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STABILITY INDICATING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF ROSUVASTATIN CALCIUM AS BULK DRUG AND IN TABLET DOSAGE FORM

Dipak R Supe, Padmanabh B. Deshpande*, Saurabh Jadhav and Sandeep Swami

All India Shri Shivaji Memorial Society's College of Pharmacy, Department of Pharmaceutical Quality Assurance, Kennedy Road, Near RTO, Pune-411001.

***Corresponding Author: Dr. Padmanabh B. Deshpande**
All India Shri Shivaji Memorial Society's College of Pharmacy, Department of Pharmaceutical Quality Assurance, Kennedy Road, Near RTO, Pune-411001.

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ABSTRACT
The present work describes the development and validation of simple, precise and accurate stability indicating high performance thin layer chromatographic method (HPTLC) method for estimation of Rosuvastatin calcium as bulk and in tablet dosage form. Best chromatographic separation was achieved with use of precoated silica gel 60 F254 aluminium plates as stationary phase and mixture of Toluene: Ethyl acetate: Methanol (5: 3: 2, v/v/v) as mobile phase. Densitometric detection was carried out at 242 nm. The drug was subjected to stress conditions of degradation and the method was validated as per ICH guidelines. The developed method shows linearity over a concentration range of 500-2500 ng band^{-1}. The drug was found to be more sensitive towards acidic and thermal stress conditions in comparison to other stress conditions. The proposed method can be effectively applied to accomplish long-term and accelerated stability studies for the determination of rosuvastatin calcium in pharmaceutical formulations.

KEYWORDS: Rosuvastatin calcium, HPTLC, Stability indicating method, Validation.

INTRODUCTION
Rosuvastatin calcium, chemically, (E)-(3R, 5S)-7-{4-(4-fluorophenyl)-6-isopropyl-2 {methyl(methyl–sulphonyl amino)] pyrimidin-5-yl}-3,5-dihydroxyhepten-6-oicacid calcium is an inhibitor of enzyme HMG-CoA reductase which is a rate limiting enzyme for conversation of the 3-hydroxy-3-methylglutarate to mevalonate which is the starting compound for cholesterol synthesis and helps to reduce harmful LDL and helps to conserve the HDL.[1] It is official in IP which describes liquid chromatographic method for its estimation.[2] In clinical trials, Rosuvastatin calcium achieved mark reduction in serum levels of LDL cholesterol, accompanied by modest increases in HDL cholesterol and reduction in triglyceride.[3] The most important related compounds for rosuvastatin are anti-isomer and lactone impurity.[4] The chemical structure of Rosuvastatin calcium is represented in Figure 1.

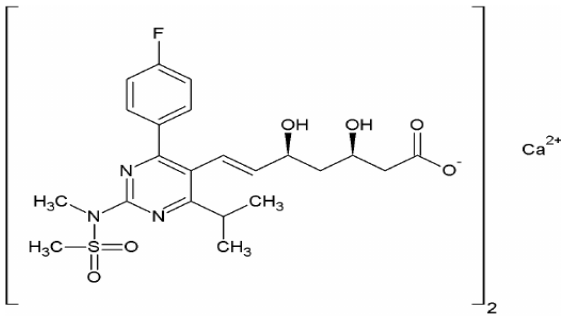

Figure 1: Chemical structure of Rosuvastatin calcium.

Extensive literature survey revealed that various analytical methods such as UV spectrophotometry[5-8] High Performance Liquid Chromatography (HPLC)[9-13] and High Performance Thin Layer Chromatography (HPTLC)[14-18] has been reported for determination of Rosuvastatin calcium either as single drug and/or in combination with other drugs in pharmaceutical dosage form. Most of the reported methods are based on hyphenated techniques and overall cost of the analysis using these techniques is more as compared to High performance thin layer chromatography. The simple, easy, high throughput and automated prospective of

HPTLC makes it a better choice over conventional analytical tool. So the main objective of current work was to develop and validate a simple, accurate and economic stability representing HPTLC method for determination of Rosuvastatin calcium in tablet dosage form as per International Conference on Harmonisation Guidelines.[19, 20]

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade working standard Rosuvastatin calcium was obtained as a gift sample from Astra Zeneca Pharma India Ltd. The pharmaceutical tablet dosage form ROSOVOS-20 labelled to contain 20 mg was used for the study. Toluene, Ethyl acetate, Methanol (all AR grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India).

Instrumentation and chromatographic conditions

Chromatographic separation of the drug was performed on Merck TLC plates precoated with silica gel 60 F_{254} (10 cm × 10 cm with 250 µm layer thickness) from E. MERCK, Darmstadt, Germany as stationary phase using a Camag Linomat V sample applicator (Switzerland). Samples were applied on the plate as a band under nitrogen stream with a 6 mm of band width using Camag 100 µL sample syringe (Hamilton, Switzerland).

Linear ascending development was carried out in 10 ×10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) by using toluene: ethyl acetate: methanol (5: 3: 2, v/v/v) as mobile phase. The mobile phase was saturated in the twin trough TLC chamber for 20 min before chromatogram development at room temperature. After development, TLC plates were removed and dried. A Camag thin layer chromatography scanner III operated by winCATS software version 1.4.2 was used for densitometric evaluation. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard stock solution

Accurately weighed 20 mg of drug was dissolved in 10 mL of acetonitrile to get a concentration of 2000 ng μL^{-1} which was further diluted to 10 mL to obtain working standard concentration 100 ng μL^{-1}.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that drug showed significant absorbance at 242 nm and hence was selected as the wavelength for detection for further analysis. The representative UV spectrum obtained is shown in Figure 2.

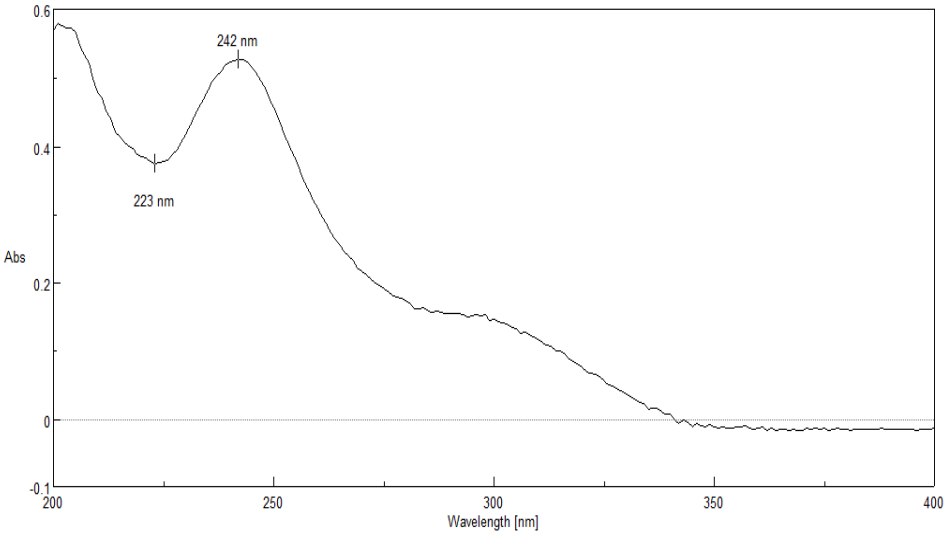

Figure 2: UV spectrum of Rosuvastatin calcium.

Tablet formulation analysis

Commercial brand of tablets namely ROSOVOS-20 containing 20 mg of drug was used to estimate the amount of Rosuvastatin in available tablet formulation. For this, 20 tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 20 mg was transferred to 10 mL volumetric flask containing 7 mL of methanol and the contents were sonicated for 15 min. The solution was filtered using Whatman paper No. 41 and the volume was made up to the mark with methanol to obtain the final concentration of 2000 ng μL^{-1}. The above solution was diluted further with methanol to get final concentration 100 ng μL^{-1}. Ten µL volume of this solution was applied on TLC plate to obtain final sample concentration of 1000 ng $band^{-1}$. After chromatographic development peak areas of the bands were measured at 242 nm and the amount of drug present in sample was estimated from the calibration curve. Procedure was repeated six times for the analysis of homogenous sample.

Stress degradation studies

In order to access the stability of bulk drug, stability studies were performed by subjecting the drug to different stress conditions (hydrolysis, peroxide, heat and light). A stressed sample at high concentration was

spotted and multi wavelength scanning was done to search for peaks of degradation product. The stress degradation studies were performed at concentration of 2000 ng μL^{-1}. The hydrolytic studies were performed by treatment of stock solution of drug separately with 0.1 N HCl and 0.1 N NaOH at room temperature for period of 1 h and 2 h, respectively. The acid and alkali stressed samples were neutralized with NaOH and HCl, respectively to furnish the final concentration of 2000 ng $band^{-1}$. Neutral hydrolytic study was performed by treatment of drug with water at room temperature for period of 24 h. The oxidative degradation was carried out in 3 % H_2O_2 at room temperature for 1 h and sample was diluted with methanol to obtain 2000 ng $band^{-1}$ solution. Thermal stress degradation was performed by keeping drug in oven at 60ºC for period of 6 h. Photolytic degradation studies were carried out by exposure of drug to UV light up to 200 watt h square $meter^{-1}$. Thermal and photolytic samples were diluted with methanol to get concentration of 2000 ng $band^{-1}$.

RESULTS AND DISCUSSION

Method development and optimization

The main purpose of existing research work was to develop stability indicating HPLTC method which would be capable to provide the acceptable resolution between Rosuvastatin and its degradation products if formed. Different solvent combinations comprising different ratios of toluene, benzene, ethyl acetate, chloroform, ethanol glacial acetic acid and methanol were examined (data not shown) in order to separate and resolve spot of Rosuvastatin calcium from its impurities and other excipients present in formulation. The optimised method involved mixture of toluene: ethyl acetate: methanol (5: 3: 2, v/v/v) which gave better resolution of drug with retention factor value 0.45 ± 0.01. The representative densitogram in depicted in Figure 3.

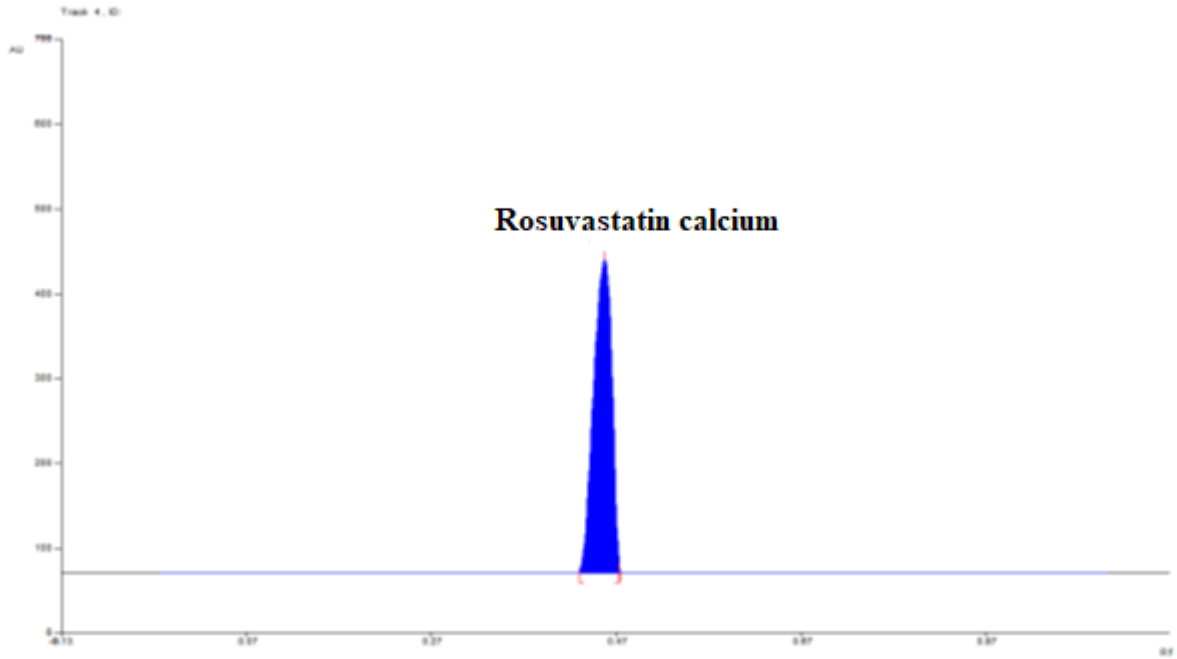

Figure 3: Representative densitogram of standard solution of Rosuvastatin calcium.

(1500 ng $band^{-1}$, Rf= 0.45 ± 0.01)

The results of stress degradation studies showed susceptibility of drug to hydrolytic, oxidative, thermal as well as photolytic stress conditions. The drug was found to be more sensitive towards acidic and thermal stress conditions in comparison to other stress conditions. Figures 4-7 denotes the densitograms of acid, alkali, neutral and oxidative degradation, while Figure 8 illustrates the densitogram of dry heat degradation. The results of stress degradation studies along with % degradation and % recovery are Table 1.

Table 1: Results of stress degradation studies.

Sr. No.	Stress conditions	% Recovery	% Degradation
1.	Acid/ 0.1 N HCl/ Kept at RT for 1h	79.23	20.74
2.	Alkali/ 0.1 N NaOH/ Kept at RT for 2 h	88.50	11.50
3.	Neutral/ H_2O/ Kept at RT for 24 h	81.00	19.00
4.	Oxidative/ 3% H_2O_2 / Kept at RT for 1h	93.62	6.38
5.	Thermal / 60ºC for 6 h	79.24	20.76
6.	Photolysis: UV light 200 watt h square $meter^{-1}$	83.12	16.88

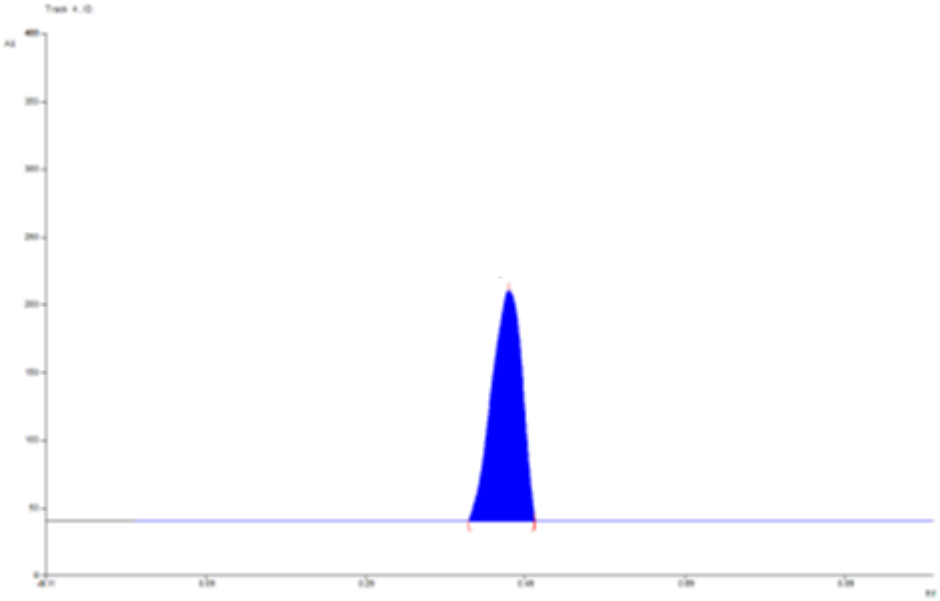

Figure 4: Densitogram after treatment with 0.1 N HCl (Kept at RT for 1 h).

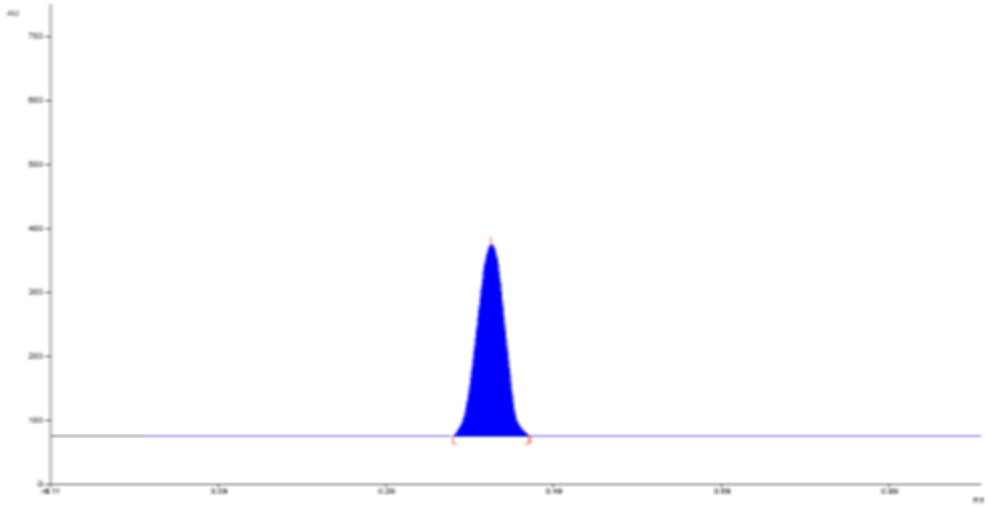

Figure 5: Densitogram after treatment with 0.1 N NaOH (Kept at RT for 2 h)

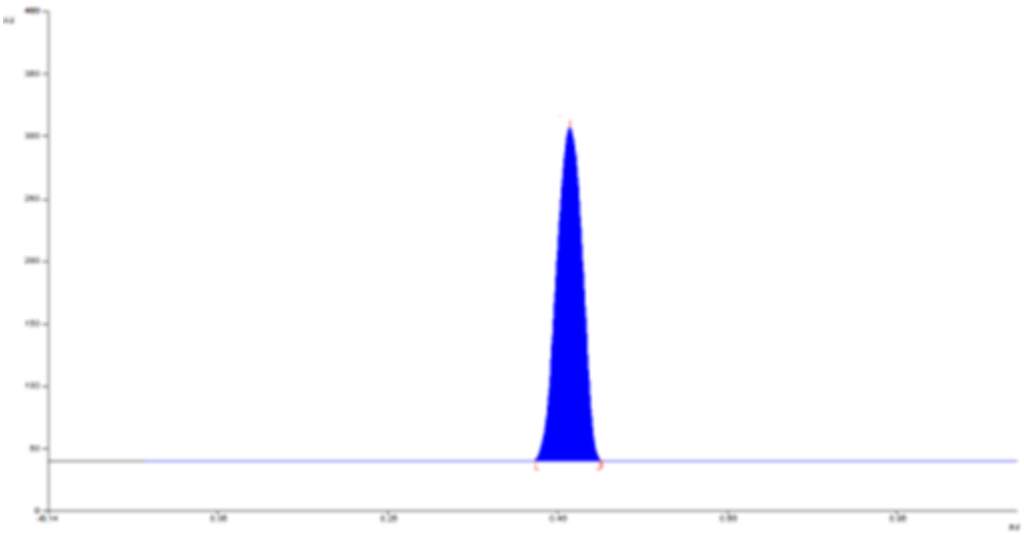

Figure 6: Neutral degradation densitogram.

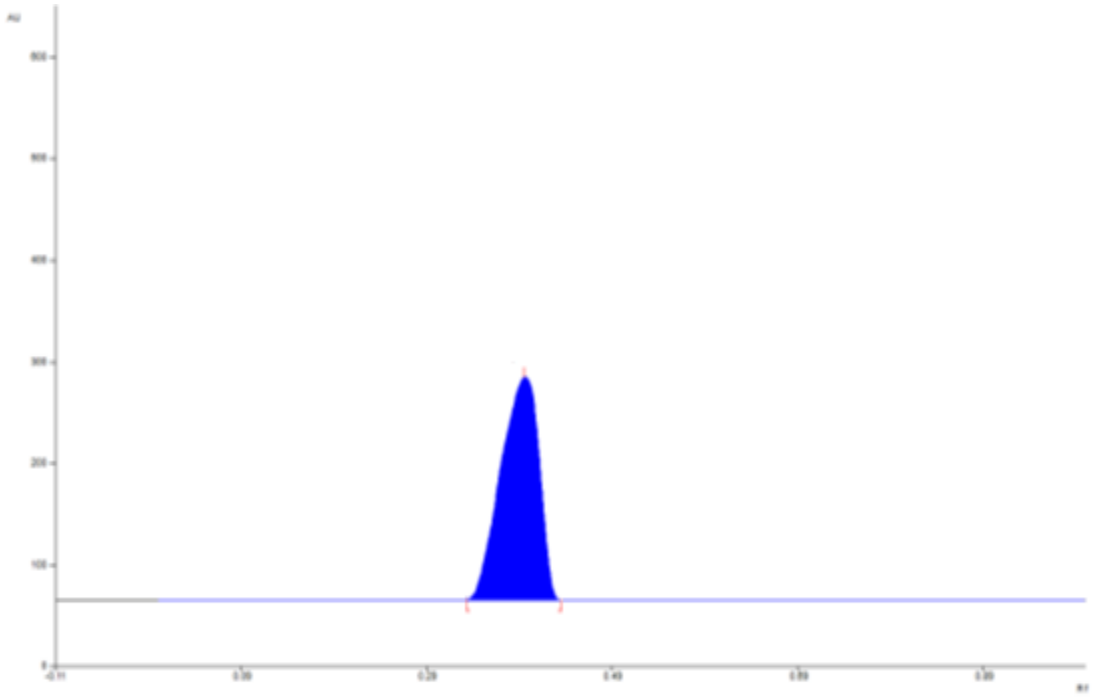

Figure 7: Densitogram after treatment with 3% H_2O_2.

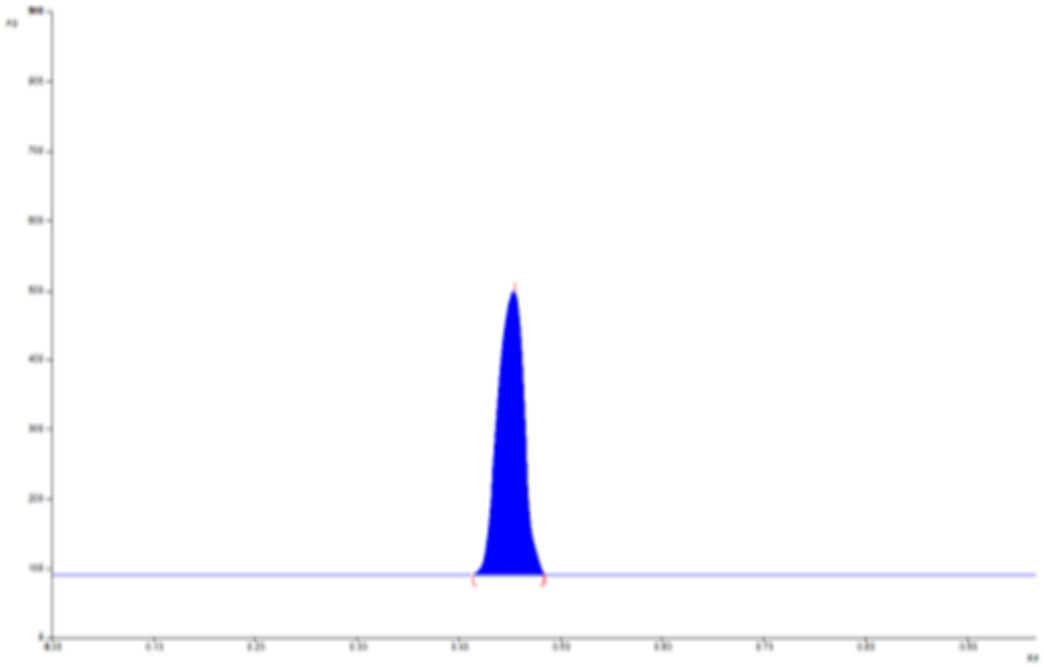

Figure 8: Densitogram after exposure of drug to 60ºC for 6 h.

Analytical method validation

The method has been validated according to the guidelines of ICH Q2 (R1) for parameters such as linearity, intra-day and inter-day precision, accuracy, limit of detection, limit of quantification, and robustness.

Linearity

To check linearity of proposed method, volumes 5, 10, 15, 20 and 25 µL of standard solution of Rosuvastatin calcium (100 ng μL^{-1}) were spotted onto the TLC plates, developed and scanned under optimized chromatographic conditions. The established method was found to be linear in the concentration range 500-2500 ng $band^{-1}$ with high correlation coefficient. The linear regression equation was found to be y =3.9635x + 1564.4 with correlation coefficient (R^2) value of 0.9976. A 3D densitogram of linearity obtained in the concentration range 500-2500 ng $band^{-1}$ is shown in Figure 9. The calibration curve achieved by plot of concentration vs peak area is depicted in Figure 10.

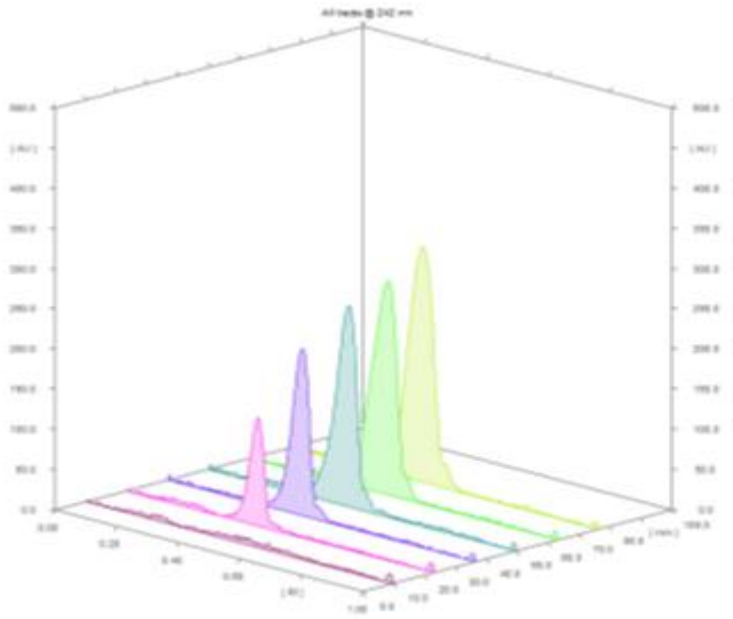

Figure 9: 3D densitogram of linearity in the concentration range 500-2500 ng band^{-1}.

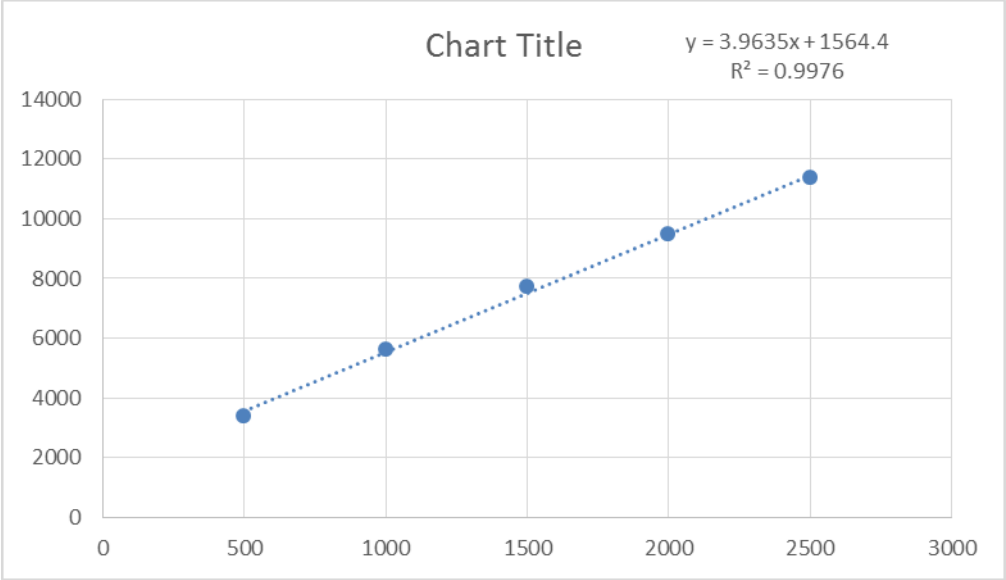

Figure 10: Calibration curve.

Precision

The method was subjected to intra-day and inter-day precision studies. Set of three different concentrations (1000, 1500, 2000 ng band^{-1}) in three replicates of standard solutions of Rosuvastatin were prepared and analysed to record intra-day and inter-day variations. The method was found to be precise as % R.S.D. was less than 2%. The results are given in Table 2 and 3.

Table 2: Intra-day precision studies.

Spotted concentration (ng band^{-1})	Average area	% R.S.D.
1000	5526.0	0.69
1500	7527.2	0.94
2000	9531.5	0.59

Table 3: Inter-day precision studies.

Spotted concentration (ng band^{-1})	Average area	% R.S.D.
1000	5552.6	0.31
1500	7573.5	0.58
2000	9561.6	0.25

Limit of Detection and Limit of Quantitation

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 9.24 ng/band and 28.02 ng/band, respectively.

Accuracy

Accuracy of developed method was checked by performing recovery studies by standard addition method. It involved addition standard drug solution to pre-analysed sample solution at three different levels 80 %, 100 % and 120 %. Basic concentration of sample chosen was 1000 ng band^{-1} from tablet solution. The drug concentrations were calculated from linear regression equation. The results of the recovery studies indicated accurateness of developed method for estimation of drug in tablet formulation.

Table 3: Recovery studies.

Drug	Concentration taken (ng band^{-1})	Concentration added (ng band^{-1})	Concentration found (ng band^{-1})	% Recovery	% R.S.D.*
Rosuvastatin	1000	800	1796.4	99.80	1.06
	1000	1000	1993.9	99.69	1.31
	1000	1200	2190.2	99.55	1.16

*Average of three determinations

Robustness

Robustness was carried out by making small and deliberate changes to optimised method parameters like change in mobile phase composition (± 1% methanol) saturation time (± 10 min) and wavelength (± 1 nm). The method was found to be robust as the areas of peaks of interest remained unaffected by small changes of the operational parameters.

CONCLUSION

A simple, precise, accurate and economic stability indicating HPTLC method for estimation of Rosuvastatin calcium in tablet formulation has been developed and validated. The suggested method was found to be less time consuming and cost effective and may be more advantageous for routine analysis of drug in marketed formulation.

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