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CHEMOMETRIC – ASSISTED UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ANTIHYPERLIPIDEMIC AGENTS IN PHARMACEUTICAL FORMULATION

Santosh V. Gandhi*, Amit S. Mutha

AISSMS College of Pharmacy, Kennedy Road, Pune- 411001, Maharashtra, India

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ABSTRACT

This presented work is based on application of two multivariate calibration methods for simultaneous UV-VIS spectrophotometric determination of active substances in combined pharmaceutical formulation composed of Atorvastatin calcium (ATV) and Ezetimibe (EZT). The methods used were Principal Component Regression (PCR) and Partial Least Square (PLS). The Spectra of ATV and EZT were recorded at concentrations within their linear range 5.0-30.0 µg/ml for both drugs. 28 set of mixtures were used for calibration and 08 set of mixtures were used for validation in the wavelength range of 230 to 260 nm with the wavelength intervals λ= 0.5 nm in methanol. The methods were validated as per International Conference on HarmonizationQ2 (R1) (ICH) guidelines. These methods were successfully applied for determination of drugs in pharmaceutical formulation (tablet) with no interference of the excipient as indicated by the recovery study results. The proposed methods are simple, rapid and can be easily used as an alternative analysis tool in the quality control as well as in process control of drugs and formulation.

INTRODUCTION

Atorvastatin calcium is[R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy-5(1methylethyl)-3-phenyl-4- [phenylamino carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt [Fig. 1(a)] is well-known member of the drug class known as statins, which are used primarily as a lipid-lowering agent that inhibits HMG-CoA reductase enzyme which is found in liver tissue for production of cholesterol [1]. It is official in Indian Pharmacopeia [2]. Ezetimibe is 1-(-4-flurophenyl)-(3S)-hydroxypropyl]-(4S)-(4-hydroxyphenyl)-2azeti-dinone [Fig. 1(b)] is a selective cholesterol absorption inhibitor, which potentially inhibits the absorption of biliary and dietary cholesterol[3]. It is official in Indian Pharmacopeia[4].Several methods are reported for quantitative determination of ATV and EZT in single and in combination such as UV, HPTLC, and RP-HPLC [5-14]. Chemometric is the science of extracting information from chemical systems. Multivariate calibration method (e.g., multiple linear regression (MLR), principle component regression (PCR) and partial least squares (PLS) utilizing spectrophotometric data are the important chemometric approach for determination of mixtures including drugs combination [15]. While working on development of simple, accurate and precise method for this combination we came across one recent report for analysis of these drugs by chemometric analysis using MATLAB software [16].We have developed method using Unscrambler X (10.3) software. Compared with reported method the results were found promising.

MATERIALS AND METHODS

Instrumentation

Double beam UV- Vis spectrophotometer (Jasco V-730) with matched pair of 1cm quartz cells were used to record spectra of all solutions. The spectra were recorded at spectral band width

***For Correspondence:** santoshvgandhi@rediffmail.com

of 2.0 nm, scanning speed 100 nm/min and data pitch 0.5 nm. Unscrambler X (10.3) (64-bit) trial version and Microsoft Excel 2013 were used for model generation and application of chemometric.

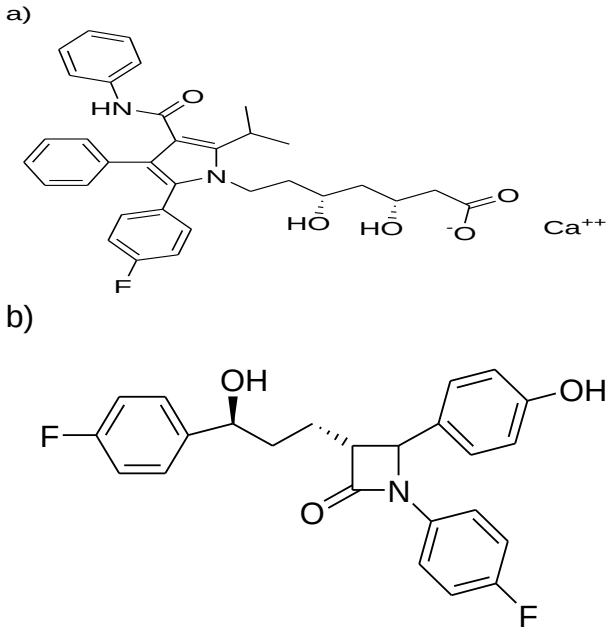

Figure 1: Structure of a) Atorvastatin calcium (ATV) and b) Ezetimibe (EZT)

Material and Reagents

Reference standard of ATV and EZT were obtained from Wockhardt Research Centre, Aurangabad as gift samples and methanol (AR grade) purchased from LOBA Chemie, India. Atorsave EZ tablets manufactured by Eris Life Sciences Pvt. Ltd. containing Atorvastatin calcium IP 10 mg and Ezetimibe IP 10 mg were procured from local pharmacy shop.

One component calibration

To find linear concentration of each drug, one component calibration was performed. Linear dynamic ranges were studied in the concentration range of 5.0-30.0 µg/ml for both ATV and EZT. Absorbance values were recorded at λ_{max} of each drug (247 nm for ATV and 233 nm for EZT) against methanol as blank. Linear dynamic range for each compound was determined by least-square linear regression of concentration and the corresponding absorbance. Fig. 2 represents Individual spectra, mixtures and sum of the spectra for ATV and EZT. According to the figures, there is no interaction between analytes as the signals appear with additive properties.

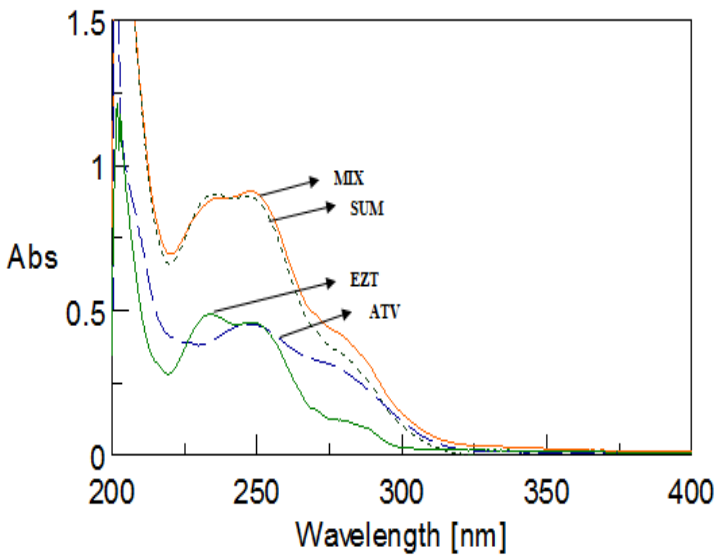

Figure 2: Individual spectra, mixtures and sum of the spectra for ATV and EZT

Preparation of standard stock solution

Stock solution of ATV and EZT were prepared by dissolving accurately weighed 10 mg of standard drug in 10 ml of methanol, separately. The concentration of ATV and EZT were 1000 µg/ml from which further 5 ml was pipetted and diluted to 50 ml to achieve final concentration of 100 µg/ml of ATV and EZT, separately.

Preparation of working stock solution

Working standard solutions were prepared from standard stock solution of 100 µg/ml by appropriate dilution with methanol to obtain final concentration of 5, 10, 15, 20, 25 and 30 µg/ml for both ATV and EZT respectively.

Construction of calibration and validation set

A total set of 36 mixtures were prepared by combining working standard of ATV and EZT in their linear concentration range of 5.0-30.0 µg/ml. (Table I). From these 28 mixtures were used for calibration set and 08 mixtures were used for validation set by random selection. The absorbance spectra were recorded in range of 230- 260 nm with 0.5 nm interval. The spectra were saved as ASCII (.txt) format which were further extracted in MS-Excel as required by Unscrambler software for model generation. The PCR and PLS models were developed utilizing absorption data using Unscrambler software. Selection of proper number of latent variables for development of model was necessary to obtain good prediction. Leave-one-out (LOO)

cross validation method was used to obtain necessary number of latent variables (LVs), as shown in Fig. 3 and calculated using formula [17],

$$\mathbf{RMSECV} = \sqrt{\sum \frac{(Cact - Cpre)^2}{Ic}}$$

Where,

RMSECV= Root mean square error of cross validation

Cact= actual concentration of calibration set

Cpre= predicted concentration of validation set

Ic= Total number of samples in calibration set

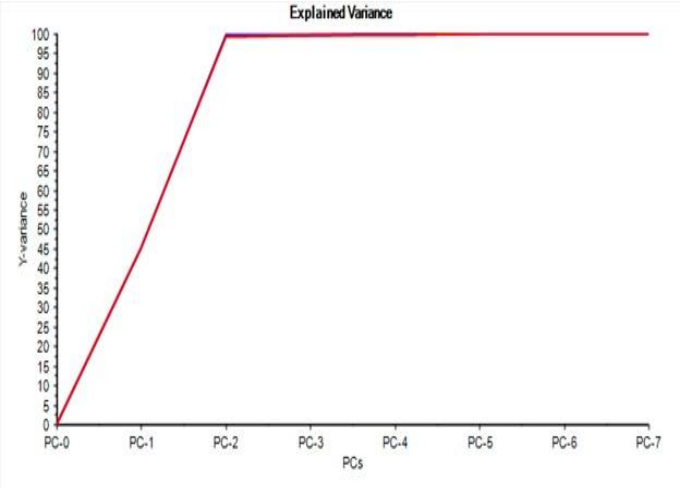

Figure 3: Explained Variance describing number of optimum PCs (Principle Components)

After the PCR and PLS models have been constructed, it was found that the optimum number of LVs were two factors for both PCR and PLS. For validation of generated models, concentration in validation set was predicted by using proposed PCR and PLS models (Table II). The validation of developed methods was performed as per ICH Q2 (R1) [18].

Assay of marketed preparation

20 tablets of AtorsaveEZ were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of ATV (10 mg of EZT) was taken and transferred to 10 ml volumetric flask and was diluted to 10 ml with methanol. The solution was sonicated for 10 minutes. This solution was then filtered with help of whatman filter paper no. 41. 1 ml of filtrate solution was diluted to 10 ml with methanol. Further 1 ml of this solution was diluted to 10 ml with methanol to get final concentration of 10 µg/ml of ATV and EZT each. The procedure was repeated 6 times for tablet formulation.The assay results are presented in Table III.

Accuracy study

The accuracy study was carried out at three levels 50 %, 100 % and 150 % of assay concentration. Calculated amount of ATV and EZT from standard solutionswere spiked into sample solution and scanned in range of 230-260 nm. Concentrations were predicted by using developed PCR and PLS models. Accuracy data is presented in Table IV and Table V.

Table I: Composition of calibration and validation sets.

MIX. NO	***ATV*** *(µg/ml)*	***EZT*** *(µg/ml)*	***MIX. NO***	***ATV*** *(µg/ml)*	***EZT*** *(µg/ml)*
1	5	15	19	25	10
2	5	20	20	25	15
3	5	25	21	25	20
4	5	30	22	25	25
5	10	5	23	25	30
6	10	10	24	30	5
7	10	15	25	30	15
8	10	20	26	30	20
9	10	25	27	30	25
10	10	30	28	30	30
11	15	15	29	5	5
12	15	20	30	5	10
13	15	25	31	15	5
14	20	5	32	15	10
15	20	10	33	15	30
16	20	15	34	20	20
17	20	25	35	20	30
18	25	5	36	30	10

*Calibration set - Mix No. 1-28

*Validation set - Mix No. 29-36

Precision

Precision was carried at three concentration levels (10, 15, 20 µg/ml for both ATV and EZT) in three replicates at each level. The results of intraday and interday precision studies which are presented in Table VI and Table VII.

LOD and LOQ

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Table II: Predicted results for validation set by PCR and PLS method.

METHOD		**PCR**				**PLS**			
ATV	***EZT***	***ATV***		***EZT***		***ATV***		***EZT***	
Actual (µg/ml)		Predicted	% R*	Predicted	% R*	Predicted	% R*	Predicted	% R*
5	5	5.046	100.9	5.013	100.2	5.046	100.9	5.016	100.3
5	10	5.070	101.4	10.129	101.2	5.077	101.5	10.122	101.2
15	5	15.108	100.7	4.902	98.0	15.108	100.7	4.903	98.0
15	10	15.560	103.7	9.882	98.8	15.559	103.7	9.884	98.8
15	30	15.106	100.7	29.127	97.0	15.105	100.7	29.128	97.0
20	20	19.942	99.7	21.488	107.4	19.946	99.7	21.484	107.4
20	30	19.626	98.1	30.412	101.3	19.627	98.1	30.411	101.3
30	10	29.965	99.8	9.895	98.9	29.966	99.8	9.894	98.9

* % R - % Recovery

Table III: Assay result for ATV and EZT in tablet (AtorsaveEZ) by proposed methods

METHOD		**PCR**				**PLS**			
ATV	***EZT***	***ATV***		***EZT***		***ATV***		***EZT***	
Actual (µg/ml)		Predicted (µg/ml)	% R	Predicted (µg/ml)	% R	Predicted (µg/ml)	% R	Predicted (µg/ml)	% R
10	10	9.716	97.1	9.877	98.7	9.700	97.0	9.878	98.7
10	10	9.969	99.6	9.922	99.2	9.968	99.6	9.927	99.2
10	10	9.959	99.5	9.975	99.7	9.954	99.5	9.975	99.7
10	10	10.004	100.0	10.162	101.6	10.001	100.0	10.164	101.6
10	10	9.979	99.7	9.892	98.9	9.975	99.7	9.899	98.9
10	10	9.995	99.9	9.916	99.1	9.994	99.9	9.917	99.1
MEAN		9.937	99.3	9.957	99.5	9.932	99.3	9.960	99.6
SD		0.109	1.095	0.105	1.057	0.114	1.148	0.105	1.050

* % R - % Recovery

Table IV: Accuracy data of ATV by PCR and PLS models.

Level %	**Sample Conc. µg/ml**	**Amount added µg/ml**	**Total Conc. µg/ml**	**Predicted Conc. µg/ml**		**% Recovery**		**% RSD**	
				PCR	**PLS**	**PCR**	**PLS**	**PCR**	**PLS**
50 %	10	5	15	14.88 14.97 14.99	14.88 14.97 14.69	99.2 99.8 99.9	99.2 99.8 97.9	0.389	0.953
100 %	10	10	20	20.08 20.15 19.85	20.07 20.14 19.85	100.4 100.7 99.2	100.3 100.7 99.2	0.774	0.773
150 %	10	15	25	24.64 25.37 24.98	24.64 25.37 24.97	98.5 101.5 99.9	98.5 101.5 99.9	1.466	1.466

Table V: Accuracy data of EZT by PCR and PLS models.

LEVEL %	**Sample Conc. µg/ml**	**Amount added µg/ml**	**Total Conc. µg/ml**	**PREDICTED CONC. µg/ml**		**% Recovery**		**% RSD**	
				PCR	**PLS**	**PCR**	**PLS**	**PCR**	**PLS**
50 %	10	5	15	14.912 15.093 14.939	14.919 15.080 14.954	99.4 100.6 99.5	99.4 100.5 99.6	0.651	0.563
100 %	10	10	20	20.285 20.281 20.088	20.297 20.292 20.099	101.4 101.4 100.4	101.4 101.4 100.4	0.557	0.558
150 %	10	15	25	24.824 25.020 24.843	24.839 25.035 24.858	99.2 100.0 99.3	99.3 100.1 99.4	0.433	0.433

Table VI: Precision results obtained using developed PCR and PLS models (Intraday Precision)

Amount taken µg/ml		**Predicted Conc. µg/ml**				**% Recovery**				**% RSD**			
		PCR		**PLS**		**PCR**		**PLS**		**PCR**		**PLS**	
ATV	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*
10	10	9.99	9.93	9.99	9.91	99.9	99.3	99.9	99.1				
10	10	10.04	10.23	10.04	10.21	100.4	102.3	100.4	102.1	1.504	1.568	1.500	1.574
10	10	10.27	10.16	10.27	10.15	102.7	101.6	102.7	101.5				
15	15	14.78	14.87	14.76	14.87	98.4	99.19	98.4	99.1				
15	15	14.86	14.99	14.86	14.99	99.1	99.9	99.1	99.9	1.326	1.381	1.324	1.389
15	15	15.14	15.27	15.14	15.28	100.9	101.8	100.9	101.8				
20	20	20.26	20.22	20.26	20.23	101.3	101.1	101.3	101.1				
20	20	19.84	19.94	19.84	19.94	99.2	99.7	99.2	99.7	1.145	1.122	1.144	1.121
20	20	19.88	19.78	19.88	19.78	99.4	98.9	99.4	98.9				

Table VII: Precision results obtained using developed PCR and PLS models (Interday Precision)

Amount Taken µg/ml		**Predicted Conc. µg/ml**				**% Recovery**				**% RSD**			
		PCR		**PLS**		**PCR**		**PLS**		**PCR**		**PLS**	
ATV	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*	*ATV*	*EZT*
10	10	10.01	10.04	10.00	10.03	100.1	100.4	100.0	100.3				
10	10	10.29	10.25	10.28	10.25	102.9	102.5	102.8	102.5	1.614	1.245	1.616	1.242
10	10	10.30	10.26	10.29	10.26	103.0	102.6	102.9	102.6				
15	15	15.58	15.33	15.57	15.32	103.9	102.2	103.8	102.1				
15	15	15.91	15.58	15.90	15.57	106.0	103.8	106.0	103.8	1.123	0.881	1.124	0.881
15	15	15.87	15.55	15.86	15.54	105.8	103.6	105.7	103.6				
20	20	21.22	20.66	21.21	20.65	106.1	103.3	106.0	103.2				
20	20	21.36	20.77	21.35	20.76	106.8	103.8	106.7	103.8	0.355	0.281	0.355	0.281
20	20	21.33	20.75	21.32	20.74	106.6	103.7	106.6	103.7				

RESULTS AND DISCUSSION

Out of 36 mixtures, 28 set of mixtures were used for calibration and 08 set of mixtures were used for validation. The models were tried to develop with varying Δ λ. The best results were obtained with the wavelengths intervals λ= 0.5 nm in methanol. The developed method found to be accurate as results are close to 100 % and precise with % RSD less than 2. Summary of results is presented in Table VIII. The limitations of the reported method [16] found to narrower range (8-14 µg/ml), less accuracy (94.1 - 108.9 %) and large scanning range (210-300 nm) as compare our developed methods.

CONCLUSION

A study of the use of UV spectrophotometric in combination with PLS and PCR for the simultaneous determination of Atorvastatin calcium (ATV) and Ezetimibe (EZT) in a binary mixture has been accomplished. The results obtained confirmed the suitability of the proposed method for simple, accurate and precise analysis of ATV and EZT in pharmaceutical preparations. The proposed methods do not need separation of ATV and EZT before analysis. In addition, the proposed methods can be applied for analysis of drugs in quality control lab as well as for in process quality control.

Table VIII: Summary of results

Parameters	**Atorvastatin calcium (ATV)**		**Ezetimibe (EZT)**	
	PCR	**PLS**	**PCR**	**PLS**
Range (µg/ml)	5.0-30.0	5.0-30.0	5.0-30.0	5.0-30.0
Wavelength, nm	230- 260	230- 260	230- 260	230- 260
Data interval , Δλ	0.5	0.5	0.5	0.5
Factors/ PC's	2	2	2	2
% Recovery	99.3	99.3	99.5	99.6
LOD	0.53	0.53	0.18	0.18
LOQ	1.61	1.61	0.57	0.57
Correlation Coefficient (r^2)	0.9968	0.9968	0.9922	0.9922
Intercept	0.0564	0.0563	0.1415	0.1415
Slope	0.9968	0.9968	0.9922	0.9922
RMSECV	0.42649	0.48623	0.7097	0.7089
RMSEP	0.4864	0.4862	0.7089	0.7089

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