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Direct determination of propranolol in urine by spectrofluorimetry with the aid of second order advantage

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Abstract

This work presented an application of the second-order advantage provided by parallel factor analysis (PARAFAC) aiming at direct determination of propranolol, a β-blocker also used as doping agent, in human urine by spectrofluorimetry. The adopted strategy combined the use of PARAFAC, for extraction of the pure analyte signal, with the standard addition method, for a determination in the presence of an individual matrix effect caused by the quenching action of the proteins present in the urine. The urine samples were previously 100 times diluted. For each sample, four standard additions were performed, in triplicates. A specific PARAFAC model was built for each triplicate of each sample, from three-way arrays formed by 231 emission wavelengths, 8 excitation wavelengths and 5 measurements (sample plus 4 additions). The models were built with three factors and always explained more than 99.87% of the total variance. The obtained loadings were related to PRO and two background interferences. The scores related to PRO were used for a linear regression in the standard addition method. The obtained determinations in the PRO concentration range from 5.0 to 20.0 μg ml^{-1} provided recoveries between 91.1 and 108.4%.

Keywords: PARAFAC; Molecular fluorescence; Clinical analysis; β-Blocker; Second-order standard addition method

1. Introduction

Propranolol (PRO), 1-[isopropylamino-3-[1-naphthyloxy]-2-propanol], is a β-adrenoceptor antagonist (β-blocker), which is widely used in the treatment of several diseases such as cardiac arrhythmia, angina pectoris, sinus tachycardia, thyrotoxicosis, hypertrophic subaortic stenosis and hypertension [1]. PRO is commercially employed in the form of hydrochloride and has also been suggested for use in a number of other conditions including dysfunctional labor and anxiety [2]. Because it is also used in low activity sports as doping agent, which acts reducing cardiac frequency and contraction force, the International Olympic Committee included it in the list of forbidden substances.

Different techniques have been used to determine PRO in pharmaceutical formulations and biological samples, including methods based on titrimetry [3], kinetics-colorimetry [4], spectrofluorimetry [2,5–7], flow injection-chemiluminescence [8], ion selective electrodes [9], chromatography [5,10–13] and electrophoresis [14]. When used for analyzing complex biological matrices, all of these methods demand tedious preliminary steps such as pre-concentration in an organic solvent or proteins precipitation. In particular, spectrofluorimetric methods are suitable for PRO determination, since it presents natural fluorescence. Nevertheless, the use of spectrofluorimetry for determining drugs in biological fluids is made difficult due to the presence of natural fluorescent interferences. In the last years, a way to circumvent this problem has been proposed, which combine spectrofluorimetric data and three-way chemometric tools, mainly parallel factor analysis (PARAFAC) [15,16]. This combination has allowed simplification of the experimental procedure and direct drug determination. Tedious preliminary steps can be eliminated, replacing the physical separation of interferences by the chemometric separation of their signals. Examples involving variants of this strategy have appeared in literature since 2002, describing determination of drugs, such as naproxen [17], ibuprofen [18], piroxicam [19], salicylic acid [17,20], doxorubicin [21], daunorubicin [22], carbamazepine [23], ciprofloxacin [24], folic acid [25], methotrexate [25], fluoroquinolone antibiotics [26,27] and phenylanthranilic acid derivatives [28,29], in

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matrices such as plasma, serum and urine. In spite of the advantages of these methodologies, some of them still have demanded previous steps, such as organic extraction [18,19,28], derivatization [25], samples UV irradiation [27] or addition of an auxiliary agent (a complexant [17,18] or a surfactant [26,29]) to improve the method.

Besides the mathematical removal of the contribution of interferences, another problem to be circumvented in the spectrofluorimetric determination of biological matrices is the significant analyte–background interactions that used to occur. These interactions may lead to the quenching of the analyte signal by blood or urine proteins and also to spectral variations from sample to sample. As the extension of these interactions is sample dependent, a matrix effect originates which prevents the use of an external calibration curve and demands the use of the standard addition method. Another possibility to cope with analyte matrix interactions would be to construct a large calibration set which includes the matrix, but this alternative is considered not feasible for the present case. The objective of this article was the direct determination of PRO in human urine, aiming at the maximum simplification of the sample manipulation. The employed methodology exploits the so-called second-order advantage of the three-way spectrofluorimetric data, through the use of PARAFAC and second-order standard addition method. Figures of merit, such as sensitivity, selectivity and limit of detection were also estimated for the proposed method.

2. Theory

2.1. PARAFAC and spectrofluorimetric data

PARAFAC is a generalization of PCA to higher order data, which was first developed by psychometricians in the early 1970s [30]. However, while PCA has only mathematical meaning, PARAFAC is of physical connotation. It might be considered a constrained version of the more general Tucker3 method [31] with a superidentity core matrix, being less flexible, using fewer degrees of freedom and providing not nested solutions. PARAFAC also provides unique solution independent of rotation (uniqueness), which is a great advantage for modelling spectroscopic data. If the data is indeed trilinear, the right number of components is used and the signal-to-noise ratio is appropriate, the true underlying spectra (or whatever constitutes the variables) will be found. One score matrix, **A**, and two loading matrices, **B** and **C**, with elements a_{if} ($i = 1, \ldots, I$), b_{jf} ($j = 1, \ldots, J$) and c_{kf} ($k = 1, \ldots, K$), give the structural model behind a trilinear PARAFAC, which is adjusted to minimize the sum of squares of the residuals e_{ijk} in the following equation:

$$X_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + e_{ijk} \quad (1)$$

where f is the number of factors. The **B** and **C** obtained matrices are columnwise normalized. The use of constraints may improve the interpretability of a PARAFAC model and the resolution of spectra used to require non-negativity constraint since negative spectral parameters do not make sense [15,16].

Chemometric three-way methods, such as PARAFAC, are suitable for modelling spectrofluorimetric data [32]. When a sample set is measured at several emission wavelengths for several excitation wavelengths, a three-way array is obtained, which must show a trilinear behavior in spite of the presence of noise, scatter (Rayleigh and Raman) and other non-trilinear phenomena. Particularly, the mathematical structural model behind PARAFAC agrees with the physicochemical model that generates spectrofluorimetric data. Recalling Eq. (1), x_{ijk} represents the fluorescence intensity measured for the ith sample at the excitation wavelength j and emission wavelength k; a_{if} is the concentration of the fth fluorophore in the sample i; b_{jf} the molar absorption coefficient of the fth fluorophore at the excitation wavelength j; c_{kf} is the relative emission coefficient of the fth fluorophore at the wavelength k; and e_{ijk} represents the residues of the model. A crucial step in this type of analysis is the choice of the appropriate number of the factors, for which there is no absolute criterion. This choice can be made based on the variance accounted for the model, the chemical knowledge of the system, split-half methods [33] or the core consistency diagnostic (CORCONDIA) [34]. Due to the easiness of generating trilinear data, spectrofluorimetric data has probably been the subject of the major number of PARAFAC applications found in the literature and practical aspects, such as the correct choice of the number of factors, the use of missing values to correct trilinearity deviations in the scattering spectral regions and the outliers' detection, have been pointed out [35].

2.2. Second-order standard addition method

The standard addition method [36] is applied to univariate data (from zeroth-order instrumentation, such as a pHmeter) as a means of overcoming matrix effects that change the instrumental response to the analyte. In comparison with an external calibration curve, this method has the disadvantage of being more time-consuming as a function of needing various standard additions. However, it is an alternative in situations where the external calibration is not feasible. Standard addition method requires two conditions: the instrumental response must (1) depend linearly on the increase of the analyte concentration and (2) be zero when the analyte concentration is zero. The estimation of the analyte concentration in the sample can be obtained through a plot of the instrumental response as a function of the amount of standard added by fitting a straight line to the data and finding its intercept on the abscissa. In 1979, Saxberg and Kowalski published an extension of standard addition method to multivariate data (from first-order instrumentation, such as a diode array spectrophotometer), named generalized standard addition method (GSAM) [37]. GSAM requires that the analyte and the interferences be sequentially added in the sample. This relaxes the constraint that the analytical method must be fully selective to the analyte of interest. However, reliable results cannot be obtained if an uncalibrated source of instrumental signal is present. Therefore, in the absence of all species included in the calibration model, the instrumental response must be zero at all channels.