Spectrophotometric Simultaneous Determination of Levamisole and Triclabendazole in Tablets by Principal Component Regression and Partial Least Squares Chemometric Methods

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Principal component regression (PCR) and partial least squares (PLS) chemometric methods were applied to the simultaneous quantitative analysis of levamisole (LVM) and triclabendazole (TCB) in tablets without using a preliminary separation, even in presence of the overlapping spectra of the above compounds. For both PCR and PLS, a concentration set containing 25 different mixtures of LVM and TCB in the linear concentration range was symmetrically prepared and then the absorbance values of the concentration set were measured at the wavelength set with $\Delta\lambda=0.1$ nm in the spectral region of 225-322.3 nm. PCR and PLS calibrations were obtained by applying the PCR and PLS algorithms to the concentration set data (y-block) and their corresponding absorbance data (x-block). The validity of PCR and PLS chemometric methods was performed by using the independent synthetic mixtures and the standard addition technique. Then, these analytical methods were applied to the commercial tablets and a good agreement was obtained between experimental results provided by the application of the PCR and PLS to the synthetic and real samples.

Keywords: partial least squares, principal component regression, levamisole, triclabendazole

Commercial formulations containing one or more active compounds in the pharmaceuticals containing a constant matrix have been used for increasing the therapeutic effect against diseases. Analysis of drug combination is very important for the commercial production of drugs. Therefore, to obtain highly precision and accurate result we need flexible, versatile and low cost methods. The chemometric analysis of complex mixtures plays an important role in analytical chemistry. During the last decades several modern mathematic techniques were used to analyze the overlapping spectra. The overlapping spectrum is an interesting topic for analytical chemistry especially when an optimal decision should be performed. In the last decades a huge effort was dedicated to apply the chemometric techniques to the simultaneous determination of active compounds in a specified mixture [1] .

PCR and PLS are two powerful standard chemometric techniques devoted to the multicomponent analysis of complex mixtures. The chemometric techniques found many applications in various areas of analytical chemistry and they were analyzed systematically in [2-8].

Brief information about general properties of LVM and TCB for these purposes is given below. Levamisole-triclabendazole combinations have been used particularly for the treatment of outbreaks of acute and subacute fasciolosis and for the strategic control of fasciola infections. LVM alone is a broad spectrum anthelmintic effective against mature and immature stages of nematodes, including strains resistant to other anthelmintics. TCB compound is a benzimidazole. It binds to tubulin impairing intracellular transport mechanisms and interferes with protein synthesis. TCB is specifically effective against all 3 stages of fasciola. In our knowledge the simultaneous determination of the binary mixture of LVM and TCB was not investigated in the literature. The determination of LVM in its different sample forms together with other active compounds and their metabolites have been performed by HPLC [9-14], LC –MS/MS [11,15]. TCB in samples in the presence of other compounds or its metabolites has been analyzed by HPLC [16,17] .

In this study PCR and PLS chemometric methods were proposed for the simultaneous quantitative prediction of LEV and TCB in synthetic mixtures and commercial oral tablets. Two chemometric approaches were successfully applied for the resolution of this problem despite the unusual shape of spectra. The accuracy and precision of the PCR and PLS approaches were validated by analyzing various synthetic mixtures and by using standard addition technique. Good accuracy and precision for the obtained results were reported in application of both chemometric approaches. As a consequence PCR and PLS chemometric methods can be applied to the routine quality control of the commercial oral formulation of LEV and TCB compounds.

Experimental Part

Instruments

A Shimadzu UV-160 double beam UV–VIS spectrophotometer equipped with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software and a LEXMARK E-320 printer were used for the

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registration of the absorption spectra. The absorption data were transformed into ASCII files and transferred to *EXCEL*. After that the transferred vectors data corresponding to the concentration set and sample set were processed by PCR and PLS approaches. Data treatments, regressions and statistical analysis were performed by using the *EXCEL* and *PLS* toolbox 3.5 in Matlab 7.0 software.

Commercial preparation

A commercial preparation (BESTAN® Oral Tablet, Vilsan Pharm. Ind.,Ankara Turkey) was investigated. Its declared content contains: 600 mg TCB, 375 mg LVM HCl per tablet. LVM and TCB compounds were obtained as a donation from Vilsan Pharm. Ind., Ankara (Turkey).

Standard solutions

Stock solution of 25 mg/100 mL LVM and TCB were prepared in methanol and 0.1 M NaOH (75:25, v/v). A concentration set of 25 mixture solutions consisting of LVM and TCB in the concentration range of 5.0–25.0 and 1.0-11.0 μg/mL for LVM and TCB in the same solvent was symmetrically prepared from the prepared stock solutions, respectively (fig. 1). To check the proposed methods we used an independent validation set consisting of the synthetic mixture solutions of LVM and TCB in the above working concentration ranges.

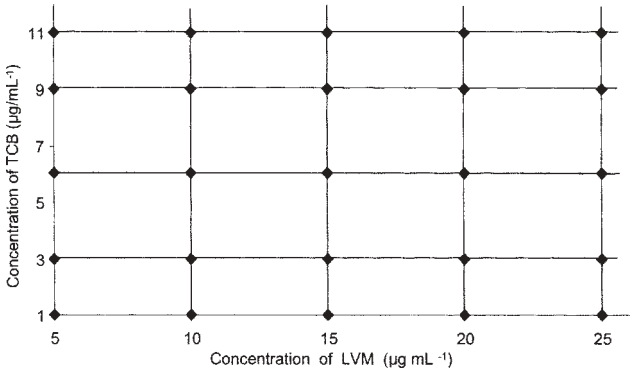

Fig. 1. Concentration set design for the preparation of the PCR and PLS calibrations

Sample solution preparation

Ten tablets were accurately weighed and powdered in a mortar. A sample containing LVM and TCB equivalent to 1/8 tablet content was dissolved in the mixture of methanol and 0.1 M NaOH (75:25, v/v) and made up in 100 mL calibrated flasks. The content of the flask was mechanically shaken for 25 min and filtrated into a 100 mL volumetric flask through a 0.45 μm membrane filter. The final solution was diluted to the working concentration range. This procedure was repeated ten times. The absorption spectra of these sample solution were recorded for the application of the PCR and PLS calibrations.

Results and discussion

PCR method

Firstly, the eigenvectors corresponding to the centered absorbance data matrix are obtained. Secondly, the multilinear regression is applied to construct PCR calibration based on the use of eigenvectors. The fundamental mathematical formulation of this approach has the form $A_{proj} = Vc^T A$, where A_{proj} denotes the matrix containing the new coordinates, Vc^T is the matrix containing the basis vectors, one column for each factor retained, whilst A is the original training set absorbance matrix. After finding the matrix A_{proj}, the unknown concentration matrix is calculated as given by the following formula $C = F A_{proj}$. Here, F represents the calibration coefficient for the obtained linear equation system.

PLS method

The PLS calibration method is obtained by the composition of both concentration and absorbance matrix into latent variables, namely $A = TP^T + E$ and $C = UQ + F$. By performing the linear regression $C = a + bA$, where the vector b is given by $b = W(PTW)^{-1}Q$, the constant a is obtained as $a = C_{mean} - A^T_{mean} b$. This equation allows us to find the unknown concentration of active compounds in samples.

Method development and applications

Figure 2 shows the absorption spectra of LVM and TCB together with their mixture. In the presence of the overlapping spectra of two compounds in the spectral range of 210-340 nm, the simultaneous determination of the related compounds in samples is not possible by using the classical spectrophotometric approaches. We have focused mainly on the quantitative resolution of the binary mixtures of LVM and TCB by using PCR and PLS chemometric approaches without any separation step and graphical procedure.

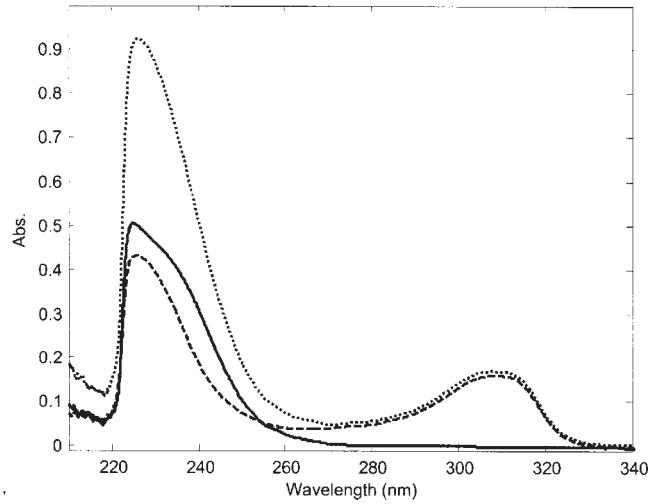

Fig. 2. Absorpstion spectra of 15.0 μg/mL LVM (-) and 3.0 μg/mL TCB (- - -) and their mixture (….) in methanol and 0.1 M NaOH

PLS and PCR methods

To build PCR and PLS calibration models, a concentration set of 25 mixtures of two compounds in the range of 5.0-25.0 μg/mL for LVM and 1.0-11.0 μg/mL for TCB in methanol and 0.1 M NaOH (75:25, v/v) was symmetrically prepared as seen in figure 1. In the case of the preparation of concentration set, a symmetric set was used to minimize the random errors. The concentration set and its composition corresponding to figure 2 were presented in table 1. The absorption values of spectra of the concentration set were measured at the wavelength set with $\Delta\lambda$=0.1 nm in the spectral region of 210-340 nm. The concentration set and absorption data were considered as y-block (25 x 2) and x-block (25x 1024) for the construction of PCR and PLS chemometric calibration. During the calibration process various factors were tested by using the cross validation procedure to obtain the best recovery results. The calculations were done within PLS Toolbox 3.5 and we have selected the optimal factor to be 6. In this case RMSEC and RMSECV values of LVM and TCB were reported as 0.367 and 0.1402; 0.1649 and 0.0956 for PCR and RMSEC and RMSECV were observed as 0.14821 and 0.0942; 0.3623 and 0.18378 for PLS. We mention that

Table 1
CONCENTRATION SET CONTAINING LVM AND TCB COMPOUNDS FOR THE PREPARATION OF THE PCR AND PLS CALIBRATIONS

Concentration set					
	Concentration (μg/mL)			Concentration (μg/mL)	
No.	LVM	TCB	No.	LVM	TCB
1	5.0	1.0	14	15.0	9.0
2	5.0	3.0	15	15.0	11.0
3	5.0	6.0	16	20.0	1.0
4	5.0	9.0	17	20.0	3.0
5	5.0	11.0	18	20.0	6.0
6	10.0	1.0	19	20.0	9.0
7	10.0	3.0	20	20.0	11.0
8	10.0	6.0	21	25.0	1.0
9	10.0	9.0	22	25.0	3.0
10	10.0	11.0	23	25.0	6.0
11	15.0	1.0	24	25.0	9.0
12	15.0	3.0	25	25.0	11.0
13	15.0	6.0			

Table 2
CONCENTRATION SET CONTAINING LVM AND TCB COMPOUNDS FOR THE PREPARATION OF THE PCR AND PLS CALIBRATIONS

			Found (μg/mL)				Recovery (%)			
	Added (μg/mL)		PCR		PLS		PCR		PLS	
No.	LVM	TCB	LVM	TCB	LVM	TCB	LVM	TCB	LVM	TCB
1	5	9.6	5.11	9.32	5.11	9.31	102.2	97.0	102.2	97.0
2	10	9.6	9.71	9.44	9.75	9.43	97.1	98.3	97.5	98.3
3	15	9.6	14.50	9.51	14.43	9.51	96.6	99.1	96.2	99.1
4	20	9.6	19.32	9.48	19.32	9.48	96.6	98.8	96.6	98.7
5	25	9.6	24.97	9.54	24.98	9.54	99.9	99.4	99.9	99.4
6	6	1	6.12	0.94	6.21	0.95	101.9	94.2	103.4	95.2
7	6	3	6.08	2.96	6.25	2.96	101.4	98.6	104.2	98.8
8	6	6	6.11	6.04	6.07	6.04	101.8	100.6	101.1	100.7
9	6	9	6.20	8.96	6.16	8.96	103.4	99.6	102.7	99.6
10	6	11	6.19	10.76	6.14	10.76	103.1	97.8	103.1	97.8
				Mean			100.4	98.3	100.7	98.5
				SD			2.67	1.75	2.98	1.52
				RSD			2.66	1.78	2.96	1.54

SD = Standard deviation, RSD = Relative standard deviation

we have checked the validity of all factors between 1 and 5 but none of them gave successful results.

Method validation

The validation of PCR and PLS methods have been done by their performance for obtaining reliable results of analysis. Therefore, ten synthetic mixtures containing LVM and TCB in different concentration levels as shown in table 2 were prepared as an independent validation set. The mean recoveries and the relative standard deviations of PCR and PLS calibrations are given in table 2. In the recovery study, the numerical values were found satisfactory for the validity of PCR and PLS. The reliable accuracy and higher precision in application of these methods were reported for the analysis of both compounds. During the process of the analysis, interference and systematical errors were absent.

To test the selectivity of the methods, the standard of LVM and TCB was added to the oral tablet solution. In this standard addition technique, three different concentration levels were used, namely 4, 8 and 12 μg/mL for LVM and 1.2, 2 and 3.5 μg/mL for TCB. This procedure was repeated six times for each concentration level. The recovery results and their standard deviations were calculated and presented in table 3. During this process no interference of the excipients formulation was reported. Therefore, PCR and PLS approaches proposed in this study are appropriate for the determination of LVM and TCB compounds in the oral tablets.

Chemometric parameters

The performance of the chemometric calibration model is defined in various ways. The most general expression is the standard error of prediction (**SEP**) and it is given in the following

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

C_i^{Added} - denotes the added concentration of drug;

C_i^{Found} - is the predicted concentration of drug;

n represents the total number of synthetic mixtures. The SEP values of PCR and PLS techniques were indicated in table 4.

Table 3
RECOVERY RESULTS OBTAINED FROM STANDARD ADDITION TECHNIQUE BY THE PROPOSED ANALYTICAL METHODS

Added to tablet (μg/mL)		Found (μg/mL)				Recovery (%)			
		PCR		PLS		PCR		PLS	
LVM	TCB	LVM	TCB	LVM	TCB	LVM	TCB	LVM	TCB
4.0	1.2	3.86	1.20	3.84	1.18	96.4	100.2	96.0	98.4
8.0	2.0	7.69	1.99	7.62	1.95	96.1	99.3	95.2	97.3
12.0	3.5	11.53	3.49	11.42	3.50	96.0	99.8	95.1	99.9
			Mean			96.2	99.8	95.5	98.6
			SD			0.20	0.45	0.48	1.29
			RSD			0.21	0.46	0.51	1.31

Results were obtained from the average of six time, for each concentration level

Table 4
STATISTICAL PARAMETERS FOR PCR AND PLS METHODS

		PLS		PCR	
Step	Parameter	LVM	TCB	LVM	TCB
Calibration	SEC	0.1683	0.0975	0.1513	5.1647
	Slope	1.0000	1.0000	1.0000	0.0000
	Intercept	-1.71E-06	7.96E-07	2.81E-07	7.77E-07
	r	0.9997	0.9997	0.9998	0.9997
Prediction	SEP	0.3200	0.1477	0.3365	0.1491
	Slope	1.0276	1.0150	1.0295	1.0163
	Intercept	-2.19E-01	-1.09E-02	-2.49E-01	-2.13E-02
	r	0.9994	0.9997	0.9993	0.9997

r = Correlation coefficient of linear regression equation

Table 5
EXPERIMENTAL RESULTS OF COMMERCIAL FORMULATION OBTAINED BY THE PROPOSED ANALYTICAL METHODS

	PCR		PLS	
No.	LVM	TCB	LVM	TCB
1	384.4	590.1	373.6	597.3
2	386.6	586.7	377.8	590.5
3	387.7	595.6	383.5	592.2
4	391.2	593.8	384.2	587.1
5	383.2	593.0	387.5	595.5
6	393.4	613.7	380.1	593.8
7	387.2	592.1	379.0	613.7
8	391.7	589.1	386.7	592.1
9	397.4	583.3	384.6	593.0
10	377.4	580.8	387.5	593.8
Mean ($\bar{x}$)	388.0	591.8	382.5	594.9
SD	5.70	8.98	4.65	7.16
RSD	1.47	1.52	1.22	1.20
SE	1.80	2.84	1.47	2.26
CL (p=0.05	$\bar{x}$ ±3.53	$\bar{x}$ ±5.57	$\bar{x}$ ±2.89	$\bar{x}$ ±4.44

Claim label: 375 mg LVM and 600 mg TCB /tablet
CL: confidence limit
SE: standard error

The standard error of calibration denoted by SEC represents another important quantity and is given us by

$$SEC = \sqrt{\frac{\sum_{i=1}^{n}(C_i^{Added} - C_i^{Found})^2}{n}}, \quad (2)$$

The values of **SEC** of **PCR** and **PLS** were calculated and the results are presented in table 4. In the same table, the statistical parameters between actual and predicted concentrations of compounds in mixtures were found by using the experimental data in the calibration and prediction steps.

Sample Analysis

PCR and PLS methods were applied to the quantitative analysis of LVM and TCB in tablets. The experimental determination results of the commercial tablets are

indicated in table 5. The analysis obtained from both methods was found satisfactory for the quantitative analysis of commercial tablet. A good coincidence was observed between determination results and the label amounts.

Conclusions

Quantitative resolution of binary mixture is not possible by using the direct absorbance measurement due to the interference of spectra in the same spectral range, but PCR and PLS methods using full absorbance measurement give us good resolution for complex mixtures containing two or more compounds. Both PCR and PLS do not require a separation step and graphical process of spectra to resolve a binary mixture as in our investigation.

In the synthetic mixtures and tablets, the simultaneous determination of LVM and TCB having overlapping spectra in the spectral range of 210-340 nm was successfully accomplished by PCR and PLS.

As in the quality control of the commercial pharmaceutical preparations, the routine quality control and analysis of the commercial preparation is a great obligation to protect the health of both animals and humans. For these reasons, the proposed PCR and PLS chemometric methods provide a good response for the quality control and routine analysis of the oral preparations consisting of LVM and TCB.

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Manuscript received: 21.11.2007