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Apremilast – A review of Analytical Methods Developed for API with its impurities, Pharmaceutical Formulations and Biological Matrices

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ABSTRACT: An extensive survey of the Literaturepublished in various analytical and pharmaceutical chemistry-related journals have been conducted for apremilast in Bulk, Laboratory mixture, Pharmaceutical Dosage form and biological fluid. Apremilast is a flourishing molecule in the field of Dermatology, it is selective enzyme phosphodiesterase 4 (PDE4) inhibitor. Initially, Apremilast was approved by United State FDAin 2014. On OCTOBER, 2017, Drug Controller General of India(CDSCO) also approved Apremilast in India for marketing. Till date, no compendia method has been reported for Apremilast. Literature survey was done by screening the papers reporting analytical techniques of Apremilast from year 2014 to 2020. Various analytical methods have been reported for the estimation of apremilast they are UV, HPLC, LC/MS, HPTLC, FTIR, DSC, NMR, XRD, SEM in bulk with its impurities, laboratory mixture, pharmaceutical dosage form as well as biological fluid.

Keywords: Apremilast, Analytical Methods, impurities

I. INTRODUCTION: -

Psoriasis is a chronic inflammatory skin disease,affecting upto 1–3% of the adult population.It is immune-mediated inflammatory disease that may be associated with the defect in proliferation and difference of keratinocytes associated with cell infiltration particularly consisting T-lymphocytes, macrophages, and neutrophils[(0)]

APREMILAST, sold under the brand name OTEZLA, is an orally administered small molecule,selectiveinhibitor of type 4 cyclic nucleotide phosphodiesterase (PDE4). Initially,approved by UNITED STATE FDA on MARCH 21, 2014(Otezla, Celgene Corporation), to treat psoriatic arthritis (PsA) in adults. Soon, on SEPTEMBER 23, 2014, FDA approved apremilast for treating patients of moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy[([2])]. Apremilast has since been approved for use in one or both of these indications in multiple countries, including Canada and the European Union. Available in following Strength: - 10 mg-20 mg-30 mg; 30 mg. On October 10, 2017,Drug Controller General of India(CDSCO) also approved Apremilast in India for marketing[(2)]. On July 19,2019 FDA approved OTEZLA(APREMILAST) for the treatment of adult patients with oral ulcers associated with Bechet's Disease[([2])]- is a systemic vasculitis, characterized by recurrent attacks of acute inflammation[([4])].

APREMILAST has shown promising result in treating patients with psoriasis and a few other dermatologic disorders[([5])]. Off label indication of APREMILAST are atopic dermatitis, alopecia areata, hidradenitis suppurativa, other variants of psoriasis, cutaneous sarcoidosis, and discoid lupus.

Due to its safety profile and its immunomodulatory effect on multiple cell. Case study reported from RCT data Alopecia areata,Atopic dermatitis,Hidradenitis suppurativa,Nail and scalp psoriasis,Palmoplantar psoriasis. From open label study case study reported wereCutaneous sarcoidosis,Discoid lupus erythematosus,Lichen planus, Rosacea, showing positive and negative result. Various case series and case reported pityriasis rubra pilaris, Hailey-Hailey disease, vitiligo, and generalized pustular psoriasis[(6)].

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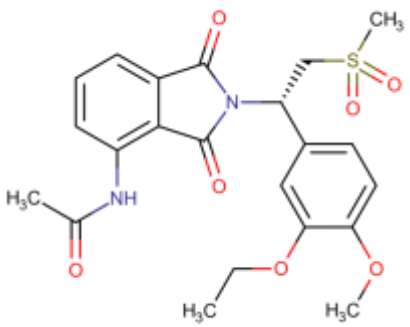
Patents on analytical method of apremilast: CN107305198 - Method For Separating And Determining Apremilast And Related Substances Through High-Performance Liquid Chromatography– patent is applicable to five process related impurities of apremilast, which can be separated and detected more accurately and effectively(Application Number: 201610242289.7)([7]).CN105628841 - Method For Separating And Measuring Apremilast And Enantiomer Of Apremilast Through Liquid Chromatography- patent for separation of enantiomer of apremilast(Application Number: 201510997147.7)([8]).

Table 1: Physiochemical properties of Apremilast ([10],[11])

PROPERTIES	DETAILS
Product Name	APREMILAST
Cas No	608141-41-9
Structure	
Chemical formula	$C_{22}H_{24}N_2O_7S$
Iupac Name	N-[2-[(1S)-1-(3ethoxy-4- methoxyphenyl)-2- methylsulfonylethyl]-1,3dioxoisoindol-4-yl] acetamide.
Molecular mass	460.501 g/mol
Characteristics	a white to pale yellow nonhygroscopic powder
Solubility	Insoluble in water, slightly soluble in ethanol, and soluble in acetone
Melting point	156.1°C
Boiling point	741.3±60.0°C
PKa	12.58
logP	1.86/1.31
logS	4.1

PHARMACOLOGY: -

Phosphodiesterase (PDE) is a group of enzymes. Till now, eleven different families of PDE enzymes have been identified, PDE-4 enzyme has been found to play important role in inflammatory diseases, because of its liberal expression in the vascular endothelium, smooth muscles, immunologic cells, and keratinocytes.

Apremilast is a small molecule, a specific PDE-4 inhibitor, works intracellularly to modulate a network of pro-inflammatory and anti-inflammatory mediators.Acts by directly targeting a central pathogenic mechanism, binds directly to the PDE-4 enzyme and bypassing complex antigen-receptor interactive immunoregulatory mechanisms. Once drug-enzyme binding occurs, a series of events follow, foremost increasing levels of cAMP, which in turn decrease the levels of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-23, IL-12, and leukotriene B4, and also increases the levels of anti-inflammatory cytokines such as IL-10.

In addition, apremilast also binds to toll-like receptor 4 **o**n peripheral blood mononuclear cells, further reducing the production of pro-inflammatory cytokines.Apremilast also reduces the activity of nitric oxide synthase, an enzyme responsible for the synthesis of nitric oxide**,** which is an important pro-inflammatory mediator, thus preventing thetransport of macrophages and

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myeloid dendritic cells to the dermis and epidermis in psoriasis-skin.In this way, apremilast plays a notable anti-inflammatory role.[5],[8].

Figure 1: Mechanism of action [5]

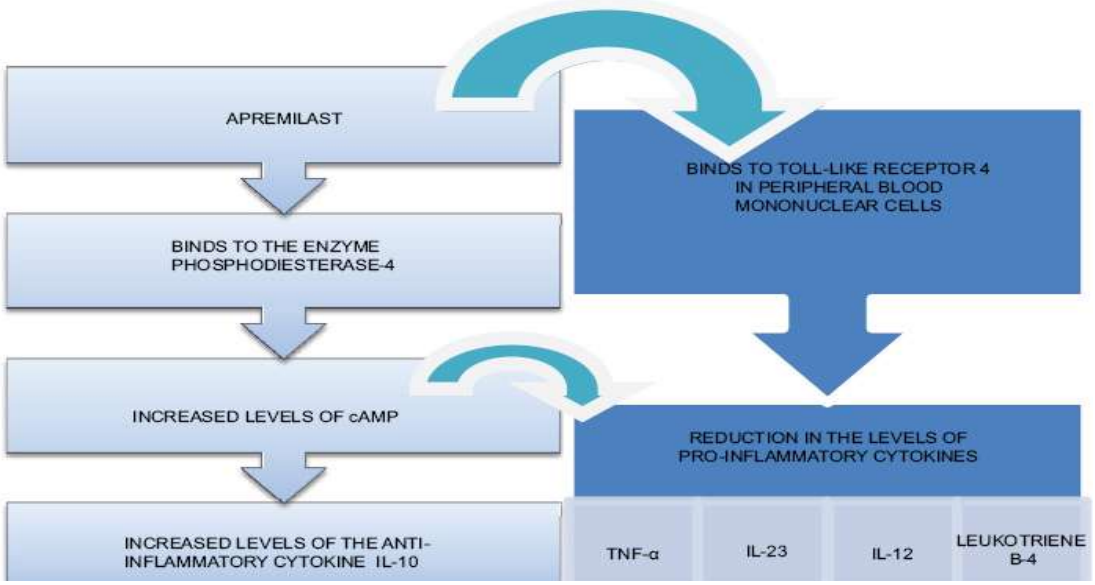

Table 2: Pharmacokinetic parameters of apremilast [10],[11]

Absorption	After oral administration, apremilast is absorbed with an absolute bioavailability of 73%, withth**e** m**a**ximumplasma concentrations (Cmax) occurring at a mean time (tmax) of 2.5 hours
Half life	5–7 hours
Distribution	Mean apparent volume of distribution (Vd) is 87 L, suggesting that apremilast is distributed in the extravascular compartment
Protein Binding	The plasma protein binding of apremilast is about 68%
Metabolism	Apremilast is heavily metabolized by various pathways, which include oxidation, hydrolysis, in addition to conjugation. Approximately 23 metabolites are produced from its metabolism. Apremilast is metabolized by both cytochrome (CYP) oxidative metabolism with subsequent glucuronidation and non-CYP mediated hydrolysis. In vitro, CYP metabolism of apremilast is primarily mediated by CYP3A4, with minor contributions from CYP1A2 and CYP2A6.
Route of elimination	Only 3% and 7% of an apremilast dose are detected in the urine and faces as unchanged drug, respectively, indicating extensive metabolism and high absorption.
Elimination Half Life	The average elimination half-life of this drug ranges from 6-9 hours
Clearance	The plasma clearance of apremilast is about 10 L/hour

ADVERSE EFFECTS:

Most common medical condition encountered with apremilast are diarrhoea, nausea, and headache. Other adverse effects include upper respiratory tract infection, vomiting, nasopharyngitis, upper abdominal pain, hypersensitivity, dyspnea, cough, and skin rash. As it has mild to moderate intensity of adverse effects, apremilast is safe drug with favourable toxicity profile [5].

DRUG INTERACTION:

No drug interaction was observed when administered with CYP3A4 inhibitor, CYP3A4 substrate.Drug Interaction was observed when administered with Strong CYP3A4 Inducer

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(rifampin, phenobarbital, carbamazepine, phenytoin), potent immunosuppressive drugs (e.g., cyclosporine, tacrolimus) and Biological Therapeutics(TNF antagonists and anti-IL-12/23 p40 antibodies). Apremilast cannot be simultaneously administered with strong CYP3A4 inducer, as it reduces level of apremilast in body[5,11].

TOXICITY:

Acute and Repeat dose toxicity studies were carried out on mice and rats.

Acute toxicity

In mice, the lowest lethal oral dose was > 2000 mg/kg, and the lowest lethal intravenous (IV) doses were 120 mg/kg and > 120 mg/kg for males and females, respectively. In rats, the lowest lethal IV dose was > 60 mg/kg and < 75 mg/kg, and the lowest lethal oral doses were 2000 mg/kg and > 300 mg/kg for males and females, respectively.

Repeat dose toxicity

studies of up to 6 months duration in mice (dose levels of 10, 100 and 1000 mg/kg/day; equivalent to 0.8-, 3.7- and 10-fold clinical exposure based on AUC), 12 months duration in monkeys (dose levels of 60, 180 and 600 mg/kg/day; equivalent to 2.3-, 3.2- and 4.8-fold clinical exposure based on AUC) and 90 days duration in rats.

From acute toxicity and repeat dose toxicity studies, apremilast was found safe for human use[11].

ANALYTICAL METHODS:

The main purpose of analytical method development and validation is to prove that the proposed analytical method is accurate, specific, precise, and robust for the particular drug[13].

Analytical Method Validation Parameters as per USP and ICH are as follow:

1.Specificity
2.Linearity
3.Precision
4.Accuracy
5.Range
6.Limit of Detection
7.Limit of Quantification
8.Robustness
9. System Suitability Test
10. Ruggedness

Literature survey reveals that various analytical method have been developed to estimate apremilast in bulk form, pharmaceutical dosage form as well as in biological sample. The method includes UV spectroscopy, HPLC, stability indicating HPLC, LC/MS(HPLC/MS, UPLC/MS), HPTLC, FTIR, DSC, NMR, X-RAY and SEM.

UV SPECTROSCOPY: -

ULTRAVIOLET(UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near infrared ranges.

Beer-Lambert Law is the principle behind absorbance spectroscopy.

BEER- LAMBERT LAW states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and path length[14].

A = abc

Where, A = Absorbance,
a = absorptivity,
b = path length,
c = concentration.

Various UV METHODS were used for analysis of APREMILASTi.e., Absorbance maxima method, Derivative method(zero order, first order) and area under curve method. Methanol was used as solvent in most of the reported article followed by acetonitrile. Detection wavelength for apremilast was selected 230nm except in few cases where detection wavelength selected was 340nm[15],220nm[21]229.3[20].Linearity was studied and Correlation coefficient was found in the range between 0.9959 to 0.9999.

Few Stability indicating UV spectrophotometric analytical method were reported where Apremilast was subjected to alkali, acid, oxidation, photolytic, UV light degradation. Result showed that Apremilast is unstable in alkaline and acidic condition whereas stable in thermal and UV light irradiation[20].

Table 3: UV Method Determination of Apremilast

Sr. No	Title	Method	Description	Ref. No
1	Method Development of Apremilast (API) in	UV	**λmax**: 340nm **Linearity**:	[15]

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	Methanol by UV-Visible Spectroscopy		10-60 µg /mL **Solvent**: Methanol	
2.	Development And Validation Of Sophisticated Analytical Method For The Estimation Of Apremilast	Double beam UV 1700 Pharmaspec (Shimadzu, Japan) METHOD A (ZERO ORDER) METHOD B (FIRST ORDER DERIVATIVE SPECTROSCOPY) METHOD C (AREA UNDER CURVE)	**λmax:** 230nm **Linearity:** 4-12 µg/mL **Solvent:** methanol **METHOD A**: **λmax:**230 nm **METHOD B:** **λmax:** 224.0 nm. **METHOD C:** **λmax:** 235-225 nm	[16]
3.	Development And Validation Of Uvspectrophotometric Method For The Estimation Of Apremilast In Bulk Form By Absorbance Maxima Method	Jasco double beam UV-visible spectrophotometer, Model: V-630,)	**λmax :** 230 nm **Linearity:** 1-7 µg/ml **Solvent:** Methanol	[17]
4.	Studies On Derivative Spectroscopy And Area Under Curve UV-Spectrophotometric Methods ForEstimation Of Apremilast In Bulk And In-House Tablets	Double beam UV-VIS Spectrophotometer (UV-2450, Shimadzu, Japan) software UV Probe 2.21) METHOD A (ZERO ORDER SPECTROPHOTOMETRY) METHOD B (ZERO ORDER SPECTROPHOTOMETRY – AUC) METHOD C (FIRST ORDER SPECTROPHOTOMETRY) METHOD D (FIRST ORDER SPECTROPHOTMETRY – AUC)	**linearity:** 2 -12 µg/mL **Solvent:** methanol **Accuracy:**98-101 % **Method A:** **λmax:**230nm **Method B:** **λmax:**226.20 - 233.20 nm **Method C:** **λmax:**233.50 nm **Method D: -** **λmax:** 233 - 234.60 nm	[18]
5.	Development and Validation of Spectrophotometric and	UV VIS Spectrophotometer (Shimadzu UV-1800)	**λmax:** 230nm **linearity:** 2-10 µg/mL **solvent:** Methanol and	[19]

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	Chromatographic Method for The Estimation of Apremilast In Bulk And Formulations	Zero Order spectrophotometric method	Water	
6	Development and Validation of Stability-Indicating UV Spectrophotometric Method for Determination Of Apremilast In Bulk And Pharmaceutical Dosage Form	ELICO Double beam SL 210 Ultra violet - Visible spectrophotometer	**λmax:** 229.3 nm **linearity:** 2 -10 µg /mL **solvent:** Acetonitrile **STRESS CONDITION** Unstable in Acidic Hydrolysis (20.1%) and Alkaline Hydrolysis (28.5%), Oxidative degradation (14.3%) Photolytic degradation (15.75%) Stable in U.V degradation (4.3%) and Thermal degradation (2.5%)	[20]
7.	Method Development And Validation Of Forced Degradation Studies Of Apremilast By Using UV Spectrophotometric Method	UV – Visible spectrophotometer (Shimadzu Model 1700)	**λmax:** 220nm **Linearity:** 20-100 µg/ml **Solvent:** methanol **STRESS CONDITION: -** Acidic (8.2%) Alkaline (13.3%) Photolytic (12.5%) Thermal (14.5%) Oxidative (10.7%)	[21]

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY(HPLC):

HPLC is the most accurate analytical technique, has the ability to separate, identify and quantify sample dissolved in liquid. It is widely used in analysis of drug product([22]).

HPLC method comprises of two mode: NORMAL PHASE HPLC, REVERSE PHASE HPLC

Common Mobile phase solvent used were acetonitrile and water followed by less common solvent used acetic acid, potassium dihydrogen phosphate. In one reported case, Buffer: Methanol was used as mobile phase where buffer was made up of 0.1% orthophosphoric acid in water adjusted pH 2.8 with potassium hydroxide. Column used were various C18 COLUMN (Cosmosil, wondersil, PrincetonSPHERE Ultima, Grace, intersil, eclipse) except in few reported articles where column used were C8 and Max-RP 80 A. Most common detector used was PDA (photodiode array) except in some cases where UV detector was used. Detection wavelength in most of the cases reported was selected 230 nm except in few cases were wavelength selected was 203nm([27]), 229nm([31]), 231nm([25],[26]),360nm([29]) respectively. Linearity was studied and correlation coefficient was found between the range 0.9981 to 0.9999. HPLC method are divided into: ISOCRATIC system and GRADIENT system.

In 2018, HPLC for determination of enantiomeric purity of apremilast was reported where 6 different chiral columns (Chiralpak AD, Chiralpak IA, Chiralpak AS, Lux Amylose-2, Chiralcel OD and Chiralcel OJ-H) were investigated, best result were obtained on Chiralpak IA column with CAN (acetonitrile). 0.1% R-enantiomer as chiral impurity in S-APR as well as quantification of the S-enantiomer were determined([24]).

Various Stability indicating RP – HPLC was performed, process related impurities of apremilast were identified, synthesized, characterized and

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quantify. Structure is elucidated with NMR, FTIR and MS techniques[23],[32],[33].

Table 4: HPLC Method Determination of Apremilast

Sr no.	Title	Method	Description	Ref no
1.	Development and Validation of Spectrophotometric and Chromatographic Method for the Estimation of Apremilast in Bulk and Formulations	HPLC (ISOCRATIC)	**Column:** Cosmosil C18 (4.6mm x 250mm, Particle size: 5µm **Detector:**UV–Visible spectrophotometer **λmax:** 230nm **Mobile phase-** Methanol: Water (80:20v/v) pH3 **Flow rate** 0.8 ml/min **Sample volume:** 20 µL **Retention time:** 4.0 min **Linearity**: 10-50 µg/mL	[19]
2	Identification, Characterization And HPLC Quantification For Impurities Of Apremilast	Agilent 1100 HPLC system (Agilent Technologies, USA) (GRADIENT)	**Column:**Wondersil C18(250mm×4.6mm, 5µm) **Detector:** PDA **λmax:** 230nm **Mobile phase(A):**0.03% TFA **Mobile phase(B):**ACN (0.03% TFA) **Flow Rate:** 1.0min/ml **Sample volume:**20µL **Linearity:** 0.03µg/ml - 0.63µg/ml	[23]
3	Validated LC Method For Determination Of Enantiomeric Purity Of Apremilast Using Polysaccharide-Type Stationary Phases In Polar Organic Mode	Agilent 1260 Infinity HPLC system	**Column:** Chiralpak IA **Detector:** UV detector **λmax:** 230nm **mobile phase:** MeOH and ACN **flow rate:** 0.7 mL min−1 **sample volume:** 10 µL **retention time:** **R-APR (**quantification as impurity)**: -** **linearity:** 2–100 µg mL−1 **S-APR** (assay): - **linearity:** 12–200 µg mL−1	[24]
4	Design Of Experiment Avenue For Development And Validation Of RP-HPLCPDA Method For Determination Of Apremilast	RP-HPLC (ISOCRATIC) UFLC HPLC system (Shimadzu Corporation, Japan)	**Column:** PrincetonSPHERE Ultima (250 mm × 4.6 mm × 5 µm) C18 column **Detector:** PDA (photodiode array) **λmax:** 231nm **Mobile Phase** - methanol:water (pH 3.50) 70:30% v/v **Flow rate**: 1ml/min	[25]

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	In Bulk And In In-House Tablet Formulation		**Sample volume:**20 µl **Retention time:**5.15 **Linearity:** 2–12 µg/ml	
5	Chromatographic Method Development And Validation Of Assay Of Apremilast In Bulk And Tablet Dosage Form	RP- HPLC (ISOCRATIC)	**Column:** Grace C 18 analytical column **λmax:** 231nm **Mobile Phase** -Methanol: Water (80:20) **Flow rate:** 0.8ml/min **Sample volume:** 20µL **Retention time:** 4.80 mins **Linearity**: 10-50µg/ml	[26]
6	A New Stability Indicating Rp-Hplc Method For The Determination Of Apremilast-An Antirheumatic Drug	RP-HPLC (ISOCRATIC) Shimadzu Model CBM-20A/20 Alite HPLC system	**Column:**Intersil ODS3 C18 column (250 mm × 4.6 mm i.d., 5 µm particle size) **Dectector:**photodiode array **λmax:** 203nm **Mobile Phase:**0.1% acetic acid and acetonitrile (20:80%, v/v) **Flow rate:** 0.8ml/min Sample volume: 20 µL **Retention time:**5.30 ± 0.02 mins **Linearity:**0.5–150 µg/ml **Stress Condition:** Acidic (7.51) Alkaline (11.72) Oxidative (8.82) Thermal (0.03)	[27]
7	Reversed Phase High Performance Liquid Chromatography Method For Determination Of Assay And Forced Degradation Study Of Apremilast From Active Pharmaceutical Dosage Form		**Column:**inertsil C8 (250 X 4.6 mm) 5µ **Detector:** diode array **λmax:** 230nm **Mobile Phase** buffer and methanol (47:53 % v/v) **Flow rate:**1.5 ml /min **Sample volume:**20 µl **Retention time:**8.3 minutes **Stress Condition** Acid (4.6%) Base (14.9% Degradation was not observed in Oxidative, Thermal, and Photolysis degradation	[28]
8	Validation of Stability Indicating Method and	Shimadzu HPLC system with UV Detector	**Column:** Analytical Technologies Limited C18 column (250 mm x 4.mm,5µm)	[29]

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	Degradation Kinetic Study of Apremilast		**Detector:** UV Detector **λmax**: 360nm **Mobile Phase:** potassium dihydrogen orthophosphate: Acetonitrile (40:60) **Flow rate:** 1 mL /min **Sample volume:** 20µl **Run time:** 10 min **Linearity:** 50-400µg/mL **STRESS CONDITION:** acidic condition (21%), alkaline condition (6.5%), oxidative condition (25.7%), photolysis degradation (3.9)	
9	Development And Validation Of Stability Indicating Rp-Hplc Method For The Estimation Of Apremilast By Forced Degradation Studies	RP-HPLC Jasco HPLC system consisting of a binary gradient system (Model no.: HPLC 3000 Series)	**Column:**Grace C18 (250mm x 4.6ID, 5µm) **Detector:**UV-3000-M detector **λmax:** 230nm **Mobile Phase:**methanol: water (70:30, v/v) **Flow rate:**0.8 ml/min **Sample volume:**20µl **Retention time:**5.203 minutes **Linearity:** 10-50 µg/ml **LOD:**0.5329µg/ml **LOQ:**1.615µg/ml **Stress condition:** Apremilast was considerably stable in acidic, photolytic and thermal as degradants were not seen In alkaline and oxidative condition , degradation was seen.	[30]
10	Development and Validation of a Stability-Indicating Reversed Phase Hplc Method for Determination of Apremilast in Bulk and Pharmaceutical Dosage Form	RP-HPLC (GRADIENT) Agilent technologies 1260 infinity system	**Column:**eclipse XDB model C18 Column (4.6 mm i.d. X 250 mm, 5 µm particle size) **Detector:**Photo diode Array 1260 DAD VL **λmax:** 229nm **Mobile Phase-:** acetonitrile **Flow rate:**1 mL/min **Sample volume:**20 µL **Retention time:**2.488 minutes **Stress condition: -** Acid (66.54%)	[31]

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			Alkaline (65.17%) Oxidative (9.01%) Thermal (18.86%) UV light (62.20%)	
11	Stability-Indicating Related Substances Method Of Apremilast By Hplc And Synthesis And Chracterization Of Related Impurities Using Mass And Nmr Spectroscopy	Shimadzu HPLC system LC-2010 CHT	**Column**: Cosmosil C-18 column 250 mm x 4.6 mm, 5.0 µm **Detector:** photodiode array **λmax:** 230nm **Mobile Phase** **Mobile Phase-A:** Buffer-1: Methanol (90:10) v/v **MobilePhase-B:** Buffer-1: Acetonitrile (10:90) v/v **Flow rate:**1.0 mL/min **Sample volume:**15 µL	[32]
12	Development and Validation of Stability Indicating Rapid RP-LC Method for Determination of Process and Degradation Related Impurities of Apremilast, an Anti-Inflammatory Drug	RP-HPLC GRADIENT METHOD (Agilent 1200, Agilent Technologies, Germany)	**Column:**Synergi Max-RP 80 A (150 × 4.6 mm ID), 4 µ **Detector:** Photodiode array **Flow rate:**1.0 mL·min−1 **Sample volume:**10 µl **Stress condition:** Acid hydrolysis (6.75), Base hydrolysis (8.47), Photolytic (0.32)	[33]

Liquid Chromatography/ Mass Spectrsoscopy(LC/MS):

LC/MS is an hyphenated analytical technique that combines the physical separation abilities of liquid chromatography with the mass analysis capabilities of mass spectrometry([34]).LC/MS is a powerful technique with high sensitivity and high selectivity.

Plasma analysis(beagle dog plasma, rabbit plasma, rat plasma) was done using LC/MS technique (HPLC/MS, UPLC/MS). Reconstituted Plasma was injected in HPLC SYSTEM. Electron Spray Ionization(ESI) was used for detection in all the cases.

All the reported article were successfully employed in pharmacokinetic study after oral administration of 10 mg(beagle dog)([35]), 1.5mg/1.9kg(male rabbits)([36]), 2mg/kg(rats)([37]), 6mg/kg(rats)([38]) of apremilast.

Table 5: LC/MS Method Determination for Apremilast

Sr no	Title	Method	Description	Ref no
1	A rapid and sensitive High-Performance Liquid Chromatography-tandem Mass Spectrometry	LC/MS (GRADIENT) Shimadzu SCL-10A HPLC system (Shimadzu, Japan) coupled to an API 3000 mass	**Column:**XTerra® MS C8 column (5 mm, 2.1 3 150 mm, Waters) **Mobile phase:** methanol and 0.1% formic acid **Flow rate:** 0.4 mL/min **Linearity:** 5–1,000 ng/mL	[35]

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	method for determining apremilast in beagle dog plasma and urine: Application in a pharmacokinetic study	spectrometer (Sciex, Framingham, MA, USA)	**m/z apremilast:** 461.3→178.2 **m/z IS (clopidogrel):** 322.2→184	
2	LC-MS/MS Determination and Pharmacokinetics Study of Apremilast after Oral Administration in Rabbits	QSight® Triple Quad UPLC-ESI-MS/MS system (Perkin Elmer) Combined with QSight LX50 UHPLC	**Column:** CORTECS C18, 2.7 µm, 4.6 mm X 150 mm **Mobile phase:**Ammonium Acetate buffer pH of 4.0 with Methanol: Acetonitrile, (20:40:40%, v/v/v) **Flow rate:** 0.5 m.L/min **Sample volume:**10 µ.L **Linearity:**0.03 to 48.00 n.g/m.L	[36]
3	Determination of apremilast in rat plasma by UPLC–MS/MS in ESI-negative mode to avoid adduct ions formation	ISOCRATIC METHOD Acquity UPLC system	**Column:**Acquity BEHTM C18 column (100 × 2.1 mm, 1.7 µm) **Moblie phase:** acetonitrile: 10 mM ammonium acetate (85: 15, v/v) **Flow rate**: 0.3 ml/min **Linearity:** 3.04 and 1000 ng/ml **Ionization technique:**ESI negative mode m/z 459.14 → 78.95 for APM and m/z 380.04 → 316.09 for IS(celecoxib) **Cone voltage: -** Apm: 42V Is: 54V **Collision energy: -** Apm: 22eV Is: 19eV	[37]
4	Determination of Apremilast in Rat Plasma by UPLC–MS-MS and Its Application to a Pharmacokinetic Study	Gradient method Acquity UPLC unit (Waters Corp., Milford, MA, USA)	**Column:** n Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 µm particle size) **Detector:** **Flow rate:** 0.40 mL/min **Linearity:** 0.1–100 ng/mL XEVO TQD triple quadruple mass spectrometer **Ionization technique:**electro-spray ionization (ESI) source **Detection:** triple quadrupole tandem mass spectrometer in the multiple reaction-monitoring mode **m/z:** 461.3 → 257.1(APR) **m/z:** 237.2 → 194.2 carbamazepine (internal standard)	[38]

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY(HPTLC):

HPTLC(High Performance Thin Layer Chromatography) is an advance form of Thin Layer

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Chromatography. HPTLC emerged as important tool in DRUG ANALYSIS[39].
DOE was based on Box Behnken design and Failure mode effect analysis.

Application of Box behken design was used to evaluate robustness. Factors like development distance, saturation time, activation time and mobile phase ratio have effect on a peak area and retention factor[40].
Failure mode effect analysis was performed for development of stability indicating assay method for estimation of apremilast through quality by design approach[42].

HPTLC system used were CAMAG TLC SYSTEM(Muttenz, Switzerland). Aluminium backed precoated silica gel was used as stationary phase. Mobile phase used were different for reported article Toluene:Methanol[40], Toluene:Ethyl Acetate[41], Toluene:Methanol:Ethyl Acetate[42] were used. Linearity was studied and regression factor was between 0.996- 0.998.

Table 6: HPTLC Method Determination for Apremilast

SR. no	Title		Description	Ref no
1.	Application of Box-Behnken Design for Validation of High-Performance Thin-Layer Chromatography/Densitometry Method for Robustness Determination of Apremilast in Bulk andinhouse Tablets	HPTLC system: Camag TLC system (Muttenz, Switzerland)	**Stationary phase:** Aluminium backed precoated silica gel 60-F254 (20 x 10 cm) **Mobile phase** -Toluene: Methanol (8:2 v/v) **Detection**: Densitometry scanning mode: Absorbance–Reflectance **Rf:**64 ± 0.05 **λmax:** 230 nm	[40]
2.	A Stability Indicating HPTLC Method For Apremilast And Identification Of Degradation Products Using MS/MS	HPTLC System: Camag TLC system (Muttenz, Switzerland)	**Stationary phase:**Aluminium plates precoated with silica gel 60 F254 plates **Mobile phase:** Toluene: Ethyl Acetate (4:6; v/v) Densitometry of scanning mod: Absorbance– Reflectance **λmax:** 236nm **Rf:** 0.55±0.02 **Linearity:** 100 - 600ng Degradation was found maximum in Acidic condition (19.38%), minimum in oxidative condition (5.15%) and stable in photolytic condition	[41]
3.	Doe Based Failure Mode Effect Analysis (FMEA) To Development Of Stability Indicating HPTLC Method For Estimation Of Apremilast	HPTLC system (Camag Switzerland)	**Stationary phase:**aluminium backed pre-coated with silica gel 60F254 **Mobile Phase:** toluene:methanol:ethyl acetate (7:2:1% v/v/v) **λmax:** 241 nm **Rf: 0**.63±0.02 **Linearity:** 200-1000 ng/band From Degradation Studies apremilast was found to be	[42]

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			more prone to alkaline hydrolysis (10.45%)but less prone to acid (23.90%)and neutral hydrolysis and was found stable in oxidative, dry heat and photolytic stress conditions Photolytic degradation (9.56%)	

Fourier-transform infrared spectroscopy (FTIR):

FTIR is a rapid and non-destructive multicomponent analysis[39].KBr pellet method was used in sample preparation except in one article were ATR crystal (Attenuated total reflection method)[44].Characteristic peak was observed.

Table 7: FTIR Method Determination for Apremilast

Sr no	Title		Description	Ref no
1	Identification, characterization and HPLC quantification for impurities of Apremilast	Thermo Scientific Nicolet iS5 FT-IR spectroscopy	**Sample preparation**: KBr pellets **Peak:** acylamino N-H stretching: 3363.3cm-1 methylene C-H: 2837 cm-1 isoindole C=O: 1764 cm-1 benzene ring: 3002 cm-1 sulphone -SO2: 1338 cm-1	[23]
2	Preparation of sustained release apremilast-loaded PLGA nanoparticles: in vitro characterization and in vivo pharmacokinetic study in rats	ALPHA-FTIR Spectrometer (OPTIK, Billerica, MA, USA)	**Sample preparation:** potassium bromide (KBr) pellets and by applying suitable pressure **Peak:** amide −C=O: 1,682 cm-1 ketone (−C=O): 1,764 cm-1 amide (−N-H): 3,363 cm-1	[43]
3	Enhancement Of Solubility And Dissolution Rate Of Apremilast By Recrystallization Technique	FTIR spectrometer (Bruker, Germany)	**Sample preparation:** ATR crystal (Attenuated Total Reflection method) **Range:** 4000-500 cm-1	[44]
4	Development and Validation of Stability Indicating Rapid RP-LC Method for Determination of Process and Degradation Related Impurities of Apremilast, an Anti-Inflammatory Drug	Perkin Elmer model-spectrum-100 (California, USA)	**Samplepreparation:** KBr pellet method	[33]
5	Formulation, optimization and in vitro evaluation of nanostructured lipid carriers for topical delivery of apremilast	IR Prestige-21, Shimadzu Corp., Tokyo, Japan	**Sample preparation:** potassium bromide disc method **Peak:** N–H stretching: 3364 cm-1 Characteristic peak: amide carbonyl (C=O): 1764 cm-	[45]

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			1 aliphatic benzene ring C–H stretching: 2837 cm-1 aromatic benzene ring C–H stretching: 3003 cm-1 amide N–H bending: 1519 cm-1 C–O stretching:1233 cm-1	
6	A Novel Apremilast Nail Lacquer Formulation for the Treatment of Nail Psoriasis	Cary 660, Agilent Technologies Santa Clara, CA	**Peak:** amide –C=O: 1687 cm−1 ketone –C=O: 1763 cm−1 amide –N–H: 3362 cm−1	[46]

Differential Scanning Calorimetry (DSC):

Differential Scanning Calorimetry (DSC) is athermo-analytical technique whichmeasures the rate of heat flow to a sample and to a standard that are at the same temperature ([46]).

DSC studies were performed comparing pure apremilast and various apremilast formulation as well as from solubility studies. Endothermic peak of pure apremilast obtained from the articles reported was between 152°Cto 166°C.

Table 8: DSC Method Determination for Apremilast

Sr.no	Title		Description	Ref no
1.	Solubility and thermodynamics of apremilast in different mono solvents: Determination, correlation and molecular interactions	DSC-60 Instrument (Shimadzu, Japan)	**Heating rate:**283.2 K min-1 **Nitrogen flow rate:** 40 mL min-1 **Endothermic peak:**432.02 K	[48]
2	Enhancement Of Solubility And Dissolution Rate Of Apremilast By Recrystallization Technique	DSC–60 Differential Scanning Calorimeter (Shimadzu, Japan)	**Heating rate:**4°C/min **Nitrogen flow rate:** 50 mL/min **Endothermic peak:**155.62 °C	[44]
3	Preparation Of Sustained Release Apremilast-Loaded PLGA Nanoparticles: In Vitro Characterization And In Vivo Pharmacokinetic Study In Rats	DSC Thermal Analyzer (DSC N-650; SINCO; Taipei, Taiwan)	**Heating rate:**10°C/minute **Nitrogen flow rate**: 20 mL/minute **Endothermic peak**: 159.4°C	[43]
4	Formulation, Optimization And In Vitro Evaluation Of Nanostructured Lipid Carriers For Topical Delivery Of Apremilast	DSC 4000, Perkin Elmer, Massachusetts, United States	**Heating rate:** 10°C/min **Nitrogen flow rate**: 20 ml/min **Endothermic peak:**159.56 °C	[45]
5	A Novel Apremilast Nail Lacquer Formulation For The	Differential scanning calorimetry; PerkinElmer,	**Heating rate:**30 °C/min **Nitrogen flow rate:** 22mL/min	45

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	Treatment Of Nail Psoriasis	California	**Endothermic peak:** 166.25°C	
6	Preparation, Characterization And In Vitro Evaluation Of Tablets Containing Microwave-Assisted Solid Dispersions Of Apremilast	Shimadzu DSC-60 differential scanning calorimeter (Shimadzu Corp.)	**Heating rate:** 10°C/min **Endothermic peak**: 157.56°C	[49]
7	Formulation And Development Modified Release Apremilast Pellets	Mettler Toledo 61000 USA, DSC system	**Heating rate:**10°C per min **Nitrogen flow rate:**20 ml/min **Endothermic peak:** 152.7°C	[50]

Nuclear Magnetic Resonance(NMR):

NMR finds its application in quantitative analysis, to determine the impurity of the drug, characterization of the composition of the drug products and in quantitation of drugs in pharmaceutical formulations and biological fluids([39]).

Few literatures were reported where Tetramethysaline(TMS) was used as internal standard. Spectra was recorded at 500MHz/400MHZ.

One-dimensional (1H NMR, 13C NMR) and 2D (distortionless enhancement by polarization transfer (DEPT), 1H-1H correlation spectroscopy (COSY), heteronuclear multiple bond correlation (HMBC), and heteronuclear singular quantum correlation (HSQC)) NMR spectra was recorded in reported case([23]).

Table 9: NMR Method Determination for Apremilast

Sr no	Title		Description	Ref no
1	Identification, characterization and HPLC quantification for impurities of Apremilast	Bruker AVANCE 500MHz NMR system (Fallanden, Switzerand)	**Spectra:** 500MHz **Sample:** dissolved in DMSO-d **IS:**tetramethylsilane	[23]
2	Development and Validation of Stability Indicating Rapid RP-LC Method for Determination of Process and Degradation Related Impurities of Apremilast, an Anti-Inflammatory Drug	Bruker AV400	**Spectra:** 400MHz **IS**: tetramethylsilane (TMS)	[33]

X - RAY Diffraction Technique (XRD):

X-RAY diffraction is performed to obtain structural information of crystalline solid([47]).APM powder is highly crystalline in nature, as clear from sharp peaks observed in the x-ray spectrum.Powder X-RAY study was carried out to investigate crystalline and amorphous form of apremilast. X-RAY diffractometer used were shimadzu, Rigaku. Target filter used was copper(Cu).

Table 10: XRAY Method Determination for Apremilast

Sr no	Title		Description	Ref no
1.	Enhancement Of Solubility And Dissolution Rate Of Apremilast	X-Ray Diffractometer (Shimadzu, Japan) using	**λ:** 1.54 Å **voltage/current:** 40 kV & 30 mA power **scan range:** 10°- 80° **scan rate:** 4°/min	[44]

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	By Recrystallization Technique	Cu-kα line as X-Ray radiation	**characteristic intense peaks** at 11.29°, 17.80°, 26.49° of pure APM	
2.	Preparation, Characterization And In Vitro Evaluation Of Tablets Containing Microwave-Assisted Solid Dispersions Of Apremilast	Smart Lab high power powder X-ray diffractometer (Rigaku Corp., Tokyo, Japan) with Cu as a target filter	**voltage/current:** 40 kV/40 mA **scan range:** 10° to 89 **scan rate:** 4°/min **characteristic diffraction peaks** observed at 10.09°, 11.87°, 13.53°, 16.34°, 26.09°, and 26.92° of pure APM	[49]
3	Preparation Of Sustained Release Apremilast-Loaded Plga Nanoparticles: In Vitro Characterization And In Vivo Pharmacokinetic Study In Rats	X-ray diffractometer (Ultima-IV, Rigaku, Tokyo, Japan) with Cu as target filter	**voltage/current:** 30 kV /25 mA **scan range**: 3°–90° (2θ) **scan rate:** 4°C/minute **characteristic intense peaks** at 10.08° 2θ, 12.38° 2θ, 13.48° 2θ, 20.82° 2θ, 22.50° 2θ, 24.10° 2θ, 24.66° 2θ, and 26.96° 2θ of pure APM	[43]
4	Formulation, Optimization And In Vitro Evaluation Of Nanostructured Lipid Carriers For Topical Delivery Of Apremilast	x-ray diffractometer (D8 Advance, Bruker, Massachusetts, United States)	**Voltage/current:** 40 mV/ 35 mA **Scan range:** 0-50° (2θ) **characteristic diffraction peaks** were observed at 21.12°, 27.29°, 26.44°, 12.61°, 13.70°, 16.50° and 24.99° in the diffractogram of pure APM	[45]

Scanning Electron Microscope(SEM):

Scanning Electron Microscope (SEM) is widely used to study the surface morphology and surface topography of powder. In reported article, sample powder was examined by placing them on a stub of metal with adhesive, coated with 40 - 60 nm of metal such as Gold/Palladium under a reduced pressure (60% vacuum). The SEM images of the pure APM and its recrystallized product showed irregular shaped crystals with different particle sizes, which infers that recrystallization, had not affected the morphology of the compound[([44])].

Apremilast and its impurities

I. Stability-Indicating Related Substances Method of Apremilast By HPLC And Synthesis AndChracterization Of Related Impurities Using Mass And NMR Spectroscopy[(31)]

Major process related impurities of

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

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

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

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

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

Column: Cosmosil C-18 (250 mm × 4.6 mm, 5 µm)

Mobile phase: mixture of 0.05%trifluoroacetic acid, methanol and acetonitrile under gradient elution

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The retention time for Apremilast is 39.0 mins and Relative Retention time (RRT) is:

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

II. Identification, Characterization and HPLC Quantification For Impurities Of Apremilast[22]

Major process related impurities are

1. 3-Acetylamino-phthalic acid Imp-C
2. N-[1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethyl]-acetamide Imp-A
3. N-(1-{1-[1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamino]-ethyl}-3-methyl-butyl)-acetamide Imp-B
4. 2-[1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethyl]-4-nitro-isoindole-1,3 -dione(Imp-D) Imp-D
5. (4-Amino-2-[1-(3-ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethyl]-isoindole1,3-dione) Imp-E
6. (N-{2'-[1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethyl]-1,3,1',3'-tetrao xo-1,3,2',3'-tetrahydro-1'H-[2,4']biisoindolyl-4-yl}-acetamide)Imp-F

Wondersil C18 column(250×4.6 mm, 5µm) , the detection wavelength was 230nm, ACN and TFA mixtures

The retention time (RT) and relative retention time (RRT) of all six impurities were

Name	RT	RRT
3-Acetylamino-phthalic acid (Imp-C)	7.87	0.25
N-[1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethyl]-acetamide Imp-A	16.94	0.54
N-(1-{1-[1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethylamino]-ethyl}-3-methyl-butyl)-acetamide Imp-B	22.13	0.71
2-[1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethyl]-4-nitro-isoindole-1,3 -dione (Imp-D)	23.71	0.76
(4-Amino-2-[1-(3-ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethyl]-isoindole1,3-dione) Imp-E	30.06	0.98
(N-{2'-[1-(3-Ethoxy-4-methoxy-phenyl)-2-methanesulfonyl-ethyl]-1,3,1',3'-tetrao xo-1,3,2',3'-tetrahydro-1'H-[2,4']biisoindolyl-4-yl}-acetamide) Imp-F:	34.12	1.10.

III. Development and Validation of Stability Indicating Rapid RP-LC Method for Determination of Process and Degradation Related Impurities of Apremilast, an Anti-Inflammatory Drug[32]

Column: Synergi Max-RP 80 A (150 × 4.6 mm ID), 4 µ HPLC

Mobile Phase:

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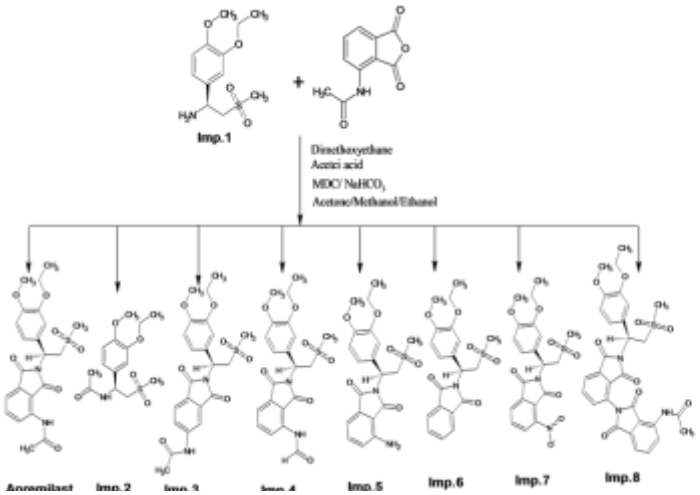
Mobile phase A- ammonium dihydrogen orthophosphate and 1 ml triethylamine in 1000 mL of Water.Mobile phase B: Acetonitrile Maximum wavelength: 230nm

Name	Retention time
Imp 1	2.68
Imp 2	3.11
Imp3	10.25
Imp 4	12.33
Imp 5	12.88
Imp 6	14.81
Imp 7	15.38
Imp 8	16.76



IV. A Stability Indicating HPTLC Method for Apremilast and Identification of degradation products using MS/MS[40].

Eleven degradation products were dentify based on MS/MS spectra.
The eleven degrading product found was DP-1, DP-2, DP-3, DP-4, DP-5, DP-6, DP-7, DP-8, DP-9, DP-10, and DP-11 respectively.

II. CONCLUSION:

An overview of the current state of the art for analytical methods for the determination of Apremilast has been presented. The literature compilation has revealed that a variety of methods are available for Apremilast. So, from all above information it can be concluded that various spectroscopic methods, chromatographic methods and other methods were used for determination of Apremilast which has been successfully used on a routine basis and allows the quantification of the drug. There is a possibility of developing Gas chromatography methods. Also, pharmacopeia might be included as an official drug.

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