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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF SIROLIMUS IN TABLET

M. S. Kalshetti*, Priti Reure, Vidyashree Kumbhar, Shraddha Jamakhandi
Dept of Pharmaceutical Quality Assurance, D.S.T.S. Mandal's College of Pharmacy, Solapur.

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ABSTRACT

Sirolimus is a macrolide compound used to prevent organ transplant rejection. A UV spectrophotometric method has been developed for the estimation of sirolimus in the tablet. Ethanol is used as a solvent. Sirolimus has shown absorbance maxima at 278 nm. The method was validated according to ICH Q2 (R1) guidelines. The method is linear in the range of 5-30 µg/ml and exhibited a good correlation coefficient (R^2=0.9992) with a recovery of 98-100% and precision. The developed analytical method is simple, accurate, precise, and can be applied for routine analysis of formulation containing Sirolimus in a short time.

Corresponding author
M. S. Kalshetti
Dept of Pharmaceutical Quality Assurance,
D.S.T.S. Mandal's College of Pharmacy,
Bijapur Road, Solapur-413004 (MS).
smkcops@gmail.com

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INTRODUCTION

Sirolimus (SRL), also known as rapamycin, is macrocyclic lactone antibiotic immunosuppressant compound produced by the soil bacterium *Streptomyces hygroscopicus* and was isolated for the first time in 1972 found on Easter Island (Fig. 1). It was initially developed as an antifungal but later it was discovered to have potent immunosuppressive and proliferative properties. It was approved by USFDA. Its molecular formula is $C_{51}H_{79}NO_{13}$ and its molecular weight is 914.2 [1].

Sirolimus is used to prevent organ transplant rejection. It is also used to treat a rare lung disease called lymphangioleiomyomatosis. It has immunosuppressant functions in humans and is especially useful in preventing the rejection of kidney transplants. It inhibits the activation of T cells and B cells by reducing their sensitivity to interleukin-2 (IL-2) through mTOR inhibition [1].

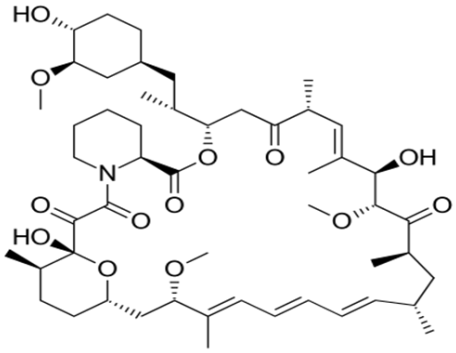

Fig.1 Sirolimus.

A literature survey revealed that estimation of sirolimus by some techniques such as UV spectrophotometric method using ethanol with phosphate buffer saline or DMSO [2,3], LC-MS [4,5], and HPLC methods with UV detection [6-15] are reported. Hence, there is a need for a UV spectrophotometric method using a common solvent, ethanol for quantification of SRL in the tablet dosage form. Hence, the present study aims to develop a validated simple, accurate, sensitive spectrophotometric method for the estimation of sirolimus in tablets using ethanol as solvent at λmax 278 nm.

MATERIALS AND METHOD

Materials

Sirolimus was obtained from Biocon Ltd., Bangalore as a gift sample. Absolute Ethanol, Rapacan Tablet of 1mg were purchased locally.

Instrument

Systronics 2201 UV-Visible spectrophotometer was used for absorbance measurement. Shimadzu AY220 electronic balance was used for weighing.

Methods

Selection of solvent

Solubility of sirolimus was checked in different solvents like methanol, ethanol, and distilled water.

Preparation of standard solution

10 mg of SRL was transferred into a 10 ml volumetric flask and dissolved in sufficient ethanol. The volume was made up to the mark with ethanol to prepare the stock solution (1000 µg/ml). The standard solution was prepared by diluting the stock solution with ethanol to get a concentration of 50 µg/ml.

Determination of absorption maxima (λmax)

SRL solution (10 µg/ml) was scanned in the range of 200-400 nm in a UV spectrophotometer to determine absorption maxima.

Method validation

Validation of the method was carried out as per the ICH guidelines [16] for the following parameter.

Linearity and Range

5, 10, 15, 20, 25, and 30 µg/ml of SRL solutions were prepared from standard solution, and absorbance was recorded at 278 nm. The calibration curve was prepared by plotting the absorbance against concentration and the correlation coefficient was determined.

Accuracy

For determination of method accuracy, SRL was spiked at 80%, 100%, and 120% levels. A known amount of standard drug concentration was added to the pre-analyzed tablet sample and percent recovery was calculated from the following formula:

$$\% \text{ Recovery} = \frac{\text{Recovered Value}}{\text{Spiked Value}} \text{ X } 100$$

Precision

Repeatability

For determination of Repeatability, six SRL solutions (20 µg/ml) were prepared and absorbance was recorded at 278 nm. %RSD was calculated.

Intermediate precision

Intermediate precision was determined by recording the absorbance of SRL solution (20 µg/ml) for three consecutive days.

Robustness

The robustness of a method is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Here, it is done by changing the wavelength of analysis, and absorbance was recorded then %RSD was calculated. The effect of detection wavelength was studied at ±1 nm.

Preparation of sample solution

10 tablets were weighed and powdered. Powder equivalent to 1 mg of SRL was transferred to a 10 ml volumetric flask, dissolved and volume was made up to the mark with ethanol. The solution was filtered through Whatman filter paper. 2 ml filtrate was pipetted out and diluted up to 10 ml using ethanol. Absorbance was measured at 278 nm and the content of SRL in the tablet was calculated.

RESULT AND DISCUSSION

Selection of solvent

SRL is insoluble in water, and soluble in methanol and ethanol. And ethanol is used as a solvent.

Determination of absorption maxima (λmax)

SRL solution (10 ug/ml) showed λmax at 278 nm (Fig. 2)

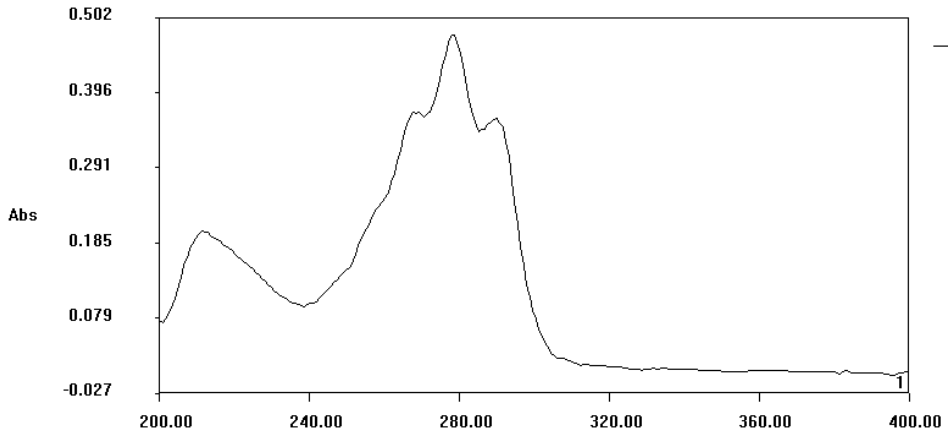

Fig. 2 UV scan of SRL (10 µg/ml).

Validation parameters

Linearity and Range

Linearity concentrations and absorbances are displayed in Table 1. Absorbance v/s concentration of SRL calibration curve was prepared by plotting graph. The method is linear for the range 5-30 µg/ml with correlation coefficient (r^2=0.9992) and linear regression equation y=0.0384x+0.0249 (Fig. 3). The overlay of UV spectra is shown in Fig. 4.

Table 1 Linearity.

Sr. No.	Concentration (µg/ml)	Absorbance
1.	5	0.207
2.	10	0.416
3.	15	0.597
4.	20	0.801
5.	25	0.994
6.	30	1.162

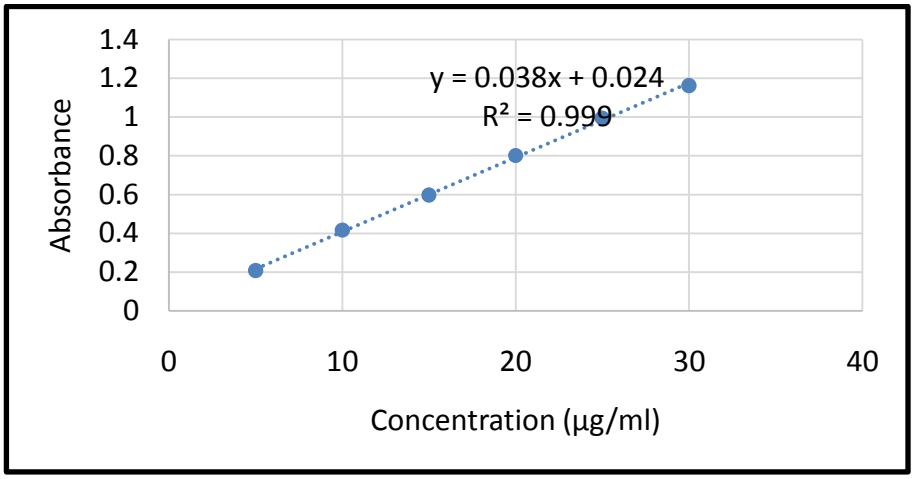

Fig. 3 Calibration curve of SRL.

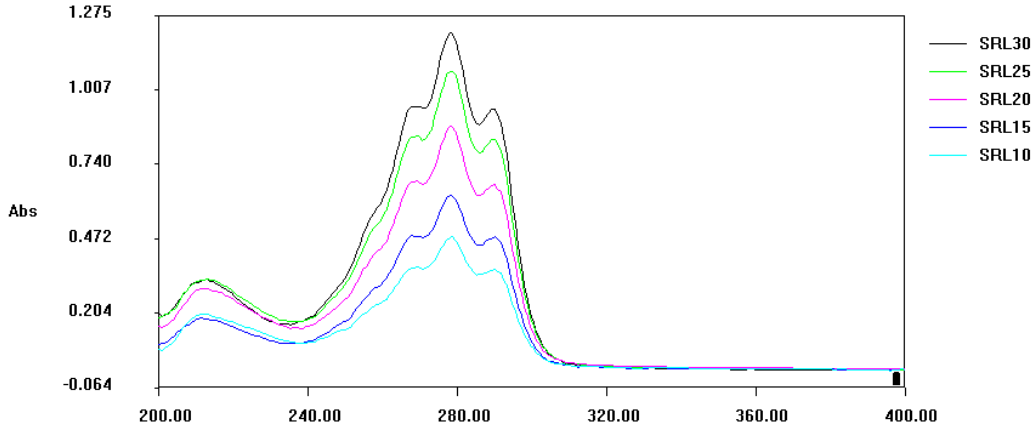

Fig. 4 Overlay UV spectra of 5-30 µg/ml solution of SRL.

Accuracy

The % recovery of spiked SRL is 98-100% as shown in Table 2.

Table 2 Results of recovery.

Sr. No.	% Spiking	Amount of std. drug added (µg/ml)	Amount of SRL recovered (µg/ml)	% Recovery
1.	80	8	8	100%
2.	100	10	9.88	98.88%
3.	120	12	12.04	100.33%

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scattering) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

The method is precise as %RSD of repeatability and intermediate precision is less than 2 as shown in Tables 3 & 4.

Repeatability

Table 3 Repeatability result.

Sr. No.	Concentration (µg/ml)	Absorbance
1.	20	0.791
2.	20	0.791
3.	20	0.793
4.	20	0.794
5.	20	0.794
6.	20	0.791
	Mean	0.792
	SD	0.002
	%RSD	0.253

Intermediate precision

Table 4 Intermediate precision result.

Sr. No.	Conc (µg/ml)	Absorbance		
		Day 1	Day 2	Day 3
1.	20	0.794	0.830	0.835
2.	20	0.794	0.832	0.835
3.	20	0.791	0.834	0.837
4.	20	0.792	0.835	0.835
5.	20	0.793	0.831	0.835
6.	20	0.793	0.832	0.837
	Mean	0.793	0.832	0.836
	SD	0.001	0.002	0.001
	%RSD	0.126	0.240	0.120

Robustness

The method was robust for change in wavelength as %RSD was less than 2, as shown in Table 5.

Table 5 Robustness result.

Sr.no.	Wavelength (nm)	Absorbance	Mean ± SD	% RSD
1.	277	0.790		
2.	278	0.797	0.788 ± 0.01	1.269
3.	279	0.778		

Assay of Rapacan tablet

Sirolimus tablet contains 98.6% of the stated amount of Sirolimus $C_{51}H_{79}NO_{13}$ (Table 6).

Table 6 SRL content in tablet.

Tablet Form	Amount taken	Amount found	% Content
RAPACAN tablet	20 µg	19.72 µg	98.6 %

CONCLUSION

The simple, cost-effective UV spectrophotometric method is developed and validated in compliance with ICH guidelines for the estimation of SRL. This method is accurate, precise, sensitive and linear in the range of 5-30 µg/ml. The analytical method can be used for routine analysis of SRL in the formulation in quality control, research, and analytical laboratories.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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