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DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC METHOD FOR ESTIMATING MONOMETHYL FUMARATE AND APREMILAST

Swati*[1], Dr. Abhinav Prasoon Mishra[2]

*[1]Advance Institute of Biotech & Paramedical Sciences, Kanpur.
[2]Professor Advance College of Pharmacy.

***Corresponding Author: Swati**

Advance Institute of Biotech & Paramedical Sciences, Kanpur.
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ABSTRACT

For the simultaneous measurement of Apremilast and Monomethyl Fumarate (MMF) in pharmaceutical dosage forms and bulk materials, A reverse-phase high-performance liquid chromatography (RP-HPLC) technique that is quick, precise, and dependable was created and verified. Using a mobile phase made up of acetonitrile and phosphate buffer (pH 3.5, adjusted with orthophosphoric acid) in a 60:40 v/v ratio, given at a flow rate of 1.0 mL/min, chromatographic separation was carried out on a C18 column (250 mm × 4.6 mm, 5 µm particle size). A UV detector tuned at 230 nm was used for detection. With correlation coefficients (R2) over 0.999, the technique demonstrated good linearity for MMF and Apremilast throughout Monomethyl Fumarate (MMF) concentrations ranged from 10 to 100 µg/mL, The concentrations of apremilast varied from 5 to 50 µg/mL. The precision, accuracy, specificity, robustness, and sensitivity of the approach were confirmed by validation in compliance with ICH Q2(R1) recommendations. The quantification limit (LOQ) and limit of detection (LOD) for MMF were found to be 2.57 µg/mL and 0.85 µg/mL, respectively, whereas the LOD and LOQ for Apremilast were 1.27 µg/mL and 0.42 µg/mL, respectively. For routine quality monitoring of Apremilast and Monomethyl Fumarate in bulk drugs and formulated products, this validated RP-HPLC technique is appropriate.

KEYWORDS: A UV detector tuned at 230 nm was used for detection.

INTRODUCTION

ANALYTICAL CHEMISTRY

The study of matter, encompassing its chemical reactivity and physical characteristics, is called chemistry, composition and structure. Chemistry can be studied in many different ways, although it is typically divided into five areas. These five fields continue to be the most basic division covering the field of chemistry, The increased interest in multidisciplinary topics like organometallic chemistry and bio-analytical chemistry is making this historical and perhaps artificial distinction less clear. Pharmaceuticals and drugs are chemicals or similar substances that can have an organic, inorganic and another origin. Whatever the source, we can measure the therapeutic agent‘s properties either via quantitatively or qualitatively.[1]

ANALYTICAL METHOD DEVELOPMENT

A set of experimental recommendations is called a technique to ensure a high quality analysis of a certain material. An analytical process consists of several steps are successions of the process from sample shipment to final product manufacture. It is possible that the process was developed domestically, Whether a technique is adapted from a reference source or acquired from a third party, it can be customised or altered based on the needs, capabilities, or intended use of the laboratory. Both the particular and generic physical characteristics of the material being studied are measured by these techniques. To ascertain whether a sample contains atomic or molecular species or their functional groups, a qualitative method is employed. A quantitative method, on the other hand, offers numerical information on the proportions of one or more of these elements.[2]

CHROMATOGRAPHY

Chromatography, which comes from the Greek word "chromos," which meaning "colour," refers to a group of scientific methods used to separate mixtures. Chromatography is a method of analysis The foundation of chromatography is the separation of molecules as a result of structural and/or compositional differences. In order to allow various components to separate based on how they interact with the mobile and stationary phases, it usually entails moving a sample through the system over a stationary phase (S.P.).

High Performance Liquid Chromatography

The most used analytical method is high-performance liquid chromatography (HPLC). As a mixture moves along a column filled with a stationary phase, it separates its constituent parts, aided by a liquid mobile phase. Prior to being forced through the column at high pressure, the analytes are first dissolved in an appropriate solvent. Separation occurs within the column according to the interactions between the analytes and the stationary and mobile phases. Altering the stationary phase's characteristics can increase the separation efficiency. characteristics or the mobile phase's composition. Based on their unique chemical and physical characteristics, HPLC is frequently used to distinguish and chemicals in organic, inorganic, and biological materials.[3]

High-resolution separations are made possible by HPLC, which forces the solvent through using high pressure generated by pumps densely packed columns that contain extremely small particles. After being injected into an injector, the sample is transported into the column and its constituent parts are separated one after the other. Individual components elute from the column. This detector's output is known as a liquid chromatogram ‘which can be obtained by linking a computer (data processor) to the system which operates all the automatic running process.[4]

Choice of Mode of Separation

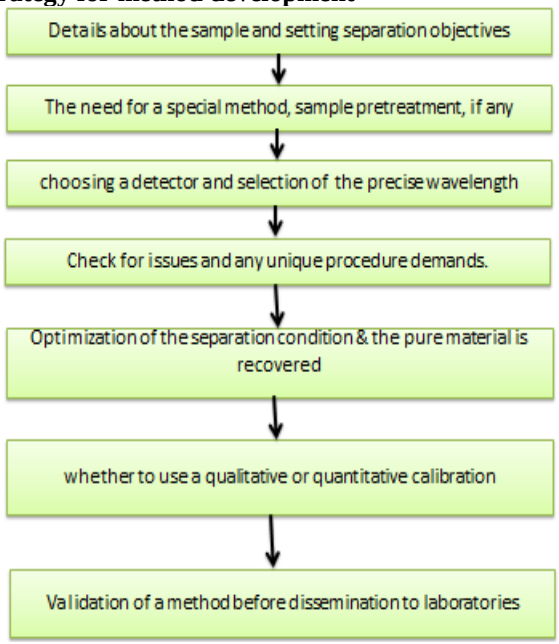
The analyst has to have a basic awareness of the physical characteristics of the sample to choose the ideal kind of column. A typical guideline for choosing a suitable chromatographic technique.

Therefore, HPLC is the method of choice for the analysis of

1. Decomposable and thermally unstable materials
2. Non-volatile substances (GC is an alternative for volatile chemicals)
3. High polarity substances or ionic samples
4. High molecular weight substances

Rapid separation, high resolving power, real-time column effluent monitoring, consistent and repeatable results, and automation of the analytical process are just a few of the benefits that HPLC offers. With careful column chemistry selection, HPLC can be used to effectively analyse a variety of compounds.[5-11]

Strategy for method development



FUNDAMENTAL PARAMETERS OF HPLC

Resolution (RS)

It measures the degree of separation between adjacent bands in a chromatogram; naturally, overlapping bands have low Rs values. It is computed using two neighbouring peaks' breadth and retention duration.

Ideally RS should be greater than 2.

$$Rs = \frac{2(t2 - t1)}{w1 + w2}$$

Capacity Factor (*k*)

It is unique to each chemical and indicates where a sample peak is located in the chromatogram. This metric shows how much a material is retained during separation. The retention factor (k) is influenced by temperature, stationary phase composition, mobile phase composition, and column packing quality.

$$k_1 = \frac{t_{r1} - t_o}{t_o} \quad \text{and} \quad k_2 = \frac{t_{r2} - t_o}{t_o}$$

Selectivity Factor

A measure is the variation in retention time between two distinct peaks of the chromatographic system's selectivity. For species A and B, a column's selectivity factor α is defined as.

$$\alpha = \frac{Kb}{Ka}$$

Number of Theoretical Plates (N)

The plate number (N) and HETP (Height equal to theoretical plate) can be used to express the column efficiency.

$$N = \frac{16}{\left[\frac{t}{w}\right]2}$$

Height Equivalent to Theoretical Plates (HETP)

HETP (H) = L/N

The retention factor (k) is influenced by temperature, the stationary and mobile phase mixture, and the column packing quality.[12-20]

$$Tf = \frac{W0.05}{2f}$$

VALIDATION OF ANALYTICAL METHOD

Validation

Documented proof that demonstrates a system, process, or facility reliably generates a product is known as validation that meets specified standards and quality attributes. It is a methodical process that entails determining, quantifying, assessing, recording, and reassessing every crucial step prior to verifying the accuracy and dependability of a technique. In addition to being an essential Current Good Manufacturing Practices (cGMP) need method validation as a component of the quality control process. There is no trustworthy method to ascertain if the procedure has produced the desired result in the absence of a validated measuring system.[21-28]

Parameters for Method Validation

The following is a summary of the method validation parameters, commonly referred to as "Analytical Performance Parameters," in compliance with the International Conference on Harmonisation (ICH) and the United States Pharmacopoeia (USP) criteria.

i. ACCURACY
ii. PRECISION
iii. REPEATIBILITY
iv. SPECIFICITY
v. LIMIT OF DETECTION (LOD)
vi. LIMIT OF QUANTITATION (LOQ)
vii. LINEARITY
viii. ROBUSTNESS

Parameters to be validated in HPLC[29-33]

S. No.	Parameters	Acceptance Criteria
1	Accuracy	**% Recovery 98-102%**
2	Precision Repeatability Intermediate Precision	**RSD ≤2%**
3	Specificity	**No Interefernce at the RT of Analytes**
4	Robustness	**RSD ≤2%**
5	Linearity	**R2 ≥ 0.999**
6	Limit of Detection	**S/N = 3/1**
7	Limit of Quantification	**S/N = 10/1**

PLAN OF WORK

1. Literature Survey
2. Procurement of pure Apremilast and Monomethyl fumarate drug samples.
3. Physical Characterization of the drug substances which includes colour, odour, solubility.
4. Analysis of drug samples with various analytical techniques (1H- NMR, Mass spectrometry, etc.) to assess their identity and purity.
5. Trials of instrumental methods on pure drug samples which includes following steps:
6. Primary screening of drugs and laboratory mixture by eluting them on various mobile phase buffers of differing pH range.
7. Selection of stationary phase.
8. Optimization of mobile phase composition by assessing and selecting solvents, additives, etc.
9. Optimization of other chromatographic conditions like flow rate, column temperature, detection wavelength, etc.
10. System suitability and solution stability studies.
11. Analysis of standard laboratory mixture to ascertain feasibility of proposed method.
12. Recovery Studies on both APIs.
13. Validation of developed method according to ICH Q2 (R1) guidelines.

MATERIALS REQUIRED

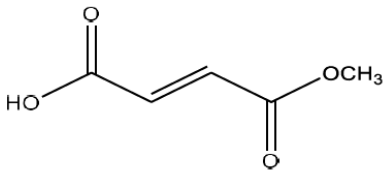
Table: List of Equipments.

S.No	Equipment(s) Used	Chemicals name	Drug(s) Used
1.	HPLC System	Acetonitrile	Monomethyl Fumarate
2.	NMR System	Methanol	Apremilast
3.	Digital pH Meter	Dimethyl sulfoxide	
4.	Digital Balance	Ammonium Acetate	
5.	Vacuum Filtration System	Ortho Phosphoric acid	
6.	Filter Unit(s)	Trifluoroacetic acid	
7.	Refrigerator	Ammonium Formate	
8.	Sonicator	Ethyl Acetate	
9.	Glasswares		
10.	Syringes		

DRUG PROFILE

MONOMETHYL FUMARATE

Structure



Pharmacokinetic data of Monomethyl fumarate

Bioavailability	60%
Metabolism	Liver (extensive) TCA cycle
Elimination half-life	1 hour
Excretion	Kidney (<16%)

APREMILAST

Structure

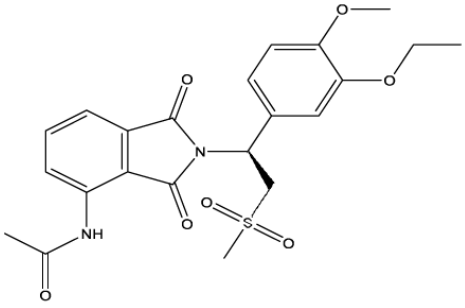


Figure.: Structure of Apremilast.

Pharmacokinetic data of apremilast

Bioavailability	73%
Protein binding	~68%
Metabolism	Liver (CYP3A4)
Onset of action	Within an hour
Elimination half-life	6 – 9 hours
Duration of action	4 – 6 hours
Excretion	Kidney

RESULTS AND DISCUSSION

Developing a suitable technique for the bulk measurement of monomethyl fumarate (MMF) and apremilast (APM) simultaneously drug form was the main goal of the current investigation. To guarantee precision, dependability, and repeatability, sophisticated and exact HPLC techniques were created, refined, and verified in compliance with ICH criteria.

ANALYSIS OF DRUGS

Authentication of Procured Drug

Determining the drug's chemical composition requires the identification of its constituent ingredients and physical characteristics before analytical method development. Procured drugs were identified by their physical properties, melting point, solubility, UV, NMR analysis.

Physical Appearance

Shows physical appearance of MMF and APM

S.No.	Drug	Reference	Observation
1	**Monomethyl Fumarate**	White to off-white powder	White powder
2	**Apremilast**	Off-white to yellowish powder	White-yellowish powder

Melting Point

Shows Melting Point of MMF and APM

S.No.	Drug	Reference	Observation
1	**Monomethyl Fumarate**	144-145˚C	142˚C
2	**Apremilast**	156.5-158.5˚C	155˚C

Solubility

Solubility of monomethyl fumarate and Apremilast was examined in several solvents. The table below shows that MMF and APM were almost soluble in each of the chosen solvents. According to this study, MMF and APM are insoluble in regular saline and easily soluble in DMSO and methanol.

Solubility data of MMF & APM with different solvents

Solvents	Solubility (W/V)	Solubility
DMSO	1 mg/mL	Highly Soluble
Methanol	1 mg/mL	Highly Soluble (MMF) Moderately Soluble (APM)
Normal Saline	1 mg/mL	Not Soluble
Acetonitrile	1 mg/mL	Freely Soluble
Water	1 mg/mL	Moderately Soluble

UV-Visible Spectroscopy of procured drug

UV-Visible spectral studies were carried out for MMF and APM in DMSO the obtained spectra are presented in **Figure** and **Table** with their absorption maxima (λmax)

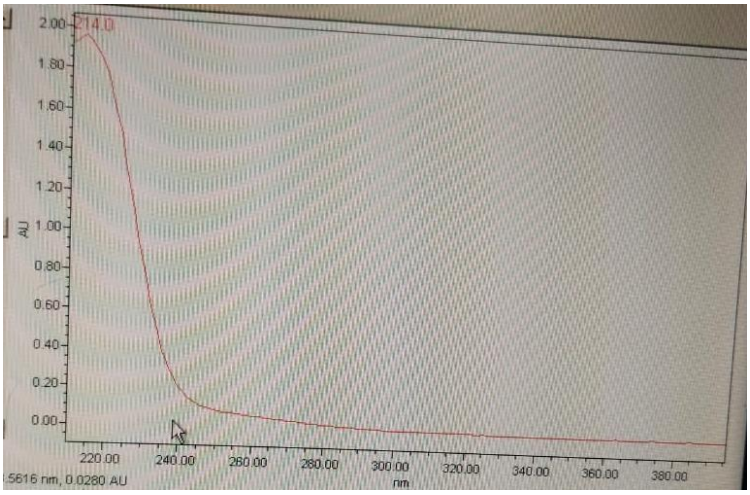


Figure: UV-Visible spectra of MMF

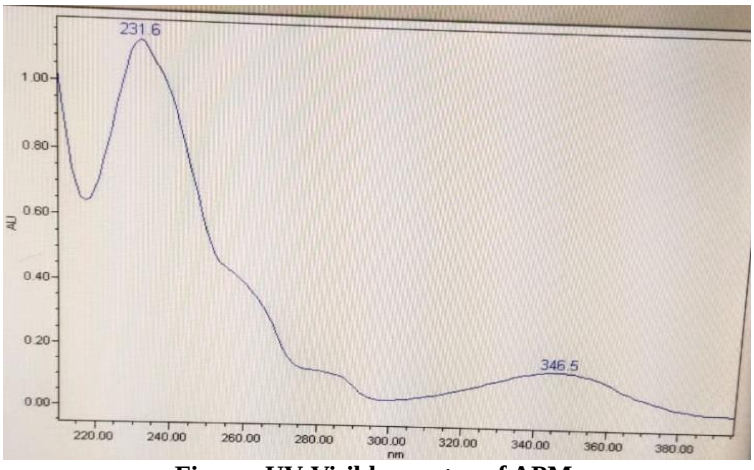


Figure: UV-Visible spectra of APM.

Table shows the absorption maxima (λmax) of MMF and APM.

S. No	Drug	Observed λmax (nm)
1	**MMF**	214
2	**APM**	231

NMR Spectroscopy of Procured Drugs

NMR spectrums of standard MMF and APM shows 06 and 24 number of protons respectively which represent the purity and identity of drug.

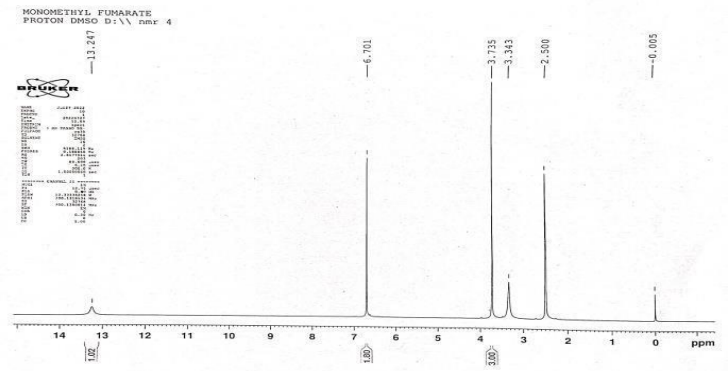


Fig: NMR spectrum of Monomethyl Fumarate API.

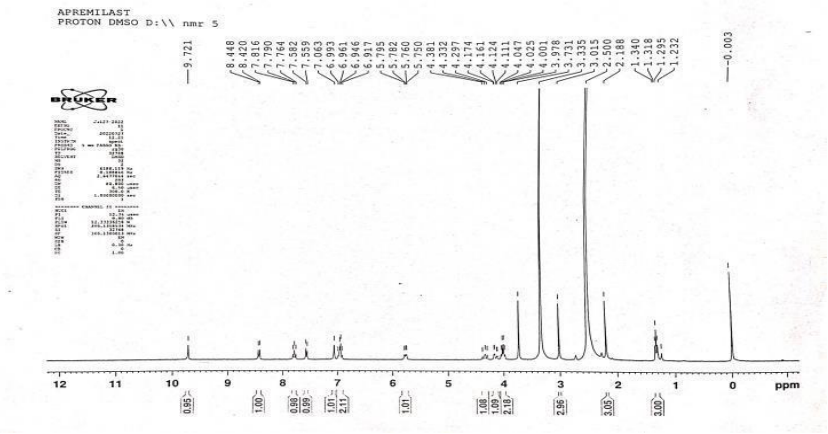


Fig: NMR spectrum of Apremilast API.

RP-HPLC APPROACH FOR METHOD DEVELOPMENT

Method Optimization Strategy

Optimization of Chromatographic Conditions

Table: Result of RP-HPLC parameters for method development first trial (T1)

Mobile phase	5mM Ammonium Formate: Methanol
Ratio	10:90
Column	Symmetry C18 (4.6 x 150mm, 3.5 µm)
Temperature	Room temperature
Wavelength (λ max)	220 nm
Detector	PDA
Injection volume	20µL
Diluents	Acetonitrile
Flow rate	1.5 mL/min.
Run time	4.00 min

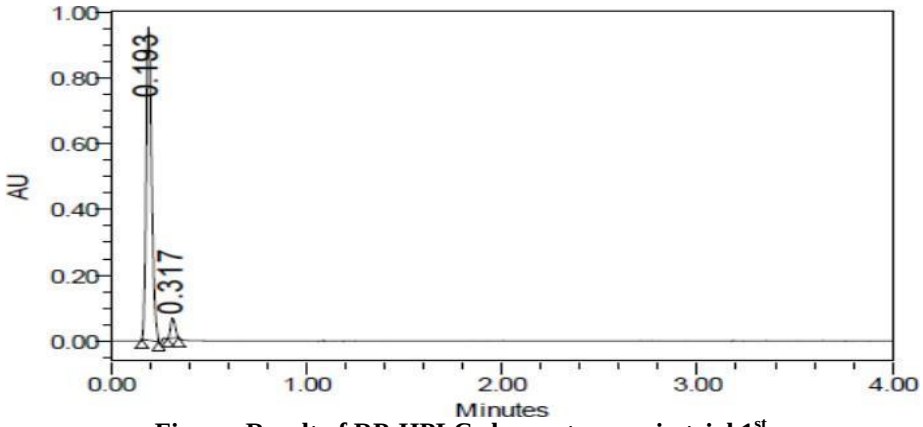


Figure: Result of RP-HPLC chromatogram in trial 1st.

Inference: The first RP-HPLC trial was unsuccessful due to the chromatographic conditions mentioned above, MMF was eluted at 0.193 and the peak of APM was eluted at 0.317 and look like merge with each other.

Table: Result of RP-HPLC parameters for method development second trial (T2)

Mobile phase	Acetonitrile: 0.1 % Orthophosphoric acid
Ratio	20:80
pH	3.0
Column	C18 column (Zorbax SB C18 150×4.6mm, 5µm)
Temperature	Room temperature
Wavelength (λ max)	230nm
Detector	PDA
Injection volume	10µL
Diluent	Acetonitrile
Flow rate	1 mL/min.

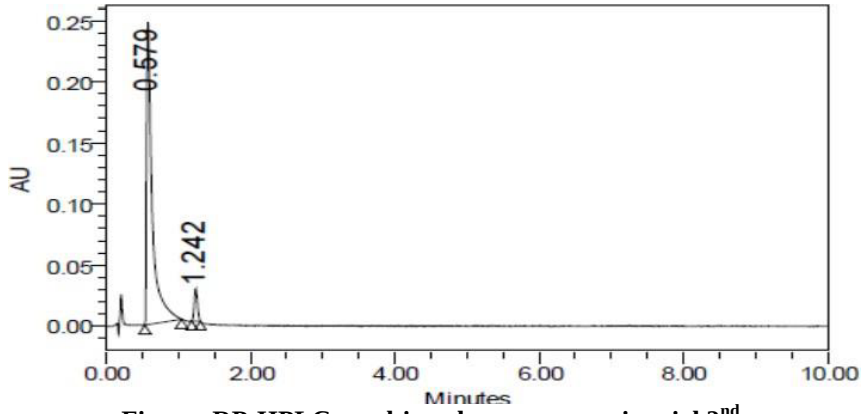


Figure: RP-HPLC resulting chromatogram in trial 2nd.

Inference: The second RP-HPLC trial was unsuccessful due to the chromatographic conditions mentioned above, MMF was eluted at 0.579 and the peak of APM was eluted at 1.242 and look like merge with each other but assymetry peak.

Table: RP-HPLC parameter for method development third trial (T3)

Mobile phase	10 mM Ammonium Acetate in water: Acetonitrile
Ratio	50:50
pH	6.5
Column	Acquity BEH RP18 (2.1 x 50mm, 1.7 µm)
Temperature	Room temperature
Wavelength (λ max)	230nm
Detector	PDA
Injection volume	10µL
Diluent	Acetonitrile
Flow rate	1 mL/min.
Run Time	10 min.

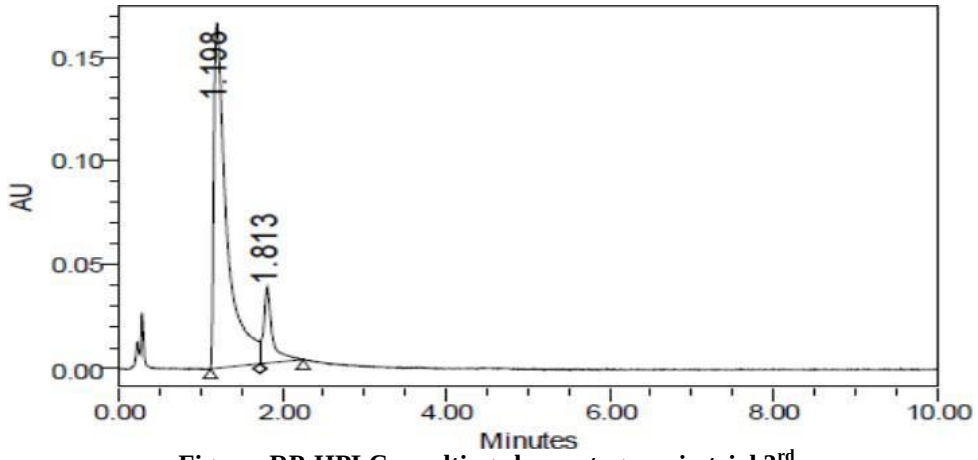


Figure: RP-HPLC resulting chromatogram in trial 3rd.

Inference: The third RP-HPLC trial was unsuccessful due to the chromatographic conditions mentioned above, MMF was eluted at 1.198 and the peak of APM was eluted at 1.813 and look like merge with each other and poor resolution.

Table: RP-HPLC parameter for method development fourth trial (T4).

Mobile phase	0.1 % trifluoroacetic acid: Acetonitrile
Mode	Gradient
pH	2.3
Column	C18 column (Zorbax SB C18 150×4.6mm, 5µm)
Temperature	Room temperature
Wavelength (λ max)	230nm
Detector	PDA
Injection volume	10µL
Diluent	Acetonitrile
Flow rate	1 mL/min.
Run Time	15 min.

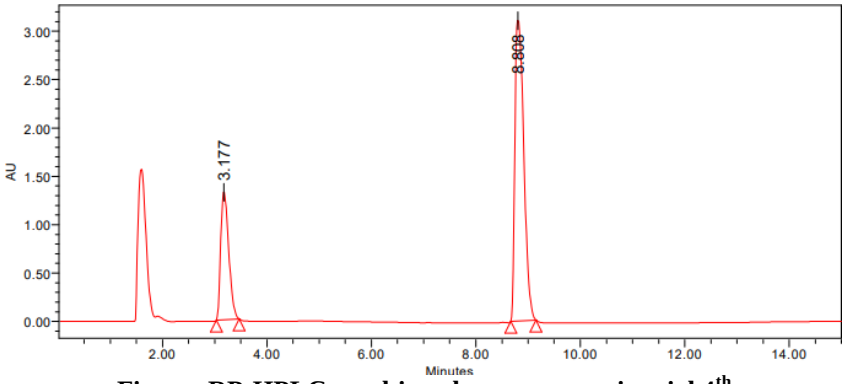


Figure: RP-HPLC resulting chromatogram in trial 4th

Inference: RP-HPLC trial 4th passed because the all above chromatographic conditions, MMF was eluted at 3.177 and the peak of APM was eluted at 8.808 and good resolution. The peaks obtained in the present study were symmetric, good and no interferencewas observed between the peaks.

*From various mobile phases tried, the mobile phase containing **0.1% trifluoroacetic acid in water (pH 2.3): ACN** showed peak purity pass, good resolution and repeatability with no elution of any impurity. This concluded that acid form buffer should be optimized to be used as a mobile phase for analytes.*

Optimization of Column

Columns of different chemistries were tried for eluting analytes so as to retain them with optimum retention time and resolution. Trials were conducted with 0.1 % trifluoroacetic acid in water buffer, with each column's mobile phase being ACN in gradient mode.

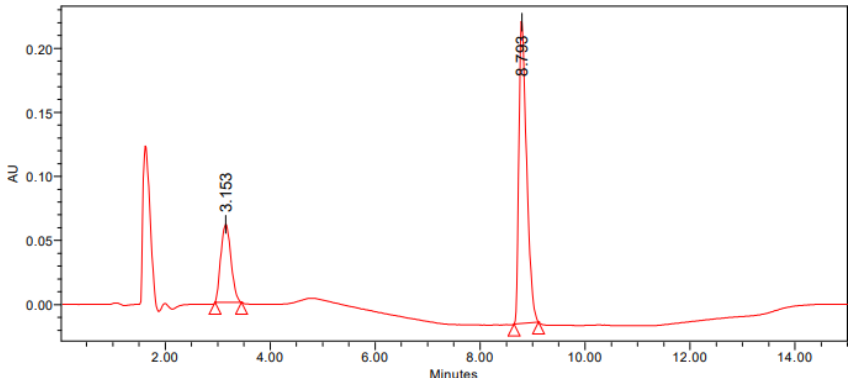


Figure: Chromatogram of trial on *Agilent HPLC.*

Organic Solvent of Mobile Phase

In this experiment, varying compositions of ACN and MeOH as organic part of mobile phase were taken and trials were conducted.

***ACN as an organic solvent** proved better in response to MeOH with the selected buffer.*

Mobile Phase Composition

It is well-known, even minor alterations in ion-pair concentration can create huge differences in the chromatographic system's capacity to separate substances.

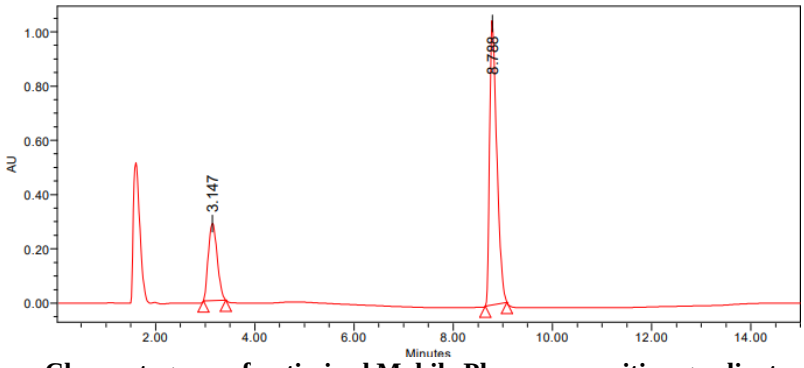


Figure: Chromatogram of optimized Mobile Phase composition gradient mode.

Table: Showing the gradient program for optimized mobile phase composition

Time(min.)	Flow(ml/min)	% A	%B
0.00	1.00	80.0	20.0
2.00	1.00	80.0	20.0
5.00	1.00	50.0	50.0
9.00	1.00	50.0	50.0
11.00	1.00	80.0	20.0
15.00	1.00	80.0	20.0

Mobile Phase: 0.1% trifluoroacetic acid gradient program in water, ***(pH 2.3): ACN*** *showed the best result for retention and separation factors of sample analytes in gradient mode composition as shown in* ***Table.***

Flow rate optimization

Chromatographic separation efficiency is significantly impacted by changes in the flow velocity of the mobile phase.

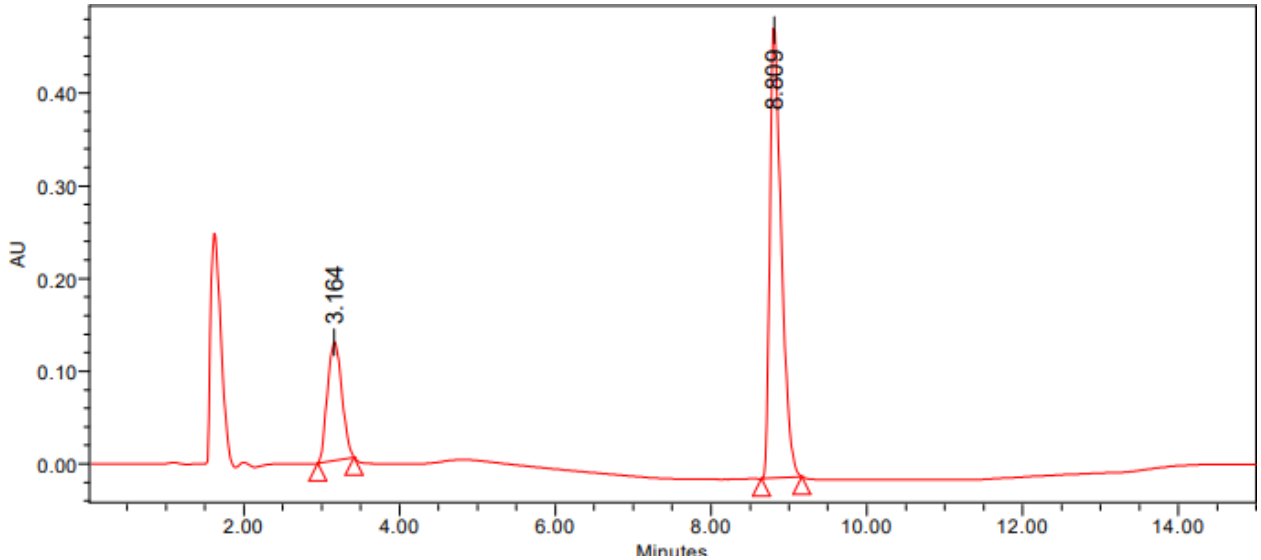


Figure: Chromatogram of optimized flow rate (1.00 mL/min).

The best retention with high resolution of both peaks was obtained under the trial involving ***1.00 mL/min****. Hence, further studies were performed at this flow rate.*

Optimization of detection wavelength

The area-to-height ratio of sample peaks may be improved by optimising the scanning wavelength, which improves detection sensitivity and peak quality even if it has little influence on selectivity.

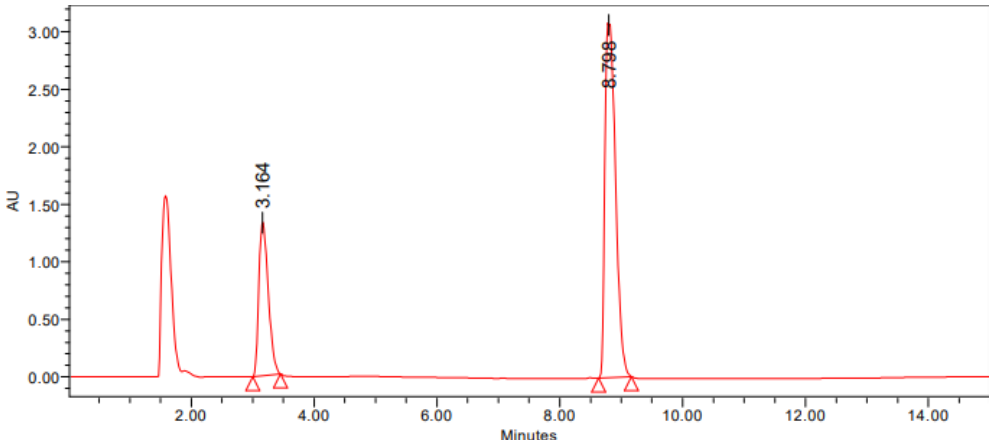


Figure: Chromatogram of optimized wavelength λmax 230 nm.

Table: The retention time of Monomethyl Fumarate and Apremilast.

S.No.	Retention time of drugs (min)	
	MMF	**APM**
1	3.167	8.814
2	3.165	8.812
3	3.153	8.800
4	3.158	8.795
5	3.162	8.805
6	3.164	8.809
Average	3.160	8.805
±SD	0.006	0.007
%RSD	**0.194**	**0.089**

The chromatograms of the blank, MMF and APM were recorded individually at the fixed chromatographic condition.

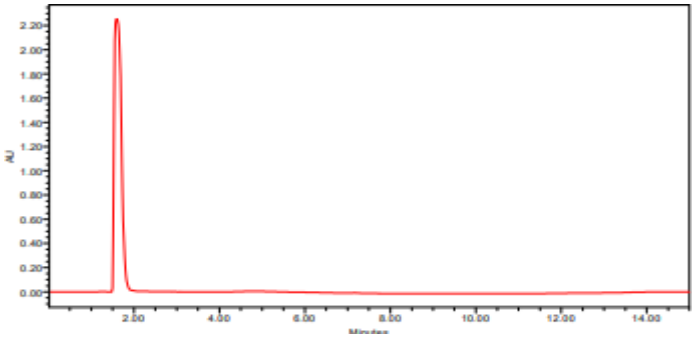


Figure: RP-HPLC Chromatogram of Blank/.

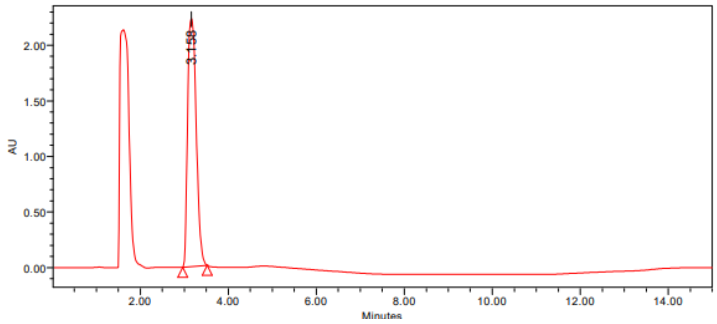


Figure: RP-HPLC Chromatogram of Monomethyl Fumarate.

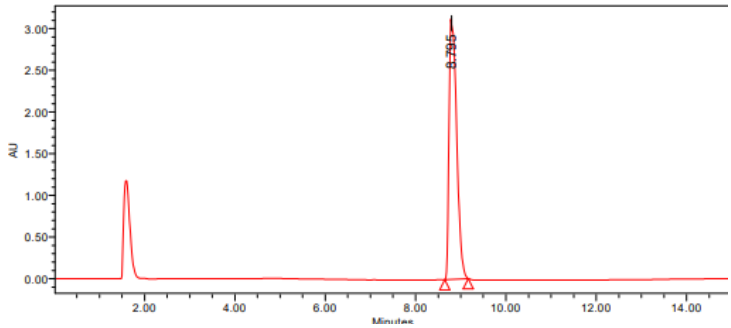


Figure: RP-HPLC Chromatogram of Apremilast.

Preparation of MMF and APM standard solutions

Preparation of MMF stock solutions

The prepared MMF stock solution has a concentration of 1.0 mg/mL (1000µg/mL).

Preparation of APM stock solution

The prepared APM stock solution has a concentration of 1.0 mg/mL (1000µg/mL).

Preparation of resolution solution

The Resolution solution containing 500 µg/mL of MMF and APM was prepared by sequential dilution of stock solution in acetonitrile (ACN).

Table: MMF and APM system suitability studies in optimised RP-HPLC in comparison to USP pharmacopoeia limitations.

S.No.	Parameters	USP Limits	MMF	APM
1	Concentration	---	500µg/mL	500µg/mL
2	Retention time	---	3.155	8.816
3	Peak area	---	6194147	18955346
4	%RSD of peak area	≤ 2.0	0.167	0.036
5	Peak Asymmetry	≤ 2.0	1.105	0.920
6	Number of Theoretical plate	≥ 2000	2607	7234
7	Final retention time	---	3.158	8.795

VALIDATION OF DEVELOPED RP-HPLC METHOD

SPECIFICITY

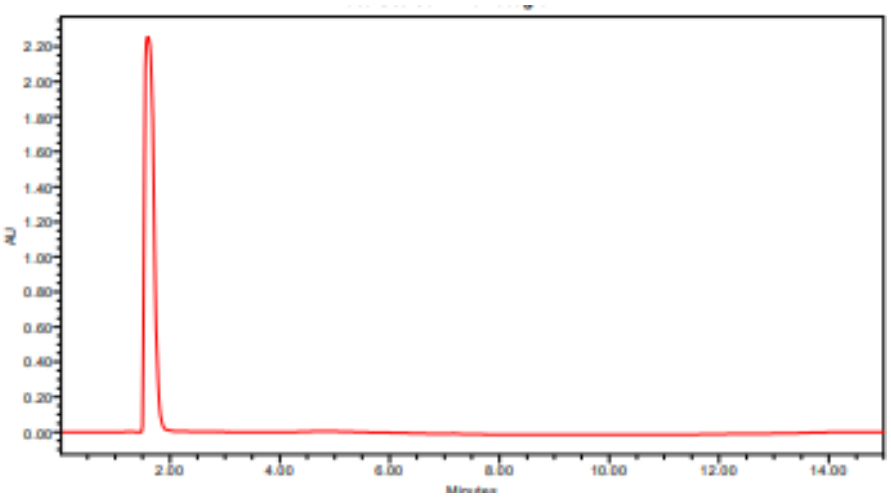


Figure: Specificity Chromatogram of Blank.

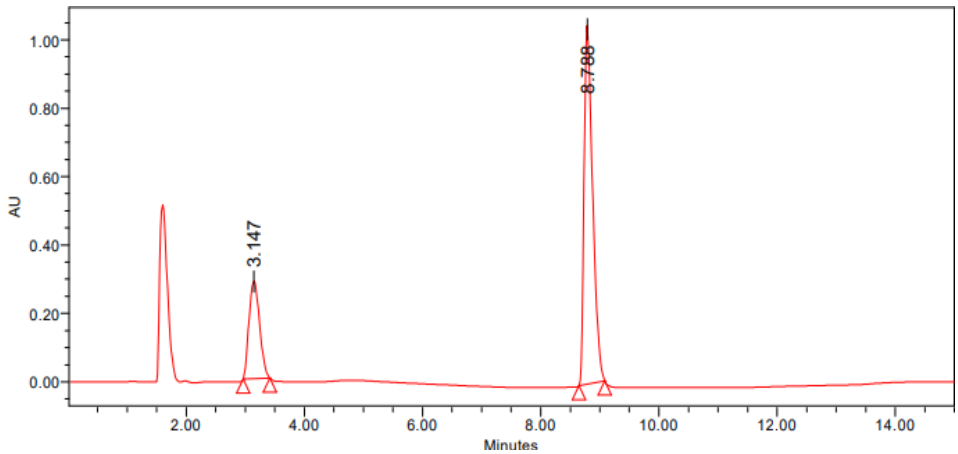


Figure: Specificity Chromatogram of MMF and APM.

LINEARITY

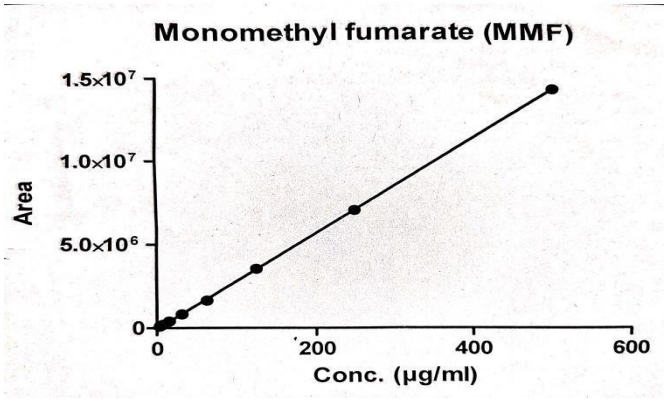


Figure: Linearity curve of MMF.

Table: Linearity results of Monomethyl fumarate.

S.No.	Concentration (ppm)	Area
1	7.812	94925
2	15.625	202233
3	31.25	394652
4	62.5	814946
5	125	1644682
6	250	3552997
7	500	7082308

The linear calibration curve for MMF, shown by R2 = 0.999, falls within the specified range to demonstrate the linearity of the technique validation.

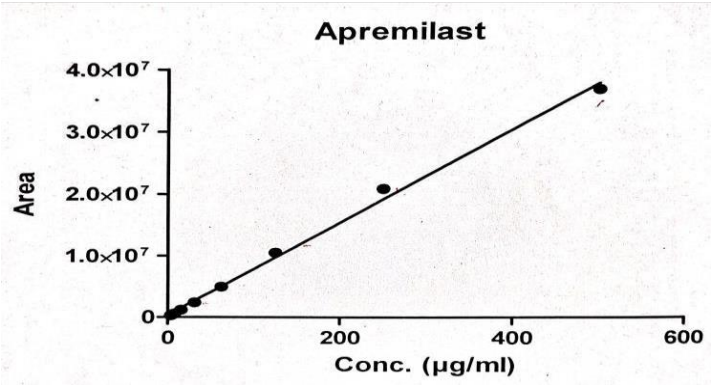


Figure: Linearity curve of APM.

Table: Linearity results of Apremilast.

S.No.	Concentration (ppm)	Area
1	7.812	283873
2	15.625	585558
3	31.25	1178055
4	62.5	2395880
5	125	4937447
6	250	10457391
7	500	20730375

The linear calibration curve for MMF, shown by R2 = 0.996, falls within the specified range to demonstrate the linearity of the technique validation. MMF and APM Overlay Chromatogram Spectra.

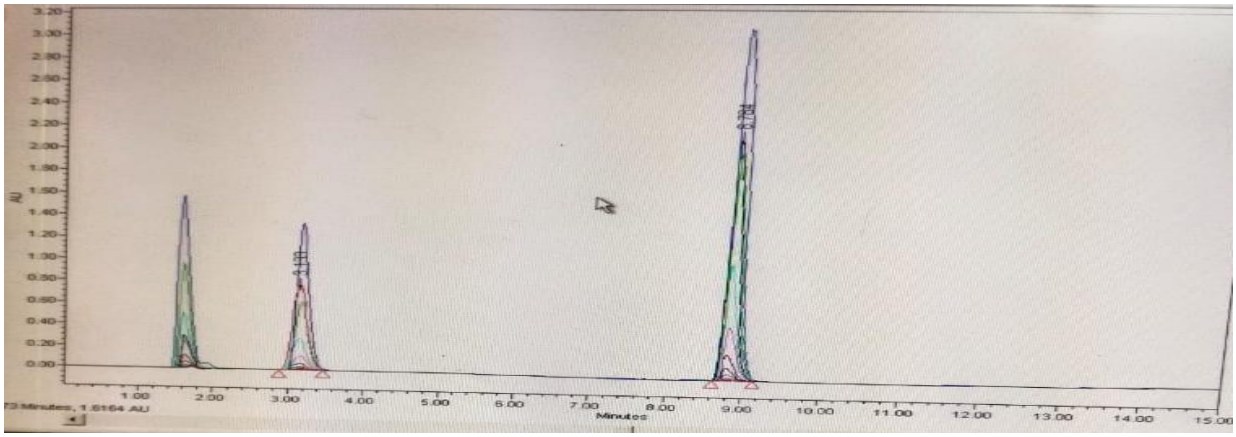


Figure: Overlay chromatogram of MMF (7.812-500 µg/mL) and APM (7.812-500 µg/mL).

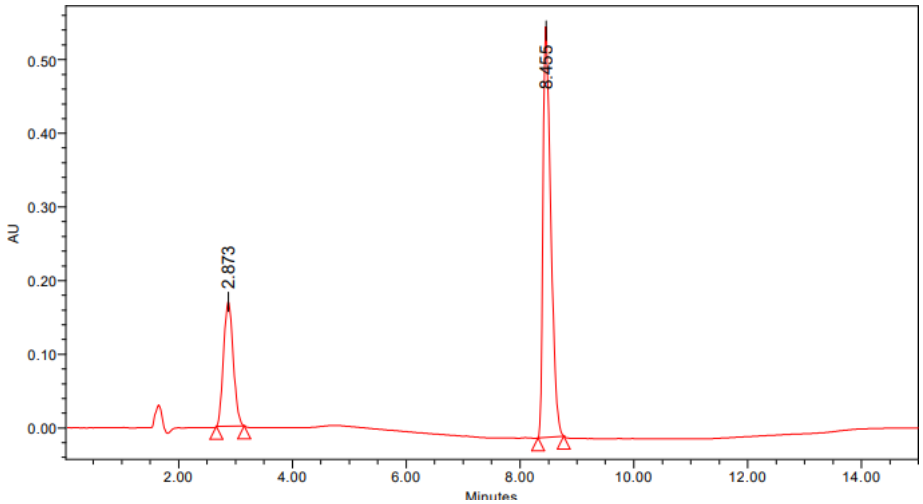


Figure: (a) Accuracy result of RP-HPLC chromatogram of 50% (250ppm) concentration.

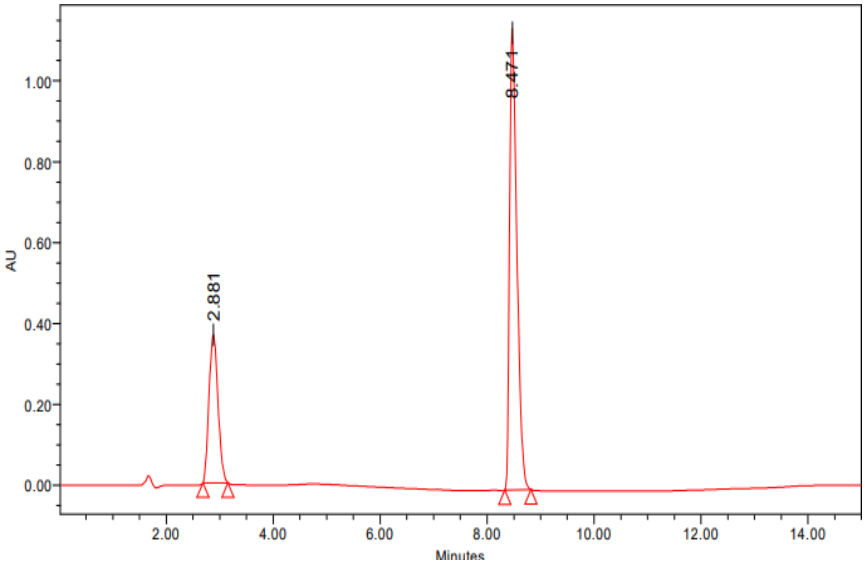


Figure: (b) Accuracy result of RP-HPLC chromatogram of 100% (500ppm) concentration.

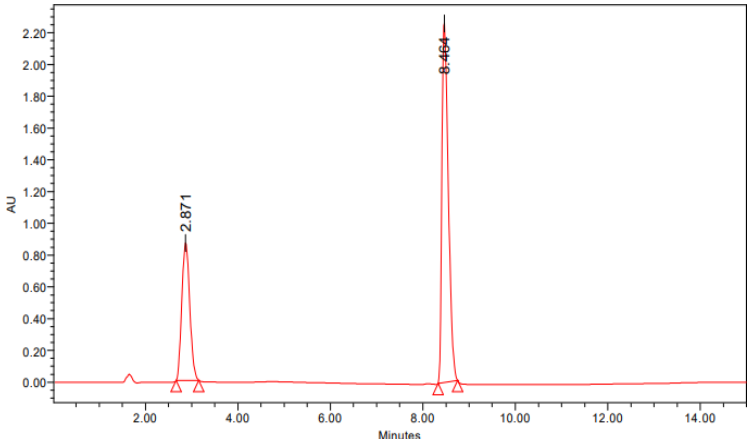


Figure: (c) Accuracy result of RP-HPLC chromatogram of 200% (1000ppm) concentration.

Table: Recovery studies of APM & MMF.

No. of Injections	Area (AU)					
	Monomethyl fumarate			Apremilast		
	50%	100%	200%	50%	100%	200%
1	2728786	5079978	9910015	5290570	10245143	19900190
2	2738851	5166728	9869270	5290121	10381062	19911998
3	2768533	5024955	9783785	5270808	10464619	20199878
Mean	2745390	5090554	9854357	5283833	10363608	20004022
%RSD	**0.754**	**1.404**	**0.653**	**0.213**	**1.069**	**0.848**
%Recovery	**99.73**	**99.79**	**102.36**	**98.46**	**101.27**	**100.39**

As stated about % R.S.D. obtained from obtained data and the mean % recovery for both drugs was found to be under the specified limits (98 – 102 %) and therefore It was discovered that the devised HPLC technique was accurate for determining the medicines.

PRECISION

Table: System Precision study of Standard Solution.

Injection	Area (mAU)	
	Monomethyl Fumarate	Apremilast
1	1597800	1705445
2	1594077	1705205
3	1538374	1669918
4	1572061	1679849
5	1528460	1675131
6	1563505	1681227
Mean	1565712.83	1686129.16
S. D.	15389.75	28335.38
%RSD	**1.81**	**0.91**

Table: Method precision & Intermediate Precision.

Precision	Area	
	Monomethyl fumarate	Apremilast
Method Precision	801065	2484669
	795938	2457251
	796067	2456565
	789161	2451316
	796844	2479037
	805582	2417552
Mean	2457731.67	797442.833
SD	23818.882	5522.15412
% RSD	**0.97**	**0.69**
Intermediate Precision	859201	2627613

	847077	2642948
	853722	2635620
	844269	2655790
	837458	2675943
	837458	2723479
Mean	846531	2660232
SD	8744.05	35313.55
% RSD	**1.03**	**1.33**

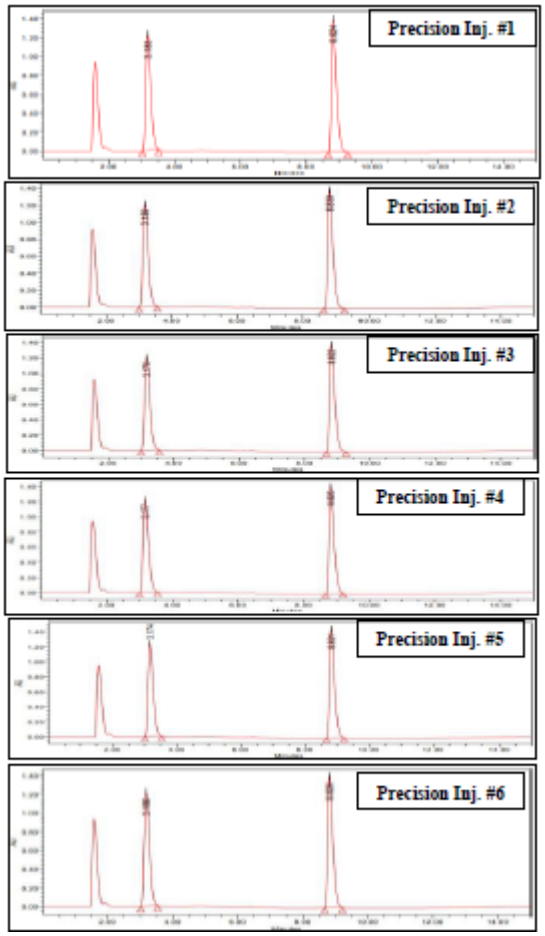


Figure: Chromatograms of precision injections (1 – 6) of standard solution.

It was concluded that ***% R.S.D.*** *for system precision study of 6 replicate injections of standard solution was estimated to be* ***1.81*** *and* ***0.91*** *for* ***MMF*** *and* ***APM*** *respectively and hence,* ***less than 2.0 %****. The precision of the suggested HPLC approach for the simultaneous determination of MMF and APM was confirmed by the observed values, which satisfied the precision study's acceptance requirements.*

Table: Robustness study of standard solution

Condition	Variation	Monomethyl Fumarate		Apremilast	
		Mean area	%RSD	Mean area	%RSD
Column	Column (Agilent, C18)	20064383	**0.341**	36106412	**0.012**
	Zorbax SB C18	19339761	**0.168**	39206668	**0.080**
Temperature	High temp (40°C)	19736506	**0.486**	38963445	**1.884**
	Actual temp (25°C)	19489761	**0.529**	37451412	**0.975**
pH	Low pH (2.0)	19557399	**0.914**	36866329	**1.301**
	Actual pH (2.3)	19339761	**0.168**	36106412	**0.080**
	High pH (3.0)	19807399	**1.255**	38866594	**1.232**

Table: Summary for results of LOD and LOQ of RP-HPLC method.

Validation Parameter	MMF	
Absorption maxima, λmax (nm)	230	
Linearity range (µg/mL)	7.81-500	7.81-500
Coefficient of determination (R 2)	0.999	0.996
Regression equation (y)	y= 74692x + 360816	y= 28581x - 55089
Limit of detection (µg/mL)	2.07	1.65
Limit of quantification (µg/mL)	6.28	5.01

CONCLUSION

A Photodiode Array (PDA) detector tuned to 230 nm was used to find the eluted chemicals. The column oven temperature was kept at 25 °C, and the flow rate was kept at 1.0 mL/min. Excellent linearity was shown by the approach, as evidenced by correlation coefficient (r2) values of 0.999 or more linearity demonstrated outstanding correlation, suggesting a strong link between analyte concentrations and the peak regions that correspond to them within the measured range. Recovery experiments revealed that accuracy fell between 98% and 102%, which is an acceptable range and its ability to separate the drug peak from the diluents and no interference was observed during analysis.

Sturdiness against little temperature changes in column ovens, composition of mobile phase buffer, etc. was also determined and for Precision analysis, six injetions of standard solutions were injected and calculated the % RSD which are found to be 1.81 for MMF and 0.91 for APM that indicates the proposed The RP-HPLC technique proved accurate and repeatable. It was determined what the Limit of Detection was using For MMF and APM, the standard deviation and standard error.

In conclusion, it has been demonstrated that the established approach for the simultaneous estimation of Apremilast and Monomethyl Fumarate is appropriate for its intended use. For both pharmaceutical substances, the approach showed accuracy, linearity, robustness, specificity, and precision. For routine analysis and quality control of mixed dose formulations including Apremilast and Monomethyl Fumarate, it may thus be used with confidence.

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