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AMD & AMV OF DRUG ELUTING STENT (SIROLIMUS & PROBUCOL) BY RP-HPLC IN BULK FORMULATION

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ABSTRACT

The aim of this study was to develop a simple, rapid and sensitive reverse phase high performance liquid chromatography (RP-HPLC) method for quantification of Sirolimus and Probucol in bulk drug formulations. The chromatographic separation was carried out on C18 Stainless Steel Column (250mm x 4.6 mm, 5µm.) with Acetonitrile and Water as Mobile phase at 263 nm. Calibration plots were linear over the concentration range 1-100 µg/ml for Sirolimus and Probucol. The method was validated over linearity, accuracy, precision and robustness. % Recoveries were found to be close to 100% with low variability. Hence method may be used for routine analysis in drug industry.

INTRODUCTION

Sirolimus, also known as Rapamycin ($C_{51}H_{79}NO_{13}$) is a lactone-lactam macrolide that is used to coat coronary stents (1, 2). Probucol ($C_{31}H_{48}O_2S_2$) is powerful antioxidant drug normally used to prevent vascular disease caused by the free radicals in the body (3). Sirolimus inhibits T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine stimulation by a mechanism that is distinct from that of other immunosuppressant (4). Sirolimus also inhibits antibody production (5). Probucol lowers plasma LDL in both animals and humans, but it also profoundly reduces HDL. In clinical testing, Probucol lowers LDL by up to 20% but can lower HDL by as much as 20–30%. Probucol lowers serum cholesterol by increasing the fractional rate of low density lipoprotein catabolism in the final metabolic pathway for cholesterol elimination from the body (6). Limited analytical methods are

available to analyze Sirolimus and Probucol individually by High Performance Liquid Chromatography (HPLC) (7, 8, 9,10). The objective of the present study was to develop and validate a simple, fast, accurate RP-HPLC method for the simultaneous estimation of Sirolimus and Probucol in bulk drug samples.

Experimental:

Chemicals:

HPLC grade acetonitrile (ACN) was purchased from Vetec (Sigma Aldrich). HPLC grade water obtained from Merck Millipore water purification system. Reference standard Sirolimus was purchased from Toronto Research Chemicals, INC and Probucol from Cayman Chemical Company of purity 95 and 100% rspectively.

Instrumentation and Analytical Conditions:

HPLC separation was performed on Shimadzu liquid chromatographic system equipped with UV and PDA detectors. The chromatographic separation was achieved on C18 Column (250 mm x 4.6 mm, 5µm) at using acetonitrile and water as mobile phase. Gradient programming was done for the elution and separation of Sirolimus and Probucol with flow rate as 1.5 ml/min and run time 40 minutes.

Preparation of Standard Solutions:

25 milligrams of standards Sirolimus and Probucol were accurately weighed, transferred to 25 ml volumetric flask separately, dissolved in acetonitrile to obtained solution containing 1000µg/ml. Aliquots of the stock solutions were appropriately diluted with acetonitrile to obtain working standards solutions of Sirolimus and Probucol.

Method Validation:

The aim of method validation is to revel that the method is suitable for its intended idea as it is stated in ICH guidelines (11). The method was validated for system suitability, specificity, linearity, precision (Repeatability and intermediate precision), accuracy and robustness. Method precision was performed with six repeated sample preparation of nominal concentration (40µg/ml) while accuracy was performed at 50%, 100% and 150% of the nominal concentration respectively. Seven point linearity was prepared by injecting mix standards of 5, 10, 20, 30, 40, 50 and 100 µg/ml. the peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. Robustness was also evaluated on 3 individual parameters i.e. flow, temperature and wavelength.

Results and Discussion:

Method Development:

The optimization of chromatographic conditions was done with a view to develop HPLC method for the simultaneous determination of Sirolimus

and Probucol in bulk drug formulations. For the selection of wavelength 20µg/ml standard solutions of Sirolimus and Probucol were scanned in the spectrum mode between 200 to 400 nm in Photo Diode Array. Although maxima for both drugs was different, after optimization 263 nm was selected as detection wavelength as both drugs showed good absorbance at this wavelength. Various mobile phase comprising different isocratic/gradient programming of acetonitrile and water were tried. Peak purity and interference from other peaks were also checked. Finally gradient program was selected with retention time of 15.1 and 25.09 for Sirolimus and Probucol respectively. Analysis was done with both C-8 and C-18 columns and better resolution was observed with C-18 column.

Method Validation:

The HPLC method was validated in terms of specificity, linearity, accuracy, precision and robustness in accordance with ICH Q2 (R1) guidelines.

System Suitability test:

System suitability was carried out by injecting six replicate of mixed standard of 40 ppm each of Sirolimus and Probucol. Various parameters like peak area, tailing factor, theoretical plates and resolution and retention time were evaluated.

Table 1: System Suitability

% RSD		Resolution		Tailing Factor		Theoretical plates	
Sirolimus	**Probucol**	**Between Sirolimus and adjacent Peak**	**Between Sirolimus and Probucol**	**Sirolimus**	**Probucol**	**Sirolimus**	**Probucol**
0.15	0.10	2.03	22.50	1.18	1.25	3413	216209

Linearity:

Linearity was evaluated by analyzing working standard solutions of Sirolimus and Probucol of different concentrations. Seven point's calibration graphs were constructed covering a concentration range 5–100 µg/ml. The linearity in terms of measured peak area versus corresponding concentration of drugs were estimated by ordinary linear regression analysis. The slope, intercept and correlation coefficient (r^2) were calculated and evaluated.

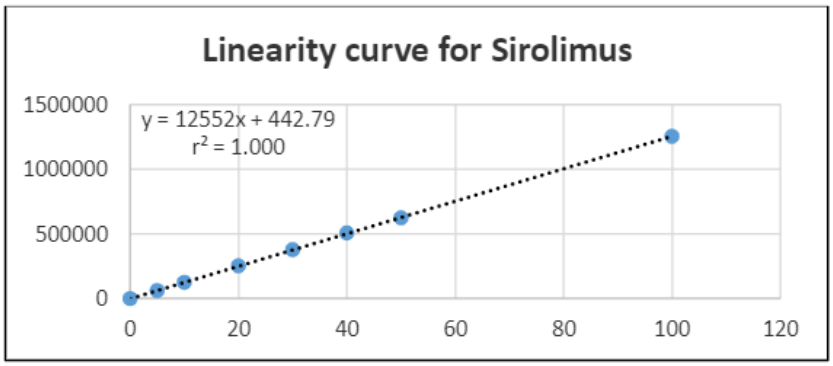

Fig 1: Linearity curve for Sirolimus

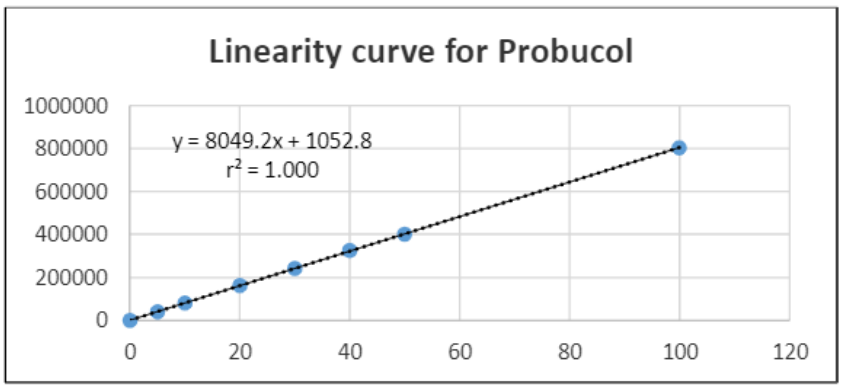

Fig 2: Linearity curve for Probucol

Precision:

The precision of the developed method was evaluated by performing intra-day and inter day precision studies. Intraday precision was carried out by performing six replicate injection of 40 µg/ml mixed standard and six different preparations of 40 µg/ml sample solution. The inter-day precision study was performed by different analyst on different day using mentioned concentrations of standard and sample solutions. Peak area measured was expressed in terms of percent relative standard deviation (%RSD) which was observed below two, that confirmed precision of the method. Table shows combined results of precision studies.

Table 2: Precision data

	% RSD Standard (40 µg/ml)		% RSD (Test 40 µg/ml)	
	Sirolimus	**Probucol**	**Sirolimus**	**Probucol**
Day 1	0.10	0.09	0.75	0.73
Day 2	0.10	0.16	0.07	0.13
Day 1 + Day 2	0.47	0.67	1.96	1.21

Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found (11).

Spiked samples were prepared at three concentrations over the range of 50, 100 and 150 % of the nominal concentration i.e. 20, 40 and 60 µg/ml levels respectively. Three individually prepared replicates at each concentration level were analysed and %RSD was recorded. The percent recovery values were found to be in range between-99.43-102.24%, which is well within acceptance criteria. Good agreement between actual and determined values were found which confirms the accuracy of the method. % RSD less than two for both drugs suggesting suitability and applicability of the method for routine drug analysis.

Robustness:

As defined by ICH guidelines the robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations in method (11). In the present study three factors were selected i.e. flow, temperature and wavelength to observe robustness of the method. Triplicate injections of mixed standard of 40 µg/ml and three different preparations of sample solution of similar concentration were injected in each variable condition. Assay of sample was calculated with reference to standard area and %RSD was calculated. %RSD for all the variable parameters was within 2% which shows the method is robust to various variable conditions.

Table-3: Summary of Results of Robustness

Variation		%RSD of Assay Sirolimus	% RSD Assay Probucol
Flow	**At flow 1.4 ml/min (a)**	**0.06**	**0.03**
	At flow 1.6 ml/min (b)	**0.15**	**0.08**
	Overall (a)+(b)	**0.13**	**0.06**
Temperature	**At temp. 38˚C (a)**	**0.32**	**0.32**
	At temp. 42˚C (b)	**0.50**	**0.47**
	Overall (a)+(b)	**0.41**	**0.37**
Wavelength	**At wavelength 261 nm**	**0.05**	**0.11**
	At wavelength 265 nm	**0.23**	**0.20**
	Overall (a)+(b)	**0.23**	**0.23**

Conclusion:

A simple, rapid, accurate, precise, specific and robust method was developed and validated for combined analysis of Sirolimus and Probucol in bulk drug formulations by RP-HPLC using C-18 column. Method was thoroughly validated, demonstrating to be linear in the studied concentration range, precise, accurate and robust in determining Sirolimus and Probucol in drug formulations. The proposed

HPLC method would be of use in routine quality control in pharmaceutical industry.

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