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D-optimal designs and N-way techniques to determine sulfathiazole in milk by molecular fluorescence spectroscopy

Rocío Morales[a], M. Cruz Ortiz[a,*], Luis A. Sarabia[b], M. Sagrario Sánchez[b]

[a] Department of Chemistry, Faculty of Sciences, University of Burgos, Pza. Misael Bañuelos s/n, 09001 Burgos, Spain
[b] Department of Mathematics and Computation, Faculty of Sciences, University of Burgos, Pza. Misael Bañuelos s/n, 09001 Burgos, Spain

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ABSTRACT

The present work proposes an analytical procedure to determine sulfathiazole in milk by using molecular fluorescence spectroscopy. For this sulfonamide the European Union in Regulation 37/2010 has established a maximum residue limit in milk of 100 μg kg^{-1}.

The study includes the effect of six factors on the recovery of sulfathiazole. The factors are: (i) The one related to the matrix depending on the heat treatment of the milk (UHT, pasteurized); (ii) Those related to the protein precipitation step, namely the ratio between the volume of trichloroacetic acid (TCA) and milk, centrifugation speed and temperature; (iii) Those affecting the derivatization reaction: derivatization time and volume of fluorescamine.

To do this, two chemometric tools are used together: a D-optimal design for studying the effect of the factors on the recovery of sulfathiazole, considerably reducing the number of needed experiments; and the second-order property of the PARAFAC (Parallel Factor Analysis) decomposition that avoids the need of fitting a new calibration model each time that the experimental conditions change.

It has been found that the type of milk, the TCA:milk ratio and the volume of fluorescamine have significant effect on the response. The rest of factors and interactions are not significant. The best recovery is obtained with UHT milk, 4:6 rate for TCA:milk volumes and 40 μL of fluorescamine. In UHT milk, the mean recovery (n = 5) in the optimal conditions is 88.7% (RSD = 12.4%).

As some non-linear behaviour may occur when using fluorescence spectroscopy, the calibration model that relates the fluorescence spectra with the concentration is computed by a partial least squares regression and a multi-layer feed-forward neural network. In both cases, the proposed procedures have been validated according to Decision 2002/657/EC, concluding that the two are accurate although the calibration model built with the neural network has better figures of merit, the decision limit (CCα) for x_0 = 100 μg L^{-1} is 103.3 μg L^{-1} and the detection capability (CCβ) is 106.5 μg L^{-1}, with the probabilities of false noncompliance (α) and false compliance (β) equal to 5%.

1. Introduction

The use of antibacterials in farms is a usual practice, essential for these farms to be economically viable. One of the reasons is that they help control the appearance or development of infectious diseases. However, this practice can lead to the existence of residues in products intended for human consumption, posing a problem of food security and public health. Besides, its presence has a number of problems, both economic and technological at an industrial level (altered fermentation processes, low quality products, etc.). Also when these drugs are metabolized by the animal, residues may occur in its excrements and their use as fertilizer contributes to the dispersal of these residues in the environment [1]. Consequently, to minimize these effects, the European Union in Regulation 37/2010 [2], taking into account the scientific evaluations about security that exist about these substances, classifies drugs in those with a maximum residue limit (MRL) and those whose presence is strictly forbidden.

Furthermore, Annex I of the last document of the Commission about the implementation of national residue monitoring plans [3] states that "*the proportion of non-compliant results for antibacterials (B1) in targeted samples was 0.29%. Antibacterials were the dominant source for noncompliant results in targeted samples of pigs (78.7%), bovines (30.4%), sheep and goats (30.3%), rabbit meat (70%), milk (79%) and honey (80%)*".

Sulfonamides are synthetic antibacterials and are employed to treat bacterial infections both in veterinary and human medicine. The compounds belonging to this group of antibacterials have a chemical structure derived from sulfanilamide. The chemical and physical properties and the antibacterial activity of each one

* Corresponding author. Fax: +34 947 258831.
E-mail address: mcortiz@ubu.es (M.C. Ortiz).

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depend on the functional groups that are attached to the amide group. The sulfonamides can have adverse effects on human health, some research works report that they are involved in the development of some diseases such as toxic epidermal necrolysis [4] and teratogenesis [5]. Within the group of sulfonamides is the sulfathiazole, widely used today, alone or in combination with some other sulfonamides, and whose toxicity is higher than in other compounds of the same group [6]. Thus, because of its toxicity, Regulation 37/2010 establishes a MRL of 100 $\mu g\,kg^{-1}$, considering the sum of all the sulfonamides that may be present in muscle, fat, liver, kidney and milk.

The presence of sulfonamides in several food matrices has been widely studied by using several analytical techniques. On the one hand, there are techniques developed to be used as screening methods, classified into three main groups: microbiological, immunoassays and, more recently, biosensors [7]. Some authors also use chromatographic techniques such as HPLC (high performance liquid chromatography) as screening methods [8]. On the other hand, for confirmatory methods, the techniques should provide information about the chemical structure of the analyte. Among these techniques, liquid chromatography with mass spectrometry is the most usual [9–14].

Other techniques used are HPLC with diode array detector [15,16], micellar liquid chromatography [17,18], capillary zone electrophoresis (CZE) [19], hydrophilic interaction chromatography tandem with mass spectrometry [20] and gas chromatography/mass spectrometry [21].

Moreover, Ref. [22] reports a study conducted to assess the stability of various antibacterials in milk (beta-lactams, tetracyclines and sulfonamides) under different storage conditions and subsequent heat treatment. The study concludes that the conditions of cooling and freezing the milk do not affect the stability of sulfonamides and that sterilization of milk in its container yields a significant reduction in antibacterials except for sulfadimethoxine and sulfathiazole. That means that these antibacterials (or their corresponding products, generated during heat treatment) can reach the consumer. It is therefore useful to develop analytical methods fast, simple and inexpensive that can be used routinely for the detection of these antibacterials in milk.

The objectives of the present work are twofold:

1 To optimize the conditions for the determination of sulfonamides by molecular fluorescence spectroscopy. In particular, the experimental conditions to determine sulfathiazole in milk are optimized so that they yield the highest recovery. The optimization stage involves the study of the effect that six factors may exert on the analytical result: (i) the one related to the matrix depending on the heat treatment of the milk (UHT, pasteurized); (ii) those related to the protein precipitation step, namely the ratio between the volume of trichloroacetic acid (TCA) and milk, centrifugation speed and temperature; (iii) those affecting the derivatization reaction: derivatization time and volume of fluorescamine, which is the derivatizing reagent.
2 To study the effect of the type of multivariate calibration model on the performance characteristics of the method according to the requirements of Decision 2002/657/EC [23] that regulates the performance of the analytical methods used in the determination of residues of veterinary treatments on foodstuffs. To take into account the possible non-linearity of the fluorescence signals in the calibration range, emission spectra are used to fit calibration curves with two different regression techniques: (i) PLS (partial least squares) that supports some degree of nonlinearity, and (ii) a MLF (multi-layer feed-forward) neural network which does not impose any condition about linearity of the relation between the spectra and the concentration of sulfathiazole in the standard calibration samples.

2. Theoretical background

2.1. Parallel Factor Analysis: PARAFAC

According to Leurgans and Ross [24], signals coming from fluorescence spectroscopy can be described by a tensor of data. For example, for the three way case, the data would be arranged into a cube, which is a tensor of three dimensions. For the case of spectroscopy, the fluorescence intensity of a i-th sample, $i = 1,\ldots, I$, irradiated with a radiation beam whose excitation wavelength is λ_j^{ex}, $j = 1, \ldots, J$, when the emission of radiation occurs at a emission wavelength λ_k^{em}, $k = 1, \ldots, K$, is the one described in Eq. (1):

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf}, \quad i = 1, \ldots, I \;\; j = 1, \ldots, J, \quad k = 1, \ldots, K \qquad (1)$$

where a_{if} is the concentration of the fluorophor f in the i-th sample, b_{jf} is the relative absorption (extinction coefficient) of fluorophor f at the excitation wavelength λ_j^{ex}, and c_{kf} is the relative emission at the λ_k^{em} wavelength.

The physical model in Eq. (1) corresponds to the trilinear PARAFAC model in Eq. (2), when the fluorescence intensities are arranged as a three-way tensor $\underline{\mathbf{X}}$ with size $(I \times J \times K)$, so that:

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + \varepsilon_{ijk}, \quad i = 1, \ldots, I \;\; j = 1, \ldots, J, \quad k = 1, \ldots, K \qquad (2)$$

where ε_{ijk} is the residual non explained by the trilinear model, x_{ijk} is the fluorescence intensity of the i-th sample at the excitation wavelength λ_j^{ex}, and emission wavelength λ_k^{em}.

When a tensor of experimental data is compatible with the structure in Eq. (2) it is said that the data are trilinear and the estimation by least squares of the coefficients in Eq. (2) is unique [25]. Experimental data are compatible with a trilinear model if the factors are the same in all samples differing only in the proportion involved in each, that is the emission and excitation spectra of the analyte are the same along the I samples. The uniqueness property is known in chemical analysis as the 'second order' property. In practice, when the data are trilinear this means that the estimates must coincide (except for a scale factor) with the sample profiles, the emission spectra and the excitation spectra of the F fluorophors in the sample. A revision of the theoretical aspects of PARAFAC as well as algorithms and analytical applications can be read in Ref. [26]. The most useful application is that it is possible to determine the analyte of interest in presence of unknown interferents because, in this case, the interferent(s) will appear as new factor(s) without affecting the rest. Another application is that it is possible to analyze the effect of changing the experimental factors on the response without the need of re-calibrating because the factor common in all the samples is the same, regardless of whether the samples are calibration samples or samples of the experimental design (different experimental conditions). This is very important in the context of experimental design to analyze the effect of experimental factors (pretreatment, clean-up of the samples) avoiding a new calibration in each case.

2.2. Neural networks

Neural networks are a general family of models that are computed by the composition of simple elements, usually known as neurons, units or processing elements. A sample, typically defined by a vector, is weighted before applying a real function (called the transfer function of the neuron) to give an output, which is a real