

SIMPLE MATHEMATICAL RESOLUTION FOR BINARY MIXTURE OF OXFENDAZOLE AND OXYCLOZANIDE IN BOLUS BY BIVARIATE AND MULTIVARIATE CALIBRATIONS BASED ON THE LINEAR REGRESSION FUNCTIONS

Erdal DİNÇ^{a*}, Dumitru BĂLEANU^{b,c} and Ferdi TOKAR^a

^aDepartment of Analytical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandoğan, Ankara, Turkey

^bDepartment of Mathematics and Computer Sciences, Faculty of Arts and Sciences, Çankaya University, 06530 Ankara, Turkey

^cNational Institute for Laser, Plasma and Radiation, Physics, Institute of Space Sciences, Măgurele-Bucharest, P.O. Box, MG-23, R 76911, Roumania

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Two simple mathematical approaches were proposed for the simultaneous determination of oxfendazole (OXF) and oxyclozanide (OXY) in their synthetic binary mixtures and commercial veterinary formulation without requiring a priori separation step. One of these mathematical calibration techniques is called the bivariate calibration (BSC) based on four linear regression functions obtained at the wavelengths (230nm and 305 nm) with two single-component regression equations for each compound. The selection of the above mentioned wavelengths was performed by using the Kaiser technique. In the second mathematical approach, the multivariate spectral calibration (MSC) was constructed by a combined use of 42 linear regression functions consisting of 21 single linear regression equations for each compound at 21-wavelength set in between 230 and 330 nm with intervals of $\Delta\lambda=5\text{nm}$. These two mathematical approaches were validated and applied to the quantitative analysis of the commercial veterinary formulation containing OXF and OXY. In this study successful results for the synthetic and real samples were reported.

INTRODUCTION

Nowadays, various chemometric resolutions have been proposed for the qualitative and quantitative analysis of mixtures of two or more compounds and other analytical problems such as de-noising and baseline correction.¹⁻⁶ These chemometric approaches have been applied to spectrophotometric,⁷⁻¹¹ chromatographic¹¹ and electrochemical¹² data having in mind to perform the quantitative analysis of complex samples. Particularly, some of the chemometric calibrations for sample analysis require data processing with additional powerful software using various mathematical manipulations, such as the abstract vector decomposition procedure, to construct the calibrations. Therefore, one of the main problems for an analytical chemist is to understand the complexity of the abstract mathematical theories used in chemometrics. In the quantitative spectral resolution of mixtures, classical derivative method

and its modified versions have been intensively applied but this derivative technique may not give desirable results in same case. Namely, the application of the higher order derivative diminishes the peak amplitudes with decreasing the signal noise-to-noise ratio and it is difficult to find an optimal zero-crossing point to obtain calibration graph. As it is known, the application of the derivative method to the analysis of ternary mixture is not possible for all cases.¹³⁻¹⁵ For the quantitative analysis of mixtures, HPLC and its modified versions based on a separation step procedure were applied to sample analysis. These analytical methods require a separation step, long periods of time and they possess complex components. In addition of that, the above chromatographic method may not provide good results in some particular cases.

For the spectrophotometric quantitative resolution of two-component mixtures, the bivariate calibration method was proposed by

* Corresponding author. Tel.: +90 312 215 4886; Fax: +90 312 213 1081; E-mail: dinc@pharmacy.ankara.edu.tr

López-de-Alba and co-workers.¹⁶⁻¹⁸ Based on this approach a new method based on the simultaneous use of multi-linear regression functions, was elaborated in¹⁸ and applied in.¹⁹⁻²²

The combined use of OXF–OXY is important in the veterinary practice and it is used as the anthelmintic drugs. Several techniques, namely the derivative and ratio-spectra derivative spectrophotometry,²³ chemometric calibration techniques,²⁴ HPLC²⁵⁻²⁶ and the continuous wavelet transform²⁷ have been used to performe the quantitation of OXF and OXY in the samples.

The aim of this study is to develop simple mathematical approaches to the simultaneous determination of OXF and OXY in their binary mixtures. These new mathematical BSC and MSC methods are based on four linear regression functions obtained at the selected two wavelengths (230 nm and 305 nm) and 42 linear regression functions obtained at 21 wavelength points in the spectral range of 230 and 330 nm with intervals of $\Delta\lambda=5\text{nm}$ respectively. The validation of two mathematical approaches was performed by analyzing various synthetic mixtures of OXF and OXY and by using the standard addition technique.

A commercial veterinary formulation was analyzed by using the applied methods and the obtained results show a good agreement with the ones obtained by other literature methods.

THEORETICAL APPROACH

1. Bivariate spectral calibration method

For a mixture of X and Y, the mathematical relation between the concentrations and their corresponding absorbance values at a two-wavelength set can be given below

$$\begin{aligned} A_{mix_1} &= b_{X_1}C_X + b_{Y_1}C_Y + a_{XY_1} \\ A_{mix_2} &= b_{X_2}C_X + b_{Y_2}C_Y + a_{XY_2} \end{aligned} \quad (1)$$

Here A_{mix_1} and A_{mix_2} denote the absorbances of the mixture of X and Y analytes at the two-wavelength set, b_{X_1} , b_{X_2} and b_{Y_1} , b_{Y_2} represent the slopes of linear regression equations of X, and Y, and a_{XY_1} and a_{XY_2} are the sums of intercepts of linear regression equations at the two wavelengths.

In the matrix form (1) is given by

$$(A_{mix} - a_{XY})_{2 \times 1} = K_{2 \times 2} * C_{2 \times 1} \quad (2)$$

where $K = \begin{bmatrix} b_{X_1} & b_{Y_1} \\ b_{X_2} & b_{Y_2} \end{bmatrix}$ and $C_{2 \times 1}$ represents the concentration matrices

As a result we obtain

$$C_{2 \times 1} = (K^{-1})_{2 \times 2} * (A_{mix} - a_{XY})_{2 \times 1} \quad (3)$$

The above presented calibration model can be applied successfully to the resolution of binary mixtures. The selection of optimal wavelength set represents the important role for the application of this mathematical procedure to a binary mixture analysis.

In order to obtain the best sensitivity and selectivity in the application of the method, Kasier's technique was applied to the selection of the optimum wavelength set. The sensitivity matrices K are formed by considering every pairs of pre-selected wavelengths for binary mixtures.

The sensitivity of a two-component mixture analysis is defined as the absolute value of the determinant of the sensitivity matrix K. The calculated maximum determinant value permits to decide the optimum wavelength set. The method is based on use the linear regression lines for each compound at two selected wavelengths.

According to the Kaiser technique²⁸⁻²⁹ the selection of the best wavelengths pair for the BSC construction was obtained from the absolute values of the determinant of the sensitivity matrices. This calculation can be done by using the following formula

$$C_n^p = \frac{p!}{(p-n)!n!} \quad (4)$$

Here C_n^p represents the number of two pairs of sensitivity matrices, p is the number of wavelength and n is the number of the compounds.

2. Multivariate spectral calibration

If the absorbance values of a mixture of two analytes are measured at n wavelengths ($\lambda_i = 1, 2, \dots, n$), the following set of equations is obtained

$$\begin{aligned} A_{mix_1} &= b_{X_1}C_X + b_{Y_1}C_Y + a_{XY_1} \\ A_{mix_2} &= b_{X_2}C_X + b_{Y_2}C_Y + a_{XY_2} \\ &\dots \quad \dots \quad \dots \quad \dots \\ &\dots \quad \dots \quad \dots \quad \dots \\ A_{mix_n} &= b_{X_n}C_X + b_{Y_n}C_Y + a_{XY_n} \end{aligned} \quad (5)$$

where A_{mix1} , A_{mix2} , ..., and A_{mixn} are the absorbances of the mixture of X and Y analytes at selected wavelengths, b_{X1} , b_{X2} ... b_{Xn} and b_{Y1} , b_{Y2} ... b_{Yn} are the slopes of n linear regression equations of X and Y, corresponding to selected wavelengths, respectively and a_{XY1} , a_{XY2} , ,and a_{XYn} represent the sums of intercepts of linear regression equations at n wavelengths.

In the matrix form the above equation can be written as

$$(A_{mix} - a_{XYZ})_{nx1} = K_{nx2} * C_{2x1}, \quad (6)$$

where the matrix of the slope values is given by

$$K_{nx2} = \begin{bmatrix} b_{X1} & b_{Y1} \\ b_{X2} & b_{Y2} \\ \dots & \dots \\ \dots & \dots \\ b_{Xn} & b_{Yn} \end{bmatrix} \quad (7)$$

Finally, the concentration of the X and Y compounds in binary mixture can be calculated as:

$$C_{2x1} = [K_{nx2}]^{-1} (A_{mix} - a_{XY})_{nx1} \quad (8)$$

RESULTS AND DISCUSSION

In the application of BSC and MSC mathematical approaches, the absorption spectra of OXF and OXY in the linear concentration range of 2-20 μ g/mL and 4-22 μ g/mL were recorded and stored in the spectral region of 210-365 nm, respectively. The similar spectral registration procedure was also applied to the other sample solutions. The absorption spectra of OXF and OXY overlap in the same spectral region of 210-365 nm as it is shown in Fig. 1.

To eliminate the disadvantages of the graphical and chromatographic methods described in the introduction, two simple mathematical bivariate and multivariate calibration models were proposed for the simultaneous spectrophotometric determination of OXF and OXY in samples. In this study, the proposed BSC and MSC have simple mathematical content and they are simple to be applied for the analysis of both compounds.

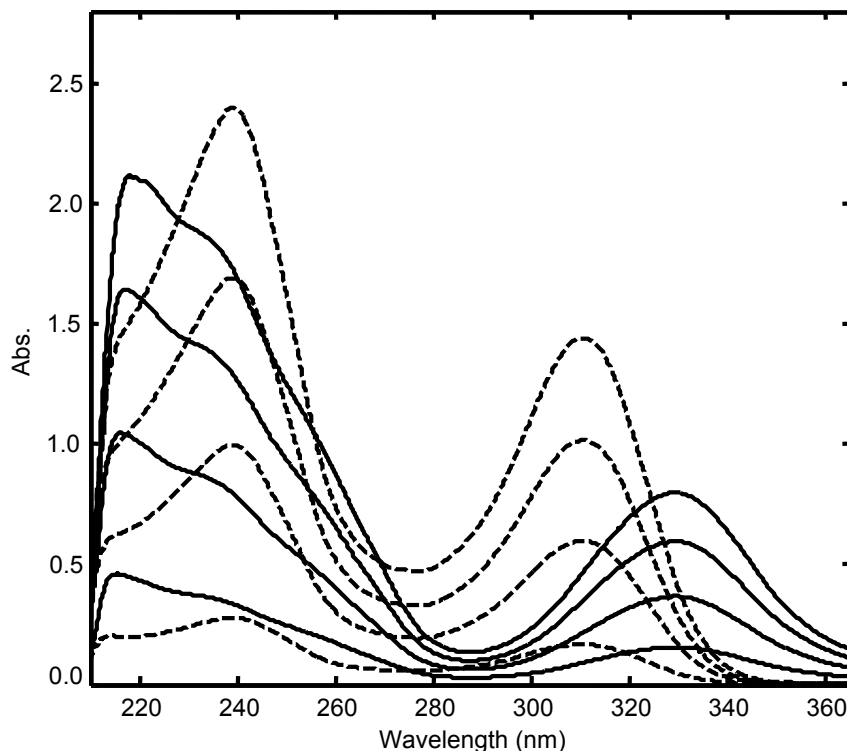


Fig. 1 – Absorption spectra of 2-20 μ g/mL OXF (....) and 4-22 μ g/mL OXY (—) in methanol 0.1 M NaOH (50:50, v/v).

1. Bivariate spectral calibration method

In our case the BSC model is based on the selection of the critical wavelengths which have higher sensitivities in the spectral range of 230-330 nm of OXF and OXY. For both compounds, 21 wavelengths with intervals of $\Delta\lambda=5$ nm in the above specified spectral range, were selected and a linear regression function at each wavelength point was obtained for each compound. The computed linear regression equations and their corresponding statistical results were summarized in Table 1. We observed that the correlation coefficients of OXF approach to 1.000, while those of OXY are smaller than 1.000 for the considered wavelengths. This may be considered as a model error between the ideal case and measurement conditions.

The sensitivity matrices were obtained from the slopes of the linear regression equations indicated in Table 1.

In the application of the BSC methods, an optimal two-wavelength set having the highest determinant value of the sensitivity matrices was found to be 230/305 wavelength pair as shown in Table 3. At the above specified two-wavelength set the individual linear regression equation for each compound were separately presented in Table 4. Based on the equation set presented in Table 4 the equation set corresponding to the BSC method was illustrated in Table 5. The BSC algorithm described in¹⁸⁻²² was applied to the equation set given in Table 5. The calculated BSC approach was used for the analysis of the synthetic mixtures and for the commercial veterinary formulation containing OXF and OXY compounds.

2. Multivariate calibration method

MSC approach is developed by using the analogy to the BSC method, but it contains in our specific case 21-wavelength procedure instead of two-wavelengths.¹⁸⁻²² Therefore, the 21-wavelengths set, as shown in Table 1, at the critical points, which correspond to the maximum, shoulder and minimum in the spectral range of 230-330 nm were used to build the MSC model for the analysis of OXF and OXY in their mixture.

As shown in Table 1, the 21 linear regression equations for each active compound were obtained by measuring the zero-order absorbance values at the wavelengths set. For the MSC approach, the equation set indicated in Table 6 was obtained from Table 1. The MSC algorithm introduced in¹⁸⁻²² was applied to the equation set shown in Table 6. The MSC approach here was used to the resolution of the binary mixture and bolus containing OXF and OXY.

3. Validation of the developed methods

In this study two proposed calibration models (BSC and MSC) were validated by analyzing the synthetic mixtures of OXF and OXY. To check the validity of the calibration models, the simultaneous resolution of the synthetic mixtures containing various concentrations of OXF and OXY was performed by the BSC and MSC. The mean recoveries and the relative standard deviations of the methods were summarized in Table 7. Their numerical values were found satisfactory to validate the accuracy and precision of all calibration methods. The standard addition technique was applied to observe the effects of the bolus excipients in the analysis of the veterinary formulation and no interference effect was reported. Recoveries and corresponding relative standard deviations were presented in Table 8. The results for each compound were obtained in the average of five replicates, which are repeated three times.

4. Analysis of the commercial veterinary formulation

The determination results of commercial veterinary formulation were depicted in Table 8. It was reported that the results of all methods were very close to each other as well as to the label value of commercial veterinary formulation. In addition of this, the statistic parameters indicate that the applied methods are suitable to determine the two compounds in a commercial veterinary formulation.

Table 1

Linear regression analysis and corresponding statistical results

OXF									OXY							
(nm)	m	n	r	SE(m)	SE(n)	SE(r)	LOD	LOQ	m	n	r	SE(m)	SE(n)	SE(r)	LOD	LOQ
230	9.98 x10 ⁻²	4.44 x10 ⁻²	1.0000	4.43 x10 ⁻⁴	5.70 x10 ⁻³	5.94 x10 ⁻³	0.30	0.99	8.56 x10 ⁻²	3.76E-02	0.9997	1.53E-03	2.24 x10 ⁻²	2.06 x10 ⁻²	1.36	4.54
235	1.12x10 ⁻¹	4.79 x10 ⁻²	1.0000	4.77 x10 ⁻⁴	6.15 x10 ⁻³	6.40 x10 ⁻³	0.29	0.95	8.29 x10 ⁻²	3.16E-02	0.9997	1.37E-03	2.00 x10 ⁻²	1.84 x10 ⁻²	1.25	4.18
240	1.17x10 ⁻¹	4.73 x10 ⁻²	1.0000	4.40 x10 ⁻⁴	5.67 x10 ⁻³	5.90 x10 ⁻³	0.25	0.84	7.60 x10 ⁻²	2.80E-02	0.9998	1.19E-03	1.73 x10 ⁻²	1.59 x10 ⁻²	1.18	3.95
245	1.04x10 ⁻¹	4.26 x10 ⁻²	1.0000	4.15 x10 ⁻⁴	5.35 x10 ⁻³	5.57 x10 ⁻³	0.27	0.89	7.60 x10 ⁻²	2.80E-02	0.9998	1.19E-03	1.73 x10 ⁻²	1.59 x10 ⁻²	1.18	3.95
250	7.98x10 ⁻²	3.37 x10 ⁻²	1.0000	2.83 x10 ⁻⁴	3.64 x10 ⁻³	3.79 x10 ⁻³	0.24	0.79	5.61 x10 ⁻²	2.49E-02	0.9998	8.59E-04	1.26 x10 ⁻²	1.15 x10 ⁻²	1.16	3.88
255	5.42 x10 ⁻²	2.34 x10 ⁻²	1.0000	2.40 x10 ⁻⁴	3.09 x10 ⁻³	3.21 x10 ⁻³	0.30	0.99	4.80 x10 ⁻²	2.25E-02	0.9998	7.42E-04	1.08 x10 ⁻²	9.95E-03	1.18	3.92
260	3.61 x10 ⁻²	1.63 x10 ⁻²	1.0000	1.93 x10 ⁻⁴	2.48 x10 ⁻³	2.59 x10 ⁻³	0.36	1.19	3.92 x10 ⁻²	1.84E-02	0.9998	5.72E-04	8.37E-03	7.68E-03	1.11	3.70
265	2.81 x10 ⁻²	1.29 x10 ⁻²	0.9999	2.20 x10 ⁻⁴	2.83 x10 ⁻³	2.95 x10 ⁻³	0.52	1.75	2.97 x10 ⁻²	1.54E-02	0.9998	4.59E-04	6.71E-03	6.16E-03	1.17	3.91
270	2.44 x10 ⁻²	1.14 x10 ⁻²	0.9999	2.06 x10 ⁻⁴	2.66 x10 ⁻³	2.77 x10 ⁻³	0.57	1.89	2.11 x10 ⁻²	1.14E-02	0.9998	2.65E-04	3.87E-03	3.55E-03	0.96	3.18
275	2.31 x10 ⁻²	1.06 x10 ⁻²	0.9999	1.91 x10 ⁻⁴	2.46 x10 ⁻³	2.56 x10 ⁻³	0.55	1.84	1.27 x10 ⁻²	8.26E-03	0.9998	1.90E-04	2.78E-03	2.55E-03	1.13	3.78
280	2.36 x10 ⁻²	1.06 x10 ⁻²	0.9999	1.79 x10 ⁻⁴	2.31 x10 ⁻³	2.40 x10 ⁻³	0.51	1.70	7.92E-03	5.90E-03	0.9998	1.10E-04	1.60E-03	1.47E-03	1.05	3.50
285	2.73 x10 ⁻²	1.24 x10 ⁻²	1.0000	1.75 x10 ⁻⁴	2.26 x10 ⁻³	2.35 x10 ⁻³	0.43	1.44	6.05E-03	5.19E-03	0.9998	8.86E-05	1.30E-03	1.19E-03	1.11	3.71
290	3.34 x10 ⁻²	1.48 x10 ⁻²	1.0000	1.51 x10 ⁻⁴	1.94 x10 ⁻³	2.02 x10 ⁻³	0.30	1.01	6.07E-03	4.89E-03	0.9998	8.63E-05	1.26E-03	1.16E-03	1.08	3.60
295	4.26 x10 ⁻²	1.75 x10 ⁻²	1.0000	1.63 x10 ⁻⁴	2.10 x10 ⁻³	2.19 x10 ⁻³	0.26	0.85	7.53E-03	4.95E-03	0.9998	1.00E-04	1.47E-03	1.35E-03	1.02	3.38
300	5.45 x10 ⁻²	2.29 x10 ⁻²	1.0000	2.11 x10 ⁻⁴	2.72 x10 ⁻³	2.83 x10 ⁻³	0.26	0.86	1.04E-02	4.52E-03	0.9998	1.48E-04	2.16E-03	1.98E-03	1.08	3.59
305	6.57 x10 ⁻²	2.66 x10 ⁻²	1.0000	2.73 x10 ⁻⁴	3.52 x10 ⁻³	3.67 x10 ⁻³	0.28	0.93	1.48E-02	5.67E-03	0.9997	2.42E-04	3.54E-03	3.24E-03	1.24	4.14
310	7.06 x10 ⁻²	2.78 x10 ⁻²	1.0000	2.89 x10 ⁻⁴	3.72 x10 ⁻³	3.87 x10 ⁻³	0.27	0.91	2.06E-02	6.22E-03	0.9998	2.72E-04	3.98E-03	3.65E-03	1.00	3.35
315	6.67 x10 ⁻²	2.60 x10 ⁻²	1.0000	2.82 x10 ⁻⁴	3.63 x10 ⁻³	3.78 x10 ⁻³	0.28	0.94	2.64E-02	7.13E-03	0.9998	3.80E-04	5.55E-03	5.09E-03	1.09	3.65
320	5.31 x10 ⁻²	2.18 x10 ⁻²	1.0000	2.24 x10 ⁻⁴	2.89 x10 ⁻³	3.01 x10 ⁻³	0.28	0.94	3.14E-02	8.93E-03	0.9997	5.13E-04	7.51E-03	6.89E-03	1.24	4.15
325	3.55 x10 ⁻²	1.42 x10 ⁻²	1.0000	1.60 x10 ⁻⁴	2.06 x10 ⁻³	2.15 x10 ⁻³	0.30	1.01	3.49E-02	1.01E-02	0.9997	5.55E-04	8.12E-03	7.45E-03	1.21	4.04
330	1.93 x10 ⁻²	7.88 x10 ⁻³	1.0000	1.30 x10 ⁻⁴	1.67 x10 ⁻³	1.74 x10 ⁻³	0.45	1.50	3.60E-02	1.11E-02	0.9998	5.51E-04	8.06E-03	7.39E-03	1.16	3.88

SE(m): Standard error of slope, SE(n): Standard error of intercept, SE(r): Standard error of regression constant, r: Regression coefficient, LOD: Limit of detection, LOQ: Limit of quantitation

Table 3

Individual linear regression equations at the selected two wavelength set

λ (nm)	OXF	OXY
230	$A = 9.98 \times 10^{-2} \times C + 4.44 \times 10^{-2}$	$A = 8.56 \times 10^{-2} \times C + 3.76 \times 10^{-2}$
305	$A = 6.57 \times 10^{-2} \times C + 2.66 \times 10^{-2}$	$A = 1.48 \times 10^{-2} \times C + 5.67 \times 10^{-3}$

Table 4

Equation set for the bivariate calibration

λ (nm)	Corresponding equation
230	$A_{\text{mix1}} = 9.98 \times 10^{-2} \times C_{\text{OXF}} + 8.56 \times 10^{-2} \times C_{\text{OXY}} + 8.20 \times 10^{-2}$
305	$A_{\text{mix2}} = 6.57 \times 10^{-2} \times C_{\text{OXF}} + 1.48 \times 10^{-2} \times C_{\text{OXY}} + 3.22 \times 10^{-2}$

Table 5

Equation set for the multivariate calibration

λ (nm)	Corresponding equations
230	$A_{k1} = 9.98 \times 10^{-2} C_{\text{OXF}} + 8.56 \times 10^{-2} C_{\text{OXY}} + 8.20 \times 10^{-2}$
235	$A_{k2} = 1.12 \times 10^{-1} C_{\text{OXF}} + 8.29 \times 10^{-2} C_{\text{OXY}} + 7.95 \times 10^{-2}$
240	$A_{k3} = 1.17 \times 10^{-1} C_{\text{OXF}} + 7.60 \times 10^{-2} C_{\text{OXY}} + 7.53 \times 10^{-2}$
245	$A_{k4} = 1.04 \times 10^{-1} C_{\text{OXF}} + 7.60 \times 10^{-2} C_{\text{OXY}} + 7.06 \times 10^{-2}$
250	$A_{k5} = 7.98 \times 10^{-2} C_{\text{OXF}} + 5.61 \times 10^{-2} C_{\text{OXY}} + 5.86 \times 10^{-2}$
255	$A_{k6} = 5.42 \times 10^{-2} C_{\text{OXF}} + 4.80 \times 10^{-2} C_{\text{OXY}} + 4.59 \times 10^{-2}$
260	$A_{k7} = 3.61 \times 10^{-2} C_{\text{OXF}} + 3.92 \times 10^{-2} C_{\text{OXY}} + 3.47 \times 10^{-2}$
265	$A_{k8} = 2.81 \times 10^{-2} C_{\text{OXF}} + 2.97 \times 10^{-2} C_{\text{OXY}} + 2.83 \times 10^{-2}$
270	$A_{k9} = 2.44 \times 10^{-2} C_{\text{OXF}} + 2.11 \times 10^{-2} C_{\text{OXY}} + 2.27 \times 10^{-2}$
275	$A_{k10} = 2.31 \times 10^{-2} C_{\text{OXF}} + 1.27 \times 10^{-2} C_{\text{OXY}} + 1.89 \times 10^{-2}$
280	$A_{k11} = 2.36 \times 10^{-2} C_{\text{OXF}} + 7.92 \times 10^{-3} C_{\text{OXY}} + 1.65 \times 10^{-2}$
285	$A_{k12} = 2.73 \times 10^{-2} C_{\text{OXF}} + 6.05 \times 10^{-3} C_{\text{OXY}} + 1.75 \times 10^{-2}$
290	$A_{k13} = 3.34 \times 10^{-2} C_{\text{OXF}} + 6.07 \times 10^{-3} C_{\text{OXY}} + 1.97 \times 10^{-2}$
295	$A_{k14} = 4.26 \times 10^{-2} C_{\text{OXF}} + 7.53 \times 10^{-3} C_{\text{OXY}} + 2.25 \times 10^{-2}$
300	$A_{k15} = 5.45 \times 10^{-2} C_{\text{OXF}} + 1.04 \times 10^{-2} C_{\text{OXY}} + 2.74 \times 10^{-2}$
305	$A_{k16} = 6.57 \times 10^{-2} C_{\text{OXF}} + 1.48 \times 10^{-2} C_{\text{OXY}} + 3.22 \times 10^{-2}$
310	$A_{k17} = 7.06 \times 10^{-2} C_{\text{OXF}} + 2.06 \times 10^{-2} C_{\text{OXY}} + 3.40 \times 10^{-2}$
315	$A_{k18} = 6.67 \times 10^{-2} C_{\text{OXF}} + 2.64 \times 10^{-2} C_{\text{OXY}} + 3.31 \times 10^{-2}$
320	$A_{k19} = 5.31 \times 10^{-2} C_{\text{OXF}} + 3.14 \times 10^{-2} C_{\text{OXY}} + 3.07 \times 10^{-2}$
325	$A_{k21} = 3.55 \times 10^{-2} C_{\text{OXF}} + 3.49 \times 10^{-2} C_{\text{OXY}} + 2.43 \times 10^{-2}$
330	$A_{k20} = 1.93 \times 10^{-2} C_{\text{OXF}} + 3.60 \times 10^{-2} C_{\text{OXY}} + 1.90 \times 10^{-2}$

Table 6

Recovery results obtained by application of the proposed mathematical methods to the synthetic mixtures

Binary mixture (µg/mL)		Found (µg/mL)				Recovery (%)			
		BSC		MSC		BSC		MSC	
OXF	OXY	OXF	OXY	OXF	OXY	OXF	OXY	OXF	OXY
2	20	2.00	19.93	1.91	19.54	100.0	99.6	95.6	97.7
8	20	8.10	19.76	8.07	19.62	101.2	98.8	100.9	98.1
14	20	14.32	18.87	14.25	19.05	102.3	94.4	101.8	95.2
20	20	20.66	19.38	20.40	19.95	103.3	96.9	102.0	99.8
10	4	10.13	3.80	10.13	3.89	101.3	94.9	101.3	97.3
10	10	9.76	9.90	9.76	9.73	97.6	99.0	97.6	97.3
10	16	9.71	15.68	9.71	15.49	97.1	98.0	97.1	96.8
10	22	10.01	21.61	9.95	21.75	100.1	98.2	99.5	98.8
Mean						100.4	97.5	99.5	97.6
SD						2.15	1.93	2.43	1.35
RSD						2.15	1.98	2.45	1.39

SD = standard deviation, RSD=relative standard deviation

Table 7

Recovery data obtained by standard addition technique

Actual Concentration (µg/mL)		Found (µg/mL)				Recovery (%)			
		BSC		MSC		BSC		MSC	
OXF	OXY	OXF	OXY	OXF	OXY	OXF	OXY	OXF	OXY
5	10	5.21	9.56	5.09	9.62	104.2	95.6	101.7	96.2
5	10	4.93	10.20	4.90	10.12	98.7	102.0	98.0	101.2
5	10	5.05	9.97	5.03	9.92	101.0	99.7	100.6	99.2
5	10	4.89	10.02	4.94	9.94	97.7	100.2	98.8	99.4
5	10	5.04	10.21	5.00	9.65	100.9	102.1	100.1	96.5
Mean						100.5	99.9	99.8	98.5
SD						2.50	2.64	1.48	2.09
RSD						2.49	2.64	1.48	2.12

Table 8

Experimental results obtained by application of the proposed mathematical methods to the commercial veterinary formulation

	mg/bolus			
	BSC		MSC	
	OXF	OXY	OXF	OXY
	308.52	588.96	304.50	594.84
	300.60	591.18	297.71	601.80
	308.55	587.61	304.41	592.92
	301.68	594.30	297.88	598.55
	298.96	599.37	297.58	598.86
Mean	303.7	592.3	300.4	597.4
SD	4.55	4.70	3.69	3.52
RSD	1.50	0.79	1.23	0.59
SE	1.72	1.78	1.39	1.33
CL (p=0.05)	3.37	3.48	2.73	2.60

Claim label: 300 mg OXF and 600 mg OXY per bolus
CL = Confidence limit

EXPERIMENTAL

1. Instruments

A Shimadzu UV-60 double beam UV-Vis spectrophotometer with a fixed slit width (2 nm) was connected to a computer loaded with Shimadzu UVPC software. LEXMARK E-320 printer has utilized to record the absorption spectra. After that, the absorption data were transformed into ASCII files and transferred to *EXCEL*. All data treatments, regressions and statistical analysis were calculated by using the *EXCEL* and *Matlab 7.0* software.

2. Commercial Veterinary Formulation

A commercial veterinary formulation (OKSAVET® bolus, Vilsan Pharm. Ind., Ankara Turkey) was analyzed by the proposed mathematical methods. Its content is given as follows: 300 mg OXF, 600 mg OXY per bolus. OXF and OXY compounds together with their commercial veterinary preparation were obtained as a kindly donation of Vilsan Pharm. Ind., Ankara (Turkey).

3. Standard Solutions

Stock solutions of 50 mg/ 100 mL for both compounds (OXY and OXF) were prepared in the solvent system consisting of 0.1 M NaOH and methanol (50:50, v/v). Calibration solutions for each drug in the linear concentration range of 2-20 µg/ mL OXF and 4-22 µg/mL OXY in the same solvent is obtained from the above stock solutions. The solutions of the synthetic mixtures containing OXF and OXY in the above working concentration ranges were used as a validation set for the proposed mathematical methods.

4. Sample Solutions Preparation

In the analysis of the commercial veterinary preparation, twenty boluses were accurately weighed and powdered in a mortar. An amount equivalent to half bolus containing OXF and OXY was dissolved in 100-mL calibration flask by using a solvent system consisting of 0.1 M NaOH and methanol (50:50, v/v). 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 obtained solution was diluted to the working concentration range for the application of the methods to the final sample solutions. This procedure was repeated five times.

CONCLUSIONS

In this study OXF and OXY show an overlapping absorption spectra in the working spectral range. As a result new simple and efficient method to analyze this binary mixture should be developed.

For these reasons we proposed two calibration models, namely BSC and MSC, and we successfully applied them for the simultaneous analysis of OXF and OXY compounds.

The developed methods do not require any graphical and separation procedure, are easy to apply and even very cheap.

Taking into consideration the results obtained for the analyzed binary mixture we strongly

recommend these new approaches to be used for the quality control and routine analysis of veterinary formulation containing OXF and OXY.

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