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Simultaneous spectrophotometric determination of ampicillin and penicillin in human plasma using multivariate calibration

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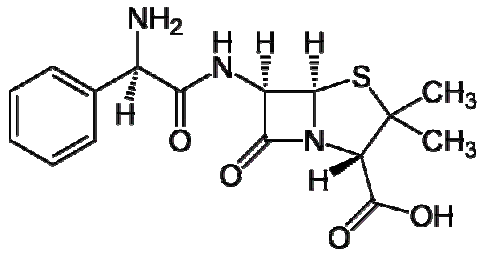
An analytical methodology based on spectrophotometric and partial least squares (PLS) algorithm for the simultaneous determination of ampicillin and penicillin in human plasma was developed and validated. The multivariate model was developed as a binary calibration model and it was built and validated with an independent set of synthesis and real samples in presence of matrix. It is shown how a developed technique for signal filtering, orthogonal signal correction (OSC), can be applied in multivariate calibration to enhance predictive power. The experimental calibration matrix was constructed with 25 samples. The concentration ranges considered were 1.0-40.0 and 0.5-20.0 μg mL^{-1} for ampicillin and penicillin, respectively. This procedure allows the simultaneous determination of ampicillin and penicillin in synthetic and human plasma good reliability of the determination was proved. The results obtained by the OSC-PLS and HPLC were statistically compared. Very similar values were found by two methods. No time consuming pretreatment was needed and this method also provides rapid, accurate and economical analysis of these drugs.

Keywords: Ampicillin; Penicillin; Spectrophotometric; Determination; OSC-PLS; HPLC

1. INTRODUCTION

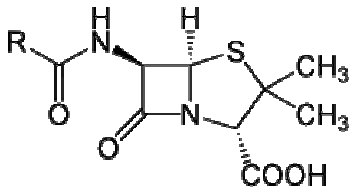
Ampicillin (Scheme I) is a beta-lactam antibiotic that is part of the aminopenicillin family and is roughly equivalent to its successor, amoxicillin in terms of spectrum and level of activity. It can sometimes result in reactions that range in severity from a rash (in the case of patients that may unwittingly have mononucleosis) to potentially lethal allergic reactions such as anaphylaxis. However, as with other penicillin drugs, it is relatively non-toxic and adverse effects of a serious nature are encountered only rarely [1].



Scheme I. Chemical structure of ampicillin.

Penicillin (Scheme II) is a group of antibiotics derived from *Penicillium* fungi. They include penicillin G, procaine penicillin, benzathine penicillin, and penicillin V. Penicillin antibiotics are historically significant because they are the first drugs that were effective against many previously

serious diseases, such as syphilis, and infections caused by staphylococci and streptococci. Penicillins are still widely used today, though many types of bacteria are now resistant. All penicillins are β-lactam antibiotics and are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms [2].



Scheme II. Chemical structure of penicillin.

Multivariate calibration is increasingly being used for developing quantitative relations between a set of calibration data (usually digitized spectra contained in a data matrix **X**) and a set of concentrations or reference values of sample properties (containing in matrix **Y**) [3]. The basic concept of PLS method was originally developed by Wold [4, 5] and application of PLS in spectrometry have been discussed by several workers [6-10]. In addition, several multicomponent determinations based on the application of these methods to spectrophotometric data have been reported [11-16]. OSC was introduced by Wold et al. [17] to remove systematic variation from the response matrix **X** that is unrelated, or orthogonal, to the property matrix **Y** [18, 19]. Therefore, one can be certain that important information regarding the analyte is retained. Since then, several groups [20-23] have published various OSC algorithms in an attempt to reduce model complexity by removing orthogonal components from the signal. In this study describes an analytical methodology for simultaneous spectrophotometric determination of ampicillin and penicillin using multivariate calibration technique (PLS) with preprocessing by OSC. The results obtained, with and without using OSC algorithm as a preprocessing treatment of original data, were compared.

2. EXPERIMENTAL

2.1. Chemical Reagents

All chemicals were reagent grade chemicals. Ampicillin and penicillin standard solutions were prepared in distilled water. These standards were obtained from Merck. Stock standard solutions of ampicillin and penicillin, 1000 μg mL^{-1}, were prepared by dissolving appropriate amount of solutes in water. Working solutions of lower concentrations were prepared by proper dilution with water from the stock standard solutions. Universal buffer solutions were prepared from boric acid, acetic acid and phosphoric acid (0.04 mol L^{-1}). The final pH was adjusted by the addition of 0.2 mol L^{-1} sodium hydroxide.

2.2. Instrument and software

A Hewlett-Packard 8453 diode array spectrometer controlled by a Hewlett -Packard computer and equipped with a quartz cell was used for recording the spectra. A centrifuge (Behdad Universal Centrifuge) was used to accelerate the phase separation process in real sample preparation. The pH was determined with a model 780 Metrohm pH-meter with combined glass-calomel electrode. PLS and OSC programs were written in MATLAB Version 6.5 (Math works Inc.). All programs were run on a personal computer (CPU 3.0 GHz and RAM 4 GB) with Windows XP operation system.

2.3. Procedure

2.3.1. Standard calibration set

An experimental design was used to maximize statistically the information content in the spectra [24]. A training set of 25 samples was taken (Table 1). The concentrations of ampicillin and penicillin were varied between 1.0-40.0 and 0.5-20.0 μg mL^{-1}, respectively. The mixed standard solutions were placed in a 10 mL volumetric flask and completed to the final volume with buffer solution. The absorption spectra were recorded between 200 and 400 nm against blank.

2.3.2. Prediction set and analysis of real samples

For prediction set, five mixtures prepared randomly that were not included in the previous set were employed as an independent test (Table 2). For real matrix samples, human plasma samples were tested. The range concentrations were added to be 1.0-40.0 and 0.5-20.0 μg mL^{-1} for ampicillin and penicillin, respectively.

3. RESULTS AND DISCUSSION

3.1. Spectrophotometric measurements

Fig. 1 shows the absorption spectra in aqueous solution of the individual ampicillin and penicillin at pH 7.0. As this figure shows, there is a clear overlapping of the two spectra. This prevents the simultaneous determination of the ampicillin and penicillin by direct UV-Vis absorbance measurements. To overcome this problem a suitable and simple technique, which presents a good recovery, is PLS regression. Spectra of mixture of ampicillin and penicillin solutions between 200 and 400 nm wavelengths by 1-nm intervals were recorded, and then the data were digitized and stored for late treatment.

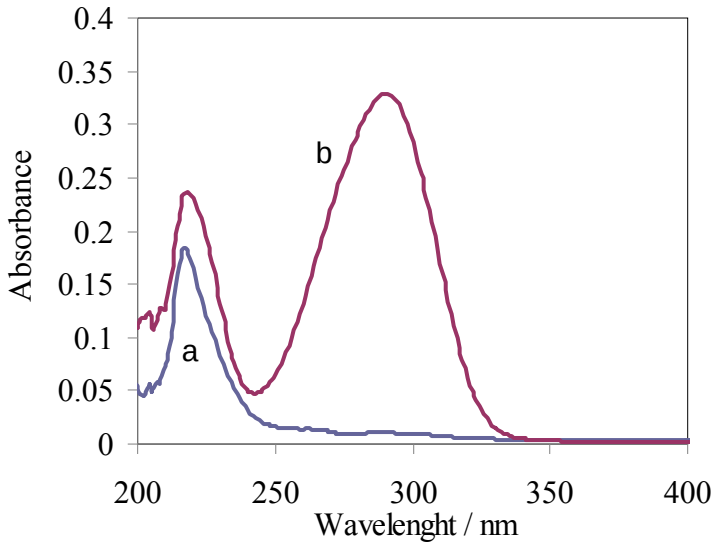


Fig. 1. Absorption spectra of (a) Ampicillin and (b) Penicilin at pH 7.0.

3.2. Optimization of experimental condition

For the finding the optimum conditions, the influence of pH values on the spectrum of each drug at a constant concentration of each drug were studied. The formed absorbance of ampicillin and penicillin were affected differently with pH. In order to select the optimum pH value at which the minimum overlap occurs, influences of the pH of the medium on the absorption spectra of ampicillin and penicillin were studied over the pH range 1.0-10.0. However pH 7.0 was chosen as the optimum pH for this work because both drugs have maximum absorbance and this pH is close to biological environment pH's. Individual calibration curves were constructed with several points

as absorbance versus drug concentration. For constructing the individual calibration lines the absorbencies were measured at 218 and 291 nm against a blank for ampicillin and penicillin, respectively. The linear regression equation for the calibration graph for ampicillin for the concentration range of 1.0-40.0 μg mL^{-1} was $A=0.0052+0.0141C_{ampicillin}$ ($r^2=0.9938$, n=20) and for penicillin for the concentration range of 0.5-20.0 μg mL^{-1} was $A=0.0097+0.0342C_{penicillin}$ ($r^2=0.9972$, n=20). The limits of detection were 0.28 and 0.11 μg mL^{-1} for ampicillin and penicillin, respectively, were calculated according to calibration lines characteristics.

3.3. Calibration and prediction data sets

The multivariate calibration is a powerful tool for determinations, because it extracts more information from the data and allows building more robust models. According to an experimental design (Table 1), 25 solutions were used to construct the models (calibration set) and another 5 solutions to validate them (prediction set) that these not included in the previous set were employed as an independent test (see Table 2).

Table 1. Concentration data of the different mixtures used in the calibration set for the determination of ampicillin and penicillin (μg mL^{-1}).

Mixture	Ampicillin	Penicillin	Mixture	Ampicillin	Penicillin	Mixture	Ampicillin	Penicillin
M1	1.0	0.5	M10	10.0	5.0	M19	30.0	15.0
M2	1.0	0.5	M11	20.0	10.0	M20	30.0	15.0
M3	1.0	0.5	M12	20.0	10.0	M21	40.0	20.0
M4	1.0	0.5	M13	20.0	10.0	M22	40.0	20.0
M5	1.0	0.5	M14	20.0	10.0	M23	40.0	20.0
M6	10.0	5.0	M15	20.0	10.0	M24	40.0	20.0
M7	10.0	5.0	M16	30.0	15.0	M25	40.0	20.0
M8	10.0	5.0	M17	30.0	15.0			
M9	10.0	5.0	M18	30.0	15.0			

All recorded data are mean-centered. The calibration matrix was experimental designed over the concentration ranges of 1.0-40.0 and 0.5-20.0 μg mL^{-1} for ampicillin and penicillin, respectively. According to the following basic rules. First, the calibration standards should be mixtures of components in order to compensate for effects on absorbance from interaction between the components. Second, the peak absorbance of each standard should be less 1.5 in the analytical wavelength range. Finally, the concentration of all of the components must be independently varied within the set of standards. To ensure that the prediction and real samples are in the subspace of training set, the score plot of first principal component versus second was sketched and all the samples are spanned with the training set scores. For the evaluation of the predictive ability of a multivariate calibration models, the root mean square error of prediction (RMSEP) and relative standard error of prediction (RSEP) [25] can be used:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(y_{pred} - y_{obs})^2}{n}}$$

$$RSEP(\%) = 100 \times \sqrt{\frac{\sum_{i=1}^{n}(y_{pred} - y_{obs})^2}{\sum(y_{obs})^2}}$$

where y_{pred} is the predicted concentration in the sample, y_{obs} is the observed value of the concentration in the sample and n is the number of samples in the prediction set.

3.4. Preprocessing by orthogonal signal correction

For calibration two-OSC components were used for filtering. Evaluation of the prediction errors for the validation set reveals that the OSC treated data give substantially lower RMSEP values than original data. Also, the OSC-filtered data give much simpler calibration models with fewer components than the ones based on original data. The results imply that the OSC method indeed removes information from UV-Visible data that is not necessary for fitting of the Y-variables. In some cases the OSC method also removes non-linear relationships between **X** and **Y**. Fig. 2 is showing the score plot for when the PLS and OSC-PLS are used. The score plots are shown for comparison of the results obtained from PLS and OSC-PLS. The results show, score plots have better results when OSC-PLS is used.

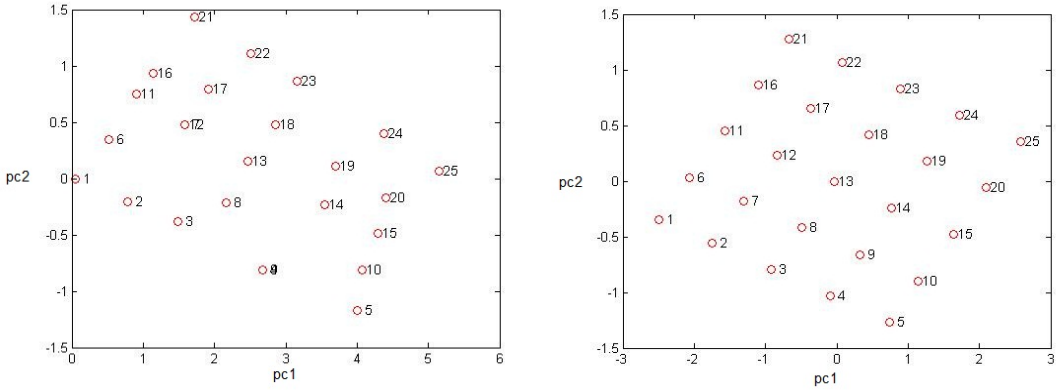


Fig. 2. Plots of first principal component against second principal component for ampicillin and penicillin determination (left) by PLS model, (right) by OSC-PLS model.

3.5. Selection of the optimum number of factors

The optimum number of factors (latent variables) to be included in the calibration model was determined by computing the RMSEC for cross-validated models using a high number of factors (half the number of total standard +1). The cross-validation method employed was to eliminate only one sample at a time and then PLS calibrate the remaining standard spectra. By using this calibration the concentration of the sample, left out was predicted. This process was repeated until each standard had been left out once. One reasonable choice for the optimum number of factors would be that number which yielded the minimum RMSEC. Since there are a finite number of samples in the training set, in many cases the minimum RMSEC value causes overfitting for unknown samples that were not included in the model. A solution to this problem has been suggested by Haaland et al. [26, 27] in which the RMSEC values for all previous factors are compared to the RMSEC value at the minimum. The F-statistical test can be used to determine the significance of RMSEC values greater than the minimum. The maximum number of factors used to calculate the optimum RMSEC was selected as 13 and the optimum number of factors obtained by the application of PLS and OSC-PLS models are summarized in Table 3. In all instances, the number of factors for the first RMSEC values whose F-ratio probability drops below 0.75 was selected as the optimum.

3.6. Determination of ampicillin and penicillin in synthetic mixtures

The predictive ability of method was determined using 5 two-component ampicillin and penicillin mixtures (their compositions are given in Table 2). The results obtained by applying PLS and OSC-PLS algorithm to five synthetic samples are listed in Table 2 and Table 3. Table 2 and Table 3 also show the recovery for prediction series of ampicillin and penicillin mixtures. As can be

seen, the percentage error was also quite acceptable. The results of RMSEP and RSEP are summarized in Table 3.

Table 2. PLS results and statistical parameters of synthetic mixtures of ampicillin and penicillin (μg mL^{-1}).

Sample	Added		Predicted		Recovery (%)	
	Ampicillin	Penicillin	Ampicillin	Penicillin	Ampicillin	Penicillin
P1	3.0	20.0	2.45	23.41	81.7	117.0
P2	14.0	1.0	15.64	0.88	111.7	88.0
P3	20.0	12.0	22.94	11.23	114.7	93.6
P4	4.0	40.0	3.21	47.61	80.2	119.0
P5	8.0	30.0	7.61	37.12	95.1	123.7
N.F.			2	2		
RMSEC			3.022	0.784		
RMSEP			1.575	4.916		
RSEP			0.134	0.199		

Table 3. OSC-PLS results and statistical parameters of synthetic mixtures of ampicillin and penicillin (μg mL^{-1}).

Sample	Added		Predicted		Recovery (%)	
	Ampicillin	Penicillin	Ampicillin	Penicillin	Ampicillin	Penicillin
P1	3.0	20.0	2.89	20.26	96.3	101.3
P2	14.0	1.0	14.13	0.96	100.9	96.0
P3	20.0	12.0	20.19	11.97	100.9	99.7
P4	4.0	40.0	3.94	40.78	98.5	101.9
P5	8.0	30.0	7.96	31.06	99.5	103.5
N.F.			2	2		
RMSEC			0.535	0.328		
RMSEP			0.118	4.068		
RSEP			0.010	0.036		

3.7. Determination of ampicillin and penicillin in real matrix samples

In order to test the applicability and matrix interferences of the proposed method to the analysis of real samples, the method was applied in a variety of situations. For this purpose, diverse spiked samples and reference materials were analyzed. Table 4 shows the results obtained for real matrix (human plasma) samples. Therefore, the OSC-PLS model is able to predict the concentrations of each ampicillin and penicillin in the real matrix sample. The results obtained by the OSC-PLS and HPLC were statistically compared. Very similar values were found by two methods.

Table 4. OSC-PLS results applied on the real matrix samples (μg mL^{-1}).

Added		Found		S.D.[a]		HPLC	
Ampicillin	Penicillin	Ampicillin	Penicillin	Ampicillin	Penicillin	Ampicillin	Penicillin
3.0	20.0	2.86	19.67	0.11	0.16	2.98	19.88
34.0	10.0	33.48	9.73	0.18	0.13	33.86	9.93
14.0	1.0	13.74	0.91	0.14	0.08	13.87	0.96

[a] Standard deviation for n=3.

4- CONCLUSION

The ampicillin and penicillin mixture is a complex system due to its high spectral overlapping between the absorption spectra of their individual component. However, a simple, easy and inexpensive method such as PLS in a very short time was applied to overcome this problem. In addition, the present study shows that the OSC can be a good method for remove systematic variation from the response matrix **X** that is unrelated, or orthogonal, to the property matrix **Y**. Therefore, one can be certain that important information regarding the analyte is retained. The good agreement clearly demonstrates the utility of this procedure for the simultaneous determination of ampicillin and penicillin mixtures without tedious pretreatment in complex samples in synthetic and human plasma samples.

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