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Simultaneous spectrophotometric determination of sulfamethoxazole and trimethoprim in pharmaceutical preparations by using multivariate calibration methods

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Abstract: The UV-spectrophotometric method of analysis was proposed for simultaneous determination of sulfamethoxazole and trimethoprim. Considering the strong spectral overlap among UV-vis spectra of these compounds, a previous separation should be carried out in order to determine them by conventional spectrophotometric techniques. Here, full-spectrum multivariate calibrations PLS and PCR methods are developed. The experimental calibration matrix was constructed with 26 samples. The concentration ranges considered were 2-15µgml-1 sulfamethoxazole and 0.4-6µgml-1 trimethoprim.Absorbance data of the calibration standards were taken between 200-400nm with UV-vis spectrophotometer. For achieving the best model, related parameters of the model were evaluated. The optimum number of factors was selected by using the cross-validation method. The relative errors in each step were calculated. The PLS and PCR calibrations based on the raw data matrix were utilized to illustrate successfully the application of the proposed methods for analysis of the drugs in synthetic mixtures and pharmaceutical tablet. The errors obtained in the PLS method were slightly better. The errors were less than 0.1% for sulfamethoxazole and 0.9% for trimethoprim in PLS method, respectively.

Keywords: *spectrophotomtry; multivariate calibration; PLS; PCR; sulfamethoxazole; trimethoprim*

Introduction

Mixture of sulfamethoxazole[1] and trimethoprim[2] which is known as co-trimoxazole tablet has been used in a wide variety of infections due to susceptible organisms, particularly those of the genito-urinary-tract infections, respiratory-tract infections such as bronchitis, and enteric infections. Its main uses now are in Pneumocystis carinii pneumonia, toxoplasmosis, and nocardiosis. Gastrointestinal disturbances (mainly nausea and vomiting) and skin reactions are the most common adverse effects. A high incidence of adverse effects has been reported in AIDS patients; desensitization may sometimes be considered.

The most recent methods found in the bibliography to determine mixtures of SMZ[1] and TMP[2] are based

1. N1-(5-methyl-3-isoxazolyl) sulfanilamide
2. 2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine

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on high-performance liquid chromatography (HPLC) [1], spectrophotometry [2], and TLC [3]. However, these methods are generally complex in nature and need expensive instruments and ultra pure solvents. In other hand, analysis of the clinical samples demands simple and fast analytical methods and therefore, finding an alternative analytical procedure or technique is crucial. Spectrophotometry combined with chemometrics methods will be a simple analytical method for quantitative analysis.

Chemometrics is a field of science that studied the application of statistical and mathematical methods in chemistry. One of the chemometrics methods is multivariate calibration technique. Multivariate calibration is a collection of powerful mathematical tools that can be applied to resolve complexity in chemical analysis.

It is useful in spectral analyses because the simultaneous inclusion of multiple spectral intensities can greatly improve the precision and applicability of quantitative spectral analysis of multi component mixtures that can not be resolved by conventional spectrometry. In recent years multivariate calibration has become an important tool in resolution of mixtures of components in many different fields including biomedical [4,5], environmental [6,7], and drug analysis [8,9]. The two most common multivariate calibration methods used for spectral analyses are [10-16]:

- Partial least-squares (PLS)
- Principal component regression (PCR)

These methods generally presume that there is a linear relationship between absorbance and component concentrations. In addition, each method has a calibration step where a model that can relate the spectral intensities to the known component concentrations from a set of standard samples. This step followed by a prediction in which the model of the calibration is used to predict or estimate the component concentrations from the "unknown" sample spectra. Both PCR and PLS are factor-based methods and involve spectral decomposition. For PCR, decomposition is based entirely on spectral variations without regard to the analyte concentrations, and the PCR decomposition is significantly influenced by variations, which have no relevance to the analyte concentrations [17].For PLS, the decomposition utilizes information from both spectral and concentration. The major difference in the predictive abilities of PLS and PCR is that PLS seems to predict better than PCR when there are random linear baselines and/or independently varying major spectral components, which overlap with the spectral features of the analytes [18].

The choice of the calibration method often depends on the particular experimental conditions. However, experience suggests that PLS seems to perform well in many circumstances.

In this paper, the performance of calibration models for PLS and PCR methods, constructed from raw spectral matrices were presented and compared.

Experimental

Apparatus and software

UV-vis spectrophotometer BIO-TEK-KONTRON (UVIKON922) equipped with 1cm pathlength quartz spectrophotometric cells was used for acquisition of spectral data. The Matlab version 6.5 software package was applied for the statistical treatment of the data.

Stock solutions

Stock solutions were obtained by dissolving of 50 mg of sulfamethoxazole and 25 mg of trimethoprim (purchased from Sobhan Daru Co.) in ethanol. Working standard solutions were prepared by suitable dilution of stock solutions.

Real sample solution

For the analysing, 10 tablets of the pharmaceutical Co-trimoxazole were weighted and ground to fine powder. Then a proportion of powder equivalent to one average tablet weighted and dissolved in ethanol. The solution was filtered and diluted to an appropriate volume with ethanol. Absorbance spectra were recorded.

Procedure

Sulfamethoxazole and trimethoprim binary mixtures were prepared. The concentrations were in the ranges 2-15 µg ml-1 sulfamethoxazole and 0.4-6 µg ml^{-1} trimethoprim. Their Uv-vis spectra recorded in 200-400 nm wavelength regions. The absorbance data and concentration were fed to PLS and PCR models as inputs and outputs, respectively. The optimized calibration model for these methods was applied to the spectra of the samples to calculate the concentration of each chemical in the mixtures.

Results and Discussion

The absorption spectra of SMZ and TMP are shown in figure 1. As can be seen, the strongly overlap between their spectra preclude the direct determination of the analyts by conventional spectrophotomtry, and physical prior separation processes could be necessary for the spectrophotomric determination of these components. In contrast, multivariate methods may resolve bands overlapping, without separations.

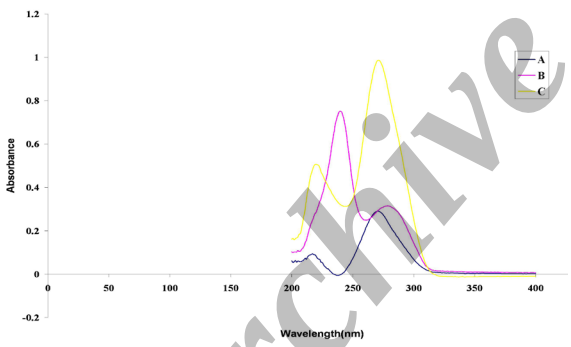

Figure1 Absorption spectra for: (A), Sulfamethoxazole; (B), Trimethoprim; (C), mixture of these components.

Calibration models based on PLS and PCR regressions were built. Two sets of samples were used to building the models as calibration and testing set. The calibration was used for design the model. The testing set, not included in the calibration was used to validate the prediction ability of the model. Therefore , 26 standard solutions were randomly divided to as calibration set composed of 20 samples and testing set contained 6 samples. Table 1 shows the composition of the binary mixtures used in the calibration and testing sets.

Table1 Composition of the samples of calibration (1-20) and testing (21-26) sets

Sample	Sulfamethoxazole ($\mu g ml^{-1}$)	Trimethoprim ($\mu g ml^{-1}$)
1	2.00	0.50
2	4.00	1.00
3	5.00	1.00
4	5.00	1.60
5	5.00	2.00
6	6.00	1.20
7	6.00	2.00
8	8.00	0.40
9	8.00	2.00
10	8.00	6.00
11	9.00	2.00
12	10.00	2.50
13	10.00	4.00
14	12.00	2.00
15	12.00	3.00
16	12.00	4.00
17	14.00	3.00
18	14.00	5.00
19	15.00	3.00
20	15.00	5.00
21	3.00	1.00
22	4.00	2.00
23	6.00	1.50
24	8.00	4.00
25	8.00	5.00
26	9.00	3.00

Optimization of the PLS and PCR Models

For achieving the optimum model, different spectral regions were evaluated and the most convenient spectral regions were selected. The optimum number of factors to be used in PLS and PCR modeling is an important step to obtain better performance in prediction stage. The cross-validation procedure used for

this purpose, consisting of systematically removing one of the calibration samples in turn, and using the remaining ones for construction of the latent factors and regression. This process was repeated until each standard had been left out once. To accomplish this work, finding a minimum or acceptably small predictive residual error sum of squares (PRESS) is used as follow:

$$PRESS = \sum_{i=1}^{\hat{n}} (X_i - X_i)^2$$

where n is the total number of calibration samples, represents the estimated concentration, and is the reference concentration for ith sample. The effective number of factors was found to be 3 for each component in PLS and 4 in PCR method. The values of root mean squares error of cross-validation (RMSECV) which is an estimate of the absolute error of prediction by cross-validation for each component in the calibration sample and also square correlation of coefficient (R2), obtained when plots of actual versus predicted concentration were constructed. The value of RMSECV is calculated as follows:

$$RMSECV = \sqrt{\sum_{i=1}^{n} (\hat{X}_i - X_i)^2 / n}$$

These parameters are summarized in Table 2. The best improvement in the accuracy of the predicted concentrations of the components was achieved when the 260-264nm wavelength range was described by PCR method . An estimation of the relative errors of prediction (REP %), using the following equation, for each component was made by cross-validation.

$$REP(\%) = \left[\frac{\sum_{i=1}^{n} (\hat{X}_i - X_i)^2}{\sum_{i=1}^{n} (X_i)^2} \right]^{1/2} \times 100$$

Tables 3 and 4, show the REP values for each component in testing set by using PLS and PCR, respectively.

Analysis of real formulation

In order to test the performance of the proposed methods, the produced model was used to predict the concentrations of the components in pharmaceutical formulation. The prediction ability of both methods was assessed by goodness of results. Results are given in Table 5.

Conclusions

Quantification of sulfamethoxazole and trimethoprim

Table 2 Statistical parameters obtained for optimum calibration model by PLS

Component	Factors	RMSECV	R2	Wavelength (nm)
Sulfamethoxazole	3	0.1640	0.9991	260-264
Trimethoprim	3	0.1070	0.9976	242-255

Table 3 Results obtained for Sulfamethoxazole and Trimethoprim in testing set by PLS

Sulfamethoxazole($\mu g ml^{-1}$)				Trimethoprim($\mu g ml^{-1}$)			
Sample	Actual	Prediction	REP%	Sample	Actual	Prediction	REP%
1	6.00	5.96	0.67	1	1.50	1.48	1.33
2	8.00	7.88	1.50	2	5.00	4.87	2.60
3	3.00	2.98	0.67	3	1.00	0.98	2.00
4	4.00	4.04	1.00	4	2.00	2.03	1.50
5	8.00	8.29	3.63	5	4.00	4.00	0.00
6	9.00	9.00	0.00	6	3.00	2.92	2.67

Table 4 Results obtained for Sulfamethoxazole and Trimethoprim in testing set by PCR

Sulfamethoxazole(μgml^{-1})				Trimethoprim(μgml^{-1})			
Sample	**Actual**	**Prediction**	**REP%**	**Sample**	**Actual**	**Prediction**	**REP%**
1	6.00	5.97	0.50	1	1.50	1.47	2.00
2	8.00	7.88	1.50	2	5.00	4.87	3.60
3	3.00	2.90	3.33	3	1.00	1.03	3.00
4	4.00	3.90	2.50	4	2.00	2.05	2.50
5	8.00	8.27	3.38	5	4.00	4.11	2.75
6	9.00	9.10	1.11	6	3.00	2.92	2.67

Table 5 Determination of Sulfamethoxazole and Trimethoprim by using PLS and PCR models in pharmaceutical formulation

Sample	**Actual (mg/tablet)**	**PLS**		**PCR**	
		Prediction (mg/tablet)	**REP%**	**Prediction (mg/tablet)**	**REP%**
Sulfamethoxazole	400.00	400.26	0.065	397.63	0.59
Trimethoprim	80.00	79.34	0.83	81.43	1.79

in pharmaceutical preparations has been accomplished from spectrophotometric spectral data, in combination with two multivariate calibration methods: partial least-squares (PLS) and principal component regression (PCR).These are rapid procedures which only require the solution of the sample and followed by measurment of its UV-vis spectrum. So they are simple, inexpensive and very fast procedures which neither need a previous separation of the analytes nor other previous sample treatments.

Although other methods such as chromatographic methods can be used to determine these components in pharmaceuticals, they are both more time consuming and expensive than the procedures here developed. PLS and PCR gave similar results for the prediction of concentrations, thus proving a high resolving power to the analysis of multi component complex mixtures. Moreover, the obtained results have a good agreement with those of standard methods. PLS seemed to be more sensitive in determination and showed slightly better results.

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