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Development and validation of chemometric assisted analytical methods for simultaneous estimation of Atorvastatin calcium and Aspirin in capsule dosage form

Keerthisikha Palur*[1], Bharathi Koganti[2], Sreenivasa Charan Archakam[3]

[1]Research Scholar, Institute of Pharmaceutical Technology, Sri Padmavathi MahilaViswavidyalayam, Tirupati-517502, India
[2]Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Viswa Vidyalayam, Tirupati, India
[3]Department of Pharmaceutical Analysis, Sri Padmavathi School of Pharmacy, Tirupati, India

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ABSTRACT

To develop two Chemometric-assisted analytical methods like UV spectrophotometry and RP-HPLC methods for the quantification of Atorvastatin calcium (ASC) and Aspirin (APN) in the capsule dosage form. Chemometric models used in UV spectrophotometry were Principal component regression model (PCRM) and Partial least-square regression (PLSR). Both the models were applied for the drugs in the calibration ranges of 4-20 and 30-150 μg/mL for ASC and APN respectively. Total of nineteen laboratory prepared mixtures were used for calibration and prediction set of the models. In addition, RP-HPLC method by using chemometric approach for was developed using C18 column at room temperature with a mobile phase of acetonitrile: methanol: triethylamine (53.1:11.9:35 v/v/v), pH- 3.0, with detection at 275 nm.PCRM and PLSR models were evaluated by statistical parameters and RP-HPLC method was optimized by using Response surface methodology. The developed methods like UV and RP-HPLC by using chemometrics showed almost similar results and both the methods can be used for their analysis.

*Corresponding Author

Name: Keerthisikha Palur
Phone: +91 9533559130
Email: keerthi8spsp@gmail.com

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INTRODUCTION

Atorvastatin Calcium (ASC) acts by inhibiting the enzyme HMG CoA reductase and used to treat hyperlipidemia. Aspirin (APN) has several multiple effects like anti-inflammatory, antipyretic and anti-platelet action. The chemical structures of the analytes ASC and APN were shown in Figure 1. The combination of ASC and APN is used to reduce Hyperlipidemia, Hypertriglyceridemia.

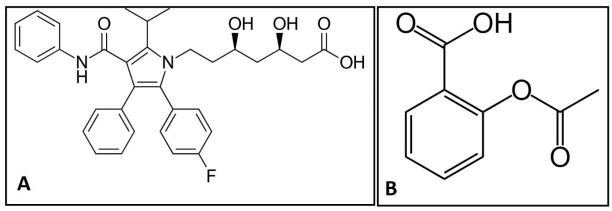

Figure 1: Chemical structure of analytes; A- Atorvastatin calcium; B- Aspirin

Literature survey revealed that few analytical methods like UV, RP-HPLC and LC-MS-MS were reported for the simultaneous determination of ASC and APN (Palur *et al.*, 2016; Shah *et al.*, 2007; Suma *et al.*, 2012; Pawar *et al.*, 2013). It was also found that no chemometric assisted RP-HPLC method and UV methods like PCRM and PLSR have been reported.

Table 1: Training and Prediction sets for PCRM and PLSR methods

Training set a			Prediction set a		
Mixture	ASC	APN	Mixture	ASC	APN
1	4	30	14	20	150
2	4	150	15	12	60
3	12	90	16	4	60
4	16	150	17	12	120
5	20	30	18	16	90
6	12	150	19	20	90
7	16	30			
8	16	60			
9	20	60			
10	8	30			
11	8	60			
12	8	90			
13	16	120			

Partial least squares (PLSR) and Principal Component Regression (PCRM) are the most widely used chemometric models for simultaneous determination of multi-component formulations (Şahin *et al.*, 2007). RP-HPLC method optimization using chemometrics has several advantages. (Sivakumar *et al.*, 2007). Most widely used design which was reported in many works is central composite design under the category of response surface methodology. Optimization uses the function of Derringer's desirability.

EXPERIMENTAL

Instrumentation and software

Spectral analysis was carried out using a double beam UV VIS spectrophotometer. The software used for spectral measurements was UV probe. Chromatographic measurements were analysed by using HPLC instrument of SHIMADZU make assisted with UV detector. The software used for chromatographic data processing is LC solutions. PLSR and PCRM models were performed using UNSCRAMBLER X version 10.5.1. In RP-HPLC, design conditions, statistical analysis, optimisation functions were performed by using Design Expert software.

Materials and Reagents

Working standards of ASC and APN (Raffles Pharmaceuticals Tirupati, India) were used without purification. ECOSPIRIN ES capsules labeled with 10 mg of Atorvastatin and 75 mg of Aspirin per capsule were purchased from local Pharmacy. Acetonitrile (ANL) and methanol (MN) were of HPLC grade, and triethylamine and orthophosphoric acid of analytical grade (SD Fine Chemicals, Mumbai, India) was used for analysis.

Standard solutions and Calibration

Standard stock solutions of ASC and APN were prepared by dissolving 10 mg of drug in 10 ml of methanol to get a concentration of 1 mg/mL. Stock solutions were further diluted with methanol to get a range of 4-20 μg/mL for ASC and 30-150 μg/mL for APN in spectrophotometry and in case of RP-HPLC diluted with mobile phase in the concentration range of 5–15 μg/mL and 37.5- 112.5 μg/mL for ASC and APN, respectively.

Sample preparation

20 capsules were taken, weighed, and the powder was removed from the capsules. An equivalent amount of 10 mg ASC and 75 mg APN was taken in a 10 ml standard flask and the volume was made up to mark with methanol. The sample solution was kept for sonication for fifteen minutes and then filtered. The solution was further diluted with methanol (PLSR and PCRM methods) and the mobile phase (for RP-HPLC) to obtain a concentration of 10 and 75 μg/mL of ASC and APN, respectively.

PCRM and PLSR methods

Nineteen mixtures containing ASC and APN in different ratios were prepared in the laboratory from their stock solutions. The mixtures were divided into two sets, as shown in Table 1. The training set consisted of thirteen mixtures, and prediction/validation set consisted of six mixtures. All the mixtures were scanned in the UV spectrophotometer. The range of wavelengths selected for PCRM and PLSR models were 240-300nm with a data interval of 1 nm. The statistical parameters obtained from

Table 2: Central composite design of experiments and responses

	Factor levels			Responses		
Run	A:A	B:B	C:C	K 2	RS 1,2	tR2
1	55	38.409	1	1.088	17.509	6.329
2	55	21.591	1	0.363	6.023	3.815
3	46.591	30	1	1.137	17.755	6.475
4	55	30	1	0.552	9.279	4.417
5	55	30	1	0.552	9.3	4.414
6	50	25	1.2	0.519	7.886	3.615
7	55	30	1	0.552	9.231	4.419
8	55	30	1	0.553	9.244	4.415
9	60	35	0.8	0.719	13.18	6.237
10	55	30	0.663641	0.554	10.729	6.537
11	63.409	30	1	0.626	10.462	4.701
12	60	25	1.2	0.424	6.566	3.333
13	55	30	1	0.554	9.209	4.411
14	60	25	0.8	0.425	7.793	4.961
15	55	30	1.33636	0.554	8.234	3.314
16	50	35	0.8	1.334	22.305	8.925
17	55	30	1	0.551	9.36	4.421
18	60	35	1.2	0.722	11.229	4.194
19	50	35	1.2	1.335	19.379	6
20	50	25	0.8	0.519	9.387	5.385

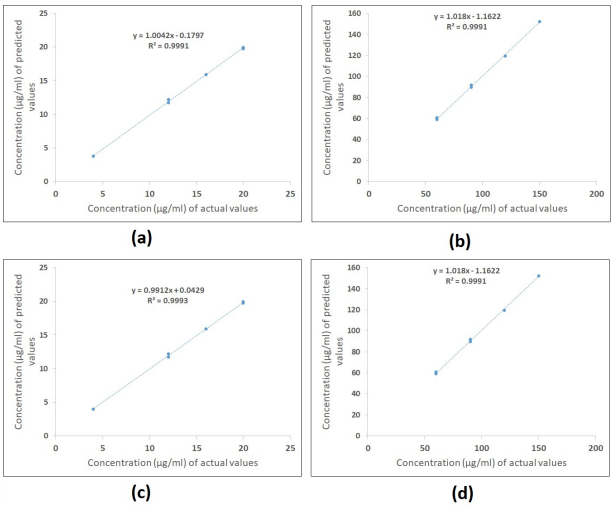

Figure 2: PCRM and PLSR methods: (a) and (b) represent the correlation coefficients obtained by PCRM for ASC and APN respectively and (c) and (d)represent the correlation coefficients obtained by PCRM for ASC and APN respectively

the training set was used to predict the concentrations of validation set and then applied to the analysis of dosage form.

Chromatographic procedure

Chromatographic measurements were performed

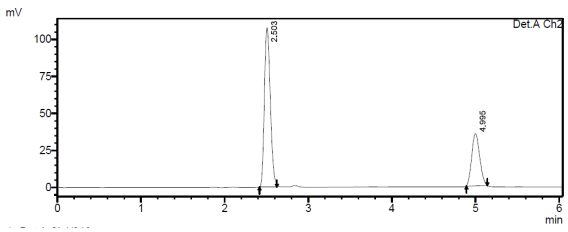

Figure 3: Assay Chromatogram

by using Phenomenex C18 analytical column. ANL-MN-0.1 %(w/v) triethyl amine (pH 3.0) was used as mobile phase. Flow rate maintained was 1.2 mL/min. Detection wavelength used for measurements was 246 nm. Response surface methodology (RSM) - Central composite design (CCD) was used for further analysis which was presented in Table 2. For the trials performed, ANL content (50-60%) triethylamine content (25-35%) and flow rate (0.8-1.2 mL/min) were selected as critical factors which are mainly affecting the separation process. 20 experiments which was given in CCD were carried out in the different ranges of selected factors and the three responses like APN capacity factor, resolution between ASC and APN and the retention time of ASC were found to have a profound effect on the selected factors. Statistical parameters were studied, and

Table 3: Results obtained by PCRM and PLSR methods for Prediction set

Validation	PCRM				PLSR			
	ASC		APN		ASC		APN	
	Predicted conc.	% Recovery	Predicted conc.	% Recovery	Predicted conc.	% Recovery	Predicted conc.	% Recovery
1. 1.	19.9398	99.70	152.2009	101.47	19.9397	99.70	152.2011	101.47
1. 2.	12.1960	101.63	59.0843	98.47	12.1964	101.64	59.0813	98.47
1. 3.	3.9931	99.83	60.7432	101.24	3.9932	99.83	60.7424	101.24
1. 4.	11.7042	97.53	119.6590	99.72	11.7041	97.53	119.6599	99.72
1. 5.	15.9086	99.43	91.7159	101.91	15.9086	99.43	91.7158	101.91
1. 6.	19.7761	98.88	89.8896	99.88	19.7764	98.88	89.8876	99.88
MEAN		99.50	100.45		99.50		100.45	
% RSD		1.34	1.301		1.34		1.303	
RMSEP		0.2037	1.2454		0.2037		1.2458	

Table 4: CCD-RSM statistical parameters

Responses	P value	% CV	Adequate Precision	Adjusted R2
K 2	< 0.0001	5.90	34.4488	0.9814
RS 1,2	< 0.0001	5.30	38.5816	0.9825
tR2	< 0.0001	3.95	37.9583	0.9793

Table 5: Assay of ECOSPIRIN-ES by developed methods

Methods	ASC			APN		
	Amount in capsule (mg)	Amount found a (mg)	Mean ±%RSD	Amount in capsule (mg)	Amount found a (mg)	Mean ± %RSD
PCRM	10	9.996	99.96±1.76	75	73.23	97.64±0.16
PLSR	10	9.993	99.93±1.80	75	73.22	97.63±0.16
RP-HPLC	10	9.89	98.99±0.99	75	75.41	100.55±0.19

optimization and validation was further carried out.

RESULTS AND DISCUSSION

Optimization and Validation of PCRM and PLSR methods

The PCRM and PLSR models were optimized by using factors and RMSECV values (root-mean-square error of cross-validation) and the no. of factors were two for both the drugs in the developed models (Haaland and Thomas, 1988; Belal *et al.*, 2018). The correlation coefficients of ASC and APN obtained were 0.9991 and 0.9991 for both PCRM and PLSR models, and the models indicated a good linear relationship, as shown in Figure 2.

The statistical parameters like root mean squares of error of calibration (RMSEC), root mean squares of error of prediction (RMSEP) and percent recoveries for the prediction set were presented in Table 3.

Optimization in RP-HPLC method

Statistical parameters for the CCD- RSM which were found from ANOVA are presented in Table 4. All the parameters like adjusted $R^{2,}$ Coefficient of variation and adequate precision were found to be within limits (Lundstedt *et al.*, 1998; Beg *et al.*, 2003). The interaction effects of factors and responses were studied by using three-dimensional response surface plots, and overall effects were shown by perturbation plots (Janardhanan *et al.*, 2016). The derringer's desirability value was found to be 0.840, and the optimum condition is given was ANL (53.16 %v/v), triethylamine (35%v/v) and flow rate of 1.2 mL/min. Assay and validation of the method was carried out using optimum condition.

Validation of optimized RP-HPLC method

According to ICH guidelines, all validation parameters were performed. The developed RP-HPLC method was found to be linear for ASC and APN, and their correlation coefficients was found to be 0.9996 and 0.9991 respectively. System precision was carried out by injecting the six replicas of standard mixture and the %RSD of parameters like retention times, tailing factor, theoretical plates were found to be less than 2.0. Method precision was performed for six test sample injections, and %RSD of peak areas was found to be less than 2.0. The percent recovery values are 99.54 and 100.49 for ASC and APN respectively indicating the method accuracy. Robustness parameter also studied in the CCD design.

Application of the developed methods

The developed and validated chemometric assisted UV and RP-HPLC methods were applied for the quantitative analysis of ECOSPIRIN –ES capsules containing ASC 10 mg and APN 75 mg. The assay results obtained by PLSR, PCRM and RP-HPLC methods were found to be within the acceptable limits for both the drugs and were presented in Table 5 and assay chromatogram was shown in Figure 3.

A comparative study has been carried out on the chemometric assisted UV and RP-HPLC methods for the quantification of ASC and APN in their capsule dosage form. The applied PCRM and PLSR models were found to be suitable for simultaneous estimation of drugs. The CCD-RSM design employed in RP-HPLC was found to have good accuracy and precision and made the optimization part easier. Both methods showed good results

CONCLUSION

The proposed chemometric assisted UV spectrophotometric methods like PCRM and PLSR and RP-HPLC methods were found to be simple, accurate, precise and robust for the analysis of ASC and APN in dosage forms. If was found to be that the results of the developed UV and RP-HPLC methods are insignificant and all the methods can be used for quantitative analysis of ASC and APN.

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