Simultaneous Determination of Chlorotetracycline and Benzocaine in Bolus by Chemometric Methods

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Two chemometric methods were applied to the simultaneous determination of chlortetracycline (CTC) and benzocaine (BC) in their binary mixture. The methods involve multivariate calibration based on principal least-squares (PCR) and partial least-squares (PLS) regressions. A concentration set consisting of binary mixtures of CTC and BC in 13 different combinations were randomly prepared in 0.011 M HCl. Both multivariate calibration models were constructed by using the relationship between the concentration set and its corresponding absorption data in the spectral region of 200-305 nm. The accuracy and the precision of the methods were validated by analyzing synthetic mixtures containing investigated drugs. The recovery results obtained by applying PCR and PLS calibrations to artificial mixtures were found between 100.2 and 101.0 %. Data treatments, regressions and statistical analysis were performed by using the Microsoft EXCEL and PLS toolbox 3.5 in Matlab 7.0 software. Satisfactory results for both artificial and commercial veterinary samples were obtained. The experimental results from two chemometric methods were compared with each other.

Keywords: PLS method; PCR method; chlortetracycline; benzocaine; veterinary powder product

CTC drug was the first tetracycline discovered and was first to be used for clinical use and it is marketed alone or in combination with other drugs [1]. It is broad-spectrum antimicrobial drugs with a long history of use in humans and animals. It inhibits the growing of a wide variety of bacteria, protozoa and many intercellular organisms such as mycoplasma, chlamydia and rickettsia [2]. BC is used as local anesthetic referred to as ethyl aminobenzoate. BC is available as a dusting powder or in oil, as an oinment for surface application [3]. It is used in cattle, sheep, swine and horses for local and prolonged low epidural anesthesia. It is also currently used as a surface anesthetic as oinments for wounds and ulcerates surfaces in horses, cattle and sheep [4].

Nowadays, chemometric calibration techniques namely as principal component regression and partial least squares regression techniques in spectral analysis are gaining importance in the quality control of drugs in mixtures and pharmaceutical formulations containing two or more drugs with overlapping spectra due to the fact that they do not need any separation procedure in the drug determinations [5-13]. In addition, these techniques can be successfully applied to all analysis methods.

In this study, two chemometric techniques PCR and PLS were applied to the determination of CTC and BC in their binary mixture and the proposed calibration techniques were validated by analyzing synthetic mixtures consisting of these drugs. The methods were subjected to the real samples and successful results were obtained. The numerical calculations were performed by using the "*Microsoft EXCEL* and *PLS toolbox 3.5* in Matlab 7.0 software".

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Methods

PLS method

The PLS calibration method is obtained by the composition of both concentration and absorbance matrix into latent variables, $A = TP^T + E$ and $C = UQ + F$. The form of the vector b is given by $b = W(PTW)^{-1}Q$. Here W denotes a weight matrix. By using the linear regression $C = a + b*A$, the constant a is obtained as $a = C_{mean} \times A^T_{mean} b$. This last equation is used to find the unknown concentration of active compounds in samples.

PCR method

This method has two parts. In the first part, the eigenvectors corresponding to the centered absorbance data matrix are calculated. In the second step, the multilinear regression is used to obtain PCR calibration based on the use of eigenvectors. The mathematical formulation of this approach is given by $A_{proj} = Vc^T A$, where A_{proj} is the matrix containing the new coordinates (the projections), Vc^T denotes the matrix containing the basis vectors, one column for each factor retained, whilst A denotes the original training set absorbance matrix. Once the matrix A_{proj} known, it leads us, after some simple manipulations, to the unknown concentration matrix given by the following formula $C = F A_{proj}$ F denotes the calibration coefficient for the obtained linear equation system.

Experimental part

Apparatus

The absorption spectra were recorded by using a Shimadzu UV-160 double beam UV-VIS spectrophotometer possesing a fixed slit width (2 nm) connected to a computer

loaded with Shimadzu UVPC software and a LEXMARK–E320 printer. The spectrum was recorded in the wavelength range of 200-305 nm against a blank (0.01 M HCl). Data treatments, regressions and statistical analysis were done by using the *EXCEL* and *PLS* toolbox 3.5 in Matlab 7.0 software.

Veterinary powder product

A commercial veterinary powder product, Devamisin® Skin Powder produced by Veta° Veterinary and Agricultural Drugs Company, Istanbul, Turkey was investigated by using the PLS and PCR approaches. The label claim of the commercial veterinary powder is that 50 mg CTC and 10 mg BC per gram powder. CTC and BC drugs were obtained kindly from Veta° Veterinary and Agricultural Drugs Company.

Standard solution

Stock solutions of 100 mg/100 mL CTC and BC for each compound were prepared in 0.01 M HCl. A concentration set of the mixture solutions containing 0.0-24.0 μg/mL CTC and 0.0-18.0 μg/mL BC was obtained from the stock solutions for recording spectra. A validation set of 10 mixture solutions containing two compounds was also prepared by using the same stock solutions.

Preparation of sample solution

An accurately weighed quantity equivalent to 500 mg powder was dissolved in 0.01 M HCl in a 100 mL volumetric flask. After that, the solution was filtered into a 100 mL volumetric flask through a 0.45-μm membrane filter. This solution was diluted to the working concentration range of 20 μg/mL for CTC and 4 μg/mL for BC in a 25 mL volumetric flask. PCR and PLS methods were applied to the original absorption spectra of final sample solutions.

Results and discussion

The absorption spectra for CTC, BC and their binary mixture in 0.01 M HCl were presented in figure 1. Therefore, we conclude that these overlapping spectra are not suitable for the quantitative determination of these drugs in tablets. As a result we focused to apply two chemometric calibrations (PCR and PLS) to the quantitative resolution of mixtures consisting CTC and BC. The application of the methods is explained below.

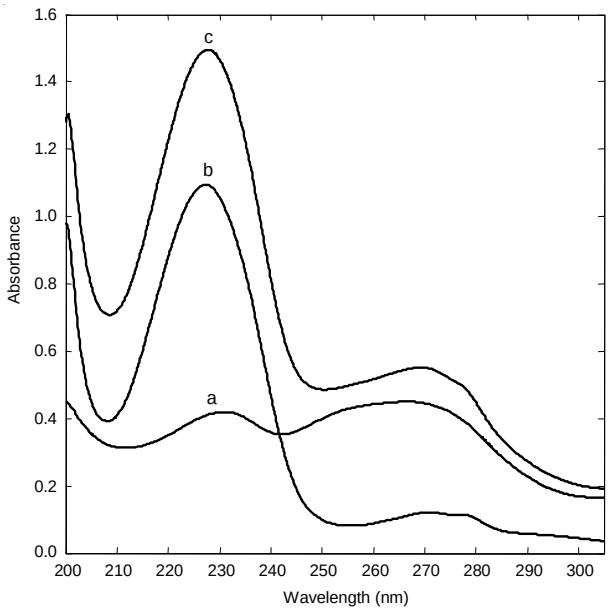

Fig. 1. Absorption spectra of a) 12 μg/mL CTC, b) 14 μg/mL BC and their mixture in 0.01 M HCl

Table 1
COMPOSITION OF THE CALIBRATION SAMPLES

Sample No.	Concentration (μg/mL)	
	CTC	BC
1	8.0	18.0
2	12.0	14.0
3	20.0	18.0
4	20.0	6.0
5	24.0	2.0
6	0.0	4.0
7	20.0	0.0
8	10.0	8.0
9	6.0	7.0
10	8.0	4.0
11	12.0	4.0
12	16.0	4.0
13	20.0	4.0

PCR and PLS methods

As it can be seen in table 1, the concentration set of the mixture solutions in the concentration range of 0.0-24.0 μg/mL for CTC, and 0.0-18.0 μg/mL BC in possible combinations was randomly prepared for both PCR and PLS methods. This concentration set was used to build the PCR and PLS calibration.

By measuring the full wavelengths with the intervals of $\Delta\lambda$= 0.1 nm in between 200-305 nm .The absorbance data matrix corresponding to the concentration set was obtained. The absorption spectra of the concentration set corresponding to table 1 are shown in figure 2.

pCR and PLS calibrations were obtained by using the concentration sets and absorbance data in the spectral range of 200-305 nm.

A calibration for each method was calculated in the *Microsoft EXCEL* and *PLS toolbox 3.5* in Matlab 7.0 software by using the training set and its measured absorbance. The PCR and PLS calibration techniques were used for the prediction of the amounts of the two drugs in the samples.

Method validation

PCR and PLS methods were tested by using the binary mixtures of CTC and BC drugs. Mean recoveries and relative standard deviations for the PCR and PLS

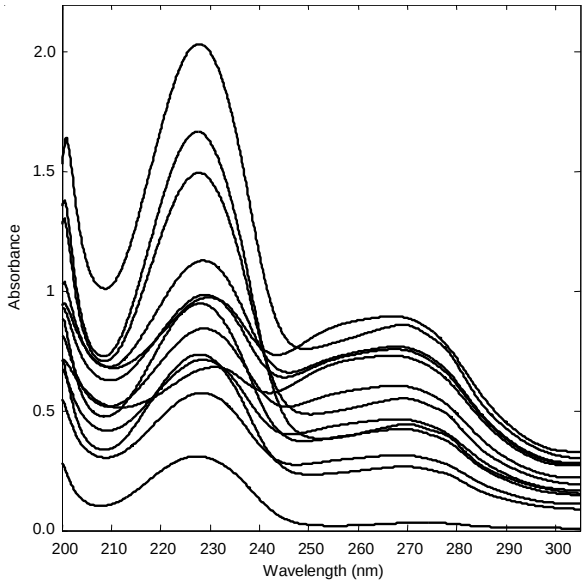

Fig. 2. Absorption spectra of the concentation set corresponding to table 1

Table 2
RECOVERY STUDY OF BC AND CTC BY USING THE PROPOSED METHODS

Mixture (μg/mL)		Found (μg/mL)				Recovery (%)			
		PCR		PLS		PCR		PLS	
CTC	BC	CTC	CTC	BC	CTC	CTC	BC	CTC	CTC
8.0	4.0	7.93	4.03	7.99	4.04	99.1	100.7	99.9	100.9
12.0	4.0	12.06	4.04	12.06	4.02	100.5	100.9	100.5	100.6
16.0	4.0	15.83	4.02	15.84	4.03	98.9	100.4	99.0	100.6
20.0	4.0	20.01	4.04	20.02	4.02	100.0	101.0	100.1	100.6
24.0	4.0	24.28	4.05	24.30	4.06	101.2	101.3	101.3	101.4
20.0	2.0	20.31	2.12	20.31	2.11	101.6	106.2	101.5	105.6
20.0	6.0	19.91	5.96	19.89	5.96	99.5	99.4	99.5	99.3
20.0	10.0	20.09	10.03	20.16	10.05	100.4	100.3	100.8	100.5
20.0	14.0	20.27	13.99	20.37	14.00	101.3	99.9	101.8	100.0
20.0	18.0	19.94	17.97	20.03	17.96	99.7	99.8	100.1	99.8
					Mean	100.2	101.0	100.5	100.9
								.87	1.65
					RSD	0.88	1.81	0.87	1.64

SD = Standard deviation, RSD = Relative standard deviation

Table 3
CHEMOMETRIC PARAMETER FOR PCR AND PLS

	PCR		PLS	
Parameter	CTC	BC	CTC	BC
SEC	0.1545	0.0558	0.1569	0.0666
SEP	0.1850	0.0535	0.2084	0.0547
slop	0.9819	1.0056	0.9821	1.0056
intercept	0.2653	0.0646	0.2264	0.0653
(r)	0.9995	1.0000	0.9994	1.0000

calibrations were calculated and presented in table 2. Therefore, these experimental results indicate that these two methods are suitable for simultaneous determination of CTC and BC in samples.

Statistical Parameters

To test the proposed calibrations, an independent set of the validation set in table 2 was analysed and used for the calculations of the standard error of prediction (SEP) and the standard error of calibration (SEC), which are given as follows

$$SEP = SEC\sqrt{\frac{C^{Added} - C^{Found}}{n-1}}$$

Where C^{Added} represents the added concentration of drug, $C_i^{Found^i}$ denotes 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=13 calibration samples and the errors of prediction (SEP) for n=10 prediction samples were found acceptable in PCR and PLS method and their values were presented in table 3. The square of the correlation coefficient (r), which indicates the quality of the straight line that fits the data, is calculated. The values of r presented in table 3 are in all cases close to 1, which represents the expression of similarity between predicted and active concentration values.

Analysis of tablet content

The obtained results by applying PCR and PLS methods to tablets were shown in table 4. A good coincidence was observed between the experimental results and label claim of the commercial veterinary product in this study. The

Table 4.
DETERMINATION RESULTS OF COMMERCIAL VETERINARY FORMULATION

	mg/bolus			
	PCR		PLS	
	CTC	BC	CTC	BC
	52.63	9.57	52.28	9.55
	50.86	10.04	50.27	10.00
	52.02	9.50	51.59	9.85
	50.13	9.75	50.10	9.78
	51.90	10.03	50.94	9.88
Mean	51.51	9.78	51.04	9.81
SD	1.00	0.25	0.91	0.17
RSD	1.94	2.56	1.79	1.72
SE	0.45	0.11	0.41	0.08
CL (P=0.05)	0.24	0.06	0.22	0.04

Claim label: 50 mg CTC and 10 mg BC per gram powder
CL = Confidence limit

numerical values of all statistical parameters calculated in table 4, are acceptable determination limits in application of two methods to the commercial veterinary samples.

Conclusion

The proposed chemometric techniques are rapid, precise and accurate for the simultaneous quantitative resolution of the veterinary formulation, as well as for the simultaneous analysis of the mixtures containing drugs having overlapped spectra. PCR and PLS techniques do not require any graphical treatment and they do not use some steps of separation and extraction etc., as in HPLC method.

In this paper, we used the interval of the working range of both techniques larger than those presented in literature.

Due to the non-existence of an official and literature methods for the investigated binary mixture, the application of PCR and PLS calibration techniques may be considered as a basic result for the future studies on these drugs.

The assay results obtained in this study strongly encourage us to apply these techniques for a routine analysis and quality control of the commercial veterinary products containing two drugs.

References

1. RIVIERE,J.E., SPOO, J. W., Tetracycline Antibiotics, in Veterinary Pharmacology and Therapeutics, Edited by Adams HR. Blackwell Publishing, 8th Ed., Iowa, 2001

2. WOUTERS, M.F.A., VERMEULEN, J:E.M., VAN LEEUWEN, F.X.R., Chlortetracycline and Tetracycline.IPCS INCHEM, http://www.inchem.org/documents/jecfa/jecmono/v36je06.htm, 2006

3. MAMA, K. R., STEFFEY, E.P., Local Anesthetics, in Veterinary Pharmacology and Therapeutics. Edited by Adams HR. Blackwell Publishing, 8th Edition, Iowa, 2001

4. ANONYMUS, Committee for Veterinary Medicinal Products, Benzocaine Summary Report, http://www.emea.eu.int/pdfs/vet/mrls/021897en.pdf, 1997

5. MARTENS, H. NAES, T. Multiseriate Calibration. J. Wiley and Sons: Chichester, UK, 1991

6. KRAMER, R., Chemometric Techniques for Quantitative Analysis, Marcel Dekker, New York, 1998

7. MARKOPOULOU, C.K., MALLIOU, E.T., KOUNDOURELLIS, J.E., J. Pharmaceut. Biomed. Anal., **37**, 2005, p.249

8. FERRARO, M.C.F., CASTELLANO, P.M., KAUFMAN, T.S., Anal. Bioanal. Chem. , 377, 2003, p. 1159

9. CASTELLANO, P.M., VIGNADUZZO, S.E., MAGGIO, R.M., KAUFMAN, T.S., Anal. Bioanal. Chem. , 382, 2005, p. 1711

10. DINÇ, E., OZDEMIR, A., ONUR, F., BĂLEANU, D., AKSOY, H., YUCESOY, C., Rev. Chim. (Bucureºti), **57**, nr. 4, 2006, p. 368

11. DINÇ, E., OZDEMIR, A., AKSOY, H., BĂLEANU, D., J. Liq. Chrom. & Rel. Techn., **29** , nr. 12, 2006, p. 1803

12. DINÇ, E., DERMIª, S., BĂLEANU, D., Rev. Chim. (Bucureºti), **57**, nr. 3, 2006, p. 229

13. DINÇ, E., ÖZDEMIR, A., BĂLEANU, D., J. Pharmaceut. Biomed. Anal. 37, nr. 3,2005, p. 569

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