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Direct Measurement of Organic Micropollutants in Water and Wastewater Using Fluorescence Spectroscopy

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Cite This: *ACS EST Water* 2023, 3, 3905−3915

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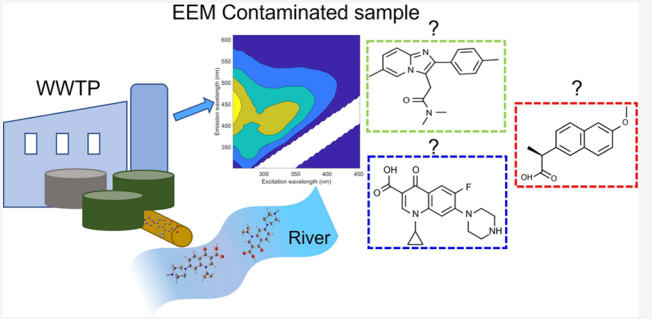
ABSTRACT: Quantifying organic micropollutants (OMPs) in aquatic environments and assessing their removal by water treatment requires expensive and time-consuming analyses typically using liquid chromatographic separation and tandem mass spectrometry (LC-MS/MS). In this study, we evaluated the potential for detecting fluorescent OMPs via spectroscopy, which is cheap, rapid, and widely accessible. The method involved using a priori PARAFAC models to eliminate interfering background fluorescence emitted by naturally occurring dissolved organic matter. Of 20 screened pharmaceutical OMPs, three (ciprofloxacin, naproxen, and zolpidem) with calculated fluorescence quantum yields 0.14, 0.21, and 0.71, respectively, could be quantified in the low μg L^{-1} range when added alone or in combination to water samples without any sample pretreatment other than filtration and pH adjustment. Limits of detection for all three OMPs were 1.0−3.3 μg L^{-1} in surface waters, while in wastewater, they were 0.6−9.0 μg L^{-1} for ciprofloxacin and naproxen and 1.0−2.6 μg L^{-1} for zolpidem. Given the high cost of pharmaceutical analyses and widespread availability of fluorometers, the new approach will improve access to rapid and cost-effective results by supporting data-intensive lab-scale studies, wherein the types of OMPs studied and their concentration ranges are under the control of the analyst.



KEYWORDS: *contaminants of emerging concern (CECs), wastewater, pharmaceutical, PARAFAC, fluorescence excitation−emission matrices, quantum yield*

1. INTRODUCTION

Organic micropollutants (OMPs) are increasingly detected at high concentrations in aquatic milieu.[1] OMPs comprise a broad spectrum of chemical compounds including pharmaceuticals, personal care products, pesticides, surfactants, and per- and polyfluoroalkyl substances (PFAS) among others.[2,3] So-called contaminants of emerging concern trigger known or potential adverse ecological and/or human health effects, yet they are usually excluded from routine monitoring programs due to the lack of regulation criteria.[4,5] Due to the high consumption of pharmaceutical OMPs and their massive environmental release, many have been detected at ng L^{-1} to μg L^{-1} concentrations in water resources, including surface water, groundwater, wastewater, and drinking water.[1,6,7] In developed countries, the main route of contamination is treated urban wastewater discharged to surface waters, which occurs because wastewater treatment plants (WWTPs) were not historically designed to remove these contaminants.[8]

The most common analytical methodology used to quantify OMPs in aquatic samples is liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).[6,9,10] While sensitive and accurate, analysis by LC-MS/MS is costly due to time-consuming sample pretreatment (e.g., extraction procedures to remove interferents, preconcentration) and advanced analytical equipment.[10] This limits data access to well-equipped chemical laboratories and well-funded projects, which places severe throughput constraints on most studies. Accordingly, for cost reasons at WWTPs, it is typical to limit OMP measurements to the influent and effluent water,[10] which hinders studying removal mechanisms inside the plant. An advantage of laboratory batch studies is the opportunity to elucidate mechanisms and processes via detailed, time-resolved experiments. However, high analysis costs encourage a low level of replication (or no replication) and small numbers of treatments and time points. Having low-cost, rapid "screening" methods for at least some OMPs would provide inexpensive models for the behavior of other OMPs with similar molecular structures.

Fluorescence and absorbance spectroscopies are widely used to study the chemical composition of water, and instruments for performing three-dimensional excitation−emission matrices

Received: June 19, 2023
Revised: October 26, 2023
Accepted: October 27, 2023
Published: November 15, 2023

(EEMs) are relatively low-cost instruments found in water quality laboratories around the world, including often in university departments of civil engineering. Many OMPs, including many pharmaceuticals, have an aromatic molecular structure that causes them to emit fluorescence when dissolved in water. Dozens of fluorescent OMPs have high detection frequencies in wastewater-impacted milieu, including certain antibiotics (e.g., ciprofloxacin), nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g., naproxen), and hypnotics (e.g., zolpidem), as well as numerous antidepressants, antiepileptics, antifungals, antihypertensives, antineoplasics, anxiolytics, and hormones.[1] The fluorescent antibiotic ciprofloxacin and the NSAID naproxen have very high detection frequencies in waterbodies, with concentrations exceeding 100 μg L^{-1} recorded in wastewater.[11,12]

Accurate measurement of fluorescent OMPs using spectroscopy without preconcentration or chemical modification is currently limited to situations in which the OMP is both intrinsically highly fluorescent and present at very high concentrations. However, in almost all natural and artificial water matrices, e.g., lakes, drinking water, and wastewater, the OMP fluorescence is obscured by fluorescence emitted by naturally occurring dissolved organic matter (DOM). DOM compounds are the major light-absorbing chemicals in all natural waters, and their emission peaks are in the range of 300−600 nm where they collide with the native fluorescence spectra for many OMPs.[10] In some cases, chemical reactions may be induced to produce fluorescent products with spectra at wavelengths unobscured by DOM,[13] but this can be technically complicated and has limited applicability. Widely accessible reagent-free methods that support high-throughput and rapid quantification of even a handful of environmentally important OMPs would help to alleviate a significant bottleneck in laboratory studies seeking to predict or evaluate the outcomes of water treatment processes.

Quantifying low-level OMP fluorescence in real samples despite the presence of interfering DOM can be achieved only if the DOM background is known a priori. At that point, the background can be simply removed by blank subtraction, leaving only the fluorescence due to OMPs plus noise. In real situations, this is normally considered impossible because DOM varies in space and time, which necessitates a new blank for each new sample. A novel way to surmount this challenge is by leveraging recent advances in the field of DOM fluorescence spectroscopy. Specifically, recent studies show that fluorescent components determined by parallel factor analysis (PARAFAC) are highly conserved across diverse aquatic environments.[14−16] This means that after a sample is collected and analyzed by fluorescence EEM spectroscopy, the data can be passed through automatic or semiautomatic postprocessing algorithms to subtract DOM fluorescence and quantitatively recover the concentration of fluorescent OMPs despite varying background water matrices.

In this study, we aimed to provide a toolbox for water quality practitioners consisting of a collection of methods that support cheap and rapid quantification of OMPs in water and wastewater at relatively low concentrations (>3 μg L^{-1}) without complicated sample pretreatment or complicated postprocessing. This is appropriate for field studies where specific fluorescent OMPs are known to be present at such concentrations or laboratory studies where OMPs can be seeded at specific concentrations. Our methodology leverages the latest advances in the field of DOM fluorescence spectroscopy to rapidly distinguish OMP signals from interfering DOM. A critical novel aspect was that blank subtraction for diverse samples was achieved using assumed spectral properties for DOM fluorescence, obviating the usual purpose of PARAFAC modeling which is to determine such properties. Furthermore, unlike most PARAFAC applications these methods do not require large data sets and can be used to quantify OMPs in single samples. The fact that this approach succeeded validates the underlying assumptions and paves the way for further novel methods leveraging our growing comprehension of DOM fluorescence.

2. MATERIALS AND METHODS

2.1. Reagents and Solutions. Analytical-grade chemical standard atenolol, carbamazepine, ciprofloxacin, citalopram, clarithromycin, diclofenac, erythromycin, β-Estradiol, 17α-Ethynylestradiol, ibuprofen, levonorgestrel, losartan, metoprolol, naproxen, oxazepam, sulfamethoxazole, tramadol, trimethoprim, venlafaxine, and zolpidem were purchased from Sigma-Aldrich (Massachusetts).

Phosphate-buffered saline tablets, sodium hydroxide, and hydrochloride acid (HCl, 37%) were purchased from Fisher Reagent (Pennsylvania), Sigma-Aldrich (Massachusets) and Fisher Chemical (U.K.), respectively. Ultrapure water was obtained from an in-lab Millipore system (Bedford). Ethanol and methanol were purchased from Fisher Scientific and Sigma-Aldrich, respectively.

Stock solutions of 100 mg L^{-1} were prepared in 0.025 mol L^{-1} HCl, 70% methanol, or 70% ethanol and stored in the dark at 4 °C. Working solutions were prepared by diluting the stock solutions with ultrapure water to reach concentrations of 100 μg L^{-1}.

2.2. Fluorescence Spectroscopy Measurements. Fluorescence and absorbance measurements were made on an AquaLog fluorometer (HORIBA) using a 10 mm quartz cuvette (Helma Analytics). Excitation−emission matrices were recorded in the emission wavelength range of 246−823 nm (increment ∼ 4.65 nm) with integration times between 0.5 and 3 s and at excitation wavelengths between 239 and 620 nm (increment 3 nm).

2.3. Screening and Optimization. Initially, screening tests were conducted to identify pharmaceuticals with high fluorescence in the range of pH expected in water treatment plants (pH 6−8). As a result of screening, all but three of the original 20 OMPs were eliminated from further studies (Supporting Information Table S1). Elimination was due to one or more of the following factors that reduce measurement precision, sensitivity, and accuracy: (1) low molar fluorescence, (2) uncertain emission spectrum due to scatter interference (Rayleigh or Raman), and (3) uncertain excitation spectrum due to excitation peaks occurring only at short wavelengths where there is high lamp-related noise (<240 nm). After screening, only ciprofloxacin (CIP), naproxen (NAP), and zolpidem (ZOL) were retained for further testing.

Since fluorescence spectra can be sensitive to pH, the effect of pH on spectral properties was investigated in detail for CIP, NAP, and ZOL. EEMs were collected for 100 μg L^{-1} solutions of each OMP standard dissolved in phosphate buffer (0.01 mol L^{-1}) after adjusting pH to specific values (6, 6.5, 7, 7.5, 8, 8.5) using NaOH or HCl. These tests indicated all three chemicals exhibited relatively high fluorescence at pH 7.5 (Supporting Section S1). Therefore, in subsequent experiments, all samples

https://doi.org/10.1021/acsestwater.3c00323
ACS EST Water 2023, 3, 3905−3915

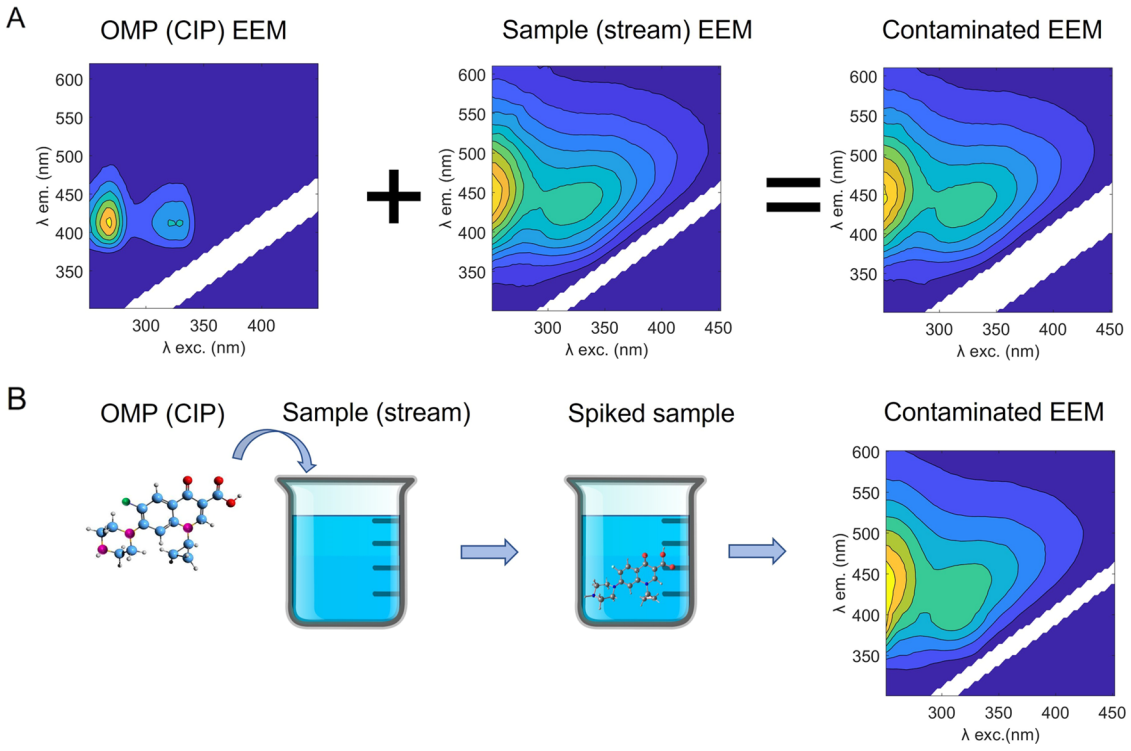


Figure 1. Methodology for creating "contaminated" samples in this study. Top row (A): in silico (simulated) data sets were created by mathematical addition of fluorescence EEMs from OMPs and water samples and bottom row (B): spiked (validation) data sets were created by physically spiking OMPs into water samples.

were adjusted to pH = 7.5 prior to measurement (see further below).

The quantum yields of the three OMPs were calculated with reference to quinine sulfate and salicylic acid standards, following the procedure of Wünsch et al.[17] as described in the Supporting Information (Supporting Section S2).

2.4. Study Area and Sampling Sites. Samples were collected from six sites in or near the city of Gothenburg, located on the west coast of Sweden, in April−November 2022. For simplicity in this paper, these samples are classified as either "natural samples" or "wastewater samples", representing samples with relatively low or high organic carbon content, respectively.

Natural samples (N = 34) included both environmental samples (river, stream, pond, or lake) and samples from a drinking water treatment plant (DWTP) in the region of Gothenburg, Sweden. The DWTP draws water from the river Göta Älv which drains water from lake Vänern 93 km upstream. At the DWTP, samples (N = 11) were collected in October from untreated (raw) river water and partially treated water from the full-scale plant and a pilot plant. Environmental samples were collected from a small stream in April (N = 1), lakes Delsjön (N = 1) and Radasjön (N = 1) in November, and 20 ponds and lakes in the wider Gothenburg area during October to November. Lakes Delsjön and Radasjön are raw water sources for additional DWTPs not sampled in this study.

Wastewater samples (N = 28) were from a wastewater treatment plant (WWTP) that treats effluent from about 970 000 person equivalents with an average flow of 4 m^3/s prior to discharging in river Göta Älv.[18,19] The WWTP has a combined sewer system receiving mainly domestic wastewater and, to a lesser extent, industrial wastewater. The treatment process includes screening, grit removal, primary settlers, high-loaded activated sludge for pre-denitrification and simultaneous precipitation, trickling filters for nitrification, secondary settlers, nitrifying, and denitrifying moving bed bioreactors (MBBRs), disk filters, and a pilot granular activated carbon (GAC) filter. The wastewater samples were collected in two periods: June 9, 2022 and November 21−25, 2022. The June samples (N = 3) were from three process stages: influent, after nitrifying MBBR, and effluent, whereas the November samples (N = 25) were collected daily for 5 days from five process stages: influent, after nitrifying MBBR, and before/after two sets of pilot GAC filters (GAC influent/GAC effluent). GAC effluent samples (N = 10) were from two process lines with different flow rates. Hereafter, the GAC influent and GAC effluent will be referred to as "GAC in" and "GAC out", respectively.

All samples were filtered after collection. Natural samples were filtered with 0.45 μm polyether sulfone (PES) syringe filters. Wastewater samples were highly turbid so were first centrifuged at 3500 rpm for 10 min, then filtered using 1.6 and 0.7 μm glass microfiber filters, and finally passed through 0.45 μm PES syringe filters. After filtration, samples were stored at 4 °C in amber glass bottles until analysis.

2.5. Calibration Samples. For each OMP, a calibration set of six standard samples was prepared in triplicate by transferring appropriate aliquots of the CIP, NAP, or ZOL working standard solutions to 5.00 mL volumetric flasks of 0.01 mol L^{-1} phosphate buffer pH 7.5. The final concentrations of the calibration samples were in the range of 0.1−50.0 $\mu g\ L^{-1}$.

2.6. Experimental Design. Two types of data sets were constructed from which OMP detection limits were determined in each natural or wastewater sample. First, in silico (simulated) data sets were constructed, by mathematical

https://doi.org/10.1021/acsestwater.3c00323
ACS EST Water 2023, 3, 3905−3915

addition of the EEM of each OMP in ultrapure water to the EEM of each water sample (Figure 1A). Second, real data sets were constructed by spiking each OMP at low concentrations into each water sample (Figure 1B). The simulation experiments represented the "best-case scenario" for quantifying each OMP, while the spiking experiments both validated this approach and provided a more realistic assessment of the detection limits for each OMP in each water matrix.

Specifically, "contaminated" water samples in simulated data sets were obtained by modifying the original water EEMs to include OMP fluorescence signals (either one OMP or all three together). First, each OMP EEM was scaled according to its calibration curve at pH 7.5 to simulate a final concentration selected randomly from the range of 1.0−20.0 μg L^{-1}. In contrast, "contaminated" water samples in spiked data sets were generated using known concentrations of OMPs (1.0−50.0 μg L^{-1}) added to the water samples either individually or as mixture of all three OMPs (Table S2). First, the filtered water samples were adjusted to pH 7.5, and then appropriate aliquots of the OMP working solutions were injected into 5.00 mL samples of natural water or wastewater. This procedure was performed in duplicate.

Wastewater samples can contain high pharmaceutical concentrations; therefore, background OMP concentrations were determined prior to spiking. The true concentrations of OMPs in these samples after spiking was subsequently determined as the sum of background concentrations plus the spiked concentration. Background OMP concentrations were determined by UPLC-MS/MS at MoLab-Environmental analytical laboratory, Kristianstad University, following the methodology of Gidstedt et al.[20] In natural samples, it was assumed (and later confirmed) that background OMP concentrations would be far lower than the detection limits for the developed method.[5,21] For these samples, the true concentrations of OMPs in spiked samples were assumed to be equal to the added concentration of OMPs.

In each case, the analysis procedure after constructing the "contaminated" data set was the same: each data set consisting of 1−25 samples was modeled using PARAFAC to identify and quantify the signals due to each OMP contaminant and separate these from signals due to natural organic matter. Signals due to natural organic matter were assumed to conform with a pre-existing PARAFAC model developed previously using either natural samples (NW model) or wastewater samples (WW model). An OMP was identified if it matched the pure spectra of the same OMP dissolved in ultrapure water with a near-perfect Tucker congruence coefficient (TCC > 0.98 in excitation and emission).[22]

2.7. Data Preprocessing. The fluorescence data sets were processed in MATLAB R2022a (v9.12, Mathworks Inc.) using the drEEM toolbox, version 0.6.5.[22] Inner filter effects were eliminated with the absorbance-based method[23] and fluorescence signals in each EEM were normalized using the Raman peak area of ultrapure water at 350 nm. Data preprocessing also included setting first- and second-order Raman and Rayleigh scatter bands to missing numbers. Thereafter, both Raman scatter bands and the second-order Rayleigh scatter band were replaced with interpolated data. Lastly, fluorescence emission wavelengths were restricted to the range of 300−620 nm, and excitation was restricted to the ranges of 250−452 nm (natural samples) and 250−620 nm (wastewater samples).

2.8. Parallel Factor Analysis. Traditionally when applying PARAFAC modeling to DOM samples, it is assumed that the number of independent natural organic matter components and their spectral properties are unknown, and a new PARAFAC model is constructed for each new data set especially if different sources are involved. The downside is that it can be difficult and time-consuming to identify a reliable model, especially when fluorescence intensities are highly correlated as is typical when sampling throughout a water treatment plant.

In the current study, a novel approach was used to capture interfering DOM fluorescence in natural samples compared to wastewater samples. Recent studies suggest that the underlying fluorescence components in natural river and lake sources around the world are highly similar[14,16,24] suggesting an opportunity to use a pre-existing PARAFAC model to capture natural DOM signals. An a priori model ("NW model") was chosen with spectra matching the Swedish "Kungalv5" model of Moona et al.[25] which itself closely matches the global SUEZ8 model of Philibert et al.[16] Reusing a model with broad applicability circumvents one of the main drawbacks of the traditional PARAFAC approach, i.e., building a reliable PARAFAC model.

For wastewater samples, no prior model was available so a new wastewater model ("WW model") was developed from a subset of samples collected from the WWTP. The data set behind the WW model consisted of 20 EEMs of samples collected from the WWTP in the MBBR and subsequent treatment steps (MBBR, GAC in, GAC out) during the November sampling period. PARAFAC modeling was conducted using the drEEM toolbox[22] in conjunction with the N-way toolbox[26] and PLS_Toolbox (Eigenvector Inc.). All models were constrained with non-negativity to fit components with positive scores and loadings. Models with three to seven components were explored, and a five-component model provided the best representation of the data set.

2.9. Recovery of OMP Concentrations Using PARAFAC. Two different modeling approaches for recovering OMP concentrations were tested, hereafter referred to as the "partially specified" and "fully specified" approaches.

In the "partially specified" approach, the spectral properties for natural organic matter components were assumed to be known a priori, while the properties of the corresponding OMP contaminants were assumed unknown. Thus, the excitation and emission loadings for five components were assumed to match the prior PARAFAC model, and n, $n + 1$, or $n + 2$ additional components were estimated, with n equaling the number of OMPs added to the samples. The additional n components were included to capture the individual pure spectra of each added OMP, while the additional "+1" components were included to capture additional non-OMP spectra or noise (Figure S2A). This approach might be used in practice to quantify one or more strongly fluorescent OMPs with unknown spectral properties.

In the "fully specified" approach, the spectral properties for each OMP was obtained by applying 1-component PARAFAC to the dilution series of the OMP dissolved in buffer solution.[27] Thereafter, the prior 5-component PARAFAC model was augmented with n additional components matching the spectral loadings of OMPs added to the sample (Figure S2B,C). From here, the data set was simply projected upon the augmented PARAFAC model to obtain the scores of each component according to a least-squares fit, and then the

https://doi.org/10.1021/acsestwater.3c00323
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Table 1. Optical Properties of Ciprofloxacin (CIP), Naproxen (NAP), and Zolpidem (ZOL) Dissolved in 0.01 mol L^{-1} Buffer Phosphate at pH 7.5

OMP	molecular weight (g mol^{-1})	λexc (nm)	λem (nm)	molar fluorescence (R U $\mu mol^{-1}L$)	molar absorbance (L mol^{1} cm^{-1})	stokes shift (eV)	QY	
							QS	SA
CIP	331.34	269	410	8.9	34022			
		329			14165	0.75	0.13 (0.01)	0.14 (0.01)
NAP	230.26	263	350	6.1	9613			
		323			2483	0.30	0.19 (0.03)	0.21 (0.02)
ZOL	307.40	308	382	11.6	6232	0.78	0.65 (0.03)	0.71 (0.02)

Molar fluorescence is reported for the absorbance maximum; molar absorbance is reported for the absorbance maximum and, when present, the secondary absorbance peak. Stokes shift and quantum yield (QY) are reported relative to the lowest energy excitation peak. QYs were determined with reference to quinine sulfate (QS) or salicylic acid (SA) with standard deviations (in parentheses) estimated using a 95% confidence interval.[17]

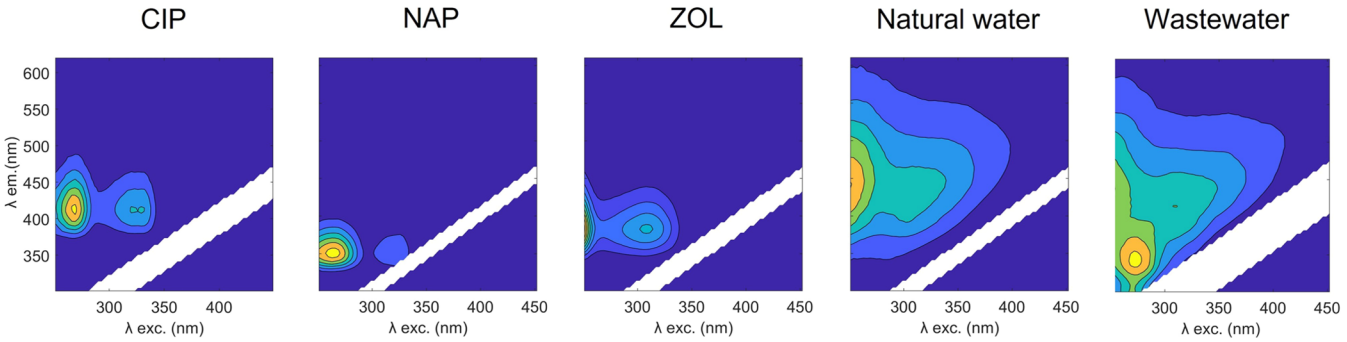

Figure 2. Excitation−emission matrices of dissolved OMPs, natural water, and wastewater.

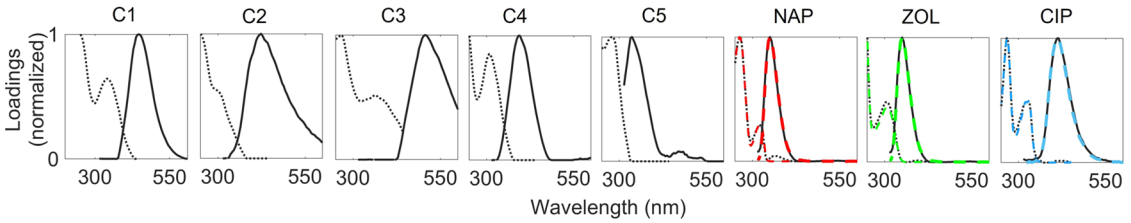

Figure 3. Spectral loadings of DOM and pharmaceuticals obtained using a "partially specified" approach, after the OMPs (CIP = ciprofloxacin, NAP= naproxen, and ZOL= zolpidem) were spiked into samples from a drinking water treatment plant. The DOM spectra (C1−C5) were presumed, whereas black CIP, NAP, and ZOL spectra were estimated. The overlaid blue, red and green lines represent true spectra of each OMP dissolved in ultrapure water.

estimated concentrations of OMPs were obtained from calibration curves. This approach might be used in practice to quantify a fluorescent OMP that is known to be present in a water sample, for example, because it has been spiked into a batch reactor.

The accuracy of the OMP recovery was calculated the same way for all data sets and modeling approaches according to eq 1.

$$\text{accuracy (\%)} = \frac{\text{OMP predicted concentration}}{\text{OMP spiked concentration}} \times 100 \quad (1)$$

3. RESULTS AND DISCUSSION

3.1. Fluorescence Characteristics of OMPs and Aquatic Samples. Table 1 compares molar fluorescence and quantum yield (ratio between the photon number emitted and photon number absorbed) for the three OMPs. Higher values for ZOL indicate that of the three OMPs, ZOL should have lowest detection limits if this would depend only on how much fluorescence it produces per unit concentration.

Fluorescence EEMs for the three OMPs are shown in Figure 2 alongside typical EEMs for NW and WW samples. Because there is near-complete spectral overlap between the OMPs and DOM fluorescence, these OMPs cannot be quantified by fluorescence spectroscopy without first subtracting the background interference due to DOM. A simple blank subtraction procedure will not work because DOM fluorescence varies in response to physical, chemical, and biological processes, i.e., an appropriate blank consisting of background fluorescence without the presence of the OMP would not exist in any real situation. As an example, consider an OMP spiked at a known concentration into a biological reactor. Fluorescence due to the background DOM can be determined prior to spiking, so it is possible to perform a normal blank subtraction for the initial time point. However, biological reactions will quickly begin to impact the abundance of both the OMP and the background DOM so that after a few hours, neither will be accurately known. Similarly, blanks are automatically missing in the case of natural samples collected from, e.g., lakes or water treatment systems. In all of these cases, blank subtraction must be performed using a chemometric approach.

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ACS EST Water 2023, 3, 3905−3915

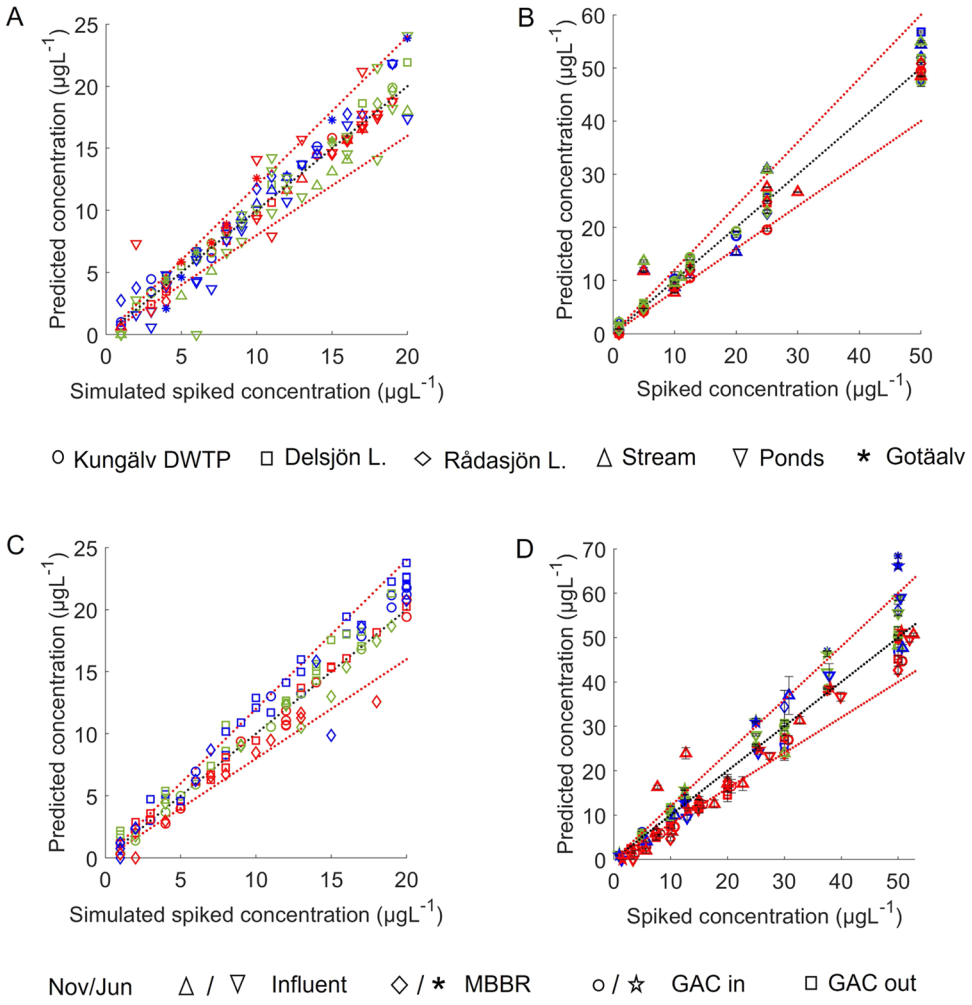

Figure 4. Correspondence between spiked concentrations of CIP (blue), NAP (red), and ZOL (green) and predicted concentrations of the same using the NW model (A, B) or WW model (C, D) in simulated samples (A, C) or real samples (B, D). A "partially specified" modeling approach was used for lake, river, and drinking water samples, and a "fully specified" approach was used for stream, pond, and wastewater samples. Samples enclosed within red dotted lines have perfect correspondence ±20%.

As described above, a five-component PARAFAC model with assumed spectral properties was used to capture the fluorescent background due to the natural sample DOM ("NW model"). That model is reproduced in the Supporting Information (Figure S2A) and is also shown as the first five components in Figure 3. Supporting Figure S3 presents the WW model derived in this study and used to capture and eliminate background fluorescence for wastewater samples.

3.2. Recovery of OMP Spectra and Concentrations from Natural Waters. Figure 3 illustrates the recovery of OMP spectra from NW samples spiked with OMPs using a "partially specified" modeling approach. The first five plots represent the five PARAFAC components from the NW model which were used as "prior knowledge", and the final three plots represent the extra components recovered by PARAFAC modeling. The extra components match the pure spectra of the three added OMPs with very high congruencies (TCC ex/em > 0.998/0.998 for CIP, NAP, and ZOL). Accurate recovery of the pure OMP spectra was achieved by PARAFAC despite a strong overlap between the excitation and emission spectra of the PARAFAC model compared to the OMPs.

Figure 4 shows the correspondence between the spiked concentrations of the OMPs and their predicted concentrations in simulated and real samples. In all natural samples with no spiked OMPs, the predicted concentrations were below the LOD and LOQ. For most natural water samples at concentrations above the LOQ (Figure 4A,B), the predicted concentrations were within 20% of actual simulated or spiked concentrations, with the most accurate recoveries (±10% for NAP and ZOL) achieved at the highest concentrations. Accuracy was lower for pond samples in the simulation than other types of samples, indicating that the NW model had difficulty capturing the natural complexity of DOM fluorescence in ponds and distinguishing this from OMP fluorescence. Like in the wastewater samples, the pond water samples often had relatively strong protein-like fluorescence having significant spectral overlap with short-wavelength fluorescence from OMPs.

Overall, LOD and LOQ (Table 2) calculated according IUPAC's recommendations[28,29] using a 95% confidence interval for the three pharmaceuticals in real samples were 1.0−3.3 and 2.9−9.3 μg L^{-1}, respectively. Similar LOD and LOQ values were achieved for all three OMPs; however, ZOL could be quantified at slightly lower concentrations due to its higher molar fluorescence (Table 1).

Surprisingly, LOD, LOQ values, and regression slopes for real spiked samples were only marginally worse than for simulated contaminated samples (Tables S3 and S4), although

https://doi.org/10.1021/acsestwater.3c00323
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Table 2. Limits of Detection and Quantification for Real Samples of Natural Water iked with individual OMPs or OMP mixtures.

	natural water real samples (μg L^{-1})					
	CIP		NAP		ZOL	
matrices	LOD	LOQ	LOD	LOQ	LOD	LOQ
DWTP[a]	1.9	5.4	1.2	3.3	1.4	4.1
Delsjön Lake[b]	1.9	5.4	1.3	3.8	1.3	3.5
Rådasjön Lake[c]	3.3	9.2	1.7	4.7	1.0	2.9
Streamwater[b]	1.5	3.2	2.1	3.5	1.3	3.5

[a]Mix of three OMPs (cip/nap/zol) [b]Each OMP individually. [c]Mix of two OMPs (cip/nap, nap/zol, cip/zol).

simulated data sets often produce overly optimiztic results. Also, although samples collected from the same drinking water treatment plant in an earlier study were used to develop the PARAFAC model, DWTP samples in this study did not produce better detection limits than other samples. This suggests that the main factor responsible for the detection limits was the OMP signal (including spectral properties, concentration, and quantum yield) rather than the DOM background and how well it corresponded to the assumptions of the a priori PARAFAC model. Results in Section 3.5, where a different chemometric technique for recovering the OMP concentrations produced detection limits in the same order of magnitude, further support this assertion.

3.3. Recovery of OMPs from Wastewater Samples. Background levels measured by UPLC-MS/MS were in the low ng L^{-1} range (1.9−4.6 ng L^{-1}) for ZOL, both in influent and effluent wastewater. CIP and NAP concentrations were <30 ng L^{-1} and <0.8 μg L^{-1} in the effluent, respectively, while for untreated wastewater, they were in the range of 0.4−0.8 μg L^{-1} for CIP and 2.0−2.7 μg L^{-1} for NAP (Table S5). In Figure 4, the horizontal axis takes into account these background OMP concentrations. In all wastewater samples with no spiked OMPs, the predicted concentrations were below the LOD and LOQ, including in influent samples where the OMP concentrations were highest.

The recovery of the concentrations of the OMP from wastewater samples, using a "fully specified" approach wherein the pure spectral loadings for each OMP were passed to the model, is also shown in Figure 4. At OMP concentrations above 10 μg L^{-1}, recoveries were within 20% of actual concentrations for simulated samples (Figure 4C) as well as real spiked samples (Figure 4D). Accuracy deteriorated below 10 μg L^{-1}, resulting in higher LOD and LOQ values than for natural samples (Table 3). This is probably due to the higher complexity of wastewater fluorescence and also higher DOC concentrations (Table S6) and may also indicate a less reliable PARAFAC model. Interestingly, OMP recoveries in influent samples (>10 μg L^{-1}) were quite similar to recoveries in other treatment steps although no influent samples were used to build the WW model.

When spiked alone, LODs and LOQs for the three OMPs ranged between 0.6 and 5.0 and 1.5−14.2 μg L^{-1}, respectively. Spiking the OMPs together reduced the sensitivity and resulted in higher detection limits (Table 3). These findings are expected since better detection limits are expected when there are fewer uncalibrated sources of fluorescence interference.

Table 3. Limits of detection and quantification for real wastewater samples spiked with individual OMPs or OMP mixtures.

		wastewater samples (μg L^{-1})					
		CIP		NAP		ZOL	
matrices		LOD	LOQ	LOD	LOQ	LOD	LOQ
influent	Nov[a]	9.0	23.1	7.7	17.9	2.6	6.1
	Nov[b]			1.6	4.1		
	June[b]	5.0	14.2	2.1	5.8	1.3	3.5
MBBR	Nov[a]	4.9	12.5	4.3	11.0	2.3	5.9
	Nov[b]			0.6	1.5		
	June[b]	3.6	10.0	1.3	3.8	1.0	2.7
GAC in	Nov[a]	3.1	8.0	2.7	6.9	1.5	4.0
	Nov[b]			0.9	2.1		
	June[b]	1.9	5.3	1.0	2.8	1.9	5.5
GAC out	Nov[a]	2.5	6.3	2.4	6.1	1.9	4.9
	Nov[b]			1.2	3.0		

[a]Mix of three OMPs (cip/nap/zol) [b]Each OMP was spiked individually.

3.4. Comparing Aquatic Matrices, PARAFAC Models, and Modeling Approaches. Figure S4 summarizes the results for the three OMPs spiked experimentally into the two aquatic matrices and modeled using the two different approaches. Specifically, it shows the accuracy of recovering the correct OMP concentrations when spiked individually or as a mixture of three OMPs, using either a "partially specified" modeling approach (fixed components = 5, estimated components = 1 or 3) or a "fully specified" approach (fixed components = 6 or 8, estimated components = 0). The fixed components were derived from the NW model in the case of the natural water samples and the WW model in the case of wastewater samples.

For natural water samples, prediction accuracies are seen to differ little by the modeling approach, although the "fully specified" approach outperformed the "partially specified" approach for stream and pond samples where there were higher DOC concentrations (>9 mg L^{-1}) compared with other samples (DOC < 7 mg L^{-1}) (Table S6). For wastewater samples, the "fully specified" approach significantly outperformed the "partially specified" approach when quantifying CIP and NAP (Figure S4). This indicates that in complex matrices with higher DOC, greater accuracy is likely to be achieved by including the spectral properties of the target OMPs in the a priori PARAFAC model.

It was also observed that recoveries were poor when switching the models, i.e., if using the NW model to capture DOM fluorescence in wastewater samples instead of the WW model and vice versa. Figure S5 in the Supporting Information compares these two models and shows that they have generally high spectral overlap but also important differences. In particular, strong secondary emission peaks are seen for tryptophan-like component C4 and microbial-like component C3 in the WW model, whereas in the NW model, the secondary peak is very small for tryptophan-like C5 and absent for microbial-like C4 (Figure S5). Wastewater DOM is generally more concentrated and more complex than natural DOM with larger contributions from microbially linked fluorescence components.[30,31] Such components are typically characterized by short-wavelength emission peaks for which there is a high potential for their emitted fluorescence to be

https://doi.org/10.1021/acsestwater.3c00323
ACS EST Water 2023, 3, 3905−3915

reabsorbed (quenched) by humic DOM. For example, tryptophan fluorescence has been shown to strongly quench humic-like fluorescence under experimental conditions,[32] in violation of the additivity and trilinearity assumptions underlying PARAFAC. These secondary emission peaks in the WW model may therefore be an attempt by PARAFAC to account for significant quenching interactions, explaining the poorer fit of wastewater samples to the NW model.

The WWTP samples in this study came from a real treatment plant in which a range of fluorescent and nonfluorescent OMPs are known to occur. Influent OMPs at this plant have previously been measured in the low $\mu g\ L^{-1}$ concentration range for NAP, compared to ng L^{-1} for CIP and ZOL. Thus it would not be possible to reliably quantify these OMPs by fluorescence spectroscopy in real samples from this plant. In particular, ZOL concentrations at the WWTP were 3 orders of magnitude lower than the LOQ in this study, e.g., 2.6−4.9 ng L^{-1} in influents and 0.9−2 ng L^{-1} in effluents.[33]

At the same time, detection limits for CIP and NAP in the low $\mu g\ L^{-1}$ concentration range may be sufficiently sensitive for quantifying these OMPs in environmental samples at some other locations. CIP and NAP are very frequently measured in WWTPs; earlier studies reported that they occurred in approximately 21 and 87.5−100% of WWTP influents and 8.3% and 100% in WWTP effluents, respectively.[7,34] In other studies, influent concentrations have frequently been reported in the range of 0.5−10.5 $\mu g\ L^{-1}$ for CIP[6,7] and 0.1−33.4 $\mu g\ L^{-1}$ for NAP,[7,35,36] although higher concentrations have also been reported, e.g., CIP (140 $\mu g\ L^{-1}$)[11] and NAP (452 $\mu g\ L^{-1}$).[12] In effluents, concentrations have been reported at 0.2−5 $\mu g\ L^{-1}$ for CIP[7] but as high as 58 $\mu g\ L^{-1}$[11] and 0.002−6.412 $\mu g\ L^{-1}$ for NAP.[7] This suggests that the new method may support direct measurement of CIP and NAP concentrations in some highly contaminated waterbodies.

3.5. Other Approaches for Quantifying Fluorescent OMPs. Previously, Qian et al.[32] used parallel factor framework-clustering analysis (PFFCA) to separate and quantify fluorescent components in a mixture including humic DOM; furthermore, they helpfully published code that allows others to apply the same method. Unlike PARAFAC, PFFCA handles nontrilinear data structures; still, when tested with samples from the current data set, PFFCA produced similar OMP detection limits compared to using the PARAFAC-based method (Table S7). Additionally, the PFFCA method had some drawbacks. First, the lack of an a priori model meant that it was necessary to test many different models although the algorithms have not yet been optimized for speed. Also, it did not work to accurately quantify more than one OMP at a time, as was possible using PARAFAC. Finally, it was not possible to quantify OMPs in a single sample because the model requires multiple samples to learn to distinguish OMPs from DOM. An advantage of the "fully specified" approach is that due to using an a priori PARAFAC model, postprocessing is rapid and it is possible to evaluate either a single sample or a large data set (hundreds of water samples) containing all three OMPs in just a few minutes.

Other earlier studies used DOM fluorescence components as proxies of various nonfluorescent OMPs based on observed correlations between DOM and OMPs in a water treatment plant.[9,10] However, this indirect approach involves a range of assumptions and has an unverified applicability outside of the specific context where the model was developed. Other methods used to quantify OMPs using fluorescence include inducing chemical reactions to enhance native fluorescence, e.g., via degradation by Fenton reactions[37,38] or photolysis,[39] or even derivatization reactions[13] to induce new fluorescent products nonoverlapped by DOM; however, this requires often complex sample pretreatment under controlled experimental conditions.

3.6. Applications and Future Challenges. The approaches developed in our study solve the primary weakness of a traditional PARAFAC approach to quantifying OMPs in water containing a fluorescent DOM background, i.e., the difficulty to obtain a reliable PARAFAC model,[40] which is complex due to the need for a large and variable data set to capture all independently varying features, and the difficulty of correctly specifying the appropriate number of model components.[15] This study shows that a generalist a priori PARAFAC model can adequately account for potential variability in the DOM background, making it unnecessary to create a site-specific model. This makes it possible to quantify the concentration of the OMP in individual samples containing unknown background DOM without reference to any other samples except a calibration curve relating the fluorescence of the OMP in pure water to concentration. In the "partially specified" approach, both the OMP spectra and scores are unknown, allowing discovery and quantification of unknown compounds, whereas in the "fully specified" approach, the compound spectra are specified and only scores (concentrations) need to be estimated. This gives enough flexibility to simplify the method if it is already known which OMPs are present, as is often the case in laboratory-scale experiments.

The detection limits for the OMPs in this study were in the low $\mu g\ L^{-1}$ range, which exceeds their expected concentrations in secondarily treated wastewater. NAP typically occurs in wastewater at 10−1000× higher concentrations than either CIP or ZOL; thus, the method may succeed in quantifying NAP in environmental levels at specific locations with a high load of pharmaceutical contaminants. However, this method is likely of greatest utility in experimental studies of OMPs fate and removal where the OMP types and initial concentrations are chosen by the analyst. For example, several prior studies used starting concentrations of 10−100 $\mu g\ L^{-1}$ when investigating CIP, NAP, and ZOL biodegradation and biotransformation at lab scale.[41−43] The current method supports high sampling intensity and rapid data analysis at low cost, which could greatly reduce the time and cost of analyses, especially at laboratories lacking in-house LC-MS/MS capability. This makes it especially suited for rapid screening to compare different experimental designs and for kinetic studies.

OMP elimination and (bio)transformation during water treatment depends on the chemical structure and physicochemical characteristics and how these interact with the treatment process and its operational parameters.[41,44] Each of the three OMPs in this study is representative of different classes of medicines and could serve as proxies for related pharmaceuticals with a similar chemical structure. Since the wastewater PARAFAC model was based on a very limited data set (20 samples all collected during the same week), there is potential to improve the quantification of OMPs in wastewater samples by improving this PARAFAC model. However, it would probably be difficult to achieve a large (i.e., order of magnitude) improvement in the detection limits for these three OMPs by improving the chemometric approach alone

https://doi.org/10.1021/acsestwater.3c00323
ACS EST Water 2023, 3, 3905−3915

because a major constraint is the fluorescence quantum yield, which is an intrinsic property of each fluorophore. However, there is a strong potential for future studies to identify additional fluorescent species that can be rapidly quantified by these methods, increasing the overall benefits of the approach.

4. CONCLUSIONS

The present study demonstrated the direct quantification via fluorescence spectroscopy of several pharmaceuticals of public health and environmental concern in water and wastewater samples at low $\mu g\ L^{-1}$ concentrations. Three OMPs (ciprofloxacin, naproxen, and zolpidem) representing three classes of medicine (antibiotic, NSAID, and sedative hypnotic) could be quantified in natural (river, lake) waters, drinking water, and wastewater by drawing upon an a priori PARAFAC model to eliminate interfering fluorescence from natural organic matter. The approach is much less sensitive than standard analytical techniques for quantifying pharmaceuticals by LC-MS/MS. However, it is also far simpler, quicker, and cheaper and thus is accessible beyond only specialist chemistry laboratories. Whereas the higher detection limits will restrict its application for quantifying environmental OMPs to specific, highly contaminated waterbodies, the approach could be widely useful in facilitating data-intensive laboratory experiments, wherein the analyst is able to specify the types of OMPs studied and their concentration ranges.

■ ASSOCIATED CONTENT

Data Availability Statement

Data from this study are available as *.mat and *.csv files at https://doi.org/10.11583/DTU.24440110.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.3c00323.

Summary of screening tests of the 20 OMPs, effect of pH on fluorescence spectra, quantum yield calculation, OMP concentrations spiked into water samples, modeling approaches used to quantify OMPs, validation of the wastewater model, OMP limits of detection and quantification in simulated samples, comparison of regression slopes of OMP concentrations in simulated and spiked samples, OMP background concentrations by UPLC-MS/MS, dissolved organic carbon and total organic carbon in water samples, prediction accuracy of OMP concentrations in spiked water samples, comparison between the NW model and WW model, and comparison between PARAFAC and PFFCA algorithms (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

L.P.F. acknowledges support from Carl Tryggers Stiftelse (Project 19:246). The authors thank Maria Neth, Ville Tanskanen, and collaborating water treatment plants for supplying samples and data.

■ REFERENCES

(1) Kosek, K.; Luczkiewicz, A.; Fudala-książek, S.; Jankowska, K.; Szopińska, M.; Svahn, O.; Tränckner, J.; Kaiser, A.; Langas, V. Implementation of Advanced Micropollutants Removal Technologies in Wastewater Treatment Plants (WWTPs) - Examples and Challenges Based on Selected EU Countries. *Environ. Sci. Policy* **2020**, *112* (April), 213−226.

(2) Bacci, F.; Campo, P. *Emerging and Less Commonly Recognized Chemical Contaminants: Organic Micropollutants*, 2nd ed.; Mehner, T.; Tockner, K., Eds.; Elsevier: Oxford, 2022; pp 247−259 DOI: 10.1016/B978-0-12-819166-8.00038-4.

(3) Rodrigues, M.; Roman, M.; ter Heijne, A.; Sleutels, T.; Cornelissen, E. R.; Verliefde, A.; Buisman, C. J. N.; Kuntke, P. Characterization of the Organic Micropollutants Behavior during Electrochemical Ammonia Recovery. *J. Environ. Chem. Eng.* **2023**, *11* (3), No. 109613.

(4) Paradina-Fernández, L.; Brasca, R.; Repetti, M. R.; Attademo, A. M.; Peltzer, P. M.; Lajmanovich, R. C.; Culzoni, M. J. Bioaccumulation of Abacavir and Efavirenz in Rhinella Arenarum Tadpoles after Exposure to Environmentally Relevant Concentrations. *Chemosphere* **2022**, *301*, No. 134631.

(5) Golovko, O.; Rehrl, A. L.; Köhler, S.; Ahrens, L. Organic Micropollutants in Water and Sediment from Lake Mälaren, Sweden. *Chemosphere* **2020**, *258*, No. 127293, DOI: 10.1016/j.chemosphere.2020.127293.

(6) Pereira, A. M. P. T.; Silva, L. J. G.; Meisel, L. M.; Lino, C. M.; Pena, A. Environmental Impact of Pharmaceuticals from Portuguese Wastewaters : Geographical and Seasonal Occurrence, Removal and Risk Assessment. *Environ. Res.* **2015**, *136*, 108−119.

(7) Paíga, P.; Correia, M.; João, M.; Silva, A.; Carvalho, M.; Vieira, J.; Jorge, S.; Gabriel, J.; Freire, C.; Delerue-matos, C. *Science of the Total Environment Assessment of 83 Pharmaceuticals in WWTP infl uent and Ef fl uent Samples by UHPLC-MS/MS* ; Hourly Variation, 2019; Vol. *648*, pp 582−600 DOI: 10.1016/j.scitotenv.2018.08.129.

(8) Verlicchi, P.; Al Aukidy, M.; Zambello, E. Occurrence of Pharmaceutical Compounds in Urban Wastewater: Removal, Mass Load and Environmental Risk after a Secondary Treatment-A Review. *Sci. Total Environ.* **2012**, *429*, 123−155.

(9) Sgroi, M.; Roccaro, P.; Korshin, G. V.; Greco, V.; Sciuto, S.; Anumol, T.; Snyder, S. A.; Vagliasindi, F. G. A. Use of Fluorescence

EEM to Monitor the Removal of Emerging Contaminants in Full Scale Wastewater Treatment Plants. *J. Hazard. Mater.* **2017**, *323*, 367−376.
(10) Guillossou, R.; Le, J.; Goffin, A.; Mailler, R.; Varrault, G.; Vulliet, E.; Morlay, C.; Nauleau, F.; Guérin, S.; Rocher, V.; Gaspéri, J. Fluorescence Excitation/Emission Matrices as a Tool to Monitor the Removal of Organic Micropollutants from Wastewater Effluents by Adsorption onto Activated Carbon. *Water Res.* **2021**, *190*, No. 116749.
(11) Archana, G.; Dhodapkar, R.; Kumar, A. Ecotoxicological Risk Assessment and Seasonal Variation of Some Pharmaceuticals and Personal Care Products in the Sewage Treatment Plant and Surface Water Bodies (Lakes). *Environ. Monit. Assess.* **2017**, *189*, No. 446, DOI: 10.1007/s10661-017-6148-3.
(12) Kibuye, F. A.; Gall, H. E.; Elkin, K. R.; Ayers, B.; Veith, T. L.; Miller, M.; Jacob, S.; Hayden, K. R.; Watson, J. E.; Elliott, H. A. Science of the Total Environment Fate of Pharmaceuticals in a Spray-Irrigation System : From Wastewater to Groundwater. *Sci. Total Environ.* **2019**, *654*, 197−208.
(13) Leitão, J. M. M.; Simões, E. F. C.; Esteves Da Silva, J. C. G. PARAFAC Based Methods for the Analysis of Diltiazem Drug Excitation Emission Matrices of Fluorescence Obtained by a Derivatization Reaction. *Anal. Methods* **2011**, *3* (12), 2758−2769.
(14) Murphy, K. R.; Timko, S. A.; Gonsior, M.; Powers, L. C.; Wünsch, U. J.; Stedmon, C. A. Photochemistry Illuminates Ubiquitous Organic Matter Fluorescence Spectra. *Environ. Sci. Technol.* **2018**, *52* (19), 11243−11250.
(15) Wünsch, U. J.; Murphy, K. A Simple Method to Isolate Fluorescence Spectra from Small Dissolved Organic Matter Datasets. *Water Res.* **2021**, *190*, No. 116730, DOI: 10.1016/j.watres.2020.116730.
(16) Philibert, M.; Luo, S.; Moussanas, L.; Yuan, Q.; Filloux, E.; Zraick, F.; Murphy, K. R. Drinking Water Aromaticity and Treatability Is Predicted by Dissolved Organic Matter Fluorescence. *Water Res.* **2022**, *220* (April), No. 118592.
(17) Wünsch, U. J.; Murphy, K. R.; Stedmon, C. A. Fluorescence Quantum Yields of Natural Organic Matter and Organic Compounds: Implications for the Fluorescence-Based Interpretation of Organic Matter Composition. *Front. Mar. Sci.* **2015**, *2* (NOV), 1−15.
(18) Neth, M.; Mattsson, A.; I'Ons, D.; Tumlin, S.; Arnell, M.; Blom, L.; Wilén, B. M.; Modin, O. A Collaborative Planning Process to Develop Future Scenarios for Wastewater Systems. *J. Environ. Manage.* **2022**, *316* (October 2021), No. 115202, DOI: 10.1016/j.jenvman.2022.115202.
(19) Neth, M.; Mattsson, A.; Wilén, B. M.; Modin, O. Weighted Score Ratios (WRS) Give Transparent Weighting in Multicriteria Sustainability Assessments - A Case Study on Removal of Pharmaceutical Residues from Wastewater. *Sci. Total Environ.* **2023**, *893* (June), No. 164792.
(20) Gidstedt, S.; Betsholtz, A.; Falås, P.; Cimbritz, M.; Davidsson, Å.; Micolucci, F.; Svahn, O. A Comparison of Adsorption of Organic Micropollutants onto Activated Carbon Following Chemically Enhanced Primary Treatment with Microsieving, Direct Membrane Filtration and Tertiary Treatment of Municipal Wastewater. *Sci. Total Environ.* **2022**, *811*, No. 152225, DOI: 10.1016/j.scitotenv.2021.152225.
(21) Tröger, R.; Köhler, S. J.; Franke, V.; Bergstedt, O.; Wiberg, K. A Case Study of Organic Micropollutants in a Major Swedish Water Source − Removal Ef Fi Ciency in Seven Drinking Water Treatment Plants and in Fl Uence of Operational Age of Granulated Active Carbon Fi Lters. *Sci. Total Environ.* **2020**, *706*, No. 135680.
(22) Murphy, K. R.; Stedmon, C. A.; Graeber, D.; Bro, R. Fluorescence Spectroscopy and Multi-Way Techniques. PARAFAC. *Anal. Methods* **2013**, *5* (23), 6557−6566.
(23) Kothawala, D. N.; Murphy, K. R.; Stedmon, C. A.; Weyhenmeyer, G. A.; Tranvik, L. J. Inner Filter Correction of Dissolved Organic Matter Fluorescence. *Limnol. Oceanogr. Methods* **2013**, *11* (DEC), 616−630.
(24) Wünsch, U. J.; Bro, R.; Stedmon, C. A.; Wenig, P.; Murphy, K. R. Emerging Patterns in the Global Distribution of Dissolved Organic Matter Fluorescence. *Anal. Methods* **2019**, *11* (7), 888−893.
(25) Moona, N.; Holmes, A.; Wünsch, U. J.; Pettersson, T. J. R.; Murphy, K. R. Full-Scale Manipulation of the Empty Bed Contact Time to Optimize Dissolved Organic Matter Removal by Drinking Water Biofilters. *ACS ES&T Water* **2021**, *1* (5), 1117−1126.
(26) Andersson, C. A.; Bro, R. The N-Way Toolbox for MATLAB. *Chemom. Intell. Lab. Syst.* **2000**, *52* (1), 1−4.
(27) Murphy, K. R.; Parcsi, G.; Stuetz, R. M. Non-Methane Volatile Organic Compounds Predict Odor Emitted from Five Tunnel Ventilated Broiler Sheds. *Chemosphere* **2014**, *95*, 423−432.
(28) Allegrini, F.; Olivieri, A. C. *2.20 - Figures of Merit☆*, 2nd ed.; Brown, S.; Tauler, R.; Walczak, B., Eds.; Elsevier: Oxford, 2020; pp 441−463 DOI: 10.1016/B978-0-12-409547-2.14612-8.
(29) Danzer, K.; Currie, L. A. Guideline for Calibration in Analytical Chemistry— Part 1. Fundamentals and Single Component Calibration. *Pure Appl. Chem.* **1998**, *70* (4), 993−1014.
(30) Chen, W.; Westerhoff, P.; Leenheer, J. A.; Booksh, K. Fluorescence Excitation-Emission Matrix Regional Integration to Quantify Spectra for Dissolved Organic Matter. *Environ. Sci. Technol.* **2003**, *37* (24), 5701−5710.
(31) Borisover, M.; Laor, Y.; Parparov, A.; Bukhanovsky, N.; Box, P. O.; Dagan, B. Spatial and Seasonal Patterns of Fluorescent Organic Matter in Lake Kinneret (Sea of Galilee) and Its Catchment Basin. *Water Res.* **2009**, *43* (12), 3104−3116.
(32) Qian, C.; Wang, L. F.; Chen, W.; Wang, Y. S.; Liu, X. Y.; Jiang, H.; Yu, H. Q. Fluorescence Approach for the Determination of Fluorescent Dissolved Organic Matter. *Anal. Chem.* **2017**, *89* (7), 4264−4271.
(33) Kim, K. Y.; Oh, J. Evaluation of Pharmaceutical Abuse and Illicit Drug Use in South Korea by Wastewater-Based Epidemiology. *J. Hazard. Mater.* **2020**, *396* (April), No. 122622.
(34) Praveenkumarreddy, Y.; Vimalkumar, K.; Bhat, K.; Narayana, H.; Balakrishna, K. Assessment of Non-Steroidal Anti-in Fl Ammatory Drugs from Selected Wastewater Treatment Plants of Southwestern India. *Emerg. Contam.* **2021**, *7*, 43−51.
(35) Elmouatezz, A.; Kermia, B.; Fouial-djebbar, D. Comptes Rendus Chimie Occurrence, Fate and Removal Ef Fi Ciencies of Pharmaceuticals in Wastewater Treatment Plants (WWTPs) Discharging in the Coastal Environment of Algiers. *C. R. Chim.* **2016**, *19* (8), 963−970.
(36) Oliveira, T. S.; Murphy, M.; Mendola, N.; Wong, V.; Carlson, D.; Waring, L. Characterization of Pharmaceuticals and Personal Care products in hospital effluent and waste water influent/effluent by direct-injection LC-MS-MS. *Sci. Total Environ.* **2015**, *519*, 459−478, DOI: 10.1016/j.scitotenv.2015.02.104.
(37) Carabajal, M. D.; Arancibia, J. A.; Escandar, G. M. Excitation-Emission Fluorescence-Kinetic Third-Order/Four-Way Data: Determination of Bisphenol A and Nonylphenol in Food-Contact Plastics. *Talanta* **2019**, *197*, 348−355.
(38) Sciscenko, I.; Garcia-ballesteros, S.; Sabater, C.; Angeles, M.; Escudero-oñate, C.; Oller, I.; Arques, A. Science of the Total Environment Monitoring Photolysis and (Solar Photo) -Fenton of Enro Fl Oxacin by a Methodology Involving EEM-PARAFAC and Bioassays : Role of PH and Water Matrix. *Sci. Total Environ.* **2020**, *719*, No. 137331.
(39) Paradina-Fernández, L.; Brasca, R.; Goicoechea, H.; Culzoni, M. J. Fluorescence-Kinetic Four-Way Data Generation and Modeling for Abacavir Determination in Water Samples. *Microchem. J.* **2020**, *159*, No. 105315.
(40) Bro, R.; Kiers, H. A. L. A New Efficient Method for Determining the Number of Components in PARAFAC Models. *J. Chemom.* **2003**, *17* (5), 274−286.
(41) Fernandez-fontaina, E.; Gomes, I. B.; Aga, D. S.; Omil, F.; Lema, J. M.; Carballa, M. Science of the Total Environment Biotransformation of Pharmaceuticals under Nitri Fi Cation, Nitratation and Heterotrophic Conditions. *Sci. Total Environ.* **2016**, *541*, 1439−1447.

(42) Park, J.; Yamashita, N.; Wu, G.; Tanaka, H. Science of the Total Environment Removal of Pharmaceuticals and Personal Care Products by Ammonia Oxidizing Bacteria Acclimated in a Membrane Bioreactor : Contributions of Cometabolism and Endogenous Respiration. *Sci. Total Environ.* **2017**, *605−606*, 18−25.
(43) Torresi, E.; Tang, K.; Deng, J.; Sund, C.; Smets, B. F.; Christensson, M.; Andersen, H. R. Science of the Total Environment Removal of Micropollutants during Biological Phosphorus Removal : Impact of Redox Conditions in MBBR. *Sci. Total Environ.* **2019**, *663*, 496−506.
(44) Alvarino, T.; Suarez, S.; Lema, J.; Omil, F. Science of the Total Environment Understanding the Sorption and Biotransformation of Organic Micropollutants in Innovative Biological Wastewater Treatment Technologies. *Sci. Total Environ.* **2018**, *615*, 297−306.

https://doi.org/10.1021/acsestwater.3c00323
ACS EST Water 2023, 3, 3905−3915