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Chemometric-Assisted UV Spectrophotometric Method for Determination of Rosuvastatin Calcium and Ezetimibe in Pharmaceutical Dosage Form

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Amit Gunge, Padmanabh Deshpande*

All India Shri Shivaji Memorial Society's College of Pharmacy, Department of Quality Assurance Techniques, Kennedy Road, Near RTO, Pune-411001

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ABSTRACT

This presented work is based on the application of two multivariate calibration methods for simultaneous UV-VIS spectrophotometric determination of active substances in combined pharmaceutical formulation composed of Rosuvastatin calcium and Ezetimibe. The methods used were Principal Component Regression (PCR) and Partial Least Square (PLS). The Spectra of Rosuvastatin calcium and Ezetimibe were recorded at concentrations within their linear range 5.0-30.0 µg/ml for both drugs. 32 set of mixtures were used for calibration and 09 set of mixtures were used for validation in the wavelength range of 220- 280nm 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 the determination of drugs in a 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

Rosuvastatin calcium (RSV), chemically, bis [(E)-7 [4-(4fluorophenyl)-6 isopropyl-2-[methyl (methylsulphonyl) amino] pyrimidin-5-yl] (3R, 5S) -3, 5dihydroxyhept-6-enoic acid],calcium salt [Figure 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 (EZT) is 1-(-4-fluorophenyl)-(3S)-hydroxypropyl]-(4S)-(4-hydroxyphenyl)-2azeti-dinone [Figure 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]. Extensive literature survey revealed that methods such as spectrophotometry [5, 6], High-performance liquid chromatography (HPLC) [7, 8] and High-performance thin layer chromatography (HPTLC) [9] has been reported for the determination of RSV in pharmaceutical formulations either as single drug or in combination with other drugs. Analytical methods reported for determination of EZT includes HPLC [10] and HPTLC [11] methods. Several methods are reported for quantitative determination of RSV and EZT in combination such as UV, RP-HPLC and HPTLC, and [12-16].

Chemometric is the science of extracting information from chemical systems. Multivariate calibration method (e.g., multiple linear regression (MLR), principal component regression (PCR) and partial least squares (PLS) utilizing spectrophotometric data are the important Chemometric approach for determination of mixtures including drugs combination [17]. While working on the development of the 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 [18].We have developed a method using Unscrambler X (10.3) software. Compared with a reported method the results were found promising. As there were no reports on a Chemometric analysis of these drugs, the work was undertaken with the aim to develop simple, accurate and reproducible multivariate spectrophotometric methods for simultaneous determination of RSV and ETZ in tablet dosage form.

a) b)

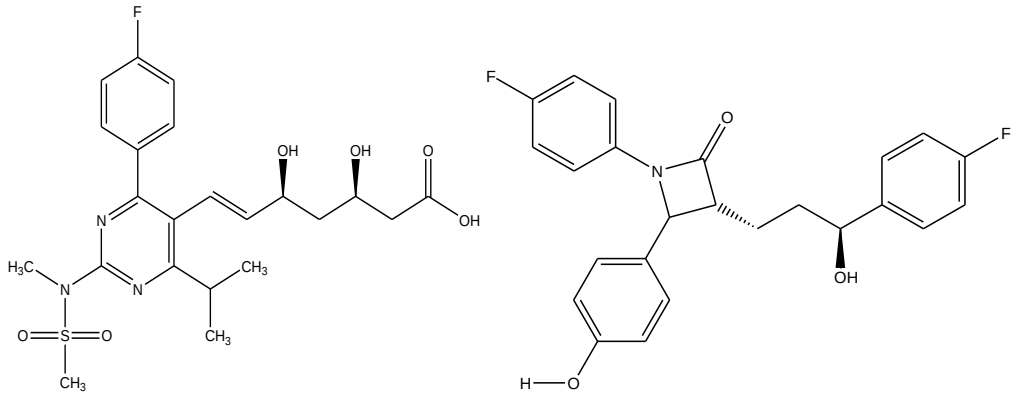

Fig.1: Structure of a) Rosuvastatin calcium (RSV) and b) Ezetimibe (EZT)

MATERIALS AND METHODS:

Instrumentation:

Double beam UV- Vis spectrophotometer (Jasco V-730) with the matched pair of 1cm quartz cells were used to record spectra of all solutions. The spectra were recorded at a spectral bandwidth of 1.0 nm, scanning speed 100 nm/min and data pitch 0.5 nm. Unscrambler X (10.5) (64-bit) trial version and Microsoft Excel 2013 were used for model generation and application of Chemometric.

Material and Reagents:

The reference standard of RSV and EZTwere obtained as gift samples from Ajanta Pharma Ltd. (Aurangabad, India) and methanol (AR grade) purchased from LOBA Chemie, India. Razel EZ tablets manufactured by Glenmark Pharmaceuticals Ltd.labeled to contain 10 mg RSV and 10 mg EZT10 mg were procured from local pharmacy shop.

One component calibration:

To find a 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 RSV and EZT. Absorbance values were recorded at λ_{max} of each drug (244 nm for RSV and 234 nm for EZT)against methanol as blank. A linear dynamic range of each compound was determined by least-square linear regression of concentration and the corresponding absorbance. Figure 2 represents Individual spectra, mixtures and some of the spectra for RSV

and EZT. According to the figures, there is no interaction between analytes as the signals appear with additive properties.

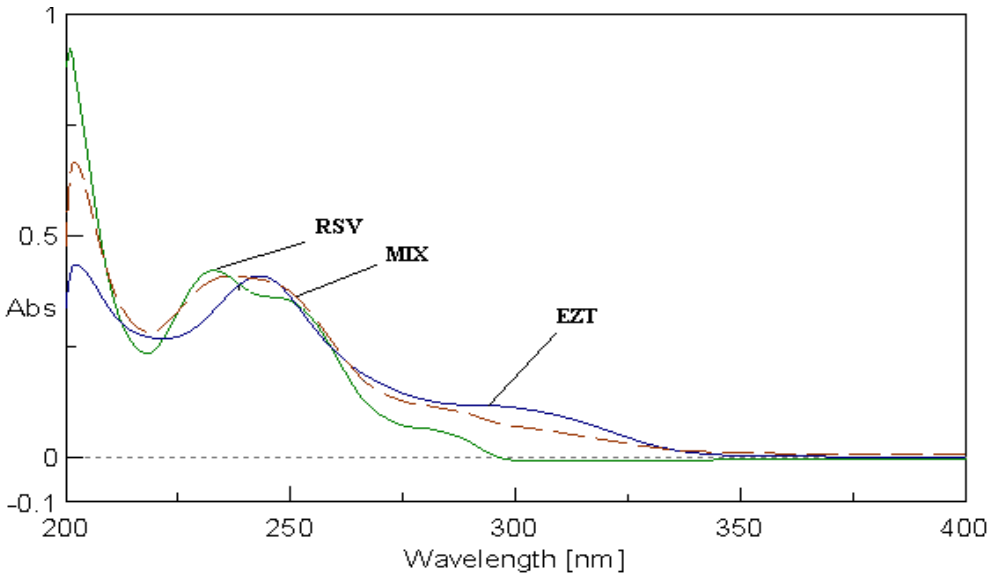

Fig. 2: Individual spectra, mixtures and sum of spectra for RSV and EZT

Preparation of standard stock solution:

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

Preparation of working stock solution:

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

Construction of calibration and validation set:

A total set of 41 mixtures were prepared by combining working standard of RSV and ETZ in their linear concentration range of 5.0-30.0 µg/ml. (Table 1). From these 32 mixtures were used for calibration set and 09 mixtures were used for the validation set by random selection. The absorbance spectra were recorded in a range of 220- 280 nm with 0.5 nm interval. The spectra were saved as ASCII (.txt) format which was 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 a proper number of latent variables for development of the model was necessary to obtain good prediction. Leave-one-out (LOO)cross-validation method was used to obtain the necessary number of latent variables (LVs), as shown in Figure 3 and calculated using formula [19],[24]

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

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 the calibration set

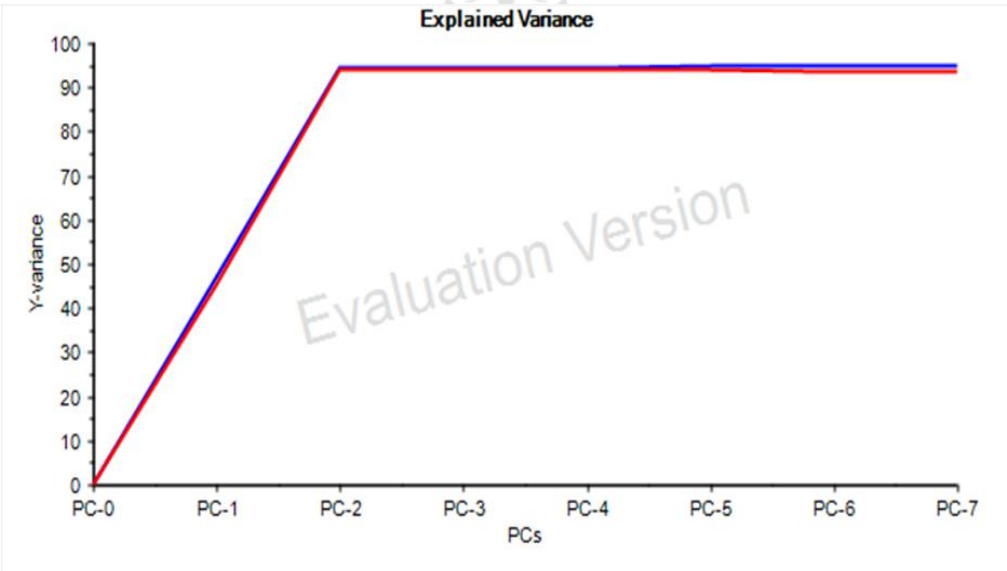

Fig. 3: Explained Variance describing a number of optimum PCs (Principal Components)

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 2). The validation of developed methods was performed as per ICH Q2 (R1) [20].

Table 1: Composition of calibration and validation sets

MIX.NO.	RSV (*µg/ml*)	EZT (*µg/ml*)	MIX.NO.	RSV (*µg/ml*)	EZT (*µg/ml*)
1	5	5	22	17.5	30
2	5	10	23	20	5
3	5	15	24	20	10
4	5	17.5	25	20	15
5	5	20	26	20	20
6	5	25	27	20	25
7	5	30	28	20	30
8	10	5	29	25	5
9	10	10	30	25	10
10	10	15	31	25	15
11	10	20	32	25	20
12	10	25	33	25	25
13	10	30	34	25	30
14	15	5	35	30	5
15	15	10	36	30	10
16	15	15	37	30	15
17	15	20	38	30	17.5
18	15	25	39	30	20
19	15	30	40	30	25
20	17.5	5	41	30	30

*Calibration set - Mix No. 1-32

*Validation set - Mix No. 33-41

Table 2: Predicted results for validation set by PCR and PLS method

METHOD		PCR				PLS			
RSV	ETZ	RSV		EZT		RSV		EZT	
Actual (µg/ml)		Predicted	%R*	Predicted	%R*	Predicted	%R*	Predicted	%R*
5	5	5.02	100.46	5.01	100.30	5.02	100.46	5.01	100.34
5	10	5.09	101.96	10.12	100.40	5.09	101.98	10.12	101.29
15	5	15.19	101.31	4.90	101.27	15.19	101.26	4.90	98.16
15	10	15.20	101.34	9.86	98.63	15.21	101.40	9.86	98.69
15	30	15.12	100.40	29.15	97.17	15.12	100.84	29.15	97.19
20	20	19.94	102.00	20.47	102.38	19.94	99.72	20.47	102.39
20	30	20.10	100.20	30.41	101.38	20.10	100.54	30.41	101.38
30	10	29.34	99.94	9.89	98.90	29.84	99.48	9.89	98.92

* % R - % Recovery

2.7 Assay of marketed preparation

20 tablets of Razel EZ were accurately weighed and finely powdered. Tablet powder equivalent to 10 mg of RSV (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 RSV and EZT each. The procedure was repeated 6 times for tablet formulation. The assay results are presented in Table 3.

Table 3: Assay result for RSV and EZT in a tablet (Razel-EZ) by proposed methods

METHOD		**PCR**				**PLS**			
RSV	**ETZ**	**RSV**		**EZT**		**RSV**		**EZT**	
Actual (µg/ml)		Predicted (µg/ml)	% R*	Predicted (µg/ml)	%R*	Predicted (µg/ml)	% R*	Predicted (µg/ml)	% R*
10	10	9.82	98.21	9.82	98.25	9.72	97.20	9.82	98.26
10	10	9.87	98.70	9.87	98.78	9.89	98.90	9.87	98.78
10	10	9.77	97.71	9.77	97.74	9.85	98.50	9.76	97.68
10	10	10.29	102.90	10.29	102.20	10.11	101.11	10.29	102.20
10	10	10.03	100.30	10.13	101.24	10.12	101.24	10.13	101.13
10	10	9.99	99.91	9.99	99.91	9.97	99.72	9.99	99.99
MEAN		10.02	99.62	9.97	99.68	9.94	99.44	9.87	99.67
SD		0.27	1.88	0.20	1.7581	0.15	1.56	0.19	1.75

* % R - % Recovery

Accuracy study:

The accuracy study was carried out at three levels 50 %, 100 % and 150 % of assay concentration. The calculated amount of RSV and ETZ from standard solutions were spiked into sample solution and scanned in the range of 220-280 nm. Concentrations were predicted by using developed PCR and PLS models. Accuracy data is presented in Table 4 and 5.

Table 4: Accuracy data of RSV 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	14.86 14.91 14.75	14.91 14.70 14.89	99.06 99.40 98.33	99.00 98.00 98.33	0.18	0.51
100%	10	10	20	20.12 19.89 20.17	20.11 19.67 20.89	100.6 99.65 100.85	100.5 98.35 99.45	0.54	1.08
150%	10	15	25	25.04 24.98 25.60	25.23 24.81 25.35	100.16 99.92 102.40	100.92 99.24 101.41	1.35	1.13

Table 5: 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	14.83 14.93 15.05	14.94 14.82 15.07	98.86 99.53 100.34	99.60 98.80 100.4	0.744	0.803
100%	10	10	20	19.82 19.78 20.03	19.78 19.87 20.08	99.10 98.90 100.15	98.90 99.35 100.40	0.675	0.773
150%	10	15	25	24.70 24.88 25.15	24.72 24.91 25.08	98.8 99.52 100.60	98.88 99.64 100.32	1.054	0.872

Precision:

Precision was carried at three concentration levels (10, 15, 20 µg/ml for both RSV and ETZ in three replicates at each level. The results of intraday and interday precision studies which are presented in Table 6 and Table 7.

Table 6: 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	
RSV	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*
10 10 10	10 10 10	9.99 10.05 10.28	9.94 10.26 10.15	9.99 10.04 10.28	9.92 10.22 10.14	99.60 100.50 102.40	99.70 102.70 101.4	99.50 101.40 102.40	99.60 102.50 101.50	1.41	1.48	1.45	1.45
15 15 15	15 15 15	14.28 14.54 15.14	14.78 14.36 15.11	14.86 14.64 15.14	14.81 14.99 15.17	98.40 99.30 100.80	100.60 99.30 101.90	101.90 99.40 100.90	101.80 99.30 100.40	1.21	1.29	1.24	1.24
20 20 20	20 20 20	21.16 20.10 20.88	21.18 20.14 20.85	21.26 20.76 20.97	21.22 20.99 20.84	102.20 100.40 100.10	102.60 100.90 100.40	102.20 100.10 100.30	102.60 100.70 100.40	1.12	1.13	1.14	1.17

Table 7: Precision results obtained using developed PCR and PLS models (Interday Precision

Amount took µg/ml		Predicted Conc.(µg/ml)				% Recovery				% RSD			
		PCR		PLS		PCR		PLS		PCR		PLS	
RSV	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*	*RSV*	*EZT*
10 10 10	10 10 10	10.02 10.28 10.29	10.03 10.26 10.16	10.00 10.23 10.22	10.10 10.22 10.18	100.10 102.40 103.10	100.30 102.50 102.40	99.60 100.40 102.70	100.20 102.30 102.60	1.54	1.22	1.59	1.28
15 15 15	15 15 15	15.28 15.82 15.14	15.76 15.76 14.91	15.56 15.89 15.14	15.33 15.85 14.92	102.10 103.00 100.00	102.20 103.80 99.40	102.00 103.10 100.00	102.10 103.80 99.46	1.20	0.86	1.25	0.88
20 20 20	20 20 20	21.23 21.77 21.76	21.22 21.76 21.35	20.64 21.29 21.98	20.56 21.56 21.67	105.00 105.80 105.30	103.30 103.50 103.80	105.20 105.80 105.10	103.20 103.90 103.80	0.34	0.29	0.34	0.29

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.

RESULTS AND DISCUSSION:

Out of 41 mixtures, 32 set of mixtures were used for calibration and 09 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 8.

Table 8: Summary of Results

Parameters	**RSV**		**EZT**	
	PCR	**PLS**	**PCR**	**PLS**
Range(µg/ml)	5-30	5-30	5-30	5-30
Wavelength(nm)	220-280	220-280	220-280	220-280
Data intervalΔλ	0.5	0.5	0.5	0.5
Factors/PC's	2	2	2	2
%Recovery	99.62	99.91	99.72	99.99
LOD	0.52	0.52	0.18	0.18
LOQ	1.54	1.54	0.57	0.57
Correlation Coefficient(r^2)	0.995	0.997	0.997	0.995
Intercept	0.036	0.036	0.0141	0.0141
Slope	0.9907	0.0382	0.9907	0.039
RMSECV	0.81617	0.86817	0.2818	0.2819
RMSEP	0.26340	0.81610	0.2818	0.2815

CONCLUSION

A study of the use of UV spectrophotometric in combination with PLS and PCR for the simultaneous determination of RSV and ETZ in a binary mixture has been accomplished. The results obtained confirmed the suitability of the proposed method for simple, accurate and precise analysis of RSV and ETZ in pharmaceutical preparations. The proposed methods do not need separation of RSV and ETZ 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.

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REFERENCES

1. https://pubchem.ncbi.nlm.nih.gov/compound/Rosuvastatin_calcium(Accessed on 16/12/2017)
2. The Indian Pharmacopoeia. Vol. III, 1676-1678,(2014)
3. https://pubchem.ncbi.nlm.nih.gov/compound/Ezetimibe(Accessed on 16/12/2017)
4. The Indian Pharmacopoeia. Vol. I, 1727-1728, (2014).
5. Gupta A, Mishra P, Shah K. Simple UV spectrophotometric determination of rosuvastatin calcium in pure form and in pharmaceutical formulations. J Chem. 2009; 6: 89-92.
6. Gupta K and Singhvi I. Development of UV spectrophotometric method for the estimation of ezetimibe from tablet formulation. Int J Chem Sci.2015; 13(2): 1051-1056.
7. Rothekar H, Telrandhe R, Jumade P. Development and validation of RP-HPLC method for simultaneous determination of rosuvastatin and clopidogrel in tablet dosage form. International Journal of Pharmaceutics & Drug Analysis. 2017; 5(12): 475-482.
8. Narayankar SM, Sakpal PH, Bhingare CL, Ingale PL. Development and validation of RP-HPLC method for the estimation of rosuvastatin calcium and niacin in combined tablet dosage form. International Journal of Pharma Research & Review. 2015; 4(6): 44-50.
9. Tank PH, Vadalia KR, Dedania ZR. Development and validation of HPTLC method for simultaneous estimation of rosuvastatin calcium and aspirin in capsule dosage form. IJPSR. 2012; 3(10): 3867-3870.
10. Dixit PR, Chandrashekar R, Nagashekar SM. Stability indicating HPLC method for simultaneous determination of ezetimibe and simvastatin. Asian J Pharm Sci. 2007; 2: 182-190.
11. Chaudhari BG, Patel NM, Shah PB, Modi KP. Development and validation of a HPTLC method for the simultaneous estimation of atorvastatin calcium and ezetimibe. Ind J Pharm Sci. 2006; 68(6): 793-796.
12. Gajjar Anuradha K and Shah VD. Simultaneous UV spectrophotometric estimation of rosuvastatin and ezetimibe in their combined dosage forms. Int J Pharm Pharm Sci. 2010; 2(1): 131-138.
13. Sireesha. D, Laksmi M, Bakshi V. Development and validation of UV spectrophotometric method for the simultaneous estimation of rosuvastatin and ezetimibe in pharmaceutical dosage form. Asian J Pharm Ana. 2017; 7(3): 135-140.
14. Swathi Sri D, Hemant Kumar T, Vara Prasada Rao K, Srinivasa Rao Y. Validated RP-HPLC method for simultaneous determination of rosuvastatin calcium and ezetimibe in pharmaceutical dosage form. Int J Pharm Pharm Sci. 2015; 7(4): 209-213.
15. Laksmi M, Sireesha D, Bakshi V. Development and validation of stability indicating RP- HPLC method for the simultaneous estimation of rosuvastatin and ezetimibe in combined dosage form.World Journal of Pharmaceutical Research. 2017; 6(8): 2369-2381.
16. Varghese SJ, Ravi TK. Determination of rosuvastatin and ezetimibe in a combined tablet dosage form using high-performance column liquid chromatography and high-performance thin-layer chromatography.J AOAC Int. 2010; 93(4): 1222-1227.
17. Dinc E, Baleanu D. Spectrophotometric quantitative determination of cilazapril and hydrochlorothiazide in tablets by chemometric methods. J Pharm Biomed Anal.2002; 30: 715-723.
18. Jahangiri A, Adibkia K, Asadpour-Zeynali, K, Javadzadeh Y, Hamishehkar H, Barzegar-Jalali M. Application of multivariate calibration methods, in dissolution testing and simultaneous determination of atorvastatin and ezetimibe in their combined solid dosage form. Pharm Sci. 2016;22: 105-111.
19. Bhaskar R, Sagar M, Saini V, Bhat M. UV spectrophotometric-assisted chemometric methods for the simultaneous determination of metformin hydrochloride and gliclazide in pharmaceutical formulations. Pharmaceut Anal Acta. 2012; 3: 1-5.

20. International Conference on Harmonization. ICH harmonized tripartite guideline validation of analytical procedures text and Methodology Q2 (R1) ICH, *Geneva*, 1-17, (2005).