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Four-way calibration applied to the processing of pH-modulated fluorescence excitation-emission matrices. Analysis of fluoroquinolones in the presence of significant spectral overlapping

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ABSTRACT

A new methodology involving four-way multivariate calibration with a balanced number of data points in all modes is presented. The method is based on fluorescence excitation-emission matrices modulated by a double pH gradient obtained in a flow injection system. This data array was employed for the quantitation of ciprofloxacin, ofloxacin and norfloxacin in unprocessed urine samples. Due to the presence of potential interfering compounds with overlapping profiles in the analyzed samples, it is required to achieve the second-order advantage. The four-way arrays obtained were processed by parallel factor analysis (PARAFAC), attaining satisfactory results with relative errors of prediction (REP%) between 3% and 7.5% in the analyzed samples for all analytes. The average limit of detection (mg L^{-1}) was 0.035 for norfloxacin and ofloxacin and 0.028 for ciprofloxacin.

1. Introduction

Chemical multi-way calibration has gained widespread acceptance by the analytical community, since it provides better quality of the results when developing analytical methods to quantify analytes of interest in complex matrices. Nowadays, the research dedicated to the development and testing of multivariate algorithms applied to progressively more complex chemical systems is very extensive, as can be seen from the literature in relevant analytical, chemometrics and applied journal [1,2]. The main reason for this continuing interest is that second-order and higher-order data exhibit the so-called "second order advantage". This property allows to accurately quantify the calibrated analytes, even in the presence of interfering compounds not included in the calibration set [1–3]. Higher-order data might also exhibit other advantages that could improve the predictive ability. They would provide richer analytical information, allowing the development of more stable methods towards concerning interference and matrix effects, and less prone to minor changes in experimental conditions [4].

A variety of second- and higher-order instrumental data can be produced using modern instrumentation. However, it is interesting to note that while the use of second-order data is widespread, only in a few cases third-order data have been recorded and used to construct quantitative calibration models and to develop analytical methodologies [1,2]. This may be attributed to the fact that the experimental acquisition of these data arrays is still difficult to implement. Examples of four-way/third-order data are bidimensional chromatographic systems equipped with detection based on time of flight mass spectrometry (TOFMS) or diode array detection (DAD), leading to GC-GC–TOFMS [5] or LC-LC–DAD, and LC–DAD as a function of reaction time [6], and more recently ultra-fast high performance liquid chromatographic data with fluorescence excitation-emission detection [7–10]. On the other hand, excitation-emission fluorescence or phosphoresce matrices as a function of reaction time [11–14] or decay time [15], and also UV spectra-time reaction-pH data [16], are also some of the examples of the use of multi-way analysis using third-order data.

In this work we present an innovative form of third-order data consisting of excitation-emission matrices (EEMs) modulated by a double pH gradient. A fast scanning spectrofluorimeter allowed recording a complete EEM in a short time, and flow injection analysis (FIA) was used to generate the pH mode. Thus excitation-emission-pH third-order data, with a reasonably balanced number of sensors in all modes, have been easily measured for each experimental sample and used to construct a four-way calibration model. This calibration was applied to quantify fluoroquinolones (FQs) in unprocessed urine samples, which contain other fluorescent compounds with significant overlapping profiles. The fluoroquinolones ciprofloxacin (CIP), ofloxacin (OFLO) and norfloxacin (NOR) were selected as a model to show the potentiality of the proposed strategy of third-order data generation and the corresponding modeling. FQs and other strongly pH-dependent

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compounds have already been determined in urine samples, using pH-modulated second-order fluorescence signal [17,18].

Several algorithms are available for the convenient processing of multidimensional data, achieving the second-order advantage. Four-data arrays have been usually processed by resorting to the well-known parallel factor analysis algorithm (PARAFAC) [19,20]. The algorithms based on latent variables, unfolded (U-PLS) and multi-way partial least-squares (N-PLS), can also be applied, combined with residual trilinealization (RTL) [7,21,22] in order to achieve the second-order advantage. Additionally, multivariate curve resolution coupled to alternating least-squares (MCR-ALS) [23] can be used to process these data by first unfolding them into matrices. The selection of the proper algorithm depends on whether the multidimensional array complies or not with the so-called multilinearity condition. This may be briefly defined as the possibility of expressing a multi-way data array for a set of samples as a linear function of component concentrations and profiles in the different data modes. Third-order data that meet this property are called quadrilinear.

When the pH mode is obtained in a flow system, the obtained four-data array might not be strictly quadrilinear due to: a) irreproducibility in the pH gradient generation, as a consequence of the lack of synchronization among samples in the flow system; b) pH evolution while measuring each EEM. In addition, in the presence of a pH gradient a closure relationship exists between pH-equilibrating species, implying that they might be mutually correlated. Correlations may complicate the resolution of the multi-way array [3].

In this report, the four-way arrays were processed with PARAFAC, which provided satisfactory predictions in all the analyzed systems. The results suggest that the pH mode, in the selected experimental conditions, does not produce a breaking of the quadrilinearity of the data. Furthermore, PARAFAC can handle the presence of different unmodeled compounds exhibiting significant spectral overlapping signal with those for the analytes.

In summary, the proposed experimental system enabled us to obtain, in a simple way and in a reasonable time, four-way data arrays useful to quantify FQs in urine samples without pretreatment and in the presence of interferent compounds.

2. Experimental

2.1. Reagents

All experiments were performed with analytical grade chemicals. The following solutions were employed: acetic acid (HAc) 0.025 mol L^{-1}, prepared from commercial HAc (Merck, Darmstadt, Germany); sodium hydrogen carbonate ($NaHCO_3$) 5×10^{-3} mol L^{-1}, prepared from commercial $NaHCO_3$ (Analar, Poole, England). Ultrapure water provided by a MilliQ purification system was used.

Ofloxacin and norfloxacin were purchased from Sigma (Seelze, Germany) and ciprofloxacin was provided by Fluka (Seelze, Germany). Fluoroquinolone stock solutions (all 200 mg L^{-1}) were prepared by dissolving the exact amount of the corresponding compound in 50 mmol L^{-1} HAc solution. These solutions were stored at 4 °C and were stable for at least a month.

Stock solutions of sodium salicylate (Merck, Darmstadt, Germany) and naproxen (Sigma, Seelze, Germany), both 200 mg L^{-1}, were prepared weighing the required amount of the corresponding compounds and dissolving them in MilliQ water.

2.2. Apparatus

Two Gilson Minipuls Evolution peristaltic pumps (Gilson, Middleton, WI, USA) were consecutively used for the propulsion of the carrier solution. 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).

Fluorescence measurements were done using a fast scanning Varian Cary Eclipse spectrofluorimeter (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. Excitation-emission fluorescence matrices were collected under the following conditions: for OFLO, excitation wavelength range, 275–375 nm, emission wavelength range 425–540 nm; for CIP, excitation wavelength range, 255–355 nm, emission wavelength range 370–485 nm and for NOR, excitation wavelength range, 250–350 nm, emission wavelength range 375–490 nm (in all cases, excitation ranges each 5.5 nm and emission ranges each 3.6 nm). The slit widths for the excitation and emission monochromators were fixed at 5 nm, and the detector voltage was set at 850 for OFLO and CIP, and 800 V for NOR. A wavelength scanning speed of 18,000 nm/min was employed, so that a complete excitation-emission fluorescence matrix was obtained in few seconds, collecting 45 successive matrices in 15 min.

The complete data were arranged into a third-order array of size $18 \times 32 \times 45$ data points, saved in ASCII format and transferred to a PC for subsequent manipulation with the multivariate program.

2.3. Calibration and validation samples

Three different calibration sets were prepared, one for each fluoroquinolone, having six duplicate concentration levels, equally spaced in the range 0.00 to 1.00 mg L^{-1}, which was established on the analysis of the linear fluorescence-concentration range for each analyte. In order to obtain the desired concentrations, appropriate aliquots of standard solutions were measured and placed in 20.00 mL volumetric flasks, completing to the mark with HAc 0.025 mol L^{-1} to be employed as carrier, or with $NaHCO_3$ 5×10^{-3} mol L^{-1} to be injected into the flow system. In order to test the method performance, a validation set was prepared for each analyte, employing different concentrations than those used for calibration and following a random design, i.e., choosing the validation concentrations by generating random numbers, equally distributed within the analyte calibration ranges.

Since test urine samples were diluted 1/200 (see below), the calibration concentration range, once converted to urine concentrations, covers the therapeutic ranges of the analytes in the urine samples of patients administered with the three studied drugs. All samples were measured in random order.

2.4. Urine samples

With the purpose of evaluating the feasibility of the method to quantify FQs in complex samples, sets of urine were prepared, one for each analyte, spiked at concentrations given by random numbers in the range of 0–200 mg L^{-1} (therapeutic range).

Besides, for OFLO a set of spiked urine samples containing the analyte was prepared with the addition of salicylate (SA) as interferent. For NOR, as well as for CIP, different sets of spiked urine samples were prepared containing the corresponding FQ, with the addition of SA or naproxen (NX) as interferent. Both analyte and interferent concentrations in all cases were in the range of 0–200 mg L^{-1}. These test samples are intended to mimic truly unknown samples composed of uncalibrated substances, where a responsive background may occur. The inclusion of known chemical components in these samples has the purpose of checking whether the multivariate algorithm is able to successfully retrieve their corresponding profiles, and accurately quantify the analytes. All spiked samples were diluted 1/200 with HAc 0.025 mol L^{-1} to be employed as carriers, or with $NaHCO_3$ 5×10^{-3} mol L^{-1} to be injected into the flow stream as calibration and validation sets, and measured in random order.