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

ABSTRACT

Two multivariate calibration methods, including principal component regression (PCR) and partial least square (PLS), have been used for the determination of rosuvastatin calcium in the presence of its acid 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 calcium in laboratory-prepared mixtures and in commercial preparations. Rosuvastatin calcium was analyzed with mean accuracies 99.93 ± 0.699 and 100.06±0.630 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.

1.0. INTRODUCTION

Rosuvastatin calcium (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..

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 calcium 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, $\Delta\lambda$ 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 acid degradation products (RAD, RAU).

The present work aims to develop feasible, sensitive and specific analytical procedures for the analysis of rosuvastatin calcium in the presence of its two acid 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.

2. Experimental

2.1. 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.

2.2. Reagents and chemicals

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

Methanol (E. Merck, Darmstadt, Germany), 1N HCl, 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 calcium, 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.

2.3. Procedure

2.3.1. Degradation of rosuvastatin

Preparation of the acid degradation products.

The drug (50 mg) was weighed in a conical flask, dissolved in 20 ml methanol, 25 ml 1 N HCl 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 of ethyl acetate: methanol: ammonia (7: 3: 0.01; v/v/v) as a developing solvent.

2.3.2 Standard stock solutions

a. RC standard solution; 100 µg/ml in methanol

b. RAD standard solution; 100 µg/ml in methanol

c. RAU standard solution; 100 µg/ml in methanol

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2.3.3. PCR and PLS chemometric models

Construction of the calibration set

Different mixtures of rosuvastatin calcium and its degradation products were prepared by transferring different volumes of their standard solutions (100 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.-1.

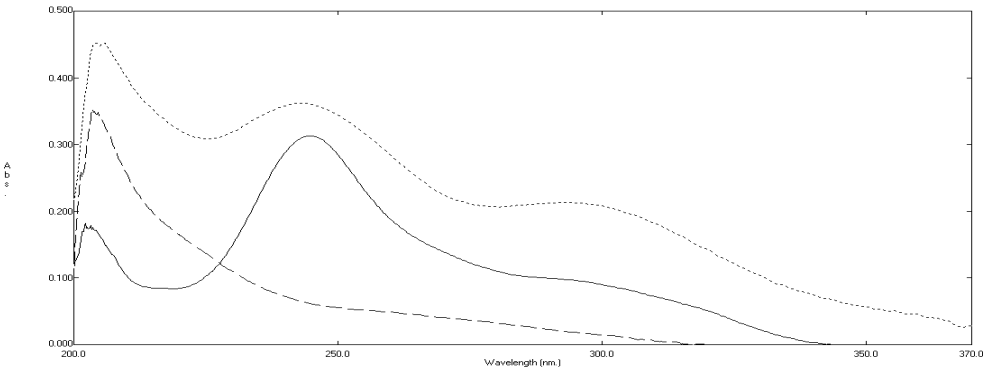

Fig. 1: Absorption spectra for RC (_____), RAD (------) and RAU (……) each is 10 µg/ml.

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

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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 calcium in certain pharmaceutical formulations

Twenty tablets were accurately weighed and powdered, an amount of the powder equivalent to 25mg of RC 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 RC concentration.

3.0. Results and Discussion

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

Rosuvastatin was found to degrade under acidic, oxidative, photolytic conditions but was comparitively stable under neutral and basic conditions [14].

In this study, RC was degraded by refluxing in 1 N HCl (methanolic solution) and the degradation process was monitored by spotting on TLC plates at 30 minutes intervals and developing using ethyl acetate : methanol : ammonia (7: 3: 0.01; v/v/v) as developing solvent, complete degradation of RC occurs after 3 hours and the suggested pathway for degradation is represented in (Figure 2). The two degradation products were separated and their structure was elucidated by IR and mass spectrometry.

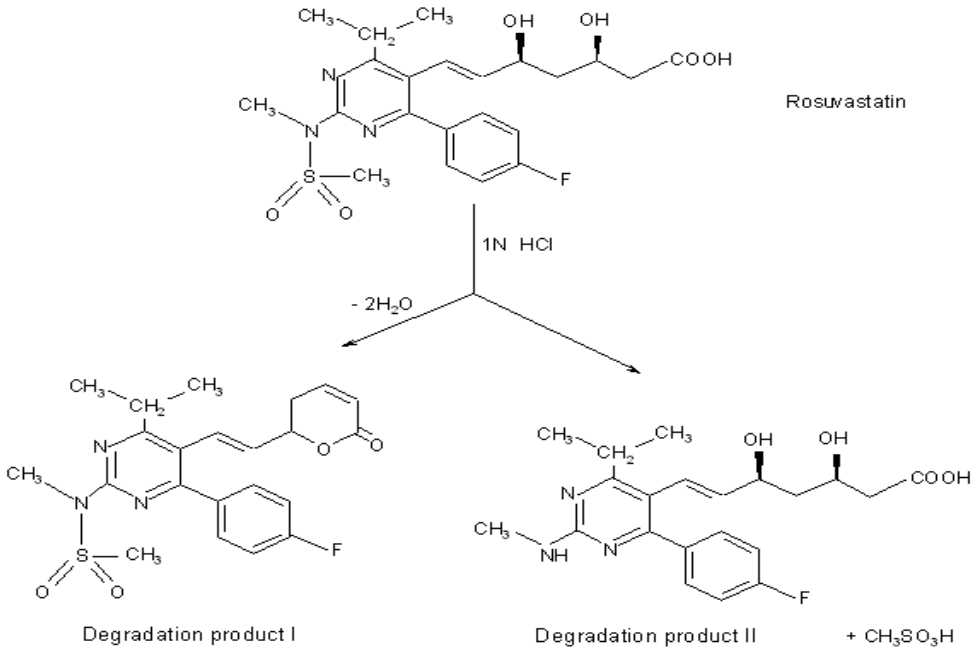

Fig. 2: Suggested scheme for the acid degradation of rosuvastatin calcium**.**

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Mass spectroscopy was able to verify the structures of the degradation products, where the parent molecular ion peaks for the RAD and RAU were identified at m/z = 514.37 and m/z =497.96, respectively, in accordance with the molecular weights of the suggested degradation products.

Mixtures with different concentrations of rosuvastatin and its acid degradation products were used as calibration samples to construct the models Table -1

Mixture no.	RC µg/ml	RAD µg/ml	RAU µg/ml	PLS		PCR	
				Found* RC µg/ml	Recovery % of RC	Found* RC µg/ml	Recovery % of RC
1	15	15	15	14.97	99.80	14.95	99.67
2	15	5	5	14.99	99.93	14.99	99.93
3	5	5	25	4.97	99.40	4.92	98.4
4	5	25	10	4.97	99.40	4.94	98.8
5	25	10	25	25.01	100.04	25.02	100.08
6	10	25	15	10.04	100.40	10.07	100.7
7	25	15	10	25.02	100.08	25.04	100.16
8	15	10	10	15.01	100.07	15.03	100.2
9	10	10	20	10.02	100.20	10.05	100.5
10	10	20	25	10.06	100.6	10.12	101.2
11	20	25	20	19.95	99.75	19.89	99.45
12	25	20	15	24.99	99.96	24.97	99.88
13	20	15	25	19.99	99.95	19.96	99.8
14	15	25	25	14.98	99.87	15.01	100.07
15	25	25	5	25.01	100.04	25.01	100.04

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Mean	99.97	99.93
S.D.	0.317	0.689
R.S.D.%	0.317	0.689

TABLE 1: The concentration of different mixtures of RC, RAD and RAU used in the calibration set**.**

*Average of three different determinations.

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.- 3a and 3b.

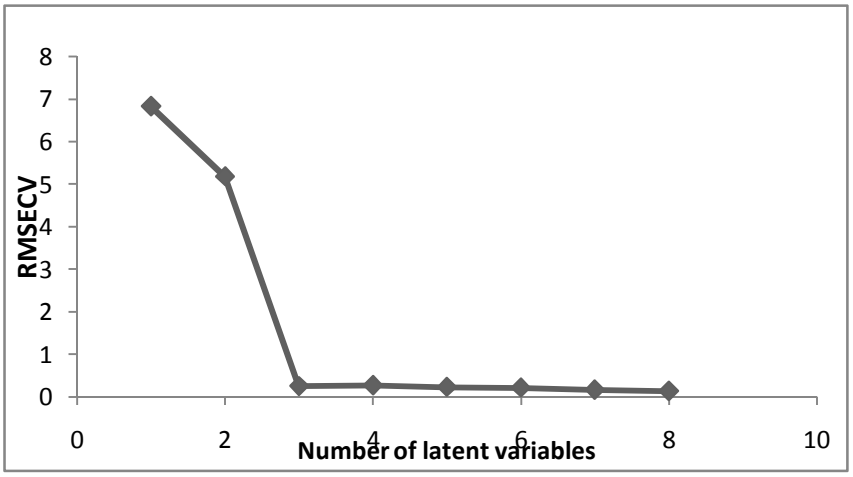

FIG. 3A: RMSECV plot of the cross validation results of the training set as a function of the number of principal components used to construct the PCR calibration of rosuvastatin and its acid degradates.

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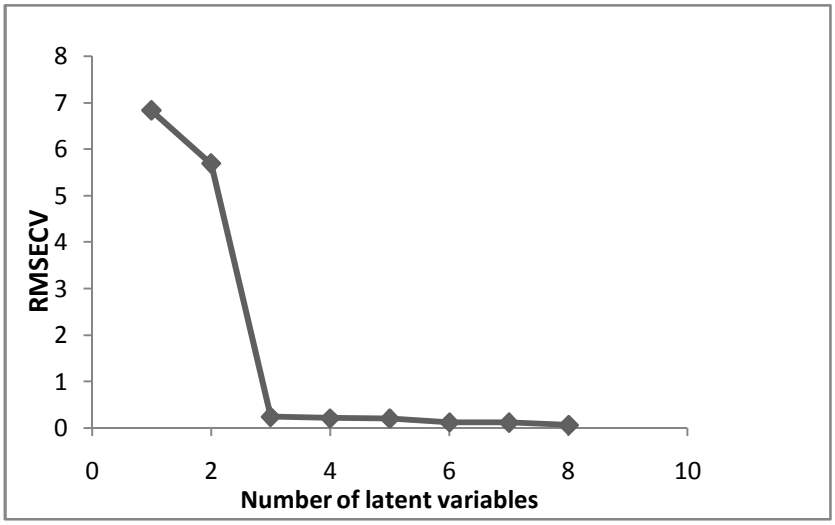

FIG. 3B: RMSECV plot of the cross validation results of the training set as a function of the number of principal components used to construct the PLS calibration of rosuvastatin and its acid degradates.

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

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Mixture. no.	Mixture Composition (µg/ml)			Recovery* % of RC	
				PLS method	PCR method
	RC	RAD	RAU	RC	RC
1	25	5	20	99.88	99.80
2	5	20	5	99.20	98.00
3	20	5	15	99.95	99.60
4	5	15	20	98.20	98.40
5	15	20	20	99.53	99.53
6	20	20	10	100.15	99.95
7	20	10	5	100.05	100.00
8	10	5	10	100.90	101.50
9	5	10	15	99.00	98.20
10	10	15	5	100.70	101.10
	Mean			**99.76**	**99.61**
	S.D.			**0.886**	**1.162**
	R.S.D.%			**0.888**	**1.167**

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

*Average of three different determinations.

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 $y = 0.9998x + 0.0025$ ($r = 0.9999$) for PCR and $y = 1.0029 x - 0.0229$ ($r = 0.9999$) for PLS. Both plots had a slope of nearly 1 and an intercept close to zero.

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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.

Pharmaceutical preparation	Claimed taken* (µg/ml)		Added (µg/ml)	In presence of acid degradates			
	PCR	PLS		PCR		PLS	
				Found (µg/ml)	Recovery%	Found (µg/ml)	Recovery%
Rosuvast 10 tablets Batch No.100333A	9.98	9.95	5.00	4.99	99.80	4.98	99.60
			10.00	9.97	99.70	9.98	99.80
			15.00	15.03	100.20	15.06	100.40
			Mean		99.90		99.93
			S.D.		0.265		0.416
			R.S.D.%		0.265		0.416
Sovikan 10 tablets Batch No.003	10.12	10.16	5.00	4.96	99.20	4.97	99.40
			10.00	9.99	99.90	10.01	100.10
			15.00	14.99	99.93	14.97	99.80
			Mean		99.68		99.77
			S.D.		0.413		0.351
			R.S.D.%		0.414		0.352
Sovikan 20 tablets Batch No.001	10.01	10.02	5.00	5.01	100.20	5.04	100.80
			10.00	10.15	101.50	10.12	101.20
			15.00	15.10	100.67	15.05	100.33
			Mean		100.79		100.78
			S.D.		0.658		0.435
			R.S.D.%		0.653		0.432

*Average of three different determinations.

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

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Statistical analysis of the results obtained by the suggested methods and the reported 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 reported method.

Value	PLS	PCR	Reported [19] method*
Mean	100.06	99.93	99.75
S.D.	0.630	0.699	1.057
R.S.D.%	0.630	0.699	1.060
n	15	15	7
Variance	0.397	0.489	1.117
Student's t test (2.086)**	0.777	0.438	-------
F value (2.850)**	2.814	2.284	-------

Table 4: Statistical analysis of the results obtained by applying the proposed chemometric methods and the reported method for the determination of rosuvastatin in pure bulk powder.

**Direct UV spectrophotometric method at 244 nm.

**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.

REFERENCES

1. Sweetman S.C. (Ed.), Martindale The Complete Drug Reference, 34th ed., Pharmaceutical Press, London, UK 2005: p.1249.

2. Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E.Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses . Am. J. Cardiol. 2003; 92(2):152-160.

3. Bergman E., Forsell P, Tevell A, Persson EM., Hedeland M, Bondesson U, et al. Biliary secretion of rosuvastatin and bile acids in humans during the absorption phase. Eur J. Pharm Sci 2006; 29:205-214.

4. Kramer R., Chemometric Techniques for Quantitative Analysis, Marcel Dekker Inc.: New York, 1998.

5. Espinosa M. A., Munoz de la Pena A., Salinas F. Simultaneous determination of 2-furfuraldehyde, 5-hydroxymethylfurfuraldehyde and malonaldehyde in mixtures by derivative spectrophotometry and partial least- squares analysis. Anal. Chim. Acta. 1993; 276(1): 141-149.

6. Haland D. M., Thomas E. V. Partial least-squares methods for spectral analyses.1. Relation to other quantitative calibration methods and the extraction of qualitative information. J. Anal. Chem. 1988; 60 (11): 1193–1202.

7. Lindberg W., Persson J. A., Wold S. Partial least squares method for spectrofluorimetric analysis of mixtures of humic acid and lignin sulphonate. J. Anal. Chem. 1983; 55: 643.

8. Haaland D. M, Thomas E V. Partial least-squares methods for spectral analyses .2. application to simulated and glass spectral data. J. Anal. Chem. 1988; 60: 1202-1208.

9. Brown C. W., Lynch P. F., Obremski R. J., Lavery D. S. Matrix representations and criteria for selecting analytical wavelengths for multicomponent spectroscopic analysis. J. Anal. Chem. 1982; 54 (9): 1472-1479.

10. Donahue M., Brown C. W., Caputo B., Modell M. D**.** Near-infrared multicomponent analysis in the spectral and Fourier domains: energy content of high-pressure natural gas J. Anal. Chem.1988; 60 (18): 1873-1878.

11. Espinosa-M.A., Salinas F., Orbepaya I. D. Simultaneous determination of sulfadiazine, doxycycline, furaltadone and trimethoprim by partial least squares multivariate calibration. Anal. Chim. Acta. 1995; 313(1-2): 103-112.

12. Goicoechea H. C., Olivier A. C. Simultaneous multivariate spectrophotometric analysis of paracetamol and minor components (diphenhydramine or phenylpropanolamine) in tablet preparations, J. Pharm. Biomed. Anal. 1999; 20 (1-2): 255-261.

13. Gong Y. Ni, X. Simultaneous spectrophotometric determination of mixtures of food colorants. Anal. Chim. Acta. 1997; 354: 163-171.

14. Mehta TN, Patel AK, Kulkarni GM, Subbaiah G. Determination of rosuvastatin in the presence of its degradation products by a stability- indicating LC method J. AOAC Int. 2005; 88(4):1142- 47.

15. Hull CK, Penman AD, Smith CK, Martin PD. Quantification of rosuvastatin in human plasma by automated solid- phase extraction using tandem mass spectrometric detection. J .Chromatogr. B. 2002; 722(2): 219-28.

16. Fabrio P. G.; Pedro L. G.; Anil K. S. Development and validation of stability Indicating HPLC methods for quantitative determination of pravastatin, fluvastatin, atrovastatin, and rosuvastatin in pharmaceuticals. Anal. Lett. 2009; 42(12):1784-1804.

17. Hull CK, Martin PD, Warwick MJ, Thomas EJ. Quantification of the N-desmethyl metabolite of rosuvastatin in human plasma by automated SPE followed by HPLC with tandem MS detection. J. Pharm Biomed Anal. 2004; (3):609-14.

18. Trivedi RK, Kallem RR, Mullani R, Srinivas NR. Simultaneous determination of rosuvastatin and fenofibric acid in human plasma by LC-MS/MS with electrospray ionization: assay development, validation and application to a clinical study. J. Pharm Biomed Anal. 2005; 39: 661-9.

19. Gupta A., Mishra P., Shah K., Simple UV spectrophotometric determination of rosuvastatin calcium in pure form and in pharmaceutical formulations E-J. Chem.; 2009 6(1): 89- 92.

20. Süslü İ., Çelebier M., Altınöz S. Determination of rosuvastatin in pharmaceutical formulations by capillary zone electrophoresis. J.Chromatographia. 2007; 66: 65-72.

21. Gajjar A. K., Shah V. D., Simultaneous UV spectrophotometric estimation of rosuvastatin and ezetimibe in their combined dosage forms. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; 2(1): 131-8.

22. Gajjar A. K., Shah V. D., Simultaneous estimation of rosuvastatin and ezetimibe by ratio spectra derivative spectrophotometry method in their fixed dosage forms. International Journal of Pharm. Tech. Research. 2010; 2(1): 404-410.

23. Smita S., Sharma M. C., Kohli D. V., Chaturvedi S. C., Micellar liquid chromatographic method development for determination of rosuvastatin calcium and ezetimibe in pharmaceutical combination dosage form. Der Pharma Chemica. 2010; 2(1): 371-377.

24. Kenneth R. B., Randy J. P., Seasholtz M. B. Chemometrics: A Practical Guide, JohnWiley & Sons, Inc.: New York, 1988.

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