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Quantification of Danofloxacin and Difloxacin in Chicken Tissues in the Presence of Sarafloxacin As Interference

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A new spectrofluorimetric method has been developed for the quantification of danofloxacin (DANO) and difloxacin (DIFLO), in the presence of the primary metabolite of difloxacin, with sarafloxacin (SARA) as interference, in chicken tissue samples. The method is based on second-order multivariate calibration, applying parallel factor analysis (PARAFAC), to the excitation−emission matrices (EEMs) of these compounds. High overlapping of the signals and influence of matrix effects were observed. To solve the problem, the standard addition method was used. Chemical variables were optimized. The measured EEMs of the analytes, as analytical signals, allowed their quantification in chicken tissue samples. Solid phase extraction was used for the extraction of the analytes in real samples. The range of concentration examined varied from 30 to 100 ng g^{-1} for danofloxacin, and from 100 to 200 ng g^{-1} for difloxacin. Both analytes can be analyzed individually, and the binary mixture can be resolved, with recoveries comprising between 88.7 and 106.6%.

KEYWORDS: Parallel factor analysis; excitation−emission matrices; danofloxacin; difloxacin; chicken tissue

1. INTRODUCTION

Drugs belonging to the quinolone derivatives are well known and widely used because of their broad-spectrum activity against many pathogenic Gram-negative and Gram-positive bacteria. The use of quinolone in food-producing animals can generate microbial resistance; thus, the European Union has established maximum residue limits (MRL) for quinolone residues in animal tissues, and this is included in the Council Regulation 2377/90 (*1*). Danofloxacin (DANO) and difloxacin (DIFLO) are fluoroquinolones, in which the presence of the fluorine in the molecule greatly enhances its activity, and produces a rapid bactericidal effect. They are used in the treatment of respiratory disease in chickens, cattles, and pigs. Difloxacin could be demethylated, and this gave its main metabolite, sarafloxacin (SARA), which also displays potent antimicrobial activity. Both DIFLO and SARA could be used as individual antimicrobials, SARA being roughly twice as active as DIFLO (*2*). Only 2−4% of DIFLO is metabolized as SARA. The MRL for those analytes are 200 ng g^{-1} for DANO and 300 ng g^{-1} for DIFLO. Although SARA is not a regulated drug, the main interest in its determination arises in that it is a metabolite of difloxacin, which is a regulated drug.

Several separation methods have been developed for the determination of these analytes in tissue samples. Thus, high performance liquid chromatography (HPLC) using different detection systems, such as ultraviolet (UV) (*3*,*4*), fluorescence, (*5*) and mass spectrometry (*3*, *6*, *7*), has been used. Furthermore, another method using capillary electrophoresis (CE) with diode-array detection (DAD) has been developed for the resolution of quinolones with strongly overlapped peaks, with the aid of partial least-squares calibration (PLS-2) (*8*). Recently, mass spectrometry has been coupled with capillary electrophoresis (*9*) and ultra performance liquid chromatography (*10*) for the determination of those analytes in meat and urine, respectively.

Because of the strong matrix effects that are present in animal tissue samples, several procedures have been used for the extraction of the analytes and the cleanup of the samples. For instance, Hermo et al. (*11*) developed a comparative study of classical and microwave extraction techniques. The authors concluded that microwave energy is a good alternative for extraction and can be used in automatic systems for routine analysis. Other alternatives are the extraction with solid phase extraction (SPE) cartridges, which could be eluted by the use of acetonitrile and aqueous solution of trifluoroacetic acid (*4*,*6*). A review, published in 2002, summarizes the analysis of quinolone residues in edible animal products. It covers most of the methods described to date and the most relevant information about the corresponding analytical procedures (*12*).

The application of luminescent techniques to the analysis of complex mixtures is particularly attractive because of the high sensitivity that can be achieved. However, its selectivity is usually reduced by extensive spectral overlap. First-order multivariate calibration methods applied to fluorescence signals have been used extensively. Among the possible first-order regression methods, partial least-squares (PLS) is the most used (*13*, *14*). The principal disadvantage of these methods is that they are sensitive to the presence of unmodeled interferents, which are usually present in real samples. To solve this problem, second-order multivariate calibration methods can be used. Multiway partial least-squares (N-PLS) (*15*), the unfolded variant (U-PLS) (*16*), or

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parallel factor analysis (PARAFAC) (*17*), among others, are decomposition methods for multiway data obtained by recording excitation−emission fluorescence matrices. N-PLS and U-PLS have the disadvantage that they do not provide second-order advantage because they first decomposes the calibration set and then project the samples to be predicted.

PARAFAC is a decomposition method, which conceptually can be compared to bilinear principal component analysis. The decomposition is made into triads or trilinear components, and each component consists of one score vector and two loading vectors. An advantage of PARAFAC is the uniqueness of the solution. This means that the true underlying spectra will be found if the right number of components is used and the signal-to-noise ratio is appropriate (*18*). The decomposition of excitation−emission matrices (EEM) into three-dimensional arrays, by PARAFAC, exploits the second-order advantage (*19*). This advantage allows direct extraction of the spectral profiles as well as the relative concentrations of individual sample components (*20*). PARAFAC, in combination with excitation−emission matrices, has been applied to several samples, such as complex biological mixtures, *N*-phenylanthranilic derivatives, sulfathiazole in honey, doxorubicin in plasma, fluoroquinolones in human urine, and so forth (*19*−*23*).

However, the standard addition method is well known among analytical chemists. It is applied to univariate data as a means of overcoming matrix effects that change the analyte signal. An extension of the standard addition methods to multivariate data (for first-order instrumentation) is the generalized standard addition method (GSAM), in which the analyte and interferences are sequentially added to the sample (*24*). Another extension, for multiway data (for second-order instrumentation), is the second-order standard addition method (SOSAM) (*25*) which has the second-order advantage; thus, it can be used with PARAFAC and other methods that fulfill this condition.

Few references have been found in the literature to the application of three-way chemometric methods combined with SOSAM. For instance, SOSAM has been combined with trilinear decomposition method (TLD) in spectroelectrochemistry (*26*). Wu et al. (*27*) used alternating trilinear decomposition (ATLD) with SOSAM in HPLC. The results obtained were compared with those obtained by other SOSAM employing direct trilinear decomposition (DTLD) and PARAFAC. The ATLD method was slightly superior to the other methods. Sena et al. (*28*) determined salicylate in undiluted human plasma, by spectrofluorimetry, combining PARAFAC and SOSAM, trying to avoid the strong matrix effect caused by the quenching effect of the proteins present in the plasma. Also, Muñoz de la Peña et al. (*29*) used the standard addition method for the determination of flufenamic and meclofenamic acids in human urine samples in micelar media and for the determination of danofloxacin in milk samples (*30*).

To date, no second-order multivariate method applied to excitation−emission matrices has been developed, as far as we know for the individual determination of DANO and DIFLO, in the presence of metabolites (SARA) as interferents. For this reason, we focus our attention in this report on the use of the EEM and the second-order multivariate method PARAFAC, combined with the standard addition method, to determine individually those fluoroquinolones in chicken tissue samples. It is well known that modeling three-way data arrays is a convenient way of simultaneously measuring the individual concentration of an analyte in the presence of others which present overlapping spectra and also in the presence of uncalibrated interferents.

2. EXPERIMENTAL PROCEDURES

2.1. Apparatus. Fluorescence spectral measurements were performed on a Fluorescence Spectrophotometer Varian Model Cary Eclipse. The instrument consists of two Czerny-Turner monochromators (excitation and emission), a Xenon light source, and a range of fixed width, selectable slits, selectable filters, attenuators, and two photomultiplier tubes as detectors. The fluorimeter is connected to a PC microcomputer via an IEE serial interface. The Cary Eclipse Version 1.0 software was used for data acquisition, data interpretation, and graphical display. All measurements were performed in 10 mm quartz cells at 20 °C, by use of a thermostatic cell holder and a Selecta Model Frigiterm thermostatic bath.

2.2. Software. All calculations were done using MATLAB 5.3. (*31*). Appropriate routines for applying PARAFAC, developed by Bro, are available on the Internet (*32*), although a useful MATLAB graphical interface was developed by Olivieri (*33*) for easy data manipulation and graphics presentation. This interface allows selecting appropriate recording spectral regions, optimizing the number of factors, and plotting emission and excitation spectral profiles and pseudounivariate calibration graphs.

2.3. Reagents. Stock 2.5×10^{-3} M solutions of danofloxacin (Riedel de-Häen), sarafloxacin hydrochloride (Riedel de-Häen), and difloxacin hydrochloride (Fort Dodge Veterinaria S.A., Girona, Spain) were prepared in 50-mL volumetric flasks, by weighing the appropriate amount and dissolving in 0.5% aqueous acetic acid. These solutions were stored in dark bottles at 4 °C, remaining stable for at least 1 month. Working solutions were prepared by appropriate dilution of the stock solutions with Milli-Q water. Isolute Env+ cartridges (International Sorbent Technology, U.K., www.ist-spe.com) were used for solid-phase extraction.

2.4. Spectrofluorimetric Studies. No references have been found in the literature about the fluorescent characteristics of the fluoroquinolones under study; for this reason, previous studies were performed.

First of all, the fluorescence characteristic of the analytes were studied using several solvents, such as, water, methanol (MeOH), ethanol (EtOH), and acetonitrile (ACN). The maximum fluorescence signal was observed in water, ACN, and MeOH, for DANO, SARA, and DIFLO, respectively. Water was chosen as the working medium, in order to simplify the preparation of the samples and taking into account that the three analytes showed a good fluorescence signal in this solvent.

Second, the influence of pH on fluorescence intensity was studied in the range comprised between 2 to 12. It could be observed in all cases that the maximum fluorescence signal was obtained in acidic media; thus, pH 3.5 was chosen for further studies. To maintain the pH at a constant value, chloroacetate buffer (0.2 M; pH 3.5) was chosen.

Also, the influence of the temperature on the fluorescence behavior of the fluoroquinolones was studied in the range 10−45 °C. In all cases, a fluorescence quenching could be observed when the temperature increased; for this reason, 20 °C was chosen for further experiments.

Finally, the influence of the organized media on the fluorescence signal was studied. Among the organized media, a variety of surfactants (sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide) and cyclodextrins (CDs) (α-CD, β-CD, dimethyl-β-CD, 2-hydroxypropyl-β-CD, and γ-CD) were tested. Only with SDS an increment of the fluorescence signal was observed. For this reason, the study of the variation of fluorescence signal with the concentration of SDS was performed. We observed that the signal increased with concentration until reaching a maximum at about 12 mM. This concentration was chosen for further studies.

In summary, the optimum conditions found were pH 3.5; buffer concentration, 0.2 M; SDS concentration, 12 mM; and temperature, 20 °C. **Figure 1** shows the excitation and emission spectra of the three analytes, in the optimized conditions. We observed that the three analytes present a high overlapping of the signals, with the same excitation wavelength (280 nm) and a difference of 15 nm in the emission wavelengths, which are 440 nm for DANO and 455 nm for SARA and DIFLO.

2.5. Procedure for the Determination of Fluoroquinolones in Chicken Tissue Samples. One gram of lyophilized chicken muscle was extracted twice (5 min) with 15 mL of 0.3% *meta*-phosphoric acid/acetonitrile (75:25, v/v) at pH 3, and the mixture was stirring magnetically. The solution was centrifuged at 4000 rpm for 10 min, and the decanted

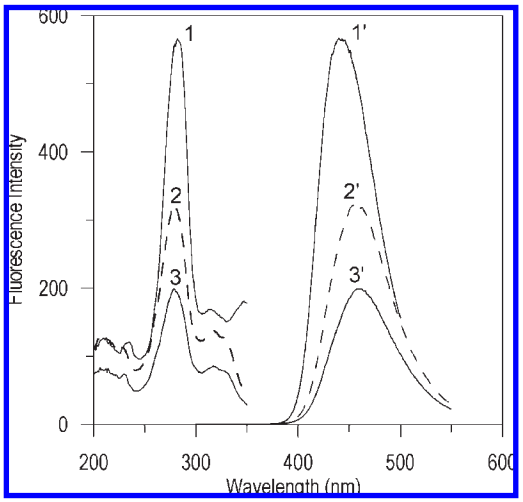

Figure 1. Excitation (1−3) and emission (1′−3′) spectra of danofloxacin (3,3′), difloxacin (2,2′), and sarafloxacin (1,1′) in optimum conditions. [DANO] = 80 ng mL^{-1}; [DIFLO] = 400 ng mL^{-1}; [SARA] = 150 ng mL^{-1}; pH 3.5; [SDS] = 12.0 mM; buffer concentration 0.2 M.

supernatant was filtered through a 0.45 μm nylon filter. It was diluted with 35 mL of water, and the resulting solution was passed through a SPE Isolute Env+ cartridge, which was previously conditioned with methanol, water, and 50 mM phosphoric acid (pH 3). After that, the cartridge was cleaned with 1 mL of water, and the analytes were eluted with 2.5 mL of 2% trifluoroacetic acid/acetonitrile (75:25, v/v) and 1 mL of acetonitrile. The eluted solution was evaporated with heat (50 °C) and under N_2. Finally, the residue was redissolved in chloroacetate buffer (pH 3.5; 0.2 M) and SDS until a final volume of 10 mL. The final solution was placed in a quartz cell, and the excitation−emission matrices were registered in the selected spectral regions.

2.6. Validation of the Proposed Method. The proposed spectrofluorimetric method was validated by RP-HPLC using the method proposed by Bailac et al. (*34*). An analytical column Eclipse XDB-C18 (4.6 × 150 mm, 5 μm) was used at room temperature. The ultraviolet detector was set a 280 nm, and in the fluorescence detector, the emission was set at 443 nm for DANO and 460 nm for DIFLO and SARA. The external standard method was used with peak area as the analytical signal.

The chromatographic studies were performed on a Hewlett-Packard Model 1200 LC instrument equipped with degasser, a 20 μL loop, a diode array detector, and the Chemstation software package to control the instrument, data acquisition, and data analysis. The mobile phase was formed by 0.01 M of citric acid (adjusted to pH 4.5 with ammonia)/acetonitrile, with a flow rate of 1.5 mL/min. The initial mobile phase contains 12% of acetonitrile; from 6.5 to 20 min, the percentage of organic modifier increases linearly to 27%; from 20 to 22 min, this percentage increases to 30%. Finally, this percentage decreases to 12% from 25 to 28 min.

3. RESULTS AND DISCUSSION

3.1. Selection of EEM Spectral Ranges for Analyte Determination by PARAFAC Calibration. As has been mentioned above, the three analytes present a high overlapping of the signals; for this reason, chemometrics tools are necessary for its determination. PARAFAC, a three-way chemometric tool, was chosen because of its second-order advantage properties. In the above-mentioned optimum chemical conditions, maximum information on the three drugs was scanned in the region λ_{em} = 400−540 nm (1 nm intervals), exciting in the range 240−351 nm (3 nm intervals). In **Figure 2**, the excitation−emission matrix of DANO is shown as an example. We observed the presence of the first-order Rayleigh and second-order diffraction signals. It is well known that these scatterings do not contain any information concerning the fluorescence properties of the analytes and that it may complicate the analysis of fluorescence data by

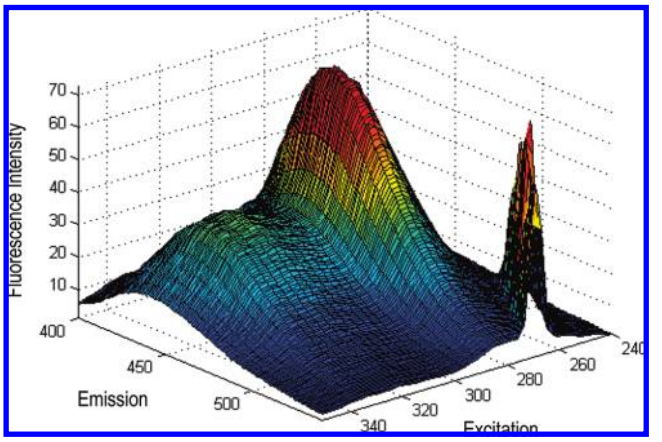

Figure 2. Excitation−emission matrix obtained for danofloxacin in the complete scanned spectral region. [DANO] = 80 ng mL^{-1}.

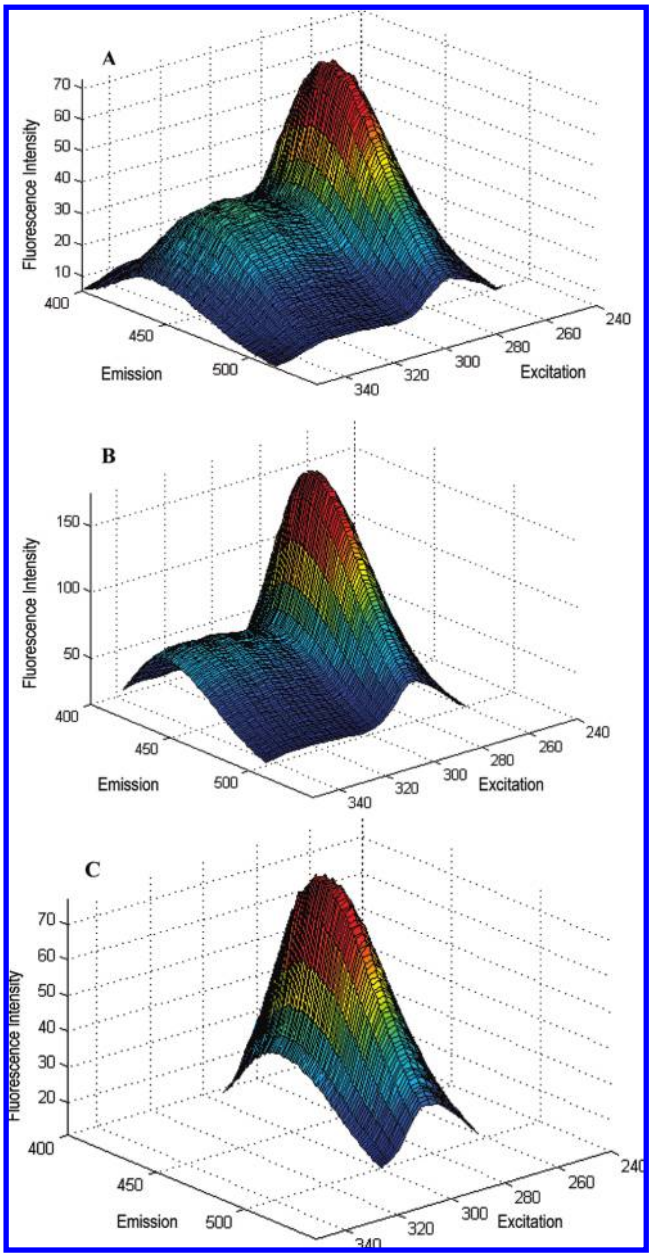

Figure 3. Excitation−emission matrices obtained for (**A**) DANO; (**B**) SARA; and (**C**) DIFLO, in the selected spectral region.

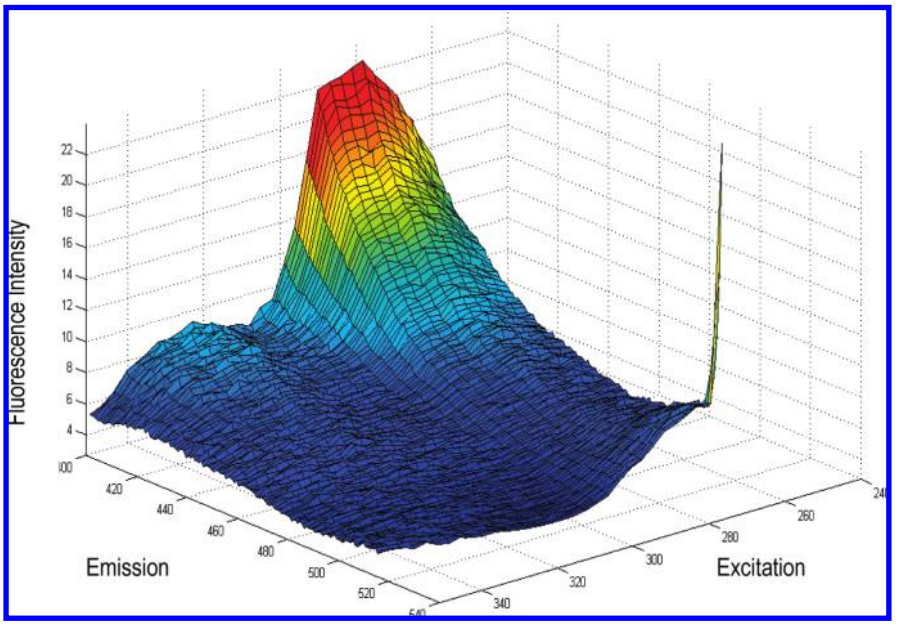

Figure 4. Excitation−emission matrix obtained for a chicken tissue sample, free of fluoroquinolones contamination.

PARAFAC since they do not conform to a low-rank trilinear mode (*35*, *36*).

In order to avoid them and to focus on the responsive spectral regions for each analyte, the wavelength ranges were appropriately reduced, as can be seen in **Figure 3**. The emission goes from 400 to 515 nm and excitation from 260 to 351 nm for DANO (115 × 30 data points); 410−500 nm and 260−345 nm for SARA (90 × 28); and 410−500 nm and 263−300 nm for DIFLO (90 × 12).

3.2. Chicken Tissue Analysis. To determine these compounds in chicken tissue samples, the first step of the analysis must be the extraction of the analytes from the real samples, using the procedure described in section 2.5 with Isolute Env+ cartridges. These cartridges are filled with a copolymer of hydroxyl-polystyrene and divinylbenzene, and because of that, they have a very strong nonpolar (hydrophobic) phase and are extraordinarily appropriate for the extraction of high polar analytes which are not retained in C_8 or C_{18} phases.

Figure 4 shows the EMM of a chicken tissue sample that is free of contamination by fluoroquinolones. It can be seen that there is a maximum from the matrix background in the range where the fluoroquinolones have the maximum signal, which can produce interference on its quantification.

The concentration range studied was assumed according to the MRL concentration ranges for the three analytes: 30−100 ng g^{-1} for DANO (MRL 200 ng g^{-1}) and 50−200 ng g^{-1} for DIFLO (MRL 300 ng g^{-1}) and SARA.

Initially, quantification of the three analytes was attempted by constructing a calibration set and applying N-PLS and U-PLS methods. In both cases, the developed methodology failed when it was applied to chicken tissue samples, with recoveries comprised between 10 and 200%. Those results could be explained as being due to the high matrix effect.

In order to solve the problem of matrix effects, another second-order multivariate method, PARAFAC, was chosen. Furthermore, the standard addition technique was employed. With this combination, it is possible to perform the calibration of one analyte considering the others as interferences, which is useful because it exploits the second-order advantage of PARAFAC.

To apply the standard addition method, a separate calibration scheme is necessary for each analyte. Thus, as an example, to an aliquot of unknown sample containing 30 ng mL^{-1} of DANO,

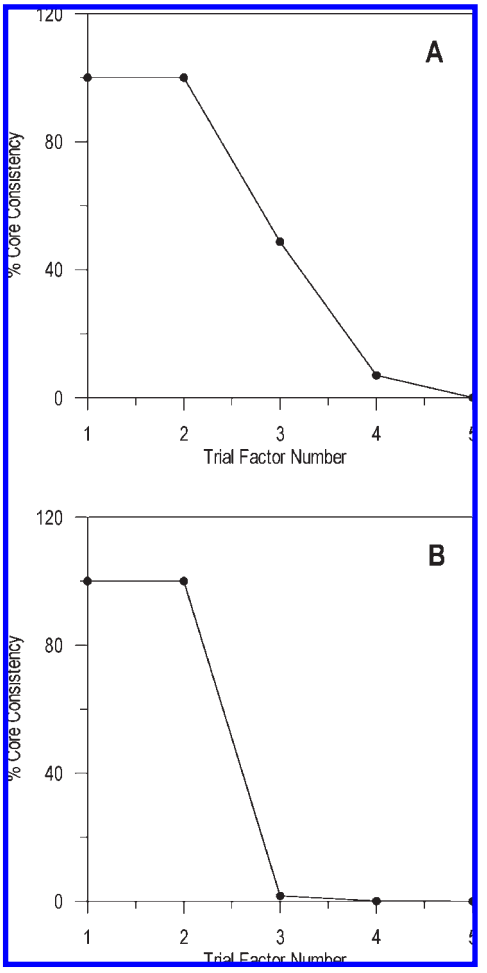

Figure 5. Plot of the PARAFAC core consistency values as a function of the trial number of components. (**A**) DANO; (**B**) DIFLO.

200 ng mL^{-1} of DIFLO, and 50 ng mL^{-1} of SARA (as interference), four different amounts of DANO were added, concretely 16.7, 33.3, 50.0, and 66.7 ng mL^{-1}; keeping the final concentration within the linear range, the EEM was then registered for

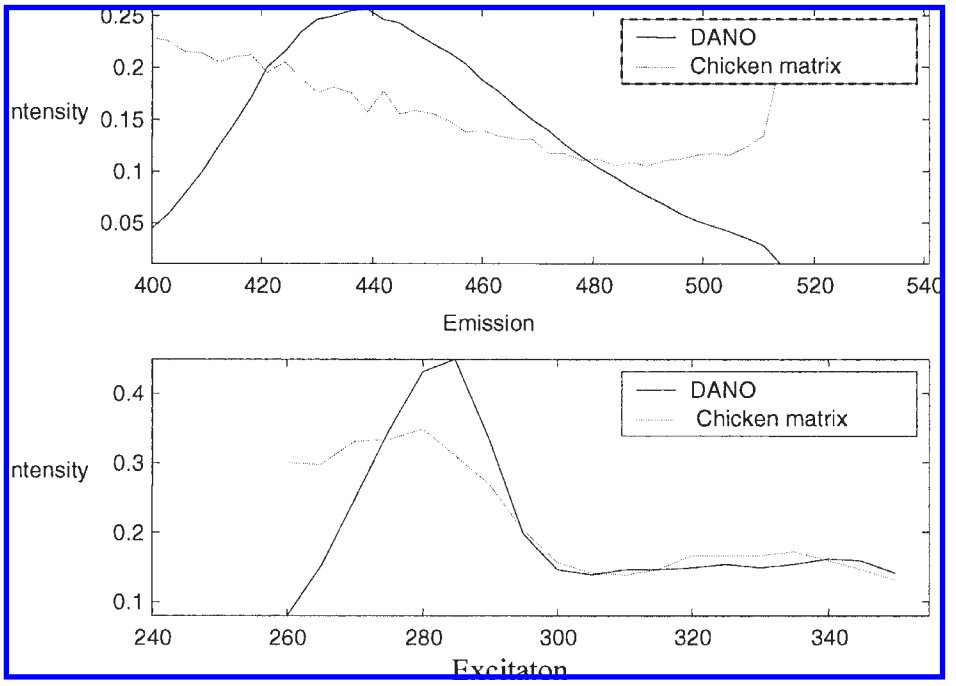

Figure 6. Excitation and emission profiles provided by a two components PARAFAC model for a chicken tissue sample spiked with danofloxacin. The components are labeled according to the contribution of the overall variance.

each of the resulting solutions. This was repeated for difloxacin, spiking this in this case with 33.3, 66.7, 100.0, and 133.3 ng mL^{-1} of difloxacin. All of the solutions were prepared and recorded in triplicate. The determinations were carried out in a situation of variable total volume, with continuous variation of the standard, and the consequent analyte dilution was taken into account according to ref *37*. Pseudounivariate calibrations were obtained where the PARAFAC loadings are used for plotting the standard addition line, instead of the pure instrumental signal. These loadings are equivalent to the filtered signal free of interferences.

In order to determine the optimal number of factors for each test chicken tissue sample, in PARAFAC, several different procedures, such as residual analysis, split-half analysis, and core consistency diagnostic (CONCORDIA) could be used. In this work, the CONCORDIA procedure (*38*) was applied, and it could be observed that the core value dropped when a number of factors higher than two were used (**Figure 5**); thus, two factors were considered as optimum for the prediction step for each of the mixture components. This is due to the fact that the increment of the signal of one of the analytes when it is spiked is considered as one factor, and the other signals (the other two analytes and the chicken matrix) are considered as another factor. The above analysis shows that the presence of chicken tissue is considered as a single extra component by PARAFAC, and this is mandatory for achieving the second-order advantage.

The PARAFAC model was built from three-way arrays formed with emission wavelengths, excitation wavelengths, and 5 measures (the original sample plus four standard additions). A specific model was constructed for every sample in triplicate. Once the number of components was estimated, the array formed by joining the EEMs for the test sample and those obtained by standard addition was subjected to decomposition. Employment of this method implies that the array decomposition should be repeated for each newly analyzed sample. The identification of the chemical constituents under investigation is required before quantification, by resorting to the pseudounivariate calibration graph provided by PARAFAC. This is done with the aid of the spectral profiles extracted by these algorithms and comparing them with those for standard solutions of the pure analytes of interest. Absolute analyte concentrations are obtained after proper standard addition calibration, starting from the known amounts of analyte added to the test samples.

Table 1. Recoveries Obtained in the Determination of Danofloxacin and Difloxacin in Chicken Tissue Samples, by the PARAFAC-Standard Addition Method, in the Presence of 215 μg kg^{-1} of SARA as Interference[a]

danofloxacin			difloxacin		
added (g kg^{-1})	found[b] (g kg^{-1})	% R	added (g kg^{-1})	found[b] (g kg^{-1})	% R
129.0	114.4 ± 3.8	88.7	430.0		
430.0			860.0	915.9 ± 2.8	106.6
215.0	206.4 ± 5.0	96.0	430.0	391.3 ± 2.2	91.0
322.5	305.7 ± 4.2	94.8	645.0	614.9 ± 4.2	95.3
RMSEP	3.03				9.62
REP (%)	5.41				5.06

[a] % R, recovery (%); RMSEP, root-mean-square error of prediction; REP, relative error of prediction (%). [b] In triplicate.

As an example, **Figure 6** plots the loadings provided by PARAFAC, when processing the array formed by the EEM of the chicken tissue sample before the standard addition of DANO. Components have been labeled with the order assigned by the model, i.e., they appear in the order of their contribution to the total of the variance. The comparison of the excitation and emission profiles extracted by the PARAFAC model, with the excitation and emission spectra, shows a satisfactory agreement. Similar success was observed upon studying the remaining samples, after the standard addition of any of the two analytes.

In chemometrics, the root-mean-square error of prediction (RMSEP) generally expresses the accuracy of the model. In **Table 1**, the RMSEP, $\mathrm{RMSEP} = (\Sigma_{i=1}^{n}(c_{\mathrm{act}} - c_{\mathrm{pred}})2/n)^{1/2}$, where n is the number of prediction samples, c_{act} and c_{pred} are the actual and predicted concentrations, respectively; and relative error of prediction $\mathrm{REP}\ (\%) = (100/\bar{c})\cdot\mathrm{RMSEP}$, where $\bar{c}$ is the average component concentration, are included. As can be seen, relative errors of prediction (REP) values are about 5% in both cases.

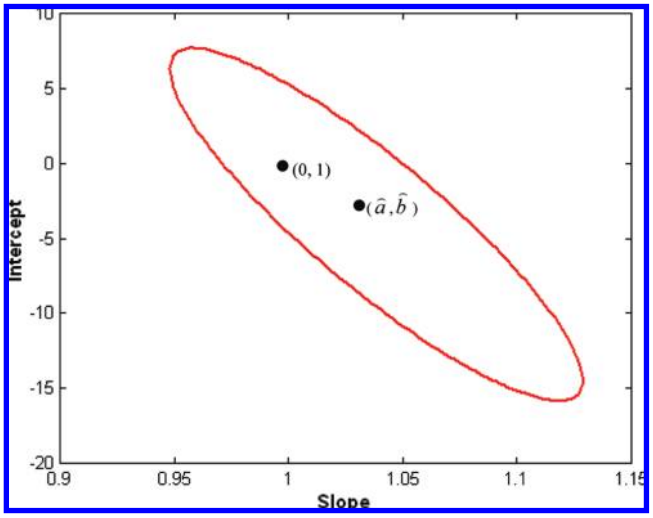

Figure 7. Elliptical joint confidence region (EJCR) for the slope and intercept, corresponding to regressions of predicted versus added concentrations of DANO, SARA, and DIFLO, when applying the PARAFAC method. The circles mark the theoretical ($a = 0$, $b = 1$) point and the experimental $(\hat{a},\hat{b})$ point. The level of significance was set at 5%.

The concentrations predicted for DANO and DIFLO, by the above-described methodology, in the spiked chicken tissue samples are also summarized in **Table 1** for the PARAFAC calibration. Each and every one of the samples was in the presence of 50 μg mL^{-1} of SARA, acting as interference and was carried out in triplicate. It can be seen that both analytes can be determined successfully in the presence of the other two as interferents. When the binary mixture was analyzed in the presence of SARA as the interference, satisfactory recoveries of around 91−96% were obtained.

The predictions are seen to be reasonable for samples of the complexity of chicken tissue. Comparison of the predicted concentrations and recoveries provided by algorithm shows a good predictive ability toward the test set of spiked chicken tissue samples and confirms the potentiality of the presently studied second-order method for the analysis of complex food samples.

In order to gain further insight into the accuracy of the method, linear regression analysis of added (spiked) versus found (predicted) concentration values ($x_{found} = a + bx_{added}$) can be performed to calculate the recoveries from spiked samples. These studies were proposed by Mandel and Linning (*39*) to study the accuracy in chemical analyses. The theory predicts a value of 1 for the slope and 0 for the intercept. However, the occurrence of systematic and random errors in the analytical procedure may produce deviations of the ideal situation. In our case, regression was performed using concentration data for the three analytes simultaneously, taken from all of the chicken tissue samples reported in **Table 1**, as recommended in the literature (*40*), in order to obtain better estimates of the experimental errors.

To calculate the confidence intervals, the equation of the elliptic joint confidence region (EJCR) for the true slope (β) and intercept (α) adopted by Mandel and Linning (*39*) is

$$n(a-\alpha)^2+2\left(\sum x_i\right)(a-\alpha)(b-\beta)+\left(\sum x_i^2\right)(b-\beta)^2 = 2s^2F_{2,\nu}$$

where n is the number of points, s^2 the regression variance, and $F_{2,\nu}$ the critical value of the Snedecor-Fisher's statistic with 2, and $\nu = n - 2$ degrees of freedom at a given P% confidence level, usually 95%.

The center of the ellipse is (a,b). Any point (α,β) which lies inside the EJCR is compatible with the data at the chosen confidence level P. In order to check bias, the values $\alpha = 0$ and $\beta = 1$ are compared with the estimates $\hat{a}$ and $\hat{b}$ using the EJCR. If the point (0, 1) lies inside the EJCR, then bias is absent, and consequently, the recovery may be taken as unity (or 100% in the percentile scale) (*41*).

In our case, as can be seen in **Figure 7**, the fitted regression parameters $(\hat{a},\hat{b})$ are included within the corresponding ellipse. Also, the theoretical point (0,1) lies inside the EJCR; this indicates that the intercept may be considered to be zero and the slope to be unity, which leads to the fact that the recovery can be considered as 100%. All data were considered for the joint confidence test; thus, a good estimate of the experimental error $\hat{s}^2$ was obtained. It could be concluded that the proposed method provides correct results when simultaneously analyzing the two fluoroquinolones in chicken tissue samples.

Also, the method was validated, by RP-HPLC, for the analysis of chicken tissues samples, obtaining in this case, a recovery medium of 93.4% of DANO and 94.8% for DIFLO. This led us to the conclusion that the fluorimetric method is adequate for the analysis of fluoroquinolones.

3.3. Conclusions. The fluorimetric determination of DANO and DIFLO in a complex matrix such as chicken tissue, using a second-order multivariate calibration technique such as PARAFAC, combined with the standard addition method, is demonstrated to be feasible. An adequate selection of the excitation and emission ranges, to avoid first-order Rayleigh and second-order diffraction grating harmonic signals, improved the predictive capacity of the model. The employed chemometric method is able to model the system in the present situation, through the unique decomposition of the three-way data array. This allows the direct extraction of excitation and emission spectral profiles, as well as the relative concentrations of the analytes of interest in the presence of unmodeled interferents, due to the exploitation of the second-order advantage inherent to this type of method. Adequate recovery values are obtained for each of the analytes analyzed individually, in the presence of the others as interferences and in the binary mixtures.

In conclusion, this work presents an application of the second-order advantage provided by PARAFAC in combination with the standard addition method for the direct determination of fluoroquinolones in chicken tissue samples by spectrofluorimetry. The analysis combines the use of PARAFAC for the extraction of the pure analyte signal, with the standard addition method, for a determination in the presence of a strong matrix effect caused by the unknown components of chicken tissue samples. In our case, the employment of PARAFAC for data analysis in conjunction with the advantages of the standard addition method is crucial to the success of analytical determinations.

ACKNOWLEDGMENT

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