SPECTROPHOTOMETRIC DETERMINATION OF ROSUVASTATIN CALCIUM WITH VANILLIN REAGENT BY USING ACETYLATION REACTION

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Abstact: *A sensitive, rapid, and accurate spectrophotometric method was proposed for the determination of rosuvastatin calcium (ROS-Ca) using nucleophilic addition reactions. The acetylation reaction is an important reaction resulting from the reaction of alcohol groups with aldehydes or ketones, which gives products with distinctive colors. This method is based on the acetylation formation from the reaction of forming hydroxyl groups in ROS-Ca with the carbonyl group in the aldehyde (vanillin) in an acidic medium of concentrated sulfuric acid to give a colored product that has a higher absorption at a 550 nm wavelength. The calibration curve gave a linear relationship in the concentration range of 1.25-15 µg/mL. The molar absorptivity was (9×104) L.mol^{-1}.cm^{-1} and Sandell's sensitivity index was (0.0011 µg.cm^{-2}.ml) with a correlation coefficient of 0.9995. The limits of detection (LOD) and quantitation (LOQ) values were 0.0375 and 0.1252 µg/mL, respectively. The method has been successfully applied to pharmaceutical preparation tablets from different origins.*
Keywords: *Spectrophotometric study, Rosuvastatin Calcium, Vanillin, Acetylation reaction*

Introduction

Rosuvastatin Calcium is one of the statin medications that work to reduce blood cholesterol and inhibit the MG-COA enzyme in the liver [1]. It reduces harmful cholesterol, increases beneficial fats, and reduces the risk of heart disease [2]. The scientific name is [(3R-5S) -7-[4-(4-fluorphenyl)-2-[methyl(methyl sulfonyl)amino]6-propan-2-yipytimidin-5-yl]-3,5-dihydroyhept-6-enoate] and the molecular weight is 1001.14g/mol [3]. Fig. 1 show structure of rosuvastatin calcium**.**

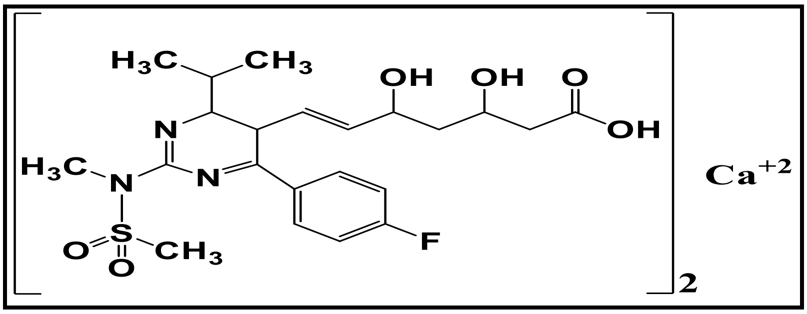

Fig. 1. Structure of calcium rosuvastatin

Rosuvastatin calcium can be determined using various analytical techniques, including spectrophotometry, chromatography, fluorimetry, flow injection analysis, and electrochemical methods [4-17].

Acetylation is an organic reaction commonly employed in nucleophilic addition processes involving alcohols and carbonyl compounds (ketones and aldehydes) in the presence of a concentrated strong acid and an organic solvent [18–20]. Vanillin (4-hydroxy-3-methoxybenzaldehyde) [21] serves as a reagent in the acetylation reaction and is widely used in spectrophotometric methods [22–24].

Experimental part

Apparatus. A Shimadzu UV-1900 UV–Visible spectrophotometer was used for the absorbance measurements, utilizing 1 cm path length quartz cuvettes. An electro-magnetic water bath was employed for heating, and sample weights were measured using a high-precision analytical balance (AE ADAM type).

The chemicals and solvent. The chemicals and analytical reagents used were all of high purity. Preparation of the solutions of the chemical compounds and drugs is listed in Tables 1 and 2.

Table 1. Preparing the chemical compounds involved in the experiments

Chemicals	Conc.	Preparation	Final dilution with organic solvent
Rosuvastatin Calcium	(50 µg/ml)	The solution was prepared by dissolving 5 mg of the pure ROS-Ca in 20 ml of acetone and completing with the same solvent	100 ml
Vanillin	(0.05 %)	A 50 mg of pure vanillin was dissolved in methanol and complete with same solvent	100 ml

Table 2. Preparation of drug solution (Rosuvastatin Calcium) from the pharmaceutical preparation

Tablets CRESTOR 10 mg	The ten tablets of ROS-Ca were weighed. One tablet, equivalent to 0.01 g, was weighed, and 20 ml of acetone (100 µg/ml) was dissolved in a 100 ml volumetric flask. The solution was filtered through filter paper (Whatman 41), and then the volume was completed with the same solvent. 50 ml was withdrawn and diluted in a 100 ml volumetric flask with a concentration of 50 µg/ml.
Tablets CRESTOR 10mg	The ten tablets of ROS-Ca were weighed. One tablet, equivalent to 0.02 g, was weighed, and 20 ml of acetone (100 µg/ml) was dissolved in a 100 ml volumetric flask. The solution was filtered through filter paper (Whatman 41), and then the volume was completed with the same solvent. 50 ml was withdrawn and diluted in a 100 ml volumetric flask with a concentration of 50 µg/ml.

The Preliminary study. This method is based on an acetylation-type reaction involving the interaction of hydroxyl groups in rosuvastatin calcium (ROS-Ca) with the carbonyl group of an aldehyde in the presence of concentrated sulfuric acid. The reaction produces a colored compound, which forms the basis of the spectrophotometric determination. A preliminary study was conducted to select the most suitable aldehyde reagent for optimal color development.

Results and discussion

Aldehyde type. A 1 ml of 50 µg/ml ROS-Ca solution was taken, 1 ml of aldehydes at 0.05% and 1 ml of concentrated sulfuric acid were added, a spectrum was taken for the whole solution, and the solutions were completed with acetone. Table 3 shows that the vanillin reagent at a concentration of 0.05% gives the highest wavelength and highest absorption compared to the other reagents.

Table 3. Types of aldehydes

Type of Aldehyde (0.05%)	λ_{max}	Absorbance
3,4-di methoxy benz aldehyde	460	0.233

4-hydroxy benz aldehyde	376	0.013
Vanilin	**550**	**0.495**
Salicy aldehyde	504.5	0.151
P-Anis aldehyde	520	0.131

Optimization of Vanillin Reagent Volume. The effect of varying volumes (0.25–3.0 mL) of vanillin reagent (0.05% w/v) was investigated by adding each to 1.0 mL of ROS-Ca solution (50 µg/mL), followed by the addition of 1.0 mL of concentrated sulfuric acid. After allowing the reaction to proceed for 5 minutes at room temperature to ensure color development, the solution volume was adjusted to the mark with acetone, and the absorbance was measured at 550 nm. As shown in Fig. 2, the highest absorbance was obtained with 1.0 mL of vanillin reagent, which was selected as the optimum volume for subsequent experiments.

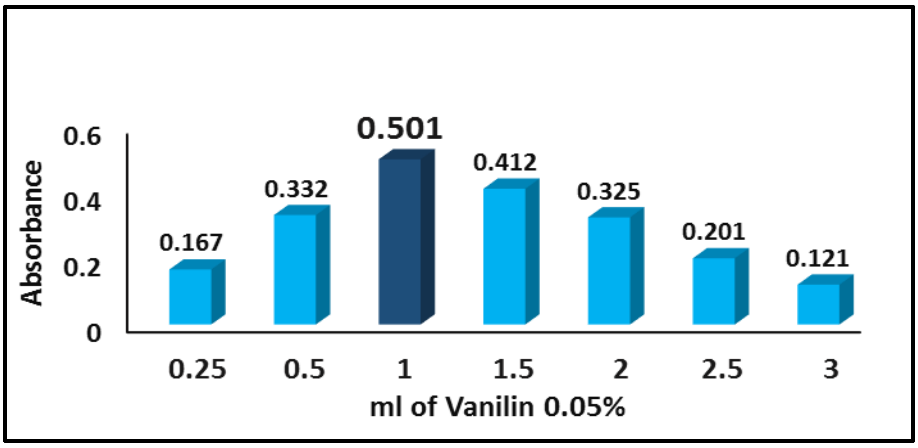

Fig. 2. The effect of vanillin volume on the absorbance

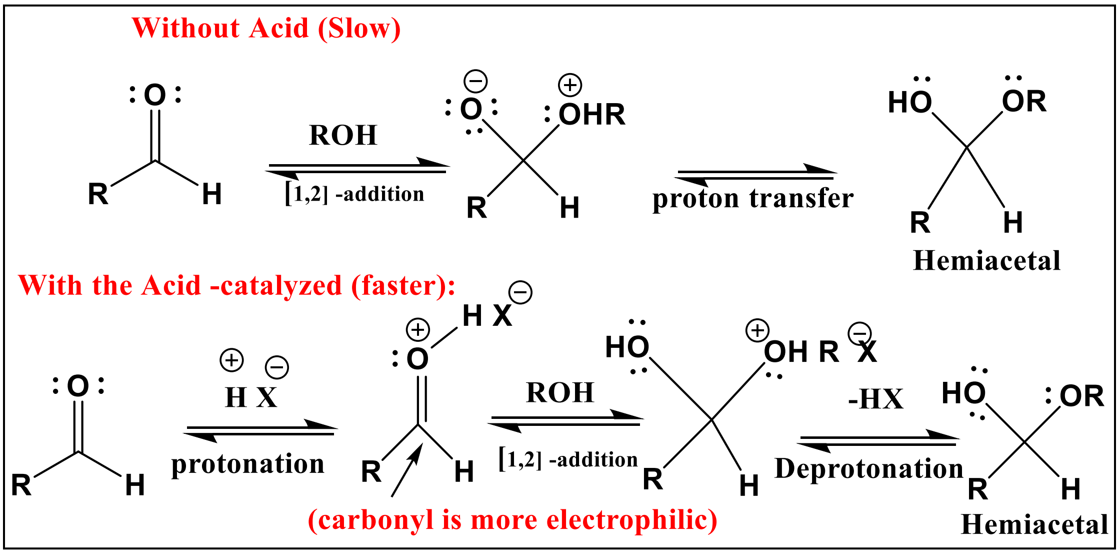

Fig. 3. The effect of acid on the resulting reaction mechanism [25]

The effect of acid type. The reaction proceeds slowly in a weakly acidic medium and does not occur under basic conditions. Therefore, a strongly acidic environment is essential to facilitate the acetylation reaction, as demonstrated in Fig. 3. To evaluate the influence of various strong acids on the acetylation reaction, 1 ml of ROS-Ca solution (50 µg/ml) was mixed with 1 ml of vanillin reagent and 1 ml of different strong acids in separate 10 ml volumetric flasks. The mixtures were allowed to stand for 5 minutes and cooled under running tap water. The volume in each flask was then completed to the mark with acetone, and the absorbance was measured at a wavelength of 550 nm (see Table 4).

As shown in Table 4, sulfuric acid was found to be the most effective acid, providing the highest absorbance of the colored product.

Table 4. Types of acid

Type of acid conc	HCl	**H_2SO_4**	HNO_3	H_3PO_4
Absorbance	0.054	**0.505**	0.014	0.112

Effect of Concentrated Sulfuric Acid Volume. Different volumes of concentrated H_2SO_4 (0.25–3.0 mL) were tested while keeping the other reaction components in the previously specified amounts. The reaction mixtures were allowed to stand for 5 minutes, and the absorbance was measured at a wavelength of 550 nm, as shown in Fig. 4. The volume of 1.5 mL of sulfuric acid gave the highest absorbance of the colored product and was therefore used in subsequent experiments.

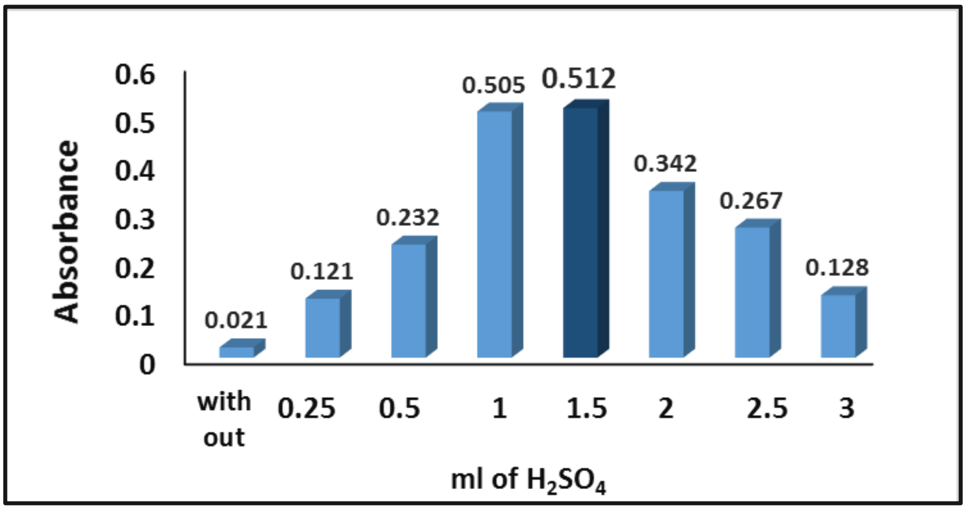

Fig. 4. Effect of concentrated H_2SO_4 volume

Study of the order of addition. The effect of the order of addition of reagents was investigated by taking 1 mL of ROS-Ca (50 µg/mL), and adding the remaining reaction components in various sequences, as previously described, in a volumetric flask. The absorbance of each mixture was measured at 550 nm (see Table 5). Sequence I showed the highest absorbance and was therefore selected for use in subsequent experiments.

Table 5. The order of addition

NO	Order of addition	Absorbance
I	**ROS-Ca+Vanillin+ H_2SO_4**	**0.513**
II	+ H_2SO_4+ Vanillin ROS-Ca	0.432
III	Vanillin + H_2SO_4+ ROS-Ca	0.454
IV	Vanillin + ROS-Ca + H_2SO_4	0.267

Study of the Effect of Reaction Time. The effect of reaction time was evaluated by adding 1 mL of ROS-Ca (50 µg/mL), 1 mL of vanillin reagent, and 1.5 mL of concentrated sulfuric acid to a volumetric flask. The mixture was shaken and allowed to react for different time intervals before dilution to volume with acetone. The absorbance was measured at a wavelength of 550 nm (see

Table 6). A reaction time of 10 minutes yielded the highest absorbance before dilution, and this condition was therefore adopted for subsequent experiments. All measurements were conducted at ambient laboratory temperature.

Table 6. The time effect on the absorbance of color product

Time, min before dilution	0	2	5	**10**	15	20	25
Absorbance	0.243	0.387	0.513	**0.521**	0.417	0.383	0.231

Study of the Effect of Solvent. The influence of various solvents on the reaction yield was investigated using 10 mL volumetric flasks. To each flask, 1 mL of ROS-Ca (50 µg/mL), 1 mL of vanillin reagent, and 1.5 mL of concentrated H_2SO_4 were added. The mixtures were allowed to react for 10 minutes to ensure completion of the reaction. Subsequently, the solutions were diluted to volume with different organic solvents. The absorbance was measured at 550 nm, and the results are illustrated in Fig. 5.

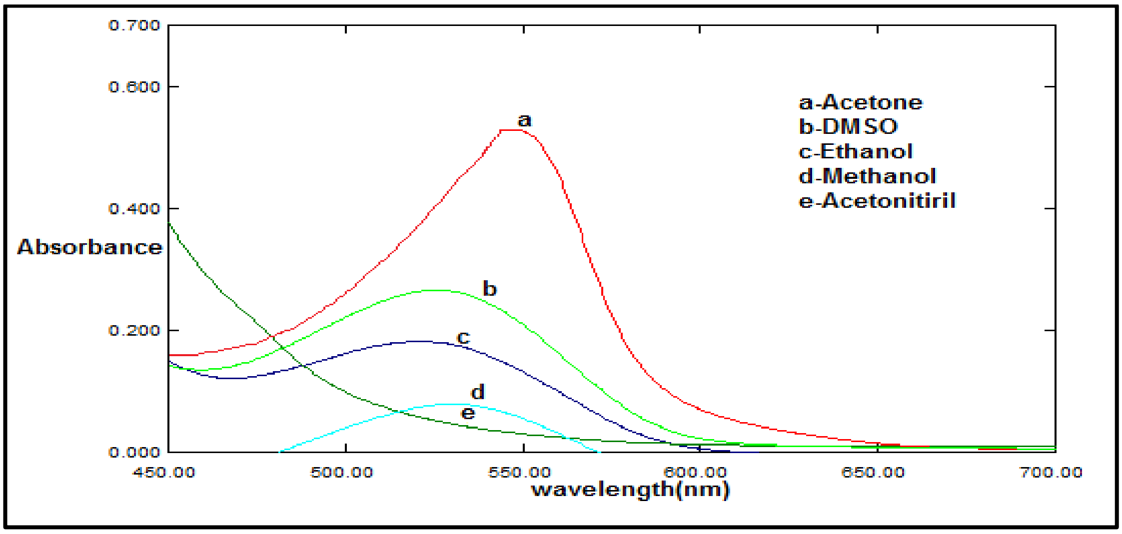

Fig. 5. Absorption spectra of 5 µg/ml of ROS with vanillin with different solvents

The effect of time on the stability of the reaction product. The effect of time on the stability of the colored reaction product formed by ROS-Ca (in the concentration range of 5–10 µg/mL) was investigated. As shown in Figure 6, the absorbance of the product remained stable from 5 to 50 minutes after the reaction, indicating that the formed chromophore is sufficiently stable and suitable for analytical determination within this time interval.

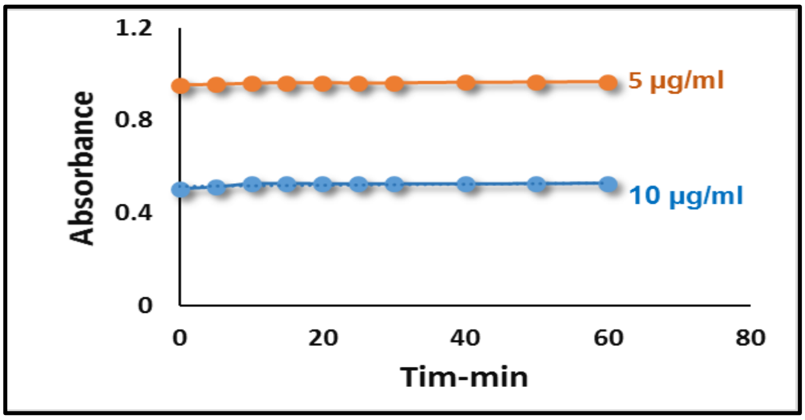

Fig. 6. The effect of time

Table 7. The optimal reaction conditions

Type of Aldehyde	Amount of reagent (ml)	Type of conc. acid	H_2SO_4 (ml)	λ_{max}	Time (min)	Solvent
Vanillin	1	H_2SO_4	1.5	550	10	Acetone

Final Absorption Spectrum. The final absorption spectrum was recorded after establishing the optimal reaction conditions. A ROS-Ca solution with a concentration of 50 µg/mL was used, and the remaining reagents were added according to the optimized conditions summarized in Table 7. The absorbance was measured at a wavelength of 550 nm. The resulting absorption spectrum is shown in Fig. 7.

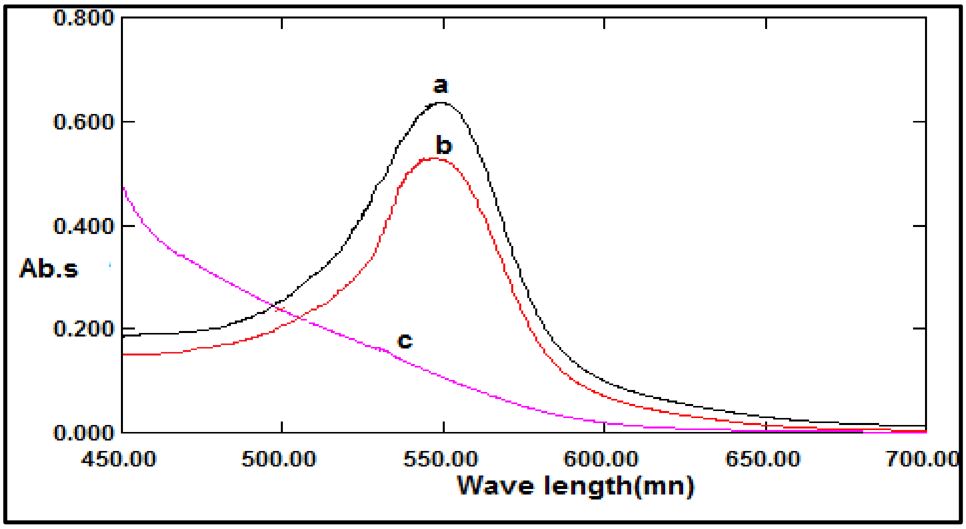

Fig. 7. Absorption spectrum of rosuvastatin calcium (ROS-Ca): (**a**) ROS-Ca solution versus acetone; (**b**) ROS-Ca solution versus blank reagent solution; (**c**) blank reagent solution versus acetone

Approved Analytical Method and Standard Calibration Curve. As shown in Table 7, after optimizing the conditions for the determination of rosuvastatin calcium (ROS-Ca), various volumes (0.25–3.0 mL) of a ROS-Ca stock solution at a concentration of 50 µg/mL were used. To each sample, 1.0 mL of vanillin reagent (0.05%) and 1.5 mL of concentrated sulfuric acid were added with gentle shaking. The reaction mixtures were allowed to stand for 10 minutes at room temperature, followed by dilution to the mark with acetone in 10 mL volumetric flasks. The absorbance was measured at 550 nm (Fig. 8).

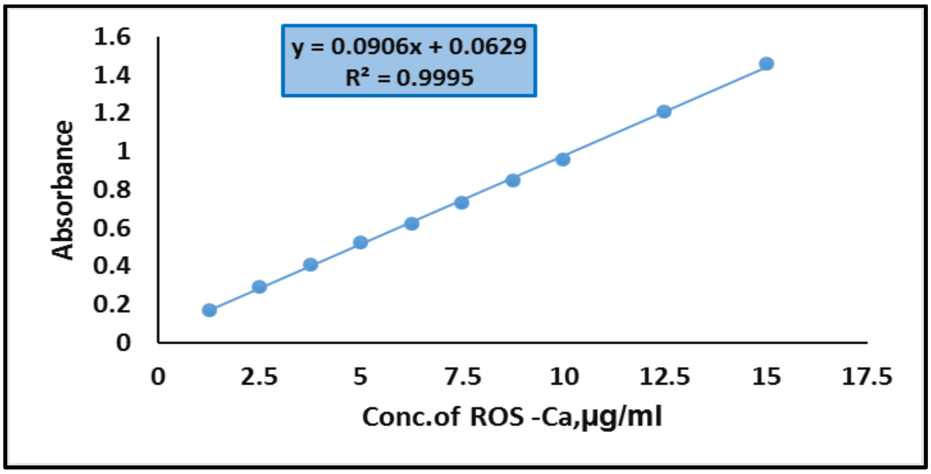

Fig. 8. Standard curve for rosuvastatin calcium

The method obeys Beer's Law in the concentration range of 1.25–15 µg/mL. The molar absorptivity (ε) of rosuvastatin calcium was found to be 9×10^4 $L \cdot mol^{-1} \cdot cm^{-1}$. The limit of detection (LOD) and the limit of quantification (LOQ) were determined to be 0.0375 µg/mL and 0.1252 µg/mL, respectively [26, 27].

Table 8. Specifications for rosuvastatin calcium

Parameters	Rosuvastatin
Linecrity range (µg/ml)	1.25-15
Molar Absorptivity (l/mol.cm)	9×10^4
Sandell's Index (µg/cm²)	0.0011
LOD and LOQ (µg/ml)	0.0375 and 0.1252
Slope	0.0906
Intercept	0.0629
Correlation Coefficient (R^2)	0.9995

Accuracy and precision of the proposed method. The accuracy and precision of the method have been calculated for the estimation of ROS-Ca based on the optimal conditions and the standard curve at three different concentrations (2.5 - 5 - 7.5 µg/ml) of ROS-Ca, each with five replicates.

Table 9. Accuracy and precision of the proposed method.

Amount Added(µg.ml^{-1}) Taken	Found	Recovery (%)	Average Recovery (%)	Relative* Error (%)	RSD* (%)	Average RSD*(%)
2.5	2.59	103.60	102.48	3.6	0.910	0.685
5	5.14	102.80		2.8	0.580	
7.5	7.58	101.06		1.06	0.565	

** Average of five measurements*

The resulting product nature. The drug compound and the reagent can also be linked in different ways continuous changes method (Job method) [28] and molar ratio method [28]: the drug compound and the reagent were prepared at the same concentration (5×10^{-5} M) adding ROS-Ca. Adding vanillin reagent, then adding concentrated sulfuric acid according to the optimal conditions, and then measuring at a wavelength of 550 nm. Figure 9 shows the sampling method and the ratio between the compound and the reagent.

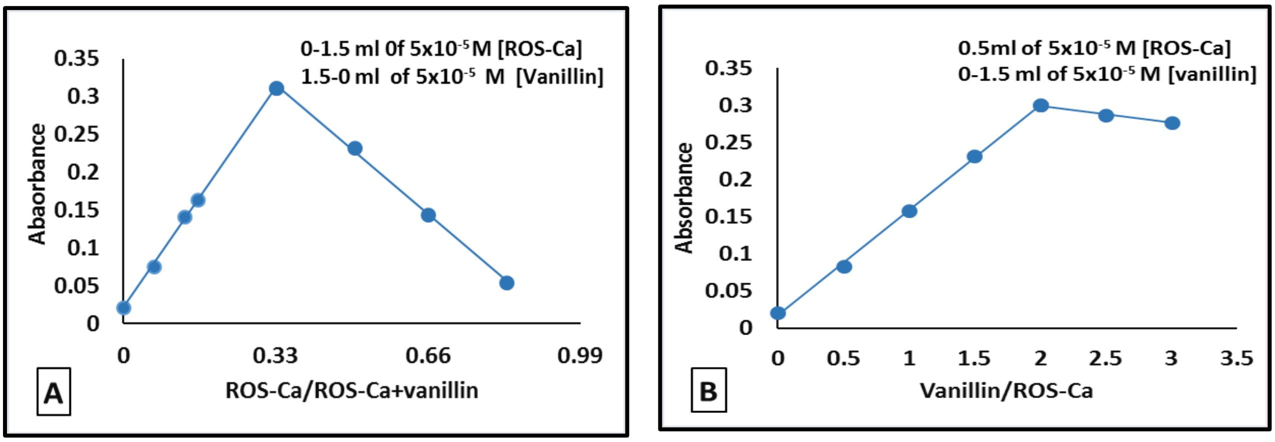

Fig. 9. Ratio between the compound and the reagent in continuous changes method (**A**) and of molar ratio method (**B**)

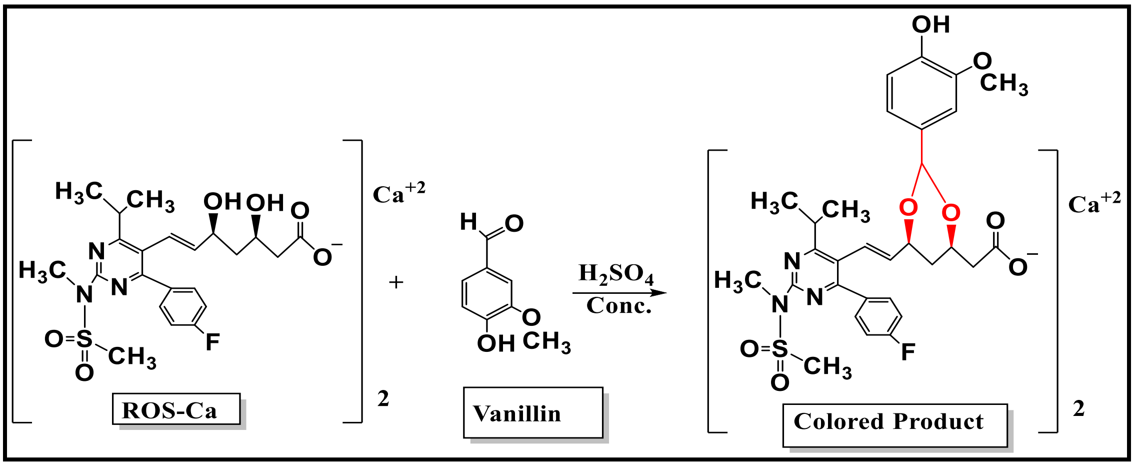

Fig. 10. The expected and proposed chemical formula for the colored product [30]

Complex stability constant. The stability constant was calculated at a ratio of 1:2 between the compound rosuvastatin and the reagent vanillin (Table 10).

Table 10. Stability constant for the colored product

Conc.of ROS-Ca		As	Am	∂	Kst (l^2/mol^2)	Kst (l^2/mol^2)
µg/ml	Mol/ml					
1.25	1.25×10^{-6}	0.085	0.114	0.254	7.28×10^{12}	4.60×10^{12}
2.5	2.5×10^{-6}	0.261	0.325	0.195	4.21×10^{12}	
5	5×10^{-6}	0.386	0.435	0.155	2.33×10^{12}	

Applying the proposed method to pharmaceutical preparations. The method was applied to estimate ROS-Ca in pharmaceutical preparations, as in Table 11, through taking different concentrations of 2.5-5-7.5 µg/ml according to the optimal conditions for the reaction on both 10 mg and 20 mg pharmaceutical preparations**.**

Table 11. Estimation of rosuvastatin calcium on pharmaceutical preparations

Prmaceutical Preparation	Certified Value	Amount Present µg/ml		Drug content found mg	Recovery* (%)	Average Recovery* (%)	Relative Error* (%)	RSD* (%)
		Taken	Found					
CRESTOR	**10mg**	2.5	2.51	10.04	100.4	100.8	0.4	0.287
		5.0	5.10	10.20	102.0		2.0	0.195
		7.5	7.51	10.01	100.1		0.1	0.183
CRESTOR	**20mg**	2.5	2.58	20.60	103.0	101.1	3.0	0.280
		5.0	5.08	20.32	101.6		1.6	0.367
		7.5	7.40	19.74	98.7		-1.3	0.263

Evaluation the proposed results with the standard additive method. To validate the accuracy and applicability of the proposed spectrophotometric method for the determination of rosuvastatin calcium (ROS-Ca) in pharmaceutical formulations, the standard addition method was employed. This technique was used to confirm the absence of matrix interferences and to assess the recovery of known amounts of ROS-Ca in the presence of excipients. The study was conducted on commercial tablet preparations (CRESTOR) containing two different labeled doses: 10 mg and 20 mg. Stock solutions were prepared at a concentration of 50 µg/mL. Two levels of ROS-Ca (2.5 µg/mL and 5 µg/mL) were added to the sample solutions. The absorbance was measured at 550 nm after applying the optimized conditions established earlier.

As shown in Fig. 11, the results obtained from the standard addition method closely match those of the proposed method, indicating high recovery rates and minimal matrix effects. This confirms the accuracy and reliability of the proposed method for routine analysis of ROS-Ca in pharmaceutical dosage forms.

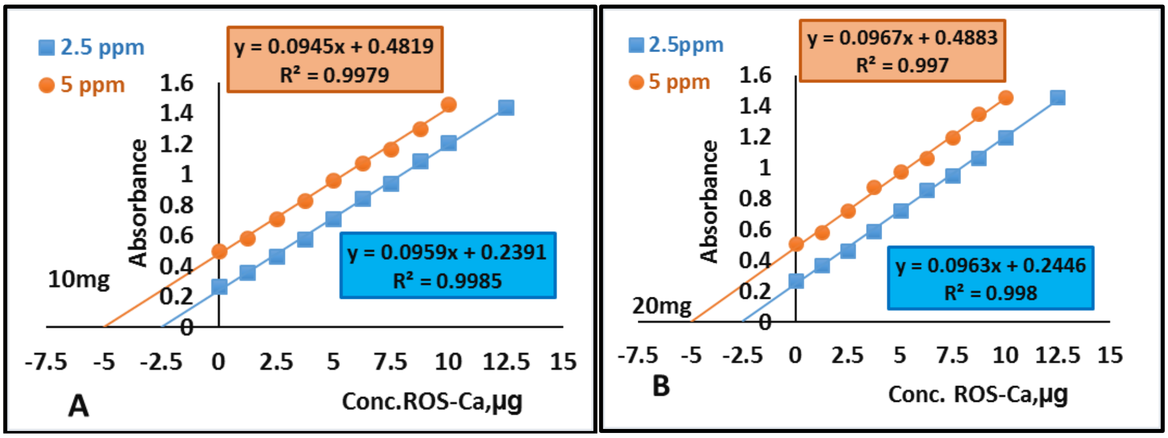

Fig. 11. (A-B) The standard addition per kilogram (10 and 20 mg) of the compound rosuvastatin

Table 12. Comparison of method

Analytical parameters	Present method	Literature method [31]	Literature method [32]
Type of reaction	nucleophilic addition	nucleophilic addition	Charge transfer
λ_{max} (nm)	550	570	579
Reagent	Vanillin	P-dimethyl aminobenzaldehyde	Quinalizarin
Beer's law (µg/mg)	(1.25-15)	(2-16)	6-15
Molar absorptivity (l/mol.cm)	9×10^4	5×10^4	1.5×10^4
Correlation Coefficient (R^2)	0.9995	0.9995	0.9989
Solvent	Acetone	Acetone	DMSO

Conclusion

A simple, accurate, and sensitive spectrophotometric method was developed for the determination of rosuvastatin calcium (ROS-Ca) in pharmaceutical preparations. The method is based on a nucleophilic addition reaction between ROS-Ca and vanillin in a strongly acidic medium (concentrated sulfuric acid), followed by dilution with acetone. The absorbance of the resulting colored product was measured at 550 nm. The method obeys Beer's law over the concentration range of 1.25–15 µg/mL. The proposed method demonstrated good sensitivity, precision, and accuracy, making it suitable for routine analysis of ROS-Ca in dosage forms.

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