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Simultaneous chemometric determination of pyridoxine hydrochloride and isoniazid in tablets by multivariate regression methods

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The sole use of pyridoxine hydrochloride during treatment of tuberculosis gives rise to pyridoxine deficiency. Therefore, a combination of pyridoxine hydrochloride and isoniazid is used in pharmaceutical dosage form in tuberculosis treatment to reduce this side effect. In this study, two chemometric methods, partial least squares (PLS) and principal component regression (PCR), were applied to the simultaneous determination of pyridoxine (PYR) and isoniazid (ISO) in their tablets. A concentration training set comprising binary mixtures of PYR and ISO consisting of 20 different combinations were randomly prepared in 0.1 M HCl. Both multivariate calibration models were constructed using the relationships between the concentration data set (concentration data matrix) and absorbance data matrix in the spectral region 200–330 nm. The accuracy and the precision of the proposed chemometric methods were validated by analyzing synthetic mixtures containing the investigated drugs. The recovery results obtained by applying PCR and PLS calibrations to the artificial mixtures were found between 100.0 and 100.7%. Satisfactory results obtained by applying the PLS and PCR methods to both artificial and commercial samples were obtained. The results obtained in this manuscript strongly encourage us to use them for the quality control and the routine analysis of the marketing tablets containing PYR and ISO drugs. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: pyridoxine hydrochloride; isoniazid; partial least squares; principal component regression

Introduction

Isoniazid (ISO) is the main drug used for the treatment of tuberculosis. On the one hand, prolonged administration of this drug may be accompanied by side effects with neurotoxic manifestations. such as psychotic states and peripheral neuritis. It is believed that isoniazid competes with pyridoxyl phosphate for the enzyme aportryptophanase which may lead to symptoms of pyridoxine (PYR) deficiency. PYR administration can prevent and reverse peripheral neuropathy, thus complicating isoniazid use. The administration of PYR hydrochloride in the course of tuberculosis treatment with ISO reduces these side effects. As a result, it was important to create a combined medicinal preparation of ISO with PYR hydrochloride in the form of granules ISO, which is chemically known as isonicotinyl hydrazine or isonicotinic acid hydrazide. The molecular structures of PYR and ISO compounds are given in Figure 1.

Several analytical methods, for example, spectrophotometry analysis, were investigated for the analysis of ISO.[1–2] Besides high performance liquid chromatography (HPLC),[3] spectrophotometry,[4] and voltammetry,[5] results were reported for the analysis of PYR and ISO alone or combined with other ingredients in samples.

Nowadays, the chemometric calibration methods in spectral analyses are very important approaches for the quality control and routine analysis of commercial pharmaceutical formulations containing two or more drugs. In spite of the closely overlapping bands of the analyzed drugs, these multivariate calibration methods do not require any separation procedure for the quantitative chemometric evaluation of the commercial dosage forms containing multiple drugs.[6–12] The chemometric calibration algorithms are applied to all data sets at the different formats such as the UV-VIS spectra, IR spectra, chromatograms, and voltammograms obtained from different analytical instrumentations in order to predict the content of the pharmaceutical samples. Partial least squares (PLS) and principal component regression (PCR) approaches are preferable calibration techniques mainly because they provide an efficient quantitative resolution of complex mixtures of drugs.[13–29]

In this study, the chemometric PCR and PLS methods were applied to the simultaneous quantitative prediction of PYR and ISO in tablets without requiring any separation step. In spite of the strong interference of PYR and ISO in the same spectral region, both PCR and PLS provide successful prediction results of the related drugs in the synthetic and tablets samples. A good agreement was reported between the results provided by both chemometric calibration approaches.

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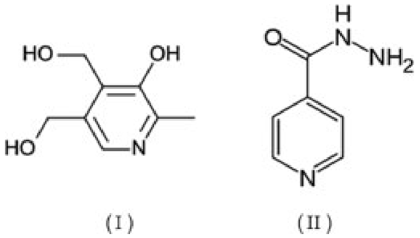

Figure 1. The molecular structure of pyridoxine (I) and isoniazid (II) compounds.

Experimental

Apparatus and Software

Shimadzu UV-1601 double-beam UV-Visible spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software, equipped with an HP OfficeJet Pro 1150C, was used for all absorbance measurements and the treatment of data was made by means of the *Microsoft EXCEL* and *PLS toolbox 3.5* in Matlab 7.0 software.

Pharmaceutical Preparation

A commercial dosage form (ISOVIT® tablet, Deva Pharm. Ind., Turkey, Batch No. 5051087), containing 25 mg PYR and 100 mg ISO per tablet, was investigated. PYR and ISO compounds were kindly obtained from Deva Pharm. Ind. and ISO compounds (Istanbul, Turkey).

Standard Solutions

Stock solutions of PYR and ISO were prepared by dissolving 25 mg of each drug in 100 mL volumetric flasks containing in 0.1 M HCl. A training set (concentration data matrix) of the standard mixture solutions containing 0–45 µg/ml of PYR and 0–45 µg/ml of ISO was randomly prepared by using the stock solutions. An independent set consisting of 10 binary mixtures in the different concentration compositions between 5–45 µg/ml for PYR and 5–45 µg/ml for ISO were obtained from these standard stock solutions as a validation set. We observed that the solutions of PYR and ISO in 0.1M HCl are stable for 8 h corresponding to their absorption spectra.

The absorbance spectra were recorded with a sampling interval of $\Delta\lambda = 0.1$ nm and at 1500 nm/min of scanning speed against a reagent blank (0.1 M HCl) and stored in a computer.

Sample Solutions

Twenty tablets of commercial preparations selected were accurately weighed and powdered in a mortar. A quantity of the powder equivalent to one tablet was dissolved in 0.1 M HCl in 50-ml calibrated flasks. After a period of 30 min of shaking, the solution was filtrated and the residue was washed three times with 10 ml solvent and the volume was completed to 100 ml with 0.1 M HCl. The obtained solution was diluted to the working concentration range with the same solvent. The tablet analysis procedure was repeated ten times.

Chemometric Methods

Principal Component Regression

This model-building procedure has two steps. The computation of the eigenvalues and their eigenvectors corresponding to the covariance square matrix of the mean-centred absorbencies represents the first step of PCR. By using the cross-validation in the calibration step, the principal components (or the eigenvectors) corresponding to the large eigenvalues are selected to compute A_{proj} (new axes) in the second step.

The mathematical formulation of the technique is given by

$$A_{proj} = V_c{}^T A \tag{1}$$

Here, A_{proj} is the matrix containing the new coordinates, A represents the original training set absorbencies matrix and $V_c{}^T$ is the matrix containing the basis vectors (principal components), one column for each factor retained. If we know the matrix A_{proj} we find, after some simple calculations, the unknown concentration matrix using the following formula:

$$C = F\, A_{proj} \tag{2}$$

where F represents the calibration coefficient for the obtained linear equation system.

Partial Least Squares

The PLS calibration technique based on the orthogonalized PLS algorithm developed by (Ref 9) Wold and extensively discussed by Martens and Naes involves simultaneously the independent and the dependent variables on the data compression and decomposition operations. The absorbance data (independent data, A) and the concentration data matrix (dependent data, C) are mean centred and denoted by the data matrix A_o and the vector C_o, respectively. Based on data matrix A_o and vector C_o, the orthogonalized PLS calibration is based on the decomposition of both concentration and the absorbance data matrix into latent variables:

$$A = T\,P^T + E \tag{3}$$
$$C = U\,Q^T + F \tag{4}$$

The matrix A is decomposed into a matrix T (the score matrix) and a matrix P^T (the absorbance loadings matrix) plus an error matrix E. In addition, the matrix C is decomposed into U and Q (the concentration loadings). The goal of the PLS method is to minimize the norm of F (error matrix) in such a way to keep the correlation between A and C by the inner relation U=BT.

The linear regression (Equation 5) is used for the quantitative estimation of the compounds in the samples.

$$C_{prediction} = B \times A_{sample} \tag{5}$$

The vector, B is given as Equation 6

$$B = W \times (P^T \times W)^{-1} \times Q \tag{6}$$

where W is a weight matrix.

Application of the PCR and PLS algorithims was performed by means of *PLS toolbox 3.0* within Matlab 6.5 software.

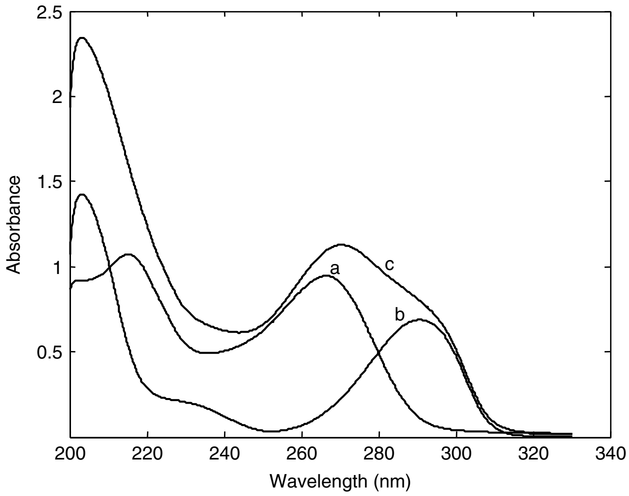

Figure 2. Absorption spectra of the solution of (a) 25 µg/mL ISO, (b) 15 µg/mL PYR and (c) their mixture in 0.1 M HCl.

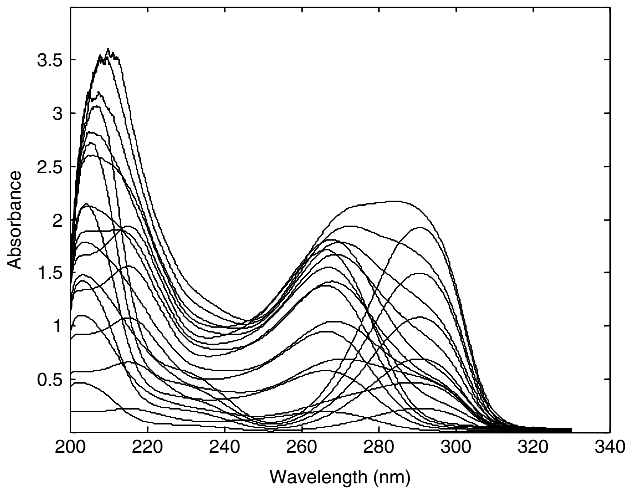

Figure 3. Absorption spectra of the training set from Table 1.

Table 1. Training set composition for PCR and PLS calibrations

	Concentration (µg/mL)			(Concentration) µg/mL	
No.	PYR	ISO	No.	PYR	ISO
1	10.0	5.0	11	5.0	40.0
2	0.0	5.0	12	25.0	0.0
3	10.0	15.0	13	15.0	40.0
4	5.0	0.0	14	0.0	35.0
5	10.0	25.0	15	25.0	40.0
6	0.0	15.0	16	35.0	0.0
7	10.0	35.0	17	35.0	40.0
8	15.0	0.0	18	0.0	45.0
9	10.0	45.0	19	45.0	40.0
10	0.0	25.0	20	45.0	0.0

Results and Discussion

Figure 2 indicates the absorption spectra for PYR, ISO, and their binary mixture in 0.1 M HCl. As it can be seen from this figure, the simultaneous quantitative evaluation of the subjected drugs in the same samples is difficult by using the conventional spectrophotometric methods due to the overlapping spectra in the wavelength range 200–340 nm.For this reason, we focused on applying PCR and PLS to the quantitative resolution of mixtures containing PYR and ISO drugs.

PCR and PLS Methods

As shown in Table 1, the training set of the mixture solutions in the concentration range of 0–45 µg/ml for PYR, and ISO in possible combinations was randomly prepared for both PCR and PLS. This training set was used to construct the PCR and PLS calibrations. The absorbance data matrix corresponding to the training set was obtained by measuring at the full wavelengths with the intervals of $\Delta\lambda = 0.1$ nm in the interval 200.0–340.0 nm. The absorption spectra of the training set corresponding to Table 1 are presented in Figure 3. A chemometric calibration based on the relationship between the training set and its measured absorbance values was calculated by using *Microsoft EXCEL* and *PLS toolbox 3.5* in Matlab 7.0 software for each technique. PCR and PLS calibration techniques were used to predict the amount of drugs in the samples.

Method Validation

The validity and applicability of the proposed PCR and PLS methods were tested by using the binary mixtures of PYR and ISO drugs. The mean recoveries and the relative standard deviations for PCR and PLS calibrations were calculated and are depicted in Table 2. These experimental results indicate that these two methods are suitable for the simultaneous determination of PYR and ISO in samples.

Statistical Parameters

The general expression of the standard error of prediction (SEP) and the standard error of calibration (SEC) are given in Equation 7.

$$SEP = SEC = \sqrt{\frac{\sum (C_i^{Added} - C_i^{Found})^2}{n-1}}, \qquad (7)$$

where C_i^{Added} denotes the added concentration of drug, C_i^{Found} represents the predicted concentration of drug and n denotes the total number of samples.

The sets of the calibration samples and the synthetic mixtures containing the two drugs were used to check both techniques.

The standard error of calibration (SEC) for $n = 20$ calibration samples and the errors of prediction (SEP) for $n = 10$ prediction samples were found acceptable for PCR and PLS methods. Their values are summarized in Table 3. The square of the correlation coefficient (r^2), which is the indication of the quality of the straight line that fits the data, was also calculated. The values of r^2 indicated in Table 3 are in all cases close to 1, which means that the predicted and the known values are much closer. Cross-validation procedure was used to find the optimal factors to construct PCR and PLS calibrations. It was found that PCR and PLS based on the first two factors provide the minimal values for SEC and SEP in the calibration and prediction steps, respectively. Therefore, we conclude that the first two factors were suitable for both chemometric calibration methods.

Table 2. Recovery results obtained by applying the proposed PCR and PLS methods to binary mixtures

	(µg/mL)		Found (µg/mL)				Recovery (%)			
	Mixture		PCR		PLS		PCR		PLS	
No.	ISO	PYR	ISO	PYR	ISO	PYR	ISO	PYR	ISO	PYR
1	5	10	5.11	9.93	5.11	9.93	102.3	99.3	102.3	99.3
2	15	10	15.19	10.40	15.19	10.38	101.2	104.0	101.3	103.8
3	25	10	24.88	10.15	24.89	10.13	99.5	101.5	99.6	101.3
4	35	10	35.27	10.19	35.28	10.16	100.8	101.9	100.8	101.6
5	45	10	44.91	10.42	44.91	10.39	99.8	104.2	99.8	103.9
6	40	5	39.69	4.94	39.69	4.92	99.2	98.7	99.2	98.4
7	40	15	38.87	15.51	38.88	15.48	97.2	103.4	97.2	103.2
8	40	25	40.00	24.85	40.00	24.84	100.0	99.4	100.0	99.4
9	40	35	40.14	33.70	40.13	33.80	100.3	96.3	100.3	96.6
10	40	45	39.79	44.10	39.90	44.17	99.5	98.0	99.8	98.2
						Mean	100.0	100.7	100.0	100.6
						SD	1.29	2.58	1.27	2.45
						RSD	1.29	2.56	1.27	2.44

RSD = Relative standard deviation

Table 3. Statistical parameters of PCR and PLS techniques in the calibration and prediction steps

	SEP n=10		SEC n=20		r^2 (SEP)		Intercept (SEP)		Slope (SEP)	
	PCR	PLS	PCR	PLS	PCR	PLS	PCR	PLS	PCR	PLS
PYR	0.41	0.38	0.97	0.79	0.9993	0.9994	−0.5674	−0.5186	1.04	1.03
ISO	0.29	0.28	0.48	0.49	0.9992	0.9992	−0.2219	−0.2178	1.01	1.01

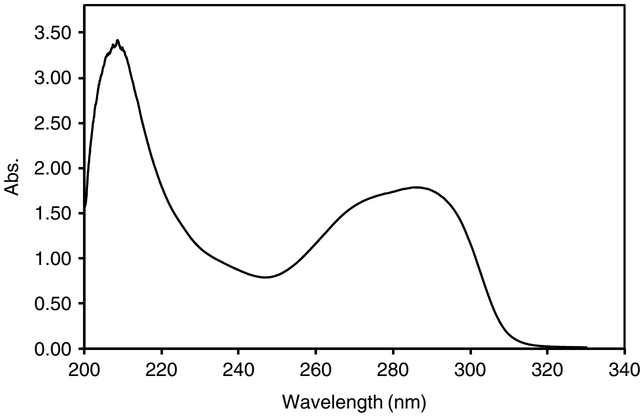

Figure 4. Absorption spectra of the sample tablet solution in 0.1 M HCl.

Analysis of Tablet Content

In the tablet analysis procedure, the absorption spectra of the tablet solution described in sample solution section were recorded in the range of 200–340 nm as it is shown in Figure 4.

The results obtained by applying PCR and PLS methods to the spectra of the tablet solutions are shown in Table 4. A good coincidence was observed between the experimental results and the label claim of the commercial tablet preparation. The numerical values of all statistical parameters calculated in Table 4 were found acceptable.

Table 4. Determination results obtained by applying the PCR and PLS methods

	mg/tablet			
	PCR		PLS	
	ISO	PYR	ISO	PYR
1	97.86	25.16	97.89	25.08
2	99.15	25.82	99.18	25.75
3	99.29	25.82	99.32	25.74
4	99.34	25.75	99.37	25.68
5	100.19	25.37	100.22	25.30
6	100.32	25.23	100.34	25.16
7	100.32	25.37	100.35	25.29
8	100.93	24.74	100.96	24.67
9	100.04	24.98	100.07	24.90
10	100.19	25.02	100.22	24.94
Mean	99.76	25.33	99.79	25.25
SD	0.87	0.37	0.87	0.37

Declared amounts: 100 mg ISO and 25 mg PYR per tablet

Conclusion

The proposed PCR and PLS chemometric methods are rapid, precise, and accurate for the simultaneous resolution of tablets containing PYR and PLS drugs having strongly overlapping spectra. PCR and PLS methods do not require any graphical treatment and they do not use some steps of separation and extraction, etc., as in the HPLC method. In this study, we have considered the interval of the working range of both techniques larger than those presented in the literature. Due to the non-existence of an official method for the investigated binary mixture, the performances of PCR and PLS calibration techniques were compared to each other as well as with those obtained by the HPLC method and capillary electrophoresis, and no significant difference was observed among the results. The assay results obtained in this study strongly encourage us to apply these methods for the routine analysis and quality control of the pharmaceutical formulation containing two drugs.

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