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Development and Validation of Chemometric Assisted UV Spectroscopic Methods for the Simultaneous Determination of Amitriptyline Hydrochloride and Propranolol Hydrochloride in Marketed Formulation

KEERTHISIKHA PALUR*, SREENIVASA CHARAN ARCHAKAM, PRIYANKA PATAKAM, KAVYA ORUPALLI, PADMAJA YADAVALI, YOGESWAR POTHAPALA and RANGANAYAKULU DIVITI

Department of Pharmaceutical Analysis, Sri Padmavathi School of Pharmacy, Mohan Gardens, Tiruchanoor-517503, India

*Corresponding author: E-mail: keerthi8spsp@gmail.com

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The present work discusses the development and validation of chemometric assisted UV spectroscopic methods for analyzing two drugs amitriptyline hydrochloride and propranolol hydrochloride in their marketed formulation. The developed methods are two prominent chemometric models namely, principal component regression (PCR) and partial least squares regression (PLS) on which several research articles have been published for their immense contribution in the quantification of multi-component formulations. Linearity of the UV spectroscopic method was in the concentration ranges of 3-15 µg/mL of amitriptyline hydrochloride and 5-45 µg/mL of propranolol hydrochloride. The PCR and PLS chemometric models were established by employing fourteen mixtures as a calibration set and five mixtures as a validation set and were executed in the wavelength range of 240-320 nm with data interval of 5 nm. The statistical parameters obtained from both the methods revealed their validity and hence allows their suitability for analysis in regular quality control laboratories.

Keywords: Chemometrics, Principal component regression, Partial least squares regression, Amitriptyline, Propranolol.

INTRODUCTION

Amitriptyline hydrochloride (AH), *N,N*-dimethyl-3-(2-tricyclo[9.4.0.03,8]pentadeca-1(15),3,5,7,11,13-hexaenylidene)propan-1-amine is a tricyclic antidepressant [1] while propranolol hydrochloride (PH), 1-naphthalen-1-yloxy-3-(propan-2-ylamino)propan-2-ol is a non-selective β-adrenergic receptor blocker [2]. The combination of amitriptyline hydrochloride and propranolol hydrochloride is available in tablet dosage form in different strengths and prescribed in the treatment of migraine. The commercial formulation TRIPTOLOL (Centaur Pharmaceuticals Pvt. Ltd.) containing 10 mg of amitriptyline hydrochloride and 40 mg of propranolol hydrochloride per tablet was used in the present study.

The quantification of amitriptyline hydrochloride and propranolol hydrochloride in formulation was determined by few HPLC methods, however simultaneous estimation of amitriptyline hydrochloride by using spectroscopic methods was not reported yet [3-5]. Hence, an attempt was made to develop the most prominent chemometric models, principal component regression (PCR) and partial least squares regression (PLS) for their simultaneous quantification in the marketed formulation.

In comparison to other widely used quantitative techniques, UV-VIS spectroscopic methods have the benefit of being easier, simple to use and relatively less expensive. It has been found that chemometrically assisted UV spectroscopic approaches can increase spectrum data quality, selectivity, sensitivity, and the ability to resolve complex spectra with a better degree of accuracy and precision. The fundamental goal of spectral analysis of multicomponent formulations is to develop a calibration model that links spectrophotometer outputs to the quantities of the analyzed samples [6,7]. Chemometric instruments used in UV spectrophotometry are based on multivariate data analysis, which draws out pertinent data from the entire spectrum to employ a greater number of signals simultaneously. The PCR and PLS chemometric models determine one or more target variables by using a number of concurrent variables [8-14].

EXPERIMENTAL

A Shimadzu electronic balance (AY 220) was used for weighing of samples. The UV spectroscopic measurements are carried out by using Shimadzu UV-1800 spectrophotometer with matched pair of 10 mm quartz cells. UV Probe software was used for spectroscopic data acquisition. Chemometric models, principal component regression (PCR) and partial least squares regression (PLS) were executed using the software Unscrambler X (Camo analytics).

The pure samples of amitriptyline hydrochloride (AH) and propranolol hydrochloride (PH) were purchased from TCI Chemicals (India) Pvt. Ltd, Chennai, India. Methanol was used as solvent in the current analysis. The marketed formulation TRIPTOLOL (Centaur Pharmaceuticals Pvt Ltd) containing 10 mg of amitriptyline hydrochloride and 40 mg of propranolol hydrochloride per tablet was purchased from local pharmacy (Tirupati, India) and was the sample selected for the study.

UV spectroscopic method development: Appropriate concentrations of amitriptyline hydrochloride and propranolol hydrochloride were prepared in methanol and their UV spectra was measured in the 200-400 nm range and the spectra revealed that the absorption maxima of amitriptyline hydrochloride and propranolol hydrochloride were 239 nm and 273 nm, respectively. The concentration ranges of the working standard solutions to establish linearity for amitriptyline hydrochloride and propranolol hydrochloride were found to be 3-15 and 5-45 μg/mL, respectively.

Application of chemometric PCR & PLS models: Standard mixture solutions with various ratios of amitriptyline hydrochloride and propranolol hydrochloride in the ranges of their calibration curve were prepared for the application of the chemometric models. Using methanol as a blank, the prepared solutions were scanned in UV-VIS spectrophotometer in the 200-400 nm wavelength range. The recorded spectral data was imported into MS-EXCEL and data modelling was done in the wavelength range of 240-320 nm with a data interval of 5 nm.

Two sets of the prepared standard mixture solutions were prepared. The first set served as a training and calibration set for the building of calibrated models, while the second set served as a validation set to predict the concentrations of standard mixture solutions that were unknown. By employing k-fold cross-validation method, the optimal number of components was chosen and the calibration models for PCR and PLS were executed. Based on the results of statistical parameters obtained, optimization of calibration set was done.

The optimized calibration set containing fourteen standard mixture solutions in both the models at the selected components was used to test the validation set containing five standard mixture solutions, which were not used in the calibration set (Table-1). The predictive ability of the optimized PCR and PLS models was defined by accuracy (% recovery) and RMSEP (root mean square error of prediction) values. Sample solution was prepared from the marketed formulation to get a concentration of 10 μg/mL of amitriptyline hydrochloride (AH) and 5-45 μg/mL of propranolol hydrochloride (PH). The optimized models were applied to the analysis of the prepared sample solution.

TABLE-1
CALIBRATION AND VALIDATION SETS FOR PCR AND PLS METHODS

Calibration set						Validation set		
Mixture	AH	PH	Mixture	AH	PH	Mixture	AH	PH
1	6	35	8	12	35	15	6	9
2	6	45	9	12	45	16	9	45
3	9	15	10	15	5	17	9	35
4	9	25	11	15	15	18	6	25
5	12	5	12	15	25	19	9	5
6	12	15	13	15	35	–	–	–
7	12	25	14	15	45	–	–	–

AH = Amitriptyline hydrochloride; PH = Propranolol hydrochloride

RESULTS AND DISCUSSION

The interaction of a few components with the absorption spectra of the target analytes makes selectivity a common issue in UV-VIS spectroscopy with complex samples. Furthermore, multicomponent analysis using conventional UV spectral approaches is less frequently encountered in the literature. Another explanation for this is that because traditional approaches are dependent on univariate calibration, they might not be able to resolve complicated spectra. Chemometric assisted UV spectrum analysis ultimately aims to replace more complex and expensive methods with quicker and less expensive ones.

PCR and PLS chemometric models: The chosen wavelength range for spectrum analysis is 240-320 nm with a 5 nm data interval. Data below 240 nm was rejected due to noise and data over 300 nm was not chosen because of the presence of extremely little absorbance. The resulting RMSECV values were used to determine the number of principal components (PCs) in PCR and latent variables (LVs) in PLS. The ideal number of PCs and LVs for the PCR and PLS methods were found to be 7 and 6 for amitriptyline hydrochloride (AH) and propranolol hydrochloride (PH), respectively as these revealed the least RMSECV values (Fig. 1). The PCR and PLS calibration models containing the 14 standard mixture solutions were constructed at the chosen PCs and LVs and the calibration set was optimized based on the obtained statistical data as depicted in Tables 2 and 3, respectively. The models were further applied to the validation set and the statistical parameters like percent recovery, RMSEP and correlation coefficient are presented in Table-4. The validation set results demonstrated that both models were judged to be reliable and depicted the accuracy and strong predictive ability of the developed PCR and PLS models. The RMSEP values from the two models were quite low, indicating only slight prediction errors.

Analysis of marketed formulation: The concentrations of amitriptyline hydrochloride (AH) and propranolol hydrochloride (PH) in the marketed formulation were predicted using the validated PCR and PLS models and the assay results are reported in Table-5. The assay results obtained for amitriptyline hydrochloride (AH) and propranolol hydrochloride (PH) in their marketed formulation by PCR and PLS models were found to be within the acceptance criteria.

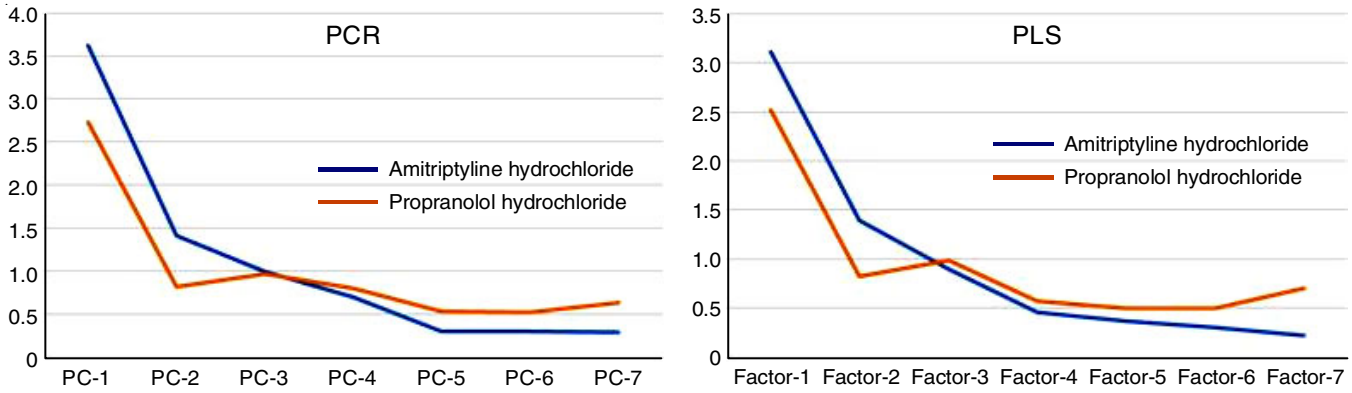

Fig. 1. RMSECV plot of the cross-validation results for amitriptyline hydrochloride and propranolol hydrochloride obtained by PCR and PLS

TABLE-2
RESULTS OBTAINED FOR CALIBRATION SET OF AMITRIPTYLINE HYDROCHLORIDE AND PROPRANOLOL HYDROCHLORIDE BY PCR

Calibration set of mixtures	Amitriptyline hydrochloride		Recovery (%)	Propranolol hydrochloride		Recovery (%)
	Actual	Predicted		Actual	Predicted	
Mixture 1	6	6.12	98.06	35	35.12	99.67
Mixture 2	6	5.92	101.35	45	44.91	100.19
Mixture 3	9	8.98	100.27	15	14.96	100.27
Mixture 4	9	9.00	99.95	25	25.04	99.84
Mixture 5	12	12.11	99.09	5	5.13	97.43
Mixture 6	12	11.86	101.16	15	14.93	100.45
Mixture 7	12	11.91	100.78	15	25.07	59.84
Mixture 8	12	12.01	99.87	35	34.32	101.97
Mixture 9	12	12.07	99.43	45	45.49	98.93
Mixture 10	15	15.06	99.62	5	5.18	96.50
Mixture 11	15	14.99	100.06	15	14.72	101.92
Mixture 12	15	14.91	100.62	25	25.11	99.57
Mixture 13	15	15.06	99.61	35	35.12	99.65
Mixture 14	15	15.01	100	45	44.90	100.22
	Mean%		99.99	Mean%		96.89
	SD		0.85	SD		10.76
	%RSD		0.85	%RSD		11.10
	RMSEC		0.07	RMSEC		0.25
	Correlation coefficient (R^2)		0.999	Correlation coefficient (R^2)		0.999
	RMSECV		0.289	RMSECV		0.524

TABLE-3
RESULTS OBTAINED FOR CALIBRATION SET OF AMITRIPTYLINE HYDROCHLORIDE AND PROPRANOLOL HYDROCHLORIDE BY PLS

Calibration set of mixtures	Amitriptyline hydrochloride		Recovery (%)	Propranolol hydrochloride		Recovery (%)
	Actual	Predicted		Actual	Predicted	
Mixture 1	6	6.00	100	35	35.10	100.28
Mixture 2	6	6.00	100	45	44.92	99.82
Mixture 3	9	9.01	99.89	15	14.98	99.83
Mixture 4	9	8.99	100.11	25	25.03	100.12
Mixture 5	12	12.07	99.42	5	5.12	102.47
Mixture 6	12	11.94	100.50	15	14.97	99.77
Mixture 7	12	11.95	100.42	25	25.06	100.22
Mixture 8	12	12.02	99.83	35	34.34	98.12
Mixture 9	12	12.02	99.83	45	45.46	101.02
Mixture 10	15	15.01	99.93	5	5.17	103.34
Mixture 11	15	15.01	99.93	15	14.73	98.20
Mixture 12	15	14.91	100.60	25	25.08	100.33
Mixture 13	15	15.07	99.50	35	35.12	100.32
Mixture 14	15	14.99	100.07	45	44.92	99.83
	Mean%		100.01	Mean%		100.26
	SD		0.33	SD		1.37
	%RSD		0.33	%RSD		1.37
	RMSEC		0.04	RMSEC		0.24
	Correlation coefficient (R^2)		0.999	Correlation coefficient (R^2)		0.999
	RMSECV		0.298	RMSECV		0.498

TABLE-4
RESULTS OBTAINED FOR VALIDATION SET OF AMITRIPTYLINE HYDROCHLORIDE (AH) AND PROPRANOLOL HYDROCHLORIDE (PH) BY PCR AND PLS

Validation mixture	PCR				PLS			
	Predicted values (µg/mL)		Recovery (%)		Predicted values (µg/mL)		Recovery (%)	
	AH	PH	AH	PH	AH	PH	AH	PH
15	5.86	15.59	97.69	103.91	5.58	15.56	92.93	103.70
16	8.93	48.44	99.20	107.65	8.89	48.42	98.73	107.60
17	8.05	34.77	89.48	99.34	8.28	34.80	92.03	99.43
18	7.06	24.97	117.74	99.87	7.18	24.99	119.62	99.97
19	8.60	5.89	95.50	111.19	8.47	5.86	94.14	117.13
Mean			99.92	104.40	Mean		99.49	105.57
SD			10.62	5.07	SD		11.54	7.25
%RSD			10.63	4.86	%RSD		11.60	6.86
Root mean square of prediction (RMSEP)			0.142	0.109	Root mean square of prediction (RMSEP)		0.147	0.113

TABLE-5
ASSAY RESULTS OBTAINED FOR VALIDATION SET OF AMITRIPTYLINE HYDROCHLORIDE (AH) AND PROPRANOLOL HYDROCHLORIDE (PH) BY PCR AND PLS

Marketed formulation	Principal component regression (PCR)				Partial least squares regression (PLS)			
	Predicted values (µg/mL)		Assay (%)		Predicted values (µg/mL)		Assay (%)	
	AH	PH	AH	PH	AH	PH	AH	PH
1	9.954	73.203	99.537	97.604	9.955	73.195	99.548	97.594
2	10.197	73.365	101.972	97.820	10.198	73.356	101.984	97.809
3	9.845	73.130	98.455	97.508	9.847	73.12344	98.465	97.498
MEAN			99.988	97.644	MEAN		99.999	97.633
SD			1.801	0.160	SD		1.802	0.159
%RSD			1.802	0.164	%RSD		1.802	0.163

Conclusion

The current study illustrated the simultaneous determination of amitriptyline hydrochloride (AH) and propranolol hydrochloride (PH) in the chosen marketed formulation using chemometric models like PCR and PLS. The generated models were able to detect the content of drugs simultaneously without any prior separation, despite the presence of extremely overlapped spectra. Development of PCR and PLS models was carried out at the chosen optimal factors in the 240-320 nm wavelength range with a 5 nm data interval. The results obtained from the models revealed the strength of the chemometric tools in easier quantification of drugs. The optimized models employed in this research work can be employed in regular and routine analysis in quality control laboratories.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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