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# Development and Validation of Rp-Hplc Method for Estimation of Apremilast (Anti Arthritis) Drug

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**ABSTRACT**

Apremilast drug is administrated by oral root and it is phosphodiesterase 4 inhibitor, now approved with significant amounts of adverse effect, newer treatment options with an efficacious outcome, sans the toxicity, is something all clinicians would implement readily in their therapeutic regimens. Apremilast is one such drug which has shown promise in the management of psoriasis, PsA, and a few other dermatologic disorders. This review will throw light on the dermatologic prespective regarding apremilast.

Apremilast is used for treatment of psoriasis and psoriatic arthritis. It may also be beneficial for other inflammayor diseases relevant to the immnune system.

**KEY WORDS :**Apremilast, Phosphodiesterase 4 inhibitor, psoriasis, psoroaticarthropathy

## I. INTRODUCTION

Apremilast,sold under brand name Otezla among others it is also used to treat the mouth related ulcers in people with bahcet'ssyndrome,psoriasis,psoriatic arthritis.

It is approved by US Food and drug Administration in 2014 and press launch done in September 23,2014.

- **Objectives:**

To develop new, simple, sensitive, accurate, and economical analytical method for the determination of assay of Anti-Arthritis Drug in tablet dosage from by RP-HPLC.

**01 DRUG PROFILE:**

Name:Apremilast

Structure:

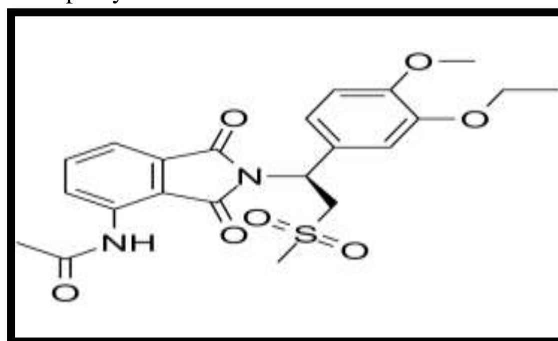

**Fig. Structure of Apremilast**

**Table General profile of Apremilast**

| **Category** | Anti- Arthritis |
|---|---|
| **Chemical Name** | N-{2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl}acetamide |
| **Molecular Formula** | $C_{22}H_{24}N_2O_7S$ |
| **Molecular Weight** | 460.5 g/mol |
| **Description** | It is a white to pale yellow, non-hygroscopic powder |

| | |
|---|---|
| **Solubility** | insoluble in water and buffer solutions in a wide pH range, but is **soluble** in lipophilic solvents such as acetone, acetonitrile, butanone, dichloromethane, and tetrahydrofuran. |
| **pKa** | 12.58 |
| **Melting point** | 156.1°C |

**Mechanism of action**

Apremilast is a small molecule inhibitor of PDE4, 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.

**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. 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.

**PLAN OF WORK:**

- Estimation of Anti-ArthritisDrug in tablet dosage from will be done by following methods.

**Selection of Drugs and Formulation**

- By literature and market survey

**Selection of analytical techniques**

- Estimation by UV-Visible spectroscopy.
- Identification by IR Spectroscopy
- Development and validation of RP-HPLC method

**Method development by RP-HPLC.**

**Validation of proposed method.**

- ✓ System suitability parameter
- ✓ Specificity
- ✓ Linearity and Range
- ✓ Accuracy
- ✓ Precision

**Drug used in experiment:**

**Table:Drug and drug product samples suppliers and manufacturers**

| Name of drug and drug product | Supplier and manufacturer by |
|---|---|
| Apremilast | Micro labs ltd |

**REAGENTS:**

**Table : List of Reagent**

| Sr.No | Chemical | Make |
|---|---|---|
| 1 | Water | Rankem |
| 2 | Acetonitrile | Merck life science |
| 3 | Methanol | Merck life science |
| 4 | Triethylamine | Merck life science |
| 5 | 0.45 µ Nylon | Mdi |

**INSTRUMENTS:**

**HPLC:**

| **Make** | Waters e2695 |
|---|---|

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| **Pump** | Reciprocating Water-510 |
|---|---|
| **Detector** | Waters 2998 PDA and 2489 UV |
| **Software** | Empower 3 |

**SPECTROPHOTOMETER:** Double beam UV-visible spectrophotometer with 10mm Matched quartz cells
**Model** UV1800,**Make**Shimadzu.

**RESULTS AND DISCUSSION**

A simple, precise and economic RP-HPLC method was developed and validated for estimation of Apremilastin bulk and tablet. The method was validated as per ICH guidelines by using various validation parameters such as Linearity, accuracy, precision, specificity

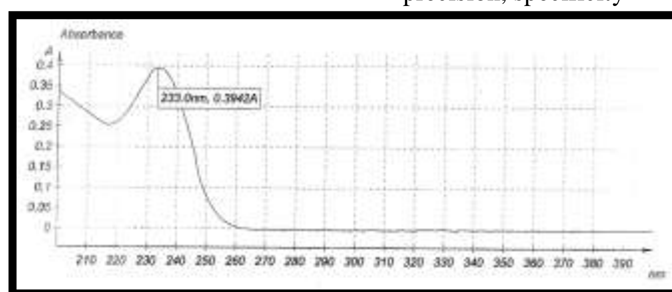


**Fig: Spectra showing λ max of Apremilast**

- **Determination of λ max of Apremilast**

| Sr. No. | Wavelength (nm) | Absorbance |
|---|---|---|
| 1. | 233 | 0.3942 $A^0$ |

**Identification by IR Spectroscopy:**

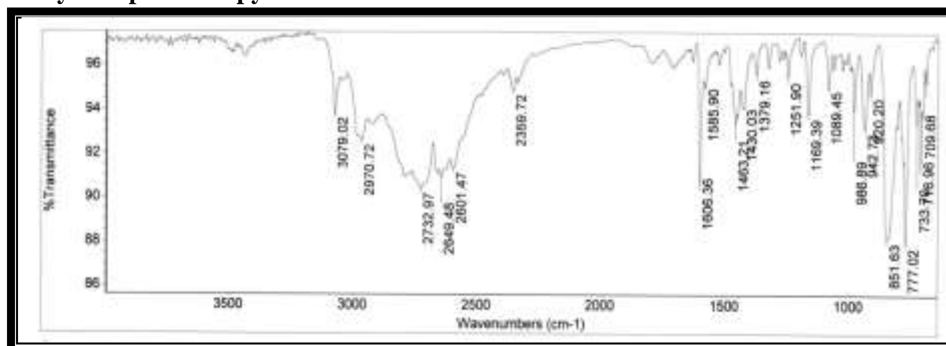


**Fig: FTIR spectrum of ApremilastStandard Solution**

**Table 7.2 IR peak Assignment value of Apremilast**

| Standard IR Ranges ($cm^{-1}$) | IR Ranges ($cm^{-1}$) | Functional Group |
|---|---|---|
| 2600-2550 | 2601.47 | **S-H Stretching** |
| 1650-1580 | 1585.90 | **N-H Bending** |
| 1410-1380 | 1379.16 | **S=O Stretching** |
| 730-665 | 716.96 | **C=C Bending** |

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**Reverse Phase High Performance Liquid Chromatography Method Development**
**Different trials taken were as follows**

**TRIAL: 1**

**Chromatographic Conditions:**

| Column | ACE C18 (250 x 4.6mm) 5µ |
|---|---|
| Mobile Phase | Water:Methanol (90:10 v/v) |
| Flow Rate | 0.8 mL/min |
| Injection Volume | 5 µL |
| Wavelength | 233 nm |
| Column Temp. | 25°C |
| Auto sampler Temp. | 25°C |
| Run time | 7.0 min. |
| Needle wash | Water: Methanol (20:80 v/v) |
| Seal wash | Water: Methanol (80:20 v/v) |

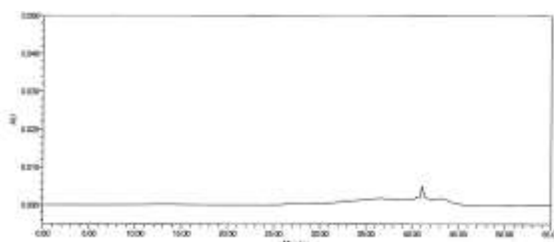

**Fig. Typical chromatogram for Trial- 1**

**Observation:** Usingwater : methanol mobile phase apremilast Standard solutioninjected. Analyte peak not observed in chromatogram. Plan baseline observed.
**Conclusion:**Mobile phase ratio need to optimize

**TRIAL: 2**

**Chromatographic Condition:**

| Column | ACE C18 (150 x 4.6mm) 5µ |
|---|---|
| Mobile Phase | Water, methanol (50:50 v/v) |
| Flow Rate | 0.8 mL/min |
| Injection Volume | 5 µL |
| Wavelength | 233 nm |
| Column Temp. | 25°C |
| Auto sampler Temp. | 20°C |
| Run time | 30.0 min. |
| Needle wash | Water: Methanol (20:80 v/v) |
| Seal wash | Water : Methanol (80:20 v/v) |

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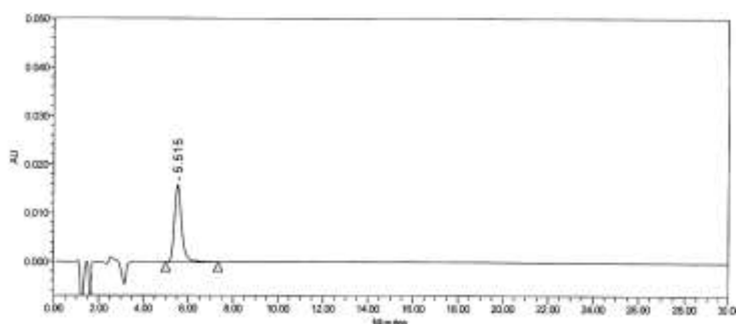


**Fig. 7.4 Typical chromatogram for Trial- 2**

**Observation:** By optimizing mobile phase ratio apremilast Standard solutioninjected. Analyte peak observed at 5.5 min in chromatogram.
**Conclusion:**Analyte volume need to optimize.

**TRIAL: 3**
**Chromatographic Condition:**

| | |
|---|---|
| Column | ACE C18 (150 x 4.6mm) 5µ |
| Mobile Phase | Water, methanol(50:50v/v) |
| Flow Rate | 1.0 mL/min |
| Injection Volume | 10 µL |
| Wavelength | 233 nm |
| Column Temp. | 25°C |
| Auto sampler Temp. | 20°C |
| Run time | 30.0 min. |
| Needle wash | Water: Methanol (20:80 v/v) |
| Seal wash | Water: Methanol (80:20 v/v) |

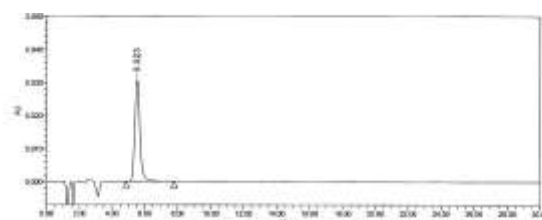

**Fig. Typical chromatogram for Trial- 3**

**Observation:** Analyte peak eluted at retention time 5.523min in chromatogram.
**Conclusion:**analyte concentration and base line needed to optimize.

**TRIAL: 4**
**Chromatographic Condition:**

| | |
|---|---|
| Column | waters symmetry C18 (150 x 4.6mm) 5µ |
| Mobile Phase | Water, methanol and Triethylamine (50:50:2v/v) |
| Flow Rate | 1.0 mL/min |
| Injection Volume | 15 µL |
| Wavelength | 233 nm |
| Column Temp. | 35 °C |
| Auto sampler Temp. | 20°C |
| Run time | 8.0 min. |
| Needle wash | Water: Methanol (20:80 v/v) |
| Seal wash | Water: Methanol (80:20 v/v) |

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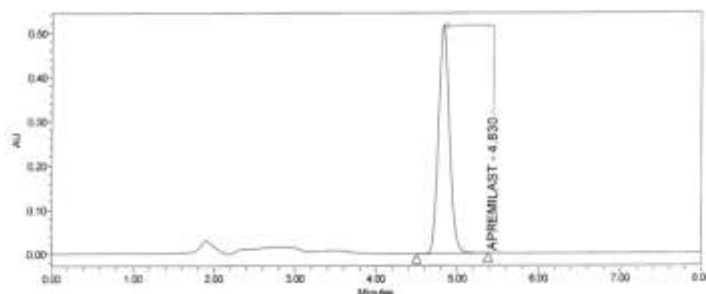


**Fig. Typical chromatogram for Trial- 4**

**Observation:** Analyte peak eluted at retention time 4.830 min in chromatogram. Plain baseline observed.
**Conclusion:**Mobile phase needed to be changedfurtherto decrease the retention time.

**TRIAL: 5**
**Chromatographic Condition:**

| Column | waters symmetry C18 (150 x 4.6mm) 5µ |
|---|---|
| Mobile Phase | Water, methanol and Triethylamine (30:70:2v/v) |
| Flow Rate | 1.2mL/min |
| Injection Volume | 15 µL |
| Wavelength | 233 nm |
| Column Temp. | 35 °C |
| Auto sampler Temp. | 20°C |
| Run time | 8.0 min. |
| Retention Time | 3.931 min. |
| Needle wash | Water: Methanol (20:80 v/v) |
| Seal wash | Water: Methanol (80:20 v/v) |

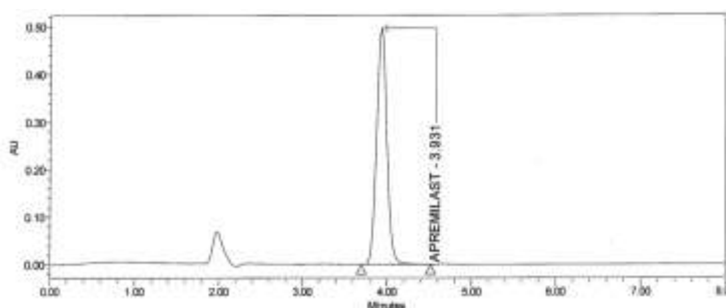


**Fig.Typical chromatogram for Trial- 5**

**Observation:**Apremilastpeakwas eluted at RT 3.931 min. Plain baseline observed.peakshowspeakpurity.Theoretical plate, symmetry was found to be satisfactory.
**Conclusion:**So conditions of trial 5 were selected as optimized chromatographic conditions

**METHOD VALIDATION**
The following parameters were considered for the analytical method validation of title ingredients.
System Suitability.

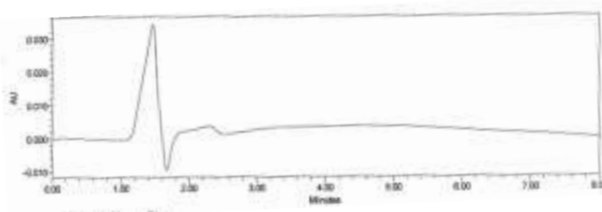
- Specificity,Linearity,Precision
- **system suitability test**

| **Tailing factor** | **1.1** |
|---|---|
| **Theoretical plates** | **12354** |
| **S. No.** | **Area** |

| 1 | 1670039 |
|---|---|
| 2 | 1650937 |
| 3 | 1662309 |
| 4 | 1669325 |
| 5 | 1673352 |
| 6 | 1656309 |
| **Mean** | 1663712 |
| **Std DEV.** | 8768.6 |
| **% RSD** | 0.5 |

**Specificity(Identification and Interference)**

| **Component** | **Retention time (min)** | **Tailing factor** | **Theoretical plates** | **Purity angle** | **Purity threshold** |
|---|---|---|---|---|---|
| Blank | - | - | - | - | - |
| Placebo solution | - | - | - | - | - |
| Standard solution | 3.350 | 1.2 | 13650 | 1.68 | 2.15 |
| Sample solution | 3.432 | 1.0 | 12037 | 1.90 | 2.67 |
| Spike solution | | | | | |
| Sample solution | 3.351 | 1.1 | 12253 | 1.84 | 2.54 |
| N-Acetyl Amine | 1.96 | 1.5 | 4563 | 1.11 | 1.94 |
| Des-Acetyl | 2.927 | 1.4 | 6306 | 0.96 | 1.56 |
| Individual Impurity Solution | | | | | |
| N-Acetyl Amine | 1.98 | 1.7 | 5036 | 0.84 | 1.20 |
| Des-Acetyl | 2.885 | 1.5 | 6096 | 0.80 | 1.49 |



**Chromatogram of Blank**

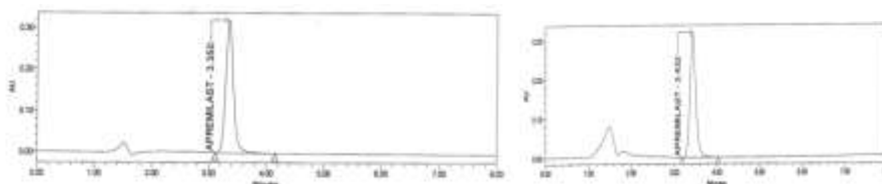


**Fig Chromatogram of StandardFig Chromatogram of Sample**

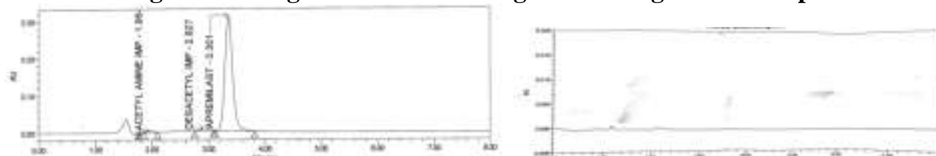


**Fig Chromatogram of Spike SampleFig Chromatogram of Placebo**

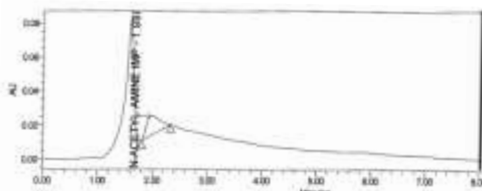


**Fig : Chromatogram of N-Acetyl AmineImpurity**

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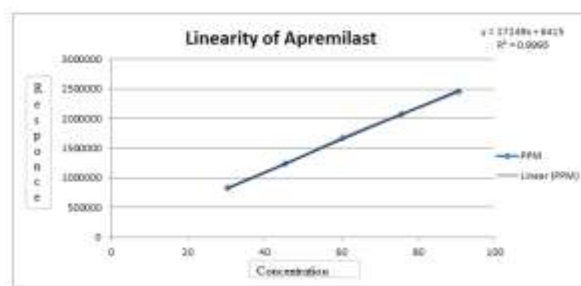
**Linearity**

| Level (%) | Concentration (ppm) | Response | | |
|---|---|---|---|---|
| | | 1 | 2 | Mean |
| 50 | 30.24 | 824036 | 819909 | 821973 |
| 75 | 45.36 | 1220938 | 1245968 | 1233453 |
| 100 | 60.48 | 1682698 | 1678630 | 1680664 |
| 125 | 75.60 | 2090638 | 2058960 | 2074799 |
| 150 | 90.72 | 2468309 | 2454367 | 2461338 |
| **Co-relation coefficient ($r^2$)** | | | | **0.999** |
| **SLOPE** | | | | **24249.18** |
| **Y-INTERCEPT** | | | | **6415** |
| **WORKING LEVEL AREA** | | | | **1680664** |
| **%LIMIT OF Y-INTERCEPT ( ± 5 OF WORKING LEVEL)** | | | | **0.38** |




**Linearity plot**

**Precision:**

**System precision:**Systemprecision parameter performed to verify system is precise to method and method parameter. The Tailing factor,Theoretical plates and reproducibility of the chromatographic system are adequate for analysis to be done.

**Table : Result of System precision**

| **Tailing factor** | **1.2** |
|---|---|
| **Theoretical plate** | **12367** |
| **Sr. No.** | **Area** |
| 1 | 1675398 |
| 2 | 1680637 |
| 3 | 1692368 |
| 4 | 1670852 |
| 5 | 1684307 |
| 6 | 1679380 |
| **Mean** | **1680490** |
| **% RSD** | **0.4** |

**Pooled Intermediate Precision data**

| **Parameter** | **Method Precision(Analyst-I)** | **Intermediate Precision(Analyst-II)** |
|---|---|---|
| **HPLC Instrument No.** | **HPLC-047** | **HPLC-028** |
| **Date of analysis** | **XXX** | **XXX** |
| **HPLC column No.** | **LC-024** | **C18-049** |
| **Sample No.** | **% Assay** | |
| 1 | 99.8 | 100.1 |

| 2 | 100 | 100.5 |
|---|---|---|
| 3 | 99.8 | 99.5 |
| 4 | 100.3 | 99.4 |
| 5 | 100.1 | 100.3 |
| 6 | 99.0 | 99.9 |
| **Mean** | **99.8** | **100** |
| **Average** | **99.9** | |
| **% RSD of all determinations** | **0.4** | |

## II. CONCLUSION

1 RP-High Performance Liquid Chromatography (HPLC) Method:

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique was employed in the present investigation for estimation of Apremilasttabletformulation. HPLC Water2469 with waters symmetry C18(150 mm X 4.6 mm), 5µm columnandUV/PDA detector with empower pro Software was used for the study. The standard and sample solution of Apremilastwere prepared in diluent.Different pure solvents of varying polarity in different proportions were tried as mobile phase for development of the chromatogram.

The mobile phase that was found to be most suitable was WaterandMethanol, Triethylaminethewavelength233 nm were selected for the evaluation of the chromatogram ofApremilastrespectively. The selection of the wavelength was based on the λmax obtained by UV scanning of standard laboratory mixture in water: methanol. This system gave good resolution and optimum retention time with appropriate tailing factor (<2).

After establishing the chromatographic conditions, standard laboratory mixture was prepared and analysed by procedure described under Materials and methods. It gave accurate, reliable results and was extended for estimation of drugs in tablet formulation.

The results from table clearly indicate that the RP-HPLC technique can be successfully applied for the estimation of above-mentioned drugs in their formulation

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