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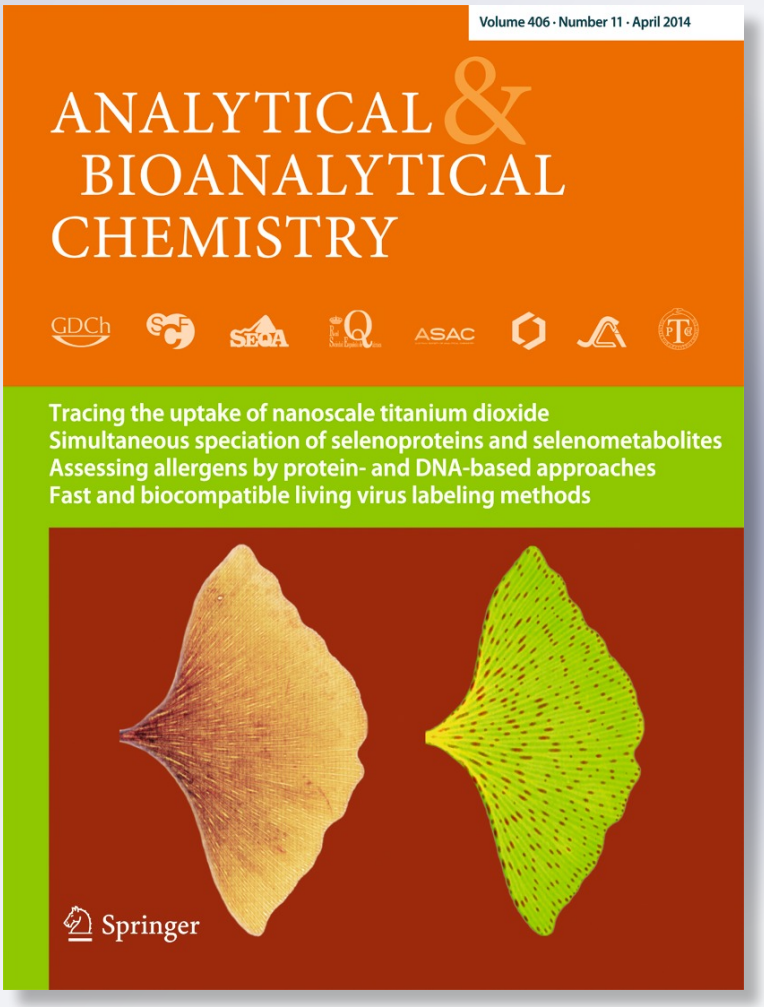

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RESEARCH PAPER

Ultrafast quantitation of six quinolones in water samples by second-order capillary electrophoresis data modeling with multivariate curve resolution–alternating least squares

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Abstract This paper presents the development of a capillary electrophoresis method with diode array detector coupled to multivariate curve resolution–alternating least squares (MCR-ALS) to conduct the resolution and quantitation of a mixture of six quinolones in the presence of several unexpected components. Overlapping of time profiles between analytes and water matrix interferences were mathematically solved by data modeling with the well-known MCR-ALS algorithm. With the aim of overcoming the drawback originated by two compounds with similar spectra, a special strategy was implemented to model the complete electropherogram instead of dividing the data in the region as usually performed in previous works. The method was first applied to quantitate analytes in standard mixtures which were randomly prepared in ultrapure water. Then, tap water samples spiked with several interferences were analyzed. Recoveries between 76.7 and 125 % and limits of detection between 5 and 18 μg L^{-1} were achieved.

Keywords Capillary electrophoresis · Chemometrics · Water

Introduction

Pharmaceuticals, as well as their synthetic precursors and transformation products, are continuously released into the environment in enormous quantities, i.e. directly into the domestic sewage system and via burial in landfills. Manufacturing, use, and disposal of unused and expired drugs constitute a variety of routes that allow the entrance of pharmaceuticals into the environment, which can cause untoward effects in biota [1–4]. Drugs are detected in the environment in the nanograms per liter to micrograms per liter concentration range. Moreover, even though individual drug concentrations might be low, the combination of drugs sharing a common mechanism of action could be substantial. Exposures in the aquatic environment are of particular concern, since aquatic organisms, in contrast to those spending at least some time in terrestrial settings, are subjected to continual unabated life cycle exposures. This consideration is particularly relevant regarding pharmaceuticals (or bioactive metabolites) that are refractory to structural transformations and are continually introduced into surface waters from sewage treatment plants. In addition, the polar, nonvolatile nature of most drugs prevents their escape from the aquatic realm. Even pharmaceuticals with relatively short environmental half-life assume the qualities of highly persistent pollutants because they are continually replenished by infusion to the aquatic environment from wastewater treatment plants. Due to particular concerns over the occurrence of these compounds in water resources, several research studies have been conducted for the detection and determination of several groups of antibiotics in different environments [5].

Quinolones (Qs) are highly useful antibacterial agents, particularly because of their broad spectrum activity against Gram-positive and Gram-negative bacteria and mycoplasma, and for their good oral intake. These compounds are administrated in large quantities to humans and animals, and they end up in wastewater coming from hospital and municipal emissions, whereas veterinary drugs are excreted by the animals and are released in the manure [2]. In this context and due to their prevalence of use, 5 to 500 ng L^{-1} Qs residues in

M. R. Alcaráz · L. Vera-Candioti · M. J. Culzoni · H. C. Goicoechea (✉)
Laboratorio de Desarrollo Analítico y Quimiometría (LADAQ), Cátedra de Química Analítica I, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral–CONICET, Ciudad Universitaria, Santa Fe 3000, Argentina
e-mail: hgoico@fbcb.unl.edu.ar

the natural environment have been reported in many countries [6]. Consequently, monitoring of low quantities of these compounds from different environmental matrices is imperative for human health protection and environmental control. A large number of methods for the determination of Qs in environmental waters have been published, especially including liquid chromatography (LC) with fluorescence detection (FD) or mass spectrometry (MS) detection [7]. Very recently, the quantitation of eight Qs in groundwater samples with ultrasound-assisted ionic liquid dispersive liquid–liquid microextraction prior to high-performance LC and FD has been published, reporting limits of detection (LOD) between 0.8 and 13 ng L^{-1} [8].

Usually, when working with capillary electrophoresis (CE), total analyte separation is expected, i.e., each peak belongs to a single compound. On the other hand, when a complete separation of the peaks could not be performed, generating second-order data and its proper modeling with chemometric algorithms can guarantee selectivity by mathematical means, allowing for resolution and quantitation of overlapped analytes [9, 10]. In this way, the information provided by second-order data adequately decomposed by suitable algorithms can be uniquely ascribed to the analyte of interest, even in the presence of unexpected components not considered in the calibration stage (a property that has been called "the second-order advantage"), and avoids the requirement of physically removing interferences [11]. Multivariate curve resolution–alternating least squares (MCR-ALS) [12] and parallel factor analysis 2 [13] are two well-known second-order algorithms able to handle third-order data arrays deviating from trilinearity, i.e., when changes in shape and/or position of component profiles from sample to sample occur. It should be stressed that this fact is commonly found in CE data [14]. To overcome this challenge, MCR-ALS is performed in the so-called extended mode, which involves building an augmented data matrix by appending calibration and test data matrices in the time direction, i.e., the rows represent spectra and the columns represent time profiles, because this alleviates the problems associated with sample-to-sample differences in this dimension [15].

Several works have been published concerning the chemometric modeling of CE–diode array detector (DAD) data [16, 17]. In most of them, MCR-ALS has been the algorithm of choice to solve the analytical problems [9, 18–20]. As discussed previously, many papers were presented regarding the combination of CE and second-order modeling. However, to the best of our knowledge, no paper has been published regarding the development of analytical methodologies to be applied in environmental analysis for resolution and quantitation of emergent contaminants. In this work, we present a CE method with DAD coupled to the MCR-ALS algorithm to conduct the resolution and quantitation of a complex mixture of six Qs in water samples.

Materials and methods

Chemicals and reagents

All standards were of analytical grade. Flumenique (FLU), enoxacin (ENO), ofloxacin (OFL), sarafloxacin (SRF), cinoxacin (CIN), difloxacin (DIF), and phenytoin (PHT) were provided by Sigma-Aldrich (Munich, Germany). Enrofloxacin (ENF), ciprofloxacin (CPF), and danofloxacin (DNF) were purchased from Fluka (St. Gallen, Switzerland), and marbofloxacin (MRF) was obtained from Molekula (Gillingham, UK).

LC grade methanol (MeOH) was obtained from J.T. Baker (Deventer, The Netherlands). Ultrapure water was obtained from a Milli-Q water purification system from Millipore (Billerica, MA, USA). Hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium phosphate (NaH_2PO_4), and sodium tetraborate ($Na_2B_4O_7$), all of analytical grade, were purchased from Cicarelli (San Lorenzo, Argentina).

Standards and samples

Stock standard solutions of pharmaceuticals were prepared in MeOH with concentration levels of 200 mg L^{-1} and were maintained under refrigeration at 4 °C in the dark. Working standard solutions were prepared daily by appropriate dilution of the stock standard solutions in water. HCl and NaOH solutions were prepared with concentrations of 1.0 and 0.1 mmol L^{-1} and were used to adjust the background electrolyte (BGE) pH and as washing solutions.

pH values of the BGE solutions were adjusted using a pH meter Hanna Instrument (Smithfield, RI, USA). All solutions and samples were filtered through 0.45 μm nylon membrane (Sartorius, Goettingen, Germany) and degassed in an ultrasonic bath (Cole Palmer 8891, Abbott, Temecula, CA, USA) before use.

Solid phase extraction (SPE) cartridges (Oasis HLB 1 cm^3/30 mg) were purchased from Waters Corporation (Milford, MA, USA).

Calibration, validation, and real samples

For calibration, a set of five standard samples of each analyte was prepared in a concentration range from 20.0 to 100.0 μg L^{-1} by dilution of known amounts of Qs stock solutions in ultrapure water. Besides, five standard mixture samples were randomly prepared in the same concentration levels in ultrapure water for model validation (samples SM 1–5 in Table 1).

Finally, to evaluate the predictive ability of the methodology, a test set containing four real tap water samples were analyzed. Three of them (samples 1 to 3) were randomly prepared in triplicate with concentrations between 40.0 and

Table 1 Prediction results when analyzing the validation mixtures set using MCR-ALS

Analyte	SM1		SM2		SM3		SM4		SM5	
	Nominal/ predicted (μg L^{-1})	Recovery/ CV[a] (%)	Nominal/ predicted (μg L^{-1})	Recovery/ CV[a] (%)	Nominal/ predicted (μg L^{-1})	Recovery/ CV[a] (%)	Nominal/ predicted (μg L^{-1})	Recovery/ CV[a] (%)	Nominal/ predicted (μg L^{-1})	Recovery/ CV[a] (%)
ENO	100.0/80	80/3.1	80.0/76	95/5.2	60.0/47	77/9.2	40.0/40	100/10.2	50.0/50	100/10.8
CPF	20.0/21	105/2.5	20.0/17	80/9.3	80.0/88	110/3.0	80.0/83	103/2.7	50.0/60	118/5.4
OFL	40.0/44	107/6.4	100.0/80	80/10.4	100.0/103	103/4.4	60.0/62	103/6.1	50.0/48	96/3.3
ENF	60.0/57	93/5.0	40.0/50	125/6.8	20.0/21	105/4.3	100.0/103	103/8.6	50.0/52	105/9.8
FLU	20.0/17	85/3.9	100.0/110	110/10.5	40.0/44	110/1.1	80.0/98	123/7.3	50.0/49	98/4.4
CIN	333.0/287	86/3.2	67.0/80	120/1.0	200.0/204	102/1.9	133.0/147	110/1.2	167.0/150	90/2.3

[a] Recovery=(c_{pred}/c_{spiked})×100; c_{pred} is predicted concentration, and c_{spiked} is spiked concentration. $CV(\%) = \left(s/\overline{C}_{pred}\right) \times 100$, where s is standard deviation and $\overline{C}_{pred}$ is the average predicted concentration (n=3)

80.0 μg L^{-1} of ENO, CPF, OFL, ENF, and FLU and from 133.0 to 260.0 μg L^{-1} of CIN. In addition, 50.0 μg L^{-1} of MRF, DNF, and PHT were incorporated as interferents. Sample number 4 containing 50.0 μg L^{-1} of ENO, CPF, OFL, ENF, and FLU and 167.0 μg L^{-1} of CIN was also prepared. This latter sample was spiked with 50.0 μg L^{-1} of five interferents (MRF, DNF, PHT, SRF, and DIF). Then, these solutions were processed in the same way as calibration standards, as explained in the "Solid phase extraction procedure" section.

All the samples were prepared in triplicate by transferring appropriate aliquots of stock solution of each Q and interferences, when necessary, to 25.0 mL volumetric flasks and completing to the mark with Milli-Q water and tap water for the calibration and validation mixtures, respectively.

CE-DAD procedure

All experiments were conducted on a CE system (Agilent Technologies, Waldbronn, Germany) equipped with a DAD. An uncoated fused silica capillary of 50 cm total length (41.5 cm effective length) and 75 μm inner diameter (MicroSolv Technology Corporation, Eatontown, NJ, USA) was used. Separation was performed by applying a voltage of 25 kV and with a typical current of approximately 65 μA. The cartridge was maintained at 25.0 °C. The electropherograms were recorded during 3 min, and the second-order data were obtained by recording UV spectra between 220 and 400 nm each 2 nm at 0.4 s steps. The hydrodynamic injection was performed in the positive electrode of the capillary by applying a pressure of 50 mbar for 10 s.

To condition and activate the capillary, daily rinses were performed with 1.0 mol L^{-1} NaOH, ultrapure water, and BGE for 10 min each. The BGE consisted of a mixture of equal amounts of $Na_2B_4O_7$ and NaH_2PO_4 with a concentration of 15 mmol L^{-1} and adjusted to pH 8.20.

To remove substances adsorbed on the capillary wall, the capillary was flushed between runs with 1.0 mol L^{-1} NaOH, ultrapure water, and BGE for 3 min each. At the end of the day, the capillary was washed with 1.0 mol L^{-1} NaOH (5 min) and ultrapure water (5 min) and then air-dried for 3 min.

Optimization of the electrophoretic conditions

Optimum electrophoretic conditions were set by experimental design and optimization. The design consisted in combining process variables (voltage and pH) and a mixture of BGE salts. After modeling responses, resolution between peak nos. 2 (corresponding to CPF) and 4 (corresponding to ENF), and analysis time, optimization was conducted using the desirability function (see the following section).

Solid phase extraction procedure

The SPE experiments were performed using commercial Oasis HLB cartridges preconditioned with 1.0 mL of MeOH and 2.0 mL of ultrapure water. Samples (25.0 mL) were introduced into the preconditioned cartridges at a flow rate of approximately 3 mL min^{-1}. After that, the cartridges were washed with 2.0 mL of ultrapure water. Since the elution was performed with 250.0 μL MeOH, a 100-fold preconcentration factor was achieved. The extract was injected directly into the electrophoretic system.

Software

The CE ChemStation software (Agilent Technologies, Waldbronn, Germany) was employed for instrument control and data acquisition. Data were saved in ASCII format and transferred to a PC for subsequent manipulation by chemometric algorithms. All the algorithms were implemented in

MATLAB 7.10 [21]. Those for applying MCR-ALS are available in the Internet at http://www.mcrals.info/. Homemade routines based on the Eilers algorithm were applied to perform second-order data baseline correction [22]. Experimental design and desirability function calculations were performed with Design-Expert 8.0.5 [23, 24]. Savitzky–Golay smoothing and differentiation filter was applied to preprocess the data [25, 26].

Results and discussion

General considerations

As previously discussed, the main objective of the present work was to develop an analytical method that combines a fast CE technique for partial resolution of several Qs with a second-order algorithm able to quantitate the target analytes in complex water samples without requiring laborious experimental work. In this sense, our research included several stages. First, a pre-concentration step was performed by using SPE cartridges. Then, the prediction ability of MCR-ALS to resolve and quantitate standard mixtures of the six studied Qs was studied. Finally, the application of the method to the resolution and determination of these compounds in tap water samples was conducted.

Optimization of the separation conditions

An optimization procedure was applied to determine the values of the most important factors that fulfill special requirements for this experiment, i.e., partial separation of the six Qs to reduce the length of the electrophoretic run, but with the requirement of total separation of peak nos. 2 and 4 because, as stated previously, these two compounds share the same spectrum (see Fig. 1). Taking into account that, in this case, the MCR-ALS resolution is based on the differences between the spectra of the analytes, the algorithm is not capable of achieving a successful temporal distinction of analytes that lack selectivity in the spectral mode. In addition, as shown in Fig. 1, the spectra are also highly overlapped.

The design was built by combining a simplex lattice design (including seven combinations of the mixture factors) with a factorial design (including nine combinations of the two process variables). As a result, 63 experiments were performed in 2 blocks (2 consecutive days). First, the amounts of three different salts used to prepare the BGE (sodium borate, NaH_2PO_4, and sodium citrate) were varied using the simplex lattice design, in the range of 0 to 15 mmol L^{-1}, with the following constraints: no pure salt should be used and with a maximum concentration of 10 mmol L^{-1}. This design consists of seven combinations: one central point, three points in the interior of the simplex, and the last three points in the center of the edges. Eventually, nine combinations of the process factors pH (between 7.00 and 9.00) and voltage (between 15 and 25 kV) were studied using the factorial design (see Fig. 2). These factors were selected based on previous experiments in which other factors (temperature, sample injection time, and capillary length) were also tested. In addition, the BGE concentration range (0 to 15 mmol L^{-1}) was also selected after a series of screening experiments.

After performing the experiments, two responses were fitted to polynomials, i.e., the resolution between peak nos. 2 (corresponding to CPF) and 4 (corresponding to ENF) and the analysis time. The model coefficients were calculated by backward multiple regression and validated by ANOVA test [24]. All the possible models, i.e., linear×linear, linear×quadratic, quadratic×linear, and quadratic×quadratic (in the case of combined models) were evaluated. Then, the model was considered satisfactory when the regression was significant for the selected confidence level (i.e., it obtained a p value$\leq$ 0.05). After that, the one which followed the principle of parsimony (or simplicity) was chosen. Thus, the resolution between peak nos. 2 and 4 was fitted by a linear×quadratic combined model, while the analysis time was fitted by a quadratic×linear combined model. For both models evaluated

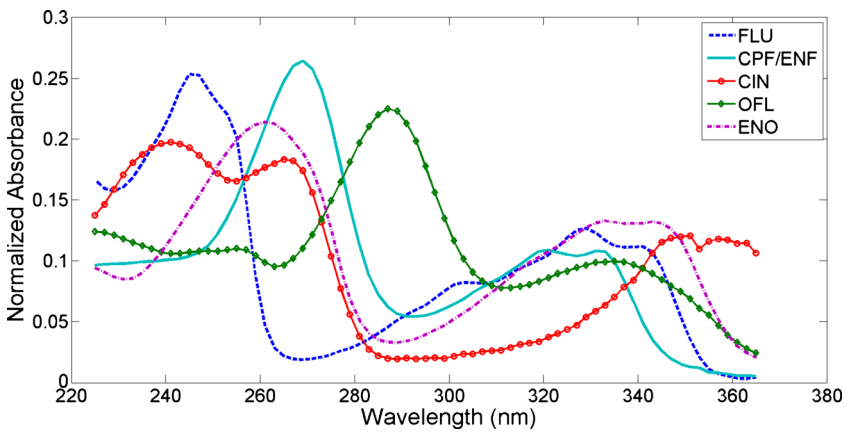

Fig. 1 Normalized spectra for the six Qs in water

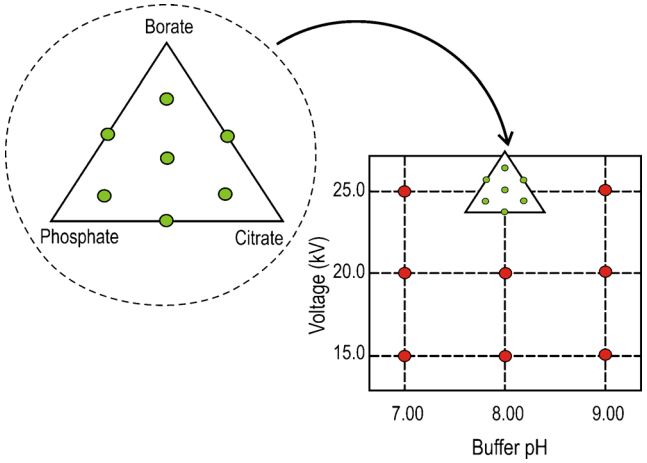

Fig. 2 Experimental design scheme. The *red dots* represent the factorial design points, and the *green dots* represent the mixture design points

in this work, the p values were <0.0001. In addition, the adjusted r^2 values were 0.8746 and 0.9283, respectively.

The optimization was conducted using the desirability function [24] with the following criteria: (a) minimization of the analysis time and (b) resolution between CPF and ENF equal to a target value of 2 (see Fig. 3A). It is important to remark that the complete separation between these two compounds is mandatory to be successful when modeling with MCR-ALS. Under these conditions, complete electropherograms were used for modeling, eliminating the need to cutting regions (see succeeding paragraphs).

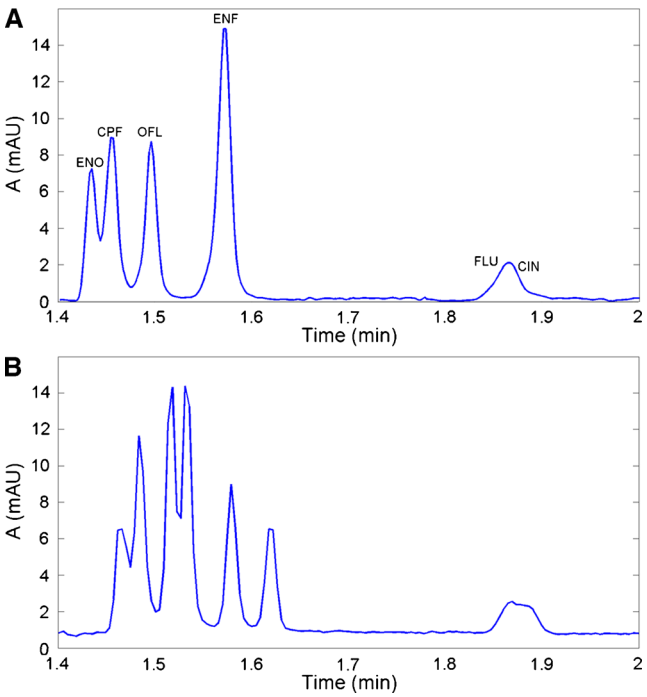

Fig. 3 (**A**) Electropherogram for a mixture after SPE containing 50.0 μg L^{-1} of each Q in Milli-Q water. (**B**) Electropherogram for real sample no. 4 after SPE. This sample contains 50.0 μg L^{-1} of ENO, CPF, OFL, ENF, and FLU and 167.0 μg L^{-1} of CIN and the following interferences: MRF, DNF, PHT, SRF, and DIF spiked at 50.0 μg L^{-1} each (both electropherograms registered at 280 nm). BGE was a mixture of 7.5 mmol L^{-1} $Na_2B_4O_7$ and 7.5 mmol L^{-1}, pH 8.20; voltage was 25 kV

Under the mentioned optimization criteria, the experimental conditions corresponding to one selected maximum in the desirability function (D=1.00) are the following: a mixture of borate (10.0 mmol L^{-1}) and phosphate (5.0 mmol L^{-1}) as BGE; pH, 8.20; and separation voltage, 25 kV. The predicted responses were as follow: resolution, 2.0±0.4; analysis time, 2.2±0.2 min. These values were checked experimentally, and the corresponding electropherogram obtained after SPE for a mixture containing 50.0 μg L^{-1} of each Q is shown in Fig. 3A. No significant differences were observed between pairs of predicted and experimental values. As shown in Fig. 3A, all analytes have a migration time higher than EOF (1.3 min). In addition, overlapping between the different peaks is evident, although the separation between peak nos. 2 and 4 was successfully furnished by performing the electrophoretic runs under the optimized conditions.

Regarding the migration order, the fluoroquinolones ENO, CPF, OFN, and ENF have two relevant ionizable functional groups: the carboxylic group (pK_{a1} in the range of 5.0–6.5) and the N4 of the piperazine ring placed at position 7 (pK_{a2} in the range of 6.0–8.5). At pH values between pK_{a1} and pK_{a2}, the fluoroquinolones are in zwitterionic form. On the other hand, CIN and FLU contain a single ionizable group (pK_a values of 5.20 and 6.60, respectively) [27]. Considering that the separation is conducted at pH 8.20, it can be analyzed if the migration order can be linked to the mass/charge ratio. pK_as, molecular weights, percentages of neutral and negative species at pH 8.20, and migration orders of all the studied analytes are shown in Table 2.

At the working pH, neutral species (zwitterionic) coexist with the corresponding negative ones of each analyte, and their proportion depends on their pK_a. The first Q that migrates is the one with higher amount of neutral species (ENO), and after that, in increasing order of migration, appear those that present a higher amount of negative species. Since CIN and FLU are totally ionized, only negative species exist at the working pH. This fact explains not only their migration in the last position but also the overlapping between them due to the minimal difference in their molecular weight, which can be overcome through further modeling with MCR-ALS.

The complexity of the analytical problem under study can also be observed in Fig. 3, which shows two electropherograms (registered at 280 nm) for a calibration standard composed of six Qs (Fig. 3A) and for a tap water sample spiked with the target analytes and several interferents (Fig. 3B). In the latter figures, peak shifts among different experimental runs can also be observed. Consequently, MCR-ALS was chosen for data processing because this algorithm achieves the second-order advantage without requiring the data to

Table 2 Physicochemical parameters of the studied analytes (pK_as, molecular weights, percentage of neutral and negative species at pH 8.20, and migration order)

Analyte	ENO	CPF	OFL	ENF	FLU	CIN
pK_{a1}	6.32	5.86	6.10	5.88	6.60	5.20
pK_{a2}	8.62	8.24	8.28	7.74	–	–
Molecular weight (g mol^{-1})	320.32	331.25	361.37	259.40	261.25	262.22
Neutral species at pH 8.20 (%)	80	50	50	20	0	0
Negative species at pH 8.20 (%)	20	50	50	80	100	100
Migration order	1	2	3	4	5	6

fulfill the trilinearity property, i.e., migration time and peak shape remain invariant among different runs for each analyte.

MCR-ALS modeling of second-order CE data to resolve standard mixtures of Qs

Owing to the occurrence of considerable peak overlapping between analytes and interferents and significant baseline drift, neither the identification of the analytes nor the application of classical univariate calibration to quantify them is possible.

Elimination of the electropherogram baseline has been shown to be a critical step for reducing the complexity of the matrix sample. With this aim, we applied the asymmetric least squares method proposed by Eilers. It is a multidimensional extension of the spline-based approach with an algorithm taking advantage of the special structure of the data as an array and the model matrix as a tensor product [28]. In addition, spline interpolation filter cube and smoothing were applied to all matrices, taking into account the high level of noise in the data.

The first modeling step consisted in building an augmented data matrix D (1,677×71) in the temporal mode by stacking baseline-corrected data matrices corresponding to each validation mixture followed by those of the standard samples for each analyte.

To build the initial estimation, the analysis of the purest spectra based on the SIMPLISMA methodology was applied to obtain each Q spectra [28]. Interestingly, the number of contributing species in the system under study when applying singular value decomposition was always equal to the real number of components, but two more components should be considered to improve the fitting quality. This fact can be due to the high overlapping in both modes and the presence of a severe background that originated during the preconcentration step (facts that probably originate rank deficiency). Therefore, based on previous knowledge of the system under study, 7 components were selected for modeling the validation mixtures, 10 for those samples presenting 3 interferents, and 12 for those having 5 interferents. After MCR-ALS decomposition of D, concentration information of standard samples contained in C (the areas under the temporal profiles of each component) was used to construct the univariate regression of areas against analyte concentrations.

Figure 4 shows the single temporal profile ($c_{CPF\text{-}ENF}$) retrieved by MCR-ALS when modeling sample number 5 for CPF and ENF. Owing to the fact that these two compounds share the same spectra, they had to be considered as a single analyte, but with two electrophoretic peaks (that is why they have to be completely separated). Therefore, the computed area under the retrieved profile for both analytes in a given sample is the sum of their contributions. To obtain the

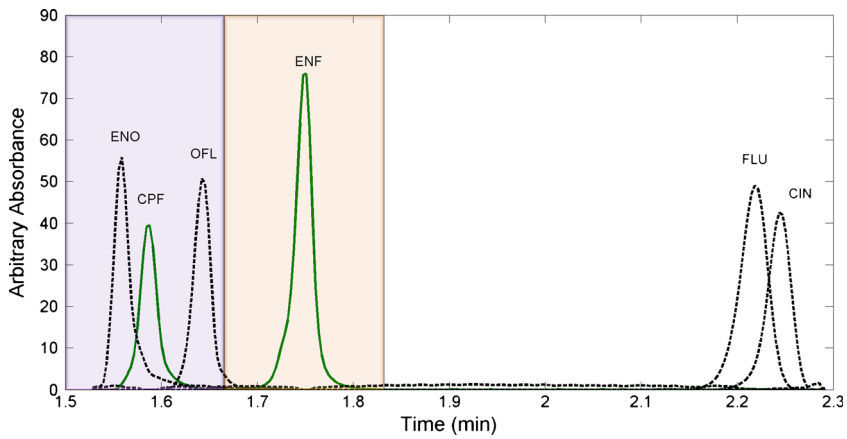

Fig. 4 Concentration profile retrieved by MCR-ALS when processing a mixture of standards (validation mixture number 5). The profiles corresponding to CPF (*left*) and ENF (*right*) in *green solid line*; the other analytes in *black dashed lines*

individual contribution of CPF and ENF and taking into account their migration time, the vector profile was split into two parts, i.e., one region between 1.55 and 1.66 min for CPF and the other region between 1.70 and 1.80 min for ENF, for this particular sample. The way in which the splitting was made can be visualized in the two boxes shown in Fig. 4.

Table 3 summarizes the figures of merit obtained when modeling the data based on the procedure discussed previously. Slope, intercept, and determination coefficient were computed from univariate regression plots after applying a linearity test recommended by IUPAC [29]. The inverse of analytical sensitivity, LOD, and limit of quantitation were calculated for each analyte using the values of SEN computed following ref. [30]. Based on the inspection of the LOD values, the proposed method becomes an alternative for routine laboratories to monitor the levels of the studied analytes in water samples, although the reported concentration levels that can be quantitated by using an MS detector in more complex laboratories (0.5 μg L^{-1}) were not reached [6]. On the other hand, it should be considered that the pre-concentration level can be enhanced by augmenting the volume of sample passed through the cartridge, for example, a tenfold concentration increase is expected if 250.0 mL of water sample was used, with LODs of approximately 0.2 μg L^{-1}. Experiments to check that the breakthrough volume is not surpassed using 250.0 mL were performed, showing that this can be conducted.

Table 1 shows the prediction results of the validation mixtures by applying the developed method. As can be seen, recoveries ranging from 80 to 125 % indicate that the recommended standards for analyzing this kind of samples can be complied [31, 32]. On the other hand, the CV values computed to assess precision show variations between 1 and 10 %. This precision could be considered highly satisfactory, considering that a pre-concentration step was implemented.

Application of MCR-ALS to the analysis of tap water samples

Several samples were prepared to evaluate the predictive ability of the present methodology, i.e., to prove that the method is able to exploit the second-order advantage. The spectra of the compounds MRF, DNF, PHT, SRF, and DIF added to the samples mostly overlapped with the spectra of the analytes under investigation, as shown in Fig. 5A. Moreover, the electrophoretic peaks are also superimposed in the whole time domain (see Fig. 5B).

Data matrices were processed following the same procedure implemented for the calibration and validation data matrices (see the previous section). As a result, MCR-ALS allowed us to obtain not only concentration information but also the spectra and temporal profiles of both analytes and interferents. The spectra of the six Qs and those for the interferents retrieved by MCR-ALS for a tap water sample (sample number 4) are shown in Fig. 5A. In this figure, the five spectra corresponding to the six analytes can be observed (it may be noted that CPF and ENF share the same spectrum); all of them completely overlapped with the five spectra of the interferents. Visual inspection of the temporal profiles extracted by MCR-ALS (Fig. 5B) reveals that there are several coeluting peaks with the analytes having the same migration time, i.e., CPF, OFL, and CIN.

An interesting way to evaluate the quality of the modeling is to compare the retrieved profiles with the normalized real profiles. To quantitate the degree of spectral overlap ($\mathbf{S}_{12}$) between the normalized real spectrum of each Q ($\mathbf{s}_1$) and those recovered by MCR-ALS ($\mathbf{s}_2$), the following expression was employed:

$$\mathbf{S}_{12} = \frac{\|\mathbf{s}_1{}^T \mathbf{s}_2\|}{\|\mathbf{s}_1\| \ \|\mathbf{s}_2\|}. \quad (1)$$

The value of $\mathbf{S}_{12}$ ranges from 0 to 1, corresponding to the extreme situation of no overlapping and complete

Table 3 Analytical figures of merit

Analyte	Slope±SD (L μg^{-1})	Intercept±SD	Determination coefficient (r^2)	Linearity test probability[a] (p>0.05)	Inverse of analytical sensitivity $(\gamma)^{-1}$ (μg L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})
CIN	3.5±0.1	−27±12	0.996	0.271	4.8	15.9	48.2
FLU	12.2±0.2	−15±12	0.997	0.323	1.7	5.0	15.2
CIP	9.9±0.6	74±37	0.976	0.321	5.0	17.5	53.0
ENO	87±4	−26±10	0.992	0.128	3.4	11.2	33.9
ENF	19±1	−54±17	0.984	0.605	3.3	16.5	50.0
OFL	12.5±0.2	−68±15	0.996	0.082	2.0	5.9	17.9

SD standard deviation, *LOD* limit of detection calculated according to ref. [30], *LOQ* limit of quantitation calculated as LOD×(10/3.3)

[a] *F* test for regression

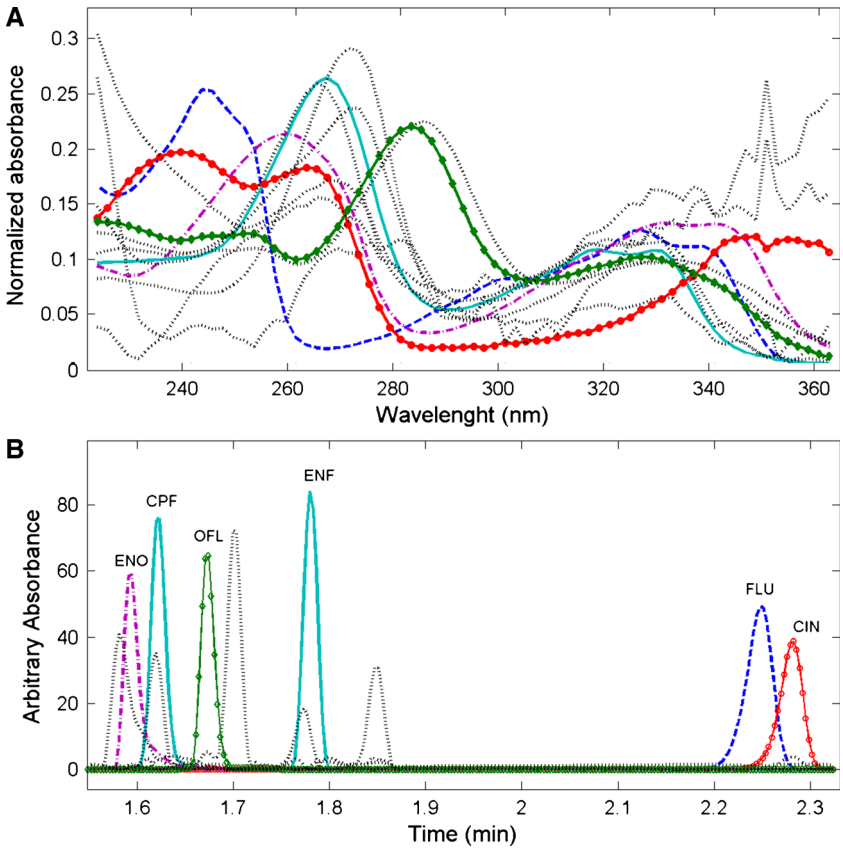

Fig. 5 (**A**) Spectral profiles retrieved by MCR-ALS for tap water sample number 4 with five spiked interferences: the profiles correspond to ENO (*violet dash and dot line*), CPF and ENF (*cyan solid line*), OFL (*green diamond line*), FLU (*blue dashed line*), CIN (*red circle line*), and several unexpected components (*gray solid lines*). (**B**) Concentration profiles retrieved by MCR-ALS for the same samples in (**A**)

overlapping, respectively [33]. Using Eq. 1, the following values were obtained for $\mathbf{S}_{12}$: 0.9968, 0.9992, 0.9997, 1.0000, 0.9992, and 0.9997 for CIN, CPF, ENF, FLU, ENO, and OFL, respectively. These figures allow us to conclude that the quality of the modeling is highly satisfactory.

Table 4 displays the prediction results by applying the present method to the real samples. The recoveries obtained range from 76 to 118 %. These values are similar to those obtained when analyzing the validation set, which contains samples without unexpected components. From this observation, it can be concluded that the second-order advantage is fully exploited. Interestingly, the most complex sample (sample number 4, containing five interferents) furnished the best recoveries for the six analytes. In addition, similar precision to that achieved for the validation mixtures was obtained. Consequently, although extremely complex samples are analyzed

Table 4 Prediction results when analyzing the real samples using MCR-ALS

Analyte	Sample 1[a]		Sample 2[a]		Sample 3[a]		Sample 4[b]	
	Nominal/predicted (μg L^{-1})	Recovery/ CV (%)	Nominal/predicted (μg L^{-1})	Recovery/ CV (%)	Nominal/predicted (μg L^{-1})	Recovery/ CV (%)	Nominal/predicted (μg L^{-1})	Recovery/ CV (%)
ENO	80.0/68	85/4.1	60.0/51	85/6.0	40.0/47	118/3.2	50.0/53	106/3.0
CPF	40.0/69	98/6.5	80.0/79	99/3.2	60.0/53	87/7.7	50.0/49	97/7.0
OFL	60.0/58	96/5.8	40.0/47	118/6.2	80.0/70	88/3.8	50.0/53	106/1.2
ENF	80.0/91	114/9.2	60.0/48	79/3.2	40.0/36	97/4.3	50.0/49	99/6.1
FLU	60.0/49	82/4.7	40.0/36	91/1.6	80.0/61	76/1.9	50.0/46	92/12.3
CIN	133.0/117	88/8.0	267.0/233	88/2.2	200.0/163	82/4.3	167.0/162	97/11.7

[a] Containing three unexpected components (MRF, DNF, and PHT at 50.0 μg L^{-1}, see the text)

[b] Containing five unexpected components (ENO, CPF, OFL, ENF, and FLU at 50.0 μg L^{-1}, see the text)

in only 2.4 min, satisfactory figures of merit were obtained. Besides, the proposed methodology does not involve the use of contaminant solvents.

Finally, following the recommendations of Vander Heyden et al. [34], a robustness study was performed with a two-level Plackett–Burman design (eight experiments), introducing small variations in the optimized conditions: pH (8.15–8.25), voltage (24–26 kV), borate concentration (9.5–10.5 mmol L^{-1}), and phosphate concentration (4.5–5.5 mmol L^{-1}). For each factor combination, the predictions on real sample number 1 were obtained for all the six analytes. From the results obtained in this study, it could be concluded that the studied factors have no significant effect when their levels are varied in the intervals specified in the robustness test ($p>0.05$).

Conclusions

Quantitation of a mixture of six Qs in the presence of unexpected compounds can be conducted by using second-order data generated with CE coupled to DAD. It has been demonstrated once again that modeling data with severe deviation from the trilinearity (as it happens with CE-DAD data) using MCR-ALS is the strategy of choice, owing to its flexibility.

Water samples containing a large number of analytes and interferents can be analyzed in only 2.4 min. By performing the strategy presented in this work, no contaminant solvents should be used in the separation step, which is highly recommended to follow the green analytical chemistry principles [35]. In addition, satisfactory figures of merit can be obtained. Hence, the proposed method becomes an alternative for routine laboratories.

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References

1. Petrovic M, González S, Barceló D (2003) Analysis and removal of emerging contaminants in wastewater and drinking water. Trends Anal Chem 22:685–696
2. Jjemba PK (2006) Excretion and ecotoxicity of pharmaceutical and personal care products in the environment. Ecotoxicol Environ Saf 63:113–130
3. Sui Q, Wang B, Zhao W, Huang J, Yu G, Deng S, Qiu Z, Lu S (2012) Identification of priority pharmaceuticals in the water environment of China. Chemosphere 89:280–286
4. Ortiz de García S, Pinto Pinto G, García Encina P, Irusta Mata R (2013) Consumption and occurrence of pharmaceutical and personal care products in the aquatic environment in Spain. Sci Total Environ 444:451–465
5. Seifrtová M, Nováková L, Lino C, Pena A, Solich P (2009) An overview of analytical methodologies for the determination of antibiotics in environmental waters. Anal Chim Acta 649:158–179
6. Tamtam F, Mercier F, Eurin J, Chevreuil M, Le Bot B (2009) Ultra performance liquid chromatography tandem mass spectrometry performance evaluation for analysis of antibiotics in natural waters. Anal Bioanal Chem 393:1709–1718
7. Speltini A, Sturini M, Maraschi F, Profumo A (2010) Fluoroquinolone antibiotics in environmental waters: Sample preparation and determination. J Sep Sci 33:1115–1131
8. Parrilla Vázquez MM, Parrilla Vázquez P, Martínez Galera M, Gil García MD (2012) Determination of eight fluoroquinolones in groundwater samples with ultrasound-assisted ionic liquid dispersive liquid–liquid microextraction prior to high-performance liquid chromatography and fluorescence detection. Anal Chim Acta 748:20–27
9. Escandar GM, Faber NM, Goicoechea HC, Muñoz de la Peña A, Olivieri AC, Poppi RJ (2007) Second- and third-order multivariate calibration: data, algorithms and applications. Trends Anal Chem 26:752–765
10. Mancha de Llanos A, Zan MM, Culzoni MJ, Espinosa-Mansilla A, Cañada-Cañada F, Muñoz de la Peña A, Goicoechea HC (2011) Determination of marker pteridines in urine by HPLC with fluorimetric detection and second-order multivariate calibration using MCR-ALS. Anal Bioanal Chem 399:2123–2135
11. Booksh KS, Kowalski BR (1994) Theory of Analytical Chemistry. Anal Chem 66:782A–791A
12. Tauler R (1995) Multivariate curve resolution applied to second order data. Chemom Intell Lab Syst 30:133–146
13. Bro R (1998) Multi-way Analysis in the Food Industry. Doctoral Thesis, University of Amsterdam, The Netherlands
14. Olivieri AC, Escandar GM, Muñoz de la Peña A (2011) Second-order and higher-order multivariate calibration methods applied to non-multilinear data using different algorithms. Trends Anal Chem 30:607–617
15. Tauler R, Maeder M, de Juan A (2009) Chapter: 2.24 –Multiset Data Analysis: Extended Multivariate Curve Resolution. In: Brown S, Tauler R, Walczak B (eds) Comprehensive chemometrics. Elsevier, Oxford, pp 473–505
16. Sentellas S, Saurina J (2003) Chemometrics in capillary electrophoresis. Part A: Methods for optimization. J Sep Sci 26:875–885
17. Sentellas S, Saurina J (2003) Chemometrics in capillary electrophoresis. Part B: Methods for data analysis. J Sep Sci 26:1395–1402
18. Vera-Candioti L, Olivieri AC, Goicoechea HC (2008) Chemometric resolution of fully overlapped CE peaks: Quantitation of carbamazepine in human serum in the presence of several interferences. Electrophoresis 29:4527–4537
19. Zhang F, Li H (2005) Resolution of overlapping capillary electrophoresis peaks by using chemometric analysis: Quantification of the components in compound reserpine tablets. Electrophoresis 26: 1692–1702
20. Godoy-Caballero MP, Culzoni MJ, Galeano-Díaz T, Acedo-Valenzuela MI (2013) Novel combination of non-aqueous capillary electrophoresis and multivariate curve resolution-alternating least squares to determine phenolic acids in virgin olive oil. Anal Chim Acta 763:11–19
21. MATLAB 7.10 (2010) The MathWorks Inc., Natick, MA, USA
22. Eilers PHC (2004) Parametric Time Warping. Anal Chem 76:404–411
23. Design Expert 8.0.5 (2010) Stat-Ease Inc., Minneapolis, MN, USA
24. Myers RH, Montgomery D, Anderson-Cook CM (2009) Response surface methodology: process and product optimization using designed experiments, 3rd edn. Wiley, Hoboken, NJ
25. Savitzky A, Golay MJE (1964) Smoothing and differentiation of data by simplified least squares procedures. Anal Chem 36: 1627–1639

26. Luo J, Ying K, Bai J (2005) Savitzky-Golay smoothing and differentiation filter for even number data. Signal Proc 85:1429–1434
27. Herrera-Herrera AV, Ravelo-Pérez LM, Hernández-Borges J, Afonso MM, Palenzuela JA, Rodríguez-Delgado MA (2011) Oxidized multiwalled carbon nanotubes for the dispersive solid-phase extraction of quinolone antibiotics from water samples using capillary electrophoresis and large volume sample stacking with polarity switching. J Chromatogr A 1218:5352–5361
28. Windig W, Guilment J (1991) Interactive self-modeling mixture analysis. Anal Chem 63:1425–1432
29. Danzar K, Currie LA (1998) Guidelines for calibration in analytical chemistry. Part I. Fundamentals and single component calibration (IUPAC Recommendations 1998). Pure Appl Chem 70:993–1001
30. Bauza MC, Ibanez GA, Tauler R, Olivieri AC (2012) Sensitivity Equation for Quantitative Analysis with Multivariate Curve Resolution-Alternating Least-Squares: Theoretical and Experimental Approach. Anal Chem 84:8697–8706
31. EPA-821-R-08-002, Method 1694: Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS. December 2007
32. De Zan MM, Gil García MD, Culzoni MJ, Siano GG, Goicoechea HC, Martinez Galera M (2008) Solving matrix-effects exploiting the second order advantage in the resolution and determination of eight tetracycline antibiotics in effluent wastewater by modelling liquid chromatography data with multivariate curve resolution-alternating least squares and unfolded-partial least squares followed by residual bilinearization algorithms: I. Effect of signal pre-treatment. J Chromatogr A 1179:106–114
33. Culzoni MJ, Goicoechea HC, Ibañez GA, Lozano VA, Marsili NR, Olivieri AC, Pagani AP (2008) Second-order advantage from kinetic-spectroscopic data matrices in the presence of extreme spectral overlapping: A multivariate curve resolution—Alternating least-squares approach. Anal Chim Acta 614:46–57
34. Vander Heyden Y, Nijhuis A, Smeyers-Verbeke J, Vandeginste BGM, Massart DL (2001) Guidance for robustness/ruggedness tests in method validation. J Pharm Biomed Anal 24:723–753
35. Gałuszka A, Migaszewski Z, Namiesnik J (2013) The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. Trends Anal Chem 50:78–84