Research Article

STABILITY-INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ROSUVASTATIN CALCIUM IN PHARMACEUTICAL DOSAGE FORM BY REVERSE PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: The present work was focused on the development and validation of the reversed-phase high-performance liquid chromatography (RP-HPLC) method, which is simple, rapid, precise, accurate, sensitive, economical, and stability-indicating for the quantification of rosuvastatin calcium in bulk and tablet formulation.

Methods: The separation was attained on reversed-phase Princeton (C18) column with dimensions (250 mm × 4.6 mm, 5μ) employing buffer, which is a mixture of water (pH 3.0, adjusted with orthophosphoric acid) and methanol in the ratio (20:80) v/v as mobile phase, at flow rate 1.0 ml/min. and detection was carried out at wavelength 240 nm. The retention time under the optimized condition of rosuvastatin calcium was found to be 2.844 min.

Results: The linearity of the method was demonstrated in the concentration range of 6-16 μg/ml for rosuvastatin calcium with a correlation coefficient (r²) of 0.9999. The percentage relative standard deviation was <2% and percentage recovery was found to be 100.12-101.37% for rosuvastatin calcium. The assay of marketed tablet formulations was found to be 98.99%.

Conclusion: The developed RP-HPLC technique was found to be simple, specific, sensitive, rapid, linear, accurate, precise, and economical and will be used for regular quality control of rosuvastatin calcium in bulk and tablet formulations.

Keywords: Rosuvastatin calcium, RP-HPLC, Validation, ICH guidelines

INTRODUCTION

Rosuvastatin calcium (RSV), is chemically (3R, 5R)-7-[4-(4-Fluorophenyl)-2-[methyl(methylsulfonyl)amino]-6-propan-2-ylpyrimidin-5-yl]-3,5-dihydroxyhept-6enoic acid (fig. 1). It is an HMG-CoA reductase inhibitor used in the treatment of hypertension, abnormal lipid [1, 2]. An extensive literature survey revealed that several HPLC methods were reported for the estimation of rosuvastatin calcium in bulk and tablet formulation [3-21]. The International Conference on Harmonization (ICH) guideline entitled "Stability testing of new drug substances and products" requires that stress testing be administered to elucidate the inherent stability characteristics of the active substance [22]. An ideal stability-indicating technique is one that resolves the drug and its degradation products efficiently. Consequently, the implementation of an analytical methodology to determine RSV in the presence of its degradation products is sort of a challenge for pharmaceutical analysts. Therefore, it was thought necessary to study the stability of RSV under acidic, alkaline, hydrolytic, oxidative, light, and thermal conditions. This paper reports validated stability-indicating reverse phase HPLC method for the simultaneous determination of RSV in the presence of their degradation products. The proposed method is simple, accurate, reproducible, stability-indicating, and suitable for routine determination of RSV in bulk and tablet formulations. The method was validated in compliance with ICH guidelines.
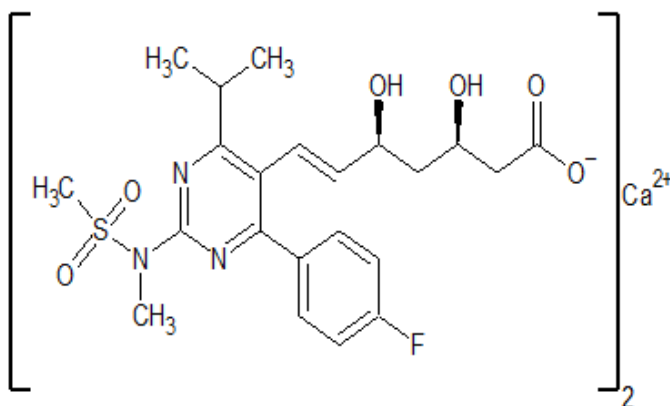

Fig. 1: Chemical structure of rosuvastatin calcium (RSV)

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade Rosuvastatin calcium was procured as a gift sample from Cadila Pharmaceuticals Ltd., Ahmedabad (India), Rosmi 10 a tablet formulation, obtained commercially.

Methanol, ortho-phosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide 30% of analytical grade were used throughout the work.

Instrumentation

The analysis was performed using reversed-phase high-performance liquid chromatography (RP-HPLC) Shimadzu LC-6AD system equipped with a PDA detector with Lab Solution software were used.

Chromatographic Conditions

Chromatographic separation was achieved on a reversed-phase Princeton (C18) column with dimensions (250 mm × 4.6 mm, 5μ) at ambient temperature using a mobile phase consisting of a mixture of buffer (pH 3.0, adjusted with orthophosphoric acid) and methanol in the ratio of (20:80) v/v at a flow rate of 1.0 ml/min. Detection was carried out at 240 nm. The pH of the mobile phase was set at 3.0; Injection volume was 10 μl. The optimized chromatographic condition is shown in table 1.

Table 1: Optimized chromatographic condition

	Chromatographic condition
Mobile phase	Water (pH adjusted to 3.0 with ortho phosphoric acid): Methanol (20:80) v/v
Flow rate	1.0 ml/min.
Column	Princeton C18 (250 mm × 4.6 mm, 5μ)
Detector wavelength	240 nm
Column temperature	30 °C
Injection volume	10 μl
Runtime	20 min
Diluent	Methanol
Retention time	2.844 min

Preparation of standard solution of RSV

For RSV, an accurately weighed 1.0 mg of RSV was transferred to a 10.0 ml volumetric flask and dissolved in 5.0 ml of methanol. The volume was completed to 10.0 ml with methanol. One milliliter of the resulting solution was pipetted in 10.0 ml volumetric flask and the volume was made up to 10.0 ml with methanol to furnish a solution of concentration 10 μg/ml of RSV.

Preparation of sample solution of RSV

Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 1.0 mg of RSV was transferred into a 10.0 ml volumetric flask. Then 5.0 ml of methanol was added in it. The flask contents were sonicated for 10 min to make the contents homogeneous. This solution was then diluted up to the mark with methanol. The resultant solution was filtered through Whatman Grade I filter paper. One milliliter of the filtrate was transferred to a 10 ml volumetric flask and then the volume was made up to the mark with methanol to furnish a sample solution containing 10 μg/ml of RSV. Six replicate of tablet powder equivalent to 1.0 mg of RSV was transferred into six 10.0 ml volumetric flask and homogenous sample solutions were prepared similarly.

Method validation

The developed method was validated following ICH guidelines (ICH Q2R1) for accuracy, precision, specificity, linearity, limit of detection (LOD), the limit of quantification (LOQ), robustness [23].

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a standard true value or an accepted reference value, and therefore, the value found. It was computed at three different levels, i.e., 80, 100, and 120% of the label claim. Standard addition and recovery experiments were conducted to determine the accuracy of RSV for the quantification of drugs in the sample.

Precision

The system precision was evaluated by measuring the area of six qualified working standards for RSV and calculating the percentage of relative standard deviation (RSD). The assay method precision was evaluated by conducting six independent assays of test samples of RSV against qualified working standards and calculating the percentage of relative standard deviation (RSD). The intermediate precision of the method was also verified using different analysts and different days.

Linearity

Linearity test solutions of RSV were prepared at concentration levels of 6-16 μg/ml. Linearity test solutions were prepared by diluting the stock solution to the required concentrations. Linearity was established by the least-squares linear regression analysis of the calibration data. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. The linear curve of rosuvastatin calcium was shown in fig. 2.

Specificity

The specificity of the developed method was established by comparing the chromatograph of the standard and sample. It was found that there was no interference due to excipients and impurities at the retention time of the drug.

LOD and LOQ

The LOD is the lowest analyte concentration that can be detected. LOQ is the lowest analyte concentration that can be quantified with acceptable accuracy and precision. The limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviation of the response and the slope of the calibration plot. LOD and LOQ were established, under ICH definitions, by use of the equations LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$, where σ is the standard deviation of the regression line and S is the slope of the calibration plot.

Robustness

To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution for RSV was evaluated. To study the effect of wavelength on the estimation, the wavelength was altered by±2 nm, i.e., 238 and 242 nm from the actual wavelength, 240 nm. To study the effect of flow rate on estimation, the flow rate was altered by±0.1 ml/min i.e., 0.9 and 1.1 ml/min from the actual flow rate, 1.0 ml/min.

Stability of the solution

The stability of both the standard and sample solutions was checked up to 24 hr. at room temperature.

Stability-indicating assay

The drug was subjected to acidic (0.1N HCl), alkaline (0.1N NaOH), oxidative (0.3% H_2O_2), photo (UV light), thermal (sand bath at 50 °C), and hydrolytic (water) condition and the percentage degradation were calculated.

RESULTS AND DISCUSSION

HPLC method development and optimization

In this work, the analytical HPLC method was used for the determination of degraded products, which was developed and validated. Initially, pure drugs solution was chromatographed using a mobile phase consisting of a mixture of buffer (pH 3.0, adjusted with orthophosphoric acid) and methanol in the ratio of (20:80) v/v at a flow rate of 1.0 ml/min. gives well-resolved peaks of drugs as well. Detection was carried out at 240 nm. The retention time under the optimized condition of rosuvastatin calcium was found to be 2.844 min. The total run time of the chromatogram was about 20 min. A typical chromatography of standard and sample of rosuvastatin calcium is shown in fig. 3 and fig. 4, respectively.
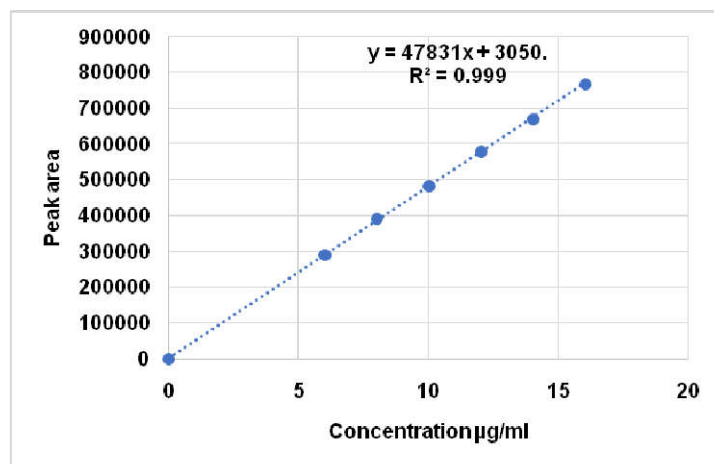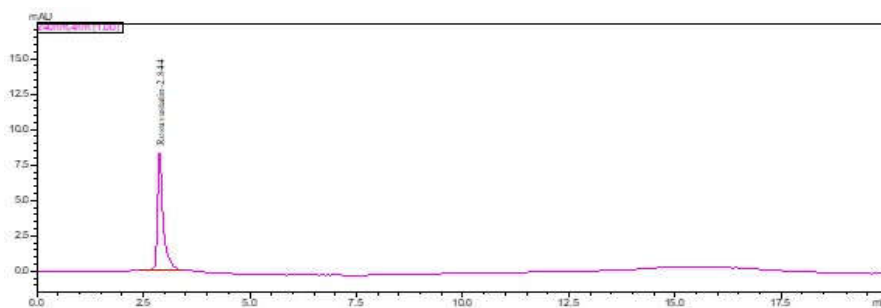

Fig. 2: Linear curve of rosuvastatin calcium (RSV)

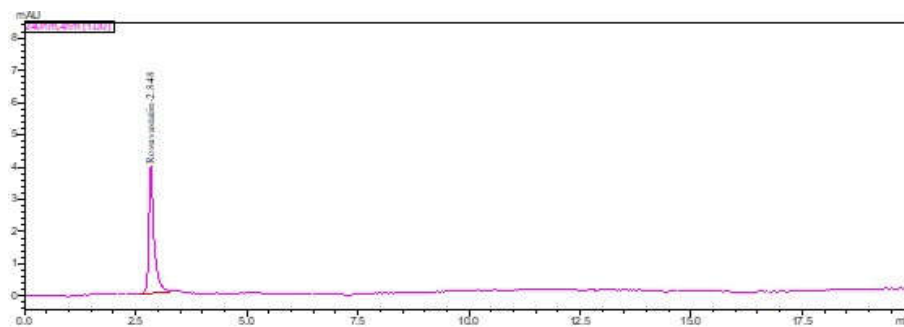Fig. 3: A typical chromatograph of standard rosuvastatin calcium (RSV)

Fig. 4: A typical chromatograph of sample rosuvastatin calcium (RSV)

Validation of the method

System suitability

The suitability of the system was demonstrated by assessing various parameters. It was established by injecting six replicate injections of the standard solution. Theoretical plates were found to be 2816, tailing factor of 1.60, and %RSD of peak area was 0.9 for RSV (table 2). All the system suitability parameters were well within limits, indicating that the system was well suitable for performing the analysis.

Table 2: **System suitability results**

Parameter	RSV
Theoretical Plate	2816
Retention Time (Rt)	2.844
Tailing Factor	1.60
% RSD	0.9

Rt: Retention time, %RSD: Percentage relative standard deviation

Linearity

Linearity was established by the least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range of 6-16 µg/ml for RSV. Peak areas were plotted against the respective concentrations, and linear regression analysis performed on the resulting curves. Equation for the calibration plots of RSV was Y = 47831x+3050.8 with correlation coefficient 0.9999. The results of linearity are shown in table 3.

Table 3: **Linearity results**

Parameter	RSV
Concentration Range (µg/ml)	6-16
Slope (m)	47831
Tailing factor	3050.8
Coefficient correlation (r^2)	0.99999

Accuracy

The percentage of recoveries was 100.12-101.37% for RSV. The %RSD value was found to be <2%. The results of recovery are shown in table 4.

Table 4: Recovery results

Level (%)	Amount taken (µg/ml)	Amount found*	% Recovery* (µg/ml)
80	8	8.11	101.37
100	10	10.01	100.12
120	12	12.05	100.41

*Average of three determinations

Precision

The precision of the method for the determination of RSV was studied using the parameters i.e. system precision, method precision, and intermediate precision. The system precision was determined by six replicate injections of standard solution injected into the HPLC system.

The relative standard deviation was less than 2%. Method precision was determined by the six individuals sample preparations injected into the HPLC system. The relative standard deviation was less than 2%. The result of intraday and interday for RSV was 0.9 and 0.6, respectively. The percentage RSD of system, method, and intermediate precision study was well within±2.0%, indicate that the method was precise.

LOD and LOQ

The LOD and LOQ of RSV were 0.65 μg/ml and 1.98 μg/ml, respectively.

Robustness

The robustness of the method was designed by changing the optimized condition adequately. To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution for RSV was evaluated. On the assessment of the result, it can be deduced that the variation in the changing wavelength, the flow rate does not affect the method significantly. %RSD<2% specifies that the developed method was robust. The results of robustness are shown in table 5.

Table 5: Robustness results

Condition		RSV	
		Amount Estimated* [%]	RSD [%]
Change in wavelength	238 nm	97.28	0.5698
(240±2 nm)	242 nm	98.21	0.5733
Change in flow rate	0.9 ml/min	99.55	0.6381
(1.0±0.1 ml/min)	1.1 ml/min	99.24	0.5078

*Average of three determinations, %RSD: Percentage relative standard deviation

The percentage assay of tablet formulation was found to be 98.99 for RSV. Forced degradation studies were performed by subjecting to the different stress conditions like acidic, alkaline, hydrolytic, oxidative, thermal, photolytic. From the chromatograms of stressed samples, it was found that no interference from degradants was observed at the retention time of RSV. Major degradation was observed in acidic conditions with 2 degraded samples at Rt values 1.784, 2.146 with percentage areas 9.29%, in alkaline condition with the degraded sample at Rt values 2.515 with percentage areas 8.72%. Substantial degradation was observed in hydrolytic conditions with the degraded samples at Rt values 2.142 with percentage areas 6.16%, in oxidative condition with 2 degraded samples at Rt values 1.122, 2.518 with percentage areas 7.82%, in light condition with the degraded sample at Rt values 2.212 with percentage areas 4.45%. No degradation was observed in the presence of heat for RSV. The results of the percentage of degradation are presented in table 6 and fig. 5-10.
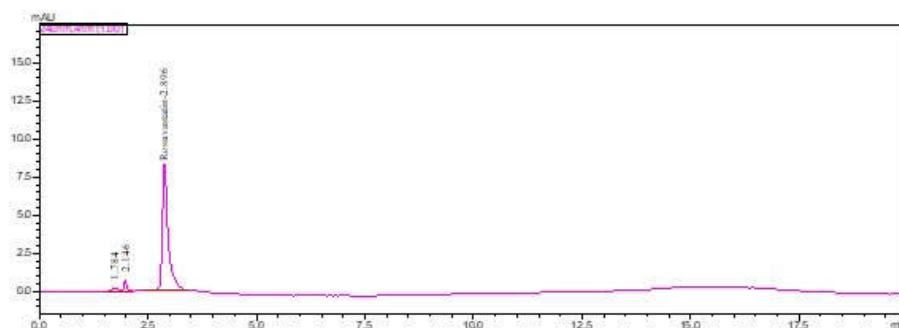

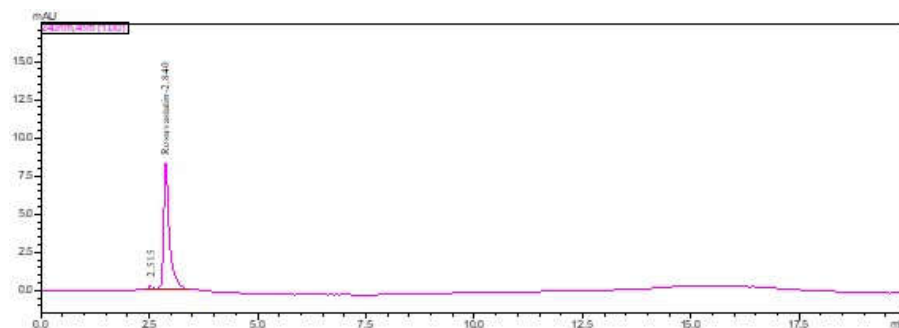**Fig. 5: A typical chromatogram of RSV degraded with acid hydrolysis**

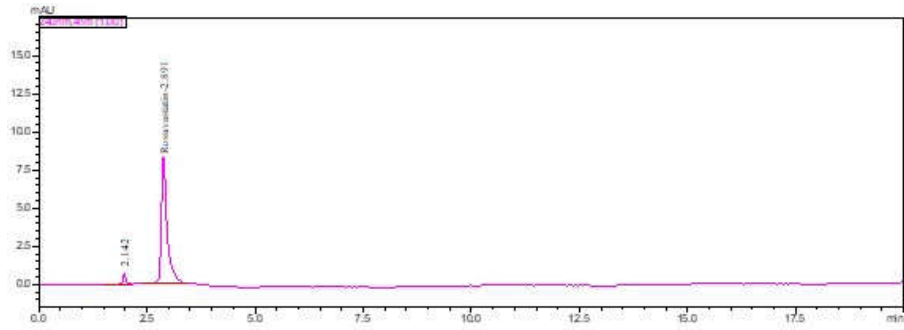**Fig. 6: A typical chromatogram of RSV degraded with alkali hydrolysis**

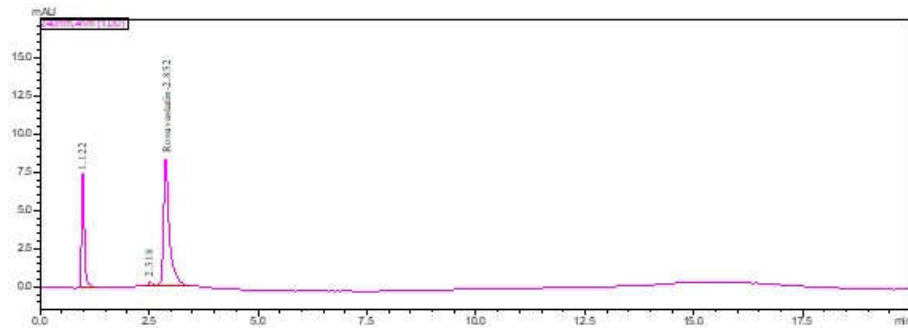**Fig. 7: A typical chromatogram of RSV degraded with neutral hydrolysis**

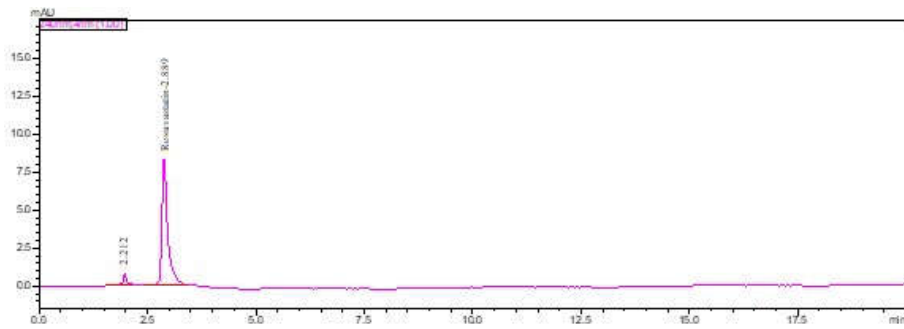**Fig. 8: A typical chromatogram of RSV degraded with oxidative hydrolysis**

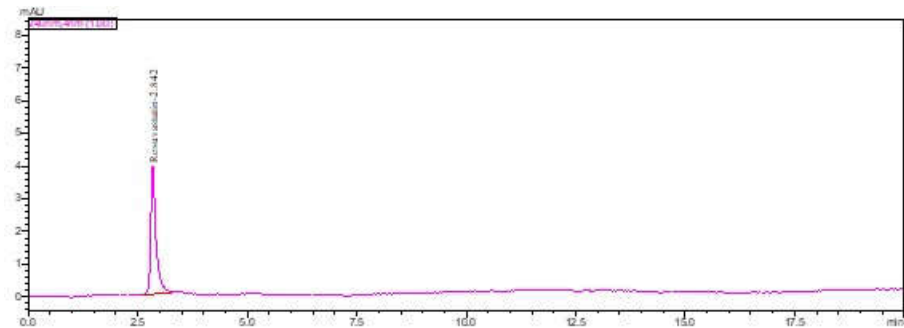**Fig. 9: A typical chromatogram of RSV degraded with exposed to direct sunlight**

Fig. 10: A typical chromatogram of RSV degraded with thermal hydrolysis

Table 6: Stability-indicating method data for RSV

Stress condition	RSV
Acidic (0.1N HCl for 24 h)	9.29
Alkaline (0.1N NaOH for 24 h)	8.72
Hydrolytic (HPLC waters for 24 h)	6.16
Oxidative (0.3% H_2O_2 for 24 h)	7.82
Photo (Sunlight for 30 d)	4.45
Thermal (Sand bath at 50 °C for 24 h)	No degradation

HPLC: High-Performance Liquid Chromatography

Table 7: Summary of validation parameter

Parameter	RSV
Calibration range (μg/ml)	6-16
Optimized wavelength (nm)	240
Retention Time	2.844
Regression equation (Y)	Y = 47831x+3050.8
Slope	47831
Intercept	3050.8
Coefficient correlation (r^2)	0.9999
Precision (% RSD)	
Intraday	0.9
Interday	0.6
% Assay*	98.99
LOD (μg/ml)	0.65
LOQ (μg/ml)	1.98

*Average of five determinations, LOD: Limit of detection, LOQ: Limit of quantification

CONCLUSION

The method enables simple, rapid, accurate, precise, specific, economical, and sensitive analysis of rosuvastatin calcium in bulk and tablet dosage form. This method was validated as per ICH guidelines. The method can, therefore, be used for routine quality-control analysis rosuvastatin calcium in bulk and tablet dosage form.

AUTHORS CONTRIBUTIONS

AP and RK designed the study. AP performed the experiment and analyzed the data and reviewed it. RK supervised the experiment, reviewed the data, and supported for writing the manuscript.

CONFLICT OF INTERESTS

Authors declare that they have no conflict of interest exists in this investigation.

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