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Simultaneous Spectrophotometric Determination of Amlodipine and Metoprolol by Principal Component Regression Multivariate Calibration and Comparison with HPLC

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Multi-drug therapy in high blood pressure is appreciated to achieve a normal blood pressure. A simple spectrophotometric method based on principal component analysis has been proposed for simultaneous determination of amlodipine (Amd) and metoprolol (Met) in pharmaceutical preparations. Calibration matrix contains 3.402 μgml^{-1} to 170.130 μg ml^{-1} amlodipine and 13.7 μg ml^{-1} to 68.5 μgml^{-1} metoprolol. The proposed method was validated by using a set of synthetic sample mixtures and subsequently applied to simultaneous determination of amlodipine and metoprolol in different pharmaceutical preparations.

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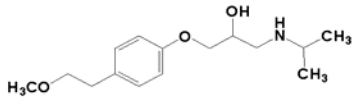
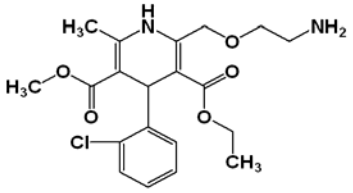
Introduction

High blood pressure, as one of the most common human diseases, can be treated with number of medications depending upon the causes which are responsible for it. It is more and more appreciated that the elusive aim of a 'normal' blood pressure is achieved only if multi-drug therapy is used[1].

Amlodipine (AMD), 2-[(2-aminoethoxy)-methyl]-4-(2-chlorophenyl) 1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylic acid-3 ethyl-5 methyl ester (Scheme 1), is a relatively new potent long-acting calcium channel blocker[2]. AMD is a third-generation dihydropyridine calcium antagonist that is applied alone or in combination with other drugs for treating high blood pressure, some types of vasospastic angina, hypertension, cardiac arrhythmias, and coronary heart failure [3].



Metoprolol



Amlodipine

Scheme 1. The chemical structures of AMD and MEP.

Various techniques including high-performance liquid chromatography (HPLC) [4], liquid chromatography (LC) [5], high-performance thin layer chromatography [6], gas chromatography (GC)[7], capillary electrophoresis[8], flow injection analysis [9], spectrofluorometric [10], spectrophotometric and voltammetric methods [11,12] have been employed for the determination of AMD in pharmaceutical and biological samples.

Metoprolol (MEP), 1-[4-(2-methoxyethyl)-phenoxy]-3-[(1-methylethylamino]-2-propanol (Scheme. 1) is a beta adrenergic blocking agent, which used in treatment of cardiovascular disorders and high blood pressure [13]. Several descriptions were presented in the literature for assaying MEP, with and without metabolites, in biological fluids and pharmaceutical samples [14-16,1].

As discussed above, there are several publications for the determination of AMD or MEP in pharmaceutical and biological samples individually. However, no publications were found for the simultaneous spectrophotometric determination of AMD and MEP by multivariate regression techniques.

Almost all of these techniques have the required sensitivity and selectivity for the analysis of AMD and MEP in various samples; however their sophisticated instrumentation and high-analysis cost limited their application in quality control laboratories for analysis of these drugs in their pharmaceutical dosage forms.

The principal component regression (PCR) technique is plainly explained as a principal component analysis (PCA) followed by a simple multivariate linear regression [17]. In PCR, the spectra are decomposed on the basis of the maximum variance between spectral data and information about the concentrations of samples is not applied.

The aim in this study is the simultaneous determination of AMD and MEP using the multivariate calibration and PCR method in pharmaceutical dosage (commercial oral tablets) using combination of PCA and UV-vis spectroscopy.

The results obtained by proposed method have been compared with the results obtained by application of a reference HPLC method (with a spectrophotometr detector)

Material and Methods

Chemicals, Instruments and software

Pure powders of AMD besylate and MEP tartarate were kindly provided by Amin Pharmacuetical Company and Tehran Daru, respectively. HPLC grade acetonitrile and methanol were obtained from Merck and Caledon. Methanolic stock solutions (10^{-3} M) were used. All other chemicals used were of analytical grade quality and bidistilled water was used too. A Perkin-Elmer Lambda spectrophotometer with 10 mm quartz cells was used. A PCA and required routines were written in Matlab package in our laboratory. The concentration of the mixture solutions were uniformly distributed over the range from 3.402 µg/ml to 170.130 µg/ml and from 13.7 µg/ml to 68.5 µg/ml for AMD and MEP, respectively. The UV–visible spectra of the mixtures

were used over the wavelength range 210–425 nm in increments of 1nm.

Univariate calibration

In order to find the linear dynamic concentration range of AMD and MEP, A univariate calibration was carried out. Different volumes of a 1×10^{-3} solution of each medication were added into different 10 ml volumetric flasks and diluted to the mark with methanol. The absorbance spectra were recorded over the 200–800 nm spectral range versus a solvent blank. The linear dynamic range for each drug was determined by plotting the absorbance at its λ_{max} (241 nmfor AMD and 210 nm for MEP) versus sample concentration.

Standard mixture solutions

A set of standard mixture solutions (i.e. training and test sets) were prepared. As shown in Table 1, the training set contained 30 standard mixtures, and 23 mixtures were employed in the test set. The respective concentrations of AMD and MEP in the standard mixtures were in their linear range. For preparation of each solution, the required volumes of stock solution were added to a 10.0 ml volumetric flask, and the contents of the flask were diluted to volume with methanol. Then, the absorbance spectra of the mixture were recorded versus the solvent blank. The spectra were recorded in the wavelength range of 200–800 nm but the range of 210–425 was used in PCR calibration step.

Training and test sets

In order to test the final model performances, about 45% of the synthetic samples (23 out of 53) were selected as external test set samples. The best situation of this step of model formation is dividing original matrix to guarantee that both training and test sets individually cover the total space occupied by original dataset. Then ideal splitting of dataset as each of samples in test set is close to at least one of the samples in the training set. Various methods were used as tools for splitting the whole original dataset to the training and test sets. According to Tropsha, the best models would be built when Kennard and Stone algorithm was used [18].

The Kennard–Stone [19] algorithm selects a set of samples in studied set of data, which are 'uniformly' distributed over the space defined by the original dataset.

This is a classic technique to extract a representative set of samples from a given dataset. In this technique the samples are selected consecutively. The first two samples are chosen by selecting the two farthest apart from each other. The third sample chosen is the one farthest from the first two samples, etc. Supposing that m samples have already been selected (m<n), the (*m+1*)th sample in the calibration set is chosen using the following criterion:

$$\max_{m<r\le n}(\min(d_{1r}, d_{2r}, ...d_{mr})$$

where *n* stands for the number of samples in the training set, d_{jr}, j=1,...,m are the squared Euclidean distances from a candidate sample *r*, not yet included in the representative set, to the *m* samples already included in the representative set. One more benefit of the Kennard–Stone method is that it may be used to any matrix of predictors; there are no restrictions regarding the matrix multicollinearity. The other advantage is that the test samples all fall inside the measured region and the training set samples map the measured region of the input variable space completely with respect to the induced metric.

Procedure of pharmaceutical preparations

Five tablets were finely powdered and diluted with 250 ml of methanol by sonication during 20 min. The mixture was filtered into a 10 ml calibrated flask; the residue was washed two times with the same solvent and diluted to the mark.

For HPLC determination, after the preparation of the methanolic solution of the five tablets (as described before), an aliquot of 5 ml of this solution was transfered into a 10 ml calibrated flask and 5 ml of internal solution (propranolol of 3.5×10^{-4} M) was added and well mixed. This solution was injected in the HPLC system. The mobile phase was a deaerated mixture of 146 µl triethylamin and 750 µl phosphoric acid to 530 ml water. Then, pH was adjusted to 3.3 using a 10% potassium hydroxide solution and finally adds 470 ml acetonitrile. Spectrophotometric detection was performed at 220 nm. The flow rate was set at 0.6 ml min^{-1}.

Results and discussion

Fig. 1 shows the absorption spectra in mathanolic solution of AMD and MEP separately. Fig. 1 shows that the absorbance maximum of AMD and MEP are 241 nm and 275 nm, respectively. As can be seen, the difference in maximum of absorbance spectra is not so large to permit simultaneous determination of the analytes using conventional univariate calibration methods. Said another way, the simultaneous determination of AMD and MEP in mixtures by conventional spectrophotometric methods is hindered by strong spectral overlap throughout the wavelength range. Such a determination could theoretically be facilitated by the application of multivariate calibration such as PCA.

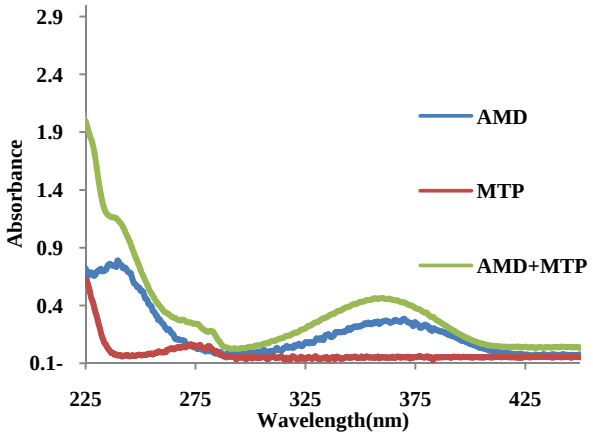


Fig. 1. UV absorbance spectra of methanolic solution of (A) 5×10^{-5} M of AMD, (B) 5×10^{-5} M of MEP and (C) mixtures of AMD and MEP each 5×10^{-5} M.

Hence, PCR as a robust multivariate regression technique was used for simultaneous analysis of AMD and MEP in pharmaceutical samples. It should be mentioned that the spectral regions employed in PCR were between 210 and 425 nm because methanol has strong absorbancies at wavelength regions lower than 210 nm.

Conventional univariate calibration

In order to establish the optimal conditions for the joint determination of AMD and MEP, the univariate calibration technique was applied to investigate the effect of experimental variables on the absorption spectra for the drugs studied. We first checked the stability of the two analytes in methalonic solution. For this purpose, the UV absorbance spectra for solutions of AMD and MEP as a function of time were examined and found that the spectra of AMD and MEP did not vary appreciably over a period of a few days provided that the solutions were kept at room temperature in the dark. It is found that the temperature doesn't have appreciable effect on the spectra of the AMD and MEP, and thus room temperature was chosen for the subsequent study.

In order to analyze the drugs, individual calibration curves were constructed with several points (Fig. 2), as absorbance vs. analytes concentration in their range of 3.402 μgml^{-1} to 170.130 μgml^{-1} and from 13.060 μgml^{-1} to 65.3 μgml^{-1} AMD and MEP, respectively. The wavelengths employed to generate calibration curves were 241and 275 nm for AMD and MET, respectively. Linear regression results, line equations, and R^2 are also shown in Fig. 2.

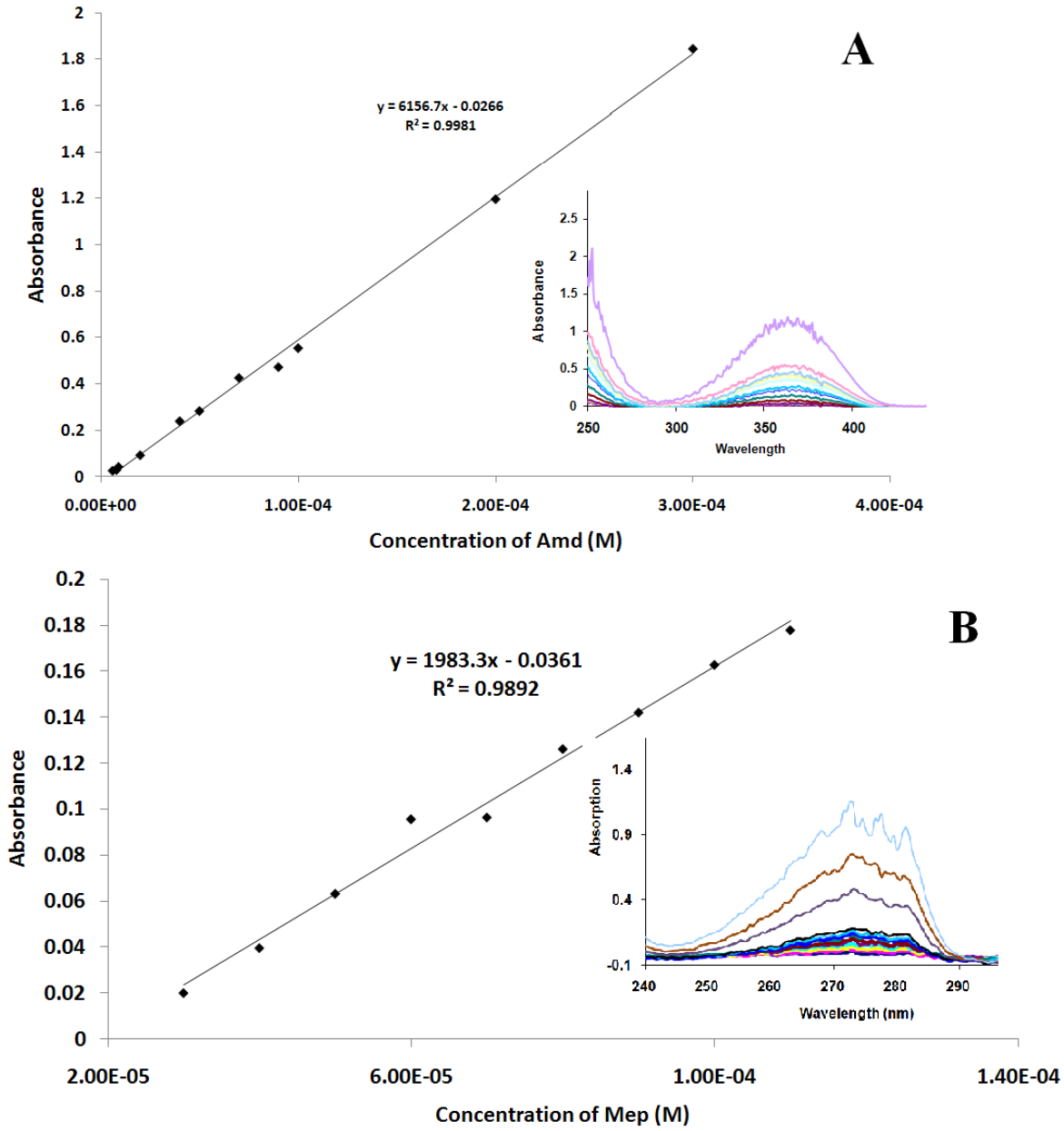


Fig. 2. Analytical curve for univariate determination of (A) AMD and (B) MEP

PCR modeling

Before application of the PCR for simultaneous determination of AMD and MEP in pharmaceutical preparation, this procedure was used to analysis of these medications in their synthestic binary mixtures. Multivariate regression techniques such as PCR require appropriate experimental design of the standard solution belonging to the calibration set in order to provide good predictions. The original data matrix was designed over the concentration linear ranges of 10-95 and 1-50 gmL^{-1} for AMD and MEP, respectively. The original data matrix used for the analysis is shown in Table 1. For the test step, 23 prepared mixtures that were not included in the calibration set were selected by Kennard and Stone algorithm and used (Table 1).

Table 1. Concentration data of the original dataset for mixture systems

Sample No.	AMD Conc. (M)	MEP Conc. (M)	Sample No.	AMD Conc. (M)	MEP Conc. (M)
1	7.10×10^{-6}	2.04×10^{-5}	28*	1.50×10^{-4}	6.13×10^{-5}
2*	7.10×10^{-6}	3.65×10^{-5}	29	1.50×10^{-4}	7.59×10^{-5}
3*	7.10×10^{-6}	5.25×10^{-5}	30	1.50×10^{-4}	9.93×10^{-5}
4	7.10×10^{-6}	6.86×10^{-5}	31*	1.85×10^{-4}	2.04×10^{-5}
5*	7.10×10^{-6}	8.47×10^{-5}	32	1.85×10^{-4}	3.65×10^{-5}
6*	3.53×10^{-5}	2.77×10^{-5}	33	1.85×10^{-4}	5.25×10^{-5}
7*	3.53×10^{-5}	4.38×10^{-5}	34	1.85×10^{-4}	6.86×10^{-5}
8	3.53×10^{-5}	6.13×10^{-5}	35*	1.85×10^{-4}	8.47×10^{-5}
9*	3.53×10^{-5}	7.59×10^{-5}	36	2.12×10^{-4}	2.77×10^{-5}
10*	3.53×10^{-5}	9.93×10^{-5}	37	2.12×10^{-4}	4.38×10^{-5}
11*	6.17×10^{-5}	2.04×10^{-5}	38	2.12×10^{-4}	6.13×10^{-5}
12	6.17×10^{-5}	3.65×10^{-5}	39	2.12×10^{-4}	7.59×10^{-5}
13*	6.17×10^{-5}	5.25×10^{-5}	40*	2.12×10^{-4}	9.93×10^{-5}
14*	6.17×10^{-5}	6.86×10^{-5}	41	2.38×10^{-4}	2.04×10^{-5}
15*	6.17×10^{-5}	8.47×10^{-5}	42	2.38×10^{-4}	3.65×10^{-5}
16*	8.82×10^{-5}	2.77×10^{-5}	43	2.38×10^{-4}	5.25×10^{-5}
17	8.82×10^{-5}	4.38×10^{-5}	44	2.38×10^{-4}	6.86×10^{-5}
18*	8.82×10^{-5}	6.13×10^{-5}	45*	2.38×10^{-4}	8.47×10^{-5}
19*	8.82×10^{-5}	7.59×10^{-5}	46	2.73×10^{-4}	2.77×10^{-5}
20*	8.82×10^{-5}	9.93×10^{-5}	47	2.73×10^{-4}	4.38×10^{-5}
21	1.23×10^{-4}	2.04×10^{-5}	48	2.73×10^{-4}	6.13×10^{-5}
22*	1.23×10^{-4}	3.65×10^{-5}	49	2.73×10^{-4}	7.59×10^{-5}
23*	1.23×10^{-4}	5.25×10^{-5}	50	2.73×10^{-4}	9.93×10^{-5}
24	1.23×10^{-4}	6.86×10^{-5}	51	3.00×10^{-4}	2.04×10^{-5}
25*	1.23×10^{-4}	8.47×10^{-5}	52	3.00×10^{-4}	3.65×10^{-5}
26	1.50×10^{-4}	2.77×10^{-5}	53	3.00×10^{-4}	5.25×10^{-5}
27	1.50×10^{-4}	4.38×10^{-5}			

PCA summarizes the information residing in the initial data, i.e., in our case the original data matrix of mixtures, into a new variables which may be more easily overviewed and applied. The original multi-dimensional space, defined by the spectral data of mixtures contracted into a few descriptive dimensions, represented principal components (PCs), which denote the main variation in the data.

The greatest amount of variability of the original data set is implied by the first PC, and the second PC describes the maximum variances of the residual dataset. Then, the third one will explain the most important variability of the next residual dataset, and so on. According to the theory of least squares, the eigenvectors of all PCs are orthogonal to each other in multi-dimension data space. Generally speaking, only *p* PCs are enough to account for the most variance in an *m*-dimensional data set, where *p* is the number of important PCs of the data set, and *m* means the number of all the PCs in the dataset of interest. It is obvious that *p* is less than *m*. So PCA is generally regarded as a data reduction method. It must be noted that a multi-dimensional dataset can be projected to a lower dimension data space without loss most of the information of the original data set by PCA [20].

To explore the structure of pool of original spectral data matrix, PCA was adopted on all the 53 mixtures, then 53 principal components (PCs) were generated. The eigenvlues, variances explained by the first ten PCs and cumulative variance are reported in Table 2. It can be found that the PC1 could explain more than 91% variance of all spectral data, and variances explained by the latter PCs gradually decreased. In total, the accumulative variance of the first ten PCs was up to 99%. So, it could be concluded that the first ten PCs could explain most of the variance of the 53 mixture of drugs.

Table 2. The results of PCA analysis

PC No.	Eigenvalue	Varainceexplaiened	Comulative variance
1	341.72	91.65	91.65
2	20.49	5.50	97.15
3	2.66	0.71	97.86
4	2.13	0.57	98.43
5	1.13	0.30	98.74
6	1.08	0.29	99.03
7	0.73	0.20	99.22
8	0.57	0.15	99.38
9	0.48	0.13	99.50
10	0.35	0.10	99.60

After PCA procedure, a series of new variables (PCs) were generated, so every sample could be denoted with the PCs, and the score plot is a description of samples in the new defined space by PCs. As the PCA had compressed most variance of the calculated descriptors into the first several PCs, the score plot of the first PCs may reveal important information of recognition. Fig. 3 is the score plot of the first two PCs. It could be seen that most samples were clustered together. So, it can be concluded that the first PCs contained the main characteristic for recognition of multi-dimentional dataset include mixture samples of AMD and MEP, and the major information of samples had been compressed into the first PCs by PCA.

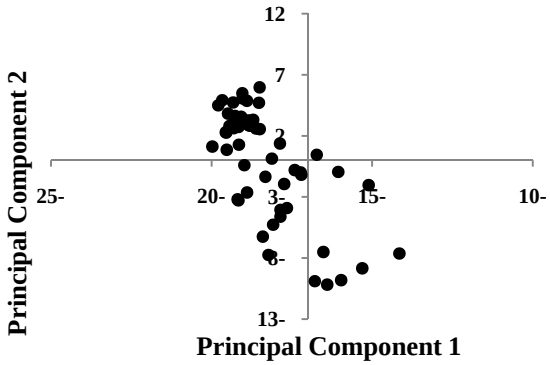


Fig. 3. Score plot of PCA on the original dataset

As discussed above, In order to use most of the standard linear and nonlinear calibration methods, the dataset should be split into the calibration and the test sets. The latter one is unavoidable for evaluation of developed models' features. Applying Kennard and Stone on the score matrix, the mixture samples are divided into the calibration set comprising 30 samples and the test set containing 23 samples (Table 1). The calibration set was used for the model development. The test set, consisting of 23 samples, was used to evaluate the developed model.

In order to choose the number of factors, a leave one out (LOO) cross-validation method was employed. The absorbance data was auto-scaled before any PCR procedure.

Given the set of 30 training mixture spectra, the PCR calibration was carried out on 29 calibration spectra and, using this model, the concentrations of the AMD and MEP in the sample left out during modelling were predicted. This procedure was repeated 30 times until each training sample had been left out once. Then, the predicted concentrations were compared with the experimental concentrations of the reference sample and the root mean square of error (*RMSE*) of *LOO* was calculated ($RMSE_{LOO}$). The $RMSE_{LOO}$ was calculated in the same manner each time a new factor was added to the PCR. As it is shown in Fig. 4, the best PCR model contained five factors. The predicted concentrations by using PCR technique are listed in Table 3 and are plotted in Fig.5. The plots of Fig.5 show that the data are distributed around a straight line.

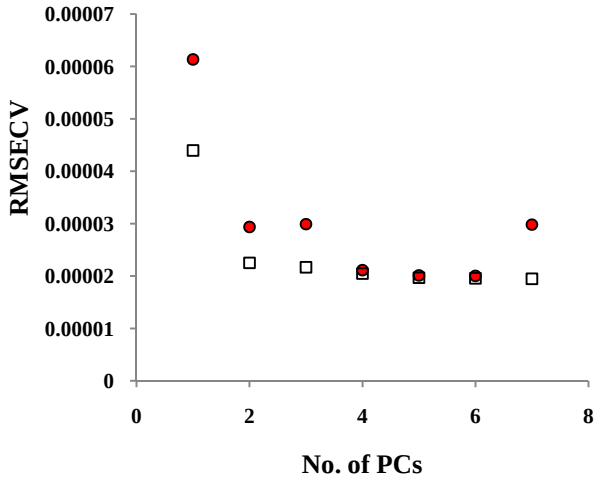


Fig. 4. Optimization of factors employed in modelling using $RMSE_{LOO.}$

The percent of recovery (*%REC*) for training and test sets are also reported in Table 3. As it is observed, there is a good agreement between the predicted and actual concentrations of AMD and MEP. The respective mean recovery for the AMD and MEP is 99.62 and 100.51, which confirm the high prediction power of the developed PCR model.

Determination of AMD and MEP in pharmaceutical formulations

The optimized PCR model has been applied to the resolution of two different formulations.

In Table 4, the obtained results by application of PCR are summarized and compared with those obtained by the HPLC method. The validation of the method has been performed by comparing with labeled amounts. As can be seen, the recovery was quantitative and there were no significant differences between the amounts obtained from developed PCR model, labeled amounts and HPLC method.

The indicated value is the mean of five different determinations of the same commercial batch.

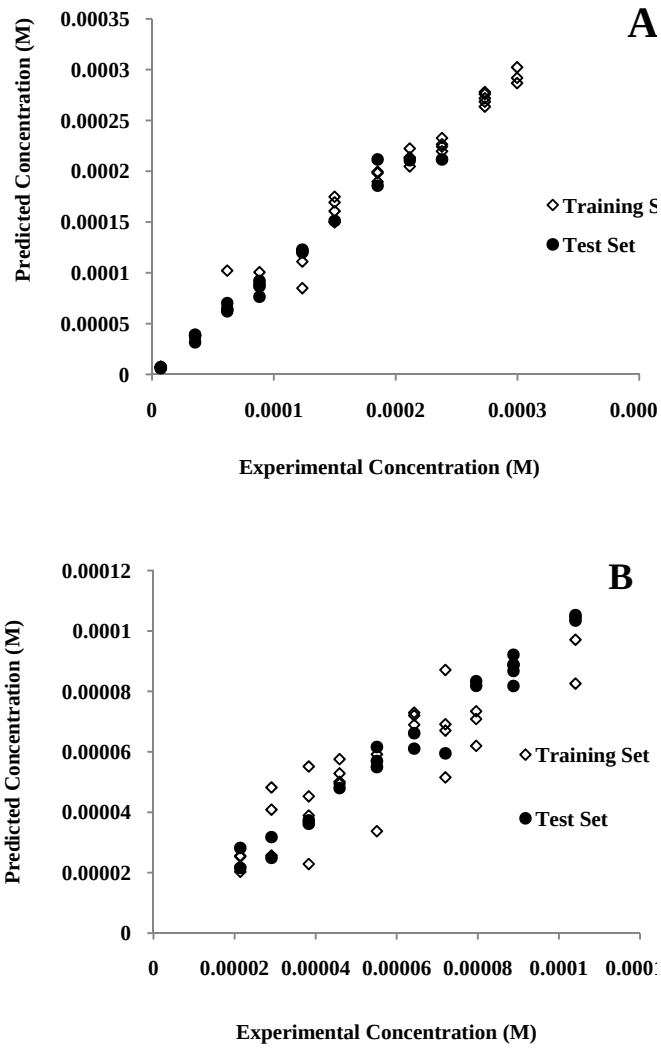


Fig. 5. Plot of predicted concentration against experimental concentration for (A) AMD and (B) MEP in synthetic mixture.

Table 3. Predicted concentration forthe original dataset for mixtures using PCR model

Sample No.	AMD Conc. (M)	REC%	MEP Conc. (M)	REC%	Sample No.	AMD Conc. (M)	REC%	MEP Conc. (M)	%Rec
1	6.84×10^{-6}	96.33	2.13×10^{-5}	104.41	28	1.51×10^{-4}	100.66	6.46×10^{-5}	105.38
2	6.40×10^{-6}	90.14	3.64×10^{-5}	99.72	29	1.75×10^{-4}	116.66	7.26×10^{-5}	95.65
3	6.19×10^{-6}	87.18	5.79×10^{-5}	110.28	30	1.61×10^{-4}	107.33	8.86×10^{-5}	89.22
4	7.67×10^{-6}	108.02	6.70×10^{-5}	97.66	31	1.86×10^{-4}	100.54	2.16×10^{-5}	105.88
5	7.25×10^{-6}	102.11	8.38×10^{-5}	98.93	32	2.00×10^{-4}	108.10	3.76×10^{-5}	103.01
6	3.91×10^{-5}	110.76	2.99×10^{-5}	107.94	33	1.90×10^{-4}	102.70	5.57×10^{-5}	106.09
7	3.89×10^{-5}	110.19	4.66×10^{-5}	106.39	34	1.98×10^{-4}	107.02	6.35×10^{-5}	92.56
8	3.84×10^{-5}	108.78	6.57×10^{-5}	107.17	35	2.12×10^{-4}	114.59	8.88×10^{-5}	104.84
9	3.73×10^{-5}	105.66	7.69×10^{-5}	101.31	36	2.05×10^{-4}	96.69	2.82×10^{-5}	101.80
10	3.16×10^{-5}	89.51	1.03×10^{-4}	103.72	37	2.22×10^{-4}	104.71	4.94×10^{-5}	112.78
11	7.03×10^{-5}	113.93	2.22×10^{-5}	108.82	38	2.14×10^{-4}	100.94	6.89×10^{-5}	112.39
12	1.02×10^{-4}	165.31	3.15×10^{-5}	86.30	39	2.13×10^{-4}	100.47	7.09×10^{-5}	93.41
13	6.48×10^{-5}	110.85	5.60×10^{-5}	106.66	40	2.11×10^{-4}	99.52	1.03×10^{-4}	103.72
14	6.36×10^{-5}	103.07	6.00×10^{-5}	87.46	41	2.20×10^{-4}	92.43	2.16×10^{-5}	105.88
15	6.22×10^{-5}	100.81	9.02×10^{-5}	106.49	42	2.24×10^{-4}	94.11	3.89×10^{-5}	106.57
16	7.64×10^{-5}	86.03	2.55×10^{-5}	92.05	43	2.33×10^{-4}	97.89	5.47×10^{-5}	104.19
17	1.01×10^{-4}	114.51	4.56×10^{-5}	104.10	44	2.27×10^{-4}	95.37	6.91×10^{-5}	100.72
18	8.66×10^{-5}	98.18	6.11×10^{-5}	99.67	45	2.12×10^{-4}	89.07	8.88×10^{-5}	104.84
19	9.21×10^{-5}	104.42	8.13×10^{-5}	107.11	46	2.69×10^{-4}	98.53	2.69×10^{-5}	97.11
20	8.85×10^{-5}	100.34	1.00×10^{-4}	100.70	47	2.64×10^{-4}	96.70	3.97×10^{-5}	90.63
21	1.11×10^{-4}	90.24	2.03×10^{-5}	99.50	48	2.76×10^{-4}	101.09	6.81×10^{-5}	111.09
22	1.20×10^{-4}	97.56	3.73×10^{-5}	102.19	49	2.72×10^{-4}	99.63	7.34×10^{-5}	96.70
23	1.23×10^{-4}	100.00	5.49×10^{-5}	104.57	50	2.78×10^{-4}	101.83	9.71×10^{-5}	97.78
24	8.49×10^{-5}	69.02	6.81×10^{-5}	99.27	51	2.87×10^{-4}	95.66	2.20×10^{-5}	107.84
25	1.22×10^{-4}	99.18	8.68×10^{-5}	102.47	52	3.02×10^{-4}	100.66	3.56×10^{-5}	97.53
26	1.50×10^{-4}	100.00	2.54×10^{-5}	93.72	53	2.92×10^{-4}	97.33	5.52×10^{-5}	105.14
27	1.69×10^{-4}	112.66	4.76×10^{-5}	108.67					

Table 4. Determination of AMD and MEP in pharmaceutical preparations using the developed PCR. These formulations contain 5 mg AMD besylate and 50 mg MEP succinate.

Commercial Formulation	AMD				MEP			
	Found (mg per tablet)		%Rec		Found (mg per tablet)		%Rec	
	HPLC	PCR	HPLC	PCR model	HPLC	PCR model	HPLC	PCR model
Tehran Daru	4.83(±0.07)	4.81(±0.11)	96.60	96.20	49.11(±0.37)	49.06(±0.81)	98.22	98.12
Mixture of Alborzdaru formulation	4.70(±0.13)	4.72(±0.12)	94.00	94.40	49.01 (±0.46)	48.83((±0.48)	98.02	97.66

Conclusion

Proposed combination of PCA and UV-Vis spectroscopy methods is specific, accurate and precise for the simultaneous determination of amlodipine besylate and metoprolol tartarate from pharmaceutical dosage form.

The explained approach is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

On the whole, this work shows that even when a complicated system is present, a well-developed chemometrics method may be capable of giving a satisfactory performance for spectroscopic calibration in pharmaceutical samples.

Conflict of Interests

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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