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Spectrophotometric multicomponent resolution of a veterinary formulation containing oxfendazole and oxyclozanide by multivariate calibration–prediction techniques

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Abstract

Four multivariate calibration–prediction techniques, classical least-squares, inverse least-squares, principal component regression and partial least-squares regression were applied to the spectrophotometric multicomponent analysis of a veterinary formulation containing oxfendazole (OXF) and oxyclozanide (OXC) without any separation step. The multivariate calibrations were constructed by measuring the absorbance values at 14 points in the 285–350 nm wavelength range and by using the training set of standard mixtures containing OXF and OXC in the different compositions. The validity of building multivariate calibrations was checked by using the synthetic mixtures of both drugs. The multivariate calibration models were successfully applied to the spectrophotometric determination of OXF and OXC in laboratory prepared mixtures and a veterinary formulation. The results obtained were statistically compared with each other.

Keywords: Oxfendazole; Oxyclozanide; Classical least-squares; Inverse least-squares; Principal component regression; Partial least-squares regression

1. Introduction

Oxfendazole–oxyclozanide (OXF–OXC) mixture is widely used in the veterinary practice as anthelmintic drugs.

The determination of OXF in the biological samples by different chromatographic methods [1–7] and its mixture with OXC in pharmaceutical formulation by HPLC [8] and by spectrophotometric methods [9] have been demonstrated in the literature.

In recent years, multivariate calibration techniques, namely classical least-squares (CLS), inverse least-squares (ILS), principal component

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regression (PCR) and partial least-squares (PLSR) techniques based on the computer-controlled instrumentation are playing a very important role in the spectrophotometric analysis of mixtures containing two or more compounds without preliminary separation [10–16]. All the multivariate approaches are useful for the resolution of spectral band overlapping in quantitative determination. In the multivariate analysis, a calibration is build from spectral response values for a set of standard samples of known concentrations corresponding to the analytes of interest. The obtained calibration is used to predict the component concentrations from the sample spectrum.

For some drugs, the application of multivariate calibration techniques to drug analysis were reported in the literature [17–25].

The multivariate calibration–prediction techniques use the full spectrum, full automation, multivariate data analysis and the reduction of noise and the advantages of the selection of the calibration model. In addition these multivariate calibrations do not need any separation procedure, they are very cheap, very easy to apply and very sensitive. For these reasons these multivariate techniques are popular today and we apply it to the subject veterinary formulation.

In this study, the CLS, ILS, PCR and PLSR calibration models were described for the spectrophotometric resolution of the samples containing OXF and OXC. A computer program, MAPLE V was used to perform the construction of multivariate calibrations–predictions. These calibrations were tested for the synthetic mixtures containing the two drugs and they were applied to the simultaneous resolution of OXF and OXC in a veterinary formulation, bolus, marketed in Turkey.

2. Experimental

2.1. Instrument

The absorbance measurements were performed by using a Shimadzu UV-1601 double beam UV–Visible spectrophotometer with a fixed slit width (2 nm) connected to a computer loaded with Shimadzu UVPC software, equipped with an HP Office Jet Pro 1150C. The additional MAPLE V software was used for mathematical computations.

2.2. Veterinary formulation

A commercial veterinary product (OKZAN® bolus, Sanofi-DIF Pharm. Ind., Turkey, Batch no. 075) was assayed. Its declared content was as follows: 112 mg OXF, 600 mg OXC and excipients (magnesium stearate, corn starch and tricalcium phosphate) per bolus.

OXF and OXC were obtained as a donation from Sanofi-DIF Pharm.Ind. (Turkey).

2.3. Standard solutions

Stock solution of 100 mg/100 ml OXC and OXF were prepared in 0.1 M NaOH. A training set containing 0–18 μg/ml OXF and 0–36 μg/ml OXC in possible proportions and ten synthetic mixture solutions as a validation set in the concentration range of 2–18 μg/ml OXF and 4–36 μg/ml OXC were prepared by using the above stock solutions.

2.4. Preparation of sample solutions

Twenty bolus were accurately weighed and powdered in a mortar. A sample containing OXF and OXC equivalent to half bolus content was dissolved in 0.1 M NaOH and made up in 100 ml calibrated flasks. The content of the flask was mechanically shaken for 20 min and filtrated into a 100 ml volumetric flask through a 0.45 μm membrane filter. The resulting solution was diluted 1:110 with the same solvent. All the techniques were applied to the final solution.

3. Multivariate calibration–prediction techniques

3.1. Classical least-squares

CLS is involved in the application of multiple linear regression (MLR) to the classical expression of the Beer–Lambert law of spectroscopy given by

$$A = K \times C \quad (1)$$

The above matrix equation can be rewritten as a linear equation system:

$$\begin{aligned} A_1 &= K_{11}C_1 + K_{12}C_2 + \cdots + K_{1c}C_c \\ A_2 &= K_{21}C_1 + K_{22}C_2 + \cdots + K_{2c}C_c \\ \cdots &\quad \cdots \quad \cdots \quad \cdots \\ A_w &= K_{w1}C_1 + K_{w2}C_2 + \cdots + K_{wc}C_c \end{aligned} \quad (2)$$

where A_w represents the absorbance at the wth wavelength, K_{wc} is the calibration coefficient corresponding to the cth component at the wth wavelength, while C_c is the concentration of the cth component.

3.2. Inverse least-squares

ILS is the application of 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 written as a linear equation system as follows:

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

Here, A_w is the absorbance at the wth wavelength, P_{cw} denotes the calibration coefficient for the cth component at the wth wavelength, while C_{cw} is the concentration of the cth component.

3.3. Principal component regression

One of the multiple regression analysis techniques is the PCR technique. In the spectral work, the fundamental concept of PCR can be explained as regression of concentration on the principal components selected in the covariance of the measured absorbances.

We used the methodology described by Martens and Naes [15] for the PCR technique. The algorithm for PCR was realized step-by-step as explained below.

The original data obtained in absorbances and concentrations of analytes were reprocessed by mean-centering. The covariance dispersion matrix of absorbances was computed and the normalized eigenvalues and eigenvectors were calculated starting from this square matrix (covariance matrix). From each eigenvector, the principal component scores were determined and the calibration model was developed by the regression analysis. The procedure is indicated in the following steps.

The ordinary linear regression equation can be written as:

$$C = a + bA \quad (3)$$

where C is the concentration of analyte, a is the constant, b is the vector calculated in the product of the principal components and the C-loadings (q) as shown below:

$$b = Pq \quad (4)$$

where P is the matrix of eigenvectors. The columns of P are the selected eigenvectors corresponding to the appropriate eigenvalues (factors) for the regression. The vector q is called as C-loadings and determined by regression of C on T (matrix of scores):

$$q = DT^{\mathrm{T}}Y_{\mathrm{o}} \quad (5)$$

Here, D is a diagonal matrix, each diagonal element is equal to the inverse of each eigenvalue. The matrix of scores t_1 would be obtained by the following expression:

$$t_1 = A_{\mathrm{o}}P_1 \quad (6)$$

The mean-centered data ($A_i - A_{\mathrm{mean}}$ and $C_j - C_{\mathrm{mean}}$) can be shown as A_{o} and C_{o}. For one factor or factors, the constant (a) can be calculated in the following expression using the ordinary linear equation:

$$a = C_{\mathrm{mean}} - A_{\mathrm{mean}}^{\mathrm{T}}b \quad (7)$$

If the values obtained in each step are replaced in Eq. (3), the amount of analyte in sample can be calculated.

3.4. Partial least-squares regression

The calibration technique called as PLSR using the orthogonalized PLSR algorithm developed by Wold [11,12] is involved in the simultaneous use of the independent and the dependent variables in the data compression and decomposition operations. Because of this, the PLSR technique is more complex than the PCR technique. In books and papers, the PLSR algorithm developed by Wold was extensively discussed by Martens and Naes [15] and other authors. Other versions of the PLSR are the PLS2 regression and non-orthogonalized PLSR algorithms.

In the spectral study, the absorbance data (A) and concentration data (C) are mean centered to give data matrix A_o and vector C_o.

The following steps were used for the orthogonalized PLSR algorithm. The loading weight vector W_k was computed from:

$$W = A'_o C_o / C'_o C_o \tag{8}$$

The scores and loadings were obtained as:

$$t_1 = A_o W_1 \qquad P_1 = (A_o^T t_1)/(t_1^T t_1)$$

$$q_1 = (C_o^T t_1)/(t_1^T t_1) \tag{9}$$

The matrix and vector of residuals in A_o and C_o were estimated by:

$$A_1 = A_o - t_1 P_1^T \qquad C_1 = C_o - t_1 q_1^T \tag{10}$$

For the general linear equation, the regression coefficients were calculated by:

$$b = W(P^T W)^{-1} q \tag{11}$$

$$a = C_{mean} - A_{mean}^T b \tag{12}$$

As with PCR, the building calibration equation can be used for the estimation of the compounds in samples.

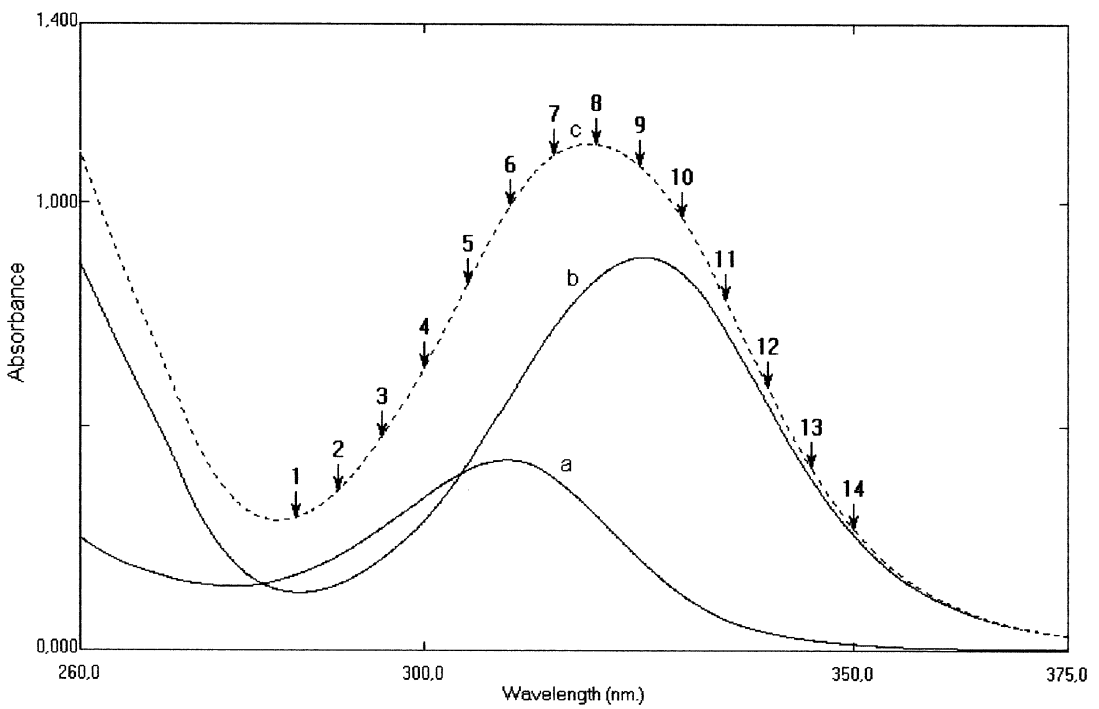

Fig. 1. Original absorption spectra of: (a) 5 μg/ml oxfendazole; (b) 25 μg/ml oxyclozanide; and (c) their mixture in 0.1 M NaOH ($\downarrow_1,\downarrow_2,\ldots,\downarrow_{14}$ corresponding to $\lambda_1,\lambda_2,\ldots,\lambda_{14}$ from 285 to 350 nm).

Table 1
Composition of a training set of standard synthetic mixtures containing two drugs

Standard no.	OXF (μg/ml)	OXC (μg/ml)
1	2.0	36.0
2	6.0	28.0
3	10.0	20.0
4	14.0	12.0
5	18.0	4.0
6	0.0	25.0
7	18.0	4.0
8	14.0	12.0
9	10.0	20.0
10	6.0	28.0
11	2.0	36.0
12	5.0	0.0

4. Results and discussion

The zero-order spectra of OXF, OXC and their mixture in 0.1 M NaOH are shown in Fig. 1. In order to build the four chemometric calibration, a training set was randomly prepared by using the standard mixture solution containing 0–18 μg/ml OXF and 0–36 μg/ml OXC in the variable proportions as shown in Table 1. The absorbances data matrix were obtained by measuring at the 14 wavelengths with the intervals of $\Delta\lambda = 5$ nm in the 285–350 nm spectral region. We observed that the solutions of two drugs in 0.1 M NaOH were soluble and remained stable during 24 h after their preparations. Linearity range was 2–18 μg/ml for OXF, 4–36 μg/ml for OXC in the multivariate calibrations proposed.

A calibration for each technique was computed in the MAPLE V software by using a training set consisting of two drugs and their absorbance data. The multivariate calibrations of four techniques were used to predict the unknown concentrations of OXF and OXC in the samples.

4.1. Classical least-squares

The regression coefficients (K) illustrated in Section 3 for CLS was calculated based on the training set and its absorbance data. By placing the K matrix and the absorbances measured at the 14 points for samples in the linear equation system, the following expressions were solved for estimating the concentration of OXF and OXC in samples.

$$\begin{bmatrix} A_1 \\ A_2 \\ A_3 \\ A_4 \\ A_5 \\ A_6 \\ A_7 \\ A_8 \\ A_9 \\ A_{10} \\ A_{11} \\ A_{12} \\ A_{13} \\ A_{14} \end{bmatrix} = \begin{bmatrix} 3.15\times10^{-2} & 5.52\times10^{-3} \\ 3.93\times10^{-2} & 6.23\times10^{-3} \\ 5.03\times10^{-2} & 8.45\times10^{-3} \\ 6.25\times10^{-2} & 1.19\times10^{-2} \\ 7.26\times10^{-2} & 1.68\times10^{-2} \\ 7.54\times10^{-2} & 2.28\times10^{-2} \\ 6.67\times10^{-2} & 2.88\times10^{-2} \\ 4.95\times10^{-2} & 3.31\times10^{-2} \\ 3.08\times10^{-2} & 3.50\times10^{-2} \\ 1.59\times10^{-2} & 3.35\times10^{-2} \\ 6.93\times10^{-3} & 2.85\times10^{-2} \\ 2.97\times10^{-3} & 2.18\times10^{-2} \\ 1.36\times10^{-3} & 1.54\times10^{-2} \\ 6.78\times10^{-4} & 1.03\times10^{-2} \end{bmatrix} \begin{bmatrix} C_{\mathrm{OXF}} \\ C_{\mathrm{OXC}} \end{bmatrix}$$

Here A is the absorbance values at 14 points corresponding to the 285–350 nm spectral range and C_{OXF} and C_{OXC} are the concentrations of OXF and OXC, respectively.

4.2. Inverse least-squares

As explained in the CLS technique, the regression coefficient matrix (P) mentioned in the multivariate calibration–prediction techniques for ILS technique was computed by means of the training set and its absorbance data for 14 points in the wavelength range of 285–350 nm. When the calculated P matrix was replaced in the expression below, the following expression was obtained:

$$\begin{bmatrix} C_{OXF} \\ C_{OXC} \end{bmatrix} =$$

$$\begin{bmatrix} 1.71 & 2.19 & 2.76 & 3.30 & 3.51 & 3.11 & 1.85 & 0.16 & -1.39 & -2.31 & -2.44 & -2.04 & -1.50 & -1.02 \\ -1.65 & -2.25 & -2.74 & -3.01 & -2.61 & -1.18 & 1.48 & 4.51 & 6.99 & 8.10 & 7.57 & 6.04 & 4.34 & 2.93 \end{bmatrix}$$

$$\begin{bmatrix} A_1 \\ A_2 \\ A_3 \\ A_4 \\ A_5 \\ A_6 \\ A_7 \\ A_8 \\ A_9 \\ A_{10} \\ A_{11} \\ A_{12} \\ A_{13} \\ A_{14} \end{bmatrix}$$

In this expression, C_{OXF} and C_{OXC} are the concentrations of OXF and OXC, respectively. The prediction of unknown concentrations of OXF and OXC in samples realized with the resolution of the above system.

Here the ILS technique is more advantageous than the CLS technique because the resolution of the linear equation system is directly given by the concentration of the compound analyzed in sample. This is a gain for the ILS technique from time point of view.

4.3. Principal component regression

The PCR calibration was constructed by using the PCR algorithm as explained in the multivariate calibration models. The calibration for two drugs was given as:

$$\begin{aligned} C_{OXF} = & -0.33 + 1.66A_1 + 2.15A_2 + 3.11A_3 \\ & + 3.20A_4 + 3.43A_5 + 3.04A_6 + 1.82A_7 \\ & + 0.21A_8 - 1.29A_9 - 2.18A_{10} - 2.32A_{11} \\ & - 1.93A_{12} - 1.41A_{13} - 0.97A_{14} \\ C_{OXC} = & -1.17 - 1.46A_1 - 2.03A_2 - 3.57A_3 \\ & - 2.68A_4 - 2.24A_5 - 0.83A_6 + 1.77A_7 \\ & + 4.68A_8 + 7.04A_9 + 8.07A_{10} + 7.50A_{11} \\ & + 5.98A_{12} + 4.30A_{13} + 2.91A_{14} \end{aligned}$$

where C_{OXF} and C_{OXC} are the concentrations of OXF and OXC, respectively.

The absorbance measured at $\lambda_1,\lambda_2,\lambda_3,\ldots,\lambda_{14}$ in the 285–350 nm spectral region for samples were replaced in the above equation and the content of each drugs in samples was computed.

Table 2
Results obtained for OXF and OXC in different synthetic mixtures by using the proposed techniques

Mixtures added (μg/ml)		Recovery (%)							
		CLS		ILS		PCR		PLSR	
OXF	OXC	OXF	OXC	OXF	OXC	OXF	OXC	OXF	OXC
2.0	25.0	102.0	99.6	102.5	101.6	102.0	101.9	101.5	102.0
6.0	25.0	101.6	101.8	102.8	102.2	101.7	101.2	101.0	102.4
10.0	25.0	99.1	102.0	99.1	102.1	100.0	102.7	100.0	102.7
14.0	25.0	100.0	101.0	100.0	100.7	100.7	102.5	100.7	102.5
18.0	25.0	98.9	101.9	98.9	101.5	99.6	102.0	99.6	102.4
5.0	4.0	102.8	102.0	102.0	100.7	102.4	101.4	102.8	103.0
5.0	12.0	102.6	99.1	102.8	99.0	101.0	97.7	102.6	98.0
5.0	20.0	101.0	100.6	100.8	101.3	102.0	99.9	102.0	99.9
5.0	28.0	102.0	101.1	101.8	100.8	101.0	100.4	101.2	100.4
5.0	36.0	98.0	100.8	101.0	100.9	102.5	101.1	102.8	101.1
Mean		100.8	101.0	101.2	101.0	101.3	101.1	101.4	101.4
RSD[a]:		1.68	1.00	1.01	0.90	0.98	1.46	1.12	1.57

[a] RSD: relative standard deviation.

4.4. Partial least-squares regression

The PLSR calibration for both drugs were constructed by using the orthogonalized PLSR algorithm and were given as:

$$C_{OXF} = -0.33 + 1.68A_1 + 2.16A_2 + 2.97A_3 + 3.23A_4 + 3.45A_5 + 3.06A_6 + 1.85A_7 + 0.21A_8 - 1.29A_9 - 2.19A_{10} - 2.33A_{11} - 1.94A_{12} - 1.43A_{13} - 0.97A_{14}$$

$$C_{OXC} = -1.18 - 1.48A_1 - 2.04A_2 - 3.45A_3 - 2.70A_4 - 2.27A_5 - 0.85A_6 + 1.74A_7 + 4.66A_8 + 7.04A_9 + 8.08A_{10} + 7.52A_{11} + 5.99A_{12} + 4.31A_{13} + 2.92A_{14}$$

Here C_{OXF} and C_{OXC} are the concentrations of OXF and OXC, respectively. The absorbances values measured at 14 points corresponding to 285–350 nm in the zero-order spectra were replaced in the above equation systems. The amount of OXF and OXC in samples was calculated.

In the application of four multivariate techniques to the synthetic mixtures containing two drugs in variable compositions, the mean recoveries and the relative standard deviations for CLS, ILS, PCR and PLSR were found to be 100.8 and 1.68%, 101.2 and 1.01%, 101.3 and 0.98% and 101.4 and 1.12%, respectively for OXF and 101.0 and 1.00%, 101.0 and 0.90%, 101.1 and 1.46% and 101.4 and 1.57%, respectively for OXC (Table 2).

Comparison of the spectra of OXF and OXC in standard and drug formulation solutions showed that the wavelength of maximum absorbances in the absorption spectra did not change. It has been decided that excipients placed in the veterinary formulation selected (magnesium stearate, corn starch and tricalcium phosphate in Bolus®) per bolus did not interfere with the quantitation of OXF and OXC in these techniques.

4.5. Statistical parameters

Some statistical parameters were given for the validation of the constructed calibrations for the training set and the synthetic binary mixtures of both drugs.

Table 3
Statistical parameters in the calibration–prediction

Parameter	Technique	OXF	OXC
SEP	CLS	0.269	0.311
	ILS	0.109	0.397
	PCR	0.264	0.404
	PLSR	0.094	0.452
PRESS	CLS	0.852	0.983
	ILS	0.343	1.255
	PCR	0.836	1.280
	PLSR	0.298	1.428
r	CLS	1.000	0.999
	ILS	1.000	1.000
	PCR	1.000	0.999
	PLSR	1.000	0.999
Intercept	CLS	0.132	−0.050
	ILS	0.165	−0.075
	PCR	0.117	−0.178
	PLSR	0.132	−0.104
Slope	CLS	0.985	1.012
	ILS	0.983	1.015
	PCR	0.992	1.020
	PLSR	0.991	1.019
RSD	CLS	0.181	0.0165
	ILS	0.185	0.175
	PCR	0.198	0.161
	PLSR	0.190	0.170

The application ability of a calibration model can be explained in various ways. The most general expression is the standard error of prediction (SEP), which is given by the following formula (13).

$$\mathrm{SEP} = \sqrt{\frac{\sum_{i=1}^{n} (C_i^{\mathrm{Added}} - C_i^{\mathrm{Found}})^2}{n-1}} \quad (13)$$

where C_i^{Added} is the added concentration of drug, C_i^{Found} is the found concentration of drug and n is the total number of the synthetic mixtures.

Another statistical parameter is the prediction residual error sum-of-squares (PRESS) in the calibration step. The PRESS value can be computed by using the following formula (14).

$$\mathrm{PRESS} = \sum_{i=1}^{n} (C_i^{\mathrm{Added}} - C_i^{\mathrm{Found}})^2 \quad (14)$$

According to the added concentration and the concentration found in the samples, the SEP ($n = 10$, synthetic mixture solution) and PRESS ($n = 12$, calibration mixture solution) values of CLS, ILS, PCR and PLSR techniques were calculated as 0.269 and 0.852, 0.109 and 0.343, 0.264 and 0.836, and 0.094 and 0.298 for OXF, respectively, and 0.311 and 0.983, 0.397 and 1.255, 0.404 and 1.280, and 0.451 and 1.428 for OXC, respectively (Table 3).

The linear regression analysis of the added concentration and the concentration found in the synthetic mixtures were realized for each drug and for each calibration technique. In this regression analysis, the correlation coefficient (r), intercept, slope and relative standard deviation (RSD) values were found satisfactory for the proposed multivariate calibration techniques as summarized in Table 3.

In this case, the detection of limit for CLS, ILS, PCR and PLSR calibrations was 0.95 μg/ml, 1.1 g/ml, 1.0 μg/ml and 0.91 μg/ml, whereas the limit of quantification was linear in the range of 2–18 μg/ml for OXF and 4–36 μg/ml for OXC, respectively.

With respect to the cross-validation procedure, the first two factors and one factor were used for the PCR and PLSR calibrations, respectively.

Table 4
Assay results for the veterinary formulation (mg/tablet)

Drug	CLS	ILS	PCR	PLS
OXF				
Mean ± SD	109.6 ± 1.06	109.6 ± 2.06	111.0 ± 1.17	110.0 ± 1.81
OXC				
Mean ± SD	593.3 ± 5.44	593.8 ± 5.70	598.5 ± 6.11	599.2 ± 5.42

Results obtained are average of eight experiments for each technique; SD: standard deviation.

Table 5
ANOVA table for the assay results obtained in application of the multivariate calibration–prediction techniques to veterinary formulation

	Among group		Within group		Total variation	
	OXF	OXC	OXF	OXC	OXF	OXC
Sum of squares	10.88	231.16	70.40	902.63	81.28	1133.79
Degrees of freedom	3	3	28	28	31	31
Mean squares	3.63	77.05	2.51	32.24		
F-test	1.45 ($F_{theor} = 2.95$) ($P = 0.05$)	2.39 ($F_{theor} = 2.95$) ($P = 0.05$)				

All the computed statistical values indicated that both the proposed techniques are suitable for the spectral simultaneous determination of two drugs in the samples.

A summary of the assay results for the commercial veterinary formulation is given in Table 4. We observed that the results obtained by the proposed calibration models are very close to each other.

The precision of four techniques was estimated by one-way analysis of variance (ANOVA) of four sets of ten sub-samples for each drug in veterinary formulation. For this estimation, Snedeccor's F values were computed and compared with the standard tabulated values using a significance level of $P = 0.05$. The same computation process was repeated for the other drug. From the standard table, for $n_1 = 3$ and $n_2 = 28$, and with a 5% level of significance, the value of F is given as 2.95. The calculated (experimental) F values did not exceed the tabulated value of F in ANOVA, indicating that there was no significant difference between the chemometric techniques as shown in Table 5.

5. Conclusions

Inspite of the interference of the original absorption spectra of OXF and OXC in the wavelength range of 260–375 nm, the multivariate calibration techniques mentioned here were applied to the spectrophotometric analysis of the veterinary formulation and the synthetic mixtures. We observed that the assay results of the multivariate techniques obtained by us are comparable with the HPLC [8] and the spectrophotometric [9] methods for the determination of OXF and OXC in the same mixture. In addition, the multivariate calibrations do not require any pretreatment such as a priori separation step as used in HPLC, a derivation of spectrum as used in derivative spectrophotometry, and a derivation and a division of the spectra as used in ratio spectra derivative spectrophotometry described in the above literature. We observed that the linear range of our techniques is larger than the HPLC method [8].

Although the techniques suggested by us for the determination of two drugs are more rapid, easy, cheap, precise and accurate than the methods given in the literature, the multivariate calibrations are required only for data processing with powerful software as well as the manipulations of the abstract vector space and its applications to the regression analysis.

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

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