EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 8
129-134
Year: 2018

MULTIVARIATE CHEMOMETRIC MODELS APPLIED FOR SIMULTANEOUS DETERMINATION OF ROSUVASTATIN CALCIUM AND TELMISARTAN IN PURE FORM AND IN PHARMACEUTICAL PREPARATION; A COMPARATIVE STUDY

Nasr M. El-Abasawy, Khalid A. M. Attia, Ahmad A. Abo-serie, Ragab A. Said and Ahmed A. Almrasy*

Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, 11751 Nasr City, Cairo, Egypt.

***Corresponding Author: Ahmed A. Almrasy**
Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, 11751 Nasr City, Cairo, Egypt.

Article Received on 11/06/2018 **Article Revised on 01/07/2018** **Article Accepted on 22/07/2018**

ABSTRACT
Objective: Experimental design of different synthetic mixtures of rosuvastatin calcium and telmisartan in different ratios were constructed. The zero-order absorption spectra of these prepared mixtures have been recorded and used for building four multivariate chemometric methods for simultaneous determination of rosuvastatin calcium and telmisartan in their pure and pharmaceutical dosage forms. **Methods:** namely; partial least squares and artificial neural network have been applied for the quantitative analysis of the studied drugs. **Results:** the application of genetic algorithm to partial least squares and artificial neural network has been done and greatly increased the precision and predictive ability of the methods. **Conclusion:** The four methods have been successfully applied for determination of both drugs in their pharmaceutical preparation without any preliminary separation steps.

KEYWORDS: Rosuvastatin calcium; telmisartan; chemometry; overlapped spectra.

INTRODUCTION

- **Rosuvastatin calcium (ROS)**, **figure (1)**, is bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl)amino] pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt. Its molecular weight is 1001.14 and its molecular formula is $(C_{22}H_{27}FN_3O_6S)_2Ca$. It is white amorphous powder that is sparingly soluble in water and methanol. It is a selective and competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol.[1]

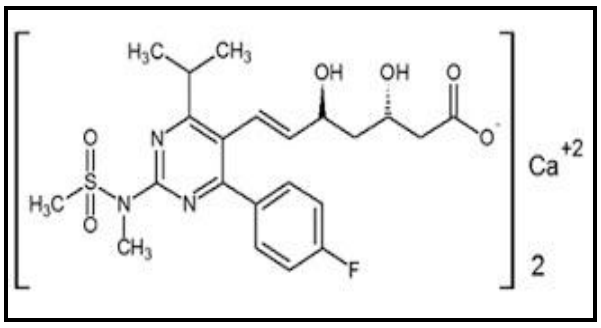

Figure 1: Structural formula of rosuvastatin calcium.

- **Telmisartan (TEL)**, **figure (2)**, is 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl) methyl]-[1,1'-biphenyl]-2-carboxylic acid. Its molecular weight is 514.63 and its molecular formula is $C_{33}H_{30}N_4O_2$. It is white or slightly yellowish crystalline powder. It is practically insoluble in water, slightly soluble in methyl alcohol and sparingly soluble in dichloromethane. It is an angiotensin II receptor antagonist with antihypertensive activity due mainly to selective blockade of AT_1 receptors and the consequent reduced pressor effect of angiotensin II.[2]

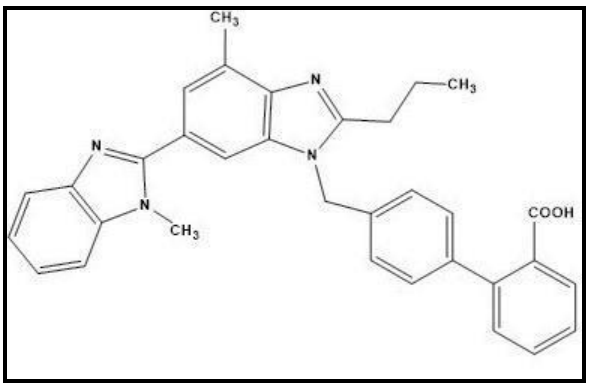

Figure (2): Structural formula of telmisartan.

Literature survey revealed that few analytical methods such as spectrophotometry,[3,4] TLC[5] HPLC[6-8], have been reported for determination of rosuvastatin calcium

and telmisartan in bulk drug formulations or combination with other drugs. Hence the objective of the present work is to develop simple, precise and accurate methods for the simultaneous determination of rosuvastatin calcium and telmisartan in tablets formulation.

To the best of our knowledge there is no reported chemometric method available for simultaneous determination of rosuvastatin calcium and telmisartan. Hence, the aim of this work was to develop accurate and precise chemometric methods for simultaneous determination of ROS and TEL in their dosage form. The developed methods are partial least squares (PLS-1) with application of genetic algorithm (GA-PLS-1) and artificial neural network (ANN) with application of genetic algorithm (GA-ANN).

EXPERIMENTAL

Instruments

Shimadzu UV-Vis. 1800 *Spectrophotometer*, (Tokyo, Japan), equipped with 10 mm matched quartz cells was used. The spectral band was 2 nm and scanning speed is 2800 nm/min with 1 nm interval.

Software

UV-Probe personal spectroscopy software version 2.1. (SHIMADZU).

All chemometric methods were implemented in Matlab R2013b (8.2.0.701).

PLS, ANN and application of GA were carried out by using PLS toolbox software version 2.1. in conjugation with neural network toolbox

The student *t*-test and F value were performed using Microsoft-Excel.

All calculations were performed using a Quad core CPU, 1.47 GHz, 4.00GB of RAM under Microsoft Windows 7™.

MATERIALS AND REAGENTS

- Pure rosuvastatin calcium (99.61 %) certified by the manufacturer was pursued as a gift sample from Copad for pharmaceuticals, Obour city, Egypt.
- Pure telmisartan (99.79 %) certified by the manufacturer was kindly supplied by Boehringer Ingelhiem International Germany.Gmbh,
- Pharmaceutical preparation: **Telrose** ® tablets nominally containing 10 mg of rosuvastatin calcium and 40 mg of telmisartan per tablet, manufactured by Micro labs for pharmaceuticals, purchased from local market of India.
- Methanol, analytical grade was purchased from (El-Nasr Pharmaceutical Chemicals Co. Abu- Zabaal, Cairo, Egypt).

STANDARD SOLUTIONS

Standard solution of rosuvastatin calcium

A standard solution of rosuvastatin calcium (100 µg/ml) was prepared by dissolving 10 mg of the drug powder in 50 ml of methanol and complete to 100 ml with the same solvent.

Standard solution of telmisartan

A standard solution of telmisartan (200 µg/mL) was prepared by dissolving 20 mg of the drug powder in 50 mL of methanol using a 100-mL volumetric flask and completing to volume with the same solvent.

PROCEDURES

Experimental design

Brereton constructed multilevel multifactor experimentaldesign was applied for the construction of the calibration and validation sets.[9] A five-levels, two factors experimental design was used in which aliquots of standard solution (100 µg/ml) of rosuvastatin calcium containing 0.8, 0.9, 1, 1.1 and 1.2 mg were combined with aliquots of standard solutions (200µg/ml) of telmisartan containing 1.6, 1.8, 2, 2.2 and 2.4 mg and diluted to 10 ml with methanol. The concentrations details are given in **table (1).** The absorption spectra of the prepared mixtures were recorded over the wavelength range 220-350 nm with 1 nm interval thus the produced spectral data matrix has 25 rows representing different samples and 131 columns representing wavelengths (25 x 131). For construction of the models, to build the CLS and PCR models, feed the computer with the absorbance and concentration matrices for the training set, use the training set absorbance and concentration matrices using Matlab® version R2013b (8.2.0.701), together with PLS-Toolbox 2.1. software for the calculations.

Table (1): The 5-level, 2-factor experimental design shown as concentrations of rosuvastatin calcium and telmisartan in µg/ml.

Mixture number	Rosuvastatin calcium (µg/ml)	Telmisartan (µg/ml)
1	10	20
2	10	16
3	8	16
4	8	24
5	12	18
6	9	24
7	12	20
8	10	18
9	9	18
10	9	22
11	11	24
12	12	22
13	11	20
14	10	24
15	12	24
16	12	16
17	8	22
18	11	16
19	8	20
20	10	22
21	11	22
22	11	18
23	9	16
24	8	18
25	9	20

The shaded rows represent the calibration set.

Application of the method to pharmaceutical preparation

Ten **Telrose®** tablets (10 mg of rosuvastatin calcium and 40 mg of telmisartan per tablet**)** were scratched, weighed and then finely powdered. Appropriate weight of the powder equivalent to 10 mg rosuvastatin calcium and 40 mg telmisartan was accurately weighed, transferred to 100 mL volumetric flask and the volume was made up to 50 mL with methanol. The mixture was shaken vigorously for 20 min then sonicated for 30 min and filtered. The volume was completed to 100 mL with methanol to produce a stock solution labeled to contain 0.1 mg/mL of rosuvastatin calcium and 0.4 mg/mL of telmisartan. Necessary dilutions of the stock solution were made using methanol.

The spectra from 220 to 350 nm of the samples were recorded and the content of tablets was determined using these models.

RESULTS AND DISCUSSION

Spectroscopic techniques can supply the analyst with a large data within a short period of time. Coupling the spectral data with chemometric models enhance the quality of the spectral information and making this combined technique into a powerful and highly convenient analytical tool. To date there is no reported spectrophotometric method for the simultaneous analysis of ROS and TEL. This has prompted the authors to apply different chemometric methods, especially PLS, GA-PLS, ANN, GA-ANN for simultaneous analysis of the studied drugs. These described methods have higher prediction power, providing maximum relevant information and analyzing a large number of samples in a short period of time with higher degree of accuracy and precision.

The UV spectra of ROS and TEL show sever overlap, as shown in **figure (3)**, which creates difficulty in the simultaneous analysis of this mixture. Therefore, multivariate calibration methods were applied to predict the concentrations of ROS and TEL in both calibration and validation sets as well as in their pharmaceutical formulation.

GA searches the solution space of a function through the use of simulated evolution. It solves the optimization problem by exploring all regions of the potential solutions and exponentially exploiting promising areas through mutation, crossover, and selection operation applied to individuals in the populations. A critical issue of successful GA performance is the adjustment of GA parameters.[10] In order to avoid the risk of over fitting, a number of independent short runs were done and the results of all the runs were taken into consideration to obtain the final model. Doing this, a much more consistent (and less over fitted) solution can be obtained.[11,12] The adjusted GA parameters with the lowest mean square error were shown in **Table (2).**

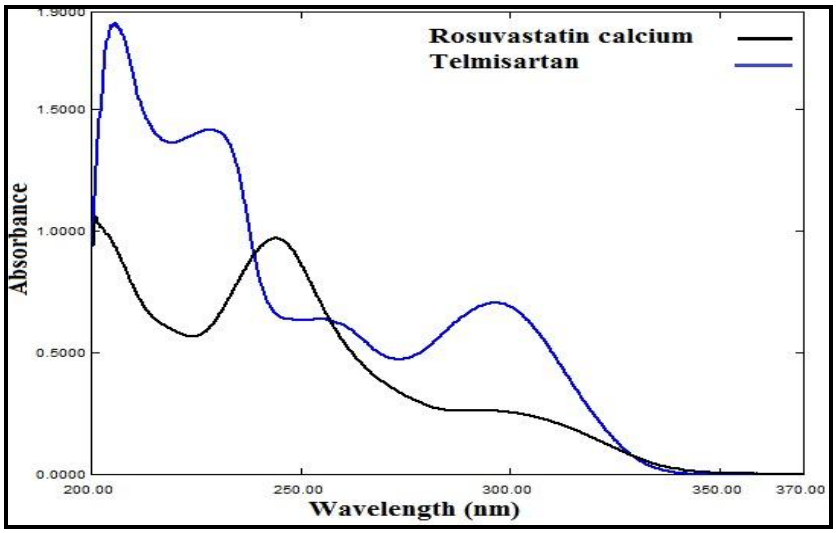

Figure (3): Absorption spectra of rosuvastatin calcium (20 µg/mL) and telmisartan (20µg/mL).

Partial least squares (PLS) and applying genetic algorithm (GA-PLS)

PLS-1 is a widely used regression method. It is known that information from the concentrations values is introduced into the calculation of the so-called latent variables, which are linear combinations of the original variables. PLS-1 method was run on the calibration data of absorption spectra. To select the number of factors in the PLS-1 algorithm, a cross validation (CV) method leaving out one sample at a time was applied using calibration set of 13 calibration spectra. RMSECV (Root Mean Squared Error of Cross Validation) was recalculated upon addition of each new factor to the PLS-1. Then number of factors was selected based on Haaland and Tomas criteria.[13] It was found that two factors were sufficient for modelling both ROS and TEL.

Table 2: Parameters of genetic algorithm.

Parameter	Value	
	ROS	TEL
Population size	36	36
Maximum generations	80	87
Mutation rate	0.005	0.005
The number of variables in a window (window width)	1	1
Percent of population the same at Convergence	80	80
% Wavelengths used at initiation	10	15
Crossover type	Double	Double
Maximum number of latent variables	2	2
Cross validation	Random	Random
Number of subsets to divide data into for cross validation	4	4
Number of iterations for cross validation at each generation	2	2

However, to increase the quality and improve the calibration, the variables selection technique namely genetic algorithm (GA) was performed; by its application the un-informative variables were excluded. The predictability of both models was tested by validation set and it was found that the PLS-1 model constructed after removing the un-informative variables is more robust and simpler with lower root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). This is surely due to the fact that the un-informative wavelengths have been excluded. The percentage % recoveries, RSD (relative standard deviation) and RMSEP values of the validation set for these models are listed in **Table 3**.

The genetic algorithm was run on 131 variables for rosuvastatin calcium and telmisartan using a partial least squares with the maximum number of latent variables determined by cross-validation on the model containing all the variables. Genetic algorithm reduced absorbance matrix to about 3.1% of the original matrix (4 for rosuvastatin calcium) and about 3.8% of the original matrix (5 for telmisartan).

Artificial neural network (ANN) and applying genetic algorithm (GA-ANN)

ANNs are a type of computational models simulating the biological neural networks. They composed of an inter-connected group of artificial neurons. To optimize a neural network, we have to use the trial and error method to find out the best neural network architecture.[14,15] Choosing the values of optimum parameters to construct the network are not an easy task because the parameters are mutually related.

The output layer resembles the concentration vector of one component. The hidden layer consists of single layer which is sufficient to solve similar or more complex problems. Moreover, more hidden layers may cause over-fitting. The hidden neurons number is one of the most important parameters among other ANN parameters that must be adjusted. This parameter is related to the converging performance of the output error function during the learning process.

Table 3: **Validation parameters of the proposed methods.**

Model	PLS		GA-PLS		ANN		GA-ANN	
Drug	ROS	TEL	ROS	TEL	ROS	TEL	ROS	TEL
	101.88	100.47	101.80	99.22	101.70	99.84	101.60	100.34
	98.54	98.85	101.75	99.69	101.88	100.31	101.75	100.52
	98.11	98.50	101.44	100.58	101.56	101.00	99.11	101.00
	98.65	100.92	98.70	98.14	98.60	98.42	98.50	98.14
	97.86	100.30	98.11	100.52	98.22	99.61	98.11	99.16
	99.14	101.32	98.92	100.64	98.83	100.18	98.92	100.18
	99.20	100.17	100.90	98.08	100.70	100.17	100.80	100.38
	99.67	98.91	99.58	98.59	99.75	98.28	99.67	101.41
	101.55	100.81	98.91	100.50	98.73	100.81	98.55	100.50
	101.50	100.34	99.20	100.11	99.40	98.30	99.60	100.57
	99.64	101.06	99.64	100.06	99.64	100.89	100.18	100.17
	98.50	101.81	101.88	99.47	101.75	99.19	98.38	99.47
Mean (%R)	99.51	100.29	100.07	99.63	100.06	99.75	99.59	100.15
%RSD	1.401	1.034	1.388	0.946	1.380	1.005	1.254	0.868
RMSEP	0.135	0.417	0.125	0.393	0.125	0.381	0.119	0.324

Transfer function pairs also an important parameter that should be adjusted carefully. Choosing of transfer function based on the nature of data to be analysed. In the present work, purelin-purelin transfer function was used due to the linear correlation between absorbance and concentration. The learning rate controls the degree at which connection weights are modified during the learning phase. The optimized parameters values of the ANN for ROS and TEL were shown in **Table 4.**

ANNs show better RMSEP than PLS-1 which may be due to the fact that ANNs is a type of artificial intelligence where there is less chance for over-fitting than that may occur in PLS calibrations. % recoveries, % RSD and RMSEP values of the validation set for ANN and GA-ANN models are listed in **Table 3**.

The application of the ANN on the raw data after using the variable selection technique GA shows improvement of the results. A large number of nodes in the input layer of the network (wavelengths) increase the CPU time for ANN modelling. GA allowed the use of less number of neurons (shorter training time) than those used in the network utilized the raw data.

Table 4: Optimized parameters of ANN.

Method	**ANN**		**GA-ANN**	
Drug	**ROS**	**TEL**	**ROS**	**TEL**
Architecture	131-5-1	131-7-1	4-2-1	5-2-1
Hidden neurons number	5	7	2	2
Transfer functions	**Purelin – Purelin**			
Learning rate	0.1	0. 1	0.1	0.1
Training function	TRAINLM			

Analysis of pharmaceutical sample

The proposed procedure was applied for determination of both ROS and TEL in Telrose® tablets. Satisfactory results were obtained in good agreement with the label claim. The obtained results were statistically compared to those obtained by the reported method.[3] No significant differences were found by applying two tail student *t*-test and F-test at 95% confidence level[16], indicating good accuracy and precision of the proposed methods for the analysis of the studied drugs in their pharmaceutical dosage form, as shown in **Table 5.**

Table 5: Statistical comparison for the results obtained by the proposed and the reported methods for the analysis of ROS and TEL in Telrose® tablets.

Method	**Drug**	**Mean**	**N***	**% RSD**	***t*****	**F****
PLS	ROS	100.19	**5**	1.098	1.553	1.673
					(2.306)	(6.388)
	TEL	99.97		1.388	0.803	2.121
					(2.306)	(6.388)
GA-PLS	ROS	99.71		1.288	0.713	2.278
					(2.306)	((6.388)
	TEL	99.95		1.128	0.891	1.401
					(2.306)	(6.388)
ANN	ROS	99.91		1.456	0.917	2.924
					(2.306)	(6.388)
	TEL	98.79		0.454	1.220	4.514
					(2.306)	(6.388)
GA-ANN	ROS	100.02		1.225	1.191	2.074
					(2.306)	(6.388)
	TEL	99.83		0.639	0.903	2.228
					(2.306)	(6.388)
Reported method[3]	ROS	99.22		0.857	-------	-------
	TEL	99.36		0.959	-------	-------

* No. of experimental.

** The values in the parenthesis are tabulated values of t and F at (p= 0.05).

(3) Rosuvastatin calcium and telmisartan in their binary mixture were determined by dual wavelength and first derivative of ratio spectra methods.

CONCLUSION

In this study, application of GA on PLS and ANN models enhance the results with respect to RMSEP. The developed methods can be applied for routine and analysis of rosuvastatin calcium and telmisartan in its pure forms and in tablets.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. U.S. Food and Drug Administration. Crestor (ROS Calcium) Tablets Drug Approval Package. https://www.accessdata.fda.gov/drugsatfda_ docs/label/2005/21366slr005lbl.pdf.
2. Sweetman S. Martindale: the complete drug reference. 36th ed. London: The Pharmaceutical Press, 2009.
3. Solanki BK, Dave JB, Raval PP. Development and validation of UV spectrophotometric methods for simultaneous estimation of rosuvastatin and telmisartan in tablet dosage form. World J Pharm Pharm Sci., 2014; 3(6): 2030-2041.
4. Doshi N, Sheth A, Patel T, DaveJB, Patel CN. Spectrophotometric absorption factor method development and validation for estimation of rosuvastatin calcium and telmisartan in solid dosage form. J Chem Pharm Res., 2010; 2(3): 15-24.
5. Patel N. Method development and validation for simultaneous estimation of rosuvastatin calcium and telmisartan in combined dosage form by high performance thin layer chromatography. In Rapid Pharm Ana Qual Assur, 2012; 1: 1-5.
6. Janardhanan VS, Manavalan R, Valliappan K. Chemometric technique for the optimization of chromatographic system: Simultaneous HPLC determination of rosuvastatin, telmisartan, ezetimibe and atorvastatin used in combined cardiovascular therapy. Arab. J. Chem., 2016; 9: 1378-1387.
7. Ketema G. Development of RP-HPLC method for the simultaneous estimation of rosuvastatin calcium and telmisartan in bulk and pharmaceutical formulations. J Anal Bioanal Tech., 2013; 4(5): 188-195.
8. Doshi N, Sheth A, Sharma A, Dave JB, Patel CN. Validated RP-HPLC method for simultaneous estimation of rosuvastatin calcium and telmisartan in pharmaceutical dosage form. J Chem Pharm Res., 2010; 2(2): 252-263.
9. Massart DL. Vandeginste BGM, Buydens LMC, De Jong S, Lewi PJ, Smeyers-Verbeke, Handbook of chemometrics and qualimetrics, Part A, Elsevier Sci, Amsterdam, 1997.
10. Beebe KR, Pell RJ, Seasholtz MB. Chemometrics: A practical guide, John Wiley & Sons, Inc., New York, 1998; 337.
11. Brereton RG, Zissis KD, Escott R. Partial least-squares calibration of two-way diode-array high-performance liquid chromatograms: influence of calibration design, noise and peak separation. Analyst, 1998; 123(6): 1165-1173.
12. Martens H, Naes T. Multivariate calibration: A user-friendly guide to multivariate calibration and classification. John Wiley & Sons, New York, 1989.
13. Haaland DM, Thomas EV. Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. Anal Chem., 1988; 60(11): 1193-1202.
14. Gerlach RW, Kowalski BR, Wold HOA. Partial least-squares path modelling with latent variables. Anal Chim Acta, 1979; 112(4): 417-421.
15. Darwish HW, Hassan SA, Salem MY, El-Zeany BA. Different approaches in partial least squares and artificial neural network models applied for the analysis of a ternary mixture of amlodipine, valsartan and hydrochlorothiazide. Spectrochim Acta A, 2014; 122: 744-750.
16. Armitage P, Berry G. Statistical methods in medical research. 3rd ed. Oxford (UK): Blackwell, 1994.