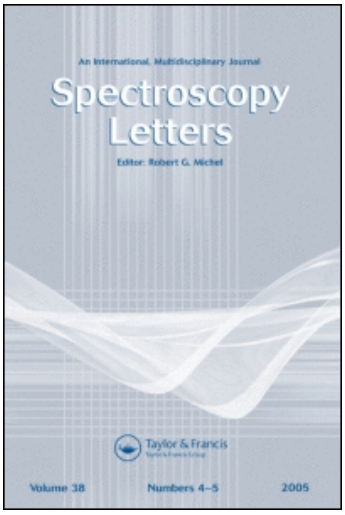

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CHEMOMETRIC QUANTITATIVE ANALYSIS OF PYRIDOXINE HCl AND THIAMINE HCl IN A VITAMIN COMBINATION BY PRINCIPAL COMPONENT ANALYSIS, CLASSICAL LEAST SQUARES, AND INVERSE LEAST SQUARES TECHNIQUES

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ABSTRACT

Three chemometric techniques were described for the analysis of pyridoxine hydrochloride and thiamine hydrochloride within a vitamin combination in the presence of spectral interferences. For these techniques, the training set was prepared by using synthetic mixtures containing two vitamins in multiple possible combinations for the range of 8-40 μ/mL in 0.1 M HCl. The absorbance

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values for the training set were obtained by direct measurements at 18 wavelengths in the region 222–305 nm for the zero order spectra. The numerical values were calculated by using the '*Maple V*' software. Mean recoveries and relative standard deviations for the principal component analysis, classical least squares and inverse least squares techniques were found as 100.7% and 0.95%; 100.5% and 1.38% and, 99.3% and 1.04 for pyridoxine hydrochloride; and 99.7% and 1.03%; 99.1% and 1.05% and, 99.6% and 1.58% for thiamine hydrochloride, respectively. These three chemometric techniques were successfully applied to vitamin tablets marketed in Turkey. The results were compared with each other and good coincidence was observed .

Key Words: Chemometric quantitative analysis; Pyridoxine hydrochloride; Thiamine hydrochloride; Principal component analysis; Classical least square and inverse least square techniques.

INTRODUCTION

Recently, the chemometric techniques of multiple linear regression (inverse least squares and classical least squares) and principal component analysis are the most important methods for the quantitative analysis of compounds in multicomponent mixtures (1–3) and for drug analysis in pharmaceutical formulations (4–6).

We have observed that chemometric quantitative analysis techniques have many applications and advantages where mixtures are analyzed without any separation procedures for drug determinations. For example, these techniques are very easy to apply, very sensitive, useful, and yet very inexpensive as compared to other analysis techniques for simultaneous determination of compounds in multicomponent mixtures. These techniques can be applied to many analytical methods, such as spectrophotometry, high performance liquid chromatography, mass spectrometry, polarography (or voltametry), and spectrofluorimetry.

Quantitative analysis of vitamins in mixtures containing pyridoxine hydrochloride or thiamine hydrochloride with other active compounds) has been described for several mixtures and vitamin combinations using different methods including spectrophotometry (7–14) and HPLC (14–18).

In this paper, three numerical methods have been used for the analysis of pyridoxine hydrochloride and thiamine hydrochloride for a vitamin combination in the presence of spectral interferences. The treatment of the analytical data was performed by using the "*Maple V*" software. We found that these three techniques

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applied to chemometric quantitative analysis for the pyridoxine hydrochloride and thiamine hydrochloride gave successful results.

EXPERIMENTAL

Apparatus

A Shimadzu UV-160 double beam UV - Visible spectrophotometer with a fixed slit width (2 nm) was connected to a computer loaded with Shimadzu UVPC software, equipped with an HP OfficeJet Pro 1150C. This instrument was used for all the absorbance measurements and the treatment of data was made by using "*Maple V*" software.

Pharmaceutical Preparation

A commercial vitamin product (Benexol® film-coated tablet, Roche Pharm. Ind., Turkey, Batch no. I0169), containing 250 mg pyridoxine hydrochloride and 250 mg thiamine hydrochloride per tablet, was investigated. Pyridoxine hydrochloride and thiamine hydrochloride were kindly donated by Roche Pharm. Ind., Turkey.

Standard Solutions

Standard solutions were prepared in 25-ml volumetric flasks containing 8–40 μg/ml of the two vitamins and their synthetic mixtures were prepared by using these stock solutions. The zero-order spectra were recorded with a sampling interval of $\Delta\lambda = 0.1$ nm and a medium level of scanning speed against a reagent blank (0.1 M HCI) and stored in the computer.

Sample Solutions

20 tablets were accurately weighed and powdered in a mortar. An amount of the powder equivalent to a tablet, was dissolved in 0.1 M HCI in 50 ml calibrated flasks. After 30 min. of shaking, the solution was filtrated and the residue was washed three times with 10 ml solvent then the volume was completed to 100 ml with 0.1 M HCI (*solution* 1). The *solution* 1 was diluted 1:500 with the same solvent.

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METHODS

a) Classical Least-Squares

Classical least-squares (LS) is involved the application of multiple linear regression (MLR) to the classical expression of the Beer-Lambert Law of spectroscopy given by

$$A = K \times C$$

This equation is a matrix equation and it can be written as a linear equation system:

$$\begin{aligned}
A_1 &= K_{11}C_1 + K_{12}C_2 + \ldots\ldots\ldots\ldots\ldots K_{1c}C_c \\
A_2 &= K_{21}C_1 + K_{22}C_2 + \ldots\ldots\ldots\ldots\ldots K_{2c}C_c \\
A_3 &= K_{31}C_1 + K_{32}C_2 + \ldots\ldots\ldots\ldots\ldots K_{3c}C_c \\
\ldots &\quad \ldots \quad \ldots\ldots\ldots \quad \ldots\ldots\ldots \\
A_w &= K_{w1}C_1 + P_{w2}C_2 + \ldots\ldots\ldots\ldots\ldots P_{wc}C_c
\end{aligned}$$

where A_w denotes the absorbance at the w^{th} wavelength, K_{wc} represents the calibration coefficient for the c^{th} component at the w^{th} wavelength and C_c is the concentration corresponding to the c^{th} component

b) Inverse Least-Squares

Another technique, called the inverse least square technique (ILS), is involved in the application of multiple linear regression (MLR) to the inverse expression of the Beer-Lambert Law of spectroscopy. The mathematical expression is given as

$$C = P \times A$$

The above equation can be rewritten as the following linear system:

$$\begin{aligned}
C_1 &= P_{11}A_1 + P_{12}A_2 + \ldots\ldots\ldots\ldots\ldots P_{1w}A_w \\
C_2 &= P_{21}A_1 + P_{22}A_2 + \ldots\ldots\ldots\ldots\ldots P_{2w}A_w \\
C_3 &= P_{31}A_1 + P_{32}A_2 + \ldots\ldots\ldots\ldots\ldots P_{3w}A_w \\
\ldots &\quad \ldots \quad \ldots\ldots\ldots \quad \ldots\ldots\ldots \\
C_c &= P_{c1}A_1 + P_{w2}A_2 + \ldots\ldots\ldots\ldots\ldots P_{cw}A_w
\end{aligned}$$

Here, A_w is the absorbance at the w^{th} wavelength, P_{cw} denotes the calibration coefficient for the c^{th} component at the w^{th} wavelength whilst C_c represents the concentration of the c^{th} component.

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c) Principal Component Analysis

This model-building procedure has two steps. In the first step we calculate the eigenvectors of the centered absorbance data matrix. The second step of PCR uses MLR to regress the concentration data matrix. We can express this procedure as

$$A_{proj} = V_c{}^T A$$

In this equation, A_{proj} indicates the matrix containing the new coordinates (the projections), $V_c{}^T$ represents the matrix containing the basis vectors, one column for each factor retained, whilst A denotes the original training set absorbance matrix. Knowing the matrix A_{proj} permits us to compute the unknown concentration matrix as indicated below

$$C = FA_{proj}$$

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

RESULTS AND DISCUSSION

Figure 1 shows the absorption spectra of pyridoxine hydrochloride and thiamine hydrochloride and their binary mixture in 0.1 M HCI. The absorbance data

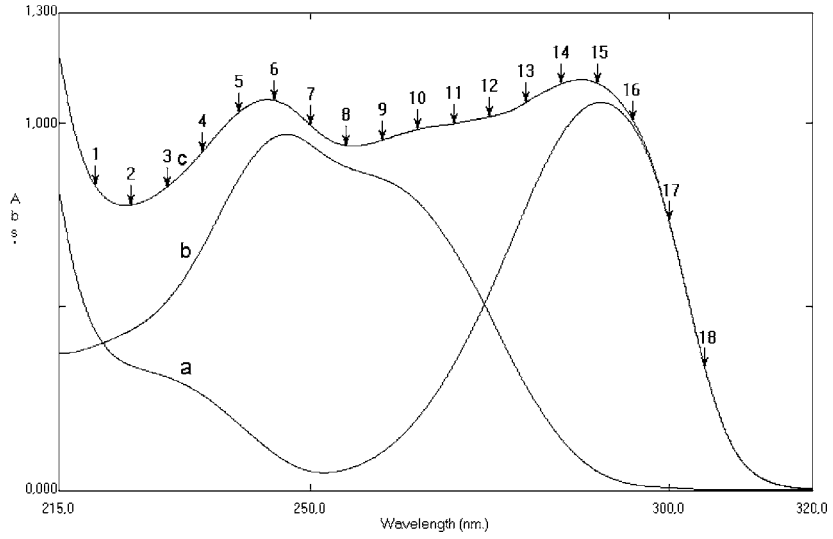

Figure 1. Absorption spectra of a) 24 μ/mL pyridoxine hydrochloride, b) 24 μ/mL thiamine hydrochloride, and c) their mixture in 0.1 M HCI. ($\overset{1}{\downarrow}, \overset{2}{\downarrow}, \ldots\ldots, \overset{18}{\downarrow}$ corresponding to $\lambda_1, \lambda_2, \ldots\ldots, \lambda_{18}$ (from 222. nm to 305 nm)).

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Table 1. Composition of the Training Set

Standard No.	Pyridoxine Hydrochloride μg/mL	Thiamine Hydrochloride μg/mL
1	24.0	8.0
2	24.0	16.0
3	0.0	24.0
4	24.0	0.0
5	24.0	24.0
6	24.0	32.0
7	24.0	40.0
8	0.0	8.0
9	8.0	0.0
10	8.0	24.0
11	16.0	24.0
12	0.0	16.0
13	32.0	0.0
14	24.0	24.0
15	32.0	24.0
16	40.0	24.0
17	0.0	40.0
18	40.0	0.0

matrix for the prepared concentration data matrix were obtained by measurements at the 18 wavelengths (in nanometers) for the intervals with $\Delta\lambda = 5$ nm in the region 222–305 nm in the original spectra.

The training set was prepared by using the synthetic mixtures containing two vitamins in possible combinations as shown in Table 1.

In the classical least squares and inverse least squares techniques, calibration and regression were obtained by using the absorbance and concentration data matrix for prediction of the amounts of pyridoxine hydrochloride, and thiamine hydrochloride in their binary mixtures and vitamin combination. In the principal component regression, the variances and covariances matrix corresponding to the absorbance data matrix were calculated for the basis vectors and matrix containing the new coordinates. Computing the calibration was used for the prediction of concentration of pyridoxine hydrochloride and thiamine hydrochloride in the above mentioned mixtures.

The predictive applicability of a model can be defined in various ways. The most general expression is the standard error of prediction (SEP) which is given the following equation:

$$\text{SEP} = \sqrt{\frac{\sum_{i=1}^{N} (C_i^{Added} - C_i^{Found})^2}{N}}$$

where C_i^{Added} is the added concentration of drug, C_i^{Found} is the predicted concentration of drug and N is the total number of synthetic mixtures. For the applied techniques, the SEP for PCA, CLS and ILS techniques was calculated as 0.26, 0.42, and 0.38 for thiamine hydrochloride and 0.25 , 0.32, and 0.39 for pyridoxine hydrochloride, respectively.

In order to test the proposed techniques, the sets of synthetic mixtures containing the two drugs in variable composition were prepared and the obtained results were indicated in Tables 2, 3 and 4.

Mean recoveries and relative standard deviations for the principal component analysis, classical least square and inverse least square techniques were found as 100.7% and 0.95%, 100.5% and 1.38%, 99.3% and 1.04 for pyridoxine hydrochloride and 99.7% and 1.03%, 99.1% and 1.05%, 99.6% and 1.58% for thiamine hydrochloride, respectively (Tables 2, 3 and 4).

Table 2. Results Obtained for the Determination Pyridoxine Hydrochloride and Thiamine Hydrochloride in Different Synthetic Mixtures by Using the Principal Component Analysis Technique

Pyridoxine Hydrochloride			Thiamine Hydrochloride		
Added μg	Found μg	Recovery %	Added μg	Found μg	Recovery %
24.0	24.03	101.3	8.0	8.0	100.0
24.0	23.7	98.8	16.0	16.1	100.6
24.0	23.5	97.9	24.0	24.4	101.7
24.0	24.1	100.4	32.0	32.3	100.9
24.0	24.2	100.8	40.0	39.7	99.3
8.0	8.0	100.0	24.0	24.1	100.4
16.0	16.0	100.0	24.0	24.4	101.7
24.0	23.7	98.3	24.0	24.4	101.7
32.0	31.8	99.4	24.0	23.8	99.2
40.0	40.0	100.0	24.0	24.3	101.3
		$\bar{X} = 100.7$			$\bar{X} = 99.7$
		RSD = 0.95			RSD = 1.03

RSD = Relative standard deviation.

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Table 3. Results Obtained for the Determination Pyridoxine Hydrochloride and Thiamine Hydrochloride in Different Synthetic Mixtures by Using the Classical Least Square Technique

Pyridoxine Hydrochloride			Thiamine Hydrochloride		
Added μg	Found μg	Recovery %	Added μg	Found μg	Recovery %
24.0	24.1	100.4	8.0	8.2	102.5
24.0	23.7	98.8	16.0	16.2	101.3
24.0	23.6	98.3	24.0	24.0	100.0
24.0	24.0	100.0	32.0	32.3	100.9
24.0	24.1	100.4	40.0	39.0	97.5
8.0	7.8	97.5	24.0	24.0	100.0
16.0	15.8	98.8	24.0	24.1	101.8
24.0	23.6	98.3	24.0	24.3	101.3
32.0	32.1	100.0	24.0	23.3	99.2
40.0	39.3	98.3	24.0	23.8	100.5
		$\bar{X} = 100.5$			$\bar{X} = 99.1$
		RSD = 1.38			RSD = 1.05

Table 4. Results Obtained for the Determination Pyridoxine Hydrochloride and Thiamine Hydrochloride in Different Synthetic Mixtures by Using the Inverse Least Square Technique

Pyridoxine Hydrochloride			Thiamine Hydrochloride		
Added μg	Found μg	Recovery %	Added μg	Found μg	Recovery %
24.0	24.0	100.0	8.0	7.9	98.8
24.0	24.1	100.4	16.0	16.0	100.0
24.0	23.7	98.3	24.0	24.1	100.4
24.0	24.2	100.8	32.0	32.0	100.0
24.0	23.8	99.2	40.0	39.0	97.5
8.0	8.2	102.5	24.0	24.0	100.0
16.0	15.8	100.0	24.0	23.6	98.3
24.0	23.6	98.8	24.0	24.0	100.0
32.0	31.0	96.9	24.0	23.5	97.9
40.0	40.1	100.3	24.	23.9	99.6
		$\bar{X} = 99.3$			$\bar{X} = 99.6$
		RSD = 1.04			RSD = 1.58

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Table 5. Results Obtained for the Pharmaceutical Formulation (mg/Tablet) by Using the Three Chemometric Techniques, Spectropotometric and HPLC Methods (8–9)

Method	Pyridoxine Hydrochloride Mean ± SD	Thiamine Hydrochloride Mean ± SD
PCA	251.2 ± 0.7	248.9 ± 0.8
CLS	250.2 ± 1.7	248.6 ± 0.8
ILS	248.9 ± 1.1	248.7 ± 1.1
Absorbance ratio (13)	251.9 ± 4.0	248.7 ± 4.3
Ratio spectra derivative (14)	249.5 ± 0.8	249.6 ± 0.8
HPLC (14)	250.0 ± 0.9	249.8 ± 0.9

When these three chemometric techniques were applied to quantitative analysis of pharmaceutical formulation containing two vitamins, It was observed that the results are in good coincidence as shown in Table 5.

CONCLUSIONS

Despite of the interferences of the spectra of pyridoxine hydrochloride and thiamine hydrochloride in the spectral range presented in Figure 1, these three approximation techniques were applied successfully to the quantitative determination of the subjected vitamins in their binary mixtures.

For the same commercial formulation, we observed that the results obtained in these chemometric techniques are very close to each other and in comparison with the other methods, such as HPLC and ratio spectra derivative methods developed by us (14), and absorbance ratio method (13) described in the literature (Table 5).

The simultaneous use of the spectrophotometry and the chemometric techniques for the analysis of the mixtures containing drugs with overlapped spectra are a suitable choice to develop new methods for the quality control of the pharmaceutical formulations without using the some prior steps of separation and extraction common to classical determination processes.

The results obtained lead us to the conclusion that the above techniques can be used for a routine analysis of pyridoxine hydrochloride and thiamine hydrochloride.

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