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Second-order multivariate models for the processing of standard-addition synchronous fluorescence–pH data. Application to the analysis of salicylic acid and its major metabolite in human urine

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ABSTRACT

In the present work, we describe the determination of salicylic acid and its major metabolite, salicyluric acid, in spiked human urine samples, using synchronous fluorescence spectra measured in a flow-injection system with a double pH gradient. Because the fluorescent urine background constitutes a potentially interfering signal, it becomes necessary to achieve the second-order advantage. Moreover, due to significant changes in the signal of the analytes in the presence of the urine matrix, mainly for salicyluric acid, standard addition was required in order to obtain appropriate quantifications. Several second-order multivariate calibration models were evaluated for this purpose: PARAFAC and MCR-ALS in two different modes, and PLS/RBL.

1. Introduction

Second- and third-order multivariate analysis has been gaining great importance in analytical applications, as can be seen from the literature in relevant analytical, chemometrics and applied journals. The combination of second-order data and multivariate algorithms allowed the development of the methods for analyte monitoring in complex biological, environmental and industrial samples [1]. This is also due to the variety of second-order instrumental data that are being employed, such as fluorescence excitation–emission spectroscopy, pH or kinetically modulated spectral information, or data from hyphenated techniques such as chromatography–mass spectrometry [1]. Furthermore, novel second-order signals based on the time evolution of chemiluminescence emission [2] and lanthanide-sensitized luminescence excitation [3] have been recently applied to determine a number of fluoroquinolones. It is important to emphasize the special properties exhibited by second-order data with respect to the presence of potential interferents. An adequate selection of data and algorithms may allow the quantification of analytes, even in the presence of unexpected sample constituents, a property known as the second-order advantage [1,4–6]. The presence of a complex matrix, which not only provides a significant interfering signal but also affects the analyte response in the sample (e.g., through inner filter effects or interactions between the analyte and the matrix, such as formation of complexes with proteins or associations), requires both second-order multivariate calibration and standard addition for successful analyte quantification, achieving the second-order advantage [7]. Instead of using standard addition, external calibration in the presence of the complex matrix could be employed [8]. However, this procedure is not always experimentally feasible.

Although several papers involving absorbance–pH data are reported in the literature [9–12], the analysis of fluorescence–pH data is not widespread. As has been discussed in the determination of fluoroquinolone in urine, second-order pH-fluorescence data can be used to quantify analytes when the fluorescence is highly pH-dependent [13]. Based on the strong pH-dependence shown by salicylic acid (SA) and its major metabolites, salicyluric (SU) and gentysic (GE) acids [14], Estevez da Silva et al. have proposed their determination in ternary mixtures using synchronous fluorescence–pH data. In this work, the changes in the pH values were obtained by acid–base titrations, using a peristaltic pump to force the solution into a flow cell, and data were analyzed using PARAFAC [15].

In the present work, we propose the determination of SA and SU in spiked human urine samples, using synchronous fluorescence spectra measured in a flow-injection system with a double pH gradient. The synchronous fluorescence mode is of particular

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interest for the analysis of complex samples because higher spectral resolution is achieved in the simultaneous determination of multiple analytes [14,16,17]. In order to generate a controlled and reproducible pH gradient, a flow-injection method was used, providing a large amount of data in a very simple and fast way. As previously employed by Borraccetti et al. [13], a fast scanning spectrofluorimeter in the synchronous fluorescence mode was used, producing fluorescence–pH gradient data with improved selectivity and sensitivity, retaining the important second-order advantage.

Even though several algorithms are available for the convenient processing of second-order data, achieving the second-order advantage [7,18], it is important whether the three dimensional array built with these data for a set of samples complies or not with the so-called trilinearity condition [19]. In general, when the three-way array of second-order data is trilinear, trilinear decomposition algorithms can be conveniently applied, such as parallel factor analysis (PARAFAC) [20]. However, when the three-way data array deviates from the trilinearity condition, non-trilinear algorithms may be applied, such as multivariate curve resolution (MCR-ALS) when bilinearity of individual matrices is preserved [21], and latent variable algorithms such as unfolded partial least-squares (U-PLS) [22] and multiway PLS (N-PLS) [23], both combined with residual bilinearization (RBL) [8,24–27]. The selection of a suitable algorithm depends on the specific data properties.

When one of the data modes is a pH gradient, as in the present report, two challenges may appear for second-order multivariate calibration algorithms: (1) linear dependency, because closure relations may exist between pH-equilibrating species of each sample constituent, and (2) lack of reproducibility in the pH gradients from sample to sample. Solving these problems may demand MCR-ALS (which takes into account the lack of reproducibility of pH profiles and linear dependency). Alternatively, suitably initialized and restricted PARAFAC or PLS/RBL may be adequate, if the lack of reproducibility is small.

In this work, data analysis was performed using several second-order calibration methods in the standard addition mode, with the purpose of correcting both a responsive background and analyte–background interactions. It should be noticed that linear dependence is present in standard addition data when more than one interferent occurs in the test sample, in addition to the linear dependence produced by the pH gradient. Second-order standard addition data can be analyzed in the classical mode (mode 1), as well as in the modified approach, in which matrix data from the test sample are subtracted from the standard addition matrices (mode 2), and quantitation is subsequently done using the external calibration methodology [28].

In summary, in the present study SA and SU were quantified in urine samples employing synchronous fluorescence–pH data in the standard addition mode. The algorithms applied were PARAFAC and MCR-ALS in both modes, and PLS/RBL necessarily only in mode 2. The performance of the different algorithms was compared, suggesting that PARAFAC and MCR-ALS in mode 1 retrieved satisfactory predictions for SA, while in the case of SU, suitable predicted concentration values were achieved using PARAFAC in mode 1, MCR in mode 2 and PLS algorithms.

2. Experimental

2.1. Apparatus

A Gilson Minipuls Evolution peristaltic pump (Gilson, Middleton, WI, USA) was used for the propulsion of the carrier solution, at a flow rate of 0.5 mL min^{-1}. All sample solutions were manually injected into the carrier system using a dual proportional Upchurch injection valve (Upchurch scientific, Oak Harbor, WA, USA). The flow was injected into a quartz Hellma flow cell model 176.752-QS, 25 μL inner volume, 1.5 mm optical path length (Hellma, Müllheim, Germany). Synchronous fluorescence measurements were done using a fast scanning Varian Cary Eclipse spectrofluorometer (Varian Inc., Mulgrave, Victoria, Australia), equipped with two Czerny-Turner monochromators and a xenon flash lamp, and connected to a PC microcomputer via an IEEE 488 (GBIP) Serial Interface. Fluorescence scanning parameters were scanning speed, 3600 nm min^{-1}; slit widths, 5 nm; detecting voltage, 830. Spectral parameters are detailed in Section 2.3.

2.2. Reagents

All experiments were performed with analytical grade chemicals. The following solutions were employed: HCl 10^{-3} M, prepared from commercial HCl (Merck, Darmstadt, Germany); NaOH 10^{-3} M, prepared from commercial NaOH (Merck, Darmstadt, Germany). Stock solutions of sodium salicylate (Merck, Darmstadt, Germany) and salicyluric acid (Sigma, St. Louis, MO, USA), both 200 mg L^{-1}, were prepared weighing the required amount of the corresponding compounds and dissolving them in MilliQ water.

2.3. Flow injection methodology

In order to generate the double pH gradient inside the flow stream, the alkaline sample was injected into the acid sample used as the carrier. Each of the studied samples was diluted with HCl 10^{-3} M and used as the carrier steam. The composition of the injected sample was identical to that of the carrier, except that the dilution was carried out with NaOH 10^{-3} M. It was verified that this mode of generating the pH gradient presents the highest sensitivity, and therefore selected for this study. The flow injection analysis (FIA) assembly used is composed of a peristaltic pump, which drives the acid sample used as carrier through a Teflon tube (0.8 nm inner diameter). After the alkaline sample (500 μL) is injected, the flow is sent to the spectrofluorometer cell flow through a Teflon tube (total length = 4 m). The spectral measurements were done 2 min after the sample injection. Synchronous fluorescence spectra were collected under the following conditions: $\Delta\lambda$ (constant difference between excitation and emission monochromators), 100 nm; emission wavelength range, 260–360 nm each 3 nm; time between successive spectra, 0.05 min; total time, 2.5 min. The spectra were arranged into a matrix of size 31×50 data points, saved in ASCII format and transferred to a PC for subsequent manipulation with the multivariate programs.

2.4. Urine samples

Urine samples were spiked at a concentration given by random numbers in the range 0–300 mg L^{-1} for both analytes (in order to test the method performance for many concentrations within the therapeutic range). The spiked samples were diluted 1:200 with HCl 10^{-3} M to be employed as carriers, or with NaOH 10^{-3} M to be injected into the flow system. Afterwards, new solutions were prepared starting from the spiked samples in order to carry out three successive additions of each analyte. Concentrations were increased by 0.50, 1.00 and 1.50 mg L^{-1} for both salicyluric and salicylic acids, on different aliquots of the original samples (values refer to the measuring cells). Each of these samples was also diluted with HCl 10^{-3} M or with NaOH 10^{-3} M as described above. We estimate the uncertainties in all these analyte concentrations to be of the order of ± 0.01 mg L^{-1}. All the concentration ranges are within the therapeutic values of the studied drugs in human urine.