Green analytical determination of emerging pollutants in environmental waters using excitation–emission photoinduced fluorescence data and multivariate calibration

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ABSTRACT

An eco-friendly strategy for the simultaneous quantification of three emerging pharmaceutical contaminants is presented. The proposed analytical method, which involves photochemically induced fluorescence matrix data combined with second-order chemometric analysis, was used for the determination of carbamazepine, ofloxacin and piroxicam in water samples of different complexity without the need of chromatographic separation. Excitation–emission photoinduced fluorescence matrices were obtained after UV irradiation, and processed with second-order algorithms. Only one of the tested algorithms was able to overcome the strong spectral overlapping among the studied pollutants and allowed their successful quantitation in very interferent media. The method sensitivity in superficial and underground water samples was enhanced by a simple solid-phase extraction with C18 membranes, which was successful for the extraction/preconcentration of the pollutants at trace levels. Detection limits in preconcentrated (1:125) real water samples ranged from 0.04 to 0.3 ng mL^{-1}. Relative prediction errors around 10% were achieved. The proposed strategy is significantly simpler and greener than liquid chromatography-mass spectrometry methods, without compromising the analytical quality of the results.

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1. Introduction

Emerging pollutants are compounds not currently covered by existing water-quality regulations, representing potential threats to ecosystems and human health because of their toxic effects [1,2]. They do not need to persist to negatively affect the exposed organisms, since their introduction into the environment is continuous, especially those belonging to the pharmaceutical group [1–7].

Pharmaceutically active compounds used in both human and veterinary medicine are excreted via feces and urine, partly transformed into glucuronides and sulphates or even unchanged, and are suspected to enter aquatic bodies through the effluents of sewage treatment plants [7–11]. Therefore, continuous efforts are devoted to develop appropriate methods for their monitoring and quantification in natural samples.

Although liquid chromatography–mass spectrometry (LC–MS) is one of the most commonly applied methods for the determination of pharmaceutical compounds and their degradation products in the aquatic environment [2,12,13], greener methodologies, i.e. without separations and clean up steps, and minimizing the use of organic solvents, are very welcome [14].
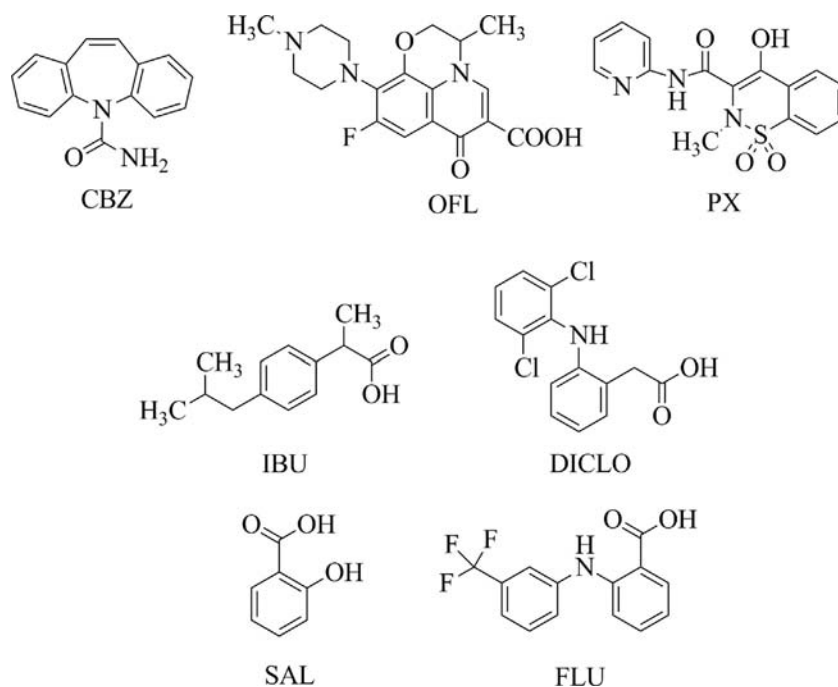
Scheme 1. Structures of carbamazepine (CBZ), ofloxacin (OFL), piroxicam (PX), ibuprofen (IBU), diclofenac (DICLO), salicylic acid (SAL) and flufenamic acid (FLU).

In the present work, three emerging pollutants, representative of different groups of therapeutic drugs, were investigated: the anticonvulsant carbamazepine (CBZ), the antibacterial fluoroquinolone ofloxacin (OFL), and the non-steroidal anti-inflammatory piroxicam (PX) (Scheme 1), because they are frequently found in environmental waters. They display photo-induced fluorescence (PIF) upon UV irradiation, which could allow their quantification. Relatively few molecules are fluorescent, and fluorescent photoproducts are even fewer, or parent compounds are photodegradated after UV irradiation. This may lead to the erroneous conclusion that PIF-based methods are free from interferences. However, as presently demonstrated, in multicomponent systems, the probability of the occurrence of interferences significantly increases and, in principle, clean-up and separation procedures are almost unavoidable.

Recently, our research group quantified CBZ, as a single analyte, in environmental waters using the PIF signals after UV irradiation of acidic solutions in a simple laboratory-constructed reactor [15]. The lack of selectivity was overcome by the second-order advantage of multi-way calibration [16] and pollutant was quantitated in the presence of unknown sample constituents. Second-order data were obtained as excitation–emission photoinduced fluorescence matrices (EEPIFMs) and processed by different algorithms, although successful results were obtained with multivariate curve resolution-alternating least-squares (MCR-ALS) [17].

The critical difference of the present report with the earlier work is that the simultaneous resolution of three usual emerging contaminants which strongly overlap their PIF spectra is presently intended, with the concomitant change in both data analysis and results interpretation. Further, the determinations are performed in solutions containing the analytes and additional pharmaceuticals, such as ibuprofen (IBU), diclofenac (DICLO), salicylic acid (SAL) and flufenamic acid (FLU) (Scheme 1). The latter are profusely employed in our geographical region and may thus be present in real waters, and showed fluorescence signals (either in native form or from their photoproducts) which significantly overlap those of the analytes.

Three chemometric algorithms achieving the second-order advantage, i.e., parallel factor analysis (PARAFAC) [18], MCR-ALS, and unfolded partial least-squares/residual bilinearization (U-PLS/RBL) [19,20], were applied to process the EEPIFMs. Noticeable differences

in the prediction capabilities of the employed algorithms were found and discussed.

To the best of our knowledge, it is the first time that the selectivity offered by the chemometric analysis is evaluated for the simultaneous determination of several analytes using EEPIFMs in very interfering media. The feasibility of determining the three emergent pollutants in real water samples using sustainable resources is demonstrated.

2. Experimental

2.1. Reagents and solutions

CBZ, OFL and PX were purchased from Sigma (St. Louis, MO, USA). Methanol (MeOH), formic acid and hydrochloric acid (HCl) were obtained from Merck (Darmstadt, Germany). IBU, DICLO, SAL and FLU were of analytical grade and were used as received. Stock standard solutions of individual analytes (404.0 µg mL^{-1} CBZ, 420.0 µg mL^{-1} PX and 510.0 µg mL^{-1} OFL) were prepared by dissolving an appropriate amount of each compound in methanol, and stored at 4 °C. Working analyte solutions of 2.0 µg mL^{-1} were daily prepared by dilution of stock standard solutions in ultrapure water. Ultra pure Milli-Q water was used throughout the work.

2.2. Instrumentation

Fluorescence measurements were performed on an Aminco Bowman (Rochester, NY, USA) Series 2 luminescence spectrophotometer, equipped with a 150 W xenon lamp. EEPIFMs were measured in the ranges 246–333 nm (each 3 nm, excitation) and 380–480 nm (each 1 nm, emission), leading to 29 × 100 matrices. Excitation and emission slit widths were of 8 nm using 1.00 cm quartz cells. The photomultiplier tube sensitivity was 600 V and the cell temperature was regulated at 20 °C using a thermostatic bath (Cole-Parmer, IL, USA). EEPIFMs were saved and transferred to a PC for subsequent chemometric analysis.

For the reference chromatographic analysis, see Supplementary material.

Table 1
Calibration samples provided by a central composite design.

Sample	CBZ[a]	OFL[a]	PX[a]
1	30.0	0.0	30.0
2	30.0	60.0	30.0
3	30.0	30.0	0.0
4	30.0	30.0	60.0
5	0.0	30.0	30.0
6	60.0	30.0	30.0
7	12.2	12.2	12.2
8	12.2	47.8	12.2
9	12.2	12.2	47.8
10	12.2	47.8	47.8
11	47.8	12.2	12.2
12	47.8	47.8	12.2
13	47.8	12.2	47.8
14	47.8	47.8	47.8
15	30.0	30.0	30.0

[a] All concentrations are given in ng mL^{-1}.

2.3. Chemometric algorithms and software

For a brief theoretical description of the applied algorithms, see Supplementary material. All routines are written in MATLAB 7.10 [21], and implemented using the graphical interface MVC2 [22], available on the Internet [23]. Design Expert 6.0 (Stat-Ease Inc.) was used for the experimental design.

2.4. Calibration, validation and test samples

A calibration set was built with a central composite design in the concentration ranges between 0.0 and 60 ng mL^{-1} for all analytes (Table 1). The corresponding volumes of the aqueous standard solutions of each analyte were transferred into 2.00 mL volumetric flasks and 2 mol L^{-1} HCl was added to the mark. These solutions were transferred to a 1.0 cm quartz cell, and irradiated during 20 min in a laboratory-constructed reactor described in a previous work [15]. Finally, solutions were cooled to 20 °C and their EEPIFMs were recorded in the conditions described in Section 2.2.

A set of 15 validation samples was prepared and processed in a similar way, having analyte concentrations different from the calibration ones and selected at random from the corresponding calibration ranges.

With the purpose of evaluating the method in the presence of the interfering pollutants IBU, DICLO, SAL and FLU, which have fluorescence signals (either native or photoinduced) overlapped with those for the analytes, 15 samples were prepared containing random analyte concentrations in the range 0–60 ng mL^{-1} and high interferent concentrations: 1000–3000, 100–300, 3000–8000 and 1000–5000 ng mL^{-1} (IBU, DICLO, SAL and FLU, respectively). Since the highest analyte concentration was about 60 ng L^{-1}, interferents were between 2 and 130 times more concentrated.

2.5. Water samples

CBZ, OFL and PX were analyzed in real waters, including river (Paraná River, Argentina), underground (Funes City and Santa Rosa City, Argentina) and tap water (Venado Tuerto City, Santa Fe, Argentina). They were prepared by spiking them with the analytes at two different concentrations between 0.08 and 14 ng mL^{-1}. All samples were sequentially filtered through paper and a 20 μm nylon membrane to remove suspended solids. To improve the sensitivity, most samples were subjected to solid-phase extraction (SPE) with C18 disks. Each disk was previously conditioned with 0.5 mL of MeOH and 1 mL of ultrapure water. Aliquots of either 100 mL (analyte concentrations > 0.2 ng mL^{-1}) or 250 mL (analyte concentrations < 0.2 ng mL^{-1}) were passed through the disks under vacuum, with a flow rate of 10 mL min^{-1}. No pre-concentration was applied for concentrations larger than 6 ng mL^{-1}.

After elution of the retained organic compounds with 500 μL of MeOH, the extract was collected in a 2.00 mL volumetric flask, the solvent was evaporated with nitrogen, and the residue was reconstituted with 2 mol L^{-1} HCl until the mark. This implies pre-concentration degrees of 1:50 or 1:125, depending on the sample volume. Finally, the samples were subjected to the procedure described above, and the analyte concentrations were estimated using second-order multivariate calibration.

Aliquots of the investigated samples were analyzed by LC–MS. A similar SPE procedure was applied, but after elution of the retained organic compounds with 500 μL of MeOH, the extract was collected in a 2.00 mL volumetric flask, reconstituted with ultra pure Milli-Q water until the mark, and injected in the chromatographic system.

3. Results and discussion

3.1. Preliminary studies

As previously reported [15], CBZ is not fluorescent, but emits fluorescence upon UV irradiation in acid media, with excitation and emission maxima at 308 and 410 nm, respectively (Fig. 1A). To obtain the largest signals, optimal working conditions were found to be 2 mol L^{-1} HCl, and 20 min irradiation time with two 4 W germicide tubes separated by 6 cm from each other.

On the other hand, OFL is natively fluorescent (excitation, 290 nm, emission, 500 nm, Fig. 1B). When OFL is irradiated in the above CBZ conditions, fluorescence is observed (excitation, 252 nm, emission, 435 nm), ascribed to fluorescent photoproducts.

Finally, in the case of PX, intense fluorescent signals are detected only at pH < 2 [24]. Under UV irradiation in 2 mol L^{-1} HCl, the PX photoproducts display excitation and emission maxima at 294 nm and 372 nm, respectively (Fig. 1C). This can be mainly ascribed to 2-aminopyridine, which exhibits maxima near the above values [25].

Linear relationships between the original analyte concentrations and the obtained fluorescence intensities were corroborated. Among the three studied analytes, CBZ shows the lowest signals (Fig. 1) and, consequently, the experimental conditions for the quantitative analyses were adjusted in order to optimize the CBZ signals. These conditions were indicated above and were maintained in the subsequent experiments.

3.2. Quantitative analysis

Fig. 2A shows the normalized fluorescence spectra for the CBZ, OFL and PX photoproducts obtained upon UV irradiation under the employed working conditions. It is clear that overlapping occurs among both the excitation and emission spectra, which hinders their direct determination though zeroth-order calibration. The selectivity situation becomes more serious if other fluorescent pollutants are also present (Fig. 2B). Therefore, with the purpose of overcoming this problem avoiding separation steps, second-order calibration of EEPIFMs and applying algorithms achieving the second-order advantage [16] was intended. As already indicated, this advantage implies analyte quantitation in the presence of unsuspected constituents in samples, avoiding the requirement of either interference removal, as in zeroth-order calibration, or the construction of a large and diverse calibration set, as in first-order calibration.
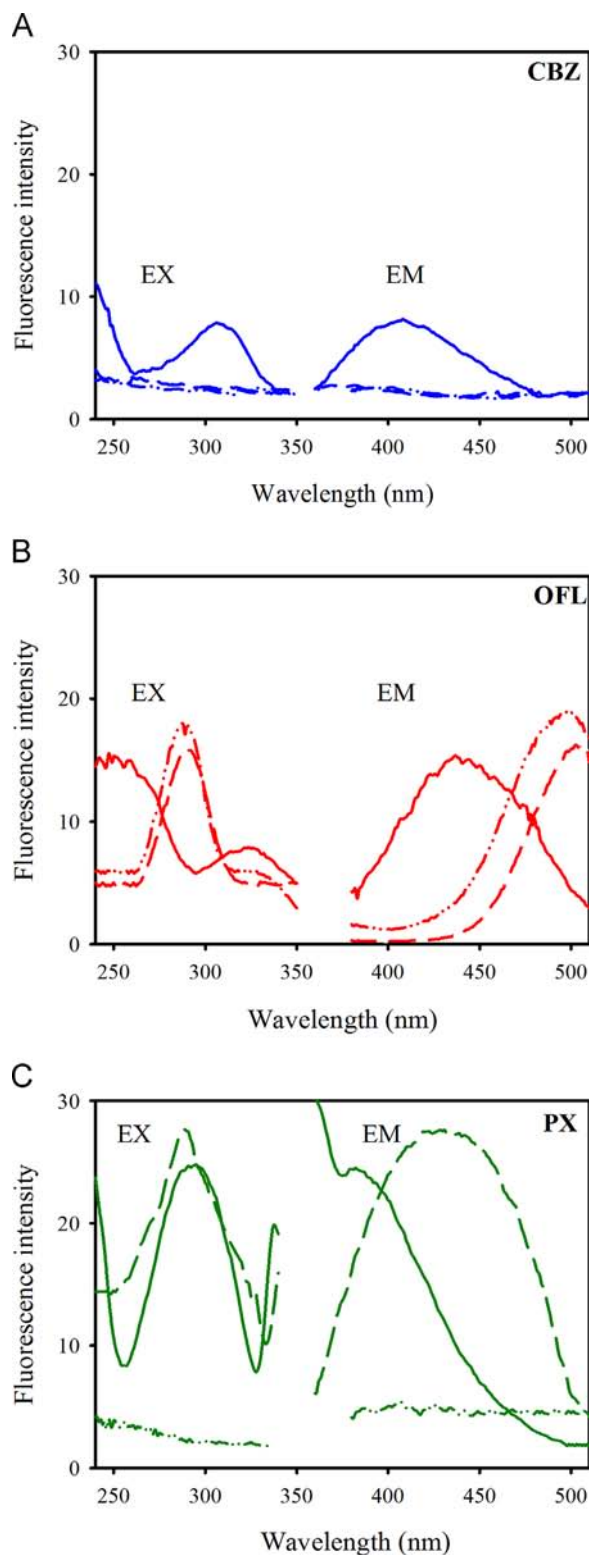
Fig. 1. Excitation and emission fluorescence spectra for aqueous CBZ (A), OFL (B) and PX (C) (dash–dot-dotted line), and in 2 mol L^{-1} HCl, before (dashed line) and after UV irradiation (solid line). $C_{CBZ}=C_{OFL}=C_{PX}=60.0$ ng mL^{-1}.

Fig. 2. (A) Normalized excitation and emission photoinduced fluorescence spectra for CBZ (blue line), OFL (red line) and PX (green line). (B) Comparison with the normalized spectra for DICLO (dashed-gray line), FLU (dashed-cyan line), IBU (dashed-pink line) and SAL (dashed-black line) after UV irradiation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2.1. Calibration and validation samples

After UV irradiation, EEPIFMs were recorded for the calibration and validation samples (Fig. 3A), where only CBZ, PX and OFL are present, and subjected to chemometric analysis. A set of EEPIFMs can be arra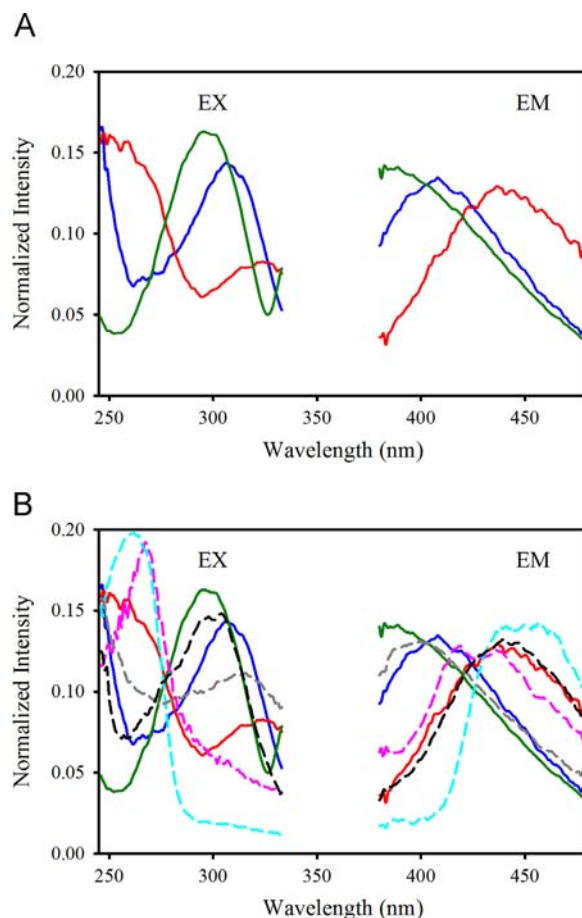nged as a three-way array, which in general complies with the trilinearity conditions [26] and, therefore, the algorithm of choice for data processing should be PARAFAC [27].

The selection of the optimum number of PARAFAC components was performed according to Supplementary material. This number was 4 in validation samples, which can be justified by the presence of three analytes and a background signal.

Fig. 4A illustrates the predicted analyte concentrations when PARAFAC was applied to the validation set. While the predictions for both OFL and PX are in good agreement with the nominal values, the results for CBZ are poor. This conclusion is reinforced by the elliptical join confidence region (EJCR) test [28], which computes the joint confidence interval for the intercept and the slope of the found vs. nominal concentration plot, and checks if the ideal values of 0 and 1 are within the ellipse. Both OFL and PX comply with the test, unlike CBZ. The poor PARAFAC recoveries for CBZ may be ascribed to lack of selectivity, i.e., significant spectral overlapping between weak CBZ signals and those for PX (Fig. 2A).

MCR-ALS, which proved to be the best algorithm for determining CBZ in natural waters by a similar EEPIFM approach [15], was also applied. Since a significant overlapping among the studied analytes occurs in excitation and emission spectra, both augmentation modes were checked, e.g. column-wise (emission spectral) and row-wise (excitation spectral). The optimum number of MCR-ALS components was estimated according to Supplementary material. For both augmentation modes, the number was four, ascribed to three analytes and a background. Non-negativity in both modes was applied, and
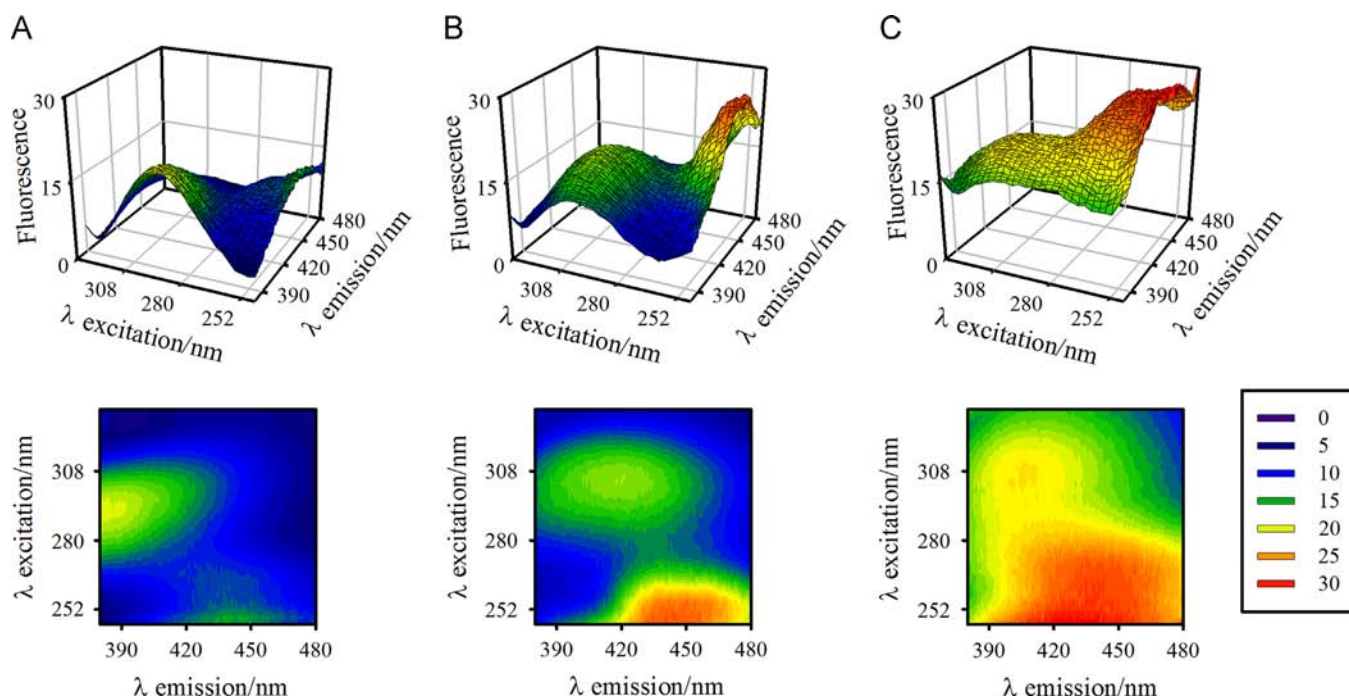
Fig. 3. Three-dimensional and contour plots of EEPIFMs for (A) a validation sample, 28.0 ng mL^{-1} CBZ, 32.0 ng mL^{-1} OFL and 31.5 ng mL^{-1} PX, (B) a test sample, 7.8 ng mL^{-1} CBZ, 8.2 ng mL^{-1} OFL, 16.8 ng mL^{-1} PX, 3000 ng mL^{-1} IBU, 1500 ng mL^{-1} DICLO, 1500 ng mL^{-1} SAL and 100 ng mL^{-1} FLU, and (C) a spiked underground water after solid-phase extraction (original concentrations are: $C_{CBZ}=0.34$ ng mL^{-1}, $C_{OFL}=0.36$ ng mL^{-1} and $C_{PX}=0.34$ ng mL^{-1}).

the convergence criterion was 0.1% (relative fit change for successive iterations).

MCR-ALS in both augmentation modes showed a similar behavior to that of PARAFAC, rendering good results for OFL and PX (Fig. 4B) but unsuitable predictions for CBZ (not shown). This fact was also ascribed to the significant spectral overlapping between CBZ and strong PX signals. Due to these results, PARAFAC and MCR-ALS were not applied to more complex samples.

U-PLS/RBL has been successfully employed in systems requiring processing flexibility [27] and was thus applied to the present system. In a first phase, validation samples were studied with U-PLS, and then considerably more complex samples were analyzed using RBL to model the regression residues as a sum of bilinear contributions from the unexpected components.

The optimum number of latent variables, estimated by cross-validation (see Supplementary material), was four for CBZ and OFL and three for PX. Apparently, due to the relatively high intensities of PX signals, U-PLS does not require an additional component to model the background.

The U-PLS analyte predictions are very good in the validation samples (Fig. 4C), even for the conflicting analyte CBZ. This fact analyte can be justified by the use of latent variables, which are flexible enough to overcome the problem of the high degree of spectral similarity among certain analytes.

From the EJCR test (Fig. 4), we conclude that all ellipses include the theoretically expected point (1,0). For OFL and PX they are significantly smaller than that corresponding to PARAFAC, suggesting better precision. Table 2 supports this conclusion with a relative error of prediction (REP) equal to or less than 10% for all analytes.

It is important to remark that the limits of detection (LODs) were calculated according to a novel IUPAC-consistent estimator [29], which adopts the form of a detection interval, as shown in Table 2.

3.2.2. Test samples

The potential interferents IBU, DICLO, SAL and FLU display signals which strongly overlap those for the target pollutants (Fig. 2B).

Therefore, with the purpose of simulating a genuine situation, test samples containing the analytes and the above compounds, which could be concomitantly present in real samples, were analysed (see Fig. 3B).

For the test samples, U-PLS required, in addition to the calibration latent variables, the RBL procedure with two components, corresponding to the unexpected constituents. Adding more components did not improve the RBL fit. Apparently, U-PLS/RBL considers the profiles of all interferences as two mathematical components, and distinguishes these combined signals from those of the analytes and the background.

Fig. 5A illustrates the excellent U-PLS/RBL predictions for the test samples, and Fig. 5B displays the corresponding EJCR tests, which denote accuracy. The analytical performance of is further appreciated in Table 2. The results are encouraging, taking into account that the simultaneous determination of three analytes is easily and rapidly performed in complex matrices. Considering that photoreactor geometry allows the simultaneous irradiation of four solutions, in about 5 min per sample, and that EEPIFM measurements are performed in 5 min, a throughput of about 6 samples per hour is achieved.

3.2.3. Real water samples

CBZ is one of the most frequently detected drugs in environmental waters all over the world [30]. Due to the fact that CBZ is recalcitrant to various wastewater treatment processes, it is considered as a hydrologic tracer of wastewaters [8]. The incomplete CBZ removal when employing on-site wastewater treatments results in a high probability of groundwater, surface water and, finally, drinking water contamination [10]. It was recently pointed out that CBZ is one of the six pharmaceuticals most often found in finished drinking waters, with levels as high as 0.6 ng mL^{-1} [11]. CBZ concentration values around 0.15 ng mL^{-1} were detected in a municipal wastewater influent in Waco (Texas, USA) [10]. A multi-residue analysis of both human and veterinary pharmaceuticals in surface and treated waters from different sites in Catalonia (Spain) revealed values ranging from 2×10^{-3} to 0.16 ng mL^{-1} for CBZ, and up to 0.31 and
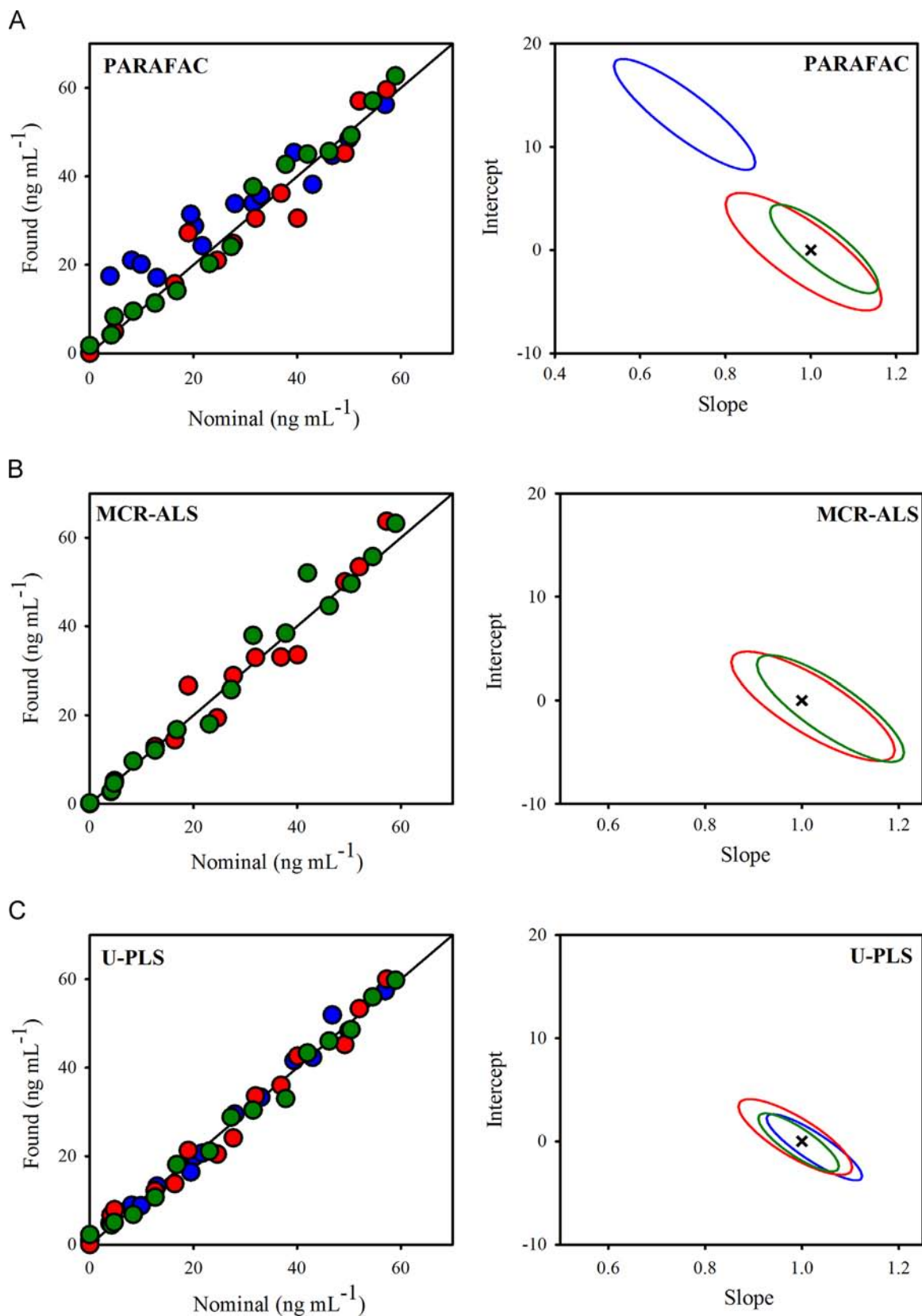
Fig. 4. Plots for the CBZ (blue), OFL (red) and PX (green) predicted concentrations in validation samples as a function of the nominal values (the solid lines are the perfect fits), and elliptical joint regions (at 95% confidence level) for the slope and intercept of the regression of the corresponding data. Black points mark the theoretical (intercept=0, slope=1) point. (A) PARAFAC, (B) MCR-ALS, and (C) U-PLS. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

0.25 ng mL^{-1} for OFL and PX, respectively [31]. CBZ concentrations up to 0.30 ng mL^{-1} have been detected in Serbian waters, similar to other European regions [30]. Likewise, values ranging from 0.22 to 0.80 ng mL^{-1} were reported for OFL and other related antibiotics.

Table 2
Statistical results for CBZ, OFL and PX in validation, test and real water samples using the proposed methodology and U-PLS/RBL.

	CBZ	OFL	PX
Validation samples			
LOD range (min–max)	4–7	3–6	2–4
RMSEP	2	3	2
REP	7	10	7
Test samples[a]			
LOD range (min–max)	4–7	4–6	3–4
RMSEP	2	3	2
REP	7	9	7
Tap water (Venado Tuerto city)			
LOD range (min–max)[b]	0.16–0.22	0.07–0.09	0.05–0.06
LOD range (min–max)[c]	0.31–0.44	0.14–0.18	0.10–0.13
RMSEP	0.03	0.07	0.03
REP	6	11	10
Underground water (Santa Rosa city)			
LOD range (min–max)[b]	0.13–0.18	0.05–0.07	0.04–0.05
LOD range (min–max)[c]	0.28–0.42	0.12–0.16	0.09–0.13
RMSEP	0.04	0.04	0.03
REP	9	10	6
Underground water (Funes city)			
LOD range (min–max)[b]	0.25–0.31	0.11–0.14	0.04–0.05
LOD range (min–max)[c]	0.43–0.57	0.17–0.21	0.13–0.17
RMSEP	0.03	0.04	0.05
REP	8	9	12
River water (Paraná River)			
LOD range (min–max)[d]	5–7	5–7	3–5
LOD range (min–max)[c]	0.1–0.2	0.1–0.2	0.05–0.08
RMSEP	1	2	2
REP	8	9	6

LOD, limit of detection calculated according to ref. 29.
RMSEP, root-mean-square error of prediction.
REP, relative error of prediction.
Values for LOD and RMSEP are given in ng mL^{-1}. Values of REP are giving in %.
The real samples results refer to the original water samples before SPE.

[a] Fifteen samples containing IBU, DICLO, SAL and FLU as interferents.
[b] Pre-concentration factor=125.
[c] Pre-concentration factor=50.
[d] Sample without pre-concentration.

CBZ and OFL were found at concentrations up to 1.2 and 0.58 ng mL^{-1}, respectively, in sewage treatment plant effluents of several European countries, although higher levels of the former (until 6.3 ng mL^{-1}) were detected in Germany and Switzerland [32]. OFL and other two fluoroquinolones have been found in hospital wastewaters (at concentrations of 0.06–120 ng mL^{-1}), in wastewater treatment plant effluents (2×10^{-3}–0.58 ng mL^{-1}) and in surface waters (5×10^{-3}–1.30 ng mL^{-1}) throughout the world, including the United States, Italy, Switzerland, Finland, Sweden, Germany, China, and Australia [4]. OFL was one of the three most reported fluoroquinolone antibiotics in Chinese surface waters, with concentrations up to 5.1 ng mL^{-1} [6].

Since the analytes are generally detected as part- and sub-part-per-billions, pre-concentration with C18 membrane-SPE was applied. The selectivity is provided by the chemometric tool, and the physical separation of target analytes from the matrix is not required, as in traditional extraction techniques.

Because the polarity of the molecules plays a crucial role to achieve efficient extraction in the C18 membrane, the working pH was selected on the basis of the pKas of the target compounds. The latter pKas (2.3 and 13.99 for CBZ [33], 6.05 and 8.11 for OFL [34], and 1.81 and 5.12 for PX [35]) suggest that the uncharged species for CBZ, OFL and PX prevail in the pH 3–13, 7, and 3, respectively. In principle, the selection of an optimal pH for the simultaneous retention of the three analytes is not possible. However, extracting 250 mL of synthetic aqueous samples at pH 3.5, 5.0 and 7.0 containing 0.30 ng mL^{-1} of each analyte demonstrated recoveries nearly 100 % for the three compounds in all cases. This result can be justified considering the small concentration of analytes in the samples, combined with the strong extraction power of the C18 membranes. Therefore, the neutral pH of real samples was not adjusted before the corresponding treatment.

A recovery study was carried out by spiking four different types of waters with the analytes, in duplicate, at two different concentration levels, following the treatment indicated above. Typical EEPIFM plots of a spiked underground water after preconcentration are shown in Fig. 3C. The strong matrix interference is evident. However, the physical removal of these interferences is not necessary when using an appropriate second-order calibration methodology, highlighting the value of the chemometric approach.

RBL was also required for real samples, with two unexpected components in most cases. Adding more components did not improve the fit. The recoveries (Table 3), are statistically
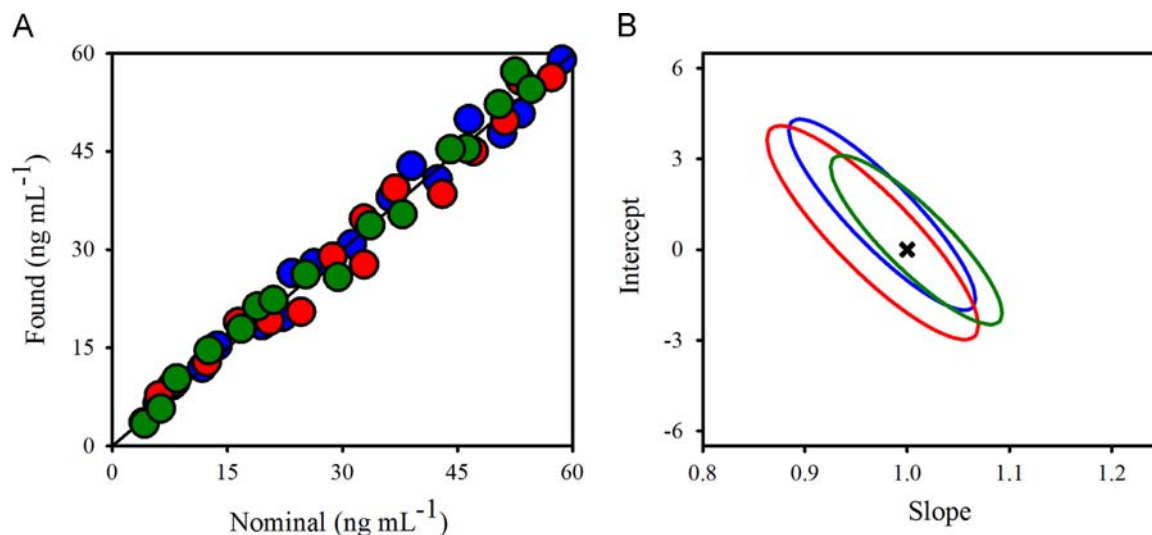
Fig. 5. (A) Plot for CBZ (blue), OFL (red) and PX (green) predicted concentrations by U-PLS/RBL in samples containing potential interferences, as a function of the nominal values (the solid line is the perfect fit). (B) Elliptical joint regions (at 95% confidence level) for the slope and intercept of the regression of the corresponding data. The black point marks the theoretical (intercept=0, slope=1) point. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Recovery study of CBZ, OFL and PX for spiked water samples using U-PLS/RBL algorithm and LC–MS method[a].

Sample	CBZ			OFL			PX		
	Taken	U-PLS/RBL	LC–MS	Taken	U-PLS/RBL	LC–MS	Taken	U-PLS/RBL	LC–MS
Tap water[b]	0.23	0.24 (0.01) [104]	0.20 (0.01) [87]	0.29	0.29 (0.01) [100]	0.28 (0.01) [97]	0.17	0.16 (0.04) [94]	0.17 (0.01) [100]
	0.49	0.54 (0.02) [110]	0.60 (0.01) [122]	0.72	0.81 (0.04) [113]	0.79 (0.04) [110]	0.42	0.38 (0.01) [90]	0.56 (0.02) [133]
Underground water[c]	0.20	0.17 (0.01) [85]	0.23 (0.01) [115]	0.16	0.18 (0.02) [113]	0.19 (0.01) [119]	0.13	0.15 (0.03) [115]	0.12 (0.01) [92]
	0.34	0.39 (0.01) [115]	0.35 (0.01) [103]	0.36	0.39 (0.06) [108]	0.38 (0.02) [106]	0.34	0.36 (0.02) [106]	0.27 (0.01) [79]
Underground water[d]	0.08	0.08 (0.01) [100]	0.10 (0.01) [125]	0.21	0.21 (0.04) [100]	0.21 (0.01) [100]	0.46	0.42 (0.01) [91]	0.43 (0.02) [93]
	0.29	0.24 (0.08) [83]	0.28 (0.01) [97]	0.92	0.9 (0.1) [98]	1.00 (0.01) [109]	0.63	0.53 (0.03) [84]	0.59 (0.02) [94]
River water[e]	13.7	14 (2) [102]	13.6 (0.1) [99]	6.14	6.1 (0.3) [99]	6.0 (0.3) [98]	10.5	11.8 (2.3) [112]	10.1 (0.4) [96]
	0.68	0.75 (0.09) [110]	0.78 (0.01) [115]	1.02	1.0 (0.2) [98]	1.1 (0.1) [108]	0.84	1.0 (0.2) [119]	0.83 (0.03) [99]

[a] Concentrations are given in ng mL^{-1}, standard deviations (mean of two determinations) are given between parentheses, and recoveries (between square brackets) are given in %.
[b] Venado Tuerto city.
[c] Santa Rosa city.
[d] Funes city.
[e] Paraná River.

Table 4
Analytical performance of selected methods recently reported for emerging contaminants in natural waters.

Pre-treatment	Method	Compounds	Other	Medium	LOD[a]	RSD, REP, REC[b]	Sample	Ref.
SPE (Oasis HLB cartridges)	LC–MS/MS	CBZ, OFL, PX	Horm, Pharm	Organic	1×10^{-3} –2.5×10^{-3}	REC=76.9–93.4	WW	[37]
SPE (HLB cartridges)	LC–MS/MS	OFL	A	Organic	2.8×10^{-3}– 6.6×10^{-3}	REC=75–112	RW, WWI, WWE	[38]
SPE (Oasis HLB cartridges)	UPLC–MS/MS	CBZ	Pharm	Organic	2.85×10^{-3}	RSD=2.2, REC=99.3	RW	[39]
SPME (silica fiber-polymeric phase)	LC–DAD	CBZ, PX	NSAI	Organic	2.6–3	RSD=4.6–8, REC=71.6–119.0	RW	[40]
SPME (PDS/DVB/polyacrylate)	LC–DAD–MCR-ALS	CBZ, PX	NSAI	Organic	LOQ=10–20	REC=72–119.3	RW, WW	[41]
SPE (Oasis HLB cartridges)	UPLC–QTOF–MS	OFL	A	Organic	0.4		WWE, WWI, WW, RW	[42]
SPE (Oasis HLB cartridges)	UPLC–MS/MS	OFLO	A	Organic	3.8×10^{-3}	RDS=3–25, REC=33–142	RW	[43]
On line SPE (Strata-X column)	LC–MS/MS	CBZ	OP	Organic	$0.7–1 \times 10^{-3}$		TW, RW	[44]
SPE (Strata-X column)	EC–LIF	OFLO	A	Organic	0.9×10^{-3}	RDS=2.6–9.8, REC=100.6–106.6	UW, TW	[45]
TF-SPME	LC–MS/MS	CBZ	Pharm	Organic	2×10^{-3}	RSD=14, REC=83.7	WW	[46]
MPs (PDS/MWCN)	CLC	OFLO	A	Organic	0.48	RSD=5.9, REC=112	MW	[47]
SPE (Oasis HLB cartridges)	UPLC–QqLIT–MS/MS	CBZ, OFL, PX	Pharm	Organic	0.1×10^{-3}–4.6×10^{-3}	RSD=1.5–15.9 REC=40–124	DW, RSW, RW, SW, WWE, WWI	[31]
SPME (modified silica gel)	LC–UV	CBZ	Pharm	Organic	2.36	REC=65.6	EW	[48]
SPE (Oasis HLB cartridges)	GC–MS	CBZ	Pharm	Organic	8×10^{-3}	REC=92.0, RSD=6.3	RW	[49]
SPE (C18 membranes)	PIF–MCR–ALS	CBZ		Aqueous	0.1–2	REC=78–117, REP=2–7	TW, RW, UW	[15]
SPE (Oasis HLB cartridges)	UPLC–QqLIT–MS/MS	CBZ, OFL, PX	Pharm	Organic	0.2×10^{-3}– 2.4×10^{-3}	RSD=2.5–15.9, REC=56–124	DW, RW, WE	[30]
SPE (Oasis HLB cartridges)	LC–TOF–MS	CBZ, OFL	EP	Organic	LOQ=2.5×10^{-3}– 3.1×10^{-3}	RSD=1–15, REC =30–128	RW, WW	[13]
SPE (C18 membranes)	PIF–U-PLS/RBL	CBZ, OFL, PX		Aqueous	0.04–0.3	REP=6–12, REC=83–119	TW, RW, UW	This work

[a] For comparison, concentration units were unified to ng mL^{-1}.
[b] Relative standard deviation (RSD), relative error of prediction (REP), and recovery (REC), all in %.

comparable to those provided by LC–MS at a 95% confidence level [36]: the experimental t-coefficients for U-PLS/RBL in the cases of CBZ ($t=0.74$), OFL ($t=0.35$) and PX ($t=1.02$) favorably compare with the tabulated value for $n-1$ degrees of freedom [t_{crit} $(0.05,7)=2.36$], suggesting that foreign compounds which may be present in the studied samples do not produce a significant

interference in our analysis. Finally, the good analytical performance for U-PLS/RBL can be appreciated from the statistical results shown in Table 2. These results indicate that the REP is not significantly affected by the fact that real samples are being studied. Besides, LODs reflect the benefits of the pre-concentration, and the possibility of determining the studied analytes at

sub-part-per-billion levels. It should be noted that an SPE employing a larger sample volume would allow to decrease the LOD even more.

In Table 4, a comparison with selected methods for the determination of the studied compounds in water samples is performed, including already cited reports and additional ones. [37–49] The great advantage of the proposed approach is that it allows the determination of the analytes using very simple equipment and without involving significant amounts of organic solvents. As a consequence, the experimental time and the errors associated with multiple experimental steps are substantially diminished, working at the same time under the green chemistry principles.

4. Conclusions

A sustainable photoinduced fluorescence method, suitable for the simultaneous determination of CBZ, OFL and PX at trace levels without the need of chromatographic separation, has been developed. The method is assisted by second-order chemometric analysis and representing a new example of the power of coupling the partial least-squares algorithm with residual bilinearization for the resolution of very complex systems. The beauty of this procedure is that it achieves an outstanding selectivity avoiding the use of toxic organic solvents, a fact which is essential for the environmental safety. In addition, the method is fast, allowing a sample throughput of about 6 samples per hour. On the basis of the obtained results, one can assert that the proposed method favorably compares with more sophisticated approaches.

Acknowledgments

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2014.11. 022.

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