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Research Article

DEVELOPMENT & VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF RAPAMYCIN IN DRUG ELUTING STENTS

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ABSTRACT

Background: Rapamycin or Sirolimus is a macrolide compound which is used to coat coronary stents for the treatment of cardiovascular disease. The release of drug from the stent is very vital in treating the patients suffering from coronary heart problems. In the past various attempts were made to estimate Rapamycin using HPLC, LCMS, etc. in biological fluids. However, no significant work has been reported for its estimation in drug eluting stents using UV spectrophotometry. Hence the present study aims to develop a simple, reliable and cost effective UV spectrophotometric method for the evaluation of release of Rapamycin in drug eluting stents over a period of time. **Method:** The absorption maxima of Rapamycin was found to be at 278 nm and the method was validated for specificity, precision, linearity, accuracy and limit of quantification (LOQ) with different concentrations. The release of rapamycin in phosphate buffer saline (PBS) was noted for day 1,2,3,4 and 10. **Results:** The method is specific as the blank showed no interference with Rapamycin. The relative standard deviation (RSD) obtained from six replicates at 1µg/ml concentration is < 2 which indicates the method is precise. The method represents correlation coefficient (R2 =0.999) at concentration range of 0.2-10 µg/ml. The recovery was found to be 97.2 %. **Conclusion:** The above method is simple, reliable and cost effective. Hence it can be used in the routine analysis for the quantification of Rapamycin from drug eluting stents.

INTRODUCTION

Rapamycin is a macrolide compound produced by a soil bacterium *Streptomyces hygroscopicus* [1]. Initially it was used as an antifungal agent but in later studies it was found that it has a very good immunosuppressive and anti tumor activities. Rapamycin is used in the coating of coronary stents, in preventing organ transplantation rejection mainly kidney transplantation [2] and also in the treatment of a rare lung disease called lymphangioleiomyomatosis [3]. It has immunosuppressant functions in humans by inhibiting the action of T cells and B cells by reducing their sensitivity to interleukin-2 (IL 2) through mammalian target of Rapamycin (mTOR) inhibition. It acts specifically on FK binding protein 12 (FKBP 12), a substance known as immunophilin because it binds to immunosuppressive drugs. In turn the Rapamycin-FKBP 12 complex binds to the mTOR, a kinase enzyme that adds phosphate group to other molecules that plays a fundamental role in regulating the progression of cell cycle. The Rapamycin complex inhibits mTOR and by doing so it disrupts the cell division and hence the proliferation of T cells [2].

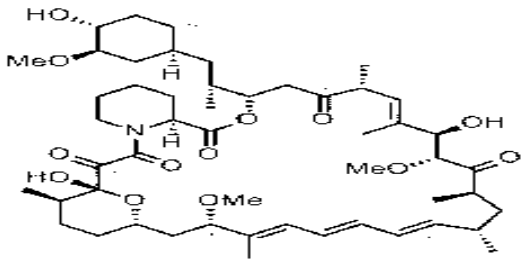

Fig 1 Chemical structure of Rapamycin

In addition Rapamycin coated stents are also used in the treatment of cardiovascular disease for lowering the rate of restenosis i.e. recurrence of blood vessels narrowing. A drug eluting stent is a peripheral or coronary stent placed into narrowed arteries that slowly releases the drug to prevent cell proliferation and thereby preventing the narrowing of the arteries again [4]. The drug eluting stents after clinical trials have been approved by the Food & Drug administration (FDA) since it showed that they are statistically superior to the bare metal stents for the treatment of coronary artery narrowing having lower rates of major adverse cardiac events [5, 6, 7]. In

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drug eluting stents a thin polymer coating is used to control drug release. A bi-layer system, with PLLA (poly lactic acid polymer) as the supporting layer and PLGA (polylactic-co-glycolic acid copolymer) as the drug-eluting layer is used to control the release of Rapamycin. The release of Rapamycin can be increased by the addition of a plasticizer like polyethylene glycol (PEG) or can be decreased by coating the stent with biodegradable polyester. So a multilayered coated stents have many options for controlling the release of Rapamycin release over months [8].

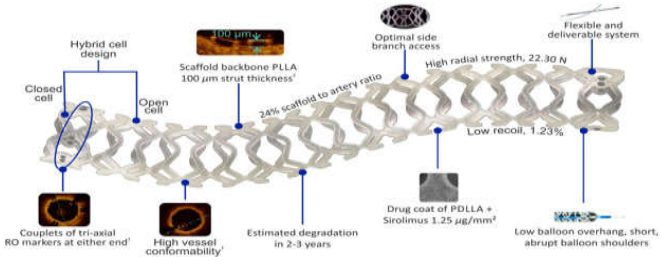

Fig 2 A drug eluting stent coated with Rapamycin

Rapamycin is not yet official in any pharmacopoeia, some analytical methods have been reported for its determination in biological fluids which involves mainly high performance liquid chromatography (HPLC) and Liquid Chromatography Mass Spectrometry (LCMS) methods [9, 10] whereas hardly reports are available for its estimation in drug eluting stents. Hence the present study aims to develop and validate a simple, accurate, sensitive and cost effective spectrophotometric method for the estimation of Rapamycin in PBS (Phosphate Buffer Saline) eluates of drug eluting stents.

MATERIALS AND METHODS

Chemical and Reagents

The standard was obtained from Sigma Aldrich India. The chemicals Sodium dihydrogen phosphate dihydrate , Disodium hydrogen phosphate dihydrate, Sodium hydroxide of A.R. quality were purchased from S.D.Fine Chemicals, Sodium chloride of analytical grade were purchased from Fisher Scientific and Ethanol of analytical grade was purchased from Changshu Hongsheng Fine chemicals. Demineralised water of HPLC grade was purchased from Vater X.

METHODOLOGY

The stents were incubated in incubator (IEC) and the Spectrophotometric experiments were performed using a UV-Visible Spectrophotometer instrument (Shimadzu UV 1700). PBS solution was prepared by dissolving 0.08 M Disodium hydrogen phosphate dihydrate, 0.02 M Sodium dihydrogen phosphate monohydrate and 0.15 M Sodium Chloride in water. The pH of the solution was adjusted to 7.4 with 1 M sodium hydroxide solution. 10 % ethanolic PBS was prepared by adding 100 ml of ethanol in 900 ml of PBS. The standard stock solution was prepared by dissolving 10 mg of Rapamycin in 10 ml of ethanol and made up to 100 ml with PBS solution. The working standard was prepared in the range of 0.2 µg/ml to10 µg/ml with 10% ethanolic PBS solution. An appropriate volume of working standard solution of Rapamycin was scanned in the UV range 220 – 700 nm. The spectrum showed absorbance maxima at 278 nm for Rapamycin (Fig.3).

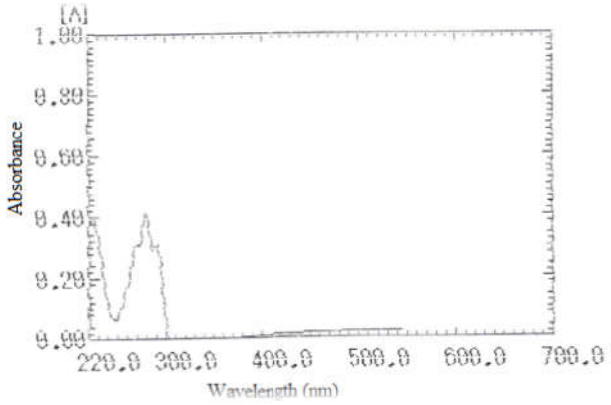

Fig 3 UV Spectrum of Rapamycin

Preparation of Sample

Each stent was taken in a vial and the stent was completely immersed by adding PBS buffer. The vials were then closed and incubated at 37°C for certain time period. After the elapse of the respective time periods the UV absorption of the solution was measured at 278 nm.

RESULTS AND DISCUSSIONS

The method development and validation study was made as discussed above and the results are discussed below.

Specificity

The specificity of the method was checked by its ability to identify the compound of interest that has no interference with blank at 278 nm. To check the specificity PBS solution was used as blank and its absorbance was noted. . The absorbance of the blank at 278nm was less than 0.005 which shows there is no interference of blank with respect to Rapamycin. Hence the method is specific.

Precision

Precision is an ability of a method to measure its repeatability and reproducibility. In this study the absorbance of Rapamycin standard solution of 1 µg/ml concentration was noted in six replicates. The method is precise as the RSD obtained from six replicates is 1.68% (Fig. 4).

Linearity

Linearity is measured by the ability of the method to obtain results which is directly proportional to the concentration of the sample. Six concentrations of Rapamycin standard in the range of 0.2 to 10 µg/ml were taken and the absorbance was plotted against their concentration. The linearity of the method was established in the range of 0.2 to10 µg/ml with a correlation coefficient of 0.999 (Fig. 5)

Accuracy

Accuracy of a method is the closeness of the measured value to its true value. In this method the extracted stent was spiked with 1 µg/ml standard solution and the absorbance at 278 nm was noted in six replicates. The recovery of the spiked sample was shown in the Fig.6. Therefore the method is accurate with 97.2% recovery.

Limit of Quantification (LOQ)

The sensitivity of the method was determined by the Limit of Quantification (LOQ) which is the lowest amount of analyte in the sample which can be quantified with suitable precision and accuracy. In this method Rapamycin standard of 0.2 µg/ml was taken and absorbance was noted in six replicates. The RSD for the limit of quantification was calculated and found to be less than 2.0% (Fig. 7). Hence the method is sensitive with a LOQ of 0.2 µg/ml.

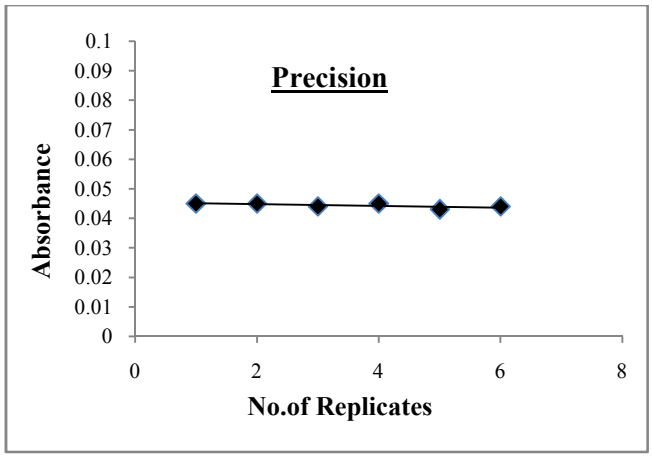

Fig 4 Precision at 1 µg/ml level

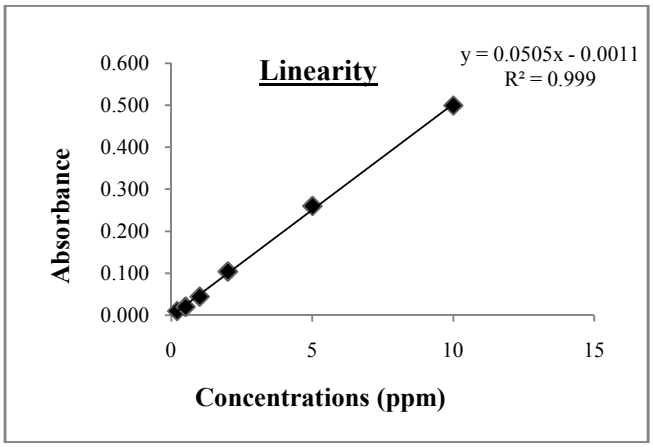

Fig 5 Calibration curve for linearity

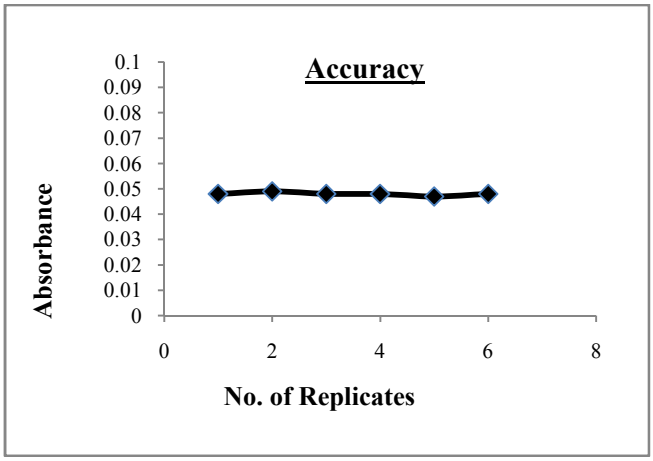

Fig 6 Accuracy at 1 µg/ml level

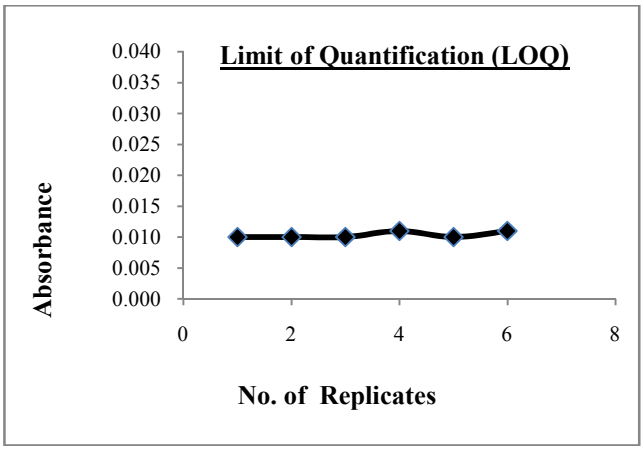

Fig 7 LOQ **at** 0.2 µg/ml level

Table 1 Summary of results for validation parameters of Rapamycin

Sl. No.	Parameters	Results
1	Specificity	No interference of blank (PBS) was observed with respect to Rapamycin
2	Precision	RSD = 1.68%
3	Linearity	Correlation coefficient (R^2) = 0.999 with a concentration range of 0.2 to10 µg/ml
4	Accuracy/Recovery	97.2 % recovery @ 1 µg/ml concentration level
5	LOQ	RSD < 2% @ 0.2 µg/ml

Sample Analysis

Release of Rapamycin in three finished stent product of same dimensions were analyzed as per the method of analysis for Day 1, 2, 3, 4 & 10 and results are shown in Fig. 8-10. The samples showed a steady increase in the release of Rapamycin from day 1 to day 10.

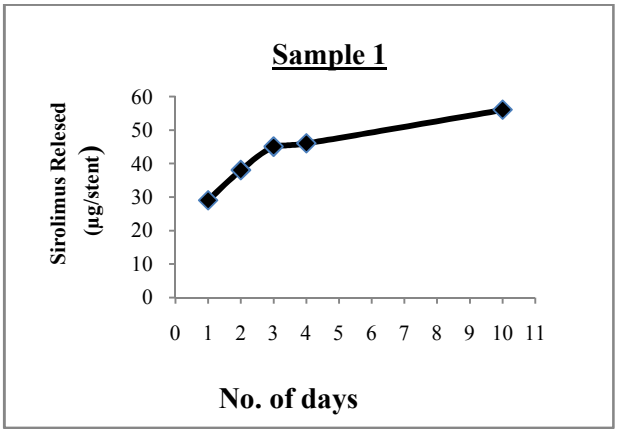

Fig 8 Release of Rapamycin of stent 1

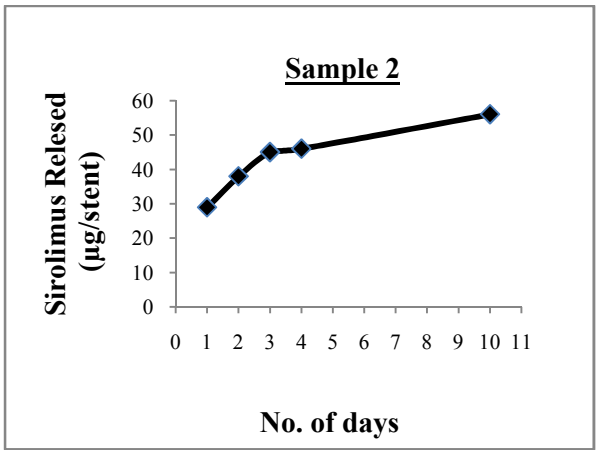

Fig 9 Release of Rapamycin of stent 2

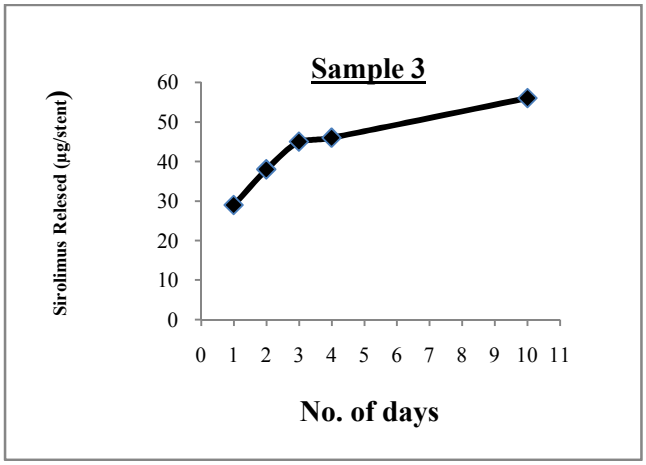

Fig 10 Release of Rapamycin of stent 3

CONCLUSION

The UV spectrophotometric developed for Rapamycin quantification in drug eluting stents is specific, precise and accurate in the range of 0.2 µg/ml to 10 µg/ml. Hence the method can be used in the routine analysis for the quantification of release of Rapamycin from drug eluting stents for a particular time period. Moreover the method is cost effective and more environment friendly than HPLC method as it uses less quantity of less hazardous solvent.

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