



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

Keerthisikha Palur^{*1}, Bharathi Koganti², Sreenivasa Charan Archakam³

¹Research Scholar, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Viswavidyalayam, Tirupati-517502, India,

²Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Viswa Vidyalayam, Tirupati-India

³Department of Pharmaceutical Analysis, Sri Padmavathi School of Pharmacy, Tirupati-India



Article History:

Received on: 13.03.2019

Revised on: 21.06.2019

Accepted on: 24.06.2019

Keywords:

Principal component regression,
Partial least-square regression,
Response surface methodology

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 (PCR) and Partial least-square regression (PLSR). Both the models were applied for the drugs in the calibration ranges of 4-20 and 30-150 $\mu\text{g}/\text{mL}$ 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. PCR 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

ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v10i3.1342>

Production and Hosted by

Pharmascope.org

© 2019 Pharmascope Publications. All rights reserved.

of ASC and APN is used to reduce Hyperlipidemia, Hypertriglyceridemia.

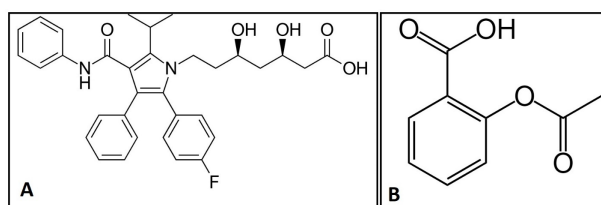


Figure 1: Chemical structure of analytes; A- Atorvastatin calcium; B- Aspirin

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

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 PCR and PLSR have been reported.

Partial least squares (PLSR) and Principal Component Regression (PCR) 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 PCR 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 PCR methods) and the mobile phase (for RP-HPLC) to obtain a concentration of 10 and 75 µg/mL of ASC and APN, respectively.

PCR 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 PCR and PLSR models were 240-300nm with a data interval of 1 nm. The statistical parameters obtained from the training set was used to predict the concentrations of validation set and then applied to the analysis of dosage form.

Table 1: Training and Prediction sets for PCR 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			

Chromatographic procedure

Chromatographic measurements were performed 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

Table 2: Central composite design of experiments and responses.

Run	Factor levels			Responses		
	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

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 optimization and validation was further carried out.

RESULTS AND DISCUSSION

Optimization and Validation of PCR and PLSR methods

The PCR 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 PCR 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

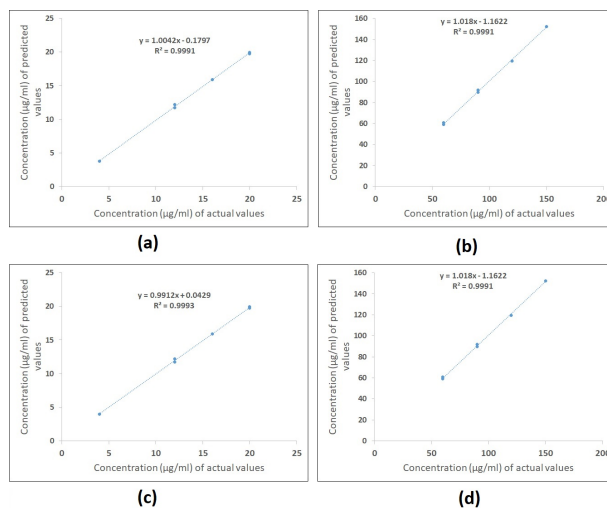


Figure 2: PCR and PLSR methods: (a) and (b) represent the correlation coefficients obtained by PCR for ASC and APN respectively and (c) and (d) represent the correlation coefficients obtained by PLSR for ASC and APN respectively.

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 der-

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

Validation	PCR			PLSR		
	ASC	APN		ASC	APN	
	Predicted conc.	% Recovery	Recovery	Predicted conc.	% Recovery	Recovery
1. 1.	19.9398	99.70		152.2009	101.47	
1. 2.	12.1960	101.63		59.0843	98.47	
1. 3.	3.9931	99.83		60.7432	101.24	
1. 4.	11.7042	97.53		119.6590	99.72	
1. 5.	15.9086	99.43		91.7159	101.91	
1. 6.	19.7761	98.88		89.8896	99.88	
MEAN		99.50		100.45		
% RSD		1.34		1.301		
RMSEP		0.2037		1.2454		

ringer'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.

Table 4: CCD-RSM statistical parameters

Re-sponses	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

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 stan-

dard 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.

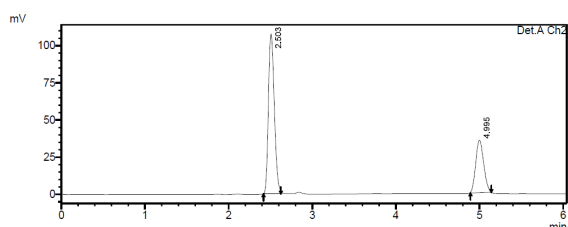
Application of the developed methods

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, PCR 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 PCR 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 preci-

Table 5: Assay of ECOSPIRIN-ES by developed methods

Methods	ASC			APN			±
	Amount in capsule (mg)	Amount found (mg)	Mean ±%RSD ^a	Amount in capsule (mg)	Amount found (mg)	Mean ±%RSD ^a	
PCRМ	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	

**Figure 3: Assay Chromatogram**

sion and made the optimization part easier. Both methods showed good results

CONCLUSION

The proposed chemometric assisted UV spectrophotometric methods like PCRМ 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. It 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.

ACKNOWLEDGEMENT

We acknowledge the management and the principal of Sri Padmavathi School of Pharmacy, Tiruchanoor, for providing the facilities to carry out this research work.

REFERENCES

- Beg, Q. K., Sahai, V., Gupta, R. 2003. Statistical media optimization and alkaline protease production from *Bacillus mojavensis* in a bioreactor. *Process Biochemistry*, 39(2):203–209.
- Belal, F., Ibrahim, F., Sheribah, Z. A., Alaa, H. 2018. New spectrophotometric/chemometric assisted methods for the simultaneous determination of imatinib, gemifloxacin, nalbuphine and naproxen in pharmaceutical formulations and human urine. *Spectrochimica Acta Part A: Molecular*

and Biomolecular Spectroscopy, 198:51–60.

Şahin, S., Demir, C., Ş. Güçer 2007. Simultaneous UV-vis spectrophotometric determination of disperse dyes in textile wastewater by partial least squares and principal component regression. *Dyes and Pigments*, 73(3):368–376.

Haaland, D. M. Thomas, E. V. 1988. Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. *Analytical Chemistry*, 60(11):1193–1202.

Janardhanan, V. S., Manavalan, R., Valliappan, K. 2016. Chemometric technique for the optimization of chromatographic system: Simultaneous HPLC determination of Rosuvastatin, Telmisartan, Ezetimibe and Atorvastatin used in combined cardiovascular therapy. *Arabian Journal of Chemistry*, 9:1378–1387.

Lundstedt, T., Seifert, E., Abramo, L., Thelin, B., Nyström, Å., Pettersen, J., Bergman, R. 1998. Experimental design and optimization. *Chemometrics and Intelligent Laboratory Systems*, 42(1–2):3–40.

Palur, K., Koganti, B., Archakam, S. C., Chenchugari, S., Nagireddy, B., Devabhaktuni, M. B., Sankranthi, M. 2016. Simultaneous Estimation of Atorvastatin and Aspirin in Bulk and Capsule Dosage Form by Chemometric Assisted Spectrophotometric Methods. *Journal of Young Pharmacists*, 8(4):424–429.

Pawar, P. Y., Bhagat, A. R., Lokhande, S. R., Bankar, A. A. 2013. Simultaneous estimation of atorvastatin calcium and aspirin in pure and capsule dosage form by using U.V. spectrophotometric method. *Der Pharma Chemica*, 5(3):98–103.

Shah, D., Bhatt, K., Mehta, R., Shankar, M., Baldania, S., Gandhi, T. 2007. Development and validation of a RP-HPLC method for determination of atorvastatin calcium and aspirin in a capsule dosage form. *Indian Journal of Pharmaceutical Sciences*, 69(4):546–10.

Sivakumar, T., Manavalan, R., Muralidharan, C., Valliappan, K. 2007. Multi-criteria decision making

approach and experimental design as chemometric tools to optimize HPLC separation of domperidone and pantoprazole. *Journal of Pharmaceutical and Biomedical Analysis*, 43(5):1842–1848.

Suma, B. V., Kannan, K., Madhavan, V., Nayar, C. R. 2012. Simultaneous Estimation and Validation of Atorvastatin Calcium and Aspirin in Combined Capsule Dosage Form by RP HPLC Method. *E-Journal of Chemistry*, 9(3):1449–1456.