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ARTICLE

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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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