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Research Article

STABILITY-INDICATING PLS AND PCR CHEMOMETRIC METHODS FOR THE DETERMINATION OF ROSUVASTATIN IN PRESENCE OF ITS TWO OXIDATIVE DEGRADATION PRODUCTS

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ABSTRACT

Two multivariate calibration methods, including principal component regression (PCR) and partial least square (PLS), have been used for the determination of rosuvastatin in the presence of its oxidative degradation products. The PCR and PLS techniques are useful in spectral analysis due to the simultaneous inclusion of many spectral wavelengths instead of the single wavelength used in derivative spectrophotometry, thus a great improvement in the precision and predictive abilities of these multivariate calibrations is observed. A calibration set was constructed for the mixture and the best model was used for the prediction of the concentration of the selected drug. The proposed procedures were applied successfully in the determination of rosuvastatin in laboratory-prepared mixtures and in commercial preparations. Rosuvastatin was analyzed with mean accuracies 99.93 ± 0.866 and 99.99±0.645 using the PCR and PLS methods respectively. The validity of the proposed methods was assessed using the standard addition technique. The proposed procedures were found to be rapid and simple and required no preliminary separation. They can therefore be used for the routine analysis of rosuvastatin in quality-control laboratories.

Keywords: Rosuvastatin, Chemometry, Stability indicating method.

INTRODUCTION

Rosuvastatin (RC), bis ((E)-7-(4-(4-flurophenyl)-6-1sopropyl-2-(methyl (methylsulfonyl) amino) pyrmidin-5yl)(3R,5S)-3,5-dihydroxyhept-6-enoic acid) calcium salt is a highly effective 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. It is widely used for the treatment of hyperlipidemia. In clinical trials, rosuvastatin achieved marked reductions in serum levels of LDL cholesterol, accompanied by modest increases in HDL cholesterol and reductions in triglycerides[1-3]. It may also be used in patients with homozygous familial hypercholesterolaemia. Rosuvastatin is given orally as the calcium salt, although the doses are expressed in terms of the base.

Chemometrics is the art of processing data with various numerical techniques in order to extract useful information, [4]. It is the application of mathematical and statistical methods to design optimum procedures and to provide maximum chemical information through the analysis of chemical data.

Quantitative spectroscopy has been greatly improved by the use of a variety of multivariate statistical methods[5-12]. Multivariate calibrations are useful in spectral analysis because of the simultaneous inclusion of multiple spectral intensities which can greatly improve the precision and applicability of quantitative spectral analysis.[13]

Despite of the wide application of rosuvastatin in the treatment of hyperlipidemia, a literature survey reveals that only few methods have been reported for the determination of RC in pharmaceutical formulation and biological samples including HPLC[14-18], spectrophotometry[19] and capillary electrophoresis[20]. Simultaneous determination of rosuvastatin and ezetimibe by spectrophotometry[21-22] and HPLC[23].

These methods depend on measuring the amplitude at one wavelength, which may be affected by several factors (for example, noise, scanning speed, Δλ and smoothing function). All these factors were overcome by the multivariate calibrations, which was the trigger for this work. While no method has been developed for the determination of RC in the presence of its two oxidative degradation products (ROD, ROU).

The present work aims to develop feasible, sensitive and specific analytical procedures for the analysis of rosuvastatin in the presence of its two oxidative degradation products. Adaptation of the proposed procedures to the analysis of the available dosage forms is also an important task in order to solve problems encountered in quality control.

MATERIALS AND METHODS

Apparatus

SHIMADZU dual beam UV-visible spectrophotometer (Kyoto/ Japan), model UV-1601 PC connected to IBM compatible and a HP1020 laser jet printer. The bundled software, UV- Probe personal spectroscopy software version 2.21 (SHIMADZU) was used. The spectral band is 2 nm and scanning speed is 2800 nm/min with 0.5 nm interval.

The absorption spectra of the reference and test solutions were carried out in a 1 cm quartz cells over the range of 210-330 nm. PLS and PCR were modeled using PLS toolbox 2.0 software under MATLAB® 6.5.

Reagents and chemicals

All chemicals used were of analytical grade and solvents are of spectroscopic grade.

Methanol (E. Merck, Darmstadt, Germany), 30% H_2O_2 ,ethyl acetate: concentrated ammonia (specific gravity 0.91) (Adwic , El-Nasr Pharmaceutical Chemicals. Co. Cairo, Egypt)

Pure RC certified to contain 99.75%, was kindly provided by Chemipharm Pharmaceutical industry, 6th October, Egypt.

Rosuvast tablets labeled to contain 10 mg/tablet rosuvastatin, batch number 100333A, manufactured by Chemipharm Pharmaceutical industry, 6th October, Egypt. Sovikan tablets, labeled to contain 10, 20 mg per tablet rosuvastatin calcium Batch numbers: 003, 001, respectively, manufactured by Hikma Pharma, 6th October, Egypt.

Procedure

Degradation of rosuvastatin

The suggested pathways for degradation are shown in Fig.-1.

Preparation of the oxidative degradation products

The drug (50 mg) was weighed in a conical flask, dissolved in 20ml methanol, 5 ml hydrogen peroxide 30% (v/v) was added and the solution was subjected to reflux at 100 °C for three hours. The degradation products were separated on preparative TLC plates using a mixture ethyl acetate: methanol: ammonia (7: 3: 0.01 by

volume) as a developing solvent. The degraded solution was applied as a band onto several preparative TLC plates. The plates were developed using the aforementioned solvent system in chromatographic tank previously saturated for 30 minutes with the developing solvents and then dried in air. The bands were visualized under UV light at 254 nm, by examining the TLC plate, three different spots were obtained, one for the intact drug (R_f =0.32) and the other two spots for two oxidative degradation products (R_f =0.1, R_f =0.75 for oxidative degradates II (ROD) and I(ROU), respectively).

Then the bands were scraped and the silica was suspended in the least amount of methanol. Filtered and filtrate was left to dry at room temperature (25°C) to obtain the two degradation products. The purity of the degradation products obtained was tested by dissolving a small portion in methanol, applying onto TLC plates and developing using the previously mentioned solvent system. The structure of the isolated degradation products was elucidated using mass spectrometry.

Standard stock solutions

a. RC standard solution; 100.0 µg/ml in methanol

b. ROD standard solution; 100.0 µg/ml in methanol

c. ROU standard solution; 100.0 µg/ml in methanol

PCR and PLS chemometric models

Construction of the calibration set

Different mixtures of rosuvastatin and its degradation products were prepared by transferring different volumes of their standard solutions (100.0 µg/ml) into 25 ml measuring flasks as shown in Table -1. The volume was completed with methanol and the absorbance of these mixtures was recorded between 210 and 330 nm at 1 nm intervals Fig.-2.

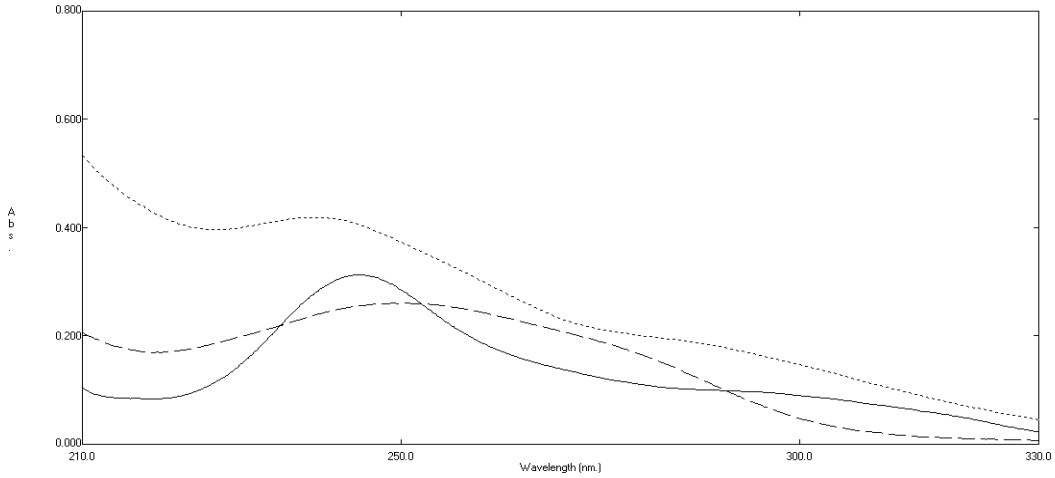

Fig. 2: Absorption spectra for RC (____) , ROD (------) and ROU (.....) each is 10 µg/ml.

Pre-processing the data

The regions from 200-210 nm and above 330 nm were rejected.

Constructing the models

To build the PCR and PLS models, the calibration set absorbance was used and concentration matrices together with PLS-Toolbox 2.0 software for the calculations.

Selection of the optimum number of factors to build the PCR and PLS models

The cross validation method, leaving out one sample at a time, was used to select the optimum number of factors[4], Given a set of fifteen calibration samples, the PCR and PLS calibrations were performed on ten samples. By using this calibration, the concentration of the sample left out was predicted. This process was repeated a total of fifteen times until each sample had been left out once. The predicted concentrations were then compared with the known concentrations and the root mean square error of calibration (RMSECV) was calculated. The RMSECV was calculated in the same manner each time a new factor was added to the model. The maximum number of factors used to calculate the optimum RMSECV was selected to be three. The method described by Haland and Thomas [6, 8] was used for selecting the optimum number of factors.

Construction of the validation set

Different mixtures of rosuvastatin and its degradation products were prepared by transferring different volumes from their standard solutions into 25 ml measuring flasks as shown in Table-2. The suggested models were applied to predict the concentrations of rosuvastatin. The predicted concentrations of the validation samples were plotted against the actual concentration values to evaluate the predictive abilities of the suggested chemometric methods.

Application of the proposed methods for the analysis of rosuvastatin in certain pharmaceutical formulations

Twenty tablets were accurately weighed and powdered, an amount of the powder equivalent to 25mg of rosuvastatin were accurately weighed into a 250-ml beaker and sonicated in 30 ml methanol for 15 minutes, filtered into 250-ml volumetric flask. The residue was washed three times each using 10 ml methanol and completed to the mark with the same solvent. 2.5 ml of the extracted solution was accurately transferred into a 25-ml measuring flask and completed to the mark using the same solvent. The spectra of the prepared solutions were measured then the developed multivariate models, PCR and PLS were applied for calculation of rosuvastatin concentration.

RESULTS AND DISCUSSION

Two chemometric methods were applied for the determination of rosuvastatin in presence of its oxidative degradation products including PCR and PLS.

Rosuvastatin was subjected to oxidation within 3 hours upon reflux in 30% H_2O_2. The proposed scheme for degradation is shown in Fig. 1.

Mass spectroscopy was able to verify the structures of the degradation products, where the parent molecular ion peaks for the ROD and ROU were identified at m/z = 514.37 and m/z =497.96, respectively, in accordance with the molecular weights of the suggested degradation products Figs- 3a, 3b, respectively.

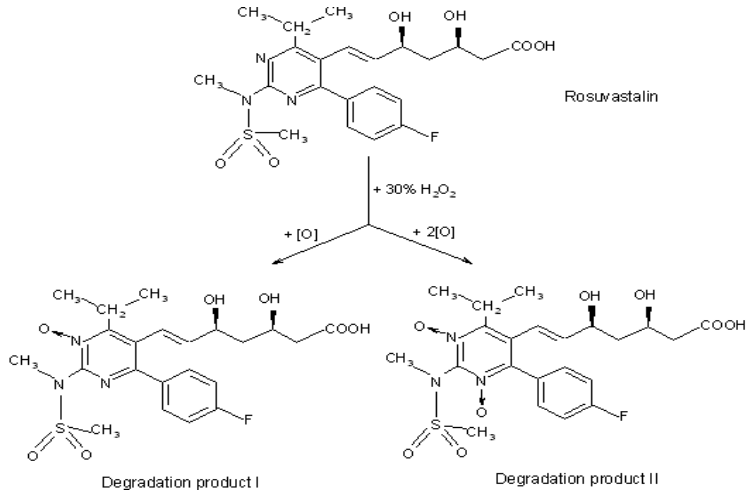

Fig. 1: Suggested scheme for the oxidative degradation of rosuvastatin

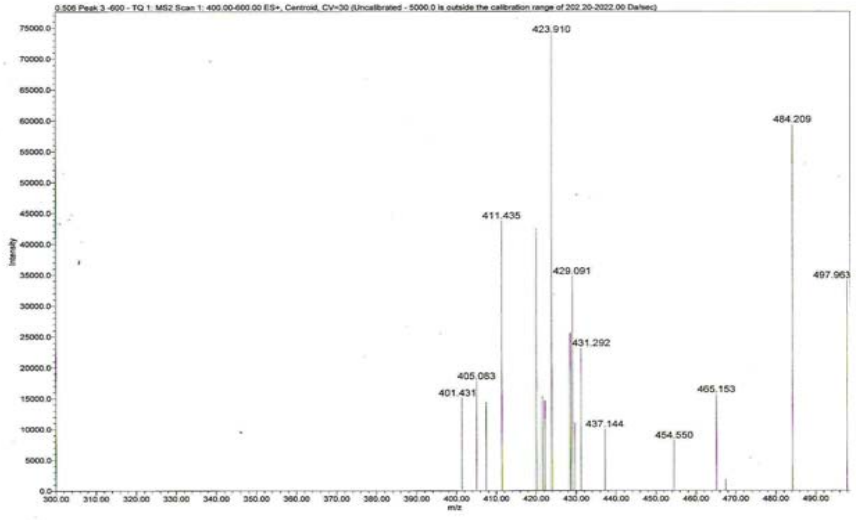

Fig. 3a: Mass spectrum of rosuvastatin oxidative degradation product I.

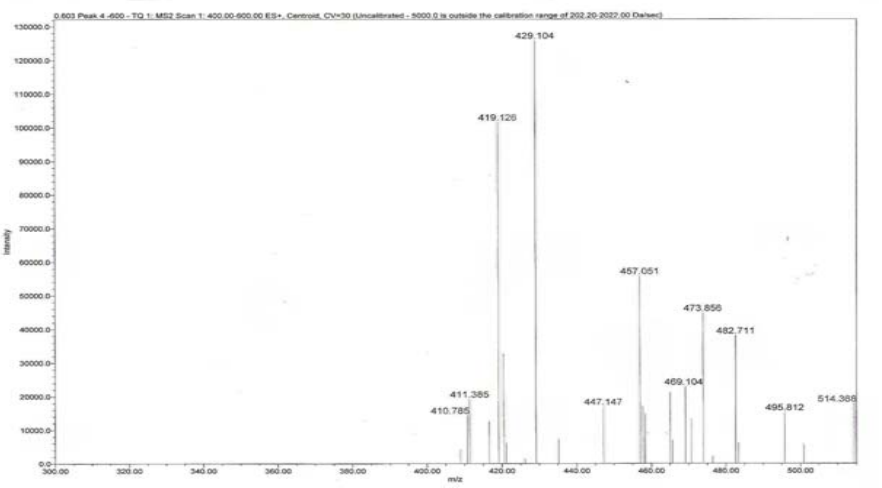

Fig. 3b: Mass spectrum of rosuvastatin oxidative degradation product II.

Table 1: The concentration of different mixtures of RC, ROD and ROU used in the calibration set

Mixture no.	RC µg/ml	ROD µg/ml	ROU µg/ml	PLS			PCR	
				Found* RC µg/ml	Recovery of RC	%	Found* RC µg/ml	Recovery % of RC
1	15	15	15	15.11	100.73		15.07	100.47
2	15	5	5	14.96	99.73		14.96	99.73
3	5	5	25	4.98	99.6		4.92	98.4
4	5	25	10	4.92	98.4		4.93	98.6
5	25	10	25	25.12	100.48		25.14	100.56
6	10	25	15	10.03	100.3		10.04	100.4
7	25	15	10	25.03	100.12		24.96	99.84
8	15	10	10	14.98	99.87		15.08	100.53
9	10	10	20	10.03	100.3		10.09	100.9
10	10	20	25	10.1	101		10.01	100.1
11	20	25	20	19.85	99.25		19.83	99.15
12	25	20	15	24.97	99.88		24.97	99.88
13	20	15	25	20.01	100.05		19.76	98.8
14	15	25	25	14.94	99.6		15.21	101.4
15	25	25	5	25.14	100.56		25.04	100.16
Mean					99.99			99.93
S.D.					0.645			0.866
R.S.D.%					0.645			0.867

*Average of three different determinations.

Mixtures with different concentrations of rosuvastatin and its oxidative degradation products were used as calibration samples to construct the models Table -1. The spectra of these mixtures were collected and examined, the noisy region from 200-210 nm and the near zero absorbance after 330 nm accounted for the rejection of these parts from the spectra.

The selection of the optimum number of factors for the PCR and PLS techniques was a very important step before constructing the models because if the number of factors retained was more than required more noise would be added to the data. On the other hand, if the number retained was too small meaningful data that could be necessary for the calibration might be discarded. Different methods could be used to determine the optimum number of factors[4,24]. In this study, the leave-one-out cross validation method was used and the RMSECV values of different developed models were compared. Three factors were found suitable for both PCR and PLS methods as in Figs.- 4a and 4b.

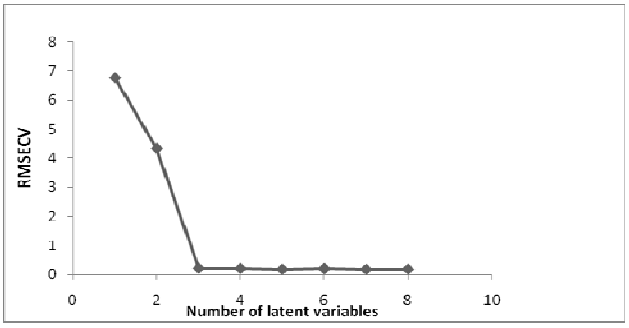

Fig. 4a: RMSECV plot of the cross validation results of the training set as a function of the number of principal components used to construct the PCR calibration of rosuvastatin and its oxidative degradates.

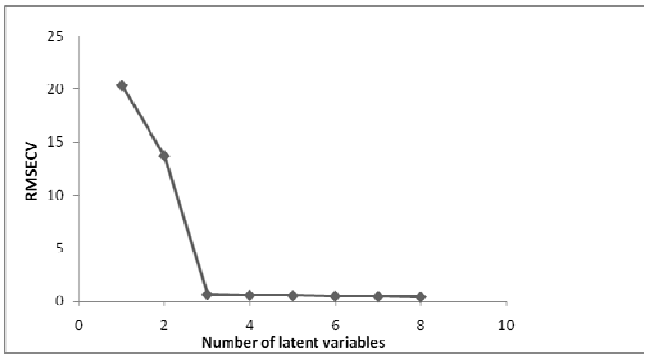

Fig. 4b: RMSECV plot of the cross validation results of the training set as a function of the number of principal components used to construct the PLS calibration of rosuvastatin and its oxidative degradates.

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Table 2: Percent recoveries of RC in the validation set using PCR and PLS methods

Mixture. no.	Mixture Composition (µg/ml)			Recovery* % of RC	
				PLS method	PCR method
	RC	ROD	ROU	RC	RC
1	25	5	20	99.96	100.04
2	5	20	5	99.80	96.60
3	20	5	15	99.80	98.15
4	5	15	20	100.20	99.40
5	15	20	20	99.73	100.67
6	20	20	10	99.80	98.10
7	20	10	5	99.90	98.55
8	10	5	10	100.20	98.60
9	5	10	15	99.60	99.40
10	10	15	5	100.80	99.20
Mean				**99.98**	**98.87**
S.D.				**0.346**	**1.141**
R.S.D.%				**0.346**	**1.154**

*Average of three different determinations.

To validate the predictive ability of the suggested models, the PCR and PLS models were employed to predict the concentration of rosuvastatin in ten laboratory prepared mixtures containing different ratios, where satisfactory results were obtained Table-2

The predicted concentrations of the validation samples were plotted against the known concentrations to determine whether the model accounted for the concentration variation in the validation set. Plots were expected to fall on a straight line with a slope of 1 and zero intercept. Rosuvastatin, in all samples, lay on a straight line and the equations of these lines were shown in table y = 0.9925x – 0.0367 (r = 0.9996) for PCR and y = 0.998 x + 0.020 (r = 0.9999) for PLS. Both plots had a slope of nearly 1 and an intercept close to zero.

The proposed PCR and PLS methods were successfully used for the determination of RC in certain pharmaceutical formulations. The results were shown in Table -3. Each value indicated is the mean of 3 determination of the same commercial batch. The validity of the proposed methods was further assessed by applying the standard addition technique.

Statistical analysis of the results obtained by the suggested methods and the comparison method [19] of analysis was carried out. Table -4 showed that the calculated t and F values were less than the theoretical ones, indicating no significant differences between the proposed methods and the comparison method.

Table 3: Determination of rosuvastatin in certain pharmaceutical formulations by the proposed chemometric methods and application of standard addition technique.

Pharmaceutical Formulation	In presence of oxidative degradates		Taken* (µg/ml)	Added (µg/ml)	In presence of oxidative degradates			
	PCR	PLS			PCR		PLS	
					Found (µg/ml)	Recovery%	Found (µg/ml)	Recovery%
Rosuvast 10 tablets	9.96	9.98	10.00	5.00	4.97	99.40	4.97	99.40
				10.00	9.92	99.20	9.90	99.00
Batch No.100333A				15.00	14.86	99.06	14.82	99.87
			Mean			99.22		99.42
			S.D.			0.171		0.435
			R.S.D.%			0.172		0.438
Sovikan 10 tablets	10.10	10.12	10.00	5.00	5.02	100.40	5.04	100.80
Batch No.003				10.00	9.99	99.90	10.00	100.00
				15.00	14.98	99.87	14.96	99.73
			Mean			100.06		100.18
			S.D.			0.298		0.556
			R.S.D.%			0.298		0.555
Sovikan 20 tablets	10.02	10.03	10.00	5.00	4.98	99.60	4.99	99.80
Batch No.001				10.00	10.01	100.10	10.02	100.20
				15.00	15.04	100.27	15.05	100.33
			Mean			99.99		100.11
			S.D.			0.348		0.276
			R.S.D.%			0.348		0.276

*Average of three different determinations.

Table 4: Statistical analysis of the results obtained by applying the proposed chemometric methods and the comparison [19] method for the determination of Rosuvastatin in pure bulk powder

Value	PLS	PCR	comparison [19] method*
	RC		RC
Mean	99.99	99.93	99.760
S.D.	0.645	0.866	1.179
R.S.D.%	0.645	0.867	1.179
n	15	15	5
Variance	0.416	0.750	1.390
Student's t test (2.086)**	0.597	0.410	-------
F value (2.850)**	2.685	1.489	-------

**Direct UV spectrophotometric method at 243nm.

**The values in parenthesis are the corresponding tabulated t and F values at P=0.05.

CONCLUSION

From the above discussion we can conclude that the proposed methods are simple, do not require complicated techniques or instruments, sensitive and selective, thus can be applied for the routine analysis of rosuvastatin in pure form and in its available dosage forms.

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