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(RESEARCH ARTICLE)

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Stability indicating RP-HPLC method development and validation for sirolimus

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Abstract

A simple, precise, and accurate stability-indicating RP-HPLC method was developed and validated for Sirolimus determination. Chromatographic separation was performed on a Chromosil C18 column using methanol:acetonitrile (80:20 v/v) as the mobile phase at a flow rate of 1.0 mL/min, with UV detection at 272 nm. The method was validated following ICH guidelines. Forced degradation studies confirmed the method's stability-indicating capability. This method is suitable for routine quality control and stability studies of Sirolimus.

Keywords: Sirolimus, RP-HPLC; Validation; Stability-indicating method; Forced degradation; ICH

1. Introduction

Sirolimus is a macrolide immunosuppressant commonly used in organ transplantation. Reliable analytical methods are essential for its quality control and stability assessment. This study aimed to develop and validate a stability-indicating RP-HPLC method for the quantitative estimation of Sirolimus.

1.1. Drug Profile

Chemical Name is 3S, 6R, 7E,9R,10R,12R,14S,15E,17E,19E, 21S, 23S, 26R, 27R, 34aS) 9,10,12,13,14, 21, 22, 23, 24, 25, 26, 27, 32, 33, 34, 34a-hexadecahydro-9,27-dihydroxy-3-[(1R)-2-[(1S,3R,4R)- 4-hydroxy-3- methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3H-pyrido[2,1-c] [1,4] oxaazacyclohentriacontine 1,5,11,28,29 (4H,6H,31H)-pentone. It is a macrocyclic lactone and is widely used as an immunosuppressive drug having molecular formula $C_{51}H_{79}NO_{13}$

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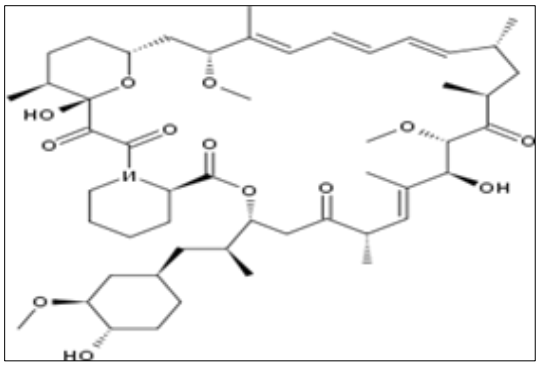

Figure 1 Sirolimus

2. Material and methods

2.1. Chemical and Reagents-

Sirolimus, acetonitrile, methanol and Distilled water

2.2. Instrumentation

Shimadzu LC-20AT HPLC system, UV–Visible detector, Chromosil C18 column (250 mm × 4.6 mm, 5 µm), ultrasonic bath sonicator (Loba), analytical balance (Denver make).

2.3. Preparation of Standard Solution

A stock solution of Sirolimus (100 µg/mL) was prepared by accurately weighing and dissolving the drug in the mobile phase. Working standard solutions were then prepared by appropriate dilution of the stock solution with the mobile phase.

2.4. Preparation of Sample Solution

Twenty RAPACAN tablets containing Sirolimus (1 mg) were finely powdered. A quantity of powder equivalent to the labeled claim was transferred to a volumetric flask and dissolved in the mobile phase to achieve an approximate concentration of 30 µg/mL The solution was sonicated, filtered through a 0.45 µm nylon membrane filter, and used for HPLC analysis.

2.5. Method Development and Optimization

The RP-HPLC method was optimized by evaluating different chromatographic parameters, including detection wavelength, stationary phase, mobile phase composition, and flow rate. The UV spectrum of Sirolimus exhibited maximum absorbance at 272 nm, which was selected as the detection wavelength.

Various reversed-phase C18 columns were investigated, and the Chromosil C18 column (250 × 4.6 mm, 5 µm) was selected because it provided sharp, symmetrical peaks with acceptable retention time and minimal peak tailing.

Several mobile phase compositions containing methanol, acetonitrile, and aqueous systems were evaluated under isocratic conditions. A mixture of methanol and acetonitrile (80:20, v/v) produced optimum peak symmetry, stable baseline, satisfactory resolution, and reproducible retention time, and was therefore selected as the mobile phase.

The flow rate was optimized in the range of 0.5–1.5 mL/min. A flow rate of 1.0 mL/min provided efficient separation, good peak symmetry, and appropriate retention time and was selected for further studies.

2.6. Optimized Chromatographic Conditions

The following were the ideal chromatographic conditions: Chromosil C18 column (250 × 4.6 mm, 5 µm); methanol (80:20, v/v) mobile phase; isocratic elution mode; pH 5.5; flow rate 1.0 mL/min; injection volume 20 µL; detection wavelength 272 nm; ambient column temperature; and run time of 6 minutes. Sirolimus was eluted under these circumstances at a retention duration of 2.740 minutes.

3. HPLC Method Validation

In terms of system applicability, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ), the RP-HPLC technique was validated in accordance with ICH criteria.

3.1. System Suitability

System suitability testing was performed prior to analysis by injecting the standard solution multiple times. Chromatographic parameters such as retention time, peak area, theoretical plates, and tailing factor were evaluated to verify the performance of the HPLC system.

3.2. Specificity

Chromatograms of blank, standard, and sample solutions were compared in order to assess the method's specificity. The method's capacity to measure Sirolimus in the presence of formulation excipients and degradation products without interfering with the analyte's retention time was evaluated.

3.3. Linearity

By creating a number of standard solutions of Sirolimus at various concentration levels within the chosen analytical range, linearity was evaluated. Plotting peak area against concentration allowed for the construction of calibration curves after each concentration was put into the HPLC system.

3.4. Precision

The method's precision was assessed in terms of intermediate precision (inter-day precision) and repeatability (intra-day precision). After analyzing several injections of reference solutions, the method's precision was represented as the percentage relative standard deviation (%RSD) of the results.

3.5. Accuracy

Recovery studies utilizing the usual addition approach were used to assess the method's accuracy. Pre-analyzed sample solutions were mixed with known concentrations of Sirolimus standard, and the percentage recovery was computed.

3.6. Robustness

By making minor, intentional changes to chromatographic parameters such flow rate, mobile phase composition, and detection wavelength, robustness was assessed. Chromatographic performance was evaluated in relation to these modifications.

3.7. Limit of Detection (LOD) and Limit of Quantification (LOQ)

By computing the limit of detection (LOD) and limit of quantification (LOQ), the method's sensitivity was ascertained. The regression equation's slope and response standard deviation were used to determine these values using the calibration curve method.

4. Forced Degradation Studies

Sirolimus was exposed to a range of stress conditions, such as acidic, alkaline, oxidative, thermal, photolytic, humidity, and hydrolytic degradation, in order to assess the stability-indicating capabilities of the established approach. To evaluate the separation of degradation products from the drug peak, the stressed samples were examined using the devised RP-HPLC method.

4.1. Acid Hydrolysis

The drug solution was treated with 0.01 N hydrochloric acid and heated to 72°C for two hours in order to achieve acidic breakdown. RP-HPLC was used to examine the strained solution after it had been neutralized and diluted with the mobile phase.

4.2. Alkaline Hydrolysis

The drug solution was treated with 0.01 N sodium hydroxide and heated at 70°C for two hours to achieve alkaline degradation. After deterioration, the solution was neutralized and subjected to the established procedure for analysis.

4.3. Oxidative Degradation

The medication solution was exposed to 5% hydrogen peroxide at 25°C for 24 hours in order to conduct oxidative stress experiments. After the deteriorated solution was suitably diluted, chromatographic analysis was performed.

4.4. Water Hydrolysis

The drug solution was treated with distilled water and heated to 70°C for two hours in order to assess hydrolytic breakdown. The new RP-HPLC method was used to examine the solution after it had cooled.

4.5. Humidity Degradation

The medication sample was exposed to a saturated potassium nitrate solution at 25°C for seven days in order to perform humidity degradation experiments. Following treatment, the material was examined under ideal chromatographic conditions.

4.6. Thermal Degradation

Sirolimus powder was subjected to 80°C for 12 days in order to conduct thermal degradation investigations. After dissolving and diluting the stressed sample to the necessary concentration, RP-HPLC was used for analysis.

4.7. Photolytic Degradation Under UV Light

Sirolimus powder was exposed to ultraviolet light for a whole day in order to study photolytic breakdown. Following exposure, the sample was prepared in the mobile phase and subjected to chromatographic analysis.

4.8. Photolytic Degradation Under Fluorescent Light

The medication powder was exposed to fluorescent light for a whole day in order to assess the impact of visible light on Sirolimus stability. The devised RP-HPLC method was then used to produce and evaluate the exposed material.

5. Results and discussion

5.1. Studies on the chromatographic behavior of Sirolimus

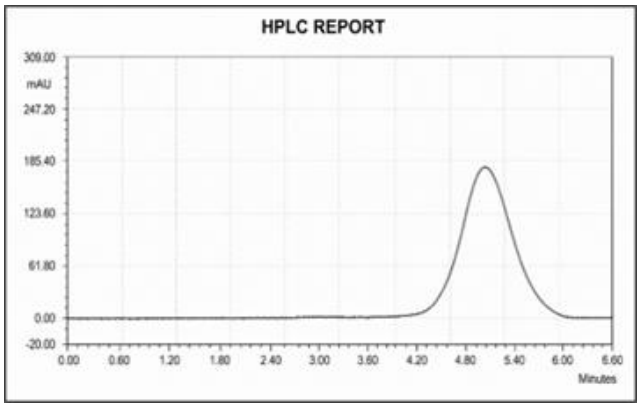

Figure 2 Trial 1 with different proportion of Mobile Phase

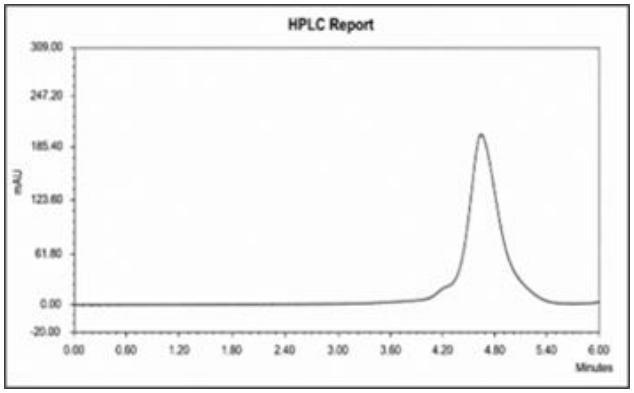

Figure 3 Trial 2 with different proportion of Mobile phase

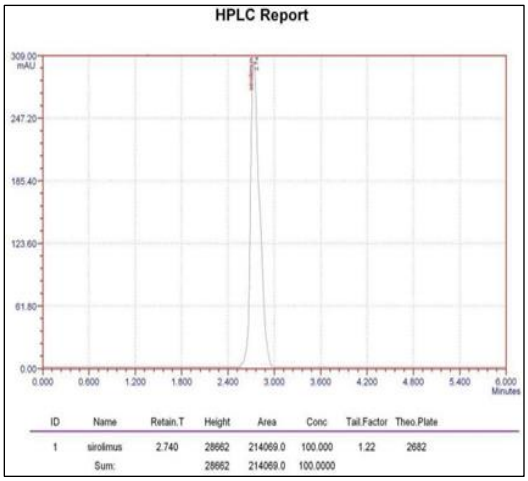

Figure 4 Standard chromatogram for sirolimus

5.2. Specificity

The chromatograms showed that:

- In the blank chromatogram, there were no interference peaks at the Sirolimus retention time.
- The retention period of the standard and sample peaks was well-resolved.
- Analyte detection was not hampered by the formulation's excipients.
- These findings support the established method's specificity for estimating Sirolimus in pharmaceutical dosage forms and bulk.

Table 1 Specificity

Name Of The Solution	Retention Time In Minutes
Blank	No Peaks
Sirolimus	2.739

5.3. Linearity

The least squares linear regression approach was used to do the regression analysis of the calibration data, and the correlation coefficient (r2), slope, and intercept were used to evaluate the method's linearity.

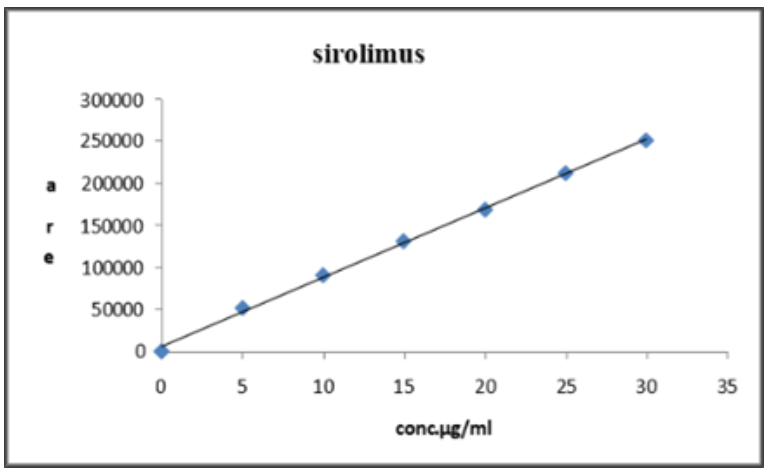

Figure 5 Linearity

Table 2 Summary of the linearity study's findings.

Level	Concentration of SIROLIMUS in µg/ml	Mean peak area
Level -1	5	50822.8
Level -2	10	89746.4
Level -3	15	130149.1
Level -4	20	168017.8
Level -5	25	211372.2
Level -6	30	249504.2
Range of target concentration i.e. 5 to 30 µg/ml	INTERCEPT SLOPE C.C	10320.16668 7978.013 0.998

5.4. Precision

The precision of the proposed RP-HPLC method for Sirolimus was evaluated in terms of intra- day precision and inter-day precision.

5.5. Intra Day Precision

The method is accurate and repeatable under the same operating conditions, as evidenced by the intra-day precision percentage RSD of 1.45%, which is within the permissible level of not more than 2.0%.

Table 3 Summary of precision study findings**.**

SAMPLE	CONC(µg/ml)	INJECTION No.	PEAKS AREA	R.S.D (Acceptance criteria 2.0)
Sirolimus	30	1	225587.7	1.45
		2	226051.2	
		3	220212.5	
		4	221302.5	
		5	223670.5	
		6	229101.8	

5.5.1. Inter Day Precision

he results of inter-day precision studies are presented in Table 4.

Table 4 Summary of inter-day precision study findings

Sample	Conc (µg/Ml)	Injection No.	Peaks Area	R.S.D (Acceptance criteria 2.0)
Sirolimus	30	1	206987.8	1.02
		2	209520.7	
		3	204944.3	
		4	206612.5	
		5	203189.3	
		6	205511.3	

5.6. Accuracy

The percentage recovery obtained ranged from **99.0% to 100%**, indicating that the method is highly accurate. The low % RSD values further confirm the reliability of the method.

The accuracy results are summarized in **Table 5**.

Table 5 Percentage recovery and %RSD

Level	Amount of Sirolimus spiked (µg/ml)	Amount of Sirolimus Recovered (µg/ml)	%Recovery	Mean % Recovery	%R.S.D	MEAN % R.S.D
50 %	15	14.84	99.01		0.239	
	15	14.91	99.34			
	15	14.91	99.47			
100%	20	19.71	98.51		0.289	
	20	19.81	99.01			
	20	19.81	99.01			
150%	25	24.79	99.21		0.232	
	25	24.79	99.22			
	25	24.89	99.62			
				99.146		0.251

5.7. Robustness

It was determined how these changes affected chromatographic parameters like retention duration, peak area, and peak shape. Under the modified conditions, it was found that the chromatographic profile did not significantly change.

The fact that the approach is unaffected by slight but intentional adjustments to the experimental settings shows that it is robust.

Table 6 displays the robustness study's findings**.**

Table 6 Robustness

Condition	Mean area	% assay	% difference
Unaltered	130149.1	100.0	0.0
Flow rate at 0.8 mL/min Flow rate at 1.2mL/min	129588.1 128986.0	99.55 99.11	0.43 0.91
Mobile phase: MEOH: Acetonitrile 82% 18% 78% 22%	131285.1 130684.4	99.16 100.04	0.84 0.04
pH of mobile phase at 5.8	130555.5	100.30	0.30
pH of mobile phase at 5.2	131027.1	100.68	0.68

5.8. System Suitability

The results obtained were found to be within the acceptable limits, indicating that the system was suitable for the analysis.

The system suitability data are summarized in 7.

Table 7 System Suitability

Parameter	Tailing factor	Theoretical plates
Specificity study	1.21	2682.16
Linearity study	1.58	3297.24
Precision study	1.55	2644.82

5.9. LOD and LOQ

In the present study, LOD and LOQ were determined by analyzing replicate solutions at the lowest detectable concentration level and evaluating the signal-to-noise ratio and precision of the response.

The LOD and LOQ values for Sirolimus were calculated and are presented in **8**.

Table 8 LOQ and LOD

Parameter	Measured volume
Limit of Quantification	35ng/ml
Limit of Detection	10ng/ml

Table 9 Stress Degradation

Stress Condition	Test Area	Assay (mg/tablet)	Assay (%)	Degradation (%)
UV	4694459	0.87	88.16	11.84
Fluorescent	4540057	0.84	85.12	14.88
Thermal	4618522	0.86	86.75	13.25
Humidity	4181361	0.77	78.43	21.57
Elevated Temperature	4650616	0.86	87.35	12.65
Acid Hydrolysis	17663	0.0032	0.34	99.66
Alkaline Hydrolysis	4554369	0.85	85.72	14.28
Oxidative Degradation	4421000	0.82	83.03	16.97
Water Hydrolysis	5275588	9.07	99.97	0.03

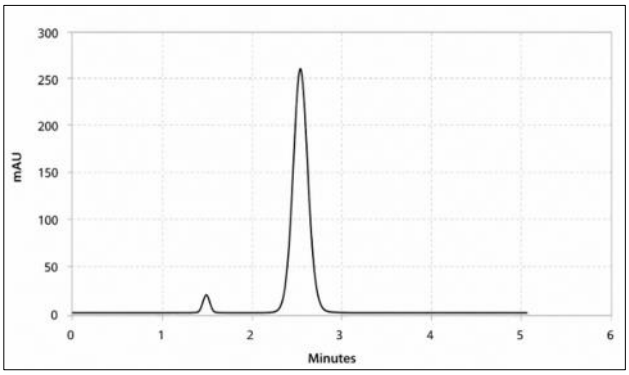

Figure 6 Chromatogram Shows UV Degradation

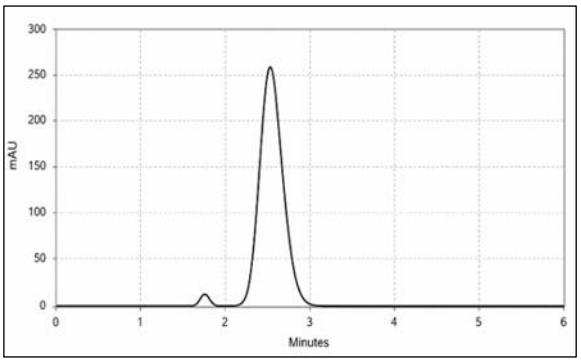

Figure 7 Chromatogram Shows Fluorescent Degradation

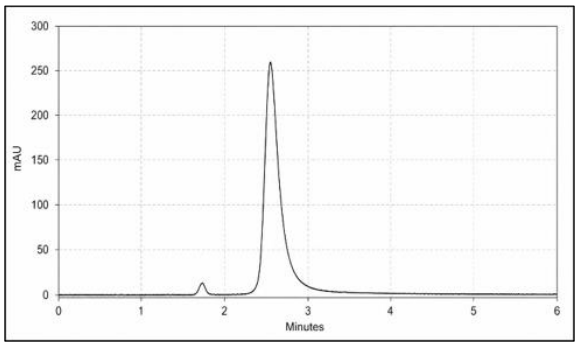

Figure 8 Chromatogram Shows Thermal Degradation

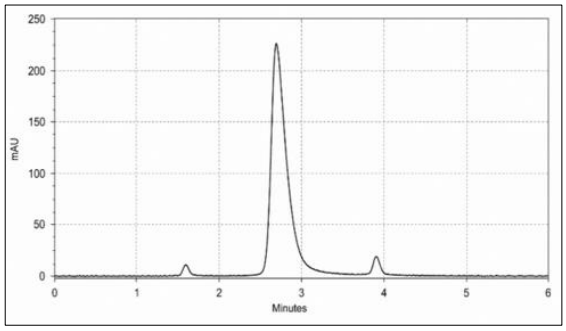

Figure 9 Chromatogram Shows Humidity Degradation

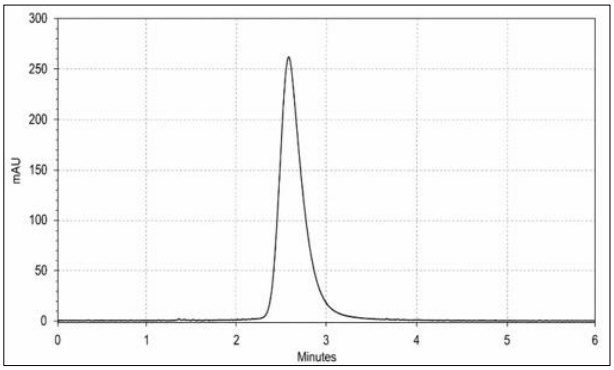

Figure 10 Chromatogram Shows Temperature Elevation

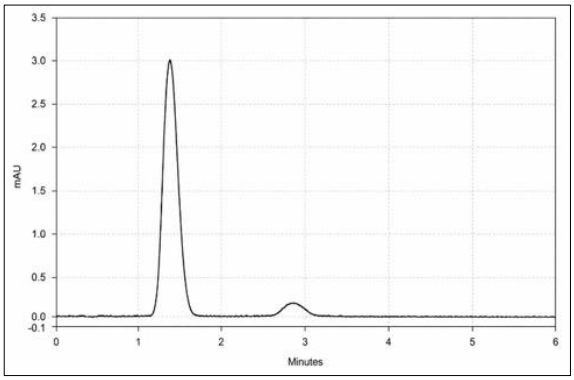

Figure 11 Chromatogram Shows Acid Hydrolysis

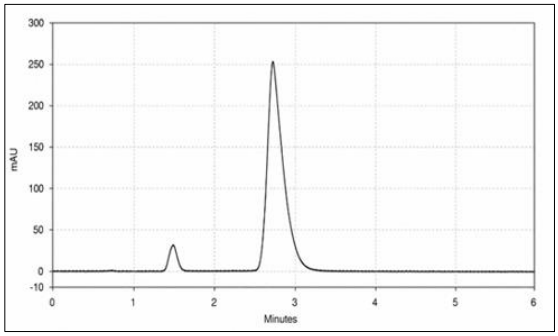

Figure 12 Chromatogram Shows Alkaline hydrolysis

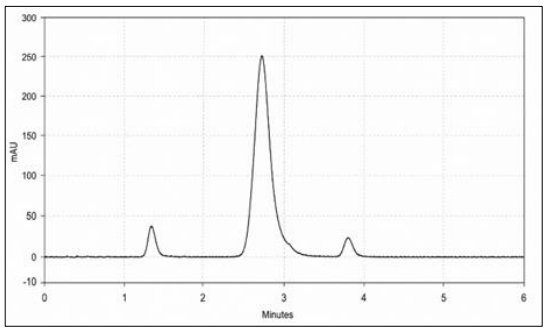

Figure 13 Chromatogram Shows Oxidation

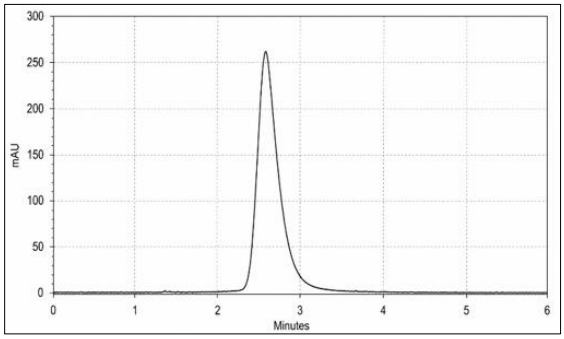

Figure 14 Chromatogram Shows Water hydrolysis

6. Conclusion

For the analysis of Sirolimus in pharmaceutical formulations, a dependable, quick, accurate, precise, and stability-indicating RP-HPLC technique was successfully designed and validated. The medication was effectively separated from

its degradation products with acceptable system suitability parameters thanks to the adjusted chromatographic settings.

The method's specificity, linearity, precision, accuracy, and robustness were confirmed by the validation findings, which met the requirements of the International Council for Harmonization. The method's capacity to indicate stability under various stress settings was shown by forced degradation studies.

For routine quality control analysis, stability investigations, and assay determination of Sirolimus in pharmaceutical dosage forms and bulk, the proposed RP-HPLC method is hence appropriate. For routine analytical applications, the technique can be successfully applied in research labs and the pharmaceutical industry.

Compliance with ethical standards

Disclosure of conflict of interest

Author declares no conflict of interest in constructing this manuscript.

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