyl}benzoic acid i.e. Impurity-C. On basis of degradation study the obvious degradants are included in the proposed related substances method. All degradant impurities are well resolved and peak

purity of all peaks is passing. Mass balance is tabulated in table-II

Table I Specificity and peak purity

Name of the compound	Relative Retention time (RRT)	Peak purity index	Peak purity
N-(1,3-dioxo-1,3-dihydroisobenzofuran -4- yl)acetamide i.e. KRM-B	0.29	0.999927	Pass
(S)-1-(3-ethoxy-4-methoxyphenyl) -2-(methyl sulfonyl)ethanamine i.e. KRM-A	0.42	0.997840	Pass
3-(acetylamino-2-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]carbamoyl}benzoic acid i.e. Impurity-B	0.78	0.999998	Pass
3-(acetylamino-6-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]carbamoyl}benzoic acid i.e. Impurity-C	0.79	1.000000	Pass
(S)-4-amino-2-(1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl) isoindoline-1,3-dione i.e. Impurity-A	0.97	0.999994	Pass
Apremilast	1.00	1.000000	Pass

Table II Force degradation study and mass balance

Sample details	Area of apremilast peak in related substances test	Total impurities in related substances test in % (A)	Assay of apremilast in % (B)	Mass Balance % (A+B)
APR/29A/III/294/34 as such	3861013	0.04	99.89	99.93
APR/29A/III/294/34 (0.1N NaOH)	1148255	78.37	29.84	108.21
APR/29A/III/294/34 (0.1N HCl)	3794009	0.42	98.76	99.18
APR/29A/III/294/34 (3% H2O2)	3790070	0.06	98.78	98.84
APR/29A/III/294/34 (UV 24 hrs)	3803345	0.04	98.95	98.99
APR/29A/III/294/34 (105°C 24 hrs)	3846581	0.04	100.43	100.47

Solution stability

The solution stability till seventeen hours of apremilast API had been checked by injecting test solution and standard solution. Apremilast and unknown/unspecified impurities were not significantly changed till nine hours. After twelve hours impurity B and impurity C were found increased. So it was decided to inject freshly prepared test solution and reference standard solution. Solutions are found stable for some more time when auto sample cooler temperature is kept 5°C. Impurity C is getting degraded in solution form and it converts in to KRM-A and KRM-B after 12 hours. The impurity is found stable in refrigerated condition at 2°C to 8°C. So the standard stock solution was prepared and stored in a refrigerator. For further precaution auto-sample cooler temperature is kept 5°C.

LOD and LOQ

Limit of detection and limit quantification of (S)-4-amino-2-(1-(3-ethoxy-4- methoxyphenyl)-2-(methylsulfonyl)ethyl)isoindoline-1,3-dione i.e. Impurity-A; 3-(acetylamino-2-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]carbamoyl}benzoicacid i.e. Impurity-B; 3- (acetylamino-6-{[1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl)ethyl]carbamoyl}benzoicacid i.e. Impurity-C; (S)-1-(3-ethoxy-4- methoxyphenyl)-2-(methylsulfonyl)ethanamine i.e. KRM-A and N-(1,3-dioxo-1,3- dihydroisobenzofuran-4-yl)acetamide i.e. KRM-B are determined by slope method and tabulated in table-III. LOD and LOQ concentration is reported in percentage with respect to apremilast test concentration.

Table III LOD and LOQ Values

Sr. No	Name of the compound	LOD in %	LOQ in %	LOQ precision (%RSD of six replicate)
1	KRM-B	0.0024%	0.008%	2.25%
2	KRM-A	0.0063%	0.021%	2.06%
3	Impurity-B	0.0033%	0.011%	1.07%
4	Impurity-C	0.0045%	0.015%	0.73%
5	Impurity-A	0.0027%	0.009%	1.00%
6	Apremilast	0.0012%	0.004%	3.51%

Linearity

Under the experimental conditions, the peak area vs. concentration plot for the proposed method was found to be linear over the range of 50%, 80%, 100% and 150% of the specified limit with a regression coefficient as tabulated in Table-IV. The regression coefficient (r^2) is > 0.99 is generally considered as evidence of acceptable fit of the data to the regression line. Impurity C is getting degraded in solution form and it converts in to KRM-A and KRM-B after 12 hours. The impurity is found stable in refrigerated condition at 2°C to 8°C. So the standard stock solution was prepared and stored in a refrigerator and linearity levels were injected with freshly prepared solutions. For further precaution auto-sample cooler temperature is kept 5°C.

Table IV Linearity

Sr. No	Name of the compound	Regression coefficient (R^2)
1	KRM-B	0.997
2	KRM-A	0.996
3	Impurity-B	0.999
4	Impurity-C	0.996
5	Impurity-A	0.999
6	Apremilast	0.999

Accuracy

Analytical method may be considered validated in terms of accuracy if the mean value is within ± 20% of the actual value. Recovery of specified impurities was found in the range of 80.0% to 120.0%, which was well within the acceptance criteria. 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. The spiked solutions were prepared immediately before analysis. Refer Table-V.

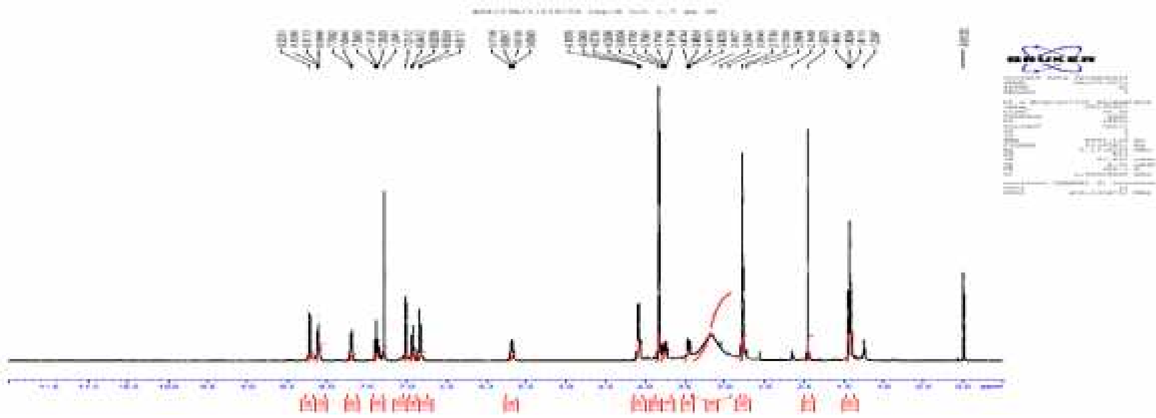


Figure VII NMR of (RRT 0.77) 3-(acetylamino-2-{[1-(3-ethoxy-4-methoxyphenyl)-2- (methylsulfonyl)ethyl]carbamoyl}benzoicacid i.e. Impurity-B

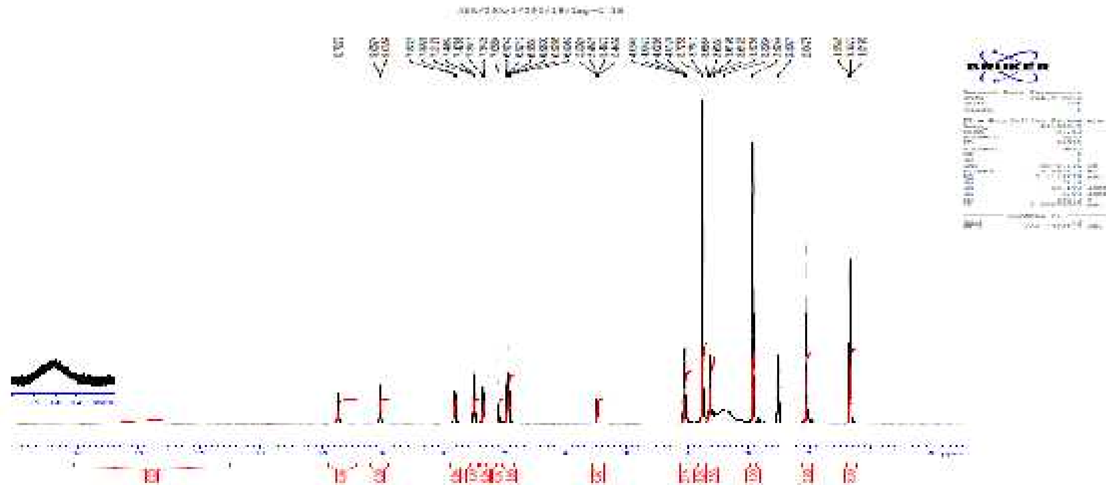


Figure VIII NMR of (RRT 0.79) 3- (acetylamino-6-{[1-(3-ethoxy-4-methoxyphenyl)- 2-(methylsulfonyl)ethyl]carbamoyl}benzoicacid i.e. Impurity-C

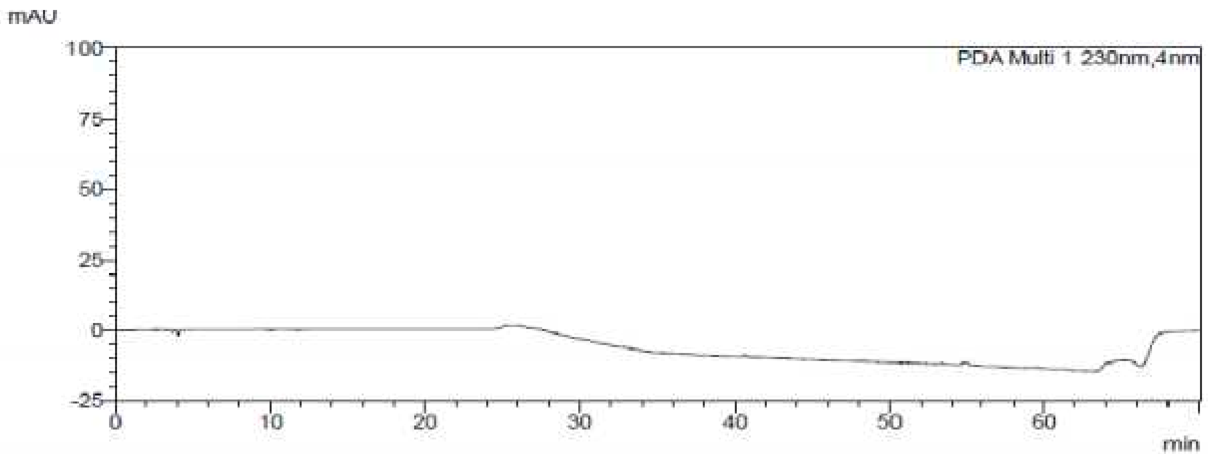


Figure IX Blank chromatogram of related substances test

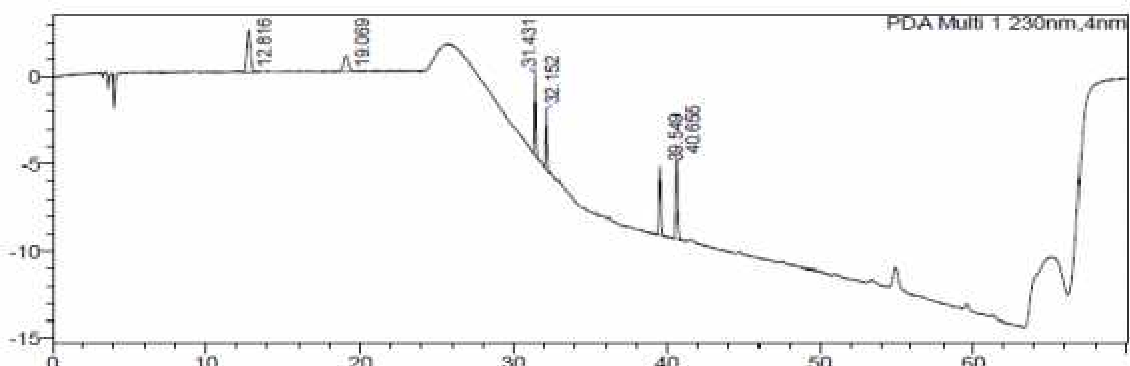


Figure X System suitability chromatogram of related substances where RT 12.8 min is KRM B, 19.0 min is KRM A, 31.4 min is impurity B, 32.1 min impurity C, 39.5 min is impurity A and 40.6 min is Apremilast

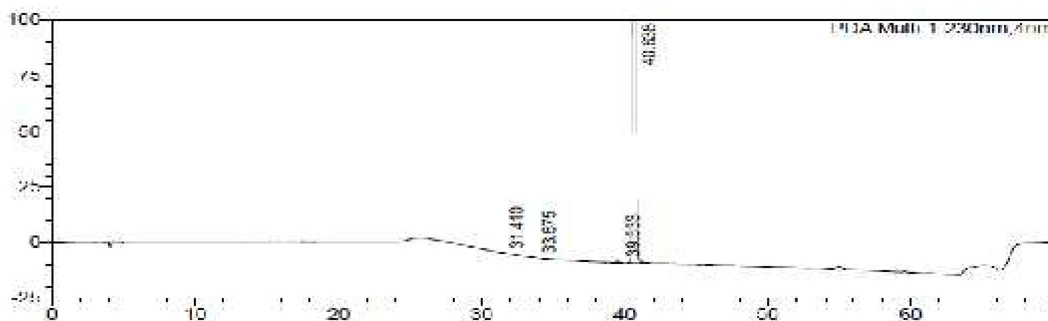


Figure XI-Test solution chromatogram of related substances test

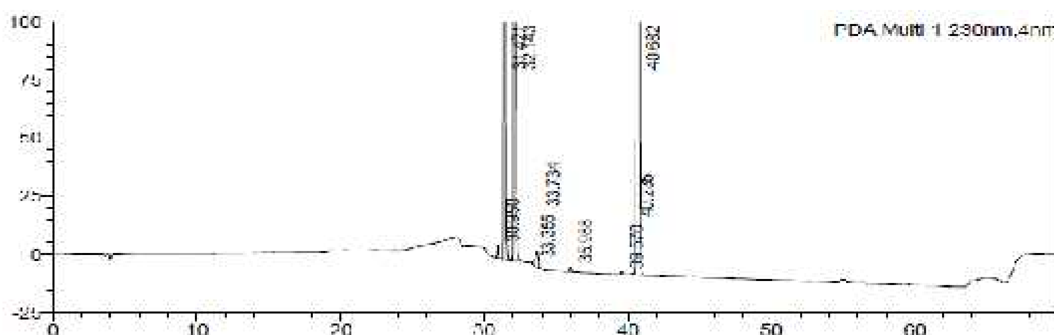


Figure XII-Base degraded apremilast sample of force degradation study of related substances where degradant observed at RRT 0.77 (impurity B) and 0.79 (impurity C)

Table V Recovery study

Sr. No	Name of the compound	LOQ level	50% level	100% level	150% level
1	KRM-B	100.0%	103.0%	102.0%	99.0%
2	KRM-A	99.0%	102.0%	101.0%	100.0%
3	Impurity-B	118.0%	100.0%	100.0%	101.0%
4	Impurity-C	114.0%	94.0%	95.0%	101.0%
5	Impurity-A	96.0%	99.0%	100.0%	100.0%

Ruggedness study

Experiment was performed by two different analysts using different HPLC instrument and columns on different days. Standard solution and three validation batches were prepared by each analyst separately. % RSD of each impurity of both analysts was observed less than 10%.

Robustness

Robustness of the method was determined by analyzing the system suitability solution and batch analysis with deliberate change in the parameters of flow rate and column temperature. Results are discussed in table VI. RSD of all impurities are found within limit below 10.0%.

Table VI Robustness study with deliberate changes in method parameters

FLOW (0.9 mL/min) and (1.1 mL/min)	%RSD for Results of standard and test solution in actual condition and changed condition is NMT:10%	High flow	Low flow
		Complies	complies
		Complies	complies
COLUMN TEMP (30°c and 40°C)	% RSD for Results of standard and test solution in actual condition and changed condition is NMT:10%	30°C	40°C
		Complies	complies
		Complies	Complies

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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